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Toward drugging the translocon: sequence determinants and cellular consequences Sec61 inhibition

Author
Maglathlin, Rebecca

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Toward drugging the translocon: sequence determinants and cellular consequences of Sec61 inhibition

by

Rebecca L. Maglathlin

DISSERTATION

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"In the discovery of secret things, and in the investigation of hidden causes, stronger reasons are obtained from sure experiments and demonstrated arguments than from probable conjectures and the opinions of philosophical speculators."

-William Gilbert, *Loadstone and Magnetic Bodies, and on The Great Magnet of the Earth,* translated from the 1600 edition of *De Magnete* by P. Fleury Mottelay (Bernard Quaritch, London, 1893)

I would like to thank my mentor, Jack Taunton, for instilling in me that "good" is never enough and that greatness is just as much a matter of hard work and perseverance as it is a function of intelligence and insight.

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The mass spectrometry was completed by Jeff Johnson and Tasha Johnson (Krogan Lab, UCSF and Gladstone Institute). The STAT5 phosphorylation blot in Figure 2.7 was performed by Geoff Smith (Taunton Lab, UCSF).

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Experiments performed by Gonzolo Ureta are cited in the figure legends when necessary.

To my family

Toward drugging the translocon: sequence determinants and cellular consequences of Sec61 inhibition

Rebecca L. Maglathlin

Abstract

Secretory and membrane proteins (secretory proteins) are the conduits through which cells communicate. Membrane proteins make up 35% of the human genome and, though greater than 60% of drug targets are secretory proteins, they remain difficult to inhibit, as many lack traditional small molecule binding sites. In eukaryotes, the biogenesis of most secretory proteins begins with cotranslational translocation into the endoplasmic reticulum (ER). Cotransins are small molecule inhibitors of cotranslational translocation and therefore inhibitors of secretory protein expression. Cotransins inhibit translocation by binding directly to the Sec61 translocon and perturbing the interaction between Sec61α and the targeting sequence of the nascent chain (either an N-terminal signal peptide or the first transmembrane domain). Remarkably, cotransins inhibit translocation of only a small subset of secretory proteins, dependent on the sequence of the targeting signal.

Here, we describe work toward both identifying the sequence determinants of cotransin selectivity and the broader cellular consequences of cotransin inhibition, including the discovery of Sec61α as a novel target in multiple myeloma. Using a quantitative membrane proteomics appraoch, we directly identify targets of a selective

cotransin analog, CT8, in an unbiased manner. We show that CT8 inhibits only 25% of the identified secreted proteome. CT8-sensitivity of signal peptides correlates with the free energy of membrane integration suggesting that 1) signal peptides and transmembrane domains gate Sec61α via a similar mechanism and 2) cotransins interfere with this signal-partitioning step. Finally, we use this metric to predict the sensitivity of novel CT8-sensitive proteins based solely on primary amino acid sequence.

To solidify Sec61α as a novel target for myeloma, we show that cotransin potently induces apoptosis in patient-derived CD138+ cells while leaving CD138- cells virtually unaffected. We begin to explore the myeloma-specific mechanism of CT8-induced apoptosis by showing that the CT8-sensitive ER-resident chaperone $p58^{ipk}$ is an essential protein in multiple myeloma cells.

This study furnishes a method for predicting new cotransin-sensitive proteins, thereby allowing for the rapid identification of clinically relevant arenas in which to begin testing these compounds. Within the context of multiple myeloma, we show that Sec61α is a viable therapeutic target and that cotransins represent a promising lead for further development. Using the information uncovered here, it will be possible to design new analogs to more specifically target proteins relevant to the anti-cancer properties of cotransins.

Table of Contents

List of Tables

List of Figures

Chapter 1

Introduction

1.1 Abstract

Secreted and membrane proteins (secretory proteins) mediate inter- and intracellular communication and are responsible for initiating numerous signaling pathways that result in cell growth, differentiation, proliferation, migration, and even death. Many of these pathways are dysregulated in disease and represent attractive targets for potential chemotherapeutics, yet they can be notoriously difficult to target with traditional small molecule inhibitors. Cotransins represent a novel class of secretory protein inhibitor as they target secretory protein maturation and trafficking rather than protein function.

Cotransins are small molecule inhibitors of the protein translocation channel Sec61α. Nearly all secretory proteins must translocate through this channel into the endoplasmic reticulum (ER) during their biogenesis. Cotransins selectively inhibit the translocation and expression of a subset of secretory proteins and this selectivity appears to be mediated by the first hydrophobic segment of the translocating protein. However, the precise sequence determinants of this selectivity and the full complement of cotransin-sensitive proteins have yet to be discovered.

Multiple myeloma is an incurable cancer of the plasma cells. These antibody-secreting cells are extremely dependent on cytokines, growth factors and adhesion molecules in their microenvironment as well as the integrity of their secretory pathway for survival. As such, myeloma represents an exciting model in which to test the therapeutic potential of Sec61α inhibition by cotransins.

1.2 Secreted and membrane proteins in human disease

Secreted and membrane proteins are the conduit through which cells communicate. They make up 35% of the human genome and over 60% of current drug targets[1]. Many secretory proteins such as cytokines and growth factors act as signaling molecules between cells, binding to cell surface receptors to elicit their effect. Targeting these interactions with small molecules remains challenging as many of these ligands and receptors lack canonical small molecule binding sites[2]. Many current therapies that target cytokine and growth factor signaling are kinase inhibitors that block the action of the intracellular domain of receptor tyrosine kinases or other downstream kinases[3]. While kinase inhibitors have been extremely successful, they suffer from rapid development of resistance by mutation of their target[4] and overexpression of upstream signaling molecules and receptors[5]. One notable example of this is the finding that hepatocyte growth factor (HGF) overexpression in lung cancer, acute myeloid leukemia (AML)[6] and melanoma[7] results in resistance to targeted therapies. Antibody therapies represent another mode of inhibiting membrane bound receptors, though protein therapeutics are hard to administer and expensive to produce[8]. Therefore, there is an increasing need to develop novel small molecules to target this important class of proteins.

1.3 Biogenesis of secreted and membrane proteins

The biogenesis of most eukaryotic secretory proteins requires cotranslational translocation across the membrane of the ER. This process begins as the first hydrophobic segment of the translating nascent polypeptide emerges from the ribosome

(**Figure 1.1**). This segment can be either an N-terminal, cleavable signal peptide (SP) as in the case of secreted, type I membrane, and some multi-spanning membrane proteins, or the first transmembrane domain (TMD) as in the case of type II, type III and the remaining multi-spanning membrane proteins. The signal recognition particle (SRP) binds to this hydrophobic segment[9], which results in a pause in translation[10] and targeting of the entire ribosome nascent chain complex (RNC) to the ER membrane, where it binds to the signal recognition particle receptor (SR) [11,12].

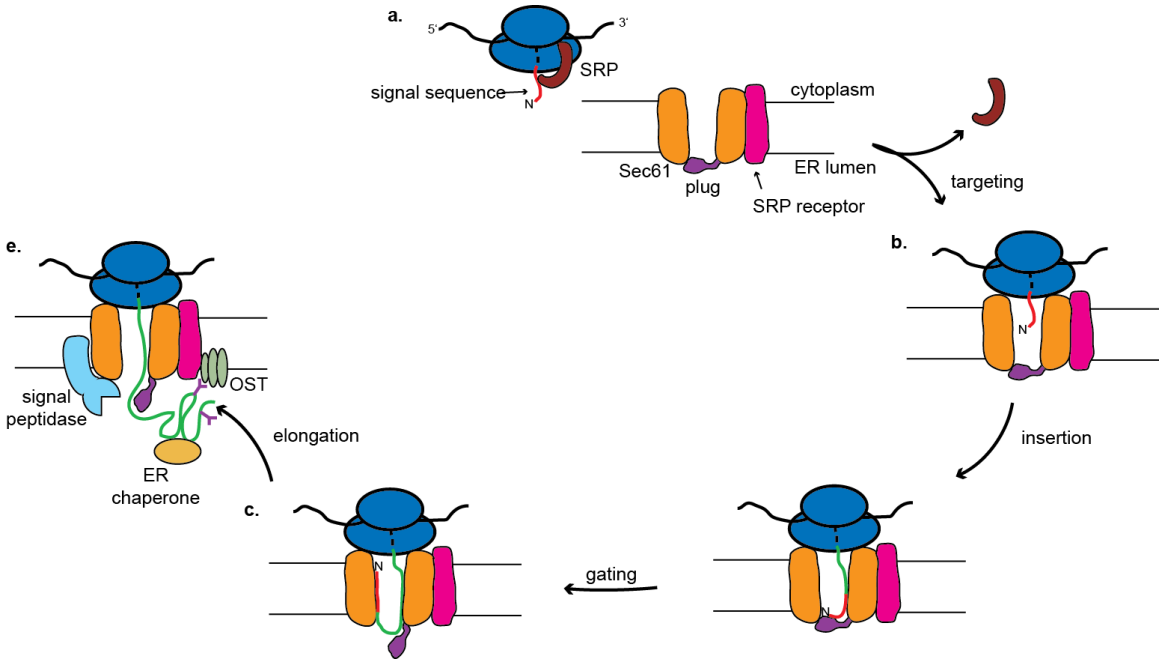

Figure 1.1 | Overview of cotranslational translocation. (a) The first hydrophobic segment of the translating protein is recognized by the signal recognition particle (SRP) and targeted to the membrane of the ER **(b)** where binding of SRP to the SRP receptor (SR) facilitates transfer of the ribosome nascent chain to the Sec61 translocon. **(c)** Continued translation and gating of the channel by the signal peptide or TMD displaces the plug and allows access to the ER lumen where **(d)** the signal peptide is cleaved by signal peptidase and the protein can be glycosylated by oligosaccharide transferases (OST) and folded with the help of ER chaperones.

Binding of SRP to SR at the ER membrane effectively transfers the RNC to an integral membrane protein complex called the translocon[13]. The translocon is primarily comprised of Sec61α, β, and γ, with Sec61α forming the protein-conducting channel[14].

Once the RNC binds to the translocon, the translational pause is lifted and elongation of the polypeptide continues into the lumen of the ER with cotranslational translocation through the pore formed by Sec61α.

Sec61α acts both as a pore through which the translating polypeptide can pass into the ER lumen, and as a gate which opens laterally, allowing translating TMDs to pass into the lipid bilayer where they remain. As translocating sequences gain access to the lumen, they are recognized by chaperones that aid in folding and disulfide bond formation, oligosaccharide transferases that append N-linked glycosides, and in the case of proteins with cleavable SPs, signal peptidase which cleaves the SP at a semi-conserved recognition site[15,16]. These processing steps, along with lateral partitioning of TMDs into the membrane, are necessary for the proper packaging of secretory proteins into vesicles. The vesicles are trafficked through the Golgi and finally fuse with the plasma membrane releasing soluble secreted proteins into the extracellular space and presenting integral membrane proteins on the surface of the cell.

1.4 Substrate recognition by the Sec61 translocon

Sec61α is a 10-helix bundle that forms an hour-glass shaped aqueous pore through the membrane[17]. In the closed state, a small α-helical plug (TM2a) blocks the pore of the channel on the lumenal side **(Figure 1.2a)**, while the interface between TM2b and TM7 forms the lateral gate through which transmembrane domains partition into the lipid bilayer[18] (**Figure 1.2b**). Several models have been proposed to explain how this occurs. In one model, a dynamic equilibrium between open and closed states of the channel allows sampling of the lipid environment by the translocating

polypeptide[19-21]. In another model, supported by persistent and specific cross-linking patterns between Sec61α and inserting TMDs[22,23], specific interactions between the signal peptide or TMD side chains and the channel α-helices are required for productive gating and integration,.

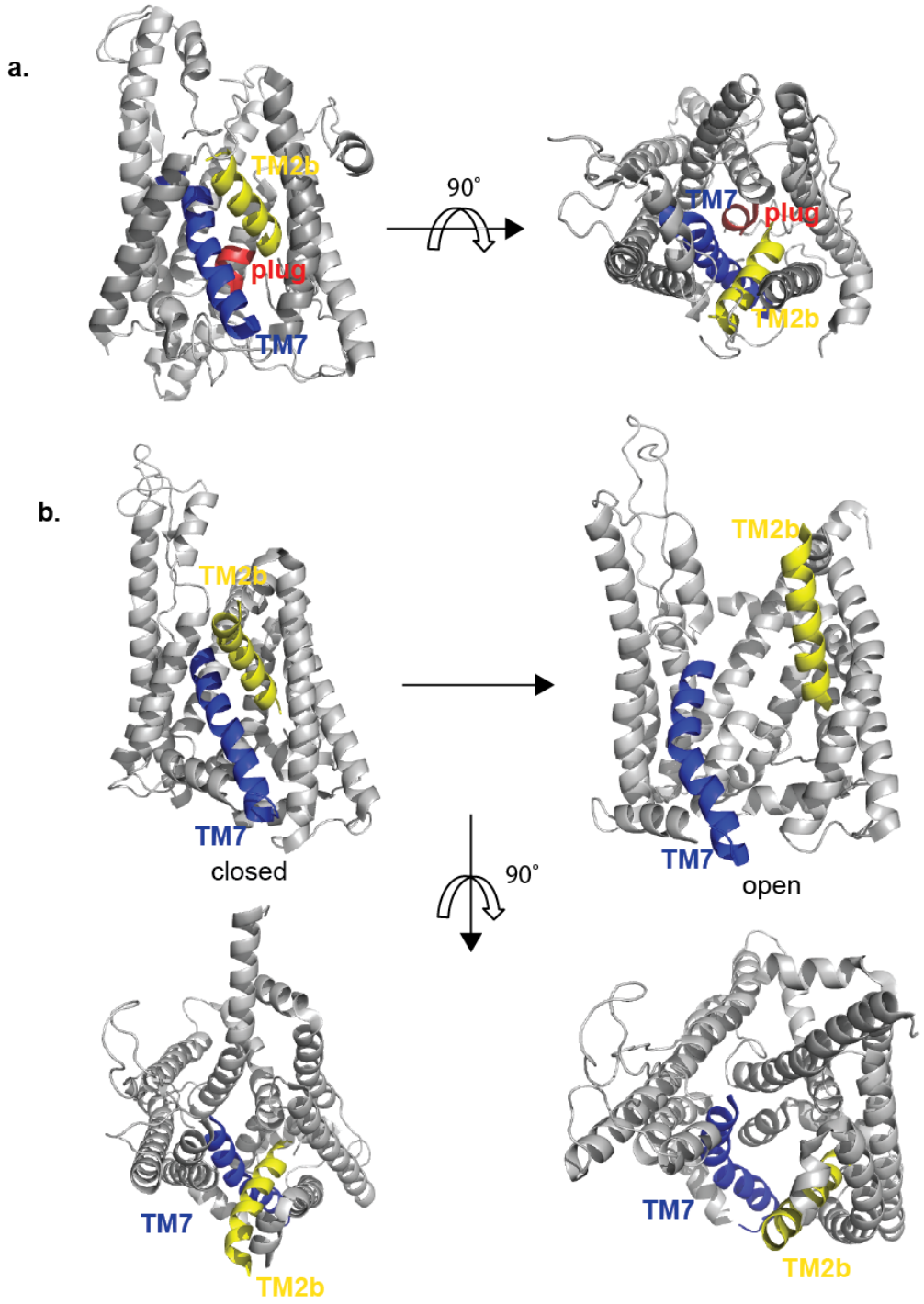

Figure 1.2 | Crystal structure of SecY. (a) SecY (bacterial homolog of Sec61α) channel from *M. jannaschii* in the plane of the membrane (left) with cytosol up and ER lumen down. TM7 (blue) and TM2b (yellow) form the lateral gate through which translating transmembrane domains and signal peptides are thought to partition. The plug (TM2a) is shown in red. On the right is a 90° rotation to show a top-down view of the pore with the plug blocking access to the ER lumen. Structures are adapted from the PDB entry 1RHZ{vandenBerg:2005un} **(b)** Transition from the closed state (left) to the open state (right) of the lateral gate with top down views in the bottom row. Structures are adapted from the PDB entry 3J45 and 3J46{Park:2013hi}.

Regardless of whether the mechanism of channel gating is an active or passive, equilibrium process, there is little doubt, at least in the case of TMDs, that the polypeptide passes through this gate into the lipid bilayer. Whether signal peptides also partition into the lipid bilayer by passing through the lateral gate is less clear. A 2D-crystal structure of SecYEG (the bacterial equivalent of Sec61αβγ) in complex with a signal peptide (as opposed to a signal anchor TMD) showed density for the short alpha-helical peptide intercalated between the lateral gate alpha helices, suggesting that these peptides also transition through this gate[24]. However, this was only a short, 40 amino acid polypeptide rather than a full nascent chain.

More recently, a cryo-EM structure of SecYEG bound to a stalled RNC again showed similar density for the alpha-helical hydrophobic segment of the signal peptide situated within an open lateral gate[25]. In this case, there was also density for the remaining polypeptide chain, looped at the plug region (**Figure 1.3**). Biochemical evidence also suggests that signal peptides have access to lipids through the lateral gate as the signal sequence of pre pro-α-factor was shown to cross-link to lipids as well as TM2 and TM7 of Sec61p during post-translational translocation in yeast[26]. These data suggest that, like TMDs, signal peptides likely partition through the lateral gate. However, whether this partitioning is necessary for the continued translation of SP-containing proteins remains unclear.

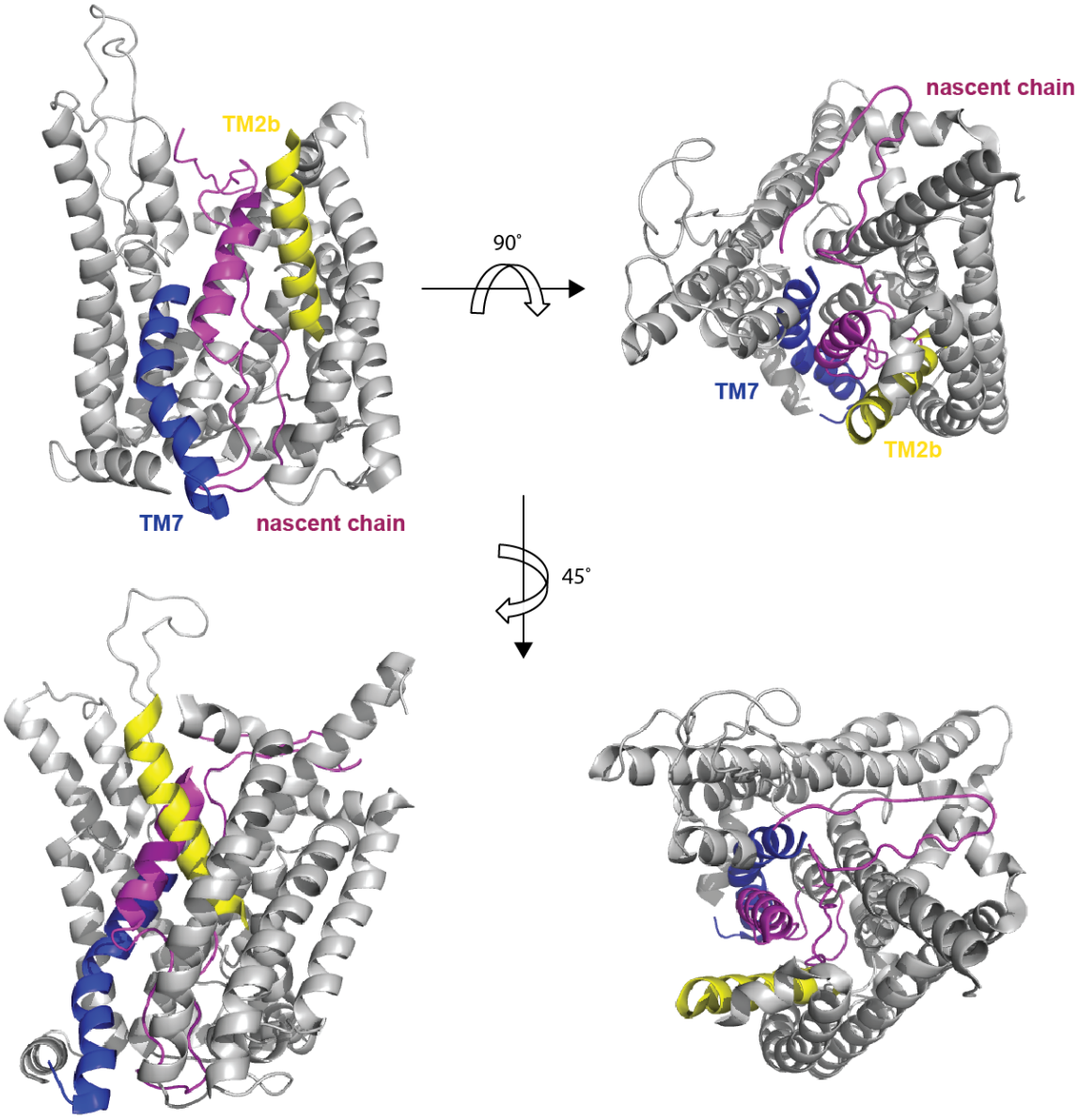

Figure 1.3 | Structure of SecY in complex with a nascent chain. SecY (grey) from *E. coli* shown in complex with a translocating nascent chain (magenta). The hydrophobic region of the signal peptide forms an alpha helix that intercalates between the lateral gate helices of TM2b (yellow) and TM7 (blue). Bottom row shows a 45° rotation to the left from the structures in the top row. Structures are adapted from the PDB entry 3J46{Park:2013hi}.22

1.5 Cotransins are substrate selective inhibitors of Sec61α

Cotransins are small molecule inhibitors of cotranslational translocation and therefore inhibitors of secretory protein biogenesis[27,28] (**Figure 1.4**). They are analogs of the fungally derived cyclodepsipeptide natural product HUN-7293, which was discovered in a screen for inhibitors of induced cellular adhesion molecule expression[29]. Incorporation of a photo-leucine residue into a cotransin analog identified Sec61α as the direct target of this class of compounds[30], and extensive crosslinking experiments suggested that they inhibit cotranslational translocation at the step of lateral gating of Sec61α[23]. Mutations in Sec61α that confer resistance to cotransins cluster at the lumenal end of the lateral gate, strongly suggesting that this may be the cotransin binding site.

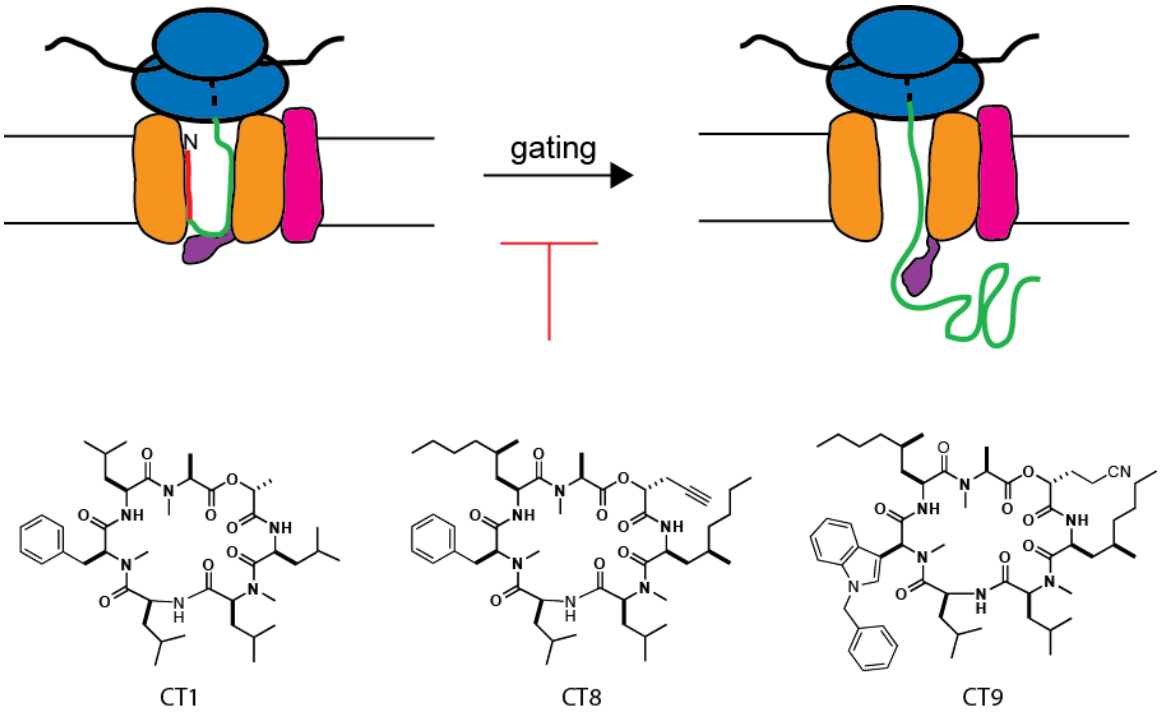

Figure 1.4 | Cotransins inhibit gating of Sec61α. Cotransin binds directly to Sec61α and inhibits the "gating" step of translocation. Three cotransin analogs, CT1, CT8 and CT9 are shown.

The most intriguing feature of cotransins is that they inhibit the translocation and therefore the functional expression of only a small subset of secretory proteins. SP swapping experiments[27,28] confined the selectivity determinants to this region of the polypeptide. Even single point mutations in sensitive SPs[31,32] and signal anchors[23,33] can render them more resistant to inhibition, shifting the dose response up to 100-fold.

The potency and selectivity can also be tuned by changing the structure of the cotransin molecule itself. Whereas the first cotransin, CT1 (**Figure 1.4)**, lacked the potency of the natural product, reintroducing the propyl-leucine side chains and the simple addition of a propargyl group in CT8 restored potency against VCAM-1 and maintained selectivity over ICAM-1, similar to HUN-7293[33]. More surprising was the near complete loss of selectivity for the compound CT9, which differed from CT8 at only two positions. Whereas CT8 significantly inhibited only VCAM-1 and TNFα at concentrations below 100 nM, nearly 80% of proteins tested were affected by CT9 at this concentration[33].

The findings that cotransins, and CT8 in particular, seem to be remarkably selective for only a subset of secretory proteins and that this specificity is dependent on the structure of the compound suggest that even though these compounds target a highly conserved, essential protein (Sec61α), they still may have a therapeutic window to exploit. To date, the only attempt to identify the full complement of proteins affected by cotransin was a targeted screen of 25 secretory proteins, biased toward proteins involved in inflammation[33]. Because cotransin sensitivity can be mapped to the relatively short sequence of the SP or signal anchor, it seems feasible that analysis of sensitive and resistant sequences would reveal selectivity determinants that would allow

prediction of cotransin sensitive proteins from primary amino acid sequence. However, with such a limited number of known sensitive sequences, this analysis has been difficult. Development of a method to predict cotransin-sensitive proteins from primary amino acid sequence would allow for rapid identification of novel targets and therefore therapeutic arenas in which Sec61α inhibition may be beneficial. One such arena where ER proteostasis modulators have had a profound impact is multiple myeloma (MM).

1.6 Multiple myeloma and protein secretion

Multiple myeloma is a hematologic cancer, with approximately 16,000 new cases reported every year in the United States alone[34]. Though treatable with combination chemotherapies and stem cell transplants, multiple myeloma remains incurable due to both innate and developed drug resistance. Thus, there is an increasing need to develop novel therapies for this devastating disease.

Multiple myeloma is characterized by the clonal proliferation of plasma cells in the bone marrow. Plasma cells are terminally differentiated B-cells that are responsible for antibody secretion as part of the humoral immune response. B-cell differentiation begins in the bone marrow where commitment to the B-cell lineage depends upon numerous transcription factors. Ikaros and PU.1 control the expression of essential cell surface proteins such as IL7-Rα. E2a and EBF initiate immunoglobulin heavy chain gene rearrangements, PAX5 limits the expression of lineage-inappropriate genes, and IRF4 promotes light-chain gene rearrangement and the exit from the bone marrow[35].

Upon exit from the bone marrow, these early, naïve B-cells travel to the spleen where they further develop into either marginal zone, follicular, germinal or memory B-

cells. Terminal differentiation to a plasma cell can occur from any of these progenitor B-cells upon stimulation with antigen. Once stimulated, the transcription factors BLIMP1 and XBP1 activate a plasma cell-specific program involving inhibition of cell cycle progression and increased immunoglobulin secretion[36]. To accommodate this ramp up in secretion, plasma cells undergo a massive expansion of their ER coupled with increased expression of numerous ER resident chaperones such as the glucose-regulated 78kD protein (Bip), protein disulfide isomerase (PDI), and the DnaJ proteins, Hsp40 and ERdj3, which act as a Bip co-chaperones along with DnaJ protein homolog 3 ($p58^{ipk}$)[37].

Multiple myeloma results when these carefully timed differentiation steps are dysregulated. Either terminal differentiation to plasma cells occurs prior to exit from the bone marrow or plasma cells from other parts of the body aberrantly home to the bone marrow. In addition, cell cycle arrest is also lifted, and rather than remaining in a non-proliferative state, typical for plasma cells, they continue to proliferate. Myeloma cells are therefore aberrantly proliferating, terminally differentiated, secretory plasma cells in the bone marrow.

Myeloma cells, like many other tumor cells, rely heavily on their microenvironment for survival and resistance to chemotherapeutics. Adhesion to bone marrow stromal cells mediated by vascular cell adhesion molecule 1 (VCAM-1, expressed on stromal cells), very late antigen 4 (VLA-4), CD44, and others triggers secretion of interleukin 6 (IL-6), vascular endothelial growth factor (VEGF) and tumor necrosis factor-α (TNFα), which mediate drug resistance, angiogenesis, and survival[34].

Binding of VLA-4 to fibronectin on stromal cells induces NFκB expression, which leads to cell adhesion-mediated drug resistance[38].

In addition to being heavily reliant on secreted proteins and membrane bound adhesion molecules and receptors for proliferation and survival, myeloma cells are also professional secretory cells. The increased secretory protein flux coupled with dysregulated cell growth likely leads to increased levels of both ER and proteotoxic stress in myeloma cells. Therefore, it is no coincidence that many current clinical treatments for myeloma target protein homeostasis.

The most recent class of MM therapeutics, including bortezomib and carfilzomib, target the 26S proteasome. The proteasome is a multi-protein complex responsible for the degradation of ubiquitinated proteins in the cytosol. Sensitivity of myeloma cells to bortezomib has been correlated to levels of the major transcription factor NFκB[39]. NFκB is activated by proteasomal degradation of its inhibitory binding partner IκB[40]. Proteasomal inhibition then leads to a sustained sequestration of NFκB and a subsequent decrease in expression of its downstream, anti-apoptotic targets. However, NFκB inhibition is not likely the only mediator of proteasome inhibitor toxicity, as susceptibility to a specific NFκB inhibitor does not mirror proteasome inhibition[41]. Therefore, there are likely other proteasome-dependent pathways that contribute to the therapeutic effects of these important molecules. What is important to note is that while their direct target is known, the downstream mechanism by which proteasome inhibitors kill cells remains a mystery and is likely to be pleiotropic in nature.

Sec61α represents a node in protein homeostasis, in some ways analogous to the 26S proteasome. Sec61α is essential for the expression of many of the cytokines,

growth factors, adhesion molecules, and cell surface receptors that myeloma cells depend on for survival within the bone marrow niche, not to mention their primary payload, immunglobulins. Upregulation of many of these secretory proteins is responsible for the development of resistance to the traditional chemotherapies. Cotransins inhibit the expression of both VCAM-1 and TNFα, proteins already implicated in the survival of myeloma cells. We therefore sought to test the hypothesis that Sec61α is a potential target in myeloma.

1.7 Conclusions

Sec61 inhibition represents a novel mechanism for perturbing protein homeostasis. Unlike proteasome inhibitors, cotransins act in a substrate-selective manner dependent on the sequence of the client protein. The studies presented here begin to classify the full complement of cotransin-sensitive substrates and reveal a predictive algorithm for identifying cotransin-sensitive proteins on the basis of their primary amino acid sequence. In addition, they show that, within the context of a cancer defined by protein secretion like multiple myeloma, Sec61α has potential as a therapeutic target both in its own right and in combination with other extremely successful proteostasis modulators.

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Chapter 2

Sequence determinants of Sec61α inhibition by cotransins

2.1 Abstract

Cotransins are a class of small-molecule Sec61 inhibitors that prevent the expression of a subset of Sec61 client proteins by inhibiting their cotranslational translocation into the ER. However, the complement of the human proteome amenable to cotransin modulation has remained largely unexplored. Using quantitative membrane proteomics, we identify targets of a selective cotransin analog, CT8, in an unbiased manner. We show that CT8 inhibits only 6.5% of the global proteome and 25% of the secreted proteome in JJN-3 cells. CT8-sensitive proteins function in a host of biochemical pathways and include multi-spanning integral membrane proteins, whose sensitivity to cotransin is likely mediated by their first transmembrane domain. CT8 sensitivity of a secretory protein correlates with the calculated free energy of membrane integration of its signal peptide (SP), suggesting that cotransins interfere with SP partitioning through the lateral gate of Sec61α. Finally, we use this metric to predict novel targets of CT8, based solely on their primary amino acid sequence.

2.2 Introduction

Cotranslational translocation into the ER, a process mediated by the Sec61 translocon, is an essential step in the biogenesis of nearly all eukaryotic secreted and membrane proteins[1]. Our analysis of a current list of drug targets[2] indicates that over 60% are Sec61 dependent (555 out of 879 proteins), making Sec61 client proteins an important therapeutic class (**Appendix A**). While 25% of Sec61-dependent drug targets are G-protein coupled receptors (GPCRs) and 4% are receptor tyrosine kinases (RTKs), two Sec61 client classes where development of small-molecule inhibitors is relatively

straightforward, many others, such as secreted cytokines, growth factors and even some of their receptors, lack canonical small molecule binding sites and are thus difficult to target with small molecules. In addition, over-expression and gain of function mutations in many of these proteins can confer resistance to targeted small-molecule therapies.

Cotransins are a class of small-molecule Sec61 inhibitors that prevent the expression of Sec61 client proteins by inhibiting their cotranslational translocation[3,4]. While they bind directly to Sec61α[5], only a subset of secretory and membrane proteins is affected and sensitivity is determined by the N-terminal signal peptide or first transmembrane domain (TMD).[4,6]. We recently reported two novel cotransin analogs, CT8 and CT9, that differ dramatically in their selectivity profiles in a targeted screen of 25 secretory proteins, the largest screen to date[7]. CT9 potently inhibited 80% of proteins tested whereas only VCAM-1 and TNFα were inhibited by CT8 at doses less than 100 nM. The selectivity of CT8 in this targeted screen suggested that it would inhibit the translocation of only a small fraction of the secretome. However, an unbiased characterization of the CT8-sensitive proteome has not been performed.

The mechanism underlying cotransin's ability to selectively inhibit protein translocation remains a mystery. While it is known that the N-terminal targeting sequence of a given secretory protein (signal peptide or transmembrane signal anchor) is a key determinant of cotransin sensitivity, the precise sequence features required are unknown. Knowledge of these features would allow for rapid identification of candidate targets from genomic sequence data and would enable identification of cellular pathways amenable to cotransin modulation for potential therapeutic benefit.

Cross-linking studies with the type II signal anchor protein, tumor necrosis factor alpha, have shown that CT8 binding results in an arrested conformation in which the TMD docks to the cytosolic vestibule of Sec61, but is unable to laterally gate the channel and is thus prevented from partitioning into the lipid bilayer[8]. Extensive mutational analysis of the signal anchor demonstrated that sensitivity roughly correlates with decreased hydrophobicity[8], suggesting that hydrophobicity of the signal is a key feature in overcoming CT8 inhibition. This work also showed that mutations in Sec61 that confer resistance to CT8 cluster in the plug region, around the lumenal base of the lateral gate. Collectively, these data suggest that CT8 inhibits lateral gating of the channel and that increasing hydrophobicity increases CT8 resistance for a given sequence. However, this study focused on a type II signal anchor, which is destined to reside in the lipid bilayer, and therefore must transit fully through the lateral gate. The fate of signal peptides and their requirement for gating the channel is less clear.

To date, the number of known CT8-sensitive sequences, coupled with their high sequence variability, has thwarted attempts to identify the sequence determinants of sensitivity and the ability to predict CT8-sensitive proteins from the proteome. In this study, we present the first proteome-wide characterization of CT8-sensitive targets, which include proteins for which therapeutics are currently being developed in the clinic. Using this, the largest dataset of CT8-sensitive and -resistant sequences to date, we show that a biological measure of hydrophobicity, the calculated free energy of integration into the lipid bilayer (ΔG_{calc})[9], can predict a given signal peptide's sensitivity to CT8, enabling the prospective identification of new, potently inhibited targets solely by analyzing the primary amino acid sequence of the predicted signal peptidome.

2.3 Proteomic analysis of CT8-treated cells

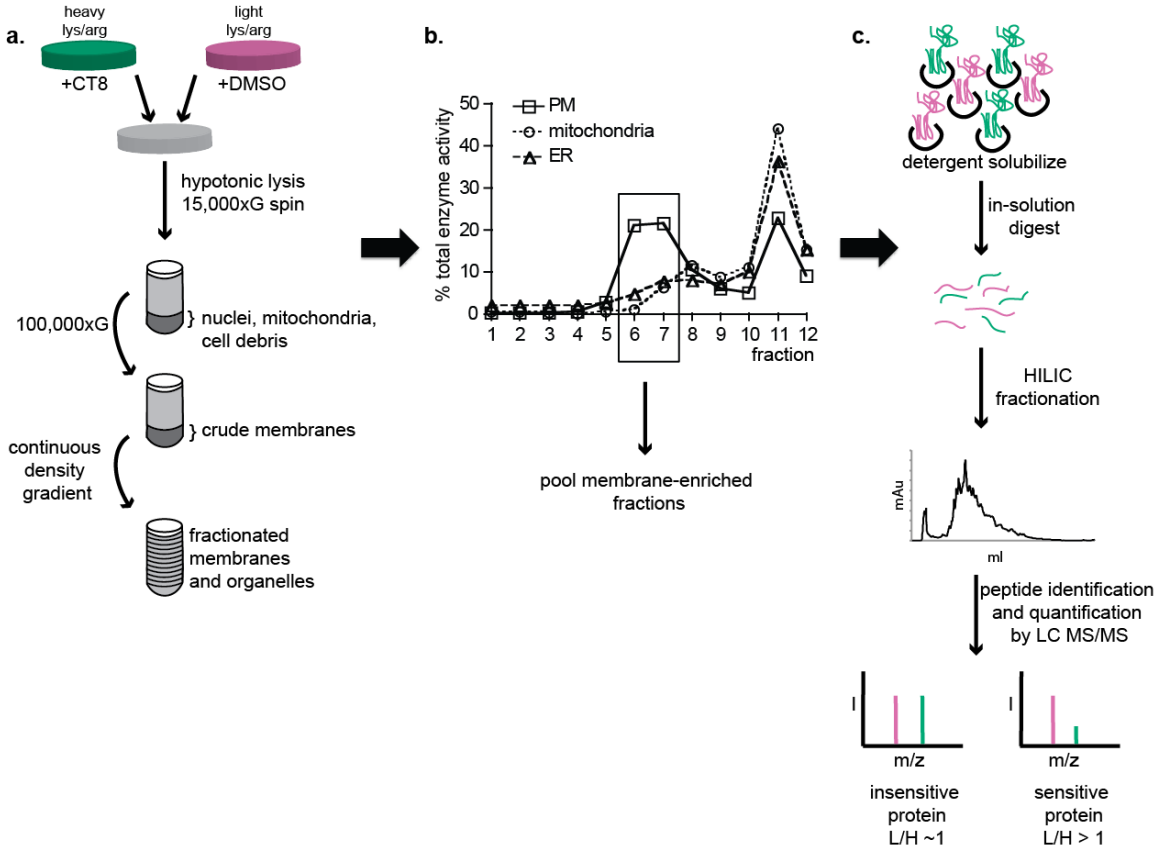

Figure 2.1 | SILAC workflow. (a) JJN-3 cells labeled with heavy arginine and lysine ($^{13}C_6$-lysine, $^{13}C_6$,$^{15}N_4$-arginine) were treated with 250 nM CT8 for 24hrs and then pooled with an equal number of DMSO-treated, light (natural lysine and arginine) labeled cells. Hypotonic lysis and fractionation resulted in 12 fractions **(b)**, which were analyzed for enzyme activity of gamma-glutamyl transferase (PM), succinate dehydrogenase (mitochondria), and cytochrome c reductase (ER). **(c)** Detergent solubilization and in-solution digest gave tryptic peptides that were separated into 12 fractions by hydrophilic interaction liquid chromatography (HILIC) and analyzed by LC MS/MS in duplicate.

To gain an unbiased view of the CT8-sensitive proteome, we determined the effect of CT8 on plasma membrane-localized proteins with Stable Isotope Labeling in Cell Culture (SILAC)[10] and mass spectrometry. JJN-3 cells were labeled in “light” media containing natural amino acids or in “heavy” media supplemented with $^{13}C_6$-lysine and $^{13}C_6$,$^{15}N_4$-arginine. Light, untreated cells and heavy, CT8-treated (250 nM CT8 for 24

hrs) cells were lysed; lysates were pooled and subjected to membrane enrichment using a coupled differential/continuous density gradient centrifugation protocol (**Figure 2.1**). Membrane-enriched fractions were detergent-solubilized, reduced with DTT, alkylated with iodoacetamide and then subjected to hydrophilic interaction chromatography (HILIC). Fractions were collected at 1.5 minute intervals over 1 hour (40 fractions) and were combined into 12 final fractions based on their absorbance at 280 nm. Each of the 12 combined fractions were separately analyzed by LC MS/MS on a Thermo Scientific LTQ Orbitrap Elite mass spectrometer. The SILAC LC MS/MS experiment was performed in in three biological replicates.

Light/heavy ratios ($(L/H)_{final}$) were determined for 2951 unique proteins that were identified with two or more peptides. $(L/H)_{final}$ for each protein was calculated as follows. For a given protein, the median L/H ratio (median $(L/H)_{pep}$) for all identified peptides of a given protein in a given experiment was normalized to the median $(L/H)_{pep}$ ratio for all identified proteins in that experiment, giving $(L/H)_{norm,exp}$. For each protein, the median $(L/H)_{norm,exp}$ value (over the three biological replicates) was termed "$(L/H)_{final}$". For proteins identified in only two replicates, the mean $(L/H)_{norm,exp}$ was used to calculate $(L/H)_{final}$. To determine the statistical significance of $(L/H)_{final}$, a p-value was calculated using a Welch's t test. Briefly, a p-value (p_{exp}) for each protein's $(L/H)_{norm,exp}$ was calculated by randomly sampling $\log((L/H)_{pep})$ from the entire data set 1,000 times and randomized distributions of these $\log((L/H)_{pep})$ values were built for proteins identified with 2 peptides, 3 peptides, and so on up to the maximum number of peptides identified for a single protein. A Welch's t-test probability was calculated to compare two samples having possibly unequal variances. The t value was calculated as:

$$t = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{s_1^2}{N_1} + \frac{s_2^2}{N_2}}}$$

X1 is the median $\log((L/H)_{norm,exp})$ for the protein of interest, X2 is the median $\log((L/H)_{norm,exp})$ for the randomly sampled protein (identified with the same number of peptides), s is the sample variance, and N is the sample size. The degrees of freedom (v) was calculated as N-1. p_{exp} was calculated from *t* and v independently for each protein in each experiment using a t-distribution and then combined over the three experiments to give the combined p-value (p_{final}). Therefore, the p-value can be thought of as the probability of getting a given $(L/H)_{norm,exp}$ for a protein identified with that many peptides at random based on the distribution of $(L/H)_{norm,exp}$ in that experiment. Low p-values ($p \leq 0.05$) are considered significant. It is important to note that, because the Welch's t-test denotes whether a given $(L/H)_{norm,exp}$ is significantly different from the median $(L/H)_{norm,exp}$ for the randomly sampled protein (which will be close to 1), anything with a L/H ratio close to 1 is, by definition going to have a higher p-value and may not reach significance, even though the L/H ratio is highly reproducible. For this reason, resistant proteins (proteins with L/H ≈ 1) were required to have EITHER a $p_{final} \leq 0.05$ OR have an $(L/H)_{norm,exp} \leq 1.36$ in two or more biological replicates.

Of the proteins whose SILAC-based $(L/H)_{final}$ were deemed statistically significant ($p_{final} \leq 0.05$, 1895 proteins), 448 were classified as being Sec61 dependent (**Figure 2.2a**), according to the following criteria. Sec61-dependent proteins were those whose Swiss-Prot entries contained one of the following key word strings: "signal peptide", "type II membrane", "type III membrane", "multi-spanning membrane". For all key word

searches, selected proteins also had to meet the criteria of: organism = human, subcellular localization NOT mitochondria. For proteins classified as "multi-spanning membrane" (multi-TMD), they also could not have “signal peptide” in their entry (multi-spanning membrane proteins with predicted signal peptides were classified as "signal peptide"). Finally, we annotated proteins as “sensitive” or “resistant” on the basis of their $(L/H)_{final}$ and p_{final}.

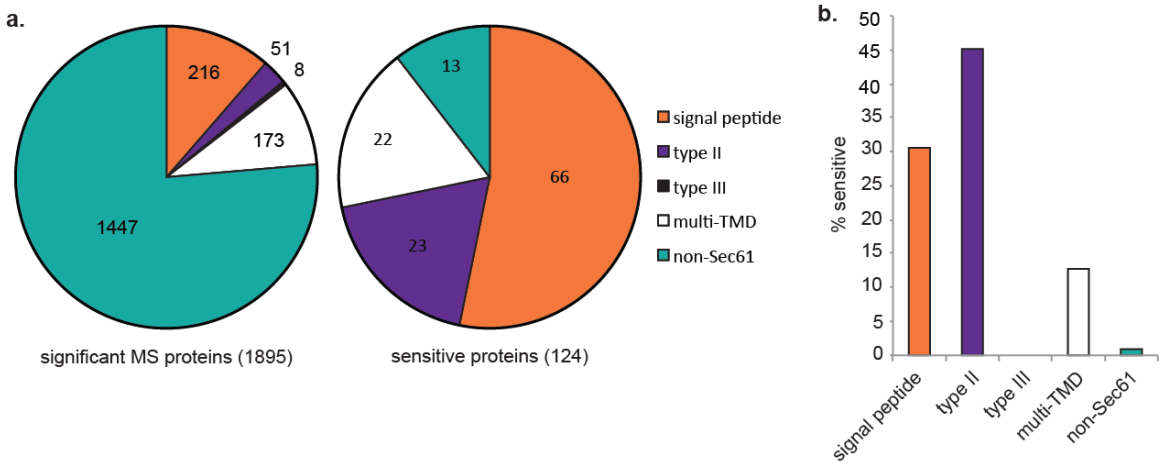

Figure 2.2 | CT8-sensitive proteins are primarily Sec61-dependent. (a) All proteins identified in the SILAC experiement with significant p_{final} ($p_{final} \leq 0.05$, left) and CT8-sensitive proteins (($L/H_{final} \geq 1.5$ and $p_{final} \leq 0.05$, right) were annotated as signal peptide-containing, type II, type III, multi-spanning transmembrane (multi-TMD) or non-Sec61 according to bioinformatics analysis of their Swiss-Prot entries as described in the text and Experimental procedures. **(b)** Percent of each Sec61-dependent class inhibited by CT8.

We wanted to identify very sensitive proteins, ideally with an apparent $EC_{50} \leq 100$ nM. To identify the lowest possible $(L/H)_{final}$ for a given protein that would correspond to this EC_{50}, we assumed no degradation of the protein and one doubling over the 24-hour treatment and plotted a theoretical inhibition curve for a protein with an EC_{50} of 100 nM (**Figure 2.3a**). Based on this dose-response curve, at a dose of 250 nM (the concentration used in the SILAC experiment), there would be a 71% loss in protein, resulting in an $(L/H)_{final} = 1.55$ which we rounded down to 1.5 for simplicity (**Figure**

2.3b). Although few proteins experience no degradation over 24 hrs, an L/H ratio of 1.5 represents the lower bound for proteins with an EC_{50} = 100 nM.

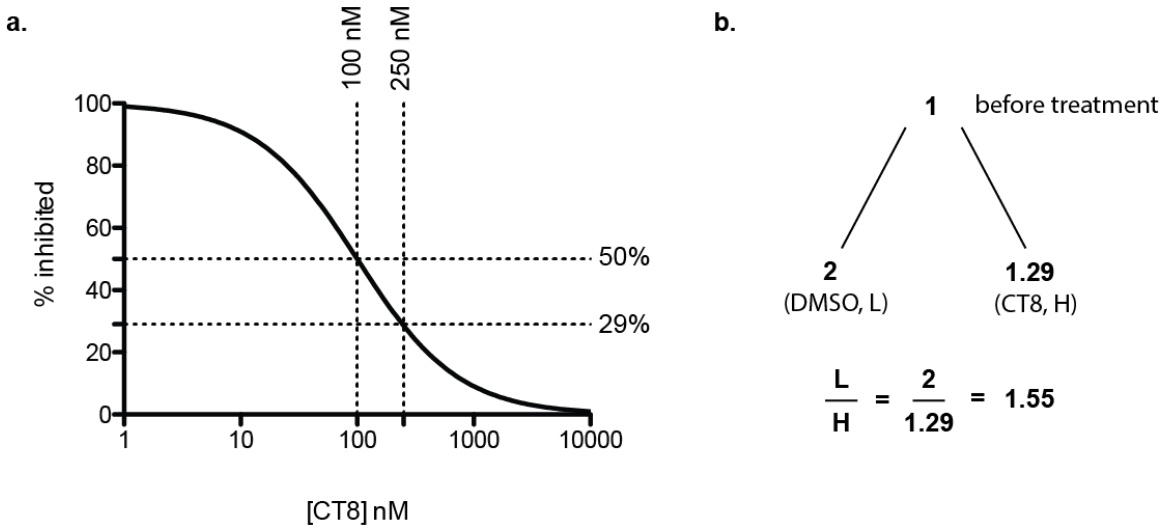

Figure 2.3 | Calculation of the L/H ratio for a theoretical protein with an EC_{50} = 100 nM. (a) A theoretical dose response curve was plotted using GraphPad Prism and a three parameter log[Ct8] vs normalized response for a protein with an EC_{50} of 100 nM and an EC_{100} of 10 µM. The % inhibition is shown at both 100 nM CT8 (50%) and 250 nM CT8 (29%). The SILAC experiment was carried out at 250 nM. **(b)** Assuming zero degradation and one doubling, after 24 hours with CT8 the L/H ratio for this protein would be 1.55.

Proteins with a $p_{final} \leq 0.05$ and $(L/H)_{final} \geq 1.5$ were defined as "sensitive" (**Figure 2.4**). Only 6.5% of all identified proteins and 25% of identified Sec61-dependent proteins met these criteria (**Table 2.1**). Proteins with an $(L/H)_{final} \leq 1.36$ (dataset median + one standard deviation) and a $p_{final} \leq 0.05$ (or with $(L/H)_{norm,exp} \leq 1.36$ in at least two biological replicates) were annotated as resistant. The majority of Sec61-dependent and nearly all Sec61-independent proteins were found to be resistant. Proteins with $(L/H)_{final}$ ratios between 1.36 and 1.5 were considered borderline sensitive and were not considered further.

Figure 2.4 | CT8 inhibits a subset of secretory proteins. Log($(L/H)_{final}$) for all proteins identified with 2 or more peptides in any of three biological replicates is plotted against p_{final}. $(L/H)_{final}$ was calculated by taking the median of all peptide L/H ratios for a given protein in a given experiment and normalizing that to the median L/H ratio for all proteins in that experiment. Calculating the median of the normalized L/H ratios for three independent biological replicates gave $(L/H)_{final}$ for a given protein. P_{final} is a combined p-value calculated using a Welch's t test of a given L/H ratio compared to a randomized distribution of L/H ratios as described in the text and Experimental procedures.

Of the 124 proteins annotated as CT8-sensitive, 111 were Sec61 dependent, suggesting their cotranslational translocation could be directly inhibited by CT8 (**Figure 2.2a**). 22 of these potential targets were multi-spanning membrane proteins with no cleavable signal peptide. Prior to this study, CT8 was known to affect only secreted, type I and type II single-spanning integral membrane proteins. While they seem to be intrinsically more sensitive (**Figure 2.2b**), the data suggest that multi-spanning membrane proteins may also be CT8-sensitive Sec61 clients as well. CT8-mediated effects on Sec61-independent proteins are likely to be indirect.

Swiss-Prot	Description	$(L/H)_{final}$	p_{final}	Sec61?
P14210	HGF Hepatocyte growth factor	6.37	8.44E-12	SP
Q9BRK5	CAB45 45 kDa calcium-binding protein	5.76	2.73E-05	SP
Q9NQX7	ITM2C Integral membrane protein 2C	5.31	2.03E-05	type II
P22794	EVI2A Protein EVI2A	3.76	2.82E-04	SP
Q6UWB1	I27RA Interleukin-27 receptor subunit alpha	3.69	2.01E-04	SP
O94779	CNTN5 Contactin-5	3.57	8.05E-05	SP
P04233	HG2A HLA class II histocompatibility antigen gamma chain	3.56	3.71E-09	type II
O60512	B4GT3 Beta-1,4-galactosyltransferase 3	3.51	5.35E-04	type II
Q86X52	CHSS1 Chondroitin sulfate synthase 1	3.49	6.53E-04	type II
Q9P2E5	CHPF2 Chondroitin sulfate glucuronyltransferase	3.18	8.16E-04	type II
P78310	CXAR Coxsackievirus and adenovirus receptor	2.90	2.35E-06	SP
Q12860	CNTN1 Contactin-1	2.82	1.93E-02	SP
Q9UKQ2	ADA28 Disintegrin and metalloproteinase domain-containing protein 28	2.79	3.59E-04	SP
P33908	MA1A1 Mannosyl-oligosaccharide 1,2-alpha-mannosidase IA	2.79	3.42E-17	type II
P01591	IGJ Immunoglobulin J chain	2.76	4.66E-04	SP
P18084	ITB5 Integrin beta-5	2.73	1.03E-05	SP
Q14165	MLEC Malectin	2.65	5.06E-03	SP
Q5VW38	GP107 Protein GPR107	2.62	1.36E-03	SP, multi-TM
Q11201	SIA4A CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase 1	2.60	1.87E-10	type II
P52333	JAK3 Tyrosine-protein kinase JAK3	2.55	5.25E-06	other
P31785	IL2RG Cytokine receptor common subunit gamma	2.55	1.76E-07	SP
Q9HBR0	S38AA Putative sodium-coupled neutral amino acid transporter 10	2.54	4.49E-04	multi-TM
P06756	ITAV Integrin alpha-V	2.52	2.26E-10	SP
Q9UKM7	MA1B1 Endoplasmic reticulum mannosyl-oligosaccharide 1,2-alpha-mannosidase	2.49	7.56E-11	type II
O95297	MPZL1 Myelin protein zero-like protein 1	2.46	1.27E-03	SP
Q9NXS2	QPCTL Glutaminyl-peptide cyclotransferase-like protein	2.44	2.86E-03	type II
Q8NBJ4	GOLM1 Golgi membrane protein 1	2.41	2.23E-04	type II
Q8IZA0	K319L Dyslexia-associated protein KIAA0319-like protein	2.37	7.30E-03	multi-TM
Q9HC07	TM165 Transmembrane protein 165	2.35	9.69E-06	SP, multi-TM
P20036	DPA1 HLA class II histocompatibility antigen, DP alpha 1 chain	2.35	4.21E-11	SP
P52799	EFNB2 Ephrin-B2	2.35	2.25E-07	SP
P54756	EPHA5 Ephrin type-A receptor 5	2.33	2.43E-08	other
Q13641	TPBG Trophoblast glycoprotein	2.32	7.78E-05	SP
Q96AP7	ESAM Endothelial cell-selective adhesion molecule	2.32	1.33E-02	SP
Q8WTV0	SCRB1 Scavenger receptor class B member 1	2.31	8.82E-06	multi-TM
P32970	CD70 CD70 antigen	2.31	3.04E-07	type II
Q8N2K0	ABD12 Monoacylglycerol lipase ABHD12	2.28	7.69E-03	type II
P27701	CD82 CD82 antigen	2.26	1.24E-08	multi-TM
P09326	CD48 CD48 antigen	2.22	6.09E-08	SP
Q9NPR9	GP108 Protein GPR108	2.22	6.38E-03	other
Q6ZQN7	SO4C1 Solute carrier organic anion transporter family member 4C1	2.19	5.37E-04	multi-TM

Swiss-Prot	Description	$(L/H)_{final}$	p_{final}	Sec61?
Q96AQ6	PBIP1 Pre-B-cell leukemia transcription factor-interacting protein 1	2.18	4.60E-06	other
P19256	LFA3 Lymphocyte function-associated antigen 3	2.14	2.70E-02	SP
P04440	DPB1 HLA class II histocompatibility antigen, DP beta 1 chain	2.10	4.79E-12	SP
P28067	DMA HLA class II histocompatibility antigen, DM alpha chain	2.05	6.19E-04	SP
Q9NZZ3	CHMP5 Charged multivesicular body protein 5	2.04	1.27E-02	other
Q9Y287	ITM2B Integral membrane protein 2B	2.04	4.86E-05	type II
Q9NUM4	T106B Transmembrane protein 106B	2.04	2.50E-04	type II
Q13740	CD166 CD166 antigen	2.03	2.19E-08	SP
P13473	LAMP2 Lysosome-associated membrane glycoprotein 2	2.03	8.05E-10	SP
Q6ZRP7	QSOX2 Sulfhydryl oxidase 2	2.02	5.91E-10	SP
P20701	ITAL Integrin alpha-L	2.01	6.97E-16	SP
Q9BY67	CADM1 Cell adhesion molecule 1	1.98	4.43E-08	SP
Q9Y282	ERGI3 Endoplasmic reticulum-Golgi intermediate compartment protein 3	1.97	1.23E-07	multi-TM
Q16706	MA2A1 Alpha-mannosidase 2	1.97	1.44E-23	type II
P01920	DQB1 HLA class II histocompatibility antigen, DQ beta 1 chain	1.97	1.14E-03	SP
Q8IW92	GLBL2 Beta-galactosidase-1-like protein 2	1.91	1.55E-13	SP
Q8NBJ9	SIDT2 SID1 transmembrane family member 2	1.90	2.97E-02	SP, multi-TM
Q08334	I10R2 Interleukin-10 receptor subunit beta	1.89	3.74E-02	SP
O60476	MA1A2 Mannosyl-oligosaccharide 1,2-alpha-mannosidase IB	1.88	8.12E-12	type II
Q9BX59	TPSNR Tapasin-related protein	1.86	1.78E-02	SP
Q8N766	K0090 Uncharacterized protein KIAA0090	1.84	5.96E-08	SP
O60449	LY75 Lymphocyte antigen 75	1.84	1.82E-14	SP
Q86UN3	R4RL2 Reticulon-4 receptor-like 2	1.84	1.59E-05	SP
P13747	HLAE HLA class I histocompatibility antigen, alpha chain E	1.83	4.10E-02	SP
P04229	2B11 HLA class II histocompatibility antigen, DRB1-1 beta chain	1.81	2.45E-02	SP
P16070	CD44 CD44 antigen	1.81	1.57E-07	SP
O95864	FADS2 Fatty acid desaturase 2	1.79	1.14E-09	multi-TM
P08069	IGF1R Insulin-like growth factor 1 receptor	1.78	4.65E-04	SP
O75787	RENR Renin receptor	1.78	4.02E-04	SP
P04062	GLCM Glucosylceramidase	1.78	4.34E-11	SP
Q9P244	LRFN1 Leucine-rich repeat and fibronectin type III domain-containing protein 1	1.76	2.30E-05	SP
O94905	ERLN2 Erlin-2	1.76	1.35E-09	type II
P12109	CO6A1 Collagen alpha-1(VI) chain	1.76	3.31E-05	SP
Q5BJF2	TMM97 Transmembrane protein 97	1.76	1.89E-04	multi-TM
Q9P273	TEN3 Teneurin-3	1.76	2.23E-11	type II
P13612	ITA4 Integrin alpha-4	1.76	2.33E-12	SP
P11117	PPAL Lysosomal acid phosphatase	1.75	2.56E-08	SP
P49768	PSN1 Presenilin-1	1.74	7.08E-06	multi-TM
O96005	CLPT1 Cleft lip and palate transmembrane protein 1	1.74	5.35E-03	multi-TM
Q5T9L3	WLS Protein wntless homolog	1.73	1.09E-02	SP, multi-TM
Q14108	SCRB2 Lysosome membrane protein 2	1.72	6.71E-16	multi-TM

Swiss-Prot	Description	$(L/H)_{final}$	p_{final}	Sec61?
O15118	NPC1 Niemann-Pick C1 protein	1.72	1.38E-09	SP, multi-TM
P29597	TYK2 Non-receptor tyrosine-protein kinase TYK2	1.72	2.17E-02	other
P04114	APOB Apolipoprotein B-100	1.69	3.84E-03	SP
P13598	ICAM2 Intercellular adhesion molecule 2	1.69	1.51E-04	SP
Q30154	DRB5 HLA class II histocompatibility antigen, DR beta 5 chain	1.69	1.72E-05	SP
Q92542	NICA Nicastrin	1.69	1.55E-08	SP
Q96G97	BSCL2 Seipin	1.68	5.87E-04	multi-TM
O43292	GPAA1 Glycosylphosphatidylinositol anchor attachment 1 protein	1.67	2.01E-04	multi-TM
P16422	EPCAM Epithelial cell adhesion molecule	1.67	9.45E-03	SP
Q12913	PTPRJ Receptor-type tyrosine-protein phosphatase eta	1.67	3.69E-03	SP
Q96RQ1	ERGI2 Endoplasmic reticulum-Golgi intermediate compartment protein 2	1.67	8.41E-06	multi-TM
Q96KC8	DNJC1 DnaJ homolog subfamily C member 1	1.66	1.30E-04	SP
Q13724	MOGS Mannosyl-oligosaccharide glucosidase	1.66	2.80E-03	type II
Q96S52	PIGS GPI transamidase component PIG-S	1.65	1.92E-07	multi-TM
Q07065	CKAP4 Cytoskeleton-associated protein 4	1.65	1.66E-02	type II
P05107	ITB2 Integrin beta-2	1.65	4.41E-11	SP
P28068	DMB HLA class II histocompatibility antigen, DM beta chain	1.65	6.65E-03	SP
Q9UH99	SUN2 SUN domain-containing protein 2	1.64	1.07E-04	other
Q8NBN7	RDH13 Retinol dehydrogenase 13	1.63	1.15E-02	other
Q96KA5	CLP1L Cleft lip and palate transmembrane protein 1-like protein	1.62	2.15E-08	multi-TM
Q8ND76	CCNY Cyclin-Y	1.62	3.07E-02	other
P08574	CY1 Cytochrome c1, heme protein, mitochondrial	1.61	1.68E-05	other
Q13795	ARFRP ADP-ribosylation factor-related protein 1	1.60	2.58E-05	other
P61009	SPCS3 Signal peptidase complex subunit 3	1.60	2.31E-05	type II
Q15223	PVRL1 Poliovirus receptor-related protein 1	1.59	5.22E-07	SP
P26572	MGAT1 Alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase	1.59	1.44E-04	type II
Q96PU8	QKI Protein quaking	1.57	3.58E-02	other
Q8N4L2	TM55A Transmembrane protein 55A	1.57	3.38E-03	multi-TM
Q8TDW0	LRC8C Leucine-rich repeat-containing protein 8C	1.56	6.20E-04	multi-TM
O60486	PLXC1 Plexin-C1	1.55	1.80E-05	SP
P22001	KCNA3 Potassium voltage-gated channel subfamily A member 3	1.55	5.88E-03	multi-TM
Q9BS26	ERP44 Endoplasmic reticulum resident protein 44	1.55	3.90E-15	SP
Q92643	GPI8 GPI-anchor transamidase	1.53	1.42E-03	SP
Q9Y2T2	AP3M1 AP-3 complex subunit mu-1	1.53	2.02E-05	other
P78536	ADA17 Disintegrin and metalloproteinase domain-containing protein 17	1.53	3.50E-02	SP
Q9BTV4	TMM43 Transmembrane protein 43	1.52	5.38E-06	multi-TM
Q96JJ7	TMX3 Protein disulfide-isomerase TMX3	1.52	8.79E-05	SP
Q8IXU6	S35F2 Solute carrier family 35 member F2	1.52	1.76E-04	multi-TM
Q969N2	PIGT GPI transamidase component PIG-T	1.52	3.07E-05	SP
P37268	FDFT Squalene synthase	1.52	3.95E-07	multi-TM
Q13217	DNJC3 DnaJ homolog subfamily C member 3	1.51	4.43E-04	SP
Q10472	GALT1 Polypeptide N-acetylgalactosaminyltransferase 1	1.51	1.61E-02	type II

Table 2.1 | CT8-sensitive proteins. All proteins with $(L/H)_{final} \geq 1.5$ and $p_{final} \leq 0.05$ are shown with their Swiss-Prot accession number. Sec61-dependent proteins are classified as signal peptide containing (SP), type II transmembrane (type II), type III transmembrane (type III), or multi-spanning transmembrane (multi-TM) according to their annotations in Swiss-Prot. Multi-spanning transmembrane proteins that also have a signal peptide are annotated as SP, multi-TM. Other = Sec61 independent.

2.4 Cellular validation of CT8-sensitive proteins

We chose four CT8-sensitive proteins and one CT8-insensitive protein to validate in cellular immunoassays based on therapeutic relevance and mechanistic interest: CD74 ($(L/H)_{final} = 3.55$), IL2RG ($(L/H)_{final} = 2.55$), GRP78 ($(L/H)_{final} = 0.96$), HGF ($(L/H)_{final} = 6.31$), and Jak3 ($(L/H)_{final} = 2.55$) (**Table 2.1**). To confirm the SILAC results, we quantified their expression by immunoblotting after a 24 hour treatment with 250 nM or 1 μM CT8. For the major histocompatibility complex (MHC) class II invariant chain (CD74) and cytokine receptor common subunit gamma (IL2RG), both identified as sensitive proteins, there was a dose-dependent loss of protein expression from whole-cell lysates (**Figure 2.5a,b**). The 78 KD glucose-regulated protein (GRP78, or Bip) was identified as an insensitive protein and was not affected by CT8 at either dose.

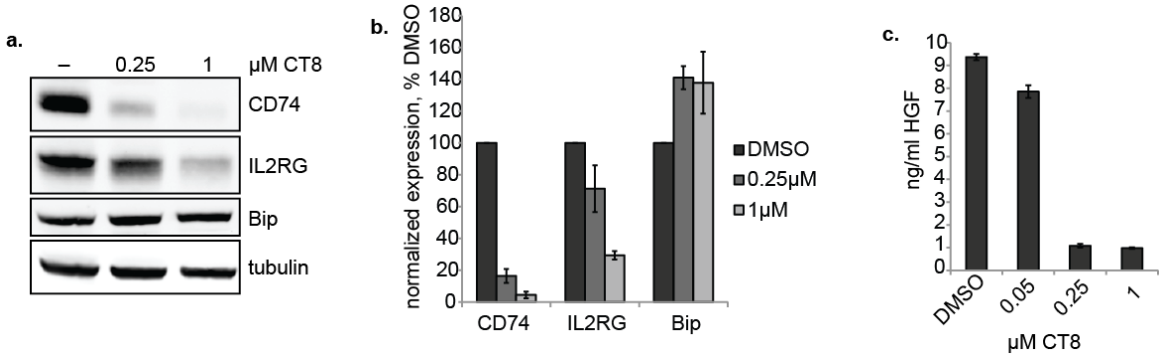

Figure 2.5 | Cellular validation of CT8-sensitive proteins identified by mass spectrometry. **(a)** JJN-3 cells were treated with DMSO or the indicated dose of CT8 for 24 hrs and whole-cell lysates were separated on 10% Tris-Tricine SDS-PAGE gels and immunoblotted for the indicated proteins. Tubulin is shown as a loading control. **(b)** Mean normalized expression from three independent replicates of **(a)** Error bars represent +/– SD. **(c)** Sandwich ELISA for HGF was performed on media collected from JJN-3 cells treated with the indicated doses of CT8 for 24hrs. Bars represent mean ng/ml of HGF for three independent replicates. Error bars represent +/– SD.

Sandwich ELISA of conditioned media confirmed potent inhibition of hepatocyte growth factor (HGF) secretion by CT8 (**Figure 2.5c**).

Of the Sec61-independent proteins affected by CT8, Janus kinase 3 (Jak3) had the highest $(L/H)_{final}$ (**Table 2.1** $(L/H)_{final}$ = 2.55). Jak3 is a cytosolic kinase that associates with IL2RG and is required for signaling downstream of many important cytokines[11]. As Jak3 expression does not require translocation through Sec61, we hypothesized that its high $(L/H)_{final}$ was due to mislocalization rather than total protein loss. Immunoblotting of whole-cell lysates from cells treated with 250 nM CT8 for 24 hrs indeed showed no loss of total Jak3 relative to DMSO (**Figure 2.6a**). However, when lysates were subjected to fractionation, Jak3 was depleted from the membrane fraction in the CT8-treated sample (**Figure 2.6a**,**b**). In addition, CT8 potently inhibits STAT5 phosphorylation in response to IL-2 stimulation, consistent with disruption of this important signaling pathway (**Figure 2.7**). Loss of Jak3 at the membrane is therefore a secondary effect, likely due the loss of IL2RG.

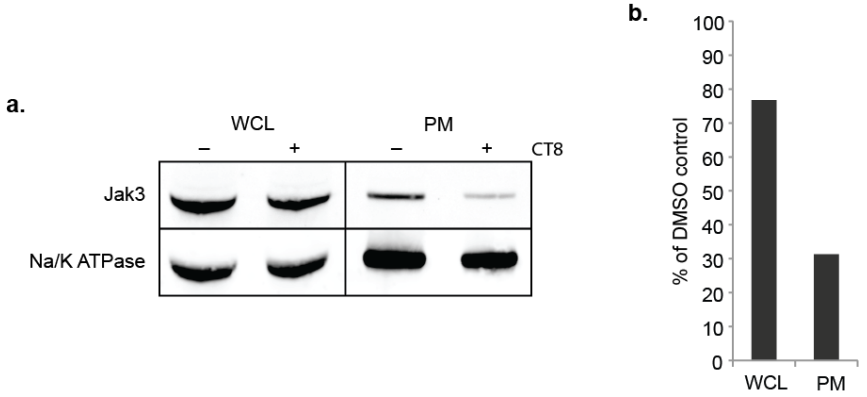

Figure 2.6 | CT8 inhibits membrane localization of Jak3. (a) Whole-cell lysates from JJN-3 cells treated with DMSO or 250 nM CT8 for 24 hrs were separated on 10% Tris-Tricine SDS-PAGE gels and immunoblotted for Jak3 (left) or subjected to the fractionation protocol outlined in Figure1. Purified membrane fractions were 10% Tris-Tricine SDS-PAGE gels and immunoblotted for Jak3 (right). The Na/K ATPase was used as a loading control and to normalize the quantification in **(b)**. **(b)** Quantification of the bands in **(a)**.

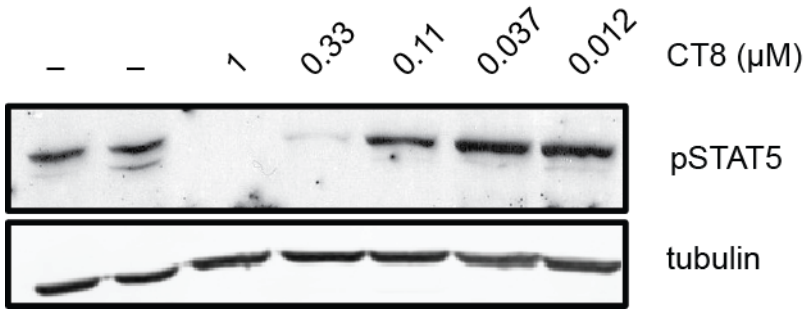

Figure 2.7 | CT8 inhibits STAT5 phosphorylation. MLA144 cells were treated for 24 hrs with DMSO or the indicated concentration of CT8. Whole-cell lysates were separated on 7.5% SDS-PAGE gels and gels were analyzed by immunoblotting for pSTAT5. Tubulin is shown as a loading control. Experiment was performed by Geoff Smith.

2.5 Validation of CT8-sensitive proteins in cotranslational translocation assays

CT8 inhibits protein expression by blocking cotranslational translocation and redirection of translation into the cytosol, where the misfolded proteins are presumably targeted to the proteasome and degraded[3]. Therefore, the best way to test whether a protein is directly affected by CT8 is in cell-free assays of cotranslational translocation.

CD74 is a single-spanning transmembrane protein that integrates into the membrane in a type II orientation, with its N-terminus in the cytosol and C-terminus in the lumen (extracellular C-terminus upon trafficking to the plasma membrane). In vitro translation of CD74 in the presence of canine rough microsomes (cRM) resulted in an increase in molecular weight due to glycosylation of its C-terminus (**Figure 2.8a**), a modification that requires cotranslational translocation. Upon treatment with proteinase K (PK), the cytosolic N-terminus of CD74 is cleaved, leaving the TMD and the glycosylated C-terminal domain protected. Increasing concentrations of CT8 caused a

dose dependent decrease in the intensity of both the glycosylated full-length CD74 and the PK-protected fragment (**Figure 2.8a,b**).

IL2RG is a type I membrane protein with an N-terminal cleavable signal peptide followed by a large luminal domain, a TMD, and a C-terminal cytosolic domain. In vitro translocation of IL2RG shows a similar gel shift to CD74, resulting from glycosylation. PK treatment resulted in cleavage of the cytosolic tail, producing a lower molecular weight fragment. Again, CT8 caused a dose dependent loss of full-length glycosylated IL2RG and the PK protected fragment (**Figure 2.8a,b**). It is interesting to note that while CT8 decreases CD74 and IL2RG expression to a similar extent in cells, in vitro translocation of IL2RG is much less sensitive. A possible explanation for this observation is that IL2RG may have a very short half life[12], resulting in a cellular EC_{50} that is lower than in the in vitro translocation assay, in which protein degradation does not occur.

HGF is a secreted protein without a transmembrane domain. Upon translocation, HGF is both glycosylated and signal-cleaved, resulting in a negligible molecular weight shift. However, PK treatment allows for unambiguous identification of the translocated fraction, as the untranslocated product is completely degraded **(Figure 2.8a,b)**. CT8 inhibited translocation of HGF with an IC_{50} below 250 nM, making it the most sensitive Sec61 client tested. As expected, cotranslational translocation of Bip was unaffected by CT8 **(Figure 2.8a,b)**.

The proteomic data highlight the overall selectivity of CT8, as it affected only 25% of Sec61-dependent proteins quantified by mass spectrometry. We have confirmed four new CT8-sensitive targets (CD74, HGF, IL2RG, and Jak3), as well as a resistant

protein (Bip). The expression of these proteins is inhibited by CT8 at the level of translocation in the case of HGF, CD74, and IL2RG, and at the level of localization for the soluble kinase Jak3.

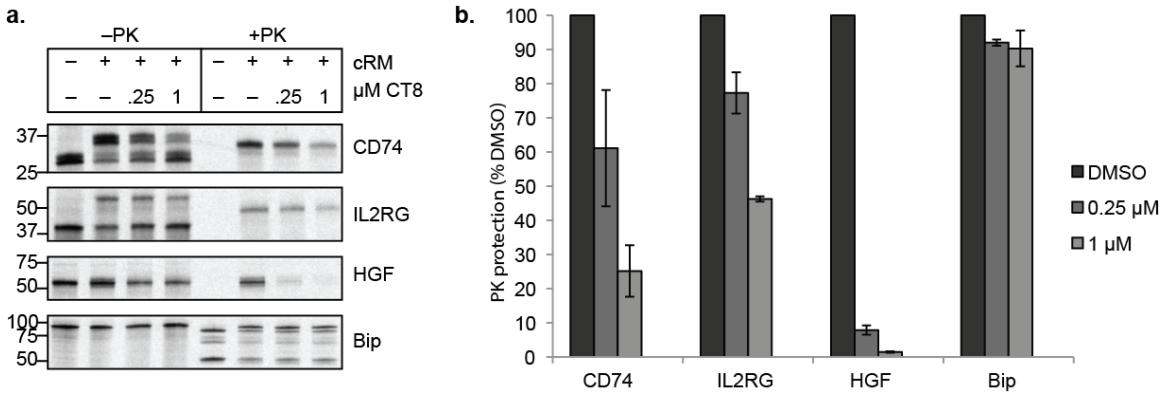

Figure 2.8 | Validation of CT8-sensitive proteins in cell-free cotranslational translocation assays. (a) In vitro translation reactions in rabbit reticulocyte lysates were programmed with the indicated mRNA and run in the presence of [35S]-Methionine, canine rough microsomes (cRM) and either DMSO or the indicated concentration of CT8. Translated material was left untreated or treated with proteinase K (PK) and separated on 10% Tris-Tricine SDS-PAGE gels, followed by analysis by autoradiography. **(b)** Intensity of the PK-protected fragment from three independent replicates plotted as a percent of the DMSO control. Error bars represent +/– SD.

2.6 Validation of TRPML1, the first identified CT8-sensitive multi-spanning integral membrane protein

To date, cotransins have only been shown to inhibit the expression of proteins with cleavable signal peptides or single TMDs, which act as Sec61-targeting signals. Our proteomic results indicated that CT8 might also inhibit multi-spanning integral membrane proteins that lack a cleavable signal peptide. Transient receptor potential mucolipin-1 (TRPML1) is one of these putatively sensitive multi-spanning integral membrane proteins (**Table 2.1**, $(L/H)_{final}$ = 2.14). TRPML1 is a lysosomal member of the TRP family of cation channels[13], human mutations in which cause the lysosomal storage disorder mucolipidosis IV[14]. TRPML1 has 6 predicted transmembrane domains,

with domains 2-6 thought to be responsible for formation of the cation channel[15]. To confirm the ability of CT8 to inhibit TRPML1 production, HEK293FRT cells stably expressing a C-terminally 3xFLAG-tagged TRPML1 under a tetracycline-inducible promoter were treated concomitantly with tetracycline and either DMSO or increasing concentrations of CT8 for 24 hrs. Immunoblotting for the FLAG epitope tag revealed potent inhibition of TRPML1 expression even at the lowest concentration of CT8 tested (**Figure 2.9**).

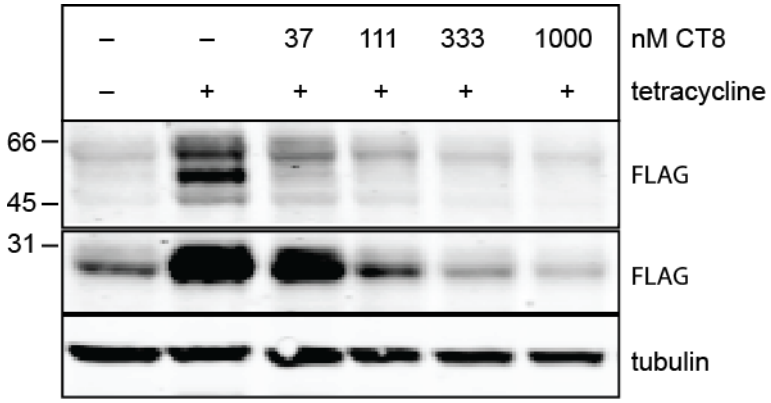

Figure 2.9 | CT8 inhibits TRPML1 expression. HEK293FRT cells stably expressing a tetracycline-inducible 3xFLAG-tagged TRPML1 were treated with DMSO or the indicated concentrations of CT8 for 24 hrs. Whole-cell lysates were separated on 7.5% Tris-Tricine SDS-PAGE gels and immunoblotted for FLAG. The upper blot indicates full length TRPML1 and the lower blot represents the C-terminal fragment after protealytic processing of the lumenal loop. Tubulin is shown as a loading control.

The first TMD of TRPML1 (TMD1) is predicted to have a type II orientation, with the subsequent ~200 amino acids forming a large, lumenal loop that contains a predicted N-linked glycosylation site at residue 230[16]. Extensive mutagenesis of the type II signal anchor from TNFα showed that mutation of polar residues to leucine tended to increase the resistance of TNFα to CT8[8]. To test whether TMD1 acts as the sensitivity determinant of TRPML1 in an analogous fashion, we made two TMD1

mutants, containing either 2 (2L) or 4 (4L) leucines (**Figure 2.10a**). In vitro translation/translocation of WT TRPML1, 2L and 4L showed increasing resistance to CT8 treatment with increasing leucine mutations, as indicated by the persistence of the higher molecular weight glycosylated band (**Figure 2.10b,c**).

a.

	TMD 1
WT	LMLQVVKILVVTVQLILFGLS
2L	LMLLVVLILVVTVQLILFGLS
4L	LMLLVVLILVVLVLLILFGLS

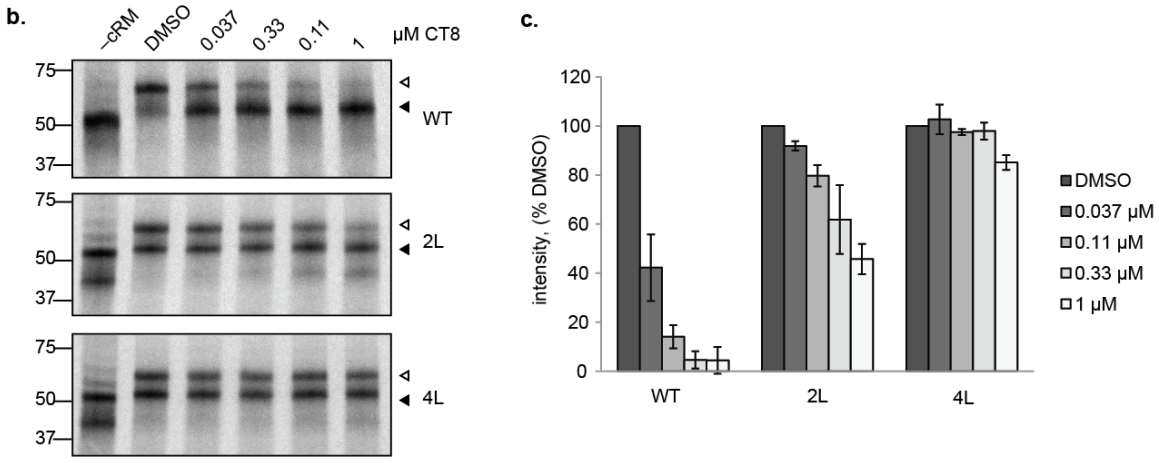

Figure 2.10 | Mutation of TMD1 in TRPML1 results in resistance to CT8. (a) The first transmembrane domain of WT TRPML1 and the subsequent leucine mutations to form 2L and 4L. **(b)** In vitro translation reactions in rabbit reticulocyte lysates were programmed with the indicated mRNA and run in the presence of [35S]-Methionine, canine rough microsomes (cRM) and either DMSO or the indicated concentration of CT8. Translated material was separated on 7.5% Tris-Tricine SDS-PAGE gels, followed by analysis by autoradiography. **c)** Quantification of the glycosylated band in **(b)**. Bars represent the average of three independent replicates with error bars representing +/– SD.

2.7 Measuring hydrophobicity of Sec61-targeting signals

AA	AA	Kyte-Doolittle	Hopp-Woods
A	Alanine	1.8	-0.5
C	Cysteine	2.5	-1
D	Aspartic Acid	-3.5	3
E	Glutamis Acid	-3.5	3
F	Phenylalanine	2.8	-2.5
G	Glycine	-0.4	0
H	Histidine	-3.2	-0.5
I	Isoleucine	4.5	-1.8
K	Lysine	-3.9	3
L	Leucine	3.8	-1.8
M	Methionine	1.9	-1.3
N	Asparagine	-3.5	0.2
P	Proline	-1.6	0
Q	Glutamine	-3.5	0.2
R	Arginine	-4.5	3
S	Serine	-0.8	0.3
T	Threonine	-0.7	-0.4
V	Valine	4.2	-1.5
W	Tryptophan	-0.9	-3.4
Y	Tyrosine	-1.3	-2.3

Table 2.2 | Amino acid values for the Kyte-Doolittle and Hopp-Woods hydrophobicity scales.

The results from mutagenesis of both TRPML1 and TNFα[8] point to a correlation between TMD hydrophobicity and CT8-sensitivity. To test whether hydrophobicity of Sec61-targeting signals and CT8-sensitivity were related in the proteomic dataset as a whole, we needed to systematically calculate a hydrophobicity score for each signal, both for signal peptides and for signal anchor TMDs. Numerous hydrophobicity scales have been developed but we chose to focus on three: Kyte-Doolittle (KD)[17], Hopp-Woods(HW)[18], and a "biological hydrophobicity" scaleΔG_{calc}, developed by Hessa, von Heijne, and colleagues[9]. All three of these scales generate a hydrophobicity score

based on assigning values to individual amino acids and summing those values over the length of the peptide being interrogated. The Hessa et al. ΔG_{calc} scale also includes a positional component.

The Kyte-Doolittle hydrophobicity scale is commonly used for predicting transmembrane helices. Developed in 1981, this scale assigns positive values to hydrophobic amino acids and negative values to polar and charged amino acids (**Table 2.2**). A good way to determine the hydrophobicity of a given amino acid is to calculate a transfer free energy ($\Delta G_{transfer}$) from a measured partition coefficient between water and a non-interacting, isotropic phase. The previous hydrophobicity scales developed by Rose et al. assigned values to amino acids based on the partition coefficient between water and ethanol[19]. Kyte and Doolittle showed that ethanol was a poor model for a non-interacting phase and instead used a water-vapor $\Delta G_{transfer}$. In addition to $\Delta G_{transfer}$, Kyte and Doolittle also took into account the tendency of an amino acid to be buried within a protein, based on values generated by Chothia[20]. The resulting values that form the Kyte-Doolittle scale are typically used to calculate the average "hydropathy value" for a moving window of 7-11 residues resulting in a hydropathy plot (**Figure 2.11**). In our case, however, we were interested in calculating a hydrophobicity score for a defined sequence, either a TMD or a signal peptide, so we simply summed the values for that sequence to give KD_{tot} or averaged over the length to give KD_{avg} (KD_{tot} divided by the number of amino acids in the sequence).

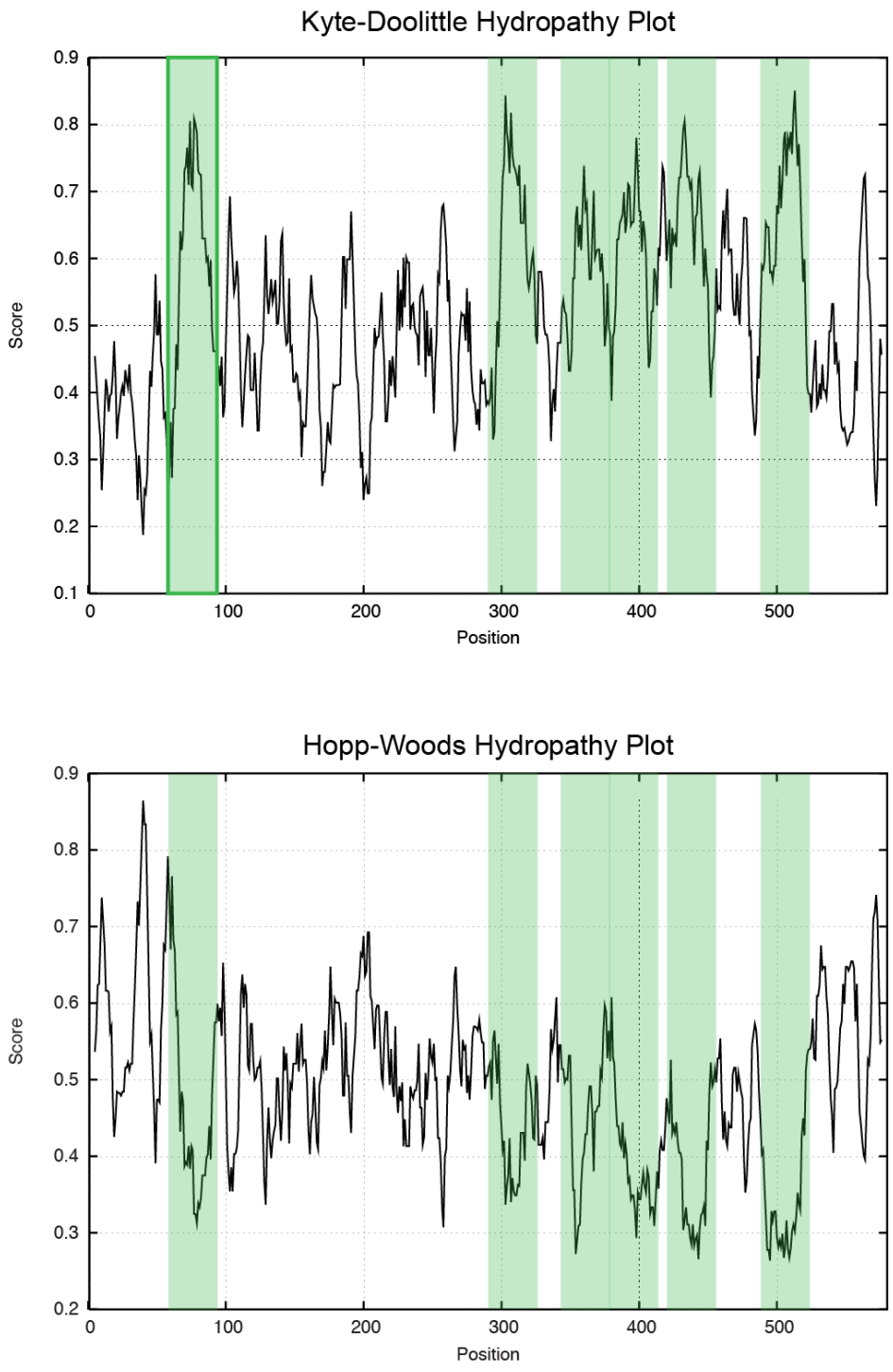

Figure 2.11 | Hydropathy plots for TRPML1. A hydropathy plot for full length TRPML1 was generated using the Kyte-Doolittle scale (upper) or the Hopp-Woods scale (lower) at http://web.expasy.org/protscale/ with a window size of 9 and 100% weight on the window edges compared to the central amino acid. The scales were normalized to 1. Green shading indicates the hydrophobic peaks and troughs. These positions correspond well to the positions of the 6 transmembrane domains of TRPML1 annotated in Swiss-Prot.

The second scale used in this study was developed by Hopp and Woods for determining antigenic sites on proteins[18]. This is another very common hydrophobicity scale, though it is the reverse of the Kyte-Doolittle in that polar and charged amino acids are given positive values while apolar resides have negative values (**Table 2.2**). Therefore, the Hopp-Woods scale can be thought of as a measure of hydrophilicity, rather than hydrophobicity.

The values of the amino acid hydrophobic parameters for the Hopp-Woods scale are modified slightly from those reported by Levitt[21], which are themselves derived from $\Delta G_{transfer}$ (water-ethanol) determined experimentally by Nozaki and Tanford[22]. Values for aspartic acid, proline and glutamic acid were determined empirically by maximizing the values for known antigenic sites. Similar to the Kyte-Doolittle scale, the Hopp-Woods scale is typically used to calculate a hydropathy plot by scanning a window of 7-11aa and averaging the values within that window. This produces a plot similar to the Kyte-Doolittle, except that the transmembrane domains are now troughs instead of peaks (**Figure 2.11**).

The final method used in our studies for calculating hydrophobicity of the Sec61-targeting signal is ΔG_{calc}, developed by Hessa et al[9] and available at http://dgpred.cbr.su.se/index.php?p=home. This scale differs from the Kyte-Doolittle and Hopp-Woods scales in two important ways. First, rather than using $\Delta G_{transfer}$ from water to ethanol or vapor, Hessa et al. empirically derived a new hydrophobicity parameter for each amino acid based on its contribution to the "free energy" of transfer from the aqueous pore of Sec61 into the lipid bilayer, presumably through the lateral gate, making it potentially more relevant to the action of CT8[23]. Note that this empirical "free

energy" value (described in more detail below) likely encompasses both thermodynamic and kinetic components (cite refs). Secondly, this ΔG_{calc} scale takes into account the position of each amino acid, not just its intrinsic hydrophobicity.

The ΔG_{calc} scale was created empirically by introducing systematically designed 19 aa H-segments into the middle of the luminal P2 domain of the bacterial protein Lep (leader peptidase from E. coli)[9]. The protein was translated in rabbit reticulocyte lysates in the presence of canine rough microsomes (cRMs) and an apparent equilibrium constant for membrane integration of the H-segment was calculated on the basis of the amount of singly versus doubly glycosylated proteins; this value serves as a proxy for the fraction nascent chains with integrated as opposed to translocated H-segments (**Figure 2.12**). This ratio is converted into a ΔG_{app} using the following equation:

$$\Delta G_{app} = -RT\ln K_{app}$$

Where $K_{app} = f_{1g}/f_{2g}$ and f_{1g} is the fraction of singly glycosylated (integrated, or "inserted") Lep protein and f_{2g} is the fraction of doubly glycosylated (translocated) Lep protein. Remarkably, ΔG_{app} was found to correlate linearly with the number of Leu residues in an H-segment that otherwise contained only Ala residues.

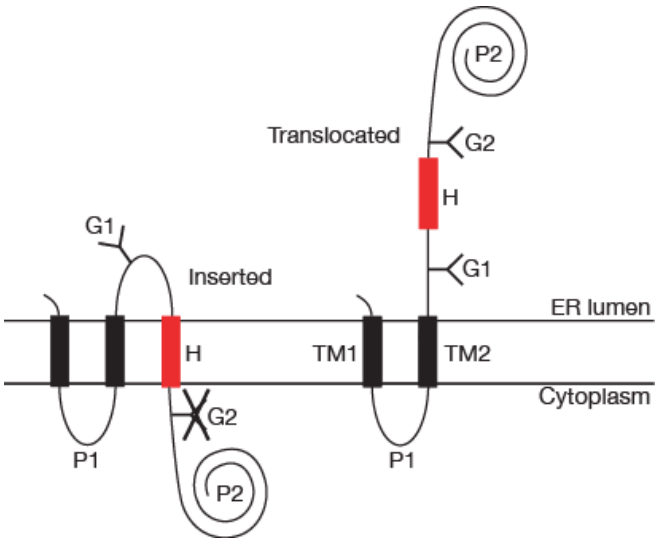

Figure 2.12 | Experimental design for determining ΔG_{app}. Systematically designed H-segments were inserted into the luminal P2 loop of Lep. H-segments that are inserted into the membrane result in a singly glycosylated species (left). H-segments that are not inserted into the membrane are pushed through the Sec61 channel and into the ER lumen, resulting in a doubly glycosylated species. ΔG_{app} is calculated based on the amount of singly versus doubly glycosylated protein. Figure taken from Hessa et al.3

To determine the positional dependence of ΔG_{app} for each amino acid ($\Delta G^{aa}{}_{app}$) each of the 20 amino acids was scanned singly through the 19 aa H-segment. $\Delta G^{aa}{}_{app}$ varied greatly with position for polar and charged residues, as well as proline, but apolar residues were virtually position independent. To generate the final ΔG_{calc} scale used in our study, ΔG_{app} values for 324 19 aa H-segments were determined. They were then fit to the model:

$$\Delta G^{pred}_{app} = \sum_{i=1}^{l} \Delta G^{aa\,(i)}_{app} + c_0\mu$$

Where l is the length of the H-segment and $c_0\mu$ is the contribution of the hydrophobic moment (which is dependent on $\Delta G^{aa}{}_{app}$)[23]. The Hessa et al. ΔG calculator (http://dgpred.cbr.su.se/index.php?p=home) calculates $\Delta G^{pred}{}_{app}$ for a given sequence. This calculated ΔG value (ΔG_{calc}) is a prediction of the transfer free energy of that segment from the pore of the Sec61 translocon into the lipid bilayer. For our purposes,

we ignored the length correction option and simply used ΔG_{calc} as the ΔG^{pred}_{app} above. For transmembrane domains, the calculator was used in the "full protein scan" mode and the segment with the lowest ΔG_{calc} was used. For signal peptides, ΔG_{calc} was calculated using the "ΔG prediction" mode for 30 aa N-terminal to the predicted cleavage site both with (ΔG_{avg}) and without (ΔG_{tot}) dividing by the length of the segment. Finally, because signal peptides are comprised of an N-terminal region, a central hydrophobic region (6-20aa) and a C-terminal region, we hypothesized that ΔG_{calc} of just the hydrophobic region may provide a more relevant measure of a signal peptides hydrophobicity. To this end, we allowed the ΔG calculator to pick the subsequence with the lowest ΔG_{calc}, giving ΔG_{sub}. A list of the various hydrophobicity measurements is given in **Table 2.3**.

Hydrophobicity Value	Description
ΔG_{calc}	Refers to any use of the ΔG calculator, also refers specifically to measurement of type II signal anchor ΔG
ΔG_{tot}	Refers to ΔG_{calc} for full signal peptide (truncated to 30aa)
ΔG_{avg}	Refers to ΔG_{calc} for full signal peptide (truncated to 30aa) divided by length
ΔG_{sub}	Refers to ΔG_{calc} for optimal subregion of full signal peptide
KD_{tot}	Refers to Kyte-Doolittle sum over the full signal peptide (truncated to 30aa)
KD_{avg}	Refers to Kyte-Doolittle sum over the full signal peptide (truncated to 30aa) divided by length
HW_{tot}	Refers to Hopp-Woods sum over the full signal peptide (truncated to 30aa)
HW_{avg}	Refers to Hopp-Woods sum over the full signal peptide (truncated to 30aa) divided by length

Table 2.3 | Summary of hydrophobicity values used to describe type II signal anchors and signal peptides

2.8 CT8-sensitive signals tend to have higher calculated free energies of integration (ΔG_{calc})

We hypothesized that the apparent relationship between TMD hydrophobicity and CT8-sensitivity seen in the TRPML1 and TNFα mutation experiments could be explained by a model in which CT8 binding interferes with the TMD's ability to laterally gate Sec61 and partition into the bilayer. In this scenario, sensitivity would correlate with the calculated free energy of membrane integration (ΔG_{calc})[23], with higher ΔG_{calc} sequences (less favorable membrane integration) favoring inhibition. To test this hypothesis, we used the ΔG calculator to determine ΔG_{calc} of all type II signal anchors (TMD in type II membrane proteins) identified in the SILAC experiment (51 proteins, 23 of which were sensitive, **Table 2.4**)[9]. Again, sensitive proteins had an $(L/H)_{final} \geq 1.5$ and $p_{final} \leq 0.05$ and resistant proteins had an $(L/H)_{final} \leq 1.36$ and $p_{final} \leq 0.05$ or $(L/H)_{norm,exp} \leq 1.36$ in two or more biological replicates. Comparing median ΔG_{calc} values shows a difference between sensitive and resistant proteins (–0.95 for sensitive and –1.7 for resistant, **Figure 2.13**), supporting the model that CT8 inhibits lateral partitioning into

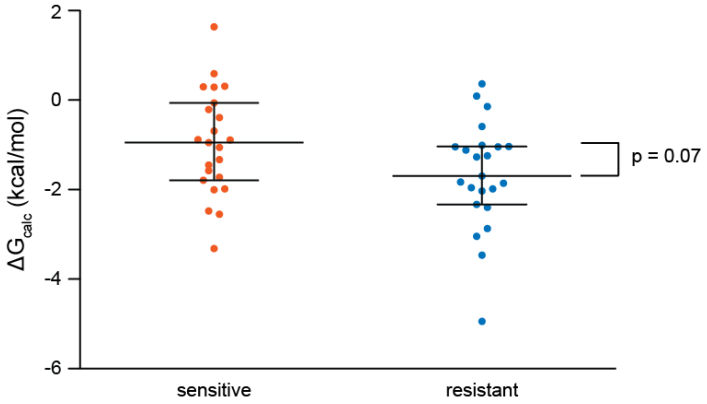

Figure 2.13 | CT8-sensitive type II signal anchors tend to have higher ΔG_{calc} values. ΔG_{calc} values for sensitive and resistant type II transmembrane proteins were plotted as groups. Long horizontal lines represent the median ΔG_{calc} with the shorter horizontal lines indicating the interquartile range. An exact p-value was calculated using a two-tailed Mann-Whitney U test.

the bilayer. However, a statistical test of this difference does not show significance ($p = 0.07$). It is likely that the sample size of type II membrane proteins is too small.

This analysis, and the previous mutational studies with TNFα and TRPML1, still suggested that TMDs with less favorable membrane integration tend to be more sensitive to CT8. This makes sense, as TMDs, by definition, must partition into the bilayer; however, it was unclear whether this would hold true for signal peptides, as the biophysical determinants of SP-mediated gating of Sec61 are poorly understood. Most importantly, the ΔG_{calc} algorithm was designed for TMDs, not signal peptides. There is no obvious way to calculate a ΔG_{app} for signal peptides as they contain relatively polar N and C-regions as well as a central hydrophobic stretch that is often much shorter than a TMD. Additionally, the SILAC-MS experiments gave a much larger dataset of SP-containing proteins than type II transmembrane proteins, possibly providing more statistical power to reveal differences in hydrophobicity (or other biophysical properties) between sensitive and resistant groups.

Swiss-Prot	ΔG	Signal Anchor	Description	$(L/H)_{final}$	p_{final}
O94905	1.640	LGAVVAVASSFFCASLFSA	ERLN2 Erlin-2	1.76	1.35E-09
O60512	0.592	CTLALLVGSQLAVMMYLSL	B4GT3 Beta-1,4-galactosyltransferase 3	3.51	5.35E-04
Q7LGA3	0.364	KLQLLAVVAFAVAMLFLEN	HS2ST Heparan sulfate 2-O-sulfotransferase 1	1.36	1.98E-06
Q13724	0.311	TAGGVALAVVVLSLALGMS	MOGS Mannosyl-oligosaccharide glucosidase	1.66	2.80E-03
P04233	0.298	ALYTGFSILVTLLLAGQAT	HG2A HLA class II histocompatibility antigen gamma chain	3.56	3.71E-09
Q9P2E5	0.289	ALPLILGLSLGCSLSLLRV	CHPF2 Chondroitin sulfate glucuronyltransferase	3.18	8.16E-04
Q9NZ08	0.090	WSLATMSFLLSSLLALLTV	ERAP1 Endoplasmic reticulum aminopeptidase 1	1.12	4.37E-04
Q07065	-0.061	FLFYLALVAAAAFSGWCVH	CKAP4 Cytoskeleton-associated protein 4	1.65	1.66E-02
Q9HDC9	-0.141	FRVTFLMLAVSLTVPLLGAMMLL	APMAP Adipocyte plasma membrane-associated protein	1.20	7.86E-06
P61009	-0.213	LFAFSLSVMAALTFGCFITTAF	SPCS3 Signal peptidase complex subunit 3	1.60	2.31E-05
P32970	-0.392	LRAALVPLVAGLVICLVVCI	CD70 CD70 antigen	2.31	3.04E-07
P15291	-0.589	RACRLLVAVCALHLGVTLVYYL	B4GT1 Beta-1,4-galactosyltransferase 1	1.26	3.22E-03
Q10472	-0.692	VLATSLIWVLLDMFLLLYF	GALT1 Polypeptide N-acetylgalactosaminyltransferase 1	1.51	1.61E-02
Q8N2K0	-0.881	LRLRKILFCVLGLYIAIPFLI	ABD12 Monoacylglycerol lipase ABHD12	2.28	7.69E-03
Q9NXS2	-0.889	RLLPLLLALAVGSAFYTIW	QPCTL Glutaminyl-peptide cyclotransferase-like protein	2.44	2.86E-03
Q9NQX7	-0.953	VCYLSMGMVVLLMGLVFASVYIY	ITM2C Integral membrane protein 2C	5.31	2.03E-05
Q9Y673	-1.012	AALAAAALVLISIVAFTTA	ALG5 Dolichyl-phosphate beta-glucosyltransferase	0.76	1.75E-04
Q86SF2	-1.040	FILRSLLVVGSFLGLVVLW	GALT7 N-acetylgalactosaminyltransferase 7	1.21	1.22E-02
Q8TC12	-1.042	LMFPLLLLLLPFLLYMAAP	RDH11 Retinol dehydrogenase 11	0.91	2.90E-02
Q10469	-1.043	RKVLILTLVVAACGFVLWS	MGAT2 Alpha-1,6-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase	1.06	4.63E-02
Q9P273	-1.058	CTALCAVGVSVLLAILLSYFI	TEN3 Teneurin-3	1.76	2.23E-11
Q6IAN0	-1.115	AILPLLFGCLGVFGLFRLL	DRS7B Dehydrogenase/reductase SDR family member 7B	0.90	1.22E-02
O00461	-1.241	TLLLLTVVFGFLYGAMLYY	GOLI4 Golgi integral membrane protein 4	1.26	3.90E-03
Q8NF37	-1.273	LFPVRLLVAAAMMLLAWPLAL	PCAT1 Lysophosphatidylcholine acyltransferase 1	1.07	1.99E-02
Q9Y287	-1.332	RAWCWCMCFGLAFMLAGVIL	ITM2B Integral membrane protein 2B	2.04	4.86E-05
Q86X52	-1.449	RAWLSVLLGLVLGFVLASRLVL	CHSS1 Chondroitin sulfate synthase 1	3.49	6.53E-04
Q9NUM4	-1.580	LYVMASVFVCLLLSGLAVFFLF	T106B Transmembrane protein 106B	2.04	2.50E-04
P02786	-1.696	ICYGTIAVIVFFLIGFMIGYL	TFR1 Transferrin receptor protein 1	1.32	8.24E-24
Q8NBJ4	-1.725	LVLAALVACIIVLGFNYWI	GOLM1 Golgi membrane protein 1	2.41	2.23E-04
Q16706	-1.789	FTVFGSAIFCVVIFSLYLML	MA2A1 Alpha-mannosidase 2	1.97	1.44E-23
P08195	-1.830	RTRWALLLLFWLGWLGMLA	4F2 4F2 cell-surface antigen heavy chain	0.74	1.13E-12
Q86UE4	-1.856	WVILVGTGALGLLLLFLLGYGWA	LYRIC Protein LYRIC	0.97	1.44E-04
P54709	-1.958	LLFYLVFYGFLAALFSFTMWVML	AT1B3 Sodium/potassium-transporting ATPase subunit beta-3	1.00	2.51E-03
P33908	-1.983	FVLLLVFSAFITLCFGAIFFL	MA1A1 Mannosyl-oligosaccharide 1,2-alpha-mannosidase IA	2.79	3.42E-17
Q9UIQ6	-1.986	TMVVCAFVIVVAVSVIMVIYLL	LCAP Leucyl-cystinyl aminopeptidase	1.23	1.66E-11
P26572	-2.003	LVLWGAILFVAWNALLLLFFWT	MGAT1 Alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase	1.59	1.44E-04
Q10471	-2.030	RMLLCFAFLWVLGIAYYMY	GALT2 Polypeptide N-acetylgalactosaminyltransferase 2	0.91	2.28E-06
Q68CQ7	-2.332	FRKVNIIILVLAVALFLLVL	GL8D1 Glycosyltransferase 8 domain-containing protein 1	0.58	1.44E-02
Q8IV08	-2.399	ARWVLLVLILAVVGFGALM	PLD3 Phospholipase D3	0.92	8.28E-02
Q11201	-2.476	TLKVLTFLVLFIFLTSFFL	SIA4A CMP-N-acetylneuraminate-beta-galactosamide-alpha-2,3-sialyltransferase 1	2.60	1.87E-10
O60476	-2.547	FILLLILSAFITLCFGAFFFL	MA1A2 Mannosyl-oligosaccharide 1,2-alpha-mannosidase IB	1.88	8.12E-12

Swiss-Prot	ΔG_{calc}	Signal Anchor	Description	$(L/H)_{final}$	p_{final}
Q9BT22	-2.871	AASCLVLLALCLLLPLLLL	ALG1 Chitobiosyldiphosphodolichol beta-mannosyltransferase	0.82	4.93E-03
Q9Y5Y6	-3.046	WVVLAAVLIGLLLVLLGIGFLVW	ST14 Suppressor of tumorigenicity 14 protein	1.22	4.90E-05
Q9UKM7	-3.317	MILFLLAFLLFCGLLFYINL	MA1B1 Endoplasmic reticulum mannosyl-oligosaccharide 1,2-alpha-mannosidase	2.49	7.56E-11
Q9BXN2	-3.461	WRLIAVILGILCLVILVIAVVL	CLC7A C-type lectin domain family 7 member A	0.92	8.73E-03
P21964	-4.943	LLLAAVLLGLVLLVVLLLLLR	COMT Catechol O-methyltransferase	0.94	1.44E-03

Table 2.4 | Type II membrane proteins identified by SILAC membrane proteomics. Proteins were annotated as being type II based on their Swiss-Prot entry as described in the text. Sensitive proteins (orange shading) have $(L/H)_{final} \geq 1.5$ and $p_{final} \leq 0.05$. Resistant proteins (blue shading) have an $(L/H)_{final} \leq 1.36$ and $p_{final} \leq 0.05$. Signal anchors were predicted using the ΔG calculator in "full protein scan" mode with no length correction and taking the segment with the lowest ΔG_{calc}. Proteins are ranked from highest ΔG_{calc} to lowest ΔG_{calc}.

Swiss-Prot	ΔG_{tot}	ΔG_{sub}	Signal Peptide	Description	$(L/H)_{final}$
Q6ZRP7	5.69	0.987	MAAAGAAVARSPGIGAGPALR	QSOX2 Sulfhydryl oxidase 2	2.02
Q9BUR5	5.893	0.39	MFKVIQRSVGPASLSLLTFKVYAAP	APOO Apolipoprotein O	0.39
Q15904	4.972	0.121	MMAAMATARVRMGPRCAQALWRMPWLPVFLSLAAAAAAAAA	VAS1 V-type proton ATPase subunit S1	1.17
P78536	1.679	0.083	MRQSLLFLTSVVPFVLA	ADA17 Disintegrin and metalloproteinase domain-containing protein 17	1.53
Q9UKQ2	1.874	0.034	MLQGLLPVSLLLSVAVSA	ADA28 Disintegrin and metalloproteinase domain-containing protein 28	2.79
P11117	5.703	-0.021	MAGKRSGWSRAALLQLLLGVNLVVMPPTRA	PPAL Lysosomal acid phosphatase	1.75
Q30154	5.924	-0.142	MVCLKLPGGSYMAKLTVTLMVLSSPLALA	DRB5 HLA class II histocompatibility antigen, DR beta 5 chain	1.69
P12109	1.531	-0.18	MRAARALLPLLLQACWTAA	CO6A1 Collagen alpha-1(VI) chain	1.76
Q96DX4	1.02	-0.237	MIVFGWAVFLASRSLG	RSPRY RING finger and SPRY domain-containing protein 1	0.88
P16070	2.617	-0.28	MDKFWWHAAWGLCLVPLSLA	CD44 CD44 antigen	1.81
Q96JJ7	3.095	-0.28	MAAWKSWTALRLCATVVVLDMVVC	TMX3 Protein disulfide-isomerase TMX3	1.52
P04229	3.917	-0.293	MVCLKLPGGSCMTALTVTLMVLSSPLALA	2B11 HLA class II histocompatibility antigen, DRB1-1 beta chain	1.81
Q5VW38	6.195	-0.311	MAALAPVGSPASRGPRLAAGLRLLPMLGLLQLLAEPGLG	GP107 Protein GPR107	2.62
P13747	2.275	-0.317	MVDGTLLLLLSEALALTQTWA	HLAE HLA class I histocompatibility antigen, alpha chain E	1.83
P22794	8.323	-0.35	MPTDMEHTGHYLHLAFLMTTVFSLSPGTKA	EVI2A Protein EVI2A	3.76
P28067	7.362	-0.407	MGHEQNQGAALLQMLPLLWLLPHSWA	DMA HLA class II histocompatibility antigen, DM alpha chain	2.05
P25445	4.854	-0.423	MLGIWTLLPLVLTSVARLSSKSVNA	TNR6 Tumor necrosis factor receptor superfamily member 6	1.31
P26885	0.85	-0.436	MRLSWFRVLTVLSICLSAVAT	FKBP2 Peptidyl-prolyl cis-trans isomerase FKBP2	1.01
Q08334	1.499	-0.445	MAWSLGSWLGGCLLVSALG	I10R2 Interleukin-10 receptor subunit beta	1.89
P28068	0.743	-0.473	MITFLPLLLGLSLGCTGA	DMB HLA class II histocompatibility antigen, DM beta chain	1.65
P31785	3.385	-0.476	MLKPSLPFTSLLFLQLPLLGVG	IL2RG Cytokine receptor common subunit gamma	2.55
Q12860	0.141	-0.479	MKMWLLVSHLVIISITTCLA	CNTN1 Contactin-1	2.82
Q13217	7.839	-0.529	MVAPGSVTSRLGSVFPFLLVLVDLQYEGAEC	DNJC3 DnaJ homolog subfamily C member 3	1.51
P19256	3.717	-0.541	MVAGSDAGRALGVLSVVCLLHCFGFISC	LFA3 Lymphocyte function-associated antigen 3	2.14
P20701	2.901	-0.547	MKDSCITVMAMALLSGFFFFAPASS	ITAL Integrin alpha-L	2.01
P06756	6.407	-0.548	MAFPPRRRLRLGPRGLPLLLSGLLLPLCRA	ITAV Integrin alpha-V	2.52
O60888	2.703	-0.561	MSGGRAPAVLLGGVASLLLSFVWMPALLPVAS	CUTA Protein CutA	0.79
P14210	2.686	-0.566	MWVTKLLPALLLQHVLLHLLLLPIAIPYAEG	HGF Hepatocyte growth factor	6.37
P55145	2.589	-0.616	MRRMWATQGLAVALALSVLPGSRA	MANF Mesencephalic astrocyte-derived neurotrophic factor	1.12
P04062	6.333	-0.618	MEFSSPSREECPKPLSRVSIMAGSLTGLLLLQAVSWASG	GLCM Glucosylceramidase	1.78
O94779	1.559	-0.632	MASSWKLMLFLSVTMCLS	CNTN5 Contactin-5	3.57
P10586	6.951	-0.684	MAPEPAPGRTMVPLVPALVMLGLVAGAHG	PTPRF Receptor-type tyrosine-protein phosphatase F	0.97
Q08722	1.032	-0.712	MWPLVAALLLGSACCGSA	CD47 Leukocyte surface antigen CD47	1.24
O43852	1.142	-0.712	MDLRQFLMCLSLCTAFALS	CALU Calumenin	0.99

Swiss-Prot	ΔG_{tot}	ΔG_{sub}	Signal Peptide	Description	$(L/H)_{final}$
O95297	7.673	-0.717	MAASAGAGAVIAAPDSRRWLWSVLAAALGLLTAGV	MPZL1 Myelin protein zero-like protein 1	2.46
P01920	5.447	-0.729	MSWKKALRIPGGLRAATVTLMLAMLSTPVAEG	DQB1 HLA class II histocompatibility antigen, DQ beta 1 chain	1.97
P52799	6.328	-0.78	MAVRRDSVWKYCWGVLMVLCRTAISKS	EFNB2 Ephrin-B2	2.35
Q8TB96	4.654	-0.781	MAAAGRLPSSWALFSPLLAGLALLGVGPVPARA	TIP T-cell immunomodulatory protein	1.30
P13598	3.927	-0.79	MSSFGYRTLTVALFTLICCPGSDE	ICAM2 Intercellular adhesion molecule 2	1.69
P40189	2.125	-0.798	MLTLQTWLVQALFIFLTTESTG	IL6RB Interleukin-6 receptor subunit beta	1.23
Q92544	3.555	-0.81	MATAMDWLPWSLLLFSLMCETSA	TM9S4 Transmembrane 9 superfamily member 4	1.32
Q9Y6N7	3.941	-0.835	MKWKHVPFLVMISLLSLSPNHLFLA	ROBO1 Roundabout homolog 1	1.14
Q9Y320	4.928	-0.88	MAVLAPLIALVYSVPRLSRWLAQPYYLLSALLSAAFLLVRKLPPLCHG	TMX2 Thioredoxin-related transmembrane protein 2	0.82
Q9Y3A6	3.852	-0.932	MGDKIWLPFPVLLLAALPPVLLPGAAG	TMED5 Transmembrane emp24 domain-containing protein 5	1.34
O60486	8.877	-0.94	MEVSRRKAPPRPPRPAAPLPLLAYLLALAAPGRG	PLXC1 Plexin-C1	1.55
P09326	2.621	-0.944	MCSRGWDSCLALELLLLPLSLLVTSI	CD48 CD48 antigen	2.22
Q5T9L3	1.669	-0.959	MAGAIIENMSTKKLCIVGGILLVFQIIAFLVGGLIAPGPTTA	WLS Protein wntless homolog	1.73
Q8NBJ7	3.773	-0.969	MARHGLPLLPLLSLLVGAWLKLGNG	SUMF2 Sulfatase-modifying factor 2	1.22
Q9H0U3	1.788	-0.978	MAARWRFWCVSVTMVVALLIVCDVPSASA	MAGT1 Magnesium transporter protein 1	1.06
Q8NDZ4	5.837	-0.995	MWRLVPPKLGRLSRSLKLAALGSLLVLMVLHSPSL	CC058 UPF0672 protein C3orf58	1.24
P30101	-0.31	-1.017	MRLRRLALFPGVALLLAAARLAAA	PDIA3 Protein disulfide-isomerase A3	0.89
Q6IEE7	4.902	-1.018	MAPGMSGRGGAALLCLSALLAHASG	T132E Transmembrane protein 132E	1.03
Q6UWB1	4.586	-1.026	MRGGRGAPFWLWPLPKLALLPLLWVLFQRTRP	I27RA Interleukin-27 receptor subunit alpha	3.69
Q14697	6.871	-1.041	MAAVAAVAARRRRSWASLVLAFLGVCLG	GANAB Neutral alpha-glucosidase AB	1.02
Q92643	4.147	-1.05	MAVTDSLSRAATVLATVLLLSFGSVAA	GPI8 GPI-anchor transamidase	1.53
P07339	2.137	-1.051	MQPSSLLPLALCLLAAPA	CATD Cathepsin D	1.13
P27797	-0.405	-1.062	MLLSVPLLLGLLGLAVA	CALR Calreticulin	0.97
Q15223	5.525	-1.081	MARMGLAGAAGRWWGLALGLTAFFLPGVHS	PVRL1 Poliovirus receptor-related protein 1	1.59
P04439	1.782	-1.084	MAVMAPRTLLLLLSGALALTQTWA	1A03 HLA class I histocompatibility antigen, A-3 alpha chain	1.13
Q14118	4.091	-1.106	MRMSVGLSLLLPLSGRTFLLLLSVVMAQS	DAG1 Dystroglycan	1.16
P20036	4.949	-1.111	MRPEDRMFHIRAVILRALSLAFLLSLRG	DPA1 HLA class II histocompatibility antigen, DP alpha 1 chain	2.35
Q8NBJ9	0.839	-1.123	MFALGLPFLVLLVASVES	SIDT2 SID1 transmembrane family member 2	1.90
P16422	1.764	-1.143	MAPPQVLAFGLLLAAATATFAAA	EPCAM Epithelial cell adhesion molecule	1.67
P05107	1.046	-1.144	MLGLRPPLLALVGLLSLGCVLS	ITB2 Integrin beta-2	1.65
Q8N766	3.406	-1.147	MAAEWASRFWLWATLLIPAAA	K0090 Uncharacterized protein KIAA0090	1.84
P29323	-0.504	-1.147	MALRRLGAALLLLPLLAA	EPHB2 Ephrin type-B receptor 2	1.15
P13612	9.729	-1.165	MAWEARREPGPRRAAVRETVMLLLCLGVPTGRP	ITA4 Integrin alpha-4	1.76
P48723	0.66	-1.174	MAREMTILGSAVLTLLLAGYLA	HSP13 Heat shock 70 kDa protein 13	0.79
P29320	2.37	-1.186	MDCQLSILLLLSCSVLDSFG	EPHA3 Ephrin type-A receptor 3	1.02
O60613	4.4	-1.189	MAAGPSGCLVPAFGLRLLLATVLQAVSA	SEP15 15 kDa selenoprotein	1.04

Swiss-Prot	ΔG_{tot}	ΔG_{sub}	Signal Peptide	Description	$(L/H)_{final}$
Q92896	4.492	-1.191	MAACGRVRRMFRLSAALHLLLLFAAGAEK	GSLG1 Golgi apparatus protein 1	
Q9BRR6	0.881	-1.202	MALWRGSAYAGFLALAVGCVFL	ADPGK ADP-dependent glucokinase	
Q96AP7	6.023	-1.21	MISLPGPLVTNLLRFLFLGLSALAPPSRA	ESAM Endothelial cell-selective adhesion molecule	
P01591	3.385	-1.213	MKNHLLFWGVLAVFIKAVHVKA	IGJ Immunoglobulin J chain	
Q9BXS4	4.605	-1.228	MAAPKGSLWVRTQLGLPPLLLLTMALAGGSGTASA	TMM59 Transmembrane protein 59	
Q8NBN3	1.278	-1.28	MAAAAWLQVLPVILLLLGAHP	TM87A Transmembrane protein 87A	
P43121	1.493	-1.284	MGLPRLVCAFLLAACCCCPRVAG	MUC18 Cell surface glycoprotein MUC18	
P14314	-0.96	-1.294	MLLPLLLLLPMCWA	GLU2B Glucosidase 2 subunit beta	
Q15084	0.054	-1.316	MALLVLGLVSCTFFLAVNG	PDIA6 Protein disulfide-isomerase A6	
P07237	-1.064	-1.318	MLRRALLCLAVAALVRA	PDIA1 Protein disulfide-isomerase	
P23470	2.713	-1.326	MRRLLEPCWWILFLKITSS	PTPRG Receptor-type tyrosine-protein phosphatase gamma	
P04844	2.269	-1.342	MAPPGSSTVFLLALTIIASTWA	RPN2 Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2	
O15533	0.828	-1.355	MKSLSLLLAVALGLATAVSA	TPSN Tapasin	
P25774	0.564	-1.356	MKRLVCVLLVCSSAVA	CATS Cathepsin S	
P05362	6.647	-1.396	MAPSSPRPALPALLVLLGALFPGPGNA	ICAM1 Intercellular adhesion molecule 1	
P61769	1.287	-1.398	MSRSVALAVLALLSLSGLEA	B2MG Beta-2-microglobulin	
Q969H8	5.37	-1.406	MAAPSGGWNGVGASLWAALLLGAVALRPAEA	CS010 UPF0556 protein C19orf10	
P08069	6.361	-1.419	MKSGSGGGSPTSLWGLLFLSAALSLWPTSG	IGF1R Insulin-like growth factor 1 receptor	
Q15043	4.488	-1.429	MKLLLLHPAFQSCLLLTLLGLWRTTPEAHA	S39AE Zinc transporter ZIP14	
Q8TEM1	1.931	-1.451	MAARGRGLLLLTLSVLLAAGPSAAAA	PO210 Nuclear pore membrane glycoprotein 210	
Q96HE7	2.104	-1.457	MGRGWGFLFGLLGAVWLLSSGHG	ERO1A ERO1-like protein alpha	
P07602	-1.003	-1.46	MYALFLLASLLGAALA	SAP Proactivator polypeptide	
Q9H8H3	0.831	-1.466	MELTIFILRLAIYILTFPLYLLNFLGLWS	MET7A Methyltransferase-like protein 7A	
O15031	0.805	-1.47	MALQLWALTLLGLLGAGAS	PLXB2 Plexin-B2	
P15586	7.941	-1.472	MRLLPLAPGRLRRGSPRHLPSCSPALLLLVLGGCLG	GNS N-acetylglucosamine-6-sulfatase	
Q9Y4L1	6.386	-1.473	MADKVRRQRPRRRVCWALVAVLLADLLALSDT	HYOU1 Hypoxia up-regulated protein 1	
P13473	1.853	-1.494	MVCFRLFPVPGSGLVLVCLVLGAVRSYA	LAMP2 Lysosome-associated membrane glycoprotein 2	
Q9P0K1	2.693	-1.494	MQAAVAVSVPFLLLCVLGTCPPARC	ADA22 Disintegrin and metalloproteinase domain-containing protein 22	
P06213	2.956	-1.497	MATGGRRGAAAAPLLVAVAALLLGAAG	INSR Insulin receptor	
P50454	-0.149	-1.507	MRSLLLLSAFCLLEAALA	SERPH Serpin H1	
Q9UHG3	4.09	-1.513	MGRVVAELVSSLLGLWLLLCSCGCPEG	PCYOX Prenylcysteine oxidase 1	
P51571	1.418	-1.522	MAAMASLGALALLLLSSLSRCSA	SSRD Translocon-associated protein subunit delta	
P18084	2.304	-1.531	MPRAPAPLYACLLGLCALLPRLA	ITB5 Integrin beta-5	
Q13641	10.143	-1.536	MPGGCSRGPAAGDGRLRLARLALVLLGWVSS	TPBG Trophoblast glycoprotein	
P04440	4.381	-1.557	MMVLQVSAAPRTVALTALLMVLLTSVVQG	DPB1 HLA class II histocompatibility antigen, DP beta 1 chain	
Q96A33	2.393	-1.565	MKAFHTFCVVLLVFGSVSEA	CCD47 Coiled-coil domain-containing protein 47	

Swiss-Prot	ΔG_{tot}	ΔG_{sub}	Signal Peptide	Description	$(L/H)_{final}$
Q92542	8.361	-1.581	MATAGGGSGADPGSRGLLRLLSFCVLLAGLCRG	NICA Nicastrin	1.69
P11717	6.951	-1.606	MGAAAGRSPHLGPAPARRPQRSLLLLQLLLLVAAPGSTQA	MPRI Cation-independent mannose-6-phosphate receptor	0.86
O00469	5.67	-1.619	MGGCTVKPQLLLLALVLHPWNPCLG	PLOD2 Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2	1.18
Q92520	0.613	-1.625	MRVAGAAKLVVAVAVFLLTFYVIS	FAM3C Protein FAM3C	0.99
P18433	0.157	-1.64	MDSWFILVLLGSGLICVSA	PTPRA Receptor-type tyrosine-protein phosphatase alpha	1.17
Q8NBQ5	-0.8	-1.65	MKFLLDILLLLPLLIVCSL	DHB11 Estradiol 17-beta-dehydrogenase 11	1.11
Q13740	4.737	-1.659	MESKGASSCRLLFCLLISATVFRPGLG	CD166 CD166 antigen	2.03
Q9BS26	4.594	-1.659	MHPAVFLSLPDLRCSLLLLVTWVFTPVTT	ERP44 Endoplasmic reticulum resident protein 44	1.55
O60568	5.524	-1.669	MTSSGPGPRFLLLLPLLLPPAASA	PLOD3 Procollagen-lysine,2-oxoglutarate 5-dioxygenase 3	1.09
Q9P244	6.156	-1.676	MAPGPFSSALLSPPPAALPFLLLLWAGASRG	LRFN1 Leucine-rich repeat and fibronectin type III domain-containing protein 1	1.76
O75787	-0.68	-1.678	MAVFVVLLALVAGVLG	RENR Renin receptor	1.78
P30040	4.738	-1.678	MAAAVPRAAFLSPLLPLLLGFLLLSAPHGGSG	ERP29 Endoplasmic reticulum resident protein 29	0.92
P11021	-0.369	-1.684	MKLSLVAAMLLLLSAARA	GRP78 78 kDa glucose-regulated protein	0.96
P16150	0.47	-1.687	MATLLLLLGVLVVSPDALG	LEUK Leukosialin	0.89
P48960	1.959	-1.693	MGGRVFLAFCVWLTLPGAET	CD97 CD97 antigen	1.15
P23284	3.658	-1.699	MLRLSERNMKVLLAAALIAGSVFFLLLPGPSAA	PPIB Peptidyl-prolyl cis-trans isomerase B	0.88
O15321	6.751	-1.715	MTVVGNPRSWSCQWLPILILLLGTGHG	TM9S1 Transmembrane 9 superfamily member 1	1.10
Q9NYU2	3.394	-1.72	MGCKGDASGACAAGALPVTGVCYKMGVLVVLTVLWLFSSVKA	UGGG1 UDP-glucose:glycoprotein glucosyltransferase 1	1.01
Q9BRK5	2.748	-1.769	MVWPWVAMASRWGPLIGLAPCCLWLLGAVLLMDASA	CAB45 45 kDa calcium-binding protein	5.76
Q15363	0.312	-1.777	MVTLAELLVLLAALLATVSG	TMED2 Transmembrane emp24 domain-containing protein 2	1.09
Q12907	5.805	-1.791	MAAEGWIWRWGWGRRCLGRPGLLGPGPGPTTPLFLLLLLGSVTA	LMAN2 Vesicular integral-membrane protein VIP36	0.93
Q92820	1.177	-1.792	MASPGCLLCVLGLLLCGAASLELS	GGH Gamma-glutamyl hydrolase	1.34
P80303	0.245	-1.797	MRWRTILLQYCFLLITCLLTALEA	NUCB2 Nucleobindin-2	1.02
P39656	6.103	-1.801	MGYFRCARAGSFGRRRKMEPSTAARAWALFWLLLPLLGAVCA	OST48 Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit	1.17
Q7Z7H5	6.698	-1.812	MAGVGAGPLRAMGRQALLLLALCATGAQG	TMED4 Transmembrane emp24 domain-containing protein 4	1.03
Q13443	6.125	-1.817	MGSGARFPSGTLRVRWLLLLGLVGPVLG	ADAM9 Disintegrin and metalloproteinase domain-containing protein 9	1.35
P32942	4.944	-1.844	MATMVPSVLWPRACWTLLVCCLLTPGVQG	ICAM3 Intercellular adhesion molecule 3	0.93
Q13445	2.853	-1.883	MMAAGAALALALWLLMPPVEVGG	TMED1 Transmembrane emp24 domain-containing protein 1	1.08
Q8WU39	0.126	-1.917	MRLSLPLLLLLLGAWAIPGGLG	PERP1 Plasma cell-induced resident endoplasmic reticulum protein	0.97
Q8NFY4	-0.614	-1.92	MRVFLLCAYILLLMVSQLRA	SEM6D Semaphorin-6D	0.67
P35613	1.24	-1.931	MAAALFVLLGFALLGTHGASG	BASI Basigin	0.85
O60462	2.76	-1.951	MDMFPLTWVFLALYFSRHQV	NRP2 Neuropilin-2	0.63
P13987	3.438	-1.973	MGIQGGSVLFGLLLVLAVFCHSGHS	CD59 CD59 glycoprotein	1.07
P34910	3.739	-1.974	MDPKYFILILFCGHLNNTFFS	EVI2B Protein EVI2B	1.21
Q86UN3	5.036	-1.979	MLPGLRRLLQAPASACLLLMLLALPLAAPSCPMLCTCYSSPPTVSC	R4RL2 Reticulon-4 receptor-like 2	1.84

Swiss-Prot	ΔG_{tot}	ΔG_{sub}	Signal Peptide	Description	$(L/H)_{final}$
P78357	1.432	-1.98	MMHLRLFCILLAAVSGAEG	CNTP1 Contactin-associated protein 1	1.24
P09923	1.565	-1.985	MQGPWVLLLLGLRLQLSLG	PPBI Intestinal-type alkaline phosphatase	0.71
Q8NC42	3.364	-1.997	MAWRRREASVGARGVLALALLALALCVPGARG	RN149 E3 ubiquitin-protein ligase RNF149	1.14
Q969P0	2.538	-2.019	MGALRPTLLPPSLPLLLLMLGMGCWA	IGSF8 Immunoglobulin superfamily member 8	0.94
P05067	-0.518	-2.021	MLPGLALLLLAAWTARA	A4 Amyloid beta A4 protein	0.91
Q9ULF5	5.246	-2.025	MKVHMHTKFCLICLLTFIFHHCNHC	S39AA Zinc transporter ZIP10	1.05
Q9BY67	4.285	-2.029	MASVVLPSGSQCAAAAAAAAPPGLRLRLLLLLFSAAALIPTGDG	CADM1 Cell adhesion molecule 1	1.98
P26010	0.595	-2.034	MVALPMVLVLLLVLSRGES	ITB7 Integrin beta-7	1.04
Q15293	1.722	-2.034	MARGGRGRRLGLALGLLLALVLAPRVLRA	RCN1 Reticulocalbin-1	0.93
Q9UBV2	-0.7	-2.126	MRVRIGLTLLLCAVLLSLASA	SE1L1 Protein sel-1 homolog 1	1.30
P27824	1.114	-2.131	MEGKWLLCMLLVLGTAIVEA	CALX Calnexin	1.10
P14625	-0.893	-2.135	MRALWVLGLCCVLLTFGSVRA	ENPL Endoplasmin	0.98
Q9Y3B3	6.479	-2.149	MPRPGSAQRWAAVAGRWGCRLLALLLLVPGPGGA	TMED7 Transmembrane emp24 domain-containing protein 7	1.13
Q14554	0.611	-2.165	MARAGPAWLLLAIWVVLPSWL	PDIA5 Protein disulfide-isomerase A5	1.17
P50897	3.125	-2.171	MASPGCLWLLAVALLPWTCASRALQHL	PPT1 Palmitoyl-protein thioesterase 1	1.27
P20645	1.953	-2.185	MFPFYSCWRTGLLLLLLAVAVRESWQ	MPRD Cation-dependent mannose-6-phosphate receptor	1.06
Q14165	2.472	-2.191	MLGAWAVEGTAVALLRLLLLLLPPAIRG	MLEC Malectin	2.65
Q9BX59	2.162	-2.191	MGTQEGWCLLLCLALSGA	TPSNR Tapasin-related protein	1.86
Q06481	1.622	-2.193	MAATGTAAAAATGRLLLLLLVGLTAPALALA	APLP2 Amyloid-like protein 2	0.45
P04843	2.64	-2.212	MEAPAAGLFLLLLLGTWAPAPGS	RPN1 Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1	1.18
Q9HC07	4.57	-2.277	MAAAAPGNGRASAPRLLLLFLVPLLWAPAAVRA	TM165 Transmembrane protein 165	2.35
Q9NPF0	1.377	-2.283	MSGGWMAQVGAWRTGALGLALLLLLGLGLGLEAAA	CD320 CD320 antigen	0.74
Q12913	6.243	-2.286	MKPAAREARLPPRSPGLRWALPLLLLLLRLGQILC	PTPRJ Receptor-type tyrosine-protein phosphatase eta	1.67
Q9Y4D7	7.194	-2.286	MAPRAAGGAPLSARAAAASPPPFQTPPRCPVPLLLLLLLGAARAGA	PLXD1 Plexin-D1	1.22
P0CW18	1.337	-2.287	MLLAVLLLLPLPSSWFAHG	PRS56 Putative serine protease 56	0.99
Q99805	6.177	-2.292	MSARLPVLSPPRWPRLLLLSLLLLGAVP	TM9S2 Transmembrane 9 superfamily member 2	1.31
Q8NBS9	4.2	-2.324	MPARPGRLLPLLARPAALTALLLLLLGHGGGG	TXND5 Thioredoxin domain-containing protein 5	0.93
O60449	4.605	-2.325	MRTGWATPRRPAGLLMLLFWFFDLAEP	LY75 Lymphocyte antigen 75	1.84
Q13162	7.148	-2.339	MEALPLLAATTPDHGRHRRLLLLPLLLFLLPAGAVQG	PRDX4 Peroxiredoxin-4	1.05
Q9BVK6	5.318	-2.345	MAVELGVLLVRPRPGTGLGRVMRTLLLVLWLATRGSA	TMED9 Transmembrane emp24 domain-containing protein 9	1.33
P78310	-0.3	-2.361	MALLLCFVLLCGVVDFARS	CXAR Coxsackievirus and adenovirus receptor	2.90
Q96CG8	9.013	-2.386	MRPQGPAASPQRLRGLLLLLLQLPAPSSA	CTHR1 Collagen triple helix repeat-containing protein 1	1.08
Q99674	0.281	-2.45	MLPLTMTVLILLLLPTGQA	CGRE1 Cell growth regulator with EF hand domain protein 1	0.89
Q04721	1.455	-2.496	MPALRPALLWALLALWLCCAAPAHA	NOTC2 Neurogenic locus notch homolog protein 2	0.91
P13667	-0.064	-2.509	MRPRKAFLLLLLLGLVQLLA	PDIA4 Protein disulfide-isomerase A4	0.97
Q96HY6	-0.132	-2.524	MVAPVWYLVAAALLVGFILFLTRSRGRA	DDRGK DDRGK domain-containing protein 1	1.04

Swiss-Prot	ΔG_{tot}	ΔG_{sub}	Signal Peptide	Description	$(L/H)_{final}$
O15118	0.114	-2.528	MTARGLALGLLLLLLCPAQVFS	NPC1 Niemann-Pick C1 protein	1.72
Q15392	0.348	-2.536	MEPAVSLAVCALLFLLWVRLKG	DHC24 Delta(24)-sterol reductase	1.30
P07686	0.292	-2.537	MELCGLGLPRPPMLLALLLATLLAAMLALLTQVALVVQVAEA	HEXB Beta-hexosaminidase subunit beta	0.97
Q13438	0.246	-2.539	MAAETLLSSLLGLLLLGLLLPASLT	OS9 Protein OS-9	1.10
P16278	0.337	-2.564	MPGFLVRILPLLLVLLLLGPTRG	BGAL Beta-galactosidase	0.80
Q86UL3	-0.093	-2.628	MFLLLPFDSLIVNLLGISLTVLFTLLLVFIIVPAIFG	GPAT4 Glycerol-3-phosphate acyltransferase 4	1.13
P53634	1.911	-2.68	MGAGPSLLLAALLLLLSGDGAVRC	CATC Dipeptidyl peptidase 1	0.98
Q02809	-1.206	-2.79	MRPLLLLALLGWLLLAEA	PLOD1 Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1	1.00
P04114	0.581	-2.825	MDPPRPALLALLALPALLLLLAGARA	APOB Apolipoprotein B-100	1.69
Q969N2	-0.199	-2.849	MAAAMPLALLVLLLLGPGGWC	PIGT GPI transamidase component PIG-T	1.52
Q9HD45	1.068	-2.857	MRPLPGALGVAAAAALWLLLLLLPRTRA	TM9S3 Transmembrane 9 superfamily member 3	1.29
Q8NBJ5	6.929	-2.86	MAAAPRAGRRRGQPLLALLLLLLAPLPPG	GT251 Procollagen galactosyltransferase 1	1.09
P49755	4.698	-2.867	MSGLSGPPARRGPFPLALLLLFLLGPRLVLA	TMEDA Transmembrane emp24 domain-containing protein 10	1.20
P04066	4.524	-2.974	MRAPGMRSRPAGPALLLLLLFLGAAESVRRA	FUCO Tissue alpha-L-fucosidase	1.16
P43307	-0.899	-3.016	MRLLPRLLLLLLVFPAT	SSRA Translocon-associated protein subunit alpha	1.02
Q8IW92	0.612	-3.031	MTTWSLRRRPARTLGLLLLVVLGFLVLRRLDW	GLBL2 Beta-galactosidase-1-like protein 2	1.91
O75976	6.058	-3.236	MASGRDERPPWRLGRLLLLMCLLLLGSSARA	CBPD Carboxypeptidase D	0.81
Q02818	0.89	-3.272	MPPSGPRGTLLLLPLLLLLLLRAVLA	NUCB1 Nucleobindin-1	1.09
Q96KC8	5.814	-3.356	MTAPCSQPAQLPGRRQLGLVPFPPPPPRTPLLWLLLLLAAVAPARG	DNJC1 DnaJ homolog subfamily C member 1	1.66
Q06136	-0.713	-3.373	MLLLAAAFLVAFVLLLYMVSPLISP	KDSR 3-ketodihydrosphingosine reductase	0.51
Q9Y624	2.625	-3.477	MGTKAQVERKLLCLFILAILLCSLALG	JAM1 Junctional adhesion molecule A	0.89
P11279	1.966	-3.888	MAAPGSARRPLLLLLLLLLLGLMHCASA	LAMP1 Lysosome-associated membrane glycoprotein 1	1.31
Q8IYS2	1.244	-3.918	MWLQQRLKGLPGLLSSSWARRLLCLLGLLLLLLWFGGSGA	K2013 Uncharacterized protein KIAA2013	1.29
Q9UIW2	-0.023	-4.403	MPLPPRSLQVLLLLLLLLLLLPGMWA	PLXA1 Plexin-A1	0.97
Q9Y394	-3.238	-4.599	MNWELLLWLLVLCALLLLLVQLLRFLRA	DHRS7 Dehydrogenase/reductase SDR family member 7	0.74

Table 2.5 | Signal peptide-containing proteins identified by SILAC mass spectrometry and used for enrichment analysis. Proteins were annotated as haivng a signal peptide based on their Swiss-Prot entry as indicated in the text. Sensitive proteins (orange shading) have a $(L/H)_{final} \geq 1.5$ and $p_{final} \leq 0.05$. Resistant proteins (blue shading) have an $(L/H)_{final} \leq 1.36$ and $p_{final} \leq 0.05$. Resistant proteins whose $p_{final} > 0.05$ were still included as long as they were identified as resistant in 2 or more experiments. Signal peptide cleavage sites were obtained from the Swiss-Prot entry and sequences were extracted from the full protein sequence using the python script in Appendix A. ΔG_{tot} was calculated by entering the 30aa N-terminal to the predicted cleavage site into the ΔG calculator in "ΔG prediction" mode with no length correction. ΔG_{sub} was calculated using the same 30aa from the predicted cleavage site but allowing the calculator to pick the subsequence with the lowest ΔG_{calc}. Proteins are ranked highest to lowest by ΔG_{sub}.

To determine whether ΔG_{calc} is the most relevant measure of hydrophobicity for signal peptides with respect to predicting CT8 sensitivity, we compared it to both the Kyte-Doolittle and Hopp-Woods scales. Signal peptide cleavage sites for sensitive and resistant proteins were obtained from Swiss-Prot (66 sensitive and 136 resistant, **Table 2.4**). To increase the size of the analyzed signal peptide-containing protein dataset as much as possible, proteins with $p_{final} > 0.05$ that were identified with an $(L/H)_{final} \leq 1.36$ in two or more biological replicates were also included in the resistant population (13 proteins). Multi-TM proteins predicted to have a signal peptide were excluded from the analysis because of the difficultly in distinguishing between a true signal peptide and a potential transmembrane domain.

The sequence of the signal peptide was extracted from the full protein sequence in FASTA format using a python script developed by Peter Malkin (Google) (**Appendix B**). Because the longest sequence allowed by the ΔG calculator is 30 aa, we truncated all signal peptides to the 30 aa N-terminal to the predicted cleavage site. Hydrophobicity of the truncated 30 aa signal peptides was calculated using the Kyte-Doolittle and Hopp-Woods scales, both as a sum over the sequence ($KD_{tot,}$ and HW_{tot}, respectively) and as an average (KD_{avg} and HW_{avg}, respectively, **Figure 2.14**). ΔG_{tot} was calculated by entering the 30 aa truncated signal sequence into the ΔG calculator in "ΔG prediction" mode with no length correction and not allowing for a subsequence with lower ΔG. To calculate ΔG_{avg}, ΔG_{tot} was divided by the length of the truncated sequence. ΔG_{avg} resulted in the most significant separation of median hydrophobicity between sensitive and resistant proteins (ΔG_{avg} = 0.15 kcal/mol/aa for sensitive and 0.086 kcal/mol/aa for resistant, **Figure 2.14**).

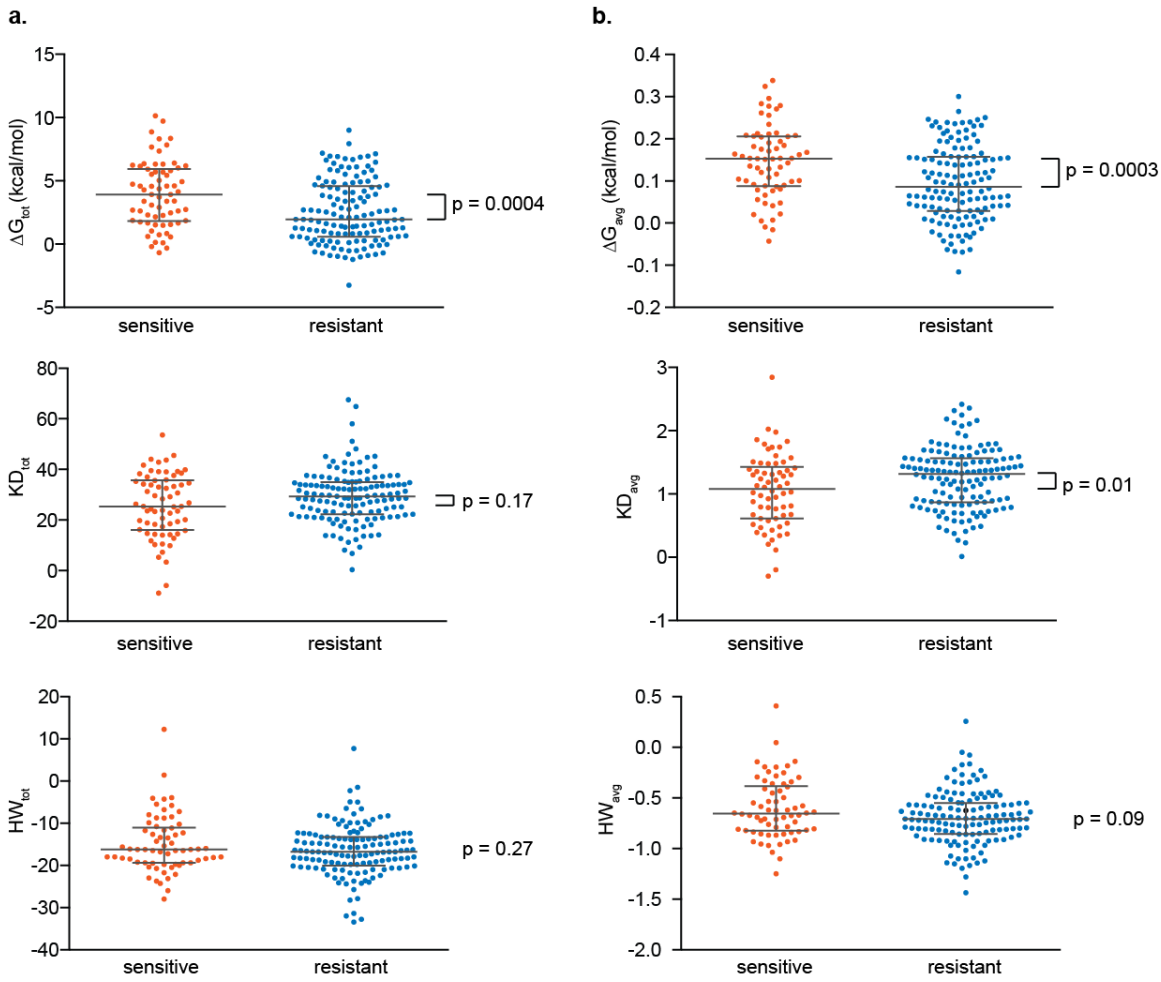

Figure 2.14 | CT8 sensitive signal peptides have significantly higher ΔG_{avg} values. (a) ΔG_{calc} (upper), Kyte-Doolittle3 (KD, middle) and Hopp-Woods3 (HW, lower) scales were used to calculate hydrophobicity values for signal peptides extending 30aa from the predicted cleavage site for sensitive ($(L/H)_{final} > 1.5$) and resistant ($(L/H)_{final} < 1.36$) proteins without normalizing for length. Long horizontal lines represent the median and the upper and lower short horizontal lines mark the interquartile range. Exact p-values were calculated using a two-tailed Mann-Whitney U test. **(b)** As in **(a)** but values were normalized by length of signal peptide.

N-terminal signal peptides are characterized by a short, positively charged N-terminal portion (N-region), followed by a stretch of hydrophobic amino acids ranging from 6-20 in length (H-region), and culminating in the polar but often uncharged C-region just prior to the signal peptidase cleavage site[24]. The H-region forms a short alpha helix that is possibly responsible for laterally gating Sec61[25]. Even so, it is still unclear whether lateral gating by the signal peptide is required for cotranslational translocation of secreted proteins, whose fate does not require them to remain imbedded in the membrane. To test whether sensitivity is more accurately described when ΔG_{calc} is calculated for an optimal subregion of the SP, rather than the entire SP, we allowed the ΔG calculator to pick the region with the lowest ΔG_{calc} in a given signal peptide (ΔG_{sub}) by choosing the "allow subsequence (if lower ΔG)" option in "ΔG prediction" mode. Plotting ΔG_{sub} for sensitive and resistant proteins gave an even more significant difference in the population medians (–1.3 vs –1.9 kcal/mol for sensitive vs resistant, respectively; $p < 0.0001$), suggesting that the sensitivity determinant is confined to a shorter, hydrophobic region within the signal peptide (**Figure 2.15**). Mechanistically, this makes sense if lateral gating is a required step in translocation of signal peptides, if the H-region of a signal peptide provides the greatest thermodynamic driving force for lateral gating, and most importantly, if this is the key step blocked by CT8 binding to Sec61. It would follow that those signal peptides with H-regions bearing low ΔG_{calc}, and therefore the greatest driving force for lateral gating, would be the most resistant to CT8. Allowing the ΔG calculator to find the optimal subregion of a SP provides a simple way to estimate this driving force.

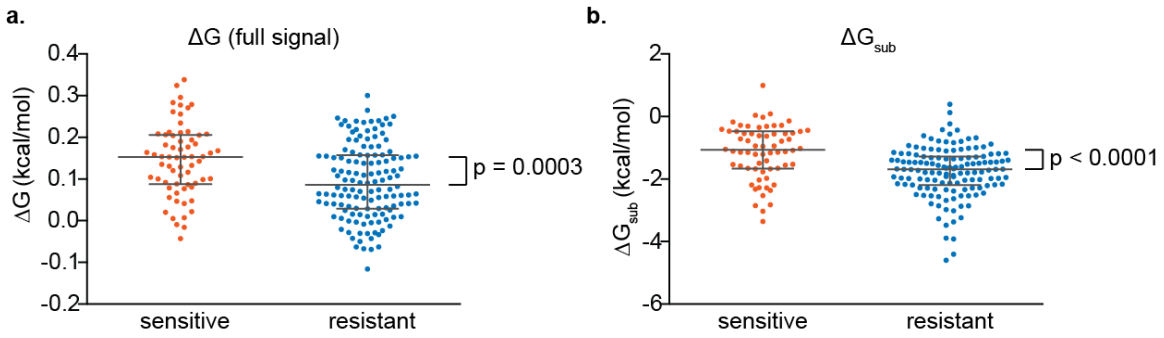

Figure 2.15 | ΔG_{sub} separates sensitive and resistant signal peptide populations better than ΔG_{avg}. ΔG_{avg} **(a)** and ΔG_{sub} **(b)** of signal peptides were plotted for sensitive (orange, L/H > 1.5) and resistant (blue, L/H < 1.36) proteins. Long horizontal lines represent the median and shorter horizontal lines indicate the interquartile range. Exact p-values were calculated using a two-tailed Mann-Whitney U test.

2.9 ΔG_{sub} can predict sensitivity to CT8

Our ultimate goal in defining sequence characteristics that correlate with CT8 sensitivity is to be able to predict CT8-sensitive secretory proteins simply by examining their primary amino acid sequence. To test whether ΔG_{sub} is sufficient to enrich for sensitive proteins, we ranked signal peptide-containing proteins by their corresponding ΔG_{sub} (**Figure 2.16**). This resulted in a significant enrichment of sensitive proteins in the top quartile of ΔG_{sub} values ($p = 0.0003$, two-tailed Fisher's exact test). While we cannot define a quantitative correlation between $(L/H)_{final}$ or apparent EC_{50} and ΔG_{sub}, this enrichment suggests that any protein with a ΔG_{sub} in the top quartile ($\Delta G_{sub} \geq -0.889$) will have greater than 50% chance of being sensitive to CT8 (opposed to only a 30% occurrence of sensitive proteins in the signal peptide-containing proteome as a whole). Confining our search to proteins with ΔG_{sub} in the top 10 percentile increases the probability to 75%. Therefore, confining our search for CT8-senstive proteins to those with high ΔG_{sub} should result in a high success rate. There is, however, the important caveat that these probabilities depend on the assumption that the distribution of sensitive and resistant proteins with respect to ΔG_{sub} is the same in the entire

secretome as it is in our sample. There are 3400 SP-containing proteins in the proteome. For a 95% confidence interval and 0.05% accuracy that the distribution of our sample matches the population it was drawn from, we would need a sample size of 350 proteins. Our sample is only 202 so the confidence interval is larger and accuracy less precise for this sample.

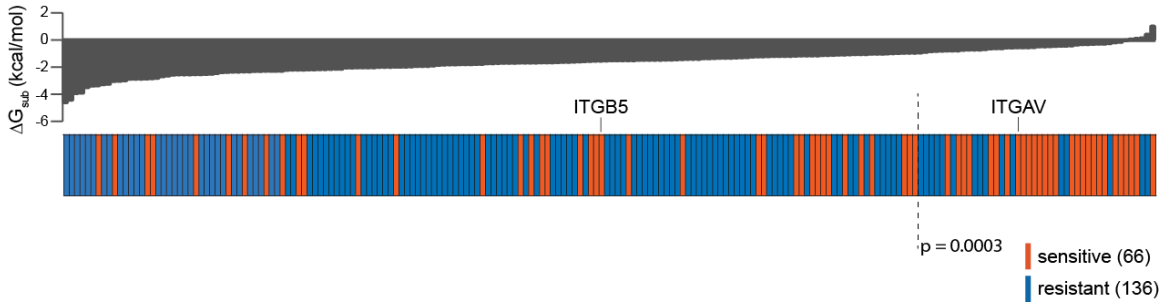

Figure 2.16 | Ranking by ΔG_{sub} significantly enriches for CT8-senstive proteins. Sensitive (orange) and resistant (blue) proteins were ranked by ΔG_{sub} (values shown in the upper panel). P-value for enrichment was determined using a two-tailed Fisher's exact test. Ranking of integrins β5 (ITGB5) and αV (ITGAV) is highlighted.

Before attempting to predict CT8-sensitive proteins, we sought to investigate the contradictory observation of numerous CT8-sensitive proteins with relatively low values of ΔG_{sub}. We surmised that, while these proteins are sensitive in cells, their translocation may not be directly inhibited by CT8; rather, their apparent sensitivity may derive from CT8-mediated inhibition of a binding partner. To test this hypothesis, we examined the integrin subunits αV (ITGAV, ΔG_{sub} = –0.548) and β5 (ITGB5, ΔG_{sub} = –1.531). Integrins are cell surface adhesion molecules that are expressed as obligate heterodimers of α and β subunits. Integrin αV is known to partner with β1, β3, β5, β6 or β8, whereas the only known partner for β5 is αV. Both integrins αV and β5 were identified as CT8-sensitive proteins, with similar $(L/H)_{final}$ of 2.4 and 2.3, respectively.

Flow cytometry of JJN-3 cells confirmed the CT8 sensitivity of integrin β5 in cells, with an EC_{50} of ~15 nM (**Figure 2.17a,b**).

To test whether the translocation of either of these proteins is affected by CT8, we employed in vitro translation/translocation assays. Integrins αV and β5 translated poorly in vitro as the full-length construct, probably due to their large size. Truncation to shorter constructs resulted in increased translation. In vitro translocation of truncated mRNA for both integrin αV (truncated to 900 bases) and β5 (truncated to 1212 bases) (in separate assays) shows dose-dependent loss of a protease-protected, cRM-dependent band, indicating direct inhibition of their cotranslational translocation by CT8 (**Figure 2.17c**). However, the IC_{50} for β5 is ~400 nM, whereas the IC_{50} for αV is ~30 nM, which more closely resembles the EC_{50} value in cells (**Figure 2.17d**). Since β5 trafficking to the plasma membrane is dependent on αV synthesis, loss of αV is predicted to result in loss of β5. Thus, the potent inhibition of β5 expression in cells is most likely an indirect effect of blocking αV translocation into the ER. It's possible that other apparently CT8-sensitive proteins are affected indirectly (e.g. Jak3, **Figure 2.6**). Despite these caveats, we hypothesized that signal peptides with the highest ΔG_{sub} would tend to be more sensitive to CT8.

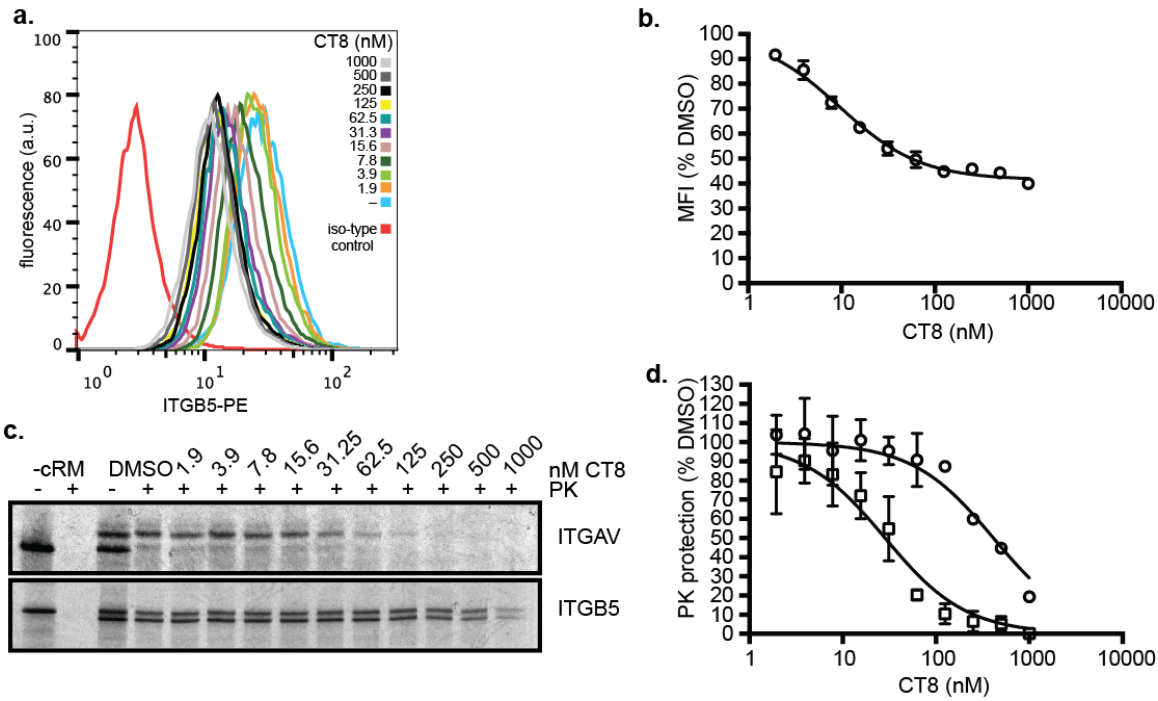

Figure 2.17 | CT8 inhibition of integrin αV expression drives loss of integrin β5. (a) JJN-3 cells were treated with DMSO or the indicated concentration of CT8 for 24hrs and then stained with a phycoerythrin (PE)-conjugated anti-integrin β5 antibody and 10,000 live cells per sample were counted by flow cytometry. **(b)** Plot of the mean fluorescence intensity (MFI) for each concentration from **(a)**. **(c)** ITGAV (upper) or ITGB5 (lower) mRNA (truncated to 900 and 1212 bases, respectively) was translated in vitro in the absence or presence of canine rough microsomes (cRM) and either DMSO or the indicated concentration of CT8. Samples translated in the presence of cRMs were treated with protease K (PK). Samples were separated on 10% Tris-Tricine SDS-PAGE gels and analyzed by autoradiography. **(d)** Intensity of the PK-protected fragment is plotted as a percent of DMSO.

Comparison of the ΔG_{sub} distributions for the secretome and our analyzed sample showed near perfect overlap (**Figure 2.18**), suggesting that the top quartile enrichment that we see in the mass spectrometry-derived dataset might reflect the secretome as a whole. To test the predictive power of ΔG_{sub}, we chose 5 proteins predicted to be resistant and 5 proteins predicted to be sensitive on the basis of their ΔG_{sub} values (**Figure 2.19**). We determined their CT8 sensitivity in in vitro translocation assays (**Figure 2.20a, b**). Of the 5 proteins predicted to be sensitive, CT8 blocked the

translocation of 4 with an IC_{50} of less than 250 nM (**Figure 2.20a, c**). All 5 proteins predicted to be resistant had an IC_{50} greater than or equal to 1 μM (**Figure 2.20b, d**).

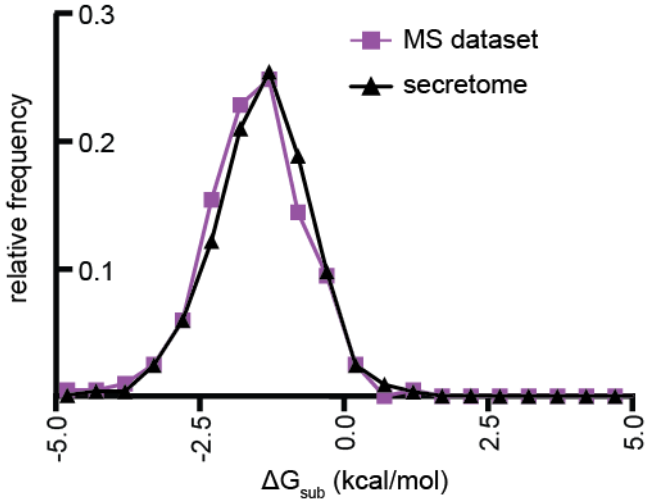

Figure 2.18 | Distribution of ΔG_{sub} for SILAC dataset matches that of the secretome. ΔG_{sub} for all signal peptides predicted in Swiss-Prot (black) and in the SILAC dataset (purple) were calculated and plotted against their relative frequencies.

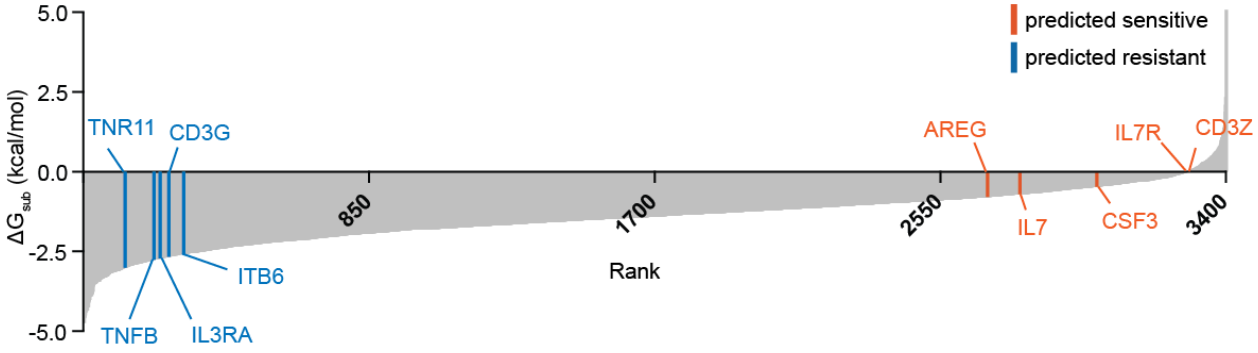

Figure 2.19 | ΔG_{sub} ranking of proteins predicted to be either sensitive or resistant to CT8. The ΔGsub for all proteins annotated with a signal peptide in Swiss-Prot, sorted from lowest (most hydrophobic) to highest (least hydrophobic), with predicted proteins in blue (resistant) and orange (sensitive).

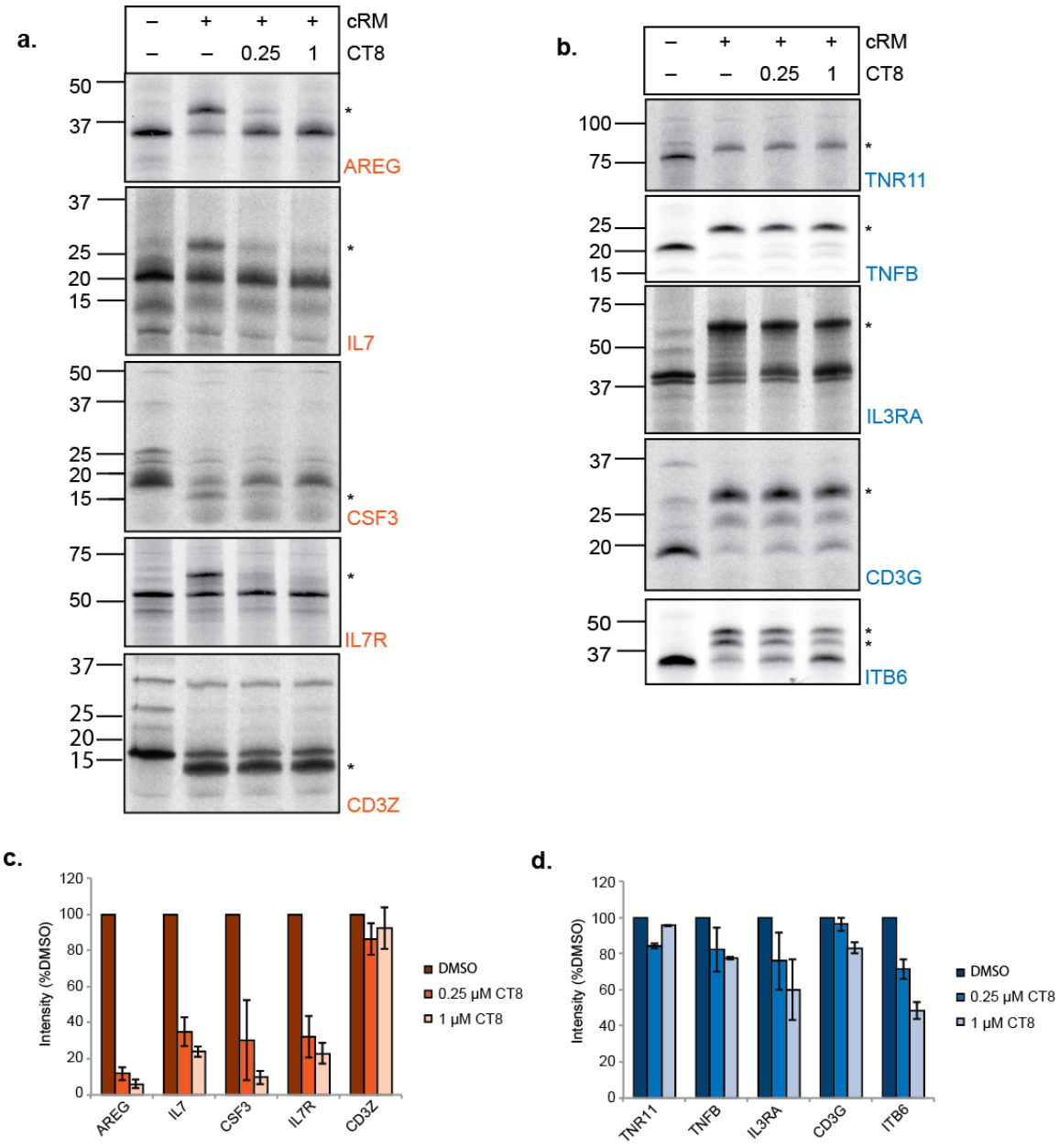

Figure 2.20 | ΔG_{sub} of signal peptides can predict sensitivity to CT8. Representative in vitro translation/translocation assays for proteins predicted to be sensitive **(a)** or resistant **(b)** to CT8. mRNA was translated in reticulocyte lysate with ^{35}S-Met in the absence or presence of canine rough microsomes (cRMs) and the indicated concentrations of CT8. The samples were separated on 7.5% (TNR11), 10% (AREG, IL7, IL7R, TNFB, IL3RA, CD3G, ITB6), or 12% (CSF3, CD3Z) Tris-Tricine SDS-PAGE gels and analyzed by autoradiography. Quantification of the intensity of the cRM-dependent band (* for AREG, IL7, IL7R, TNR11, TNFB, IL3RA, CD3G, and ITB6 indicates the glycosylated forms, * for CSF3 and CD3Z indicates signal-cleaved forms) from predicted sensitive proteins is shown in **(c)** and predicted resistant in **(d)**. Intensity is shown as a percent of DMSO. Bars represent the mean intensity from three independent experiments with error bars representing +/– SD.

2.10 Discussion

The ability to pair drug-induced cellular phenotypes with distinct biological targets is at the core of chemical biology. Inhibitors of protein machines responsible for the biogenesis of thousands of cellular proteins like Sec61α, provide an extra challenge in that there may be distinct targets responsible for cellular phenotypes in different contexts. With this study, we have identified 124 novel CT8-sensitive proteins. These targets consist primarily of Sec61 client proteins, though 13 are cytosolic proteins whose membrane localization is likely perturbed by loss of an integral membrane interaction partner, as in the case of JAK3 and IL2RG. Many of these targets, such as HGF and CD74 suggest potential disease contexts in which Sec61 inhibitors like CT8 may be effective tools and/or leads for development of novel therapies.

HGF has recently been implicated in acute myeloid leukemia[26]. Both small molecule kinase inhibitors of its receptor c-Met (tivantinib, crizotinib) and antibody-based therapeutics (rilotumumab and MetMAb) are being investigated for clinical applications in a range of both solid and non-solid tumors[27]. Despite the therapeutic promise, resistance mechanisms are already being detected, ranging from up-regulation of c-Met expression to over-expression of HGF itself. Additionally, HGF has been shown to mediate both innate and acquired drug resistance in lung cancer[28,29] and melanoma[30]. Cotransins provide a novel mechanism of blocking HGF signaling by inhibiting the synthesis of the growth factor, thereby addressing the main mechanism of resistance. The ability to inhibit the synthesis of this growth factor represents a novel point of access to this important pathway.

CD74 has been implicated in the survival of chronic lymphocytic leukemia[31]. CD74 acts primarily as a chaperone for the MHC class II molecules, responsible for shepherding them through the secretory pathway to their final destination on the plasma membrane[32]. A very small amount of chondroitin sulfate-modified CD74 remains on the surface of the cell and acts as a survival receptor[33]. CT8 affects the plasma membrane expression of both CD74 and a number of its client MHC class II molecules, as well as chondroitin sulfate synthase I, an enzyme required for conjugation of the glycan chondroitin sulfate. Careful dissection of this pathway will be required to pinpoint whether just one or all of these molecules are directly inhibited by CT8, yet our results point to Sec61 inhibition as a novel way to inhibit plasma membrane expression of CD74.

Along with identifying new CT8-sensitive proteins, proteomic analysis provided the largest dataset yet of sensitive and resistant proteins. Initial analysis of type II signal anchors in this dataset showed a difference in median ΔG_{calc} values between sensitive and resistant proteins possibly supporting our existing model that cotransins inhibit TMD partitioning through the lateral gate of Sec61. A more surprising result was the striking enrichment of CT8-sensitive sequences among signal peptides with high ΔG_{calc}. Because it is still unclear whether signal peptides must fully partition through the lateral gate into the lipid bilayer, it was not obvious that the transfer free energy between the Sec61 translocon and the membrane, estimated by ΔG_{calc}, would relate to CT8 sensitivity of signal peptides. The fact that it does suggests that 1) signal peptides and TMDs share a common translocational mechanism that requires lateral gating of Sec61,

partially described by ΔG_{calc} and 2) this gating represents the CT8-senstive step in cotranslational translocation.

There is structural evidence to support a model in which signal peptides at least partially partition through the lateral gate to facilitate translocation of type I membrane and secreted proteins. Structures of *E. coli* SecYEG either in complex with a signal peptide (2D electron crystallography)[25] or, more recently, in complex with a stalled ribosome-nascent chain (cryo-EM)[34] show density for the short, α-helical hydrophobic region of a signal peptide in the open lateral gate of SecY. In addition, translating nascent chains show cross-linking between signal peptide amino acids as well as lipids of the bilayer. Therefore it is likely that CT8 blocks SP-mediated translocation by a mechanism similar to its inhibition of type II signal anchor integration.

We have previously shown that simple changes to cotransin structure can lead to dramatic effects on potency and selectivity[7]. Our results with CT8 suggest that, rather than distinguishing between specific sequences per se, cotransins instead raise the thermodynamic energy barrier to integration. Only sequences with a high enough intrinsic thermodynamic driving force are able to overcome CT8 binding and laterally gate Sec61. Therefore, it is likely that CT9, the more potent and promiscuous cotransin analog, increases the energy barrier to a greater extent than CT8. Thus, a SP would require an even higher intrinsic driving force (captured by a low ΔG_{sub}) to counteract the effect of CT9 binding. Since fewer proteins have signal peptides with low enough ΔG_{sub} values to satisfy this requirement, more proteins are sensitive to CT9. It should therefore be possible to make cotransins that are even more selective than CT8 by synthesizing compounds whose binding to Sec61 increase the energy barrier to

integration less than CT8. More sequences would have the intrinsic driving force necessary to overcome binding of these new compounds, resulting in translocation inhibition of fewer proteins.

While our predictions were relatively successful, there is still a level of complexity in cotransin sensitivity that has yet to be explained. We can explain some of the more hydrophobic (lower ΔG_{sub}) yet sensitive sequences as indirect effects, as in the case of integrin αVβ5. We currently cannot explain why proteins with relatively hydrophilic signal peptides like CD3Z are resistant. In addition, previous mutational studies of signal peptides showed that even mutations in the mature domain of the protein, C-terminal to the signal peptidase cleavage site, can result in increased resistance to the CT8-related compound, CAM741[6]. Lastly, our results show that there is a spectrum of sensitivity and resistance along the spectrum of ΔG_{sub} values and the correlation is far from perfect. A much larger survey of signal peptides and possibly signal anchors will be required to determine the extent to which ΔG_{sub} predicts sensitivity. It is likely that other sequence features such as helical propensity, charges, presence of consecutive polar and/or non-polar residues, residues adjacent to the H-region, and the distance between the H-region and the signal peptide cleavage site all play a role in determining CT8 sensitivity.

In spite of the imperfections, the fact that SPs with high ΔG_{sub} tend to be CT8-sensitive allowed for the identification of novel CT8-sensitive targets based solely on primary amino acid sequence. The ability to predict sensitive targets should greatly increase our ability to find new therapeutic applications for Sec61 modulators. For example, 9% of patients with T-cell acute lymphoblastic leukemia (T-ALL) exhibit gain of function mutations in IL7R that cause cytokine-independent proliferation of lymphoid

progenitor cells[3536]. Based on our analysis of signal peptide ΔG_{sub} across the entire secretome, we predicted IL7R would be inhibited by CT8. In vitro translation/translocation reactions bore this out. These results suggest that Sec61α inhibition by CT8-like compounds may be an attractive therapeutic strategy for T-ALL driven by IL7R mutations. Interestingly, in non-cancer settings, IL7R requires IL2RG for downstream signaling. Moreover, translocation of the IL7R ligand, IL7, was also potently inhibited by CT8. Thus, CT8 affects three members of the IL7 signaling complex. CT8 may therefore be profoundly immunosuppressive, as $IL7^{-/-}$ and $IL7R^{-/-}$ knockout mice show significant repression of T-cell development[37,38].

2.11 Experimental Procedures

Antibodies, cells, and reagents

JJN-3 cells were a kind gift from Peter Walter (UCSF). The transcription reaction mix, rabbit reticulocyte lysates and canine rough microsomes[39] were provided by Ramanujan Hegde (MRC, LMB, Cambridge). CT8 was prepared as previously described[5]. The following primary antibodies were used: anti-CD74 (Santa Cruz, sc-6262), anti-IL2Rγ (Santa Cruz, sc-3659), anti-Bip (Cell Signaling, #3177), anti-Jak3 (Cell Signaling, #8827), anti-Na/K ATPase (Ab-cam, ab7671), anti-pSTAT5 (Cell Signaling, #4322), anti-β-tubulin (Sigma, T6199), anti-Flag M2 antibody (Sigma, F1804), anti-integrin β5-PE (BioLegend, 345203). The sources of other reagents are listed as they are described. All cDNAs used to make PCR templates for in vitro translocation assays were purchased from DNASU or the Harvard plasmid database except for CD3Z

and CD3G, which were kind gifts from Art Weiss (UCSF). See **Appendix C** for a full list of cDNAs and catalog numbers/origin lab.

Cell culture, SILAC labeling and membrane purification

HEK293/FRT/Trex cells (Invitrogen) were cultured in DMEM (Gibco) supplemented with 10% FBS (Axxenia) and 1,000 units/ml penicillin and streptomycin (Gibco) (growth media) in a humidified incubator at 37°C and 10% CO_2. JJN-3 cells were cultured in RPMI-1640 (Gibco) supplemented with 10% FBS (Axxenia), 1,000 units/ml penicillin and streptomycin (Gibco) and GlutaMAX^{TM} (growth media) in a humidified incubator at 37°C and 5% CO_2.

SILAC labeling of JJN-3 cells was carried out by culturing cells in growth media containing either light arginine and lysine (arginine-$^{12}C_6$ and lysine-$^{12}C_6$) or heavy arginine and lysine (arginine-$^{13}C_6$,$^{15}N_4$ and lysine-$^{13}C_6$) (Thermo Scientific) for 6 doublings to ensure complete labeling. Labeled cells were treated with either 0.1% DMSO (light) or 250nM CT8 (heavy) at $7.5x10^5$ cells/ml in 20 ml of media in 5 x 15 cm dishes per treatment for 24hrs at 37°C. Cells were collected by centrifugation at 500 x *g* for 5 min at room temperature. Cell pellets were washed once with PBS, pelleted again and frozen in 20 x 10^6 cell aliquots.

Frozen cell pellets of 20 x 10^6 cells were thawed in 400 µl hypotonic lysis buffer (10mM Tris pH 8.0, 10mM NaCl, 1.5mM $MgCl_2$) for 30 min on ice and heavy and light samples were pooled 1:1 to give 800 µl samples. For samples that were not SILAC labeled, cell pellets were kept separate and processed in parallel. Complete protease inhibitor, EDTA-free (Roche) was added to 1x (from a 50x stock solution in water).

Swelled cell pellets were sonicated using a miniature probe sonication wand: 10 x 10 s bursts with 10 s rests in between. Complete homogenization was confirmed by microscope visualization.

Lysates (800 µl) were centrifuged at 15,000 x *g* for 10 minutes at 4°C followed by ultra-centrifugation of the supernatant at 43,000 rpm in a TLA100.3 Beckman rotor for 30 min at 4°C using an Optima TLX Ultracentrifuge (Beckman Coulter). The crude membrane pellet from 40 x 10^6 cells was resuspended in 100 µl 40% Optiprep (Sigma) in membrane buffer (250 mM sucrose, 10 mM Tris pH 8.0, 1.5 mM $MgCl_2$, 10 mM NaCl) and layered at the bottom of a 0-22% continuous Optiprep gradient. Gradients were prepared by layering 1.25 ml 0% Optiprep on top of 1.25 ml 22% Optiprep in 11 x 34 mm polyallomer centrifuge tubes (Beckman, 347357) and allowing diffusion to occur horizontally over 1 hour. For an experiment with 5 plates for each treatment, 4 gradient tubes were prepared.

The gradients were ultra-centrifuged at 55,000 rpm for 3 hrs at 4°C in a TLA-55 Beckman swinging bucket rotor. 12 x 208 µl fractions were collected by pipetting from the top. Fractions with the highest enrichment of gamma-glutamyl transferase activity (over succinate dehydrogenase or cytochrome c reductase activity, see following section for details) were pooled, diluted 1:1 with membrane buffer and pelleted for 1 hour at 49,000 rpm and 4°C in a TLA100.3 Beckman rotor. The pellet, corresponding to 2-3 combined fractions, was resuspended in 50 µl 0.1% Rapigest detergent in 50 mM NH_4HCO_3 and the protein concentration was measured by BCA analysis. For samples that were not SILAC labeled and were destined for immunoblotting, final solubilized pellets were diluted to 1x in Laemlli sample buffer and separated by SDS-PAGE.

Analysis of gradient fractions for membrane enrichment

Gamma-glutamyl transferase activity (plasma membrane marker) was measured by mixing 20 µl of each fraction with 60 µl of GGT working solution (1 mM gamma-glutamyl-p-nitroanalide (Sigma), 20 mM glycyl glycine (Sigma), 0.1 M Tris pH 7.6). Reactions were incubated at room temperature for 10 minutes and then the absorbance at 405 was measured in 1:30 minute intervals for 15 minutes.

Succinate dehydrogenase activity (mitochondrial marker) was measured by mixing 20 µl each fraction with 80 µl di H_2O and 100 µl SDH working solution (0.05 M sucrose, 0.1M sodium succinate (Sigma), 2 mg/ml p-iodonitrotetrazolium (Sigma). Reactions were incubated at room temperature for 10 min and then the absorbance at 490 nm was measured at 30-second intervals for 5 min.

Cytochrome c reductase activity (endoplasmic reticulum) was measured using the cytochrome c reductase (NADPH) assay kit (Sigma, CY0100) according to the manufacture's instructions. Briefly, 20 µl of each fraction was mixed with 190 µl cytochrome c solution (5.4 mg cytochrome c in 12 ml assay buffer), 4 µl cytochrome c oxidase inhibitor solution (50 mM potassium cyanide in H_2O) and 20 µl NADPH in H_2O

Analysis of membrane enriched samples by mass spectrometry

Membrane pellets were solubilized in 0.1% Rapigest (50 µl for a pellet arising from 2-3 combined gradient fractions). Solubilized pellets were combined to give 100 µl containing ~150-200 µg of protein determined by BCA analysis. Samples were reduced by adding 2.5 µl 200 mM DTT in H_2O at 60°C for 30 min Reduced samples were

alkylated by addition of 1.5 µl 1 M iodoacetamide in the dark for 30 min at room temperature. Reduced and alkylated samples were trypsinized with 1:100 m/m sequence grade modified trypsin (0.5 µg/µl, Promega, V511C) at 37°C overnight. Following proteolysis, 25 µl 10% TFA in H_2O was added and samples were incubated for 30 min at 37°C to destroy the Rapigest detergent prior to analysis by LC MS/MS.

Digested peptides (100 µg) were fractionated using hydrophilic interaction chromatography (HILIC). The samples were injected onto a TSKgel amide-80 column (Tosoh Biosciences, 2.0 mm x 15 cm packed with 5 um particles) using 10% HILIC buffer A (2% ACN, 0.1% TFA) and 90% HILIC buffer B (98% ACN, 0.1% TFA) using an AKTA P10 purifier system. The samples were then separated by a one-hour gradient from 90% HILIC buffer B to 55% HILIC buffer B at a flow rate of 0.3 ml/min. Fractions were collected every 1.5 minutes and combined into 12 fractions based on the 280 nm absorbance chromatogram. Fractions were evaporated to dryness and reconstituted in 20 µl 0.1% formic acid for mass spectrometry analysis.

Each combined fraction (2 µl, in duplicate) was analyzed separately by a Thermo Scientific LTQ Orbitrap Elite mass spectrometry system equipped with an Easy-nLC 1000 HPLC and autosampler. Samples were injected onto a pre-column (2 cm x 100 µm I.D. packed with 5 um C18 particles) in 100% buffer A (0.1% formic acid in water) and separated by a 120 minute reverse phase gradient from 5% to 30% buffer B (0.1% formic acid in 100% ACN) at a flow rate of 400 nl/min. The mass spectrometer continuously collected spectra in a data-dependent manner, acquiring a full scan in the Orbitrap (at 120,000 resolution with an automatic gain control target of 1,000,000 and a maximum injection time of 100 ms) followed by collision-induced dissociation spectra for

the 20 most abundant ions in the ion trap (with an automatic gain control target of 10,000, a maximum injection time of 10 ms, a normalized collision energy of 35.0, activation Q of 0.250, isolation width of 2.0 m/z, and an activation time of 10.0). Singly and unassigned charge states were rejected for data-dependent selection. Dynamic exclusion was enabled to data-dependent selection of ions with a repeat count of 1, a repeat duration of 20.0 s, an exclusion duration of 20.0 s, an exclusion list size of 500, and exclusion mass width of + or - 10.00 ppm.

SILAC quantitation and statistical analysis

Raw mass spectrometry data was converted to a pearl list using the PAVA algorithm. Data were searched using the Protein Prospector suite of algorithms[40]. The data were searched against the Swiss-Prot Human protein sequence database (downloaded March 6, 2012) with a concatenated decoy database comprised of all sequences with their amino acids randomized. The algorithm searched for fully tryptic peptides with up to 2 missed cleavages using a parent mass tolerance of 20 ppm and a fragment mass tolerance of 0.8 Da. The algorithm indicated a static modification for carboxyamidomethyl of cysteine residues, and for variable modifications acetylation of protein N-termini, glutamine to pyroglutamate conversion, and methionine oxidation. SILAC light and heavy searches were performed separately with the heavy search including $^{13}C_6$-lysine and $^{13}C_6$,$^{15}N_4$-arginine static modifications. Light and heavy results were combined and filtered using a Protein Prospector expectation value necessary to obtain a false positive rate of 1%. The data were further filtered to remove any peptides that mapped to multiple protein sequence and proteins identified by fewer than 2

peptides.

For quantification of SILAC ratios, data were converted to mzXML using the ReAdW program. SILAC ratios were calculated by extracted precise ion chromatograms using an in-house algorithm written in the C programming language. The algorithm extracted an ion chromatogram for light and heavy peptide m/z values with a mass accuracy of + or – 10 ppm and smoothed the data by averaging every three data points. The algorithm picked peaks by starting at the retention time at which each peptide was identified by MS/MS and defining the beginning and end of the peak as either the retention times where the intensity was half the maximum or for which a local minimum was detected in either the light or heavy channels. The SILAC ratio was calculated as the area under the light peak divided by the area under the heavy peak.

Light/heavy ratios ($(L/H)_{final}$) were determined for 2951 unique proteins that were identified with two or more peptides. $(L/H)_{final}$ for each protein was calculated as follows. For a given protein, the median L/H ratio (median $(L/H)_{pep}$) for all identified peptides of a given protein in a given experiment was normalized to the median $(L/H)_{pep}$ ratio for all identified proteins in that experiment, giving $(L/H)_{norm,exp}$. The median of all $(L/H)_{norm,exp}$ was calculated over the three biological replicates to give $(L/H)_{final}$.

The median and standard deviation of the log of all $(L/H)_{pep}$ for all peptides of each protein were calculated. In order to rapidly identify statistically significant changes, log SILAC ratios were randomly sampled from the entire data set 1,000 times and randomized distributions were built for proteins identified with 2 peptides, 3 peptides, and so on up to the maximum number of peptides identified for a single protein. A Welch's t-test probability was then calculated to compare to samples having possibly

unequal variances. The t value was calculated as below:

$$t = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{s_1^2}{N_1} + \frac{s_2^2}{N_2}}}$$

X1 is the median of log SILAC ratios for the protein of interest, X2 is the median of log SILAC ratios for the randomly sampled protein, s is the sample variance, and N is the sample size. The degrees of freedom was calculated as N-1.

Statistical comparison of the difference in median hydrophobicity values (**Table 2.3**) for sensitive and resistant proteins was done using a two-tailed Mann Whitney U test with an exact p-value in Graphpad Prism 6. Statistical significance was defined as $p \leq 0.05$. The nonparametric Mann Whitney U test was used because, assuming sensitive proteins are enriched in high ΔG_{calc} and resistant proteins enriched in low ΔG_{calc}, the overall distribution of ΔG_{calc} in these two populations would not be Gaussian.

Statistical enrichment of sensitive signal peptide-containing proteins in the top quartile of ΔG_{sub} when ranked by ΔG_{sub} was determined using a Fisher's exact test with a two-tailed p-value using GraphPad QuickCalcs (http://graphpad.com/quickcalcs/contingency1/). Outcome 1 = sensitive (28 proteins), Outcome 2 = resistant (22 proteins), Group 1 = top quartile ΔG_{sub} (50 proteins), Group 2 = all sensitive and resistant SP-containing proteins (202 proteins).

Bioinformatics and hydrophobicity analysis of identified proteins

Lists of proteins whose Swiss-Prot entries contained the following key words were generated, one list per key word search: "signal peptide", "type II membrane",

“type III membrane”, “multi-spanning membrane”. For all key word searches, selected proteins also had to meet the criteria of: organism = human, subcellular localization NOT mitochondria. To be labeled as a multi-spanning membrane protein, they also could not have “signal peptide” in their entry. The list of identified proteins was compared to these four, Sec61-dependent protein lists to annotate each entry as signal peptide-containing (SP), type II, type III, or multi-spanning membrane protein (multi-TM). For the drug target lists in Chapter 4 and Appendix A, proteins were also compared to lists of Swiss-Prot entries with the subcellular location “single-pass type I transmembrane” and the GO terms 0004714 (transmembrane receptor tyrosine kinase activity) and 0004930 (G-protein coupled receptor activity) to annotate for type I transmembrane proteins (type I), receptor tyrosine kinases (RTK) and G-protein coupled receptors (GPCR) respectively. The sequence features (GFF) file retrieved from batch search of Swiss-Prot accession numbers for all proteins annotated as “SP” were manually examined for entries containing more than one transmembrane domain. These proteins were labeled “SP, multi-TM” and were excluded from the ΔG_{calc} analysis of signal peptides due to the difficulty in distinguishing between true signal peptides and transmembrane domains.

To obtain amino acid sequences of type II signal anchors, the entire protein sequence was entered into the ΔG calculator (http://dgpred.cbr.su.se/index.php?p=home) using the “Full Scan” function to pick the lowest ΔG transmembrane domain with a window size of 19-23 amino acids and no length correction. For the few proteins that were annotated as type II in Swiss-Prot, yet the ΔG calculator identified more than one TMD, the one with the lowest ΔG_{calc} was

chosen for analysis. In these cases, there was typically one ΔG_{calc} with a negative value and one with a positive value. The ΔG_{calc} associated with the chosen sequence was the value used for analysis.

To obtain the sequences of signal peptides, the sequence feature file was batch retrieved from Swiss-Prot for accession numbers annotated “SP”. This file was sorted for the sequence feature “signal peptide” to give the N-terminal amino acid position (always 1) and the amino acid position for the predicted cleavage site. These two numbers, along with the corresponding accession number, were transferred to an Excel file with three columns with the headings: Accession, From, To. The Accession column contained accession numbers, the “From” column contained the N-terminal position (1) and the “To” column contained the cleavage site prediction (varied for each signal peptide). This file was saved as a Windows compatible .CSV file. Additionally, FASTA sequences for each accession number in the .CSV file were batch retrieved from Swiss-Prot. The .FASTA was changed to .TXT by changing the file name. These two files, the .CSV containing the from and to designations for the beginning and end of the signal peptide and the .TXT file containing the sequences for all proteins being interrogated were given as input to the fasta_segment.py script written by Peter Malkin (Google) in the Python programming language (**Appendix B**). The output contains the amino acid sequence beginning at the “From” position and ending at the “To” position.

To calculate the hydrophobicity values for signal peptides, each signal peptide was truncated to 30 amino acids from the predicted cleavage site, as the maximum input length for the ΔG calculator in “ΔG prediction” mode is 30aa. Hydrophobicity was calculated for signal peptides using the Kyte-Doolittle[17] and Hopp-Woods[18] scales

(**Table 2.2**) by summing the value for each amino acid in the sequence across the truncated signal peptide and either dividing by the length (KD_{avg} and HW_{avg}) or not (KD_{tot} and HW_{tot}). For ΔG_{calc} analysis of signal peptides, the truncated signal peptide was entered into the ΔG calculator (http://dgpred.cbr.su.se/index.php?p=home) using "ΔG prediction" mode with no length correction to give ΔG_{tot}. This value was divided by the length of the truncated sequence to give ΔG_{avg}. Finally, ΔG_{sub} was calculated by entering the truncated sequence into the ΔG calculator in "ΔG prediction" mode with no length correction AND allowing for a subsequence (if lower ΔG).

Cell-based assays

JJN-3 cells were seeded at 750,000/well in 1.5 ml growth media in a 6-well plate in the presence of either 0.1% DMSO or the indicated concentrations of CT8 and 0.1% DMSO. Cells were incubated with drug for 24 hrs after which they were collected, pelleted at 900xG for 5 min at room temperature and washed twice with PBS. Final cell pellets were flash frozen and then thawed in Celytic M (Sigma) supplemented with 1x Complete EDTA-Free Protease Inhibitor Cocktail (Roche). Whole-cell lysates were normalized for protein content by Bradford analysis (Bio-Rad) and 20 µg (10 µg for TRPML1 gels) was separated by SDS-PAGE.

SDS-PAGE, autoradiography, and Immunoblotting

7.5, 10, or 12% Tris-Tricine or traditional SDS-SAGE gels were run as indicated. For autoradiography, gels were stained with Coomassie Brilliant Blue before drying with a gel drier. Quantitative autoradiography was performed after exposing the dried gels to

a storage phosphor screen (GE Healthcare) and imaged on a Typhoon 9400 scanner. Images were quantified using ImageJ (NIH). Qualitative images were obtained by exposing the gels to BioMax MR film (Kodak).

For immunoblotting, whole-cell lysates or solubilized membrane enriched fractions were run on 7.5 or 10% Tris-Tricine cells and transferred to nitrocellulose. Membranes were blocked with LI-COR blocking buffer, incubated with the appropriate primary antibodies at the following dilutions: α-IL2Rγ (1:500), α-CD74 (1:500), α-Bip (1:1000), α-Flag (1:10,000) at 4°C overnight. Tubulin was used a 1:2000 at room temperature for 1 hr. Following primary antibody incubation, membranes were washed three times with TBST and then incubated with infrared dye-labeled secondary antibodies (IR680 or IR800, LI-COR) and visualized using the LI-COR Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE).

HGF ELISA

JJN-3 cells were washed once with fresh growth media and were seeded at $1x10^6$ cells per well in 1.5 ml growth media supplemented with 0.1% DMSO and the indicated concentrations of CT8 and incubated for 24 hrs. Media and cells were collected and cells were pelleted at 900xG. Media was diluted 1:10 with assay diluent (R&D Systems) and ELISA was performed according to manufacturer's instructions (R&D Systems Quantikine ELISA Human HGF DHG00).

Flow cytometry

JJN-3 cells were seeded at 750,000/well in 1.5 ml growth media supplemented with 0.1% DMSO and the indicated concentrations of CT8. Cells were collected by centrifugation at 500xG for 5 min, washed once with PBS containing 5% FBS (FACs buffer) and then incubated in 100 µl 1:20 α-integrin-β5-PE (Bio Legend) or isotype control (Bio Legend) in FACs buffer for 30 min at room temperature. Cells were then washed once with FACs buffer and resuspended 350 µl FACs buffer and analyzed by flow cytometry using a FACSCalibur (BD Biosciences), counting 10,000 cells per sample. Data were analyzed using FlowJo 10.0.6.

Plasmid and DNA template preparation

To generate WT-TRPML1-3xFLAG, WT TRPML1 was PCR amplified from the pDONR221 vector purchased from the Harvard Plasmid Database (clone ID HsCD00040401) with forward and reverse primers introducing 5'-EcoRI and 3'-XbaI restriction sites using the following primers:

TRPML1_EcoRI_fwd: 5'-CGCGGAATTCATGACAGCCCCGGCGGGTCCG-3'

TRPML1_XbaI_rev: 5'-CGCGTCTAGACAAATTCACCAGCAGCGAA-3'

The product was PCR purified and doubly digested with EcoRI-HF and XbaI (NEB) in CutSmart™ buffer for 2 hours at 37°. The empty pCMV-3xFLAG™-13 expression vector (Sigma, E7783) was digested in the same way. The gel purified 5'-EcoRI-TRPML1-XbaI-3' insert and the digested vector were ligated at 4°C o/n with T4 DNA ligase (NEB) followed by transformation into DH5α competent cells and plating on ampicillin. Colonies were confirmed by DNA sequencing.

Point mutations in TRPML1 TMD1 were generated using the Quickchange method (Stratagene) with the following primers:

TRPML1 2L fwd: 5'-TGCAAGCTGATGCTGCTGGTGGTCCTGATCCTGGTGGTCACG-3'

TRPML1 2L rev: 5'-CGTGACCACCAGGATCAGGACCACCAGCAGCATCAGCTTGCA-3'

TRML1 4L fwd: 5'-CTGATCCTGGTGGTCCTGGTGCTGCTCATCCTGTTTGG-3'

TRPML1 4L rev: 5'-CCAAACAGGATGAGCAGCACCAGGACCACCAGGATCAG-3'

2L TRPML1 was generated using the WT-TRPML1-3xFLAG plasmid as template. 4L TRPML1 was generated using 2L TRPML1 as template. All mutations were confirmed by DNA sequencing.

DNA templates for in vitro translation were prepared by PCR amplification of the indicated gene (see **Appendix C** for a full list of cDNAs) using a forward primer that included the T7 promoter (bold) followed by a Kozak consensus sequence (underlined) and ending with a gene specific sequence that begins immediately after the ATG start codon (highlighted):

5'-gcc**TAATACGACTCACTATAGGG**AGACCATG-Gene_specific_sequence-3'.

The reverse primer was comprised of a gene-specific sequence corresponding to the 3' end including a stop codon followed by 6 random bases (see **Appendix D** for full list of forward and reverse primers used to make in vitro translation templates). Integrins αV and β5 did not translate well in vitro as the full-length protein. Therefore, templates for these two genes were truncated to 900 and 1212 bases, respectively. For truncated constructs, the reverse primer was comprised of a gene specific sequence corresponding to 15 bases at the point of truncation, followed by a stop codon and 6 random bases. All DNA templates were purified by PCR purification (Quigen).

In vitro translation/translocation assays

In vitro transcription/translation/translocation was carried out as previously described[39]. Briefly, DNA templates encoding full-length or truncated versions of the indicated constructs were transcribed with T7 polymerase (NEB) for 1 hr at 37°C. Transcripts were used immediately in subsequent translation reactions which were assembled at 0°C in the presence of CT8 (from a 100x stock in DMSO) or the equivalent amount of DMSO. Reactions were run at 32°C for 1 hour and then moved to ice for further processing. All samples contained ^{35}S-Methionine and where indicated canine rough microsomes (cRM). Samples not subjected to protease protection were diluted 1:10 with 0.1 M Tris pH 8.0, 1% SDS (quench buffer) followed by 1:1 dilution with 2x Laemmli sample buffer and separation by SDS-PAGE. Protease protection was performed as previously described[39].

Stable cell line generation

The C-terminally 3xFlag TRPML1 construct (WT-TRPML1-3xFlag) was inserted the pCDNA5/FRT/TO donor vector using Gateway technology (Life Technologies) to generate TRPML1-3xFlag-pCDNA5/FRT/TO. Stable cells expressing TRPML1-3xFlag under tetracycline control were generated using the Invitrogen Flp-in HEK293 T-REx in-cell FRT recombination system according to manufacturer's instructions. Briefly, TRPML1-pCDNA5/FRT/TO was transfected HEK293/FRT/TO cells along with the pOG44 plasmid containing the Flp recombinase (Life Technologies) using Lipofectamine 2000 (Life Technologies). Transfected cells were continuously selected

with 150 µg/ml Hygromycin B in DMEM supplemented with 10% FBS and pen/strp.

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Chapter 3

Sec61α as a novel therapeutic target for multiple myeloma

3.1 Abstract

Sec61α mediates translocation of secretory proteins across the membrane of the endoplasmic reticulum. Cotransins are a class of small molecules that bind to Sec61α and inhibit the translocation, and therefore the expression, of a subset of secretory proteins including ER-resident chaperones, growth factors, cytokines, adhesion molecules and cell surface receptors. Multiple myeloma is a cancer of the plasma cells that is largely incurable but has been shown to be particularly sensitive to proteostasis modulators like proteasome inhibitors. We therefore sought to determine the efficacy of Sec61α inhibition by cotransins in multiple myeloma. The studies presented here show that the selective cotransin analog CT8 preferentially induces apoptosis in myeloma cells, including cells derived from patients. We identify the CT8-sensitive ER-resident chaperone $p58^{ipk}$ as an essential protein in myeloma. Despite this, CT8 does not robustly induce the UPR. Finally, we confirm cotransin's mechanism of action by conferring resistance to CT8 in a myeloma cell line by introducing a point mutation in the plug region of Sec61α, previously identified as the putative CT8 binding site. These studies identify Sec61α as a novel target in myeloma and point to cotransins as interesting leads for pre-clinical development.

3.2 Introduction

Multiple myeloma is a largely incurable cancer of the plasma cells. With nearly 16,000 new cases presenting yearly in the United States alone, the demand for novel therapeutics is high[1]. Current treatment regimens include classical, non-selective chemotherapies like the nitrogen alkylating mustard melphalan, anti-inflammatory

steroids like dexamethasone, immunomodulatory drugs (IMiDs) such as lenalidomide and pomalidomide, and proteasome inhibitors like bortezomib and carfilzomib. Myeloma's particular sensitivity to the proteasome inhibitors has been attributed both to the dependence of myeloma on signaling within its tumor microenvironment[2] as well as the perturbations these drugs have on protein homeostasis[3].

Myeloma cells are plasma cells and as such secrete massive amounts of monoclonal antibodies. Levels of serum M (monoclonal) protein serve as a diagnostic for disease progression. To accommodate the increase in protein synthesis and secretion upon differentiation, plasma cells massively increase the size and folding capacity of their ER, primarily driven by the transcription factor XBP1[4].

The ER is the site of posttranslational modification and folding of all secreted and membrane (secretory) proteins. Synthesis of secretory proteins, like antibodies, require cotranslational translocation from the cytosol through the Sec61 translocon into the ER[5]. Upon entry into the ER lumen, proteins are recognized by chaperones that aid in disulfide bond formation and protein folding. In the event that a protein is not properly folded, it is rapidly exported back into the cytosol, where it is ubiquitinated and degraded by the proteasome in a process termed ER-associated degradation (ERAD)[6]. An inability of ERAD to deal with an accumulation of unfolded proteins in the ER triggers the unfolded protein response (UPR), led by three ER-resident membrane proteins: inositol requiring enzyme 1 (IRE-1), PKR-like endoplasmic reticulum-resident kinase (PERK), and activating transcription factor 6 (ATF6). Activation of these sensors results in a series of responses that lead to increased expression of chaperones and ERAD

machinery, inhibit translation, and ultimately trigger apoptosis in the event that homeostasis cannot be restored[7,8].

As professional secretory cells, it has been hypothesized that myeloma would be particularly sensitive to compounds that inhibit the UPR and other major proteostatic pathways. Consistent with this notion, increased immunoglobulin secretion has been found to correlate with sensitivity to proteasome inhibition[9]. This intrinsic sensitivity may account for the dramatic clinical success of the proteasome inhibitors. Piggy-backing on this success, compounds are currently being developed as PERK[10], IRE1[11], HSP70[12] and HSP90 inhibitors[13].

Cotransins are a new class of proteostasis modulators that inhibit the expression of a subset of secretory proteins by inhibiting their cotranslational translocation into the ER[14,15]. By analogy to the strategies mentioned above, we hypothesized that myeloma cells would be uniquely sensitive to cotransin inhibition. The studies presented in this chapter identify Sec61 inhibition by cotransins as a potentially promising strategy in multiple myeloma. In addition, these studies begin to uncover the mechanisms by which these pleiotropic compounds kill cells.

3.3 CT8 specifically induces apoptosis in multiple myeloma cells

Because myeloma cells are particularly sensitive to perturbations in protein homeostasis, we hypothesized that they may also be sensitive to inhibition of cotranslational translocation by CT8. Treatment of the myeloma cell lines JJN-3 and RPMI-8226 caused a potent decrease in proliferation as measured by metabolism of the Alamar blue dye (**Figure 3.1a**). The inhibition of proliferation was specific to myeloma

cells as the epithelial cell line HEK293FRT was unaffected. Other cancer cell lines are also affected by CT8, although several are relatively resistant (data not shown).

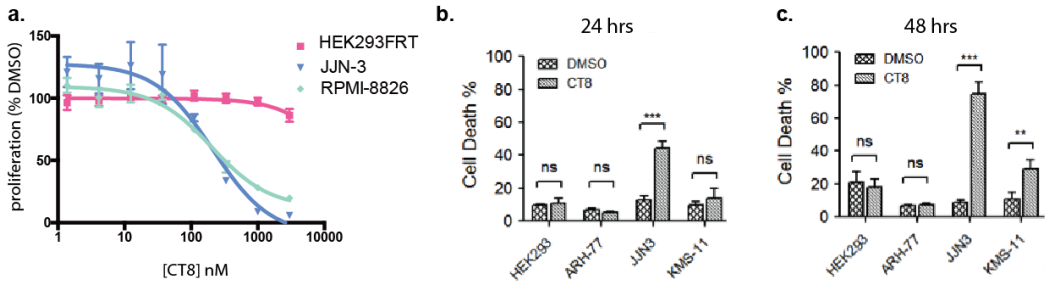


Figure 3.1 | CT8 inhibits cell proliferation and induces cell death in myeloma cells. (a) HEK293FRT (non-myeloma), JJN-3 (myeloma) or RPMI-8226 (myeloma) cells were treated with the indicated concentration of CT8 for 72 hrs and then incubated with Alamar blue for 6 hrs. Fluorescence was measured and is plotted as the % of DMSO. Measurements were done in triplicate and error bars represent +/– SD. **(b)** HEK293 (non-myeloma), ARH-77 (non-myeloma), JJN-3 (myeloma) and KMS-11 (myeloma) cells were treated with either DMSO or 1 μM CT8 for 24 hrs. Cells were stained with trypan blue and the percent stained cells was plotted. Average of 3 independent experiments is shown with error bars representing +/– SD. Significance was tested using a student's t test. **(c)** As in **(b)** but treatment was for 48hrs. Experiments in **(b)** and **(c)** were performed by Gonzalo Ureta (Fundacion Ciencia de la Vida, Chile).

To confirm that the decrease in proliferation was due to a cytotoxic rather than cytostatic effect, cells were treated with 1 μM CT8 for 24 and 48 hrs and then stained with trypan blue (**Figure 3.1b**). Both the myeloma cell lines JJN-3 and KMS-11 showed a significant increase in trypan blue staining after CT8 treatment, with JJN-3 cells reaching nearly 80% cell death after 48 hrs. Again, HEK293 cells were unaffected. Also unaffected was the EBV-transformed lymphoblastoid cell line ARH-77. This cell line is also of B-cell lineage but is not a plasma cell and therefore it synthesizes far less immunoglobulins. The observed cell death in JJN-3 cells was concomitant with an increase in surface annexin V and propidium iodide staining, indicating that CT8 induced apoptosis (**Figure 3.2a,b**). As predicted by the trypan blue assay, there was no increase in annexin V staining of ARH-77 cells treated with CT8.

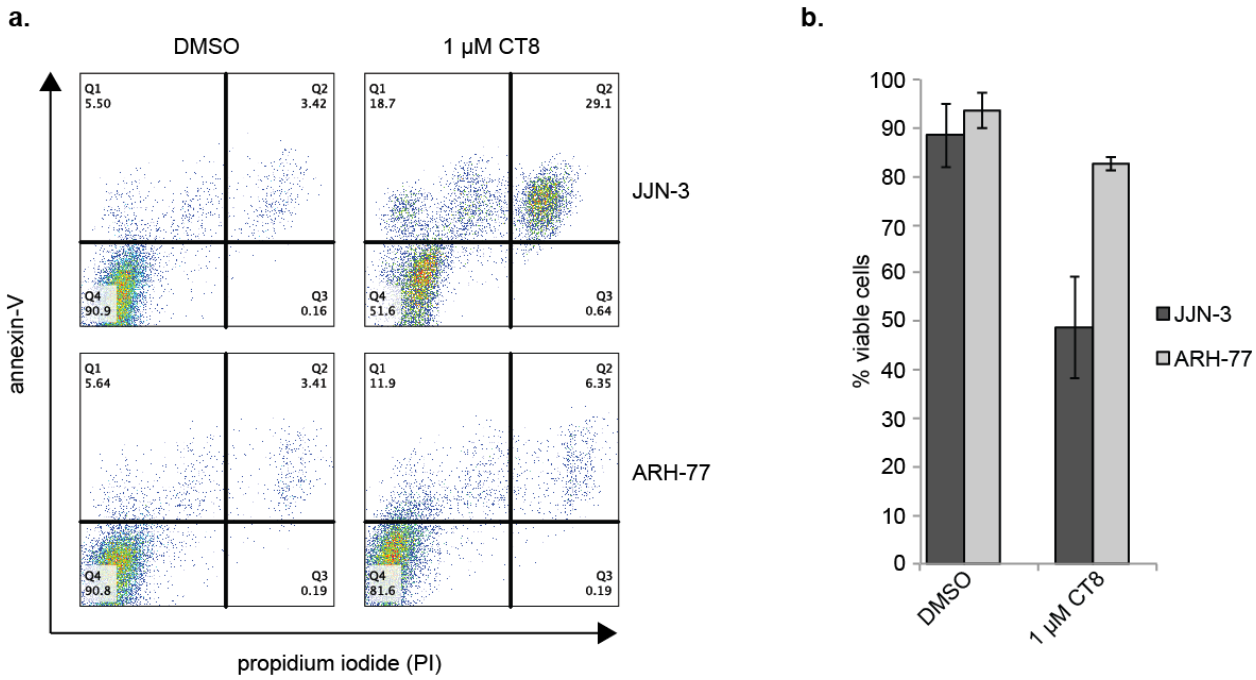
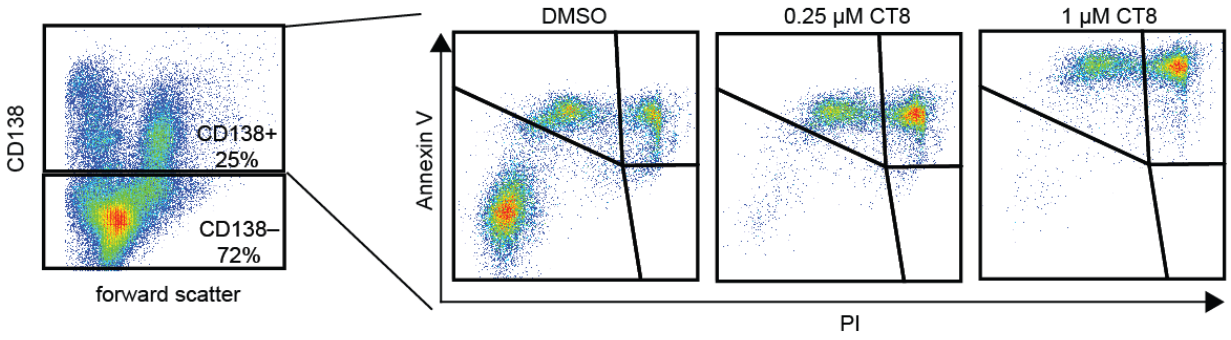


Figure 3.2 | CT8 specifically induces apoptosis in the myeloma cell line JJN-3. (a) JJN-3 and ARH-77 cells were treated with DMSO (left) or 1 μM CT8 (right) for 48hrs and then stained with annexin V-APC and propidium iodide (PI). 10,000 cells were counted using flow cytometry. **(b)** Quantification of viable cells (annexin V/PI negative, bottom left quadrant of plots in **(a)**) for 3 independent experiments for JJN-3 cells and two independent experiments for ARH-77 cells. Bars represent the average % viable cells and error bars represent +/– SD for JJN-3 cells and the range for ARH-77 cells.

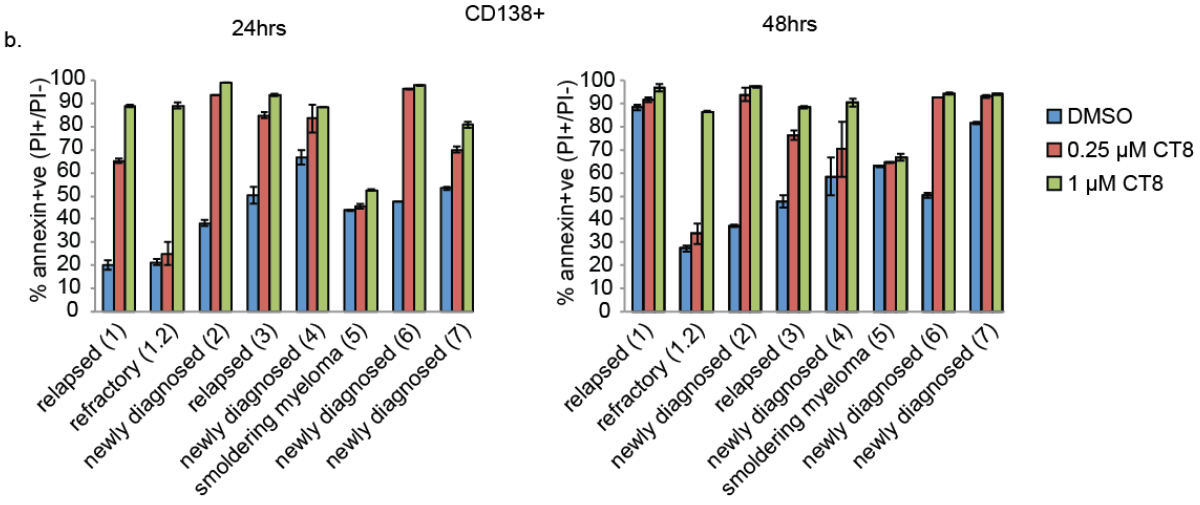
To test CT8 in a more clinically relevant setting, total bone marrow mononuclear cells were isolated from patients with various stages of multiple myeloma. These cells were treated with CT8 for 24 and 48 hrs and then stained for the plasma cell marker CD138, as well as annexin V and propidium iodide (PI). For nearly all patients tested, apoptosis was selectively induced in the CD138+ fraction (**Figure 3.3a**) while having no effect on the non-plasma cell, CD138– fraction (**Figure 3.3b**). Cells from patients that were newly diagnosed, relapsed, or refractory all displayed similar sensitivity. It is interesting to note that the only sample that did not respond to CT8 was from a case of smoldering myeloma. Smoldering myeloma is a clinical category defined as having serum M protein levels > 3 μg/L and greater than 10% clonal bone marrow plasma cells

in the blood but lacking characteristic end-organ damage attributed to unchecked plasma cell proliferation[16]. This might reflect a less aggressive state of disease and may hint again at the selectivity of CT8 toward myeloma cells over other types of cells.

a.


CD138
CD138+
25%
CD138–
72%
forward scatter
DMSO
0.25 μM CT8
1 μM CT8
Annexin V
PI

b.


CD138+
24hrs
48hrs
% annexin+ve (PI+/PI-)
relapsed (1)
refractory (1.2)
newly diagnosed (2)
relapsed (3)
newly diagnosed (4)
smoldering myeloma (5)
newly diagnosed (6)
newly diagnosed (7)
DMSO
0.25 μM CT8
1 μM CT8

c.

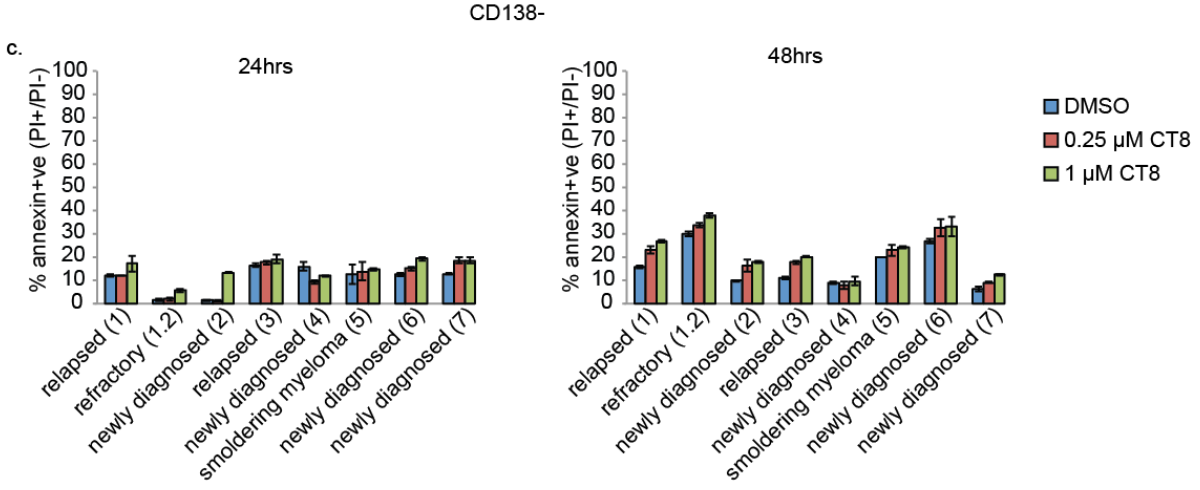

CD138-
24hrs
48hrs
% annexin+ve (PI+/PI-)
relapsed (1)
refractory (1.2)
newly diagnosed (2)
relapsed (3)
newly diagnosed (4)
smoldering myeloma (5)
newly diagnosed (6)
newly diagnosed (7)
DMSO
0.25 μM CT8
1 μM CT8

Figure 3.3 | CT8 induces apoptosis in patient-derived primary CD138+ myeloma cells. (a) Total bone marrow mononuclear cells isolated from a bone marrow aspirate of a newly diagnosed patient (Patient 2 from **(b)** and **(c)**) were treated with DMSO or the indicated concentrations of CT8 for 24 hrs. Cells were stained with anti-CD138-FITC, annexin V-APC and propidium iodide (PI). The CD138+ gate was analyzed for annexin V/PI staining. **(b)** Total bone marrow mononuclear cells isolated from bone marrow aspirates of patients diagnosed with the indicated stages of multiple myeloma were treated for 24 (left) and 48 (right) hours with DMSO or the indicated concentration of CT8. Cells were stained with anti-CD138-FITC, annexin V-APC and propidium iodide (PI) and subjected to the gating scheme illustrated in **(a).** Annexin V-positive cells (both PI positive and negative) were counted in the CD138+ fraction of mononuclear cells and are presented as a % of total CD138+ cells. Numbers in parentheses next to the disease stage indicate patient number. Samples 1 and 1.2 were isolated from the same patient, 2 months apart. **(c)** As in **(b)** but annexin V-positive cells were counted in the CD138- fraction. Bars represent mean of three independent treatments with error bars representing +/– SD.

3.4 CT8 inhibits the expression of ER chaperones ERdj3 and p58^{ipk}

Primary myeloma cells and myeloma cell lines (JJN-3, KMS-11, RPMI-8226 and primary patient cells) are more sensitive to CT8 than two non-myeloma cell lines (HEK293 and ARH-77). The difference in sensitivity between JJN-3 and ARH-77 cells was especially intriguing since these two cell lines are both derived from B-cells. As the basic mechanism of CT8 inhibition, namely inhibition of cotranslational translocation by direct binding to Sec61α, must be identical in all cells, one possible explanation for the difference in sensitivity is that there are one or more CT8-sensitive clients on which myeloma cells are particularly dependent. As previously mentioned, myeloma cells have massively increased the size of their ER compared to progenitor B-cells to accommodate the ramp-up in immunoglobulin synthesis and secretion. Both JJN-3 and ARH-77 cells synthesize IgG kappa light chain (κLC). Because ARH-77 cells secrete nearly 5-fold less κLC than JJN-3 cells[17], we hypothesized that the relative levels of ER resident proteins required to properly fold secreted proteins would be lower in these cells.

Immunoblotting for ER-resident chaperones confirmed this hypothesis, showing much lower expression of 78-kDa glucose-regulate protein (Bip), calnexin (CNX), the DnaJ protein ERdj3, protein disulfide isomerase (PDI), and calreticulin (CRT) in the ARH-77 cells (**Figure 3.4a)**. Interestingly, another DnaJ protein, p58^{ipk}, was expressed at similar levels in the two cell lines, while the expression of the UPR sensor PERK was lower in the JJN-3 cells.

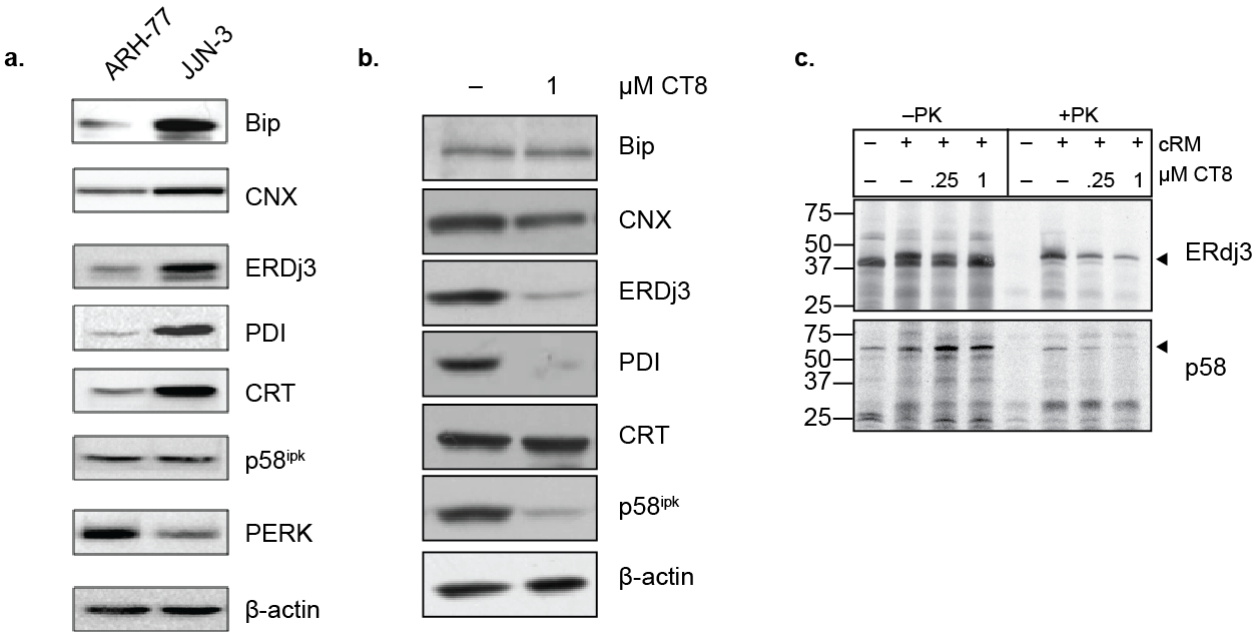


Figure 3.4 | JJN-3 cells have increased expression of ER-resident proteins, some of which are directly inhibited by CT8. (a) Whole-cell lysates from ARH-77 and JJN-3 cells were separated on 10% SDS-PAGE gels and analyzed by immunoblot for the indicated ER-resident proteins. β-actin is shown as a loading control. **(b)** JJN-3 cells were treated with DMSO or 1 μM CT8 for 24 hrs and whole-cell lysates were separated on 10% SDS-PAGE gels and analyzed by immunoblot for the indicated ER-resident proteins. **(c)** mRNA encoding ERdj3 or p58^{ipk} was translated in vitro in the presence of canine rough microsomes (cRMs) and either DMSO or 1 μM CT8. Translation reactions were either left alone or treated with proteinase K (PK) and then separated on 10% Tris-Tricine SDS-PAGE gels and analyzed by autoradiography. Image is representative of three independent experiments. Experiments in **(a)** and **(b)** were performed by Gonzalo Ureta.

The SILAC-based proteomics survey of JJN-3 cells treated with 250 nM CT8 discussed in Chapter 2 showed that expression of both ERdj3 and p58^{ipk} was decreased after 24 hrs ($(L/H)_{final}$ of 1.48 and 1.51 respectively). Consistent with this result, immunoblot analysis of whole-cell lysates from JJN-3 cells treated with 1 μM CT8 for 24 hrs revealed a significant decrease in p58^{ipk} and ERdj3 protein levels (**Figure 3.4b**). Calreticulin and calnexin were unaffected by CT8 treatment, a result that was also reflected in the SILAC analysis ($(L/H)_{final}$ of 0.98 and 1.09 respectively). To determine whether translocation of ERdj3 and p58^{ipk} is directly inhibited by CT8, mRNA encoding each protein was translated in the presence of canine rough microsomes. Translocated protein is protected from treatment with proteinase K (PK). Treatment with 1 μM CT8 caused a dose-dependent loss in this PK-protected fragment for both proteins, indicating a direct effect on cotranslational translocation (**Figure 3.4c**). p58^{ipk} was more sensitive than ERdj3 with complete inhibition observed at 1 μM CT8.

DnaJ proteins, like p58^{ipk} and Erdj3, act as co-chaperones for the ER-lumenal Hsp70 protein Bip[18]. Binding of these proteins to Bip facilitates ATP hydrolysis required for chaperone mediated protein folding. A major Bip client protein in both the ARH-77 and JJN-3 cells is κLC[19]. We hypothesized that, because JJN-3 cells secrete much higher levels of κLC than the ARH-77 cells and p58^{ipk} levels are similar in the two cell lines (whereas many other chaperones, including Bip, were over expressed in the JJN-3 cells), JJN-3 myeloma cells would be particularly sensitive to the loss of p58^{ipk} due to CT8 inhibition.

To test if loss of p58^{ipk} is sufficient to induce apoptosis in either cell line, both JJN-3 and ARH-77 cells were transduced with lentiviruses containing either a non-

targeting control shRNA or one of two different shRNAs against p58^{ipk}. All constructs also contained a constitutively expressed eGFP for selection. Cells were sorted for eGFP expression 48 hrs after transduction and immunoblot analysis of sorted, eGFP-positive cells confirmed knockdown of p58^{ipk} protein (**Figure 3.5a**). Four days post-sorting, only 5% of JJN-3 cells expressing either of the two p58^{ipk} shRNAs were viable compared to over 75% of cells expressing a control shRNA, indicating that loss of p58^{ipk} is sufficient to induce apoptosis in these cells (**Figure 3.5b**). The ARH-77 cells transduced with the same shRNAs showed no loss in viability, despite showing knockdown in p58^{ipk} levels (**Figure 3.5a,c**). We hypothesize that a lower secretory flux in ARH-77 cells, in part due to lower κLC levels, allows them to withstand the decrease in p58^{ipk} levels better than JJN-3 cells.

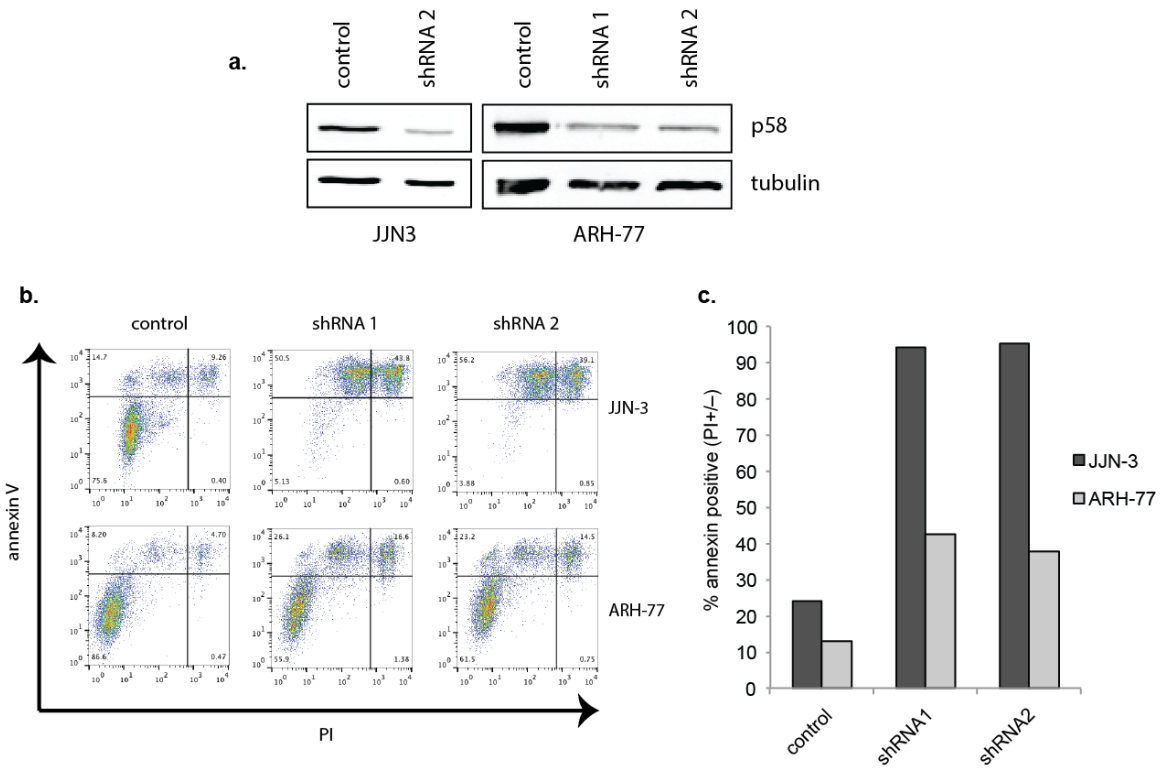


Figure 3.5 | Knockdown of p58ipk is sufficient to selectively induce apoptosis in myeloma cells. (a) JJN-3 and ARH-77 cells were transduced with lentiviruses containing either a scrambled control shRNA of one of two shRNAs against p58^{ipk} and a constitutive GFP. Cells were sorted for GFP expression 48 hrs post-transduction and whole cell lysates from cells 4 days post-sort were separated on 10% Tris-Tricine SDS-PAGE gels and analyzed by immunoblot for p58^{ipk}. Tubulin is shown as a loading control. **(b)** Cells 4 days post-sort from **(a)** were stained with annexin V-APC and propidium iodide (PI) and counted by flow cytometry. **(c)** Quantification of total annexin V-positive cells (both PI positive and negative) from **(b)**.

3.5 CT8 does not robustly induce the UPR in myeloma cells

Loss of ER chaperones and a subsequent decrease in the folding capacity of the ER would presumably lead to an induction of the UPR. One measure of UPR induction is splicing of mRNA encoding the transcription factor X-box binding protein 1 (XBP1). Upon sensing misfolded proteins in the ER lumen, inositol requiring enzyme 1 (IRE1) oligomerizes, activating its cytosolic RNAse domain, which in turn splices *XBP1u* to yield *XBP1s*[20]. *XBP1s* encodes a transcription factor that regulates the expression of numerous genes that increase the folding capacity of the ER, including p58^{ipk} [21]. *XBP1*

splicing in both JJN-3 and ARH-77 cells treated with CT8 for 4 hrs was much lower compared to cells treated with the protein glycosylation inhibitor, tunicamycin (Tm) (**Figure 3.6**). CT8 partially induced *XBP1* splicing after 24 hrs in both cells. This result is consistent with a model in which $p58^{ipk}$ synthesis must first be inhibited and the existing pool allowed to turn over, before unfolded proteins can accumulate in the ER at a sufficient level to trigger *XBP1* splicing.

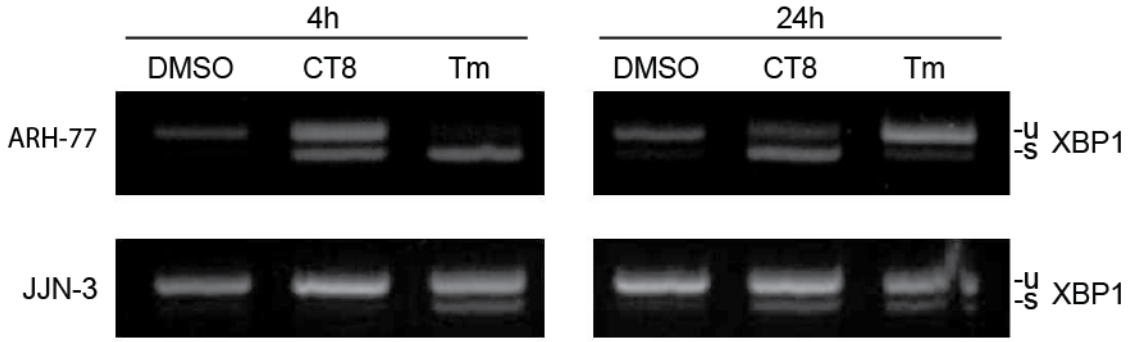


Figure 3.6 | CT8 induction of *XBP1u* splicing is less robust than tunicamycin in both ARH-77 and JJN-3 cells. JJN-3 and ARH-77 cells were treated with DMSO, 1 μM CT8 or 300 nM tunicamycin (TM) for 4 or 24 hrs. Total mRNA was isolated, converted to cDNA and amplified using primers specific *XBP1*. PCR products were separated on 3% agarose gels at 100 V. Experiment was performed by Gonzalo Ureta.

While activation of IRE-1 by misfolded proteins results in the production of an active transcription factor responsible for the expression of proteins that increase the folding capacity of the ER, another function of the UPR is to decrease the protein burden on the stressed ER by attenuating translation. PERK mediates this arm through phosphorylation of the eukaryotic translation initiation factor 2 alpha subunit (eIF2α). While the primary effect of phosphorylated eIF2α (eIF2α-P) is to limit translation by inhibiting initiation, a few select mRNAs are preferentially translated, such as *ATF4*[22]. The ATF4 protein is a transcription factor that upregulates several genes, including the pro-apoptotic transcription factor, CHOP. The eIF2α-P/ATF4 pathway is called the

integrated stress response (ISR) and is required to facilitate activation of the third arm of the UPR, the membrane-bound transcription factor, ATF6[23].

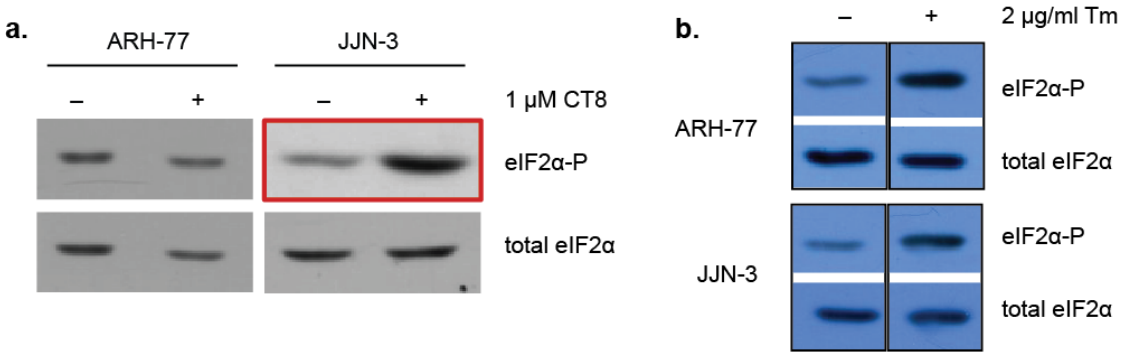


Figure 3.7 | CT8 induces eIF2α phosphorylation in JJN-3 cells preferentially. (a) ARH-77 and JJN-3 cells were treated with 1 μM CT8 for 4 hrs. Whole-cell lysates were separated on 10% SDS-PAGE gels and analyzed by immunoblot for phosphorylated eIF2α (eIF2α-P) and total eIF2α. **(b)** As in **(a)** but cells were treated with 2 μg/ml tunicamycin (Tm) for 4hrs instead. Experiments were performed by Gonzalo Ureta.

Treatment of JJN-3 cells with 1 μM CT8 for 4 hrs resulted in a marked increase in eIP2α-P, which was not seen in the ARH-77 cells (**Figure 3.7a**). As a positive control, treatment with 2 μg/ml Tm for 4 hrs caused an increase in eIF2α-P in both cell lines (**Figure 3.7b**). To examine the downstream consequences of eIF2α-P, we measured induction of CHOP by q-PCR. 24-hr treatment of JJN-3 cells with 1 μM CT8 caused a 5-fold increase in CHOP mRNA, whereas there was no increase in the ARH-77 cells, consistent with the eIF2α-P results (**Figure 3.8a**). In contrast, Tm treatment caused upregulation of CHOP in both cell lines, albeit to a greater extent in the JJN-3 cells (**Figure 3.8a**). Interestingly, CHOP induction in JJN-3 cells did not correlate with apoptotic markers (**Figure 3.8b**). CT8 treatment resulted in a modest 5-fold increase in CHOP mRNA, but it robustly induced apoptosis, as shown by the 50% annexin V-positive cells. In contrast, Tm treatment caused a much larger, 20-fold increase in

CHOP, but a more limited induction of apoptosis, with only 40% annexin V-positive cells. ARH-77 cells behaved more consistently. CT8 induced a small increase in CHOP expression which resulted in ~20% annexin positive cells, whereas Tm induced a 10-fold increase in CHOP and 30% annexin positive cells (**Figure 3.8b**).

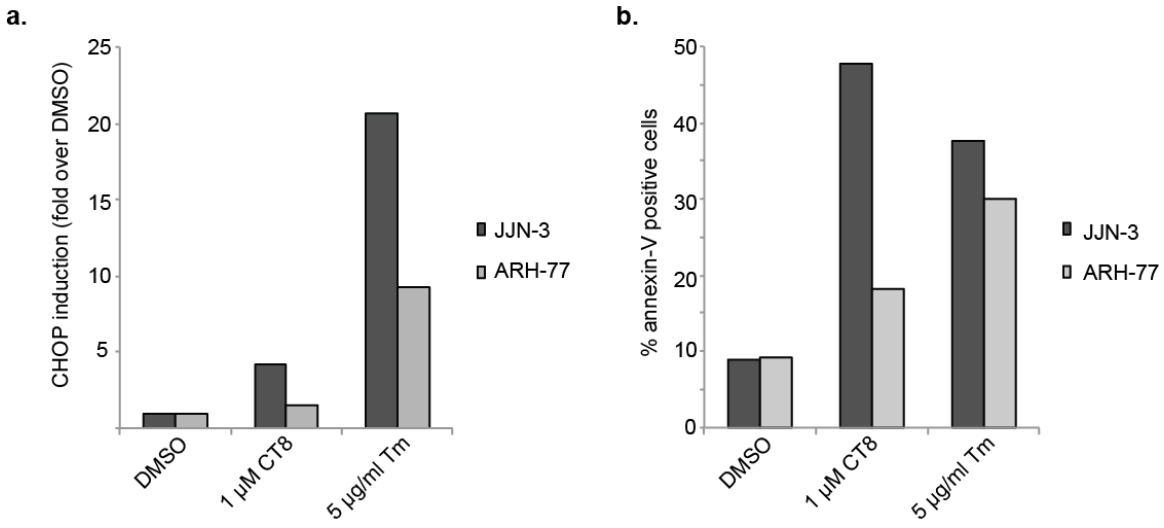


Figure 3.8 | CHOP induction does not correlate with induction of apoptosis in response to CT8 and tunicamycin in JJN-3 cells. (a) JJN-3- (dark grey) and ARH-77 (light grey) cells were treated with DMSO or the indicated concentrations of CT8 or tunicamycin (Tm) for 24 hours. cDNA was prepared from total RNA isolated from lysed cells and qPCR for CHOP was performed. Cq values were normalized to the ribosomal protein RPL0 and then normalized to DMSO. Data is presented as fold over DMSO. Bars represent the mean of three technical replicates. **(b)** Cells were treated for 48 hrs and then stained with annexin-V-APC and PI. % Annexin-V positive (PI positive and negative) cells of 10,000 total counted cells is presented. Bars represent one experiment.

The fact that lower CHOP levels are associated with higher levels of apoptosis in JJN-3 cells treated with CT8 as compared to tunicamycin suggests that CT8 likely induces other pro-apoptotic pathways that are 1) not induced in ARH-77 cells and 2) not due to ER stress. In preliminary support of this hypothesis, CT8 still induced apoptosis in JJN-3 cells after CHOP knockdown by RNAi (data not shown).

To determine whether PERK is the kinase responsible for eIF2α phosphorylation and subsequent CHOP activation, we treated JJN-3 cells with both CT8 and a selective

PERK inhibitor ($PERK^{inhib}$)[10,24]. EIF2α phosphorylation was unaffected by $PERK^{inhib}$, suggesting that another kinase must be responsible (**Figure 3.9a,b**). As a control, Tm-induced eIF2α-P was completely blocked by treatment with $PERK^{inhib}$ (**Figure 3.9a,b**). CT8-mediated CHOP induction was also unaffected by PERK inhibition, whereas the Tm-induced CHOP increase was completely blocked (**Figure 3.9c**). Finally, apoptosis of JJN-3 cells in response to CT8 was also unaffected by PERK inhibition, consistent with the previous observations.

Interestingly, while Tm treatment along with PERK inhibition in the ARH-77 cells resulted in a decrease in CHOP levels, there was no decrease in apoptosis. Closer examination of CHOP levels in these cells indicates that they do not reach baseline, suggesting that there may be some PERK/eIF2α-P independent CHOP induction in the presence of Tm. It is likely that PERK activation does not play a role in apoptosis induction by CT8 or tunicamycin. In fact, the PERK/eIF2α-P/CHOP axis may serve a protective role as the data show that apoptosis in response to tunicamycin actually increases in the presence of $PERK^{inhib}$. Since the primary role of eIF2α-P is as a translation inhibitor, loss of this species results in continued protein synthesis, increasing the levels of ER stress which likely leads to the greater induction of apoptosis in cells treated with tunicamycin in conjunction with $PERK^{inhib}$.

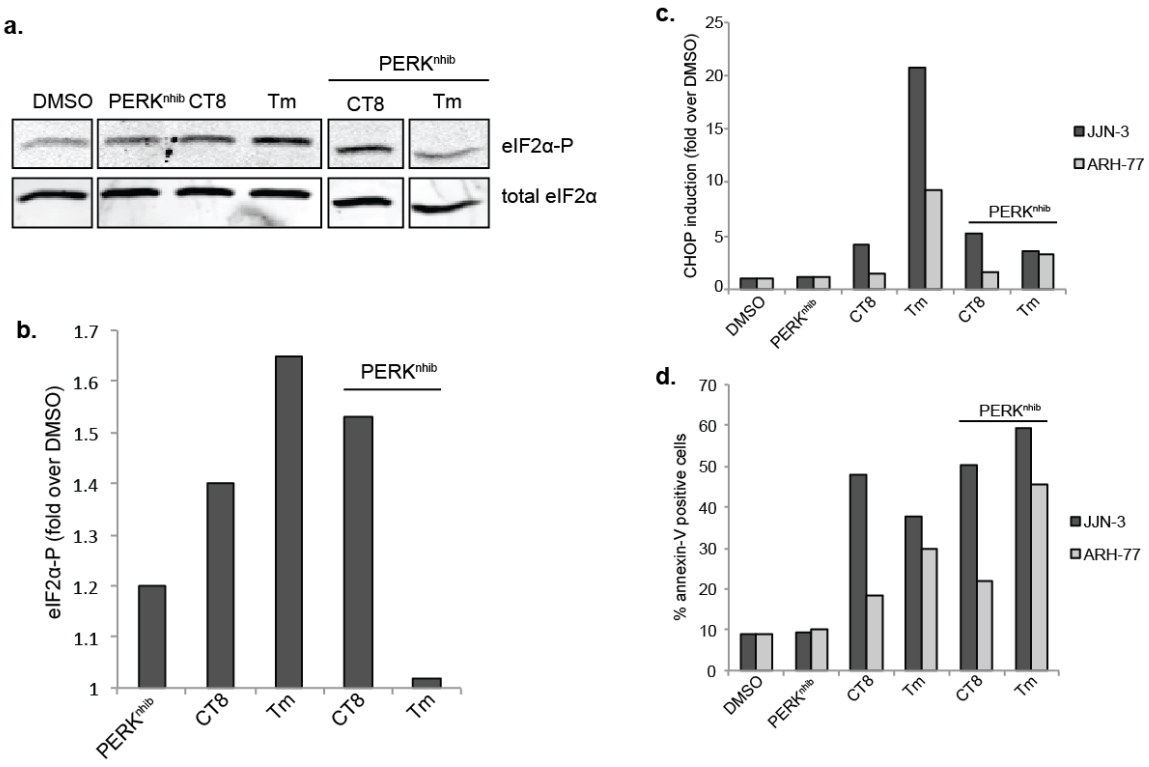


Figure 3.9 | eIF2α phosphorylation, CHOP induction and subsequent apoptosis in response to CT8 is PERK-independent. (a) JJN-3 cells were treated with DMSO, PERK^{inhib} (100 nM), CT8 (1 μM), Tm (5 g/ml) or a combination of PERK^{inhib}/CT8 or PERK^{inhib}/Tm for 4 hrs. Whole-cell lysates were separated on 10% Tris-Tricine SDS-PAGE gels and analyzed by immunoblot for eIF2α-P and total eIF2α. **(b)** Quantification of eIF2α-P from **(a)** presented as fold increase over DMSO. **(c)** JJN-3 and ARH-77 cells were treated with the indicated compounds, at the doses mentioned in **(a)** for 24 hrs. CHOP mRNA was quantified by qPCR and is presented as fold induction over DMSO. **(d)** JJN-3 and ARH-77 cells were treated with the indicated compounds at the concentrations in **(a)** for 48hrs. Cells were stained with annexin V-APC analyzed by flow cytometry.

3.6 R66I Sec61α mutant partially rescues proliferation of myeloma cells treated with CT8

CT8 has pleiotropic effect on cells, causing a 50% decrease in expression of over 50 proteins in JJN-3 cells after treatment with 250 nM CT8 for 24 hrs, and presumably more in response to the 48-hr 1 μM treatment required to see robust induction of apoptosis (See **Chapter 2**). Because of this, identification of a single CT8-sensitive protein responsible for the apoptotic response is highly unlikely. However, CT8

must bind to and inhibit Sec61α to elicit its effects, so equipping cells with a CT8-resistant Sec61α should result in resistance to CT8. To test this hypothesis, we generated JJN-3 cell lines stably expressing either WT Sec61α or a CT8-resistant mutant of Sec61α where arginine 66 has been mutated to isoleucine (R66I Sec61α)[25]. This mutant was generated by exposing the DNA repair-defective colon carcinoma cell line HCT-116[26] to cytotoxic levels of the more potent CT8 analog, CT9 (**Chapter 1, Figure 1.4**). Colonies that survived after 9-12 days had varying levels of resistance to both CT8 and CT9 in proliferation assays. Sanger sequencing revealed that all resistant colonies contained one of five different single point mutations (all heterozygous) in the *SEC61A1* coding sequence. These mutations clustered to the lumenal base of the plug region of Sec61α, between the plug and the C-terminal end of TM3.

HEK293T (whose proliferation is unaffected by CT8 or CT9) stably expressing R66I Sec61α showed little effect of CT8 or CT9 on TNFα expression compared to cells expressing WT Sec61α, where TNFα expression was inhibited with an EC_{50} of 50 nM. Additionally, cross-linking experiments with R66I Sec61α and the photo-affinity probe, CT7[27], showed little to no binding to the mutant channel as compared to wild-type, suggesting that the rescue is caused by an inability to bind cotransins rather than a gain of function. These results indicate that the R66I mutant assembles into a functional translocon and that expression is sufficient to cause dominant resistance to cotransin.

To generate stable myeloma cell lines, we generated lentivirus (LV) particles carrying a bidirectional expression cassette with either WT or R66I Sec61α[25] in the forward direction and mCherry in the reverse. The bidirectional promoter allows for simultaneous transcription of two different mRNAs using the same promoter and

response elements, resulting in equal levels of mRNA production for the two constructs[28]. LV for both the WT and R66I Sec61α were produced to a titer of $1.1x10^8$/ml. JJN-3 cells were infected at an MOI of 22 and FACs analysis 48 hrs post-infection showed ~100% infection efficiency for both constructs as determined by mCherry fluorescence (**Figure 3.10a**).

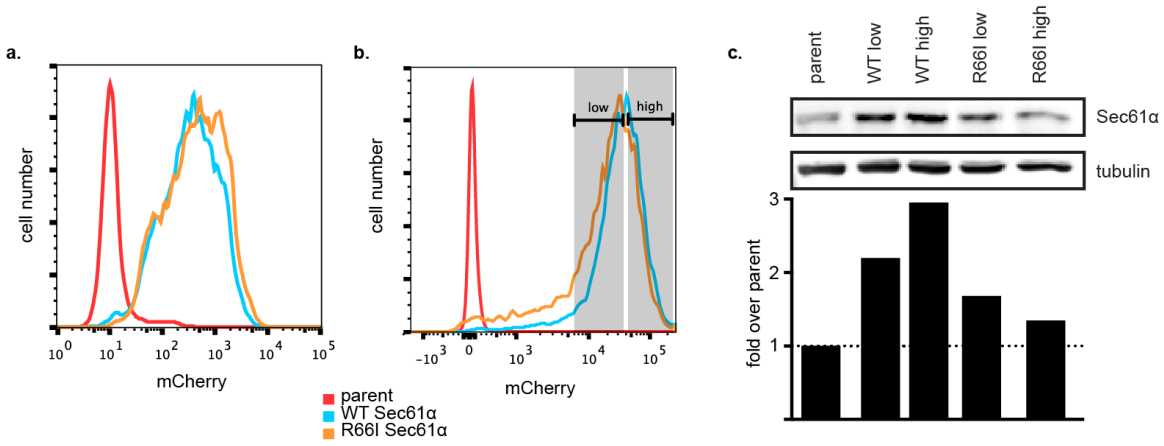


Figure 3.10 | Lentiviral infection of JJN-3 cells results in overexpression of both WT and R66I Sec61α. a) JJN-3 cells were infected with lentiviral particles carrying a bidirectional expression cassette that encodes for mCherry in the forward direction and either WT or R66I Sec61α in the reverse. Cells were analyzed by FACs 48 hrs after infection. mCherry expression is a proxy for expression of the Sec61α construct. Parent, uninfected cells were analyzed as a negative control. **b)** Infected cells were sorted into low and high expressing populations based on mCherry expression. Again, uninfected parent cells were analyzed as a negative control. The shaded grey bars represent populations that were collected for low and high. **c)** Whole-cell lysates from the indicated cell populations were separated on 10% SDS-PAGE gels and analyzed by immunoblot for Sec61α. Tubulin is shown as a loading control. Intensity of each band was normalized to the loading control and then plotted as fold over the parent cell expression, which was set to 1 (bottom graph). The bars represent one experiment.

To ensure that all cells were expressing the Sec61 constructs, infected cells were sorted into low and high expressing populations based on mCherry fluorescence 6 days post-infection (**Figure 3.10b**). Western blot for Sec61α in the four different cell lines (WT low, WT high, R66I low and R66I high) showed a clear increase in expression for all

infected cell lines, but no significant difference between the low and high mCherry expressers (**Figure 3.10c**).

Cells from the low mCherry-expressing populations were chosen for follow up experiments, because their Sec61α expression levels were most similar in the WT and R66I cell lines. Cell proliferation of the WT and R66I Sec61α-expressing JJN-3 cells, along with the uninfected parent cells, was measured using the Alamar blue assay following 72 hrs of treatment with increasing doses of CT8. Over-expression of WT Sec61α had no effect on CT8 sensitivity. However, over-expression of the R66I mutant Sec61α resulted in a 2.5-fold increase in the IC_{50}, from 250 to 650 nM (**Figure 3.11**). To test whether R66I Sec61α conferred resistance to the effects of CT8 on secretory protein biogenesis, we measured the expression of the CT8-sensitive secreted protein hepatocyte growth factor (HGF). HGF ELISA of conditioned media from R66I cells confirmed resistance to CT8 relative to cells expressing WT Sec61α, showing a similar increase in EC_{50} (**Figure 3.12**).

These results further support Sec61α as the primary target of cotransins and point to potential resistance mechanisms that may develop in response to chronic treatment with these compounds. In contrast to HCT-116 colon carcinoma cells, in which a spontaneous R66I mutation in Sec61α appears to confer near complete resistance to CT8 (EC_{50} ~ 10 μM vs. 250 nM in the parental cells)[25], resistance was only partial in R66I-expressing JJN-3 cells. This may reflect a cell type-dependent difference in the mechanism of cell death induced by CT8. We hypothesize that cell death induced by CT8 in MM cells is due in part to a toxic “gain of function”, an increased accumulation of misfolded proteins in the ER and cytoplasm, to which MM cells are extremely

sensitive. This mechanism predicts that mutations in only one allele of Sec61α will not confer complete resistance, as the remaining WT allele will still be sensitive and capable of generating toxic misfolded secretory proteins (e.g. κLC) in the cytosol, similar to proteasome inhibitors. By contrast, CT8-induced cell death in HCT-116 cells may involve inhibition of the biosynthesis of one or more essential secretory proteins. In this context, a heterozygous R66I mutation is predicted to be dominant. It is also possible that ectopically expressed R66I Sec61α is not efficiently incorporated into functional Sec61 translocons in the JJN-3 cells.

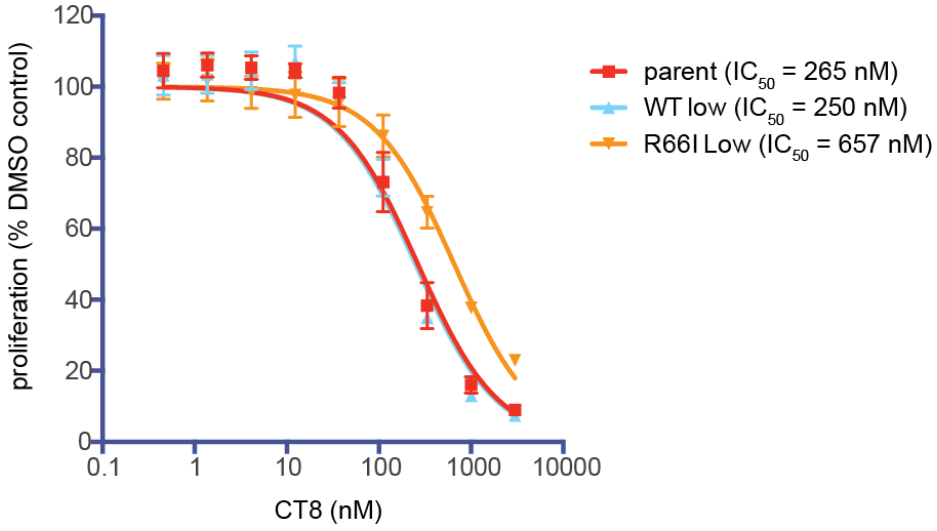


Figure 3.11 | R66I mutation in Sec61α partially rescues proliferation in the presence of CT8. JJN-3 cells, expressing WT Sec61α, R66I Sec61α, or the uninfected parent cell line were treated with the indicated concentrations of CT8 for 72 hrs. Proliferation was measured as a function of Alamar blue metabolism and is presented as a % of DMSO control. Points represent the mean of three replicates with error bars representing +/– SD.

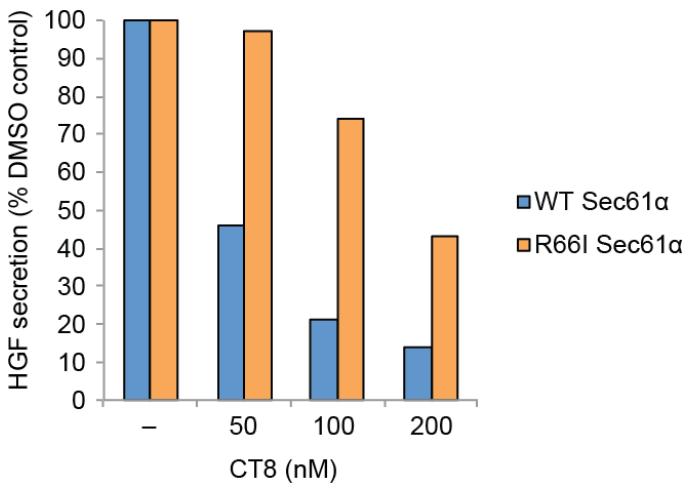


Figure 3.12 | R66I Sec61α rescues the ability of JJN-3 cells to secrete HGF in the presence of CT8. JJN-3 cells expressing either WT or R66I Sec61α were treated with CT8 for 24 hrs and the conditioned media was subjected to a sandwich ELISA for HGF. HGF concentration is presented as a % DMSO control. Bars represent results from one experiment.

3.7 Discussion

Multiple myeloma is a disease that benefits from a multi-pronged approach for intervention. Therefore, compounds that have pleiotropic effects because they target members of the proteostasis network, like the proteasome and Sec61α, may be more effective than single-agent, targeted therapies. Moreover, as highly secreting, antibody-producing cells, myeloma cells require an increased capacity of the ER to cope with the secretory burden. Therefore, we hypothesized that modulation of protein secretion by direct inhibition of the Sec61 translocon would uniquely affect myeloma cells. Indeed, CT8, a potent and selective inhibitor of Sec61α, specifically induces apoptosis in both primary multiple myeloma cells and derived cell lines. Induction of cell death is at least partially specific to myeloma cells, with the compound showing little effect on $CD138^{-}$ cells, HEK293 cells, or ARH-77 cells, derived from a B-cell leukemia.

A defining difference between the sensitive JJN-3 cell line and the resistant ARH-77 cell line is the level of κLC expressed. JJN-3 cells express 5-fold greater levels of κLC as compared to ARH-77 cells. We show that the increased secretory burden in JJN-3 cells is associated with higher levels of all ER chaperones examined, except for $p58^{ipk}$. Knockdown of $p58^{ipk}$ was sufficient to induce apoptosis in the JJN-3 cells, but had no effect on ARH-77 cells. This differential sensitivity toward $p58^{ipk}$ levels further supports the hypothesis that myeloma cells are especially sensitive to perturbations in the proteostasis network. Consistent with this notion, κLC expression levels correlate with sensitivity to CT8. A similar correlation has been reported with proteasome inhibition[9]. However, the converse has also been reported in the case of brefeldin A, which blocks secretion by a different mechanism, leading to collapse of the Golgi and expansion of the ER. RPMI-8226 cells accumulate 2-fold higher levels of lambda light chain (λLC) in response to brefeldin A than MM1.S cells, suggesting a higher secretory burden and yet, MM1.S cells are 10-fold more sensitive to bortezomib[29]. A more comprehensive assessment of light chain production in established myeloma cell lines will help to elucidate whether an increased secretory burden correlates with sensitivity to proteostasis modulators. A major challenge lies in quantifying secretory protein burden and the quantitative and qualitative aspects of protein misfolding.

One potential result of CT8 inhibition of $p58^{ipk}$ expression would be an increase in unfolded proteins in the ER, possibly triggering the UPR. Indeed, *XBP1u* splicing does occur in both JJN-3 and ARH-77 cells, albeit on different timescales. However, this splicing is slower and to a lesser extent in response to CT8 as compared to tunicamycin. However, it seems that this unfolded protein burden does not result in an

activation of the PERK arm of the UPR as evidenced both by the total lack of eIF2α-P in ARH-77 cells and the independence of eIF2α-P of PERK in the JJN-3 cells.

Proteasome inhibition also results in PERK-independent phosphorylation of eIF2α[30,31]. EIF2α-P has been proposed to lead to proteasome inhibitor resistance as translation inhibition by eIF2α leads to a decrease in protein synthesis, a subsequent decrease in cytosolic, misfolded proteins and therefore a decrease in the load on the proteasome. These studies point to heme-regulated eIF2α kinase (HRI) as the relevant kinase. However, recent work has pointed to general control nonrepressed 2 (GCN2) as the responsible kinase, which phosphorylates eIF2α in response to a disruption in amino acid homeostasis[32]. In this model, proteasome inhibitors lead to a decrease in the pool of available amino acids, triggering the integrated stress response and activating GCN2. Since CT8 does not seem to elicit a robust UPR and seems to mimic proteasome inhibition with respect to PERK-independent eIF2α-P, it is possible that, rather than accumulating in the ER, unfolded proteins are instead rapidly exported to the cytosol via the ER-associated degradation pathway (ERAD). In this scenario, unfolded proteins would be ubiquitinated in the cytosol and then degraded, potentially overloading the proteasome. In support of this hypothesis, CT8 potently synergizes with the proteasome inhibitor carfilzomib[33] (unpublished data from Eric Lowe at Onyx Pharmaceuticals).

Regardless of the downstream mechanisms of CT8-induced apoptosis, which are most likely pleiotropic, they all start with Sec61α inhibition. Therefore, mutations in Sec61α represent a potential mode of resistance to these compounds. The R66I mutation utilized in this study conferred total resistance to CT8 in a proliferation assay in

the HCT-116 colon cancer cell line. However, we observed only a modest 2-fold increase in resistance in the JJN-3 cells. This resistance is paralleled by a similar increase in resistance to CT8 effects on HGF secretion, further supporting the claim that Sec61α is the relevant target of CT8. The parallel increase in resistance between the proliferation effects and the specific effects on a sensitive target also suggest that resistance conferred by R66I Sec61α in JJN-3 cells may not be as dominant as initially observed HCT-116 cells.

Taken together, our results provide impetus for the exploration of Sec61α inhibitors in the context of multiple myeloma. They potently inhibit the proliferation of these cells, leading to apoptosis. Much like the clinically validated proteasome inhibitors, bortezomib and carfilzomib, CT8 inhibits a highly conserved, essential protein complex within the proteostasis network, and myeloma cells seem particularly sensitive. As with proteasome inhibition, Sec61α inhibition likely has many downstream effects leading to cell death. Therefore, pinpointing a single pathway or CT8-sensitive client will likely be impossible. However, we have shown that loss of expression of one CT8-sensitive target, the ER chaperone $p58^{ipk}$, is sufficient to induce apoptosis on its own, further supporting the concept that myeloma cells are poised to succumb to perturbations in protein homeostasis. Further characterization of proteins sensitive to CT8 in the context of myeloma, and careful characterization of CT8 effects on specific secretory proteins that overload the ER in MM cells (e.g., κLC), will provide a more complete picture of how these compounds kill cancer cells.

3.8 Experimental procedures

Antibodies, cells, and reagents

JJN-3, RPMI-8226, and ARH-77 cells were a generous gift from Peter Walter (UCSF). HEK293T cells were a generous gift from Kevan Shokat (UCSF). Patient-derived total bone marrow mononuclear cells were obtained through the UCSF MMTI Tissue Bank. CT8 was prepared as previously described[27]. Stock solutions were prepared in dimethyl sulfoxide (DMSO, Sigma) and used as indicated, with a final vehicle concentration of 0.1% vol/vol. The following primary antibodies were used: anti-p58^{ipk} (Cell Signaling, #2940S) , anti-calreticulin, anti-calnexin, anti-Bip (Cell Signaling, #3177), anti-PDI, anti-eIF2α (Cell Signaling, #2103S), anti-eIF2α-P (Cell Signaling, #3597S), anti-PERK (Cell-signaling,), anti-β-actin (Sigma), anti-β-tubulin (Sigma, T6199), anti-ERdj3 (Santa Cruz, sc-271240), anti-Sec61α (Novus Biologicals, NB-120-15575), anti-CD138-FITC (BD Biosciences, 552723), and annexin V-APC (BD Biosciences, 550474). Mission shRNA plasmids were purchased from Sigma (**Appendix C**).

Cell culture and viability assays

JJN-3, ARH-77 and patient-derived mononuclear cells were cultured in RPMI-1640 media (Gibco) supplemented with GlutaMax^{TM}, 10% FBS (Axxenia), and 1,000 units/ml penicillin and streptomycin (Gibco) (growth media) at 37°C and 5% CO_2. HEK293T cells were cultured in DMEM (Gibco) supplemented with 10% FBS (Axxenia), and 1,000 units/ml penicillin and streptomycin (Gibco) (growth media) at 37°C and 5%

CO_2. Patient-derived total bone marrow mononuclear cells (see below) were treated immediately upon receipt.

For proliferation assays, cells were plated at 10,000/well in 100 µl in 96-well plates (Corning CellBIND surface, black with clear bottom) and incubated with drug (addition of 50 µl 3x drug dilution) for 72 hrs prior to addition of 17 µl Alamar Blue and reading of fluorescence (ex. 545, em. 590) 6-8 hours after addition. Dose response curves were fit and EC_{50}'s determined using a log(inhibitor) vs. normalized response nonlinear regression GraphPad Prism 6.

For trypan blue assays, cells were seeded at 2 x 10^5/ml of growth media in 12-well cell culture plates (Falcon), 1 ml/well and incubated for 24 hrs at 37°C and 5% CO_2. 1 µl of a 1000x DMSO stock of CT8 or DMSO was added to each well and cells were harvested after 24 or 48 hrs of incubation. Harvested cells were washed 1x with PBS, mixed with trypan blue and counted using a Luna™ Automated Cell Counter. Graphs were made using GraphPad Prism 5.1 and p-values were calculated using an unpaired student's t test with significance at $p \leq 0.05$.

Isolation of total bone marrow mononuclear cells (BMMCs)

Total bone marrow mononuclear cells were isolated from bone marrow aspirates by the MMTI Tissue Bank at UCSF. Briefly, aspirates were centrifuged at 500 x *g* without braking for 10 min followed by removal of the top plasma layer. The plasma volume was replaced with RPMI-1640 culture media supplemented with 10% FBS (RPMI) and the RPMI/marrow mixture was transferred in 5 ml aliquots to separate 50 ml conical tubes. Red blood cells were lysed by addition of 45 ml ACK lysing buffer (Life

Technologies) and incubation at room temperature for 5 min. Mononuclear cells were pelleted at 400 x *g* for 5 min followed by aspiration of the supernatant. All pellets were resuspended in a single 5 ml of RPMI in one 50 ml conical tube, which was then brought to 50 ml and spun at 400 x *g* for 5 min. The supernatant was removed and the cells were resuspended in 1-10 ml of RPMI depending on the size of the pellet. Cells were filtered through a 30 μm cell strainer into a 15 ml conical tube. Viable cells were counted by trypan blue staining using a hemocytometer. Final samples of total BMMCs were received from the tissue bank in 20 x 10^6 cells per patient at 10 x 10^6/ml in RPMI. Immediately upon receipt, cells were diluted to 1 x 10^6/ml in RPMI.

Flow cytometry

Cells were seeded at 1x10^6/well in 1.5 ml growth media supplemented with 0.1% DMSO and the indicated concentrations of CT8. Cells were collected by centrifugation at 500xG for 5 min, washed once with PBS containing 5% FBS (FACs buffer) and then resuspended in 1:100 Fc Block (anti-mouse CD16/CD32, AM002 UCSF Antibody Core) and incubated at 4°C for 10 minutes. For CD138 staining, anti-CD138-FITC (1:5) was added directly to cells in Fc Block and incubated for 30 minutes at 4°C. After incubation, cells were washed once with FACs buffer and incubated with annexin V-APC (1:20) in BD Binding Buffer (BD Biosciences) for 15 minutes at room temperature in the dark followed by 1:2 dilution with FACs buffer and addition of 1:1000 propidium iodide (PI, Sigma). Cells were analyzed with a FACSCalibur (BD Biosciences), counting 10,000 CD138+ cells per sample. Data were analyzed using FlowJo 10.0.6.

SDS-PAGE, autoradiography, and immunoblotting

10% Tris-Tricine or traditional SDS-PAGE gels were run where indicated. For autoradiography, gels were stained with Coomassie Brilliant Blue before drying with a gel drier. Quantitative autoradiography was performed after exposing the dried gels to a storage phosphor screen (GE Healthcare) and imaged on a Typhoon 9400 scanner. Images were quantified using ImageJ (NIH). Qualitative images were obtained by exposing the gels to BioMax MR film (Kodak).

For immunoblotting, whole-cell lysates were run on 10% SDS-PAGE gels and transferred to nitrocellulose or PVDF. Membranes were blocked with 5% milk in TBST or LI-COR blocking buffer (LI-COR Biosciences), incubated with the appropriate primary antibodies at the following dilutions: anti-p58^{ipk} (1:1000), anti-calreticulin (1:1000), anti-calnexin (1:1000), anti-ERdj3 (1:1000), and α-Bip (1:1000), anti-β-actin (1:1000), anti-β-tubulin (1:2000) at room temperature for 1 hour. Anti-PDI (1:1000), anti-eIF2α (1:1000), anti-eIF2α-P (1:1000), anti-PERK (1:1000) and anti-Sec61α (1:10,000) were used at 4°C overnight. Following primary antibody incubation, membranes were washed three times with TBST and then incubated with either HRP-conjugated (1:5000, Rockland) or infrared (IR) dye-labeled secondary antibodies (1:5000, IR680 or IR800, LI-COR Biosciences). Membranes incubated with HRP-conjugated secondary antibodies were incubated with ECL (Thermo) and visualized using a Gel Logic 6000 (CareStream MI Software). IR dye-labeled membranes were visualized using the LI-COR Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE).

Plasmid and DNA template preparation

DNA templates for in vitro translocation assays were prepared by PCR amplification of the indicated gene using a forward primer that included the T7 promoter (bold) followed by a Kozak consensus sequence (underlined) and ending with a gene specific sequence that begins immediately after the ATG start codon (highlighted): 5'-gcc**TAATACGACTCACTATAGGG**AGACC-Gene_specific_sequence-3'. The reverse primer was comprised of a gene specific sequence corresponding to the 3' end including a stop codon followed by 6 random bases (see **Appendix D** for sequences of all primers). DNA templates were purified by PCR purification (Quigen).

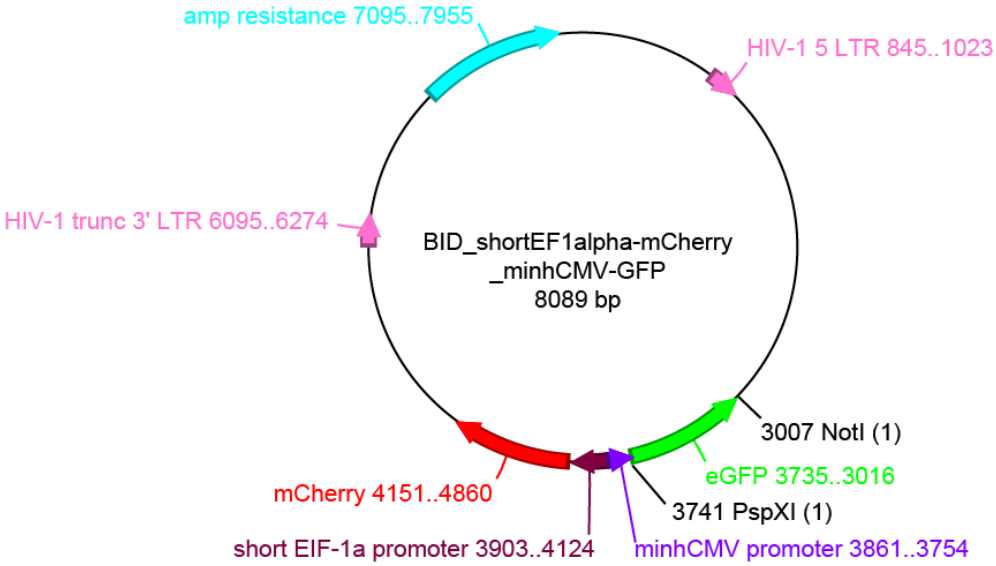
To replace the puromycin selection gene of the pLKO.1 plasmids containing shRNA sequences against $p58^{ipk}$ (Mission, Sigma) with eGFP, each shRNA plasmid was double digested with BamHI/KpnI (NEB) for 2 hrs at 37°C followed by gel purification. 5'-BamHI-eGFP-KpnI-3' was generated by PCR amplification of the pInducer-11 vector[34] using the following primers:

BamHI-eGFP-fwd: 5'- GCGCGGATCCATGGTGAGCAAGGGCGAGGAG-3'

KpnI-eGFP-rev: 5'- GCGCGGTACCTTACTTGTACAGCTCGTCCAT-3'

PCR purified 5'-BamHI-eGFP-KpnI-3' was ligated into the doubly digested pLKO.1 shRNA vectors with T4 DNA ligase (NEB) at 4°C overnight followed by transformation into DH5α competent cells and plating on ampicillin. All constructs were confirmed by DNA sequencing. The shRNA sequences against $p58^{ipk}$ were as follows: shRNA 1: GAGCCAAGCATTGCTGAATAT shRNA 2: CAGTCGCAGAAACGAGATTAT. The control shRNA (Mission, Sigma, SHC016) is a non-targeting shRNA. Its sequence is 5'-GCGCGATAGCGCTAATAATTT-3'.

WT Sec61α and R66I Sec61α coding sequences[25] were inserted in place of eGFP in the bidirectional expression vector BID_shortEF1alpha-mCherry_minhCMV-GFP, which was purchased from the ViraCore at UCSF and was developed in the McManus Lab (see map below).



5'-PspXI-Sec61α-NotI-3' inserts were generated for WT and R66I Sec61α coding sequences by PCR amplification with the following primers:

PspXI-Sec61α-fwd: 5'- GCGCGCCTCGAGATGGCGATCAAATTTCTGGAAGTT-3'

NotI-Sec61α-rev: 5'- GCGCGCGCGGCCGCTCAGAACAGAAGGGCGCCCATGCT-3'

BID_shortEF1alpha-mCherry_minhCMV-GFP was sequentially digested with NotI (NEB) followed by PspXI (NEB), both for 2 hrs at 37°C followed by gel extraction to make BID_shortEF1alpha-mCherry_minhCMV-empty. PCR purified, sequentially digested Sec61α inserts were ligated into BID_shortEF1alpha-mCherry_minhCMV-empty with T4 DNA ligase at 4°C overnight followed by transformation in DH5α competent cells and plating on ampicillin to give BID_shortEF1alpha-

mCherry_minhCMV-Sec61α-WT and BID_shortEF1alpha-mCherry_minhCMV-Sec61α-R66I. Constructs were confirmed by DNA sequencing.

Lentiviral shRNA and stable cell line generation

For shRNA knockdown, lentiviral particles were generated in HEK293T cells using a 2nd generation packaging system as previously described[35]. Briefly, $1x10^6$ HEK293T cells in 2 ml DMEM supplemented with 10% FBS and pen/step in 6-well plates (Falcon) were co-transfected with the shRNA-containing plasmid, plasmid p8.91 (Mullins lab, encoding gag, pol, rev, and tat HIV genes, for reference see Harvard Plasmid Database, clone ID EvNO00438081) and plasmid pMD2.G (Dyche Mullins Lab, UCSF, encoding VSV-G envelope protein, for reference see Addgene, 12259) in a 2:3:1 ratio using *Trans*IT (Mirus) according to manufacture's recommendations. Transfected 293T cells were incubated at 37°C for 3 days, after which the lentivirus-containing media (~1.5 ml) was collected, syringe filtered and added to pellets of $1x10^6$ JJN-3 or ARH-77 cells in the presence of 8 μg/ml polybrene (Santa Cruz) (final volume of 1.5 ml). The cells were diluted 1:6 with DMEM containing 10% FBS and pen/strep media 24 hrs and cells were sorted for GFP fluorescence using a FACSAria (BD Biosciences) cell sorter 48 hrs after transduction. Annexin V and PI (Sigma) staining were performed 4 days after sorting.

For generation of Sec61α stable cell lines, 200 μl of concentrated lentivirus was obtained from the ViraCore at UCSF at a titer of ~$1x10^8$ particles/ml. The entire concentrated stock was added 1:1 (v/V) to $1x10^6$ JJN-3 cells suspended in 200 μl RPMI-1640 with 10% FBS and pen/strep, supplemented with 16 μg/ml polybrene in a 12-well cell culture plate to a final concentration of 8 μg/ml polybrene. Cells were spun

at 2500 rpm at room temperature for 90 min followed by addition of 1 ml growth media and incubation at 37°C for 24 hrs. Cells were then diluted with 2.5 ml media and transferred to a T75 cell culture flask (Greiner). Infection efficiency was determined by mCherry fluorescence 48 hrs post-transduction using a FACSCalibur. Cells were sorted based on mCherry fluorescence 6 days post-transduction using a FACSAria cell sorter. Stable cell lines were maintained in normal growth media.

In vitro translocation assays

In vitro transcription/translation/translocation was carried out as previously described[36]. Briefly, DNA templates encoding full-length versions of the indicated constructs were transcribed with T7 polymerase (NEB) for 1 hr at 37°C. Transcripts were used immediately in subsequent translation reactions which were assembled at 0°C in the presence of CT8 (from a 100x stock in DMSO) or the equivalent amount of DMSO. Reactions were run at 32°C for 1 hour and then moved to ice for further processing. All samples contained ^{35}S-Methionine and where indicated canine rough microsomes (cRM). Non-PK treated samples were diluted 1:10 with 0.1 M Tris pH 8.0, 1% SDS (quench buffer) followed by 1:1 dilution with 2x Laemmli sample buffer and separation by SDS-PAGE. Protease protection was performed as previously described[36].

XBP1 splicing

Cells were seeded at 1x10^6/well in 2 ml growth media containing drug or DMSO in a 6-well cell culture plate and incubated for the indicated time. Total RNA was

extracted from cell pellets using the RNeasy RNA purification kit (Quigen). One microgram RNA was used as input into an annealing reaction containing 1 µl oligo dT (NEB) and 10 µl H_2O and run at 70°C for 10 minutes. DNTPs (2 µl, NEB), Mulv Reverse Transcriptase (1 µl, NEB), 5X reverse transcriptase buffer (4 µl), and H_2O (3 µl) were added and the reaction was heated to 42°C for 1 hour followed by 10 minutes are 70°C to produce cDNA.

CDNA (2 µl) was incubated with 1 µl 10 µM forward primer, 1 µl 10µM reverse primer and 10 µl GoTaq green mix 2x (Promega) + 6 µl ultrapure dH_2O (total volume 20 µl per tube) using the following PCR protocol: 95°C/5 min, 28 cycles [95°/30sec – 55°C/30sec – 72°C/30sec], 72°C/5 min, 4°C end. 10 µl of sample were run directly on a 3% agarose gel at 100V until bands are separated.

Forward primer = 5'-TTA CGA GAG AAA ACT CAT GGC-3'

Reverse primer = 5'-TCC AAG TTG TCC AGA ATG C-3'

CHOP real-time PCR

Cells were seeded at 500,000 cells/ml in 1 ml growth media containing drug or DMSO and incubated at 37°C for 24 hrs. Cells were harvested and RNA was isolated from cell pellets using the RNeasy RNA isolation kit (Qiagen). cDNA was generated using iScript (Bio-Rad) according to the manufacturer's instructions. RT-PCR was performed on a CFX Connect Real-Time PCR machine (Bio-Rad) using SsoAdvanced™ SYBR® Green Supermix (Bio-Rad) and the following primers for CHOP and the house keeping gene, ribosomal protein large, P0 (RPLP0).

Chop Fwd: 5'- CAG AAC CAG CAG AGG TCA CA -3'

Chop Rev: 5'- AGC TGT GCC ACT TTC CTT TC -3'

Rplp0 Fwd: 5’ – GCT GCT GCC CGT GCT GGT G – 3’

Rplp0 Rev: 5’ – TGG TGC CCC TGG AGA TTT TAG TGG – 3’

Reactions were run in triplicate and C_q values were averaged. The fold induction (FI) was calculated as[37]:

$$FI = 2^{(-\Delta\Delta C_q)}$$

Where $\Delta C_q = C_q^{CHOP} - C_q^{RPLP0}$ and $\Delta\Delta C_q = \Delta C_q^{DMSO} - \Delta C_q^{treatment}$.

HGF ELISA

JJN-3 cells expressing WT or R66I Sec61α were washed once with fresh growth media and were seeded at $1x10^6$ cells per well in 1.5 ml growth media supplemented with 0.1% DMSO and the indicated concentrations of CT8 and incubated for 24 hrs. Media and cells were collected and cells were pelleted at 900xG. Media was diluted 1:10 with assay diluent (R&D Systems) and ELISA was performed according to the manufacturer’s instructions (R&D Systems Quantikine ELISA Human HGF DHG00).

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Chapter 4

Conclusions and future directions

4.1 Conclusions and future directions

The full complement of CT8-senstive proteins, as well as the extent of its substrate selectivity was unknown prior to these studies. Using SILAC and membrane proteomics, we showed that CT8 inhibits 6.5% of the identified proteome and only 25% of the Sec61-dependent secretome in the model cell line JJN-3, confirming its selectivity. We validated HGF, CD74, IL2RG and ITAV as direct, CT8-sensitive proteins. Additionally, we showed that while plasma membrane expression of ITB5 and the cytosolic kinase Jak3 are potently inhibited by CT8, these are most likely secondary effects due to loss of ITAV and IL2RG, respectively. Finally, we characterize TRPML1 as the first multi-spanning integral membrane protein sensitive to CT8.

Proteomics not only identified new CT8-sensitive proteins but also provided the largest list of sensitive and resistance sequences to date. Comparison of sensitive and resistance signal peptide sequences identified the simple, calculable metric ΔG_{sub} that was then used to predict four new CT8-senstive proteins from primary amino acid sequence: AREG, CSF3, IL7, and IL7R. These proteins were chosen from a list of all signal peptide-containing proteins, sorted from highest to lowest by ΔG_{sub}. Since the top 25% highest ΔG_{sub} values from the SILAC dataset were highly enriched in CT8-sensitive proteins, we hypothesized that the proteins in the top 25% highest ΔG_{sub} for the whole secretome would likely be CT8-sensitive. **Appendix E** provides a list of 850 proteins with the highest ΔG_{sub} values (top 25%, 850/3400 total signal peptide-containing proteins). To date, we have only tested five proteins from this list, four of which, listed above, were extremely sensitive to CT8, with IC_{50} values less than 250 nM.

Swiss-Prot	ΔG_{sub}	Signal Peptide	Protein names	Type
Q13324	1.22	MDAALLHSLLEANCSLA	Corticotropin-releasing factor receptor 2 (CRF-R-2) (CRF-R2) (CRFR-2) (Corticotropin-releasing hormone receptor 2) (CRH-R-2) (CRH-R2)	GPCR
P05019	0.993	MGKISSLPTQLFKCCFCDFLK	Insulin-like growth factor I (IGF-I) (Mechano growth factor) (MGF) (Somatomedin-C)	SP
Q02643	0.799	MDRRMWGAHVFCVLSPLPTVLG	Growth hormone-releasing hormone receptor (GHRH receptor) (Growth hormone-releasing factor receptor) (GRF receptor) (GRFR)	GPCR
P49763	0.443	MPVMRLFPCFLQLLAGLA	Placenta growth factor (PlGF)	SP
O00220	0.423	MAPPPARVHLGAFLAVTPNPGSA	Tumor necrosis factor receptor superfamily member 10A (Death receptor 4) (TNF-related apoptosis-inducing ligand receptor 1) (TRAIL receptor 1) (TRAIL-R1) (CD antigen CD261)	Type I
O00206	0.35	MMSASRLAGTLIPAMAFLSCVRP	Toll-like receptor 4 (hToll) (CD antigen CD284)	Type I
P03951	0.31	MIFLYQVVHFILFTSVSG	Coagulation factor XI (FXI) (EC 3.4.21.27) (Plasma thromboplastin antecedent) (PTA) [Cleaved into: Coagulation factor XIa heavy chain; Coagulation factor XIa light chain]	SP
P43235	0.211	MWGLKVLLLPVVSFA	Cathepsin K (EC 3.4.22.38) (Cathepsin O) (Cathepsin O2) (Cathepsin X)	SP
P18505	0.107	MWTVQNRESLGLLSFPVMITMVCCA	Gamma-aminobutyric acid receptor subunit beta-1 (GABA(A) receptor subunit beta-1)	SP
O15455	0.104	MRQTLPCIYFWGGLLPFGMLCAS	Toll-like receptor 3 (CD antigen CD283)	Type I
P78536	0.083	MRQSLLFLTSVVPFVLA	Disintegrin and metalloproteinase domain-containing protein 17 (ADAM 17) (EC 3.4.24.86) (Snake venom-like protease) (TNF-alpha convertase) (TNF-alpha-converting enzyme) (CD antigen CD156b)	Type I
P20963	0.051	MKWKALFTAAILQAQLPITEA	T-cell surface glycoprotein CD3 zeta chain (T-cell receptor T3 zeta chain) (CD antigen CD247)	Type I
P05556	0.028	MNLQPIFWIGLISSVCCVFA	Integrin beta-1 (Fibronectin receptor subunit beta) (Glycoprotein IIa) (GPIIA) (VLA-4 subunit beta) (CD antigen CD29)	Type I
P16871	-0.008	MTILGTTFGMVFSLLQVVSG	Interleukin-7 receptor subunit alpha (IL-7 receptor subunit alpha) (IL-7R subunit alpha) (IL-7R-alpha) (IL-7RA) (CDw127) (CD antigen CD127)	Type I
O60939	-0.021	MHRDAWLPRPAFSLTGLSLFFSLVPPGRS	Sodium channel subunit beta-2	Type I
Q14957	-0.121	MGGALGPALLLTSLFGAWA	Glutamate receptor ionotropic, NMDA 2C (GluN2C) (Glutamate [NMDA] receptor subunit epsilon-3) (N-methyl D-aspartate receptor subtype 2C) (NMDAR2C) (NR2C)	SP
P20151	-0.142	MWDLVLSIALSVGCTGAV	Kallikrein-2 (EC 3.4.21.35) (Glandular kallikrein-1) (hGK-1) (Tissue kallikrein-2)	SP
Q14832	-0.162	MKMLTRLQVLTLALFSKGFLLS	Metabotropic glutamate receptor 3 (mGluR3)	GPCR
P16444	-0.193	MWSGWWLWPLVAVCTA	Dipeptidase 1 (EC 3.4.13.19) (Dehydropeptidase-I) (Microsomal dipeptidase) (Renal dipeptidase) (hRDP)	SP
P23415	-0.194	MYSFNTLRLYLWETIVFFSLAASKEAEA	Glycine receptor subunit alpha-1 (Glycine receptor 48 kDa subunit) (Glycine receptor strychnine-binding subunit)	SP
P07202	-0.213	MRALAVLSVTLVMACTEA	Thyroid peroxidase (TPO) (EC 1.11.1.8)	Type I
Q14626	-0.229	MSSSCSGLSRVLVAVATALVSA	Interleukin-11 receptor subunit alpha (IL-11 receptor subunit alpha) (IL-11R subunit alpha) (IL-11R-alpha) (IL-11RA)	Type I
P01579	-0.258	MKYTSYILAFQLCIVLGSLGCYC	Interferon gamma (IFN-gamma) (Immune interferon)	SP
P16109	-0.26	MANCQIAILYQRFQRVVFGISQLLCFSALISELTNQKEVAA	P-selectin (CD62 antigen-like family member P) (Granule membrane protein 140) (GMP-140) (Leukocyte-endothelial cell adhesion molecule 3) (LECAM3) (Platelet activation dependent granule-external membrane protein) (PADGEM) (CD antigen CD62P)	Type I
P40933	-0.274	MRISKPHLRSISIQCYLCLLLNSHFLTEA	Interleukin-15 (IL-15)	SP
Q9H239	-0.279	MVARVGLLLRALQLLLWGHLDA	Matrix metalloproteinase-28 (MMP-28) (EC 3.4.24.-) (Epilysin)	SP
Q9UGM1	-0.303	MNWSHSCISFCWIYFAASRLRAAET	Neuronal acetylcholine receptor subunit alpha-9 (Nicotinic acetylcholine receptor subunit alpha-9) (NACHR alpha-9)	SP
P40198	-0.315	MGPPSASPHRECIPWQGLLLTASLLNFWNPPTTA	Carcinoembryonic antigen-related cell adhesion molecule 3 (Carcinoembryonic antigen CGM1) (CD antigen CD66d)	Type I
P51512	-0.329	MILLTFSTGRRLDFVHHSGVFFLQTLLWILC	Matrix metalloproteinase-16 (MMP-16) (EC 3.4.24.-) (MMP-X2) (Membrane-type matrix metalloproteinase 3) (MT-MMP 3) (MTMMP3) (Membrane-type-3 matrix metalloproteinase) (MT3-MMP) (MT3MMP)	Type I
P47870	-0.36	MWRVRKRGYFGIWSFPLIIAAVCAQ	Gamma-aminobutyric acid receptor subunit beta-2 (GABA(A) receptor subunit beta-2)	SP
P03952	-0.362	MILFKQATYFISLFATVSC	Plasma kallikrein (EC 3.4.21.34) (Fletcher factor) (Kininogenin) (Plasma prekallikrein) [Cleaved into: Plasma kallikrein heavy chain; Plasma kallikrein light chain]	SP
P21860	-0.389	MRANDALQVLGLLFSLARG	Receptor tyrosine-protein kinase erbB-3 (EC 2.7.10.1) (Proto-oncogene-like protein c-ErbB-3) (Tyrosine kinase-type cell surface receptor HER3)	Type I
P28335	-0.404	MVNLRNAVHSFLVHLIGLLVWQCDISVSPVAA	5-hydroxytryptamine receptor 2C (5-HT-2C) (5-HT2C) (5-HTR2C) (5-hydroxytryptamine receptor 1C) (5-HT-1C) (5-HT1C) (Serotonin receptor 2C)	GPCR
P02749	-0.404	MISPVLILFSSFLCHVAIA	Beta-2-glycoprotein 1 (APC inhibitor) (Activated protein C-binding protein) (Anticardiolipin cofactor) (Apolipoprotein H) (Apo-H) (Beta-2-glycoprotein I) (B2GPI) (Beta(2)GPI)	SP

Table 4.1 | Drug targets from Okada et al.[1] with ΔG_{sub} in the top 10%. Official gene symbols from Okada et al. were converted to Swiss-Prot accession numbers using the DAVID Gene ID Conversion Tool (http://david.abcc.ncifcrf.gov/conversion.jsp){Huang:2009be}{Huang:2008gk}. Proteins were annotated as signal peptide-containing (SP), type I, type II, type III, multi-TM (with no signal peptide), G-protein coupled receptor (GPCR) or receptor tyrosine kinase (RTK) according to the procedures outlined in Chapter 2. The full signal peptide is shown, though ΔG_{sub} was calculated using sequences truncated to 30 aa N-terminal to the predicted signal peptidase cleavage site. Proteins with ΔG_{sub} values are ranked from highest to lowest ΔG_{sub}. Only proteins with ΔG_{sub} in the top 10% (based on ΔG_{su} analysis of the entire human signal peptidome) are shown. Proteins discussed in the text are highlighted in green.

Continued mining of this list with special attention to specific disease-related targets will guide future exploration of Sec61α inhibition in new disease contexts.

As a first attempt to prioritize potentially sensitive proteins, we annotated a comprehensive list of current drug targets[3] based on their Sec61 dependence (**Appendix A**). Proteins were annotated as signal peptide-containing (SP), type I, type II, type III or multi-spanning membrane (multi-TM). Multi-TM proteins were annotated as either G-protein coupled receptors (GPCR), and Type I proteins as protein tyrosine kinases (RTK) where appropriate. Of the 850 drug targets, 555 are Sec61 dependent. Of the Sec61-dependent drug targets, 400 are neither GPCRs nor RTKs, and 322 are predicted to have an N-terminal signal peptide. To identify which of these 322 signal peptide-containing proteins are likely to be sensitive to CT8, we extracted signal peptide sequences as previously described and calculated ΔG_{sub} for the 30-aa truncated sequences. **Table 4.1** shows the list of Sec61-dependent drug targets with signal peptides in the top 10% of ΔG_{sub} (based on ΔG_{sub} values calculated for the entire human signal peptidome). Based on our SILAC results and enrichment analysis, the proteins on this list have a ~75% chance of being sensitive to CT8 (EC_{50} < 250 nM).

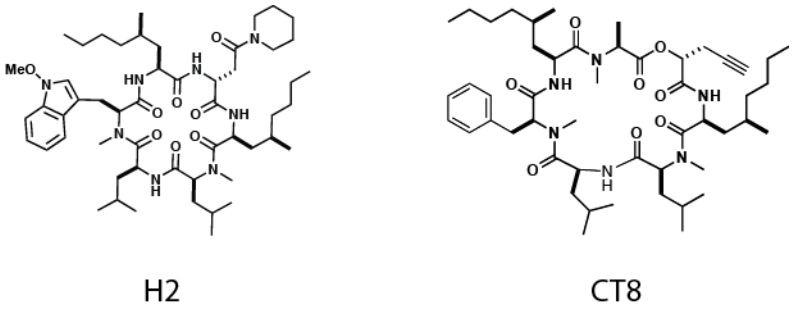

Figure 4.1 | Structures of H2 and CT8.

According to our model of cotransin action, these proteins have the best chance of remaining sensitive to new cotransin analogs that only slightly raise the energy barrier to membrane integration. H2[4] is a cyclic hexapeptide that potently inhibits VCAM-1 expression with no effect on other secretory proteins in an ELISA-based screen (**Figure 4.1**, data not shown). We hypothesize that H2 inhibits cotranslational translocation by binding to Sec61α, like cotransins, but that binding of H2 has a minimal effect on the barrier to integration, resulting in much more selective inhibition of translocation than CT8. To test whether H2 had an effect on the most sensitive secretory protein identified in the SILAC experiment, HGF, JJN-3 cells were treated with 0.5, 1, and 5 μM H2 for 24 hrs and conditioned media was subjected to an HGF ELISA. H2 was much less effective at blocking HGF secretion than CT8, as 5 μM H2 was required for a similar effect elicited by 250 nM CT8 (**Figure 4.2a**). Additionally, while 1 μM CT8 results in a complete loss of integrin αV (ITAV) translocation, 5 μM H2 was required to achieve significant inhibition (**Figure 4.2b**). While HGF and ITAV both fall within the top 25% of ΔG_{sub}, they do not fall within the top 10%. We hypothesize that H2 may inhibit the expression of proteins with higher ΔG_{sub}, including some of the drug targets listed in **Table 4.1**.

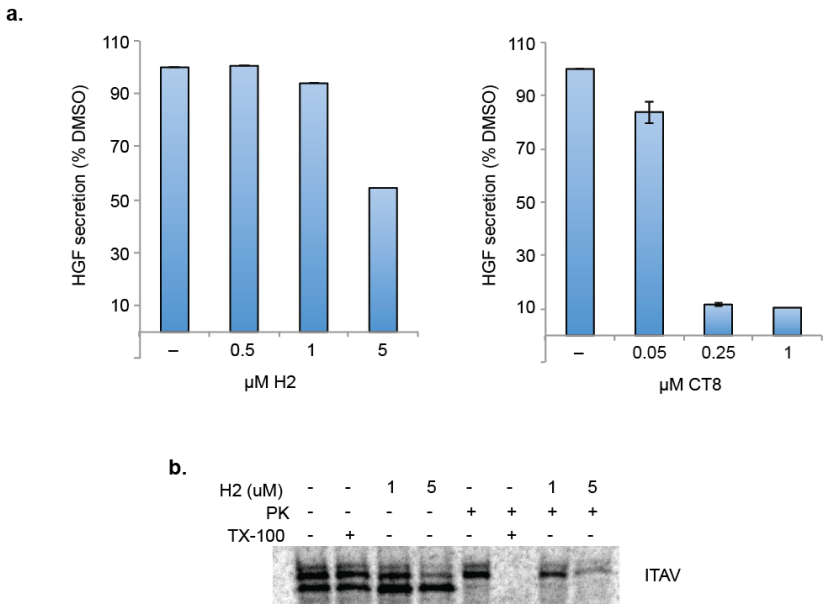

Figure 4.2 | H2 is not as potent as CT8 in blocking expression of HGF or ITAV. (a) JJN-3 cells were treated for 24 hrs with indicated concentrations of H2 (left) or CT8 (right). HGF in conditioned media from treated cells was quantified by ELISA. Bars represent the average of three replicates with error bars representing +/– SD. **(b)** ITAV mRNA truncated to 300aa was translated in the presence of canine rough microsomes (cRM), detergent (TX-100) and the indicated concentrations of H2. Translation reactions were either left alone or treated with proteinase K (PK) and then separated on 10% Tris-Tricine SDS-PAGE gels and analyzed by autoradiography.

Future studies will prioritize this list for testing sensitivity to CT8 and H2 as well as newly developed compounds. Receptor tyrosine pseudokinase erbB3 (Her3, **Table 4.1**, P21860) is an exciting potential target as it has been implicated in numerous cancers including breast, lung, head and neck, skin and colon[5]. Her3 is a member of the Her family of receptor tyrosine kinases, which all possess an extracellular ligand binding domain as well as an intracellular kinase domain. Her receptors are activated by binding of a growth factor ligand to the extracellular domain, which induces dimerization and subsequent transphosphorylation and activation of the intracellular kinase domain. Interestingly, the intracellular domain of Her3 is a psuedokinase that lacks kinase

activity. Therefore, Her3 requires heterodimerization with other Her family members for activation. Because Her3 has no active kinase domain, this protein has been very difficult to drug with small molecules. Additionally, 11% of colon and gastric cancers have mutations in Her3. The Her3 mutants, when in complex with Her2, promote oncogenic signaling in a ligand-independent manner[6]. Therefore, blocking expression of Her3 with cotransins may provide a way to inhibit Her signaling by destroying the formation of these essential, potentially ligand-independent heterodimers. Finally, Her3 amplification has been shown to provide a mechanism of resistance to BRAF/MEK inhibitors in melanoma[7]. Resistance to BRAF inhibitors has also been shown to be dependent on HGF, so cotransins may provide a way to target both mechanisms of resistance at once[8].

Plasma kallikrein is another interesting, potentially sensitive protein. pKAL is formed by cleavage of the zymogen, plasma prekallikrein (**Table 4.1**, P03952) by coagulation factor XII. pKAL is a serine protease responsible for the production of bradykinin, a potent vasodilator. Patients with hereditary angioedema have mutations in the C1-esterase inhibitor, which inhibits pKAL activity. Therefore, these patients have unrestrained pKAL activity resulting in overproduction of bradykinin, leading to excessive vasodilatation, swelling, inflammation and edema. Ecallantide is a recombinant protein-based inhibitor of pKAL that has shown promise in the clinic[9]. However, there have been no reports of small-molecule inhibitors of pKAL in clinical development.

One final interesting protein from this list is cathepsin K (**Table 4.**1, P43235). Cathepsin K is a cysteine protease essential for bone remodeling and resorption. As

such, inhibitors of cathepsin K have been developed to treat osteoporosis, the most successful of which is odanacatib in Phase III clinical trials[10]. While cathepsin K is primarily thought of in the context of bone remodeling, it has also been implicated in autoimmune inflammation and arthritis[11]. In this study, cathepsin K inhibition resulted in defective toll-like receptor 9 signaling in dendritic cells, leading to decreased induction of helper T-cells, with no deleterious effect on antigen-presentation. These results point to cathepsin K as a potential target in autoimmune disease. Interestingly, toll-like receptor 9 is also predicted to be sensitive to cotransins as it has a ΔG_{sub} in the top 25% (**Appendix E**, ΔG_{sub} = –0.473). Therefore, cotransins might target two members of this pathway.

While cotransins may prove useful in the indications outlined above, the studies presented here already identify Sec61α as a promising new therapeutic target in multiple myeloma, a largely incurable cancer that is notoriously susceptible to both innate and developed drug resistance. Future work towards unraveling the mechanisms through which cotransin inhibition specifically leads to apoptosis in myeloma cells will likely uncover additional novel targets. For example, the work presented here uncovered the ER-resident chaperone $p58^{ipk}$ as an essential protein in myeloma JJN-3 cells, whereas knockdown was tolerated in non-myeloma ARH-77 cells. While CT8-induced apoptosis is likely to be caused through a myriad of pathways, dissecting each one will provide opportunities for development of novel therapies. Additionally, careful characterization of the association between secretory protein load and sensitivity to cotransin may provide insights into how these compounds function. For example, if the

high expression of kappa light chain (κLC) in JJN-3 cells is in part responsible for their sensitivity to CT8, then over expression of κLC in ARH-77 cells should sensitize them.

The studies here suggest a therapeutic potential of cotransins, as well as point toward new areas of exploration. Medicinal chemistry will be required to develop analogs with better pharmaceutical properties to move these compounds into animal models. Additionally, development of screening techniques to systematically define the cotransin sensitivity determinants will provide a more accurate model for prediction of new sensitive substrates and potential off-target effects as these and related compounds move into the clinic.

4.2 Experimental procedures

Bioinformatics

The sequence feature list of accession numbers whose Swiss-Prot entries contained the key word “signal peptide” was sorted for “signal peptide” and only accessions with signal peptides were kept. This list was compared to lists of Swiss-Prot entries with the subcellular location “single-pass type I transmembrane” and the GO terms 0004714 (transmembrane receptor tyrosine kinase activity) and 0004930 (G-protein coupled receptor activity) to annotate for type I transmembrane proteins (type I), receptor tyrosine kinases (RTK) and G-protein coupled receptors (GPCR) respectively.

To obtain the sequences of signal peptides, the sequence feature file was batch retrieved from Swiss-Prot. This file was sorted for the sequence feature “signal peptide” to give the N-terminal amino acid position (always 1) and the amino acid position for the predicted cleavage site. These two numbers, along with the corresponding accession

number, were transferred to an Excel file with three columns with the headings: Accession, From, To. The Accession column contained accession numbers, the “From” column contained the N-terminal position (1) and the “To” column contained the cleavage site prediction (varied for each signal peptide). This file was saved as a Windows compatible .CSV file. Additionally, FASTA sequences for each accession number in the .CSV file were batch retrieved from Swiss-Prot. The .FASTA was changed to .TXT by changing the file name. These two files, the .CSV containing the from and to designations for the beginning and end of the signal peptide and the .TXT file containing the sequences for all proteins being interrogated were given as input to the fasta_segment.py script written by Peter Malkin (Google) in the Python programming language (**Appendix B**). The output contains the amino acid sequence beginning at the “From” position and ending at the “To” position.

To calculate the hydrophobicity values for signal peptides, each signal peptide was truncated to 30 amino acids from the predicted cleavage site, as the maximum input length for the ΔG calculator in “ΔG prediction” mode is 30aa. ΔG_{sub} was calculated by entering the truncated sequence into the ΔG calculator in “ΔG prediction” mode with no length correction AND allowing for a subsequence (if lower ΔG).

HGF ELISA

JJN-3 cells were washed once with fresh growth media and were seeded at $1x10^6$ cells per well in 1.5 ml growth media supplemented with 0.1% DMSO and the indicated concentrations of H2 or CT8 and incubated for 24 hrs. Media and cells were collected and cells were pelleted at 900 x *g*. Media was diluted 1:10 with assay diluent

(R&D Systems) and ELISA was performed according to manufacturer's instructions (R&D Systems Quantikine ELISA Human HGF DHG00).

In vitro translation/translocation assays

In vitro transcription/translation/translocation was carried out as previously described[12]. Briefly, a DNA template encoding truncated ITAV were transcribed with T7 polymerase (NEB) for 1 hr at 37°C. Transcripts were used immediately in subsequent translation reactions which were assembled at 0°C in the presence of CT8 (from a 100x stock in DMSO) or the equivalent amount of DMSO. Reactions were run at 32°C for 1 hour and then moved to ice for further processing. All samples contained ^{35}S-Methionine and where indicated canine rough microsomes (cRM) or 1% detergent (Triton x-100, Sigma). Samples not subjected to protease protection were diluted 1:10 with 0.1 M Tris pH 8.0, 1% SDS (quench buffer) followed by 1:1 dilution with 2x Laemmli sample buffer and separation by 10% Tris-Tricine SDS-PAGE. Protease protection was performed as previously described[12].

4.3 References

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Appendix A

List of drug targets

Swiss-Prot	Description	Sec61?	dG_{sub}
Q13324	Corticotropin-releasing factor receptor 2 (CRF-R-2) (CRF-R2) (CRFR-2) (Corticotropin-releasing hormone receptor 2) (CRH-R-2) (CRH-R2)	GPCR	1.22
P05019	Insulin-like growth factor I (IGF-I) (Mechano growth factor) (MGF) (Somatomedin-C)	SP	0.99
Q02643	Growth hormone-releasing hormone receptor (GHRH receptor) (Growth hormone-releasing factor receptor) (GRF receptor) (GRFR)	GPCR	0.80
P49763	Placenta growth factor (PlGF)	SP	0.44
O00220	Tumor necrosis factor receptor superfamily member 10A (Death receptor 4) (TNF-related apoptosis-inducing ligand receptor 1) (TRAIL receptor 1) (TRAIL-R1) (CD antigen CD261)	SP	0.42
O00206	Toll-like receptor 4 (hToll) (CD antigen CD284)	SP	0.35
P03951	Coagulation factor XI (FXI) (EC 3.4.21.27) (Plasma thromboplastin antecedent) (PTA) [Cleaved into: Coagulation factor XIa heavy chain; Coagulation factor XIa light chain]	SP	0.31
P43235	Cathepsin K (EC 3.4.22.38) (Cathepsin O) (Cathepsin O2) (Cathepsin X)	SP	0.21
P18505	Gamma-aminobutyric acid receptor subunit beta-1 (GABA(A) receptor subunit beta-1)	SP	0.11
O15455	Toll-like receptor 3 (CD antigen CD283)	SP	0.10
P78536	Disintegrin and metalloproteinase domain-containing protein 17 (ADAM 17) (EC 3.4.24.86) (Snake venom-like protease) (TNF-alpha convertase) (TNF-alpha-converting enzyme) (CD antigen CD156b)	SP	0.08
P20963	T-cell surface glycoprotein CD3 zeta chain (T-cell receptor T3 zeta chain) (CD antigen CD247)	SP	0.05
P05556	Integrin beta-1 (Fibronectin receptor subunit beta) (Glycoprotein IIa) (GPIIA) (VLA-4 subunit beta) (CD antigen CD29)	SP	0.03
P16871	Interleukin-7 receptor subunit alpha (IL-7 receptor subunit alpha) (IL-7R subunit alpha) (IL-7R-alpha) (IL-7RA) (CDw127) (CD antigen CD127)	SP	-0.01
O60939	Sodium channel subunit beta-2	SP	-0.02
Q14957	Glutamate receptor ionotropic, NMDA 2C (GluN2C) (Glutamate [NMDA] receptor subunit epsilon-3) (N-methyl D-aspartate receptor subtype 2C) (NMDAR2C) (NR2C)	SP	-0.12
P20151	Kallikrein-2 (EC 3.4.21.35) (Glandular kallikrein-1) (hGK-1) (Tissue kallikrein-2)	SP	-0.14
Q14832	Metabotropic glutamate receptor 3 (mGluR3)	GPCR	-0.16
P16444	Dipeptidase 1 (EC 3.4.13.19) (Dehydropeptidase-I) (Microsomal dipeptidase) (Renal dipeptidase) (hRDP)	SP	-0.19
P23415	Glycine receptor subunit alpha-1 (Glycine receptor 48 kDa subunit) (Glycine receptor strychnine-binding subunit)	SP	-0.19
P07202	Thyroid peroxidase (TPO) (EC 1.11.1.8)	SP	-0.21
Q14626	Interleukin-11 receptor subunit alpha (IL-11 receptor subunit alpha) (IL-11R subunit alpha) (IL-11R-alpha) (IL-11RA)	SP	-0.23
P01579	Interferon gamma (IFN-gamma) (Immune interferon)	SP	-0.26
P16109	P-selectin (CD62 antigen-like family member P) (Granule membrane protein 140) (GMP-140) (Leukocyte-endothelial cell adhesion molecule 3) (LECAM3) (Platelet activation dependent granule-external membrane protein) (PADGEM) (CD antigen CD62P)	SP	-0.26
P40933	Interleukin-15 (IL-15)	SP	-0.27
Q9H239	Matrix metalloproteinase-28 (MMP-28) (EC 3.4.24.-) (Epilysin)	SP	-0.28
Q9UGM1	Neuronal acetylcholine receptor subunit alpha-9 (Nicotinic acetylcholine receptor subunit alpha-9) (NACHR alpha-9)	SP	-0.30
P40198	Carcinoembryonic antigen-related cell adhesion molecule 3 (Carcinoembryonic antigen CGM1) (CD antigen CD66d)	SP	-0.32
P51512	Matrix metalloproteinase-16 (MMP-16) (EC 3.4.24.-) (MMP-X2) (Membrane-type matrix metalloproteinase 3) (MT-MMP 3) (MTMMP3) (Membrane-type-3 matrix metalloproteinase) (MT3-MMP) (MT3MMP)	SP	-0.33
P47870	Gamma-aminobutyric acid receptor subunit beta-2 (GABA(A) receptor subunit beta-2)	SP	-0.36
P03952	Plasma kallikrein (EC 3.4.21.34) (Fletcher factor) (Kininogenin) (Plasma prekallikrein) [Cleaved into: Plasma kallikrein heavy chain; Plasma kallikrein light chain]	SP	-0.36
P21860	Receptor tyrosine-protein kinase erbB-3 (EC 2.7.10.1) (Proto-oncogene-like protein c-ErbB-3) (Tyrosine kinase-type cell surface receptor HER3)	SP	-0.39
P28335	5-hydroxytryptamine receptor 2C (5-HT-2C) (5-HT2C) (5-HTR2C) (5-hydroxytryptamine receptor 1C) (5-HT-1C) (5-HT1C) (Serotonin receptor 2C)	GPCR	-0.40
P02749	Beta-2-glycoprotein 1 (APC inhibitor) (Activated protein C-binding protein) (Anticardiolipin cofactor) (Apolipoprotein H) (Apo-H) (Beta-2-glycoprotein I) (B2GPI)	SP	-0.40

P27930	Interleukin-1 receptor type 2 (IL-1R-2) (IL-1RT-2) (IL-1RT2) (CD121 antigen-like family member B) (CDw121b) (IL-1 type II receptor) (Interleukin-1 receptor beta) (IL-1R-beta) (Interleukin-1 receptor type II) (CD antigen CD121b) [Cleaved into: Interleukin-1 receptor type 2, membrane form (mIL-1R2) (mIL-1RII); Interleukin-1 receptor type 2, soluble form (sIL-1R2) (sIL-1RII)]	SP	-0.43
P42261	Glutamate receptor 1 (GluR-1) (AMPA-selective glutamate receptor 1) (GluR-A) (GluR-K1) (Glutamate receptor ionotropic, AMPA 1) (GluA1)	SP	-0.43
O15123	Angiopoietin-2 (ANG-2)	SP	-0.45
Q9NR96	Toll-like receptor 9 (CD antigen CD289)	SP	-0.47
P31785	Cytokine receptor common subunit gamma (Interleukin-2 receptor subunit gamma) (IL-2 receptor subunit gamma) (IL-2R subunit gamma) (IL-2RG) (gammaC) (p64) (CD antigen CD132)	SP	-0.48
P26992	Ciliary neurotrophic factor receptor subunit alpha (CNTF receptor subunit alpha) (CNTFR-alpha)	SP	-0.48
P24347	Stromelysin-3 (SL-3) (ST3) (EC 3.4.24.-) (Matrix metalloproteinase-11) (MMP-11)	SP	-0.49
P28472	Gamma-aminobutyric acid receptor subunit beta-3 (GABA(A) receptor subunit beta-3)	SP	-0.50
O60894	Receptor activity-modifying protein 1 (Calcitonin-receptor-like receptor activity-modifying protein 1) (CRLR activity-modifying protein 1)	SP	-0.50
P25942	Tumor necrosis factor receptor superfamily member 5 (B-cell surface antigen CD40) (Bp50) (CD40L receptor) (CDw40) (CD antigen CD40)	SP	-0.51
P25092	Heat-stable enterotoxin receptor (STA receptor) (hSTAR) (EC 4.6.1.2) (Guanylyl cyclase C) (GC-C) (Intestinal guanylate cyclase)	SP	-0.52
P02751	Fibronectin (FN) (Cold-insoluble globulin) (CIG) [Cleaved into: Anastellin; Ugl-Y1; Ugl-Y2; Ugl-Y3]	SP	-0.54
P36888	Receptor-type tyrosine-protein kinase FLT3 (EC 2.7.10.1) (FL cytokine receptor) (Fetal liver kinase-2) (FLK-2) (Fms-like tyrosine kinase 3) (FLT-3) (Stem cell tyrosine kinase 1) (STK-1) (CD antigen CD135)	RTK	-0.55
P20701	Integrin alpha-L (CD11 antigen-like family member A) (Leukocyte adhesion glycoprotein LFA-1 alpha chain) (LFA-1A) (Leukocyte function-associated molecule 1 alpha chain) (CD antigen CD11a)	SP	-0.55
P06756	Integrin alpha-V (Vitronectin receptor subunit alpha) (CD antigen CD51) [Cleaved into: Integrin alpha-V heavy chain; Integrin alpha-V light chain]	SP	-0.55
Q9NRE1	Matrix metalloproteinase-26 (MMP-26) (EC 3.4.24.-) (Endometase) (Matrilysin-2)	SP	-0.55
P00734	Prothrombin (EC 3.4.21.5) (Coagulation factor II) [Cleaved into: Activation peptide fragment 1; Activation peptide fragment 2; Thrombin light chain; Thrombin heavy chain]	SP	-0.55
P22894	Neutrophil collagenase (EC 3.4.24.34) (Matrix metalloproteinase-8) (MMP-8) (PMNL collagenase) (PMNL-CL)	SP	-0.56
P49190	Parathyroid hormone 2 receptor (PTH2 receptor)	GPCR	-0.56
P14210	Hepatocyte growth factor (Hepatopoietin-A) (Scatter factor) (SF) [Cleaved into: Hepatocyte growth factor alpha chain; Hepatocyte growth factor beta chain]	SP	-0.57
O75311	Glycine receptor subunit alpha-3	SP	-0.59
Q96IY4	Carboxypeptidase B2 (EC 3.4.17.20) (Carboxypeptidase U) (CPU) (Plasma carboxypeptidase B) (pCPB) (Thrombin-activable fibrinolysis inhibitor) (TAFI)	SP	-0.59
Q8TCU5	Glutamate receptor ionotropic, NMDA 3A (GluN3A) (N-methyl-D-aspartate receptor subtype 3A) (NMDAR3A) (NR3A) (NMDAR-L)	SP	-0.60
P19022	Cadherin-2 (CDw325) (Neural cadherin) (N-cadherin) (CD antigen CD325)	SP	-0.61
A8MPY1	Gamma-aminobutyric acid receptor subunit rho-3 (GABA(A) receptor subunit rho-3) (GABA(C) receptor)	SP	-0.61
P04062	Glucosylceramidase (EC 3.2.1.45) (Acid beta-glucosidase) (Alglucerase) (Beta-glucocerebrosidase) (Beta-GC) (D-glucosyl-N-acylsphingosine glucohydrolase) (Imiglucerase)	SP	-0.62
P22888	Lutropin-choriogonadotropic hormone receptor (LH/CG-R) (Luteinizing hormone receptor) (LHR) (LSH-R)	GPCR	-0.62
P24394	Interleukin-4 receptor subunit alpha (IL-4 receptor subunit alpha) (IL-4R subunit alpha) (IL-4R-alpha) (IL-4RA) (CD antigen CD124) [Cleaved into: Soluble interleukin-4 receptor subunit alpha (Soluble IL-4 receptor subunit alpha) (Soluble IL-4R-alpha) (sIL4Ralpha/prot) (IL-4-binding protein) (IL4-BP)]	SP	-0.64
P36544	Neuronal acetylcholine receptor subunit alpha-7	SP	-0.67
P32418	Sodium/calcium exchanger 1 (Na(+)/Ca(2+)-exchange protein 1) (Solute carrier family 8 member 1)	SP	-0.68
P02671	Fibrinogen alpha chain [Cleaved into: Fibrinopeptide A; Fibrinogen alpha chain]	SP	-0.68
P31644	Gamma-aminobutyric acid receptor subunit alpha-5 (GABA(A) receptor subunit alpha-5)	SP	-0.68

P56817	Beta-secretase 1 (EC 3.4.23.46) (Aspartyl protease 2) (ASP2) (Asp 2) (Beta-site amyloid precursor protein cleaving enzyme 1) (Beta-site APP cleaving enzyme 1) (Memapsin-2) (Membrane-associated aspartic protease 2)	SP	-0.68
P48551	Interferon alpha/beta receptor 2 (IFN-R-2) (IFN-alpha binding protein) (IFN-alpha/beta receptor 2) (Interferon alpha binding protein) (Type I interferon receptor 2)	SP	-0.68
Q99542	Matrix metalloproteinase-19 (MMP-19) (EC 3.4.24.-) (Matrix metalloproteinase RASI) (Matrix metalloproteinase-18) (MMP-18)	SP	-0.69
P54760	Ephrin type-B receptor 4 (EC 2.7.10.1) (Hepatoma transmembrane kinase) (Tyrosine-protein kinase TYRO11)	RTK	-0.70
P47872	Secretin receptor (SCT-R)	GPCR	-0.72
Q15303	Receptor tyrosine-protein kinase erbB-4 (EC 2.7.10.1) (Proto-oncogene-like protein c-ErbB-4) (Tyrosine kinase-type cell surface receptor HER4) (p180erbB4) [Cleaved into: ERBB4 intracellular domain (4ICD) (E4ICD) (s80HER4)]	RTK	-0.74
P00742	Coagulation factor X (EC 3.4.21.6) (Stuart factor) (Stuart-Prower factor) [Cleaved into: Factor X light chain; Factor X heavy chain; Activated factor Xa heavy chain]	SP	-0.74
P33681	T-lymphocyte activation antigen CD80 (Activation B7-1 antigen) (BB1) (CTLA-4 counter-receptor B7.1) (B7) (CD antigen CD80)	SP	-0.74
P50281	Matrix metalloproteinase-14 (MMP-14) (EC 3.4.24.80) (MMP-X1) (Membrane-type matrix metalloproteinase 1) (MT-MMP 1) (MTMMP1) (Membrane-type-1 matrix metalloproteinase) (MT1-MMP) (MT1MMP)	SP	-0.75
P61812	Transforming growth factor beta-2 (TGF-beta-2) (BSC-1 cell growth inhibitor) (Cetermin) (Glioblastoma-derived T-cell suppressor factor) (G-TSF) (Polyergin) [Cleaved into: Latency-associated peptide (LAP)]	SP	-0.75
Q13093	Platelet-activating factor acetylhydrolase (PAF acetylhydrolase) (EC 3.1.1.47) (1-alkyl-2-acetylglycerophosphocholine esterase) (2-acetyl-1-alkylglycerophosphocholine esterase) (Group-VIIA phospholipase A2) (gVIIA-PLA2) (LDL-associated phospholipase A2) (LDL-PLA(2)) (PAF 2-acylhydrolase)	SP	-0.76
Q9ULX7	Carbonic anhydrase 14 (EC 4.2.1.1) (Carbonate dehydratase XIV) (Carbonic anhydrase XIV) (CA-XIV)	SP	-0.78
P34998	Corticotropin-releasing factor receptor 1 (CRF-R-1) (CRF-R1) (CRFR-1) (Corticotropin-releasing hormone receptor 1) (CRH-R-1) (CRH-R1)	GPCR	-0.79
P29317	Ephrin type-A receptor 2 (EC 2.7.10.1) (Epithelial cell kinase) (Tyrosine-protein kinase receptor ECK)	RTK	-0.79
P40189	Interleukin-6 receptor subunit beta (IL-6 receptor subunit beta) (IL-6R subunit beta) (IL-6R-beta) (IL-6RB) (CDw130) (Interleukin-6 signal transducer) (Membrane glycoprotein 130) (gp130) (Oncostatin-M receptor subunit alpha) (CD antigen CD130)	SP	-0.80
P06729	T-cell surface antigen CD2 (Erythrocyte receptor) (LFA-2) (LFA-3 receptor) (Rosette receptor) (T-cell surface antigen T11/Leu-5) (CD antigen CD2)	SP	-0.82
P06280	Alpha-galactosidase A (EC 3.2.1.22) (Alpha-D-galactosidase A) (Alpha-D-galactoside galactohydrolase) (Melibiase) (Agalsidase)	SP	-0.82
P07098	Gastric triacylglycerol lipase (GL) (Gastric lipase) (EC 3.1.1.3)	SP	-0.82
P54802	Alpha-N-acetylglucosaminidase (EC 3.2.1.50) (N-acetyl-alpha-glucosaminidase) (NAG) [Cleaved into: Alpha-N-acetylglucosaminidase 82 kDa form; Alpha-N-acetylglucosaminidase 77 kDa form]	SP	-0.83
P05186	Alkaline phosphatase, tissue-nonspecific isozyme (AP-TNAP) (TNSALP) (EC 3.1.3.1) (Alkaline phosphatase liver/bone/kidney isozyme)	SP	-0.84
P17181	Interferon alpha/beta receptor 1 (IFN-R-1) (IFN-alpha/beta receptor 1) (Cytokine receptor class-II member 1) (Cytokine receptor family 2 member 1) (CRF2-1) (Type I interferon receptor 1)	SP	-0.84
P16233	Pancreatic triacylglycerol lipase (PL) (PTL) (Pancreatic lipase) (EC 3.1.1.3)	SP	-0.87
Q02763	Angiopoietin-1 receptor (EC 2.7.10.1) (Endothelial tyrosine kinase) (Tunica interna endothelial cell kinase) (Tyrosine kinase with Ig and EGF homology domains-2) (Tyrosine-protein kinase receptor TEK) (Tyrosine-protein kinase receptor TIE-2) (hTIE2) (p140 TEK) (CD antigen CD202b)	RTK	-0.88
Q9BYF1	Angiotensin-converting enzyme 2 (EC 3.4.17.23) (ACE-related carboxypeptidase) (Angiotensin-converting enzyme homolog) (ACEH) (Metalloprotease MPROT15) [Cleaved into: Processed angiotensin-converting enzyme 2]	SP	-0.90
P16473	Thyrotropin receptor (Thyroid-stimulating hormone receptor) (TSH-R)	GPCR	-0.91
Q03405	Urokinase plasminogen activator surface receptor (U-PAR) (uPAR) (Monocyte activation antigen Mo3) (CD antigen CD87)	SP	-0.91
P08637	Low affinity immunoglobulin gamma Fc region receptor III-A (CD16a antigen) (Fc-gamma RIII-alpha) (Fc-gamma RIII) (Fc-gamma RIIIa) (FcRIII) (FcRIIIa) (FcR-10) (IgG Fc receptor III-2) (CD antigen CD16a)	SP	-0.92
O75015	Low affinity immunoglobulin gamma Fc region receptor III-B (Fc-gamma RIII-beta) (Fc-gamma RIII) (Fc-gamma RIIIb) (FcRIII) (FcRIIIb) (FcR-10) (IgG Fc receptor III-1) (CD antigen CD16b)	SP	-0.92

P11597	Cholesteryl ester transfer protein (Lipid transfer protein I)	SP	-0.93
P15248	Interleukin-9 (IL-9) (Cytokine P40) (T-cell growth factor P40)	SP	-0.93
P40238	Thrombopoietin receptor (TPO-R) (Myeloproliferative leukemia protein) (Proto-oncogene c-Mpl) (CD antigen CD110)	SP	-0.95
P25101	Endothelin-1 receptor (Endothelin A receptor) (ET-A) (ETA-R) (hET-AR)	GPCR	-0.96
P34903	Gamma-aminobutyric acid receptor subunit alpha-3 (GABA(A) receptor subunit alpha-3)	SP	-0.96
P46098	5-hydroxytryptamine receptor 3A (5-HT3-A) (5-HT3A) (5-hydroxytryptamine receptor 3) (5-HT-3) (5-HT3R) (Serotonin receptor 3A) (Serotonin-gated ion channel receptor)	SP	-0.97
P10912	Growth hormone receptor (GH receptor) (Somatotropin receptor) [Cleaved into: Growth hormone-binding protein (GH-binding protein) (GHBP) (Serum-binding protein)]	SP	-0.97
O14764	Gamma-aminobutyric acid receptor subunit delta (GABA(A) receptor subunit delta)	SP	-0.97
Q15822	Neuronal acetylcholine receptor subunit alpha-2	SP	-0.97
Q03431	Parathyroid hormone/parathyroid hormone-related peptide receptor (PTH/PTHrP type I receptor) (PTH/PTHr receptor) (Parathyroid hormone 1 receptor) (PTH1 receptor)	GPCR	-0.98
P08887	Interleukin-6 receptor subunit alpha (IL-6 receptor subunit alpha) (IL-6R subunit alpha) (IL-6R-alpha) (IL-6RA) (IL-6R 1) (Membrane glycoprotein 80) (gp80) (CD antigen CD126)	SP	-0.99
P0C0L4	Complement C4-A (Acidic complement C4) (C3 and PZP-like alpha-2-macroglobulin domain-containing protein 2) [Cleaved into: Complement C4 beta chain; Complement C4-A alpha chain; C4a anaphylatoxin; C4b-A; C4d-A; Complement C4 gamma chain]	SP	-0.99
P0C0L5	Complement C4-B (Basic complement C4) (C3 and PZP-like alpha-2-macroglobulin domain-containing protein 3) [Cleaved into: Complement C4 beta chain; Complement C4-B alpha chain; C4a anaphylatoxin; C4b-B; C4d-B; Complement C4 gamma chain]	SP	-0.99
Q9Y251	Heparanase (EC 3.2.1.166) (Endo-glucoronidase) (Heparanase-1) (Hpa1) [Cleaved into: Heparanase 8 kDa subunit; Heparanase 50 kDa subunit]	SP	-0.99
P05106	Integrin beta-3 (Platelet membrane glycoprotein IIIa) (GPIIIa) (CD antigen CD61)	SP	-0.99
P61278	Somatostatin (Growth hormone release-inhibiting factor) [Cleaved into: Somatostatin-28; Somatostatin-14]	SP	-0.99
P01589	Interleukin-2 receptor subunit alpha (IL-2 receptor subunit alpha) (IL-2-RA) (IL-2R subunit alpha) (IL2-RA) (TAC antigen) (p55) (CD antigen CD25)	SP	-0.99
P19235	Erythropoietin receptor (EPO-R)	SP	-1.00
P32927	Cytokine receptor common subunit beta (CDw131) (GM-CSF/IL-3/IL-5 receptor common beta subunit) (CD antigen CD131)	SP	-1.00
P04234	T-cell surface glycoprotein CD3 delta chain (T-cell receptor T3 delta chain) (CD antigen CD3d)	SP	-1.01
O00591	Gamma-aminobutyric acid receptor subunit pi (GABA(A) receptor subunit pi)	SP	-1.01
O60603	Toll-like receptor 2 (Toll/interleukin-1 receptor-like protein 4) (CD antigen CD282)	SP	-1.03
Q9NYK1	Toll-like receptor 7	SP	-1.04
Q07699	Sodium channel subunit beta-1	SP	-1.08
Q8IWT1	Sodium channel subunit beta-4	SP	-1.09
P22607	Fibroblast growth factor receptor 3 (FGFR-3) (EC 2.7.10.1) (CD antigen CD333)	RTK	-1.09
P07766	T-cell surface glycoprotein CD3 epsilon chain (T-cell surface antigen T3/Leu-4 epsilon chain) (CD antigen CD3e)	SP	-1.12
P10253	Lysosomal alpha-glucosidase (EC 3.2.1.20) (Acid maltase) (Aglucosidase alfa) [Cleaved into: 76 kDa lysosomal alpha-glucosidase; 70 kDa lysosomal alpha-glucosidase]	SP	-1.12
P10747	T-cell-specific surface glycoprotein CD28 (TP44) (CD antigen CD28)	SP	-1.14
P10646	Tissue factor pathway inhibitor (TFPI) (Extrinsic pathway inhibitor) (EPI) (Lipoprotein-associated coagulation inhibitor) (LACI)	SP	-1.14
P04070	Vitamin K-dependent protein C (EC 3.4.21.69) (Anticoagulant protein C) (Autoprothrombin IIA) (Blood coagulation factor XIV) [Cleaved into: Vitamin K-dependent protein C light chain; Vitamin K-dependent protein C heavy chain; Activation peptide]	SP	-1.15
P15509	Granulocyte-macrophage colony-stimulating factor receptor subunit alpha (GM-CSF-R-alpha) (GMCSFR-alpha) (GMR-alpha) (CDw116) (CD antigen CD116)	SP	-1.16
P13612	Integrin alpha-4 (CD49 antigen-like family member D) (Integrin alpha-IV) (VLA-4 subunit alpha) (CD antigen CD49d)	SP	-1.17
P05231	Interleukin-6 (IL-6) (B-cell stimulatory factor 2) (BSF-2) (CTL differentiation factor) (CDF) (Hybridoma growth factor) (Interferon beta-2) (IFN-beta-2)	SP	-1.17
P23141	Liver carboxylesterase 1 (Acyl-coenzyme A:cholesterol acyltransferase) (ACAT) (Brain carboxylesterase hBr1) (Carboxylesterase 1) (CE-1) (hCE-1) (EC 3.1.1.1) (Cocaine carboxylesterase) (Egasyn) (HMSE) (Methylumbelliferyl-acetate deacetylase 1) (EC 3.1.1.56) (Monocyte/macrophage serine esterase) (Retinyl ester hydrolase) (REH) (Serine esterase 1)	SP	-1.19

	(Triacylglycerol hydrolase) (TGH)		
P24530	Endothelin B receptor (ET-B) (ET-BR) (Endothelin receptor non-selective type)	GPCR	-1.19
P01730	T-cell surface glycoprotein CD4 (T-cell surface antigen T4/Leu-3) (CD antigen CD4)	SP	-1.20
Q16602	Calcitonin gene-related peptide type 1 receptor (CGRP type 1 receptor) (Calcitonin receptor-like receptor)	GPCR	-1.20
P43681	Neuronal acetylcholine receptor subunit alpha-4	SP	-1.20
P32241	Vasoactive intestinal polypeptide receptor 1 (VIP-R-1) (Pituitary adenylate cyclase-activating polypeptide type II receptor) (PACAP type II receptor) (PACAP-R-2) (PACAP-R2) (VPAC1)	GPCR	-1.21
P21802	Fibroblast growth factor receptor 2 (FGFR-2) (EC 2.7.10.1) (K-sam) (KGFR) (Keratinocyte growth factor receptor) (CD antigen CD332)	RTK	-1.23
P08514	Integrin alpha-IIb (GPalpha IIb) (GPIIb) (Platelet membrane glycoprotein IIb) (CD antigen CD41) [Cleaved into: Integrin alpha-IIb heavy chain; Integrin alpha-IIb light chain, form 1; Integrin alpha-IIb light chain, form 2]	SP	-1.24
Q15389	Angiopoietin-1 (ANG-1)	SP	-1.24
Q8N119	Matrix metalloproteinase-21 (MMP-21) (EC 3.4.24.-)	SP	-1.25
P04629	High affinity nerve growth factor receptor (EC 2.7.10.1) (Neurotrophic tyrosine kinase receptor type 1) (TRK1-transforming tyrosine kinase protein) (Tropomyosin-related kinase A) (Tyrosine kinase receptor) (Tyrosine kinase receptor A) (Trk-A) (gp140trk) (p140-TrkA)	RTK	-1.26
P54289	Voltage-dependent calcium channel subunit alpha-2/delta-1 (Voltage-gated calcium channel subunit alpha-2/delta-1) [Cleaved into: Voltage-dependent calcium channel subunit alpha-2-1; Voltage-dependent calcium channel subunit delta-1]	SP	-1.26
P15260	Interferon gamma receptor 1 (IFN-gamma receptor 1) (IFN-gamma-R1) (CDw119) (CD antigen CD119)	SP	-1.27
P39086	Glutamate receptor ionotropic, kainate 1 (GluK1) (Excitatory amino acid receptor 3) (EAA3) (Glutamate receptor 5) (GluR-5) (GluR5)	SP	-1.27
P01024	Complement C3 (C3 and PZP-like alpha-2-macroglobulin domain-containing protein 1) [Cleaved into: Complement C3 beta chain; Complement C3 alpha chain; C3a anaphylatoxin; Acylation stimulating protein (ASP) (C3adesArg); Complement C3b alpha' chain; Complement C3c alpha' chain fragment 1; Complement C3dg fragment; Complement C3g fragment; Complement C3d fragment; Complement C3f fragment; Complement C3c alpha' chain fragment 2]	SP	-1.29
P23416	Glycine receptor subunit alpha-2	SP	-1.30
P16234	Platelet-derived growth factor receptor alpha (PDGF-R-alpha) (PDGFR-alpha) (EC 2.7.10.1) (Alpha platelet-derived growth factor receptor) (Alpha-type platelet-derived growth factor receptor) (CD140 antigen-like family member A) (CD140a antigen) (Platelet-derived growth factor alpha receptor) (Platelet-derived growth factor receptor 2) (PDGFR-2) (CD antigen CD140a)	RTK	-1.30
Q9NY72	Sodium channel subunit beta-3	SP	-1.31
P45452	Collagenase 3 (EC 3.4.24.-) (Matrix metalloproteinase-13) (MMP-13)	SP	-1.31
O00253	Agouti-related protein	SP	-1.32
Q01344	Interleukin-5 receptor subunit alpha (IL-5 receptor subunit alpha) (IL-5R subunit alpha) (IL-5R-alpha) (IL-5RA) (CDw125) (CD antigen CD125)	SP	-1.33
Q13332	Receptor-type tyrosine-protein phosphatase S (R-PTP-S) (EC 3.1.3.48) (Receptor-type tyrosine-protein phosphatase sigma) (R-PTP-sigma)	SP	-1.33
P12318	Low affinity immunoglobulin gamma Fc region receptor II-a (IgG Fc receptor II-a) (CDw32) (Fc-gamma RII-a) (Fc-gamma-RIIa) (FcRII-a) (CD antigen CD32)	SP	-1.33
P12821	Angiotensin-converting enzyme (ACE) (EC 3.2.1.-) (EC 3.4.15.1) (Dipeptidyl carboxypeptidase I) (Kininase II) (CD antigen CD143) [Cleaved into: Angiotensin-converting enzyme, soluble form]	SP	-1.34
P09237	Matrilysin (EC 3.4.24.23) (Matrin) (Matrix metalloproteinase-7) (MMP-7) (Pump-1 protease) (Uterine metalloproteinase)	SP	-1.35
P02768	Serum albumin	SP	-1.35
P00533	Epidermal growth factor receptor (EC 2.7.10.1) (Proto-oncogene c-ErbB-1) (Receptor tyrosine-protein kinase erbB-1)	RTK	-1.35
P25774	Cathepsin S (EC 3.4.22.27)	SP	-1.36
P06126	T-cell surface glycoprotein CD1a (T-cell surface antigen T6/Leu-6) (hTa1 thymocyte antigen) (CD antigen CD1a)	SP	-1.36
P00746	Complement factor D (EC 3.4.21.46) (Adipsin) (C3 convertase activator) (Properdin factor D)	SP	-1.36
P14679	Tyrosinase (EC 1.14.18.1) (LB24-AB) (Monophenol monooxygenase) (SK29-AB) (Tumor rejection antigen AB)	SP	-1.37
Q95460	Major histocompatibility complex class I-related gene protein (MHC class I-related gene	SP	-1.38

	protein) (Class I histocompatibility antigen-like protein)		
P01009	Alpha-1-antitrypsin (Alpha-1 protease inhibitor) (Alpha-1-antiproteinase) (Serpin A1) [Cleaved into: Short peptide from AAT (SPAAT)]	SP	-1.40
P05362	Intercellular adhesion molecule 1 (ICAM-1) (Major group rhinovirus receptor) (CD antigen CD54)	SP	-1.40
P16581	E-selectin (CD62 antigen-like family member E) (Endothelial leukocyte adhesion molecule 1) (ELAM-1) (Leukocyte-endothelial cell adhesion molecule 2) (LECAM2) (CD antigen CD62E)	SP	-1.40
Q13255	Metabotropic glutamate receptor 1 (mGluR1)	GPCR	-1.41
O95264	5-hydroxytryptamine receptor 3B (5-HT3-B) (5-HT3B) (Serotonin receptor 3B)	SP	-1.41
P25116	Proteinase-activated receptor 1 (PAR-1) (Coagulation factor II receptor) (Thrombin receptor)	GPCR	-1.41
Q12879	Glutamate receptor ionotropic, NMDA 2A (GluN2A) (Glutamate [NMDA] receptor subunit epsilon-1) (N-methyl D-aspartate receptor subtype 2A) (NMDAR2A) (NR2A) (hNR2A)	SP	-1.42
P08069	Insulin-like growth factor 1 receptor (EC 2.7.10.1) (Insulin-like growth factor I receptor) (IGF-I receptor) (CD antigen CD221) [Cleaved into: Insulin-like growth factor 1 receptor alpha chain; Insulin-like growth factor 1 receptor beta chain]	RTK	-1.42
P16471	Prolactin receptor (PRL-R)	SP	-1.43
P00750	Tissue-type plasminogen activator (t-PA) (t-plasminogen activator) (tPA) (EC 3.4.21.68) (Alteplase) (Reteplase) [Cleaved into: Tissue-type plasminogen activator chain A; Tissue-type plasminogen activator chain B]	SP	-1.45
Q9Y5R2	Matrix metalloproteinase-24 (MMP-24) (EC 3.4.24.-) (Membrane-type matrix metalloproteinase 5) (MT-MMP 5) (MTMMP5) (Membrane-type-5 matrix metalloproteinase) (MT5-MMP) (MT5MMP) [Cleaved into: Processed matrix metalloproteinase-24]	SP	-1.47
Q16552	Interleukin-17A (IL-17) (IL-17A) (Cytotoxic T-lymphocyte-associated antigen 8) (CTLA-8)	SP	-1.47
P08581	Hepatocyte growth factor receptor (HGF receptor) (EC 2.7.10.1) (HGF/SF receptor) (Proto-oncogene c-Met) (Scatter factor receptor) (SF receptor) (Tyrosine-protein kinase Met)	RTK	-1.49
P06213	Insulin receptor (IR) (EC 2.7.10.1) (CD antigen CD220) [Cleaved into: Insulin receptor subunit alpha; Insulin receptor subunit beta]	RTK	-1.50
O60391	Glutamate receptor ionotropic, NMDA 3B (GluN3B) (N-methyl-D-aspartate receptor subtype 3B) (NMDAR3B) (NR3B)	SP	-1.50
P04626	Receptor tyrosine-protein kinase erbB-2 (EC 2.7.10.1) (Metastatic lymph node gene 19 protein) (MLN 19) (Proto-oncogene Neu) (Proto-oncogene c-ErbB-2) (Tyrosine kinase-type cell surface receptor HER2) (p185erbB2) (CD antigen CD340)	RTK	-1.50
O60896	Receptor activity-modifying protein 3 (Calcitonin-receptor-like receptor activity-modifying protein 3) (CRLR activity-modifying protein 3)	SP	-1.50
P43489	Tumor necrosis factor receptor superfamily member 4 (ACT35 antigen) (OX40L receptor) (TAX transcriptionally-activated glycoprotein 1 receptor) (CD antigen CD134)	SP	-1.50
P05121	Plasminogen activator inhibitor 1 (PAI) (PAI-1) (Endothelial plasminogen activator inhibitor) (Serpin E1)	SP	-1.51
P20273	B-cell receptor CD22 (B-lymphocyte cell adhesion molecule) (BL-CAM) (Sialic acid-binding Ig-like lectin 2) (Siglec-2) (T-cell surface antigen Leu-14) (CD antigen CD22)	SP	-1.52
Q05586	Glutamate receptor ionotropic, NMDA 1 (GluN1) (Glutamate [NMDA] receptor subunit zeta-1) (N-methyl-D-aspartate receptor subunit NR1) (NMD-R1)	SP	-1.52
P12314	High affinity immunoglobulin gamma Fc receptor I (IgG Fc receptor I) (Fc-gamma RI) (FcRI) (Fc-gamma RIA) (FcgammaRIa) (CD antigen CD64)	SP	-1.52
Q92637	High affinity immunoglobulin gamma Fc receptor IB (IgG Fc receptor IB) (Fc-gamma RIB) (FcRIB) (hFcgammaRIB)	SP	-1.52
P08253	72 kDa type IV collagenase (EC 3.4.24.24) (72 kDa gelatinase) (Gelatinase A) (Matrix metalloproteinase-2) (MMP-2) (TBE-1) [Cleaved into: PEX]	SP	-1.53
P17948	Vascular endothelial growth factor receptor 1 (VEGFR-1) (EC 2.7.10.1) (Fms-like tyrosine kinase 1) (FLT-1) (Tyrosine-protein kinase FRT) (Tyrosine-protein kinase receptor FLT) (FLT) (Vascular permeability factor receptor)	RTK	-1.55
P14784	Interleukin-2 receptor subunit beta (IL-2 receptor subunit beta) (IL-2R subunit beta) (IL-2RB) (High affinity IL-2 receptor subunit beta) (p70-75) (p75) (CD antigen CD122)	SP	-1.56
P17787	Neuronal acetylcholine receptor subunit beta-2	SP	-1.56
P31995	Low affinity immunoglobulin gamma Fc region receptor II-c (IgG Fc receptor II-c) (CDw32) (Fc-gamma RII-c) (Fc-gamma-RIIc) (FcRII-c) (CD antigen CD32)	SP	-1.57
P31994	Low affinity immunoglobulin gamma Fc region receptor II-b (IgG Fc receptor II-b) (CDw32) (Fc-gamma RII-b) (Fc-gamma-RIIb) (FcRII-b) (CD antigen CD32)	SP	-1.57
O60882	Matrix metalloproteinase-20 (MMP-20) (EC 3.4.24.-) (Enamel metalloproteinase) (Enamelysin)	SP	-1.57
Q9NR97	Toll-like receptor 8 (CD antigen CD288)	SP	-1.58
P22748	Carbonic anhydrase 4 (EC 4.2.1.1) (Carbonate dehydratase IV) (Carbonic anhydrase IV) (CA-IV)	SP	-1.58
P35916	Vascular endothelial growth factor receptor 3 (VEGFR-3) (EC 2.7.10.1) (Fms-like tyrosine kinase 4) (FLT-4) (Tyrosine-protein kinase receptor FLT4)	RTK	-1.59

P12259	Coagulation factor V (Activated protein C cofactor) (Proaccelerin, labile factor) [Cleaved into: Coagulation factor V heavy chain; Coagulation factor V light chain]	SP	-1.59
Q16568	Cocaine- and amphetamine-regulated transcript protein [Cleaved into: CART(1-39); CART(42-89)]	SP	-1.60
P11362	Fibroblast growth factor receptor 1 (FGFR-1) (EC 2.7.10.1) (Basic fibroblast growth factor receptor 1) (BFGFR) (bFGF-R-1) (Fms-like tyrosine kinase 2) (FLT-2) (N-sam) (Proto-oncogene c-Fgr) (CD antigen CD331)	RTK	-1.60
P08185	Corticosteroid-binding globulin (CBG) (Serpin A6) (Transcortin)	SP	-1.61
P48169	Gamma-aminobutyric acid receptor subunit alpha-4 (GABA(A) receptor subunit alpha-4)	SP	-1.62
P02741	C-reactive protein [Cleaved into: C-reactive protein(1-205)]	SP	-1.62
P37023	Serine/threonine-protein kinase receptor R3 (SKR3) (EC 2.7.11.30) (Activin receptor-like kinase 1) (ALK-1) (TGF-B superfamily receptor type I) (TSR-I)	SP	-1.62
P29279	Connective tissue growth factor (CCN family member 2) (Hypertrophic chondrocyte-specific protein 24) (Insulin-like growth factor-binding protein 8) (IBP-8) (IGF-binding protein 8) (IGFBP-8)	SP	-1.63
P41439	Folate receptor gamma (FR-gamma) (Folate receptor 3)	SP	-1.63
P41180	Extracellular calcium-sensing receptor (CaSR) (Parathyroid cell calcium-sensing receptor 1) (PCaR1)	GPCR	-1.64
P06858	Lipoprotein lipase (LPL) (EC 3.1.1.34)	SP	-1.65
P09871	Complement C1s subcomponent (EC 3.4.21.42) (C1 esterase) (Complement component 1 subcomponent s) [Cleaved into: Complement C1s subcomponent heavy chain; Complement C1s subcomponent light chain]	SP	-1.66
P00749	Urokinase-type plasminogen activator (U-plasminogen activator) (uPA) (EC 3.4.21.73) [Cleaved into: Urokinase-type plasminogen activator long chain A; Urokinase-type plasminogen activator short chain A; Urokinase-type plasminogen activator chain B]	SP	-1.66
P06881	Calcitonin gene-related peptide 1 (Alpha-type CGRP) (Calcitonin gene-related peptide I) (CGRP-I)	SP	-1.67
P01258	Calcitonin [Cleaved into: Calcitonin; Katacalcin (Calcitonin carboxyl-terminal peptide) (CCP) (PDN-21)]	SP	-1.67
P43220	Glucagon-like peptide 1 receptor (GLP-1 receptor) (GLP-1-R) (GLP-1R)	GPCR	-1.68
P20138	Myeloid cell surface antigen CD33 (Sialic acid-binding Ig-like lectin 3) (Siglec-3) (gp67) (CD antigen CD33)	SP	-1.68
P51511	Matrix metalloproteinase-15 (MMP-15) (EC 3.4.24.-) (Membrane-type matrix metalloproteinase 2) (MT-MMP 2) (MTMMP2) (Membrane-type-2 matrix metalloproteinase) (MT2-MMP) (MT2MMP) (SMCP-2)	SP	-1.68
P29459	Interleukin-12 subunit alpha (IL-12A) (Cytotoxic lymphocyte maturation factor 35 kDa subunit) (CLMF p35) (IL-12 subunit p35) (NK cell stimulatory factor chain 1) (NKSF1)	SP	-1.69
P23284	Peptidyl-prolyl cis-trans isomerase B (PPIase B) (EC 5.2.1.8) (CYP-S1) (Cyclophilin B) (Rotamase B) (S-cyclophilin) (SCYLP)	SP	-1.70
P00740	Coagulation factor IX (EC 3.4.21.22) (Christmas factor) (Plasma thromboplastin component) (PTC) [Cleaved into: Coagulation factor IXa light chain; Coagulation factor IXa heavy chain]	SP	-1.70
O14786	Neuropilin-1 (Vascular endothelial cell growth factor 165 receptor) (CD antigen CD304)	RTK	-1.71
P05164	Myeloperoxidase (MPO) (EC 1.11.2.2) [Cleaved into: Myeloperoxidase; 89 kDa myeloperoxidase; 84 kDa myeloperoxidase; Myeloperoxidase light chain; Myeloperoxidase heavy chain]	SP	-1.72
P23945	Follicle-stimulating hormone receptor (FSH-R) (Follitropin receptor)	GPCR	-1.73
P22455	Fibroblast growth factor receptor 4 (FGFR-4) (EC 2.7.10.1) (CD antigen CD334)	RTK	-1.74
Q9UHF1	Epidermal growth factor-like protein 7 (EGF-like protein 7) (Multiple epidermal growth factor-like domains protein 7) (Multiple EGF-like domains protein 7) (NOTCH4-like protein) (Vascular endothelial statin) (VE-statin) (Zneu1)	SP	-1.75
P20594	Atrial natriuretic peptide receptor 2 (EC 4.6.1.2) (Atrial natriuretic peptide receptor type B) (ANP-B) (ANPR-B) (NPR-B) (Guanylate cyclase B) (GC-B)	SP	-1.76
Q13224	Glutamate receptor ionotropic, NMDA 2B (GluN2B) (Glutamate [NMDA] receptor subunit epsilon-2) (N-methyl D-aspartate receptor subtype 2B) (NMDAR2B) (NR2B) (N-methyl-D-aspartate receptor subunit 3) (NR3) (hNR3)	SP	-1.78
P00451	Coagulation factor VIII (Antihemophilic factor) (AHF) (Procoagulant component) [Cleaved into: Factor VIIIa heavy chain, 200 kDa isoform; Factor VIIIa heavy chain, 92 kDa isoform; Factor VIII B chain; Factor VIIIa light chain]	SP	-1.78
P39900	Macrophage metalloelastase (MME) (EC 3.4.24.65) (Macrophage elastase) (ME) (hME) (Matrix metalloproteinase-12) (MMP-12)	SP	-1.79
P42081	T-lymphocyte activation antigen CD86 (Activation B7-2 antigen) (B70) (BU63) (CTLA-4 counter-receptor B7.2) (FUN-1) (CD antigen CD86)	SP	-1.80
P08246	Neutrophil elastase (EC 3.4.21.37) (Bone marrow serine protease) (Elastase-2) (Human leukocyte elastase) (HLE) (Medullasin) (PMN elastase)	SP	-1.80

P24046	Gamma-aminobutyric acid receptor subunit rho-1 (GABA(A) receptor subunit rho-1) (GABA(C) receptor)	SP	-1.80
P08254	Stromelysin-1 (SL-1) (EC 3.4.24.17) (Matrix metalloproteinase-3) (MMP-3) (Transin-1)	SP	-1.80
P04054	Phospholipase A2 (EC 3.1.1.4) (Group IB phospholipase A2) (Phosphatidylcholine 2-acylhydrolase 1B)	SP	-1.80
P08648	Integrin alpha-5 (CD49 antigen-like family member E) (Fibronectin receptor subunit alpha) (Integrin alpha-F) (VLA-5) (CD antigen CD49e) [Cleaved into: Integrin alpha-5 heavy chain; Integrin alpha-5 light chain]	SP	-1.81
P15692	Vascular endothelial growth factor A (VEGF-A) (Vascular permeability factor) (VPF)	SP	-1.81
O15399	Glutamate receptor ionotropic, NMDA 2D (GluN2D) (EB11) (Glutamate [NMDA] receptor subunit epsilon-4) (N-methyl D-aspartate receptor subtype 2D) (NMDAR2D) (NR2D)	SP	-1.82
P78334	Gamma-aminobutyric acid receptor subunit epsilon (GABA(A) receptor subunit epsilon)	SP	-1.82
P04275	von Willebrand factor (vWF) [Cleaved into: von Willebrand antigen 2 (von Willebrand antigen II)]	SP	-1.83
P01008	Antithrombin-III (ATIII) (Serpin C1)	SP	-1.83
P19961	Alpha-amylase 2B (EC 3.2.1.1) (1,4-alpha-D-glucan glucanohydrolase 2B) (Carcinoid alpha-amylase)	SP	-1.87
P04746	Pancreatic alpha-amylase (PA) (EC 3.2.1.1) (1,4-alpha-D-glucan glucanohydrolase)	SP	-1.87
P35354	Prostaglandin G/H synthase 2 (EC 1.14.99.1) (Cyclooxygenase-2) (COX-2) (PHS II) (Prostaglandin H2 synthase 2) (PGH synthase 2) (PGHS-2) (Prostaglandin-endoperoxide synthase 2)	SP	-1.89
Q9ULZ9	Matrix metalloproteinase-17 (MMP-17) (EC 3.4.24.-) (Membrane-type matrix metalloproteinase 4) (MT-MMP 4) (MTMMP4) (Membrane-type-4 matrix metalloproteinase) (MT4-MMP) (MT4MMP)	SP	-1.89
Q9NPF7	Interleukin-23 subunit alpha (IL-23 subunit alpha) (IL-23-A) (Interleukin-23 subunit p19) (IL-23p19)	SP	-1.91
Q13477	Mucosal addressin cell adhesion molecule 1 (MAdCAM-1) (hMAdCAM-1)	SP	-1.91
P14867	Gamma-aminobutyric acid receptor subunit alpha-1 (GABA(A) receptor subunit alpha-1)	SP	-1.91
P35968	Vascular endothelial growth factor receptor 2 (VEGFR-2) (EC 2.7.10.1) (Fetal liver kinase 1) (FLK-1) (Kinase insert domain receptor) (KDR) (Protein-tyrosine kinase receptor flk-1) (CD antigen CD309)	RTK	-1.92
P01133	Pro-epidermal growth factor (EGF) [Cleaved into: Epidermal growth factor (Urogastrone)]	SP	-1.93
Q8N1C3	Gamma-aminobutyric acid receptor subunit gamma-1 (GABA(A) receptor subunit gamma-1)	SP	-1.95
O60895	Receptor activity-modifying protein 2 (Calcitonin-receptor-like receptor activity-modifying protein 2) (CRLR activity-modifying protein 2)	SP	-1.95
P14780	Matrix metalloproteinase-9 (MMP-9) (EC 3.4.24.35) (92 kDa gelatinase) (92 kDa type IV collagenase) (Gelatinase B) (GELB) [Cleaved into: 67 kDa matrix metalloproteinase-9; 82 kDa matrix metalloproteinase-9]	SP	-1.96
P03956	Interstitial collagenase (EC 3.4.24.7) (Fibroblast collagenase) (Matrix metalloproteinase-1) (MMP-1) [Cleaved into: 22 kDa interstitial collagenase; 27 kDa interstitial collagenase]	SP	-1.97
P02766	Transthyretin (ATTR) (Prealbumin) (TBPA)	SP	-1.98
P0DJD8	Pepsin A-3 (EC 3.4.23.1) (Pepsinogen-3)	SP	-1.98
P0DJD9	Pepsin A-5 (EC 3.4.23.1) (Pepsinogen-5)	SP	-1.98
P10696	Alkaline phosphatase, placental-like (EC 3.1.3.1) (ALP-1) (Alkaline phosphatase Nagao isozyme) (Germ cell alkaline phosphatase) (GCAP) (Placental alkaline phosphatase-like) (PLAP-like)	SP	-1.99
P32881	Interferon alpha-8 (IFN-alpha-8) (Interferon alpha-B) (LeIF B) (Interferon alpha-B2)	SP	-1.99
P08709	Coagulation factor VII (EC 3.4.21.21) (Proconvertin) (Serum prothrombin conversion accelerator) (SPCA) (Eptacog alfa) [Cleaved into: Factor VII light chain; Factor VII heavy chain]	SP	-2.00
P07333	Macrophage colony-stimulating factor 1 receptor (CSF-1 receptor) (CSF-1-R) (CSF-1R) (M-CSF-R) (EC 2.7.10.1) (Proto-oncogene c-Fms) (CD antigen CD115)	RTK	-2.01
P18507	Gamma-aminobutyric acid receptor subunit gamma-2 (GABA(A) receptor subunit gamma-2)	SP	-2.01
P05067	Amyloid beta A4 protein (ABPP) (APPI) (APP) (Alzheimer disease amyloid protein) (Cerebral vascular amyloid peptide) (CVAP) (PreA4) (Protease nexin-II) (PN-II) [Cleaved into: N-APP; Soluble APP-alpha (S-APP-alpha); Soluble APP-beta (S-APP-beta); C99; Beta-amyloid protein 42 (Beta-APP42); Beta-amyloid protein 40 (Beta-APP40); C83; P3(42); P3(40); C80; Gamma-secretase C-terminal fragment 59 (Amyloid intracellular domain 59) (AICD-59) (AID(59)) (Gamma-CTF(59)); Gamma-secretase C-terminal fragment 57 (Amyloid intracellular domain 57) (AICD-57) (AID(57)) (Gamma-CTF(57)); Gamma-secretase C-terminal fragment 50 (Amyloid intracellular domain 50) (AICD-50) (AID(50)) (Gamma-	SP	-2.02

	CTF(50)); C31]		
O14763	Tumor necrosis factor receptor superfamily member 10B (Death receptor 5) (TNF-related apoptosis-inducing ligand receptor 2) (TRAIL receptor 2) (TRAIL-R2) (CD antigen CD262)	SP	-2.02
P00797	Renin (EC 3.4.23.15) (Angiotensinogenase)	SP	-2.03
P14207	Folate receptor beta (FR-beta) (Folate receptor 2) (Folate receptor, fetal/placental) (Placental folate-binding protein) (FBP)	SP	-2.03
P26010	Integrin beta-7 (Gut homing receptor beta subunit)	SP	-2.03
P30988	Calcitonin receptor (CT-R)	GPCR	-2.04
P30926	Neuronal acetylcholine receptor subunit beta-4	SP	-2.04
P48167	Glycine receptor subunit beta (Glycine receptor 58 kDa subunit)	SP	-2.05
Q9UHC9	Niemann-Pick C1-like protein 1	SP	-2.07
P07225	Vitamin K-dependent protein S	SP	-2.07
P38484	Interferon gamma receptor 2 (IFN-gamma receptor 2) (IFN-gamma-R2) (Interferon gamma receptor accessory factor 1) (AF-1) (Interferon gamma transducer 1)	SP	-2.07
P36955	Pigment epithelium-derived factor (PEDF) (Cell proliferation-inducing gene 35 protein) (EPC-1) (Serpin F1)	SP	-2.09
Q9UBS5	Gamma-aminobutyric acid type B receptor subunit 1 (GABA-B receptor 1) (GABA-B-R1) (GABA-BR1) (GABABR1) (Gb1)	GPCR	-2.12
P08842	Steryl-sulfatase (EC 3.1.6.2) (Arylsulfatase C) (ASC) (Steroid sulfatase) (Steryl-sulfate sulfohydrolase)	SP	-2.13
Q9UN88	Gamma-aminobutyric acid receptor subunit theta (GABA(A) receptor subunit theta)	SP	-2.14
P12319	High affinity immunoglobulin epsilon receptor subunit alpha (Fc-epsilon RI-alpha) (FcERI) (IgE Fc receptor subunit alpha)	SP	-2.15
P20645	Cation-dependent mannose-6-phosphate receptor (CD Man-6-P receptor) (CD-MPR) (46 kDa mannose 6-phosphate receptor) (MPR 46)	SP	-2.19
P31358	CAMPATH-1 antigen (CDw52) (Cambridge pathology 1 antigen) (Epididymal secretory protein E5) (Human epididymis-specific protein 5) (He5) (CD antigen CD52)	SP	-2.19
Q16445	Gamma-aminobutyric acid receptor subunit alpha-6 (GABA(A) receptor subunit alpha-6)	SP	-2.24
P10909	Clusterin (Aging-associated gene 4 protein) (Apolipoprotein J) (Apo-J) (Complement cytolysis inhibitor) (CLI) (Complement-associated protein SP-40,40) (Ku70-binding protein 1) (NA1/NA2) (Testosterone-repressed prostate message 2) (TRPM-2) [Cleaved into: Clusterin beta chain (ApoJalpha) (Complement cytolysis inhibitor a chain); Clusterin alpha chain (ApoJbeta) (Complement cytolysis inhibitor b chain)]	SP	-2.24
Q99928	Gamma-aminobutyric acid receptor subunit gamma-3 (GABA(A) receptor subunit gamma-3)	SP	-2.25
P05546	Heparin cofactor 2 (Heparin cofactor II) (HC-II) (Protease inhibitor leuserpin-2) (HLS2) (Serpin D1)	SP	-2.27
P02656	Apolipoprotein C-III (Apo-CIII) (ApoC-III) (Apolipoprotein C3)	SP	-2.32
P41594	Metabotropic glutamate receptor 5 (mGluR5)	GPCR	-2.33
P35225	Interleukin-13 (IL-13)	SP	-2.33
P13500	C-C motif chemokine 2 (HC11) (Monocyte chemoattractant protein 1) (Monocyte chemotactic and activating factor) (MCAF) (Monocyte chemotactic protein 1) (MCP-1) (Monocyte secretory protein JE) (Small-inducible cytokine A2)	SP	-2.37
P30273	High affinity immunoglobulin epsilon receptor subunit gamma (Fc receptor gamma-chain) (FcRgamma) (Fc-epsilon RI-gamma) (IgE Fc receptor subunit gamma) (FceRI gamma)	SP	-2.37
P16410	Cytotoxic T-lymphocyte protein 4 (Cytotoxic T-lymphocyte-associated antigen 4) (CTLA-4) (CD antigen CD152)	SP	-2.39
P08138	Tumor necrosis factor receptor superfamily member 16 (Gp80-LNGFR) (Low affinity neurotrophin receptor p75NTR) (Low-affinity nerve growth factor receptor) (NGF receptor) (p75 ICD) (CD antigen CD271)	SP	-2.39
P47869	Gamma-aminobutyric acid receptor subunit alpha-2 (GABA(A) receptor subunit alpha-2)	SP	-2.41
P09238	Stromelysin-2 (SL-2) (EC 3.4.24.22) (Matrix metalloproteinase-10) (MMP-10) (Transin-2)	SP	-2.41
P02818	Osteocalcin (Bone Gla protein) (BGP) (Gamma-carboxyglutamic acid-containing protein)	SP	-2.41
P01031	Complement C5 (C3 and PZP-like alpha-2-macroglobulin domain-containing protein 4) [Cleaved into: Complement C5 beta chain; Complement C5 alpha chain; C5a anaphylatoxin; Complement C5 alpha' chain]	SP	-2.42
P55157	Microsomal triglyceride transfer protein large subunit	SP	-2.43
P09603	Macrophage colony-stimulating factor 1 (CSF-1) (M-CSF) (MCSF) (Lanimostim) [Cleaved into: Processed macrophage colony-stimulating factor 1]	SP	-2.46

Q9GZZ6	Neuronal acetylcholine receptor subunit alpha-10 (Nicotinic acetylcholine receptor subunit alpha-10) (NACHR alpha-10)	SP	-2.48
P14778	Interleukin-1 receptor type 1 (IL-1R-1) (IL-1RT-1) (IL-1RT1) (CD121 antigen-like family member A) (Interleukin-1 receptor alpha) (IL-1R-alpha) (Interleukin-1 receptor type I) (p80) (CD antigen CD121a) [Cleaved into: Interleukin-1 receptor type 1, membrane form (mIL-1R1) (mIL-1RI); Interleukin-1 receptor type 1, soluble form (sIL-1R1) (sIL-1RI)]	SP	-2.50
Q99062	Granulocyte colony-stimulating factor receptor (G-CSF receptor) (G-CSF-R) (CD antigen CD114)	SP	-2.54
P10721	Mast/stem cell growth factor receptor Kit (SCFR) (EC 2.7.10.1) (Piebald trait protein) (PBT) (Proto-oncogene c-Kit) (Tyrosine-protein kinase Kit) (p145 c-kit) (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) (CD antigen CD117)	RTK	-2.58
P09619	Platelet-derived growth factor receptor beta (PDGF-R-beta) (PDGFR-beta) (EC 2.7.10.1) (Beta platelet-derived growth factor receptor) (Beta-type platelet-derived growth factor receptor) (CD140 antigen-like family member B) (Platelet-derived growth factor receptor 1) (PDGFR-1) (CD antigen CD140b)	RTK	-2.59
P15391	B-lymphocyte antigen CD19 (B-lymphocyte surface antigen B4) (Differentiation antigen CD19) (T-cell surface antigen Leu-12) (CD antigen CD19)	SP	-2.64
P09693	T-cell surface glycoprotein CD3 gamma chain (T-cell receptor T3 gamma chain) (CD antigen CD3g)	SP	-2.66
P08311	Cathepsin G (CG) (EC 3.4.21.20)	SP	-2.67
P00747	Plasminogen (EC 3.4.21.7) [Cleaved into: Plasmin heavy chain A; Activation peptide; Angiostatin; Plasmin heavy chain A, short form; Plasmin light chain B]	SP	-2.69
P26951	Interleukin-3 receptor subunit alpha (IL-3 receptor subunit alpha) (IL-3R subunit alpha) (IL-3R-alpha) (IL-3RA) (CD antigen CD123)	SP	-2.71
P28476	Gamma-aminobutyric acid receptor subunit rho-2 (GABA(A) receptor subunit rho-2) (GABA(C) receptor)	SP	-2.72
O75899	Gamma-aminobutyric acid type B receptor subunit 2 (GABA-B receptor 2) (GABA-B-R2) (GABA-BR2) (GABABR2) (Gb2) (G-protein coupled receptor 51) (HG20)	GPCR	-2.72
P32297	Neuronal acetylcholine receptor subunit alpha-3	SP	-2.73
P01374	Lymphotoxin-alpha (LT-alpha) (TNF-beta) (Tumor necrosis factor ligand superfamily member 1)	SP	-2.76
P04114	Apolipoprotein B-100 (Apo B-100) [Cleaved into: Apolipoprotein B-48 (Apo B-48)]	SP	-2.83
P19875	C-X-C motif chemokine 2 (Growth-regulated protein beta) (Gro-beta) (Macrophage inflammatory protein 2-alpha) (MIP2-alpha) [Cleaved into: GRO-beta(5-73) (GRO-beta-T) (Hematopoietic synergistic factor) (HSF) (SB-251353)]	SP	-2.86
Q99835	Smoothened homolog (SMO) (Protein Gx)	GPCR	-2.87
P16066	Atrial natriuretic peptide receptor 1 (EC 4.6.1.2) (Atrial natriuretic peptide receptor type A) (ANP-A) (ANPR-A) (NPR-A) (Guanylate cyclase A) (GC-A)	SP	-2.89
P07949	Proto-oncogene tyrosine-protein kinase receptor Ret (EC 2.7.10.1) (Cadherin family member 12) (Proto-oncogene c-Ret) [Cleaved into: Soluble RET kinase fragment; Extracellular cell-membrane anchored RET cadherin 120 kDa fragment]	RTK	-2.89
P14555	Phospholipase A2, membrane associated (EC 3.1.1.4) (GIIC sPLA2) (Group IIA phospholipase A2) (Non-pancreatic secretory phospholipase A2) (NPS-PLA2) (Phosphatidylcholine 2-acylhydrolase 2A)	SP	-2.90
P06276	Cholinesterase (EC 3.1.1.8) (Acylcholine acylhydrolase) (Butyrylcholine esterase) (Choline esterase II) (Pseudocholinesterase)	SP	-2.98
Q14416	Metabotropic glutamate receptor 2 (mGluR2)	GPCR	-2.99
P47871	Glucagon receptor (GL-R)	GPCR	-3.11
Q9NPA2	Matrix metalloproteinase-25 (MMP-25) (EC 3.4.24.-) (Leukolysin) (Membrane-type matrix metalloproteinase 6) (MT-MMP 6) (MTMMP6) (Membrane-type-6 matrix metalloproteinase) (MT6-MMP) (MT6MMP)	SP	-3.14
Q9H306	Matrix metalloproteinase-27 (MMP-27) (EC 3.4.24.-)	SP	-3.15
P22303	Acetylcholinesterase (AChE) (EC 3.1.1.7)	SP	-3.16
Q96NY8	Poliovirus receptor-related protein 4 (Ig superfamily receptor LNIR) (Nectin-4) [Cleaved into: Processed poliovirus receptor-related protein 4]	SP	-3.17
Q8NBP7	Proprotein convertase subtilisin/kexin type 9 (EC 3.4.21.-) (Neural apoptosis-regulated convertase 1) (NARC-1) (Proprotein convertase 9) (PC9) (Subtilisin/kexin-like protease PC9)	SP	-3.45
P23219	Prostaglandin G/H synthase 1 (EC 1.14.99.1) (Cyclooxygenase-1) (COX-1) (Prostaglandin H2 synthase 1) (PGH synthase 1) (PGHS-1) (PHS 1) (Prostaglandin-endoperoxide synthase 1)	SP	-4.10
P08908	5-hydroxytryptamine receptor 1A (5-HT-1A) (5-HT1A) (G-21) (Serotonin receptor 1A)	GPCR	N/A
P28222	5-hydroxytryptamine receptor 1B (5-HT-1B) (5-HT1B) (S12) (Serotonin 1D beta receptor) (5-HT-1D-beta) (Serotonin receptor 1B)	GPCR	N/A

P28221	5-hydroxytryptamine receptor 1D (5-HT-1D) (5-HT1D) (Serotonin 1D alpha receptor) (5-HT-1D-alpha) (Serotonin receptor 1D)	GPCR	N/A
P30939	5-hydroxytryptamine receptor 1F (5-HT-1F) (5-HT1F) (Serotonin receptor 1F)	GPCR	N/A
P28223	5-hydroxytryptamine receptor 2A (5-HT-2) (5-HT-2A) (Serotonin receptor 2A)	GPCR	N/A
P41595	5-hydroxytryptamine receptor 2B (5-HT-2B) (5-HT2B) (Serotonin receptor 2B)	GPCR	N/A
Q13639	5-hydroxytryptamine receptor 4 (5-HT-4) (5-HT4) (Serotonin receptor 4)	GPCR	N/A
P50406	5-hydroxytryptamine receptor 6 (5-HT-6) (5-HT6) (Serotonin receptor 6)	GPCR	N/A
P34969	5-hydroxytryptamine receptor 7 (5-HT-7) (5-HT7) (5-HT-X) (Serotonin receptor 7)	GPCR	N/A
P30542	Adenosine receptor A1	GPCR	N/A
P29274	Adenosine receptor A2a	GPCR	N/A
P29275	Adenosine receptor A2b	GPCR	N/A
P33765	Adenosine receptor A3	GPCR	N/A
P11229	Muscarinic acetylcholine receptor M1	GPCR	N/A
P08172	Muscarinic acetylcholine receptor M2	GPCR	N/A
P20309	Muscarinic acetylcholine receptor M3	GPCR	N/A
P08173	Muscarinic acetylcholine receptor M4	GPCR	N/A
P08912	Muscarinic acetylcholine receptor M5	GPCR	N/A
Q01718	Adrenocorticotropic hormone receptor (ACTH receptor) (ACTH-R) (Adrenocorticotropin receptor) (Melanocortin receptor 2) (MC2-R)	GPCR	N/A
P35348	Alpha-1A adrenergic receptor (Alpha-1A adrenoreceptor) (Alpha-1A adrenoceptor) (Alpha-1C adrenergic receptor) (Alpha-adrenergic receptor 1c)	GPCR	N/A
P35368	Alpha-1B adrenergic receptor (Alpha-1B adrenoreceptor) (Alpha-1B adrenoceptor)	GPCR	N/A
P25100	Alpha-1D adrenergic receptor (Alpha-1A adrenergic receptor) (Alpha-1D adrenoreceptor) (Alpha-1D adrenoceptor) (Alpha-adrenergic receptor 1a)	GPCR	N/A
P08913	Alpha-2A adrenergic receptor (Alpha-2 adrenergic receptor subtype C10) (Alpha-2A adrenoreceptor) (Alpha-2A adrenoceptor) (Alpha-2AAR)	GPCR	N/A
P18089	Alpha-2B adrenergic receptor (Alpha-2 adrenergic receptor subtype C2) (Alpha-2B adrenoreceptor) (Alpha-2B adrenoceptor) (Alpha-2BAR)	GPCR	N/A
P18825	Alpha-2C adrenergic receptor (Alpha-2 adrenergic receptor subtype C4) (Alpha-2C adrenoreceptor) (Alpha-2C adrenoceptor) (Alpha-2CAR)	GPCR	N/A
P08588	Beta-1 adrenergic receptor (Beta-1 adrenoreceptor) (Beta-1 adrenoceptor)	GPCR	N/A
P07550	Beta-2 adrenergic receptor (Beta-2 adrenoreceptor) (Beta-2 adrenoceptor)	GPCR	N/A
P13945	Beta-3 adrenergic receptor (Beta-3 adrenoreceptor) (Beta-3 adrenoceptor)	GPCR	N/A
P30556	Type-1 angiotensin II receptor (AT1AR) (AT1BR) (Angiotensin II type-1 receptor) (AT1)	GPCR	N/A
P50052	Type-2 angiotensin II receptor (Angiotensin II type-2 receptor) (AT2)	GPCR	N/A
P30411	B2 bradykinin receptor (B2R) (BK-2 receptor)	GPCR	N/A
P32238	Cholecystokinin receptor type A (CCK-A receptor) (CCK-AR) (Cholecystokinin-1 receptor) (CCK1-R)	GPCR	N/A
P32246	C-C chemokine receptor type 1 (C-C CKR-1) (CC-CKR-1) (CCR-1) (CCR1) (HM145) (LD78 receptor) (Macrophage inflammatory protein 1-alpha receptor) (MIP-1alpha-R) (RANTES-R) (CD antigen CD191)	GPCR	N/A
P41597	C-C chemokine receptor type 2 (C-C CKR-2) (CC-CKR-2) (CCR-2) (CCR2) (Monocyte chemoattractant protein 1 receptor) (MCP-1-R) (CD antigen CD192)	GPCR	N/A
P51677	C-C chemokine receptor type 3 (C-C CKR-3) (CC-CKR-3) (CCR-3) (CCR3) (CKR3) (Eosinophil eotaxin receptor) (CD antigen CD193)	GPCR	N/A
P51679	C-C chemokine receptor type 4 (C-C CKR-4) (CC-CKR-4) (CCR-4) (CCR4) (K5-5) (CD antigen CD194)	GPCR	N/A
P51681	C-C chemokine receptor type 5 (C-C CKR-5) (CC-CKR-5) (CCR-5) (CCR5) (CHEMR13) (HIV-1 fusion coreceptor) (CD antigen CD195)	GPCR	N/A
Q9Y271	Cysteinyl leukotriene receptor 1 (CysLTR1) (Cysteinyl leukotriene D4 receptor) (LTD4 receptor) (G-protein coupled receptor HG55) (HMTMF81)	GPCR	N/A
Q9NS75	Cysteinyl leukotriene receptor 2 (CysLTR2) (G-protein coupled receptor GPCR21) (hGPCR21) (G-protein coupled receptor HG57) (HPN321)	GPCR	N/A
P21554	Cannabinoid receptor 1 (CB-R) (CB1) (CANN6)	GPCR	N/A

P34972	Cannabinoid receptor 2 (CB-2) (CB2) (hCB2) (CX5)	GPCR	N/A
P25024	C-X-C chemokine receptor type 1 (CXC-R1) (CXCR-1) (CDw128a) (High affinity interleukin-8 receptor A) (IL-8R A) (IL-8 receptor type 1) (CD antigen CD181)	GPCR	N/A
P25025	C-X-C chemokine receptor type 2 (CXC-R2) (CXCR-2) (CDw128b) (GRO/MGSA receptor) (High affinity interleukin-8 receptor B) (IL-8R B) (IL-8 receptor type 2) (CD antigen CD182)	GPCR	N/A
P49682	C-X-C chemokine receptor type 3 (CXC-R3) (CXCR-3) (CKR-L2) (G protein-coupled receptor 9) (Interferon-inducible protein 10 receptor) (IP-10 receptor) (CD antigen CD183)	GPCR	N/A
P61073	C-X-C chemokine receptor type 4 (CXC-R4) (CXCR-4) (FB22) (Fusin) (HM89) (LCR1) (Leukocyte-derived seven transmembrane domain receptor) (LESTR) (NPYRL) (Stromal cell-derived factor 1 receptor) (SDF-1 receptor) (CD antigen CD184)	GPCR	N/A
P21728	D(1A) dopamine receptor (Dopamine D1 receptor)	GPCR	N/A
P14416	D(2) dopamine receptor (Dopamine D2 receptor)	GPCR	N/A
P35462	D(3) dopamine receptor (Dopamine D3 receptor)	GPCR	N/A
P21917	D(4) dopamine receptor (D(2C) dopamine receptor) (Dopamine D4 receptor)	GPCR	N/A
P21918	D(1B) dopamine receptor (D(5) dopamine receptor) (D1beta dopamine receptor) (Dopamine D5 receptor)	GPCR	N/A
O14842	Free fatty acid receptor 1 (G-protein coupled receptor 40)	GPCR	N/A
P32239	Gastrin/cholecystokinin type B receptor (CCK-B receptor) (CCK-BR) (Cholecystokinin-2 receptor) (CCK2-R)	GPCR	N/A
Q92847	Growth hormone secretagogue receptor type 1 (GHS-R) (GH-releasing peptide receptor) (GHRP) (Ghrelin receptor)	GPCR	N/A
P30968	Gonadotropin-releasing hormone receptor (GnRH receptor) (GnRH-R)	GPCR	N/A
Q96P88	Putative gonadotropin-releasing hormone II receptor (GnRH II receptor) (GnRH-II-R) (Type II GnRH receptor)	GPCR	N/A
Q8TDV5	Glucose-dependent insulinotropic receptor (G-protein coupled receptor 119)	GPCR	N/A
Q8TDU6	G-protein coupled bile acid receptor 1 (G-protein coupled receptor GPCR19) (hGPCR19) (Membrane-type receptor for bile acids) (M-BAR) (hBG37) (BG37)	GPCR	N/A
P30550	Gastrin-releasing peptide receptor (GRP-R) (GRP-preferring bombesin receptor)	GPCR	N/A
P35367	Histamine H1 receptor (H1R) (HH1R)	GPCR	N/A
P25021	Histamine H2 receptor (H2R) (HH2R) (Gastric receptor I)	GPCR	N/A
Q9Y5N1	Histamine H3 receptor (H3R) (HH3R) (G-protein coupled receptor 97)	GPCR	N/A
Q969F8	KiSS-1 receptor (KiSS-1R) (G-protein coupled receptor 54) (G-protein coupled receptor OT7T175) (hOT7T175) (Hypogonadotropin-1) (Kisspeptins receptor) (Metastin receptor)	GPCR	N/A
Q15722	Leukotriene B4 receptor 1 (LTB4-R 1) (LTB4-R1) (Chemoattractant receptor-like 1) (G-protein coupled receptor 16) (P2Y purinoceptor 7) (P2Y7)	GPCR	N/A
P41968	Melanocortin receptor 3 (MC3-R)	GPCR	N/A
P32245	Melanocortin receptor 4 (MC4-R)	GPCR	N/A
Q99705	Melanin-concentrating hormone receptor 1 (MCH receptor 1) (MCH-R1) (MCHR-1) (G-protein coupled receptor 24) (MCH-1R) (MCH1R) (MCHR) (SLC-1) (Somatostatin receptor-like protein)	GPCR	N/A
Q01726	Melanocyte-stimulating hormone receptor (MSH-R) (Melanocortin receptor 1) (MC1-R)	GPCR	N/A
O43193	Motilin receptor (G-protein coupled receptor 38)	GPCR	N/A
P48039	Melatonin receptor type 1A (Mel-1A-R) (Mel1a receptor)	GPCR	N/A
P49286	Melatonin receptor type 1B (Mel-1B-R) (Mel1b receptor)	GPCR	N/A
P25103	Substance-P receptor (SPR) (NK-1 receptor) (NK-1R) (Tachykinin receptor 1)	GPCR	N/A
P21452	Substance-K receptor (SKR) (NK-2 receptor) (NK-2R) (Neurokinin A receptor) (Tachykinin receptor 2)	GPCR	N/A
P29371	Neuromedin-K receptor (NKR) (NK-3 receptor) (NK-3R) (Neurokinin B receptor) (Tachykinin receptor 3)	GPCR	N/A
P25929	Neuropeptide Y receptor type 1 (NPY1-R)	GPCR	N/A
P49146	Neuropeptide Y receptor type 2 (NPY2-R) (NPY-Y2 receptor) (Y2 receptor)	GPCR	N/A
P50391	Neuropeptide Y receptor type 4 (NPY4-R) (Pancreatic polypeptide receptor 1) (PP1)	GPCR	N/A
Q15761	Neuropeptide Y receptor type 5 (NPY5-R) (NPY-Y5 receptor) (NPYY5-R) (Y5 receptor)	GPCR	N/A
P30989	Neurotensin receptor type 1 (NT-R-1) (NTR1) (High-affinity levocabastine-insensitive neurotensin receptor) (NTRH)	GPCR	N/A

O95665	Neurotensin receptor type 2 (NT-R-2) (NTR2) (Levocabastine-sensitive neurotensin receptor)	GPCR	N/A
P41143	Delta-type opioid receptor (D-OR-1) (DOR-1)	GPCR	N/A
P41145	Kappa-type opioid receptor (K-OR-1) (KOR-1)	GPCR	N/A
P35372	Mu-type opioid receptor (M-OR-1) (MOR-1) (Mu opiate receptor) (Mu opioid receptor) (MOP) (hMOP)	GPCR	N/A
O43613	Orexin receptor type 1 (Ox-1-R) (Ox1-R) (Ox1R) (Hypocretin receptor type 1)	GPCR	N/A
P30559	Oxytocin receptor (OT-R)	GPCR	N/A
P41231	P2Y purinoceptor 2 (P2Y2) (ATP receptor) (P2U purinoceptor 1) (P2U1) (P2U receptor 1) (Purinergic receptor)	GPCR	N/A
Q9H244	P2Y purinoceptor 12 (P2Y12) (ADP-glucose receptor) (ADPG-R) (P2T(AC)) (P2Y(AC)) (P2Y(cyc)) (P2Y12 platelet ADP receptor) (P2Y(ADP)) (SP1999)	GPCR	N/A
Q13258	Prostaglandin D2 receptor (PGD receptor) (PGD2 receptor) (Prostanoid DP receptor)	GPCR	N/A
P34995	Prostaglandin E2 receptor EP1 subtype (PGE receptor EP1 subtype) (PGE2 receptor EP1 subtype) (Prostanoid EP1 receptor)	GPCR	N/A
P43116	Prostaglandin E2 receptor EP2 subtype (PGE receptor EP2 subtype) (PGE2 receptor EP2 subtype) (Prostanoid EP2 receptor)	GPCR	N/A
P43115	Prostaglandin E2 receptor EP3 subtype (PGE receptor EP3 subtype) (PGE2 receptor EP3 subtype) (PGE2-R) (Prostanoid EP3 receptor)	GPCR	N/A
P35408	Prostaglandin E2 receptor EP4 subtype (PGE receptor EP4 subtype) (PGE2 receptor EP4 subtype) (Prostanoid EP4 receptor)	GPCR	N/A
P43088	Prostaglandin F2-alpha receptor (PGF receptor) (PGF2-alpha receptor) (Prostanoid FP receptor)	GPCR	N/A
P43119	Prostacyclin receptor (Prostaglandin I2 receptor) (PGI receptor) (PGI2 receptor) (Prostanoid IP receptor)	GPCR	N/A
P25105	Platelet-activating factor receptor (PAF-R) (PAFr)	GPCR	N/A
P21453	Sphingosine 1-phosphate receptor 1 (S1P receptor 1) (S1P1) (Endothelial differentiation G-protein coupled receptor 1) (Sphingosine 1-phosphate receptor Edg-1) (S1P receptor Edg-1) (CD antigen CD363)	GPCR	N/A
P30872	Somatostatin receptor type 1 (SS-1-R) (SS1-R) (SS1R) (SRIF-2)	GPCR	N/A
P30874	Somatostatin receptor type 2 (SS-2-R) (SS2-R) (SS2R) (SRIF-1)	GPCR	N/A
P32745	Somatostatin receptor type 3 (SS-3-R) (SS3-R) (SS3R) (SSR-28)	GPCR	N/A
P35346	Somatostatin receptor type 5 (SS-5-R) (SS5-R) (SS5R)	GPCR	N/A
P31391	Somatostatin receptor type 4 (SS-4-R) (SS4-R) (SS4R)	GPCR	N/A
P21731	Thromboxane A2 receptor (TXA2-R) (Prostanoid TP receptor)	GPCR	N/A
Q96RJ0	Trace amine-associated receptor 1 (TaR-1) (Trace amine receptor 1)	GPCR	N/A
P47901	Vasopressin V1b receptor (V1bR) (AVPR V1b) (AVPR V3) (Antidiuretic hormone receptor 1b) (Vasopressin V3 receptor)	GPCR	N/A
P37288	Vasopressin V1a receptor (V1aR) (AVPR V1a) (Antidiuretic hormone receptor 1a) (Vascular/hepatic-type arginine vasopressin receptor)	GPCR	N/A
P30518	Vasopressin V2 receptor (V2R) (AVPR V2) (Antidiuretic hormone receptor) (Renal-type arginine vasopressin receptor)	GPCR	N/A
Q6P2N6	Protein ADORA3, isoform 3	multi-TM	N/A
O95342	Bile salt export pump (ATP-binding cassette sub-family B member 11)	multi-TM	N/A
Q09428	ATP-binding cassette sub-family C member 8 (Sulfonylurea receptor 1)	multi-TM	N/A
O00767	Acyl-CoA desaturase (EC 1.14.19.1) (Delta(9)-desaturase) (Delta-9 desaturase) (Fatty acid desaturase) (Stearoyl-CoA desaturase)	multi-TM	N/A
Q08828	Adenylate cyclase type 1 (EC 4.6.1.1) (ATP pyrophosphate-lyase 1) (Adenylate cyclase type I) (Adenylyl cyclase 1) (Ca(2+)/calmodulin-activated adenylyl cyclase)	multi-TM	N/A
P20292	Arachidonate 5-lipoxygenase-activating protein (FLAP) (MK-886-binding protein)	multi-TM	N/A
Q8WW43	Gamma-secretase subunit APH-1B (APH-1b) (Aph-1beta) (Presenilin-stabilization factor-like)	multi-TM	N/A
P05023	Sodium/potassium-transporting ATPase subunit alpha-1 (Na(+)/K(+) ATPase alpha-1 subunit) (EC 3.6.3.9) (Sodium pump subunit alpha-1)	multi-TM	N/A
P98194	Calcium-transporting ATPase type 2C member 1 (ATPase 2C1) (EC 3.6.3.8) (ATP-dependent Ca(2+) pump PMR1)	multi-TM	N/A
P20648	Potassium-transporting ATPase alpha chain 1 (EC 3.6.3.10) (Gastric H(+)/K(+) ATPase subunit alpha) (Proton pump)	multi-TM	N/A
O00555	Voltage-dependent P/Q-type calcium channel subunit alpha-1A (Brain calcium channel I) (BI)	multi-TM	N/A

	(Calcium channel, L type, alpha-1 polypeptide isoform 4) (Voltage-gated calcium channel subunit alpha Cav2.1)		
Q00975	Voltage-dependent N-type calcium channel subunit alpha-1B (Brain calcium channel III) (BIII) (Calcium channel, L type, alpha-1 polypeptide isoform 5) (Voltage-gated calcium channel subunit alpha Cav2.2)	multi-TM	N/A
Q13936	Voltage-dependent L-type calcium channel subunit alpha-1C (Calcium channel, L type, alpha-1 polypeptide, isoform 1, cardiac muscle) (Voltage-gated calcium channel subunit alpha Cav1.2)	multi-TM	N/A
Q01668	Voltage-dependent L-type calcium channel subunit alpha-1D (Calcium channel, L type, alpha-1 polypeptide, isoform 2) (Voltage-gated calcium channel subunit alpha Cav1.3)	multi-TM	N/A
O60840	Voltage-dependent L-type calcium channel subunit alpha-1F (Voltage-gated calcium channel subunit alpha Cav1.4)	multi-TM	N/A
O43497	Voltage-dependent T-type calcium channel subunit alpha-1G (Cav3.1c) (NBR13) (Voltage-gated calcium channel subunit alpha Cav3.1)	multi-TM	N/A
O95180	Voltage-dependent T-type calcium channel subunit alpha-1H (Low-voltage-activated calcium channel alpha1 3.2 subunit) (Voltage-gated calcium channel subunit alpha Cav3.2)	multi-TM	N/A
Q9P0X4	Voltage-dependent T-type calcium channel subunit alpha-1I (Voltage-gated calcium channel subunit alpha Cav3.3) (Ca(v)3.3)	multi-TM	N/A
Q13698	Voltage-dependent L-type calcium channel subunit alpha-1S (Calcium channel, L type, alpha-1 polypeptide, isoform 3, skeletal muscle) (Voltage-gated calcium channel subunit alpha Cav1.1)	multi-TM	N/A
P49069	Calcium signal-modulating cyclophilin ligand (CAML)	multi-TM	N/A
Q06432	Voltage-dependent calcium channel gamma-1 subunit (Dihydropyridine-sensitive L-type, skeletal muscle calcium channel subunit gamma)	multi-TM	N/A
P11836	B-lymphocyte antigen CD20 (B-lymphocyte surface antigen B1) (Bp35) (Leukocyte surface antigen Leu-16) (Membrane-spanning 4-domains subfamily A member 1) (CD antigen CD20)	multi-TM	N/A
Q16739	Ceramide glucosyltransferase (EC 2.4.1.80) (GLCT-1) (Glucosylceramide synthase) (GCS) (UDP-glucose ceramide glucosyltransferase) (UDP-glucose:N-acylsphingosine D-glucosyltransferase)	multi-TM	N/A
P51788	Chloride channel protein 2 (ClC-2)	multi-TM	N/A
Q96D31	Calcium release-activated calcium channel protein 1 (Protein orai-1) (Transmembrane protein 142A)	multi-TM	N/A
Q494W8	CHRNA7-FAM7A fusion protein (CHRNA7-DR1) (D-10)	multi-TM	N/A
Q53GD3	Choline transporter-like protein 4 (Solute carrier family 44 member 4)	multi-TM	N/A
O75907	Diacylglycerol O-acyltransferase 1 (EC 2.3.1.20) (ACAT-related gene product 1) (Acyl-CoA retinol O-fatty-acyltransferase) (ARAT) (Retinol O-fatty-acyltransferase) (EC 2.3.1.76) (Diglyceride acyltransferase)	multi-TM	N/A
Q96PD7	Diacylglycerol O-acyltransferase 2 (EC 2.3.1.20) (Acyl-CoA retinol O-fatty-acyltransferase) (ARAT) (Retinol O-fatty-acyltransferase) (EC 2.3.1.76) (Diglyceride acyltransferase 2)	multi-TM	N/A
Q14534	Squalene monooxygenase (EC 1.14.13.132) (Squalene epoxidase) (SE)	multi-TM	N/A
O95864	Fatty acid desaturase 2 (EC 1.14.19.-) (Delta(6) fatty acid desaturase) (D6D) (Delta(6) desaturase) (Delta-6 desaturase)	multi-TM	N/A
Q01362	High affinity immunoglobulin epsilon receptor subunit beta (FcERI) (Fc epsilon receptor I beta-chain) (IgE Fc receptor subunit beta) (Membrane-spanning 4-domains subfamily A member 2)	multi-TM	N/A
P37268	Squalene synthase (SQS) (SS) (EC 2.5.1.21) (FPP:FPP farnesyltransferase) (Farnesyl-diphosphate farnesyltransferase)	multi-TM	N/A
P04035	3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) (EC 1.1.1.34)	multi-TM	N/A
P78508	ATP-sensitive inward rectifier potassium channel 10 (ATP-dependent inwardly rectifying potassium channel Kir4.1) (Inward rectifier K(+) channel Kir1.2) (Potassium channel, inwardly rectifying subfamily J member 10)	multi-TM	N/A
Q14654	ATP-sensitive inward rectifier potassium channel 11 (IKATP) (Inward rectifier K(+) channel Kir6.2) (Potassium channel, inwardly rectifying subfamily J member 11)	multi-TM	N/A
Q14500	ATP-sensitive inward rectifier potassium channel 12 (Inward rectifier K(+) channel Kir2.2) (IRK-2) (Inward rectifier K(+) channel Kir2.2v) (Potassium channel, inwardly rectifying subfamily J member 12)	multi-TM	N/A
P48048	ATP-sensitive inward rectifier potassium channel 1 (ATP-regulated potassium channel ROM-K) (Inward rectifier K(+) channel Kir1.1) (Potassium channel, inwardly rectifying subfamily J member 1)	multi-TM	N/A
P48544	G protein-activated inward rectifier potassium channel 4 (GIRK-4) (Cardiac inward rectifier) (CIR) (Heart KATP channel) (Inward rectifier K(+) channel Kir3.4) (IRK-4) (KATP-1) (Potassium channel, inwardly rectifying subfamily J member 5)	multi-TM	N/A
Q15842	ATP-sensitive inward rectifier potassium channel 8 (Inward rectifier K(+) channel Kir6.1) (Potassium channel, inwardly rectifying subfamily J member 8) (uKATP-1)	multi-TM	N/A
Q14643	Inositol 1,4,5-trisphosphate receptor type 1 (IP3 receptor isoform 1) (IP3R 1) (InsP3R1) (Type 1 inositol 1,4,5-trisphosphate receptor) (Type 1 InsP3 receptor)	multi-TM	N/A

Q14571	Inositol 1,4,5-trisphosphate receptor type 2 (IP3 receptor isoform 2) (IP3R 2) (InsP3R2) (Type 2 inositol 1,4,5-trisphosphate receptor) (Type 2 InsP3 receptor)	multi-TM	N/A
Q14573	Inositol 1,4,5-trisphosphate receptor type 3 (IP3 receptor isoform 3) (IP3R 3) (InsP3R3) (Type 3 inositol 1,4,5-trisphosphate receptor) (Type 3 InsP3 receptor)	multi-TM	N/A
Q12791	Calcium-activated potassium channel subunit alpha-1 (BK channel) (BKCA alpha) (Calcium-activated potassium channel, subfamily M subunit alpha-1) (K(VCA)alpha) (KCa1.1) (Maxi K channel) (MaxiK) (Slo-alpha) (Slo1) (Slowpoke homolog) (Slo homolog) (hSlo)	multi-TM	N/A
Q09470	Potassium voltage-gated channel subfamily A member 1 (Voltage-gated K(+) channel HuKI) (Voltage-gated potassium channel HBK1) (Voltage-gated potassium channel subunit Kv1.1)	multi-TM	N/A
P22460	Potassium voltage-gated channel subfamily A member 5 (HPCN1) (Voltage-gated potassium channel HK2) (Voltage-gated potassium channel subunit Kv1.5)	multi-TM	N/A
Q9UK17	Potassium voltage-gated channel subfamily D member 3 (Voltage-gated potassium channel subunit Kv4.3)	multi-TM	N/A
Q12809	Potassium voltage-gated channel subfamily H member 2 (Eag homolog) (Ether-a-go-go-related gene potassium channel 1) (ERG-1) (Eag-related protein 1) (Ether-a-go-go-related protein 1) (H-ERG) (hERG-1) (hERG1) (Voltage-gated potassium channel subunit Kv11.1)	multi-TM	N/A
O95069	Potassium channel subfamily K member 2 (Outward rectifying potassium channel protein TREK-1) (TREK-1 K(+) channel subunit) (Two pore domain potassium channel TREK-1) (Two pore potassium channel TPKC1)	multi-TM	N/A
O14649	Potassium channel subfamily K member 3 (Acid-sensitive potassium channel protein TASK-1) (TWIK-related acid-sensitive K(+) channel 1) (Two pore potassium channel KT3.1) (Two pore K(+) channel KT3.1)	multi-TM	N/A
Q9NPC2	Potassium channel subfamily K member 9 (Acid-sensitive potassium channel protein TASK-3) (TWIK-related acid-sensitive K(+) channel 3) (Two pore potassium channel KT3.2) (Two pore K(+) channel KT3.2)	multi-TM	N/A
O15554	Intermediate conductance calcium-activated potassium channel protein 4 (SK4) (SKCa 4) (SKCa4) (IKCa1) (IK1) (KCa3.1) (KCa4) (Putative Gardos channel)	multi-TM	N/A
P51787	Potassium voltage-gated channel subfamily KQT member 1 (IKs producing slow voltage-gated potassium channel subunit alpha KvLQT1) (KQT-like 1) (Voltage-gated potassium channel subunit Kv7.1)	multi-TM	N/A
O43525	Potassium voltage-gated channel subfamily KQT member 3 (KQT-like 3) (Potassium channel subunit alpha KvLQT3) (Voltage-gated potassium channel subunit Kv7.3)	multi-TM	N/A
O43526	Potassium voltage-gated channel subfamily KQT member 2 (KQT-like 2) (Neuroblastoma-specific potassium channel subunit alpha KvLQT2) (Voltage-gated potassium channel subunit Kv7.2)	multi-TM	N/A
P56696	Potassium voltage-gated channel subfamily KQT member 4 (KQT-like 4) (Potassium channel subunit alpha KvLQT4) (Voltage-gated potassium channel subunit Kv7.4)	multi-TM	N/A
P21439	Multidrug resistance protein 3 (EC 3.6.3.44) (ATP-binding cassette sub-family B member 4) (P-glycoprotein 3)	multi-TM	N/A
P33527	Multidrug resistance-associated protein 1 (ATP-binding cassette sub-family C member 1) (Leukotriene C(4) transporter) (LTC4 transporter)	multi-TM	N/A
Q92887	Canalicular multispecific organic anion transporter 1 (ATP-binding cassette sub-family C member 2) (Canalicular multidrug resistance protein) (Multidrug resistance-associated protein 2)	multi-TM	N/A
Q99572	P2X purinoceptor 7 (P2X7) (ATP receptor) (P2Z receptor) (Purinergic receptor)	multi-TM	N/A
Q14432	cGMP-inhibited 3',5'-cyclic phosphodiesterase A (EC 3.1.4.17) (Cyclic GMP-inhibited phosphodiesterase A) (CGI-PDE A)	multi-TM	N/A
Q9H237	Protein-cysteine N-palmitoyltransferase porcupine (EC 2.3.1.-) (Protein MG61)	multi-TM	N/A
Q04671	P protein (Melanocyte-specific transporter protein) (Pink-eyed dilution protein homolog)	multi-TM	N/A
P21817	Ryanodine receptor 1 (RYR-1) (RyR1) (Skeletal muscle calcium release channel) (Skeletal muscle ryanodine receptor) (Skeletal muscle-type ryanodine receptor) (Type 1 ryanodine receptor)	multi-TM	N/A
Q15413	Ryanodine receptor 3 (RYR-3) (RyR3) (Brain ryanodine receptor-calcium release channel) (Brain-type ryanodine receptor) (Type 3 ryanodine receptor)	multi-TM	N/A
Q92736	Ryanodine receptor 2 (RYR-2) (RyR2) (hRYR-2) (Cardiac muscle ryanodine receptor) (Cardiac muscle ryanodine receptor-calcium release channel) (Type 2 ryanodine receptor)	multi-TM	N/A
Q13621	Solute carrier family 12 member 1 (Bumetanide-sensitive sodium-(potassium)-chloride cotransporter 2) (Kidney-specific Na-K-Cl symporter)	multi-TM	N/A
P55017	Solute carrier family 12 member 3 (Na-Cl cotransporter) (NCC) (Na-Cl symporter) (Thiazide-sensitive sodium-chloride cotransporter)	multi-TM	N/A
P55011	Solute carrier family 12 member 2 (Basolateral Na-K-Cl symporter) (Bumetanide-sensitive sodium-(potassium)-chloride cotransporter 1)	multi-TM	N/A
Q9UP95	Solute carrier family 12 member 4 (Electroneutral potassium-chloride cotransporter 1) (Erythroid K-Cl cotransporter 1) (hKCC1)	multi-TM	N/A
Q9H2X9	Solute carrier family 12 member 5 (Electroneutral potassium-chloride cotransporter 2) (K-Cl cotransporter 2) (hKCC2) (Neuronal K-Cl cotransporter)	multi-TM	N/A

Q4U2R8	Solute carrier family 22 member 6 (Organic anion transporter 1) (hOAT1) (PAH transporter) (hPAHT) (Renal organic anion transporter 1) (hROAT1)	multi-TM	N/A
Q8TCC7	Solute carrier family 22 member 8 (Organic anion transporter 3) (hOAT3)	multi-TM	N/A
Q9NSA0	Solute carrier family 22 member 11 (Organic anion transporter 4)	multi-TM	N/A
Q96S37	Solute carrier family 22 member 12 (Organic anion transporter 4-like protein) (Renal-specific transporter) (RST) (Urate anion exchanger 1)	multi-TM	N/A
Q9UGH3	Solute carrier family 23 member 2 (Na(+)/L-ascorbic acid transporter 2) (Nucleobase transporter-like 1 protein) (Sodium-dependent vitamin C transporter 2) (hSVCT2) (Yolk sac permease-like molecule 2)	multi-TM	N/A
P18405	3-oxo-5-alpha-steroid 4-dehydrogenase 1 (EC 1.3.1.22) (SR type 1) (Steroid 5-alpha-reductase 1) (S5AR 1)	multi-TM	N/A
P31213	3-oxo-5-alpha-steroid 4-dehydrogenase 2 (EC 1.3.1.22) (5 alpha-SR2) (SR type 2) (Steroid 5-alpha-reductase 2) (S5AR 2) (Type II 5-alpha reductase)	multi-TM	N/A
P13866	Sodium/glucose cotransporter 1 (Na(+)/glucose cotransporter 1) (High affinity sodium-glucose cotransporter) (Solute carrier family 5 member 1)	multi-TM	N/A
P31639	Sodium/glucose cotransporter 2 (Na(+)/glucose cotransporter 2) (Low affinity sodium-glucose cotransporter) (Solute carrier family 5 member 2)	multi-TM	N/A
P23975	Sodium-dependent noradrenaline transporter (Norepinephrine transporter) (NET) (Solute carrier family 6 member 2)	multi-TM	N/A
P30531	Sodium- and chloride-dependent GABA transporter 1 (GAT-1) (Solute carrier family 6 member 1)	multi-TM	N/A
Q01959	Sodium-dependent dopamine transporter (DA transporter) (DAT) (Solute carrier family 6 member 3)	multi-TM	N/A
P31645	Sodium-dependent serotonin transporter (5HT transporter) (5HTT) (Solute carrier family 6 member 4)	multi-TM	N/A
P48029	Sodium- and chloride-dependent creatine transporter 1 (CT1) (Creatine transporter 1) (Solute carrier family 6 member 8)	multi-TM	N/A
P48067	Sodium- and chloride-dependent glycine transporter 1 (GlyT-1) (GlyT1) (Solute carrier family 6 member 9)	multi-TM	N/A
Q99250	Sodium channel protein type 2 subunit alpha (HBSC II) (Sodium channel protein brain II subunit alpha) (Sodium channel protein type II subunit alpha) (Voltage-gated sodium channel subunit alpha Nav1.2)	multi-TM	N/A
P35498	Sodium channel protein type 1 subunit alpha (Sodium channel protein brain I subunit alpha) (Sodium channel protein type I subunit alpha) (Voltage-gated sodium channel subunit alpha Nav1.1)	multi-TM	N/A
Q9NY46	Sodium channel protein type 3 subunit alpha (Sodium channel protein brain III subunit alpha) (Sodium channel protein type III subunit alpha) (Voltage-gated sodium channel subtype III) (Voltage-gated sodium channel subunit alpha Nav1.3)	multi-TM	N/A
P35499	Sodium channel protein type 4 subunit alpha (SkM1) (Sodium channel protein skeletal muscle subunit alpha) (Sodium channel protein type IV subunit alpha) (Voltage-gated sodium channel subunit alpha Nav1.4)	multi-TM	N/A
Q14524	Sodium channel protein type 5 subunit alpha (HH1) (Sodium channel protein cardiac muscle subunit alpha) (Sodium channel protein type V subunit alpha) (Voltage-gated sodium channel subunit alpha Nav1.5)	multi-TM	N/A
Q15858	Sodium channel protein type 9 subunit alpha (Neuroendocrine sodium channel) (hNE-Na) (Peripheral sodium channel 1) (PN1) (Sodium channel protein type IX subunit alpha) (Voltage-gated sodium channel subunit alpha Nav1.7)	multi-TM	N/A
Q9Y5Y9	Sodium channel protein type 10 subunit alpha (Peripheral nerve sodium channel 3) (PN3) (hPN3) (Sodium channel protein type X subunit alpha) (Voltage-gated sodium channel subunit alpha Nav1.8)	multi-TM	N/A
Q9UI33	Sodium channel protein type 11 subunit alpha (Peripheral nerve sodium channel 5) (PN5) (Sensory neuron sodium channel 2) (Sodium channel protein type XI subunit alpha) (Voltage-gated sodium channel subunit alpha Nav1.9) (hNaN)	multi-TM	N/A
P51168	Amiloride-sensitive sodium channel subunit beta (Beta-NaCH) (Epithelial Na(+) channel subunit beta) (Beta-ENaC) (ENaCB) (Nonvoltage-gated sodium channel 1 subunit beta) (SCNEB)	multi-TM	N/A
P51172	Amiloride-sensitive sodium channel subunit delta (Delta-NaCH) (Epithelial Na(+) channel subunit delta) (Delta-ENaC) (ENaCD) (Nonvoltage-gated sodium channel 1 subunit delta) (SCNED)	multi-TM	N/A
P51170	Amiloride-sensitive sodium channel subunit gamma (Epithelial Na(+) channel subunit gamma) (ENaCG) (Gamma-ENaC) (Gamma-NaCH) (Nonvoltage-gated sodium channel 1 subunit gamma) (SCNEG)	multi-TM	N/A
P37088	Amiloride-sensitive sodium channel subunit alpha (Alpha-NaCH) (Epithelial Na(+) channel subunit alpha) (Alpha-ENaC) (ENaCA) (Nonvoltage-gated sodium channel 1 subunit alpha) (SCNEA)	multi-TM	N/A
O94956	Solute carrier organic anion transporter family member 2B1 (Organic anion transporter B) (OATP-B) (Organic anion transporter polypeptide-related protein 2) (OATP-RP2) (OATPRP2) (Solute carrier family 21 member 9)	multi-TM	N/A

P35610	Sterol O-acyltransferase 1 (EC 2.3.1.26) (Acyl-coenzyme A:cholesterol acyltransferase 1) (ACAT-1) (Cholesterol acyltransferase 1)	multi-TM	N/A
Q7L0J3	Synaptic vesicle glycoprotein 2A	multi-TM	N/A
P24557	Thromboxane-A synthase (TXA synthase) (TXS) (EC 5.3.99.5) (Cytochrome P450 5A1)	multi-TM	N/A
Q7Z2W7	Transient receptor potential cation channel subfamily M member 8 (Long transient receptor potential channel 6) (LTrpC-6) (LTrpC6) (Transient receptor potential p8) (Trp-p8)	multi-TM	N/A
Q8NER1	Transient receptor potential cation channel subfamily V member 1 (TrpV1) (Capsaicin receptor) (Osm-9-like TRP channel 1) (OTRPC1) (Vanilloid receptor 1)	multi-TM	N/A
Q8NET8	Transient receptor potential cation channel subfamily V member 3 (TrpV3) (Vanilloid receptor-like 3) (VRL-3)	multi-TM	N/A
P38435	Vitamin K-dependent gamma-carboxylase (EC 4.1.1.90) (Gamma-glutamyl carboxylase) (Peptidyl-glutamate 4-carboxylase) (Vitamin K gamma glutamyl carboxylase)	multi-TM	N/A
Q8N0U8	Vitamin K epoxide reductase complex subunit 1-like protein 1 (VKORC1-like protein 1)	multi-TM	N/A
Q9BQB6	Vitamin K epoxide reductase complex subunit 1 (EC 1.1.4.1) (Vitamin K1 2,3-epoxide reductase subunit 1)	multi-TM	N/A
P54219	Chromaffin granule amine transporter (Solute carrier family 18 member 1) (Vesicular amine transporter 1) (VAT1)	multi-TM	N/A
Q05940	Synaptic vesicular amine transporter (Monoamine transporter) (Solute carrier family 18 member 2) (Vesicular amine transporter 2) (VAT2)	multi-TM	N/A
Q9UPY5	Cystine/glutamate transporter (Amino acid transport system xc-) (Calcium channel blocker resistance protein CCBR1) (Solute carrier family 7 member 11) (xCT)	multi-TM	N/A
P52895	Aldo-keto reductase family 1 member C2 (EC 1.-.-.-) (3-alpha-HSD3) (Chlordecone reductase homolog HAKRD) (Dihydrodiol dehydrogenase 2) (DD-2) (DD2) (Dihydrodiol dehydrogenase/bile acid-binding protein) (DD/BABP) (Trans-1,2-dihydrobenzene-1,2-diol dehydrogenase) (EC 1.3.1.20) (Type III 3-alpha-hydroxysteroid dehydrogenase) (EC 1.1.1.357)	other	N/A
P37231	Peroxisome proliferator-activated receptor gamma (PPAR-gamma) (Nuclear receptor subfamily 1 group C member 3)	other	N/A
Q99720	Sigma non-opioid intracellular receptor 1 (Aging-associated gene 8 protein) (SR31747-binding protein) (SR-BP) (Sigma 1-type opioid receptor) (SIG-1R) (Sigma1-receptor) (Sigma1R) (hSigmaR1)	other	N/A
P14060	3 beta-hydroxysteroid dehydrogenase/Delta 5-->4-isomerase type 1 (3 beta-hydroxysteroid dehydrogenase/Delta 5-->4-isomerase type I) (3-beta-HSD I) (Trophoblast antigen FDO161G) [Includes: 3-beta-hydroxy-Delta(5)-steroid dehydrogenase (EC 1.1.1.145) (3-beta-hydroxy-5-ene steroid dehydrogenase) (Progesterone reductase); Steroid Delta-isomerase (EC 5.3.3.1) (Delta-5-3-ketosteroid isomerase)]	other	N/A
P26439	3 beta-hydroxysteroid dehydrogenase/Delta 5-->4-isomerase type 2 (3 beta-hydroxysteroid dehydrogenase/Delta 5-->4-isomerase type II) (3-beta-HSD II) (3-beta-HSD adrenal and gonadal type) [Includes: 3-beta-hydroxy-Delta(5)-steroid dehydrogenase (EC 1.1.1.145) (3-beta-hydroxy-5-ene steroid dehydrogenase) (Progesterone reductase); Steroid Delta-isomerase (EC 5.3.3.1) (Delta-5-3-ketosteroid isomerase)]	other	N/A
Q13542	Eukaryotic translation initiation factor 4E-binding protein 2 (4E-BP2) (eIF4E-binding protein 2)	other	N/A
Q9Y478	5'-AMP-activated protein kinase subunit beta-1 (AMPK subunit beta-1) (AMPKb)	other	N/A
Q13131	5'-AMP-activated protein kinase catalytic subunit alpha-1 (AMPK subunit alpha-1) (EC 2.7.11.1) (Acetyl-CoA carboxylase kinase) (ACACA kinase) (EC 2.7.11.27) (Hydroxymethylglutaryl-CoA reductase kinase) (HMGCR kinase) (EC 2.7.11.31) (Tau-protein kinase PRKAA1) (EC 2.7.11.26)	other	N/A
P42684	Abelson tyrosine-protein kinase 2 (EC 2.7.10.2) (Abelson murine leukemia viral oncogene homolog 2) (Abelson-related gene protein) (Tyrosine-protein kinase ARG)	other	N/A
P00519	Tyrosine-protein kinase ABL1 (EC 2.7.10.2) (Abelson murine leukemia viral oncogene homolog 1) (Abelson tyrosine-protein kinase 1) (Proto-oncogene c-Abl) (p150)	other	N/A
O00763	Acetyl-CoA carboxylase 2 (EC 6.4.1.2) (ACC-beta) [Includes: Biotin carboxylase (EC 6.3.4.14)]	other	N/A
P00813	Adenosine deaminase (EC 3.5.4.4) (Adenosine aminohydrolase)	other	N/A
P07327	Alcohol dehydrogenase 1A (EC 1.1.1.1) (Alcohol dehydrogenase subunit alpha)	other	N/A
P00325	Alcohol dehydrogenase 1B (EC 1.1.1.1) (Alcohol dehydrogenase subunit beta)	other	N/A
P00326	Alcohol dehydrogenase 1C (EC 1.1.1.1) (Alcohol dehydrogenase subunit gamma)	other	N/A
P55263	Adenosine kinase (AK) (EC 2.7.1.20) (Adenosine 5'-phosphotransferase)	other	N/A
Q06278	Aldehyde oxidase (EC 1.2.3.1)	other	N/A
P12235	ADP/ATP translocase 1 (ADP,ATP carrier protein 1) (ADP,ATP carrier protein, heart/skeletal muscle isoform T1) (Adenine nucleotide translocator 1) (ANT 1) (Solute carrier family 25 member 4)	other	N/A

P05141	ADP/ATP translocase 2 (ADP,ATP carrier protein 2) (ADP,ATP carrier protein, fibroblast isoform) (Adenine nucleotide translocator 2) (ANT 2) (Solute carrier family 25 member 5) [Cleaved into: ADP/ATP translocase 2, N-terminally processed]	other	N/A
P12236	ADP/ATP translocase 3 (ADP,ATP carrier protein 3) (ADP,ATP carrier protein, isoform T2) (ANT 2) (Adenine nucleotide translocator 3) (ANT 3) (Solute carrier family 25 member 6)	other	N/A
P14550	Alcohol dehydrogenase [NADP(+)] (EC 1.1.1.2) (Aldehyde reductase) (Aldo-keto reductase family 1 member A1)	other	N/A
P51857	3-oxo-5-beta-steroid 4-dehydrogenase (EC 1.3.1.3) (Aldo-keto reductase family 1 member D1) (Delta(4)-3-ketosteroid 5-beta-reductase) (Delta(4)-3-oxosteroid 5-beta-reductase)	other	N/A
P31749	RAC-alpha serine/threonine-protein kinase (EC 2.7.11.1) (Protein kinase B) (PKB) (Protein kinase B alpha) (PKB alpha) (Proto-oncogene c-Akt) (RAC-PK-alpha)	other	N/A
P15121	Aldose reductase (AR) (EC 1.1.1.21) (Aldehyde reductase) (Aldo-keto reductase family 1 member B1)	other	N/A
P10275	Androgen receptor (Dihydrotestosterone receptor) (Nuclear receptor subfamily 3 group C member 4)	other	N/A
P04083	Annexin A1 (Annexin I) (Annexin-1) (Calpactin II) (Calpactin-2) (Chromobindin-9) (Lipocortin I) (Phospholipase A2 inhibitory protein) (p35)	other	N/A
P21397	Amine oxidase [flavin-containing] A (EC 1.4.3.4) (Monoamine oxidase type A) (MAO-A)	other	N/A
P27338	Amine oxidase [flavin-containing] B (EC 1.4.3.4) (Monoamine oxidase type B) (MAO-B)	other	N/A
P27695	DNA-(apurinic or apyrimidinic site) lyase (EC 3.1.-.-) (EC 4.2.99.18) (APEX nuclease) (APEN) (Apurinic-apyrimidinic endonuclease 1) (AP endonuclease 1) (APE-1) (REF-1) (Redox factor-1) [Cleaved into: DNA-(apurinic or apyrimidinic site) lyase, mitochondrial]	other	N/A
P54710	Sodium/potassium-transporting ATPase subunit gamma (Na(+)/K(+) ATPase subunit gamma) (FXYD domain-containing ion transport regulator 2) (Sodium pump gamma chain)	other	N/A
O14965	Aurora kinase A (EC 2.7.11.1) (Aurora 2) (Aurora/IPL1-related kinase 1) (ARK-1) (Aurora-related kinase 1) (hARK1) (Breast tumor-amplified kinase) (Serine/threonine-protein kinase 15) (Serine/threonine-protein kinase 6) (Serine/threonine-protein kinase aurora-A)	other	N/A
Q96GD4	Aurora kinase B (EC 2.7.11.1) (Aurora 1) (Aurora- and IPL1-like midbody-associated protein 1) (AIM-1) (Aurora/IPL1-related kinase 2) (ARK-2) (Aurora-related kinase 2) (STK-1) (Serine/threonine-protein kinase 12) (Serine/threonine-protein kinase 5) (Serine/threonine-protein kinase aurora-B)	other	N/A
Q9UQB9	Aurora kinase C (EC 2.7.11.1) (Aurora 3) (Aurora/IPL1-related kinase 3) (ARK-3) (Aurora-related kinase 3) (Aurora/IPL1/Eg2 protein 2) (Serine/threonine-protein kinase 13) (Serine/threonine-protein kinase aurora-C)	other	N/A
Q92843	Bcl-2-like protein 2 (Bcl2-L-2) (Apoptosis regulator Bcl-W)	other	N/A
Q07817	Bcl-2-like protein 1 (Bcl2-L-1) (Apoptosis regulator Bcl-X)	other	N/A
P10415	Apoptosis regulator Bcl-2	other	N/A
P53004	Biliverdin reductase A (BVR A) (EC 1.3.1.24) (Biliverdin-IX alpha-reductase)	other	N/A
O15392	Baculoviral IAP repeat-containing protein 5 (Apoptosis inhibitor 4) (Apoptosis inhibitor survivin)	other	N/A
P30043	Flavin reductase (NADPH) (FR) (EC 1.5.1.30) (Biliverdin reductase B) (BVR-B) (EC 1.3.1.24) (Biliverdin-IX beta-reductase) (Green heme-binding protein) (GHBP) (NADPH-dependent diaphorase) (NADPH-flavin reductase) (FLR)	other	N/A
P15056	Serine/threonine-protein kinase B-raf (EC 2.7.11.1) (Proto-oncogene B-Raf) (p94) (v-Raf murine sarcoma viral oncogene homolog B1)	other	N/A
P15538	Cytochrome P450 11B1, mitochondrial (CYPXIB1) (Cytochrome P-450c11) (Cytochrome P450C11) (Steroid 11-beta-hydroxylase) (EC 1.14.15.4)	other	N/A
Q02641	Voltage-dependent L-type calcium channel subunit beta-1 (CAB1) (Calcium channel voltage-dependent subunit beta 1)	other	N/A
Q08289	Voltage-dependent L-type calcium channel subunit beta-2 (CAB2) (Calcium channel voltage-dependent subunit beta 2) (Lambert-Eaton myasthenic syndrome antigen B) (MYSB)	other	N/A
P54284	Voltage-dependent L-type calcium channel subunit beta-3 (CAB3) (Calcium channel voltage-dependent subunit beta 3)	other	N/A
O00305	Voltage-dependent L-type calcium channel subunit beta-4 (CAB4) (Calcium channel voltage-dependent subunit beta 4)	other	N/A
P00915	Carbonic anhydrase 1 (EC 4.2.1.1) (Carbonate dehydratase I) (Carbonic anhydrase B) (CAB) (Carbonic anhydrase I) (CA-I)	other	N/A
P00918	Carbonic anhydrase 2 (EC 4.2.1.1) (Carbonate dehydratase II) (Carbonic anhydrase C) (CAC) (Carbonic anhydrase II) (CA-II)	other	N/A
P07451	Carbonic anhydrase 3 (EC 4.2.1.1) (Carbonate dehydratase III) (Carbonic anhydrase III) (CA-III)	other	N/A
P43166	Carbonic anhydrase 7 (EC 4.2.1.1) (Carbonate dehydratase VII) (Carbonic anhydrase VII) (CA-VII)	other	N/A
P62158	Calmodulin (CaM)	other	N/A
Q9NYX4	Neuron-specific vesicular protein calcyon	other	N/A

Q96LZ3	Calcineurin subunit B type 2 (Calcineurin B-like protein) (CBLP) (Calcineurin BII) (CNBII) (PPP3R1-like) (Protein phosphatase 2B regulatory subunit 2) (Protein phosphatase 3 regulatory subunit B beta isoform)	other	N/A
P04040	Catalase (EC 1.11.1.6)	other	N/A
P35520	Cystathionine beta-synthase (EC 4.2.1.22) (Beta-thionase) (Serine sulfhydrase)	other	N/A
Q16204	Coiled-coil domain-containing protein 6 (Papillary thyroid carcinoma-encoded protein) (Protein H4)	other	N/A
P24385	G1/S-specific cyclin-D1 (B-cell lymphoma 1 protein) (BCL-1) (BCL-1 oncogene) (PRAD1 oncogene)	other	N/A
P06493	Cyclin-dependent kinase 1 (CDK1) (EC 2.7.11.22) (EC 2.7.11.23) (Cell division control protein 2 homolog) (Cell division protein kinase 1) (p34 protein kinase)	other	N/A
P11802	Cyclin-dependent kinase 4 (EC 2.7.11.22) (Cell division protein kinase 4) (PSK-J3)	other	N/A
P24941	Cyclin-dependent kinase 2 (EC 2.7.11.22) (Cell division protein kinase 2) (p33 protein kinase)	other	N/A
Q00534	Cyclin-dependent kinase 6 (EC 2.7.11.22) (Cell division protein kinase 6) (Serine/threonine-protein kinase PLSTIRE)	other	N/A
P50613	Cyclin-dependent kinase 7 (EC 2.7.11.22) (EC 2.7.11.23) (39 kDa protein kinase) (p39 Mo15) (CDK-activating kinase 1) (Cell division protein kinase 7) (Serine/threonine-protein kinase 1) (TFIIH basal transcription factor complex kinase subunit)	other	N/A
P50750	Cyclin-dependent kinase 9 (EC 2.7.11.22) (EC 2.7.11.23) (C-2K) (Cell division cycle 2-like protein kinase 4) (Cell division protein kinase 9) (Serine/threonine-protein kinase PITALRE) (Tat-associated kinase complex catalytic subunit)	other	N/A
Q02224	Centromere-associated protein E (Centromere protein E) (CENP-E) (Kinesin-related protein CENPE)	other	N/A
O14646	Chromodomain-helicase-DNA-binding protein 1 (CHD-1) (EC 3.6.4.12) (ATP-dependent helicase CHD1)	other	N/A
O14757	Serine/threonine-protein kinase Chk1 (EC 2.7.11.1) (CHK1 checkpoint homolog) (Cell cycle checkpoint kinase) (Checkpoint kinase-1)	other	N/A
O96017	Serine/threonine-protein kinase Chk2 (EC 2.7.11.1) (CHK2 checkpoint homolog) (Cds1 homolog) (Hucds1) (hCds1) (Checkpoint kinase 2)	other	N/A
Q9Y259	Choline/ethanolamine kinase (Choline kinase beta) (CK) (CKB) (EC 2.7.1.32) (Choline kinase-like protein) (Ethanolamine kinase) (EK) (EC 2.7.1.82) (Ethanolamine kinase beta) (EKB) (choline/ethanolamine kinase beta) (CKEKB)	other	N/A
Q96MW5	Conserved oligomeric Golgi complex subunit 8 (COG complex subunit 8) (Component of oligomeric Golgi complex 8)	other	N/A
P05108	Cholesterol side-chain cleavage enzyme, mitochondrial (EC 1.14.15.6) (CYPXIA1) (Cholesterol desmolase) (Cytochrome P450 11A1) (Cytochrome P450(scc))	other	N/A
P05093	Steroid 17-alpha-hydroxylase/17,20 lyase (EC 1.14.99.9) (EC 4.1.2.30) (17-alpha-hydroxyprogesterone aldolase) (CYPXVII) (Cytochrome P450 17A1) (Cytochrome P450-C17) (Cytochrome P450c17) (Steroid 17-alpha-monooxygenase)	other	N/A
P11511	Aromatase (EC 1.14.14.14) (CYPXIX) (Cytochrome P-450AROM) (Cytochrome P450 19A1) (Estrogen synthase)	other	N/A
O43174	Cytochrome P450 26A1 (EC 1.14.-.-) (Cytochrome P450 retinoic acid-inactivating 1) (Cytochrome P450RAI) (hP450RAI) (Retinoic acid 4-hydroxylase) (Retinoic acid-metabolizing cytochrome)	other	N/A
P10635	Cytochrome P450 2D6 (EC 1.14.14.1) (CYPIID6) (Cytochrome P450-DB1) (Debrisoquine 4-hydroxylase)	other	N/A
P08684	Cytochrome P450 3A4 (EC 1.14.13.-) (1,8-cineole 2-exo-monooxygenase) (EC 1.14.13.157) (Albendazole monooxygenase) (EC 1.14.13.32) (Albendazole sulfoxidase) (CYPIIIA3) (CYPIIIA4) (Cytochrome P450 3A3) (Cytochrome P450 HLp) (Cytochrome P450 NF-25) (Cytochrome P450-PCN1) (Nifedipine oxidase) (Quinine 3-monooxygenase) (EC 1.14.13.67) (Taurochenodeoxycholate 6-alpha-hydroxylase) (EC 1.14.13.97)	other	N/A
Q16850	Lanosterol 14-alpha demethylase (LDM) (EC 1.14.13.70) (CYPLI) (Cytochrome P450 51A1) (Cytochrome P450-14DM) (Cytochrome P45014DM) (Cytochrome P450LI) (Sterol 14-alpha demethylase)	other	N/A
P31327	Carbamoyl-phosphate synthase [ammonia], mitochondrial (EC 6.3.4.16) (Carbamoyl-phosphate synthetase I) (CPSase I)	other	N/A
P50416	Carnitine O-palmitoyltransferase 1, liver isoform (CPT1-L) (EC 2.3.1.21) (Carnitine O-palmitoyltransferase I, liver isoform) (CPT I) (CPTI-L) (Carnitine palmitoyltransferase 1A)	other	N/A
Q92523	Carnitine O-palmitoyltransferase 1, muscle isoform (CPT1-M) (EC 2.3.1.21) (Carnitine O-palmitoyltransferase I, muscle isoform) (CPT I) (CPTI-M) (Carnitine palmitoyltransferase 1B) (Carnitine palmitoyltransferase I-like protein)	other	N/A
P23786	Carnitine O-palmitoyltransferase 2, mitochondrial (EC 2.3.1.21) (Carnitine palmitoyltransferase II) (CPT II)	other	N/A
P19113	Histidine decarboxylase (HDC) (EC 4.1.1.22)	other	N/A
P27707	Deoxycytidine kinase (dCK) (EC 2.7.1.74)	other	N/A

P11926	Ornithine decarboxylase (ODC) (EC 4.1.1.17)	other	N/A
Q96C86	m7GpppX diphosphatase (EC 3.6.1.59) (DCS-1) (Decapping scavenger enzyme) (Hint-related 7meGMP-directed hydrolase) (Histidine triad nucleotide-binding protein 5) (Histidine triad protein member 5) (HINT-5) (Scavenger mRNA-decapping enzyme DcpS)	other	N/A
P20711	Aromatic-L-amino-acid decarboxylase (AADC) (EC 4.1.1.28) (DOPA decarboxylase) (DDC)	other	N/A
Q9NX09	DNA damage-inducible transcript 4 protein (HIF-1 responsive protein RTP801) (Protein regulated in development and DNA damage response 1) (REDD-1)	other	N/A
Q9HBH1	Peptide deformylase, mitochondrial (EC 3.5.1.88) (Polypeptide deformylase)	other	N/A
P14061	Estradiol 17-beta-dehydrogenase 1 (EC 1.1.1.62) (17-beta-hydroxysteroid dehydrogenase type 1) (17-beta-HSD 1) (20 alpha-hydroxysteroid dehydrogenase) (20-alpha-HSD) (E2DH) (Placental 17-beta-hydroxysteroid dehydrogenase)	other	N/A
O14521	Succinate dehydrogenase [ubiquinone] cytochrome b small subunit, mitochondrial (CybS) (CII-4) (QPs3) (Succinate dehydrogenase complex subunit D) (Succinate-ubiquinone oxidoreductase cytochrome b small subunit) (Succinate-ubiquinone reductase membrane anchor subunit)	other	N/A
P26358	DNA (cytosine-5)-methyltransferase 1 (Dnmt1) (EC 2.1.1.37) (CXXC-type zinc finger protein 9) (DNA methyltransferase HsaI) (DNA MTase HsaI) (M.HsaI) (MCMT)	other	N/A
Q07864	DNA polymerase epsilon catalytic subunit A (EC 2.7.7.7) (DNA polymerase II subunit A)	other	N/A
P56282	DNA polymerase epsilon subunit 2 (EC 2.7.7.7) (DNA polymerase II subunit 2) (DNA polymerase epsilon subunit B)	other	N/A
Q9NRF9	DNA polymerase epsilon subunit 3 (EC 2.7.7.7) (Arsenic-transactivated protein) (AsTP) (Chromatin accessibility complex 17 kDa protein) (CHRAC-17) (HuCHRAC17) (DNA polymerase II subunit 3) (DNA polymerase epsilon subunit p17)	other	N/A
Q9NR33	DNA polymerase epsilon subunit 4 (EC 2.7.7.7) (DNA polymerase II subunit 4) (DNA polymerase epsilon subunit p12)	other	N/A
P09884	DNA polymerase alpha catalytic subunit (EC 2.7.7.7) (DNA polymerase alpha catalytic subunit p180)	other	N/A
P06746	DNA polymerase beta (EC 2.7.7.7) (EC 4.2.99.-)	other	N/A
P00374	Dihydrofolate reductase (EC 1.5.1.3)	other	N/A
Q96KS0	Egl nine homolog 2 (EC 1.14.11.29) (Estrogen-induced tag 6) (HPH-3) (Hypoxia-inducible factor prolyl hydroxylase 1) (HIF-PH1) (HIF-prolyl hydroxylase 1) (HPH-1) (Prolyl hydroxylase domain-containing protein 1) (PHD1)	other	N/A
Q99814	Endothelial PAS domain-containing protein 1 (EPAS-1) (Basic-helix-loop-helix-PAS protein MOP2) (Class E basic helix-loop-helix protein 73) (bHLHe73) (HIF-1-alpha-like factor) (HLF) (Hypoxia-inducible factor 2-alpha) (HIF-2-alpha) (HIF2-alpha) (Member of PAS protein 2) (PAS domain-containing protein 2)	other	N/A
P62508	Estrogen-related receptor gamma (ERR gamma-2) (Estrogen receptor-related protein 3) (Nuclear receptor subfamily 3 group B member 3)	other	N/A
Q92731	Estrogen receptor beta (ER-beta) (Nuclear receptor subfamily 3 group A member 2)	other	N/A
P09467	Fructose-1,6-bisphosphatase 1 (FBPase 1) (EC 3.1.3.11) (D-fructose-1,6-bisphosphate 1-phosphohydrolase 1)	other	N/A
O00519	Fatty-acid amide hydrolase 1 (EC 3.5.1.99) (Anandamide amidohydrolase 1) (Oleamide hydrolase 1)	other	N/A
O60427	Fatty acid desaturase 1 (EC 1.14.19.-) (Delta(5) fatty acid desaturase) (D5D) (Delta(5) desaturase) (Delta-5 desaturase)	other	N/A
P49327	Fatty acid synthase (EC 2.3.1.85) [Includes: [Acyl-carrier-protein] S-acetyltransferase (EC 2.3.1.38); [Acyl-carrier-protein] S-malonyltransferase (EC 2.3.1.39); 3-oxoacyl-[acyl-carrier-protein] synthase (EC 2.3.1.41); 3-oxoacyl-[acyl-carrier-protein] reductase (EC 1.1.1.100); 3-hydroxyacyl-[acyl-carrier-protein] dehydratase (EC 4.2.1.59); Enoyl-[acyl-carrier-protein] reductase (EC 1.3.1.39); Oleoyl-[acyl-carrier-protein] hydrolase (EC 3.1.2.14)]	other	N/A
P05230	Fibroblast growth factor 1 (FGF-1) (Acidic fibroblast growth factor) (aFGF) (Endothelial cell growth factor) (ECGF) (Heparin-binding growth factor 1) (HBGF-1)	other	N/A
P09038	Fibroblast growth factor 2 (FGF-2) (Basic fibroblast growth factor) (bFGF) (Heparin-binding growth factor 2) (HBGF-2)	other	N/A
P62942	Peptidyl-prolyl cis-trans isomerase FKBP1A (PPIase FKBP1A) (EC 5.2.1.8) (12 kDa FK506-binding protein) (12 kDa FKBP) (FKBP-12) (Calstabin-1) (FK506-binding protein 1A) (FKBP-1A) (Immunophilin FKBP12) (Rotamase)	other	N/A
P49354	Protein farnesyltransferase/geranylgeranyltransferase type-1 subunit alpha (EC 2.5.1.58) (EC 2.5.1.59) (CAAX farnesyltransferase subunit alpha) (FTase-alpha) (Ras proteins prenyltransferase subunit alpha) (Type I protein geranyl-geranyltransferase subunit alpha) (GGTase-I-alpha)	other	N/A
P14324	Farnesyl pyrophosphate synthase (FPP synthase) (FPS) (EC 2.5.1.10) ((2E,6E)-farnesyl diphosphate synthase) (Dimethylallyltranstransferase) (EC 2.5.1.1) (Farnesyl diphosphate synthase) (Geranyltranstransferase)	other	N/A
P02794	Ferritin heavy chain (Ferritin H subunit) (EC 1.16.3.1) (Cell proliferation-inducing gene 15	other	N/A

	protein) [Cleaved into: Ferritin heavy chain, N-terminally processed]		
P02792	Ferritin light chain (Ferritin L subunit)	other	N/A
P06241	Tyrosine-protein kinase Fyn (EC 2.7.10.2) (Proto-oncogene Syn) (Proto-oncogene c-Fyn) (Src-like kinase) (SLK) (p59-Fyn)	other	N/A
P80404	4-aminobutyrate aminotransferase, mitochondrial (EC 2.6.1.19) ((S)-3-amino-2-methylpropionate transaminase) (EC 2.6.1.22) (GABA aminotransferase) (GABA-AT) (Gamma-amino-N-butyrate transaminase) (GABA transaminase) (GABA-T) (L-AIBAT)	other	N/A
Q14353	Guanidinoacetate N-methyltransferase (EC 2.1.1.2)	other	N/A
P04150	Glucocorticoid receptor (GR) (Nuclear receptor subfamily 3 group C member 1)	other	N/A
P33402	Guanylate cyclase soluble subunit alpha-2 (GCS-alpha-2) (EC 4.6.1.2)	other	N/A
O95749	Geranylgeranyl pyrophosphate synthase (GGPP synthase) (GGPPSase) (EC 2.5.1.-) ((2E,6E)-farnesyl diphosphate synthase) (Dimethylallyltranstransferase) (EC 2.5.1.1) (Farnesyl diphosphate synthase) (Farnesyltranstransferase) (EC 2.5.1.29) (Geranylgeranyl diphosphate synthase) (Geranyltranstransferase) (EC 2.5.1.10)	other	N/A
P34896	Serine hydroxymethyltransferase, cytosolic (SHMT) (EC 2.1.2.1) (Glycine hydroxymethyltransferase) (Serine methylase)	other	N/A
P00390	Glutathione reductase, mitochondrial (GR) (GRase) (EC 1.8.1.7)	other	N/A
P49841	Glycogen synthase kinase-3 beta (GSK-3 beta) (EC 2.7.11.26) (Serine/threonine-protein kinase GSK3B) (EC 2.7.11.1)	other	N/A
P69905	Hemoglobin subunit alpha (Alpha-globin) (Hemoglobin alpha chain)	other	N/A
P68871	Hemoglobin subunit beta (Beta-globin) (Hemoglobin beta chain) [Cleaved into: LVV-hemorphin-7; Spinorphin]	other	N/A
Q13547	Histone deacetylase 1 (HD1) (EC 3.5.1.98)	other	N/A
Q92769	Histone deacetylase 2 (HD2) (EC 3.5.1.98)	other	N/A
O15379	Histone deacetylase 3 (HD3) (EC 3.5.1.98) (RPD3-2) (SMAP45)	other	N/A
P56524	Histone deacetylase 4 (HD4) (EC 3.5.1.98)	other	N/A
Q9UBN7	Histone deacetylase 6 (HD6) (EC 3.5.1.98)	other	N/A
Q9UKV0	Histone deacetylase 9 (HD9) (EC 3.5.1.98) (Histone deacetylase 7B) (HD7) (HD7b) (Histone deacetylase-related protein) (MEF2-interacting transcription repressor MITR)	other	N/A
P13716	Delta-aminolevulinic acid dehydratase (ALADH) (EC 4.2.1.24) (Porphobilinogen synthase)	other	N/A
P22830	Ferrochelatase, mitochondrial (EC 4.99.1.1) (Heme synthase) (Protoheme ferro-lyase)	other	N/A
Q16665	Hypoxia-inducible factor 1-alpha (HIF-1-alpha) (HIF1-alpha) (ARNT-interacting protein) (Basic-helix-loop-helix-PAS protein MOP1) (Class E basic helix-loop-helix protein 78) (bHLHe78) (Member of PAS protein 1) (PAS domain-containing protein 8)	other	N/A
P09601	Heme oxygenase 1 (HO-1) (EC 1.14.99.3)	other	N/A
P50135	Histamine N-methyltransferase (HMT) (EC 2.1.1.8)	other	N/A
P32754	4-hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27) (4-hydroxyphenylpyruvic acid oxidase) (4HPPD) (HPD) (HPPDase)	other	N/A
P00492	Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) (HGPRTase) (EC 2.4.2.8)	other	N/A
Q14568	Putative heat shock protein HSP 90-alpha A2 (Heat shock 90 kDa protein 1 alpha-like 3)	other	N/A
P07900	Heat shock protein HSP 90-alpha (Heat shock 86 kDa) (HSP 86) (HSP86) (Renal carcinoma antigen NY-REN-38)	other	N/A
P11142	Heat shock cognate 71 kDa protein (Heat shock 70 kDa protein 8)	other	N/A
P04792	Heat shock protein beta-1 (HspB1) (28 kDa heat shock protein) (Estrogen-regulated 24 kDa protein) (Heat shock 27 kDa protein) (HSP 27) (Stress-responsive protein 27) (SRP27)	other	N/A
P35557	Glucokinase (EC 2.7.1.2) (Hexokinase type IV) (HK IV) (Hexokinase-4) (HK4) (Hexokinase-D)	other	N/A
P14902	Indoleamine 2,3-dioxygenase 1 (IDO-1) (EC 1.13.11.52) (Indoleamine-pyrrole 2,3-dioxygenase)	other	N/A
P14735	Insulin-degrading enzyme (EC 3.4.24.56) (Abeta-degrading protease) (Insulin protease) (Insulinase) (Insulysin)	other	N/A
P06730	Eukaryotic translation initiation factor 4E (eIF-4E) (eIF4E) (eIF-4F 25 kDa subunit) (mRNA cap-binding protein)	other	N/A
O15111	Inhibitor of nuclear factor kappa-B kinase subunit alpha (I-kappa-B kinase alpha) (IKK-A) (IKK-alpha) (IkBKA) (IkappaB kinase) (EC 2.7.11.10) (Conserved helix-loop-helix ubiquitous kinase) (I-kappa-B kinase 1) (IKK1) (Nuclear factor NF-kappa-B inhibitor kinase alpha) (NFKBIKA) (Transcription factor 16) (TCF-16)	other	N/A

O14920	Inhibitor of nuclear factor kappa-B kinase subunit beta (I-kappa-B-kinase beta) (IKK-B) (IKK-beta) (IkBKB) (EC 2.7.11.10) (I-kappa-B kinase 2) (IKK2) (Nuclear factor NF-kappa-B inhibitor kinase beta) (NFKBIKB)	other	N/A
Q14164	Inhibitor of nuclear factor kappa-B kinase subunit epsilon (I-kappa-B kinase epsilon) (IKK-E) (IKK-epsilon) (IkBKE) (EC 2.7.11.10) (Inducible I kappa-B kinase) (IKK-i)	other	N/A
Q14116	Interleukin-18 (IL-18) (Iboctadekin) (Interferon gamma-inducing factor) (IFN-gamma-inducing factor) (Interleukin-1 gamma) (IL-1 gamma)	other	N/A
P01584	Interleukin-1 beta (IL-1 beta) (Catabolin)	other	N/A
P20839	Inosine-5'-monophosphate dehydrogenase 1 (IMP dehydrogenase 1) (IMPD 1) (IMPDH 1) (EC 1.1.1.205) (IMPDH-I)	other	N/A
P12268	Inosine-5'-monophosphate dehydrogenase 2 (IMP dehydrogenase 2) (IMPD 2) (IMPDH 2) (EC 1.1.1.205) (IMPDH-II)	other	N/A
P49895	Type I iodothyronine deiodinase (EC 1.97.1.10) (5DI) (DIOI) (Type 1 DI) (Type-I 5'-deiodinase)	other	N/A
O60674	Tyrosine-protein kinase JAK2 (EC 2.7.10.2) (Janus kinase 2) (JAK-2)	other	N/A
P52333	Tyrosine-protein kinase JAK3 (EC 2.7.10.2) (Janus kinase 3) (JAK-3) (Leukocyte janus kinase) (L-JAK)	other	N/A
P05412	Transcription factor AP-1 (Activator protein 1) (AP1) (Proto-oncogene c-Jun) (V-jun avian sarcoma virus 17 oncogene homolog) (p39)	other	N/A
P12277	Creatine kinase B-type (EC 2.7.3.2) (B-CK) (Creatine kinase B chain)	other	N/A
P06732	Creatine kinase M-type (EC 2.7.3.2) (Creatine kinase M chain) (M-CK) [Cleaved into: Creatine kinase M-type, N-terminally processed]	other	N/A
P17540	Creatine kinase S-type, mitochondrial (EC 2.7.3.2) (Basic-type mitochondrial creatine kinase) (Mib-CK) (Sarcomeric mitochondrial creatine kinase) (S-MtCK)	other	N/A
P12532	Creatine kinase U-type, mitochondrial (EC 2.7.3.2) (Acidic-type mitochondrial creatine kinase) (Mia-CK) (Ubiquitous mitochondrial creatine kinase) (U-MtCK)	other	N/A
P52732	Kinesin-like protein KIF11 (Kinesin-like protein 1) (Kinesin-like spindle protein HKSP) (Kinesin-related motor protein Eg5) (Thyroid receptor-interacting protein 5) (TR-interacting protein 5) (TRIP-5)	other	N/A
P17252	Protein kinase C alpha type (PKC-A) (PKC-alpha) (EC 2.7.11.13)	other	N/A
P05771	Protein kinase C beta type (PKC-B) (PKC-beta) (EC 2.7.11.13)	other	N/A
Q05655	Protein kinase C delta type (EC 2.7.11.13) (Tyrosine-protein kinase PRKCD) (EC 2.7.10.2) (nPKC-delta) [Cleaved into: Protein kinase C delta type regulatory subunit; Protein kinase C delta type catalytic subunit (Sphingosine-dependent protein kinase-1) (SDK1)]	other	N/A
Q02156	Protein kinase C epsilon type (EC 2.7.11.13) (nPKC-epsilon)	other	N/A
P05129	Protein kinase C gamma type (PKC-gamma) (EC 2.7.11.13)	other	N/A
Q04759	Protein kinase C theta type (EC 2.7.11.13) (nPKC-theta)	other	N/A
P23443	Ribosomal protein S6 kinase beta-1 (S6K-beta-1) (S6K1) (EC 2.7.11.1) (70 kDa ribosomal protein S6 kinase 1) (P70S6K1) (p70-S6K 1) (Ribosomal protein S6 kinase I) (Serine/threonine-protein kinase 14A) (p70 ribosomal S6 kinase alpha) (p70 S6 kinase alpha) (p70 S6K-alpha) (p70 S6KA)	other	N/A
P43405	Tyrosine-protein kinase SYK (EC 2.7.10.2) (Spleen tyrosine kinase) (p72-Syk)	other	N/A
P06239	Tyrosine-protein kinase Lck (EC 2.7.10.2) (Leukocyte C-terminal Src kinase) (LSK) (Lymphocyte cell-specific protein-tyrosine kinase) (Protein YT16) (Proto-oncogene Lck) (T cell-specific protein-tyrosine kinase) (p56-LCK)	other	N/A
P09960	Leukotriene A-4 hydrolase (LTA-4 hydrolase) (EC 3.3.2.6) (Leukotriene A(4) hydrolase)	other	N/A
P09917	Arachidonate 5-lipoxygenase (5-LO) (5-lipoxygenase) (EC 1.13.11.34)	other	N/A
P53582	Methionine aminopeptidase 1 (MAP 1) (MetAP 1) (EC 3.4.11.18) (Peptidase M 1)	other	N/A
P78559	Microtubule-associated protein 1A (MAP-1A) (Proliferation-related protein p80) [Cleaved into: MAP1A heavy chain; MAP1 light chain LC2]	other	N/A
P50579	Methionine aminopeptidase 2 (MAP 2) (MetAP 2) (EC 3.4.11.18) (Initiation factor 2-associated 67 kDa glycoprotein) (p67) (p67eIF2) (Peptidase M)	other	N/A
P27448	MAP/microtubule affinity-regulating kinase 3 (EC 2.7.11.1) (C-TAK1) (cTAK1) (Cdc25C-associated protein kinase 1) (ELKL motif kinase 2) (EMK-2) (Protein kinase STK10) (Ser/Thr protein kinase PAR-1) (Par-1a) (Serine/threonine-protein kinase p78)	other	N/A
Q07820	Induced myeloid leukemia cell differentiation protein Mcl-1 (Bcl-2-like protein 3) (Bcl2-L-3) (Bcl-2-related protein EAT/mcl1) (mcl1/EAT)	other	N/A
P08235	Mineralocorticoid receptor (MR) (Nuclear receptor subfamily 3 group C member 2)	other	N/A
Q00987	E3 ubiquitin-protein ligase Mdm2 (EC 6.3.2.-) (Double minute 2 protein) (Hdm2) (Oncoprotein Mdm2) (p53-binding protein Mdm2)	other	N/A
Q99707	Methionine synthase (EC 2.1.1.13) (5-methyltetrahydrofolate--homocysteine	other	N/A

	methyltransferase) (Vitamin-B12 dependent methionine synthase) (MS)		
P14174	Macrophage migration inhibitory factor (MIF) (EC 5.3.2.1) (Glycosylation-inhibiting factor) (GIF) (L-dopachrome isomerase) (L-dopachrome tautomerase) (EC 5.3.3.12) (Phenylpyruvate tautomerase)	other	N/A
P28482	Mitogen-activated protein kinase 1 (MAP kinase 1) (MAPK 1) (EC 2.7.11.24) (ERT1) (Extracellular signal-regulated kinase 2) (ERK-2) (MAP kinase isoform p42) (p42-MAPK) (Mitogen-activated protein kinase 2) (MAP kinase 2) (MAPK 2)	other	N/A
P27361	Mitogen-activated protein kinase 3 (MAP kinase 3) (MAPK 3) (EC 2.7.11.24) (ERT2) (Extracellular signal-regulated kinase 1) (ERK-1) (Insulin-stimulated MAP2 kinase) (MAP kinase isoform p44) (p44-MAPK) (Microtubule-associated protein 2 kinase) (p44-ERK1)	other	N/A
P45983	Mitogen-activated protein kinase 8 (MAP kinase 8) (MAPK 8) (EC 2.7.11.24) (JNK-46) (Stress-activated protein kinase 1c) (SAPK1c) (Stress-activated protein kinase JNK1) (c-Jun N-terminal kinase 1)	other	N/A
P53778	Mitogen-activated protein kinase 12 (MAP kinase 12) (MAPK 12) (EC 2.7.11.24) (Extracellular signal-regulated kinase 6) (ERK-6) (Mitogen-activated protein kinase p38 gamma) (MAP kinase p38 gamma) (Stress-activated protein kinase 3)	other	N/A
Q16539	Mitogen-activated protein kinase 14 (MAP kinase 14) (MAPK 14) (EC 2.7.11.24) (Cytokine suppressive anti-inflammatory drug-binding protein) (CSAID-binding protein) (CSBP) (MAP kinase MXI2) (MAX-interacting protein 2) (Mitogen-activated protein kinase p38 alpha) (MAP kinase p38 alpha) (Stress-activated protein kinase 2a) (SAPK2a)	other	N/A
P34949	Mannose-6-phosphate isomerase (EC 5.3.1.8) (Phosphohexomutase) (Phosphomannose isomerase) (PMI)	other	N/A
P11137	Microtubule-associated protein 2 (MAP-2)	other	N/A
P42345	Serine/threonine-protein kinase mTOR (EC 2.7.11.1) (FK506-binding protein 12-rapamycin complex-associated protein 1) (FKBP12-rapamycin complex-associated protein) (Mammalian target of rapamycin) (mTOR) (Mechanistic target of rapamycin) (Rapamycin and FKBP12 target 1) (Rapamycin target protein 1)	other	N/A
P22033	Methylmalonyl-CoA mutase, mitochondrial (MCM) (EC 5.4.99.2) (Methylmalonyl-CoA isomerase)	other	N/A
P10242	Transcriptional activator Myb (Proto-oncogene c-Myb)	other	N/A
P01106	Myc proto-oncogene protein (Class E basic helix-loop-helix protein 39) (bHLHe39) (Proto-oncogene c-Myc) (Transcription factor p64)	other	N/A
Q15274	Nicotinate-nucleotide pyrophosphorylase [carboxylating] (EC 2.4.2.19) (Quinolinate phosphoribosyltransferase [decarboxylating]) (QAPRTase) (QPRTase)	other	N/A
P19838	Nuclear factor NF-kappa-B p105 subunit (DNA-binding factor KBF1) (EBP-1) (Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1) [Cleaved into: Nuclear factor NF-kappa-B p50 subunit]	other	N/A
Q00653	Nuclear factor NF-kappa-B p100 subunit (DNA-binding factor KBF2) (H2TF1) (Lymphocyte translocation chromosome 10 protein) (Nuclear factor of kappa light polypeptide gene enhancer in B-cells 2) (Oncogene Lyt-10) (Lyt10) [Cleaved into: Nuclear factor NF-kappa-B p52 subunit]	other	N/A
P40261	Nicotinamide N-methyltransferase (EC 2.1.1.1)	other	N/A
P29475	Nitric oxide synthase, brain (EC 1.14.13.39) (Constitutive NOS) (NC-NOS) (NOS type I) (Neuronal NOS) (N-NOS) (nNOS) (Peptidyl-cysteine S-nitrosylase NOS1) (bNOS)	other	N/A
P35228	Nitric oxide synthase, inducible (EC 1.14.13.39) (Hepatocyte NOS) (HEP-NOS) (Inducible NO synthase) (Inducible NOS) (iNOS) (NOS type II) (Peptidyl-cysteine S-nitrosylase NOS2)	other	N/A
P29474	Nitric oxide synthase, endothelial (EC 1.14.13.39) (Constitutive NOS) (cNOS) (EC-NOS) (Endothelial NOS) (eNOS) (NOS type III) (NOSIII)	other	N/A
Q8NFA2	NADPH oxidase organizer 1 (NADPH oxidase regulatory protein) (Nox organizer 1) (Nox-organizing protein 1) (SH3 and PX domain-containing protein 5)	other	N/A
P06748	Nucleophosmin (NPM) (Nucleolar phosphoprotein B23) (Nucleolar protein NO38) (Numatrin)	other	N/A
P15559	NAD(P)H dehydrogenase [quinone] 1 (EC 1.6.5.2) (Azoreductase) (DT-diaphorase) (DTD) (Menadione reductase) (NAD(P)H:quinone oxidoreductase 1) (Phylloquinone reductase) (Quinone reductase 1) (QR1)	other	N/A
P16083	Ribosyldihydronicotinamide dehydrogenase [quinone] (EC 1.10.99.2) (NRH dehydrogenase [quinone] 2) (NRH:quinone oxidoreductase 2) (Quinone reductase 2) (QR2)	other	N/A
O75469	Nuclear receptor subfamily 1 group I member 2 (Orphan nuclear receptor PAR1) (Orphan nuclear receptor PXR) (Pregnane X receptor) (Steroid and xenobiotic receptor) (SXR)	other	N/A
Q96RI1	Bile acid receptor (Farnesoid X-activated receptor) (Farnesol receptor HRR-1) (Nuclear receptor subfamily 1 group H member 4) (Retinoid X receptor-interacting protein 14) (RXR-interacting protein 14)	other	N/A
Q14994	Nuclear receptor subfamily 1 group I member 3 (Constitutive activator of retinoid response) (Constitutive active response) (Constitutive androstane receptor) (CAR) (Orphan nuclear receptor MB67)	other	N/A
Q9NXE4	Sphingomyelin phosphodiesterase 4 (EC 3.1.4.12) (Neutral sphingomyelinase 3) (nSMase-3) (nSMase3) (Neutral sphingomyelinase III)	other	N/A

P04637	Cellular tumor antigen p53 (Antigen NY-CO-13) (Phosphoprotein p53) (Tumor suppressor p53)	other	N/A
P47712	Cytosolic phospholipase A2 (cPLA2) (Phospholipase A2 group IVA) [Includes: Phospholipase A2 (EC 3.1.1.4) (Phosphatidylcholine 2-acylhydrolase); Lysophospholipase (EC 3.1.1.5)]	other	N/A
P09874	Poly [ADP-ribose] polymerase 1 (PARP-1) (EC 2.4.2.30) (ADP-ribosyltransferase diphtheria toxin-like 1) (ARTD1) (NAD(+) ADP-ribosyltransferase 1) (ADPRT 1) (Poly[ADP-ribose] synthase 1)	other	N/A
Q15154	Pericentriolar material 1 protein (PCM-1) (hPCM-1)	other	N/A
Q9Y233	cAMP and cAMP-inhibited cGMP 3',5'-cyclic phosphodiesterase 10A (EC 3.1.4.17) (EC 3.1.4.35)	other	N/A
P54750	Calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase 1A (Cam-PDE 1A) (EC 3.1.4.17) (61 kDa Cam-PDE) (hCam-1)	other	N/A
Q01064	Calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase 1B (Cam-PDE 1B) (EC 3.1.4.17) (63 kDa Cam-PDE)	other	N/A
Q14123	Calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase 1C (Cam-PDE 1C) (EC 3.1.4.17) (hCam-3)	other	N/A
P27815	cAMP-specific 3',5'-cyclic phosphodiesterase 4A (EC 3.1.4.53) (DPDE2) (PDE46)	other	N/A
Q07343	cAMP-specific 3',5'-cyclic phosphodiesterase 4B (EC 3.1.4.53) (DPDE4) (PDE32)	other	N/A
Q08493	cAMP-specific 3',5'-cyclic phosphodiesterase 4C (EC 3.1.4.53) (DPDE1) (PDE21)	other	N/A
Q08499	cAMP-specific 3',5'-cyclic phosphodiesterase 4D (EC 3.1.4.53) (DPDE3) (PDE43)	other	N/A
O76074	cGMP-specific 3',5'-cyclic phosphodiesterase (EC 3.1.4.35) (cGMP-binding cGMP-specific phosphodiesterase) (CGB-PDE)	other	N/A
Q13946	High affinity cAMP-specific 3',5'-cyclic phosphodiesterase 7A (EC 3.1.4.53) (HCP1) (TM22)	other	N/A
Q9NP56	cAMP-specific 3',5'-cyclic phosphodiesterase 7B (EC 3.1.4.53)	other	N/A
O95263	High affinity cAMP-specific and IBMX-insensitive 3',5'-cyclic phosphodiesterase 8B (HsPDE8B) (EC 3.1.4.53) (Cell proliferation-inducing gene 22 protein)	other	N/A
O60658	High affinity cAMP-specific and IBMX-insensitive 3',5'-cyclic phosphodiesterase 8A (EC 3.1.4.53)	other	N/A
Q15118	[Pyruvate dehydrogenase (acetyl-transferring)] kinase isozyme 1, mitochondrial (EC 2.7.11.2) (Pyruvate dehydrogenase kinase isoform 1) (PDH kinase 1)	other	N/A
O00764	Pyridoxal kinase (EC 2.7.1.35) (Pyridoxine kinase)	other	N/A
P00439	Phenylalanine-4-hydroxylase (PAH) (EC 1.14.16.1) (Phe-4-monooxygenase)	other	N/A
P48736	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit gamma isoform (PI3-kinase subunit gamma) (PI3K-gamma) (PI3Kgamma) (PtdIns-3-kinase subunit gamma) (EC 2.7.1.153) (Phosphatidylinositol 4,5-bisphosphate 3-kinase 110 kDa catalytic subunit gamma) (PtdIns-3-kinase subunit p110-gamma) (p110gamma) (Phosphoinositide-3-kinase catalytic gamma polypeptide) (Serine/threonine protein kinase PIK3CG) (EC 2.7.11.1) (p120-PI3K)	other	N/A
Q15111	Inactive phospholipase C-like protein 1 (PLC-L1) (Phospholipase C-deleted in lung carcinoma) (Phospholipase C-related but catalytically inactive protein) (PRIP)	other	N/A
O60664	Perilipin-3 (47 kDa mannose 6-phosphate receptor-binding protein) (47 kDa MPR-binding protein) (Cargo selection protein TIP47) (Mannose-6-phosphate receptor-binding protein 1) (Placental protein 17) (PP17)	other	N/A
P53350	Serine/threonine-protein kinase PLK1 (EC 2.7.11.21) (Polo-like kinase 1) (PLK-1) (Serine/threonine-protein kinase 13) (STPK13)	other	N/A
O60733	85/88 kDa calcium-independent phospholipase A2 (CaI-PLA2) (EC 3.1.1.4) (Group VI phospholipase A2) (GVI PLA2) (Intracellular membrane-associated calcium-independent phospholipase A2 beta) (iPLA2-beta) (Patatin-like phospholipase domain-containing protein 9) (PNPLA9)	other	N/A
Q96GD0	Pyridoxal phosphate phosphatase (PLP phosphatase) (EC 3.1.3.3) (EC 3.1.3.74) (Chronophin)	other	N/A
Q6P1K2	Polyamine-modulated factor 1 (PMF-1)	other	N/A
P29590	Protein PML (Promyelocytic leukemia protein) (RING finger protein 71) (Tripartite motif-containing protein 19)	other	N/A
P00491	Purine nucleoside phosphorylase (PNP) (EC 2.4.2.1) (Inosine phosphorylase) (Inosine-guanosine phosphorylase)	other	N/A
Q07869	Peroxisome proliferator-activated receptor alpha (PPAR-alpha) (Nuclear receptor subfamily 1 group C member 1)	other	N/A
Q03181	Peroxisome proliferator-activated receptor delta (PPAR-delta) (NUCI) (Nuclear hormone receptor 1) (NUC1) (Nuclear receptor subfamily 1 group C member 2) (Peroxisome proliferator-activated receptor beta) (PPAR-beta)	other	N/A
P62937	Peptidyl-prolyl cis-trans isomerase A (PPIase A) (EC 5.2.1.8) (Cyclophilin A) (Cyclosporin A-binding protein) (Rotamase A) [Cleaved into: Peptidyl-prolyl cis-trans isomerase A, N-terminally processed]	other	N/A

P30044	Peroxiredoxin-5, mitochondrial (EC 1.11.1.15) (Alu corepressor 1) (Antioxidant enzyme B166) (AOEB166) (Liver tissue 2D-page spot 71B) (PLP) (Peroxiredoxin V) (Prx-V) (Peroxisomal antioxidant enzyme) (TPx type VI) (Thioredoxin peroxidase PMP20) (Thioredoxin reductase)	other	N/A
P06401	Progesterone receptor (PR) (Nuclear receptor subfamily 3 group C member 3)	other	N/A
P55786	Puromycin-sensitive aminopeptidase (PSA) (EC 3.4.11.14) (Cytosol alanyl aminopeptidase) (AAP-S)	other	N/A
P20618	Proteasome subunit beta type-1 (EC 3.4.25.1) (Macropain subunit C5) (Multicatalytic endopeptidase complex subunit C5) (Proteasome component C5) (Proteasome gamma chain)	other	N/A
P49721	Proteasome subunit beta type-2 (EC 3.4.25.1) (Macropain subunit C7-I) (Multicatalytic endopeptidase complex subunit C7-I) (Proteasome component C7-I)	other	N/A
P28074	Proteasome subunit beta type-5 (EC 3.4.25.1) (Macropain epsilon chain) (Multicatalytic endopeptidase complex epsilon chain) (Proteasome chain 6) (Proteasome epsilon chain) (Proteasome subunit MB1) (Proteasome subunit X)	other	N/A
Q99460	26S proteasome non-ATPase regulatory subunit 1 (26S proteasome regulatory subunit RPN2) (26S proteasome regulatory subunit S1) (26S proteasome subunit p112)	other	N/A
Q13200	26S proteasome non-ATPase regulatory subunit 2 (26S proteasome regulatory subunit RPN1) (26S proteasome regulatory subunit S2) (26S proteasome subunit p97) (Protein 55.11) (Tumor necrosis factor type 1 receptor-associated protein 2)	other	N/A
Q16647	Prostacyclin synthase (EC 5.3.99.4) (Prostaglandin I2 synthase)	other	N/A
P18031	Tyrosine-protein phosphatase non-receptor type 1 (EC 3.1.3.48) (Protein-tyrosine phosphatase 1B) (PTP-1B)	other	N/A
P29350	Tyrosine-protein phosphatase non-receptor type 6 (EC 3.1.3.48) (Hematopoietic cell protein-tyrosine phosphatase) (Protein-tyrosine phosphatase 1C) (PTP-1C) (Protein-tyrosine phosphatase SHP-1) (SH-PTP1)	other	N/A
P22102	Trifunctional purine biosynthetic protein adenosine-3 [Includes: Phosphoribosylamine--glycine ligase (EC 6.3.4.13) (Glycinamide ribonucleotide synthetase) (GARS) (Phosphoribosylglycinamide synthetase); Phosphoribosylformylglycinamidine cyclo-ligase (EC 6.3.3.1) (AIR synthase) (AIRS) (Phosphoribosyl-aminoimidazole synthetase); Phosphoribosylglycinamide formyltransferase (EC 2.1.2.2) (5'-phosphoribosylglycinamide transformylase) (GAR transformylase) (GART)]	other	N/A
P31939	Bifunctional purine biosynthesis protein PURH [Includes: Phosphoribosylaminoimidazolecarboxamide formyltransferase (EC 2.1.2.3) (5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase) (AICAR transformylase); IMP cyclohydrolase (EC 3.5.4.10) (ATIC) (IMP synthase) (Inosinicase)]	other	N/A
P11217	Glycogen phosphorylase, muscle form (EC 2.4.1.1) (Myophosphorylase)	other	N/A
Q02127	Dihydroorotate dehydrogenase (quinone), mitochondrial (DHOdehase) (EC 1.3.5.2) (Dihydroorotate oxidase)	other	N/A
P04049	RAF proto-oncogene serine/threonine-protein kinase (EC 2.7.11.1) (Proto-oncogene c-RAF) (cRaf) (Raf-1)	other	N/A
P10276	Retinoic acid receptor alpha (RAR-alpha) (Nuclear receptor subfamily 1 group B member 1)	other	N/A
P10826	Retinoic acid receptor beta (RAR-beta) (HBV-activated protein) (Nuclear receptor subfamily 1 group B member 2) (RAR-epsilon)	other	N/A
P13631	Retinoic acid receptor gamma (RAR-gamma) (Nuclear receptor subfamily 1 group B member 3)	other	N/A
Q969G6	Riboflavin kinase (EC 2.7.1.26) (ATP:riboflavin 5'-phosphotransferase) (Flavokinase)	other	N/A
P23921	Ribonucleoside-diphosphate reductase large subunit (EC 1.17.4.1) (Ribonucleoside-diphosphate reductase subunit M1) (Ribonucleotide reductase large subunit)	other	N/A
Q7LG56	Ribonucleoside-diphosphate reductase subunit M2 B (EC 1.17.4.1) (TP53-inducible ribonucleotide reductase M2 B) (p53-inducible ribonucleotide reductase small subunit 2-like protein) (p53R2)	other	N/A
P31350	Ribonucleoside-diphosphate reductase subunit M2 (EC 1.17.4.1) (Ribonucleotide reductase small chain) (Ribonucleotide reductase small subunit)	other	N/A
Q13464	Rho-associated protein kinase 1 (EC 2.7.11.1) (Renal carcinoma antigen NY-REN-35) (Rho-associated, coiled-coil-containing protein kinase 1) (Rho-associated, coiled-coil-containing protein kinase I) (ROCK-I) (p160 ROCK-1) (p160ROCK)	other	N/A
P19793	Retinoic acid receptor RXR-alpha (Nuclear receptor subfamily 2 group B member 1) (Retinoid X receptor alpha)	other	N/A
P28702	Retinoic acid receptor RXR-beta (Nuclear receptor subfamily 2 group B member 2) (Retinoid X receptor beta)	other	N/A
P48443	Retinoic acid receptor RXR-gamma (Nuclear receptor subfamily 2 group B member 3) (Retinoid X receptor gamma)	other	N/A
Q96EB6	NAD-dependent protein deacetylase sirtuin-1 (hSIRT1) (EC 3.5.1.-) (Regulatory protein SIR2 homolog 1) (SIR2-like protein 1) (hSIR2) [Cleaved into: SirtT1 75 kDa fragment (75SirT1)]	other	N/A
Q9NXA8	NAD-dependent protein deacylase sirtuin-5, mitochondrial (EC 3.5.1.-) (Regulatory protein SIR2 homolog 5) (SIR2-like protein 5)	other	N/A

Q9NWM0	Spermine oxidase (EC 1.5.3.16) (Polyamine oxidase 1) (PAO-1) (PAOh1)	other	N/A
P60880	Synaptosomal-associated protein 25 (SNAP-25) (Super protein) (SUP) (Synaptosomal-associated 25 kDa protein)	other	N/A
P00441	Superoxide dismutase [Cu-Zn] (EC 1.15.1.1) (Superoxide dismutase 1) (hSod1)	other	N/A
Q9NYA1	Sphingosine kinase 1 (SK 1) (SPK 1) (EC 2.7.1.91)	other	N/A
P52788	Spermine synthase (SPMSY) (EC 2.5.1.22) (Spermidine aminopropyltransferase)	other	N/A
P12931	Proto-oncogene tyrosine-protein kinase Src (EC 2.7.10.2) (Proto-oncogene c-Src) (pp60c-src) (p60-Src)	other	N/A
P51649	Succinate-semialdehyde dehydrogenase, mitochondrial (EC 1.2.1.24) (Aldehyde dehydrogenase family 5 member A1) (NAD(+)-dependent succinic semialdehyde dehydrogenase)	other	N/A
P42224	Signal transducer and activator of transcription 1-alpha/beta (Transcription factor ISGF-3 components p91/p84)	other	N/A
P40763	Signal transducer and activator of transcription 3 (Acute-phase response factor)	other	N/A
Q8N9I0	Synaptotagmin-2 (Synaptotagmin II) (SytII)	other	N/A
Q71U36	Tubulin alpha-1A chain (Alpha-tubulin 3) (Tubulin B-alpha-1) (Tubulin alpha-3 chain)	other	N/A
P68363	Tubulin alpha-1B chain (Alpha-tubulin ubiquitous) (Tubulin K-alpha-1) (Tubulin alpha-ubiquitous chain)	other	N/A
P68366	Tubulin alpha-4A chain (Alpha-tubulin 1) (Testis-specific alpha-tubulin) (Tubulin H2-alpha) (Tubulin alpha-1 chain)	other	N/A
Q9H4B7	Tubulin beta-1 chain	other	N/A
Q13509	Tubulin beta-3 chain (Tubulin beta-4 chain) (Tubulin beta-III)	other	N/A
P07437	Tubulin beta chain (Tubulin beta-5 chain)	other	N/A
Q9UJT1	Tubulin delta chain (Delta-tubulin)	other	N/A
Q9UJT0	Tubulin epsilon chain (Epsilon-tubulin)	other	N/A
P23258	Tubulin gamma-1 chain (Gamma-1-tubulin) (Gamma-tubulin complex component 1) (GCP-1)	other	N/A
Q99973	Telomerase protein component 1 (Telomerase-associated protein 1) (Telomerase protein 1) (p240) (p80 telomerase homolog)	other	N/A
Q92734	Protein TFG (TRK-fused gene protein)	other	N/A
P21980	Protein-glutamine gamma-glutamyltransferase 2 (EC 2.3.2.13) (Tissue transglutaminase) (Transglutaminase C) (TG(C)) (TGC) (TGase C) (Transglutaminase H) (TGase H) (Transglutaminase-2) (TGase-2)	other	N/A
P10827	Thyroid hormone receptor alpha (Nuclear receptor subfamily 1 group A member 1) (V-erbA-related protein 7) (EAR-7) (c-erbA-1) (c-erbA-alpha)	other	N/A
P10828	Thyroid hormone receptor beta (Nuclear receptor subfamily 1 group A member 2) (c-erbA-2) (c-erbA-beta)	other	N/A
P24752	Acetyl-CoA acetyltransferase, mitochondrial (EC 2.3.1.9) (Acetoacetyl-CoA thiolase) (T2)	other	N/A
P10599	Thioredoxin (Trx) (ATL-derived factor) (ADF) (Surface-associated sulphydryl protein) (SASP)	other	N/A
P29401	Transketolase (TK) (EC 2.2.1.1)	other	N/A
P63316	Troponin C, slow skeletal and cardiac muscles (TN-C)	other	N/A
P02585	Troponin C, skeletal muscle	other	N/A
Q969P6	DNA topoisomerase I, mitochondrial (TOP1mt) (EC 5.99.1.2)	other	N/A
P11387	DNA topoisomerase 1 (EC 5.99.1.2) (DNA topoisomerase I)	other	N/A
P11388	DNA topoisomerase 2-alpha (EC 5.99.1.3) (DNA topoisomerase II, alpha isozyme)	other	N/A
Q02880	DNA topoisomerase 2-beta (EC 5.99.1.3) (DNA topoisomerase II, beta isozyme)	other	N/A
P17752	Tryptophan 5-hydroxylase 1 (EC 1.14.16.4) (Tryptophan 5-monooxygenase 1)	other	N/A
Q9H3S4	Thiamin pyrophosphokinase 1 (hTPK1) (EC 2.7.6.2) (Placental protein 20) (PP20) (Thiamine pyrophosphokinase 1)	other	N/A
P51580	Thiopurine S-methyltransferase (EC 2.1.1.67) (Thiopurine methyltransferase)	other	N/A
P29144	Tripeptidyl-peptidase 2 (TPP-2) (EC 3.4.14.10) (Tripeptidyl aminopeptidase) (Tripeptidyl-peptidase II) (TPP-II)	other	N/A
P12270	Nucleoprotein TPR (Megator) (NPC-associated intranuclear protein) (Translocated promoter region protein)	other	N/A

Q16881	Thioredoxin reductase 1, cytoplasmic (TR) (EC 1.8.1.9) (Gene associated with retinoic and interferon-induced mortality 12 protein) (GRIM-12) (Gene associated with retinoic and IFN-induced mortality 12 protein) (KM-102-derived reductase-like factor) (Thioredoxin reductase TR1)	other	N/A
B1AH88	Putative peripheral benzodiazepine receptor-related protein	other	N/A
P30536	Translocator protein (Mitochondrial benzodiazepine receptor) (PKBS) (Peripheral-type benzodiazepine receptor) (PBR)	other	N/A
P07101	Tyrosine 3-monooxygenase (EC 1.14.16.2) (Tyrosine 3-hydroxylase) (TH)	other	N/A
P04818	Thymidylate synthase (TS) (TSase) (EC 2.1.1.45)	other	N/A
Q8TBC4	NEDD8-activating enzyme E1 catalytic subunit (EC 6.3.2.-) (NEDD8-activating enzyme E1C) (Ubiquitin-activating enzyme E1C) (Ubiquitin-like modifier-activating enzyme 3) (Ubiquitin-activating enzyme 3)	other	N/A
P23763	Vesicle-associated membrane protein 1 (VAMP-1) (Synaptobrevin-1)	other	N/A
P63027	Vesicle-associated membrane protein 2 (VAMP-2) (Synaptobrevin-2)	other	N/A
P38606	V-type proton ATPase catalytic subunit A (V-ATPase subunit A) (EC 3.6.3.14) (V-ATPase 69 kDa subunit) (Vacuolar ATPase isoform VA68) (Vacuolar proton pump subunit alpha)	other	N/A
P21281	V-type proton ATPase subunit B, brain isoform (V-ATPase subunit B 2) (Endomembrane proton pump 58 kDa subunit) (HO57) (Vacuolar proton pump subunit B 2)	other	N/A
P45880	Voltage-dependent anion-selective channel protein 2 (VDAC-2) (hVDAC2) (Outer mitochondrial membrane protein porin 2)	other	N/A
Q9Y277	Voltage-dependent anion-selective channel protein 3 (VDAC-3) (hVDAC3) (Outer mitochondrial membrane protein porin 3)	other	N/A
P11473	Vitamin D3 receptor (VDR) (1,25-dihydroxyvitamin D3 receptor) (Nuclear receptor subfamily 1 group I member 1)	other	N/A
P30291	Wee1-like protein kinase (WEE1hu) (EC 2.7.10.2) (Wee1A kinase)	other	N/A
P47989	Xanthine dehydrogenase/oxidase [Includes: Xanthine dehydrogenase (XD) (EC 1.17.1.4); Xanthine oxidase (XO) (EC 1.17.3.2) (Xanthine oxidoreductase) (XOR)]	other	N/A
P98170	E3 ubiquitin-protein ligase XIAP (EC 6.3.2.-) (Baculoviral IAP repeat-containing protein 4) (IAP-like protein) (ILP) (hILP) (Inhibitor of apoptosis protein 3) (IAP-3) (hIAP-3) (hIAP3) (X-linked inhibitor of apoptosis protein) (X-linked IAP)	other	N/A
O95477	ATP-binding cassette sub-family A member 1 (ATP-binding cassette transporter 1) (ABC-1) (ATP-binding cassette 1) (Cholesterol efflux regulatory protein)	SP	N/A
P05091	Aldehyde dehydrogenase, mitochondrial (EC 1.2.1.3) (ALDH class 2) (ALDH-E2) (ALDHI)	SP	N/A
P05120	Plasminogen activator inhibitor 2 (PAI-2) (Monocyte Arg-serpin) (Placental plasminogen activator inhibitor) (Serpin B2) (Urokinase inhibitor)	SP	N/A
Q9NY47	Voltage-dependent calcium channel subunit alpha-2/delta-2 (Voltage-gated calcium channel subunit alpha-2/delta-2) [Cleaved into: Voltage-dependent calcium channel subunit alpha-2-2; Voltage-dependent calcium channel subunit delta-2]	type I	N/A
P03372	Estrogen receptor (ER) (ER-alpha) (Estradiol receptor) (Nuclear receptor subfamily 3 group A member 1)	type I	N/A
P15382	Potassium voltage-gated channel subfamily E member 1 (Delayed rectifier potassium channel subunit IsK) (IKs producing slow voltage-gated potassium channel subunit beta Mink) (Minimal potassium channel)	type I	N/A
Q16853	Membrane primary amine oxidase (EC 1.4.3.21) (Copper amine oxidase) (HPAO) (Semicarbazide-sensitive amine oxidase) (SSAO) (Vascular adhesion protein 1) (VAP-1)	type II	N/A
P32970	CD70 antigen (CD27 ligand) (CD27-L) (Tumor necrosis factor ligand superfamily member 7) (CD antigen CD70)	type II	N/A
P21964	Catechol O-methyltransferase (EC 2.1.1.6)	type II	N/A
P28845	Corticosteroid 11-beta-dehydrogenase isozyme 1 (EC 1.1.1.146) (11-beta-hydroxysteroid dehydrogenase 1) (11-DH) (11-beta-HSD1)	type II	N/A
P09172	Dopamine beta-hydroxylase (EC 1.14.17.1) (Dopamine beta-monooxygenase) [Cleaved into: Soluble dopamine beta-hydroxylase]	type II	N/A
P27487	Dipeptidyl peptidase 4 (EC 3.4.14.5) (ADABP) (Adenosine deaminase complexing protein 2) (ADCP-2) (Dipeptidyl peptidase IV) (DPP IV) (T-cell activation antigen CD26) (TP103) (CD antigen CD26) [Cleaved into: Dipeptidyl peptidase 4 membrane form (Dipeptidyl peptidase IV membrane form); Dipeptidyl peptidase 4 soluble form (Dipeptidyl peptidase IV soluble form)]	type II	N/A
Q04609	Glutamate carboxypeptidase 2 (EC 3.4.17.21) (Cell growth-inhibiting gene 27 protein) (Folate hydrolase 1) (Folylpoly-gamma-glutamate carboxypeptidase) (FGCP) (Glutamate carboxypeptidase II) (GCPII) (Membrane glutamate carboxypeptidase) (mGCP) (N-acetylated-alpha-linked acidic dipeptidase I) (NAALADase I) (Prostate-specific membrane antigen) (PSM) (PSMA) (Pteroylpoly-gamma-glutamate carboxypeptidase)	type II	N/A
P05981	Serine protease hepsin (EC 3.4.21.106) (Transmembrane protease serine 1) [Cleaved into: Serine protease hepsin non-catalytic chain; Serine protease hepsin catalytic chain]	type II	N/A
O43451	Maltase-glucoamylase, intestinal [Includes: Maltase (EC 3.2.1.20) (Alpha-glucosidase); Glucoamylase (EC 3.2.1.3) (Glucan 1,4-alpha-glucosidase)]	type II	N/A

O75900	Matrix metalloproteinase-23 (MMP-23) (EC 3.4.24.-) (Femalysin) (MIFR-1) (Matrix metalloproteinase-21) (MMP-21) (Matrix metalloproteinase-22) (MMP-22) [Cleaved into: Matrix metalloproteinase-23, soluble form]	type II	N/A
P08473	Neprilysin (EC 3.4.24.11) (Atriopeptidase) (Common acute lymphocytic leukemia antigen) (CALLA) (Enkephalinase) (Neutral endopeptidase 24.11) (NEP) (Neutral endopeptidase) (Skin fibroblast elastase) (SFE) (CD antigen CD10)	type II	N/A
P14410	Sucrase-isomaltase, intestinal [Cleaved into: Sucrase (EC 3.2.1.48); Isomaltase (EC 3.2.1.10)]	type II	N/A
Q9Y275	Tumor necrosis factor ligand superfamily member 13B (B lymphocyte stimulator) (BLyS) (B-cell-activating factor) (BAFF) (Dendritic cell-derived TNF-like molecule) (TNF- and APOL-related leukocyte expressed ligand 1) (TALL-1) (CD antigen CD257) [Cleaved into: Tumor necrosis factor ligand superfamily member 13b, membrane form; Tumor necrosis factor ligand superfamily member 13b, soluble form]	type II	N/A
O14788	Tumor necrosis factor ligand superfamily member 11 (Osteoclast differentiation factor) (ODF) (Osteoprotegerin ligand) (OPGL) (Receptor activator of nuclear factor kappa-B ligand) (RANKL) (TNF-related activation-induced cytokine) (TRANCE) (CD antigen CD254) [Cleaved into: Tumor necrosis factor ligand superfamily member 11, membrane form; Tumor necrosis factor ligand superfamily member 11, soluble form]	type II	N/A
P01375	Tumor necrosis factor (Cachectin) (TNF-alpha) (Tumor necrosis factor ligand superfamily member 2) (TNF-a) [Cleaved into: Tumor necrosis factor, membrane form (N-terminal fragment) (NTF); Intracellular domain 1 (ICD1); Intracellular domain 2 (ICD2); C-domain 1; C-domain 2; Tumor necrosis factor, soluble form]	type II	N/A
P07947	Tyrosine-protein kinase Yes (EC 2.7.10.2) (Proto-oncogene c-Yes) (p61-Yes)	other	N/A

Appendix A | Drug targets from Okada et al.[1] Official gene symbols from Okada et al. were converted to Swiss-Prot accession numbers using the DAVID Gene ID Conversion Tool (http://david.abcc.ncifcrf.gov/conversion.jsp).[2,3] Proteins were annotated as signal peptide-containing (SP), type I, type II, type III or multi-TM (with no signal peptide) according to the procedures outlined in Chapter 2. Other = Sec61 independent. ΔG_{sub} was calculated for proteins with signal peptides. Red text indicates proteins whose ΔG_{sub} value falls in the top 10% and blue text indicates proteins whose ΔG_{sub} value falls in the top 25%. Proteins with ΔG_{sub} values are ranked from highest to lowest ΔG_{sub}. The rest was organized by type.

References

1. Okada, Y., Wu, D., Trynka, G., Raj, T. & Terao, C. Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* online (2013).

2. Huang, D. W., Sherman, B. T. & Lempicki, R. A. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Research* **37,** 1–13 (2009)

3. Huang, D. W., Sherman, B. T. & Lempicki, R. A. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* **4,** 44–57 (2008).

Appendix B

Source code for fasta_segment.py

```
import sys
import re
import csv

def Main():

      if (len(sys.argv) < 3):
            print 'I take two files. The first: .CSV file containing
Accession, From and To columns. The second: .TXT file containing full
protein sequences in FASTA format. '
            print 'Example: python fasta_segment.py Accession_From_To.csv
sequences_FASTA.txt'
            exit(0)

      fastaData = {}
      fastaline = ""
      fastaacc  = ""

      with open(sys.argv[2], "r") as infile:
            for line in infile:
                  match = re.match(r"\>sp\|([A-Z0-9]+)\|", line)
                  if (match != None):
                        if ( len(fastaacc) > 0 and len(fastaline) > 0 ):
                              fastaData[fastaacc] = fastaline
                        fastaacc = match.group(1)
                        fastaline = ""
                  else:
                        fastaline += line.strip()

      fastaData[fastaacc] = fastaline

      # read sample from csv file to detect its dialect
      with open(sys.argv[1], "rt") as f:
            header = f.readline()

      csvDialectSniffer = csv.Sniffer()
      dialect = csvDialectSniffer.sniff(header, delimiters=' ,\t')

      f = open(sys.argv[1], "rt")

      csvReader = csv.DictReader(f, dialect=dialect)

      outFileName = "output.csv"
      if (len(sys.argv)>3):
            outFileName = sys.argv[3]

      fields = csvReader.fieldnames
      fields = fields + ["Sequence"]

      csvWriter = csv.DictWriter( open(outFileName,'w'), dialect=dialect,
delimiter='\t', quotechar='"', fieldnames=fields, escapechar='\\',
quoting=csv.QUOTE_ALL )
      csvWriter.writerow(dict((fn,fn) for fn in fields))
```

```
      for row in csvReader:
            From = int(row["from"])
            From -= 1
            To = int(row["to"])
            seq = fastaData[row["Accession"]]
            if ( (len(seq) >= To > From >= 0) ):
                  row["Sequence"] = seq[From:To]
            else:
                  row["Sequence"] = ""
                  print "Warning: Not found sequence for
"+str(row["Accession"])
            csvWriter.writerow(row)

      f.close()



Main()
```

Appendix C

List of plasmids

Plasmid #	Protein	Vector	Source
pRLM-5	Bip	clontech N1	Eric Snapp, Albert Einstein
pRLM-3	ITAV	pBJ-1	Yoshikazu Takada, M.D., Ph.D., UC Davis
pRLM-8	ERdj3	clontech N1	Eric Snapp, Albert Einstein
pRLM-10	$p58^{ipk}$	pCDNA	Ramanujan Hegde, MRC, LMB, Cambridge
pRLM-18	pCMV-3XFLAG	p3XFLAG-CMV	Sigma (E7783)
pRLM-28	ITB5	pWzl	Dean Sheppard, UCSF
pRLM-31	TRPML1	pDONR221	Harvardplasmid database (HSCD00040401)
pRLM-36	CD74	pDONR221	Harvard plasmid database (HsCD00040956)
pRLM-58	HGF	pBABE puro	Addgene (http://www.addgene.org/10901/)
pRLM-61	p8.91 (encodes gag, pol, rev, tat HIV genes)		Dyche Mullins, UCSF, Harvard plasmid database EvNO00438081
pRLM-62	pMD2.G (encodes VSV-G envelope protein)		Dyche Mullins, UCSF, Addgene 12259
pRLM-65	IL2RG	pLX304	DNASU (HsCD00438048)
pRLM-68	control shRNA	pKLO.1-puro	Kevan Shokat, UCSF, Sigma (SHC016)
pRLM-79	Sec61a R66I	pCDNA5- FRT-TO	Jack Taunton, UCSF
pRLM-80	Sec61a WT	pCDNA5- FRT-TO	Jack Taunton, UCSF
pRLM-83	BID_shortEF1alpha-mCherry_minhCMV-GFP	pSicoR/mCherry	UCSF Viracore/McManus Lab
pRLM-151	CD3Z	pGEM	Art Weiss, UCSF
pRLM-153	IL3RA	pDONR221	DNASU (HsCD00076065)
pRLM-154	TNR11	pENTR223.1	DNASU (HsCD00297094)
pRLM-156	IL7R	pANT7_cGST	DNASU (HsCD00356394)
pRLM-158	IL7	pANT7_cGST	DNASU (HsCD00305386)
pRLM-159	CD3G		Art Weiss, UCSF
pRLM-163	AREG	pANT7_cGST	DNASU (HsCD00078799)
pRLM-164	TNFB	pANT7_cGST	DNASU (HsCD00077224)
pRLM-165	ITB6	pANT7_cGST	DNASU (HsCD00302707)
pRLM-167	CSF3	pANT7_cGST	DNASU (HsCD00305526)
pRLM-63	$p58^{ipk}$ shRNA (shRNA 2)	pLKO.1-puro	Sigma Mission shRNA (NM_006260.2-1192s1c1)
pRLM-64	$p58^{ipk}$ shRNA (shRNA 1)	pLKO.1-puro	Sigma Mission shRNA (NM_006260.2-919s1c1)

Appendix D

Primers for in vitro transcription templates

Gene	Forward	Reverse
CD74	gcctaatacgactcactatagggagaccATGCACAGGAGGAGAAGCA	tgatgacagctaTCACAACATGGGGACTGGGCC
IL2RG	gcctaatacgactcactatagggagaccATGTTGAAGCCATCATTACC	ttcaacttctgaacaCTGTCAGGTTTCAGGCTTTAGGG
HGF	gcctaatacgactcactatagggagaccATGTGGGTGACCAAACTC	ttcaacttctgaacactgTCATGGTTCCCAGAAGATAT
Bip	gcgtaatacgactcactatagggagaccATGAAGCTCTCCCTGGTGG	TGGTCTTTGTAGTCAGCCCGGG (anneals to plasmid 3' to gene)
TRPML1	gcctaatacgactcactatagggagaccATGACAGCCCCGGCGGGT	tgatgacagctaTCACAAATTCACCAGCAGCGA
ITAV	gcctaatacgactcactatagggagaccATGGCTTTTCCGCCGCGG	tgatgacagctaTCACTCGCCAGTAAAATTGTATAAGGAGG
ITB5	gcctaatacgactcactatagggagaccATGCCGCGGGCCCCGGCG	gcgtgatgacagTCAAGCAGTAAAGAAGAGATT
AREG	gcctaatacgactcactatagggagaccATGAGAGCCCCGCTGCTACCG	gcgcgcttaattaaTTACATCATCATCAATGCTATAGCATGTACATT
IL7	gcctaatacgactcactatagggagaccATGTTCCATGTTTCTTTT	gcgcgcttaattaaTTACATCATCATCAAGTGTTCTTTAGTGCC
CSF3	gcctaatacgactcactatagggagaccATGGCTGGACCTGCCACCCAG	gcgcgcttaattaaTTACATCATCATTCAGGGCTGGGCAAGGTGGCG
IL7R	gcctaatacgactcactatagggagaccATGACAATTCTAGGTACA	gcgcgcttaattaaTTACATCATCATCTGGTTTTGGTAGAAGCT
CD3Z	gcctaatacgactcactatagggagaccATGAAGTGGAAGGCGCTT	gcgcgcttaattaaTTACATCATCATGCGAGGGGGCAGGGCCTG
TNR11	gcctaatacgactcactatagggagaccATGGCCCCGCGCGCCCGG	gcgcgcttaattaaTTACATCATCATAGCCTTGGCCCCGCCTTG
TNFB	gcctaatacgactcactatagggagaccATGACACCACCTGAACGTCTC	gcgcgcttaattaaTTACATCATCATCAACAGAGCGAAGGCTCCAAA
IL3RA	gcctaatacgactcactatagggagaccATGGTCCTCCTTTGGCTC	gcgcgcttaattaaTTACATCATCATCATAGTTTTCTGCACGAC
CD3G	gcctaatacgactcactatagggagaccATGGAACAGGGGAAGGGC	gcgcgcttaattaaTTACATCATCATATTCCTCCTCAACTGGTT
ITB6	gcctaatacgactcactatagggagaccATGGAACAGGGGAAGGGC	gcgcgcttaattaaTTACATCATCATATTCCTCCTCAACTGGTT
p58^{ipk}	gcctaatacgactcactatagggagaccATGGTGGCCCCCGGCTCGGTG	gcgtgatgacagttaATTGAAGTGGAACTTAAA
Erdj3	gcctaatacgactcactatagggagaccATGGCTCCGCAGAA	gcgtgatgacagTCAATATCCTTGCAGTCCATTGTA

Appendix D | Primers used to generate in vitro translation PCR templates. Blue = T7 promoter sequence, Red = anneals to gene, Underline = 3xMet to increase signal strength in translation reactions. All primers are listed in the 5' → 3' direction.

Appendix E

List of signal peptide-containing proteins with ΔG_{sub} values in the top 25%

Swiss-Prot	Signal Peptide	Description	ΔG_{sub}
Q5TF21	MSQPPIGGAAPATAAASPAAA	Protein SOGA3	2.34
P34925	MRGAARLGRPGRSCLPGPALRAAAA	Tyrosine-protein kinase RYK (EC 2.7.10.1)	2.10
Q8IZW8	MSQVMSSPLLAGGHAVSL	Tensin-4 (C-terminal tensin-like protein)	1.88
Q9UF72	MCLLSSSAASDLAATSLTA	Putative TP73 antisense gene protein 1 (TP73 antisense RNA 1) (p53-dependent apoptosis modulator)	1.45
Q4G0I0	MNRVLCAPAAGAVRA	Protein CCSMST1	1.39
Q86TW2	MARKALKLASWTSMALA	Uncharacterized aarF domain-containing protein kinase 1 (EC 2.7.11.-)	1.38
Q2TV78	MAPAPVTLLAPGAASSMSCS	Putative macrophage stimulating 1-like protein (Brain rescue factor 1) (BRF-1) (Hepatocyte growth factor-like protein homolog)	1.30
P58397	MPCAQRSWLANLSVVAQLLNFGALC	A disintegrin and metalloproteinase with thrombospondin motifs 12 (ADAM-TS 12) (ADAM-TS12) (ADAMTS-12) (EC 3.4.24.-)	1.26
P0CAP1	MLRSTSTVTLLSGGAARTPG	Myocardial zonula adherens protein (GRINL1A upstream protein) (Gup)	1.25
Q13324	MDAALLHSLLEANCSLA	Corticotropin-releasing factor receptor 2 (CRF-R-2) (CRF-R2) (CRFR-2) (Corticotropin-releasing hormone receptor 2) (CRH-R-2) (CRH-R2)	1.22
Q6PB30	MSATTACWPAFTVLGEARG	Putative chondrosarcoma-associated gene 1 protein (Cancer/testis antigen 24.1) (CT24.1) (Cancer/testis antigen CSAGE)	1.08
Q96MS0	MLRYLLKTLLQMNLFADSLA	Roundabout homolog 3 (Roundabout-like protein 3)	1.08
Q70Z44	MQKHSPGPPALALLSQSLLTTGNG	5-hydroxytryptamine receptor 3D (5-HT3-D) (5-HT3D) (Serotonin receptor 3D)	1.07
P05019	MGKISSLPTQLFKCCFCDFLK	Insulin-like growth factor I (IGF-I) (Mechano growth factor) (MGF) (Somatomedin-C)	0.99
Q6ZRP7	MAAAGAAVARSPGIGAGPALR	Sulfhydryl oxidase 2 (EC 1.8.3.2) (Neuroblastoma-derived sulfhydryl oxidase) (Quiescin Q6-like protein 1)	0.99
Q9H2X8	MMKRAAAAAVGGALAVGAVPVVLS	Interferon alpha-inducible protein 27-like protein 2 (Interferon-stimulated gene 12b protein) (ISG12(b)) (Protein TLH29) (pIFI27-like protein)	0.90
Q96I85	MCRETAGYGWLLASTELLSLLEPLSP	Putative uncharacterized protein C14orf144	0.86
Q96PB7	MSPPLLKLGAVLSTMAMISNWMS	Noelin-3 (Olfactomedin-3) (Optimedin)	0.82
Q02643	MDRRMWGAHVFCVLSPLPTVLG	Growth hormone-releasing hormone receptor (GHRH receptor) (Growth hormone-releasing factor receptor) (GRF receptor) (GRFR)	0.80
Q9NWH7	MPKVKALQCALALEISSVTC	Spermatogenesis-associated protein 6	0.80
Q6NUJ2	MSARAPKELRLALPPCLLNRTFA	Uncharacterized protein C11orf87	0.78
Q5TIE3	MPGLLNWITGAALPLTAS	von Willebrand factor A domain-containing protein 5B1	0.74
Q86T20	MYRRKSGWTGCAITCSPCTA	Uncharacterized protein C6orf1 (Protein LBH)	0.73
Q86XR5	MLLRDLVLRRGCCWSSLLLHCALHPLWGFVQVTHG	Proline-rich membrane anchor 1 (PRiMA)	0.72
Q8N816	MVGILPLCCSGCVPSLCCS	Transmembrane protein 99	0.70
Q6ZRU5	MRCVTRTRNWWRRAARMPRAGSSAWWVAVCKQVCT	Putative uncharacterized protein FLJ46089	0.68
P60153	MMRTLITTHPLPLLLLPQQLLQLVQF	Inactive ribonuclease-like protein 9	0.66
Q9H972	MSFSATILFSPPSGSEA	Uncharacterized protein C14orf93	0.66
Q8N2X6	MPAVFMLASSSALQCGRG	Uncharacterized protein C5orf55	0.66
Q6UXV4	MAAIRMGKLTTMPAGLIYASVSVHA	Apolipoprotein O-like (Protein FAM121A)	0.63
Q7Z3S7	MVCGCSALLPLPNPRPTMP	Voltage-dependent calcium channel subunit alpha-2/delta-4 (Voltage-gated calcium channel subunit alpha-2/delta-4) [Cleaved into: Voltage-dependent calcium channel	0.63

		subunit alpha-2-4; Voltage-dependent calcium channel subunit delta-4]	
Q9HCM2	MKAMPWNWTCLLSHLLMVGMGSS	Plexin-A4	0.60
Q8N2U0	MAGPAAAFRRLGALSGAAALGFASYGAHG	Transmembrane protein 256	0.60
Q6UWP2	MARPGMERWRDRLALVTGASGGIGAAVARA	Dehydrogenase/reductase SDR family member 11 (EC 1.-.-.-)	0.59
Q5VUM1	MTPSRLPWLLSWVSATAWRAARS	UPF0369 protein C6orf57	0.56
P0C854	MQSHLAPLACAAAAGRAGGSCQA	Putative cat eye syndrome critical region protein 9	0.56
P40200	MEKKWKYCAVYYIIQIHFVKG	T-cell surface protein tactile (Cell surface antigen CD96) (T cell-activated increased late expression protein) (CD antigen CD96)	0.53
Q99748	MQRWKAAALASVLCSSVLS	Neurturin	0.51
Q96HP4	MACAAVMIPGLLRCSVG	Oxidoreductase NAD-binding domain-containing protein 1 (EC 1.-.-.-)	0.51
Q8TAV5	MLTRLVLSAHLSSTTSPPWTHA	Putative uncharacterized protein C11orf45	0.51
Q8NFM7	MAPWLQLCSVFFTVNA	Interleukin-17 receptor D (IL-17 receptor D) (IL-17RD) (IL17Rhom) (Interleukin-17 receptor-like protein) (Sef homolog) (hSef)	0.50
Q6JVE9	MPGAAEALPTVTVTLVAGAVPPASG	Epididymal-specific lipocalin-8	0.50
O43300	MGLHFKWPLGAPMLAAIYAMSMVLKMLPALGMA	Leucine-rich repeat transmembrane neuronal protein 2 (Leucine-rich repeat neuronal 2 protein)	0.47
Q96FE7	MLLAWVQAFLVSNMLLAEAYG	Phosphoinositide-3-kinase-interacting protein 1 (Kringle domain-containing protein HGFL)	0.47
Q6UW88	MALGVPISVYLLFNAMTALTEE	Epigen (Epithelial mitogen) (EPG)	0.46
P49763	MPVMRLFPCFLQLLAGLA	Placenta growth factor (PlGF)	0.44
Q6ZVS6	MGLQFSQVISICWAAMGSLYA	Putative uncharacterized protein FLJ42147	0.44
O00220	MAPPPARVHLGAFLAVTPNPGSA	Tumor necrosis factor receptor superfamily member 10A (Death receptor 4) (TNF-related apoptosis-inducing ligand receptor 1) (TRAIL receptor 1) (TRAIL-R1) (CD antigen CD261)	0.42
Q99784	MSVPLLKIGVVLSTMA	Noelin (Neuronal olfactomedin-related ER localized protein) (Olfactomedin-1)	0.42
Q6UXN2	MAWGGVHTCCFHLCCCCSWPQGAVP	Trem-like transcript 4 protein (TLT-4) (Triggering receptor expressed on myeloid cells-like protein 4)	0.42
A6NCI4	MKKYRKISIGCFAMATQTSHVFHG	von Willebrand factor A domain-containing protein 3A	0.42
Q9BUR5	MFKVIQRSVGPASLSLLTFKVYAAP	Apolipoprotein O (Protein FAM121B)	0.39
Q9NVR0	MAAAAVAAAAAAAAA	Kelch-like protein 11	0.38
Q9NXC2	MLPGVGVFGTSLTARVIIPLL	Glucose-fructose oxidoreductase domain-containing protein 1 (EC 1.-.-.-)	0.38
Q8N755	MEAALLGLCNWSTLGVCAA	PQ-loop repeat-containing protein 3	0.37
P08236	MARGSAVAWAALGPLLWGCALG	Beta-glucuronidase (EC 3.2.1.31) (Beta-G1)	0.36
O00206	MMSASRLAGTLIPAMAFLSCVRP	Toll-like receptor 4 (hToll) (CD antigen CD284)	0.35
Q8IYD9	MAKSKTKHRLCSQESSVSALLASCTLSGSNS	Lung adenoma susceptibility protein 2	0.35
Q8NDA2	MMPGAPLLRLLTAVSAAVAVAVA	Hemicentin-2	0.34
Q9Y2I2	MYLSRFLSIHALWVTVSSVMQPYPLVWG	Netrin-G1 (Laminet-1)	0.34
Q5VYX0	MAQVLIVGAGMTGSLCA	Renalase (EC 1.4.-.-) (Monoamine oxidase-C) (MAO-C)	0.32
A6ND01	MACWWPLLLELWTVMPTWA	Probable folate receptor delta (FR-delta) (Folate receptor 4)	0.32
Q86TE4	MKFSPAHYLLPLLPALVLS	Leucine zipper protein 2	0.31
P03951	MIFLYQVVHFILFTSVSG	Coagulation factor XI (FXI) (EC 3.4.21.27) (Plasma thromboplastin antecedent) (PTA) [Cleaved into: Coagulation factor XIa heavy chain; Coagulation factor XIa light chain]	0.31

Q96S42	MHAHCLPFLLHAWWALLQAGAATVAT	Nodal homolog	0.31
Q8TAT8	MTERRRALSLAAVVDSINL	Putative uncharacterized protein LOC644613	0.30
Q8N8R5	MWGFRLLRSPPLLLLLPQLGIGNA	UPF0565 protein C2orf69	0.30
Q15116	MQIPQAPWPVVWAVLQLGWR	Programmed cell death protein 1 (Protein PD-1) (hPD-1) (CD antigen CD279)	0.30
Q3B7J2	MKMLPGVGVFGTGSSARVLVPLLRA	Glucose-fructose oxidoreductase domain-containing protein 2 (EC 1.-.-.-)	0.30
Q2M3V2	MALAAAAAAAAAGVSQA	Ankyrin repeat domain-containing protein SOWAHA (Ankyrin repeat domain-containing protein 43) (Protein sosondowah homolog A)	0.28
Q96QE4	MSWLRFWGPWPLLTWQLLSLLVKEAQP	Leucine-rich repeat-containing protein 37B (C66 SLIT-like testicular protein)	0.27
O95156	MRLRPLPLVVVPGLLQLLFCDS	Neurexophilin-2	0.26
O43915	MYREWVVVNVFMMLYVQLVQG	Vascular endothelial growth factor D (VEGF-D) (c-Fos-induced growth factor) (FIGF)	0.24
P29376	MGCWGQLLVWFGAAGA	Leukocyte tyrosine kinase receptor (EC 2.7.10.1) (Protein tyrosine kinase 1)	0.23
Q9BRK3	MALPSRILLWKLVLLQSSA	Matrix-remodeling-associated protein 8 (Limitrin)	0.22
A8MZH6	MKTILGFKGLFYLHSLIWTCAGDWSA	Oocyte-secreted protein 1 homolog	0.22
P43235	MWGLKVLLLPVVSFA	Cathepsin K (EC 3.4.22.38) (Cathepsin O) (Cathepsin O2) (Cathepsin X)	0.21
O95866	MAVFLQLLPLLLSRAQG	Protein G6b	0.20
Q96II8	MAAAGLVAVAAAAEYSGTVASG	Leucine-rich repeat and calponin homology domain-containing protein 3	0.19
Q9C0K3	MFESFNVPGLYIAVQAVLALA	Actin-related protein 3C (Actin-related protein 11)	0.18
P02790	MARVLGAPVALGLWSLCWSLAIA	Hemopexin (Beta-1B-glycoprotein)	0.17
Q13822	MARRSSFQSCQIISLFTFAVGVNICLG	Ectonucleotide pyrophosphatase/phosphodiesterase family member 2 (E-NPP 2) (EC 3.1.4.39) (Autotaxin) (Extracellular lysophospholipase D) (LysoPLD)	0.16
Q86TM6	MFRTAVMMAASLALTGAVVAHA	E3 ubiquitin-protein ligase synoviolin (EC 6.3.2.-) (Synovial apoptosis inhibitor 1)	0.16
Q5VST6	MNNLSFSELCCLFCCPPCPG	Alpha/beta hydrolase domain-containing protein 17B (EC 3.-.-.-)	0.16
Q96GS6	MNGLSLSELCCLFCCPPCPG	Alpha/beta hydrolase domain-containing protein 17A (EC 3.-.-.-)	0.16
Q9BXW7	MAAWGCVAALGAARGLCWRAARA	Cat eye syndrome critical region protein 5	0.15
P17050	MLLKTVLLLGHVAQVLM	Alpha-N-acetylgalactosaminidase (EC 3.2.1.49) (Alpha-galactosidase B)	0.14
P24001	MCFPKVLSDDMKKLKARMVMLLPTSAQGLG	Interleukin-32 (IL-32) (Natural killer cells protein 4) (Tumor necrosis factor alpha-inducing factor)	0.13
Q8NB37	MASERLPNRPACLLVASGAAEGVSAQSFLHCFTMASTA	Parkinson disease 7 domain-containing protein 1	0.13
Q6UXR6	MQCWQQPFLRFLQQPFFLATASLAGSSSS	Putative uncharacterized protein UNQ6494/PRO21346	0.12
Q15904	MMAAMATARVRMGPRCAQALWRMPWLPVFLSLAAAAAAAAA	V-type proton ATPase subunit S1 (V-ATPase subunit S1) (Protein XAP-3) (V-ATPase Ac45 subunit) (V-ATPase S1 accessory protein) (Vacuolar proton pump subunit S1)	0.12
Q9H5Y7	MKLWIHLFYSSLLACISLHSQTPVLS	SLIT and NTRK-like protein 6	0.12
Q96E22	MTGLYELVWRVLHALLCLHRTLT	Nogo-B receptor (NgBR) (Nuclear undecaprenyl pyrophosphate synthase 1 homolog)	0.11
P18505	MWTVQNRESLGLLSFPVMITMVCCA	Gamma-aminobutyric acid receptor subunit beta-1 (GABA(A) receptor subunit beta-1)	0.11
O15455	MRQTLPCIYFWGGLLPFGMLCAS	Toll-like receptor 3 (CD antigen CD283)	0.10
Q69YU5	MPAGVPMSTYLKMFAASLLAMCAGA	Uncharacterized protein C12orf73	0.10
P59510	MWVAKWLTGLLYHLSLFITRS	A disintegrin and metalloproteinase with thrombospondin motifs 20 (ADAM-TS 20)	0.09

		(ADAM-TS20) (ADAMTS-20) (EC 3.4.24.-)	
P78536	MRQSLLFLTSVVPFVLA	Disintegrin and metalloproteinase domain-containing protein 17 (ADAM 17) (EC 3.4.24.86) (Snake venom-like protease) (TNF-alpha convertase) (TNF-alpha-converting enzyme) (CD antigen CD156b)	0.08
Q96EG1	MGWLFLKVLLAGVSFS	Arylsulfatase G (ASG) (EC 3.1.6.-)	0.08
O95633	MRPGAPGPLWPLPWGALAWAVGFVSS	Follistatin-related protein 3 (Follistatin-like protein 3) (Follistatin-related gene protein)	0.07
O43240	MRAPHLHLSAASGARALAKLLPLLMAQLWA	Kallikrein-10 (EC 3.4.21.-) (Normal epithelial cell-specific 1) (Protease serine-like 1)	0.06
Q00888	MGPLSAPPCTQRITWKGVLLTASLLNFWNPPTTA	Pregnancy-specific beta-1-glycoprotein 4 (PS-beta-G-4) (PSBG-4) (Pregnancy-specific glycoprotein 4) (Pregnancy-specific beta-1-glycoprotein 9) (PS-beta-G-9) (PSBG-9) (Pregnancy-specific glycoprotein 9)	0.05
P20963	MKWKALFTAAILQAQLPITEA	T-cell surface glycoprotein CD3 zeta chain (T-cell receptor T3 zeta chain) (CD antigen CD247)	0.05
P13762	MVCLKLPGGSCMAALTVTLTVLSSPLALA	HLA class II histocompatibility antigen, DR beta 4 chain (MHC class II antigen DRB4)	0.04
Q9BXJ2	MFVLLYVTSFAICASG	Complement C1q tumor necrosis factor-related protein 7	0.04
Q9UKQ2	MLQGLLPVSLLLSVAVSA	Disintegrin and metalloproteinase domain-containing protein 28 (ADAM 28) (EC 3.4.24.-) (Epididymal metalloproteinase-like, disintegrin-like, and cysteine-rich protein II) (eMDC II) (Metalloproteinase-like, disintegrin-like, and cysteine-rich protein L) (MDC-L)	0.03
Q8NEW7	MAGWPGAGPLCVLGGAALGVCLAGVAG	Transmembrane inner ear expressed protein	0.03
P11465	MGPLSAPPCTEHIKWKGLLVTASLLNFWNLPTTA	Pregnancy-specific beta-1-glycoprotein 2 (PS-beta-G-2) (PSBG-2) (Pregnancy-specific glycoprotein 2) (Pregnancy-specific beta-1 glycoprotein E) (PS-beta-E)	0.03
P05556	MNLQPIFWIGLISSVCCVFA	Integrin beta-1 (Fibronectin receptor subunit beta) (Glycoprotein IIa) (GPIIA) (VLA-4 subunit beta) (CD antigen CD29)	0.03
Q7Z6M3	MWSHLNRLLFWSIFSSVTC	Allergin-1 (Allergy inhibitory receptor 1) (Mast cell antigen 32) (MCA-32) (Mast cell immunoglobulin-like receptor 1)	0.02
C9J442	MLLSLLGACAVVGPFHG	Uncharacterized protein C22orf46	0.02
P32004	MVVALRYVWPLLLCSPCLL	Neural cell adhesion molecule L1 (N-CAM-L1) (NCAM-L1) (CD antigen CD171)	0.02
Q6UY09	MGPADSWGHHWMGILLSASLCTVWSPPAAA	Carcinoembryonic antigen-related cell adhesion molecule 20	0.02
O95388	MRWFLPWTLAAVTAAAASTVLA	WNT1-inducible-signaling pathway protein 1 (WISP-1) (CCN family member 4) (Wnt-1-induced secreted protein)	0.00
P16871	MTILGTTFGMVFSLLQVVSG	Interleukin-7 receptor subunit alpha (IL-7 receptor subunit alpha) (IL-7R subunit alpha) (IL-7R-alpha) (IL-7RA) (CDw127) (CD antigen CD127)	-0.01
Q6P5S2	MAFLPSWVCVLVGSFSASLA	UPF0762 protein C6orf58	-0.01
P12872	MVSRKAVAALLVVHVAAMLASQTEA	Promotilin [Cleaved into: Motilin; Motilin-associated peptide (MAP)]	-0.01
Q5FYB1	MHTLTGFSLVSLLSFGYLSWDWA	Arylsulfatase I (ASI) (EC 3.1.6.-)	-0.02
A8MXB1	MVPVLLSLLHLLGPAIP	Putative zinc-alpha-2-glycoprotein-like 2	-0.02
Q13219	MRLWSWVLHLGLLSAALGCGLA	Pappalysin-1 (EC 3.4.24.79) (Insulin-like growth factor-dependent IGF-binding protein 4 protease) (IGF-dependent IGFBP-4 protease) (IGFBP-4ase) (Pregnancy-associated plasma protein A) (PAPP-A)	-0.02
Q8TB22	MLGARAWLGRVLLLPRAGAGLA	Spermatogenesis-associated protein 20 (Sperm-specific protein 411) (Ssp411)	-0.02
O60939	MHRDAWLPRPAFSLTGLSLFFSLVPPGRS	Sodium channel subunit beta-2	-0.02

P11117	MAGKRSGWSRAALLQLLLGVNLVVMPPTRA	Lysosomal acid phosphatase (LAP) (EC 3.1.3.2)	-0.02
Q14314	MKLANWYWLSSAVLATYGFLVVA	Fibroleukin (Fibrinogen-like protein 2) (pT49)	-0.02
O75473	MDTSRLGVLLSLPVLLQLATG	Leucine-rich repeat-containing G-protein coupled receptor 5 (G-protein coupled receptor 49) (G-protein coupled receptor 67) (G-protein coupled receptor HG38)	-0.02
P04436	IFASLLRAVIASICVVSSMA	T-cell receptor alpha chain V region HPB-MLT (Fragment)	-0.03
Q8TAL6	MVFLKFFCMSFFCHLCQG	Fin bud initiation factor homolog	-0.04
Q86VH5	MGFNVIRLLSGSAVALVIAPTVLLTMLSSA	Leucine-rich repeat transmembrane neuronal protein 3	-0.04
A3KMH1	MQSRLLLLGAPGGHG	von Willebrand factor A domain-containing protein 8	-0.06
P01744	MDWTXXXXFLVAAATRVHS	Ig heavy chain V-I region ND (Fragments)	-0.06
Q9Y4X1	MLNNLLLFSLQISLIGTTLG	UDP-glucuronosyltransferase 2A1 (UDPGT 2A1) (EC 2.4.1.17)	-0.06
Q9UGM3	MGISTVILEMCLLWGQVLS	Deleted in malignant brain tumors 1 protein (Glycoprotein 340) (Gp-340) (Hensin) (Salivary agglutinin) (SAG) (Surfactant pulmonary-associated D-binding protein)	-0.06
Q8N5I4	MSPLSAARAALRVYAVGAAVILAQLLRRCRG	Dehydrogenase/reductase SDR family member on chromosome X (EC 1.1.-.-) (DHRSXY)	-0.06
Q7Z3Q1	MKILFVEPAIFLSAFAMTLTGPLTT	Solute carrier family 46 member 3	-0.07
Q9UNE0	MAHVGDCTQTPWLPVLVVSLMCSARA	Tumor necrosis factor receptor superfamily member EDAR (Anhidrotic ectodysplasin receptor 1) (Downless homolog) (EDA-A1 receptor) (Ectodermal dysplasia receptor) (Ectodysplasin-A receptor)	-0.07
Q8N1Y9	MAKWVPALLLRRVPLFSLRFRPASS	Putative uncharacterized protein FLJ37218	-0.07
P13611	MFINIKSILWMCSTLIVTHA	Versican core protein (Chondroitin sulfate proteoglycan core protein 2) (Chondroitin sulfate proteoglycan 2) (Glial hyaluronate-binding protein) (GHAP) (Large fibroblast proteoglycan) (PG-M)	-0.07
Q76B58	MIWRSRAGAELFSLMALWEWIALSLHCWVLAVA	Protein FAM5C (DBCCR1-like protein 1)	-0.08
A2VDJ0	MAGLRRPQPGCYCRTAAAVNLLLGVFQVLLPCCRPGGAQG	Transmembrane protein 131-like	-0.08
Q96LC7	MLLPLLLSSLLGGSQA	Sialic acid-binding Ig-like lectin 10 (Siglec-10) (Siglec-like protein 2)	-0.09
O43157	MPALGPALLQALWAGWVLT	Plexin-B1 (Semaphorin receptor SEP)	-0.09
Q99538	MVWKVAVFLSVALGIGA	Legumain (EC 3.4.22.34) (Asparaginyl endopeptidase) (Protease, cysteine 1)	-0.09
Q6UW78	MDSLRKMLISVAMLGAGAGVGYA	UPF0723 protein C11orf83	-0.09
Q9Y5F8	MGGSCAQRRRAGPRQVLFPLLLPLFYPTLC	Protocadherin gamma-B7 (PCDH-gamma-B7)	-0.09
Q9Y5F9	MGGSCAQRRRAGPRQVLFPLLLPLFYPTLS	Protocadherin gamma-B6 (PCDH-gamma-B6)	-0.09
P14151	MIFPWKCQSTQRDLWNIFKLWGWTMLCC	L-selectin (CD62 antigen-like family member L) (Leukocyte adhesion molecule 1) (LAM-1) (Leukocyte surface antigen Leu-8) (Leukocyte-endothelial cell adhesion molecule 1) (LECAM1) (Lymph node homing receptor) (TQ1) (gp90-MEL) (CD antigen CD62L)	-0.09
P78423	MAPISLSWLLRLATFCHLTVLLAG	Fractalkine (C-X3-C motif chemokine 1) (CX3C membrane-anchored chemokine) (Neurotactin) (Small-inducible cytokine D1) [Cleaved into: Processed fractalkine]	-0.11
P13385	MDCRKMARFSYSVIWIMAISKVFELGLVAG	Teratocarcinoma-derived growth factor 1 (Cripto-1 growth factor) (CRGF) (Epidermal growth factor-like cripto protein CR1)	-0.11
P0C7L1	MKGICSDAILVLATSMWMAFA	Serine protease inhibitor Kazal-type 8	-0.11
O75054	MKCFFPVLSCLAVLGVVSA	Immunoglobulin superfamily member 3 (IgSF3) (Glu-Trp-Ile EWI motif-containing	-0.12

		protein 3) (EWI-3)	
Q13683	MAGARSRDPWGASGICYLFGSLLVELLFSRAVA	Integrin alpha-7 [Cleaved into: Integrin alpha-7 heavy chain; Integrin alpha-7 light chain; Integrin alpha-7 70 kDa form]	-0.12
Q14957	MGGALGPALLLTSLFGAWA	Glutamate receptor ionotropic, NMDA 2C (GluN2C) (Glutamate [NMDA] receptor subunit epsilon-3) (N-methyl D-aspartate receptor subtype 2C) (NMDAR2C) (NR2C)	-0.12
P20151	MWDLVLSIALSVGCTGAV	Kallikrein-2 (EC 3.4.21.35) (Glandular kallikrein-1) (hGK-1) (Tissue kallikrein-2)	-0.14
Q30154	MVCLKLPGGSYMAKLTVTLMVLSSPLALA	HLA class II histocompatibility antigen, DR beta 5 chain (DR beta-5) (DR2-beta-2) (Dw2) (MHC class II antigen DRB5)	-0.14
Q96N03	MGAPLAVALGALHYLALFLQLGGA	V-set and transmembrane domain-containing protein 2-like protein	-0.14
P20827	MEFLWAPLLGLCCSLAAA	Ephrin-A1 (EPH-related receptor tyrosine kinase ligand 1) (LERK-1) (Immediate early response protein B61) (Tumor necrosis factor alpha-induced protein 4) (TNF alpha-induced protein 4) [Cleaved into: Ephrin-A1, secreted form]	-0.15
Q7RTX1	MLLCTARLVGLQLLISCCWA	Taste receptor type 1 member 1 (G-protein coupled receptor 70)	-0.15
O15146	MRELVNIPLVHILTLVAFSGTEK	Muscle, skeletal receptor tyrosine-protein kinase (EC 2.7.10.1) (Muscle-specific tyrosine-protein kinase receptor) (MuSK) (Muscle-specific kinase receptor)	-0.15
Q7Z4B0	MINLHRLCIIHVVATLLSTLLSLISVAIS	Putative uncharacterized protein encoded by LINC00305	-0.15
Q96BF3	MGSPGMVLGLLVQIWALQEASS	Transmembrane and immunoglobulin domain-containing protein 2 (Immunoglobulin and proline-rich receptor 1) (IGPR-1)	-0.15
Q6HA08	MEGVGGLWPWVLGLLSLPGVILG	Astacin-like metalloendopeptidase (EC 3.4.-.-) (Oocyte astacin) (Ovastacin)	-0.15
O95868	MKPQFVGILLSSLLGAALG	Lymphocyte antigen 6 complex locus protein G6d (Protein Ly6-D) (Megakaryocyte-enhanced gene transcript 1 protein)	-0.16
Q9BZM4	MAAAASPAILPRLAILPYLLFDWSGTGRA	NKG2D ligand 3 (N2DL-3) (NKG2DL3) (ALCAN-gamma) (Retinoic acid early transcript 1N)	-0.16
O43567	MLLSIGMLMLSATQVYTILTVQLFAFLNLLPVEA	E3 ubiquitin-protein ligase RNF13 (EC 6.3.2.-) (RING finger protein 13)	-0.16
Q14832	MKMLTRLQVLTLALFSKGFLLS	Metabotropic glutamate receptor 3 (mGluR3)	-0.16
Q8NI32	MLYKSSDRPAHKVSMLLLCHALAIAVVQIVIFSESWAFA	Ly6/PLAUR domain-containing protein 6B	-0.16
P22004	MPGLGRRAQWLCWWWGLLCS	Bone morphogenetic protein 6 (BMP-6) (VG-1-related protein) (VG-1-R) (VGR-1)	-0.17
P48357	MICQKFCVVLLHWEFIYVITA	Leptin receptor (LEP-R) (HuB219) (OB receptor) (OB-R) (CD antigen CD295)	-0.17
P28799	MWTLVSWVALTAGLVAG	Granulins (Proepithelin) (PEPI) [Cleaved into: Acrogranin; Paragranulin; Granulin-1 (Granulin G); Granulin-2 (Granulin F); Granulin-3 (Granulin B); Granulin-4 (Granulin A); Granulin-5 (Granulin C); Granulin-6 (Granulin D); Granulin-7 (Granulin E)]	-0.18
Q49AH0	MWCASPVAVVAFCAGLLVSHPVLT	Cerebral dopamine neurotrophic factor (ARMET-like protein 1) (Conserved dopamine neurotrophic factor)	-0.18
P12109	MRAARALLPLLLQACWTAA	Collagen alpha-1(VI) chain	-0.18
Q7Z443	MFFKGGSWLWLYIRTSIILGSEL	Polycystic kidney disease protein 1-like 3 (PC1-like 3 protein) (Polycystin-1L3)	-0.18
Q96BH3	MTRWSSYLLGWTTFLLYSYESSGG	Epididymal sperm-binding protein 1 (Epididymal secretory protein 12) (hE12)	-0.19
Q9UPX0	MIWYVATFIASVIGTRGLAA	Protein turtle homolog B (Immunoglobulin superfamily member 9B) (IgSF9B)	-0.19
Q5TEV5	MLCCCPLADALLIFLETGSC	Putative uncharacterized protein C1orf134	-0.19
P48060	MRVTLATIAWMVSFVSNYSHT	Glioma pathogenesis-related protein 1 (GliPR	-0.19

		1) (Protein RTVP-1)	
Q8IVK1	MKFFMVLLPASLASTSLA	Putative glycosylation-dependent cell adhesion molecule 1 (GlyCAM-1)	-0.19
P16444	MWSGWWLWPLVAVCTA	Dipeptidase 1 (EC 3.4.13.19) (Dehydropeptidase-I) (Microsomal dipeptidase) (Renal dipeptidase) (hRDP)	-0.19
Q8NEB7	MRKPAAGFLPSLLKVLLLPLAPAAA	Acrosin-binding protein (Cancer/testis antigen 23) (CT23) (Cancer/testis antigen OY-TES-1) (Proacrosin-binding protein sp32)	-0.19
P23415	MYSFNTLRLYLWETIVFFSLAASKEAEA	Glycine receptor subunit alpha-1 (Glycine receptor 48 kDa subunit) (Glycine receptor strychnine-binding subunit)	-0.19
Q9H1M3	MKLLFPIFASLMLQYQVNT	Beta-defensin 129 (Beta-defensin 29) (DEFB-29) (Defensin, beta 129)	-0.20
P08217	MIRTLLLSTLVAGALS	Chymotrypsin-like elastase family member 2A (EC 3.4.21.71) (Elastase-2A)	-0.20
P08218	MIRTLLLSTLVAGALS	Chymotrypsin-like elastase family member 2B (EC 3.4.21.71) (Elastase-2B)	-0.20
E5RQL4	MSSSRVGLRLAACLLNVSEA	Formiminotransferase N-terminal subdomain-containing protein (Formiminotransferase-cyclodeaminase N-terminal-like protein)	-0.20
Q6UXP8	MNGNLDGWVVVLAAPLLPAAQ	Putative uncharacterized protein UNQ6975/PRO21958	-0.20
P13765	MGSGWVPWVVALLVNLTRLDSSMTQG	HLA class II histocompatibility antigen, DO beta chain (MHC class II antigen DOB)	-0.21
A8MTL9	MSISSALAMVFMGAKGNTAA	Serpin-like protein HMSD (Minor histocompatibility protein HMSD) (Minor histocompatibility serpin domain-containing protein)	-0.21
Q9ULW2	MQRPGPRLWLVLQVMGSCAA	Frizzled-10 (Fz-10) (hFz10) (FzE7) (CD antigen CD350)	-0.21
O95206	MSPVRRWGSPCLFPLQLFSLCWVLSVAQS	Protocadherin-8 (Arcadlin)	-0.21
P07202	MRALAVLSVTLVMACTEA	Thyroid peroxidase (TPO) (EC 1.11.1.8)	-0.21
Q9HBT6	MWTSGRMSNAKNWLGLGMSLYFWGLMDLTTTVLS	Cadherin-20	-0.21
Q9NPE2	MAVTLSLLLGGRVCA	Neugrin (Mesenchymal stem cell protein DSC92) (Neurite outgrowth-associated protein) (Spinal cord-derived protein FI58G)	-0.21
P20333	MAPVAVWAALAVGLELWAAAHA	Tumor necrosis factor receptor superfamily member 1B (Tumor necrosis factor receptor 2) (TNF-R2) (Tumor necrosis factor receptor type II) (TNF-RII) (TNFR-II) (p75) (p80 TNF-alpha receptor) (CD antigen CD120b) (Etanercept) [Cleaved into: Tumor necrosis factor receptor superfamily member 1b, membrane form; Tumor necrosis factor-binding protein 2 (TBP-2) (TBPII)]	-0.22
Q8IWV2	MRLPWELLVLQSFILCLA	Contactin-4 (Brain-derived immunoglobulin superfamily protein 2) (BIG-2)	-0.22
Q9NZ20	MGVQAGLFGMLGFLGVALG	Group 3 secretory phospholipase A2 (EC 3.1.1.4) (Group III secretory phospholipase A2) (GIII sPLA2) (sPLA2-III) (Phosphatidylcholine 2-acylhydrolase 3)	-0.22
Q8WUJ3	MGAAGRQDFLFKAMLTISWLTLTCFPGATS	Protein KIAA1199	-0.22
P29016	MLLLPFQLLAVLFPGGN	T-cell surface glycoprotein CD1b (CD antigen CD1b)	-0.22
Q9UF33	MGGCEVREFLLQFGFFLPLLTA	Ephrin type-A receptor 6 (EC 2.7.10.1) (EPH homology kinase 2) (EHK-2) (EPH-like kinase 12) (EK12)	-0.22
Q9BZQ6	MSEAGGRGCGSPVPQRARWRLVAATAAFCLVSATSV WTAGA	ER degradation-enhancing alpha-mannosidase-like protein 3 (EC 3.2.1.113) (Alpha-1,2-mannosidase EDEM3)	-0.22
Q8IUW5	MAPRALPGSAVLAAAVFVGGAVS	RELT-like protein 1	-0.23
Q14626	MSSSCSGLSRVLVAVATALVSA	Interleukin-11 receptor subunit alpha (IL-11 receptor subunit alpha) (IL-11R subunit alpha) (IL-11R-alpha) (IL-11RA)	-0.23

Q96KX0	MKASVVLSLLGYLVVPSGA	Lysozyme-like protein 4 (Lysozyme-4)	-0.23
Q9NWM8	MRLFLWNAVLTLFVTSLIG	Peptidyl-prolyl cis-trans isomerase FKBP14 (PPIase FKBP14) (EC 5.2.1.8) (22 kDa FK506-binding protein) (22 kDa FKBP) (FKBP-22) (FK506-binding protein 14) (FKBP-14) (Rotamase)	-0.23
Q16099	MPRVSAPLVLLPAWLVMVAC	Glutamate receptor ionotropic, kainate 4 (GluK4) (Excitatory amino acid receptor 1) (EAA1) (Glutamate receptor KA-1) (KA1)	-0.23
P55082	MKLHCCLFTLVASIIVPA	Microfibril-associated glycoprotein 3	-0.24
P59827	MWMAWCVAALSVVAVCGT	BPI fold-containing family B member 4 (Ligand-binding protein RY2G5) (Long palate, lung and nasal epithelium carcinoma-associated protein 4)	-0.24
Q96DX4	MIVFGWAVFLASRSLG	RING finger and SPRY domain-containing protein 1	-0.24
P29322	MAPARGRLPPALWVVTAAAAAATCVSA	Ephrin type-A receptor 8 (EC 2.7.10.1) (EPH- and ELK-related kinase) (EPH-like kinase 3) (EK3) (hEK3) (Tyrosine-protein kinase receptor EEK)	-0.24
O94910	MARLAAVLWNLCVTAVLVTSATQG	Latrophilin-1 (Calcium-independent alpha-latrotoxin receptor 1) (CIRL-1) (Lectomedin-2)	-0.24
Q6NSX1	MATPPFRLIRKMFSFKVSRWMGLACFRSLAAS	Coiled-coil domain-containing protein 70	-0.24
A6NJ69	MCSYYHMKKRSVSGCNITIFAVMFSHLSAG	IgA-inducing protein homolog	-0.24
Q6MZW2	MKPGGFWLHLTLLGASLPAALG	Follistatin-related protein 4 (Follistatin-like protein 4)	-0.25
Q8N7C0	MSLASGPGPGWLLFSFGMGLVSG	Leucine-rich repeat-containing protein 52 (BK channel auxiliary gamma subunit LRRC52)	-0.26
Q6UXU1	MYPLRAGRRAMLSELRARPAPLLLLTSVLSETLA	Putative uncharacterized protein UNQ6490/PRO21339	-0.26
Q86V40	MSPWSWFLLQTLCLLPTGA	Metalloprotease TIKI1 (EC 3.4.-.-) (TRAB domain-containing protein 2A)	-0.26
P01579	MKYTSYILAFQLCIVLGSLGCYC	Interferon gamma (IFN-gamma) (Immune interferon)	-0.26
Q9H6Y7	MHPAAFPLPVVVAAVLWGAAPTRG	E3 ubiquitin-protein ligase RNF167 (EC 6.3.2.-) (RING finger protein 167) (RING105)	-0.26
P16109	MANCQIAILYQRFQRVVFGISQLLCFSALISELTNQKEVAA	P-selectin (CD62 antigen-like family member P) (Granule membrane protein 140) (GMP-140) (Leukocyte-endothelial cell adhesion molecule 3) (LECAM3) (Platelet activation dependent granule-external membrane protein) (PADGEM) (CD antigen CD62P)	-0.26
Q86WA6	MVAVLGGRGVLRLRLLLSALKPGIHVPRAGPAAAFGT	Valacyclovir hydrolase (VACVase) (Valacyclovirase) (EC 3.1.-.-) (Biphenyl hydrolase-like protein) (Biphenyl hydrolase-related protein) (Bph-rp) (Breast epithelial mucin-associated antigen) (MCNAA)	-0.27
Q9H336	MKCTAREWLRVTTVLFMARAIPA	Cysteine-rich secretory protein LCCL domain-containing 1 (CocoaCrisp) (Cysteine-rich secretory protein 10) (CRISP-10) (LCCL domain-containing cysteine-rich secretory protein 1) (Trypsin inhibitor HI)	-0.27
O00634	MPGWPWGLLLTAGTLFAALSPGPPAPA	Netrin-3 (Netrin-2-like protein)	-0.27
P40933	MRISKPHLRSISIQCYLCLLLNSHFLTEA	Interleukin-15 (IL-15)	-0.27
P21781	MHKWILTWILPTLLYRSCFHIICLVGTISLA	Fibroblast growth factor 7 (FGF-7) (Heparin-binding growth factor 7) (HBGF-7) (Keratinocyte growth factor)	-0.28
Q71RG6	MGTGGSLLCGCSLVLSCLCPSAS	Putative chemokine-related protein FP248 (Protein N73)	-0.28
Q9H239	MVARVGLLLRALQLLLWGHLDA	Matrix metalloproteinase-28 (MMP-28) (EC 3.4.24.-) (Epilysin)	-0.28
Q96MU5	MDELALSFSLTCLLPENRA	Uncharacterized protein C17orf77	-0.28
P16070	MDKFWWHAAWGLCLVPLSLA	CD44 antigen (CDw44) (Epican) (Extracellular matrix receptor III) (ECMR-III) (GP90 lymphocyte homing/adhesion receptor)	-0.28

		(HUTCH-I) (Heparan sulfate proteoglycan) (Hermes antigen) (Hyaluronate receptor) (Phagocytic glycoprotein 1) (PGP-1) (Phagocytic glycoprotein I) (PGP-I) (CD antigen CD44)	
Q96JJ7	MAAWKSWTALRLCATVVVLDMVVC	Protein disulfide-isomerase TMX3 (EC 5.3.4.1) (Thioredoxin domain-containing protein 10) (Thioredoxin-related transmembrane protein 3)	-0.28
Q7Z5A9	MAMVSAMSWVLYLWISACA	Protein FAM19A1 (Chemokine-like protein TAFA-1)	-0.29
P48058	MRIISRQIVLLFSGFWGLAM	Glutamate receptor 4 (GluR-4) (GluR4) (AMPA-selective glutamate receptor 4) (GluR-D) (Glutamate receptor ionotropic, AMPA 4) (GluA4)	-0.29
Q4G0T1	MRAALWTLGLGPLLLNLWA	Putative scavenger receptor cysteine-rich domain-containing protein LOC619207	-0.29
Q9NX61	MAVLGVQLVVTLLTATLMHRLAPHCSFA	Transmembrane protein 161A	-0.29
Q86TG1	MTAWILLPVSLSAFSITGIWTVYA	Transmembrane protein 150A (Transmembrane protein 150)	-0.29
P15018	MKVLAAGVVPLLLVLHWKHGAG	Leukemia inhibitory factor (LIF) (Differentiation-stimulating factor) (D factor) (Melanoma-derived LPL inhibitor) (MLPLI) (Emfilermin)	-0.29
A6NNL5	MEALRRAHEVALRLLLCRPWASRAAA	Uncharacterized protein C15orf61	-0.29
Q8WXS8	MAPLRALLSYLLPLHCALCAAA	A disintegrin and metalloproteinase with thrombospondin motifs 14 (ADAM-TS 14) (ADAM-TS14) (ADAMTS-14) (EC 3.4.24.-)	-0.29
Q6ZWJ8	MAGVGAAALSLLLHLGALALAAGAEG	Kielin/chordin-like protein (Cysteine-rich BMP regulator 2) (Cysteine-rich motor neuron 2 protein) (CRIM-2) (Kielin/chordin-like protein 1) (KCP-1)	-0.29
P01911	MVCLKLPGGSCMTALTVTLMVLSSPLALS	HLA class II histocompatibility antigen, DRB1-15 beta chain (DW2.2/DR2.2) (MHC class II antigen DRB1*15)	-0.29
P04229	MVCLKLPGGSCMTALTVTLMVLSSPLALA	HLA class II histocompatibility antigen, DRB1-1 beta chain (MHC class II antigen DRB1*1) (DR-1) (DR1)	-0.29
P13760	MVCLKFPGGSCMAALTVTLMVLSSPLALA	HLA class II histocompatibility antigen, DRB1-4 beta chain (MHC class II antigen DRB1*4) (DR-4) (DR4)	-0.29
P13761	MVCLKLPGGSCMAALTVTLMVLSSPLALA	HLA class II histocompatibility antigen, DRB1-7 beta chain (MHC class II antigen DRB1*7) (DR-7) (DR7)	-0.29
Q29974	MVCLKLPGGSCMTALTVTLMVLSSPLALA	HLA class II histocompatibility antigen, DRB1-16 beta chain (MHC class II antigen DRB1*16) (DR-16) (DR16)	-0.29
Q9TQE0	MVCLKLPGGSCMAALTVTLMVLSSPLALA	HLA class II histocompatibility antigen, DRB1-9 beta chain (MHC class II antigen DRB1*9) (DR-9) (DR9)	-0.29
P23229	MAAAGQLCLLYLSAGLLSRLGAA	Integrin alpha-6 (CD49 antigen-like family member F) (VLA-6) (CD antigen CD49f) [Cleaved into: Integrin alpha-6 heavy chain; Integrin alpha-6 light chain]	-0.30
Q02509	MIAFLLTSVLMIPHAGG	Otoconin-90 (Oc90) (Phospholipase A2 homolog)	-0.30
Q16671	MLGSLGLWALLPTAVEA	Anti-Muellerian hormone type-2 receptor (EC 2.7.11.30) (Anti-Muellerian hormone type II receptor) (AMH type II receptor) (MIS type II receptor) (MISRII) (MRII)	-0.30
Q9UGM1	MNWSHSCISFCWIYFAASRLRAAET	Neuronal acetylcholine receptor subunit alpha-9 (Nicotinic acetylcholine receptor subunit alpha-9) (NACHR alpha-9)	-0.30
P11678	MHLLPALAGVLATLVLA	Eosinophil peroxidase (EPO) (EC 1.11.1.7) [Cleaved into: Eosinophil peroxidase light chain; Eosinophil peroxidase heavy chain]	-0.31
E0CX11	MLQFLLGFTLGNVVGMYLA	Uncharacterized protein C7orf73	-0.31

P08F94	MTAWLISLMSIEVLLLAVRHLSL	Fibrocystin (Polycystic kidney and hepatic disease 1 protein) (Polyductin) (Tigmin)	-0.31
P56159	MFLATLYFALPLLDLLLSAEVSGG	GDNF family receptor alpha-1 (GDNF receptor alpha-1) (GDNFR-alpha-1) (GFR-alpha-1) (RET ligand 1) (TGF-beta-related neurotrophic factor receptor 1)	-0.31
P06340	MALRAGLVLGFHTLMTLLSPQEAGA	HLA class II histocompatibility antigen, DO alpha chain (MHC DN-alpha) (MHC DZ alpha) (MHC class II antigen DOA)	-0.31
Q6UXU6	MSQAWVPGLAPTLLFSLLAGPQKIAA	Transmembrane protein 92	-0.31
P23327	MGHHRPWLHASVLWAGVASLLLPPAMTQ	Sarcoplasmic reticulum histidine-rich calcium-binding protein	-0.31
Q8TE60	MECALLLACAFPAAGSGPPRGLAGLGRVAKALQLCCLC CASVAAALA	A disintegrin and metalloproteinase with thrombospondin motifs 18 (ADAM-TS 18) (ADAM-TS18) (ADAMTS-18) (EC 3.4.24.-)	-0.31
Q5VW38	MAALAPVGSPASRGPRLAAGLRLLPMLGLLQLLAEPGL G	Protein GPR107 (Lung seven transmembrane receptor 1)	-0.31
Q9Y5G3	MQRAREAEMMKSQVLFPFLLSLFCGAIS	Protocadherin gamma-B1 (PCDH-gamma-B1)	-0.31
P14616	MAVPSLWPWGACLPVIFLSLGFGLDT	Insulin receptor-related protein (IRR) (EC 2.7.10.1) (IR-related receptor) [Cleaved into: Insulin receptor-related protein alpha chain; Insulin receptor-related protein beta chain]	-0.31
P11464	MGTLSAPPCTQRIKWKGLLLTASLLNFWNLPTTA	Pregnancy-specific beta-1-glycoprotein 1 (PS-beta-G-1) (PSBG-1) (Pregnancy-specific glycoprotein 1) (CD66 antigen-like family member F) (Fetal liver non-specific cross-reactive antigen 1/2) (FL-NCA-1/2) (PSG95) (Pregnancy-specific beta-1 glycoprotein C/D) (PS-beta-C/D) (CD antigen CD66f)	-0.32
P40198	MGPPSASPHRECIPWQGLLLTASLLNFWNPPTTA	Carcinoembryonic antigen-related cell adhesion molecule 3 (Carcinoembryonic antigen CGM1) (CD antigen CD66d)	-0.32
Q00887	MGPLPAPSCTQRITWKGLLLTASLLNFWNPPTTA	Pregnancy-specific beta-1-glycoprotein 9 (PS-beta-G-9) (PSBG-9) (Pregnancy-specific glycoprotein 9) (PS34) (Pregnancy-specific beta-1 glycoprotein B) (PS-beta-B) (Pregnancy-specific beta-1-glycoprotein 11) (PS-beta-G-11) (PSBG-11) (Pregnancy-specific glycoprotein 11) (Pregnancy-specific glycoprotein 7) (PSG7)	-0.32
Q00889	MGPLSAPPCTQHITWKGLLLTASLLNFWNLPTTA	Pregnancy-specific beta-1-glycoprotein 6 (PS-beta-G-6) (PSBG-6) (Pregnancy-specific glycoprotein 6) (Pregnancy-specific beta-1-glycoprotein 10) (PS-beta-G-10) (PSBG-10) (Pregnancy-specific glycoprotein 10) (Pregnancy-specific beta-1-glycoprotein 12) (PS-beta-G-12) (PSBG-12) (Pregnancy-specific glycoprotein 12)	-0.32
Q13046	MGPLSAPPCTQHITWKGLLLTASLLNFWNPPTTA	Putative pregnancy-specific beta-1-glycoprotein 7 (PS-beta-G-7) (PSBG-7) (Pregnancy-specific glycoprotein 7)	-0.32
Q15238	MGPLSAPPCTQHITWKGLLLTASLLNFWNLPITA	Pregnancy-specific beta-1-glycoprotein 5 (PS-beta-G-5) (PSBG-5) (Pregnancy-specific glycoprotein 5) (Fetal liver non-specific cross-reactive antigen 3) (FL-NCA-3)	-0.32
Q9UQ74	MGLLSAPPCTQRITWKGLLLTASLLNFWNPPTTA	Pregnancy-specific beta-1-glycoprotein 8 (PS-beta-G-8) (PSBG-8) (Pregnancy-specific glycoprotein 8)	-0.32
Q8NBL3	MEPRALVTALSLGLSLCSLGLLVTA	Transmembrane protein 178A	-0.32
P13747	MVDGTLLLLLSEALALTQTWA	HLA class I histocompatibility antigen, alpha chain E (MHC class I antigen E)	-0.32
P01912	MVCLRLPGGSCMAVLTVTLMVLSSPLALA	HLA class II histocompatibility antigen, DRB1-3 chain (Clone P2-beta-3) (MHC class II antigen DRB1*3)	-0.32
P20039	MVCLRLPGGSCMAVLTVTLMVLSSPLALA	HLA class II histocompatibility antigen, DRB1-11 beta chain (DR-5) (DR5) (DRw11) (MHC class II antigen DRB1*11)	-0.32

Q30134	MVCLRLPGGSCMAVLTVTLMVLSSPLALA	HLA class II histocompatibility antigen, DRB1-8 beta chain (MHC class II antigen DRB1*8) (DR-8) (DR8) (DRw8)	-0.32
Q30167	MVCLRLPGGSCMAVLTVTLMVLSSPLALA	HLA class II histocompatibility antigen, DRB1-10 beta chain (DRw10) (MHC class II antigen DRB1*10)	-0.32
Q5Y7A7	MVCLRLPGGSCMAVLTVTLMVLSSPLALA	HLA class II histocompatibility antigen, DRB1-13 beta chain (MHC class II antigen DRB1*13) (DR-13) (DR13)	-0.32
Q95IE3	MVCLRLPGGSCMAVLTVTLMVLSSPLALA	HLA class II histocompatibility antigen, DRB1-12 beta chain (MHC class II antigen DRB1*12) (DR-12) (DR12)	-0.32
Q9GIY3	MVCLRLPGGSCMAVLTVTLMVLSSPLALA	HLA class II histocompatibility antigen, DRB1-14 beta chain (MHC class II antigen DRB1*14) (DR-14) (DR14)	-0.32
A6NMB1	MLLLPLLLPVLGAGSL	Sialic acid-binding Ig-like lectin 16 (Siglec-16) (Siglec-P16)	-0.32
Q8IUX8	MPLPWSLALPLLLSWVAGGFG	Epidermal growth factor-like protein 6 (EGF-like protein 6) (MAM and EGF domains-containing gene protein)	-0.32
Q9Y5G2	MKASSGRCGLVRWLQVLLPFLLSLFPGALP	Protocadherin gamma-B2 (PCDH-gamma-B2)	-0.33
P51512	MILLTFSTGRRLDFVHHSGVFFLQTLLWILC	Matrix metalloproteinase-16 (MMP-16) (EC 3.4.24.-) (MMP-X2) (Membrane-type matrix metalloproteinase 3) (MT-MMP 3) (MTMMP3) (Membrane-type-3 matrix metalloproteinase) (MT3-MMP) (MT3MMP)	-0.33
Q8N3J6	MIWKRSAVLRFYSVCGLLLQGSQG	Cell adhesion molecule 2 (Immunoglobulin superfamily member 4D) (IgSF4D) (Nectin-like protein 3) (NECL-3) (Synaptic cell adhesion molecule 2) (SynCAM 2)	-0.33
Q16363	MALSSAWRSVLPLWLLWSAACSRA	Laminin subunit alpha-4 (Laminin-14 subunit alpha) (Laminin-8 subunit alpha) (Laminin-9 subunit alpha)	-0.33
O00222	MVCEGKRSASCPCFFLLTAKFYWILTMMQRTHS	Metabotropic glutamate receptor 8 (mGluR8)	-0.34
Q5JU69	MAAATRGCRPWGSLLGLLGLVSAAAA	Torsin-2A (Torsin family 2 member A) (Torsin-related protein 1)	-0.34
Q8N2E6	MAAATRGCRPWGSLLGLLGLVSAAAA	Prosalusin (Torsin family 2 member A) (Torsin-2A) [Cleaved into: Salusin-alpha; Salusin-beta]	-0.34
Q9UBX1	MAPWLQLLSLLGLLPGAVA	Cathepsin F (CATSF) (EC 3.4.22.41)	-0.34
P15088	MRLILPVGLIATTLA	Mast cell carboxypeptidase A (MC-CPA) (EC 3.4.17.1) (Carboxypeptidase A3)	-0.34
Q9NP55	MFQTGGLIVFYGLLAQTMA	BPI fold-containing family A member 1 (Lung-specific protein X) (Nasopharyngeal carcinoma-related protein) (Palate lung and nasal epithelium clone protein) (Secretory protein in upper respiratory tracts) (Tracheal epithelium-enriched protein) (Von Ebner protein HI)	-0.35
Q6UX82	MKGILVAGITAVLVAAVES	Ly6/PLAUR domain-containing protein 8	-0.35
P22794	MPTDMEHTGHYLHLAFLMTTVFSLSPGTKA	Protein EVI2A (Ecotropic viral integration site 2A protein homolog) (EVI-2A)	-0.35
Q9C0A0	MGSVTGAVLKTLLLLSTQNWNRVEA	Contactin-associated protein-like 4 (Cell recognition molecule Caspr4)	-0.35
Q9Y215	MVVLNPMTLGIYLQLFFLSIVS	Acetylcholinesterase collagenic tail peptide (AChE Q subunit) (Acetylcholinesterase-associated collagen)	-0.35
Q9H293	MRERPRLGEDSSLISLFLQVVAFLAMVMGTHT	Interleukin-25 (IL-25) (Interleukin-17E) (IL-17E)	-0.36
P03971	MRDLPLTSLALVLSALGA	Muellerian-inhibiting factor (Anti-Muellerian hormone) (AMH) (Muellerian-inhibiting substance) (MIS)	-0.36
Q9UQV4	MPRQLSAAAALFASLAVILHDGSQMRA	Lysosome-associated membrane glycoprotein 3 (LAMP-3) (Lysosomal-associated membrane protein 3) (DC-lysosome-associated membrane glycoprotein) (DC LAMP) (Protein TSC403) (CD antigen CD208)	-0.36

Q6UWY2	MGLGLRGWGRPLLTVATALMLPVKPPAGSWG	Serine protease 57 (EC 3.4.21.-) (Serine protease 1-like protein 1)	-0.36
P47870	MWRVRKRGYFGIWSFPLIIAAVCAQ	Gamma-aminobutyric acid receptor subunit beta-2 (GABA(A) receptor subunit beta-2)	-0.36
Q075Z2	MGSLMLLFVETTRNSSA	Binder of sperm protein homolog 1 (Bovine seminal plasma protein homolog 1) (Bovine seminal plasma protein-like 1)	-0.36
P03952	MILFKQATYFISLFATVSC	Plasma kallikrein (EC 3.4.21.34) (Fletcher factor) (Kininogenin) (Plasma prekallikrein) [Cleaved into: Plasma kallikrein heavy chain; Plasma kallikrein light chain]	-0.36
Q9BXR5	MRLIRNIYIFCSIVMTAEG	Toll-like receptor 10 (CD antigen CD290)	-0.36
P37173	MGRGLLRGLWPLHIVLWTRIAS	TGF-beta receptor type-2 (TGFR-2) (EC 2.7.11.30) (TGF-beta type II receptor) (Transforming growth factor-beta receptor type II) (TGF-beta receptor type II) (TbetaR-II)	-0.36
P41271	MLRVLVGAVLPAMLL	Neuroblastoma suppressor of tumorigenicity 1 (DAN domain family member 1) (Protein N03) (Zinc finger protein DAN)	-0.36
Q6B9Z1	MVPRISAAIFIFELLGSNS	Insulin growth factor-like family member 4	-0.37
Q8NDY8	MARGPLAARGLRLLLPLLPLLPLLPLPQVALG	Transmembrane protein 52	-0.37
O94907	MMALGAAGATRVFVAMVAAALGGHPLLGVSA	Dickkopf-related protein 1 (Dickkopf-1) (Dkk-1) (hDkk-1) (SK)	-0.37
Q8IWL1	MWLCPLALNLILMAASGAAC	Pulmonary surfactant-associated protein A2 (PSP-A) (PSPA) (SP-A) (SP-A2) (35 kDa pulmonary surfactant-associated protein) (Alveolar proteinosis protein) (Collectin-5)	-0.37
Q8IWL2	MWLCPLALNLILMAASGAVC	Pulmonary surfactant-associated protein A1 (PSP-A) (PSPA) (SP-A) (SP-A1) (35 kDa pulmonary surfactant-associated protein) (Alveolar proteinosis protein) (Collectin-4)	-0.37
P07498	MKSFLLVVNALALTLPFLAV	Kappa-casein	-0.37
Q8IX05	MLRAALPALLLPLLGLAAAAVA	CD302 antigen (C-type lectin BIMLEC) (C-type lectin domain family 13 member A) (DEC205-associated C-type lectin 1) (Type I transmembrane C-type lectin receptor DCL-1) (CD antigen CD302)	-0.37
Q9Y4C0	MSSTLHSVFFTLKVSILLGSLLGLCLG	Neurexin-3 (Neurexin III-alpha) (Neurexin-3-alpha)	-0.37
Q6UWN0	MGPQHLRLVQLFCLLGAISTLPRAGA	Ly6/PLAUR domain-containing protein 4	-0.38
Q96RL6	MVPGQAQPQSPEMLLLPLLLPVLGAGS	Sialic acid-binding Ig-like lectin 11 (Sialic acid-binding lectin 11) (Siglec-11)	-0.38
Q7Z2R9	MWGFLVLKARWLVTPVRT	Putative uncharacterized protein C1orf191	-0.38
Q13145	MDRHSSYIFIWLQLELCAMA	BMP and activin membrane-bound inhibitor homolog (Non-metastatic gene A protein) (Putative transmembrane protein NMA)	-0.38
Q9H8L6	MILSLLFSLGGPLGWGLLGAWA	Multimerin-2 (EMILIN-3) (Elastin microfibril interface located protein 3) (Elastin microfibril interfacer 3) (EndoGlyx-1 p125/p140 subunit)	-0.39
P12544	MRNSYRFLASSLSVVVSLLLIPEDVC	Granzyme A (EC 3.4.21.78) (CTL tryptase) (Cytotoxic T-lymphocyte proteinase 1) (Fragmentin-1) (Granzyme-1) (Hanukkah factor) (H factor) (HF)	-0.39
P21860	MRANDALQVLGLLFSLARG	Receptor tyrosine-protein kinase erbB-3 (EC 2.7.10.1) (Proto-oncogene-like protein c-ErbB-3) (Tyrosine kinase-type cell surface receptor HER3)	-0.39
Q13072	MAARAVFLALSAQLLQA	B melanoma antigen 1 (B melanoma antigen) (Antigen MZ2-BA) (Cancer/testis antigen 2.1) (CT2.1)	-0.39
A6NHM9	MAHDLLFRLFPLLALG	Putative DBH-like monooxygenase protein 2 (EC 1.14.17.-) (DBH-like monooxygenase protein 2 pseudogene)	-0.39
Q96A83	MKLALLLPWACCCLCGSALA	Collagen alpha-1(XXVI) chain (Alpha-1 type XXVI collagen) (EMI domain-containing protein 2) (Emilin and multimerin domain-	-0.40

		containing protein 2) (Emu2)	
P04808	MPRLFLFHLLEFCLLLNQFSRA	Prorelaxin H1 [Cleaved into: Relaxin B chain; Relaxin A chain]	-0.40
Q8N907	MLLGQLSTLLCLLSGALPTGSG	DAN domain family member 5 (Cerberus-like protein 2) (Cerl-2) (Cysteine knot superfamily 1, BMP antagonist 3) (Gremlin-3)	-0.40
O60667	MDFWLWPLYFLPVSGAL	Fas apoptotic inhibitory molecule 3 (Regulator of Fas-induced apoptosis Toso)	-0.40
Q9H6B9	MPELVVTALLAPSRLSLKLLRAFMWSLVFSVALVA	Epoxide hydrolase 3 (EC 3.3.-.-) (Abhydrolase domain-containing protein 9)	-0.40
Q96QH8	MKAWGTVVVTLATLMVVTVDA	Sperm acrosome-associated protein 5 (EC 3.2.1.17) (Lysozyme-like protein 5) (Sperm-specific lysozyme-like protein X) (SLLP-X)	-0.40
Q86YB8	MSQGVRRAGAGQGVAAAVQLLVTLSFLRSVVEA	ERO1-like protein beta (ERO1-L-beta) (EC 1.8.4.-) (Endoplasmic oxidoreductin-1-like protein B) (Oxidoreductin-1-L-beta)	-0.40
O75445	MNCPVLSLGSGFLFQVIEMLIFAYFASISLT	Usherin (Usher syndrome type IIa protein) (Usher syndrome type-2A protein)	-0.40
P02749	MISPVLILFSSFLCHVAIA	Beta-2-glycoprotein 1 (APC inhibitor) (Activated protein C-binding protein) (Anticardiolipin cofactor) (Apolipoprotein H) (Apo-H) (Beta-2-glycoprotein I) (B2GPI) (Beta(2)GPI)	-0.40
Q9UKJ1	MGRPLLLPLLPLLLPPAFL	Paired immunoglobulin-like type 2 receptor alpha (Cell surface receptor FDF03) (Inhibitory receptor PILR-alpha)	-0.40
P28335	MVNLRNAVHSFLVHLIGLLVWQCDISVSPVAA	5-hydroxytryptamine receptor 2C (5-HT-2C) (5-HT2C) (5-HTR2C) (5-hydroxytryptamine receptor 1C) (5-HT-1C) (5-HT1C) (Serotonin receptor 2C)	-0.40
P05060	MQPTLLLSLLGAVGLAAVNS	Secretogranin-1 (Chromogranin-B) (CgB) (Secretogranin I) (SgI) [Cleaved into: GAWK peptide; CCB peptide]	-0.41
P28067	MGHEQNQGAALLQMLPLLWLLPHSWA	HLA class II histocompatibility antigen, DM alpha chain (MHC class II antigen DMA) (Really interesting new gene 6 protein)	-0.41
P0CG01	MKHLVASSILGVFVLTPSLA	Gastrokine-3	-0.41
Q9P0V8	MVMRPLWSLLLWEALLPITVTG	SLAM family member 8 (B-lymphocyte activator macrophage expressed) (BCM-like membrane protein) (CD antigen CD353)	-0.41
Q8WYK1	MDSLPRLTSVLTLLFSGLWHLGLT	Contactin-associated protein-like 5 (Cell recognition molecule Caspr5)	-0.41
Q8J025	MSWPRRLLLRYLFPALLLHGLGEGSA	Protein APCDD1 (Adenomatosis polyposis coli down-regulated 1 protein)	-0.41
Q9NZN1	MKAPIPHLILLYATFTQS	Interleukin-1 receptor accessory protein-like 1 (IL-1-RAPL-1) (IL-1RAPL-1) (IL1RAPL-1) (Oligophrenin-4) (Three immunoglobulin domain-containing IL-1 receptor-related 2) (TIGIRR-2) (X-linked interleukin-1 receptor accessory protein-like 1)	-0.42
Q96D42	MHPQVVILSLILHLADSVAG	Hepatitis A virus cellular receptor 1 (HAVcr-1) (Kidney injury molecule 1) (KIM-1) (T-cell immunoglobulin and mucin domain-containing protein 1) (TIMD-1) (T-cell immunoglobulin mucin receptor 1) (TIM) (TIM-1) (T-cell membrane protein 1)	-0.42
P11047	MRGSHRAAPALRPRGRLWPVLAVLAAAAAAGCA	Laminin subunit gamma-1 (Laminin B2 chain) (Laminin-1 subunit gamma) (Laminin-10 subunit gamma) (Laminin-11 subunit gamma) (Laminin-2 subunit gamma) (Laminin-3 subunit gamma) (Laminin-4 subunit gamma) (Laminin-6 subunit gamma) (Laminin-7 subunit gamma) (Laminin-8 subunit gamma) (Laminin-9 subunit gamma) (S-laminin subunit gamma) (S-LAM gamma)	-0.42
O75951	MTKALLIYLVSSFLALNQA	Lysozyme-like protein 6 (EC 3.2.1.17)	-0.42
P42262	MQKIMHISVLLSPVLWGLIFGVSS	Glutamate receptor 2 (GluR-2) (AMPA-selective glutamate receptor 2) (GluR-B)	-0.42

		(GluR-K2) (Glutamate receptor ionotropic, AMPA 2) (GluA2)	
Q5ZPR3	MLRRRGSPGMGVHVGAALGALWFCLTGA	CD276 antigen (4Ig-B7-H3) (B7 homolog 3) (B7-H3) (Costimulatory molecule) (CD antigen CD276)	-0.42
P54855	MSLKWTSVFLLIQLSCYFSSGSC	UDP-glucuronosyltransferase 2B15 (UDPGT 2B15) (EC 2.4.1.17) (HLUG4) (UDP-glucuronosyltransferase 2B8) (UDPGT 2B8) (UDPGTh-3)	-0.42
Q2L4Q9	MKWCWGPVLLIAGATVLMEGLQA	Serine protease 53 (EC 3.4.21.-) (Polyserine protease 3) (Polyserase-3)	-0.42
Q16820	MDLWNLSWFLFLDALLVISGLA	Meprin A subunit beta (EC 3.4.24.63) (Endopeptidase-2) (Meprin B) (N-benzoyl-L-tyrosyl-P-amino-benzoic acid hydrolase subunit beta) (PABA peptide hydrolase) (PPH beta)	-0.42
Q8N6P7	MRTLLTILTVGSLAA	Interleukin-22 receptor subunit alpha-1 (IL-22 receptor subunit alpha-1) (IL-22R-alpha-1) (IL-22RA1) (Cytokine receptor class-II member 9) (Cytokine receptor family 2 member 9) (CRF2-9) (ZcytoR11)	-0.42
P25445	MLGIWTLLPLVLTSVARLSSKSVNA	Tumor necrosis factor receptor superfamily member 6 (Apo-1 antigen) (Apoptosis-mediating surface antigen FAS) (FASLG receptor) (CD antigen CD95)	-0.42
P27930	MLRLYVLVMGVSA	Interleukin-1 receptor type 2 (IL-1R-2) (IL-1RT-2) (IL-1RT2) (CD121 antigen-like family member B) (CDw121b) (IL-1 type II receptor) (Interleukin-1 receptor beta) (IL-1R-beta) (Interleukin-1 receptor type II) (CD antigen CD121b) [Cleaved into: Interleukin-1 receptor type 2, membrane form (mIL-1R2) (mIL-1RII); Interleukin-1 receptor type 2, soluble form (sIL-1R2) (sIL-1RII)]	-0.43
P42261	MQHIFAFFCTGFLGAVVG	Glutamate receptor 1 (GluR-1) (AMPA-selective glutamate receptor 1) (GluR-A) (GluR-K1) (Glutamate receptor ionotropic, AMPA 1) (GluA1)	-0.43
P12272	MQRRLVQQWSVAVFLLSYAVPSCG	Parathyroid hormone-related protein (PTH-rP) (PTHrP) (Parathyroid hormone-like protein) (PLP) [Cleaved into: PTHrP[1-36]; PTHrP[38-94]; Osteostatin (PTHrP[107-139])]	-0.43
P06133	MSMKWTSALLLIQLSCYFSSGSC	UDP-glucuronosyltransferase 2B4 (UDPGT 2B4) (EC 2.4.1.17) (HLUG25) (Hyodeoxycholic acid-specific UDPGT) (UDPGTh-1)	-0.43
Q6UXH9	MELGCWTQLGLTFLQLLLISS	Inactive serine protease PAMR1 (Peptidase domain-containing protein associated with muscle regeneration 1) (Regeneration-associated muscle protease homolog)	-0.43
Q9H3U7	MLLPQLCWLPLLAGLLPPVPA	SPARC-related modular calcium-binding protein 2 (Secreted modular calcium-binding protein 2) (SMOC-2) (Smooth muscle-associated protein 2) (SMAP-2)	-0.43
Q8IZC6	MGAGSARGARGTAAAAAARGGGFLFSWILVSFACHLA STQG	Collagen alpha-1(XXVII) chain	-0.43
P34810	MRLAVLFSGALLGLLAAQGTG	Macrosialin (Gp110) (CD antigen CD68)	-0.43
P26885	MRLSWFRVLTVLSICLSAVAT	Peptidyl-prolyl cis-trans isomerase FKBP2 (PPIase FKBP2) (EC 5.2.1.8) (13 kDa FK506-binding protein) (13 kDa FKBP) (FKBP-13) (FK506-binding protein 2) (FKBP-2) (Immunophilin FKBP13) (Rotamase)	-0.44
O43699	MQGAQEASASEMLPLLLPLLWAGALA	Sialic acid-binding Ig-like lectin 6 (Siglec-6) (CD33 antigen-like 1) (CDw327) (Obesity-binding protein 1) (OB-BP1) (CD antigen CD327)	-0.44
O00478	MKMASSLAFLLLNFHVSLFLVQLLTPCSA	Butyrophilin subfamily 3 member A3	-0.44
P78410	MKMASSLAFLLLNFHVSLLLVQLLTPCSA	Butyrophilin subfamily 3 member A2	-0.44

P04141	MWLQSLLLLGTVACSIS	Granulocyte-macrophage colony-stimulating factor (GM-CSF) (Colony-stimulating factor) (CSF) (Molgramostin) (Sargramostim)	-0.44
Q9ULB4	MRTYHYIPLFIWTYMFHTVDT	Cadherin-9	-0.44
P18627	MWEAQFLGLLFLQPLWVAPVKPLQPGAE	Lymphocyte activation gene 3 protein (LAG-3) (Protein FDC) (CD antigen CD223)	-0.44
O43692	MIAISAVSSALLFSLLCEA	Peptidase inhibitor 15 (PI-15) (25 kDa trypsin inhibitor) (p25TI) (Cysteine-rich secretory protein 8) (CRISP-8) (SugarCrisp)	-0.44
Q8NDX9	MKVHMLVGVLVMVGFTVG	Lymphocyte antigen 6 complex locus protein G5b	-0.44
P80108	MSAFRLWPGLLIMLGSLCHRGSP	Phosphatidylinositol-glycan-specific phospholipase D (PI-G PLD) (EC 3.1.4.50) (Glycoprotein phospholipase D) (Glycosyl-phosphatidylinositol-specific phospholipase D) (GPI-PLD) (GPI-specific phospholipase D)	-0.44
Q08334	MAWSLGSWLGGCLLVSALG	Interleukin-10 receptor subunit beta (IL-10 receptor subunit beta) (IL-10R subunit beta) (IL-10RB) (Cytokine receptor class-II member 4) (Cytokine receptor family 2 member 4) (CRF2-4) (Interleukin-10 receptor subunit 2) (IL-10R subunit 2) (IL-10R2) (CD antigen CDw210b)	-0.45
Q6UWE3	MAAALALVAGVLSGAVLPLWS	Colipase-like protein 2	-0.45
O15123	MWQIVFFTLSCDLVLAAA	Angiopoietin-2 (ANG-2)	-0.45
P23471	MRILKRFLACIQLLCVCRLDWANG	Receptor-type tyrosine-protein phosphatase zeta (R-PTP-zeta) (EC 3.1.3.48) (Protein-tyrosine phosphatase receptor type Z polypeptide 1) (Protein-tyrosine phosphatase receptor type Z polypeptide 2) (R-PTP-zeta-2)	-0.45
P23469	MEPLCPLLLVGFSLPLARA	Receptor-type tyrosine-protein phosphatase epsilon (Protein-tyrosine phosphatase epsilon) (R-PTP-epsilon) (EC 3.1.3.48)	-0.45
Q9H4F8	MLPARCARLLTPHLLLVLVQLSPARG	SPARC-related modular calcium-binding protein 1 (Secreted modular calcium-binding protein 1) (SMOC-1)	-0.45
Q5VU13	MRVGGAFHLLLVCLSPALLSA	V-set and immunoglobulin domain-containing protein 8	-0.45
C9JL84	MLGFLSRGPSMKLCMGLACVLSLWNTVSG	HERV-H LTR-associating protein 1	-0.45
P05113	MRMLLHLSLLALGAAYVYA	Interleukin-5 (IL-5) (B-cell differentiation factor I) (Eosinophil differentiation factor) (T-cell replacing factor) (TRF)	-0.46
Q13201	MKGARLFVLLSSLWSGGIG	Multimerin-1 (EMILIN-4) (Elastin microfibril interface located protein 4) (Elastin microfibril interfacer 4) (Endothelial cell multimerin) [Cleaved into: Platelet glycoprotein Ia*; 155 kDa platelet multimerin (p-155) (p155)]	-0.46
Q86Y27	MAAGAVFLALSAQLLQA	B melanoma antigen 5 (Cancer/testis antigen 2.5) (CT2.5)	-0.46
Q86Y28	MAAGAVFLALSAQLLQA	B melanoma antigen 4 (Cancer/testis antigen 2.4) (CT2.4)	-0.46
Q8TDF5	MIHGRSVLHIVASLIILHLSGA	Neuropilin and tolloid-like protein 1 (Brain-specific transmembrane protein containing 2 CUB and 1 LDL-receptor class A domains protein 1)	-0.46
P07288	MWVPVVFLTLSVTWIGA	Prostate-specific antigen (PSA) (EC 3.4.21.77) (Gamma-seminoprotein) (Seminin) (Kallikrein-3) (P-30 antigen) (Semenogelase)	-0.46
P79483	MVCLKLPGGSSLAALTVTLMVLSSRLAFA	HLA class II histocompatibility antigen, DR beta 3 chain (MHC class II antigen DRB3)	-0.46
Q9NYU1	MAPAKATNVVRLLLGSTALWLSQLGSG	UDP-glucose:glycoprotein glucosyltransferase 2 (UGT2) (hUGT2) (EC 2.4.1.-) (UDP--Glc:glycoprotein glucosyltransferase 2) (UDP-glucose ceramide glucosyltransferase-like 1)	-0.46
Q9Y5X9	MSNSVPLLCFWSLCYCFAAG	Endothelial lipase (EC 3.1.1.3) (Endothelial cell-derived lipase) (EDL) (EL)	-0.47

Q9BZA8	MFRVGFLIISSSSSLSPLLLVSVVRVNT	Protocadherin-11 Y-linked (Protocadherin-11) (Protocadherin on the Y chromosome) (PCDH-Y) (Protocadherin prostate cancer) (Protocadherin-PC) (Protocadherin-22)	-0.47
Q96NU0	MASVAWAVLKVLLLLPTQTWSPVGA	Contactin-associated protein-like 3B (Cell recognition molecule Caspr3b)	-0.47
Q9BZ76	MASVAWAVLKVLLLLPTQTWSPVGA	Contactin-associated protein-like 3 (Cell recognition molecule Caspr3)	-0.47
P09919	MAGPATQSPMKLMALQLLLWHSALWTVQE	Granulocyte colony-stimulating factor (G-CSF) (Pluripoietin) (Filgrastim) (Lenograstim)	-0.47
P28068	MITFLPLLLGLSLGCTGA	HLA class II histocompatibility antigen, DM beta chain (MHC class II antigen DMB) (Really interesting new gene 7 protein)	-0.47
O75795	MSLKWMSVFLLMQLSCYFSSGSC	UDP-glucuronosyltransferase 2B17 (UDPGT 2B17) (EC 2.4.1.17) (C19-steroid-specific UDP-glucuronosyltransferase) (C19-steroid-specific UDPGT)	-0.47
Q14641	MASLFRSYLPAIWLLLSQLLRESLA	Early placenta insulin-like peptide (EPIL) (Insulin-like peptide 4) (Placentin) [Cleaved into: Early placenta insulin-like peptide B chain; Early placenta insulin-like peptide A chain]	-0.47
Q9NR96	MGFCRSALHPLSLLVQAIMLAMTLA	Toll-like receptor 9 (CD antigen CD289)	-0.47
Q8N7I0	MPNFLGLHRARSFTVLCFWSTADVLNA	Protein GVQW1 (GVQW motif-containing protein 1) (Tigger transposable element-derived 1-like protein 2)	-0.48
P31785	MLKPSLPFTSLLFLQLPLLGVG	Cytokine receptor common subunit gamma (Interleukin-2 receptor subunit gamma) (IL-2 receptor subunit gamma) (IL-2R subunit gamma) (IL-2RG) (gammaC) (p64) (CD antigen CD132)	-0.48
Q9UNW1	MLRAPGCLLRTSVAPAAALAAALLSSLARC	Multiple inositol polyphosphate phosphatase 1 (EC 3.1.3.62) (2,3-bisphosphoglycerate 3-phosphatase) (2,3-BPG phosphatase) (EC 3.1.3.80) (Inositol (1,3,4,5)-tetrakisphosphate 3-phosphatase) (Ins(1,3,4,5)P(4) 3-phosphatase)	-0.48
Q12860	MKMWLLVSHLVIISITTCLA	Contactin-1 (Glycoprotein gp135) (Neural cell surface protein F3)	-0.48
P06319	MAWAPLLLTLLAHCTDCWA	Ig lambda chain V-VI region EB4	-0.48
P35555	MRRGRLLEIALGFTVLLASYTSHGADA	Fibrillin-1	-0.48
Q15256	MRRAVCFPALCLLLNLHAAGC	Receptor-type tyrosine-protein phosphatase R (R-PTP-R) (EC 3.1.3.48) (Ch-1PTPase) (NC-PTPCOM1) (Protein-tyrosine phosphatase PCPTP1)	-0.48
P26992	MAAPVPWACCAVLAAAAAVVYA	Ciliary neurotrophic factor receptor subunit alpha (CNTF receptor subunit alpha) (CNTFR-alpha)	-0.48
Q9BXJ3	MLPLLLGLLGPAACWA	Complement C1q tumor necrosis factor-related protein 4	-0.48
Q3KNT9	MWRLALGGVFLAAAQA	Transmembrane protein 95	-0.49
P58499	MRPLAGGLLKVVFVVFASLCAWYSGYLLA	Protein FAM3B (Cytokine-like protein 2-21) (Pancreatic-derived factor) (PANDER)	-0.49
P24347	MAPAAWLRSAAARALLPPMLLLLLQPPPLLA	Stromelysin-3 (SL-3) (ST3) (EC 3.4.24.-) (Matrix metalloproteinase-11) (MMP-11)	-0.49
P35318	MKLVSVALMYLGSLAFLGADT	ADM [Cleaved into: Adrenomedullin (AM); Proadrenomedullin N-20 terminal peptide (ProAM N-terminal 20 peptide) (PAMP) (ProAM-N20)]	-0.49
Q9H156	MLSGVWFLSVLTVAGILQTES	SLIT and NTRK-like protein 2	-0.49
O94898	MAPAPLGVPEEQLLGCRSRVLSRLLFIAQTALLLLPAAGA	Leucine-rich repeats and immunoglobulin-like domains protein 2 (LIG-2)	-0.49
P05000	MALLFPLLAALVMTSYSPVGS	Interferon omega-1 (Interferon alpha-II-1)	-0.49
Q9H756	MKVTGITILFWPLSMILLSDKIQS	Leucine-rich repeat-containing protein 19	-0.49
P36537	MALKWTTVLLIQLSFYFSSGSCG	UDP-glucuronosyltransferase 2B10 (UDPGT	-0.50

		2B10) (EC 2.4.1.17)	
Q30KQ6	MRIFYYLHFLCYVTFILPATCTLVNA	Beta-defensin 114 (Beta-defensin 14) (DEFB-14) (Defensin, beta 114)	-0.50
Q6YHK3	MQGPPLLTAAHLLCVCTAALA	CD109 antigen (150 kDa TGF-beta-1-binding protein) (C3 and PZP-like alpha-2-macroglobulin domain-containing protein 7) (Platelet-specific Gov antigen) (p180) (r150) (CD antigen CD109)	-0.50
P22003	MHLTVFLLKGIVGFLWSCWVLVGYAKGGLG	Bone morphogenetic protein 5 (BMP-5)	-0.50
Q9UKF5	MKMLLLLHCLGVFLSCSG	Disintegrin and metalloproteinase domain-containing protein 29 (ADAM 29) (Cancer/testis antigen 73) (CT73)	-0.50
P28472	MWGLAGGRLFGIFSAPVLVAVVCCA	Gamma-aminobutyric acid receptor subunit beta-3 (GABA(A) receptor subunit beta-3)	-0.50
Q8IU57	MAGPERWGPLLLCLLQAAPG	Interferon lambda receptor 1 (IFN-lambda receptor 1) (IFN-lambda-R1) (Cytokine receptor class-II member 12) (Cytokine receptor family 2 member 12) (CRF2-12) (Interleukin-28 receptor subunit alpha) (IL-28 receptor subunit alpha) (IL-28R-alpha) (IL-28RA) (Likely interleukin or cytokine receptor 2) (LICR2)	-0.50
P49767	MHLLGFFSVACSLLAAALLPGPREAPAAAAA	Vascular endothelial growth factor C (VEGF-C) (Flt4 ligand) (Flt4-L) (Vascular endothelial growth factor-related protein) (VRP)	-0.50
P61109	MMLFKVLVITVFCGLTVA	Kidney androgen-regulated protein (ARP) (KAP)	-0.50
O60894	MARALCRLPRRGLWLLLAHHLFMTTA	Receptor activity-modifying protein 1 (Calcitonin-receptor-like receptor activity-modifying protein 1) (CRLR activity-modifying protein 1)	-0.50
P20933	MARKSNLPVLLVPFLLCQALVRC	N(4)-(beta-N-acetylglucosaminyl)-L-asparaginase (EC 3.5.1.26) (Aspartylglucosaminidase) (Glycosylasparaginase) (N4-(N-acetyl-beta-glucosaminyl)-L-asparagine amidase) [Cleaved into: Glycosylasparaginase alpha chain; Glycosylasparaginase beta chain]	-0.51
P33151	MQRLMMLLATSGACLGLLAVAAVAA	Cadherin-5 (7B4 antigen) (Vascular endothelial cadherin) (VE-cadherin) (CD antigen CD144)	-0.51
P40199	MGPPSAPPCRLHVPWKEVLLTASLLTFWNPPTTA	Carcinoembryonic antigen-related cell adhesion molecule 6 (Non-specific crossreacting antigen) (Normal cross-reacting antigen) (CD antigen CD66c)	-0.51
Q13873	MTSSLQRPWRVPWLPWTILLVSTAAA	Bone morphogenetic protein receptor type-2 (BMP type-2 receptor) (BMPR-2) (EC 2.7.11.30) (Bone morphogenetic protein receptor type II) (BMP type II receptor) (BMPR-II)	-0.51
P25942	MVRLPLQCVLWGCLLTAVHP	Tumor necrosis factor receptor superfamily member 5 (B-cell surface antigen CD40) (Bp50) (CD40L receptor) (CDw40) (CD antigen CD40)	-0.51
Q9BT76	MGLPWGQPHLGLQMLLLALNCLRPSLSLG	Uroplakin-3b (UP3b) (Uroplakin IIIb) (UPIIIb) (p35)	-0.51
Q6UXQ6	MARVPPVGALLLLRGSRQ	Putative uncharacterized protein UNQ6125/PRO20090	-0.51
Q6UXB0	MFLATLSFLLPFAHPFGTVSC	Protein FAM131A	-0.51
Q6P7N7	MKVLATSFVLGSLGLAFYLPLVVTTPKTLA	Transmembrane protein 81	-0.51
Q96MU8	MAPPAARLALLSAAALTLA	Kremen protein 1 (Dickkopf receptor) (Kringle domain-containing transmembrane protein 1) (Kringle-containing protein marking the eye and the nose)	-0.51
O75594	MSRRSMLLAWALPSLLRLGAA	Peptidoglycan recognition protein 1 (Peptidoglycan recognition protein short) (PGRP-S)	-0.52

O43897	MGLGTLSPRMLVWLVASGIVFYGELWVCAG	Tolloid-like protein 1 (EC 3.4.24.-)	-0.52
O95185	MRKGLRATAARCGLGLGYLLQMLVLPALALLSASGTGSAA	Netrin receptor UNC5C (Protein unc-5 homolog 3) (Protein unc-5 homolog C)	-0.52
P34059	MAAVVAATRWWQLLLVLSAAGMGASG	N-acetylgalactosamine-6-sulfatase (EC 3.1.6.4) (Chondroitinsulfatase) (Chondroitinase) (Galactose-6-sulfate sulfatase) (GalN6S) (N-acetylgalactosamine-6-sulfate sulfatase) (GalNAc6S sulfatase)	-0.52
P08861	MMLRLLSSLLLVAVA	Chymotrypsin-like elastase family member 3B (EC 3.4.21.70) (Elastase IIIB) (Elastase-3B) (Protease E)	-0.52
P09093	MMLRLLSSLLLVAVA	Chymotrypsin-like elastase family member 3A (EC 3.4.21.70) (Elastase IIIA) (Elastase-3A) (Protease E)	-0.52
P47972	MLALLAASVALAVAA	Neuronal pentraxin-2 (NP2) (Neuronal pentraxin II) (NP-II)	-0.52
P43629	MSLMVVSMACVGLFLVQRAGP	Killer cell immunoglobulin-like receptor 3DL1 (CD158 antigen-like family member E) (HLA-BW4-specific inhibitory NK cell receptor) (MHC class I NK cell receptor) (Natural killer-associated transcript 3) (NKAT-3) (p70 natural killer cell receptor clones CL-2/CL-11) (p70 NK receptor CL-2/CL-11) (CD antigen CD158e)	-0.52
Q14943	MSLMVVSMACVGLFLVQRAGP	Killer cell immunoglobulin-like receptor 3DS1 (MHC class I NK cell receptor) (Natural killer-associated transcript 10) (NKAT-10)	-0.52
Q96CP7	MPRLLHPALPLLLGATLTFRALRRALCRLPLPVHV	TLC domain-containing protein 1	-0.52
Q08629	MPAIAVLAAAAAAWCFLQVES	Testican-1 (Protein SPOCK)	-0.52
P25092	MKTLLLDLALWSLLFQPGWLSFS	Heat-stable enterotoxin receptor (STA receptor) (hSTAR) (EC 4.6.1.2) (Guanylyl cyclase C) (GC-C) (Intestinal guanylate cyclase)	-0.52
Q8NI17	MMWTWALWMLPSLCKFSLA	Interleukin-31 receptor subunit alpha (IL-31 receptor subunit alpha) (IL-31R subunit alpha) (IL-31R-alpha) (IL-31RA) (Cytokine receptor-like 3) (GLM-R) (hGLM-R) (Gp130-like monocyte receptor) (Gp130-like receptor) (ZcytoR17)	-0.52
P39877	MKGLLPLAWFLACSVPAVQG	Calcium-dependent phospholipase A2 (EC 3.1.1.4) (Group V phospholipase A2) (PLA2-10) (Phosphatidylcholine 2-acylhydrolase 5)	-0.52
Q01151	MSRGLQLLLLSCAYSLAPA	CD83 antigen (hCD83) (B-cell activation protein) (Cell surface protein HB15) (CD antigen CD83)	-0.53
Q9BXJ7	MGVLGRVLLWLQLCALTQA	Protein amnionless	-0.53
Q8TE59	MRLTHICCCCLLYQLGFLSNG	A disintegrin and metalloproteinase with thrombospondin motifs 19 (ADAM-TS 19) (ADAM-TS19) (ADAMTS-19) (EC 3.4.24.-)	-0.53
Q9BQY6	MGLSGLLPILVPFILLGDIQEPGHA	WAP four-disulfide core domain protein 6 (Putative protease inhibitor WAP6)	-0.53
Q9Y5K2	MATAGNPWGWFLGYLILGVAGSLVSG	Kallikrein-4 (EC 3.4.21.-) (Enamel matrix serine proteinase 1) (Kallikrein-like protein 1) (KLK-L1) (Prostase) (Serine protease 17)	-0.53
Q13217	MVAPGSVTSRLGSVFPFLLVLVDLQYEGAEC	DnaJ homolog subfamily C member 3 (Endoplasmic reticulum DNA J domain-containing protein 6) (ER-resident protein ERdj6) (ERdj6) (Interferon-induced, double-stranded RNA-activated protein kinase inhibitor) (Protein kinase inhibitor of 58 kDa) (Protein kinase inhibitor p58)	-0.53
O75310	MTLKWTSVLLLIHLSCYFSSG	UDP-glucuronosyltransferase 2B11 (UDPGT 2B11) (EC 2.4.1.17)	-0.53
Q9NPG4	MMQLLQLLLGLLGPGGYLFLLGDC	Protocadherin-12 (Vascular cadherin-2) (Vascular endothelial cadherin-2) (VE-cad-2) (VE-cadherin-2)	-0.54

P35443	MLAPRGAAVLLLHLVLQRWLAAGAQA	Thrombospondin-4	-0.54
Q9NTN9	MWGRLWPLLLSILTATA	Semaphorin-4G	-0.54
Q6UWJ1	MKVLGRSFFWVLFPVLPWAVQA	Transmembrane and coiled-coil domain-containing protein 3 (Putative LAG1-interacting protein)	-0.54
P00751	MGSNLSPQLCLMPFILGLLSGGVTT	Complement factor B (EC 3.4.21.47) (C3/C5 convertase) (Glycine-rich beta glycoprotein) (GBG) (PBF2) (Properdin factor B) [Cleaved into: Complement factor B Ba fragment; Complement factor B Bb fragment]	-0.54
Q6UXK5	MARMSFVIAACQLVLGLLMTSLTES	Leucine-rich repeat neuronal protein 1 (Neuronal leucine-rich repeat protein 1) (NLRR-1)	-0.54
P19256	MVAGSDAGRALGVLSVVCLLHCFGFISC	Lymphocyte function-associated antigen 3 (Ag3) (Surface glycoprotein LFA-3) (CD antigen CD58)	-0.54
Q15113	MLPAATASLLGPLLTACALLPFAQG	Procollagen C-endopeptidase enhancer 1 (Procollagen COOH-terminal proteinase enhancer 1) (PCPE-1) (Procollagen C-proteinase enhancer 1) (Type 1 procollagen C-proteinase enhancer protein) (Type I procollagen COOH-terminal proteinase enhancer)	-0.54
P02751	MLRGPGPGLLLLAVQCLGTAVPSTGASKSKR	Fibronectin (FN) (Cold-insoluble globulin) (CIG) [Cleaved into: Anastellin; Ugl-Y1; Ugl-Y2; Ugl-Y3]	-0.54
Q9ULH4	METLLGGLLAFGMAFAVVDA	Leucine-rich repeat and fibronectin type-III domain-containing protein 2 (Synaptic adhesion-like molecule 1)	-0.55
P08922	MKNIYCLIPKLVNFATLGCLWISVVQC	Proto-oncogene tyrosine-protein kinase ROS (EC 2.7.10.1) (Proto-oncogene c-Ros) (Proto-oncogene c-Ros-1) (Receptor tyrosine kinase c-ros oncogene 1) (c-Ros receptor tyrosine kinase)	-0.55
P20701	MKDSCITVMAMALLSGFFFFAPASS	Integrin alpha-L (CD11 antigen-like family member A) (Leukocyte adhesion glycoprotein LFA-1 alpha chain) (LFA-1A) (Leukocyte function-associated molecule 1 alpha chain) (CD antigen CD11a)	-0.55
P36888	MPALARDGGQLPLLVVFSAMIFGTIT	Receptor-type tyrosine-protein kinase FLT3 (EC 2.7.10.1) (FL cytokine receptor) (Fetal liver kinase-2) (FLK-2) (Fms-like tyrosine kinase 3) (FLT-3) (Stem cell tyrosine kinase 1) (STK-1) (CD antigen CD135)	-0.55
P06756	MAFPPRRRLRLGPRGLPLLLSGLLLPLCRA	Integrin alpha-V (Vitronectin receptor subunit alpha) (CD antigen CD51) [Cleaved into: Integrin alpha-V heavy chain; Integrin alpha-V light chain]	-0.55
Q6PCB0	MLPWTALGLALSLRLALARSGA	von Willebrand factor A domain-containing protein 1	-0.55
Q9UQC9	MTQRSIAGPICNLKFVTLLVALSSELPFLGA	Calcium-activated chloride channel regulator 2 (Calcium-activated chloride channel family member 2) (hCLCA2) (Calcium-activated chloride channel protein 3) (CaCC-3) (hCaCC-3) [Cleaved into: Calcium-activated chloride channel regulator 2, 109 kDa form; Calcium-activated chloride channel regulator 2, 35 kDa form]	-0.55
Q9NRE1	MQLVILRVTIFLPWCFA	Matrix metalloproteinase-26 (MMP-26) (EC 3.4.24.-) (Endometase) (Matrilysin-2)	-0.55
A8K4G0	MWLPPALLLLSLSGCFS	CMRF35-like molecule 7 (CLM-7) (CD300 antigen-like family member B) (CMRF35-A2) (Immune receptor expressed on myeloid cells 3) (IREM-3) (Leukocyte mono-Ig-like receptor 5) (Triggering receptor expressed on myeloid cells 5) (TREM-5) (CD antigen CD300b)	-0.55
A4D1T9	MKYVFYLGVLAGTFFFADS	Probable inactive serine protease 37 (Probable inactive trypsin-X2)	-0.55

Q99795	MVGKMWPVLWTLCAVRVTVDA	Cell surface A33 antigen (Glycoprotein A33)	-0.55
Q9Y6Y9	MLPFLFFSTLFSSIFTEA	Lymphocyte antigen 96 (Ly-96) (ESOP-1) (Protein MD-2)	-0.55
P00734	MAHVRGLQLPGCLALAALCSLVHS	Prothrombin (EC 3.4.21.5) (Coagulation factor II) [Cleaved into: Activation peptide fragment 1; Activation peptide fragment 2; Thrombin light chain; Thrombin heavy chain]	-0.55
P22894	MFSLKTLPFLLLLHVQISKA	Neutrophil collagenase (EC 3.4.24.34) (Matrix metalloproteinase-8) (MMP-8) (PMNL collagenase) (PMNL-CL)	-0.56
Q9NYY1	MKASSLAFSLLSAAFYLLWTPSTG	Interleukin-20 (IL-20) (Cytokine Zcyto10)	-0.56
Q9UK05	MCPGALWVALPLLSLLAGSLQG	Growth/differentiation factor 2 (GDF-2) (Bone morphogenetic protein 9) (BMP-9)	-0.56
Q7RTX0	MLGPAVLGLSLWALLHPGTG	Taste receptor type 1 member 3 (Sweet taste receptor T1R3)	-0.56
Q14C87	MCPSEMGTLWHHWSPVLISLAALFSKVTEG	Transmembrane protein 132D (Mature oligodendrocytes transmembrane protein) (Mature OL transmembrane protein)	-0.56
Q8NCS4	MALLLSVLRVLLGGFFALVGLA	Uncharacterized protein ZMYM6NB (ZMYM6 neighbor protein)	-0.56
O60888	MSGGRAPAVLLGGVASLLLSFVWMPALLPVAS	Protein CutA (Acetylcholinesterase-associated protein) (Brain acetylcholinesterase putative membrane anchor)	-0.56
P49190	MAGLGASLHVWGWLMLGSCLLARA	Parathyroid hormone 2 receptor (PTH2 receptor)	-0.56
P04003	MHPPKTPSGALHRKRKMAAWPFSRLWKVSDPILFQMT LIAALLPAVLG	C4b-binding protein alpha chain (C4bp) (Proline-rich protein) (PRP)	-0.56
P06316	MTCSPLLLTLLIHCTGSWA	Ig lambda chain V-I region BL2	-0.57
Q9NPH6	MKTLFLGVTLGLAAA	Odorant-binding protein 2b (Odorant-binding protein IIb) (OBPIIb)	-0.57
Q9NY56	MKTLFLGVTLGLAAA	Odorant-binding protein 2a (Odorant-binding protein IIa) (OBPIIa)	-0.57
Q9BY64	MALKWTSVLLLIHLGCYFSSGSCG	UDP-glucuronosyltransferase 2B28 (UDPGT 2B28) (EC 2.4.1.17)	-0.57
P14210	MWVTKLLPALLLQHVLLHLLLLPIAIPYAEG	Hepatocyte growth factor (Hepatopoietin-A) (Scatter factor) (SF) [Cleaved into: Hepatocyte growth factor alpha chain; Hepatocyte growth factor beta chain]	-0.57
Q68DV7	MSGGHQLQLAALWPWLLMATLQA	E3 ubiquitin-protein ligase RNF43 (EC 6.3.2.-) (RING finger protein 43)	-0.58
Q2M2E5	MLAPLFLCCLRNLFRKLIS	Uncharacterized protein C5orf64	-0.58
Q14246	MRGFNLLLFWGCCVMHSWEG	EGF-like module-containing mucin-like hormone receptor-like 1 (EGF-like module receptor 1) (EMR1 hormone receptor)	-0.58
Q9BQ16	MLKVSAVLCVCAAAWCSQSLA	Testican-3 (SPARC/osteonectin, CWCV, and Kazal-like domains proteoglycan 3)	-0.58
Q8WVF2	MTWRQAVLLSCFSAVVLLSMLREGTS	Unique cartilage matrix-associated protein [Cleaved into: Unique cartilage matrix-associated protein C-terminal fragment (Ucma-C) (Gla-rich protein) (GRP)]	-0.58
P20916	MIFLTALPLFWIMISASRG	Myelin-associated glycoprotein (Siglec-4a)	-0.58
Q9HBB8	MGSWALLWPPLLFTGLLVRPPGTMA	Cadherin-related family member 5 (Mu-protocadherin) (Mucin and cadherin-like protein)	-0.58
Q9BX73	MVLGGCPVSYLLLCGQAALLLGNLLLLHCVSRSHS	TM2 domain-containing protein 2 (Beta-amyloid-binding protein-like protein 1) (BBP-like protein 1)	-0.58
Q8IVL8	MKPLLETLYLLGMLVPGGLG	Carboxypeptidase O (CPO) (EC 3.4.17.-)	-0.58
Q00604	MRKHVLAASFSMLSLLVIMGDTDS	Norrin (Norrie disease protein) (X-linked exudative vitreoretinopathy 2 protein)	-0.58
Q6UWV2	MQQRGAAGSRGCALFPLLGVLFFQGVYIVFS	Myelin protein zero-like protein 3	-0.59
O75311	MAHVRHFRTLVSGFYFWEAALLLSLVATKETDS	Glycine receptor subunit alpha-3	-0.59
A8MTB9	MDLSRPRWSLWRRVFLMASLLACGICQASG	Carcinoembryonic antigen-related cell adhesion molecule 18	-0.59

Q9Y5Z0	MGALARALLLPLLAQWLLRA	Beta-secretase 2 (EC 3.4.23.45) (Aspartic-like protease 56 kDa) (Aspartyl protease 1) (ASP1) (Asp 1) (Beta-site amyloid precursor protein cleaving enzyme 2) (Beta-site APP cleaving enzyme 2) (Down region aspartic protease) (DRAP) (Memapsin-1) (Membrane-associated aspartic protease 1) (Theta-secretase)	-0.59
Q92876	MKKLMVVLSLIAAAWA	Kallikrein-6 (EC 3.4.21.-) (Neurosin) (Protease M) (SP59) (Serine protease 18) (Serine protease 9) (Zyme)	-0.59
Q9ULK0	MEALTLWLLPWICQCVSVRA	Glutamate receptor ionotropic, delta-1 (GluD1) (GluR delta-1 subunit)	-0.59
Q96IY4	MKLCSLAVLVPIVLFCEQHVFA	Carboxypeptidase B2 (EC 3.4.17.20) (Carboxypeptidase U) (CPU) (Plasma carboxypeptidase B) (pCPB) (Thrombin-activable fibrinolysis inhibitor) (TAFI)	-0.59
Q5T1H1	MTDKSIVILSLMVFHSSFING	Protein eyes shut homolog (Epidermal growth factor-like protein 10) (EGF-like protein 10) (Epidermal growth factor-like protein 11) (EGF-like protein 11) (Protein spacemaker homolog)	-0.60
Q8TCU5	MRRLSLWWLLSRVCLLLPPPCAL	Glutamate receptor ionotropic, NMDA 3A (GluN3A) (N-methyl-D-aspartate receptor subtype 3A) (NMDAR3A) (NR3A) (NMDAR-L)	-0.60
P19835	MGRLQLVVLGLTCCWAVASA	Bile salt-activated lipase (BAL) (EC 3.1.1.13) (EC 3.1.1.3) (Bile salt-stimulated lipase) (BSSL) (Bucelipase) (Carboxyl ester lipase) (Cholesterol esterase) (Pancreatic lysophospholipase) (Sterol esterase)	-0.60
Q969Z4	MKPSLLCRPLSCFLMLLPWPLATLT	Tumor necrosis factor receptor superfamily member 19L (Receptor expressed in lymphoid tissues)	-0.60
P52798	MRLLPLLRTVLWAAFLGSPLRGGSS	Ephrin-A4 (EPH-related receptor tyrosine kinase ligand 4) (LERK-4)	-0.60
Q15485	MELDRAVGVLGAATLLLSFLGMAWA	Ficolin-2 (37 kDa elastin-binding protein) (Collagen/fibrinogen domain-containing protein 2) (EBP-37) (Ficolin-B) (Ficolin-beta) (Hucolin) (L-ficolin) (Serum lectin p35)	-0.60
Q16880	MKSYTPYFILLWSAVGIAKA	2-hydroxyacylsphingosine 1-beta-galactosyltransferase (EC 2.4.1.45) (Ceramide UDP-galactosyltransferase) (Cerebroside synthase) (UDP-galactose-ceramide galactosyltransferase)	-0.60
P16662	MSVKWTSVILLIQLSFCFSSGNC	UDP-glucuronosyltransferase 2B7 (UDPGT 2B7) (EC 2.4.1.17) (3,4-catechol estrogen-specific UDPGT) (UDP-glucuronosyltransferase 2B9) (UDPGT 2B9) (UDPGTh-2)	-0.61
Q9Y258	MMGLSLASAVLLASLLSLHLGTA	C-C motif chemokine 26 (CC chemokine IMAC) (Eotaxin-3) (Macrophage inflammatory protein 4-alpha) (MIP-4-alpha) (Small-inducible cytokine A26) (Thymic stroma chemokine-1) (TSC-1)	-0.61
Q8WW62	MSPLLFGAGLVVLNLVTSARS	Transmembrane emp24 domain-containing protein 6 (p24 family protein gamma-5) (p24gamma5)	-0.61
Q9HAW9	MARTGWTSPIPLCVSLLLTCGFAEA	UDP-glucuronosyltransferase 1-8 (UDPGT 1-8) (UGT1*8) (UGT1-08) (UGT1.8) (EC 2.4.1.17) (UDP-glucuronosyltransferase 1-H) (UGT-1H) (UGT1H) (UDP-glucuronosyltransferase 1A8)	-0.61
Q9N2K0	MIFAGKAPSNTSTLMKFYSLLLYSLLFSFPFLCHP	HERV-H_2q24.3 provirus ancestral Env polyprotein (Env protein HERV-H/p62) (Env protein HERV-H19) (Env protein HERV-Hcl.3) (Envelope polyprotein) (HERV-H/env62) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)]	-0.61
Q9N2J8	MILAGRAPSNTSTLMKFYSLLLYSLLFSFPFLYHP	HERV-H_2q24.1 provirus ancestral Env polyprotein (Env protein HERV-H/p59)	-0.61

		(Envelope polyprotein) (HERV-H/env59) [Cleaved into: Surface protein (SU); Transmembrane protein (TM)]	
P02748	MSACRSFAVAICILEISILTA	Complement component C9 [Cleaved into: Complement component C9a; Complement component C9b]	-0.61
P19022	MCRIAGALRTLLPLLAALLQASVEA	Cadherin-2 (CDw325) (Neural cadherin) (N-cadherin) (CD antigen CD325)	-0.61
Q6UX71	MARFPKADLAAAGVMLLCHFFTDQFQFADG	Plexin domain-containing protein 2 (Tumor endothelial marker 7-related protein)	-0.61
Q7Z4H4	MARIPTAALGCISLLCLQLPGSLS	ADM2 (Intermedin) [Cleaved into: Adrenomedullin-2 (AM2) (Intermedin-long) (IMDL); Intermedin-short (IMDS)]	-0.61
A8MPY1	MVLAFQLVSFTYIWIILKPNVCA	Gamma-aminobutyric acid receptor subunit rho-3 (GABA(A) receptor subunit rho-3) (GABA(C) receptor)	-0.61
P55145	MRRMWATQGLAVALALSVLPGSRA	Mesencephalic astrocyte-derived neurotrophic factor (Arginine-rich protein) (Protein ARMET)	-0.62
P28827	MRGLGTCLATLAGLLLTAAG	Receptor-type tyrosine-protein phosphatase mu (Protein-tyrosine phosphatase mu) (R-PTP-mu) (EC 3.1.3.48)	-0.62
P02763	MALSWVLTVLSLLPLLEA	Alpha-1-acid glycoprotein 1 (AGP 1) (Orosomucoid-1) (OMD 1)	-0.62
P19652	MALSWVLTVLSLLPLLEA	Alpha-1-acid glycoprotein 2 (AGP 2) (Orosomucoid-2) (OMD 2)	-0.62
Q6UWQ5	MKAAGILTLIGCLVTGAES	Lysozyme-like protein 1 (EC 3.2.1.17)	-0.62
Q7Z4W2	MKAAGILTLIGCLVTGAES	Lysozyme-like protein 2 (Lysozyme-2) (EC 3.2.1.17)	-0.62
P04062	MEFSSPSREECPKPLSRVSIMAGSLTGLLLLQAVSWASG	Glucosylceramidase (EC 3.2.1.45) (Acid beta-glucosidase) (Alglucerase) (Beta-glucocerebrosidase) (D-glucosyl-N-acylsphingosine glucohydrolase) (Imiglucerase)	-0.62
Q6UWW9	MSRSRLFSVTSAISTIGILCLPLFQLVLS	Transmembrane protein 207	-0.62
P53708	MSPGASRGPRGSQAPLIAPLCCAAAALGMLLWSPACQA	Integrin alpha-8 [Cleaved into: Integrin alpha-8 heavy chain; Integrin alpha-8 light chain]	-0.62
P48304	MAQTNSFFMLISSLMFLSLSQG	Lithostathine-1-beta (Pancreatic stone protein 2) (PSP-2) (Regenerating islet-derived protein 1-beta) (REG-1-beta) (Regenerating protein I beta)	-0.62
P22888	MKQRFSALQLLKLLLLLQPPLPRALR	Lutropin-choriogonadotropic hormone receptor (LH/CG-R) (Luteinizing hormone receptor) (LHR) (LSH-R)	-0.62
Q86YL7	MWKVSALLFVLGSASLWVLAEG	Podoplanin (Aggrus) (Glycoprotein 36) (Gp36) (PA2.26 antigen) (T1-alpha) (T1A)	-0.62
Q92729	MARAQALVLALTFQLCAP	Receptor-type tyrosine-protein phosphatase U (R-PTP-U) (EC 3.1.3.48) (Pancreatic carcinoma phosphatase 2) (PCP-2) (Protein-tyrosine phosphatase J) (PTP-J) (hPTP-J) (Protein-tyrosine phosphatase pi) (PTP pi) (Protein-tyrosine phosphatase receptor omicron) (PTP-RO) (Receptor-type protein-tyrosine phosphatase psi) (R-PTP-psi)	-0.63
A6NHS7	MHVAEVAVNVILLLSMGWTSDSLC	MANSC domain-containing protein 4	-0.63
Q9BZM2	MKKFFTVAILAGSVLSTAHG	Group IIF secretory phospholipase A2 (GIIF sPLA2) (sPLA2-IIF) (EC 3.1.1.4) (Phosphatidylcholine 2-acylhydrolase 2F)	-0.63
O94779	MASSWKLMLFLSVTMCLS	Contactin-5 (Neural recognition molecule NB-2) (hNB-2)	-0.63
Q9P2N4	MQFVSWATLLTLLVRDLA	A disintegrin and metalloproteinase with thrombospondin motifs 9 (ADAM-TS 9) (ADAM-TS9) (ADAMTS-9) (EC 3.4.24.-)	-0.63
Q96FM1	MAGLAARLVLLAGAAALASG	Post-GPI attachment to proteins factor 3 (COS16 homolog) (hCOS16) (Gene coamplified with ERBB2 protein) (PER1-like domain-containing protein 1)	-0.63
Q969D9	MFPFALLYVLSVSFRKIFILQLVGLVLT	Thymic stromal lymphopoietin	-0.64

P35542	MRLFTGIVFCSLVMGVTS	Serum amyloid A-4 protein (Constitutively expressed serum amyloid A protein) (C-SAA)	-0.64
P55000	MASRWAVQLLLVAAWSMGCGEA	Secreted Ly-6/uPAR-related protein 1 (SLURP-1) (ARS component B) (ARS(component B)-81/S) (Anti-neoplastic urinary protein) (ANUP)	-0.64
Q13214	MGRAGAAAVIPGLALLWAVGLGSA	Semaphorin-3B (Sema A(V)) (Semaphorin-V) (Sema V)	-0.64
Q13634	MKITSTSCICPVLVCLCFVQRCYG	Cadherin-18 (Cadherin-14)	-0.64
P24394	MGWLCSGLLFPVSCLVLLQVASSGN	Interleukin-4 receptor subunit alpha (IL-4 receptor subunit alpha) (IL-4R subunit alpha) (IL-4R-alpha) (IL-4RA) (CD antigen CD124) [Cleaved into: Soluble interleukin-4 receptor subunit alpha (Soluble IL-4 receptor subunit alpha) (Soluble IL-4R-alpha) (sIL4Ralpha/prot) (IL-4-binding protein) (IL4-BP)]	-0.64
O00144	MAVAPLRGALLLWQLLAAGGAA	Frizzled-9 (Fz-9) (hFz9) (FzE6) (CD antigen CD349)	-0.64
O00175	MAGLMTIVTSLLFLGVCAHHIIPTGS	C-C motif chemokine 24 (CK-beta-6) (Eosinophil chemotactic protein 2) (Eotaxin-2) (Myeloid progenitor inhibitory factor 2) (MPIF-2) (Small-inducible cytokine A24)	-0.64
Q92752	MGADGETVVLKNMLIGINLILLGSMIKPSEC	Tenascin-R (TN-R) (Janusin) (Restrictin)	-0.64
Q13349	MTFGTVLLLSVLASYHG	Integrin alpha-D (ADB2) (CD11 antigen-like family member D) (Leukointegrin alpha D) (CD antigen CD11d)	-0.65
P01266	MALVLEIFTLLASICWVSA	Thyroglobulin (Tg)	-0.65
P29460	MCHQQLVISWFSLVFLASPLVA	Interleukin-12 subunit beta (IL-12B) (Cytotoxic lymphocyte maturation factor 40 kDa subunit) (CLMF p40) (IL-12 subunit p40) (NK cell stimulatory factor chain 2) (NKSF2)	-0.65
Q9GZP0	MHRLIFVYTLICANFCSC	Platelet-derived growth factor D (PDGF-D) (Iris-expressed growth factor) (Spinal cord-derived growth factor B) (SCDGF-B) [Cleaved into: Platelet-derived growth factor D, latent form (PDGFD latent form); Platelet-derived growth factor D, receptor-binding form (PDGFD receptor-binding form)]	-0.65
P02743	MNKPLLWISVLTSLLEAFA	Serum amyloid P-component (SAP) (9.5S alpha-1-glycoprotein) [Cleaved into: Serum amyloid P-component(1-203)]	-0.65
Q4LDE5	MWPRLAFCCWGLALVSG	Sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1 (CCP module-containing protein 22) (Polydom) (Selectin-like osteoblast-derived protein) (SEL-OB) (Serologically defined breast cancer antigen NY-BR-38)	-0.65
Q9HBW9	MKRLPLLVVFSTLLNCSYT	EGF, latrophilin and seven transmembrane domain-containing protein 1 (EGF-TM7-latrophilin-related protein) (ETL protein)	-0.66
Q05707	MKIFQRKMRYWLLPPFLAIVYFCTIVQG	Collagen alpha-1(XIV) chain (Undulin)	-0.66
P58335	MVAERSPARSPGSWLFPGLWLLVLSGPGGLLRA	Anthrax toxin receptor 2 (Capillary morphogenesis gene 2 protein) (CMG-2)	-0.66
Q32P28	MAVRALKLLTTLLAVVAAASQA	Prolyl 3-hydroxylase 1 (EC 1.14.11.7) (Growth suppressor 1) (Leucine- and proline-enriched proteoglycan 1) (Leprecan-1)	-0.66
O75487	MARFGLPALLCTLAVLSA	Glypican-4 (K-glypican) [Cleaved into: Secreted glypican-4]	-0.66
P52961	MQMPAMMSLLLVSVGLMEALQA	GPI-linked NAD(P)(+)--arginine ADP-ribosyltransferase 1 (EC 2.4.2.31) (ADP-ribosyltransferase C2 and C3 toxin-like 1) (ARTC1) (Mono(ADP-ribosyl)transferase 1) (CD antigen CD296)	-0.66
Q9UKX5	MDLPRGLVVAWALSLWPGFTDT	Integrin alpha-11	-0.66
Q9NPD7	MGLKLNGRYISLILAVQIAYLVQAVRA	Neuritin	-0.66

P11226	MSLFPSLPLLLLSMVAASYS	Mannose-binding protein C (MBP-C) (Collectin-1) (MBP1) (Mannan-binding protein) (Mannose-binding lectin)	-0.67
Q9NZU0	MISAAWSIFLIGTKIGLFLQVAPLSVMA	Leucine-rich repeat transmembrane protein FLRT3 (Fibronectin-like domain-containing leucine-rich transmembrane protein 3)	-0.67
P36544	MRCSPGGVWLALAASLLHVSLQ	Neuronal acetylcholine receptor subunit alpha-7	-0.67
A4D0S4	MQFQLTLFLHLGWLSYSKA	Laminin subunit beta-4 (Laminin beta-1-related protein)	-0.67
Q96CW9	MLHLLALFLHCLPLASG	Netrin-G2 (Laminet-2)	-0.67
O75121	MDRLKSHLTVCFLPSVPFLILVSTLATA	Microfibrillar-associated protein 3-like (Testis development protein NYD-SP9)	-0.67
Q7Z304	MLLRGVLLALQALQLAGA	MAM domain-containing protein 2 (MAM domain-containing proteoglycan) (Mamcan)	-0.67
P32418	MYNMRRLSLSPTFSMGFHLLVTVSLLFSHVDHVIA	Sodium/calcium exchanger 1 (Na(+)/Ca(2+)-exchange protein 1) (Solute carrier family 8 member 1)	-0.68
P04438	MDILCSTLLLLTVPSGVLS	Ig heavy chain V-II region SESS	-0.68
Q9UKY3	MWLPALVLATLAASAAWA	Putative inactive carboxylesterase 4 (Inactive carboxylesterase 1 pseudogene 1) (Placental carboxylesterase 3) (PCE-3)	-0.68
P02671	MFSMRIVCLVLSVVGTAWT	Fibrinogen alpha chain [Cleaved into: Fibrinopeptide A; Fibrinogen alpha chain]	-0.68
P31644	MDNGMFSGFIMIKNLLLFCISMNLSSHFGFS	Gamma-aminobutyric acid receptor subunit alpha-5 (GABA(A) receptor subunit alpha-5)	-0.68
P56817	MAQALPWLLLWMGAGVLPAHG	Beta-secretase 1 (EC 3.4.23.46) (Aspartyl protease 2) (ASP2) (Asp 2) (Beta-site amyloid precursor protein cleaving enzyme 1) (Beta-site APP cleaving enzyme 1) (Memapsin-2) (Membrane-associated aspartic protease 2)	-0.68
Q9Y625	MPSWIGAVILPLLGLLLSLPAGA	Glypican-6 [Cleaved into: Secreted glypican-6]	-0.68
P48551	MLLSQNAFIFRSLNLVLMVYISLVFG	Interferon alpha/beta receptor 2 (IFN-R-2) (IFN-alpha binding protein) (IFN-alpha/beta receptor 2) (Interferon alpha binding protein) (Type I interferon receptor 2)	-0.68
P17213	MRENMARGPCNAPRWASLMVLVAIGTAVTAA	Bactericidal permeability-increasing protein (BPI) (CAP 57)	-0.68
P01891	MAVMAPRTLVLLLSGALALTQTWA	HLA class I histocompatibility antigen, A-68 alpha chain (Aw-68) (HLA class I histocompatibility antigen, A-28 alpha chain) (MHC class I antigen A*68)	-0.68
P01892	MAVMAPRTLVLLLSGALALTQTWA	HLA class I histocompatibility antigen, A-2 alpha chain (MHC class I antigen A*2)	-0.68
P05534	MAVMAPRTLVLLLSGALALTQTWA	HLA class I histocompatibility antigen, A-24 alpha chain (Aw-24) (HLA class I histocompatibility antigen, A-9 alpha chain) (MHC class I antigen A*24)	-0.68
P10316	MAVMAPRTLVLLLSGALALTQTWA	HLA class I histocompatibility antigen, A-69 alpha chain (Aw-69) (HLA class I histocompatibility antigen, A-28 alpha chain) (MHC class I antigen A*69)	-0.68
P18462	MAVMAPRTLVLLLSGALALTQTWA	HLA class I histocompatibility antigen, A-25 alpha chain (HLA class I histocompatibility antigen, A-10 alpha chain) (MHC class I antigen A*25)	-0.68
P30447	MAVMAPRTLVLLLSGALALTQTWA	HLA class I histocompatibility antigen, A-23 alpha chain (HLA class I histocompatibility antigen, A-9 alpha chain) (MHC class I antigen A*23)	-0.68
P30450	MAVMAPRTLVLLLSGALALTQTWA	HLA class I histocompatibility antigen, A-26 alpha chain (MHC class I antigen A*26)	-0.68
P30453	MAIMAPRTLVLLLSGALALTQTWA	HLA class I histocompatibility antigen, A-34 alpha chain (Aw-34) (HLA class I histocompatibility antigen, A-10 alpha chain) (MHC class I antigen A*34)	-0.68
P30456	MAVMAPRTLVLLLSGALALTQTWA	HLA class I histocompatibility antigen, A-43 alpha chain (Aw-43) (MHC class I antigen	-0.68

		A*43)	
P30457	MAVMAPRTLVLLLSGALALTQTWA	HLA class I histocompatibility antigen, A-66 alpha chain (Aw-66) (HLA class I histocompatibility antigen, A-10 alpha chain) (MHC class I antigen A*66)	-0.68
Q9GZU5	MKGRGMLVLLLHAVVLGLPSAWA	Nyctalopin	-0.68
P10586	MAPEPAPGRTMVPLVPALVMLGLVAGAHG	Receptor-type tyrosine-protein phosphatase F (EC 3.1.3.48) (Leukocyte common antigen related) (LAR)	-0.68
P22897	MRLPLLLVFASVIPGAVL	Macrophage mannose receptor 1 (MMR) (C-type lectin domain family 13 member D) (C-type lectin domain family 13 member D-like) (Macrophage mannose receptor 1-like protein 1) (CD antigen CD206)	-0.69
Q99542	MNCQQLWLGFLLPMTVSG	Matrix metalloproteinase-19 (MMP-19) (EC 3.4.24.-) (Matrix metalloproteinase RASI) (Matrix metalloproteinase-18) (MMP-18)	-0.69
Q9UBD9	MDLRAGDSWGMLACLCTVLWHLPAVPA	Cardiotrophin-like cytokine factor 1 (B-cell-stimulating factor 3) (BSF-3) (Novel neurotrophin-1) (NNT-1)	-0.69
P55287	MKENYCLQAALVCLGMLCHSHA	Cadherin-11 (OSF-4) (Osteoblast cadherin) (OB-cadherin)	-0.69
O14960	MFSTKALLLAGLISTALA	Leukocyte cell-derived chemotaxin-2 (LECT-2) (hLECT2)	-0.69
Q5DX21	MTSQRSPLAPLLLLSLHGVAAS	Immunoglobulin superfamily member 11 (IgSF11) (Brain and testis-specific immunoglobulin superfamily protein) (Bt-IGSF) (V-set and immunoglobulin domain-containing protein 3)	-0.69
Q96HD1	MAPWPPKGLVPAMLWGLSLFLNLPGPIWL	Cysteine-rich with EGF-like domain protein 1	-0.70
Q6PCB8	MRALPGLLEARARTPRLLLLQCLLAAARPSSA	Embigin	-0.70
Q99650	MALFAVFQTTFFLTLLSLRTYQSEVLA	Oncostatin-M-specific receptor subunit beta (Interleukin-31 receptor subunit beta) (IL-31 receptor subunit beta) (IL-31R subunit beta) (IL-31R-beta) (IL-31RB)	-0.70
O00755	MNRKARRCLGHLFLSLGMVYLRIGGFSSVVA	Protein Wnt-7a	-0.70
Q99470	MAVVPLLLLGGLWSAVGA	Stromal cell-derived factor 2 (SDF-2)	-0.70
Q9HD43	MAGAGGGLGVWGNLVLLGLCSWTGARA	Receptor-type tyrosine-protein phosphatase H (R-PTP-H) (EC 3.1.3.48) (Stomach cancer-associated protein tyrosine phosphatase 1) (SAP-1) (Transmembrane-type protein-tyrosine phosphatase type H)	-0.70
A8MTI9	MGYCQGVSQVAVVLLMFPKEKEA	Putative serine protease 47 (EC 3.4.21.-)	-0.70
Q3MIP1	MSVHYTLNLRVFWPLVTGLCTALVCLYHVLRGSGGARA	Inositol 1,4,5-trisphosphate receptor-interacting protein-like 2	-0.70
P0CG36	MTWRHHVRLLFTVSLALQIINLGNS	Cryptic family protein 1B	-0.70
P0CG37	MTWRHHVRLLFTVSLALQIINLGNS	Cryptic protein (Cryptic family protein 1)	-0.70
Q9NQ38	MKIATVSVLLPLALCLIQDAAS	Serine protease inhibitor Kazal-type 5 (Lympho-epithelial Kazal-type-related inhibitor) (LEKTI) [Cleaved into: Hemofiltrate peptide HF6478; Hemofiltrate peptide HF7665]	-0.70
O95631	MMRAVWEALAALAAVACLVGAVRG	Netrin-1 (Epididymis tissue protein Li 131P)	-0.70
Q5GFL6	MPPFLLLEAVCVFLFSRVPPSLP	von Willebrand factor A domain-containing protein 2 (A domain-containing protein similar to matrilin and collagen) (AMACO) (Colon cancer secreted protein 2) (CCSP-2)	-0.70
Q4KMG0	MHPDLGPLCTLLYVTLTILCSSVSS	Cell adhesion molecule-related/down-regulated by oncogenes	-0.70
P54760	MELRVLLCWASLAAA	Ephrin type-B receptor 4 (EC 2.7.10.1) (Hepatoma transmembrane kinase) (Tyrosine-protein kinase TYRO11)	-0.70
Q86WD7	MASYLYGVLFAVGLCAPIYCVSP	Serpin A9 (Centerin) (Germinal center B-cell-	-0.70

		expressed transcript 1 protein)	
Q9GZN4	MVVSGAPPALGGGCLGTFTSLLLLASTAILNA	Brain-specific serine protease 4 (BSSP-4) (EC 3.4.21.-) (Serine protease 22) (Serine protease 26) (Tryptase epsilon)	-0.70
Q9H3W5	MKDMPLRIHVLLGLAITTLVQA	Leucine-rich repeat neuronal protein 3 (Neuronal leucine-rich repeat protein 3) (NLRR-3)	-0.71
P13232	MFHVSFRYIFGLPPLILVLLPVASS	Interleukin-7 (IL-7)	-0.71
Q9Y5F6	MGPKTLPQLAGKWQVLCMLSLCCWGWVSG	Protocadherin gamma-C5 (PCDH-gamma-C5)	-0.71
Q8N4T0	MKCLGKRRGQAAAFLPLCWLFLKILQPGHS	Carboxypeptidase A6 (EC 3.4.17.1) (Carboxypeptidase B)	-0.71
P36894	MPQLYIYIRLLGAYLFIISRVQG	Bone morphogenetic protein receptor type-1A (BMP type-1A receptor) (BMPR-1A) (EC 2.7.11.30) (Activin receptor-like kinase 3) (ALK-3) (Serine/threonine-protein kinase receptor R5) (SKR5) (CD antigen CD292)	-0.71
Q92823	MQLKIMPKKKRLSAGRVPLILFLC	Neuronal cell adhesion molecule (Nr-CAM) (Neuronal surface protein Bravo) (hBravo) (NgCAM-related cell adhesion molecule) (Ng-CAM-related)	-0.71
P31997	MGPISAPSCRWRIPWQGLLLTASLFTFWNPPTTA	Carcinoembryonic antigen-related cell adhesion molecule 8 (CD67 antigen) (Carcinoembryonic antigen CGM6) (Non-specific cross-reacting antigen NCA-95) (CD antigen CD66b)	-0.71
Q17R55	MPPMLWLLLHFAAPALG	Protein FAM187B (Transmembrane protein 162)	-0.71
Q99075	MKLLPSVVLKLFLAAVLSA	Proheparin-binding EGF-like growth factor [Cleaved into: Heparin-binding EGF-like growth factor (HB-EGF) (HBEGF) (Diphtheria toxin receptor) (DT-R)]	-0.71
Q9NQ30	MKSVLLLTTLLVPAHLVAA	Endothelial cell-specific molecule 1 (ESM-1)	-0.71
Q7Z5J1	MKVLLLTGLGALFFA	Hydroxysteroid 11-beta-dehydrogenase 1-like protein (EC 1.1.1.-) (11-beta-hydroxysteroid dehydrogenase type 3) (11-DH3) (11-beta-HSD3) (Short-chain dehydrogenase/reductase 10)	-0.71
Q5GAN3	MAPAVTRLLFLQLVLGPTLV	Probable inactive ribonuclease-like protein 13	-0.71
O75871	MGPPSAAPRGGHRPWQGLLITASLLTFWHPPTTVQ	Carcinoembryonic antigen-related cell adhesion molecule 4 (Carcinoembryonic antigen CGM7) (Non-specific cross-reacting antigen W236)	-0.71
Q08722	MWPLVAALLLGSACCGSA	Leukocyte surface antigen CD47 (Antigenic surface determinant protein OA3) (Integrin-associated protein) (IAP) (Protein MER6) (CD antigen CD47)	-0.71
O43852	MDLRQFLMCLSLCTAFALS	Calumenin (Crocalbin) (IEF SSP 9302)	-0.71
Q14767	MRPRTKARSPGRALRNPWRGFLPLTLALFVGAGHA	Latent-transforming growth factor beta-binding protein 2 (LTBP-2)	-0.71
P13521	MAEAKTHWLGAALSLIPLIFLISGAEA	Secretogranin-2 (Chromogranin-C) (Secretogranin II) (SgII) [Cleaved into: Secretoneurin (SN)]	-0.71
Q17RW2	MHLRAHRTRRGKVSPTAKTKSLLHFIVLCVAGVVV	Collagen alpha-1(XXIV) chain	-0.71
P47872	MRPHLSPPLQQLLLPVLLACAA	Secretin receptor (SCT-R)	-0.72
P08572	MGRDQRAVAGPALRRWLLLGTVTVG	Collagen alpha-2(IV) chain [Cleaved into: Canstatin]	-0.72
Q13508	MKTGHFEIVTMLLATMILVDIFQVKA	Ecto-ADP-ribosyltransferase 3 (EC 2.4.2.31) (ADP-ribosyltransferase C2 and C3 toxin-like 3) (ARTC3) (Mono(ADP-ribosyl)transferase 3) (NAD(P)(+)--arginine ADP-ribosyltransferase 3)	-0.72
O95297	MAASAGAGAVIAAPDSRRWLWSVLAAALGLLTAGV	Myelin protein zero-like protein 1 (Protein zero-related)	-0.72
Q8IWF2	MGLSAAAPLWGPPGLLLAIALHPALS	FAD-dependent oxidoreductase domain-containing protein 2 (Endoplasmic reticulum	-0.72

		flavoprotein associated with degradation)	
Q8N967	MLAPGSSPGQRGRLALQWRQVSWITCWIALYAVEA	Leucine-rich repeat and transmembrane domain-containing protein 2	-0.72
Q14831	MVQLRKLLRVLTLMKFPCCVLEVLLCALAAAARG	Metabotropic glutamate receptor 7 (mGluR7)	-0.72
Q08830	MAKVFSFILVTTALTMGREISA	Fibrinogen-like protein 1 (HP-041) (Hepassocin) (Hepatocyte-derived fibrinogen-related protein 1) (HFREP-1) (Liver fibrinogen-related protein 1) (LFIRE-1)	-0.72
A6NJW4	MRMTSSSFVSYCTPGLCQFMAMLPTAGHLLPLLLVIGTGGT	Leucine-rich repeat-containing protein 3C	-0.72
Q96RI0	MWGRLLLWPLVLGFSLS	Proteinase-activated receptor 4 (PAR-4) (Coagulation factor II receptor-like 3) (Thrombin receptor-like 3)	-0.72
Q9HCB6	MRLSPAPLKLSRTPALLALALPLAAALA	Spondin-1 (F-spondin) (Vascular smooth muscle cell growth-promoting factor)	-0.73
Q75N90	MTLEGLYLARGPLARLLLAWSALLCMAGGQG	Fibrillin-3	-0.73
Q9H106	MPIPASPLHPPLPSLLLYLLLELAGVTHV	Signal-regulatory protein delta (SIRP-delta) (Protein tyrosine phosphatase non-receptor type substrate 1-like 2)	-0.73
Q8IWU5	MGPPSLVLCLLSATVFSLLGGSSA	Extracellular sulfatase Sulf-2 (hSulf-2) (EC 3.1.6.-)	-0.73
P01920	MSWKKALRIPGGLRAATVTLMLAMLSTPVAEG	HLA class II histocompatibility antigen, DQ beta 1 chain (MHC class II antigen DQB1)	-0.73
P05538	MSWKMALQIPGGFWAAAVTVMLVMLSTPVAEA	HLA class II histocompatibility antigen, DQ beta 2 chain (HLA class II histocompatibility antigen, DX beta chain) (MHC class II antigen DQB2)	-0.73
Q13591	MKGTCVIAWLFSSLGLWRLAHP	Semaphorin-5A (Semaphorin-F) (Sema F)	-0.73
Q13478	MNCRELPLTLWVLISVST	Interleukin-18 receptor 1 (IL-18R-1) (IL-18R1) (CD218 antigen-like family member A) (CDw218a) (IL1 receptor-related protein) (IL-1Rrp) (IL1R-rp) (CD antigen CD218a)	-0.73
Q8NBM8	MARAAPLLAALTALLAAAAAGG	Prenylcysteine oxidase-like (EC 1.8.3.-)	-0.73
Q15303	MKPATGLWVWVSLLVAAGTVQPSDS	Receptor tyrosine-protein kinase erbB-4 (EC 2.7.10.1) (Proto-oncogene-like protein c-ErbB-4) (Tyrosine kinase-type cell surface receptor HER4) (p180erbB4) [Cleaved into: ERBB4 intracellular domain (4ICD) (E4ICD) (s80HER4)]	-0.74
Q96J42	MVPAAGRRPPRVMRLLGWWQVLLWVLGLPVRG	Thioredoxin domain-containing protein 15	-0.74
Q14CZ8	MKRERGALSRASRALRLAPFVYLLLIQTDPLEG	Hepatocyte cell adhesion molecule (Protein hepaCAM)	-0.74
P07093	MNWHLPLFLLASVTLPSIC	Glia-derived nexin (GDN) (Peptidase inhibitor 7) (PI-7) (Protease nexin 1) (PN-1) (Protease nexin I) (Serpin E2)	-0.74
P27658	MAVLPGPLQLLGVLLTISLSSIRLIQA	Collagen alpha-1(VIII) chain (Endothelial collagen) [Cleaved into: Vastatin]	-0.74
P00742	MGRPLHLVLLSASLAGLLLLGESLFIRREQA	Coagulation factor X (EC 3.4.21.6) (Stuart factor) (Stuart-Prower factor) [Cleaved into: Factor X light chain; Factor X heavy chain; Activated factor Xa heavy chain]	-0.74
P33681	MGHTRRQGTSPSKCPYLNFFQLLVLAGLSHFCSG	T-lymphocyte activation antigen CD80 (Activation B7-1 antigen) (BB1) (CTLA-4 counter-receptor B7.1) (B7) (CD antigen CD80)	-0.74
Q8TCT8	MGPQRRLSPAGAALLWGFLLQLTAA	Signal peptide peptidase-like 2A (SPP-like 2A) (SPPL2a) (EC 3.4.23.-) (Intramembrane protease 3) (IMP-3) (Presenilin-like protein 2)	-0.74
Q9BRN9	MAGGVLPLRGLRALCRVLLFLSQFCILSG	TM2 domain-containing protein 3 (Beta-amyloid-binding protein-like protein 2) (BBP-like protein 2)	-0.74
Q9BQ49	MIGDILLFGTLLMNAGA	Small integral membrane protein 7	-0.74
Q9Y240	MQAAWLLGALVVPQLLGFGHG	C-type lectin domain family 11 member A (C-type lectin superfamily member 3) (Lymphocyte secreted C-type lectin) (Stem	-0.74

		cell growth factor) (p47)	
Q8IWB1	MAMGLFRVCLVVVTA	Inositol 1,4,5-trisphosphate receptor-interacting protein (Protein DANGER)	-0.75
Q9BY76	MSGAPTAGAALMLCAATAVLLSAQG	Angiopoietin-related protein 4 (Angiopoietin-like protein 4) (Hepatic fibrinogen/angiopoietin-related protein) (HFARP)	-0.75
Q5DID0	MLRTSGLALLALVSAVGPSQA	Uromodulin-like 1 (Olfactorin)	-0.75
P08118	MNVLLGSVVIFATFVTLCNA	Beta-microseminoprotein (Immunoglobulin-binding factor) (IGBF) (PN44) (Prostate secreted seminal plasma protein) (Prostate secretory protein of 94 amino acids) (PSP-94) (PSP94) (Seminal plasma beta-inhibin)	-0.75
A6BM72	MVLSLTGLIAFSFLQATLA	Multiple epidermal growth factor-like domains protein 11 (Multiple EGF-like domains protein 11)	-0.75
Q53EL9	MRPVALLLLPSLLALLAHG	Seizure protein 6 homolog (SEZ-6) (hSEZ-6)	-0.75
P50281	MSPAPRPPRCLLLPLLTLGT	Matrix metalloproteinase-14 (MMP-14) (EC 3.4.24.80) (MMP-X1) (Membrane-type matrix metalloproteinase 1) (MT-MMP 1) (MTMMP1) (Membrane-type-1 matrix metalloproteinase) (MT1-MMP) (MT1MMP)	-0.75
P61812	MHYCVLSAFLILHLVTVALS	Transforming growth factor beta-2 (TGF-beta-2) (BSC-1 cell growth inhibitor) (Cetermin) (Glioblastoma-derived T-cell suppressor factor) (G-TSF) (Polyergin) [Cleaved into: Latency-associated peptide (LAP)]	-0.75
Q8WUJ1	MLRCGGRGLLLGLAVAAAAVMA	Neuferricin (Cytochrome b5 domain-containing protein 2)	-0.75
Q9H3G5	MVGAMWKVIVSLVLLMPGPCDG	Probable serine carboxypeptidase CPVL (EC 3.4.16.-) (Carboxypeptidase, vitellogenic-like) (Vitellogenic carboxypeptidase-like protein) (VCP-like protein) (hVLP)	-0.75
Q9H3Y0	MPLLPSTVGLAGLLFWAGQAVNAL	Peptidase inhibitor R3HDML (Cysteine-rich secretory protein R3HDML)	-0.75
Q15762	MDYPTLLLALLHVYRALC	CD226 antigen (DNAX accessory molecule 1) (DNAM-1) (CD antigen CD226)	-0.76
P12110	MLQGTCSVLLLWGILGAIQA	Collagen alpha-2(VI) chain	-0.76
Q13093	MVPPKLHVLFCLCGCLAVVYP	Platelet-activating factor acetylhydrolase (PAF acetylhydrolase) (EC 3.1.1.47) (1-alkyl-2-acetylglycerophosphocholine esterase) (2-acetyl-1-alkylglycerophosphocholine esterase) (Group-VIIA phospholipase A2) (gVIIA-PLA2) (LDL-associated phospholipase A2) (LDL-PLA(2)) (PAF 2-acylhydrolase)	-0.76
P18509	MTMCSGARLALLVYGIIMHSSVYS	Pituitary adenylate cyclase-activating polypeptide (PACAP) [Cleaved into: PACAP-related peptide (PRP-48); Pituitary adenylate cyclase-activating polypeptide 27 (PACAP-27) (PACAP27); Pituitary adenylate cyclase-activating polypeptide 38 (PACAP-38) (PACAP38)]	-0.76
Q765I0	MNKILSSTVCFGLLTLLSVLSFLQSVHG	Urotensin-2B (Urotensin II-related peptide) (Urotensin IIB) (U-IIB) (UIIB) (Urotensin-2 domain-containing protein)	-0.76
Q5T4W7	MELGLGGLSTLSHCPWPRQQPALWPTLAALALLSSVAEA	Artemin (Enovin) (Neublastin)	-0.76
Q9H4D0	MLPGRLCWVPLLLALGVGSG	Calsyntenin-2 (Alcadein-gamma) (Alc-gamma)	-0.76
P60568	MYRMQLLSCIALSLALVTNS	Interleukin-2 (IL-2) (T-cell growth factor) (TCGF) (Aldesleukin)	-0.76
Q8N7Q2	MFCLLHLCFYLANFASSIKRTHA	Putative uncharacterized protein CELF2-AS1 (CELF2 antisense RNA 1) (CELF2 antisense gene protein 1)	-0.76
Q86Y29	MAAGVVFLALSAQLLQA	B melanoma antigen 3 (Cancer/testis antigen 2.3) (CT2.3)	-0.76
Q86Y30	MAAGVVFLALSAQLLQA	B melanoma antigen 2 (Cancer/testis antigen	-0.76

		2.2) (CT2.2)	
Q8N387	MLALAKILLISTLFYSLLSGSHG	Mucin-15 (MUC-15)	-0.77
Q86WI0	MRSSLTMVGTLWAFLSLVTA	Lipoma HMGIC fusion partner-like 1 protein	-0.77
Q8WXA2	MDKSLLLELPILLCCFRALSG	Prostate and testis expressed protein 1	-0.77
Q8N271	MKHTLALLAPLLGLGLGLALSQLAAG	Prominin-2 (PROM-2) (Prominin-like protein 2) (hPROML2)	-0.77
Q96KJ4	MAAAVTIPGPRIGALQSSGLTLLLSLAAHCSGPQA	Mesothelin-like protein (Pre-pro-megakaryocyte-potentiating-factor-like)	-0.77
Q13519	MKVLLCDLLLLSLFSSVFS	Prepronociceptin [Cleaved into: Neuropeptide 1; Nociceptin (Orphanin FQ) (PPNOC); Neuropeptide 2]	-0.77
A8MVW0	MPPASGPSVLARLLPLLGLLLGSASRAPG	Protein FAM171A2	-0.77
P05408	MVSRMVSTMLSGLLFWLASGWTPAFA	Neuroendocrine protein 7B2 (Pituitary polypeptide) (Secretogranin V) (Secretogranin-5) (Secretory granule endocrine protein I) [Cleaved into: N-terminal peptide; C-terminal peptide]	-0.77
Q9UQP3	MSLQEMFRFPMGLLLGSVLLVASAPATL	Tenascin-N (TN-N)	-0.77
Q9NS71	MLAYSSVHCFREDKMKFTIVFAGLLGVFLAPALA	Gastrokine-1 (18 kDa antrum mucosa protein) (AMP-18) (Protein CA11)	-0.78
P01909	MILNKALMLGALALTTVMSPCGG	HLA class II histocompatibility antigen, DQ alpha 1 chain (DC-1 alpha chain) (DC-alpha) (HLA-DCA) (MHC class II DQA1)	-0.78
P58550	MEVVLIFVYSLLVPVVLA	Putative FXYD domain-containing ion transport regulator 8	-0.78
P51460	MDPRLPAWALVLLGPALVFA	Insulin-like 3 (Leydig insulin-like peptide) (Ley-I-L) (Relaxin-like factor) [Cleaved into: Insulin-like 3 B chain; Insulin-like 3 A chain]	-0.78
K9M1U5	MRPSVWAAVAAGLWVLCTVIA	Interferon lambda-4 (IFN-lambda-4)	-0.78
Q9ULX7	MLFSALLLEVIWILA	Carbonic anhydrase 14 (EC 4.2.1.1) (Carbonate dehydratase XIV) (Carbonic anhydrase XIV) (CA-XIV)	-0.78
A2RRL7	MQRLPAATRATLILSLAFASLHSACSA	Transmembrane protein 213	-0.78
Q08397	MALARGSRQLGALVWGACLCVLVHG	Lysyl oxidase homolog 1 (EC 1.4.3.-) (Lysyl oxidase-like protein 1) (LOL)	-0.78
P52799	MAVRRDSVWKYCWGVLMVLCRTAISKS	Ephrin-B2 (EPH-related receptor tyrosine kinase ligand 5) (LERK-5) (HTK ligand) (HTK-L)	-0.78
Q8TB96	MAAAGRLPSSWALFSPLLAGLALLGVGPVPARA	T-cell immunomodulatory protein (Protein TIP) (Integrin-alpha FG-GAP repeat-containing protein 1)	-0.78
P18075	MHVRSLRAAAPHSFVALWAPLFLLRSALA	Bone morphogenetic protein 7 (BMP-7) (Osteogenic protein 1) (OP-1) (Eptotermin alfa)	-0.78
Q8WUF8	MSISLSSLILLPIWINMA	Protein FAM172A	-0.78
Q96LB8	MLPWLLVFSALGIQAWG	Peptidoglycan recognition protein 4 (Peptidoglycan recognition protein I-beta) (PGLYRPIbeta) (PGRP-I-beta) (Peptidoglycan recognition protein intermediate beta)	-0.79
Q8NEX6	MVSLMKLWIPMLMTFFCTVLLSVLG	Protein WFDC11	-0.79
P22891	MAGCVPLLQGLVLVLALHRVEPS	Vitamin K-dependent protein Z	-0.79
Q7Z7G0	MRGGKCNMLSSLGCLLLCGSITLALGNA	Target of Nesh-SH3 (Tarsh) (ABI gene family member 3-binding protein) (Nesh-binding protein) (NeshBP)	-0.79
P34998	MGGHPQLRLVKALLLLGLNPVSA	Corticotropin-releasing factor receptor 1 (CRF-R-1) (CRF-R1) (CRFR-1) (Corticotropin-releasing hormone receptor 1) (CRH-R-1) (CRH-R1)	-0.79
O95965	MRPPGFRNFLLLASSLLFAGLSA	Integrin beta-like protein 1 (Osteoblast-specific cysteine-rich protein) (Ten integrin EGF-like repeat domain-containing protein)	-0.79
P13598	MSSFGYRTLTVALFTLICCPGSDE	Intercellular adhesion molecule 2 (ICAM-2) (CD antigen CD102)	-0.79

P15514	MRAPLLPPAPVVLSLLILG	Amphiregulin (AR) (Colorectum cell-derived growth factor) (CRDGF)	-0.79
Q9NPG1	MAMTWIVFSLWPLTVFMGHIGG	Frizzled-3 (Fz-3) (hFz3)	-0.79
Q9P232	MMFPWKQLILLSFIGCLGG	Contactin-3 (Brain-derived immunoglobulin superfamily protein 1) (BIG-1) (Plasmacytoma-associated neuronal glycoprotein)	-0.79
P29317	MELQAARACFALLWGCALAAAAA	Ephrin type-A receptor 2 (EC 2.7.10.1) (Epithelial cell kinase) (Tyrosine-protein kinase receptor ECK)	-0.79
Q9UPU3	MEAARTERPAGRPGAPLVRTGLLLLSTWVLAGA	VPS10 domain-containing receptor SorCS3	-0.79
Q9UM22	MPGRAPLRTVPGALGAWLLGGLWAWTLCGLCSLGAVG	Mammalian ependymin-related protein 1 (MERP-1) (Upregulated in colorectal cancer gene 1 protein)	-0.79
Q6UXL0	MQTFTMVLEEIWTSLFMWFFYALIPCLLT	Interleukin-20 receptor subunit beta (IL-20 receptor subunit beta) (IL-20R-beta) (IL-20RB) (IL-20R2)	-0.79
P55773	MKVSVAALSCLMLVTALGSQA	C-C motif chemokine 23 (CK-beta-8) (CKB-8) (Macrophage inflammatory protein 3) (MIP-3) (Myeloid progenitor inhibitory factor 1) (MPIF-1) (Small-inducible cytokine A23) [Cleaved into: CCL23(19-99); CCL23(22-99); CCL23(27-99); CCL23(30-99)]	-0.80
Q8IZF5	MVCSAAPLLLLATTLPLLGSPVAQA	Probable G-protein coupled receptor 113 (G-protein coupled receptor PGR23)	-0.80
P13671	MARRSVLYFILLNALINKGQA	Complement component C6	-0.80
Q99985	MAFRTICVLVGVFICSICVK	Semaphorin-3C (Semaphorin-E) (Sema E)	-0.80
Q4KMZ8	MGKCSGRCTLVAFCCLQLVAA	Sodium/potassium-transporting ATPase subunit beta-1-interacting protein 1 (Na(+)/K(+)-transporting ATPase subunit beta-1-interacting protein 1) (Protein FAM77C)	-0.80
Q86U17	MGPAWLWLLGTGILASVHC	Serpin A11	-0.80
P40189	MLTLQTWLVQALFIFLTTESTG	Interleukin-6 receptor subunit beta (IL-6 receptor subunit beta) (IL-6R subunit beta) (IL-6R-beta) (IL-6RB) (CDw130) (Interleukin-6 signal transducer) (Membrane glycoprotein 130) (gp130) (Oncostatin-M receptor subunit alpha) (CD antigen CD130)	-0.80
Q9UK55	MKVVPSLLLSVLLAQVWLVPG	Protein Z-dependent protease inhibitor (PZ-dependent protease inhibitor) (PZI) (Serpin A10)	-0.80
Q8N4V1	MAPSLWKGLVGIGLFALAHA	Membrane magnesium transporter 1 (ER membrane protein complex subunit 5) (Transmembrane protein 32)	-0.80
P22352	MARLLQASCLLSLLLAGFVS	Glutathione peroxidase 3 (GPx-3) (GSHPx-3) (EC 1.11.1.9) (Extracellular glutathione peroxidase) (Plasma glutathione peroxidase) (GPx-P) (GSHPx-P)	-0.80
O75339	MVGTKAWVFSFLVLEVTSVLG	Cartilage intermediate layer protein 1 (CILP-1) (Cartilage intermediate-layer protein) [Cleaved into: Cartilage intermediate layer protein 1 C1; Cartilage intermediate layer protein 1 C2]	-0.81
Q86WN2	MIIKHFFGTVLVLLASTTIFS	Interferon epsilon (IFN-epsilon) (Interferon epsilon-1)	-0.81
Q9H1A3	MRLLAGWLCLSLASVWLA	Methyltransferase-like protein 9 (DORA reverse strand protein) (DREV) (DREV1)	-0.81
Q86YC3	MELLPLWLCLGFHFLTVG	Leucine-rich repeat-containing protein 33	-0.81
P43627	MSLMVVSMACVGFFLLQGAWP	Killer cell immunoglobulin-like receptor 2DL2 (CD158 antigen-like family member B1) (MHC class I NK cell receptor) (Natural killer-associated transcript 6) (NKAT-6) (p58 natural killer cell receptor clone CL-43) (p58 NK receptor CL-43) (CD antigen CD158b1)	-0.81
P43630	MSLTVVSMACVGFFLLQGAWP	Killer cell immunoglobulin-like receptor 3DL2 (CD158 antigen-like family member K) (MHC class I NK cell receptor) (Natural killer-	-0.81

		associated transcript 4) (NKAT-4) (p70 natural killer cell receptor clone CL-5) (p70 NK receptor CL-5) (CD antigen CD158k)	
P43631	MSLMVVSMACVGFFLLQGAWP	Killer cell immunoglobulin-like receptor 2DS2 (CD158 antigen-like family member J) (MHC class I NK cell receptor) (NK receptor 183 ActI) (Natural killer-associated transcript 5) (NKAT-5) (p58 natural killer cell receptor clone CL-49) (p58 NK receptor CL-49) (CD antigen CD158j)	-0.81
Q14954	MSLTVVSMACVGFFLLQGAWP	Killer cell immunoglobulin-like receptor 2DS1 (CD158 antigen-like family member H) (MHC class I NK cell receptor Eb6 ActI) (CD antigen CD158h)	-0.81
Q8N743	MSLMVVSMACVGFFLLEGPWPHVGG	Killer cell immunoglobulin-like receptor 3DL3 (CD158 antigen-like family member Z) (Killer cell inhibitory receptor 1) (CD antigen CD158z)	-0.81
P30464	MRVTAPRTVLLLLSGALALTETWA	HLA class I histocompatibility antigen, B-15 alpha chain (MHC class I antigen B*15)	-0.81
P30484	MRVTAPRTVLLLLSGALALTETWA	HLA class I histocompatibility antigen, B-46 alpha chain (Bw-46) (MHC class I antigen B*46)	-0.81
Q9H9K5	MGSLSNYALLQLTLTAFLTILVQP	HERV-MER_4q12 provirus ancestral Env polyprotein (Endogenous retrovirus group MER34 member 1)	-0.81
Q92544	MATAMDWLPWSLLLFSLMCETSA	Transmembrane 9 superfamily member 4	-0.81
Q9NSA1	MDSDETGFEHSGLWVSVLAGLLLGACQA	Fibroblast growth factor 21 (FGF-21)	-0.81
P25189	MAPGAPSSSPSPILAVLLFSSLVLSPAQA	Myelin protein P0 (Myelin peripheral protein) (MPP) (Myelin protein zero)	-0.81
A8MWS1	MSLMVISMACVGFFLLQGAWT	Putative killer cell immunoglobulin-like receptor like protein KIR3DP1 (CD antigen CD158c)	-0.81
Q8N109	MSLMVISMACVGFFLLQGAWT	Killer cell immunoglobulin-like receptor 2DL5A (CD antigen CD158f1)	-0.81
Q8NHK3	MSLMVISMACVGFFLLQGAWT	Killer cell immunoglobulin-like receptor 2DL5B (CD158 antigen-like family member F2) (Killer cell immunoglobulin-like receptor 2DLX) (CD antigen CD158f2)	-0.81
Q5JTB6	MRPLLCALTGLALLRAAGSLAA	Placenta-specific protein 9	-0.82
O95157	MQLTRCCFVFLVQGSLYLVICG	Neurexophilin-3	-0.82
Q9NQE7	MAVWLAQWLGPLLLVSLWGLLAPA	Thymus-specific serine protease (EC 3.4.-.-) (Serine protease 16)	-0.82
A6NE02	MPRRGYSKPGSWGSFWAMLTLVGLVTHA	BTB/POZ domain-containing protein 17 (Galectin-3-binding protein-like)	-0.82
Q08431	MPRPRLLAALCGALLCAPSLLVA	Lactadherin (Breast epithelial antigen BA46) (HMFG) (MFGM) (Milk fat globule-EGF factor 8) (MFG-E8) (SED1) [Cleaved into: Lactadherin short form; Medin]	-0.82
P06729	MSFPCKFVASFLLIFNVSSKGAVS	T-cell surface antigen CD2 (Erythrocyte receptor) (LFA-2) (LFA-3 receptor) (Rosette receptor) (T-cell surface antigen T11/Leu-5) (CD antigen CD2)	-0.82
A6NLX4	MAPGPWPVSCLRGGPLGLTYLSLLLIPAAAG	Transmembrane protein 210	-0.82
Q99895	MLGITVLAALLACASS	Chymotrypsin-C (EC 3.4.21.2) (Caldecrin)	-0.82
P07098	MWLLLTMASLISVLGTTHG	Gastric triacylglycerol lipase (GL) (Gastric lipase) (EC 3.1.1.3)	-0.82
P06280	MQLRNPELHLGCALALRFLALVSWDIPGARA	Alpha-galactosidase A (EC 3.2.1.22) (Alpha-D-galactosidase A) (Alpha-D-galactoside galactohydrolase) (Melibiase) (Agalsidase)	-0.82
Q9NZW4	MKIITYFCIWAVAWA	Dentin sialophosphoprotein [Cleaved into: Dentin phosphoprotein (Dentin phosphophoryn) (DPP); Dentin sialoprotein (DSP)]	-0.82
Q6UWJ8	MEAPGPRALRTALCGGCCCLLLCAQLAVA	CD164 sialomucin-like 2 protein	-0.82

P00739	MSDLGAVISLLLWGRQLFA	Haptoglobin-related protein	-0.83
P11684	MKLAVTLTLVTLALCCSSASA	Uteroglobin (Clara cell phospholipid-binding protein) (CCPBP) (Clara cells 10 kDa secretory protein) (CC10) (Secretoglobin family 1A member 1) (Urinary protein 1) (UP-1) (UP1) (Urine protein 1)	-0.83
P43146	MENSLRCVWVPKLAFVLFGASLFSA	Netrin receptor DCC (Colorectal cancer suppressor) (Immunoglobulin superfamily DCC subclass member 1) (Tumor suppressor protein DCC)	-0.83
Q5W186	MSSPQRRKAMPWALSLLLMGFQLLVTYA	Cystatin-9 (Cystatin-like molecule)	-0.83
P20849	MKTCWKIPVFFFVCSFLEPWASA	Collagen alpha-1(IX) chain	-0.83
P54802	MEAVAVAAAVGVLLLAGAGGAAG	Alpha-N-acetylglucosaminidase (EC 3.2.1.50) (N-acetyl-alpha-glucosaminidase) (NAG) [Cleaved into: Alpha-N-acetylglucosaminidase 82 kDa form; Alpha-N-acetylglucosaminidase 77 kDa form]	-0.83
Q9P0W0	MSTKPDMIQKCLWLEILMGIFIAGTLS	Interferon kappa (IFN-kappa)	-0.83
Q6X4U4	MLPPAIHFYLLPLACILMKSCLA	Sclerostin domain-containing protein 1 (Ectodermal BMP inhibitor) (Ectodin) (Uterine sensitization-associated gene 1 protein) (USAG-1)	-0.83
O75715	MTTQLRVVHLLPLLLACFVQT	Epididymal secretory glutathione peroxidase (EC 1.11.1.9) (Epididymis-specific glutathione peroxidase-like protein) (EGLP) (Glutathione peroxidase 5) (GPx-5) (GSHPx-5)	-0.83
P06731	MESPSAPPHRWCIPWQRLLLTASLLTFWNPPTTA	Carcinoembryonic antigen-related cell adhesion molecule 5 (Carcinoembryonic antigen) (CEA) (Meconium antigen 100) (CD antigen CD66e)	-0.83
P13688	MGHLSAPLHRVRVPWQGLLLTASLLTFWNPPTTA	Carcinoembryonic antigen-related cell adhesion molecule 1 (Biliary glycoprotein 1) (BGP-1) (CD antigen CD66a)	-0.83
Q3KPI0	MGPPSACPHRECIPWQGLLLTASLLTFWNAPTTA	Carcinoembryonic antigen-related cell adhesion molecule 21	-0.83
Q14002	MGSPSACPYRVCIPWQGLLLTASLLTFWNLPNSAQ	Carcinoembryonic antigen-related cell adhesion molecule 7 (Carcinoembryonic antigen CGM2)	-0.83
P05186	MISPFLVLAIGTCLTNS	Alkaline phosphatase, tissue-nonspecific isozyme (AP-TNAP) (TNSALP) (EC 3.1.3.1) (Alkaline phosphatase liver/bone/kidney isozyme)	-0.84
H3BS89	MAAGRLLLYTGLSLALCALGMLA	Transmembrane protein 178B	-0.84
Q9Y6N7	MKWKHVPFLVMISLLSLSPNHLFLA	Roundabout homolog 1 (Deleted in U twenty twenty) (H-Robo-1)	-0.84
A6NM11	MSSAQCPALVCVMSRLRFWGPWPLLMWQLLWLLVK	Leucine-rich repeat-containing protein 37A2	-0.84
A6NMS7	MSSAQCPALVCVMSRLRFWGPWPLLMWQLLWLLVK	Leucine-rich repeat-containing protein 37A	-0.84
O60309	MTSAQCPALACVMSPLRFWGPWPLLMWQLLWLLVK	Leucine-rich repeat-containing protein 37A3	-0.84
O94919	MGTARWLALGSLFALAGLLEG	Endonuclease domain-containing 1 protein (EC 3.1.30.-)	-0.84
O75051	MEQRRPWPRALEVDSRSVVLLSVVWVLLAPPAAG	Plexin-A2 (Semaphorin receptor OCT)	-0.84
P17181	MMVVLLGATTLVLVAVAPWVLSAAAGG	Interferon alpha/beta receptor 1 (IFN-R-1) (IFN-alpha/beta receptor 1) (Cytokine receptor class-II member 1) (Cytokine receptor family 2 member 1) (CRF2-1) (Type I interferon receptor 1)	-0.84
Q96B86	MQPPRERLVVTGRAGWMGMGRGAGRSALGFWPTLAFLLCSFPAATSP	Repulsive guidance molecule A (RGM domain family member A)	-0.84
Q9BX74	MAAAWPSGPSAPEAVTARLVGVLWFVSVTTGPWGAVA	TM2 domain-containing protein 1 (Beta-amyloid-binding protein) (hBBP)	-0.84
A6NFA1	MHAALAGPLLAALLATARA	Metalloprotease TIKI2 (EC 3.4.-.-) (Heart, kidney and adipose-enriched transmembrane protein homolog) (TRAB domain-containing protein 2B)	-0.85

Q14952	MSLMVISMACVGFFWLQGAWP	Killer cell immunoglobulin-like receptor 2DS3 (MHC class I NK cell receptor) (Natural killer-associated transcript 7) (NKAT-7)	-0.85
Q5JZY3	METCAGPHPLRLFLCRMQLCLALLLGPWRPGTA	Ephrin type-A receptor 10 (EC 2.7.10.1)	-0.85
Q9HB29	MWSLLLCGLSIALPLSVTA	Interleukin-1 receptor-like 2 (IL-36 receptor) (Interleukin-1 receptor-related protein 2) (IL-1Rrp2) (IL1R-rp2)	-0.85
Q9Y264	MLSQLAMLQGSLLLVVATMSVAQQ	Angiopoietin-4 (ANG-4) (Angiopoietin-3) (ANG-3)	-0.85
Q9UNK4	MELALLCGLVVMAGVIPIQG	Group IID secretory phospholipase A2 (GIID sPLA2) (sPLA2-IID) (EC 3.1.1.4) (PLA2IID) (Phosphatidylcholine 2-acylhydrolase 2D) (Secretory-type PLA, stroma-associated homolog)	-0.85
Q9UKP5	MEILWKTLTWILSLIMASSEF	A disintegrin and metalloproteinase with thrombospondin motifs 6 (ADAM-TS 6) (ADAM-TS6) (ADAMTS-6) (EC 3.4.24.-)	-0.85
Q3ZCN5	MIPWSIFLLHVLLFSLQEYICA	Otogelin-like protein	-0.85
P51690	MLHLHHSCLCFRSWLPAMLAVLLSLAPSASS	Arylsulfatase E (ASE) (EC 3.1.6.-)	-0.85
Q9BY15	MQGPLLLPGLCFLLSLFGAVT	EGF-like module-containing mucin-like hormone receptor-like 3 (EGF-like module receptor 3) [Cleaved into: EGF-like module-containing mucin-like hormone receptor-like 3 subunit alpha; EGF-like module-containing mucin-like hormone receptor-like 3 subunit beta]	-0.85
Q86YD5	MWLLGPLCLLLSSAAES	Low-density lipoprotein receptor class A domain-containing protein 3	-0.86
Q6PKH6	MARLLGLCAWARKSVRLASS	Dehydrogenase/reductase SDR family member 4-like 2 (EC 1.1.-.-)	-0.86
A6NC51	MWGYLSLMPVFLAVWAISGVWIVFAIA	Transmembrane protein 150B (Transmembrane protein 224)	-0.86
Q03692	MLPQIPFLLLVSLNLVHG	Collagen alpha-1(X) chain	-0.86
P17342	MPSLLVLTFSPCVLLGWALLAGGTGG	Atrial natriuretic peptide receptor 3 (Atrial natriuretic peptide clearance receptor) (Atrial natriuretic peptide receptor type C) (ANP-C) (ANPR-C) (NPR-C)	-0.86
P09848	MELSWHVVFIALLSFSCWG	Lactase-phlorizin hydrolase (Lactase-glycosylceramidase) [Includes: Lactase (EC 3.2.1.108); Phlorizin hydrolase (EC 3.2.1.62)]	-0.86
P01222	MTALFLMSMLFGLTCGQAMS	Thyrotropin subunit beta (Thyroid-stimulating hormone subunit beta) (TSH-B) (TSH-beta) (Thyrotropin beta chain) (Thyrotropin alfa)	-0.86
Q6W4X9	MVQRWLLLSCCGALLSAGLANT	Mucin-6 (MUC-6) (Gastric mucin-6)	-0.86
Q8N0Z9	MAAGGSAPEPRVLVCLGALLAGWVAVGLEA	V-set and immunoglobulin domain-containing protein 10	-0.86
Q6NUS6	MRTPQLALLQVFFLVFPDGVRP	Tectonic-3	-0.86
P12107	MEPWSSRWKTKRWLWDFTVTTLALTFLFQAREVRG	Collagen alpha-1(XI) chain	-0.86
P01275	MKSIYFVAGLFVMLVQGSWQ	Glucagon [Cleaved into: Glicentin; Glicentin-related polypeptide (GRPP); Oxyntomodulin (OXM) (OXY); Glucagon; Glucagon-like peptide 1 (GLP-1) (Incretin hormone); Glucagon-like peptide 1(7-37) (GLP-1(7-37)); Glucagon-like peptide 1(7-36) (GLP-1(7-36)); Glucagon-like peptide 2 (GLP-2)]	-0.86
P38567	MGVLKFKHIFFRSFVKSSGVSQIVFTFLLIPCCLT	Hyaluronidase PH-20 (Hyal-PH20) (EC 3.2.1.35) (Hyaluronoglucosaminidase PH-20) (Sperm adhesion molecule 1) (Sperm surface protein PH-20)	-0.86
Q9BYJ0	MKFVPCLLLVTLSCLGTLG	Fibroblast growth factor-binding protein 2 (FGF-BP2) (FGF-binding protein 2) (FGFBP-2) (37 kDa killer-specific secretory protein) (Ksp37) (HBp17-related protein) (HBp17-RP)	-0.86
Q9HBV2	MSPRGTGCSAGLLMTVGWLLLAGLQSARG	Sperm acrosome membrane-associated protein 1 (Sperm acrosomal membrane-	-0.87

		associated protein 32)	
Q9BXJ5	MIPWVLLACALPCAA	Complement C1q tumor necrosis factor-related protein 2	-0.87
Q8TF66	MPLKHYLLLLVGCQAWGAGLA	Leucine-rich repeat-containing protein 15 (Leucine-rich repeat protein induced by beta-amyloid homolog) (hLib)	-0.87
P54793	MRPRRPLVFMSLVCALLNTCQA	Arylsulfatase F (ASF) (EC 3.1.6.-)	-0.87
Q86YD3	MALPPGPAALRHTLLLLPALLSSGWG	Transmembrane protein 25	-0.87
O95976	MGTASRSNIARHLQTNLILFCVGAVGA	Immunoglobulin superfamily member 6 (IgSF6) (Protein DORA)	-0.87
O75325	MRLLVAPLLLAWVAGATA	Leucine-rich repeat neuronal protein 2 (Glioma amplified on chromosome 1 protein) (Leucine-rich repeat neuronal protein 5)	-0.87
Q9UKI3	MACRCLSFLLMGTFLSVSQT	Pre-B lymphocyte protein 3 (N27C7-2) (Protein VPreB3)	-0.87
Q4W5P6	MASDLIRTILAVALISKLGTAVDA	Protein TMEM155	-0.87
Q9P0T7	MKLLSLVAVVGCLLVPPAEA	Transmembrane protein 9 (Dermal papilla-derived protein 4)	-0.87
Q96QU1	MFRQFYLWTCLASGIILGSLFEICLG	Protocadherin-15	-0.87
Q76MJ5	MASAVRGSRPWPRLGLQLQFAALLLGTLSPQVHT	Serine/threonine-protein kinase/endoribonuclease IRE2 (Endoplasmic reticulum-to-nucleus signaling 2) (Inositol-requiring protein 2) (hIRE2p) (Ire1-beta) (IRE1b) [Includes: Serine/threonine-protein kinase (EC 2.7.11.1); Endoribonuclease (EC 3.1.26.-)]	-0.87
P28039	MQSPWKILTVAPLFLLLSLQSSA	Acyloxyacyl hydrolase (EC 3.1.1.77) [Cleaved into: Acyloxyacyl hydrolase small subunit; Acyloxyacyl hydrolase large subunit]	-0.87
P01138	MSMLFYTLITAFLIGIQA	Beta-nerve growth factor (Beta-NGF)	-0.87
Q93033	MAGISYVASFFLLLTKLSIG	Immunoglobulin superfamily member 2 (IgSF2) (Cell surface glycoprotein V7) (Glu-Trp-Ile EWI motif-containing protein 101) (EWI-101) (CD antigen CD101)	-0.87
P01737	MLLLLVPVLEVIFTLGGTRA	T-cell receptor alpha chain V region PY14	-0.87
P16233	MLPLWTLSLLLGAVAG	Pancreatic triacylglycerol lipase (PL) (PTL) (Pancreatic lipase) (EC 3.1.1.3)	-0.87
Q14982	MGVCGYLFLPWKCLVVVSLRLLFLVPT	Opioid-binding protein/cell adhesion molecule (OBCAM) (OPCML) (Opioid-binding cell adhesion molecule) (IgLON family member 1)	-0.87
Q8N3T6	MRSEGAAPGPAAPLCGALSLLLGALLG	Transmembrane protein 132C	-0.87
P14543	MLASSSRIRAAWTRALLLPLLLAGPVGC	Nidogen-1 (NID-1) (Entactin)	-0.87
Q9P121	MGVCGYLFLPWKCLVVVSLRLLFLVPTGVPVRS	Neurotrimin (hNT) (IgLON family member 2)	-0.87
P09668	MWATLPLLCAGAWLLGVPVCGA	Pro-cathepsin H [Cleaved into: Cathepsin H mini chain; Cathepsin H (EC 3.4.22.16); Cathepsin H heavy chain; Cathepsin H light chain]	-0.88
Q5VWK5	MNQVTIQWDAVIALYILFSWCHG	Interleukin-23 receptor (IL-23 receptor) (IL-23R)	-0.88
Q5W188	MWSLPPSRALSCAPLLLLFSFQFLVTYA	Putative cystatin-9-like protein CST9LP1 (Cystatin-9-like pseudogene 1)	-0.88
Q8TE57	MKPRARGWRGLAALWMLLAQVAEQ	A disintegrin and metalloproteinase with thrombospondin motifs 16 (ADAM-TS 16) (ADAM-TS16) (ADAMTS-16) (EC 3.4.24.-)	-0.88
Q6IED9	MLAVLYLLVKTAKLGTS	Putative diacylglycerol O-acyltransferase 2-like protein DGAT2L7P (EC 2.3.1.-) (Diacylglycerol O-acyltransferase 2-like 7 pseudogene)	-0.88
Q6UWU2	MAPKKLSCLRSLLLPLSLTLLLPQADT	Beta-galactosidase-1-like protein (EC 3.2.1.-)	-0.88
P13726	METPAWPRVPRPETAVARTLLLGWVFAQVAGA	Tissue factor (TF) (Coagulation factor III) (Thromboplastin) (CD antigen CD142)	-0.88
A2VEC9	MLLPALLFGMAWALADG	SCO-spondin	-0.88

P54764	MAGIFYFALFSCLFGICDA	Ephrin type-A receptor 4 (EC 2.7.10.1) (EPH-like kinase 8) (EK8) (hEK8) (Tyrosine-protein kinase TYRO1) (Tyrosine-protein kinase receptor SEK)	-0.88
Q9Y2E5	MGQLCWLPLLAPLLLLRPPGVQS	Epididymis-specific alpha-mannosidase (EC 3.2.1.24) (Mannosidase alpha class 2B member 2)	-0.88
P17693	MVVMAPRTLFLLLSGALTLTETWA	HLA class I histocompatibility antigen, alpha chain G (HLA G antigen) (MHC class I antigen G)	-0.88
Q9Y320	MAVLAPLIALVYSVPRLSRWLAQPYYLLSALLSAAFLLV RKLPPLCHG	Thioredoxin-related transmembrane protein 2 (Cell proliferation-inducing gene 26 protein) (Thioredoxin domain-containing protein 14)	-0.88
Q8WU66	MSALLSLCFVLPLAAPGHG	Thrombospondin-type laminin G domain and EAR repeat-containing protein (TSP-EAR)	-0.88
Q9Y693	MASSLTCTGVIWALLSFLCAA	Lipoma HMGIC fusion partner	-0.88
Q02763	MDSLASLVLCGVSLLLSGTVEG	Angiopoietin-1 receptor (EC 2.7.10.1) (Endothelial tyrosine kinase) (Tunica interna endothelial cell kinase) (Tyrosine kinase with Ig and EGF homology domains-2) (Tyrosine-protein kinase receptor TEK) (Tyrosine-protein kinase receptor TIE-2) (hTIE2) (p140 TEK) (CD antigen CD202b)	-0.88
Q9UQF0	MALPYHIFLFTVLLPSFTLT	HERV-W_7q21.2 provirus ancestral Env polyprotein (Endogenous retrovirus group W member 1) (Env-W) (Envelope polyprotein gPr73) (Enverin) (HERV-7q Envelope protein) (HERV-W envelope protein) (Syncytin) (Syncytin-1) [Cleaved into: Surface protein (SU) (gp50); Transmembrane protein (TM) (gp24)]	-0.88
O75493	MGAAARLSAPRALVLWAALGAAA	Carbonic anhydrase-related protein 11 (CA-RP XI) (CA-XI) (CARP XI) (Carbonic anhydrase-related protein 2) (CA-RP II) (CARP-2)	-0.88
A6NGN9	MPPPAPGARLRLLAAAALAGLAVISRGLLS	IgLON family member 5	-0.88
Q6UX41	MALMLSLVLSLLKLGSG	Butyrophilin-like protein 8	-0.89
Q96K78	MASCRAWNLRVLVAVVCGLLTGIILG	Probable G-protein coupled receptor 128	-0.89
P00736	MWLLYLLVPALFCRAGG	Complement C1r subcomponent (EC 3.4.21.41) (Complement component 1 subcomponent r) [Cleaved into: Complement C1r subcomponent heavy chain; Complement C1r subcomponent light chain]	-0.89
Q9UBT3	MVAAVLLGLSWLCSPLGA	Dickkopf-related protein 4 (Dickkopf-4) (Dkk-4) (hDkk-4) [Cleaved into: Dickkopf-related protein 4 short form]	-0.89
Q9BX93	MKLASGFLVLWLSLGGGLA	Group XIIB secretory phospholipase A2-like protein (Group XIII secretory phospholipase A2-like protein) (GXIII sPLA2-like) (sPLA2-GXIIB) (GXIIB)	-0.89
Q86WK6	MHPHRDPRGLWLLLPSLSLLLFEVARA	Amphoterin-induced protein 1 (AMIGO-1) (Alivin-2)	-0.89
O75443	MNYSSFLRIWVSFIFALVQHQA	Alpha-tectorin	-0.89
O60883	MRWLWPLAVSLAVILAVGLSRVSGG	Endothelin B receptor-like protein 2 (ETBR-LP-2) (G-protein coupled receptor 37-like 1)	-0.89
Q6UXZ3	MWLSPSLLLLILPGYSIA	CMRF35-like molecule 4 (CLM-4) (CD300 antigen-like family member D) (CMRF35-A4) (CD antigen CD300d)	-0.89

Appendix E | Signal peptide-containing proteins with ΔG_{sub} in the top quartile. Proteins were extracted from Swiss-Prot with the key word search “signal peptide”. The sequence feature file was sorted for “signal peptide” and accession numbers with a signal peptide sequence feature were kept. Amino acid positions for the beginning and end of the signal peptide were used as input into the fasta_segment.py program to extract signal peptide sequences from full protein sequences. The full signal peptide is given. To calculate ΔG_{sub} the full signal peptide was truncated to 30aa N-terminal to the predicted cleavage site. The truncated sequence was entered into the ΔG calculator in “ΔG prediction” mode with no length correction. The option to allow for subsequence (if lower ΔG) was chosen. Proteins were sorted from highest to lowest ΔG_{sub}. Of the 3400 signal peptide-containing proteins analyzed, the 25% (850 proteins) with the highest ΔG_{sub} are shown. These proteins are the most likely to be sensitive to CT8. Orange text indicates proteins validated as sensitive to CT8. Blue text indicates proteins validated as resistant to CT8

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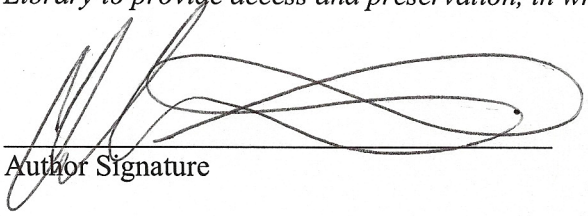

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