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Blencowe, Montgomery

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Integrative Genomics Analysis Reveals Tissue-specific Pathways and Gene Networks for Type 1 Diabetes

A thesis submitted in satisfaction of the requirements for the degree Master of Science in

Physiological Science

by

Montgomery Charles Thomas Blencowe

2019

ABSTRACT OF THE THESIS

Integrative Genomics Analysis Reveals Tissue-specific Pathways and Gene Networks for Type 1 Diabetes

by

Montgomery Charles Thomas Blencowe

Master of Science in Physiological Science

University of California, Los Angeles, 2019

Professor Xia Yang, Chair

Type 1 diabetes (T1D) is a complex disease, involving a genetic predisposition that interacts with environmental triggers, leading to the loss of insulin producing beta cells in the pancreas. However, the molecular cascades underlying T1D are poorly understood and remain to be explored. We hypothesize that genetic risk factors of T1D perturb tissue-specific biological pathways and gene networks, which ultimately leads to the pathogenic end point in beta cells. We sought to identify the gene networks and key regulators for T1D by conducting a comprehensive, data-driven multi-omics analysis that integrates human genome-wide association studies (GWAS) of T1D, tissue-specific genetic regulation of gene expression in the form of expression quantitative trait loci (eQTLs), and tissue-specific gene network models using a computational pipeline Mergeomics. Our integrative genomics approach revealed immune pathways such as adaptive immune system, cytokines and inflammatory response, ZAP70 translocation, primary immunodeficiency and immunoregulatory interactions between a lymphoid and non-lymphoid cell, across various tissues. We also identified tissue-specific signals such as regulation of complement cascade in adipose tissue, macrophages, and monocytes, NOTCH signaling in adipose tissue and macrophages, protein folding, calcium signaling, chemotaxis and lysosomal pathways in the pancreas, adipose, and monocytes, and viral infection in macrophages and monocytes. Network modelling of these pathways highlights a number of key regulator genes such as GBP1, USP18, STAT1, RPL17, HLA genes (HLA-A,-B,-C, and –G), and

immunomodulatory genes (LCK, VAV1, ZAP-70), each of which has suggestive roles in the pathophysiology of T1D or other autoimmune disorders. Together, our integrative genomics approach offers comprehensive insights into the tissue-specific molecular networks and regulators as well potential between-tissue interactions underlying T1D, which may guide future development of therapeutic strategies targeting the disease.

The thesis of Montgomery Charles Thomas Blencowe is approved by:

Elaine Yih-Nien Hsiao

Stephanie Correa Van Veen

Xia Yang, Committee Chair

University of California, Los Angeles

2019

DEDICATION

This thesis is dedicated to my family and more particularly my parents for supporting my academic

endeavours throughout.

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ACKNOWLEDGEMENTS

INTRODUCTION

Type 1 diabetes (T1D) is characterized as the autoimmune loss of pancreatic beta cells and resultant impairment of glucose homeostasis. Currently, T1D accounts for 5-10% of all diabetic individuals worldwide, approximately 40 million cases, with an increasing incidence rate of 2-3% per year (Maahs, West, Lawrence, & Mayer-Davis, 2010). Risk of developing T1D is increased by ~5.6% and ~50% with a diseased parent or diseased monozygotic twin, respectively, when compared to the general population. Parental heritability estimates predict that diabetic fathers confer an increased risk of T1D development of about 12% while mothers at around 6% (Steck et al., 2005). Thus, there is a strong genetic component predisposing T1D pathogenesis. However, T1D is not usually present in individuals with a family history, with only ~10% of patients having a first/second-degree relative with the disease, and a significant environmental contribution has been highlighted (Knip & Simell, 2012). Interestingly, there seems to be an increased disposition for developing T1D if one lives in regions of Northern Europe, independent of genetic background which is highlighted by an increase in T1D incidence in migrants living within these regions (Oilinki, Otonkoski, Ilonen, Knip, & Miettinen, 2012; Söderström, Åman, & Hjern, 2012). This environmental contribution has several potential manifestations, with alterations in gut microbiota (Kostic et al., 2015) or pre and post-natal dietary factors, including intake of gluten (Norris et al., 2003), vitamin D (Weets et al., 2004), and polyunsaturated fatty acids (Sørensen, Joner, Jenum, Eskild, & Stene, 2012) as suggested factors. Furthermore, a longstanding hypothesis predicts that the exposure to viral infection may also be a causal factor (Gamble, Kinsley, FitzGerald, Bolton, & Taylor, 1969), particular enteroviruses (Coppieters, Wiberg, Tracy, & von Herrath, 2012) which seem to target pancreatic islet cells (Yeung, Rawlinson, & Craig, 2011). Therefore, both genetic and environmental components contribute to T1D incidence and progression, yet a large gap exists in understanding the complex genetic and environmental architectures as well as the interaction between the two.

From the genetics perspective, with the ease and power of Genome-Wide Association Studies (GWAS) we have uncovered ~60 T1D genetic risk loci. The main genes predisposing T1D patients are located within the HLA region on chromosome 6, encoding the major histocompatibility complex (MHC) which is critical for adaptive immunity. While HLA-encoding genes have the strongest association and account for up to 50% of the total genetic T1D risk (Lambert et al., 2004; Noble et al., 1996), loci outside of the HLA region including protein tyrosine phosphatase, non-receptor type 22 (*PTPN22*), the interleukin 2 receptor, alpha (*IL2RA*), insulin gene (*INS*) and cytotoxic T-lymphocyte-associated protein 4 (*CTLA4*), have also been associated with disease development (Noble & Erlich, 2012).

While GWAS have played an essential role in identifying T1D-associated candidate genes, typically only the top loci reaching genome-wide association significance of p<5e-8 are reported, and these top loci cannot fully explain the total genetic heritability of T1D. Identifying the missing genetic risks, or the "dark matter", is important to gain a full understanding of the genetic underpinnings of T1D pathogenesis. In addition, growing lines of evidence support an "omnigenic" disease model (Boyle, Li, & Pritchard, 2017), which states that a large proportion of genes on the genome may contribute to disease development through intimate gene-gene interactions in networks within and between tissues, and key network regulators likely play more central roles than other peripheral disease genes in the networks. More interestingly, GWAS loci for complex diseases have been found to be more concentrated in the periphery of gene networks and less likely to be network regulators. Therefore, simply focusing on the top GWAS hits will likely miss crucial regulatory genes, and understanding how T1D genetic risks interact in gene networks and identifying key regulators will offer novel insights

into T1D pathogenesis and help prioritize regulators for targeted therapeutic strategies (Chan et al., 2014; Chella Krishnan et al., 2018; Makinen et al., 2014; Shu et al., 2017).

Integration of GWAS data with functional data such as tissue-specific expression quantitative trait loci (eQTLs) and gene networks has proven to be a powerful tool to pinpoint causal genes and their associated pathogenic mechanisms and regulators within the context of particular tissue type (Chella Krishnan et al., 2018; Foroughi Asl et al., 2015; Hauberg et al., 2017; Makinen et al., 2014; Shu et al., 2017; Zhong, Yang, Kaplan, Molony, & Schadt, 2010). Our lab has previously developed a computational pipeline, Mergeomics, to facilitate such analysis (Shu et al., 2016). We hypothesize that T1D genetic risks with a wide spectrum of effect sizes (strong, moderate to subtle) interact and perturb tissue-specific gene networks through a select set of regulatory genes, resulting in variations in T1D susceptibility, and application of Mergeomics to T1D GWAS datasets in conjunction with other orthogonal functional genomics data will help reveal these networks and regulators.

Though the integration of the largest T1D GWAS studies to date with tissue-specific functional information such as genetics of gene expression and gene regulatory networks, our analysis confirmed the importance of immune pathways across various tissues, such as adaptive immune system, cytokines and inflammatory response, ZAP70 translocation, primary immunodeficiency, and immunoregulatory interactions between a lymphoid and non-lymphoid cell. Signals demonstrating certain tissue specificity include regulation of complement cascade (adipose, macrophage, and monocytes), NOTCH signalling (adipose tissue and macrophages), chemotaxis and lysosomal pathways (pancreas, adipose tissue, and monocytes), and genes involved in viral infection/interferon signaling (macrophages and monocytes). The finding related to infection suggests a potential interaction between genetic predisposition and environmental perturbation. Importantly, we found

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key genes associated with pathways that are not directly associated with immune function, such as spliceosome, proteasome, calcium, Wnt, and cell cycle related pathways, however they may have modulatory or interactive effects on the immune system within the context of T1D. Of these, we find cell cycle to be enriched in the pancreatic tissue which highlights the potential for tissue specific dysregulation of this key cellular process directly leading to a local environment which promotes beta cell death. These results provide us with a novel area to further explore in disease progression and exploit as potential therapeutic targets.

MATERIALS AND METHODS

Overview of study design

We utilized an integrative genomics approach that leverages multiple large-scale human genetic and genomic datasets to elucidate the genetic networks and regulators of T1D pathogenesis (Figure 1). The datasets utilized included T1D GWAS from two independent cohorts, tissue specific eQTLs from diverse human tissues or cell types, various network models including gene coexpression networks, Bayesian gene regulatory networks and protein-protein interaction networks, and biological pathway information (detailed in subsequent sections). To address reproducibility, we ran the integrative analysis on each GWAS study independently and then focused on the findings that are consistent between the two cohorts. For each GWAS, we mapped the single nucleotide polymorphisms (SNPs) to genes using tissue/cell-specific eQTL data. The use of eQTLs helps inform on the most likely genes affected by GWAS SNPs based on functional evidence. Next, we grouped the genes based on whether they belong to the same biological pathways or show coexpression, which indicates functional relevance, in data-driven gene co-expression networks. We then assessed which pathways or gene coexpression modules (a module contains genes that show coexpression patterns) demonstrated

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stronger genetic associations with T1D compared to randomly generated gene sets using a Marker Set Enrichment Analysis (MSEA). After carrying out the MSEA process for each T1D GWAS dataset, we subsequently used a Meta-MSEA to meta-analyze the two independent GWAS data sets to look for shared pathways/modules that showed significant T1D associations, which we further simplified into independent "supersets" to reduce redundancy between pathways/modules. Integrating these T1D supersets with gene regulatory networks (Bayesian) and protein-protein interaction networks, we carried out the weighted key driver analysis (wKDA) to identify key drivers (KDs), which are central network genes whose network neighborhoods are highly enriched for genes in the T1D pathways and coexpression modules. These KDs were then visualized in tissue-specific networks. Furthermore, we carried out *in silico* validation via literature mining (T-HOD, PolySearch 2.0, DisGeNET) to seek if these key drivers are novel, suggestive, or known to be linked with T1D (Cheng et al., 2008; Dai, Wu, Tsai, Pan, & Hsu, 2013; Pinero et al., 2017).

T1D GWAS datasets

The summary statistics of GWAS for T1D was obtained from the JDRF/Wellcome Diabetes and Inflammation Laboratory, University of Oxford (Barrett et al., 2009; Cooper et al., 2017).

The study is comprised of 5913 T1D individuals of European descent (Barrett et al., 2009; Cooper et al., 2017), among which 3983 were genotyped using Illumina HumanHap550v3 (550k) Infinium Beadchip from the UK GRID and 1930 T1D individuals genotyped using Affymetrix 500K from the WTCCC. There were a total 8828 Controls, with 3999 genotyped using Illumina HumanHap550v3 (550k) Infinium Beadchip from the 1958 Birth Cohort (1958BC), 1490 genotyped using Affymetrix 500K (1958BC), 1455 genotyped using Affymetrix 500k from the UK Blood Services (UKBS) and 1884 genotyped using Affymetrix 500K from a cohort of Bipolar disorders.

Inclusion criteria for the UK GRID are: T1D diagnosed between 6 months to 16 years of age, insulin dependent for greater than 6 months, a UK resident and self-identified as white European (average diagnosis age = 7.8 years of age, SD = 3.9, 47% female). Inclusion criteria for the WTCCC are: T1D diagnosed less than 17 years of age, insulin dependent for greater than 6 months and self-identified white European (average diagnosis age = 7.2 years of age, SD = 3.8, 49% female). Control inclusion criteria for UKBS and 1958BC included being residents in the UK and self-identified white Europeans. For the Bipolar cohort, control individuals greater than 16 years old and resident in the UK and of European descent were included.

The above T1D and control individuals were partitioned into two independent cohorts based on matching genotyping platforms (i.e., Illumina or Affymetrix) between cases and controls. Cohort 1 was comprised of 1930 T1D patients and 4830 Controls. Cohort 2 was comprised of 3983 T1D patients and 3999 Controls.

SNPs genotyped were imputed to ~10 million SNPs (1000 Genomes Phase III) using IMPUTE2, and routine quality controls were conducted as described in Cooper et al. (Cooper et al., 2017). Statistical association between SNPs and T1D was carried out using a Bayesian analysis. All statistical association p values for all imputed SNPs that passed quality control, regardless of significance level for T1D association, were used in our downstream analyses.

Mapping SNPs to genes

In order to link GWAS SNPs to their potential target genes, tissue-specific eQTLs were used as they can provide functional insight for the role of SNPs in gene expression regulation within a given tissue.

Thirteen eQTLs sets were obtained from the GTEx database including subcutaneous adipose, visceral omentum adipose, blood, brain, colon, heart, liver, lymphocyte, muscle, pancreas, pituitary, spleen and stomach (Consortium, 2015). Additionally, we obtained macrophage and monocyte eQTLs from the Cardiogenics Consortium (Rotival et al., 2011). A broader spectrum of tissues was considered at this step to help objectively infer which tissues might be more informative for T1D association. GWAS was mapped to each tissue eQTL set separately to derive individual SNP-gene mapping sets reflecting tissue origins to allow assessment of tissue-specific signals.

A high degree of linkage disequilibrium (LD) was observed in the eQTL data, which may cause biases in the downstream analysis. For this reason, we removed redundant SNPs that had LD of $r^2 > 0.7$ with a chosen SNP. Briefly, a GWAS SNP was compared against other SNPs for LD and T1D association. If the SNP was in LD of $r^2 > 0.7$ with other SNPs, the one with the strongest T1D associations was chosen. This process was repeated until all remaining SNPs were not in LD based on the $r^2 > 0.7$ cut-off. These non-redundant SNPs were used for downstream analyses.

Data-driven modules of co-expressed genes

In order to assess whether T1D GWAS signals are enriched in specific gene subnetworks, we derived gene co-expression networks from transcriptomic data sets from genomic studies of subcutaneous adipose, visceral omentum adipose, blood and pancreas (Shu et al., 2016). These tissues were chosen due to their relevance to T1D. The WGCNA (Weighted Gene Correlation Network Analysis) package was used to reconstruct co-expression networks based on gene expression profiles (Langfelder & Horvath, 2008). Each tissue network contains multiple "modules" and each module is comprised of tens to hundreds or thousands of genes that show coexpression. A total of 272 co-expression modules were curated.

Knowledge-based biological pathways

We used a total of 1827 canonical pathways from Reactome (Version 45) (Croft et al., 2014), Biocarta (Nishimura, 2001) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) databases (Kanehisa & Goto, 2000) . In addition to the knowledge-based pathways, we constructed a T1D positive control gene set based on candidate causal genes curated in GWAS catalog (p<5.0E-8) (MacArthur et al., 2017). Similar control gene sets for coronary heart disease (CHD), type 2 diabetes (T2D), and height were also constructed to compare with the T1D positive control set.

Marker Set Enrichment Analysis (MSEA)

To identify co-expression modules and pathways that show evidence for genetic association with T1D, we applied MSEA from the Mergeomics (Shu et al., 2016) package on each of the GWAS cohorts separately in conjunction with the eQTL sources. MSEA employs a chi-square-like statistic with multiple quantile thresholds to assess whether a co-expression module or pathway shows enrichment of functional disease SNPs (i.e., those likely regulate gene expression as captured in eQTLs) compared to random chance. 10,000 permuted gene sets were generated for each co-expression module and pathway. As detailed in Shu et al., the enrichment statistics from the permutations were used to approximate a Gaussian distribution from which enrichment P-values were determined. Benjamini-Hochberg (BH) false discovery rate (FDR) was estimated across all co-expression modules and pathways tested for each GWAS. Gene sets were considered to be statistically significant if FDR < 5% in at least one SNP-gene mapping set. To evaluate gene sets across both GWAS studies, we followed up with a meta-analysis at the module/pathway level using the meta-MSEA function in Mergeomics, to retrieve robust gene sets across both cohorts. Stouffer's Z score method was used to calculate meta P-values based on the P-values from the multiple MSEA runs. Meta-FDR was

calculated using the Benjamini-Hochberg method, as described above.

Merging overlapping pathways into supersets

The curated pathways and gene coexpression modules may carry redundant information. For example, a KEGG pathway "insulin signalling" can have largely overlapping genes with a Reactome pathway "insulin receptor signalling". To reduce redundancy, we compared the significant modules and pathways associated with T1D at FDR <5% and merged the overlapping ones using a merging algorithm in Mergeomics to produce independent, non-overlapping "supersets". The algorithm employs an overlap ratio r between two gene sets A and B as $r = (r_{AB} \times r_{BA})^{0.5}$, where r_{AB} is the proportion of genes in A that are also present in B and r_{BA} is the proportion of genes in B which are also in A. The overlap ratio cut-off was set to $r >= 0.33$ and Fisher's exact test was used for assessing the statistical significance of gene overlap between modules/pathways. BH FDR < 5% was considered significant. Resultant supersets containing more than 500 genes were trimmed down to contain core genes shared among the overlapping gene sets.

Tissue-specific gene regulatory networks and key driver analysis (KDA)

Tissue-specific Bayesian gene regulatory networks of adipose, blood, and pancreas tissue were obtained (Shu et al., 2016) as well as protein-protein interaction (PPI) networks obtained from the Human protein reference database (Keshava Prasad et al., 2008). We chose to focus on these tissue networks due to our MSEA results showing the strongest statistical significance for these tissues. With these networks, we performed a key driver analysis using a KDA algorithm in Mergeomics to identify potential key drivers (KDs) whose network neighbors are enriched for genes within the T1D associated supersets uncovered by MSEA. The algorithm employed a chi-square like statistic similar to that described for MSEA, and FDR < 5% was used to focus on top robust KDs.

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In silico validation of KDs

To determine the relevance of the top ranked KDs to T1D, we used literature-mining methods such as PolySearch 2.0 (Liu, Liang, & Wishart, 2015), DisGeNET (Pinero et al., 2017) and T-HOD (Dai et al., 2013).

RESULTS

Identification of T1D-associated knowledge-based biological pathways

We first assessed which knowledge-based biological pathways were enriched for T1D GWAS signals. The use of tissue-specific eQTLs served to guide SNP-to-gene mapping, allowing us to capture tissue specific results. From the 15 eQTLs sets used, we found the following six to be the most informative in terms of whether and how many significant pathways were identified: blood, macrophage, monocyte, pancreas, subcutaneous adipose and visceral omentum adipose. We therefore focused on reporting the results from these six informative tissues only, with other tissues serving to supplement the main results.

Out of the 1827 curated canonical pathways, we identified 224 pathways enriched from Cohort 1 at an FDR <5%. Of these 224 pathways, 154 were significant at FDR <5% in at least one of the six chosen tissues based on MSEA and the remaining 70 significant pathways passed FDR <5% when meta-analyzed across tissues using meta-MSEA. The top pathways enriched from Cohort 1 included T1D positive control group, CHD positive control, Adaptive Immune system, G-protein coupled receptor related processes, and positive control set for Height. In the remaining 70 pathways that collectively passed meta-MSEA significance across tissues, Influenza life cycle, Nuclear envelope breakdown and PLC-gamma-1 signalling showed top significance. From the Cohort 2 dataset we

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found a total of 158 pathways enriched at an FDR <5%, with 129 pathways significant in at least one tissue in MSEA and the remaining 29 passed FDR<0.05 in meta-MSEA across tissues. The top pathways were confirmatory with Cohort 1, with T1D, CHD, and Height positive control sets all showing strong statistical significance. Additionally, Adaptive Immune system, interactions between lymphoid and non-lymphoid cells, ER-Phagosome pathways, Antigen processing, Wnt signaling, transfer in endocytosis and recycling, and SNARE interactions in vesicular transport were our top pathways.

When comparing Cohort 1 and Cohort 2 for shared pathways, as expected we found largely confirmatory and overlapping pathways heavily related to immune processes and importantly the T1D positive control gene set. Between the two datasets we found 111 pathways significant in both, 113 unique for Cohort 1 and 47 unique for Cohort 2 (Figure 2A). Some unique pathways for Cohort 1 include Influenza life cycle, HIV life cycle, and nuclear envelope breakdown. For Cohort 2 the unique pathways included purine and inositol phosphate metabolism and RNA degradation.

We found largely confirmatory results with the other tissue types tested across both Cohorts, with the spleen, adrenal, liver and lymphocyte revealing largely immune related signals. The pituitary gland also showed significance in Wnt signaling and insulin receptor signalling.

Identification of T1D associated gene co-expression modules

The above biological pathways were curated based on known knowledge. To increase the chance to uncover novel biology, we collected data-driven gene coexpression modules which reflect functionally related gene sets based on gene expression patterns. This approach has previously helped derived

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novel biological insights into various diseases (Chella Krishnan et al., 2018; Makinen et al., 2014; Shu et al., 2017).

From our WGCNA networks we generated 272 co-expression modules, with which we ran our Cohort 1 and Cohort 2 data sets independently. Cohort 1 showed enrichment in a total of 40 unique modules of which 36 modules passed an FDR <5% in at least one tissue and four modules were deemed significant collectively across the six tissues (Figure 2B). The top annotated modules for Cohort 1 included interferon signaling, translocation of ZAP70, Stress related pathways, destabilization of mRNA, focal adhesion, and diabetes pathways. On the other hand, the Cohort 2 dataset showed significance in 46 modules (FDR <5%), with 37 significant in at least one tissue and 9 collectively across the six tissues (Figure 2B). The top modules included cytokine signaling, interferon signalling, antigen processing, DNA repair, metabolism, pre-NOTCH processing in golgi, and Pitx2 pathway.

When comparing the two cohorts we found that the majority of the modules reach significance in both, with a total of 30 overlapping modules. The top modules found in both were consistent with immune related pathways such as interferon signaling, and Antigen processing/presentation. In terms of unique pathways, we saw 16 unique modules for Cohort 2 including mTOR pathways, extracellular matrix (ECM) organization and DNA repair. In Cohort 1 we find 10 unique modules consisting of AKT signaling, complement pathway and ion transport (Figure 2B).

Comparing these 30 overlapping modules to the 111 canonical pathways uncovered in our pathway-based analysis, we see similar signals enriched across both. These signals include complement and

coagulation cascade, DNA repair, cell cycle, NOTCH signaling, metabolism of amino acids and derivatives, transcription, and translation.

Merging of pathways and co-expression modules into independent supersets

We chose to focus on the shared 141 significant gene sets uncovered in our meta-analysis between Cohort 1 and Cohort 2 (30 co-expression modules and 111 canonical pathways) as they reflect reproducible signals for T1D association (Figure 2A, 2B). As the co-expression modules and pathways were obtained from various sources, the gene sets may share a high number of overlapping gene members. To decrease the redundancy, we merged 83 overlapping gene sets into 12 independent supersets (Table 1). Interestingly, canonical pathways tend to merge with canonical pathways and coexpression modules tend to merge with coexpression modules, suggesting different biological properties of the two types of gene sets. The supersets represented diverse biological pathways including activation of adaptive immune system, antigen processing, ubiquitination & proteasome degradation, HIV infection, protein folding, RNA Polymerase I/III, mitochondrial transcription, signaling by GPCR, signaling by NOTCH, signaling by the B-cell receptor (BCR) and tRNA aminoacylation. The other 58 non-overlapping gene sets were kept intact, producing a total of 70 non-overlapping supersets.

Second round of MSEA on the 70 supersets

After using our merging algorithm of similar pathways, we ran a second round of MSEA to confirm whether our merged modules retained significance for T1D association. We confirmed 40 supersets derived from canonical pathways to show statistical significance in our combined Cohort 1 and Cohort 2 datasets (Figure 3A). Our results from the monocyte and macrophage-based analysis showed the strongest and most consistent enrichment of pathways including Natural Killer Cell mediated

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cytotoxicity, lymphoid and non-lymphoid cell interaction, and mitotic G1/S phase. The adipose pathways included systemic lupus erythematosus, complement cascade, Natural Killer Cell mediated cytotoxicity and signaling by NOTCH. The blood tissue-derived pathways contained ABC transporters, Antigen processing and presentation, Secretin receptors, cell cycle and neuroactive ligand-receptor interaction. The pancreas showed pathways involved in Natural Killer Cell mediated cytotoxicity, secretin receptors, protein folding, calcium signaling and Wnt signaling. When looking at the remaining 30 supersets derived from our co-expression modules (Figure 3B). Again, monocyte and macrophage were informative across most of the listed supersets (Figure 3B). Pancreas and subcutaneous adipose tissue were also informative for 14 and 25, respectively, of the 30 supersets (FDR <5%). The blood appeared to be the least informative for these supersets, with only a few supersets related to DNA replication, interferon signaling, mitotic cell cycle, and TCR signalling being significant. Across all six tissues we found that translocation of ZAP70, calcium signaling, interferon signalling, and Stress pathways were significant.

Identification of central regulators for T1D via a weighted key driver analysis

To identify central regulatory genes, or key drivers (KDs), among the potential T1D associated supersets, we performed a weighted key driver analysis (wKDA) on our independent supersets using tissue-specific Bayesian gene regulatory networks as well as using the PPI networks (Figure 4).

The total number of unique KDs found from our Bayesian network included 299 across blood, monocyte, macrophage, adipose and pancreatic islet networks at an FDR <5% (Supplement Table). To focus on the most central regulators, for each superset in each tissue-specific network, we chose the top five ranked KDs satisfying an FDR <5%, to derive 60 KDs, of which 13 were shared among >=2 supersets and two (*RPS29* and *RPS18)* were shared across the adipose, blood, monocyte and

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macrophage networks. In terms of tissue specific KDs we found that the adipose network contained the most with 45 KDs, and 13 unique KDs were shared between blood, monocyte and macrophage networks (Supplement Table). There were no KDs discovered for our pancreatic tissue Bayesian network, potentially due to the sparseness of this network. The key drivers uncovered can generally be categorized under cell cycle, metabolism and immune related pathways. Interestingly, many of the KDs have been found to be involved in viral infections, autoimmune and childhood onset disease, all of which are associated with T1D. These KDs include *RPS29, RPLP2, CD19, OAS2, IFIH1* and *PTPN6.*

To expand our search for KDs and look for consistencies between networks, we ran the PPI network for adipose, blood, monocyte. macrophage and pancreatic tissues. Focusing on the top five ranked KDs in each tissue specific network within each superset, we found a total of 113 KDs, five specific to adipose (*CASP9, FLNA, BRF1, CD74, HLA-DRA*), three specific to blood (*PTPRC, MYC, GNA11*), one for the pancreas (*JAK1*), two for monocyte (*AKT1, ESR1*) and two for macrophage (*POLR2G, PROS1*).

When comparing the PPI network with the Bayesian network we find 17 overlapping KDs for tissue specific networks (15 for adipose, two for blood) including complement related genes such as *CD19* and *CD74*, DNA replication genes such as *MCM2* and *MCM6*, as well as those linked to autoimmunity such as *STAT1, SERPINE1* and *GZMB*. Those shared in the Top 5 KDs (FDR <5%) of the same supersets for both the Bayesian and PPI networks include *LCK, VAV1, PTPN6* for Natural Killer Cell Cytotoxicity and *F2* and *PLG* for Complement and Coagulation pathways (Table 2).

In silico validation of the Key Drivers

To determine the relevance and functional significance of the unique KDs from our Bayesian and PPI network analysis to T1D from our study, we cross-validated the genes by performing a comprehensive *in silico* analysis using three literature mining methods: PolySearch 2.0, DisGeNET, and T-HOD.

Our search yielded 6 KDs that are known T1D GWAS hits (*HLA-DRB1, IFIH1, PGM1, HLA-DQB1, HLA-B* and *HLA-DRA*) as well as 43 KDs such as *GC, PLG, LCK, STAT1* and *PPARG* which were suggested to be associated with T1D due to previously being implicated in one or more studies to play a potential role in T1D pathogenesis.

Our study further uncovered a total of 107 potential novel KDs for T1D, many of which have viral infection associations (*OAS2, IFIH1, ISG15, SLC15A3* and *RTP4*). Additionally, we find large overlap of KDs with genes previously associated with autoimmune diseases such as Lupus Erythematosus, Psoriasis and Rheumatoid Arthritis (*PTPN6, MCM2, LCK, OAS2* and *CD3G*).

DISCUSSION

At present, genetic studies have uncovered >60 loci linked with T1D development, yet our understanding of the intricate mechanisms underlying these associations is still lacking. In addition, GWAS loci alone cannot provide the level of insights required for intervention. To this end, we utilised a multi-omics integrative approach to advance our understanding of T1D etiology and prioritize potential therapeutic targets among the large numbers of disease associated signals. Through the use of multiple T1D GWAS data sets, functional genomics data represented as tissue-specific eQTLs, knowledge-driven pathways, and data-driven networks, the perturbed pathways and key regulators which potentially drive the etiology of T1D were further elucidated.

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This computational strategy confirmed known genes and biological pathways in T1D and revealed novel genes and their corresponding networks and biological processes. Among the biological networks which are perturbed to initiate or exacerbate T1D, are a significant number of immune and apoptosis related processes which are known to be involved in T1D, and several novel pathways such as viral infection, NOTCH signaling, Wnt signaling, protein folding, and calcium signaling. Our tissue specific analyses reveal consistent enrichment of antigen processing/presentation and INF-α/β/γ signalling across tissues, supporting the immune origin of T1D highlighted in the literature and emphasizing the presence of systemic dysregulation of immune function beyond insulitis. For the multi-tissue immune pathways for T1D, top KDs predicted by our analyses are HLA-DR/DQ alleles (Hu et al., 2015). Although HLA genes have been known to confer the greatest genetic risk for T1D, our network analysis uniquely highlights the critical regulatory roles of these genes in T1D development.

Of the immune related pathways, a notable process which shows perturbation in macrophage and monocyte is ubiquitination and proteasome degradation within the context of Class I MHC mediated antigen processing. Many of the key drivers which were highlighted in our analysis relate directly to the immunoproteasome and the maturation of the MHC I complexes, including *GBP1* (Lundberg, Krogvold, Kuric, Dahl-Jorgensen, & Skog, 2016)*, PSMA6* (Sjakste et al., 2016) , and *PSMA4* (Jin et al., 2013), each of which have been previously implicated in T1D susceptibility. Additionally we identified several HLA genes, including HLA-A, -B (Nejentsev et al., 2007), -C (Genetic Analysis of Psoriasis et al., 2010), and -G (Eike, Becker, Humphreys, Olsson, & Lie, 2009), which have been previously associated with T1D or autoimmune disease susceptibility. Dysregulation of the MHC class I binding peptides produced by the cytosolic multicatalytic proteasome may play a fundamental role in the development of T1D causing autoimmunity. These MHC class I molecules are a major inducer

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of the host immune response and alterations in MHC I peptide presentation have a putative role in autoimmune pathogenesis, with links to ankylosing spondylitis (Evans et al., 2011), multiple sclerosis (Guerini et al., 2012), and Crohn's disease (Barrett et al., 2008). Previous evidence also supports the role of the immunoproteasome in the induction of T1D through the generation of aberrant polypeptide derived epitopes which act as β-cell neo-autoantigens (Thomaidou, Zaldumbide, & Roep, 2018), which is another potential avenue of disease development.

Furthermore, a number of the identified KDs relate to the modulation of immune response and autoimmunity, most notably *IFIH1*, a DEAD box RNA helicase which has been linked to T1D susceptibility. Additionally, we identify *JAK1* via our PPI KDA, which has previously been shown to regulate pediatric autoimmunity and local inflammatory response via its interactions with STAT and INF signaling, thus acting as an intriguing target for further study. Moreover, the inhibition of *JAK1* levels via AZD1480 blocked MHC class I upregulation via cytokine signaling within both mouse and human beta cells (Trivedi et al., 2017). Through this, the infiltration of immune cells into the pancreatic islets was dramatically slowed, as the T cells were no longer able to directly associate with beta cells, protecting NOD mice from insulitis. These results affirm the previous predictions made regarding the etiology of T1D, with significant emphasis on the failure of the immune system to recognize beta cell autoantigens as self, thus resulting in the destruction of these cells. Our results here further emphasize the potential of these immune related genes as central regulators and targets to ameliorate the pathogenesis of T1D.

Our results also support the importance of T cells and cytokine signalling in T1D pathogenesis. *FYN* (Figure 4A) is one of the most central key regulators in the T1D tissue networks and is connected a number of previously identified T1D associated GWAS loci (*IL7R*, *MAPT*, *TYK2*). Importantly, this

gene has been shown to be essential in T cell signaling and related processes, through its interaction with *ZAP-70* (Michel, Grimaud, Tuosto, & Acuto, 1998) and *VAV1* (Garcia-Bernal et al., 2005), which are both key components of T cell mediated immune response and have been identified as key drivers in our network analysis. The role of T-cell signaling and autoimmunity within the context of T1D progression is further highlighted by KD *LCK* (Stanley, Trivedi, Sutherland, Thomas, & Gurzov, 2017), which has also been previously shown to preclude autoimmunity through its interaction with DUSP22, serving as a negative regulator of T-cell activation. Also, the KDA identifies *PTPN6,* which has been shown to modulate cytokine signaling within pancreatic beta cells, in coordination with the action of *PTPN1* to connect the beta cells with the immune system. Finally, several other KDs were noted using the Bayesian network blood tissue data, including *SEMA4A* (Chapoval, Vadasz, Chapoval, & Toubi, 2017) and *TCF7* (Noble et al., 2003) . *SEMA4*, coding for Semaphorin 4A, has been shown to play a role in a number of autoimmune diseases including multiple sclerosis, Systemic lupus erythematosus, and Rheumatoid arthritis through its action in activating regulatory T cells (Delgoffe et al., 2013). Furthermore, it may have a potential role in inhibiting angiogenesis which may further perpetuate the T1D disease progression (Toyofuku et al., 2007). Similarly, polymorphisms within *TCF7,* a T cell transcription factor, have also been identified as being associated with T1D and may be an essential locus in the etiology of T1D through its downstream action of modulating immune response. Our analysis thus provides confirmation for the importance of T cell genes and pathways, as well as pinpointing the essential regulators to explore further within the context of T1D.

More globally, we identified several pathways which imply a pathogenic change in the molecular machinery coordinating protein production and processing, including spliceosome, mRNA metabolism, tRNA aminoacylation, gene expression, translation, and proteasome in our meta-MSEA. Kracht et al. reported that the production of a non-conventional products due to mRNA processing errors produces an autoimmune polypeptide that is detected by T cells in T1D patients (Kracht et al.,

2017). It is also predicted that the formation of hybrid insulin peptides within beta cells activates CD4 T cells in NOD mice (Delong et al., 2016), thus further supporting the association between T1D and the failure of proteins to be processed correctly. It is plausible that variations within genes governing protein formation and processing components induce antigenic protein products within the pancreatic tissue itself or within immune cells, which results in the activation of an autoimmune response, beta cell death, and development of T1D.

Viral infection (Allen, Kim, Rawlinson, & Craig, 2018) and its association with T1D has been suggestive as the potential causal environmental trigger, particularly with regard to antenatal maternal infection and subsequent incidence of T1D. In support of this theory we found a number of pathways associated with HIV and influenza virus infection across multiple tissues tested. Given that these pathways are genetically perturbed as informed by our T1D GWAS datasets, our finding implies that genetic variants in genes involved in viral infection may confer vulnerability to infections and/or promote over-reactive viral response that induces autoimmunity, which explains how viral infection may trigger T1D pathogenesis. We have identified several KDs which may be susceptible to viral perturbation resulting in modulation of immune response, which includes *PSMA7* (Apcher et al., 2003), *TRAF6* (Yoboua, Martel, Duval, Mukawera, & Grandvaux, 2010)*, CD3G* (Willard-Gallo, Furtado, Burny, & Wolinsky, 2001) *, PIK3R2* (Ylosmaki, Schmotz, Ylosmaki, & Saksela, 2015), and *MCM6* (Gautier et al., 2009). Of these, proteasomal alpha subunit *PSMA7* enables the human immunodeficiency virus-1 (HIV-1) Tat protein to disrupt proteasome function through its interaction with the subunit, thus highlighting a potential region which is susceptible to viral modulation (Apcher et al., 2003). The association with this protein and potential T1D pathogenesis goes further as it may modulate both cellular stress response (Li, Zhang, Zhang, & Wei, 2011) and antigen processing (Du et al., 2009), which may collectively impact beta cell survivability. Also, *TRAF6*, a TNF receptor, has

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been previously shown to modulate viral load in hepatitis C virus patients (Pu et al., 2017) as well as the immunogenic inflammatory response in dengue virus infected patients (Motaleb, Nabih, Mohamed, & Elhalim, 2017), thus serving as a connection between viral infection and immune response (Fang et al., 2017). Additionally, there is evidence suggesting that viral infection alters the expression and local chromatin landscape of the T cell receptor complex subunit *CD3G* (Akl et al., 2007; H. Yu et al., 2015), with further reports associating this subunit with autoimmunity (Tokgoz et al., 2013). The other two predicted key driver genes, *MCM6* (Tan et al., 2017) and *PIK3R2* (Kim, Hollenbaugh, Kim, & Kim, 2011) are predicted to influence antiviral and inflammatory response after viral infection.

Another immune-related pathway is the SNARE interaction with vesicular transport pathway (Offenhauser et al., 2011), which is significant in that the protein complex seems to act as a negative regulator of macrophage activity and is associated with familial hemophagocytic lymphohistiocytosis type 4 (*FHL-4*) (zur Stadt et al., 2005), a hyper-inflammatory disease. Moreover, SNARE also regulates cross presentation of antigens within dendritic cells through the action of Sec22b (Cebrian et al., 2011) which enables the delivery of antigens from the phagosome into the cytosol, thus further corroborating this complex's role within immune response.

Further, we identify NOTCH signaling as a potential contributor to T1D, which has been implicated in rheumatoid arthritis (Yabe, Matsumoto, Tsurumoto, & Shindo, 2005), another proposed autoimmune disease. There is evidence that Notch receptors are constitutively expressed on macrophages and dendritic cells, thus play a direct role in immunity (Zhang et al., 2012). NOTCH signaling has also been identified within the context of viral response (Ito et al., 2011), with its signal integral in influenza pathogenesis. These previous results highlight the integral role Notch signals play

within many of the proposed mechanisms of T1D initiation, thus our predictions serve as further evidence of this mechanism as a potentiation route to explore further mechanistically and therapeutically. Our KDA results also support this finding, with NOTCH1, 2, 3, and 4 all arising as significant KDs within blood, adipose, macrophage, and monocyte tissue/cell types. Furthermore, in a recent publication by Bartolome et al., chronic NOTCH activation is shown to impair beta cell function through a loss of maturity combined with increased proliferation rate during T2D related physiological stress (Bartolome, Zhu, Sussel, & Pajvani, 2018). Our results suggest that this may also contribute to the loss of beta cells function throughout T1D disease progression.

Finally, we highlight calcium signaling as a perturbed pathway in the pancreatic tissue. Dysregulation of this signaling process has been previously identified as a pathogenic mechanism in acute pancreatitis (Frick, 2012) and its perturbation is generally predicted to play a significant role in apoptotic pathways (Orrenius, Zhivotovsky, & Nicotera, 2003). Moreover, this pathway was identified within the macrophage and blood tissues which may further link calcium signaling and immune dysfunction. Previous reports show that modulation of the calcium signal transducer, calmodulin, mitigates the development of systemic lupus erythematosus in mice and suppresses INFγ production in human SLE cells (Ichinose, Juang, Crispín, Kis-Toth, & Tsokos, 2011). Furthermore, calcium alters B cell reactivity and anergy, thus critically regulating the development of autoreactivity (Bouillet et al., 1999) and inflammatory response (P. Yu et al., 2005). Interestingly, the B cell adaptor protein *CD19* which integrates calcium signalling and B cell mediated immunity (Tedder, Haas, & Poe, 2002), was identified as a KD within the blood tissue, which has a protective role in slowing the decline in beta cell function within T1D models (Vonberg et al., 2018).

Overall, our integrative genomics analysis recapitulates previously known pathways, processes and genes associated with T1D pathogenesis, primarily components of the immune system, confirming the robustness of our study. We additionally uncover possible avenues to explore further with suggested pathways and key genes involving the pancreas to be potentially contributing significantly to the T1D outcome through genetic perturbations, including those that can interact with environmental factors such as viral infections. The KDs prioritized through our comprehensive integrative analyses may serve as putative T1D targets for therapeutic development. Future efforts to validate the predicted novel regulators and pathways are warranted.

Table 1. Top pathways associated with T1D identified across multiple tissues at an FDR <5%.

Blood: 1, Macrophage: 2, Monocyte: 3, Pancreas: 4, Subcutaneous Adipose: 5 and Visceral Omentum Adipose: 6.

Supersets	Module Size	Tissues	Pathways
T1D positive control	60	1, 2, 3, 4, 5, 6	Positive control gene set for T1DM
S1: Mitotic G1-G1/S phases	186	1, 2, 3, 5, 6	Mitotic G1-G1/S phases; Cell Cycle Checkpoints and Regulation; DNA Replication; Vpu mediated degradation of CD4; Disease; Antigen Processing-Cross presentation
S2: HIV Infection	222	2, 3	HIV Infection; Host Interactions of HIV factors
S3: Signaling by NOTCH	122	3, 5, 6	Signaling by NOTCH Pre-NOTCH Expression and Processing
S4: Protein folding	53	3, 4, 5	Protein folding; Chaperon-mediated protein folding; Association of TriC/CCT with target proteins during biosynthesis
S5: Signaling by B Cell Receptor (BCR)	170	2, 3	Signaling by the B Cell Receptor (BCR); Downstream Signaling Events of B Cell Receptor (BCR)
S6: RNA Polymerase I, RNA Polymerase III, and Mitochondrial Transcription	95	3, 5	RNA Polymerase I, RNA Polymerase III, and Mitochondrial Transcription; RNA Polymerase I Transcription; RNA Polymerase I Promoter Clearance; RNA Polymerase I Chain Elongation
S7: Antigen processing: Ubiquitination & Proteasome degradation	211	2, 3	Antigen processing: Ubiquitination & Proteasome degradation; Class I MHC mediated antigen processing & presentation
S8: Adaptive Immune System	261	2, 3	Adaptive Immune System; Immune System
S9: tRNA Aminoacylation	46	2, 3, 5	tRNA Aminoacylation; Aminoacyl-tRNA biosynthesis; Mitochondrial tRNA aminoacylation
S10: Mitotic M-M/G1 phases	172	2, 3	Mitotic M-M/G1 phases; Mitotic Metaphase and Anaphase; Cell Cycle; Cell Cycle, Mitotic; M Phase; Mitotic Anaphase; Separation of Sister Chromatids
S11: Signaling by GPCR	230	3	Signaling by GPCR; GPCR downstream signaling GPCR ligand binding
S12: Activation of GABAB receptors	39	3	Activation of GABAB receptors; Activation of GABA B receptors

Table 2. T1D genetic supersets with KDs for both Bayesian and PPI networks. Only supersets with at least one KD (FDR <5%) were shown. For supersets with more than five KDs, only the top 5 KDs are shown.

Supersets	Bayesian Network		PPI Network		
	Adipose	Blood	Adipose	Blood	Pancreas
Metabolism of mRNA	RPL31, FAU, RPS29, RPS18, RPS27	RPL38, RPS29, RPL7A, RPLP2, RPL7	UPF2, EXOSC6, NDRG1, EXOSC8, EXOSC4	UPF2, EXOSC6, NDRG1, EXOSC8, EXOSC4	
Signal Transduction	BC052328, LGMN, EMR1, EVI2B, SLC15A3		DOK1, PIK3R2, MMP9, GNAI2, CCR5		
Gene Expression	RPL31, RPS29, RPS18, RPS27, FAU		NCOA6, RXRG, NR0B2, PPARGC1A, MED1		
Systemic Lupus Erythematosus	HIST1H2BM, HIST1H2BC		PTMA	PTMA, ASF1A	
Mitotic M-M/G1 phases	RAD51, MCM2, CDCA8, MKI67, FIGNL1		CDK2, PLK1, PCNA, CDKN1A, MCM7	CDK2, PLK1, PCNA, CDKN1A, MCM7	
Natural Kill Cell Cytoxicity	PTPN6, FERMT3, ARHGAP30, NCKAP1L, LCK		LCK, SHC1, SYK, PTPN6, VAV1	LCK, SHC1, SYK, PTPN6, VAV1	LCK, SHC1, SYK, PTPN6, VAV1
RNA Polymerase I, RNA Polymerase III, and Mitochondrial Transcription	HIST1H2BM, HIST1H2BC		TBP, ERCC3, CDK7, RB1, BRF1		
Complement & Coagulation	F2, FGG, PLG, AHSG, GC		F2, SERPINA5, PLG, C3, F10	F2, SERPINA5, PLG, C3, F10	
Disease	RPS18, RPL27, MYO1F, ACTR3, FAU		PIK3R2, FGFR1, ERBB4, NOTCH1, STAT5B	PIK3R2, FGFR1, ERBB4, NOTCH1, STAT5B	
Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell	CD3G, LCK		HLA-C, HLA-G, CD3D, HLA-B, ITGA4	HLA-C, HLA-G, CD3D, HLA-B, ITGA4	
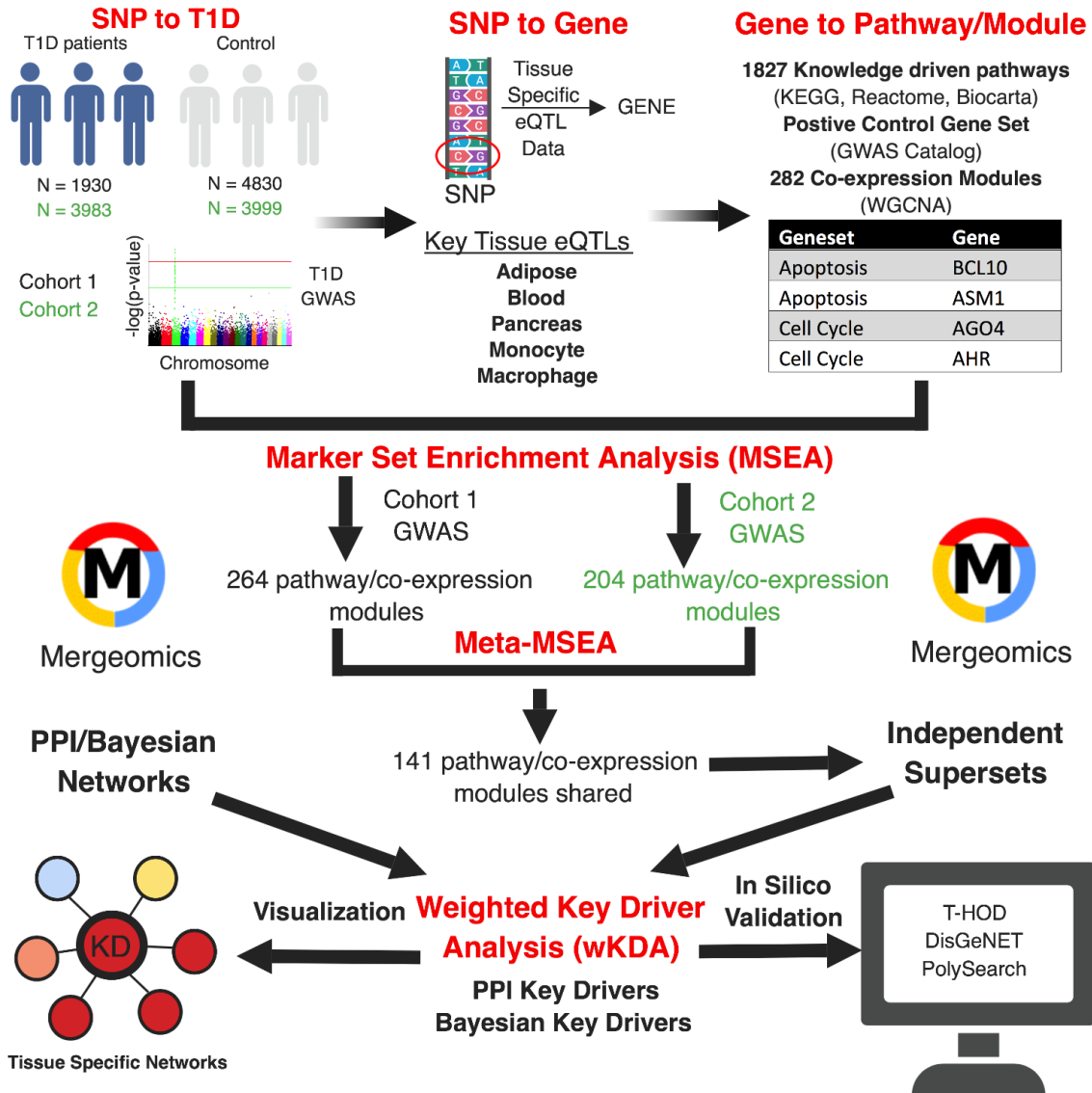
Figure 1. Overview of Study. 1) T1D GWAS SNPs are mapped onto genes using tissue specific eQTLs. Genes found are then linked to canonical pathways and co-expression modules. 2) MSEA of Cohort 1 and Cohort 2 are carried out independently for pathway/co-expression module enrichment. 3) Meta-MSEA of the combined cohorts for pathway/co-expression module enrichment. Similar modules are then categorized into independent supersets and input into a wKDA. 4) wKDA implemented using both protein-protein interaction (PPI) and Bayesian networks independently for key driver gene identification, followed by *in silico* validation.

Figure 2. Venn Diagram of enriched canonical pathways and co-expression modules for both T1D GWAS cohorts. A) Venn diagram of the independent and overlapping knowledge driven biological pathways for both cohorts (FDR <5%). B) Venn diagram of the independent and overlapping co-expression modules for both cohorts (FDR <5%).

A

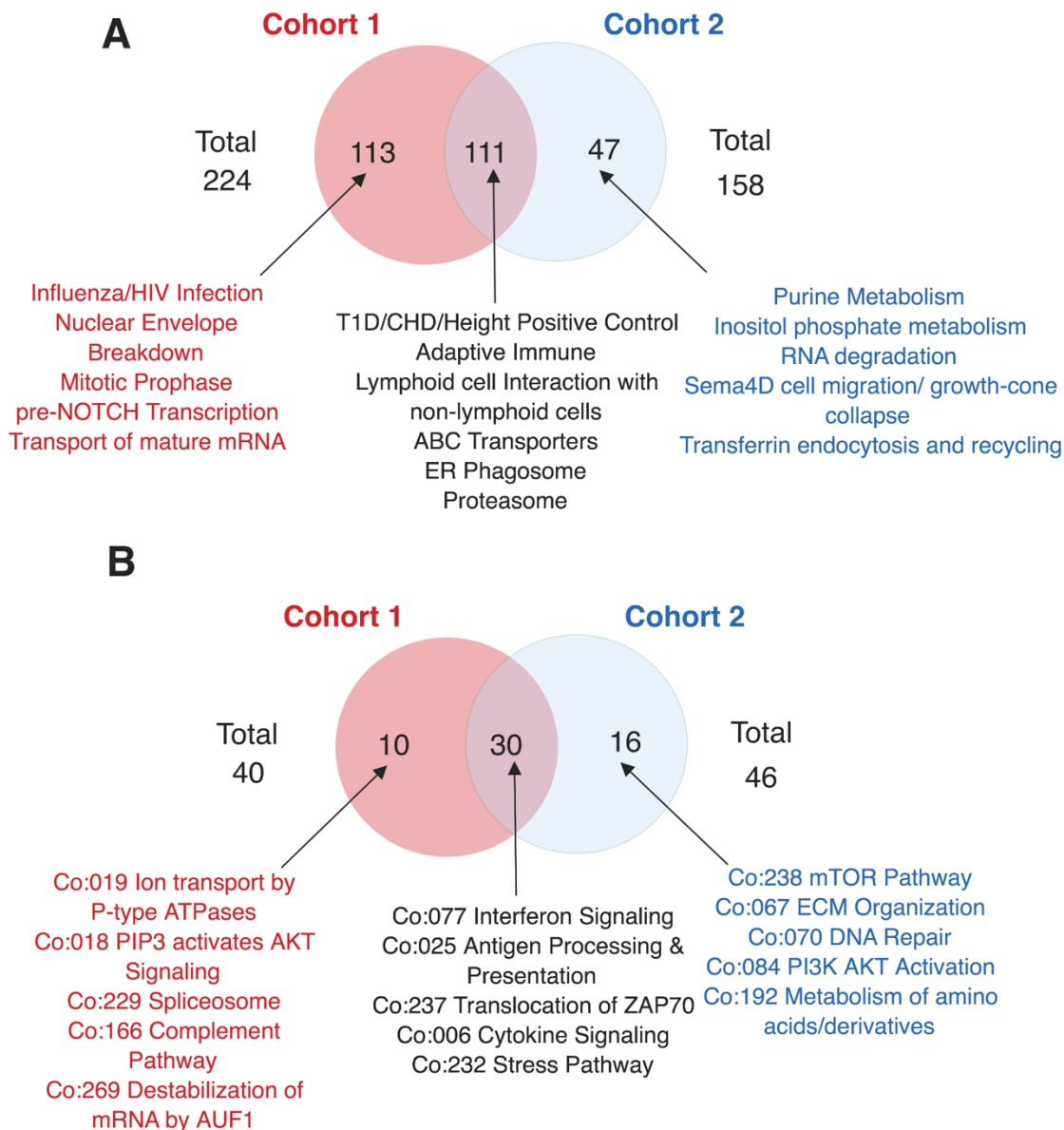|	Blood	Macrophage	Monocyte	Pancreas	Sub.adipose	Vis.adipose	META FDR
Positive control gene set for CHD							
Positive control gene set for Height							
ABC transporters							
Neuroactive ligand-receptor interaction							
Antigen processing and presentation							
Complement and coagulation cascades							
RNA polymerase							
Spliceosome							
Gap junction							
Primary immunodeficiency							
Systemic lupus erythematosus							
Natural killer cell mediated cytotoxicity							
Notch signaling pathway							
Long-term depression							
Activation of GABAB receptors							
Adaptive Immune System							
Antigen Presentation							
Antigen processing							
Apoptosis							
Class B/2 (Secretin family receptors)							
Complement cascade							
Disease							
G alpha (s) signalling events							
Gene Expression							
HIV Infection							
Lymphoid and a non-Lymphoid cell interaction							
Metabolism of amino acids and derivatives							
Metabolism of mRNA							
Mitotic G1-G1/S phases							
Mitotic M-M/G1 phases							
Protein folding							
RNA Polymerase I, III & Mitochondrial Transcription							
Regulation of Complement cascade							
Signal Transduction							
Signaling by GPCR							
Signaling by NOTCH							
Signaling by Wnt							
Signaling by the B Cell Receptor (BCR)							
tRNA Aminoacylation							
Positive control gene set for T1DM							

B

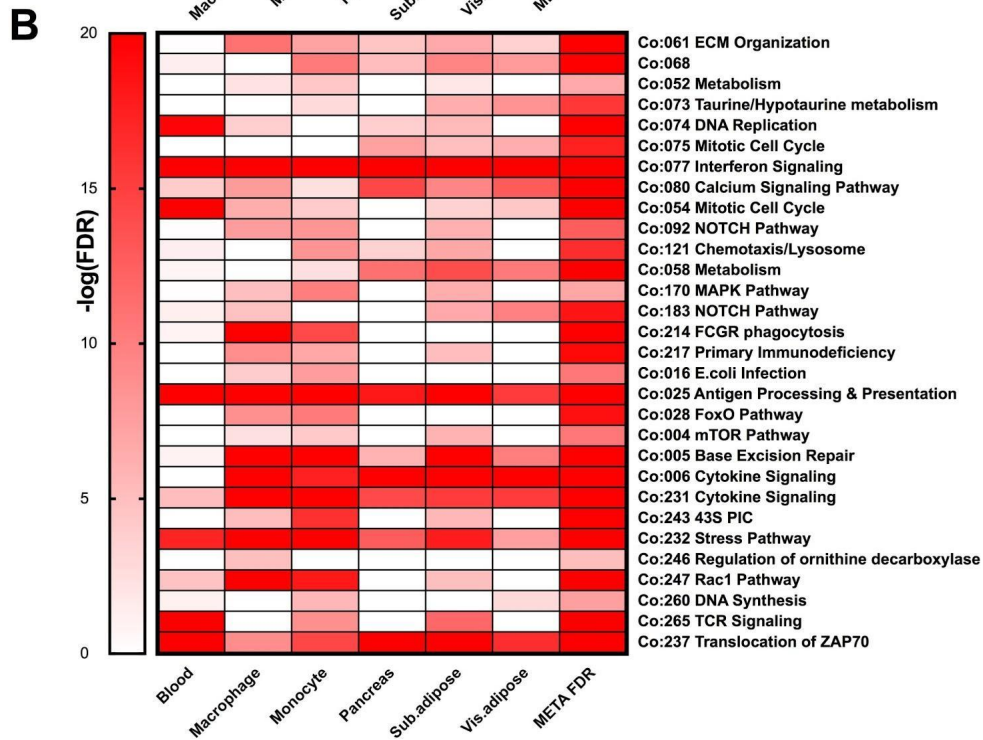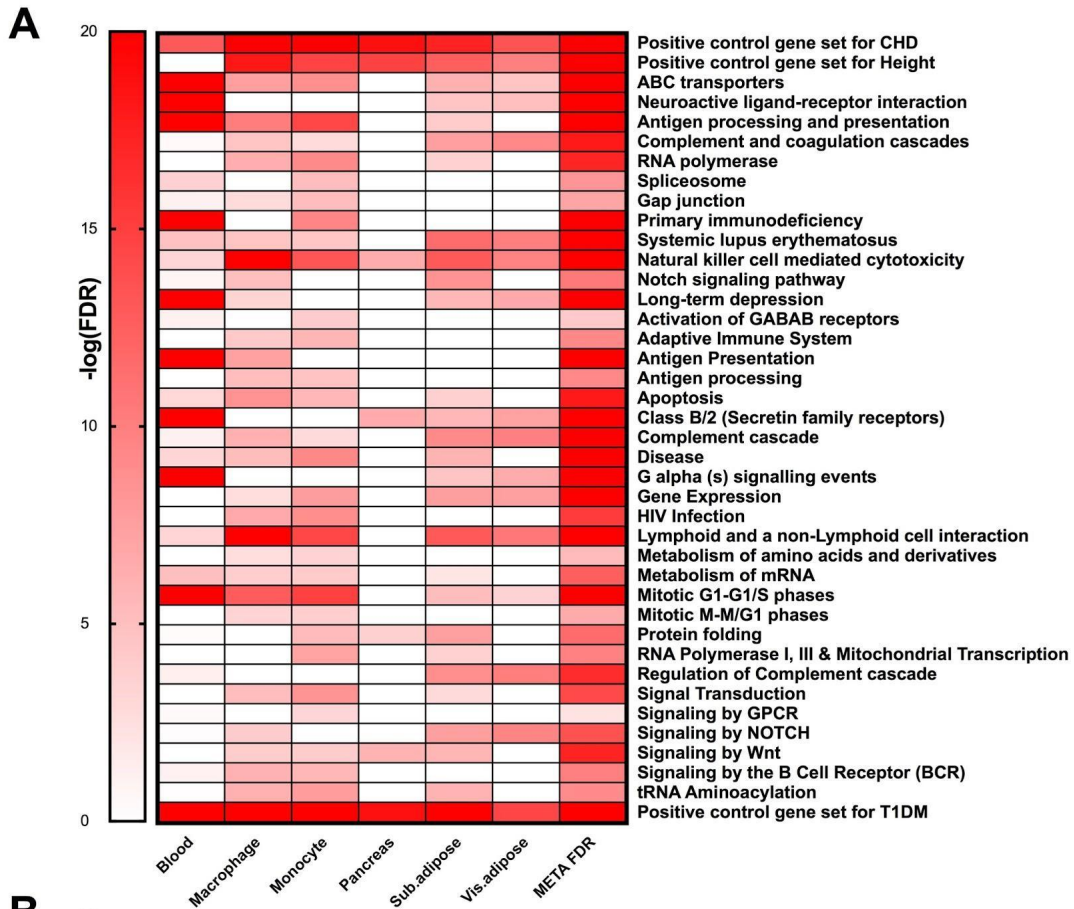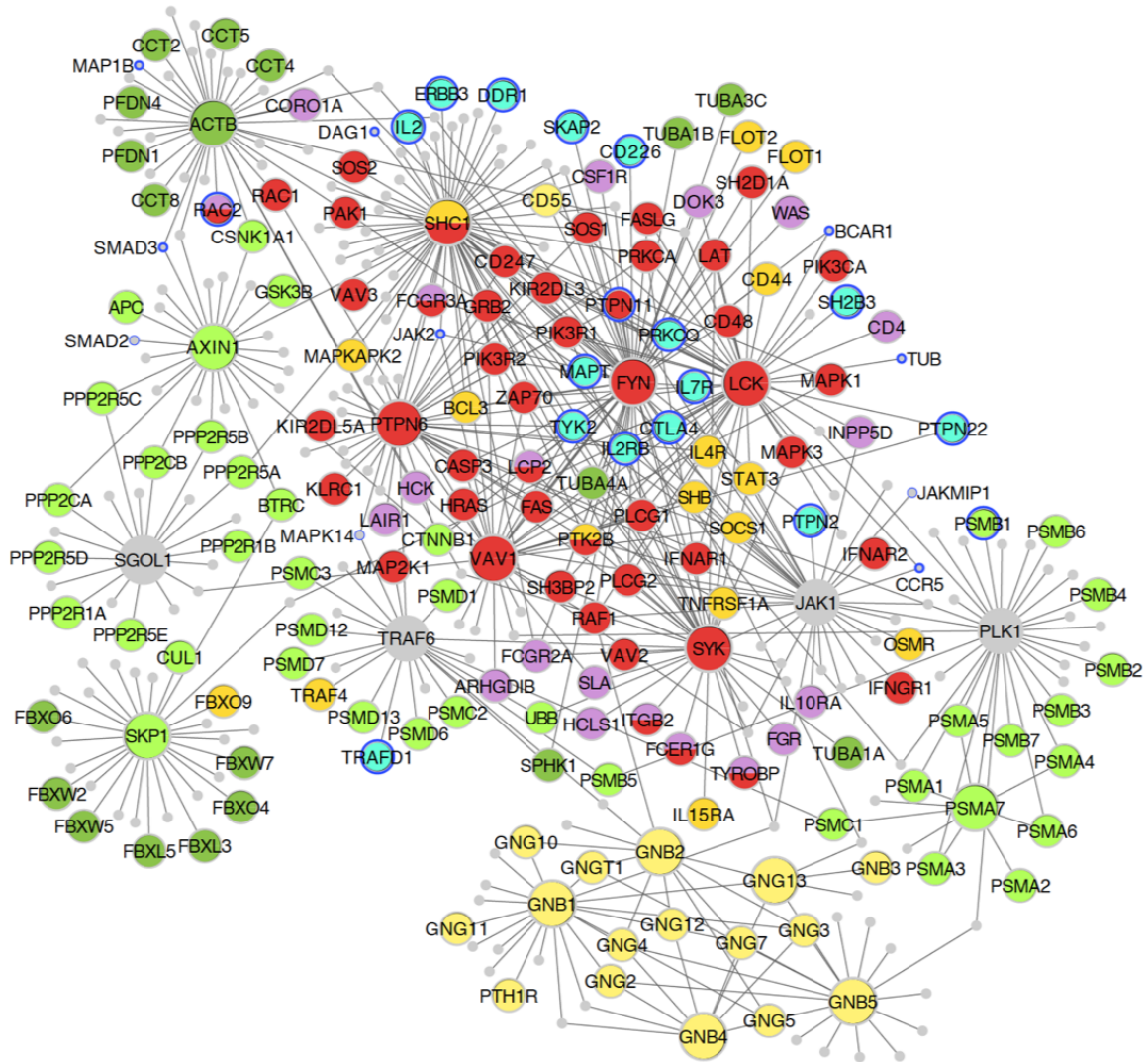|	Blood	Macrophage	Monocyte	Pancreas	Sub.adipose	Vis.adipose	META FDR
Co:061 ECM Organization							
Co:068							
Co:052 Metabolism							
Co:073 Taurine/Hypotaurine metabolism							
Co:074 DNA Replication							
Co:075 Mitotic Cell Cycle							
Co:077 Interferon Signaling							
Co:080 Calcium Signaling Pathway							
Co:054 Mitotic Cell Cycle							
Co:092 NOTCH Pathway							
Co:121 Chemotaxis/Lysosome							
Co:058 Metabolism							
Co:170 MAPK Pathway							
Co:183 NOTCH Pathway							
Co:214 FCGR phagocytosis							
Co:217 Primary Immunodeficiency							
Co:016 E.coli Infection							
Co:025 Antigen Processing & Presentation							
Co:028 FoxO Pathway							
Co:004 mTOR Pathway							
Co:005 Base Excision Repair							
Co:006 Cytokine Signaling							
Co:231 Cytokine Signaling							
Co:243 43S PIC							
Co:232 Stress Pathway							
Co:246 Regulation of ornithine decarboxylase							
Co:247 Rac1 Pathway							
Co:260 DNA Synthesis							
Co:265 TCR Signaling							
Co:237 Translocation of ZAP70							

-log(FDR)

28

Figure 3. Heatmap of the tissue-specific meta-MSEA from the combined Cohort 1 and Cohort 2 datasets for the supersets derived from the canonical pathways and co-expression network modules. A) Heatmap for the statistical significance of T1D genetic association across the supersets derived from the Canonical pathways (FDR <5%) in the tissue-specific and cross-tissue analyses. B) Heatmap for the statistical significance of T1D genetic association across the supersets derived co-expression modules (FDR <5%) in the tissue-specific and cross-tissue analyses.

A

B
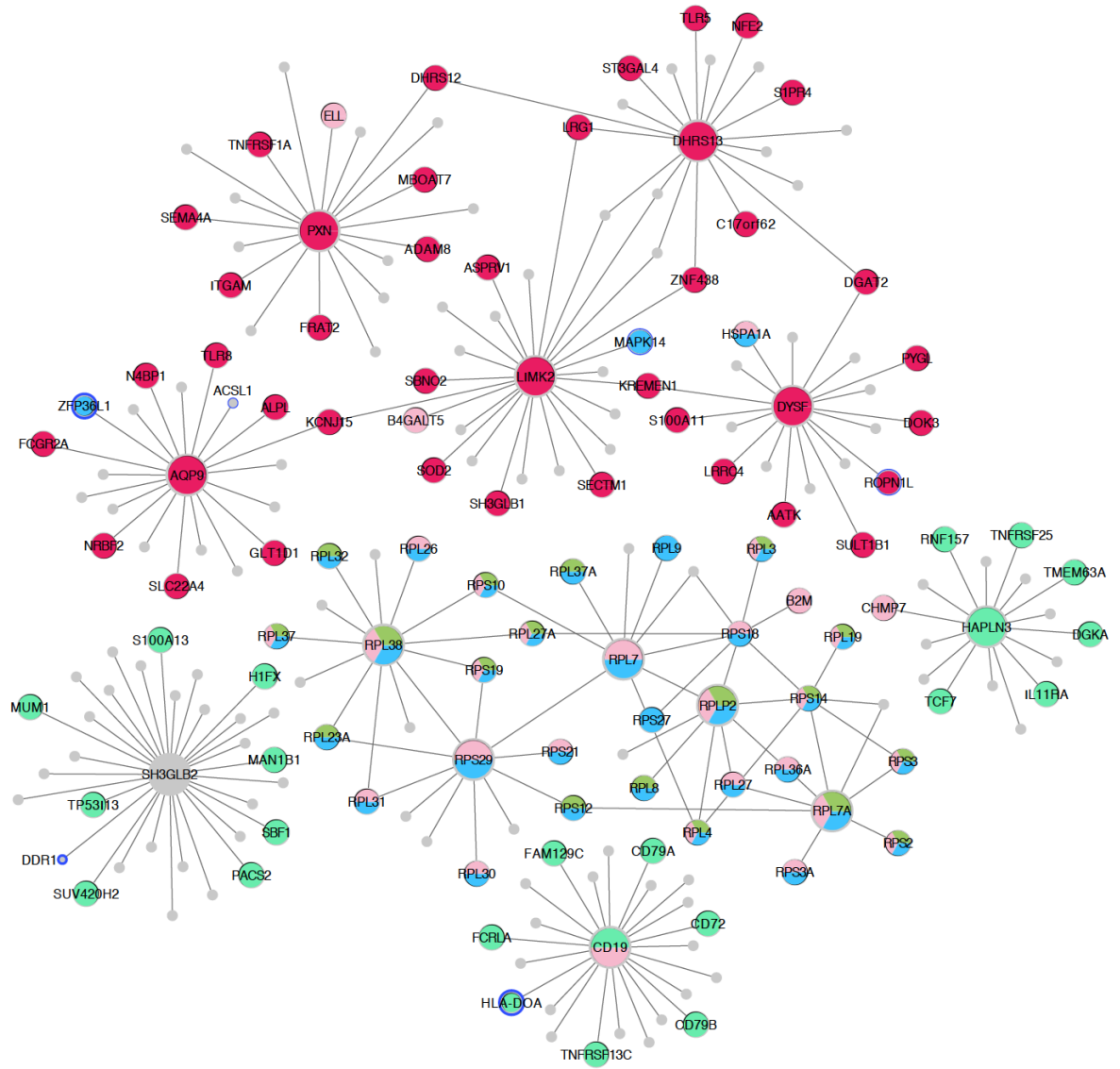
C

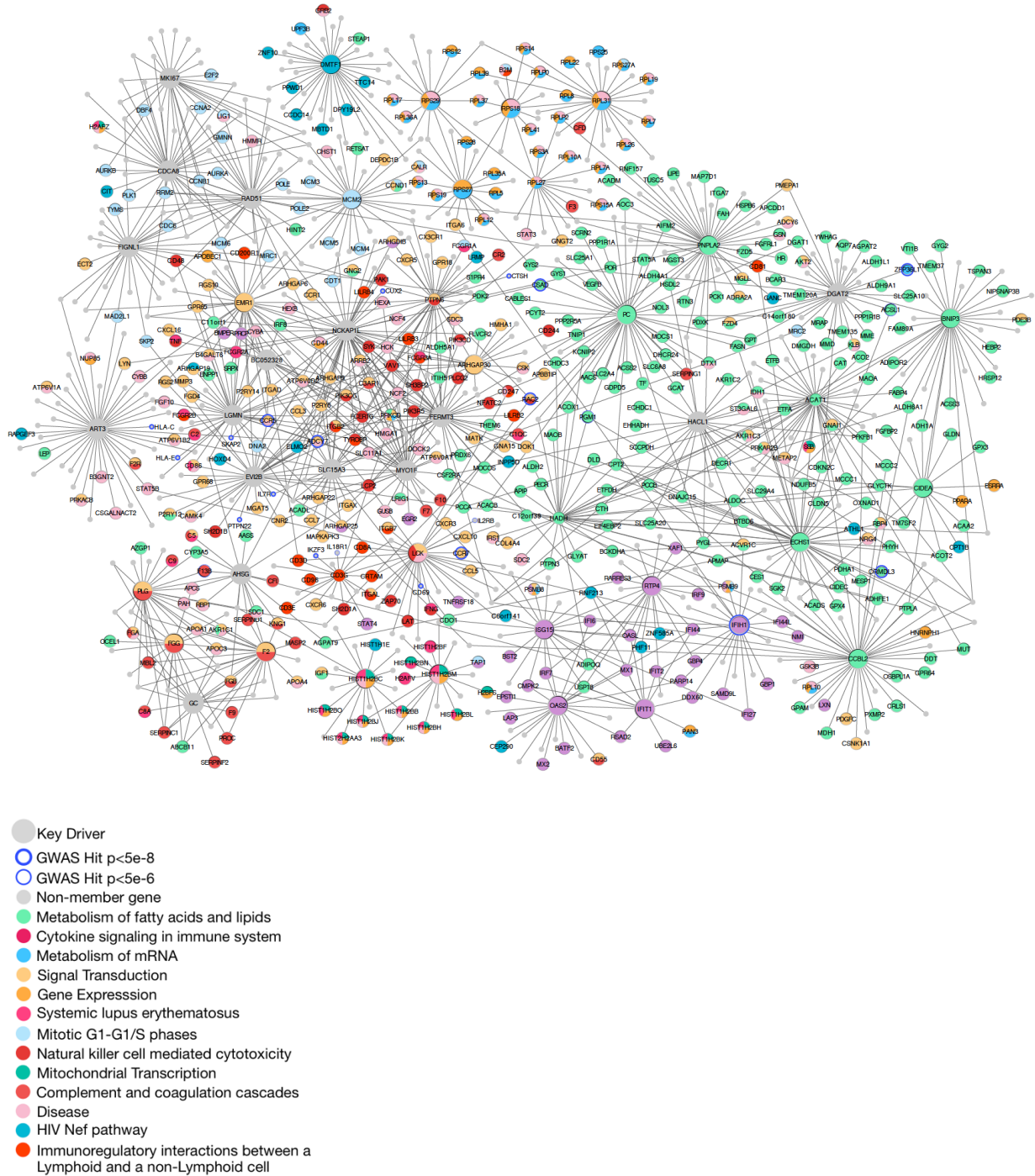**Figure 3. Tissue specific Key Driver Subnetworks.** A) Pancreas PPI network. B) Blood Bayesian

Network. C) Adipose Bayesian Network.

BIBLIOGRAPHY

Akl, H., Badran, B., Dobirta, G., Manfouo-Foutsop, G., Moschitta, M., Merimi, M., . . . Willard-Gallo, K. E. (2007). Progressive loss of CD3 expression after HTLV-I infection results from chromatin remodeling affecting all the CD3 genes and persists despite early viral genes silencing. *Virology journal, 4*(1), 85.

Allen, D. W., Kim, K. W., Rawlinson, W. D., & Craig, M. E. (2018). Maternal virus infections in pregnancy and type 1 diabetes in their offspring: Systematic review and meta-analysis of observational studies. *Rev Med Virol, 28*(3), e1974. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/29569297. doi:10.1002/rmv.1974

Apcher, G. S., Heink, S., Zantopf, D., Kloetzel, P.-M., Schmid, H.-P., Mayer, R. J., & Krüger, E. (2003). Human immunodeficiency virus-1 Tat protein interacts with distinct proteasomal α and β subunits. *FEBS letters, 553*(1-2), 200-204.

Barrett, J. C., Clayton, D. G., Concannon, P., Akolkar, B., Cooper, J. D., Erlich, H. A., . . . Type 1 Diabetes Genetics, C. (2009). Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nat Genet, 41*(6), 703-707. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/19430480. doi:10.1038/ng.381

Barrett, J. C., Hansoul, S., Nicolae, D. L., Cho, J. H., Duerr, R. H., Rioux, J. D., . . . Daly, M. J. (2008). Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet, 40*(8), 955-962. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/18587394. doi:10.1038/ng.175

Bartolome, A., Zhu, C., Sussel, L., & Pajvani, U. B. (2018). Notch signaling dynamically regulates adult β cell proliferation and maturity. *The Journal of clinical investigation, 129*(1).

Bouillet, P., Metcalf, D., Huang, D. C., Tarlinton, D. M., Kay, T. W., Köntgen, F., . . . Strasser, A. (1999). Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. *Science, 286*(5445), 1735-1738.

Boyle, E. A., Li, Y. I., & Pritchard, J. K. (2017). An Expanded View of Complex Traits: From Polygenic to Omnigenic. *Cell, 169*(7), 1177-1186. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/28622505. doi:10.1016/j.cell.2017.05.038

Cebrian, I., Visentin, G., Blanchard, N., Jouve, M., Bobard, A., Moita, C., . . . Savina, A. (2011). Sec22b regulates phagosomal maturation and antigen crosspresentation by dendritic cells. *Cell, 147*(6), 1355-1368. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/22153078. doi:10.1016/j.cell.2011.11.021

Chan, K. H., Huang, Y. T., Meng, Q., Wu, C., Reiner, A., Sobel, E. M., . . . Liu, S. (2014). Shared molecular pathways and gene networks for cardiovascular disease and type 2 diabetes mellitus in women across diverse ethnicities. *Circ Cardiovasc Genet, 7*(6), 911-919. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25371518. doi:10.1161/CIRCGENETICS.114.000676

Chapoval, S. P., Vadasz, Z., Chapoval, A. I., & Toubi, E. (2017). Semaphorins 4A and 4D in chronic inflammatory diseases. *Inflamm Res, 66*(2), 111-117. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27554682. doi:10.1007/s00011-016-0983-5

Chella Krishnan, K., Kurt, Z., Barrere-Cain, R., Sabir, S., Das, A., Floyd, R., . . . Yang, X. (2018). Integration of Multi-omics Data from Mouse Diversity Panel Highlights Mitochondrial Dysfunction in Non-alcoholic Fatty Liver Disease. *Cell Syst, 6*(1), 103-115 e107. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/29361464. doi:10.1016/j.cels.2017.12.006

Cheng, D., Knox, C., Young, N., Stothard, P., Damaraju, S., & Wishart, D. S. (2008). PolySearch: a web-based text mining system for extracting relationships between human diseases, genes, mutations, drugs and metabolites. *Nucleic Acids Res, 36*(Web Server issue), W399-405. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/18487273. doi:10.1093/nar/gkn296

Consortium, G. T. (2015). Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science, 348*(6235), 648-660. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25954001. doi:10.1126/science.1262110

Cooper, N. J., Wallace, C., Burren, O., Cutler, A., Walker, N., & Todd, J. A. (2017). Type 1 diabetes genome-wide association analysis with imputation identifies five new risk regions. *bioRxiv*, 120022. Retrieved from https://www.biorxiv.org/content/biorxiv/early/2017/04/23/120022.full.pdf. doi:10.1101/120022

Coppieters, K., Wiberg, A., Tracy, S., & von Herrath, M. (2012). Immunology in the clinic review series: focus on type 1 diabetes and viruses: the role of viruses in type 1 diabetes: a difficult dilemma. *Clinical & Experimental Immunology, 168*(1), 5-11.

Croft, D., Mundo, A. F., Haw, R., Milacic, M., Weiser, J., Wu, G., . . . D'Eustachio, P. (2014). The Reactome pathway knowledgebase. *Nucleic Acids Res, 42*(Database issue), D472-477. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/24243840. doi:10.1093/nar/gkt1102

Dai, H. J., Wu, J. C., Tsai, R. T., Pan, W. H., & Hsu, W. L. (2013). T-HOD: a literature-based candidate gene database for hypertension, obesity and diabetes. *Database (Oxford), 2013*, bas061. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/23406793. doi:10.1093/database/bas061

Delgoffe, G. M., Woo, S. R., Turnis, M. E., Gravano, D. M., Guy, C., Overacre, A. E., . . . Vignali, D. A. (2013). Stability and function of regulatory T cells is maintained by a neuropilin-1-semaphorin-4a axis. *Nature, 501*(7466), 252-256. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/23913274. doi:10.1038/nature12428

Delong, T., Wiles, T. A., Baker, R. L., Bradley, B., Barbour, G., Reisdorph, R., . . . Kumar, N. (2016). Pathogenic CD4 T cells in type 1 diabetes recognize epitopes formed by peptide fusion. *Science, 351*(6274), 711-714.

Du, H., Huang, X., Wang, S., Wu, Y., Xu, W., & Li, M. (2009). PSMA7, a potential biomarker of diseases. *Protein and peptide letters, 16*(5), 486-489.

Eike, M. C., Becker, T., Humphreys, K., Olsson, M., & Lie, B. A. (2009). Conditional analyses on the T1DGC MHC dataset: novel associations with type 1 diabetes around HLA-G and confirmation of HLA-B. *Genes Immun, 10*(1), 56-67. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/18830248. doi:10.1038/gene.2008.74

Evans, D. M., Spencer, C. C., Pointon, J. J., Su, Z., Harvey, D., Kochan, G., . . . Wellcome Trust Case Control, C. (2011). Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility. *Nat Genet, 43*(8), 761-767. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/21743469. doi:10.1038/ng.873

Fang, J., Bolanos, L. C., Choi, K., Liu, X., Christie, S., Akunuru, S., . . . Greis, K. D. (2017). Ubiquitination of hnRNPA1 by TRAF6 links chronic innate immune signaling with myelodysplasia. *Nature immunology, 18*(2), 236.

Foroughi Asl, H., Talukdar, H. A., Kindt, A. S., Jain, R. K., Ermel, R., Ruusalepp, A., . . . Consortium, C. A. (2015). Expression quantitative trait Loci acting across multiple tissues are enriched in inherited risk for coronary artery disease. *Circ Cardiovasc Genet, 8*(2), 305-315. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25578447. doi:10.1161/CIRCGENETICS.114.000640

Frick, T. W. (2012). The role of calcium in acute pancreatitis. *Surgery, 152*(3), S157-S163.

Gamble, D., Kinsley, M., FitzGerald, M., Bolton, R., & Taylor, K. (1969). Viral antibodies in diabetes mellitus. *Br Med J, 3*(5671), 627-630.

Garcia-Bernal, D., Wright, N., Sotillo-Mallo, E., Nombela-Arrieta, C., Stein, J. V., Bustelo, X. R., & Teixido, J. (2005). Vav1 and Rac control chemokine-promoted T lymphocyte adhesion mediated by the integrin alpha4beta1. *Mol Biol Cell, 16*(7), 3223-3235. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/15872091. doi:10.1091/mbc.e04-12-1049

Gautier, V. W., Gu, L., O'Donoghue, N., Pennington, S., Sheehy, N., & Hall, W. W. (2009). In vitro nuclear interactome of the HIV-1 Tat protein. *Retrovirology, 6*, 47. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/19454010. doi:10.1186/1742-4690-6-47

Genetic Analysis of Psoriasis, C., the Wellcome Trust Case Control, C., Strange, A., Capon, F., Spencer, C. C., Knight, J., . . . Trembath, R. C. (2010). A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1. *Nat Genet, 42*(11), 985-990. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/20953190. doi:10.1038/ng.694

Guerini, F. R., Cagliani, R., Forni, D., Agliardi, C., Caputo, D., Cassinotti, A., . . . Sironi, M. (2012). A functional variant in ERAP1 predisposes to multiple sclerosis. *PLoS One, 7*(1), e29931. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/22253828. doi:10.1371/journal.pone.0029931

Hauberg, M. E., Zhang, W., Giambartolomei, C., Franzen, O., Morris, D. L., Vyse, T. J., . . . Roussos, P. (2017). Large-Scale Identification of Common Trait and Disease Variants Affecting Gene Expression. *Am J Hum Genet, 101*(1), 157. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/28686855. doi:10.1016/j.ajhg.2017.06.003

Hu, X., Deutsch, A. J., Lenz, T. L., Onengut-Gumuscu, S., Han, B., Chen, W.-M., . . . Rich, S. S. (2015). Additive and interaction effects at three amino acid positions in HLA-DQ and HLA-DR molecules drive type 1 diabetes risk. *Nature genetics, 47*(8), 898.

Ichinose, K., Juang, Y. T., Crispín, J. C., Kis-Toth, K., & Tsokos, G. C. (2011). Suppression of autoimmunity and organ pathology in lupus-prone mice upon inhibition of calcium/calmodulin-dependent protein kinase type IV. *Arthritis & Rheumatism, 63*(2), 523-529.

Ito, T., Allen, R. M., Carson, W. F. t., Schaller, M., Cavassani, K. A., Hogaboam, C. M., . . . Kunkel, S. L. (2011). The critical role of Notch ligand Delta-like 1 in the pathogenesis of influenza A virus (H1N1) infection. *PLoS Pathog, 7*(11), e1002341. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/22072963. doi:10.1371/journal.ppat.1002341

Jin, Y., Sharma, A., Carey, C., Hopkins, D., Wang, X., Robertson, D. G., . . . She, J. X. (2013). The expression of inflammatory genes is upregulated in peripheral blood of patients with type 1 diabetes. *Diabetes Care, 36*(9), 2794-2802. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/23637351. doi:10.2337/dc12-1986

Kanehisa, M., & Goto, S. (2000). KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res, 28*(1), 27-30. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10592173. doi:10.1093/nar/28.1.27

Keshava Prasad, T., Goel, R., Kandasamy, K., Keerthikumar, S., Kumar, S., Mathivanan, S., . . . Venugopal, A. (2008). Human protein reference database—2009 update. *Nucleic acids research, 37*(suppl_1), D767-D772.

Kim, Y., Hollenbaugh, J. A., Kim, D.-H., & Kim, B. (2011). Novel PI3K/Akt inhibitors screened by the cytoprotective function of human immunodeficiency virus type 1 Tat. *PloS one, 6*(7), e21781.

Knip, M., & Simell, O. (2012). Environmental triggers of type 1 diabetes. *Cold Spring Harbor perspectives in medicine, 2*(7), a007690.

Kostic, A. D., Gevers, D., Siljander, H., Vatanen, T., Hyötyläinen, T., Hämäläinen, A.-M., . . . Mattila, I. (2015). The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. *Cell host & microbe, 17*(2), 260-273.

Kracht, M. J., van Lummel, M., Nikolic, T., Joosten, A. M., Laban, S., van der Slik, A. R., . . . Hoeben, R. C. (2017). Autoimmunity against a defective ribosomal insulin gene product in type 1 diabetes. *Nature medicine, 23*(4), 501.

Lambert, A. P., Gillespie, K. M., Thomson, G., Cordell, H. J., Todd, J. A., Gale, E. A., & Bingley, P. J. (2004). Absolute risk of childhood-onset type 1 diabetes defined by human leukocyte antigen class II genotype: a population-based study in the United Kingdom. *J Clin Endocrinol Metab, 89*(8), 4037-4043. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/15292346. doi:10.1210/jc.2003-032084

Langfelder, P., & Horvath, S. (2008). WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics, 9*, 559. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/19114008. doi:10.1186/1471-2105-9-559

Li, N., Zhang, Z., Zhang, W., & Wei, Q. (2011). Calcineurin B subunit interacts with proteasome subunit alpha type 7 and represses hypoxia-inducible factor-1α activity via the proteasome pathway. *Biochemical and biophysical research communications, 405*(3), 468-472.

Liu, Y., Liang, Y., & Wishart, D. (2015). PolySearch2: a significantly improved text-mining system for discovering associations between human diseases, genes, drugs, metabolites, toxins and more. *Nucleic Acids Res, 43*(W1), W535-542. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25925572. doi:10.1093/nar/gkv383

Lundberg, M., Krogvold, L., Kuric, E., Dahl-Jorgensen, K., & Skog, O. (2016). Expression of Interferon-Stimulated Genes in Insulitic Pancreatic Islets of Patients Recently Diagnosed With Type 1 Diabetes. *Diabetes, 65*(10), 3104-3110. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27422384. doi:10.2337/db16-0616

Maahs, D. M., West, N. A., Lawrence, J. M., & Mayer-Davis, E. J. (2010). Epidemiology of type 1 diabetes. *Endocrinology and Metabolism Clinics, 39*(3), 481-497.

MacArthur, J., Bowler, E., Cerezo, M., Gil, L., Hall, P., Hastings, E., . . . Parkinson, H. (2017). The new NHGRI-EBI Catalog of published genome-wide association studies (GWAS Catalog). *Nucleic Acids Res, 45*(D1), D896-D901. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27899670. doi:10.1093/nar/gkw1133

Makinen, V. P., Civelek, M., Meng, Q., Zhang, B., Zhu, J., Levian, C., . . . Assimes, T. L. (2014). Integrative genomics reveals novel molecular pathways and gene networks for

coronary artery disease. *PLoS Genet, 10*(7), e1004502. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25033284. doi:10.1371/journal.pgen.1004502

Michel, F., Grimaud, L., Tuosto, L., & Acuto, O. (1998). Fyn and ZAP-70 are required for Vav phosphorylation in T cells stimulated by antigen-presenting cells. *J Biol Chem, 273*(48), 31932-31938. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9822663. doi:10.1074/jbc.273.48.31932

Motaleb, F. I. A., Nabih, E. S., Mohamed, S. M., & Elhalim, N. S. A. (2017). Up-regulation of circulating miRNA146a correlates with viral load via IRAK1 and TRAF6 in hepatitis C virus-infected patients. *Virus research, 238*, 24-28.

Nejentsev, S., Howson, J. M., Walker, N. M., Szeszko, J., Field, S. F., Stevens, H. E., . . . Wellcome Trust Case Control, C. (2007). Localization of type 1 diabetes susceptibility to the MHC class I genes HLA-B and HLA-A. *Nature, 450*(7171), 887-892. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/18004301. doi:10.1038/nature06406

Noble, J. A., & Erlich, H. A. (2012). Genetics of type 1 diabetes. *Cold Spring Harb Perspect Med, 2*(1), a007732. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/22315720. doi:10.1101/cshperspect.a007732

Noble, J. A., Valdes, A. M., Cook, M., Klitz, W., Thomson, G., & Erlich, H. A. (1996). The role of HLA class II genes in insulin-dependent diabetes mellitus: molecular analysis of 180 Caucasian, multiplex families. *Am J Hum Genet, 59*(5), 1134-1148. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8900244.

Noble, J. A., White, A. M., Lazzeroni, L. C., Valdes, A. M., Mirel, D. B., Reynolds, R., . . . Erlich, H. A. (2003). A polymorphism in the TCF7 gene, C883A, is associated with type 1 diabetes. *Diabetes, 52*(6), 1579-1582. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12765974. doi:10.2337/diabetes.52.6.1579

Norris, J. M., Barriga, K., Klingensmith, G., Hoffman, M., Eisenbarth, G. S., Erlich, H. A., & Rewers, M. (2003). Timing of initial cereal exposure in infancy and risk of islet autoimmunity. *Jama, 290*(13), 1713-1720.

Offenhauser, C., Lei, N., Roy, S., Collins, B. M., Stow, J. L., & Murray, R. Z. (2011). Syntaxin 11 binds Vti1b and regulates late endosome to lysosome fusion in macrophages. *Traffic, 12*(6), 762-773. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/21388490. doi:10.1111/j.1600-0854.2011.01189.x

Oilinki, T., Otonkoski, T., Ilonen, J., Knip, M., & Miettinen, P. (2012). Prevalence and characteristics of diabetes among Somali children and adolescents living in Helsinki, Finland. *Pediatric diabetes, 13*(2), 176-180.

Orrenius, S., Zhivotovsky, B., & Nicotera, P. (2003). Calcium: Regulation of cell death: the calcium–apoptosis link. *Nature reviews Molecular cell biology, 4*(7), 552.

Pinero, J., Bravo, A., Queralt-Rosinach, N., Gutierrez-Sacristan, A., Deu-Pons, J., Centeno, E., . . . Furlong, L. I. (2017). DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. *Nucleic Acids Res, 45*(D1), D833-D839. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27924018. doi:10.1093/nar/gkw943

Pu, J., Wu, S., Xie, H., Li, Y., Yang, Z., Wu, X., & Huang, X. (2017). miR-146a Inhibits dengue-virus-induced autophagy by targeting TRAF6. *Archives of virology, 162*(12), 3645-3659.

Rotival, M., Zeller, T., Wild, P. S., Maouche, S., Szymczak, S., Schillert, A., . . . Blankenberg, S. (2011). Integrating genome-wide genetic variations and monocyte expression data reveals

trans-regulated gene modules in humans. *PLoS Genet, 7*(12), e1002367. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/22144904. doi:10.1371/journal.pgen.1002367

Shu, L., Chan, K. H. K., Zhang, G., Huan, T., Kurt, Z., Zhao, Y., . . . Yang, X. (2017). Shared genetic regulatory networks for cardiovascular disease and type 2 diabetes in multiple populations of diverse ethnicities in the United States. *PLoS Genet, 13*(9), e1007040. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/28957322. doi:10.1371/journal.pgen.1007040

Shu, L., Zhao, Y., Kurt, Z., Byars, S. G., Tukiainen, T., Kettunen, J., . . . Yang, X. (2016). Mergeomics: multidimensional data integration to identify pathogenic perturbations to biological systems. *BMC Genomics, 17*(1), 874. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27814671. doi:10.1186/s12864-016-3198-9

Sjakste, T., Paramonova, N., Osina, K., Dokane, K., Sokolovska, J., & Sjakste, N. (2016). Genetic variations in the PSMA3, PSMA6 and PSMC6 genes are associated with type 1 diabetes in Latvians and with expression level of number of UPS-related and T1DM-susceptible genes in HapMap individuals. *Mol Genet Genomics, 291*(2), 891-903. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/26661414. doi:10.1007/s00438-015-1153-0

Söderström, U., Åman, J., & Hjern, A. (2012). Being born in Sweden increases the risk for type 1 diabetes–a study of migration of children to Sweden as a natural experiment. *Acta paediatrica, 101*(1), 73-77.

Sørensen, I., Joner, G., Jenum, P., Eskild, A., & Stene, L. (2012). Serum long chain n-3 fatty acids (EPA and DHA) in the pregnant mother are independent of risk of type 1 diabetes in the offspring. *Diabetes/metabolism research and reviews, 28*(5), 431-438.

Stanley, W. J., Trivedi, P. M., Sutherland, A. P., Thomas, H. E., & Gurzov, E. N. (2017). Differential regulation of pro-inflammatory cytokine signalling by protein tyrosine phosphatases in pancreatic beta-cells. *J Mol Endocrinol, 59*(4), 325-337. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/28827413. doi:10.1530/JME-17-0089

Steck, A. K., Barriga, K. J., Emery, L. M., Fiallo-Scharer, R. V., Gottlieb, P. A., & Rewers, M. J. (2005). Secondary attack rate of type 1 diabetes in Colorado families. *Diabetes Care, 28*(2), 296-300. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/15677782. doi:10.2337/diacare.28.2.296

Tan, P., He, L., Cui, J., Qian, C., Cao, X., Lin, M., . . . Yu, X. (2017). Assembly of the WHIP-TRIM14-PPP6C mitochondrial complex promotes RIG-I-mediated antiviral signaling. *Molecular cell, 68*(2), 293-307. e295.

Tedder, T., Haas, K., & Poe, J. (2002). CD19-CD21 complex regulates an intrinsic Src family kinase amplification loop that links innate immunity with B-lymphocyte intracellular calcium responses. In: Portland Press Limited.

Thomaidou, S., Zaldumbide, A., & Roep, B. O. (2018). Islet stress, degradation and autoimmunity. *Diabetes, Obesity and Metabolism, 20*, 88-94.

Tokgoz, H., Caliskan, U., Keles, S., Reisli, İ., Guiu, I. S., & Morgan, N. V. (2013). Variable presentation of primary immune deficiency: Two cases with CD 3 gamma deficiency presenting with only autoimmunity. *Pediatric Allergy and Immunology, 24*(3), 257-262.

Toyofuku, T., Yabuki, M., Kamei, J., Kamei, M., Makino, N., Kumanogoh, A., & Hori, M. (2007). Semaphorin-4A, an activator for T-cell-mediated immunity, suppresses angiogenesis via Plexin-D1. *EMBO J, 26*(5), 1373-1384. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/17318185. doi:10.1038/sj.emboj.7601589

Trivedi, P. M., Graham, K. L., Scott, N. A., Jenkins, M. R., Majaw, S., Sutherland, R. M., . . . Thomas, H. E. (2017). Repurposed JAK1/JAK2 Inhibitor Reverses Established Autoimmune Insulitis in NOD Mice. *Diabetes, 66*(6), 1650-1660. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/28292965. doi:10.2337/db16-1250

Vonberg, A. D., Acevedo-Calado, M., Cox, A. R., Pietropaolo, S. L., Gianani, R., Lundy, S. K., & Pietropaolo, M. (2018). CD19+ IgM+ cells demonstrate enhanced therapeutic efficacy in type 1 diabetes mellitus. *JCI insight, 3*(23).

Weets, I., Kaufman, L., Van der Auwera, B., Crenier, L., Rooman, R., De Block, C., . . . Laron, Z. (2004). Seasonality in clinical onset of type 1 diabetes in Belgian patients above the age of 10 is restricted to HLA-DQ2/DQ8-negative males, which explains the male to female excess in incidence. *Diabetologia, 47*(4), 614-621.

Willard-Gallo, K. E., Furtado, M., Burny, A., & Wolinsky, S. M. (2001). Down-modulation of TCR/CD3 surface complexes after HIV-1 infection is associated with differential expression of the viral regulatory genes. *Eur J Immunol, 31*(4), 969-979. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11298321. doi:10.1002/1521-4141(200104)31:4<969::AID-IMMU969gt;3.0.CO;2-2

Yabe, Y., Matsumoto, T., Tsurumoto, T., & Shindo, H. (2005). Immunohistological localization of Notch receptors and their ligands Delta and Jagged in synovial tissues of rheumatoid arthritis. *J Orthop Sci, 10*(6), 589-594. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/16307184. doi:10.1007/s00776-005-0943-3

Yeung, W. C., Rawlinson, W. D., & Craig, M. E. (2011). Enterovirus infection and type 1 diabetes mellitus: systematic review and meta-analysis of observational molecular studies. *BMJ, 342*, d35. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/21292721. doi:10.1136/bmj.d35

Ylosmaki, L., Schmotz, C., Ylosmaki, E., & Saksela, K. (2015). Reorganization of the host cell Crk(L)-PI3 kinase signaling complex by the influenza A virus NS1 protein. *Virology, 484*, 146-152. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/26099693. doi:10.1016/j.virol.2015.06.009

Yoboua, F., Martel, A., Duval, A., Mukawera, E., & Grandvaux, N. (2010). Respiratory syncytial virus-mediated NF-kappa B p65 phosphorylation at serine 536 is dependent on RIG-I, TRAF6, and IKK beta. *J Virol, 84*(14), 7267-7277. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/20410276. doi:10.1128/JVI.00142-10

Yu, H., Khalid, M., Heigele, A., Schmökel, J., Usmani, S. M., van der Merwe, J., . . . Kirchhoff, F. (2015). Lentiviral Nef proteins manipulate T cells in a subset-specific manner. *Journal of virology, 89*(4), 1986-2001.

Yu, P., Constien, R., Dear, N., Katan, M., Hanke, P., Bunney, T. D., . . . Schröder, A. (2005). Autoimmunity and inflammation due to a gain-of-function mutation in phospholipase Cγ2 that specifically increases external Ca2+ entry. *Immunity, 22*(4), 451-465.

Zhang, Q., Wang, C., Liu, Z., Liu, X., Han, C., Cao, X., & Li, N. (2012). Notch signal suppresses Toll-like receptor-triggered inflammatory responses in macrophages by inhibiting extracellular signal-regulated kinase 1/2-mediated nuclear factor kappaB activation. *J Biol Chem, 287*(9), 6208-6217. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/22205705. doi:10.1074/jbc.M111.310375

Zhong, H., Yang, X., Kaplan, L. M., Molony, C., & Schadt, E. E. (2010). Integrating pathway analysis and genetics of gene expression for genome-wide association studies. *Am J Hum*

Genet, 86(4), 581-591. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/20346437. doi:10.1016/j.ajhg.2010.02.020

zur Stadt, U., Schmidt, S., Kasper, B., Beutel, K., Diler, A. S., Henter, J. I., . . . Hennies, H. C. (2005). Linkage of familial hemophagocytic lymphohistiocytosis (FHL) type-4 to chromosome 6q24 and identification of mutations in syntaxin 11. *Hum Mol Genet, 14*(6), 827-834. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/15703195. doi:10.1093/hmg/ddi076