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Proximity Proteomics Revealed Aberrant mRNA Splicing Elicited by ALS-Linked Profilin-1 Mutants

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Abstract

Profilin 1 (PFN1) is a cytoskeleton protein that modulates actin dynamics through binding to monomeric actin and polyproline-containing proteins. Mutations in PFN1 have been linked to the pathogenesis of familial amyotrophic lateral sclerosis (ALS). Here, we employed an unbiased proximity labeling strategy in combination with proteomic analysis for proteome-wide profiling of proteins that differentially interact with mutant and wild-type (WT) PFN1 proteins in human cells. We uncovered 11 mRNA splicing proteins that are preferentially enriched in the proximity proteomes of the two ALS-linked PFN1 variants, C71G and M114T, over that of wild-type PFN1. We validated the preferential interactions of the ALS-linked PFN1 variants with two mRNA splicing factors, hnRNPC and U2AF2, by immunoprecipitation, followed with immunoblotting. We also found that the two ALS-linked PFN1 variants promoted the exonization of *Alu* elements in the mRNAs of *MTO1*, *TCFL5*, *WRN* and *POLE* genes in human cells. Together, we showed that the two ALS-linked PFN1 variants interacted preferentially with mRNA splicing proteins, which elicited aberrant exonization of the *Alu* elements in mRNAs. Thus, our work provided pivotal insights into the perturbations of ALS-linked PFN1 variants in RNA biology and their potential contributions to ALS pathology.

Graphical Abstract

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ASSOCIATED CONTENT

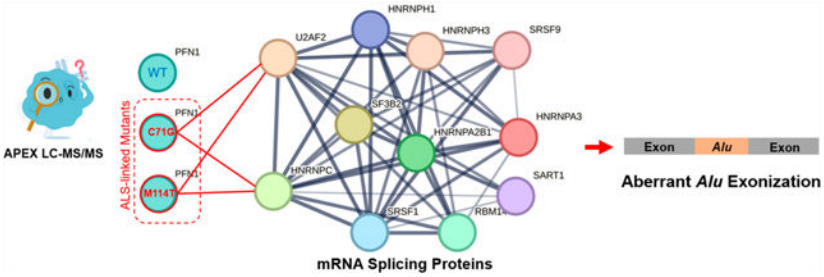
Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.3c03734.

Detailed experimental procedures, list of PCR primers, optimized conditions for the IP-IB assay, and lists of proteins preferentially interact with ALS-linked PFN1 variants (C71G and/or M114T) identified by APEX-MS (PDF)

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.analchem.3c03734

The authors declare no competing financial interest.



Proteins often function in cellular processes through interactions with other proteins.[1] Immunoprecipitationmass spectrometry (IP-MS) is a powerful approach for elucidating the interaction partners of a protein of interest. However, immunoprecipitation of protein complexes might only capture stable interaction partners and miss transient and/or weak binding partners, resulting in false negatives.[1–4]

Proximity labeling is an enzymatic biotinylation approach that can capture spatial and temporal interactions of a protein of interest in its native cellular environment.[2–4] In one of these approaches, a second-generation engineered ascorbate peroxidase (APEX) can be fused with a bait protein and introduced into cells through ectopic expression.[2–4] Because endogenous proximal proteins are covalently tagged with biotin after a short (1 min) reaction and the biotinylated proteins are enriched with streptavidin-coated beads, harsh washing conditions can be employed to minimize nonspecific binding.[4] The purified proteins are then digested into peptides and analyzed by LC-MS/MS.[4] The approach allows for capturing weak and transient interactions.[4]

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease primarily affecting motor neurons.[5] About 10% of ALS cases are familial and arise from known genetic defects.[5] The approximately 25 known ALS-linked proteins cluster into three biological processes: cytoskeletal dynamics, proteostasis, and RNA processing.[5,6] These functional categorizations are not mutually exclusive because some ALS-linked proteins can affect more than one process.[7–15] For instance, mutations in the *PFN1* gene, which encodes the cytoskeleton protein profilin 1 (PFN1), were recently reported to be implicated in familial ALS by disrupting cytoskeletal dynamics.[7–11] In addition, ALS-linked PFN1 variants led to destabilization of tertiary structures of the protein, thereby inducing protein aggregation and impairing proteostasis.[7,12–15] Little, however, is known about whether ALS-linked PFN1 variants can perturb RNA processing.

Recently, perturbation of mRNA splicing has been identified as an important mechanism for ALS pathogenesis.[16–18] While interacting proteins of ALS-linked PFN1 variants have been studied recently to uncover the link between PFN1 and ALS through an IP-MS approach,[12] it remains elusive whether ALS-linked PFN1 variants can interact preferentially with proteins involved in RNA processing.[7–15] Understanding these interactions is crucial since such interactions may perturb downstream cellular processes, e.g., mRNA splicing, transport, localization and translation.[19,20]

In this study, we employed an unbiased APEX-based proximity labeling strategy coupled with mass spectrometry (APEX-MS) for proteome-wide profiling of proteins that interact differentially with two ALS-linked PFN1 variants (i.e., C71G and M114T) and the wild-type (WT) counterpart. Our results revealed 55 and 14 mRNA splicing proteins exhibiting preferential binding toward the ALS-linked PFN1 variants of C71G and M114T, respectively, over the corresponding WT protein, where 11 proteins were commonly enriched for both variants. In addition, we employed IP followed by immuno-blotting (IP-IB) to validate the preferential interactions of the ALS-linked PFN1 variants with two mRNA splicing proteins, hnRNPC and U2AF2. Importantly, we found that the ALS-linked PFN1 variants promoted exonization of *Alu* elements in the mRNAs of multiple human genes. Our results laid a strong foundation for understanding the biological consequences of ALS-linked PFN1 variants in RNA processing and its potential implications in ALS pathogenesis.

Previous attempts to identify proteins that differently interact with ALS-linked PFN1 variants and WT PFN1 using IP-MS have led to the discovery of several proteins exhibiting augmented interactions with ALS-linked PFN1 variants of M114T and G118V.[15] All of these proteins are implicated in the biological process of cytoskeletal regulation.[15] We hypothesized that there are other important proteins that interact preferentially with the ALS-linked PFN1 variants, which may contribute to ALS pathogenesis by perturbing other biological processes, especially RNA processes.

To test the above hypothesis, we employed the APEX-based MS approach for a systematic and unbiased screening of cellular proteins that interact preferentially with ALS-linked PFN1 variants over the WT protein. To this end, we constructed three plasmids by fusing the APEX2 sequence to the C termini of WT PFN1 and the two ALS-linked variants, i.e., C71G and M114T. A short V5 epitope tag was inserted between PFN1 and APEX2 for monitoring the relative expression levels of the PFN1 variants. The PFN1-V5-APEX2 fusions were introduced into HEK293T cells through ectopic expression or lentiviral transduction. We first conducted a pilot experiment with cells in a 6-well plate to test the efficiency of proximity labeling by biotinylation followed with immunoblotting. We verified the expression of PFN1-V5-APEX2 fusion proteins and assessed their biotinylation efficiencies using Western blot analysis (Figure 1), where we observed that the expression levels of the ectopically expressed PFN1-V5-APEX2 variants were similar or lower than that of endogenous PFN1 (Figure S1).

We then scaled up the experiment by culturing the cells in T75 flasks to obtain sufficient cells for preparing proteomic samples (Figures 1 and S1). As described previously,[21] after biotin-phenol incubation and H_2O_2 induction, cells were washed with a quenching buffer 5 times. After centrifugation and cell lysis in a 7 M urea buffer, streptavidin-conjugated agarose beads were added to incubate with the bait-prey protein mixture. Subsequently, the beads were washed with the 7 M urea buffer vigorously to remove nonbiotinylated proteins. Bound proteins were eluted from the beads by boiling in sodium dodecyl sulfate (SDS) sample loading buffer and briefly separated on a 15% SDS-PAGE gel to remove streptavidin. After cysteine reduction and alkylation, proteins were digested in-gel with trypsin, and the resulting peptides were subjected to LC-MS/MS analysis (Figure 1).

By imposing an average fold change cutoff of 1.5 ($p < 0.05$, two-tailed, unpaired Student's *t*-test), our results from LC-MS/MS analysis (three biological replicates) revealed 1118 and 238 proteins displaying preferential enrichment in the proximity proteomes of the two ALS-linked PFN1 variants, C71G and M114T, respectively, relative to WT PFN1 (Tables S2 and S3). Among these proteins, 213 exhibited preferential interactions with both C71G and M114T (Figure 2 a and b and Table S4). Gene ontology (GO) analysis showed that many of these proteins are involved in protein stabilization and protein folding (Figure 2c and Table S4), which is consistent with the known mechanisms that ALS-linked PFN1 variants can cause familial ALS through inducing protein destabilization and aggregation.[7,12,15,22] In this vein, the C71G variant displays more severe destabilization in protein structure than the M114T variant,[12,15] which, together with our APEX-MS proteomic data, suggests that elevated aggregation of the former PFN1 variant results in augmented enrichment of cellular protein in its proximity proteome. Remarkably, all of the top ten biological pathways identified here (Figure 2c) have been linked with the pathogenesis of neurodegenerative diseases.[7,16,23,24] Specifically, disruption of heterochromatin structure led to neuron loss as well as motor and memory impairments that are commonly observed in ALS patients.[23] Glycolysis dysfunction in the central nervous system can contribute to ALS through reducing mitochondrial energy generation and increasing oxidative stress.[24] Interestingly, six proteins from the proximity proteomes of the ALS-linked PFN1 variants were involved in substantia nigra development that is commonly undermined in Parkinson's disease.[25] More importantly, growing lines of evidence supported perturbation in mRNA splicing as crucial to ALS pathogenesis.[16–18] Notably, 55 and 14 proteins, which are preferentially enriched for the C71G and M114T variants over wild-type PFN1, respectively, are involved in mRNA splicing via spliceosome (Figures S2 and S3). Among them, 11 mRNA splicing proteins are enriched for both ALS-linked PFN1 variants, including heterogeneous nuclear ribonucleoproteins C1/C2 (hnRNPC) and splicing factor U2AF 65 kDa (U2AF2) (Figure 2c, d).

hnRNPC belongs to the heterogeneous nuclear ribonucleoprotein (hnRNP) family of proteins and is involved in multiple aspects of RNA metabolism, including pre-mRNA splicing, mRNA stability, and transport.[26–30] In particular, hnRNPC is known to protect the human transcriptome from the exonization of *Alu* elements.[26] In this vein, recent studies revealed that dysfunctions in hnRNP protein play a fundamental role in driving the pathogenesis of ALS and related diseases such as frontotemporal dementia (FTD).[31]

U2AF2 is a component of the spliceosome, a complex responsible for pre-mRNA splicing, and it plays a crucial role in splice site recognition, contributing to the accuracy and specificity of splicing events.[26] Binding of U2AF2 upstream of the 3′ splice site is crucial for recruiting the small nuclear ribonucleoprotein (snRNP) U2, an integral component of the spliceosome, thus comprising a major regulatory event in 3′ splice-site recognition.[26] Interestingly, SF3B2, which is a component of snRNP U2, is also among the 11 mRNA splicing proteins enriched for ALS-linked PFN1 variants (Figure 2d).

Representative MS/MS of tryptic peptides derived from hnRNPC and U2AF2 confirmed the identification of these two proteins (Figures S4–S5). The average fold changes of a tryptic peptide from hnRNPC between the ALS-linked PFN1 variants (i.e., C71G and M114T)

and WT PFN1 were 2.83 and 1.85, respectively (Figure S4). The average fold changes of a tryptic peptide from U2AF2 between the ALS-linked PFN1 variants (i.e., C71G and M114T) and WT PFN1 were 4.08 and 2.22, respectively (Figure S5).

To further substantiate the preferential interactions of the two ALS-linked PFN1 variants with the two mRNA splicing proteins, we employed an IP-IB assay. We recognized that these preferential interactions, which were not uncovered previously,[15] may not be very strong and thus required optimized washing conditions to preserve the binding. Meanwhile, we also included a blocking step using a mixture of BSA, glycogen, and ssDNA to reduce nonspecific binding to the FLAG beads. Moreover, we included a step of benzonase treatment to remove nucleic acids from the protein-containing lysates, eliminating the possibility that these interactions might be mediated by nucleic acids.

Our results from three biological replicates showed that both hnRNPC and U2AF2 were enriched for the ALS-linked PFN1 variants over the WT PFN1 in HEK293T cells; the average enrichment fold changes for the C71G and M114T variants were 12.8 and 7.5, respectively, for hnRNPC and 31.3 and 28.8, respectively, for U2AF1, which are consistent with our APEX-MS results (Figures 3 and S6). We also conducted the IP-IB experiments using mouse N2a cells, which were derived from mouse neural crests and have been widely used for studying neurodegeneration.[7] It turned out that we again observed pronounced enrichments of hnRNPC and U2AF2 in the pull-down mixtures of the C71G and M114T variants over WT PFN1 (Figures 3 and S6).

Deficiency in hnRNPC can lead to aberrant splicing through promoting exonization of *Alu* elements in human cells.[26] By employing reverse transcription-polymerase chain reaction (RT-PCR) coupled with agarose gel electrophoresis, we found that the ALS-linked PFN1 variants significantly promoted the exonization of *Alu* elements in *MTO1*, *TCFL5*, *WRN* and *POLE* genes in human cells, presumably through sequestering hnRNPC (Figure 4). Interestingly, all four genes encode nucleic-acid binding proteins and are associated with human genetic disorders or diseases.[32–35] For example, aberrant splicing of *MTO1* affects mitochondrial tRNA modification, resulting in mitochondrial dysfunction, which is commonly observed in ALS etiology.[36] Abnormal splicing of *TCFL5*, *WRN* and *POLE* transcripts may impact the binding affinities of their encoded proteins toward nucleic acids, which can lead to genome or transcriptome instability.[33–35]

Emerging evidence has shown that dysfunctional mRNA splicing is at the core of ALS pathogenesis.[16–18] ALS-linked PFN1 variants were implicated in ALS through perturbing cytoskeletal dynamics and protein aggregation.[7,15,37–39] However, the biological relevance of ALS-linked PFN1 variants in RNA biology remains largely unexplored.[7–15] Our study reveals for the first time a plausible mechanism through which ALS-linked PFN1 variants contribute to ALS pathogenesis through sequestering mRNA splicing proteins, thereby leading to dysregulation in mRNA splicing.

Taken together, we utilized APEX-MS to comprehensively investigate the proteome-wide interactions of two ALS-linked PFN1 variants (C71G and M114T) compared with the WT protein. Our findings revealed 11 mRNA spicing proteins displaying preferential interactions

with both C71G and M114T over WT PFN1, including hnRNPC, which is known to protect the human transcriptome from *Alu* element exonization. We confirmed, by employing IP-IB, enhanced binding between the ALS-linked PFN1 variants and these two mRNA splicing proteins. We also found that the ALS-linked variants perturbed the splicing patterns of the *MTO1*, *TCFL5*, *WRN* and *POLE* genes, likely through sequestering hnRNPC, in human cells. Thus, our findings revealed, for the first time, the functions of the ALS-linked PFN1 variants in perturbing mRNA splicing, which provides new insights into the pathogenic mechanisms of the ALS-linked PFN1 mutants.

Although hnRNPC is an important member of the hnRNP family and is deeply involved in RNA regulation, little is known about the relationship between hnRNPC dysfunction and ALS pathogenesis.[31,40] Additionally, hnRNPC protects the human transcriptome from the exonization of *Alu* elements.[26] Our study suggests that ALS-linked mutants of PFN1 perturb hnRNPC-mediated protection of *Alu* element exonization through sequestration of hnRNPC. It will be interesting to explore, in the future, whether ALS-linked PFN1 variants cause ALS by sequestering hnRNPC and thus disrupting N^6-methyladenosine (m^6A)-mediated RNA regulation.[40] It will also be important to validate the aberrant interactions of other proteins with ALS-associated variants of PFN1, and to examine how such interactions contribute to ALS pathology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

(1). Phizicky EM; Fields S Microbiol. Rev 1995, 59, 94–123. [PubMed: 7708014]

(2). Hung V; Zou P; Rhee H-W; Udeshi ND; Cracan V; Svinkina T; Carr SA; Mootha VK; Ting AY Mol. Cell 2014, 55, 332–341. [PubMed: 25002142]

(3). Rhee HW; Zou P; Udeshi ND; Martell JD; Mootha VK; Carr SA; Ting AY Science 2013, 339, 1328–1331. [PubMed: 23371551]

(4). Hung V; Udeshi ND; Lam SS; Loh KH; Cox KJ; Pedram K; Carr SA; Ting AY Nat. Protoc 2016, 11, 456–475. [PubMed: 26866790]

(5). Brown RH; Al-Chalabi AN Engl. J. Med 2017, 377, 162–172.

(6). Bartoletti M; Bosco DA; Da Cruz S; Lagier-Tourenne C; Liachko N; Markmiller S; Webster KM; Wharton KA J. Neurosci 2019, 39, 8217–8224. [PubMed: 31619490]

(7). Wu C-H; Fallini C; Ticozzi N; Keagle PJ; Sapp PC; Piotrowska K; Lowe P; Koppers M; McKenna-Yasek D; Baron DM; Kost JE; Gonzalez-Perez P; Fox AD; Adams J; Taroni F; Tiloca C; Leclerc AL; Chafe SC; Mangroo D; Moore MJ; Zitzewitz JA; Xu Z-S; van den Berg LH; Glass JD; Siciliano G; Cirulli ET; Goldstein DB; Salachas F; Meininger V; Rossoll W; Ratti A; Gellera C; Bosco DA; Bassell GJ; Silani V; Drory VE; Brown RH; Landers JE Nature 2012, 488, 499–503. [PubMed: 22801503]

(8). Smith BN; Vance C; Scotter EL; Troakes C; Wong CH; Topp S; Maekawa S; King A; Mitchell JC; Lund K; Al-Chalabi A; Ticozzi N; Silani V; Sapp P; Brown RH; Landers JE; Al-Sarraj S; Shaw CE Neurobiol. Aging 2015, 36, 1602.e17–1602.e27.

(9). Chen Y; Zheng Z; Huang R; Chen K; Song W; Zhao B; Chen X; Yang Y; Yuan L; Shang H Neurobiol. Aging 2013, 34, 1922.e1–1922.e5.

(10). Burk K; Pasterkamp RJ Acta Neuropathol. 2019, 137, 859–877. [PubMed: 30721407]

(11). Morfini GA; Burns M; Binder LI; Kanaan NM; LaPointe N; Bosco DA; Brown RH; Brown H; Tiwari A; Hayward L; Edgar J; Nave K-A; Garberrn J; Atagi Y; Song Y; Pigino G; Brady ST J. Neurosci 2009, 29, 12776–12786. [PubMed: 19828789]

(12). Boopathy S; Silvas TV; Tischbein M; Jansen S; Shandilya SM; Zitzewitz JA; Landers JE; Goode BL; Schiffer CA; Bosco DA Proc. National Acad. Sci 2015, 112, 7984–7989.

(13). Nekouei M; Ghezellou P; Aliahmadi A; Arjmand S; Kiaei M; Ghassempour A Metab. Brain Dis. 2018, 33, 1975–1984. [PubMed: 30203378]

(14). Figley MD; Bieri G; Kolaitis R-M; Taylor JP; Gitler AD J. Neurosci 2014, 34, 8083–8097. [PubMed: 24920614]

(15). Schmidt EJ; Funes S; McKeon JE; Morgan BR; Boopathy S; O'Connor LC; Bilsel O; Massi F; Jégou A; Bosco DA Proc. Natl. Acad. Sci. U. S. A 2021, 118, e2024605118.

(16). Brown A-L; Wilkins OG; Keuss MJ; Hill SE; Zanovello M; Lee WC; Bampton A; Lee FCY; Masino L; Qi YA; Bryce-Smith S; Gatt A; Hallegger M; Fagegaltier D; Phatnani H; Phatnani H; Kwan J; Sareen D; Broach JR; Simmons Z; Arcila-Londono X; Lee EB; Van Deerlin VM; Shneider NA; Fraenkel E; Ostrow LW; Baas F; Zaitlen N; Berry JD; Malaspina A; Fratta P; Cox GA; Thompson LM; Finkbeiner S; Dardiotis E; Miller TM; Chandran S; Pal S; Hornstein E; MacGowan DJ; Heiman-Patterson T; Hammell MG; Patsopoulos; Nikolaos A; Butovsky O; Dubnau J; Nath A; Bowser R; Harms M; Aronica E; Poss M; Phillips-Cremins J; Crary J; Atassi N; Lange DJ; Adams DJ; Stefanis L; Gotkine M; Baloh RH; Babu S; Raj T; Paganoni S; Shalem O; Smith C; Zhang B; Harris B; Broce I; Drory V; Ravits J; McMillan C; Menon V; Wu L; Altschuler S; Lerner Y; Sattler R; Van Keuren-Jensen K; Rozenblatt-Rosen O; Lindblad-Toh K; Nicholson K; Gregersen P; Lee J-H; Kokos S; Muljo S; Newcombe J; Gustavsson EK; Seddighi S; Reyes JF; Coon SL; Ramos D; Schiavo G; Fisher EMC; Raj T; Secrier M; Lashley T; Ule J; Buratti E; Humphrey J; Ward ME; Fratta P Nature 2022, 603, 131–137. [PubMed: 35197628]

(17). Ma XR; Prudencio M; Koike Y; Vatsavayai SC; Kim G; Harbinski F; Briner A; Rodriguez CM; Guo C; Akiyama T; Schmidt HB; Cummings BB; Wyatt DW; Kurylo K; Miller G; Mekhoubad S; Sallee N; Mekonnen G; Ganser L; Rubien JD; Jansen-West K; Cook CN; Pickles S; Oskarsson B; Graff-Radford NR; Boeve BF; Knopman DS; Petersen RC; Dickson DW; Shorter J; Myong S; Green EM; Seeley WW; Petrucelli L; Gitler AD Nature 2022, 603, 124–130. [PubMed: 35197626]

(18). Ling JP; Pletnikova O; Troncoso JC; Wong PC Science 2015, 349, 650–655. [PubMed: 26250685]

(19). Boccaletto P; Stefaniak F; Ray A; Cappannini A; Mukherjee S; Purta E; Kurkowska M; Shirvanizadeh N; Destefanis E; Groza P; Avşar G; Romitelli A; Pir P; Dassi E; Conticello SG; Aguilo F; Bujnicki JM Nucleic Acids Res. 2022, 50, D231–D235. [PubMed: 34893873]

(20). Levi O; Arava YS Nucleic Acids Res. 2021, 49, 432–443. [PubMed: 33305314]

(21). Chen X; He X; Yang Y-Y; Wang Y ACS Chem. Biol 2022, 17, 3450–3457. [PubMed: 36475596]

(22). Blokhuis AM; Groen EJN; Koppers M; van den Berg LH; Pasterkamp RJ Acta Neuropathol. 2013, 125, 777–794. [PubMed: 23673820]

(23). Zhang Y-J; Guo L; Gonzales PK; Gendron TF; Wu Y; Jansen-West K; O'Raw AD; Pickles SR; Prudencio M; Carlomagno Y; Gachechiladze MA; Ludwig C; Tian R; Chew J; DeTure M; Lin W-L; Tong J; Daughrity LM; Yue M; Song Y; Andersen JW; Castanedes-Casey M; Kurti A; Datta A; Antognetti G; McCampbell A; Rademakers R; Oskarsson B; Dickson DW; Kampmann M; Ward ME; Fryer JD; Link CD; Shorter J; Petrucelli L Science 2019, 363, eaav2606.

(24). Tefera TW; Steyn FJ; Ngo ST; Borges K Cell Biosci. 2021, 11, 14. [PubMed: 33431046]

(25). Damier P; Hirsch EC; Agid Y; Graybiel AM Brain 1999, 122, 1437–1448. [PubMed: 10430830]

(26). Zarnack K; König J; Tajnik M; Martincorena I; Eustermann S; Stévant I; Reyes A; Anders S; Luscombe NM; Ule J Cell 2013, 152, 453–466. [PubMed: 23374342]

(27). Cieniková Z; Damberger FF; Hall J; Allain FH-T; Maris CJ Am. Chem. Soc 2014, 136, 14536–14544.

(28). Rajagopalan LE; Westmark CJ; Jarzembowski JA; Malter JS Nucleic Acids Res. 1998, 26, 3418–3423. [PubMed: 9649628]

(29). McCloskey A; Taniguchi I; Shinmyozu K; Ohno M Science 2012, 335, 1643–1646. [PubMed: 22461616]

(30). König J; Zarnack K; Rot G; Curk T; Kayikci M; Zupan B; Turner DJ; Luscombe NM; Ule J Nat. Struct. Mol. Biol 2010, 17, 909–915. [PubMed: 20601959]

(31). Purice MD; Taylor JP Front. Neurosci 2018, 12, 326. [PubMed: 29867335]

(32). Wang X; Yan Q; Guan M-X Mitochondrion 2009, 9, 180–185. [PubMed: 19460296]

(33). Wimmer K; Beilken A; Nustede R; Ripperger T; Lamottke B; Ure B; Steinmann D; Reineke-Plaass T; Lehmann U; Zschocke J; Valle L; Fauth C; Kratz CP Fam. Cancer 2017, 16, 67–71. [PubMed: 27573199]

(34). Siep M; Sleddens-Linkels E; Mulders S; Eenennaam H; van Wassenaar E; Cappellen WAV; Hoogerbrugge J; Grootegoed JA; Baarends WM Nucleic Acids Res. 2004, 32, 6425–6436. [PubMed: 15585666]

(35). Orren DK; Brosh RM; Nehlin JO; Machwe A; Gray MD; Bohr VA Nucleic Acids Res. 1999, 27, 3557–3566. [PubMed: 10446247]

(36). Mehta AR; Walters R; Waldron FM; Pal S; Selvaraj BT; Macleod MR; Hardingham GE; Chandran S; Gregory JM Brain Commun. 2019, 1, fcz009.

(37). Giampetruzzi A; Danielson EW; Gumina V; Jeon M; Boopathy S; Brown RH; Ratti A; Landers JE; Fallini C Nat. Commun 2019, 10, 3827. [PubMed: 31444357]

(38). Henty-Ridilla JL; Juanes MA; Goode BL Curr. Biol 2017, 27, 3535–3543. [PubMed: 29129529]

(39). Teyssou E; Chartier L; Roussel D; Perera ND; Nemazanyy I; Langui D; Albert M; Larmonier T; Saker S; Salachas F; Pradat P-F; Meininger V; Ravassard P; Côté F; Lobsiger CS; Boillée S; Turner BJ; Seilhean D; Millecamps S Int. J. Mol. Sci 2022, 23, 5694. [PubMed: 35628504]

(40). Liu N; Dai Q; Zheng G; He C; Parisien M; Pan T Nature 2015, 518, 560–564. [PubMed: 25719671]

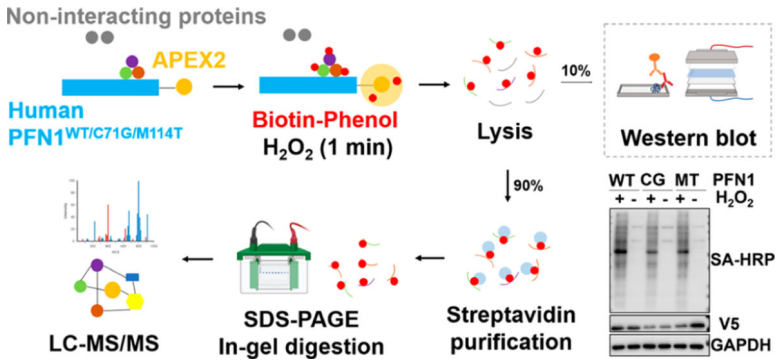

Figure 1.

Workflow of using APEX-MS for proteome-wide profiling of proteins that differentially interact with two ALS-linked PFN1 variants (C71G and M114T) and WT PFN1. In the pilot experiment, cell lysates collected from a 6-well plate were subjected to Western blot to assess the labeling efficiency (bottom right). Control groups without the addition of H_2O_2 showed minimal endogenous biotinylation. Proteomic samples were prepared with cell lysates collected from T75 flasks. Approximately 10% of the lysates were subjected to Western blot to examine the expression levels and labeling efficiencies of the PFN1-V5-APEX2 fusion proteins. The remaining 90% of lysates were used for streptavidin affinity purification, in-gel digestion, and LC-MS/MS analysis.

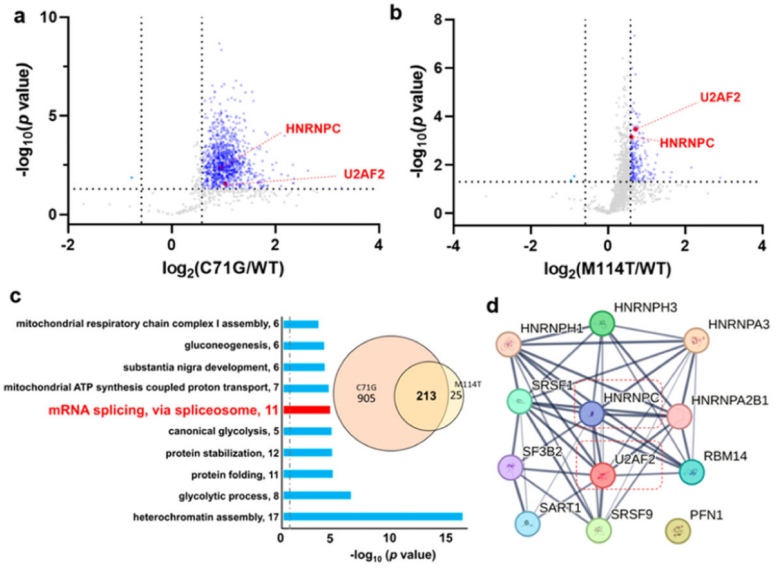

Figure 2.

APEX-MS uncovered preferential interactions of mRNA splicing proteins with the two ALS-linked PFN1 variants. (a, b) Volcano plots showing the preferential interactions of 1118 and 238 proteins with the C71G and M114T PFN1 variants, respectively, over wild-type PFN1. The *p* values were calculated by using paired, two-tailed Student's *t* test. Colored dots designate those with statistically significant fold changes ($p < 0.05$). (c) Gene ontology analysis of biological processes (top ten were shown) for the 213 proteins interacting preferentially with both ALS-linked PFN1 variants. Eleven mRNA splicing proteins are commonly enriched in the proximity proteomes of the two ALS-linked PFN1 variants. The numbers on the *y* axis denote the numbers of proteins identified for the biological processes. The vertical dotted line denotes the *p* value of 0.05. (d) Interconnected nodes based on the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database are shown for WT PFN1 and the 11 mRNA splicing proteins. The result is consistent with our findings that the ALS-linked PFN1 variants interact preferentially with these mRNA splicing proteins overWT PFN1.

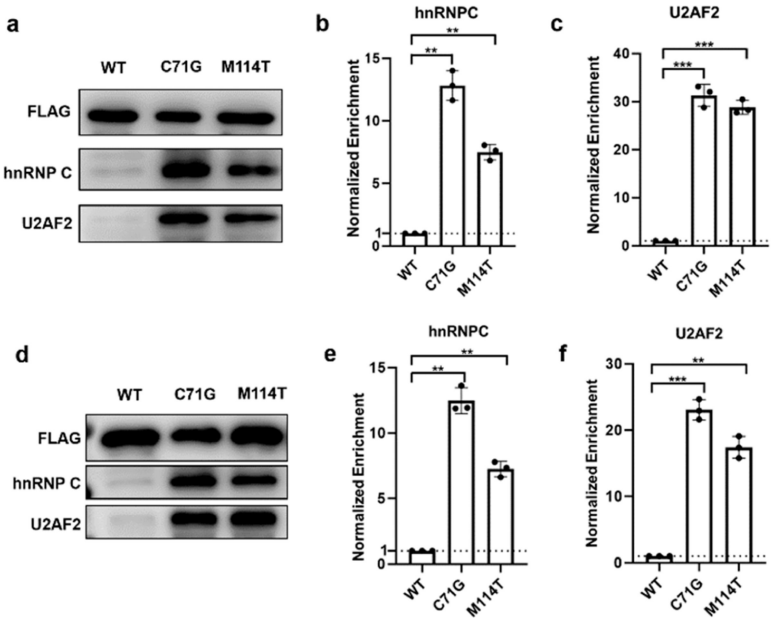

Figure 3.

IP-IB substantiated that hnRNPC and U2AF2 interacted preferentially with both ALS-linked PFN1 variants (C71G and M114T) in HEK293T cells (a–c) and N2a mouse neuroblastoma cells (d–f). The *p* values were calculated using unpaired, two-tailed Student's *t* test: **, $0.001 \leq p < 0.01$; ***, $p < 0.001$.

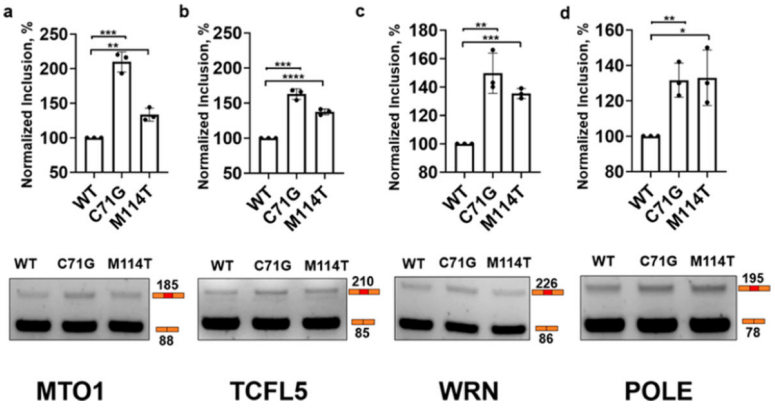

Figure 4.

ALS-linked PFN1 variants promoted exonization of *Alu* elements in mRNAs of *MTO1* (a), *TCFL5* (b), *WRN* (c) and *POLE* (d) genes in HEK293T cells. The *p* values were calculated using unpaired, two-tailed Student's *t* test: *, $0.05 < p < 0.001$; **, $0.001 \leq p < 0.01$; ***, $0.0001 \leq p < 0.001$; ****, $p < 0.0001$; $n = 3$.