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Supplementary Materials for

TMEM106B core deposition associates with TDP-43 pathology and is increased in risk SNP carriers for frontotemporal dementia

Jordan D. Marks *et al.*

Corresponding author: Mercedes Prudencio, Prudencio.mercedes@mayo.edu; Casey N. Cook, Cook.casey@mayo.edu; Leonard Petrucelli, Petrucelli.leonard@mayo.edu

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Materials and Methods

Human samples

Human postmortem brain tissue from patients with FTLD-TDP or AD was provided by the Mayo Clinic Florida Brain Bank, with neuropathological diagnosis determined by a single neuropathologist (D.W.D.). Written informed consent was obtained from all subjects (or their legal next of kin if they were unable to give written consent) prior to study entry, and all protocols were approved by the Mayo Clinic Institutional Review Board (IRB).

gDNA extraction and rs3173615 genotyping

gDNA was extracted from 10-20 mg of postmortem frontal cortex tissue using the Wizard® Genomic DNA Purification Kit (Promega) following manufacturer's instructions. SNP genotyping for the coding TMEM106B variant p.T185S (rs3173615) was performed using 30ng of isolated gDNA and the TaqMan™ SNP Genotyping Assay (ID C__27465458_10, ThermoFisher) with TaqMan™ Genotyping Master Mix (ThermoFisher) per manufacturer's instructions. Genotyping was performed on the QuantStudio™ 7 Pro Real-Time PCR System (ThermoFisher) and QuantStudio™ 7 Pro Real-Time PCR Software used for analysis. TMEM106B risk and protective haplotypes were determined depending on the presence of C or G, respectively, in the following reference sequence: GGAAAGGCACGCTTAAACAACATAA[C/G] CATTATTGGTCCACTTGATATGAAA.

Cloning and construct generation

Human cDNA encoding sequence-verified full-length TMEM106B (S185 allele) (Horizon Discovery MHS6278-202759497) was cloned into pcDNA 3.1 (−)/Myc−His C expression vector (ThermoFisher #V80020). Site-directed mutagenesis was performed to generate the T185 risk allele (QuikChange® II XL Site-Directed Mutagenesis Kit Agilent #200521) following manufacturer's instructions. In addition, TMEM106B S185 and T185 coding sequences were subcloned into the vector pAG3 using HindIII and BamHI restriction enzymes. The TMEM106BΔ191-206 plasmid was made using wild-type TMEM106B plasmid as a template for site-directed mutagenesis using the QuikChange II XL Site-Directed Mutagenesis Kit (Agilent) according to the manufacturer's directions. The sequence of mutagenesis primers was 5' TAAACAACATAACCATTATTGGTCCACTTATGAGTTATATGTATGATTTCTGTAC-3' (sense) and 5' GTACAGAAATCATACATATAACTCATAAGTGGACCAATAATGGTTATGTTGTTTA-3' (antisense). All constructs were sequence-verified using ABI3730 with Big Dye chemistry (Applied Biosystems, Foster City, CA, USA) prior to use.

Antibodies

The TMEM106B core antibody was generated with Labcorp by immunizing rabbits with a peptide corresponding to residues 191-206 of human TMEM106B (used at 1:1000 for Western blot, 1:10,000 for IHC, and 1:2500 for IF). In addition, we purchased N-terminal TMEM106B antibodies from Cell Signaling Technology (#93334; 1:1000 for Western Blot), Invitrogen (PA5-63558; 1:1000 for Western blot), Atlas (HPA058342; attempted as low as 1:200 for IHC), ProteinTech (60333-1-Ig; 1:500 for IHC), and Millipore Sigma (MABN473; attempted as low as 1:50 for IHC), as well as C-terminal TMEM106B antibodies from ProteinTech (20995-1-AP; attempted as low as 1:200 for IHC) and Millipore Sigma (SAB2106773; positive staining at 1:200 for IHC). Other antibodies purchased were anti-GAPDH from Meridian Life Science (H86504M; 1:10,000 for Western blot), anti-cathepsin D from R&D systems (AF1014; 1:100 for IF), anti-Iba1 from Millipore-Sigma (MABN92; 1:50 for IF), anti-MAP2 from Millipore-Sigma (M1406; 1:1000 for IF), and anti-GFAP from Santa Cruz (sc-58766; 1:200 for IF), anti-KIF5B from ProteinTech (21632-1-AP, 1:2000 for Western blot), anti-VPS35 from Santa Cruz (sc-374372, 1:1000 for Western blot), ATP8A1 from ProteinTech (21565-1-AP, 1:1000 for Western blot), and VPS8 from ProteinTech (15079-1-AP, 1:1000).

Cell culture
Human embryonic kidney 293T (HEK293T) cells were maintained in Opti-Mem plus 10% FBS and 1% penicillin–streptomycin. Cells plated in 6-well plates were transfected with the indicated plasmids using Lipofectamine 2000 (Thermo Fisher Scientific) and harvested for Western blot analysis 24 hours post-transfection. Kolf2.1J and isogenic TMEM106B knockout iPSC lines with a stably integrated, tetracycline-inducible hNGN2 promoter were dissociated using StemPro Accutase (ThermoFisher Scientific) and plated at a concentration of $1.5x10^6$ cells per well in 6-well plates coated with Matrigel. Cells were incubated for 72 hours in neuronal induction media (Knockout DMEM/F12, Thermofisher Scientific), 1X N2 Supplement (LifeTech), 1X Non-Essential Amino Acids (Thermofisher Scientific), 1X Glutamax (Thermofisher Scientific), 10 μM Rock inhibitor Y-27632 (Millipore) and 2 μg/mL doxycycline (Sigma). After 72 hours, the cells were dissociated and plated on poly-L-ornithine coated plates with neuronal maturation media (BrainPhys neuronal medium [Stem Cell Technologies], 1X N21 max [R&D Systems], 10 ng/mL GDNF [Peprotech], 10 ng/mL BDNF [Peprotech], 10 ng/mL NT3 [Peprotech], 1 μg/mL laminin [ThermoFisher], 2 μg/mL doxycycline [Sigma], 1 μM 5-fluro-2'uridine [Sigma] and 1 μM uridine [Sigma]) for 21 days for neuronal differentiation with a half media change for every 3 days. All cell pellets were lysed in co-immunoprecipitation (co-IP) buffer (50 mM Tris–HCl, pH 7.4, 300 mM NaCl, 1% Triton X-100, 5 mM EDTA) with protease and phosphatase inhibitors, sonicated on ice, and then centrifuged at 16,000 × g for 20 minutes. Supernatants were saved as cell lysates with protein concentration determined by BCA assay (Thermo Fisher Scientific). To induce deglycosylation, cell lysates (20μg) were incubated with N-glycosidase F (PNGase, NEB# P0704S) following manufacturer's protocols for 16 hours at 37 °C prior to Western blot analysis.

RIPA extraction method
To generate RIPA-soluble extracts to measure full-length TMEM106B, tissues were first homogenized in 5 volumes (w/v) of ice-cold RIPA buffer (25mM Tris-HCl pH 7.5, 150mM NaCl, 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% SDS, with protease and phosphatase inhibitors). Homogenates were then sonicated (1 sec on/1 sec off for 10 sec) and centrifuged at 100,000 x g for 30 minutes at 4 °C. The resulting supernatants were collected as the RIPA-soluble fraction. To induce deglycosylation, samples were incubated in the presence or absence of N-glycosidase F (PNGase, NEB #P0704S) following manufacturer's protocols for 16 hours at 37 °C prior to Western blot analysis.

Sarkosyl extraction method
Human postmortem frontal cortex tissue was homogenized in 5 volumes (w/v) of cold buffer consisting of 10 mM Tris-HCI (pH 7.4), 80 mM NaCI, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM EDTA, 1 mM PMSF, 1 mM Dithiothreitol and a protease and phosphatase inhibitor cocktail. Following homogenization, 400 µL of homogenate (corresponding to 80 mg brain tissue) was ultracentrifuged at 150,000 g for 40 minutes at 4 °C in a TLA110 rotor at 60,000 rpm. The resulting pellet was resuspended in one volume of cold buffer containing 10 mM Tris [pH 7.4], 0.85 M NaCl, 10% sucrose, 1 mM EGTA, and subsequently centrifuged at 14,000 g for 10 minutes at 16 °C. The supernatant was then incubated with sarkosyl at a final concentration of 1% for 1 hour at room temperature with continuous agitation. Following this incubation, samples were ultracentrifuged at 150,000 g for 40 minutes at 4 °C in a TLA110 at 60,000 rpm. The resulting pellet (sarkosyl-insoluble P3 fraction) was resuspended in 50 µL TE buffer (10 mM Tris-HCl, 1 mM EDTA), frozen on dry ice, and stored at -80 °C until use. To induce deglycosylation, 10 µL of the sarkosyl-insoluble fraction was incubated in the presence or absence of N-glycosidase F (PNGase, NEB #P0704S) following manufacturer's protocols for 16 hours at 37 °C prior to Western blot analysis.

Immunoblotting
Sarkosyl-insoluble fraction (10 µL) or cell lysate (20 µg) was diluted with 2× SDS gel loading buffer at a 1:1 ratio (v/v), and then heated at 95 °C for 5 minutes. Samples were loaded into 10- or 20-well 4–20%

Tris-glycine gels (Novex), and after running gels, were transferred to PVDF membranes. RIPA soluble extracts were diluted 1:1 (v/v) in 2x SDS gel loading buffer while being kept cold on ice. Samples were then run on 10% Tris-glycine gels with ice surrounding the tank to maintain cold conditions. All membranes were blocked with 5% nonfat dry milk in TBS plus 0.1% Triton X (TBST) for 1 hour, and then incubated with primary antibody overnight rocking at 4 °C. The next day, membranes were washed in TBST and incubated with donkey anti-rabbit or anti-mouse IgG antibodies conjugated to horseradish peroxidase (1:5000; Jackson ImmunoResearch) for 1 hour. Protein expression was visualized by enhanced chemiluminescence treatment and exposure to film. Blot quantitation was performed using Scion Image.

Immunoelectron microscopy

Sarkosyl-insoluble fractions were diluted 1:10 in TBS (pH. 7.4) and absorbed onto carbon/formvar-coated 400 mesh copper grids for 60 seconds. Non-specific binding was blocked by incubating grids with blocking buffer (1 mg/mL of BSA in TBS) for 30 minutes at room temperature in a humidified chamber, followed by incubating in TMEM106B core antibody (diluted 1:50 in blocking buffer) at room temperature for 60 minutes. Grids were washed three times in blocking buffer (40 µL drops), followed by incubation with gold-labeled secondary antibody (diluted 1:20 in blocking buffer) for 60 minutes in the same humidified chamber at room temperature. Grids were washed six times with TBS (40 µL drops), stained with 2% uranyl acetate (Electron Microscopy Sciences, Hatfield, PA) for 60 seconds, and examined with a Transmission Electron Microscope JEM-1400Flash and Matataki^{TM} 4M Flash camera.

Immunoprecipitation of TMEM106B and on-bead digestion for MS analysis

Nine CC/TT185 cases with high accumulation of sarkosyl-insoluble 29 kDa TMEM106B filaments were chosen for immunoprecipitation (IP). All rounds of Dynabead washes were performed with 1 mL of co-IP buffer (50 mM Tris pH 7.4, 300 mM NaCl, 5 mM EDTA, 1% Triton-X 100), rotation at 4 ^{o}C for 10 minutes, followed by spinning at 100 g × 2 minutes at room temperature, isolation of the beads on a magnet, and aspiration of the wash buffer. To begin the experiment, the total required volume of Dynabeads™ Protein G (Invitrogen #10004D) (30 µL per case) was pipetted into a single Eppendorf tube and washed 3 times. After the final wash, the Dynabeads were incubated with sarkosyl-insoluble extracts diluted in co-IP buffer (210 µL of sarkosyl-insoluble extracts and 390 µL of chilled co-IP buffer) for 30 minutes on the rotor at 4 ^{o}C to eliminate proteins binding non-specifically to beads. Following removal of beads containing non-specific protein interactors, the pre-cleared sarkosyl-insoluble lysates were split between two tubes and incubated with 2.3 µg of either the in-house rabbit TMEM106B antibody or normal rabbit IgG (2729S, Cell Signalling) rocking at 4 ^{o}C overnight. The next morning, the total volume of Dynabeads required was again prepared by three 10-minute washes with 1 mL of co-IP buffer. The beads were then incubated with the sample/antibody solutions rocking at 4 ^{o}C for 4 hours. Following this incubation, beads were spun at 100 g x 2 minutes at room temperature and isolated via magnet, and the remaining solution in each tube was discarded. The beads were subsequently washed twice with co-IP buffer, three times with chilled PBS and once with 50 mM ammonium bicarbonate (ABC) (Sigma, Cat# A6141). During the last wash, 20 µg of lyophilized trypsin/LysC powder (MS Grade - Promega, Cat # V5073) was dissolved in 1 mL of the 50 mM ABC solution. Following bead washes, 100 µL of this solution was added to the beads and shaken on a Thermomixer at 1200 RPM at 37 ^{o}C overnight. The next morning, the supernatants were separated from the beads and mixed with 10 µL of 5% Trifluoroacetic acid (ThermoScientific, Cat # 28904) in a chemical safety hood to acidify the samples, which were then frozen at -30 ^{o}C prior to analysis by mass spectrometry.

Affinity purification-mass spectrometry-based proteomics

The liquid chromatography (LC) separation was performed on an UltiMate 3000 nano-HPLC system (Thermo Scientific) coupling an EASY-Spray 75 µm × 50 cm column packed with 2 µm C_{18} particles and a nano trap column (75 µm × 2 cm, 3 µm C_{18} particle). The tryptic peptides were separated using a 90-minute linear gradient with 2%-35% of solvent B (0.1% formic acid in 5% DMSO/95% ACN). The nano

columns were heated to 60 °C to reduce the back pressure. For the mass spectrometry analyses, we used the single-shot and direct-data independent acquisition (DIA) method reported previously on a hybrid Orbitrap Eclipse mass spectrometer (Thermo Scientific) (*64*). Briefly, two experiments, full MS1 scan and DIA MS2 scan, were applied to the peptide sequencing and quantification. The MS1 resolution was set to 120,000 with AGC target and auto maximum injection time. The DIA scans were acquired with 8 m/z isolation window of 400-1000 m/z scan range in the orbitrap with resolution set to 30,000 and 30% HCD collision energy. The cycle time of each experiment was set to 3 seconds to maintain the ion peak shape. For the database search of DIA results, we applied a direct-DIA approach using Spectronaut (v17.5). Briefly, the MS/MS spectra from MS raw files were aligned to the reviewed Uniprot human proteome reference containing 20,382 protein sequence entries. False discovery rates (FDRs) for both peptides and proteins were set to 1%. The methionine oxidation and N-terminal acetylation were set as variable modifications. Trypsin and lysC were selected as digestion enzymes with up to two mis-cleavages. Cross-run normalization was disabled. The differential abundance analyses to identify TMEM106B-specific interactors (enriched in 9 TMEM106B IP samples compared to 9 IgG IP samples) was performed using an in-house ProtPipe package including vector-based selection for bona-fide interaction proteins.

Immunohistochemistry

Paraffin-embedded tissue sections were cut at 5 μm, mounted on positively charged glass slides and dried overnight in a 60 ºC oven. Tissue sections were deparaffinized in xylene, rehydrated in a graded series of alcohols, and then incubated with 98% formic acid (EMD Chemicals Catalog, #FX0440-6) for 30 minutes. Following several washes, antigen retrieval was performed by steaming in de-ionized water for 30 minutes. Slides were then incubated with 3% hydrogen peroxide to quench endogenous peroxidase activity, and subsequently rinsed in distilled water prior to a 5-minute incubation in TBS with 0.05% tween 20. Immunostaining was then performed using the Thermo Scientific, Lab Vision Autostainer 480S (Kalamazoo, MI) and the DAKO EnVision + HRP system with the TMEM106B core antibody diluted 1:10,000. Stained slides were then counterstained with hematoxylin, dehydrated, cover-slipped, and scanned with the Leica Aperio AT2 Slide Scanner (Aperio, Vista, CA).

Immunofluorescence and confocal microscopy

Paraffin-embedded frontal cortex tissue sections (5 μm) were deparaffinized, rehydrated, and incubated in water 2.5 inches from a Mars Hydro TS 600W LED Grow Light with 2x2x3 Grow Tent (Amazon, ASIN #B09MFFQYN4) at 4 ºC for 16 hours to photobleach and quench autofluorescence without overheating the tissue. Slides were then incubated with 98% formic acid (EMD Chemicals Catalog, #FX0440-6) for 10 minutes, followed by steaming in citrate buffer (pH = 6.0) for 30 minutes. After several washes in distilled water (20 minutes total to allow slides to cool), slides were blocked with Dako All Purpose Blocker for 1 hour and incubated with primary antibody overnight in a humidified chamber at 4 ºC. The next day, slides were washed in TBST (TBS with 0.01% Triton-X) and incubated with Alexa Fluor 488 donkey anti-mouse (Molecular Probes, 1:200), Alexa Fluor 568 donkey anti-rabbit (Molecular Probes, 1:500), and Alexa Fluor 647 donkey anti-goat (Molecular Probes, 1:500) for 2 hours. After washing in TBST, slides were coverslipped with Fluoromount G. Images were obtained on a Zeiss LSM 980 laser scanning confocal microscope.

pTDP-43 and tSTMN2 quantification

pTDP-43 protein burden in the urea fraction from the frontal cortex of FTLD-TDP patients was available from our previous study (*17*) where pTDP-43 burden was measured using a sandwich immunoassay based on electrochemiluminescence detection by Meso Scale Discovery (MSD) technology. In addition, *tSTMN2* RNA data from the frontal cortex of FTLD-TDP cases was also available from a previous study (*16*), where a NanoString PlexSet was used for calculation.

qRT-PCR for TMEM106B RNA expression

RNA was extracted from post-mortem frozen frontal cortex tissue using the RNAeasy Plus Mini Kit (Qiagen) according to manufacturer's directions. RNA integrity number (RIN) was determined using an Agilent 2100 bioanalyzer (Agilent Technologies), with samples having a RIN value < 6 excluded from the study. A total of 500 ng of RNA was transcribed into complementary DNA (cDNA) using the High-Capacity cDNA Transcription Kit (Applied Biosystems) per manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) was then performed using SYBR GreenER qPCR SuperMix (Invitrogen) on QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems). All samples were run in triplicate, and relative quantification was determined using the ΔΔCt method and normalized to the endogenous controls *GAPDH* and *RPLP0*. The following primers and their sequences were used: *TMEM106B* forward: 5′- AGAGATAGTGTCACCTGCCCT-3′, *TMEM106B* reverse: 5′-GCCATCACATACAGCTTTGTTCTT-3′, *GAPDH* forward: 5′-GTTCGACAGTCAGCCGCATC-3′, *GAPDH* reverse: 5′-GGAATTTGCCATGGGTGGA-3′; and *RPLP0* forward: 5′-TCTACAACCCTGAAGTGCTTGAT-3′; *RPLP0* reverse: 5′-CAATCTGCAGACAGACACTGG-3'.

Pathway and STRING Analysis

Lollipop plots of KEGG and GO pathways were retrieved using the ShinyGo web application with default search settings. Functional STRING networks of the illustrated pathways were generated in Cytoscape with the publicly available stringApp and curated manually. The textmining filter was increased to 0.300 for the Ribosome network to enhance visibility of edges. Markov clustering was used with a granularity/inflation parameter of 3 for both networks to facilitate clear observation of closely related proteins within each category.

Digestion of the P3 Fraction for Proteomic Analysis

P3 pellets were resuspended in 50 µL of 8 M urea lysis buffer (8 M urea, 10 mM Tris, 100 mM NaH2PO4, pH 8.5) with HALT protease and phosphatase inhibitor cocktail (ThermoFisher) using a Bullet Blender (NextAdvance). The samples were sonicated for 3 cycles consisting of 5 seconds of active sonication at 30% amplitude, followed by 15 seconds on ice. Samples were reduced with 5 mM dithiothreitol (DTT) at room temperature for 30 minutes, followed by 10 mM iodoacetamide (IAA) alkylation in the dark for another 30 minutes. Lysyl endopeptidase (Wako) at 1:25 (w/w) was added, and digestion allowed to proceed overnight. Samples were then diluted 7-fold with 50 mM ammonium bicarbonate. Trypsin (Promega) was then added at 1:25 (w/w) and digestion proceeded overnight. The peptide solutions were acidified to a final concentration of 1% (vol/vol) formic acid (FA) and 0.1% (vol/vol) trifluoroacetic acid (TFA) and desalted with a 30 mg HLB column (Oasis). Each HLB column was first rinsed with 1 mL of methanol, washed with 1 mL 50% (vol/vol) acetonitrile (ACN), and equilibrated with 2×1 mL 0.1% (vol/vol) TFA. The samples were then loaded onto the column and washed with 2×1 mL 0.1% (vol/vol) TFA. Elution was performed with 2 volumes of 0.5 mL 50% (vol/vol) ACN.

Isobaric Tandem Mass Tag (TMT) Peptide Labeling

Each sample was re-suspended in 100 mM TEAB buffer (100 µL). The TMT labeling reagents were equilibrated to room temperature, and anhydrous ACN (256 µL) was added to each reagent channel. Each channel was gently vortexed for 5 minutes, and then 41 µL from each TMT channel was transferred to the peptide solutions and allowed to incubate for 1 hour at room temperature. The reaction was quenched with 5% (vol/vol) hydroxylamine (8 µl) (Pierce). All channels were then combined and dried by SpeedVac (LabConco) to approximately 150 µL and diluted with 1 mL of 0.1% (vol/vol) TFA, then acidified to a final concentration of 1% (vol/vol) FA and 0.1% (vol/vol) TFA. Labeled peptides were desalted with a 200 mg C18 Sep-Pak column (Waters). Each Sep-Pak column was activated with 3 mL of methanol, washed with 3 mL of 50% (vol/vol) ACN, and equilibrated with 2×3 mL of 0.1% TFA. The

samples were then loaded, and each column was washed with 2×3 mL 0.1% (vol/vol) TFA, followed by 2 mL of 1% (vol/vol) FA. Elution was performed with 2 volumes of 1.5 mL 50% (vol/vol) ACN. The eluates were then dried to completeness using a SpeedVac. Off-line high pH fractionation was performed as described (*65*)

Liquid Chromatography Coupled to Tandem Mass Spectrometry

All fractions were resuspended in an equal volume of loading buffer (0.1% FA, 0.03% TFA, 1% ACN) and analyzed by liquid chromatography coupled to tandem mass spectrometry. Peptide eluents were separated on a custom in-house packed CSH 1.7 µm (20 cm × 150 µM internal diameter (ID) by an Ultimate U3000 RSLCnano (ThermoFisher Scientific). Buffer A was water with 0.1% (vol/vol) formic acid, and buffer B was 80% (vol/vol) acetonitrile in water with 0.1% (vol/vol) formic acid. Elution was performed over a 30-minute gradient with flow rate at 1250 nL/minute. The gradient was from 1% to 99% solvent B. Peptides were monitored on a Orbitrap Eclipse mass spectrometer with a high-field asymmetric waveform ion mobility spectrometry (FAIMS Pro Duo) ion mobility source (ThermoFisher Scientific). Two compensation voltages (CV) were chosen for the FAIMS. For each CV (-45 and -65) top speed cycle of 1.5 seconds, the full scan (MS1) was performed with an m/z range of 410-1600 at 60,000 resolution at standard settings. The higher energy collision-induced dissociation (HCD) tandem scans were collected at 35% collision energy with an isolation of 0.7 m/z, a resolution of 30,000 with TurboTMT on, an AGC setting of 250% normalized agc target, and a maximum injection time of 54 milliseconds. Dynamic exclusion was set to exclude previously sequenced peaks for 20 seconds within a 10-ppm isolation window.

Database Search Parameters and Peptide Quantification

The built-in FragPipe (FP, version 18.0) workflow for "TMT16" was used essentially as described (*66*). First, mzML files were generated from the original MS .raw files of the 4 batches that each included 96 high pH fractions (n = 384 total raw files) using the ProteoWizard MSConvert tool and then searched using MSFragger (version 3.7) using default parameters. The human proteome database used comprised of 20,402 sequences (Swiss-Prot, downloaded 2/11/2019) and their corresponding decoys, including common contaminants. MSFragger search results were processed using Percolator(*67*) for peptide-spectrum match validation, followed by Philosopher (*68*) for protein inference (using ProteinProphet (*69*)) and false discovery rate (FDR) filtering. The reports of the quantified proteins and peptides with FDR < 1% were generated. All raw files, the database, the sample to TMT channel information, and the FP search parameter settings are provided on https://www.synapse.org/#!Synapse:syn51448575. Following quantification, peptide amounts were first scaled by dividing each peptide intensity by all reporter ion intensities of the TMT channel (each sample) followed by multiplying the maximum channel-specific peptide intensity in the channel. A tunable median polish approach (TAMPOR) was used to adjust technical batch variance as previously described (*70*). Peptides with more than 50% of samples with missing values were removed from the matrix prior to the process. No imputation of missing values was performed (samples were excluded from analyses).

Supplementary Figures

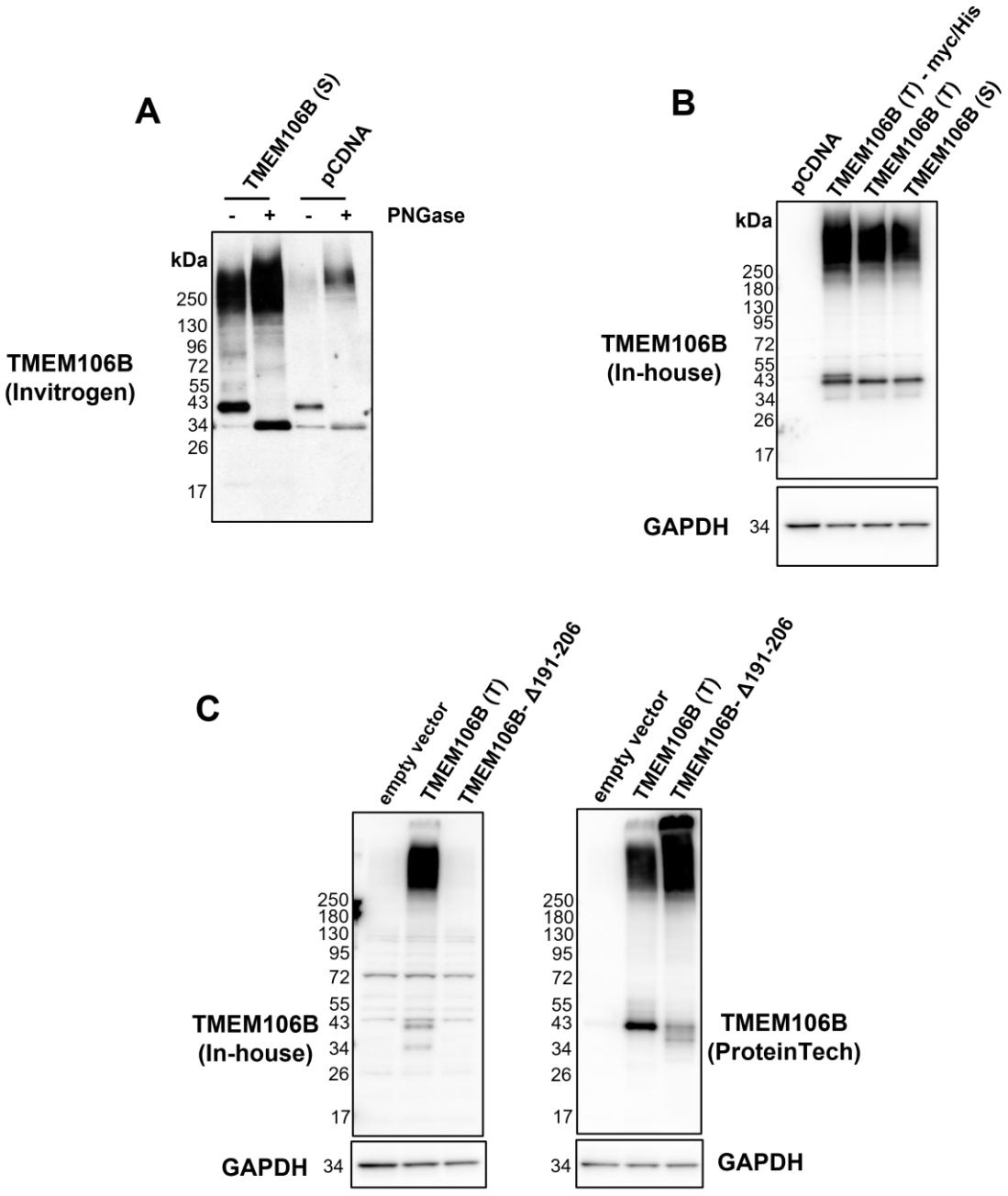

Fig. S1. Validation of TMEM106B expression constructs and core antibody.
(**A**) HEK293T cells were transiently transfected with full-length TMEM106B (p.S185) or empty vector (pcDNA) for 24 hours. Cell lysates were collected and subsequently incubated in the presence or absence of PNGase (N-glycosidase F) to induce deglycosylation of TMEM106B (*20*). Lysates were then evaluated by immunoblotting with a commercially available TMEM106B antibody (Invitrogen).

(**B**) HEK293T cells were transfected with myc/His-tagged TMEM106B (p.T185), untagged TMEM106B (p.T185) or TMEM106B (p.S185), or empty vector (pcDNA). 24 hours following transfection, cells were harvested, and lysates separated by SDS-PAGE followed by immunoblotting to evaluate specificity of the TMEM106B core antibody. GAPDH was used to control for protein loading.
(**C**) HEK293T cells were transfected with empty vector, TMEM106B (p.T185), or TMEM106B (p.T185) lacking amino acids 191-206 (the epitope for the in-house TMEM106B core antibody). Cells were collected as in (B), and immunoblot analysis revealed absence of immunoreactivity for the new TMEM106B core antibody in cells transfected with the deletion construct compared to the full-length TMEM106B construct (arrow). In contrast, the TMEM106B ProteinTech antibody was able to detect both the full-length and deletion constructs.

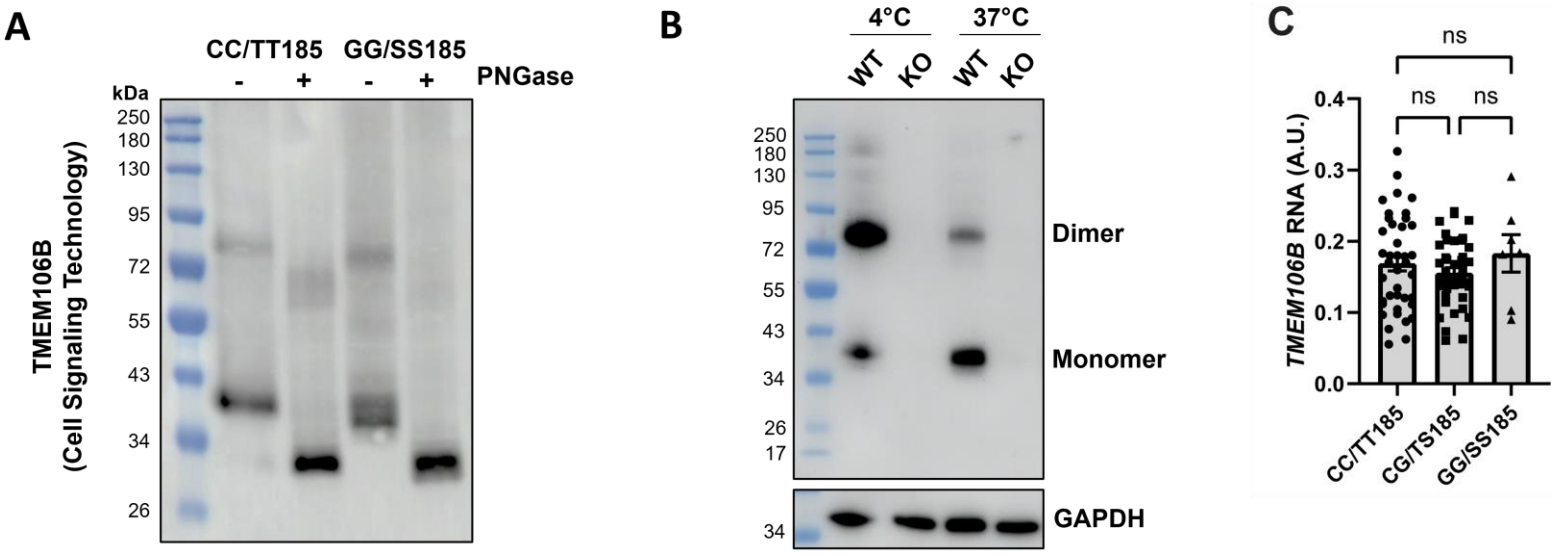

Figure S2. Characterization of full-length TMEM106B protein and RNA levels.

(**A**) RIPA soluble frontal cortex lysates from CC/TT185 and GG/SS185 donors were incubated in the presence or absence of PNGase (N-glycosidase F) to induce deglycosylation of TMEM106B (*20*). Lysates were then evaluated by immunoblotting under cold conditions and probed with a commercially-available TMEM106B antibody (Cell Signaling Technology).

(**B**) Further validation of the anti-TMEM106B antibody (Cell Signaling Technology) using wildtype and knockout TMEM106B i3Neurons. Samples and gels were kept at 4 ^{o}C or 37 ^{o}C to confirm the temperature sensitivity of the TMEM106B dimer.

(**C**) *TMEM106B* RNA expression was quantified by qRT-PCR and normalized to the endogenous controls GAPDH and RPLP0 (n=87 total; CC/TT185=40, CG/TS185=40, GG/SS185=7). Data is presented as mean $\pm$ SEM, with statistical analysis performed by one-way ANOVA with multiple comparisons (n.s. = not significant).

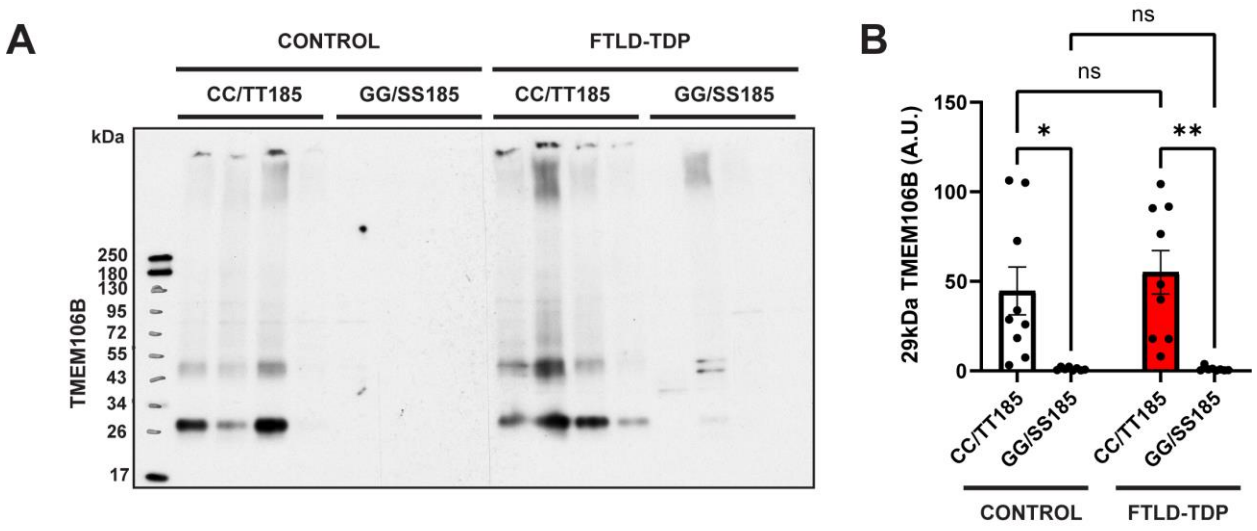

Figure S3. Evaluation of sarkosyl-insoluble 29 kDa TMEM106B fragment in FTLD-TDP and control cases.

(**A-B**) The sarkosyl-insoluble fraction was extracted from the frontal cortex of control or FTLD-TDP patients (17 control [9 CC, 8 GG], 17 FTLD-TDP [9 CC, 8 GG]), and separated by SDS-PAGE. TMEM106B was examined by immunoblotting (**A**), and quantification performed on the 29 kDa fragment (**B**). Data presented as mean ± SEM, with statistical analysis performed using two-way ANOVA with Tukey's multiple comparisons (*p<0.05, **p<0.01, ns = not significant).

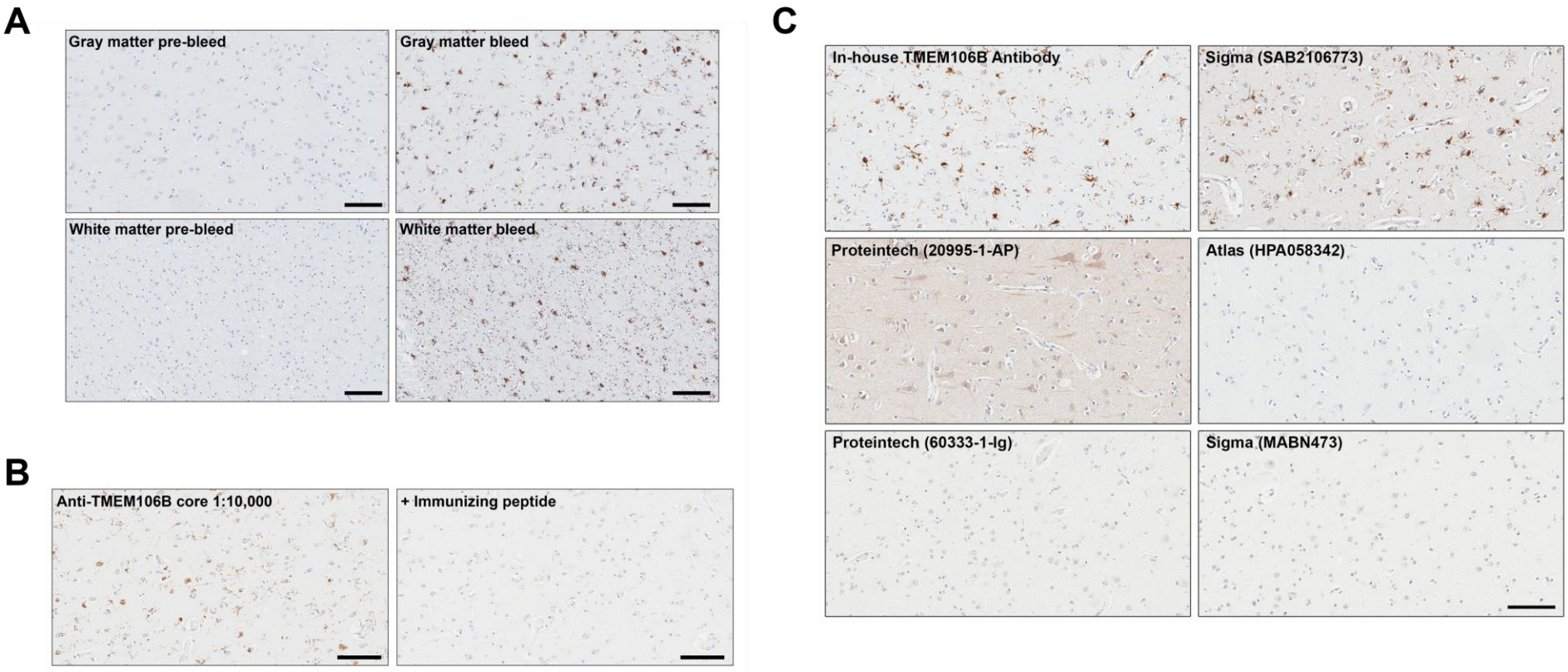

Figure S4. IHC validation of custom TMEM106B core domain antibody.

(**A**) Staining patterns in frontal cortex and white matter using serum from pre-immunized host animals (pre-bleed, left) vs. staining using serum from animals immunized with the 191-206 peptide fragment of TMEM106B (bleed, right).

(**B**) Incubation of TMEM106B core antibody without immunizing peptide preincubation (left) and with 200 molar excess of immunizing peptide preincubation (right).

(**C**) Comparison of staining with our in-house TMEM106B antibody with commercially available TMEM106B antibodies as indicated. Scale bars, 100 μm.

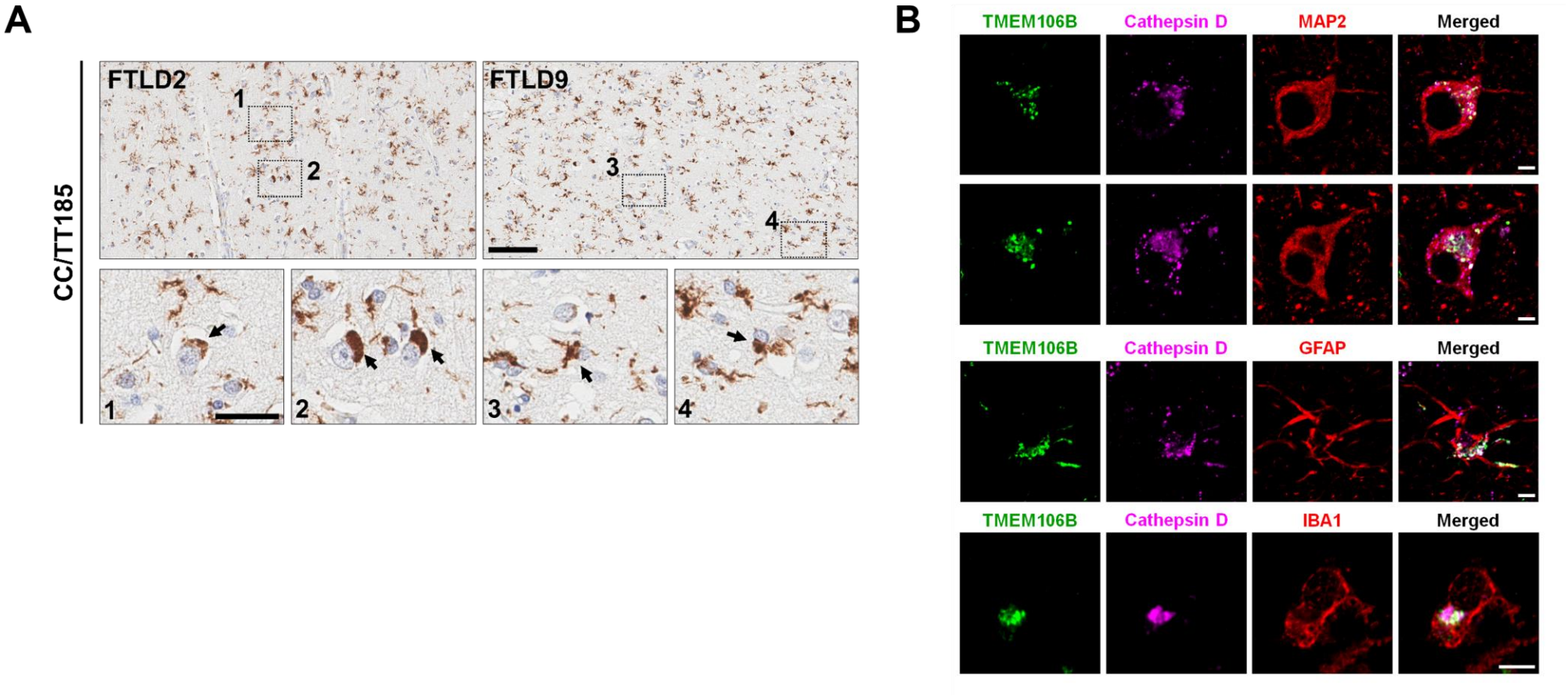

Figure S5. TMEM106B core antibody identifies TMEM106B accumulation in lysosomes of multiple cell types.

(A) Representative immunohistochemical staining of TMEM106B in frontal cortex of two FTLD-TDP cases. Two different neuronal staining patterns from one case are depicted (arrows, panels 1 and 2), though the same neuronal staining patterns were observed in both cases. Panels 3 and 4 depict examples of glial staining patterns. Top panels: 20x magnification, scale 100 µm. Bottom panels: 40x magnification, scale 30 µm.

(**B**) Representative triple-label immunofluorescence of TMEM106B, lysosomal marker cathepsin D, and cell-type specific markers for neurons, astrocytes, and microglia in frontal cortex of FTLD-TDP brain. Scale, 5 µm.

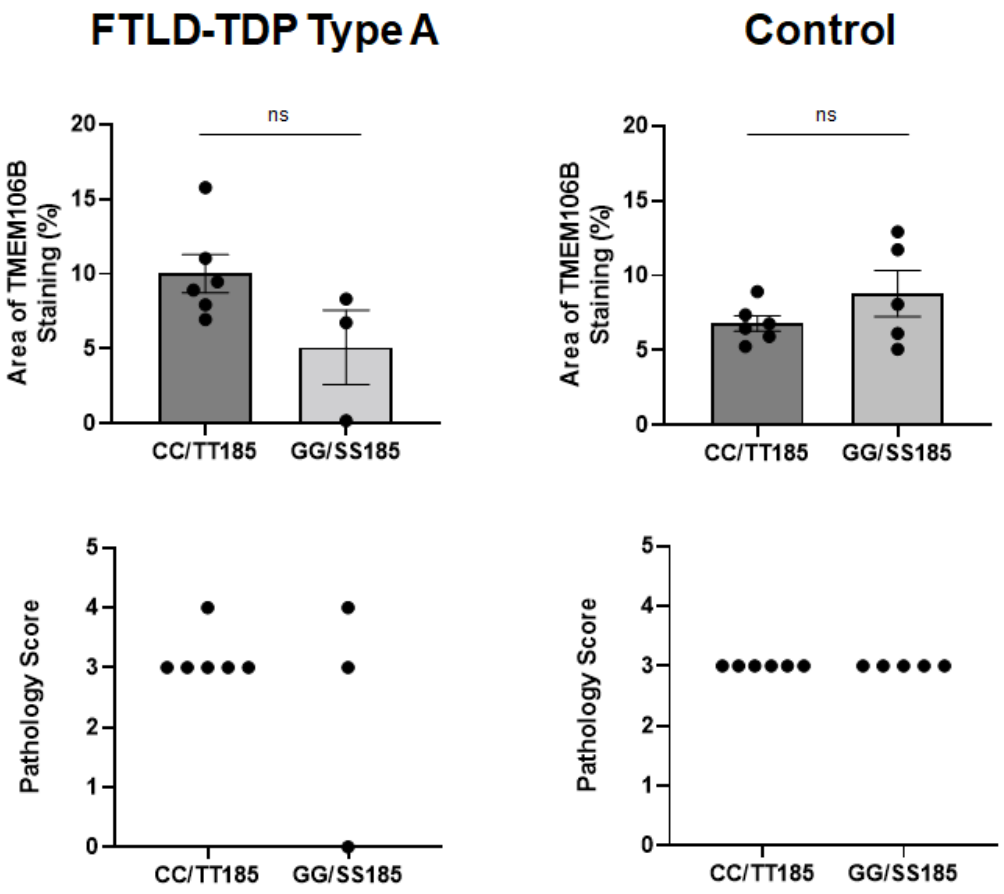

Figure S6. Evaluation of TMEM106B core detection in frontal cortex by IHC.
TMEM106B staining burden in the frontal cortex of FTLD-TDP Type A cases or controls quantified by a custom image analysis algorithm (top row) or scored semi-quantitatively by a neuropathologist blinded to disease status and genotype. Data in top panels presented as mean ± SEM, with statistical analysis performed via unpaired t-test (ns: not significant). Bottom panels were not statistically analyzed. FTLD-TDP Type A n=6 CC/TT185, 3 GG/SS185; Control n=6 CC/TT185, 5 GG/SS185.

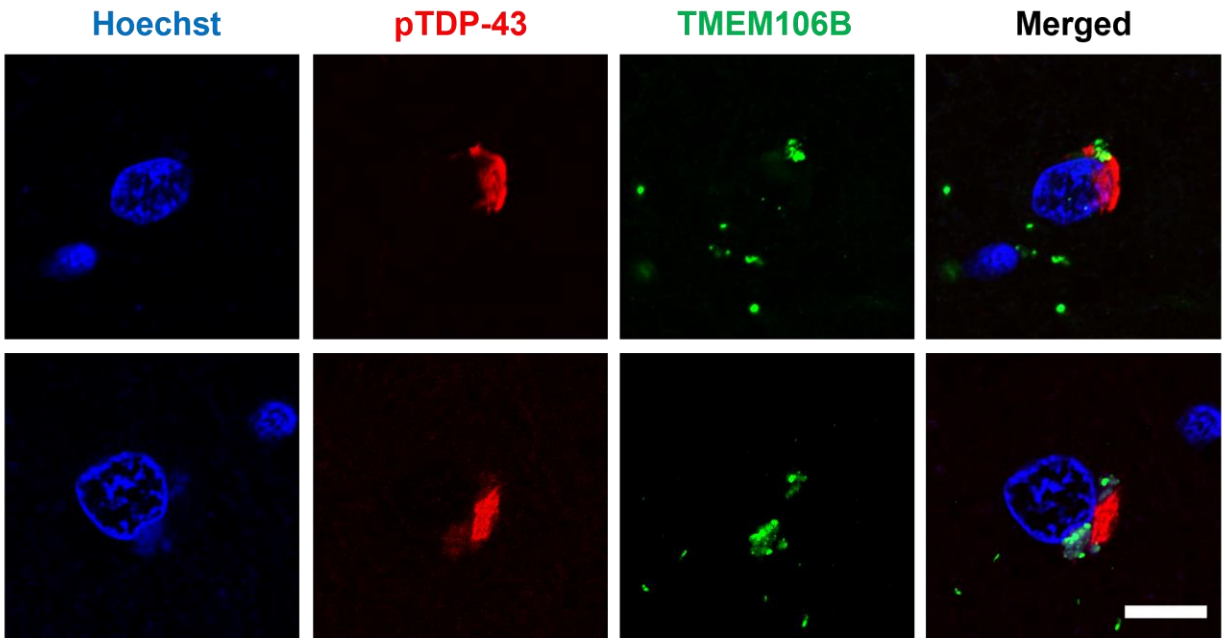

Figure S7. Co-immunofluorescence of TMEM106B and pTDP-43 in FTLD-TDP frontal cortex.

Representative cells double positive for TMEM106B and pTDP-43 from 2 FTLD-TDP cases (1 case per row) with CC/TT185 genotype. Scale, 20 µm.

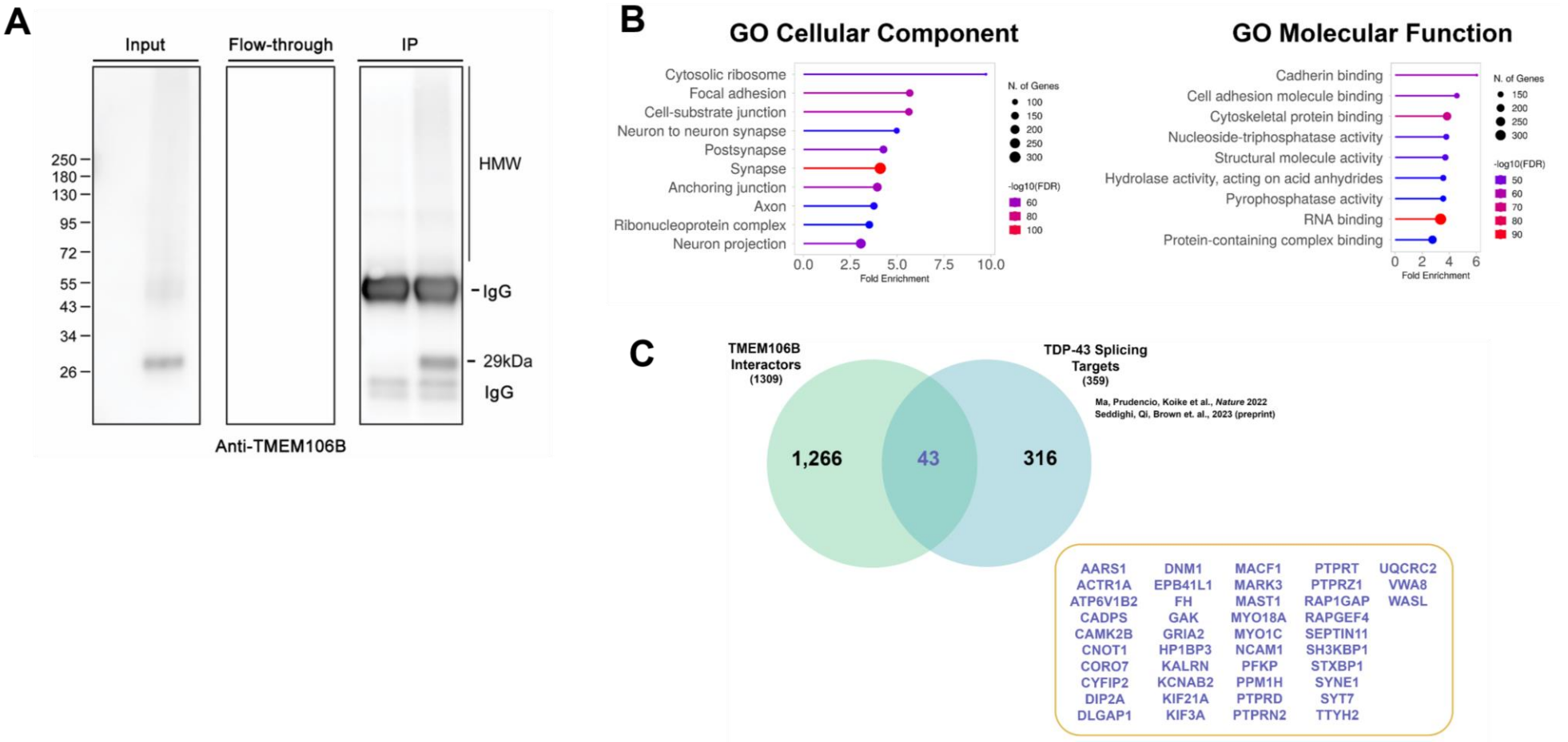

Figure S8. Pathway analysis of the insoluble TMEM106B interaction network.

(A) Optimization of TMEM106B IP from the P3 fraction. 2 lanes are depicted in each image, the left lane showing a case that had no 29-kDa band in the P3 and the right a 29-kDa positive case. Middle panel shows an absence of the 29 kDa band in the right case in the flow-through. The right panel confirms successful IP of the 29-kDa TMEM106B core (right lane).

(B) GO Cellular Component and Molecular Function analysis of the TMEM106B IP-MS dataset (n=9 cases, see Supplementary File 1 for case information). Lollipop plots of the top 10 terms in each GO category, sorted by fold enrichment.

(C) Venn diagram of overlapping hits between TMEM106B core interactors and TDP-43 cryptic splicing targets. Citations denote the original reports from which the hits for each TDP-43 study were derived. Overlapping hits are highlighted in blue.

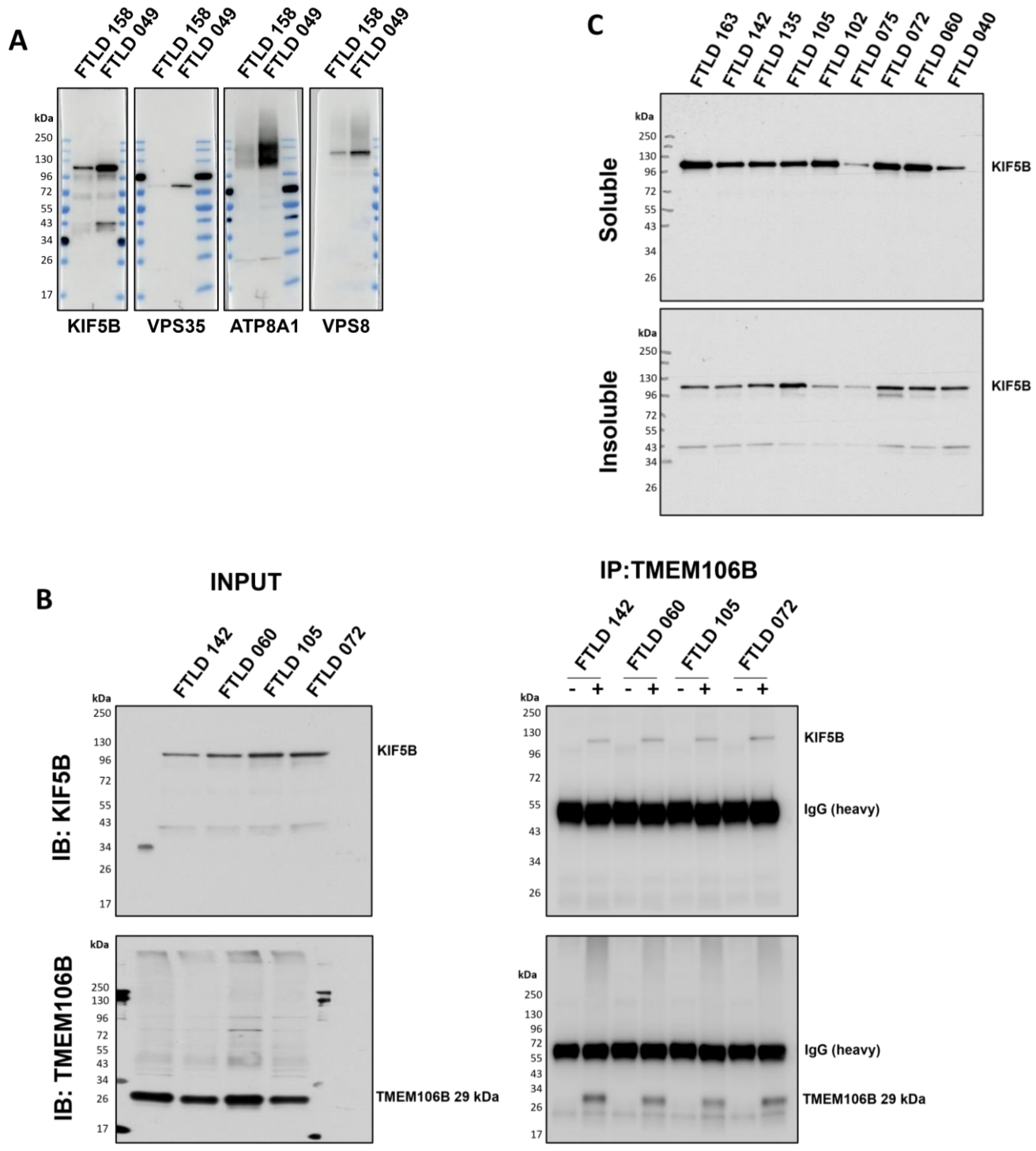

Figure S9. Validation of core domain interacting proteins identified via IP-MS.
(**A**) Antibody validation for selected core domain interactors in P3 fractions from 2 FTLD-TDP cases.
(**B**) Immunoblot of KIF5B and TMEM106B following immunoprecipitation from the P3 fraction in 4 FTLD-TDP cases included in IP-MS experiments. Symbols denote antibody used for immunoprecipitation (- for anti-GFP, + for anti-TMEM106B).
(C) Immunoblots of KIF5B in sarkosyl soluble and insoluble fractions.
All FTLD case data included in Supplementary File 1.

Table S1. Patient demographic information for antibody validation and immunolabeling studies

ID	PathDx	rs3173615	TDP-43 type	Sex	Age at death (yrs)	Use	Jiang et al., 2022 (18)
FTLD1	FTLD-TDP	CG/TS185	A	M	63	WB	
FTLD2	FTLD-TDP	CC/TT185	A	M	86	iEM/WB/IHC/IF	Donor 3
FTLD3	FTLD-TDP	CC/TT185	C	M	75	WB	
FTLD4	FTLD-TDP	CG/TS185	C	M	65	WB	Donor 1
FTLD5	FTLD-TDP	CC/TT185	C	M	65	WB	
FTLD6	FTLD-TDP	CG/TS185	C	M	66	iEM/WB	
FTLD7	FTLD-TDP	CG/TS185	B	F	76	WB	Donor 2
FTLD8	FTLD-TDP	CG/TS185	D	F	64	WB	Donor 4
FTLD9	FTLD-TDP	CC/TT185	A	M	66	IHC/IF	
AD1	AD	CC/TT185	B	F	86	iEM	
AD2	AD	CC/TT185	A	M	86	WB	
AD3	AD	CC/TT185	0	M	72	WB	

PathDx = pathological diagnosis; AD = Alzheimer's disease; FTLD-TDP = frontotemporal lobar degeneration with TDP-43 inclusions; M = male; F = female; WB = Western blot; iEM = immuno-electron microscopy; IHC = immunohistochemistry; IF = immunofluorescence

Table S2. Association between 29kDa TMEM106B accumulation and rs3173615 TMEM106B SNP status in FTLD-TDP cases

		Unadjusted analysis		Adjusting for age at death and sex	
	Median (minimum, maximum) TMEM106B 29kDa levels	**β (95% CI)**	**P-value**	**β (95% CI)**	**P-value**
C/C (T185T)	50.68 (0.358, 470.6)	0.00 (reference)	NA	0.00 (reference)	NA
C/G (T185S)	9.39 (0.001, 230.6)	-0.5531 (-0.740, -0.366)	<0.0001	-0.4857 (-0.659, -0.313)	<0.0001
G/G (S185S)	2.19 (0.126, 10.3)	-1.334 (-1.655, -1.014)	<0.0001	-1.300 (-1.596, -1.004)	<0.0001

β: regression coefficient; CI: confidence interval; β values, 95% CIs, and *P* values result from unadjusted linear regression models or linear regression models adjusted for age at death and sex, where TMEM106B 29kDa levels were considered on the base 10 logarithmic scale. β values are interpreted as the difference in the TMEM106B 29kDa levels in FTLD-TDP cases carrying the TMEM106B risk (CC/TT185) and protective (CG/TS185 or GG/SS185) coding rs3173615 SNP. *P*-values < 0.025 are considered statistically significant after correcting for multiple testing.