Fronto-temporal dementia risk gene *TMEM106B* has opposing effects in different lysosomal storage disorders

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Running title: *TMEM106B effects in lysosomal disorders*

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ABSTRACT
TMEM106B is a transmembrane protein localized to the endo-lysosomal compartment. Genome-wide association studies have identified TMEM106B as a risk modifier of Alzheimer’s disease and frontotemporal lobar degeneration, especially with progranulin (PGRN) haploinsufficiency. We recently demonstrated that TMEM106B loss rescues PGRN null mouse phenotypes including lysosomal enzyme dysregulation, neurodegeneration and behavioral alterations. However, whether TMEM106B is involved in other neurodegenerative lysosomal diseases is unknown. Here, we evaluate the potential role of TMEM106B in modifying the progression of lysosomal storage disorders using PGRN-independent models of Gaucher disease and neuronal ceroid lipofuscinosis. To study Gaucher disease, we employ a pharmacological approach using the inhibitor conduritol B epoxide in wild-type and hypomorphic Tmem106b-/- mice. TMEM106B depletion ameliorates neuronal degeneration and some behavioral abnormalities in the pharmacological model of Gaucher disease, similar to its effect on certain PGRN null phenotypes. In order to examine the role of TMEM106B in neuronal ceroid lipofuscinosis, we crossbred Tmem106b-/- mice with Ppt1-/-, a genetic model of the disease. In contrast to its conduritol B epoxide-rescuing effect, TMEM106B loss exacerbates Purkinje cell degeneration and motor deficits in Ppt1-/- mice. Mechanistically, TMEM106B is known to interact with subunits of the vacuolar ATPase and influence lysosomal acidification. In the pharmacological Gaucher disease model, the acidified lysosomal compartment is enhanced and TMEM106B loss rescues in vivo phenotypes. In contrast, gene-edited neuronal loss of Ppt1 causes a reduction in vacuolar ATPase levels and impairment of the acidified lysosomal compartment, and TMEM106B deletion exacerbates the mouse Ppt1-/- phenotype. Our findings indicate that TMEM106B differentially modulates the progression of the lysosomal storage disorders Gaucher disease and neuronal ceroid lipofuscinosis. The effect of TMEM106B in neurodegeneration varies depending on vacuolar ATPase state and modulation of lysosomal pH. These data suggest TMEM106B as a target for correcting lysosomal pH alterations, and in particular for therapeutic intervention in Gaucher disease and neuronal ceroid lipofuscinosis.

KEYWORDS (5)
Lysosome, TMEM106B, Gaucher, Neuronal ceroid lipofuscinosis, palmitoyl-protein thioesterase 1

ABBREVIATIONS
CBE conduritol B epoxide
GCase glucocerebrosidase
GD Gaucher disease
FTLD frontotemporal lobar degeneration

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NCL neuronal ceroid lipofuscinosi
PGRN progranulin
PPT1 palmitoyl-protein thioesterase 1
V-ATPase vacuolar ATPase

INTRODUCTION
Lysosomes are membrane-bound organelles containing more than 50 acid hydrolases that function in the degradation of macromolecules. Lysosomal dysfunction in neurons is closely tied to neurodegeneration and cell death mechanisms (Nixon and Yang, 2012; Česen et al., 2012). Growing genetic and biochemical evidence implicates dysfunction of the endosomal-lysosomal and autophagic lysosomal pathways in the pathogenesis of a number of neurodegenerative disorders, including Alzheimer’s Disease (AD), Progranulin (GRN) gene-related frontotemporal lobar degeneration (FTLD) and Parkinson’s disease (PD) (Nixon, 2013; Ghavami et al., 2014; Menzies et al., 2015).

TMEM106B is a transmembrane lysosomal protein expressed in neurons, glial and endothelial cells (Lang et al., 2012; Busch et al., 2013; Schwenk et al., 2013; Stagi et al., 2014). Genome-wide association study (GWAS) identified TMEM106B as a risk modifier of FTLD-TDP (trans-activation response element [TAR] DNA-binding protein) (Nicholson and Rademakers, 2016). SNPs in TMEM106B reduce disease penetrance in FTLD-TDP with GRN mutations (Van Deerlin et al., 2010; Finch et al., 2011). TMEM106B SNPs may also modify the pathological presentation of AD (Rutherford et al., 2012). Several genetic studies have also reported a significant underrepresentation of the TMEM106B protective allele in hippocampal sclerosis patients (Murray et al., 2014; Nelson et al., 2015). Furthermore, TMEM106B genotype predicts the rate of cognitive decline in PD patients (Tropea et al., 2019).

Haploinsufficiency of progranulin (PGRN) mRNA and protein is a common genetic etiology for FTLD-TDP, a progressive neurodegenerative disease and one of the most common dementias in subjects younger than 65 years (Vieira, 2013). We previously characterized the role of TMEM106B depletion in a PGRN-deficient (Grn-/-) mouse model (Stagi et al., 2014; Klein et al., 2017). Transcriptomic and proteomic analyses revealed an early lysosomal dysregulation in Grn-/- mouse brain, while Tmem106b-/- mouse brain showed opposite changes in several lysosomal enzymes. Remarkably, TMEM106B deficiency normalized lysosomal protein dysregulation and rescued FTLD-related behavioral deficits and retinal degeneration in Grn-/- mice. Mechanistically, TMEM106B interacts with the vacuolar ATPase (V-ATPase), a multimeric pump that transports protons from the cytosol into the lysosomal lumen and maintains pH gradient across the lysosomal membrane. TMEM106B deficiency causes downregulation of V-ATPase V0 domain, impairment in lysosomal acidification, and thereby normalizes lysosomal enzyme activity in Grn-/- neurons (Klein et al., 2017). TMEM106B also plays a role in the axonal transport of
lysosomes (Stagi et al., 2014; Lüningschrör et al., 2020) and myelination (Simons et al., 2017, Feng et al., 2020b, Zhou et al., 2020b).

In contrast to PGRN haploinsufficiency in FTLD-TDP, homozygous null mutations in the GRN gene have been identified as a cause of neuronal ceroid lipofuscinosis (NCL), a lysosomal storage disease (Smith et al., 2012). This devastating disease causes blindness, seizures, motor symptoms and early death, and mice lacking PGRN exhibit retinal neurodegeneration (Hafler et al., 2014). A hallmark of NCLs is the aberrant accumulation of autofluorescent pigment in the lysosomal compartment in neuronal cell bodies. While TMEM106B deficiency rescues certain phenotypes of young Grn-/- mice, lipofuscinosis is not altered by TMEM106B levels (Klein et al., 2017). In addition, recent studies have reported severe motor deficits and lysosomal deficits and motor neuronal loss in spinal cord of older Tmem106b-/-;Grn-/- double knockout mice and Grn-/- mice with alternate Tmem106b loss-of-function alleles (Feng et al., 2020a, Zhou et al., 2020a). Thus, the specificity and mechanistic determinants of TMEM106B modulation of neurodegeneration remain unclear.

Genetic studies have also suggested PGRN as a risk factor for the most prevalent lysosomal storage disorder, Gaucher disease (GD) (Jian et al., 2016b). GD is caused by loss-of-function mutations in GBA1, which encodes the lysosomal enzyme glucocerebrosidase (GCase) (Hruska et al., 2008). The resulting GCase deficiency causes accumulation of the glycosphingolipid (GSLs) glucosylceramide (GlcCer) within lysosomes. GD is characterized by hepatosplenomegaly, haematological defects and bone disease (Cox and Schofield, 1997; Vitner et al., 2015). The neurological forms, type 2 (acute) and type 3 (chronic), display central nervous system (CNS) involvement in addition to systemic disease (Vitner and Futerman, 2013). PGRN directly binds to and functions as a chaperone of the lysosomal enzyme GCase (Jian et al., 2016b, a) and PGRN mutations result in reduced GCase activity (Arrant et al., 2019; Valdez et al., 2019).

Here, we investigate the potential roles of TMEM106B in lysosomal storage diseases in which PGRN is implicated, GD and NCL. We utilize a pharmacological model to induce GD, the GCase inhibitor conduritol B epoxide (CBE), and a genetic model of NCL, Ppt1-/- mice. While TMEM106B depletion protects against neuronal degeneration and certain behavioral abnormalities in GD, TMEM106B deficiency exacerbates Purkinje cell loss and motor performance in Ppt1-/- mice NCL model. Mechanistically, TMEM106B may mediate these opposing effects by influencing lysosomal acidification through V-ATPase. Cultured neurons treated with CBE show increased lysosomal acidification as opposed to the decrease reported in Tmem106b-/- neurons. On the other hand, CRISPR-cas9 editing of Ppt1 in cultured neurons confirms impairment of the acidified lysosome compartment. We propose TMEM106B protein as a regulator of lysosomal physiology with TMEM106B/V-ATPase
interactions as a potential therapeutic target for certain lysosome-dependent neurodegenerative conditions.

MATERIALS AND METHODS

Mice

*Tmem106b*-/- and *Grn/-/-* mice were generated previously (Klein et al., 2017). *Ppt1/-/-* mice (Gupta et al., 2001) were kindly provided by Dr. Sreeganga Chandra. All mice were maintained on a 12 hr light-dark schedule with access to standard mouse chow and water ad libitum. Yale Institutional Animal Care and Use Committee approved all animal studies. Both male and female mice were included the studies, unless otherwise specified.

CBE treatment in mice

In order to induce GD, wild-type (WT) and *Tmem106b/-/-* mice were treated with CBE (Millipore, 234599), which is an irreversible inhibitor of GCase (Kanfer et al., 1975). In cohort 1, two months old mice were injected i.p. with 50 mg CBE per kg body weight or vehicle (PBS) per day for 30 days. Since no sign of pathology was observed using this dose, the amount of drug was increased to 100 mg CBE per kg body weight or vehicle (PBS) per day for 15 additional days, when some mice started to show body weight loss and tremors (data not shown). This cohort included males and females (*n* = 5-6 mice per group). In cohort 2, two months old WT and *Tmem106b/-/-* females directly received 100 mg CBE per kg body weight or vehicle (PBS) per day for 40 days. No poor body condition or apparent signs of motor impairment were observed at the end point of this treatment (*n* = 6-8 mice per group). The second cohort was sacrificed prior to systemic signs in order to allow optimal tissue collection and to assess neurodegeneration prior to and separate from any consequence of general decline.

Immunoprecipitation

The brains were dissected out from male 6-7-month-old WT and *Tmem106b/-/-* mice and homogenized in five-fold volume of ice-cold TBST (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Trion X-100) supplemented with cOmplete Mini (Roche). After ultracentrifugation at 100,000 x g for 30 min at 4°C, the supernatant was precleared with Protein A-Sepharose CL-4B (GE Healthcare 17-0780-01) for at least 3 hr at 4°C. The precleared lysate was incubated overnight at 4°C with anti-TMEM106B antibody (Abcam ab140185) that is covalently conjugated with Protein A-Sepharose CL-4B using BS3 (ThermoScientific 21580). The immunoprecipitates were washed six times with ice-cold TBST and proteins were eluted with 2x Laemmli buffer without 2-mercaptoethanol on ice for 50 min.

Immunofluorescence

For CBE and double knockout *Ppt1/-/-;Tmem106b/-/-* cohorts, mice were perfused in PBS and brains were immediately post-fixed in 4% PFA for 24 hr at 4°C. Coronal (for CBE cohort) and sagittal (for double
knockout Ppt1/-;Tmem106b/- cohort) 40 µm free-floating sections were prepared using a vibratome (Leica WT1000S). Sections were blocked with 1% BSA (Sigma), 1% Triton X-100 in PBS for 1 hr, followed by incubation with primary antibody for 2 days at 4°C. The following primary antibodies were used: mouse anti-NeuN (Millipore, MAB377, 1:200), rabbit anti-Iba1 (Wako, 019-19741, 1:250), rat anti-CD68 (AbD Serotec, MCA1957, 1:1000), rabbit anti-GFAP (Dako, Z-0334, 1:1000), rat anti-Lamp1 (Santa Cruz, 1:250, sc-199929), rabbit anti-Calbindin D28k (Invitrogen, 711443, 1:100). The sections were washed three times with PBS and incubated in secondary fluorescent antibody (Invitrogen Alexa Fluor, 1:1000) overnight at 4°C. For the Iba1 staining in the Ppt1/- mice, autofluorescence was quenched by treating the stained sections with 10 mM CuSO₄ in ammonium acetate for 15 min (Schnell et al., 1999). Typically, a total of 4 images from two sections per each mouse were captured using LSM800 confocal microscopy with a 10x or 40x objective lens or Zeiss AxioImager Z1 epifluorescence microscopy with a 5x objective lens (for Calbindin). Images were analyzed using ImageJ (version 1.50i) software with a single automated macro script to quantitate areas and intensity. Imaging and quantification were performed without knowledge of genotype or treatment.

For double knockout Grn/-;Tmem106b/- cohort, immunohistochemistry and image quantification were performed as previously reported (Takahashi et al., 2017) with modifications. The fixed brain hemispheres from 11-month-old WT, Grn/-, Tmem106b/-, and Grn/-;Tmem106b/- mice (both male and female with approximately 1:1 ratio) were embedded in 10% gelatin (Sigma G1896) and fixed in 4% PFA (Sigma 158127) for another 3 days at 4°C. Sagittal 50 µm sections were prepared using a Leica WT1000S vibratome. For GFAP/NeuN staining, two free-floating sections (approximately lateral 1.1 and 1.3 mm) were used. For pTDP-43/TDP-43 staining, one free-floating section (approximately lateral 1.2) was used and heat-mediated antigen retrieval was performed in 10 mM citrate buffer pH 6.0 for 30 min at 95°C. The sections were permeabilized and blocked with 10% normal donkey serum, 0.2% Trion X-100 in PBS for 1 hr at room temperature (RT), followed by incubation in primary antibody in 1% normal donkey serum, 0.2% Triton X-100 in PBS overnight at RT. The following primary antibodies were used: chicken anti-GFAP antibody (Abcam, ab4674, 1:500), rabbit anti-NeuN antibody (Abcam, ab177487, 1:500), mouse anti-phospho-TDP-43 (pS409/410) antibody (COSMO BIO, TIP-PTD-M01, 1:500), rabbit anti-TDP-43 antibody (Abcam, ab133547, 1:340). The sections were washed three times with PBS and incubated in Alexa Fluor secondary antibody (ThermoFisher, all 1:500) in 1% normal donkey serum, 0.2% Triton X-100 in PBS for 3 hr at RT. To quench autofluorescence, the sections were incubated in 10 mM CuSO₄ in 50 mM ammonium acetate pH 5.0 for 15 min (Schnell et al., 1999).

For GFAP staining, the images were taken using the Zeiss AxioImager Z1 epifluorescence microscopy with a 5x objective lens. For NeuN staining, the single-plane images were taken using LSM800 confocal microscopy with a 10x objective lens and 3 x 3 tile scan function in Zen software. For

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pTDP-43/TDP-43 staining, the single-plane images were taken using LSM800 confocal microscopy with a 20x objective lens.

Quantitative analyses of the GFAP/NeuN images were performed using Fiji/ImageJ (Version 1.0). All images were uniformly thresholded and binarized. GFAP area was calculated using “analyze particle” function. The number of NeuN+ cells was calculated using “analyze particle” function with “watershed” algorithm after specifying the particle size and ROI (0.6 mm x width of cortical layers). The mean of two sections was used to represent for each animal. The number of pTDP-43 inclusions in in the motor cortex was examined manually under LSM800 confocal microscopy with a 20x objective lens and the number of the inclusions in the hypothalamus was examined manually using three confocal images with high number of the inclusions and the mean was used to represent for each animal. All procedures were performed by an investigator who was blinded to the genotypes.

For spinal cord staining, 9-month-old WT and Tmem106b/-/- mice (n = 5 mice per group) were perfused in 4% PFA and spinal cords were dissected and immediately post-fixed in 4% PFA for 24 hr at 4°C. Spinal cords were cryoprotected in 30% sucrose. Thirty micron transverse sections from the lumbar enlargement were prepared using a cryostat (Leica CM1850). Six to eight sections per mouse were blocked with 1% BSA (Sigma), 1% Triton X-100 in PBS for 1 hr, followed by incubation with primary antibody (rat anti-Lamp1, Santa Cruz, sc-199929, 1:250; rabbit anti-GFAP, Dako, Z-0334, 1:1000), overnight at 4°C. The sections were washed three times with PBS and incubated in secondary fluorescent antibody (Invitrogen Alexa Fluor, 1:1000) overnight at 4°C. A total of 8 images per mouse were acquired using a Leica TCS SP8 confocal. Images were analyzed using ImageJ (version 1.50i) software with a single automated macro script to quantitate areas and particle number. Imaging and quantification were performed without knowledge of genotype.

Brain homogenization and immunobLOTS
For immunoblot, brain cortex was homogenized in RIPA buffer (50 mM Tris, 150 mM NaCl, 1 mM EDTA, 0.5 % sodium deoxycholate, 1% Triton X-100, 0.1% SDS) supplemented with PhosSTOP and 1x cOmplete Mini protease inhibitor cocktail (Roche). BCA assay (ThermoFisher Scientific) was used to determine the protein concentration in the RIPA soluble fraction. Proteins in the RIPA soluble fraction were resolved by SDS-PAGE using precast 4%–20% Tris-glycine gels (Bio-Rad) and transferred onto nitrocellulose membranes (Invitrogen) with an iBlot Dry Blotting System (Invitrogen). Membranes were incubated in blocking buffer (Rockland, MB-070-010) for 1 hr at RT and then in primary antibodies overnight at 4°C. The following primary antibodies were used: mouse anti-ATP6AP1 (Santa Cruz, 85.1, sc-81886, 1:500), rabbit anti-ATP6V1A (GeneTex, GTX110815, 1:500), rabbit anti-ATP6v0a1 (Synaptic Systems, 109003, 1:500), rabbit anti-TMEM106B (Abcam, ab140185, 1:1000) and mouse anti- β -actin (Sigma, A1978, 1:2000). Membranes were washed three times with TBST. Secondary antibodies were
applied for 1 hr at RT (Li-Cor Biosciences, 1:10,000 donkey anti-mouse, donkey anti-rabbit, donkey anti-
rat and donkey anti-goat, IRDye 680 or 800). Membranes were then washed and proteins visualized using
an Odyssey Infrared imaging system (Li-Cor Biosciences). Immunoreactive bands were quantified using
ImageJ (version 1.50i).

**Primary Neuronal cultures**

Primary cortical cultured neurons were prepared from E16-17 WT embryos as previously reported (Hu et
al., 2010) and plated onto PDL-coated 96 well plates (20,000 cells/well) and 15 mm glass coverslips.
Neurons were maintained in Neurobasal-A media (Gibco) supplemented with B27 (Gibco). For CBE
treatment, 7 DIV neurons were treated with 200, 500 or 1000 µM CBE for 7 days. CBE was added to the
media every other day.

**DNA constructs**

The SpCas9 (pX551) and single guide RNA (sgRNA, pX552) expression plasmids developed by the
Zhang lab (Swiech et al., 2015) were obtained from Addgene. To generate Gba1 and Ppt1 sgRNA, 20-nt
target sequences were chosen using the CRISPR design tool (https://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design). sgRNA sequences were selected to precede a 5’-NGG protospacer-adjacent motif sequence and prioritized based on minimal
off-target effects. The primers used to design the sgRNA targets are as follows: Gba1-1 (5’ to 3’) ACCCGTTACGAGAGCACTCGACG; Gba1-2 (5’ to 3’) ACCGGATAACTGGAAGTCGTTAG; Ppt1-1 (5’ to 3’) ACCTGTTAATGTCCAAGTCAACA; Ppt1-2 (5’ to 3’) ACCCCATGCCAGATCACCAGCGG. To generate sgRNA-expressing constructs, pX552 was digested
using SapI Fast Digest (ThermoFisher Scientific, D1934) and annealed oligos were ligated using T7 DNA
Ligase (NEB, M0318). Transformation was performed using One Shot Stbl3 Chemically Competent E.
coli (Thermo, 737303). Following maxi prep (Qiagen, 12662), Sanger sequencing confirmed correct
sgRNA insertion using the U6 promoter sequencing primer (5’ to 3’) GAGGGCCTATTTCCCATGATTC.

**AAV production**

AAV particles were produced as described (Konermann et al., 2013). Briefly, HEK293T cells were
maintained in DMEM (Gibco, 11965-092) supplemented with 10% fetal bovine serum (Gibco, 10437028)
and 1% penicillin/streptomycin (Gibco, 15140-122). Cells were passaged the day before transfection at a
density of 10^7 cells per 15 cm plate and were transfected at approximately 80% confluency. For AAV
production, 18 µg of DF6 helper plasmid, 6 µg of sgRNA expression plasmid and 6 µg of 2/1 serotype
packaging plasmid were combined in 3 ml of serum-free DMEM. After addition of 150 µl of the
transfection reagent polyethylenimine (Polysciences Inc., 23966-1), the DNA:PEI transfection mixture
was incubated at room temperature for 15 min before adding it to HEK293T cells contained in supplemented DMEM. Transfection supernatant was collected 72 hr later, filtered using a 0.22 µm cellulose acetate filter (Corning, 430769), and stored at -80°C. Viral titers were determined using iQ SYBR Green Supermix (Bio-Rad, 1708882) and quantitative PCR (Bio-Rad CFX96). Samples were compared against a standard curve derived from a virus of known titer diluted from 10^{13} to 10^{8} copies per ml.

**AAV infection in neuronal cultures**

Three DIV cortical cultured neurons were co-transduced with AAV2/1-cas9 and AAV2/1-sgRNA for \textit{Ppt1}. Neurons transduced with AAV2/1-cas9 were used as a control. Briefly, 50 µl of AAV2/1 was added to each well. The average virus titer was 10^7-10^9 GC/50µl. Media was partially changed and fresh Neurobasal media was added 48 hr after infection. Neurons were analyzed two weeks after infection.

**LysoTracker Red DND-99 staining**

Neurons plated on PDL-coated 96 well plates were stained with LysoTracker Red DND-99 (Invitrogen, L7528, 100 nM) for 10 min, washed once with Neurobasal media and then immediately imaged as previously described (Klein \textit{et al.}, 2017). MAP2 staining was performed with the same batch of neurons prepared for LysoTracker staining. Neurons were fixed with 4% PFA for 12 min and blocked with 2% BSA, 0.1% Triton X-100 in PBS for 1 hr, followed by incubation with anti-MAP2 antibody (Millipore, AB5622, 1:1000) overnight at 4°C. After washing the plates three times with PBS, neurons were incubated in Alexa Fluor 488 donkey anti-rabbit antibody (Invitrogen, 1:1000, A11034) for 1 hr. Images of LysoTracker and MAP2 staining were automatically taken using ImageXpress Micro XLS (Molecular Devices) (40x objective lens). LysoTracker-positive area, mean fluorescence intensity and integrated fluorescence intensity and MAP2-positive areas were analyzed using ImageJ (version 1.50i). A threshold for LysoTracker and MAP2 images was applied to quantify intensity and positive area.

**DNA editing confirmation using T7EI enzyme**

DNA was extracted from cultured neurons using QuickExtract DNA Extraction Solution (Epicentre, QE09050). Experimental target site was amplified by PCR using KAPA HiFi HotStart PCR Kit (Kapa Biosystems, KK2501) in a C1000 Touch Thermal Cycler system (Bio-rad). The following primer sequences, designed using the PrimerQuest Tool (IDT, http://www.idtdna.com/PrimerQuest), were used for \textit{Ppt1} sg1: GGGAAAGAACATGATGGAGGTAA (sense), GGGTGGAGAGAGATGATTTAGTG (antisense); and for \textit{Ppt1} sg2: AGAAGGCAAGGATTTCCGATGG (sense), TCACACCTGAGGCTCTATCT (antisense).
Then the Alt-R Genome Editing Detection Kit with T7 endonuclease I (T7EI) (IDT, 1075932) was used to determine on-target genome editing and estimate editing efficiency following manufacturer instructions. Digestion was visualized in a 1% agarose gel.

RNA extraction and quantitative real-time PCR (qRT-PCR)

RNA was isolated from cortical cultured neurons using Trizol reagent (Invitrogen, 15596026) extraction and Purelink RNA kit (Ambion, 10296010). Retrotranscription to first strand cDNA was performed using Maxima H Minus First Strand cDNA Synthesis Kit with dsDNase (ThermoFisher Scientific, K1681). The TaqMan gene expression assay and iQ supermix (Bio-rad, 170-8862) were used for PCR amplification and real time detection of PCR products. The following probes were used: Ppt1 (Mm00477078_m1) and Gapdh (Mm99999915_g1) from ThermoFisher Scientific. Real-time quantitative PCR was performed using the C1000 Thermal Cycler and quantified using CFX96 Real-Time System (Bio-Rad). Ppt1 mRNA expression values were normalized to the Gapdh expression level.

Behavioral tests

Mice were allowed to habituate in the testing room for at least 1 hr prior to initiating various tests. All behavioral experiments were conducted and analyzed by personnel unaware of genotype and treatment group.

Rotarod

Motor testing was performed with an accelerating rotarod apparatus (Columbus Instruments), on which the mice were trained for 2 days at a constant speed: four times at 4 rpm for 1 min on the first day and four times at 8 rpm for 1 min on the second day. On the third day, the rotarod was set to progressively accelerate from 4 to 40 rpm over 5 min, and the mice were tested four times. During the accelerating trials, the latency to fall from the rod was measured.

Elevated Plus Maze

The elevated plus maze was performed as in Klein et al, 2017. Briefly, mice were individually placed at the center of the apparatus and behavior was recorded for 10 min. Between trials, all arms were wiped with 70% ethanol and allowed to dry. Behavior was video-recorded (JVC Everio, Yokohama, Japan) and tracked by Panlab (Barcelona, Spain) Smart software.

Open field

The open field test was performed as in Klein et al, 2017. Briefly, mice were individually placed in the center of a 50-cm-wide × 50-cm-long × 40-cm-high arena with an open top. Mice were allowed to explore and move freely for 10 minutes. Total distance traveled was assessed with the Panlab software described above. The box was cleaned with 70% ethanol between trials.

Novel object recognition

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The novel object recognition test was performed as in Salazar et al., 2017 with modifications. After a one hour habituation period, an 8 min acquisition trial was conducted with two identical objects. Mice were randomly assigned to access either two 15 ml conical tubes with blue caps or two wrapped 5 ml plastic syringes (label side down) placed one inch from the edge the long axis of the cage. The test trial was performed one hour after the acquisition period. Each mouse was exposed to one 15 ml conical tube with blue cap and one wrapped 5 ml plastic syringe (label side down), wherein one was familiar and one novel. Mice were allowed to explore the cage for 5 min. All trials were recorded using a videocamera (Canon FS400). Orofacial exploration, defined as whisking or sniffing near the objects, was quantified for each object until a combined total of 30 s exploration was reached.

Wire hang
Mice were individually positioned in the center of a metal grid and then the grid was inverted. The grid was placed at a 40 cm height. The latency to fall over the course of two trials (>1 min rest between each trial) was measured. Trials were concluded for 2 min if a mouse was still hanging. For each mouse the longest hang time was selected.

Statistical analysis
Two-tailed unpaired T-test (for simple comparisons), One-way ANOVA with Tukey’s correction (for multiple comparisons), Two-way ANOVA with Sidak’s correction (for NOR test) and correlation analysis were performed using Prism (version 7.0a) software. Based on previous studies, Gaussian distributions were assumed. Data are shown as mean ± SEM. Specific N values reflecting different mice are indicated by separate dots on each Figure. Data are reported as statistically significant for p < 0.05 (*p<0.05; **p<0.005; ***p<0.001).

Data availability
The data supporting this study are available in the manuscript and Supplementary Material. Raw data will be made available on request following publication. Summary statistics including exact P-values, F-values and degrees of freedom are included in the Supplementary Material (Supplementary Table 1).

RESULTS
Hypomorphic Tmem106b allele with variable rostral-caudal effects and PGRN interaction.

The Tmem106b-/- mice are generated based on a gene trap insertion (Klein et al., 2017). While most protein expression is lost, a sensitive immunoprecipitation/blot combination experiment demonstrates a small (~10%) residual amount of immunoreactive TMEM106B protein in the Tmem106b-/- brain (Supplementary Fig. 1A). The identity of TMEM106B peptides accounting for the faint immunoreactivity was verified by liquid-chromatography-mass-spectrometric characterization of tryptic fragments (data not shown). Thus, the Tmem106b-/- gene trap line is a hypomorph, consistent with recent description (Zhou...
et al., 2020a). As reported previously, and of greatest relevance for the current study, this line shows no significant increase in astrocytosis in the cerebral cortex or hippocampus (Supplementary Fig. 1B, C) and no cortical neuronal loss at 11 months of age (Supplementary Fig. 1D, E). With regard to an interaction of the hypomorphic Tmem106b allele with PGRN deficiency in the forebrain, the Tmem106b genotype has no detectable effects on GFAP immunoreactive area or neuronal loss. In addition, we found prominent phospho-TDP-43 inclusions in the hypothalamus, but no other regions of the forebrain, of 11-month-old Grn-/- mice, which are decreased by TMEM106B reduction (Supplementary Fig. 1F, G), consistent with the rescue of 9-month-old retinal neurodegeneration (Klein et al., 2017).

In more caudal regions of the CNS, complete elimination of TMEM106B protein with CRISPR-cas9 technique causes spinal cord motoneuron vacuolation (Lüningschrör et al., 2020), and synergizes with PGRN loss to create a lethal phenotype by 5 months of age characterized by spinal cord pathology (Feng et al., 2020a, Zhou et al., 2020a). In contrast, the hypomorphic gene trap allele exhibits mild anti-LAMP1 positive lysosomal vacuolization of motoneuron initial segments at 9 months of age (Supplementary Fig., 2A, B, C) with mild spinal cord astrocytosis (Supplementary Fig. 2D, E), but there is no motor phenotype even at 20 months of age (data not shown). The hypomorphic Tmem106b-/- mice lacking PGRN survive until 11 months of age, but then develop a rapidly progressive lethal motor syndrome with ataxia and weakness, consistent with a recent report (Zhou et al., 2020a). At 11 months of age, the double knockout Grn-/-;Tmem106b-/- brainstem shows significantly greater astrocytosis than the Grn-/- samples (Supplementary Fig. 2F, G). Overall, TMEM106B loss-of-function and its interaction with PGRN are sensitive to residual protein amounts, to assay, and to CNS region.

**TMEM106B loss rescues neuronal degeneration and microgliosis induced by GCase inhibition**

In order to study the role of TMEM106B in GD, we induced the disease pharmacologically. CBE is a covalent active site-directed inhibitor of GCase that recapitulates neuronopathic GD (Kanfer et al., 1975; Farfel-Becker et al., 2011). Two cohorts of WT and Tmem106b-/- mice received daily i.p. injections of CBE at different doses (Fig. 1A). First, we focused on analyzing neuronal degeneration in layer V of the cortex (Vitner et al., 2014; Rocha et al., 2015). NeuN (Neuronal Nuclei) staining confirmed CBE-induced neuronal death in WT mice, while Tmem106b-/- mice show resistance to cell loss in both cohorts (Fig. 1B, C). In parallel to neuronal degeneration, CBE treatment induces an increase in the number and activation of microglial cells (Vitner et al., 2014; Rocha et al., 2015), measured by Iba1 and CD68, which is partially rescued in Tmem106b-/- mice (Fig. 2). These data indicate that TMEM106B depletion is protective against CBE-induced neuronal loss and microglia activation.

**CBE-induced astrocyte proliferation is not recovered by TMEM106B loss**

Astrocyte activation is a known feature of GD, and we assessed this feature in the same cohorts. GFAP (glial fibrillary acid protein) staining shows a robust increase in astrocyte number in layer V of the cortex
in both WT and Tmem106b-/- mice (Supplementary Fig. 3A, B), indicating that TMEM106B depletion does not protect against astrocytosis induced by CBE. Interestingly, GFAP positive cells show a prominent signal for the lysosomal marker Lamp1, which we designated as “Lamp1 rings” (Supplementary Fig. 3C). These Lamp1 rings do not colocalize with neurons (Supplementary Fig. 3C) or with microglial cells (Supplementary Fig. 3D).

Lipofuscin accumulation is a common feature of lysosomal storage disorders. In order to investigate a possible role of TMEM106B depletion in lipofuscin deposition we measured autofluorescent signal in different brain regions of WT and Tmem106b-/- mice treated or not with CBE. Within the time frame of this experiment, CBE treatment does not have a significant effect on lipofuscin accumulation in the cortex, hippocampus and thalamus in WT or Tmem106b-/- mice (Supplementary Fig. 4A, B).

Taken together, these results indicate that CBE treatment induces a robust astrocytosis characterized by intense Lamp1 lysosomal accumulation, which is independent of Tmem106b genotype. In contrast, lipofuscin accumulation is not characteristic of this pharmacological model of GD.

**TMEM106B deficiency partially rescues behavioral phenotypes induced by CBE treatment**

The data above show that TMEM106B depletion rescues neurodegeneration and microgliosis, but not astrocytosis, induced by CBE. Therefore, we sought to evaluate the functional consequences by assessing motor and memory behavior. In cohort 1, WT mice treated with CBE show a shorter latency to fall from the rotarod that the controls, indicating motor impairment, while Tmem106b-/- mice have normal motor performance independent of treatment (Fig. 3A). No further behavioral tests were conducted in this cohort due to the poor condition of some CBE-treated mice, indicating an end-stage of the disease. In cohort 2, treatment was discontinued before the appearance of pronounced motor deficits. CBE-treated mice in cohort 2 did not show motor impairment in the rotarod test (data not shown), confirming an earlier stage of the disease compared to cohort 1. We decided to further explore behavior with the open field test. Both WT and Tmem106b-/- mice treated with CBE travel greater distances when compared to non-treated controls (Fig. 3B), indicating hyperactivity. In order to explore whether this behavior correlated with disinhibition we performed the elevated plus maze test. Again, both WT and Tmem106b-/- mice treated with CBE show disinhibited behavior as measured by the distance traveled in the open arms (Fig. 3C), while the distance traveled was similar in the closed arms (Supplementary Fig. 5). The number of entries in the open arms was also higher in CBE-treated mice than in controls (Supplementary Fig. 5). Given the neuronal loss and gliosis observed in the cortex of CBE-treated mice, we sought to investigate whether they presented cognitive impairment using the novel object recognition test. WT and Tmem106b-/- controls prefer novelty, demonstrated by more time spent with the novel object. Interestingly, CBE-treated WT mice are unable to distinguish between novel and familiar objects and their time with each object is similar, reflecting significant memory impairment. Critically, memory performance is rescued in
Tmem106b-/- mice exposed to CBE treatment (Fig. 3D), paralleling the rescue of neurodegeneration and microgliosis described above. These data show that TMEM106B removal corrects certain behavioral phenotypes in GD mice.

**TMEM106B depletion accelerates Purkinje cell degeneration in Ppt1-/- mice**

As mentioned above, null mutations in GRN gene cause NCL and TMEM106B deficiency compensates lysosomal, behavioral, and degenerative Grn-/- phenotypes at early age. To study a potential role of TMEM106B in other NCL conditions, we used the genetic mouse model Ppt1 knockout mice (Ppt1-/-). Ppt1-/- mice develop progressive motor abnormalities, autofluorescent material storage (lipofuscin) and neuronal loss, leading to early death by 10 months of age (Gupta et al., 2001). PPT1 (palmitoyl-protein thioesterase 1) is a lysosomal thioesterase that catalyzes the hydrolysis of long chain fatty acyl CoAs. In addition to this function, PPT1 hydrolyzes fatty acids from modified cysteine residues in proteins that are undergoing degradation in the lysosome.

To investigate whether TMEM106B removal modulates PPT1-dependent NCL progression we crossbred Tmem106b-/- mice with Ppt1-/- mice. Typically, Ppt1-/- mice show motor symptoms and brain pathology at 6 months of age (Macauley et al., 2009). However, we observed some decline and early death in the double knockout Ppt1-/-;Tmem106b-/- mice starting at 4 months of age (Fig. 7A). Therefore, we collected tissue and performed histological and biochemical analyses at 5 months. First, we analyzed neuronal degeneration in the cerebellum of WT, Ppt1-/-, Tmem106b-/- and double knockout Ppt1-/-;Tmem106b-/- mice. As Purkinje cell loss is a hallmark of Ppt1-/- mice (Macauley et al., 2009), we quantified the number of Purkinje cells in sections stained for calbindin, a canonical Purkinje cell marker. Double knockout Ppt1-/-;Tmem106b-/- mice show significantly greater Purkinje cell loss compared to Ppt1-/- mice in anterior and mid cerebellar regions, indicating an acceleration of the Ppt1-/- phenotype induced by TMEM106B depletion (Fig. 4A, B). We next sought to analyze the lysosomal status of the remaining Purkinje cells. Co-staining of Purkinje cells and the lysosomal protein Lamp1 revealed a significant lysosomal enlargement in both Ppt1-/- and double knockout Ppt1-/-;Tmem106b-/- mice (Fig. 4C, D). In addition to the cerebellum, we further analyzed neuronal death in other brain regions. NeuN staining and neuronal counting revealed a significant neuronal loss in double knockout Ppt1-/-; Tmem106b-/- mice in the cortex (Fig. 4E, F) and CA1 region of the hippocampus (Fig. 4G, H). In line with observations in the cerebellum, a significant Lamp1-particle enlargement is also present in hippocampal CA1 neurons in both Ppt1-/- and double knockout Ppt1-/-;Tmem106b-/- mice (Supplementary Fig. 6A, B), indicating that TMEM106B depletion does not exacerbate lysosomal accumulation. Consistent with these observations, lipofuscin accumulation (Gupta et al., 2001) in different brain regions (cortex, hippocampus and cerebellum) is similarly increased in Ppt1-/- and double knockout Ppt1-/-; Tmem106b-/- mice (Supplementary Fig. 7A, B). Altogether, these data show that
TMEM106B deficiency accelerates Purkinje cell loss and neuronal death in the cortex and hippocampus without causing an increase in lysosomal histopathology in Ppt1-/- mice.

**Gliosis is exacerbated in different brain regions of Ppt1-/- mice after TMEM106B depletion**

Given that TMEM106B depletion accelerated neurodegeneration in Ppt1-/- mice without affecting lysosomal histopathology, we examined astrocytosis and microgliosis (Macauley et al., 2009) in the cortex, hippocampus and cerebellum of double knockout Ppt1-/-;Tmem106b-/- mice. GFAP staining shows significant astrocytosis in Ppt1-/- mice compared to WT and Tmem106b-/- controls in the different brain regions analyzed. Interestingly, astrocytosis is exacerbated in double knockout Ppt1-/-;Tmem106b-/- mice in certain brain regions (Fig. 5). Parallel to astrocyte activation, Iba1 and CD68 staining confirms significant microglia activation in Ppt1-/- mice. Microgliosis is also worsened by TMEM106B depletion in some brain regions (Fig. 6). Interestingly, astrocytosis and microgliosis correlate with neurodegeneration in most of the brain regions analyzed, being this correlation higher in the cerebellum and hippocampus than cortex (Supplementary Fig. 8). Similar to the robust lysosomal Lamp1 signal observed within astrocytes in CBE-treated mice (Supplementary Fig. 3), Ppt1-/- astrocytes display substantial Lamp1 clusters (Supplementary Fig. 9A, C, D) that are not found in microglial cells (Supplementary Fig. 9B). Quantification of Lamp1 clusters within GFAP-positive cells (astrocytes) shows equal significant increase in lysosomal pathology in Ppt1-/- and double knockout Ppt1-/-;Tmem106b-/- mice (Supplementary Fig. 6C, D). These results confirm that TMEM106B depletion exacerbates astrocytosis and microgliosis in several brain regions of Ppt1-/-, which correlate with neuronal loss.

**TMEM106B loss worsens motor performance in Ppt1-/- mice**

We next sought to evaluate whether the acceleration of neurodegeneration and gliosis in double knockout Ppt1-/-;Tmem106b-/- mice translates into a functional decline. As described above, we observed a decrease in survival rate in double knockout Ppt1-/-;Tmem106b-/- mice starting at around 4 months of age (Fig. 7A). At 5 months of age, Ppt1-/- mice do not show motor impairment in the rotarod (Fig. 7B) or wire hang (Fig. 7C) test compared to WT and Tmem106b-/- controls. Interestingly, double knockout Ppt1-/-;Tmem106b-/- mice exhibit reduced latency to fall from the rotarod and wire hang test. These observations confirm the acceleration of motor impairment in Ppt1-/- mice driven by TMEM106B depletion.

**Altered lysosomal acidification in GCase-inhibited versus PPT1 null neurons**

Given the opposing effects of TMEM106B depletion in GD and NCL, we investigated the mechanisms by which TMEM106B deficiency orchestrates the divergent effects. TMEM106B protein levels are not altered in WT CBE-treated or Ppt1-/- mice (Supplementary Fig. 10A-D). We previously showed that TMEM106B interacts with V-ATPase and participates in lysosomal acidification (Klein et al., 2017).
Therefore, we imaged the acidic lysosomal compartment with the pH-sensitive dye LysoTracker red DND-99 (Chen-Plotkin et al., 2012; Busch et al., 2016; Fassio et al., 2018). CBE treatment in WT cultured neurons significantly increases LysoTracker-positive integrated fluorescence intensity despite a comparable cell density between control and CBE-treated neurons (Fig. 8A,B). Thus, lysosomal acidification is not impaired, and appears to be enhanced after GCase inhibition. The increased LysoTracker red DND-99 staining after CBE treatment is opposes the Tmem106b-/- neuronal phenotype (Klein et al., 2017), providing a potential explanation for the rescue of some CBE phenotypes by TMEM106B deletion.

We sought to determine whether PPT1 loss might have an opposite effect to CBE treatment with regard to lysosomal acidification. In order to study the effects of PPT1 loss specifically in neurons prior to the development of in vivo pathologies, we edited the Ppt1 locus in tissue culture via CRISPR-cas9. WT cultured neurons were transduced with AAV2/1 virus expressing two single guide (sg) RNAs against Ppt1. T7EI digestion confirmed Ppt1 DNA editing (Fig. 8C) and qPCR analyses revealed a decrease in Ppt1 mRNA with both sgRNAs (Fig. 8D). The Ppt1 reduction via CRISPR-cas9 editing significantly reduces V-ATPase levels (Fig. 8E, F). Examination of brain tissue from Ppt1-/- mice revealed a similar lowering of V-ATPase levels compared to WT controls (Fig. 8G, H). These data are consistent with recent observations pointing to V-ATPase processing and trafficking alterations in Ppt1-/- mice (Bagh et al., 2017). Therefore, we assessed the acidic lysosome compartment of PPT1-deficient neurons. Ppt1 downregulation with CRISPR-cas9 editing in neurons leads to significant reduction in LysoTracker red DND-99 positive area, suggesting impairment in lysosomal acidification in the absence of PPT1 (Fig. 8I, J). Significant reduction in V-ATPase subunits was also observed in cortical brain extracts from double knockout Ppt1-/-;Tmem106b-/- mice (Fig. 8K, L).

These results support the hypothesis that CBE-induced alterations in lysosomal acidification are partially counteracted by TMEM106B depletion, similar to the interaction of TMEM106B and PGRN deficiencies (Klein et al., 2017). On the other hand, PPT1 deficiency destabilizes V-ATPase levels, causing a lysosomal acidification deficit that is further exacerbated by TMEM106B depletion.

DISCUSSION
The major finding of the present study is that TMEM106B deficiency rescues neurodegeneration in a GD model while exacerbating neurodegeneration in a PPT1-dependent NCL model. The GD rescue by TMEM106B deletion parallels a previous study in PGRN-dependent neurodegeneration (Klein et al., 2017). The rescue of GD-related neurodegeneration is accompanied by improved memory and motor function, as well as a reversal of microgliosis, despite continued astrocytosis and disinhibition behavior. A distinguishing feature of the different neurodegenerative conditions is their effect on V-ATPase levels
and lysosomal pH. For GCase inhibition, the acidic lysosomal compartment is increased, and the consequences are counteracted by TMEM106B loss, which suppresses V-ATPase (Klein et al., 2017). For PPT1 neuronal deficiency, V-ATPase levels are suppressed and this is exacerbated by TMEM106B deficiency. Overall, these studies reveal a modulating effect of the TMEM106B/V-ATPase interaction on neurodegeneration, with the net benefit or detriment of TMEM106B loss varying as a function of V-ATPase function in different neurodegenerative conditions.

For the GD model, the rescuing effect of TMEM106B loss was restricted to neurodegeneration, microgliosis, memory and motor function, but not astrogliosis or disinhibition. This suggests differences in GCase-dependent cellular dysfunction for neurons and astrocytes. Interestingly, some type 1 GD (non-neuronopathic) patients show astrogliosis without significant neuronal loss (Wong et al., 2004). The astrocytic phenotype of the CBE-treated mice included striking LAMP1-positive lysosomal rings. While the basis of this cellular morphology will require future studies, it is not TMEM106B-dependent. In the PPT1 model, astrogliosis is affected by TMEM106B loss to the same extent as microgliosis and neurodegeneration. Thus, the TMEM106B-independence of astrocyte pathology is unique to the GD model.

The reduced memory, motor and microglial phenotypes in CBE-treated Tmem106b/-/- mice may be secondary to neuronal sparing. While sustained microglial activation is observed in GD patients and in mouse models of the disease, the role that microglial cells play in GD is not well understood. Traditionally, microglia have been considered harmful in several neurodegenerative diseases due to cytokine release and neuroinflammation. However, recent data indicate that microglial cells can perform different tasks and function in both positive and negative ways in different mouse disease models (Song and Colonna, 2018). A GD mouse model with GCase deficiency restricted to neuronal and glial precursors and their progeny, but not to microglia, shows symptoms similar to those observed in mice with reduced GCase activity in all tissues except the skin but with a slower phenotypic progression (Enquist et al., 2007). Both models present neuronal loss and microgliosis, supporting the idea that GCase function is critical for neuronal survival. In addition, these results suggest that GCase-deficient microglia may not be the primary determinants of the CNS pathogenesis in GCase-deficient mice, but they contribute by influencing the onset and progression of the disease (Enquist et al., 2007). Conversely, a previous study has shown that GCase deficiency in dopaminergic neurons is not sufficient to induce neurodegeneration, but it causes sustained microglia activation, pointing to GCase deficiency in other cell types as a potential pathological mechanism (Soria et al., 2017). A cytotoxic role for microglia is also suggested after a critical threshold of GlcCer storage is reached in neurons, inducing the release of inflammatory cytokines by microglia that amplifies the inflammatory response and contributes to
neuronal death (Vitner et al., 2012). Further studies are needed in order to clarify the role of microglia in neurodegeneration in GD.

Deficiency of several different lysosomal genes can result in NCL, including PPT1 and PGRN. Despite shared NCL neuropathology (Ward et al., 2017), certain aspects of PGRN-dependent neurodegeneration are rescued by TMEM106B loss (Klein et al., 2017), whereas PPT1-dependent neurodegeneration is strongly exacerbated. These conditions share lipofuscinosis, but the presence of lipofuscin does not correlate with outcome, consistent with the notion that the accumulated proteolipid itself is not directly responsible for degeneration. This is consistent with the observation of lipofuscin in healthy aged brain without neurodegeneration (Moreno-García et al., 2018).

The opposite effects of TMEM106B deficiency in the GD and PPT1-NCL models are striking, but are also present in the PGRN deficient state. For example, PGRN-null lipofuscinosis is not rescued by TMEM106B deficiency while retinal neurodegeneration, behavioral deficits and lysosomal dysregulation are reversed in young adult mice (Klein et al., 2017). In addition, after 11 months of age, double knockout Grn-/-;Tmem106b-/- mice develop synthetic phenotypes with brainstem and spinal cord gliosis coupled to weakness and ataxia leading to poor feeding and death (Zhou et al., 2020a). These opposing effects are consistent with TMEM106B modulating a pathway such as lysosomal acidification that participates differentially in various degenerative mechanisms and at different disease stages. Importantly, these phenotypes are all manifest with low levels of residual TMEM106B protein in hypomorphic Tmem106b-/- mice. TMEM106B protein levels have non-linear effect, such that experimentally complete loss of TMEM106B and PGRN leads to a severe and earlier lysosomal deficits and neurodegeneration in spinal cord (Feng et al., 2020a, Zhou et al., 2020a). Partial reduction of TMEM106B may have greater relevance to common variants of TMEM106B in humans and any prospects for TMEM106B-directed therapeutic approaches.

Previously, we found that TMEM106B physically associates with subunits of V-ATPase, including subunit AP1, and participates in maintaining expression levels of V-ATPase and lysosomal acidification. The dysregulation of V-ATPase and lysosomal pH predicts the opposing action of TMEM106B null state on neurodegeneration in GD and PPT1-dependent NCL. For the GCase inhibition model of GD, the acidic lysosome compartment of neurons is enhanced, and V-ATPase is not lost even with neurodegeneration. As for PGRN deficiency, TMEM106B reduction has neurodegeneration-rescuing effect. In contrast, PPT1 deficiency leads to a reduction in V-ATPase and impairment of lysosomal acidification, which is exacerbated by TMEM106B deletion. Thus, disease-specific changes in lysosomal pH and V-ATPase level appear to predict whether the effect of reduced V-ATPase caused by TMEM106B deletion corrects a deficit or moves lysosomal pH further from normal with degenerative consequences.
In this study and previously, we have considered the role of TMEM106B in lysosomal storage disorders and FTLD, but the gene is implicated genetically in other degenerative conditions, including AD. Hippocampal sclerosis is negatively associated with the FTLD-protective TMEM106B allele. The linkage of GCase heterozygosity with PD risk implies that TMEM106B loss-of-function may ameliorate PD as well.

The mechanistic studies implicating TMEM106B ability to maintain steady state protein levels of V-ATPase and lysosomal acidification suggest a potential therapeutic intervention site. Pharmacological agents blocking the TMEM106B interaction with V-ATPase, or partially reducing the catalytic function of the V-ATPase itself, are predicted to mimic TMEM106B reduction. In this sense, such compounds may have therapeutic utility to reduce neurodegeneration in PGRN deficiency, GCase deficiency, AD and PD. For the neurodegeneration of PPT1 deficiency, the same compounds or other agents with V-ATPase inhibiting activity may be deleterious. Overall, our molecular studies of TMEM106B provide new insights into disease-specific neurodegenerative mechanisms.

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Competing interests
The authors report no competing interests.

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Figure legends

Figure 1. TMEM106B deficiency protects against neuronal death induced by CBE.
A Schematic representation of experimental design.
B Representative images of cortex in WT and Tmem106b-/- mice from cohort 1 (top panels) and cohort 2 (bottom panels), treated or not with CBE, stained with anti-NeuN antibody (neurons). Blue lines indicate layer V. Scale bars = 100 µm.
C Graphs show mean ± SEM of number of positive cells or area in the layer V of the cortex. For cohort 1, n = 5-6 mice per group; for cohort 2, n = 6-8 mice per group.

Figure 2. TMEM106B loss is protective against microgliosis induced by CBE.
A Representative images of cortex in WT and Tmem106b-/- mice from cohort 1, treated or not with CBE, stained with anti-Iba1 and CD68 antibodies (microglia). Scale bars = 50 µm.
B Graphs show mean ± SEM of number and area of the soma of Iba1+ cells and CD68+ area in the layer V of the cortex. n = 5-6 mice per group.
C Representative images of cortex in WT and *Tmem106b*−/− mice from cohort 2, treated or not with CBE, stained with anti-Iba1 antibody (microglia). Scale bars = 50 µm.

D Graphs show mean ± SEM of number and area of the soma of Iba1+ cells in the layer V of the cortex. n = 6-8 mice per group.

Figure 3. TMEM106B depletion protects against some behavioral phenotypes induced by CBE.

A Graph shows mean ± SEM of time spent in the rotarod test in WT and *Tmem106b*−/− mice from cohort 1 treated or not with CBE. n = 5-6 mice per group.

B Graph shows mean ± SEM of distance traveled in the open field test in WT and *Tmem106b*−/− mice from cohort 2 treated or not with CBE. n = 6-8 mice per group.

C Graph shows mean ± SEM of distance traveled in the open arms of the elevated plus maze in WT and *Tmem106b*−/− mice from cohort 2 treated or not with CBE. n = 6-8 mice per group.

D Graphs show mean ± SEM of exploration time of familiar (F) and novel (N) objects and discrimination index calculated as follows: (novel − familiar)/(novel + familiar); in the novel object recognition test in WT and *Tmem106b*−/− mice from cohort 2 treated or not with CBE. n = 6-8 mice per group.

Figure 4. TMEM106B deletion accelerates neuronal death in *Ppt1*−/− mice, a model of infantile neuronal ceroid lipofuscinosis.

A Representative images of anterior (I-V), mid (VI-VIII) and posterior (IX-X) cerebellar lobes stained with anti-calbindin D28k antibody in 5-month-old WT, *Ppt1*−/−, *Tmem106b*−/− and *Ppt1*−/−;*Tmem106b*−/− mice. Scale bars = 200 µm.

B Graph shows mean ± SEM of number of calbindin positive cells in the different cerebellar regions. n = 4-9 mice per group.

C Representative images of Purkinje cells in 5-month-old WT, *Ppt1*−/−, *Tmem106b*−/− and *Ppt1*−/−;*Tmem106b*−/− mice stained with anti-Calbindin D28k and Lamp1 antibodies. Scale bar = 10 µm. n = 4-9 mice per group.

D Graphs show mean ± SEM of Lamp1 area, number of particles and particle size in Purkinje cells. n = 4-7 mice per group.

E Representative images of cerebral cortex in 5-month-old WT, *Ppt1*−/−, *Tmem106b*−/− and *Ppt1*−/−;*Tmem106b*−/− mice stained with anti-NeuN antibody. Scale bar = 100 µm.

F Graph shows mean ± SEM of NeuN+ cells per area in the cortex. n = 5-12 mice per group.

G Representative images of hippocampus (CA1) in 5-month-old WT, *Ppt1*−/−, *Tmem106b*−/− and *Ppt1*−/−;*Tmem106b*−/− mice stained with anti-NeuN antibody. Scale bar = 20 µm.
H Graph shows mean ± SEM of NeuN+ cells per area in the hippocampus (CA1). n = 5-12 mice per group.

Figure 5. TMEM106B deletion exacerbates astrogliosis in the cortex of Ppt1/-/- mice.
A Representative images of cortex in 5-month-old WT, Ppt1/-/-, Tmem106b/-/- and Ppt1/-/-;Tmem106b/-/- mice stained with anti-GFAP antibody. Scale bar = 50 µm.
B Graphs show mean ± SEM of number of GFAP+ cells per area (right) or GFAP+ area (left) in the cortex. n = 6-12 mice per group.
C Representative images of hippocampus (CA1) in 5-month-old WT, Ppt1/-/-, Tmem106b/-/- and Ppt1/-/-;Tmem106b/-/- mice stained with anti-GFAP antibody. Scale bar = 50 µm.
D Graphs show mean ± SEM of number of GFAP+ cells per area (right) or GFAP+ area (left) in the hippocampus. n = 6-12 mice per group.
E Representative images of cerebellum in 5-month-old WT, Ppt1/-/-, Tmem106b/-/- and Ppt1/-/-;Tmem106b/-/- mice stained with anti-GFAP antibody. Scale bar = 100 µm.
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Figure 6. TMEM106B deletion exacerbates microgliosis in different brain regions in Ppt1/-/- mice.
A Representative images of cortex in 5-month-old WT, Ppt1/-/-, Tmem106b/-/- and Ppt1/-/-;Tmem106b/-/- mice stained with anti-Iba1 and CD68 antibodies. Scale bars = 50 µm.
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A Survival curves of WT, Ppt1/-/-, Tmem106b+/-, Tmem106b-/-, and Ppt1/-/-;Tmem106b+/- and Ppt1/-/-;Tmem106b/-/- mice. n = 8-13 mice per group.
B Graph shows mean ± SEM of time spent in the Rotarod test. n = 8-12 mice per group.
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**Figure 8. Opposite effect of CBE and PPT1 deficiency on lysosomal acidification.**

A Representative images of primary cortical cultured neurons treated with CBE for 7 days at different concentrations and stained with anti-MAP2 antibody and LysoTracker Red DND-99. Scale bar = 50 µm.

B Graphs show mean ± SEM LysoTracker-red-DND-99 integrated fluorescence intensity and MAP2 positive area from one representative experiment. n = 36-54 images.

C Representative agarose gel showing confirmation of *Ppt1* DNA editing by T7EI digestion in cultured neurons two weeks after AAV2/1 infection. Cortical cultured neurons were infected with AAV2/1 expressing two single guide *Ppt1* RNA sequences (sg) at 3 DIV.

D *Ppt1* mRNA expression in cultured neurons two weeks after AAV2/1 infection. n = 3 independent experiments.

E Representative immunoblots with anti-ATP6V1A and β-actin using cortical cultured neuronal lysates two weeks after AAV2/1 infection. Full blots for this other panels are in Supplementary Figure 11.

F Graph shows mean ± SEM of the immunoblot signal from E. n = 3 independent experiments.

G Representative immunoblots with anti-ATP6AP1, ATP6V1A and β-actin using cortical lysates from WT and *Ppt1*−/− mice.

H Graphs show mean ± SEM of the immunoblot signal from G. n = 6-9 mice per group.

I Representative images of primary cortical cultured neurons infected with AAV2/1. Two weeks later neurons were stained with anti-MAP2 antibody and LysoTracker Red DND-99. Scale bar = 100 µm.

J Graphs show mean ± SEM LysoTracker-red-DND-99 integrated fluorescence intensity and MAP2 positive area from one representative experiment. n = 50-58 images.

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The role of TMEM106B in modifying the progression of lysosomal storage disorders is investigated here. TMEM106B depletion ameliorates neuronal degeneration and some behavioral abnormalities in a pharmacological model of Gaucher disease. On the other hand, TMEM106B loss exacerbates Purkinje cell degeneration and motor deficits in a neuronal ceroid lipofuscinosis genetic mouse model.