

Supplemental Information

**The FTLD Risk Factor TMEM106B
Regulates the Transport of Lysosomes
at the Axon Initial Segment of Motoneurons**

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SUPPLEMENTAL INFORMATIONS

Figure S1

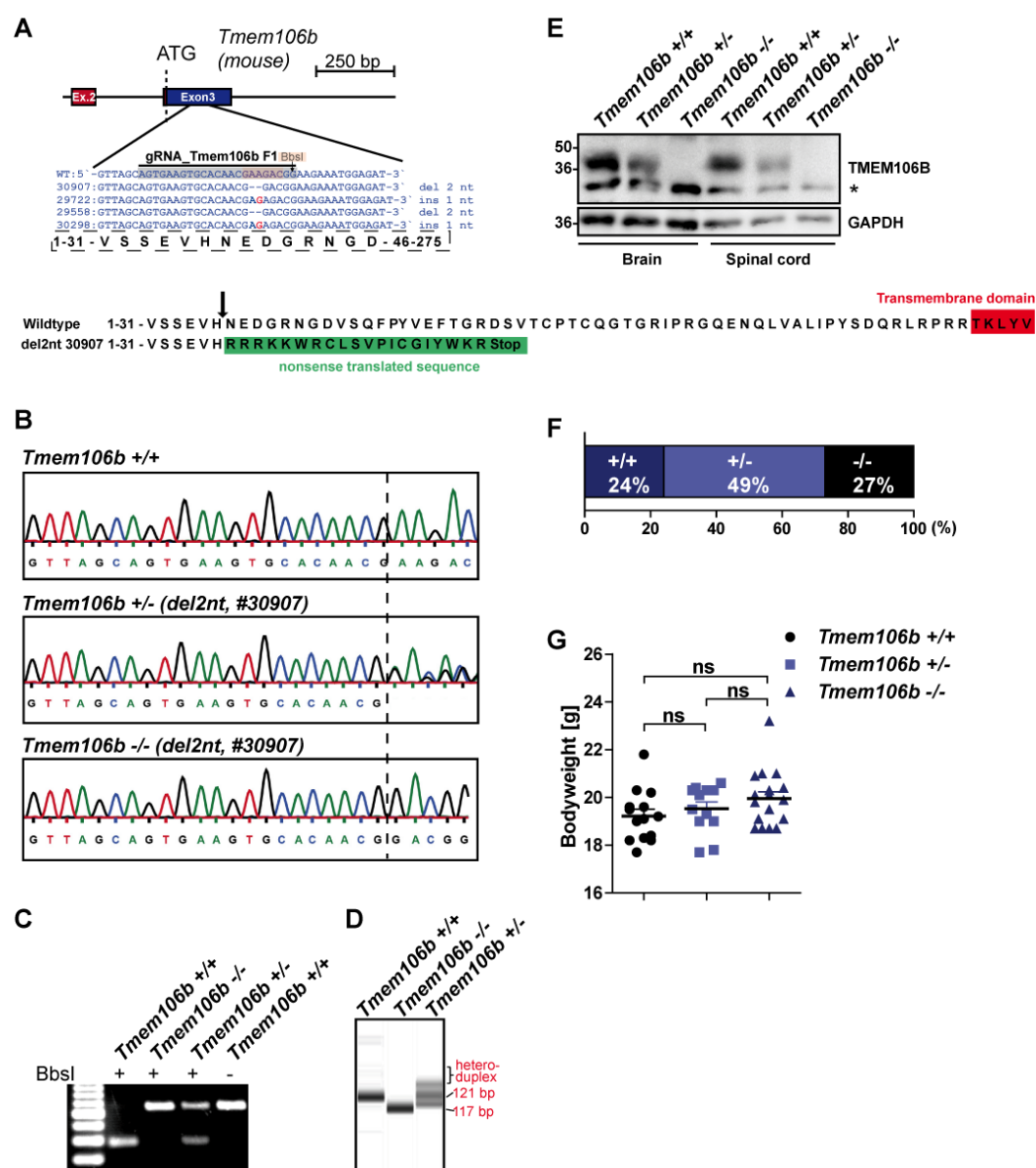


Figure S1. Related to Figure 1. CRISPR/Cas9-mediated generation and genotyping of *Tmem106b* knockout mice. (A) Schematic representation of the murine *Tmem106b* locus covering exon 2 and the first coding exon 3 containing the ATG start codon. Excerpt of the sequence of genomic tail DNA of several founder lines (30907; 29722; 29558; 30298) with the resulting genomic modifications covering the target sequence of the guide (g)RNA is shown. Recognition site of the restriction enzyme BbsI is indicated. The coded amino acid sequence is indicated below the nucleotide sequence. The predicted amino acid sequence of the strain 30907 with the 2 nucleotide deletion leading to a frameshift and premature stop-codon is depicted below in comparison to the wildtype sequence. **(B)** Representative sequencing chromatogram of founder line 30907 containing a 2

nucleotide deletion (del2nt) either heterozygous or homozygous is shown in comparison to the non-edited wildtype sequence. **(C)** Agarose gel of the PCR product for initial genotyping generating a 552bp fragment (right lane) that is further digested with the restriction enzyme BbsI. In the case of an intact recognition site for BbsI, two fragments are generated after restriction digest (274 & 276bp) (left lane). Homozygous CRISPR/Cas9-mediated genomic changes lead to the complete loss of the BbsI cleavage site. Heterozygous animals show both, undigested (552bp) and digested bands (274 and 276bp). **(D)** Electrophoresis chromatogram of the capillary-electrophoresis-based genotyping-PCR. A smaller PCR-fragment (121bp in wildtype mice) covering the CRISPR/Cas9-edited genomic sequence is generated with genomic tail-DNA as template. Capillary electrophoresis efficiently separates the 2bp size-difference between homozygous wildtype and knockout animals. In heterozygous animals, an additional band is observed, presumably due to the formation of a heteroduplex of the small fragments. **(E)** Immunoblot analysis of *Tmem106b* $+/+$, $+/-$ and $-/-$ brain- and spinal cord lysates with an antibody against TMEM106B (Bethyl-Laboratories; A303-439A) and GAPDH as loading control. **(F)** Genotype distribution of litters from *Tmem106b* heterozygote couples. (n = 102 animals in total). **(G)** Total bodyweight of 3 months old *Tmem106b* $+/+$, $+/-$ and $-/-$ animals. N = 12-17. Student's t-test; ns = not significant.

Figure S2

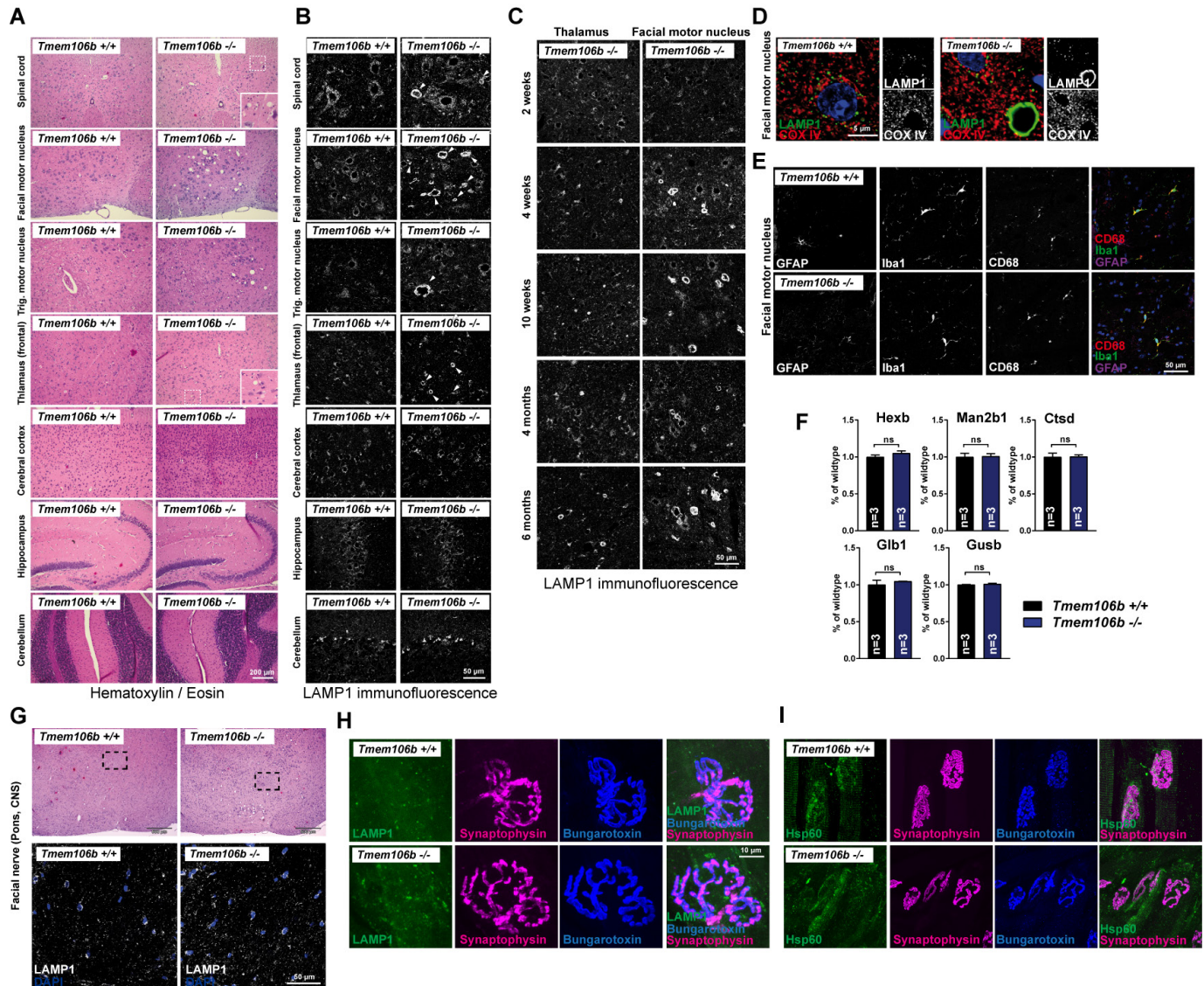


Figure S2. Related to Figure 1. Vacuolization phenotype of *Tmem106b* KO mice. (A) Hematoxylin / Eosin staining of sagittal sections of 4-months-old wildtype and *Tmem106b* KO mice of the indicated brain regions. **(B)** Immunofluorescence staining of wildtype and *Tmem106b* KO mice of the corresponding regions to (A) for LAMP1. Age: 4 months. **(C)** Immunofluorescence staining of the FMN of *Tmem106b* KO mice for LAMP1 at indicated the age. **(D)** Immunofluorescence staining for LAMP1 (green) and the mitochondria-marker COX IV (red). Nuclei are stained with DAPI (blue). The large vacuole is negative for Cox IV. **(E)** Immunofluorescence staining of CD68 (red), GFAP (magenta) and Iba-1 (green) of the FMN of 4-months-old wildtype and *Tmem106b* KO mice. Nuclei are stained with DAPI (blue). **(F)** Transcript levels of *Hexb*, *Man2b1*, *Ctsd*, *Glb1*, and *Gusb* determined by qPCR from wildtype and *Tmem106b* KO mice. The mean of the wildtype was set as 1 and the ratio of the average of the KO animals is depicted. Age: 6 months. (Mean \pm SEM, n = 3). **(G)** Hematoxylin / Eosin staining of the Pons / Medulla of wildtype and *Tmem106b* KO mice. The compact facial

nerve is boxed. Immunofluorescence staining of the compact facial nerve for LAMP1 does not show any vacuoles in *Tmem106b* KO mice. Nuclei are stained with DAPI (blue). Age: 4 months. **(H)** (Immuno-)fluorescence staining of neuromuscular junctions (NMJ) of the orbicularis oculi muscle of six months-old *Tmem106b* KO and control animals with fluorescently labelled Bungarotoxin (blue) and antibodies against synaptophysin (magenta) and LAMP1 (green). **(I)** (Immuno-)fluorescence staining of neuromuscular junctions (NMJ) of the orbicularis oculi muscle of six months-old *Tmem106b* and control animals with fluorescently labelled Bungarotoxin (blue) and antibodies against synaptophysin (magenta) and Hsp60 (green).

Figure S3

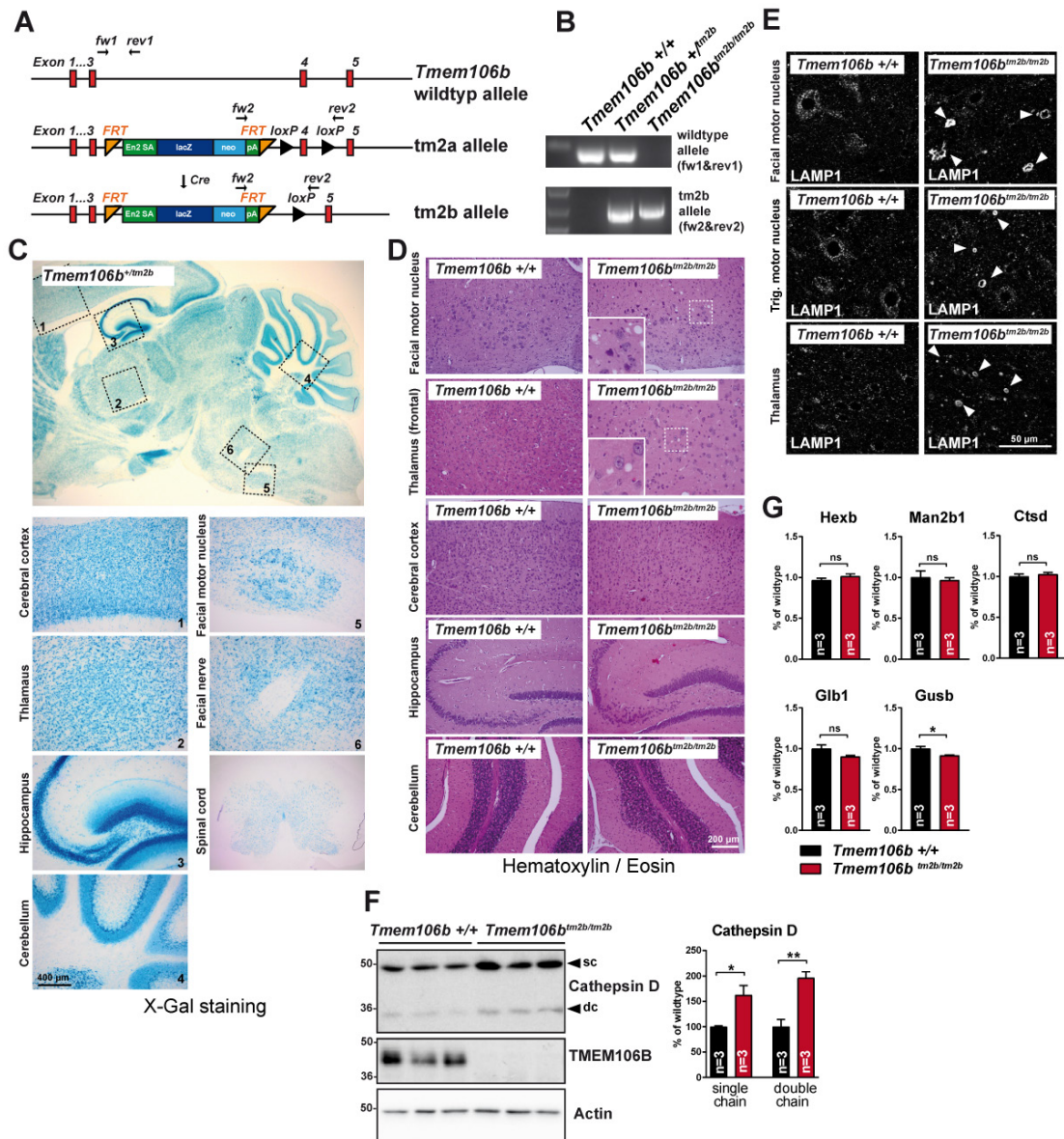


Figure S3. Related to Figure 1. Generation and characterization of *Tmem106b* KO mice generated by targeted ES-cells (*Tmem106b*^{tm2b}). (A) Schematic representation of the *Tmem106b* tm2a-targeting construct. The coding exons, recombinase sites (FRT for Flippase, Cre for Cre-recombinase) and the knockout construct containing the artificial splice-acceptor site (EN SA), LacZ, neo-cassette for selection and the polyadenylation site (pA) are indicated. The conversion of the tm2a allele to tm2b by crossing with Cre-recombinase expressing mice is indicated. (B) X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside)-staining of the brain of an adult (3-month-old) old heterozygote *Tmem106b* tm2a-mouse expressing the LacZ reporter gene under the control of the endogenous *Tmem106b* promoter. (C) Genotyping PCR for the tm2b allele. The primer binding site is indicated in (A). (D) Hematoxylin / Eosin staining of sagittal sections of the indicated brain regions. Age: 6 months. (E) Immunofluorescence staining of the indicated brain regions for LAMP1. Age: 6 months. (F)

Immunoblot analysis of brain lysates from 4-months-old *Tmem106b^{tm2b}* KO and wildtype control mice for cathepsin D, TMEM106B and actin as a loading control. Quantification of the cathepsin D signal (Mean \pm SEM, n = 3). (G) Transcript levels of *Hexb*, *Man2b1*, *Ctsd*, *Glb1*, and *Gusb* determined by qPCR from wildtype and *Tmem106b^{tm2b}* KO mice. The mean of the wildtype was set as 1 and the ratio of the average of the KO animals is depicted. Age: 4 months. (Mean \pm SEM, n = 3).

Figure S4

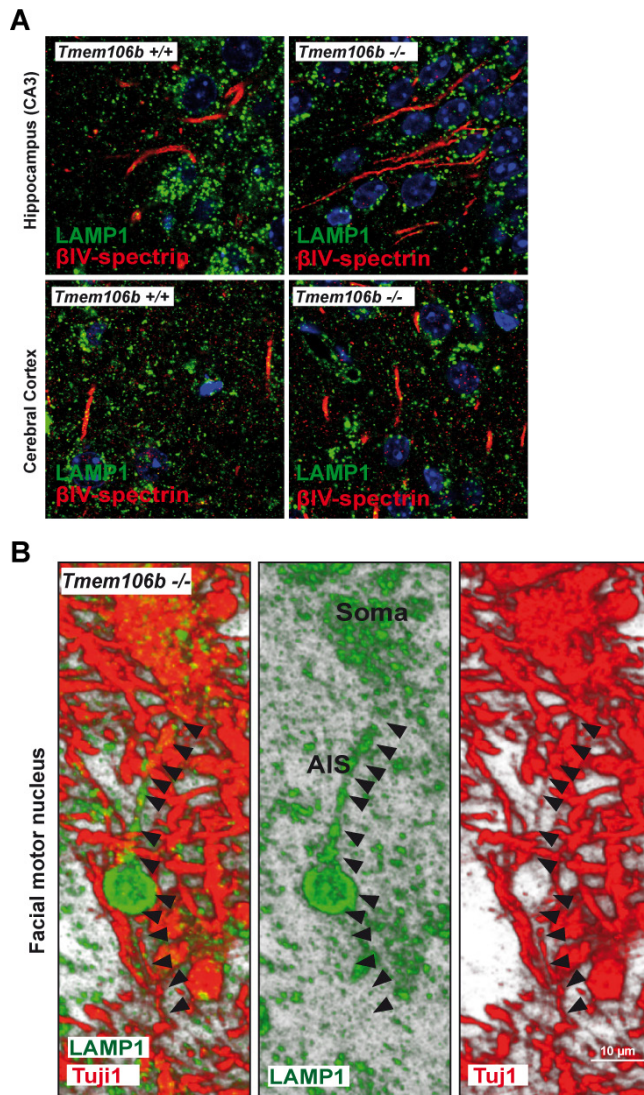


Figure S4. Related to Figure 1. Vacuolisation of facial motor neurons does not lead to axonopathy and axonal vacuoles are absent in the cerebral cortex and the hippocampus. (A) Immunofluorescence of LAMP1 (green) and β -VI Spectrin (red) of the hippocampus (CA3-region) and the cerebral cortex. Nuclei are stained with DAPI (blue). Age: two months. **(B)** Maximum intensity Z-projection of confocal stacks of LAMP1 (green) and β III-tubulin (red) staining of the FMN. The axon of the depicted motoneuron is marked by arrowheads. Age: two months.

Figure S5

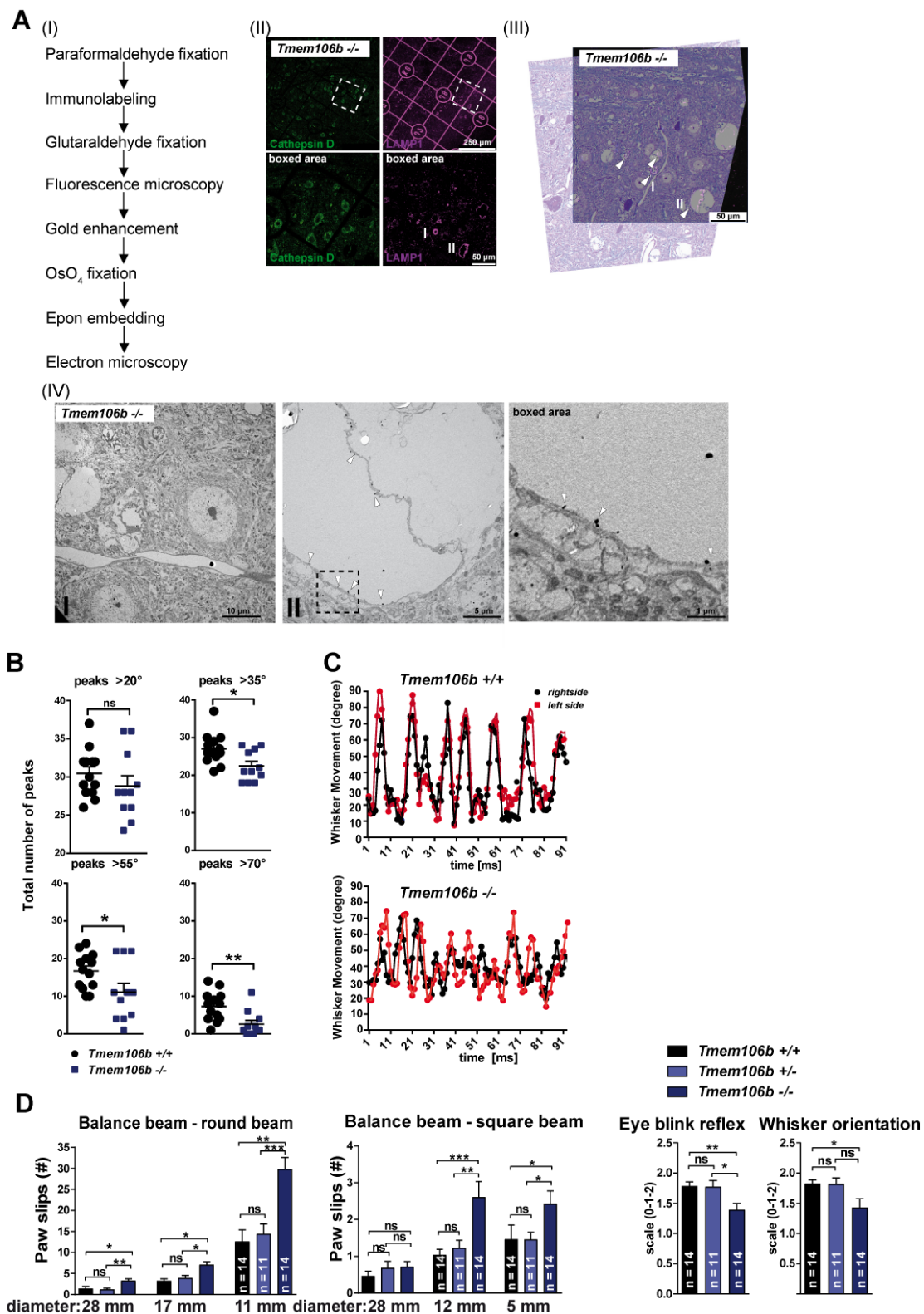


Figure S5. Related to Figure 2. Correlative light and electron microscopy and additional whisker movement data. (A) (I) Schematic overview of the workflow for correlative light and electron microscopy (CLEM). (II) Co-immunofluorescence for cathepsin D (green) and LAMP1 (magenta). The fluorescent grid for

CLEM is depicted in the magenta channel. (III) Merge of the toluidine-blue stained section and the corresponding fluorescent image. Vacuoles are labeled with arrow heads. Two vacuoles analyzed by EM are labeled with I and II. (IV) EM of the vacuoles depicted in (II). Cathepsin D immunolabeling after gold enhancement is labelled with arrowheads. **(B)** Quantification of whisker movement expressed as the total number of peaks $>20^\circ$, $<35^\circ$, $<35^\circ$ and $<70^\circ$ from *Tmem106B* KO mouse and wildtype control mice. The total number of peaks is depicted ($n = 11-13$; each point represents one animal; age: 6 months). **(C)** The whisker movement of the right and left whisker (expressed as the movement in degree over time) of a single, representative control and *TMEM106B* KO mouse is depicted. **(D)** Quantification of the eye blink and whisker-orienting reflex in *Tmem106b* $+/+$, $+/-$ and $-/-$ mice ($n = 11-16$). The average of 6 assessments was used for analysis. 3-point scale (0: absent – 1: reduced – 2: normal). Age: 14 months Quantification of neuromotor function: number of paw slips during balance beam traversal (square and round beams of *Tmem106b* $+/+$, $+/-$ and $-/-$ mice (Age 14 months); (Mean \pm SEM, $n = 11-16$).

Figure S6

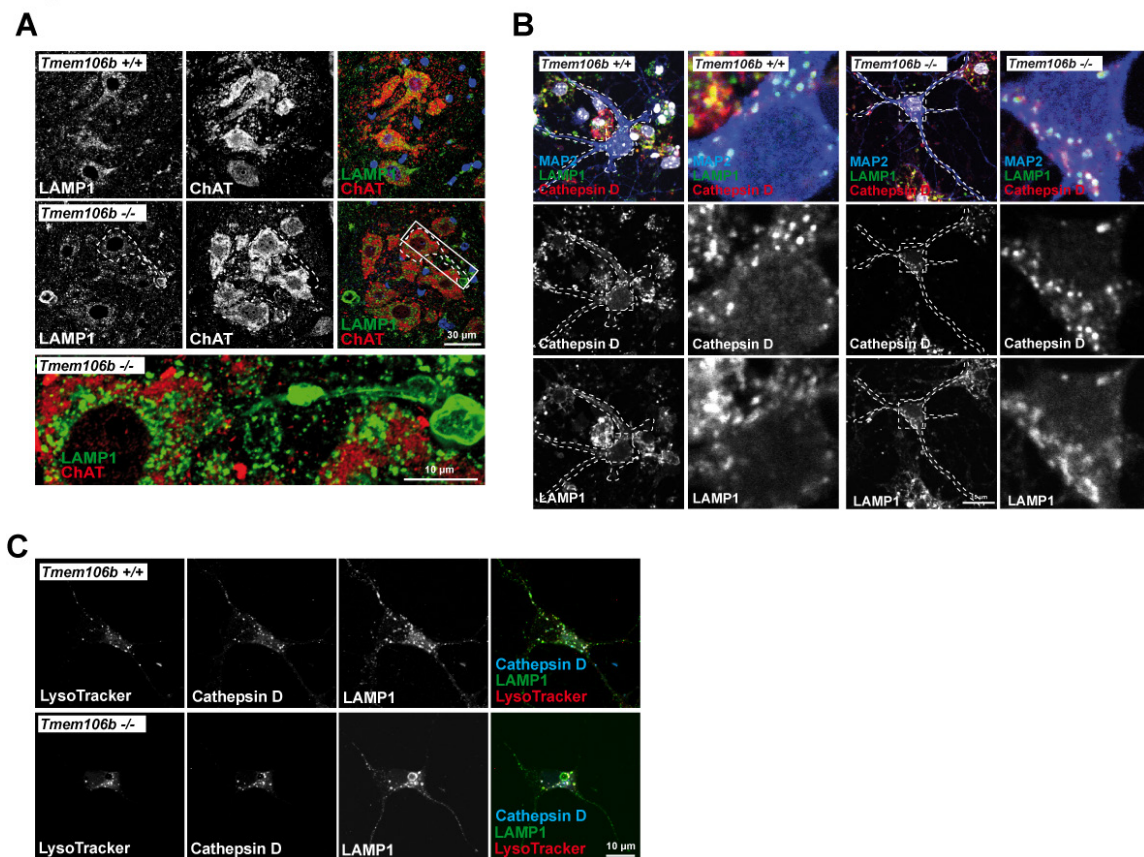


Figure S6. Related to Figure 3. Vacuoles in the spinal cord are found in ChAT-positive lower motoneurons. (A) Co-immunofluorescence staining of spinal cord sections (age: 10 weeks) from LAMP1

(green) and Choline acetyltransferase (ChAT) (red) from control and *Tmem106b* knockout mice. **(B)** Co-immunofluorescence staining of neurosphere-derived neurons for cathepsin D (red), LAMP1 (green) and MAP2 (blue) as a marker for neurons from control and *Tmem106b* KO mice. **(C)** Co-immunofluorescence staining of primary MN (DIV5) stained for LAMP1 (green), cathepsin D (blue) and LysoTracker (red). Nuclei are stained with DAPI (blue).

Figure S7

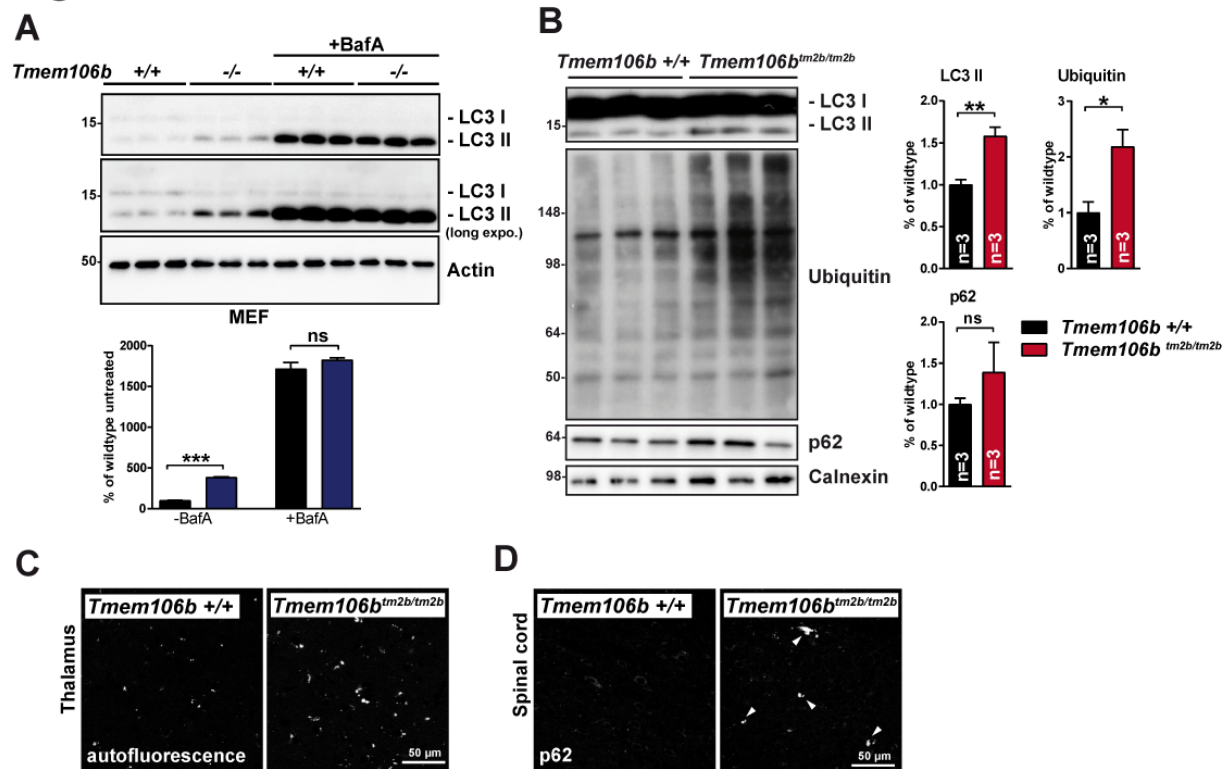


Figure S7. Related to Figure 5. Analysis of autophagic flux in wildtype and *Tmem106b* KO mouse embryonic fibroblasts and altered autophagy in *Tmem106b*^{tm2b} KO mice. (A) Immunoblot analysis of lysates from mouse embryonic fibroblasts (MEFs) prepared from CRISPR/Cas9-mediated KO mice for LC3. Three replicates of untreated and Bafilomycin A (25 nm for 4 hours) treated wildtype and *Tmem106b* KO cells are shown. (B) Immunoblot analysis of total brain lysates from wildtype and *Tmem106b*^{tm2b} KO mice for LC3, ubiquitin and, p62. Calnexin is depicted as loading control. Age: 4-5 months. A quantification is depicted on the right (mean \pm SEM, n = 3). (C) Representative confocal images of autofluorescence (488 nm laser excitation) in the thalamus of *Tmem106b*^{tm2b} KO and control mice. Age: 6 months. (D) Immunofluorescence staining of the spinal cord for p62 of *Tmem106b*^{tm2b} KO and control mice. Age: 6 months. p62-positive aggregates are marked with arrowheads.