Online Supplemental Materials

The FTLD risk factor TMEM106B and MAP6 control dendritic trafficking of lysosomes

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Legends for the Supplementary Figures

Supplementary Figure S1: Rescue of lysosomal clustering with siRNA TMEM106B

HeLa cells cotransfected with either control siRNA or siRNA against TMEM106B and either empty vector control or an siRNA resistant HA-TMEM106B (HA-hT106b*) expressing construct. Immunostaining with antibodies against HA (red) and LAMP2 (green). Scale bar represents 50 µm.

Supplementary Figure S2 Quantitative analysis of colocalization of TMEM106B with organelle marker

(A) Primary hippocampal neurons (DIV12) were immunostained with antibodies against TMEM106B (red) and either LAMP1, SV2 or transferrin receptor Tfr (green). Scale bar represents 50µm. In at least 15 confocal fields the amount of colocalization was analyzed using the JaCoP plugin of ImageJ. (B) Quantitative analysis of colocalization using the Pearson’s coefficient. Mean +/- SEM).
Supplementary Figure S3: Validation of TMEM106B knockdown in neurons

(A) Primary hippocampal neurons were transfected with TMEM106B shRNAs (shT106b#1 or #2) or a control shRNA and GFP as transfection marker. Immunostaining with an antibody against TMEM106B (red) confirms TMEM106B knockdown in the two dimensional projection. Scale bar represents 50 µm or 10 µm (magnification). (B) Primary rat hippocampal neurons (DIV7+5) were cotransfected with shCtrl, shT106b#1 or shT106#2 and GFP as transfection marker. Immunofluorescence of neurons stained with an antibodies against LAMP1 (red). Scale bar represents 50 µm or 10 µm (magnification).

Supplementary Figure S4: TMEM106B knockdown leads to changes in dendritic length and synaptic protein and dominant-negative Rab7a impairs dendritic arborization

(A) Primary hippocampal neurons (DIV7+5) were transfected with either TMEM106B shRNA #2 or a Ctrl shRNA and GFP to visualize the cell morphology. In confocal images the length of the longest dendrite of each cell was measured using ImageJ software blinded to the experimental condition. 40 neurons per condition were analyzed. Mean +/- SEM, one-way ANOVA: *** denotes p<0.001. (B, C) Primary rat hippocampal neurons (DIV7+5) were cotransfected with the indicated Rab7a constructs and GFP to visualize the whole cell. The dendritic arborization was quantified manually and blinded to the experimental condition by Sholl analysis. Neurons transfected with Rab7a dominant negative T22N (dn) are significantly different from Rab7a wildtype (wt) or constitutive active Q67L (ca) transfected cells (n>40 neurons per condition, three independent experiments, mean +/- SEM, two-way ANOVA: Rab7a wt vs. Rab7a dn: from 50µm to 75µm radius p<0.05). Scale bar represents 100 µm. (D) Hippocampal neurons
transfected with the indicated shRNAs and GFP (DIV14+5). Immunofluorescence showing thinner and less dense dendritic protrusions upon TMEM106B knockdown. (E, F) Primary cortical neurons were transduced with the indicated shRNA-expressing lentivirus. Immunoblot with antibodies against presynaptic synaptophysin and the postsynaptic PSD-95. (F) Quantification of the immunoblots from (E) normalized to β-actin. (n=4, mean +/- SEM, Student’s t-test, ** denotes p<0.01). Reduction of synaptic marker proteins corroborates synapse loss at the biochemical level.

**Supplementary Figure S5: Axonal Phenotype of TMEM106B knockdown**

(A) Primary rat hippocampal neurons (DIV4) immunostained with antibodies against TMEM106B (red) and Tau-1 (green) or LAMP1 (green). Scale bar represents 50 µm.
(B, C) Primary rat hippocampal neurons were nucleofected with either TMEM106B shRNA #2 or control shRNA together with YFP prior to plating. Immunostaining on DIV4 with anti-YFP (green) and axonal marker anti-Tau-1 (red). The axonal length was measured using AxioVision software blinded to the experimental condition. (n>80 per condition, three independent experiments; Student’s t-test, ** denotes p<0.01). Scale bar represents 100 µm. Axon elongation in TMEM106B knockdown cells indicates that TMEM106B knockdown does not cause unspecific toxicity. (D) Primary hippocampal neurons (DIV0+4) were nucleofected with either TMEM106B shRNA #2 or a Ctrl shRNA and Rab7a-GFP and live imaged every second for 5 minutes to visualize late endosomal / lysosomal trafficking in axons. Axonal segments and kymographs of axonal movement of Rab7a-GFP labeled vesicles. Scale bar represents 60 s (vertical) and 25 µm (horizontal). (E) Quantitative analysis of vesicle movement from 5 minute kymographs. Vesicles were manually classified according to their motility. At least seven
Supplementary Figure S6: Identification of MAP6 in TMEM106B immunoprecipitates and analysis of MAP6 in neurons

(A) Proteins coimmunoprecipitating with TMEM106B in rat brain (P15) were analyzed by LC-MS/MS. MAP6 was identified by 11 peptides highlighted in green throughout the protein sequence. The region with five tandem repeats binding microtubules is underlined. (B) Primary rat hippocampal neurons (DIV12) immunostained with antibodies against MAP6 (green) and TMEM106B (red) or LAMP1 (red). A commercial mouse monoclonal and home-made rabbit polyclonal anti-MAP6 antibody were used in the upper and lower panel, respectively. Scale bar represents 50 µm. (C) Primary hippocampal neurons (DIV7+5) were transduced with MAP6 shRNA or a Ctrl shRNA lentivirus. Immunoblot with the indicated antibodies indicates specific MAP6 knockdown.

Supplementary Figure S7: Rescue experiments confirm specificity of TMEM106B and MAP6 shRNA constructs

(A, B) Primary rat hippocampal neurons (DIV7+5) were cotransfected with combinations of control shRNA (shCtrl), shRNA targeting rat MAP6 (shMAP6) and human MAP6 (hMAP6) or an empty vector (Ctrl) together with GFP. Sholl analysis to quantify dendritic complexity. MAP6 knockdown increases distal branching while overexpression of human MAP6 prevents this effect (n=25 per condition; 3 independent experiments, mean +/- SEM, two-way ANOVA: shCtrl+Ctrl
Supplementary Materials: TMEM106B and MAP6 control lysosomal trafficking

vs shMAP6+Ctrl: 62.5 µm: p<0.05, from 75 µm to 112.5 µm radius p<0.001; shCtrl+Ctrl vs. shCtrl+hMAP6 at 25 µm, 50 µm and 62.5 µm radius p<0.05, at 37.5 µm radius p<0.001; shMAP6+Ctrl vs shMAP6+hMAP6: from 12.5 µm to 112.5 µm radius p<0.001). Scale bar represents 100 µm. (C) Primary rat hippocampal neurons were virally infected (DIV5) with either mCherry (RFP) or shRNA-resistant TMEM106B mutant (T106b*) and cotransfected (DIV6) with either a control shRNA or the indicated TMEM106B shRNA and Rab7a-GFP to visualize late endosomal/lysosomal trafficking. Dendrite segment and kymographs of dendritic movement of Rab7a-GFP-labeled vesicles on DIV9. Scale bars represent 60 s and 15 µm. (D) Quantitative analysis of vesicle movement from 5 min kymographs. Vesicles were manually classified according to their movement. Expression of shRNA-resistant TMEM106B prevents the induction of retrograde lysosomal transport. Between five and nine neurons per condition were analyzed per experiment in at least three independent experiments, mean +/- SEM, unpaired t-test: *** denotes p<0.001.

Supplementary Figure S8: TMEM106B knockdown enhances retrograde movement of late endosomes / lysosomes but not of mitochondria

(A-C) Primary hippocampal neurons (DIV6+3) were transfected with either TMEM106B shRNA #2 or a Ctrl shRNA and Rab7a-GFP. Live cell imaging of Rab7a-GFP labeled vesicles was used to visualize late endosomal / lysosomal trafficking in dendrites. Velocity (A) and total run length (B) of individual moving vesicles was analyzed in kymographs (compare Figure 6). At least 80 moving vesicles per condition were analyzed. The speed of lysosomal movement is in the expected range for organelle transport in dendrites (Bannai et al, 2004; Kwinter et al, 2009; van Spronsen et al, 2013). The number of vesicles per 100 µm of dendrite length did not change (C).
(D-E) Live imaging experiments in hippocampal neurons (DIV6+3) transfected with either TMEM106B shRNA #2 or a Ctrl shRNA and mito-dsRed to visualize mitochondrial trafficking in dendrites. TMEM106B had no effect on mitochondrial density (D) and movement (E) in dendrites. (F) MAP6 overexpression had no effect on the density of Rab7a-GFP labeled vesicles in dendrites. Mean +/- SEM, unpaired t-test: * denotes p<0.05 *** denotes p<0.001.

**Supplementary Figure S9: Live imaging of MAP6-GFP and LAMP1-RFP**

Primary hippocampal neurons were transfected with MAP6-GFP and LAMP1-RFP (DIV6+3). Live cell imaging for 5 min (1 image/second) to visualize movement of the fluorescently tagged proteins. Merged Kymograph shows of excess MAP6 bound to moving LAMP1-positive late-endosomes/lysosomes in dendrites. Scale bars represent 60 s and 15 µm.
Supplementary Movie S1: Dendritic trafficking of Rab7a-GFP labeled vesicles in controls

Live imaging of primary hippocampal neurons (DIV6+3) transfected with Ctrl shRNA and Rab7a-GFP to visualize late endosomal / lysosomal trafficking in dendrites. Most vesicles remain in place. The soma is on the right.

Supplementary Movie S2: Dendritic trafficking of Rab7a-GFP labeled vesicles upon TMEM106B knockdown

Live imaging of primary hippocampal neurons (DIV6+3) transfected with TMEM106B shRNA #2 and Rab7a-GFP to visualize late endosomal / lysosomal trafficking in dendrites. Note that vesicles are more mobile than in controls (Supplementary Movie S1) and preferentially move towards the soma on the right.

Supplemental References

