**A**

Bar graph showing the longest dendrite length in [µm] with group comparisons:
- shCtrl
- shT106b#1
- shT106b#2

**B**

Images of neurons with bar graphs showing:
- wt Rab7a
- dn Rab7a
- ca Rab7a

**C**

Line graph showing the number of crossings with distance from soma [µm], with group comparisons:
- wt Rab7a
- dn Rab7a (T22N)
- ca Rab7a (Q67L)

**D**

Images of DIV14+5 with group comparisons:
- shCtrl
- shT106b#1
- shT106b#2

**E**

Western blot analysis showing:
- PSD-95
- synapto
dyns
- β-actin
- TMEM106B

**F**

Bar graph showing relative protein levels:
- PSD-95
- synapto
dyns

Schwenk et al., Supplementary Figure S4
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.