# Supplementary Material

Membrane orientation and subcellular localization of transmembrane protein 106B (TMEM106B), a major risk factor for frontotemporal lobar degeneration*

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*Running title: *TMEM106B – membrane orientation and subcellular localization*

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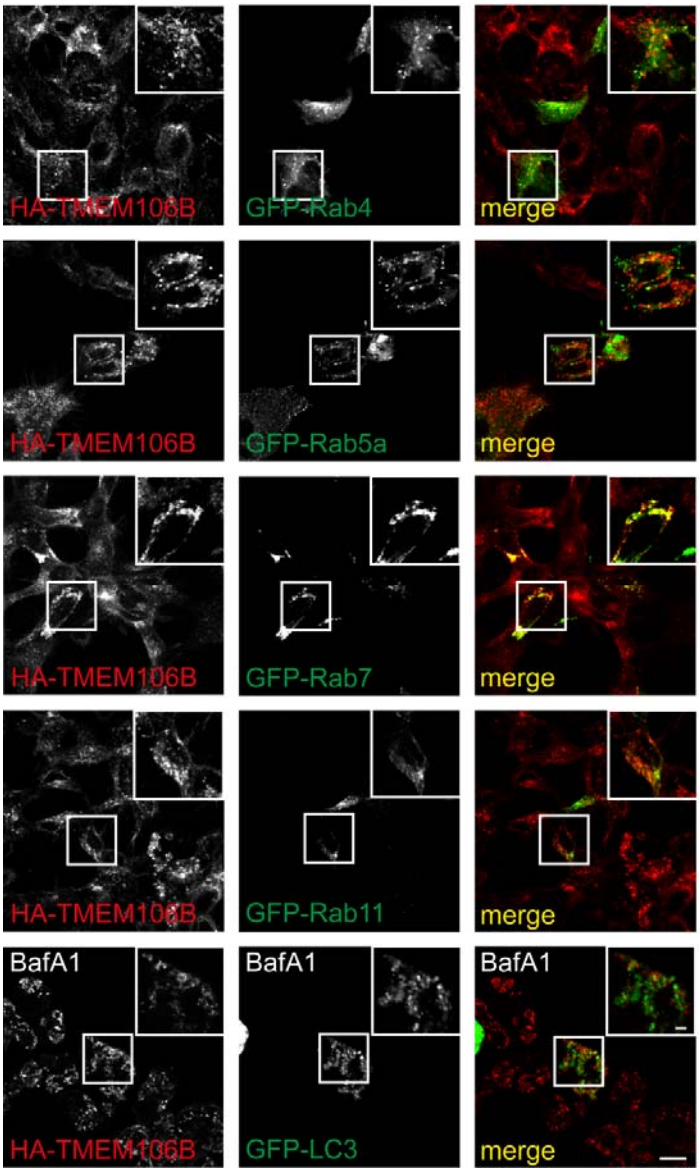


**FIGURE S1. TMEM106B is located in late endosomes and lysosomes.** The stable tetracycline inducible wt HA-TMEM106B expressing T-REx™ 293 cell line was transiently transfected with carboxy-terminal GFP fusion cDNA constructs of Rab4, Rab5a, Rab7 or Rab11 (1) or a GFP-LC3 (2) cDNA construct as indicated. 8 hours after transfection, TMEM106B expression was induced for 16 hours. TMEM106B was stained with an anti-HA antibody (red). Cells transfected with GFP-LC3 were additionally treated with 30 nM BafA1 for 16 hours to inhibit autophagy and to obtain accumulation of autophagosomes. Scale bars represent 10 µm and 2.5 µm (inlay). Coexpression of these proteins revealed little localization of TMEM106B within early (Rab5a) and recycling endosomes (Rab4 and Rab11) (1) and almost no colocalization of TMEM106B with LC3-positive autophagosomes (2). However, significant colocalization of TMEM106B with Rab7-positive late endosomes was observed (1).

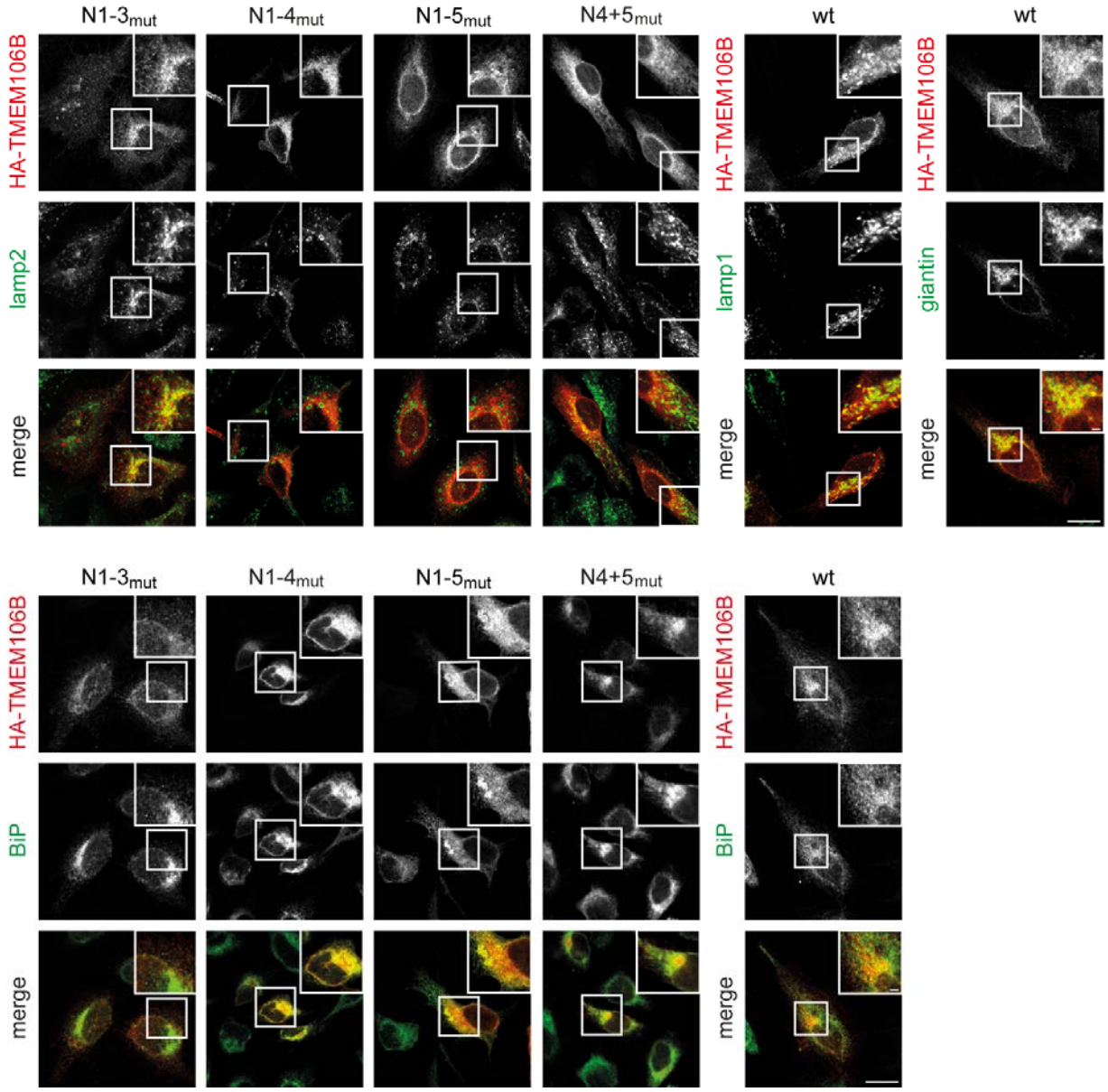


**FIGURE S2. Impaired transport to late endosomal/ lysosomal compartments of mutants lacking the N4 complex glycosylation site.** HeLa cells were transiently transfected with wt HA-TMEM106B cDNA or glycosylation site mutants N1-$3_{mut}$, N1-$4_{mut}$, N1-$5_{mut}$ and N4+$5_{mut}$. TMEM106B was stained with an anti-HA antibody (red) and costained with the cell marker antibodies for giantin (only for wt; Golgi, green), lamp1/2 (lysosomes, green) and BiP (ER, green). Scale bars represent 10 µm and 2.5 µm (inlay). Note that HA-TMEM106B N1-$3_{mut}$, which still contains both complex glycosylation sites, is similarly distributed as the wt, whereas HA-TMEM106B N1-$4_{mut}$, N1-$5_{mut}$ and N4+$5_{mut}$ are retained in the ER.

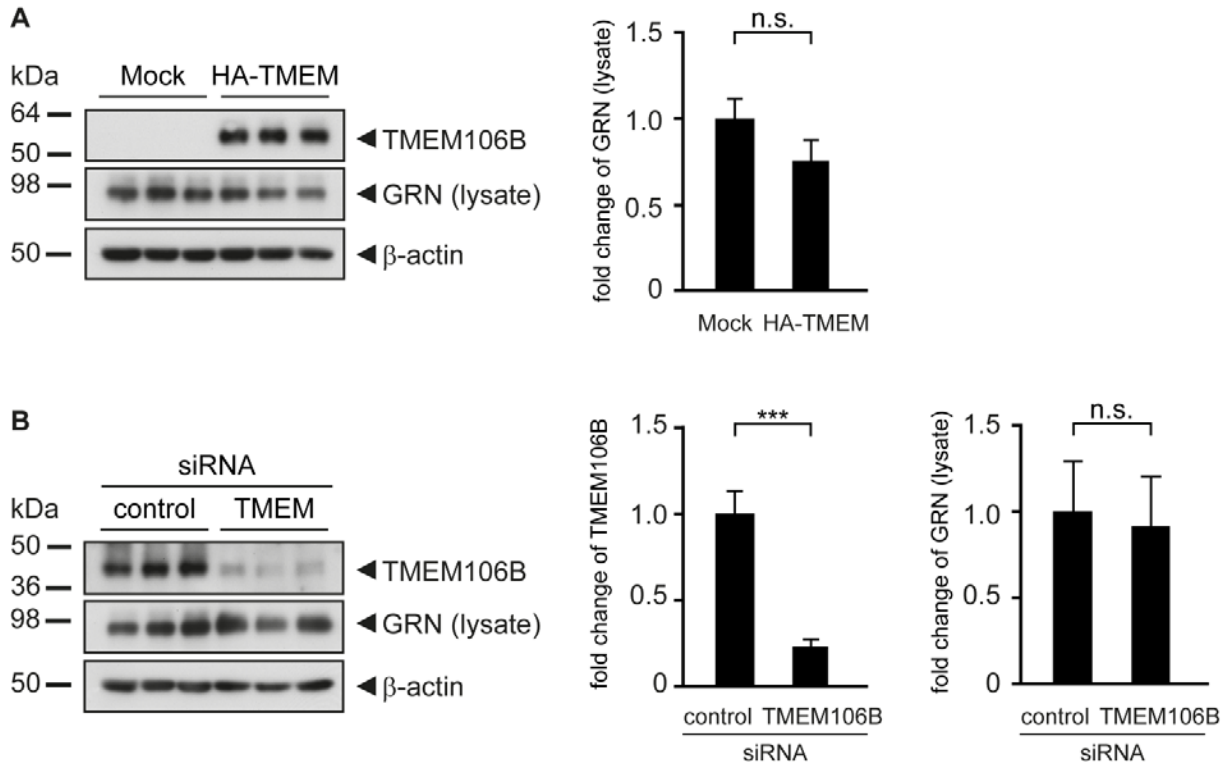


**FIGURE S3. TMEM106B expression does not affect GRN levels in SH-SY5Y cells**. *A*, GRN levels in SH-SY5Y cells transiently transfected with HA-TMEM106B. SH-SY5Y cells were transiently transfected with the HA-TMEM106B construct. Subsequently, lysates were analyzed for TMEM106B and endogenous GRN expression by immunoblotting. β-actin serves as a loading control. The bar graph represents the quantification of cell lysates for GRN expression by measuring the chemiluminescence on the immunoblot. Data are shown as fold change in GRN levels normalized to the mock control, means ± s.d. (n=3) are depicted (n.s. by unpaired student's t-test). *B*, GRN levels after *TMEM106B* knockdown in SH-SY5Y cells. SH-SY5Y cells were reversely transfected with non-targeting siRNA (control) and a pool of TMEM106B siRNAs. Cell lysates were analyzed by immunoblotting 72 hours post transfection. β-actin serves as a loading control. The bar graphs represent the quantification of cell lysates for TMEM106B as well as GRN expression by measuring the chemiluminescence on the immunoblot. Data are shown as fold changes in TMEM106B or GRN levels, means ± s.d. (n=3) are depicted (***: $p < 0.001$ and n.s. by unpaired student's t-test).

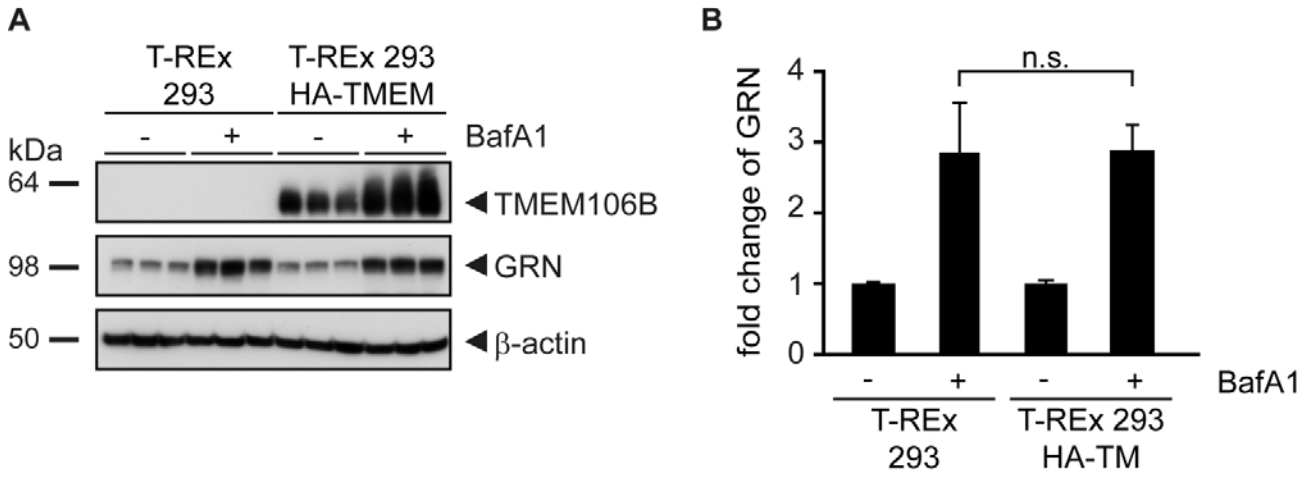


**FIGURE S4. TMEM106B overexpression does not affect the BafA1-mediated increase in GRN levels**. *A*, GRN levels in T-REx™ 293 cells stably expressing wt HA-TMEM106B after BafA1 treatment. T-REx™ 293 cells and T-REx™ 293 cells stably transfected with HA-TMEM106B were incubated with 0.2 µg/ml tetracycline and treated with/without 30 nM BafA1 for 16 hours. Subsequently, lysates were analyzed for TMEM106B and GRN expression by immunoblotting. β-actin serves as a loading control. *B*, Data are shown as fold change in GRN levels normalized to the untreated controls, means ± s.d. (n=3) are depicted (n.s. by one-way ANOVA post hoc Tukey's test).

**REFERENCES**

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