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## Supplemental information

# TMEM106B reduction does not rescue GRN deficiency in iPSC-derived human microglia and mouse models

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**Figure S1. Characterization of maturation and enrichment of iMG cultures used for multi-omic studies in Fig. 1&2.**

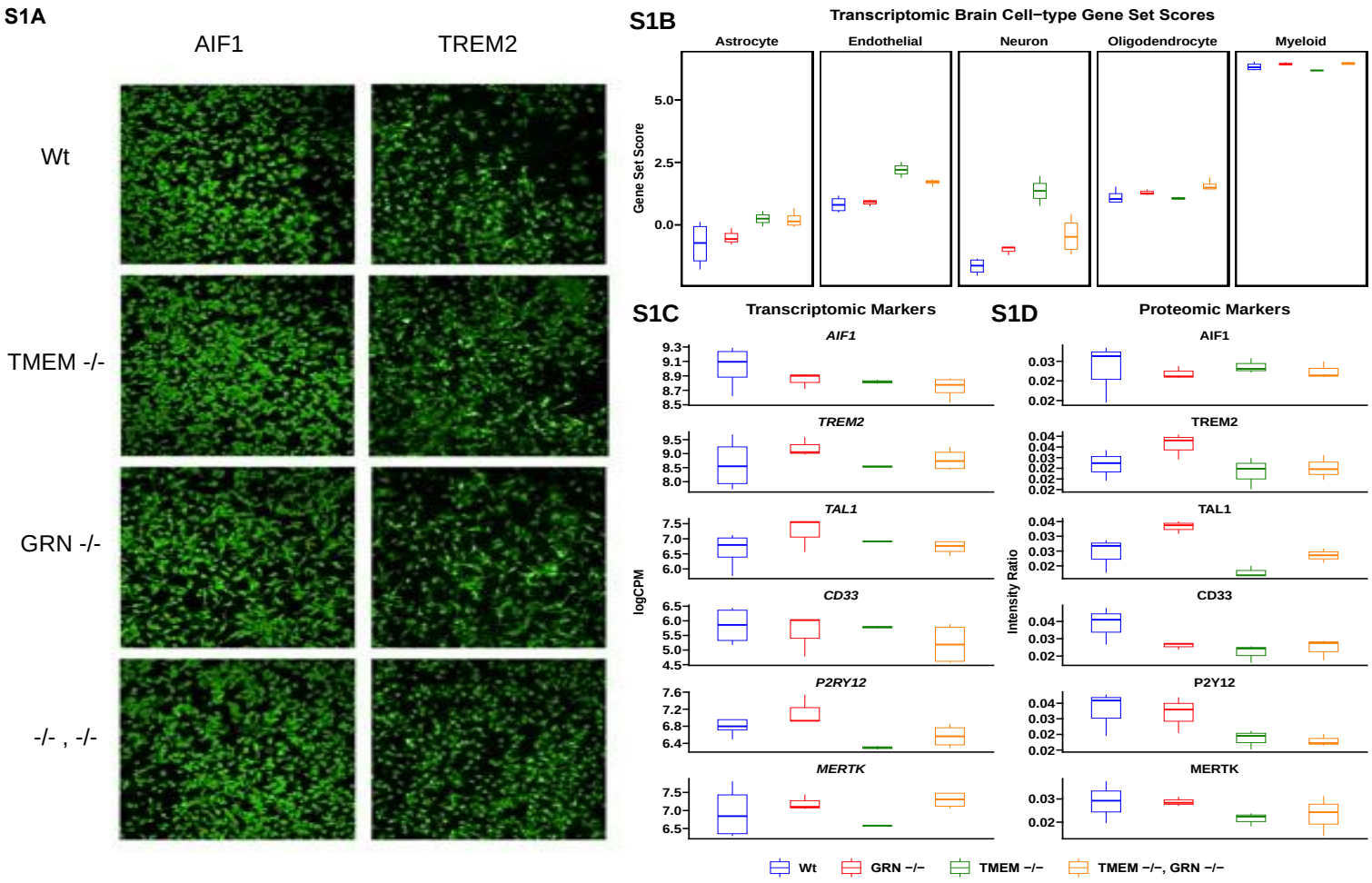



**Figure S1.** iMicroglia display expected morphology and maturation markers. **(S1A)** Immunocytochemistry of AIF1 and TREM2 expression at day 40 of iMicroglia maturation. **(S1B)** Transcriptional analysis reveals iMicroglia most closely resemble myeloid cell type lineages in contrast to endothelial and neuroectoderm cell types. **(S1C,D)** Transcriptomic and proteomic analysis of microglia lineage and maturation markers across lines.

**Figure S2. Phagocytic activity of iMG used for multi-omic studies in Fig. 1&2.**

**S2A** **S2B**

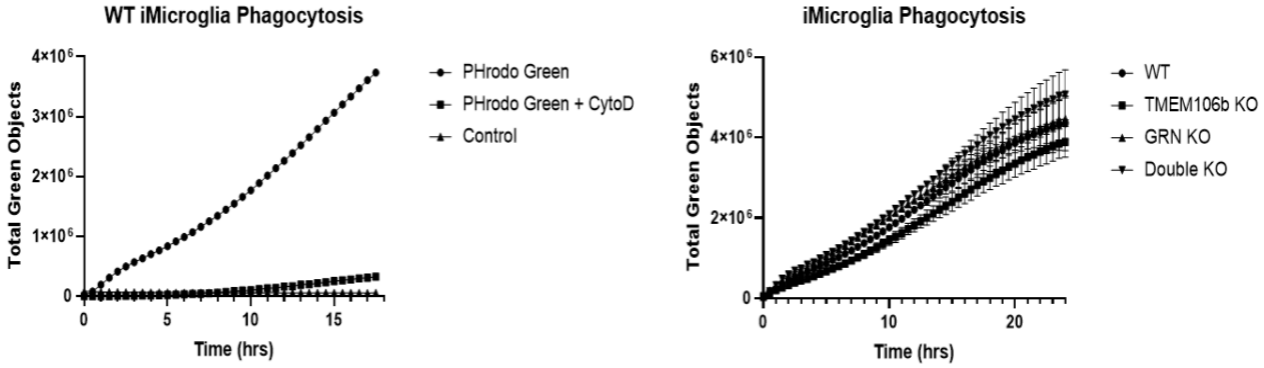



**Figure S2.** No significant changes in phagocytosis activity between WT, GRN -/-, TMEM -/- and TMEM-/-, GRN -/- iMicroglia. **(S2A)** Phagocytosis stimulated by 10ug/ml PHrodo Green bioparticles (Thermofisher). Phagocytosis was measured as the total integrated intensity within a cell boundary every hour for 24 hours. Three technical replicates from each biological replicate were averaged. Each value is expressed as the mean ± SEM for two independent biological replicates and three technical replicates. Cells treated with 10uM cytochalasinD as a negative control to validate reagent specificity for stimulating phagocytic activity. (**S2B**) iMG Phagocytosis in TMEM106B and GRN KO cells.

**Figure S3. Confirmation of Tmem106b deletion in knock-out mice used in Fig. 3 – 6.**

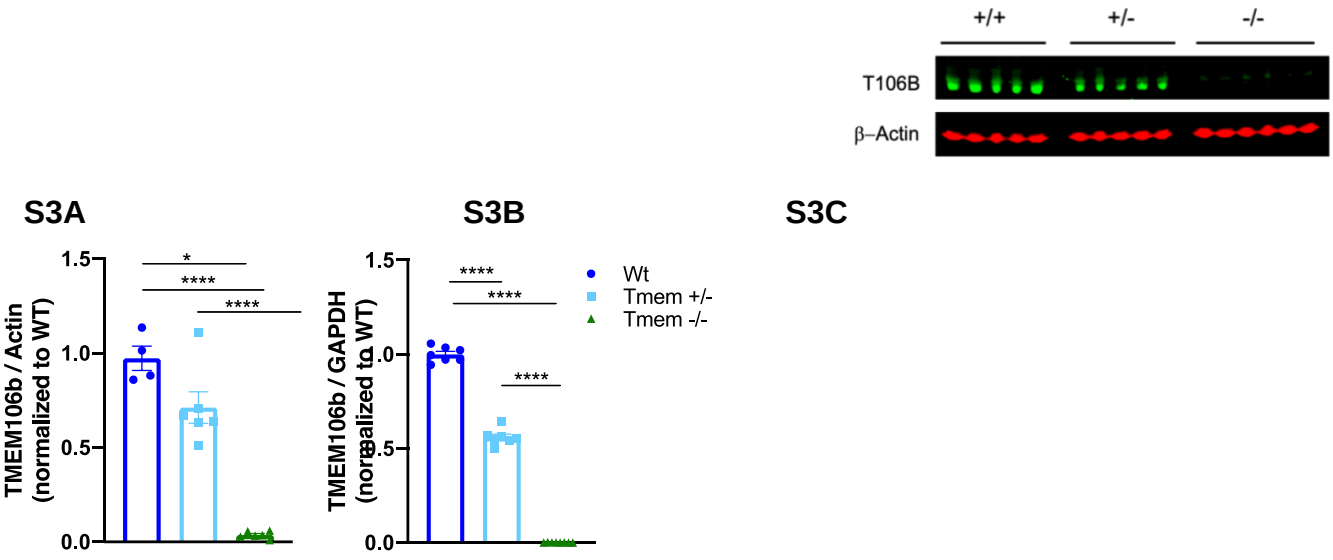



**Figure S3A-C.** Protein and mRNA Tmem106b levels are reduced in the brains of *Tmem106b* CRISPR knock out mice in a dose dependent manner. **(S3A)** Western blot showing mTmem106b protein levels in brain for *Tmem106b* +/+, +/- and -/- mice. (**S3B)** Tmem106b protein levels are significantly reduced in Tmem +/- and -/-. One-way ANOVA reveals an overall effect on genotype p <0.0001 and post hoc student t-test shows that as Tmem106b protein levels in brain decrease with copy number loss, when compared to Wt. n = 4-6 animals/genotype. **(S3C)** *Tmem106b* mRNA levels are significantly reduced in *Tmem* +/- and -/- by One-way ANOVA (p <0.0001) and post hoc student t-test. n = 7-8 animals/genotype. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. All error bars represent S.E.M.

**Figure S4. Immunostaining for inflammation markers quantified in Fig. 3.**

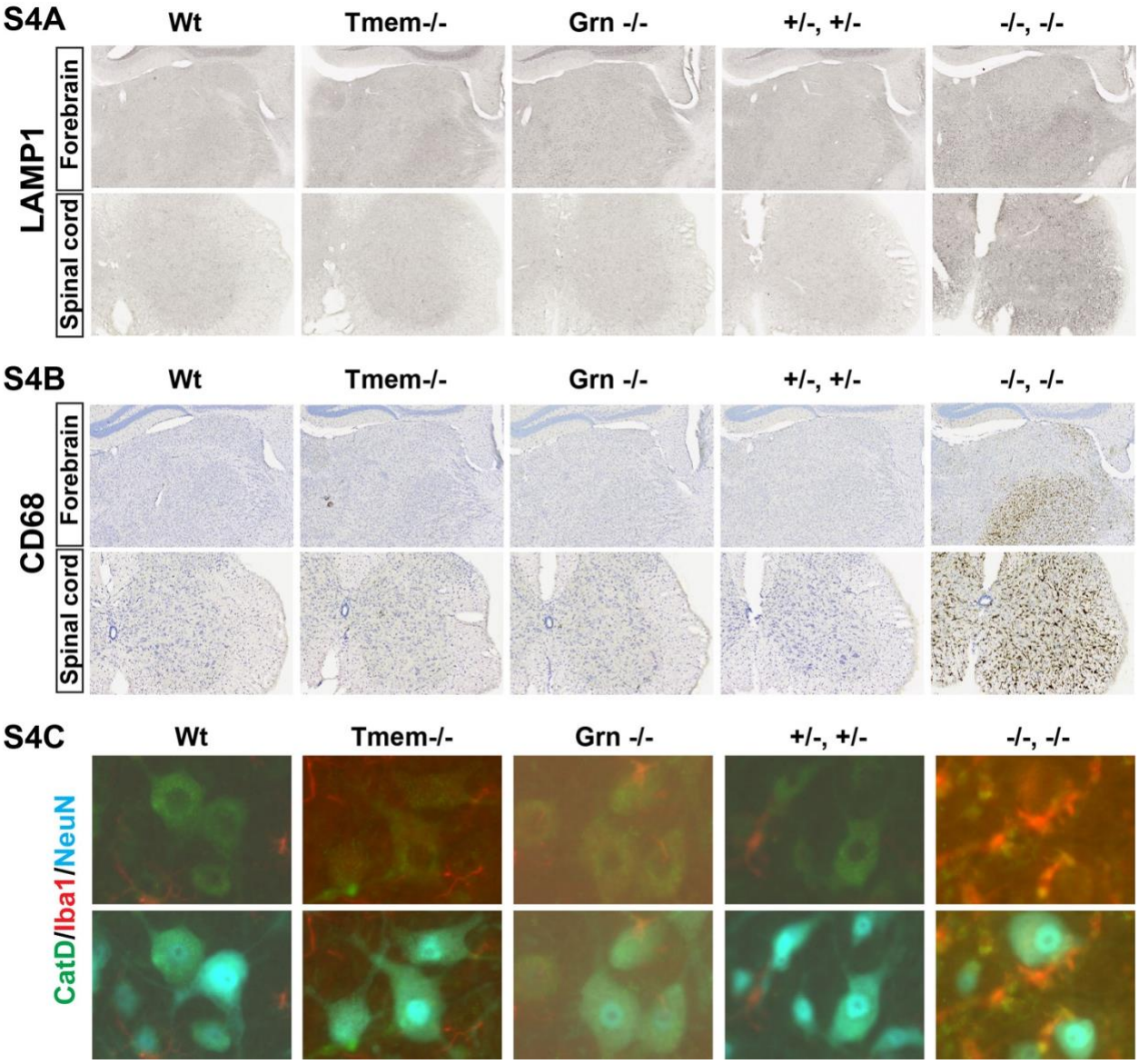
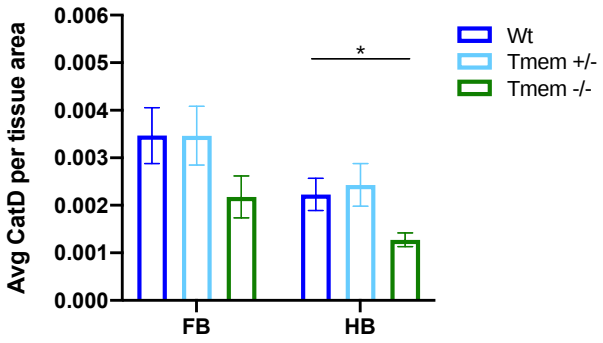



**Figure S4A-C.** Representative images of forebrain and spinal cord immunostaining for LAMP1 (S4A) and CD68 (S4B) quantified in Fig 3D,E. Representative images of immunofluorescent staining of CatD (green), microglia (Iba1, red) and neurons (NeuN, blue) in spinal cord ventral horn. Notice increased lysosomal and inflammation markers in dKO mice and reduction of CatD staining in neurons of the *Tmem-/-* and dKO mice.

**Figure S5. Cathepsin D and ChaT staining in *Tmem106b* and *Grn* knock-out mice from study shown in Fig.3.**

## S5A




## S5B

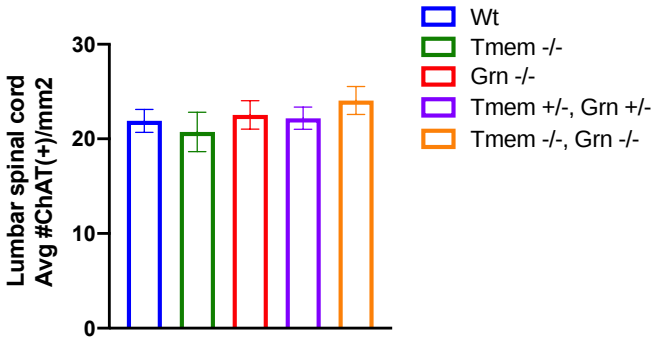
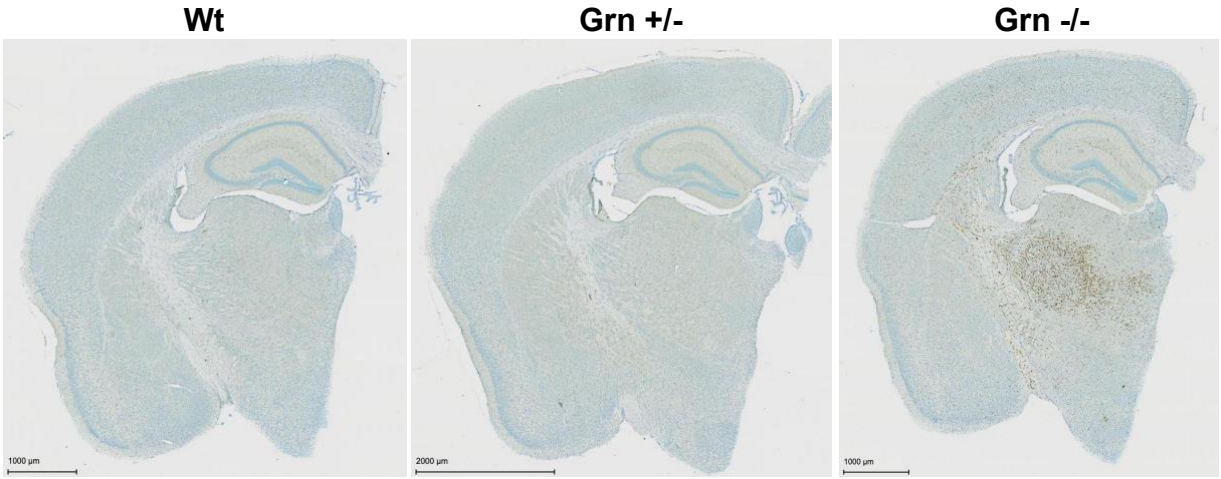


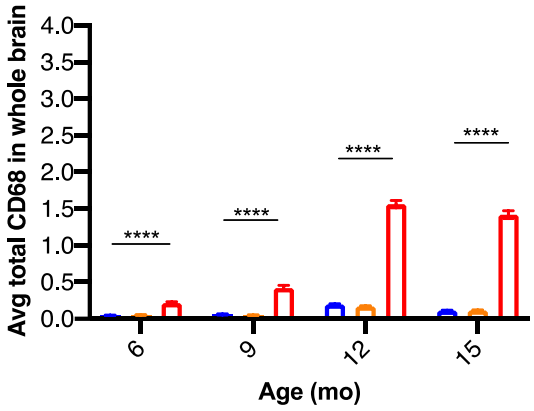

**Figure S5. (S5A)** *Tmem106b* -/- mice have reduced Cathepsin D staining in brain. One-way ANOVA shows a significant effect of genotype, p = 0.0403* for HB but not FB analysis. Post hoc student T-test reveals that *Tmem* -/- mice have significantly less Cathepsin D staining in the HB region, when compared to both *Wt* and *Tmem* +/- in HB but not FB. n = 7-8 animals/genotype and 4-5 sections/animal/tissue area. **(S5B)** No changes in motor neuron numbers were observed in lumbar spinal cord of Wt, *Tmem106b* -/-, and *Grn* -/- mice with ChAT staining by One-Way ANOVA and post hoc student t-test n = 11-16 animals/genotype and 10-12 sections/animal. All error bars represent S.E.M. n = 6-8 animals/genotype. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

**Figure S6. CD68 staining with age in *Grn* knock-out mouse brain as referred to in Fig. 3-6.**

**S6A**




**S6B**




**S6C**

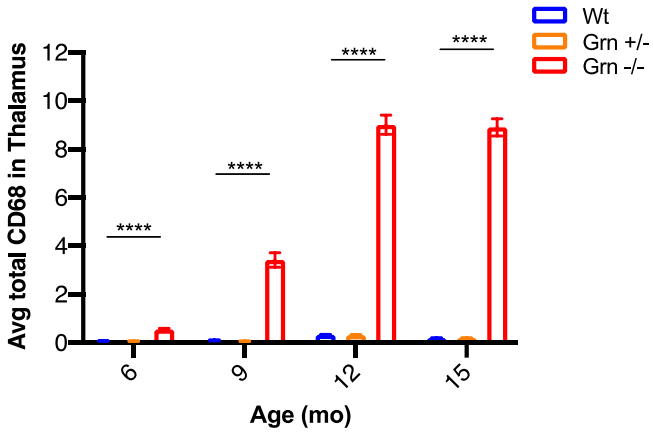



**Figure S6.** CD68 increases with age in brains of *Grn* -/- mice and is highly expressed in thalamus. **(S6A)** Images of CD68 staining for each genotype at 15 months of age. **(S6B-C)** Individual t-tests at each time point reveals increased levels of CD68 in the brains of *Grn* -/- mice **(S6B)** with striatum most highly affected **(S6C).** n = 8-20 animals/genotype/timepoint and 4-5 sections/animal. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. All error bars represent S.E.M.

**Figure S7. Lipidomics in WT, *Tmem106b*, and *Grn* KO mice from study shown in Fig. 5 & 6.**

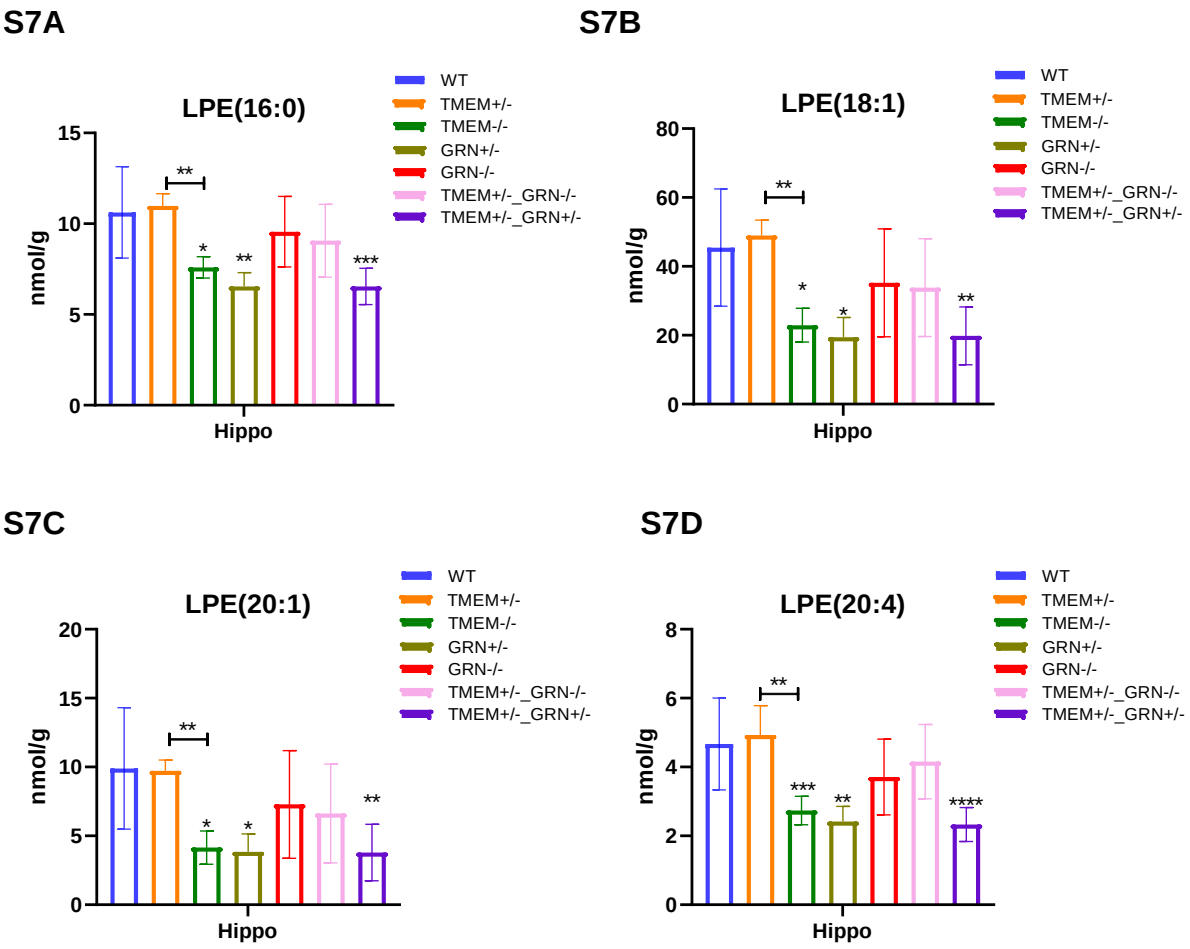
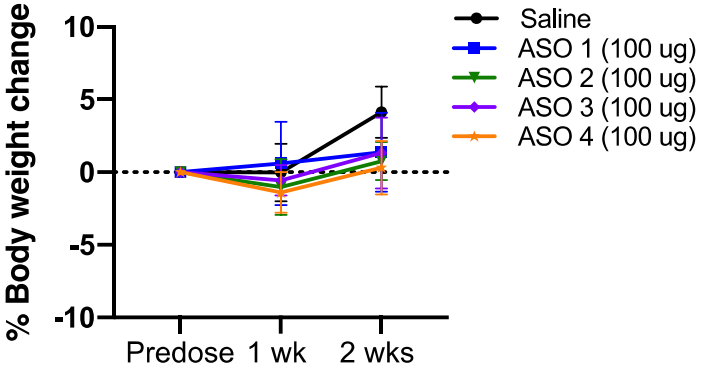



**Figure S7.** Deletion of *Tmem106b* alters lipids (LPEs) in brain hippocampus. LPE species (**S7A-D**) decreased in Tmem-/-, Grn+/- and Tmem+/-_Grn+/- compared to Wt control. LPE species (**S7A-D**) decreased in Tmem-/- group compared to Tmem+/- group. All error bars represent standard deviation. n = 5-10 animals/genotype. Mann-Whitney U *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001

**Fig. S8. *Tmem106b* ASO treatment effects in WT mice, pilot study prior to study shown in Fig. 7.**

**S8A**



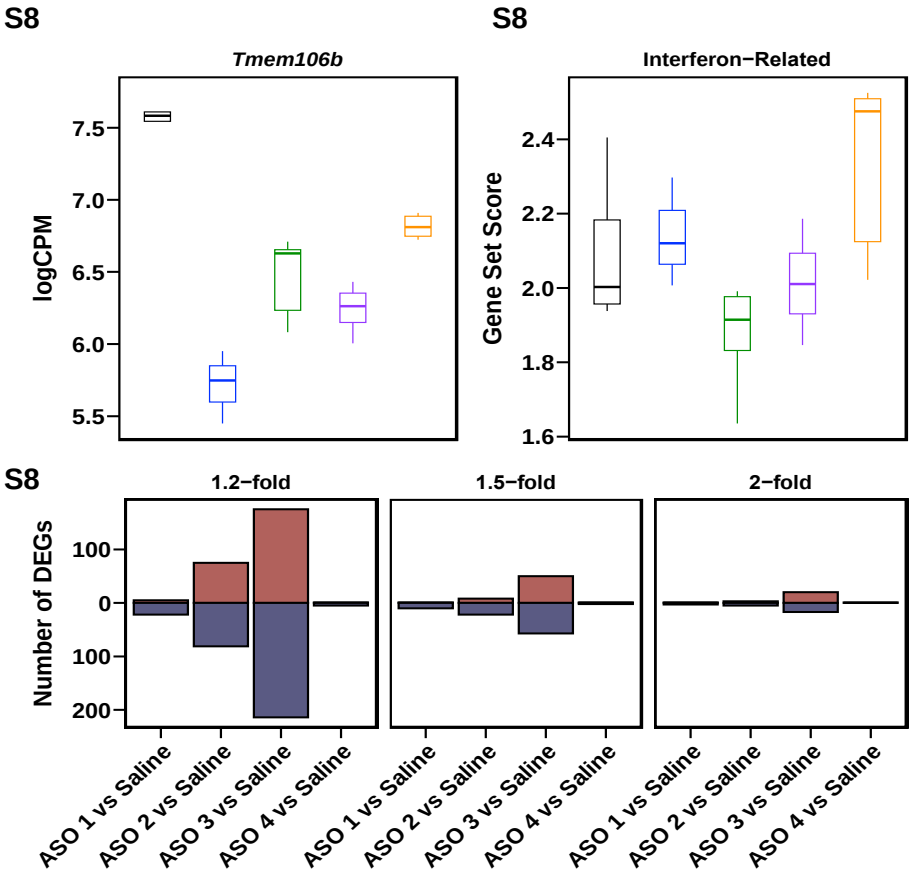




**Figure S8.** All ASOs were well-tolerated in C57Bl/6J mice out to 2 weeks post-dose and had minimal effects on other transcripts including inflammation gene sets. **(S8A)** No weight loss was observed in Wt mice out to 2 weeks post ICV injection with *Tmem106b* targeting ASOs. (**S8B-D)** RNA sequencing of brain tissue confirmed knockdown of target, minimal changes to interferon related genes and minimal effects on other transcripts.

**Figure S9. Electron microscopy of brain sections from mice treated with *Tmem106b* ASO related to study in Fig. 7.**

**S9**

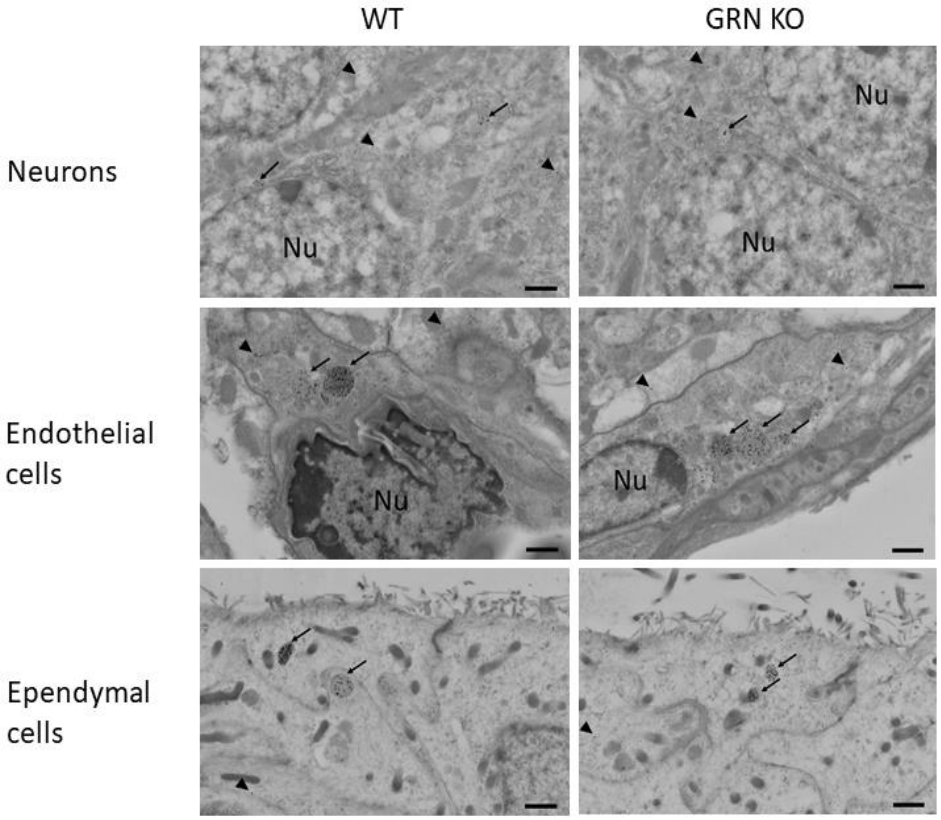



**Figure S9.** Clusters of anti-ASO antibody-bound gold particles are seen within the cytoplasm of neurons, endothelial cells, and ependymal cells in both WT and KO animals. Clusters of particles are present within spherical electron-dense organelles consistent with lysosomes (arrows). Individual free particles are seen within the cytoplasm of cells (arrowheads). Nu- Nucleus. Size bar=1 micron.