Supplementary Information

Lysosomal TMEM106B interacts with galactosylceramidase to regulate myelin lipid metabolism

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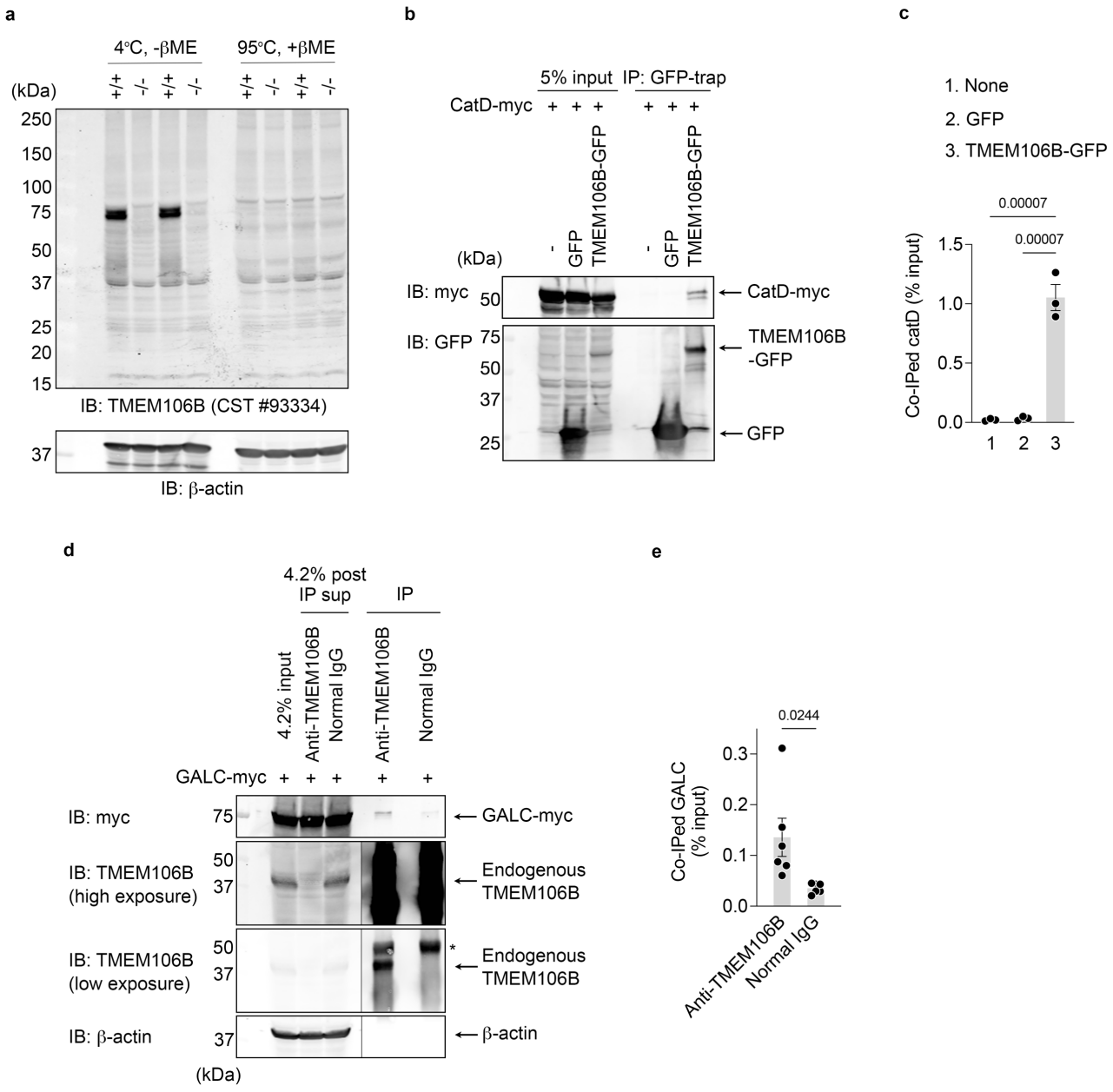
Supplementary Figures 1-5



Supplementary Fig. 1: TMEM106B interacts with cathepsin D or GALC

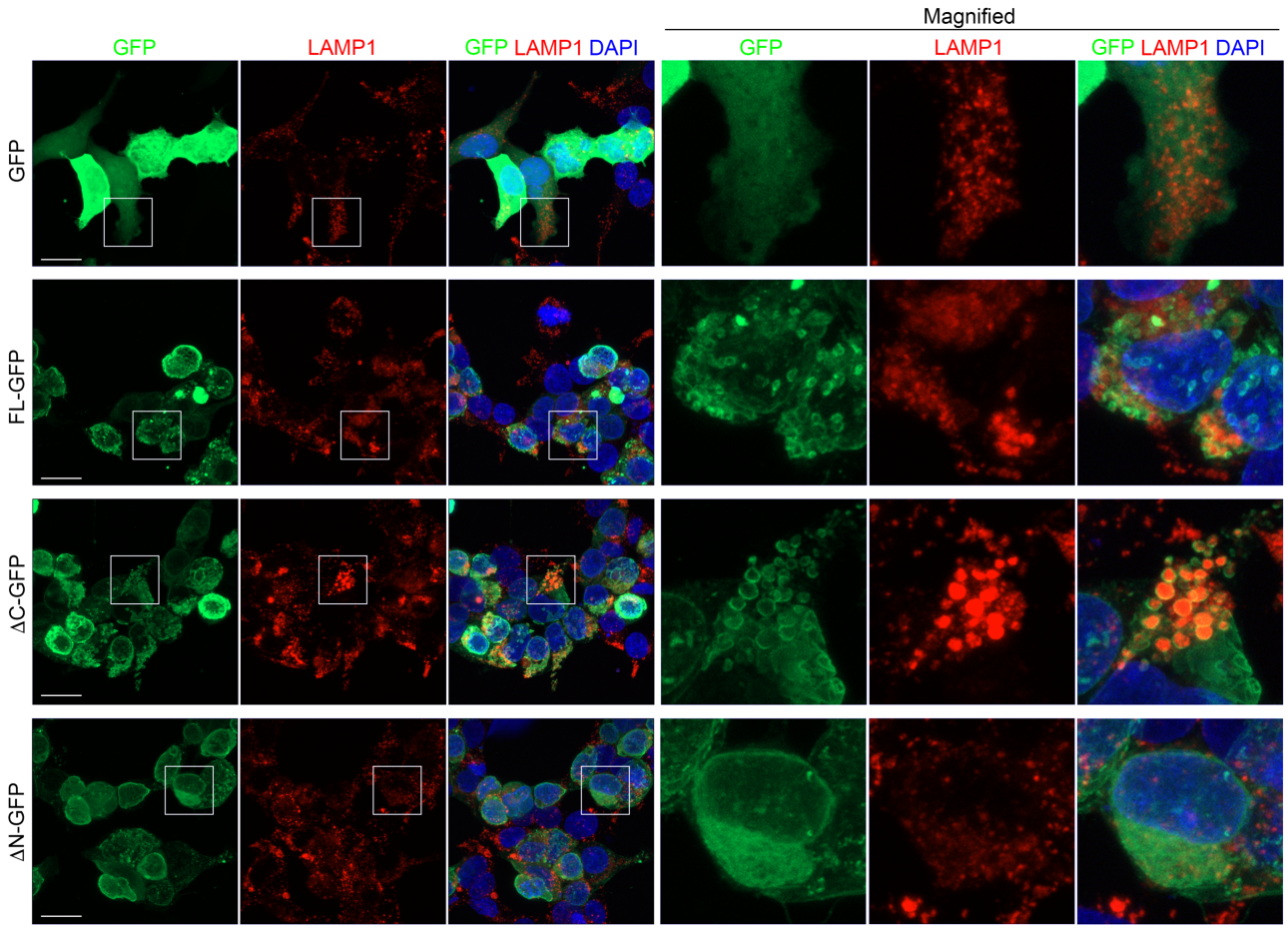
a) Representative blots showing TMEM106B expression in WT and TMEM106B-deficient brains. The samples were prepared with or without heat and 2-mercaptoethanol treatment.

b) Representative blots of co-IP assays using HEK293T cells expressing GFP or TMEM106B-GFP, together with myc-DDK-tagged cathepsin D (catD). Note that all samples were treated with 2-mercaptoethanol and heat (95°C) before running a gel.

c) Quantification of co-IP in (**a**). Mean ± SEM, n = 3 experiments. P-values obtained from one-way ANOVA with Tukey's multiple comparisons test are shown in the graph.

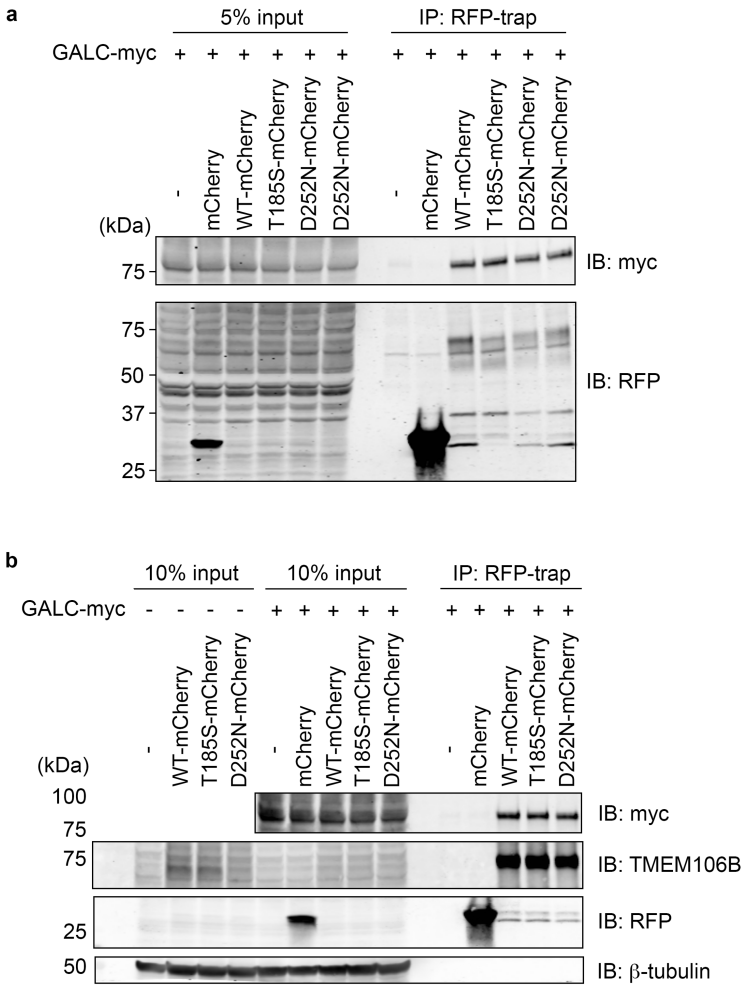
d) Representative blots of co-IP assays using HEK293T cells expressing myc-DDK-tagged human GALC. Anti-TMEM106B antibody (Cell Signaling #93334) was used to immunoprecipitate endogenous TMEM106B in HEK293T cells. Note that all samples were treated with 2-mercaptoethanol and heat (95°C) before running a gel. The asterisk indicates heavy chains of IgG.

e) Quantification of co-IP in (**c**). Mean ± SEM, n = 6 experiments, p = 0.0244; two-tailed unpaired t-test.



Supplementary Fig. 2: Subcellular localization of GFP-tagged TMEM106Bs in HEK293T cells

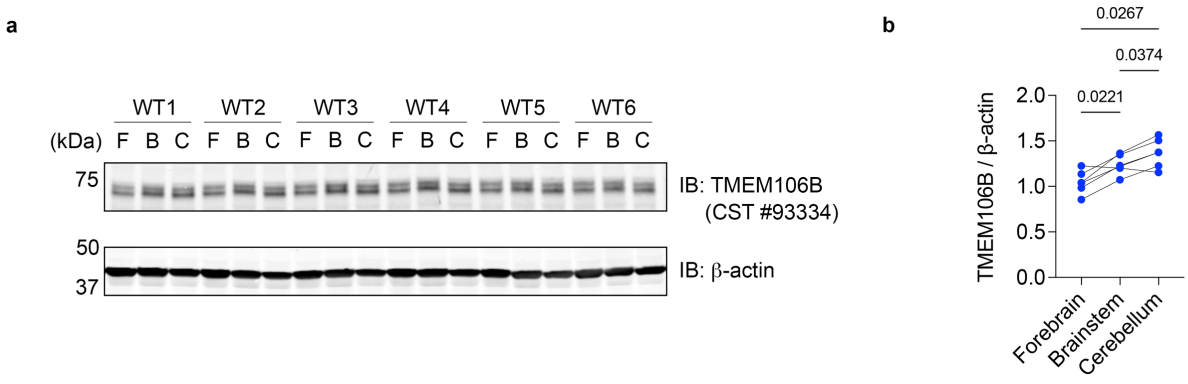
Representative immunostaining of HEK293T cells expressing GFP, FL TMEM106B-GFP, ΔC-TMEM106B-GFP, or ΔN-TMEM106B-GFP using anti-GFP and anti-LAMP1 antibodies. Bar, 20 μm. Note that, consistent with many previous studies, overexpression of TMEM106B increased/altered the size and number of LAMP1-positive lysosomes. In addition, the lysosomal changes were also observed in HEK293T cells expressing the other two deletion mutants. GFP alone was expressed throughout the cytoplasm. Overexpressed GFP-tagged FL and ΔC-TMEM106B localized at the LAMP1-positive lysosomes to some extent but did not show complete colocalization likely due to overexpression of the proteins. GFP-tagged ΔN-TMEM106B appeared to localize nonspecifically to any membrane-like structures but was also localized to organelles weakly positive for LAMP1, which are likely enlarged lysosomes caused by overexpression of the protein.



Supplementary Fig. 3: T185S and D252N mutations have no significant effects on TMEM106B binding to GALC

a) Representative blots of co-IP assays using HEK293T cells expressing mCherry, FL TMEM106B-mCherry, T185S TMEM106B-mCherry, or D252N TMEM106B-mCherry, together with myc-DDK-tagged mouse GALC. Note that all samples were treated with 2-mercaptoethanol and heat (95°C) before running a gel.

b) Immunoblots of a replication co-IP of **a)** using HEK293T cells expressing mCherry, FL TMEM106B-mCherry, T185S TMEM106B-mCherry, or D252N TMEM106B-mCherry, together with or without myc-tagged mouse GALC. In this replication experiment, input samples with single transfection of mCherry-tagged TMEM106Bs were also included as controls. In addition, anti-TMEM106B antibody was used to confirm expression of mCherry-tagged TMEM106Bs. Note that co-transfection of GALC decreased expression of mCherry-tagged TMEM106Bs, but still significant amounts of mCherry-tagged TMEM106Bs were immunoprecipitated by RFP-trap from the co-transfected HEK293T cells. Note that all samples were treated with 2-mercaptoethanol and heat (95°C) before running a gel.



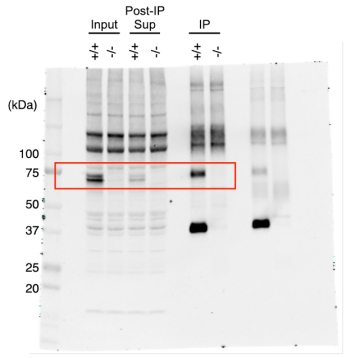
Supplementary Fig. 4: TMEM106B levels in the forebrain, brainstem, and cerebellum

a) Immunoblot analysis of the Triton X-100-soluble fraction from the forebrain, brainstem, and cerebellum of WT mice using ant-TMEM106B antibody (CST #93334). Note that immunoblot with anti-TMEM106B antibody and anti-β-actin antibody was performed using samples without and with 2-mercaptoethanol plus heat (95°C) treatment, respectively. F, forebrain; B, brainstem; C, cerebellum.

b) Quantification of immunoblot analysis in **a)**. n = 6 mice. Values shown in the graph are p-values from Tukey's multiple comparisons test after repeated measures one-way ANOVA with the Geisser-Greenhouse correction.

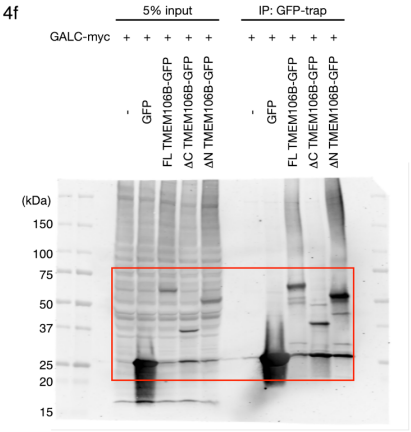
Supplementary Fig. 5: Uncropped blots

Fig. 4b

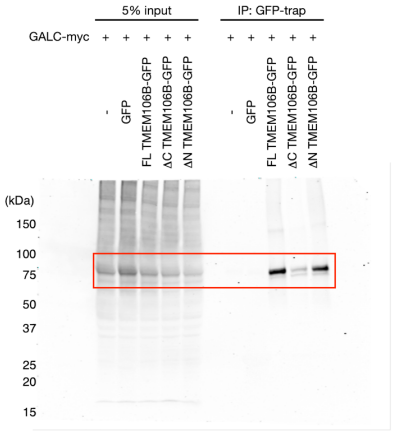


IB: TMEM106B (Abcam #ab140185)

Fig. 4f

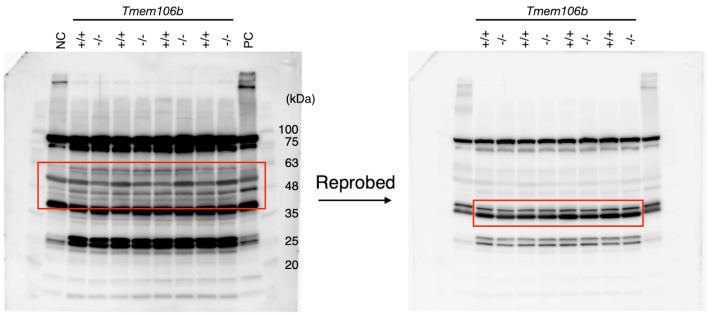


IB: GFP (Santa Cruz #sc-9996)



IB: Myc-Tag (CST #2278)

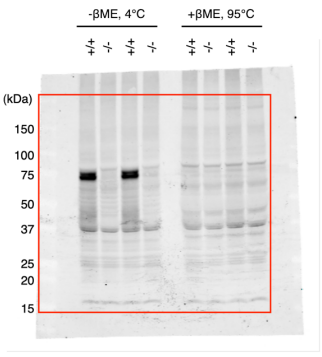
Fig. 5e



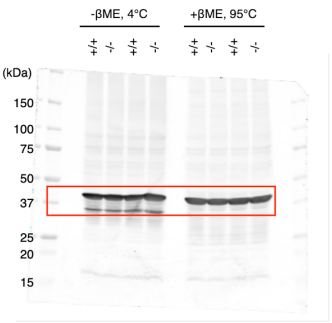
IB: GALC (CL1021AP)

IB: GAPDH (Proteintech #60004-1-Ig)

Supplementary Fig. 1a

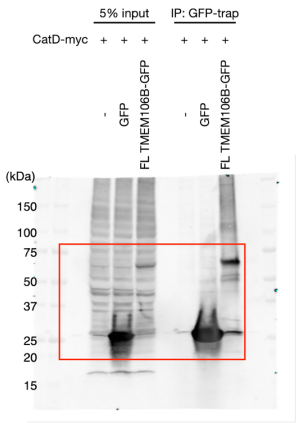


IB: TMEM106B (CST #93334)

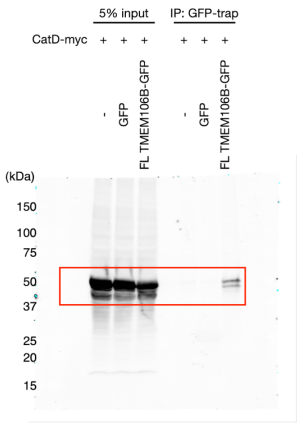


IB: β-actin (CST #3700)

Supplementary Fig. 1b

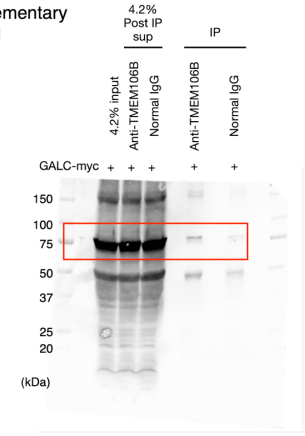


IB: GFP (Santa Cruz #sc-9996)



IB: Myc-Tag (CST #2278)

Supplementary Fig. 1d



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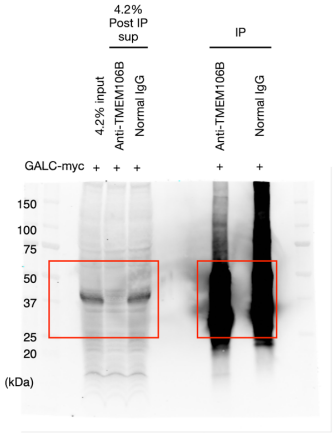
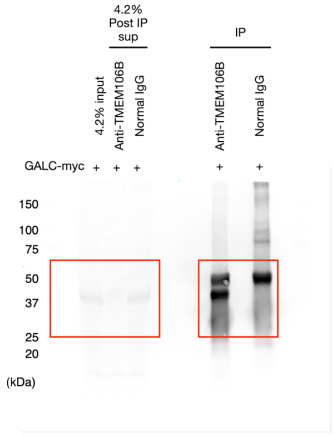


IB: TMEM106B (CST #93334)
High exposure



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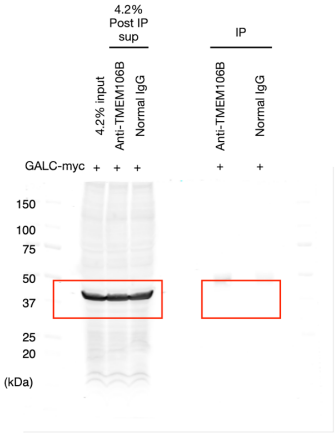
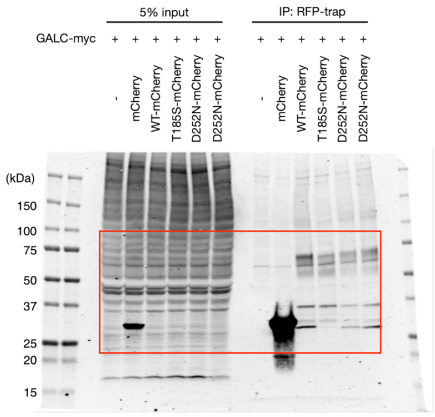
Low exposure

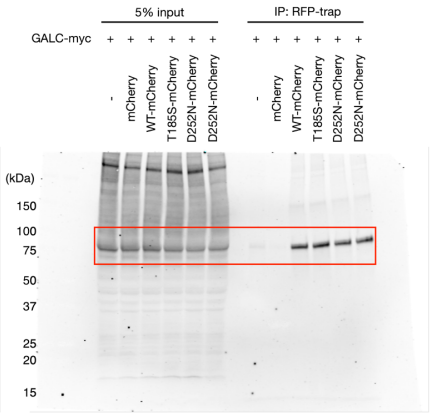


IB: β-actin (CST #3700)

Supplementary Fig. 3a



IB: RFP (ThermoFisher #MA5-15257)



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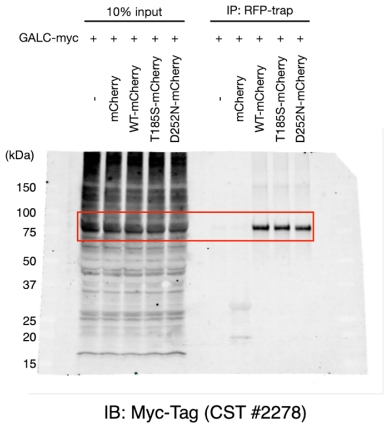
Supplementary Fig. 3b



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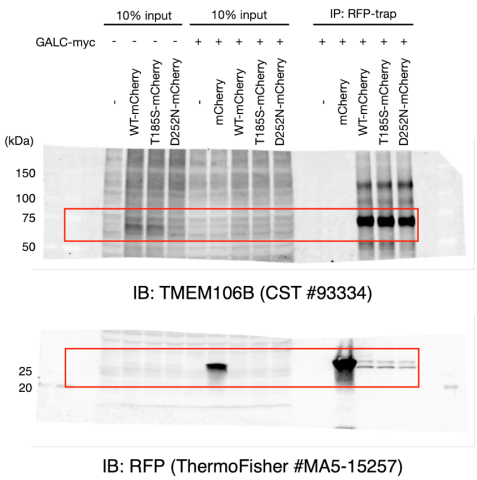
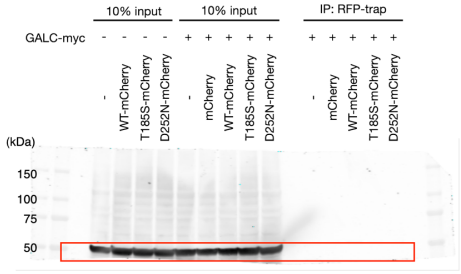


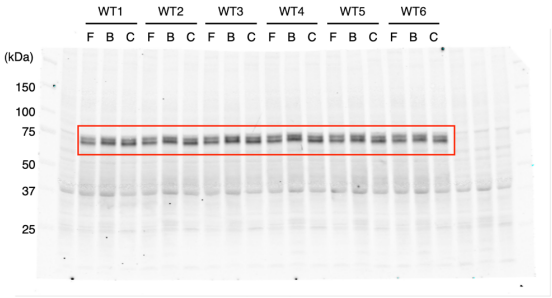
IB: TMEM106B (CST #93334)

IB: RFP (ThermoFisher #MA5-15257)



IB: β-tubulin (CST #86298)

Supplementary Fig. 4a



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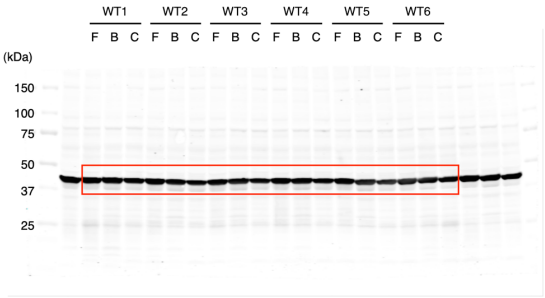

IB: β-actin (CST #3700)