Supplemental Materials

Molecular Biology of the Cell

Khakurel *et al*.

Supplementary Fig. S1. GARP-KO alters glycosylation in secreted glycoproteins and total lysates. **A,** WB of cathepsin D secreted from WT, GARP-KO and rescued RPE1 cells. Representative images from three independent experiments are shown. **B**, GNL-647 staining of secretory proteins from WT, GARP-KO and rescued RPE1 cells. **C**, HPA-647 staining of secretory proteins from WT, GARP-KOs and rescued RPE1 cells. **D, E,** GNL-647 (**D**) and HPA-647 (**E**) staining of whole-cell lysates from WT, GARP-KO HEK293T cells (left) and quantification of staining relative to WT HEK293T cells (right). Bars represent the mean ± SD of values from three independent experiments. Statistical significance was calculated using one-way ANOVA. ** P≤ 0.01, *** P ≤ 0.001.

Supplementary Fig. S2. **GARP KO alters the glycosylation of Golgi and lysosomal glycoproteins in HEK293T cells**. **A-D,** WB of endogenous GPP130 (**A**), TMEM165 (**B**), TGN46 (**C**) and LAMP2 (**D**) from cell lysates of WT, VPS53-KO and VPS54-KO cells. The same blot was used for incubation with rabbit anti-GPP130 and mouse anti-LAMP2 antibody; therefore, the same actin control was used.

Supplementary Fig. S3. GARP-KO alters the stability of N- and O-Golgi glycosylation enzymes in HEK293T cells. **A-C,** WB (top) and quantification (bottom) of MGAT1 (**A**), B4GalT1 (**B**) and ST6Gal1 (**C**). Bars represent the mean ± SD of values from three independent experiments. Statistical significance was calculated using one-way ANOVA. **** P ≤ 0.0001, ** P ≤ 0.01. (**D,E**) WB analysis of GalNacT2 (**D**) and ST6Gal1 (**E**) in GARP-KO HeLa cells (top) and quantification (bottom).

Supplementary Fig. S4. Localization of COG3 and COG8 is not altered in GARP-KO cells. (**A**) RPE1 cells were stained for COG3 (top row) or COG8 (bottom row) together with GM130, and images were taken by Airyscan microscopy. (**B**) WB of COG3 and COG8 in GARP-KO and rescued cells.

Supplementary Fig. S5. (A) RUSH assay reveals mislocalization of B4GalT1 in GARP-KO RPE1 and HeLa cells. RPE1 WT and VPS54-KO cells were transfected with plasmids encoding a B4GalT1 RUSH construct and chased with biotin/cycloheximide mix for 6 h. Cells were then stained for the Golgi marker GM130. Differences in co-localization of B4GalT1 with GM130 in cells with high B4GalT1-mCh expression or low B4GalT1-mCh expression was measured in approximately 30 cells using Pearson's correlation coefficient. Statistical significance was calculated using two-way ANOVA. *** P ≤ 0.001. No significant difference was observed between high B4GalT1-mCh expressed RPE1 cells and low B4GalT1-mCh expressed cells. (**B,C**) WT and VPS54-KO HeLa cells were co-transfected with plasmids encoding a B4GalT1-mCherry and MAN2A-GFP RUSH constructs and chased with biotin/cycloheximide mix

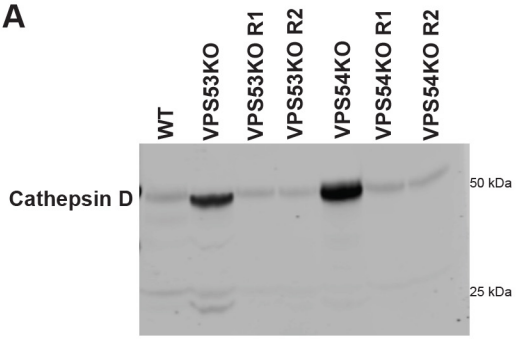
for 1 h (**B**) and 6 h(**C**), followed by staining for giantin. The arrows on **C** indicate the co-localization of B4GalT1 and MAN2A in putative endolysosomal puncta.

Supplementary Fig. S6. B4GalT1 localization is altered after 90 min exposure to chloroquine in HeLa and RPE1 cells. HeLa cells expressing endogenously tagged B4GalT1-GFP were treated or not treated with 0.1 mM chloroquine (CQ) for 90 min. Cells were stained for giantin and GPP130. **B,** RPE1 cells treated for 90 min with 0.1 mM chloroquine (CQ). Cells were stained for giantin, GPP130 and B4GalT1. **C,** Quantification of co-localization of B4GalT1-GFP with giantin (left panel) and giantin with GPP130 (right panel) in 40 HeLa cells using Pearson's correlation coefficient. Statistical analysis was done using paired t test in GraphPrism 8, **** $P \leq 0.0001$, ns, not significant. **D,** Quantification of co-localization of B4GalT1 with giantin in 40 RPE1 cells using Pearson's correlation coefficient. Statistical analysis was done using paired t test in GraphPrism 8, * $P \leq 0.05$. ns, not significant.

Supplementary Fig. S7. B4GalT1 recycles back to the Golgi upon chloroquine wash-out. A, HeLa cells expressing endogenously tagged B4GalT1-GFP were treated for 3 h with 0.1 mM chloroquine (CQ) (top panel) and washed for 3 h in normal culture medium (bottom panel). Cells were stained for giantin and GPP130. **B,** RPE1 cells treated for 3 h with 0.1 mM chloroquine (CQ) (top panel) or washed for 3 h in normal culture medium (bottom panel). Cells were stained for giantin, GPP130 and B4GalT1. **C,** Quantification of co-localization of B4GalT1-GFP with giantin in 40 HeLa cells using Pearson's correlation coefficient. Each dot on the bar graph indicates the co-localization in cells per field. *** $P \leq 0.001$. **D,** Quantification of co-localization of B4GalT1 with giantin in 40 RPE1 cells using Pearson's correlation coefficient. Each dot on the bar graph indicates the co-localization in cells per field. Statistical analysis was done using GraphPad Prism (paired t test). ** $P \leq 0.01$.

Supplementary 1

S1A



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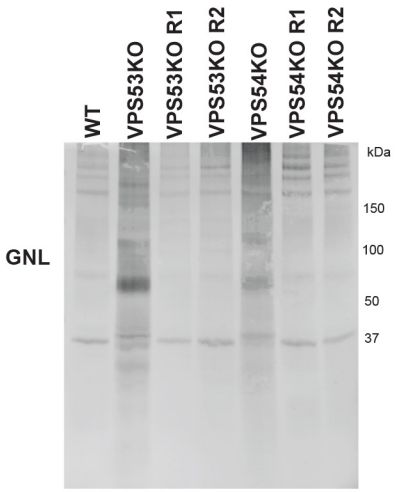

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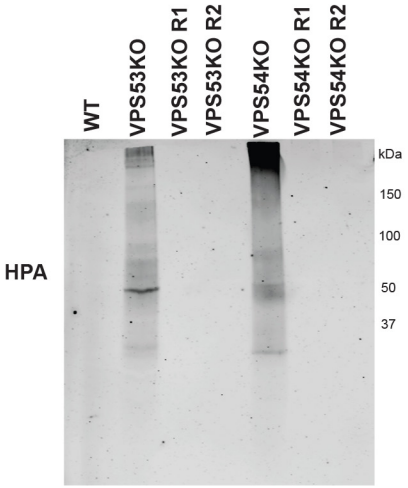

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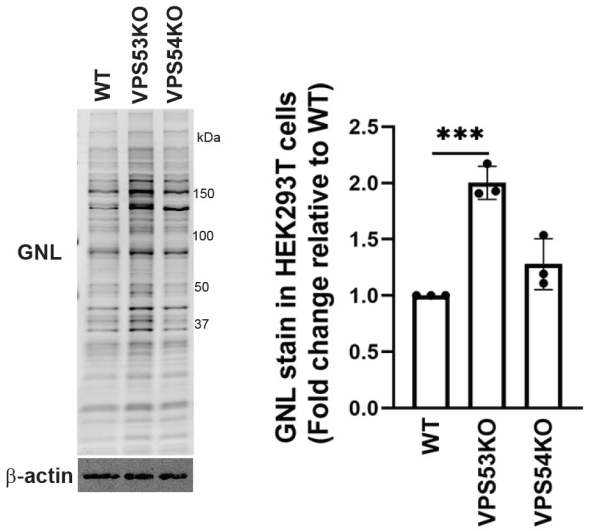

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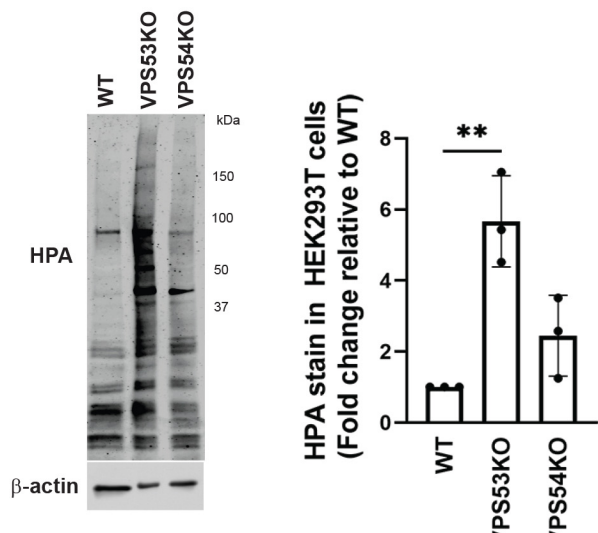

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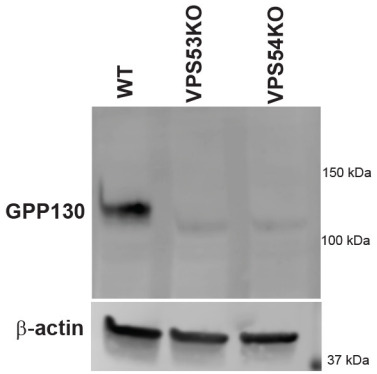

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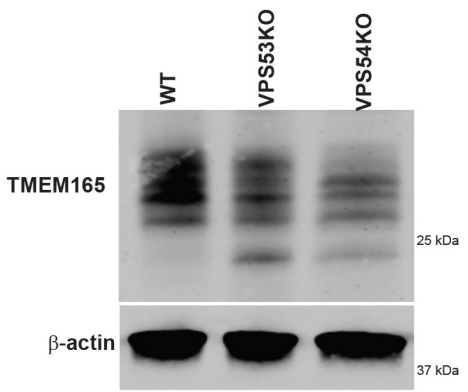

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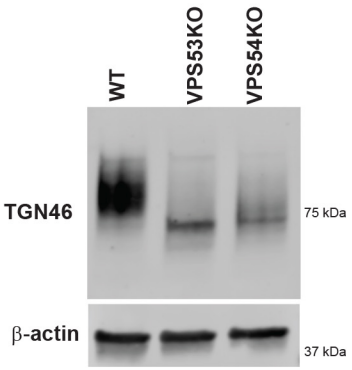

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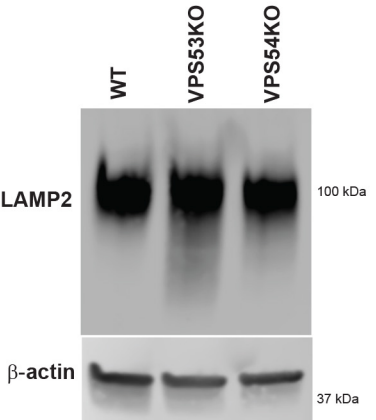

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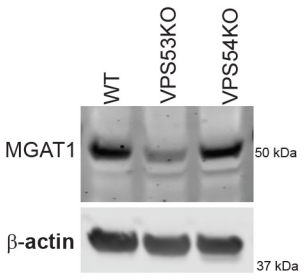

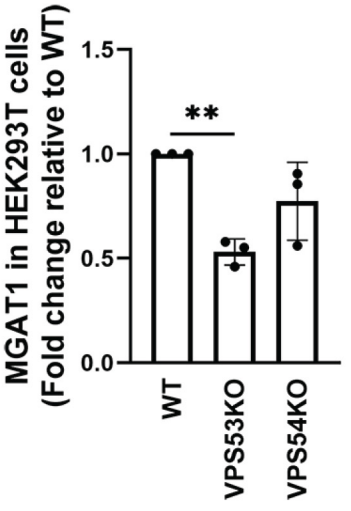

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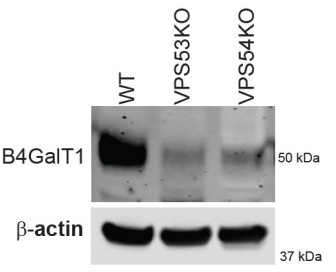

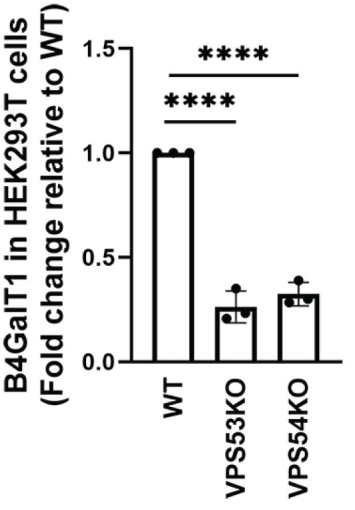

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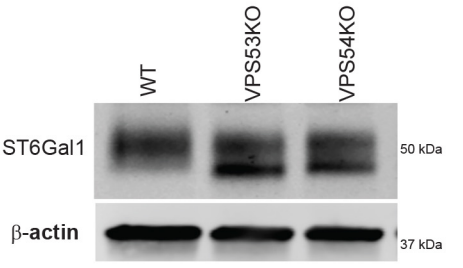

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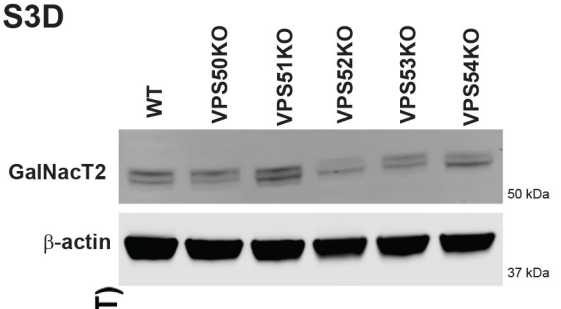

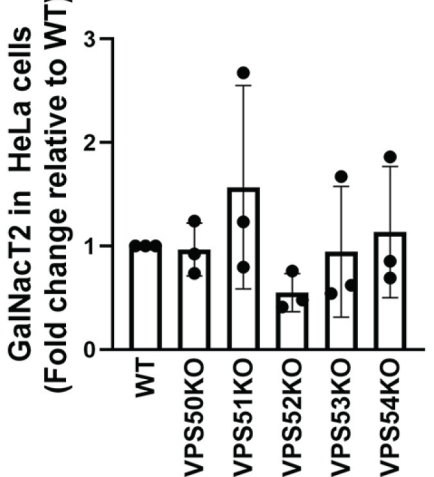

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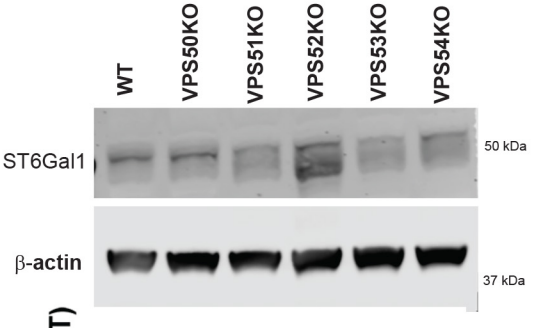

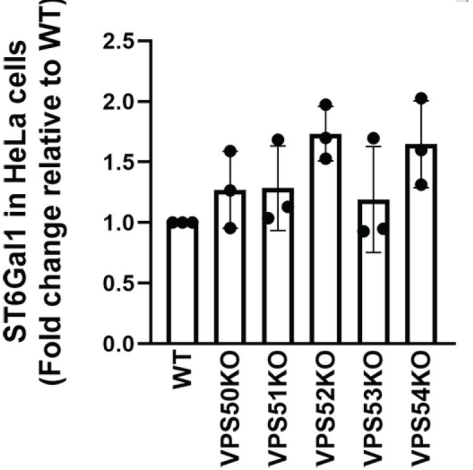

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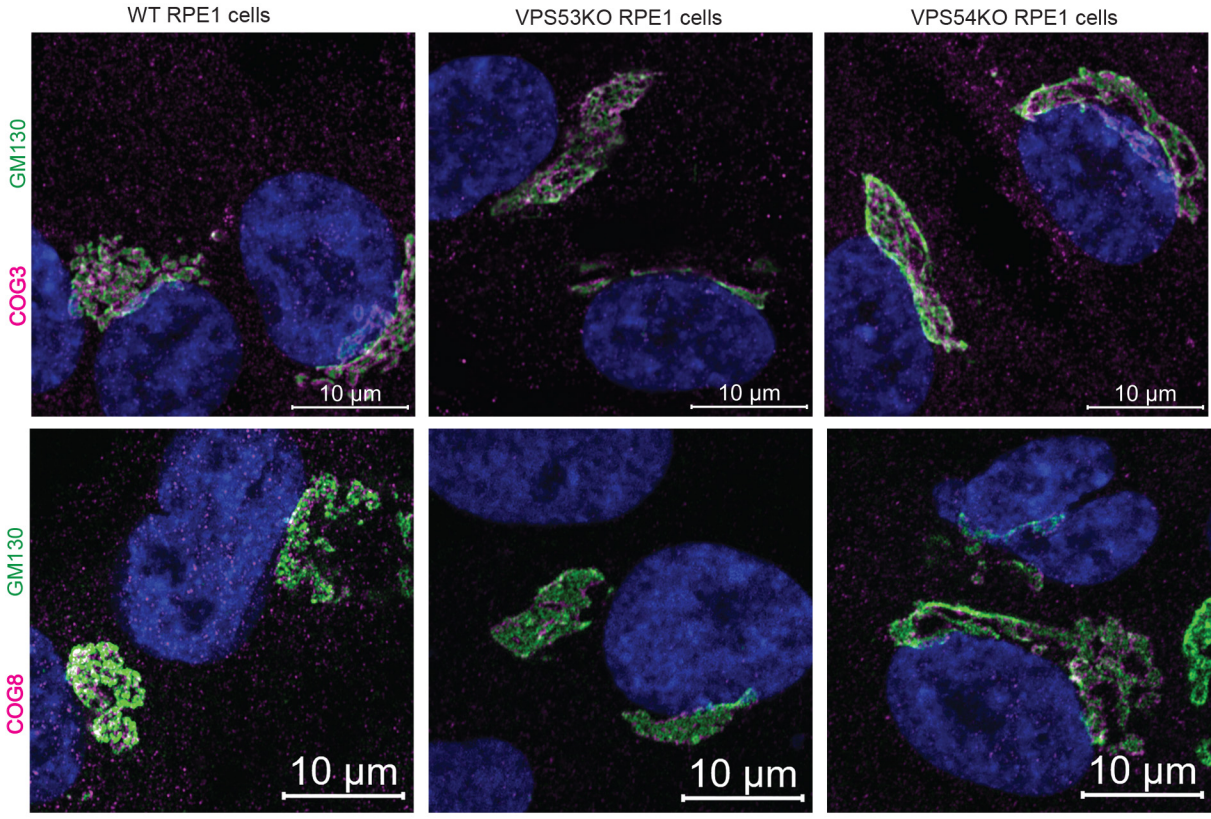

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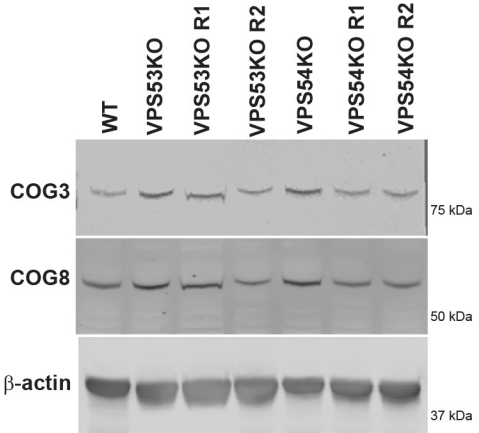

Supplementary 5

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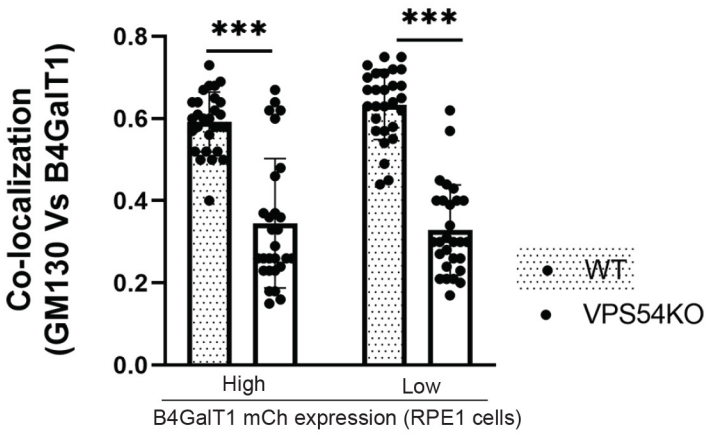

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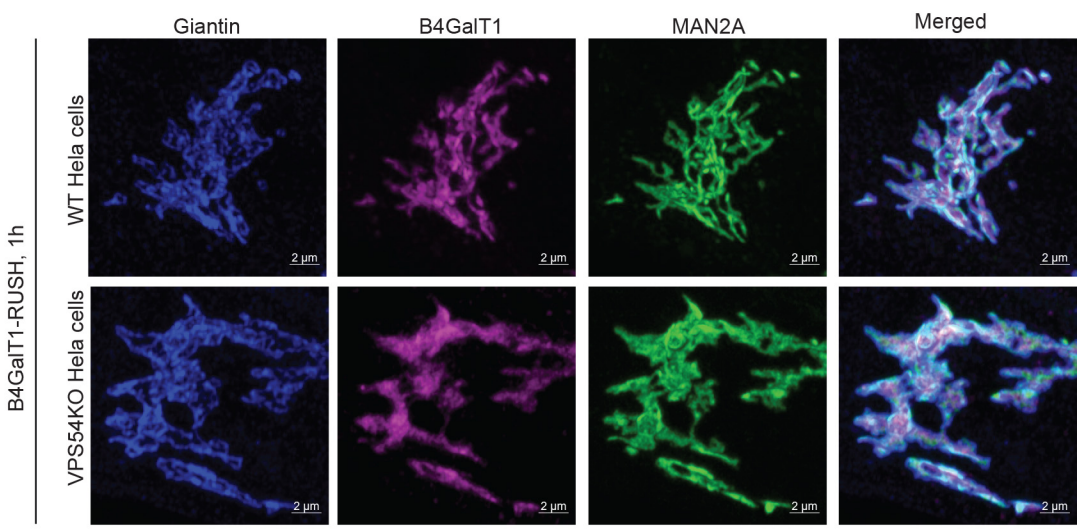

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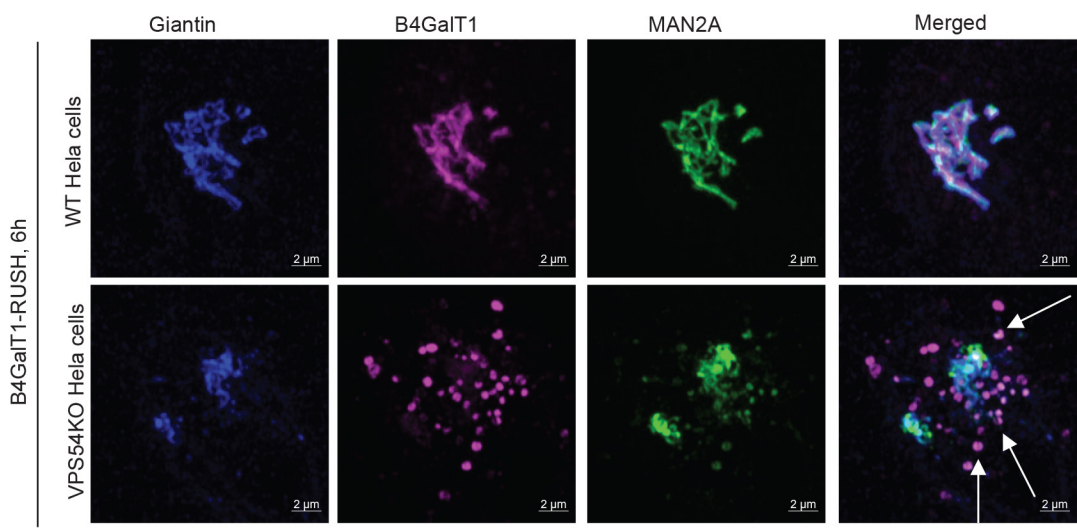

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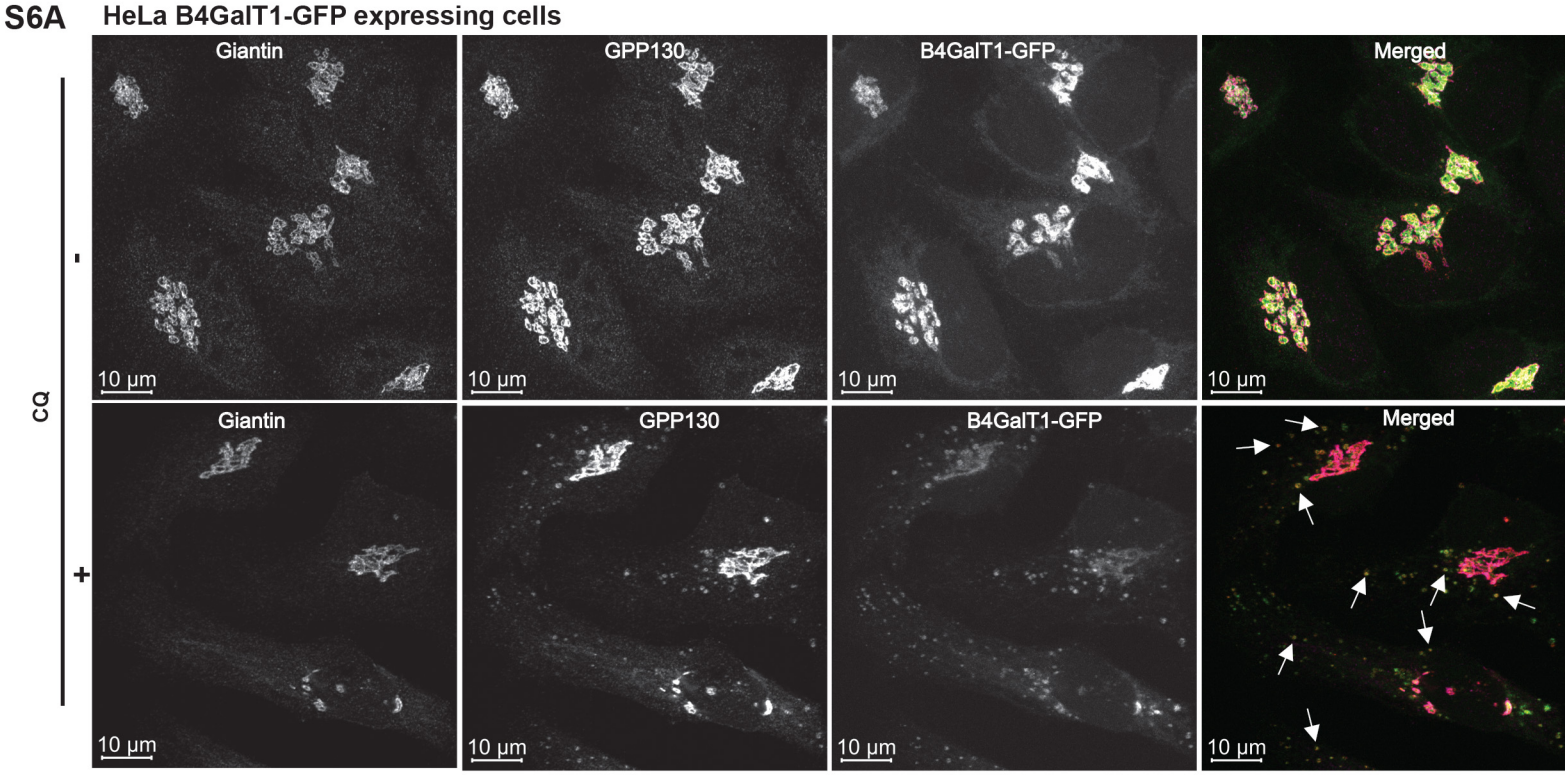

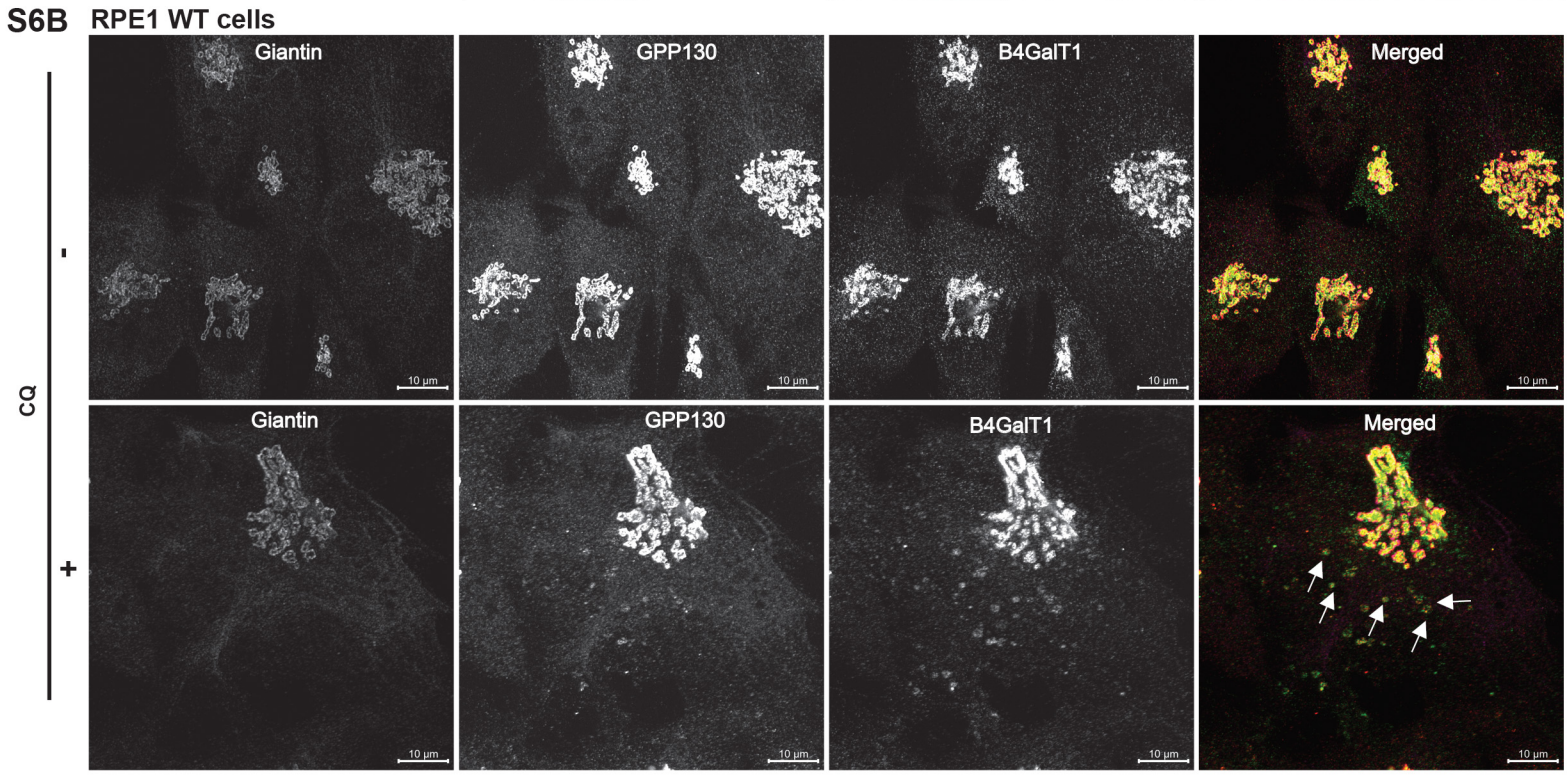

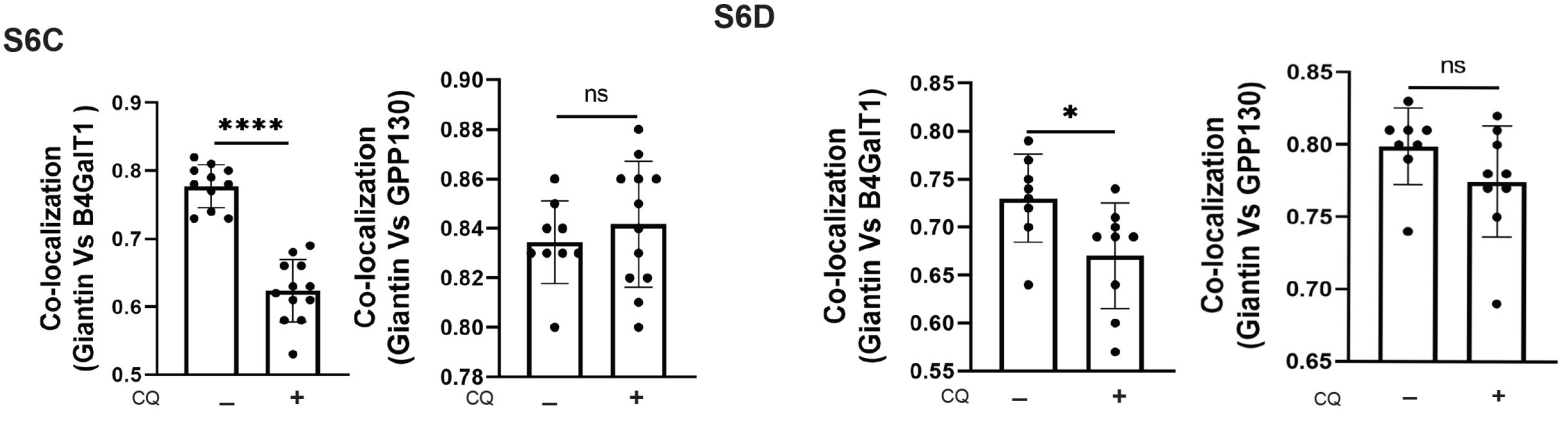

Supplementary figure 7