Supplemental material

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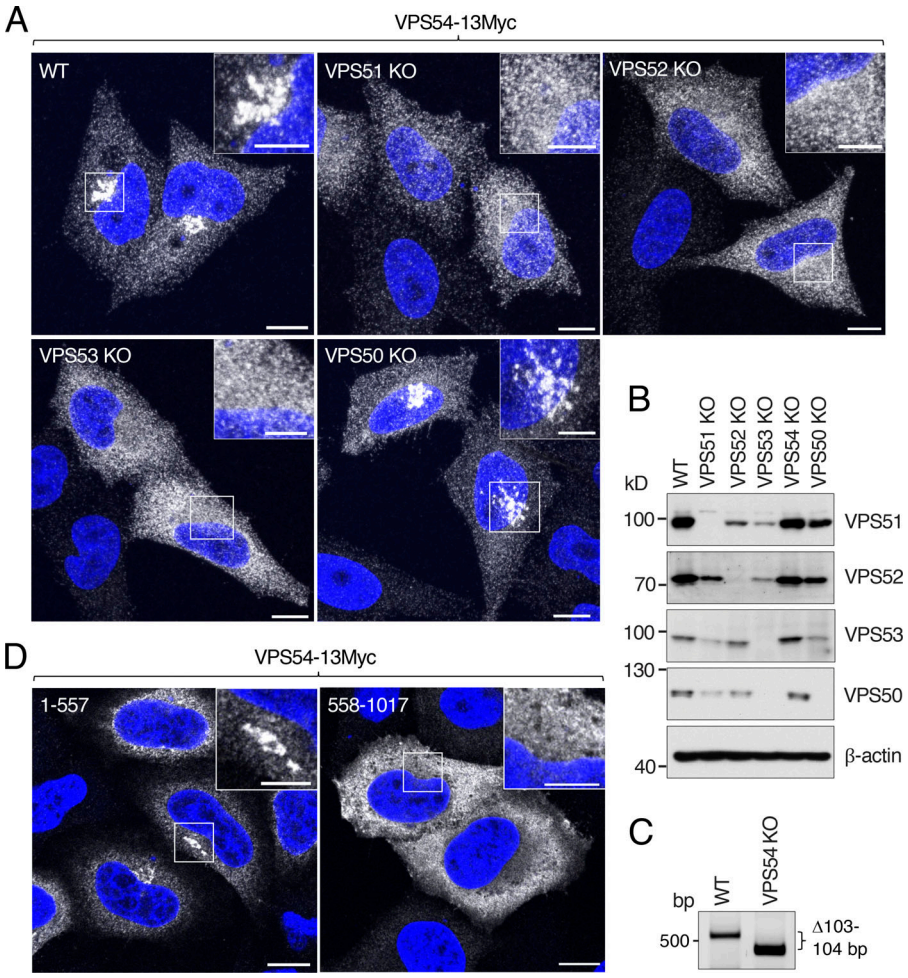

Figure S1. **Validation of epitope-tagged VPS54 as a surrogate for GARP in immunofluorescence microscopy experiments.** Genes encoding the VPS50, VPS51, VPS52, VPS53, and VPS54 subunits of GARP/EARP (see Fig. 1 B) were knocked out in HeLa cells using CRISPR/Cas9. **(A)** Immunofluorescence microscopy of WT and KO HeLa cells transfected with a plasmid encoding VPS54-13Myc and stained for the Myc epitope and nuclei (DAPI; blue). Scale bars: 10 µm. Insets are magnified views of the boxed areas. Inset scale bars: 5 µm. Notice that KO of VPS51, VPS52, or VPS53, but not VPS50, abrogated the localization of VPS54-13Myc to the TGN, yielding a cytosolic pattern of staining. These observations indicated that localization of VPS54-13Myc to the TGN requires the whole GARP complex and that transgenic VPS54-13Myc can be used as a surrogate for GARP in immunofluorescence microscopy experiments. **(B)** Confirmation of the KO of GARP and EARP subunits in HeLa cells by SDS-PAGE and immunoblotting with antibodies to the proteins indicated on the right. β-actin was used as a loading control. The positions of molecular mass markers are indicated on the left. **(C)** Due to the lack of good antibodies to VPS54, 103-bp and 104-bp deletions in the *VPS54* gene were detected by genomic PCR and agarose gel electrophoresis, with confirmation by Sanger sequencing. These deletions are predicted to result in frameshifts causing premature termination at amino acid positions 22 and 11, respectively. **(D)** Demonstration that the N-terminal half of VPS54 is sufficient for association with the TGN. VPS54 fragments comprising amino acids 1–557 or 558–1,017 appended with 13 copies of the Myc epitope were expressed in H4 cells and detected by immunostaining for Myc. Nuclei were stained with DAPI. Scale bars: 10 µm. Insets are magnified views of the boxed areas. Inset scale bars: 5 µm. Notice the localization of the 1–557 fragment, but not the 558–1,017 fragment, to the TGN.

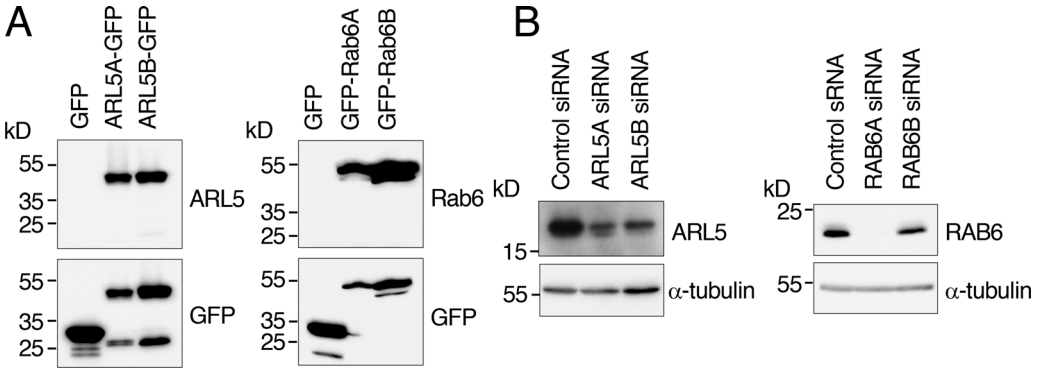

Figure S2. **Specificity of antibodies to ARL5 and RAB6 paralogs. (A)** Analysis of the paralog specificity of antibodies to ARL5 and RAB6 used in this study. pGFP-N1, pGFP-N1-ARL5A, pGFP-N1-ARL5B, pGFP-C1-Rab6A, or pGFP-C1-Rab6B (mouse orthologues of human RAB6A and RAB6B) were expressed by transfection into HEK293T cells, and 48 h after transfection, the cells were harvested and lysed in 1% Triton X-100; 50 mM Tris, pH 7.4; 150 mM NaCl; and cOmplete EDTA-free Protease Inhibitor. Because the antibodies nonspecifically bound to a protein of ∼55 kD, which overlapped with ARL5A-GFP and ARL5B-GFP, these GFP-tagged proteins were immunoprecipitated using magnetic GFP-trap beads (ChromoTek) before immunoblotting. The immunoprecipitates were analyzed by SDS-PAGE followed by immunoblotting with anti-ARL5 and anti-GFP antibodies. The lysates containing GFP, GFP-Rab6A, or GFP-Rab6B were directly analyzed by SDS-PAGE followed by immunoblotting with anti-Rab6 and anti-GFP antibodies. Note that the anti-ARL5 antibody recognized both ARL5A and ARL5B, and that the anti-Rab6 antibody recognized both Rab6A and Rab6B, but did not recognize GFP. **(B)** KDs of endogenous ARL5 or RAB6 proteins in HeLa cells by specific siRNAs are shown. The siRNAs targeting ARL5A, ARL5B, RAB6A, or RAB6B were transfected into HeLa cells, and 72 h after transfection, the cells were harvested and lysed with the buffer described above. The lysates were analyzed by SDS-PAGE followed by immunoblotting with antibodies to ARL5, RAB6, and α-tubulin (loading control). Note that the treatment with siRNAs to ARL5A, ARL5B, RAB6A, or RAB6B reduced the protein levels, indicating that all of these isoforms are expressed in this HeLa cell line. The positions of molecular mass markers are indicated on the left.

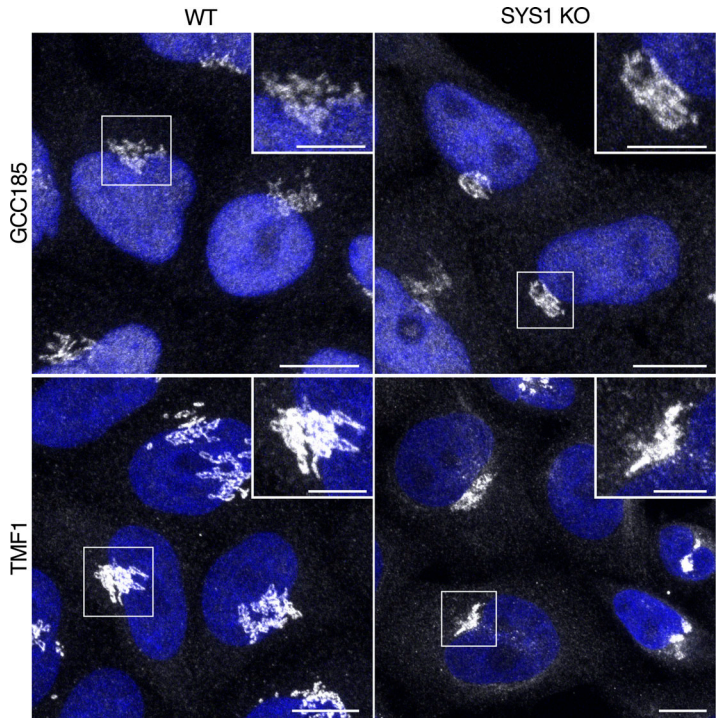

Figure S3. **Intracellular localization of GCC185 and TMF1 in WT and SYS1-KO cells.** Immunofluorescence microscopy of endogenous GCC185 or TMF1 in WT and SYS1-KO cells counterstained with DAPI (blue). Scale bars: 10 µm. Insets are magnified views of the boxed areas. Inset scale bars: 5 µm. Notice that the localization of GCC185 and TMF1 to the Golgi complex is unaffected by KO of SYS1.

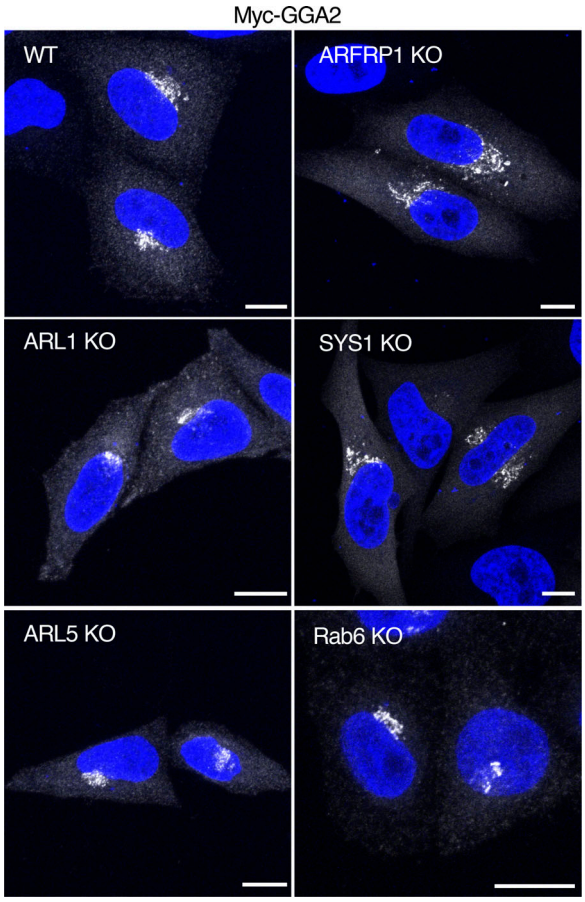

Figure S4. **Intracellular localization of Myc-GGA2 in WT, ARL1-KO, ARL5-KO, ARFRP1-KO, RAB6-KO, and SYS1-KO cells.** Myc-tagged GGA2 (Dell'Angelica et al., 2000) was expressed by transient transfection in WT, ARL1-KO, ARL5-KO, ARFRP1-KO, RAB6-KO, and SYS1-KO cells. Cells were then stained for the Myc epitope and counterstained with DAPI (blue). Scale bars: 10 µm. Notice the presence of Myc-GGA2 at the TGN of all the transfected cells. Together with data in Fig. 2 B and Fig. 5 A, these data demonstrate that KO of SYS1 or downstream GTPases or effectors does not cause complete disruption of the TGN.