Simonetti et al., https://doi.org/10.1083/jcb.201703015

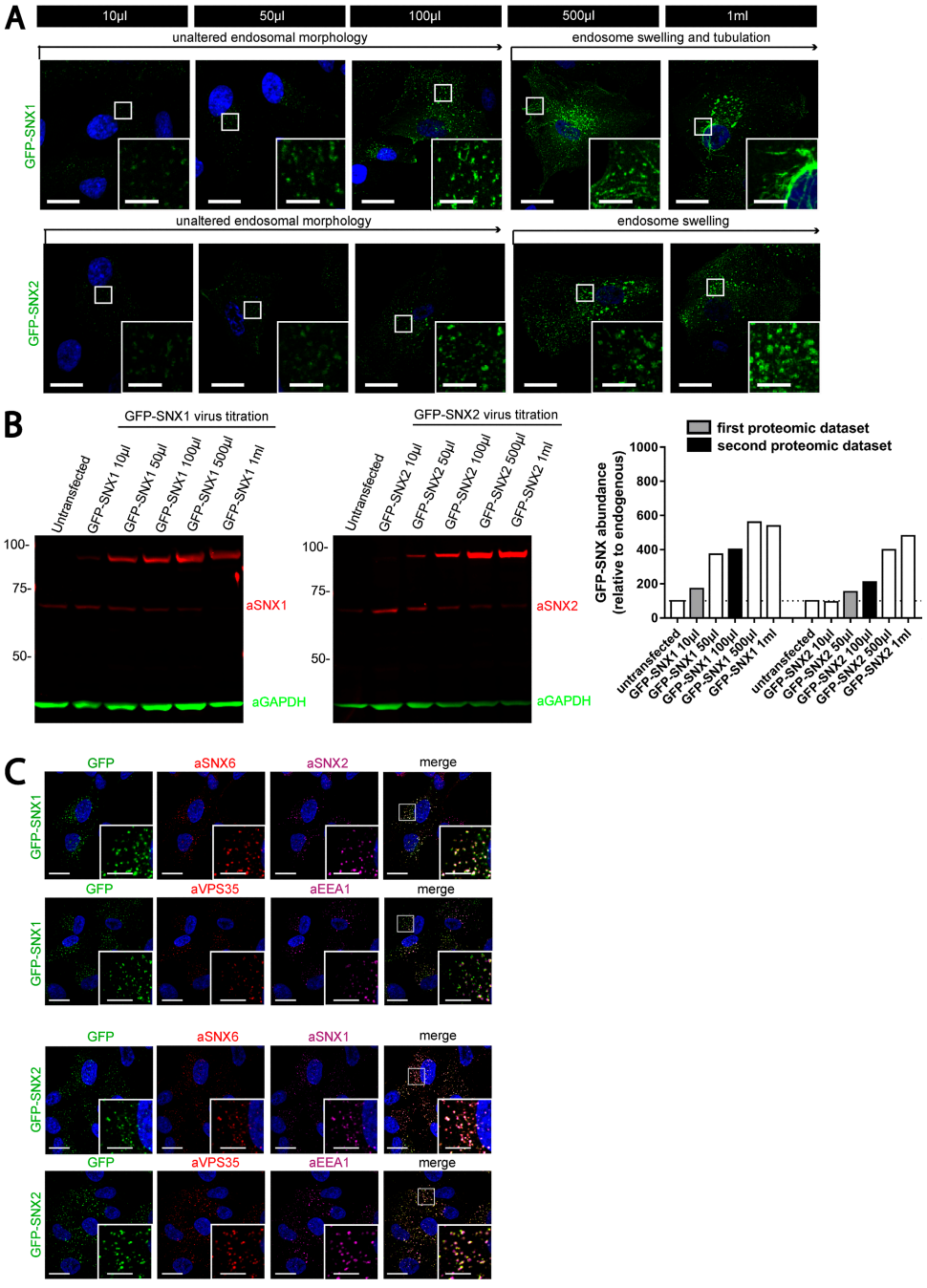


Figure S1. **Titration of GFP-SNX1 and GFP-SNX2 lentivirally transduced RPE1 cells.** (A) GFP-SNX1 and GFP-SNX2 show a punctate localization and have an increased tubular profile as the level of overexpression increases. RPE1 cells were lentivirally transduced with increasing concentration of GFP-SNX1 or GFP-SNX2 viral particles. Transduced cells were then fixed and imaged. Low titrations show a punctate localization similar to the appearance of endogenous SNX1- and SNX2-positive endosomes. GFP-SNX1 also localized on extended tubules at higher viral titrations. Bars: (main images) 20 µm; (insets) 5 µm. (B) Quantification of GFP-SNX1 and GFP-SNX2 expression level over the respective endogenous protein. Cell extracts derived from RPE1 cells lentivirally transduced with GFP-SNX1 and GFP-SNX2 were blotted for endogenous SNX1, endogenous SNX2, and GAPDH as the loading control. The amount of protein was quantified using an Odyssey scanning system and normalized over the loading control. Data are expressed as fold overexpression relative to the endogenous SNX1 or SNX2 proteins. The dotted line highlights the normalized level of endogenous SNX1 and SNX2. Molecular masses are given in kilodaltons. (C) Colocalization of GFP-tagged forms of SNX1 and SNX2 is not affected. Lentivirally transduced GFP-SNX1 and GFP-SNX2 RPE1 cells were fixed and stained for the endosome markers EEA1, VPS35, SNX6, SNX1, and SNX2. Bars: (main images) 20 µm; (insets) 10 µm.

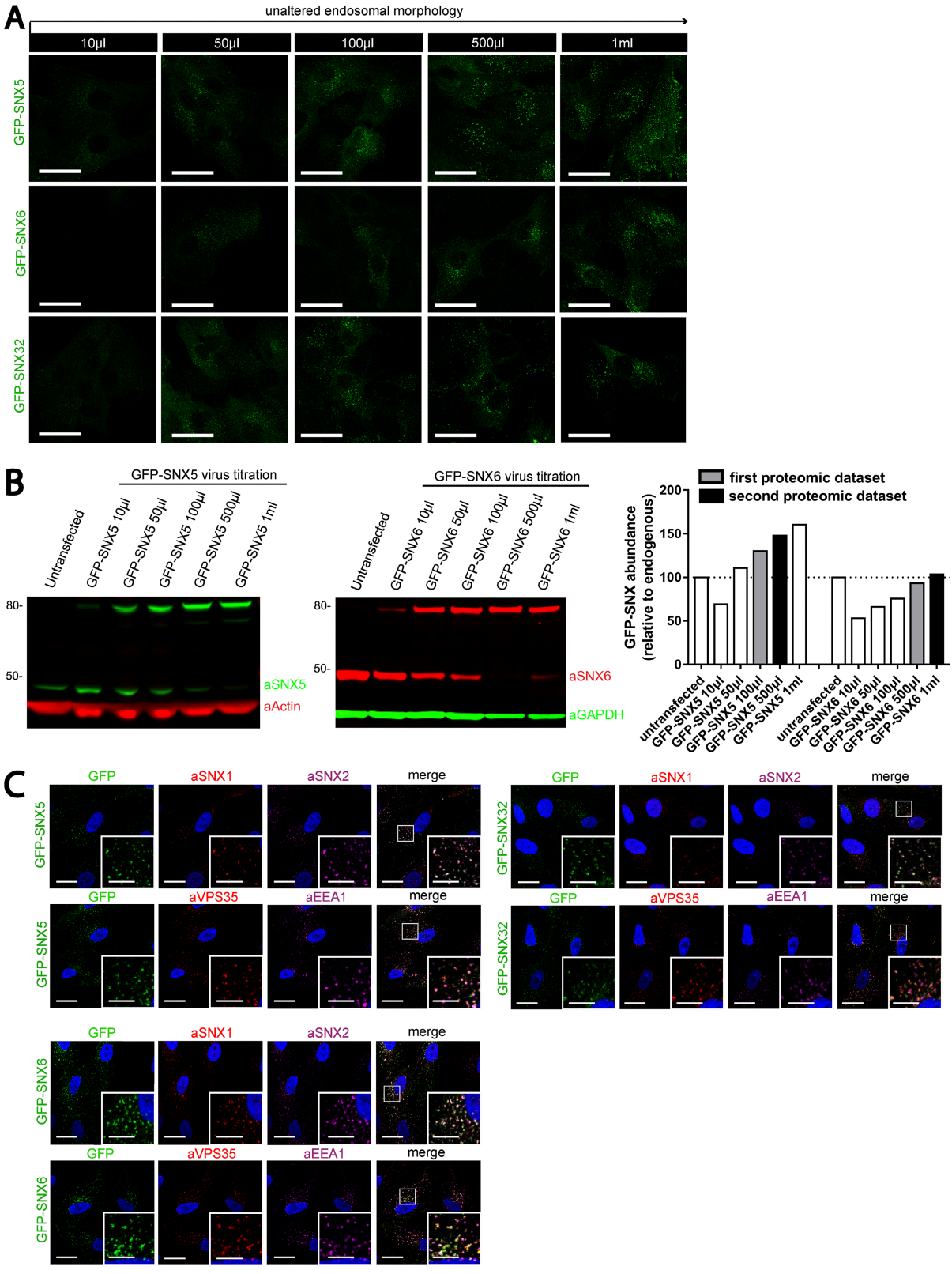


Figure S2. **Titration of GFP-SNX5, GFP-SNX6, and GFP-SNX32 lentivirally transduced RPE1 cells.** (A) GFP-SNX5, GFP-SNX6, and GFP-SNX32 show a cytosolic and punctate localization. RPE1 cells were lentivirally transduced with increasing concentrations of GFP-SNX5, GFP-SNX6, or GFP-SNX32 viral particles. Transduced cells were then fixed and imaged. Bars, 20 µm. (B) Quantification of GFP-SNX5 and GFP-SNX6 expression levels over their corresponding endogenous proteins. Cell extracts derived from RPE1 cells lentivirally transduced were blotted for SNX5, SNX6, and actin. The amount of protein was quantified using the Odyssey scanning system and normalized over the loading control. Data are expressed as percent overexpression relative to the endogenous SNX5 and SNX6 proteins. (C) Colocalization of GFP-tagged forms of SNX5, SNX6, and SNX32 is not affected. Lentivirally transduced RPE1 cells were fixed and stained for the endosome markers EEA1, VPS35, SNX1, and SNX2. Bars: (main images) 20 µm; (insets) 10 µm.

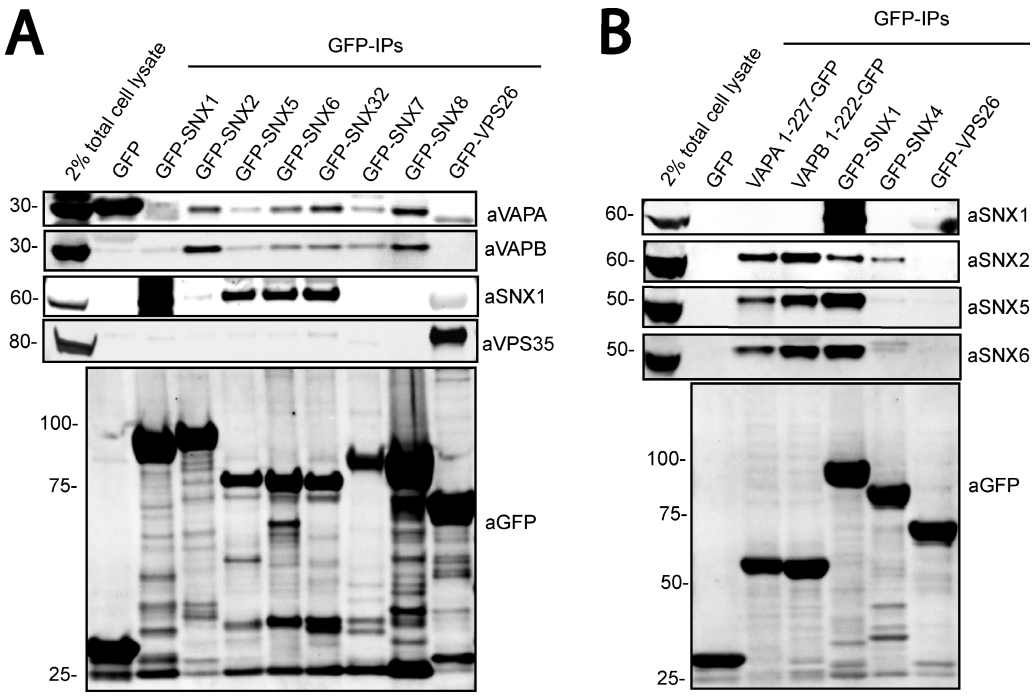


Figure S3. **Validation of retromer-linked SNX-BAR interactors.** (A) GFP-tagged SNX-BAR constructs transiently transfected in HEK293T cells showing the binding of SNX2 with VAPA and VAPB. (B) Expression of the GFP-tagged cytosolic domain of VAPA and VAPB transiently transfected in HEK293T cells. The cytosolic domain of VAP proteins is sufficient to interact with SNX2–SNX5/6 heterodimers. Molecular masses are given in kilodaltons. IP, immunoprecipitation.

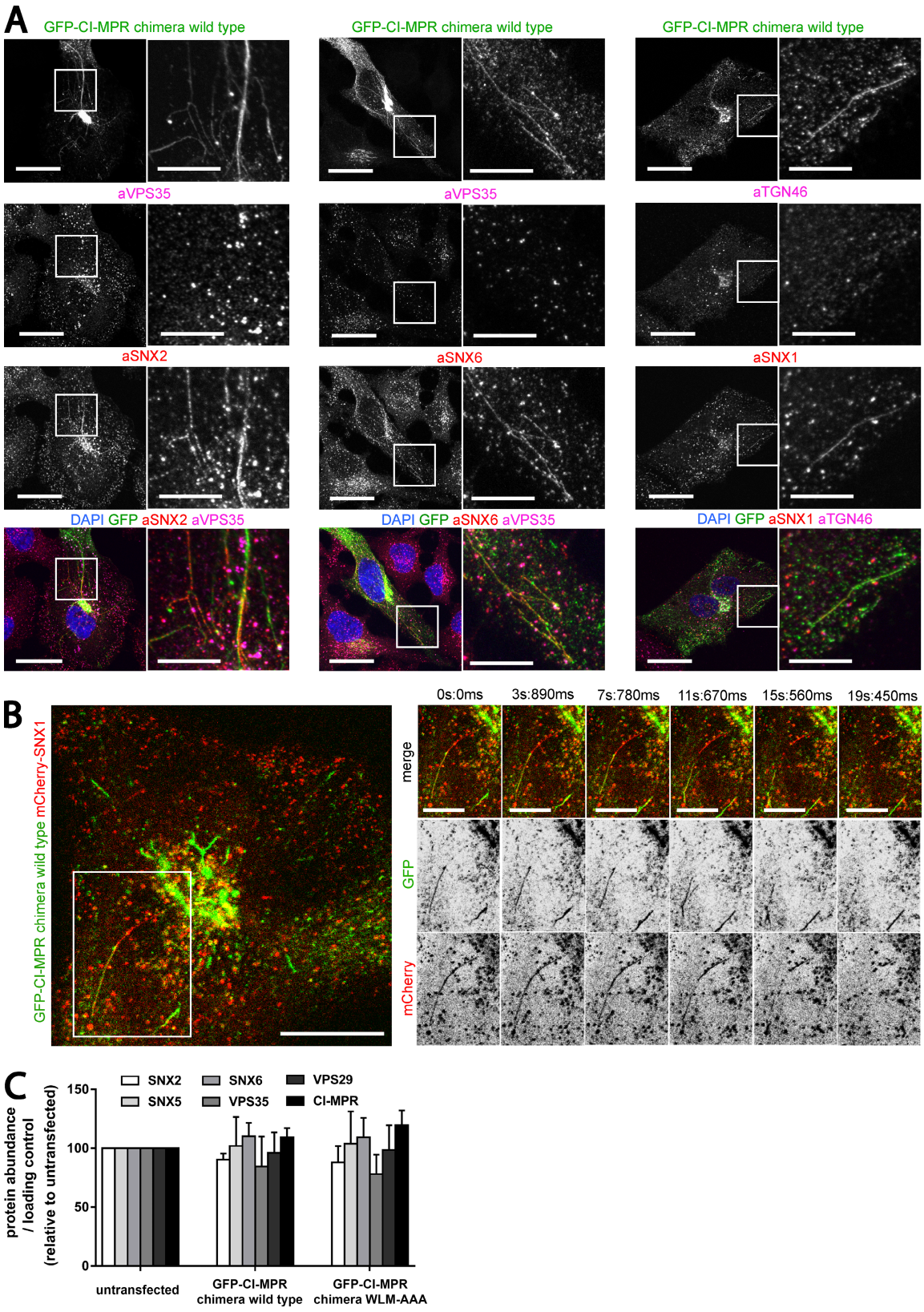


Figure S4. **The WT GFP–CI-MPR chimera induces the formation of tubules decorated with SNX1/2–SNX5/6 but not with retromer.** (A) HeLa cells were transfected with the GFP–CI-MPR chimera WT construct and immunostained for endogenous SNX1, SNX2, SNX6, VPS35, and TGN46 after 48 h. (B) HeLa cells were transfected with GFP–CI-MPR chimera WT and mCherry-SNX1 and live imaged after 48 h. Bars: (main images) 20 µm; (zooms [A] and time series [B]) 10 µm. (C) The GFP–CI-MPR chimera WT and the GFP–CI-MPR chimera WLM-AAA mutant do not affect retromer or SNX-BARs levels. HeLa cells were transfected with CI-MPR chimeras. 48 h after transfection, endogenous protein levels were analyzed by Western blotting. n = 3 independent experiments (means ± SEM).

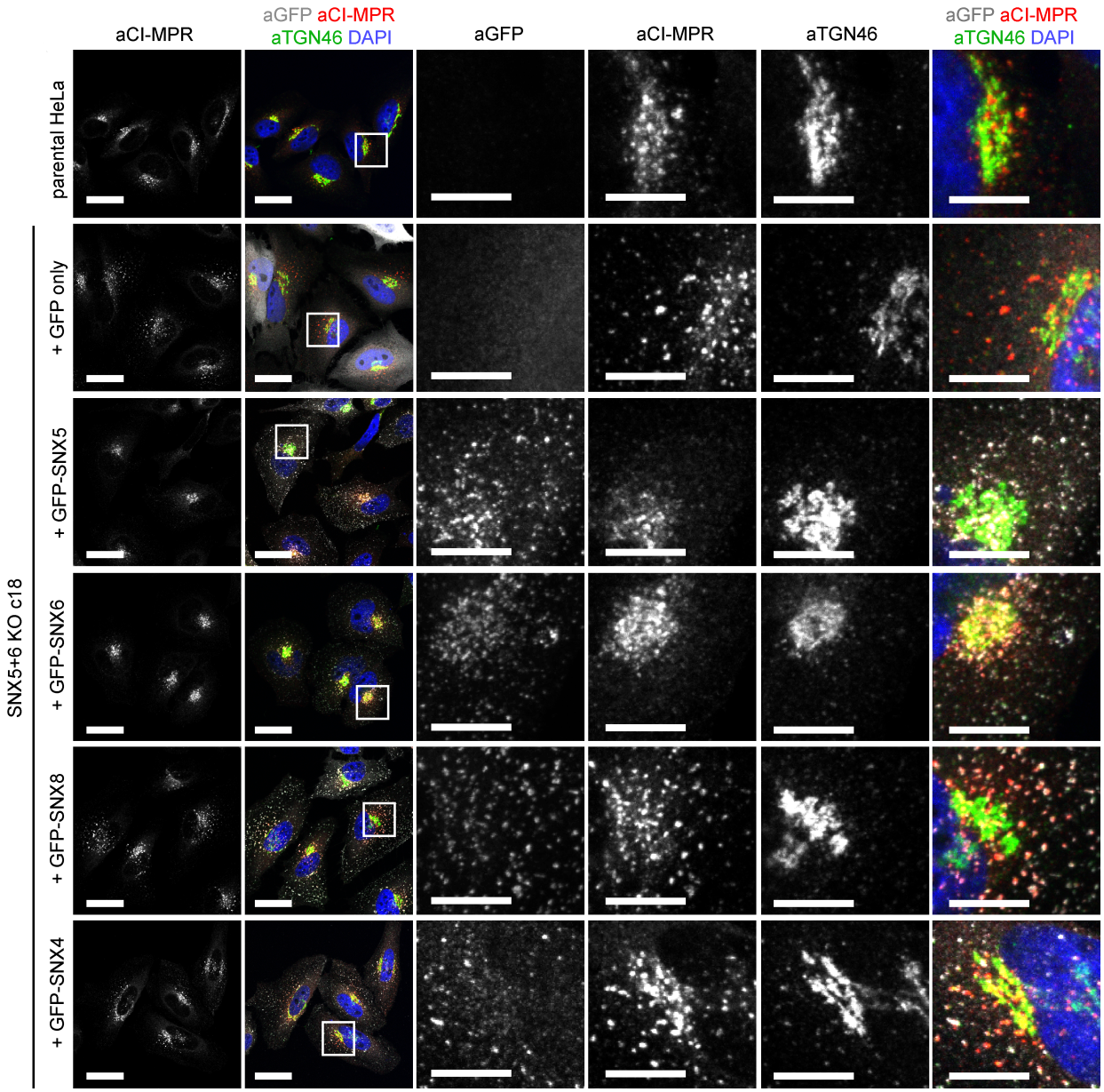
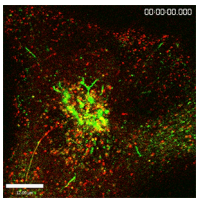


Figure S5. **Reexpression of GFP-tagged SNX5 or SNX6 rescues the normal steady-state distribution of CI-MPR in the SNX5+6 KO c18 clonal line.** The SNX5+6 KO clonal line c18 was lentivirally transduced with GFP-SNX5, GFP-SNX6, GFP-SNX4, GFP-SNX8, or GFP alone. Colocalization analysis of endogenous CI-MPR and TGN marker TGN46 allowed comparison of CI-MPR distribution between the transduced lines and parental HeLa. Bars: (main images) 20 µm; (zooms) 10 µm.



Video 1. **SXN1-decorated GFP–CI-MPR chimera tubules are highly dynamic.** SXN1-decorated GFP–CI-MPR chimera tubules are highly dynamic HeLa cells and were transfected with GFP–CI-MPR chimera WT and mCherry-SNX1 and then live imaged after 48 h. GFP signal (green) and mCherry signal (red) were imaged using a confocal laser-scanning microscope at 37°C. "Adaptive Focus Control" was used to correct focus drift during time courses. Frames were collected every 3.890 s for 03:30:06 min. Playback rate is 3.5 frames per second. Representative frames from Video 1 showing the dynamics of a GFP-positive and mCherry-positive tubules are displayed in Fig. S4 B.