Figure S1

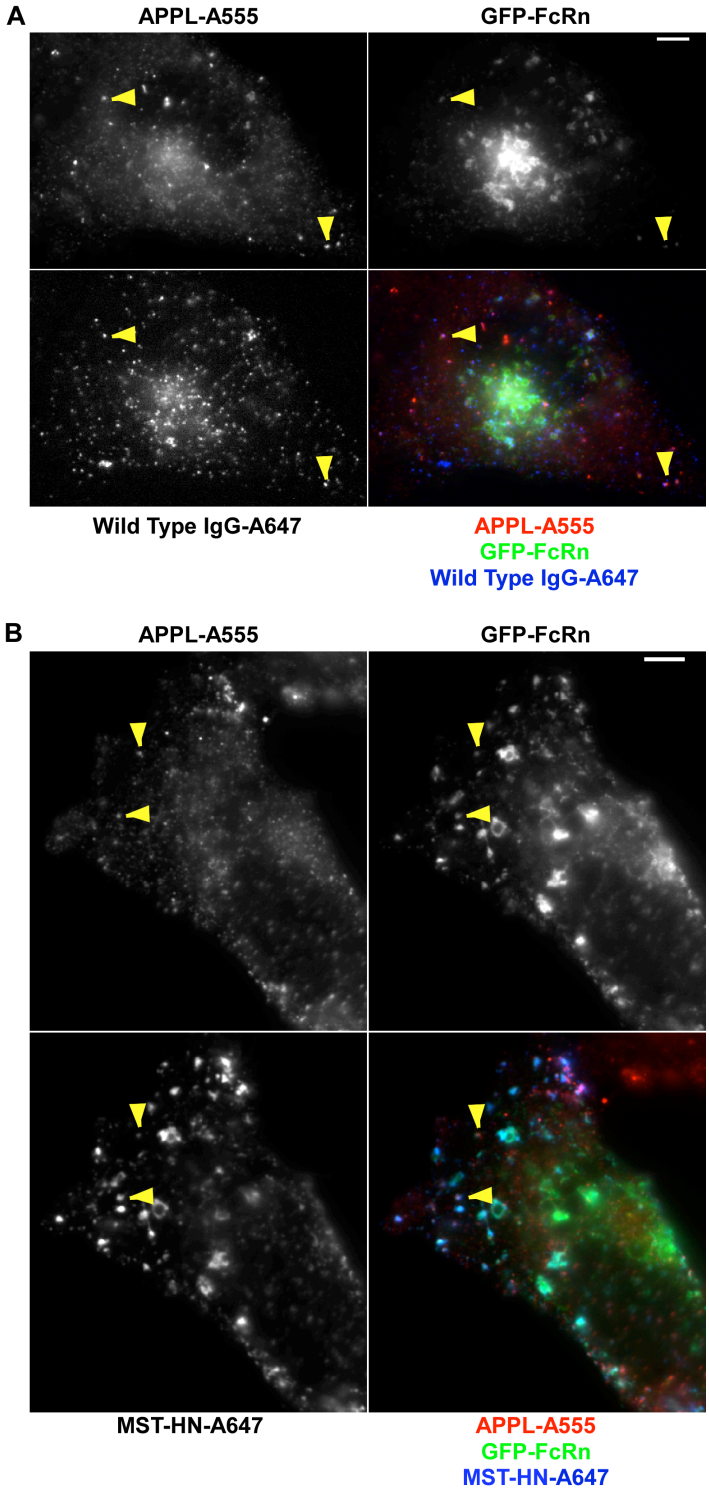


Figure S1. Wild type IgG and FcRn colocalize in APPL+ TCs. HMEC-1 cells were cotransfected with GFP-FcRn and β_2m. Transfected cells were incubated with 200 µg/mL Alexa 647-wild type IgG (A) or 5 µg/mL MST-HN IgG (B) for 10 min. Cells were then fixed and stained with anti-APPL antibody. Yellow arrowheads indicate examples of FcRn+IgG+APPL+ TCs. Scale bars = 5 µm.

Figure S2

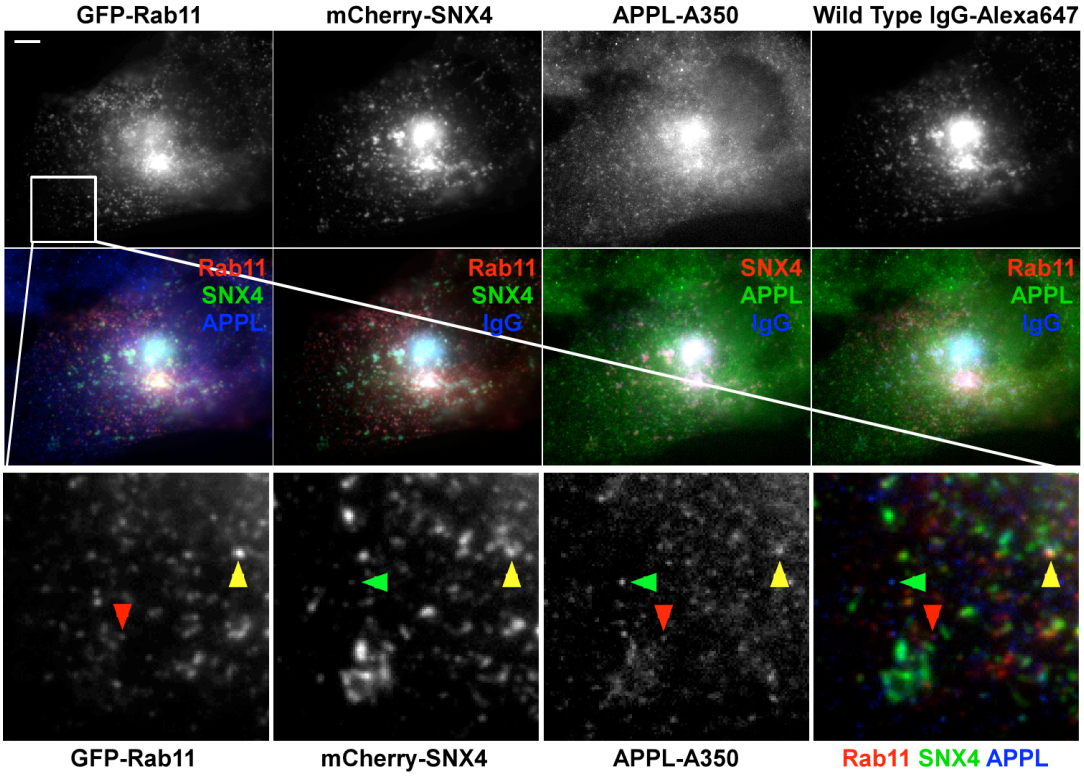


Figure S2. Analyses of the distribution of SNX4, Rab4 and Rab11 in the presence of wild type IgG1. HMEC-1 cells were cotransfected with FcRn/β_2m/GFP-Rab11/mCherry-SNX4. Transfected cells were pulsed with 25 μg/mL Alexa 647-labeled wild type IgG for 30 min prior to washing and fixation. Cells were then stained with anti-APPL antibody. The boxed region in the upper rows is presented as cropped images in the lower row. The upper (or middle) row shows the single color (or overlay) data. The red, green or yellow arrowheads indicate examples of TCs that are APPL+Rab11+, APPL+SNX4+ or APPL+SNX4+Rab11+, respectively. Scale bars = 5 μm.

Figure S3

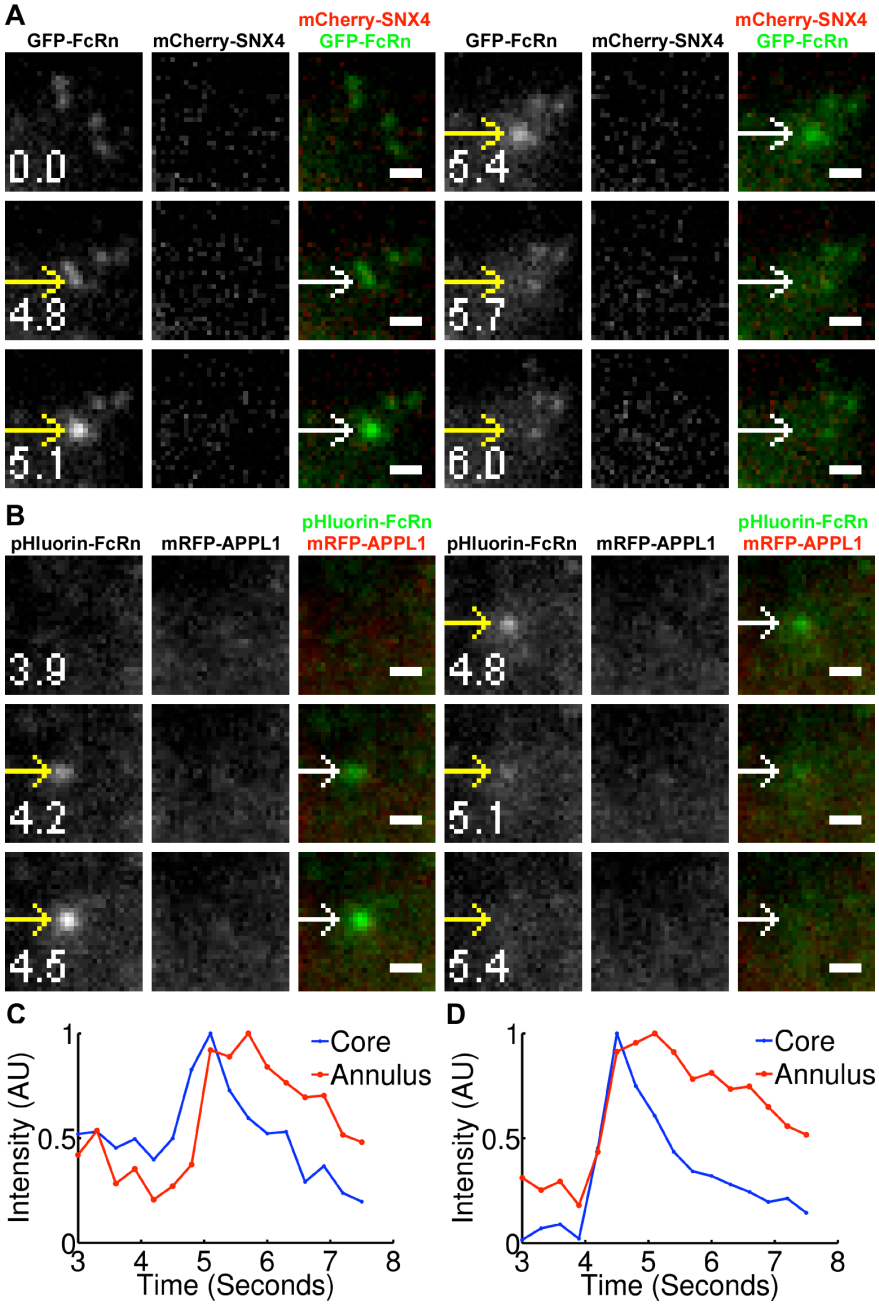


Figure S3. **SNX4 and APPL1 are not associated with FcRn during exocytosis.** HMEC-1 cells were cotransfected with mCherry-SNX4/GFP-FcRn/β_2m (A) or mRFP-APPL1/pHluorin-FcRn/β_2m (B). Individual TIRFM images are presented with the time (in seconds) at which each image was acquired (first image is arbitrarily set to time 0). White arrows in the overlay images show the events of interest that are also indicated in the single color data by yellow arrows. Scale bars = 1 μm. A, an exocytic event involving FcRn without detectable levels of SNX4 (4.8-5.7 s). B, an exocytic event involving FcRn without detectable levels of APPL1 (4.2-5.1 s). C and D, the core/annulus intensity plots of the changes in fluorescence intensity for the exocytic events displayed in A and B, respectively.

Figure S4

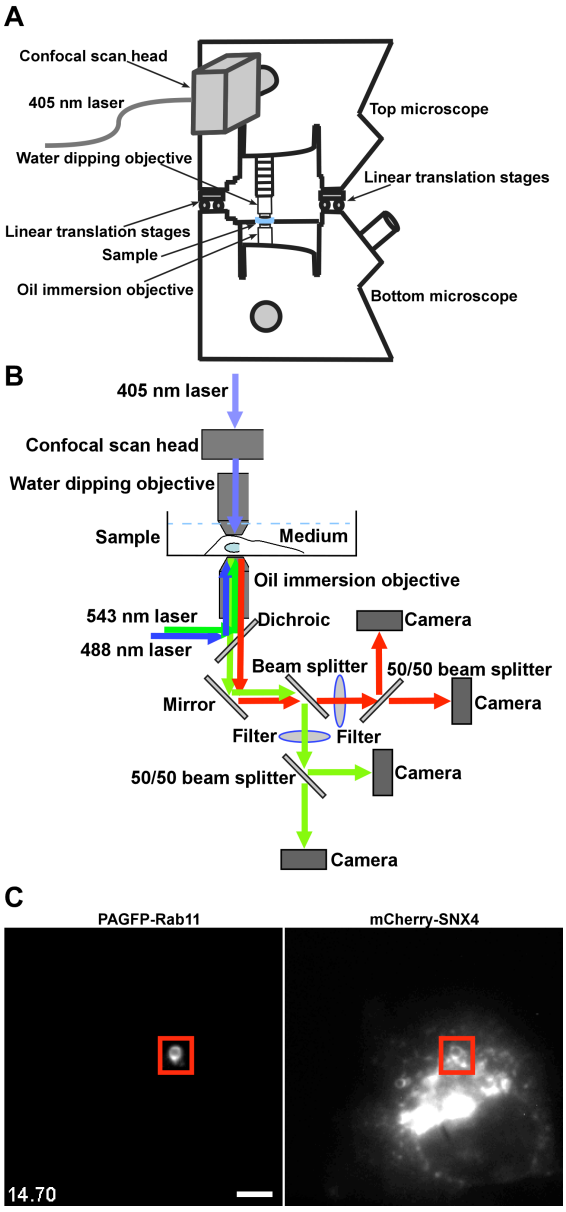
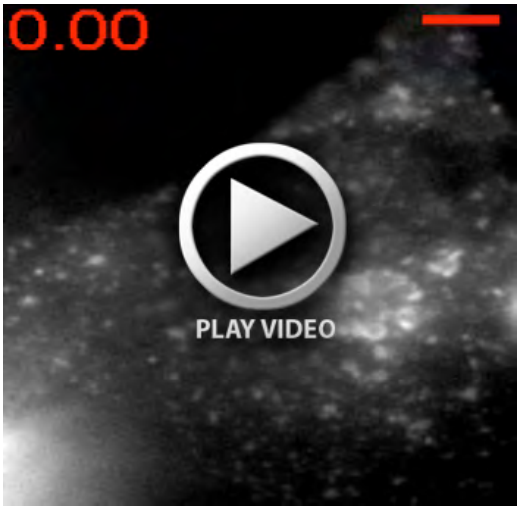
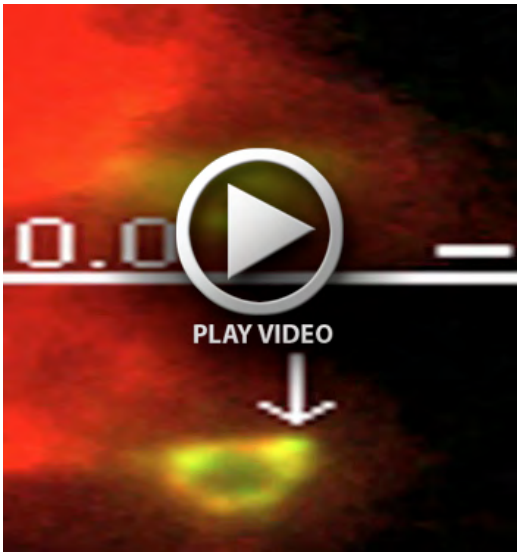


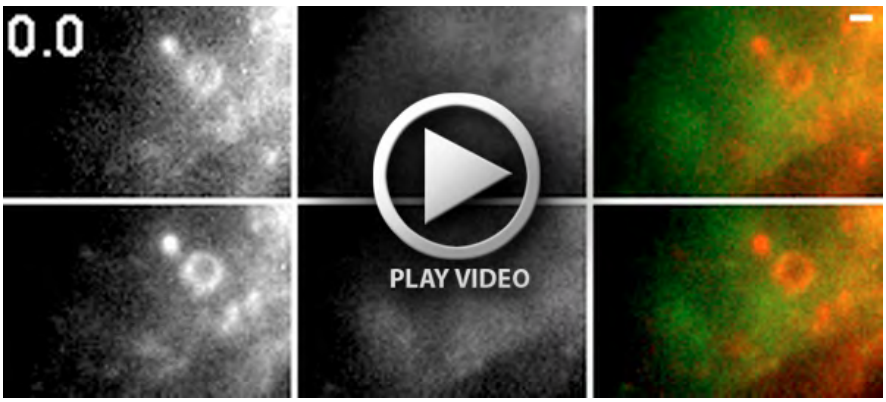
Figure S4. The LP-MUM configuration. A, The LP-MUM imaging station is composed of two inverted microscope bodies. A confocal scan head and a water dipping objective were mounted on the upper microscope to introduce the 405 nm photoactivation laser from the top of the sample. The sample is placed on the stage of the lower microscope. The sample is excited and fluorescence signal is collected by an oil immersion objective mounted on the lower microscope body. B, the excitation and emission light paths of the LP-MUM setup. C, HMEC-1 cells were cotransfected with PAGFP-Rab11/mRFP-SNX4. A 405 nm laser beam was focused onto a SNX4+ sorting endosome for ~1 s to photoactivate PAGFP (associated with Rab11). The left panel shows that PAGFP-Rab11 signal was detected only from the photoactivated sorting endosome (red rectangle). The right panel shows the mCherry-SNX4 image of the complete cell. Scale bar = 1 μm.



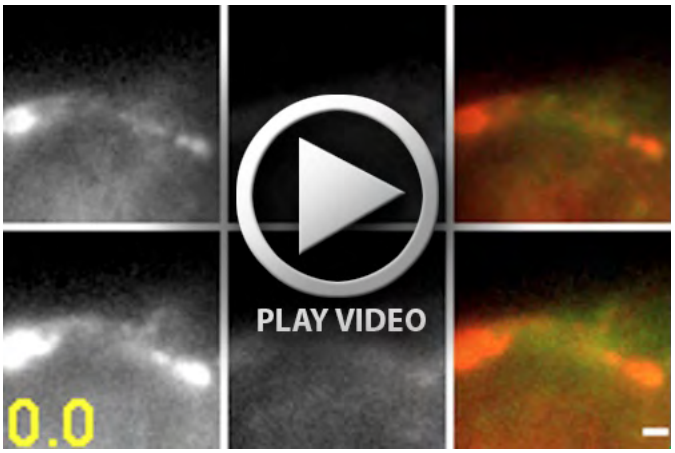
Movie 1. FcRn can be seen in circular sorting endosomes and tubulovesicular TCs. The movie corresponds to Figure 1A. Movie plays at 4 times the acquisition speed. Scale bar = 1 μm.



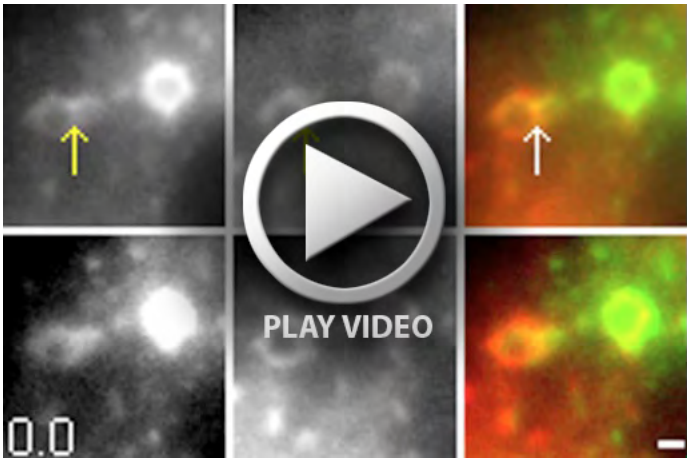
Movie 2. A FcRn+/Rab4+ looping event. The movie corresponds to Figure 1D. FcRn-PAGFP and mRFP-Rab4 are shown as green and red in the overlay data, respectively. The top row shows images from the upper plane and the bottom row shows images from the lower plane. Arrows indicate the event of interest as described in the figure legend. Movie plays at 3 times the acquisition speed. Scale bar = 1 μm.



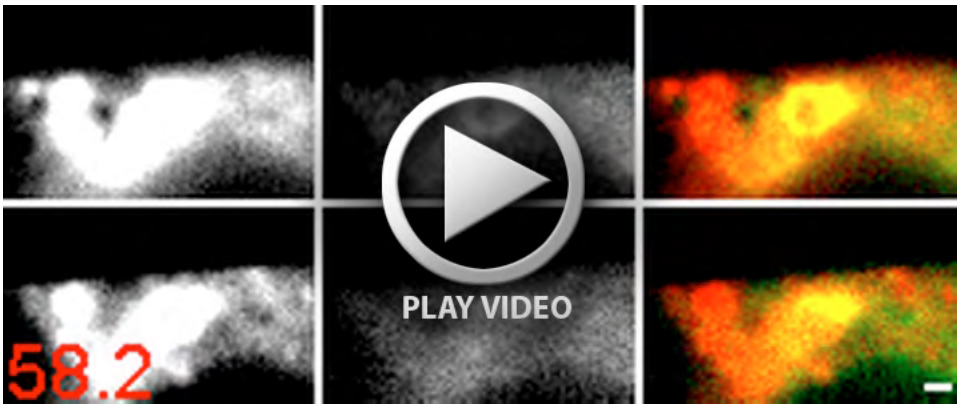
Movie 3. A FcRn+/APPL1+ TC merges with a sorting endosome. The movie corresponds to Figure 2A. The first and second columns show the single color data of mRFP-FcRn (red in overlay data) and GFP-APPL1 (green in overlay data), respectively. The top row shows images from the upper plane and the bottom row shows images from the lower plane. Arrows indicate the event of interest as described in the figure legend. Movie plays at 2 times the acquisition speed. Scale bar = 1 μm.



Movie 4. A MST-HN+/APPL1+ TC merges with a sorting endosome. The movie corresponds to Figure 2B. The first and second columns show the single color data of MST-HN-A555 (red in overlay data) and GFP-APPL1 (green in overlay data), respectively. The top row shows images from the upper plane and the bottom row shows images from the lower plane. Arrows indicate the event of interest as described in the figure legend. Movie plays at 2 times the acquisition speed. Scale bar = 1 μm.



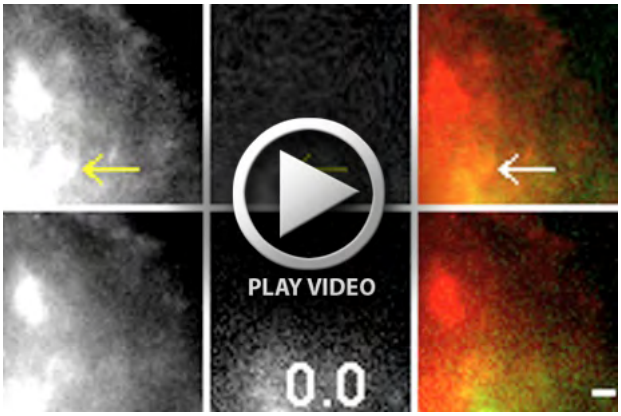
Movie 5. A SNX4+/FcRn+ TC transfers between sorting endosomes. The movie corresponds to Figure 4A. The first and second columns show the single color data of GFP-SNX4 (green in overlay data) and mRFP-FcRn (red in overlay data), respectively. The top row shows images from the upper plane and the bottom row shows images from the lower plane. Arrows indicate the event of interest as described in the figure legend. Movie plays at 2 times the acquisition speed. Scale bar = 1 μm.



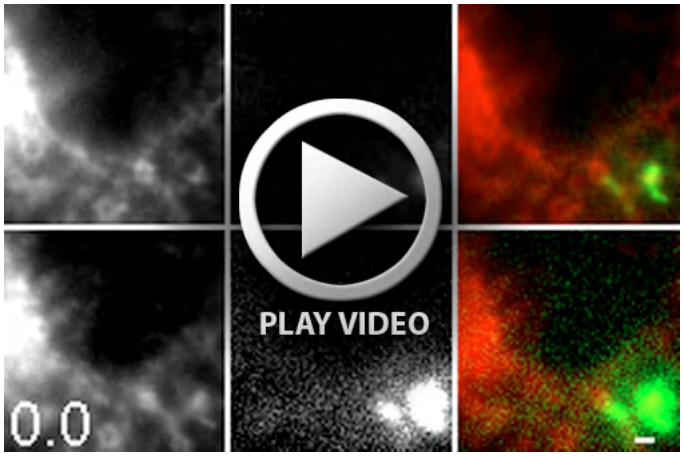
Movie 6. A SNX4+/Rab4+ TC transfers between sorting endosomes. The movie corresponds to Figure 5B. The first and second columns show the single color data of mCherry-SNX4 (red in overlay data) and PAGFP-Rab4 (green in overlay data), respectively. The top row shows images from the upper plane and the bottom row shows images from the lower plane. Arrows indicate the event of interest as described in the figure legend. Movie plays at 2 times the acquisition speed. Scale bar = 1 μm.

Movie 7. A SNX4+/Rab4+ TC returns to a sorting endosome in a looping event. The movie corresponds to Figure 6A. PAGFP-Rab4 and mCherry-SNX4 are shown as green and red in the overlay data, respectively. The top row shows images from the upper plane and the bottom row shows images from the lower plane. Arrows indicate the event of interest as described in the figure legend. Movie plays at 2 times the acquisition speed. Scale bar = 1 μm.

Movie 8. A SNX4+/Rab11+ TC returns to a sorting endosome in a looping event. The movie corresponds to Figure 6B. The first and second columns show the single color data of mCherry-SNX4 (red in overlay data) and PAGFP-Rab11 (green in overlay data), respectively. Arrows indicate the event of interest as described in the figure legend. Movie plays at 5 times the acquisition speed. Scale bar = 1 μm.



Movie 9. A FcRn+/Rab11+ TC leaves a sorting endosome and migrates towards the plasma membrane. The movie corresponds to Figure 7A. The first and second columns show the single color data of mRFP-FcRn (red in overlay data) and PAGFP-Rab11 (green in overlay data), respectively. The top row shows images from the upper plane and the bottom row shows images from the lower plane. Arrows indicate the event of interest as described in the figure legend. Movie plays at 2 times the acquisition speed. Scale bar = 1 μm.



Movie 10. A SNX4-/Rab11+ TC leaves a sorting endosome and migrates towards the plasma membrane. The movie corresponds to Figure 7B. The first and second columns show the single color data of mCherry-SNX4 (red in overlay data) and PAGFP-Rab11 (green in overlay data), respectively. The top row shows images from the upper plane and the bottom row shows images from the lower plane. Arrows indicate the event of interest as described in the figure legend. Movie plays at 2 times the acquisition speed. Scale bar = 1 μm.