Supplemental Figure Legends:

Supplemental Figure 1: GFP-FIP1B and mCherry-FIP1C overlap in the pericentriolar recycling compartment. Time lapse images were collected for two minutes (Video 7). Perinuclear localization was observed in dual expression studies of FIP1B and FIP1C in two independent experiments. Bar, 10μm.

Supplemental Figure 2: Rab11-FIP2 accumulates in the pericentriolar region of live HeLa cells in the presence of FIP1B, FIP1C, or FIP3. HeLa cells transfected with EGFP-Rab11-FIPs (FIP1B, FIP1C, or FIP3) and mCherry-Rab11-FIP2 were imaged with time-lapse deconvolution microscopy (Video 9). The mCherry-Rab11-FIP2 accumulated in pericentriolar compartments in the presence of FIP1B, FIP1C, and FIP3. Data are representative of 3 independent experiments. Bars, 10 μm.

Supplemental Videos:

Video 1. EGFP-Rab11 and EGFP-Rab11-FIP1 protein localization and movement in live HeLa cells. HeLa cells transfected with EGFP-Rab11-FIPs were analyzed by time-lapse deconvolution microscopy. Frames were taken at least every 2 seconds for 2 minutes and the display rates are set to 2 frames per second. Representative still images are presented in Figure 1. Panels from left to right: EGFP-Rab11a, EGFP-FIP1A, EGFP-FIP1B, and EGFP-FIP1C.

Video 2. EGFP-FIP1A and Cherry-Rab11a colocalization and movement in live HeLa cells. HeLa cells transfected with EGFP-FIP1A and Cherry-Rab11a were

analyzed by time-lapse deconvolution microscopy. Frames were taken at least every 2 seconds for 2 minutes and the display rates are set to 2 frames per second. Representative still images are presented in Figure 2. Panels from left to right: EGFP-FIP1A, Cherry-Rab11a, and Merged.

Video 3. EGFP-FIP1B and EGFP-FIP1C with Cherry-Rab11a colocalization and movement in live HeLa cells. HeLa cells transfected with EGFP-FIP1B or EGFP-FIP1C and Cherry-Rab11a were analyzed by time-lapse deconvolution microscopy. Frames were taken at least every 2 seconds for 2 minutes and the display rates are set to 2 frames per second. Representative still images are presented in Figure 2. Top Panels from left to right: EGFP-FIP1B, Cherry-Rab11a, and Merged. Bottom Panels from left to right: EGFP-FIP1C, Cherry-Rab11a, and Merged.

Video 4. EGFP-FIP1A or EGFP-FIP1C and transferrin-Alexa-568 in HeLa cells. HeLa cells transfected with EGFP-FIP1A or EGFP-FIP1C, incubated with transferrin-Alexa-568 for 5 minutes, and chased for 1 hour were analyzed by time-lapse deconvolution microscopy. Frames were taken every 5 minutes for 1 hour and the display rates are set to 2 frames per second. Representative still images are presented in Figure 4. Top panels left to top right: EGFP-FIP1A, transferrin-568, and Merged. Bottom panels left to bottom right: EGFP-FIP1C, transferrin-568, and Merged.

Video 5. EGFP-Rab11-FIP1B and Cherry-FIP1A tubular compartments in live HeLa cells. HeLa cells transfected with EGFP-FIP1B and Cherry-FIP1A were analyzed by

time-lapse deconvolution microscopy. Frames were taken at least every 2 seconds for 2 minutes and the display rates are set to 2 frames per second. Representative still images are presented in Figure 6. Panels from left to right: EGFP-FIP1B, Cherry-FIP1A, and Merged.

Video 6. EGFP-FIP1C and Cherry-FIP1A tubular compartments in live HeLa cells. HeLa cells transfected with EGFP-FIP1C and Cherry-FIP1A were analyzed by time-lapse deconvolution microscopy. Frames were taken at least every 2 seconds for 2 minutes and the display rates are set to 2 frames per second. Representative still images are presented in Figure 6. Panels from left to right: EGFP-FIP1C, Cherry-FIP1A, and Merged.

Video 7. EGFP-FIP1B and mCherry-FIP1C overlap on static centralized compartments. HeLa cells transfected with EGFP-FIP1B and mCherry-FIP1C were analyzed by time lapse deconvolution microscopy for 2 minutes. Frames were collected every 2 seconds and the display rate is set to 2 frames per second. Representative still images are presented in supplemental Figure 1. Panels from left to right: EGFP-FIP1B, mCherry-FIP1C, and Merged.

Video 8. Transferrin passage through endosomal tubules labeled by both Cerulean-FIP1C and Venus-FIP1A. HeLa cells transfected with Cerulean-FIP1C and Venus-FIP1A were treated with transferrin-568 and analyzed by time lapse deconvolution microscopy for 1 hr. Frames were collected every 5 minutes and the

display rate is set to 2 frames per second. Representative still images are presented in Figure 7. Panels from left to right: Cerulean-FIP1C, Venus-FIP1A, transferrin-568, and Merged.

Video 9. mCherry-FIP2 accumulates in the perinuclear region of HeLa cells in the presence of EGFP-FIP1B, FIP1C, and FIP3. HeLa cells transfected with EGFP-FIP1B, FIP1C, or FIP3 and mCherry-FIP2 were analyzed by time lapse deconvolution microscopy for 2 minutes. Frames were collected every 2 seconds and the display rate is set to 2 frames per second. Representative still images are presented in supplemental Figure 2. Top Panels from left to right: EGFP-FIP1B, mCherry-FIP2, and Merged. Middle Panels from left to right: EGFP-FIP1C, mCherry-FIP2, and Merged. Bottom Panels from left to right: EGFP-FIP3, mCherry-FIP2, and Merged.

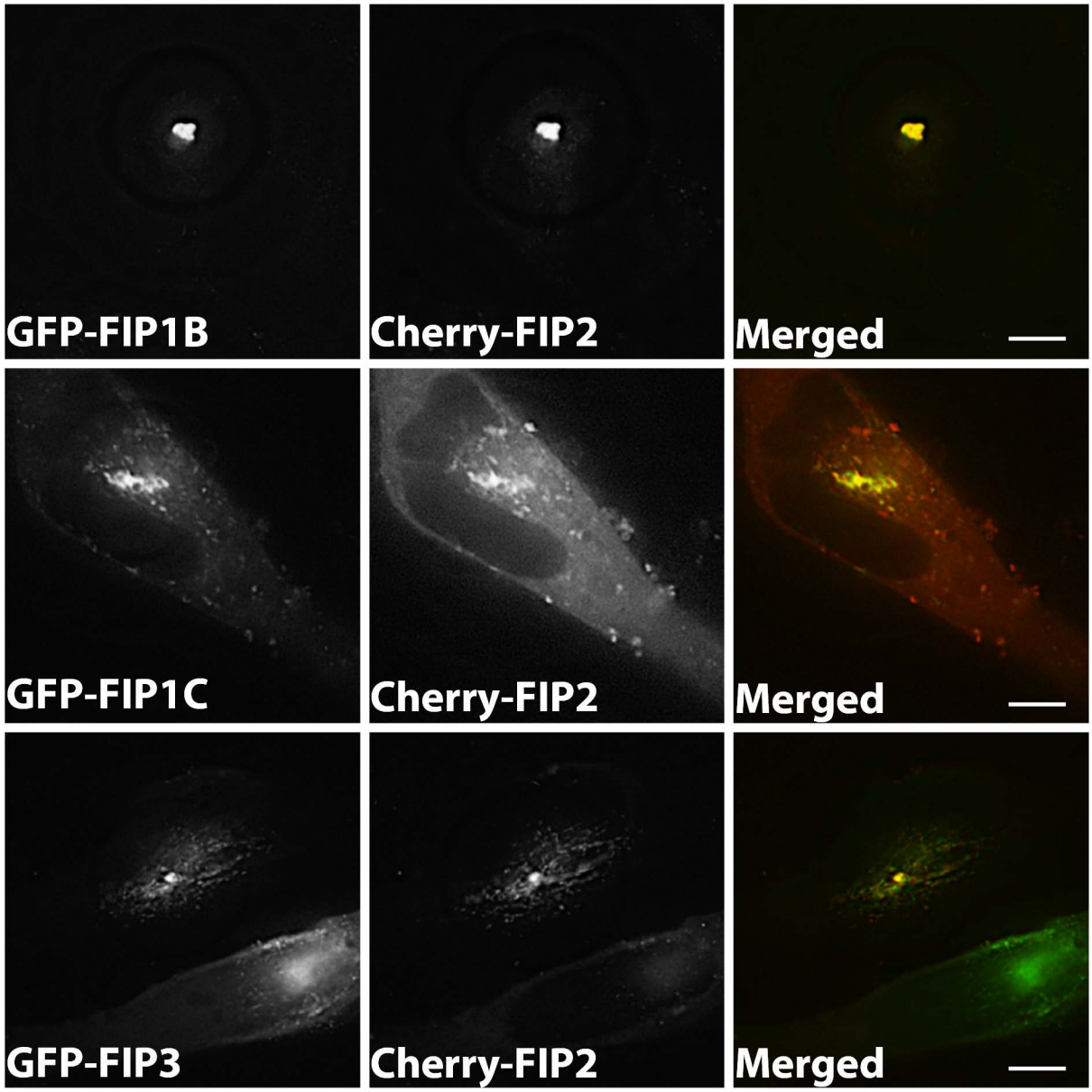
Video 10. EGFP-FIP2 or EGFP-FIP5 and Cherry-FIP1A separation in live HeLa cells. HeLa cells transfected with EGFP-FIP2 or EGFP-FIP5 and Cherry-Rab11a were analyzed by time-lapse deconvolution microscopy. Frames were taken at least every 2 seconds for 2 minutes and the display rates are set to 2 frames per second. Representative still images are presented in Figure 8. Top panels from left to right: EGFP-FIP2, Cherry-FIP1A, and Merged. Bottom panels from left to right: EGFP-FIP5, Cherry-FIP1A, and Merged.

Video 11. EGFP-FIP5 and Cherry-FIP2 overlap in live HeLa cells. HeLa cells transfected with EGFP-FIP5 and Cherry-FIP2 were analyzed by time-lapse

deconvolution microscopy. Frames were taken at least every 2 seconds for 2 minutes and the display rates are set to 2 frames per second. Representative still images are presented in Figure 8. Panels from left to right: EGFP-FIP5, Cherry-FIP2, and Merged.

Video 12. EGFP-FIP3 and Cherry-FIP1A on tubular compartments in live HeLa cells. HeLa cells transfected with EGFP-FIP3 and Cherry-FIP1A were analyzed by time-lapse deconvolution microscopy. Frames were taken at least every 2 seconds for 2 minutes and the display rates are set to 2 frames per second. Representative still images are presented in Figure 11A. Panels from left to right: EGFP-FIP3, Cherry-FIP1A, and Merged.

Video 13. EGFP-FIP3 and Cherry-FIP1C overlap on tubular compartments in live HeLa cells. HeLa cells transfected with EGFP-FIP3 and Cherry-FIP1C were analyzed by time-lapse deconvolution microscopy. Frames were taken at least every 2 seconds for 2 minutes and the display rates are set to 2 frames per second. Representative still images are presented in Figure 11B. Panels from left to right: EGFP-FIP3, Cherry-FIP1A, and Merged.


GFP-FIP1B
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Merged
GFP-FIP1C
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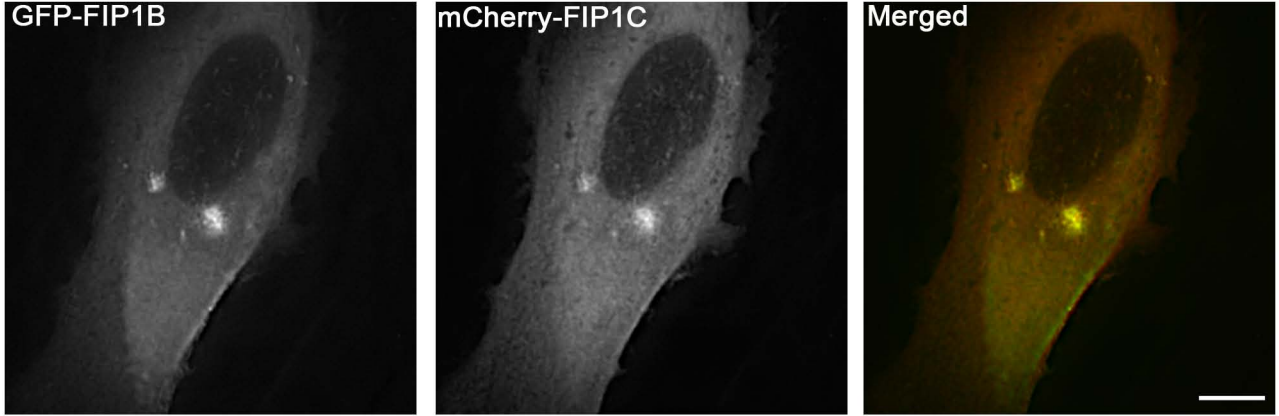
GFP-FIP1B
mCherry-FIP1C
Merged