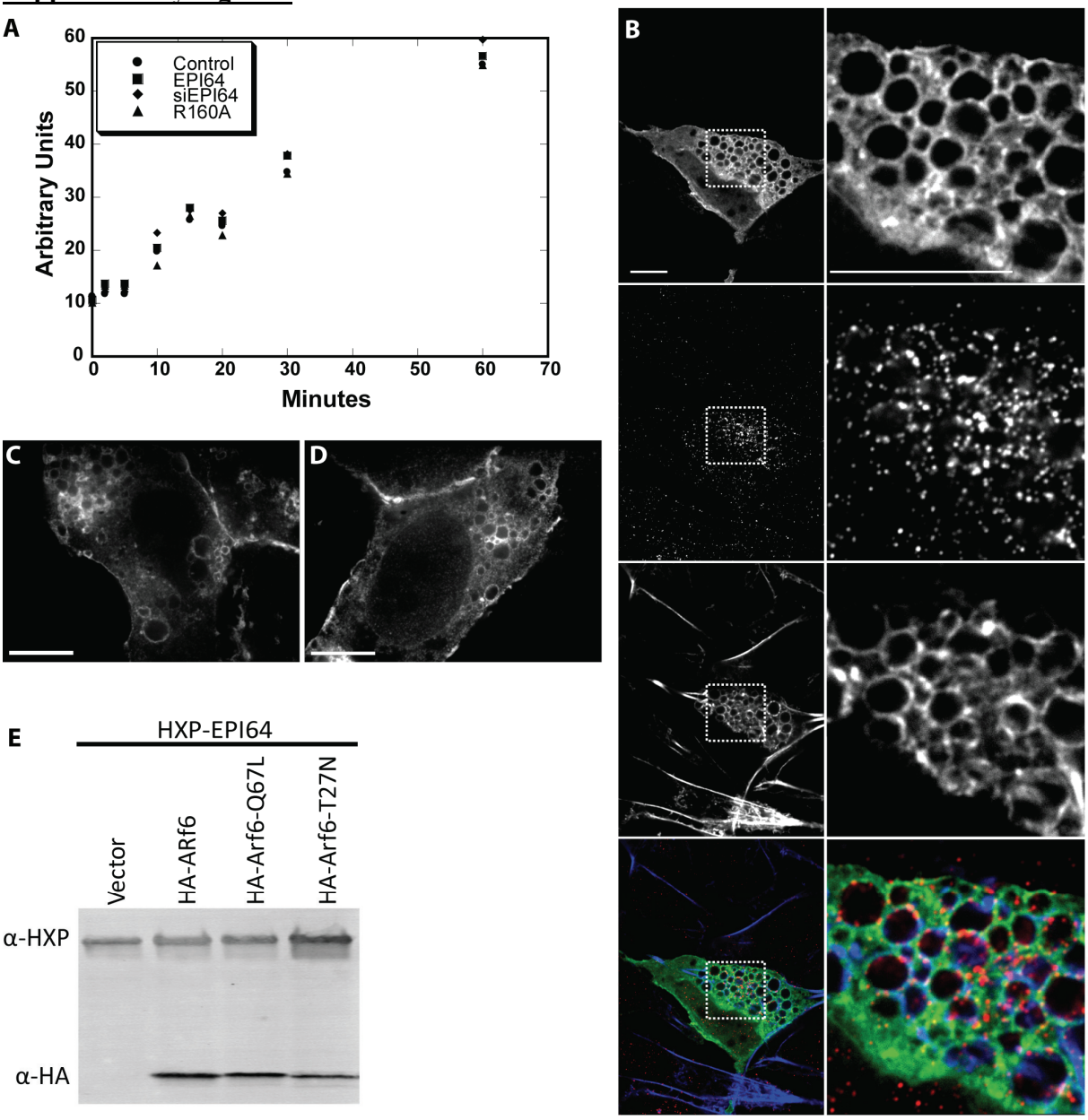
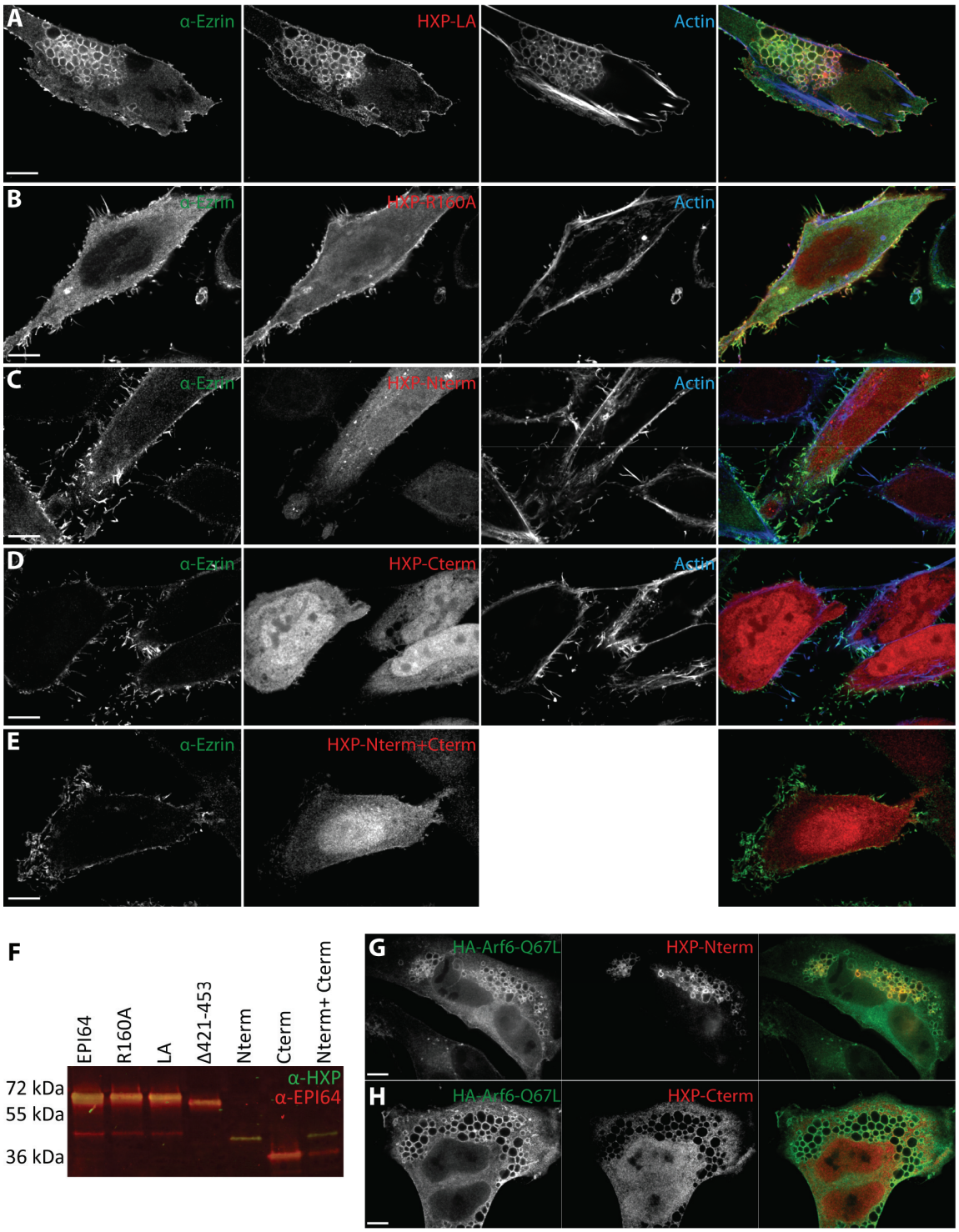
Supplementary Figures:

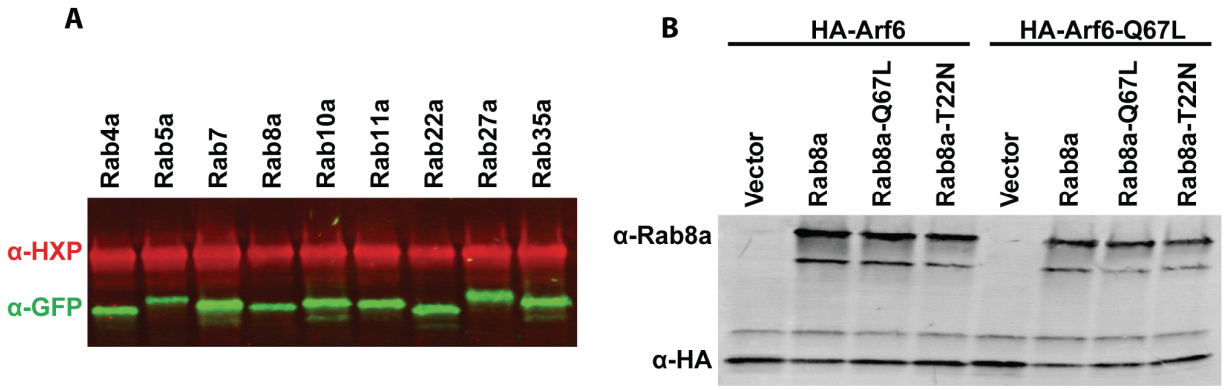


Supplementary Figure 1. A MHC1 internalization was monitored using a flow cytometer. HeLa cells transfected to express control vector, HXP-EPI64, siEPI64, or HXP-EPI64-R160A were brought up to 37 °C for 0-60 minutes to allow for internalization of surface bound fluorescent MHC1 antibody. Average fluorescence of > 12,000 cells is plotted on Y-axis as arbitrary units. Each data point is an average from 2-4 experiments. **B** JEG-3 cell transfected to express HA-Arf6-Q67L (top row, green) and stained for endogenous EPI64 (second row, red) and f-actin (third row, blue). Boxed region is magnified on right to show EPI64 localization on the vacuoles. Scale bars are 10 μm. **C-D** JEG-3 cells transfected to express siGL2 as a control (C) or siEPI64 (D), followed by a transfection to express HA-Arf6-Q67L were stained for HA-Arf6-Q67L. Scale bars are10 μm. **E** Western blot of HeLa cells lysates from cells in figure 2I probed with antibodies against HXP-tag and HA-tag.

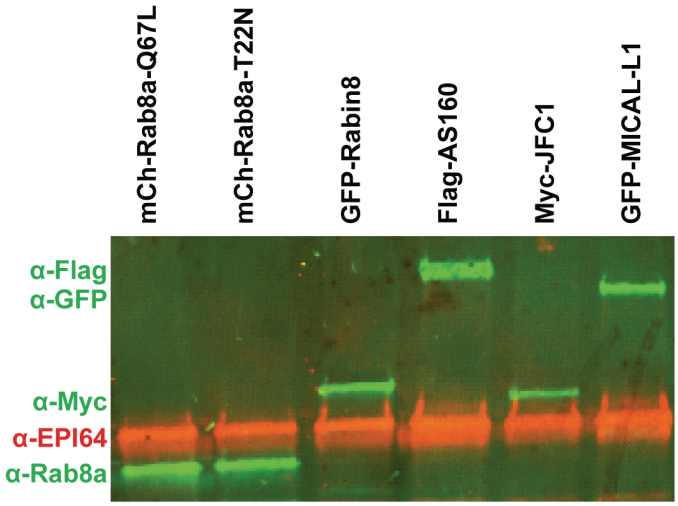


Supplementary Figure 2. A-E HeLa cells were transfected to express HXP-EPI64 constructs and stained for ezrin (left column, green), HXP-tag (2nd column, red), and F-actin (3rd column, blue). Scale bars are 10 μm. **F** Fluorescent Western blot of HeLa cells lysates transfected to express HXP-EPI64 constructs probed with antibodies against HXP-tag in green and EPI64 in

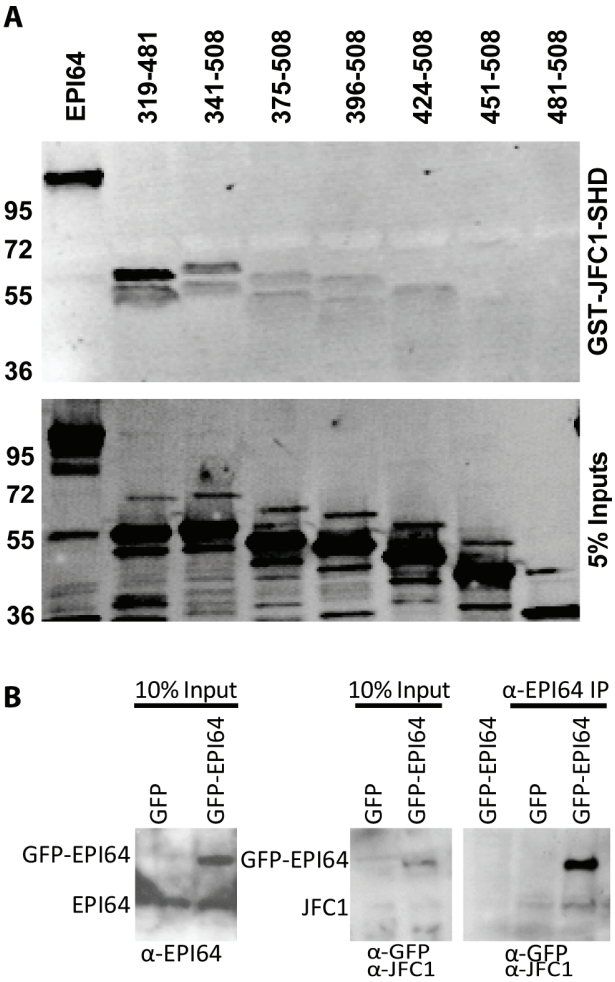
red. **G-H** HeLa cells cotransfected to express HA-Arf6-Q67L (left column, green) and either HXP-EPI64-Nterm (G) or HXP-EPI64-Cterm (H) (middle column, red). Scale bars are 10 μm.



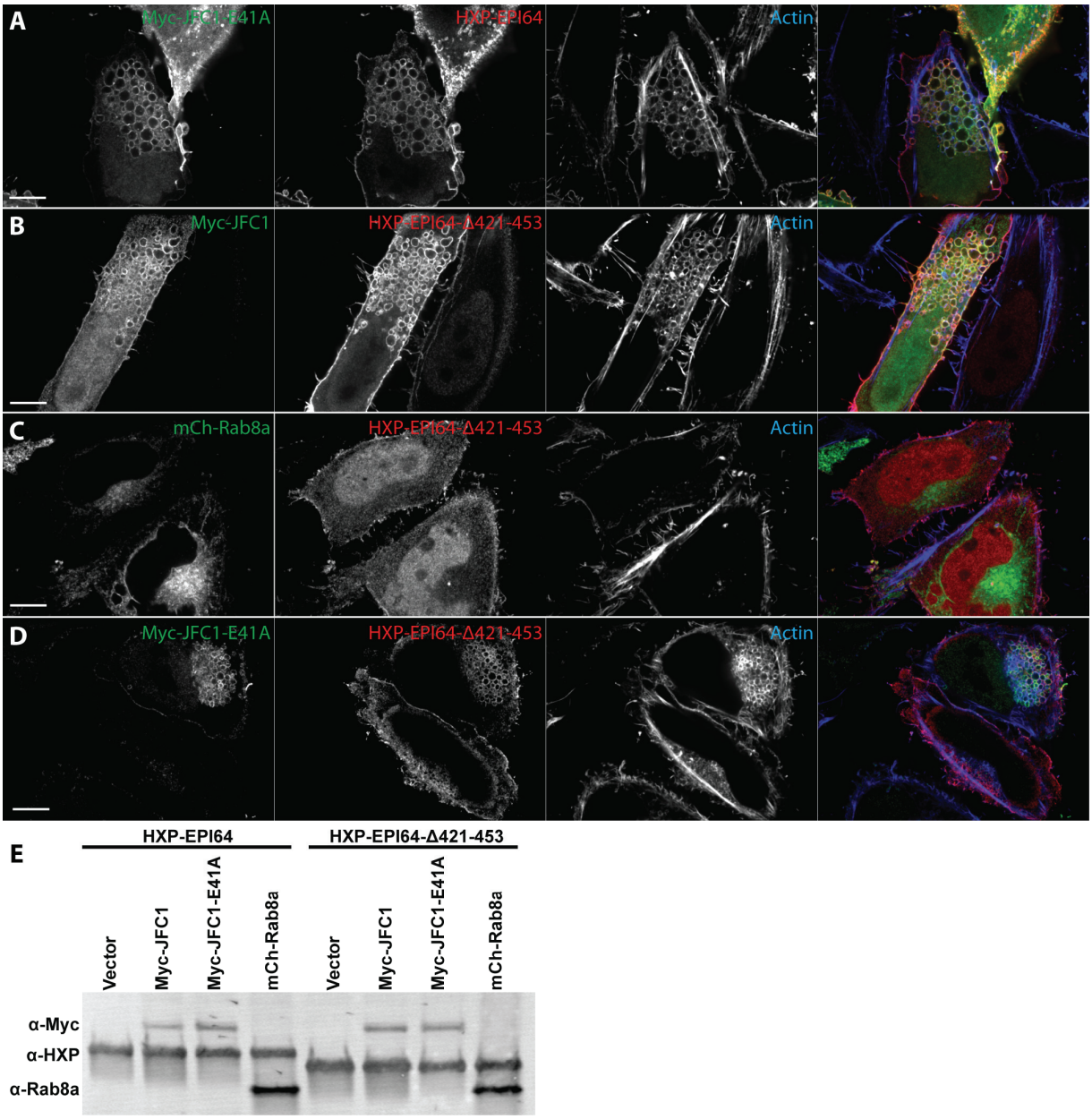
Supplementary Figure 3. A Fluorescent Western blot of HeLa cells lysates from cells in figure 4J probed with antibodies against HXP-tag in red and GFP-tag in green. **B** Western blot of HeLa cells lysates from cells in figure 4K probed with antibodies against Rab8a and HA-Tag.



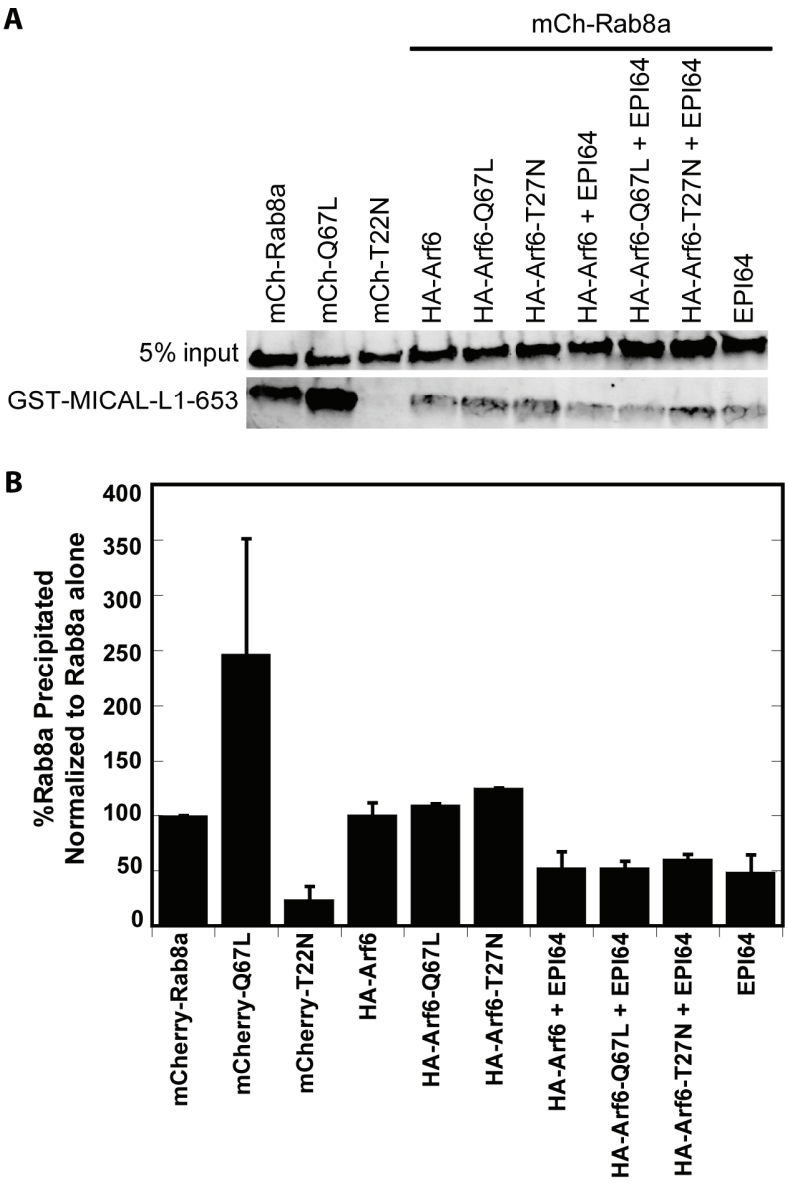
Supplementary Figure 4. Fluorescent Western blot of HeLa cells lysates from cells in figure 5F probed with antibodies against Flag-tag, Myc-Tag, GFP, and Rab8a in green and EPI64 in red.



Supplementary Figure 5. A GST-JFC1-SHD was used to precipitate overexpressed GFP-EPI64 truncations from HeLa cells lysate. Western blot was probed with antibodies against GFP. **B** Control antibody (first lane of IP) or an antibody against EPI64 (last two lanes of IP) were used to immunoprecipitate endogenous or expressed GFP-EPI64 from JEG-3 cell lysate. Membranes were blotted with antibodies against EPI64 to show endogenous EPI64 and GFP-EPI64 inputs (left blot), or with antibodies against GFP and JFC1 to show GFP-EPI64 and endogenous JFC1 inputs (middle blot) and immunoprecipitates (right blot).



Supplementary Figure 6. A-D HeLa cells were cotransfected to express HXP-EPI64 (red) and myc-JFC1-E41A (green) (A), HXP-EPI64-Δ421-453 (red) and myc-JFC1 (green) (B), HXP-EPI64-Δ421-453 (red) and mCherry-Rab8a (green) (C), or HXP-EPI64-Δ421-453 (red) and myc-JFC1-E41A (green) (C), and stained for f-actin (blue). Scale bars are 10 μm. **E** Western blot of HeLa cells lysates from cells in figure 7B probed with antibodies against HXP-tag, Myc-tag, and Rab8a.



Supplementary Figure 7. A GST-MICAL-L1-653 was used to precipitate over-expressed mCherry-Rab8a, mCherry-Rab8a-Q67L, Rab8a-T22N, or mCherry-Rab8a coexpressed with the other proteins listed. MICAL-L1 binds specifically to GTP-bound Rab8a. Membrane was blotted with antibodies against Rab8a. **B** Quantification of the amount of Rab8a-GTP precipitated by GST-MICAL-L1-653 was normalized to the amount of Rab8a-GTP precipitated with only Rab8a overexpressed. Error bars are the standard deviations from 2-5 experiments.