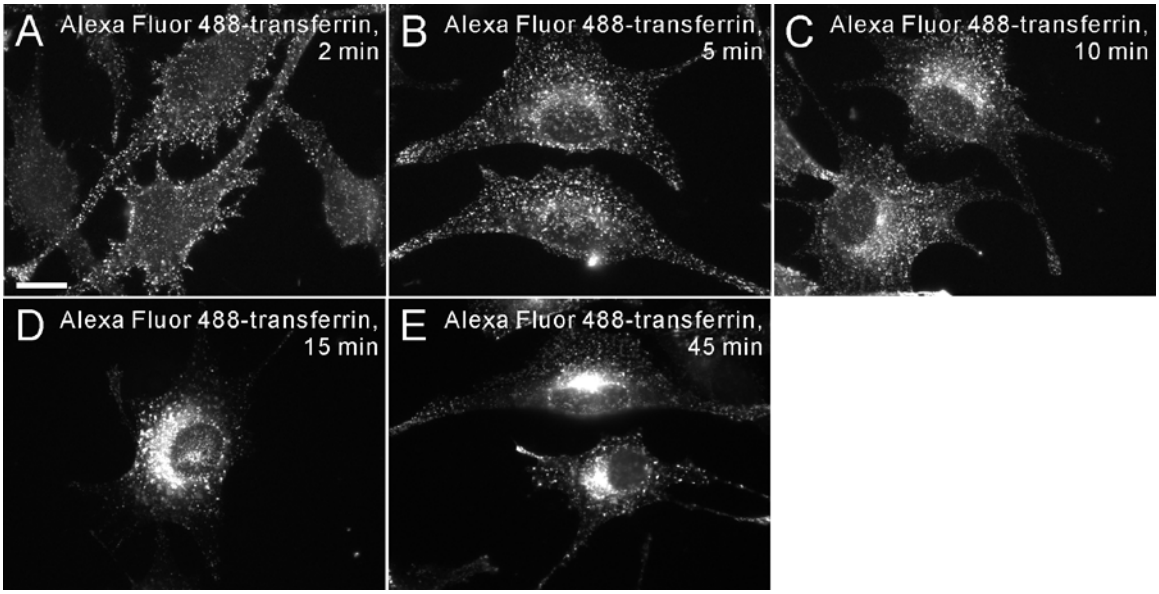

Figure S1. Time course of Alexa Fluor 488-transferrin uptake in SK-MEL-28 cells. (A-E) Cells were serum-starved for 1 hour and incubated with 25 μg/ml Alexa Fluor 488-transferrin for 2, 5, 10, 15, and 45 minutes at 37 ^{o}C, respectively. At 2 minutes, the fluorescent transferrin was localized at the cell periphery. By 5, 10 and 15 minutes, the transferrin was trafficked and concentrated at the peri-nuclear region of the cell. After 45 minutes, the fluorescent transferrin accumulated in a dense region near to the nucleus. Bar, 10 μm.

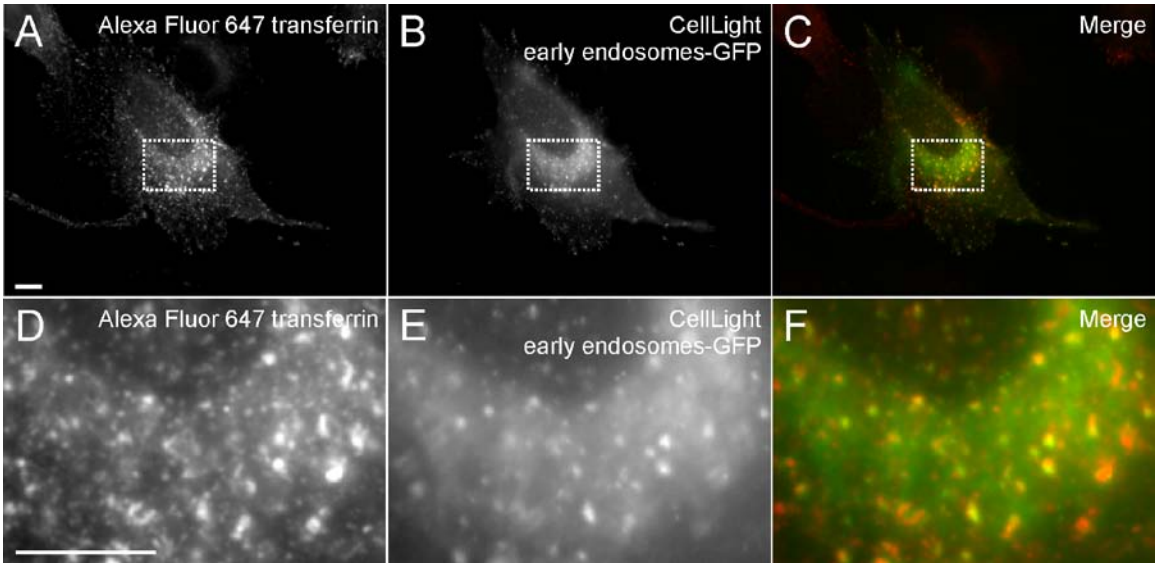

Figure S2. The co-localization of internalized Alexa fluor 647-transferrin with an early endosome marker. SK-MEL-28 cells were infected with baculovirus expressing early endosomes-GFP (CellLight Rab5a-GFP) for 24 h and then incubated with Alexa Fluor 647-transferrin for 10 min. (A-C) The internalized transferrin (A) colocalizes with the CellLight early endosome-GFP (B) at the perinuclear region. (C) The merge of (A) and (B). (D-F). A higher magnification of A-C at the perinuclear region. Bars, 10 μm.

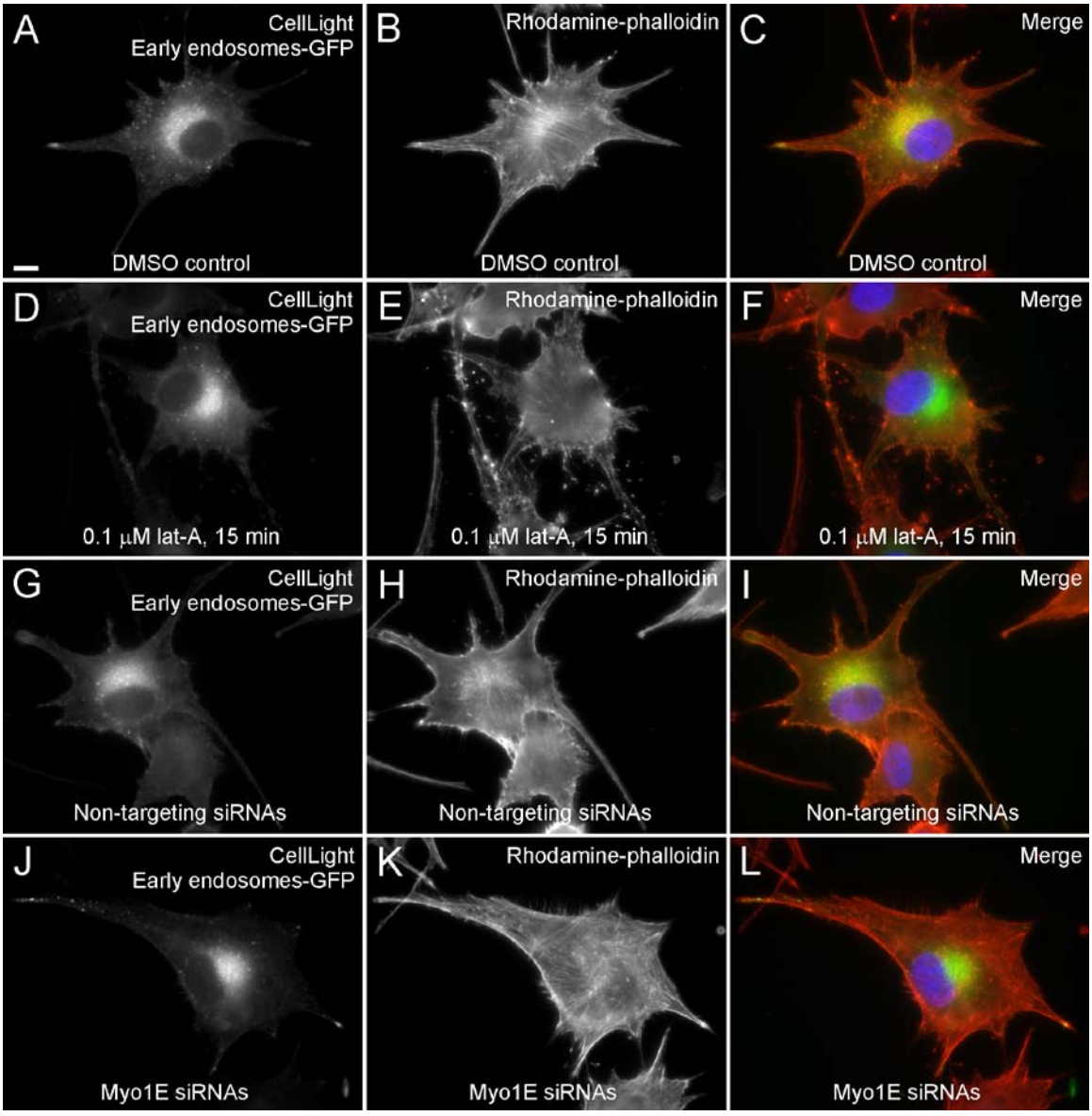

Figure S3. Disruption of actin with lat-A or depletion of Myo1E does not affect the distribution of early endosomal marker. SK-MEL-28 cells were treated with 0.01% DMSO (A-C) or 0.1 μM lat-A (D-F) showed a perinuclear localization of early endosomal marker, CellLight Rab5a-GFP. Similarly, SK-MEL-28 cells treated with either non-targeting siRNAs (G-I) or Myo1E siRNAs (J-L) does not affect the distribution of Rab5a-GFP. Bar, 10 μm.

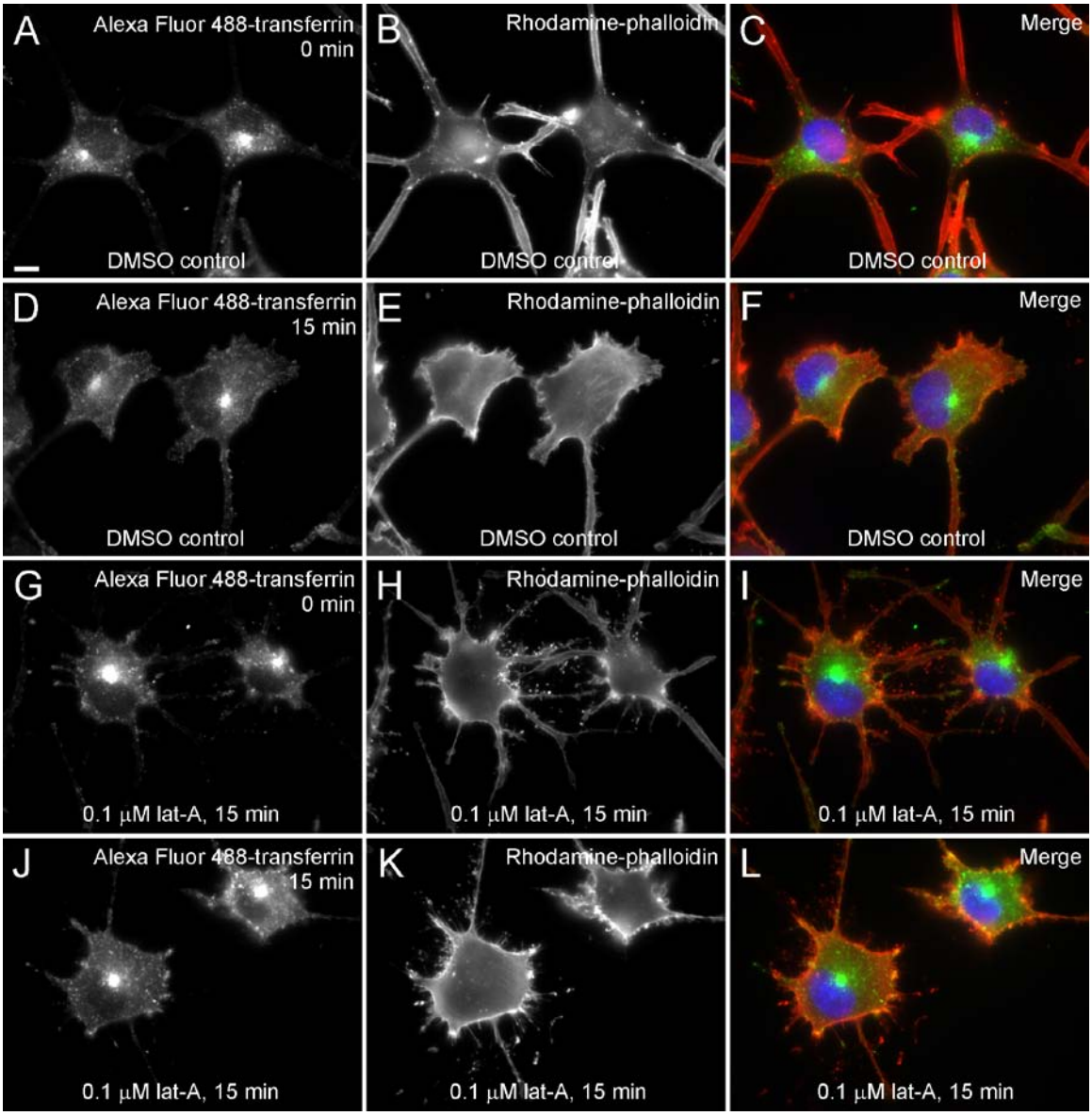

Figure S4. Disruption of actin with lat-A does not affect the recycling of internalized transferrin. Serum-starved SK-MEL-28 cells were pre-incubated with Alexa Fluor 488-transferrin at 37°C for 1 h. The cells were cooled on ice and then acid-washed to remove surface-bound Alexa Fluor 488-transferrin. The cells were treated with either 0.01% DMSO (A-F) or 0.1 μM lat-A (G-L) for 15 minutes on ice. The treated cells were then washed and fixed with 4% PFA (A-C and G-I) or incubated at 37°C for 15 minutes before fixation (D-F and J-L). The treatment of lat-A (G-L) does not result in apparent changes in the distribution of recycling transferrin compared to the DMSO-treated control. Bar, 10 μm.

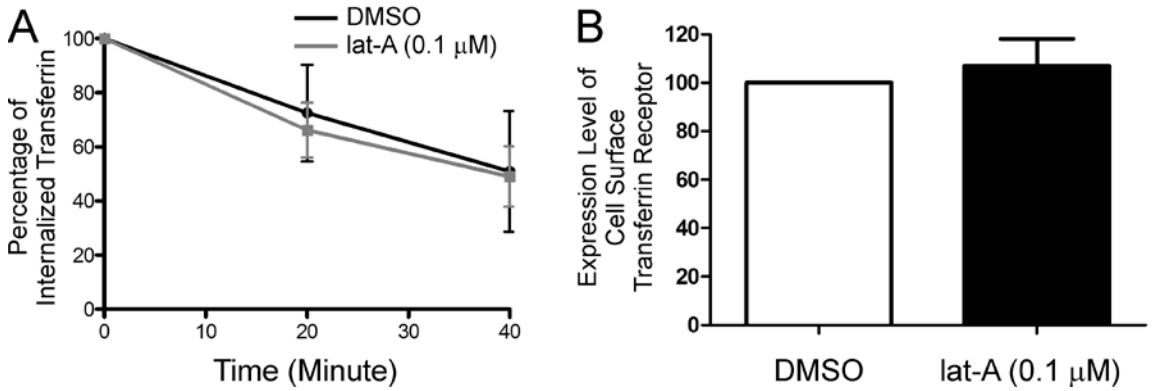

Figure S5. (A) Disruption of actin with lat-A does not affect the recycling of internalized Alexa Fluor 647-conjugated transferrin using a quantitative FACS assay. (B) The expression of transferrin receptor at the cell surface does not affected by the lat-A treatment.

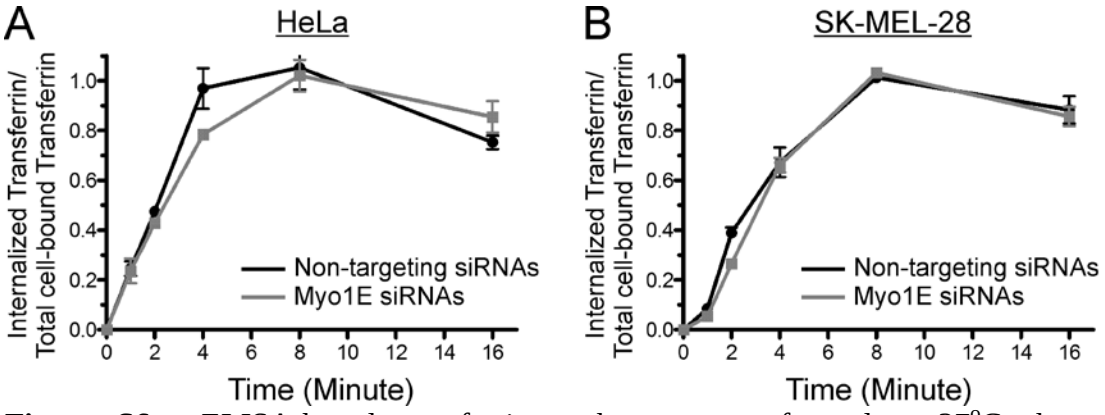

Figure S6. ELISA-based transferrin-uptake assay performed at 37^{o}C showed a diminished defect in the internalization of biotinylated transferrin in both (A) HeLa and (B) SK-MEL-28 cells compared to the same assay performed at 31^{o}C (Compared to Fig. 4B).

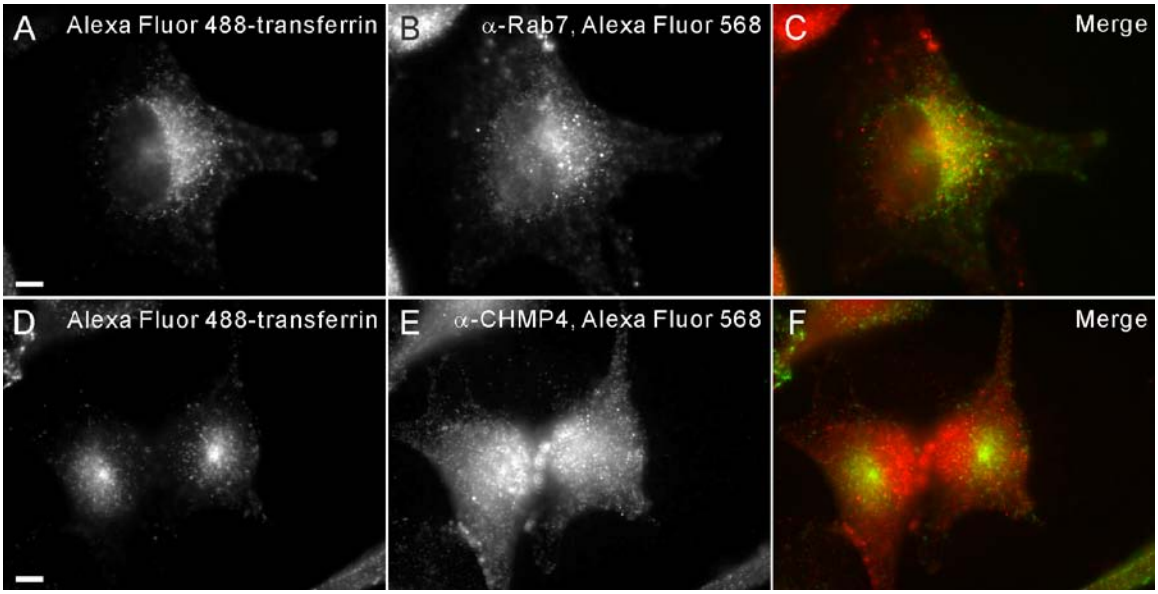

Figure S7. Perinuclear internalized transferrin does not co-localize with late endosomal markers. (A-F) Perinuclear internalized Alexa Fluor 488-transferrin-containing puncta (A and D) do not co-localize with late endosomal markers Rab7 (B) and CHMP4 (E) by immunofluorescence. (C) The merge of (A) and (B). (F) The merge of (D) and (E). Bars, 10 μm.

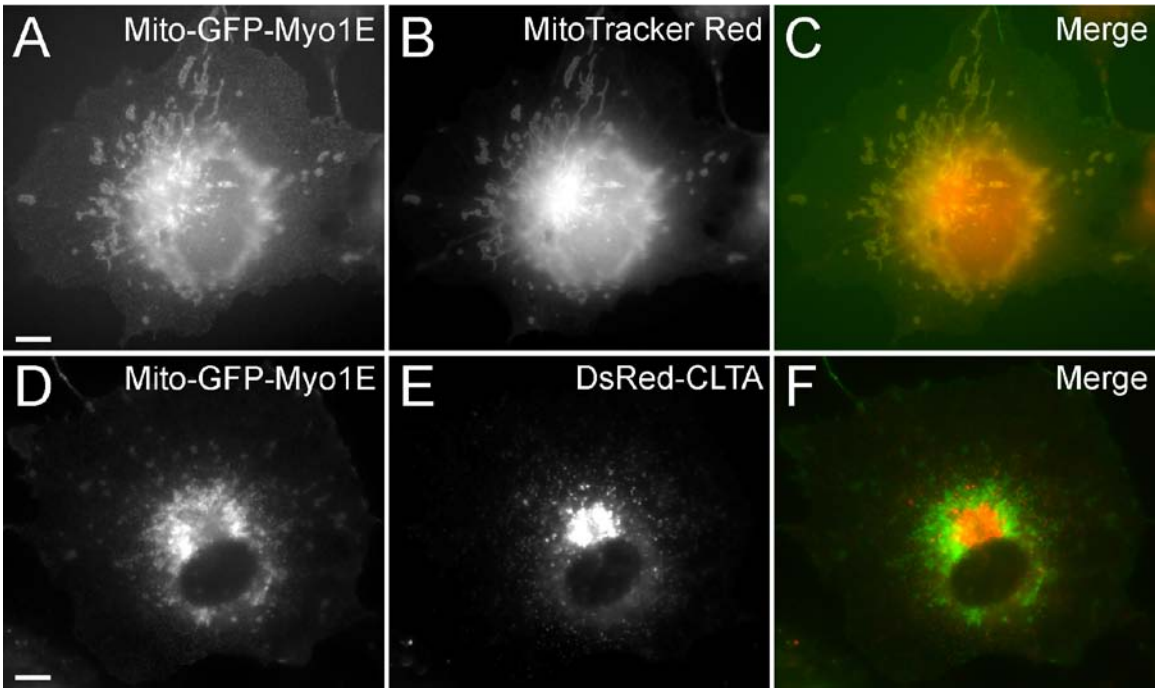

Figure S8. Mis-targeting of GFP-Myo1E to mitochondria. (A-C) Transiently transfected Mito-GFP-Myo1E co-localizes with the mitochondria marker MitoTracker Red in Cos-7 cells. (C) The merge of (A) and (B). (D-F) Mito-GFP-Myo1E and DsRed-CLTA do not co-localize in the transfected cell. (F) The merge of (D) and (E). Bars, 10 μm.

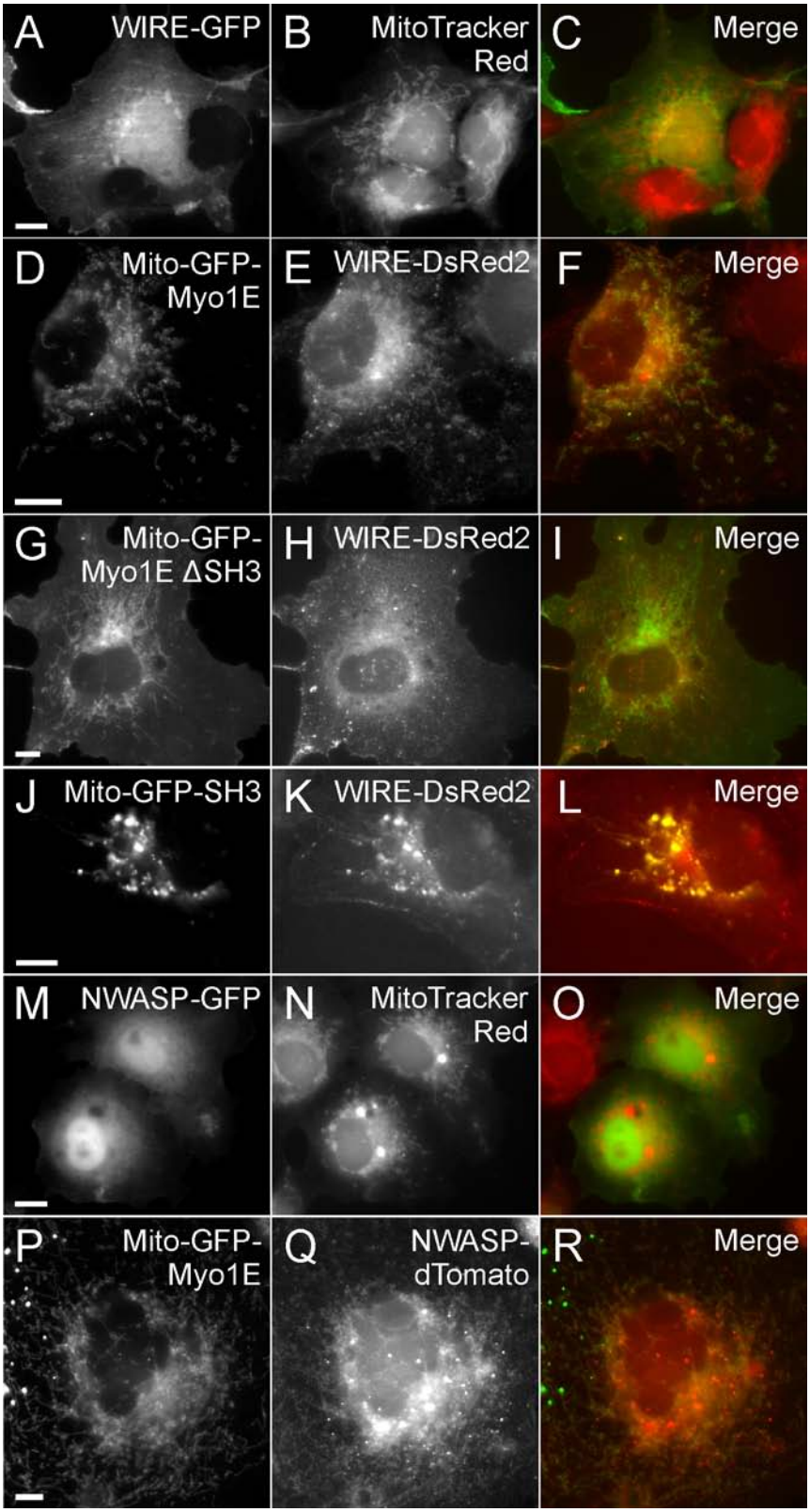

Figure S9. Mis-targeting of GFP-Myo1E to mitochondria recruits WIRE-DsRed2 and N-WASP-dtomato. (A-C) Transient expression of WIRE-GFP in control Cos-7 cells labeled with MitoTracker Red. WIRE-GFP does not localize to mitochondria. (C) The

merge of (A) and (B). (D-F) In cells co-transfected with Mito-GFP-Myo1E and WIRE-DsRed2, WIRE-DsRed2 is recruited to the GFP-Myo1E-decorated mitochondria. (F) The merge of (D) and (E). (G-L) Myo1E SH3 domain is necessary and sufficient to recruit WIRE-DsRed2. (G-I) In cells co-transfected with Mito-GFP-Myo1E ΔSH3 and WIRE-DsRed2, WIRE-DsRed2 is not recruited to the mitochondria. (I) The merge of (G) and (H). (J-L) In cells co-transfected with Mito-GFP-SH3 and WIRE-DsRed2, the recruitment of WIRE-DsRed2 to the mitochondria is restored. (L) The merge of (J) and (K). (M-O) Transient expression of N-WASP-GFP in control cells labeled with MitoTracker Red. N-WASP-GFP does not localize to mitochondria. (O) The merge of (M) and (N). (P-R) In cells co-transfected with Mito-GFP-Myo1E and N-WASP-dtomato, N-WASP-dtomato is recruited to the GFP-Myo1E-decorated mitochondria. (R) The merge of (P) and (Q). Bars, 10 μm.

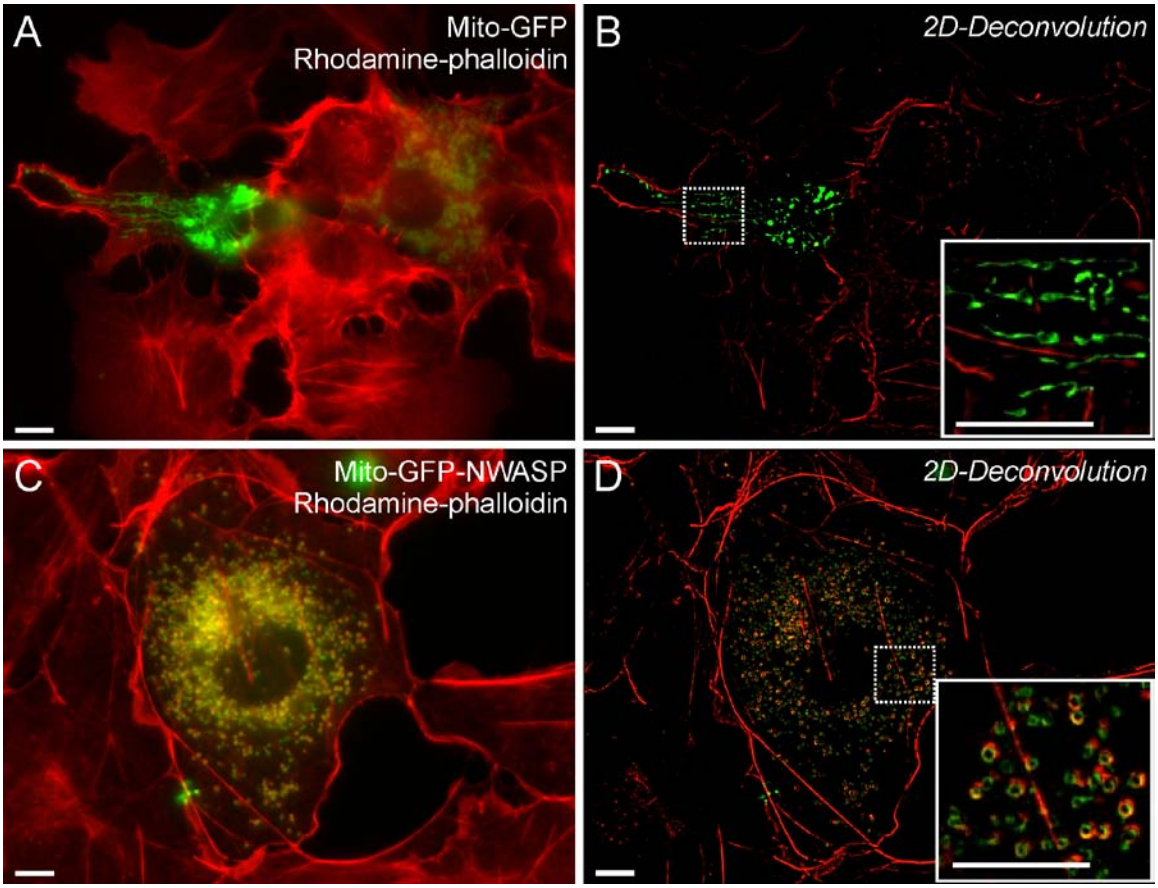

Figure S10. Actin assembly at GFP-N-WASP-decorated mitochondria. Cos-7 cells were transfected with either Mito-GFP or Mito-GFP-N-WASP (green) and labeled with rhodamine-phalloidin (red). (A and B) Expression of Mito-GFP does not induce actin assembly at the mitochondria. (B) 2D-deconvolved image of (A). Inset is a magnified view of the indicated region. (C and D) Expression of Mito-GFP-N-WASP induces the formation of actin structures at the mitochondria. (D) 2D-deconvolved image of (C). Bars, 10 μm.

Movie 1. Time-lapse movie of Swiss 3T3 DsRed-clathrin cells transiently expressing GFP-Myo1E. Exposure time is 900 ms. Image sequences were captured at 2-sec intervals. Bar, 10 μm.

Movie 2. Time-lapse movie of Swiss 3T3 DsRed-clathrin cells transiently expressing GFP-Dynamin 2aa. Exposure time is 900 ms. Image sequences were captured at 2-sec intervals. Bar, 10 μm.

Movie 3. Time-lapse movie of Swiss 3T3 DsRed-clathrin cells transiently expressing GFP-N-WASP. Exposure time is 900 ms. Image sequences were captured at 2-sec intervals. Bar, 10 μm.

Movie 4. Time-lapse movie of Swiss 3T3 DsRed-clathrin cells transiently expressing GFP-labeled F-actin binding domain of utrophin. Exposure time is 900 ms. Image sequences were captured at 2-sec intervals. Bar, 10 μm.

Movie 5. Time-lapse movie of Swiss 3T3 cells transiently expressing Myo1E-GFP and Dynamin 2aa-mCherry. Exposure time is 900 ms. Image sequences were captured at 1-sec intervals. Bar, 10 μm.

Movie 6. Time-lapse movie of Swiss 3T3 cells transiently expressing Myo1E-GFP and WIP-dtomato. Exposure time is 900 ms. Image sequences were captured at 1-sec intervals. Bar, 10 μm.

Movie 7. Time-lapse movie of Swiss 3T3 cells transiently expressing Myo1E-GFP and WIRE-DsRed2. Exposure time is 900 ms. Image sequences were captured at 1-sec intervals. Bar, 10 μm.

Movie 8. 3-D kymograph for SK-MEL-2 $CLTA^{EN}/DNM2^{EN}$ cells treated with non-targeting siRNA. Exposure time is 500 ms. Image sequences were captured at 2-sec intervals.

Movie 9. 3-D kymograph for SK-MEL-2 $CLTA^{EN}/DNM2^{EN}$ cells treated with Myo1E siRNA. Exposure time is 500 ms. Image sequences were captured at 2-sec intervals.