Supplemental Materials

Molecular Biology of the Cell

Xie et al.

Supplemental Figure Legends

Supplemental Figure 1:The disengagement defects in EHD1 knockout cells are rescued by transienttransfectionof EHD1.

(A-C)Wild-typeNIH3T3 cells were transientlytransfected with GFP-EHD1 and treated with RO-3306 for 18 h, and immunostained for c-Nap1 (red) and Centrin 1 (magenta).DNA, blue. Dashed region of interest marks transfected cells.

(D-F) CRISPR/Cas9 EHD1-knockout NIH3T3 cells were transientlytransfected with GFP-EHD1and treated with RO-3306 for 18 h, and immunostained for c-Nap1 (red) and Centrin 1 (magenta). DNA, blue.Transfected cells are indicated with dashed lines marking their borders. Insets show magnification of centrioles. Scale, 10μm.

(G) Graph shows the percentage of NIH3T3 cells containing either disengaged/engaged centrioles with the indicated treatments and genotypes. *n* = 3 independent experiments, ≥30 cells analyzed per experiment. Error bars, SD. *p<0.01.

Supplemental Figure 2: MICAL-L1, Vps26a, and Vps35regulate Cep215 centrosomal levels, whereas Rab11a does not.HeLa cells were either mock-treated (A) or siRNA-treated with oligonucleotides targeting Rab11a (B), MICAL-L1 (C), Vps26a (D), or Vps35 (E) for 72 h according to manufacturer's instruction, and then subjected to methanol fixation andimmunostainingwith antibodies against Cep215 (green) and γ-tubulin (red). DNA, blue.

(F and G) Immunoblotting demonstrates the efficiency of siRNA-mediated depletion of Vps26a, Vps35, Rab11a and MICAL-L1 from HeLa cells.Equal total protein amounts were loaded. α-tubulin and actin were used as loading controls.

(H-J) Graphs showingthe ratiometric intensity of Cep215/γ-tubulincentrosomal levels during cytokinesis for each indicated treatment. *p<0.001; *n* = 3 independent experiments. At least 53 cells were counted for each experiment in (H), at least 55 cells were counted for each experiment in (I), and at least 26 cells were counted for each experiment in (J).

Supplemental Figure 3: Sas6 and STIL recruitment is not affected by EHD1 knockdown. HeLa cells were treated with EHD1-siRNA and synchronized to S phase. Mock- and EHD1-siRNA treated cells were immunostained for Cep215 (green) and either Sas6 (A and B) or STIL (C and D) (red)**.**

(E and F) Graphs show the percentage of cells displayingcentriolar Sas6 (E) or STIL (F). The presence of two Sas6/STIL foci in a cell is considered as positive Sas6/STIL recruitment. *n* = 3 independent experiments, ≥58cells analyzed per experiment. Error bars, SD.

(G) Immunoblot of U2OS cell lysates demonstrating the specificity of the anti-STIL antibody used in this study. As expected, STIL migrates as a >150 kDa polypeptide on SDS-PAGE and a smaller proteolytic fragment (>95 kDa). Note, both polypeptides are dramatically reduced in lysates prepared from STIL-depleted cells. α-tubulin, loading control.

Supplemental Figure 4: Cep215 is localized to vesicles of endocytic origin. U2OS cells transfected with pEGFP-Cep215 were pulsed for 15 min with Alexa 568-conjugated transferrin and imaged every 2 s for 10 min. Yellow arrows indicate Cep215 on transferrin-containing vesicles. Insets highlight two vesicles (dashed yellow rectangle) at higher magnification. Blue and magenta arrows mark the movement of these vesicles.

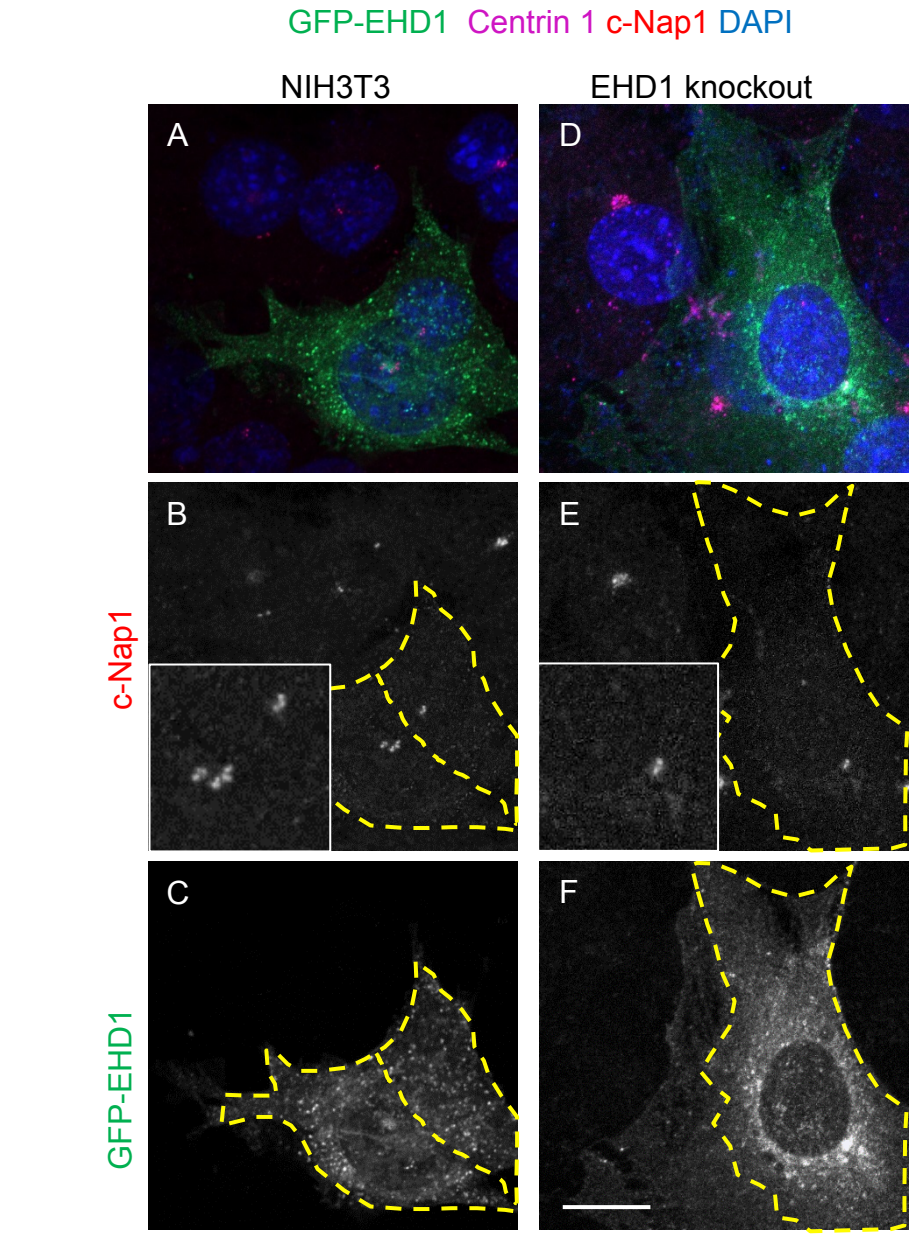
Supplemental Figure 5: Cep215 and Vps35 localize to transferrin-containing endocytic vesicles in the proximity of the centrosome.HeLa cells were incubated with transferrin-568 for 15 min., washed and incubated with fresh DMEM for an additional 15 min., fixed in paraformaldehyde and immunostained with antibodies to detect Cep215 (blue) and Vps35 (green). The white dashed region of interest outlines the borders of the dividing cell, and yellow arrows mark the likely position of the Cep215-containing centrosome. Blue arrows

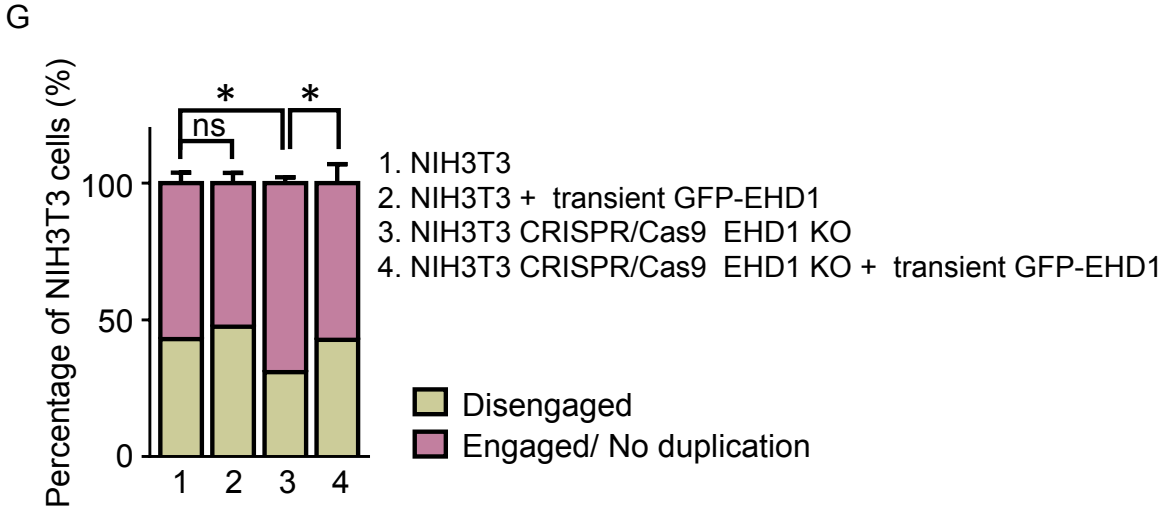
denote vesicles positive for cep215, internalized transferrin and Vps35 in the proximity of the centrosome.Scale, 10 μm.

Supplemental Video 1: U2OS cells were transfected with GFP-Cep215 and dTomato-γ-tubulin for 18 h. On the day of imaging, cells were incubated in fresh phenol-red free DMEM and immediately transferred to a heated chamber with 5% CO_2 for live cell imaging. Cells were imaged in dual channels every 5 s for 10 min. For each image, 5 z-slices were obtained, each with a 1.5 μm interval. This video demonstrates the z-projection of images at 15 frames per second.

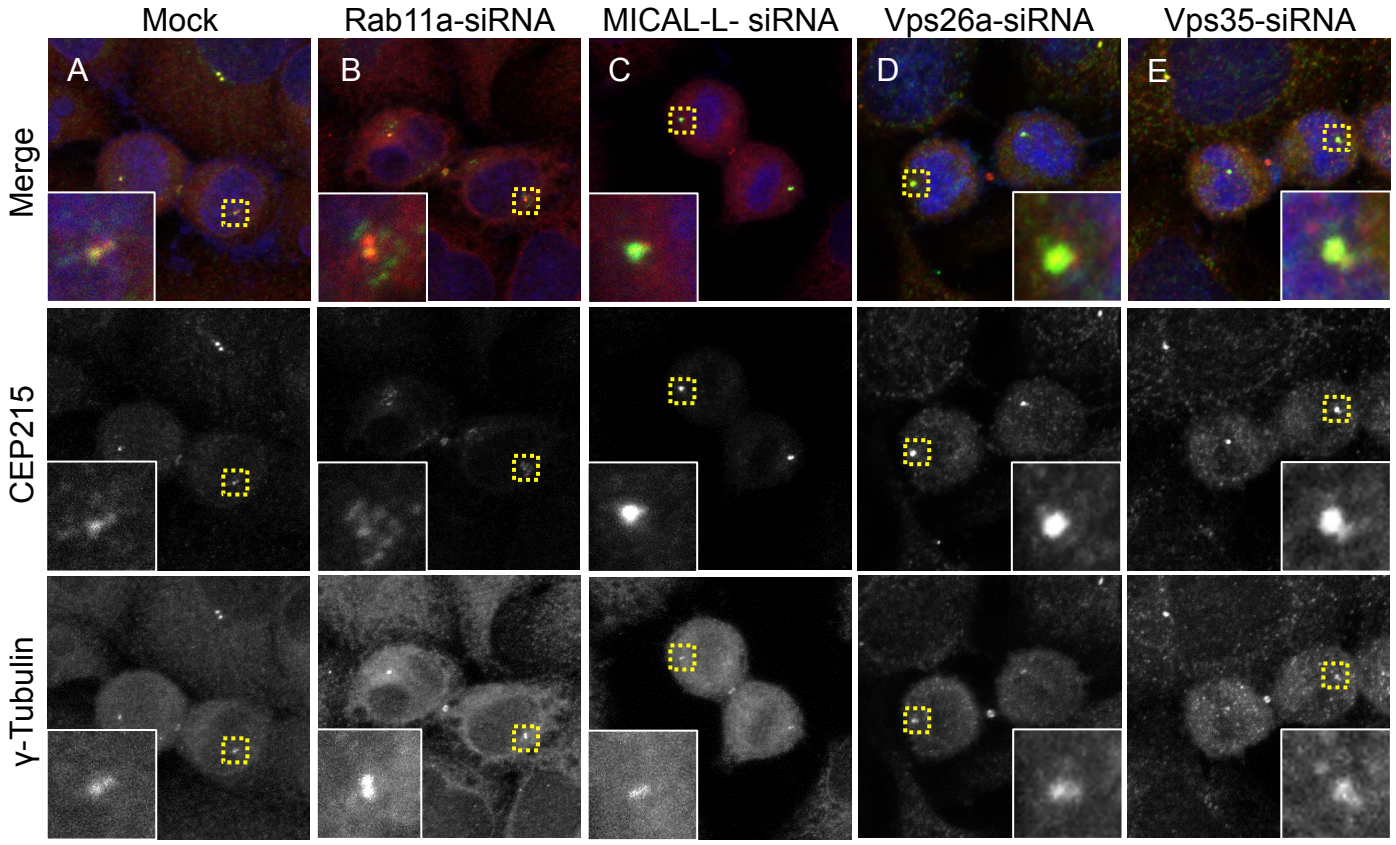
Supplemental Video 2. U2OS cells were transfected with GFP-Cep215 for 18 h. On the day of imaging, the cells were incubated in freshly prepared phenol red-free DMEM along with Alexa Fluor568-conjugated transferrin for 15 min. The medium was then replaced with fresh phenol red-free DMEM and cells were transferred to a heated chamber with 5% CO_2 for live cell imaging. Images were taken every 2 s for 10 min. The processed video shows images at 15 frames per second.

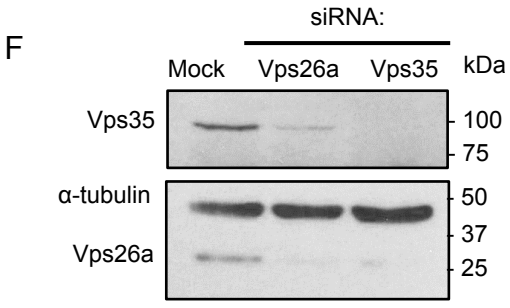
Supplemental Figure 1

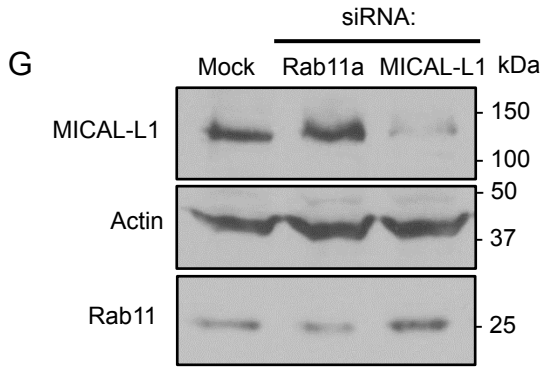


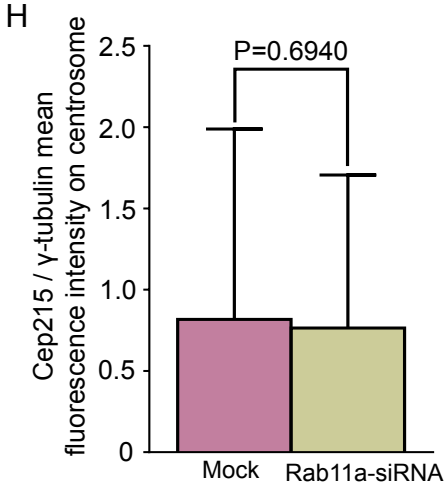


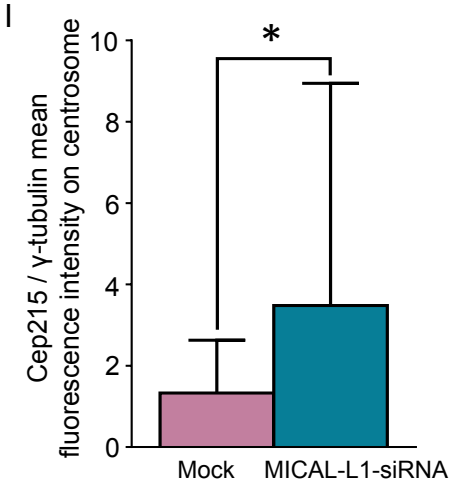
Supplemental Fig. 2

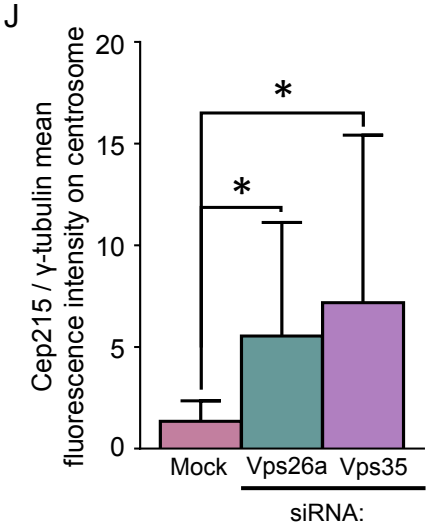




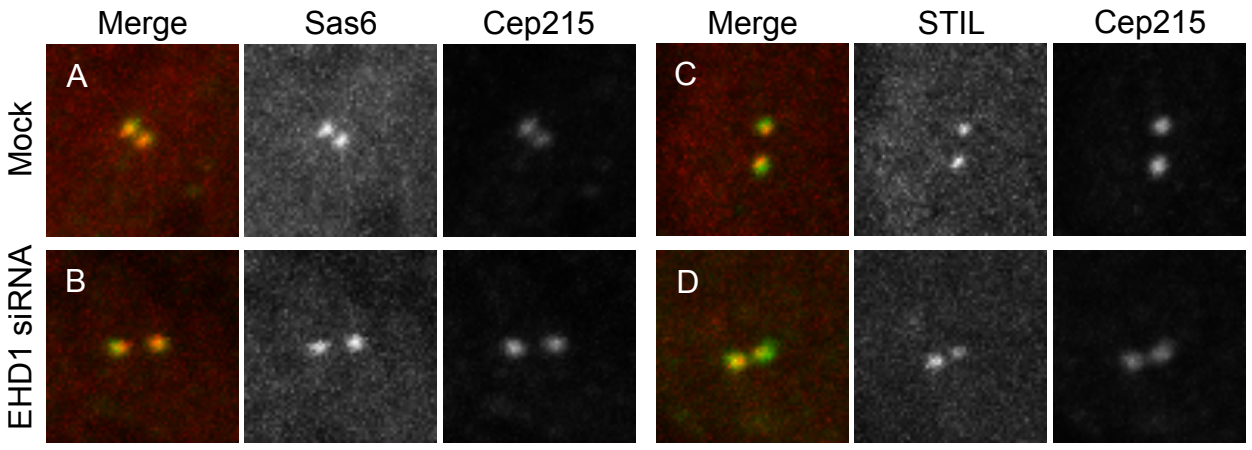


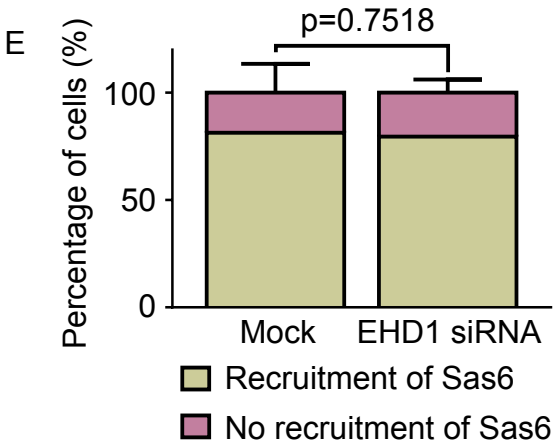


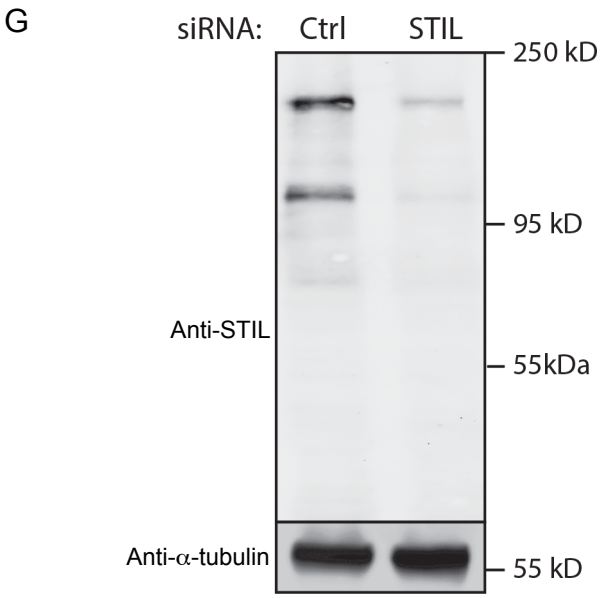




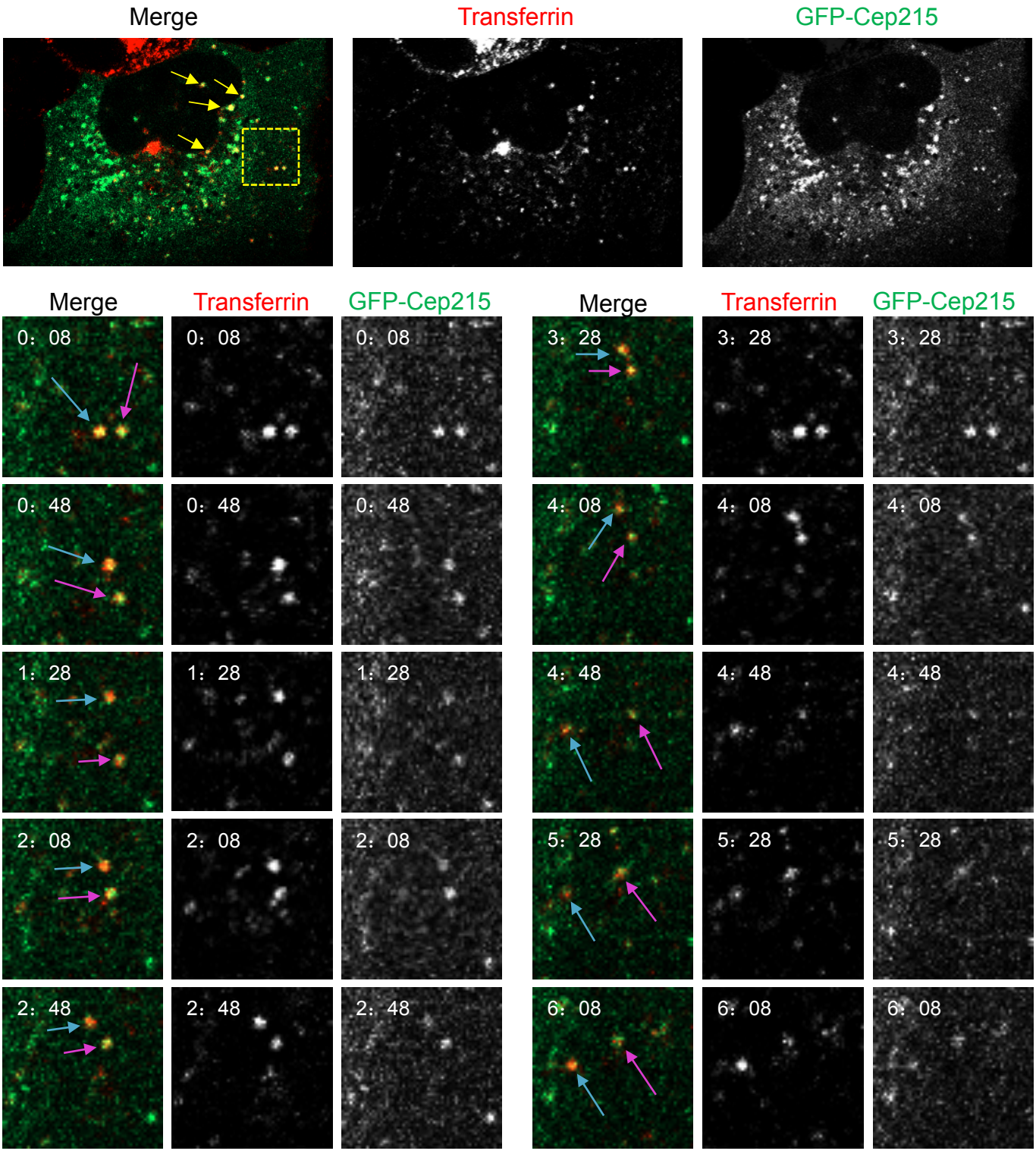
Supplemental Fig. 3







Supplemental Fig. 4



Supplemental Fig. 5