

# Supplemental Materials

*Molecular Biology of the Cell*

Xie et al.

## SUPPLEMENTARY FIGURES

### Supplementary Figure S1. dSTORM visualization of CD59 and TfR in the cell periphery.

**A-C** The processed images acquired in Figure 4D were convolved to validate the processing. HeLa cells plated on glass bottom Mattek dishes were stained for CD59 and TfR as described in Figure 4D. dSTORM was performed, with a 642 nm wavelength laser for TfR, and then a 488 nm wavelength laser for CD59. Approximately 10,000 frames were obtained for each channel. For channel drift alignment, 100 nm-gold bead fiducial markers were placed on the plate prior to image acquisition. Gold beads are depicted by the larger dots in the upper left and bottom right quadrants of the micrographs. The pixel information collected was reconstructed into normalized Gaussian images with 200 nm pixel size using ThunderSTORM in ImageJ (for details of parameters, please refer to Methods). After shift correction, filters were applied so that pixels with uncertainty values larger than 60 nm were filtered out. The images were then convolved by a Gaussian Blur filter with the mean point spread function (PSF). The PSF was 0.179  $\mu\text{m}$  for CD59, and 0.257  $\mu\text{m}$  for TfR.

**D-F** The acquired data from dSTORM were processed using ThunderSTORM into pixel information (for details of parameters, please refer to Methods), shift-corrected and filtered as described above, and then reconstructed to normalized Gaussian images with 10 nm pixel size. Yellow boxes represent CD59 (D) and TfR (E) in the cell periphery. The boxed region is displayed in a larger image as Figure 4D. Bar, 10  $\mu\text{m}$ .

**G** The degree of co-localization between CD59 and TfR was determined by ImageJ. The histogram images of CD59 and TfR with 10 nm pixel size were generated respectively, and then aligned as described above. The pixels from each images were converted to “0” (no pixel intensity) or “1” (pixel intensity from 1-255). Co-localization was defined as a pixel with values of “1” in each channel. Total 2-channel represents the number of pixels, and Total co-localized is the number of those pixels that have values of 1 in each channel. The degree of co-localization was determined by the ratio of “total co-localized” to “total 2 channel.” Results from the entire image as well as the zoomed regions are given.

**H** Histograms representing the calculated uncertainty for each channel were plotted on frequency distribution graphs with 30 bins, and the mode of the distribution was taken as the precision for each channel.

**I** Nyquist resolution for the two boxed region was calculated. The number of pixels (n) within the region of interest (ROI) and the area (A,  $\text{nm}^2$ ) of ROI were calculated. The Nyquist resolution (r) can be calculated as below:  $r = 2 \times \frac{1}{\sqrt{n/A}}$ .

**Supplementary Figure S2. dSTORM visualization of CD59 and TfR in the perinuclear area.**

**A-C** The processed images acquired in Figure 4E were convolved to validate the processing. HeLa cells plated on glass bottom Mattek dishes were stained for CD59 and TfR as described in Figure 4E. dSTORM was performed and acquired data were processed as described in Supplementary Figure S1 and Methods. Approximately 25,000 frames were obtained for each channel. The PSF was 0.265  $\mu\text{m}$  for CD59, and 0.256  $\mu\text{m}$  for MICAL-L1.

**D-F** The acquired data from dSTORM were processed into normalized Gaussian images with 10 nm pixel size as described in Supplementary Figure S1 and Methods. Yellow boxes represent CD59 (D) and TfR (E) in the perinuclear area. The boxed region in D-F is enlarged and presented in Figure 4E. Bar, 10  $\mu\text{m}$ .

**G** The degree of co-localization between CD59 and TfR was determined by ImageJ as described in Supplementary Figure S1. Calculations from the entire image and the boxed regions are given.

**H** Histograms representing the calculated uncertainty for each channel were plotted on frequency distribution graphs with 30 bins, and the mode of the distribution was taken as the precision for each channel.

**I** Nyquist resolution for the two boxed region was calculated as described in Supplementary Figure S1.

**Supplementary Figure S3. Myosin Vb tail overexpression selectively traps CME but not CIE cargos at the ERC.**

**A-B** HeLa cells were transfected with GFP-Myosin Vb tail and treated with transferrin or anti-LDLR antibody as described in Figure 5 A-F. SIM images were taken using a Zeiss Elyra PS.1 microscope and processed by Zen Black. Maximal projections were generated from SIM slices spaced 110 nm apart. The intensity profile along the white arrow for both channels was tracked using RGB Profiler in ImageJ.

**C-D** HeLa cells were transfected with GFP-Myosin Vb tail and treated with anti-CD59 or anti- $\beta$ 1-integrin antibody as described in Figure 5 G-L. The image acquisition, processing, and intensity profile tracking were performed as described in A-B. Bar, 10  $\mu\text{m}$ .

**E-G** HeLa cells transfected with GFP-Myosin Vb tail were fixed, and incubated with anti-MICAL-L1 antibody for 1 h at room temperature, followed by appropriate secondary antibody staining. Confocal microscopy images were acquired with a Zeiss LSM5 Pascal microscope as described in the Methods.

**H-J** HeLa cells transfected with GFP-Myosin Vb tail and HA-Syndapin2 were fixed,

and observed by confocal microscopy as described in the Methods. Bar, 10  $\mu\text{m}$ .

**Supplementary Figure S4. dSTORM visualization of Rab11a and MICAL-L1-labeled recycling endosomes.**

**A-C** The processed images acquired in Figure 7D were convolved to validate the processing. HeLa cells plated on glass bottom Mattek dishes were stained for Rab11 and MICAL-L1 as described in Figure 7D. dSTORM was performed and acquired data were processed as described in Supplementary Figure S1 and Methods. Approximately 25,000 frames were obtained for each channel. The PSF was 0.21  $\mu\text{m}$  for MICAL-L1 and 0.277  $\mu\text{m}$  for Rab11a.

**D-F** The acquired data from dSTORM were processed into normalized Gaussian images with 10 nm pixel size as described in Supplementary Figure S1 and Methods. Yellow rectangles represent Rab11a and MICAL-L1 in the perinuclear area (box a) and periphery (b), respectively. Bar, 10  $\mu\text{m}$ .

**G** The degree of co-localization between Rab11a and MICAL-L1 was determined by ImageJ as described in Supplementary Figure S1. Calculations from the entire image and the boxed regions are given.

**H** Nyquist resolution for the two boxed region was calculated as described in Supplementary Figure S1.

**I, J** Histograms representing the calculated uncertainty for each channel were plotted on frequency distribution graphs with 30 bins, and the mode of the distribution was taken as the precision for each channel.

**Supplementary Figure S5. Rabenosyn-5 partially colocalizes with MICAL-L1 to TRE in untreated cells.**

**A-C** HeLa cells plated on coverslips were fixed, and then stained with anti-MICAL-L1 and anti-Rabenosyn-5 together with the corresponding secondary antibody, and imaged by SIM. Dashed boxes denote the peripheral cell area, where a higher degree of co-localization between MICAL-L1 and Rabenosyn-5 was observed, and are shown with higher magnification in the inset. Scale bar, 10  $\mu\text{m}$ .

**Supplementary Figure S6. MICAL-L1-decorated TRE do not originate from the PM.**

**A-C** HeLa cells were incubated with Alexa-488 conjugated WGA for 5 min at 37°C to label the PM (A). After fixation, cells were stained with anti-MICAL-L1 antibody and Alexa-568 conjugated secondary antibody (B) and examined by confocal microscopy. Yellow boxes

indicate a representative region of the cell periphery that contains MICAL-L1 and WGA. The inset panels show the boxed area with higher magnification. Scale bar, 10  $\mu\text{m}$ .

**D-F** HeLa cells were incubated with Alexa-488 conjugated WGA (D) and mouse anti-CD59 antibody (E) for 5 min at 37°C, and subsequently fixed and stained with Alexa-568 conjugated anti-mouse antibody. Yellow boxes focus on the region of the cell where CD59 is internalized and localized beneath the PM. The inset panels show the boxed area with higher magnification. Scale bar, 10  $\mu\text{m}$ .

**Supplementary Figure S7. MICAL-L1 displays limited co-localization with transferrin.**

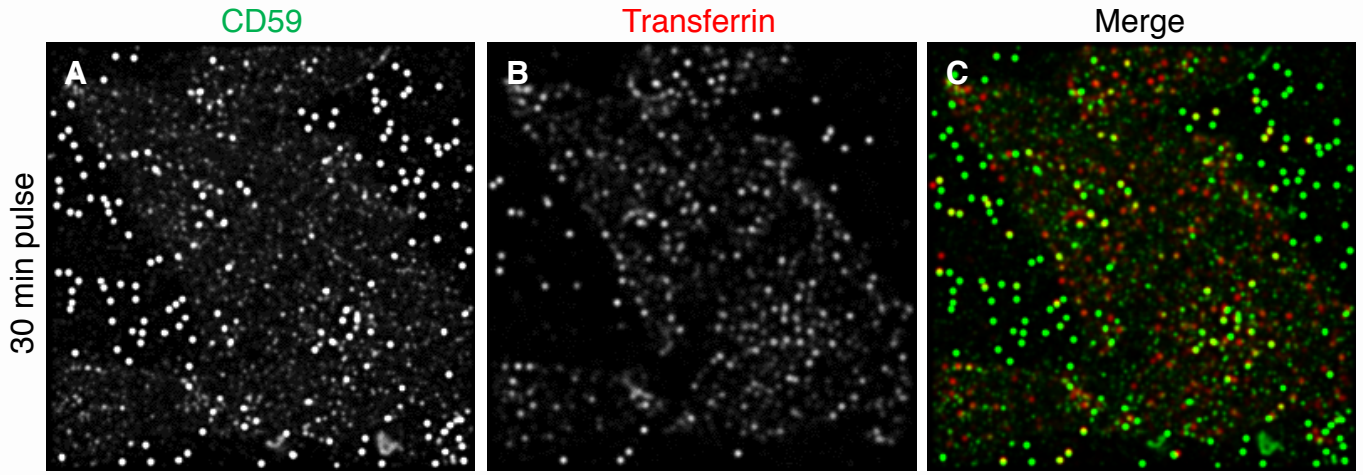
**A-C** HeLa cells growing on coverslips were serum-starved, incubated with Alexa-568 conjugated transferrin for 9 min at 37 °C, and fixed (B). Endogenous MICAL-L1 was detected with mouse anti-MICAL-L1 antibody and Alexa-488 conjugated anti-mouse antibody with saponin (A). Insets depict the boxed areas in higher magnification. Scale bar, 10  $\mu\text{m}$ .

**D-E** HeLa cells were treated and imaged by SIM as described in Figure 9G-N. The degree of co-localization between different cargos and Rabenosyn-5 (D) or MICAL-L1 (E) was measured using ImageJ with Manders' overlap coefficients. 10 cells from three independent experiments were measured and subjected to statistical analysis. Error bars represent standard deviation. \*\*\*\*,  $p < 0.0001$ . \*\*,  $p < 0.01$ .

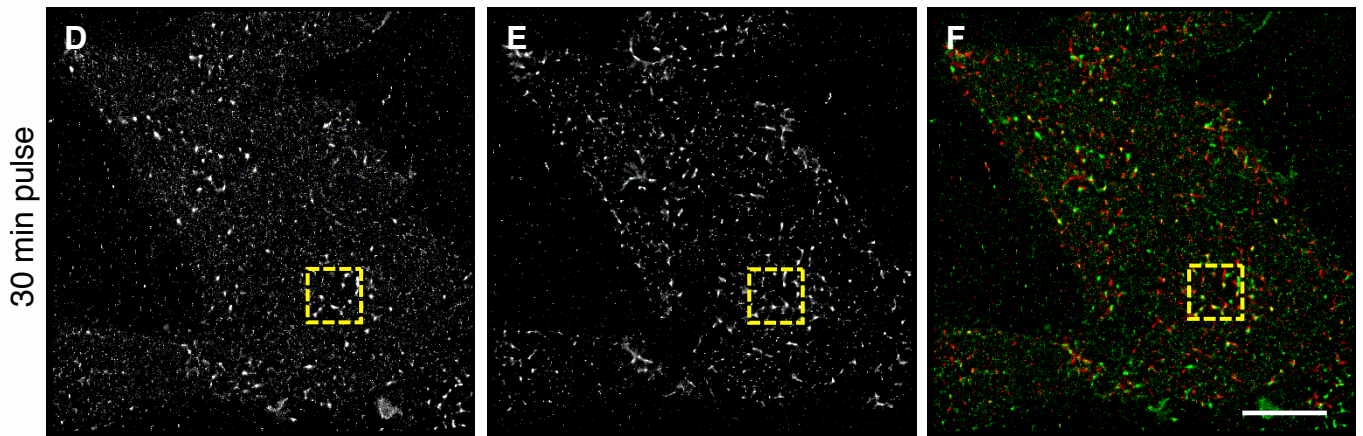
**CONFLICT OF INTEREST STATEMENT**

The authors declare that they have no conflict of interest.

# Supplementary Figure S1



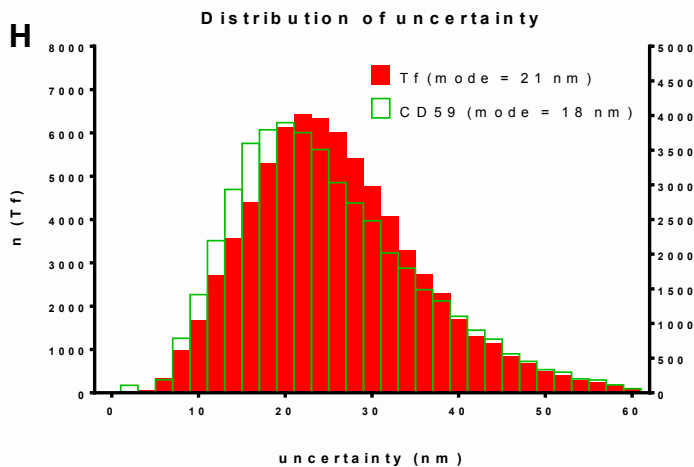
Convolved dSTORM (200 nm pixels)



dSTORM (10 nm pixels)

**G**

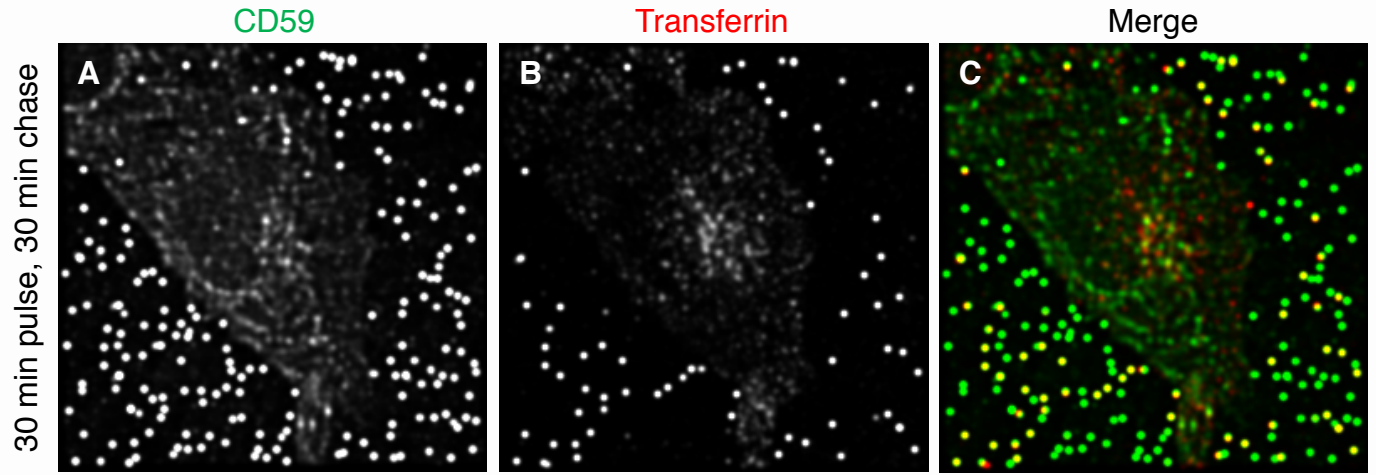
	Cell	Zoomed region
Total 2-channel	1147178	48929
Total co-localized	48983	5441
Co-localization	4.27%	11.12%



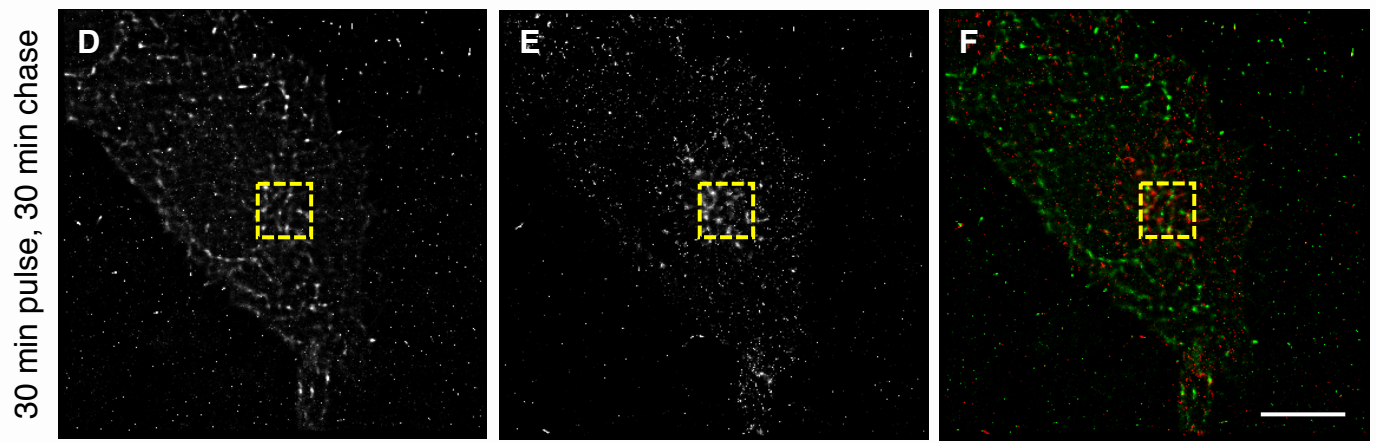
**I**

Nyquist resolution (nm)	
CD59	Transferrin
61	48.5

# Supplementary Figure S2



Convolved dSTORM (200 nm pixels)

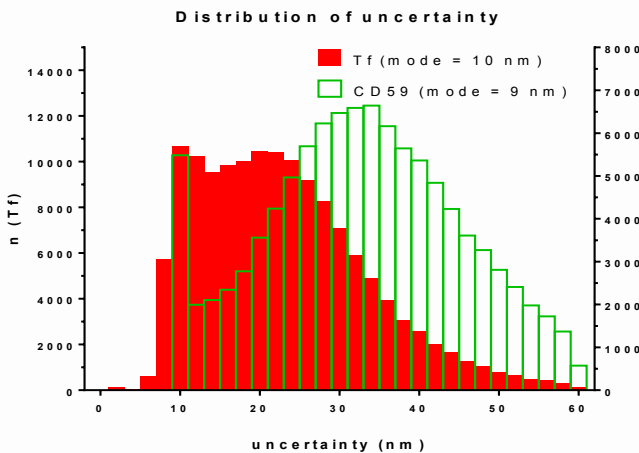


dSTORM (10 nm pixels)

**G**

	Cell	Zoomed region
Total 2-channel	2205235	158520
Total co-localized	96434	26415
Co-localization	4.37%	16.66%

**H**

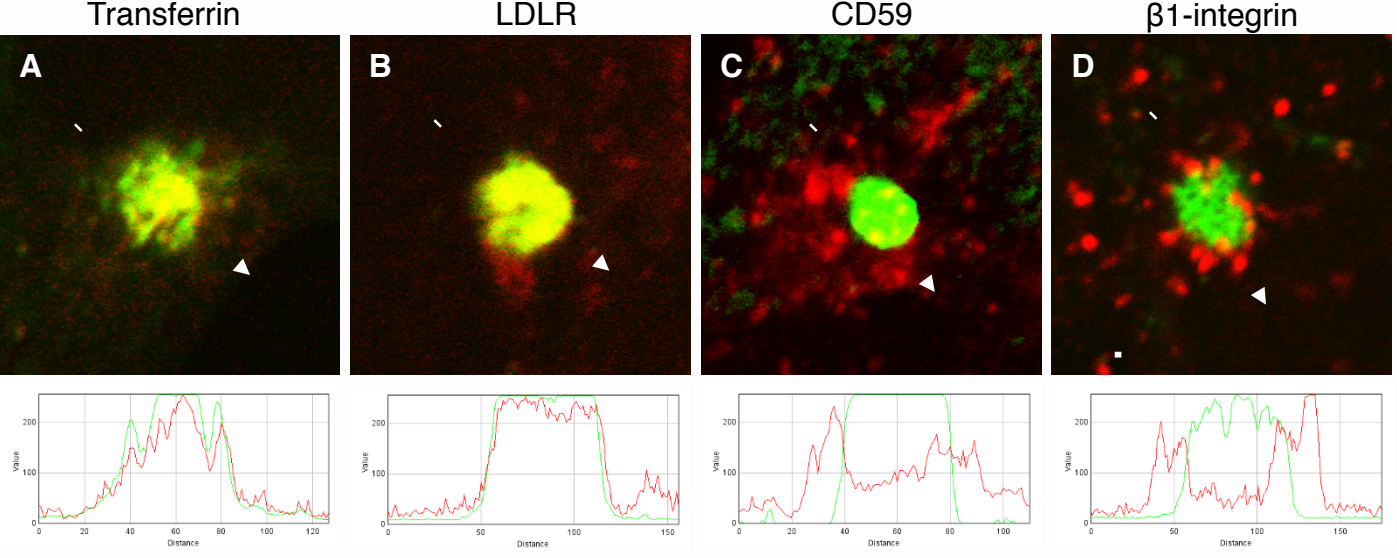


**I**

Nyquist resolution (nm)	
CD59	Transferrin
38.7	33

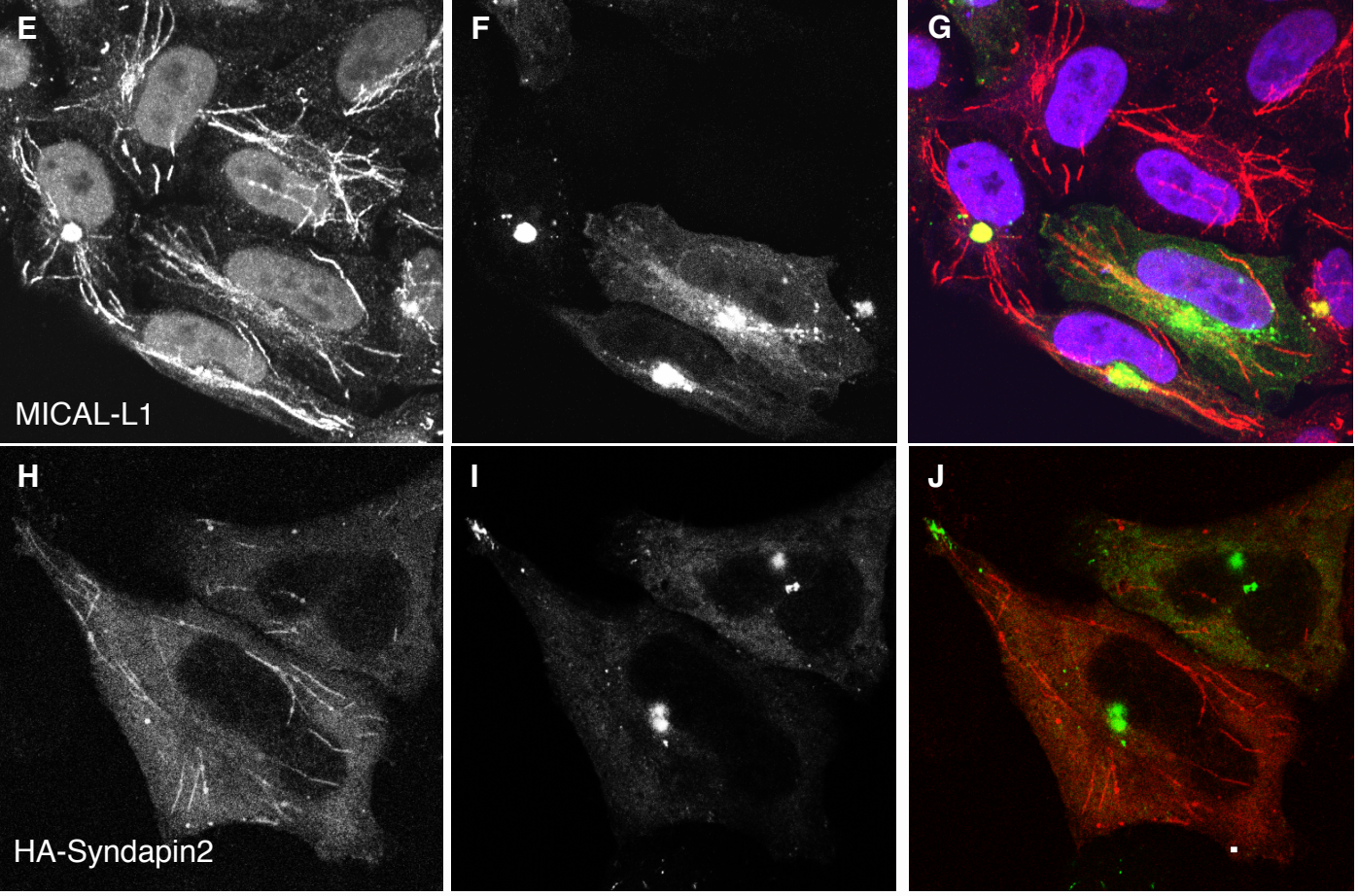
# Supplementary Figure S3

30 min pulse, 2 h chase  
GFP-Myosin Vb tail overexpression



GFP-Myosin Vb tail

Merge





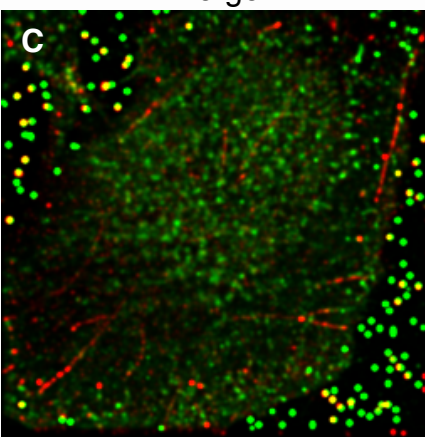
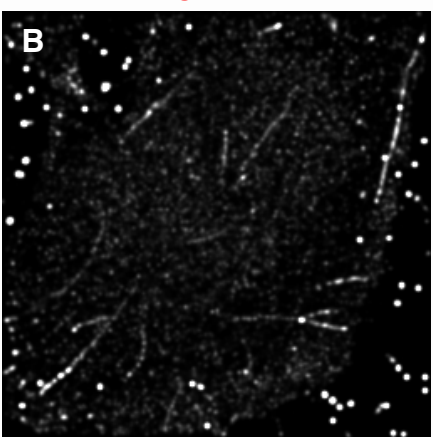
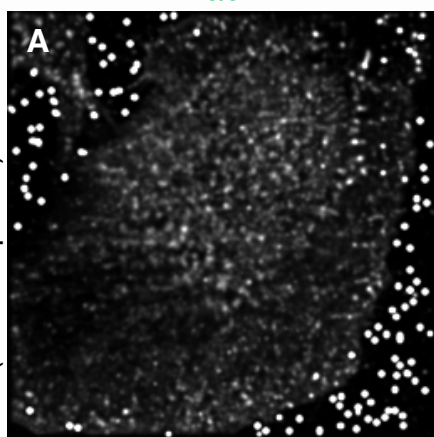
# Supplementary Figure S4

Rab11

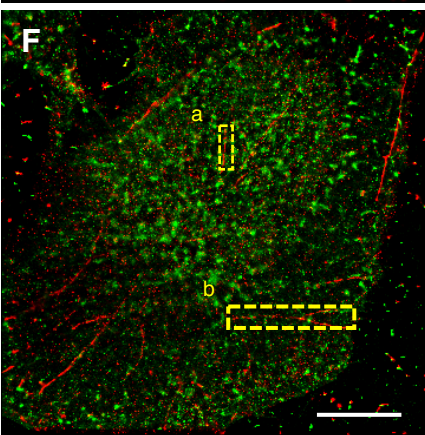
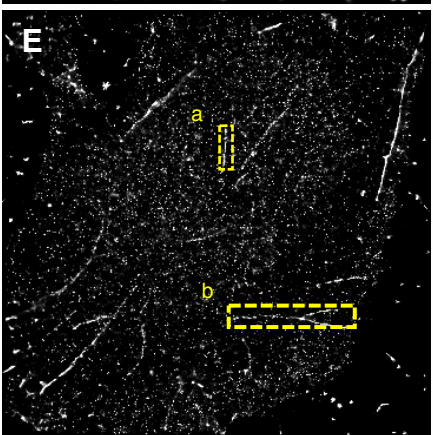
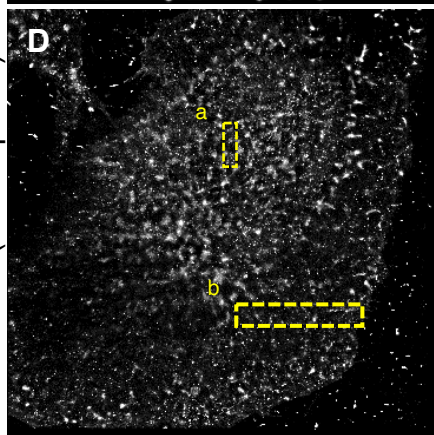
MICAL-L1

Merge

Convolved dSTORM  
(200 nm pixels)



dSTORM (10 nm pixels)



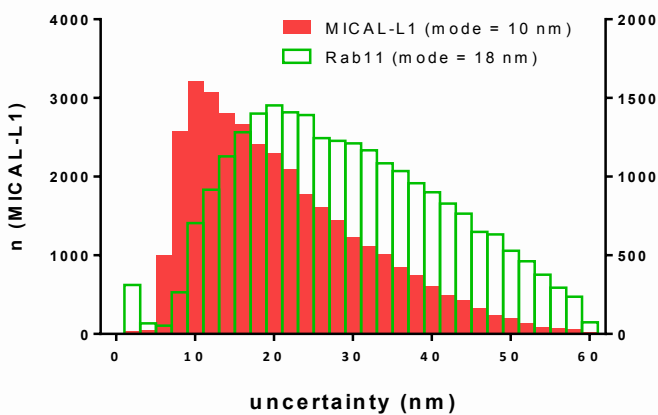
G

	Cell	Zoomed region (a)	Zoomed region (b)
Total 2-channel	4884462	21543	95859
Total co-localized	320600	3302	8135
Co-localization	6.56%	15.3%	8.49%

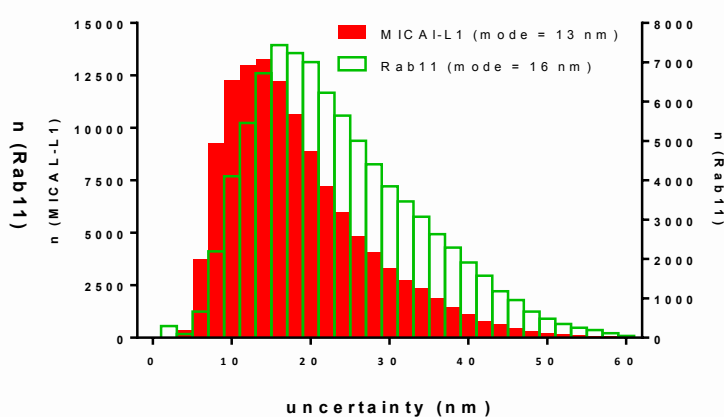
H

	MICAL (a)	Rab11 (a)	MICAL (b)	Rab11 (b)
Nyquist resolution (nm)	31.6	38	39.8	47.5

I Distribution of uncertainty (Region a)

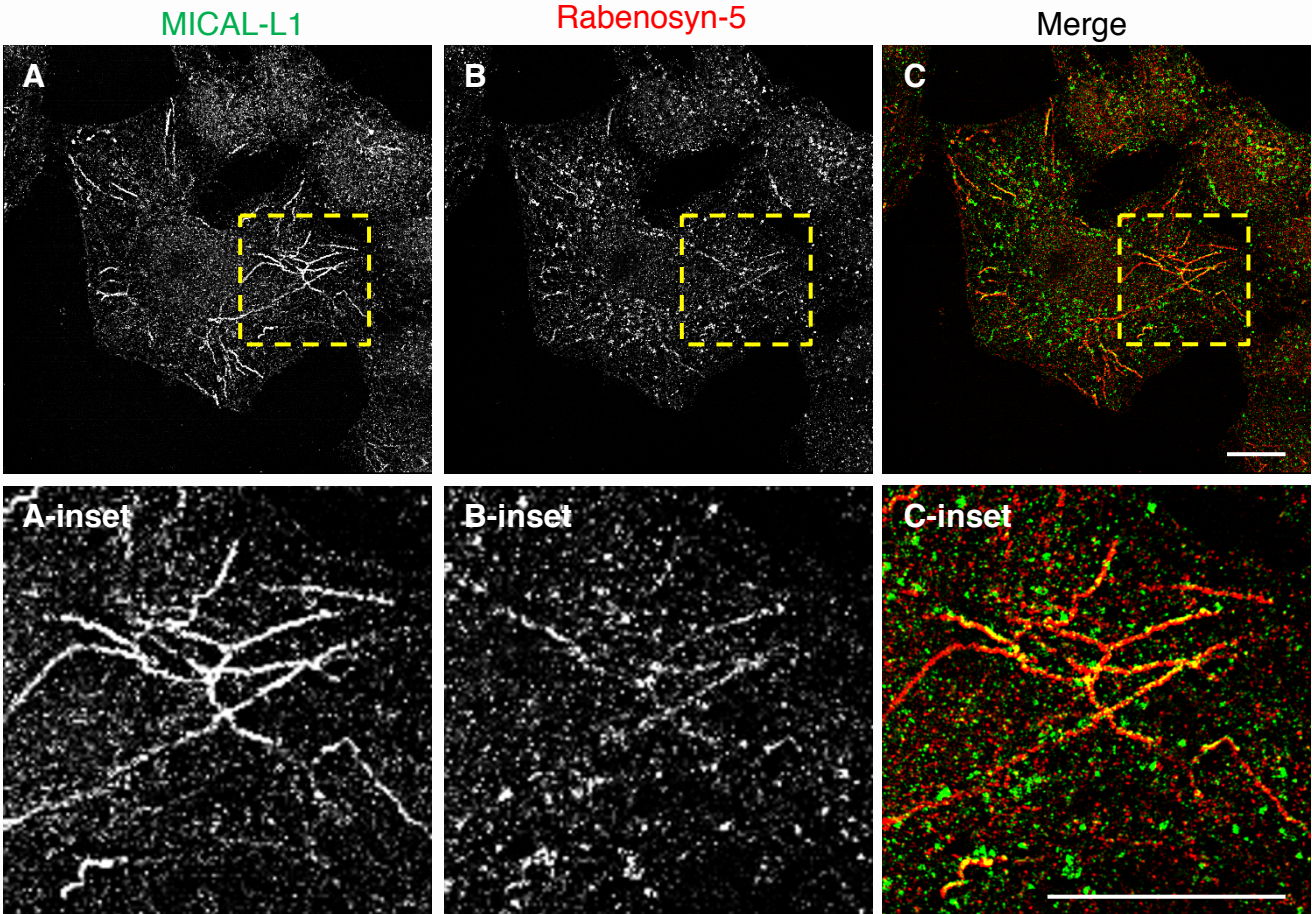


J Distribution of uncertainty (Region b)

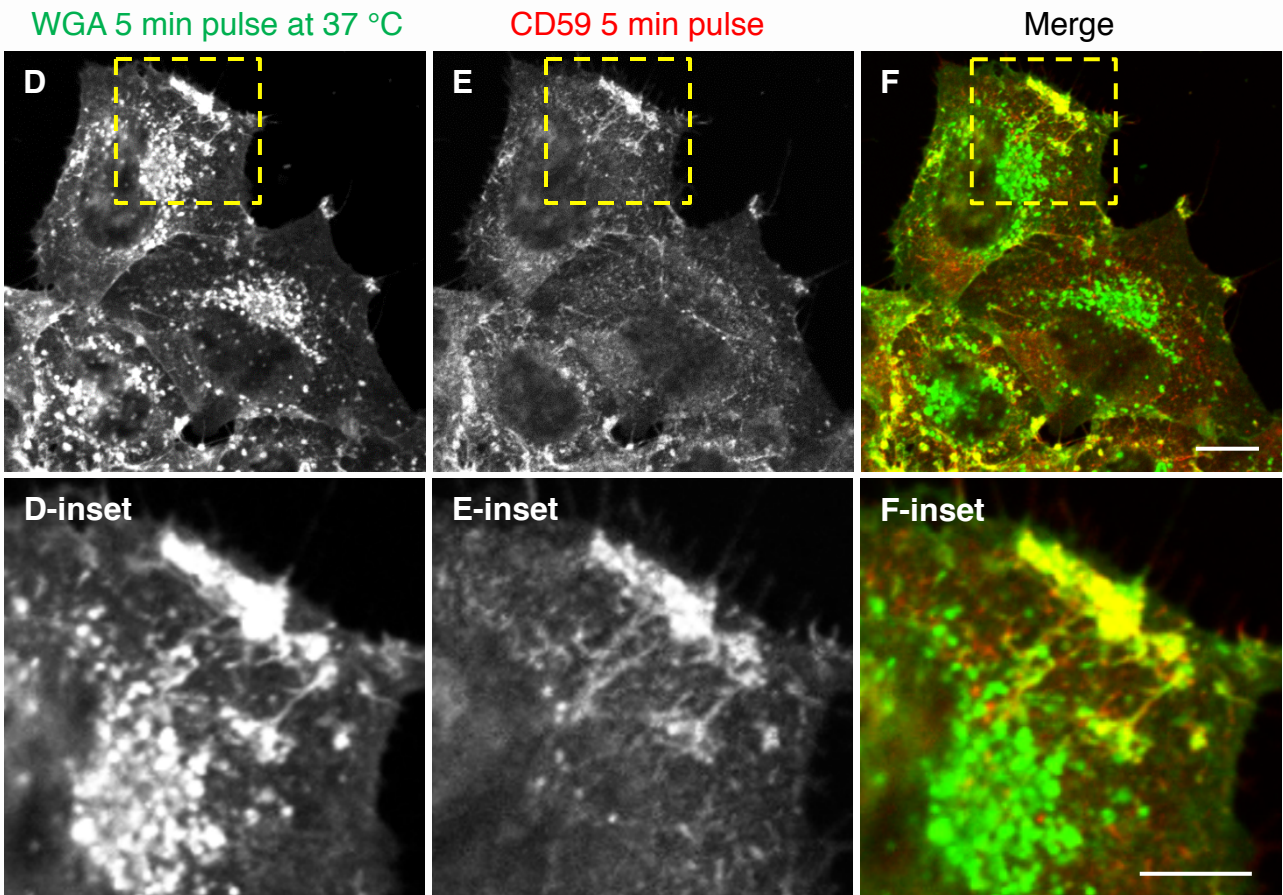
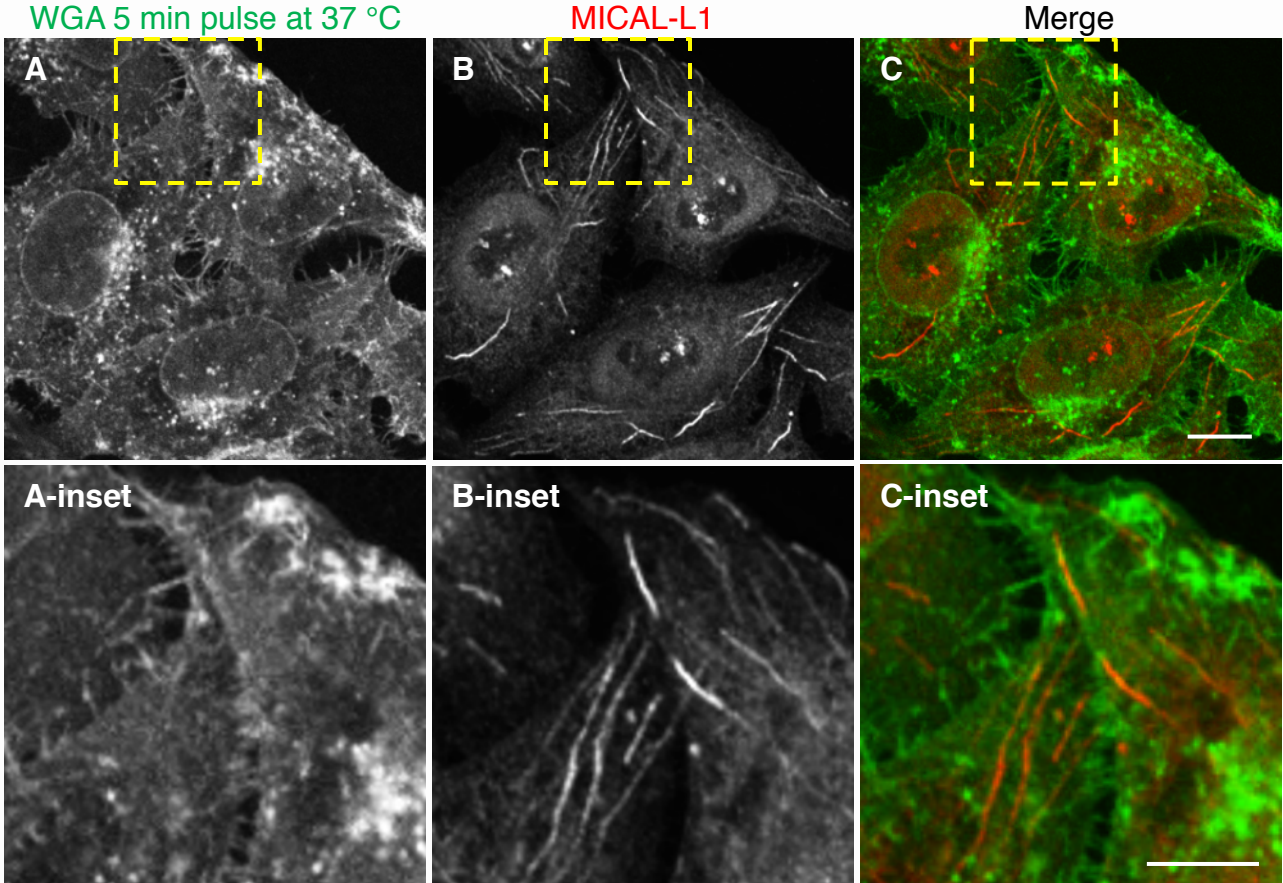


# Supplementary Figure S5

(Structured Illumination Microscopy) SIM



# Supplementary Figure S6



# Supplementary Figure S7

