

Light-inducible generation of membrane curvature in live cells with engineered BAR domain proteins

Supplementary Information

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This PDF file includes:

Supplementary Figures S1-S10

Other supplementary materials for this manuscript include the following:

opto-FBAR.avi

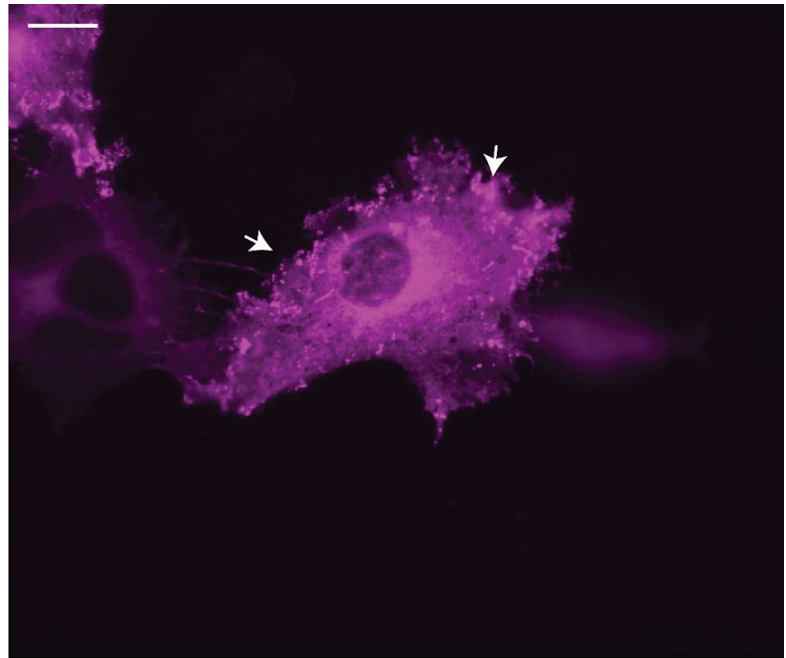
opto-IBAR Bleach Corrected.avi

opto-IBAR uncorrected.avi

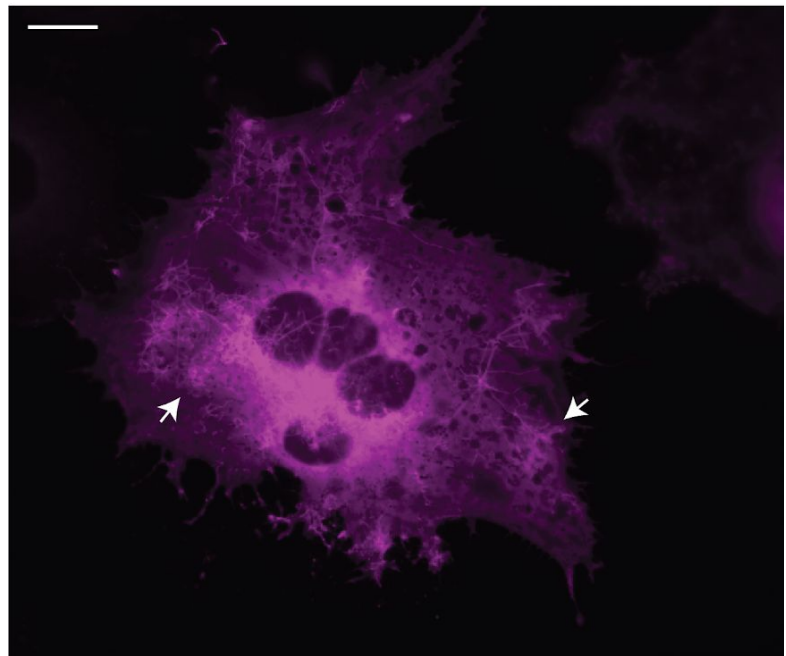
TubuleTracer1.3.txt

Supplementary Figure S1

tdTomato-micro-FBP17(FL)
+
iLID-EGFP-CAAX

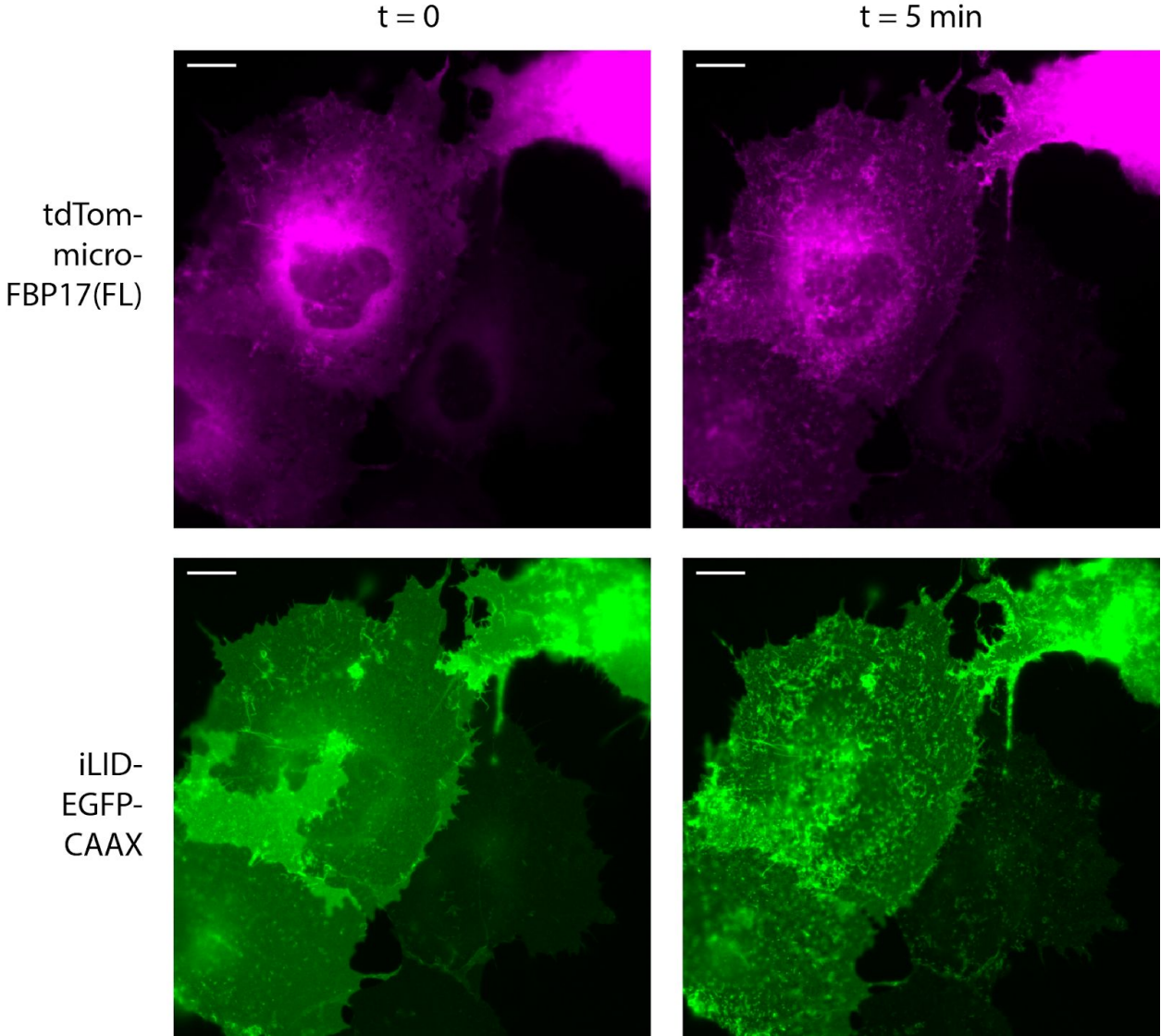


tdTomato-micro-FBP17(FL)
only



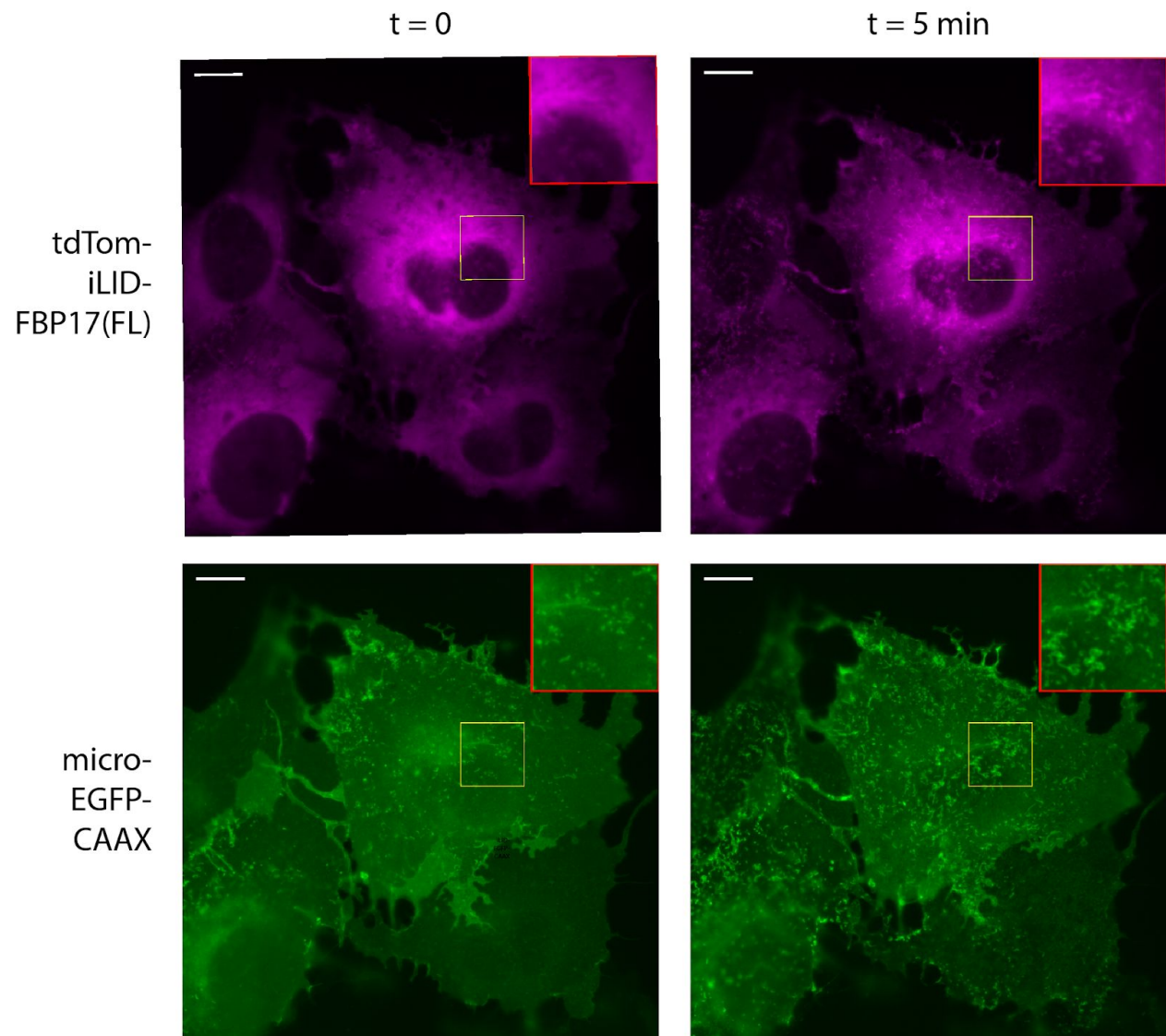
Supplementary Figure S1: opto-FBAR with FBP17(FL) exhibits high levels of tubulation (arrows) in the dark. *Top:* COS7 cells expressing tdTom-micro-FBP17(FL) with iLID-EGFP-CAAX in the dark. *Bottom:* COS7 cells expressing tdTom-micro-FBP17(FL) only. Images show the red channel (tdTom-micro-FBP17(FL)). Scale bar = 10 μm .

Supplementary Figure S2



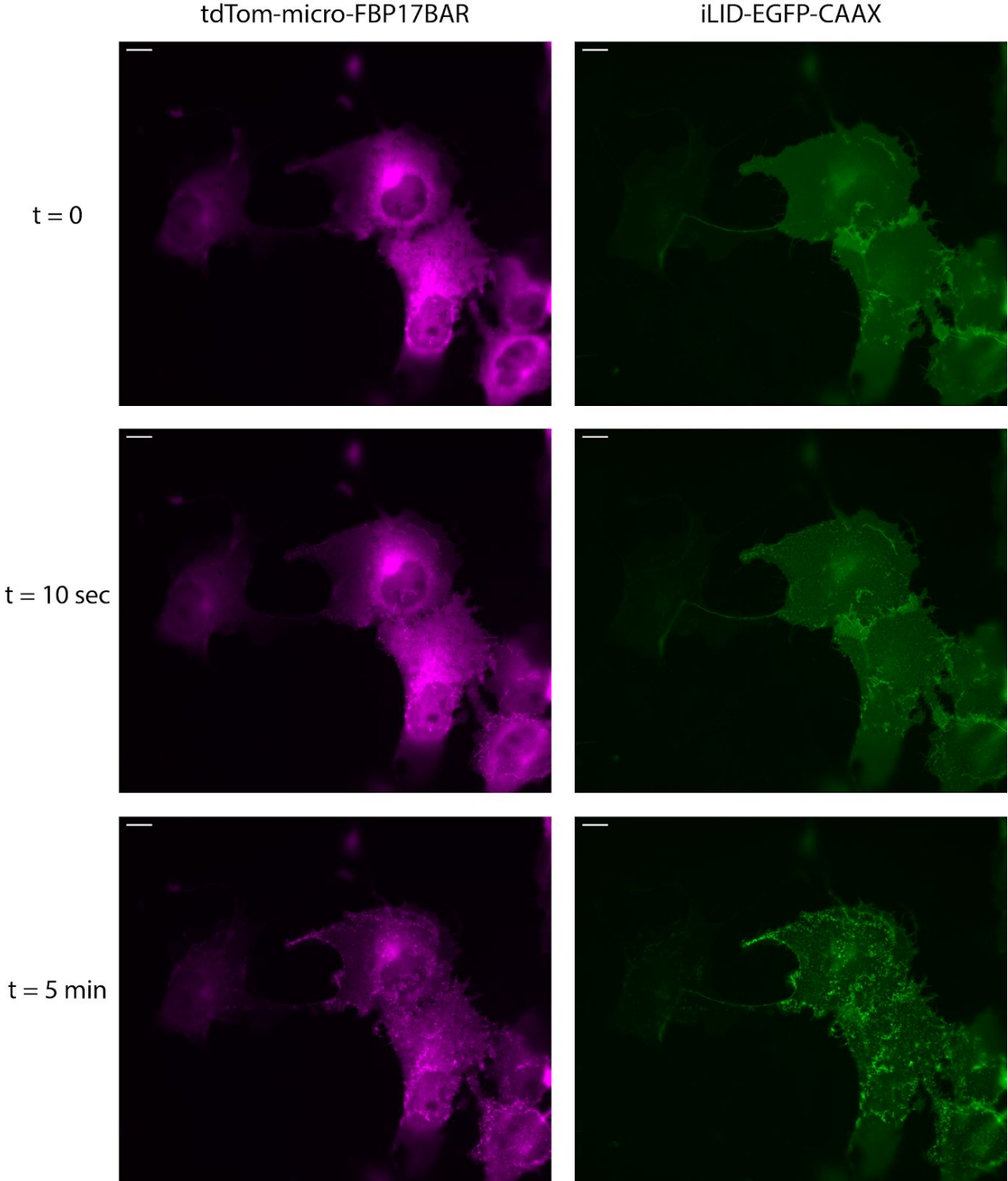
Supplementary Figure S2: Full field images of cells shown in Figure 2B. Scale bar = 10 μm .

Supplementary Figure S3



Supplementary Figure S3: COS7 cells expressing tdtom-iLID-FBP17(FL) and micro-EGFP-CAAX, i.e. the bait and prey positions are switched. Cells were kept in the dark prior to 5 minutes of blue light exposure. Scale bar = 10 μ m.

Supplementary Figure S4



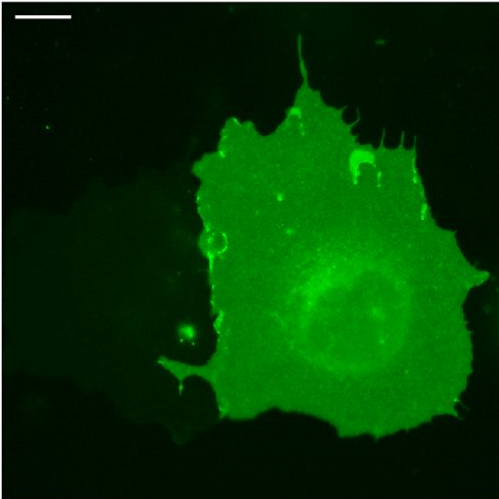
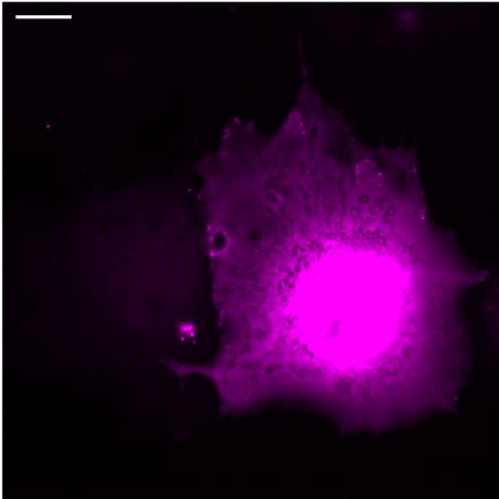
Supplementary Figure S4: Full field images of cells shown in Figure 2D. Scale bar = 10 μ m.

Supplementary Figure S5

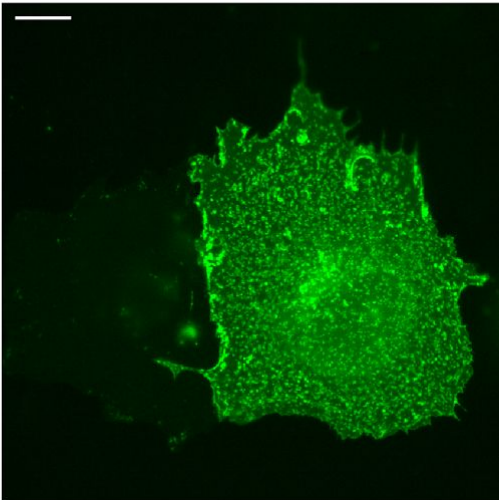
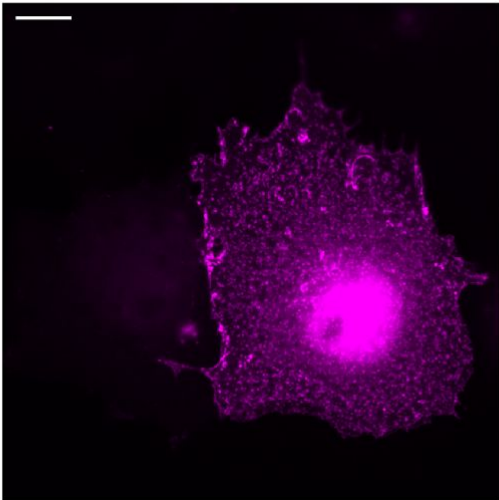
tdTom-micro-FBP17BAR

iLID-EGFP-CAAX

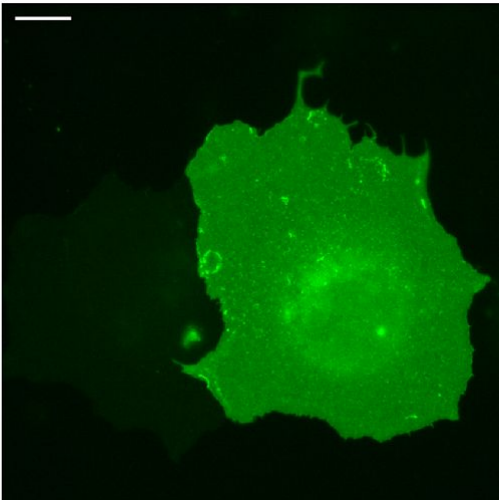
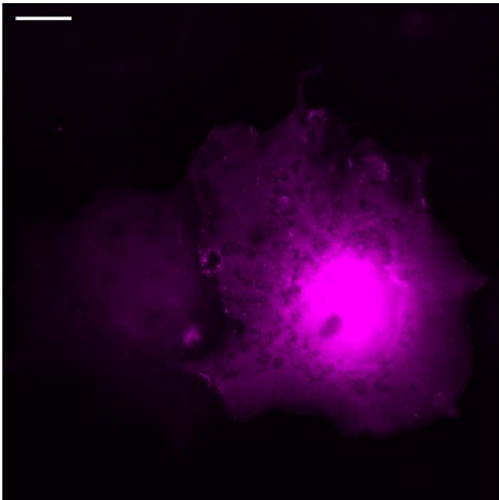
t = 0



t = 5 min

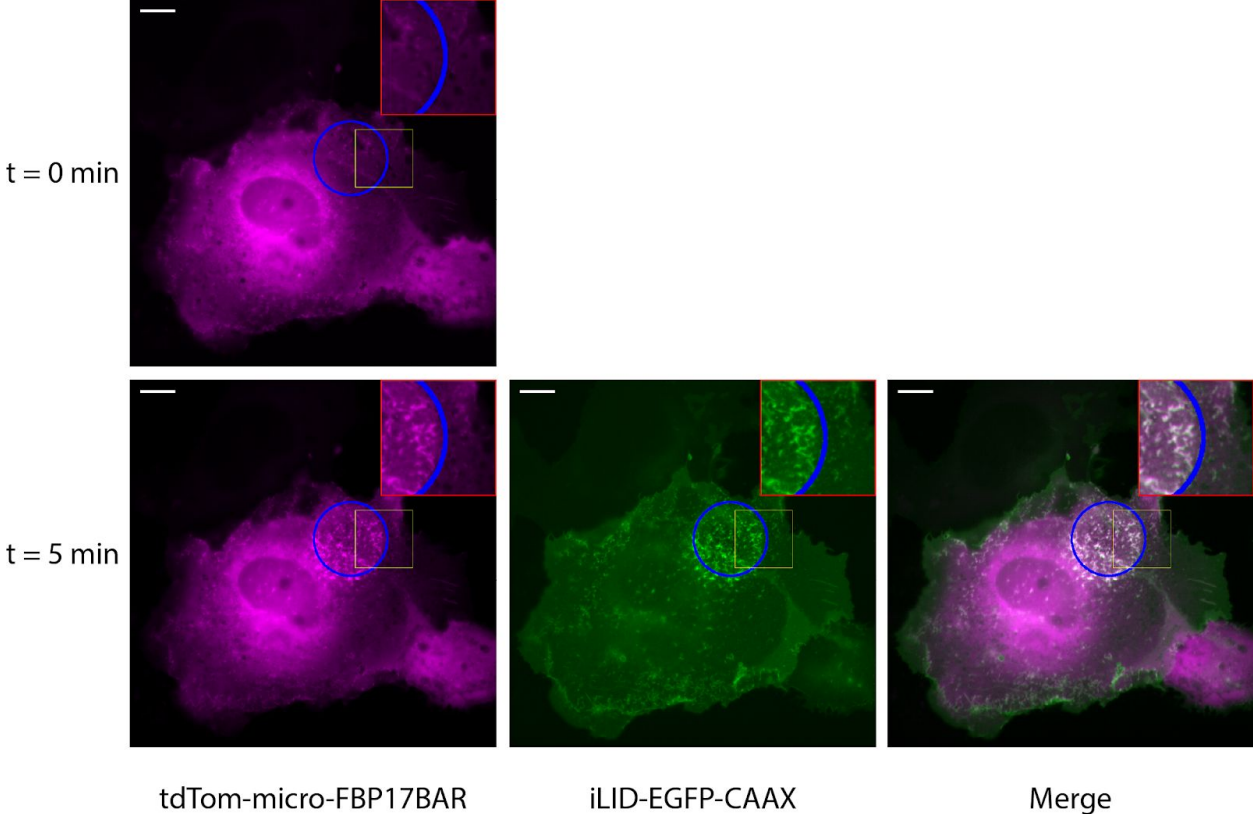


t = 35 min



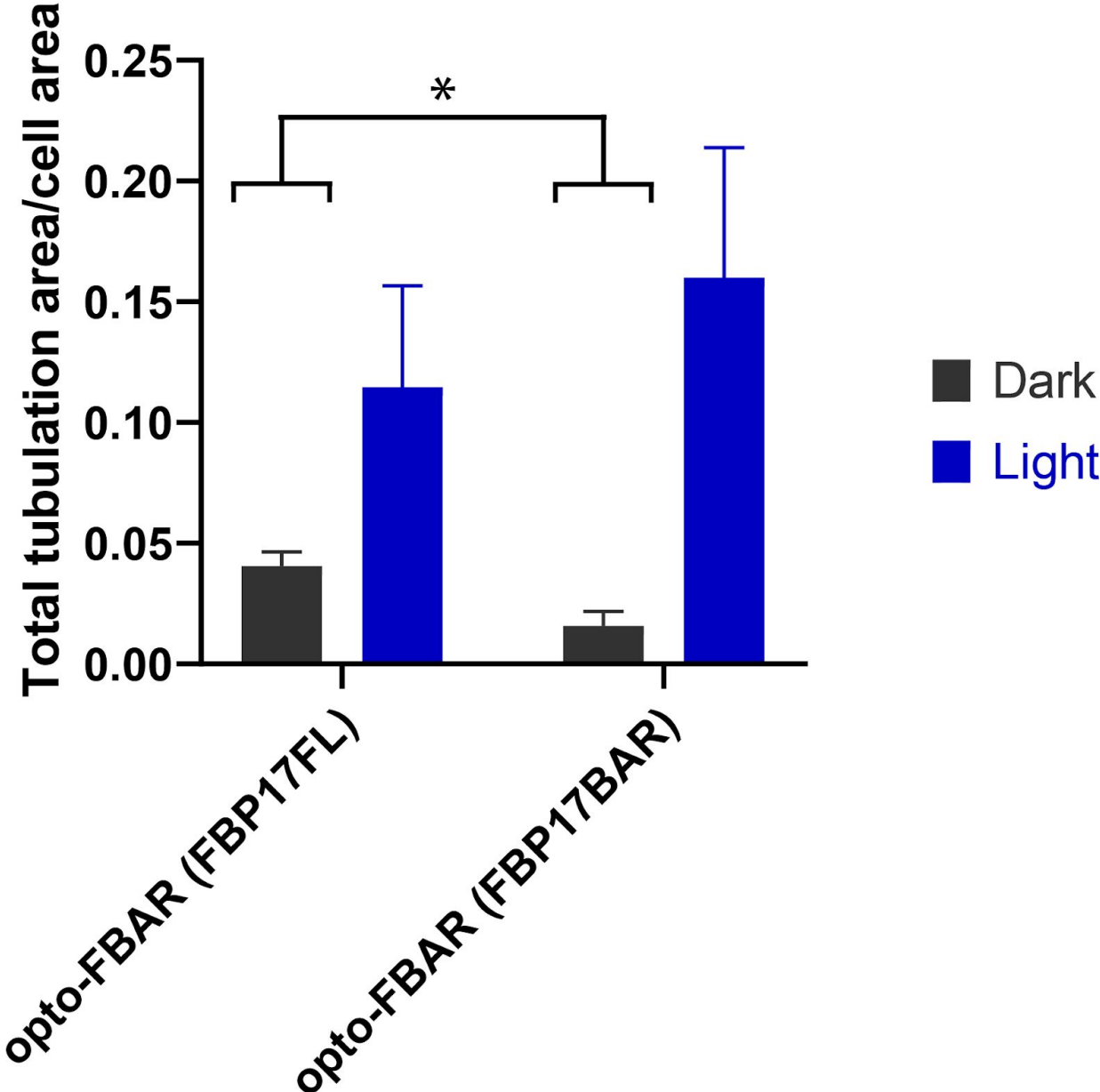
Supplementary Figure S5: Full field images of cells shown in Figure 3A. Scale bar = 10 μ m.

Supplementary Figure S6



Supplementary Figure S6: Full field images of cells shown in Figure 3B. Blue light illumination for 5 minutes was limited to a small area of the cell (blue circle). Yellow box indicates the area shown in Figure 3B. Scale bar = 10 μ m.

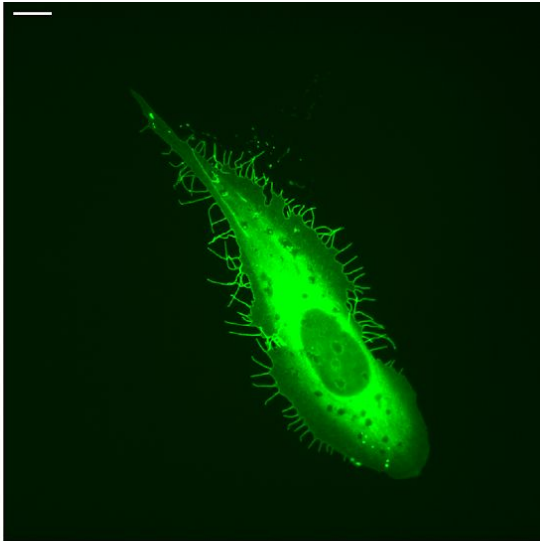
Supplementary Figure S7



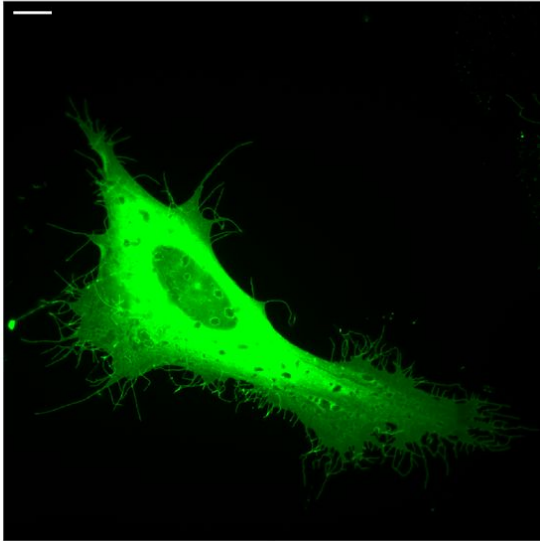
Supplementary Figure S7: TubuleTracer analysis of opto-FBAR design steps. iLID-EGFP-CAAX images acquired at t=0 were used for the “Dark” group and images acquired after 5 minutes of blue light activation were used for the “Light” group. Image acquisition was performed as stated in the Methods section. *p < 0.05

Supplementary Figure S8

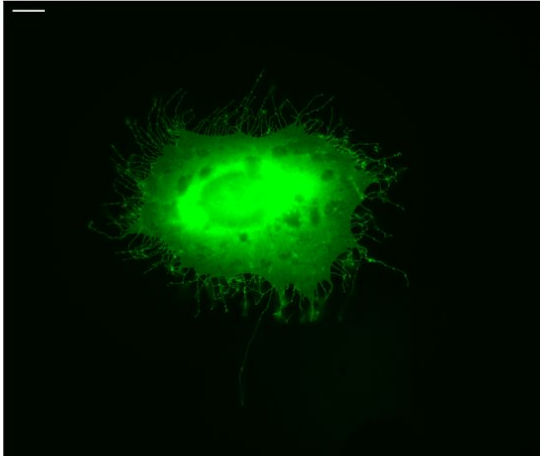
IRSp53(FL)-mEGFP



IRSp53(hv)-mEGFP



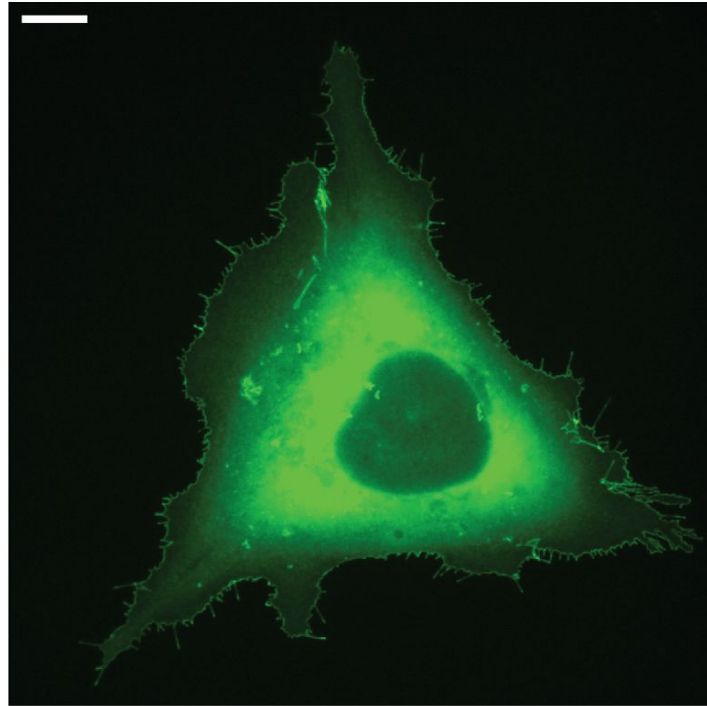
IRSp53BAR-mEGFP



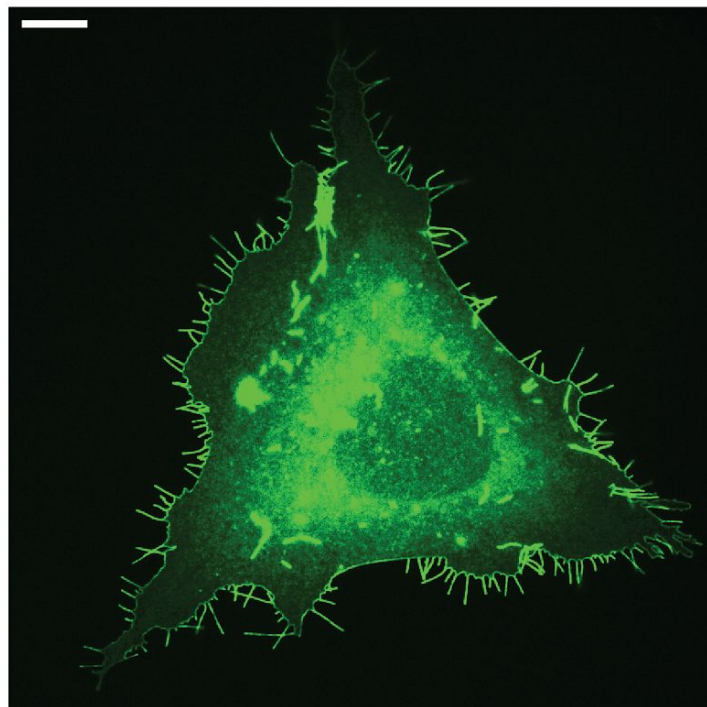
Supplementary Figure S8: Full field images of cells shown in Figure 4A. Scale bar = 10 μ m.

Supplementary Figure S9

t = 0

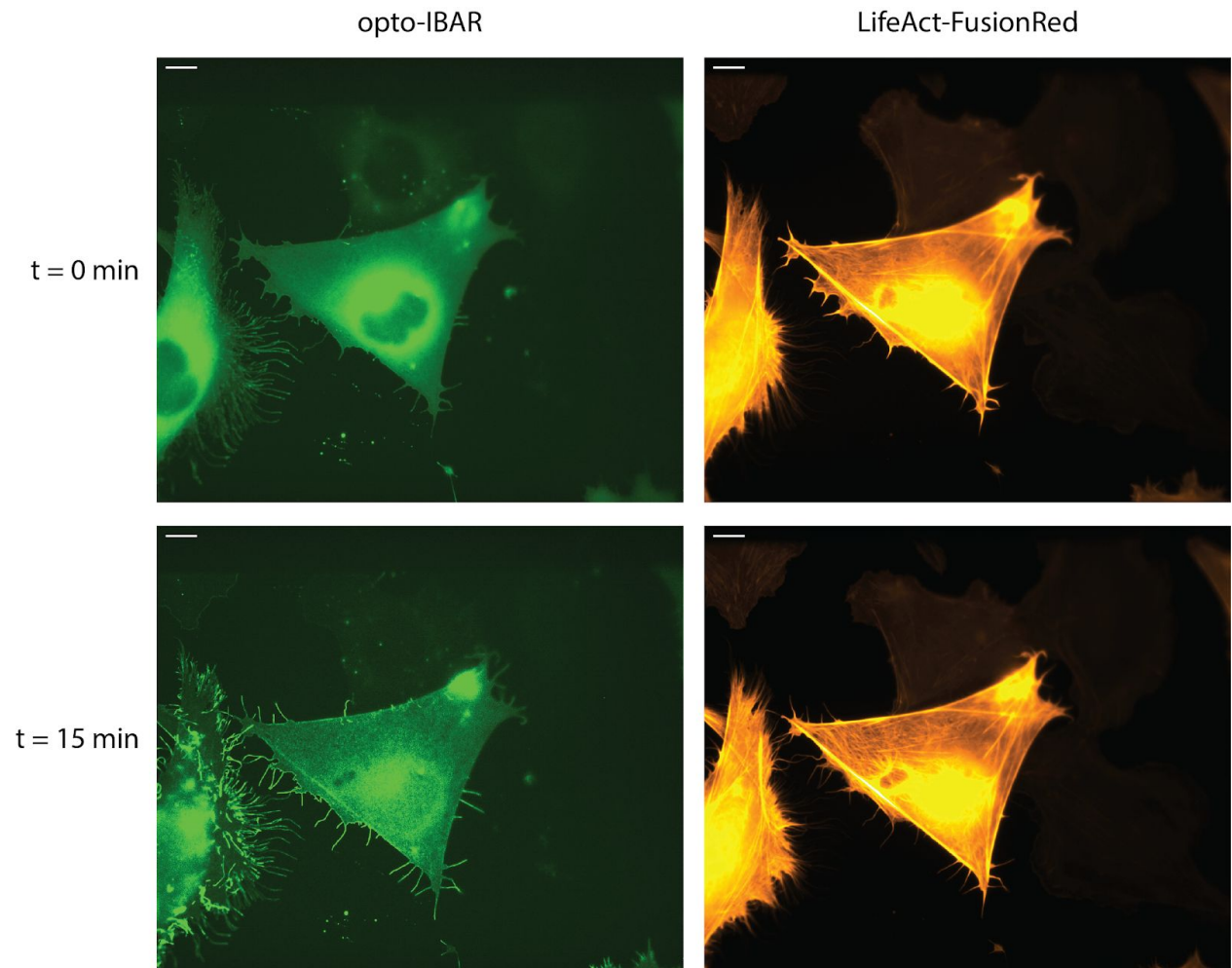


t = 5 min



Supplementary Figure S9: Full field images of cells shown in Figure 4D. Scale bar = 10 μm . *Note:* Images were bleach corrected using the histogram matching method in ImageJ.

Supplementary Figure S10



Supplementary Figure S10: Full field images of cells shown in Figure 5B. Scale bar = 10 μm . *Note:* Green channel Images were bleach corrected using the histogram matching method in ImageJ.

Additional File Contents:

opto-FBAR.avi: COS7 cells expressing the final opto-FBAR construct. Left frame: tdTom-SspBmicro-FBP17. Right frame: iLID-EGFP-CAAX. Time (min:sec).

opto-IBAR Bleach Corrected.avi: U2OS cells expressing the final opto-IBAR construct. Time (min:sec). *Note:* images were bleach corrected using the histogram matching method in ImageJ.

opto-IBAR uncorrected.avi: Cells shown in *opto-IBAR Bleach Corrected.avi* without bleach correction. Time (min:sec).

TubuleTracer1.3.txt: ImageJ macro designed for quantification of membrane tubulation.