Supplemental Material

Condon et al., https://doi.org/10.1083/jcb.201804137

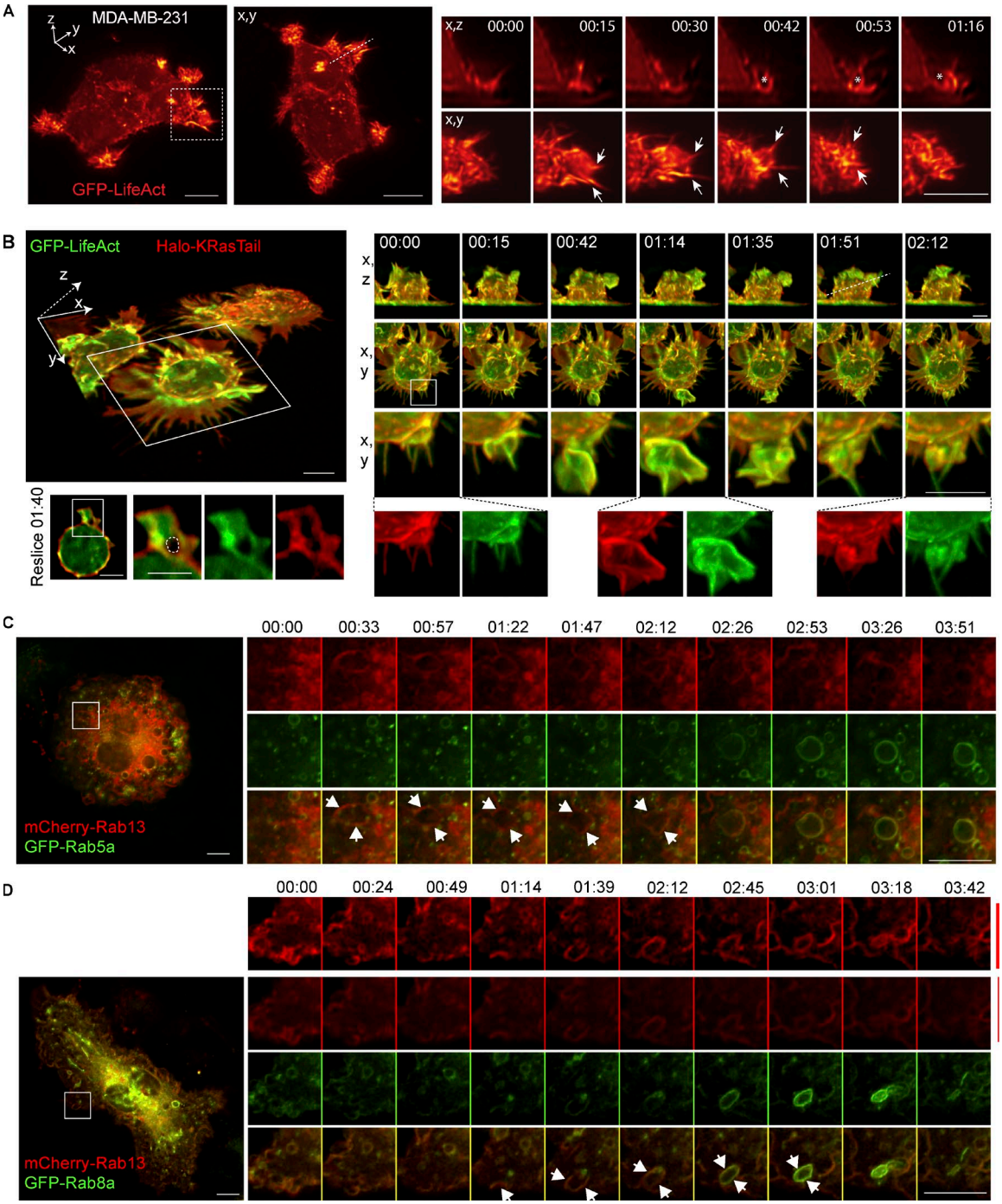


Figure S1. **Imaging tent pole ruffles and Rab13. (A)** LLSM of GFP-LifeAct in MDA-MB-231 adenocarcinoma cells. GFP-LifeAct fluorescence is visualized using the Orange-Hot LUT from the Amira data visualization software. Arrows indicate tent poles. **(B)** LLSM imaging of RAW 264.7 cells stably expressing GFP-LifeAct and Halo-KRas-Tail in the presence of LPS. Inset panels show whole cell examples from Video 7 in x,z and x,y orientations. Key frames show separate channels and resliced through ruffle at 1:51. **(C and D)** RAW 264.7 macrophages transiently expressing mCherry-Rab13 with GFP-Rab5c (C) or GFP-Rab8a (D) stimulated with LPS. Cells were imaged using a Nikon deconvolution microscope over a time-course and inset frames are displayed every 33 s as single slices. Arrows indicate the presence of Rab13 on the ruffles giving rise to macropinosomes. For mCherry-Rab13 in D, two levels are represented for clarity of Rab13 on ruffles. Time stamps, min:s. Bars, 10 µm.

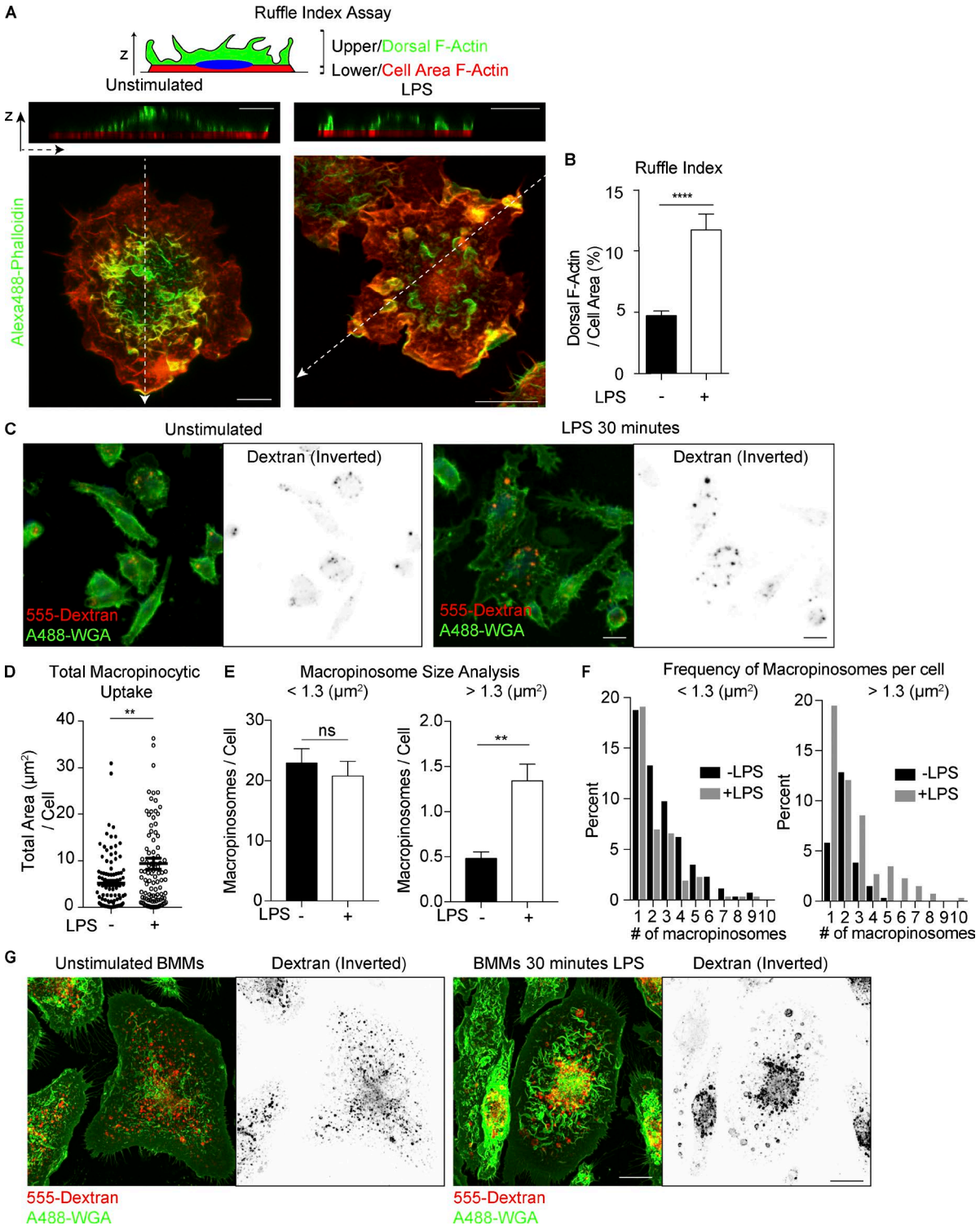


Figure S2. **LPS-induced ruffling and macropinocytosis. (A)** Ruffle index assay. Macrophages incubated ± LPS for 30 min were fixed and probed with Alexa Fluor 488–phalloidin and full z stacks acquired. **(B)** Upper and lower F-actin staining was thresholded to produced area measurements to generate the Ruffle Index (RI), where RI = Dorsal F-Actin/Cell Area (%). Images were acquired using Personal DeltaVision microscope; *n* = 30 individual cells. **(C)** Macropinocytosis assay. RAW 264.7 macrophages pretreated ± LPS for 15 min before a 15-min incubation in the presence of Alexa Fluor 555–Dextran (100 µg/ml). Cell membranes were stained with Alexa Fluor 488–WGA post-fixation. **(D)** Macropinocytosis was quantified as total area of Alexa Fluor 555–Dextran/cell. *n* = 150 cells/group. **(E)** Size distribution analysis in cells before and after LPS treatment. Macropinosomes are classed as small (<1.3 µm²) or large (>1.3 µm²). Data presented as mean ± SEM. *n* = 150 macropinosomes analyzed. **(F)** Frequency distribution analysis of cells that contain small and large macropinosomes. **(G)** A total of G, Dextran uptake assay in BMMs treated with or without LPS. Histograms are generated from three independent experiments under no LPS and LPS treated conditions. A total of 255 cells were analyzed in both no LPS and LPS treated conditions. Test for statistical significance was calculated using unpaired *t* tests. Bars, 10 µm. **, P < 0.01; ****, P < 0.0001; ns, not significant.

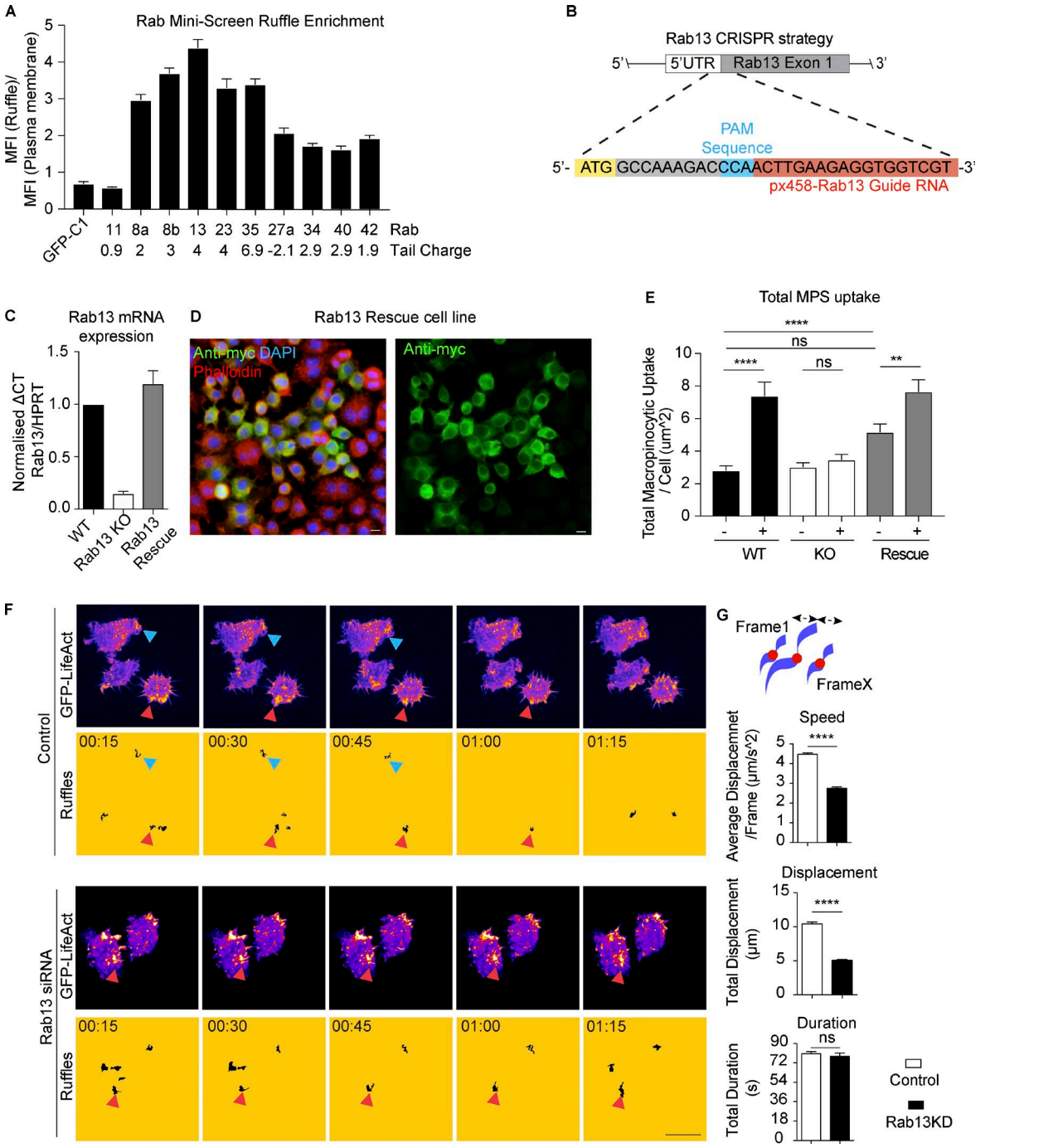
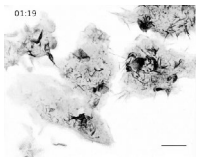
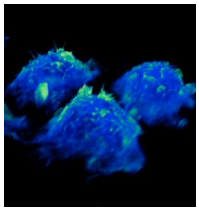


Figure S3. **Analysis of Rab13 on tent pole ruffles. (A)** Rab GTPase mini-screen for ruffle enrichment. Enrichment value was calculated for *n* = 20 cells for each Rab GTPase as described in the Materials and methods. C-terminal tail charge is also shown for each Rab. Rab13 has the highest enrichment, but not the highest polybasic tail charge. **(B)** Rab13 CRISPR targeting strategy. Diagram of exon 1 illustrates the position of the CRISPR target site relative to the first ATG. A donor vector was generated using a neomycin selection cassette flanked by 1.5-kb 5′ and 3′ homology arms. **(C)** A stable rescue cell line was generated by reexpression of an exogenous myc-Rab13 constructs in the Rab13 KO background. Rab13 mRNA quantification by qRT-PCR in control, Rab13 KO, and Rab13 rescue cell lines. Data are normalized to HPRT and represented as mean ± SEM; *n* = 3 individual experiments. **(D)** Detection of myc-Rab13 in the Rab13 rescue cell line. Cells were counterstained with phalloidin and DAPI. **(E)** Total uptake by macropinocytosis in Control, CRISPR Rab13 KO, and Rab13 rescue cell lines. Total uptake is matched data from Fig. 5 (D and E). Data are displayed as mean ± SEM with *n* ≥ 400 macropinosomes per group and from three independent experiments. **(F)** RAW 264.7 cells stably expressing GFP-LifeAct were treated with control or Rab13 siRNA before analysis of ruffling by LLSM. Top panel of control and Rab13 siRNA treated cells shows MIP depicted using the Fire LUT from ImageJ. The second panel shows thresholded ruffles that were used for tracking analysis using the Imaris particle tracking software. Blue and red arrowheads indicate two independent events for both control and Rab13 siRNA treated cells. **(G)** Ruffle tracking analysis. Schematic diagram of ruffle and center of mass (red circle) that is tracked for each ruffle from frame 1 to frame X to generate the ruffling metrics. Ruffles (prethresholded) were detected using the Imaris object tracking, and three measurements were recorded: the displacement (distance from first detected frame – last detected frame), distance (track length), and duration (number of frames/time ruffle was detected). Quantified data are displayed as mean ± SEM from *n* = 250 ruffle events per group. Bars, 10 µm. **, P < 0.01; ****, P < 0.0001; ns, not significant. Time stamps, min:s.

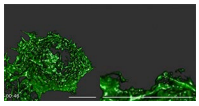
Video 1. **LLSM of macrophage ruffling.** RAW 264.7 macrophages stably expressing GFP-LifeAct stimulated with LPS. Video of Fig. 1 A visualized in 3D (tilted) to visualize top and side of the cell. GFP-LifeAct fluorescence is visualized using the Orange-Hot LUT using Amira visualization software. Frames were collected every 1.38 s and are displayed at 25 frames per second (fps). Bar, 10 µm. Time stamp, min:s.



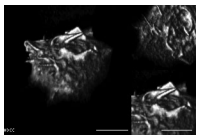
Video 2. **LLSM of macrophage ruffling.** RAW 264.7 macrophages stably expressing GFP-LifeAct stimulated with LPS. Video of Fig. 1 B visualized as an MIP. GFP-LifeAct fluorescence is visualized using an inverted Gray LUT using ImageJ software. Frames were collected every 2.84 s and are displayed at 25 fps. Bar, 10 µm. Time stamp, min:s.



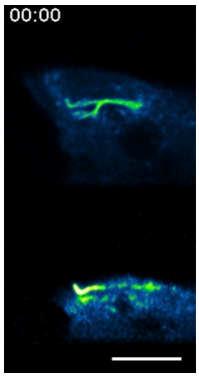
Video 3. **LLSM of tent pole ruffles.** RAW 264.7 macrophages stably expressing GFP-LifeAct stimulated with LPS. Video of Fig. 2 A visualized in 3D (tilted) to visualize top and side of the cell. GFP-LifeAct fluorescence is visualized using the Blue-Green LUT using Amira visualization software. Frames were collected every 3.10 s and are displayed as 20 fps. Bars, 10 µm. Time stamp, min:s.



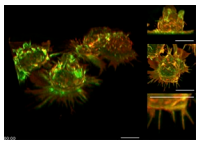
Video 4. **LLSM of tent pole ruffles.** RAW 264.7 macrophages stably expressing GFP-LifeAct stimulated with LPS. Video of Fig. 2 B visualized in 3D (tilted) to visualize top and side of the cell. GFP-LifeAct fluorescence is visualized by 3D volume rendering (green) using Amira visualization software. Frames were collected every 4.59 s and are displayed as 20 fps. Bars, 10 µm. Time stamp, min:s.



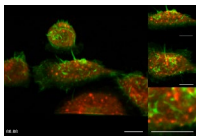
Video 5. **LLSM of tent pole ruffles.** RAW 264.7 macrophages stably expressing GFP-LifeAct stimulated with LPS. Video of Fig. 2 C visualized in 3D (side and top) to visualize tent pole ruffle formation and collapse. GFP-LifeAct fluorescence visualized by 3D vole rendering (gray) using Arivis Vision 4D visualization software. Left Panel shows side view, upper right shows inset from top, lower right shows inset from side. Frames were collected every 1.32 s and are displayed as 20 fps. Bars, 10 µm. Time stamp, min:s.



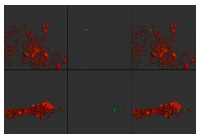
Video 6. **LLSM of tent pole–associated ruffle conversion to a macropinosome.** RAW 264.7 macrophages stably expressing GFP-LifeAct stimulated with LPS (100 ng/ml). GFP-LifeAct is displayed using the Cyan Hot LUT from ImageJ. Videos are from inset panels in Fig. 3 A visualized from the top (MIP) and a z slice through the ruffle to macropinosome conversion. Frames were collected every 2.3 s and are displayed as 20 fps. Time stamp, min:s. Bar, 5 µm.



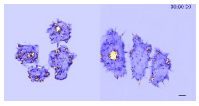
Video 7. **Dual color LLSM of tent pole ruffle formation (Halo-KRasTail/GFP-LifeAct).** RAW 264.7 macrophages stably expressing GFP-LifeAct (green) and Halo-KRasTail (red, labeled with 10 nm $_{JF}$549) stimulated with LPS. Video of Fig. S1 B visualized in 3D (side and top) to visualize tent pole ruffle formation and collapse. Left panel shows overview of acquisition FOV, right upper panel shows side view, right middle panel shows top view, and right lower panel shows top inset. Frames were collected every 5.40 s and are displayed as 10 fps. Time stamp, min:s. Bars, 10 µm.



Video 8. **Dual color LLSM of tent pole ruffle formation (Halo-Rab5c/GFP-LifeAct).** RAW 264.7 macrophages stably expressing GFP-LifeAct (green) and Halo-Rab5c (red, labeled with 10 nm $_{JF}$549) stimulated with LPS. Video of Fig. 3 B visualized in 3D (side and top) to visualize tent pole ruffle formation and collapse into macropinosomes. Left panel shows overview of acquisition FOV, right upper panel shows side view, right middle panel shows top view, and right lower panel shows top inset. Frames were collected every 5.40 s and are displayed as 10 fps. Time stamp, min:s. Bars, 10 µm.



Video 9. **Dual color rendering of LLSM of tent pole ruffle formation (Halo-KRasTail/GFP-2×FYVE).** RAW 264.7 macrophages transiently expressing GFP-2×FYVE (green) and Halo-KRasTail (red, labeled with 10 nm $_{JF}$549) stimulated with LPS. Video is from inset panel in Fig. 3 C. Channels were rendered using Imaris and visualized from the top (x,y; upper panels) and side (y,z; lower panels). Frames were collected every 24 s and displayed at 4 fps. Time stamp, min:s.



Video 10. **LLSM of Rab13 CRISPR KO macrophages.** CRISPR control and Rab13 KO RAW 264.7 macrophages stably expressing GFP-LifeAct stimulated with LPS were imaged using LLSM. Video of Fig. 5 F visualized as MIP. GFP-LifeAct is visualized using ICA LUT in ImageJ. Left panel shows control; right panel shows Rab13 CRISPR KO. Frames were collected every 3 s and are displayed as 25 fps. Bars, 10 µm. Time stamp, h:min:s.