Supplemental Materials

Molecular Biology of the Cell

Faust et al.

Supplemental Materials

An actin-based protrusion originating from a podosome-enriched region initiates macrophage fusion

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Video 1: A phase-dense protrusion initiates macrophage fusion. Short phase-dense protrusions at the leading edge initiate fusion.

Video 2: A long phase-dense protrusion initiates macrophage fusion. Occasionally (~10 %) long protrusions initiate fusion.

Video 3: Fusion between the leading edge and cell body.

Video 4: Fusion between the leading edge and cell rear.

Video 5: Fusion between leading edges.

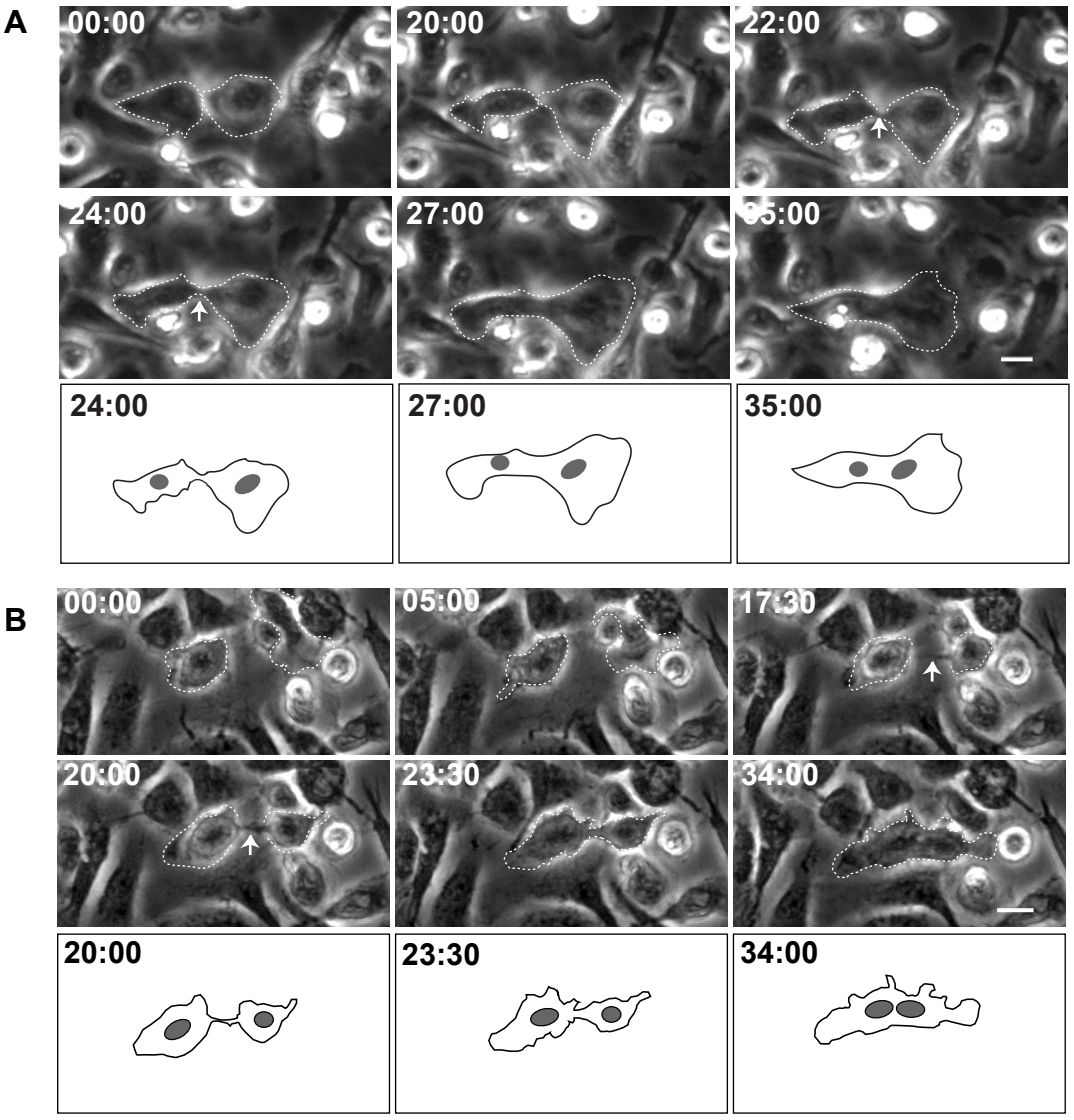
Video 6: Lattice light sheet microscopy of fusing macrophages. High spatiotemporal view of a MGC undergoing multiple fusion events is shown. A wave of eGFP-LifeAct puncta (white) emanate from the interior of the MGC and enrich at the cell periphery before fusion.

Video 7: Maximum intensity isosurface render of eGFP-LifeAct. Surface render show actin-based protrusions during the fusion process.

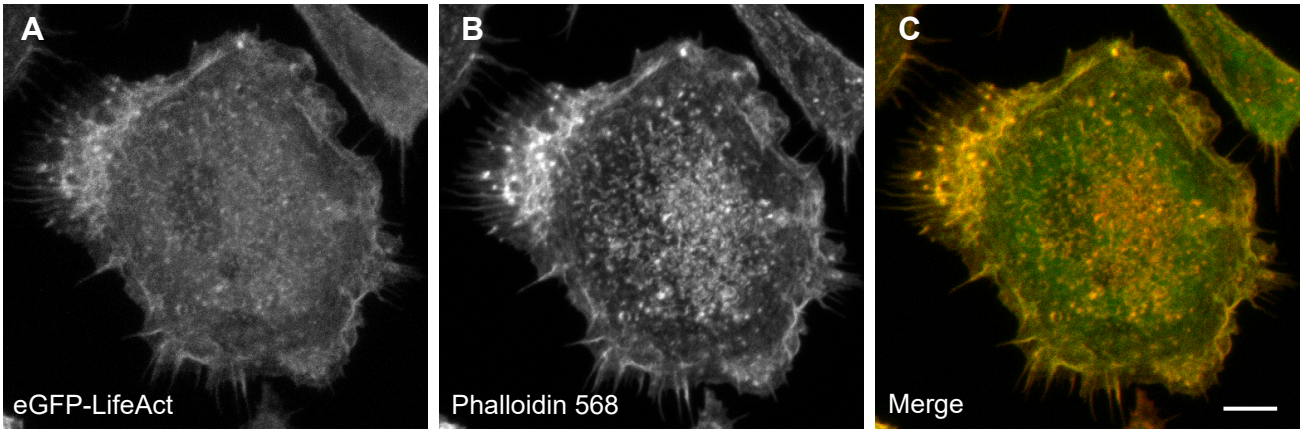
Video 8: Maximum intensity isosurface render of eGFP-LifeAct. When there is adequate space between fusion macrophages fusion-competent protrusions can be observed. The protrusion appear to become stabilized and subsequently expand as fusion progresses.

Video 9: Lattice light sheet microscopy of mixed populations of eGFP- and mRFP-LifeAct. Integration of LifeAct signal appears to be asymmetric.

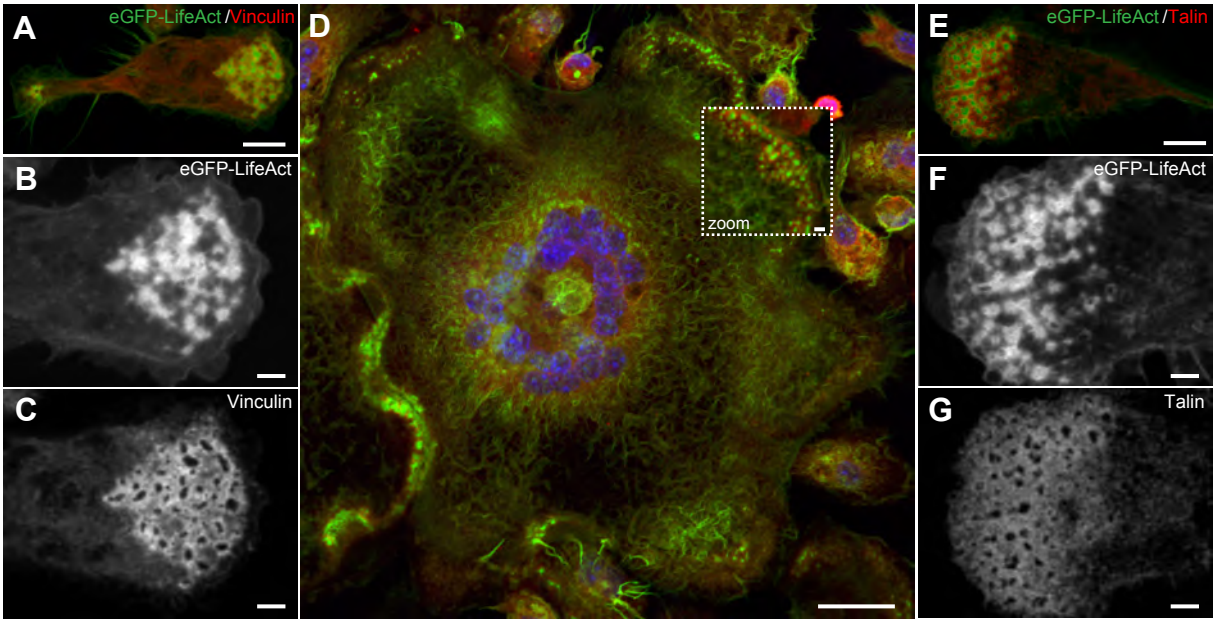
Video 10: Lattice light sheet microscopy of mixed populations of eGFP- and mRFP-LifeAct. eGFP and mRFP channels are separated to observe integration of eGFP- and mRFP-LifeAct.



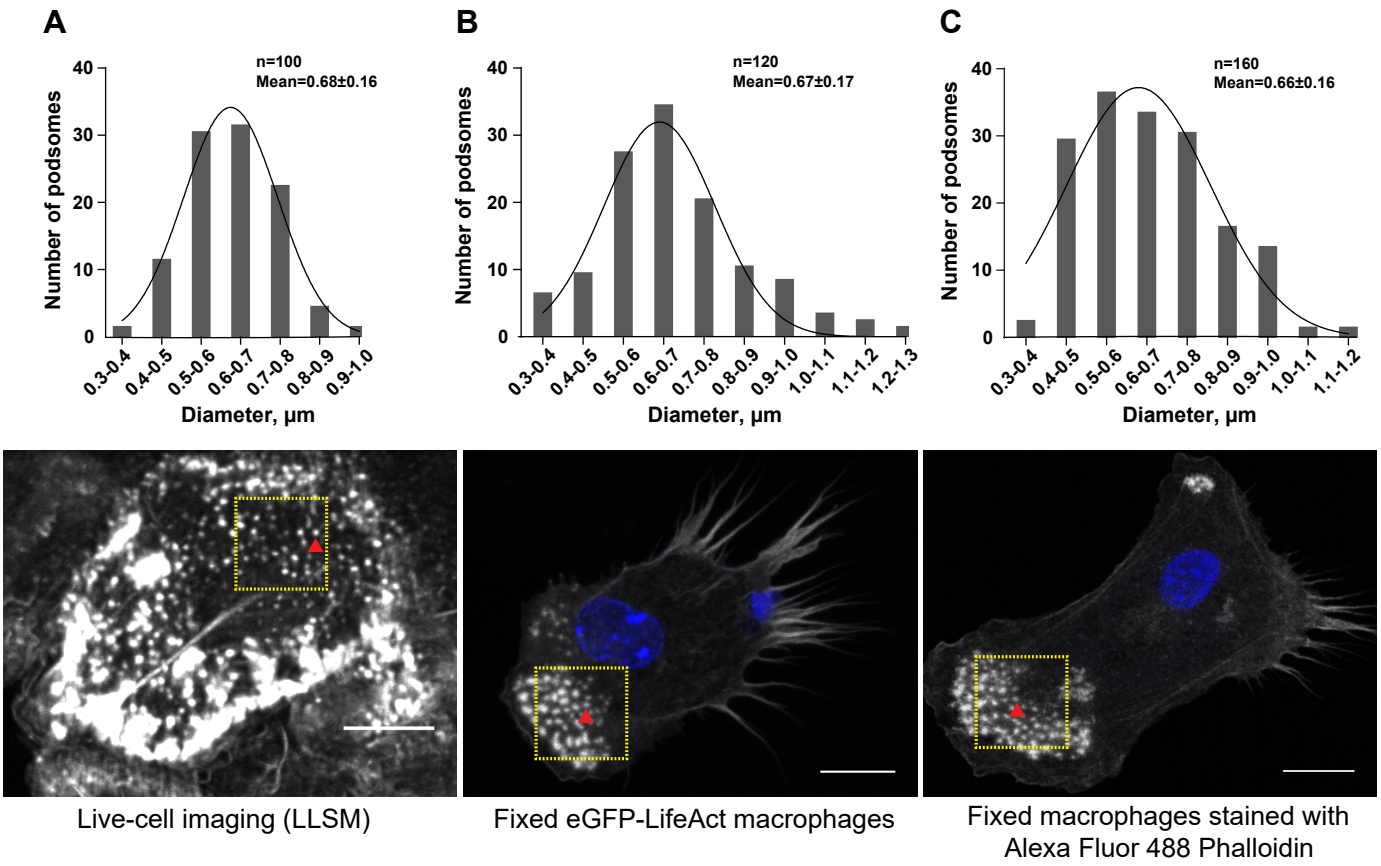
Supplemental Figure 1. Phase-dense protrusions initiating Type 1 fusion. Live imaging of mononuclear macrophages undergoing fusion. Macrophages were isolated from the mouse peritoneum 3 days after TG injection, plated on a 35-mm Fluorodish and fusion was induced by IL-4. (A) A mononuclear macrophage extends a short phase-dense protrusion (white arrow) toward another macrophage before fusion. The lower panel is a diagram of frames at 24:00, 27:00 and 35:00 min illustrating morphological aspects of the fusion process. (B) Macrophage fusion mediated by a long protrusion (white arrows at 17:30-20:00 min). The lower panels show diagrams of frames at 20:00, 23:30 and 34:00 min. In each micrograph, time is shown in minutes:seconds. The scale bars are 10 μm.



Supplemental Figure 2: eGFP-LifeAct faithfully reports the distribution of F-actin in TG-elicited macrophages. (A) The distribution of eGFP-LifeAct in a fixed and permeabilized macrophage 24 h after plating. (B) Alexa 568-conjugated phalloidin labeled structures. (C) The panel is an overlay of eGFP-LifeAct (green) and Alexa 568-Phalloidin (red). The majority of phalloidin-labeled structures appear to contain eGFP-LifeAct in fixed specimens. The scale bar is 10 μm.



Supplemental Figure 3. eGFP-LifeAct puncta in macrophages are podosomes. (A) eGFP-LifeAct macrophage 48 h after the application of IL-4. Punctate eGFP-LifeAct structures (green) at the leading edge of a mononuclear macrophage contain vinculin (red). The scale bar is 7.5 μm. (B) High magnification view of eGFP-LifeAct puncta shows the typical interconnected network with a core of eGFP-LifeAct and fine fibrils radiating from a central point. The scale bar is 2.5 μm. (C) High magnification view of vinculin enriched around a core devoid of vinculin signal. The scale bar is 2.5 μm. (D) Low magnification view of a MGC shows similar podosomes at the cell periphery. Nuclei are stained with DAPI (blue). The scale bar is 25 μm. The scale bar for the zoomed box region is 2 μm.(E) Punctuate eGFP-LifeAct structures (green) at the leading edge of a mononuclear macrophage contain talin (red).The scale bar is 7.5 μm. (F) High magnification view of eGFP-LifeAct puncta. The scale bar is 2.5 μm. (G) High magnification view of talin enriched around a core devoid of talin signal. The scale bar is 2.5 μm.



Supplemental Figure 4. Distribution of the podosome size in samples of live and fixed macrophages. Gaussian distribution of the podosome size obtained from live cell imaging using LLSM (A), from images of fixed eGFP-LifeAct-expressing macrophages (B) and samples of fixed cells stained with Alexa Fluor 488-conjugated phalloidin (C). Only individual podosomes (red arrowheads) situated at some distance from the front of actin in the LLSM movies and away from the cell periphery in fixed samples were included in analyses. The scale bar is 10 µm. Nuclei were stained with DAPI (blue).

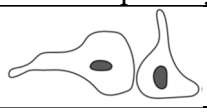
Supplemental Table 1

Characteristics of macrophages undergoing fusion on Permanox

Number of analyzed protrusions	Type 1 fusion	Type 2 fusion	Type 3 fusion
Short	6 (75%)	29 (97%)	14 (100%)
Long	2 (25%)	1 (3%)	0 (0%)
Length of protrusions, μm	Type 1 fusion	Type 2 fusion	Type 3 fusion
Short	2.7 ± 1.4	2.3 ± 0.6	2.4 ± 0.9
Long	13 ± 3.8	12.3 ± N/A	N/A
Total fusion time from the first cell-cell contact to full integration, min	Type 1 fusion	Type 2 fusion	Type 3 fusion
Short	43 ± 18	67 ± 21	N/A
Long	36 ± 23	47.5 ± N/A	N/A

Supplemental Table 2

Patterns of macrophage fusion on Permanox

Pattern mediated by short protrusions	Schematic showing cell polarity	Type 1 fusion	Type 2 fusion
Leading edge to cell body		12.5%	33%
Leading edge to rear edge		50%	23%
Leading edge to leading edge		37.5%	37%
Other		0%	7%