Carroll-Portillo et al., http://www.jcb.org/cgi/content/full/jcb.201412074/DC1

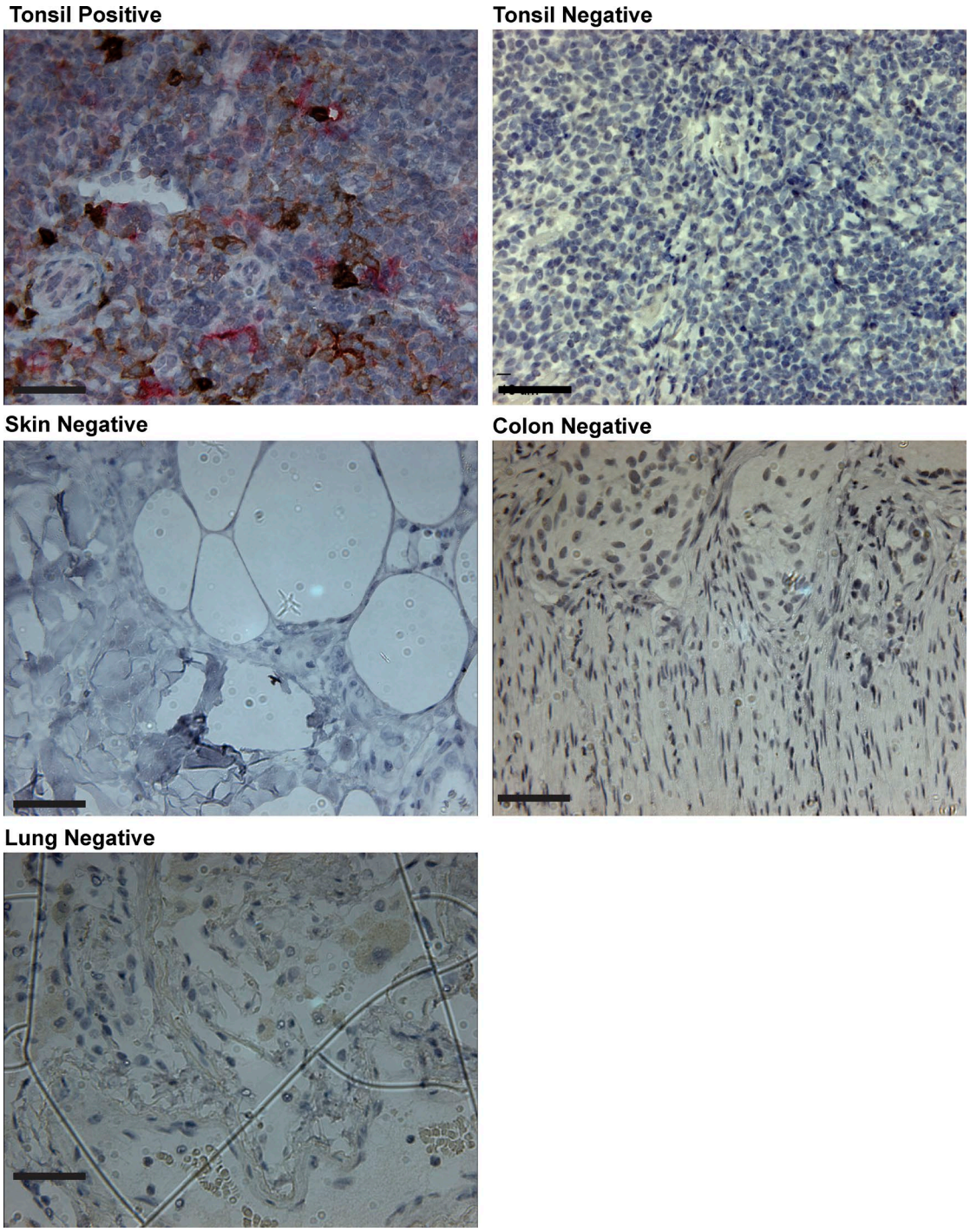


Figure S1. **IHC of tonsil controls (positive and negative) and skin, colon, and lung negative controls.** Controls were performed with each set of immunohistochemical experiments represented in Fig. 1 A. Images are brightness and contrast enhanced. Bars, 50 µm.

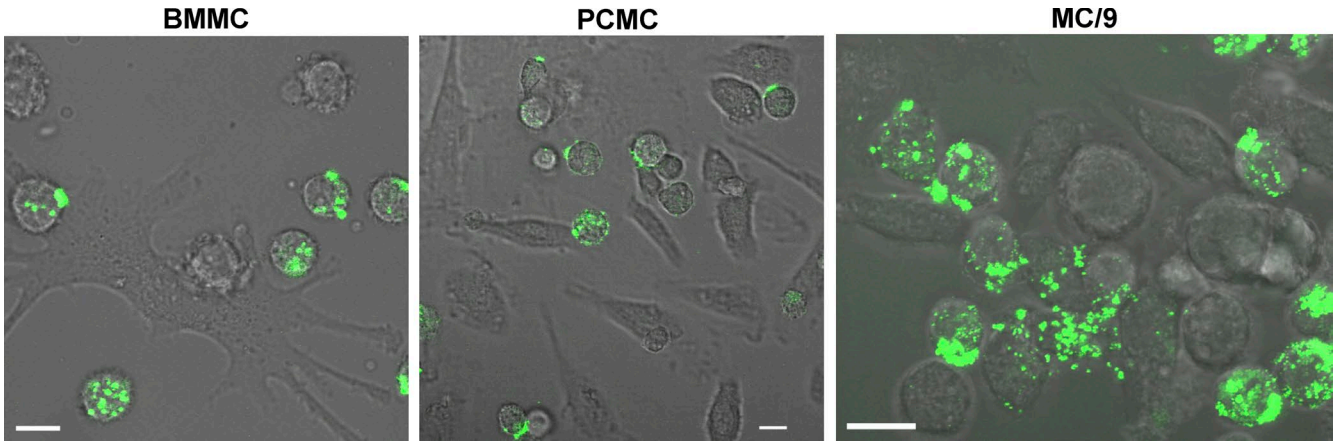


Figure S2. **Single images from live-cell imaging of different types of actMC–DC interactions.** BMMC, PCMC, and MC/9 cells all demonstrate polarized accumulation of internalized AF488-IgE (green) after stimulation with DNP-BSA and in the presence of imDCs. Images are brightness and contrast enhanced. Bars, 10 µm.

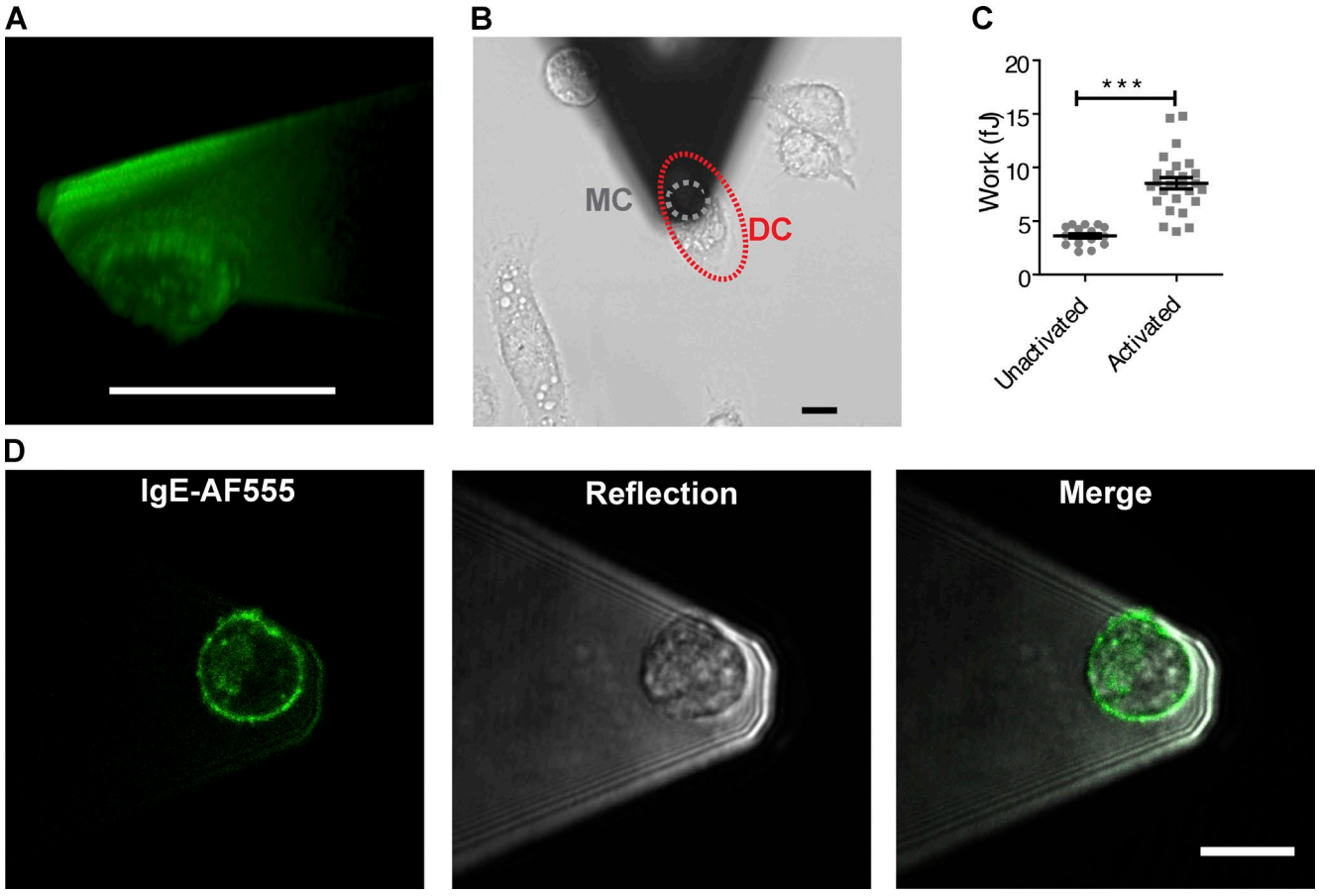


Figure S3. **AFM MC–imDC interactions.** (A) 3D reconstruction of a z stack by confocal scanning light microscopy of an MC labeled with AF555 IgE on the cantilever (image is pseudocolored). Close to the cantilever, reflected light gives a ghost image of the apex. (B) Bright-field image of an MC on the cantilever interacting with a DC during an adhesion measurement. Outlines of cells are indicated with dotted lines. (C) The work in femtojoule needed to detach unactivated or activated MCs from DCs (unactivated: $n = 5$ experiments; $n = 21$ MC–imDC contacts; $n = 12$ F–D curves, activated; $n = 4$ experiments; $n = 24$ MC–imDC contacts; $n = 140$ F–D curves). ***, $P < 0.001$ (by Mann–Whitney test). Error bars are SEM. (D) Single section of the z stack in the center of an MC labeled with AF555 IgE and reflection of the laser to image the cantilever are shown. Image is pseudocolored. Bars, 10 µm.

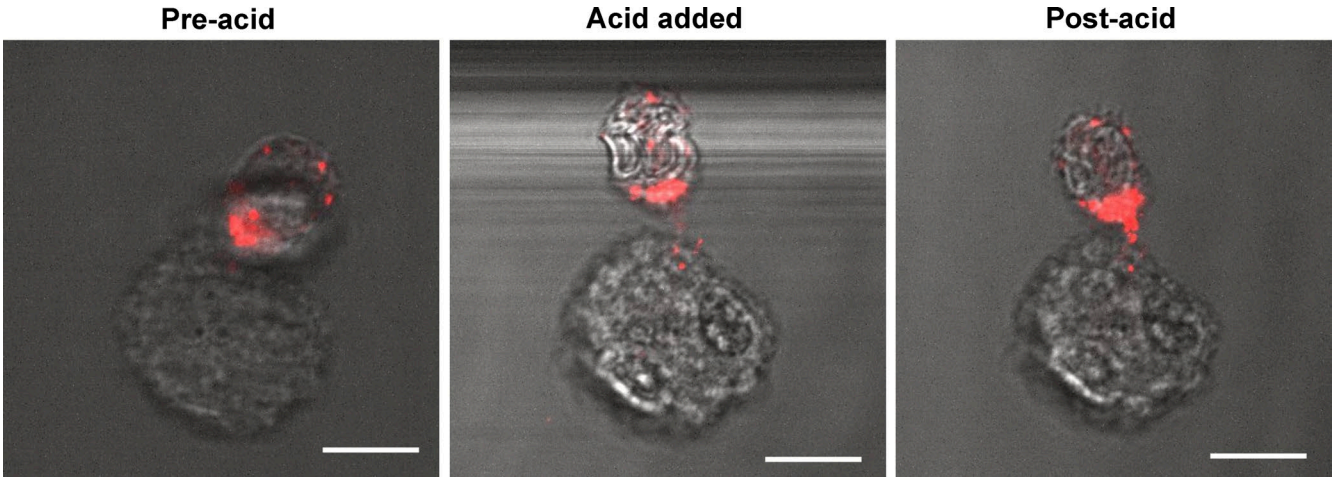


Figure S4. **Images from live-cell imaging of acid strip on an actMC–DC conjugate.** IgE–FcεRI (red) is polarized at the cell junction and remains present after exposure to low pH buffer (third image), confirming that the antigen cross-linked IgE–FcεRI complexes are internalized. Images are brightness and contrast enhanced. Bars, 10 µm.

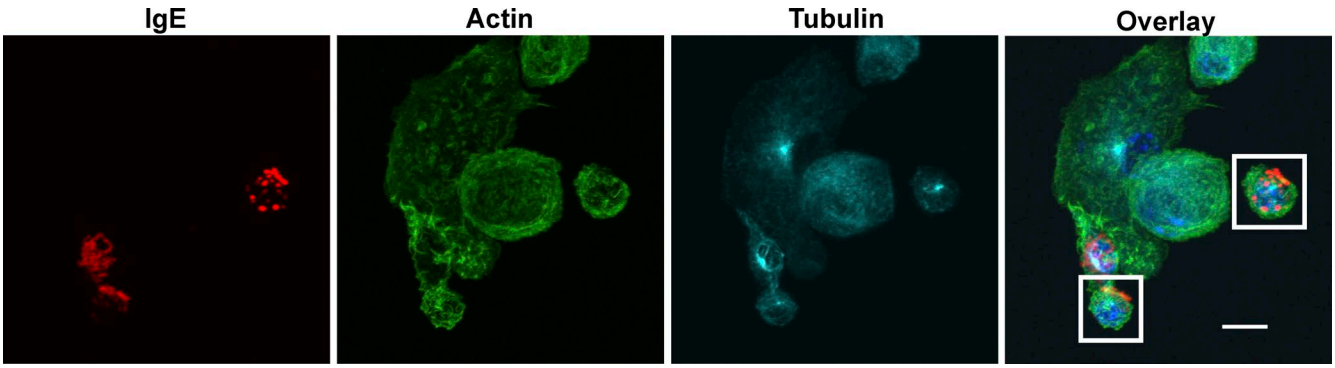
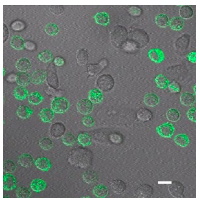
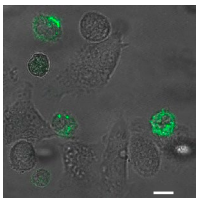


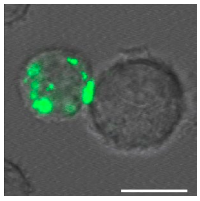
Figure S5. **Examples of MC polarization.** MC polarization is maintained even when DC contact is lost (boxed MCs in overlay). Images are from a maximum projection of a fixed actMC–DC sample. The MTOC (tubulin, cyan) is located internal to the polarized IgE (red) vesicles and region where F-actin (green) is reduced at the membrane. Nuclei (Hoechst, blue) labeling is included in the overlay. Images are brightness and contrast enhanced. Bar, 10 µm.



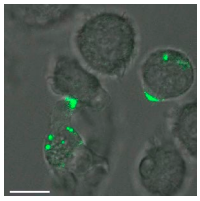
Video 1. **Interactions between unactivated MCs and imDCs.** Unactivated MCs do not demonstrate polarization in the presence of imDCs (Fig. 3 A, Unactivated). Live-cell, time-lapse confocal imaging (LSM510-META; Carl Zeiss) at 37°C of BMMCs primed with AF488 IgE (green) in co-culture with imDCs. Image acquisition was at one frame every 4 s for a total time of 1 min and 48 s; playback is four frames per second. Bar, 10 µm.



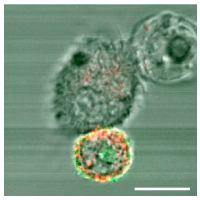
Video 2. **Interactions between actMCs and imDCs.** Example of long-lived interactions between actMCs and imDC early after activation (imaging initiated within 5 min). BMMCs were primed with AF488-IgE (green) and then activated through cross-linking of FceRI with DNP-BSA. Image acquisition was one frame every 10 s for a total time of 8 min and 20 s. Playback is five frames per second. Bar, 10 µm. Images acquired with live-cell, time-lapse confocal imaging (LSM510-META; Carl Zeiss) at 37°C.



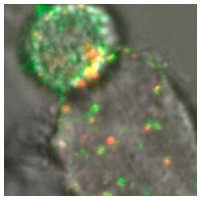
Video 3. **Polarization of actMCs.** Activated MCs form long-lived interactions and polarize when incubated with imDCs (Fig. 3 A, Activated). Example of long-lived interaction (synapse) between an actBMMC and DC. The BMMC was primed with AF488-IgE (green) and then activated by cross-linking of FcεRI with DNP-BSA. Image acquisition was one frame every 4 s for a total time of 3 min and 16 s. Playback is five frames per second. Bar, 10 mm. Images acquired with live-cell, time-lapse confocal imaging (LSM510-META; Carl Zeiss) at 37°C.



Video 4. **Activated MC/9s with imDCs.** Activated MC/9s become polarized and form uropod-like structures when in the presence of imDCs. Live-cell imaging with time-lapse confocal imaging (LSM510-META; Carl Zeiss) at 37°C of AF488-IgE (green) primed MC/9 cells contacting an imDC after MC/9 activation via cross-linking with DNP-BSA. The uropod structure that forms at the cellular junction is unique to the MC/9 cell line. Images acquired at one frame every 10 s for a total time of 16 min and 30 s; playback is 15 frames per second. Bar, 10 µm.



Video 5. **GPI-GFP during BMMC material transfer.** GPI-GFP is not transferred from actMCs to imDCs (Fig. 3 B, top). Live imaging of an actMC (BMMC) expressing GPI-GFP (green) in contact with an imDC. Transfer of internalized AF555-FcεRI-IgE (red) occurs in a rapid manner at the site of actMC-DC contact. The transferred material does not contain GPI-GFP. Image acquisition at one frame every 9 s for a total time of 13 min and 33 s. Playback, five frames per second. Images acquired with live-cell, time-lapse confocal imaging (LSM510-META; Carl Zeiss) at 37°C. Bar, 10 µm.



Video 6. **CD9-GFP during BMMC material transfer.** An exosome marker (CD9) is transferred with IgE from actMCs to imDCs (Fig. 3 B, bottom). Live-cell, time-lapse confocal imaging (LSM510-META; Carl Zeiss) of an actMC (BMMC) expressing CD9-GFP (green) in contact with an imDC. Transfer of AF555-IgE (red) from the actMC to the imDC is seen to colocalize with CD9-GFP (yellow). Images acquired at one frame every 16 s with a total acquisition time of 2 min and 23 s. Playback, two frames per second. Images acquired at 37°C. Bar, 5 µm.