

Supplemental Materials

Molecular Biology of the Cell

Hui et al.

Supplementary Figure S1: **Effect of inhibitors on actin structure and dynamics.** a) Fluorescence intensity (per pixel) in Jurkat cells spreading on anti-CD3 coated glass coverslips and fixed at 15 min. after activation and labeled with rhodamine-phalloidin. The before case shows the intensity/pixel at 10 min. Drugs were added at 10 min after activation. (b) Ratio of retrograde flow measured before and after addition of 100 μ M CK666 or 50 μ M Blebbistatin in Jurkat cells spreading on anti-CD3 coated glass coverslips (for CK-666) and on 1kPa gels (Blebb). Drugs were added 15 min. after activation. (*, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$).

Supplementary Figure S2. **Loss of F-actin dynamics reduces cellular force generation.** Comparison of the after-to-before ratios of traction stresses for application of Lat-A (N = 20 cells), CK666 (N = 17 cells) and Jasp (N = 10 cells) with control (DMSO carrier, N = 20 cells). The average stresses in a 3 min time interval just before addition of drug, and the average stresses in the indicated time intervals following addition of drugs were used to compute the ratios. (*, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$).

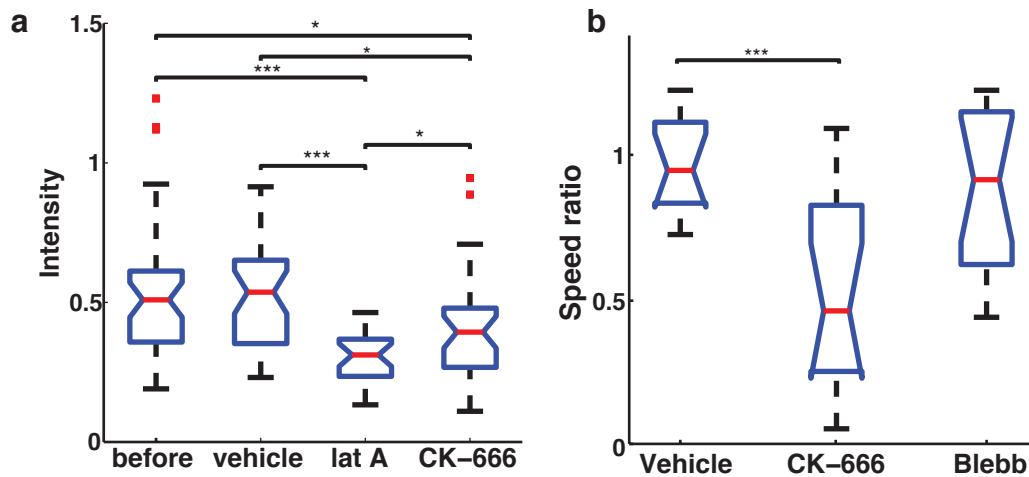
Supplementary Figure S3: **Microtubules and dynein motors do not play in role in force generation.** Comparison of the after-to-before ratios of traction stresses for application of the microtubule inhibitor, 1 μ M nocodazole (N = 12 cells), and the dynein inhibitor, 50 μ M HPI4 (N = 19 cells), with control (DMSO carrier, N = 20 cells). The average stresses in a 3 min time interval just before addition of drug, and in the time interval 3 min – 9 min after addition of drugs were used to compute the ratios. Both nocodazole and HPI-4 treatment resulted in stress ratios that were not significantly different from the control case.

Supplementary Figure S4: **Myosin activity is required for maintenance of actin rings.** (a) TIRF images of tagRFP-T-actin labeled Jurkat cell spreading on an anti-CD3 coated glass surface showing formation of a robust actin ring which persists for longer than 8 min. (b) TIRF images of a tagRFP-T-actin cell shows the formation of an actin ring which is disrupted upon application of 50 μ M blebbistatin after 5 min of spreading (arrow). All scale bars are 5 μ m.

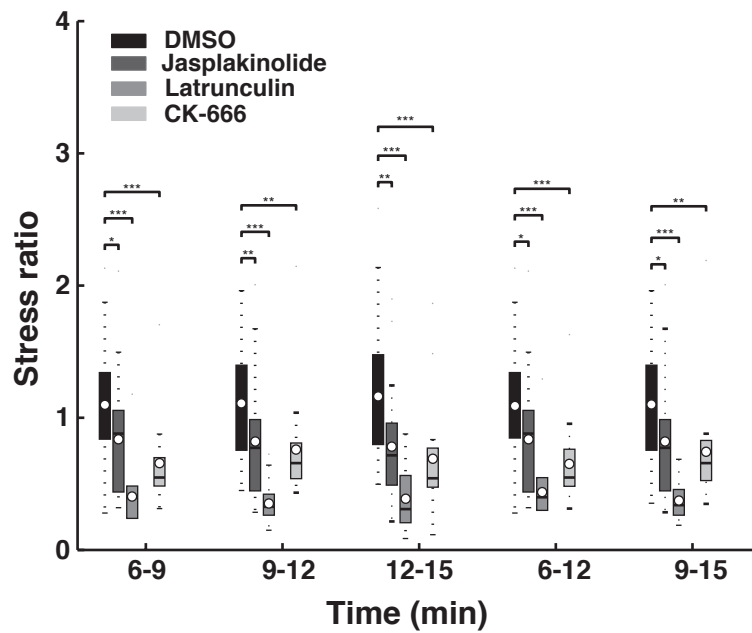
Supplementary Figure S5: **TCR activation is required for signaling.** (a) TIRF images of EGFP-actin labeled Jurkat cell spreading on a poly-L-lysine coated glass surface with phosphotyrosine staining (top) and actin fluorescence image (bottom). (b-d) TIRF images of EGFP actin cells

spreading on anti-CD3 coated substrates of indicated stiffness. Gel compositions were (3:0.1, 5:0.1 and 10:0.1 of acrylamide:BIS). All scale bars are 10 μm .

Supplementary Figure S6. **Particle tracking velocimetry can detect bead displacements on stiff gels.** A. Jurkat cell expressing EGFP-actin spreading on a stiff (6kPa) gel. B. Image of 200 nm fluorescent bead. C. Zoomed in view of ROI shown in A,B with bead tracks superimposed on binary image of cell. Tracks for beads under the cell are shown in red while external beads are shown in white. D. Cumulative histogram of multiple beads tracked over 15 min. of cell spreading.

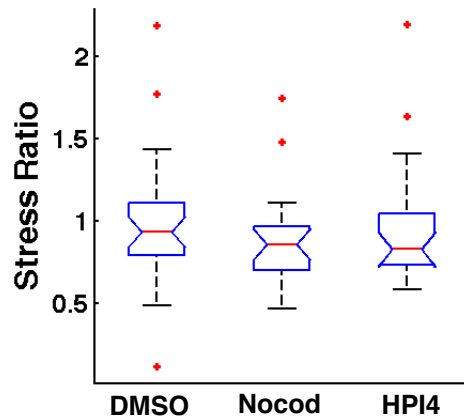


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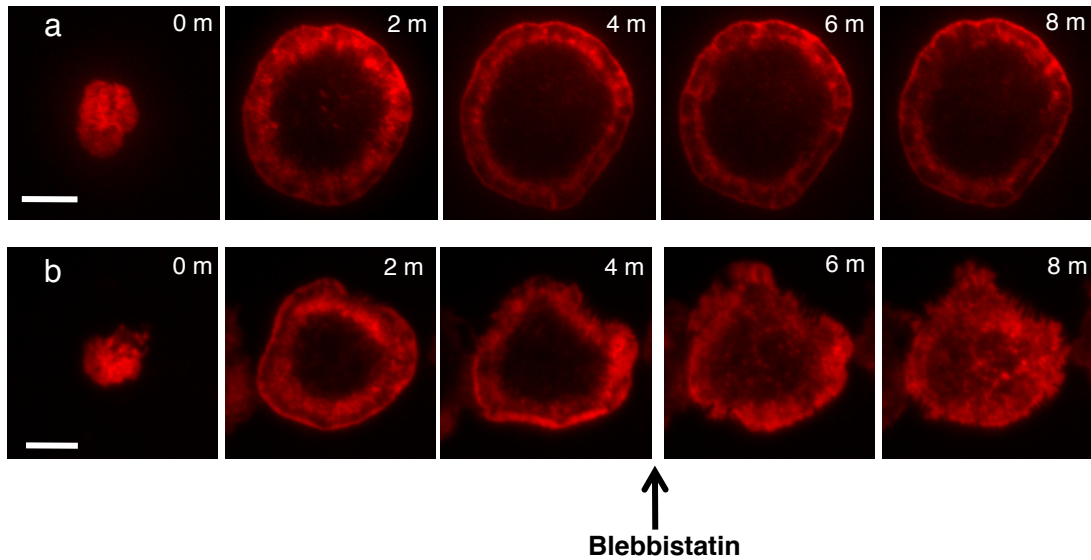


Supplementary Figure S2. Loss of F-actin dynamics reduces cellular force generation.

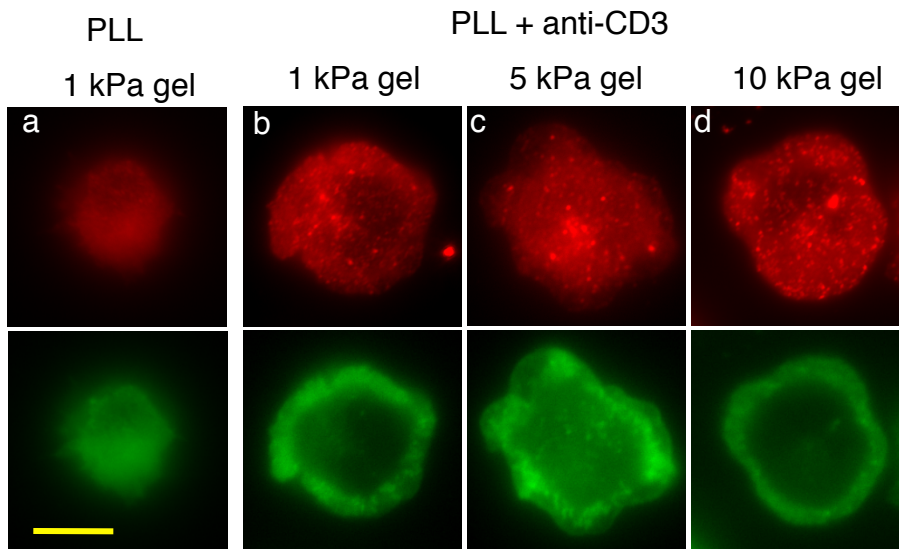
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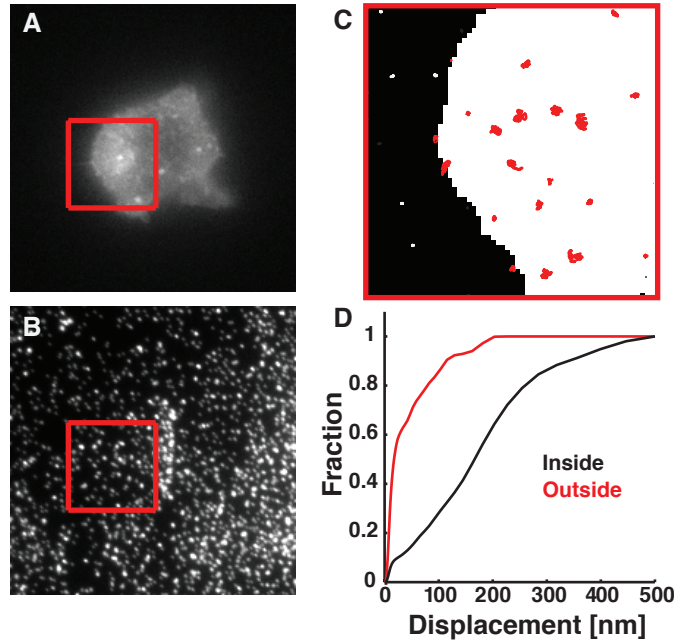
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