Supplemental Materials

Molecular Biology of the Cell

Murrell et al.

Movie Captions

Movie 1: Contraction of a Disordered F-actin Network.
1.32 μM F-actin (red) is crowded to the surface of a 91% EPC / 8.6%NTA / 0.4% DHPE membrane (R_{xlink}=0, R_{adh}=0). Skeletal muscle myosin II thick filaments (green) assemble and contract the network. Scale bar is 10 μm. Time is in min:sec.

Movie 2: Quantification of F-actin Velocity during Network Contraction.
1.32 μM F-actin is crowded to the surface of a 91% EPC /8.6% NTA/0.4% DHPE membrane and contracted by skeletal muscle myosin II (not seen) (R_{xlink}=0, R_{adh}=0). Scale bar is 10 μm. Time is in min:sec. Vectors corresponding to the velocity of the F-actin are shown as red arrows.

Movie 3: Correlated F-actin and Myosin Motions in a Highly Contractile Network
1.32 μM F-actin is crowded to the surface of a 91% EPC /8.6% NTA/ 0.4% DHPE membrane. Skeletal muscle myosin II is added and contracts the network. (left) F-actin with overlayed velocity vectors (red) (R_{xlink}=0, R_{adh}=0). Scale bar is 10 μm. Time is in min:sec. (middle) Myosin Thick filaments with overlayed velocity vectors (green). (right) Velocity vectors for both F-actin (red) and myosin (green).

Movie 4: Uncorrelated F-actin and Myosin Motions in a Weakly Contractile Network Formed by Membrane Coupling
10 μM FimA2 couples 1.32 μM F-actin to a 91% EPC / 8.6% NTA / 0.4% DHPE membrane. 30 nM α-actinin has been added to crosslink the F-actin. Skeletal muscle myosin II thick filaments assemble and move throughout the network. (left) F-actin with overlaid velocity vectors (red). Scale bar is 10 μm. Time is in min:sec. (middle) Myosin Thick filaments with overlaid velocity vectors (green). (right) Velocity vectors for both F-actin (red) and myosin (green).

Supplemental Information for:

Actomyosin Sliding is Attenuated in Contractile Biomimetic Cortices

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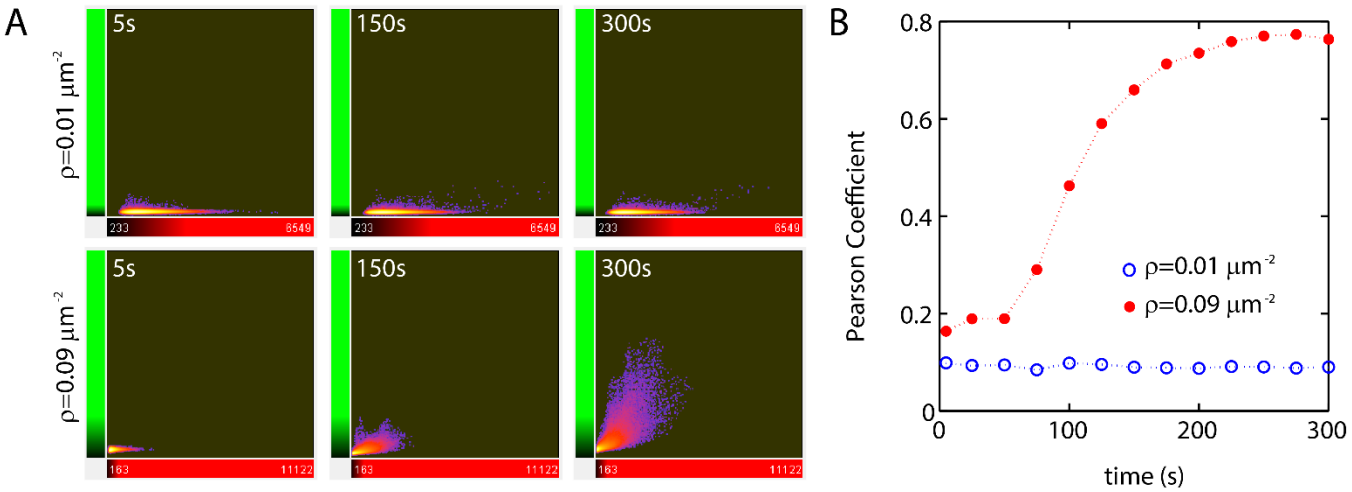
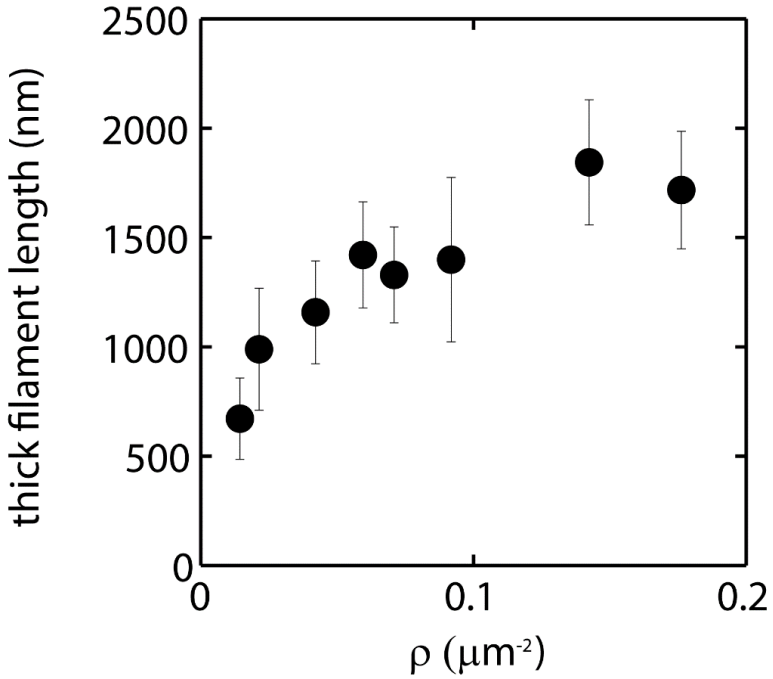


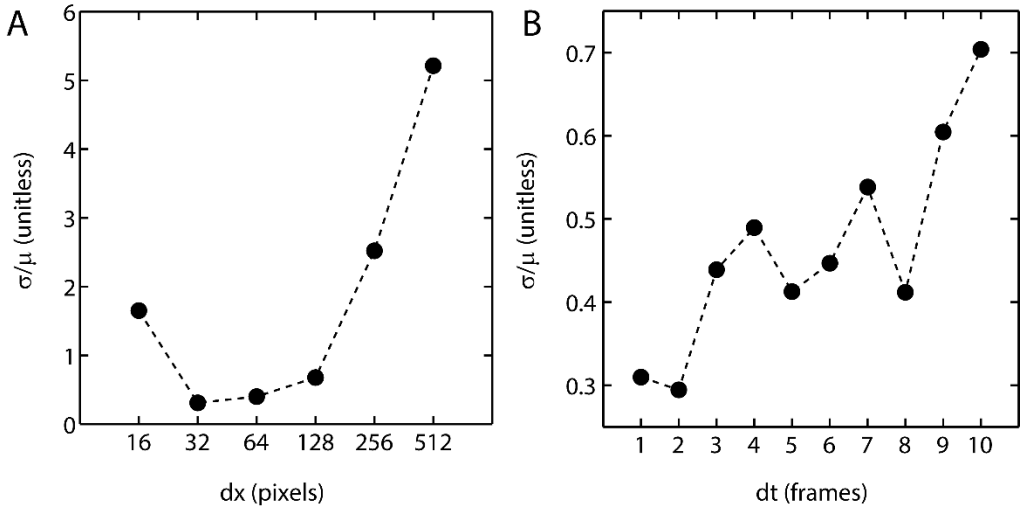
Supplemental Figure 1: **Actin and Myosin Colocalize During Contraction.**

(A) 2D histogram of actin and myosin fluorescence intensities over time for a non-contractile network (top) and a contractile network (bottom). This analysis pertains to the data shown in Fig 4 and 5. The vertical axis (green) is the fluorescence intensity of myosin at every pixel within the image. The horizontal axis is the fluorescence intensity of F-actin. When the network is not contractile, there are only small intensities for myosin, as they remain thick filaments and never aggregate, which would increase their signal. However, when the network is contractile, the fluorescence intensity of myosin increases concomitantly with that of the F-actin as both aggregate together. This is shown by the Pearson Coefficient in (B) which quantifies the total colocalization of both actin and myosin via the strength of correlation in the two fluorescence channels. 1 is a perfect colocalization, and 0 is random localization. The contractile network has a value of approximately 0.8 indicating the actin and myosin have the same structure.



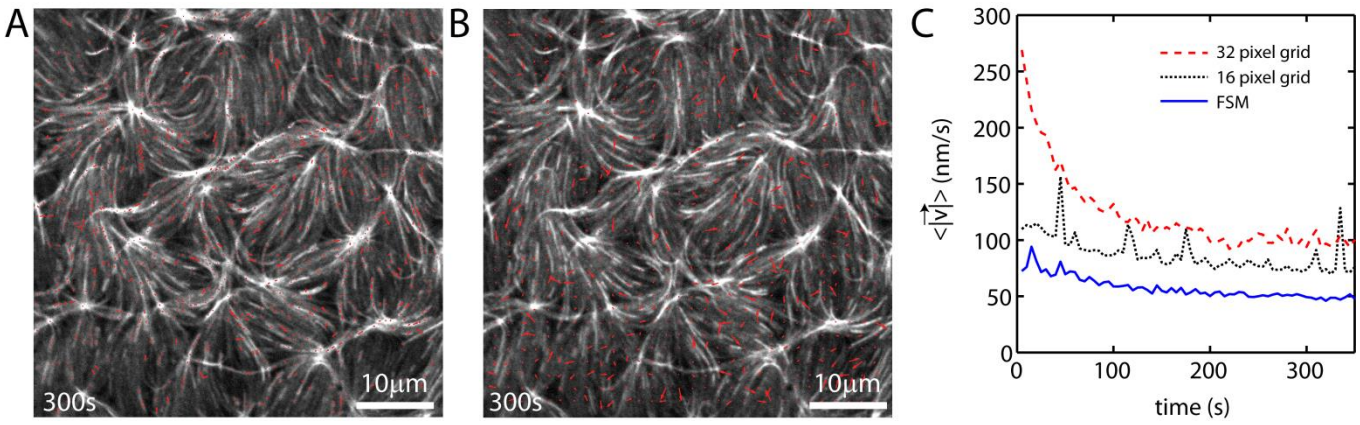
Supplemental Figure 2: **Thick Filament Size Varies with Myosin Dimer Concentration.**

Thick filament length versus thick filament density. Low myosin densities result in short thick filaments. Conversely, myosin densities above 0.1 μm^{-2}, reach a maximum thick filament length of approximately 2 μm.



Supplemental Figure 3: **Parameter Choice Minimizes Deviation in Network Divergence.**

The contractility is measured by the divergence of the vector field taken between two images, reflecting displacement of the F-actin network. The grid size used to create the vector field is 32 pixels square, and is taken between two successive images. Calculation of the standard deviation σ divided by the mean, μ of the divergence of a highly contractile network with varying either (A) the grid size, or (B) the number of frames between which the divergence is calculated. The standard deviation is minimized at 32 pixels (3 μm), and 1-2 frames.



Supplemental Figure 4: **Velocity Baseline Varies with Measurement Method.**

Velocity calculations for the displacement of stabilized F-actin during its sedimentation to the surface of a phospholipid bilayer and subsequent entanglement. After sedimentation, movement of F-actin is purely thermal. The velocity is calculated via (A) FSM and (B) FTTC. (C) Speed of F-actin during sedimentation to the surface shows that 32-pixel window over-estimates the speed of thermal motion, as it decreases with decreased grid size, and decreases further with FSM.