SUPPLEMENTARY MATERIAL

FIGURES

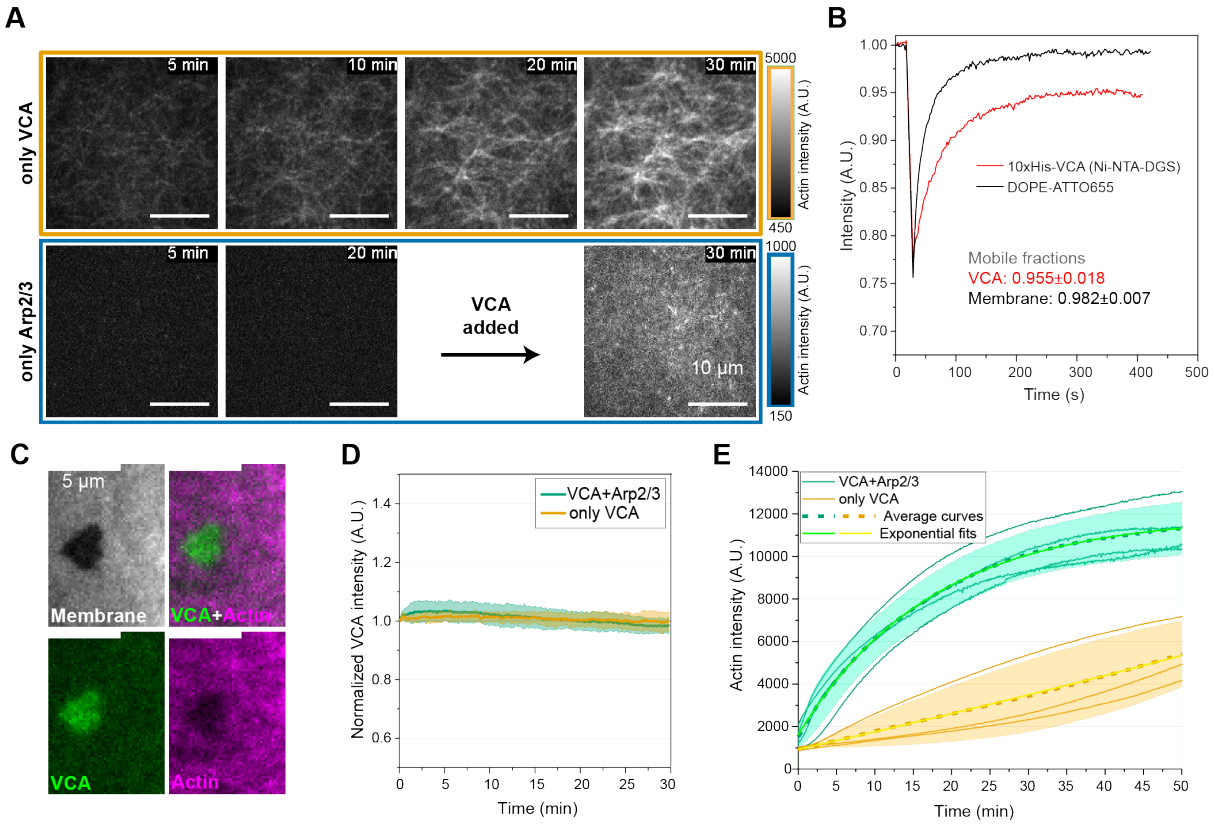


Figure S1. Characterization of actin assembly on SLBs.

(A) Frames from actin growth curves of control conditions in Figure 1B. Upper panel shows images for actin growth in the presence of only VCA; lower panel for growth in the presence of only Arp2/3. Since no intensity change was observed in the latter condition, VCA was added at 20 min. Ten minutes later, filaments and monomers could be observed on the membrane. Note that intensities are set differently between conditions to highlight structural details. **(B)** Fluorescence recovery after photobleaching (FRAP) analysis of labeled 10xHis-tagged VCA on an SLB containing 1 mol% DGS-NTA. Inset shows the mean±s.d. of mobile fractions calculated from 3 experiments. **(C)** Although strong accumulation of VCA (green) was seen on membrane holes or defects, actin (magenta) growth largely excluded these regions. **(D)** Time profile of the normalized intensity of labeled VCA on the surface. VCA intensity is normalized to the value before the addition of actin. Solid lines depict average curves from 3 independent experiments for each condition, with shaded region depicting ±s.d. **(E)** Long-term growth curves of actin in presence and absence of Arp2/3. Solid lines depict time profiles of actin intensity from individual samples: 4 for VCA+Arp2/3 and 3 for only VCA. Dotted line represents average profile with ±s.d. as shaded region. Fit parameters from an exponential growth model are shown in Table S2. Fitting excluded the initial 3 minutes for better fit and is drawn by extrapolation in this region.

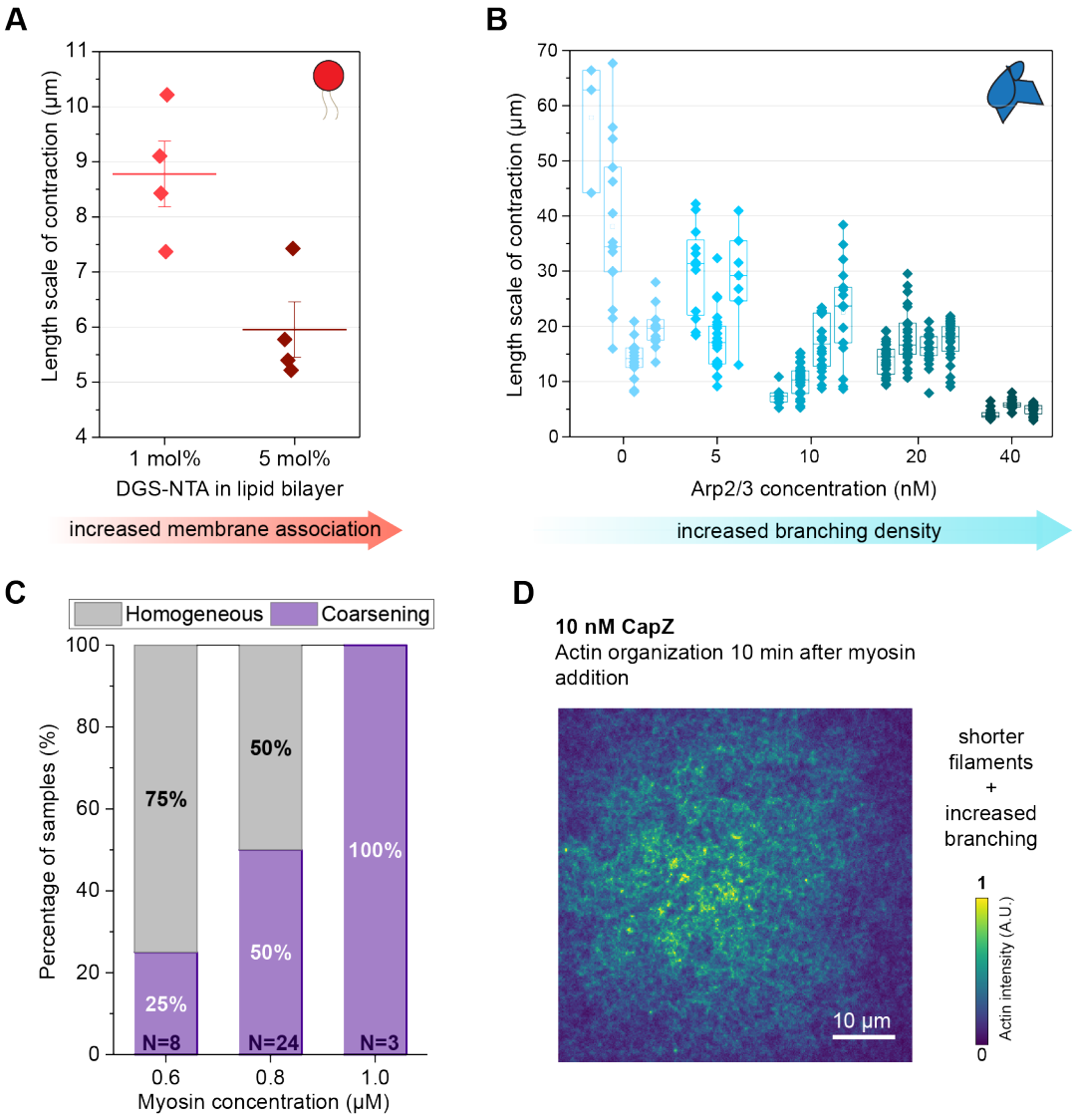


Figure S2. Factors influencing length scale of contraction.

(A) Increased mol% of nickelated lipid DGS-NTA in the SLB results in a lowering of the measured length scale. This would result in a higher surface density of VCA and consequently a higher net membrane adhesion of the actin network. Individual data points depict mean values from 4 independent samples each. Line with whiskers depicts mean±s.e.m. $p<0.05$ (Mann-Whitney test). **(B)** Increasing Arp2/3 concentration correlates with reduced length scale of contraction. Plot shows 25-75 percentile as box, range within 1.5 interquartile region as whiskers, median as line and mean as square. Individual data points depict pairwise distances between foci. More variability is seen in concentrations 0, 5 and 10 nM due to instances of coarsening. Higher Arp2/3 corresponds to increased branching density in the actin network. **(C)** Higher myosin concentrations resulted in more incidences of coarsening during contraction. Sample size for each data set is mentioned. **(D)** Inclusion of capping protein during branched network assembly shifts the balance towards increasing branching at the expense of filament elongation. When myosin was added to a network assembled in the presence of CapZ, at a concentration equal to that of Arp2/3, no distinct foci could be identified in the resulting contraction pattern (N=3 out of 4). This suggests that the length scales might be too short for observable foci formation.

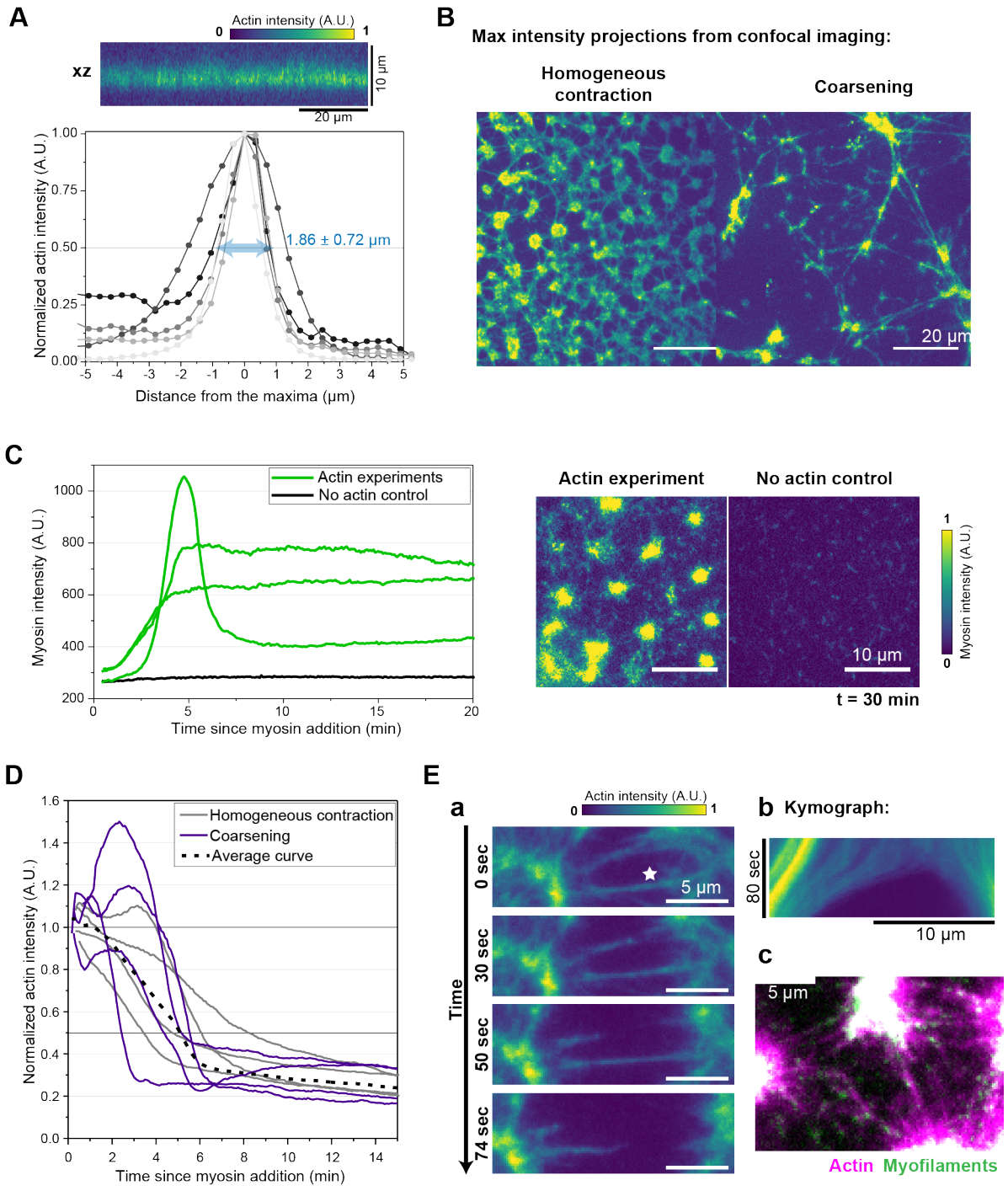


Figure S3. Myosin activity drives the breakdown of membrane-associated actin networks.

(A) Representative image (xz plane) from spinning disk confocal microscopy shows thickness of the actin network on the membrane. The thickness was roughly estimated as the width at half maxima from z-axis profiles of actin intensity on SLBs. Blue arrow depicts mean thickness with mean±s.d calculated from 5 samples. **(B)** Maximum intensity projections of spinning disk confocal image stacks of the actomyosin network on the membrane show both coarsening and homogeneous contraction in independent samples. Intensities are set differently between the images to highlight actin distribution. **(C)** Time profiles of myosin on the membrane in experiments where an actin network was present on the membrane (green; n = 3), and a control experiment where myofilaments were added to the membrane in the absence of actin (black; n = 1). Images show distribution of labelled myosin on the membrane 30 minutes after it was added for the two conditions. **(D)** Time profiles of actin intensity on the membrane from different samples showing homogeneous contraction (gray) or coarsening (purple). Actin intensity is normalized to the value before the addition of myosin. An average curve was calculated (dashed black line) to show the net decrease in surface actin intensities. **(E)** Rupturing and recoil of actin bundles observed during contraction. **(a)** Image sequence shows two actin bundles that break under extensile stress. **(b)** Kymograph of the bundle marked with a star is in

panel a. **(c)** The actin bundles are dotted with myofilaments. The two-channel image is background subtracted.

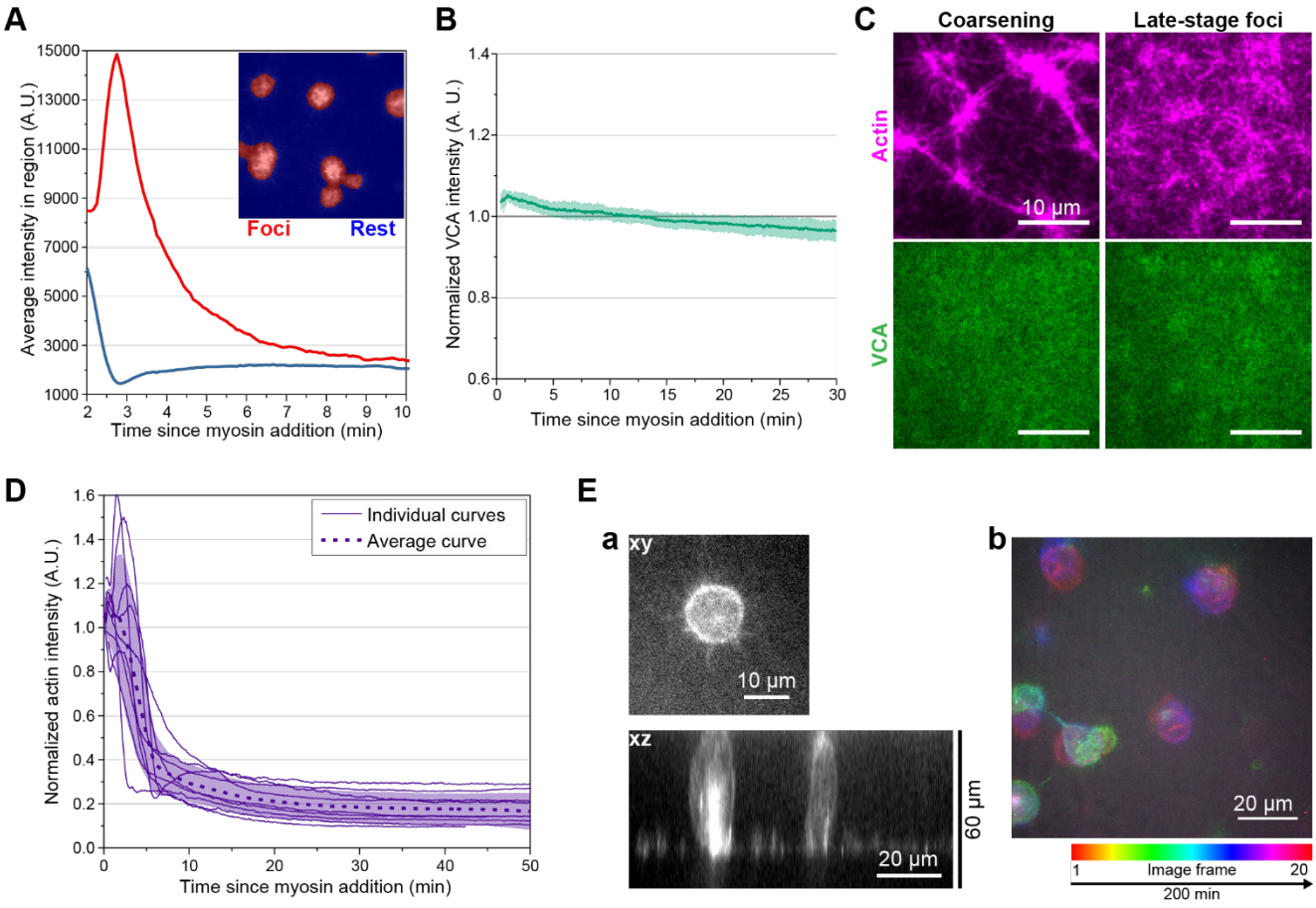


Figure S4. Factors affecting redistribution of actin on the membrane after coarsening.

(A) Time course of surface actin intensity in the region of the condensates formed during coarsening (red), contrasted with the actin intensity in the remaining region (blue). The regions were separately measured using image segmentation. Data representative of 3 samples. **(B)** Time profile of VCA intensity on the membrane after the addition of myosin. VCA intensity is normalized to the value before the addition of myosin. The initial increase is possibly due to the compression of actin against the lipid bilayer caused by myosin. The graph shows the average (solid line) with shaded region representing ±s.d. from 3 samples. **(C)** VCA distribution on the membrane during coarsening (left panel) and in the presence of late-stage foci (right panel) in the same sample. VCA intensity is comparable between images. Actin intensity has been enhanced in the right panel to highlight its distribution. **(D)** Time profiles of surface actin intensity over longer periods of observation. Actin intensity is normalized to the value before the addition of myosin. The graph shows curves from 8 individual experiments, overlaid with the average curve (dashed line) and ±s.d. (shaded region). **(E)** **(a)** Large actomyosin aggregates appear in solution concurrent with the loss of actin from the membrane (xy). These aggregates appear to be attached to the actin on the surface (xz). **(b)** These aggregates show shape dynamics over long periods of time, as can be seen in the maximum intensity projection with temporal color coding over a 200-minute time window.

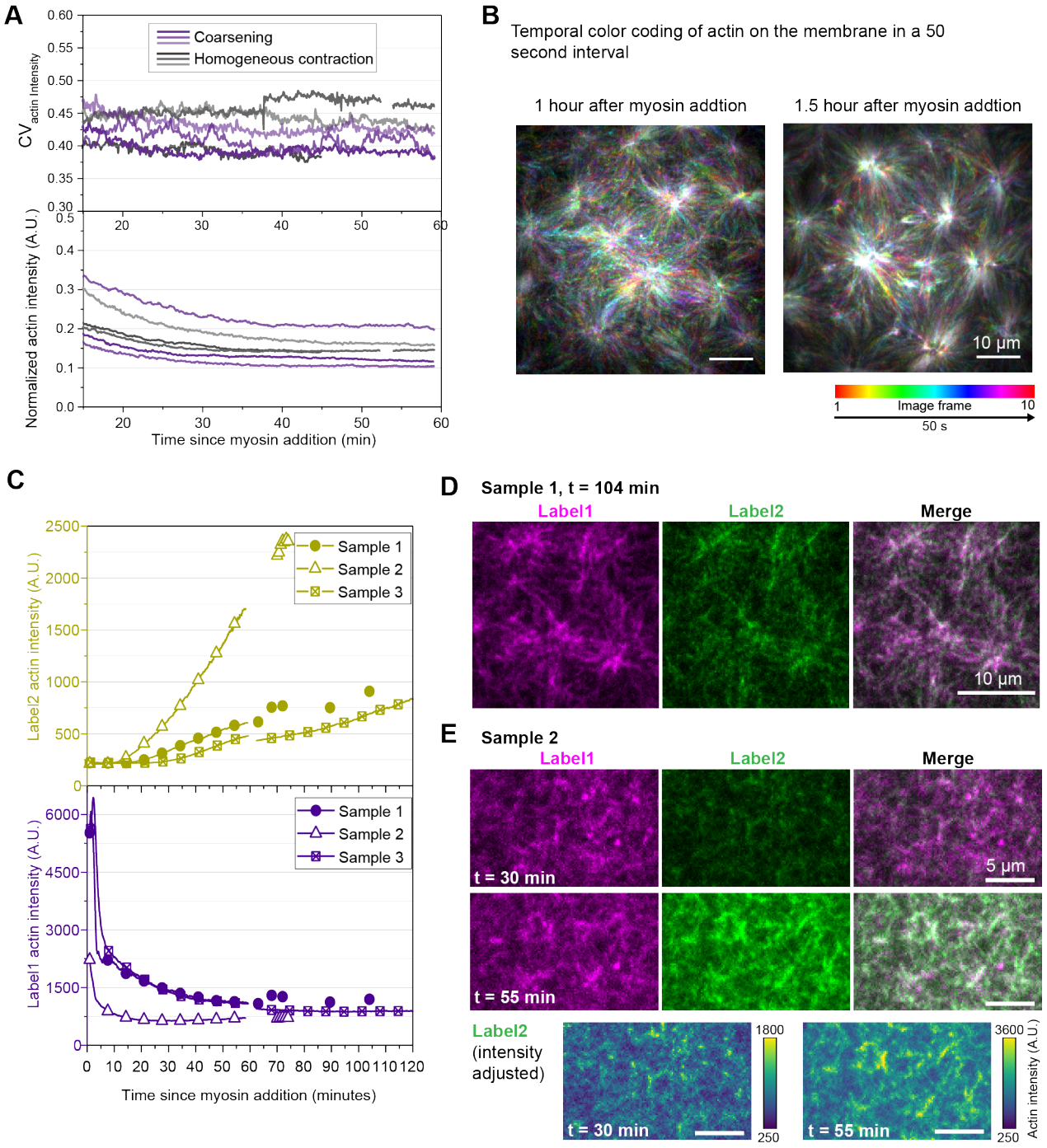


Figure S5. Characterization of the dynamic steady state and actin network turnover.

(A) Coefficient of Variation (CV; upper panel) of actin intensities and normalized actin intensities (lower panel) calculated from 6 experiments in the time period designated as dynamic steady state (between 15 and 60 minutes since the addition of myosin). Purple lines depict samples with coarsening and gray lines depict homogeneous contraction. Actin intensity is normalized to the value before the addition of myosin. **(B)** Actin dynamics on the membrane observed 1 hour (left panel) and 1.5 hours (right panel) after the addition of myosin (different regions) are visualized by maximum intensity projections with temporal color coding in a 50 second time window. Static points appear white in this color coding since all colors overlap there. **(C)** Time profiles of Label1 and Label2 actin intensities for 3 repeats of the experiment described in Figure 5A, where Label2 monomers are added along with myosin on a washed Label1 actin network. Sample 1 corresponds to Figure 5B and Sample 2 corresponds to Figure 5C. The symbols mark every 200th data point on each curve to be able to identify individual curves. Disconnected data points for each curve show data from different regions within the same sample. **(D)** Images of actin on the membrane 104 minutes after the addition of myosin and Label2 in Sample 1 from the plot in C show that the distribution of Label2 is still speckled in comparison with that of Label1. **(E)** Time frames from Sample 2 from the plot in C show how the distribution of the two labels in the actin structures on the membrane evolve. Intensities are comparable for the two time

points in the upper panel. In the lower panel, intensities are adjusted differently to highlight the distribution of the Label2 fluorophores.

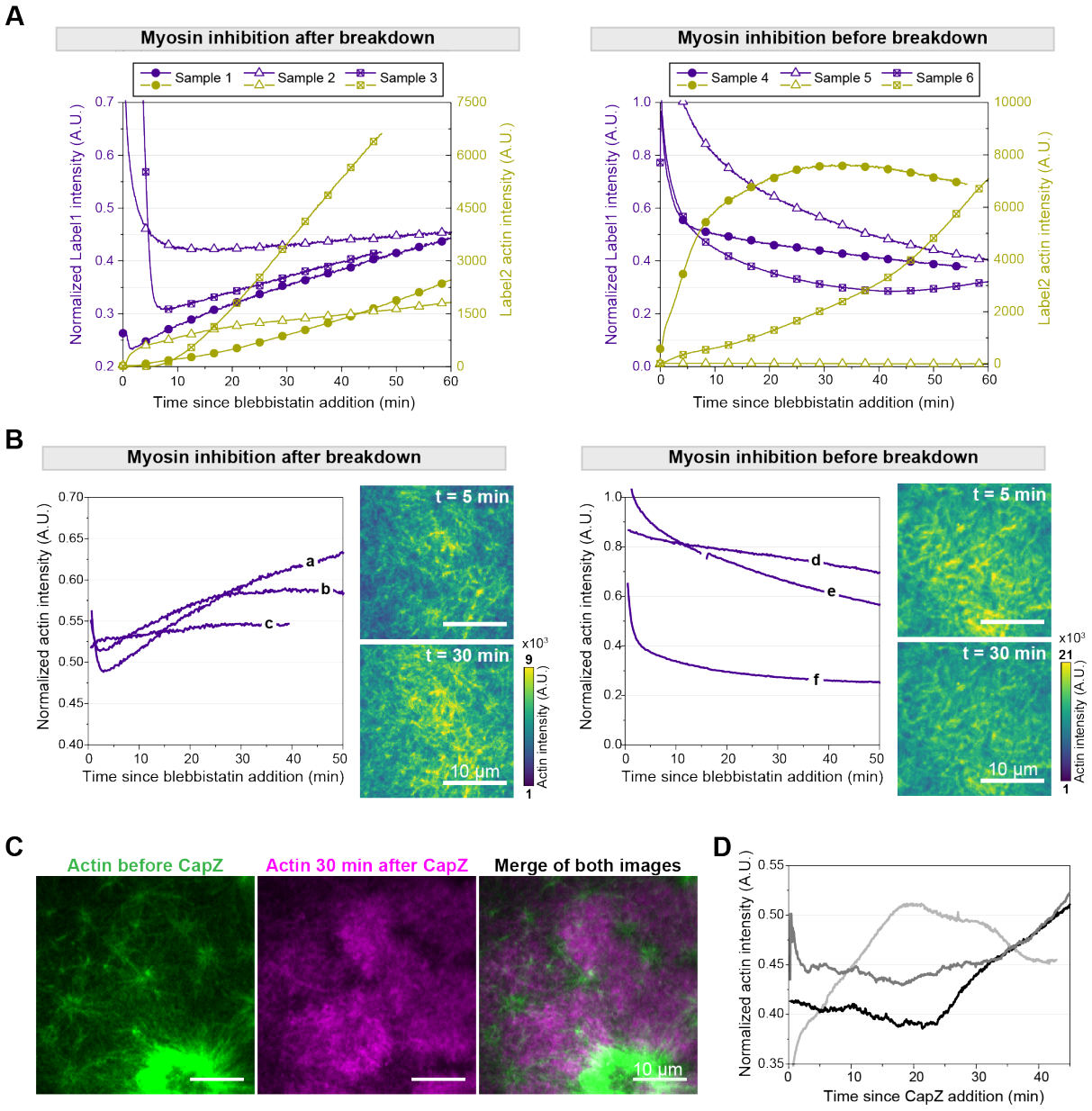


Figure S6. Myosin-driven breakdown facilitates new assembly in the actin network.

(A) Time profiles of Label1 and Label2 actin intensities from experiments testing the significance of myosin-assisted breakdown in the growth of actin on the membrane (Fig. 6 A, B). Data from 3 samples each for experiment and control conditions are compiled in the graph. When the myosin inhibitor blebbistatin was added after Label1 network breakdown, both Label1 and Label2 intensities increased (left panel). When myosin was inhibited before network breakdown, only Label2 intensity increased while the Label1 network showed photobleaching (right panel). Label1 intensity is normalized to the intensity before the addition of myosin. Label2 intensity is background subtracted. Sample 1 and Sample 4 are also shown in Figure 6A and 6B, respectively. In Sample 5, blebbistatin and myosin were added simultaneously and no increase of Label2 was observed indicating that the actin network on the membrane is saturated in the complete absence of myosin activity. Symbols mark every 50th data point on the curves to serve as identifiers for individual data sets. **(B)** Time profiles of actin intensity when myosin activity was blocked before (right panel) or after (left panel) breakdown of the actin network by myosin. No additional monomers were added in these experiments, in contrast with A. Each line represents an independent sample. The trends were similar, with actin intensity increasing if myosin was blocked after network breakdown. Photobleaching of the actin network was observed when myosin was blocked before network breakdown. Actin intensity is normalized to the value before the addition of myosin. The data is compiled from experiments with slightly different imaging setting. Samples c, e and f were imaged with two labels even though only data from one label were used for analysis.

Additionally, sample f was imaged at 5 s time intervals, as opposed to 10 s intervals for other samples. This explains the higher photobleaching observed in samples e and f. The concentrations of components in the reaction mix were otherwise identical. Selected time frames for samples a and d are shown. The intensities are comparable within a sample, but not between samples. **(C)** The actin distribution in the actomyosin cortex before the addition of CapZ (green) is compared with the actin distribution 30 minutes after addition of CapZ (magenta) in the same sample. The merge shows that the two distributions are largely mutually exclusive. Data representative of 3 samples. **(D)** Time profiles of normalized actin intensities from three different CapZ experiments show some increase in actin intensity in all cases. In 2 experiments, the rise occurs with a delay, while in one case the intensity rises shortly after addition of CapZ but decreases later. Actin intensity is normalized to the value before the addition of myosin.

TABLES

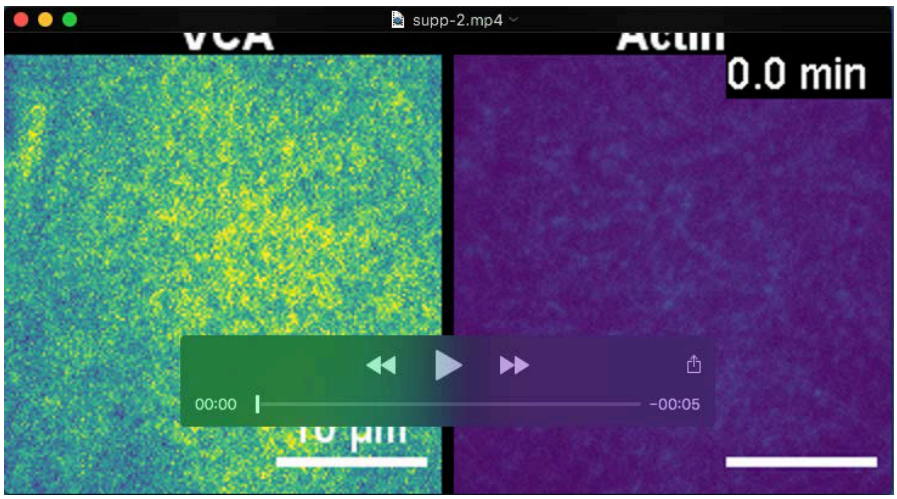
Equation	y = A1*exp(x/t1) + y0	
	VCA+Arp2/3	Only VCA
y0	21.7 ± 0.04	-1.8 ± 0.1
A1	-21.8 ± 0.03	3.5 ± 0.1
t1	-18.4 ± 0.1	56.7 ± 1.3
Reduced Chi-Sqr	0.00782	7.25E-04
R-Square(COD)	0.99958	0.9989
Adj. R-Square	0.99958	0.99889

Table S1. Fit parameters for growth curves in Figure 1B.

Equation	y = A1*exp(x/t1) + y0	
	VCA+Arp2/3	Only VCA
y0	11904 ± 3.6	-2367.7 ± 848.5
A1	-10294.1 ± 3.6	24604.1 ± 847.8
t1	-17.3 ± 0.0	302.8 ± 9.8
Reduced Chi-Sqr	4.46E-04	6.41E-04
R-Square(COD)	0.9999	0.99965
Adj. R-Square	0.9999	0.99965

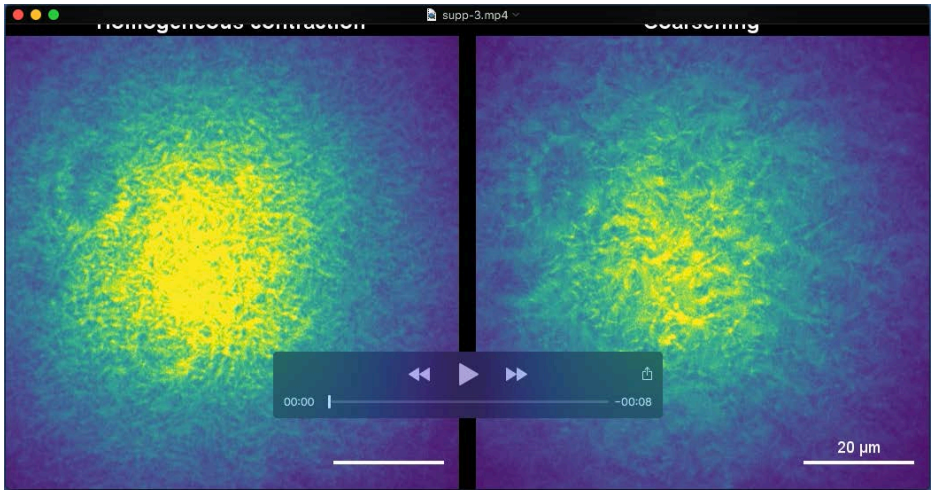
Table S2. Fit parameters for average growth curves in Figure S1D.

MOVIES



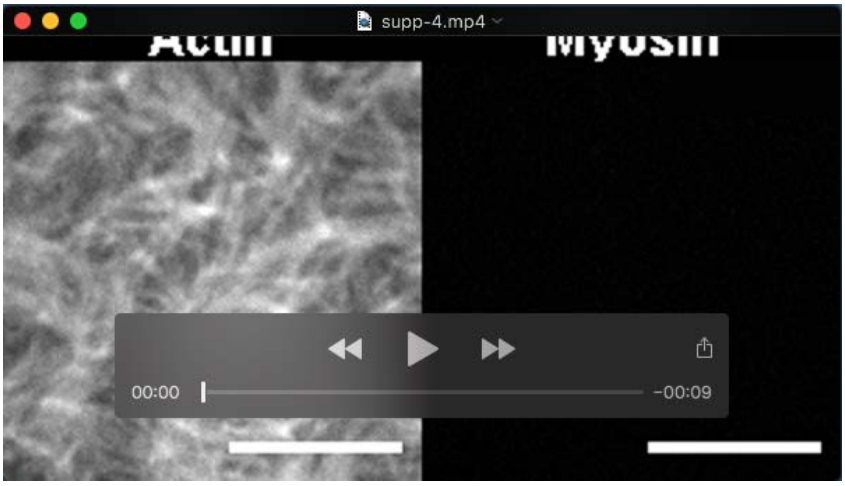
Movie 1. Actin network assembly on a VCA-functionalized SLB.

Growth of actin (1 μM) on an SLB functionalized with VCA in the presence of 10 nM Arp2/3 and 100 μM ATP is visualized using TIRF microscopy. The movie spans a time period of 20 minutes and shows VCA intensity (left) and actin intensity (right) on the surface. Time is measured from the mixing of reaction components. Intensities are color-coded.



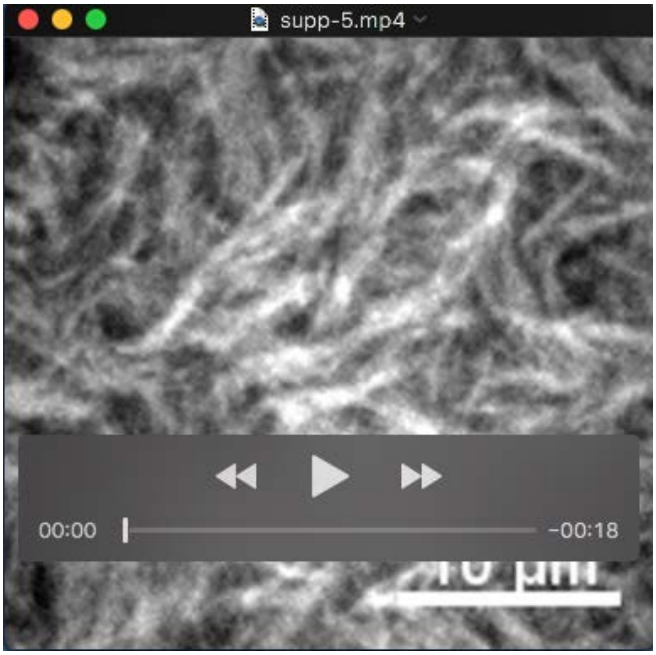
Movie 2. Two modes of actomyosin contraction in a minimal dynamic cortex.

Two different modes of actomyosin contraction are observed in our experiments under the same experimental conditions: 1) Homogeneous contraction (left panel), and 2) Coarsening (right panel). The addition of myosin is indicated by flashing text and the movie depicts a time period of approximately 6 minutes for two samples. Time is measured from the addition of myosin. Actin intensity is color-coded.



Movie 3. Myosin breaks down membrane-associated actin networks.

The flash indicates the addition of myosin (right panel) to an actin network (left panel) grown on an SLB. The movie depicts a time period of 3 minutes, with time measured from the addition of myosin. Scale bars are 10 μm.



Movie 4. ATP is required for myosin-driven breakdown of actin networks.

Myosin-driven breakdown of actin network stalls at bundling in the absence of the ATP regeneration system. 100 μM ATP was provided as in a standard reaction mix. The first flash indicates the addition of myosin and the second flash indicates the addition of the ATP regeneration system. Video shows actin intensity in grayscale and time is measured from the addition of myosin.

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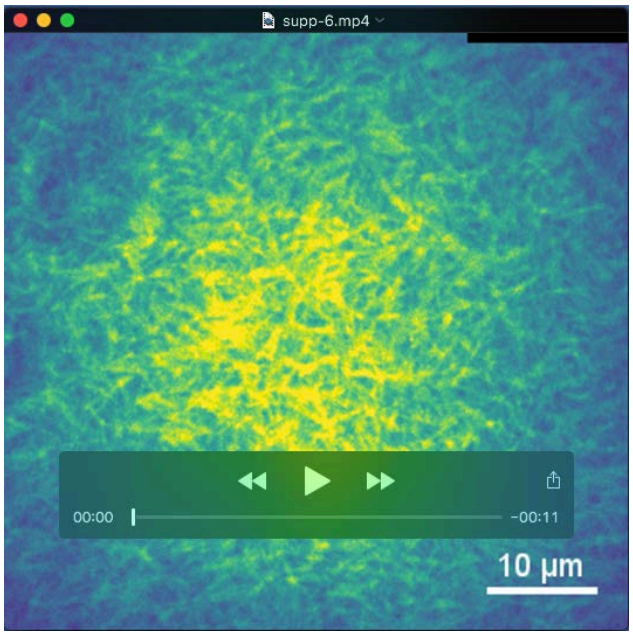
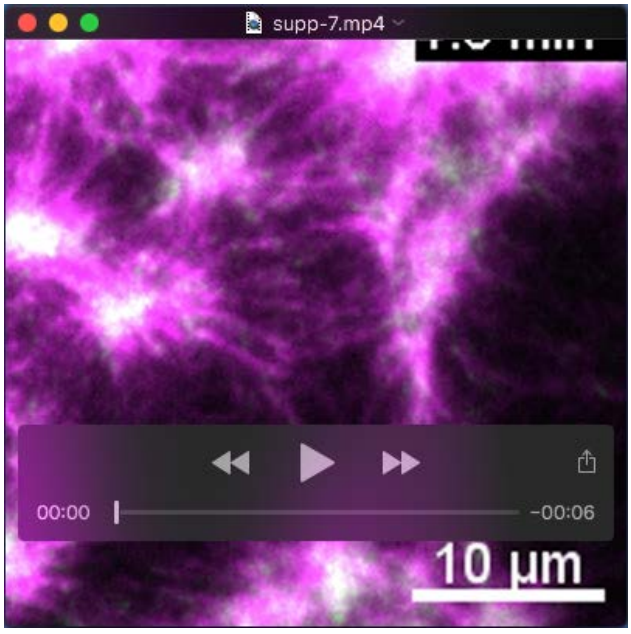


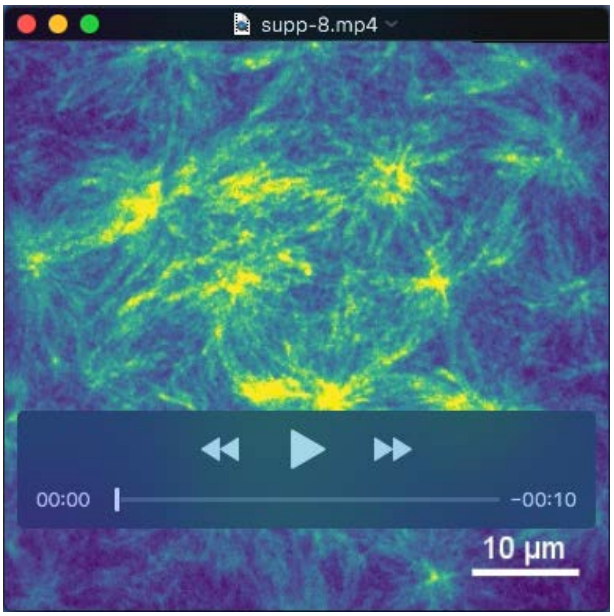
Movie 5. Redistribution of actin after condensation restores a more homogeneous actin distribution.

Flash indicates addition of myosin, after which the actin network undergoes coarsening, followed by redistribution of actin. Movie shows a time window of 10 minutes, with time measured from the addition of myosin. Actin intensity is color-coded.



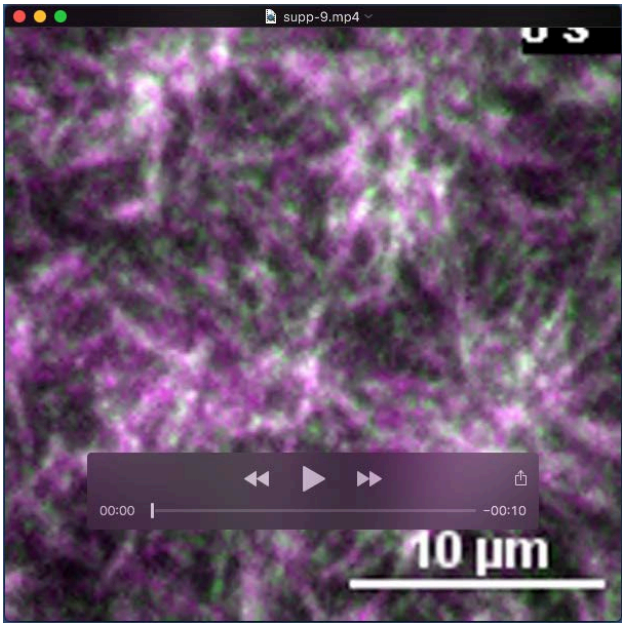
Movie 6. Formation of new actomyosin nodes after coarsening.

New nodes of actin (magenta) and myosin (green) appear in the region between large condensates during coarsening. Movie shows a time period of approximately 10 minutes. Time is measured from the addition of the myosin.



Movie 7. Dynamics in the steady state actin distribution in the minimal cortex.

Actin dynamics on the membrane 30 minutes after the addition of myosin. Movie depicts a time window of 5 minutes, with time measured from the addition of myosin. Actin intensity is color-coded.



Movie 8. Buckling and rupture of actin bundles in the dynamic steady state distribution

Actin dynamics observed in the steady state actin distribution in a pulse-chase experiment (Fig. 5B). Actin bundles with homogeneous distribution of Label1 (magenta) are speckled with Label2 (green) monomers. The movie shows a 40 second time window, captured at 104 minutes after the addition of myosin. Images have been background subtracted by using a walking average over 3 frames.