Mechanical Stress and Network Structure Drive Protein Dynamics during Cytokinesis

Highlights

- Equatorially enriched proteins have reduced mobility at the cleavage furrow
- Mechanical stress felt by the cytoskeleton inhibits equatorial protein dynamics
- Structural changes in cytoskeletal network are sufficient to alter protein dynamics
- Cortical protein dynamics are robust to genetic and mechanical perturbations

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In Brief

Srivastava and Robinson examine how protein dynamics can explain spatiotemporal changes in protein localization during cytokinesis. The reduced cleavage furrow mobility of many mechanoresponsive, equatorially enriched proteins is dependent on mechanical stress and cytoskeletal structure and is robust to genetic and mechanical perturbations.
Mechanical Stress and Network Structure Drive Protein Dynamics during Cytokinesis

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Summary

Cell-shape changes associated with processes like cytokinesis and motility proceed on several-second timescales but are derived from molecular events, including protein-protein interactions, filament assembly, and force generation by molecular motors, all of which occur much faster [1–4]. Therefore, defining the dynamics of such molecular machinery is critical for understanding cell-shape regulation. In addition to signaling pathways, mechanical stresses also direct cytoskeletal protein accumulation [5–7]. A myosin-II-based mechanosensory system controls cellular contractility and shape during cytokinesis and under applied stress [6, 8]. In Dictyostelium, this system tunes myosin II accumulation by feedback through the actin network, particularly through the crosslinker cortexillin I. Cortexillin-binding IQGAPs are major regulators of this system. Here, we defined the short timescale dynamics of key cytoskeletal proteins during cytokinesis and under mechanical stress, using fluorescence recovery after photobleaching and fluorescence correlation spectroscopy, to examine the dynamic interplay between these proteins. Equatorially enriched proteins including cortexillin I, IQGAP2, and myosin II recovered much more slowly than actin and polar crosslinkers. The mobility of equatorial proteins was greatly reduced at the furrow compared to the interphase cortex, suggesting their stabilization during cytokinesis. This mobility shift did not arise from a single biochemical event, but rather from a global inhibition of protein dynamics by mechanical-stress-associated changes in the cytoskeletal structure. Mechanical tuning of contractile protein dynamics provides robustness to the cytoskeletal framework responsible for regulating cell shape and contributes to cytokinesis fidelity.

Results and Discussion

Equatorial Proteins Have Slower Recovery Times Than Polar Crosslinkers and Exhibit Reduced Mobility at the Cleavage Furrow

The short timescale dynamics of proteins regulate their recruitment and localization. Actin-associated proteins may be classified into two groups: the equatorially enriched cleavage furrow proteins and the polar or globally distributed proteins ([9], Figure 1A). We used fluorescence recovery after photobleaching (FRAP) to examine the dynamics of these groups in interphase and dividing Dictyostelium cells, to explain differences in their spatiotemporal localization. We measured the fluorescence intensity in the bleached region until the recovery curve saturated (10–25 s), allowing accurate calculation of recovery times and immobile fractions for key cytoskeletal proteins (see Supplemental Experimental Procedures; Figures S1A–S1C). The characteristic recovery time is dominated by binding-unbinding rates, while the immobile fraction represents the population that does not turn over during the experiment (Figure 1B).

As Dictyostelium cells are highly motile, longer acquisitions can show additional long-scale recovery due to cellular motility instead of protein dynamics. Thus, for this study we only measure the fast dynamic recovery and mobility. GFP-actin recovers within a second, establishing the dynamic of the actin network (Figures 1C and S1D). The cortical actin recovery times and immobile fractions were significantly higher than for GFP or cytoplasmic GFP-actin (Figures 1C and S1D). Thus, cortical GFP-actin dynamics reported by FRAP are dominated by actin filaments, even though ~70% of the total actin (250 μM) in Dictyostelium cells is monomeric [11].

Polar crosslinkers, including dynacortin and fimbrin, mediate cell mechanics and cortical tension, while equatorial proteins—myosin II and cortexillin I—regulate contractility during cytokinesis [9, 12]. As cytokinesis is largely a mechanical shape change process, several equatorial proteins also mediate cellular responses to externally applied mechanical stresses. The mechanoenzyme, myosin II, is the major driver of contractility and accumulates in response to internally or externally generated mechanical stresses [5–7]. This stress-dependent myosin II accumulation results from cooperative interactions between the actin-bound myosin heads and the actin-bundling protein cortexillin I [4]. Scaffolding proteins IQGAP1 and IQGAP2 bind to cortexillin I [13–15] and regulate myosin II accumulation [6]. IQGAP1 inhibits myosin II recruitment, while IQGAP2 relieves this inhibition. Consequently, the iqgap1/2 double-null mutant (i1g1/2) exhibits enhanced myosin II accumulation under stress. IQGAP2 also transmits mechanical signals to spindle signaling proteins (Kif12/INCENP), promoting symmetric cell division.

Genetic, biochemical, and mechanical studies demonstrated crosstalk between the polar and equatorial modules [10, 16]. However, how these proteins interact dynamically to control these processes is unknown. The molecular events governing cytokinesis, including motor activity, actin filament turnover and rearrangement, and crosslinker interactions, occur at much faster timescales than the associated cell-shape changes. Hence, these short timescale cytoskeletal dynamics must be defined to develop a mechanistic understanding of how cells respond to physical forces.

Interestingly, we observed that actin dynamics changed during cytokinesis as the recovery time increased and the immobile fraction decreased in the furrow (Figures 1D and S1E). In comparison, the dynacortin and fimbrin recovery times at the furrow increased significantly, while their mobility was unaffected (Figure 1D [9]). The polar cortex dynamics of dynacortin and fimbrin were similar to interphase phases, while actin showed increased mobility at the poles (data not shown). Myosin II, cortexillin I, and IQGAP2, which localize to the cleavage furrow, recovered more slowly (1.5–5 s) than actin or polar
crosslinkers in the interphase cortex (Figures 1D, S2A, and S2B). Their much slower recovery than that of soluble GFP demonstrates that the fluorescence recovery is dominated by unbinding events at the cortex instead of diffusion. Further, their cytoplasmic recovery times are significantly faster than those in the cortex (Figures 1C and S1D), indicating that the equatorial proteins form stable complexes at the cortex with slower unbinding, as compared to polar crosslinkers, which recover more quickly. Cortexillin I recovery was slower at the furrow than in interphase, while myosin II and IQGAP2 showed no change. In contrast, myosin II recovery slows in anaphase as compared to metaphase in Drosophila S2 cells [17].

The equatorial proteins were much more mobile during interphase compared to other proteins (Figure 1D). The mobility of cortexillin I and IQGAP2 in the interphase cortex was comparable to those in the cytoplasm (Figures 1C and S1D). However, the immobile fractions for these proteins increased significantly at the cleavage furrow (Figures 1D and S1C). The magnitude of mobility shift for cortexillin I and IQGAP2 was higher than for myosin II [9, 10, 18]. Thus, we focused on cortexillin I and IQGAP2 dynamics for the remainder of this study. The reduction in protein mobility at the furrow suggests that these proteins are stabilized at the cortex during furrow ingression, consistent with their slower recovery times. The high immobile fractions also likely promote their furrow enrichment. Therefore, determining the factors that cause this mobility shift is essential to explaining how the contractile proteins accumulate and remodel during furrow ingression.

Genetic Control of Protein Dynamics Is Suppressed at the Furrow

Previous genetic studies established the functional interplay between myosin II, cortexillin I, and the IQGAPs in governing protein accumulation and contractility at the cleavage furrow and in responding to mechanical stress [6] (Figure 2A). Thus, we tested whether the same genetic relationships also dictate protein dynamics, regulating their furrow accumulation. We conducted FRAP on cortexillin I and IQGAP2 at the interphase cortex and the furrow in cell lines lacking key components of this mechanoresponsive system.

Cortexillin I recovery time increased at the furrow compared to the interphase cortex in wild-type (WT) cells (Figures 2B and 2C). However, this slower recovery was not observed in
myosin II (myoII) and iqgap2 (iqg2) -null cells (Figures 2C and S2A). In contrast, while IQGAP2’s recovery time was unaltered at the furrow in WT cells, IQGAP2 had significantly higher recovery time at the furrow in myoII cells (Figures 2D and S2B). Both cortexillin I and IQGAP2 had >2-fold higher immobile fractions at the cleavage furrow (Figures 2B–2D, S2A, and S2B). Cortexillin I immobile fractions were higher in interphase myoII and iqg2 compared to WT but were unchanged at the furrow in these mutants (Figures 2C and S2A). This demonstrates that while myosin II and IQGAP2 are important for maintaining a mobile pool of cortexillin I, additional factors such as mechanical stress could dominate cortexillin I mobility.
at the furrow, ensuring its recruitment during cytokinesis, as cortexillin I also shows mechanical-stress-dependent accumulation [5, 7]. Consistently, the cleavage furrow localization of cortexillin I was not affected in any of the mutants tested. IQGAP2 immobile fraction was also higher in interphase myoII as compared to WT (Figures 2D and S2B), suggesting that myosin II drives the dynamic remodeling of the cytoskeletal network. Myosin II’s full power stroke is required for this mobility regulation, as the 10-fold slower S456L mutant myosin II, which only takes a 2-nm step (1/4 of WT) [7, 9, 19], fails to rescue the IQGAP2 and cortexillin I mobility defects seen in myoII cells (Figures S2C and S2D). Although myosin II regulates the actin cortex dynamics in epithelial cells [20, 21], deletion of myosin II, cortexillin I, or IQGAP2 had no impact on actin dynamics (Figures 2E and S2E).

The deletion of IQGAP1 (iqg1) did not affect the interphase or furrow dynamics of either cortexillin I or IQGAP2 (Figures 2C and 2D), in agreement with its role as a damper of stress-dependent protein accumulation [5]. Because IQGAP1 and IQGAP2 interact with distinct domains of cortexillin I [13–15], we also studied cortexillin I dynamics in the iqg1/2 double mutant. Here, cortexillin I still showed faster recovery at the furrow as compared to WT, similar to iqg2 (Figures S2F and S2G). However, the immobile fraction at the furrow was higher in the double mutant compared to the WT or iqg2 mutant (Figures S2F and S2G). The iqg1/2 cells show enhanced stress-dependent protein accumulation, while iqg2 cells are unresponsive due to IQGAP1 inhibition [5]. Thus, the reduced mobility of cortexillin I at the furrow is likely due to mechanical stresses locking in the cytoskeletal network of these highly mechanoresponsive cells.

To examine molecular scale events driving the protein dynamics changes, we used fluorescence correlation spectroscopy (FCS) to measure the in vivo diffusion of cortexillin I and IQGAP2 across various mutant backgrounds (see Supplemental Experimental Procedures; Figures S1F–S1H). FCS experiments were performed in the cytoplasm as cell movement precluded positioning the confocal volume at the cortex. We compared the diffusion time for cortexillin I and GFP in the cytoplasm to that of purified proteins in vitro. GFP had 5-fold reduction in diffusion time in cells while cortexillin I showed >8-fold slower diffusion (Figure S1H), confirming that cortexillin I is a part of large molecular assemblies. The deletion of myosin II did not impact the cytoplasmic diffusion of either cortexillin I or IQGAP2 though it increased the immobile fraction of both proteins at the cortex (Figures 2F and 2G), implying that myosin II affects protein dynamics by regulating contractility and cytoskeletal structure. The diffusion time for cortexillin I was increased by ~30% in iqg2 cells (Figure 2F). This suggests that without IQGAP2, the effective mass of the cortexillin I complex roughly doubles as diffusion time is approximately proportional to the cube root of the effective molecular weight of the diffusing species. As expected, cortexillin I diffusion also showed a similar trend in the iqg1/2 double mutant. Thus, the changes in cortexillin I mobility could arise from changes in biochemical interactions in the absence of IQGAP2 (Figures 2C and S2F).

Collectively, the FRAP and FCS experiments enabled us to attribute changes in cortexillin I mobility to either protein-protein interactions (in iqg2 and iqg1/2) or to cortex restructuring (in myoII). We demonstrated that the dynamics of cortexillin I and IQGAP2 at the cleavage furrow are well conserved across mutants, though differences emerge during interphase (Figure 2H). As cleavage furrow contractility is common to all cells, we hypothesized that mechanical stresses acting at the furrow could override the biochemical signals to define cleavage furrow protein dynamics. Physical mechanisms such as myosin-II-mediated force generation, Laplace pressure-mediated furrow thinning, and protrusive forces from the polar cortex drive furrow ingestion [22]. Thus, we next examined whether mechanical stresses at the cleavage furrow were sufficient to shift the dynamics of these mechanoresponsive proteins.

**Mechanical Stress Drives the Reduction in Cleavage Furrow Mobility of Cortexillin I and IQGAP2**

In addition to enrichment at the cleavage furrow, myosin II, cortexillin I, and IQGAP2 accumulate to sites of externally applied mechanical stress, thereby allowing the cell to retract against this stress [5–7]. Hence, we applied compression using agarose overlay to test if mechanical stress, as compared to biochemical signaling, affects protein dynamics changes at the cleavage furrow. Flattening of the cells drives the accumulation of the mechanoresponsive proteins studied here to the cell cortex to counter this stress [3, 6]. The ratio of fluorescence intensity in the cortex to that in the cytoplasm is dependent on the thickness of agarose and plating density (T. Luo and D.N.R., unpublished data), confirming that the increase in cortical intensity is driven by mechanical stress and is not simply due to volume effects. Further, soluble GFP does not change in cortical intensity upon compression [5].

We examined cortexillin I and IQGAP2 interphase dynamics in presence or absence of compression across mutants studied above (Figure 3A). Cortexillin I exhibited a slower recovery time under compression, but IQGAP2’s recovery time was unaffected (Figures 3B, 3C, 3A, and S3B). Both cortexillin I and IQGAP2 showed a >2-fold increase in the immobile fraction under compression, similar to the observation at the furrow (Figures 3B, 3C, 3A, and S3B). By FCS, the cortexillin I diffusion time also doubled under compression, while IQGAP2 diffusion was unaffected (Figure 3D). Both the recovery time and immobile fraction for IQGAP2 increased in compressed myoII compared to WT (Figures 3C and S3B). Cortexillin I and IQGAP2 dynamics under compression did not change in other mutants as compared to WT (Figures 3B, 3C, 3F, 3A, and S3B). The cortexillin I mobility shift in iqg2 cells under compression was higher than that observed at the furrow (Figure 2C), suggesting that under compression cortexillin I directly responds to mechanical stress, compared to the cleavage furrow where biochemical signals through IQGAP2 also contribute to cortexillin I mobility. Importantly, both compression and cleavage furrow showed a consistent, >2-fold increase in immobile fractions of both cortexillin I and IQGAP2 compared to the unstressed, interphase cortex across various mutants (Figures 2C, 2D, 3B, and 3C). This validates the importance of mechanical stress in driving the dynamics of equatorially enriched proteins at the cleavage furrow, thereby ensuring their robust localized accumulation. In addition, by measuring dynamics of cortexillin I and IQGAP2 in cells lacking the small GTPase racE (racE), we assessed the contribution of cortical tension on protein dynamics, as racE is a major regulator of cortical mechanics [9, 23, 24]. For cortexillin I, the immobile fraction was higher and the recovery time was shorter in racE cells, while IQGAP2 dynamics were unchanged (Figures S3D and S3E). Thus, cortexillin dynamics are not only affected by mechanical stress, but also by general cortical mechanics. Furthermore, the mobility and recovery times of GFP-actin were not affected by compression (Figure S1E). Overall, cortexillin I dynamics are more sensitive
to compressive stresses than the dynamics of IQGAP2, actin, and GFP are.

As compression reduced cell height by up to 4-fold, we measured GFP dynamics to examine the impact of altered protein transport and cellular structure upon compression. The immobile fraction and diffusion time for GFP nearly doubled (Figures 3D, 3E, and S3C), suggesting sieving effects may become significant under compression. The altered GFP dynamics under compression confirm that network structure and intracellular environment are important contributors to mechanical-stress-dependent protein dynamics. However, in myoII cells, GFP FRAP dynamics did not change upon compression; rather, GFP diffusion was faster in compressed myoII cells (Figures 3D, 3E, and S3C), suggesting that myosin II is important for stabilizing the cortex under mechanical stress, and in its absence the cortical dynamics are dominated by passive diffusive behaviors [25]. As the actin cytoskeleton forms a highly dense meshwork in Dictyostelium, structural changes between interphase and furrow cortex cannot be resolved by confocal and electron microscopy [9]. Thus, we
next chemically perturbed the cytoskeleton to determine how these network properties affect protein mobility and dynamics.

Alterations to Cortical Structure and Mechanics Shift Mobility of Cortexillin I

To test the effect of cytoskeletal structure on protein dynamics, we perturbed the actin cytoskeleton by treating the cells with either latrunculin-A or jasplakinolide. Latrunculin-A prevents F-actin assembly by sequestering free G-actin monomers, while jasplakinolide enhances actin filament nucleation. We quantified changes in F-actin amount upon treatment with latrunculin-A and jasplakinolide by measuring the relative fluorescence intensity of cells stained with phalloidin 15 min post-drug treatment [4] (Figures 4A, 4B, and S4A). Anti-actin staining was also used to visualize changes in actin level and cytoskeletal morphology (Figures 4A, 4B, and S4A). Interestingly, even with 5 μM latrunculin-A, cells still had ~50% residual F-actin (~35 μM) (Figure 4B), suggesting sufficient F-actin binding sites for the ~1 μM actin crosslinkers [3, 26, 27]. The residual F-actin mostly concentrated in puncta illustrating discontinuity of the cytoskeletal network (Figure 4A), also reflected by the increase in the recovery time and immobile fraction of GFP even though its diffusion is unaffected (Figures 4D, 4E, and S4D). Latrunculin-A had a drastic effect on cellular mechanics, as 1 μM latrunculin-A-treated cells had 12-fold lower cortical tension as measured by micropipette aspiration (Figures 4C, S4B, and S4C), consistent with the 85% reduction in viscoelasticity previously reported for latrunculin-B treatment [12]. Latrunculin-A-treated (5 μM) cells were too soft for mechanical measurements. In contrast, jasplakinolide enhanced the cellular F-actin levels ~4-fold inducing the formation of F-actin clusters (Figures 4A, 4B, and S4A) and increased cortical tension slightly (Figures 4C, S4B, and S4C). Jasplakinolide also increased the recovery time of soluble GFP while not affecting its immobile fraction or diffusion (Figures 4D and S4D). Thus, we were able to directly probe the impact of changes in cytoskeletal structure and mechanics on protein dynamics by using these two compounds.

Latrunculin-A also increased actin mobility and recovery rate, while jasplakinolide had no effect (Figures 4D and S4D). The increased actin mobility with latrunculin-A is quantitatively similar to that at the furrow (Figures 1D and 4D), further validating the importance of cytoskeletal restructuring during cytokinesis (Figure 4H). These dynamic features also explain why actin does not show a significant accumulation at the cleavage furrow or upon micropipette aspiration [3, 9]. Overall, perturbations to the cytoskeletal structure are sufficient to affect changes in the dynamics of cytoskeletal proteins. Similarly, protein dynamics are also affected by mechanical stress, which leads to accumulation of equatorial proteins during cytokinesis (Figure 4H).

Conclusions

Mechanical stresses are important for driving cellular processes like cell division and motility and play a major role in determining cell fate [6, 28, 29]. Understanding the effect of mechanical stress on protein dynamics is critical for having predictive power over these cellular behaviors. Here, we identified that equatorial protein mobility significantly reduces at the cleavage furrow, while that of polar crosslinkers is unchanged (Figure 1). Both biochemical associations and myosin II-mediated remodeling affect protein dynamics (Figure 2). Compressive stress applied externally also leads to reduced mobility (Figure 3). The molecular mechanisms that result in this drastic reduction in protein mobility need to be examined. Even when key contractile proteins are eliminated, the cytoskeleton is capable of maintaining fairly normal dynamics (Figures 2 and 3). Interestingly, most of the mutant phenotypes in protein dynamics are seen in the unstressed, interphase cortex, while the dynamics are unchanged across mutants during cytokinesis or upon compression (Figures 2 and 3). Thus, the cell’s contractile system is built as a highly adaptive machine, maintaining fairly normal dynamics under mechanical stress ensuring fidelity of protein recruitment. In contrast, in other scenarios mechanical stress can exaggerate many mutant phenotypes. For example, myoII-null cells cannot perform cytokinesis without substrate adhesion or when challenged by mechanical stress [5, 30]. Further, changes to cytoskeletal structure are sufficient to drive similar changes in protein dynamics, highlighting the importance of network properties in governing protein and cellular behaviors (Figure 4). Myosin II emerges as the major driver of active processes in the cortex, in accordance with previous studies (Figures 2 and 3) [25].

The mechanical tuning of protein dynamics and recruitment is an important mode of regulating cellular responses to physical stimuli and requires protein-protein interactions to be stabilized or disrupted under mechanical load. Protein-protein stabilization can induce protein clustering and provide signal amplification, while disruption can lead to signal dissipation. This is the classical paradigm for any signal transduction system. Basic molecular mechanisms for protein clustering in response to mechanical stress include catch bond formation and structural cooperativity, while slip bonds allow for force-induced disassembly. These fundamnetals are important in directing macromolecular assembly of actin crosslinking proteins [3, 4, 31, 32]. Here we have demonstrated that these mechanisms are also applicable to scaffolding proteins like IQGAP2, emphasizing the importance of network structure and higher order self-assembly in governing cellular behavior. Indeed, cellular systems are engineered as smart materials where many of the constituents are mechanoresponsive.
Experimental Procedures

Experimental procedures for Dictyostelium cell culture, agarose overlay, FRAP analysis, FCS analysis, latrunculin-A and jasplakinolide treatment, F-actin quantification by phalloidin staining, and cortical tension measurements by micropipette aspiration are given in Supplemental Experimental Procedures. All curve fitting and statistical analysis was done using KaleidaGraph (Synergy Software). Significance of difference was determined using ANOVA with Fisher’s LSD post-test.
Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, four figures, and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.01.025.

Author Contributions

V.S. and D.N.R. designed the experiments, analyzed the data, and prepared the manuscript. V.S. conducted the experiments.

Acknowledgments

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References

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Supplemental Information

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Supplementary Figure S1, Related to Figure 1: FRAP and FCS calibration. (A) An example confocal image of a GFP-cortexillin I-expressing cell illustrating the bleach, reference and background region used for FRAP measurements. (B) Example fluorescence intensity curves showing changes in intensity of bleached and reference regions during a FRAP experiment. (C) Example traces showing fluorescence recovery of GFP-cortexillin I in interphase and cleavage furrow cortex. (D) Distribution of recovery times and immobile fractions for various proteins in the cell cortex and the cytoplasm. (E) Distribution of recovery times and immobile fractions for GFP-actin and GFP in interphase, dividing, and compressed cells. (F) Example autocorrelation curves for rhodamine 6G diffusion as measured by FCS. The autocorrelation data was fitted to a 1-component 3D diffusion model with triplet state dynamics. The panel on right is the residual curve showing the goodness of fit. (G) The distribution of diffusion times and structural parameter measured for rhodamine 6G by FCS. (H) Diffusion times for purified mCherry and GFP-cortexillin I in vitro, and GFP and GFP-cortexillin I in cellular cytoplasm as measured by FCS. Calculated diffusion coefficients are provided in Table S3.
Supplementary Figure S2, Related to Figure 2: Changes in cortexillin I and IQGAP2 dynamics at the cleavage furrow. (A, B) Distribution of recovery times and immobile fractions for GFP-cortexillin I (A) and GFP-IQGAP2 (B) in different genetic mutants in interphase and dividing cell cortex. (C, D) Distribution of recovery times and immobile fractions for GFP-cortexillin I (C) and GFP-IQGAP2 (D) in WT, myoII and S456L myoII cells. (E) Distribution of recovery times and immobile fractions of GFP-actin in the interphase cortex of different mutants. (F) Mean recovery times and immobile fractions for GFP-cortexillin I in WT, iqg2 single, and iqg1/2 double mutants. (G) Distribution of recovery times and immobile fractions for GFP-cortexillin I in WT, iqg2 single, and iqg1/2 double mutants. Values plotted are mean ± SEM; sample sizes are listed on the bars. p values are represented as ns: p >0.05, *: p <0.05, **: p <0.005, ***: p <0.0005 based on ANOVA with Fischer’s LSD post-test.
Supplementary Figure S3, Related to Figure 3: Changes in cortexillin I and IQGAP2 dynamics under compression. (A, B) Distribution of recovery times and immobile fractions for GFP-cortexillin I (A) and GFP-IQGAP2 (B) in different genetic mutants in control and compressed cells. (C) Distribution of recovery times and immobile fractions for GFP in WT and myoII cells without or with compression. (D) Average recovery times and immobile fractions of GFP-cortexillin I and GFP-IQGAP2 in WT and racE null cells. (E) Distribution of recovery times and immobile fractions of GFP-cortexillin I and GFP-IQGAP2 in WT and racE null cells. Values plotted are mean ± SEM; sample sizes are listed on the bars. p values are represented as ns: p >0.05, *: p <0.05, **: p <0.005, ***: p <0.0005 based on ANOVA with Fischer’s LSD post-test. #: The immobile fraction for cortexillin I in racE null cells is bimodal (see panel E); hence SEM is not shown.
Supplementary Figure S4, Related to Figure 4: Changes in the actin network structure result in altered protein dynamics. (A) Distribution of normalized fluorescence intensity for phalloidin and anti-actin staining in cells treated with DMSO, 5 µM latrunculin-A and 2 µM jasplakinolide (B) DIC images of micropipette aspiration experiments with DMSO, 1 µM latrunculin-A and 2 µM jasplakinolide (C) Cortical tension measurements for DMSO, 1 µM latrunculin-A and 2 µM jasplakinolide-treated cells (D) Distribution of recovery times and immobile fractions for GFP, GFP-actin, GFP-cortexillin I and GFP-IQGAP2 in DMSO, 5 µM latrunculin-A or 2 µM jasplakinolide-treated cells. (E) Distribution of recovery times and immobile fractions of GFP-cortexillin I in interphase and dividing cells with or without 5 µM latrunculin-A.
Table S1: Mean recovery times (τ) and mean immobile fractions (Fᵢ) for proteins in interphase cells, at the cleavage furrow and in compressed cells; FRAP Analysis

**GFP-cortexillin I**

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**GFP-IQGAP2**

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**GFP-actin**

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<td>myoII</td>
<td>0.68 ± 0.12 s, 0.43 ± 0.05 (11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ctxA</td>
<td>0.61 ± 0.12 s, 0.35 ± 0.05 (10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iqg2</td>
<td>0.46 ± 0.03 s, 0.34 ± 0.05 (15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>racE</td>
<td>0.61 ± 0.18 s, 0.25 ± 0.07 (10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**GFP**

<table>
<thead>
<tr>
<th></th>
<th>Interphase</th>
<th>Furrow</th>
<th>Compression</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>0.26 ± 0.03 s, 0.12 ± 0.02 (27)</td>
<td>0.27 ± 0.07 s, 0.12 ± 0.03 (12)</td>
<td>0.66 ± 0.12 s, 0.26 ± 0.04 (14)</td>
</tr>
<tr>
<td>myoII</td>
<td>0.35 ± 0.03 s, 0.14 ± 0.03 (15)</td>
<td></td>
<td>0.50 ± 0.06 s, 0.11 ± 0.02 (15)</td>
</tr>
</tbody>
</table>

The values represent mean ± SEM for recovery times and immobile fractions. The number of measurements is given in parentheses.
Table S2: Mean recovery times (τ) and mean immobile fractions (F_i) for proteins latrunculin-A or jasplakinolide treated cells; FRAP Analysis

<table>
<thead>
<tr>
<th></th>
<th>DMSO</th>
<th>5 μM Latrunculin-A</th>
<th>2 μM Jasplakinolide</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFP cortl (interphase)</td>
<td>2.5 ± 0.4 s, 0.25 ± 0.04 (14)</td>
<td>3.5 ± 0.6 s, 0.53 ± 0.05 (13)</td>
<td>2.2 ± 0.4 s, 0.46±0.06 (15)</td>
</tr>
<tr>
<td>GFP cortl (furrow)</td>
<td>4.6 ± 0.8 s, 0.53 ± 0.10 (9)</td>
<td>6.2 ± 1.1 s, 0.60 ± 0.04 (16)</td>
<td></td>
</tr>
<tr>
<td>GFP IQGAP2</td>
<td>1.5 ± 0.2 s, 0.19 ± 0.03 (14)</td>
<td>2.0 ± 0.2 s, 0.26 ± 0.03 (19)</td>
<td>1.1 ± 0.1 s, 0.14±0.02 (23)</td>
</tr>
<tr>
<td>GFP actin</td>
<td>0.66 ± 0.05 s, 0.36 ± 0.02 (68)</td>
<td>1.1 ± 0.13 s, 0.25 ± 0.03 (41)</td>
<td>0.82 ± 0.08 s, 0.44±0.03 (22)</td>
</tr>
<tr>
<td>GFP</td>
<td>0.26 ± 0.03 s, 0.12 ± 0.02 (27)</td>
<td>0.40 ± 0.03 s, 0.20 ± 0.02 (15)</td>
<td>0.39 ± 0.03 s, 0.10±0.02 (15)</td>
</tr>
</tbody>
</table>

The values represent mean ± SEM for recovery times and immobile fractions. The number of measurements is given in parentheses.
Table S3: Cytosolic diffusion times ($\tau_D$) and diffusion coefficients ($D_{eff}$) for proteins as measured by FCS

### Diffusion in PBS at 22°C

<table>
<thead>
<tr>
<th>Molecular weight (kDa)</th>
<th>Diffusion time ($\tau_D$)</th>
<th>Diffusion Coefficient ($D_{eff}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodamine 6G</td>
<td>0.48 kDa</td>
<td>0.033 ± 0.002 ms (34)</td>
</tr>
<tr>
<td>His-mCherry</td>
<td>28 kDa</td>
<td>0.15 ± 0.01 ms (11)</td>
</tr>
<tr>
<td>His-GFP-cortexillin-I</td>
<td>80 kDa</td>
<td>0.28 ± 0.02 ms (11)</td>
</tr>
</tbody>
</table>

### Diffusion in cytoplasm at 22°C

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cortexillin I</th>
<th>IQGAP2</th>
<th>GFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT control</td>
<td>2.4 ± 0.2 ms (20), $D_{eff} = 5.8$ µm²/s</td>
<td>2.9 ± 0.3 ms (18), $D_{eff} = 4.8$ µm²/s</td>
<td>0.78 ± 0.05 ms (20), $D_{eff} = 18$ µm²/s</td>
</tr>
<tr>
<td>WT + compression</td>
<td>4.0 ± 0.3 ms (17), $D_{eff} = 3.5$ µm²/s</td>
<td>3.4 ± 0.4 ms (16), $D_{eff} = 4.1$ µm²/s</td>
<td>1.5 ± 0.1 ms (16), $D_{eff} = 9.4$ µm²/s</td>
</tr>
<tr>
<td>myoll</td>
<td>2.6 ± 0.2 ms (18), $D_{eff} = 5.4$ µm²/s</td>
<td>2.9 ± 0.2 ms (11), $D_{eff} = 4.8$ µm²/s</td>
<td>1.4 ± 0.2 ms (15), $D_{eff} = 10$ µm²/s</td>
</tr>
<tr>
<td>myoll + compression</td>
<td>3.9 ± 0.4 ms (12), $D_{eff} = 3.6$ µm²/s</td>
<td>-</td>
<td>0.69 ± 0.06 ms (14), $D_{eff} = 20$ µm²/s</td>
</tr>
<tr>
<td>iqq2</td>
<td>3.1 ± 0.2 ms (16), $D_{eff} = 4.5$ µm²/s</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>iqq1/2</td>
<td>3.7 ± 0.3 ms (16), $D_{eff} = 3.8$ µm²/s</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ctxA</td>
<td>-</td>
<td>2.6 ± 0.3 ms (12), $D_{eff} = 5.4$ µm²/s</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cortexillin I</th>
<th>IQGAP2</th>
<th>GFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>2.8 ± 0.4 ms (12), $D_{eff} = 5.0$ µm²/s</td>
<td>2.7 ± 0.4 ms (15), $D_{eff} = 5.2$ µm²/s</td>
<td>0.75 ± 0.10 ms (13), $D_{eff} = 19$ µm²/s</td>
</tr>
<tr>
<td>5 µM latrunculin-A</td>
<td>P1:2.4 ± 0.1 ms (8), $D_{eff} = 5.8$ µm²/s</td>
<td>P2:2.1 ± 0.5 ms (7), $D_{eff} = 1.7$ µm²/s</td>
<td>3.6 ± 0.4 ms (16), $D_{eff} = 3.9$ µm²/s</td>
</tr>
<tr>
<td></td>
<td>3.6 ± 0.4 ms (16), $D_{eff} = 3.9$ µm²/s</td>
<td>1.1 ± 0.1 ms (25), $D_{eff} = 13$ µm²/s</td>
<td></td>
</tr>
<tr>
<td>2 µM jasplakinolide</td>
<td>3.2 ± 0.2 ms (20), $D_{eff} = 4.4$ µm²/s</td>
<td>3.2 ± 0.4 ms (18), $D_{eff} = 4.4$ µm²/s</td>
<td>0.86 ± 0.07 ms (18), $D_{eff} = 16$ µm²/s</td>
</tr>
</tbody>
</table>

The values represent mean ± SEM for diffusion times. The numbers of measurements is given in parentheses.

* Diffusion coefficients for rhodamine 6G and GFP were reported in Petrášek and Schwille (2008) [S1].
**Table S4: Cell strains used in this study**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Experimental Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>Ax3(Rep orf+)</td>
<td>Phalloidin staining/F-actin quantification; MPA (cortical tension)</td>
</tr>
<tr>
<td>WT::GFP</td>
<td>Ax3(Rep orf+):Hyg^R^::pDRH; GFP, G418^R^::DM181</td>
<td>FRAP, FCS</td>
</tr>
<tr>
<td>WT::GFP</td>
<td>myoll(HS1)::mCH-myoll, Hyg^R^::pDRH; GFP, G418^R^::DM181</td>
<td>FRAP</td>
</tr>
<tr>
<td>WT::GFP-actin</td>
<td>KAx3(RF)::Hyg^R^::pDRH; GFP-actin, G418^R^::DM181</td>
<td>FRAP</td>
</tr>
<tr>
<td>WT::GFP-actin</td>
<td>myoll(HS1)::mCH-myoll, Hyg^R^::pDRH; GFP-actin, G418^R^::DM181</td>
<td>FRAP</td>
</tr>
<tr>
<td>WT::GFP-cortexillin I</td>
<td>KAX3(RF)::GFP-cortl, Hyg^R^::pDRH; G418^R^::DM181</td>
<td>FRAP, FCS</td>
</tr>
<tr>
<td>WT::GFP-IQGAP2</td>
<td>KAX3::RFP-α-tubulin, Hyg^R^::pDRH; GFP-IQGAP2, G418^R^::pEXP4</td>
<td>FRAP, FCS</td>
</tr>
<tr>
<td>myoll::GFP-actin</td>
<td>myoll(HS1)::RFP-α-tubulin, Hyg^R^::pDRH; GFP-actin, G418^R^::DM181</td>
<td>FRAP</td>
</tr>
<tr>
<td>myoll::GFP-cortexillin I</td>
<td>myoll(HS1)::GFP-cortl, Hyg^R^::pDRH; G418^R^::DM181</td>
<td>FRAP</td>
</tr>
<tr>
<td>myoll::GFP-IQGAP2</td>
<td>myoll(HS1)::RFP-α-tubulin, Hyg^R^::pDRH; GFP-IQGAP2, G418^R^::pEXP4</td>
<td>FRAP, FCS</td>
</tr>
<tr>
<td>myoll::GFP</td>
<td>myoll(HS1)::Hyg^R^::pDRH; GFP, G418^R^::DM181</td>
<td>FRAP</td>
</tr>
<tr>
<td>S456L::GFP-cortexillin I</td>
<td>myoll(HS1)::GFP-cortl, Hyg^R^::pDRH; myoll(S456L), G418^R^::pBIG</td>
<td>FRAP</td>
</tr>
<tr>
<td>S456L::GFP-IQGAP2</td>
<td>myoll(HS1)::CFP-myoll(S456L), Hyg^R^::pDRH; GFP-IQGAP2, G418^R^::pEXP4</td>
<td>FRAP</td>
</tr>
<tr>
<td>ctxA::GFP-actin</td>
<td>cortl(RF)::RFP-α-tubulin, Hyg^R^::pDRH; GFP-actin, G418^R^::DM181</td>
<td>FRAP</td>
</tr>
<tr>
<td>ctxA::GFP-IQGAP2</td>
<td>cortl(RF)::RFP-α-tubulin, Hyg^R^::pDRH; GFP-IQGAP2, G418^R^::pEXP4</td>
<td>FRAP, FCS</td>
</tr>
<tr>
<td>iqg2::GFP-actin</td>
<td>iqgap2(RF)::RFP-α-tubulin, Hyg^R^::pDRH; GFP-actin, G418^R^::DM181</td>
<td>FRAP</td>
</tr>
<tr>
<td>iqg2::GFP-cortexillin I</td>
<td>iqgap2(RF)::GFP-cortl, Hyg^R^::pDRH; G418^R^::DM181</td>
<td>FRAP, FCS</td>
</tr>
<tr>
<td>iqg1::GFP-cortexillin I</td>
<td>iqgap1(RF)::GFP-cortl, Hyg^R^::pDRH; G418^R^::DM181</td>
<td>FRAP</td>
</tr>
<tr>
<td>iqg1/2::GFP-cortexillin I</td>
<td>iqgap1/2(RF)::GFP-cortl, Hyg^R^::pDRH; G418^R^::DM181</td>
<td>FRAP, FCS</td>
</tr>
<tr>
<td>racE::GFP-actin</td>
<td>racE^{24EH6}::RFP-α-tubulin, Hyg^R^::pDRH; GFP-actin, G418^R^::DM181</td>
<td>FRAP</td>
</tr>
<tr>
<td>racE::GFP-cortexillin I</td>
<td>racE^{24EH6}::GFP-cortl, Hyg^R^::pDRH; G418^R^::DM181</td>
<td>FRAP</td>
</tr>
<tr>
<td>racE::GFP-IQGAP2</td>
<td>racE^{24EH6}::RFP-α-tubulin, Hyg^R^::pDRH; GFP-IQGAP2, G418^R^::pEXP4</td>
<td>FRAP</td>
</tr>
</tbody>
</table>
Supplemental Experimental Procedures

Cell strains and Culture
A complete list of the strains used is provided in **Supplementary Table S4**. Cells were grown in Hans’ enriched 1.5X HL-5 media (enriched with 8% FM) containing penicillin and streptomycin at 22°C on polystyrene petri dishes. Wild type strains used were KAx3 [S2], Ax3:Rep orf+ (HS1000) [S3] and rescued strains. Mutant cell lines used have been described previously – myoII [2], ctxA, ctxB, and ctxA/B [S3, S4], iqg1, iqg2 and iqg1/2 [S4], and racE [S3]. The plasmids for RFP-tubulin, GFP-cortexilllin-I, GFP-IQGAP2, GFP-actin, mCherry-myosin-II and GFP have been described previously [S4-S7]. Cells were transformed with expression plasmids by electroporation using a GenePulser-II electroporator (Bio-Rad, Hercules, CA). Cells were then grown in selection medium containing 15 µg/mL G418 or 40 µg/mL hygromycin or both drugs when transforming two plasmids. Expression levels were checked by fluorescence imaging or Western blotting. Cells with comparable fluorescent protein expression were used for the experiments.

Compression by Agarose Overlay
Agarose overlay has been established as a method for applying uniform global mechanical stress, and has previously been shown to drive mechanosensitive accumulation of certain proteins at the cell cortex [S6]. For compression, thin sheets of 2% agarose in MES starvation buffer (50 mM MES pH 6.8, 2 mM MgCl₂ and 0.2 mM CaCl₂) were prepared according to the protocol described by Fukui et al. [8, 9] and modified by Kee et al. [S6]. The cells were plated in imaging chambers for 1 hour. The culture medium was aspirated and cells were washed with MES starvation buffer twice to reduce the background fluorescence. The buffer was removed completely and a sheet of agarose was carefully placed to cover the cells. Imaging was started after the cells were completely flattened (about 2 minutes). The slide was replaced every 10 minutes to ensure proper cell health.

Latrunculin-A and Jasplakinolide Treatment
Latrunculin-A and Jasplakinolide were obtained from Sigma-Aldrich. All cells were pre-treated with 0.1% DMSO for 4-6 hours. For phalloidin and anti-actin staining, the cells were incubated with the drugs for 15 minutes. For live cell imaging, drug stocks were freshly made in MES starvation buffer. The cells plated in imaging chambers were washed with MES starvation buffer + 0.1% DMSO, followed by the addition of the drug-containing buffer. Imaging was performed after 10 minute incubation, and each slide was imaged for 15 minutes before it was replaced with a new slide.
**Fluorescence Recovery after Photobleaching (FRAP)**

FRAP experiments were performed using a Zeiss Axiovert 200 inverted microscope with LSM510-Meta confocal module, with a 63x (NA 1.4) objective. Cells expressing GFP-tagged proteins were plated in glass-bottom imaging chambers for an hour. The culture medium was replaced with MES starvation buffer immediately before imaging. A small region of the cell cortex was bleached using a 488 nm Argon laser, and the fluorescence recovery was recorded until recovery saturated (150 frames, 45-150 ms/frame depending on the protein). The size and placement of the bleach region was kept relatively constant across measurements.

For each frame, the average intensity of the bleached cortical region, reference (unbleached) region, and background was quantified using ImageJ (National Institutes of Health, Bethesda, MD) (Fig. S1A-B). For photobleaching correction, the reference theoretical intensity (RTI) was calculated by fitting the background subtracted reference intensity to an exponential decay equation as follows:

\[ RTI (t) = A - B \cdot e^{-Ct} \]  

Where, A, B and C are fitting parameters.

The intensity of the bleached region was background subtracted and normalized to RTI (Fig. S1C). The normalized intensity (NI) was obtained by normalizing this to the pre-bleach intensity (average of 4 pre-bleach images), and was fitted to a single exponential as follows:

\[ NI (t) = m_1 (1 - m_2 \cdot e^{-kt}) \]  

Where, \( m_1, m_2 \) are fitting parameters and \( k \) is the recovery rate.

The recovery time, \( \tau \), and the immobile fraction, \( F_i \) were measured as:

\[ \text{Recovery time, } \tau = \frac{1}{k} \]  

\[ \text{Immobile fraction, } F_i = \frac{1-m_1}{1-m_1+m_2} \]  

We also plotted the derivative of normalized intensity for each protein to confirm that our data fit a single-exponential, and did not require more complicated models.

**Fluorescence Correlation Spectroscopy (FCS)**

FCS experiments were performed using a Zeiss AxioObserver with 780-Quasar confocal module & FCS, with a C-Apochromat 40x (NA 1.2) water objective. For purified proteins and dyes, the imaging plane was set 200 μm above the coverslip. 10 repetitions of 5 seconds each were collected and the
average spectrum was used for measuring diffusion times. For diffusion measurements in cells, the imaging plane was set through the middle of the cell. The acquisition time was reduced to 2 seconds to avoid complications from long distance cellular and intracellular movement. The average from 2-7 repetitions was used to calculate diffusion times. Any trace showing a persistent deviation from the mean or significant photobleaching was discarded. The autocorrelation data was then fit to a single component, 3D-diffusion model with triplet state dynamics using the following equation:

\[ g(\tau) = G_{\text{triplet}} \cdot \frac{1}{N} \cdot (1 + \frac{\tau}{\tau_D} )^{-1} \cdot (1 + \frac{\tau}{\frac{\gamma}{\tau_D}})^{-0.5} \]  

(5)

Where,
\( \tau \) = correlation time  
\( \tau_D \) = diffusion time  
N = number of particles  
\( \gamma \) = structural parameter

\[ G_{\text{triplet}} = \frac{1 - \theta_T + \theta_T \cdot e^{-\tau/\tau_T}}{\theta_T} \]

(\( \theta_T \) = fraction of particles in triplet state and \( \tau_T \) = relaxation time for triplet state).

100 nM Rhodamine 6G was used for pinhole alignment and structural parameter calculation (Fig. S1F-H). The measured value of the structural parameter was used for diffusion time calculation of other proteins. All imaging was done in uncoated 35 mm-glass bottom dishes (Coverslip No. 1.5) (MatTek Corp., Ashland, MA).

In compressed cells, the cell height is comparable to the z-dimension of the FCS confocal volume. Therefore, we also analyzed the FCS data using a 2D-diffusion model and observed no significant differences in diffusion times as compared to those calculated using the 3D-diffusion model.

**Calculation of Diffusion Coefficients**

Rhodamine 6G was used as a standard for calculating diffusion coefficients. As the diffusion coefficient is inversely proportional to the diffusion time, we used the published value of the diffusion coefficient for rhodamine 6G and measured diffusion times for rhodamine 6G and the proteins of interest to calculate effective diffusion coefficients (\( D_{\text{eff}} \)).

\[ D_{\text{eff}} = \frac{D_{\text{Re}} \cdot \tau_{D,\text{Re}}}{\tau_D} \]  

(6)

Where,
\( D_{\text{Re}} \) = diffusion coefficient for rhodamine 6G (= 426 \( \mu \text{m}^2/\text{s} \) [S1])
\( \tau_{D,R6} = \) measured diffusion time for rhodamine 6G = 33 ± 2 μs

\( \tau_D = \) measured diffusion time.

We used purified mCherry to validate our diffusion coefficient calculation. Our measured diffusion coefficient for mCherry (94 μm²/s) matched closely the published value for purified GFP (95 μm²/s [S1]) under the same conditions (Supplemental Table S3).

**Phalloidin and Anti-actin Staining for F-actin Quantification**

For quantifying the relative amount of F-actin, the cells were fixed and stained with TRITC-phalloidin (Sigma Aldrich) or anti-actin monoclonal antibody (Developmental Studies Hybridoma Bank, University of Iowa) as described in Luo et al. [10]. Dictyostelium cells were plated on sterile 22x22 mm glass coverslips in 6-well polystyrene dishes at 70-80% confluency for 1 hour. The media was aspirated and replaced with 2 mL drug-containing media for 15 minutes. The cells were washed with 1X PBS, and immediately fixed on ice using acetone at -20°C for 3 minutes. The coverslips were transferred to a new 6-well dish and washed once with 1X PBS, followed by blocking in blocking buffer (1X PBS + 0.05% Triton X-100 + 0.5% BSA) for 30 min. The cells were stained with 0.16 μM TRITC-phalloidin for 1 hour or with anti-actin antibody overnight followed by 2 hour incubation with TRITC goat-anti-mouse secondary. All coverslips were washed 4 times with 1X PBT (1X PBS + 0.05% Triton X-100) for 5 minutes each, and then once with 1X PBS. The coverslips were then mounted on glass slides using 10 μL mounting buffer (90% glycerol in 1X PBS).

To quantify the relative amount of F-actin, all coverslips were imaged under identical conditions on a motorized Olympus IX71 microscope using a 40x (NA 1.3) objective with a 1.6x optovar (Olympus, Center Valley, PA). The integrated fluorescence intensity of the cells was quantified using ImageJ. At least 100 cells from more than 10 different fields were quantified. The intensity was normalized to the average fluorescence intensity of the untreated control for a given experiment. The data shown represents three biological replicates.

**Cortical Tension Measurement Using Micropipette Aspiration**

The experimental set-up has been previously described in detail in Effler et al. (2006) [S5]. 0.01-0.6 nN/μm² pressures were applied to a smooth region of the cell cortex through a ~5 μm internal diameter glass micropipette (R_p = 2.3-3 μm). A low pressure was first applied to form a pressure seal. The cell protrusion was allowed to stabilize for 30 seconds before imaging. Subsequently, the pressure was gradually increased and imaging was resumed after the protrusion stabilized. This
was continued until the protrusion length became large ($L_p > R_p$) or the cell blebbed. At each pressure, the protrusion length from five consecutive frames was averaged. The critical pressure ($\Delta P_{\text{crit}}$) was identified as the pressure where $L_p = R_p$, and the cortical tension ($T_{\text{eff}}$) was calculated using the following equation:

$$\Delta P_{\text{crit}} = 2 T_{\text{eff}} \cdot \left( \frac{1}{R_p} - \frac{1}{R_c} \right)$$  \hspace{1cm} (7)

**Supplemental References**


