

Molecular mechanisms of cellular mechanosensing

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Mechanical forces direct a host of cellular and tissue processes. Although much emphasis has been placed on cell-adhesion complexes as force sensors, the forces must nevertheless be transmitted through the cortical cytoskeleton. Yet how the actin cortex senses and transmits forces and how cytoskeletal proteins interact in response to the forces is poorly understood. Here, by combining molecular and mechanical experimental perturbations with theoretical multiscale modelling, we decipher cortical mechanosensing from molecular to cellular scales. We show that forces are shared between myosin II and different actin crosslinkers, with myosin having potentiating or inhibitory effects on certain crosslinkers. Different types of cell deformation elicit distinct responses, with myosin and α -actinin responding to dilation, and filamin mainly reacting to shear. Our observations show that the accumulation kinetics of each protein may be explained by its molecular mechanisms, and that protein accumulation and the cell's viscoelastic state can explain cell contraction against mechanical load.

Cells are the ultimate smart material, being capable of self-renewal, self-repair and self-defence through mechanisms that include the regulation of the cells' physical properties¹. To accomplish these features, cells must be able to sense and respond to mechanical inputs. Tremendous effort has been invested in understanding how they sense mechanical cues from substrates through focal adhesions^{2–4}. However, not all mechanosensation is mediated through focal adhesions. As an active material, the actin cytoskeleton is a highly dynamic network, which senses mechanical stimuli, remodels its own microstructures and activates associated signalling pathways^{5,6}. These properties are essential for many cellular events including cell division, differentiation, migration, morphogenesis and stem cell fate determination^{3,7–12}. In *in vitro* assembled actin networks, the evolution of the microstructures is a result of active forces due to actin polymerization and myosin II contractility¹³. The force-dependent behaviours of the main cytoskeletal proteins have been well characterized in single-molecule assays¹⁴, and the mechanical properties of *in vitro* assembled actin networks with different concentrations of cytoskeletal proteins have been systematically explored^{15–21}. However, the kinetic mechanisms of the mechanosensory behaviours of the proteins, the quantitative links between the different hierarchical levels (from molecules to cells to tissues), and how these networks sense forces in living cells are largely unknown. These unresolved issues not only limit our understanding of mechanosensing at different scales but also hamper our ability to design smart materials using cellular components.

Here, by combining molecular and mechanical experimental perturbations of the social amoeba *Dictyostelium discoideum* with multiscale modelling, we identify a complex molecular landscape for force sensing and transmission through the cortical cytoskeleton. We demonstrate experimentally that the magnitude of myosin II mechanosensitive accumulation is modulated by the presence of actin crosslinkers, which we interpret as force sharing between myosin II and the crosslinkers. Furthermore, we reveal that myosin II, α -actinin and filamin react to different deformations, and demonstrate how their molecular mechanisms

account for the cellular-scale response of these proteins. Finally, we show how the mechanosensitive accumulation of myosin and actin-crosslinking proteins as well as the cell's viscoelastic properties account for the dynamics of monotonic and oscillatory contractility in cells, which could explain the oscillatory contractile behaviours observed in some tissues^{22,23}.

The actin cytoskeleton is composed of myosin II motors, actin filaments and actin crosslinkers, which are physically linked to the membrane by anchoring proteins. To determine the spectrum of force transmission in the cell cortex in the absence of focal adhesions, we analysed 37 proteins, each tagged with a fluorescent protein, for changes in their localization in response to mechanical stress (Supplementary Table S1). To apply this stress, we primarily used micropipette aspiration, which allows precisely controlled forces to be applied to specific regions along the cell surface²⁴. Micropipette aspiration has proved to be a useful approach for studying myosin II mechanosensitive accumulation in several systems, including *Dictyostelium*^{7,25–27}, *Drosophila* embryos²⁸ and mammalian stem cells (D. E. Discher, personal communication). Twenty-three of these proteins, most of which are components of the cortical cytoskeleton–membrane composite (Fig. 1a), were identified as being of interest for further characterization in wild-type (WT) cells and selected genetic mutants. This analysis also uncovered several key features of cellular mechanosensitivity, which we then evaluated theoretically.

We established a baseline by characterizing the mechanosensitive response of myosin II in interphase WT cells and comparing it with that in mitotic cells^{7,26}. When interphase cells were aspirated by a micropipette, we observed that myosin II accumulated in the tip inside the pipette, where the highest dilation of the cortex occurs²⁹ (Supplementary Movie S1). Under constant aspiration pressure, myosin II accumulation accelerated until reaching its peak (Fig. 1b), suggesting the presence of cooperativity in this type of mechanosensory response. Greater applied pressures also led to higher levels of accumulation (Fig. 1c). Moreover, at the same pressures, the accumulation of myosin II increased with increased lever-arm lengths for engineered myosins (Fig. 1c). The lever-arm length, not motor speed, was the critical determinant of

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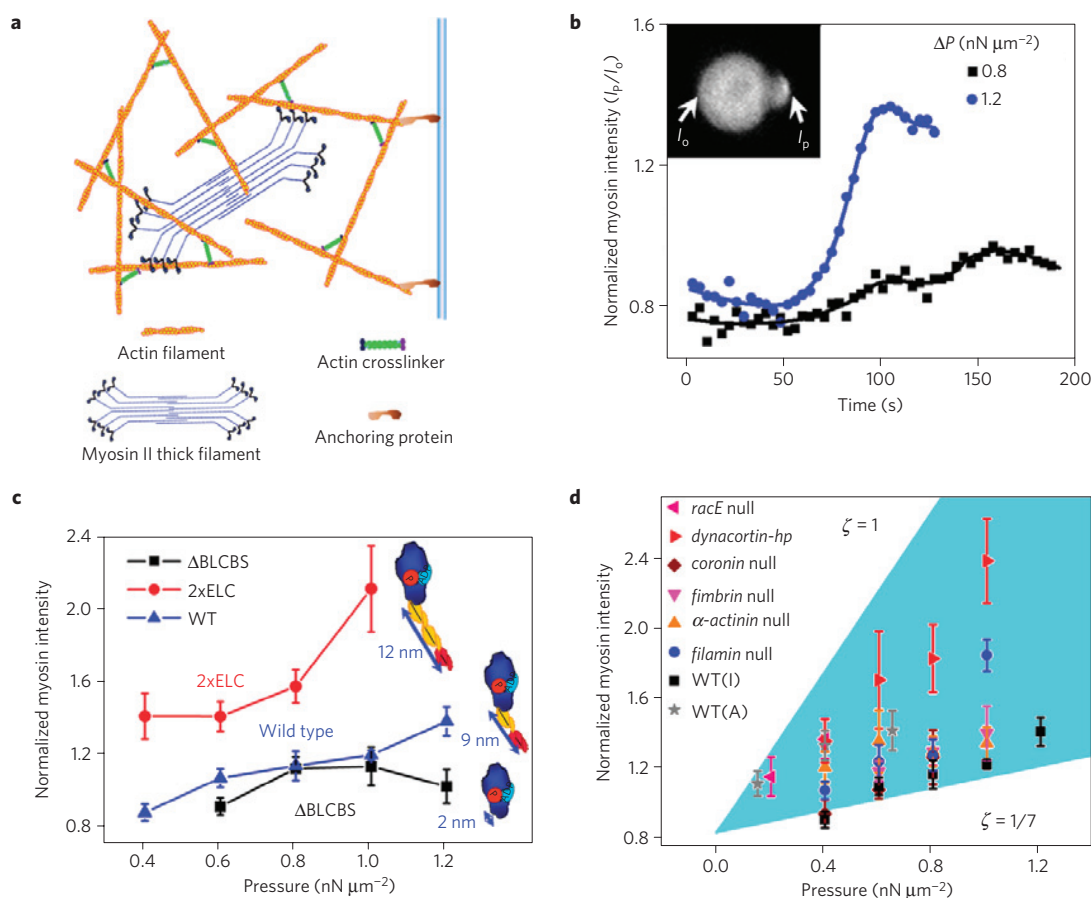


Figure 1 | Mechanosensitivity of myosin II in interphase *Dictyostelium* cells. **a**, Schematic diagram of the cytoskeleton-membrane complex. **b**, Time course of myosin II accumulations during micropipette aspiration. The data are from one representative cell at two different pressures. **c**, Force dependency of the accumulation for myosin II motors with WT and altered lever-arm lengths. In this and other panels, normalized intensity is taken at its peak. Dot plots of all data are provided in Supplementary Fig. S2. **d**, Normalized intensity of the aspirated region for various *Dictyostelium* strains. The shaded region is bounded by the lines where $\zeta = |\mathbf{F}_{\text{myosin}}|/|\mathbf{F}_{\text{internal}}|$ equals 1 and 1/7. I and A represent interphase and anaphase, respectively. Dot plots of all data are provided in Supplementary Fig. S3. A schematic graph for force-sharing and force-transmission is shown in Supplementary Fig. S5. Error bars in **c,d** correspond to s.e.m. and are based typically on $n \geq 10$ at each point.

mechanosensitivity. This is demonstrated by the fact that the S456L uncoupler motor (a mutant with shorter step size, slower ADP release and 10-fold slower unloaded actin-filament sliding velocity, but normal ATP hydrolysis and lever-arm length) showed strong mechanosensitive accumulation (Supplementary Fig. S2). Finally, only intact myosin II accumulated because neither the myosin motor domain (myosin S1) nor the long coiled-coil tail domain (BTF) assembly are both essential for mechanosensitive accumulation of myosin (Supplementary Fig. S2). These results, especially the myosin lever-arm-length dependency, demonstrate that mechanical stress is directly felt by the cortex where it promotes myosin II accumulation, rather than acting through a signalling pathway that is activated by mechanical stress acting on the plasma membrane.

The force-dependent accumulation of non-muscle myosin II at the cellular scale may be explained by using a molecular level catch-bond model. This class of models has been proposed as a simplification of the actin-myosin interaction during the myosin cross-bridge cycle^{30–33}. In the model, the effective off-rate of the myosin head from the actin filament, k_{off} , is a function of force applied to the head $k_{\text{off}} \propto k_{\text{off}}^0 \exp(-f\Delta x/k_B T)$, where k_{off}^0 is the off-rate in the absence of force, f is the force applied on myosin, and Δx is the bond length. Smaller values of k_{off}^0 imply that

myosin heads bind longer to F-actin, leading to greater myosin accumulation. Thus, the relation $\Delta x_{2\text{xELC}} > \Delta x_{\text{WT}} > \Delta x_{\Delta\text{BLCBS}}$ between the bond lengths of these myosin proteins with different lever-arm lengths³⁴ indicates that 2xELC (extra essential light chain binding site) myosin should have the highest accumulation of these motors, whereas ΔBLCBS (deletion of both light chain binding sites) myosin should have the lowest accumulation, provided that the applied force is the same for all three myosin II motors. This prediction matched the experimental observations (Fig. 1c). Although the mechanosensitive accumulation of myosin II in interphase and mitotic cells is qualitatively similar^{7,26}, interphase cells required a higher pressure range (see Supplementary Fig. S2a for comparison).

The difference in the mechanosensitive response between interphase and mitotic cells could be a result of the depletion of actin crosslinkers in the polar regions of mitotic cells³⁵. Consistent with this view, we further observed that myosin II accumulation in various mutants, especially *dynacortin* and *racE* mutants, in which actin crosslinkers were deleted was higher than that in WT cells over a wide force range (Fig. 1d). In contrast, the mechanosensitive accumulation of myosin II was reduced in mutants where the physical link between the actin cortex and the plasma membrane was disrupted. To this end, we studied cells in which anchoring proteins (cortexillin I, myosin I D, E and

F isoforms, or enlazin, which is the *Dictyostelium* ezrin–radixin–moesin family protein) were deleted, or cells missing PTEN, which catalyses the formation of the lipid phosphatidylinositol 4,5-bisphosphate (Fig. 1a). These data suggest that the applied force is transmitted by anchoring proteins from the plasma membrane to the actin cortex where it is shared among myosin II and the cortical actin crosslinkers. The force sharing here is analogous to that proposed for cell adhesion^{31,36,37}.

To test this force sharing further, we plotted the myosin II mechanosensitive response for a number of strains (Fig. 1d and Supplementary Figs S3a and S5). All of the data fell in a region that is bounded by two lines that correspond to different assumptions regarding the fraction (ζ) of the total internal force in the actin cytoskeleton borne by myosin II. These two lines were obtained by solving reaction–diffusion equations describing the mechanosensitive accumulation of myosin II thick filaments²⁵ (Supplementary Information). In the graph, WT cells fall near the line corresponding to $\zeta = 1/7$ consistent with the fact that myosin II contributes ~ 10 – 20% of the cortical tension of WT cells (Supplementary Fig. S1c). At the other extreme, if $\zeta = 1$, then 100% of the force would be carried by myosin II and the actin crosslinkers would bear no force. This would correspond to a hypothetical mutant in which all actin crosslinkers are inactivated or deleted. The *dynacortin*-depleted cells and *racE* null cells, which have reduced levels of several actin crosslinkers at the cortex³⁸, come close to this limit (Fig. 1d).

These data suggest a simplified picture where the cytoskeleton–membrane composite can be viewed structurally as an assembly of dynamic elastic components (Supplementary Fig. S5). The membrane is coupled to the actin network through membrane-anchoring proteins, which function in series with the cytoskeleton. Myosin II and actin crosslinkers then act in a parallel fashion with each other. Thus, disruption of the link between the membrane and the cytoskeleton reduced force transmission and hence myosin II experienced less force, leading to lower mechanosensitive accumulation. On the other hand, depletion of any actin crosslinkers redistributed the force that would be borne by that actin crosslinker onto the remaining proteins, resulting in greater myosin II mechanosensitive accumulation. Consistent with this observation, a plot of the myosin mechanosensitive accumulation as a function of the measured cortical tensions for each of the actin crosslinker mutant cell lines revealed a negative correlation between these parameters (Supplementary Figs S1c and S3b).

Having established that actin crosslinkers modulate the response of myosin II to external force, we next studied the effect of myosin II on the mechanosensory responses of actin crosslinkers. Cells expressing a number of fluorescent-protein-labelled proteins in the presence and absence of myosin II were aspirated at a fixed applied pressure of $1.0 \text{ nN } \mu\text{m}^{-2}$ and the protein accumulation at the tip was quantified (Fig. 2a and Supplementary Fig. S6). These data showed that myosin had different effects on the accumulation at the tip of various actin crosslinker proteins. For example, cortaxillin I accumulation correlated with and depended on myosin II, as previously observed in mitotic cells (Supplementary Fig. S7). However, myosin II was antagonistic with α -actinin as the latter accumulated only in the absence of myosin II (Supplementary Movie S4). Further, α -actinin accumulation increased with time (Fig. 2b) and with increasing applied force (Fig. 2c). Most actin crosslinkers showed weak myosin II dependency (Supplementary Fig. S6). The accumulations of anchoring proteins, such as talin B and myosin IE, was markedly affected by myosin II. We also imaged actin polymers using fluorescent-protein-labelled actin, Lifeact and LimE- Δ coil, all of which exhibited transient, low-level accumulations in WT cells. These accumulations were more dynamic and did not correlate with the myosin II increase but

were instead similar to the patterns of actin waves documented by others³⁹ (Supplementary Movies S2 and S3). These probes failed to accumulate in *myoII* null cells, indicating that actin accumulation by itself is not mechanosensitive to the applied pressure and does not provide the driving force for the myosin II accumulations in the various mutants described here.

So far, we have described protein accumulations only in the tip region of the aspirated cortex. However, as we analysed the accumulation of proteins in other mutant strains, we found that in *racE* null cells, filamin alone (of 20 proteins tested) localized along the neck of the pipette (Supplementary Movies S5 and Figs S9 and S10 and Fig. 3). Filamin also accumulated at the neck in cells depleted of dynacortin using an RNA-mediated interference hairpin plasmid (*dyn-hp* cells³⁸; data not shown), but not in the other tested *Dictyostelium* strains. We conjectured that the different accumulations could be due to responses to varying types of deformation. To test this, we calculated the strain field of a deformed cell in a micropipette using coarse-grained molecular simulations (Supplementary Fig. S8). The deformation due to dilation had its highest value at the tip region (Fig. 3a), and the deformation due to shear is greatest at the neck region adjacent to the entrance of the micropipette (Fig. 3b). Thus, the spatial accumulation patterns of myosin II (in various cell lines) and that of α -actinin in *myosin II* null cells coincided with the dilation strain field suggesting that myosin II and α -actinin were sensitive to dilation strain/stress (Fig. 3c). On the other hand, filamin localization along the neck of the pipette suggests that filamin is sensitive to shear deformation. As multiple actin crosslinking and bundling proteins (including dynacortin) are downregulated from the cortex in *racE* null cells⁴⁰ and dynacortin depletion itself allowed filamin to accumulate at shear-strained domains, these results suggest that the actin network structure and composition in these mutant cells is more conducive for filamin accumulation in response to shear stress^{19,20,41}. We further tested the hypothesis that these proteins respond to specific deformations using a cell compression assay. As expected, we observed that myosin II and α -actinin tracked the dilated regions whereas filamin accumulated at the sheared regions (Supplementary Fig. S11).

To test quantitatively the hypothesis that different proteins are sensitive to different types of cellular deformation, we identified different mechanisms for the force-dependent binding of myosin II (Fig. 3e, Supplementary Fig. S12 and ref. 25), α -actinin, and filamin to F-actin. We then carried out simulations in a three-dimensionally deformed cell shape based on experimentally measured kinetic values for diffusion, binding and assembly of their functional units (Supplementary Information). The functional unit of myosin II is the assembled BTF, which generates contractile force, whereas the actin crosslinkers α -actinin and filamin form rod-shaped⁴² and V-shaped dimers⁴³, respectively. In these simulations, the dilation and shear strain/stress profiles (Fig. 3a,b) obtained from coarse-grained simulations were used as inputs. Previously, we demonstrated that the catch-bond model together with a model of myosin BTF assembly can account for force-dependent myosin II accumulation²⁵. Here, to account for the force-dependent accumulations of α -actinin and filamin, we tested four types of bond formation: force-independent bonds, slip bonds, simplified catch bonds, and structural cooperativity (Supplementary Figs S13–S17). We found that a simplified catch-bond model based on measured force-dependent bond parameters for α -actinin⁴⁴ reproduced the accumulation levels and accounted for the deformation-type specificity (Fig. 3f and Supplementary Fig. S13). For filamin accumulation, we found that structural cooperativity resulting in increased on-rates during binding was required along with the catch-bond behaviour observed in single-molecule studies⁴⁴ (Fig. 3g and Supplementary Fig. S16f,g). The structural cooperativity proposed here could result from the

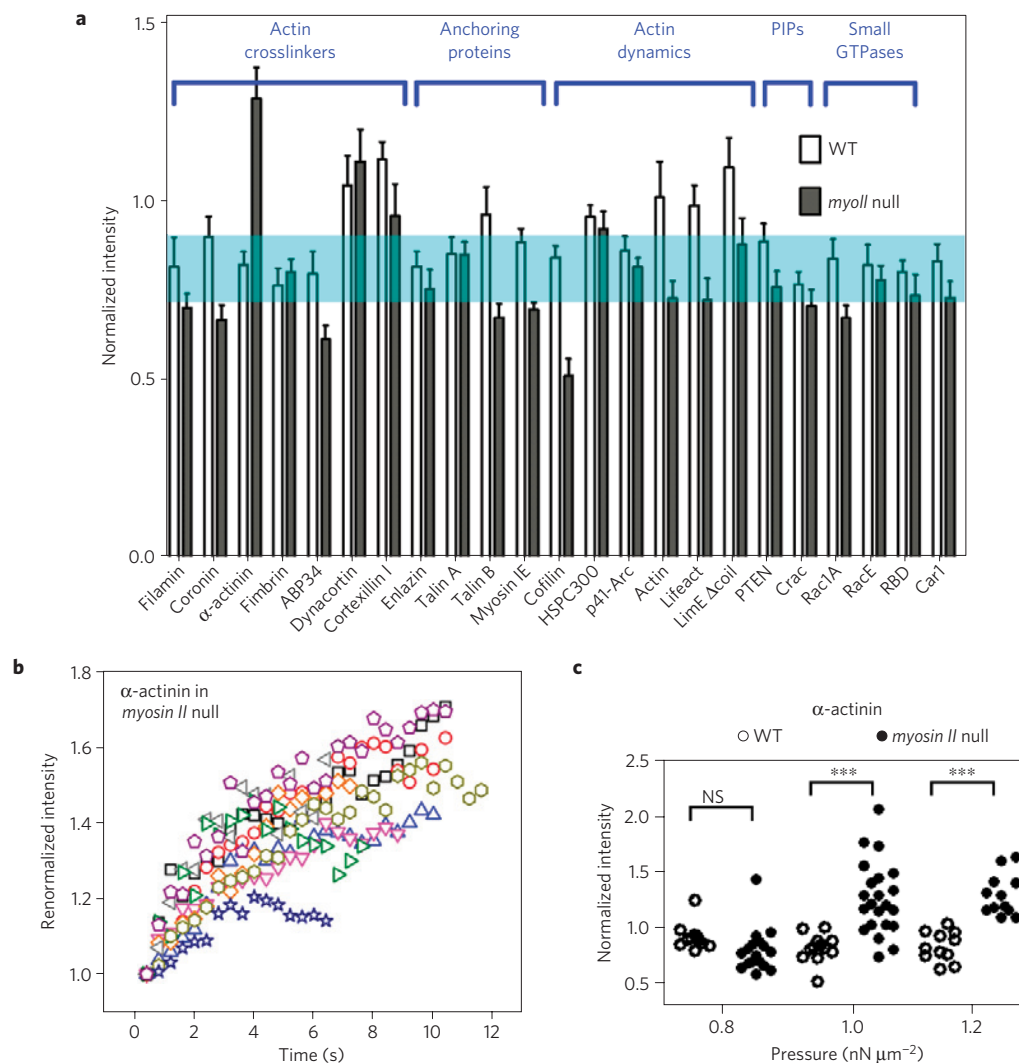


Figure 2 | The responses of different actin-associated proteins to aspiration pressure. a, Different actin-associated proteins have distinctive mechanosensitive accumulations in the presence and absence of myosin II ($n > 10$ cells for each measurement; details in Supplementary Fig. S6). The shaded region shows the mean \pm s.e.m. of a soluble green fluorescent protein (GFP) volume marker⁸. **b**, The kinetics of α -actinin accumulation in *myosin II* null cells. **c**, The pressure-dependence of α -actinin accumulation. NS and *** denote not significantly different and $p < 0.005$, respectively.

V-shape of the filamin dimer (Supplementary Information). The simplified catch bond is sufficient for these simulations because our experimental approach probes only low- to mid-range forces. However, a model (Supplementary Information) similar to those used in studies of muscle myosin II (ref. 45) and selectin^{46,47} that incorporates a catch-slip transition and accounts for the rupturing of bonds at higher force regimes also reproduces the mechanosensitive accumulation observed in our experimental data (Supplementary Figs S15 and S17).

Our experiments and simulations showed that myosin II and α -actinin accumulate in the pipette tip whereas filamin accumulates in the neck region (Fig. 3d and Supplementary Movies S6–S8). More importantly, both the magnitude and kinetics obtained from simulations are in good agreement with experimental observations (Fig. 3e–g), indicating that different proteins are indeed sensitive to different types of cytoskeletal deformation (Fig. 3a). The proposed molecular mechanisms for dilation and shear (dilation could occur through the sliding between parallel and anti-parallel actin filaments, and shear could involve angle changes between actin filaments) might be responsible for the force-induced accumulations. As the catch-bond properties and

actin-binding domains associated with these proteins are relatively similar^{44,48,49}, the observed deformation specificity of these proteins probably comes from the overall crosslinker architecture (anti-parallel rods versus V-shaped).

One consequence of the force-induced accumulation of these proteins is the increase of local cortical tension and therefore the resistance to further deformation. Myosin II, in particular, provides local contractility and generates active force to counteract external force. When the contractile force is large enough, cells pull themselves back from the micropipette without forming blebs⁵⁰ (Fig. 4). We tested this model of contractility-driven cell retraction using simulations (Supplementary Information). We used the experimentally measured myosin II intensities to account for forces. These forces, along with the viscoelastic properties of each particular cell type, led to simulated cell lengths that matched the monotonic contractility in WT and most mutant cell types (Fig. 4a and Supplementary Figs S18 and S19) as well as the myosin II-driven oscillations observed in *racE* null cells (Fig. 4b and Supplementary Fig. S18 and Movie S9). Although other cytoskeletal proteins such as α -actinin also accumulated in the tip region, their accumulation in the absence of myosin II did not cause noticeable

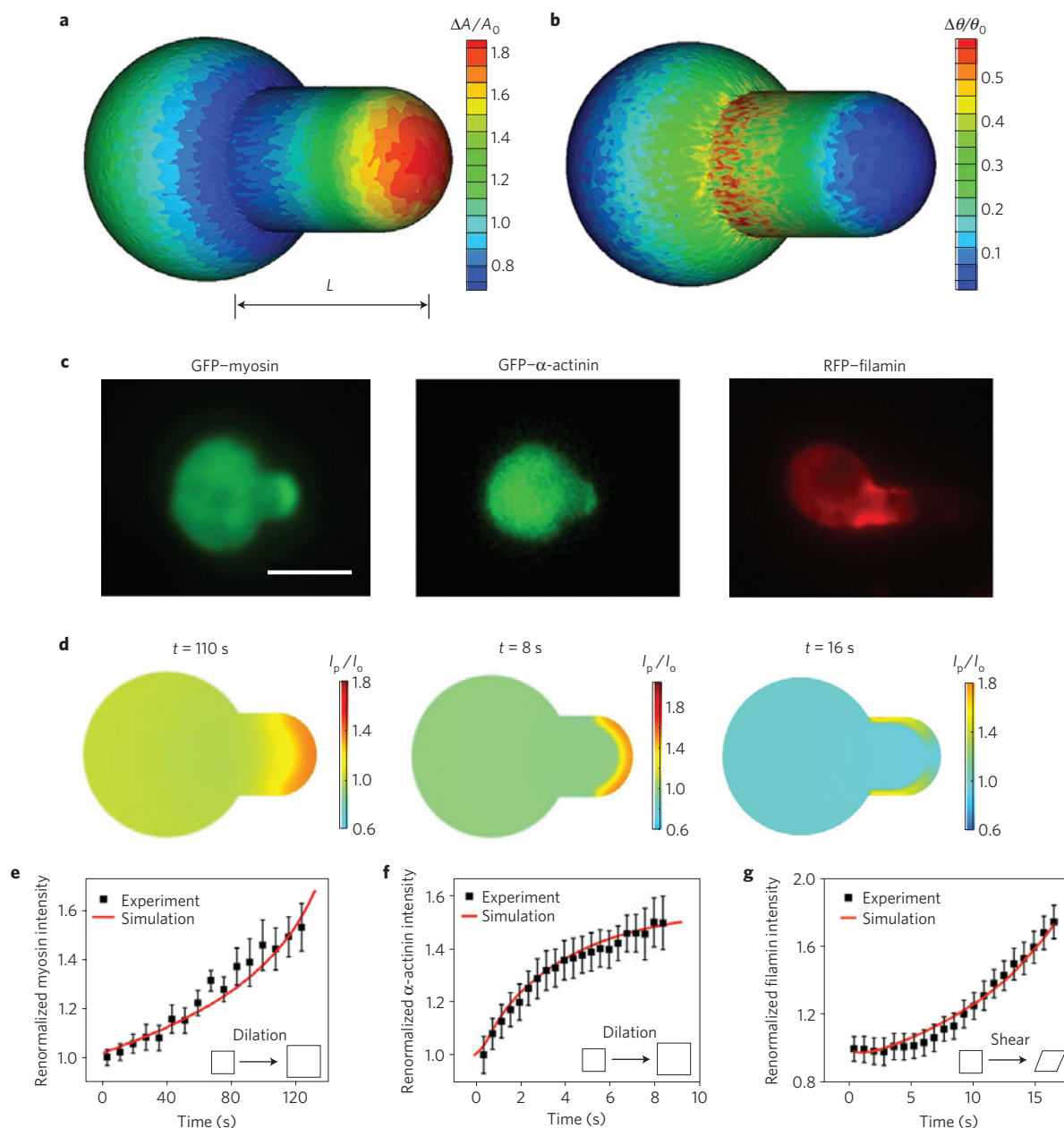


Figure 3 | Deformations and corresponding protein accumulation during micropipette aspiration. **a,b**, The dilation and angle-change of the cytoskeleton-membrane complex, respectively, calculated using coarse-grained molecular mechanics. **c,d**, The experimentally observed and simulated accumulations of myosin II, α -actinin and filamin. **e-g**, Comparisons between the measured kinetics of the accumulations and the ones simulated using the corresponding force-dependent binding models for these proteins (Supplementary Information). The insets in **e-g** show the type of deformation to which each protein responds. Error bars in **e-g** correspond to the s.e.m. Scale bar in **c**, 10 μm . Model parameters can be found in Supplementary Tables S2 and S3.

retractions (Supplementary Movie S4), indicating that myosin II is necessary for cell retraction.

By combining molecular, genetic and mechanical perturbations with multiscale modelling, we accounted for the cell's response to mechanical deformation on the basis of molecular mechanisms (Fig. 4c). We found that some cytoskeletal proteins accumulate to deformation sites in response to mechanical stimuli, and these different proteins are responsive to specific types of deformation. Both cooperative and antagonistic interactions exist between myosin II and actin crosslinkers, and these mechanosensitive protein accumulations can be predicted quantitatively by their corresponding force-dependent binding reactions.

Our data suggest that the crosslinking proteins may be separated on the basis of three different categories of behaviour. These categories are the types of deformation (dilation versus shear) to which they respond (for example, α -actinin versus filamin), whether they show myosin II dependency in their mechanosensitive accumulation (for example, α -actinin versus cortexillin I), and the magnitude of impact they have on cortical tension and the mechanosensitive accumulation of myosin II (for example, dynactin versus fimbrin). Different isoforms of a given crosslinker might have considerably different actin affinities, which might translate into further variations in a cell's mechanosensitivity⁴⁹. Thus, actin-crosslinking

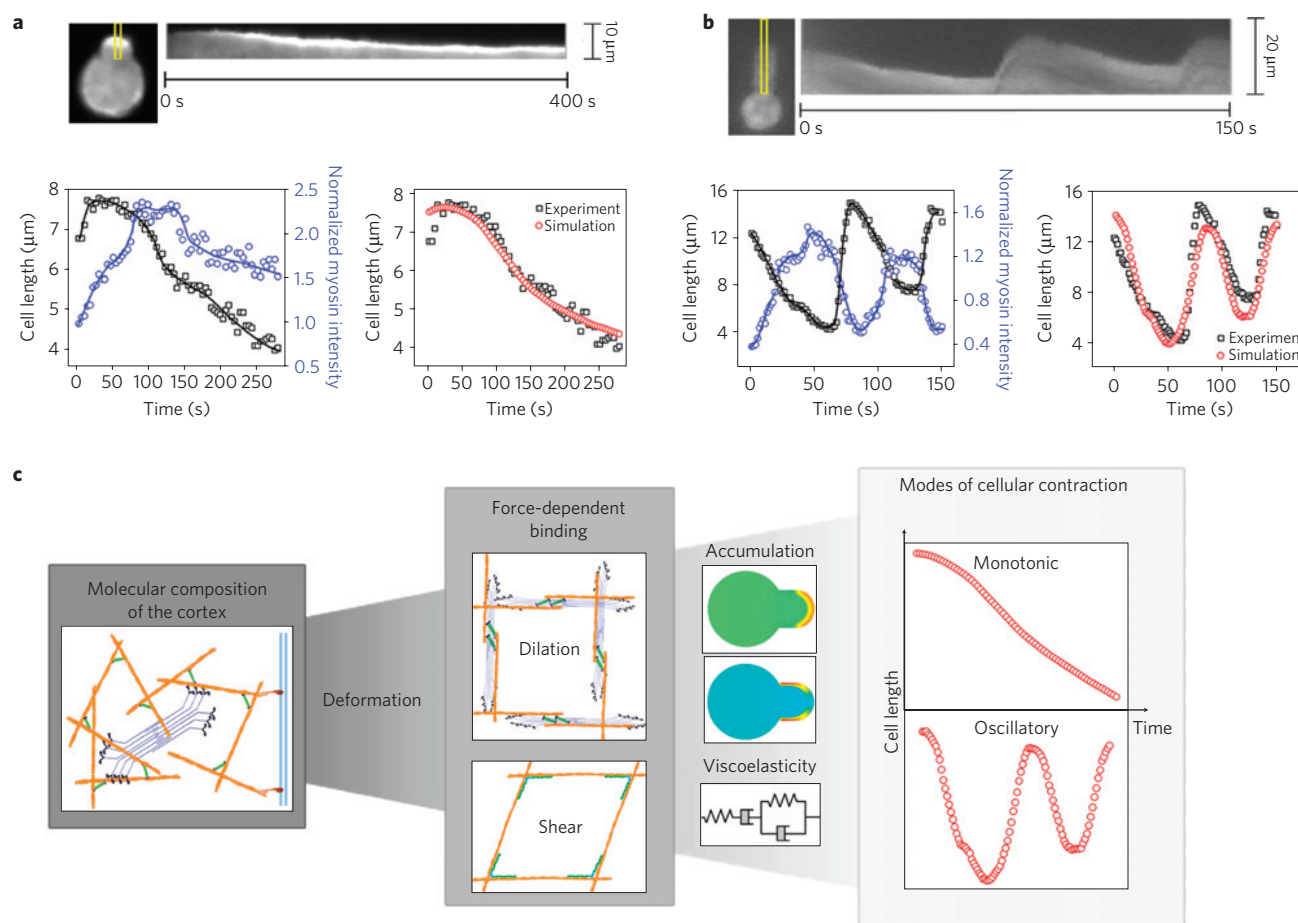


Figure 4 | Retraction of cells due to the accumulation of cytoskeletal proteins. a, b, Retractions of *filamin* null (**a**), and *racE* null (**b**) cells. Kymographs show the accumulation of myosin II along the marked region. Lower left panels show the cell length (squares) and myosin II intensity (circles). The lines depict the trends. Lower right panels compare the experimentally measured aspiration length to that calculated from the viscoelastic model using the measured myosin II intensity as an input. **c,** The flow diagram summarizes the linking of the hierarchical levels of cell shape change. From the molecular (composition and properties of cortical cytoskeletal proteins) to network (types of deformation and force-dependent binding) to cellular scale (protein accumulation and viscoelastic state), the modes and kinetics of cellular contractility may be explained quantitatively. Model parameters can be found in Supplementary Tables S2–S5.

proteins provide considerable richness in how cells can respond to mechanical stress inputs, and much work will be required to discern how the specific crosslinker structures and regulation tune these features.

Further, the antagonistic interactions between myosin and α -actinin might help explain the segregation of myosin II from α -actinin in self-organized actin structures such as stress fibres and sarcomeres^{51,52}. Moreover, in addition to filamin GAP, which is a downstream effector of filamin and is sensitive to shear stress⁶, our results demonstrate that filamin itself can accumulate in response to shear stress when other major crosslinking proteins are absent. As the proteins studied here are important for the differentiation and migration of *Dictyostelium discoideum* as well as other organisms^{5,6,11}, we expect that these mechanisms will greatly impact the understanding of these biological systems. Finally, the deformation specificities of α -actinin and filamin follow the different structures of their dimers (rod-shaped versus V-shaped), suggesting a potential strategy for designing new strain sensors for filamentous biomaterials such as collagen and fibronectin.

Overall, we have demonstrated how protein accumulations proceed and contractility is generated in an active material, the actin cytoskeleton–membrane composite, on the basis of molecular mechanisms and the local microstructures of the cell cortex. We

can now directly interpret how the active force generated by the cell cortex and the viscoelasticity of the cells themselves govern the dynamics of cell shape changes during many other biological events, such as cell division⁸, cell–cell adhesive interactions in tissues^{22,23}, and epithelial tube formation^{8,53}. The molecular mechanisms of cellular mechanosensing revealed here not only confer on the cell a self-defence mechanism for rejecting unwanted deformations, but may also allow for the artificial tuning of mechanosensitivity by genetic and pharmacological manipulation for medical purposes. From a materials research perspective, this quantitative understanding may provide guidance for the design of smart materials and strain sensors using cellular components.

Methods

Cell culture and plasmids. WT and mutant *Dictyostelium* strains were grown at 22 °C in Hans' enriched HL-5 media. Plasmids were transformed using electroporation and transformants were selected using the appropriate selection medium, which included either G418 or hygromycin. A complete list of fluorescent protein expression plasmids can be found in Supplementary Table S1. The strains used in this study are: WT, which includes Ax3(replicase orf+) and *myosin II* null rescued with myosin II; *myosin II* null (*mhcA*[−]; strain ID DBS0236379); *cortexillin I* null (*ctxA*[−]; strain ID DBS0235598); *filamin* null (*abpC*[−]; strain ID DBS0236167); α -actinin null (*abpA*[−]; strain ID DBS0235459); *fimbrin* null (*fimA*[−]; strain ID DBS0236175); *coronin* null (*corA*[−]; strain ID DBS0236174); *racE* null (3G1; strain

ID DBS0235415); *pten* null (*pten*^{-/-}; strain ID DBS0349884); *myosin I DEF* null (*myoI D*^{-/-}; ref. 54).

Measurements of mechanosensory response of proteins using micropipette aspiration. Micropipette aspiration was performed as described previously^{7,26}. In short, to apply aspiration pressure, the pressure difference was generated by adjusting the height of a motor-driven water manometer. The *Dictyostelium* cells expressing desired fluorescent proteins were loaded into the observation chamber filled with sterile filtered MES buffer (50 mM MES at pH 6.5, 2 mM MgCl₂ and 0.2 mM CaCl₂). The images were collected using an Olympus IX81 microscope equipped with Metamorph software and analysed using ImageJ (<http://rsbweb.nih.gov/ij/>). After background correction, the fluorescence intensity at the accumulation sites inside the micropipette was normalized against the opposite cortex in each frame to account for photobleaching. The fluorescence signals are assumed to be linearly proportional to the concentrations of the corresponding protein.

Measurements of mechanosensory response of proteins using agar overlay. The compression assay was conducted as described previously⁸. The images were collected on an Olympus IX81 microscope or a Zeiss 510 Meta confocal microscope.

Statistical analysis. Statistical analysis was performed with the software Graph Prism (www.graphpad.com) or Kaleidagraph (Synergy Software). The Mann–Whitney test was used for the non-parametric comparisons for different data sets. In the figures, the asterisks *, ** and *** indicate that the calculated *P* value was less than 0.05, 0.01 and 0.005, respectively. Samples not significantly different are denoted with the abbreviation NS. Data sets were also analysed by analysis of variance with a Fisher's least significant difference comparison, which led to nearly identical conclusions.

Models. Details of the models can be found in the Supplementary Information. A flow diagram describing the relationship between each model type, figures and parameters can be found in Supplementary Fig. S20. Model parameters can be found in Supplementary Tables S2–S5.

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References

- LeDuc, P. R. & Robinson, D. N. Using lessons from cellular and molecular structures for future materials. *Adv. Mater.* **19**, 3761–3770 (2007).
- Grashoff, C. *et al.* Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics. *Nature* **466**, 263–266 (2010).
- Geiger, B., Spatz, J. P. & Bershadsky, A. D. Environmental sensing through focal adhesions. *Nature Rev. Mol. Cell Biol.* **10**, 21–33 (2009).
- Tamada, M., Sheetz, M. P. & Sawada, Y. Activation of signaling cascade by cytoskeleton stretch. *Dev. Cell* **7**, 709–718 (2004).
- Chowdhury, F. *et al.* Material properties of the cell dictate stress-induced spreading and differentiation in embryonic stem cells. *Nature Mater.* **9**, 82–88 (2010).
- Ehrlicher, A. J., Nakamura, F., Hartwig, J. H., Weitz, D. A. & Stossel, T. P. Mechanical strain in actin networks regulates FilGAP and integrin binding to filamin A. *Nature* **478**, 260–263 (2011).
- Effler, J. C. *et al.* Mitosis-specific mechanosensing and contractile protein redistribution control cell shape. *Curr. Biol.* **16**, 1962–1967 (2006).
- Kee, Y.-S. *et al.* A mechanosensory system governs myosin II accumulation in dividing cells. *Mol. Biol. Cell* **23**, 1510–1523 (2012).
- Johnson, C. P., Tang, H.-Y., Carag, C., Speicher, D. W. & Discher, D. E. Forced unfolding of proteins within cells. *Science* **317**, 663–666 (2007).
- DuFort, C. C., Paszek, M. J. & Weaver, V. M. Balancing forces: Architectural control of mechanotransduction. *Nature Rev. Mol. Cell Biol.* **12**, 308–319 (2011).
- Engler, A. J., Sen, S., Sweeney, H. L. & Discher, D. E. Matrix elasticity directs stem cell lineage specification. *Cell* **126**, 677–689 (2006).
- Raab, M. *et al.* Crawling from soft to stiff matrix polarizes the cytoskeleton and phosphoregulates myosin-II heavy chain. *J. Cell Biol.* **199**, 669–683 (2012).
- Köhler, S., Schaller, V. & Bausch, A. R. Structure formation in active networks. *Nature Mater.* **10**, 462–468 (2011).
- Del Rio, A. *et al.* Stretching single talin rod molecules activates vinculin binding. *Science* **323**, 638–641 (2009).
- Gardel, M. L. *et al.* Elastic behavior of cross-linked and bundled actin networks. *Science* **304**, 1301–1305 (2004).
- Shin, J. H., Gardel, M. L., Mahadevan, L., Matsudaira, P. & Weitz, D. A. Relating microstructure to rheology of a bundled and cross-linked F-actin network *in vitro*. *Proc. Natl Acad. Sci. USA* **101**, 9636–9641 (2004).
- Chaudhuri, O., Parekh, S. H. & Fletcher, D. A. Reversible stress softening of actin networks. *Nature* **445**, 295–298 (2007).
- Koenderink, G. H. *et al.* An active biopolymer network controlled by molecular motors. *Proc. Natl Acad. Sci. USA* **106**, 15192–15197 (2009).
- Wagner, B., Tharmann, R., Haase, I., Fischer, M. & Bausch, A. R. Cytoskeletal polymer networks: The molecular structure of cross-linkers determines macroscopic properties. *Proc. Natl Acad. Sci. USA* **103**, 13974–13978 (2006).
- Schmoller, K. M., Lieleg, O. & Bausch, A. R. Cross-linking molecules modify composite actin networks independently. *Phys. Rev. Lett.* **101**, 118102 (2008).
- Murrell, M. *et al.* Spreading dynamics of biomimetic actin cortices. *Biophys. J.* **100**, 1400–1409 (2011).
- He, L., Wang, X., Tang, H. L. & Montell, D. J. Tissue elongation requires oscillating contractions of a basal actomyosin network. *Nature Cell Biol.* **12**, 1133–1142 (2010).
- Solon, J., Kaya-Çopur, A., Colombelli, J. & Brunner, D. Pulsed forces timed by a ratchet-like mechanism drive directed tissue movement during dorsal closure. *Cell* **137**, 1331–1342 (2009).
- Bao, G. & Suresh, S. Cell and molecular mechanics of biological materials. *Nature Mater.* **2**, 715–725 (2003).
- Luo, T. *et al.* Understanding the cooperative interaction between myosin II and actin cross-linkers mediated by actin filaments during mechanosensation. *Biophys. J.* **102**, 238–247 (2012).
- Ren, Y. *et al.* Mechanosensing through cooperative interactions between myosin II and the actin crosslinker cortexillin I. *Curr. Biol.* **19**, 1421–1428 (2009).
- Uyeda, T. Q. P., Iwamoto, Y., Umeki, N., Nagasaki, A. & Yumura, S. Stretching actin filaments within cells enhances their affinity for the myosin II motor domain. *PLoS ONE* **6**, e26200 (2011).
- Fernandez-Gonzalez, R., Simoes Sde, M., Roper, J. C., Eaton, S. & Zallen, J. A. Myosin II dynamics are regulated by tension in intercalating cells. *Dev. Cell* **17**, 736–743 (2009).
- Discher, D. E. & Mohandas, N. Kinematics of red cell aspiration by fluorescence-imaged microdeformation. *Biophys. J.* **71**, 1680–1694 (1996).
- Evans, E. Probing the relation between force-life-time-and chemistry in single molecular bonds. *Annu. Rev. Biophys. Biomol. Struct.* **30**, 105–128 (2001).
- Bell, G. I. Models for the specific adhesion of cells to cells. *Science* **200**, 618–627 (1978).
- Veigel, C., Molloy, J. E., Schmitz, S. & Kendrick-Jones, J. Load-dependent kinetics of force production by smooth muscle myosin measured with optical tweezers. *Nature Cell Biol.* **5**, 980–986 (2003).
- Thomas, W. E., Vogel, V. & Sokurenko, E. Biophysics of catch bonds. *Annu. Rev. Biophys.* **37**, 399–416 (2008).
- Uyeda, T. Q., Abramson, P. D. & Spudich, J. A. The neck region of the myosin motor domain acts as a lever arm to generate movement. *Proc. Natl Acad. Sci. USA* **93**, 4459–4464 (1996).
- Robinson, D. N., Kee, Y. S., Luo, T. & Surcel, A. in *Comprehensive Biophysics* (ed. Edward, H. E.) 48–72 (Elsevier, 2012).
- Seifert, U. Rupture of multiple parallel molecular bonds under dynamic loading. *Phys. Rev. Lett.* **84**, 2750–2753 (2000).
- Erdmann, T. & Schwarz, U. S. Stability of adhesion clusters under constant force. *Phys. Rev. Lett.* **92**, 108102 (2004).
- Girard, K. D., Chaney, C., Delannoy, M., Kuo, S. C. & Robinson, D. N. Dynactin contributes to cortical viscoelasticity and helps define the shape changes of cytokinesis. *EMBO J.* **23**, 1536–1546 (2004).
- Gerisch, G. *et al.* Mobile actin clusters and traveling waves in cells recovering from actin depolymerization. *Biophys. J.* **87**, 3493–3503 (2004).
- Robinson, D. N. & Spudich, J. A. Dynactin, a genetic link between equatorial contractility and global shape control discovered by library complementation of a *Dictyostelium discoideum* cytokinesis mutant. *J. Cell Biol.* **150**, 823–838 (2000).
- Nakamura, F., Osborn, T. M., Hartemink, C. A., Hartwig, J. H. & Stossel, T. P. Structural basis of filamin A functions. *J. Cell Biol.* **179**, 1011–1025 (2007).
- Meyer, R. K. & Aebi, U. Bundling of actin filaments by alpha-actinin depends on its molecular length. *J. Cell Biol.* **110**, 2013–2024 (1990).
- Hock, R. S. & Condeelis, J. S. Isolation of a 240-kilodalton actin-binding protein from *Dictyostelium discoideum*. *J. Biol. Chem.* **262**, 394–400 (1987).
- Ferrer, J. M. *et al.* Measuring molecular rupture forces between single actin filaments and actin-binding proteins. *Proc. Natl Acad. Sci. USA* **105**, 9221–9226 (2008).
- Guo, B. & Guilford, W. H. Mechanics of actomyosin bonds in different nucleotide states are tuned to muscle contraction. *Proc. Natl Acad. Sci. USA* **103**, 9844–9849 (2006).
- Pereverzev, Y. V., Prezhdo, O. V., Forero, M., Sokurenko, E. V. & Thomas, W. E. The two-pathway model for the catch-slip transition in biological adhesion. *Biophys. J.* **89**, 1446–1454 (2005).
- Evans, E., Leung, A., Heinrich, V. & Zhu, C. Mechanical switching and coupling between two dissociation pathways in a P-selectin adhesion bond. *Proc. Natl Acad. Sci. USA* **101**, 11282–11286 (2004).
- Gimona, M., Djinnovic-Carugo, K., Kranewitter, W. J. & Winder, S. J. Functional plasticity of CH domains. *FEBS Lett.* **513**, 98–106 (2002).
- Yao, N. Y. *et al.* Stress-enhanced gelation: A dynamic nonlinearity of elasticity. *Phys. Rev. Lett.* **110**, 018103 (2013).

50. Maugis, B. *et al.* Dynamic instability of the intracellular pressure drives bleb-based motility. *J. Cell Sci.* **123**, 3884–3892 (2010).
51. Hotulainen, P. & Lappalainen, P. Stress fibers are generated by two distinct actin assembly mechanisms in motile cells. *J. Cell Biol.* **173**, 383–394 (2006).
52. Friedrich, B. M., Friedrich, E. F., Gov, N. S. & Safran, S. A. Sacromeric pattern formation by actin cluster coalescence. *PLoS Comput. Biol.* **8**, e1002544 (2012).
53. Dickinson, D. J., Robinson, D. N., Nelson, W. J. & Weis, W. I. α -Catenin and IQGAP regulate myosin localization to control epithelial tube morphology in *Dictyostelium* morphogenesis. *Dev. Cell* **23**, 533–546 (2012).
54. Chen, C.-L., Wang, Y., Sesaki, H. & Iijima, M. Myosin I Links PIP3 signaling to remodeling of the actin cytoskeleton in chemotaxis. *Sci. Signal.* **5**, ra10 (2012).

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Author contributions

T.L. and D.N.R. conceived, designed and wrote the paper. T.L. performed the experiments and analysed the data. T.L. conducted the coarse-grained molecular simulations. K.M. and P.A.I. carried out the continuum simulations. T.L., K.M., P.A.I. and D.N.R. proposed the strain-specific molecular mechanisms for different cytoskeletal proteins and the one-dimensional retraction model. All authors discussed the results and commented on the manuscript.

Additional information

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Competing financial interests

The authors declare no competing financial interests.

Molecular Mechanisms of Cellular Mechanosensing

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Supplementary Materials

Supplementary Materials includes Supplementary Text, Supplementary Tables 1-5, Supplementary Figures 1-20, and Supplementary Movies S1-S9.

Supplementary Text

The force-sharing between actin cytoskeletal proteins during cell deformation

A popular structural model for cells assumes that they have a mostly elastic cortical shell, consisting of the actin cortex and plasma membrane, encapsulating the largely viscous cytoplasm (with organelles). During deformation, the actin cortex bears external loads while the plasma membrane mainly provides the resistance to bending deformation^{1,2}. Besides myosin II and actin, other load-bearing elements include the actin crosslinkers (ACs) and force-transmitting anchoring proteins (**Supplementary Fig. 1b**). The ACs organize individual actin filaments into structures such as bundles and meshwork^{3,4}, whose relative abundance is determined, in part, by the relative concentrations of two classes of ACs⁵. Anchoring proteins then bridge the actin cortex to the plasma membrane. Finally, the mechanical properties of the actin cortex depend on the force-dependent affinities of all of these proteins for F-actin and their concentrations^{6,7}. Two kinds of experimental evidence support this idea. First, the deletion or depletion of different ACs reduced the cortical tension to different degrees as compared to that of WT cells (**Supplementary Fig. 1c**). Additionally, depletion of ACs in *myoII* null cells further reduced cortical tension as compared to *myoII* null alone⁸. Second, the viscoelastic moduli of both cells and artificially assembled actin gels measured by particle tracking methods increased with higher concentrations of ACs across a wide force range^{3,6-9}. To further illustrate how force is transmitted and shared at the cellular level, it is useful to recognize the relationships between the proteins in the cytoskeleton-membrane composite (**Supplementary Fig. 5**). Myosin II and actin crosslinkers act somewhat in parallel with each other as they constitute the cortical network, which acts in series with the anchoring proteins that link the meshwork to the plasma membrane.

Superposition of the contribution of cytoskeleton proteins to the mechanical properties of cells

The localization of the actin cytoskeletal proteins was assessed using live cell imaging of cells expressing FP-fusion proteins (**Supplementary Fig. 1a; Supplementary Table 1**). These proteins are enriched in the actin cortex next to the plasma membrane but depleted in the cytoplasm. The Young's modulus or the stiffness of the cells is the summation of the contributions from all cytoskeletal proteins, and the reduction of any cytoskeletal protein concentration generally leads to reduced cell stiffness. Cortical tension measured by micropipette aspiration is usually considered an equivalent physical parameter of the cell. A schematic graph of the molecular structure of an aspirated cell is shown in **Supplementary Fig. 1b**. The reduction of cortical tension compared to WT cells due to the deletion of

myosin II and ACs is shown in **Supplementary Fig. 1c**. Comparison of the cortical tension between WT and *myosin II* null indicates that myosin II contributes 10~20% to the cortical tension. In *racE* null cells, multiple ACs, such as coronin and dynacortin, are inactivated and the cortical tension is only 1/3 of that of WT cells.

Myosin II does not affect many cytoskeletal proteins in the micropipette

Importantly, myosin II did not have an effect on the mechanosensitive accumulation of many cytoskeletal proteins (**Fig. 2a; Supplementary Fig. 6**), including two anchoring proteins, myosin I (a PH-domain-containing protein) and enlazin (a FERM-domain-containing protein). The proteins involved in adhesion also did not show much accumulation, indicating that the so-called “inside-out” signaling found in focal adhesion proteins was not prominent at the cortex during micropipette aspiration. Although probes for actin, GFP-actin, LimeΔcoil, and Lifeact-mRFP, displayed transient accumulation in WT cells (**Supplementary Movie S2 and 3**), they did not show accumulation in *myosin II* null cells, implying that actin accumulation itself is not mechanosensitive. Actin’s transient accumulation in WT cells is probably a result of waves that have been observed in various *Dictyostelium* mutants¹⁰⁻¹². Other probes for the molecules that act upstream of actin polymerization, including cofilin, HSPC300 (a SCAR subunit), p41-Arc (an Arp2/3 complex subunit), small GTPases and regulators of phosphatidylinositol phosphates, similarly did not accumulate due to cortical deformation, and most were insensitive to the presence of myosin II (**Fig. 2a; Supplementary Fig. 6**). However, a few proteins such as cofilin appeared to be depleted from the cortex of *myoII* null cells in response to mechanical stress (**Fig. 2a; Supplementary Fig. 6**). Finally, a general transmembrane protein, the cAMP-receptor car1, also showed no force-induced accumulation (**Fig. 2a; Supplementary Fig. 6**).

Coarse-grained molecular mechanics model (Fig. 3a,b)

The cell membrane and the underlying actin cortex may be considered a membrane-cortex composite. The cell membrane, composed of a lipid bilayer, contributes to the bending rigidity¹ while the actin cortex, with a thickness ranging from a few hundred nanometers to one micron, dominates the in-plane stretch modulus of the composite. The cortical actin network includes actin, ACs, and myosin II.

The actin filaments are nearly undeformable when their lengths are below 500 nm¹³. Therefore, at low force regimes, most of the deformations occur at the binding interface between ACs and actin filaments although the ACs and myosin II themselves are deformable at relatively high force regimes.

From single molecule measurements, it is known that the binding of ACs and myosin II to actin filaments is force-dependent¹⁴: some binding life-times increase with force while others may decrease with force. Thus, the microstructures and corresponding mechanical properties of the membrane-cortex composite are mainly governed by the force-dependent binding affinities of actin binding proteins.

The whole membrane-cortex composite can be discretized into inter-connected triangle elements where the average length of sides of the triangles is an indicator of the mesh size of the actin cytoskeleton model (**Supplementary Fig. 8**). The nodes of these triangles represent the crosslinking positions and the triangles mimic the meshes in the actin network. As Discher *et al.* suggested^{1,2}, the total Helmholtz free energy of the composite at the coarse-grained molecular level can be calculated by

$$E_{total} = E_{bending} + E_{in-plane} + E_{surface} + E_{volume}. \quad (S1)$$

The bending energy, $E_{bending}$, mainly contributed by the plasma membrane, is written as

$$E_{bending} = \frac{1}{2} \sum_{i,j} k_{bend} (1 - \cos(\theta_i - \theta_i^0)), \quad (S2)$$

where k_{bend} is the bending modulus, $\theta_{i,j}$ is the angle between the surface normal to the elements i and j , and θ_i^0 is the reference value of θ_i at equilibrium. The in-plane free energy $E_{in-plane}$ has the form

$$E_{in-plane} = \sum_i V_{WLC}(l_i) + \frac{1}{2} \sum_i k_{dilation} (A_i - A_i^0)^2. \quad (S3)$$

The first term $V_{WLC}(l_i)$ is the worm-like-chain energy due to the intermolecular and intramolecular deformations of actin cytoskeletal proteins associated with edge, l_i . The second term is the energy due to the dilation/shrinking of individual mesh of area A_i with initial value A_i^0 and a dilation modulus, $k_{dilation}$. Specifically, the force in the worm-like-chain model is

$$f_{WLC}(l) = -\frac{\partial V_{WLC}(l)}{\partial x} = -\frac{nk_B T}{p} \left(\frac{1}{4(1-x)^2} - \frac{1}{4} + x \right), \quad x = \frac{l}{l_{max}} \in (0,1), \quad (S4)$$

where k_B is the Boltzmann constant, T is the temperature, $2n$ is the number of functional ACs between two connected nodes, l_{max} is the maximum length of edge l , and p is the average persistence length of the ACs. Here, the deformation of actin filaments is neglected because they are too rigid to deform since their length is much shorter (about 100 nm in *Dictyostelium* cells⁸) than their persistence length ($\sim 5 \mu m$). $E_{surface}$ is the energy associated with the conservation of global surface area and is written as

$$E_{surface} = \frac{1}{2} k_{surface} (A_{total} - A_{total}^0)^2, \quad (S5)$$

where $k_{surface}$ is the global area modulus, A_{total} is the total area of membrane-cortex composite and A_{total}^0 is the initial total area. Similarly, E_{volume} is the energy associated with the conservation of global volume and has the form of

$$E_{volume} = \frac{1}{2} k_{volume} (V_{total} - V_{total}^0)^2, \quad (S6)$$

where k_{volume} is the global volume modulus and V_{total} and V_{total}^0 are the total volume of the cell and its corresponding equilibrium value, respectively. Note that there are two terms associated with area change. The area change term in **Eq. S3** comes from a local perspective, *i.e.*, short-range propagation of elastic deformation in individual mesh; in contrast, the area terms in **Eq. S5** come from a global perspective, namely, the long-range feedback of the deformation in the whole actin network.

We used a surface mesh with 10,000 nodes and 19,996 triangles representing a sphere of radius 5 μm (the size of a *Dictyostelium* cell). The resultant edges are, on average, 70 nm long, which is close the average length of F-actin in *Dictyostelium* cells⁸. For **Eq. S4**, the average persistence length of ACs is on the order of 40 nm as measured experimentally^{15, 16}. The average number of ACs, n , is assumed to be one for the time being. The values of the remaining parameters are: $k_{bend} = 100 k_B T$, $\theta_i^0 = 0$, $k_{dilation} = 100 k_B T$, $k_{surface} = 1000 k_B T$, $k_{volume} = 1000 k_B T$, which are similar to those used in other coarse-grained simulations for cells and actin networks^{1, 2, 17}.

The motion of the node at position \vec{r} is described by the Brownian dynamics equation (or over-damped Langevin dynamics equation)

$$\zeta M \frac{d\vec{r}}{dt} = -\frac{\partial \Delta E_{total}}{\partial \vec{r}} + \vec{f}_{external} + \vec{f}_{contractile} + \sqrt{2\zeta M k_B T} R(t), \quad (S7)$$

where ζ is the damping coefficient, M is the mass, $\vec{f}_{external}$ is localized external force, $\vec{f}_{contractile}$ is the contractile force-dipole due to myosin II pulling, and $R(t)$ is zero-mean Gaussian noise. $R(t)$ satisfies $\langle R(t) \rangle = 0$ and $\langle R(t)R(t') \rangle = \delta(t-t')$ where $\delta(t)$ is the delta function. Here, the term ζM has the value of $6\pi\eta a$, where η is the viscosity of the cytoplasm, for which the relevant value is $\sim 300 \text{ Pa}\cdot\text{s}$ for *Dictyostelium* cells^{18, 19}, a is the average size of the ACs, which is $\sim 30 \text{ nm}$ ^{20, 21}, and $\vec{f}_{contractile}$ is a function of the local myosin II concentration.

The initial configuration of the system was obtained by thermal annealing at room temperature until the fluctuation of the system energy was less than 10^{-3} . This configuration was then mapped to an aspirated shape with the cell length in the pipette, L , equal to the desired values¹. The final configuration of the system was achieved after 20 seconds of Brownian dynamics simulation with a 10^{-5} second time-step, according to **Eq. S7**. Meanwhile, $\bar{f}_{contractile}$ was set to 0 since the experimentally observed local accumulation of myosin II did not happen in this moment and the isotropic coarse-grained myosin contraction at basal level was already grouped into $k_{dilation}$. The 20-second simulation was long enough to relax the system to its equilibrium state.

The process described above was performed for three lengths of micropipette aspiration: $L=2R_p$, $3R_p$, and $4R_p$, where R_p is the radius of the micropipette. For each case, the area dilation of each node was calculated through averaging of the area dilation of the triangles with which the node of interest is associated. Similarly, the angle change of each node was determined. The area dilation and angle change along the axis of symmetry of the pipette (**Supplementary Fig. 8b, c**) were evaluated by averaging the corresponding values of the nodes that have the same coordinate on the axis. The contour plots of the deformations on the cell surface (**Supplementary Fig. 8d, e**) were created using the software Tecplot (www.tecplot.com). The maximum dilation deformation occurred in the tip region while the maximum shear deformation was in the neck region of the aspirated cells.

The deformation fields for compressed cells were calculated in a similar manner after the triangle mesh was mapped to a pancake shape while maintaining volume conservation. Unlike the micropipette case, for a compressed cell, the maximum dilation and the maximum shear almost coincided with each other while the dilation deformation had a sharper gradient (**Supplementary Fig. 11a**).

Myosin II is sensitive to dilation deformation

The functional unit of myosin II is the bipolar thick filament (BTF) (**Supplementary Fig. 12**). In *Dictyostelium* cells, BTFs are typically ~200-400 nm in length^{22, 23}. Myosin II heads extend out from the two opposite ends and bind to actin filaments with opposite orientations. Using the energy from ATP hydrolysis, myosin heads pull the actin filament towards its plus end. Myosin II pulling is most effective when the motors interact with two anchored antiparallel actin filaments, which conversely lock the motor heads in their isomeric state, increasing their binding lifetimes on F-actin. Any deviations from antiparallel orientation reduce the mechanical power generated by the myosin II heads²⁴ and therefore the increase of binding lifetimes is reduced.

During dilation deformation, the distance between two adjacent crosslinking points in the actin network increases through displacement between two nearby antiparallel actin filaments. This displacement raises the tension along the actin filaments, helps the motor heads to be locked and thus enhances their F-actin binding affinities. On the other hand, shear deformation associated with the angle change between actin filaments does not promote locking of the motor heads in the isomeric state and therefore leads to a smaller effect on the actin-binding affinity of myosin II. Therefore, myosin II is mostly sensitive to dilation, which is provided by the displacement of two antiparallel actin filaments (**Fig. 3e; Fig. 4c**).

In cells, the BTFs undergo constant assembly and disassembly. This turnover dynamics can be described by a force-dependent myosin II BTF assembly/disassembly scheme that is able to quantitatively account for the accumulation of local myosin II at the cellular level (**Supplementary Fig. 12** and Ref. ²⁵). This model was used to account for the two lines in Fig. 1d corresponding to different values of ζ . In the first case, all the applied stress ($\zeta=1$) was used to simulate myosin II accumulation. In the second case, only one seventh of the applied stress ($\zeta=1/7$) was used to calculate the corresponding myosin II accumulation, which was lower.

Model of force-dependent crosslinking mechanism of dimerized α -actinin in response to dilation (Fig. 3d,f)

The functional unit of some ACs, such as α -actinin, is a dimer, and the two ends of the dimer have equivalent actin binding domains. The binding of the dimers can be visualized as a two-step process that is sensitive to dilation (**Supplementary Fig. 12a, b**). In the first step, one end of the freely diffusing AC dimer binds to an actin filament in a nearby region while the other end still moves freely without binding to anything. Once the first step finishes, the AC dimer and the actin filament form a complex whose diffusivity is much reduced (as compared to the freely diffusing unattached AC) due to tethering of the AC to the actin network. Consequently, fast motion of the other end is restricted to a 3D sphere whose radius is determined by the length of the dimer. In the second step, the free end of the dimer searches for an available actin filament in this spherical volume and binds to it. Similarly, the diffusivity of the final complex consisting one AC dimer and two actin filaments is also very small. This two-step binding reaction can be written as $[Cr]+[F_1]\leftrightarrow[CrF_1]+[F_2]\leftrightarrow[CrF_1F_2]$, where Cr , F_1 and F_2 represent the crosslinker, the actin filament in step one and the actin filament in step two, respectively, and square brackets denote concentrations. The reaction-diffusion equations corresponding to this binding reaction are

$$\begin{aligned}
\frac{\partial[Cr]}{\partial t} &= D_1 \nabla^2 [Cr] - (k_{on_1}[Cr][F_1] - k_{off_1}[CrF_1]) \\
\frac{\partial[CrF_1]}{\partial t} &= D_2 \nabla^2 [CrF_1] + (k_{on_1}[Cr][F_1] - k_{off_1}[CrF_1]) - (k_{on_2}[CrF_1][F_2] - k_{off_2}[CrF_1F_2]), \\
\frac{\partial[CrF_1F_2]}{\partial t} &= D_3 \nabla^2 [CrF_1F_2] + (k_{on_2}[CrF_1][F_2] - k_{off_2}[CrF_1F_2]),
\end{aligned} \tag{S8}$$

where D_1 , D_2 and D_3 are the diffusion coefficients of Cr , CrF_1 and CrF_1F_2 , respectively. The Stokes-Einstein equation was used to calculate D_1 based on the viscosity of *Dictyostelium* cytoplasm and the size of monomeric α -actinin²⁰. Since D_2 and D_3 are intrinsically associated with tethered diffusion due to the connectivity of actin network, their values are much smaller than those calculated by the Stokes-Einstein equation. The parameters k_{on_i} and k_{off_i} ($i=1,2$) are the on- and off-rates, respectively. The above partial differential equations obey mass conservation over the complete cell:

$$\int [Cr_{total}] dV = \int ([Cr] + [CrF_1] + [CrF_1F_2]) dV \tag{S9}$$

because there is no protein exchange between cells and the culture media, nor is there any net production/degradation of proteins in the time window of the experiments. Note, however, that at any point in the cell, the total concentration of the crosslinker can vary over time. In our model, $[F_1]$ is the F-actin concentration in the cytoplasm, which is $\sim 72 \mu\text{M}$ ²⁵. $[F_2]$ is the effective concentration of actin filament binding sites in the spherical volume accessible to the AC dimers. This sphere size is defined by the length of the rod-like α -actinin dimer, which is $\sim 30 \text{ nm}$. If one actin filament is found in this volume the local actin concentration of filaments (not actin monomers in the filament form) is $\sim 5 \mu\text{M}$. We assumed that each α -actinin can reach ~ 2 possible binding sites on the actin filament, raising the effective concentration to $\sim 8\text{-}10 \mu\text{M}$. Significantly, sensitivity analysis (not shown) confirmed that this is the effective concentration range for which simulations recapitulate the experimental data.

To account for the observed accumulations of α -actinin during micropipette aspiration, we considered several bond-types, including 1) force-independent bond, 2) slip-bond, 3) catch-bond, 4) structural cooperativity due to force-induced local reorganization of actin filaments, and 5) catch-slip transition.

For the different force-dependent behaviors, we assumed that k_{off_2} is the only force-dependent parameter since the AC dimers can feel the force in the actin network only when both ends are bound to F-actin. Although k_{on_2} could, in principle, also be force-dependent, there is no reported experimental evidence to support this. More specifically, this force-dependency has the form of $k_{off_2} = k_{off_2}^0 \exp(-f\Delta x/k_B T)$, where $k_{off_2}^0$ is zero-force off-rate, f is the force applied on the AC dimer

and Δx is the phenomenological bond length in a Bell-type model ²⁶. Based on the strain field calculation from the coarse-grained simulation (**Fig. 3a**; **Supplementary Fig. 8**), we assumed the force f changes gradually as a function of the azimuth angle θ . The simulation results based on simple catch-bond produce an accumulation pattern that is in good agreement with the experimental observation (**Supplementary Fig. 13d**; **Fig. 3d**). The simulations based on different bond models (catch-bond, slip-bond and force-independent bond) suggest that the catch-bond, but not force-independent or slip-bond, model is able to reproduce the accumulation kinetics measured experimentally (**Supplementary Fig. 13e**; **Fig. 3g**). Sensitivity analysis of k_{on_2} narrowed down the range of its value for the catch-bond model (**Supplementary Fig. 13f**). Different forces from 40 to 100 pN do not affect the accumulation significantly (**Supplementary Fig. 13g**).

In considering structural cooperativity, we hypothesized that in response to force, neighboring parallel actin filaments undergo translation and rotation such that the distance between these filaments favors more efficient binding of α -actinin thereby affecting the on-rate. More specifically, $k_{on_2} = k_{on_2}^0 (1 + [CrF_1F_2]/[F_2])^{\chi \cos \theta}$, where θ is the azimuth angle in the tip region. In the absence of force-dependent mechanisms, the structural cooperativity in the region of the cell experiencing dilation is sufficient to generate local accumulation of α -actinin, reproducing the experimental observations (**Supplementary Fig. 14**). However, at a molecular level, it is unclear how dilation strain, but not shear strain, would lead to the structural cooperativity.

Finally, the catch-slip transition is a more complete model that covers multiple force-dependent behaviors. In this model, we adopt the formula proposed by Pereverzev *et al.* ²⁷⁻²⁹:

$$k_{off_2} = k_{off_2}^{c0} \exp(-f\Delta x_c/k_B T) + k_{off_2}^{s0} \exp(-f\Delta x_s/k_B T), \quad (\text{S10})$$

where the superscripts and subscripts c and s represent the catch-bond and slip-bond, respectively. Here, the values for the catch-bond pathway ($k_{off_2}^{c0}$ and Δx_c) remain the same as in the simple catch-bond model. The values for the slip-bond pathway ($k_{off_2}^{s0}$ and Δx_s) are calculated from the ratios $k_{off_2}^{s0}/k_{off_2}^{c0}$ and $\Delta x_s/\Delta x_c$ used for muscle myosin II ²⁸ or L/P selectin ²⁷. **Equation S10** allows k_{off_2} to increase with the force f in the low force regime and decrease in high force regime (**Supplementary Fig. 15a**). The simulated accumulation of α -actinin in the force range of 0 to 100 pN also displayed a rise-fall transition (**Supplementary Fig. 15b**). The maximum accumulation occurred at $f \approx 50$ pN where the kinetics using the ratios from muscle myosin II resembled more closely the experimental observations

(**Supplementary Fig. 15c**). Nevertheless, the catch-slip transition model is more general than the catch and slip models alone.

In summary, both the catch-bond and the structural cooperativity models were able to reproduce the experimentally observed accumulation of α -actinin in the micropipette. However, the molecular basis for which dilation, but not shear deformation, would induce structural cooperativity is not clear. The catch-slip transition model is a more general treatment that accounts for larger force ranges.

Force-dependent crosslinking mechanism of dimerized filamin in response to shear (Fig. 3d,g)

The kinetics of filamin accumulation measured experimentally (**Fig. 3g**; **Supplementary Fig. 10**) show two important features: 1) a flat initial stage lasting for about 5 seconds; followed by 2) continuous acceleration afterwards. Since the hyperbolic kinetics observed in the binding mechanism of α -actinin are different from those of filamin, the binding mechanism of filamin must also be different from those of α -actinin (**Fig. 2c**; **Fig. 3f, g**; **Supplementary Fig. 10**). Moreover, the accelerated accumulation seen indicates the possibility of cooperativity.

Like α -actinin, filamin also forms dimers and undergoes two-step binding reactions during crosslinking (**Supplementary Fig. 16a**). However, unlike the parallel actin bundles crosslinked by α -actinin, filamin forms an actin meshwork in which the orientation of two crosslinked actin filaments may be at non-zero angles. In the absence of shear deformation, the four angles associated with one crosslinking point of two crosslinked actin filaments are about 90° due to the four-fold rotational symmetry. After shearing, two angles become larger and two become smaller (the corresponding binding sites are defined as α and β sites, respectively; **Supplementary Fig. 16b**). Based on single molecule measurements¹⁴, the different angles at α and β sites are expected to lead to different off-rates for filamin from F-actin. These two different binding affinities only exist when the two filamin monomers within a dimer are bound to F-actin at the same time, *i.e.*, in the second step of binding reactions (**Supplementary Fig. 16a**). Otherwise, the filamin dimer does not feel the force and force-induced affinity change does not occur. Using Cr , F_1 and F_2 to represent the crosslinker, the actin filament in the first step and the actin filament in the second step, respectively, the corresponding reaction-diffusion equations for filamin are written as

$$\begin{aligned}
\frac{\partial[Cr]}{\partial t} &= D_1 \nabla^2 [Cr] - (k_{on_1} [Cr][F_1] - k_{off_1} [CrF_1]) \\
\frac{\partial[CrF_1]}{\partial t} &= D_2 \nabla^2 [CrF_1] + (k_{on_1} [Cr][F_1] - k_{off_1} [CrF_1]) - (k_{on_2} [CrF_1][F_2] - k_{off_2}^\alpha [Cr^\alpha F_1 F_2]) \\
&\quad - (k_{on_2} [CrF_1][F_2] - k_{off_2}^\beta [Cr^\beta F_1 F_2]) \\
\frac{\partial[Cr^\alpha F_1 F_2]}{\partial t} &= D_3 \nabla^2 [Cr^\alpha F_1 F_2] + (k_{on_2} [CrF_1][F_2] - k_{off_2}^\alpha [Cr^\alpha F_1 F_2]) \\
\frac{\partial[Cr^\beta F_1 F_2]}{\partial t} &= D_3 \nabla^2 [Cr^\beta F_1 F_2] + (k_{on_2} [CrF_1][F_2] - k_{off_2}^\beta [Cr^\beta F_1 F_2]).
\end{aligned} \tag{S11}$$

As above, D_1 , D_2 and D_3 are the diffusion coefficients of Cr , CrF_1 and CrF_1F_2 , respectively, and k_{on_i} and k_{off_i} ($i=1,2$) are the on- and off-rates, respectively. The Stokes-Einstein equation was again used to calculate D_1 based on the viscosity of *Dictyostelium* cytoplasm and the size of filamin²¹. D_2 and D_3 are assumed to be smaller than D_1 because they are intrinsically associated with tethered diffusion due to the connectivity of actin network. Here, $[F_1]$ is $\sim 72 \mu\text{M}$. The effective concentration $[F_2]$ is $\sim 6 \mu\text{M}$ since the length of a filamin is $\sim 25 \text{ nm}$, based on similar assumptions for $[F_2]$ as described for α -actinin (above).

Once again, we considered a number of cases to account for the observed accumulations of filamin in response to shear strain: 1) force-independent bond, 2) slip-bond, 3) catch-bond; 4) catch-bond mechanism plus the structural cooperativity due to force-induced local reorganization of actin filaments; and 5) catch-slip transition plus the structural cooperativity.

For different force-dependent behaviors, again we used the form $k_{off_2} = k_{off_2}^0 \exp(-f\Delta x/k_B T)$. The values of the parameters can be found in **Supplementary Table 3**. After applying a force-profile in the tip region with catch-bond assumption, we observed a hyperbolic curve for filamin accumulation (**Supplementary Fig. 16e**) that is different from the experimental observations. This indicates that catch-bond alone does not give rise to the accelerated accumulation found in the experiments. Simulations of slip-bond and force-independent models failed to display any accumulation (not shown). Based on the binding kinetics of myosin II and cofilin to F-actin in *in vitro* assays^{30,31}, it is known that accelerated binding usually comes from cooperative interactions at the molecular level.

For the fourth case, in addition to the Bell-type catch-bond formula for k_{off_2} , we incorporated structural cooperativity into k_{on_2} :

$$k_{on_2} = k_{on_2}^0 \left(1 + \frac{[Cr^\alpha F_1 F_2] + [Cr^\beta F_1 F_2]}{[F_2]} \right)^{\chi \cos \theta}, \tag{S12}$$

where $k_{on_2}^0$ is the on-rate of the second step without cooperativity, χ is the cooperativity index and θ is the azimuth angle in the tip region (**Supplementary Fig. 16c**). The rationale for the cooperativity is that when a CrF_1F_2 complex forms, it creates two non-parallel actin filaments with four V-shape binding sites for filamins (illustrated in **Supplementary Fig. 16a**). These four V-shape binding sites may allow more than four filamins to bind simultaneously because filamin dimers at the same binding site may stagger themselves with small relative displacements while maintaining their V-shape. Therefore, the formation of one CrF_1F_2 complex may promote more filamin molecules to bind the same junction of two actin filaments, F_1 and F_2 . As a result, CrF_1F_2 complex inherently has cooperativity in the on-rate of filamin, k_{on_2} , during the crosslinking process. We proposed that the structural cooperativity is proportional to the local concentration of total CrF_1F_2 and the difference of on-rates between α and β sites may be neglected for simplification. Additionally, this cooperativity is limited by the local F-actin concentration, F_2 , which leads to the normalization factor $[F_2]$. The cooperativity index χ possibly has direct physical implications on the local structures of filamin binding to F-actin³². $\chi=0$ corresponds to the case of no cooperativity. The exact value of χ can be determined by comparing the kinetics of the simulated accumulations to the experimental observations. **Equation S12** is, of course, not the only form for cooperativity in k_{on_2} , and other possible forms could also work. Nevertheless, the cooperative forms should have three properties: (1) When there is no cooperativity (*i.e.*, $\chi=0$), $k_{on_2}=k_{on_2}^0$ must be satisfied; (2) k_{on_2} is a power function of the concentration of crosslinked Cr , (*i.e.*, $[Cr^\alpha F_1 F_2] + [Cr^\beta F_1 F_2]$); and (3) cooperativity is limited by the local effective concentration of actin filaments (*i.e.*, $[F_2]$). Taking the structural difference between α and β sites into account, we further suggest that the terms for $k_{off_2}^i = k_{off_2}^0 \exp(-f^i \Delta x / k_B T)$ for $i=(\alpha, \beta)$ have $\Delta x^\alpha > 0$ and $\Delta x^\beta < 0$. Mass conservation was maintained as described by **Eq. S9**. The values of the parameters may be found in **Supplementary Table 3**.

As shown in **Supplementary Fig. 16f**, the cooperativity index χ was determined by sensitivity analysis when both structural cooperativity and catch-bond were considered. If a difference of k_{off_2} exists for α and β sites, the agreement between simulation and experiment improves (**Supplementary Fig. 16g**). However, the model of structural cooperativity alone did not produce filamin accumulation kinetics observed experimentally (**Supplementary Table 3; Supplementary Fig. 16h**).

For the catch-slip transition model, we used **Eq. S10** where $k_{off_2}^{c0}$ and Δx_c had the same values as in the catch-bond. The values for $k_{off_2}^{s0}$ and Δx_s were calculated from the ratios of $k_{off_2}^{s0}/k_{off_2}^{c0}$ and $\Delta x_s/\Delta x_c$ assessed for muscle myosin II²⁸ and L/P selectin²⁷. We also include the difference between α and β sites. The exact values of all the parameters are listed in **Supplementary Table 3**. Simulations showing the resulting k_{off_2} values at α and β sites for 0-120 pN are provided (**Supplementary Fig. 17a, b**). The simulated accumulation of filamin in this force range displayed an increase-decrease transition with the peak located at about 90 pN (**Supplementary Fig. 17c**). The kinetics of accumulation at $f=90$ pN indicate that the ratios of $k_{off_2}^{s0}/k_{off_2}^{c0}$ and $\Delta x_s/\Delta x_c$ used for muscle myosin II are suitable for filamin (**Supplementary Fig. 17d**).

Overall, to reproduce the experimentally-observed filamin accumulation in the micropipette, a minimal model must consider both catch-bond and structural cooperativity. By themselves, neither model reproduced the experimental observations. The catch-slip transition model is a more complete treatment that accounts for broader force ranges, including those not accessible in these experiments presented here.

Estimate of the molecular forces during cellular deformation

The applied pressure in this study is on the order of $1 \text{ nN}/\mu\text{m}^2$, and the cellular extension in the pipette is around a few μm . However, the physiological force on each protein is usually smaller than 100 pN and the maximum extension of each protein is usually a few tens of nm. To use the molecular mechanisms to quantitatively interpret the force-induced protein accumulation at the cellular level, it is necessary to link the cellular forces and deformations to those that individual proteins experience at the molecular level.

In experiments, the cell length L in the pipette increases up to $10 \mu\text{m}$, equivalent to $4R_p$. Based on the deformations calculated by the coarse-grained molecular mechanics model, the area change corresponding to $L=4R_p$ is about 100% in the tip region of deformed cells, *i.e.*, the area dilation is about two fold. This results in an edge-stretch ratio $l/l_0 = (l_0 + \Delta l)/l_0 = \sqrt{2}$, where l_0 is the initial edge length and Δl is the change of the edge length. If l_0 is 70 nm, then the resulting deformation, Δl , is $\sim 30 \text{ nm}$. On the other hand, when $L=4R_p$, the angle change associated with the shear deformation in the neck region of deformed cells is $\Delta\theta = (\Delta\theta/\theta_0) \times \theta_0 = 0.5 \times 90^\circ = 45^\circ$.

The deformation at the molecular level is attributed to both inter-molecular and intra-molecular displacements. The inter-molecular deformation occurs at the interface between the actin-binding domains of proteins and F-actin. Based on single molecule deformations, the rupture forces for the actin-binding domains of mammalian filamin A and α -actinin are about 60 pN and 40 pN, respectively ¹⁴. Since their counterparts in *Dictyostelium* cells share the same actin-binding domains, these values presumably may be applied to *Dictyostelium* filamin and α -actinin. The intra-molecular deformations include unfolding of protein domains and extension of flexible regions. The unfolding of one spectrin domain in α -actinin requires a force of 30 pN and gives rise to a corresponding extension of 32 nm ¹⁵. This extension is close to the edge length change, Δl , calculated above using the coarse-grained molecular mechanics model. The unfolding of one Ig domains in filamin requires a force of 50 pN and gives rise to a corresponding extension of 17 nm ¹⁶. This extension is also close to the edge length change, Δl . Additionally, the intra-molecular deformations can also occur by rigid rotation between domains in the same molecule, for example, the rotation between the two monomers within the same filamin dimer. Since the half-length of the *Dictyostelium* filamin dimer is ~ 25 nm, the end-to-end distance change of filamin is around +10.8 nm at α sites and is close to -16.2 nm at β sites when the angles have a change, $\Delta\theta$, of 45° with respect to its initial angle 90° . The force associated with angle change is usually a product of the bending modulus of the molecule and the angle change value. So far, there has been no measured value for the bending modulus of a filamin dimer. Therefore, the reported values are based on either estimation or simulations: the bending moduli being used in some computational research are in the range of 100 to 1000 $k_B T$ ^{17,33}, which leads to a force of a few hundred pN. In contrast, molecular dynamics simulations from other researchers also demonstrated that a pair of forces about 5 pN at the two ends of *Dictyostelium* filamin is large enough to cause significant angle change ³³.

Based on the published values ¹⁴ and the above discussion, we used 40 pN and 60 pN for the parameter f in the Bell-type off-rates for α -actinin (**Supplementary Table 2**) and filamin (**Supplementary Table 3**), respectively, to account for the force-dependency in their binding affinities. These forces roughly result in 10-fold changes in k_{off_2} . Nevertheless, the simulations with catch-slip transition (**Supplementary Tables 2, 3; Supplementary Figs. 15, 17**) provide insight into how the accumulations change over a wider force range.

Simulation of the accumulations of cytoskeletal proteins (Fig. 1d, 3d-g)

The accumulation of myosin II, α -actinin and filamin were simulated by solving the corresponding reaction-diffusion equations in a 3D-aspirated cell shape using COMSOL Multiphysics (Burlington, MA) (**Supplementary Fig. 13-17**). In the simulations, the cell diameter is 10 μm , and the pipette radius is 2.5 μm . The reaction-diffusion equations for myosin II are based on the BTF assembly scheme of myosin II, which was described in detail previously²⁵. The reaction-diffusion equations for α -actinin and filamin are listed above in **Eq. S8** and **Eq. S11**, respectively, and the corresponding values of the parameters are summarized in **Supplementary Tables 2 and 3**. The input force-profiles for dilation and shear strains are based on the coarse-grained simulations (**Fig. 3a**; **Supplementary Fig. 8**), and the estimations of molecular force are described in the previous sections. The force-profile for dilation sensitivity is $f_{\max} \sin \theta$ where θ defines the azimuth angle in the tip region, and f_{\max} is 40 pN for α -actinin (**Supplementary Fig. 13c**). The force-profile for shear sensitivity is $f_{\max} \cos \theta$ for the tip region and f_{\max} for the neck region (**Supplementary Fig. 16c**). f_{\max} is 60 pN for filamin. Reasonable values of k_{on_2} for both α -actinin and filamin were found by sensitivity analysis to identify the values that provided good agreement between simulations and experiments (**Supplementary Fig. 13f**; **Supplementary Fig. 16f**). The measured diffusion coefficient of α -actinin in solution is $\sim 30 \mu\text{m}^2/\text{s}$ ³⁴. Considering the diffusion coefficient of GFP is 3-fold slower in *Dictyostelium* cells than that in solution³⁵, we set the cellular diffusion coefficient of α -actinin to be $10 \mu\text{m}^2/\text{s}$. When the CrF_1 and CrF_1F_2 complexes are tethered to the cortical actin network, their diffusion must be much slower than that of free α -actinin. Here, we assumed that $D_2 = D_3 = D_1/50$ for α -actinin. Considering the size difference between α -actinin and filamin, we used $D_1 = 5.0 \mu\text{m}^2/\text{s}$, $D_2 = D_1/100$, and $D_3 = D_1/1000$ for filamin. Outside the cortical region, we set $D_1 = D_2 = D_3$. The simulation results of α -actinin and filamin for different mechanisms are provided (**Fig. 3**; **Supplementary Fig. 13-17**).

Model for cell retraction due to accumulations of cytoskeletal proteins in the tip region (Fig. 4a,b)

The cytoskeleton-membrane composite is a viscoelastic material. In general, we describe this using a one-dimensional viscoelastic model (**Supplementary Fig. 19a**) consisting of a Voigt element (with elastic spring constant k_1 and viscosity η_1 in parallel) in series with a Maxwell element (with elastic spring constant k_2 and viscosity η_2 in series), in which case the position of the tip is given by:

$$L(t) = L(0) + \frac{1}{\eta_1} \int_0^t e^{-(k_1/\eta_1)(t-\tau)} \sigma_{\text{total}}(\tau) d\tau + \frac{1}{k_2} \sigma_{\text{total}}(t) + \frac{1}{\eta_2} \int_0^t \sigma_{\text{total}}(\tau) d\tau, \quad (\text{S13})$$

where σ_{total} is the stress acting on the viscoelastic material. To obtain the length of cell inside the pipette requires the parameters of the viscoelastic model (k_1 , η_1 , k_2 , η_2) as well as the total stress acting on the cell.

The viscoelastic parameters can be obtained during creep tests, in which a constant aspiration stress is applied at time 0. In this time scale, $\sigma_{\text{total}} = P_{\text{app}}$, for $t \geq 0$ and the length simplifies to:

$$L(t) = L(0^-) + P_{\text{app}} \left[\frac{1}{k_1} (1 - \exp(-k_1 t / \eta_1)) + \frac{1}{k_2} \left(1 + \frac{k_2}{\eta_2} t \right) \right], \quad (\text{S14})$$

where $L(0^-)$ is the length immediately preceding the application of the aspiration pressure. The four parameters can be determined by fitting experimental data to this equation. For example, measurements of k_2 and η_2 are straightforward since the instantaneous cell length immediately after application of the aspiration pressure is $L(0^+) = L(0^-) + P_{\text{app}}/k_2$, and the elongation rate at long time scale is $\dot{L}(\infty) = P_{\text{app}}/\eta_2$. For *filamin* null and *α -actinin* null cells, these parameters were sufficient to fit the creep data, suggesting that the Voigt model is unnecessary in these strains (equivalently, that η_1/k_1 is negligible). For *racE* null cells, however, the characteristic time $\tau = \eta_1/k_1$ is on the order of a few seconds, and hence cannot be ignored. These were obtained by least squares minimization between measured and simulated trajectories (**Supplementary Table 4**).

Our experiments and simulations of cell retraction (**Fig. 4a,b** and **Supplementary Fig. S18**) take place over a considerably longer time scale (~ 100 - 250 s vs. 20 - 30 s) than the creep experiments. In this time scale, the viscous (η_2) properties dominate the elastic (k_2) component in the Maxwell model. Thus, the simulations for the *filamin* null, *α -actinin* null and *dynacortin-hp* cells use the model

$$L(t) \approx L(0) + \frac{1}{\eta_2} \int_0^t \sigma_{\text{total}}(\tau) d\tau. \quad (\text{S15})$$

For *racE* null cells, the elastic-Voigt model obtained in the creep experiments is appropriate.

For the cell retraction simulations, we also require a measure of the total stress acting on the cell:

$$\sigma_{\text{total}} = P_{\text{app}} - \sigma_{\text{myo}} - \sigma_{\text{cross}} + \sigma_{\text{ten}}. \quad (\text{S16})$$

The four components on the right-hand side refer to the applied pressure from the micropipette, the stress exerted by the accumulated myosin and cross-linkers, and the cortical tension outside the pipette, respectively. The cortical tension measurements show that the contribution of the membrane is much smaller and hence is neglected in this study (**Supplementary Fig. 1c**).

To compute the contractile pressure due to accumulated myosin, we take the fluorescence intensity measured at the tip, $I_{\text{myo}}(t)$, and normalize this for volume using a factor ($I_0=0.82$) determined empirically by measuring soluble GFP³⁶. We multiply this ratio by the concentration of myosin II in the cell ($3.4 \mu\text{M}$ ³⁷) and use this as the concentration of myosin II at the aspirated tip. This value is then converted into a stress. To this end we take this concentration and compute the number of myosin II molecules per unit area by multiplying by the thickness of the cortex, assumed to be $0.5 \mu\text{m}$ and by Avogadro's number (N_A). We multiply this by the fraction of myosin II in BTF form (k_{BTF} ; between 20-60%, as reported previously³⁸), and by two to account for the number of heads in a myosin II motor. Finally, we multiply by the force per head (4 pN)^{39,40}, and by the duty ratio (within 10-fold of its zero-force value; *i.e.*, between 0.006 and 0.06³⁷). Thus, a measured intensity $I_{\text{myo}}(t)$ is converted to a contractile stress according to the following formula:

$$\sigma_{\text{myo}} = (I_{\text{myo}} / I_0) \times (3.4 \mu\text{M}) \times (0.5 \mu\text{m}) \times 2k_{\text{BTF}} \times N_A \times (\text{duty ratio}) \times (\text{force / head}). \quad (\text{S17})$$

Note that this stress is contractile and thus works against the applied micropipette pressure, hence the negative sign in Equation S16 above.

To compute the passive pressures due to the accumulation of the active crosslinkers, we use the formula for Laplace pressure: $\sigma_{\text{cross}} = 2\gamma_{\text{cross}}/R_p$, where R_p is the radius of the pipette (assumed to be $2 \mu\text{m}$). As observed in experiments, ACs, such as cortexillin I, α -actinin, and dynacortin, exhibit different levels of accumulation in the tip region over time. We define:

$$\gamma_{\text{cross}}(t) = \gamma_{\text{cross}}^0 + \Delta\gamma(I_{\text{cross}}(t)/I_0 - 1). \quad (\text{S18})$$

The term γ_{cross}^0 is the crosslinker contribution in the absence of force, $\Delta\gamma$ is the change of cortical tension due to accumulation of crosslinkers (assumed to be $0.3 \text{ nN}/\mu\text{m}$ based on cortexillin I's contribution to cortical tension since cortexillin I accumulates along with myosin II³⁶), and $I_{\text{cross}}(t)$ is the fluorescence intensity of accumulated crosslinkers.

Finally, we include the passive cortical tension (at the other end of the cell): $\sigma_{\text{ten}} = 2\gamma_{\text{cross}}^0 / R_c$, where R_c is the radius of the cell outside the pipette (assumed to be $5.0 \mu\text{m}$). Parameter values used for *filamin* null, *α -actinin* null, *dynacortin-hp* and *racE* null cells are listed in **Supplementary Table 5**.

It is worth pointing out that the retractions we studied are due to myosin II accumulation, unlike the ones associated with blebbing⁴¹, which is the process of the rupture and healing of the linkages between actin cortex and plasma membrane. In comparison to the study of bleb-associated retractions

which uses a viscoelastic model similar to ours (**Eq. S15**)⁴², we used measured myosin II signals rather than constant contractile stresses.

Supplementary References

1. Discher, D.E., Boal, D.H. & Boey, S.K. Simulations of the erythrocyte cytoskeleton at large deformation. II. Micropipette aspiration. *Biophys. J.* **75**, 1584-1597 (1998).
2. Li, J., Dao, M., Lim, C.T. & Suresh, S. Spectrin-level modeling of the cytoskeleton and optical tweezers stretching of the erythrocyte. *Biophys. J.* **88**, 3707-3719 (2005).
3. Wagner, B., Tharmann, R., Haase, I., Fischer, M. & Bausch, A.R. Cytoskeletal polymer networks: The molecular structure of cross-linkers determines macroscopic properties. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 13974-13978 (2006).
4. Gardel, M.L. *et al.* Prestressed F-actin networks cross-linked by hinged filamins replicate mechanical properties of cells. *Proc. Natl. Acad. Sci. USA* **103**, 1762-1767 (2006).
5. Schmoller, K.M., Lieleg, O. & Bausch, A.R. Cross-linking molecules modify composite actin networks independently. *Phys. Rev. Lett.* **101**, 118102 (2008).
6. Shin, J.H., Gardel, M.L., Mahadevan, L., Matsudaira, P. & Weitz, D.A. Relating microstructure to rheology of a bundled and cross-linked F-actin network in vitro. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 9636-9641 (2004).
7. Gardel, M.L. *et al.* Elastic behavior of cross-linked and bundled actin networks. *Science* **304**, 1301-1305 (2004).
8. Reichl, E.M. *et al.* Interactions between myosin and actin crosslinkers control cytokinesis contractility dynamics and mechanics. *Curr. Biol.* **18**, 471-480 (2008).
9. Girard, K.D., Chaney, C., Delannoy, M., Kuo, S.C. & Robinson, D.N. Dynacortin contributes to cortical viscoelasticity and helps define the shape changes of cytokinesis. *EMBO J.* **23**, 1536-1546 (2004).
10. Gerisch, G. *et al.* Mobile actin clusters and traveling waves in cells recovering from actin depolymerization. *Biophys. J.* **87**, 3493-3503 (2004).
11. Diez, S., Gerisch, G., Anderson, K., Müller-Taubenberger, A. & Bretschneider, T. Subsecond reorganization of the actin network in cell motility and chemotaxis. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 7601-7606 (2005).
12. Taniguchi, D. *et al.* Phase geometries of two-dimensional excitable waves govern self-organized morphodynamics of amoeboid cells. *Proc. Natl. Acad. Sci. U. S. A.* (2013).
13. Palmer, J.S. & Boyce, M.C. Constitutive modeling of the stress/strain behavior of F-actin filament networks. *Acta Biomater.* **4**, 597-612 (2008).
14. Ferrer, J.M. *et al.* Measuring molecular rupture forces between single actin filaments and actin-binding proteins. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 9221-9226 (2008).
15. Rief, M., Pascual, J., Saraste, M. & Gaub, H.E. Single molecule force spectroscopy of spectrin repeats: low unfolding forces in helix bundles. *J. Mol. Biol.* **286**, 553-561 (1999).
16. Schwaiger, I., Kardinal, A., Schleicher, M., Noegel, A.A. & Rief, M. A mechanical unfolding intermediate in an actin-crosslinking protein. *Nat. Struct. Mol. Biol.* **11**, 81-85 (2004).
17. Kim, T., Hwang, W., Lee, H. & Kamm, R.D. Computational analysis of viscoelastic properties of crosslinked actin networks. *PLoS Comput. Biol.* **5**, e1000439 (2009).
18. Feneberg, W., Westphal, M. & Sackmann, E. Dictyostelium cells' cytoplasm as an active viscoplastic body. *Eur. Biophys. J.* **30**, 284-294 (2001).
19. Zhang, W. & Robinson, D.N. Balance of actively generated contractile and resistive forces controls cytokinesis dynamics. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 7186-7191 (2005).
20. Wachsstock, D.H., Schwarz, W.H. & Pollard, T.D. Affinity of α -actinin determines the structure and mechanical properties of actin filament gels. *Biophys. J.* **65**, 205-214 (1993).

21. Popowicz, G.M. *et al.* Molecular structure of the rod domain of Dictyostelium filamin. *J. Mol. Biol.* **342**, 1637-1646 (2004).
22. Mahajan, R.K. & Pardee, J.D. Assembly mechanism of *Dictyostelium* myosin II: Regulation by K^+ , Mg^{2+} , and actin filaments. *Biochemistry* **35**, 15504-15514 (1996).
23. Mahajan, R.K., Vaughan, K.T., Johns, J.A. & Pardee, J.D. Actin filaments mediate Dictyostelium myosin assembly in vitro. *Proceedings of the National Academy of Sciences of the United States of America* **86**, 6161-6165 (1989).
24. Tanaka, H., Ishijima, A., Honda, M., Saito, K. & Yanagida, T. Orientation dependence of displacements by a single one-headed myosin relative to the actin filament. *Biophys. J.* **75**, 1886-1894 (1998).
25. Luo, T. *et al.* Understanding the cooperative interaction between myosin II and actin cross-linkers mediated by actin filaments during mechanosensation. *Biophys. J.* **102**, 238-247 (2012).
26. Bell, G.I. Models for the specific adhesion of cells to cells. *Science* **200**, 618-627 (1978).
27. Pereverzev, Y.V., Prezhdo, O.V., Forero, M., Sokurenko, E.V. & Thomas, W.E. The two-pathway model for the catch-slip transition in biological adhesion. *Biophys. J.* **89**, 1446-1454 (2005).
28. Guo, B. & Guilford, W.H. Mechanics of actomyosin bonds in different nucleotide states are tuned to muscle contraction. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 9844-9849 (2006).
29. Luo, T. & Robinson, D. The role of the actin cytoskeleton in mechanosensation, in *Mechanosensitivity and Mechanotransduction*, Vol. 4. (eds. A. Kamkin & I. Kiseleva) 25-65 (Springer Netherlands, 2011).
30. Greene, L.E. & Eisenberg, E. Cooperative binding of myosin subfragment-1 to the actin-troponin-tropomyosin complex. *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2616-2620 (1980).
31. De La Cruz, E.M. & Sept, D. The kinetics of cooperative cofilin binding reveals two states of the cofilin-actin filament. *Biophys. J.* **98**, 1893-1901 (2010).
32. Nakamura, F., Osborn, T.M., Hartemink, C.A., Hartwig, J.H. & Stossel, T.P. Structural basis of filamin A functions. *J. Cell. Biol.* **179**, 1011-1025 (2007).
33. Kolahi, K.S. & Mofrad, M.R.K. Molecular mechanics of filamin's rod domain. *Biophys. J.* **94**, 1075-1083 (2008).
34. Wachsstock, D.H., Schwarz, W.H. & Pollard, T.D. Cross-linker dynamics determine the mechanical properties of actin gels. *Biophys. J.* **66**, 801-809 (1994).
35. Potma, E.O. *et al.* Reduced protein diffusion rate by cytoskeleton in vegetative and polarized Dictyostelium cells. *Biophys. J.* **81**, 2010-2019 (2001).
36. Kee, Y.-S. *et al.* A mechanosensory system governs myosin II accumulation in dividing cells. *Mol. Biol. Cell* **23**, 1510-1523 (2012).
37. Robinson, D.N., Kee, Y.S., Luo, T. & Surcel, A. 7.5 Understanding how dividing cells change shape, in *Comprehensive Biophysics*. (ed. H.E. Edward) 48-72 (Elsevier, Amsterdam; 2012).
38. Zhou, Q. *et al.* 14-3-3 coordinates microtubules, rac, and myosin II to control cell mechanics and cytokinesis. *Curr. Biol.* **20**, 1881-1889 (2010).
39. Kovacs, M., Thirumurugan, K., Knight, P.J. & Sellers, J.R. Load-dependent mechanism of nonmuscle myosin 2. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 9994-9999 (2007).
40. Veigel, C., Molloy, J.E., Schmitz, S. & Kendrick-Jones, J. Load-dependent kinetics of force production by smooth muscle myosin measured with optical tweezers. *Nat. Cell Biol.* **5**, 980-986 (2003).
41. Charras, G.T., Yarrow, J.C., Horton, M.A., Mahadevan, L. & Mitchison, T.J. Non-equilibration of hydrostatic pressure in blebbing cells. *Nature* **435**, 365-369 (2005).

42. Brugués, J. *et al.* Dynamical organization of the cytoskeletal cortex probed by micropipette aspiration. *Proc. Natl. Acad. Sci. U. S. A.* **107**, 15415-15420 (2010).
43. Goldmann, W.H. & Isenberg, G. Analysis of filamin and α -actinin binding to actin by the stopped flow method. *FEBS Lett.* **336**, 408-410 (1993).
44. Ren, Y. *et al.* Mechanosensing through cooperative interactions between myosin II and the actin crosslinker cortexillin I. *Curr. Biol.* **19**, 1421-1428 (2009).

Supplementary Table 1. List of proteins studied and the corresponding plasmids.

| <i>Protein</i> | <i>Plasmid</i> |
|----------------|--|
| myosin II | GFP-myosin II-pDRH, GFP-myosin II-pBIG, mCherry-myosin II-pDRH |
| cortexillin I | mCherry-cortexillin I-pDM181 |
| MHCKA | GFP-MHCKA-pTX |
| MHCKB | GFP-MHCKB-pTX |
| MHCKC | GFP-MHCKC-pTX |
| pakA | PakA-GFP-pMSG |
| PTEN | PTEN-GFP-pCV5 |
| filamin | filamin-mRFP-pDXA |
| ACA | ACA-YFP-pCV5 |
| PH-CRAC | PH-CRAC-GFP-pMSG |
| RBD | GFP-RBD-pDM323 |
| PI3K2 | PI3K2-GFP-pEXP4 |
| actin | GFP-actin-pDM181 |
| enlazin | GFP-enlazin-pLD1 |
| fimbrin | fimbrin-GFP-pDRH, fimbrin-GFP-pLD1 |
| dynacortin | GFP-dynacortin-pLD1 |

| | |
|----------------------|------------------------------------|
| coronin | GFP-coronin-pLD1 |
| α -actinin | GFP- α -actinin-pDN |
| ABP34 | GFP-ABP34-pLD1 |
| limE- Δ -coil | mCherry-limE- Δ coil-pDM181 |
| Lifeact | Lifeact-mRFP-pDM181 |
| p41-Arc | GFP-p41-Arc-pLD1 |
| HSPC300 | GFP-HSPC300-pDM459 |
| cofilin | GFP-cofilin-pLD1 |
| car1 | mCherry-car1-pDM181 |
| myosin IE | GFP-myosin IE-p1S1 |
| myosin VII | GFP-myosin VII- pDTi112 |
| rac1A | mCherry-rac1A-pDM181 |
| racE | mCherry2-racE-pDM181 |
| IQGAP2 | GFP-IQGAP2-pEXP4 |
| clcD | GFP-clcD-pDM181 |
| 14-3-3 | 14-3-3-GFP-pLD1 |
| PP2A | YFP-PP2A-pTX |
| talin A | mCherry-talinA-pDXA |
| talin B | GFP-talinB-pDXA |
| sad A | sadA-GFP-pTX |
| paxillin B | GFP-paxB-pBIG |

Supplementary Table 2. Parameters used in simulation of α -actinin dimer binding based on various assumptions.

| <i>Parameter</i> | <i>Value</i> | <i>(units)</i> | <i>Resource</i> |
|---|--------------|---------------------------------|--|
| <i>Common Parameters</i> | | | |
| $[F_1]$ | 72.0 | μM | 37 |
| $[F_2]$ | 8.0 | μM | estimated |
| D_1 | 10.0 | $\mu\text{m}^2/\text{s}$ | estimated (Stokes-Einstein) |
| $^{\#}D_2$ | 0.12-0.2 | $\mu\text{m}^2/\text{s}$ | estimated |
| $^{\#}D_3$ | 0.12-0.2 | $\mu\text{m}^2/\text{s}$ | estimated |
| k_{on_1} | 1.1 | $\mu\text{M}^{-1}\text{s}^{-1}$ | 34 |
| k_{off_1} | 5.2 | s^{-1} | 34 |
| $k_{on_2}^0$ | 1.1 | $\mu\text{M}^{-1}\text{s}^{-1}$ | estimated |
| $k_{off_2}^0$ | 5.2 | s^{-1} | 34 |
| <i>Parameters for simulations using force-dependent mechanisms (Fig. 3d,f; Sup. Fig. 13d-g)</i> | | | |
| f | 40 | pN | 14 |
| Δx (catch bond) | 0.275 | nm | 14 |
| Δx (slip bond) | -0.275 | nm | 14 |
| <i>Parameters for simulations using structural cooperativity with force-dependency (Sup. Fig. 14)</i> | | | |
| χ | 16 | | estimated |
| <i>Parameters for simulations using catch-slip transition mechanism (Sup. Fig. 15)</i> | | | |
| $k_{off_2}^{c0}$ | 5.2 | s^{-1} | 34 |
| $^*k_{off_2}^{s0}$ | 0.42 | s^{-1} | estimated based on muscle myosin II behavior |
| | 1.09 | s^{-1} | estimated based on L/P selectin behavior |
| Δx_c | 0.275 | nm | 14 |
| $^*\Delta x_s$ | -0.04 | nm | estimated based on muscle myosin II behavior |
| | -0.03 | nm | estimated based on L/P selectin behavior |

[#] Diffusion coefficients D_2 and D_3 apply to complexes formed in the cortex. For the comparable complexes in the cytoplasm, we let $D_1 = D_2 = D_3$.

^{*} $k_{off_2}^{c0}$ and Δx_c are experimentally measured values ^{14, 34}. The values of $k_{off_2}^{s0}$ and Δx_s were calculated from the ratios $k_{off_2}^{s0} / k_{off_2}^{c0}$ and $\Delta x_s / \Delta x_c$ used for muscle myosin II ²⁸ and L/P selectin ²⁷.

Supplementary Table 3. Parameters used in simulation of filamin dimer binding based on various assumptions.

| <i>Parameter</i> | <i>Value</i> | <i>(units)</i> | <i>Resource</i> |
|---|--------------|---------------------------------|--|
| <i>Common Parameters</i> | | | |
| $[F_1]$ | 72.0 | μM | 37 |
| $[F_2]$ | 6.0 | μM | estimated |
| D_1 | 5.0 | $\mu\text{m}^2/\text{s}$ | estimated (Stokes-Einstein) |
| $^{\#}D_2$ | 0.05 | $\mu\text{m}^2/\text{s}$ | estimated |
| $^{\#}D_3$ | 0.005 | $\mu\text{m}^2/\text{s}$ | estimated |
| k_{on_1} | 1.3 | $\mu\text{M}^{-1}\text{s}^{-1}$ | 43 |
| k_{off_1} | 0.6 | s^{-1} | 43 |
| $k_{on_2}^0$ | 0.01 | $\mu\text{M}^{-1}\text{s}^{-1}$ | estimated |
| $k_{off_2}^0$ | 0.6 | s^{-1} | 43 |
| <i>Parameters for simulations using force-dependency; no cooperativity (Fig. 3d,g; Sup. Fig. 16e)</i> | | | |
| f^{α} | 60 | pN | 14 |
| f^{β} | 60 | pN | estimated |
| Δx (catch bond) | 0.194 | nm | 14 |
| Δx (slip bond) | -0.194 | nm | 14 |
| <i>Parameters for simulations using structural cooperativity (Sup. Fig. 16f-h)</i> | | | |
| χ | 9-13 | | estimated |
| <i>Parameters for simulations using catch-slip transition mechanism (Sup. Fig. 17)</i> | | | |
| $k_{off_2}^{c0}$ (α sites) | 0.61 | s^{-1} | 43 |
| * $k_{off_2}^{s0}$ (α sites) | 0.05 | s^{-1} | estimated based on muscle myosin II behavior |
| | 0.128 | s^{-1} | estimated based on L/P selectin behavior |
| Δx_c (α sites) | 0.194 | nm | 14 |
| * Δx_s (α sites) | -0.03 | nm | estimated based on muscle myosin II behavior |
| | -0.02 | nm | estimated based on L/P selectin behavior |
| $k_{off_2}^{c0}$ (β sites) | 0.05 | s^{-1} | estimated based on muscle myosin II behavior |
| | 0.128 | s^{-1} | estimated based on L/P selectin behavior |
| * $k_{off_2}^{s0}$ (β sites) | 0.61 | s^{-1} | 43 |
| Δx_c (β sites) | 0.03 | nm | estimated based on muscle myosin II behavior |
| | 0.02 | nm | estimated based on L/P selectin behavior |
| * Δx_s (β sites) | -0.03 | nm | 14 |

[#] Diffusion coefficients D_2 and D_3 apply to complexes formed in the cortex. For the comparable complexes in the cytoplasm, we let $D_1 = D_2 = D_3$.

* $k_{off_2}^{c0}$ and Δx_c had the values measured experimentally^{14, 34}. The values of $k_{off_2}^{s0}$ and Δx_s were calculated from the ratios of $k_{off_2}^{s0}/k_{off_2}^{c0}$ and $\Delta x_s/\Delta x_c$ used for muscle myosin II²⁸ and L/P selectin²⁷.

Supplementary Table 4. Values of the viscoelastic parameters obtained by fitting creep tests (Supplementary Fig. 19) to Eq. S14.

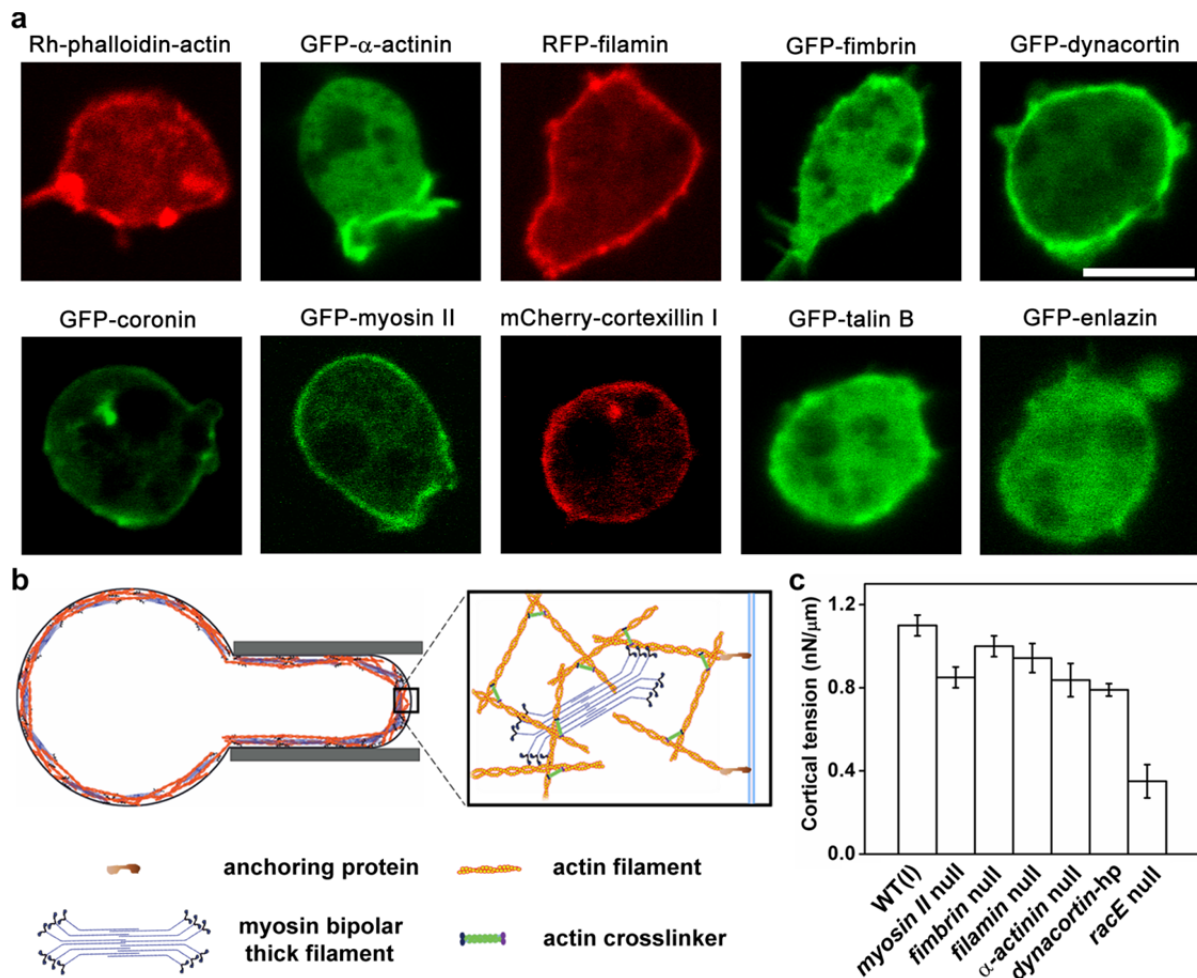
| | k_1 (nN/ μm^3) | η_1 (nN•s/ μm^3) | k_2 (nN/ μm^3) | η_2 (nN•s/ μm^3) |
|---|------------------------------|-----------------------------------|------------------------------|-----------------------------------|
| <i>filamin</i> null | N/A | N/A | 0.35±0.05 | 39.7±6.0 |
| <i>α-actinin</i> null | N/A | N/A | 0.26±0.09 | 13.5±4.7 |
| <i>racE</i> null | 0.03±0.003 | 0.12±0.01 | 0.058±0.005 | ∞ |

Supplementary Table 5. Values of the parameters used in the simulations of cell retractions with Eqs. S15-S18.

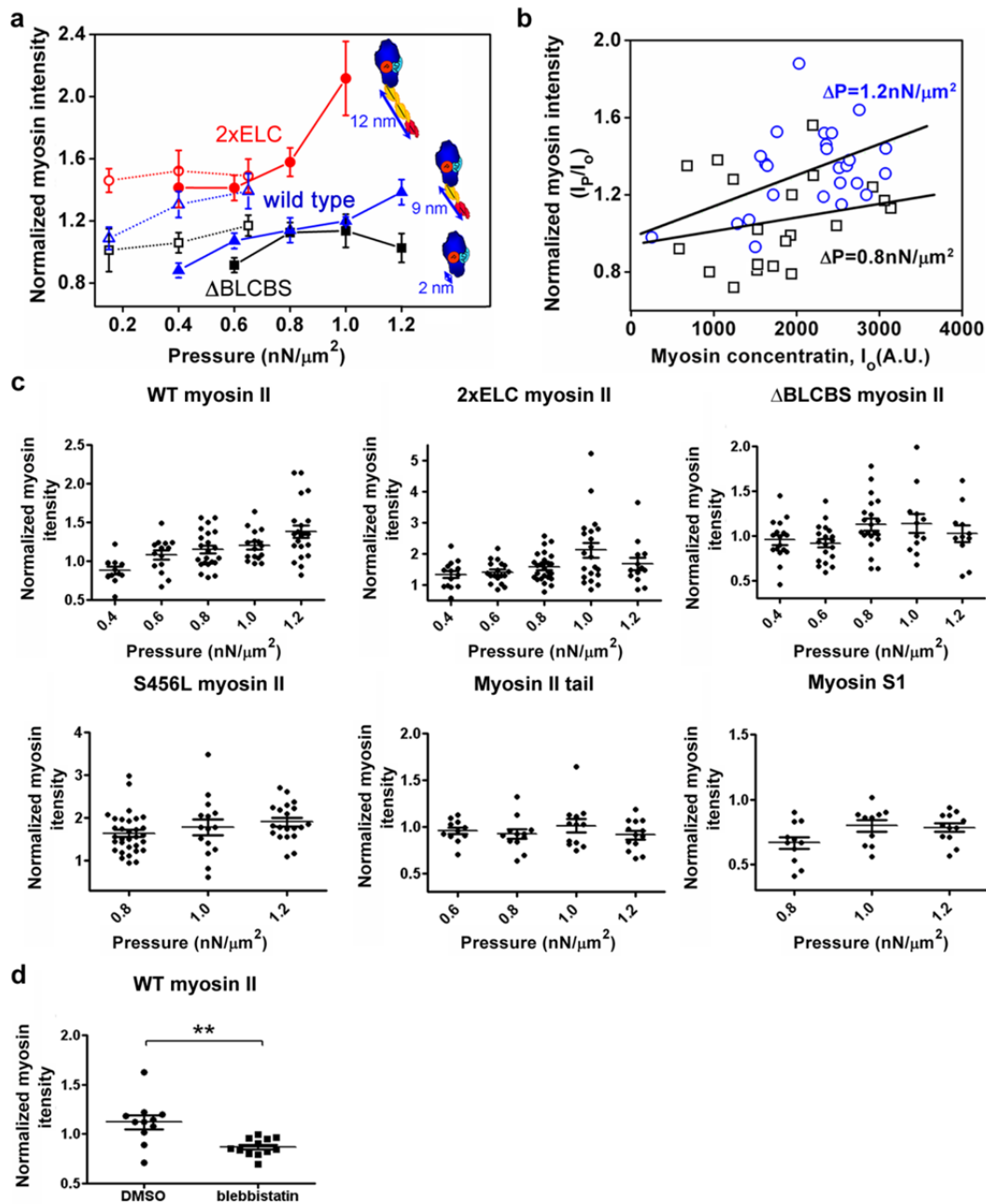
| | <i>force per myosin head</i> (pN) | <i>duty ratio*</i> | <i>BTF fraction**</i> | <i>Cortical thickness</i> (μm) | η_2 ($\text{nN}\cdot\text{s}/\mu\text{m}^3$) | <i>Applied pressure</i> ($\text{nN}/\mu\text{m}^2$) |
|---|--|------------------------|---------------------------|--|--|--|
| <i>filamin</i> null | 4.0 | 0.06 | 50% | 0.5 | 33.7 | 1.0 |
| <i>α-actinin</i> null | 4.0 | 0.06 | 30% | 0.5 | 8.8 | 1.0 |
| <i>dynacortin-hp</i> | 4.0 | 0.06 | 20% | 0.5 | 31.4 | 1.0 |
| <i>racE</i> null | 4.0 | 0.06 | 20% | 0.5 | 5.0 (η_1) | 0.4 |

* The duty ratio of *Dictyostelium* myosin II is about 0.006 at zero force, but may increase up to 10-fold with applied opposing force, resulting in force-dependent range of 0.006-0.06.

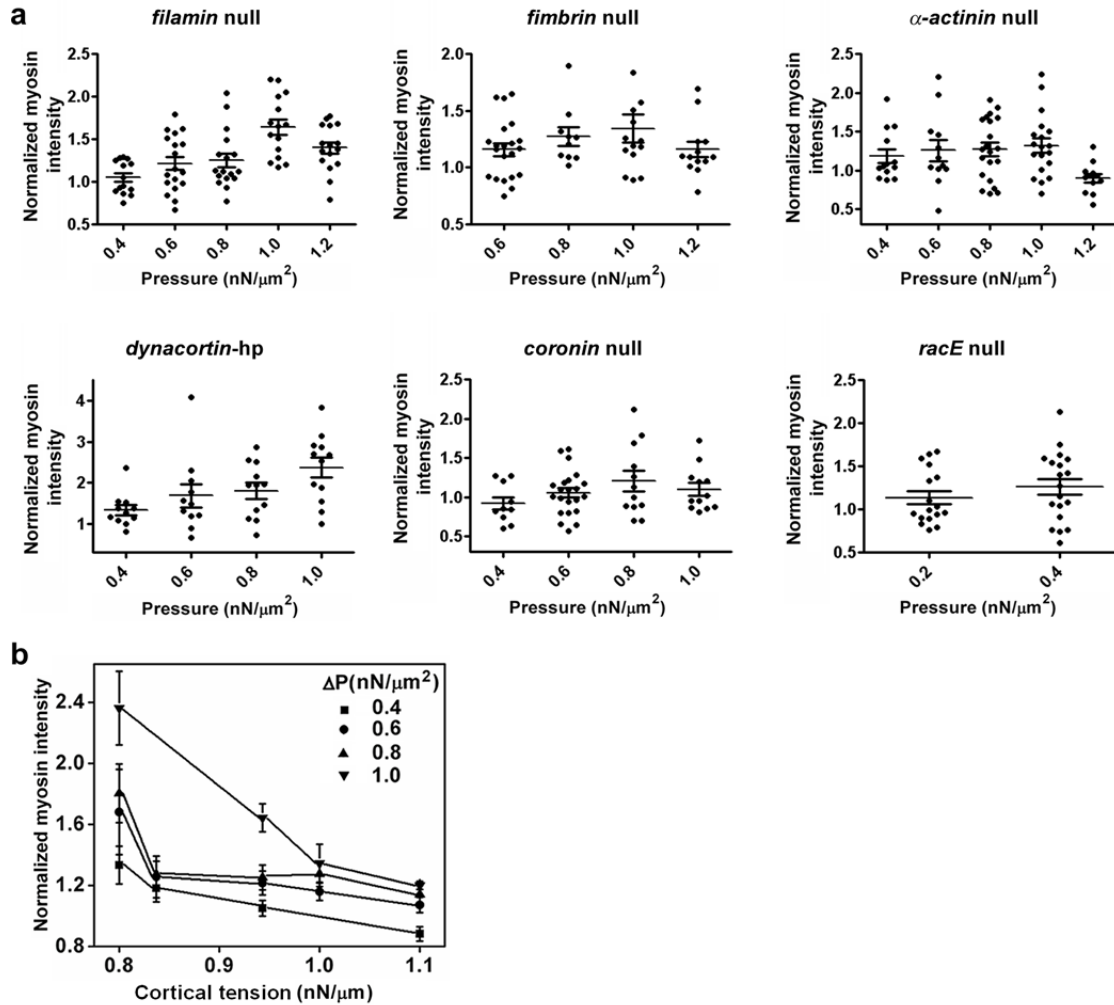
** The immobile fraction of *Dictyostelium* myosin II has an average of 40% with a range of ~20-60% in the cleavage furrow of a dividing cell (where the network is under mechanical stress)³⁸. The immobile fraction appears to reflect the BTF fraction.



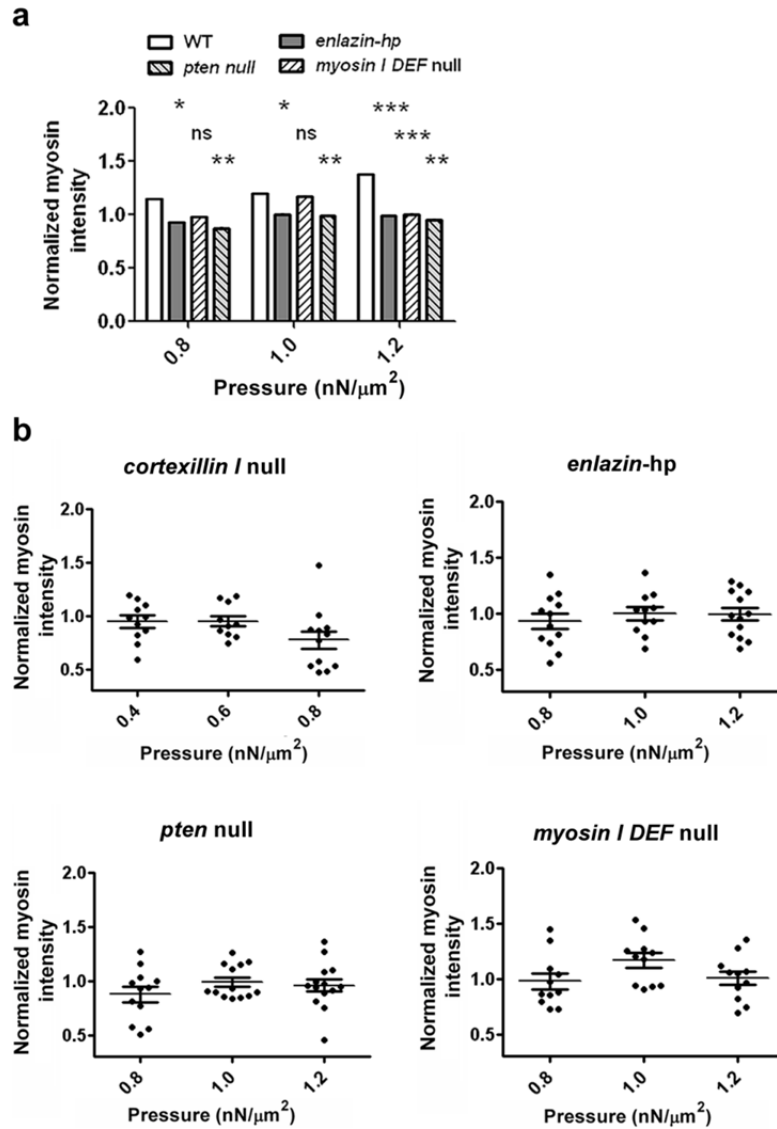
Supplementary Figure 1. The distribution of cytoskeletal proteins and the cortical tension of different mutants of *Dictyostelium*. **a**, The localization of ten cytoskeletal proteins taken by confocal microscopy. Scale bar, 10 μ m. **b**, A schematic diagram of a cell aspirated by micropipette and the zoom-in of actin cortex and plasma membrane. **c**, The cortical tension of different mutants measured by micropipette aspiration. Except for *filamin* null and *α -actinin* null cells, the cortical tensions of other mutants were obtained from previous studies^{8,9}.



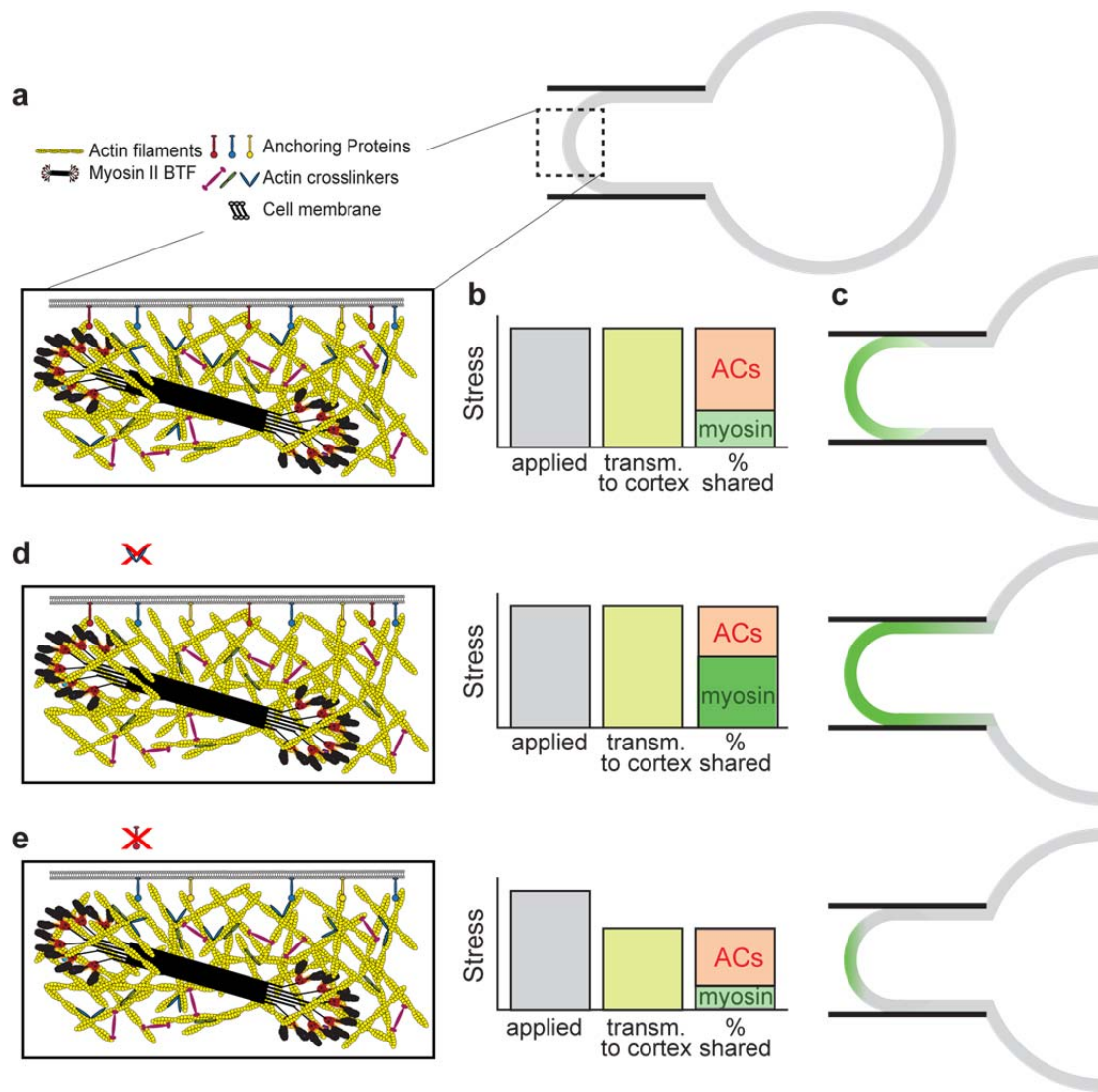
Supplementary Figure 2. The accumulation of different myosin II mutants at different pressures and the corresponding scatter plots. **a**, The accumulation of different myosin II mutants in mitosis (dotted lines) ⁴⁴ and interphase (solid lines) at different pressures. **b**, Dependence of myosin II accumulation on its concentration at different pressures. **c**, The scatter plots of the myosin II accumulations. **d**, Blebbistatin (6 μM in 0.2% DMSO) inhibits myosin II accumulation. Asterisks (**) indicates the p -value < 0.01 , calculated using a Mann-Whitney test.



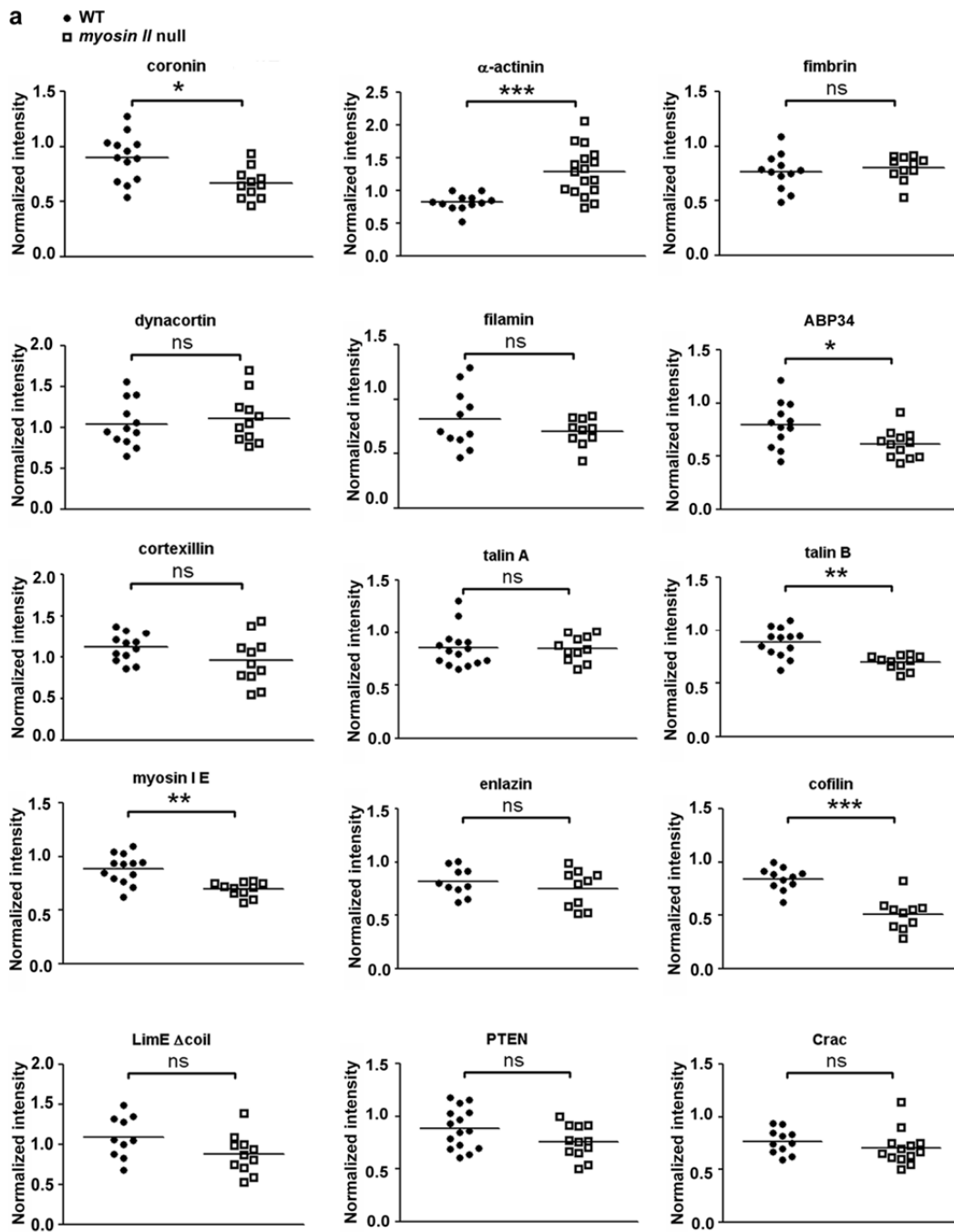
Supplementary Figure 3. The scatter plots of the accumulations of myosin II in different mutant cells and negative correlation between the cortical tension and myosin II accumulation. **a**, Myosin II accumulation in AC deletion mutants (*filamin* null, *fimbrin* null, α -actinin null, *dynacortin-hp*, *coronin* null and *racE* null) is higher than observed in WT cells in low and medium pressure region. But, myosin II accumulation levels decrease at high pressures possibly due to the rupture of the actin network with reduced connectivity, which impairs the force transmission to myosin II. **b**, Cortical tension and myosin II mechanosensitive accumulation are negatively correlated. Five strains tested here are WT, *filamin* null, *fimbrin* null, α -actinin null and *dynacortin-hp* (see **Supplementary Fig. 1c**), and the lines connect the accumulation values from the five stains measured at each pressure.



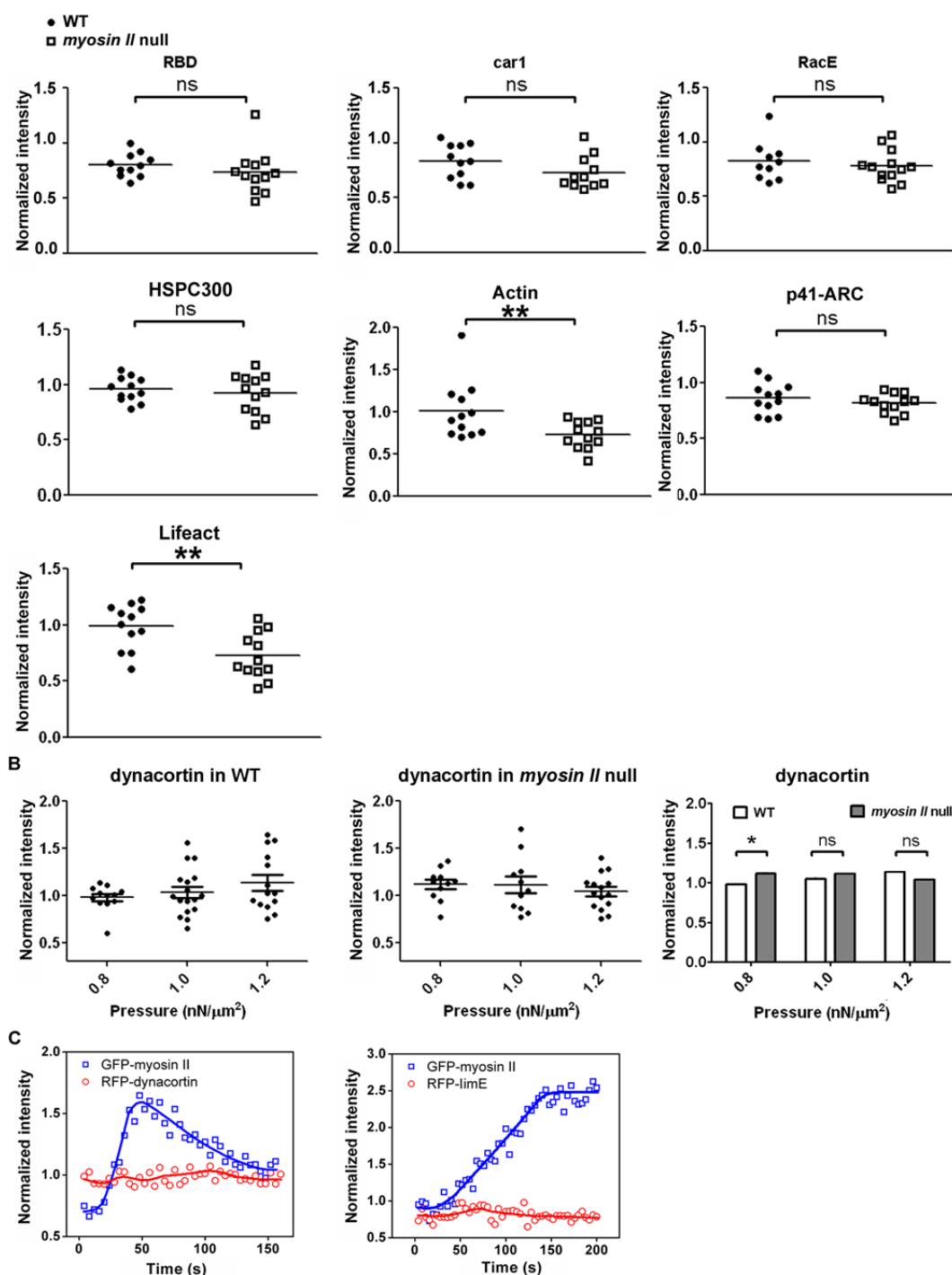
Supplementary Figure 4. Damage to the physical bridges between plasma membrane and actin cytoskeleton reduces myosin II accumulation. **a**, For simplicity, a bar graph of the means with the asterisks denoting statistical significance from a Mann-Whitney test is presented. Dot plots of composite data are in panel **b**, except for WT, which is shown in **Supplementary Fig. 2c**. **b**, Dot plots of all data for each strain and pressure are shown. Myosin II accumulation in the anchoring protein deletion mutants (*cortexillin I* null, *enlazin-hp*, *myosin I DEF* null and *pten* null) remained low across the entire pressure region.



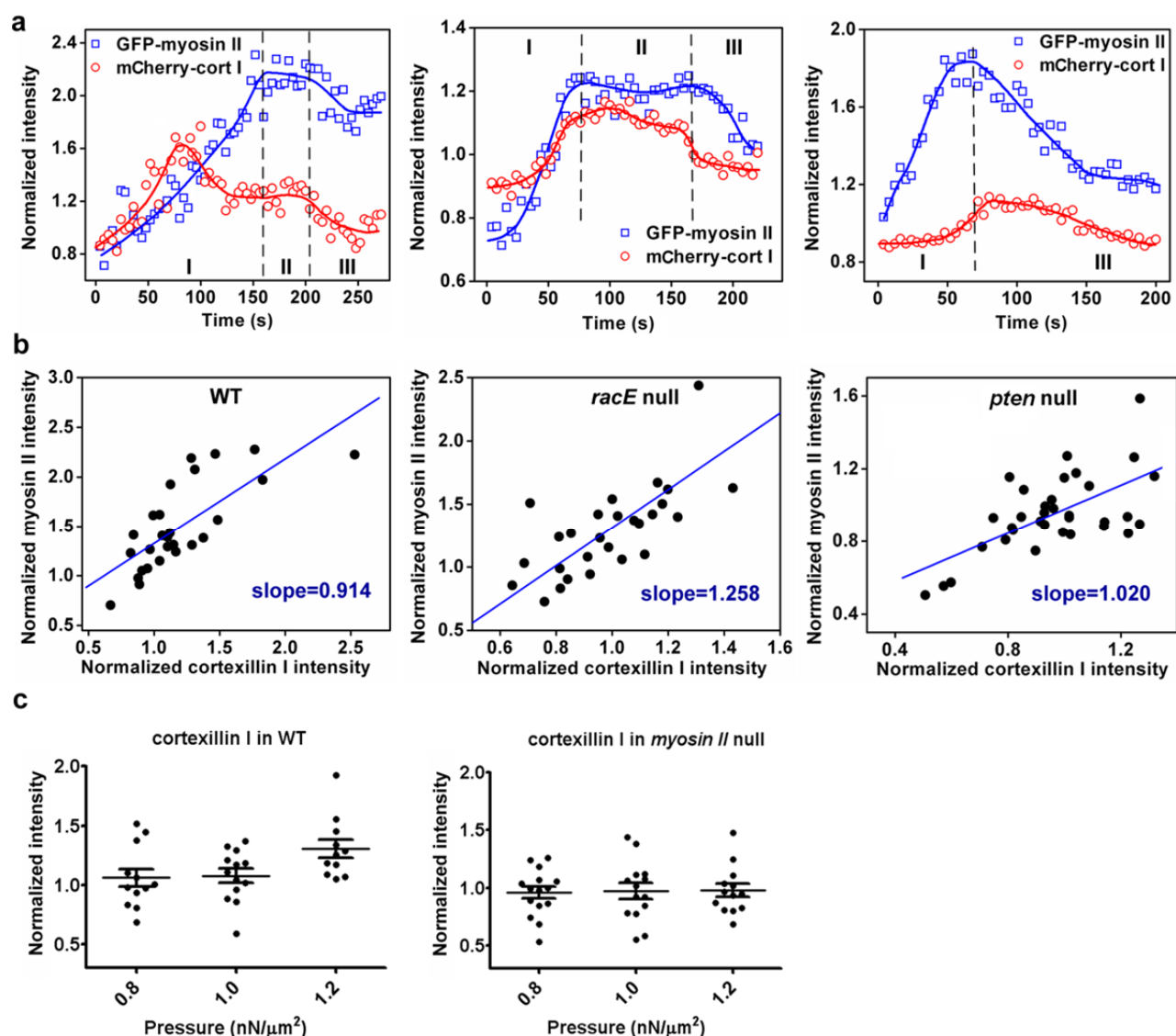
Supplementary Figure 5. Force sharing and the mechanosensory response. **a**, In this cartoon, force applied at the tip of the aspirated cell is transmitted through the membrane, membrane-anchoring and cortical (myosin II and crosslinkers) proteins. In this composite meshwork, the membrane-anchoring proteins act in series with the cortical proteins. **b**, Applied stresses (grey) must be transmitted through the membrane to the cortex (yellow) where they are shared by myosin II (green) and the other crosslinking proteins (red). **c**, This stress leads to a mechanosensory accumulation of myosin II at the tip. **d**, The effect of reducing/deleting some crosslinkers is to increase the amount of applied stress borne by myosin II, leading to greater accumulation at the tip. **e**, Reducing/deleting some of the anchoring proteins leads to lower accumulation, consistent with the view that the stress transmitted to the cortex is reduced. Further indicating a weakening in the membrane-cortex linkage, mutants deficient in anchoring proteins often form blebs at high applied pressures. Thus, the level of stress transmitted to the cortex is reduced, especially at these higher pressure regimes.



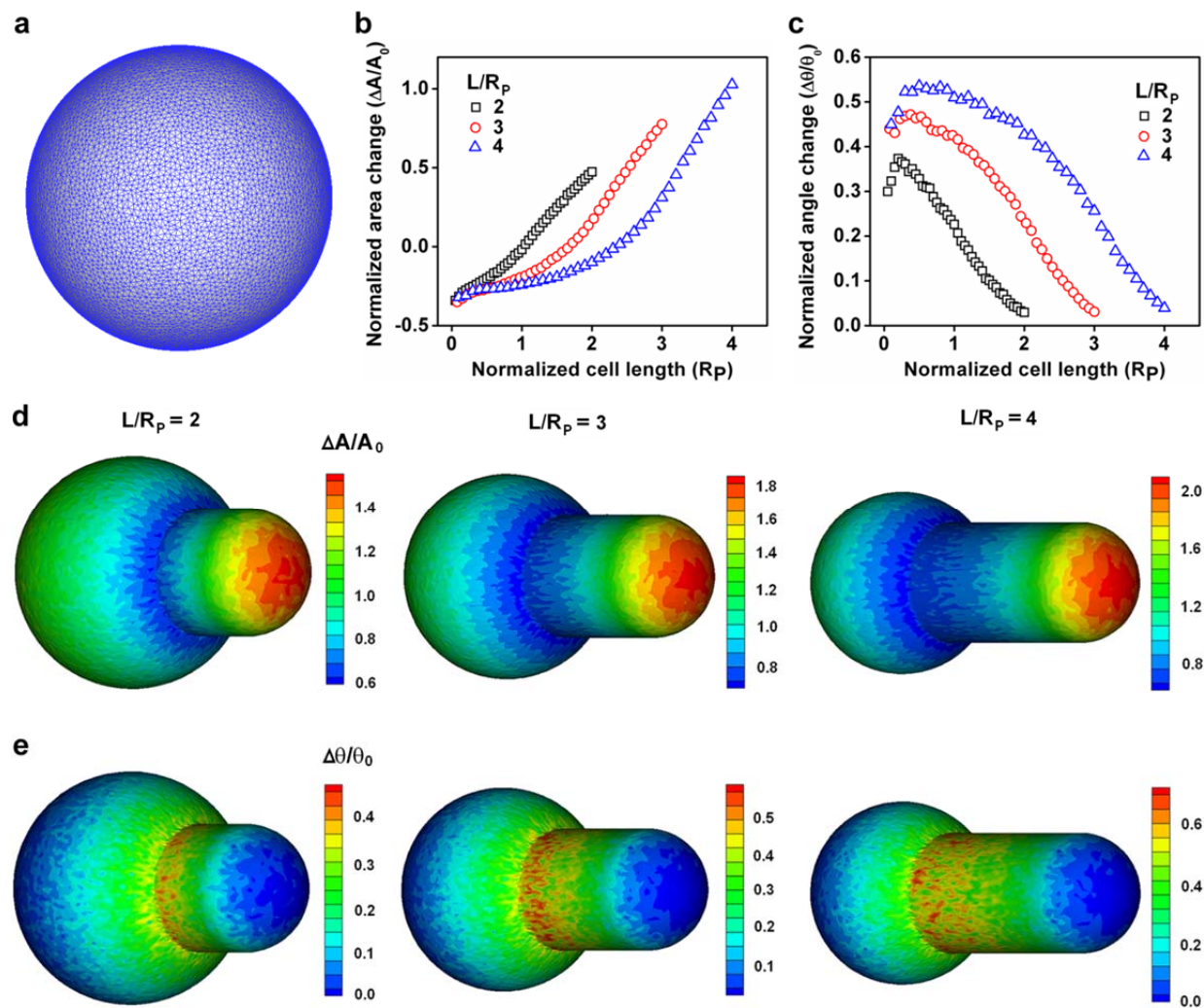
(Supplementary Figure 6; continued)



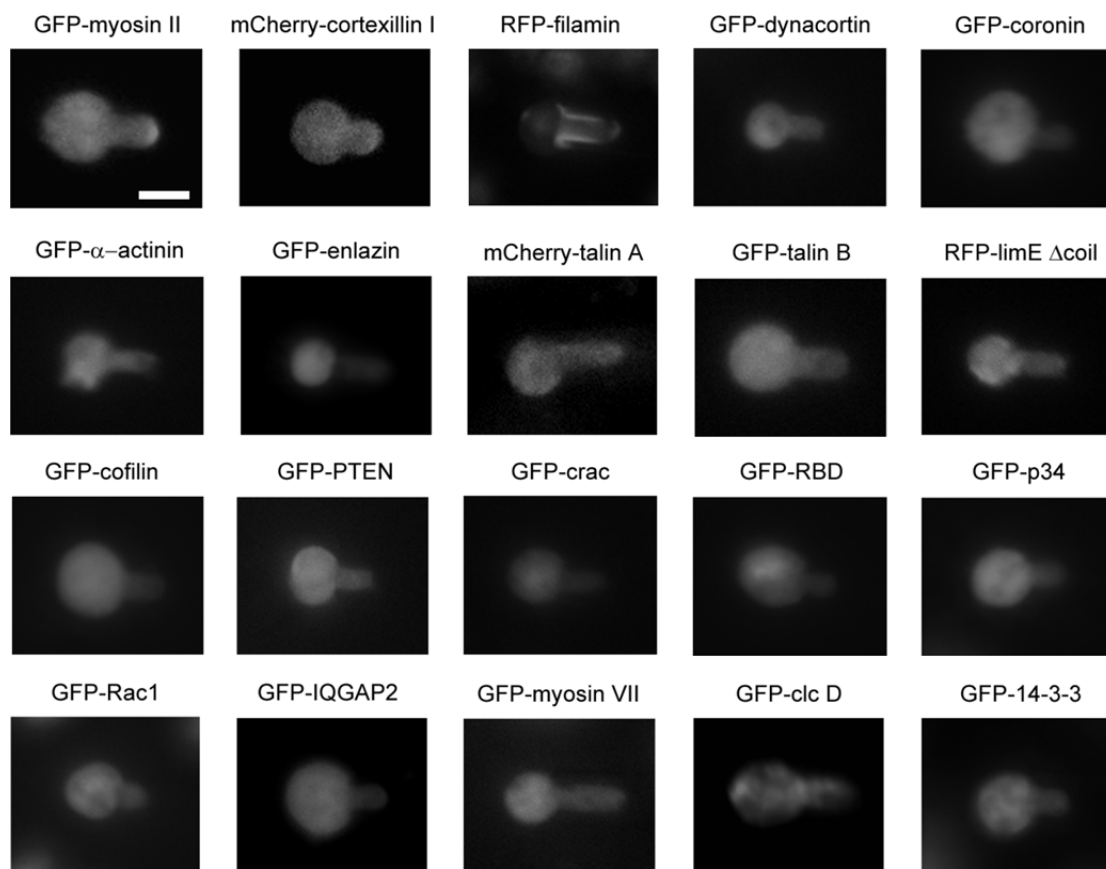
Supplementary Figure 6. Accumulation of cytoskeletal proteins in WT and *myosin II* null cells. **a**, Results of 18 proteins are shown for WT (filled circle) and *myosin II* null (open squares) cells at a pressure of $1.0 \text{ nN}/\mu\text{m}^2$. Panel **b**, shows data from three pressures for dynacortin accumulation in WT and *myosin II* null cells, respectively. **c**, Examples of the accumulation of dynacortin and limE- Δ coil. Asterisks *, ** and *** indicate p -values <0.05 , <0.01 , and <0.005 , respectively, which were calculated using a Mann-Whitney test. Not significant is abbreviated “ns”.



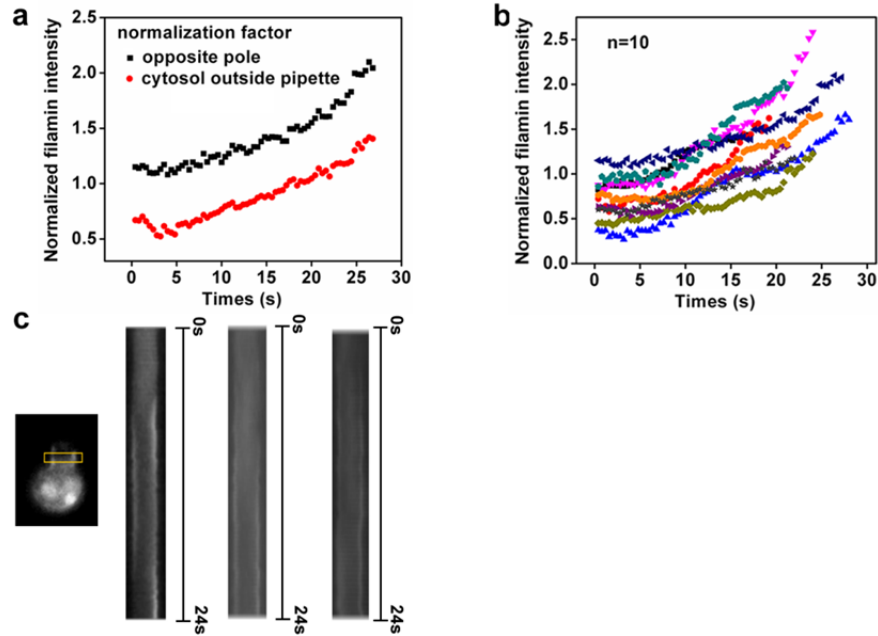
Supplementary Figure 7. Cooperative accumulation of myosin II and corticillin I. **a**, Examples of the cooperative accumulation of myosin II and corticillin I in WT cells. In general, the kinetics of the cooperative accumulation can be divided into three phases: (I) cooperative rise; (II) plateau; (III) cooperative decay. **b**, Correlations between the peak intensities of myosin II and corticillin I accumulations in different mutants. Solid lines were fitted to the scattered data points. Measurements in **a**, and **b**, were conducted in cells co-expressing GFP-myosin II and mCherry-corticillin I. **c**, The scatter plots of the corticillin I accumulation in WT and *myosin II* null cells.



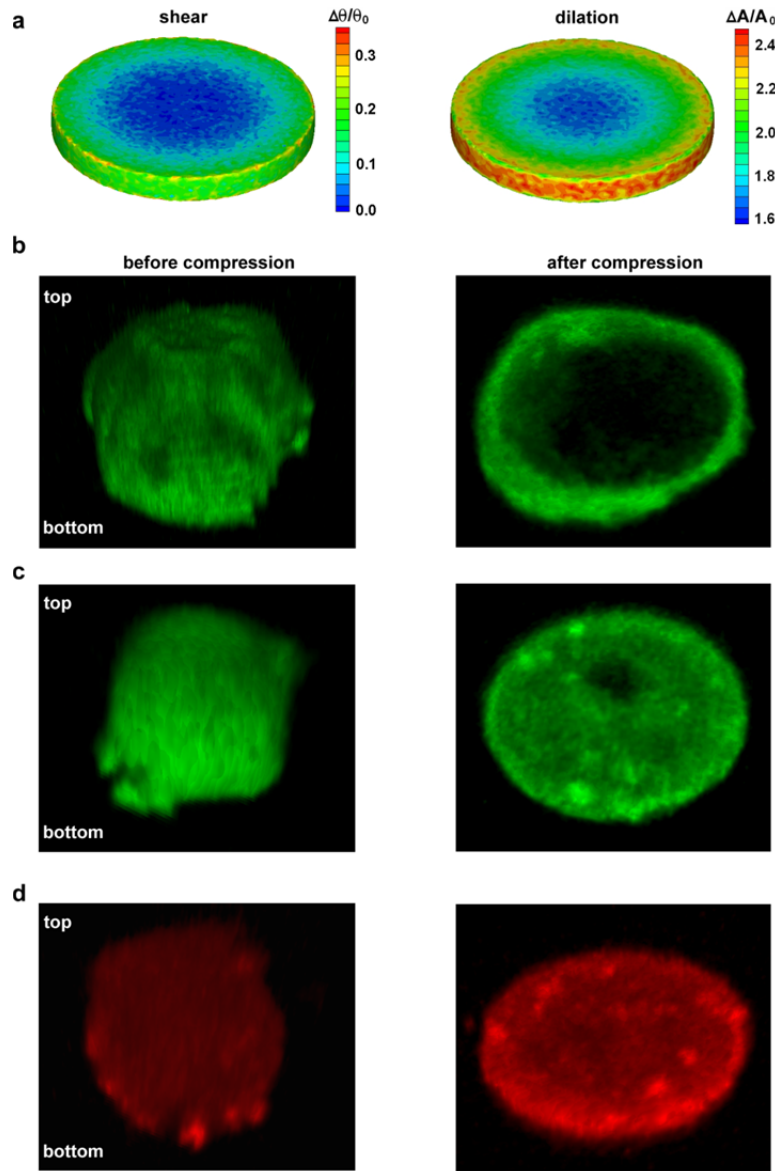
Supplementary Figure 8. The spatial profile of the deformation of actin cytoskeletal proteins calculated by the coarse-grained molecular mechanics model. **a**, The triangle mesh of the cytoskeleton-membrane composite. Panels **b**, and **c**, show the average dilation and shear strains over the rotation angle from the entrance of the pipette to the tip of cell for three different deformation states. Panels **d**, and **e**, show the corresponding detailed contour plots of the strains in each triangle element.



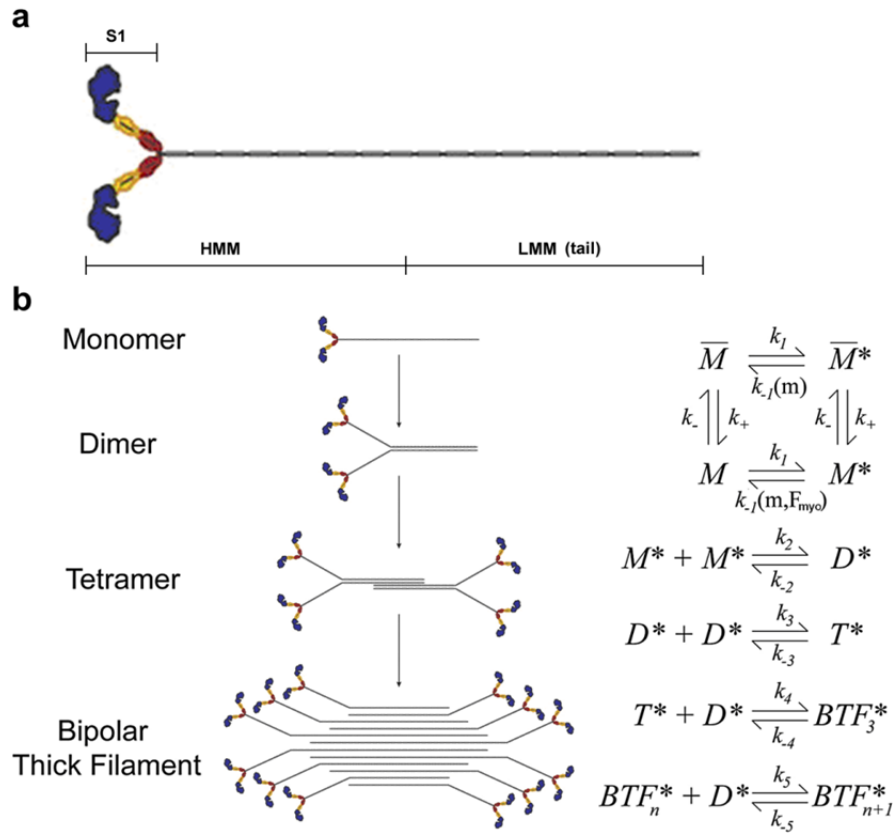
Supplementary Figure 9. The spatial distribution of different cytoskeletal proteins in *raceE* null cells during micropipette aspiration. Scale bar, 10 μ m.



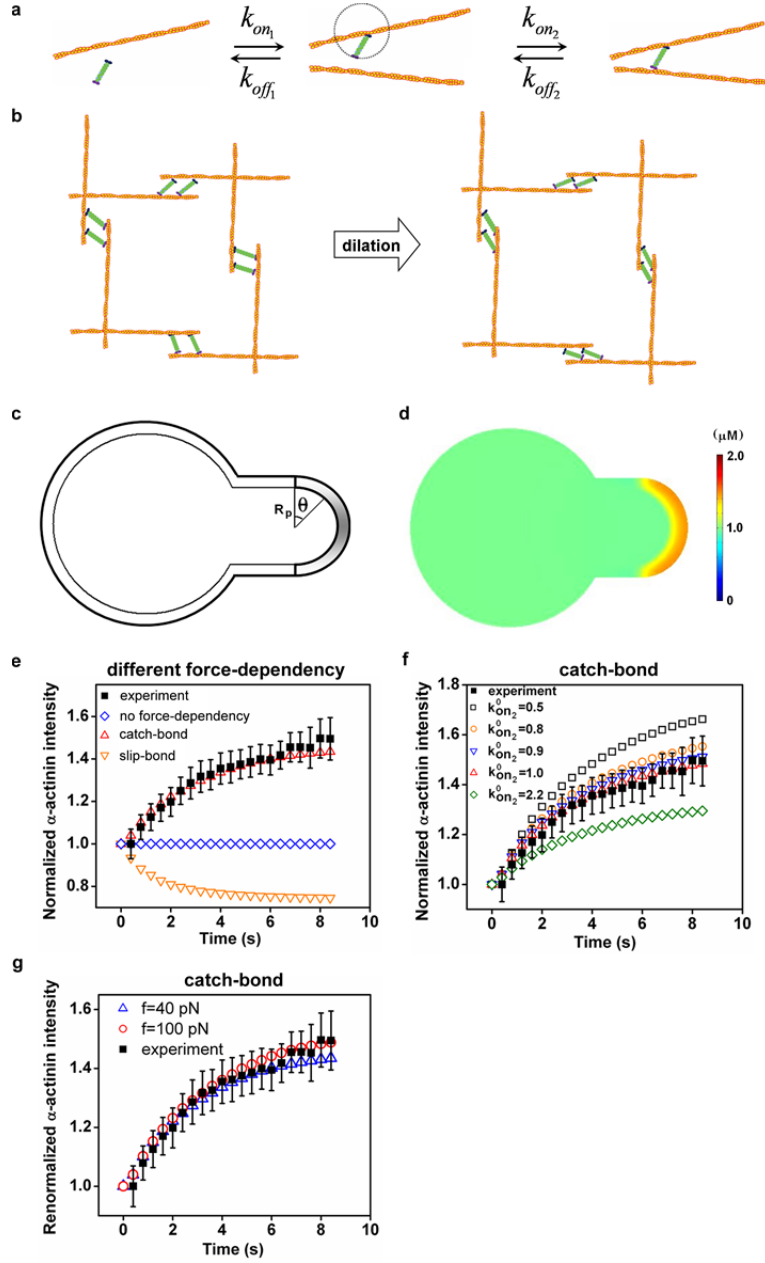
Supplementary Figure 10. The accumulation of filamin in *racE* null cells. **a**, The filamin intensity at the neck region normalized to the intensities at the opposite pole and the cytosol outside pipette show similar trends during accumulation although the absolute values are different. **b**, The kinetics of filamin accumulation in 10 different cells. **c**, The kymographs of filamin accumulation in the neck region in three different cells. The yellow box indicates the region where the signals were extracted.



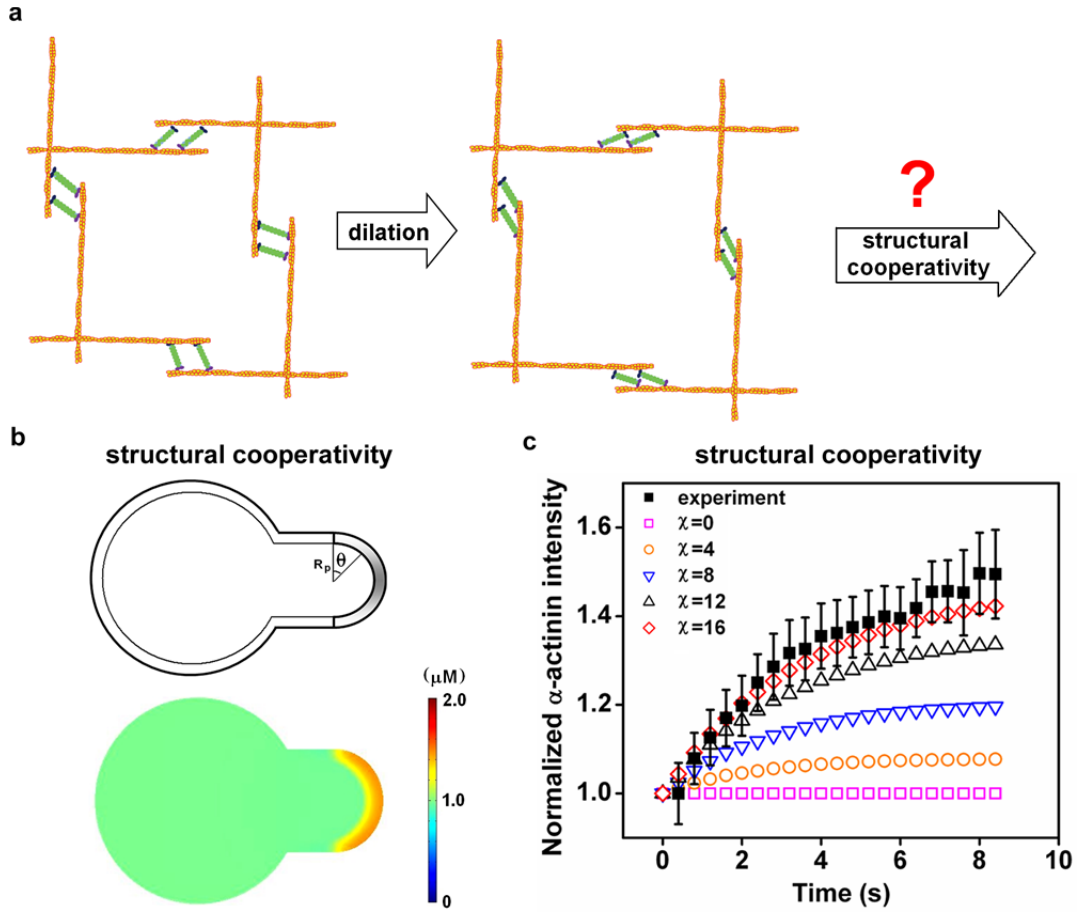
Supplementary Figure 11. Mechanosensitive accumulation of proteins in a compression assay. **a**, Simulated shear and dilation deformations for cells being compressed to a height of $\sim 2\mu\text{m}$ (**Supplementary Text**). Both deformations have the highest values on the lateral surfaces. But, dilation deformation has a sharper gradient than shear deformation near the lateral surfaces. 3D-reconstructed confocal images for GFP-myosin II in WT cells, GFP- α -actinin in *myosin* II null cells, and RFP-filamin in *racE* null cells before and after compression are shown in **b**, **c** and **d** respectively. Due to the sharper gradient of dilation deformation than shear deformation, myosin II shows more significant accumulation than filamin along the lateral edges. In comparison, relative faster accumulations of α -actinin and filamin (~ 10 s) make it challenging to capture the strong accumulations of these proteins in the compression assay combined with confocal imaging.



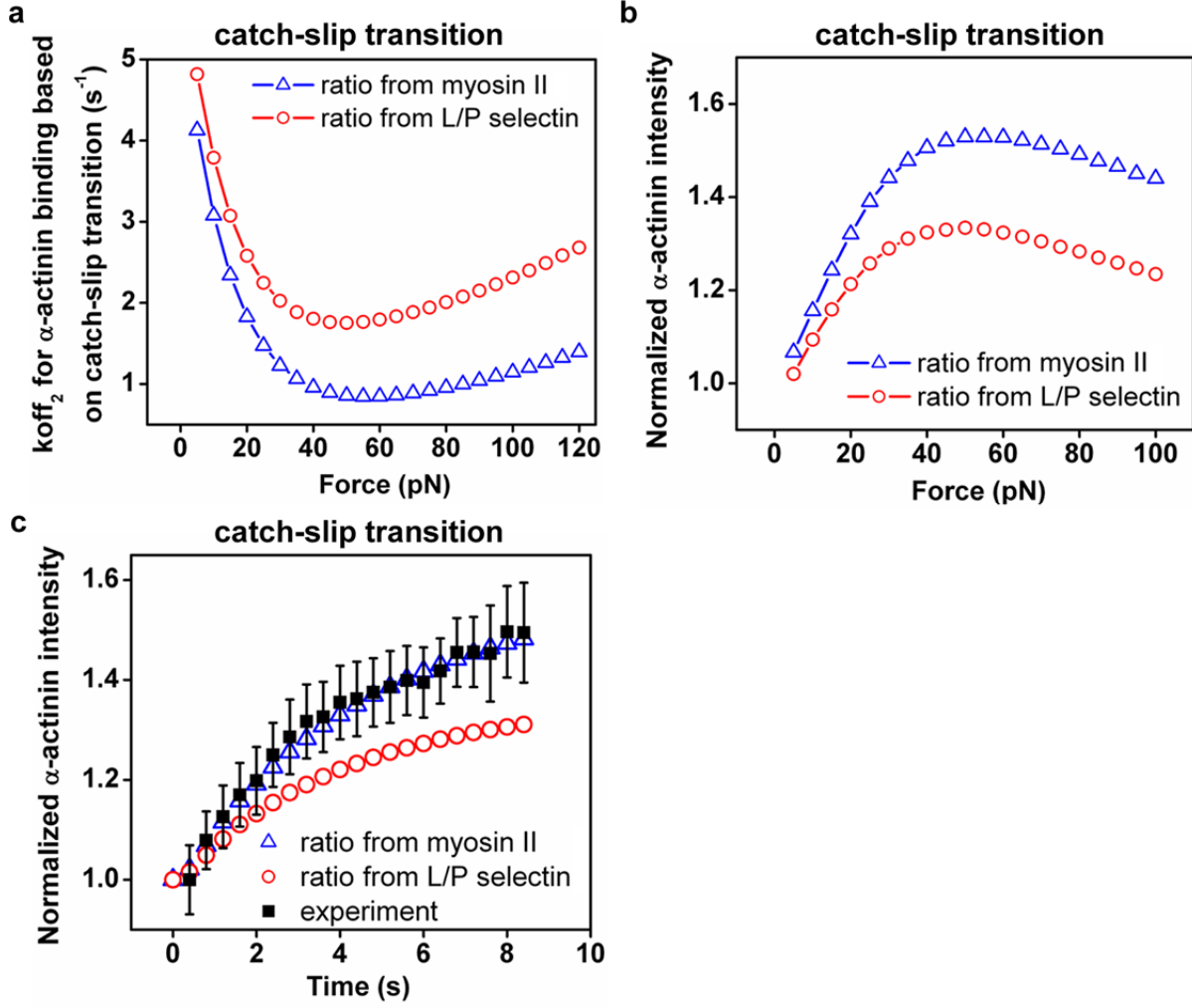
Supplementary Figure 12. The molecular structure of myosin II and its bipolar thick filament assembly scheme. a, The schematic graph of a myosin II monomer. **b,** The assembly scheme of a myosin II bipolar thick filament (adapted from Ref.²⁵).



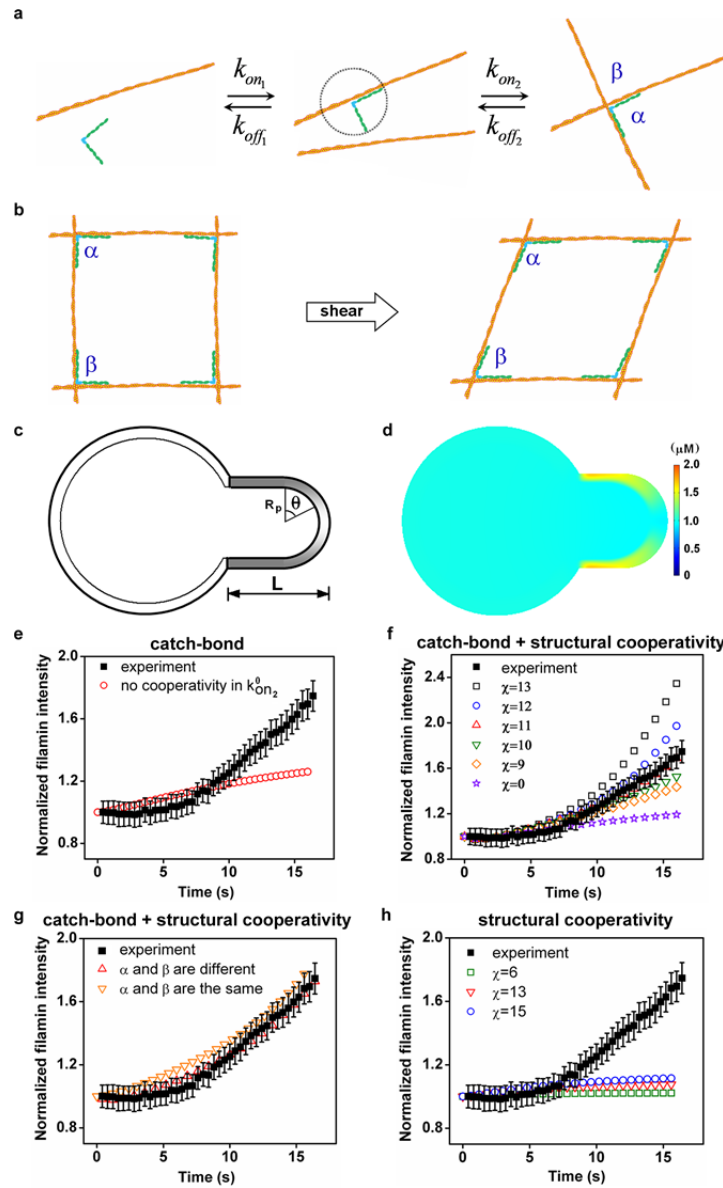
Supplementary Figure 13. The simulations of the accumulation of α -actinin in *myosin II* null cells with catch-bond or slip-bond models. **a**, The two-step force-dependent binding model of α -actinin to F-actin. **b**, The schematic graph of the dilation-induced deformation of bound α -actinin. Green structures represent the α -actinin dimers. **c**, The geometry used in the 3D simulation of α -actinin accumulation. The input force-profile is $f_{\max} \sin \theta$ where θ is the azimuth angle in the tip region, and f_{\max} is 40 pN. The force is zero in other places. **d**, An example of simulated α -actinin accumulation at 8 s for the case of catch-bond model with $k_{on_2} = 1.1 \mu\text{M}^{-1}\text{s}^{-1}$. **e**, Comparison of simulation results using different models to experimental observations suggests that the catch-bond model captures the essential characteristics of the α -actinin behaviors. **f**, Sensitivity analysis of k_{on_2} for catch-bond model. **g**, Simulations with different forces f_{\max} suggest that force does not have a dramatic effect on α -actinin when a catch-bond mechanism is used. **Supplementary Table 2** provides the other parameters.



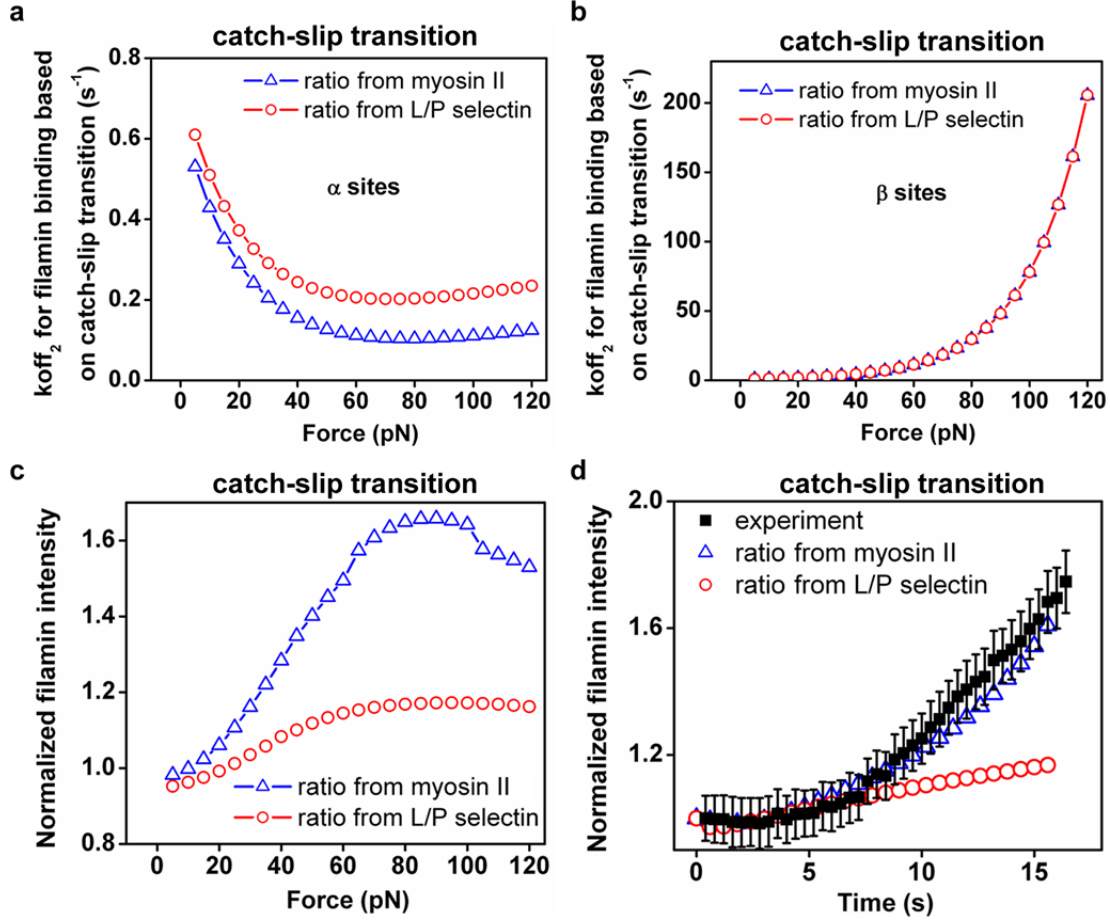
Supplementary Figure 14. Simulations of the accumulation of α -actinin in *myosin II* null cells based on structural cooperativity independent of force-dependent bond models. **a**, The schematic graph of the dilation-induced deformation of bound α -actinin and consequently leading to structural cooperativity which favors more α -actinin binding by increasing the on-rate. However, exactly how dilation induces the structural cooperativity at a molecular level remains unclear. **b**, The top panel shows the input cooperativity-profile $\chi \sin \theta$ where θ is the azimuth angle in the tip region. The on-rate in the second binding step is $k_{on_2} = k_{on_2}^0 (1 + [CrF_1F_2]/[F_2])^{\chi \sin \theta}$. The bottom is one example of the simulated accumulation pattern for α -actinin at 8 s with the cooperativity index $\chi = 16$. **c**, Simulated α -actinin accumulations for various χ . For structural cooperativity model, **Supplementary Table 2** provides the other parameters.



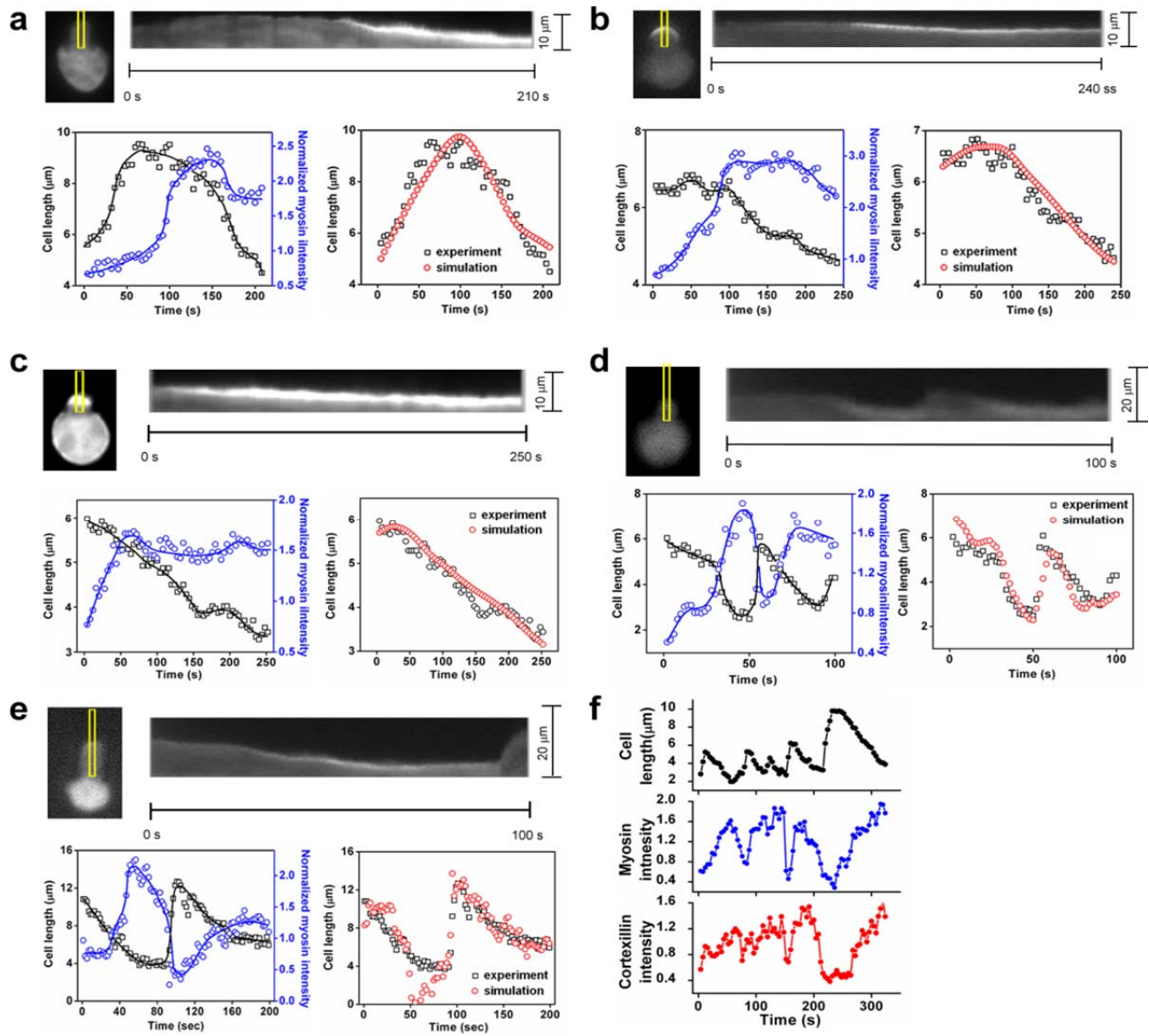
Supplementary Figure 15. The simulations of the accumulation of α -actinin in *myosin II* null cells based on catch-slip transition model. **a**, Plot shows the off-rate k_{off_2} as a function of force. $k_{off_2}^{c0}$ and Δx_c had the values measured experimentally^{14, 34}. The values of $k_{off_2}^{s0}$ and Δx_s were calculated from the ratios of $k_{off_2}^{s0}/k_{off_2}^{c0}$ and $\Delta x_s/\Delta x_c$ used for muscle myosin II²⁸ and L/P selectin²⁷. **b**, Simulated accumulations of α -actinin for 0-100 pN force displayed a rise-fall transition. **c**, The comparison between simulations and experiments suggests that the parameters derived from the ratio used for muscle myosin II accounts for α -actinin accumulation better than the ratios derived from L/P selectin. **Supplementary Table 2** provides the parameters for catch-slip transition model.



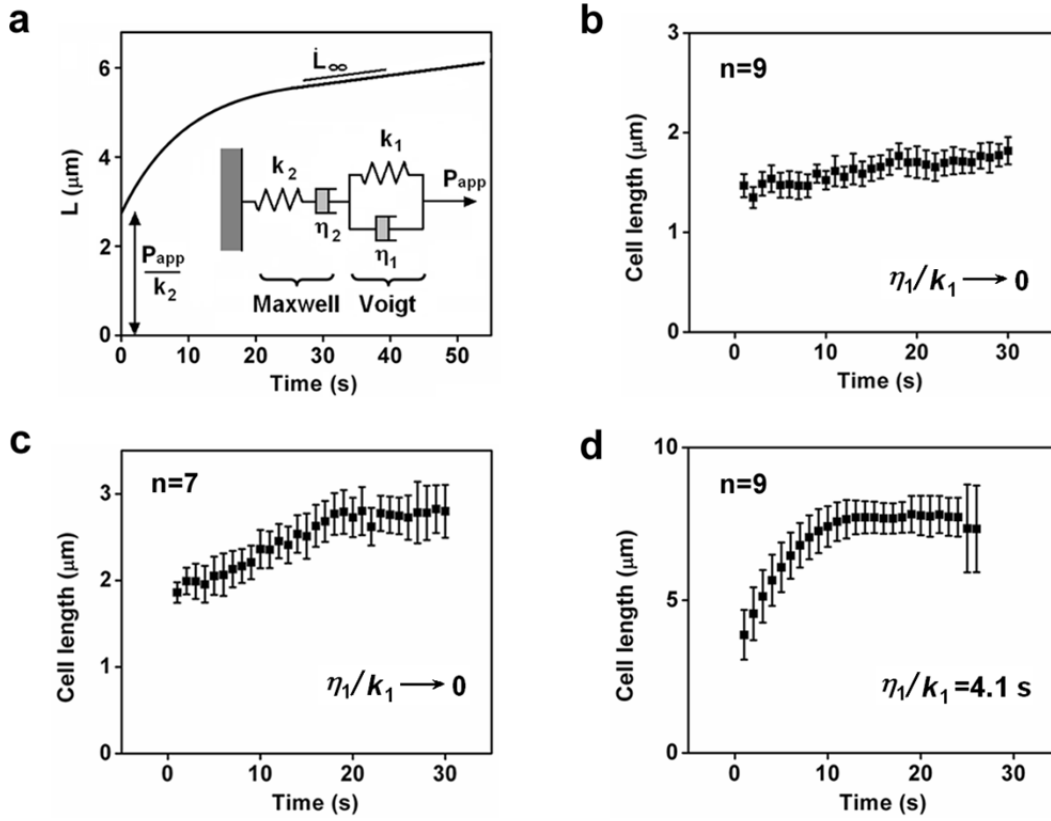
Supplementary Figure 16. The simulations of the accumulation of filamin (in *racE* null and *dyn-hp* cells) with the implementations of catch-bond model and structural cooperativity. **a, The two-step force-dependent binding model of filamin to F-actin. **b**, The schematic graph of the shear-induced deformation of bound filamin. Green structures represent the filamin dimers. **c**, The geometry used in the 3D simulation of filamin accumulation. For catch-bond simulations, the input force-profile in the actin cortex is $f_{\max} \cos \theta$ where θ is the azimuth angle in the spherical tip region and f_{\max} in the whole neck region. Here, f_{\max} is 60 pN. The force is zero in other places. **d**, An example of simulated filamin accumulation at 12 s for the case of $\chi=11$ and $k_{on_2}^0=0.01\mu\text{M}^{-1}\text{s}^{-1}$. The remaining parameter values may be found in **Supplementary Table 3**. **e**, The sensitivity analysis of χ when $k_{on_2}^0=0.01\mu\text{M}^{-1}\text{s}^{-1}$. **f**, Sensitivity analysis of $k_{on_2}^0$ when $\chi=11$. **g**, Treating filamin proteins at α and β sites differently produces better simulation results as compared to the experiments. **h**, Simulations with structural cooperativity alone do not capture the kinetics of accumulation observed experimentally. The parameter values may be found in **Supplementary Table 3**.**



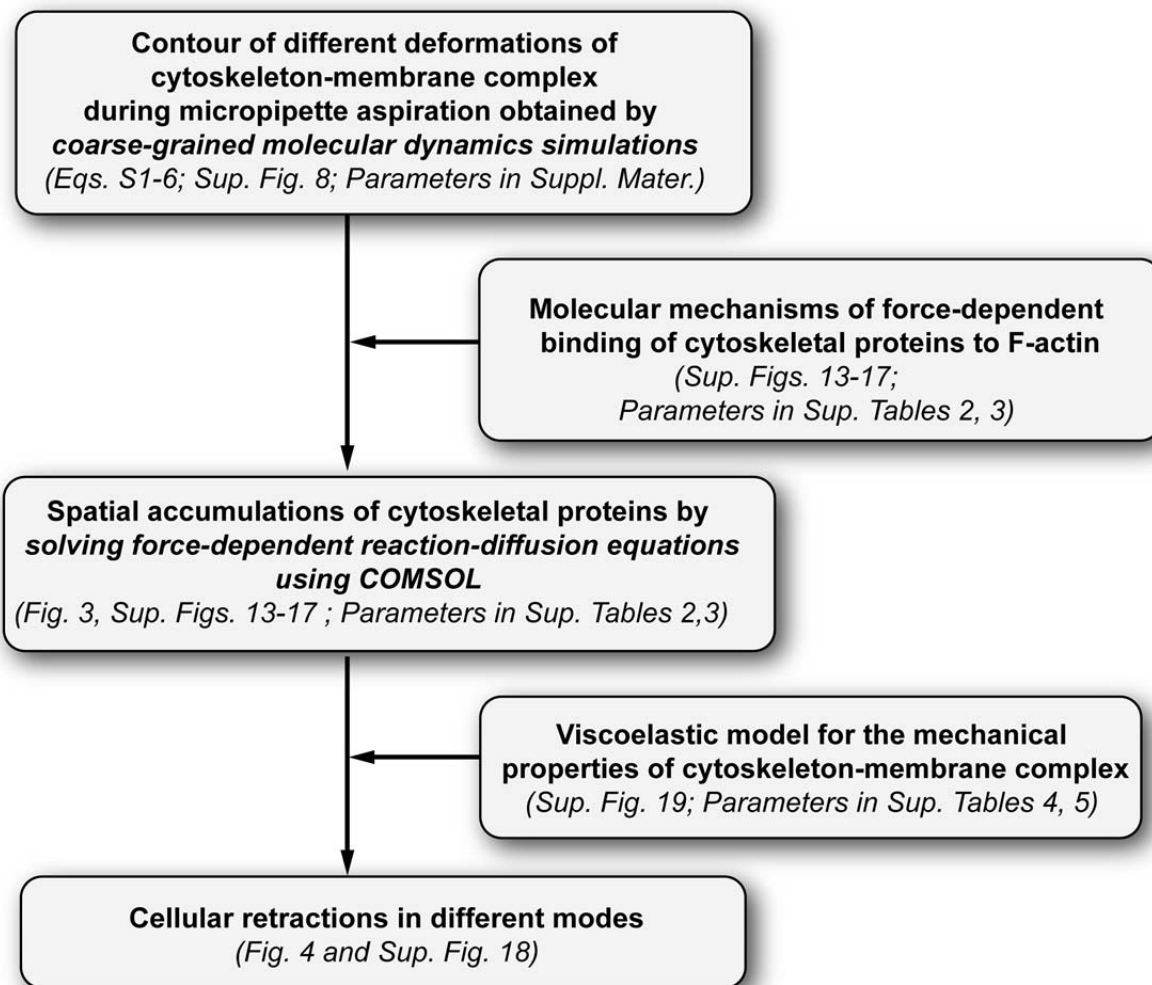
Supplementary Figure 17. The simulations of filamin accumulation in *racE* null cells based on the catch-slip transition model. **a, b,** The off-rates k_{off_2} for α and β sites as functions of force are presented. Values of $k_{off_2}^{c0}$ and Δx_c have been experimentally measured¹⁴. The values of $k_{off_2}^{s0}$ and Δx_s were calculated from the ratios of $k_{off_2}^{s0}/k_{off_2}^{c0}$ and $\Delta x_s/\Delta x_c$ used for muscle myosin II²⁸ and L/P selectin²⁷. **c,** Simulated accumulations of filamin with 0-120 pN force displayed a rise-fall transition. **d,** The comparison between simulations and experiments suggests that the parameters derived from the ratio measured for muscle myosin II more readily accounts for filamin accumulation than those derived from L/P selectin. **Supplementary Table 3** provides the parameters for catch-slip transition model.



Supplementary Figure 18. Additional examples of the retraction of cells due to the accumulation of cytoskeletal proteins. **a**, The retraction of an α -actinin null cell. **b**, and **c**, are the retractions of *dynacortin-hp* cells. Panels **d**, and **e**, show examples of retractions of *racE* null cells. The upper panels of **a**, **b**, **c**, **d**, and **e**, are kymographs. The cell length (empty squares) and myosin II intensity (empty circles) were quantified and displayed in the lower left panels. The lower right panels show the comparison between the experimentally observed cell length and the one calculated from the viscoelastic model using the measured myosin II intensity as the input (**Supplementary Text**). **f**, The cell length measured in a *racE* null cell is negatively correlated with myosin II and cortactin I intensities. **Supplementary Tables 4 and 5** provide the other parameters.



Supplementary Figure 19. The viscoelasticity of *Dictyostelium* cells. **a**, A schematic graph of creep test of a viscoelastic cell. The insert shows organization of the elastic and viscous components in this model. The initial transition has a characteristic time, $\tau = \eta_1/k_1$. When $\tau = \eta_1/k_1 \rightarrow 0$, the transition disappears. Panels **b**, **c**, and **d**, show the creep tests of *filamin* null, α -actinin null and *racE* null cells, respectively. The micropipettes had a diameter of $4 \mu\text{m}$. The pressures were $0.5 \text{ nN}/\mu\text{m}^2$ for *filamin* null and α -actinin null cells and $0.15 \text{ nN}/\mu\text{m}^2$ for *racE* null cells. Extracted values of viscoelastic parameters are listed in **Supplementary Table 4**.



Supplementary Figure 20. The flow chart of the multi-scale simulations, including references to the figures and tables.

Supplementary Movie Descriptions

Movie S1. Myosin II accumulation in a WT cell during micropipette aspiration. Movies collected at 4 seconds between frames. Movies played at 2 frames/s.

Movie S2. The dynamics of fluorescent labeled actin in WT cells during micropipette aspiration. Movies collected at 0.4 seconds between frames. Movies played at 2 frames/s.

Movie S3. The dynamics of fluorescent labeled Lifeact in WT cells during micropipette aspiration. Movies collected at 0.8 seconds between frames. Movies played at 2 frames/s.

Movie S4. α -actinin accumulation in a *myosin II* null cell during micropipette aspiration. Movies collected at 0.4 seconds between frames. Movies played at 2 frames/s.

Movie S5. Filamin accumulation in a *racE* null cell during micropipette aspiration. Movies collected at 0.4 seconds between frames. Movies played at 2 frames/s.

Movie S6. Simulation result of myosin II accumulation in a WT cell during micropipette aspiration.

Movie S7. Simulation result of α -actinin accumulation in a *myosin II* null cell during micropipette aspiration.

Movie S8. Simulation result of filamin accumulation in a *racE* null cell during micropipette aspiration.

Movie S9. Oscillations of a *racE* null cell due to myosin II accumulation during micropipette aspiration. Movies collected at 1.5 seconds between frames. Movies played at 2 frames/s.