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Large-scale curvature sensing by directional actin flow drives cellular migration mode switching

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Abstract

Cell migration over heterogeneous substrates during wound healing or morphogenetic processes leads to shape changes driven by different organizations of the actin cytoskeleton and by functional changes including lamellipodial protrusions and contractile actin cables. Cells distinguish between cell-sized positive and negative curvatures in their physical environment by

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^mData and code availability

All data and custom written code are available upon request.

Author contributions

B.L., R.V., T.C. and A.R. conceived the project. B.L. and R.V. supervised the project. T.C. performed the experiments. A.B. and X.T. helped with traction force experiments and data analysis. Y.T. established the laser ablation system and helped with the related experiments. T.C. performed image and data analysis with the help of H.T.O. The theoretical model was developed by A.C.J. and R.V. while E.F. and T.S. performed the numerical simulation. B.C.L., A.R. and Y.T. contributed resources to the project. The article was written by T.C., A.C.J., R.V. and B.L., read by all authors who all contributed to the interpretation of the results.

forming protrusions at positive ones and actin cables at negative ones; however, the cellular mechanisms remain unclear. Here, we report that concave edges promote polarized actin structures with actin flow directed towards the cell edge, in contrast to well-documented retrograde flow at convex edges. Anterograde flow and contractility induce a tension anisotropy gradient. A polarized actin network is formed, accompanied by a local polymerization-depolymerization gradient, together with leading-edge contractile actin cables in the front. These cables extend onto non-adherent regions while still maintaining contact with the substrate through focal adhesions. The contraction and dynamic reorganization of this actin structure allows forward movements enabling cell migration over non-adherent regions on the substrate. These versatile functional structures may help cells sense and navigate their environment by adapting to external geometric and mechanical cues.

Keywords

cell migration; curvature sensing; actin flow; mechanobiology; biophysical modelling

Cell migration requires force generation by the actomyosin cytoskeleton, which can self-organize into specialized structures such as lamellipodial/filopodial protrusions and contractile cables^{1–4}. Protrusions are driven by actin polymerization pushing against the membrane⁵, while retrograde flow generated by polymerizing actin at the leading edge of the cell and myosin contractility in the lamella are coupled to traction forces exerted through adhesion complexes onto the substrate^{1,6}. During collective cell migration involved in multiple processes such as morphogenesis⁷, wound healing^{3,8} and tumor progression⁹, the migrating front displays variations in cell shape associated with large-scale changes of curvature^{10,11}, resulting in either protrusions or contractile actomyosin cables^{8,10,12}.

Although the mechanisms involved remain unclear, studies have pointed to the ability of cells to choose their migration mode by sensing the curvature of the extracellular environment, in both collectively migrating epithelial cells^{2,10} and single cells plated on micro-patterns^{13–15}. At the molecular scale, curvature sensors such as the BAR domain proteins and septins respond to membrane curvature at the nanometer-to-micrometer scales^{16,17}. At the cell scale and beyond, however, curvature-sensing mechanisms are not well understood, though studies have suggested that cells can sense their own shapes¹⁸ and local curvature at this scale through actomyosin¹⁹. In particular, concave edges of the cell are associated with low substrate adhesiveness and the formation of actin cables^{15,20–22}. Although the regulation of the cytoskeleton has been well studied in lamellipodium-based mechanisms^{23–25}, its turnover and dynamic flow during actomyosin cable formation remain unclear.

Here, we report that the dynamic reorganization of actin filaments can act as a sensor of the sign of large-scale curvature. Concave edges promote the formation of polarized actin structures in which actomyosin flow is directed towards the cell edge in contrast to observations made at convex leading edge protrusions^{25,26}. The switching of the organization and dynamics of actin cytoskeleton in response to either positive or negative curvature suggests that similar functional cytoskeletal components can give rise to different

migration mechanisms, which help cells sense and navigate their environment and adapt to external physical cues.

We first observed the actin organization at the leading edge of epithelial cells (Madin-Darby canine kidney cells, MDCK) with model wound closure assays^{27,28}. The migrating front displayed either convex (positive curvature, Fig. 1a, empty arrow head) or concave edges (negative curvature, Fig. 1a, filled arrow head) showing lamellipodia-based crawling or actin cable assembly, respectively^{2,10}. This actin cable at high negative curvature is connected to a continuous actomyosin structure, which extends into the cell and is connected to the substrate at the rear by focal adhesions (FAs) (Fig. 1b and Supplementary Fig. 1a, b).

To test whether geometrical constraint suffices to produce such actomyosin organization observed at negative curvatures, we designed flower-shaped patterns for a model wound assay²⁷ (Fig. 1c, f; see Supplementary Fig. 2a for details on the pattern geometry). At regions of negative curvature, we observed similar organizations of actomyosin and focal adhesion structures (Fig. 1e, h), whereas at regions of positive curvature the cells showed lamellipodia protrusions (Fig. 1d, g). The structures at negative curvatures displayed a polarized organization of strong leading edge actomyosin cable, immediately followed by a region of network-like actin fibers and myosin II, and FAs at the rear connected to radially oriented actin fibers, reminiscent of a variation of lamellipodium and lamella actin organization^{26,29,30}. With live cell structured illumination microscopy (SIM), we observed an anterograde flow of actin fibers towards the leading edge, in contrast to the retrograde flow usually observed in lamellipodium and lamella at positive curvature (Fig. 1i, j)^{25,26}. We thus hypothesized that the actomyosin system could serve as a cell scale curvature sensor, establishing an anterograde flow at negatively-curved edges that accumulate actin fibers and contractile cables at the front (Fig. 1k), while lamellipodium formation is promoted by the retrograde flow of actin fibers at positively curved edges^{26,29}.

Migration over heterogeneous substrates³¹ and the cooperative nature of lamellipodia crawling and actin cable contraction during gap closure² suggest that negative curvature-induced actin structures could facilitate monolayer migration over non-adhesive regions. To test this, MDCK cells were plated on micro-patterned substrates composed of fibronectin coated surfaces dotted with non-adherent circles (see Methods)²⁸. When the MDCK monolayer migrated over non-adhesive regions, actin structures were formed as negatively-curved cell edges were forced by cell crawling on either side of the circle (76.5 min-178.5 min in Fig. 11). These structures actively promoted forward cellular movements over the non-adherent region (Fig. 11, Supplementary Fig. 1e and Supplementary Video 1). As the cell encountered the non-adherent circle and the edge of the cell front adopted negative curvature, the migration mode switched from lamellipodium (Fig. 1m, filled arrowhead) to actomyosin cable (Fig. 1m, empty arrowhead). This was accompanied by a switch from retrograde to an anterograde flow of myosin (Fig. 1m, yellow dashed lines). These processes were not confined to collective cell migration processes, but were also observed within single cells such as Hela and U2OS when responding to negative curvature induced by heterogeneous micro-patterned substrates (Fig. 1n and Supplementary Fig. 1c, d). We also created interconnected electro-spun fiber networks to mimic 3D fibrous *in vivo* environments³² and seeded REF52 (Rat embryonic fibroblast) cells onto the network

(Supplementary Fig. 1f). In addition to attaching to the fibers, cells were also able to extend into empty spaces at cross-sections where a suspended (non-adherent) actin structure similar to the ones induced by negative curvature was observed (Fig. 1o).

Next, we confined cells on flower-shaped patterns to immobilize cells and control cell geometry (Fig. 2a and Supplementary Fig. 2a). Cells responded to the curvature imposed by the pattern by consistently forming lamellipodial protrusions at positive curvatures and suspended actin cables at negative ones (Fig. 2b and Supplementary Video 2). The side view shows a difference in the 3D structure between opposite curvatures: a suspended cell part over the non-adherent region, with actin accumulation at its tip and continuous actomyosin staining along the ventral surface, for negative curvatures; and a lamellipodial protrusion close to the pattern edge for positive curvatures (Fig. 2c).

We then analyzed the organization and the dynamics of the actin cytoskeleton at sub-cellular levels within regions abutting these negatively curved edges. Using SIM, we confirmed our previous observation that a polarized actin organization with radially oriented actin fibers (RFs) at the rear, transversely oriented actin fibers (TFs) near the front, and a suspended actin cable at the leading edge emerge in concave regions (Fig. 2d, see also Fig. 3a and Fig. 5q). As the actin structure occupies only part of the cell, the front and rear here and henceforth refer only to the actin structure and not the whole cell. This actin organization was quantified by the nematic order parameter $S = \langle \cos(2\theta) \rangle$ (where θ denotes the angle between the normal to the cell edge and the local actin filament orientation), which ranged from positive values at the rear to negative values at the front (Fig. 2d, e and Supplementary Fig. 2b, c), suggesting anisotropic actin organization³³. TFs exhibited sarcomeric patterns of α -actinin and myosin IIA (Fig. 2f, g and Supplementary Fig. 2d), whereas RFs were associated with α -actinin and connected to focal adhesions (FAs) at the rear (Supplementary Fig. 2e). This structure recalls the lamella, where transverse arcs glide on radially oriented fibers^{30,34}, yet presents a reversed organization. Similarly, the RFs connected to the FAs at the rear remained non-contractile as shown by the absence of myosin IIA in this region, but the front part displayed concentration gradients of both actin and myosin IIA, suggesting increased contractility (Fig. 2h, i).

At negative curvatures anterograde flow of TFs was observed in the cell, and which coalesced at the front with the leading edge actin cable (Fig. 3a, b and Supplementary Fig. 3a, b and Supplementary Video 3). This contrasts with the retrograde flow observed at positive curvature regions (Supplementary Fig. 3c). The flow velocity of the TFs appeared to depend on the curvature magnitude, which was varied by changing the size of the pattern (Supplementary Fig. 3d). In order to investigate the relationship between flow velocity and curvature magnitude, we designed patterned surfaces with edges comprised of semicircular shapes with a set of positive and negative curvatures separated by regions of flat edges (Supplementary Fig. 3e, f). By imaging cells transfected with mEos3.2 myosin IIA (see Methods), we measured the myosin flow velocity at different curvatures. This quantity showed a bi-phasic behavior with an optimum value at curvature radii 15 and -20 μm , which is of the order of the cell size (Fig. 3c). This non-monotonic dependence of flow velocity on curvature can be explained in the framework of the physical model based on active gels (SI). The anterograde flow at negative curvature was not restricted to the TFs, but occurred

throughout the entire actin structure. First, photo-conversion of RFs in mEos2-actin transfected cells revealed that RFs were engaged in anterograde flow (Fig. 3d-f and Supplementary Video 4). Then, by combining fluorescent speckle microscopy (FSM)³⁵ (Fig. 3g, h and Supplementary Video 5) and direct TF tracking, we measured the flow profile across the entire actin structure. The actin flow velocity decreased from the rear ($0.38 \pm 0.05 \mu\text{m}/\text{min}$ starting from the RF region) to the front ($0.17 \pm 0.04 \mu\text{m}/\text{min}$ starting from the TF region) of the structure leading to the contractile cable at the edge (Fig. 3i). In comparison, the actin flow at positive curvature, where lamellipodia formation is preferred, increased from the rear to the front (Supplementary Fig. 3g-i and Supplementary Video 6). Altogether, these results show that concave regions promoted reverse actin flow patterns with rear-to-front actomyosin gradients, suggesting that these polarized structures may result from cell-generated active forces²⁴.

To investigate the role of mechanics in the establishment and the maintenance of these actin structures, we laser ablated actin fibers at different locations. Ablation of TFs resulted in recoil (Fig. 4a, b and Supplementary Video 7), suggesting that they were under tension. Contractility is necessary for directed actomyosin flow as blebbistatin ($25 \mu\text{M}$) treatment stopped the myosin flow in MDCK cells (transfected with mApple Myosin IIA), which can be rescued by washout of the drug (Fig. 4c and Supplementary Video 8). Point ablations within the myosin-rich transition region allowed us to assess the local tension, based on the recoil velocity of the actomyosin meshwork (Fig. 4d, e and Supplementary Fig. 4a-d). Tension in the radial direction decreased from rear to front whereas it increased in the transverse direction, resulting in a tension anisotropy gradient (Fig. 4f, g and Supplementary Fig. 4e, f) correlating well with the variation of actin order parameter (Fig. 2e). This anisotropy gradient has also been shown to couple to actin flow^{36–38}. We then used traction force microscopy (TFM)³⁹ to visualize forces exerted by cells on the substrate along the edges of the pattern (Fig. 4h). We observed large traction forces along the actin cables at the edge (Fig. 4i, filled arrow heads). In contrast, outward pointing forces were generated at highly negative curvatures (Fig. 4i, empty arrow head) resulting from the tension within the actin gel exerting pulling forces on FAs localized at the edge of the pattern. In comparison, inward pointing forces are usually observed for cells crawling or spreading with positively curved outer edges (Fig. 4h)^{6,40}. Here, the distinctive geometry of the actin structure led to cell traction forces at FA sites that are directed outward towards the cell edge, in agreement with force patterns observed during *in vitro* wound healing³⁹.

To further test our hypothesis of a coupling between tension anisotropy at negative curvatures and actin flow, we developed a model that describes the actin cytoskeleton as a 2D active nematic gel⁴¹ (see Supplementary Information (SI) for details). We summarize here a 1-dimensional analysis along the rear/front axis x of the suspended region (along the radial direction in Fig. 2d); a 2D numerical analysis of the stress field in the actin structure was also performed (Fig. 4j). The rear (i.e., edge of the adhesive pattern) is given by $x = 0$, and the front (i.e., edge of the suspended cell) is $x = l$. Following active gel theory^{38,41}, the action of myosin II motors leads to an active contractile stress parameterized by positive constants ζ and ζ' . We allow the isotropic part ζ , which can be understood as a negative pressure⁴², to depend on the bound density of myosin motors c_m , whereas, for simplicity, the anisotropic part ζ' is assumed constant. Since the actin structure is suspended and non-

adherent, we neglect friction forces with both membrane and cytosol as compared to viscous forces, as justified in43 (see also SI). Thus, calling σ the tension along x in the gel, force balance is given by $\partial_x \sigma = 0$. As a result, we find $\sigma = \lambda/R$, where λ is the line tension of the front stress fiber and R the radius of curvature of the actin structure front. Next, σ can be related to contractile activity and to the viscous stress $\eta \partial_x v$, where $v(x)$ is the actin flow field along x ; furthermore, due to fast actin turnover, pressure forces are neglected38; see SI. Thus, we find the following linear equation that determines the flow profile (see SI):

$$\partial_x v = - \frac{\zeta - \frac{\lambda}{R}}{\eta}. \quad (1)$$

In addition, our active gel model provides a prescription for determining the nematic order parameter $S(x)$, as described in SI.

This model reproduces key experimental features. (i) Equation (1) shows that the actin flow is generated by myosin activity, and as long as isotropic contractility is dominant, one finds $\partial_x v < 0$. This, together with the boundary condition $v(l) = 0$ — which assumes no boundary-specific sources of actin turnover — shows that contractility induces an anterograde flow in the actin structure, as observed experimentally. Quantitatively, flow measurements reveal that the flow profile is fitted well by a linear dependence on x (Fig. 3i), which suggests that contractile terms in Eq. (3) can be assumed constant. The maximal flow speed can then be estimated as $l\zeta/\eta$, which gives $0.4 \mu\text{m}/\text{min}$ with standard values of $\zeta \sim 10^3 \text{ Pa}$ and $\eta \sim 10^5 \text{ Pa.s}$ 44,45, in agreement with observations. (ii) This estimate of the maximal flow speed captures the effect of geometry through the dependence on l , which can be estimated as $l \sim L_c^2 |C|$ for $L_c < 1/|C|$, where C is the substrate curvature and L_c the typical cell size, and $l \sim 1/|C|$ for $L_c > 1/|C|$. This predicted non-monotonic dependence on curvature is observed experimentally (Fig 3c). (iii) It is shown in SI that $S(x)$ can be analytically determined, yielding a linear behavior in the limit of a large nematic correlation length, in agreement with observations (Fig. 2e). (iv) Last, the model also predicts the observed tension anisotropy (Fig. 4f) as shown in SI. This theoretical analysis finally indicates that the hallmarks of the actin structure at negative curvature—tension anisotropy gradient, actin order parameter gradient, and anterograde flow—result mainly from the contractile behavior of the cytoskeleton and of the geometry characterized by the negative curvature of the forward edge.

We next sought to find mechanisms of actin dynamics regulation different from the one previously observed in lamellipodium-based protrusions25,46. Photo-conversion of mEos2-Actin (Fig. 5a-c and Supplementary Video 9) within the structure at negative curvature (see Methods) revealed that actin depolymerization increased from the rear to the front, whereas polymerization peaked in the center of the structure behind the depolymerization region. It resulted in a local polymerization-depolymerization gradient, accompanying the anterograde actin flow and in contrast to polymerization gradient previously described in cell protrusions at positive curvatures25 (Fig. 5d, e). Correspondingly, Arp2/3 was mainly localized in the transition region between radial and transverse fibers (Fig. 5f, h), whereas cofilin, an actin

depolymerization factor, co-localized with the actomyosin contractile cable at the cell front (Fig. 5g, h). In addition, N-WASP clusters were observed in the same transition region (Supplementary Fig. 5a), where we also observed highly dynamic random motion of actin (Fig. 5i, left panel). The inhibition of Arp2/3 activity (200 μ M CK666 treatment) immediately abolished such activity (Fig. 5i, middle panel) and eventually led to the depletion of transverse fibers, which could be rescued by CK666 washout (Fig. 5i, right panel and Supplementary Video 10). The inhibition of the upstream regulator of the Arp2/3 complex, N-WASP, with wiskostatin led to the same observation (Supplementary Fig. 5b). To perturb actin depolymerization, we treated cells with 1 nM jasplakinolide, which caused the actin structure to collapse (Supplementary Fig. 5e and Supplementary Video 11), suggesting that actin turnover is important in maintaining the dynamic stability of the structure. The effect is irreversible and could not be rescued by washout.

We then asked how Arp2/3-dependent actin polymerization could be localized to the transition region. Actin polymerization in the lamellipodia is reported to be downstream of FA signaling, where FAK activates N-WASP and Arp2/3⁴⁷. Noticing that radial fibers were connected to FAs and also underwent anterograde flow, we hypothesized that the polymerization signal could be transported by actin flow along radial fibers from FAs to the transition region^{23,37,48,49}. To test this, laser ablation of radial fibers was performed, Arp2/3 was subsequently recruited to the rear of the ablation site (Fig. 5j, k and Supplementary Video 12, right panel). This was followed by local polymerization of actin which then engaged in anterograde flow (Supplementary Fig. 5c, d and Supplementary Video 12, left panel). Hence, actin polymerization signals could be transported from the rear of the cell by the anterograde flow of radial fibers. Since the radial fibers are connected to FAs, we wondered whether the radial fibers also could mediate the transport of signaling protein complexes. Indeed, with the YFP-PBD biosensor⁵⁰, we observed that the active small GTPases Rac/cdc42 can be transported forward at negative curvature (Fig. 5l, m). FAK localizes along radial fibers (Fig. 5n) and FAK clusters remained mostly stationary on FN patterns (Fig. 5p, empty arrow head) but could be mobilized and then flow towards the front with the radial fibers over non-adherent regions (Fig. 5p, filled arrow head).

Since the polarized actin structure can form in both single cells (Fig. 1n and Supplementary Fig. 1c, d) and individual cells within an epithelial monolayer (Fig. 2a-d), we hypothesized that cell-cell junctions may enable the formation of multicellular actin structures at negative curvatures with large curvature radii. To test this, we seeded either MDCK WT or MDCK cells with stable α -catenin knockdown (KD) where cell-cell junctions are weakened (Fig. 6d-f)⁵¹ on fibronectin patterns with varying curvatures (Fig. 6a-f and Supplementary Fig. 6a). Both WT and α -catenin KD cells could form suspended actin structures on patterns with radii of curvature equal to or smaller than 15 μ m (Fig. 6b, e). However, while the WT cells could form suspended multicellular actin structures connected by E-cadherin adhesions (Fig. 6c, white arrowhead) on patterns with curvature radii 15 to 100 μ m (Supplementary Fig. 6a), α -catenin KD cells could not and failed to extend out of the fibronectin pattern (Fig. 6f and Supplementary Fig. 6a). Laser ablation of the actin cables at the edge (Fig. 6g and Supplementary Video 13) led to their retraction, indicating that the mechanical tension exerted by the actin cables are important for the multicellular actin structures to extend over non-adherent areas. The retraction was significantly larger when the ablation was performed

in the non-adherent region than when it was done in the adherent region (Fig. 6h), suggesting that the actin structures were mechanically coupled in the non-adherent region but were uncoupled in the adherent one, potentially due to the connection of actin cables to focal adhesions in the adherent regions.

There could be a maximum non-adherent circle size beyond which the cells could not migrate over it as a coherent monolayer²². Indeed, for 100 μm diameter non-adherent circles, although the cells could occasionally extend partially into the non-adherent region with multicellular actin structures (Fig. 6l, filled arrowheads), the monolayer failed to migrate over the non-adherent circle even after 45 hours (Fig. 6l and Supplementary Video 14). On the other hand, when the size of the non-adherent pattern is small compared to the size of single cells, α -catenin KD cells could also migrate over it, albeit with less coordination compared to the WT cells (Supplementary Fig. 6b and Supplementary Video 15). These results suggest that the cell-cell junction is not required for curvature sensing and actin structure formation for single cells or individual cells in a monolayer when the radius of curvature is small, but is essential for the formation and maintenance of multicellular actin structures when the radius of curvature is larger than the size of individual cells.

Our study demonstrates that migrating cells sense large-scale curvatures through the spatio-temporal adaptation of the flow and tension distribution within the actin cytoskeleton as a direct result of the physical constraint imposed by the geometry of the cell and substrate. The unique range of curvature sensing at the scale of 10 μm to tens of microns (Fig. 3c) is distinct from that of molecular curvature sensors such as BAR domain proteins and septins. Nevertheless, curvature sensing mechanisms at scales beyond tens of microns remains elusive and may involve tissue tension sensing⁵². Apart from sensing the sign of the curvature, the cells also show different actin flow velocities in response to different magnitudes of curvatures (Fig. 3c). Our theoretical analysis suggests that this dependence could reflect the size of the actin structure, characterized by the length of the suspended part l , which naturally varies with the change of curvature (see Model).

Formins are also important actin polymerization factors; however, we did not observe noticeable differences in actin dynamics and organization when cells were treated with the formin inhibitor SMIFH2 (25 μM concentration, data not shown). Nevertheless, formins have been shown to help polymerize actin in the radial fibers³⁰ and the difference between measured actin polymerization activity (Fig. 5d) and Arp2/3 profile (Fig. 5h) at the rear of the actin structure indicates that formins could play a minor role here. The inhibition of actin depolymerization by Jasplakinolide resulted in the rapid break-up of the actin structure. This could be due to the high contractility inside the suspended structure, which in the absence of depolymerization builds up tension inside the actin network and leads to its collapse. On the other hand, the stabilization of F-actin by Jasplakinolide treatment could also reduce G-actin concentration in the cytosol and in turn inhibit the polymerization rate of actin⁵³. This suggests that the active maintenance of the actin structure depends on the dynamic turnover of the cytoskeleton.

A former study showed that after laser severing of the actin cable at the edge of a MDCK monolayer, it can transform into a lamellipodium protrusion¹². However, in our setup, the

actin cable could repair rapidly after laser ablation and repeated laser ablation induced the collapse of the actin structure which can be explained by the fact that non-adherent pattern may serve to prevent lamellipodium protrusion formation after ablation. In addition, the switching of migration mode after laser ablation of the actin cable could also be regulated by curvature. Indeed, when we ablated actin cables at the edge of a MDCK monolayer on a continuously adhesive substrate, 71% (5 out of 7) of the cells transformed into lamellipodium protrusion when the edges were of positive curvature whereas 89% (8 out of 9) of the cells repaired the cable when the edges were of negative curvature (Supplementary Fig. 4g, Supplementary Table 1 and Supplementary Video 16).

In light of these results, curvature sensing by actin flow appears to be the fundamental mechanism of migration mode switching, although the curvature itself might be generated by diverse environmental and cellular conditions *in vivo*.

Materials and Methods

a Cell culture, plasmids and transfection

MDCK WT, MDCK GFP actin, MDCK GFP MRLC, MDCK α -catenin KD cells are a kind gift from James Nelson. MDCK PBD YFP cell line is a gift from Fernando Martín-Belmonte 50. REF52 cells, GFP- α -Actinin and mCherry- α -Actinin constructs are a gift from Alexander Bershadsky. The GFP-Talin, N-WASP-GFP and GFP-FAK constructs are a gift from Michael Sheetz. The mEos3.2-MyosinIIA-C-18, mEmerald-Myosin IIA-N, mApple-Myosin IIA-N, ARP-p34-N-mEmerald, mEos2-Actin-C-18, mEmerald-Actin-C-18 and mEmerald-ARPP34-N-14 constructs are from Michael Davidson reserve and kindly distributed by Pakorn Kanchanawong.

Cells were cultured in DMEM medium (Invitrogen) supplied with 10% FBS (Invitrogen) and changed to Leibovitz imaging medium (Invitrogen) supplied with 10% FBS before imaging.

Transfection of plasmids was performed with a Neon electroporation system (Invitrogen) as per the manufacturer's instructions.

b Drug treatment

Drug treatments were performed on stage by exchanging normal imaging medium with the same amount of medium containing drugs pre-warmed to 37°C. Concentrations of drugs used were as follows: (-) Blebbistatin (Selleckchem) 25 μ M, CK666 (Millipore) 200 μ M, Wiskostatin (Sigma) 50 μ M, Jasplakinolide (Sigma) 1 nM. Washout of drugs was performed on stage by rinsing with 10 ml imaging medium 3 times. The interval between consecutive time-lapse imagings due to drug addition or washout is generally between 5 min to 10 min.

c Model wound closure assay and MDCK monolayer expansion assay

Model wound closure experiment was performed on a continuous fibronectin-coated substrate as described before 2,27 with the flower-shaped block to induce positive and negative curvatures at the edge of the gap. Cells were fixed 15 min after the removal of the block followed by immunostaining. For the freely expanding MDCK monolayer assay, a

rectangular Polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning) block (approx. 0.5 cm by 1 cm) was placed in the center of the dish and MDCK cells were allowed to grow to confluence around the block before the block was removed and the expansion of the monolayer into the empty space was initiated. Images in Fig. 1a, b were taken approx. 10 hours after the removal of the block.

d Micro-patterning of glass substrate

Silicon wafers with custom designed micro patterns were made with soft-lithography. PDMS pillars were cast from the wafers and used to pattern glass surfaces to make islands of fibronectin (20 µg/ml human plasma fibronectin mixed with 1-5% Atto-674 conjugated fibronectin in MQ water) surrounded by PLL-g-PEG (1mg/ml PLL(20)-g[3.5]- PEG(2), SuSoS, dissolved in PBS) with a modified protocol based on plasma micro-contact patterning (PµCP)⁵⁴. Briefly, PDMS pillars were placed with their features down in contact with glass, and the unblocked part of the glass was activated with a low power oxygen plasma (Harrick Plasma) for 20min. 20ul of PLL-g-PEG solution was then added and incubated at room temperature for 1 hour, after which the stencil was removed vertically with a tweezer and the glass was rinsed once with MQ water to remove the excess PLL-g-PEG. Then, fibronectin solution was added to coat the previously blocked surface at 37°C for 30 min and rinsed 3 times with MQ water. Cells were then seeded on the patterned substrate and allowed to attach for 30-35 minutes, rinsed with DMEM to remove unattached cells, and imaged the following day. Patterns with near-confluent colonies were selected for imaging to minimize movement of cells within patterns as well as over-crowding effect.

e Immunostaining and image acquisition

Actin filaments were stained with Alexa Fluor 568 Phalloidin (Life Technologies). Myosin IIA was stained with either Rabbit Anti-Myosin IIA (Sigma M8064) or Mouse anti Myosin IIA (abcam ab55456). Paxillin was stained with Rabbit Monoclonal anti-Paxillin [Y113] (abcam ab32084). Cofilin was stained with Rabbit anti cofilin (abcam ab11062). E-cadherin was stained with Rat anti E-cadherin (Sigma U3254). Secondary antibodies used were Alexa Fluor 488 goat anti rabbit (Life Technologies A11011), Alexa Fluor 568 Goat anti Rabbit (Life Technologies A11008), Alexa Fluor 488 Goat anti Mouse (Life Technologies A11001), Alexa Fluor 568 Goat anti-Mouse (Life Technologies A11004), Alexa Fluor 488 Donkey anti Rat (Life Technologies A21208).

Cells were fixed with either methanol at -20°C for cofilin or with 4% PFA at 37°C for others, permeablized with 0.2% Triton X-100 in TBS, blocked with 1% BSA in TBS and stained with the aforementioned antibodies. Quantitative imaging was performed on a Nikon A1R scanning confocal microscope with either a 1.27 N.A. 60X water immersion objective or a 1.49 N.A. 100X oil immersion objective. For E-cadherin staining, images were taken with an Olympus spinning disk confocal microscope with a super-resolution module installed, 1.49 N.A. 100X objective.

f Structure illumination microscopy

Live and fixed sample SIM imaging was performed on a Nikon NSIM microscope with a 1.49 N.A. oil immersion objective. For slow dynamics of transverse fiber flow, cells were

imaged with 100 ms exposure and 1-2 minutes per frame. For fast dynamics of actin, cells were imaged with 30-50ms exposure and 10-15 seconds per frame. Image reconstruction was done with the NIS Elements software as per the manufacturer's instruction.

g Particle image velocimetry (PIV) analysis

Velocity measurement of myosin flow at different negative curvature patterns was performed by imaging cells transfected with mEos3.2 Myosin IIA. Before the acquisition, a 405 laser was used to photo-switch a fraction of the fluorophores from green to red fluorescence, such that the images of Myosin IIA display locally distinctive patterns that can be tracked by the image cross-correlation through PIV. Images were acquired with a Nikon NSTORM microscope in TIRF mode with a 1.49 N.A. 100X oil immersion objective. The open source software MatPIV was used to process the images with a 16-by-16 pixel sampling window and 75% overlap. The results were further passed through a median filter to reduce noise. A custom written MATLAB (mathworks) code was used to define an analysis ROI in the center of the actin structure for each experiment and average the signal over space and time to produce the mean radial flow velocity. Outliers, defined as 3 times standard deviation from medium, were removed.

h Fluorescent speckle microscopy

Cells were transfected with low concentration of mEmerald-actin plasmid (0.1 μ g DNA for a 100 μ l electroporation experiment) and imaged on a Nikon Nstorm microscope with a 1.49 N.A. 100X oil immersion objective with TIRF illumination. Images were acquired with 500 ms exposure and a frame rate of 3 seconds per frame to measure the flow of actin. Images were processed with the openly available QFSM software provided by Gaudenz Danuser lab55, to measure the flow velocity. Then the output data was collected and analyzed to compute the averaged radial velocity with MATLAB.

i Laser ablation

An ultraviolet laser (355 nm, 300 ps pulse duration, 1 kHz repetition rate, PowerChip PNV-0150-100, team photonics) was interfaced to a Nikon A1R MP confocal microscope. The UV laser was integrated into a Nikon ECLIPSE Ti microscope body through a customized optical path and a customized dichroic filter, and were co-aligned with the optical axis of the microscope. The position of the laser was controlled by a mirror mounted on two linear actuators (TRA12CC, Newport), and the exposure time of the laser was controlled by a mechanical shutter (VS25S2ZM0, Uniblitz). The actuators (through the actuator controller, ESP301-3G, Newport) and shutter were controlled by custom ImageJ plug-ins from a PC. The laser ablation system, which is independent from imaging microscope, allows us to perform an ablation during imaging. MDCK GFP MRLC cells were used and for measurement of tension anisotropy, a point ablation (with laser power of 25nW at the back aperture of the objective, 300 ms duration) was performed at different positions within the myosin-rich region. Images were then analyzed with a custom-written MATLAB code to calculate the recoil in radial and transverse direction 5 seconds after the ablation. The relative distance of the ablation site was calculated with respect to the rear of the region as 0 and front of it as 1. For ablation of TFs or RFs, a ROI line was drawn and a line ablation was performed during continuous image acquisition. For measurement of actin

structure retraction after laser ablation of actin cables either on or out of fibronectin pattern, a point ablation was performed to sever the actin cables at different locations and a custom-written MATLAB code was used to calculate the retraction distance, which was defined by $d_{\text{before}} - d_{\text{after}}$, while d_{before} and d_{after} are the furthest distance from the edge of the cell to the fibronectin pattern before and 10 seconds after the ablation.

j Traction force microscopy

Traction force microscopy was performed as described before⁵⁶ with some modifications, using soft PDMS gels (1:1 mixture of CY52-276A and CY52-276B (Dow Corning Toray) resulting in a elastic modulus of ~8 kPa) spin-coated (700 rpm) on glass bottom dishes (Iwaki) and cured at 80 °C for 2 hours. The substrate was then functionalized with 5% aminopropyl triethoxysilane (Sigma) in methanol for 5 min, rinsed 3 times with ethanol, and cured at 80 °C for 20 min. Then a diluted (1:500) solution of 100 nm carboxylated red fluorescent beads (Invitrogen) in MQ water was added, incubated for 15 min and rinsed 4 times with MQ water to remove excessive beads. For fibronectin patterning of the gel, PDMS (Sylgard 184, Dow Corning) stencils with pillars of the given shapes were treated with UV for 20 min and coated with a solution of 50 µg/ml human plasma fibronectin mixed with 10% Atto647 conjugated fibronectin in MQ water for 1 hour. Afterwards, the solution was removed with suction and stencil was inverted and directly stamped on a previously prepared Polyvinyl Alcohol (PVA) membrane (Sigma). Then the PVA membrane was gently placed face down on the soft PDMS substrate. After 1 hour, a 0.2% solution of Pluronics F-127 (Sigma) in MQ water was added to dissolve the PVA and block the uncoated area.

Cells were then seeded on the patterned substrate, let attach for 1 hour, rinsed with DMEM to remove unattached cells, and imaged on a Zeiss LSM780 confocal microscope with a 1.2 N.A. 60X water immersion objective the next day. After live imaging, a 1% SDS solution in PBS was added to lyse the cells and a resting beads image (null-force) was captured.

Analysis of traction forces based on beads displacement was performed as described previously.³⁹

k Measurement of actin polymerization and depolymerization by photo-conversion

Cells were first transfected with mEos2-Actin-C-18 construct and seeded on the patterns. We performed photo-conversion of mEos2-Actin within the structure at negative curvature with a 405 laser in a rectangular ROI to measure the polymerization and depolymerization dynamics (Fig. 5a and Supplementary Video 9). After the photo-conversion, hotspots of fluorescence in the green channel reappeared within the center region at the negative curvature in the green channel, suggesting newly polymerized actin filaments (Fig. 5b). In the mean time, decreasing fluorescence in the red channel was driven by depolymerization of actin filaments (Fig. 5c). Actin polymerization and depolymerization measurements based on photo-conversion experiments are calculated as follows: polymerization is computed as local average of fluorescence intensity between 20-40 seconds after photo-conversion subtracting fluorescence intensity before photo-conversion in the green channel, normalized by the difference between the fluorescence intensity immediately before and after photo-conversion; depolymerization is computed as local average of fluorescence intensity

between 20–40 seconds after photo-conversion subtracted from fluorescence intensity immediately after photo-conversion in the red channel, normalized by the difference between the fluorescence intensity immediately before and after photo-conversion. Assuming photo-bleaching is independent of the spatial location, there is no need to correct for photo-bleaching in order to compare the relative values between different spatial locations. We do not claim that the numerical values obtained from this measurement necessarily represent a linear mapping to the molecules of actin monomers incorporated into F-actin per unit time.

I Image processing and analysis

Images shown in the figures were background subtracted and contrast enhanced for visualization. Intensity measurements were performed on raw images. Due to the non-linearity of structure illumination microscopy images, only confocal images were used for intensity measurements, hence only structural but not intensity information should be interpreted from the SIM images.

Due to the 3D shape of the cell, intensity measurements over the whole actin structure were done by taking a 2 μm z-stack and averaging along z direction after segmentation of each slice to calculate the local fluorescence concentration, except for live imaging experiments where high time resolution is needed and a single slice was taken but with a larger pinhole size to image the entire structure.

Profiles with relative distances were calculated by measuring intensity at each point within a central region at positive or negative curvature, and its position along the radial direction (perpendicular to the edge of the fibronectin pattern), taking the rear of the radial fibers as 0 and the frontal edge of the cell as 1.

Fiber orientation analysis was performed with a custom-built MATLAB code to measure the local fiber orientation based on edge-detection of F-actin images. The order parameter was then calculated as $S = \langle \cos(2\theta) \rangle$ where θ is the angle at each point between the actin fiber and the radial direction.

Actin dynamics index (ADI) is measured by taking a time-lapse (15 seconds per frame) movie of GFP actin with live SIM microscopy, and calculated as $\text{ADI}(i) = (I(i) - I(i-1)) / \text{std}(I(i))$, with image of frame i as $I(i)$ and the standard deviation of frame i as $\text{std}(I(i))$. An averaged ADI for all frames is shown in the figures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Negative curvature induces polarized actin structure formation during cell migration
a, DIC image showing the rough edge of an advancing MDCK monolayer emerging from a flat edge 10 h after a rectangular PDMS block removal where protruding fingers are seen (empty arrow head) and regions in between where negative curvature is generated (filled arrow head). Scale bar, 20 μ m. **b**, Enlarged view of square inset in (a) showing actin structure forming at negative curvature connected to focal adhesions at the rear. Scale bar, 5 μ m. **c**, Immunostaining of F-actin (with phalloidin in green and myosin IIA in magenta) during a gap closure assay with MDCK cells on a continuous fibronectin substrate, 15 min after the

removal of flower-shaped PDMS pattern. Cells form lamellipodial protrusions in response to the positive curvature (+) and form actin cables at negative curvatures (-). Scale bar, 15 μm . **d**, Zoom in view of cells forming lamellipodium protrusion at positive curvature in panel (c). Scale bar, 5 μm . **e**, High resolution SIM imaging of cells at negative curvature in gap closure experiments showing actomyosin structure (different sample from panel (c) and (d)). Scale bar, 5 μm . **f**, Immunostaining of F-actin (with phalloidin in green and paxillin in magenta) during a gap closure assay with MDCK cells on a continuous fibronectin substrate, 15 min after the removal of flower-shaped PDMS pattern. Scale bar, 15 μm . **g**, Zoom in view of cells forming lamellipodium protrusion at positive curvature in panel (f). Scale bar, 5 μm . **h**, High resolution SIM imaging of cells at negative curvature in gap closure experiments showing actin and focal adhesion structure (different sample from panel (f) and (g)). Scale bar, 5 μm . **i**, Live-SIM imaging of actin in a spontaneously formed negative or positive curvature region at the leading edge of a GFP-actin expressing MDCK monolayer on a continuous fibronectin substrate. Magenta line shows the edge of the cell and white arrow shows the direction of migration. Scale bar, 2 μm . **j**, Kymographs along yellow dashed lines in (g) showing (top) anterograde flow of actin fibers at negative curvature (horizontal scale bar, 1 μm ; vertical scale bar, 10 min) and (bottom) retrograde flow at positive curvature (horizontal scale bar, 1 μm ; vertical scale bar, 5 min). Magenta dashed line shows the leading edge of the cell. **k**, Schematic showing sign of the curvature at cell leading edge determines actin flow direction, resulting in different migration mechanism (above: anterograde flow at negative curvature results in suspended actin cable formation; below: retrograde flow at positive curvature results in lamellipodial protrusion). **l**, Time lapse TIRF images showing MDCK monolayer migrating over a non-adherent circle (yellow dashed line, 30 μm diameter) while switching from lamellipodium protrusions to actomyosin cable in response to negative curvature induced by the non-adherent pattern, GFP-MRLC (myosin regulatory light chain) stable cells were transfected with mApple-actin. Scale bar, 15 μm . **m**, Kymograph along white dashed line in (j), showing the transition from lamellipodium protrusion (filled arrowhead) to actomyosin cable (empty arrowhead). Horizontal scale bar, 5 μm ; vertical scale bar, 20 min. **n**, Single hela cell migrating over a non-adherent circle (30 μm diameter), cells were transfected with mApple-actin and GFP-talin. Scale bar, 15 μm . **o**, Immunostaining of REF52 fibroblast migrating on electro-spun fiber networks coated with Atto647-fibronectin (magenta), phalloidin in green. Scale bar, 5 μm .

Figure 2. Micro-patterned substrate reproduces cellular curvature sensing polarized actin structure

a, Phase contrast image (left) of MDCK cell colony showing lamellipodia formation at positive curvatures and suspended cell parts over non-adherent area at negative curvature, edge of fibronectin pattern (right) is shown with magenta dotted line. Scale bars, 10 μm . **b**, Immunostaining of MDCK cells on the patterned surface, left-phalloidin, middle-myosinIIA, right-merge (phalloidin in red, myosinIIA in green, nucleus in blue and fibronectin pattern in cyan). Filled arrowhead shows suspended cable formation at negative curvature, empty arrowhead shows lamellipodial protrusion at positive curvature. Scalebars, 10 μm . **c**, xz view of cells at either negative (above) or positive (below) curvature. Notice the extension of cell body over the non-adherent region at negative curvature due to the forward pulling of suspended actin cable. Horizontal scale bar, 2 μm ; vertical scale bar, 2 μm . **d**, Left, illustration with phalloidin staining image showing local radial and transverse direction for a given point within the actin structure, green line shows the fitted circle to the edge of fibronectin pattern, red star shows the center of fitted circle. Radial direction is taken as the direction of the vector pointing from the point towards the center of the fitted circle; right, local orientation of actin fibers color-coded for the angle between the fiber and local radial direction. Scale bar, 2 μm . **e**, Order parameter, S , decreases from the rear to the front of the cell. Error bars indicate s.e.m. $n=5$. Red line shows fitted model. **f**, Live SIM image showing sarcomeric organization of mApple-myosin IIA (green) and GFP- α -actinin (magenta).

Scale bar, 1 μm . **g**, Intensity profile of alpha-actinin and myosin IIA along white dashed line in (f). **h**, Myosin IIA concentration profile from the rear to the front. Error bars indicate s.e.m. $n=10$. **i**, F-actin (phalloidin) concentration profile from the rear to the front. Error bars indicate s.e.m. $n=10$.

Figure 3. Negative curvature induces anterograde flow of actin

a, Representative 3D live SIM image showing radial fiber (RF) and transverse fiber (TF) organization at negative curvature. Color-coded for cell height (0 μm being the surface of the coverslip). Magenta dashed line marks the edge of fibronectin pattern. Scale bar, 5 μm . **b**, Kymograph along white dashed line in (a) showing anterograde flow of transverse fibers and merging of TF with actin cable at the edge. Horizontal scale bar, 1 μm ; vertical scale bar, 10 min. **c**, Radial flow velocity of myosin flow at different substrate curvatures on patterned surfaces. Error bars indicate s.e.m. $n=10, 6, 8, 9, 13, 13, 9, 9, 6, 6, 9, 6, 6, 8, 10$ for curvature values $-0.200, -0.100, -0.0667, -0.0500, -0.0333, -0.0200, -0.0100, 0, 0.0100, 0.0200, 0.0333, 0.0500, 0.0667, 0.100, 0.200$ respectively. Curvature value of 0 corresponds to

regions of flat edges. Individual data points are shown with black dots, red dots show outliers. Red line shows model fit to the negative curvature data (see text and SI for detail).

d-f, Photo conversion of mEos2-actin on RF shows anterograde flow. (d) shows mEos2-actin before photo-conversion (green), edge of fibronectin pattern is marked by white dashed line, (e) shows after photo-conversion of a segment (magenta) on RF with a 405 laser and (f) shows kymograph along photo-converted RF within the yellow box shown in (d), white dashed lines marks the edge of fibronectin pattern. Scale bar, 2 μm (d and e); for (f) horizontal scale bar, 1 μm , vertical scale bar, 1 min. **g**, Velocity vector map from fluorescent speckle microscopy (FSM) measurement. Magenta dashed line in (g) and (h) marks the edge of the fibronectin pattern. Scale bar, 5 μm . **h**, Heat map showing magnitude of the actin flow velocity component along the radial direction in a representative cell at negative curvature measured by FSM. Scale bar, 5 μm . **i**, Decreasing flow profile of radial velocity at negative curvature measured by FSM (blue, $n=3$) and TF-tracking (green, $n=5$). Error bars indicate s.e.m. Red line shows fitted model (see SI for detail).

Figure 4. Contractility and tension anisotropy gradient are coupled to anterograde flow of actin at negative curvature

a, Laser ablation along the radial direction (green dashed line) in the center of actin structure (GFP-MRLC in white overlaid with fibronectin pattern in magenta) at negative curvature reveals that the actin cables are under tension. Scale bar, 5 μm . **b**, Kymograph along yellow dashed line in (a) shows recoil of actin cables after each ablation (6 ablation time points are indicated by filled arrow head). Horizontal scale bar, 3 μm , vertical scale bar, 1 min. **c**, Flow of TFs depends on myosin II contractility, anterograde flow of myosin stops after 25 μM

blebbistatin treatment and recovers after washout. Above shows images of MDCK cell transfected with mApple Myosin IIA in each condition. Scale bars, 5 μm . Below shows corresponding kymographs along yellow dashed line in each image above. Horizontal scale bars, 1 μm ; vertical scale bars, 5 min. Note that the same cell was imaged for the three conditions. **d**, Point laser ablation near the rear of the actin structure. Scale bar, 5 μm . Yellow circle shows recoil after 5 sec. **e**, Point laser ablation at the front of the actin structure. Scale bar, 5 μm . Yellow circle shows recoil after 5 sec. **f-g**, Tension anisotropy increases from the rear to the front. Relative distances within 0.2-0.4 are measured for rear ($n=6$), and 0.6-0.8 for front ($n=9$). Red line in (i) shows fitted model (see SI for detail). For (g), two-sided t-test was used, $p=0.0027$. Normal distribution was tested and confirmed with Anderson-Darling test at 0.01 confidence interval. **h**, Traction force microscopy (TFM) reveals geometry-induced traction force pattern at negative curvature. Scale bar, 5 μm . **i**, Image showing enlarged view at negative curvature, corresponding to the yellow dashed line box in (h). Filled arrowheads show traction forces parallel to the edge of the cell and empty arrowhead shows forward-pointing traction forces at the rear of the actin structure. Scale bar, 5 μm . **j**, Simulation of tension within the transition region. Fitted traction forces of 450 pN applied at the front (red arrows), and fixed boundary condition applied at the rear (red curve). Brown arrows indicate the directions of the calculated principal stresses. Color-map indicates the magnitude of the first principal stress. See SI for detailed description of the simulation.

Figure 5. Directional actin flow is accompanied by a local polymerization-depolymerization gradient

a-e, Photo-conversion of mEos2-actin to measure actin polymerization and depolymerization dynamics. (a), Left panel shows actin structure before photo-conversion (in green) in a square ROI region (yellow dashed line), edge of fibronectin pattern is marked by white dashed line. Right panel shows immediately after photo-conversion (in magenta). Scalebars, 5 μ m. (b), Kymograph along white arrow in (a) of the green channel shows hotspots of actin polymerization. (c), Kymograph along white arrow in (a) of the red channel shows actin depolymerization. Horizontal scale bar, 1 μ m, vertical scale bar, 10 sec. (d),

Actin polymerization measured from photo-conversion experiments ($n=47$). Error bars indicate s.e.m. **(e)**, Actin depolymerization measured from photo-conversion experiments ($n=47$). Error bars indicate s.e.m. **f**, mEmerald-arp-p34 in transfected cells shows Arp2/3 localization. Scale bar, 2 μm . **g**, Cofilin staining shows depolymerization activity in the front. Scale bar, 2 μm . For **(f)** and **(g)**, white dashed line marks the edge of fibronectin pattern and green dashed line marks the edge of the cell. **h**, Polymerization-depolymerization gradient at negative curvature. $n=8$ for Arp2/3 and $n=3$ for cofilin. Error bars indicate s.e.m. **i**, Arp2/3 dependent dynamic actin movement within the transition region in control, CK666 treatment and washout conditions. GFP actin shown on the left and actin dynamics index (ADI) shown on the right for each experimental condition. Magenta dotted line marks the edge of fibronectin pattern, yellow dotted line marks the frontal edge of the cell, and green dotted line marks the center region. Scale bars, 5 μm . **j-k**, Laser ablation of RFs reveals flow-dependent forward transport of actin polymerization signal. **(j)** Left: mEmerald-arp-p34 intensity distribution before laser ablation, white dashed line marks the edge of fibronectin pattern; middle: immediately after laser ablation of RFs; yellow dashed line marks the position of the line ablation. Right: 1.35 minutes after laser ablation, recruitment of Arp2/3 at the rear of ablation site (filled arrow head). Scale bar, 2 μm . **(k)** Kymograph along green dashed line in **(j, left)**, white asterisks marks the time points and positions of 3 consecutive ablations. Horizontal scale bar, 2 μm ; vertical scale bar, 1 min. **l**, Cells transfected with PBD-YFP biosensor shows active cdc42/Rac around the edge of fibronectin pattern (filled arrow) and in the actin structure (filled arrow head) at negative curvature. Notice the typical edge localization of the signal at the front of lamellipodium (empty arrow head) formed by the same cell on the positive curvature part of the pattern. White dashed line shows edge of fibronectin pattern. Scale bar, 5 μm . **m**, Kymograph showing anterograde flow of active cdc42/Rac in the suspended actin structure along green dashed line in **(l)**. Horizontal scale bar, 1 μm ; vertical scale bar, 2 min. **n**, GFP-FAK localizes on RFs. Magenta dashed line marks the edge of fibronectin pattern and green dashed line marks the edge of the cell. Scale bar, 2 μm . **o**, SIM-TIRF imaging of GFP-FAK near the substrate at edge of adhesive pattern (magenta dashed line) shows mobilization of particles of GFP-FAK on radial actin fibers. Scale bar, 2 μm . **p**, Kymograph along yellow dashed line in **(o)**, magenta dashed line marks edge of fibronectin pattern. GFP-FAK particles engage in continuous flow (filled arrow head) in non-adherent region on the left side while most particles stay immobile (empty arrowhead) in adherent region on the right side. Horizontal scale bar, 1 μm ; vertical scale bar, 1 min. **q**, Schematic showing the polarized organization of actin cytoskeleton at negative curvature.

Figure 6. Role of cell-cell junctions in curvature sensing and mechanics of actin structure
a-f, Cell-cell junctions are dispensable for suspended actin structure formation at small single-cell scales but indispensable at large multi-cellular scales. (a), Image showing immunostaining of MDCK WT cells on fibronectin pattern, E-cadherin in green and phalloidin in magenta. Scale bar, 100 μm . (b), A single WT cell can form actin suspended structure at the negative curvature with 15 μm radius. White dashed line marks the edge of fibronectin pattern (same for panel c, e and f). Scale bar, 10 μm . (c), Multicellular actin structure forms at negative curvature with 50 μm radius. Actin cables are joined by E-

cadherin adhesions (filled arrowhead). Scale bar, 10 μm . (d), Image showing immunostaining of α -catenin stable knockdown MDCK cells on fibronectin pattern, E-cadherin in green and phalloidin in magenta. Notice the weak staining of E-cadherin, indicating weakened cell-cell junctions. Scale bar, 100 μm . (e), A single α -catenin KD cell can form suspended actin structure at the negative curvature with 15 μm radius. Scale bar, 10 μm . (f), α -catenin KD cells fail to form multicellular suspended actin structures at negative curvature with 50 μm radius. Scale Bar, 10 μm . **g**, Image of actin-structure at negative curvature (30 μm radius) before (magenta) and 10 seconds after (green) laser ablation of the cables outside the fibronectin pattern (at the point of the filled arrowhead), showing retraction of the actin structure. White dashed line marks the edge of the fibronectin pattern. Scale Bar, 15 μm . **h**, Retraction of the actin structure is significantly larger when ablating actin cables out of the fibronectin pattern than when ablating actin cables on the fibronectin pattern. Two-sided t-test was used, $p=0.000014$. Normal distribution was tested and confirmed with Anderson-Darling test at 0.01 confidence interval. $n=19$ for on pattern and $n=20$ for out of pattern. **i**, MDKC monolayer cannot migrate over non-adherent circles with diameter of 100 μm , despite their ability to extend into the non-adherent region with suspended actin structure (filled arrowheads). Scale Bar, 20 μm .