Mechanical interplay between cell shape and actin cytoskeleton organization
Supplementary information

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I. DERIVATION OF EQS. (5) AND (6)

In this Section, we show how Eqs. (5) and (6) in the main text follow from Eq. (4). Without loss of generality, we orient the reference frame such that the stress fibers are parallel to the y−axis. Thus, $\theta_{\rm SF} = \pi/2$ and $\boldsymbol{n} = \hat{\boldsymbol{y}}$ (see Fig. 2 in the main text). Since we assume α, σ and $\boldsymbol{n}$ to be constant along an arc, Eq. (4) can be expressed as a total derivative and integrated directly. This yields

$$\lambda \boldsymbol{T} + (\sigma \hat{\boldsymbol{I}} + \alpha \boldsymbol{n}\boldsymbol{n}) \cdot \boldsymbol{r}^{\perp} = \boldsymbol{C}_1 \,, \tag{S1}$$

where $\boldsymbol{C}_1 = (C_{1x}, C_{1y})$ is an integration constant. Decomposing Eq. (S1) into $x-$ and $y-$directions yields

$$\lambda \cos\theta = C_{1x} + \sigma y \tag{S2a}$$

$$\lambda \sin\theta = C_{1y} - (\alpha + \sigma) x \,. \tag{S2b}$$

Next, taking the ratio of Eqs. (S2), using $\tan\theta = {\rm d}y/{\rm d}x$ and integrating, we obtain a general solution for the shape of the cellular arc subject to a non-vanishing isotropic stress (i.e., $\sigma \neq 0$), namely

$$\frac{1}{\gamma}(x - x_c)^2 + (y - y_c)^2 = C_2 \,, \tag{S3}$$

where C_2 is another integration constant and we have set

$$x_c = \frac{C_{1y}}{\sigma + \alpha} \,, \qquad y_c = -\frac{C_{1x}}{\sigma} \,, \qquad \gamma = \frac{\sigma}{\sigma + \alpha} \,.$$

Eq. (S3) describes an ellipse centered at (x_c, y_c) and whose minor and major semi-axis are $a = \sqrt{\gamma C_2}$ and $b = \sqrt{C_2}$. Using again Eqs. (S2), we further obtain an expression for the line tension λ as a function of x and y:

$$\lambda^2 = \sigma^2 (y - y_c)^2 + (\sigma + \alpha)^2 (x - x_c)^2 \,. \tag{S4}$$

Using Eqs. (S2) and (S3), this can be also expressed as a function of the turning angle θ, namely

$$\frac{\lambda^2}{\sigma^2} = C_2 \, \frac{1 + \tan^2\theta}{1 + \gamma \tan^2\theta} \,. \tag{S5}$$

This expression highlights the physical meaning of the integration constant C_2. The right-hand side of Eq. (S5) attains its minimal value (C_2) where $\theta = 0$, hence when the tangent vector is perpendicular to the stress fibers (i.e., $\boldsymbol{n} \cdot \boldsymbol{T} = 0$). Thus $C_2 = \lambda_{\rm min}^2/\sigma^2$, where $\lambda_{\rm min}$ is the minimal tension withstood by the cortical actin. Substituting C_2 in Eq. (S5) then yields Eq. (5) in the main text. The maximum value of the line tension is found at $\theta = \pi/2$, where the stress fibers are parallel to the arc, and is given by $\lambda_{\rm max} = \lambda_{\rm min}/\sqrt{\gamma}$.

Substituting C_2 in Eq. (S3) yields an implicit representation of the plane curve approximating individual cellular arcs, namely

$$\frac{\sigma^2}{\gamma \lambda_{\rm min}^2} \, (x - x_c)^2 + \frac{\sigma^2}{\lambda_{\rm min}^2} \, (y - y_c)^2 = 1 \,. \tag{S6}$$

This equation describes an ellipse centered at the point (x_c, y_c) and oriented along the $y-$direction, whose minor and major semi-axes are $a = \lambda_{\rm min}\sqrt{\gamma}/\sigma$ and $b = \lambda_{\rm min}/\sigma$ respectively (Fig. 2). For arbitrary stress fiber orientation $\theta_{\rm SF}$, Eq. (S6) can be straightforwardly generalized to find Eq. (6) in the main text.

II. ANGULAR COORDINATES OF THE ADHESION SITES

With reference to the schematic representation of Fig. 2 in the main text, the coordinates of the center of the ellipse can be expressed as:

$$x_c = \frac{d}{2} \cos\phi - \gamma \rho \sin\phi \,, \tag{S7a}$$

$$y_c = \frac{d}{2} \sin\phi + \rho \cos\phi \,, \tag{S7b}$$

where the length scale ρ is defined in Eq. (17) in the main text. From Eqs. (S7), standard algebraic manipulations allow us to express the angular coordinate ψ of the adhesion sites in the frame of the ellipse (Fig. 2a), namely

$$\tan\psi_0 = \frac{d\sin\phi + 2\rho\cos\phi}{d\cos\phi - 2\gamma\rho\sin\phi}\ , \tag{S8a}$$

$$\tan\psi_1 = \frac{d\sin\phi - 2\rho\cos\phi}{d\cos\phi + 2\gamma\rho\sin\phi}\ . \tag{S8b}$$

III. ESTIMATE OF THE NEMATIC ORDER PARAMETER VIA ORIENTATIONJ

In this Section, we demonstrate how the nematic director and order parameter can be estimated using ImageJ plugin OrientationJ (http://bigwww.epfl.ch/demo/orientation). Given the intensity $I(x_0, y_0)$ of the image (channel with TRITC-Phalloidin) at the point (x_0, y_0), we defined the symmetric 2×2 matrix $\hat{\boldsymbol{J}} = \langle\nabla I \nabla I\rangle$, where $\langle\cdots\rangle = \int w(x,y)\mathrm{d}x\,\mathrm{d}y\,(\cdots)$ represents a weighted average with $w(x,y)$ a Gaussian with a standard deviation of five pixels ($0.69\,\mu$m) centered at (x_0, y_0). The $\hat{\boldsymbol{J}}$ matrix can be expressed as:

$$\hat{\boldsymbol{J}} = (\Lambda_{\min} - \Lambda_{\max})\left(\boldsymbol{e}_{\min}\boldsymbol{e}_{\min} - \frac{1}{2}\hat{\boldsymbol{I}}\right) + \frac{\Lambda_{\max}+\Lambda_{\min}}{2}\,\hat{\boldsymbol{I}}\ , \tag{S9}$$

where $\Lambda_{\max}$ and $\Lambda_{\min}$ are the largest and smallest eigenvalues of $\hat{\boldsymbol{J}}$, $\boldsymbol{e}_{\min}$ the eigenvector corresponding to $\Lambda_{\min}$, and $\hat{\boldsymbol{I}}$ the two-dimensional identity matrix. The $\hat{\boldsymbol{J}}$ matrix was then used to estimate the average stress fiber direction $\boldsymbol{u}$:

$$\frac{\langle\nabla I \nabla I\rangle}{\langle|\nabla I|^2\rangle} = \hat{\boldsymbol{I}} - \langle\boldsymbol{u}\boldsymbol{u}\rangle\ . \tag{S10}$$

Here, the quantity $\hat{\boldsymbol{I}} - \langle\boldsymbol{u}\boldsymbol{u}\rangle$ reflects that the largest gradients in intensity are perpendicular to the orientation of the stress fibers and $\langle|\nabla I|^2\rangle = \mathrm{tr}\,\hat{\boldsymbol{J}} = \Lambda_{\max} + \Lambda_{\min}$. Combining Eqs. (S9) and (S10), we obtain

$$\left\langle \boldsymbol{u}\boldsymbol{u} - \frac{1}{2}\hat{\boldsymbol{I}}\right\rangle = \frac{\Lambda_{\max}-\Lambda_{\min}}{\Lambda_{\max}+\Lambda_{\min}}\left(\boldsymbol{e}_{\min}\boldsymbol{e}_{\min} - \frac{1}{2}\hat{\boldsymbol{I}}\right)\ . \tag{S11}$$

Comparing this with the definition of the nematic tensor:

$$\hat{\boldsymbol{Q}} = \left\langle \boldsymbol{u}\boldsymbol{u} - \frac{1}{2}\hat{\boldsymbol{I}}\right\rangle = S\left(\boldsymbol{n}\boldsymbol{n} - \frac{1}{2}\hat{\boldsymbol{I}}\right)\ , \tag{S12}$$

we found the nematic order parameter S and the nematic director n at each pixel:

$$S = \frac{\Lambda_{\max}-\Lambda_{\min}}{\Lambda_{\max}+\Lambda_{\min}}\ , \qquad \boldsymbol{n} = (\cos\theta_{\mathrm{SF}}, \sin\theta_{\mathrm{SF}}) = \boldsymbol{e}_{\min}\ . \tag{S13}$$

If a pixel has zero actin expression, $I(x_0, y_0) = 0$, and consequently $S = 0$.

IV. NUMERICAL METHODS

A. Cell contour update

In order to update the position of the cell contour, we first calculate the line tension λ by discretizing Eq. (23) as follows:

$$\lambda_k = \lambda_0 - \alpha_0 \sum_{n=1}^{k} \Delta s_n\, \boldsymbol{T}_n \cdot \left\langle \hat{\boldsymbol{Q}}_n \right\rangle \cdot \boldsymbol{N}_n\ , \quad k = 1, 2 \ldots N_{\mathrm{arc}}\ , \tag{S14}$$

where λ_0 is the line tension at the adhesion site at $s = 0$ (position $\boldsymbol{r}_0$) and λ_k is the line tension at vertex k (position $\boldsymbol{r}_k$). N_{arc} is the total number of vertices in which cellular arcs are discretized, and $\lambda_{N_{\mathrm{arc}}}$ represents the line tension at the other adhesion site. Furthermore, $\Delta s_n = |\boldsymbol{r}_n - \boldsymbol{r}_{n-1}|$, $\boldsymbol{T}_n = (\boldsymbol{r}_n - \boldsymbol{r}_{n-1})/\Delta s_n$, $\boldsymbol{N}_n = \boldsymbol{T}_n^{\perp}$ and

$$\left\langle \hat{\boldsymbol{Q}}_n \right\rangle = \frac{\hat{\boldsymbol{Q}}_n + \hat{\boldsymbol{Q}}_{n-1}}{2}\ , \tag{S15}$$

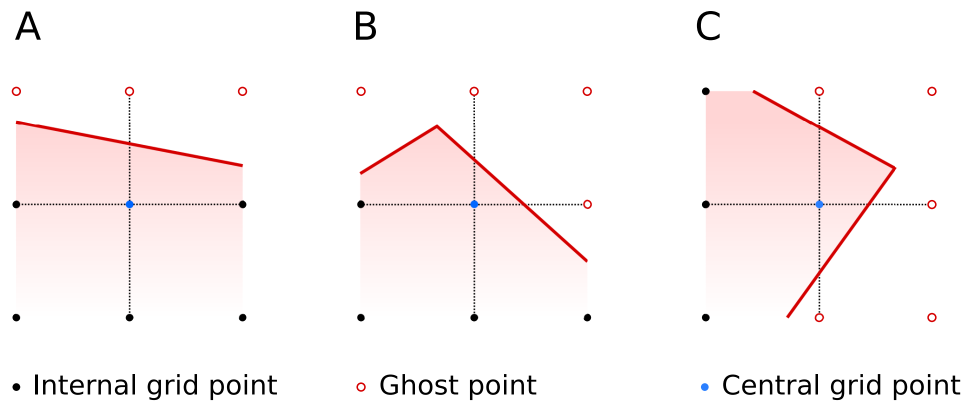

FIG. S1. Schematic overview of the three geometrical situations described in Sec. IV B. (A) There is a single ghostpoint on the $x-$ or $y-$axis. (B) There are two ghost points, one on each axis. (C) There are two ghost points on the same axis and possibly a third one on the other axis.

with $\hat{\boldsymbol{Q}}_n$ and $\hat{\boldsymbol{Q}}_{n-1}$ the nematic tensor at the vertices n and $n-1$. These are set equal to $\hat{\boldsymbol{Q}}$ at the closest bulk lattice point inside the cell among the four points, delimiting the unit cell of the bulk lattice, containing the edge vertices n and $n-1$ respectively. If none of these is inside the cell, we set $Q_{xx,n} = Q_{xy,n} = 0$. The quantity λ_0 is calculated in such a way that the minimal λ value along an arc equates the input parameter $\lambda_{\rm min}$, representing the minimal tension withstood by the cortical actin.

Next, the position of the vertices $\boldsymbol{r}_k$, $k = 0, 1 \ldots N_{\rm arc}$ is updated upon integrating Eq. (24a) using the forward Euler method with time step Δt. The curvature and normal vector at vertex k, κ_k and $\boldsymbol{N}_k$, are found by constructing a circle with radius R through vertices $k-1$, k, and $k+1$. The vector from vertex k to the center of the circle is then equated to $\pm R\boldsymbol{N}_k$, with the sign such that $\boldsymbol{N}_k$ is an inward pointing normal vector, and $\kappa_k = \pm 1/R$, with a negative sign for a concave shape and a positive sign for a convex shape. Along each arc, $\boldsymbol{r}_0$ and $\boldsymbol{r}_{N_{\rm arc}}$ represent the positions of the adhesion sites and are kept fixed during simulations.

B. Cell bulk update

Eq. (24b) is numerically solved at each lattice point inside the cell via a finite-difference scheme. Time integration is performed using the forward Euler method with time step Δt, whereas spatial derivatives are calculated using the centered difference approximation. In order to calculate derivatives at lattice points located in proximity of the edge, we use the boundary conditions, specified in Eq. (20) in the main text, to express the values of Q_{xx} and Q_{xy} in a number of *ghost points* located outside the cells. This is conveniently done upon identifying three possible scenarios, illustrated in Fig. S1. *1)* There is a single ghost point on the $x-$ or $y-$axis (Fig. S1A). *2)* There are two ghost points, one on each axis (Fig. S1B). *3)* There are two ghost points on the same axis and possibly a third one on the other axis (Fig. S1C). In the following, we explain how to address each of these cases.

1) Using the centered difference approximation for the first derivative yields the following expression of the nematic tensor at a ghost point located at $(x \pm \Delta x, y)$ or $(x, y \pm \Delta y)$, with $\Delta x = \Delta y$ the lattice spacing:

$$Q_{ij}(x \pm \Delta x, y) = Q_{ij}(x \mp \Delta x, y) \pm 2\Delta x\, \partial_x Q_{ij}(x, y)\ , \tag{S16a}$$

$$Q_{ij}(x, y \pm \Delta y) = Q_{ij}(x, y \mp \Delta y) \pm 2\Delta y\, \partial_y Q_{ij}(x, y)\ . \tag{S16b}$$

The derivative with respect to x in Eq. (S16a) can be calculated from Eq. (20), upon taking $\boldsymbol{N} = \pm\hat{\boldsymbol{x}}$, where the plus (minus) sign correspond to a ghost point located on the left (right) of the central edge point. Thus $\boldsymbol{N}\cdot\nabla Q_{ij} = \pm\partial_x Q_{ij}$. Analogously, the derivative with respect to y in Eq. (S16b), is approximated as $\boldsymbol{N}\cdot\nabla Q_{ij} = \pm\partial_y Q_{ij}$, where the plus (minus) sign corresponds to a ghost point located below (above) the central edge point. Combining this with Eq. (20), yields:

$$Q_{ij}(x \pm \Delta x, y) = Q_{ij}(x \mp \Delta x, y) - 4\Delta x\, \frac{W}{K}\left[Q_{ij}(x, y) - Q_{0,ij}(x, y)\right]\ , \tag{S17a}$$

$$Q_{ij}(x, y \pm \Delta y) = Q_{ij}(x, y \mp \Delta y) - 4\Delta y\, \frac{W}{K}\left[Q_{ij}(x, y) - Q_{0,ij}(x, y)\right]\ . \tag{S17b}$$

The tensor $Q_{0,ij}$ is evaluated via Eq. (18) in the main text using the local orientation of the cell edge.

2) If a given lattice point is linked to ghost points in both the $x-$ and $y-$directions, we evaluate equation (S17) for both directions independently as explained in the previous paragraph.

3) If a given lattice point is linked to two ghost points in either the $x-$ or $y-$direction, we employ a forward or backward finite difference approximation for the first spatial derivative of Q_{ij} to evaluate Q_{ij} at the ghost points. This yields:

$$Q_{ij}(x \pm \Delta x, y) = Q_{ij}(x, y) - 2\Delta x \frac{W}{K} \left[Q_{ij}(x, y) - Q_{0,ij}(x, y)\right] \ , \tag{S18a}$$

$$Q_{ij}(x, y \pm \Delta y) = Q_{ij}(x, y) - 2\Delta y \frac{W}{K} \left[Q_{ij}(x, y) - Q_{0,ij}(x, y)\right] \ . \tag{S18b}$$

Finally, if the given lattice point is also linked to a ghost point on the other axis, this is evaluated independently using Eq. (S17).

V. SUPPORTING FIGURES

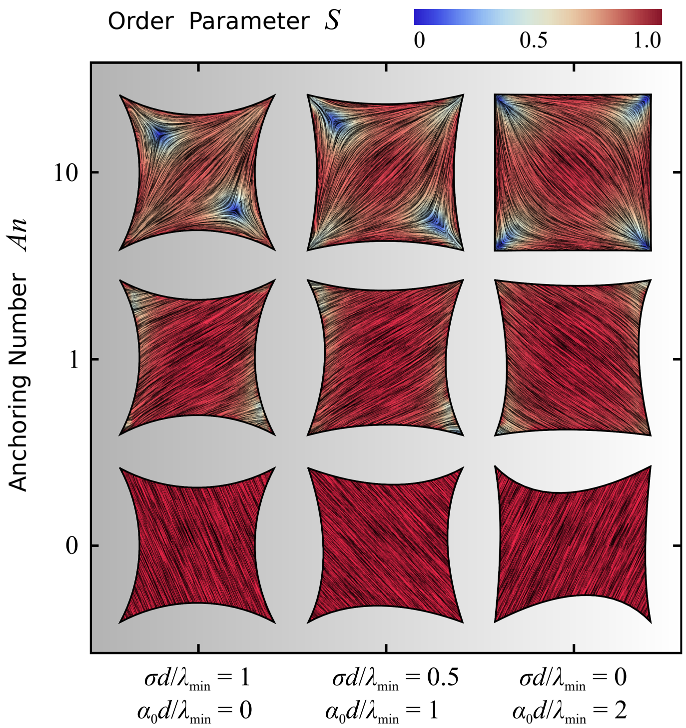

FIG. S2. Configurations of cells whose adhesion sites are located at the vertices of a square. The thick black line represents the cell boundary, the black lines in the interior of the cells represent the orientation field $\boldsymbol{n} = (\cos\theta_{\mathrm{SF}}, \sin\theta_{\mathrm{SF}})$ of the stress fibers and the background color indicates the local nematic order parameter S. The area averages of the order parameter S are given, from left to right, by: $0.74; 0.76; 0.80$ (top row), $0.90; 0.91; 0.92$ (middle row), and $1.0; 1.0; 1.0$ (bottom row). On the vertical axis the anchoring number $An = WR/K$ is varied ($An = 0, 1, 10$, with R the length of the square side) and on the horizontal axis the ratio between the isotropic bulk stress σ and the directed bulk stress α_0 (($\sigma d/\lambda_{\mathrm{min}} = 1, \alpha_0 d/\lambda_{\mathrm{min}} = 0$), ($\sigma d/\lambda_{\mathrm{min}} = 0.5, \alpha_0 d/\lambda_{\mathrm{min}} = 1$), and ($\sigma d/\lambda_{\mathrm{min}} = 0, \alpha_0 d/\lambda_{\mathrm{min}} = 2$), while λ_{min} is constant, and with d equal to the square side. The ratios $\lambda_{\mathrm{min}}\Delta t/(\xi_t R^2) = 2.8 \cdot 10^{-6}$ and $K\Delta t/(\xi_r R^2) = 2.8 \cdot 10^{-6}$, and the parameters $\delta = 0.15R$, $N_{\mathrm{arc}} = 20$, and $\Delta x = R/19$ are the same for all cells. The number of iterations is $5.5 \cdot 10^5$.

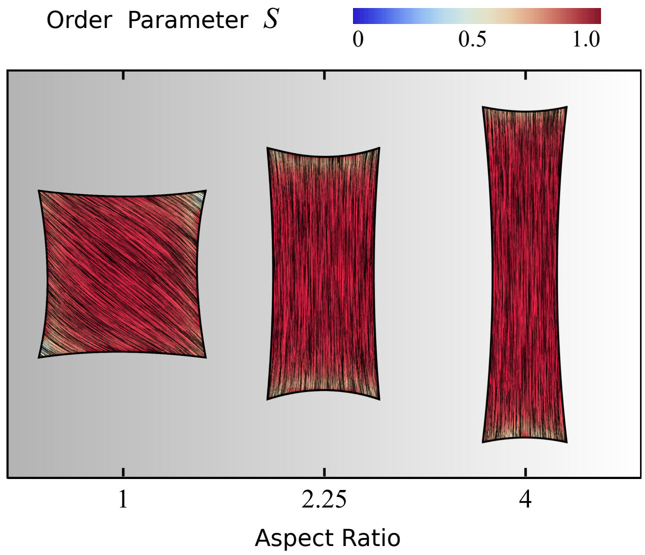

FIG. S3. Effect of the aspect ratio, ranging from 1 to 4, of the cell on cytoskeletal organization for cells, whose four adhesion sites are located at the vertices of rectangles with the same area A. The thick black line represents the cell boundary, the black lines in the interior of the cells represent the orientation field $\boldsymbol{n} = (\cos\theta_{\mathrm{SF}}, \sin\theta_{\mathrm{SF}})$ of the stress fibers and the background color indicates the local nematic order parameter S. The area averages of the order parameter S are given, from left to right, by: $0.92; 0.95; 0.96$. The simulations shown are performed with $An = WR/K$ equal to 1, 0.67, and 0.5 respectively, where R is equal to the short side of the rectangle, and $Co = \sigma d/\lambda_{\mathrm{min}}$ equal to 0.125, 0.1875, and 0.25 respectively, where d is equal to the long side of the rectangle. The ratios $\sigma/(\sigma+\alpha_0) = 1/9$, $\lambda_{\mathrm{min}}\Delta t/(\xi_t A) = 2.8 \cdot 10^{-6}$, and $K\Delta t/(\xi_r A) = 2.8 \cdot 10^{-6}$, and the parameters $\delta = 0.15R$ and $\Delta x = R/19$ are the same for all cells. $N_{\mathrm{arc}} = 20, 30, 40$ from left to right and the number of iterations is $5.5 \cdot 10^5$.

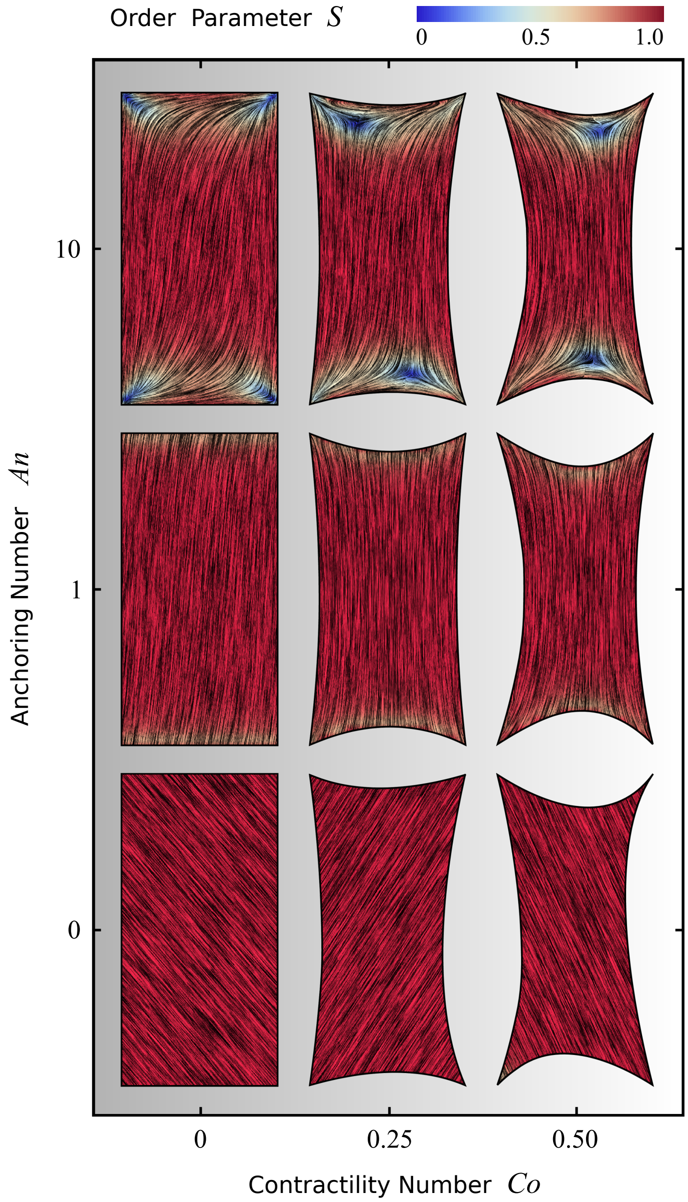

FIG. S4. Configurations of cells whose adhesion sites are located at the vertices of a rectangle of aspect ratio 2. The thick black line represents the cell boundary, the black lines in the interior of the cells represent the orientation field $\boldsymbol{n} = (\cos\theta_{\rm SF}, \sin\theta_{\rm SF})$ of the stress fibers and the background color indicates the local nematic order parameter S. The area averages of the order parameter S are given, from left to right, by: $0.88; 0.86; 0.87$ (top row), $0.97; 0.96; 0.96$ (middle row), and $1.0; 1.0; 1.0$ (bottom row). The vertical axis corresponds to the anchoring number $An = WR/K$ and the horizontal axis to the contractility number $Co = \sigma d/\lambda_{\rm min}$. The cells shown correspond to values of $An = 0, 1, 10$ and $Co = 0, 0.25, 0.50$, with R the short side of the rectangle and d the long side of the rectangle. The ratios $\sigma/(\sigma + \alpha_0) = 1/9$, $\lambda_{\rm min}\Delta t/(\xi_t R^2) = 2.8 \cdot 10^{-6}$, and $K\Delta t/(\xi_r R^2) = 2.8 \cdot 10^{-6}$, and the parameters $\delta = 0.15R$, $N_{\rm arc} = 40$, and $\Delta x = R/19$ are the same for all cells. The number of iterations is $5.5 \cdot 10^5$.

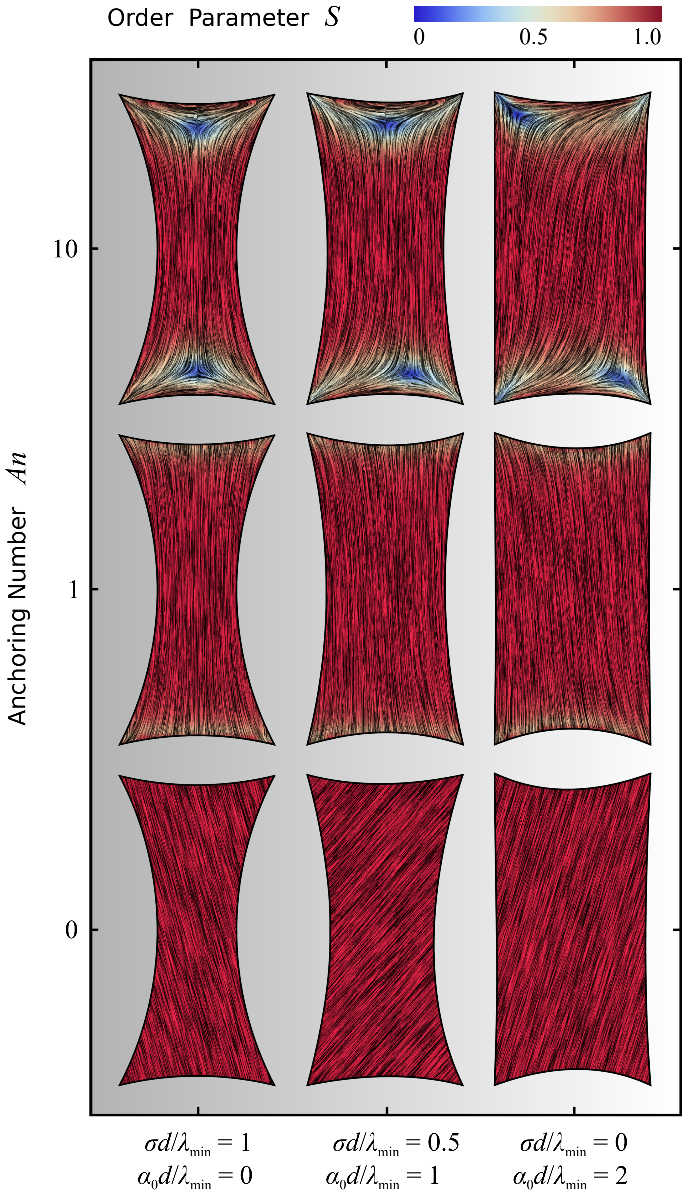

FIG. S5. Configurations of cells whose adhesion sites are located at the vertices of a rectangle of aspect ratio 2. The thick black line represents the cell boundary, the black lines in the interior of the cells represent the orientation field $\boldsymbol{n} = (\cos\theta_{\mathrm{SF}}, \sin\theta_{\mathrm{SF}})$ of the stress fibers and the background color indicates the local nematic order parameter S. The area averages of the order parameter S are given, from left to right, by: $0.84; 0.85; 0.87$ (top row), $0.94; 0.96; 0.96$ (middle row), and $1.0; 1.0; 1.0$ (bottom row). On the vertical axis the anchoring number $An = WR/K$ is varied ($An = 0, 1, 10$, with R the short side of the rectangle) and on the horizontal axis the ratio between the isotropic bulk stress σ and the directed bulk stress α_0 (($\sigma d/\lambda_{\min} = 1, \alpha_0 d/\lambda_{\min} = 0$), ($\sigma d/\lambda_{\min} = 0.5, \alpha_0 d/\lambda_{\min} = 1$), and ($\sigma d/\lambda_{\min} = 0, \alpha_0 d/\lambda_{\min} = 2$), while $\lambda_{\min}$ is constant, and with d equal to the long side of the rectangle. The ratios $\lambda_{\min}\Delta t/(\xi_t R^2) = 2.8 \cdot 10^{-6}$ and $K\Delta t/(\xi_r R^2) = 2.8 \cdot 10^{-6}$, and the parameters $\delta = 0.15R$, $N_{\mathrm{arc}} = 40$, and $\Delta x = R/19$ are the same for all cells. The number of iterations is $5.5 \cdot 10^5$.

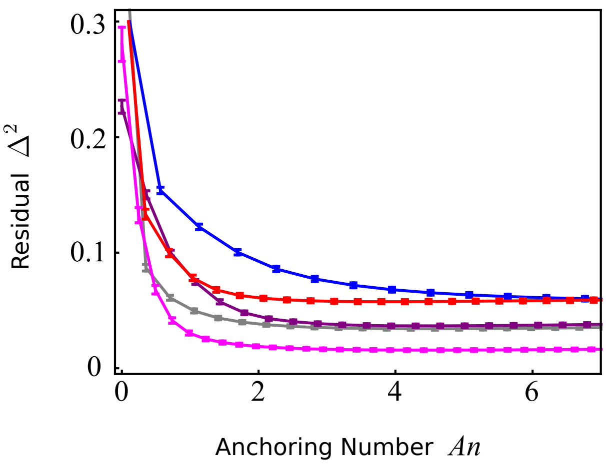

FIG. S6. Residual function Δ^2, defined in Eq. (34) in the main text, as a function of the anchoring number An (Eq. 33) for the cells displayed in Fig. 8A-E, which correspond to the magenta, red, blue, grey, and purple data respectively. The minima are given by $\Delta^2 = 0.016; 0.058; 0.057; 0.034; 0.037$ for the cells displayed in Fig. 8A-E, at values of $An = 4.4; 4.1; 19; 4.6; 4.7$, where $R = 17.3; 24.4; 39.9; 24.9; 25.3\ \mu$m is defined as the square root of the cell area. These An values correspond to $K/W = 3.9; 5.9; 2.1; 5.4; 5.4\ \mu$m. Error bars display the standard deviation obtained using jackknife resampling. For large An values the residual flattens for all cells, indicating that the actual value of An becomes unimportant once the anchoring torques (with magnitude W), which determine the tangential alignment of the stress fibers in the cell's periphery, outcompete the bulk elastic torques (with magnitude K).