SUPPORTING MATERIAL

MODEL

Surface Tension in a Contractile Elastic Sheet

We begin by considering a very simple three-element model of an adherent cell as a linear contractile elastic sheet coupled to a linear elastic substrate via focal adhesions. We use the constitutive relation for the internal stress, $\sigma = E_{\rm cell}\epsilon + \sigma_a$, where $\sigma_a > 0$ represents the uniform negative pressure due to actomyosin contractility, $E_{\rm cell}$ is an elastic modulus and ϵ is the cellular strain. The passive elastic substrate has the constitutive relation for substrate stress $\sigma_s = E_s\epsilon_s$, where E_s is the substrate elastic modulus and ϵ_s is the strain in the substrate. Focal adhesion complexes are modeled as linearly elastic material with stress $\sigma_f = E_f\epsilon_f$, where E_f is the focal adhesion's elastic modulus and ϵ_f is its strain. We assume that the cell, the substrate and the adhesion complex deform in series (Supplemental Fig. S3a). Neglecting non-local elasticity in the cell and assuming only in-plane compressional deformations, the stress-balance in equilibrium yields

$$E_{\rm cell}\epsilon + \sigma_a = E_s\epsilon_s = E_f\epsilon_f \ . \tag{1}$$

The condition of incompressibility of the total system yields $\epsilon = -(\epsilon_s + \epsilon_f)$. Using this and the stress balance condition, the substrate strain is given by $\epsilon_s = \sigma_a \frac{E_{\rm eff}}{E_s(E_{\rm eff}+E_{\rm cell})}$, where $E_{\rm eff} = (E_s^{-1} + E_f^{-1})^{-1}$ is the effective modulus of the substrate and the focal adhesion. The substrate displacement thus depends inversely on the elastic modulus of the substrate as shown in Fig. 1d. The traction stress T is then given by $T = E_s\epsilon_s = E_{\rm eff}\sigma_a/(E_{\rm cell}+E_{\rm eff})$, thus showing a monotonous rise and eventually a saturation with increasing substrate stiffness. The strain energy generated by a cell of planar area A and average thickness h in deforming the elastic substrate is given by

$$W = \frac{1}{2}T\epsilon_s hA = \frac{h\sigma_a^2 E_{\rm eff}^2}{2E_s(E_{\rm cell}+E_{\rm eff})^2}A \ . \tag{2}$$

Thus, the strain energy of a uniformly contracting elastic sheet scales linearly with its area and is constant when the area is held fixed. The characteristic surface tension $\gamma = W/A$ is then given by

$$\gamma = \frac{h\sigma_a^2 E_{\rm eff}^2}{2E_s(E_{\rm cell}+E_{\rm eff})^2} \ . \tag{3}$$

The substrate strain, the traction stress and the strain energy obtained from the model are plotted in Supplemental Fig. S3b as functions of the substrate stiffness. Assuming that $E_s \ll E_f$, as consistent with experimental parameters, the effective stiffness E_{eff} is controlled entirely by the substrate, $E_{\text{eff}} \sim E_s$. It is then apparent that while the substrate strain and the traction stress are monotonically decreasing and increasing functions, respectively, of substrate stiffness E_s, the strain energy exhibits a crossover from $W \sim E_s$ for $E_s \ll E_{\text{cell}}$ to $W \sim 1/E_s$ for $E_s \gg E_{\text{cell}}$ and goes through a maximum when the cell elasticity matches that of the substrate. The dependence of the strain energy on substrate stiffness is, however, very weak in the range $E_s > E_{\text{cell}}$ probed in the experiments. The model suggests that the location of the weak maximum in the behavior of strain energy versus substrate stiffness could be used experimentally as a way for estimating the cell stiffness. Using the estimates, $h \sim 1$ μm, $E_{\text{cell}} \sim 10$ kPa, $E_{\text{eff}} \sim E_s \sim 10$ kPa, $\sigma_a \sim 1$ kPa, we find $\gamma \sim 1.25 \times 10^{-5}$ N/m, consistent in order of magnitude with the surface tension estimates in Fig. 1. Although this simple mechanical model captures the experimental trend for the dependence of strain energy on the cell spread area, the model is incapable of predicting the spatial distribution and the geometric dependence of the traction stresses applied to the substrate. To describe these features of the experiments we need to go beyond the linear three-element model and consider a continuum mechanical model of the adherent cell.

Continuum Model

Minimizing the total energy in Eq. 3 of the manuscript with respect to the cellular displacement field $\mathbf{u}$, we get two conditions describing in-plane force-balance at the bulk and at the boundary, respectively,

$$h\partial_j \sigma_{ij}^{el} = Y u_i \quad \text{(bulk)} \,, \tag{4}$$

$$h\sigma_{ij}^{el} n_j = -(h\sigma_a + \lambda\kappa) n_i \quad \text{(boundary)} \,, \tag{5}$$

where i and j denote in-plane coordinates, κ is the curvature and $\mathbf{n}$ is the outward unit normal at the cell boundary. The constitutive relation for the elastic stress tensor is given by,

$$\sigma_{ij}^{el} = \frac{E_{\text{cell}}}{2(1+\nu)} \left(\frac{2\nu}{1-2\nu} u_{kk} \delta_{ij} + 2u_{ij} \right). \tag{6}$$

Combining Eqs. (4) and (6) yields a length scale, $l_p = \sqrt{E_{\rm cell} h\,(1-\nu)\,/Y\,(1+\nu)\,(1-2\nu)}$, characterizing the spatial variation of traction stresses and substrate deformations. The substrate rigidity parameter, Y, has contributions from focal adhesions as well as the substrate. If the substrate thickness is small compared to the lateral extent of the cell, Y is given by [1]

$$\frac{1}{Y} = \frac{1}{Y_a} + \frac{1}{Y_s}\ , \tag{7}$$

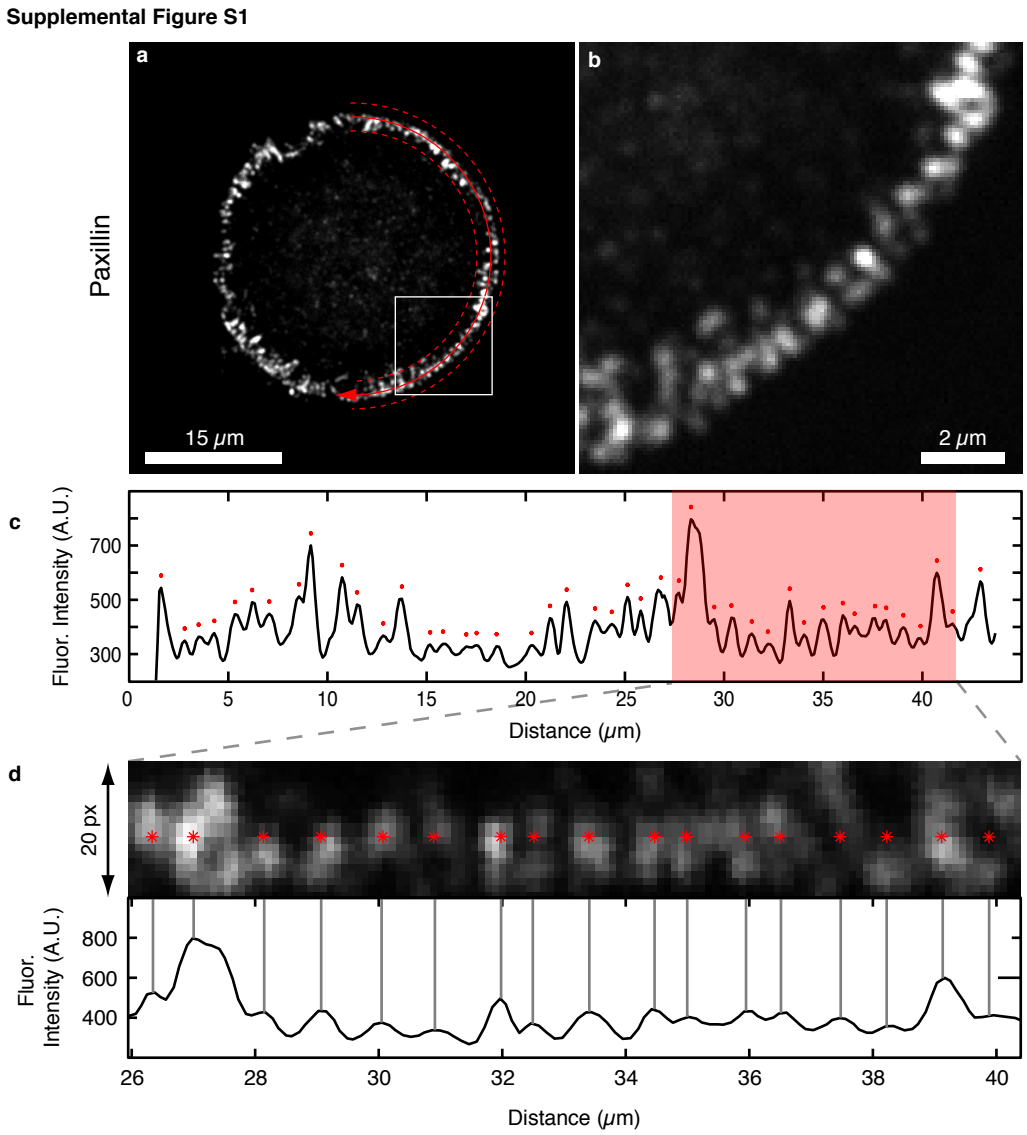
where Y_a is the effective stiffness of the focal adhesions, and $Y_s = \mu_s/h_s$ with h_s the height of the substrate and μ_s its shear modulus. We solve the resultant force-balance equations using the MATLAB finite element package for structural mechanics (MATLAB pde toolbox, The Mathworks, Natick, MA). The reference shape for the finite element calculations is taken to be the shape of the micropattern. The traction stress vector is given by $\mathbf{T} = Y\mathbf{u}$ and the strain energy is calculated as

$$W = \frac{1}{2}\int dA\ \mathbf{T}\cdot\mathbf{u_s} = \frac{Y^2 h_s}{2\mu_s}\int dA\ \mathbf{u}^2\ . \tag{8}$$

where the substrate displacement $\mathbf{u}_s$ is given by $\mathbf{u}_s = \mathbf{T}/Y_s$. For the finite element calculations, we set the maximum edge size for the triangles in the (triangulated) mesh to be $R/25$, where R is the stadium radius. For the unconstrained shapes the maximum edge size is chosen to be 0.1 μm. We set the height of the cell to be $h = 3$ μm and assume a compressible cytoskeleton with $\nu = 0.43$. The substrate shear modulus and height are taken to be 16 kPa and 80 μm, whereas the focal adhesion stiffness is set to $Y_a = 10^9$ N/m^3. We run the finite element code treating the Youngs modulus $E_{\rm cell}$, the active pressure σ_a and the tension f_m as tunable parameters, to obtain the experimentally observed values for the traction stress and strain energy.

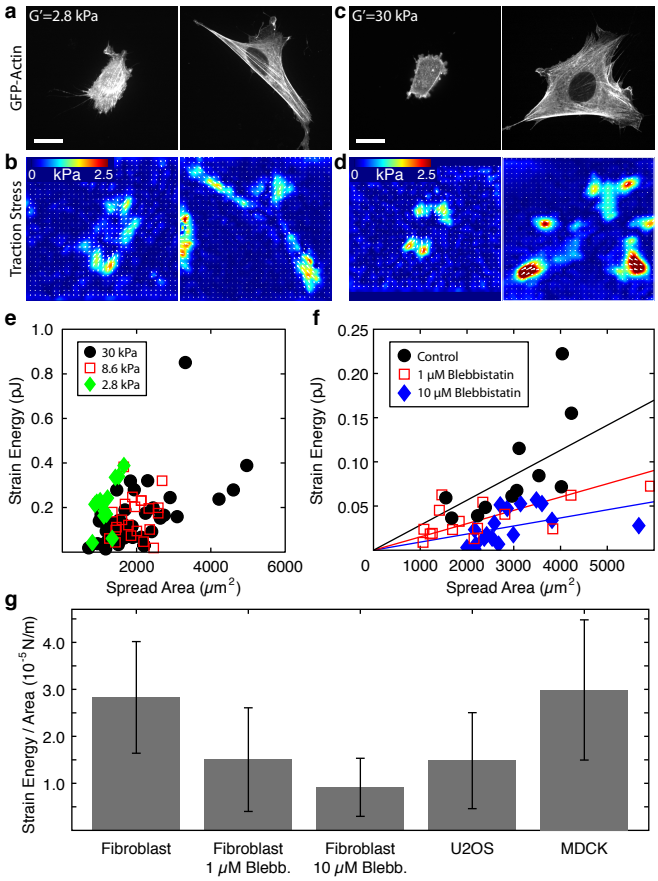
REFERENCES

[1] Banerjee, S., and M. C. Marchetti, 2012. Contractile stresses in cohesive cell layers on finite-thickness substrates. *Phys Rev Lett* 109:108101.

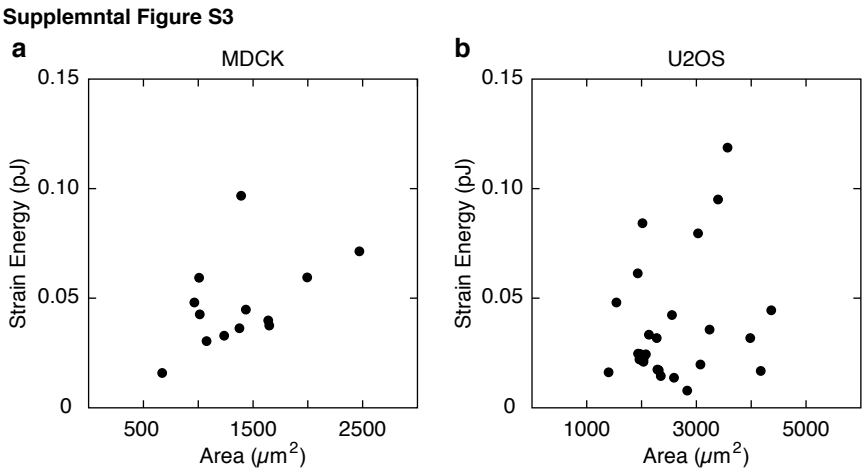


Supplemental Figure S1: Method of counting focal adhesions. **a** A representative paxillin immunoflourescence image showing the focal adhesions (taken from Fig. 2a). The red line and arrow indicates the line and direction used for the linescan, while the dotted lines indicate the width over which the linescan was averaged. **b** A magnified view of the boxed region in **a**. **c** The linescan from the region indicated in **a**. The intensity was averaged across 20 pixels (between the dotted lines) and plotted as a function of distance along the perimeter. The linescan was smoothed with a running average filter and peaks marking focal adhesions (red asterisks) were counted. **d** The 20 px thick linescan for the inset shown in **b** and the accompanying plot of the smoothed average intensity with the indicated peaks (the region shaded in red from **c**).

Supplemental Figure S2

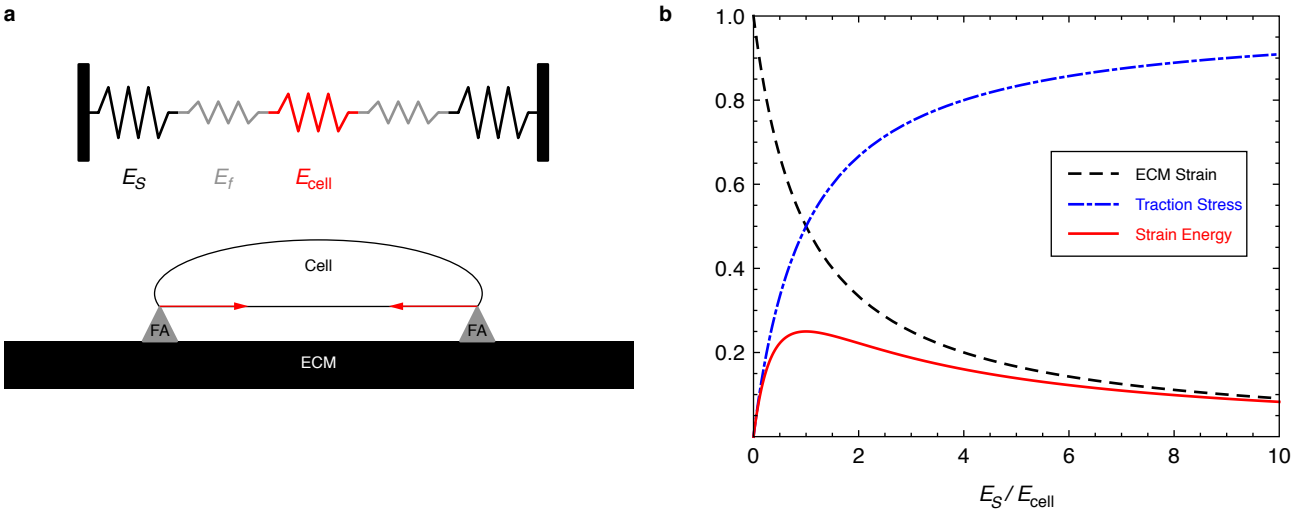


Supplemental Figure S2: Adherent cells can be characterized by an inherent surface tension. **a-b** Representative GFP-actin and traction stress images for poorly and well spread fibroblasts plated on a soft substrate (shear modulus = 2.8 kPa) uniformly coated with fibronectin. **c-d** Representative GFP-actin and traction stress images for poorly and well spread fibroblasts plated on a stiff substrate (shear modulus = 30 kPa) uniformly coated with fibronectin. **e** The strain energy as a function of spread area for individual 3T3 fibroblasts plated on polyacrylamide gels uniformly coated with fibronectin (black circles = 30 kPa; red squares = 8.6 kPa; green diamonds = 2.8 kPa shear modulus). **f** 3T3s on 30 kPa gels were incubated for 30 minutes with blebbistatin and the strain energy was measured as a function of spread area (black circles = control; red square = 1 μM blebbistatin; blue diamond = 10 μM blebbisatin). **g** The ratio of strain energy per spread area defines a characteristic inherent surface tension ($n > 12$ for each condition; error bars represent standard deviation). Scale bar is 20 μm.

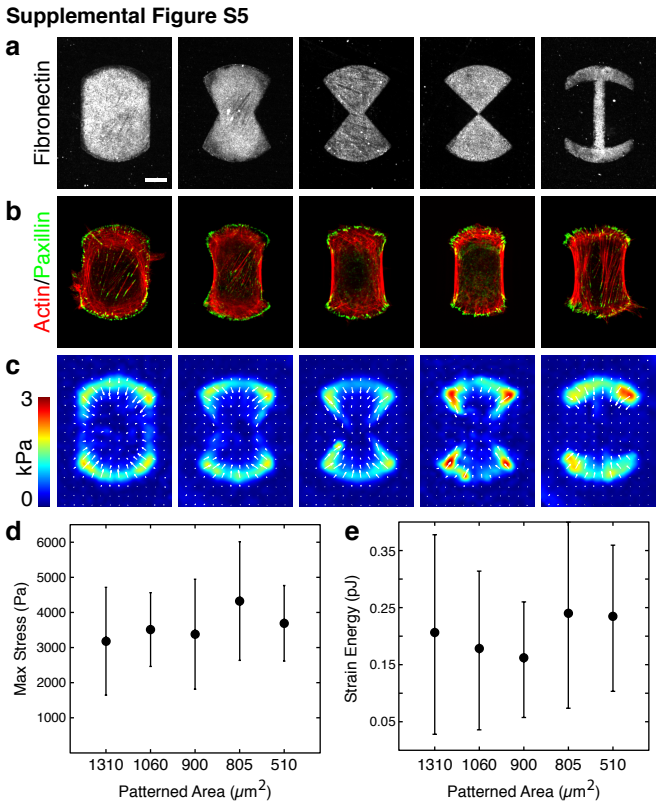


Supplemental Figure S3: Strain energy vs area for U2OS and MDCK cells. **a** Strain energy is plotted as a function of area for MDCK cells plated on polyacrylamide substrates (shear modulus 2.8 kPa) uniformly coated with collagen. **b** Strain energy is plotted as a function of area for U2OS cells plated on polyacrylamide substrates (shear modulus 2.8 kPa) uniformly coated with fibronectin.

Supplemental Figure S4

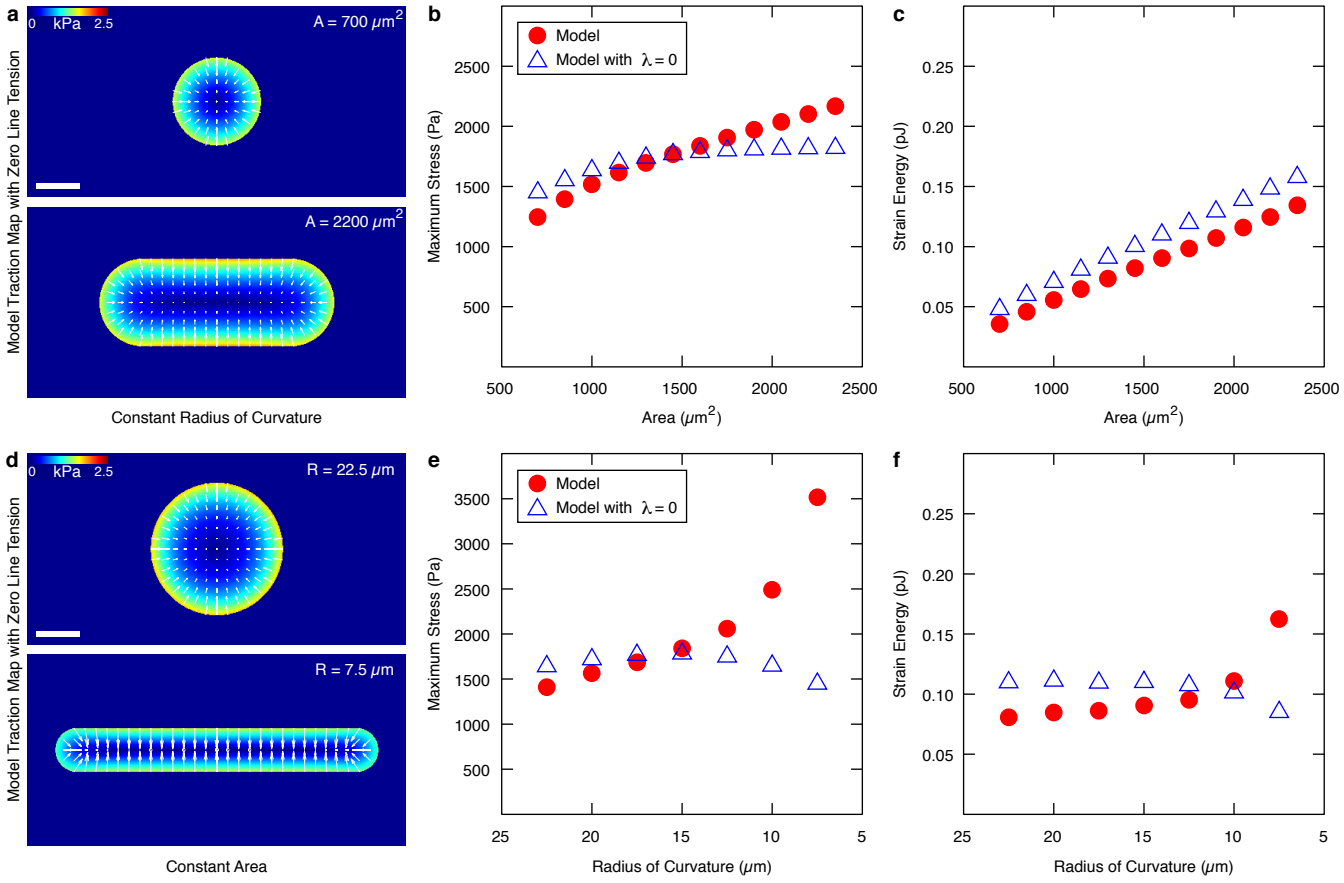


Supplemental Figure S4: Relationships of substrate strain, traction stress and strain energy in the uniform model. **a** A cartoon representing the model assumption of the cell, adhesion complexes and substrate deforming in series. **b** The substrate strain, traction stress and strain energy obtained from the model are plotted as functions of the substrate stiffness. Substrate strain ϵ_s is expressed in units of σ_a/E_{cell}, traction stress in units of σ_a, and strain energy W in units of $h\sigma_a^2 A/2E_{\text{cell}}$.



Supplemental Figure S5: Strain energy is independent of adhesive area. **a** Representative FITC-conjugated fibronectin images of the micropatterns. Scale bar is 10 μm. **b** Immunofluorescence of actin (red) and paxillin (green) in fibroblasts plated on the each pattern. **c** Average experimental traction maps of the cells on each pattern ($n > 7$ for each image). **d** The maximum stress is plotted as a function of the pattern area. Error bars represent the standard deviation with $n > 7$ for each point. **e** Strain energy is plotted as a function of pattern area. Error bars represent the standard deviation for $n > 7$ for each point.

Supplemental Figure S6



Supplemental Figure S6: Comparing models of an isotropically contracting cell with and without line tension. **a** Traction maps for the case of increasing area with a constant curvature at each end of the pattern produced with a model with $\lambda = 0$. Scale bar = 15 μm. **b-c** Plots comparing the maximum stress and strain energy in the case of a constant radius of curvature for the model (red circles) and the model with $\lambda = 0$ (blue open triangles). **d** Traction maps for the case of patterns with a constant area produced with a model with $\lambda = 0$. Scale bar = 15 μm. **e-f** Plots comparing the maximum stress and strain energy in the case of constant area for the model (red circles) and the model with $\lambda = 0$ (blue open triangles). Model Parameters (red circles): $E_{\text{cell}} = 5.4$ kPa, $\nu = 0.43$, $\sigma_a = 2.4$ kPa, $f_m = 0.7$ nN/μm; Model Parameters (blue open triangles): $E_{\text{cell}} = 5.4$ kPa, $\nu = 0.43$, $\sigma_a = 4.65$ kPa, $f_m = 0$.