Supplementary information for

Cellular nanoscale stiffness patterns governed by intracellular forces

Nicola Mandriota¹, Claudia Friedsam², John. A. Jones-Molina¹, Kathleen V. Tatem¹,³, Donald E. Ingber²,⁴,⁵ and Ozgur Sahin¹,³

¹ Department of Biological Sciences, Columbia University, New York, NY 10027, USA
² Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115, USA
³ Department of Physics, Columbia University, NY USA 10027, USA
⁴ Harvard John A. Paulson School of Engineering and Applied Sciences, Harvard University,
Cambridge, MA, 02139
⁵ Vascular Biology Program and Departments of Surgery, Boston Children’s Hospital and Harvard
Medical School
Corresponding author: O.S. (sahin@columbia.edu)
Supplementary Figures

Supplementary Figure 1 | Cantilever design. A false-colored scanning electron microscopy image of the T-shaped cantilevers designed for cell stiffness imaging. The in-plane dimensions of the cantilever are shown on the right. We used cantilevers with three overall lengths along the main axis. Shorter ones were found to provide more stable imaging conditions. The cantilever body is made of silicon nitride and has a thickness of approximately 600 nm.
Supplementary Figure 2 | Stiffness transitions and topography. a-c Effective elastic modulus maps presented in Fig. 2(a-c), color coded and overlaid onto the corresponding 3D-rendered topography images. Scale bar, 5 µm. d, Topography image that corresponds to (b). e, Comparison between topography (in purple) and elastic modulus (in yellow) across the profiles lines indicated in b and d. Note that while the elastic modulus changes abruptly around the 5th micrometer, the apparent surface height is increasing gradually. This observation implies that the changes in elastic modulus are not caused by an abrupt increase in cell thickness.
**Supplementary Figure 3| Comparison of stiffness and actin fluorescence at a leading edge.** A living fibroblast cell is tagged with fluorescent actin (a) and imaged with AFM for stiffness (b). Scale bars, 5 μm. The color bar indicates effective elastic modulus values.
Supplementary Figure 4 | Actin concentration and stiffness in actin bundles. Actin fluorescence images (a, c, e) and corresponding effective elastic modulus maps (b, d, f) acquired from living fibroblast cells. g, Plot shows actin fluorescence and effective elastic modulus values corresponding to the bundles indicated by the symbols in (a-f). Data were averaged along the respective bundles. Marker shapes represent data from different cells. Scale bars, 5µm. Note that the scale bar in (f) is black.
Supplementary Figure 5 | An observation of temporal changes in stiffness independent of actin concentration. a - b, Actin localization in a fibroblast cell, respectively at the beginning and at the end of the experiment. c - d, Effective elastic modulus maps corresponding to the dashed square regions near the centers of (a, b) acquired 17 minutes apart. e - f, Effective elastic modulus maps corresponding to the dashed squares at the lower left regions of (a, b) acquired 30 minutes apart. g, Fluorescence intensity profiles along the (h) lines within the dashed square regions near the centers of (a, b). h, Elastic modulus profiles along the (h) lines in (c, d). i, Fluorescence intensity profiles along the (q) lines within the dashed squares in the lower left regions in (a, b). j, Elastic modulus profiles along the (q) lines in (e, f). By comparing the fluorescence and effective elastic modulus images, we infer that dynamic changes in the stiffness of actin fibers (c, d) and confined cortex regions (e, f), occur independently of actin redistribution (a, b). Scale bars, 25 μm.
Supplementary Figure 6 | Changes in stiffness with myosin-II inhibition. a, Actin localization in a living fibroblast cell. Scale bar, 25µm. (b) Effective elastic modulus map acquired from the squared region in (a). (c) Comparison between actin signal (green) and elastic modulus (yellow) along the profiles shown in a and b. d, Actin localization corresponding to the squared region in (a) after blebbistatin treatment. Scale bar, 5 µm. e, Effective elastic modulus map corresponding to the same region after blebbistatin treatment. f, Comparison between actin signal (green) and elastic modulus (yellow) along the profiles shown in d and e. g, Actin localization corresponding to the same region after drug washout. Scale bar, 5 µm. h, Effective elastic modulus map corresponding to the same region after washout. i, Comparison between actin signal (green) and elastic modulus (yellow) along the profiles shown in g and h.
Supplementary Figure 7 | Changes in stiffness with myosin-II inhibition. 

- **a, c** Actin localization in a living fibroblast cell before and after blebbistatin treatment. Scale bars, 5µm.
- **b, d** Effective elastic modulus maps acquired from the same region as in (a, c).
- **e** Distribution of effective elastic modulus values measured within the cell boundary, before and after blebbistatin treatment. Note that elastic modulus values drop substantially after blebbistatin treatment, as majority of values fall below 10 kPa after the treatment.
Supplementary Figure 8 | Changes in stiffness with myosin-II inhibition. a-b, Actin localization (a) and effective elastic modulus map (b) in a living fibroblast before blebbistatin treatment. c-d, Actin localization (c) and effective elastic modulus map (d) of the same region after blebbistatin treatment. Arrows in a-d point to actin bundles visible by fluorescence before and after blebbistatin, but only visible in elastic modulus maps before blebbistatin. Scale bars, 5µm. e-h, Comparison of actin signal (green) and elastic modulus (yellow) along profiles shown in a-d, before (e,f) and after (g,h) blebbistatin treatment. e and g correspond to the profile at the top left of a-d (near left facing arrows), while f and h correspond to the profile near the center of a-d (near right facing arrows). Arrows in e-h point to region of graph that corresponds to actin bundles highlighted by arrows in a-d.
Supplementary Figure 9 | Changes in stiffness with myosin-II inhibition. a-b, Actin localization (a) and effective elastic modulus map (b) in a living fibroblast before blebbistatin treatment. c-d, Actin localization (c) and effective elastic modulus map (d) of the same region after blebbistatin treatment. Arrows in a-d point to actin bundles visible by fluorescence before and after blebbistatin, but visible clearly in elastic modulus maps only before blebbistatin. Scale bars, 5µm. e-f, Comparison of actin signal (green) and elastic modulus (yellow) along profiles shown in a-d, before (e) and after (f) blebbistatin treatment. Arrows in e,f point to region of graph that corresponds to actin bundles highlighted by arrows in a-d.
Supplementary Figure 10 | Stiffness measurements at peripheral bundles. a, Stiffness maps acquired from living fibroblast cells. Scale bars in (i-v) are 5, 15, 10, 5, and 10 μm, respectively. The
stiff regions corresponding to peripheral actin bundles have the shape of a circular arc. Solid orange lines display the arcs corresponding to circles that best fit the bundle trajectory and are shown with an offset for clarity. Green lines mark boundaries of regions investigated for the analysis of stiffness gradients near the peripheral actin bundles. In Fig. 3d and Fig. 4e, the square, circle, diamond, triangle, and pentagon markers correspond to cells i-v, respectively. b, each square shows a digital zoom into the regions marked with the orange and green lines in (a). Data presented for cells (i-v) in Fig. 5 are calculated from the stiffness images (i-v), respectively and bars are placed in the same order as the respective bundles appear in each image, i.e. left to right for cells i - iv, and top to bottom force cell v. c, Spring constant profiles within the area enclosed by the green lines in (a,b). The profiles are plotted starting from the bundle along the radial direction away from the centers of the fitted circular arcs. See methods for details on averaging. Dashed lines are fit to data according to Eq (13).
Supplementary Figure 11 | A sequence of AFM images (a-c) shows the edge of a fibroblast cell retracting over time (stiffness is color coded, with brighter colors correspond to stiffer regions, and topography is 3-d rendered). The color bar ranges are 10 KPa to 1 MPa. The cell gets thicker and reaches more than 500 nm in height and becomes more compliant in the retracting region (marked with an arrow in c). In contrast, the bundles studied in Fig. 3 and 4 have clearly higher stiffness values from their surroundings and they are much thinner, around 100 nm.
Supplementary Figure 12 | Comparison of elastic modulus values with the conventional force-distance curve method. a, Topography image of a living fibroblast cell obtained using the conventional force-distance curve method by employing the T-shaped cantilevers. b, Topography image of the same region of the cell obtained using the torsional deflections of T-shaped cantilevers in tapping mode. c,
Effective elastic modulus map acquired simultaneously with (a). d, Effective elastic modulus map acquired simultaneously with (b). e, Numerical values of effective elastic modulus across the dashed lines indicated in (c), yellow curve, and in (d), blue curve. Approximate locations of the thin region of the cell near the edge and a thicker region towards cell center are marked with black lines. Scan sizes are 50 x 50 µm (a-d). Color bars correspond to 0 - 5 µm (a), 0 - 3.5 µm (b), 0.1 kPa – 10MPa (c), and 3.68 kPa to 3.68 MPa (d). Note that conventional force-distance curves give values around 50 – 500 kPa near the cell edge. Measurements with the torsional deflections of T-shaped cantilevers in the tapping mode result in a slightly narrower distribution, in the range of 50 – 200 KPa. While these values broadly agree with each other, both approaches result in large values of stiffness near the edge. The conventional force-distance curve method uses large indentation forces (around 1 nN in the present case). As a result, thin regions of the cells appear much stiffer, due to substrate effects. In the case of measurements using the torsional deflections of T-shaped cantilevers in tapping mode, however, the DMT model leads to an overestimation of elastic modulus due to the unaccounted tensional forces. The use of the models described in Fig. 3,4 to account for tensional forces addresses this issue and provides elastic modulus values on the order of 1 kPa. Additionally, the differences in stiffness measurements near the cell center (around the nucleus) reflect the difference in probing depth of the two methods, with conventional force-distance curves probing the compliant nucleus and our platform mostly sampling the stiffer tensioned cortex.
Supplementary Notes

Supplementary Note 1. Scaling behavior of stiffness with curvature radius in the bending-modulus-dominated regime.

If the mechanical response of an actin bundle to external forces is dominated by its bending modulus rather than tension, the stiffness probed by AFM will be determined by the bending modulus of the beam, $B$, and the foundation modulus, $K_{1D}$. We explored the scaling behavior of bundle stiffness with curvature radius at the cell edge that could potentially arise in this regime. The resulting differential equation governing the profile of the beam resting on an elastic foundation can be written as follows:

$$B \frac{d^4z}{dr^4} + K_{1D} z = 0.$$  \hspace{1cm} (S.1)

For a point force applied by the AFM tip, the above equation can be solved to determine the AFM-measured stiffness, $k_b^\ast$ (here the asterisk indicates the bending-modulus dominated case):

$$k_b^\ast = \left(\frac{64BK_{1D}^2}{3}\right)^{1/4}$$  \hspace{1cm} (S.2)

If tension across the actin bundle is negligible, the Laplace equation that relates plasma membrane tension to the curvature radius of the actin bundle is no longer applicable. However, the curved shape of the bundle could still be related to the plasma membrane tension. In this regime, we can approximate the actin bundle as a doubly clamped beam under uniform loading, $\sigma_m$ (plasma membrane tension). For small displacements, the resulting deflection profile of the beam is given with a fourth order polynomial:

$$y = -\frac{\sigma_m}{24B}(x^4 - 2Lx^3 + L^3x)$$  \hspace{1cm} (S.3)

Here $L$ is the length of the beam spanning the distance between clamps at $x = 0$ and $x = L$. Although the resulting deflection profile is not an exact circular arc, curvature radius at the center of the beam ($x = L/2$) can be used as an approximation. Accordingly, the approximate curvature radius $R$ is given by

$$R \simeq \frac{8B}{\sigma mL^2}$$  \hspace{1cm} (S.4)

Equations S.2 and S.4 show that in the tension-free regime, both the curvature radius and AFM-measured stiffness depend on the bending modulus of the actin bundle. By eliminating $B$ from the two equations, we obtain the following scaling behavior of the curvature radius:

$$R \sim k_b^\ast^4$$  \hspace{1cm} (S.5)

The above relationship would imply that the fourth power of the AFM-measured stiffness would scale linearly with the curvature radius of the actin bundle. However, Fig. 3d rules this possibility out, because the data agree well with $R \sim k_b^2$. 
Supplementary Note 2. Probability analysis of equation 5.

To test whether the level of agreement seen in Fig. 4f is unlikely to occur by chance, we performed a probability analysis. We have used the following representation of Eq. (5):

\[ k_b l_c - i \cdot k_c l_b = \varepsilon \]  

(S6)

Here \( \varepsilon \) is the error term, which is equal to zero if Eq. (5) is accurate. The factor \( i \) is 1 for actin bundles at the cell edge and 2 for those away from the edge. Note that the factor 2 is due to the cortices on either side of these bundles, because they both contribute to \( K_{1D} \). For simplicity, we used the average \( k_c \) and \( l_c \) values determined on either side of the bundles away from the edge. The above relationship allows comparing evaluations of the left-hand side for separate bundles.

The data in Fig. 4f consists of four sets of parameters (\( k_b, k_c, l_b, \) and \( l_c \)) measured at different actin bundles (three at the cell edge and one away from the edge). When the left-hand side of Eq. (S6) is evaluated with these parameter sets, the results span a range of values from -1.25 to 0.27 nN. To assess whether this result is unlikely to occur by chance, we estimated the probability distribution of the outcomes, \( \varepsilon \), if the four parameters measured at a given bundle were uncorrelated with each other. Therefore, we evaluated the left-hand side of Eq. (S6) for parameters randomly selected from different sets of \( k_b, k_c, l_b, \) and \( l_c \). In this case, the results show a broader distribution of values, ranging from -4.85 to 9.17 nN. Based on the resulting distribution of outcomes, the probability of obtaining four results within the narrow range found in the experiments (-1.25 to 0.27 nN) is approximately 0.2%. Note that Eq. (S6) predicts that the results should be close to 0, which falls within the range spanned by the experimental results.
Supplementary Note 3. Estimation of the elastic modulus of the cytoplasm at the tapping frequency

It is possible to estimate the elastic modulus of the cytoplasm from the analysis of cell stiffness images, because the foundation modulus, $K_{2D}$, depends on the elastic modulus of the cytoplasm. $K_{2D}$ represents the compressibility of a unit area of the cytoplasm. If the thickness of the cytoplasm, $h$, is less than the coupling distance $l_c$, $K_{2D}$ can be approximately related to the elastic modulus $E$ via Eq. (10): $K_{2D} \approx E/h$. By substituting $K_{2D}$ with experimentally measured quantities using Eqs. (3,4), we obtain the following expression for $E$:

$$E \approx \frac{hk_c}{2l_c^2} \quad (S7)$$

A more general expression can be obtained by using Eqs. (4,11)

$$E \approx \frac{hk_cK_0(a/l_c)}{2\pi l_c^2} \quad (S8)$$

Using this equation, we estimated the elastic modulus values corresponding to the cells analyzed in in Fig. 3,4 and found that they range from 0.3 to 0.6 kPa. While these values are close to the elastic modulus values determined in earlier experiments, the earlier measurements were typically done at frequencies generally in the range of 1 Hz to 1 kHz, well below the cantilever drive frequencies employed in the present work (between 5 and 15 kHz). Indeed, the elastic modulus derived from Eq. (S6) can exhibit frequency dependency. However, the limited frequency tuning range of the tapping mode currently prevents exploring this possibility.

Another quantity that is useful in characterizing the mechanical properties of the cell is the loss modulus, which increases with frequency (Supplementary Ref. 1) and can be readily detected in AFM experiments when large indentation depths are used (Supplementary Ref. 2). In principle, loss modulus can be incorporated into the model presented in this work. Because the differential equations describing the deformations of bundles (Eq. (7)) and the cortex (Eq. (9)) are linear in time, the analysis can be carried out in the frequency domain using complex quantities for $K_{1D}$ and $K_{2D}$. However, the stiffness of the cell (Eqs. (1,3)) depends weakly on $K_{1D}$ and $K_{2D}$. Therefore, force-distance curves exhibit small hysteresis.

Note that the elastic modulus being referred here is different from the effective elastic modulus values mapped in Figs. 1,2. We calculated the effective elastic modulus values by fitting the force-distance curves with the equations of the DMT model. The resulting effective elastic modulus values plotted in Fig. 2 are significantly higher than the elastic modulus values derived using Eq. S7. This is because the DMT model does not account for the small thickness of the cell, as well as the contribution of intracellular forces to the local stiffness of the cell. Therefore, the DMT model significantly overestimates the elastic modulus of the cell. Furthermore, depending on the magnitude of the intracellular forces, contributions of the substrate, and the tip shape-dependence of the DMT model, the values estimated by the DMT model can display significant variations, which is evident in Figs. 1,2.
In addition to contributing additional forces towards the AFM tip, intracellular forces exacerbate substrate effects that increase the apparent stiffness of cells. Substrate effects are due to the finite thickness of the cell and they become significant when the contact diameter becomes comparable to the cell thickness at the measurement location. Because mechanical coupling spreads the forces of the AFM tip to a larger area, the contact diameter effectively becomes larger. However, this behavior also makes it possible to account for the substrate effects more easily via Eq. (10): \( K_{2D} \equiv E/h \). As a result, Eq. (S7) directly accounts for the substrate effects.

S4. Supplementary references

1. Rigato A., Miyagi, A., Scheuring., S. & Rico, F. High-frequency microrheology reveals cytoskeleton dynamics in living cells. *Nature Physics*, 13, 771-775 (2017)

2. Garcia, P.D., Guerrero, C.R., & Garcia, R. Time-resolved nanomechanics of a single cell under the depolymerization of the cytoskeleton. *Nanoscale*, 9, 12051-12059 (2017)