Supplementary Information for: The keratin cortex stabilizes cells at high strains

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Estimation of the Cortex Thickness

To estimate the cortex thickness, we cultivate MDCK II cells as cysts. This procedure enables us to image the cross-section of a cell along the xy-plane of the microscope, which considerably increases the resolution. Figure S1a shows an example image of actin (magenta) in a WT cyst. We manually determine the intensity profile over the cortex at multiple positions (Fig. S1a, white line) and fit each profile (Fig. S1b, black dots) with a Gaussian function (Fig. S1b, black line). Finally, we calculate the full width at half maximum (FWHM) (Fig. S1b, dashed pink line) as the cortex thickness.

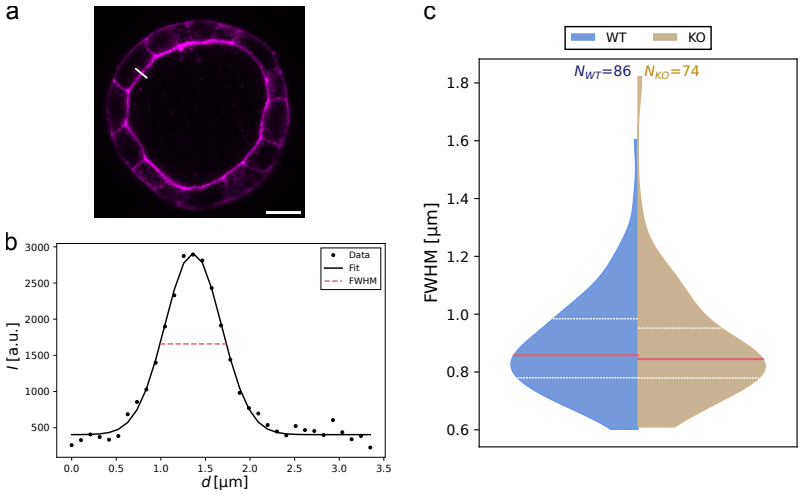

Figure S1: Estimation of the cortex thickness in MDCK II cysts. (a) Fixed MDCK II cyst cells with phalloidin-stained actin (magenta). The white line shows an example for the line taken for the intensity profile. Scale bar corresponds to 10 μm. (b) Intensity profile I (black dots) over the actin cortex of a cyst d, the corresponding Gaussian fit (black line) and the resulting full width at half maximum (FWHM, dashed pink line). (c) FWHM of the actin cortex. Data for WT cells are shown in blue, data for KO cells in yellow. The lines on the violins represent the median (solid line), the upper and lower quartiles (dashed lines). The number of intensity profiles N is displayed in the figure from 7 independent experiments each.

Fig. S1c shows these results for WT and KO cells from 7 independent experiments each. The WT cells are displayed in blue, the KO cells in yellow. The solid pink lines on the violins represent the median of each distribution and the dashed lines the quartiles. The number of intensity profiles is displayed in the respective color at the top of the figure.

We find no difference in cortex thickness between the cell lines. However, the quantitative analysis of microscopy images includes many difficulties, especially when comparing intensity values as performed here. Those values vary a lot with different microscopy settings, staining methods and photobleaching of the sample, hence complicating the comparability between samples.[1] The real differences could be marginal and not resolvable with our techniques.

Cultivation of MDCK Cysts

To cultivate the cysts, we coat gridded Petri dishes (μ-dish, 35mm, low, Ibidi, Gräfelfing, Germany) by incubating them with laminin ($20\,\mu g/cm^2$) for 1 h at 37°C and 5% $C0_2$. For the following steps, all material including the cells are cooled down to 4°C. 50 000 cells are prepared in 450 mL medium and 50 μL Matrigel (Corning Inc., Corning, NY, US) is gently mixed into the cells. The solution is then transferred into the dish and incubated at 37°C and 5% $C0_2$ for 7–9 days to allow for cyst formation.

The cysts are chemically fixed and stained for actin using phalloidin. Instead of mounting the sample, we fill the Petri dish with PBS. We image the sample with an inverse confocal microscope at 100× magnification.

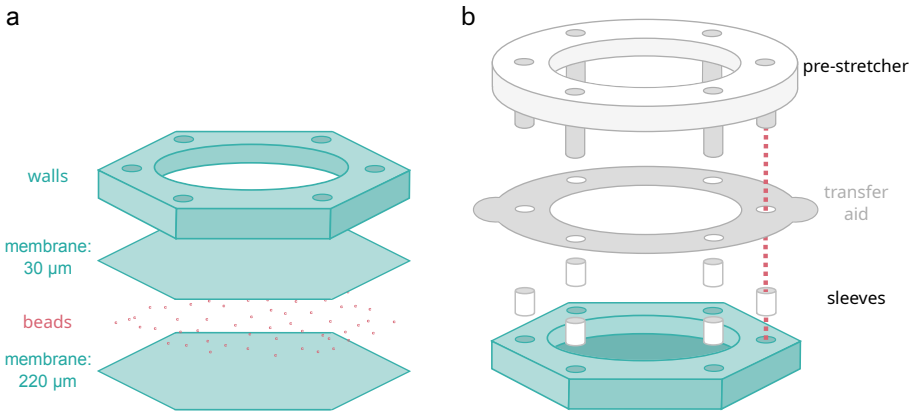

Figure S2: Construction of the PDMS devices and pre-stretcher. (a) The hexagonal PDMS device consists of a membrane and wall attached to it. The membrane is made of two layers of PDMS with fluorescent beads in-between. The lower part of the membrane is approximately 220 μm thick, the top layer 30 μm. The PDMS walls form a well for the cells. Six holes are punched through the finished device on all six corners of the hexagon. (b) The pre-stretcher keeps the device flattened out during cell culture. The six holes in the devices are stabilized by PTFE sleeves. The pre-stretcher inserts into the sleeves with six pins. A transfer aid is inserted between the pre-stretcher and the device enabling easy transfer from the pre-stretcher on the stretcher for experiments.

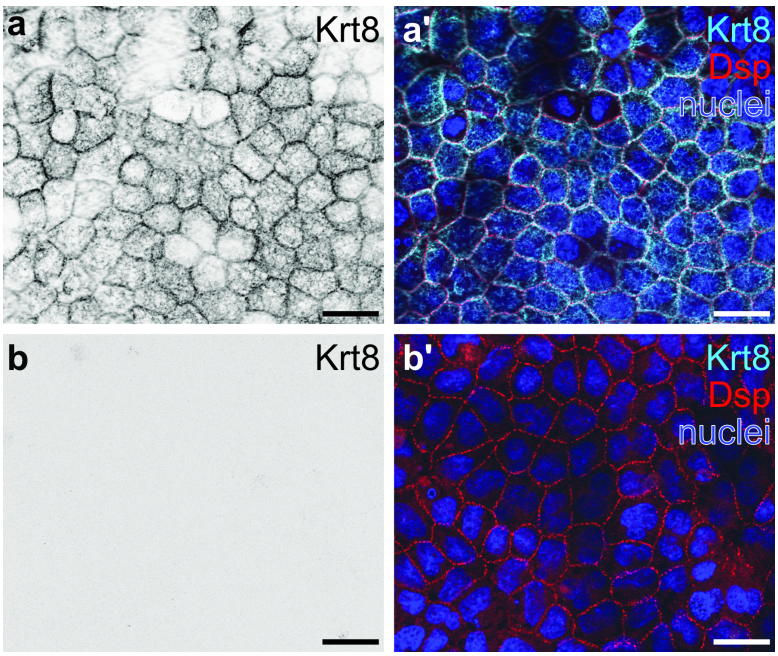

Figure S3: Confirmation of successful keratin 8 knock-out. Staining of keratin 8 (black) in (a) wildtype MDCKII (WT) and (b) keratin 8 knock-out cells (KO) confirm the absence of keratin 8 in KO cells. Corresponding composite images show nuclei (blue), desmoplakin (red) and keratin 8 (cyan) in (a') WT and (b') KO cells. Scale bars correspond to 20 μm.

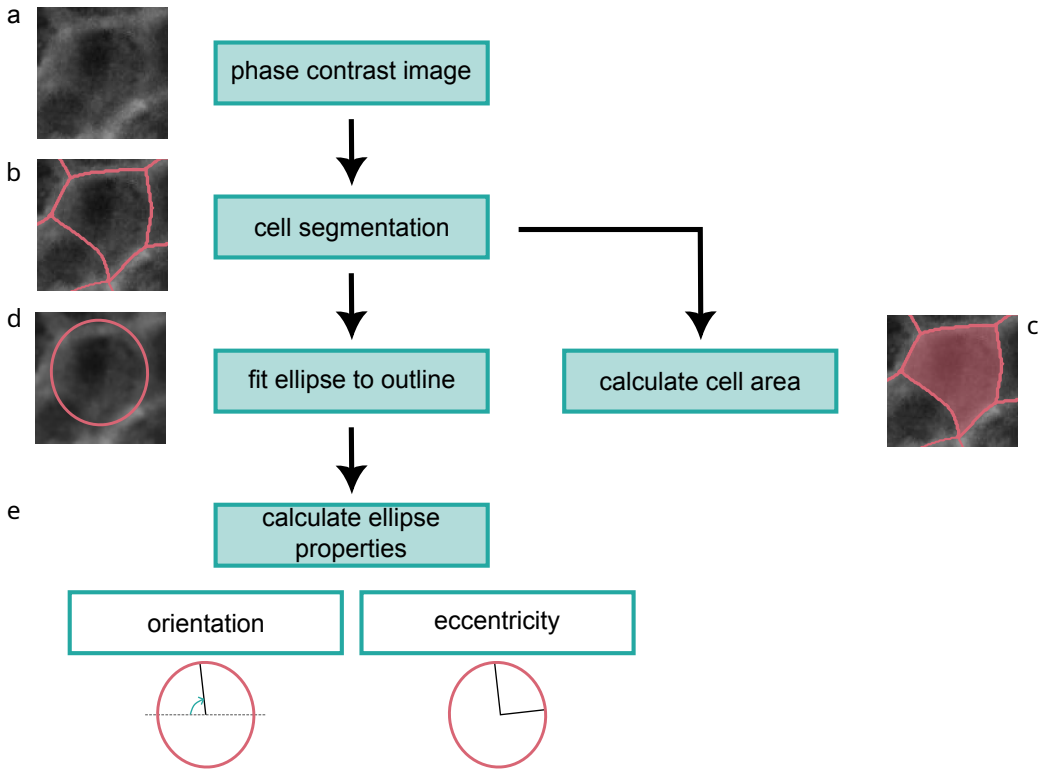

Figure S4: Workflow for the analysis of the cell shape. (a) The phase contrast image of the cells is (b) segmented to get the outline of each cell and (c) the area per cell is determined. (d) An ellipse is fitted to the outline and (e) the orientation and eccentricity are calculated.

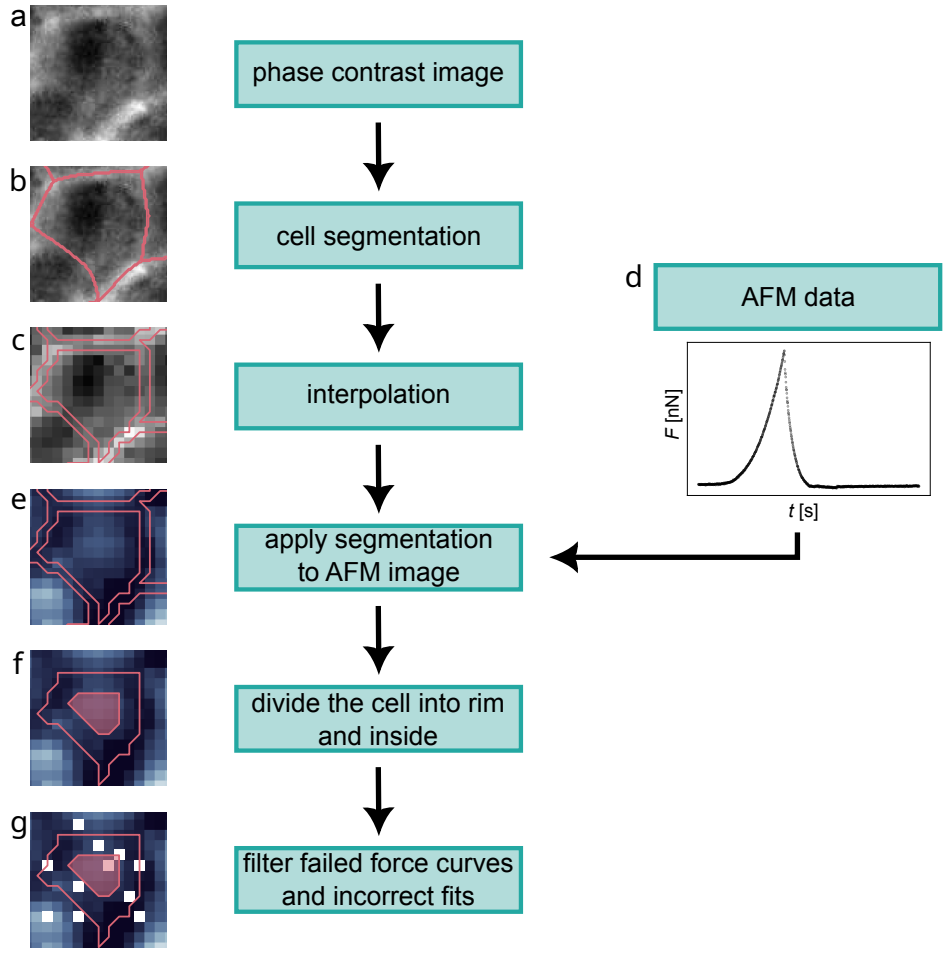

Figure S5: Workflow for the analysis of the force mapping data. (a) The phase contrast image of the cells is (b) segmented to obtain the outline of each cell. (c) The outline is interpolated to a bigger pixel size and (e) overlayed to the AFM image, (d) consisting of the force spectroscopy curves at each pixel. (f) The cell is divided into the rim and the inside regions and an interim section is excluded. (g) Failed curves and outliers are excluded.

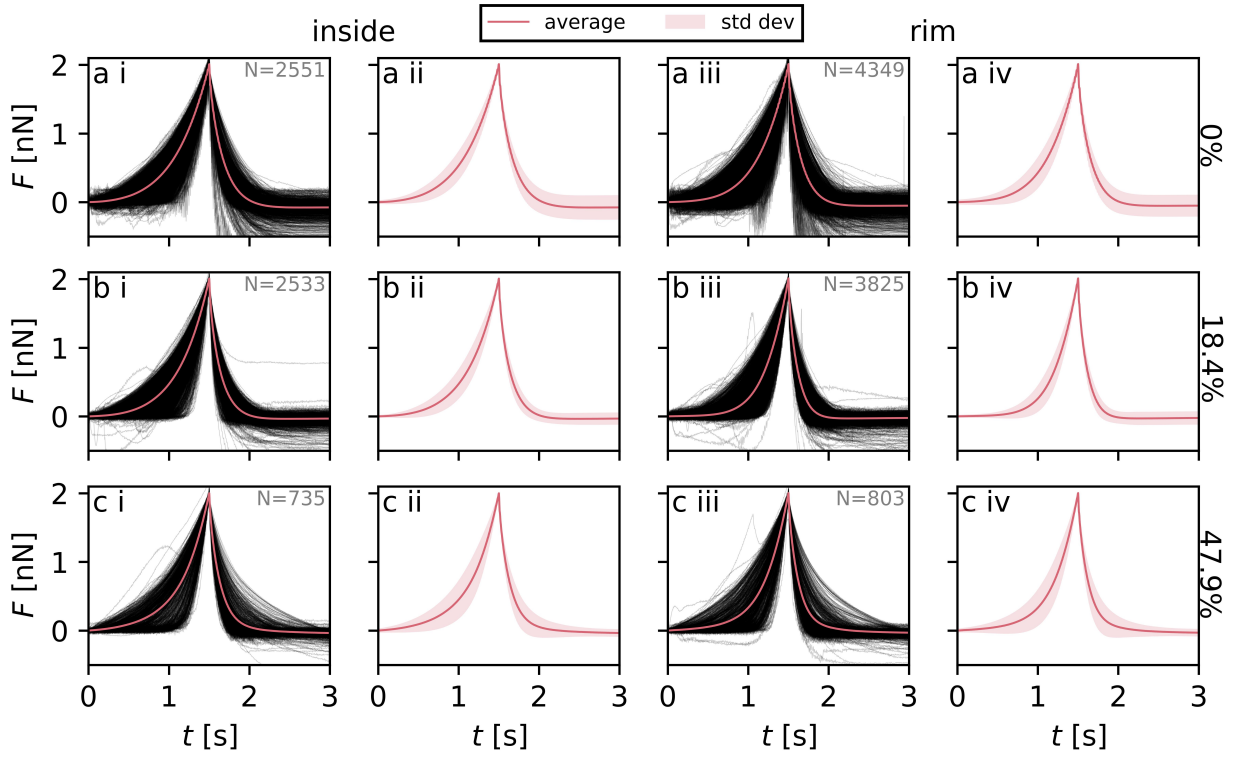

Figure S6: Force-time curves of the WT cells for an area strain of (a) 0%, (b) 18.4% and (c) 47.9%, for (i, ii) the inside region and (iii, iv) rim region. (i, iii) All single force curves (black) and their average (pink). (ii, iv) The same averaged force curve as in i and iii respectively (pink) and the standard deviation (pink shaded area). The number of force curves N is provided in the figure.

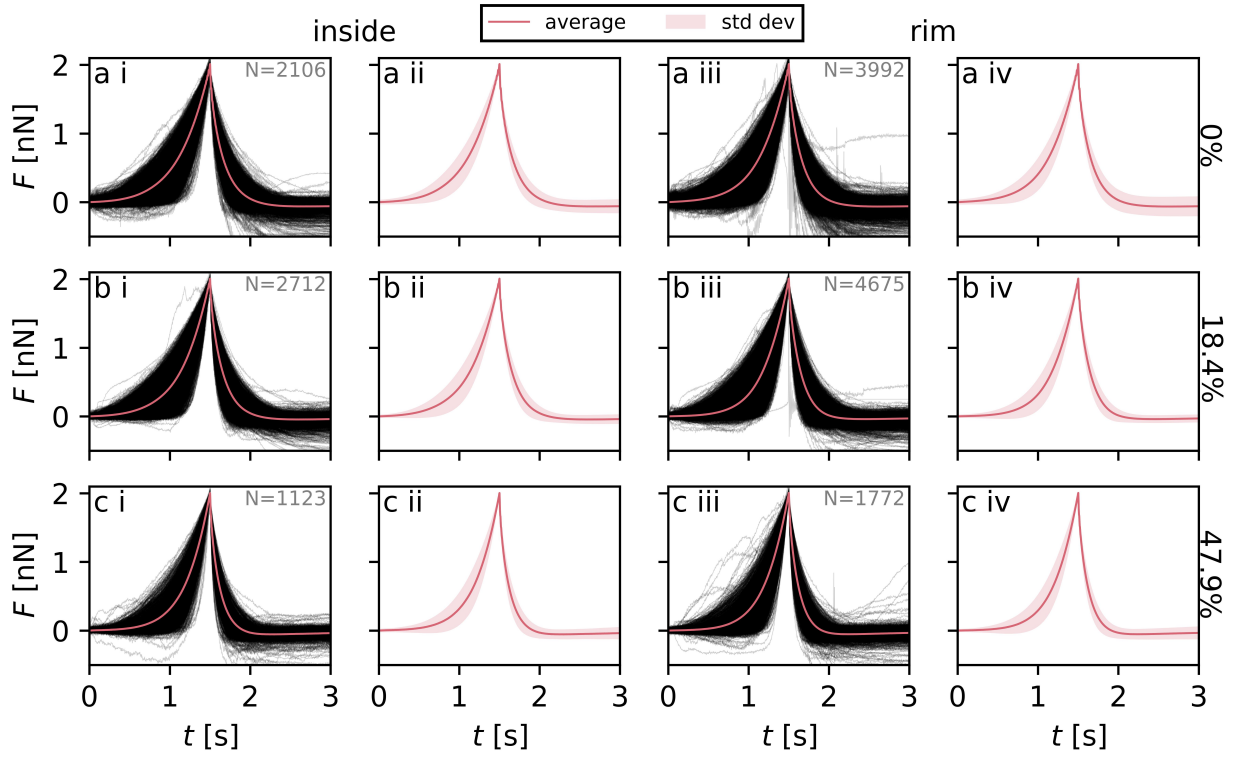

Figure S7: Force-time curves of the KO cells for an area strain of (a) 0%, (b) 18.4% and (c) 47.9%, for (i, ii) the inside region and (iii, iv) rim region. (i, iii) All single force curves (black) and their average (pink). (ii, iv) The same averaged force curve as in i and iii respectively (pink) and the standard deviation (pink shaded area). The number of force curves N is provided in the figure.

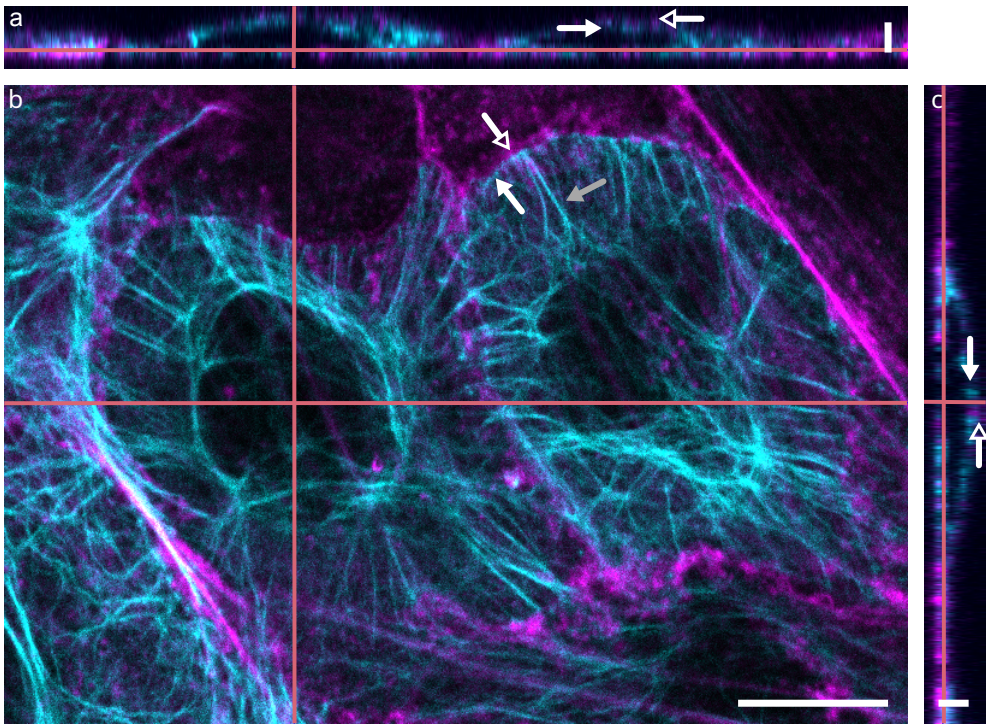

Figure S8: Example 3D confocal microscopy image of keratin tagged with EGFP (cyan) and lifeact actin tagged with mCherry (magenta) in fixed MDCK II WT cells. Pink lines indicate the displayed x, y, and z slices. (a) xz-view. Scale bar shows the z-scale and corresponds to $2\,\mu$m. The keratin cortex (closed arrow head) lies underneath the actin cortex (open arrow head). (b) xy-view. Actin cortex (open arrow head) and keratin rim (closed arrow head, white) and spokes (closed arrow head, grey). Scale bar shows the x- and y-scale for all three images and corresponds to $10\,\mu$m. (c) yz-view. Scale bar shows the z-scale and corresponds to $2\,\mu$m. The keratin cortex (closed arrow head) lies underneath the actin cortex (open arrow head). Note that the cells appear particularly flat, because they are chemically fixed and prepared between two glass slides.

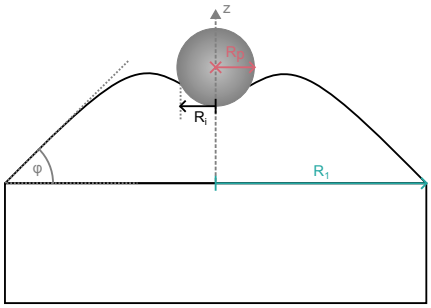

Figure S9: Schematic of the geometry for the Evans model. A cell in a monolayer is described as a spherical cap with radius R_1 and contact angle φ. During indentation with a sphere with radius R_p by indentation depth z, the contact can be described by the contact radius R_i.

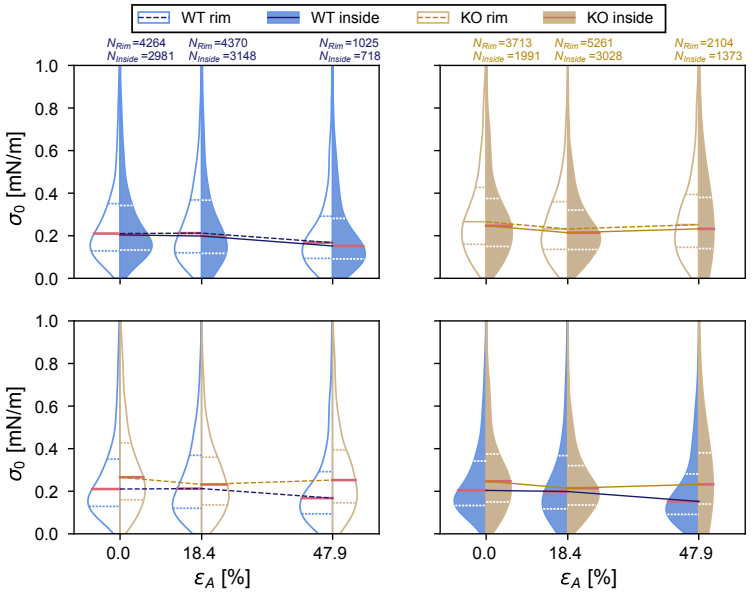

Figure S10: Pre-stress σ_0 of cells under strain ε_A. Data for WT cells are shown in blue, data for KO cells in yellow. Filled violins correspond to the inside-region of the cells, open violins with colored outlines to the rim-region. The lines on the violins represent the median (solid pink line), the upper and lower quartiles (dashed lines). The medians are connected via lines: dashed lines for the rim-region and solid lines for the inside-region. The number of force curves N is displayed in the figure. Top row and bottom row are showing the exact same data in different combinations for comparison.

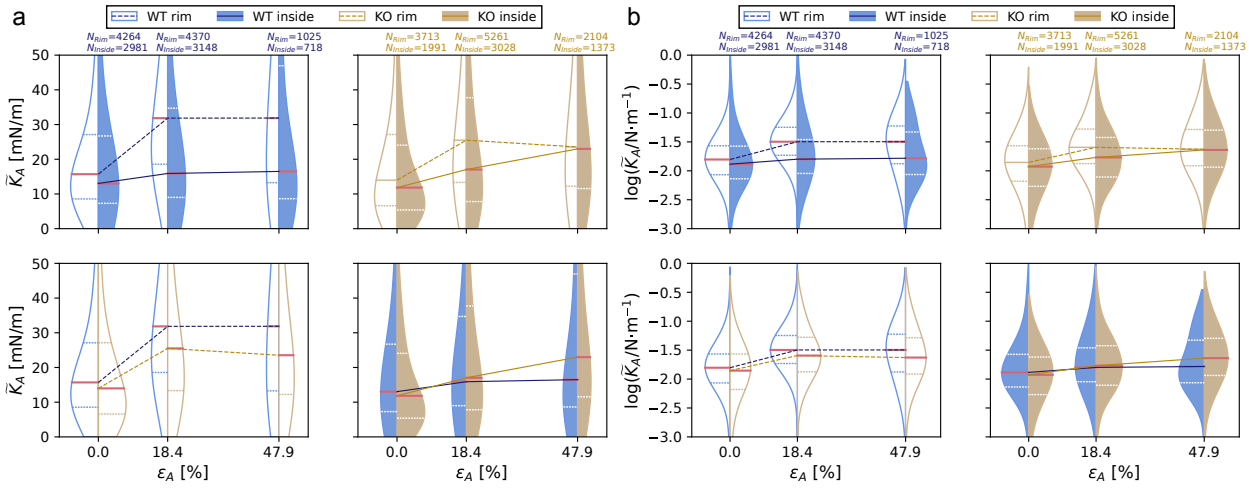

Figure S11: Area compressibility modulus $\widetilde{K}_A$ of cells under strain ε_A. Data for WT cells are shown in blue, data for KO cells in yellow. Filled violins correspond to the inside-region of the cells, open violins with colored outlines to the rim-region. The lines on the violins represent the median (solid pink line), the upper and lower quartiles (dashed lines). The medians are connected via lines: dashed lines for the rim-region and solid lines for the inside-region. The number of force curves N is displayed in the figure. Top row and bottom row are showing the exact same data in different combinations for comparison. (a) Area compressibility modulus $\widetilde{K}_A$. (b) The decadal logarithm of the same data as in (a).

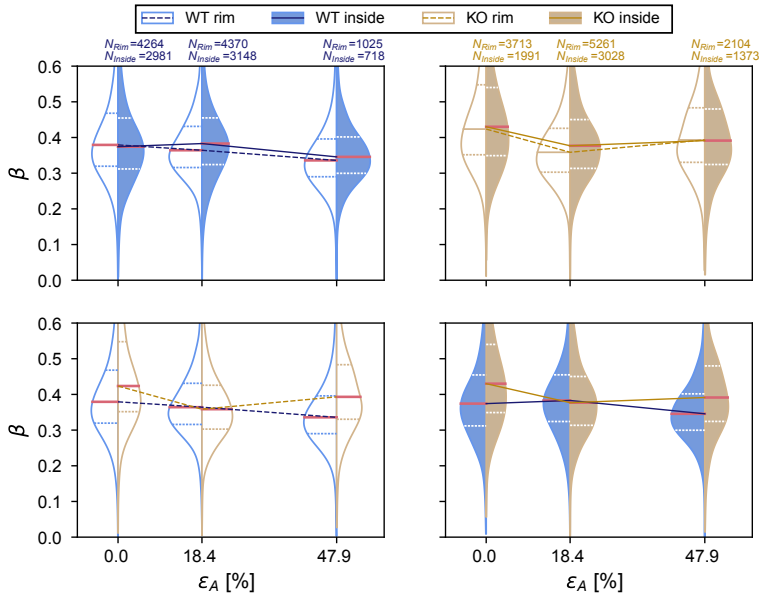

Figure S12: Fluidity β of cells under strain ε_A. Data for WT cells are shown in blue, data for KO cells in yellow. Filled violins correspond to the inside-region of the cells, open violins with colored outlines to the rim-region. The lines on the violins represent the median (solid pink line), the upper and lower quartiles (dashed lines). The medians are connected via lines: dashed lines for the rim-region and solid lines for the inside-region. The number of force curves N is displayed in the figure. Top row and bottom row are showing the exact same data in different combinations for comparison.

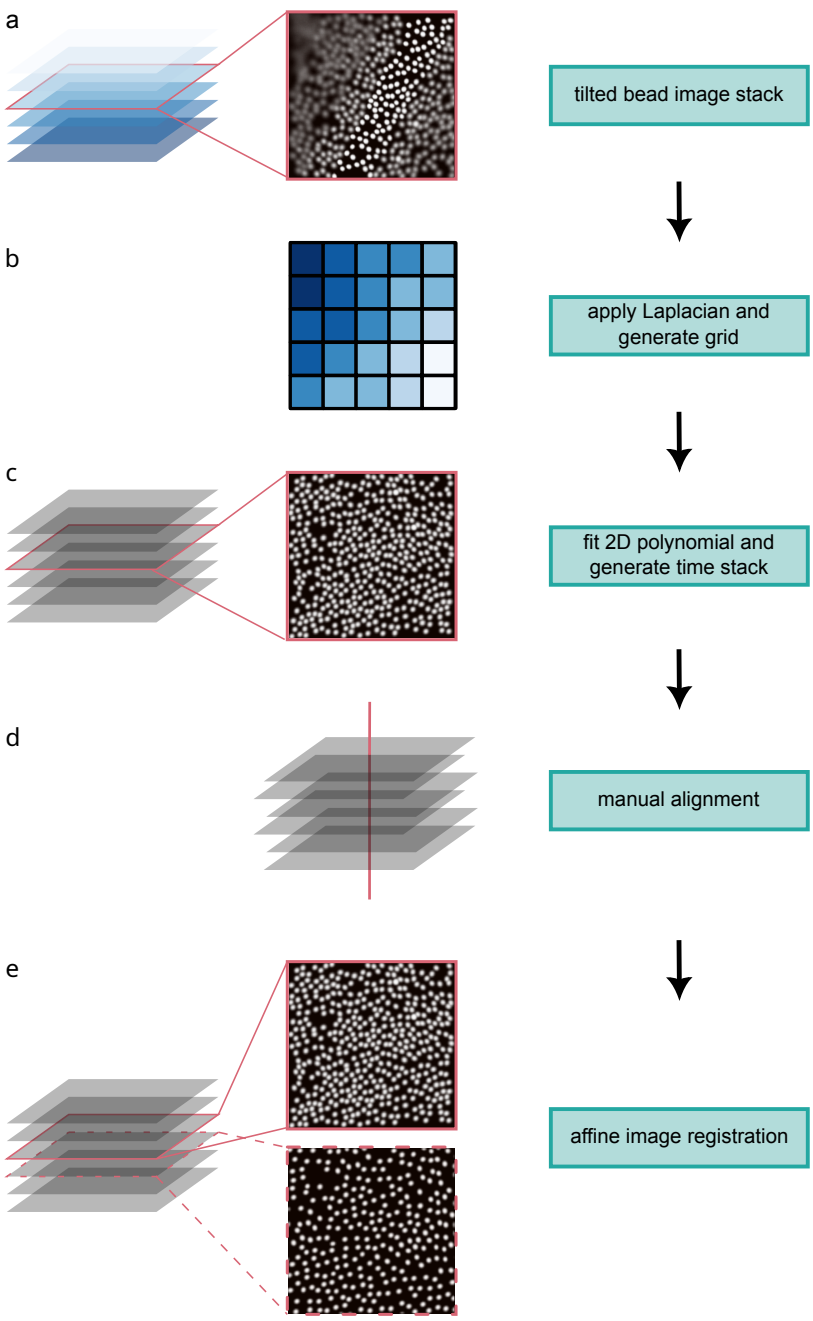

Figure S13: Workflow for the characterization of the PDMS devices in the equibiaxial stretcher. (a) Fluorescence image stack (different z-positions) of the beads in the slightly tilted membrane. (b) To compute the in-focus regions of the stack, a Laplacian is calculated for each frame and the indices of the frames in the stack where the beads are in focus are saved on a coarse grid. (c) A 2D polynomial is fitted to the grid and the original stack is interpolated to this fit to create a tilt-corrected image. By repeating this procedure for all image stacks with increasing strain, we generate a time stack with one tilt-corrected image per strain. (d) The images in the time-stack are manually aligned at the center of the image. (e) The images in the time stack are registered using an affine transformation to determine the strain.

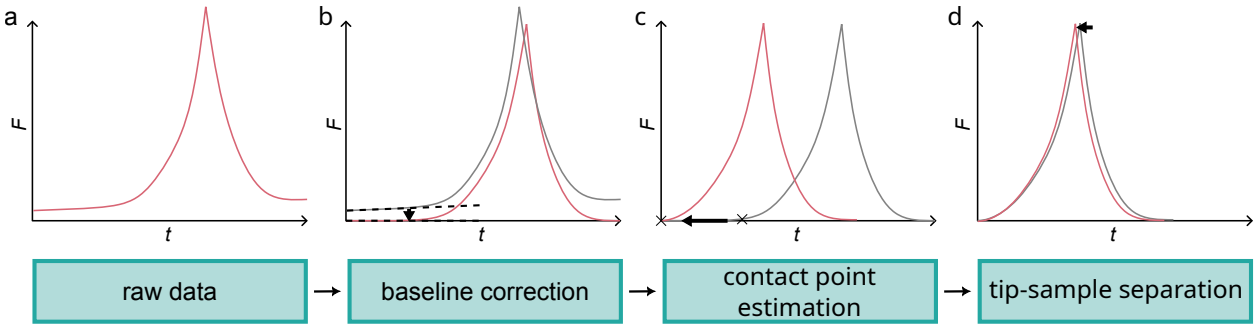

Figure S14: Schematic of the pre-processing of the force-time curves. (a) The baseline of the original force curve is (b) tilt and offset corrected by subtracting the baseline from the curve. (c) The contact point is estimated using the nanite-function "deviation from baseline" [2] and the curve is shifted such that the contact point lies at time point 0. (d) Finally, a tip-sample separation is applied to separate the distance of the cantilever moved towards the sample from the deflection of the cantilever in the opposite direction.

References

(1) Waters, J. C. Accuracy and precision in quantitative fluorescence microscopy. *Journal of Cell Biology* **2009**, *185*, 1135–1148.

(2) Müller, P.; Abuhattum, S.; Möllmert, S.; Ulbricht, E.; Taubenberger, A. V.; Guck, J. nanite: using machine learning to assess the quality of atomic force microscopy-enabled nano-indentation data. *BMC Bioinformatics* **2019**, *20*, 465.