Atomic Force Microscopy Mechanical Mapping of Micropatterned Cells Shows Adhesion Geometry-Dependent Mechanical Response on Local and Global Scales

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Figure SI1. Elasticity distribution of averaged mechanical maps

The box plot on top reports the medians and quartiles (25 and 75%), the circle corresponds to the mean value and the crosses the minimum and maximum values.

From the superposed histograms, a region at low values can be identified that is unique for ∇ -cells, while the average Y-cell showed a unique region at high elasticity (above 4.7 log (Pa)). The average T-cell shared mechanical features of both Y- and ∇ -cells.



Figure SI2. Elasticity repartition of cell areas.

Elasticity histograms of whole cell (black line), cell edges (blue line), corners (yellow line) and central region (purple line) of ∇ -, T- and Y- cells, from left to right.



Figure SI3. AFM topography of cells plated on ∇-, T-, and Y- shaped micropatterns, from left to right, before (A) and after (B) Latrunculin-A addition.

Disruption of actin filaments by Latrunculin-A causes a collapse of the cell structure, with particular strong effects on non-adherent cell borders. These undergo an increase of their concavity due to the release of tension, which counterbalances inward pulling force of the cell membrane. The cell-substrate contact area is thus reduced to the adhesive area exclusively. The symmetry-breaking of forces results in some cases in the repositioning of the nucleus, e.g. from the center to one corner in the case of ∇ -cells.



Figure SI4. Topography of fibronectin micropatterns obtained by AFM.

Height profile correspond to the section indicated by the red bar in the images, averaged over 5 μ m. Scale bars = 10 μ m, color scales 0 – 20 nm.



Figure SI5. Main figure: bright-field image of RPE1 cells on Y-micropatterns and AFM probe. Scale bar = $50 \ \mu m$. Inset: SEM image of a CSG11 AFM probe. Scale bar = $1 \ \mu m$.



Figure SI6. Dependence of Young's modulus measurements on the tip velocity.

Main figure: Young's modulus vs velocity plot. Values are obtained from conventional forcedistance curves by averaging measurements performed on the nuclear region of 4 cells plated on ∇ -pattern at 5, 25, 50, 100 µm/s (1, 5, 10, 20 Hz with 2.5 µm ramp size). The white dot corresponds to the average value obtained in Peak Force mode on the nucleus of ∇ -cells. The PeakForce velocity of 1200 µm/s is the average velocity in the region of the oscillation cycle used for fitting the Young's modulus, *i.e.* 30 to 90% of the maximum deflection.



Figure SI7. Young's modulus of non-patterned RPE1 cells.

Average histogram and single-cell mechanical map of RPE1 cells grown on a culture dish. Nonpatterned cells present a great variability of shape and size and higher elasticity the patterned ones. Moreover, inversely than patterned cells, the nuclear region is the softest while cell peripheries the stiffest.



Figure SI8. SEM images of a CSG11 AFM probe.

- A. 6000x magnification, scale bar = 1 μ m
- B. 18000x magnification, scale bar = 1 μ m



Figure SI9. Force-distance curves obtained on a Y-cell in PeakForce-QNM mode

The three curves, from left to right, were acquired on a corner, on the nuclear region and on a soft region of the cell (between the cell nucleus and the border). Young's moduli obtained from the AFM control software were of 20, 38 and 91 kPa, respectively. Such values are calculated fitting the conical contact elastic model to the curve region between the 30 and 90% of the maximum force. Young's moduli obtained fitting the same curves with a custom algorithm based on Matlab were 24, 36, 88 kPa when fitting the whole force curve, and 30, 31, and 97 when fitting the range 30-90% of the maximum force.



Figure SI10. Control time-lapse experiment with DMSO.

A. Young's modulus maps of an RPE1 cell before (0 min) and after DMSO injection. Full image size is $50 \ \mu m$.

B. Elasticity histogram of the maps reported in a.

No significant variation is observed during the 43 minutes following DMSO injection.