Electronic Supplementary Information

Cellular Orientation Is Guided by Strain Gradients

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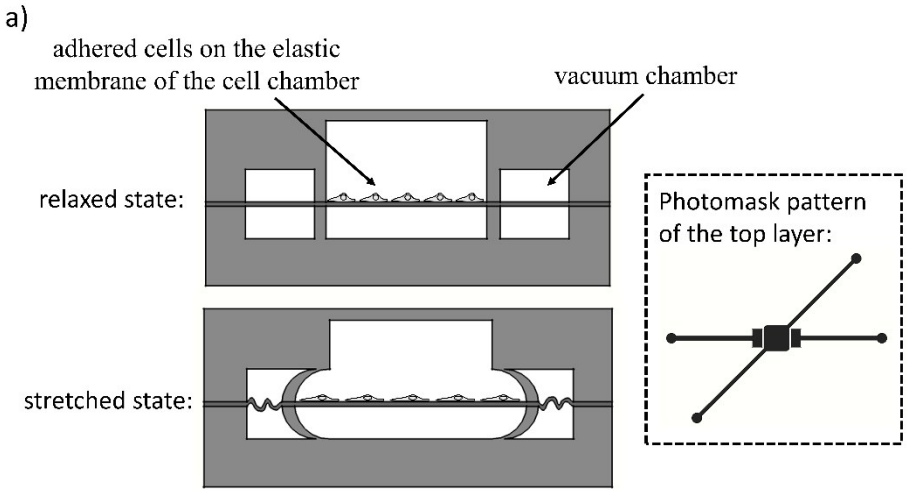
1. Additional details on the microdevice working principle and fabrication

As presented in Fig. S1a, when the device is in the relaxed state, the cells are not subjected to external stretch (no strain). The sinusoidal activation of the vacuum chambers cyclically deforms the thin PDMS walls separating the vacuum chambers from the cell chamber, which in turn pull on the elastic PDMS membrane. Additional schemes of the device are presented in Fig. S1b. As mentioned in the section Materials and methods of the article, the microdevice is composed of three PDMS layers, namely a thin membrane bounded between a top and a bottom pieces. They were fabricated and assembled in the following way. The membrane was made by spin coating PDMS on a silicon wafer prior to curing it. The top and the bottom parts were molded by soft lithography using PDMS on a micro-fabricated master mold. The latter was obtained through UV photolithography process, which allowed the transfer of a pattern from a printed photomask (see Fig. S1a) onto a light sensitive SU-8 2050 negative photoresist (Microchem, MA, USA). Two photoresist layers were deposited onto a clean silicon wafer to achieve the thickness required to mold the channels (340 µm). The cell chamber was further extended to a height of 2 mm, using additional PDMS sub-molds, to facilitate direct cell access during the staining and imaging procedures, once the stretching experiments were completed. The complete fabrication procedure and description of the advantages of this technique are described in details elsewhere (article in preparation). Finally, the three separate PDMS layers (top, bottom, and membrane) underwent an air-plasma treatment prior to being aligned and assembled using a mask aligner (OAI, CA, USA).



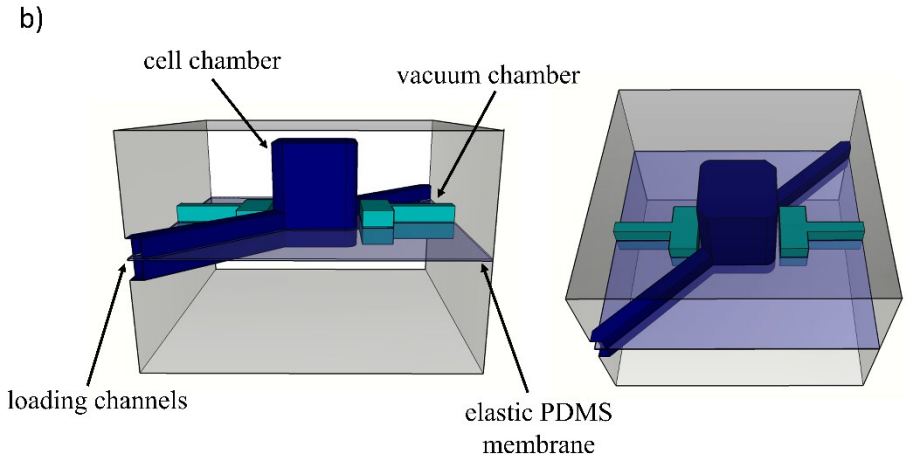


Fig. S1 Microdevice geometry. a) Left: simplified scheme of the device (cross section) demonstrating its working principle. Right: photomask pattern of the top layer. b) 3D schemes of the device. The dimensions of the cell chamber's membrane are 1.6 mm x 1.6 mm x 10 µm. Note that the device's cell chamber can be opened at the end of the stretching experiment to facilitate fluorescent staining and high resolution imaging.

2. Addition details on the membrane strain field calculation

The beads displacements were tracked with a homemade Matlab program in which the Green strain matrix elements (ε_{xx}, ε_{yy}, and ε_{xy}) are calculated for each initial bead position. The $\varepsilon_{xx}(x,y)$, $\varepsilon_{yy}(x,y)$, and $\varepsilon_{xy}(x,y)$ fields were interpolated and boxcar smoothed throughout the membrane area (the membrane was divided into cells of ~0.6 µm^2). The extracted strain fields of five devices were averaged together, as well as with their mirror images, to provide accurate strain fields representations of the overall device collection used for the experiments. The standard deviation of the devices characterized (averaged over the membrane surface) was approximately 9%. The maximum and the minimum principal strains $\varepsilon_1(x,y)$ and $\varepsilon_2(x.y)$ were computed by diagonalizing the position-dependent Green strain matrices $\boldsymbol{\Sigma}(x,y)$. For each point of the membrane, this process allows the determination of a coordinate system for which there is no shear strain, through the extraction of the eigenvectors. The eigenvalues are calculated from the matrices $\boldsymbol{\Sigma}(x,y)$ and correspond to the principal strain amplitudes $\varepsilon_1(x,y)$ (maximum strain) and $\varepsilon_2(x.y)$ (minimum strain). The direction of the maximum stretch $\theta_{strain}(x,y)$ (see Figure 1c) at each point, which is called principal strain direction, is given by the direction of the eigenvector $\boldsymbol{\varepsilon_1}(x,y)$. Finally, the gradient of $\varepsilon_1(x,y)$ was then calculated to obtain the gradient amplitude $|\nabla\varepsilon_1(x,y)|$ and gradient angle $\theta_{gradient}(x,y)$ (see Figure 1c) maps. Both $\theta_{gradient}(x,y)$ and $\theta_{strain}(x,y)$ were kept between 0 and 180 degrees. The strain field analysis was also performed using a homemade Matlab program.

3. Immunofluorescence straining in a microdevice

Since the cells were contained in microdevices, two effects must be taken into account during the staining procedures. First, the fluid mixing is low in the cell chambers due to the microscale nature of the fluid volumes. Second, the loss of photons from PDMS scattering and surface reflection can weaken the fluorescent signal during the image acquisition. We found that doubling the incubation time of every straining step was sufficient to obtain good image quality. In between every step, the samples were also incubated in washing buffer (PBS solution with 5% horse serum) for 30 min.

4. Membrane regions, number of cells, and number of FAs analysed in different figures of the manuscript

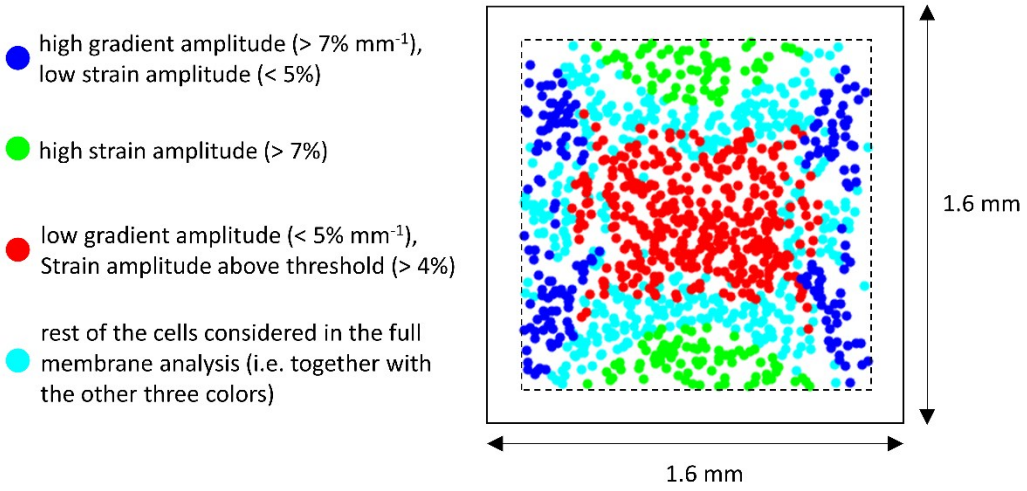


Fig. S2 Regions analyzed in the histograms displayed in Fig. 2 of the manuscript and in Fig. S3 of the ESI. The location of the cells corresponding to 6 cyclically stretched experiments are superimposed together. The legend on the left specifies the strain and strain gradient amplitudes conditions associated with each color. Fig. 2A and Fig. S3A were generated with the cells of all 4 colors; Fig. 2B was generated with the cells shown in green; Fig. 2C,D were generated with the cells shown in dark blue; Fig. S3B was generated with the cells shown in red.

Pharmacological treatments	**Regions of the membrane**	**Corresponding figures**	**Number of cells in varying strain field (2-10%)**	**Number of cells in control (0% strain)**
Control (DMSO 0.1%)	Full membrane	Fig. 2A, Fig. 3A-C	1078	1244
	High strain	Fig. 2B	132	200
	High gradient, low strain	Fig. 2C,D	181	187
	Low gradient	Fig. S2	366	409
Blebbistatin (10µM)	Full membrane	Fig. 4B	1319	1091
CalA (2 nM)	Full membrane	Fig. 4A	1231	1255

Table S1 Number of cells analysed in different figures.

Pharmacological treatments	Regions of the membrane	Corresponding figures	Number of FAs in varying strain field (2-10%)	Number of FAs in control (0% strain)
Control (DMSO 0.1%)	Full membrane	Fig. 5A	7568 (61 cells)	2607 (35 cells)
Blebbistatin (10µM)	Full membrane	Fig. 5C	1904 (62 cells)	889 (29 cells)
CalA (2 nM)	Full membrane	Fig. 5B	5164 (71 cells)	4119 (44 cells)

Table S2 Number of FAs analysed in different figures.

5. Cell orientations reported with respect to the x-axis

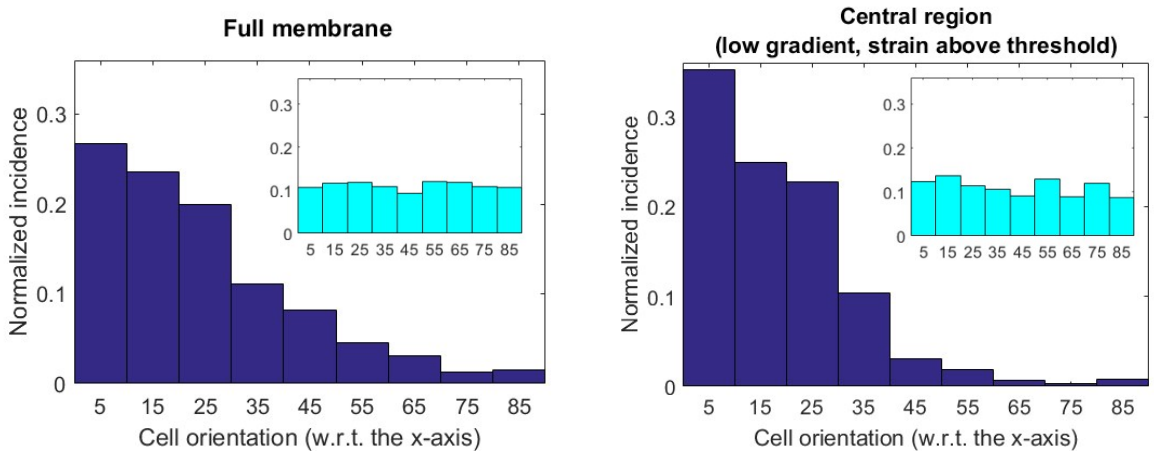


Fig. S3 Reorientation analysis of the HFF cells after 11 hours of cyclic stretching along the y-axis. Normalized incidence histograms of the cell body orientation with respect to the x-axis considering the full membrane (left) and the central region (right) (more specifically the region for which the strain gradient amplitude is lower than 5 %mm^{-1} and for which the strain amplitude is greater than 4 %). It should be emphasized that in contrast to the histograms presented in the manuscript, it is the cell body orientations which are reported, rather than the cell normals, and a unique axis is considered rather than reporting each cell with respect to its local principal strain direction. Each histogram comprises the combination of 6 unstretched control experiments (insets, shown in turquoise) as well as the combination of 6 cyclically stretched experiments (shown in dark blue).