Supplementary information

## S.1 Optical deformation measurement

For the optical deformation measurement, a customized LabVIEW program was realized to probe cell's real-time dimension during optical stretching process. An example is shown in Fig.S1: cell contour recognized from an edge-detection algorithm is superimposed on the original phase contrast microscope image. The major and minor axes of the cell, *x* and *y* as indicated in Fig.S1, are in the laser beam and flux direction respectively.

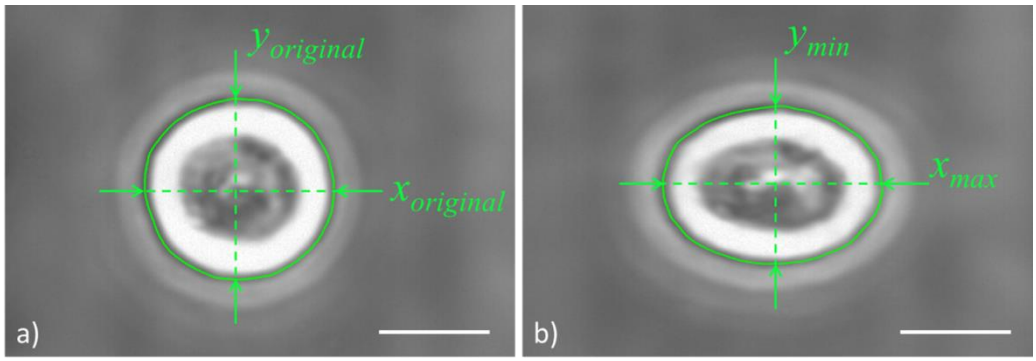



Fig.S1 Phase contrast microscope images of (a) trapped and (b) stretched cell with overlapped border recognition. Scale bar: 10μm

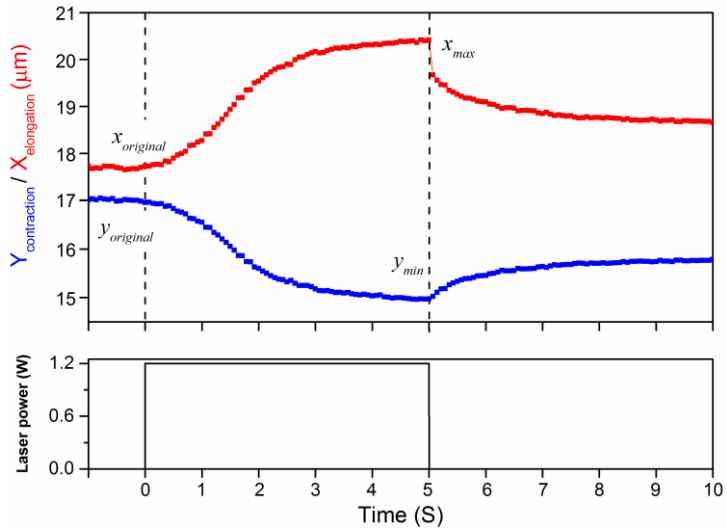



Fig.S2 Optical deformation of a single cell in terms of $X_{elongation}$ and $Y_{contraction}$ under 5 seconds of optical stretching

A typical creep compliance behavior of optical cell stretching is reported in Fig.S2: $x_{original}$ and $y_{original}$ are the horizontal and vertical dimensions of the cell obtained before optical stretching, whereas $x_{max}$ and $y_{min}$ after 5 seconds of optical stretching. Cell's maximum relative deformation, reported in Fig.3 of the manuscript, is evaluated as *Var* in the following equation:

$$Var(\%) = \left( \frac{x_{max}}{y_{min}} \Big/ \frac{x_{original}}{y_{original}} - 1 \right) \cdot 100 \cdot Corr$$

where the *Corr* factor is a correction term obtained by numerical simulations, as reported in Ref. S1, which allows to properly take into account the different force distribution caused by different cell.

## S.2 Sample enrichment

To check the effectiveness of the device, the different deformability exhibited by metastatic (A365P) and highly metastatic (A365MC2) cells has been exploited to obtain a cell sample enriched of highly metastatic (A365MC2) cells. Here we report the results obtained by choosing 10% as the deformation threshold, above which the cells are collected.

According to the measured deformability of the two populations, this threshold is expected to give 80% of A365MC2 in the collected sample, starting from a 50% mixture. The enrichment is verified by exploiting, after cells' collection, fluorescence microscopy, indeed only A365MC2 were stained (before mixing them with A375P) with a fluorescent dye (LDS 751). One example is shown in Fig. S3. According to the images, which derive from the overlapping between bright field and fluorescent images of the same microscopic field, A365MC2 ratio is increased from 51% to about 75%, which is slightly lower than the theoretical value. On the left side we report the pictures taken using a drop of the original mixture.

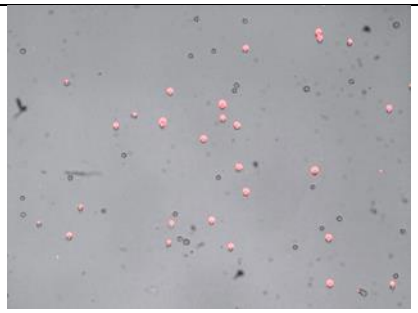
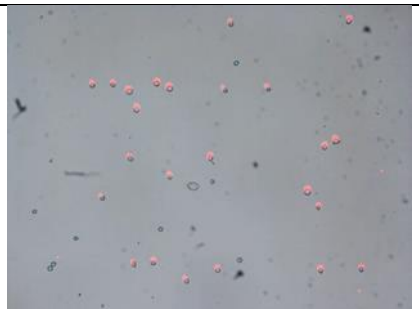
| STARTING MIXTURE | SELECTED SAMPLE |
|---|---|
|  |  |
| Fluorescent cells/ total number of cells: 33/64 (51%) | Fluorescent cells/ total number of cells: 24/32 (75%) |

Fig.S3 Merge between bright field and fluorescent images of (left) 50% starting mixture and (right) A375MC2 enriched sample obtained by sorting cells with deformability higher than 10% deformation threshold.

## S.3 Cellular viability

Cellular viability after stretching and sorting was checked on A372MC2, both staining cells with Trypan blue just after the recovery from the chip and culturing the cells *in vitro*.

The Trypan blue staining allows distinguishing viable cells from dead cells because the dye can penetrate the membrane of dead cells, while it is excluded by live cells, thus, dead cells appear blue after staining while live cells appear clear (Ref. S2). The sorted cell suspension was mixed in a 1:1 ratio with a 0.5% Trypan blue solution in PBS and cells were then counted using a burker chamber (Fig.S4). We found that the percentage of dead cells in stretched and sorted samples varied between 2 and 6% and was in the same range as that found in samples of the same cells maintained on the bench during the stretching procedure.

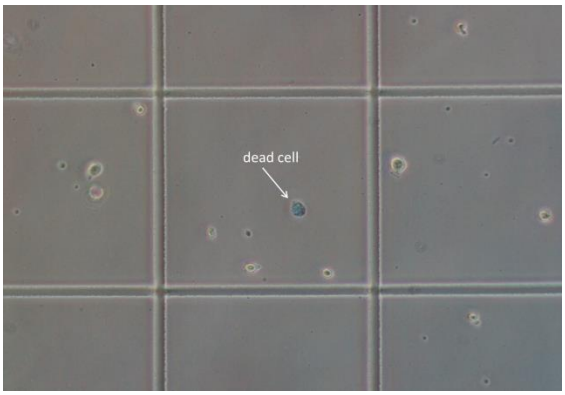



Fig.S4 A375MC2 viability checked with Trypan Blue staining. Cells were counted in a burker chamber.

To test the long termed viability of stretched cells, sorted cells were plated in a tissue culture well and incubated at 37°C for 5 days. Cells attached to the dish and were able to proliferate as control cells. These results confirm that the optical power used in the experiments does not damage cells, in agreement with data from the literature (see Ref. S3).

**References:**

[S1] J. Guck, R. Ananthakrishnan, H. Mahmood, T. J. Moon, C. C. Cunningham, and J. Käs, Biophys. J., (2001) 81:767–784.

[S2] Strober, W. 2001. Trypan Blue Exclusion Test of Cell Viability. Current Protocols in Immunology. 21:3B:A.3B.1–A.3B.2.

[S3] F. Wetzel, S. Ronicke, K. Muller, M. Gyger, D. Rose, M. Zink, J. Käs, Eur Biophys J. (2011) 40:1109–1114.