Supplemental Materials

Molecular Biology of the Cell

Rianna et al.

Supplementary Figures

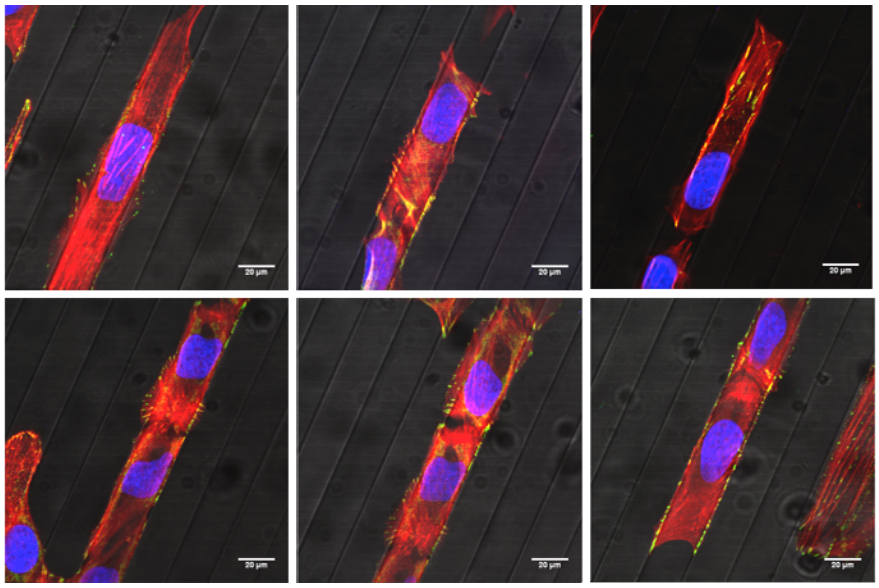

Figure S1. Confinement of U2OS cells within 20 µm PDMS channels.Confocal images of several U2OS cells on 20µm spaced channels of PDMS, seeded on different locations. Cells are fixed after 24 h from cell seeding and stained for actin (red), vinculin (green) and nuclei (blue).

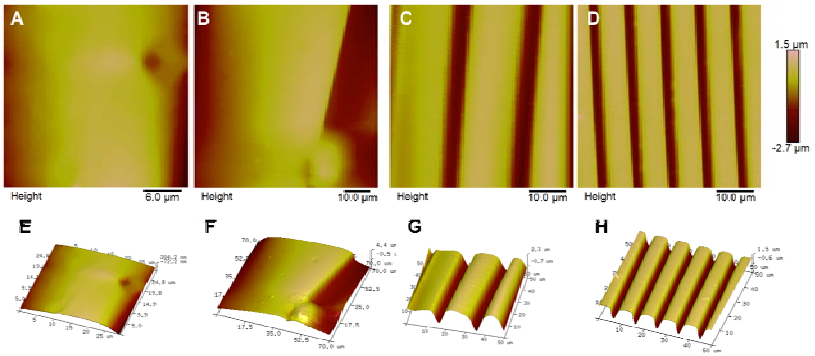

Figure S2. AFM images of PDMS channels.2D Contact-mode AFM height images of (A) flat, (B) 50 µm, (C) 10 µm and (D) 5 µm patterned PDMS with (E-H) the respective 3D representations. In panel (B) a U2OS cell is seen adjacent to the channel wall.

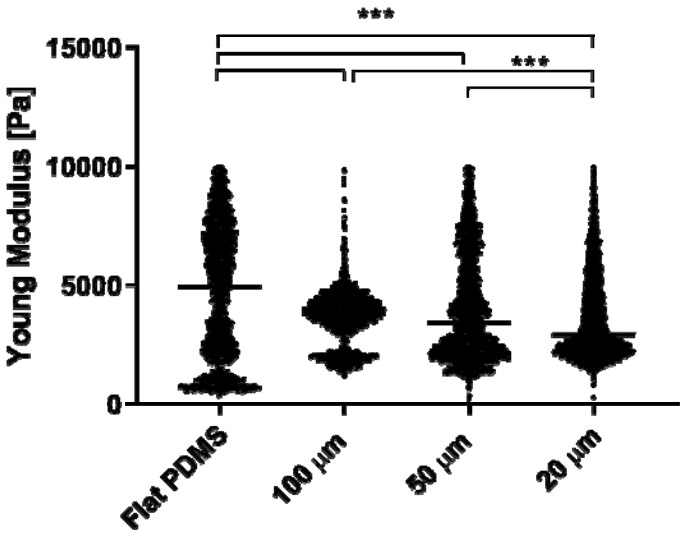

Figure S3. Dot plot graph of AFM U2OS cell mechanics. Young's modulus values of U2OS cells seeded on flat PDMS and 20-100 µm wide channels (see Figure 2D of the main text). Statistical significance was determined using the Mann-Whitney test between each pair of categories (***p<0.0001, N=2560, 2304, 3072, 3328 force curves on 10, 9, 12, 13 cells across three independent experiments. For each cell one 16x16 map was recorded on the supranuclear region of cell body).

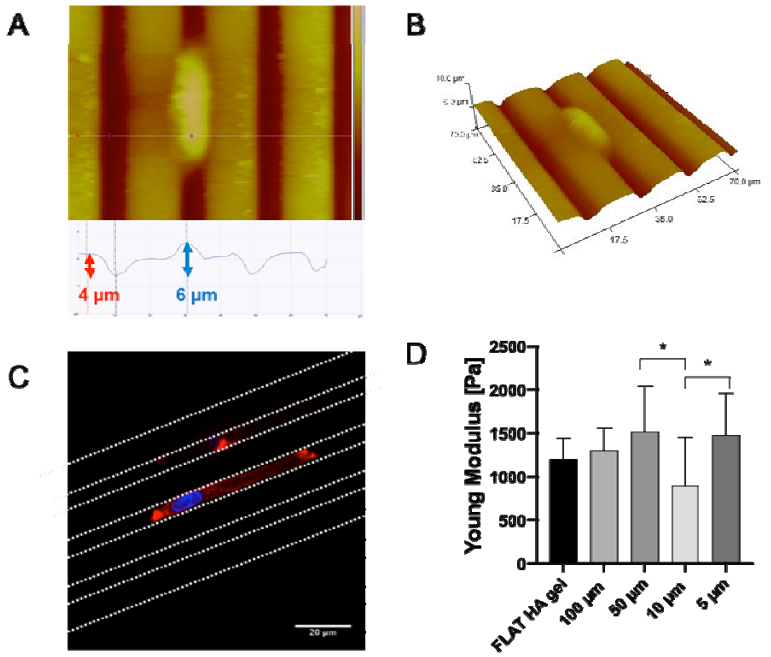

Figure S4. Confinement of U87 glioblastoma cells within channels of hyaluronic acid (HA) gels. (A) AFM image of a U87 cell confined in 10 µm HA gel channels (gel stiffness: 14 kPa) with a cross section below showing the height of the channel and the cell, 4 µm and 6 µm, respectively. (B) AFM 3D image of U87 cell. (C) Confocal image of U87 cells within 10 µm channels; cells were stained for actin (red) and nuclei (blue); channels are represented with white dashed lines. (D) Young´s modulus values of U87 cells seeded on flat HA gels and on HA channels ranging from 100 µm to 5 µm. Each bar represents the median value of all the values extracted from all the force maps collected on cells with interquartile range (10-15cells were measured for each category, with 256 force curves recorded on each cell, for a total of ~3850 total curves, *p<0.05, as calculated by Mann-Whitney test between each pair of categories).

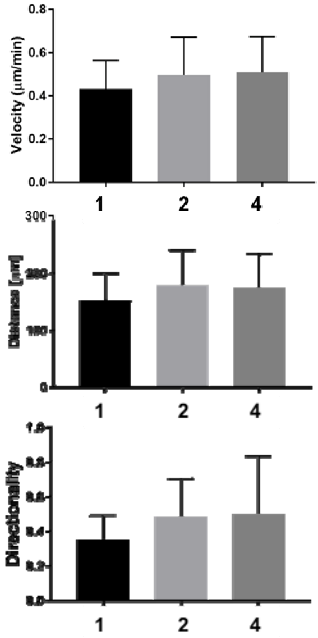

Figure S5. Cell migration study on U2OS cells on Y-shaped PDMS channels. U2OS were observed for 12 h with optical microscope, recording one image every 15 minutes. Manual tracking and chemotaxis tools of *ImageJ* were used for studying cell migratory properties, in terms of velocity, distance and directionality. The numbers 1, 2 and 4 on x-axis represent the categories in which cells were sorted: (1) Unconfined; (2) Attached to one wall; and (4) fully confined within channels (as showed inFigure 3 of the main text). We could not measure cells of category 3 (Associated with two walls but not fully confined), because this was a temporary condition of transition between unconfined and confined states and therefore did not allow measurementwithin a 12 h window.

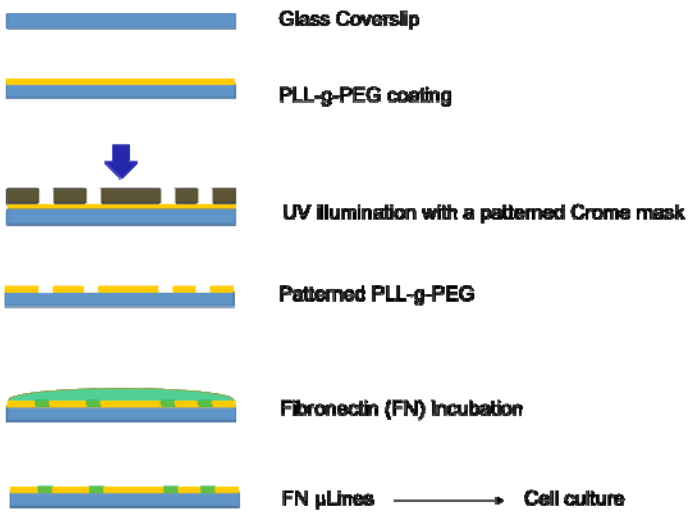

Figure S6. Schematic of microline fabrication.From top to bottom: a cover glass was coated with PLL-g-PEG for 1 h and then treated with 180 nm UV through a quartz-chrome mask with microline patterns. FN solution was then absorbedto the exposed parts of layer of PLL, at which stage the cover glasswasused in cell culture.

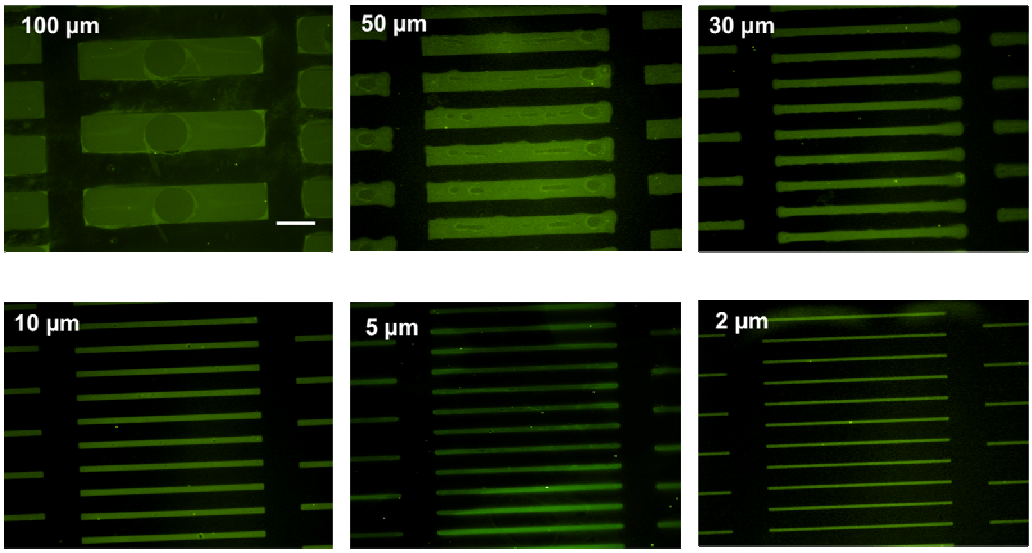

Figure S7. FNmicrolines on PLL-g-PEG coated cover slip.Optical images of fluorescently labeled FN on photopatterned microlines. Line width ranges from 100 µm to 2 µm.Scale bar: 100 µm.

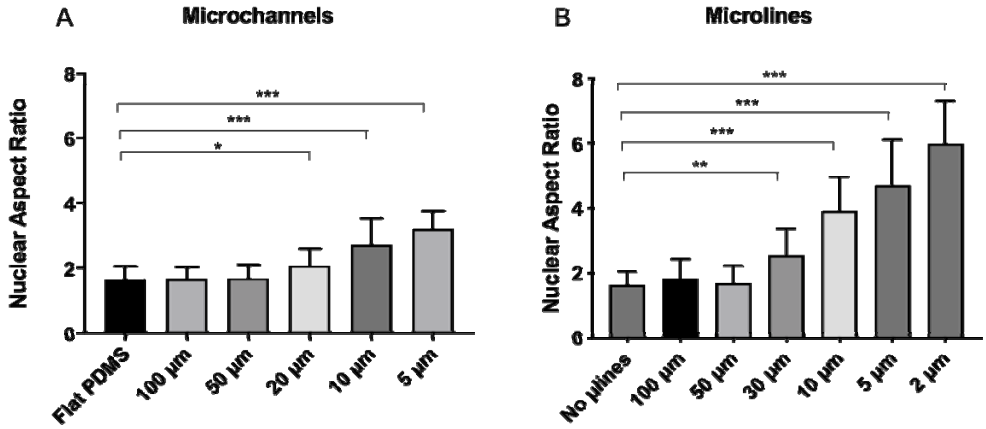

Figure S8. Quantification of nuclear aspect ratio on U2OS cells seeded on (A) topographical microchannels and (B) FN microlines. All error bars depict standard deviations (n=10 across three independent experiments, ***p<0.0001, **p<0.001, *p<0.01, as calculated by Mann-Whitney test between each pair of categories).

Movie Legends

Movie S1. Time-lapse movie of U2OS cells migrating within Y-shaped PDMS channels. Confined migration was achieved passivating the mesas between the channels by microcontact-printing PEGprior to fibronectin-coating the device.Cells were observed for 12 h with optical microscope, recording one image every 15 minutes.

Movie S2. U2OS cell migration within Y-shaped PDMS channels. A U2OS cell is seen migrating into the central channel of the device, going from unconfined to confined spaces.Cells were observed for 12 h with optical microscope, recording one image every 15 minutes.

Movie S3. Confocal z-stack movie of a U2OS cell confined in channels. Staining is for actin (red); vinculin (green) and nuclei (blue).Movie starts at the bottom of the cell and ends at the top.

Movie S4. Confocal z-stack movie of a U2OS cell confined in channels. Staining is for actin (red); vinculin (green) and nuclei (blue).Movie starts at the bottom of the cell and ends at the top.