Supporting Information

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Tunable Three-dimensional Hydrogel Microchannel Networks to Study Confined Mammalian Cell Migration

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**Tunable three-dimensional hydrogel microchannel networks to study confined mammalian cell migration**

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**SI Figure 1.** Phase contrast images showing both controls for cell seeding (HT1080) within a 24-well plate as well as 2D collagen I functionalized hydrogels (1, 17 and 50kPa) after 5 day incubation. Cells were treated with the same volume of medium and the 2D hydrogels were incubated into trans wells, similar to the 3D microstructured hydrogel. Scale: 50µm

**SI Figure 2.** Merged phase contrast and fluorescent images show a control of cell seeding (HT1080) within a 24-well plate after 5 days of incubation. Cell nuclei are mainly located in the center of the adhering cells. Scale: 100µm
**SI Figure 3.** Systematic image about cell location and hydrogel stiffness. Image represent overlays of phase contrast and NLS-GFP (green) and H2B-RFP (red) channels. Scale: 50µm

**SI Figure 4.** Analysis of channel architecture based on fluorescent 3D image stacks. a) 3D projections of channels, skeletonized channels, the respective binary image and the skeletonized z-slide, derived from hydrogel volumes of $3.5 \times 10^6$ $\mu$m$^3$ (41 z-slices, distance 1.99µm). Scale bar 50µm. Green: FITC-dextran. b) Number of intersections and channels identified as a function of Young’s modulus. The bar graph shows mean ± standard deviation. c) Ratio of the number of intersections to channels in the microstructured hydrogels, as a function of the hydrogels’ Young’s modulus. Graphs shows mean ± standard deviation. Differences were not statistically significant.