

**Supplementary figure 1. Microgrooved PDMS characterization.** Microgrooved PDMS were characterized by means of (A) scanning electron microscope (SEM) and (B) atomic force microscope (AFM). Both pictures correspond to the characterization of 200 nm deep and 10  $\mu\text{m}$  wide microgrooved PDMS. Scale bars=10  $\mu\text{m}$ .

**Supplementary figure 2. Analysis of actin stress fibers orientation.** (A) The macro builds 48x48 pixel squares throughout the whole image and (B) analyzes segments of the actin stress fibers. (C) Fast Fourier Transforms (FFT) are performed in each of these segments and (D) an ellipse fitting the Fourier spectra is fitted. (E) The major axis of the fitted ellipse is rotated 90° and (F) the angle between the major axis and the reference is calculated for all the segments.

**Supplementary figure 3. Analysis of significance of cell morphological parameters between ECs on different substrate conditions.** A statistical comparison between ECs on different substrate conditions (flat, 350 nm deep, 10  $\mu\text{m}$  and 2  $\mu\text{m}$  wide grooves) was performed at different time points of spreading, for each morphological parameter ((A, D) cell area, (B, E) elongation and (C, F) orientation) and for (A-C) non-treated as well as (D-F) Blebbistatin-treated ECs. A total of 150 cells ( $N_{\text{cells}}=150$ ) from 3 experiments ( $N_{\text{exp}}=3$ ) were analyzed.

**Supplementary figure 4. Schematic illustrating cells grown upright and upside down.** (A-B) Views from above and (C-D) the side show the cell culture setup. (E-G) The different forces acting on the cells are also illustrated. (G) A cell suspended in media usually sediments downward due to the minute net force given by the gravity ( $F_g$ ) and buoyance force ( $F_b$ ). Since the density of cells is larger than the media,  $F_g$  is slightly larger than  $F_b$ . (E-F) When cells adhere ( $F_a$ ) on a substrate all the forces equilibrate resulting in a 0 net force.