

**Supplementary Figure 1. ECs form confluent monolayers on matrices of varying stiffness.** Representative phase contrast images show that HMEC-1 grown on synthetic matrices of varying stiffness (for 2d) form confluent monolayers. Scale bar: 100 µm



Supplementary Figure 2. HMEC-1s express low baseline levels of ICAM-1. Surface expression of endothelial ICAM-1 was determined by flow cytometry. Representative histogram of ICAM-1 fluorescence intensity indicates that unstimulated HMEC-1s (HMEC con) express low levels of baseline ICAM-1 expression, which is markedly enhanced by TNF- $\alpha$  treatment (HMEC TNF). IgG was used as an isotype control.



Supplementary Figure 3. Abnormal matrix stiffness enhances monocyte-EC adhesion in the absence of pro-inflammatory cytokine. Fluorescently-labeled U937 cells were added (for 30 min) to unstimulated EC monolayers grown on different matrices for 2d. Quantification (average  $\pm$  SEM; per mm<sup>2</sup>) of adherent U937 cells from multiple (n≥8) images reveals the same biphasic trend in U937 cell-EC adhesion (\*\*\*, p<0.001) as seen with TNF- $\alpha$  stimulation.



Supplementary Figure 4. Human venous ECs also exhibit matrix stiffness-dependent biphasic regulation of monocyte-EC adhesion. Representative images show fluorescently-labeled U937 cells bound to TNF- $\alpha$ -stimulated HUVEC monolayers grown on synthetic matrices of varying stiffness for 2d. Scale bar: 100 µm. Quantification (average ± SEM ; per mm<sup>2</sup>) of adherent U937 cells from multiple (n≥8) images reveals the same biphasic trend in U937 cell-EC adhesion as seen with microvascular ECs (HMEC-1) (\*, p<0.05; \*\*\*, p<0.001).



**Supplementary Figure 5. Secondary antibody labeling control.** Labeling of U937-EC cocultures with FITC-labeled anti-rabbit polyclonal antibody alone shows no non-specific binding of secondary antibody during immunolabeling for ICAM-1.



**Supplementary Figure 6. Effect of matrix stiffness on ICAM-1 expression in long-term** (**5d**) **EC cultures.** qPCR analysis of ICAM-1 mRNA (three replicates/condition) shows that long-term (5d) culture of ECs on soft and stiff matrices leads to significant increase in ICAM-1 expression (\*, p<0.05).



**Supplementary Figure 7. Effect of matrix stiffness on ROCK2 mRNA expression.** qPCR analysis of ECs grown on different stiffness matrices (for 2d) shows that mRNA levels of ROCK2 follows the same biphasic trend as that exhibited by ROCK2 protein levels and Rho activity.