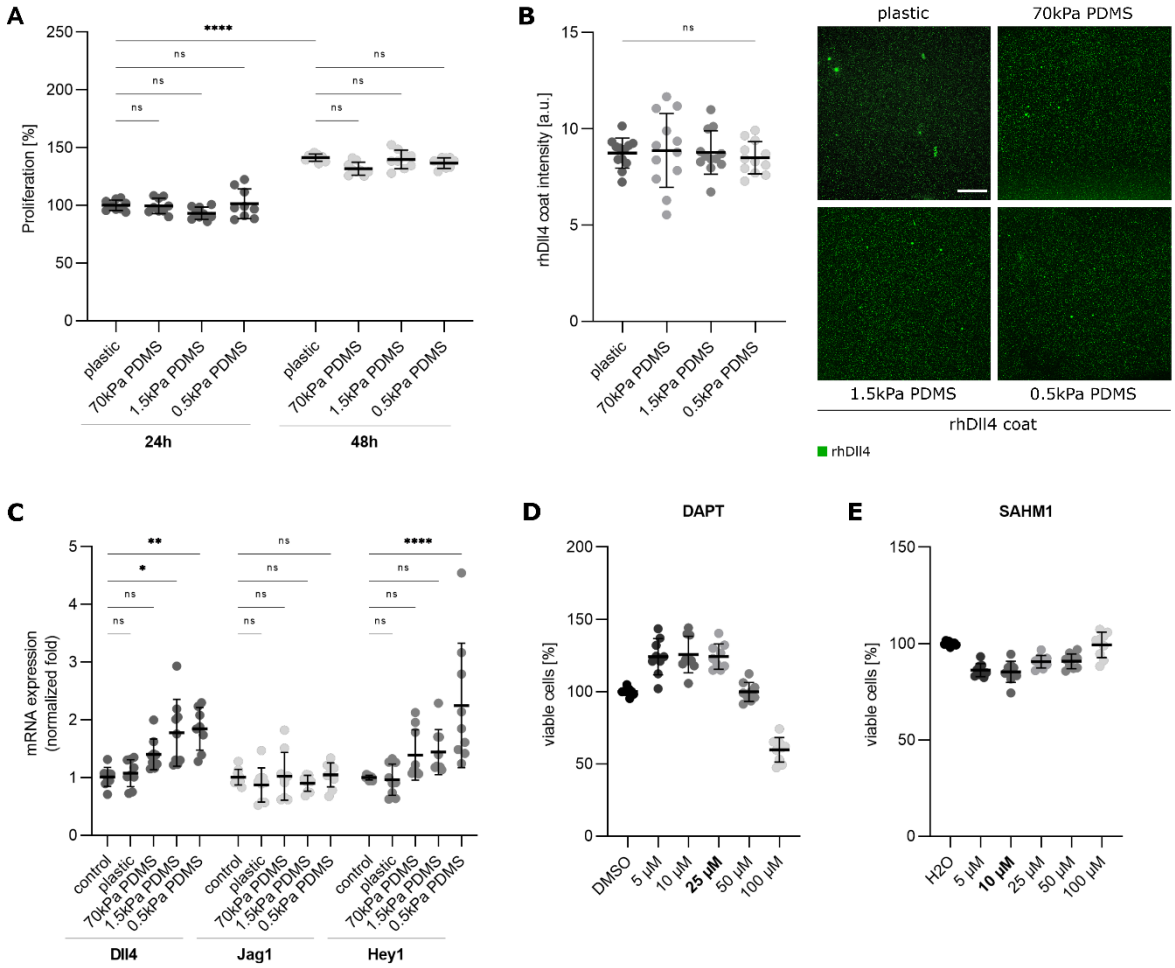

Fig. S1. General proliferation and protein coating on the PDMS substrates are not stiffness dependent and the applied inhibitor concentrations do not have a cytotoxic effect on the cells.

(A) Proliferation of HUVEC cells on substrates with different stiffnesses, incubated for 24h and 48h. Data from three independent experiments, each with triplicates, are shown in scatter plots as mean values ± SD (two-way ANOVA followed by Tukey's multiple comparison test, ns ≙ not significant, ****P<0.0001). (B) PDMS substrates were coated with rhDll4 and stained for Dll4 (shown in green). rhDll4 binding efficiency was compared by evaluation of intensity and the number of particles, summarized in a scatter plot on the left panel (three independent experiments, mean ± SD, one-way ANOVA followed by Tukey's multiple comparison test, ns ≙ not significant). (C) Normalized fold mRNA expression of the Notch ligands Dll4, Jag1 and the Notch target gene Hey1 in HUVECs, outlined in a scatter plot (three independent experiments, each with triplicates, mean ± SD, one-way ANOVA followed by Tukey's multiple comparison test, ns ≙ not significant, *P<0.1, **P<0.01, ****P<0.0001). Induction of Notch activity by coating with rhDll4. Data was normalized to cells on plastic without Notch activation. (D, E) CellTiter-Blue viability assay in HUVEC cells. Cells were treated with the Notch inhibitors DAPT (D) or SAHM1 (E) at different concentrations for 24h. Cell viability is shown in a scatter plot as mean values ± SD (three independent experiments, each with triplicates). The concentrations used for Notch inhibition are indicated in bold.

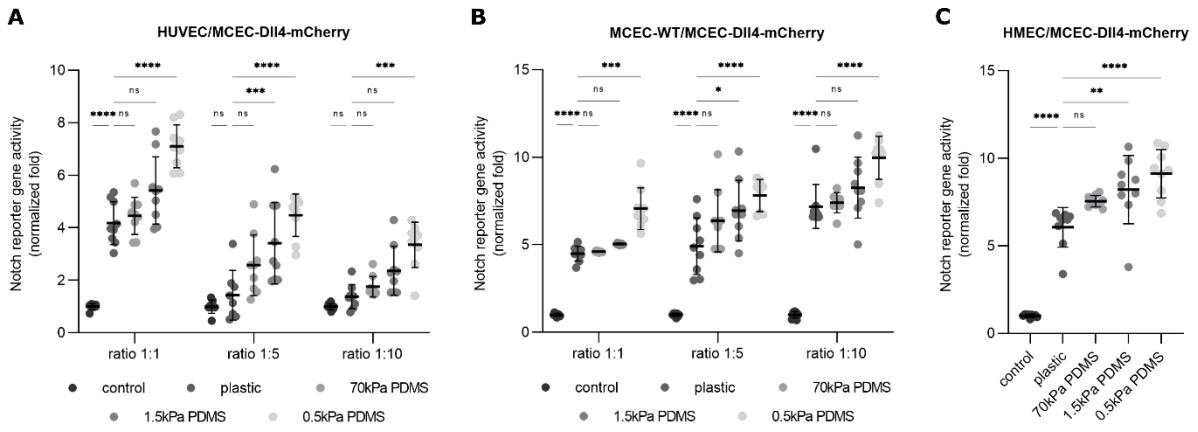

Fig. S2. Notch activation by co-culture of Notch sender and receiver cells increases in soft substrates in all Notch receiver cells but is dependent on the seeding ratio.

(A, B) Normalized fold Notch activity in endothelial co-cultures of HUVEC/MCEC-Dll4-mCherry cells (A) and MCEC-WT/MCEC-Dll4-mCherry cells (B) in seeding ratios of 1:1, 1:5 and 1:10. Scatter plots were generated by evaluation of reporter gene assays on substrates with different stiffnesses (three independent experiments, each with triplicates, two-way ANOVA followed by Tukey's multiple comparison test, ns ≙ not significant, *P<0.1, ****P<0.0001). Data was normalized to cells on plastic without Notch activation (in co-culture with untransfected HUVEC/MCEC-WT cells). (C) Normalized fold Notch activity in HMEC/MCEC-Dll4-mCherry co-culture ratio 1:1, outlined in a scatter plot (three independent experiments, each with triplicates, mean ± SD, one-way ANOVA followed by Tukey's multiple comparison test, ns ≙ not significant, **P<0.01, ****P<0.0001). Data was normalized to cells on plastic without Notch activation (in co-culture with untransfected HMEC cells).

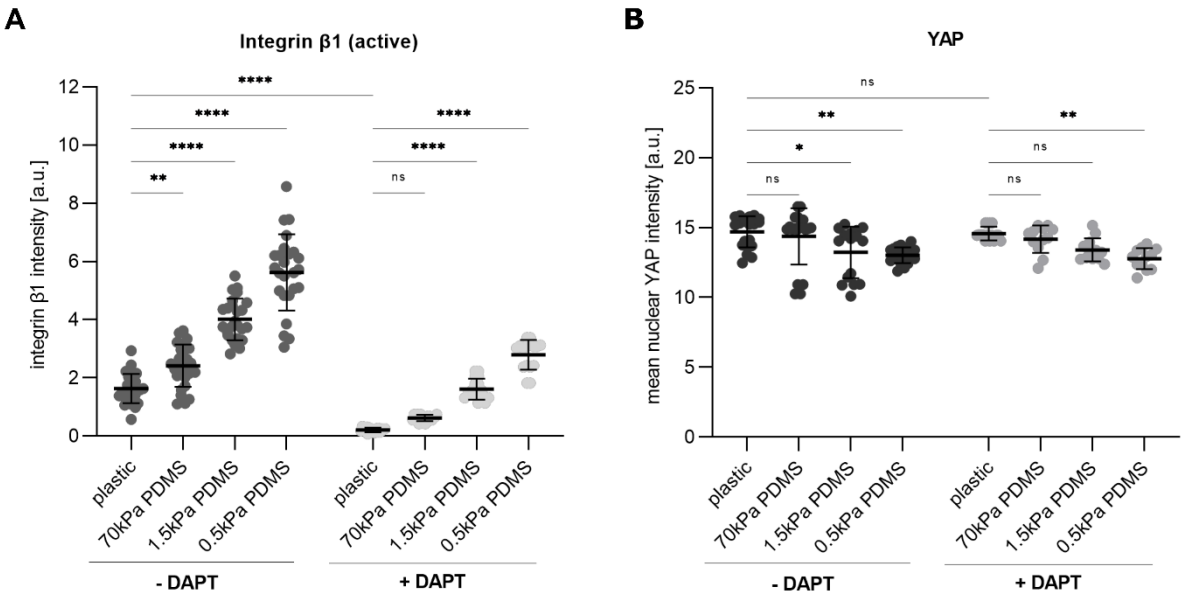

Fig. S3. Integrin β1 intensity in MCEC-WT cells is dependent on substrate stiffness as well as the Notch signaling pathway, whereas the nuclear intensity of YAP does not change after Notch inhibition by DAPT.

(A, B) MCEC-WT cells were seeded on varying substrate stiffness, treated with 25μM DAPT for 24h and stained for either the activated form of integrin β1 or YAP. The mean overall intensity for integrin β1 and the nuclear intensities for YAP ± SD of ≤ 240 untreated and treated cells derived from three independent experiments are summarized in scatter graphs (two-way ANOVA followed by Tukey's multiple comparison test, ns ≙ not significant, **P<0.01, ****P<0.0001). Integrin intensities were analyzed in segmented images. YAP intensities were analyzed with the Intensity Ratio Nuclei Cytoplasm Tool plugin for ImageJ.

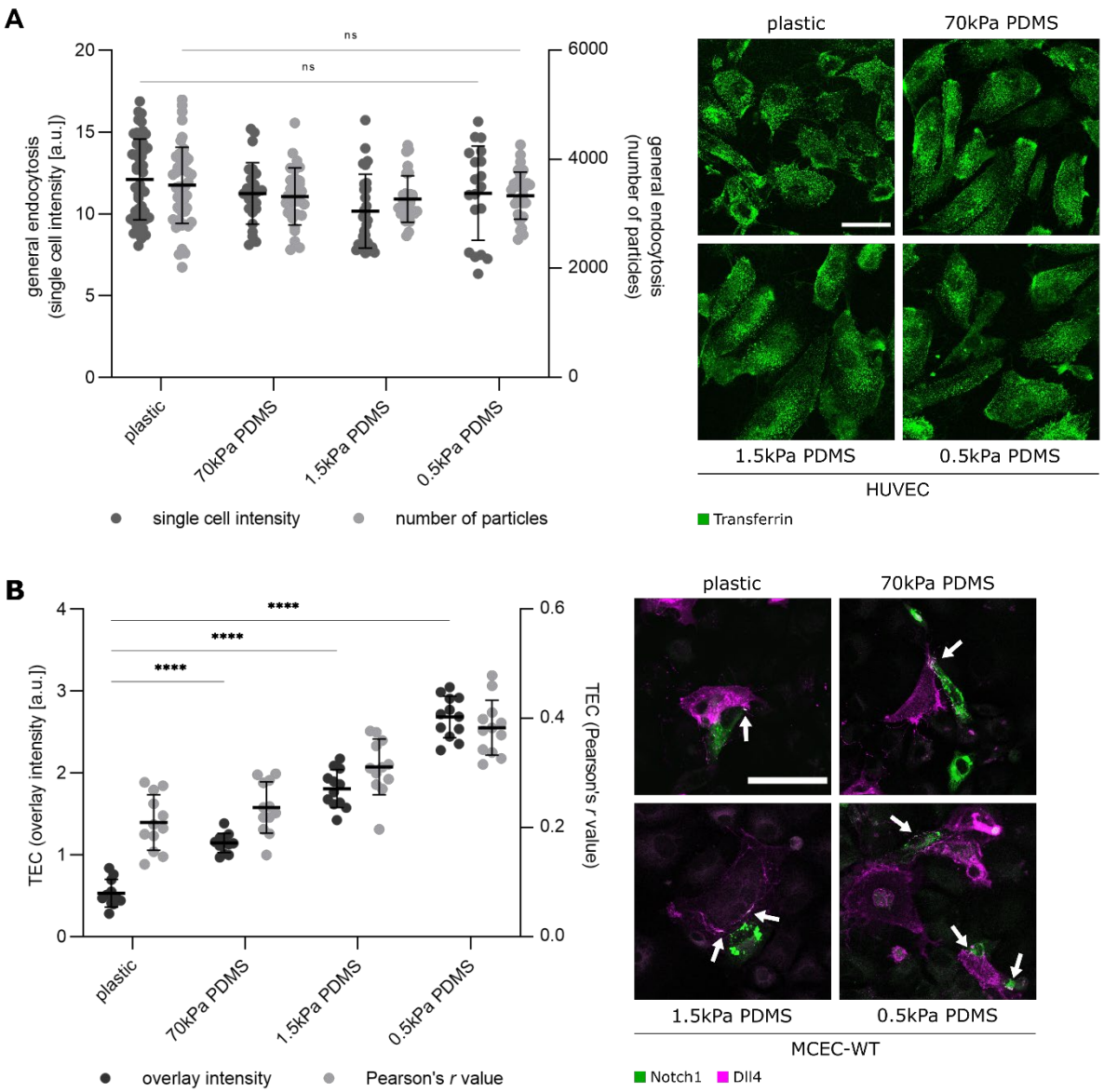

Fig. S4. General endocytosis is not affected by stiffness and trans-endocytosis in MCEC-WT cells is also increased on softer substrates.

(A) General endocytosis in HUCEC. Cells were seeded on substrates with different stiffness and a transferrin endocytosis assay was conducted. Intensity and number of particles in individual cells are presented in a scatter plot as means ± SD of ≥ 240 cells per substrate condition, derived from three independent experiments (two-way ANOVA followed by Tukey's multiple comparison test, ns ≙ not significant) Both intensity and particle number were determined in segmented images and ROIs of the cells. (B) Overlay intensity and Pearson's r value in areas of Notch receptor ligand interactions in the course of trans-endocytosis. Overlay areas are indicated by the white arrows. MCEC-WT cells were transfected separately with a citrine-coupled Notch1 plasmid and a mCherry-coupled Dll4 plasmid. Notch1 expressing Notch receiver cells are shown in green, Dll4 expressing Notch sender cells are shown in magenta. Trans-endocytosis was quantified at cell-cell contacts in ≥ 12 cells per substrate condition in three independent experiments. Data is presented as a scatter plot (mean value ± SD, two-way ANOVA with Tukey's multiple comparison test, ****P<0.0001).