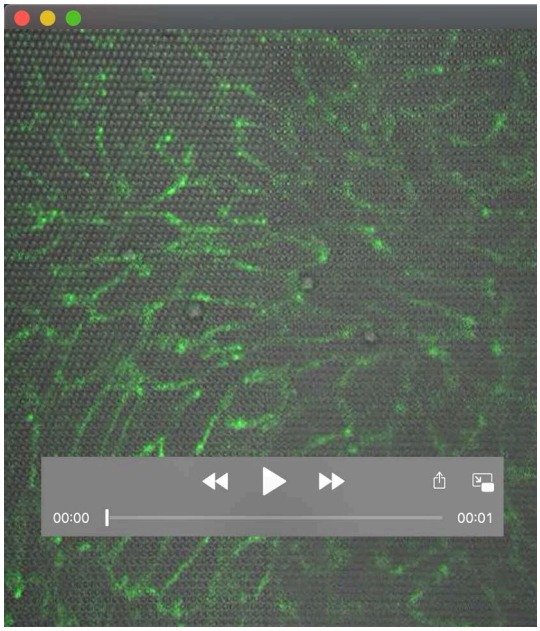
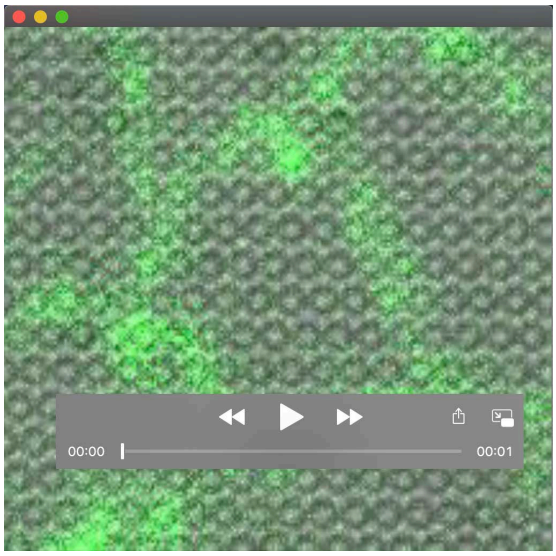
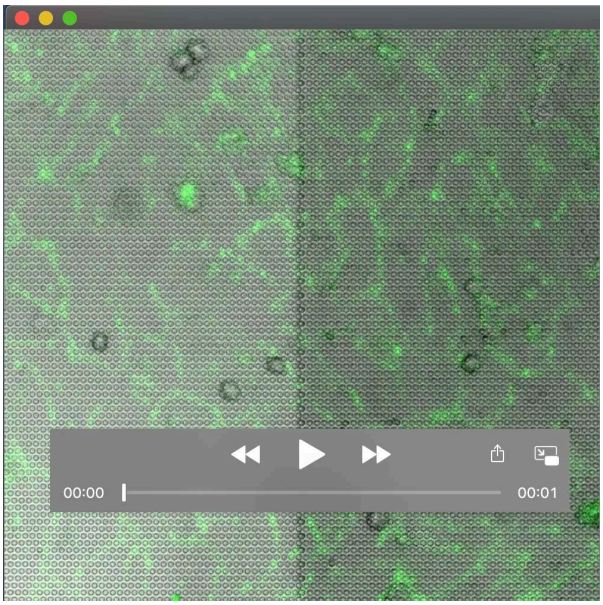


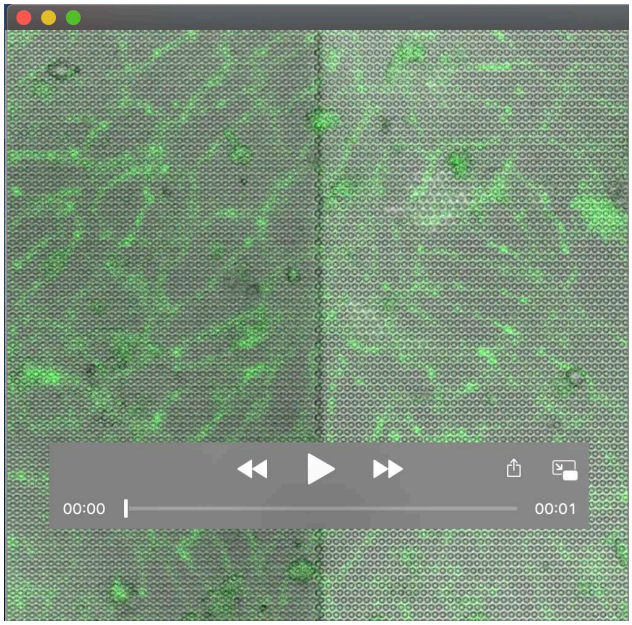
Movie 1: Neutrophil transendothelial migration. Timelapse of neutrophils transmigrating through TNF-α-stimulated human umbilical vein endothelial cell (HUVEC) monolayers on the single interface micropillar system (E_{eff} = 1.3 / 8.6 kPa), with VE-cadherin (green) and micropillars (grey). VE-cadherin was labeled by a non-function blocking antibody.



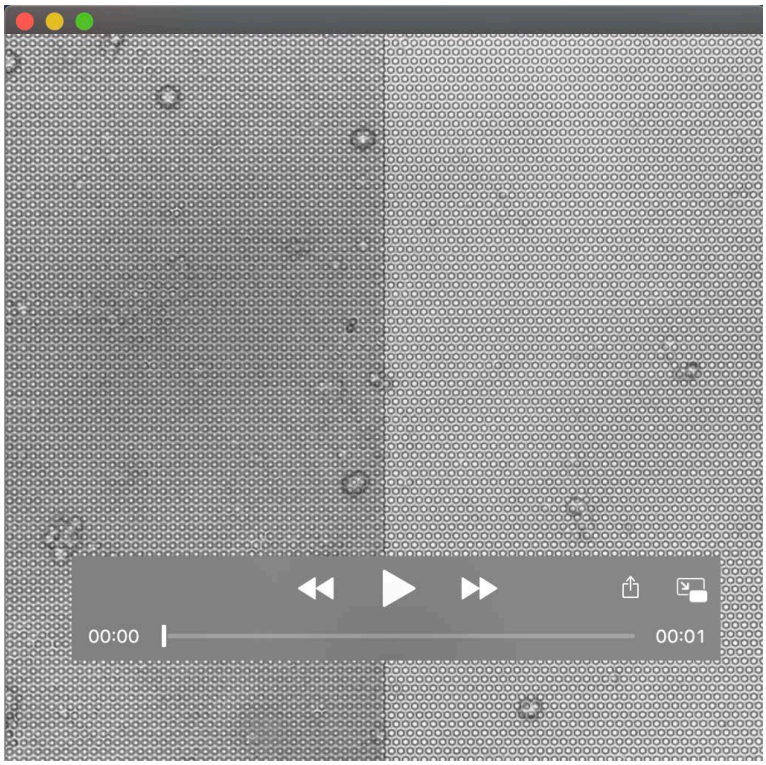
Movie 2: Region of interest during a single neutrophil transendothelial migration event, highlighting junction opening and closing. Timelapse of a single neutrophil transendothelial migration event through a TNF-α-stimulated human umbilical vein endothelial cell (HUVEC) monolayer on the single interface micropillar system (E_{eff} = 1.3 / 8.6 kPa), with VE-cadherin (green) and micropillars (grey). VE-cadherin was labeled by a non-function blocking antibody.



Movie 3: Neutrophil transendothelial migration through Rho-activated HUVEC monolayers. Timelapse of neutrophils transmigrating through TNF-α-stimulated HUVEC monolayers on interface substrates (E_{eff} = 1.3 / 8.6 kPa) that were pre-treated with Rho-activator (3 µg/mL) for 4 h prior to adding neutrophils, with VE-cadherin (green) and micropillars (grey). VE-cadherin was labeled by a non-function blocking antibody. Treatments were washed out prior to neutrophil addition.



Movie 4: Neutrophil transendothelial migration through Y-27632-treated HUVEC monolayers. Timelapse of neutrophils transmigrating through TNF-α-stimulated HUVEC monolayers on interface substrates (E_{eff} = 1.3 / 8.6 kPa) that were pre-treated with Y-27632 (10 µM) for 45 min prior to adding neutrophils, with VE-cadherin (green) and micropillars (grey). VE-cadherin was labeled by a non-function blocking antibody. Treatments were washed out prior to neutrophil addition.



Movie 5: Neutrophil transendothelial migration through blebbistatin-treated HUVEC monolayers. Timelapse of neutrophils transmigrating through TNF-α-stimulated HUVEC monolayers on interface substrates (E_{eff} = 1.3 / 8.6 kPa) that were pre-treated with blebbistatin (50 µM) for 1 h prior to adding neutrophils. Treatments were washed out prior to neutrophil addition.

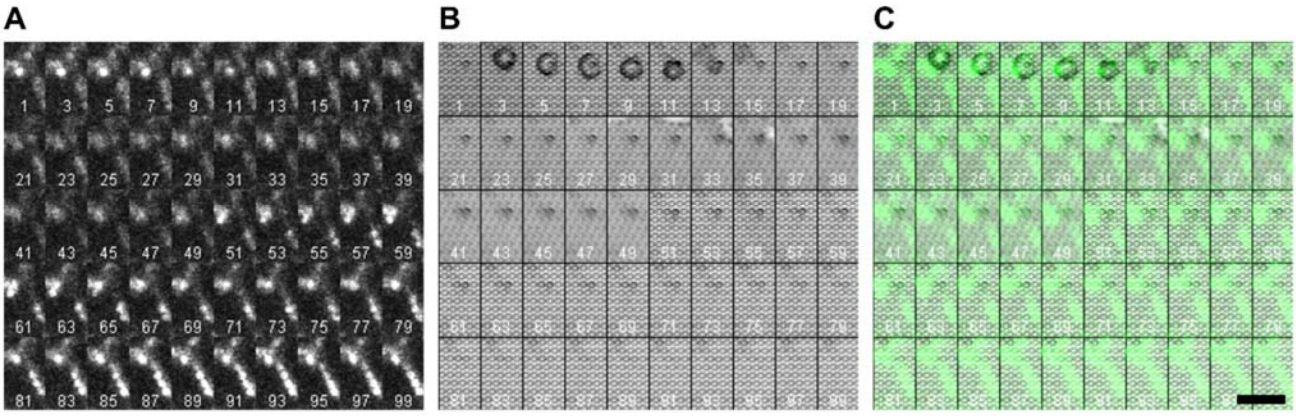


Fig. S1: Montage view of a neutrophil transendothelial migration event. (A) Montage of fluorescent images of a VE-cadherin tricellular junction during a neutrophil transendothelial migration event. VE-cadherin was labeled with a non-function-blocking antibody. (B) Montage of brightfield images depicting micropillars and a neutrophil during a neutrophil transendothelial migration event. (C) Merged montage of VE-cadherin and brightfield images. Frames were acquired every 20 s (timelapse length of 99 frames corresponds to 33 min). Scale bar: 20 µm

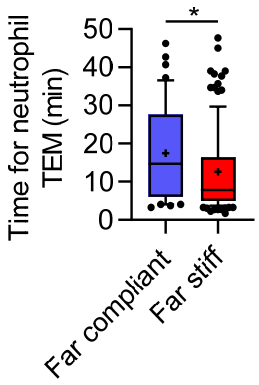


Fig. S2: Neutrophil transendothelial migration time. Far from the interface (E_{eff} = 1.3 / 8.6 kPa) in matrix stiffness (> 1 mm), neutrophil transendothelial migration events were slightly quicker to occur through monolayer regions on stiff matrix relative to compliant matrix (*n* = 159 neutrophil TEM events from 7 substrates and 8 substrates for far compliant and far stiff, respectively). Data shown as median ± interquartile range (box), 10^{th}-90^{th} percentiles (whiskers) and mean (+). Significance tested using two-tailed Mann-Whitney test. * $p < 0.05$.

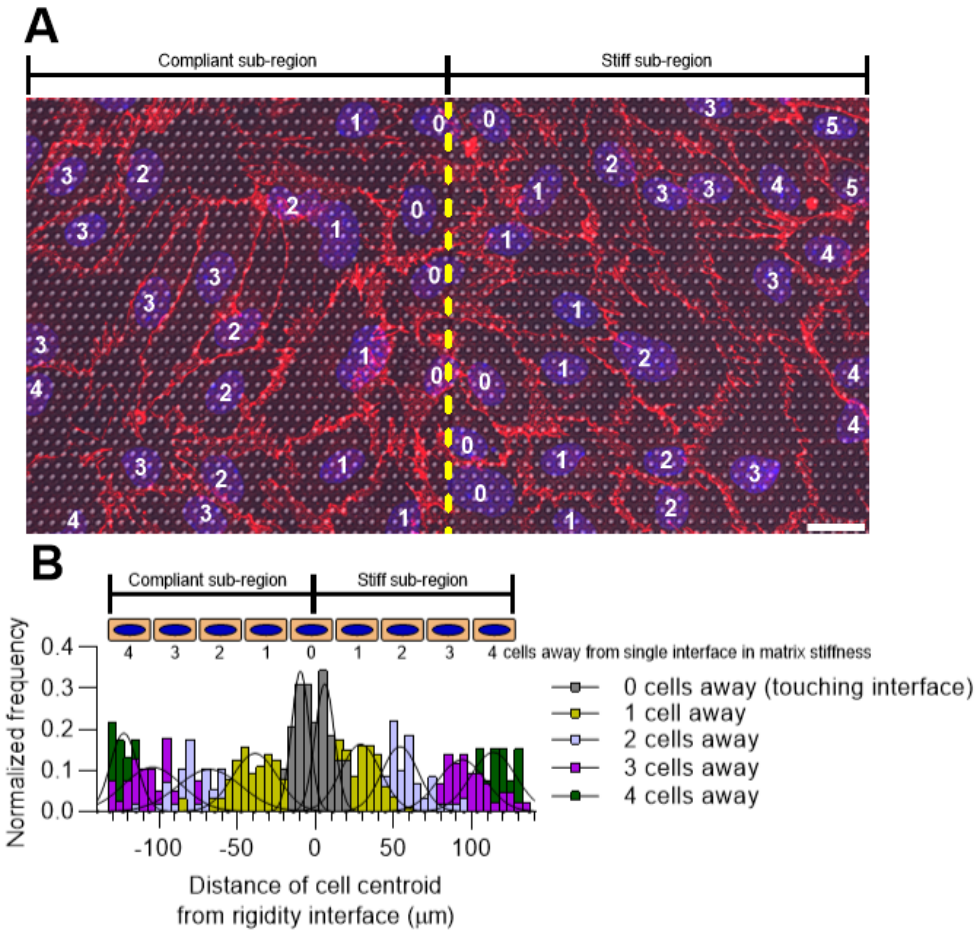


Fig. S3: Endothelial cell proximity to interface in matrix rigidity. (A) Representative image depicting the proximity of endothelial cells within a monolayer to the interface in matrix stiffness (E_{eff} = 1.3 / 8.6 kPa), with VE-cadherin (red), nuclei (blue), and micropillars (grey). The numbers overlaid depict the proximity of each cell to the interface which was defined by the closest neighbor to the interface. A proximity of zero was defined as touching the interface. The yellow dotted line highlights the interface separating the stiff and compliant sub-regions. (B) Quantification of cell proximity to the interface with respect to the distance of the cell centroid from the interface (*n* = 493 cells from 3 substrates). Each proximity value was fit to a standard Gaussian curve to produce the overlaid curve-fits.

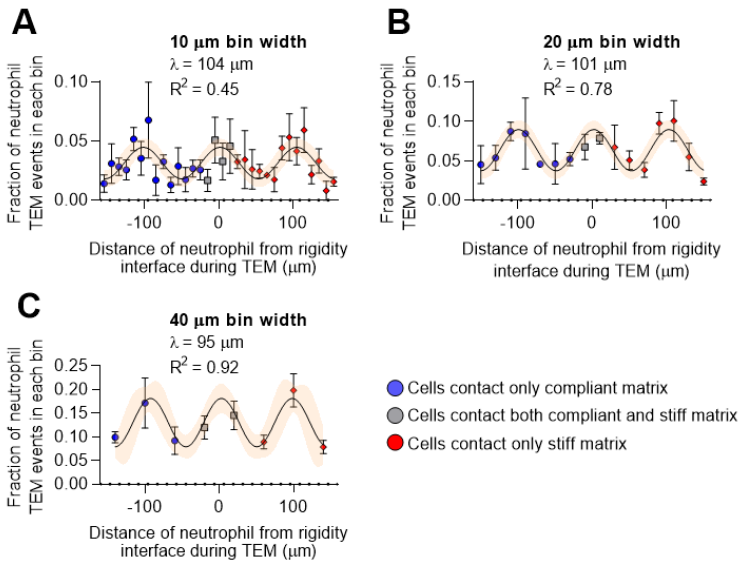


Fig. S4: Oscillatory pattern in neutrophil transmigration is not an artifact of bin size. Neutrophil transendothelial migration (TEM) fractions were binned by distance from the interface in matrix rigidity (E_{eff} = 1.3 / 8.6 kPa). Despite varying bin size from (A) 10 µm, (B) 20 µm, and (C) 40 µm, an oscillatory pattern in transmigration frequencies was observed (n = 225 neutrophil TEM events from 14 substrates). Shaded region of curve-fits represents 95% confidence intervals of the curve-fit. Data shown as mean ± S.E.M.

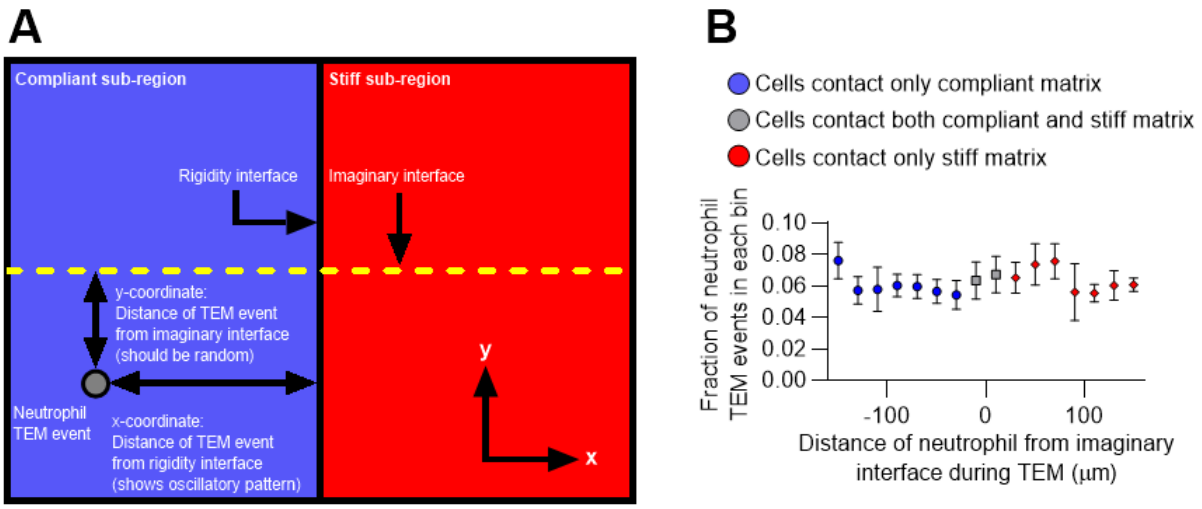


Fig. S5: Oscillatory pattern in neutrophil transmigration does not appear in a random parameter. (A) Schematic depicting how neutrophil transendothelial migration (TEM) events are catalogued. An oscillatory pattern in neutrophil TEM frequencies is observed when binning by the distance of the neutrophil from the rigidity interface (x-coordinate). However, the y-coordinate of neutrophil TEM events should be randomly distributed. (B) Quantification of fraction of neutrophil TEM events binned by the y-coordinate, which represents the distance of the neutrophil from an imaginary interface (n = 787 neutrophil TEM events from 40 substrates). Data shown as mean ± S.E.M.

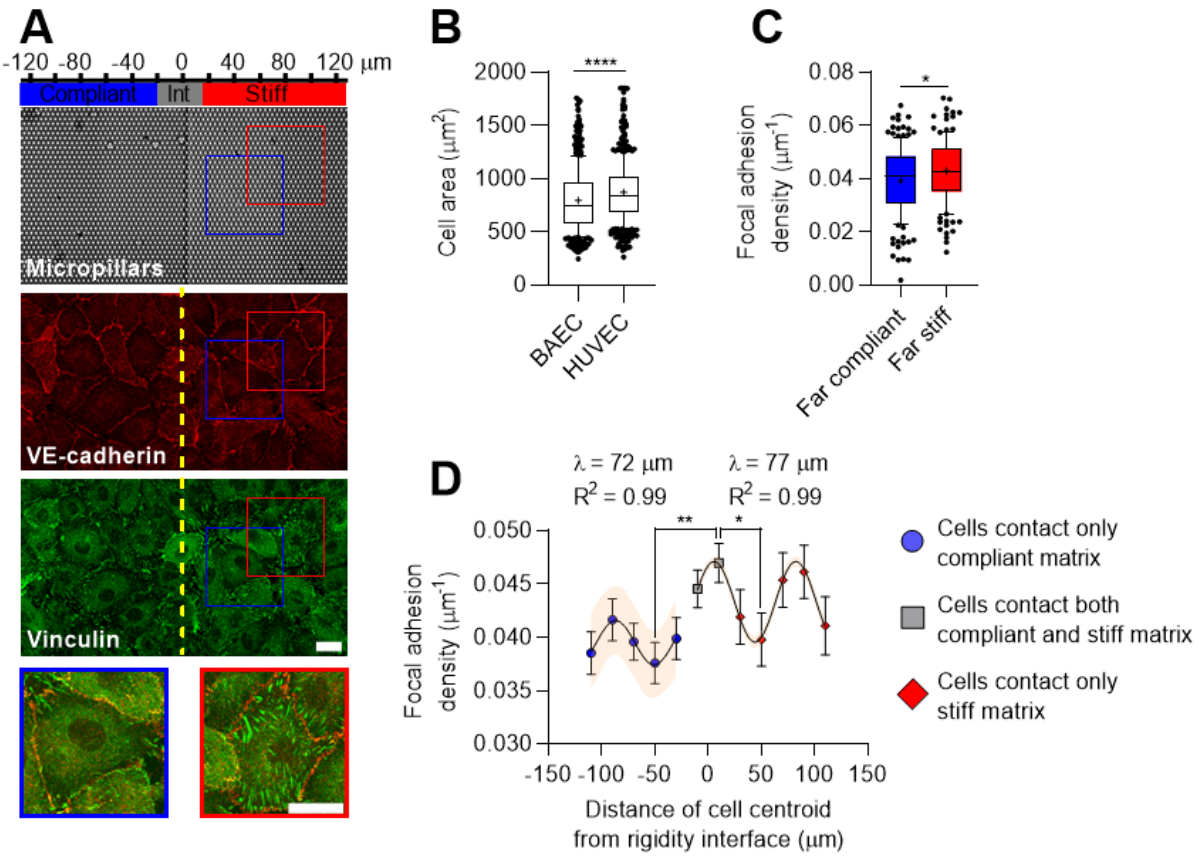


Fig. S6: Focal adhesion density also displays oscillatory pattern within bovine aortic endothelial cell (BAEC) monolayers nearby interface in matrix rigidity. (A) Representative images of BAEC monolayers immunostained for VE-cadherin (red) and vinculin (green) with micropillars (grey). (B) Quantification of BAEC and HUVEC area (n = 404 cells from 3 substrates for BAEC and 461 cells from 4 substrates for HUVEC). (C) Quantification of focal adhesion density within cells in the monolayer far (> 1mm) from the interface in matrix stiffness (n = 157 cells from 3 substrates for far compliant and 130 cells from 3 substrates for far stiff). (D) Quantification of focal adhesion density binned by distance of cell centroid from the interface in matrix rigidity (n = 389 cells from 3 substrates). Data shown as median ± interquartile range (box), 10th–90th percentiles (whiskers), and mean (+) (B, C) or mean ± S.E.M. (D). Significance tested using two-tailed student's t-test (C), one-way ANOVA followed by Holm-Sidak's multiple comparisons test (D), or two-tailed Mann-Whitney testing (B). Shaded region of curve-fits represents 95% confidence intervals of the curve-fit. * $p < 0.05$, ** $p < 0.01$. Scale bars: 20 µm

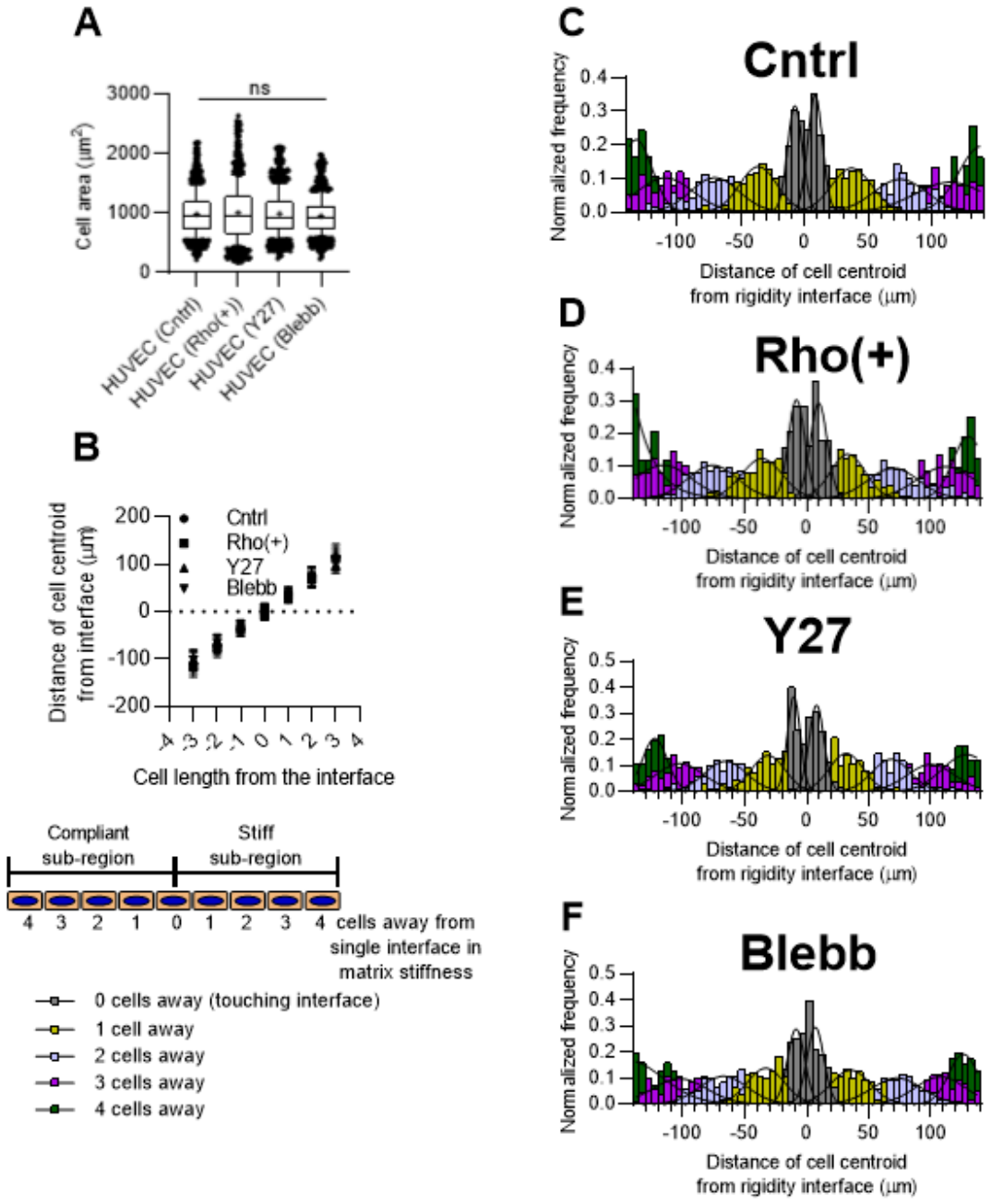


Fig. S7: Pharmacological modulation of contractility does not alter HUVEC cell area or cell positioning within the monolayer. (A) Quantification of HUVEC area in response to contractility treatments (n = 925, 901, 897, 983 cells from 3 substrates for Cntrl, Rho(+) , Y27, and Blebb, respectively). (B) Quantification of cell length and cell centroid distance from the rigidity interface. Cells touching the interface are assigned a proximity (# of cells away) of zero and proximity increments by the number of neighboring cells (see Fig. S2 for visual depiction). Parameters are extracted from Gaussian fits from histograms depicted in (C-F). (C-F) Histogram depiction of cell length and cell centroid distance from the rigidity interface in response to (C) control, (D) Rho-activator, (E) Y-27632, and (F)

Blebbistatin. Each proximity value was fit to a standard Gaussian curve to produce the overlaid curve-fits. Data shown as median ± interquartile range (box), 10th–90th percentiles (whiskers), and mean (+) (A) or mean ± S.E.M. (B). Significance tested using Kruskis-Wallis one-way ANOVA with Dunn's post-hoc testing (A).