Supplemental Figure Legends

Figure S1. VE-Cadherin Adhesions Trigger Cell Stiffening in Proportion to Locally Applied Force. (A) MTC experiments were performed with VE-cadherin-Fc coated beads bound to ECs. Where indicated, cells were pretreated with cytochalasin D (4 μM, 20 min). The magnetic field was increased stepwise (10 s intervals from 0.3 - 75 Gauss, equivalent to a shear stress of 0.036 - 9 Pa), with no pause between successive increases in the field, and hence in the applied bond shear ($n > 300$ different bead-cell pairs/condition from four independent experiments, $^*p < 0.001$). **(B)** Hysteresis does not contribute to force-induced increases in VE-cadherin junction stiffness. The magnetic field strength was first increased stepwise and then decreased back to the initial field strength, with no pause between successive increases in the field. For clarity, successive increases (black) and decreases (gray) in the field strength are presented as separate curves. ECs were bound with VE-cadherin-Fc coated beads and treated with cytochalasin D (cyto D) or not treated (no Tx) ($n > 300$ different bead-cell pairs from four independent experiments, $^*p < 0.001$).

Figure S2. Force-Actuated Protein Accumulation at Local VE-Cadherin Junctions Requires An Organized, Contractile Actomyosin Cytoskeleton. Immunofluorescenc images indicate the VE-cadherin (green), vinculin (purple), and F-actin (red) in regions of interest surrounding VE-cadherin-Fc coated beads at the apical EC surface, before (top) and after (bottom) applying bond shear. **(A)** EC monolayers were treated with 4 μM cytochalasin D for 20 min, **(B)** 100 μM blebbistatin for 20 min, **(C)** 20 μM nocodazole for 30 min, **(D)** 10 μM Y-27632 for 1 h, or **(E)** 30 μM LY294002 for 20 min. All beads were coated with VE-cadherin-Fc. Images represent > 30 bead-cell pairs/condition from > two independent experiments. Scale bars represent 5 μm.

Figure S3. VE-Cadherin Mechanotransduction Alters the Distribution and Number of Focal Adhesions. (A) ECs laden with VE-cadherin-Fc coated beads, were subject to 2.4 Pa of shear stress for 2 min. ECs were stained with anti-paxillin antibody (top left) to display focal adhesions. The figures display representative images and corresponding graphs of extracted focal adhesions for analysis ($n > 8$ images/condition from two independent experiments; scale bar represents 20 μm, $^*p < 0.001$). Background-subtracted images displaying ‘extracted focal

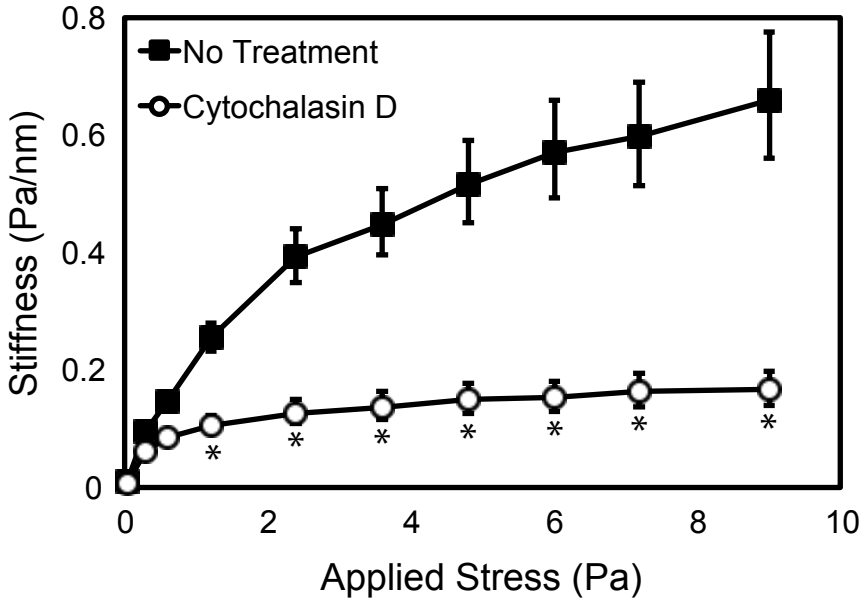
adhesions' (top right) were created by setting an intensity threshold, and the focal adhesions were then analyzed using a Matlab algorithm provided by Prof. Brenton D. Hoffman at Duke University (based on (Zamir et al., 1999)) and the "analyze particles" function in ImageJ. The bar graphs (bottom) show the normalized focal adhesion sizes (bottom left) and focal adhesion numbers (right), before and after bond shear. Values were normalized to the average values, determined before applying shear. **(B)** Quantitative analysis of focal adhesions in ECs laden with blocking anti-VE-cadherin antibody-coated beads. The bar graphs show the normalized focal adhesion size (left) and focal adhesion number (right), before and after bond shear. Values were normalized by the averages determined before applying shear. The error bars indicate the standard error of the mean.

Figure S4. Epitope Specific PECAM-1 and VE-Cadherin Cell Stiffening Disrupts Endothelial Monolayers. (A) Bar graphs of quantified gap areas between ECs in monolayers of cells laden with anti-PECAM-1.3 antibody coated beads, after 2min of applied bond shear ($n > 8$ images/condition from three independent experiments). The bars show the mean ± SEM, and $*p < 0.05$. **(B)** Blocking anti-PECAM-1 antibody-coated beads do not disrupt endothelial monolayers within 2min of applied shear. Representative confocal immunofluorescence images at the basal plane show adherens junction and cell morphology (VE-cadherin, green), focal adhesions (vinculin, purple), and F-actin (red) organization. Here $n > 12$ images/condition from three independent experiments. Scale bars are 5 μm.

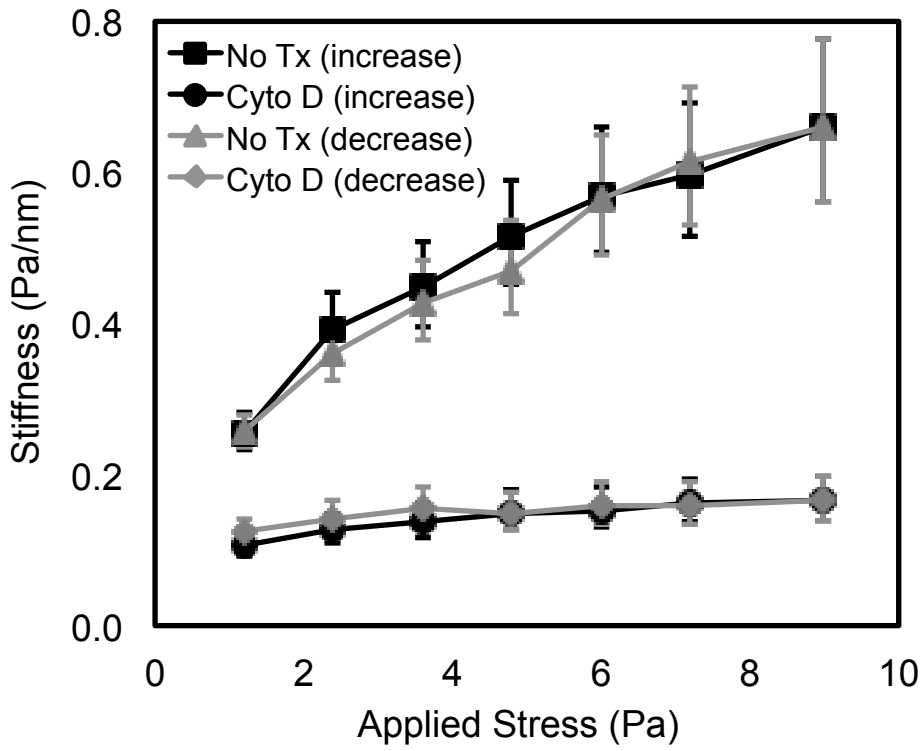
SUPPLEMENTAL REFERENCES

Zamir, E., B.Z. Katz, S. Aota, K.M. Yamada, B. Geiger, and Z. Kam (1999). Molecular diversity of cell-matrix adhesions. *J Cell Sci*. 112 (Pt 11):1655-1669.

A


No Treatment
Cytochalasin D
Stiffness (Pa/nm)
Applied Stress (Pa)
0
0.2
0.4
0.6
0.8
0
2
4
6
8
10
*

B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Cyto D (increase)
No Tx (decrease)
Cyto D (decrease)
Stiffness (Pa/nm)
Applied Stress (Pa)
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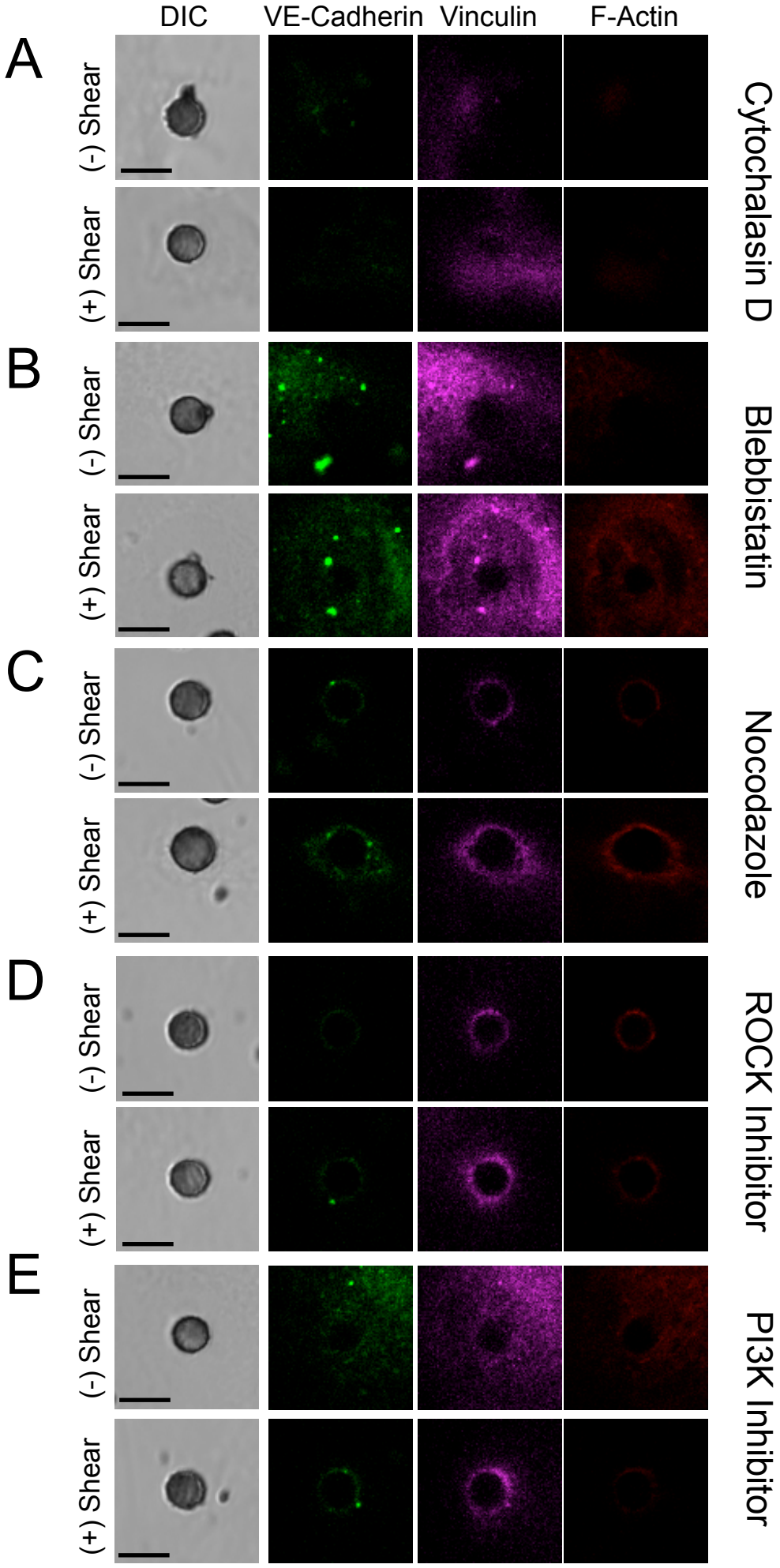
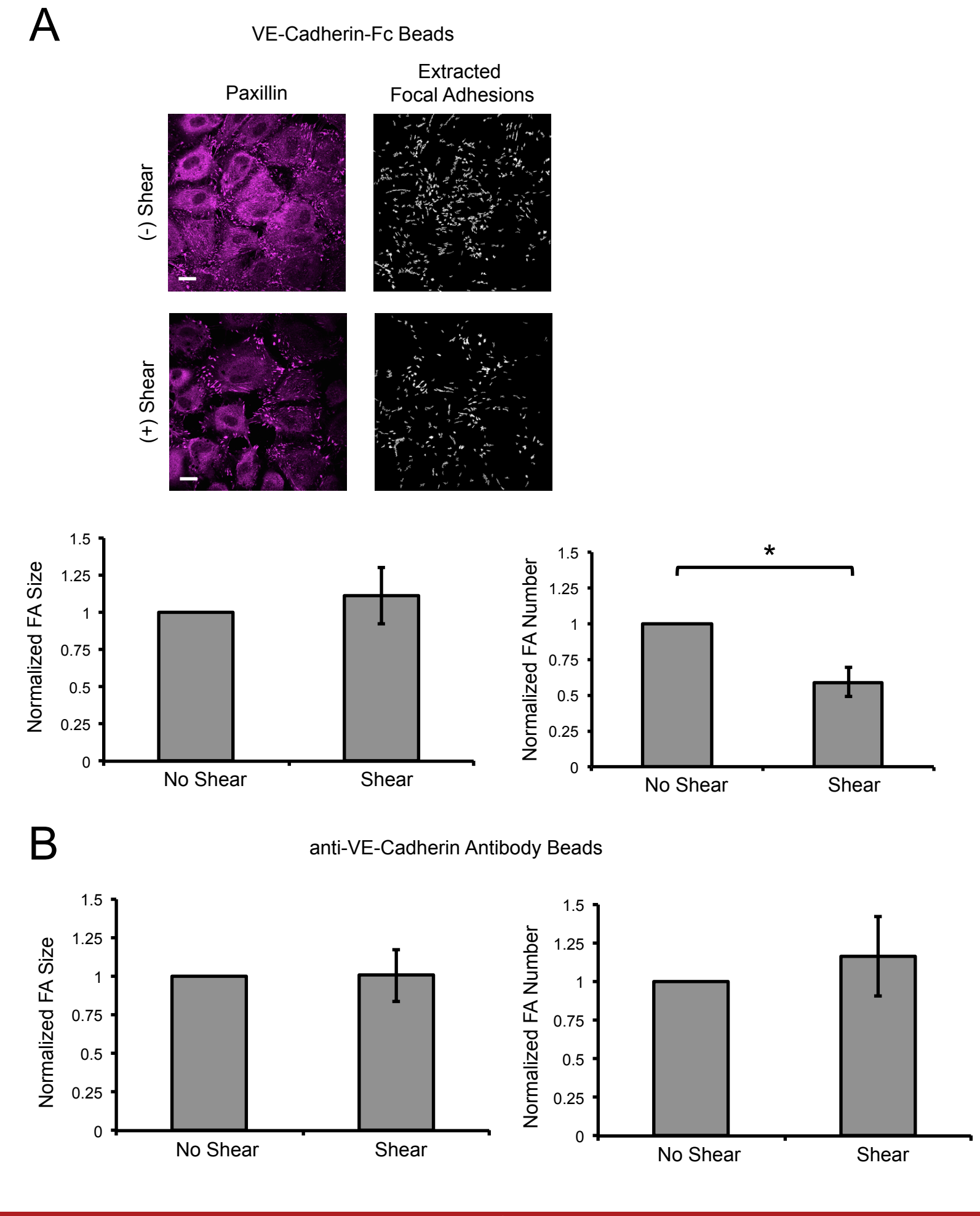
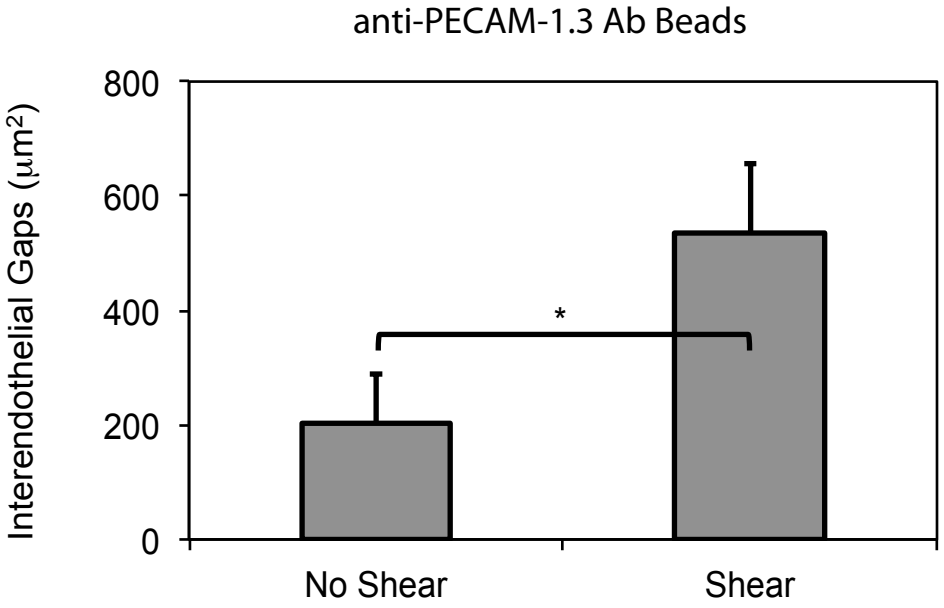
0.2
0.4
0.6
0.8
0
2
4
6
8
10

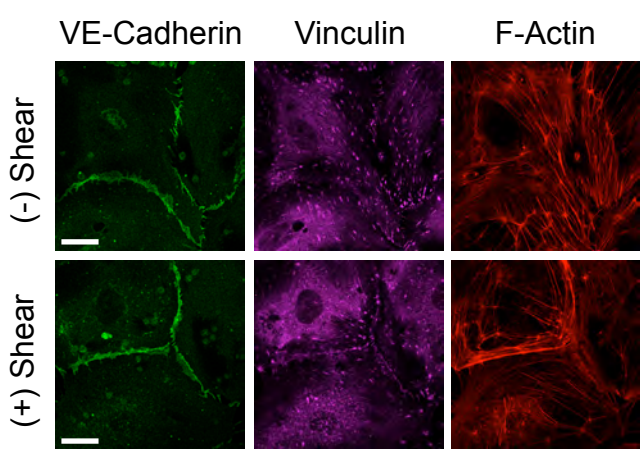

DIC
VE-Cadherin
Vinculin
F-Actin
A
(-) Shear
(+) Shear
Cytochalasin D
B
(-) Shear
(+) Shear
Blebbistatin
C
(-) Shear
(+) Shear
Nocodazole
D
(-) Shear
(+) Shear
ROCK Inhibitor
E
(-) Shear
(+) Shear
PI3K Inhibitor


A
VE-Cadherin-Fc Beads
Paxillin
Extracted
Focal Adhesions
(-) Shear
(+) Shear
Normalized FA Size
1.5
1.25
1
0.75
0.5
0.25
0
No Shear
Shear
Normalized FA Number
*
B
anti-VE-Cadherin Antibody Beads

A


anti-PECAM-1.3 Ab Beads
Interendothelial Gaps (μm²)
800
600
400
200
0
*
No Shear
Shear

B


anti-PECAM-1 Ab Beads
VE-Cadherin
Vinculin
F-Actin
(-) Shear
(+) Shear