Supporting Information

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SI Text

Preparation of Substrates. Elastomeric microneedle array substrates were fabricated via polydimethylsiloxane (PDMS; Sylgard 184, Dow-Corning, Midland, MI) based replica-molding and patterned with fibronectin by micro-contact printing as described previously (1). Subsequently, microneedles were fluorescently labeled with 5 μg/ml Δ^9-DiI (1,1′-dioleyl-3,3,3′,3′- tetramethylindocarbocyanine methanesulfonate; Invitrogen, Carlsbad, CA). Cell adhesion was restricted to the bowties by blocking the unprinted surface with 0.1% Pluronics F127 (BASF, Mount Olive, NJ). Glass coverslips that had been coated with a thin layer of PDMS were similarly patterned with bowtie-shaped fibronectin islands. Bare glass coverslips were uniformly coated with fibronectin by adsorbing 5 μg/ml fibronectin (BD Biosciences, San Jose, CA) for 1 hour.

Cell Culture. Human pulmonary artery endothelial cells (HPAECs, Lonza, Basel, Switzerland) were cultured in EGM-2 complete medium (Lonza) supplemented with 10% fetal bovine serum (Sigma, St. Louis). Cells were seeded onto substrates in normal serum-containing media and allowed to spread overnight before fixation or further treatment. For monolayer experiments, HPAECs were grown to confluence over 1–2 days on glass coverslips prior to fixation or additional treatment.

Reagents. Recombinant adenovirus encoding human vascular endothelial (VE)-cadherin lacking the β-catenin binding domain (VE-Δ) was prepared using the AdEasy XL system (Stratagene, La Jolla, CA) as previously described (2). Recombinant adenoviruses encoding RacV12 and GFP-tagged VE-cadherin were generous gifts of Drs. Anne Ridley and Sunil Shaw, respectively. To induce expression of VE-Δ or RacV12, HPAECs were infected with virus for 6 hours prior to seeding onto substrates. To express GFP-tagged VE-cadherin, HPAECs were infected with virus for 5 days prior to seeding onto substrates (see Live-cell imaging section). siRNA duplexes for myosin IIA, IIB, and cyclophilin were purchased from MWG Operon, based on previously validated sequences (3). HPAECs were transfected with siRNA duplexes (100 nM) using Lipofectamine RNAiMAX (Invitrogen, Carlsbad, CA). For experiments involving thrombin or S1P, cells were starved in 1% serum for 8 ∼ 10 hours, and then stimulated with thrombin (0.1 μM, 10 units/ml, Enzyme Research Lab, South Bend, IN) or S1P (1 μM, Sigma, St. Louis, MO) for 10 minutes prior to fixation. To disrupt cytoskeletal tension, cells were exposed to blebbistatin (30 μM, Tocris, Ellisville, MO) for 2 hours, or Y-27632 (25 μM, EMD Biosciences, San Diego, CA) for 1 hour. To increase cytoskeletal tension, cells were treated with nocodazole (1 μM, Sigma, St. Louis, MO) for 10 minutes, Calyculin-A (1 nM, Cell Signaling Technology, Danvers, MA) for 10 minutes, or RhoA-Q63L (see Live-cell imaging section; Cytoskeleton, Denver, CO). For inhibition of Rac1, cells were treated with NSC23766 (10 μM, Calbiochem, San Diego, CA) for 1 hour.

Generation of Lentiviral Myosin Light Chain. The cDNA encoding human myosin light chain 2 (MGC clone 3505) was purchased from ATCC (Manassas, VA). This cDNA was subcloned into the lentiviral plasmid, pRRL, using 5′ EcoRI and 3′ XhoI sites. Subsequently, an IRES-GFP cassette was added 3′ to the MLC2 cDNA (5′ XhoI and 3′ NheI sites). The phosphomimetic mutations (T18D, S19D) were introduced using Quikchange mutagenesis kit (Stratagene, La Jolla, CA). PCR oligos were as follows, with mutated nucleotides capitalized: 5′aagcggccacagcgggccGATGAcaatgtcttcgcaatgtttgac3′ (sense) and 5′gtcaaacattgcgaagacattgTCATCggcccgctgtggccgctt3′ (antisense). Lentivirus was prepared in 293x cells using the Lenti-X system (Clontech, Mountain View, CA). HPAEC infections were done with a spinfection protocol, 1200 g for 90 minutes. Viruses were titered based on GFP expression.

Measurement of Tugging Forces. To measure the bending of the microneedles, the tips and base of DiI-labeled microneedles were visualized by epifluorescence imaging using Apotome optical sections (Zeiss Axiovert 200 M microscope, Axiocam HRM camera, Zeiss MicroImaging, Thornwood, NY). The centroids of the microneedles at both the tip and base were determined by localized thresholding using an automated Matlab program (4) (Mathworks, Natick, MA), to yield the deflected and undeflected positions, respectively. After performing image registration on the tip and base centroids, the force on each needle is computed by multiplying the deflection by the spring constant of the microneedle, which is 32 nN/μm. AJ staining was used to identify which microneedles were attached to each cell in a bowtie, and the force vectors on the two subsets of microneedles were summed to calculate the tugging force experienced by each cell.

Immunofluorescence Imaging and Analysis. Cells were fixed in 4% paraformaldehyde, blocked in goat serum, incubated with antibody against β-catenin (BD Biosciences, San Jose, CA) and then detected with fluorophore-conjugated isotype-specific anti-IgG antibodies (Invitrogen, Carlsbad, CA). Fluorescence images were acquired on a Zeiss Axiovert 200M (Zeiss MicroImaging, Thornwood, NY) and processed in Matlab to quantify junctional area. AJ staining was binarized (for both pairs of cells on microneedles and monolayers) by thresholding to eliminate the dimmest pixels (25% and 10%, respectively). This approach for AJ quantification was validated using confocal microscopy. Measurement of AJ size in epifluorescence gave comparable results to a more rigorous volume-metric analysis (see Fig. S1). For monolayers, a single layer erosion filter also was applied to remove speckles with diameters of less than two pixels. It should be noted that in endothelial cells, area is a reasonable estimate of the size of AJs given that electron microscopy analyses show that the endothelial cell–cell interface forms obliquely, nearly coplanar with the underlying matrix (5). Adherens junctions were also quantified for mean pixel intensity as follows. Total pixel intensity was determined by summing the intensity of each pixel present at a junction in the thresholded images (nonbinarized). Mean pixel intensity was calculated by dividing the total pixel intensity by the total number of pixels comprising the junction. Mean pixel intensities for AJs are reported in Fig. S1 *B–J*.

Live-Cell Imaging. HPAECs expressing GFP-tagged VE-cadherin were seeded onto bowtie-patterned substrates (either microneedle substrates or PDMS-coated coverglass) and imaged with a 60x objective on a Nikon TE2000 (Nikon Instruments Inc., Melville, NY) equipped with a temperature and CO_2-controlled cage incubator. Fluorescence images at 488 nm (GFP) and 594 nm (DiI-labeled microneedles), and phase images were collected at one-minute intervals. Bowties were monitored for at least 10 minutes after which one cell in the bowtie pair was either injected with constitutively active RhoA Q63L, or mechanically pulled by bringing a rigid fibronectin-coated microcapillary tip into contact with the cell and dragging the tip away from the junction. Cell–cell junctions were imaged for an additional 30 minutes following

microinjection or pulling. Measurements of live-cell traction and tugging forces on microneedle substrates were performed as described for fixed samples. AJ size in microinjected/micropulled bowties on flat PDMS were measured by quantitative image analysis of the GFP-VE-cadherin. Fluorescence images were processed using built-in functions in IPLab software (BD Biosciences, Rockville, MD), namely a background subtraction, followed by a medium blur function to remove pixelation. Matlab was then used to determine AJ size from the processed images.

RhoA and Rac1 Activity Assay. RhoA and Rac1 activity were assayed using the RhoG-LISA and RacG-LISA kits, respectively (Cytoskeleton). Briefly, HPAECs were grown to confluence in 60 mm dishes, and starved overnight in medium containing 0.5% FBS. Thrombin (0.1 μM) and S1P (1 μM) were added to the HPAECs monolayers for 1 minute, followed by cell lysis, and Rho GTPase activity measurement as per the manufacturer's protocol.

1. Tan JL et al. (2003) Cells lying on a bed of microneedles: An approach to isolate mechanical force. *Proc Natl Acad Sci USA* 100(4):1484–1489.
2. Nelson CM, Pirone DM, Tan JL, Chen CS (2004) Vascular endothelial-cadherin regulates cytoskeletal tension, cell spreading, and focal adhesions by stimulating RhoA. *Mol Biol Cell* 15(6):2943–2953.
3. Ivanov AI et al. (2007) A unique role for nonmuscle myosin heavy chain IIA in regulation of epithelial apical junctions. *PLoS One* 2(7):e658.
4. Lemmon CA et al. (2005) Shear force at the cell–matrix interface: enhanced analysis for microfabricated post array detectors. *Mech Chem Biosyst* 2(1):1–16.
5. Dejana E (2004) Endothelial cell–cell junctions: Happy together. *Nat Rev Mol Cell Biol* 5 (4):261–270.

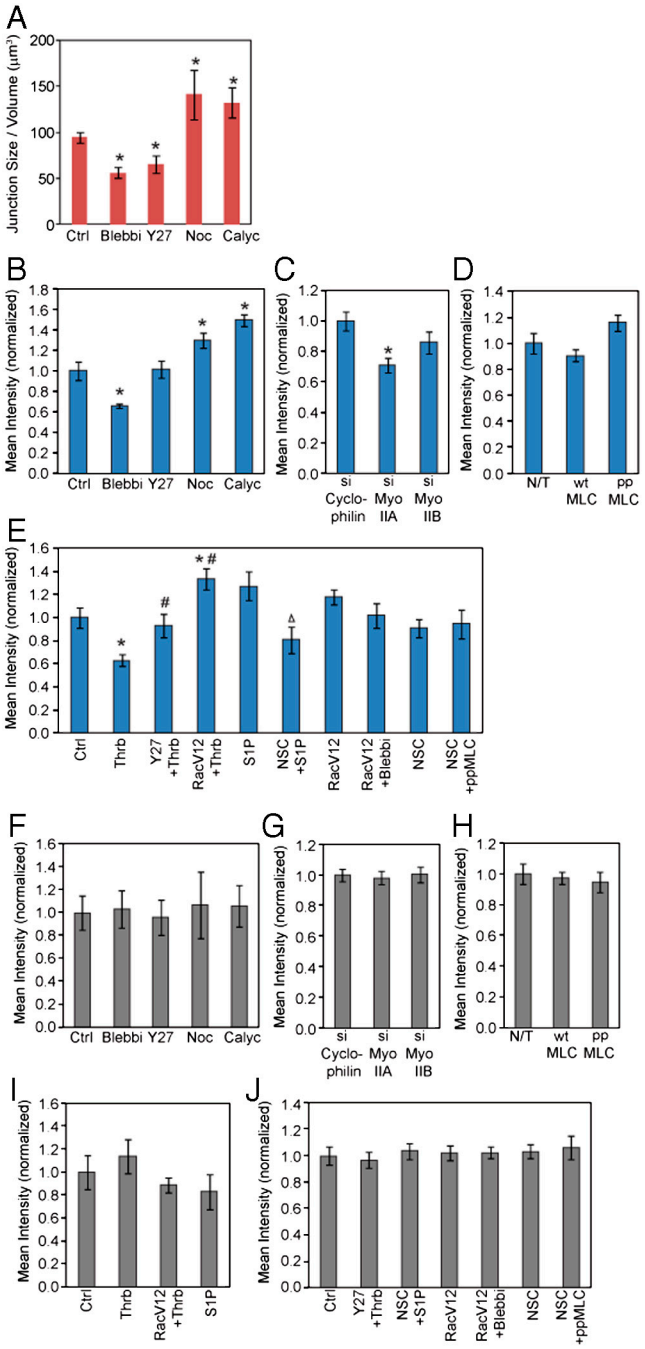
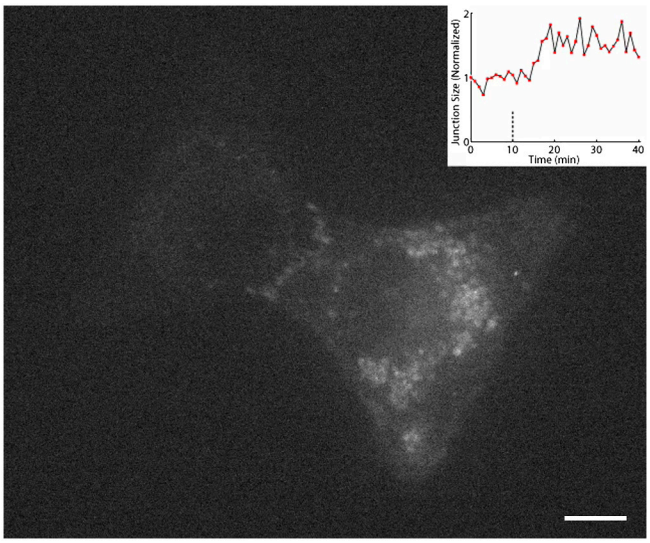
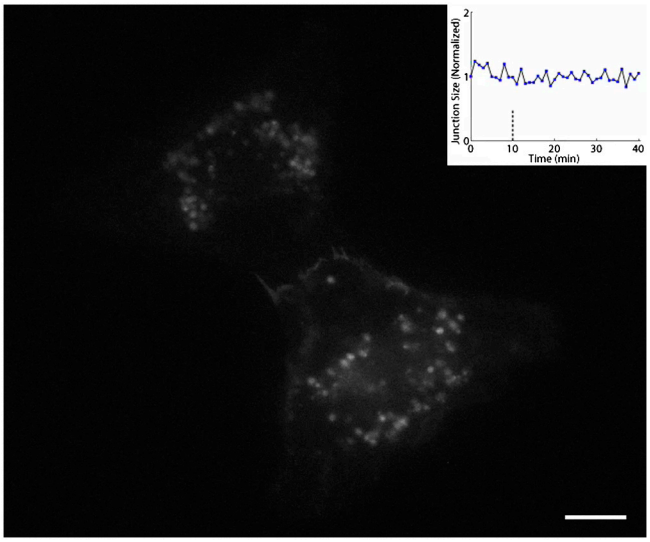


Fig. S1. Volume and intensity-based metrics of adherens junctions (*A*) Confocal imaging of bowties was done using a Zeiss LSM-510 Meta confocal microscope (Penn Biomedical Imaging Core). AJs were visualized by β-catenin staining and the volumes of AJs were analyzed with Zeiss LSM image analysis software. Bar graphs showing average junction size (volume) in bowtie pairs exposed to vehicle control (Ctrl), blebbistatin (30 μM, Blebbi), Y27632 (25 μM, Y27), nocodazole (1 μM, Noc) calyculin-A (1nM, Calyc). * $p < 0.05$, indicates comparison against vehicle control. Error bars denote standard error of the mean. (*B–E*) Mean pixel intensity of β-catenin staining in bowties-patterned cells across conditions reported in Figure 2 and 3. * $p < 0.05$, indicates comparison against the unstimulated control (*B*, *C*, *E*). # $p < 0.05$, relative to thrombin-treated cells, Δ $p < 0.05$, relative to S1P-treated cells. Error bars denote standard error of the mean. (*F–J*) Mean pixel intensity of β-catenin staining in monolayers across conditions reported in Figs. 2 and 3. Since the variation in mean pixel intensity in monolayers was too small to be of biological significance, statistical comparisons of the means were not made. Error bars denote standard deviation. Mean pixel intensities were analyzed for at least 10 images in each condition.



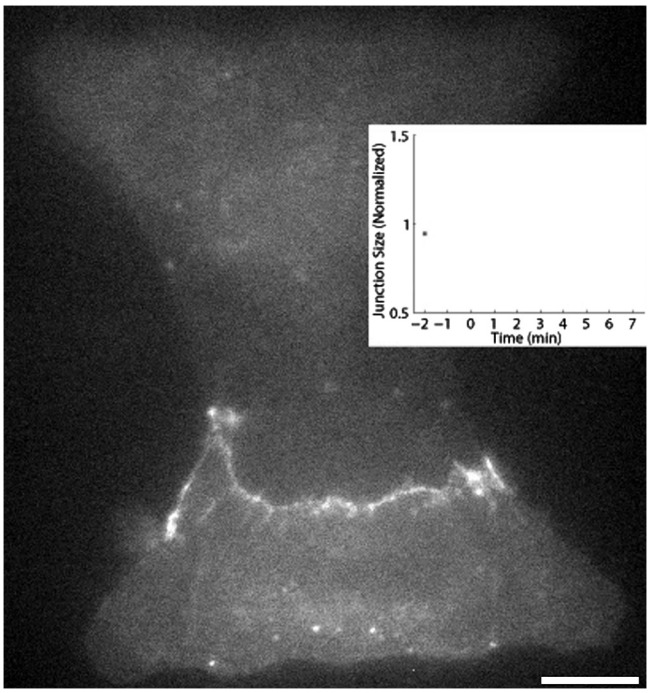
Movie S1. Activated RhoA induces immediate upregulation of F_c and VE-cadherin assembly HPAECs expressing GFP-VE-cadherin were patterned in bowties on flat PDMS and imaged at one-minute intervals to reveal adherens junction (AJ) dynamics before and after microinjection of constitutively active RhoA (RhoA-Q63L, 20 μg/mL). Inset at top right corner shows quantification of AJ size normalized to frame 1. Injection occurs at frame 10, as indicated by "Injected–>" text and dotted line on animated inset. Scale bar is 10 μm.

Movie S1 (MOV)

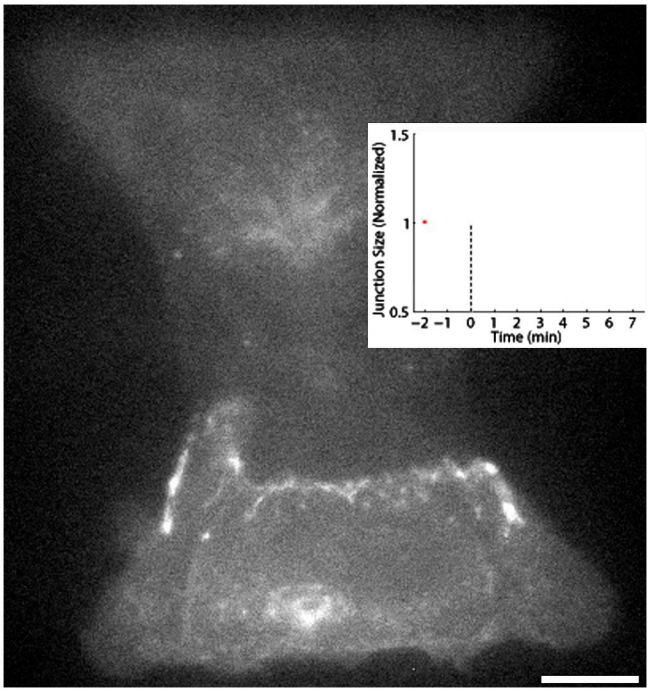


Movie S2. Activated RhoA induces AJ growth through cytoskeleton tension Fluorescent video-microscopy of AJ dynamics in bowtie-patterned HPAECs pre-treated with 25 μM Y27632. Junctional dynamics visualized and quantified by GFP-VE-cadherin localization as in Movie S1. Note that pretreatment with Y27632 completely eliminates changes in AJ size following microinjection of activated RhoA.

Movie S2 (MOV)



Movie S3. GFP-VE-cadherin dynamics in the absence of mechanical tugging. HPAECs expressing GFP-VE-cadherin were patterned in bowties on flat PDMS and imaged at one-minute intervals. Note that AJs are quite stable under these experimental conditions.
Movie S3 (MOV)



Movie S4. Exogenous pulling force stimulates VE-cadherin assembly HPAECs expressing GFP-VE-cadherin were patterned in bowties and imaged to reveal adherens junction (AJ) dynamics before and after mechanically pulling one cell of the bowtie. Inset at top right corner shows quantification of AJ size normalized to $t = 0$ min. Pulling occurs right after $t = 0$ min, as indicated by "Pull" text and dotted line and arrow on Fig 4G.
Movie S4 (MOV)

Table S1. *p*-values for statistical comparisons of tugging force and AJ area measurements

p-Values for Comparisons in Ellipses:

Fig. 2D

AJ size, *p*=	Blebbi	Y27	**F_c, *p*=**	Blebbi	Y27
Ctrl	4.45021E-09	0.000273588	Ctrl	5.59295E-05	0.004664873

Fig. 2E

AJ size, *p*=	si-MyoIIA	si-MyoIIB	**F_c, *p*=**	si-MyoIIA	si-MyoIIB
si-Cyclophilin	0.024175956	8.52762E-05	Si-cyclophilin	0.038240084	5.76525E-05

Fig. 2F

AJ size, *p*=	Noc	Calyc	**F_c, *p*=**	Noc	Calyc
Ctrl	6.93848E-06	8.98101E-07	Ctrl	6.35502E-09	2.45532E-08

Fig. 2G

AJ size, *p*=	wtMLC	ppMLC	**F_c, *p*=**	wtMLC	ppMLC
Naïve Ctrl	0.577276134	0.005020741	Naïve Ctrl	0.302437479	0.001755686
wtMLC		0.004823772	wtMLC		0.004863822

Fig. 3E

AJ size, *p*=	Thrb	S1P	**F_c, *p*=**	Thrb	S1P
Ctrl	6.60318E-05	2.55174E-06	Ctrl	1.83799E-06	0.360920818

Fig. 3I

AJ size, *p*=	Y27+Thrb	RacV12+Thrb	**F_c, *p*=**	Y27+Thrb	RacV12+Thrb
Ctrl	0.045286639	1.46416E-06	Ctrl	0.435023158	9.4955E-11
Thrb	0.086917735	6.32899E-08	Thrb	5.46316E-05	0.01788032
AJ size, *p*=	S1P	NSC+S1P	**F_c, *p*=**	S1P	NSC+S1P
Ctrl	2.55174E-06	0.762527051	Ctrl	0.360920818	0.143370237
S1P		0.000103741	S1P		0.603301025

Fig. 3L

AJ size, *p*=	RacV12	V12+Blebbi	**F_c, *p*=**	RacV12	V12+Blebbi
Ctrl	1.40523E-05	0.527471793	Ctrl	0.758601337	0.425777816
Blebbi		5.3113E-05	Blebbi		0.000791409
RacV12		0.011115842	RacV12		0.277700469

Fig. 3M

AJ size, *p*=	NSC	NSC+ppMLC	**F_c, *p*=**	NSC	NSC+ppMLC
Ctrl	0.088853066	0.657872074	Ctrl	0.598959172	0.000144062
ppMLC		0.005070349	ppMLC		0.825107507

Student's *t*-tests were applied to the mean tugging force and AJ area measurements reported as elliptical plots in Fig. 2 *D*–*G* and Fig. 3 *E*, *I*, *L*, and *M*).