SUPPORTING INFORMATION

SI Materials & Methods

Cell culture.

Both human bone marrow stromal cells (hBMSCs) (Lonza) and human lung fibroblasts (hFs) were cultured in Dulbecco's Modified Eagle Medium (low glucose DMEM; Thermo Fisher Scientific) supplemented with 10% (v/v) Fetal bovine serum (FBS; Thermo Fischer Scientific) and 1% (v/v) Penicillin Streptomycin (P/S; Thermo Fisher Scientific). To achieve labelled Green Fluorescent Protein hBMSCs (GFP-hBMSCs) cells, the hBMSCs were transduced with a lentiviral construct pCSCG-EGFP (Addgene). Human umbilical vein endothelial cells (HUVECs) (Lonza) were cultured in endothelial cell growth medium EGM-2. All experiments performed with HUVECs at passage 4 to 5, hBMSCs at passage 2 to 3, and hFs at passage 4 to 6. Human aortic smooth muscle cells (HASMCs) (Lonza) (passage 2 to 4) and human kidney pericytes (PCs) (passage 2 to 4) were cultured in smooth muscle cell media (SmGM-2 Growth Medium) (Lonza) and pericyte medium (ScienCell), respectively. Chinese hamster ovary (CHO) (Lonza) were cultured in DMEM/F12 (Thermo Fischer Scientific) supplemented with 10% (v/v) FBS and 1% (v/v) P/S. To achieve labelled CHO-N cells, the CHO were transduced with an adenoviral construct carrying N-cadherin-EGFP (1, 2). For the two-dimensional (2D) co-culture experiment, HUVECs and hBMSCs were plated on a fibronectin coated tissue culture dish (20 μg/ml) at density 30,000 cells/cm^2 for 4 h.

Microfluidic platform for vascular permeability.

Microfluidic devices were fabricated using soft lithography as described previously (3). Polydimethylsiloxane (PDMS, Sylgard 184, Dow-Corning, Midland, Michigan) devices were

treated with 0.01% (v/v) poly-L-lysine and 0.5% (v/v) glutaraldehyde to promote collagen adhesion. After washing overnight in water, steel acupuncture needles (160 μm diameter, Seirin, Kyoto, Japan) were introduced into the devices and a solution of 3 mg/ml type 1 collagen (Thermo Fisher Scientific, Waltham, MA), 1x M199 medium (Thermo Fisher Scientific), 1 mM HEPES, 0.1 M NaOH and $NaHCO_3$ (0.035% (w/v)), was infused and allowed to polymerize for 40 min at 37 °C. After needles were removed to create 160 μm diameter channels within collagen gel, a suspension of 0.5 million/ml HUVECs was introduced into devices. The cells were allowed to adhere to the top surface of the channel for 5 min and then flipped to allow cells to adhere to the bottom surface of the channel for another 5 min. The non-adherent cells were washed out with PBS and fresh media was replaced into the device. In co-culture cell experiments, devices were first seeded with hBMSCs and were incubated for 4 h at 37 °C, followed by HUVECs seeding. Media (EGM2) was immediately added thereafter. Devices were placed on a platform rocker (BenchRocker, BR2000) for 24 h before performing permeability assay.

Vascular permeability measurement.

To measure the permeability of the endothelium in the microfluidic platform, fluorescent dextran (10 kDa Cascade Blue & 70 kDa Texas Red, Thermo Fisher) was introduced into perfusion media (EGM2) at a concentration of 12.5 μg/mL. Diffusion of the dextran was imaged in real time with a confocal microscope (LSM 710, Carl Zeiss) at 10x magnification. The diffusive permeability coefficient (P_d) was calculated by measuring the flux of dextran into the collagen gel and fitting the resulting diffusion profiles to a dynamic mass conservation equation as described previously (4). Regarding the inflammation experiments, the formed microvessels were treated with LPS (100 ng/ml), thrombin (THBN) (0.3 U/ml) or TNFα (50 ng/ml) for 1 h. Similarly, the

microvessels were treated with 10 μg/ml N-cadherin blocking antibody or relative isotype control Ab (Sigma) for 1 h. Finally, for the activation of Rho GTPases in hBMSCs, the microvessels were exposed to rapalog A/C Heterodimerizer at concentration 25 nM (Clontech Laboratories #635055) and/or gibberellic acid acetoxymethyl ester (GA_3-AM) at concentration 12.5 μM (Toronto Research Chemicals #G377500). Next, 70 kDa dextran was perfused through the vessel lumens and extravasation of the dextran was measured as a function of time to quantify the diffusive permeability.

Cloning and Lentiviral vector preparation.

The CFP-GAI_{1-92} sequence in Lyn- CFP-GAI_{1-92} (Addgene) was amplified by PCR with primers: CFP-GAI-F:5'-ACAACAAGCTAGCGCTACCGGTGAATTCC-3'
and CFP-GAI-R: 5'-ACAACAAGTTAACAACAACAATTGCATTCATTTTATGT-3'. The PCR product was ligated into pCSCG (Addgene) via NheI and HpaI

The RhoA sequence in RhoA-YFP-FKBP (Addgene) was amplified by PCR with primers: RhoA-F: 5'- ACAACAAACCGGTATGGCTGCCATCCGGAAG-3'
and RhoA-R: 5'-ACAACAAACCGGTTCACAAGACAAGGCACCCAGATTT-3'. The PCR product was ligated into pCSCG (Addgene) via AgeI.

The GID1 sequence in TIAM-YFP-GID1 (Addgene) was amplified by PCR with primers:
GID1-F: 5'-ACAACAATGTACAATGGCTGCGAGCGATGAAGT-3'
and GID1-R: 5'-ACAACAAGGTACCTTAACATTCCGCGTTTACAAACGC-3. The PCR product was ligated into pCSCG-RhoA-mApple via BsrGI and KpnI.

The Rac1-YFP-FKBP sequence in Rac1-YFP-FKBP (Addgene) was digested and ligated into pCSCG (Addgene) containing multiple cloning site (MCS) vial NheI and HpaI. All PCR reactions were performed by using Phusion™ High-Fidelity DNA Polymerase according to the

manufacturer's instructions (New England Biolabs). All ligation reactions were performed based on the standard ligation protocol for T4 DNA ligase (New England Biolabs).

N-cadherin (CDH2) was knocked out by using the vector LentiCRISPRv2 (Addgene). The two complementary oligos: Forward:5'- CTTCACTGACTCCTCAGTTA -3' and reverse: 5'-TAACTGAGGAGTCAGTGAAG-3' were mixed at 1:1 ratio to a final concentration of 50 μM and annealed as followed: 37 °C for 30 min, 95 °C for 5 min, and then ramped down to 25°C at 5 °C/min. All cloning products were confirmed by sequencing from QuintaraBio.

For lentivirus production, HEK293T/17 cells were transfected with three plasmids: (15 μg each lentiviral vector, 15 μg psPAX2, and 5 μg pMD2G), using the standard calcium phosphate precipitation method. Virus was harvested 24hr post transfection, filtered through 0.45 μm filter (Millipore), pelleted by PEG-it solution (System Biosciences) and resuspended in fresh medium.

Immunostaining and Western Blot.

Devices were fixed in 4% (v/v) paraformaldehyde (PFA) for 20 min at 37 °C, washed twice in phosphate buffered saline (PBS; Thermo Fischer Scientific), permeabilized with 0.1% (v/v) Triton X 100 (Thermo Fisher Scientific) in PBS for 20 minutes at room temperature, and treated with blocking solution (0.01% (v/v) Triton X 100, 1% (w/v) bovine serum albumin (BSA; EMD Chemicals) in PBS) overnight at 4 °C. Primary antibodies (1:100 dilution, mouse anti-human CD31; Clone JC70A; Dako; rabbit anti-human laminin; Abcam; rabbit anti-human VE-cadherin; Cell Signaling; mouse anti-human JAM-A; Santa Cruz) were incubated overnight at 4 °C. Secondary antibodies (1:300, Alexa 568-conjugated goat-anti-mouse IgG, Alexa 647-conjugated goat-anti-rabbit IgG, Thermo Fisher Scientific); goat anti-GFP (FITC) antibody (1:300, FITC-conjugated, Abcam), DAPI (1:300, Sigma), and APC-anti human CD140b [Platelet Growth

Factor receptor beta (PDGFRβ)] antibody (1:100, Biolegend) were incubated overnight at 4 °C. For the two dimensional (2D) co-culture of hBMSCs and HUVECs, the cells were fixed in 4% (v/v) PFA for 5 min at room temperature, washed twice in PBS, permeabilized with 0.1% (v/v) Triton X 100 in PBS for 10 min at room temperature, and treated with blocking solution (0.01% (v/v) Triton X 100, 1% (w/v) BSA in PBS) overnight at 4 °C. Primary antibodies (1:100 dilution, mouse anti-human N-cadherin; Clone 32; BD Bioscience) were incubated overnight at 4 °C. Secondary antibodies (1:300, Alexa 568-conjugated goat-anti-mouse IgG); goat anti-GFP antibody (1:300, FITC-conjugated, Abcam), DAPI (1:300); were incubated for 1 h at room temperature. Devices and 2D coculture were imaged using confocal microscope (LSM 710, Carl Zeiss) and Leica SP5 X MP Inverted Confocal Microscope (SP5XMP), and image analysis made by Image J by performing a maximum intensity z projection and merging the channels. Using Imaris for the JAM-A staining, 3 dimensional surface was created and used as a mask for the red channel. The resulting image was further processed in Image J, by performing a maximum intensity z projection, and merging the red channel and blue (DAPI). For the images demonstrating in permeability analysis, the gray-scale images were converted into 16 color images. For GST-tagged Rhotekin-RBD and GST-tagged PAK-PBD active pulldown experiments, hBMSCs were plated at a density of 3000 cells/cm^2. Cells were lysed in radioimmunoprecipitation assay (RIPA) buffer (Thermo Scientific), protease and phosphatase inhibitor cocktails (Cell Signaling). Lysates were equalized for protein content and volume and rotated at 4 °C for 1 h with 30 μg GST–RBD or 20 μg GST-PBD (Cytoskeleton). Bead pellets were washed three times with lysis buffer and analyzed by SDS–PAGE. Polyacrylamide gels (Thermo Fischer Scientific) were transferred to nitrocellulose membranes (Biorad) and were probed with the following antibodies: rabbit anti-human RhoA (Clone 67B9) (1:1000 in 5% (w/v) BSA; Cell Signaling Technology); mouse anti-human Rac1 (Clone 102) (1:1000 in 5% (w/v) BSA; BD Bioscience); rabbit anti-human GAPDH

(Clone D16H11) (1:2000 in 5% (w/v) BSA; Cell Signaling Technology) overnight in 4 ^{o}C. Proteins of interest were detected with rabbit or mouse IgG antibody, HRP linked antibody (1:1000, Cell Signaling Technology) and visualized with the chemiluminescent substrate (Thermo Scientific), according to manufacturer instructions. All western blots were quantified by Image J according to standard procedures.

qRT-PCR analysis

Total mRNA was isolated using the RNeasy Mini Kit (Qiagen) and cDNA was synthesized using the Superscript III cDNA Synthesis Kit (Thermo Scientific). The cDNA was subjected to RT-PCR using a LightCycler® 480 SYBR Green I Master (Roche) and primers as listed in **Table 1**. The expression level of each gene was quantified using the DCT method and normalized to the expression level of the housekeeping gene RPL32.

Table 1: Primers for qRT-PCR assay

Gene	**Sense 5'-3'**
NG2	CAGACCATCAGCCGGATCTT ATCTACGGCCACGATACTGC
PDGFRβ	TCCGCTCCATCCTGCACATC ACGTAGCCGCTCTCAACCAC
Desmin	CTGGAGCGCAGAATTGAATC AGTGAGGTCTGGCTTAGACA
CD146	GGTCATCGTGGCTGTGATTG GGTAGCGTGATCTCCTGCTT
RGS5	CTGGATTGCCTGTGAGGATT AAGGTTCCACCAGGTTCTTC
RPL32	AGCGTAACTGGCGGAAAC CGTTGTGGACCAGGAACTTC

In Vivo experiments.

All animal procedures were performed at the Charles River campus animal Facility, Boston University, under a protocol approved by the institutional IACUC. All experiments pertaining to this investigation conformed to the "Guide for the Care and Use of Laboratory Animals," published by the US National Institutes of Health (Eighth Edition, 2011). Seven to ten weeks male C57Black6 mice (Taconic) were anesthetized with 1–3% (v/v) isoflurane at flow rate of 1 l/ min and body temperature was maintained on a circulating heated water pad. A subcutaneous injection of 2.5 µg of LPS (50 µl solution) was performed in the right corner of the abdominal epigastric region. Thirty minutes after injection, mice were sacrificed and a skin biopsy was dissected and fixed in PFA 4% (v/v) for 1 h. The samples were permeabilized in 0.1% (v/v) Triton X 100 in PBS for 1 h at room temperature, and blocked in 0.01% (v/v) Triton X 100, 1% (w/v) BSA overnight at 4 °C. Staining with rabbit polyclonal anti-mouse α-SMA (1:100, Abcam) was incubated overnight at 4 °C, followed by secondary antibody (1:300, Alexa 488-conjugated goat-anti-Rb IgG); DyLight 649 GSL I-B4 (IB4) (1:200, Vector Labs) and DAPI (1:300). Imaging by confocal microscope (LSM 710, Carl Zeiss), image analysis performed by Image J.

Statistical analysis.

Statistical analysis of the data was performed using ANOVA one-way. P value set to be significant if < 0.05 unless differently stated in the text.

References

1. 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.
2. Gao L, McBeath R, & Chen CS (2010) Stem cell shape regulates a chondrogenic versus myogenic fate through Rac1 and N-cadherin. *Stem Cells* 28(3):564-572.

3. Nguyen DH, *et al.* (2013) Biomimetic model to reconstitute angiogenic sprouting morphogenesis in vitro. *Proc Natl Acad Sci U S A* 110(17):6712-6717.
4. Adamson RH, Lenz JF, & Curry FE (1994) Quantitative laser scanning confocal microscopy on single capillaries: permeability measurement. *Microcirculation* 1(4):251-265.

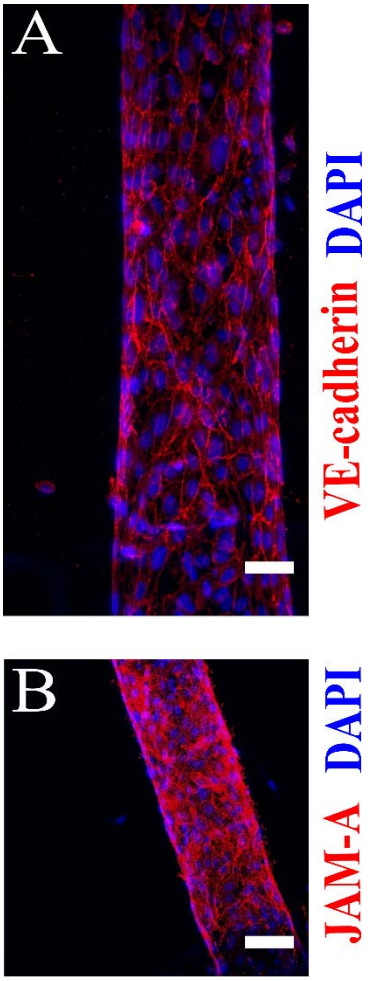

Fig.S1: ***Endothelial cell-cell interaction in microvessels.*** Representative images of engineered microvessels; (A) HUVECs were stained for adherens junctions, VE-cadherin (red) and nuclei with DAPI (blue) (B) HUVECs were stained for JAM-A (red), a marker of tight junctions, and nuclei with DAPI (blue). (Scale bar, 50 μm.)

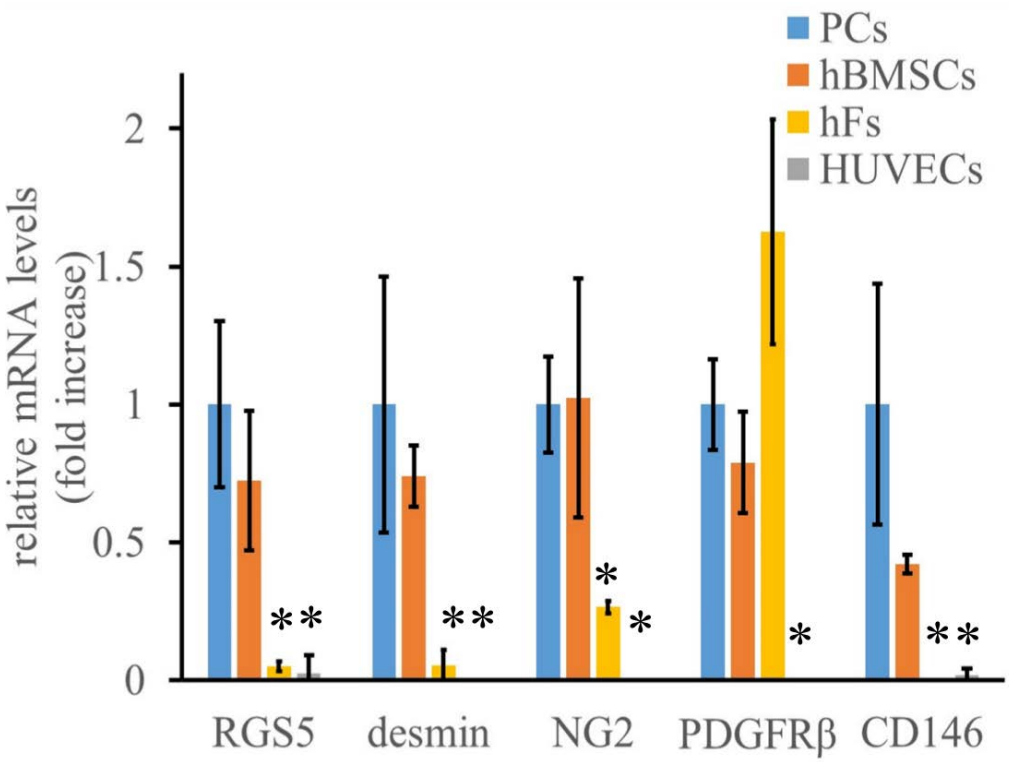

Fig.S2: ***Quantification of mural cell marker genes*****.** RT-PCR for RGS5, desmin, NG2, PDGFRβ, and CD146 in HUVECs, hFs, hBMSCs and PCs. RPL32 served as housekeeping gene. Data are expressed as mean ±SEM. $N = 3$. *, P value < 0.05.

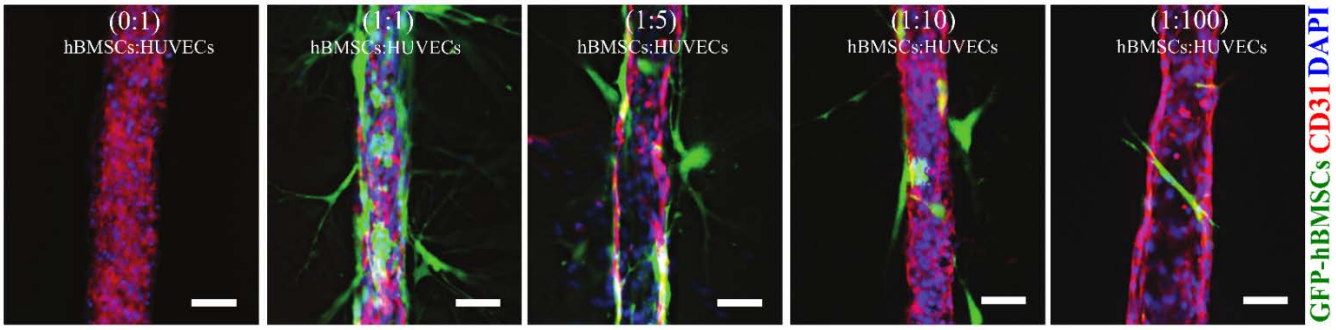

Fig.S3: ***Bi-cellular microvessel comprised of different ratios of hBMSCs:HUVECs.*** Representative immunofluorescence images; HUVECs were stained for CD31 (red) and GFP-hBMSCs stained for anti-GFP antibody (green) and nuclei for DAPI (blue). (Scale bar, 100 μm.)

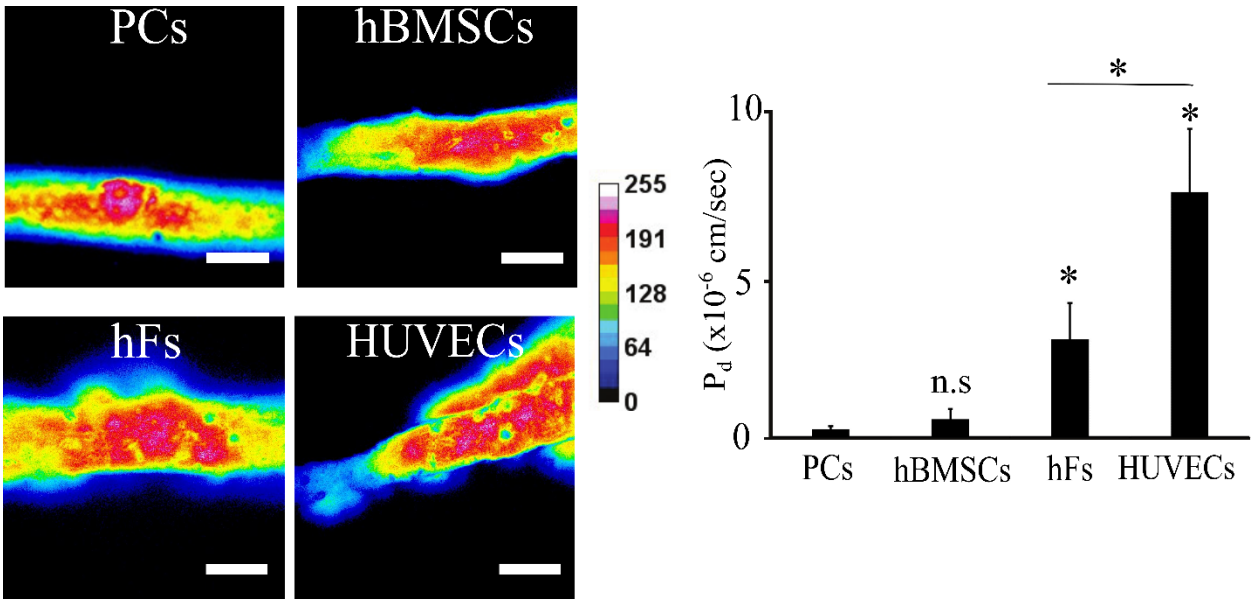

Fig.S4: ***Quantification of permeability in different stromal cells.*** Representative heat map panel of 70 kDa Texas Red dextran perfused into the engineered bicellular microvessels with ratio hFs:HUVECs (1:5), hBMSCs: HUVECs (1:5), and PCs: HUVECs (1:5) and microvessels holding only HUVECs performed as negative control. (Scale bar, 100 μm.) Histogram reports the diffusive permeability coefficient (P_d) for different bicellular microvessels. Data expressed as mean ±SEM. *N* = *6*. *, P value < 0.05; n.s. indicates non-significant.

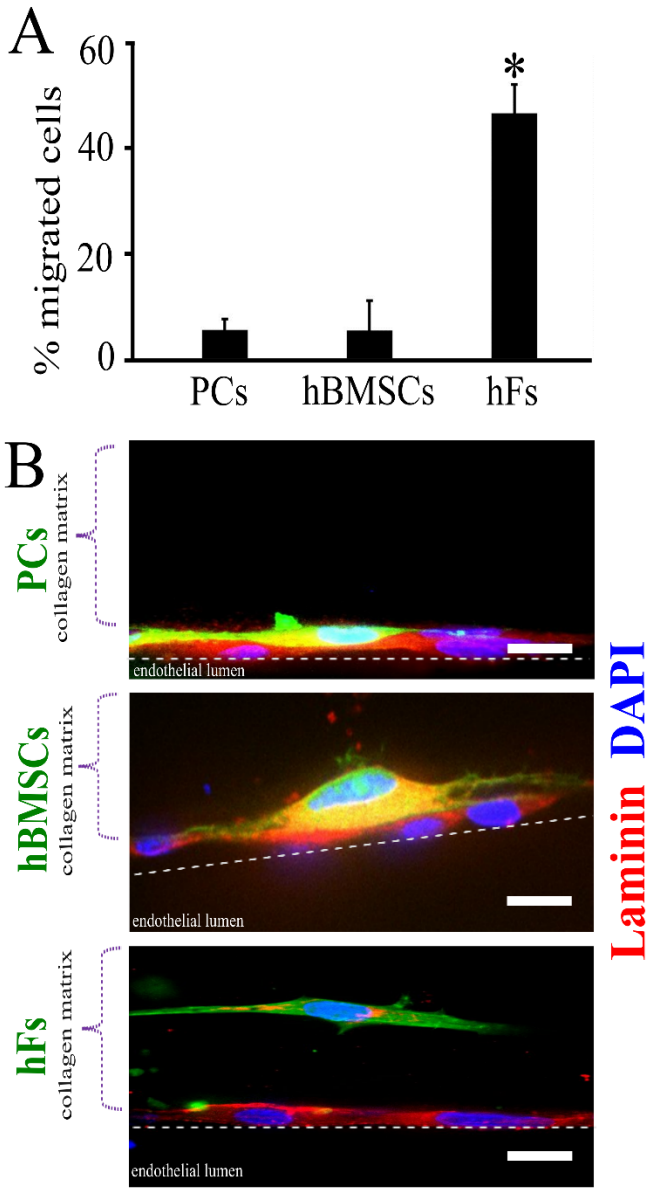

Fig.S5: ***Functional characterization of hFs, hBMSCs and PCs in the microvessels.*** (A) Histograms report migrated hFs, hBMSCs and PCs (% out of total cells per field of view) in the different groups. Data expressed as mean ±SEM. *N = 3-6 devices*. *, P value < 0.05. (B) Representative images of engineered microvessels holding hFs, hBMSCs and PCs; Basal membrane was stained for laminin (red) and GFP-hFs, GFP-hBMSCs and GFP-PCs cells were stained for anti-GFP antibody (green) and nuclei for DAPI (blue); Dotted line indicates the endothelial lumen area. (Scale bar, 20 μm.)

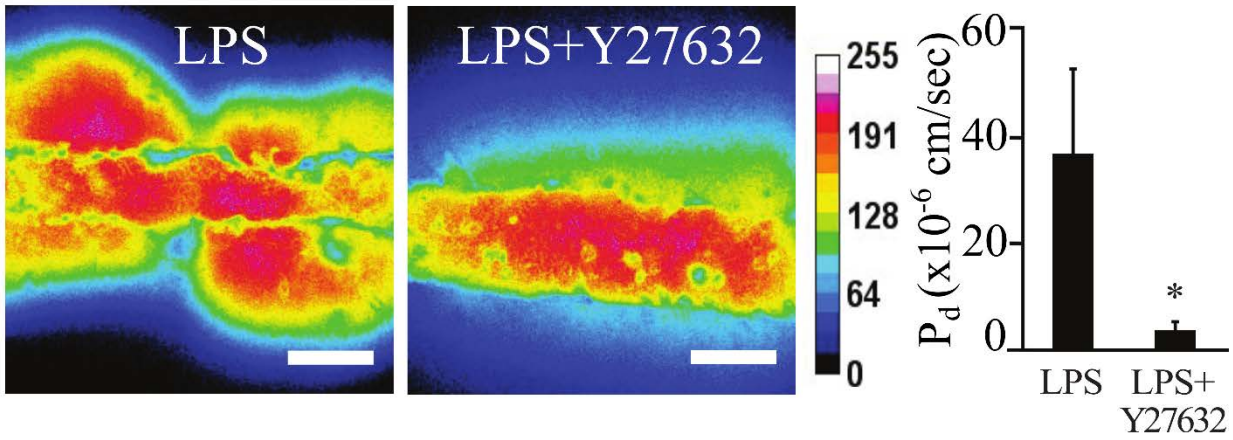

Fig.S6: ***Effect of ROCK inhibitor in LPS-induced vascular permeability.*** Representative heat map panel of 70 kDa Texas Red dextran perfused into the engineered microvessels under LPS (100 ng/ml) and LPS+Y27632 (25 μM) treatment. (Scale bar, 100 μm.) Histogram reports the diffusive permeability coefficient (P_d) for the considered different treatments; Data expressed as mean ±SEM. *N = 10*. *, P value $<$ 0.05.

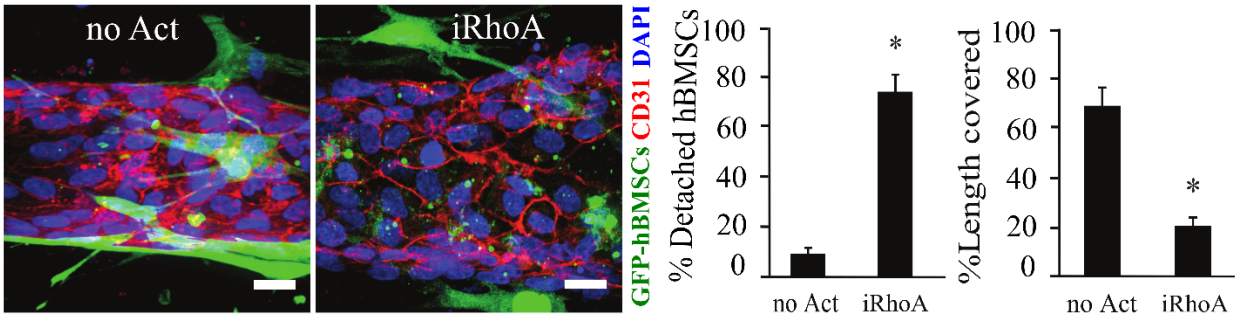

Fig.S7: ***RhoA controls mural cell detachment.*** Representative images of engineered microvessels with no activation (no Act) or RhoA activation (iRhoA; 12.5μM GA_3-AM); HUVECs were stained for CD31 (red) and GFP-hBMSCs cells were stained for anti-GFP antibody (green) and nuclei for DAPI (blue). (Scale bar, 20 μm.) Histograms report detached hBMSCs (% out of total hBMSCs per field of view) and vascular length covered by hBMSCs (% out of total length) in the different groups. Data expressed as mean ±SEM. *N = 12-20 fields (3-6 devices)*. *, P value < 0.05.

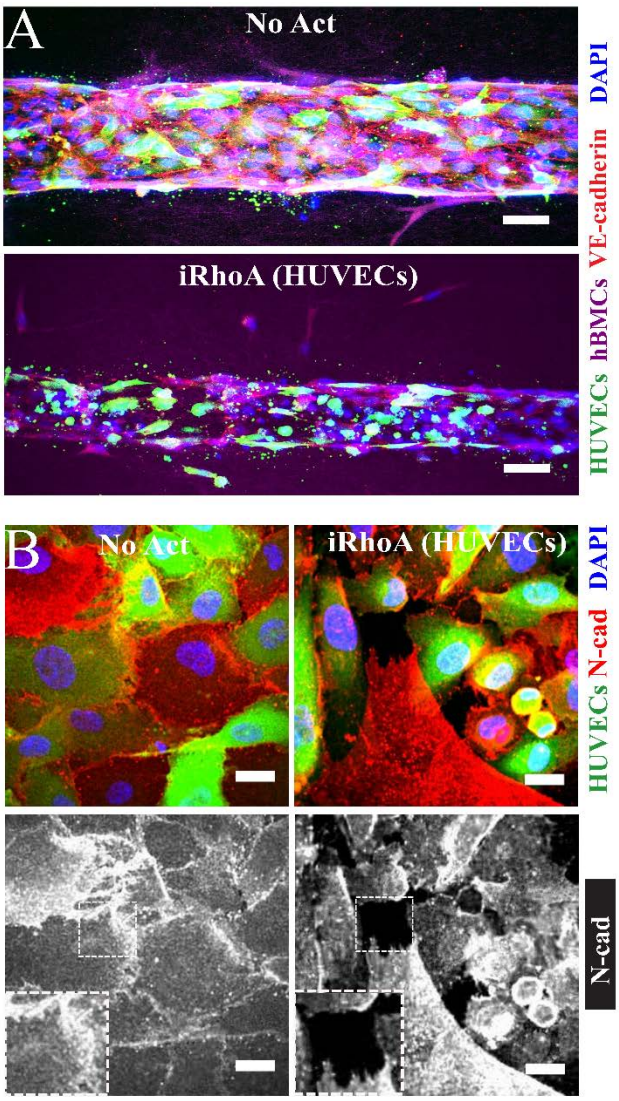

Fig.S8: ***RhoA activation in endothelial cells results in loss of heterotypic N-cadherin junctions and detachment of hBMSCs*** (A) Representative images of engineered microvessels with no activated (no Act) or RhoA activated (iRhoA; 12.5 μM GA_3-AM) HUVECs; HUVECs were stained for VE-cadherin (red) and anti-GFP antibody (green), hBMSCs cells were stained for PDFGRβ (magenta) and nuclei for DAPI (blue). (Scale bar, 50 μm.) (B) Representative confocal immunofluorescence images showing the N-cadherin mediated interaction between hBMSCs and no activated (no Act) or RhoA activated (iRhoA) HUVECs. HUVECs and hBMSCs were stained for N-cadherin (N-cad) (red) and HUVECs were stained for anti - GFP antibody (green) and nuclei for DAPI (blue). (Scale bar, 20 μm.)

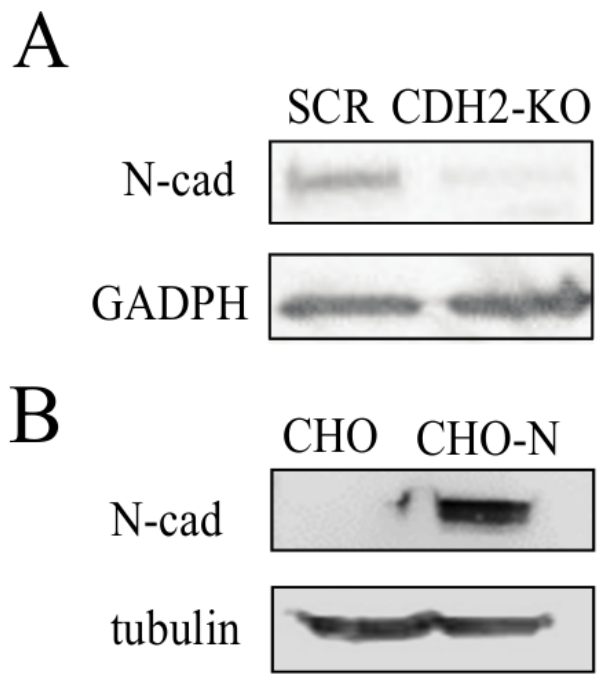

Fig.S9: ***Western Blot*** for (A) CDH2 in scrambled (SCR) and CDH2-KO GFP-hBMSCs. (B) for CDH2 in CHO and CHO cells overexpression N-cadherin (CHO-N).

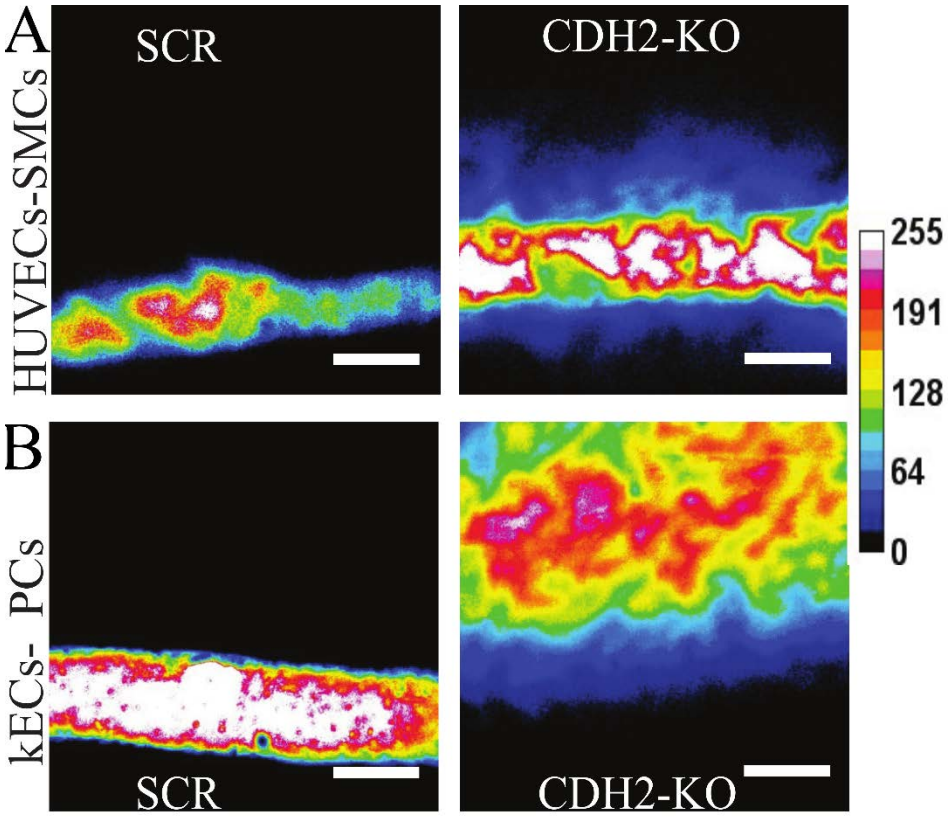

Fig.S10: ***N-cadherin regulates barrier function in perivascular cells***. Representative heat map panel of 70 kDa Texas Red dextran perfused into the engineered microvessels holding scrambled (SCR) and CDH2 knockout (CDH2-KO) (A) smooth muscle cells (SMCs) and (B) kidney pericytes (PCs). (Scale bar, 100 μm.)