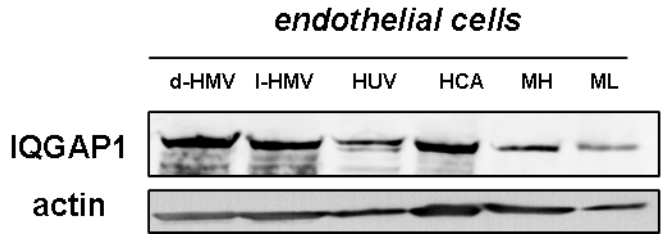
SUPPLEMENTAL MATERIAL

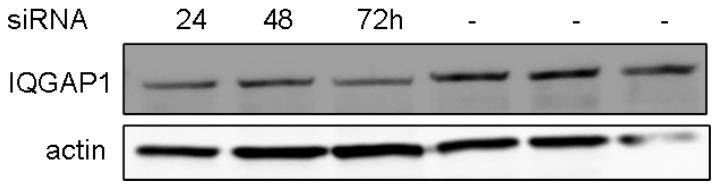
Supplementary Figure I.A

(A) Immunoblotting for IQGAP1 in multiple endothelial cell types (EC): human microvascular ECs from dermis (d-HMV) and lung (l-HMV), human umbilical cord (HUV) ECs, human coronary artery (HCA) ECs, primary mouse heart (MH) ECs, and primary mouse lung (ML) ECs.



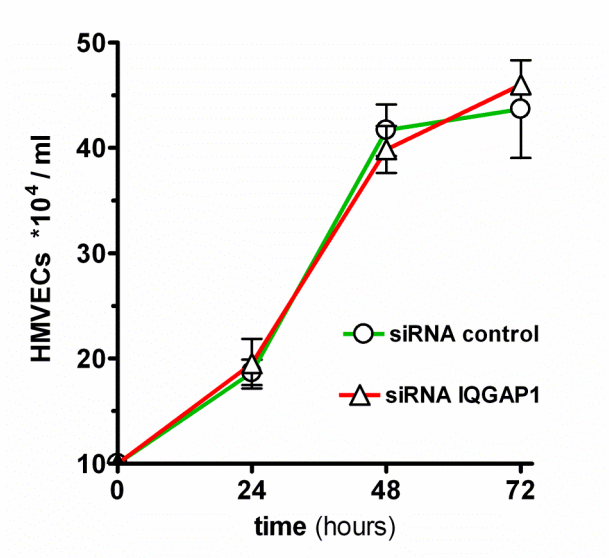
Supplementary Figure I.B

Immunoblot for IQGAP1 and actin 24, 48 and 72 hours after control or IQGAP1 siRNA transfection in HMVECs showing efficient knockdown of endogenous IQGAP1 protein.



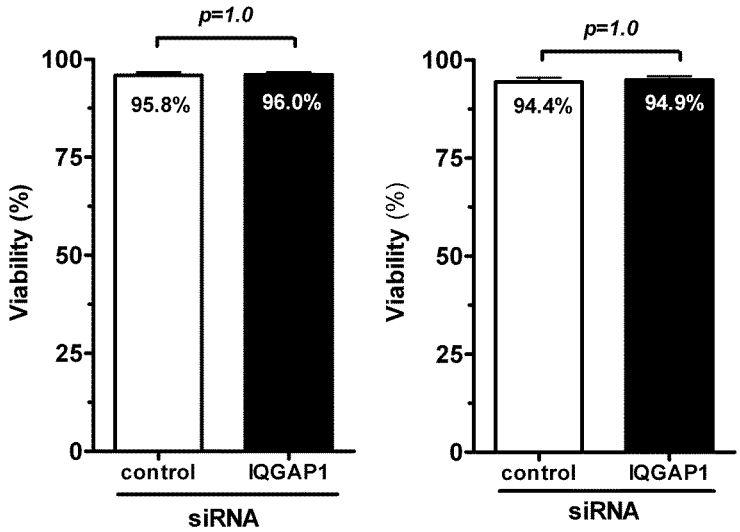
Supplementary Figure I.C

HMVECs have been counted 24, 48, and 72 hrs after treatment with IQGAP1 (red) or control (green) siRNA (n=3/condition).



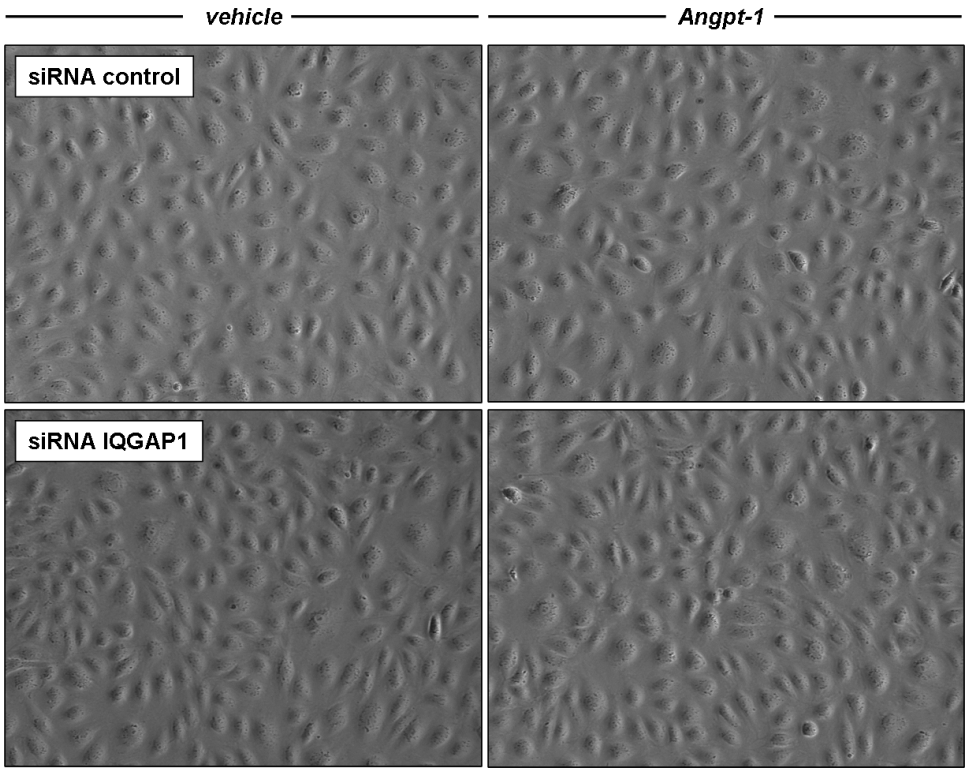
Supplementary Figure I.D

Bar graph of *"Trypan Blue Viability Assay"* 48 (left) and 72 h (right) after IQGAP1 siRNA transfection (n=6/per condition)



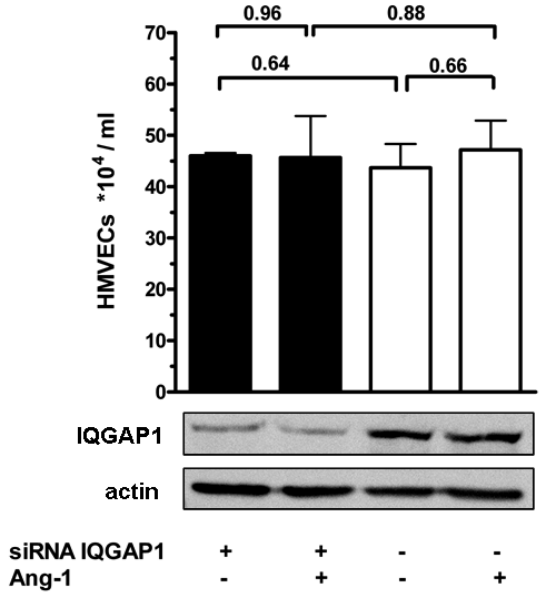
Supplementary Figure I.E

Bright-field light microscopy from control or IQGAP1 siRNA treated cells, 72 hours after transfection. These cells have additionally been treated with Angiopoietin-1 (Angpt-1) or control vehicle for 72 hrs.



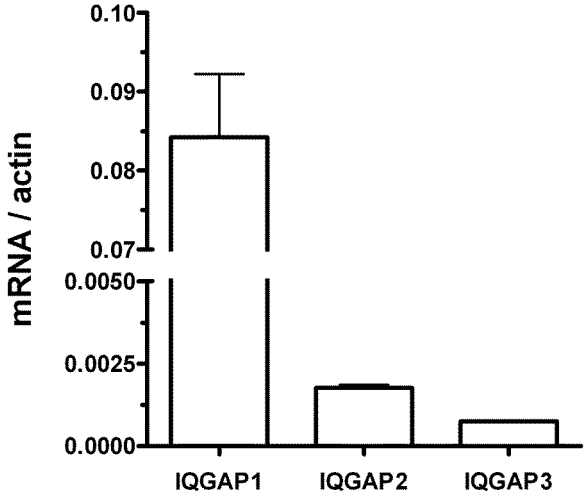
Supplementary Figure I.F

HMVECs were lysed 72h after siRNA transfection and immunoblotted for IQGAP1 and actin to demonstrate efficient protein knockdown and no change in IQGAP1 expression by Angpt-1 treatment.



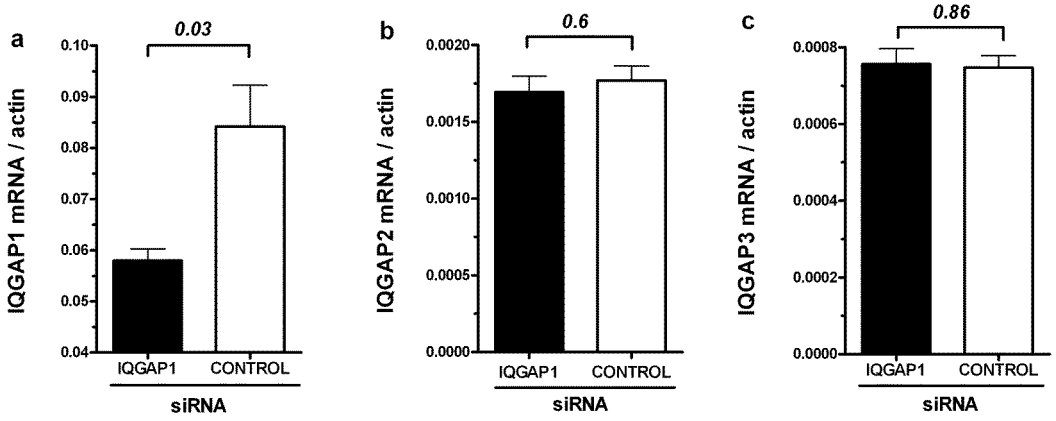
Supplementary Figure I.G

Quantitative Real-time PCR (qPCR) for different IQGAP isoforms in HMVEC lysates (n=4/condition).



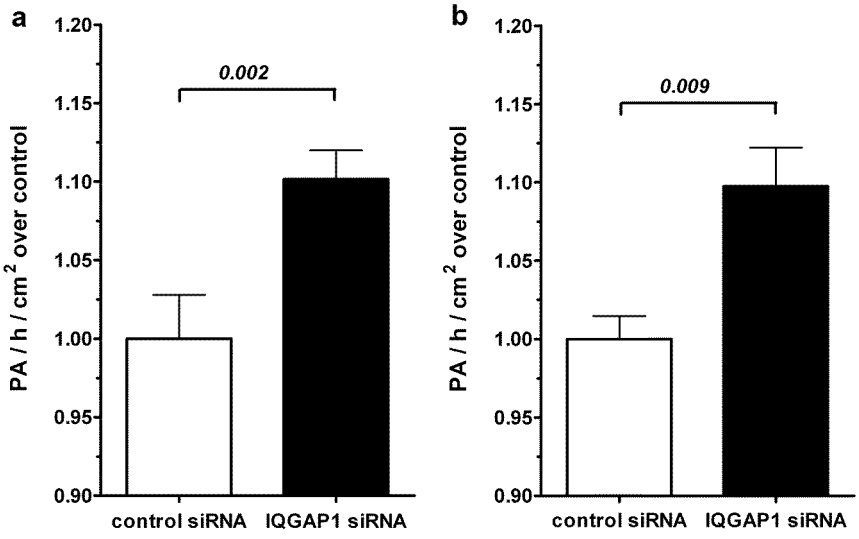
Supplementary Figure I.H

Quantitative Real-time PCR (qPCR) for (a) IQGAP1, (b) IQGAP2, and (c) IQGAP3 in control and IQGAP1 silenced HMVECS (n=4/condition).



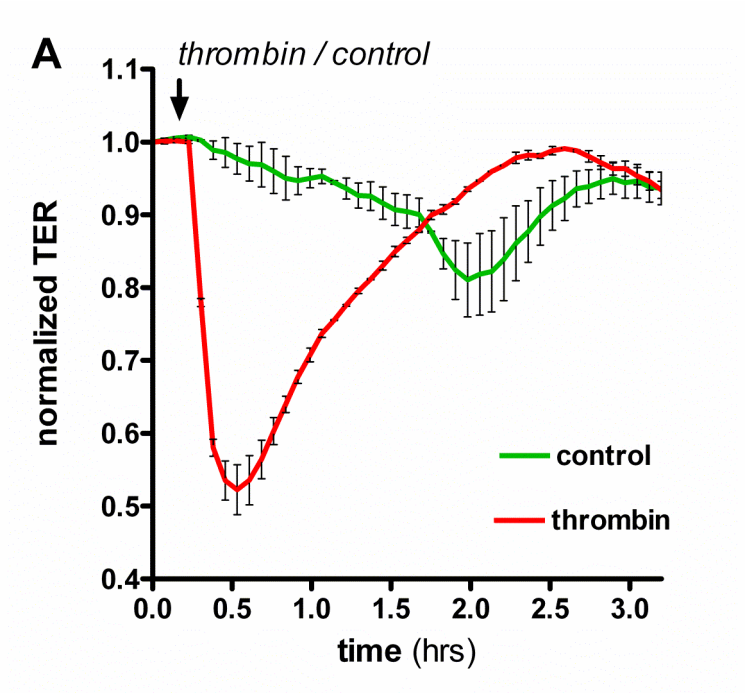
Supplementary Figure I.H

Transwell Permeability Assay. The luminometric reading of FITC-labeled albumin in the abluminal and luminal:chambers (mean ± S.E.M.) was taken (a) 24h and (b) 48 h after IQGAP1 or control siRNA transfection (n=6 per condition).



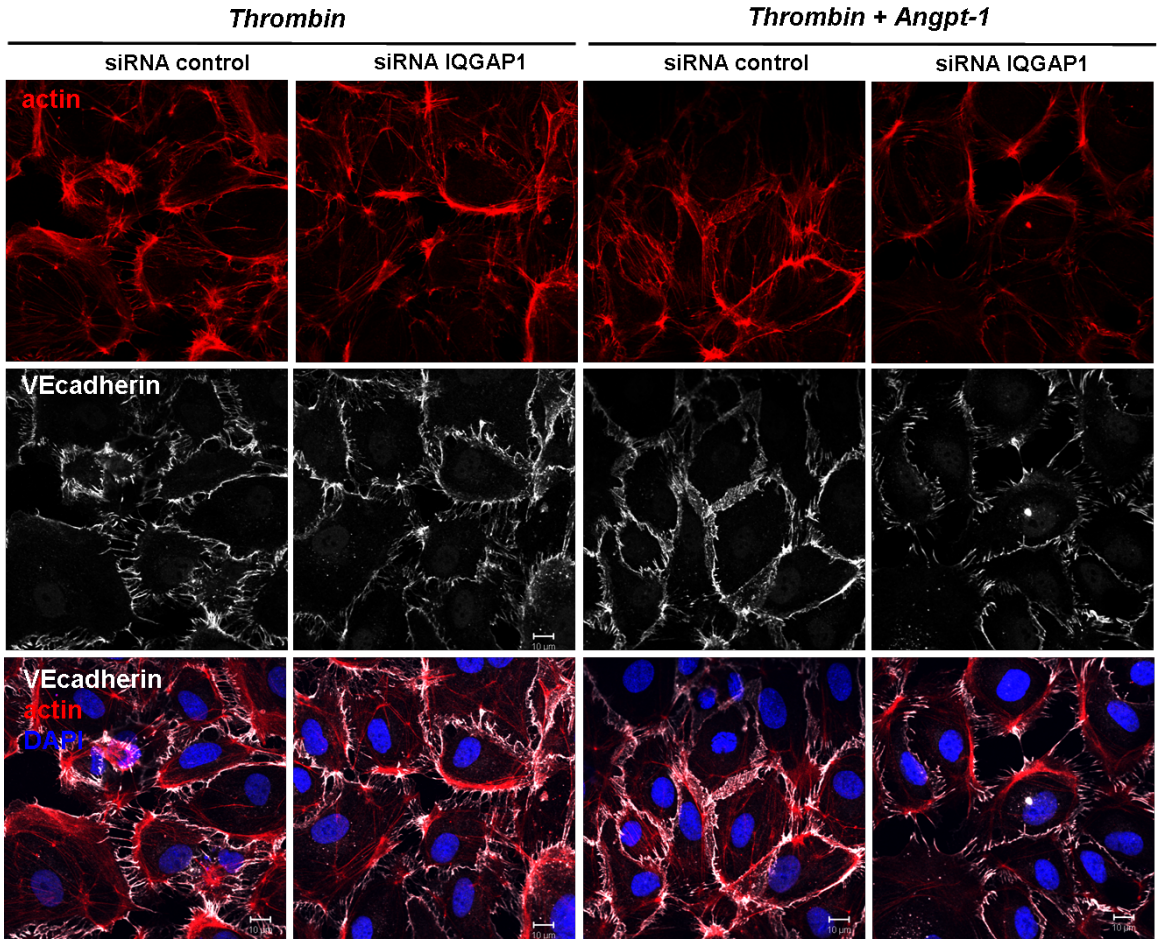
Supplementary Figure II.A

HMVECs were plated on gold microelectrodes to measure transendothelial electrical resistance (TER) and were grown to confluence. After equilibration and stabilization, thrombin (1U/mL) (*red*) or control vehicle (*green*) were applied and TER was measured in real time (n=2 per condition).



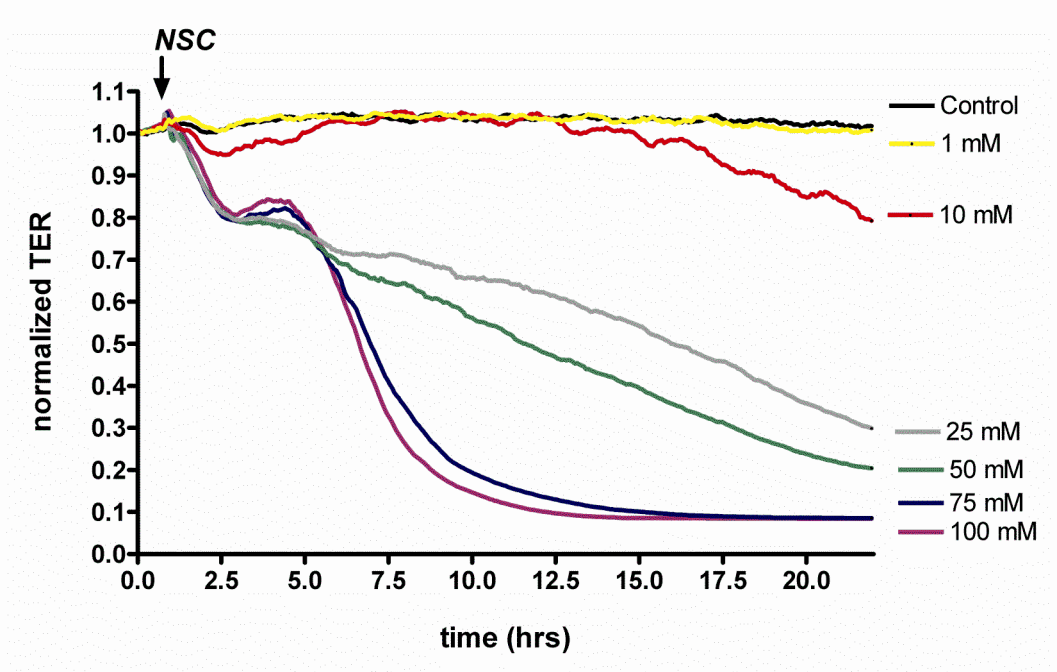
Supplementary Figure II.B

Confocal fluorescent immunocytochemistry for VE-cadherin (white), actin (red) and DAPI (blue) 72 hrs after control or IQGAP1 siRNA transfection. The first two columns consist of HMVECs challenged for 15 minutes with thrombin (1 U/mL). Both control- and IQGAP1-silenced cells show severe junctional disassembly with gap formation between adjacent cells. In the last two columns, cells have been co-treated for 15 min with thrombin (1U/mL) and Angpt-1 (300 ng/mL). In control-silenced cells, Angpt-1 abolished the paracellular gaps, but completely failed to do so if IQGAP1 was silenced. Results are representative for 3 independent sets of experiments (scale bar 10 μm).



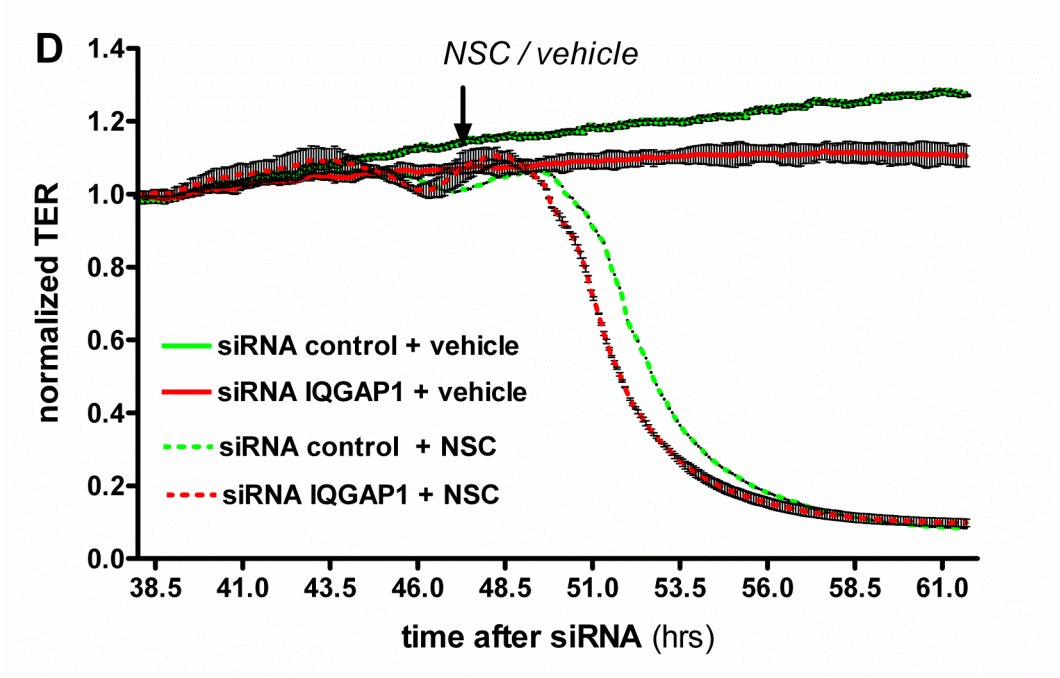
Supplementary Figure III

HMVECs were plated on gold microelectrodes and grown to confluence to measure transendothelial electrical resistance (TER). Cells were then treated with different doses of *NSC 23677* (1, 10, 25, 50, 75 and 100 mM) or control vehicle (in black) and resistance was measured in real time.



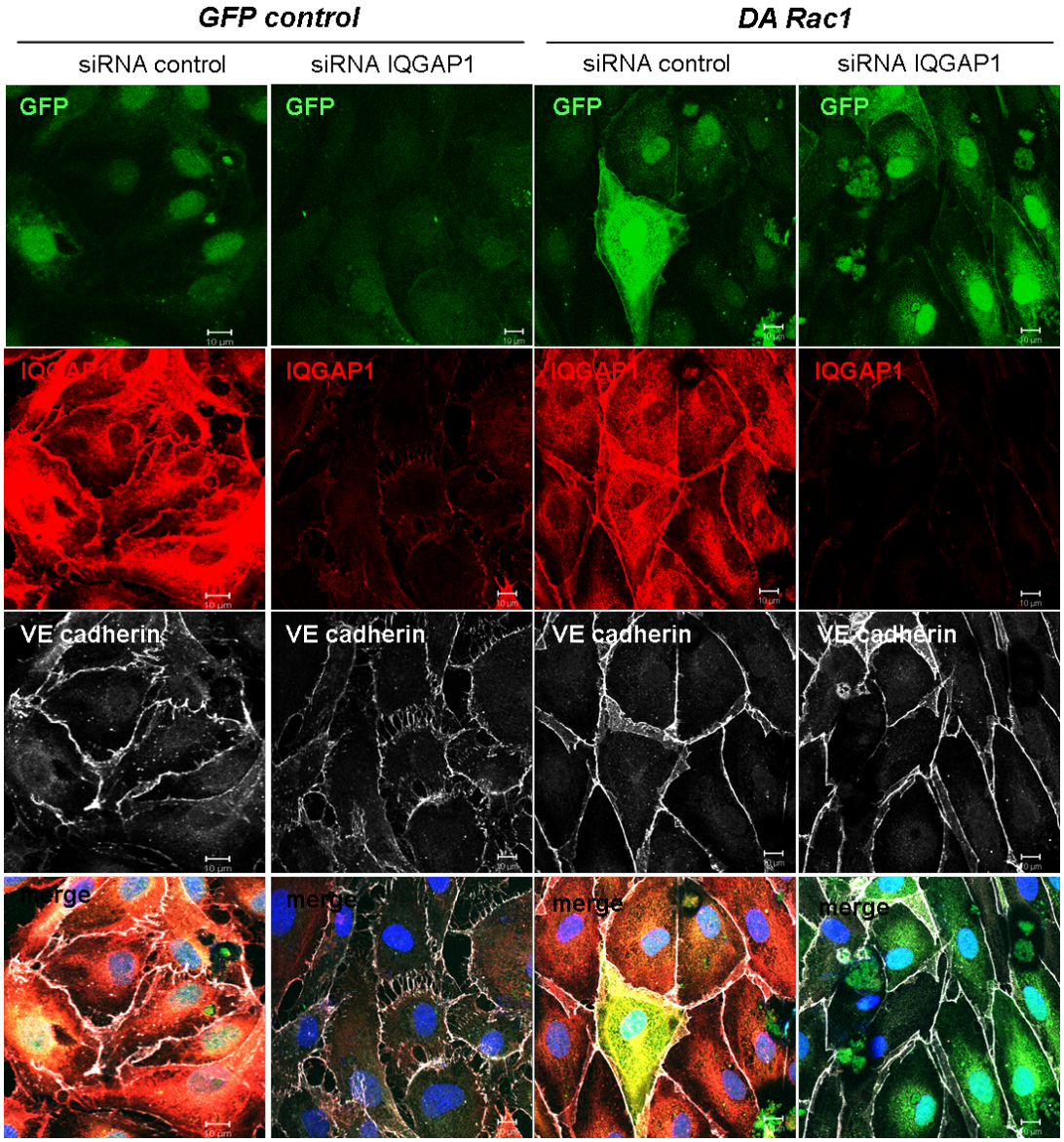
Supplementary Figure IV

HMVECs were plated on gold microelectrodes and grown to confluence to measure transendothelial electrical resistance (TER). HMVECs were treated with control or IQGAP1 siRNA and *NSC 23677* (25mM) or control vehicle. NSC-induced permeability was not aggravated by silencing of IQGAP1 (n=2 per condition).



Supplementary Figure V

Confocal fluorescent immunocytochemistry for IQGAP1 (red), VE-cadherin (white), and DAPI (blue) from HMVECs stably expressing GFP control (1st and 2nd column) or DA Rac1 (3rd and 4th column). After three consecutive retroviral transductions followed by antibiotic selection, DA Rac1 or GFP was expressed in 100% of cells (see GFP channel). Confocal images were captured 72 hours after control- or IQGAP1 siRNA transfection. Results are representative for 3 independent sets of experiments (scale bar 10 µm).



Supplementary Table I

IQGAP1-3 primers for quantitative real-time PCR.

	Forward	Reverse
IQGAP1	AGTGCTGCCCAGCAATATCAGAGA	TGATAGAGATCGGCGGCAAATGGA
IQGAP2	AGAGGTGTACAAGGCTTGGGTGAA	TTCAGGACTTTGTCGGTGACCCTT
IQGAP3	ATGCTCTCAGCTGTGGTCCTGATT	GCATCGAAGTAACGCTGGGCATTT

Supplemental Methods

Transwell Permeability Assay. Confluent HMVEC monolayers were grown on type I collagen-coated Costar transwell membranes (Corning) and permeability determined by measurement of fluorometric signal in the luminal and abluminal chambers at the indicated time point after luminal addition of 1 mg/mL FITC-labeled human serum albumin (Sigma).