

SUPPLEMENTAL FIGURES AND TABLES

Nuclear SUN2 coordinates endothelial cell-matrix interactions to regulate blood vessel homeostasis and barrier function

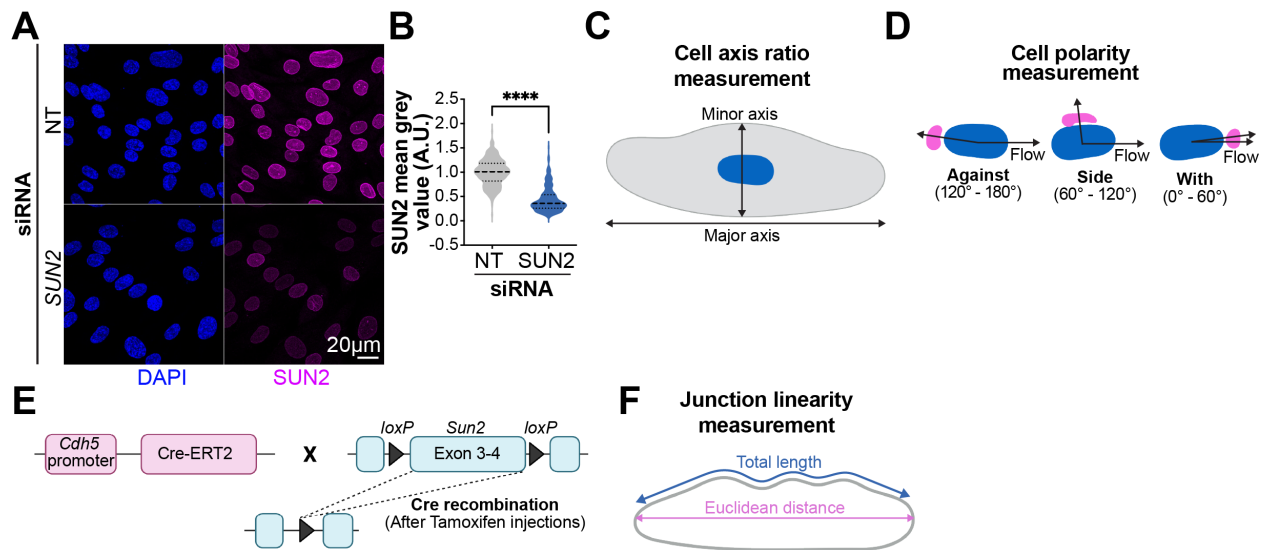
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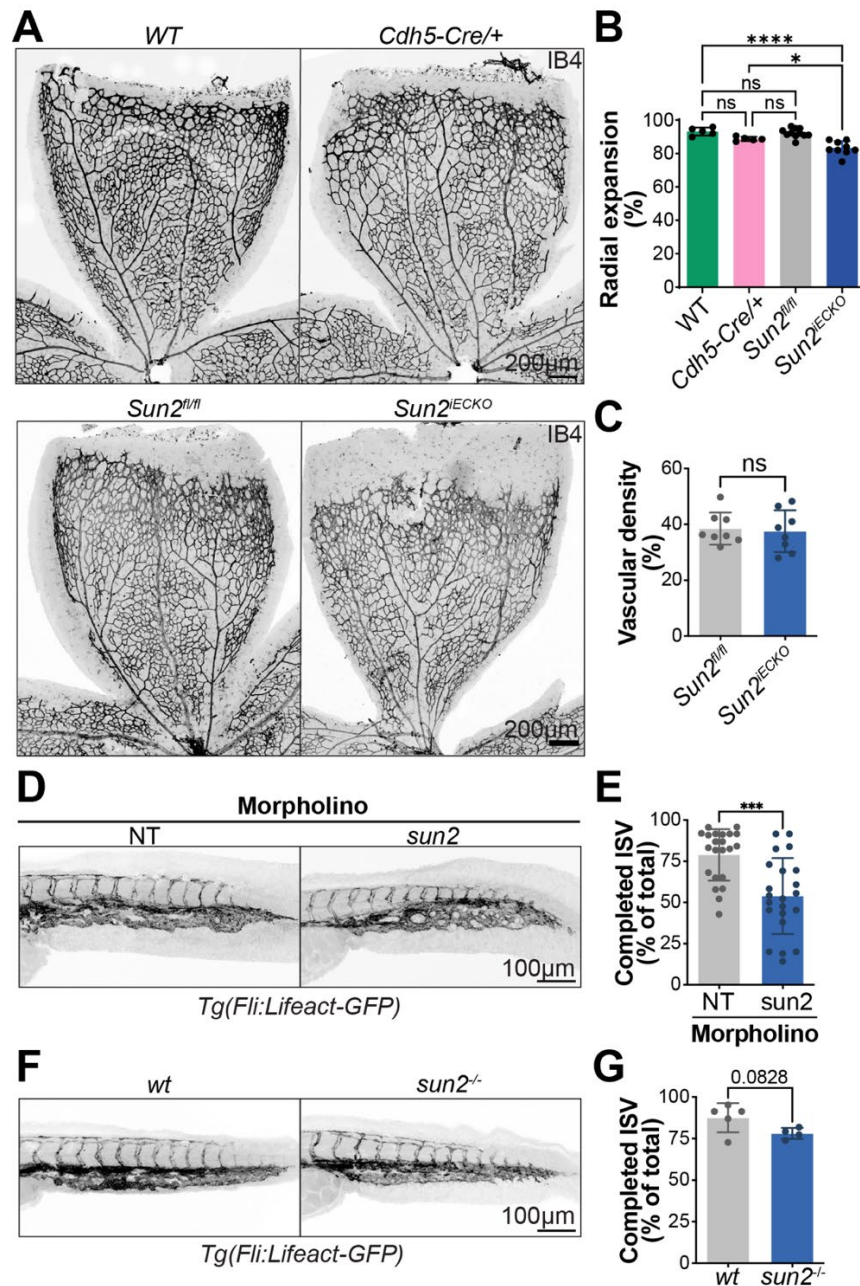
Supplemental Movie Legends: Page 9

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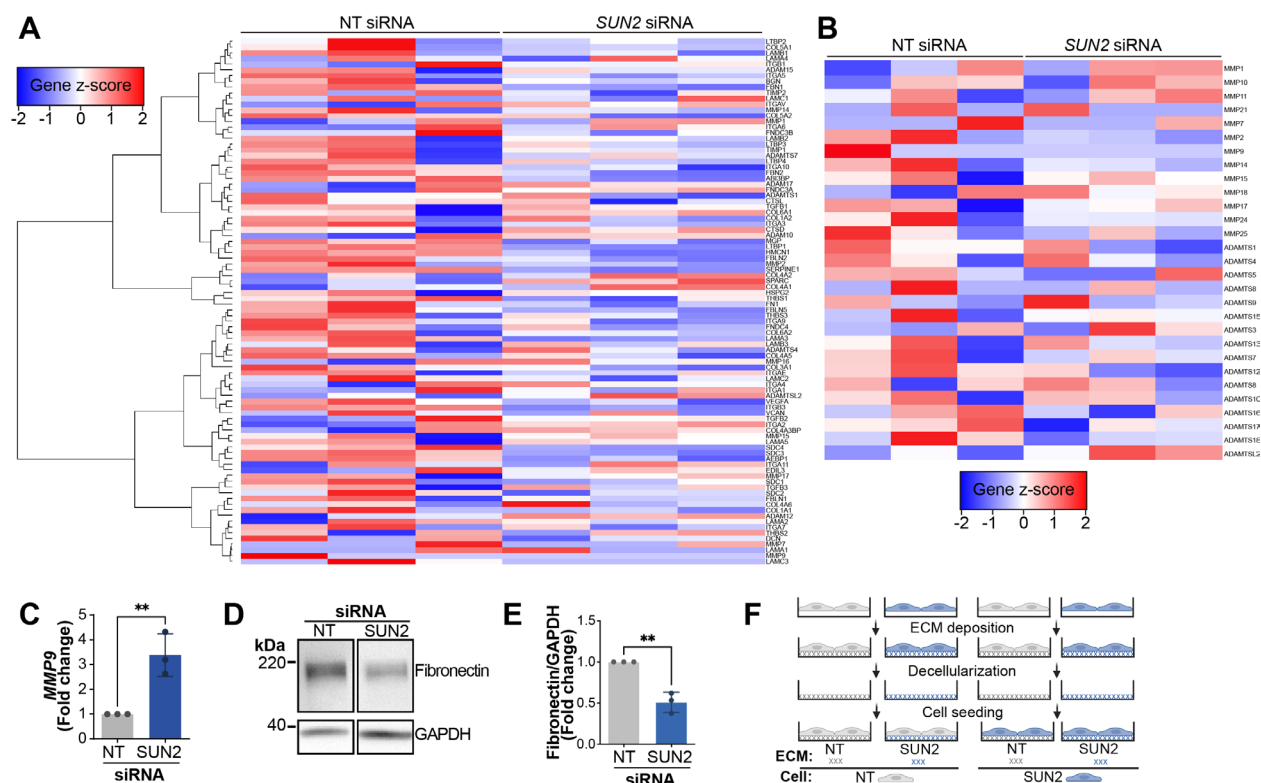
SUPPLEMENTAL FIGURES



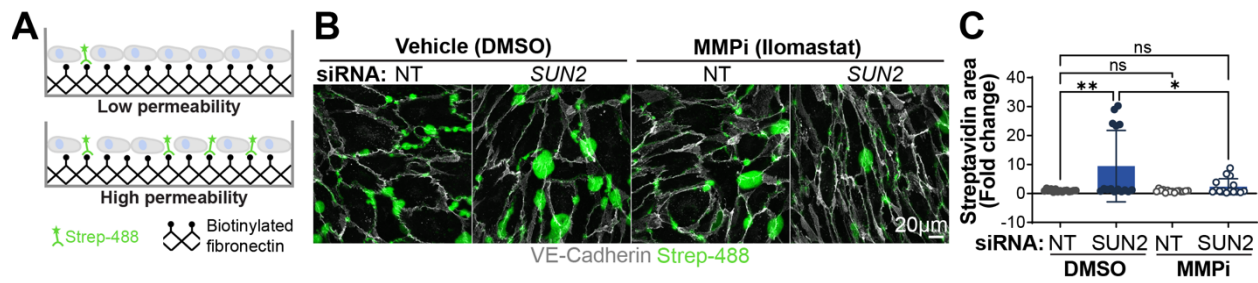
Supplementary Figure 1 (For Fig 1). Validation of SUN2 depletion and measurements in endothelial cells. (A) Confocal images (maximum intensity projections) of HUVEC with indicated siRNA and stained as indicated. Scale bar, 20µm. **(B)** Quantification of SUN2 nuclear intensity in HUVEC with indicated siRNA. **(C)** Diagram illustrating cell axis ratio quantification. **(D)** Schematic illustrating cell polarity quantification, based on nucleus and Golgi staining. **(E)** Schematic showing the breeding strategy to generate *Sun2^{IECKO}* mice. **(F)** Schematic illustrating EC junction measurement quantification. Statistical analyses: two-tailed Student's t-tests. ****, p≤0.0001.



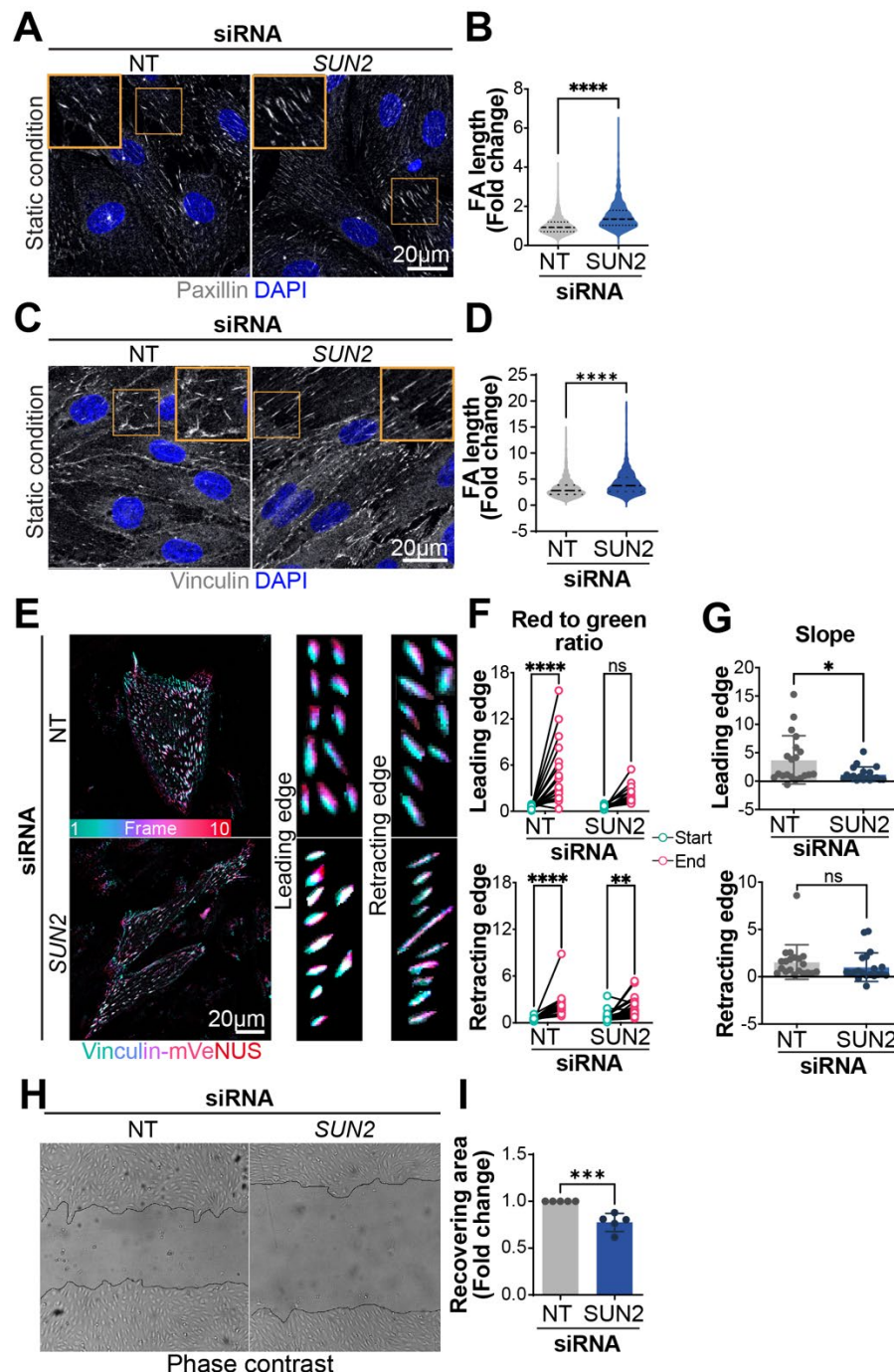
Supplementary Figure 2 (For Fig 1). Nuclear SUN2 regulates vascular development. (A) Representative confocal images (maximum intensity projections) of P7 mouse retinas from indicated genotypes, stained as indicated. Scale bar, 200 μ m. **(B, C)** Quantification of the indicated parameters (WT, n=5, *Cdh5-Cre/+*, n=5, *Sun2^{fl/fl}*, n=11; *Sun2^{IECKO}*, n=9). **(D, E)** Confocal images (*Tg(fli:LifeAct-GFP)*) and quantification of zebrafish embryos with indicated morpholino (MO) treatments at 36hpf. Scale bar: 100 μ m. (NT MO, n=22; *sun2* MO, n=22). **(F, G)** Confocal images (*Tg(fli:LifeAct-GFP)*) and quantification of zebrafish embryos with indicated genotypes at 36hpf. Scale bar, 100 μ m. (*wt*, n=5; *sun2^{-/-}*, n=4). Statistical analyses were performed using two-tailed Student's t-tests, except for **(B)**, which was analyzed using one-way ANOVA with Tukey multiple comparisons test. *, p<0.05; ***, p<0.001; ****, p<0.0001; ns, not significant.



Supplementary Figure 3 (For Fig 2). SUN2 regulates extracellular matrix gene expression and composition. (A, B) Relative expression levels of ECM-associated genes and ECM-degradation associated genes plotted using bulk RNA seq data of HUVEC treated as indicated and cultured under laminar shear stress (n=3 replicates). (C) RT-qPCR analysis of MMP9 in HUVEC treated as indicated and exposed to laminar flow (n=3 biological replicates). (D, E) Western blot analysis of fibronectin and GAPDH expression in HUVEC treated with siRNA, seeded onto gelatin matrix, and exposed to laminar shear stress (15 dyn/cm², 24h) (n=3 biological replicates). (F) Diagram of matrix swap experiments.

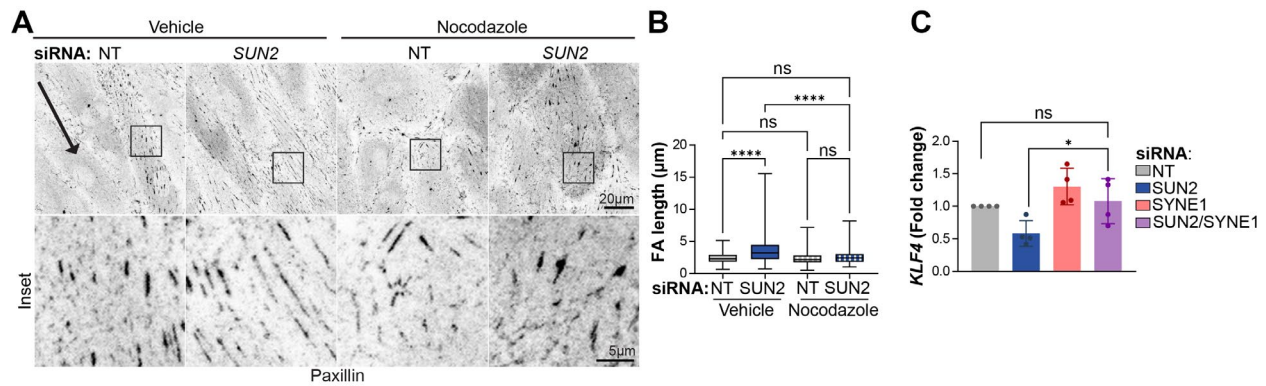


Supplementary Figure 4 (For Fig.3). Nuclear SUN2 regulates endothelial barrier function via MMP activity. (A) Schematic illustrating biotin-streptavidin labeling assay to assess monolayer integrity. (B) Confocal images (maximum intensity projections) of HUVEC with indicated siRNAs, on biotinylated-fibronectin, and treated as indicated for 24h, followed by incubation with Strep-488, fixation, and staining as indicated. Scale bar, 20µm. (C) Quantification of streptavidin area in HUVEC with indicated siRNAs and treatments (n=3 biological replicates).



Supplementary Figure 5 (For Fig.4). Nuclear SUN2 regulates focal adhesion dynamics and collective cell migration in endothelial cells. (A) Confocal images (maximum intensity projections) of HUVEC treated with siRNA, cultured under static conditions, then fixed and stained for paxillin. Scale bar, 20µm. (B) Quantification of indicated parameters (n=3 biological replicates). (C) Confocal images (maximum intensity projections) of HUVEC treated with siRNA, cultured under static conditions, then fixed and stained for vinculin. Scale bar, 20µm. (D) Quantification of indicated parameters (n=3 biological replicates). (E) Temporal projections of vinculin-mVenus expressing HUVEC treated with siRNA, with insets showing representative individual FA at the leading edge (a,c) and retracting edge (b,d). Images

were acquired at 4 min intervals over 40 min. **(F, G)** Quantification of FA dynamics in vinculin-mVenus expressing HUVEC treated with siRNA (n=2 biological replicates). **(F)** Green-to-red ratio quantification at the start and at the end of individual FA showing the temporal progression of the adhesion (time-color gradient) at the leading (top) and retracting (bottom) edges. **(G)** Slope quantification of the time-color gradient for individual FA at the leading (top) and retracting (bottom) edges. **(H)** Scratch wound healing assay of HUVEC treated with siRNA as indicated, and **(I)** quantification of the recovered area after 8h (n=5 biological replicates). Statistical analyses were performed using two-tailed Student's t-tests, except for **(F)**, which was analyzed using Multiple paired t-tests. ns, non-significant; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$.



Supplementary Figure 6 (For Fig.5 and Fig.6). Nuclear SUN2 regulates focal adhesion morphology via its effect on the microtubule cytoskeleton. (A) Top: Confocal images (maximum intensity projections) of HUVEC with the indicated siRNAs, exposed to laminar orbital flow for 24h and treated with DMSO (vehicle) or nocodazole for the last 4h, followed by fixation and staining as indicated. Scale bar: 20µm. **Bottom:** Higher magnification images of the boxed regions shown in the corresponding images above. Black arrow: Flow direction. Scale bar: 5µm **(B)** Quantification of the focal adhesion length. (n=3 biological replicates). **(C)** RT-qPCR of KLF4 in HUVEC treated as indicated and exposed to laminar flow. (15 dyn/cm², 24h, orbital). Statistical analyses were performed using one-way ANOVA with Tukey multiple comparisons test. *, p≤0.05; ****, p≤0.0001; ns, not significant.

SUPPLEMENTAL MOVIE LEGENDS

Supplementary Movie 1 (For Fig. 4E). Focal adhesion dynamics in control endothelial cells. Movies acquired by confocal imaging of paxillin–GFP expressing HUVEC treated with NT siRNA. Images acquired at 4 min intervals over 40 min. Scale bar: 5µm.

Supplementary Movie 2 (For Fig. 4E). SUN2 depletion affects focal adhesion dynamics in endothelial cells. Movies acquired by confocal imaging of paxillin–GFP expressing HUVEC treated with SUN2 siRNA. Images acquired at 4 min intervals over 40 min. Scale bar: 5µm.

Supplementary Movie 3 (For Fig. 4K). Coordinated migration of control endothelial cells. Movies acquired by phase-contrast confocal imaging of HUVEC treated with NT siRNA. Images acquired at 5 min intervals over 240 min. Scale bar: 20µm.

Supplementary Movie 4 (For Fig. 4K). SUN2 depletion disrupts coordinated migration of endothelial cells. Movies acquired by phase-contrast confocal imaging of HUVEC treated with SUN2 siRNA. Images acquired at 5 min intervals over 240 min. Scale bar: 20µm.

Supplementary Movie 5 (For Fig. 5E). Microtubule dynamics in control endothelial cells. Movies acquired using spinning disc microscopy on EB3-mNeonGreen expressing HUVEC treated with NT siRNA. Images acquired at 470ms intervals over 60 sec. Black arrows indicate shrinking microtubules. Scale bar: 5µm.

Supplementary Movie 6 (For Fig. 5E). SUN2 depletion increases microtubule stability in endothelial cells. Movies acquired using spinning disc microscopy on EB3-mNeonGreen expressing HUVEC treated with SUN2 siRNA. Images acquired at 470ms intervals over 60 sec. Black arrows indicate shrinking microtubules. Scale bar: 5µm.

SUPPLEMENTAL TABLES**Supplementary Table S1: Primers**

Primer	Application	Forward sequence (5'-3')	Reverse sequence (5'-3')
<i>Sun2 tm1a</i>	PCR (Mouse genotyping)	ACTATCCCGACCGCCTTACT	TAGCGGCTGATGTTGAACTG
<i>Sun2 floxed</i>	PCR (Mouse genotyping)	ATTGGGTGTCTCTACATCAAAGCTC	CCCCAGACAGAAGTGCCTAA
<i>FlpO</i>	PCR (Mouse genotyping)	TGAGCTTCGACATCGTGAAC	TCAGCATCTTCTTGCTGTGG
<i>Cdh5- CreERT2</i>	PCR (Mouse genotyping)	TCCTGATGGTGCCTATCCTC	CCTGTTTTGCACGTTACCG
<i>sun2</i>	PCR (Zebrafish genotyping)	CTGAGAGCAGAGCTGTCTGATT	TGCTCCCTTTCTCATGTGCT
<i>MGP</i>	RT-qPCR (HUVEC)	CCTCAGCAGAGATGGAGAGCTA	ATGGCGTAGCGTTCGCAAAGTC
<i>LTBP1</i>	RT-qPCR (HUVEC)	TGAATGCCAGCACCGTCATCTC	CTGGCAAACACTCTTGTCTCTCC
<i>ABI3BP</i>	RT-qPCR (HUVEC)	CCTTCTACACCTAAACGACGCC	GGTGTGTCCATGTAGGTTTCAGG
<i>FBLN5</i>	RT-qPCR (HUVEC)	CTGCTGGATGACAACCGAAGCT	GATAAGGCTCCTCACAGCGGAT
<i>ITGA9</i>	RT-qPCR (HUVEC)	TCAGGTCACAGAGAAGCTGCAG	CACATGCTCACTGAGGCTGTAG
<i>ITGB3</i>	RT-qPCR (HUVEC)	CATGGATTCCAGCAATGTCCTCC	TTGAGGCAGGTGGCATTGAAGG
<i>COL3A1</i>	RT-qPCR (HUVEC)	TGGTCTGCAAGGAATGCCTGGA	TCTTTCCCTGGGACACCATCAG
<i>FBN1</i>	RT-qPCR (HUVEC)	GGATACACAGGTGATGGCTTCAC	GTCGCATTACAGCGGTATCCT
<i>UNC5B</i>	RT-qPCR (HUVEC)	GCTCGACTCTAAGAACTGCACAG	TGAGGATTGCCACGACCACGAA
<i>ADAMTSL2</i>	RT-qPCR (HUVEC)	CACATCCTGCAAGCTCACTGAC	CAGATGCCACACTTGTCCAGTG
<i>MMP9</i>	RT-qPCR (HUVEC)	GCCACTACTGTGCCTTTGAGTC	CCCTCAGAGAATCGCCAGTACT
<i>GAPDH</i>	RT-qPCR (HUVEC)	CAGCAAGAGCACAAGAGGAAGAGA	TTGATGGTACATGACAAGGTGCGG

Supplementary Table S2: Antibodies

Primary antibodies	Application	Source	Catalog number
anti-VE-Cadherin	Mouse aorta staining	R&D Systems	AF1002
anti-SUN2	Mouse aorta staining	Abcam	ab124916
anti-KLF4	Mouse aorta staining, Western Blot	R&D Systems	AF3158
anti-VE-Cadherin	HUVEC staining	Cell signaling	2500S
anti-VE-Cadherin	HUVEC staining	Santa Cruz Biotechnology	sc-9989
anti-GM130	HUVEC staining	abcam	ab52649
anti-SUN2	HUVEC staining	Sigma-Aldrich	MABT880
anti-Paxillin	HUVEC staining, Western Blot	abcam	ab32084
anti-PECAM1	HUVEC staining	Cell signaling	3528S
anti-acetylated- α -tubulin	HUVEC staining	Sigma-Aldrich	T7451
anti- α -tubulin	HUVEC staining	Cell signaling	3873S
anti-Vinculin	Western Blot	Sigma-Aldrich	V9131
anti-Fibronectin	Western Blot	Cell signaling	26836S
anti-FBN1	Western Blot	abcam	ab314547
anti-GAPDH	Western Blot	Cell signaling	2118S

Secondary antibodies	Application	Source	Catalog number
Donkey anti-Rabbit IgG 488, 594, 647	Immunofluorescence	Invitrogen	A21206, A21207, A31573

Donkey anti-mouse IgG 488, 594, 647	Immunofluorescence	Invitrogen	A21202, A21203, A31571
Donkey anti-goat IgG 488, 594, 647	Immunofluorescence	Invitrogen	A11055, A11058, A21447
Donkey anti-goat IgG HRP	Western Blot	Invitrogen	PA1-28664
Goat anti-rabbit IgG HRP	Western Blot	Invitrogen	31460
Goat anti-mouse IgG HRP	Western Blot	Invitrogen	31430

Supplementary Table S3: siRNA and morpholinos

siRNA/Morpholino	Application	Sequence (5'-3')	Source	Catalog number
NT morpholino	Zebrafish injection	CCTCTTACCTCAGTTACAATTTATA	GeneTools, LLC	N/A
<i>sun2</i> morpholino	Zebrafish injection	ATCTTGTGCTTCGTCTTGACATC	GeneTools, LLC	N/A
NT siRNA	HUVEC transfection	N/A	Dharmacon	D-001810-10-20
<i>SUN2</i> siRNA	HUVEC transfection	N/A	Life technologies	4392420, s24467
<i>SYNE1</i> siRNA	HUVEC transfection	N/A	Dharmacon	M-014039-02-0005