

Supplemental Material

Experiments in human aortic endothelial cells and human microvascular endothelial cells

Human aortic endothelial cells (HAECs, passages 5 to 7) and microvascular endothelial cells (HMECs, passages 5 to 7) were cultured in EBM-2 (growth factor-free medium) with 2% FBS and exposed for 48 hrs either to normal glucose (5 mmol/L) or high glucose concentrations (25 mmol/L), in the presence or in the absence of (*R*)-PFI-2 (20 μ M) or the inactive enantiomer (*S*)-PFI-2 (20 μ M). Mannitol at the final concentration of 20 mmol/L was used as an osmotic control. After 48 h cells were harvested and used for angiogenesis assays or molecular analyses.

In vitro scratch assay

HAECs (50,000/well) were seeded 24 h before treatment in 24-well plates. A uniform scratch was introduced in the middle of the confluent cell layer with a 200 μ l pipette filter tip. The plate was transferred to a live cell imaging microscope (Olympus IX81) fitted with an incubator (humidified atmosphere, 37°C, 5% CO₂) and a motorized stage. Phase-contrast images were taken at 6.4x magnification every 30 minutes for 8 hours, with the Hamamatsu (C11440) detector at 1-megapixel (1024*1024 pixel) 16bit. Cell migration was quantified during live-cell imaging recording using the TScratch Software.

Tube formation assay

Matrigel (Corning® Matrigel® Matrix - 356234) was thawed overnight and 100 μ l were pipetted into 48-well plates [standard TC-treated (734-0028) -VWR]] and centrifuged at 12'000 rpm (1 minute at 4 °C) followed by incubation in a sterile incubator (humidified atmosphere, 37°C, 5% CO₂) for 30 minutes. Subsequently, HAECs (25,000/well) were plated at a sub-confluent level on top of Matrigel. The plate was then transferred to a live cell imaging microscope (Olympus IX81) fitted with an incubator (humidified atmosphere, 37°C, 5% CO₂) and a motorized stage. Phase-contrast images were taken at 6.4x magnification every 30 minutes for 8 hours, with the Hamamatsu (C11440) detector at 1-megapixel (1024*1024 pixel) 16bit. Tube formation and length of tubule in each well was considered and quantified by using the Image J software.

RNA-seq

RNA-sequencing (RNA-seq) was performed in NG and HG-treated HAECs, in the presence or in the absence of SETD7 siRNA or scrambled siRNA (n=5/group). Samples were sequenced as a 75 bp paired end with a NextSeq 500 using the Truseq LT kit. Before the alignment, a pre-

alignment QA/QC was done to check the quality of the input data. The raw FASTQ files were trimmed at the 3' end based on their quality score (Phred score). The unaligned reads were aligned to the *Homo sapiens* hg38 reference genome using the software STAR version 2.5.3. The default parameters were used. After the mapping step, a new quality checking was done (post-alignment QA/QC). The mapped reads were then quantified against the annotation from Gencode version 26 with the Partek Expectation/Maximization (E/M) algorithm. The transcript counts were normalized by FPKM (fragments per kilobase of exon model per million mapped reads) and by TPM (transcripts per millions).

MTT assay

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide, Thermo Fischer Scientific, M6494) was used to assess cell viability according to the manufacturer's instructions. Briefly, for adherent cells the medium was removed and replaced with 200 μ L of fresh culture medium without phenol red, followed by the addition of 20 μ L of a 12 mM MTT stock solution to each well. A negative control of 10 μ L of the MTT stock solution was added to the 100 μ L of medium alone. Cells were then incubated at 37°C for 4 hours. Next, 50 μ L of DMSO were added to each well and mixed thoroughly with a pipette, followed by incubation at 37°C for 10 minutes. Each sample was mixed again and read using a spectrophotometer at 540 nm.

Small interfering RNA transfection

Transient transfection with commercially available human SETD7, SEMA3G, PPAR γ and SOX-18 small interfering RNA (siRNA, Santa Cruz Biotechnology, Nunningen, Switzerland) was performed using Lipofectamine Reagent (Invitrogen, Basel, Switzerland). A predesigned scrambled siRNA (Microsynth, 5' UAC ACA CUC UCG UCU CU dTdT 3') was used as a negative control.

SETD7-CRISPR Activation Plasmid Transfection

CRISPR Activation Plasmid products enable the identification and upregulation of specific genes by utilizing a D10A and N863A deactivated Cas9 (dCas9) nuclease fused to a VP64 activation domain, in conjunction with sgRNA (MS2), a target-specific sgRNA engineered to bind the MS2-P65-HSF1 fusion protein. This synergistic activation mediator (SAM) transcription activation system provides a robust system to maximize the activation of endogenous gene expression. To overexpress SETD7, we used the UltraCruz Transfection Reagent (sc-395739), Plasmid Transfection Medium (sc-108062), Control CRISPR Activation Plasmid (sc-437275) and SETD7

CRISPR Activation Plasmid (h) (sc-405251-ACT). The detailed protocol is available at: https://datasheets.scbt.com/protocols/CRISPR_Activation_Protocol.pdf

Real time PCR

Total RNA was extracted from HAECs as well as mouse and human gastrocnemius muscle specimens using TRIzol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's recommendations. Before extraction, mouse and human samples were lysed by using a Precellys homogenizer. Conversion of total cellular RNA to cDNA was carried out with Moloney murine leukaemia virus reverse transcriptase and random hexamers (Amersham Bioscience, Piscataway, USA) in a final volume of 33 μ l, using 1 μ g of cDNA. Real-time PCR was performed using the SYBR Select Master Mix (Applied biosystems, Thermo Fischer Scientific, Zug, Switzerland) on a Quant Studio 7 cycler (Life Technologies, Thermo Fischer Scientific, Zug, Switzerland) according to the manufacturer's instructions. β -actin or TBP were used as endogenous control for normalizing RNA concentration. The amplification program consisted of 1 cycle at 95 °C for 10 min, followed by 40 cycles with a denaturing phase at 95°C for 30 s and an annealing and elongation phase of 1 min at 60°C. A melting curve analysis was performed after amplification to verify the accuracy of the amplicon. Differences in Ct values between test gene and endogenous controls (TBP, Δ Ct) were calculated and used for statistical analysis. All primers used in this study are reported in **Table S1**.

Co-immunoprecipitation

HAECs were lysed in ice-cold lysis buffer containing 50 mmol/L Tris-HCl, pH 7.4, 100 mmol/L NaF, 15 mmol/L Na₄P₂O₇, 1 mmol/L Na₃VO₄, 1% Triton X-100, and 1 mmol/L phenylmethylsulfonyl fluoride. Lysates were centrifuged at 10'000 g to remove insoluble material. For immunoprecipitation, precleared lysates were incubated with anti-SETD7 (SC-390823, Santa Cruz Biotechnology, Switzerland) and negative isotype control IgG antibodies (Cat. 02-6102, Invitrogen) overnight at 4°C. Lysates were precleared by incubation with 50 μ L of protein A/G-agarose (Santa Cruz Biotechnology, Nunningen, Switzerland) for 2 h at 4°C with rocking. Agarose beads were pelleted by centrifugation at 1000 g. Immunoprecipitated proteins were eluted from the beads by boiling for 5 min in SDS sample buffer and immunoblotted with both anti-SETD7 (1:1000; SC-390823, Santa Cruz Biotechnology, Nunningen, Switzerland) and anti-SEMA3G (1:1000; ab197108, Abcam, Switzerland). Bound antibody was visualized using an enhanced chemiluminescence system (Millipore, Billerica, USA) after incubation of the blot with peroxidase-conjugated secondary antibody for 1 h.

Chromatin immunoprecipitation (ChIP) assay

Chromatin immunoprecipitation was performed in HAECs and mouse gastrocnemius muscle specimens by using the Magna ChIP Assay Kit (Millipore, Billerica, USA), according to the manufacturer's instructions. Briefly, HAECs were fixed for 10 minutes with 37% paraformaldehyde. After stopping cross-linking by addition of 0.1 M glycine, cells were sonicated and centrifuged. ChIP was performed by using 10 µg of anti-H3K4me1 antibody (07-436, Merck KGaA, Darmstadt, Germany), anti-H3K4me3 antibody (P/N 49-1005, Thermo Fischer Scientific) and equivalent amount of mouse IgG as negative control (Cat.02-6102, Invitrogen). Washes and elution of the IP DNA were performed according to the Magna ChIP protocol (17-610, Millipore, Billerica, USA). ChIP quantifications of gene promoters (SEMA3G) were performed by real time PCR (primers are shown in **Table S2**). Quantifications were performed using the comparative cycle threshold method and are reported as the n fold difference in antibody-bound chromatin against the input DNA, as previously reported.¹

Western blot

HAECs and mouse tissues were lysed for immunoblotting (150 mmol/L NaCl, 50 mmol/L Tris pH 7.5, 1 mmol/L EDTA, 0.5% NP-40, 1 mmol/L sodium fluoride, 1 mmol/L DTT, 10 µg/µL Leupeptin, 10 µg/µL Aprotinin, 0.1 mmol/L sodium orthovanadate and 1 mmol/L PMSF). Equal amounts (20 µg) of samples were subjected to electrophoresis followed by semidry transfer onto Polyvinylidene difluoride (PVDF) membrane. The membrane was blocked for 1 h in 5% bovine serum albumin - TBST buffer (0.1% Tween 20, pH 7.4) and incubated overnight at 4°C with the following primary antibodies: anti-SETD7 (1:1000, SC-390823, Santa Cruz Biotechnology, Nunningen, Switzerland), anti-H3K4me1 antibody (1:10,000 dilution, 07-436, Merck KGaA, Darmstadt, Germany), anti-H3K4me3 antibody (1:10,000 dilution, P/N 49-1005, Thermo Fischer Scientific), anti-SEMA3G (1:1000 dilution, ab197108, Abcam, Switzerland), anti-PPARγ (1:1000 dilution, sc-7273, Santa Cruz Biotechnology, Nunningen, Switzerland), anti-SOX18 (1:1000 dilution, sc-376166, Santa Cruz Biotechnology, Nunningen, Switzerland), anti-HO-1 (1:1000 dilution, ADI-OSA-110, Enzo Life Sciences, Inc), anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody (1:10,000 dilution, MAB374, Merck Millipore, Billerica, Massachusetts, USA), anti-Histone 3 (1:10,000 dilution, ab9050, Abcam), and anti-Vinculin (1:10,000 dilution, V4505, Merck). The membranes were subsequently washed 3 times for 10 minutes on a shaker with TBST Buffer (0.1% Tween 20, pH 7.4) and incubated for 1 h with either anti-rabbit (1:10,000 dilution, Cat. 4050-05, Southern Biotechnology, Birmingham, AL, USA) or anti-mouse secondary antibody (1:10,000 dilution, Cat. 1031-05, Southern Biotechnology, Birmingham, AL, USA). Bands were detected by adding HRP substrate (Luminata Forte, Cat.

WBLUF0500, Merck Millipore, Billerica, Massachusetts, USA) and visualized using Amersham Imager 600 (General Electric; Healthcare Europe GmbH, Glattbrugg, Switzerland).

Immunocytochemistry

HAECs were plated at a density of 50.000 cells per chamber slide (4-well Permanox Slide, Cat. 177437, Lab-Tek, Thermo Fisher Scientific NY, USA). Cells were fixed with 4% buffered paraformaldehyde for 10 minutes at room temperature. Following washing with PBS (3 times), cells were permeabilized with 0.1% triton for 10 minutes, blocked with 5% BSA and dissolved in 0.1% Triton followed by incubation with primary antibodies for 1 hour at room temperature. After washing 3 times with PBS, cells were incubated with secondary antibodies for 1 hour, washed again with PBS (x3) and eventually incubated with DAPI for 1 minute (10 µg/mL). The following primary antibodies were used: anti-SETD7 primary antibody (1:300 dilution, sc-7273, Santa Cruz Biotechnology, Nunningen, Switzerland) and anti-IgG antibodies (Alexa 488 labelled anti-rabbit IgG and Alexa 546 labelled anti-mouse IgG secondary antibodies at 1:500 dilution) were used. Images were acquired with an epifluorescence (Leica DMI 6000B, Leica, Wetzlar, Germany) or a confocal (Leica TCS-SP8) microscope.

Interrogation of the UCSC genome browser

The enrichment of different histone marks on SEMA3G promoter was investigated by using the UCSC genome browser (<https://genome.ucsc.edu/>). ChIP-seq histograms were generated and stored as a single session on UCSC genome browser: https://genome-euro.ucsc.edu/s/shafeeq.mohammed%40uzh.ch/hg38_SET7_SHAFEEQ.

Streptozotocin-induced diabetic mice

Diabetes was induced in 8-week-old male mice by a single high dose of streptozotocin (STZ, 175 mg/Kg, via intraperitoneal injection) dissolved in sterile 0.025 M citrate buffer (PH 4.5) and injected within 10 minutes. Control animals received an equal volume of citrate buffer. Mice were housed in temperature-controlled cages (20–22°C), fed ad libitum, and maintained on a 12:12-h light/dark cycle. Blood glucose was measured using a point-of-care glucometer (Freestyle; Abbott, Abbott Park, IL). Diabetes onset was confirmed for blood glucose \geq 300 mg/dL 48 hours after STZ injection, but only mice with persistent hyperglycaemia in the subsequent 2 weeks were used for induction of hindlimb ischemia. In our study, four groups of mice were investigated: 1) non-diabetic mice treated with vehicle (control group); 2) diabetic mice treated with vehicle; 3) diabetic mice treated with (*R*)-PFI-2, and 4) non-diabetic mice treated with (*R*)-PFI-2 (n=8-12/group). Animal experiments were conducted in accordance with the guidelines approved by the Institutional Animal Care Committee of the University of Padua, Italy.

(R)-PFI-2 treatment in mice

(R)-PFI-2 hydrochloride (Cat. HY-18627A, MedChemExpress) at the final dose of 95 mg/Kg or vehicle (DMSO:Corneal oil at 1:1 ratio) were given orally in mice 5 days before the induction of hindlimb ischemia and continued for the following 14 days. Body weight and blood glucose levels were monitored before and after treatment. At the end of the study, mice were harvested, and liver specimens were tested for possible toxicity by the ALT (Alanine transaminase) assay.

Hindlimb ischemia

Animals were sedated with 5% inhaled Isoflurane (Iso-Vet, Piramal Healthcare, UK) and kept at 2-3% for maintenance. Analgesia was achieved with 5 mg/kg Tramadol. The femoral artery and the vein were surgically dissected from the femoral nerve, then cauterized by applying an electric current with a bipolar tweezer end excised from the proximal end to popliteal bifurcation. Hindlimb perfusion was measured with Perimed PeriscanPim II Laser Doppler System (PerimedAB, Sweden) at 1, 7 and 14 days after ischemia. Muscles, blood, and tissues were collected for analysis 14 days after surgery. The ratio of perfusion between the ischemic limb and the contralateral limb was calculated and used as an index of % blood flow recovery, as previously reported.²

Aortic ring assay in *db/db* mice

The mouse aortic ring assay was used as a model for the ex-vivo angiogenesis study. Dorsal aorta from a freshly sacrificed male C57BKS/Lepr^{db} (*db/db*) mice (purchased from The Jackson Laboratory) was taken out in a sterile manner and rinsed in ice-cold PBS. Aorta rings were cut into 1 mm-long pieces using a sterile surgical blade. Each ring was placed in a matrigel-pre-coated 24-well plate and treated with the SETD7 inhibitor (R)-PFI-2 (40 μM) or the inactive enantiomer (S)-PFI-2. On day 7, the rings were photographed by phase-contrast microscopy and the microvessel outgrowth was quantified as previously reported.³

In vivo matrigel plug assay

Neo-vascularization was evaluated by in vivo Matrigel plug assay. 12-week old *db/db* mice were injected with 200 μl of Matrigel subcutaneously in presence of the SETD7-inhibitor (R)-PFI-2 (Cat. HY-18627A, MedChemExpress, at the final dose of 95 mg/Kg) or vehicle (DMSO:Corneal oil at 1:1 ratio). After 14 days, mice were euthanized and the Matrigel plug was excised and collected for immunofluorescence. Matrigel plus assay experiments were approved by the Kommission für Tierversuche des Kantons Zürich, Switzerland (ZH169/2020).

Alanine transaminase (ALT) assay

ALT assay (ab105134, Abcam) was employed to investigate liver toxicity in mice treated orally with (*R*)-PFI-2. The assay was performed following the manufacturer's instructions. Briefly, liver was harvested from diabetic and non-diabetic mice treated with (*R*)-PFI-2 or vehicle. Liver specimens (50 mg) were washed with cold PBS twice, incubated with 500 μ l of ALT buffer and immediately snap-frozen in liquid nitrogen.

Detection of SEMA3G levels in EC conditioned media

SEMA3G levels in EC conditioned media and mouse plasma were assessed by using a commercially available ELISA kit from mybiosource, UK (Human SEMA3G: Cat.no MBS3803045; Mouse SEMA3G: MBS3803045). Mouse plasma samples were collected and centrifuged for 30 minutes at 3000 \times g at 4°C within 30 minutes of collection. Cell culture conditioned media was collected and centrifuged at 1200 RPM for 5 min at 4°C to remove cell debris and particulates. The ELISA assay for SEMA3G was performed following the manufacturer's recommendation (available at <https://www.mybiosource.com/human-elisa-kits/sema3g/3803045>).

RNA-seq in specimens from DM patients with PAD (Cohort 1)

Human vascular tissue samples were collected from DM patients who underwent open surgical interventions in our Department of Vascular Surgery (USZ/UZH, Cohort 1, **Table S3**). Healthy control aortas were obtained during kidney transplantation and provided by the Department of Visceral Surgery (USZ/UZH). The local ethics committee (Cantonal Ethics Committee Zurich, Switzerland; BASEC-Nr. 2020-00378) approved the tissue sample collection and analysis procedure. For RNA-seq, the RNA was extracted using the TRIZOL reagent (ThermoFisher). The quality and quantity of the isolated RNA was determined with TapeStation 4150 (Agilent, Switzerland). The library preparation and RNA sequencing were performed in collaboration with the Functional Genomic Center Zurich (FGCZ). Briefly, RNA library preparation and depletion of ribosomal RNA was performed using the SMARTer Stranded Total RNA-seq Kit from Clontech/Takara Bio (USA) according to the manufacturer's protocol. The fragmented samples were reverse transcribed to cDNA, end-repaired, polyadenylated, TruSeq adapters were ligated, and selectively enriched by PCR. The quality of the enriched libraries was validated on a TapeStation 2200 (Agilent, Germany). RNA sequencing was performed on the Illumina NovaSeq 6000 (Illumina, Germany) with 200M reads in 100 cycles. The raw data for the RNAseq are reported in the **Appendix**.

Collection of skeletal muscle specimens from DM patients with PAD (Cohort 2)

Collection of human samples from Cohort 2 (**Table S4**) was approved by the MultiMedica Research Ethics Committee and was conducted according to the principles outlined in the Declaration of Helsinki. Specimens were collected from: i) different anatomical locations of the lower extremities from non-diabetic control participants, referred for investigations/therapeutic interventions related to leg varicosity; or ii) foot muscle from T2D participants at the occasion of minor amputation for chronic limb ischemia (defined as TcPO₂ < 30 mmHg or ankle pressure >70 mmHg according to the Trans-Atlantic Inter-Society Consensus Document on Management of Peripheral Arterial Disease [TASC]. **Table S5** summarizes the molecular analyses done in each cohort.

Immunofluorescence in mouse and human sections

Formalin-fixed paraffin-embedded (FFPE) tissue samples (human) and OCT-embedded tissue samples (mice) were employed for immunofluorescence analyses. Slides (5 µm thick) were dried overnight at room temperature and rehydrated by dipping in xylene (mixed isomers) 2 times for 10 minutes each, followed by dipping in different concentrations of ethanol (100%, 95%, 70% and 50%) for 5 min each. Slides were next rehydrated with distilled water for 10 minutes followed by blocking (1% horse serum in PBS) for 30 minutes at room temperature and incubation with the following primary antibodies (1:250 dilution overnight at 4 degrees): anti-SETD7 (SC-390823, Santa Cruz Biotechnology, Nunningen, Switzerland), anti-SEMA3G (ab197108, Abcam, Switzerland), anti-H3K4me1 antibody (07-436, Merck KGaA, Darmstat, Germany), anti-CD31 (MA5-13188, Thermo Fisher Scientific). The following secondary antibodies were used: Alexa 546 labeled anti-rabbit IgG (2086712, Thermo Fischer Scientific) and Alexa 488 labeled anti-mouse IgG (2127435, Thermo Fischer Scientific). Images were acquired using a fluorescent microscope (Olympus BX51, Olympus Corporation, Tokyo, JP).

***In silico* prediction of SEMA3G-target genes**

In silico prediction analysis for SEMA3G-target genes was performed by using the CiiDER software (www.ciiider.org). Homo sapiens GRCh38.94 (standard genome reference) and Ref seq fir SEMA3G; >NM_020163.3 Homo sapiens were employed to identify putative binding sites.

Statistical analysis

Comparisons of continuous variables were performed using unpaired two-sample t-test. Multiple comparisons between normally distributed variables were performed by one-way analysis of variance (ANOVA), followed by Bonferroni correction or Benjamini and Hochberg Discovery

Rate (FDR). The latter was used when the n number was equal or lower than the number of experimental groups. Probability values <0.05 were considered statistically significant. All analyses were performed with GraphPad Prism Software (version 7.03).

Primers		
Gene	Forward	Reverse
<i>Semaphorin 3A</i>	5'-GGTTGCCCAGCTCCCTTTAC-3'	5'-TATCTTGTCGTCTTGTCGTCTCT-3'
<i>Semaphorin 3B</i>	5'-CCAGTGCCAAGAGGCGGTTC-3'	5'-AGCACCTGGGTGTGGGCTGT-3'
<i>Semaphorin 3B</i>	5'-GGATTTTTGTAGTTGGTGGAGT-3'	5'-GAGAGAGGAGTTAGATAGATTTGA-3'
<i>Semaphorin 3C</i>	5'-ATCGCAGCGCTGAGATTCCTT TAC-3'	5'- GATGCGCTTGTCCTCCAGTCC -3'
<i>Semaphorin 3D</i>	5'-TGGGACATCGAAGACAGCAT-3'	5'-AAAGTGTGCTCCTGGGCTTT-3'
<i>Semaphorin 3E</i>	5'-CTGTTTCACCTGGAATCACCC-3'	5'-GTGCGGATATGGGCCAGTC-3'
<i>Semaphorin 3F</i>	5'-CGCGCCCAGGCCACACCA-3'	5'-CATCGGGCGGAGGGCACCAT-3'
<i>Semaphorin 3G</i>	5'-CTGAGGAAGTGGTTCTGGAGGA-3'	5'-GCCGTAAGTCTCACATTGGTGC-3'
<i>Plexin -D1</i>	5'-ACCGAGCAGTGGATGATTCT-3'	5'- TCCTGGTGAACGACACAGAC-3'
<i>NRP1-1</i>	5'-TGAGCCCTGTGGTTTATTCC-3'	5'-CGTACTCCTCTGGCTTC GG-3'
<i>NRP-2</i>	5'-GGTCGCCGGCGGGGATTGG-3'	5'-TCGGTGGGGTAGGGGGTGGTTGTC-3'
<i>CCL2</i>	5'-AGAATCACCAGCAGCAAGTGTC-3'	5'-TCCTGAACCCACTTCTGCTTGG-3'
<i>CX3CL1</i>	5'-ACAGCACCACGGTGTGACGAAA-3'	5'-AACAGCCTGTGCTGTCTCGTCT-3'
<i>TFRC</i>	5'-ATCGGTTGGTGCCACTGAATGG-3'	5'-ACAACAGTGGGCTGGCAGAAAC-3'
<i>VCAM1</i>	5'-GATTCTGTGCCACAGTAAGGC-3'	5'-TGGTCACAGAGCCACCTTCTTG-3'
<i>CXCL8</i>	5'-GAGAGTGATTGAGAGTGGACCAC-3'	5'-CACAAACCCTCTGCACCCAGTTT-3'
<i>TAGLN</i>	5'-TCCAGGTCTGGCTGAAGAATGG-3'	5'-CTGCTCCATCTGCTTGAAGACC-3'
<i>RASD1</i>	5'-CACCGCAAGTTCTACTCCATCC-3'	5'-GGTTGTCCAGACTGAACACCAG-3'
<i>TROAP</i>	5'-TAAGGTCACTGGAGGGTTCTGG-3'	5'-CCTCTACTTCTGTCTCAACAGC-3'
<i>ZWINT</i>	5'-AGGCATCTTGGAACCTGTAGGC-3'	5'-GGAAATCCGCTACCTGAAGCTG-3'
<i>SETD7</i>	5'-CGTATGTAGACGGAGAGCTGAAC-3'	5'-CTCCTACAAGGCTTCTCCATC-3'
<i>ADM5</i>	5'-CGGCTCGGTTCCAGGATTCC-3'	5'-GATAGGCAAATGAAGCCGAGG-3'
<i>LRRC4</i>	5'-CGTGAAGTGGTTGCTGCCAAT-3'	5'-GTTGCCTGCAACATTGGTCACC-3'
<i>SNORD17</i>	5'-GCTGCACTGACCTTCTTCCA-3'	5'-TGCAGGGCATTTCAGGCTA-3'
<i>BCL9L</i>	5'-CCGCTCTACCACAATGCCATCA-3'	5'-CTGAGTTCAGGTGCATCTGGCT-3'
<i>MGARP</i>	5'-CCGCTCTACCACAATGCCATCA-3'	5'-CTGAGTTCAGGTGCATCTGGCT-3'
<i>BGN</i>	5'-TTGAACCTGGAGCCTTCGATGG-3'	5'-TTGGAGTAGCGAAGCAGGTCCT-3'
<i>PTGS1</i>	5'-GATGAGCAGCTTTTCCAGACGAC-3'	5'-AACTGGACACCGAACAGCAGCT-3'
<i>DUSP23</i>	5'-AGATCGACCGCTTCGTGCAGAT-3'	5'-CCGCTCCTTACCAGGTAACAG-3'
<i>AGFG2</i>	5'-TACTGACCTGCTGGCTGACATC-3'	5'-GTTGGCAAAGCCTCCTTGGGAA-3'
<i>MMP1</i>	5'-ATGAAGCAGCCAGATGTGGAG-3'	5'-TGGTCCACATCTGCTCTTGGCA-3'
<i>HMOX1</i>	5'-CCAGGCAGAGAATGCTGAGTTC-3'	5'-AAGACTGGGCTCTCCTTGTTC-3'
<i>SULF2</i>	5'-GGACTCCTTCTTGGTGGAGAGA-3'	5'-TACTCAGCACGCTGACACAGGT-3'
<i>JUP</i>	5'-ACCAGCATCCTGCACAACCTCT-3'	5'-GGTGATGGCATAGAACAGGACC-3'
<i>LYVE1</i>	5'-GCCGACAGTTTGAGCCTATTG-3'	5'-CCGAGTAGGTACTGTCACTGAC-3'
<i>HLX</i>	5'-ACGAGGCTTCTGCAATCCTGAG-3'	5'-GTGTCCTTCGTGAGCACAGCAT-3'
<i>NID2</i>	5'-GAGCTGTACCACTACTCCGACT-3'	5'-TGTCTGGTGGATGCGGTAGGA-3'
<i>CCL23</i>	5'-CCGTGTTCACTCCTGGAGAGTT-3'	5'-GCTTCAGATTCTCACGAAACC-3'
<i>PGF</i>	5'-GGCGATGAGAATCTGCACTGTG-3'	5'-ATTCGACGGAACGTGCTGAGA-3'
<i>CXCR4</i>	5'-CTCCTCTTTGTCATCAGCTTCC-3'	5'-GGATGAGGACACTGCTGTAGAG-3'
<i>FAM74A3</i>	5'-GCAACAAAAGCAGGGTAGGG-3'	5'-GGGGTAGCTGTCTTGGTTT-3'
<i>SETD7 (M)</i>	5'-TTGACGGAGAGATGCTCGAAGG -3'	5'-GAAGGAGAGCATCGCTGGAGAT-3'
<i>SEMA 3G (M)</i>	5'-GAAGAGGTGGTTTTGGAGGAGC-3'	5'-CTGCCGTAAGTCTCACTGGT-3'

Table S1. Primers used for real-time PCR

List of primers		
Target	Forward	Reverse
<i>Promoter</i>	5'-TCCAGCAAAGTGACAAGTGG-3'	5'-AGAGTCTGGCCAAAGCTTCA-3'
<i>Initiator</i>	5'-CTCAGTCCCATTTCCTTT-3'	5'-CCCAGGTCAGTGATGGTTCT-3'
<i>CAT box</i>	5'-GCCAGTTTGGAAGGTCTCTG-3'	5'-GAGATGTCCACAAATGCAGGT-3'

Table S2. Primers used for ChIP-PCR assays.

	Controls ^a (n=13)	PAD with T2D ^c (n=20)	<i>P value</i>
Clinical phenotype			
Age (years)	52 [8]	75 [16]	<i>4.99*10⁻⁵</i>
Gender (F) (%)	46	35	<i>5.22*10⁻¹</i>
Smokers (%)	0	47	<i>3.42*10⁻³</i>
Hypertension (%)	0	100	<i>1.54*10⁻⁸</i>
Hypercholesterolemia (%)	0	79	<i>1.11*10⁻⁵</i>
CKD (%)	0	79	<i>1.11*10⁻⁵</i>
CVD (%)	0	53	<i>1.61*10⁻³</i>
PAD (%)	0	100	<i>1.54*10⁻⁸</i>
Stroke (%)	0	11	<i>2.27*10⁻¹</i>
Medications			
Antiplatelet agents (%)	0	58	<i>7.08*10⁻⁴</i>
ACE-i/ARBs (%)	0	47	<i>3.42*10⁻³</i>
Beta-blockers (%)	0	74	<i>3.68*10⁻⁵</i>
Diuretics (%)	0	58	<i>7.08*10⁻⁴</i>
Statins (%)	0	74	<i>3.68*10⁻⁵</i>
Insulin (%)	0	50	<i>3.11*10⁻³</i>
Metformin (%)	0	45	<i>2.31*10⁻³</i>
DPP4 inhibitors (%)	0	10	<i>5.58*10⁻¹</i>
Suphonylureas (%)	0	20	<i>4.33*10⁻²</i>
Histopathological scores			
Infiltrates (n)	1 [0]	1 [0]	<i>5.58*10⁻¹</i>
Smooth muscle cells (n)	2 [0]	2 [1]	<i>5.62*10⁻³</i>
Neovessels (n)	1 [1]	1 [1.25]	<i>2.85*10⁻¹</i>
Thrombus (n)	0 [0]	1 [2]	<i>1.75*10⁻⁴</i>
Atherosclerosis (n)	0 [0]	1 [1]	<i>9.89*10⁻⁵</i>
Calcification (n)	0 [0]	0 [0.25]	<i>5.85*10⁻²</i>
Adventitia (n)	2 [1]	1 [2]	<i>1.21*10⁻¹</i>

Table S3. Clinical characteristics of Cohort 1. Data are presented as median [IQR] or % and compared by the Mann-Whitney U test for continuous variables and the χ^2 test for categorical variables. Statistical significance is highlighted in italic. *ACE-i: angiotensin-converting enzyme inhibitors; ARBs: angiotensin receptor blockers; CKD: chronic kidney disease; CVD: cardiovascular disease; PAD: peripheral artery disease; T2D: type 2 diabetes.*

	Controls (n=6)	PAD with T2D (n=6)	p-value
Age (mean \pm SD)	56 \pm 14.09	63.83 \pm 15.12	0.5738
Sex (% of female)	4 (66.67%)	2 (33.33%)	0.5671
BMI (mean \pm SD)	25.23 \pm 3.75	30.71 \pm 6.42	0.2298
Creatinine (mean \pm SD)	0.71 \pm 0.08	1.17 \pm 0.52	0.0651
Smoking			0.7143
No	4 (66.67%)	1 (25%)	
Yes	1 (16.67%)	1 (25%)	
Previous	1 (16.67%)	2 (50%)	
Comorbidities			
PAD (n, %)	1 (16.67%)	6 (100%)	0.0152
Hypertension (n, %)	2 (33.33%)	5 (83.33%)	0.2424
CAD (n, %)	0 (0%)	3 (50%)	0,1818
MI (n, %)	0 (0%)	1 (16.67%)	1.0000
CABG (n, %)	0 (0%)	1 (16.67%)	1.0000
PTA (n, %)	1 (16.67%)	5 (83.33%)	0.0801
PTCA (n, %)	0 (0%)	1 (16.67%)	1.0000
CKD (n, %)	0 (0%)	1 (16.67%)	1.0000
Retinopathy (n, %)	0 (0%)	1 (16.67%)	1.0000
Neuropathy (n, %)	0 (0%)	5 (83.33%)	0.0152
Medical therapy			
Insulin (n, %)	0 (0%)	4 (66.67%)	0.0606
Statin (n, %)	0 (0%)	3 (50%)	0.1818
Beta blockers (n, %)	1 (16.67%)	5 (83.33%)	0.0801
Aspirin (n, %)	1 (16.67%)	5 (83.33%)	0.0801
Clopidogrel (n, %)	0 (0%)	6 (100%)	0.0022

Table S4. Clinical characteristics of Cohort 2. BMI: body mass index, PAD: peripheral artery disease, CAD: coronary artery disease, MI: myocardial infarction, CABG: coronary artery bypass graft, PTA: percutaneous angioplasty, PTCA: percutaneous transluminal coronary angioplasty. Statistics: Wilcoxon for continuous variables, Fisher for dichotomic variables.

	Cohort 1*	Cohort 2**
RNAseq analysis	X	
Validation of SETD7/SEMA3G gene expression	X	X
Immunofluorescence for SETD7/SEMA3G in gastrocnemius muscle specimens		X
ChIP assays in gastrocnemius muscle specimens		X

Table S5. Molecular analyses performed in the 2 cohorts of DM patients with PAD. The table summarizes the molecular analyses done in each cohort. *Vascular tissue samples were collected from DM patients with PAD who underwent open surgical interventions at the Department of Vascular Surgery at the University Hospital Zurich; ** DM patients with PAD recruited at the Diabetic Foot Department, IRCCS MultiMedica, Milan, Italy.

Supplemental figures

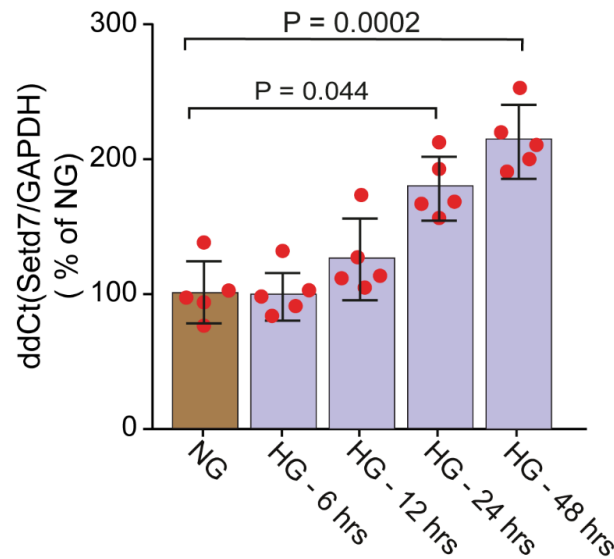


Figure S1. HAECs were cultured in growth factor-free medium and exposed to normal glucose (NG, 5 mM/L) or high glucose (HG, 25 mM/L) concentration. Real time PCR showing SETD7 expression in HAECs exposed to NG and HG at different time points (6, 12, 24 and 48h). Data are expressed as mean \pm SD and shown as a percentage of control. Multiple comparisons were performed by one-way ANOVA followed by Bonferroni post hoc test where appropriate. A p value <0.05 was considered significant.

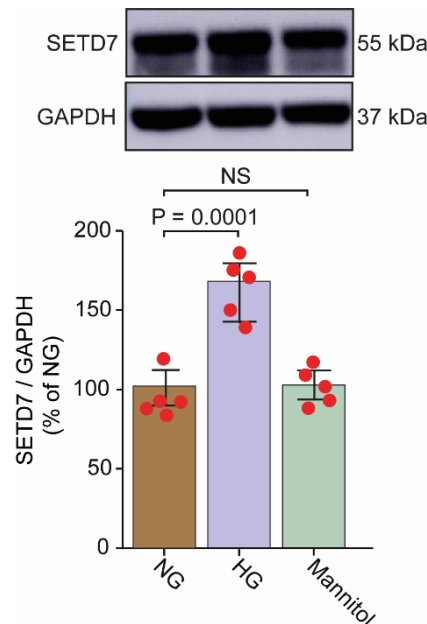


Figure S2. HAECs were cultured in growth factor-free medium and exposed to normal glucose (NG, 5 mM/L), high glucose (HG, 25 mM/L) or mannitol (20 mM/L). Representative Western blot and relative quantification showing SETD7 protein levels in HAECs exposed to NG, HG and Mannitol (used as an osmotic control). Data are expressed as mean \pm SD and shown as a percentage of control. Comparisons were performed by one-way ANOVA followed by Bonferroni post hoc test or Student's t test where appropriate. A p value <0.05 was considered significant.

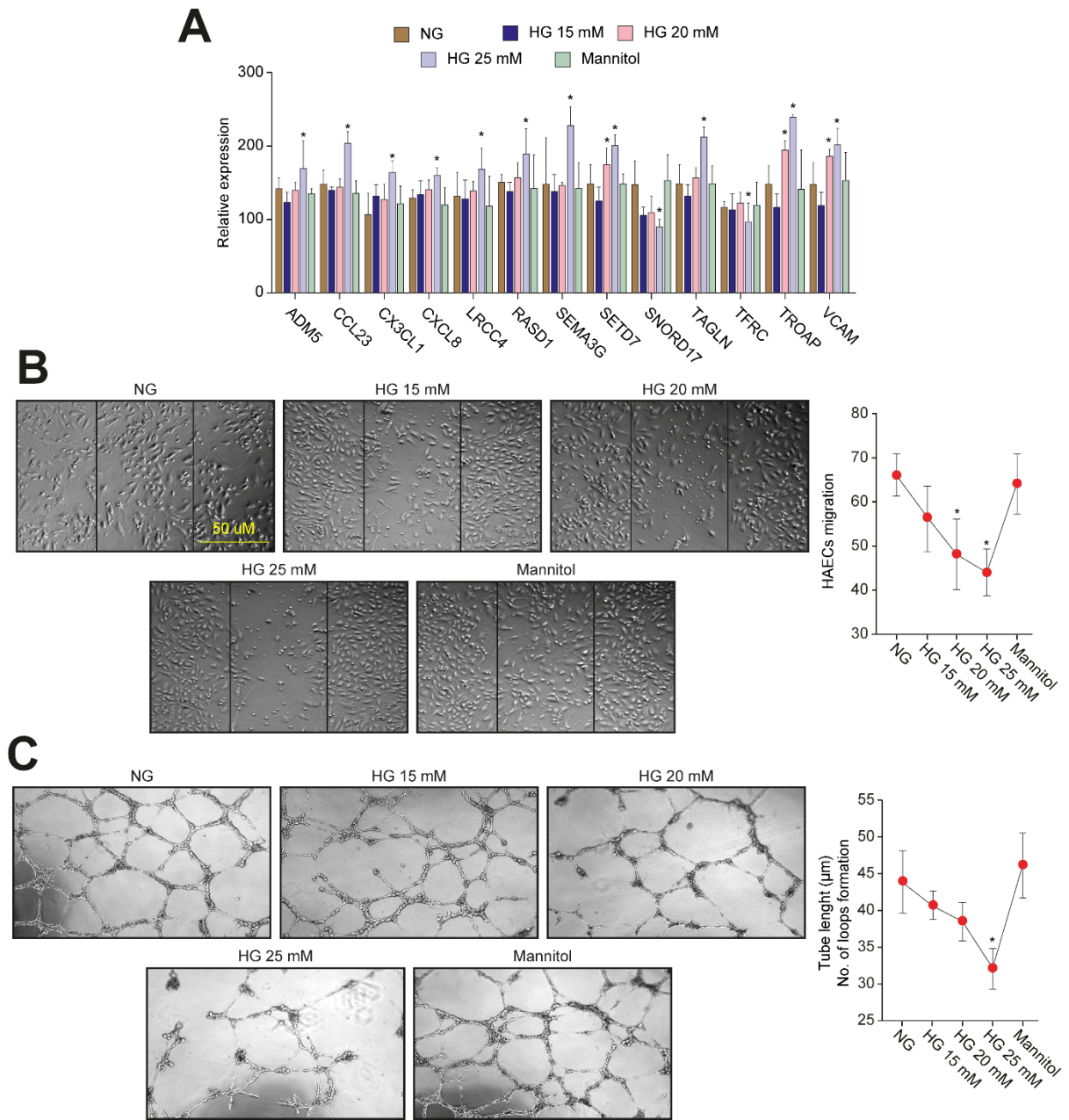


Figure S3. A) Real time PCR showing expression of top-ranking gene transcripts (based on RNAseq) in HAECs exposed to different glucose concentrations (15-25 mM). Mannitol was used as an osmotic control; **B-C)** Endothelial cell migration and tube formation assays performed at different glucose concentrations. Mannitol was used as an osmotic control. Data are expressed as mean \pm SD. Multiple comparisons were performed by one-way ANOVA followed by Bonferroni post hoc test where appropriate. A p value <0.05 was considered significant.

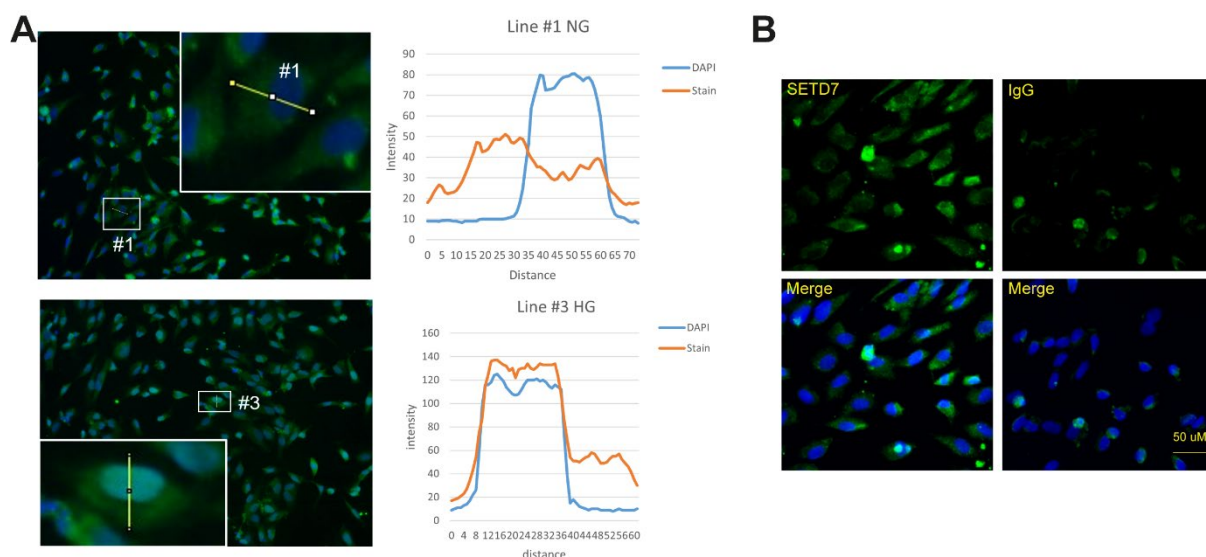


Figure S4. A) Immunostaining and Pearson's coefficient showing SETD7 cellular localization (green) in HAECs treated with NG and HG. Nuclei are stained with DAPI (blue). **B)** Immunostaining showing specificity of SETD7 signal (green) in HAECs. IgG were used as a control. A p value <0.05 was considered significant. HG, high glucose; NG, normal glucose.

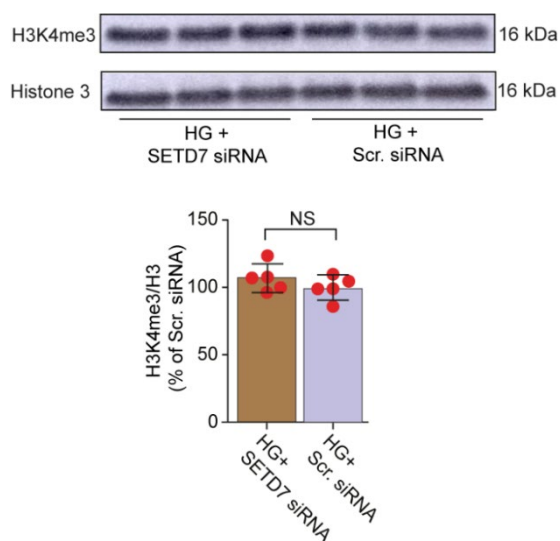


Figure S5. Representative Western blot and relative quantification showing H3K4me3 levels in HAECs treated with HG, in presence of SETD7-siRNA or scramble-siRNA. Histone 3 was used as loading control. Comparisons were performed by Student's t test. A p value <0.05 was considered significant.

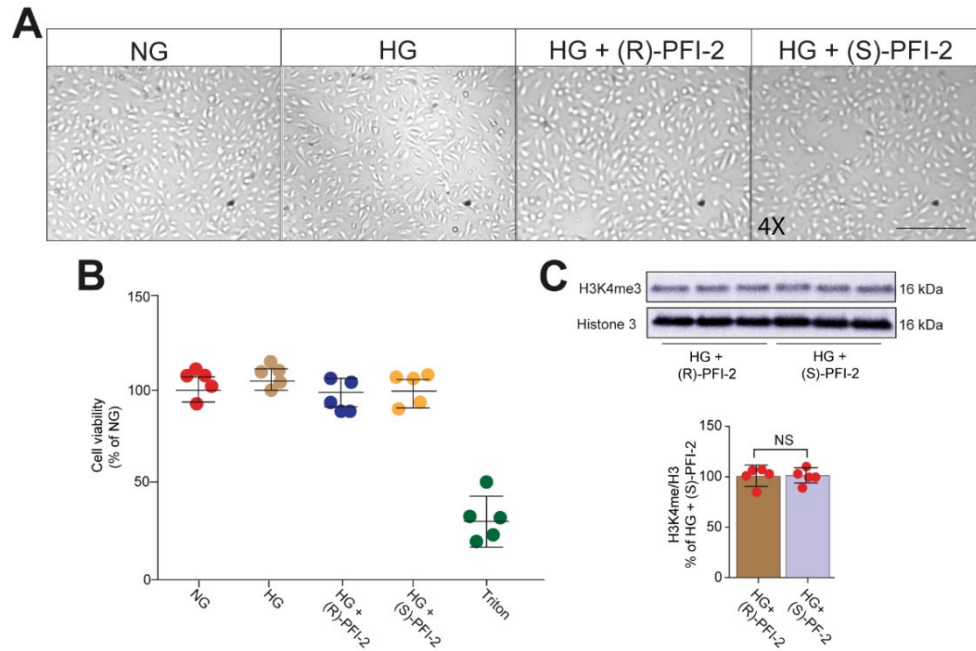


Figure S6. **A)** Microscopic images showing HAECs morphology across the different experimental groups. **B)** MTT assay showing cell viability in HAECs treated with NG and HG, in presence of the selective SETD7 inhibitor (R)-PFI-2 or its inactive enantiomer (S)-PFI-2. Triton was used as a positive control. **C)** Representative Western blot and relative quantification showing H3K4me3 levels in HAECs treated with HG, in presence of SETD7 inhibitor (R)-PFI-2 or the inactive enantiomer (S)-PFI-2. Histone 3 was used as a loading control. Data are expressed as mean \pm SD and shown as a percentage of control; n=5/group. Comparisons were performed by one-way ANOVA followed by Bonferroni post hoc test or Student's t test where appropriate. A p value <0.05 was considered significant.

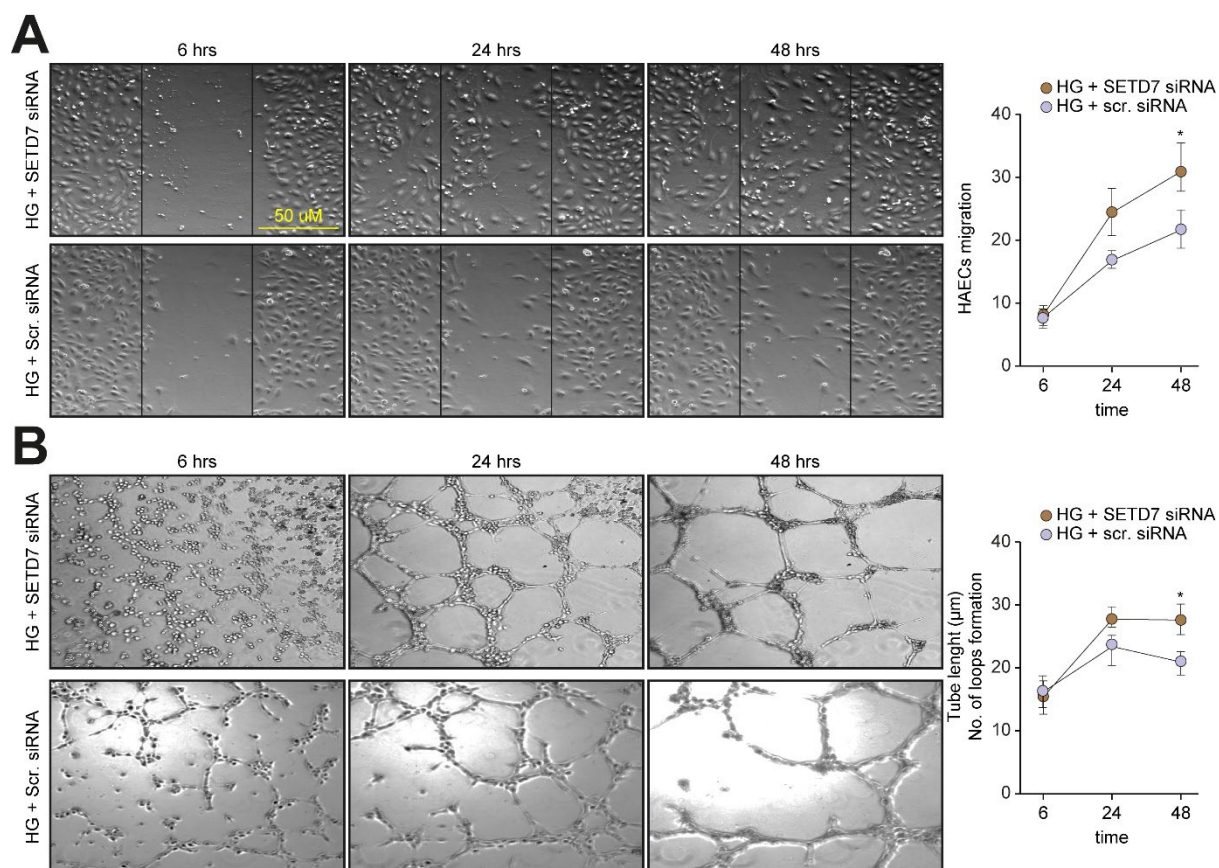


Figure S7. HAECs were cultured in growth factor-free medium and exposed to high glucose (HG, 25 mM/L), in the presence of SETD7 siRNA or scrambled siRNA (scr.siRNA). **A-B)** Endothelial cell migration and tube formation assays in HAECs with and without SETD7 depletion. Images were acquired at three different time points (6, 24 and 48h). Data are expressed as mean \pm SD and shown as a percentage of control; n=5/group. Comparisons were performed by one-way ANOVA followed by Bonferroni post hoc test or Student's t test where appropriate. A p value <0.05 was considered significant.

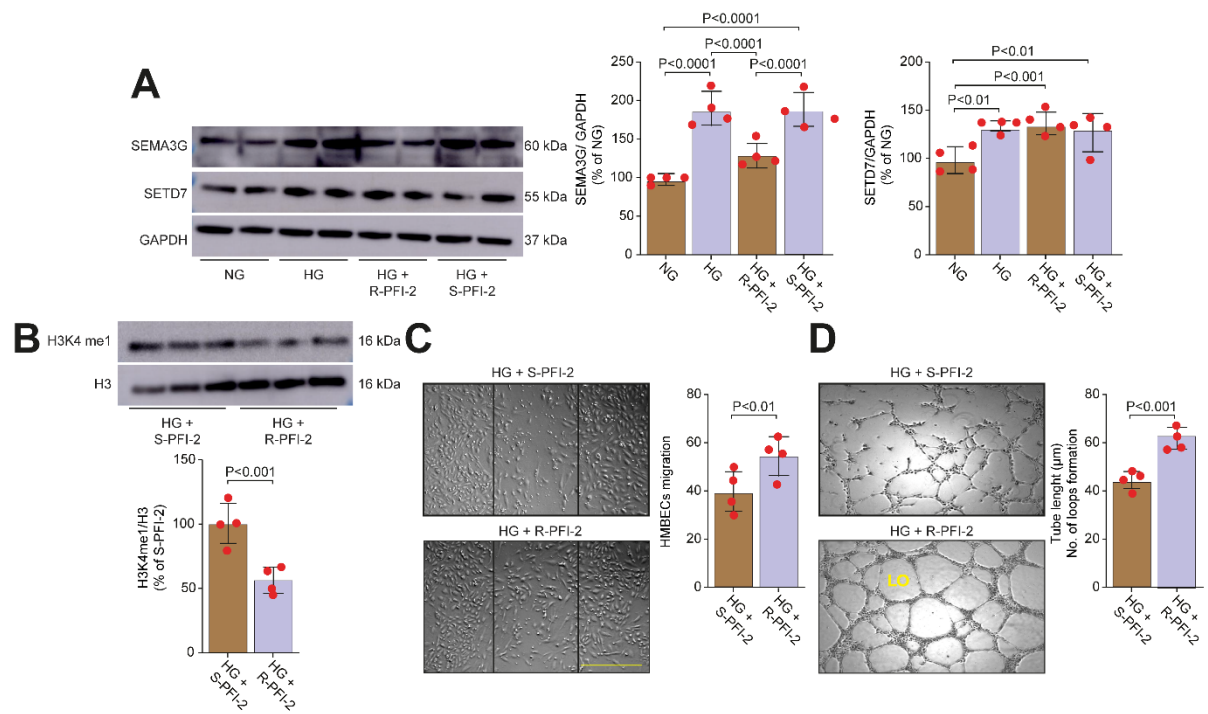


Figure S8. Human microvascular endothelial cells (HMECs) were cultured in growth factor-free medium and exposed to normal glucose (NG, 5 mM/L) or high glucose (HG, 25 mM/L), in the presence of R-PFI-2 or the inactive enantiomer S-PFI-2. **A)** Western blot and densitometric quantification showing upregulation of SETD7 and SEMA3G in HG-treated HMECs. SEMA3G expression was reduced following SETD7 inhibition by R-PFI-2. **B)** Representative Western blot and relative quantification showing H3K4me1 levels in HMECs treated with HG, in presence of the SETD7 inhibitor (R)-PFI-2 or the inactive enantiomer (S)-PFI-2. Histone 3 was used as a loading control. **C-D)** Treatment with R-PFI-2 rescues HG-induced impairment of angiogenic properties in HMECs, as shown by EC migration and tube formation assays. Data are expressed as mean \pm SD; n=4/group. Comparisons were performed by one-way ANOVA followed by Bonferroni post hoc test or Student's t test where appropriate. A p value < 0.05 was considered significant.

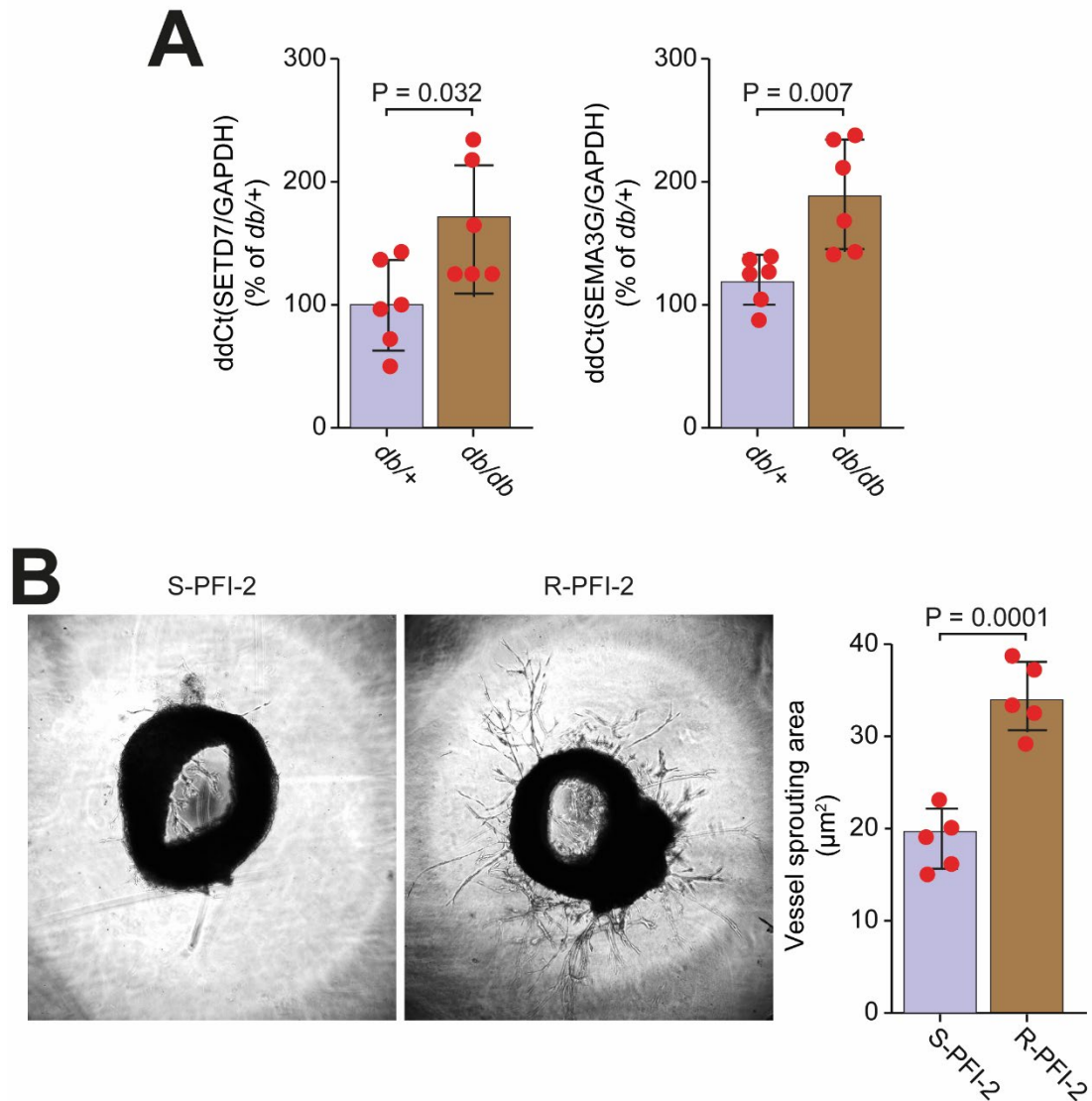


Figure S9. A) Real time PCR show increased gene expression of SETD7 and SEMA3G in aortas from *db/db* mice as compared to *db/+* animals (control group). **B)** Ex vivo aortic rings assay and relative quantification in *db/db* mice, in the presence of the SETD7 inhibitor R-PFI-2 or the inactive enantiomer S-PFI-2. SETD7 inhibition by R-PFI-2 enhanced microvessel outgrowth as compared to S-PFI-2. Data are expressed as mean \pm SD. Comparisons were performed by Student's t test. A p value <0.05 was considered significant.

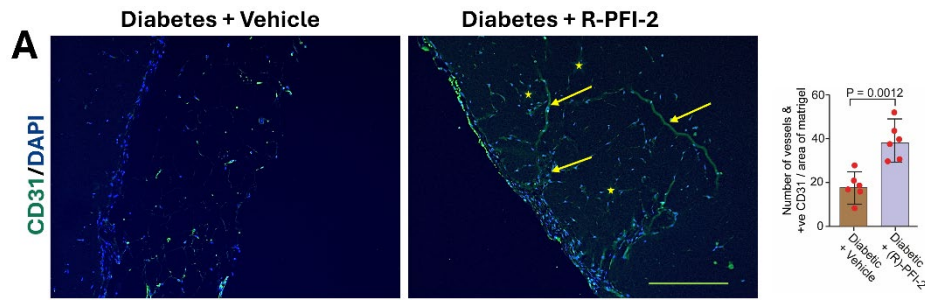


Figure S10. A) Immunofluorescence staining and relative quantification of frozen Matrigel section with antibody against endothelial marker (CD31) (green fluorescent) and DAPI (blue). Large vessels are indicated by arrowheads, small vessels-by asterisks. Scale bars-100 μ m. Neo-vascularization was evaluated by in vivo Matrigel plug assay. Diabetic mice were injected with 200 μ l of Matrigel subcutaneously in presence of SETD7-inhibitor (R)-PFI-2 or vehicle. After 14 days, mice were euthanized and the Matrigel was excised and analyzed.

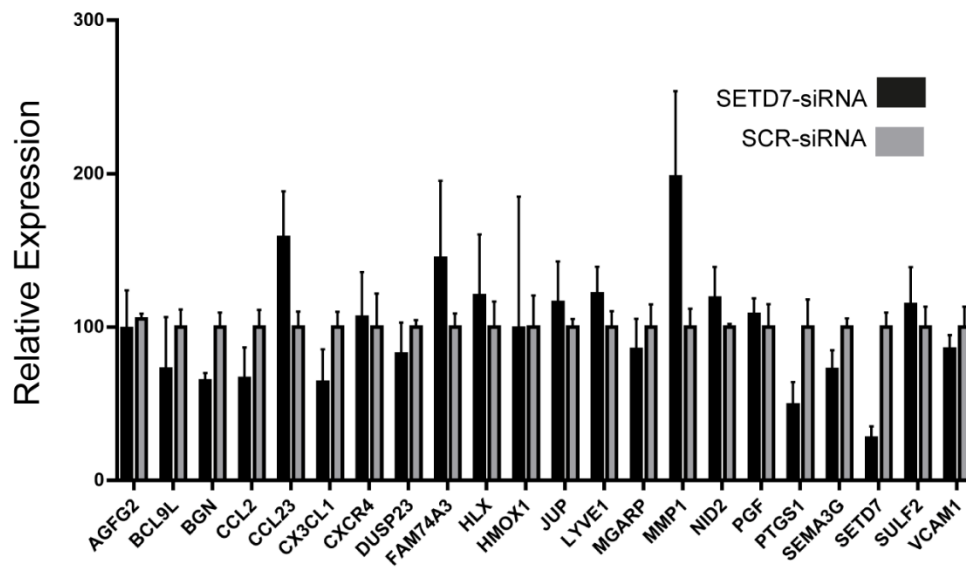


Figure S11. Real-time PCR validation of top-ranking genes (based on RNAseq data) in HG-treated HAECs treated with SETD7-siRNA or Scr.sRNA. Comparisons were performed by Student's t test.

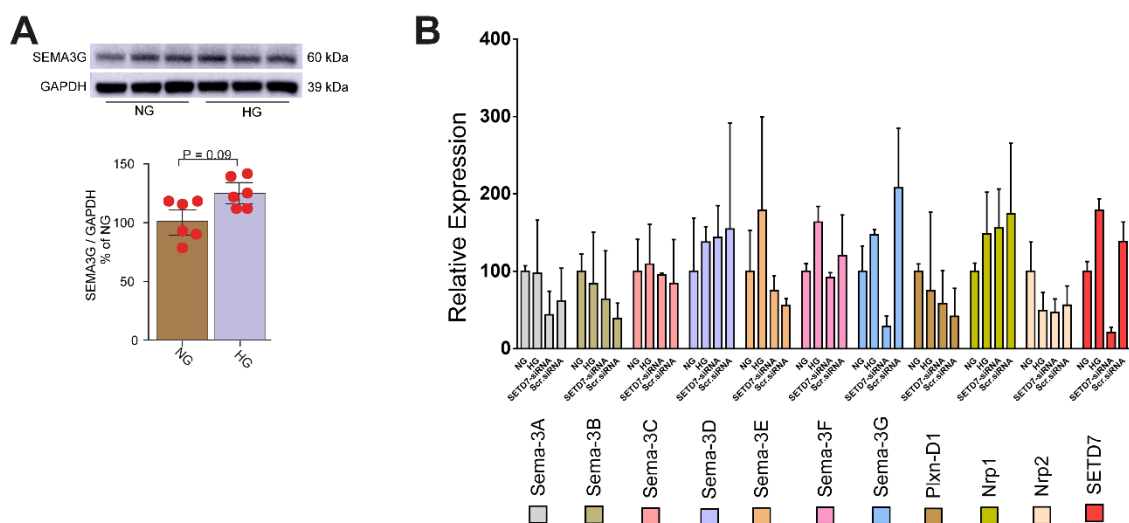


Figure S12. A). Representative Western blot and relative quantification showing SEMA3G protein levels in HAECs treated with NG and HG. **B)** Real-time PCR showing the expression of sema3 family genes in HAECs treated with HG in presence of SETD7-siRNA and Scr.sirRNA. SETD7 depletion preferentially affects *sema3g* expression.

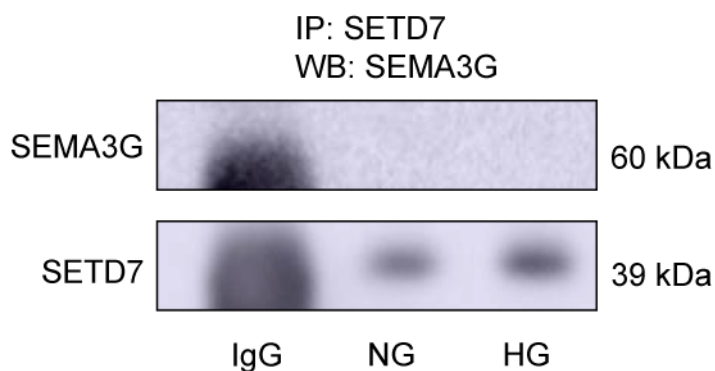


Figure S13. Coimmunoprecipitation showing the interaction of SETD7 with SEMA3G in NG and HG-treated HAECs. IgG were used as control.

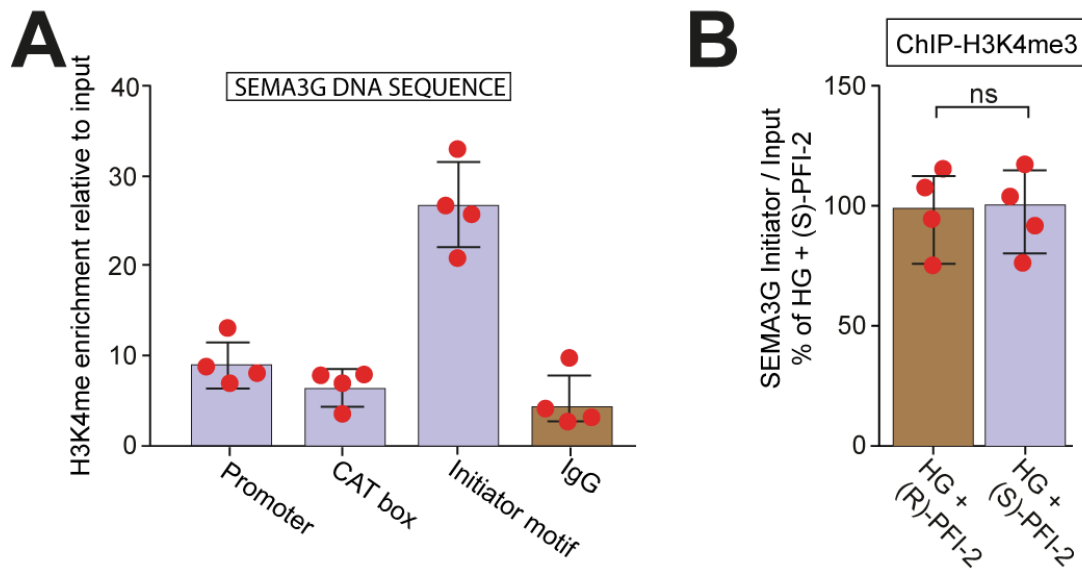


Figure S14. A) ChIP assay showing enrichment of H3K4me1 on different sites of SEMA3G. **B)** ChIP assay showing enrichment of H3K4me3 on SEMA3G initiator region in HG-treated HAECs in presence of (R)-PFI-2 or (S)-PFI-2. Comparisons were performed by Student's t-test. A p value <0.05 was considered significant. HG, high glucose.

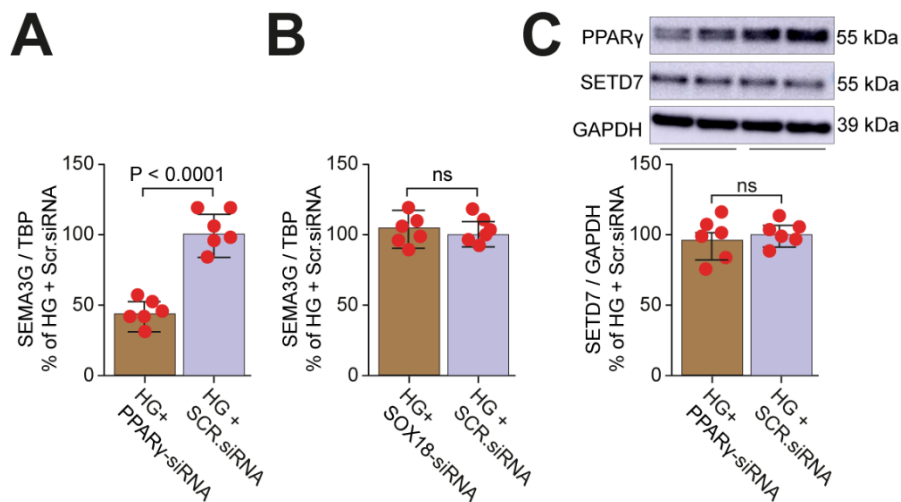


Figure S15. A-B). Real time PCR showing SEMA3G expression in HAECs exposed to HG in presence of PPAR γ -sirRNA, SOX-18 siRNA and SCR.sirRNA. **C)** Representative Western blot and relative quantification showing PPAR γ , SETD7 protein levels in HAECs treated with HG, in presence of PPAR γ -sirRNA and Scr.sirRNA. GAPDH was used as a loading control. Comparisons were performed by Student's t-test. A p value <0.05 was considered significant. HG, high glucose.

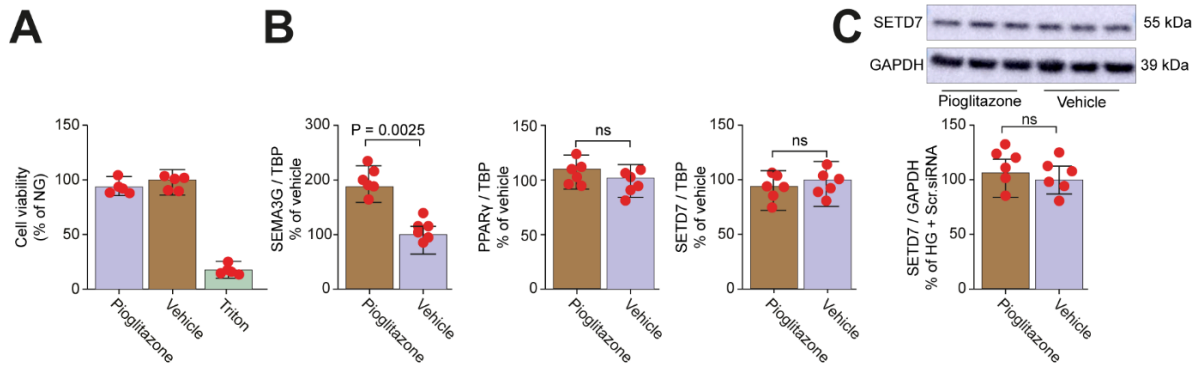


Figure S16. **A)** MTT experiment showing cell viability in HAECs treated with pioglitazone, vehicle and Triton (positive control). **B)** Real time PCR showing SEMA3G, PPAR γ and SETD7 expression in HAECs treated with pioglitazone or vehicle. **C)** Representative Western blot and relative quantification showing SETD7 protein levels in HAECs treated with pioglitazone or vehicle. Comparisons were performed by Student's t-test. A p value <0.05 was considered significant. HG, high glucose.

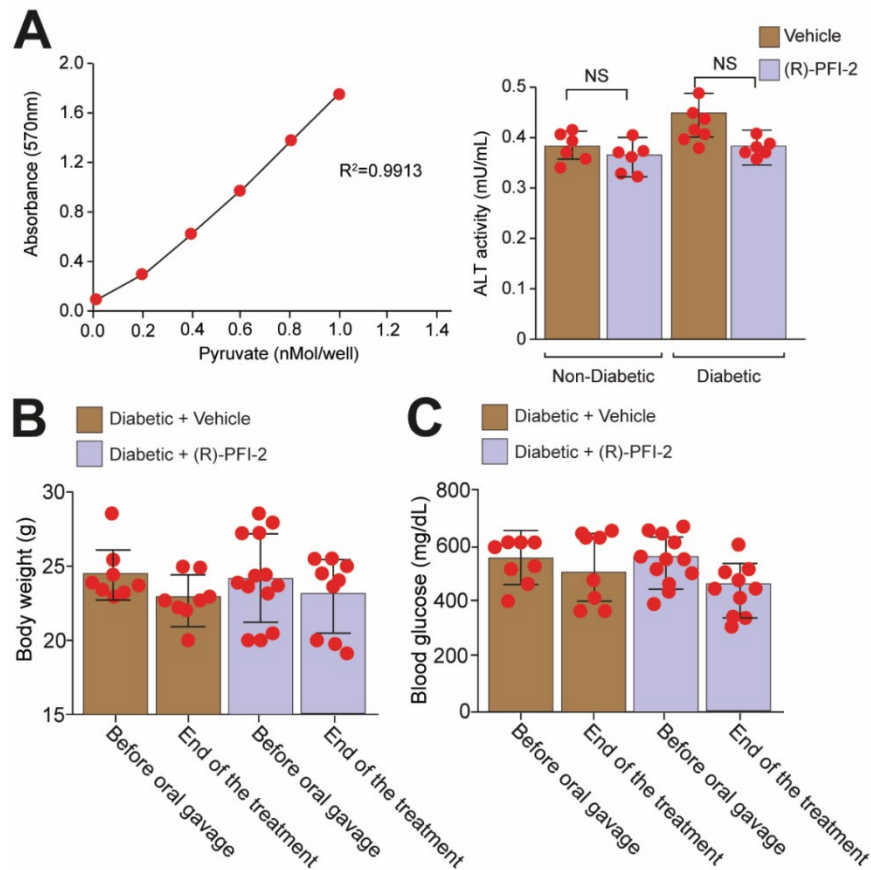


Figure S17. **A)** Alanine transaminase assay (ALT) shows the absence of liver toxicity in diabetic and non-diabetic mice orally treated with APA as compared to vehicle; n=7/group. **B-C)** Bar graphs show glucose levels and body weight in diabetic and non-diabetic mice before and after treatment with (R)-PFI-2 or vehicle; n=7/group. Diabetes leads to hyperglycemia and body weight loss, and these effects are not affected by treatment with (R)-PFI-2. Statistical comparisons were performed by one-way analysis of variance (ANOVA) followed by Bonferroni correction. A p-value <0.05 was considered significant.

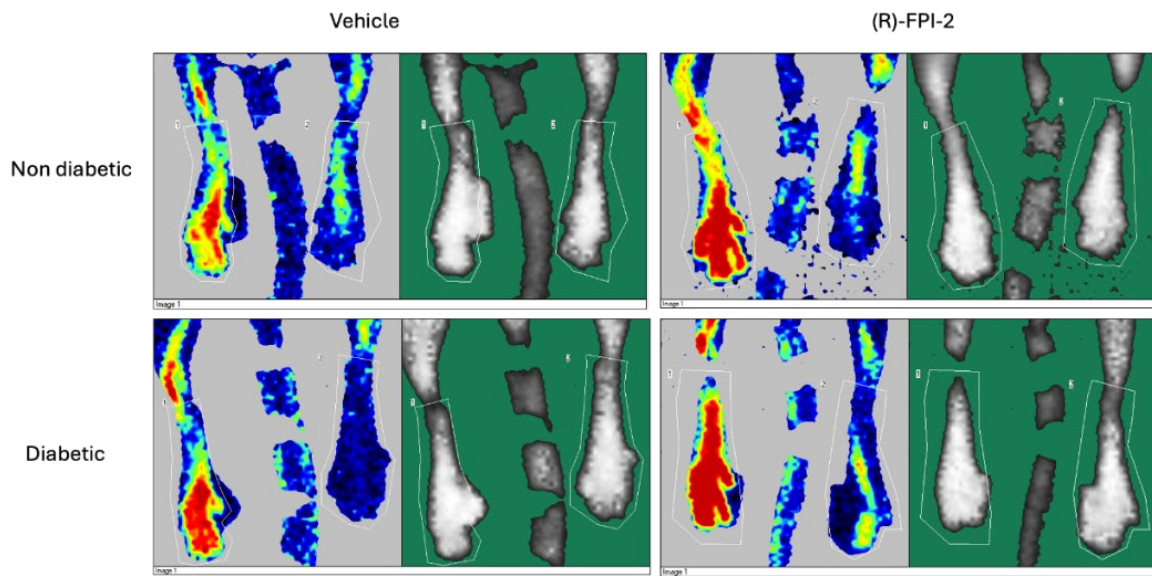


Figure S18. Region of interest (ROIs) used for the analysis of limb perfusion by laser Doppler Imaging in the different experimental groups.

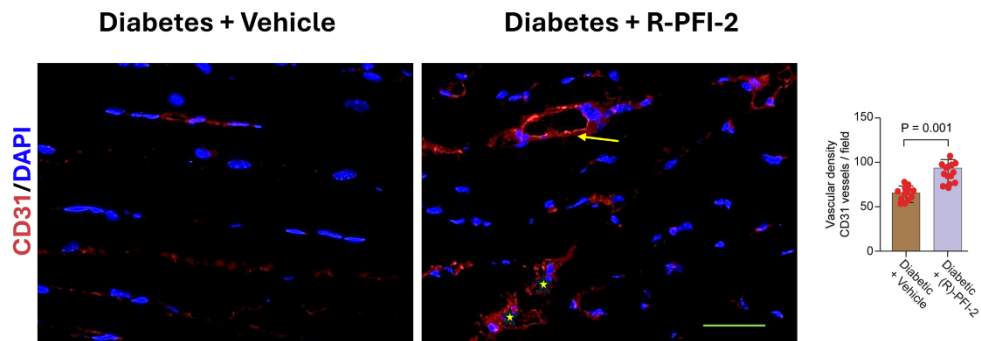


Figure S19. Immunostaining (CD31) and relative quantification showing neo-vascularization in gastrocnemius muscle samples from diabetic mice treated with R-PFI-2 or vehicle. Large vessels are indicated by arrowheads, small vessels-by asterisks. Scale bar = 50 μ m.

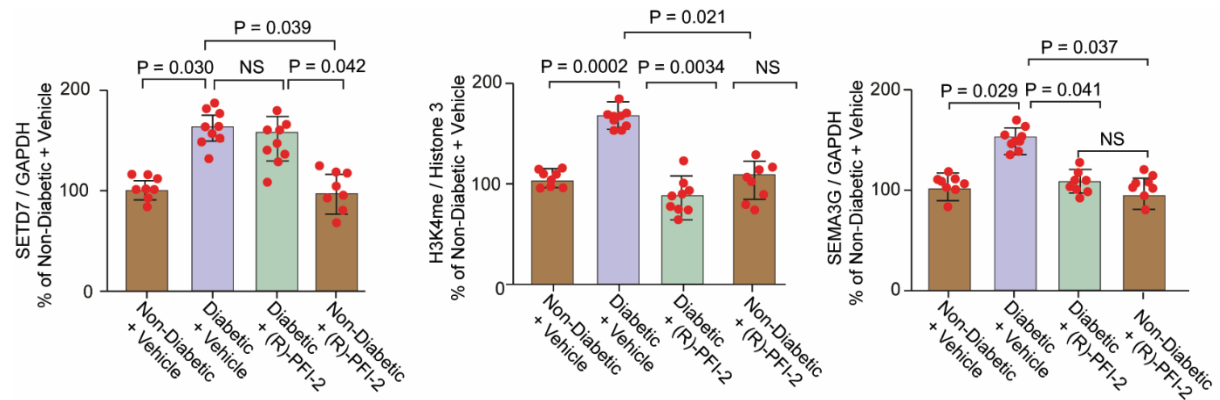


Figure S20. Western blot quantification of SETD7, SEMA3G and H3K4me1 expression in gastrocnemius muscle specimens obtained from non-diabetic and diabetic mice treated with vehicle and (R)-PFI-2. GAPDH and histone 3 were used as a loading controls. Multiple comparisons were performed by one-way ANOVA followed by Bonferroni correction. A p value <0.05 was considered significant.

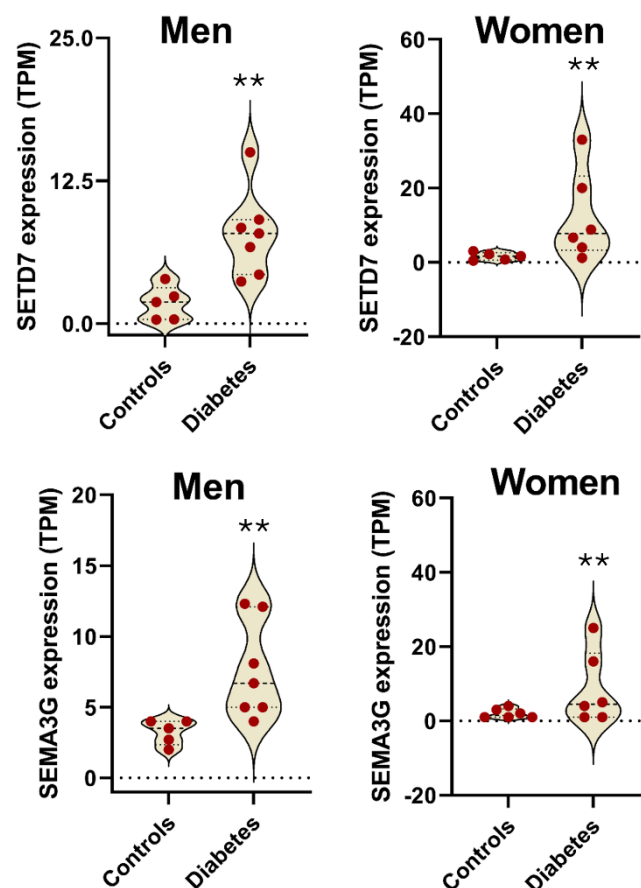
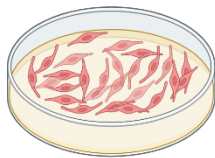
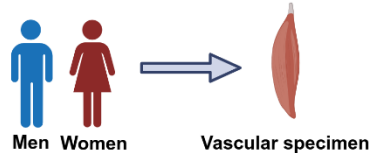


Figure S21. RNA-seq data showing SETD7 and SEMA3G gene expression in vascular specimens from male and female T2D patients with PAD as compared to age-matched healthy controls (cohort 1). **, p<0.001 vs. controls.

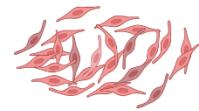
A) HAECs treated with NG vs HG for 48 hrs



B) DM patients with PAD



C) Primary Diabetic ECs

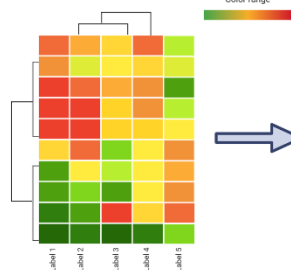


Primary human
ECs (D-HAECs)



Validation of
RNA-seq data

RNA-seq



Control vs HG/Diabetes

HAECs

- SETD7
- SEMA3G
- VCAM1
- TFRC
- CCL2
- ADM5
- RASD1
- LRRC4
- ZWINT
- TROAP
- TAGLN
- CX3CL1

Patients

- SETD7
- SEMA3G
- VCAM
- CCL2
- MMP13
- HMOX1
- CTSK
- TNNC1
- PANDAR
- TNNT1
- CMYA5
- MYH1

Figure S22. Schematic summarizing RNAseq data across the different experimental settings. ECs, endothelial cells. Common dysregulated genes in HAECs and in patients specimens are highlighted in red.

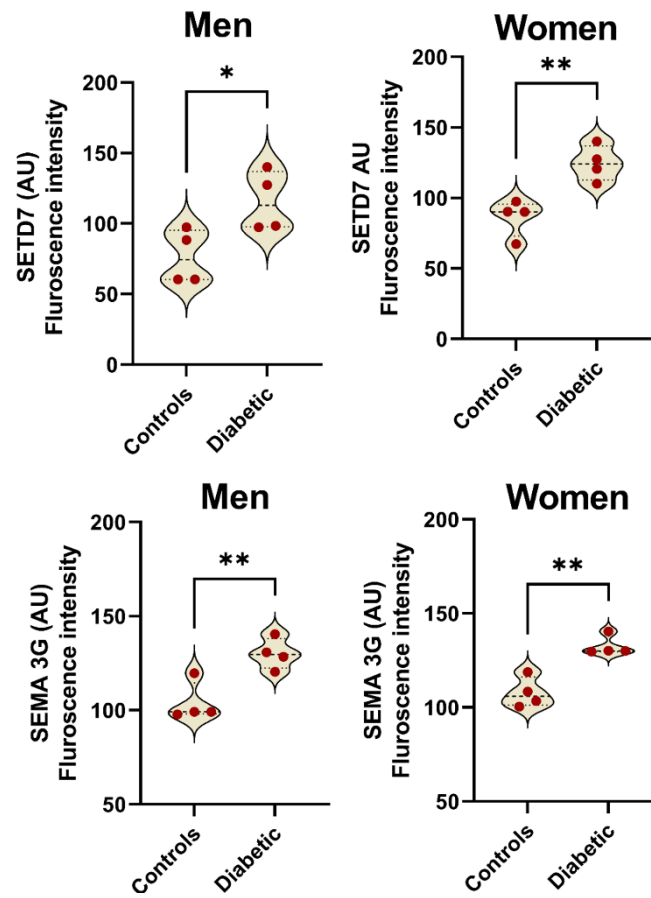


Figure S23. Relative quantification of immunofluorescence showing SETD7 and SEMA3G expression in lower limb muscle specimens from male and female T2D patients with PAD as compared to age-matched healthy controls (cohort 2). **, $p < 0.001$ vs. controls.

References

1. Paneni F, Costantino S, Battista R, Castello L, Capretti G, Chiandotto S, Scavone G, Villano A, Pitocco D, Lanza G, et al. Adverse epigenetic signatures by histone methyltransferase Set7 contribute to vascular dysfunction in patients with type 2 diabetes mellitus. *Circ Cardiovasc Genet*. 2015;8:150-158.
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3. Baker M, Robinson SD, Lechertier T, Barber PR, Tavora B, D'Amico G, Jones DT, Vojnovic B, Hodivala-Dilke K. Use of the mouse aortic ring assay to study angiogenesis. *Nat Protoc*. 2011;7:89-104.