## Online Methods

**Clinical samples.** Patients with peripheral artery disease (defined as ankle-brachial index < 0.9 or prior revascularization) and control participants without peripheral artery disease (ankle-brachial index >1.0 and <1.4) were enrolled from outpatient clinics at Boston Medical Center. The Boston Medical Center Institutional Review Board approved the study, and all participants provided written informed consent. Ankle-brachial index was measured using a Doppler probe to record systolic pressure in the brachial, posterior tibial and dorsalis pedis arteries and obtaining the ratio of the ankle to arm pressure. The lowest leg ankle-brachial index is reported.[1] Fasting blood samples were obtained on the day of visit to measure lipids and glucose. Peripheral blood mononuclear cells were isolated by density gradient centrifugation in Vacutainer cell preparation tubes (CPT tubes with sodium citrate, Becton-Dickinson), treated with an RNA stabilizer (RNAlater, Qiagen), and stored at -80 $^{o}$C prior to RNA isolation.

**Enzyme-Linked ImmunoSorbent Assay (ELISA).** Total circulating levels of VEGF-A from PAD and healthy subjects and in mouse serum following hind limb ischemia were measured with a quantitative colorimetric sandwich ELISA (R&D Systems, human: DVE00, mouse: MMV00). Circulating level of mouse soluble-Flt-1 in WT, LysM-Wnt5a$^{GOF}$ and Sfrp5-KO mice was measured with a quantitative colorimetric sandwich ELISA (R&D Systems, MVR100). For all ELISAs, protein concentrations were calculated using a standard curve generated with recombinant standards provided by the manufacturer. Optical density was measured by microtiter plate reader at 450 nm. Each sample was measured in duplicate and averaged.

**Human monocyte isolation.** The Human Monocyte Isolation Kit II (Miltenyi Biotech) was used to magnetically separate monocytes from non-monocytes cells following manufacturer recommendations. Non-monocyte cells in PBMC samples were magnetically labeled using a cocktail of antibodies against the non-monocyte antigens CD3, CD7, CD16, CD19, CD56,

CD123 and glycophorin A. Monocyte (non-labeled) and non-monocyte (labeled) fractions were then separated with Miltenyi MS columns and a MiniMacs separator.

**Animal model.** Mice with myeloid-restricted Wnt5a overexpression were generated by crossing Lysozyme M-Cre (LysM-Cre) mice (Jackson Laboratories) with knock-in mice carrying a Cre-inducible Wnt5a transgene (Supplementary Fig. 3a). Mice were fed either a standard chow diet (Harlan Teklad global 18% protein rodent diet, #2018) or a HF/HS diet (Bio-Serv, #F1850),[2] as indicated. The composition of the HF/HS diet was 35.8% fat (primarily lard), 36.8% carbohydrate (primarily sucrose), and 20.3% protein. For the high caloric diet feeding, 4 week old mice were maintained on a HF/HS diet for 8 weeks. Mice lacking Sfrp5 were described previously.[2] Sfrp5-KO mice and littermate wild-type (WT) C57BL/6J mice were used in this study. Ob/ob mice were purchased from Jackson Laboratory. Study protocols were approved by the Boston University Institutional Animal Care and Use Committee.

**Reagents.** Recombinant human VEGF-$A_{165}$, human VEGF-$A_{165}$b, mouse VEGF-$A_{164}$, human/mouse Sfrp5 and human/mouse Wnt5a were purchased from R&D systems. Poly-histidine tagged VEGF-$Ax_{Ala}$ was prepared from HEK292-6E cells as described previously.[3] Alpha-tubulin antibody (11H10) was purchased from Cell Signaling Technology. Wnt5a antibody was purchased from R&D Systems. Lamin A/C, JNK, phospho-JNK (Thr183/Tyr185), VEGF Receptor 2 and Phospho-VEGF Receptor 2 (Tyr951) antibodies were purchased from Cell Signaling Technology. SC35 antibody was purchased from BD Pharmingen™. VEGF-A (A-20), (P-20), phosphotyrosine (PY99), Flk-1 (VEGFR2, A-3) and galectin-3 (H-160) antibodies were purchased from Santa Cruz Biotechnology. The immunogen for VEGF-A antibody (A-20), sc-152 maps within amino acids 1-50 at the N-terminus of VEGF-A of human origin (protein accession #P15692). This antibody cross-reacts with all human and mouse VEGF-A isoforms. The neutralizing monoclonal antibody against human VEGF-$A_{165}$b was generated by immunizing

6-8 week old female Balb/c mice with a synthetic peptide fragment of the 9 amino acid C-terminal sequence of VEGF-$A_{165}$b (TCRSLTRKD) coupled to keyhole limpet hemocyanin (KLH). Specificity was determined by immunoblotting to recombinant VEGF-$A_{165}$b and VEGF-$A_{165}$a. C-terminal (exon 8b) specific antibody, was purchased from Abcam. The polyclonal antibody against human VEGF-$A_{165}$a, C-terminal (exon 8a) specific antibody was generated by immunization of a rabbit with a KLH conjugated peptide TCRCDKPRR, corresponding to the last 9 amino acids of VEGF-$A_{165}$a using standard immunization techniques. IgG was affinity purified from plasma, and selectivity determined by western blotting. The polyclonal antibody against mouse VEGF-$A_{165}$b, C-terminal (exon 8b) specific antibody was generated by immunizing rabbits with two synthetic peptides (CRPLTGKTD-OH conjugated to KLH, Acetyl-QSKNILMQYIKANSFIGITELCRPLTGKTD -OH) (21st Century Biochemicals). The polyclonal antibody against VEGF-Ax antibody was generated by immunizing rabbit with the synthetic KLH-conjugated peptide, AGLEEGASLRVSGTR, that is not present in the VEGF-$A_{165}$b isoform.[3]

**Conventional PCR and qRT-PCR.** mRNA was extracted from mononuclear cells from human samples and gastrocnemius muscle tissues of mice using an RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. cDNA was produced using 1 µg of starting mRNA and a QuantiTect Reverse Transcription Kit (Qiagen) as directed by the manufacturer. Conventional PCR was performed to differentiate between exon 8a and exon 8b-containing isoforms. Taking advantage of the fact that splicing of exon 8b results in a 66bp shorter mRNA, human and mouse primers were designed to amplify the region spanning the proximal and distal splice sites in human *$VEGFA_{165}a$* and *$VEGFA_{165}b$*, and in mouse *$Vegfa_{164}a$* and *$Vegfa_{165}b$* (Supplementary Fig. 4). Conventional PCR analysis was performed following conditions: 96 $^{o}$C for 2 minutes followed by 45 cycles at 96 $^{o}$C for 30 seconds, 60 $^{o}$C for 30 seconds and 72 $^{o}$C for 30 seconds. Primers were: 5'- AAGGCGAGGCAGCTTGAGTTA-3' and 5'-TCAGTCTTTCCTGGTGAGAG-3' for human *$VEGFA_{165}a$* and *$VEGFA_{165}b$*. 5'- CAGAAAATCACTGTGAGCCTTGTT -3' and 5'-

ATTAAGGACTGTTCTGTCAA-3' for mouse *Vegfa*$_{164}$*a* and *Vegfa*$_{165}$*b*. Exon-specific qRT-PCR was performed with an Applied Biosystems VIIA7 real-time PCR detection system using SYBR Green I as a double-strand DNA-specific dye according to the manufacturer's instructions (Applied Biosystems). Primers were designed to recognize the exon 5/7 junction and the 7/8a or 7/8b junction. mRNA levels were expressed relative to the levels of *GAPDH*. Real-time RT-PCR analysis was performed following conditions: 95 $^{o}$C for 10 minutes followed by 45 cycles at 95 $^{o}$C for 15 seconds, 64 $^{o}$C for 30 seconds and 72 $^{o}$C for 30 seconds. Other primer sequences are described in Supplementary Table 2.

**Western blot analysis.** Clinical serum samples were resolved by SDS-PAGE. Mouse gastrocnemius muscle tissue samples obtained on postoperative day 3 or 7 were homogenized in lysis buffer containing 20 mM Tris-HCl (pH 8.0), 1% Nonidet P-40, 150 mM NaCl, 0.5% deoxycholic acid, 1 mM sodium orthovanadate, and protease & phosphatase inhibitor cocktail (Thermo Scientific). Protein content was determined by the Bradford method. Equal amounts of protein lysates were resolved by SDS-PAGE. The membranes were immunoblotted with the indicated antibodies at a 1:1000 dilution followed by the secondary antibody conjugated to horseradish peroxidase at a 1:1000 - 5000 dilution. An ECL western blotting Detection kit (Thermo Scientific) was used for detection. Immunoblots were normalized to total loaded protein.

**Immunoprecipitation.** Immunoprecipitation was performed using magnetic beads coated with sheep anti-rabbit IgGs (Dynabeads M-280, Dynal) or immunoprecipitation kit-Dynabeads protein G (10007D, Life Technologies). Beads were incubated overnight at 4 $^{o}$C with rabbit anti mouse VEGF-A$_{165}$b antibody (1 μg antibody for 4 X $10^{7}$ beads) in PBS/1% skim milk, followed by overnight incubation at 4 $^{o}$C with 100 μg protein in the presence of protease and phosphatase

inhibitor cocktail (Thermo Scientific). Bound proteins were eluted from beads by boiling in 25 µl of SDS/PAGE sample buffer.

**PAD model of revascularization.** Wild-type (WT), LysM-Wnt5a$^{GOF}$ and Sfrp5-KO in a C57BL/6 background, HF/HS-fed, and ob/ob mice were used for this study. Mice between 10 and 12 weeks of age were subjected to unilateral hind limb surgery under anesthesia with sodium pentobarbital (50 mg/kg intraperitoneally). The left femoral artery and vein were gently excised from the proximal portion of the femoral artery to the distal portion of the saphenous artery. The remaining arterial branches including the perforator arteries were also excised. Following excision of the femoral artery as previously described,[4] hind limb blood flow recovery was monitored in real-time by Laser Doppler Imaging (LDI) (Moor LDI; Moor Instruments)[5] before surgery and on post-operative days 0, 3, 7, 14, 21 and 28 by an investigator blinded to strain identity. To avoid data variability due to ambient light and temperature, the hind limb blood flow was expressed as the ratio of left (ischemic) to right (non-ischemic) LDBF.

**Immunohistochemistry.** Muscle samples were imbedded in Tissue Tek OCT compound (VWR) and snap-frozen in liquid nitrogen. Tissue slices (5 µm in thickness) were prepared. The gastrocnemius muscles were processed for immunohistochemistry of CD31, Wnt5a and galectin-3 (Mac2). The primary antibody for CD31 (1:50; PECAM-1: Becton Dickinson), Wnt5a (1:200; R&D Systems) and galectin-3 (1:500; Santa Cruz Biotechnology) were applied to the sections overnight at 4 $^{o}$C. After washing, the sections were sequentially incubated with proper fluorescein isothiocyanate (FITC)- or PE-conjugated secondary antibodies (Invitrogen) for 1 hour. Digital images of five microscopic fields from four different sections from each animal were scored.

**Anatomical analyses.** Capillary density within thigh gastrocnemius muscle was quantified by histological analysis as previously described.[4] Capillary density was expressed as the number of CD31-positive cells per high power field. Arteriole density analysis was quantified in fresh-frozen sections of hind limb gastrocnemius muscles at day 28 after ischemic surgery. Arteriole density was expressed as the number of alpha-smooth muscle actin (aSMA) positive cells per high power field. Immunostains were visualized by using DAB.

**Generation of adenoviral Vectors and experiment.** Full-length mouse *Vegfa$_{164}$a* and *Vegfa$_{165}$b* cDNA was subcloned into an adenoviral shuttle-CMV vector, and the adenoviral (Ad) vectors expressing mouse VEGF-A$_{164}$a and VEGF-A$_{165}$b (Ad-*Vegfa$_{164}$a* and Ad-*Vegfa$_{165}$b*) were constructed under the control of the CMV promoter.[4] Ad-m*Vegfa$_{165}$b* or Ad-*β-gal* (control) was injected into the jugular vein of WT mice (2 X $10^9$ pfu in each group) at 3 days prior to ischemic surgery.

**Neutralizing antibody experiment.** Mice received intraperitoneal anti-human VEGF-A$_{165}$b neutralizing monoclonal antibody (100 μg) on postoperative days 0, 3 and 7. As a control, nonspecific mouse IgG was administered in a similar manner.

**Cell culture.** HEK293A cells (ATCC) were grown in DMEM (10% FBS). HEK293A cells were trypsinized and counted and virus (Ad-m*Vegfa$_{164}$a* and Ad-*mVegfa$_{165}$b*) added at the indicated multiplicity of infection. RNA was isolated from infected cells after 48 hours of infection. RAW264.7 cells (TIB-71, American Type Culture Collection) were grown in DMEM medium supplement with 10% fetal bovine serum and antibiotics. RAW264.7 cells were treated with recombinant Wnt5a (500 ng/ml) for the indicated lengths of time. Human umbilical vein endothelial cells (HUVECs; American Type Culture Collection) of passages 3 through 5 were cultured in EGM-2 (Lonza). HUVECs were trypsinized and counted, and virus (Ad-m*Vegfa164a*

and Ad-*mVegfa165b*) was added at a multiplicity of infection of 100. Protein was isolated from the transfected cells after 72 hours of infection. For some experiments, HUVECs were infected with an adenoviral vector encoding a mouse VEGF-$A_{165}$b (Ad-m*Vegfa*$_{165}$*b*) at a multiplicity of infection of 100 for 24 hours, followed by a media change and the collection of "conditioned media". The conditioned media was concentrated by Centrifugal Filter Units (Amicon Ultra-15 Centrifugal Filter Devices, Millipore). A lentiviral plasmid expressing a ROR2-specific shRNA (5′-CCGGCGTGGTGCTTTACGCAGAATACTCGAGTATTCTGCGTAAAGCACCACGTTTTT-3′) or SC35-specific shRNA (5′-CCGGAGTCCAGATCTGCCCGAAGATCTCGAGATCTTCGGGCAGATCTGGACTTTTTTG-3′) in the pLKO.1-puro vector were purchased from Sigma-Aldrich. Lentiviruses expressing a scrambled shRNA were used as a negative control. Recombinant lentiviruses were produced by co-transfecting the pLKO.1-scramble or pLKO.1-mouse ROR2, -mouse SC35 shRNA plasmids with MISSION® Lentiviral Packaging Mix (SHP001, Sigma-Aldrich) into HEK293T cells (American Type Culture Collection) using Lipofectamine 2000 (Invitrogen). The medium was replaced after 24 hours, and viral supernatants were harvested 2 days post-transfection and stored at -80 °C. For infection, RAW264.7 cells were seeded at a density of 2.5 X $10^5$cells/well of a 6-well plate coated poly-L-lysine and infected with 100-200 multiplicity of infection of viral supernatant containing 5 μg/ml polybrene. The medium was replaced next day. Cells were cultured for an additional 72 hours and used for experiments.

**Statistical analysis.** Statistical analyses were completed with SPSS 20.0. Distribution of VEGF-A and clinical characteristics were evaluated for normality by examining histogram and q-q plots. Variables that deviated from normality were log-transformed for analysis or nonparametric tests were used. Clinical characteristics were compared between patients with PAD and controls using independent samples t-tests or chi-square tests as appropriate. Circulating VEGF-$A_{165}$b expression was compared with PBMC expression and ankle-brachial

index with Spearman correlations. Additional analytic methods are included in the Figure legends. Data are presented as mean± SEM as indicated in the figure legends. Two-sided *P*-values of < 0.05 were considered statistically significant. No statistical method was used to pre-determine sample size. No randomization was used in the animal experiments.

References


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