SUPPLEMENTARY METHODS

Isolation of mouse endothelial cells and preparation of human circulating CD34$^+$ cells

Endothelial cells (ECs) were isolated and purified from mouse lung and heart as reported in our previous work [1]. Briefly, fresh tissues were placed on ice and minced with sterilized microscissors. The resultant tissue homogenates were then digested with collagenase (200 U/mL, Thermo Fisher Scientific, Shanghai, China) at 37°C for 45 minutes, followed by filtration through a 70-μm cell strainer (BD Medical Technology, Bedford, MA, USA). Cell pellets were resuspended in pre-cold Dulbecco's phosphate-buffered saline, followed by sequential incubation at room temperature (RT) for 15 minutes with anti-CD31 or anti-intercellular adhesion molecule 2 (ICAM-2) monoclonal antibody-coated beads (Thermo Fisher Scientific). Final separation was achieved with the aid of a magnetic separator (Bangs Lab, Fishers, IN, USA). The purity of isolated ECs was confirmed using fluorescence activated cell sorting (FACS)-based on sorting live/CD45$^-$/CD31$^+$ cells [2,3]. ECs, maintained in EGM2-MV media with 5% of fetal bovine serum (Gibco, Shanghai, China), were either immediately harvested to represent the endothelial compartment of mouse organs, or cultured to passage 2 or 3 for cell migration and other assays [4].

Human circulating CD34$^+$ cells were isolated from peripheral blood samples from 10 healthy volunteers and 15 patients with T2DM in our hospital, by FACS using an EasySep Human CD34 Positive Selection Kit (STEMCELL Technologies, Shanghai, China) [1]. Human endothelial colony forming cells (ECFCs) were prepared from mononuclear cell fractions of cord blood using CD31-coated magnetic beads (Sino Biological, Shanghai, China) [5]. Written informed consent was obtained from all individual participants. The use and handling of the human samples was strictly conformed to the standards set by 2008 Revised Declaration of Helsinki and approved by the local Human Research Committee (Approval #: XAFH-2016-0081b).

Cell treatment and transfection

Human umbilical vein endothelial cells (HUVECs) and human dermal fibroblast (hDFs) were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA), and were routinely cultured in endothelial basal medium (EBM-2), supplemented with 1 mg/mL hydrocortisone, 12 mg/mL bovine brain extract, 50 mg/mL gentamycine, 50 ng/mL amphothericin-B, 10 ng/mL epidermal growth factor, and 10% fetal calf serum (FCS), at 37°C in 5% CO_2 and 95% humidified air [1]. To study the effects of hyperglycemia on SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 5 (*SMARCA5*)/*Smarca5* expression, primary ECs or CD34$^+$ cells were incubated with different doses of glucose, in the presence or absence of co-incubation with 2-deoxyglucose (2DG, Sigma-Aldrich, Shanghai, China), for different durations as indicated, followed by quantitative reverse transcription polymerase chain reaction (RT-qPCR) analysis as described below. To investigate the potential regulation of *SMARCA5* expression by angiogenic stimuli, HUVECs were challenged with 100 nM of sphingosine 1-phosphate (S1P, Sigma-Aldrich) for different durations, followed by RT-qPCR analysis. Stable knockdown of *SMARCA5* was induced in HUVECs by short hairpin RNA (shRNA) lentivirus infection with SMARCA5 shRNA (VectorBuilder, Guangzhou, China), as described elsewhere [6]. Briefly, 70% confluent HUVECs were seeded in a 6-well plate at density of 6×10^4 cells/well and cultured in normal condition for 24 hours, followed by transfection in media containing 8 μg/mL polybrene (Beyotime Biotechnology, Shanghai, China) at MOI of 10 for another 48 hours. HUVECs successfully transfected were subsequently screened out using 2 μg/mL puromycin (Sigma-Aldrich) for consecutive 10 days. To stably overexpress the exogenous vascular endothelial growth factor (VEGF), HUVECs$^{SMARCA5-/-}$ were transfected with the promoter of human cytomegalovirus (pCMV3)-VEGF or empty vector (Sino Biological, Beijing, China) using FuGENE HD for 48 hours, followed by selection with 200 μg/mL of hygromycin (Sigma-Aldrich). To stably overexpress the exogenous SMARCA5, HUVECs were infected with pAdenoG-HA-SMARCA5 or empty vectors (Abcam, Shanghai, China) in media containing 8 μg/mL polybrene, as described above. Positive clones were then selected by incubating cell with 200 μg/mL of hygromycin. To provide functional evidence that signal transducer and activator of transcription 3 (STAT3) signaling is actually necessary for oxidative stress's action, HUVECs cells were transfected with WT STAT3 (STAT3 Flag pRc/CMV) or DN STAT3 (STAT3 Y705F Flag pRc/CMV) (Addgene, Watertown, MA, USA) using FuGENE. Forty-eight hours after transfection, cells were starved for 10 hours and were then treated for 12 hours with 1 mM of diamide, followed by immunoblotting analysis. To answer whether moderate oxidative stress was involved in the modulation of SMARCA5, HUVECs were treated with H_2O_2

or diamide (Sigma-Aldrich) for various concentrations, in the presence of or absence of the STAT3 inhibitor WP1066 (1.25 μM, Selleck, Shanghai, China), for 6 or 12 hours.

Cell migration

Scratch migration assay

Confluent HUVECs monolayer, cultured under normal or hyperglycemic conditions (incubation with 25 mM glucose), was streaked at 1-mm width with a rubber scraper. The wound edge was marked accordingly. Following a 24 hour-migration, the migrated cells beyond the edge were counted in five random fields (×40).

Transwell migration assay

Subconfluent HUVECs were detached by trypsinization and suspended in EBM-2 containing 0.5% bovine serum albumin for 3 hours. Cells were then applied to an 8-μm-pore Transwell (1×10^5 cells/insert) pre-coated with gelatin (Sigma-Aldrich), which was then inserted into a well containing the same media with or without 100 nM of S1P. The cells were allowed to migrate across the membrane for 12 hours. Finally, the migrated cells on the lower side of the membrane were stained with 4′,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich) and calculated for endothelial migration in 16 high-power (×200) fields.

***In vitro* tube formation assay**

HUVECs (1×10^4 cells), cultured under normal or hyperglycemic conditions (incubation with 25 mM glucose), were suspended in 100 μL of M199 medium containing EBM-2 and were then seeded on top of ECMatrix gel (BD Biosciences, Shanghai, China) in each well of a 96-well plate. After a 24-hour incubation at 37°C, each well was photographed. Branching points were subsequently quantified with the aid of Image J software (http://rsb.info.nih.gov/ij/).

***In vivo* vasculogenesis assay**

In vivo vasculogenesis assay was performed as described earlier [5]. In brief, human cord blood-derived ECFCs were transfected with SMARCA5 adenovirus (Applied Biological Materials, Beijing, China) as described above. Forty-eight hours later, a total of 1×10^6 cells consisting of ECFCs and hDFs at the ratio of 1:1, along with 500 ng/mL of VEGFA, were mixed with 100 mL Matrigel (BD Biosciences) and implanted subcutaneously onto the dorsal side of adult nude mice. Two weeks after implantation, the Matrigel was harvested and subjected to immunostaining of CD31 (Supplementary Table 1) as described below. Final vasculogenesis *in vivo* was evaluated by measuring numbers of $CD31^+$ vessels per analyzed areas (mm^2) [5].

***In situ* overexpression of SMARCA5 and *in vivo* wound healing assay**

The Ad-SMARCA5 incorporated hydrogel was prepared as described elsewhere [7]. Briefly, under sterilized conditions, Matrigel was thawed overnight at 4°C 1 day in advance. Acrylamide (AM, Sigma-Aldrich) was dissolved in double distilled water to form 28% wt./vol AM solution. Subsequently, same volumes of liquid Matrigel and AM were mixed and stirred regularly for 2 minutes. Following a 20-minute of ultrasonic defoaming, SMARCA5 adenovirus or vector was added to the solution (2 μg DNA per 20 μL solution), followed by vacuum degassing for 1 hour to obtain the final transparent hydrogel. Wound healing assay *in vivo* was then carried out according to a previously reported protocol [8]. Following administration of intraperitoneal anesthesia (ketamine and xylazine, 100 and 10 mg/kg, respectively), Ten-week-old Balb/c male mice were shaved in the back, wiped with ethanol, and wounds were made by using a skin biopsy punch (5 mm, Head Biotechnology, Beijing, China). After establishment of wound healing model, the Ad-SMARCA5 incorporated hydrogel was immediately applied to the skin wound on daily basis for consecutive 14 days. To monitor wound healing, the mice were anesthetized every 2 to 3 days, and the digital pictures of wounds were acquired. Wound area measurements were conducted using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

***In vivo* 5-bromodeoxyuridine (BrdU) labeling and BrdU immunofluorescence staining**

Labeling with BrdU was performed to assess cell proliferation during wound healing [9]. Six days after establishment of wound healing model, mice were injected intraperitoneally with 50 mg/kg BrdU (Abcam) on a daily basis. At 14 days after establishment of wound healing model, mice were sacrificed, and skin tissues were harvested. Tissues were fixed in 4% paraformaldehyde (PFA, Sigma-Aldrich) solution for 20 hours, routinely dehydrated and subjected to paraffin embedding procedures. Five-μm-thick skin sections were then subjected to BrdU immunofluorescence staining as described below using an anti-BrdU monoclonal antibody (Supplementary Table 1).

The number of BrdU-positive cells were counted accordingly and cell densities were calculated by dividing cell numbers by the area [9].

Assessment of VEGF concentration in supernatants by enzyme-linked immunosorbent assay

HUVECs with different transfections were stimulated with 100 nM of S1P for different durations as indicated, followed by measurement of VEGF concentration in the supernatants using a human VEGF enzyme-linked immunosorbent assay (ELISA) Kit (Beyotime Biotechnology), according to the manufacturer's instructions. Final absorbance values were determined at 450 nm and the ELISA values were given in relative units (pg/10^5 cells/mL).

Quantitative polymerase chain reaction

Quantitative polymerase chain reaction (qPCR) was performed according to our previous work [1]. Total RNA was isolated using MagMAX Nucleic Acid Isolation Kit (Thermo Fisher Scientific). Following a routine RNA cleanup with Dnase Digestion using RNase-free DNase I (20 Kunitz unites/μL, Qiagen, Shanghai, China), complementary DNA (cDNA) was synthesized by reverse transcription reaction using Superscript III Synthesis System (Thermo Fisher Scientific), following the manufacturer's instructions. qPCR was then done according to the Promega protocol (Promega, Shanghai, China). PCR products were then quantified by using the comparative $^{\Delta\Delta}$Ct method, with *18S* serving as the internal control [10]. The primers used were: *Smarca5* (NM_053124.2), 5′-GACTGTCCGAGTGTTTCGCT-3′ and 5′-TCTGATCTACAAGCCTCCCTTG-3′; *SMARCA5* (NM_003601.4), 5′-GACAGTCAGAGTGTTCCGCT-3′ and 5′-CCACAAGCCTCCCTTGTTGA-3′; *18S* [1], 5′-CTCGCCGCGCTCTACCTACCTA-3′ and 5′-ATGAGCCATTCGCAGTTTCACTGTA-3′. The detailed primers used for qPCR analysis of expression levels of angiopoietin-I (*Ang-I*), basic fibroblast growth factor (*bFGF*), *VEGF*, connective tissue growth factor (*CTGF*), and macrophage colony-stimulating factor (*M-CSF*) have been reported elsewhere [11].

Western blot

Western blot was performed according to our previous work [1]. Total protein was isolated from cells or tissues using radioimmunoprecipitation assay (RIPA) buffer (Roche, Shanghai, China). Approximate 35 μg of total protein samples were separated on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), followed by sequential electrophoresis and transfer to polyvinylidene difluoride membrane (PVDF, Thermo Fisher Scientific). Nonspecific binding was blocked with 5% non-fatty milk in Tris-buffered saline with 0.1% Tween-20 and incubated with different antibodies (Supplementary Table 1) overnight at 4°C. Final immunocomplexes were developed using a Chemiluminescent Western blots Imager (LI-COR Biotechnology, Lincoln, NE, USA), with β-actin serving as the loading control.

Histological examination

Four percentage PFA-fixed paraffin-embedded skin sections (5 μm) were dewaxed with xylene, rehydrated and stained with hematoxylin and eosin using a H&E Staining Kit (Abcam).

BrdU immunofluorescence staining was performed as described previously [12]. Following routine dewaxing and rehydration, 4% PFA-fixed skin sections were treated at RT for 30 minutes with blocking solution (10% donkey serum 0.5% bovine serum albumin, 0.3% triton X-100 in phosphate buffered saline [PBS]) and then incubated with BrdU monoclonal antibody (Supplementary Table 1) at 4°C overnight. After three thorough rinses with PBS, sections were incubated with 200 μL of fluorescein isothiocyanate (FITC)-labeled secondary antibody (Supplementary Table 1) at RT for 30 minutes. The slides were then rinsed thoroughly in PBS, stained with DAPI for 5 minutes and sealed in anti-fade fluorescence mounting medium (Thermo Fisher Scientific). BrdU immunofluorescence were finally observed and photographed using a confocal microscopy (Zeiss LSM/510 Upright 2 photon, Carl Zeiss, Shanghai, China).

Immunostaining for CD31 was approached differently. The aforementioned Matrigel implants were fixed in 10% buffered formalin overnight, embedded in paraffin, and sliced into 7-μm-thick sections. Following routine dewaxing and rehydration, sections were incubated with 3% H_2O_2 in methanol for 10 minutes to eliminate endogenous peroxidase activity. Sections were then incubated with human-specific CD31 monoclonal antibody (Supplementary Table 1) at 4°C overnight. Final peroxidase activity was developed with the aid of a VECTASTAIN Elite Kit (Vector Laboratories, Shenzhen, China).

Luciferase reporter assay

pGL4-SMARCA5 reporter plasmid was obtained from VectorBuilder. pGL4.10-VEGF was a gift from David Mu (Addgene plasmid # 66128; http://n2t.net/addgene:66128; RRID:

Addgene_66128) [13]. For reporter assay, 0.5 μg pGL4.10-VEGF reporter plasmid and pRL-TK Renilla reporter plasmid were co-transfected into scramble shRNA or SMARCA5 shRNA-transfected HUVECs using FuGENE HD. Forty-eight hours later, cells were challenged with 100 nM of S1P for 6 hours, followed by measurement of the relative luciferase activities using a dual luciferase reporter assay kit (Promega), as per the manufacturer's instrcutions. In another experimental setting, 0.5 μg pGL4-SMARCA5 reporter plasmid and pRL-TK Renilla reporter plasmid were co-transfected into HEK-293T cells using FuGENE HD. Forty-eight hours after transfection, cells were challenged with 1 mM of diamide for 12 hours, followed by measurement of the relative luciferase activities as described above.

Non-radioactive electrophoretic mobility shift assay

HUVECs were transfected with STAT3 Flag pRc/CMV which renders the STAT3 molecule constitutively active [14]. Forty-eight hours later, HUVECs were treated with 1 mM of diamide for another 12 hours. Subsequently, cells were harvested and nuclear protein extracts were prepared using a Nuclear Extraction Kit (Cayman Chemical, Ann Arbor, MI, USA) following the vendor's protocol. Subsequently, 1 μg of STAT3-enriched nuclear extracts was incubated with 30 mM of custom digoxigenin (DIG)-labeled probes corresponding to the putative STAT3 binding motifs. Upon completion of gel electrophoresis and nylon membrane transfer, protein-bound DIG-labeled probes were immunologically detected with anti-DIG–alkaline phosphatase conjugate and CSPD chemiluminescent substrate (Roche), as per the manufacturer's instructions. The oligonucleotide probe used was AGCGCAAAACCCAGACAAGTTTTCCGGTAGTGCCG. Competitive inhibition consisted of incubation with a 50-fold molar excess of unlabeled/cold probe. Validation of the pSTAT3-specific binding of DIG-labeled probe was performed using pre-incubation with 2 μg anti-pSTAT3 antibody at RT for 20 minutes at RT prior to the addition of DIG-labeled probe.

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) assay was performed as described elsewhere [15,16]. HUVECs with different transfections were dosed with 100 nM of S1P for 6 hours. HUVECs were fixed with 1% formaldehyde (Sigma-Aldrich) at RT for 10 minutes to cross-link protein-DNA, followed by cell lysis in lysis buffer (10 mM Tris-HCl pH 7.5, 10 mM NaCl, 3 mM $MgCl_2$, 1 mM $CaCl_2$, and 4% NP-40). The resultant cross-linked chromatin was sonicated with three 10 seconds pulses at 10% maximum power. The sonicated lysates were then pre-cleared with protein A agarose beads followed by incubation with primary antibodies, or their respective isotype control immunoglobulin G (Supplementary Table 1). Samples were then washed with elution buffer (0.1 M $NaHCO_3$, 1% SDS) and cross-linked protein-DNA complexes were purified using a Pierce Cross-link IP Kit (Thermo Fisher Scientific). Purified DNA fragments were examined for chromatin occupancy of SMARCA5, Brahma-related gene 1 (BRG1), Brahma homologue (BRM), metastasis-associated protein 1 (MTA1), and enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) by qPCR. The primer sequences used within *VEGF* promoter were: SMARCA5, 5′-TCGCCCTAATGAAGGT-GCTCA-3′ and 5′-TGGTGGTACCCAGCAAGGTGTGT-3′; BRG1, 5′-ATCAGCCTAGAGCATGGAGCCCAG-3′ and 5′-CAACCCTCCCTTTCCATCATTCGT-3′; BRM, 5′-AGGATGGGGCTGACTAGGTAAGCT-3′ and 5′-GACCAGTCAGTCTGATTATC-3′; MTA1, 5′-TCATAAGGGCCTTAGGACACCA-3′ and 5′-AGCTTACCTAGTCAGCCCCAT-CCT-3'; and EZH2, 5'-GAAGATGTGGAGAGTTGGAGGAA-3′ and 5′-TGTAGGCCAGCTGCCCAGAGCT-3′. The primer sequences used within *bFGF* promoter were: SMARCA5, 5′-ATGTAGAAGATGTGACGCCGCG-3′ and 5′-GA-CCTGGGGTTCACGGATGGGT-3′; BRG1, 5′-ATCTTCTC-TTCTGAGGCAGAC-3′ and 5′-CTGTGATCTAATGGTAATAAAC-3′; BRM, 5′-AGTCACGGCTGGTTGCGCA-3′ and 5′-TTGGCATTCCCTGGGCTCCA-3′; MTA1, 5′-TGGCATCAGATTTGGCATCTC-3′ and 5′-TATAGCAGGTGCCACAGATG-3′; and EZH2, 5′-TCGAGACCAGCCTGACCAAC-3′ and 5′-CTCCTGGGTTCAAGCGATTCT-3′.

Statistical analysis

Quantitative data from at least three independent experiments, presented as mean±standard deviation, were analyzed for statistically significant differences using the Student's *t*-test or analysis of variance (ANOVA) with *post hoc* tests wherever appropriate. All statistical analyses were performed by GraphPad Prism 7 (GraphPad Software Inc., San Diego, CA, USA). A *P* value less than 0.05 (typically ≤0.05) was considered statistically significant.