**Supplemental Materials and Methods**

**Patient samples and patient skin biopsies**

Full-thickness skin biopsies were obtained from the clinically involved skin of the distal forearm of nine SSc patients (8 female, 1 male) affected by the diffuse cutaneous form (dcSSc) in the early phase of the disease, recruited from the Scleroderma clinic within the Leeds Institute of Rheumatic and Musculoskeletal Medicine (UK) and the Rheumatology Unit in L’Aquila (IT). Control skin samples were from the same forearm region of age and gender matched healthy donors that had undergone surgical treatment for trauma. Each biopsy sample was divided into specimens for immunohistochemistry (IHC), biochemical analyses and fibroblast isolation. Specimens for Real-Time PCR (RT-PCR) and Western blot analyses were immediately immersed in liquid nitrogen and stored at -80°C until use. Biopsies were taken with full informed consent and the study was approved by NRES Committee Yorkshire and the Humber (REC 10/H1306/88) and the local ethical committee in University of L’Aquila. Patients with SSc fulfilled the 2013 ACR/EULAR Classification criteria [18].

**Cell culture**

Dermal fibroblasts (FBs) were isolated from excisional biopsy samples from 9 early diffuse SSc patients (dcSSc) and 9 controls. The skin biopsy samples were minced with a scalpel, enzymatically disassociated with 1mg/ml trypsin at 37°C in a humidified atmosphere of 5% CO2 for 2 hours and centrifuged at 220g for 10 minutes. After centrifugation the supernatant was discarded, the tissue was placed in plastic culture dishes and covered with DMEM medium (Sigma, USA) supplemented with 20% FCS (FCS, Sigma, USA), 100units/ml penicillin, 100ng/ml streptomycin (Sigma, USA). Fresh growth medium was added after 4 days and subsequently replenished on day 6 when a visible outgrowth of cells was obtained. Fibroblasts, used at passage 3-5, were cultured in DMEM supplemented with 10% FCS (Sigma, USA), penicillin (100U/ml) and streptomycin (100ng/ml).

**hTERT immortalization and Transduction with lentiviral shRNA**

For immortalization pBabe hTERT puromycin retrovirus supernatants were used to transduce human FBs at 50% confluence. After 24h cell media were changed and cells were cultured for a further 48 h. Selection was carried out for 4 days with 1m/ml puromycin (Life Technologies). Cav-1 and PEDF expression were silenced by transduction with shRNAmir GIPZ lentiviruses (Open Biosystems, Surrey, UK). FBs were seeded at 50% confluence and infected with lentiviral particles in serum free DMEM and incubated for 6h, after which an additional 1ml of DMEM containing 10% FCS was added and the cells were incubated for a further 72h. Stably transduced FBs were selected in media containing 1.0μg/ml puromycin (Life Technologies) for 10 days and positively sorted for GFP fluorescence.

**Immunoblotting and immuno-depletion**

For detection of secreted PEDF by immunoblotting, 30µl of cell culture supernatant from confluent HC- and SSc-FBs was electrophoresed through a 10% polyacrylamide gel and then transferred to PVDF (polyvinyl difluoride) membranes. Following an overnight block with 5% non-fat dry milk, membranes were incubated with anti-PEDF antibody (Chemicon-Millipore, USA) or polyclonal rabbit anti human Collagen-I antibody (Santa-Cruz, USA) in TBS 0.1% Tween 20 for 1h at room temperature. After incubation with horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG (Sigma-Aldrich, USA), immune complexes were detected with the enhanced chemiluminescence detection system (Amersham Biosciences, UK). Densitometric analysis of the bands was performed using ImageJ software (NIH, Bethesda, Maryland, USA). For immune-depletion experiments, the primary antibody was incubated with Dynabeads Protein G (Novex, LifeTechnologies) for 15 minutes and the Dynabeads-antibody complex was incubated with tissue culture supernatants for 2h at room temperature. Immuno-depletion was conducted three times and the loss of PEDF was verified by western blotting.

**Angiogenesis assays**

The organotypic co-culture assay [21-23] was performed with primary human microvascular endothelial cells (MVEC) or human umbilical vein endothelial cells (HUVEC) and hTERT immortalised fibroblasts stably infected with lentivirus coding for either non-silencing control or Cav-1 shRNAs. Primary SSc and healthy control (HC) fibroblasts (FBs) were stably infected with lentivirus coding for either non-silencing control or PEDF shRNAs. Fibroblasts were seeded at 2x104 cells per well in a 24-well plate and grown to confluence over 7 days. Endothelial cells (MVECs or HUVEC) were seeded onto the confluent fibroblasts at 8.5x103 cells per well in a 1:1 mixture of Human Endothelial cell Medium (TCS Cellworks, UK) and DMEM, 10% FCS medium and incubated at 37ºC in a humidified atmosphere of 5% CO2. Recombinant human PEDF or VEGF were added at 200ng/ml and 25ng/ml respectively, at the time of seeding. Tubule formation was visualized by immunohistochemistry 5 days after plating of endothelial cells onto the confluent fibroblasts. The co-cultures were fixed with ice-cold 70% ethanol for 30 min, and successively labelled with mouse monoclonal anti-CD31 antibody (0.25*μ*g/ml) in 1% (w/v) bovine serum albumin (BSA) for 1h at 37oC followed by alkaline phosphatase-conjugated secondary antibody (0.6 *μ*g/ml) in 1% BSA for 1h at 37oC. The co-cultures were washed with PBS between fixation and antibody treatments and with water before adding the BCIP (5-bromo-4-chloroindol-3-yl phosphate)/NBT substrate (Sigma), and the stain allowed to develop for 15–30 min at 37oC. For quantifications, the number of tubules and total tubule length were analysed using the Angiosys software (TCS Cellworks, UK).

For matrigel assays, reduced growth factor (RGF) matrigel (VWR, UK) was thawed overnight on ice at 4°C. The wells of a 12 well plate were washed with PBS prior to adding 120μl of matrigel diluted 2:1 (matrigel: PBS) on ice. Where indicated, rhPEDF or rhVEGF were mixed with matrigel at 200ng/ml or 25ng/ml respectively. Matrigel was allowed to set at 37°C for 30 minutes, and cells were plated at a density of 5 x 104/well in HUVEC media and incubated at 37°C with 5% CO2 for a further 24h. Tubule formation was imaged by phase contrast microscopy using an EVOS Digital Inverted Microscope (Thermofisher Scientific, USA). The assays were quantified by counting the number of loops from four fields of view, acquired using a 10X objective from each condition at the 24-hour time point. Where fibroblast supernatants were used, HUVEC were cultured in a 1:1 mix of fibroblast supernatant and HUVEC medium.

**FACS analysis and proliferation assay**

For PEDF detection using FACS analysis, fibroblasts were incubated in presence of a protein transport inhibitor GolgiPlugTM (BD Biosciences) for 12h according to manufacturer’s instructions, to increase accumulation of PEDF in the Golgi and enhance the detection of PEDF-producing cells. The cells were then fixed and permeabilised using Fixation & Permeabilisation Buffer (eBioscience) for 30 minutes, washed once with diluted Permeabilisation Buffer (eBioscience) and stained on ice with rabbit polyclonal anti-PEDF-PECy5.5 (Bioss Inc.). Cells were then washed once with Permeabilization Buffer and analysed against the corresponding isotype control using BD FACSDiva software version 6.0 BDTM LSR II flow cytometer.

For determination of HUVEC proliferation, cells were labelled with the carbocyfluorescein succinimidyl ester dye analogue, CellTrace™ Violet (Invitrogen). Prior to co-culture experiments, in order to track cell division following co-culture and for accurate gating, CD90-PEvio770 and CD31-APC (Miltenyi biotec) were used to exclude potential contamination of HUVEC with co-cultured fibroblasts; 7-Aminoactinomycin D (7-AAD) was used as a viability marker. Cell division frequency and proliferation indices from list mode data were determined using proliferation wizard of ModFit software version 3.2 (Verity Software House, Topsham, ME. USA).

**Immunohistochemistry**

Immunohistochemistry (IHC) analysis of human skin biopsy was performed on 3µm paraffin sections. Antigen retrieval was performed according to the suppliers’ instructions. The immunoreaction was detected using a goat anti-mouse-HRP and goat anti-rabbit-ALP (Menarini Diagnostics, UK) followed by 3,3-diaminobenzidine tetrahydrochloride (DAB, Vector Laboratories, UK) for visualization of PEDF, and Fast Red staining (Menarini Diagnostics, UK) which produces a bright fuchsin-red fluorescence precipitate, for visualization of Cav-1, CD31, and SMA. Twenty five microscopic fields obtained using a 40X objective from nine skin biopsy samples were analysed by two observers blinded to the tissue source. The number of PEDF positive fibroblasts was counted and expressed as the mean of two observations for each sample. Quantification of blood vessels (clearly identified lined by endothelial cell nuclei) was from at least 5 representative microscopic fields from 3 different sections of each biopsy stained for CD31.

For mouse skin biopsies staining was performed on 3µm paraffin sections. Antigen retrieval was performed according to the suppliers’ instructions. The immunoreaction was detected using a secondary HRP antibody and 3,3-diaminobenzidine tetrahydrochloride (DAB) substrate (Vector Laboratories, UK). The sections were imaged using an Axioplan Zeiss light microscope equipped with an AxioCam digital camera. Three fields obtained with a 40X objective, from 3 sections from each biopsy sample were analysed by two independent observers blinded to the mouse strain.