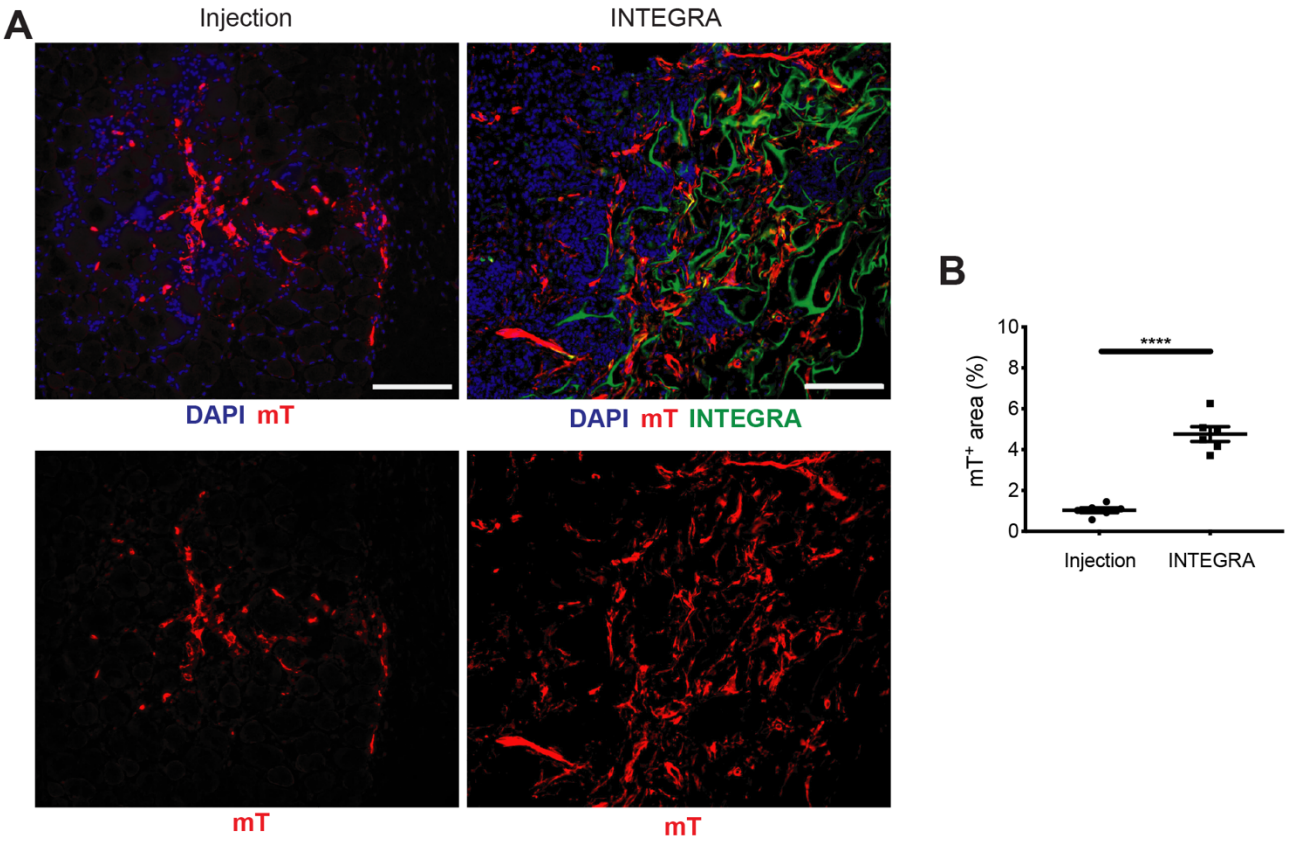
Supplementary information

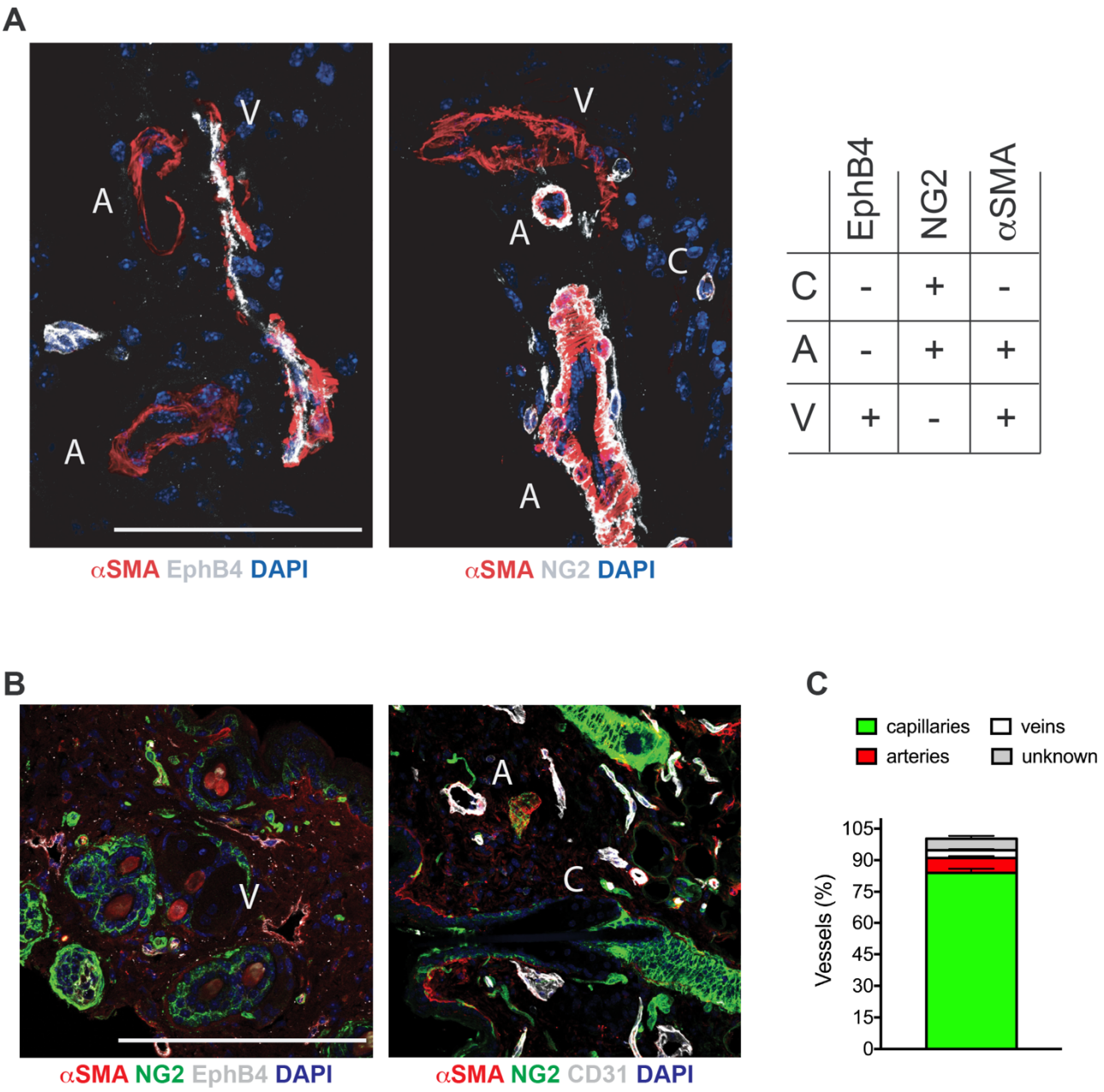
Supplementary Figures



Supplementary Fig. 1. SVF seeding on INTEGRA scaffolds promotes cell engraftment.

A. Representative images of mT^+ SVF cells either injected in the subcutaneous tissue surrounding the wound perimeter (left panels) or applied to an INTEGRA scaffold prior to wound dressing (right panels) at day 3 after cell implantation. Scale bar, 100 µm.

B. Quantification of the area occupied by the mT^+ SVF cells in the conditions described in panel A. Data are shown as mean ± S.E.M. n ≥ 3 per group. Statistical significance was determined using unpaired Student's t-test. ****P < 0.0001,



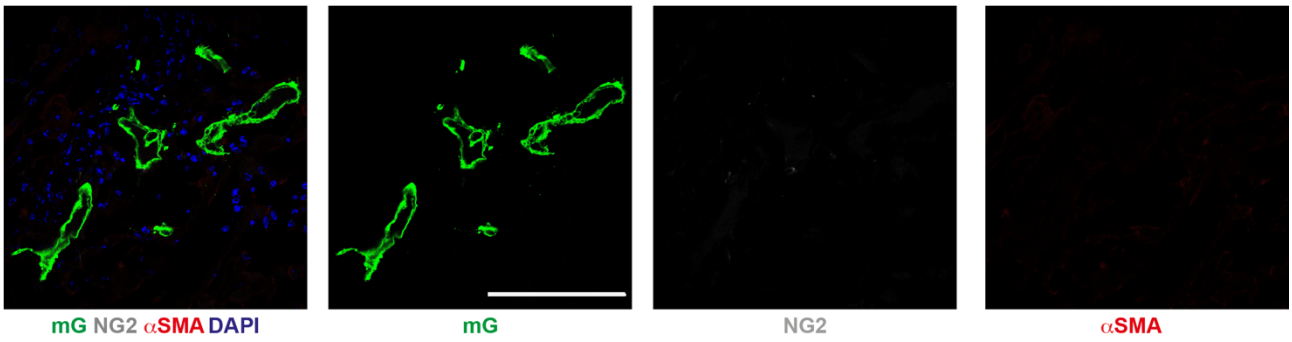
Supplementary Fig. 2. Immunophenotyping of vessel types.

A. The three major vessel types composing any vascular network, namely arteries (A), veins (V) and capillaries (C), are identified by a combined immunostaining for α-SMA, NG2 and EphB4, as detailed in the matrix on the right.

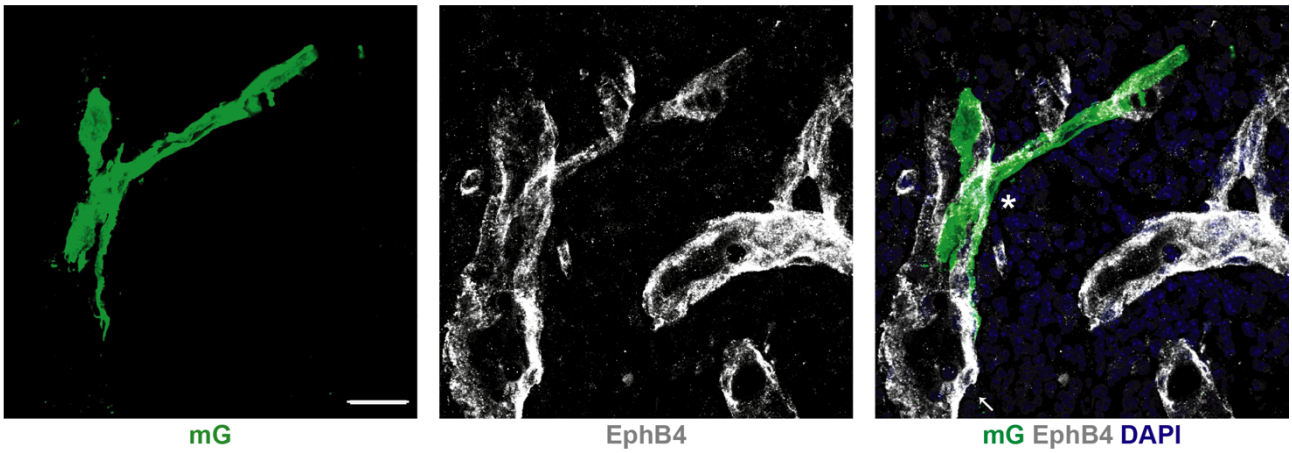
B. Representative image of healthy mouse skin stained for the indicated markers to visualize the various vessel types, as identified in panel A.

C. Quantitative analysis of the different vessel types composing the mouse healthy skin.

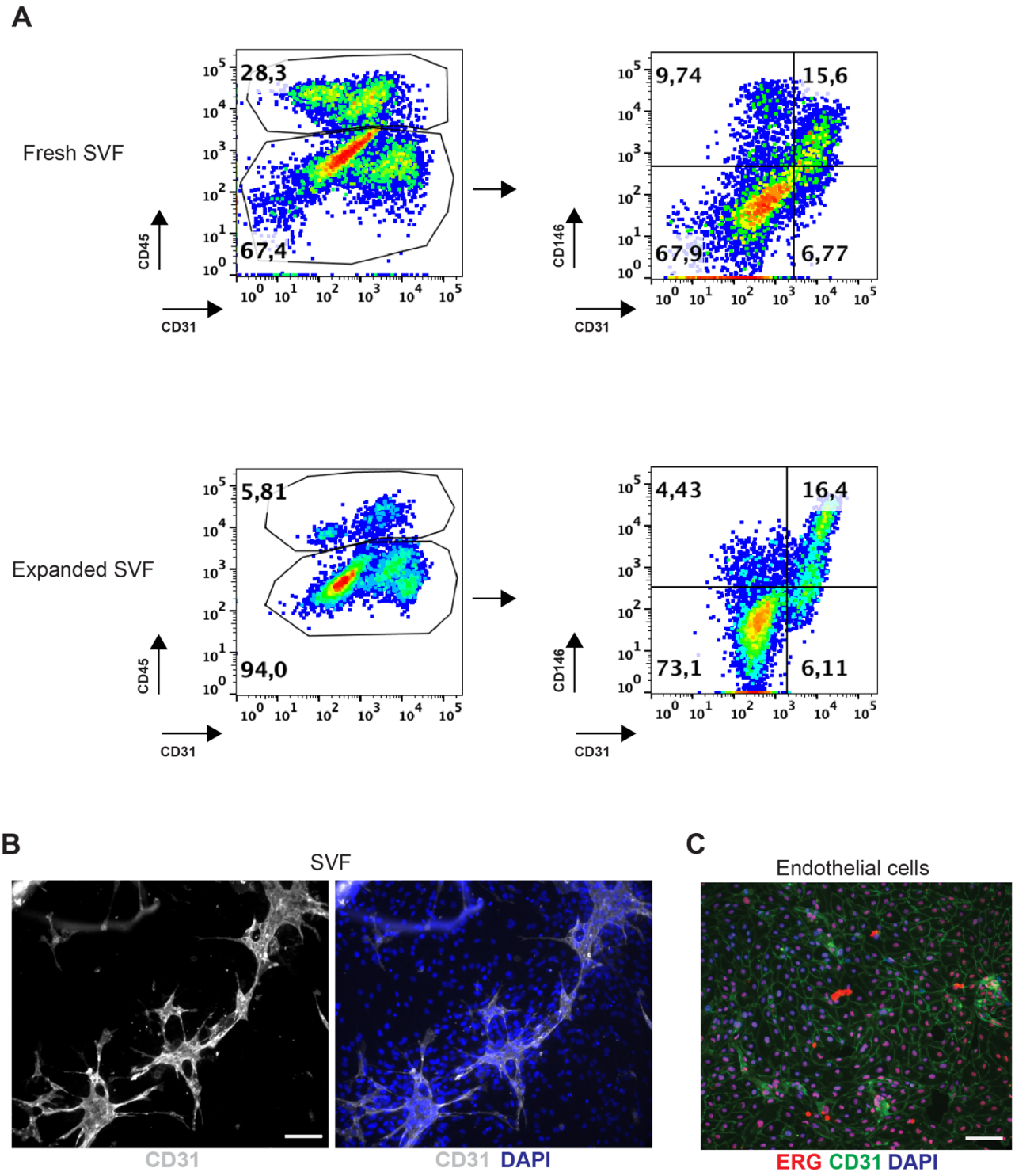
Scale bar in A, B, 100 µm.



Supplementary Fig. 3. Vessels formed by pure ECs are structurally immature.
Representative image of a wound bed covered by INTEGRA seeded with purified ECs from Cdh5-CreER/mTmG mice, treated with tamoxifen for 7 days, stained for the perivascular cell markers α-SMA and NG2. Scale bar, 100 µm.



Supplementary Fig. 4. Hybrid vessels formed by both SVF-derived and host ECs.
Representative image of a venous network labeled in white by Eph4, composed of both SVF-derived mG^+ ECs (asterisk) and host mG^- EC (arrow). Scale bar, 25 µm.



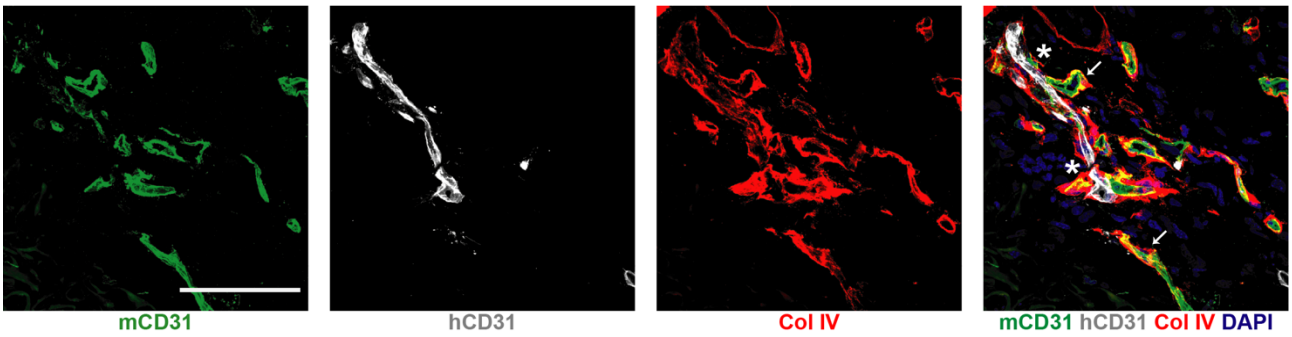
Supplementary Fig. 5. Human SVF phenotyping.

A. Flow cytometry plots showing the different populations composing the SVF either immediately after harvesting (upper panels) or after five days of *ex vivo* expansion (lower panels).

B. Representative image of human SVF in culture, with ECs (labeled by CD31) forming tubular structures over the other cell types

C. Representative image of a pure EC culture (labeled by ERG and CD31), showing a monolayer of cuboidal ECs.

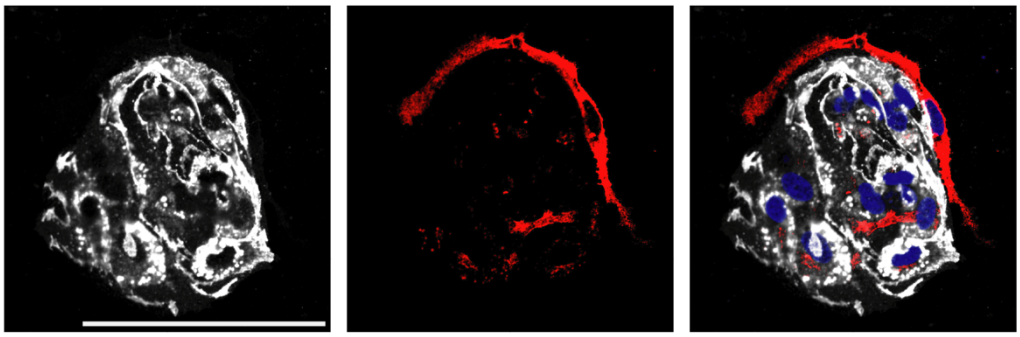
Scale bar in B, C, 100 µm.



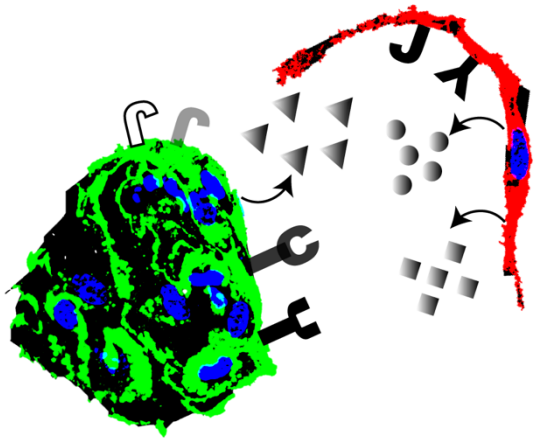
Supplementary Fig. 6. Hybrid vessels formed by both SVF-derived (human) and host (mouse) ECs are surrounded by a basal membrane.

Representative image of a vascular network composed of both host ECs (mCD31$^+$, arrows) and SVF-derived ECs (hCD31$^+$, asterisks), which form hybrid vessels covered by a layer of Collagen IV$^+$ basal membrane. Scale bar, 100 µm.

A

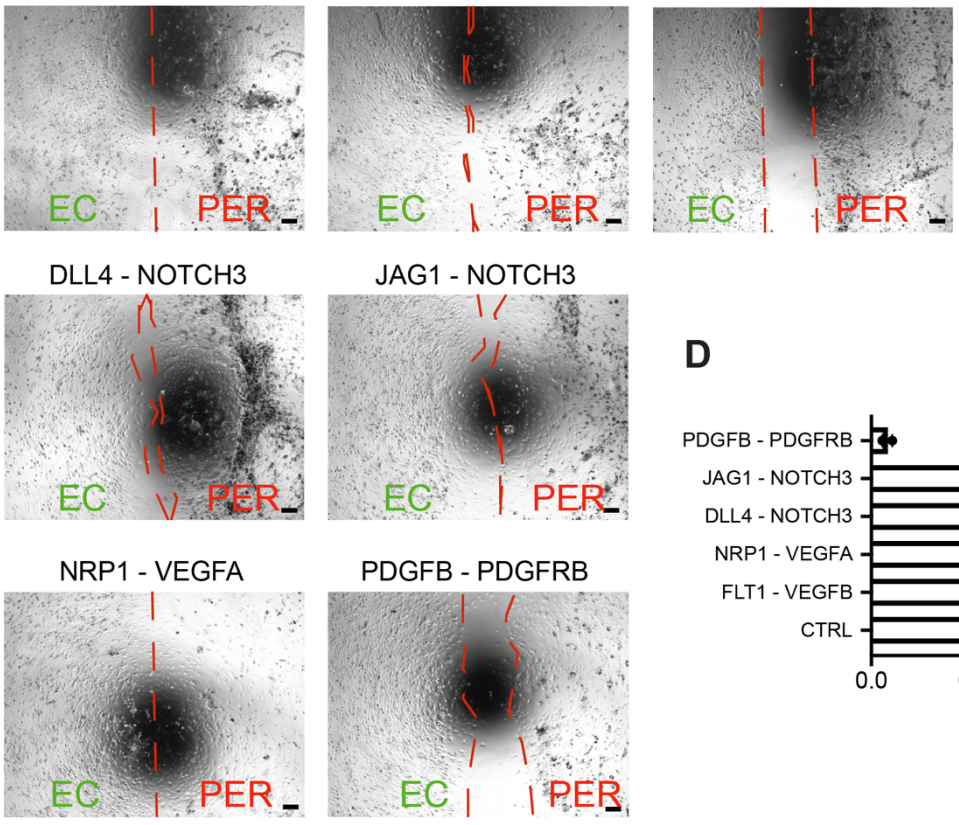


B



EC	PER
PDGFB	PDGFRB
FLT1	VEGFB
NRP1	VEGFA
DLL4	NOTCH3
JAG1	

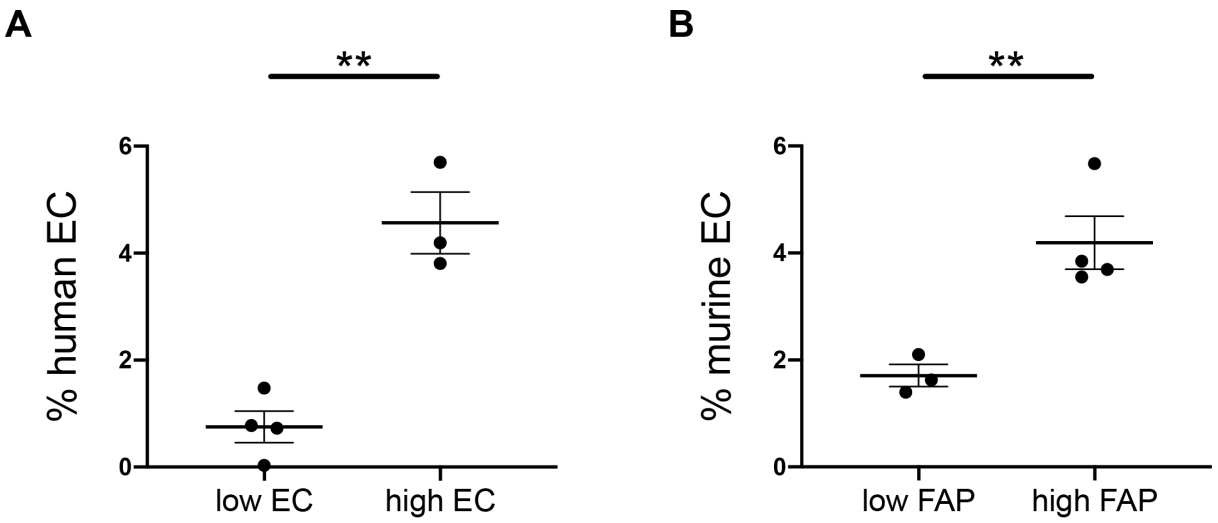
C



D

Supplementary Fig. 7. PDGFB-PDGFRB is the major ligand-receptor pair mediating the interaction between ECs and pericytes from mouse SVF.

A. High magnification of SVF-derived ECs, labeled by anti-CD31 antibodies, and SVF-derived pericytes, labeled by anti-NG2 antibodies, establishing close cell-cell interactions.
B. Schematic representation of green ECs and red pericytes, reproduced as binary images from real cells shown in panel A, with indication of the major ligand-receptor pairs identified by interactome analysis.
C. Representative brightfield images of ECs and pericytes seeded on the two halves of a well, transfected with siRNAs specific for the ligand-receptor pair indicated on top of each image, and let to migrate to close the wound for 16 hours.
D. Quantification of gap closure, expressed as the percentage of the original gap area (immediately after insert removal) covered by cells at 16 hours. Data are shown as mean ± S.E.M. n = 2 per group.

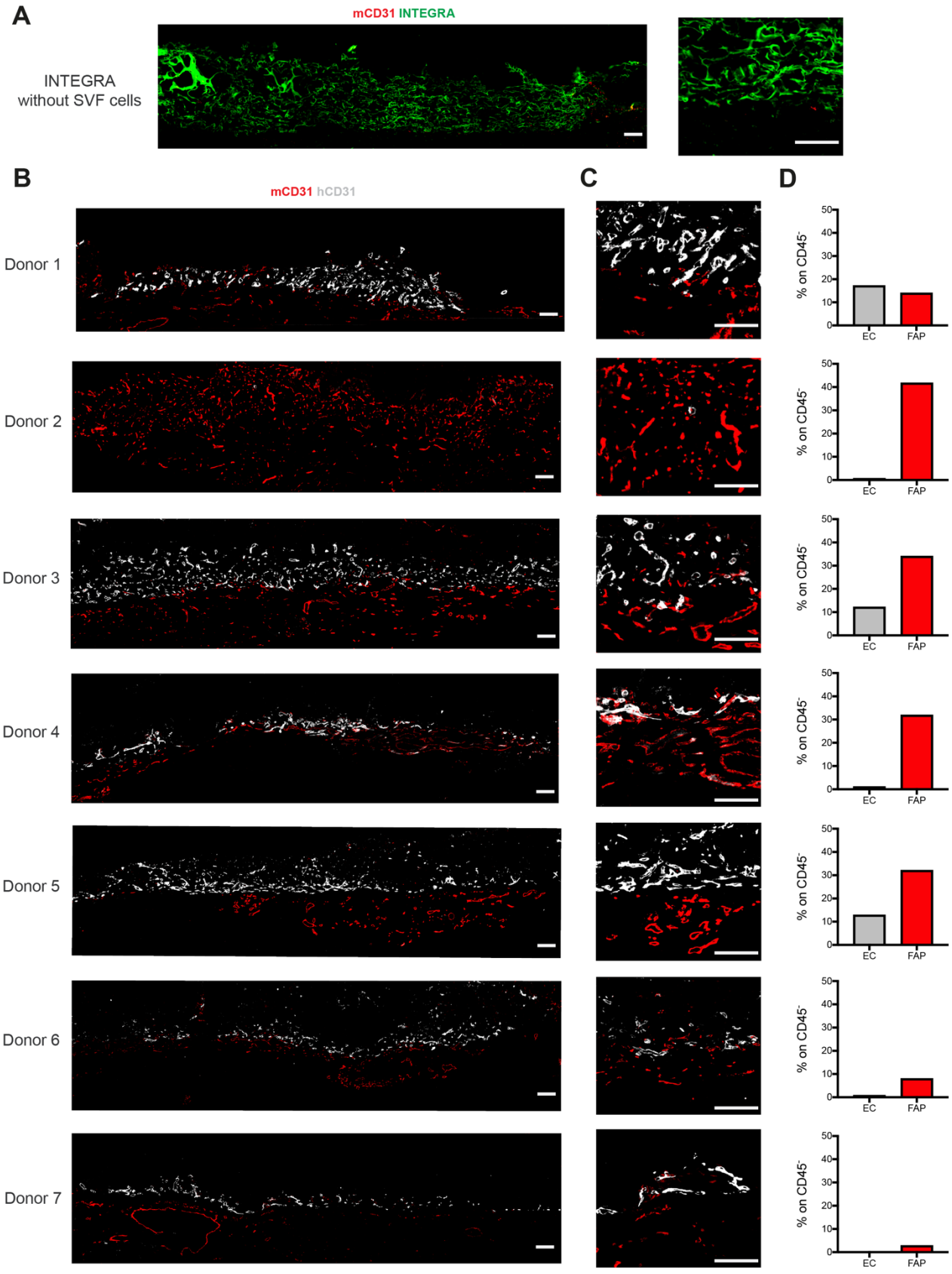


Supplementary Fig. 8. SVF-derived ECs and FAPs form new vessels through direct incorporation and paracrine activity, respectively.

A. Quantification of the wound area covered by human ECs, recognized by human-specific anti-CD31 antibodies, upon implantation of INTEGRA seeded with human SVF containing either high or low number of ECs.

B. Quantification of the wound area covered by mouse ECs, recognized by mouse-specific anti-CD31 antibodies, upon implantation of INTEGRA seeded with human SVF containing either high or low number of FAPs.

Data are shown as mean ± S.E.M. n ≥ 3 per group. Statistical significance was determined using unpaired Student's t-test. **P < 0.01.

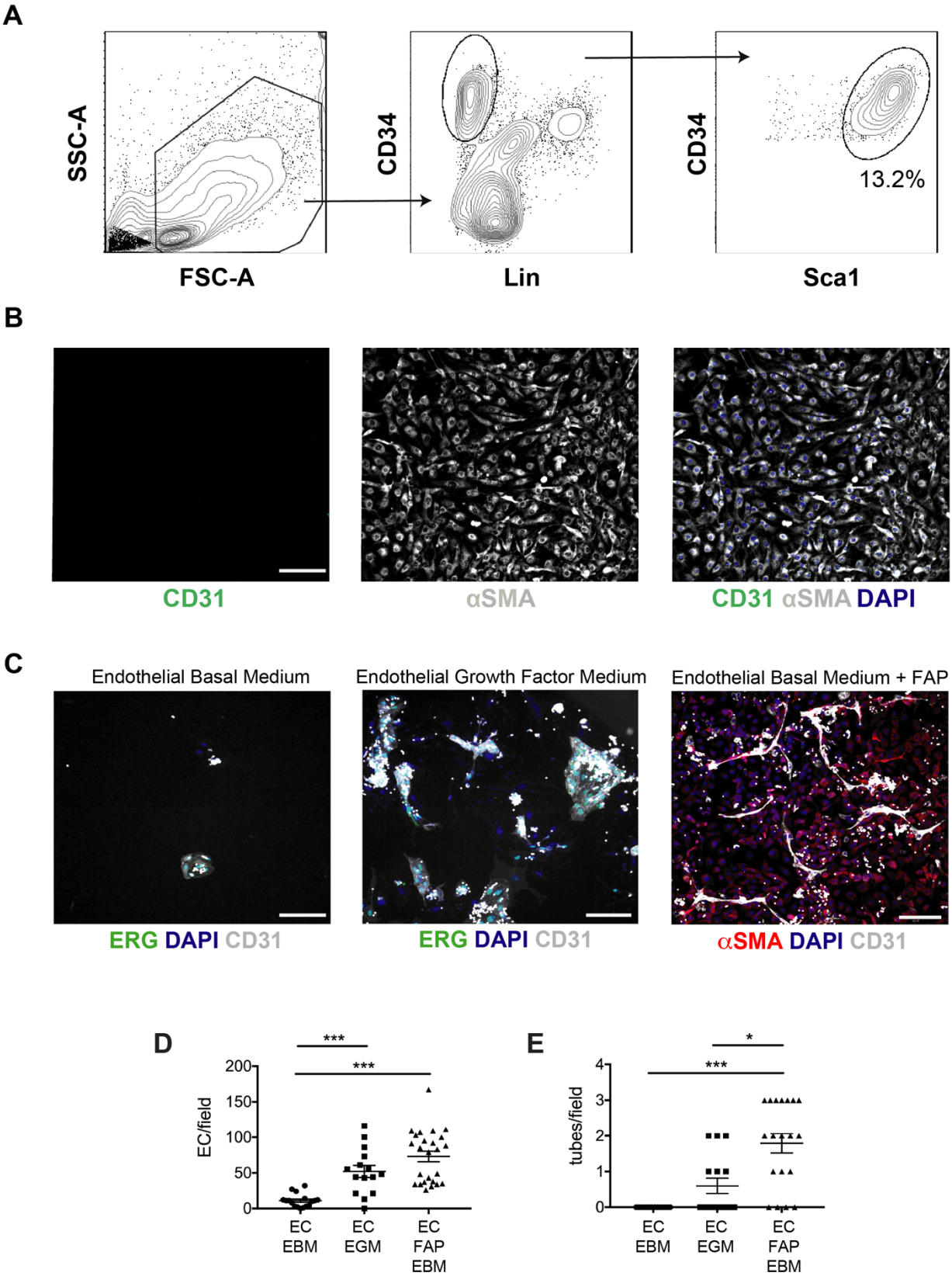


Supplementary Fig. 9. Wound revascularization exerted by SVF from seven donors.

A. Low magnification image of an ischemic wound bed covered by INTEGRA without application of SVF.

B. Low magnification images of ischemic wound beds covered by INTEGRA seeded with the SVF isolated from seven human donors.
C. High magnification images showing human (white) and mouse (red) vessels colonizing the INTEGRA scaffold.
D. Quantification of the relative abundance of ECs and FAPs in the expanded SVF of each donor prior to its seeding on INTEGRA and *in vivo* implantation.

Scale bar in A, B, C, 100 µm.



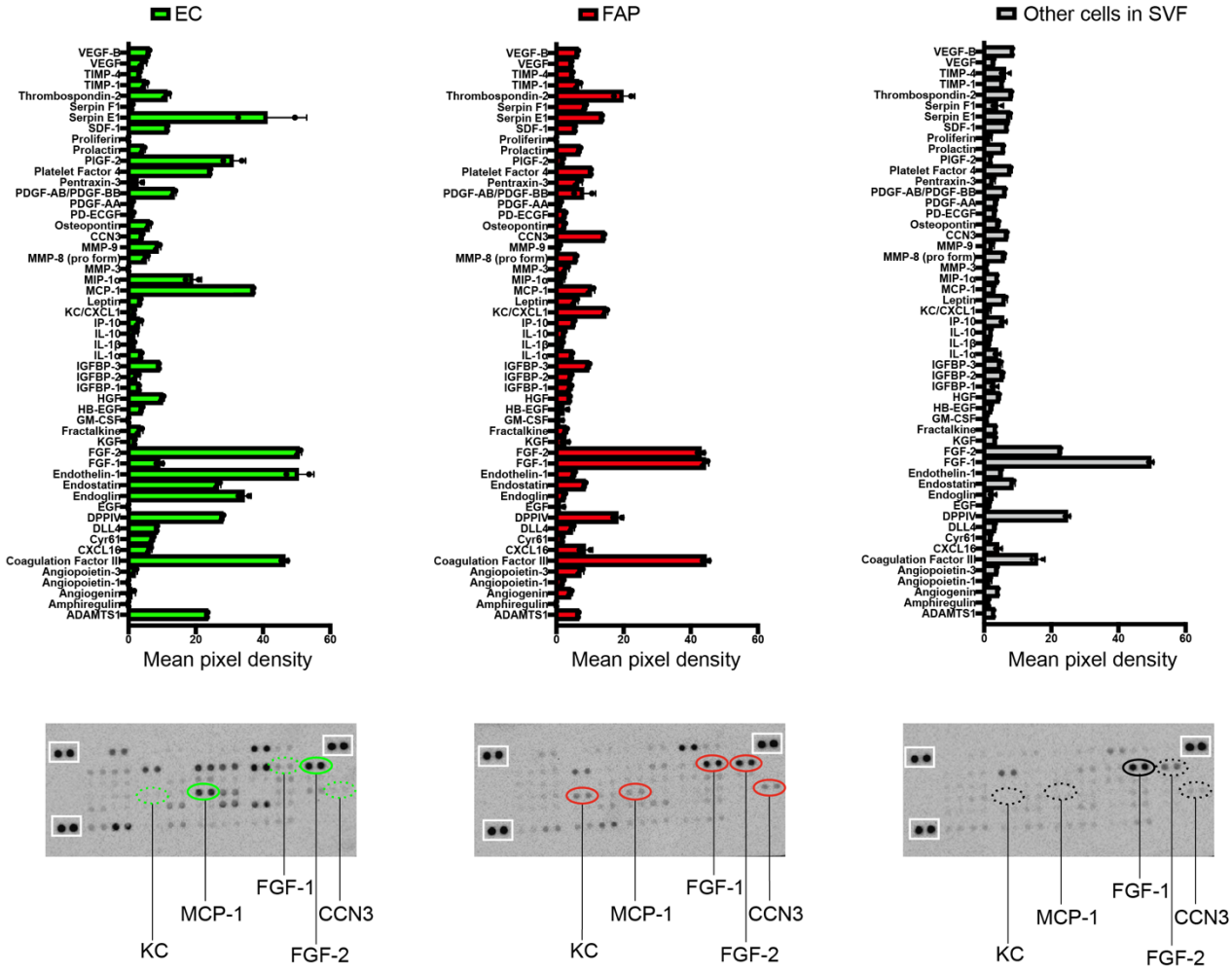
Supplementary Fig. 10. Purified mouse FAPs provide trophic support to ECs.

A. Gating strategy used for the identification and sorting of mouse FAPs. Lin, lineage (CD31 and CD45).

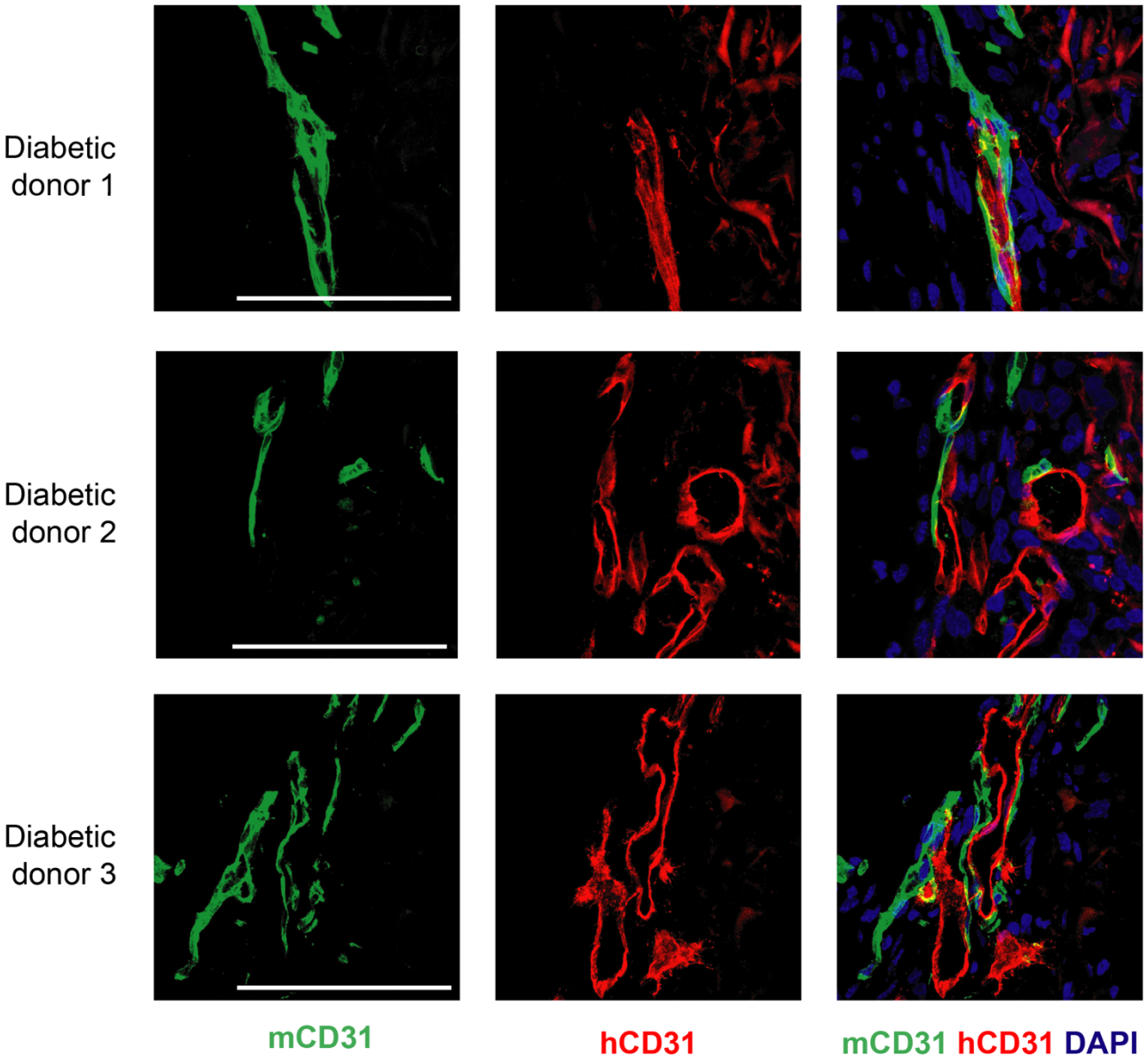
B. Sorted FAPs were cultured for 24 hours and stained for α-SMA. The absence of $CD31^+$ cells confirmed FAP purity and excluded contamination by ECs. Scale bar, 100 µm.

C. SVF-derived ECs were cultured in basal medium (EBM), growth factor-enriched medium (EGM) or basal medium in combination with SVF-derived FAPs. ECs are labeled by ERG and CD31, while FAPs are stained for α-SMA.
D. Quantification of $CD31^+$ ECs after 5 days of *ex vivo* expansion using the indicated culture media.
E. Quantification of vascular tubes formed by ECs cultured in the indicated media

Data in figure D and E are shown as mean ± S.E.M. n ≥ 3 per group. Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test. *P < 0.05, **P < 0.01, ***P < 0.001.



Supplementary Fig. 11. Multiple pro-angiogenic factors are secreted by FAPs.
Quantification and representative images of sandwich immunoassay for the analysis of 53 angiogenesis-related proteins secreted by mouse ECs (green), FAPs (red) and the remaining cell types in the SVF (gray). Multiple pro-angiogenic factors are produced most abundantly by FAPs compared to the other cell populations. Red regular circles indicate the most abundant pro-angiogenic factors produced by FAPs. The same factors are expressed at either comparable (regular green and black circles) or lower (dashed green and black circles) levels by both ECs and other SVF cells. White boxes indicate loading controls. Data are shown as mean ± S.E.M. n = 2 per group.



Supplementary Fig. 12. The SVF derived from three diabetic donors forms hybrid vessels with host (mouse) ECs.

Representative images of three vascular networks composed of both host ECs (mCD31$^+$, green) and diabetic SVF-derived ECs (hCD31$^+$, red), forming hybrid vessels similar to what observed with SVF derived from healthy donors. Scale bar, 100 µm.

Legend to Supplementary Movies

Supplementary Movie 1. The movie shows a representative 3D reconstruction of an INTEGRA sample colonized by SVF cells, in which ECs are in green and SVF remaining cells are in red, at 7 days after implantation. From the original image, isosurfaces were derived to show the composition and extension of the newly formed vascular network.

Supplementary Movie 2. The movie shows a high magnification of a representative 3D reconstruction of an INTEGRA sample colonized by SVF cells, in which ECs are in green and SVF remaining cells are in red, at 7 days after implantation. From the original image, isosurfaces were derived to show red perivascular cells wrapping around SVF-derived green ECs.

Supplementary Movie 3. The movie shows a representative 3D reconstruction of human and mouse ECs, labelled in white and red, respectively. From the original image, isosurfaces were derived to highlight the formation of hybrid vessels composed of both human and mouse ECs.

Supplementary Movie 4. The movie shows a representative 3D reconstruction of human and mouse ECs, labelled in white and red, respectively. From the original image, isosurfaces were derived to highlight the high number of filopodia emitted by a human EC sprouting to reach a mouse EC.

Supplementary Movie 5. The movie shows a representative 3D reconstruction of the vessels formed by the SVF, in which ECs are in green and SVF remaining cells are in red, upon injection of lectin (in white) in the recipient syngeneic animal. From the original image, isosurfaces were derived to highlight the presence of lectin inside vessels derived from SVF cells.

Supplementary Movie 6. The movie shows a representative 3D reconstruction of the vessels formed by human SVF, in which human ECs are in red and mouse ECs are in green, upon injection of lectin (in white) in the recipient animal. From the original image, isosurfaces were derived to highlight the presence of lectin inside vessels composed of cells of both human and mouse origin.

Supplementary Table 1

Information on key clinical features of the seven donors considered in this study, together with the relative abundance of the three main SVF sub-populations (ECs, FAPs and pericytes).

	Donor	Sex	Age	BMI class	Sampling site	EC (%)	FAP (%)	PER (%)
●	Donor 1	F	27	Normal weight	Thigh	17.2	14.0	14.1
●	Donor 2	F	56	Obese	Abdomen	0.6	41.7	0.9
●	Donor 3	F	40	Normal weight	Thigh	12.2	34.0	2.8
●	Donor 4	F	61	Normal weight	Abdomen	1.0	31.9	12.0
●	Donor 5	F	61	Normal weight	Hip	12.8	32.0	15.4
●	Donor 6	F	44	Normal weight	Hip	0.8	8.0	28.9
●	Donor 7	F	74	Normal weight	Hip	0.3	2.8	33.1

Supplementary Methods

Isolation and culture of mouse and human Stromal Vascular Fraction (SVF)

SVF isolation. Mouse SVF was isolated from inguinal white adipose tissue of adult BALB/C (Envigo), mTmG (The Jackson Laboratory, 007576), Cdh5-CreER/mTmG [1] or Apln-CreER/mTmG mice [2]. Human SVF was isolated from subcutaneous lipoaspirate of thigh, abdomen or hip. The SVF of diabetic patients was isolated from the abdominal subcutaneous adipose tissue. The collection and manipulation of human samples was performed upon approval by the competent Ethical Committee (authorization n. 15569/P/GEN/ARCS) and obtainment of informed consent by the patient.

The adipose tissue was rinsed with calcium- and bicarbonate-free Hank's solution with HEPES (CBFHH) and minced using fine scissors until the tissue suspension appeared homogeneous. The tissue was transferred in gentleMACS C Tubes (Miltenyi Biotec, 130-093-237, 130-096-334) containing a pre-warmed digestion solution composed of 1 mg/mL Collagenase NB4 Standard Grade (Nordmark Biochemicals, S1745401), 0.5 mg/mL DNase II (Sigma, D8764) and 100 µg/mL antibiotics (gentamicin, penicillin/streptomycin) dissolved in BFHH (Bicarbonate-Free Hanks´solution with HEPES) supplemented with 2mM $CaCl_2$. GentleMACS™ Octo Dissociator with Heaters (Miltenyi Biotec, 130-096-427) was used to digest the tissue running the program *37C_mr_ATDK_1*. The digested tissue was then diluted in Dulbecco's Modified Eagle Medium (DMEM, Sigma) supplemented with 10% foetal bovine serum (FBS) and centrifuged at 700 x g for 10 minutes. After removing the oily and liquid layers, the pellet was resuspended in DMEM with 10% FBS and filtered using a 70 µm pore size cell strainer (Falcon, 352350). The cell suspension was centrifuged at 500 x g for 8 minutes. Finally, SVF cells were resuspended in Endothelial Cell Growth Medium-2 (EGM-2, Lonza, CC-3162).

Murine EC isolation and culture. Mouse ECs were positively selected from the SVF by magnetic separation using Dynabeads® Sheep anti-Rat IgG (ThermoFisher, 65305) in combination with primary rat IgG anti-mouse CD31 (BD Pharmingen, 557355) following manufacturer's instructions. To assess the trophic support of FAPs on ECs, 3 x 10^4 SVF-derived $CD31^+$ cells were plated in primary 96-well plates coated with fibronectin/gelatin using either Endothelial Basal Medium (EBM)-2 or EGM-2. FAPs were then plated together with $CD31^+$ cells at a 1:1 ratio in EBM-2 and kept in culture for 5 days.

EC depletion from mouse SVF. Mouse ECs were depleted from the SVF by magnetic separation using Dynabeads® as described above. EC-depleted SVF was centrifuged at 500 x g for 8 minutes and the pellet was resuspended in EGM-2 for *in vivo* application.

Immune cell depletion from mouse SVF. Mouse immune cells were depleted from the SVF using mouse CD45 MicroBeads (Miltenyi Biotec, 130-052-301) following manufacturer's instructions. After the negative selection, the $CD45^-$ cells were centrifuged at 500 x g for 8 minutes. The pellet was then resuspended in EGM-2 for *in vivo* application.

Isolation of pericytes from mouse SVF. Mouse pericytes were positively selected from the SVF by magnetic separation using Dynabeads® M-270 Streptavidin (ThermoFisher, 11035) in combination with goat polyclonal biotinylated antibody anti-mouse PDGFR-beta (R&D/Bio-Techne, BAF1042) following manufacturer's instructions [3].

Isolation of FAPs from mouse SVF. SVF cells were centrifuged at 500 g for 5 minutes and resuspended in FACS buffer (PBD, 2mM EDTA, 2% FBS). Cells were first incubated with murine Fc blocking antibody (dilution 1:100, Biolegend, 101320) for 10 minutes at room temperature in FACS buffer. Later, they were centrifuged and incubated with staining-cocktail for 25 minutes on ice protected from light. The staining cocktail contained: PE anti-CD45 (dilution 1:200, Biolegend, 103105), BV421 anti-CD31 (dilution 1:200, BD Biosciences, 562939), PECy7 anti-Sca1 (dilution 1:2000, Invitrogen, 25-5981-81). After incubation, cells were rinsed twice, resuspended in FACS buffer and sorted using

Aria cytometer (FACS ARIA II BD). To confirm the purity of the sorted FAP population, 3 x 10^4 FAPs were plated in primary 96-well plates coated with fibronectin/gelatin in EGM-2 for 24 hours.
Ex vivo expansion of human SVF. The isolated SVF was plated at the density of 5.2 x 10^4 cells/cm^2 in primary plates coated with fibronectin/gelatin for 5 days in EGM-2. Cells were detached and collected using TrypLE™ Select CTS™ (A1285901, ThermoFischer). A similar protocol was used to expand the human SVF in the bioreactor NANT 001 (VivaBioCell).

Angiogenic protein array on purified SVF sub-populations
$CD45^-CD31^-$ SVF cells, $CD31^+$cells and FAPs were purified from the adult mouse SVF as described. The expression profile of angiogenesis-related proteins was analyzed using Proteome Profiler Mouse Angiogenesis Array Kit (R&D/Bio-Techne, ARY015,) as per manufacturer's instructions. The membranes were incubated with SuperSignal West Dura kit (Thermo Scientific, 34577) and images were acquired with BioRad ChemiDoc Touch. Pixel density was quantified using ImageJ/Fiji software (NIH).

Gene silencing in EC-pericyte co-culture
To test the crosstalk between SVF-derived ECs and pericytes, we set up a migration assay by placing a 2 well silicone insert with a defined cell-free gap (Ibidi, 80209) into 24-well plates coated with fibronectin/gelatin. Each cell type was then seeded at the density of 1,5 x 10^4 cells in one of two halves of the well. The day after, cells were transfected with 50 nM siRNA (Dharmacon- Horizon, siGENOME Smart pool siRNA) using RNAiMAX (Invitrogen, 13778-150) according to manufacturer's instructions, as indicated in **Supplementary Table 2**. The silicon inserts were removed 72 hours after transfection and fresh EBM-2 was added to the culture. Cells were fixed using 4% PFA after additional 16 hours.

Supplementary Table 2

siRNA smart pool	Catalog number	Silenced in
siGENOME Mouse Flt1 siRNA	M-040636-01-0005	Endothelial cells
siGENOME Mouse Nrp1 siRNA	M-040787-00-0005	Endothelial cells
siGENOME Mouse Dll4 siRNA	M-045947-02-0005	Endothelial cells
siGENOME Mouse Jag1 siRNA	M-041922-01-0005	Endothelial cells
siGENOME Mouse Pdgfb siRNA	M-050604-01-0005	Endothelial cells
siGENOME Mouse Vegfb siRNA	M-047407-01-0005	Pericytes
siGENOME Mouse Vegfa siRNA	M-040812-01-0005	Pericytes
siGENOME Mouse Notch3 siRNA	M-047867-01-0005	Pericytes
siGENOME Mouse Pdgfrb siRNA	M-048218-00-0005	Pericytes

Mouse model of ischemic, non-healing wound
Animals were housed in compliance with institutional guidelines, national and international laws and policies. All experimental procedures were approved by the ICGEB Animal Welfare Board, as required by the EU Directive 2010/63/EU, and by the Italian Ministry of Health (authorization n. 213/2022-PR).

To activate Cre-mediated recombination, 4-hydroxytamoxifen (Sigma, H6278) was dissolved in corn oil (20 mg/ml) and injected intraperitoneally (20 mg/Kg) into donor Cdh5-CreER/mTmG mice 7 days

before SVF isolation. Syngeneic recipient mice were similarly treated with Tamoxifen at 1 and 2 days after implantation of the SVF derived from Apln-CreER/mTmG SVF.
Surgical induction of unilateral hind limb ischemia was performed in adult BALB/C (8 weeks old), C57BL/6 (48 weeks old) and NSG (48 weeks old) mice under general anesthesia with intraperitoneal injection of ketamine 40 mg/kg (Imalgene 1000) / xylazine 100 mg/kg (Sigma). Mice were placed in a supine position on a heated plate, with the left limb extended and immobilized. An incision of the skin, approximately 1.5 cm long, was made at the level of the knee towards the medial thigh under dissection microscope. The femoral artery was separated from the femoral vein and nerve and occluded using a 7-0 silk suture at two sites: close to the inguinal ligament (proximal ligation) and under the knee (distal ligation). The segment between distal and proximal ligations was eventually excised. After having sutured the skin incision, mice were placed in a prone position and a full-thickness skin lesion was induced at the level of the posterior *femoralis biceps* muscle using a disposable punch for skin biopsies (6 mm in diameter). Animals were kept on the heated plate in a recovery cage and monitored until awake.
In vivo cell implantation. SVF cells (5 x 10^5) were either injected into the subcutaneous tissue surrounding the wound perimeter or seeded on INTEGRA Dermal Regeneration Template with silicone layer (Integra Life Science Corporation) in EGM-2 for 1 hour at 37°C and 5% pCO_2. The INTEGRA scaffold (6 mm x 5 mm) seeded with SVF cells was directly applied to the wound and sutured using a 5-0 wire.
Lectin perfusion. Biotinylated lectin (VectorLab / DBA, B-1175-1, 5 µg) was intravenously injected 10 minutes prior to animal sacrifice.
Perfusion scintigraphy. Perfusion scintigraphy with pinhole collimator was performed under general anesthesia as described before. Either BALB/C or NSG mice were intravenously injected with 3.7 mBq of 99mTc-Tetrofosmin (MioViewTM GE Healthcare). After 10 minutes, mice were placed in prone position with both ischemic left limb and non-ischemic right limb extended and immobilized. Images were acquired for 10 minutes using a gamma camera equipped with a pinhole collimator (Siemens Ecam) [4].

Immunofluorescence
For histological analysis, mice were systemically perfused with 10 ml of 1% v/v PFA (Santa Cruz) and PBS. The INTEGRA scaffold and tissue underneath were collected and fixed overnight at 4°C in 2% PFA. Tissues were equilibrated in 30% sucrose overnight at 4°C before embedding in OCT (Bio-Optica). Sections were permeabilized with 0,5% v/v Triton X-100 (Sigma-Aldrich) in PBS for 20 minutes and blocked in 5% BSA (Bovine Serum Albumin – Roche) in PBS for 1 hour. The samples were incubated overnight at 4°C with the primary antibody diluted 1:100 in 1% BSA, 0.1% Tween-20 (Sigma-Aldrich) in PBS. Subsequently, secondary antibody was diluted 1:500 in 1% BSA, 0,1% Tween-20 in PBS for 2 hours at room temperature. Nuclei were counterstained with Hoechst 33342 (Invitrogen, H3570) diluted 1:5000 in PBS. Finally, slides were mounted using Mowiol mounting medium (Sigma-Aldrich).
The same protocol was performed on cells that were previously fixed with 4% PFA for 20 minutes and permeabilized with 0.5% Triton X-100 (Sigma) in PBS for 2 minutes.

Supplementary Table 3. Primary antibodies used for immunofluorescence.

Antibody	**Company**	**Catalog number**
Goat monoclonal antibody anti-mouse CD31/PECAM-1	R&D/Bio-Techne	#AF3628

Mouse monoclonal antibody anti-human CD31/PECAM-1	Dako	#M0823
Rabbit polyclonal antibody anti-laminin	Sigma-Aldrich	#L9393
Monoclonal antibody Anti-Actin, alpha-Smooth Muscle - Cy3	Sigma-Aldrich	#C6198
Mouse monoclonal antibody anti- Actin, alpha-Smooth Muscle	Dako	#M0823
Goat polyclonal biotinylated antibody anti-mouse PDGF R beta	R&D/Bio-Techne	#BAF1042
Rabbit polyclonal antibody anti- NG2 Chondroitin Sulfate Proteoglycan	Merck	#AB5320
Rabbit monoclonal recombinant anti-LYVE1 antibody	Abcam	#ab218535
Goat polyclonal IgG antibody anti-mouse EphB4	R&D/Bio-Techne	#AF446
Lectin byotin conjugate from Lycopersicon esculentum	VectorLab / DBA	#B-1175-1
Goat polyclonal antibody anti-GFP	Abcam	#ab5450

Supplementary Table 4. Secondary antibodies used for immunofluorescence.

Antibody	**Company**	**Catalog number**
Alexa Fluor 647 Donkey anti-mouse IgG (H+L)	Invitrogen	#A-31571
Alexa Fluor 594 Donkey anti-mouse IgG (H+L)	Invitrogen	#A-32740
Alexa Fluor 647 Donkey anti-goat IgG (H+L) (#A-21447 Invitrogen)	Invitrogen	#A-21447
Alexa Fluor 594 Donkey anti-goat IgG (H+L) (# A-11058 Invitrogen)	Invitrogen	#A-11058
Alexa Fluor 488 Donkey anti-goat IgG (H+L) (# A-11055 Invitrogen)	Invitrogen	#A-11055

Alexa Fluor 647 Donkey anti-rabbit IgG (H+L) (#A-31573 Invitrogen)	Invitrogen	#A-31573
Alexa Fluor 594 Donkey anti-rabbit IgG (H+L) (# A-21207 Invitrogen)	Invitrogen	#A-21207
Alexa Fluor 488 Donkey anti-rabbit IgG (H+L) (# A-21206 Invitrogen)	Invitrogen	#A-21206
Streptavidin APC Conjugate	eBioscience™	#17-4317-82
Streptavidin, Alexa Fluor® 546 conjugate	ThermoFischer	#S11225

Microscopy and image analysis

Images were acquired with a Nikon Eclipse Ti-E inverted fluorescent microscope equipped with DC-152Q-C00-FI using NIS V4.30 software (Nikon) and a ZEISS LSM 880 with Airyscan.

At least four images per sample were acquired for both *ex vivo* and *in vivo* experiments. Images were analysed using ImageJ2 (Fiji) software. Z projection was performed on maximum intensity. Stitched images were acquired using NIS V4.30 software (Nikon). The analysis of host and donor vasculature was limited to the central area of each stitch (5120 x 2160 pixels)

Flow cytometry

Cells were centrifuged at 500 x g for 5 minutes and resuspended in FACS buffer with human Fc blocking antibody (dilution 1:100, BD Pharmigen, 564219). After 10 minutes, the cells were centrifuged at 500 x g for 5 minutes and resuspended in staining-cocktail for 25 minutes on ice, protected from light. The staining-cocktail contained: CD34 PE (1:500, clone 4H11, Invitrogen, 12-0349-42), CD146 Ax647 (1:500, clone P1H12, Biolegend, 361013), CD45 BV421 (1:500, clone H130, BD Horizon, 563880), CD31 FITC (1:500, clone WM59, Invitrogen 11-039-42), CD90 BV786 (1:500, clone 5E10, BD OptiBuild, 740986). After incubation, cells were rinsed twice with FACS buffer and fixed in 4% PFA for acquisition with Influx cytometer (FACS Celesta BD).

Interactome analysis

The interactome was generated from the public available dataset of Hildreth et al. [5] (GEO accession: GSE155960) to collect scRNA-Seq data of Smooth Muscle Cells (n=292) and ECs (n=394). The 'DaMiRseq' R package was used to select expressed genes and to perform the log2CPM normalization and the exploratory analysis [6]. Ligand-Receptor interactions were identified by CellPhoneDB (v.2.1.7) [7].

Statistical analysis

Data are represented as mean ± standard error of the mean (SEM) and the sample number is indicated in each figure legend. Data were obtained from at least 3 independent experiments. The significance of differences was assessed using the GraphPad Prism 8 software. The normal distribution of data sets was tested and, depending on the results, multiple comparisons were performed with the parametric one- or two-way analysis of variance (ANOVA) or with the nonparametric Kruskal–Wallis test, while single comparisons were analyzed with the nonparametric Mann–Whitney test or the parametric one-tailed t-test. Results with p values of less than 0.05 were considered statistically significant (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

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