SUPPLEMENTARY MATERIAL

DETAILED METHODS

Animal procedures - The experiments were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (the Institute of Laboratory Animal Resources, 1996) and with approval of the British Home Office. Type 1 diabetes (T1D) was induced in male CD1 mice (Charles River) by streptozotocin (STZ).[1] Non diabetic controls consisted in age-matched CD1 male mice injected with the vehicle of STZ. Measurements of glycemia at fast and glycosuria were performed during follow-up to confirm the persistence of diabetes.

Benfotiamine (BFT) supplementation - At 4 wk from diabetes induction, T1D mice were randomly assigned to receive BFT (70mg/kg body weight daily) or vehicle (1mmol/L HCl) in drinking water for 24 wk. Non-diabetic age-matched vehicle-treated mice were studied as a control reference. BFT concentration in drinking water was modified according to consumption. The effect of diabetes and BFT treatment on the activity of the thiamine-dependent enzyme transketolase and glucose-6-phosphate dehydrogenase (G6PDH) was measured in enriched bone marrow (BM) mononuclear cells (MNCs), as described.[2, 3]

Measurement of marrow blood flow (BF) - BF to the femur and tibia BM was assessed by fluorescent microspheres as reported.[4]

Bone fixation, decalcification and sectioning - Femoral bones were cleaned from muscle and connective tissue and fixed with 4% buffered formalin for 24 h at room temperature (RT). Bones were decalcified in 10% formic acid for 24 h at RT and then kept in PBS and processed for paraffin-embedding. Paraffin sections of marrow were cut at 3 μm thickness for histological analyses.

Morphometric measurements - Total volume of the marrow was computed from longitudinal and cross BM sections on an Olympus BX40 microscope using an ocular objective provided with a 42 points grid (Wild Heerbrugg Instruments Inc.), which defines an area of 0.2 mm^2. The following parameters were obtained using Giemsa, Trichrome Masson and Gomori staining:
-Fractional Volume (%) of bone, hematopoietic component, fatty tissue and collagen
-Bone Thickness (μm)

Immunofluorescence on marrow and analysis of vascular profiles - Paraffin embedded BM sections (3μm thick) were kept at 60°C for 10 min and deparaffinized in xylene and rehydrated through passages in alcohol (100% to 70%) and distilled water. Heat-induced antigen retrieval was carried out in 10 mM sodium citrate buffer (pH 6) for 15 min in a microwave oven. Sections were cooled down to RT, washed in distilled water and then rinsed in PBS. In order to block unspecific binding of antibodies, samples were incubated for 30 min with 1% bovine serum albumin (BSA) or 10% goat serum in PBS. For isolectin staining of endothelial cells (ECs), sections of BM obtained from mice, which received an intracardiac (i.c.) injection of biotin-conjugated isolectin IB_4 20 min before sacrifice, were incubated with streptavidin-AlexaFluor-488 or -AlexaFluor-568 for 1 h at RT. A double staining for Isolectin IB_4 and α-smooth muscle actin (α-SMA) was performed to recognize arterioles, by adding to the above an additional 2h-incubation step with a monoclonal α-SMA-Cy3 antibody at RT. To recognize erythrocytes, BM sections were incubated with a rat anti-mouse monoclonal antibody against TER119, followed by goat anti-rat secondary antibody Alexa-488-conjugated. The antibody identifies also erythroblasts, but the two cell types can be distinguished because erythroblasts are nucleated whereas erythrocytes are non-nucleated. Perfused vessels were visualized with Isolectin IB_4 as above. In all staining procedures, nuclei were visualized by DAPI (4',6-diamidino-2-phenylindole) staining. Slides were finally mounted with Fluoromount-G mounting medium.

Analysis of BM cells by immunofluorescence - In order to reduce non-specific antibody binding, BM sections were exposed to 10%-20% serum of the species in which secondary antibodies were developed. Quenching of autofluorescence was achieved by immersion of sections in an alcoholic solution of Sudan Black. In addition, each primary antibody solution contained 10% BSA and 10% specific serum. Sections were incubated with isotype-matched controls or specific primary antibodies to detect marrow hematopoietic cells (CD45) and Sca-1^{pos} c-Kit^{pos} (SK) cells. The reactions were visualized by Fluorescein isothiocyanate-IgG (FITC), tetramethyl rhodamine isothiocyanate -IgG (TRITC) and Cy5-IgG conjugated secondary antibodies to allow the simultaneous detection of multiple antigens. Nuclei were stained by DAPI. The analysis of putative SC niches was conducted on the entire femur. Moreover, LK cells were found as individual elements or nested in clusters of two or more (range 2 to 5; average 2.3 cell/cluster) closely adjacent cells, predominantly located in the epiphysis and metaphysis. This cellular configuration, strongly suggestive of a niche, was confirmed by the documentation of the engagement of LK cells with the osteoblastic and vascular aspects of the BM through the adherens junctions, N-cadherin and VE-cadherin, respectively. A list of Abs and reagents used for immunohistochemistry can be found in the **Supplementary Table I**.

Detection of DNA double-strand breaks - DNA damage was assessed by immunohistochemistry exposing BM sections to anti-phospho histone 2AX (p-H2AX) antibody followed by biotin/strepatavidin reaction and visualized by DAB precipitation (ABC system).[5] Nuclei were counterstained with hematoxylin.

Culture and characterization of BMECs used for functional studies - Freshly harvested BM cells were immunomagnetically depleted of CD11b-expressing cells to eliminate myeloid/monocyte fraction and cultured on 0.1% gelatin in DMEM 20% FBS, supplemented with tetrapeptide Ac-Ser-Asp-Lys-Pro (AcSDKP) in order to avoid SCs and fibroblasts contamination.[6] Cells were then analyzed by flow cytometry and immunocytochemistry to assess the percentage of cells expressing endothelium-specific markers (vWF, VCAM-1 or NOS3, isolectin IB_4, DiI-acLDL).

FACS Sorting of BMECs- BM cells were stained with anti MECA-32 (Biotin) followed by Streptavidin-PE and anti CD45 (APC). Sorting was performed on FACS vantage cell sorter (BD Biosciences). MECA-$32^{pos}CD45^{neg}$ BMECs were separated on a FACS vantage cell sorter using the following gating procedure. Total BM cells were first gated for propidium iodide (P1, PI^{neg}) and then PI^{neg} BM cells were divided in MECA-32^{pos} $CD45^{neg}$ (P2) and MECA-32^{pos} $CD45^{pos}$ (P3). Only MECA-$32^{pos}CD45^{neg}$ BMECs were used.

Immunofluorescence on BMECs - BMECs were seeded on 8-well chamber slides coated with 0.1% gelatin at a density of $4x10^4$ cells/well and incubated overnight in a humidified incubator at 37°C in an atmosphere containing 5% CO_2 to allow adhesion. Cells were then fixed in 2% paraformaldehyde in PBS for 15 min and incubated in 5% normal serum of the same species as secondary antibody diluted in 0.1% TritonX-100/PBS for 30 min at RT. Cells were then incubated overnight with primary antibodies against vWF, VCAM-1 or NOS3, followed by AlexaFluor 488-conjugated goat anti-rabbit secondary antibodies for 1 h at RT, in the dark. Matched isotype controls were included in each staining to check for unspecific binding of primary antibodies. After staining for vWF, cells were incubated in 1% BSA/PBS for 20 min at RT and then exposed to biotin-conjugated isolectin IB_4 for 1 h at 37°C, followed by Alexa Fluor 568-conjugated streptavidin for 1 h at RT. Slides were mounted with fluorescence mounting medium containing DAPI and analyzed using an Olympus BX40 fluorescence microscope.

Adherent cells growing in 8-well slides were also assessed for the uptake of 1,1-dioctadecyl-3,3,3',3'-tetra-methyl-indo-carbo-cyanine labelled acetylated low density lipoproteins (DiI-acLDL) and binding of FITC-labelled isolectin IB_4 and then mounted as described above.

Detection of senescent BMECs by staining for β-Galactosidase (β-Gal) activity - BMECs were seeded on 8-well chamber slides as described above and assessed for β-Gal activity at pH 6 using Senescence Detection Kit (Calbiochem) according to the manufacturer's instructions. Briefly, cells were fixed in

fixative solution and then incubated with a staining solution containing 1 mg/mL X-gal in N-N-dimethylformamide at 37°C overnight. Cells were then observed under a day light microscope for development of blue colour. Five microphotographs/well were taken at x200 magnification and the number of positive cells per microphotograph (tot area: 0.2 mm^2) was quantified and expressed as percentage of the total number of cells in the same microphotograph. Three separate experiments in triplicates were analyzed and averaged.

MitoTracker assay - BMECs were seeded on 8-well chamber slides as described above and then assessed for reactive oxygen species (ROS) production using MitoTracker® Red CM-H_2XROS, a mitochondria-selective ROS scavenger and fluorescent probe (Molecular Probes), according to manufacturer's instruction. MitoTracker® Red CM-H_2XROS is a reduced, non-fluorescent compound that fluoresces when oxidized by ROS after uptake into the mitochondrial matrix of respiring cells.[7] Fluorescent images were captured using an Olympus BX40 fluorescence microscope applying identical exposure settings to all conditions. Fluorescence intensity was evaluated by Image Pro® Plus software. Three separate experiments in triplicates were analysed and averaged.

Migration assay - Migration was assayed using a 24-well transwell setup (Costar) using polycarbonate filters (membrane pore size: 8 μm).[8] Three separate experiments in triplicates were analysed and averaged.

Matrigel assay - Cultured BMECs ($3x10^4$ cells in a total volume of 100μL EBM-2) were added on top of 100μL gelified, growth-factors-enriched matrigel. After 16 h at 37°C, gels were washed gently with PBS and fixed with 2% paraformaldehyde, followed by H&E staining (to visualize cells) and then mounting with glycerol. Five random view fields were photographed for each sample (40X magnification) in phase contrast (Olympus). A number of n=3 samples per group was analyzed in triplicate.[8]

Static adhesion of BMMNCs to BMECs - Non diabetic (C) or T1D murine BMECs were cultured to confluence on 0.1% gelatin-coated glass covers and treated with TNF-α (10 ng/mL) overnight. Next, C murine BMMNCs, pre-labelled with Calcein-AM, resulting in green-fluorescence, were allowed to adhere for 30 min on BMECs. Samples were then washed 5 times carefully with PBS supplemented with 1mM $CaCl_2$ and 1mM $MgCl_2$. Samples were fixed, permeabilized and stained for F-actin using fluorescent phalloidin. Adherent BMMNCs were counted in 5 random view-fields using confocal fluorescent microscopy.[9]

Adhesion of BMMNCs to BMECs-under flow - C or T1D BMECs were cultured to confluence and stimulated with TNF-α, as described above. Next, cells were mounted onto the microscope stage using a POC-mini chamber system (LaCon) and connected to a perfusion pump. Using low physiological flow conditions, $1x10^6$ C BMMNCs per mL were perfused over the BMECs. Next, fluid-flow was increased every 5 min. Adhesion was visualized by phase-contrast microscopy and recorded in real-time. From each experiment, 5 view-fields were analyzed.[9]

Electric Cell-substrate Impedance Sensing (ECIS) - C or T1D BMECs were added at $1x10^4$ cells per well (0.8 cm^2) to a 0.1% gelatin-coated electrode-array, containing 10 gold-electrodes per array, and grown to confluence. After the electrode-check of the array and the basal transendothelial electrical resistance (TER) of the endothelial monolayer, N-Acetyl cysteine (N-Ac) or DMSO (vehicle) were added and TER was measured on line at 37°C at 5% CO_2 with the ECIS-Model-100 Controller from Applied BioPhysics, Inc. (Troy, NY, USA). After 8 h, data were collected and changes in resistance of endothelial monolayer were analyzed as described previously.[10]

Western blotting of BMECs - BMECs were grown to confluence on 0.1% gelatin-coated dishes (50 cm^2), washed twice gently with ice-cold Ca^{2+}- and Mg^{2+}-containing PBS, and lysed in lysis buffer (25 mM Tris, 150 mM NaCl, 10 mM $MgCl_2$, 2mM EDTA, 0.02% (w/v) SDS, 0.2% (w/v) deoxycholate, 1% Nonidet P-40, 0.5 mM orthovanadate with the addition of fresh protease-inhibitor-mixture tablets (Roche Applied

Science). After 30 min on ice, cell lysates were collected and the supernatant was separated from the insoluble fraction by centrifugation (14,000 *g*, 10 min at 4°C). Supernatant was diluted with boiling 2x SDS-sample buffer containing 4% 2-mercaptoethanol (Bio-Rad). The samples were analyzed by SDS-PAGE. Proteins were transferred to 0.45-µm nitrocellulose and the blots were blocked with 5% BSA in Tris-buffered saline with Tween-20 (TBS-T) for 1 h and subsequently incubated at RT with phospho-specific antibodies against VE-cadherin-Y731 and Pyk2-Y402 and with a monoclonal antibody against Tubulin, followed by incubation with goat-α-rabbit-IgG-horseradish peroxidase for 1 h at RT. Between the various incubation steps, the blots were washed 5 times with TBS-T and finally developed with an enhanced chemiluminescence detection system (Amersham Biosciences).

Trans-endothelial migration (TEM) - BMMNC TEM was performed using transwell cell culture inserts equipped with 3µm pore size filters (BD Biosciences).[11] Inserts were pre-coated with 0.5 µg/mL fibronectin in 0.1% gelatin. BMECs were seeded on coated transwell filters ($8x10^4$/well) and cultured for 24 h before the experiment was performed in a humidified atmosphere (37°C, 5% CO^2). SDF-1 (100ng/mL) or vehicle alone was added to the lower compartment of the transwell system in DMEM containing 0.1% BSA. Freshly isolated BMMNCs from C and T1D mice were labeled with PKH67 (Green Fluorescent Cell marker) following the manufacturer´s instruction, re-suspended at 10^6 cell/mL and then added to the top compartment ($3x10^5$ cells per well in 300 µL). After 24 h incubation at 37°C, non-migrated cells on the upper side of the membrane were removed by scraping, all inserts were fixed for 10 min in methanol, and mounted on slides using Vectashield with DAPI. The number of fluorescent BMMNCs transmigrated to the lower side of the membrane was quantified in duplicates. Five random viewfields at x200 magnification were captured and the number of PKH67-positive BMMNCs was determined. In addition, green fluorescent PKH67-stained BMMNCs migrated to the lower chamber were counted using flow cytometry. Two separate experiments in triplicates were analyzed and averaged.

Isolation of marrow cells from trabecular bone - Hematopoietic Stem Cell Isolation Kit (Millipore UK) was used for isolation of marrow cells from trabecular bone of femurs and tibia. Briefly, the marrow-flushed bones were ground thoroughly with pestle in PBS with 2% FBS so that the bones were opened and broken into small fragments. The bone fragments were washed twice and filtered through a 40µm nylon cell strainer. Bone fragments were incubated in 2 mL of enzyme solution (3 mg/mL Collagenase I & 4 mg/mL Dispase II) for 5 min at 37°C in an orbital shaker at 750 rpm. The bone fragments were then washed with PBS containing 2% FBS and filtered through a 40 µm nylon cell strainer. All collected cells were washed by centrifuging at 400*g* for 5 min at 4°C, resuspended in PBS containing 2% FBS, filtered through a 40 µm nylon cell strainer and pooled.

Mouse Colony-Forming Cell Assays - BM cells harvested from trabecular bone were washed with PBS containing 2 % FBS and plated in 35mm tissue culture dishes in 1.1mL Methylucellulose-Based Medium (StemCell Technologies) according to manufacturer's instructions. Cells were seeded at $1x10^4$ cells/dish and cultured for 14d before scoring colonies. Colonies were distinguished as colony forming unit-erythroid (CFU-E), burst forming unit-erythroid (BFU-E), colony forming unit-granulocyte (CFU-G), colony forming unit-macrophage (CFU-M), colony forming unit-granulocyte-macrophage (CFC-GM), or colony forming unit-granulocyte-erythroid-macrophage-megakaryocyte (CFC-GEMM) based on their morphological appearance.

Flow cytometry analysis of freshly isolated total BM cells - Total BM cells were washed with ice-cold Hank's balanced salt solution containing 0.5% bovine serum albumin and 0.02% sodium azide. BM cells were then stained in the same buffer with anti Lineage Mixture (Alexa 488), anti Sca-1 (PE) and anti c-Kit (Alexa 647). To recognize ECs, BM cells were stained with anti-MECA-32 (Biotin) followed by Streptavidin-APC. After washing, stained cells were examined by flow cytometry on FACSCanto II (BD Biosciences) equipped with FACSDiva software (BD Biosciences). Data were represented using "Logical" displays, as previously reported.[12]

Annexin V staining: To detect apoptosis, BM cells were stained with Annexin V (FITC) together with PI. Total BM cells ($1x10^6$) were resuspended in Annexin V binding buffer (BD Biosciences) prior to adding 2.5μg Annexin V and were incubated for 30 min at 37°C in the dark.

ROS staining: Total BM cells were incubated with 100 nM CM-H_2DCFDA for 30 min at 37°C in the dark. Cells were washed and resuspended in PBS, containing 1mM $CaCl^2$, 0.5mM $MgCl^2$, 0.1% (Wt/vol) D-glucose, and 50μM L-arginine.

Flow cytometry analysis of BMEC cultures - Adherent cells were detached by trypsinization and washed in PBS. The staining method described above was carried out. A list of the primary and secondary Abs can be found in the **Supplementary Table II.**

Measurement of in *vivo* BM cell perfusion using Hoechst gradient - The Hoe dye perfusion gradient in murine BM was evaluated as described previously.[13] Briefly, mice were injected with Hoechst 33342 dye (Hoe, Sigma-Aldrich, 0.8mg/mouse, *via* the tail vein) and then sacrificed exactly 10 min after Hoe injection, a time sufficient to avoid that cells extrude the dye.[13] Marrow cells were immediately flushed out from bones, filtered and suspended in cold Hank's balanced salt solution (HBSS) containing 2 % FBS and 10 mM Hepes buffer. The dual emission wavelengths were assessed on a logarithmic scale by FACSLSRII (BD Biosciences).

Real-time quantitative RT-PCR (qPCR) - Total RNA was isolated from murine BMMNCs (RNeasy, Qiagen) and RNA quality confirmed using the RNA Nano LabChip in a bioanalyzer (Agilent). RNA was reverse transcribed (Sensiscript reverse transcriptase, Qiagen) and quantitative PCR was performed in a LightCycler (Roche). Primers for PCR amplification for *ATM* gene are: forward - GATCTGCTCATTTGCTGCCG; reverse - GTGTGGTGGCTGATACATTTGAT. 18SrRNA was used as housekeeping gene. The ΔC_t obtained was used to find the gene relative expression according to the formula: relative expression=$2^{-\Delta\Delta C}{}_t$, where $\Delta\Delta C_t$=ΔC_t of those genes in experimental groups—ΔC_t of the same genes in control group. The analyses were performed on at least 4 samples per time and repeated three times.

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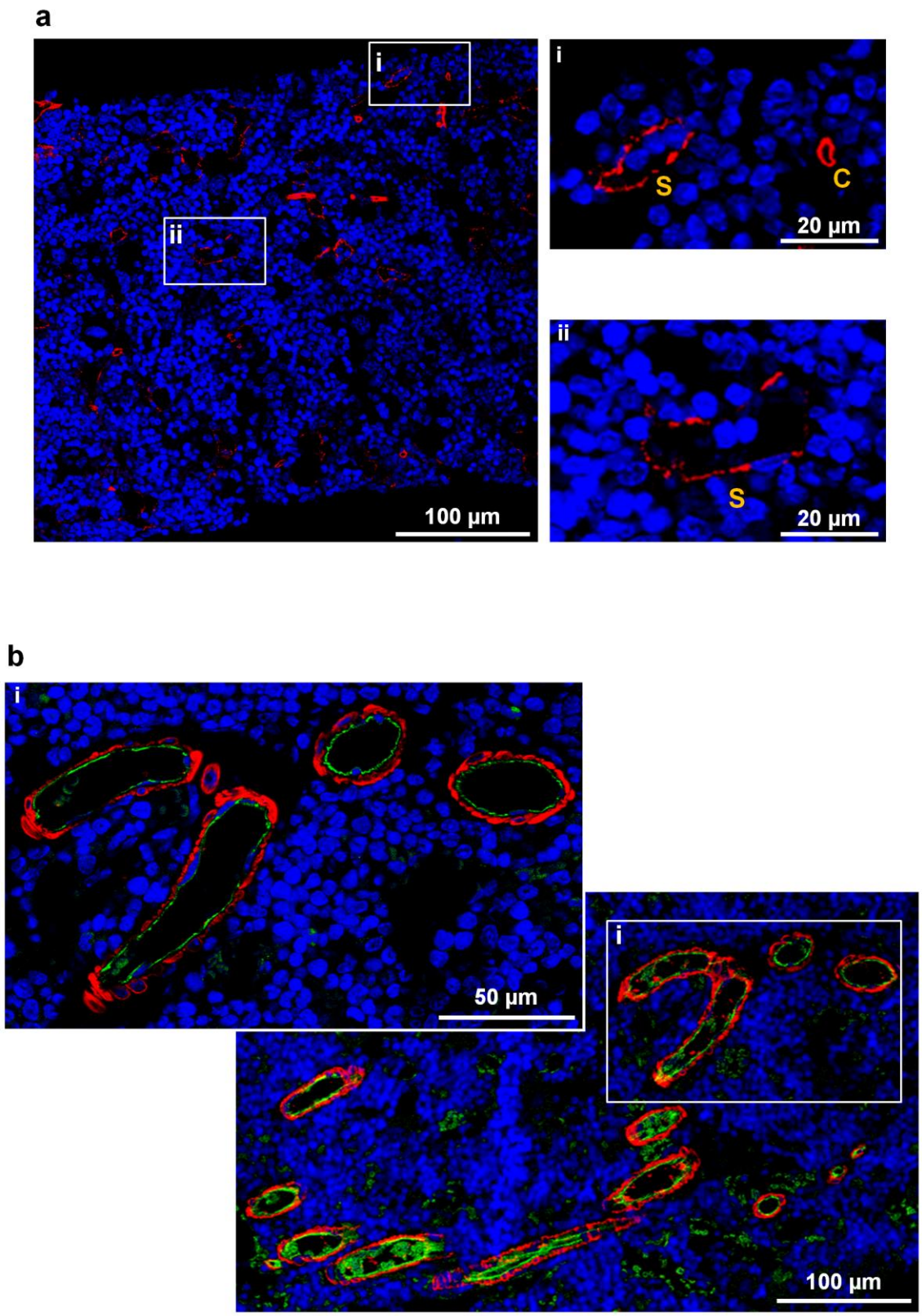
Supplemental Tables and Figures with Legends

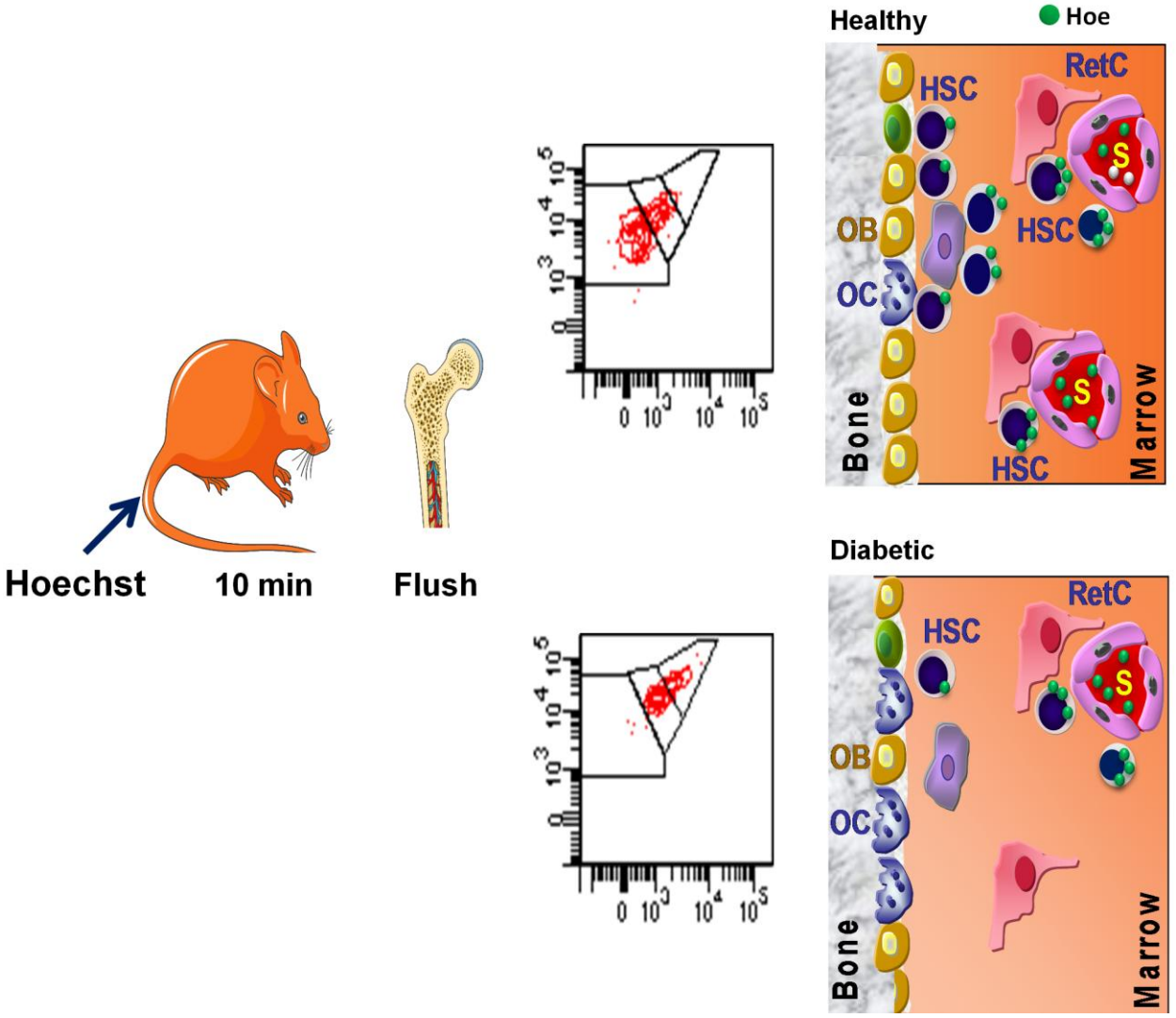
Supplementary Table I: Antibodies and reagents.		
Antibodies/reagents	**Label**	**Supplier**
Isolectin IB_4 (i.c. injected)	biotin-conjugated	Invitrogen
Isolectin IB_4	FITC	Vector Laboratories
vWF	unconjugated	Dako
α-SMA	Cy-3	Sigma-Aldrich
VCAM-1	unconjugated	S.C. Biotechnology
NOS3	unconjugated	S.C. Biotechnology
DiI-acLDL		Invitrogen
c-Kit	unconjugated	R&D
Sca-1	unconjugated	Cedarlane
CD45	unconjugated	R&D and Abcam
TER119	unconjugated	Lifespan Bioscience
VE-cadherin-2	unconjugated	S.C. Biotechnology
N-cadherin	unconjugated	Calbiochem
CD34	unconjugated	Novocastra
Anti-pH2AX	unconjugated	Bethyl
Streptavidin	AlexaFluor-488 or 568	Invitrogen
Goat anti-rabbit IgG	AlexaFluor-488 or 568	Invitrogen
donkey anti-goat	FITC or TRITC	Jakson Labs
goat anti-rat	AlexaFluor-488	Invitrogen
donkey anti-rabbit	FITC	Jakson Labs
donkey anti-rat	Cy5	Jakson Labs
donkey anti-mouse	Cy5	Jakson Labs
Vectashield with DAPI		Vector Laboratories
DAPI		Sigma-Aldrich
Fluoromount-G		SouthernBiotech
Fibronectin		Sigma-Aldrich
SDF-1		R&D Systems
VEGF-A		StemCells Technologies
Matrigel		BD Bioscience
Glycerol		Sigma-Aldrich
Calcein-AM		Invitrogen
Phalloidin		Invitrogen
VE-cadherin-Y731		Biosource

Pyk2-Y402		Biosource
Tubulin		Sigma-Aldrich
Gelatin		Sigma-Aldrich
PKH67		Sigma-Aldrich
Fluorescent microspheres		Molecular Probes

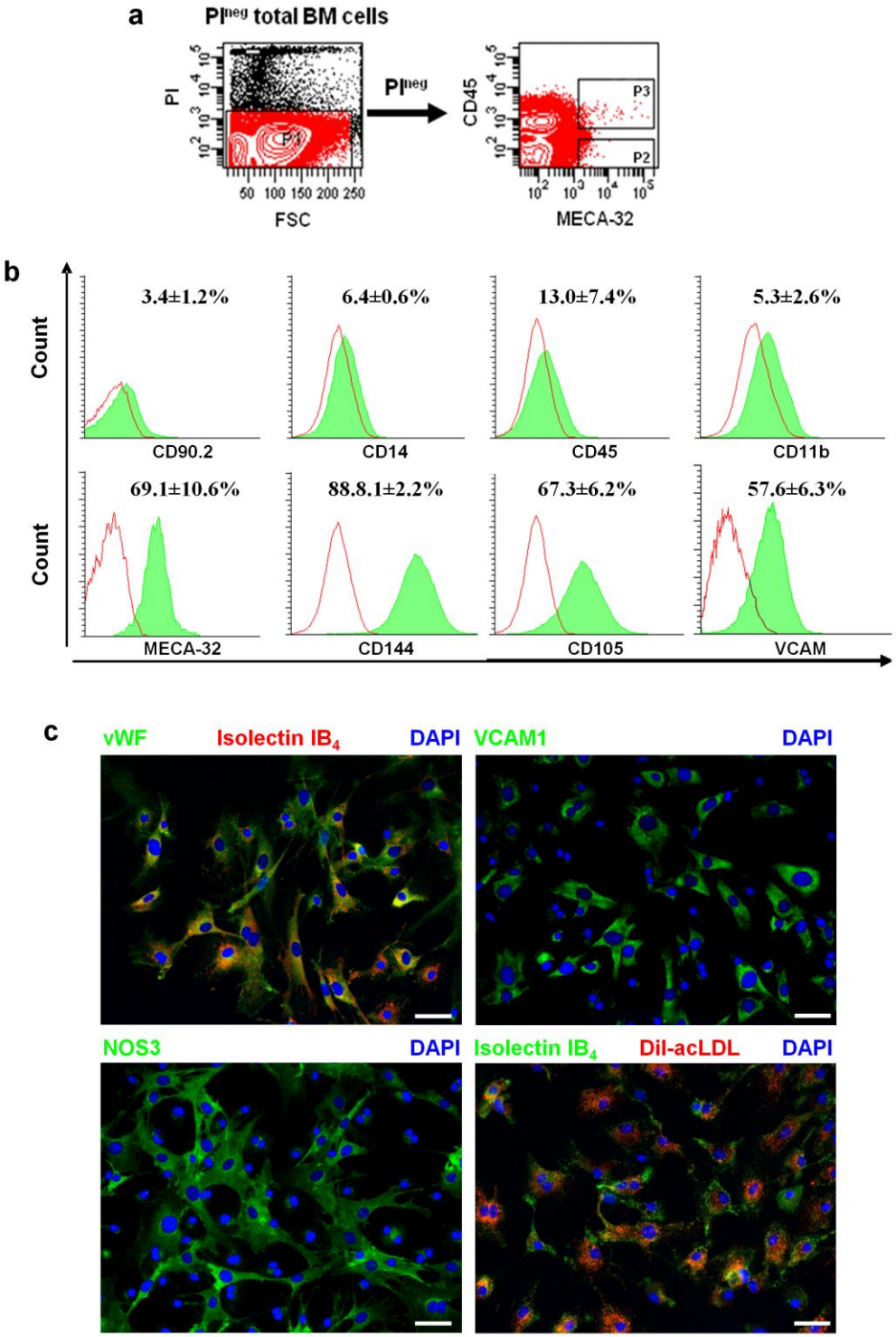
Supplementary Table II: Flow cytometry antibodies		
Antibodies/reagents	**Label**	**Supplier**
anti-Lineage Mixture	Alexa 488	Caltag
anti-Ly-6A/E (Sca-1)	PE	Caltag
anti-CD117 (c-Kit);	Alexa 647 or Alexa 750	Caltag
anti-pan-endothelial cell antigen (MECA-32)	Biotin	BD Biosciences
Streptavidin	APC	BD Biosciences
Streptavidin	PE	BD Biosciences
Annexin V	FITC	BD Biosciences
CM-H_2DCFDA	FITC	Invitrogen
anti-CD90.2	FITC	BD Biosciences
anti-CD106 (VCAM-1)	FITC	BD Biosciences
anti-CD14	PE	BD Biosciences
anti-CD105 (Endoglin)	PE	BD Biosciences
anti-CD45	APC	BD Biosciences
anti-CD144	Alexa 647	eBioscience
anti-CD11b	APC-Cy7	BD Biosciences
Anti-CD34	Alexa 647	BD Biosciences



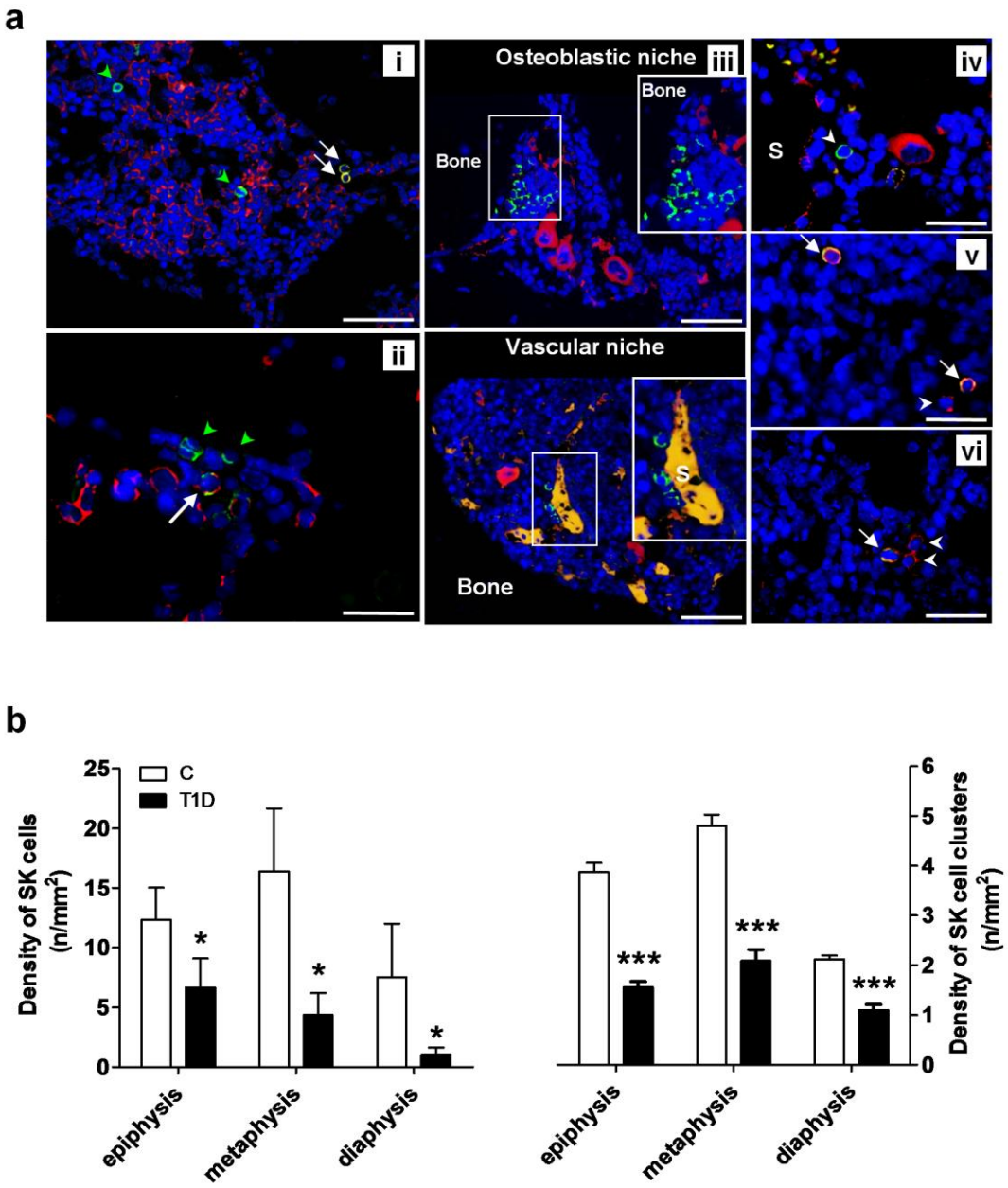
Supplementary Figure I. Immunofluorescence identification of marrow microvasculature. (**a**) Microphotographs showing vascular sinusoids (S, panel i and ii) and capillaries (C, panel i) stained red with Isolectin IB_4 (**b**) Microphotographs showing marrow arterioles, whose endothelial cell layer is stained green with Isolectin IB_4 and smooth muscle cell layer is stained red with α-SMA.



Supplementary Figure II. Distribution of BM cells across the Hoe gradient. Hoe was injected through the tail vein and the animals sacrificed 10min later to collect the hindlimb BM. Cells in microenvironments that are well perfused by blood are those exposed to the highest concentrations of Hoe, whereas cells in microenvironments that are less perfused are exposed to much lower concentrations of Hoe. Flow cytometry identification of LSK cells staining with high (Hoe^{high}) or low levels of Hoe (Hoe^{low}) allowed for recognition of hematopoietic cell abundance in high-perfused vs. low-perfused regions of BM. Sinusoid (S), hematopoietic stem cell (HSC), osteoblast (OB), osteoclast (OC).



Supplementary Figure III. Isolation and characterization of ECs from murine BM. Freshly collected BM cells were either FACS-sorted to isolate MECA-32^{pos} CD45^{neg} cells (**a**) or depleted of CD11b cells by immunomagnetic columns to eliminate the myeloid/monocyte fraction and then cultured in DMEM 10% FBS in the presence of AcSDKP to avoid SC and fibroblast contamination. The purity of culture-isolated cells was analysed by flow cytometry (**b**). Isolated BMECs were also characterized using immunofluorescence microscopy analysis of endothelial markers (**c**). Scale bars: 50μm.



Supplementary Figure IV. Diabetes alters the abundance of SK cells. (**a**) Immunofluorescence staining of c-Kit^{pos} (green) and CD45^{pos} (red) cells in BM: arrowheads point to c-Kit^{pos} cells and arrows point to c-Kit/CD45 double positive cells. c-Kit^{pos} are randomly distributed in the marrow (**i**) or clustered in groups (**ii**). (**iii**) Localization of c-Kit^{pos} cell clusters (green) in the osteoblastic (upper panel) and vascular niche (lower panel). High-magnification inserts show clusters of c-Kit^{pos} cells in contact with the paratrabecular bone or a vWF-labeled sinusoid (S, red). Megakaryocytes are also stained in red by vWF. (**iv**) Immunofluorescence staining of Sca-1^{pos} (green, arrowhead) and vWF^{pos} (red). (**v,vi**) Arrows indicate Sca-1 (green) c-Kit (red) double positive cells (SK, yellow fluorescence); arrowheads indicate Sca-1^{neg} c-Kit^{pos} cells. About 80% of c-Kit^{pos} cells coexpress Sca-1, whereas 60% of Sca-1^{pos} cells coexpress c-Kit. Scale bars: i,iii: 50 μm; ii,iv,v,vi: 20 μm. (**b**) Bar graphs show the distribution of SK cells at the level of epiphysis, metaphysis and diaphysis Values are mean±s.e.m.; n=7 per group. *P<0.05 and ***P<0.001 vs. C.