**Materials and Methods**

**Induction of diabetes in mice.**
Animal studies were carried out with the approval of the Institutional Animal Care and Use Committees of New York University and Columbia University. Male homozygous Ager<sup>−/−</sup> mice, backcrossed >12 generations into C57BL6/J were bred in our laboratory<sup>1</sup> and male C57BL6/J (Wild-type, WT) mice were purchased from Jackson Laboratories (Bar Harbor, Maine, USA). Transgenic mice overexpressing *Glo1* [Tg(*Glo1*)] (hemizgotes) in the C57BL/6 background were generously provided by Dr. Abraham Palmer (University of California at San Diego School of Medicine)<sup>2</sup> and expanded in our animal facilities and used with their littermate controls. All mice were housed in the same vivaria as their respective controls for the entire duration of their study including induction of diabetes, maintenance of diabetes for two months prior to surgery, induction of femoral artery ligation and euthanasia. Mice were rendered diabetic by intraperitoneal injections of streptozotocin, 55 mg/kg in citrate buffer (0.1 mol/L; pH 4.5) (Sigma, St. Louis, MO, USA) for five consecutive days beginning at age 7 weeks<sup>3</sup>. Diabetes was confirmed by documentation of plasma glucose concentration >250 mg/dl at age 8 weeks and then every third week until sacrifice. Mice were diabetic for two months prior to surgery in order to verify that in basal conditions, blood flow was reduced (as assessed by Laser Doppler imaging). In that way, conditions clinically relevant to the diabetic state would be in place prior to frank ischemia-inducing injury.

**Mouse model of hind limb ischemia.**
Ischemia was induced in the mouse’s left hind limb by blocking blood flow in the femoral artery, above the origin of the natural collateral vessels<sup>4</sup>. Animals were anesthetized by isoflurane inhalation or by ketamine (80 mg/kg) and xylazine (10 mg/kg) by intraperitoneal injection. The femoral artery was dissected free and ligated with two suture knots (about 0.2 mm distance between two knots) using 8-0 non-absorbable sutures. The vein and the artery were then cut between the proximal and the distal ligatures. The contralateral right limb served as the sham control in each experiment. Skin incisions were closed with sterile 5-0 surgical suture.

**Laser Doppler perfusion imaging.**
To provide functional evidence for ischemia-induced changes in vascularization, laser Doppler perfusion imaging (LDPI) experiments were performed as previously described<sup>5</sup>. Hind limb perfusion was measured using a LDPI system (Moor Instruments, Inc. Wilmington, DE). The stored perfusion values behind the color-coded pixels were then available for analysis. Consecutive measurements were obtained after scanning the same region of interest (leg and foot) with LDPI. Color photographs were recorded and analysis performed by calculating the average perfusion of the ischemic and non-ischemic foot. The results are expressed as the ratio of perfusion in the left (ischemic) versus right (non-ischemic) limb.

**Histological assays.**
After anesthesia, mice were sacrificed and whole ischemic and non-ischemic limbs were immediately fixed in 10% formalin for 48 h. After bones were carefully removed, 3-mm thick muscle tissue sections were cut and paraffin embedded. 5 μm sections were subjected to immunohistochemistry analysis. Capillary sprouting or macrophage infiltration in the muscle tissues was identified by immunostaining with rat monoclonal antibodies directed against mouse CD31 (BD Bioscience-Pharmingen, San Diego, CA, USA; catalogue #558736; 1:30 dilution) or CD68 (Abcam, Cambridge, MA, USA; catalogue #53444; 1:100 dilution) as primary antibodies, and with a biotinylated goat anti-rat IgG (Vector Laboratories Inc., Burlingame, CA, USA; 1:200 dilution) as a secondary antibody, followed by incubation with fluorescein-avidin D or Texas Red-avidin D. The signals of individual images for antigen detection were performed using a
Zeiss fluorescent microscope and Axiovision 4.8 software. CD31+ capillaries or CD68+ infiltrating macrophages were identified and counted in 10 randomly chosen fields in multiple sections using Axiovision software 4.8.

Bone marrow-derived macrophages.
Bone marrow-derived macrophages (BMDMs) were generated from 6–12 week old C57BL/6 (WT), Ager−/− and Tg(Glo1) mice and grown immediately after retrieval from the femora in either 5.5M (LG) or 25M (HG) D-glucose DMEM with L929-cell conditioned medium (LCCM) as a source of granulocyte/macrophage colony stimulating factor6, 7. Culture medium was replaced on day 5 with DMEM growth medium. Mature adherent cells were analyzed after 7 days in culture. D-mannitol (Sigma-Aldrich, St. Louis, MO) was used as the osmotic control in which 19.5 mM D-mannitol was added to 5.5 mM D-glucose media. For BMDMs used for gene expression studies of macrophage inflammatory signatures, cells were retrieved from non-diabetic mice and grown in either 5.5 mM (LG) or 25 mM (HG) D-glucose LCCM or DMEM for 7 days as above.

Pathway-focused gene expression profiling using real-time PCR (PCR array analysis).
Total RNA extracted from snap-frozen mouse muscle tissues using RNeasy® fibrous tissue mini kit (Qiagen, Valencia, CA; catalogue #74704). RNA (1 μg) was converted to cDNA by reverse transcription (RT) and genomic DNA was eliminated using the RT2 First Strand Kit (SABiosciences, Frederick, MD, USA). Gene expression profiling was performed using the RT2 Profiler PCR Array Kit, the RT2 SYBR Green/ROX qPCR Master Mix, and Mouse Angiogenesis RT2 Profiler™ PCR Array (SABiosciences, Frederick, MD, USA). This array profiles the expression of 84 stress-responsive genes related to angiogenesis. Real-time PCR cycling was performed using the ABI PRISM 7900HT Sequence Detection System. Results from PCR array showed no contamination from genomic DNA given that the cycle threshold value in a kinetic PCR amplification curve in well H6 was always undetectable. Data analysis was performed using the PCR array data analysis web portal [http://www.superarray.com/pcrarraydataanalysis.php].

Real-time PCR analysis.
Total RNA (0.5 μg) from mouse muscle tissue or cultured mouse BMDMs was processed directly to cDNA synthesis using the TaqMan Reverse Transcription Reagents kit (Applied Biosystems, Branchburg, NJ, USA) according to the manufacturer’s protocol. Real-time PCR was performed using TaqMan Universal PCR Master Mix (Applied Biosystems). The primers and probe mixture were obtained from ThermoFisher Scientific (Egr1, Mm00656724, Il1b, Mm00434228; Tnfa, Mm01300094; Nos2, Mm00440502; Ccr7, Mm99999130; Arg1, Mm00475988; Ccl2, Mm00441242 and Il10, Mm01288386). Real-time PCR analysis of Egr1 expression was performed as previously published8. Data were analyzed by the 2−ΔΔCT method9. Expression levels were normalized using TaqMan ribosomal RNA control reagents (18s rRNA, Applied Biosystems, Branchburg, NJ, USA; catalogue #4319413E). All reactions were performed in triplicate in ABI PRISM 7900HT Sequence Detection System or ABI 7500 Fast Real-Time PCR System.

Western Blotting.
Total protein was prepared from mouse muscles and subjected to Western blot using primary antibodies against carboxymethyl lysine (CML), a specific methylglyoxal-derived AGE adduct, (R&D Systems, Minneapolis, MN, USA; catalogue #MAB3247; 1:500 dilution); RAGE (GeneTex, Inc. Imine, CA, USA; catalogue #GTX23611; 1:1,000 dilution), GAPDH (Abcam, Cambridge, MA, USA; catalogue#ab8245; 1:10,000 dilution), S100B (Abcam; catalogue#ab34686; 1:500 dilution), GLO1 (Abcam; catalogue#ab81461; 1:1,000 dilution) or β-ACTIN (Sigma, St. Louis
MO; catalogue #A5441; 1:10,000 dilution). Protein signals were visualized by either: 1) HRP-conjugated donkey anti-rabbit or rat anti-mouse secondary antibody (Amersham Pharmacia Biotechnology Inc, Piscataway, NJ, USA) and Thermo Scientific Pierce enhanced chemiluminescence (ECL) (Thermo Fisher Scientific Inc. Rockford, IL USA) or 2) IRDye 680RD goat anti-mouse (LICOR, Lincoln NE) and IRDye 800 CW goat anti-rabbit (LICOR) using the Odyssey Infrared Imaging System Model 9120 (LICOR).

**Macrophage-Endothelial Cell Adhesion Assay**

Quantitative adhesion assays were performed using murine aortic endothelial cells (MAECs) and BMDMs from WT and Ager-/- mice. MAECs were seeded at 8x10^6 cells per well in collagen type I-coated 48-well plates. Both cell types were initially cultured in LG or HG medium and serum-starved overnight prior to the adhesion assay. BMDMs were lifted up with StemPro® Accutase® Cell Dissociation Reagent, washed with PBS, resuspended at 1x10^6 cells per ml in serum-free LG or HG DMEM containing 0.5% BSA, 2mM CaCl_2 and 2mM MgCl_2, and then labeled with Leuko Tracker™ (Cell Biolabs Inc. San Diego, CA). The labeled BMDMs were added at 1.6x10^5 cells per well to confluent MAEC monolayers and incubated at 37°C 5% CO_2 incubator for 1h to allow maximum adherence. Non-adherent cells were removed by washing with PBS. After lysing adherent cells, fluorescence measurements were performed on a fluorescence plate reader (Beckman Coulter, DTX 880 Multimode Detector) at 480 nm/520 nm.

**Phlebotomy, plasma separation and cytokine secretion.**

Murine blood was collected either by submandibular bleeding (Baseline and Day 7) or from the aorta of anesthetized animals (Day 28) into eppendorf tubes containing EDTA. Blood was either centrifuged to yield plasma at 5,000xg during 10 minutes at 4°C or processed to perform flow cytometry assays. Plasma levels of MCP1 were performed via enzyme linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (ThermoScientific).

**Flow Cytometry.**

Red blood cells were lysed from total blood in RBC Lysis Buffer (eBiosciences) at room temperature. Lysed blood was pelleted by centrifugation and resuspended in FACS buffer (PBS containing EDTA, 2 mM; and BSA, 0.2%). Single cell suspensions were blocked with anti-mouse CD16/CD32 (Biolegend; clone 93, catalogue #101302) for 30 min at 4°C, and then labeled with antibodies for 30 min at 4°C and washed in FACS buffer. The anti-mouse antibodies used were as follows: CD45 AF700 (Biolegend; clone 30-F11, catalogue #103128), CD115 PE (eBioscience; clone AFS98, catalogue #12-1152) and Gr-1 (Ly-6G/Ly-6C) BV421 (Biolegend; clone RB6-8C5, catalogue #108434). Monocytes were identified as DAPI<sup>lo</sup>CD45<sup>hi</sup>CD115<sup>lo</sup> and either Ly6C<sup>lo</sup> or Ly6C<sup>hi</sup>. Neutrophils were identified as DAPI<sup>lo</sup>CD45<sup>lo</sup>CD115<sup>lo</sup>Ly6C<sup>hi</sup>. Flow cytometry was performed using a LSRII analyzer (BD Bioscience), processed by FACS DIVA and analyzed using FlowJO software (v10.1r5).

**Statistical Analysis.**

All data are expressed as the mean ± the standard error of the mean (Mean ± SEM). Statistical analysis was performed using Statview software (SAS Institute) or PROC GLM performing ANOVA with interaction and repeated ANOVA analyses. Pairwise group comparisons with Tukey multiple comparison correction were followed. Probability values of <0.05 were considered statistically significant.

**References**