SUPPLEMENTAL MATERIALS AND METHODS

Immunohistochemistry

For histological analyses, fat pads were removed and fixed in zinc formalin for ≥24 hours. Macrophages in adipose cross-sections were identified with a rat anti-mouse Mac-3 monoclonal antibody (1:100 dilution; BD Biosciences, San Jose, CA), followed by detection with biotin-goat anti-rat IgG (Accurate Chemical & Scientific Corp., Westbury, NY). Stained cells were counted manually from four randomly chosen fields using Image-Pro Plus software (Media Cybernetics) and expressed as a percentage of total cells per field.

Real-Time Polymerase Chain Reaction (RTPCR)

Mice were sacrificed via cardiac puncture, and then perfused with PBS. RNA was isolated from 100 mg of perigonadal fat using 1 mL of QIAzol Lysis Reagent (QIAGEN Inc., Valencia, CA) and following the RNeasy Lipid Tissue Mini Handbook protocol (QIAGEN Inc., Valencia, CA). RNA was isolated from 30 mg of lung tissue using a QIAGEN RNeasy Mini Kit. RTPCR was performed using an ABI Prism 7000 Sequence Detection System from Applied Biosystems, Foster City, CA. 100 ng of RNA and 1µl of primer were used per reaction. P-sel, E-sel, MCP-1, and Tumor necrosis factor-alpha (TNFα) RTPCR primers were used (Applied BioSystems, Foster City, CA). 7000 System SDS Software and the $2^{-\Delta\Delta C}{}_{T}$ method [1] were used to analyze the results.

Bone marrow transplantation

Bone marrow transplantation (BMT) was performed as previously described [2]. 8-10 week-old *Lepr*$^{db/db}$*,Psgl-1*$^{+/+}$ mice were used as recipients for BMT from *Psgl-1*$^{+/+}$*, Psgl-1*$^{-/-}$ or *Psel-1*$^{-/-}$ donors. *Psgl-1*$^{-/-}$ mice received marrow from *Psgl-1*$^{+/+}$ donors, and *Psgl-1*$^{+/+}$ mice received marrow from *Psgl-1*$^{-/-}$ donors. *IL-1R* $^{+/+}$ mice received marrow from *IL-1R* $^{-/-}$ donors, and *IL-1R* $^{-/-}$ mice received marrow from *IL-1R*$^{+/+}$ donors. Bone marrow was harvested from the donor mice by flushing their femurs and tibias with RPMI medium (Gibco/Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum (Gibco/Invitrogen, Carlsbad, CA). Cells were then centrifuged at 300 x *g* and resuspended in PBS before injection. Each recipient mouse was irradiated (2 x 650 rad [0.02 x 6.5 Gy]) and injected with 4 x 10^6 bone marrow cells via the tail vein. Four weeks after transplantation, blood was drawn from the retro-orbital sinus.

Intravital microscopy

The intravital microscopy model consisted of a Nikon FN1 fixed stage microscopy system with X-cite for epi-fluorescence, Photometrics Coolsnap Cacade 512B color digital camera system, and MetaMorph premier software package and computer system. For analysis of cremaster vessels, male mice were anesthetized with pentobarbital (67mg/g) and positioned supine securely with tape. An incision was made in the scrotal skin to expose the left cremaster muscle, which was then removed from the surrounding fascia. A lengthwise incision was made on the ventral surface of the cremaster muscle, and the testicle and epididymis were separated from the underlying muscle and reintroduced into the abdominal cavity. The muscle was then spread over an optically clear viewing pedestal and secured along the edges with 3-0 suture. The exposed tissue was superfused with warm bicarbonate-buffered saline (pH 7.4). The cremaster microcirculation was observed through the intravital microscope with a 10x eyepiece and 40x objective lens. To visualize white blood cells, rhodamine 6G (0.3 mg/kg) (Sigma Chemical, St Louis, MO) was injected into the tail vein immediately prior to visualization. At this dose, rhodamine 6G labels leukocytes and allows detection of all rolling leukocytes. Rhodamine

6G-associated fluorescence was visualized by epi-illumination with a 510-560 nm emission filter. Single unbranched venules (20-40 µm in diameter) were selected for study and images of the microcirculation were digitally recorded.

Rolling leukocytes were defined as leukocytes that rolled at a velocity slower than red blood cells. Firm leukocyte adhesion was detected if leukocytes remained stationary for 30 seconds or longer. The number of rolling and firmly adherent leukocytes during each 35 second video was counted and expressed as cells/mm length of vessel. Three venules were analyzed from each mouse. Intravital microscopy was performed on diet-induced and genetically obese mice.

For adoptive transfer experiments, leukocytes were isolated from whole blood of *Psgl-1*$^{+/+}$ mice with Histopaque-1077 (Sigma-Aldrich, St. Louis, MO). Whole blood was layered onto histopaque brought to room temperature in a 1:1 ratio. Tubes were centrifuged at 400 x *g* for 30 minutes at room temperature. The upper layer was discarded and the opaque interface was transferred to another tube. 10mL of PBS was added to the tube and mixed by gentle aspiration. The solution was centrifuged at 250 x *g* for 10 minutes. The supernate was aspirated and the pellet was resuspended in 5mL of PBS. The solution was centrifuged at 250 x *g* for 10 minutes. The supernate was discarded and the pellet was resuspended in 1mL of PBS and then incubated with 100ul of 10X stock rhodamine 6G for 30 minutes. White blood cells were centrifuged at 250 x *g* for 10 minutes. Cells were washed twice in PBS and ultimately resuspended in 1mL PBS. 1x10^6 leukocytes were then injected into *Lepr*$^{db/db}$*,Psgl-1*$^{-/-}$ and *Lepr*$^{db/db}$*,Psgl-l*$^{+/+}$ mice.

Recombinant IL-1β

Recombinant IL-1β (Peprotech, Rocky Hill, NJ) was injected into *IL-1R* $^{-/-}$*, Psgl-1*$^{-/-}$*, Psgl-1*$^{+/+}$, *Lepr*$^{db/db}$*,Psgl-1*$^{+/+}$, and *Lepr*$^{db/db}$*,Psgl-1*$^{-/-}$ mice (500ng in 200µl 1X PBS) via tail vein. Recombinant IL-1β was also injected into the following mice four weeks after BMT: *Psgl-1*$^{-/-}$ mice receiving marrow from *Psgl-1*$^{+/+}$ mice; *Psgl-1*$^{+/+}$ mice receiving marrow from *Psgl-1*$^{-/-}$ mice; *IL-1R*$^{+/+}$ mice receiving marrow from *IL-1R* $^{-/-}$ mice; and *IL-1R*$^{-/-}$ mice receiving marrow from *IL-1R*$^{+/+}$ mice. Mice were bled for serum 5 hours post IL-1β injection.

Immunoblotting

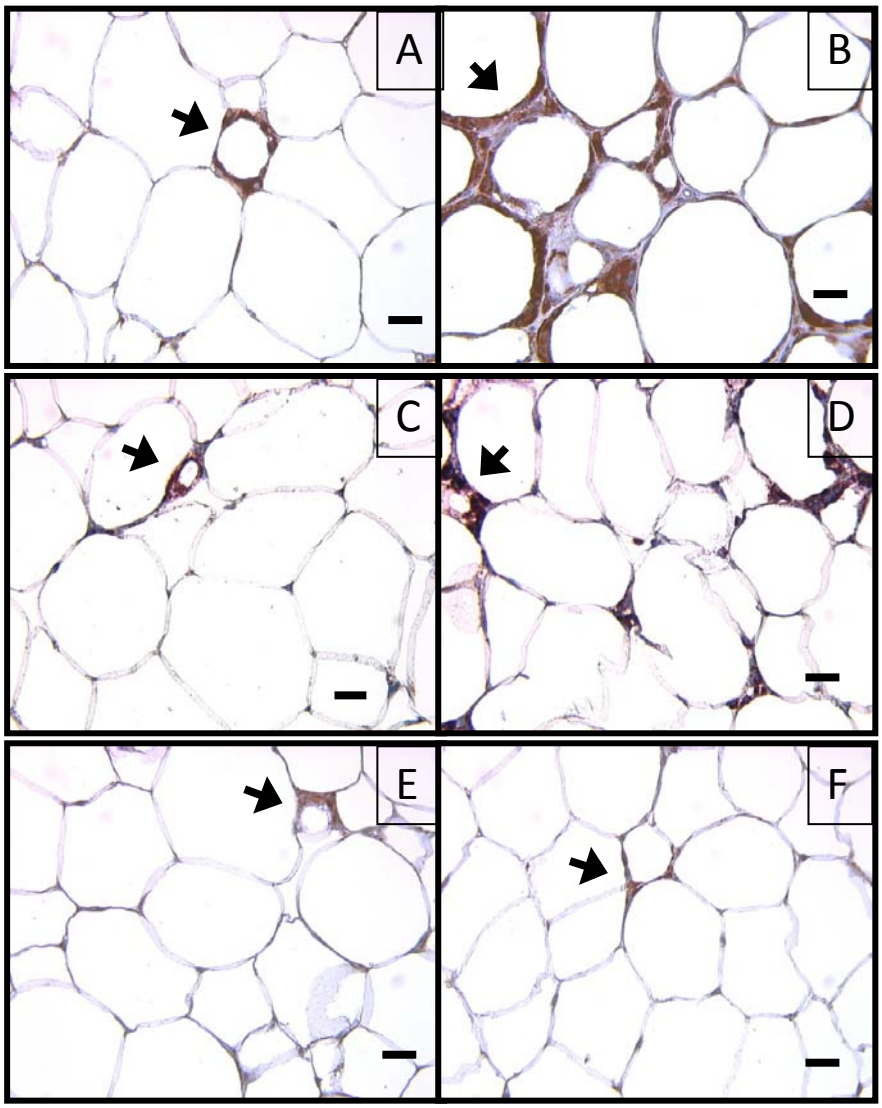
Lung was homogenized and lysed in a buffer containing 150mM NaCl, 5mM EDTA, 50mM Tris-HCl pH 7.4, 1mM DTT, 1% NP-40 (Ipagel), 1% Triton X-100, supplemented with protease inhibitor cocktail (Roche), PMSF, and phosphatase inhibitor cocktail 1 and 2 (SIGMA, St. Louis, MO). Lysates were resolved by SDS-PAGE and transferred to PVDF membranes by electro-blotting. The antibodies for mouse IκBα, phospho-IκBα, and p38 were from Cell Signaling Technology, Danvers, MA.

SUPPLEMENTAL ONLINE FIGURE

Legend for Online Figure I.

Online Figure I. Macrophage content of perigonadal, pericardial, and subcutaneous adipose tissue. Representative cross sections of perigonadal adipose tissue from **A)** *Lepr*$^{db/db}$*,Psgl-1*$^{-/-}$ and **B)** *Lepr*$^{db/db}$*,Psgl-1*$^{+/+}$ mice, pericardial adipose tissue from **C)** *Lepr*$^{db/db}$*,Psgl-1*$^{-/-}$ and **D)** *Lepr*$^{db/db}$*,Psgl-1*$^{+/+}$ mice, and subcutaneous adipose tissue from **E)** *Lepr*$^{db/db}$*,Psgl-1*$^{-/-}$ and **F)** *Lepr*$^{db/db}$*,Psgl-1*$^{+/+}$ mice. Staining with Mac3 antibody, magnification 40X, scale bar 200 µm, arrows showing stained cells. *p<0.05, **p<0.01, ***p<0.001.

Online Figure I.



SUPPLEMENTAL REFERENCES

1. Livak KJ, Schmittgen TD. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-[Delta][Delta]CT Method. *Methods.* 2001;25:402-408.

2. Bodary PF, Westrick RJ, Wickenheiser KJ, Shen Y, Eitzman DT. Effect of leptin on arterial thrombosis following vascular injury in mice. *JAMA.* 2002;287:1706-1709.