Supplemental Methods

Wound model and treatment
All surgical procedures were performed in accordance with the American Heart Association's Guidelines for Animal Use and were approved by the Institutional Animal Care and Use Committee of Northwestern University. Cutaneous wounds were created as described previously (Asai et al., 2006a; Asai et al., 2006b; Greenhalgh et al., 1990; Maruyama et al., 2007). Briefly, mice were anesthetized with an intraperitoneal injection of ketamine (90-120 mg/kg) and xylazine (5-10 mg/kg) or with 2-4% isoflurane, then the dorsal surface was shaved, washed with povidone-iodine solution and alcohol, and a disposable skin punch biopsy tool (0.8-cm diameter) (Acuderm Inc., Fort Lauderdale, FL, USA) was used to create one full-thickness excisional skin wound (extending down to the fascia) on the dorsal surface. Immediately after wounding, 6 mg/kg AMD3100 octahydrochloride (Sigma-Aldrich Co., St. Louis, MO, USA) in 30 μL saline or saline alone was topically applied to the wound bed, and then a semipermeable transparent dressing (Tegaderm; 3M Health Care, St. Paul, MN, USA) was placed over the wound, secured to the surrounding skin and muscle with 6-0 Prolene sutures, and left in place until subsequent evaluations were performed (i.e., for up to 14 days after wounding). Mice were kept on a heating pad until fully recovered from the anesthesia.

Additional cell populations
Primary isolates of dermal fibroblasts were harvested from 6- to 8-week-old db/db mice as previously described (Asai et al., 2006a; Lerman et al., 2003). Mice were sacrificed, and trunk skin was removed by sharp dissection; special care was taken to remove the underlying adipose tissue before culture. The harvested skin was minced and digested with 0.20% collagenase I solution (Sigma-Aldrich Co.) in serum-free Dulbecco’s modified Eagle’s medium (DMEM, Mediatech, Inc., Manassas, VA, USA) at 37°C for 2 hours, then the dissociated cells were isolated by centrifugation, resuspended in DMEM culture medium containing 10% FBS and 1% antibiotic/antimycotic solution (100 U/ml penicillin and 100 μg/ml streptomycin; Mediatech, Inc.), and cultured under 5% CO2 at 37°C and 100% humidity; the culture medium was refreshed every other day. Cells were passaged before attaining confluence, and experiments were performed after 5-10 passages.

Bone marrow–derived macrophages were cultured as described previously (Asai et al., 2006b; Maruyama et al., 2007; Maruyama et al., 2005). Total MNCs were isolated from the tibias and femurs of 8- to 10-week-old female db/db mice by gradient centrifugation with Histopaque-1083 (Sigma-Aldrich Co.). The isolated MNCs were plated on cell-culture dishes at a density of 5×10^5 cells/cm^2 and cultured under 5% CO2 at 37°C and 100% humidity for 4 days; then, the nonadherent cells were removed and the adherent cells were reseeded at a density of 5×10^4 cells/cm^2 and cultured for 3 additional days. The culture medium consisted of RPMI 1640 medium (Lonza Group Ltd, Basel, Switzerland) containing 10% heat-inactivated FBS (Sigma-Aldrich Co.), 1×10^{-5} mol/L 2-mercaptoethanol (Sigma-Aldrich Co.), 10 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Sigma-Aldrich Co.), 0.1 mmol/L nonessential amino acids (Lonza Group Ltd), 1 mmol/L sodium pyruvate (Lonza Group Ltd), 1% antibiotic/antimycotic solution (100 U/ml penicillin and 100 μg/ml streptomycin, Mediatech, Inc.), and 10 ng/mL of recombinant mouse macrophage colony stimulating factor (mMCSF).
3T3 fibroblasts were obtained from ATCC (Manassas, VA, USA) and cultured in DMEM culture medium containing 10% FBS and 1% antibiotic/antimycotic solution (100 U/ml penicillin and 100 μg/ml streptomycin; Mediatech, Inc.) at 5% CO₂, 37°C, and 100% humidity; the culture medium was refreshed every other day.

Cell migration
Cell migration was evaluated as described previously (Asai et al., 2006a; Asai et al., 2006b) via a modified Boyden’s chamber assay. Briefly, a polycarbonate filter (5-µm pore size) (GE Infrastructure, Fairfield, CT, USA) was inserted between the upper and lower chambers, and cell suspensions (5×10⁴ cells/well) were placed in the upper chamber; the lower chamber was filled with medium containing AMD3100 (0 or 2 µg/mL) or mouse recombinant platelet-derived growth factor BB (PDGF-BB) (50 ng/mL) (BioVision, Inc., Mountain View, CA, USA). Assays with fibroblasts isolated from db/db mice were performed in 5 mmol/L or 25 mmol/L D-glucose, or in 25 mmol/L D-mannitol to serve as an osmotic control for the high-glucose condition. The chamber was incubated for 20 hours at 37°C and 5% CO₂, and then the number of cells that had migrated to the lower chamber were counted in 5 HPFs (40× magnification) per chamber. Assays were performed in triplicate, and migration was reported as the mean number of migrated cells per HPF.

Proliferation assay
Cell proliferation was evaluated as described previously (Asai et al., 2006a; Asai et al., 2006b) via the CellTiter 96 nonradioactive cell proliferation assay (Promega Corporation, Madison, WI, USA) as directed by the manufacturer’s instructions. Briefly, subconfluent cells were seeded (fibroblasts: 5×10³ cells/well; EPCs or macrophages: 10⁴ cells/well) on 96-well, flat-bottomed plates with 100 µL of growth medium; then, the cells were treated with AMD3100 (0, 2, or 10 µg/mL) or basic fibroblast growth factor (bFGF) (50 ng/mL) (R&D Systems Inc.) and incubated for 50 hours at 37°C. Assays with fibroblasts isolated from db/db mice were performed in 5 mmol/L or 25 mmol/L D-glucose, or in 25 mmol/L D-mannitol to serve as an osmotic control for the high-glucose condition. Absorbance at 490-nm wavelength was recorded with a 96-well ELISA plate reader (Bionetics Laboratory, Kensington, MD). Assays were performed in triplicate, and proliferation was reported as mean absorbance per well.

Phagocytosis assay
Macrophage phagocytosis was evaluated via the CytoSelect™ 96-well phagocytosis assay (red blood cell, colorimetric format; Cell Biolabs, Inc., San Diego, CA, USA) as directed by the manufacturer’s instructions. Briefly, subconfluent cells were seeded on 96-well (10⁴ cells/well), flat-bottomed plates with 100 µL of culture medium, incubated overnight at 37°C, 5% CO₂, treated with AMD3100 (0 or 2μg/mL), and then incubated for 24 hours. IgG opsonized erythrocyte suspension (washed and preserved sheep red blood cells, 10% suspension; MP Biomedicals, Solon, OH, USA) was added to each well and incubated for 1 hour; then, the non-phagocytosed erythrocytes were removed, and the remaining cells were washed with PBS and incubated with cell-lysis buffer and substrate solution. The prepared cell lysate was examined via a colorimetric detection method; absorbance at 630-nm wavelength was recorded with a 96-well
ELISA plate reader (Bionetics Laboratory). Assays were performed in triplicate, and phagocytosis was reported as mean absorbance per well.

**Quantitative real-time reverse-transcriptase polymerase chain reaction (qRT-PCR)**

RNA was isolated as described previously (Asai et al., 2006a; Asai et al., 2006b; Maruyama et al., 2007) from homogenized skin samples or from $8 \times 10^5$ cells per experimental condition by using RNA-Stat (Tel-Test, Inc., Friendswood, TX, USA) as directed by the manufacturer's instructions. Total RNA was reverse transcribed with an iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA), and amplification was performed on a Taqman 7300 (Applied Biosystems, Foster City, CA, USA); primer and probe sequences are listed in the Supplemental Table. The PCR procedure consisted of a 2-minute hold at 50°C, then a 10-minute hold at 95°C followed by 40 2-step cycles between 95°C for 15 seconds and 60°C for 60 seconds. Relative mRNA expression was calculated with the comparative CT method (relative expression=$2^{\Delta CT}$) and normalized to the expression of the endogenous 18S gene.
Supplemental References

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### Table S1. Primers and probes used for real-time RT-PCR analyses.

| Gene | Primer/Probe | Sequence                  |
|------|--------------|---------------------------|
| CD68 | Forward primer | 5'-AGCTGCTGACAAGGGACACT3' |
|      | Reverse primer | 5'-TGAGAGGCAGCAAGAGGGA3'  |
|      | Probe         | 5'-CCATGTCTCTGTCAACGGTGACCA3' |
| SDF-1α | Forward primer | 5'-CCTCCAACGCAATGCTTCA3'  |
|      | Reverse primer | 5'-CCTTCCATTGCAAGGTGTA3'  |
|      | Probe         | 5'-CTGACTTCTTCCTCCTCTGTAGCCT3' |
| PDGF-B | Forward primer | 5'-ACCTCGCTGCAAAGTGTA3'   |
|      | Reverse primer | 5'-TGCTCCTGATGTCCTCA3'    |
|      | Probe         | 5'-AGTGAAGCCTCGCTGTGACTAGAAGTC3' |
| CXCR4 | Forward primer | 5'-CGCCTCTTCTCCAGTGTTC3'  |
|      | Reverse primer | 5'-GGGAGAGCTTTGAAACTTGGC3' |
|      | Probe         | 5'-ACCACATCTCCTATGCTTTCTCGG3' |