

## **SUPPLEMENTAL MATERIALS AND METHODS**

**Endothelial cell assays and culture.** Primary mouse heart endothelial cells were used for experiments 2 weeks following isolation. For cell proliferation assays, 50,000 endothelial cells were plated and treated with 5 $\mu$ g/ml AMD3100 (Sigma), 50ng/mL rmCXCL12 (R&D Systems), 250ng/mL rhCCL2, CM from SVF/EV or SVF/CCL2 cells, or SVF/CCL2 CM + 30 $\mu$ g/mL CCL2 neutralizing antibody (R&D Systems) for 36 hours and counted. For wound healing assays, 300,000 endothelial cells were plated and serum starved overnight. Confluent cells were scratched with a 200 $\mu$ l pipet tip, treated with CM as described above and imaged at 0 hour and 6 hour timepoints. Wound closure was calculated using ImageJ software.

**Adipocyte measurement and crown-like structure quantification.** Sections from reduction mammoplasty tissues were stained for CD11c using immunohistochemistry as described below. Images were captured using Spot Imaging software, and 3 images of mammary adipose tissue were used for quantification. For each image, the diameters of 10 adipocytes were measured. Data for BMI was blinded until the analysis was complete. Crown like structure (CLS) index was quantified as described (1).

**Adipose tissue whole mounts and quantification of blood vessels.** Whole glands were collected from obese and lean mice and fixed and stained as described (2). Images were captured using Spot Imaging software, and 5 images from glands of 5 mice were used for quantification. For each image, the number of CD31 $^{+}$  vessels, total adipocytes, and adipocyte diameters were quantified.

**Adipocyte differentiation assay.** Stromal cells were grown two days post confluence, then the growth media was supplemented with 5mM IBMX (Sigma), 0.1 $\mu$ M dexamethasone (Sigma) and 0.5 $\mu$ g/mL insulin (Sigma) three times weekly for 21 days. Cells were stained with Oil Red O, which was extracted and quantified as previously described (3) or stained for 10 min with 100nM BODIPY 558/568 (Invitrogen) and 4',6- diamidino-2-phynylindole (DAPI). Images were visualized using a Nikon Eclipse 80i inverted microscope and captured with Spot Imaging software (Diagnostic Instruments).

**Lentivirus production.** Lentiviral constructs (CSCG vector, pLenti-Kras<sup>G12V</sup> and pLenti-SV40er) used for gene transduction into human mammary epithelial cells for tumorigenesis studies have been described previously (4). The gene for CCL2/MCP-1 was cloned with standard cloning techniques into the self-inactivating CSCG viral vector. Lentiviral particles were generated and concentrated as described (5).

**Macrophage migration assays.** For migration assays, 3x10<sup>5</sup> RAW 264.7 cells were seeded onto 8 $\mu$ m pore size inserts in serum-free DMEM and the inserts were placed in wells containing serum free DMEM containing vehicle, 50ng/mL rmCSF-1 (R&D Systems), 250ng/mL rhCCL2, CM from SVF/EV or SVF/CCL2 cells, or CM from SVF/CCL2 cells containing 30 $\mu$ g/mL of CCL2 neutralizing antibody for 3 hours. Migrating cells were fixed and stained with crystal violet. Each condition was plated in triplicate, and three experiments were averaged.

**Flow cytometry of glands, humanized glands and bone marrow.** DTGR EGFP-Mac SCID mice (DTGR/SCID) were intraperitoneally injected with 25ng/kg diphtheria toxin (DT, Sigma) at 1 hour or 24 and 48 hours prior to euthanasia. Fourth mammary glands were dissociated to a single-cell suspension and bone marrow cells were isolated. Cells were blocked with a CD16/32 antibody (eBiosciences) and bound with fluorescently-conjugated antibodies to PE-CD31, APC-F4/80, or APC-CD11b (BD Biosciences). Data was collected on a FACSCalibur flow cytometer (BD Biosciences), and data was analyzed with the Flowjo software package (TreeStar).

**Quantitative PCR analyses.** RNA was isolated utilizing RNeasy mini kit with on-column DNase digestion (Qiagen). RNA samples were reverse transcribed using iScript cDNA kit (Bio-Rad), and quantitative PCR (qPCR) was performed with SYBR Green (Bio-Rad) on a CFX96 Real-Time System (Bio-Rad). Data was analyzed as a fold change utilizing  $\Delta\Delta Ct$  method for *in vitro* experiments or by relative expression with  $\Delta Ct$  for tissue samples. Samples were run in duplicate, and three experiments were analyzed.

**Immunohistochemistry and Immunofluorescence.** F4/80 (1:1000, Caltag) or CD11c (1:500, Abcam) immunohistochemistry was performed as described (6). Crown-like structures (CLS) are defined as F4/80<sup>+</sup> or CD11c<sup>+</sup> macrophages surrounding a single adipocyte and were quantified from 5 images of glands from 5 individual mice or sections from reduction mammoplasty tissue from 25 patients.

CD31 (1:100, BD Pharmingen) or F4/80 (1:1000) immunofluorescence on frozen tissue was performed as described (6). Images were captured with Spot imaging software system (Diagnostic Instruments, Inc.). CD31<sup>+</sup> and F4/80<sup>+</sup> cells were quantified from 5 images taken

from 10 stained glands, tumor sections, or Matrigel plugs and quantification was performed using the cell counter plug-in for ImageJ software.

**Western blotting.** HL-60 cells or homogenized whole mammary glands were lysed, and protein concentrations were measured as described (6). Immunoblotting was performed as described (6), and membranes were cut and incubated with pERK 1/2 antibody (1:1000, Cell Signaling Technologies), mouse CCL2 (1:100, Biolegend), or mouse IL-1 $\beta$  (1:100, Biolegend). The membranes were stripped and re-hybridized with total ERK 1/2 antibody (1:1000, Cell Signaling Technologies) or lamin A/C (1:1000, Cell Signaling Technologies). For detection of proteins in SVF/EV and SVF/CCL2 CM, confluent cells were grown for 48 hours prior to media collection. CM was centrifuged through 50kDa column (Millipore), and the eluate was passed through a 3kDa column. Protein levels were quantified in the retentate from the 3kDa column. Membranes were cut and incubated with antibodies for human CCL2 (1:500, R&D Systems) and IL-1 $\beta$  (1:100, Novus Biologicals).

## **Supplemental References**

- (1) Morris PG, Hudis CA, Giri D, Morrow M, Falcone DJ, Zhou XK, et al. Inflammation and increased aromatase expression occur in the breast tissue of obese women with breast cancer. *Cancer Prev Res (Phila)* 2011; 4: 1021-9.
- (2) Xue Y, Lim S, Brakenhielm E, Cao Y. Adipose angiogenesis: quantitative methods to study microvessel growth, regression and remodeling in vivo. *Nat Protoc* 2010; 5: 912-20.
- (3) Ramirez-Zacarias JL, Castro-Munozledo F, Kuri-Harcuch W. Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with Oil red O. *Histochemistry* 1992; 97: 493-7.
- (4) Wu M, Jung L, Cooper AB, Fleet C, Chen L, Breault L, et al. Dissecting genetic requirements of human breast tumorigenesis in a tissue transgenic model of human breast cancer in mice. *Proc Natl Acad Sci U S A* 2009; 106: 7022-7.
- (5) Keller PJ, Arendt LM, Skibinski A, Logvinenko T, Klebba I, Dong S, et al. Defining the cellular precursors to human breast cancer. *Proc Natl Acad Sci U S A* 2012; 109: 2772-7.
- (6) Iyer V, Klebba I, McCready J, Arendt LM, Betancur-Boissel M, Wu MF, et al. Estrogen promotes ER-negative tumor growth and angiogenesis through mobilization of bone marrow-derived monocytes. *Cancer Res* 2012; 72: 2705-13.