Figure S1

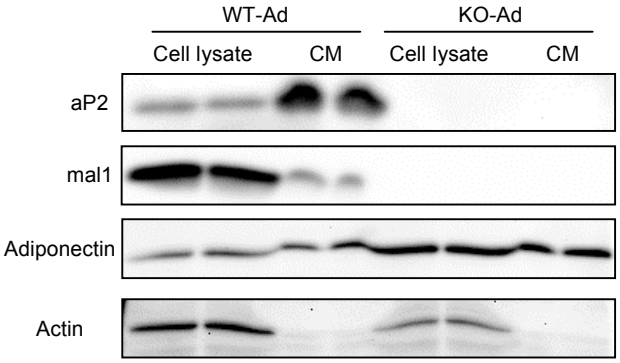

Figure S1. Detection of FABPs in adipocyte cultures.
For detection of aP2, 1 µg of cell lysate or 20 µl out of total 500 µl conditioned medium (CM) was used for western blot analysis. For detection of mal1, adiponectin (as a control of secreted protein), and actin (as a control of non-secreted protein), 25 µg of cell lysate or 45 µl of the CM was used.

Figure S2

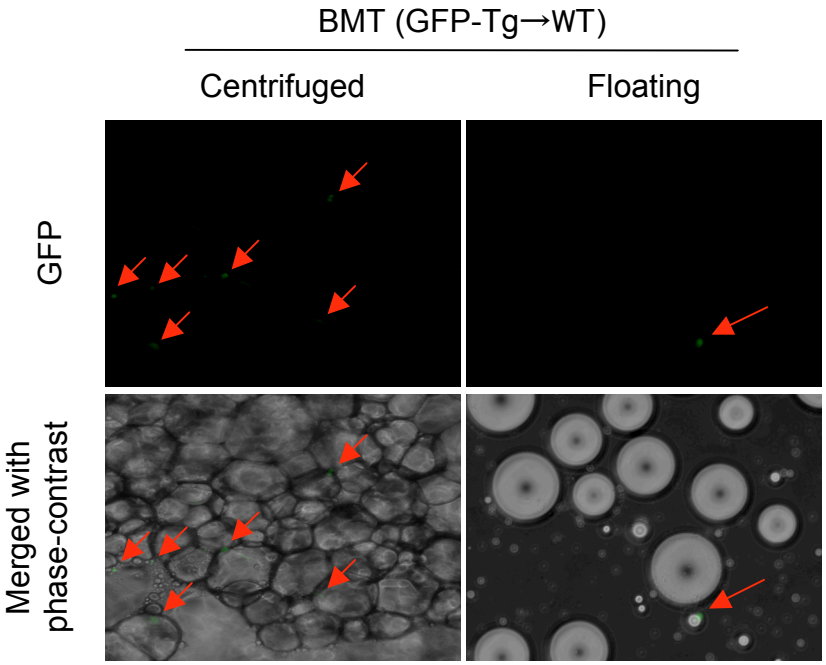

Figure S2. Occasional GFP-positive cells detected in the adipocyte fraction from BMT (GFP-Tg→WT) mice.
Very small numbers of green cells were detected in the adipocyte fraction from BMT (GFP-Tg→WT) mice. These cells were much smaller in size than adipocytes, potentially contaminating cells from the SV fraction which contained GFP-labeled cells

Figure S3

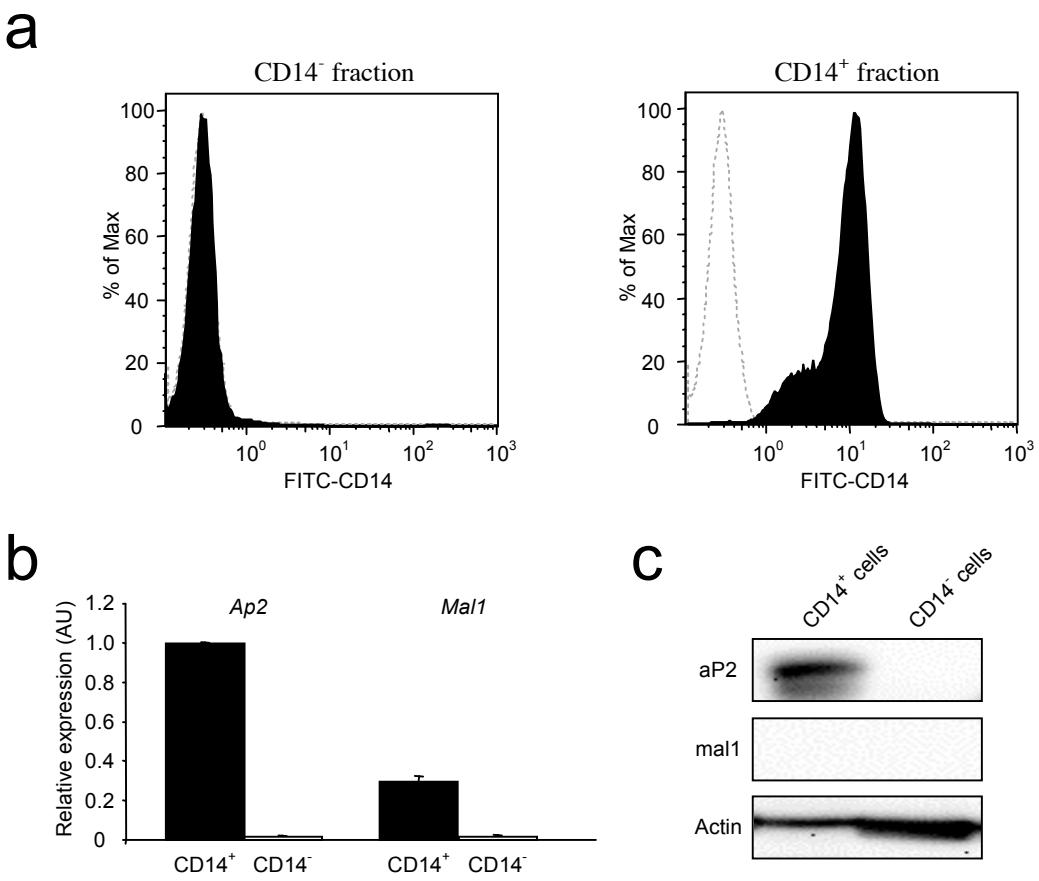

Figure S3. FABPs in hematopietic compartment.
a, Confirmation of CD14 (monocyte/macrophage) population from human blood samples by FACS analysis. **b**, Expression of FABPs in the $CD14^+$ and $CD14^-$ fractions. The expression of *Ap2* and *Mal1* mRNA was only detectable in the $CD14^+$ but not in the $CD14^-$ fraction by quantitative PCR, suggesting that aP2 and mal1 expression in the hematopoietic compartment are mainly restricted to monocyte/macrophage population. Data are shown as the mean ± s.e.m. AU, arbitrary units. **c**, Protein expression of FABPs in the $CD14^+$ and $CD14^-$ fractions. Western blot analysis using 100 µg total cellular protein extract readily revealed the presence of aP2 in the $CD14^+$ fraction, but mal1 protein was not detectable, suggesting that the levels of mal1 are much lower in these cells compared to aP2.

Figure S4

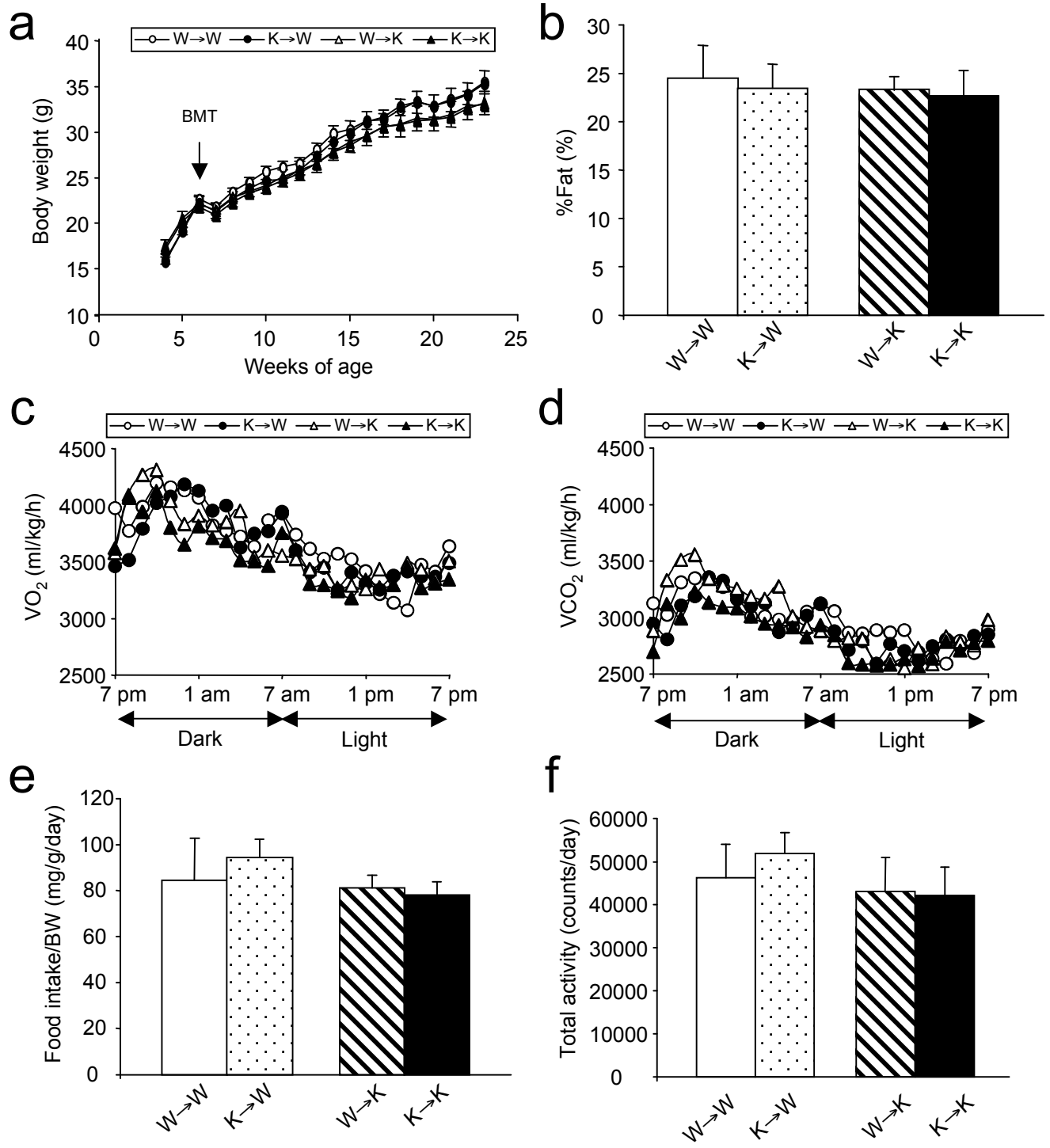

Figure S4. Metabolic studies in bone marrow-transplanted mice.
a-f, Metabolic studies were performed in all groups of bone marrow-transplanted mice (W→W, *n* = 6; K→W, *n* = 7; W→K, *n* = 7; and K→K, *n* = 5). **a**, Change in body weight. **b**, Percent body fat (%fat) measured using dual energy X-ray absorptiometry (DEXA). **c** and **d**, Rates of oxygen consumption (VO_2) (**c**) and carbon dioxide production (VCO_2) (**d**) determined by indirect calorimetry. ***e***, Daily food intake. ***f***, Total physical activity of mice determined by automatic sensors on x, y, and z axes. Data are shown as the mean ± s.e.m.

Figure S5

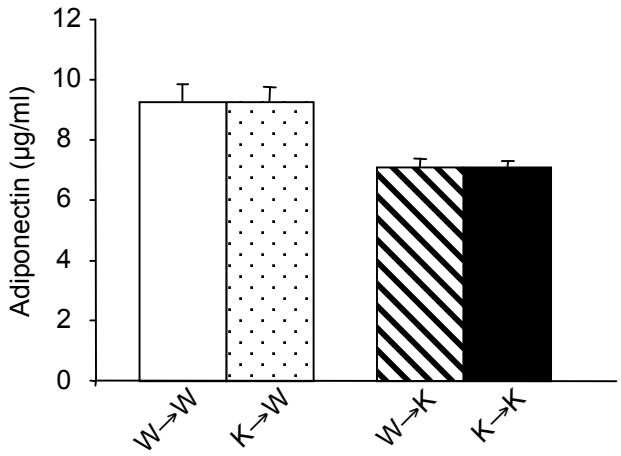

Figure S5. Serum adiponectin levels in bone marrow-transplanted mice.
Total adiponectin was measured in all groups of mice. Serum levels of adiponectin in FABP-deficient-recipient groups were lower than WT-recipient groups, but there was no significant difference in adiponectin levels between the chimeric groups. Data are shown as the mean ± s.e.m. AU, arbitrary units.

Figure S6

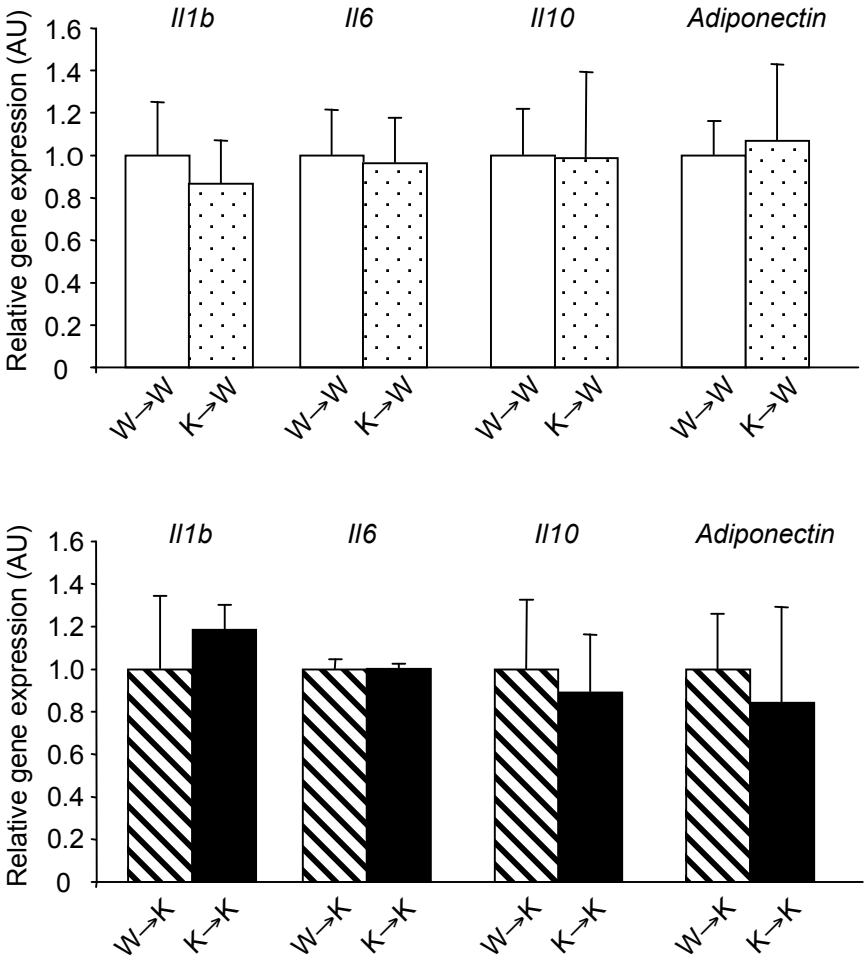

Figure S6. Expression of pro- and anti-inflammatory mediators in adipose tissue. Expression of *Il1b*, *Il6*, *Il10*, and *Adiponectin* in the adipose tissues of bone marrow-transplanted mice was analyzed. Data are shown as the mean ± s.e.m. AU, arbitrary units.

Figure S7

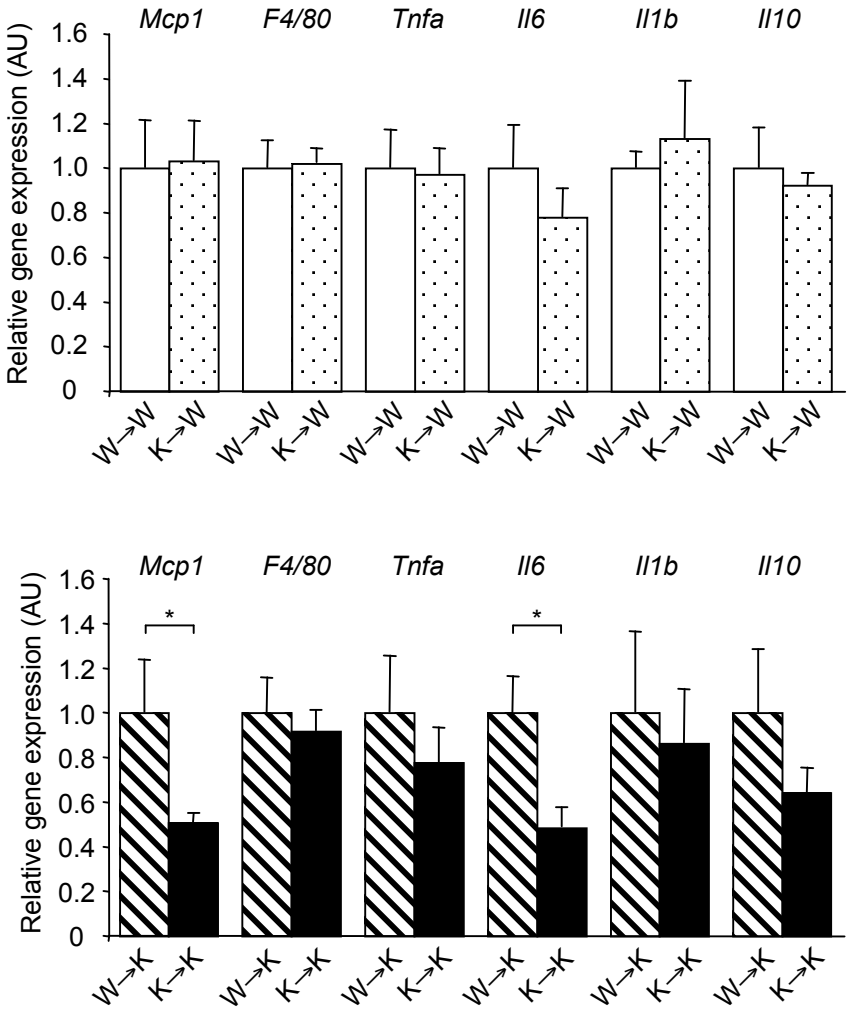

Figure S7. Expression of pro- and anti-inflammatory mediators in skeletal muscle. Expression of *Mcp1*, *F4/80*, *Tnfa*, *Il6*, *Il1b*, and *Il10* in the soleus muscle tissues of bone marrow-transplanted mice was analyzed. Data are shown as the mean ± s.e.m. **P* < 0.05. AU, arbitrary units.

Table S1

Table S1. Primers for quantitative real-time PCR or PCR

Genes	Species	Accession #	Forward primer	Reverse primer
18s	Mouse	X00686	5'- AGT CCC TGC CCT TTG TAC ACA -3'	5'- CGA TCC GAG GGC CTC ACT A -3'
	Human	M10098	5'- GTA ACC CGT TGA ACC CCA TT -3'	5'- CCA TCC AAT CGG TAG TAG CG -3'
Mcp1	Mouse	NM_011333	5'- CCA CTC ACC TGC TGC TAC TCA -3'	5'- TGG TGA TCC TCT TGT AGC TCT CC -3'
F4/80	Mouse	NM_010130	5'- CCC CAG TGT CCT TAC AGA GTG -3'	5'- GTG CCC AGA GTG GAT GTC T -3'
Tnfa	Mouse	NM_013693	5'- CCC TCA CAC TCA GAT CAT CTT CT -3'	5'- GCT ACG ACG TGG GCT ACA G -3'
Il1b	Mouse	NM_008361	5'- GCA ACT GTT CCT GAA CTC AAC T -3'	5'- ATC TTT TGG GGT CCG TCA ACT -3'
Il6	Mouse	NM_031168	5'- ACA ACC ACG GCC TTC CCT ACT T -3'	5'- CAC GAT TTC CCA GAG AAC ATG TG -3'
Il10	Mouse	NM_010548	5'- GCT CTT ACT GAC TGG CAT -3'	5'- CGC AGC TCT AGG AGC ATG TG -3'
Adiponectin	Mouse	NM_009605	5'- GAT GGC AGA GAT GGC ACT CC -3'	5'- CTT GCC AGT GCT GCC GTC AT -3'
Pecam1	Mouse	NM_008816	5'- CTG CCA GTC CGA AAA TGG AAC -3'	5'- CTT CAT CCA CCG GGG CTA TC -3'
Ap2	Human	NM_001442	5'- AGC ACC ATA ACC TTA GAT GGG G -3'	5'- CGT GGA AGT GAC GCC TTT CA -3'
Mal1	Human	NM_001444	5'- ATG AAG GAG CTA GGA GTG GGA -3'	5'- TGC ACC ATC TGT AAA GTT GCA G -3'
Gfp			5'- AAG TTC ATC TGC ACC ACC G -3'	5'- TCC TTG AAG AAG ATG GTG CG -3'

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SUPPLEMENTAL METHODS

Fluorescence-activated cell sorter (FACS) analysis

Peripheral blood cells collected from recipient mice after 4 weeks of bone marrow transplantation were stained with antibodies against the cell-surface markers, PE-CD45.1 and FITC-CD45.2 (eBioscience, San Diego, CA). Following incubation with primary antibodies, FACS Lysing Solution (BD Bioscience, San Jose, CA) was added to the cells. Cells were centrifuged at 1000 *g* for 2 min and resuspended in 1 ml FACSflow (BD Bioscience). Cells were analyzed on a FACSCalibur (BD Bioscience), and analysis was performed using CellQuest software (BD Bioscience).

Isolation of adipocyte and stromal-vascular fractions

Freshly collected epididymal fat pads were placed in Krebs-Ringer Hepes (KRH) buffer supplemented with 2.5% bovine serum albumin (BSA), 50 mM adenosine, and 1% liberase blendzyme 3 (Roche Diagnostics, Indianapolis, IN) and cut into small pieces. The tissue was incubated at 37°C with continuous shaking for 20 min, and the cell suspensions were filtered through 250 μm nylon mesh. The cells were centrifuged at 600 rpm for 10 min. Floating adipocyte layer was transferred into 10 ml of phosphate-buffered saline (PBS) containing 5 mM EDTA and 2% BSA. After centrifugation at 1000 rpm for 1 min, floating cells were collected as the adipocyte fraction. The bottom layer of filtered cells was centrifuged again at 1700 rpm for 10 min to collect the stromal-vascular (SV) fraction.

Hematopoietic compartment analysis

Heparinized venous blood samples from healthy donors were obtained after written informed consent. Mononuclear cells were separated by Ficoll-Hypaque density gradient centrifugation. The cells were magnetically labeled with monocyte/macrophage marker CD14 MicroBeads (Miltenyi Biotec, Auburn, CA), and loaded onto a MACS Column (Miltenyi Biotec) in the magnetic field of a MACS separator (Miltenyi Biotec). The magnetically labeled $CD14^+$ cells were retained in the column, and the unlabeled run-through cells collected as the CD14 depleted ($CD14^-$) fraction. The magnetically retained $CD14^+$ cells were then eluted as the positively selected cell fraction. Purity of the fractions was further confirmed by FACS analysis using a FITC-CD14 antibody (BD Bioscience). For analysis of T ($CD4^+$/$CD8^+$) and B ($CD19^+$) cell fractions, a commercially available MTC multiple tissue cDNA panel (Clontech, Mountain View, CA) was used.

Metabolic analysis *in vivo*

Blood samples were collected from animals that had fasted for 6 hours at 22 weeks of age. Blood glucose concentration was determined with 3-μl whole blood using Ascensia BREEZE blood glucose meter (Bayer Co., Mishawaka, IN). Serum levels of insulin and adiponectin were measured with ELISA (Crystal Chem Inc., Downers Grove, IL; Linco Research, St. Charles, MO). Total cholesterol and triglycerides were determined with colorimetric assay systems (Wako chemicals, Richmond, VA; and Sigma-Aldrich) adapted for microtiter plate assay. Glucose tolerance test was performed by an intraperitoneal glucose injection (2 g/kg) on conscious mice after an overnight (15 h) fasting at 19 weeks of age. Insulin tolerance test was performed by an intraperitoneal insulin injection (0.75 IU/kg) after a 6 h fast at 20 weeks of age.

For calorimetric analysis, mice were placed in an indirect open circuit calorimeter (Oxymax System; Columbus Instruments, Columbus, OH) at 23 weeks of age. Oxygen and carbon dioxide concentrations by volume were monitored at the inlet and outlet parts of a partially sealed chamber, through which a known flow of ambient air was forcibly ventilated. The concentration difference measured between the parts was used to compute oxygen consumption (VO_2) and carbon dioxide production (VCO_2). The consumption and production information were presented in units of ml/kg/h and normalized to 25°C and 760 mmHg. Food intake was investigated by using the Oxymax Feed Scale Device (Columbus Instruments). The physical activity of the mice was monitored with OPTO-M3 Activity Application Device (Columbus Instruments). The movements (other than scratching, grooming, digging, etc.) of each animal were determined by infrared beams in x, y, and z axes. Total body fat was assessed by dual energy X-ray absorptiometry (DEXA; PIXImus, GE Healthcare, Fairfield, CT).

Hyperinsulinemic-euglycemic clamp studies

Hyperinsulinemic-euglycemic clamps were performed in bone marrow-transplanted mice at 20 weeks of age by a modification of a described procedure (22). Three days before experiments, bone marrow-transplanted mice were anesthetized with an intraperitoneal injection of ketamine (80 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (40 mg/kg; Phoenix Scientific, St. Joseph, MO), and the right jugular vein was catheterized with a PE-10 polyethylene tube (inside and outside diameters, 0.28 mm and 0.61 mm, respectively; Becton, Dickinson and Co., Franklin Lakes, NJ) filled with heparin solution (100 USP U/ml, American Pharmaceutical Partners, Los Angeles, CA). On the day of experiment following an overnight fast, HPLC purified ^{3}H-glucose (0.05 μCi/min; PerkinElmer Life and Analytical Sciences, Boston, MA) was infused during the 2-h basal period, and blood samples were collected at the end to estimate the rate of basal hepatic glucose production. After the basal period, a 120-min hyperinsulinemic-euglycemic clamp was conducted with a primed infusion of human insulin (Humulin R; Eli Lilly, Indianapolis, IN) at the dose of 300 mU/kg and a sequential continuous infusion at a rate of 2.5 mU/kg/min. Blood samples were collected at 20-min intervals for the immediate measurement of glucose concentration, and 25% glucose was infused at variable rates to maintain glucose at basal concentrations. Insulin-stimulated whole-body glucose disposal was estimated with a continuous infusion of ^{3}H-glucose throughout the clamps (0.1 μCi/min). All infusions were performed using microdialysis pumps (CMA/Microdialysis,

North Chelmsford, MA). To estimate insulin-stimulated glucose uptake in individual tissues, 2-^{14}C-deoxyglucose (2-^{14}C-DG; PerkinElmer Life and Analytical Sciences) was administered as a bolus (10 μCi) 75 min after the start of clamps. Because 2-deoxyglucose is a glucose analog that is phosphorylated but not metabolized, insulin-stimulated glucose uptake in individual tissues can be estimated by determining the tissue content of 2-deoxyglucose-6-phosphate. Blood samples were collected at 80, 85, 90, 100, 110, and 120 min after the start of clamps for the determination of plasma ^{3}H-glucose, $^{3}H_2O$, and 2-^{14}C-DG concentrations. At the end of clamps, animals were sacrificed. Within 5 min, gastrocnemius muscles from both hindlimbs and epididymal adipose tissue were harvested. Each tissue was frozen immediately using liquid N_2 and stored at –80°C until further analysis.

For the determination of plasma ^{3}H-glucose and 2-^{14}C-DG concentrations, plasma was deproteinized with $ZnSO_4$ and $Ba(OH)_2$, dried to remove $^{3}H_2O$, resuspended in water, and counted in scintillation fluid (Ecoscint H; National Diagnostics, Atlanta, GA) on channels for ^{3}H and ^{14}C. The plasma concentration of $^{3}H_2O$ was determined by the difference between ^{3}H counts without and with drying. For the determination of tissue 2-^{14}C-DG-6-phosphate content, tissue samples were homogenized, and the supernatants were subjected to an ion-exchange column to separate 2-^{14}C-DG-6-phosphate from 2-^{14}C-DG. Rates of basal hepatic glucose production and insulin-stimulated whole-body glucose uptake were determined as the ratio of the ^{3}H-glucose infusion rate to the specific activity of blood glucose at the end of basal period and during the final 30 min of clamps, respectively. Hepatic glucose production during the hyperinsulinemic-euglycemic clamps was determined by subtracting the glucose infusion rate from the whole-body glucose uptake. Glucose uptake in individual tissues was calculated from plasma 2-^{14}C-DG profile, which was fitted with an exponential curve, and tissue 2-^{14}C-DG-6-phosphate content.

Macrophage content in adipose tissue

The sections of paraffin-embedded epidydimal fat were deparaffinized and incubated overnight at 4°C with monoclonal rat anti-F4/80 (AbD Serotec, Raleigh, NC). The sections were treated with rabbit biotinylated antibodies against rat IgG for 1 h at room temperature followed by incubation with avidin-biotin complex (Vectastain Elite ABC; Vector Laboratories, Burlingame, CA) and visualized with ImmPACT DAB peroxidase substrate. Slides were counterstained with hematoxylin. The images were generated 3 representative fields per slide in a blinded manner using a digital color camera (DP70; Olympus, Melville, NY) mounted on a microscope (VistaVision; VWR, West Chester, PA). The F4/80-positive crown-like structures (CLSs) were counted, and the percentage of CLSs compared to total adipocyte number was used as quantification of the adipose tissue macrophage content.

Portal vein insulin infusion

Following 6 hours of food withdrawal, mice were anesthetized with an intraperitoneal injection of tribromoethanol (250 mg/kg), and insulin (1 IU/kg) or phosphate buffered saline (PBS) was injected through the portal vein. Three minutes after infusion, tissues were removed and frozen in liquid nitrogen and kept at -80°C until processing.

Protein extraction and western blot analysis

For protein extraction, tissues or cells were placed in a cold lysis buffer containing 50 mM Tris-HCl (pH 7.0), 2 mM EGTA, 5 mM EDTA, 30 mM NaF, 10 mM Na_3VO_4, 10 mM $Na_4P_2O_7$, 40 mM β-glycerophosphate, 0.5% NP-40, and 1% protease inhibitor cocktail. After homogenization on ice, the lysates were centrifuged, and the supernatants were used for western blot analysis. Total protein content of the samples was assessed by microplate protein assay (Bio-Rad, Hercules, CA), and equal amounts of protein per sample and known molecular weight markers were subjected to SDS–polyacrylamide gel electrophoresis. Proteins were electrophoretically transferred onto PVDF membranes (Whatman, Florham Park, NJ) and incubated 1 h at room temperature with a blocking solution, 3% BSA in Tris-buffered saline buffer containing 0.1% Tween 20 (TBST). The blocked membranes were incubated with anti-aP2 (homemade), anti-mal1 (homemade), anti-IRb-pTyr1162/1163 (Calbiochem, San Diego, CA), anti-IRβ (Santa Cruz Biotechnology, Santa Cruz, CA), anti-Akt-pSer473 (Santa Cruz Biotechnology), anti-Akt (Santa Cruz Biotechnology), anti-adiponectin (BioVendor, Candler, NC), anti-GFP (Abcam, Cambridge, MA), anti-perilipin (Santa Cruz Biotechnology), anti-PECAM-1 (Santa Cruz Biotechnology), or anti-β-actin (Santa Cruz Biotechnology) for overnight at 4°C or 2 h at room temperature, and washed three times with TBST. The membranes were incubated with the secondary antibody conjugated with horseradish peroxidase (Amersham Biosciences, Piscataway, NJ) for 1 h at room temperature and washed. Immunodetection analyses were accomplished using the enhanced chemiluminescence (ECL) Kit (Roche Diagnostics).

Cells

We generated $aP2^{+/+}mal1^{+/+}$ (WT-Ad) and $aP2^{-/-}mal1^{-/-}$ (KO-Ad) preadipocytes from mouse models. The KO preadipocytes were also reconstituted by lentivirus with exogenous aP2 (KO+aP2-Ad) or with control empty vector including green fluorescent protein (KO+GFP-Ad). Mouse 3T3-L1 cells were obtained from American Type Culture Collection (ATCC; Manassas, VA). These cell lines were maintained and propagated in Dulbeco's Modified Eagle's Media (DMEM) (Invitrogen) with heat-inactivated 10% cosmic calf serum (CCS) (Hyclone, Logan, UT), 50 U/ml penicillin (Invitrogen) and 50 mg/ml streptomycin (Invitrogen) at 37°C in 10% CO_2. Differentiation was initiated (day 0) by incubation in induction medium (1 mM dexamethasone, 0.5 mM isobutylmethy xanthine, 1 mM rosiglitazone, and 5 mg/ml insulin). Following a 4-day induction period (two 48-h incubations), the medium was changed to a post-induction medium (1 mM rosiglitazone and 5 mg/ml insulin) for an additional 2 days. Thereafter, the medium was replaced with a medium supplemented only with 0.5 mg/ml of insulin for 2 days. Finally, the medium was replaced with a fresh medium.

Immortalized $aP2^{+/+}mal1^{+/+}$ and $aP2^{-/-}mal1^{-/-}$ mouse macrophage cell lines (WT-Mac and KO-Mac, respectively) were generated in our laboratory. These lines were incubated in RPMI 1640 supplemented with heat-inactivated 10% fetal bovine serum (FBS) (Hyclone) or 5% lipoprotein-deficient serum (LPDS) (Biomedical Technologies, Stoughton, MA) including 50 U/ml penicillin and 50 mg/ml streptomycin at 37°C in 5% CO_2. Peritoneal

macrophages were harvested 4th day after intraperitoneal injection of 1 ml thioglycollate medium (BD Diagnostic Systems, Sparks, MD). Primary macrophages from $aP2^{+/+}mal1^{+/+}$ (WT-pMac) and $aP2^{-/-}mal1^{-/-}$ (KO-pMac) mice were incubated in RPMI1640 supplemented with heat-inactivated 10% FBS, 50 U/ml penicillin, and 50 mg/ml streptomycin at 37°C in 5% CO_2. After 4 h incubation, nonadherent cells were removed by washing.

Glucose uptake and lipolysis in adipocytes

After overnight starvation in DMEM supplemented with 0.5% BSA, adipocytes in contact with 1 x 10^5 macrophages in a 12 well plate were washed by warm KRH buffer, followed by treatment with or without 100 nM insulin in warm KRH buffer. In some wells, 50 μM cytochalasin B was added as a control to inhibit glucose transporter-mediated glucose uptake. Cells were incubated for 20 min at 37°C. ^{3}H-2-deoxyglucose (PerkinElmer Life and Analytical Sciences) was then added to each well and incubated for an additional 5 min. Experiment was terminated by placing cells on ice and washing with ice-cold KRH buffer. After washing cells, cells were lysed in 0.1% SDS, and glucose uptake was determined by scintillation counting and normalized to cellular protein content. To examine lipolysis, differentiated adipocytes were incubated in DMEM supplemented with 0.5% BSA in the absence or presence of 0.5 mM dibutyryl cAMP for 4 hours. Release of free fatty acid into the medium was quantified by a colorimetric assay (Wako chemicals), and data were normalized to total cellular protein content and expressed as the fold changes from basal levels. The basal free fatty acid release was also measured in the conditioned medium with adipocytes for 16 h which was the same condition used in the co-culture experiments and similarly normalized to cellular protein content.