SUPPLEMENTARY DATA

Western blotting to determine inflammatory, insulin and AMPK signaling.
Frozen tissue samples and isolated/cultured cells were lysed in modified RIPA lysis buffer. The samples were separated on 8-10% sodium dodecyl sulfate-polyacrylamide gels and were then transferred to polyvinylidene fluoride membranes. The membranes were blocked with 5% non-fat milk in Tris-buffered saline with 0.1% Tween 20 for 1 h at room temperature and were then probed overnight at 4°C with primary antibodies, followed by incubation with alkaline phosphatase-conjugated secondary antibody for 1 h at room temperature. The membranes were developed with a chemifluorescence reagent and were scanned by the Storm 860 imager (GE, Healthcare, Piscataway, NJ). The band intensities were quantified with the NIH Image J program and were then incubated with antibodies diluted in blocking buffer. The blots were incubated with alkaline phosphatase-conjugated secondary antibodies, developed with a chemifluorescence reagent, and scanned by the Storm 860 imager (GE Healthcare). The antibodies against IκB, p65, arginase, AMPKα1 and α2, and ACC, as well as their phosphorylated forms, were from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). The antibodies against various units of MAPKs, Akt, and their phosphorylated forms were from Cell Signaling Technology (Beverly, MA).

Stable AMPK knock-down in adipocytes
3T3-L1 cells were maintained in high-glucose DMEM supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin as previously described (1). To differentiate the 3T3-L1 cells, the 2-d post-confluent cells were incubated in growth medium supplemented with 10 μg/ml insulin, 1 μM dexamethasone, and 0.5 mM 3-isobutyl-1-methyl-xanthine for 48 h, followed by incubation for an additional 6-8 days in growth medium supplemented with 10 μg/ml insulin. To knock down AMPKα1, predifferentiated 3T3-L1 cells were transfected with a plasmid containing shRNA against mouse AMPKα1 (Santa Cruz Biotechnology, Santa Cruz, CA) with Lipofectamine™ 2000 Transfection Reagent (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. Similarly, predifferentiated 3T3-L1 cells were transfected with an shRNA vector and served as the control (Ctrl). After transfection for 24 hours, the transfected predifferentiated 3T3-L1 cells were selected with puromycin (5 μg/ml). The stable AMPKα1-KD and AMPKα1-Ctrl cells were then induced for differentiation for 8 days and were used for the following assays.
To verify AMPKα1 knockdown, cell lysates were prepared and used to determine the AMPKα1 amount using Western blotting. Out of four selected lines, two exhibited a knockdown efficiency that was greater than 75% than that of the control. These two cell lines were then used for all adipocyte-related assays.

Methodology and analysis of the arrays
Labeling and hybridization: The total RNA was extracted from the macrophage samples using an acid-phenol reagent (TRIZol; Invitrogen Corp., Carlsbad, California) The integrity of the total RNA samples was confirmed using the Agilent Bioanalyzer 2100 Lab-on-chip system (Agilent Technologies, Palo Alto, CA). A total of 400 ng of total RNA was reverse-transcribed to cDNA, during which a T7 sequence was introduced into the cDNA. T7 RNA polymerase-driven RNA synthesis was used for the preparation and labeling of the RNA with Cy3 (or Cy5) dye. The fluorescent cRNA probes were purified using the Qiagen RNeasy Mini Kit (Qiagen, Valencia, CA), and an equal amount (825 ng) of Cy3- and Cy5-labeled cRNA probes was hybridized on a 44 K chicken Agilent array. The hybridized slides were washed using a commercial kit package (Agilent Technologies) and were then scanned using a Genepix 4100A scanner (Molecular Devices Corporation, Sunnyvale, CA) with a tolerance of saturation setting of 0.005%.
Microarray data collection and analysis: For each channel, the median of the signal intensity and the local background values was used. A locally weighted linear regression (LOWESS) normalization was applied to remove signal intensity-dependent dye bias for each array using the R program. A Student's t

test was used to identify differentially expressed genes, and a false discovery rate of 5% was used to adjust the P values derived from the t test.

Analysis of adipose macrophages with flow cytometry

Isolation of adipose tissue macrophages and SVCs: The adipose tissue was obtained from mice immediately after euthanization. The tissues were minced into small (5-10 mg) pieces. The minced samples were placed in HEPES-buffered DMEM (Invitrogen Corp.) supplemented with 10 mg/ml fatty acid-poor BSA (FAP-BSA; Sigma-Aldrich, St. Louis, MO) and were centrifuged at 1,000 *g* for 10 minutes at room temperature to pellet the erythrocytes and other blood cells. An LPS-free collagenase cocktail (Liberase 3; Roche Applied Science, Indianapolis, IN) at a concentration of 0.03 mg/ml and 50 U/ml DNase I (Sigma-Aldrich) were added to the tissue suspension, and the samples were incubated at 37°C on an orbital shaker (215 Hz) for 45-60 minutes. Following digestion, the samples were filtered through a 250-μm nylon mesh (Sefar America Inc., Depew, NY). The suspension was centrifuged at 1000 g for 10 minutes. The pelleted cells were collected as the SVCs. The SVCs were suspended in erythrocyte lysis buffer and incubated at room temperature for 7 minutes. The erythrocyte-depleted SVCs were centrifuged at 500 *g* for 5 minutes, and the pellet was resuspended in FACS buffer (PBS containing 5 mM EDTA and 0.2% [wt/vol] FAP-BSA).

Immunophenotyping with FACS: SVCs isolated from adipose tissue samples were cooled on ice and counted using a hemocytometer. The cell survival rates ranged from 80 to 90%. Following cell counting, the SVCs were centrifuged at 500 *g* for 5 minutes and resuspended in FACS buffer at a concentration of 7×10^6 cells/ml. The cells were incubated in the dark at 4°C for 30 minutes in FcBlock (20 μg/ml) (BD Pharmingen, San Jose, CA) and then for an additional 50 minutes with fluorophore-conjugated primary antibodies or isotype control antibodies. The antibodies used in these studies included F4/80-FITC (AbD Serotec, Raleigh, NC) and CD11C-APC (BD Pharmingen, San Jose, CA). Following incubation, 1 ml FACS buffer was added to the cells. The cells were centrifuged at 500 *g* for 5 minutes and were resuspended in 1 ml FACS buffer. The wash was repeated twice. The cells were analyzed on a FACSCalibur, and analysis was performed using the CellQuest software (Becton, Dickinson and Co., Franklin Lakes, NJ). To determine the percentage of CD11c+ cells, gates were set on F4/80 positive macrophage populations and percentages of CD11c+ cell were displayed as histograms.

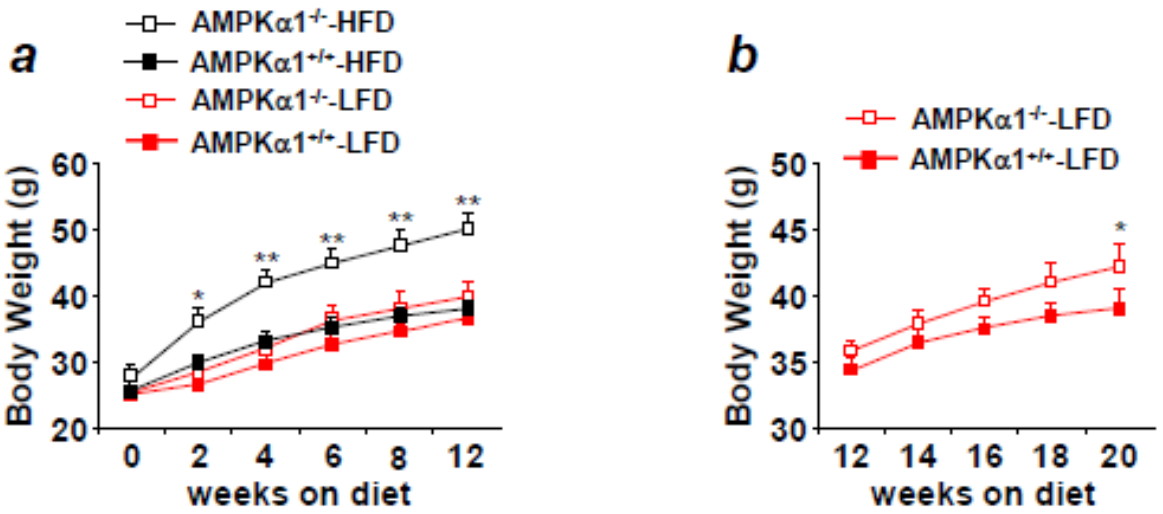
SUPPLEMENTARY DATA

Supplementary Table 1. Primer sequences for realtime RT-PCR

gene name	Sequence (5'-3')	gene name	Sequence (5'-3')
GPADH	ATCGTGGAAGGGCTAATGACCACA	Leptin	AAAGAACCTGAGCTGAGGGTGACA
	TCCTCTGTGTAAGCAAGGATGCCA		ATGCTAATGTGCCCTGAAATGCGG
F4/80	TGTCTGACAATTGGGATCTGCCCT	ACC1	GCCATTGGTATTGGGGCTTAC
	ATAGCTTCCGAGAGTGTTGTGGCA		GAGTGGGTAACCCCATTGTTG
IL-1β	AAGGGCTGCTTCCAAACCTTTGAC	FAS	TTCCAAGACGAAAATGATGC
	ATACTGCCTGCCTGAAGCTCTTGT		AATTGTGGGATCAGGAGAGC
IL-6	ATCCAGTTGCCTTCTTGGGACTGA	Glucokinase	CCCTGAGTGGCTTACAGTTC
	TAAGCCTCCGACTTGTGAAGTGGT		ACGGATGTGAGTGTTGAAGC
TNFα	AGCCGATGGGTTGTACCTTGTCTA	G6Pase	CTTCTCTGCCAAGGTCATCC
	TGAGATAGCAAATCGGCTGACGGT		TTTTGGGGATGGGCAC
IL-10	TGCACTACCAAAGCCACAAAGCAG	PEPCK	ATACACTGACTATGATCCCAC
	AGTAAGAGCAGGCAGCATAGCAGT		CCTTGTCTGAGGTATTGAGG
IL-12	AAAGCTGTCTTCTGCTTGGTTGGC	CPT1α	CAGTGGGAGCGACTCTTCAAT
	CTGGCTCTGCGGGCATTTAACATT		TCCTCGTGCAAAACAGGTCTG
MCP1	TCACCTGCTGCTACTCATTCACCA	ACC2	TCCCTGGATGACAACTCCTCT
	TACAGCTTCTTTGGGACACCTGCT		ACCAGATGGAGTCCAGACATG
AP2	TCAACCGACAACATTCCGATCCCA	PPARδ	ATGGAACAGCCACAGGAGGA
	TGAAGTGGGTCAAGCAACTCTGGA		ATCACAGCCCATCTGCAGCT
PPARγ	GGAAGACCACTCGCATTCCTT	CPT1β	CTCAAGTCATGGTGGGCAACT
	TCGCACTTTGGTATTCTTGGAG		ATCATGGCGTGAACGGCATTG
C/EBPα	AGAAGTCGGTGGACAAGAACAGCA	PGC1β	ATACACTGACTATGATCCCAC
	TGCGTTGTTTGGCTTTATCTCGGC		CCTTGTCTGAGGTATTGAGG
Adionectin	GATGGCAGAGATGGCACTCC	GLUT4	GGAAGGAAAAGGGCTATGCTG
	CTTGCCAGTGCTGCCGTCAT		TGAGGAACCGTCCAAGAATGA

SUPPLEMENTARY DATA

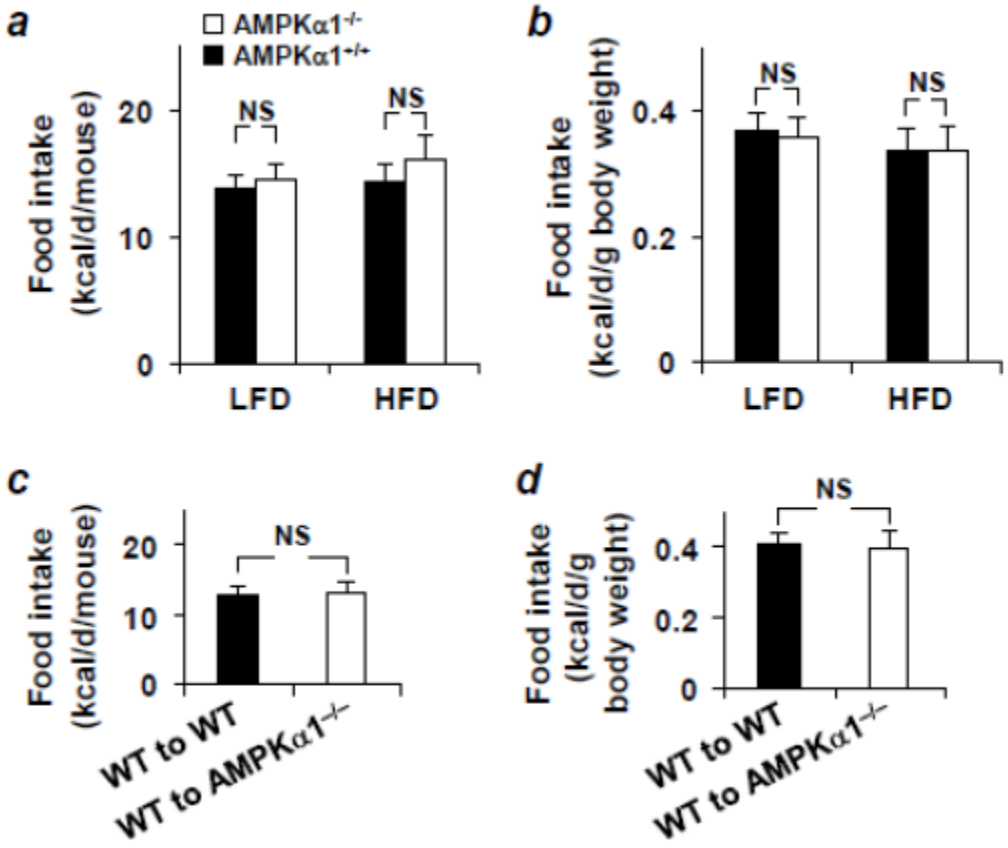
Supplementary Figure 1. LFD-induced adiposity in AMPKα1$^{-/-}$ mice.
At the age of 10 weeks, the AMPKα1$^{-/-}$ mice and their wild-type littermates were fed an LFD or HFD. *a*, Body weight of mice on a LFD or HFD for 12 weeks. *B*, Body weight of mice on a LFD for 20 weeks. Data are the means ± SE; n = 12 mice per group for (*a*) and n = 8 mice per group for (*b*), * $P <$ 0.05 and ** $P < 0.01$ for AMPKα1$^{-/-}$ vs. wild-type mice on the same diet.



SUPPLEMENTARY DATA

Supplementary Figure 2. Food intake in AMPKα1$^{-/-}$ mice

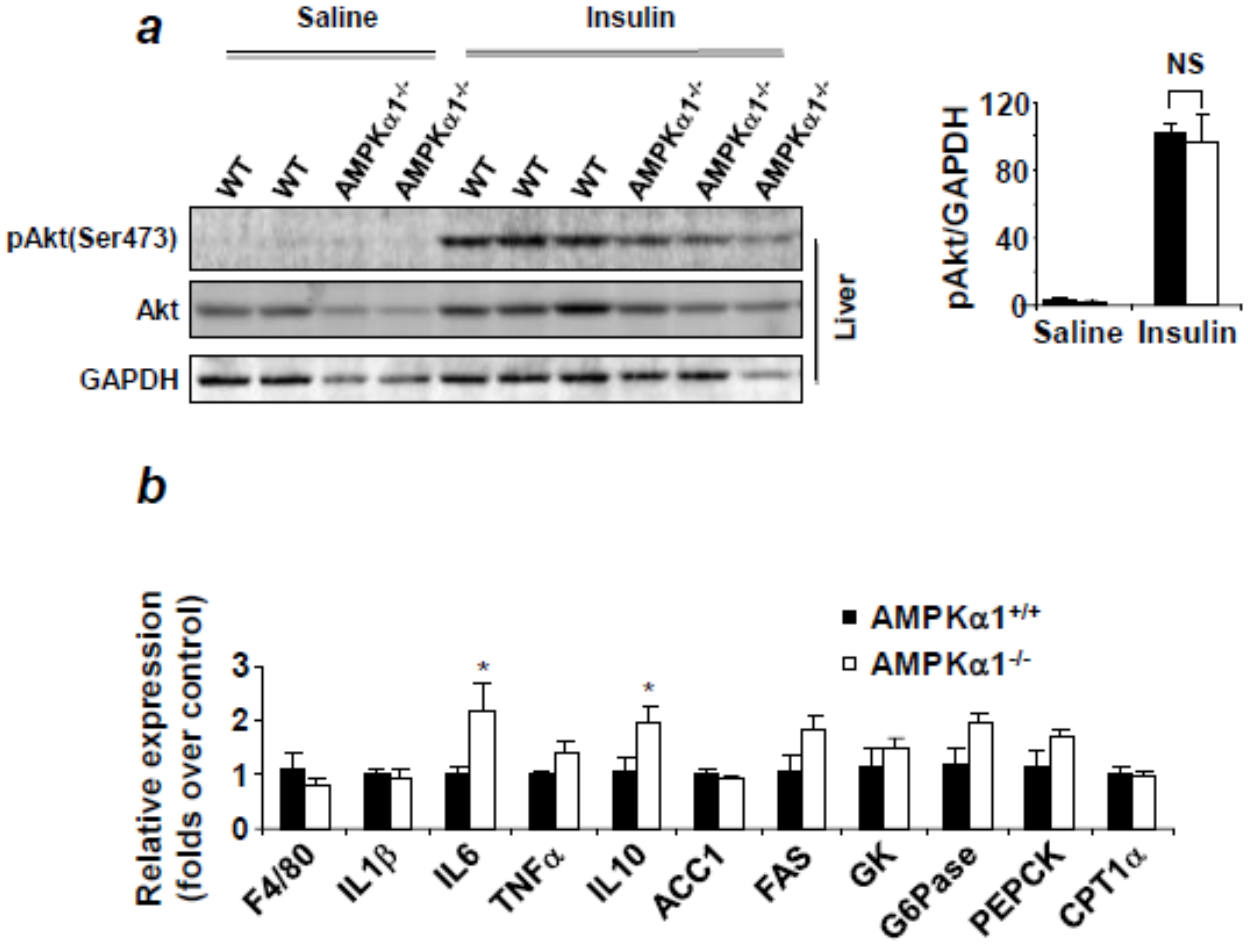
At the age of 10 weeks, mice, including the AMPKα1$^{-/-}$ mice and their wild-type littermates, the chimeric AMPKα1$^{-/-}$ mice with the bone marrow of wild-type mice and the control wild-type mice with the bone marrow of wild-type mice, were fed a HFD for 12 weeks. The food intake and body weight were measured twice a week for a 12-wk period. The food intake was analyzed for the AMPKα1$^{-/-}$ mice and their wild-type littermates (***a, b***), as well as for the chimeric AMPKα1$^{-/-}$ mice and their controls (***c, d***). The amount of food intake (grams or kcal intake/d/mouse) for the AMPKα1-/- and the chimeric AMPKα1$^{-/-}$ mice tended to be high compared to that of the control mice (***a***, ***c***). However, this difference did not reach statistical significance. Upon normalizing the data by body weight, the difference in the food intake (kcal intake/d/g) was decreased (***b***, ***d***). Data are the means ± SE; n = 12 or 6 mice per group for LFD- and HFD-feeding mice.



SUPPLEMENTARY DATA

Supplementary Figure 3. Status of insulin signaling and inflammation in the livers of HFD-fed AMPKα1$^{-/-}$ mice

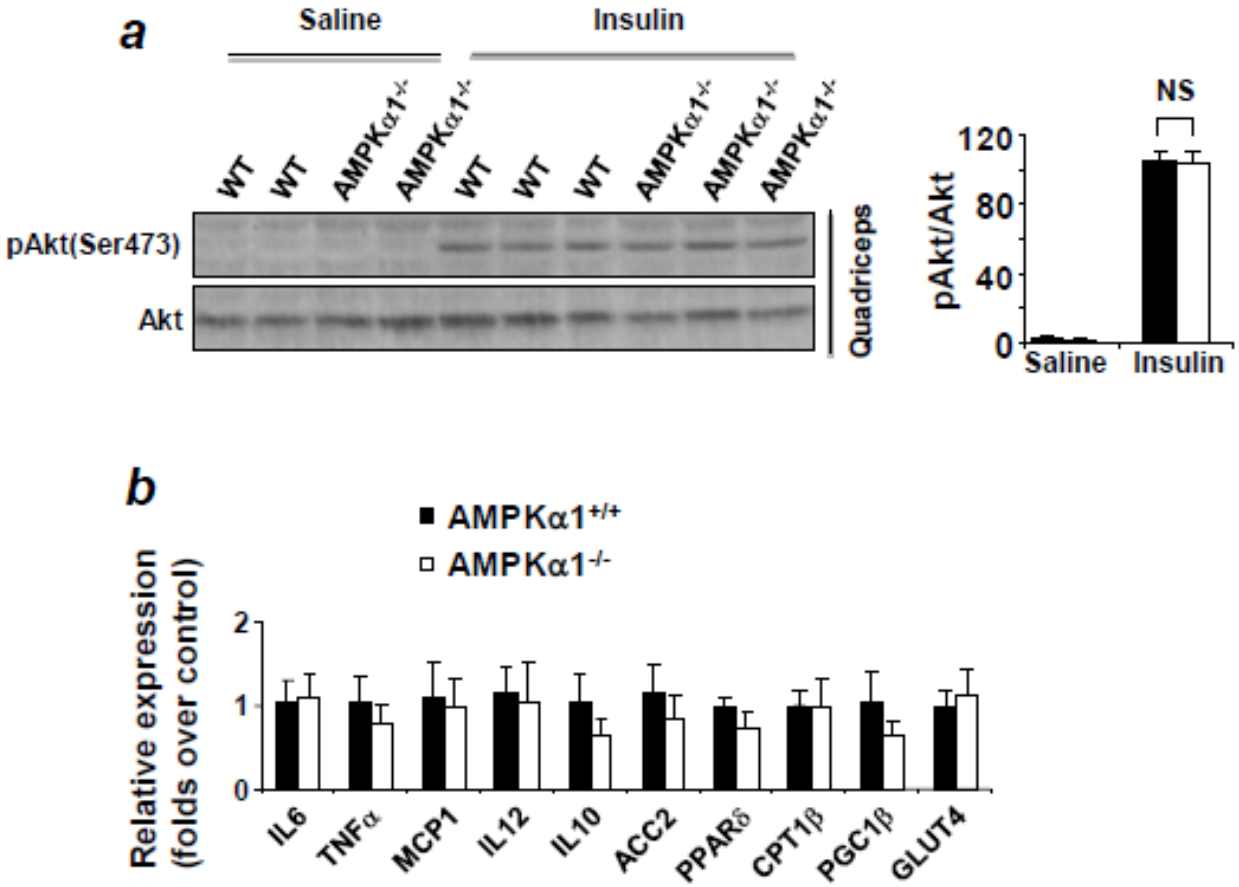
At the age of 10 weeks, the AMPKα1$^{-/-}$ mice and their wild-type littermates were fed a HFD, and *in vivo* experiments were conducted in the mice at the end of the 12-week HFD period. *a*, Levels of insulin-induced Akt phosphorylation in the liver. Liver samples were collected at 5 min after an injection of insulin (1 U/kg) or PBS into the inferior vena cava (i.v.). *b*, Levels of proinflammatory cytokines and other molecules related to hepatic metabolism. Data are the means ± SE; n = 6 mice per group (*a*) and n = 12 mice per group (*b*). * $P < 0.05$ for HFD-AMPKα1$^{-/-}$ vs. HFD-wild-type mice.



SUPPLEMENTARY DATA

Supplementary Figure 4. Status of insulin signaling and inflammation in the skeletal muscle of HFD-fed AMPK$\alpha1^{-/-}$ mice

At the age of 10 weeks, the AMPK$\alpha1^{-/-}$ mice and their wild-type littermates were fed a HFD. At the end of 12-week HFD, *in vivo* experiments were conducted in the mice. *a*, Levels of insulin-induced Akt phosphorylation in skeletal muscle. Muscle samples were collected at 5 min after an injection of insulin (1 U/kg) or PBS into the inferior vena cava (i.v.). *b*, Levels of proinflammatory cytokines and other molecules related to muscle metabolic activities. Data are the means ± SE; n = 6 mice per group (*a*) and n = 12 per group (*b*). $P > 0.05$ (NS) for HFD-AMPK$\alpha1^{-/-}$ vs. HFD-wild-type mice.



SUPPLEMENTARY DATA

Supplementary Figure 5. Increased inflammatory response of AMPKα1-deficient macrophages
The bone marrow cells of wild-type and AMPKα1$^{-/-}$ mice were isolated and were differentiated into macrophages. The cells, without LPS (10 ng/mL) treatment for 2 hours, were lysed for RNA extraction and were then analyzed with a standard microarray. *a*, Levels of chemokines and chemokine receptors. *b*, Levels of cytokines and cytokine receptors. n = 5 samples per group (*a* and *b)*, ** significant *P* values in the comparison of a certain molecule between the wild-type and AMPKα1-deficient macrophages under both LPS and resting conditions. * Just one was significant. C-WT and LPS-WT: WT macrophages treated with vehicle or LPS. C-A and LPS-A: AMPKα1-deficient macrophages treated with vehicle or LPS.