Supplementary Methods

Animal experiments Sirt1$^{flox/flox}$ mice (B6;129-Sirt1^{tm1Ygu}/J) and Lysozyme-Cre mice (B6.129P2-lyz2$^{tm1(cre)Ifo}$/J) were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). Sirt1$^{flox/flox}$ and homozygous LysM-Cre mice were crossed to obtain myeloid cell–specific Sirt1 knockout mice. To avoid potential variations stemming from gender and genetic background, male mice from the F2 generation, Sirt-1$^{flox/flox}$;LysM-Cre^{+} (mS1KO) and Sirt-1$^{flox/flox}$;LysM-Cre^{-} (WT), were used for studies. mS1KO mice and age-matched littermates older than 4 weeks of age were fed *ad libitum* either standard laboratory chow diet or a 60% high-fat diet (HFD, Research Diet, New Brunswick, NJ, USA) for 16 weeks. Oral glucose tolerance tests (1 g/kg of body weight) and insulin tolerance tests (0.75 U/kg of body weight) were performed after 14 h of fasting. All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 2011). The study protocol was approved by the Institutional Animal Care and Use Committee of Chonbuk National University.

Biochemical analysis Blood samples were collected after overnight fasting. TNF-α and IL-1β (Invitrogen, Carlsbad, CA, USA), insulin (Millipore Billerica, MA, USA), and MCP-1 (R&D Systems, Minneapolis, MN, USA) were measured using specific ELISA kits. Plasma levels of total cholesterol, triglyceride (TG), AST, and ALT (Asan Pharmaceutical, Seoul, Korea) were measured using commercially available kits. For liver TG quantification, liver tissues were homogenized and extracted in a mixture of chloroform, methanol, and DW (2/1/1 ratio).

Percentage body fat was determined using a Bruker Minispec mq 7.5 NMR analyzer (Bruker Optics, Ettlingen, Germany). Mice were placed in a clear, plastic cylinder (50 mm diameter) and kept immobile by insertion of a tight-fitting plunger. Fat and lean mass were recorded

within 2 min. The accuracy and precision of instruments was determined by crosscalibration by measuring the same groups of mice with different adiposities.

Cell culture For bone marrow-derived macrophage (BMM) culture, bone marrow was isolated from femurs and tibias of wild type and mS1KO mice and cultured in α-MEM supplemented with 10% fetal bovine serum (FBS). Cells were plated and cultured overnight in the presence of macrophage colony-stimulating factor (M-CSF, 10 ng/ml). Nonadherent cells were then collected and cultured for three days in the presence of M-CSF (10 ng/ml). Floating cells were removed, and adherent cells were used as BMMs. For M1 and M2 differentiation, BMMs were treated with 10 ng/ml LPS + IFN-γ (50 U/ml, Invitrogen) or IL-4 (10 ng/ml, Invitrogen), respectively. Murine macrophage line RAW264.7 cells were cultured in DMEM supplemented with 10% FBS. Before migration assays, RAW264.7 macrophage cells were treated with Sirt1 activator (10 nM SRT1720) or inhibitor (2.5 μM sirtinol) for 24 h. For targeted knock-down of FAK, RAW264.7 cells were transfected with si-FAK (Santa Cruz Biochemicals, Santa Cruz, CA, USA) or control siRNA (si-Control) using Lipofectamine 2000 (Invitrogen). To prepare adipocyte-conditioned medium (CM), 3T3-L1 fibroblasts were grown in DMEM containing 10% FBS. Confluent cells were treated with differentiation medium (MDI: DMEM, 10% FBS, 1 μM dexamethasone, 10 μg/ml insulin, and 0.5 mM isobutylmethylxanthine) for 2 days. Medium was then replaced with DMEM containing 10 μg/ml insulin for 2 days and maintained for 2 days without insulin. All conditioned media (CM) from adipocytes were collected at day 6 after initial exposure to a differentiation cocktail.

In vitro migration assay For migration assays, BMMs (5×10^5) or RAW264.7 (5×10^5) cells were seeded in the 24-well upper chamber of a cell culture insert with 8-μm pore membrane (BD Life Sciences, Franklin Lakes, NJ, USA) in FBS-free media. Adipocyte-conditioned

media or MCP-1 was added to the lower chamber, and cells were incubated for 3 h. Polyethylene terephthalate membranes separating the upper and lower chambers were fixed with 4% paraformaldehyde in PBS for 10 min and stained with crystal violet for 30 min.

Western blot Tissues and cells were homogenized in Tissue Protein Extraction Reagent or Mammalian Protein Extraction Reagent (Thermo, Waltham, MA, USA). Homogenates containing 20 μg of total protein were separated by SDS-PAGE and transferred to nitrocellulose membranes. For immunoprecipitation, 250 μg of protein precleared with protein G-agarose was incubated with anti-FAK or anti-Lys overnight at 4^{o}C, then with protein G-agarose at 4^{o}C for 2 h. Blots were probed with primary antibody against Sirt1 (Abcam, Cambridge, UK), acetyl-lysine, or FAK (Cell Signaling), or p50, p65, Ac-p65, or PCNA (Santa Cruz Biochemicals), and signals were detected with a Las-4000 imager (GE Healthcare Life Science, Pittsburgh, PA, USA).

RNA isolation and real-time RT-PCR Total RNA was extracted from islets or tissues using an RNA Iso kit (TaKaRa, Japan). RNA was precipitated with isopropanol and dissolved in diethylpyrocarbonate-treated distilled water. First-strand cDNA was generated with oligo dT-adaptor primers by reverse transcriptase (TaKaRa). Specific primers were designed using qPrimerDepot (http://mouseprimerdepot.nci.nih.gov, Table S1). Real-time RT-PCR reactions comprised a final volume of 10 μl, containing 10 ng of reverse-transcribed total RNA, 200 nM of forward and reverse primers, and PCR master mixture. RT-PCR was performed in 384-well plates using an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA).

Histology Tissues were removed and immediately placed in fixative (10% formalin solution in 0.1 M PBS). Histological sections (4 μm for liver and pancreas and 6 μm for adipose tissue) were cut from formalin-fixed paraffin-embedded tissue blocks. Tissue sections were

stained with hematoxylin-eosin under standard conditions. Staining of fibrillar collagen with Sirius red was performed on paraffin sections. Stained sections were quantified by iSolution DT 36 software (Carl Zeiss), and results were expressed as percentage of area. Immunohistochemical staining was performed using the DAKO Envision system (DAKO, Carpinteria, CA, USA), which uses dextran polymers conjugated with horseradish peroxidase to avoid contamination with endogenous biotin. After deparaffinization, tissue sections were treated using a microwave antigen-retrieval procedure in 0.01 M sodium citrate buffer. After blocking endogenous peroxidase, sections were incubated with Protein Block Serum-Free (DAKO) to block nonspecific staining, and anti-insulin (Santa Cruz Biochemicals) or anti-F4/80 (Abcam, Cambridge, UK). Peroxidase activity was detected with 3-amino-9-ethyl carbazole. To measure adipocyte area and islet size, sections were observed under an Axiovert 40 CFL microscope (Carl Zeiss, Oberkochen, Germany) and measured using iSolution DT 36 software (Carl Zeiss). Liver inflammation was graded on liver biopsies using a modified histologic activity index {Kleiner, 2005 #32}. Liver inflammation was defined as high grade if mice had more than 4 foci in a 200× field (inflammation score 3), or 2 to 4 foci in a 200× field (inflammation score 2). Inflammation was defined as low grade if mice had no foci (inflammation score 0), or fewer than 2 foci in a 200× field (inflammation score 1). For each animal, 3 to 5 areas in 4 different sections each were analyzed. The data were first averaged per section and then per animal.

TUNEL assay TUNEL assays were used according to the manufacturer's instructions (Promega, Madison, WI, USA) to detect apoptotic cells. Apoptotic cells were counted under a microscope (100×) and expressed as apoptotic index (AI = number of apoptotic bodies/1,000 cells). Each group was assessed in triplicate, and data were averaged.

Islet isolation Pancreatic islets were isolated from 12-week-old mice using a collagenase

digestion method as previously described [15]. Following isolation, islets were cultured overnight in RPMI-1640 supplemented with 2 mM L-glutamine, 10% heat-inactivated FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin in humidified air containing 5% CO_2 at 37°C. Prior to experiments, islets were washed three times in RPMI-1640 and cultured overnight.

Stromal vascular fraction (SVF) isolation and FACS analysis Epididymal fat pads were excised from 16-week HFD-fed wild type and mS1KO mice, rinsed three times in KR buffer (119 mM NaCl, 4.74 mM KCl, 1.19 mM KH_2PO4, 1.19 mM $MgSO_4 \cdot 7H_2O$, 20 mM $NaHCO_3$, 10 mM HEPES, 2.54 mM $CaCl_2 \cdot 2H_2O$, pH 7.4), and then homogenized in KRBA buffer containing type II collagenase, 3.5% BSA, and 6 mM glucose. Tissues were shaken for 1 h at 37°C and filtered through a 100 μm filter and then centrifuged at 1,000 rpm for 5 min. SVF pellets were then incubated with ACK lysis buffer (Invitrogen) for 3 min prior to centrifugation at 1,000 rpm for 5 min and resuspension in KRBA buffer. Stromal vascular cells (SVCs) were incubated in FACS buffer containing 2% FBS with Fc Block (BD Biosciences, San Jose, CA, USA) for 30 min at 4°C prior to staining with fluorescently labeled primary antibodies or control IgGs for 30 min at 4°C. F4/80-fluorescein isothiocyanate, CD11b-allophycocyamin, and CD11c-phycoerythrin conjugated primary antibodies were obtained from eBioscience. The cells were gently washed 3 times and resuspended in FACS buffer. SVCs were analyzed using FACS Calibrator (BD Biosciences) and Flowjo. Unstained, single stained, and fluorescence minus one controls were used for setting compensation and gates.