Extended Experimental Procedures

Animals and treatments

To induce adipocyte-specific genetic ablation, mice with homozygous loxP recombinase recognition site-flanked *HIF-1α* or/and *HIF-2α* alleles were bred to mice expressing Cre recombinase driven by *aP2* promoter (Li et al., 2011). Mice were housed in colony cages in 12 h light/12 h dark cycles. For HFD study, mice were subjected to 60% HFD for the indicated time periods (Research Diets, Inc; USA). For oral glucose tolerance test, the mice were fasted for 6 h and basal blood samples were taken, followed by oral glucose gavage (2 g/kg). Blood samples were drawn at 10, 20, 30, 45, 60, 90 and 120 min after gavage. For ITT, mice were fasted for 6 h, and basal blood samples were taken, followed by intraperitoneal injection of insulin (0.6 U/kg). Mouse clamp experiments were performed as described previously (Lee et al., 2011b). During the clamps, insulin was infused at a constant rate of 8.0 mU/kg/min. All animal procedures were in accordance with the research guidelines for the use of laboratory animals of University of California, San Diego.

Adipose tissue FFA measurement

FFA concentration in adipose tissue was measured by Lipomics, Inc. (West Sacramento, CA). The lipids were extracted from plasma and tissues in the presence of authentic internal standards using chloroform mixed with methanol (2:1 vol/vol) (Folch et al., 1957) and individual lipid classes were separated by HPLC (Cao et al., 2008). Lipid class fractions were transesterified in 1% sulfuric acid (in methanol) in a sealed vial with nitrogen at 100°C for 45 min. Fatty acid methyl esters were extracted from the mixture

with hexane containing 0.05% butylated hydroxytoluene, and readied for gas chromatography under nitrogen. Finally, fatty acid methyl esters were separated and quantified by capillary gas chromatography equipped with a 30 m DB-88MS capillary column and a flame-ionization detector.

In vitro pimonidazole adduct formation assay

Differentiated 3T3-L1 adipocytes were pre-incubated in 8% oxygen chamber, similar to in vivo oxygen level in adipose tissue, for 24h for equilibration. Pimonidazole was added to the adipocytes and incubated with or without 100 μM palmitate in the same condition for further 6 h, which was followed by immunocytochemistry analysis of pimonidazole adduct formation.

ANT Knockdown

To knockdown of ANT1 or ANT2 in 3T3-L1 adipocytes, duplex siRNAs against mouse ANT1 or ANT2 purchased from Integrated DNA Technology were introduced into 5 day differentiated 3T3-L1 adipocytes using electroporation. After 2 days of recovery, the cells were detached from the plate and subjected to oxygen consumption measurement or RNA isolation. For in vivo knockdown of ANT2 in adipose tissue, we used an AAV vector system. DNA oligomers for ANT2 shRNA were synthesized according to the "stem-loop" sequences (Valuegene), annealed and cloned into an AAV plasmid. The duplex DNA oligomer was placed downstream of a U6 promoter and the insertion was confirmed by sequencing. A control vector expressing shRNA against firefly *luciferase* was also constructed. The insert DNA sequence is as follows: ANT2-shRNA-sense, 5'-

GATCCGGGCAGATAAGCAATACAAGGTTCAAGAGACCTTGTATTGCTTATCT GCTTTTTC-3'; ANT2-shRNA-antisense, 5'-AATTGAAAAAGCAGATAAGCAATACAAGGTCTCTTGAACCTTGTATTGCTTA TCTGCCCG-3'. C57BL/6 mice were injected with AAVs encoding shRNAs against *luciferase* (Lu) or *ANT2* into epididymal adipose tissue. 3 weeks later, the mice were switched to HFD or remained on NCD for 3 days, and sacrificed for histology analysis of epididymal adipose tissue. For administration of AAV8-ANT2 shRNA vector to epididymal adipose tissue, mice were anesthetized with ketamine (25 mg/kg), acepromazine (2mg/kg) and xylazine (10 mg/kg), and laparotomy was performed. To distribute the vector in the whole depot, each epididymal fat pad was given 5 injections of 2 ul ($2x10^{13}$ gc/ml) of AAV solution using a Hamilton syringe.

Flow cytometry

Flow cytometry analysis of adipose tissue macrophages was performed as described previously (Lee et al., 2011b). Epididymal adipose tissue were weighed, rinsed three times in PBS, and then minced in FACS buffer (PBS supplemented with 1% low endotoxin BSA). Tissue suspensions were centrifuged at 500*g* for 5 min and then collagenase-treated (1 mg/mL; Sigma-Aldrich) for 30 min at 37°C with shaking. Cell suspensions were filtered through a 100 μm mesh and centrifuged at 500*g* for 5 min. SVC pellets were then incubated with erythrocyte lysis buffer (ebioscience) for 5 min before centrifuge (300*g*; 5 min) and resuspended in FACS buffer. SVCs were incubated with Fc block for 20 min at 4°C before staining with fluorescence labeled primary antibodies or

control IgGs for 30 min at 4°C. F4/80-APC FACS antibody was purchased from AbD Serotec (Raleigh, NC); CD11b-fluorescein isothiocyanate and CD11c-PE FACS antibodies were from BD Biosciences. Cells were gently washed twice and resuspended in FACS buffer with propidium iodide (PI) (Sigma-Aldrich). SVCs were analyzed using a FACS Aria flow cytometer (BD Biosciences). Unstained, single stains and Fluorescence Minus One controls were used for setting compensation and gates. The events are first gated based on forward versus side scatter area, as well as side scatter height versus width for a total of three dual parameter plots to gate out aggregates and debris. We used single color controls to calculate compensation using the FACSDiva software. A plot of forward scatter versus PI fluorescence was used as the fourth gate to identify individual, live cells. To measure markers with the maximum sensitivity, each fluorochrome was plotted versus PI and polygons were drawn, angled with the aid of the Fluorescence Minus One controls. This excluded dead and autofluorescent cells, but included dim positives. By using polygon gates in combination with logical gates, inclusion of false-positive cells in the gates was reduced.

Macrophage migration assays

In vitro migration of macrophages was measured as described previously (Lee et al., 2011b). For preparation of ACM, primary adipocytes from HFD WT or HFD HAKO were incubated in Dulbecco's Modified Eagle's media supplemented with 0.2% BSA for 12 h. In vivo macrophage tracking experiments was performed as described previously (Oh et al., 2012). Leukocyte pools from C57BL/6 male 12-week-old mice, bled by retro-orbital sinus, were subjected to erythrocyte lysis, and monocyte subsets were enriched

with the EasySep mouse monocyte enrichment kit (Stemcell Tech, Vancouver, BC, Canada), following the manufacturer's instructions. Isolated monocytes (5×10^6 to 10×10^6) were washed once in serum-free medium (RPMI-1640) and suspended in 2 mL diluent solution C (included in the PKH26 labeling kit). A total of 2 mL PKH26 (Sigma Chemical, St. Louis, MO) at 2×10^{-3} mol/L in diluent C was added and mixed, and the cells were incubated for 10 min at room temperature in the dark. The staining reaction was halted by the addition of an equal volume (2 mL) of medium supplemented with 10% FBS. The mixture was centrifuged, and the cells were washed once and resuspended in serum-containing medium. Subsequent to labeling with PKH26, the monocytes were counted and ~1×10^6 viable cells were suspended in 0.2 mL PBS and injected into the femoral vein of the each group of mice. 7 days after the injection, the ATMs were immediately isolated from visceral fat tissue and analyzed by flow cytometry.

Measurement of protein nitrosylation

To measure protein S-nitrosylation, total lysates of epididymal adipose tissues from WT or HAKO mice fed normal chow or HFD were subjected to the biotin-switch assays (Cayman) according to the manufacturer's protocol. To assess total protein S-nitrosylation, samples with the biotinylated nitrosocysteines were subjected SDS-PAGE electrophoresis, and immunoblottings with streptavidin conjugated with horseradish phosphatase. To assess Akt S-nitrosylation, samples with the biotinylated nitrosocysteines were immunoprecipitated with streptavidin-coated sepharose beads, and then subjected to immunoblottings with anti-Akt antidody.

Supplemental Materials

Figure S1. Related to Figure 1. (**A**) Pimonidazole adduct staining of epididymal adipose tissue from mice fed normal chow (NCD) or HFD for 1 day (1d HFD). Ratio of pimonidazole adduct positive area to BODIPY positive area was calculated and graphed (left panel). n=4 per group. (**B**) Western blot analysis of HIF-1α and HIF-2α protein expression in epididymal adipose tissues of mice fed normal chow (NCD) or HFD for 1 day. (**C**) FFA level in serum of NCD or HFD (15 weeks) mice. (**D**) Diacylglycerol and triacylglycerol levels in eWAT of WT mice fed NCD or HFD for 15 weeks. (**E**) Palmitate induces HIF-1α target gene expression in 3T3-L1 adipocytes. 3T3-L1 adipocytes were incubated in serum free media in the presence or absence of 100 uM palmitate for different period as indicated in the figure. mRNA expression of HIF-1α target genes in 3T3-L1 adipocytes were measured by quantitative realtime RT-PCR. (**F**) HFD increases mitochondria mass without change in mitochondrial DNA content in adipose tissue. Citrate synthase (CS) activity (left), an enzyme marker of mitochondrial mass, and mitochondrial DNA content (right) were measured in eWAT of mice fed chow or HFD.

Figure S2. UCP expression is not changed in BAT of HAKO mice (related to Figure 2). Realtime RT-PCR analysis of UCP mRNA expression in BAT of WT or HAKO mice fed NCD or HFD (15 weeeks).

Figure S3. Fat-specific HIF-1α KO mice exhibit increased epididymal fat mass and fat cell size (related to Figure 3). (**A**) DNA genotyping (exon2 region) of HIF-1α in WT or HAKO mouse tissues. Genomic DNAs were isolated from primary adipocytes or SVCs from epididymal white adipose tissue, or interscapular brown adipose tissue, brain, liver, skeletal muscle or IP macrophages of WT or HAKO mice, and subjected to realtime RT-PCR analysis to measure deletion efficiency of the floxed exon 2 region of *HIF-1α* gene. Each genomic DNA sample was amplified with 2 separate sets of primers in separate tubes: one primer set was designed to amplify exon2 region of *HIF-1α* (targeted region), and the other set was designed to amplify non-targeted *HIF-1α* gene (control region). Relative ratio of target region to control region in WT mice was considered to be 1. (**B**) mRNA expression of HIF-1α and HIF-2α in eWAT or isolated primary adipocytes (Adipo) and SVCs from WT or HAKO mice. N, NCD; H, HFD. Mean+/-SEM. (**C**) Body weight of WT and HAKO mice on NCD (n=7) or HFD (n=26). Mean+/-SEM. (**D**) Trichrome staining of adipose tissue from WT and HAKO mice fed HFD for 15 weeks. (**E**) eWAT mass on NCD and HFD (15 weeks). Mean+/-SEM. n=15 per group. (**F**) H&E staining of adipose tissue from WT and HAKO mice fed HFD for 15 weeks. (**G)** Average fat cell size of HFD WT or HAKO mice. Mean+/-SEM. n=5 or 8 per group. (**H**) Fat cell size distribution of HFD WT or HAKO mice. Mean+/-SEM. (**I**) Relative oxygen consumption rate. Mean+/-SEM. n=4 per group. (**J**) eWAT vascular density as measured by CD31 positive area in adipose tissue section. Mean+/-SEM. n=4 per group. (**K**) Liver mass. Mean+/-SEM. n=14 per group. (**L**) Hepatic TG content. Mean+/-SEM. n=7 or 8 per group. (**M**) Quantitative realtime RT-PCR analysis of mRNA expression in the liver of HFD WT or HFD HAKO mice.

Figure S4. HIF-1α mediates hypoxia-dependent activation of inflammatory pathways in 3T3-L1 adipocytes (related to Figure 4). (**A**) mRNA levels of inflammatory genes are induced in adipocytes by hypoxia. 3T3-L1 adipocytes were incubated in normoxia (Nor; 21% O_2) or hypoxia (Hypo; 1% O_2) for 24h, and mRNA levels of inflammatory genes were measured by realtime RT-PCR. (**B**) Knockdown of HIF-1α blocks hypoxia-dependent increase in FLAP, MCP-1, iNOS, and Glut1 expression. Nor, normoxia; Hypo, hypoxia; scrb, scrambled. (**C**) ChIP-Seq analysis of HIF-1α binding to inflammatory gene promoters in 3T3-L1 adipocytes. Differentiated 3T3-L1 adipocytes were incubated in normoxic (21% O^2 chamber) or hypoxic condition (1% O^2 chamber) for 24 h, fixed with formaldehyde and subjected to ChIP assays with anti-HIF-1α antibodies (Abcam; ab199) and ChIP-IT high sensitivity ChIP assay kit (Active Motif; 53040). Immunoprecipitated DNA fragments were sequenced using HiSeq2000 sequencing system (Illumina). Binding intensity of HIF-1α to the promoters of immune response-related genes (selected from genes which showed increased binding of HIF-1α on hypoxia) was expressed as a heat map. (**D**) HIF-1α knockdown reduces adipocyte chemoattractive activity. 3T3-L1 adipocytes were transfected with mock scrambled siRNA (scr) or HIF-1α siRNA. 24h after transfection, cells were washed twice with fresh media and started to be incubated in serum free media. 24h later, adipocyte-conditioned media were harvested for macrophage chemotaxis assays using Transwell plates.

Figure S5. Related to Figure 6. (**A**) DNA genotyping (exon2 region) of HIF-2α in WT or H2AKO mouse tissues. Genomic DNAs were isolated from primary adipocytes or SVCs from epididymal white adipose tissue, or interscapular brown adipose tissue, brain, liver, skeletal muscle or IP macrophages of WT or H2AKO mice, and subjected to realtime RT-PCR analysis to measure deletion efficiency of the floxed exon 2 region of *HIF-2α* gene. Each genomic DNA sample was amplified with 2 separate sets of primers in separate tubes: one primer set was designed to amplify exon2 region of *HIF-2α* (targeted region), and the other set was designed to amplify non-targeted *HIF-2α* gene (control region). Relative ratio of target region to control region in WT mice was considered to be 1. (**B-C**) mRNA expression of inflammatory (**B**) and fibrosis (**C**) genes in WT and H2AKO adipose tissue on 15 weeks of HFD. (**D**) Schematic representation of the proposed mechanism for initiation of adipose tissue hypoxia and inflammation.