Supplementary Data: Table of Contents

MATERIALS AND METHODS FOR SUPPLEMENTAL FIGURES

Transfections and Infections

Retroviral infection and selection procedures were performed essentially as described (77). Briefly, 293T cells were transfected by calcium phosphate coprecipitation. About 16 hours after transfection virus-containing media was collected, passed through a 0.45-μm syringe filter, and combined with polybrene (hexadimethrine bromide; Sigma-Aldrich, St. Louis, MO) to a final concentration of 8 μg/ml. This media was then applied to subconfluent (25-40% confluent) 3T3-L1 preadipocytes. The infection protocol was repeated every 12 hours until cells were approximately 80% confluent. 3T3-L1 cells were then split 1:5 in Dulbecco's modified Eagle's medium (GIBCO) supplemented with 10% calf serum and appropriate selection agents (400 μg/ml neomycin or 2 μg/ml puromycin). Once fully selected, stably infected cells were used for the appropriate assays.

Localization of Sfrp5

Mouse 3T3-L1 and human embryonic kidney 293T cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen, GIBCO®; Carlsbad, CA) containing 10% calf serum (Atlanta Biologicals; Laurenceville, GA). 293T cells were transiently transfected with pcDNA3.1+ (*Sfrp5-Myc*) using the Lipofectamine Plus reagent (Invitrogen) according to the manufacturer's instructions. Separation of whole cell, extracellular matrix (ECM), and conditioned media fractions was performed as previously described (Finch et al., 1997; Lee et al., 2004; Uren et al., 2000; Zhong et al., 2007). Briefly, 293T cells were treated with or without 50 μg/ml heparin (Sigma-Aldrich, St. Louis MO) for 3 hours. The conditioned media were then concentrated by centrifuging at 3000 x g for 20 min using Amicon® Ultra-15 Centrifugal filter devices (Millipore, Billerica, MA). Cells were released from the culture dishes by incubating with 10 mM EDTA in PBS. The ECM components remaining on the culture dishes were washed and extracted with SDS Laemmli sample buffer with 5% β-mercaptoethanol. 3T3-L1 adipocytes stably expressing *Sfrp5-Myc* or control vector cells were induced to differentiate into adipocytes two days after confluence as described in Materials and Methods for the main text. On day 6 after differentiation, cells were treated with or without heparin, and then released from the culture dishes by EDTA. The subsequent methods were as described for HEK293T cells.

Total body fat content

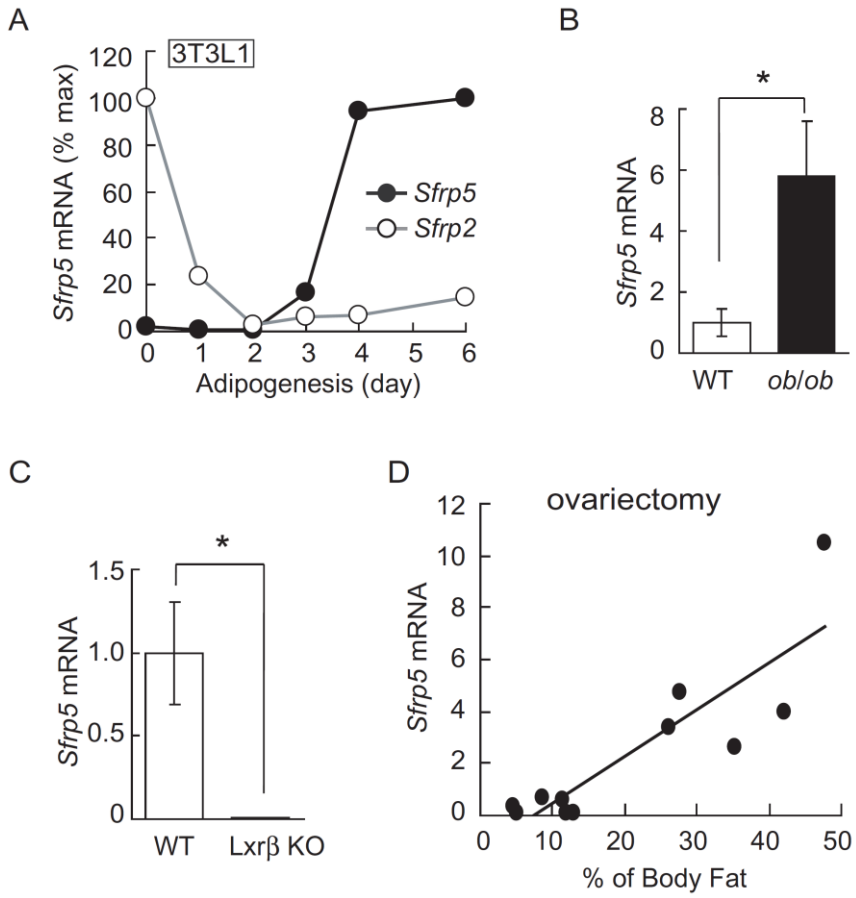
Total body fat content was determined using a dual energy X-ray absorptiometry (DEXA) scanner (GE Medical Systems Lunar) according to the manufacturer's instructions.

Measurement of Adipocyte Volume

Following quantification of cross-sectional adipocyte area with ImageJ as discussed in Materials and Methods for the main text, we assumed that adipocytes are spherical and thus calculated the volume of each adipocyte using the formula $4/3\pi r^3$.

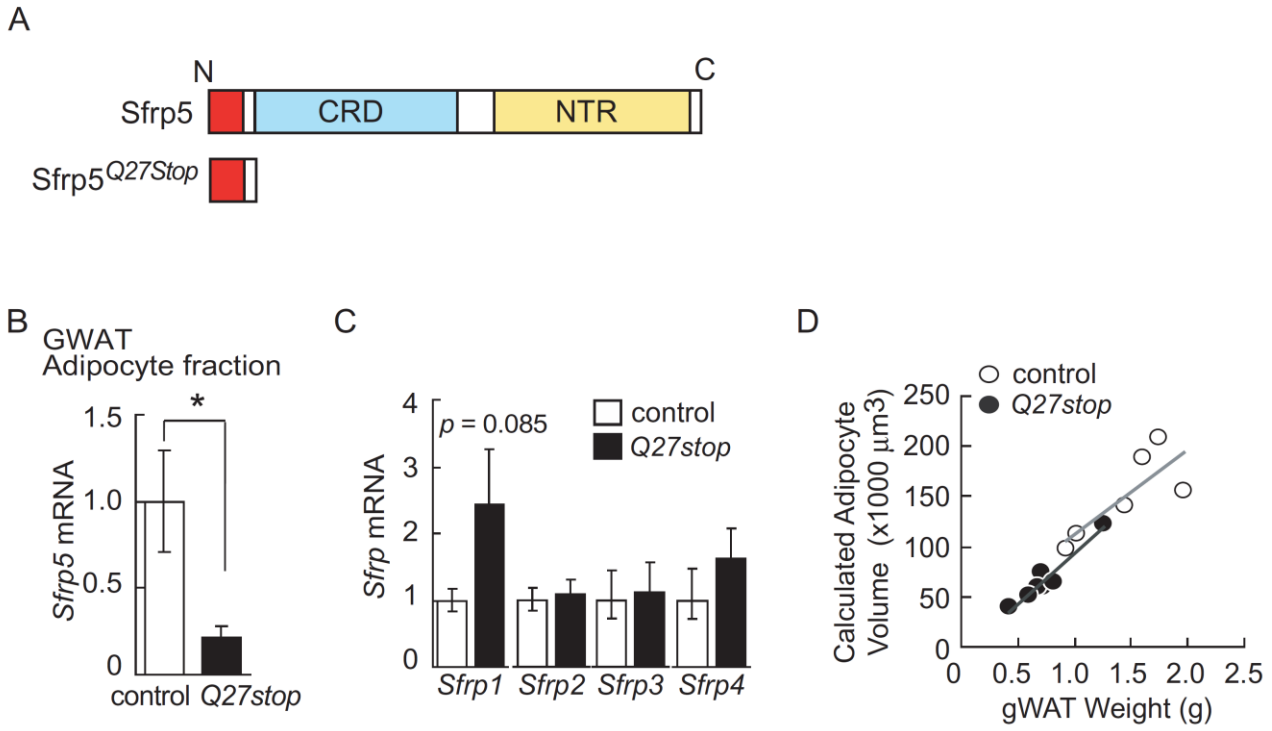
Microarray Analysis

RNA was isolated from gWAT of male control and *Sfrp5*^{Q27stop} mice. cDNA was amplified and purified using the WT Pico assay (NuGen Inc.) following the manufacturer's standard protocol. Four micrograms of cDNA was converted to sense orientation using the Exon Module (NuGen Inc.) and subsequently fragmented and biotinylated using the Ovation FL Module (NuGen Inc.) following the manufacturers standard protocol. The probe was then hybridized to Affymetrix Mouse Gene ST 1.0 GeneChips for 20 hours at 45°C, stained, and washed using a Fluidics FS450 instrument, and then scanned with the Affymetrix 7G Scanner 3000. Data were analyzed using Ingenuity Pathway Analysis software (Ingenuity Systems, Redwood City, CA) to identify relevant biological networks. Gene profiling data are available from the GEO database (http://ncbi.nlm.nih.gov/geo) with the accession number: GSE37514.



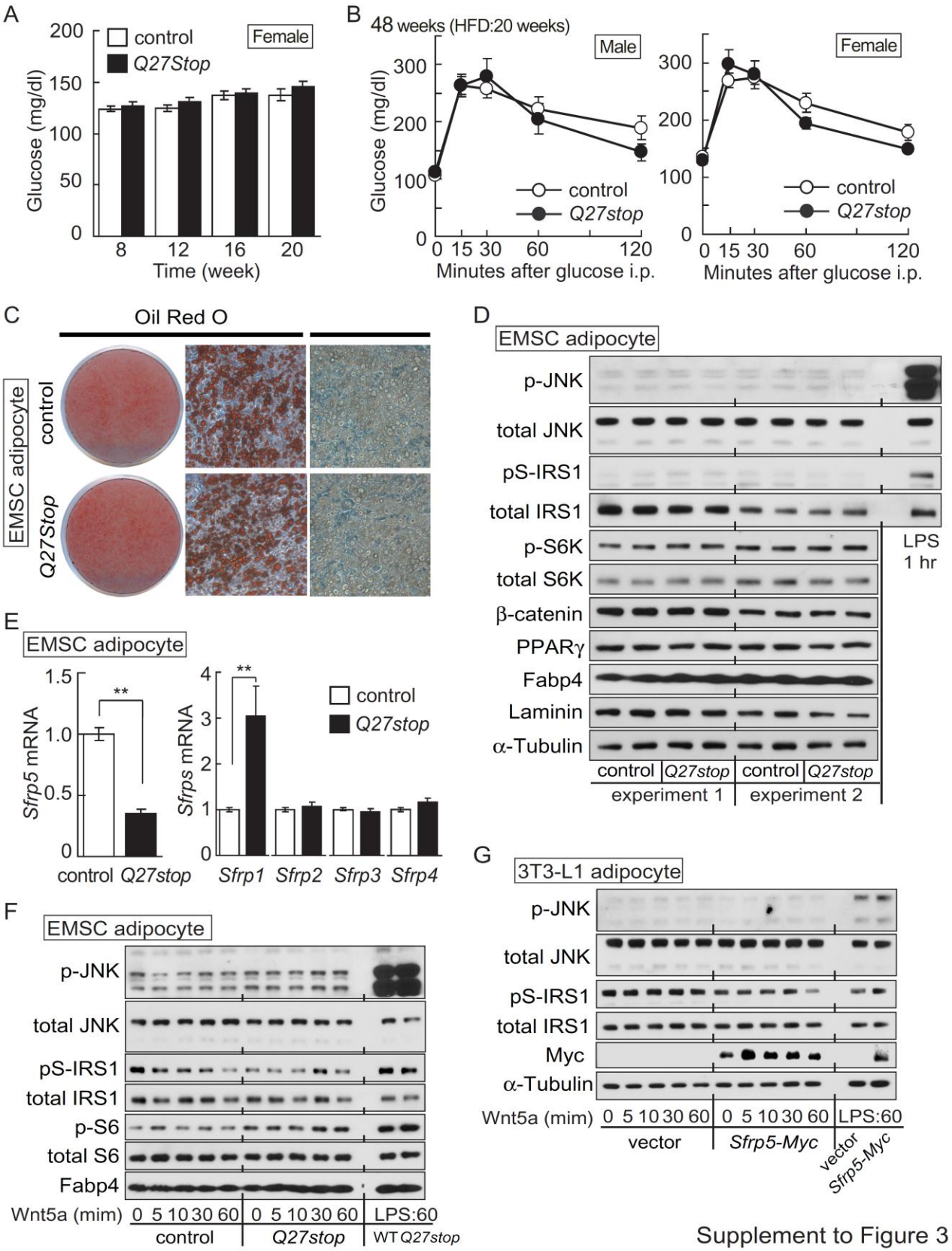
Supplement to Figure 1

(**A**) *Sfrp5* mRNA expression is induced during adipocyte differentiation *in vitro.* RNA was isolated from day-two post-confluent 3T3-L1 cells (Day 0) and at the indicated time points after induction of differentiation. Values normalized to *Tbp* mRNA are expressed as percent maximum expression (*n* = 3). (**B**) Increased *Sfrp5* mRNA expression in eWAT from *Lep*$^{ob/ob}$ mice. *Sfrp5* mRNA levels in eWAT were measured by real-time RT-PCR in 12-week-old *Lep*$^{ob/ob}$ and control mice (*Lep*$^{+/+}$). Average Ct = 21.8 from *Lepr*$^{ob/ob}$ eWAT and = 24.58 from control mice. Values normalized to *18S* mRNA are expressed relative to control mice. (*Lep*$^{+/+}$; *n* = 5*; Lep*$^{ob/ob}$, *n* = 3). (**C**) *Sfrp5* is suppressed in eWAT from mice that are resistant to HFD-induced obesity. *Sfrp5* mRNA levels in eWAT from control or *Lxrβ* KO mice under HFD for six months were measured by real-time RT-PCR. Values were normalized to *18S* mRNA and are expressed relative to control mice (*n* = 4 per genotype). (**D**) Increased *Sfrp5* expression is associated with elevated adiposity. The graph shows relationship between % body fat measured by DEXA and *Sfrp5* mRNA levels in WAT from OVX or sham-operated female mice (shown in Figure 1C). For panels (**B**) and (**C**), values are mean ± s.e.m. *p<0.05.



Supplement to Figure 2

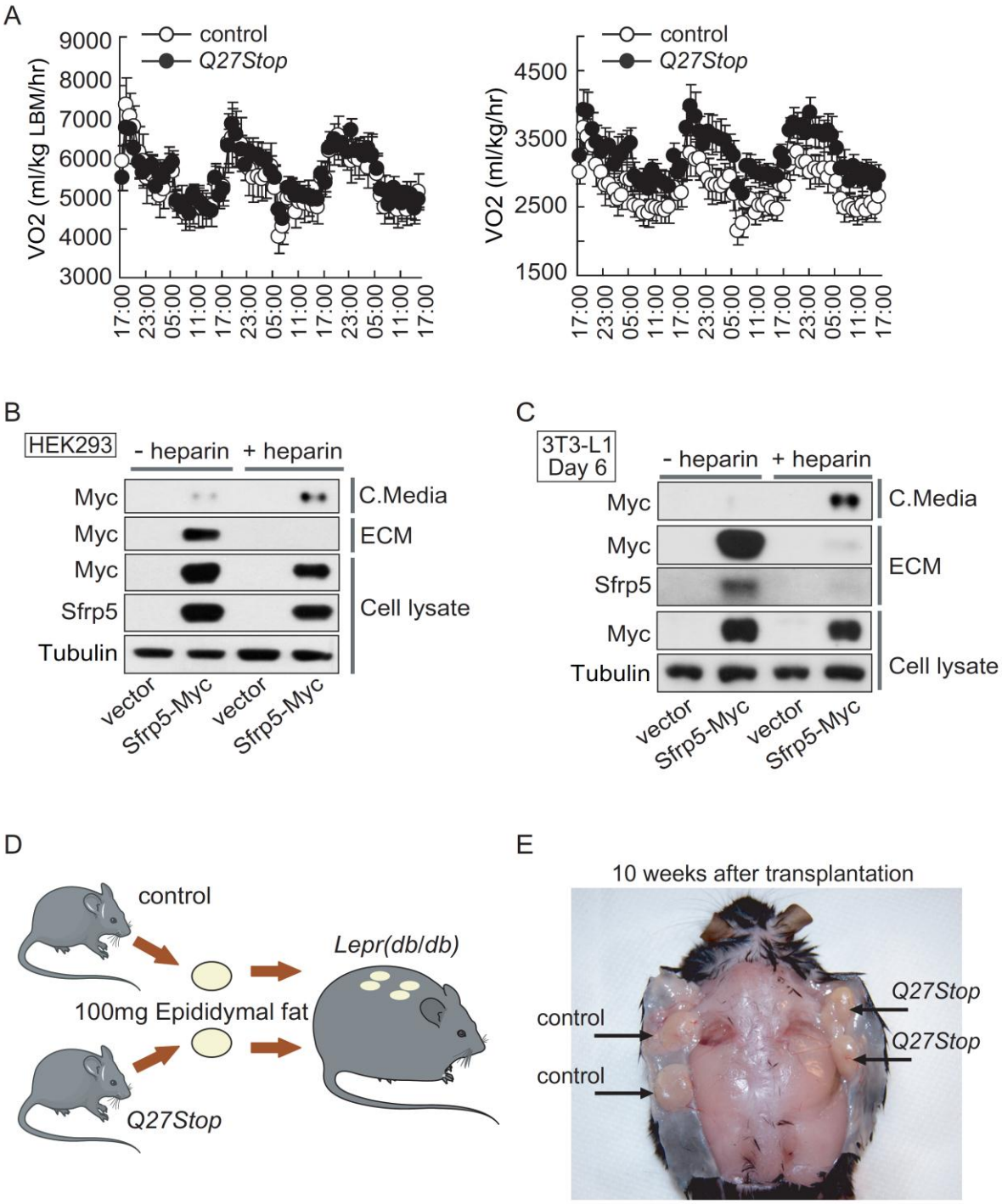
(**A**) Diagram depicting the full-length Sfrp5 protein and the predicted protein fragment in *Sfrp5*^{Q27stop} mice. CRD, cysteine-rich domain; NTR, netrin domain. (**B**) Decreased *Sfrp5* in adipocytes of *Sfrp5*^{Q27stop} mice. *Sfrp*5 mRNA expression levels in the adipocyte fraction of eWAT from control or *Sfrp5*^{Q27stop} mice under HFD were measured by real-time RT-PCR. Values were normalized to *Tbp* mRNA and are expressed relative to control mice (*n* = 8). (**C**) Compensatory increased *Sfrp1* mRNA in *Sfrp5*^{Q27stop} gWAT. mRNA for *Sfrp5* (left panel) and other Sfrp family members (right panels) in gWAT from control or *Sfrp5*^{Q27stop} mice under HFD. Values were normalized to *Tbp* mRNA and are expressed relative to control mice. (*n* = 17). (**D**) Reduction in weight of WAT in Q27stop mice can be attributed to decreased size of adipocytes. The correlation coefficient for the combined data is 0.89, indicating that most variation in weight of adipose tissue can be explained by differences in adipocyte volume. Thus changes in total number of adipocytes per WAT depot will be negligible between genotypes. Adipocyte volume was calculated from cross-sectional area, and is plotted relative to weight of ovarian WAT.



Supplement to Figure 3

Supplement to Figure 3

(**A**) Blood glucose levels in *ad libitum*-fed female *Sfrp5*^{Q27stop} and control mice (*n* = 13-19). (**B**) No change in glucose tolerance in female *Sfrp5*^{Q27stop} mice under HFD. Blood glucose levels after intraperitoneal (i.p.) injection of glucose (1 g/kg body weight) in 48-week-old female mice (*n* = 8 per each gender and per genotype). (**C**) SFRP5-deficiency does not affect adipocyte differentiation. Pictures of Oil Red-O (ORO) staining (left), photomicrographs of ORO (middle) and phase-contrast pictures (right) from control and *Sfrp5*^{Q27stop} EMSC adipocytes (Day 12 after differentiation). (**D**) No change in expression of adipocyte markers in *Sfrp5*^{Q27stop} EMSC adipocytes. Immunoblot analysis of markers of adipocytes (PPARγ and FABP4), insulin signaling components (pS-IRS1, p-S6K) or other proteins in *Sfrp5*^{Q27stop} and control EMSC adipocytes (Day 12 after differentiation). Laminin and α-tubulin were used as loading controls. Experiment 1 and 2 are two independent experiments. EMSC adipocytes stimulated with LPS were used as a control for p-JNK and pS-IRS1. (**E**) Decreased *Sfrp5* and increased *Sfrp1* in *Sfrp5*^{Q27stop} adipocytes. *Sfrp5* (left panel) and other *Sfrp* mRNAs (right panel) in *Sfrp5*^{Q27stop} EMSC adipocytes (Day 12). Values were normalized to *Tbp* mRNA and are expressed relative to control mice (*n* = 22/genotype). Values are expressed as mean ± s.e.m. **p*<0.01. (**F**) Ablation of SFRP5 does not affect phosphorylation of JNK or S-IRS1 in EMSC adipocytes after WNT5a treatment. Immunoblot analyses of control and *Sfrp5*^{Q27stop} EMSC adipocytes (Day12) after treatment with recombinant WNT5A (100 ng/mL). EMSC adipocytes stimulated with LPS were used as a positive control. (**G**) Ectopic SFRP5 does not affect phosphorylation of JNK or S-IRS1 in 3T3-L1 adipocytes after WNT5a treatment. Immunoblot analysis using indicated antibodies after recombinant WNT5a treatment of 3T3-L1 adipocytes (Day 6) stably expressing either *Sfrp5-Myc* or Ad–β-gal (vector). EMSC adipocytes in (A-D) were at day 12 post-induction. In (B), (D) and (E), adipocytes stimulated with LPS were used as a control for p-JNK and pS-IRS1. For all panels, *Sfrp5*^{Q27stop} is represented as *Q27Stop*.



Supplement to Figure 4

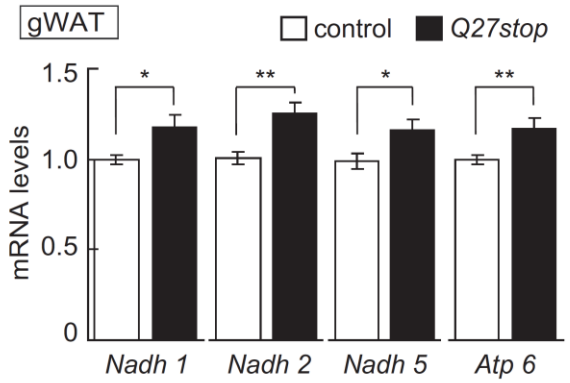
Supplement to Figure 4

(**A**) Metabolic rate in control and *Sfrp5*^{Q27stop} mice. Male control and *Sfrp5*^{Q27stop} mice (*n* = 8 each) were placed in a comprehensive lab animal monitoring system (CLAMS) for three days. Oxygen consumption rate, as normalized to lean mass (LBM, left panel) and body weight (right), is shown. (**B**) Sfrp5 is a secreted protein tightly associated with the extracellular matrix. HEK293T cells were transiently transfected with pcDNA3.1+ empty vector or vector containing a *Sfrp5-Myc* fusion construct. Twenty four hours after transfection, cells were treated with or without 10 µg/ml heparin for six hours. Cell lysates, conditioned medium (C. Media), and extracellular matrix (ECM) were then collected. Samples were analyzed by SDS-PAGE followed by immunoblotting with anti-MYC and anti-SFRP5 antibody. (**C**) 3T3-L1 preadipocytes stably expressing *Sfrp5-Myc* or control vector were induced to differentiate into adipocytes. On day 6 after differentiation, cells were treated with or without heparin (10 µg/ml), then released from the culture dishes by EDTA. The subsequent methods were the same as for HEK 293T cells. (**D**) Schema of eWAT transplantation from control and *Sfrp5*^{Q27stop} mice to *Lepr*$^{db/db}$ mice, as described in Figures 4C and 4D and in Materials and Methods for the main text. eWAT from control or *Sfrp5*^{Q27stop} donors (*n* = 6 each) was transplanted subcutaneously into *Lepr*$^{db/db}$recipients (*n* = 3) at four weeks of age. To control for host differences in hyperphagia, vasculature formation, or other variables, two pieces of tissue (100 mg) were transplanted from each genotype into each *Lepr*$^{db/db}$ recipient. This panel was produced using Servier Medical Art. (**E**) Ten weeks after transplantation, the donor tissues had grown as shown in this picture. *Sfrp5*^{Q27stop} is represented as *Q27Stop*.

A

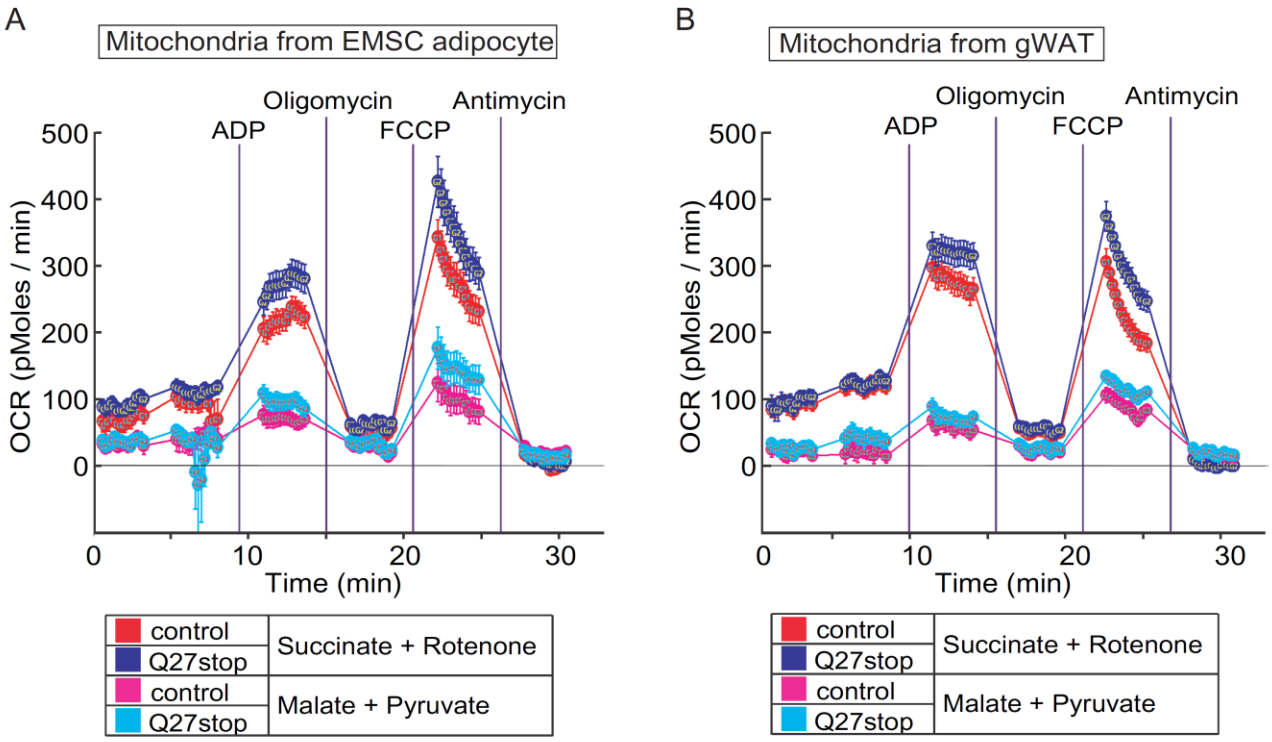
pathway	-Log(P-value)	Molecules
Oxidative Phosphorylation	3.9000	COX7B, COX17, COX6B1, ATP6V1D, COX6A1, COX6C, COX5B, NDUFB5, ATP5L, ATP5G2, UQCRB, NDUFA1, NDUFA13, NDUFA6, ATP5H (includes EG:10476), NDUFS6 (includes EG:4726), NDUFB6, COX2, ATP6V1G1, ATP6V1G2, ATP5F1, COX7C
Protein Ubiquitination Pathway	2.5000	B2M, UBB, PSMA6, PSMA3, PSMA7, UBE2N, UBE2D2, BIRC6, THOP1, USP1, PSMB6, SKP1, HSPA8, USP31, PSMC1, PSMD10, PSMA5, HSP90AA1, PSMD1, BTRC, UBE2D3, HLA-C, ANAPC1
Interferon Signaling	2.3300	IFIT3, JAK1, IFIT1L, PIAS1, IFNA7, IFITM1
Mitochondrial Dysfunction	1.6300	COX7B, COX17, COX6B1, COX6A1, COX6C, NDUFB5, COX5B, UQCRB, NDUFA13, NDUFA6, COX2, NDUFB6, SNCA, COX7C
Complement System	1.5600	C1R, SERPING1, ABPZ, C1S, C4A, CR1
Hypoxia Signaling in the Cardiovascular System	1.3500	UBB, JUN, EDN1, UBE2N, UBE2D2, BIRC6, HSP90AA1, UBE2D3, SUMO1
Antigen Presentation Pathway	1.2400	B2M, HLA-DRA, PSMB6, HLA-C

B



Supplement to Figure 5

(A) Table showing results of ingenuity pathway analysis for gene profiling of WAT from control and *Sfrp5*^{Q27stop} mice on a HFD. **(B)** Increased mitochondrial OXPHOS complex genes in *Sfrp5*^{Q27stop} WAT under HFD. NADH dehydrogenase subunit 1 (*Nadh1*), NADH dehydrogenase subunit 2 (*Nadh2*), NADH dehydrogenase subunit 5 (*Nadh5*) and ATP synthase FO subunit 6 (*Atp6*) mRNA in eWAT from control or *Sfrp5*^{Q27stop} mice under HFD for 12 weeks were measured by real-time RT-PCR; these were from the same sample set as used for gene profiling. Values normalized to *Hprt* mRNA are expressed relative to control mice (n = 6).



Supplement to Figure 6

Sfrp5 regulates mitochondrial function**.** (**A**) Mitochondria from *Sfrp5*^{Q27stop} adipocytes have higher maximal OCR. Isolated mitochondria (5 μg) from control or *Sfrp5*^{Q27stop} EMSC adipocytes were seeded to XF24 Capture Microplates. Succinate (2.5 mM) + Rotenone (1 μM) were used to assay for complex-II-dependent respiration (left panel). Malate (2.5 mM) plus pyruvate (2.5 mM) were used to assay complex-I-dependent respiration (right panel). Data show basal OCR and OCR after serial injection of ADP (1 mM), Oligomycin (2 μM), FCCP (4 μM) and Antimycin A (1.5 μg/mL). (**B**) Mitochondria from *Sfrp5*^{Q27stop} gWAT show higher maximal OCR. Mitochondria were isolated from gWAT of control or *Sfrp5*^{Q27stop} mice under HFD for 12 weeks. Mitochondria were then seeded to XF24 Capture Microplates and assayed as described in (A).