SUPPLEMENTARY FIGURE LEGENDS

Figure S1. PRDM16 Deletion in Adipocytes Results in Altered Gene Expression (Related to Figure 1).

(A) and (B) qPCR analysis of *Ucp1* mRNA from multiple adipose tissues from 8-10 week old male wild-type mice (n=9), normalized to mRNA expression in brown adipose tissue (A) or from *in vitro* differentiated primary adipocytes, normalized to mRNA expression in inguinal cells (n=3) (B).

(C) Targeting construct for PRDM16.

(D) Metabolic characterization of male Adiponectin-Cre^{+} and wild-type control mice, n=7 per group.

(E) Normalized gene expression of thermogenic, brown adipose, and mitochondrial genes in visceral adipose tissue (F) from Adipo-PRDM16 KO and control mice at ambient temperature. Mice were males, 6-8 weeks old, n=5 per group.

Data are presented as mean ± SEM.

Figure S2. PRDM16 Regulates Thermogenic Gene Expression *in Vivo* and *in Vitro* (Related to Figure 2).

(A) and (B) Normalized thermogenic gene expression in brown adipose tissue and subcutaneous adipose tissue from Adipo-PRDM16 KO and control mice either at room temperature or following 6 days at 4°C. Cold exposure was done with male 6-8 week old mice, n=4 per group.

(C) Normalized thermogenic gene expression in visceral adipose tissue from Adipo-PRDM16 KO and control mice either untreated or following five daily injections of CL 316,243 at a dose of 1 mg/kg. CL treatment was done with female 6-8 week old mice, n=5-6 per group.

Data are presented as mean ± SEM. *, $p < 0.05$ knockout vs. control cold exposed or CL 316,243 treated. #, $p < 0.05$ knockout vs. control room temperature or untreated.

(D) Core body temperature over a time course of acute cold exposure at 4°C in control and Adipo-PRDM16 KO mice. 6-8 week old male mice were used, n=3-4 per group.

(E) Gene expression in subcutaneous adipocytes from control and Adipo-PRDM16 KO mice, differentiated *in vitro*. *, $p < 0.05$ knockout vs. control.

(F) and (G) Subcutaneous adipocytes from control and Adipo-PRDM16 KO mice, differentiated *in vitro* and treated with isoproterenol (C) or FGF21 (D). #, $p < 0.05$ control vs. knockout untreated. *, $p < 0.05$ control vs. knockout treated. N=3 per group.

Data are presented as mean ± SEM.

Figure S3. Metabolic Analysis in Adipo-PRDM16 KO Mice (Related to Figure 3).

(A) Food intake (g/24 hr)

(B) Activity in day and night

(C) Oxygen consumption

(D) Respiratory exchange ratio (RER)

Data are from 10-12 week old, male control and Adipo-PRDM16 KO and mice.

Data are presented as mean ± SEM. *, $p < 0.05$.

Figure S4. Metabolic Analysis in Adipo-PRDM16 KO Mice at Thermoneutrality or on High Fat Diet (Related to Figure 4).

(A) and (B) Weight curves of control and Adipo-PRDM16 KO mice at thermoneutrality and fed either a standard chow (A) or high fat diet (B). Experiments were done with male mice, 6-8 weeks old at the start of the experiment. N=4-6 per group.

(C) Food intake (g/24 hr)

(D) Activity in day and night

(E) Oxygen consumption

(F) Respiratory exchange ratio (RER)

Data in C-F are from control and Adipo-PRDM16 KO and mice after 2 weeks on high fat diet. The experiment was done with male mice, 8 weeks old at the start of the experiment, N=8 per group.

Data are presented as mean ± SEM. *, $p < 0.05$.

Figure S5. Altered cell size and immune cell infiltration in subcutaneous adipose tissue in Adipo-PRDM16 KO mice (Related to Figure 5).

(A) Expression levels of adipocyte differentiation genes in subcutaneous adipose tissue from male control and Adipo-PRDM16 KO mice after 18 weeks on high fat diet. N=11 per group.

(B) Cell size in epididymal visceral adipose tissue from Adipo-PRDM16 KO and control mice following 18 weeks on high fat diet. Representative images from hematoxylin and eosin (H&E) strained sections. Images are shown at 5x magnification.

(C) $CD11b^{+}F4/80^{+}$ macrophages in visceral fat and spleen from high fat fed control and Adipo-PRDM16 KO mice.

(D) $Foxp3^{+}$ T regulatory cells in subcutaneous and visceral fat and spleen from high fat fed control and Adipo-PRDM16 KO mice.

For (C) and (D), experiment was done with male mice 8-10 weeks old at start of high fat feeding, n=5-6 per group.

Figure S6. Hyperinsulinemic-euglycemic clamp studies in Adipo-PRDM16 KO mice (Related to Figure 6).

(A) Fasting and clamped glucose values.

(B) Glucose infusion rate.

(C) Fasting and clamped insulin levels.

(D) mRNA expression levels in brown adipose tissue and subcutaneous adipose tissue.

The clamp studies were done with male mice, 8 weeks old at the start of high fat feeding. N=7-8 per group.

(E) Total adiponectin levels by ELISA. Levels measured in make mice after 6 weeks on high fat diet, n=10 per group.

For (A)-(E), data are presented as mean ± SEM. *, $p< 0.05$. #, 0.06 for *Pgc1a* and 0.10 for *Ucp1*.

(F) Western blot for total and phosphorylated Akt in primary subcutaneous adipocytes

Data are from control and Adipo-PRDM16 KO primary subcutaneous adipocytes.

(G) Intraperitoneal glucose tolerance test (1.5 mg/kg) in high fat fed recipients of Adipo-PRDM16 KO, control, or PRDM16 transgenic subcutaneous adipose tissue. The GTT was done 6 weeks following transplantation. N=5 per group.

Data are presented as mean ± SEM. *, $p < 0.05$ by ANOVA.

Figure S7. Cell Autonomous Induction of a Visceral Adipose Tissue Gene Expression Profile in Adipo-PRDM16 KO Subcutaneous Adipocytes (Related to Figure 7).

(A) Normalized expression of pro-inflammatory genes and (B) transcription factors in visceral adipose tissue from high fat fed control and Adipo-PRDM16 KO mice. Experiments were done in male mice after 18 weeks on high fat diet. N=11 per group.

(C) Normalized expression of pro-inflammatory genes and (D) transcription factors in primary subcutaneous adipocytes differentiated *in vitro* from control and Adipo-PRDM16 KO mice. N=3 per group.

(E) Differentiation time course showing PRDM16 and Wt1 mRNA levels in primary subcutaneous adipocytes from control and Adipo-PRDM16 KO mice. *, $p<0.05$ vs. day 4 mRNA expression level.

Data are presented as mean ± SEM. *, $p < 0.05$. # in (C), $p=0.057$.

EXTENDED EXPERIMENTAL PROCEDURES

Animals

To generate *PRDM16*$^{lox/lox}$ mice, BAC recombineering was used to retrieve ~16 kb of genomic sequence encompassing exon 9 of *Prdm16* from a 129Sv BAC clone (RPCIB731F20213Q from 129s7/AB2.2 BAC library) into plasmid PL253 (Adams et al., 2005; Liu et al., 2003). A floxed *PGK-Neo* cassette from PL452 was integrated ~280 bp upstream of exon 9, followed by Cre mediated excision of *PGK-Neo* in bacteria, leaving a single *LoxP* site upstream of exon 9. A cassette containing *FRT-PGK-Neo-FRT-LoxP* was integrated 300 bp downstream of Exon 9. The resulting targeting construct (PL253-Prdm16Ex9Neo) was electroporated into 129Sv ES cells (courtesy of Dr. Brad Lowell). ES clones were screened for homologous recombination by Southern blotting using probes 5' and 3' of the targeting area. Positive clones were injected into C57Bl6 blastocysts to derive chimeric mice. Germline transmission of the targeted allele was confirmed by Southern blotting. The *PGK-Neo* selection cassette was excised by breeding with *Rosa26-Flp* deleter mice (Jackson Laboratory). *PRDM16*$^{lox/lox}$ mice were backcrossed to C57Bl6 mice for at least 8 generations. *PRDM16*$^{lox/lox}$ mice were then crossed to either *Zp3-cre* (Jackson Laboratory) or *Adiponectin-cre* mice (provided by Dr. Evan Rosen). Wt1$^{lox/lox}$ mice were provided by Dr. Vicki Huff.

Molecular Studies

Total RNA from cultured cells or tissues was isolated using TRIzol (Invitrogen) along with QIAGEN RNeasy mini kits. For qPCR analysis, RNA was reverse transcribed using the ABI high capacity cDNA synthesis kit. cDNA was used in qPCR reactions containing SYBR-green fluorescent dye (ABI). Relative mRNA expression was determined by

normalization with TBP levels using the ΔΔ Ct method. Primer sequences are available on request. For Western blotting, nuclear extracts or whole cell lysates were prepared, separated by SDS-PAGE, and transferred to ImmobilonP membranes (Millipore).

Immunohistochemistry

For UCP1 immunohistochemistry, slides were deparaffinized in xylene, hydrated in 95%, 80% and 70% ethanol, and rinsed in water before heat-mediated antigen retrieval in 10mM pH 6.0 sodium citrate buffer. Quenching of endogenous peroxidases was performed using peroxidase quenching solution (Invitrogen). Slides were blocked in 10% goat serum and incubated with rabbit polyclonal UCP1 antibody (Abcam, ab10983) at 2 μg/ml in PBS-T/1% BSA overnight at 4°C. Slides were washed in PBS-T and incubated with 1:500 donkey anti-rabbit IgG HRP-linked antibody (GE healthcare) before developing using SuperPicture 3rd Gen IHC Detection Kit (Invitrogen). Hematoxylin was used as counterstain. Crown-like structures were detected by immunohistochemistry using mouse monoclonal anti-MAC2 (Cederlane Labs) as described (Giordano et al., 2013).

Metabolic Phenotyping

Mice were maintained on standard chow or 60% HFD (Research Diets) with 12 hour light cycles. Energy expenditure was analyzed using a Comprehensive Lab Animal Monitoring System (Columbus Instruments). Mice were acclimated for at least 24 hours before measurements were taken. Hyperinsulinemic euglycemic clamps were performed as described (Jurczak et al., 2012). After an overnight fast, mice were infused for 120 min with 3-^{3}H-glucose to determine basal glucose turnover. Next, mice were given a primed/constant infusion of insulin [(3mU/kg-min)] and 3-^{3}H-glucose (0.1 μCi/min) and

variable infusion of 20% dextrose to maintain euglycemia. A bolus injection of ^{14}C-2-deoxyglucose was given during the last 50 min to assess tissue-specific glucose uptake. Plasma samples were collected by tail bleed at set time points to determine plasma glucose, tracer, insulin and fatty acid concentrations. Calculations of glucose turnover were made at steady state during the last 40 min of the clamp.

References

Adams, D.J., Quail, M.A., Cox, T., van der Weyden, L., Gorick, B.D., Su, Q., Chan, W.I., Davies, R., Bonfield, J.K., Law, F., *et al.* (2005). A genome-wide, end-sequenced 129Sv BAC library resource for targeting vector construction. Genom. *86*, 753-758.

Giordano, A., Murano, I., Mondini, E., Perugini, J., Smorlesi, A., Severi, I., Barazzoni, R., Scherer, P.E., and Cinti, S. (2013). Obese adipocytes show ultrastructural features of stressed cells and die of pyroptosis. J. Lipid Res. *54*, 2423-2436.

Jurczak, M.J., Lee, A.H., Jornayvaz, F.R., Lee, H.Y., Birkenfeld, A.L., Guigni, B.A., Kahn, M., Samuel, V.T., Glimcher, L.H., and Shulman, G.I. (2012). Dissociation of inositol-requiring enzyme (IRE1alpha)-mediated c-Jun N-terminal kinase activation from hepatic insulin resistance in conditional X-box-binding protein-1 (XBP1) knock-out mice. J. Biol. Chem. *287*, 2558-2567.

Liu, P., Jenkins, N.A., and Copeland, N.G. (2003). A highly efficient recombineering-based method for generating conditional knockout mutations. Genome Res. *13*, 476-484.