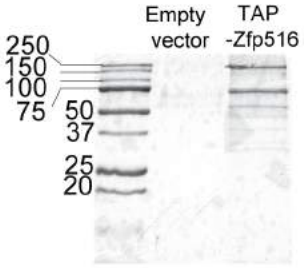
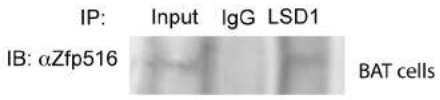
Figure S1

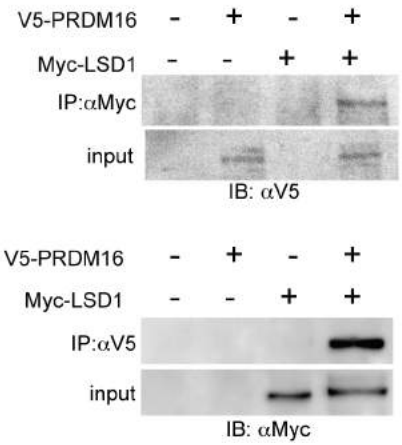
A



B



C



D

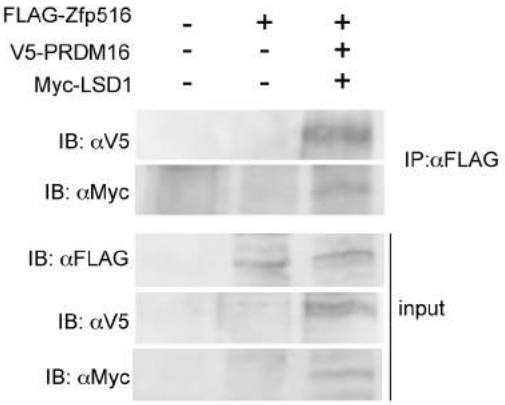
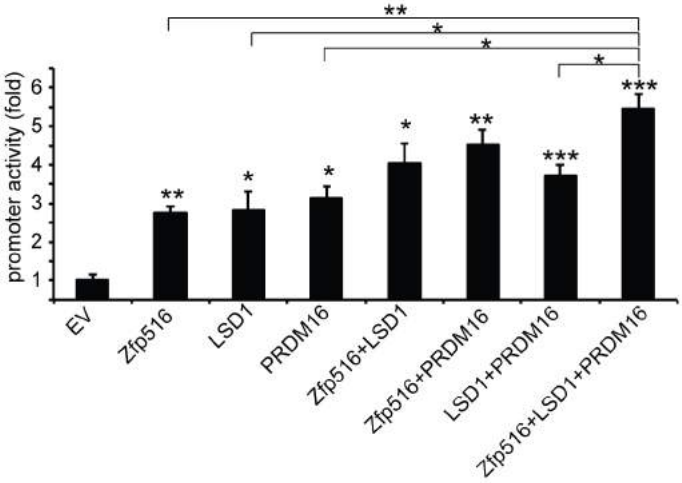


Figure S1. LSD1 interacts with Zfp516, **related to Figure 1.** A. Identification of Zfp516 interacting partners by TAP purification. SDS-PAGE of purified Zfp516 after Coomassie staining, control empty vector (left lane) and TAP-Zfp516 (right lane). TAP was performed as described previously (Griffin et al., 2007). B. Immunoblot for endogenous Zfp516 after immunoprecipitation of lysates from differentiated BAT cells using αLSD1. C Immunoblot using αV5 (Top) or αMyc (bottom) after immunoprecipitation with either αMyc or αV5, respectively, of 293FT cell lysates transfected with V5-PRDM16 and c-Myc-LSD1, either together or individually. D. Immunoblotting using αV5 (Top) or αMyc (center) after immunoprecipitation with αFLAG of 293FT cell lysates transfected with FLAG-Zfp516 alone, or in combination with Myc-LSD1 and V5-PRDM16. Immunoblots of input for the corresponding lysate are shown (bottom).

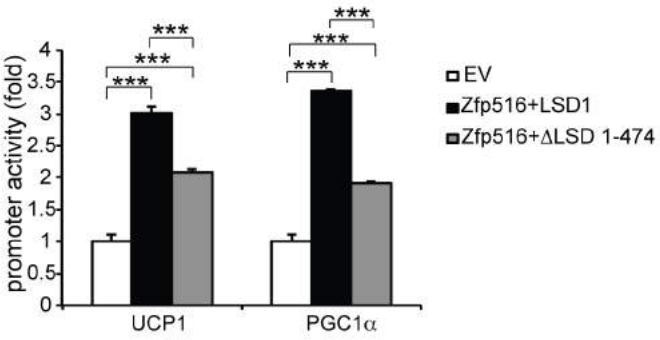
We previously reported that Zfp516 interacts with PRDM16 (Dempersmier et al., 2015). Here, we tested if there is an interaction between LSD1 and PRDM16. 293FT cells were transfected with either FLAG-Zfp516 alone or together with LSD1 and PRDM16 and the cell lysates were immunoprecipitated using FLAG antibody (Figure S1 D). We detected that Zfp516 coimmunoprecipitated with both PRDM16 and LSD1. We next transfected 293FT cells with either Myc-LSD1 or V5-PRDM16 alone, or together (Figure S1 C). Immunoprecipitation with Myc and V5 antibodies detected an interaction between PRDM16 and LSD1 when coexpressed. Overall, these results suggest that, by interacting with Zfp516, PRDM16 and LSD1 are both recruited to the promoter region of UCP1 or other BAT-enriched genes.

Figure S2

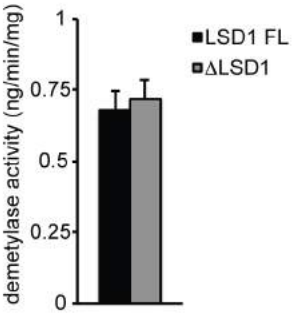
A



B



C



D

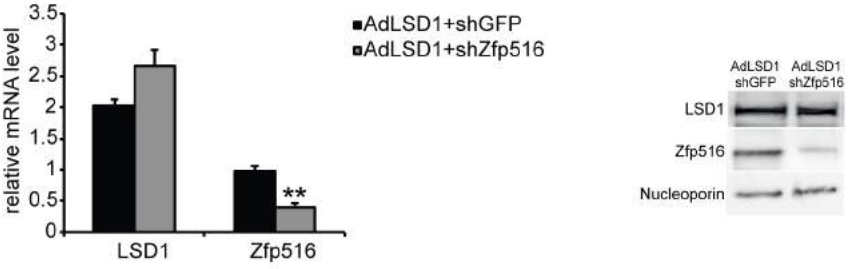
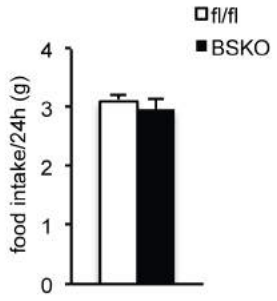


Figure S2. LSD1 activates UCP1 promoter by interacting with Zfp516, r**elated to Figure 2.** A. Luciferase activity in 293FT cells cotransfected with the -5.5 kb UCP1 promoter and empty vector (EV), along with Zfp516, LSD1 or PRDM16 expression vectors either together or individually. B. Luciferase activity in 293FT cells cotransfected with the -5.5 kb UCP1 promoter (left panel) or -2.4 kb PGC1α (right panel) and empty vector (EV), Zfp516 and either LSD1 full length (FL) or deleted ΔLSD1 1-474. C. Histone demethylase activity of LSD1 full length (FL) and deleted ΔLSD1 1-474 constructs measured using 10mg of nuclear extracts from transfected 293FT cells. D. RT-qPCR for LSD1 and Zfp516 (left) and immunoblots (right) for indicated proteins in lysates from BAT cells. Data are presented as mean ± SEM. *p<0.05; **p<0.01; ***p<0.001.

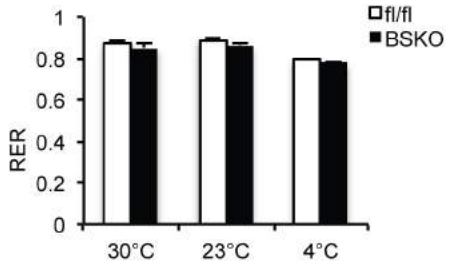
In C, enzymatic activity of LSD1 FL and Δ LSD1 1-474 was determined in 10μg of nuclear extracts of 293FT cells transfected with either LSD1 FL or Δ LSD1 1-474 using the Epigenase^{TM} LSD1 Demethylase Activity/Inhibition Assay kit (Epigentek) according to manufacturer protocol. We did not observe any significant difference in demethylase activity between LSD1 FL and Δ1-474.

Figure S3

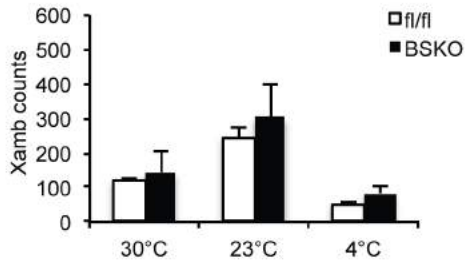
A



B



C



D

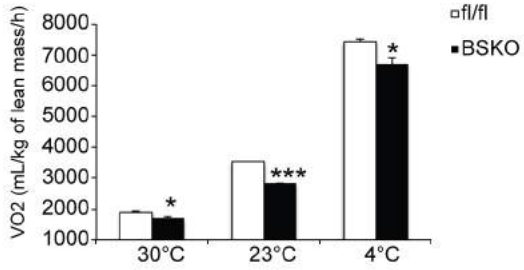


Figure S3: **LSD1 promotes a BAT gene program *in vivo,* related to Figure 5.** A. Average food intake of control (fl/fl) and BSKO mice on normal chow diet (NCD) at 10-14 wks of age. B. Resting energy expenditure rate of control and BSKO mice on NCD at 14 wks of age. C. Physical activity measured by indirect calorimetry in control and BSKO mice. D. VO_2 measured by indirect calorimetry in control and LSD1 BSKO mice on NCD at 30°C, 23°C and 4°C. (n=7 mice per group). Data are presented as mean ± SEM. *p<0.05; **p<0.01; ***p<0.001.

Figure S4

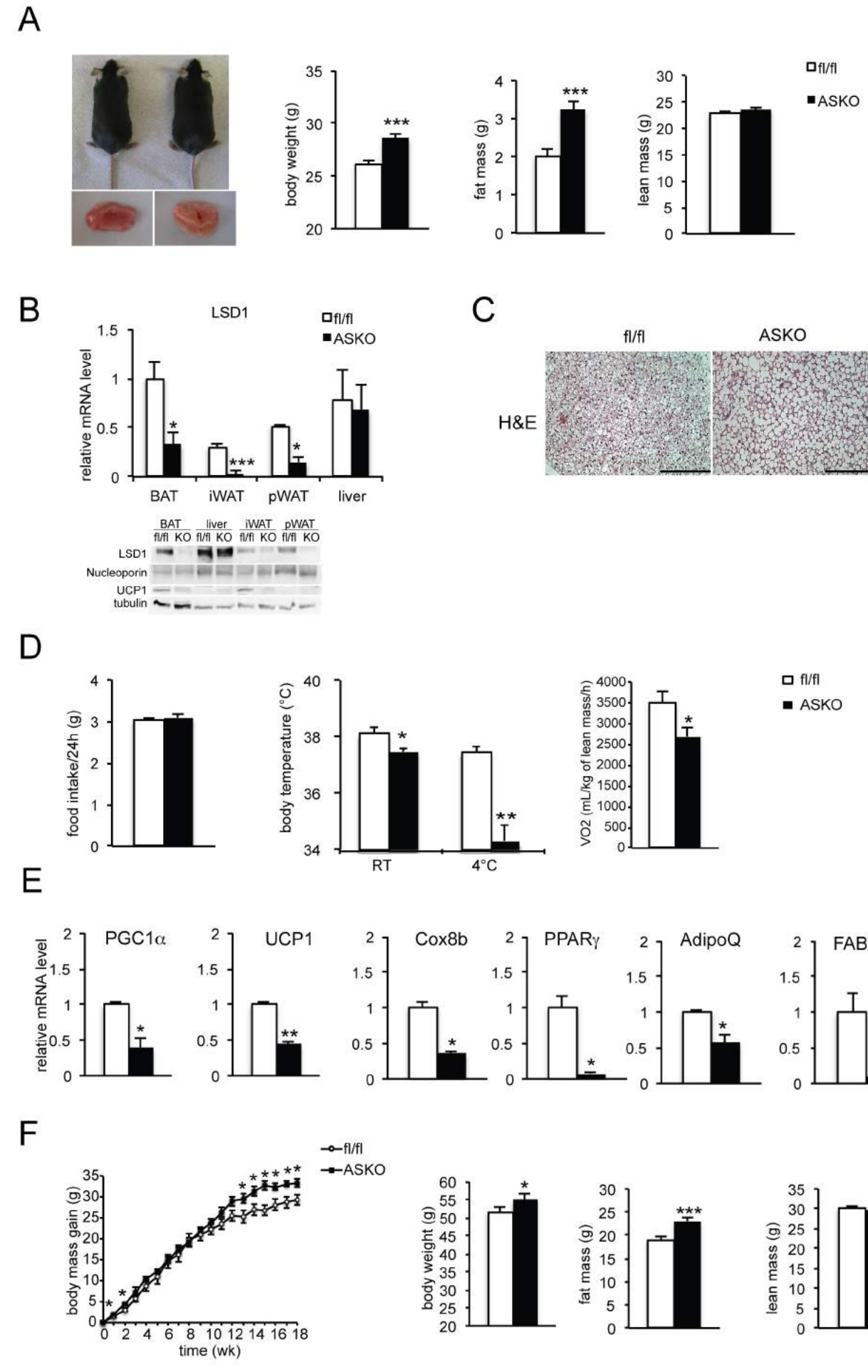

A
body weight (g)
fat mass (g)
lean mass (g)
fl/fl
ASKO
B
LSD1
relative mRNA level
BAT
iWAT
pWAT
liver
Nucleoporin
UCP1
tubulin
C
fl/fl
ASKO
H&E
D
food intake/24h (g)
body temperature (°C)
RT
4°C
VO2 (mL/kg of lean mass/h)
E
PGC1α
UCP1
Cox8b
PPARγ
AdipoQ
FABP4
relative mRNA level
F
body mass gain (g)
time (wk)
body weight (g)
fat mass (g)
lean mass (g)

Figure S4: **LSD1 promotes a BAT gene program in iWAT *in vivo*, related to Figure 5 and 6.** A. Left, representative photograph of 12 wk-old control and LSD1 ASKO mice and their BAT depots. Center, body weight of 14 wk-old control and LSD1 ASKO mice. Right, fat and lean mass of 14 wk-old control and LSD1 ASKO mice determined by EchoMRI. B. Top, RT-qPCR for LSD1 mRNA in adipose tissue depots and liver of control and LSD1 ASKO mice. Bottom, immunoblot for indicated proteins in lysates from BAT, liver, iWAT and pWAT of control and LSD1 ASKO mice. C. H&E staining of BAT from control and LSD1 ASKO mice. Scale bars represent 200μm. D. Left, food intake (g /24 h) of control and LSD1 ASKO mice. Center, body temperature from control and LSD1-BSKO mice at 23°C or 4°C after 6h exposure. Right, VO_2 measured by indirect calorimetry in control and LSD1-ASKO mice on NCD at room temperature (23°C) (n=7 mice per group). E. RT-qPCR for BAT-enriched or common adipose genes in BAT of control and LSD1-ASKO mice. F. Left, body mass gain (g) of control and ASKO mice under HFD feeding starting at 6 wks of age (n=6-8 mice per group). Right, body weight, fat and lean mass determined by EchoMRI of control and LSD1 ASKO mice after 10 wk of HFD (n=6-8 mice per group). Data are presented as mean ± SEM. *p<0.05; **p<0.01; ***p<0.001.

To evaluate the role of LSD1 in BAT function, we generated BAT specific KO mice (BSKO) using UCP1-Cre to exclude any potential compensatory or masking effect that could occur when LSD1 was deleted in both BAT and WAT. We also generated a LSD1 conditional KO mouse model in which Cre recombinase was under the control of Adiponectin (AdipoQ) promoter (ASKO mice) leading to LSD1 knockout in all adipose depots, BAT, iWAT and pWAT. Unlike previously characterized ASKO mice using aP2 promoter which were shown to be devoid of WAT (Duteil et al., 2014), our ASKO mice exhibited increased WAT mass. Our results indicate that a tissue-specific invalidation of LSD1 in adipose tissue causes mice to gain more weight with an increased fat mass associated with an impaired BAT morphology. This impaired energy balance is associated with decreased thermogenic capacity of BAT as shown by reduced body temperature and VO2 consumption. Expression of BAT-enriched genes is decreased as well in BAT of ASKO mice compared to their control littermates. Finally, ASKO mice are more sensitive to HFD-induced obesity accumulating more fat mass than control mice. Collectively, we observed in ASKO mice a phenotype very similar to the one in BSKO mice corroborating the essential role of LSD1 for BAT formation and function.

Figure S5

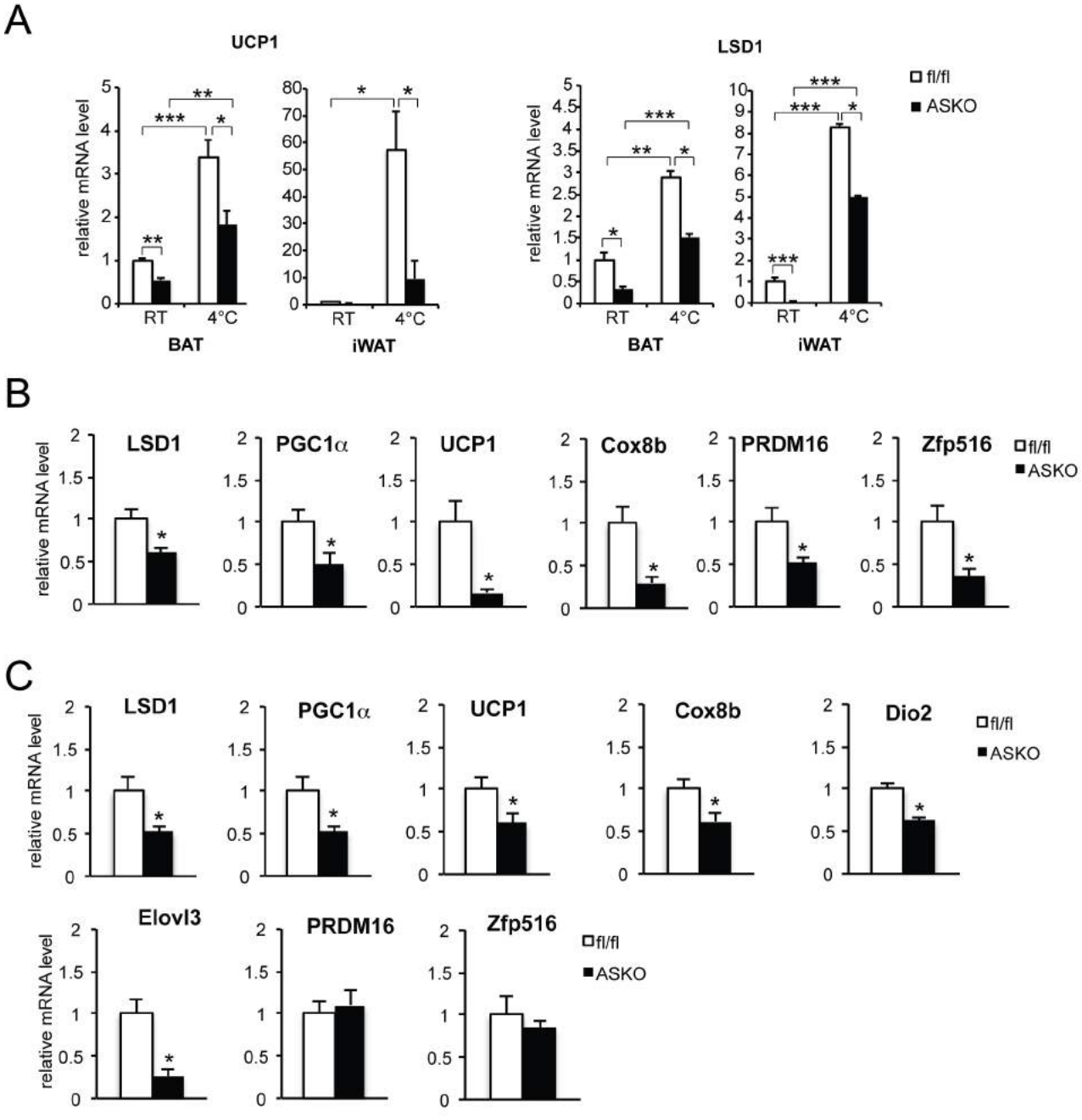
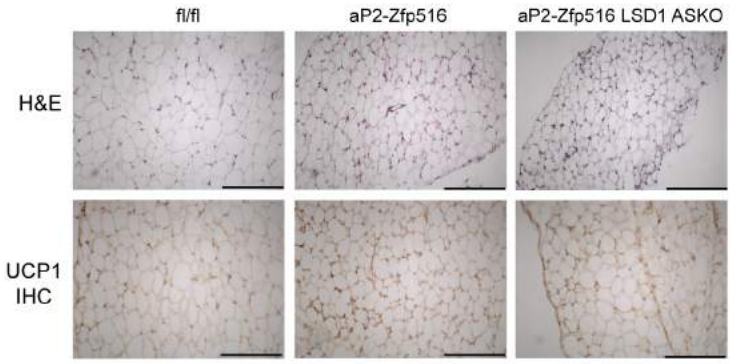

A
UCP1
LSD1
relative mRNA level
fl/fl
ASKO
RT
4°C
BAT
iWAT
B
LSD1
PGC1α
UCP1
Cox8b
PRDM16
Zfp516
C
Dio2
Elovl3

Figure S5: **LSD1 is required for browning of iWAT upon cold exposure, related to Figure 6.**
A. RT-qPCR for UCP1 and LSD1 mRNA in BAT and iWAT of control (fl/fl) and ASKO mice at 23°C (RT) and after a 6h cold exposure at 4°C (n=8 mice per group). B. RT-qPCR for BAT-enriched genes in iWAT of control and LSD1 ASKO mice after a 6h cold exposure at 4°C (n=8 mice per group). C. RT-qPCR for BAT-enriched genes in BAT of control and LSD1 ASKO mice after a 6h cold exposure at 4°C (n=8 mice per group). D. Immunoblot for indicated proteins in lysates from iWAT and liver from fl/fl, aP2-Zfp516, and aP2-Zfp516 ASKO mice. Data are presented as mean ± SEM. *p<0.05; **p<0.01; ***p<0.001.

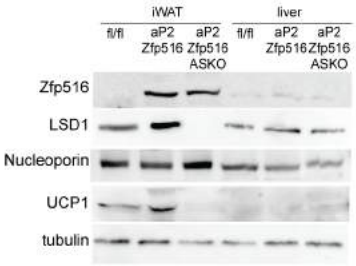
We observed that, when subjected to an acute cold exposure of 6h at 4°C, BSKO (Figure 5D) and ASKO (Figure S5 D) mice have a body temperature 4-5°C lower than control littermates indicating reduced thermogenic capacity in LSD1 KO mice. Here, we determined that upon cold exposure, UCP1 expression is still significantly inducible in BAT but not in iWAT (Figure S5 A left). Regardless, UCP1 level is reduced in cold-exposed ASKO mice by 50% and 75% in BAT and iWAT respectively. In line with these results, we checked that, albeit inducible, LSD1 expression is substantially reduced by 50-45% in BAT and iWAT of cold-exposed ASKO mice (Figure S5 A right). Subsequently, we showed a significant decrease of BAT-enriched gene expression such as PGC1α, and Cox8b in iWAT (B) and BAT (C) of ASKO mice after 6h cold exposure at 4°C. Taken together, these data indicate that LSD1 KO impairs thermogenic capacity in BAT and iWAT resulting in decreased cold-resistance.

Figure S6

A



B



C

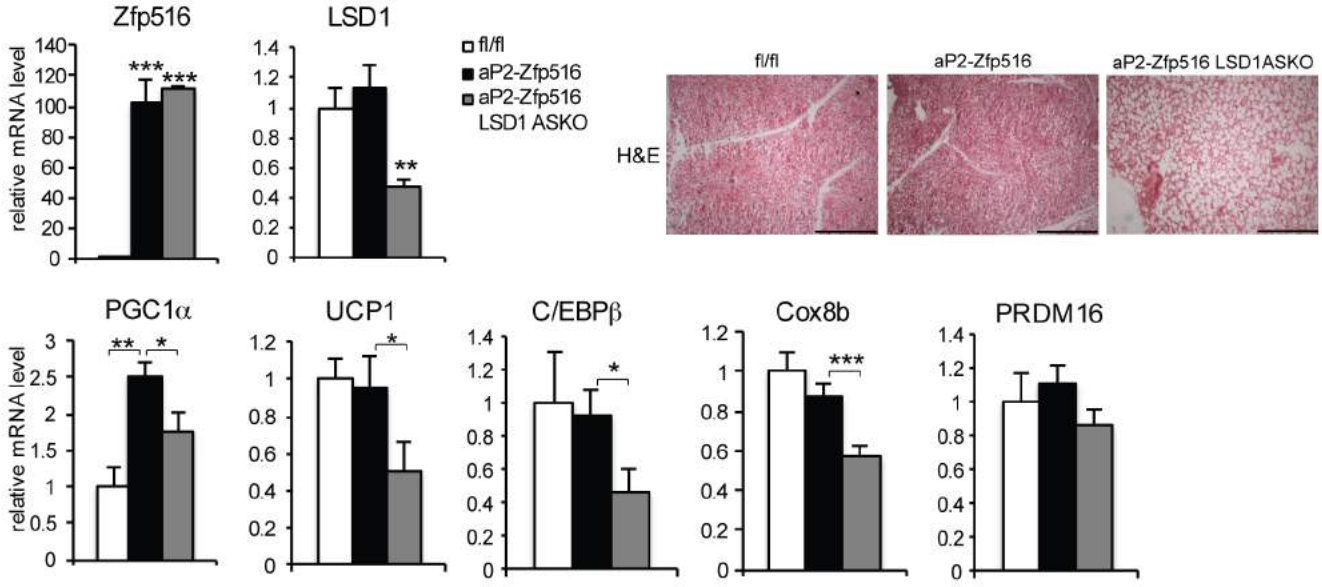


Figure S6: **LSD1 is required for Zfp516-induced browning of iWAT and BAT program in aP2-Zfp516 mice, related to Figure 6.**

A. H&E staining (upper panels) and immunostaining for UCP1 (lower panels) in sections of pWAT from 8 wk-old control (fl/fl), aP2-Zfp516 and aP2-Zfp516 ASKO mice. B. Immunoblot for indicated proteins in lysates from iWAT and liver from fl/fl, aP2-Zfp516, and aP2-Zfp516 ASKO mice. C. Top-Left, RT-qPCR for LSD1 and Zfp516 mRNA in BAT from mice described above. Top-Right, H&E staining from sections of BAT. Bottom, RT-qPCR for BAT-enriched genes in BAT. (n=6-8 mice per group). Scale bars represent 200 μm. Data are represented as mean ± SEM.*p<0.05; **p<0.01; ***p<0.001.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Plasmid constructs and antibodies

The following antibodies were used: Anti-Flag M2 and Anti-c-Myc-Peroxidase antibodies and affinity gels (Sigma), Anti-V5 (Invitrogen); Anti-UCP1, Anti-Zfp516, Anti-KDM1/LSD1 and IgG mouse and rabbit (Sigma); Anti-KDM1/LSD1 (abcam, ab17721), Anti-Histone H3K9-2me (ab1220), Anti-Histone H3K9-1me (ab9045), Anti-Histone H3K9-3me (ab8898), Anti-Histone H3K4-2me (ab32356), and Anti-tubulin (Abcam); anti-Znf516 [p-12 sc85244x], Anti-PGC1α, and Anti-PPARγ (Santa Cruz); Anti-Nucleoporin p62 (BD). The cMyc-LSD1 expression vector was from obtained from Sinobiological. The FLAG-Zfp516 expression vector, the -5.5kb UCP1-Luc, -70bp UCP1-Luc, -45bp UCP1-Luc and -2.4bp PGC1α-Luc were previously described (Dempersmier et al., 2015).

Animals

Mice carrying floxed LSD1 alleles (KDM1a $^{fl/fl}$), UCP1-Cre, Adiponectin-Cre (AQ-Cre), Myf5-Cre transgenic and Swiss SWR/J mice were obtained from The Jackson Laboratories. KDM1a $^{fl/fl}$ were crossed with either UCP1-Cre, Myf5-Cre or AQ-Cre mice and the progeny was intercrossed to generate KDM1a $^{fl/fl}$ mice carrying Cre transgene, that were used as conditional BAT specific LSD1 KO (BSKO) and LSD1 Myf5KO, and conditional adipose specific LSD1 KO (ASKO) mice respectively. KDM1a $^{fl/fl}$ mice (fl/fl) were used as control. aP2-Zfp516 transgenic mice were described previously (Dempersmier et al., 2015). All protocols for mice studies were approved from the University of California at Berkeley Animal Care and Use Committee. Mice were fed a chow diet or a high fat diet (45% calorie from fat, Research Diet) ad libitum. Body weight and food intake were measured weekly.

Cell culture and viral transduction

Brown adipocyte cell line (BAT cells) was obtained from Prof. S Kajimura (UCSF), 3T3-L1 were obtained from ATCC. Brown adipocyte differentiation was induced by treating confluent cells in DMEM containing 10% FBS, 850nM insulin, 0.5mM isobutyl-methylxanthine, 1μM dexamethasone, 1nM T3, 125nM indomethacin and 1μM rosiglitazone. After 48h of induction, cells were switched to a maintenance medium containing 10% FBS, 850nM insulin, 1nM T3 and 1μM rosiglitazone. Adenoviruses scrambled (shscr) Ad-U6-RNAi-GFP; Ad-U6-mKDM1-shRNA: Ad-GFP-mKDM1 were obtained from Vector Biolabs. shGFP and shZfp516 recombined lentiviruses were purchased from Santa Cruz; AdGFP and AdZfp516 recombined adenoviruses were obtained from Viraquest. The following inhibitors were used throughout differentiation: LSD1i 489476 (EMD Millipore), 2PCPA (*trans*-2-Phenylcyclopropylamine hydrochloride, Sigma). For Oil red O staining, cells were washed once with phosphate-buffered saline and subsequently fixed for 1h in 10% formalin. Cells were then stained with freshly prepared 0.5% Oil Red O solution for 1h for lipid visualization.

RT-qPCR and Western blotting

Total RNA was extracted using TRIzol reagent (Invitrogen). Reverse transcription was performed with 1μg of total RNA using SuperScript II (Invitrogen). RT-qPCR was performed using SYBR green fluorescent dye with an ABI PRISM 7500 sequence detection system (Applied Biosystems). Statistical analysis of the qPCR was obtained using the $2^{-\Delta\Delta Ct}$ method with U36B4 as the internal control. Experiments were performed in triplicates (biological replicates) for each sample. Primer sets used are listed in Supplemental Table. For western blot analysis, total cell lysates were prepared using Ripa buffer and nuclear/cytosolic extracts were isolated using the NE-PER Nuclear and Cytoplasmic Extraction kit (Thermo). Proteins were separated by SDS-PAGE, transferred to nitrocellulose membrane (Bio-Rad) and probed with the indicated antibodies.

Reporter assays

293FT cells were transfected with 150ng of Zfp516 or empty vector and/or 150ng of LSD1, LSD1 1-474, or empty vector together with 50ng of indicated luciferase reporter construct and 0.5ng of renilla construct pRL-CMV in 48-well plates using Lipofectamin (Invitrogen). Cells were lysed 48 h post-transfection and assayed for luciferase activity using Dual-Luciferase Reporter assay system (Promega). Firefly luciferase reporter gene measurements were normalized to Renilla luciferase activity.

Co-Immunoprecipitation

For CoIP experiments using tagged constructs, 293FT cells were transfected using Lipofectamine (Invitrogen) to express FLAG-tagged Zfp516 and/or Myc-tagged LSD1 or ΔLSD1 1-474 construct. Cells were lysed in IP buffer containing 20 mM Tris, pH 7.4, 150 mM NaCl, 1mM EDTA, 10% glycerol, 1% NP-40 supplemented with proteases inhibitors. Total cell lysates were incubated with indicated antibodies of affinity gel. For CoIP in BAT cells and BAT tissue, nuclear extraction was carried out using the NE-PER Nuclear and Cytoplasmic

Extraction kit (Thermo). Equal amounts of nuclear extracts were incubated with indicated antibodies and protein A/G agarose beads. Bound proteins were eluted and analysed by immunoblotting using the indicated antibodies.

***In vitro* Binding Assays**
GST-fused to various LSD1 fragments were expressed in BL21 by IPTG induction for 3 h at 37°C, purified on glutathione sepharose beads and eluted with elution buffer containing reduced glutathione (Porro et al., 2014). [^{35}S]-labeled Zfp516 protein was produced by using TNT coupled transcription/translation kit (Promega). Twenty µg of GST fusion proteins were incubated overnight at 4°C with *in vitro* translated proteins and glutathione sepharose beads in a binding buffer containing 20mM Hepes, pH 7.7, 300mM KCl, 2.5mM $MgCl_2$, 0.05% NP40, 1mM DTT, and 10% glycerol. The sepharose beads were then washed 3 times with binding buffer. Bound proteins were eluted, separated by SDS-PAGE and analysed by autoradiography.

ChIP and reChip
For ChIP analysis, at 48 h post-transfection, cells were crosslinked for 10 min by adding 1% formaldehyde in DMEM at room temperature. Cross-linking was stopped by the addition of glycine to a final concentration of 0.125 M. After sonication, DNA sizes were 0.3–0.9kb. For BAT, tissues were minced on ice and crosslinked using 1% formaldehyde for 10 min. After stopping crosslinking, dounced samples were resuspended in RSB buffer, containing 10mM Tris pH 7.4, 10mM NaCl, 3mM $MgCl_2$, prior to sonication. Nuclei were collected and were lysed in buffer containing 50mM Tris, pH 8.0, 1% SDS0mM EDTA supplemented with protease inhibitors, followed by sonication. Chromatin samples were diluted 1:10 with the dilution buffer, containing 16.7mM Tris, pH 8.1, 0.01% SDS 1.1 % Triton X-100 1.2mM EDTA and 1.67mM NaCl and proteinase inhibitors. Soluble chromatin was quantified by absorbance at 260nm, and equivalent amounts of input DNA were immunoprecipitated using 2.5µg of indicated antibodies or mouse IgG and protein A/G magnetic beads. After the beads were washed and cross-linking was reversed, DNA fragments were extracted (Thermo) and analyzed by PCR or qPCR using the primer sets in Table 1. ReChIP was performed as in (Truax and Greer, 2012) using 10 µg of chromatin isolated from BAT cells and immunoprecipitation was performed using anti-LSD1 (Abcam), anti-Znf516 (Santa Cruz) or Rabbit IgG (Sigma) as indicated.

SUPPLEMENTAL REFERENCES

Porro, A., Feuerhahn, S., and Lingner, J. (2014). TERRA-reinforced association of LSD1 with MRE11 promotes processing of uncapped telomeres. Cell reports *6*, 765-776.

Truax, A.D., and Greer, S.F. (2012). ChIP and Re-ChIP assays: investigating interactions between regulatory proteins, histone modifications, and the DNA sequences to which they bind. Methods in molecular biology (Clifton, NJ) *809*, 175-188.