SUPPLEMENTARY INFORMATION

FAD-dependent lysine-specific demethylase-1 regulates cellular energy expenditure

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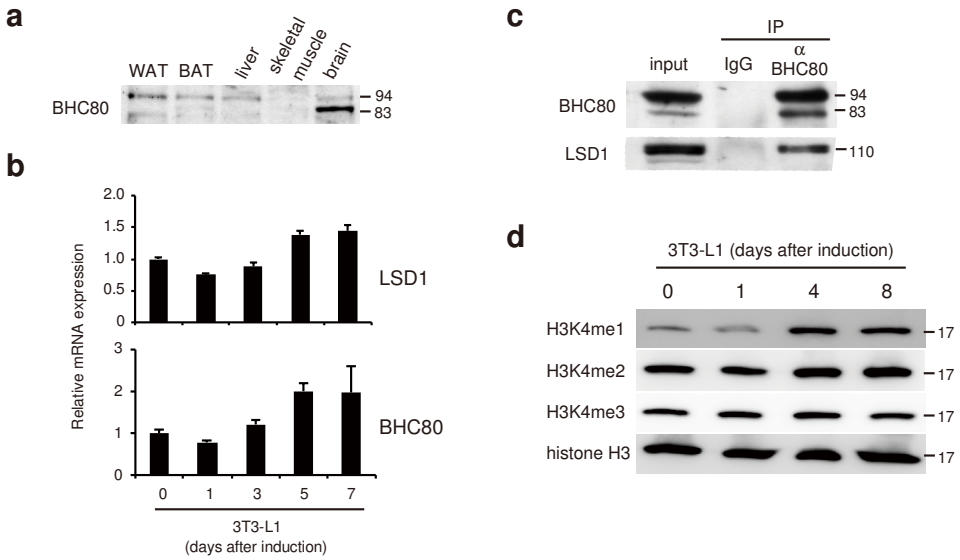
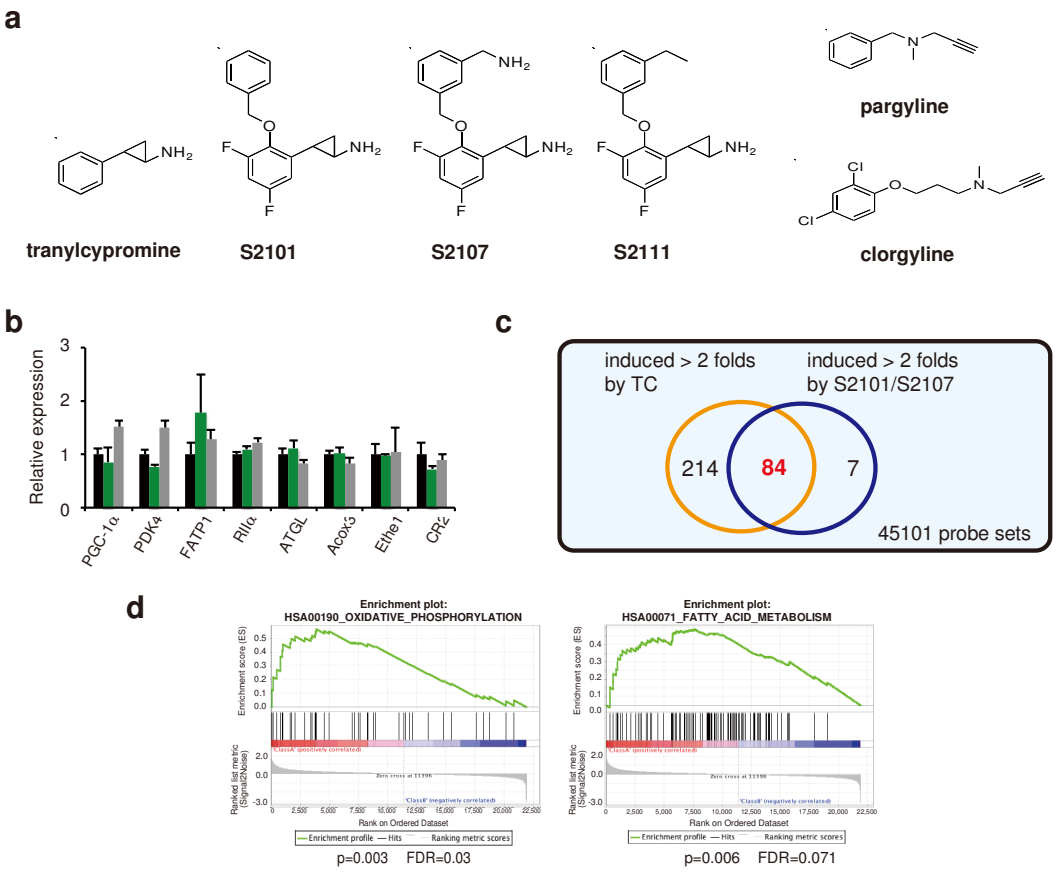
SUPPLEMENTARY FIGURES



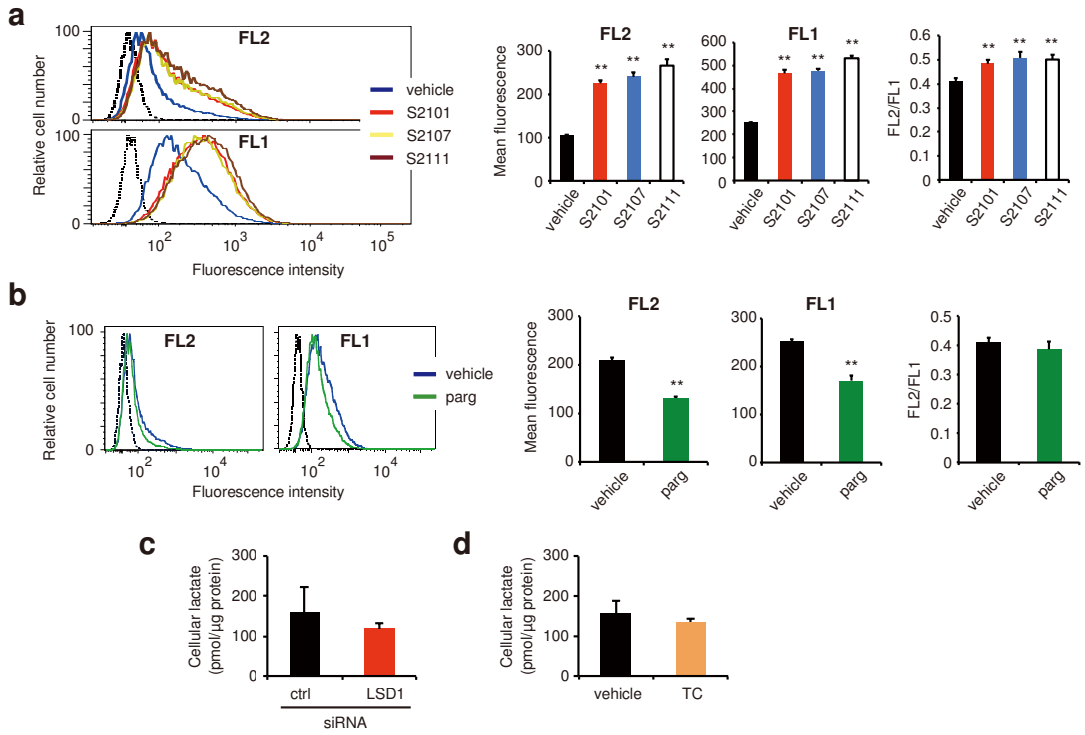
Supplementary Figure S1. Expression of LSD1 and BHC80 in adipose tissues and cells.

(**a**) Expression of BHC80 protein in mouse tissues. Size markers indicate the two splice variants of this protein. The longer form of BHC80 was dominantly expressed in WAT. (**b**) The mRNA expression of LSD1 and BHC80 in adipogenic 3T3-L1 cells. Values are means ± SD of triplicate results. (**c**) Interaction between LSD1 and BHC80 proteins. Co-immunoprecipitation experiment was performed using 3T3-L1 adipocytes. Input lane contains the 10 % of the total amount of whole cell extract relative to IP lanes. (**d**) The time course of histone H3K4 methylation levels during the adipogenesis of 3T3-L1 cells. Acid extracted nuclei were used for the western blot analyses.



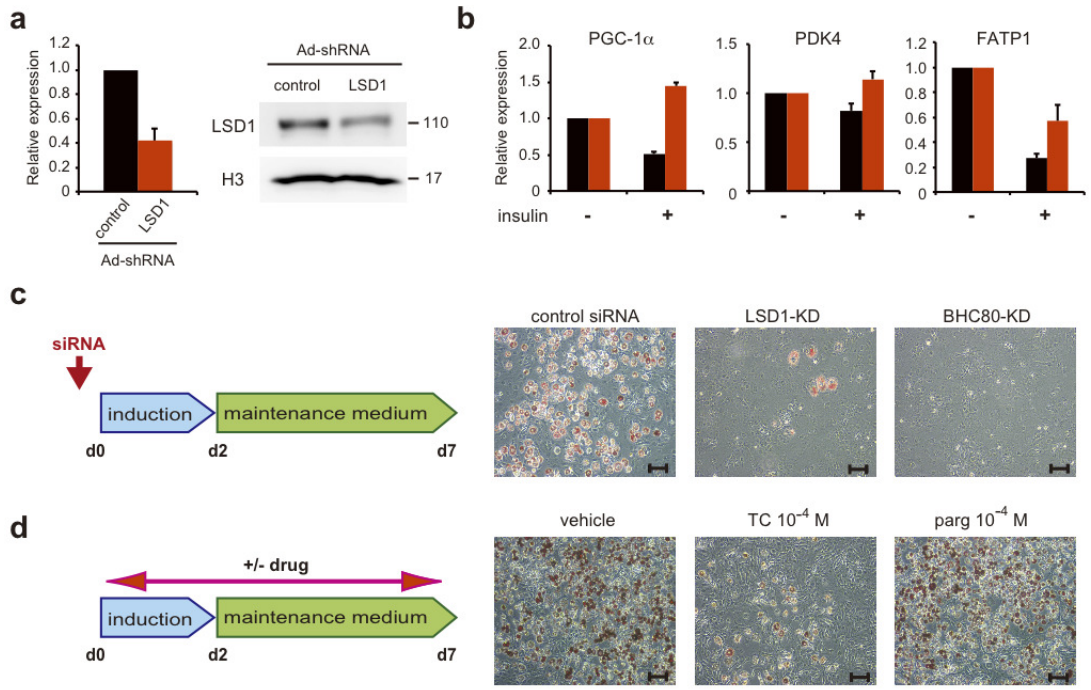
Supplementary Figure S2. Selective LSD1 inhibitors and monoamine oxidase inhibitors.

(**a**) Chemical structures of tranylcypromine (TC; non-selective) and SLIs (S2101, S2107 S2111) are shown. The structures of MAO inhibitors, pargyline (parg) and clorgyline (clorg) are also shown. (**b**) The effects of MAO inhibitors on LSD1-target gene expression in differentiating adipocytes. Parg (green bars) was used at the concentration of 10^{-4} M, and clorg (gray bars) at 10^{-5} M. The relative mRNA expression is presented as the fold difference against vehicle control (black bars). Values are means ± SD of triplicate results. (**c**) Overlapping effect of TC and SLIs on the gene expression profile. Venn diagram shows the probe sets up-regulated by TC or S2101/S2107. S2101/S2107 subgroup is composed of the probe sets, which were up-regulated both by S2101 and S2107. TC was used at the concentration of 10^{-4} M, whereas S2101 and S2107 were at 10^{-5} M. (**d**) Gene Set Enrichment Analysis of commonly up-regulated genes by TC and S2101/S2107 treatments.



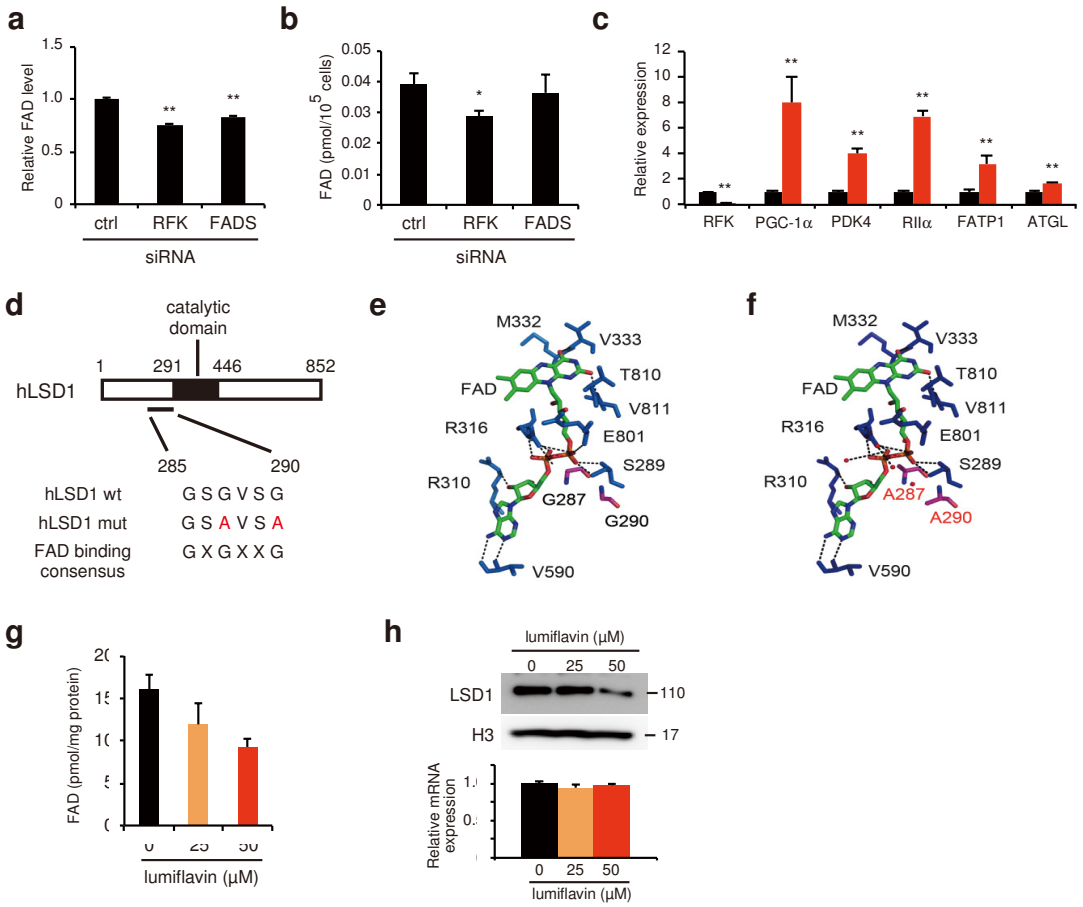
Supplementary Figure S3. LSD1 inhibition enhances energy mobilization in adipocytes.

(**a** and **b**) Mitochondrial energy metabolism was assessed in differentiating 3T3-L1 cells under 10^{-5} M SLI treatment (**a**) and 10^{-4} M parg treatment (**b**). Cells were stained with JC-1 as described in **Fig. 5**. **p<0.01 vs vehicle by Student's *t*-test. (**c** and **d**) Effect of LSD1 inhibition on the glycolytic pathway. Glycolytic activity was determined by measuring the cellular lactate production. After siRNA introduction (**c**) or TC addition (**d**), 3T3-L1 cells were subjected to adipogenic induction for 24 hours. Cells were collected and used for lactate measurement and total protein measurement. Values are means ± SD of triplicate samples.



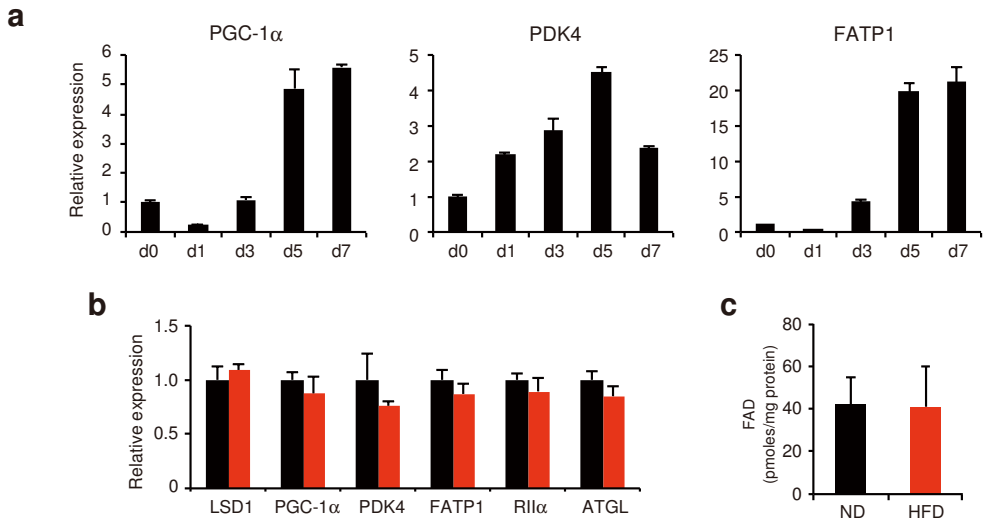
Supplementary Figure S4. LSD1 inhibition in mature and differentiating adipocytes.

(**a**) Adenovirus-mediated knockdown of *LSD1* gene in mature adipocytes. 3T3-L1 adipocytes were collected for RNA and protein analyses at four days after AdV infection. Values are means ± SD of triplicate results. (**b**) LSD1-KD attenuates the insulin-dependent repression of energy expenditure genes in mature adipocytes. Fully differentiated 3T3-L1 cells were treated with insulin and infected with control (black bars) or LSD1 (red bars) shRNA-containing adenoviruses as indicated in **Figure 5d**. Values are indicated as fold difference relative to untreated (insulin(-)) samples. Values are means ± SD of triplicate results. (**c**) Effect of LSD1 or BHC80-knockdown (KD) on lipid accumulation in differentiating adipocytes. siRNA-introduced 3T3-L1 cells were subjected to adipogenic induction for 48 hours and then cultured for an additional 5 days, followed by oil red O staining. (**d**) Effect of tranylcypromine (TC) treatment on lipid accumulation. 10^{-4} M of TC or pargyline (parg) was added to differentiating 3T3-L1 cells, as indicated on the diagram. Scale bars are 100 μm.



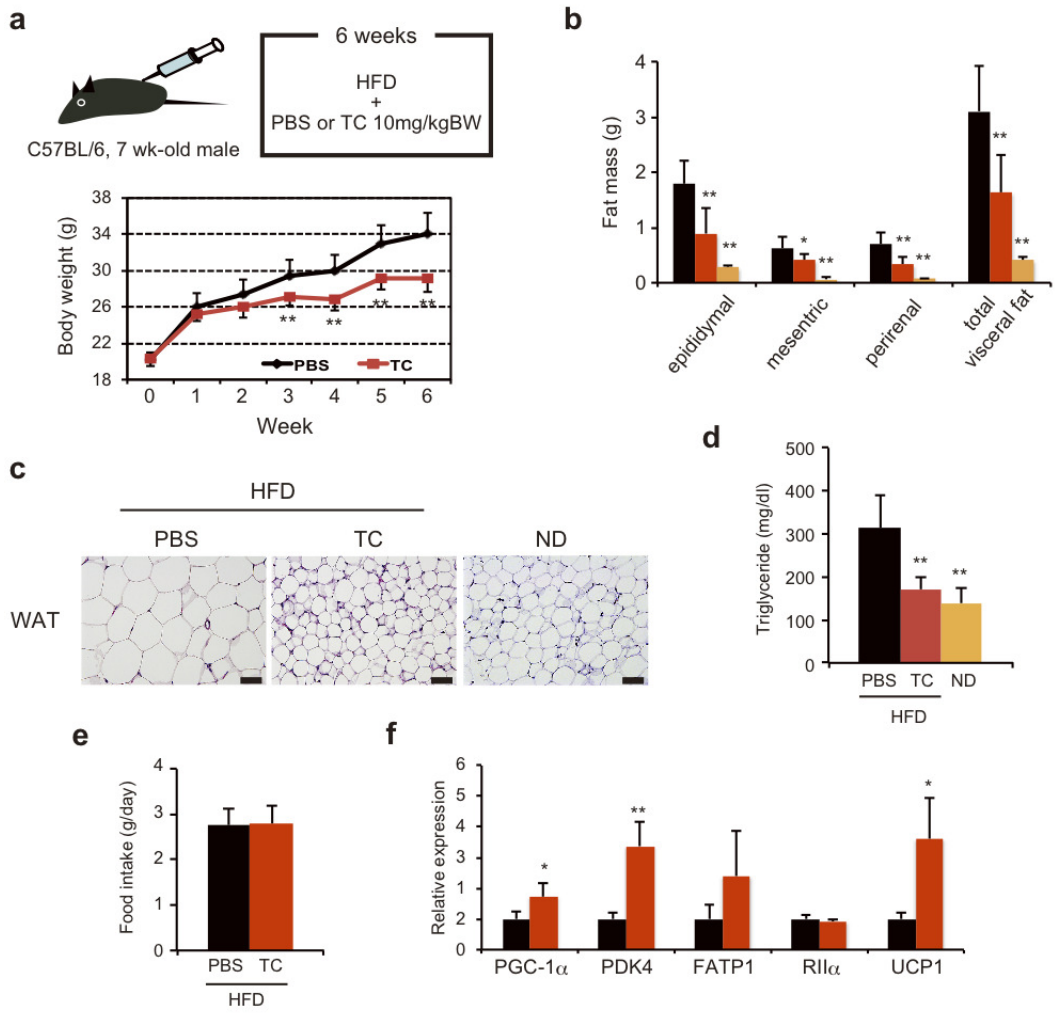
Supplementary Figure S5. Cellular FAD-dependent activities of LSD1.

(**a** and **b**) Measurement of cellular FAD content in RFK-KD and FADS-KD cells. Values are means ± SD of triplicate samples. *p<0.05, **p<0.01 vs control siRNAs by Student's *t*-test. (**a**) Relative FAD content was assessed by measuring the autofluorescence of cells by flow cytometry analysis. Specific siRNA-introduced 3T3-L1 preadipocytes were trypsinized and suspended in PBS for FACS analysis. (**b**) FAD content determined using a FAD Assay Kit (BioVision). Values are expressed as pmols per 10^5 cells. (**c**) Up-regulation of LSD1-target genes by RFK-KD was extensively confirmed using an alternative siRNA against the *RFK* gene (RFK#2, red bars). Values are shown as the fold difference against control siRNA-introduced samples (black bars). Values are means ± SD of triplicate samples. **p<0.01 vs control siRNA by Student's *t*-test. (**d**) The FAD-binding motif of LSD1. FAD-binding sequences were point-mutated as indicated in red. (**e** and **f**) Structural model of the wild- and mutant-type FAD binding site of human LSD1. Wild type (**e**) and GxAxxA mutant type (**f**) of LSD1 are shown. Mutated amino acids are indicated in red. (**g**) Decrease in cellular FAD content by lumiflavin treatment. 3T3-L1 cells were treated with the indicated concentrations of lumiflavin for 48 hours, followed by FAD measurement. Values are means ± SD of triplicate samples. **p<0.01 vs vehicle by Student's *t*-test. (**h**) LSD1 protein and mRNA levels in lumiflavin treated cells. Values are means ± SD of triplicate samples.



Supplementary Figure S6. LSD1-target gene expression in FAD-increasing conditions.

(**a**) The transition of LSD1 target gene expression during adipogenesis. Values are means ± SD of triplicate samples. (**b**) Effect of palmitate treatment on the expression of LSD1-target genes. Mature 3T3-L1 adipocytes were treated either with BSA (black bars) or 500 μM sodium palmitate (red bars) for 24 hours before RNA analysis. Values are means ± SD of triplicate samples. (**c**) FAD concentrations in epididymal adipose tissues from normal diet (ND)- and high fat diet (HFD)- fed mice. Six-week-old C57BL/6 male mice were fed with ND or HFD for four weeks, and were subjected to FAD measurement. Values are means ± SD of four mice.



Supplementary Figure S7. Administration of tranylcypromine attenuates high fat diet-induced obesity and activates energy expenditure genes in white adipose tissue.

(**a**) Body weight change during the test period. Seven-week old C57BL/6J mice were fed with HFD for six weeks in combination with alternate-day administration of tranylcypromine (TC) (10 mg/kg body weight) or PBS-control (n=8 for each group). (**b**) Effect of LSD1 inhibition on visceral fat mass. Individual and total fat mass at three depots were measured in HFD/PBS (black bars, n=8), HFD/TC (red bars, n=8) and ND mice (orange bars, n=4). Values are means ± SD. (**c**) Histological sections of epididymal WAT from the HFD/PBS, HFD/TC and age-matched ND-fed mice. Scale bars indicate 50 μm. (**d**) Concentration of triglyceride in blood. Samples were obtained on the final day after 16-hour starvation. Values are means ± SD (n=8 for HFD, n=4 for ND). (**e**) No effect of TC administration on food intake. Food intake in mice tested was repeatedly determined by measuring the amount of food remaining after 24-hour feeding. There were no abnormal behavior and neurological aspects in TC-treated mice. Values are means ± SD of eight mice. (**f**) Effect of TC administration on expression of LSD1-target genes in epididymal WAT of HFD-fed mice. HFD/PBS (black bars), HFD/TC (red bars). Values are means ± SD of eight mice. All histogram values are indicated as the fold difference against HFD/PBS. *p<0.05, **p<0.01 vs. HFD/PBS by Student's *t*-test.

Supplementary Table S1 - The expression levels of genes associated with adipogenic differentiation

Probe set	Description	Common name	Relative expression compared to control		
			LSD1-KD	BHC80-KD	TC 10^{-4} M
1418982_at	CCAAT/enhancer binding protein (C/EBP), alpha	CEBPα	1.4	0.3	1.2
1418901_at	CCAAT/enhancer binding protein (C/EBP), beta	CEBPβ	1.0	1.1	0.9
1423233_at	CCAAT/enhancer binding protein (C/EBP), delta	CEBPδ	1.2	1.1	0.8
1427683_at	early growth response 2	Krox20	0.7	0.8	0.6
1451021_a_at	Kruppel-like factor 5	KLF5	2.3	1.0	2.1
1420715_a_at	peroxisome proliferator activated receptor gamma	PPARγ	0.9	1.7	0.7
1440870_at	PR domain containing 16	Prdm16	Flag "absent" in all samples		

Supplementary Table S2 - IC50 values of LSD1 and MAO inhibitors

IC_{50} (μM)*	LSD1	MAO-A	MAO-B
clorgyline	>500	0.053 ± 0.011	5.2 ± 0.13
pargyline	>500	15 ± 3.5	0.23 ± 0.016
tranylcypromine	110 ± 11	18 ± 2.0	0.90 ± 0.094
S2101	1.3 ± 0.52	37 ± 1.9	15 ± 7.0

*Inhibition assays were performed using the peroxidase-coupled reaction method.

Supplementary Table S3 - siRNAs target sequences

Genes	siRNA target sequence
GL3	5'-GATTTCGAGTCGTCTTAAT-3'
	(control, targeting pGL3 luciferase plasmid)
lamin A/C, human	5'-CTGGACTTCCAGAAGAACA-3'
	(control, used in luciferase assays)
BHC80	5'-GTTCCAGATACAGCCATTG-3'
LSD1	5'-CACAAGGAAAGCTAGAAGA-3'
LSD1 #2	5'-CAGAGAGATCCAACGGCAT-3'
RFK	5'-TCTTCCAGCTGATGTGTGT-3'
RFK #2	5'-GTCCCTTACCTGCCTACCA-3'
FADS	5'-GAGCCCTTGGAGGAATGTC-3'

Supplementary Table S4 - Primers used in the study

Gene Name		Forward (5'-3')	Reverse (5'-3')
Quantitative RT-PCR			
36B4		GCGTCCTGGCATTGTCTGT	GCAAATGCAGATGGATCAGCC
Acox3		AAGAAGCTGGGGCAGAATGG	GCCCTCAGAGGTGATATTCC
AdipoQ		TCTGTACGATTGTCAGTGGA	AGTAACGTCATCTTCGGCAT
ATGL		GCCAACGCCACTCACATCTA	GCCTCCTTGGACACCTCAAT
BHC80		GAAGCAGGATCCACAGAATG	TTGCGCAGCTGCTCAACTA
CR2		AAGCTGAATTTCAGTGGCCT	CTTCACAGTGTCCCGTCCAA
FABP4		GGAAGCTTGTCTCCAGTGAA	TTACGCTGATGATCATGTTG
FADS		TAGCCTCAGCCATTTCAGTC	CCAGATGTTTCTGTAGGTCC
FATP1		TTTCTGCGTATCGTCTGCAA	CCGAACACGAATCAGAACA
Ethe1		GCCCAGGCTGATTTGCATAT	GTGACACAGCCTGGAGTGTG
Glut4		GTCGGGTTTCCAGCAGATC	AACTGAAGGGAGCCAAGCA
LSD1		ATGGATGTCACACTTCTGGA	CAAGACCTGTTACAACCATG
PDK4		CAAGGAGATCTGAATCTCTA	GATAATGTTTGAAGGCTGAC
PGC-1α		AAGTGTGGAACTCTCTGGAACTG	GGGTTATCTTGGTTGGCTTTATG
PPARγ2		TCGCTGATGCACTGCCTATGAG	GAGGTCCACAGAGCTGATTCC
RFK		CATTGGATGGAACCCGTACT	CCAACAATGGCCACATTGAG
RIIα		AACTGATGAGCAGAGATGCC	AACATGGCATCCAGAACTTG
UCP1		GGCCCTTGTAAACAACAAAATAC	GGCAACAAGAGCTGACAGTAAAT
ChIP-quantitative PCR			
ATGL		TGCCCAAGCTGTGGGATTGA	AGCCATCTGAGAGACCTGGA
FATP1		CGCCCAGGACTCTGCAAAG	CACAGAAGTCTGGACTGGGA
PDK4		CTGGCTAGGAATGCGTGACA	GATCCCAGGTCGCTAGGACT
PGC-1α	1	GTCTAATTGAGACTGGCTGTG	CAACATGTTGAGCAACTCAGC
	2	GTTGACTGTGTGGAAAGTAGA	TAGTAGCCTTACTCTGTACTCC
	3	CAGTGTGATGCTTGAAGCCT	TCCTGACGTCACTCCAAGG
	4	AAGCTTGACTGGCGTCATTC	GCTCCGGTCCTGCAATACTC
	5	TCAAAGATGCCTCCTGTGAC	CAAGGAGAGACCTGCTTGCT
	6	CACAAACACCTGGCACTCAC	CCACTTGCCTCATGCTGACA
MyoD		TTTGCCTGGTCTCCAGAGTG	CCTCTCCAGTGTCTACTCCT
Major satellite		GACGACTTGAAAAATGACGAAATC	CATATTCCAGGTCCTCAGTGTGC
PGC-1α/Luc-5'		AAGTGCAGGTGCCAGAACAT	TGTCCCAACGTATAGGCGTG
PGC-1α/Luc-3'		AGTGTGTGCTGTGTGTCAGA	AACAGTACCGGAATGCCAAG
Site directed mutagenesis			
LSD1, GxAxxA		ATTATAGGCTCTGCGGTC--TCAGCCTTGGCAGCAGC	GCTGCTGCCAAGGCTGAG--ACCGCAGAGCCTATAAT

Letters with underlines indicate the mutated nucleotides

SUPPLEMENTARY METHODS

Measurement of cellular and tissue FAD content

FAD content was determined using a FAD Assay Kit (BioVision) according to the manufacturer's protocol. Protein-bound FAD was dissociated using a Deproteinizing Sample Preparation Kit (BioVision) prior to FAD measurements. Alternatively, relative FAD content was determined by measuring the autofluorescence of cells by FACS [60]. Cells were trypsinized and suspended in PBS for immediate analysis. FAD autofluorescence was detected with the FL1 setting of a FACSCanto flow cytometer (excitation and emission wavelength of 488 and 525 nm, respectively). Values were calculated based on the mean fluorescence intensity of each sample. FAD concentrations were normalized to either cell numbers or protein concentrations determined using the Protein Assay Reagent (Bio-Rad).

Animal studies

Animal experiments were conducted in accordance with the guidelines of the Animal Care and Use Committee of Kumamoto University. For obesity induction experiments, seven-week old male C57BL/6J mice were fed a high fat diet (HFD21: Japan Clea) for six weeks in combination with intraperitoneal injections of PBS-control or 10mg/kg body weight of tranylcypromine every other day. Body weight was monitored weekly throughout the test period. Twenty four-hour food intake was assessed five times during weeks 4 to 6. Before being sacrificed, mice were fasted for 16 hours before blood was

collected to analyze the triglyceride concentrations. Collected tissues were weighed and dissected for RNA extraction and histological analyses.

SUPPLEMENTARY REFERENCE

60 Smelt, M. J., Faas, M. M., de Haan, B. J. & de Vos, P. Pancreatic beta-cell purification by altering FAD and NAD(P)H metabolism. *Exp Diabetes Res* **2008**, 165360, (2008).