

Supplementary materials

Indirect calorimetry

The mice were housed in individual chambers (150 cm² floor area, 8 cm in height). After at least 24 h of acclimatization, gas analysis was performed for 24 h at 23 °C using an open-circuit metabolic gas analysis system connected directly to a mass spectrometer (Arco2000; ArcoSystem, Chiba, Japan). Air was pumped into the chambers at a rate of 0.3 L/min. The expired air was directed to an O₂/CO₂ analyzer for mass spectrometry. Motor activity was measured in each chamber using an infrared sensor (NS-AS01; Neuroscience Inc., Tokyo, Japan).

Blood analysis

Blood samples were collected by decapitation without anesthesia, and then centrifuged at 2,000 × g for 10 min at 4 °C. Serum triglyceride (TG), non-esterified fatty acid (NEFA), cholesterol, and insulin levels were measured using LabAssay Triglyceride, NEFA, and cholesterol kits (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) and an Insulin ELISA kit (Morinaga Institute of Biological Science, Yokohama, Japan), respectively.

Glucose tolerance and insulin tolerance tests

For the oral glucose tolerance test, basal blood glucose levels were measured after fasting for 16 h, and glucose was orally administered to the mice (1 g/kg body weight). Subsequently, blood glucose concentrations were determined for 120 min. The insulin tolerance test was performed after a 2-hour fast. After measuring basal blood glucose levels, mice were intraperitoneally injected with insulin (0.75 U/kg body weight, Humulin-R; Eli Lilly, Indianapolis, IN, USA), and blood glucose levels were measured for 60 min.

Quantitative real-time PCR

Total RNA was extracted using phenol/guanidine-based reagents (Roche, Basel, Switzerland or Qiagen, Hilden, Germany) and then reverse-transcribed into cDNA using ReverTra Ace qRNA RT Master Mix (Toyobo, Osaka, Japan). The synthesized cDNA was diluted using EASY Dilution (Takara Bio Inc., Shiga, Japan). Gene expression levels were measured using specific primers (Supplementary Table 1) and THUNDERBIRD Next SYBR qPCR Mix (Toyobo). Expression levels of target genes were normalized to those of an internal control *Hprt*, *Rn18s* or *Rplp0*. The gene that was minimally affected by genotype and intervention was selected as the internal control.

Supplementary Table 1. Primers used for real-time PCR

Gene		Sequence (5'-3')
<i>Ppargc1a Total</i>	Forward	TGATGTGAATGACTTGGATACAGACA
	Reverse	GCTCATTGTTGTACTGGTTGGATATG
<i>Ppargc1a-a</i>	Forward	GGGACATGTGCAGCCAAGA
	Reverse	AAGAGGCTGGTCCTCACCAA
<i>Ppargc1a-b</i>	Forward	GACATGGATGTTGGGATTGTCA
	Reverse	ACCAACCAGAGCAGCACATTT
<i>Ppargc1a-c</i>	Forward	TGAGTAACCGGAGGCATTCTCT
	Reverse	TGAGGACCGCTAGCAAGTTTG
<i>Ucp1</i>	Forward	TGCACCACACTCCTGGCCTCT
	Reverse	GGCCGTCGGTCCTTCCTTGG
<i>Ucp2</i>	Forward	ATGGTTGGTTTCAAGGCCACA
	Reverse	CGGTATCCAGAGGGAAAGTGAT
<i>Ucp3</i>	Forward	CTGCACCGCCAGATGAGTTT
	Reverse	ATCATGGCTTGAAATCGGACC
<i>Prdm16</i>	Forward	TGCTGACGGATACAGAGGTGT
	Reverse	CCACGCAGAACTTCTCGCTAC
<i>Dio2</i>	Forward	AATTATGCCTCGGAGAAGACCG
	Reverse	GGCAGTTGCCTAGTGAAAGGT
<i>Cidea</i>	Forward	TGACATTCATGGGATTGCAGAC
	Reverse	GGCCAGTTGTGATGACTAAGAC

<i>Irf4</i>	Forward	AAAGGCAAGTTCCGAGAAGGG
	Reverse	CTCGACCAATTCCTCAAAGTCA
<i>Cs</i>	Forward	GGACAATTTTCCAACCAATCTGC
	Reverse	TCGGTTCATTCCCTCTGCATA
<i>Cyes</i>	Forward	CCAAATCTCCACGGTCTGTTC
	Reverse	ATCAGGGTATCCTCTCCCCAG
<i>Uqcrc2</i>	Forward	AAAGTTGCCCCGAAGGTTAAA
	Reverse	GAGCATAGTTTTCCAGAGAAGCA
<i>Cox4i1</i>	Forward	GAGCCATTTCTACTTCGGTGTGC
	Reverse	ACATCAGGCAAGGGGTAGTCAC
<i>Atp5b</i>	Forward	GGTTCATCCTGCCAGAGACTA
	Reverse	AATCCCTCATCGAACTGGACG
<i>Agt</i>	Forward	TCTCCTTTACCACAACAAGAGCA
	Reverse	CTTCTCATTACAGGGGAGGT
<i>Retn</i>	Forward	AAGAACCTTTTCATTTCCCCTCCT
	Reverse	GTCCAGCAATTTAAGCCAATGTT
<i>Slc2a4 (Glut4)</i>	Forward	GTGACTGGAACACTGGTCCTA
	Reverse	CCAGCCACGTTGCATTGTAG
<i>Hk2</i>	Forward	TGATCGCCTGCTTATTCACGG
	Reverse	AACCGCCTAGAAATCTCCAGA
<i>Cd36</i>	Forward	GGAGCCATCTTTGAGCCTTCA
	Reverse	GAACCAAACCTGAGGAATGGATCT
<i>Cpt1a</i>	Forward	CTCCGCCTGAGCCATGAAG
	Reverse	CACCAGTGATGATGCCATTCT
<i>Cpt1b</i>	Forward	GCACACCAGGCAGTAGCTTT
	Reverse	CAGGAGTTGATTCCAGACAGGTA
<i>Acadm</i>	Forward	AGGGTTTAGTTTTGAGTTGACGG
	Reverse	CCCCGCTTTTGTTCATATTCCG
<i>Atp2a1 (Serca1)</i>	Forward	TGTTTGTCTATTTCGGGGTG
	Reverse	AATCCGCACAAGCAGGTCTTC
<i>Atp2a2 (Serca2)</i>	Forward	GAGAACGCTCACACAAAGACC
	Reverse	CAATTCGTTGGAGCCCCAT
<i>Sln</i>	Forward	TCCTCGTGAGGTCCTACCAA
	Reverse	TAGAGCATTGGAAGCTCGGG
<i>Fasn</i>	Forward	GGAGGTGGTGATAGCCGGTAT
	Reverse	TGGGTAATCCATAGAGCCCAG
<i>Acaca (Acc1)</i>	Forward	ATGGGCGGAATGGTCTCTTTC
	Reverse	TGGGGACCTTGTCTTCATCAT
<i>Acly</i>	Forward	CAGCCAAGGCAATTCAGAGC
	Reverse	CTCGACGTTTGATTAAGTGGTCT

<i>Scd1</i>	Forward	TTCTTGCGATACACTCTGGTGC
	Reverse	CGGGATTGAATGTTCTTGTCGT
<i>Dgat1</i>	Forward	GTGCCATCGTCTGCAAGATTC
	Reverse	GCATCACCACACACCAATTCAG
<i>Apob</i>	Forward	AAGCACCTCCGAAAGTACGTG
	Reverse	CTCCAGCTCTACCTTACAGTTGA
<i>Mttp</i>	Forward	AGCCAGTGGGCATAGAAAATC
	Reverse	GGTCACTTTACAATCCCCAGAG
<i>Acox1</i>	Forward	CCGCCACCTTCAATCCAGAG
	Reverse	CAAGTTCTCGATTTCTCGACGG
<i>Pparg</i>	Forward	CTCCAAGAATACCAAAGTGCGA
	Reverse	GCCTGATGCTTTATCCCCACA
<i>Srebfl</i>	Forward	GCAGCCACCATCTAGCCTG
	Reverse	CAGCAGTGAGTCTGCCTTGAT
<i>Mlxipl (Chrebpα)</i>	Forward	CGACACTCACCCACCTCTTC
	Reverse	TTGTTTCAGCCGGATCTTGTC
<i>Mlxipl (Chrebpβ)</i>	Forward	TCTGCAGATCGCGTGGAG
	Reverse	CTTGTCCCGGCATAGCAAC
<i>Nr1h3 (LXRα)</i>	Forward	CTCAATGCCTGATGTTTCTCCT
	Reverse	TCCAACCCTATCCCTAAAGCAA
<i>Nr1h2 (LXRβ)</i>	Forward	ATGTCTTCCCCCACAAGTTCT
	Reverse	GACCACGATGTAGGCAGAGC
<i>Pnpla2 (ATGL)</i>	Forward	ATGTTCCCGAGGGAGACCAA
	Reverse	GAGGCTCCGTAGATGTGAGTG
<i>Lipe (HSL)</i>	Forward	CCAGCCTGAGGGCTTACTG
	Reverse	CTCCATTGACTGTGACATCTCG
<i>Lpl</i>	Forward	GGGAGTTTGGCTCCAGAGTTT
	Reverse	TGTGTCTTCAGGGGTCTTAG
<i>Rplp0</i>	Forward	AGATTCGGGATATGCTGTTGGC
	Reverse	TCGGGTCTTAGACCAGTGTC
<i>Hprt</i>	Forward	TCAGTCAACGGGGGACATAAA
	Reverse	GGGGCTGTACTGCTTAACCAG
<i>Rn18s</i>	Forward	GTAACCCGTTGAACCCCAT
	Reverse	CCATCCAATCGGTAGTAGCG

Western blotting

Tissues were lysed in ice-cold lysis buffer supplemented with a protease and phosphatase inhibitor cocktail. After centrifugation, the protein concentration of the supernatant was determined using the bicinchoninic acid assay. Equal volumes of the lysates were subjected to SDS-PAGE. The separated proteins were transferred onto polyvinylidene difluoride membranes. After blocking, the target proteins were probed with specific primary (Supplementary Table 2) and secondary antibodies (1:3,000; Cell Signaling Technology, Danvers, MA, USA). Digital images were obtained using the chemiluminescence method. The signal intensities of the target proteins on the immunoblots were normalized using Coomassie Brilliant Blue staining.

Supplementary Table 2. Primary antibodies used for western blotting

Name of antibody	Dilution	Company	Catalog Number
UCP1	1:20000	Cell Signaling Technology	72298
HSL	1:20000	Cell Signaling Technology	18381
phospho-HSL S563	1:5000	Cell Signaling Technology	4139
ATGL	1:5000	Cell Signaling Technology	2439
FASN	1:5000	Cell Signaling Technology	3180
ACC	1:5000	Cell Signaling Technology	3676
ACLY	1:5000	Cell Signaling Technology	13390
SCD1	1:5000	Cell Signaling Technology	2794
CS	1:5000	Cell Signaling Technology	14309
VDAC	1:5000	Cell Signaling Technology	4661
SERCA1	1:5000	Cell Signaling Technology	12293
SERCA2	1:5000	Cell Signaling Technology	9580

UCP1: uncoupling protein 1; HSL: hormone-sensitive lipase; ATGL: adipose triglyceride lipase; FASN: fatty acid synthase; ACC: acetyl-CoA carboxylase; ACLY: ATP-citrate lyase; SCD1: stearoyl-CoA desaturase 1; CS: citrate synthase; VDAC: voltage-dependent anion channel; SERCA: Sarcoplasmic/endoplasmic reticulum calcium ATPase.