Supporting Information

Duteil et al. 10.1073/pnas.1702641114

SI Methods

Mouse Studies. All mice were housed in a pathogen-free barrier facility of the University Medical Center Freiburg in accordance with institutional guidelines and approved by the regional board. Mice were maintained in a temperature- and humidity-controlled animal facility with a 12-h light/dark cycle, free access to water, and standard rodent chow (breeding, no. 3807; Kliba). For cold exposure experiments, mice were maintained at 10 °C with a 12-h light/dark cycle, free access to water, and standard rodent chow for 10 or 25 d. Animals were killed by cervical dislocation, and tissues were immediately collected, weighted, frozen in liquid nitrogen, or processed for further analyses. Nomenclature for dissected adipose depots was used as described previously (28). In vivo experiments including food consumption, serum analysis, glucose tolerance tests, temperature measurements, energy expenditure, and Lsd1 inhibitor treatment were described previously (15, 21). GW9578 and GW6471 were injected to mice i.p. at 2 and 20 mg/kg, respectively, for 3 d.

Generation of Conditional Lsd1-Overexpressing Mice. For the generation of the targeting vector, WT human Lsd1 was cloned by Gateway recombination in a pβ-actin-att-IRES-lo vector. This vector expresses EmGFP (referred to as GFP in the text) flanked by LoxP sites under the control of the chicken β-actin promotor. A polyadenylation signal was inserted between the 3′ UTR of EmGFP and the distal loxP site preventing downstream transcription of Lsd1. A second polyadenylation site was inserted downstream of the 3′ UTR of Lsd1 ORF. pβ-actin-att-IRES-lo_LSD1 vector was linearized, resuspended in microinjection buffer, and injected into the male pronuclei to be randomly inserted into mouse genome at a concentration of 2.0 ng/μL. The injected eggs were transferred into the oviducts of 0.5 days postcoitum pseudopregnant CD1 foster mice. The injection background strain used was C57Bl6/NCrl (Charles River). A total of 102 animals were produced. PCR (Table S2) for transgenic DNA identified eight positive founders. The founder with the strongest overexpression was used for further analyses.

Mitochondrial Respiration. Mitochondrial respiration was measured by high-resolution respiratory imaging using Oxygraph-2K (Oroboros Instruments) as previously described (21).

Histological and Immunofluorescence Analysis. Tissues were fixed in 10% buffered formalin and embedded in paraffin. Paraffin sections (5 μm) were deparaffinized and rehydrated. H&E staining, immunohistochemistry, and immunofluorescence analyses were performed as described previously (15, 40) with anti-Lsd1 (R.S. laboratory [15], 1/1,000), anti-Ucp1 (ab10983, 1/500; Abcam), or anti-GFP (ab13970, 1/500; Abcam) antibodies. Chicken or rabbit IgG were used as controls.

Protein Analyses. Western blot analyses were performed as described previously (15, 41). Western blot membranes were decorated by using the following antibodies: anti-Lsd1 [R.S. laboratory (15), 1/1,000], anti-Ucp1 (ab10983, 1/1,000; Abcam), anti-Ppara (sc-9000, H-98, 1/500; Santa Cruz), anti-Parp (ab32138, 1/500; Abcam), anti–Caspase-3 (9662, 1/500; Cell Signaling), anti–β-tubulin (T6074, 1/10,000; Sigma), and anti–β-Actin (A1978, 1/10,000; Sigma). Secondary antibodies conjugated to HRP (GE Healthcare) were detected by using an enhanced chemiluminescence detection system (GE Healthcare).

Cell Culture. C3H-10T1/2 cells were cultured in Eagle's minimum essential medium supplemented with sodium bicarbonate (10 mM), L-glutamine (2 mM), and 10% FCS. Immortalized cell lines derived from the stromal-vascular fraction from inguinal depot of 129SVE mice were generated following the 3T3 immortalization protocol (42). Cells showing adipogenic capacity and baseline low expression levels of Ucp1, but higher cellular binding to Irisin, were selected and used for further experiments. These cells were grown in DMEM/F12 GlutaMAX (Thermo Scientific) supplemented with 10% FCS and 0.1% Normocin (Invitrogen). Differentiation of was induced by treatment by addition of 10 μg/mL insulin (Gibco), 1 μM dexamethasone (Calbiochem), 10 μM rosiglitazone (Cayman), and 500 μM isobutyl methylxanthine (Serva) in the presence of 10% FCS in DMEM. Differentiation of immortalized preadipocytes was induced by treatment with 0.5 μg/mL insulin (Gibco), 5 μM dexamethasone (Calbiochem), 1 μM rosiglitazone (Cayman), 500 μM isobutyl methylxanthine (Serva), and 1 nM T3 (Sigma) in the presence of 10% FCS in DMEM. For white adipogenesis, the differentiation medium was replaced 3 d later with medium supplemented with 10% FCS, and insulin for 2 d. For beige adipogenesis, the differentiation medium was replaced 3 d later with medium supplemented with 10% FCS, rosiglitazone, T3, and insulin for 2 d. Subsequently, cells were cultured in the same medium for a further 4 d in their corresponding medium.

Lsd1 inhibitor QC6688 was applied to differentiated cells at 100 nM for 3 d. CL316,243 (Sigma) was applied at 1 mM for 24 h. Adipocytes were harvested and snap-frozen for RNA and protein experiments or fixed for 5 min with 1% PFA for ChIP experiments.

Data Analysis. Data are presented as mean ± SEM. Significance was calculated by (*i*) a two-tailed Student's *t* test for Figs. 1*A* and 3*C* and Figs. S2 *A* and *I*, S3*E*, and S4 *E* and *G* or (*ii*) a two-way ANOVA for Figs. 1*E*, 2*B*, and 4 *A* and *D* and Figs. S1*E*, S2*E*, S3 *C* and *D*, and S4 *C*, *D*, *F*, and *K*.

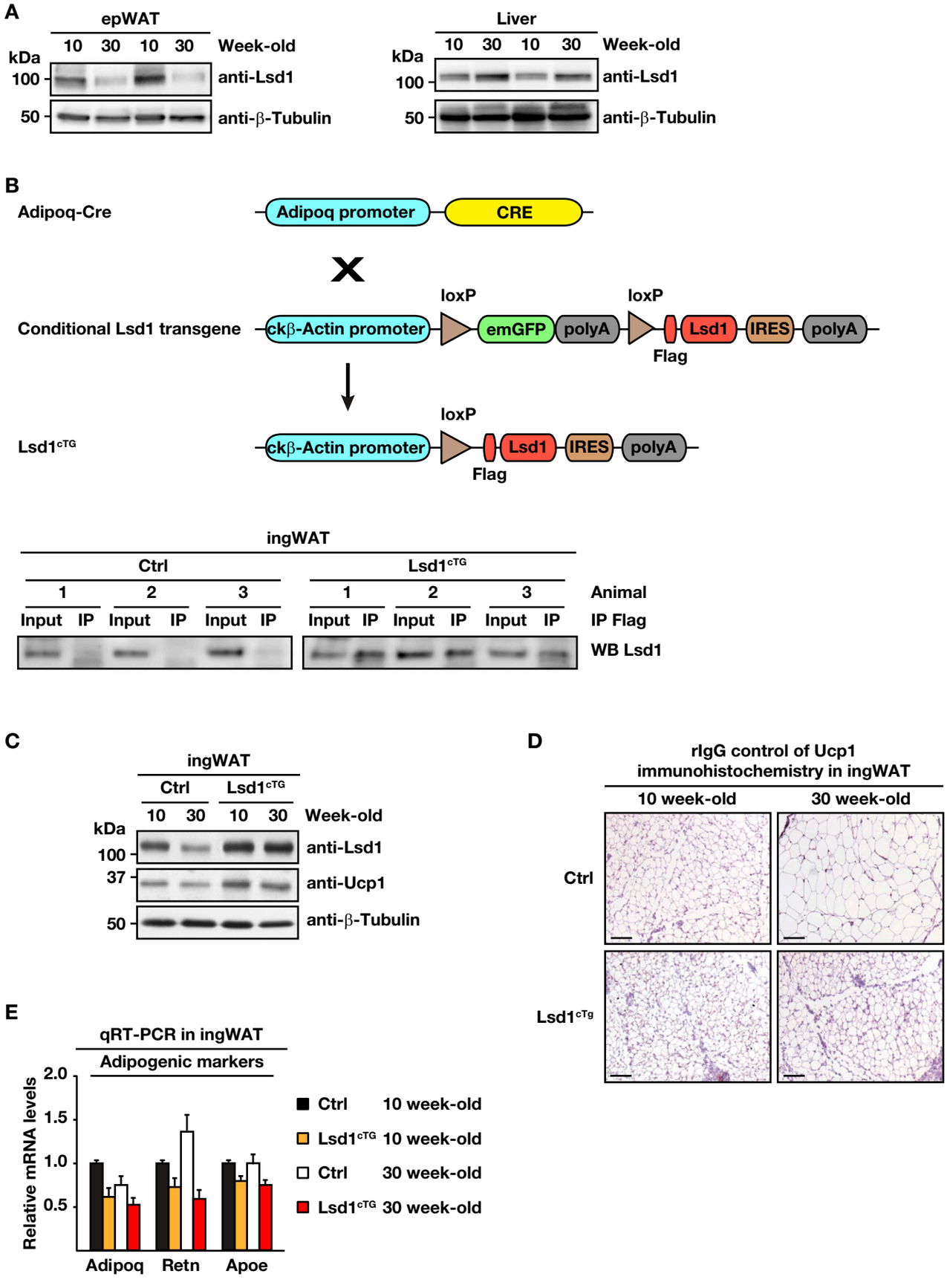

Fig. S1. Lsd1 prevents the age-programmed loss of beige adipocytes (Fig. 1). (*A*) Western blot analysis of Lsd1 protein levels in epWAT and liver of 10- and 30-wk-old WT mice. β-Tubulin was used as a loading control. (*B*) Scheme depicting generation of adipocyte-specific Lsd1-overexpressing mice (Lsd1cTg). To confirm expression of the Lsd1 transgene, we performed immunoprecipitations (IP) in ingWAT of control (Ctrl) and Lsd1cTg mice with anti-Flag antibody followed by Western blot analysis using anti-Lsd1 antibody. (*C*) Western blot analysis of Lsd1 and Ucp1 protein levels in ingWAT of control and Lsd1cTg mice at 10 and 30 wk of age. β-Tubulin was used as a loading control. (*D*) Rabbit IgG (rIgG) control for Ucp1 immunohistochemical detection shown in Fig. 1*C* performed on representative sections of ingWAT of 10- and 30-wk-old control and Lsd1cTg mice. (*E*) qRT-PCR analysis showing relative mRNA levels of indicated genes in ingWAT of control and Lsd1cTg mice at 10 and 30 wk of age (mean ± SEM; $*P < 0.05$; $n = 12$).

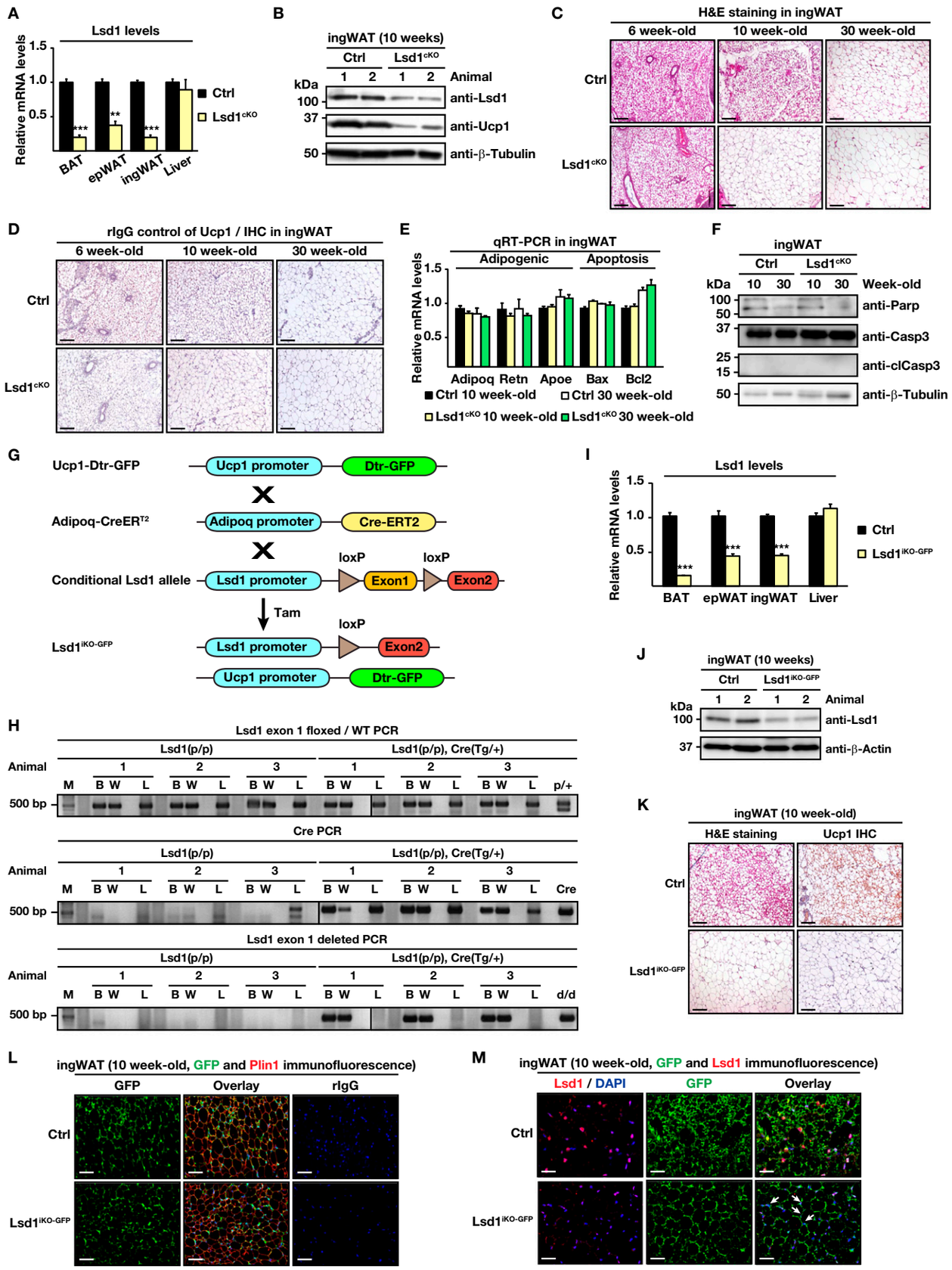

Fig. S2. Loss of Lsd1 in adipocytes accelerates the beige-to-white transition of ingWAT (Fig. 2). (*A*) qRT-PCR analysis showing relative Lsd1 mRNA levels in BAT, epWAT, ingWAT, and liver of control (Ctrl) and adipocyte-specific Lsd1cKO mice (mean ± SEM; $^{**}P < 0.01$ and $^{***}P < 0.001$; $n = 10$). (*B*) Western blot analysis of Lsd1 and Ucp1 in ingWAT of 10 wk-old control and Lsd1cKO mice. β-Tubulin was used as a loading control. (*C*) H&E staining and (*D*) rabbit IgG (rIgG) control for the Ucp1 immunohistochemical detection shown in Fig. 2*A* performed on representative sections of ingWAT of 10- and 30-wk-old control and Lsd1cKO mice. (Scale bars: 200 μm.) (*E*) qRT-PCR analysis showing relative mRNA levels of indicated genes in ingWAT of control and Lsd1cKO mice at 10 and 30 wk of age (mean ± SEM; $^{*}P < 0.05$; $n = 10$). (*F*) Western blot analysis of Parp and Casp3 in ingWAT of 10- and 30-wk-old control and Lsd1cKO mice. β-Tubulin was used as a loading control. (*G*) Cartoon depicting the Ucp1-Dtr-GFP knock-in construct, Adipoq-CreERT2 transgene, and conditional and deleted Lsd1 alleles. Lsd1iKO-GFP mice were obtained after Tam injection. (*H*) Genotyping of mouse BAT (marked "B"), ingWAT ("W"), and liver ("L") biopsies of control and inducible adipose-specific Lsd1iKO-GFP mice for the presence of Lsd1 conditional ("p") allele (*1*), Adipoq-CreERT2 recombinase (Cre) (*2*), or Lsd1 recombined ("d") allele (*3*) by semiquantitative PCR. (*I*) qRT-PCR and (*J*) Western blot analyses showing Lsd1 mRNA and protein levels in ingWAT of 10-week-old control and Lsd1iKO-GFP mice. β-Tubulin was used as a loading control (*G*) (mean ± SEM; $^{***}P < 0.001$; $n = 10$). (*K*) H&E staining and immunohistochemical detection of Ucp1 on ingWAT from Lsd1p/p control and inducible adipocyte-specific Lsd1 KO mice (Lsd1iKO-GFP), both containing the Ucp1-Dtr-GFP reporter. construct. (Scale bars: 200 μm.) (*L* and *M*) Immunofluorescence detection using (*L*) anti-GFP and anti-Plin1 or (*M*) anti-GFP and anti-Lsd1 antibodies on representative sections of ingWAT from control and Lsd1iKO-GFP mice. (Scale bars: *L*, 100 μm; *M*, 50 μm.) (*M*) Arrows indicate the GFP-positive and Lsd1-negative adipocytes.

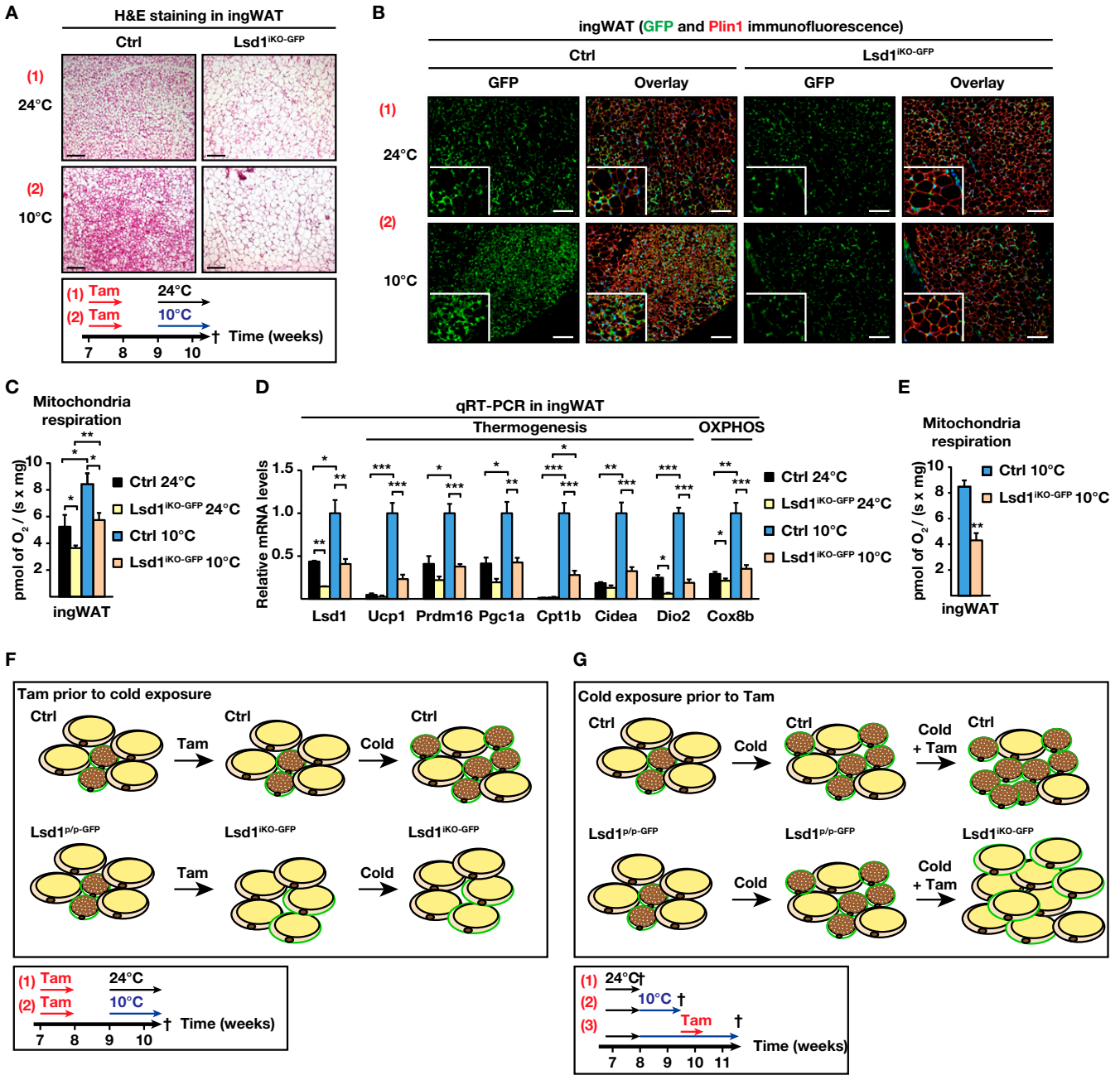

Fig. S3. Lsd1 is required for development and maintenance of cold-induced beige adipocytes (Fig. 3). (*A*) H&E staining and (*B*) immunofluorescence detection using anti-GFP and anti-Plin1 antibodies on representative sections of ingWAT of Lsd1p/p/Ucp1-Dtr-GFP control (Ctrl) and Lsd1iKO-GFP mice treated with Tam for 5 d and maintained at 24 °C or 10 °C for 10 d. Time scale indicates age of the mice and start and end of Tam and cold treatment. Dagger indicates the time point at which mice were killed (Scale bars: 200 μm.) (*C*) Mitochondrial respiration measurement performed on ingWAT extracts from control and Lsd1iKO-GFP mice maintained at 24 °C or 10 °C for 10 d (mean ± SEM; $*P < 0.05$ and $**P < 0.01$; $n = 9$). (*D*) qRT-PCR analysis showing relative mRNA levels of indicated genes in ingWAT of control and Lsd1iKO-GFP mice maintained at 24 °C or 10 °C for 10 d (mean ± SEM; $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$; $n = 9$). (*E*) Mitochondrial respiration measurement in ingWAT extracts from control and Lsd1iKO-GFP mice treated as described in Fig. 3*A* (mean ± SEM; $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$; $n = 7$). (*F* and *G*) Scheme illustrating the role of Lsd1 in the development and maintenance of beige adipocytes. (*F*) Beige adipocytes prevail in ingWAT of young rodents. In contrast, upon Lsd1 ablation in Lsd1iKO-GFP mice, beige adipocytes are not maintained and undergo a beige-to-white adipocyte transition. ingWAT of control mice responds to cold exposure by the formation of additional, inducible beige adipocytes, whereas, in ingWAT from Lsd1iKO-GFP mice, no beige adipocytes emerge upon cold stimulation. (*G*) Mice were preexposed to cold for 10 d, which results in the emergence of inducible beige adipocytes. While the cold exposure was continued, Lsd1 ablation was induced by Tam. ingWAT of control mice responds to prolonged cold stimulation by increased appearance of inducible beige adipocytes. In contrast, upon Lsd1 ablation, cold-induced beige adipocytes cannot be maintained and transition to a white adipocyte morphology despite the presence of the cold stimulus. Time scale indicates age of the mice and the start and end of Tam and cold treatment. Dagger indicates the time point at which mice were killed.

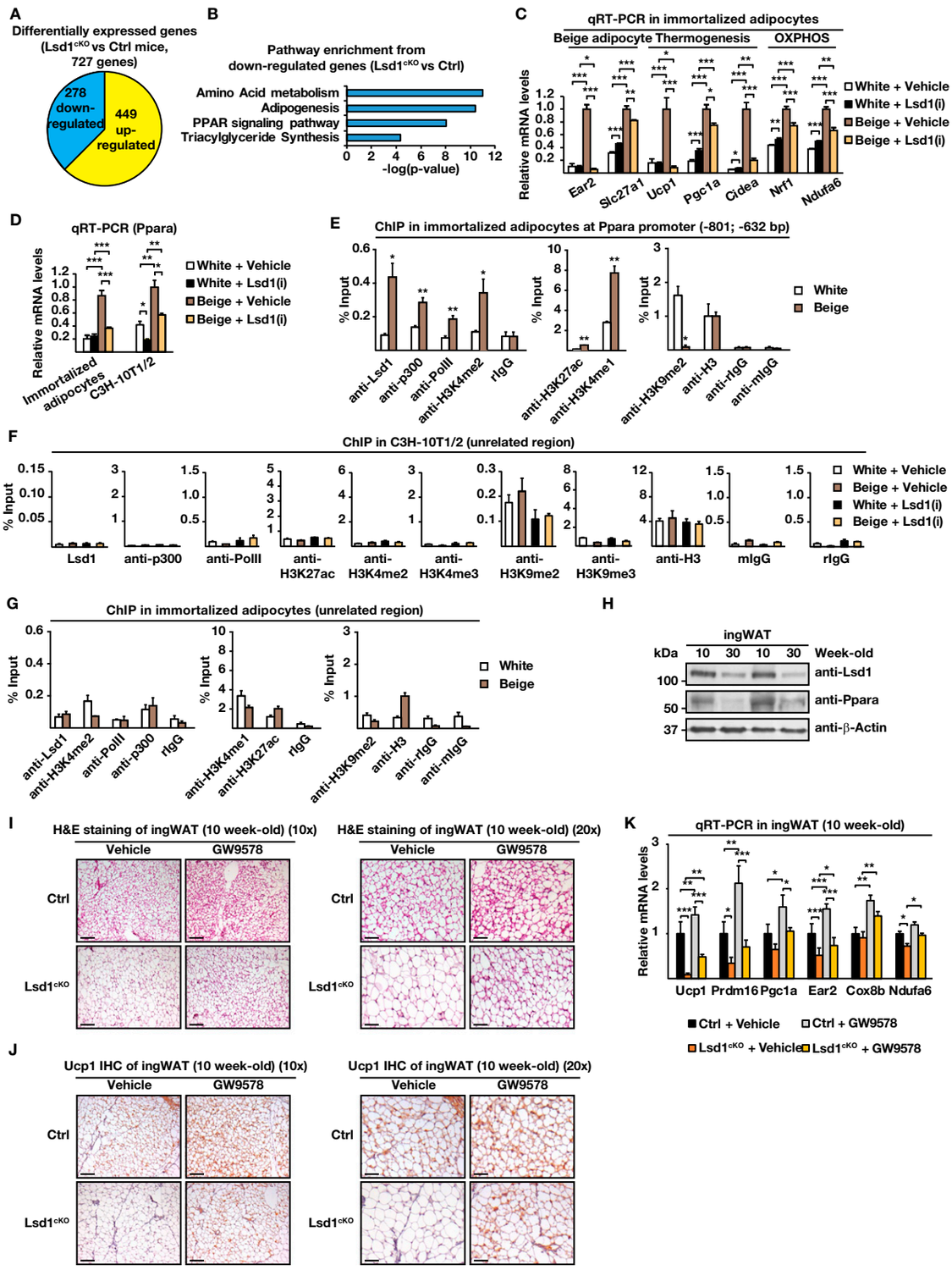

Fig. S4. Lsd1 targets Ppara to maintain beige adipocytes (Fig. 4). (*A*) Pie chart depicting the ratio of differentially expressed up- and down-regulated genes obtained from RNA-seq of ingWAT from control (Ctrl) and Lsd1cKO mice at 6 wk of age. (*B*) Pathway enrichment analysis of down-regulated genes from RNA-seq of ingWAT from control and Lsd1cKO mice at 6 wk of age. (*C* and *D*) qRT-PCR analysis showing relative mRNA levels of indicated genes in differentiated (*C* and *D*) immortalized adipocytes and (*D*) C3H-10T1/2 cells induced for white or beige adipogenesis and treated with vehicle or Lsd1-specific inhibitor QC6688 [Lsd1(i)] (mean + SEM; $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$; $n = 3$). (*E*) ChIP-qPCR analysis of the Ppara promotor (−801 to −632 bp) performed with anti-Lsd1, anti-p300, anti-PolII, anti-H3K27ac, anti-H3K4me2, anti-H3K4me3, H3K9me2, H3K9me3, and anti-H3 antibodies, and rabbit (rIgG) or mouse IgG (mIgG) in differentiated immortalized adipocytes induced for white or beige adipogenesis. The precipitated chromatin was quantified by qPCR by using primers flanking the −801 to −632 bp region of the Ppara gene (mean ± SEM; $*P < 0.05$ and $**P < 0.01$; $n = 3$). (*F* and *G*) ChIP analysis performed with anti-Lsd1, anti-p300, anti-PolII, anti-H3K27ac, anti-H3K4me2, anti-H3K4me3, H3K9me2, H3K9me3, and anti-H3 antibodies, and rabbit (rIgG) or mouse IgG (mIgG) in differentiated (*F*) C3H-10T1/2 adipocytes or (*G*) immortalized adipocytes induced for white or beige adipogenesis and treated with vehicle or Lsd1(i). The precipitated chromatin was quantified by qPCR by using primers flanking an unrelated region (mean ± SEM; $n = 3$). (*H*) Western blot analysis of Lsd1 and Ppara protein levels in ingWAT of 10- and 30 wk-old WT mice. β-Actin was used as a loading control. (*I*) H&E staining and (*J*) immunohistochemical detection of Ucp1 on representative sections and (*K*) qRT-PCR analysis showing relative mRNA levels of indicated genes performed on ingWAT of control and Lsd1cKO mice at 10 wk of age treated for 3 d with vehicle or the Ppara agonist GW9578 (mean ± SEM; $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$; $n = 6$). (Scale bars: *Left*, 200 μm; *Right*, 50 μm.)

Table S1. Pathway analysis on genes up- or down-regulated upon Lsd1 ablation

Index	Gene symbol	Gene name	EntrezGene	Ensembl
Glycolysis/Gluconeogenesis*				
1	*Eno3*	enolase 3, β-muscle	13808	ENSMUSG00000060600
2	*Pgam2*	phosphoglyceratemutase 2	56012	ENSMUSG00000020475
3	*Aldoa*	aldolase A, fructose-bisphosphate	11674	ENSMUSG00000030695
4	*Pfkm*	phosphofructokinase, muscle	18642	ENSMUSG00000033065
5	*Tpi1*	triosephosphate isomerase 1	21991	ENSMUSG00000023456
6	*Ldhb*	lactate dehydrogenase B	16832	ENSMUSG00000030246
7	*Pfkp*	phosphofructokinase, platelet	56421	ENSMUSG00000021196
8	*Aldh3b1*	aldehyde dehydrogenase 3 family, member B1	67689	ENSMUSG00000024885
9	*Hk1*	hexokinase 1	15275	ENSMUSG00000037012
Fructose and mannose metabolism*				
1	*Tpi1*	triosephosphate isomerase 1	21991	ENSMUSG00000023456
2	*Pfkm*	phosphofructokinase, muscle	18642	ENSMUSG00000033065
3	*Akr1b8*	aldo-keto reductase family 1, member B8	14187	ENSMUSG00000029762
4	*Aldoa*	aldolase A, fructose-bisphosphate	11674	ENSMUSG00000030695
5	*Pfkp*	phosphofructokinase, platelet	56421	ENSMUSG00000021196
6	*Gmds*	GDP-mannose 4, 6-dehydratase	218138	ENSMUSG00000038372
Galactose metabolism*				
1	*Pfkm*	phosphofructokinase, muscle	18642	ENSMUSG00000033065
2	*Glb1*	galactosidase, β-1	12091	ENSMUSG00000045594
3	*Akr1b8*	aldo-keto reductase family 1, member B8	14187	ENSMUSG00000029762
4	*Pfkp*	phosphofructokinase, platelet	56421	ENSMUSG00000021196
5	*Galk1*	galactokinase 1	14635	ENSMUSG00000020766
6	*Hk1*	hexokinase 1	15275	ENSMUSG00000037012
PPAR signaling pathway†				
1	*Slc27a1*	solute carrier family 27 (fatty acid transporter), member 1	26457	ENSMUSG00000031808
2	*Pck1*	Phosphoenolpyruvate carboxykinase 1, cytosolic	18534	ENSMUSG00000027513
3	*Sorbs1*	sorbin and SH3 domain containing 1	20411	ENSMUSG00000025006
4	*Lpl*	lipoprotein lipase	16956	ENSMUSG00000015568
5	*Acox1*	acyl-CoA oxidase 1, palmitoyl	11430	ENSMUSG00000020777
6	*Ppara*	peroxisome proliferator activated receptor α	19013	ENSMUSG00000022383
7	*Fabp4*	fatty acid binding protein 4, adipocyte	11770	ENSMUSG00000062515
8	*Acadl*	acyl-CoA dehydrogenase, long-chain	11363	ENSMUSG00000026003
9	*Ehhadh*	enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase	74147	ENSMUSG00000022853

For glycolysis/gluconeogenesis, (ID: 00010); C = 62; O = 9; E = 0.38; R = 23.40; raw $P = 1.87 \times 10^{-10}$; adjusted $P = 8.70 \times 10^{-9}$. For fructose and mannose metabolism, (ID: 00051); C = 37; O = 7; E = 0.23; R = 30.49; raw $P = 2.93 \times 10^{-9}$; adjusted $P = 9.08 \times 10^{-8}$. For galactose metabolism, (ID: 00052); C = 27; O = 6; E = 0.17; R = 35.82; raw $P = 1.45 \times 10^{-8}$; adjusted $P = 3.37 \times 10^{-7}$. For PPAR signaling pathway, (ID: 03320); C = 80; O = 9; E = 0.39; R = 23.33; raw $P = 2.13 \times 10^{-10}$; adjusted $P = 3.11 \times 10^{-9}$.

*Up-regulated upon Lsd1 ablation.

†Down-regulated upon Lsd1 ablation.

Table S2. Primers used for genotyping

Gene	Sense	Primer 5′–3′
Ck-βAct-*LSD1*	Forward	AATGCCTTCGAATTCAGCAC
Ck-βAct-*LSD1*	Reverse	CCTTGTCATCGTCGTCCTTG
Lsd1 WT/p	Forward	CCTCAGTAGGCCTGGTTTGT
Lsd1 WT/p	Reverse	TTGGTTTTGGTTGACCCTTC
Lsd1 del	Forward	CCGTGGAAATTCGTGCACTC
Lsd1 del	Reverse	GCAGGCGGTTTGAAATGTATTC
Ucp1-Dtr-GFP	Forward	ACGTAAACGGCCACAAGTTC
Ucp1-Dtr-GFP	Reverse	TGCTCAGGTAGTGGTTGTCG
Adipoq-Cre	Forward	TTCCCGCAGAACCTGAAGATGTTCG
Adipoq-Cre	Reverse	GGGTGTTATAAGCAATCCCCAGAAATGC
Adipoq-CreER^{T2}	Forward	TGGTGCATCTGAAGACACTACA
Adipoq-CreER^{T2}	Reverse	TGCTGTTGGATGGTCTTCACAG

Table S3. Primers used for qPCR analysis

Gene	Sense	Primer 5′–3′
36b4	Forward	GCGTCCTGGCATTGTCTGT
36b4	Reverse	GCAAATGCAGATGGATCAGCC
Hprt	Forward	AGGGCATATCCAACAACAAACTT
Hprt	Reverse	GTTAAGCAGTACAGCCCCAAA
Lsd1	Forward	GTGTTCTGGGACCCAAGTGT
Lsd1	Reverse	TAATGCCAGCAGCTTCTCCT
Ucp1	Forward	GTGAACCCGACAACTTCCGAA
Ucp1	Reverse	TGCCAGGCAAGCTGAAACTC
Prdm16	Forward	CCCCCAACGCTCTCGGATCC
Prdm16	Reverse	CCGAAGCAGCGGTTGCACAG
Pgc1a	Forward	AAGTGTGGAACTCTCTGGAACTG
Pgc1a	Reverse	GGGTTATCTTGGTTGGCTTTATG
Dio2	Forward	TCACAAGGTCTTGGGGTAGG
Dio2	Reverse	ATGCAGAAAGGCAGACTCGT
Cox8b	Forward	GAACCATGAAGCCAACGACT
Cox8b	Reverse	GCGAAGTTCACAGTGGTTCC
Ndufa6	Forward	GTCACAGACCCCAGAGTGGT
Ndufa6	Reverse	TAACATGCACCTTCCCATCA
Cpt1b	Forward	CAGCTGGCTGGTTGTTGTCA
Cpt1b	Reverse	TTGTCGGAAGAAGAAAATGC
Nrf1	Forward	TGGAGTCCAAGATGCTAATG
Nrf1	Reverse	AGAGCTCCATGCTACTGTTC
Cidea	Forward	TGCTCTTCTGTATCGCCCAGT
Cidea	Reverse	GCCGTGTTAAGGAATCTGCTG
Ear2	Forward	CCTGTAACCCCAGAACTCCA
Ear2	Reverse	CAGATGAGCAAAGGTGCAAA
Slc27a1	Forward	CTGGGACTTCCGTGGACCT
Slc27a1	Reverse	TCTTGCAGACGATACGCAGAA
Klhl13	Forward	AGAATTGGTTGCTGCAATACTCC
Klhl13	Reverse	AAGGCACAGTTTCAAGTGCTG
Apoe	Forward	GGTTCGAGCCAATAGTGGAA
Apoe	Reverse	TATTAAGCAAGGGCCACCAG
Adipoq	Forward	GCACTGGCAAGTTCTACTGCAA
Adipoq	Reverse	GTAGGTGAAGAGAACGGCCTTGT
Retn	Forward	CTGTCCAGTCTATCCTTGCAC
Retn	Reverse	CAGAAGGCACAGCAGTCTTGA
Tfam	Forward	AGGCCCGGCAGAGACGGTTAA
Tfam	Reverse	CCTGAGCCGAATCATCCTTTGCC
Bax	Forward	TGCAGAGGATGATTGCTGAC
Bax	Reverse	GATCAGCTCGGGCACTTTAG
Blc2	Forward	GGACTTGAAGTGCCATTGGT
Blc2	Reverse	CAGGCTGGAAGGAGAAGATG

Table S4. Primers used for ChIP-qPCR analysis

Gene	Sense	Primer 5′–3′
Ppara (−801; −632 bp)	Forward	TCTCCCCATTTCTCATCCTG
Ppara (−801; −632 bp)	Reverse	GCCAGGACTGAAGTTCAAGG
Ppara (unrelated region)	Forward	GTAGCTGTGACCACCCACCT
Ppara (unrelated region)	Reverse	GACCCTGCATGAACTTTGGT