Supplemental Information

Supplemental Figures

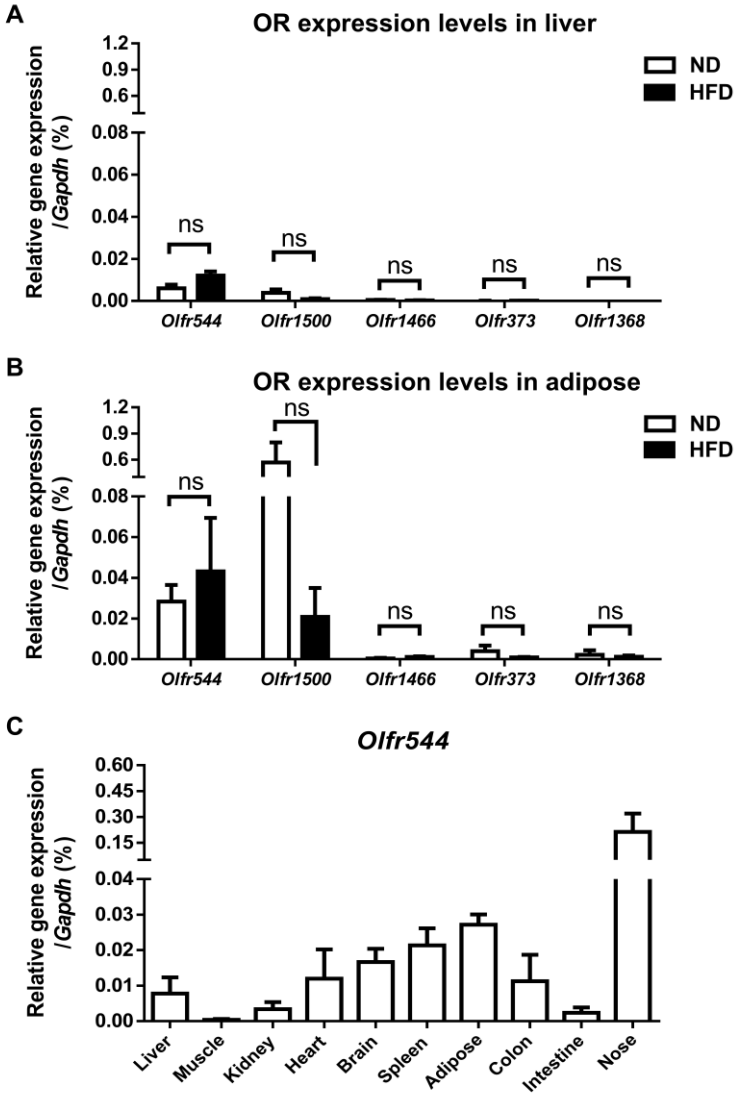

Figure S1. Olfactory receptors (ORs) are expressed in mouse liver and adipose tissues. (**A**) Expression of ORs in mouse livers (**B**) and adipose tissue assessed by qPCR (n=3–4). The expression of ORs was normalized to that of *Gapdh*. C57BL/6J mice were fed a normal (ND) or a high-fat diet (HFD) for 4 weeks prior to tissue sampling and cDNA preparation. ORs were selected from microarray experiments (data not shown). (**C**) Tissue distribution of *Olfr544* expression (n=3–5). Tissues were isolated from C57BL/6J mice on ND prior to tissue sampling and cDNA preparation. Gene expression levels were normalized to *Gapdh* expression. OR, olfactory receptor; ND, normal diet; HFD, high-fat diet (60% of total calories from fat). Data are presented as mean ± SEM and Student's t-test was used for comparing two groups (ns means there is no significant difference between two groups).

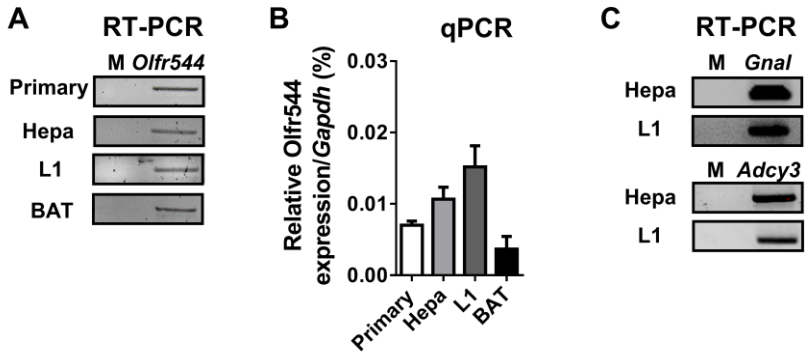

Figure S2. *Olfr544* is expressed in hepatocytes and adipocytes. (**A**) RT-PCR analyses and (**B**) qPCR analyses of *Olfr544* gene expression in primary mouse hepatocytes, cultured Hepa1c1c-7 mouse hepatocytes, cultured 3T3-L1 mouse adipocytes, and mouse brown adipose tissue (n=3). (**C**) Golfα (*Gnal*) and adenylate cyclase-3 (*Adcy3*) gene expression in Hepa1c1c-7 hepatocytes and 3T3-L1 adipocytes. M, mock RT-PCR; Primary, primary hepatocytes; Hepa, cultured Hepa1c1c-7 mouse hepatocytes; L1, cultured 3T3-L1 mouse adipocytes; BAT, brown adipose tissue. Data are presented as mean ± SEM.

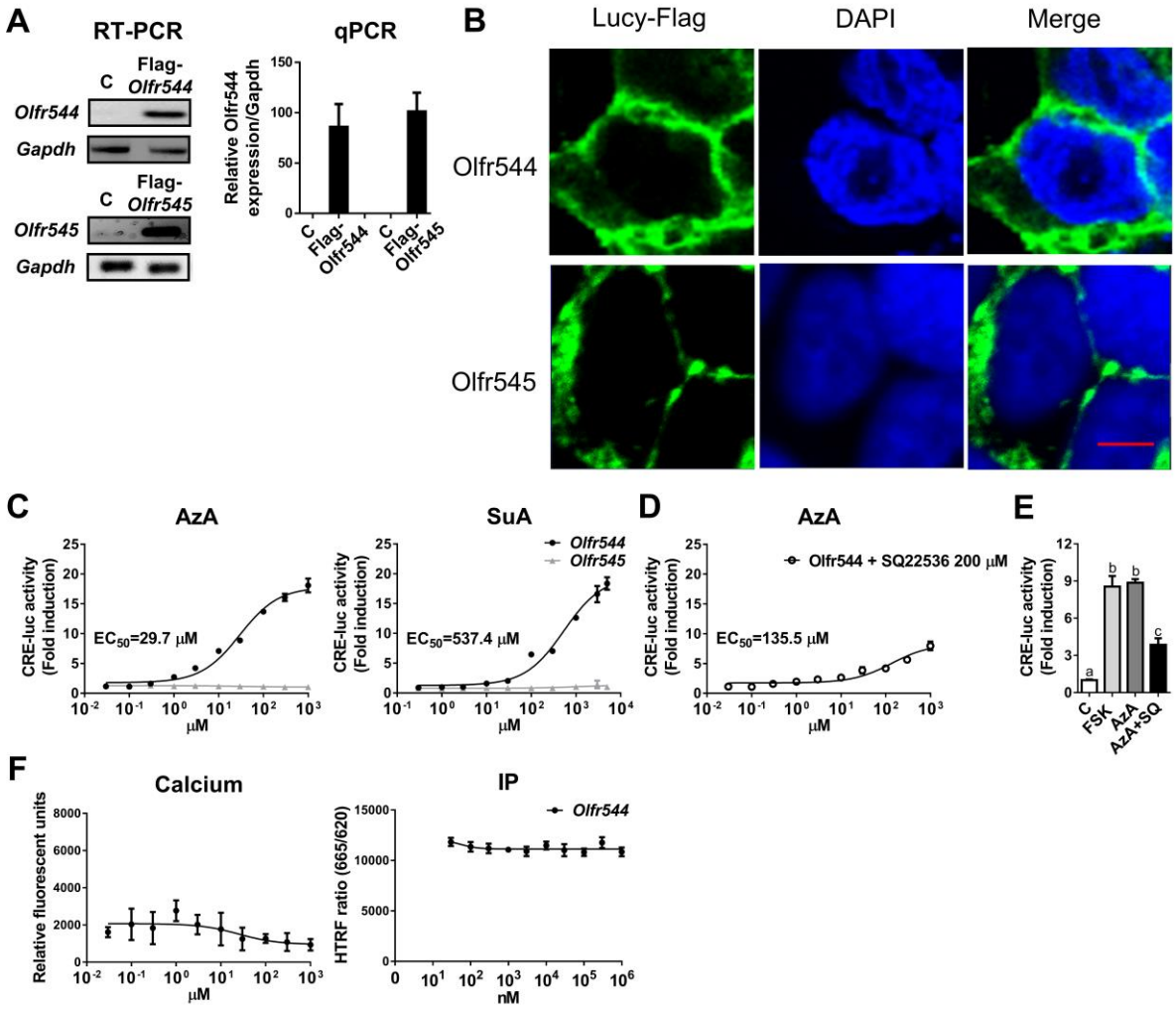

Figure S3. Olfr544 activation by AzA stimulates cAMP response element-binding protein (CREB) activity. (**A**) RT-PCR and qPCR analyses of *Olfr544* and *Olfr545* gene expression in Hana3A cells transfected with N-terminal Lucy-Flag-tagged Olfr544 or Olfr545 expression vector (n=3). (**B**) Immunocytochemistry of Hana3A cells transfected with the N-terminal Lucy-Flag-tagged Olfr544 or Olfr545 expression vector. Scale bar: 5 μm. (**C**) CRE-luciferase activity with AzA and suberic acid in Hana3A cells transfected with N-terminal Lucy-Flag-tagged Olfr544 or Olfr545 expression vector (n=4). **(D)** CRE-luciferase assay with AzA and SQ22536 (200 μM), **(E)** CRE-luciferase assay with AzA (30 μM), FSK (forskolin 1 μM) and SQ22536 (200 μM) in Hana3A cells transfected with N-terminal Lucy-Flag-tagged Olfr544 expression vector (n=3). **(F)** Intracellular calcium (n=3), and inositol phosphates assays (n=6). C, control; AzA, azelaic acid; SuA, suberic acid; SQ, adenylate cyclase inhibitor SQ22536; FSK, adenylyl cyclase activator forskolin; IP, inositol phosphates; HTRF ratio, homogeneous time-resolved fluorescence ratio (665 nm/620 nm). Data are presented as mean ± SEM. One-way ANOVA followed by Tukey's HSD test was performed for multiple group comparisons. Different letters indicate a significant difference at $P < 0.05$.

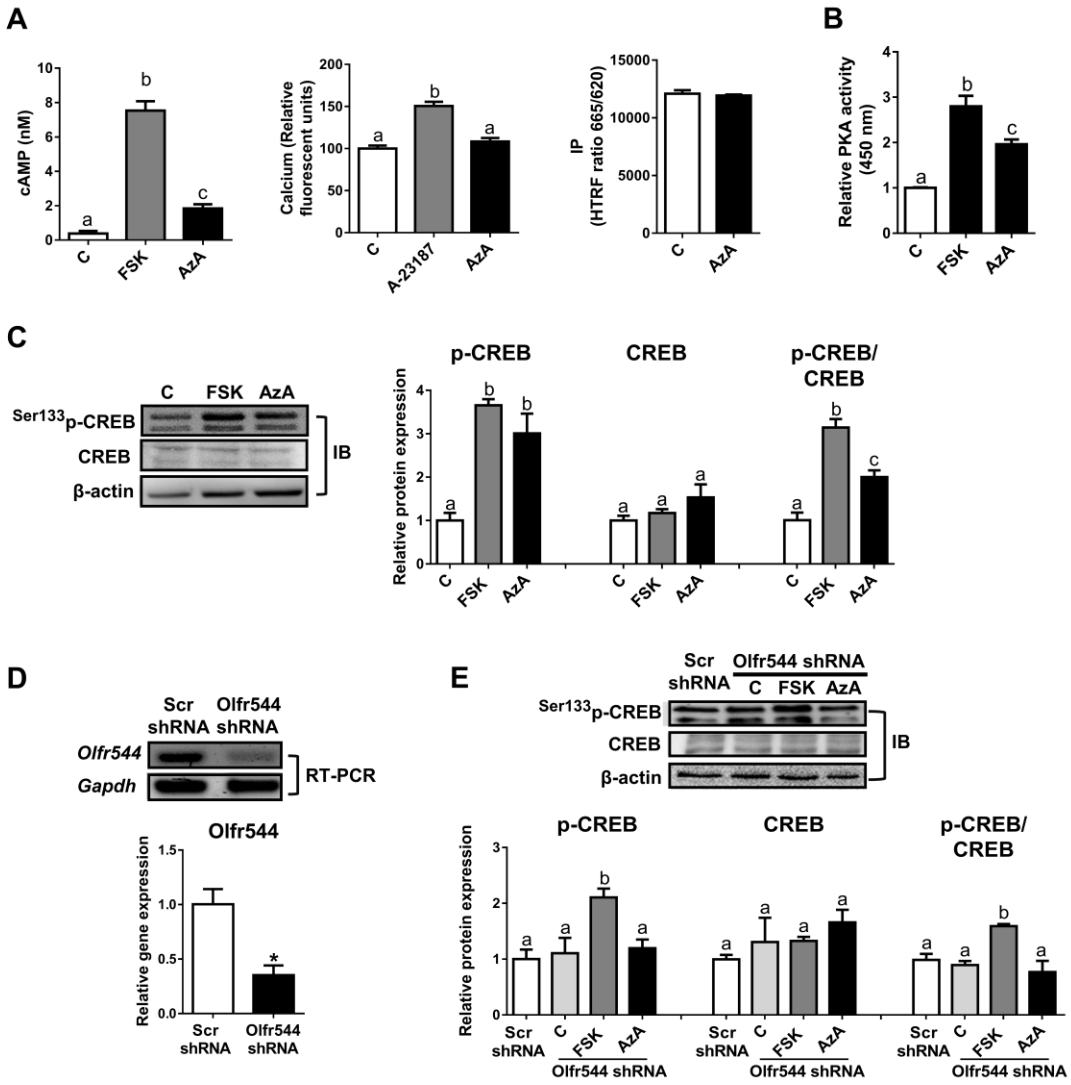

Figure S4. Olfr544 activation by AzA stimulates cAMP-PKA signaling pathway in cultured Hepa1c1c-7 mouse hepatocytes. (**A**) AzA increases cAMP concentrations (n=4), but not intracellular calcium (n=10) and inositol phosphates (IP) levels (n=3). (**B**) PKA activity (n=4). Relative PKA activity was measured using absorbance at 450 nm and normalized with protein concentration. (**C**) Immunoblot analysis of phospho-CREB, CREB, and p-CREB/CREB ratio (n=3). (**D**) *Olfr544* knockdown in Hepa1c1c-7 hepatocytes transfected with shRNA against *Olfr544*. The mRNA level of Olfr544 was determined by RT-PCR analysis (n=3). (**E**) CREB, p-CREB expression, and p-CREB/CREB ratio in Hepa1c1c-7 hepatocytes with *Olfr544* gene knockdown (n=3). C, control; FSK, forskolin 1 μM; AzA, azelaic acid 50 μM; A23187, 10 μM, a calcium-ionophore positive control; HTRF ratio, homogeneous time-resolved fluorescence ratio (665 nm/620 nm). Scr, scrambled shRNA; IB, immunoblot. Data are presented as mean ± SEM. One-way ANOVA followed by Tukey's HSD test and Student t-test were performed for multiple- and two group comparisons. Different letters and asterisk indicate a significant difference at $P < 0.05$ in multiple- and two-group comparisons, respectively.

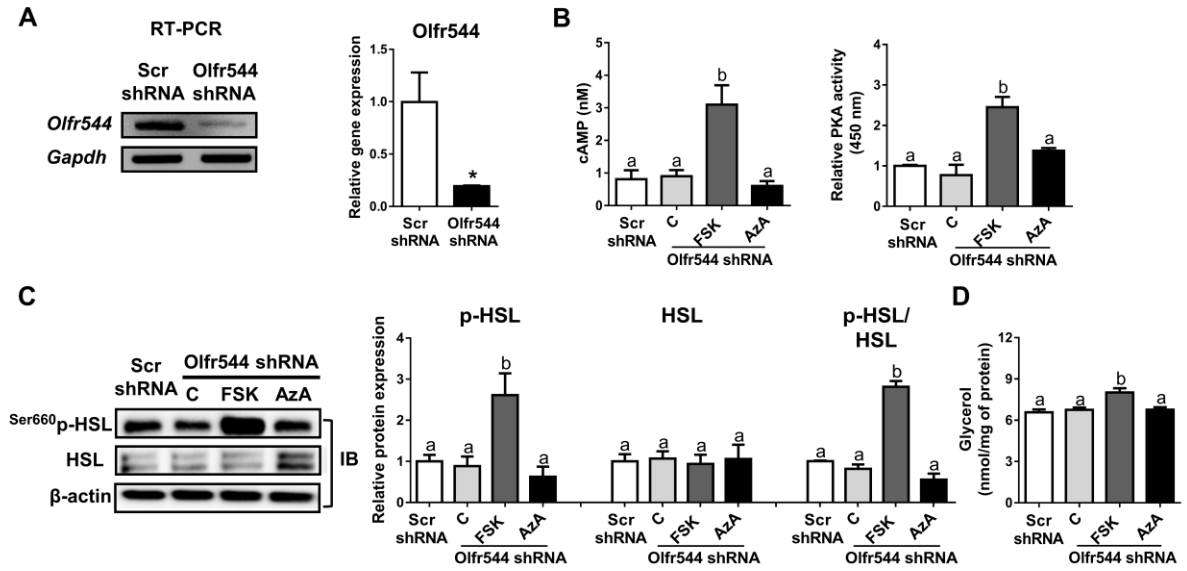

Figure S5. The effect of AzA on lipolysis is dependent on Olfr544 in 3T3-L1 adipocytes. (**A**) *Olfr544* knockdown in 3T3-L1 adipocytes transfected with shRNA against *Olfr544*. The mRNA level of Olfr544 was determined by RT-PCR analysis (n=3). (**B**) Intracellular cAMP levels (n=4) and PKA activity (n=3) in 3T3-L1 adipocytes with *Olfr544* gene knockdown. Relative PKA activity was measured for the absorbance at 450 nm and normalized with protein concentration. (**C**) HSL, p-HSL expression, and p-HSL/HSL ratio (n=3), and (**D**) glycerol release in 3T3-L1 adipocytes with *Olfr544* gene knockdown (n=4). Scr, scrambled shRNA; C, control; FSK, forskolin 1 μM; AzA, azelaic acid 50 μM; IB, immunoblot. Data are presented as mean ± SEM. One-way ANOVA followed by Tukey's HSD test and Student t-test were performed for multiple- and two group comparisons. Different letters and asterisk indicate a significant difference at $P < 0.05$ in multiple- and two-group comparisons, respectively.

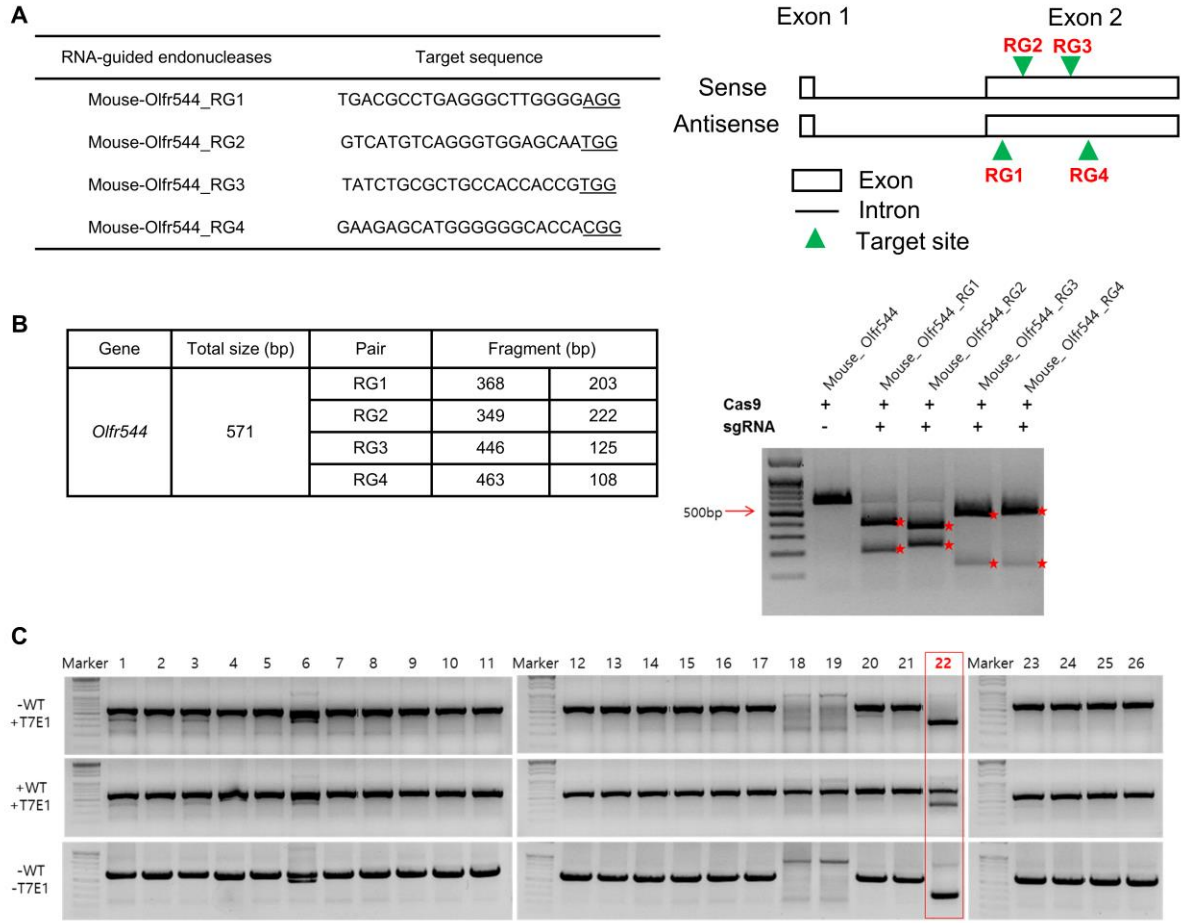

Figure S6. Generation of *Olfr544*$^{-/-}$ mice by CRISPR/Cas9. (**A**) Four single-guide (sg) RNA sequences specific to exon 2 of the *Olfr544* gene were designed, and the underlined sequences indicated the PAM sequences for Cas9 (cleavage site indicated by arrowhead shown on the right side). (**B**) Mouse *Olfr544* in vitro digestion assay with the designed sgRNA and Cas9 protein. The cleaved products by each sgRNA were analyzed by agarose gel electrophoresis. The sizes of the cleaved products are listed in the table (left side). The gel electrophoresis results (right side) revealed that all of these four sgRNA worked well cutting the double-stranded *Olfr544* DNA helix into two fragments. RG1 and RG4 were associated with more cleavage than RG2 and RG3 (larger cleavage, shown in Figure S6A right side) in *Olfr544* gene. Subsequently, the Cas9 protein and sgRNAs (RG1 and RG4) were injected into one-cell embryos and transferred into pseudopregnant recipient mice (C57BL/6J). (**C**) Genotyping scheme of *Olfr544*$^{-/-}$ mice by a T7E1 assay to screen 26 F_0 mice. T7E1 was used to digest heteroduplexes for recognizing and cleaving mismatched DNA. The PCR products were analyzed by agarose gel electrophoresis. Line 6 mouse showed a small deletion, and Line 22 mouse with a large deletion was selected for further experiments.

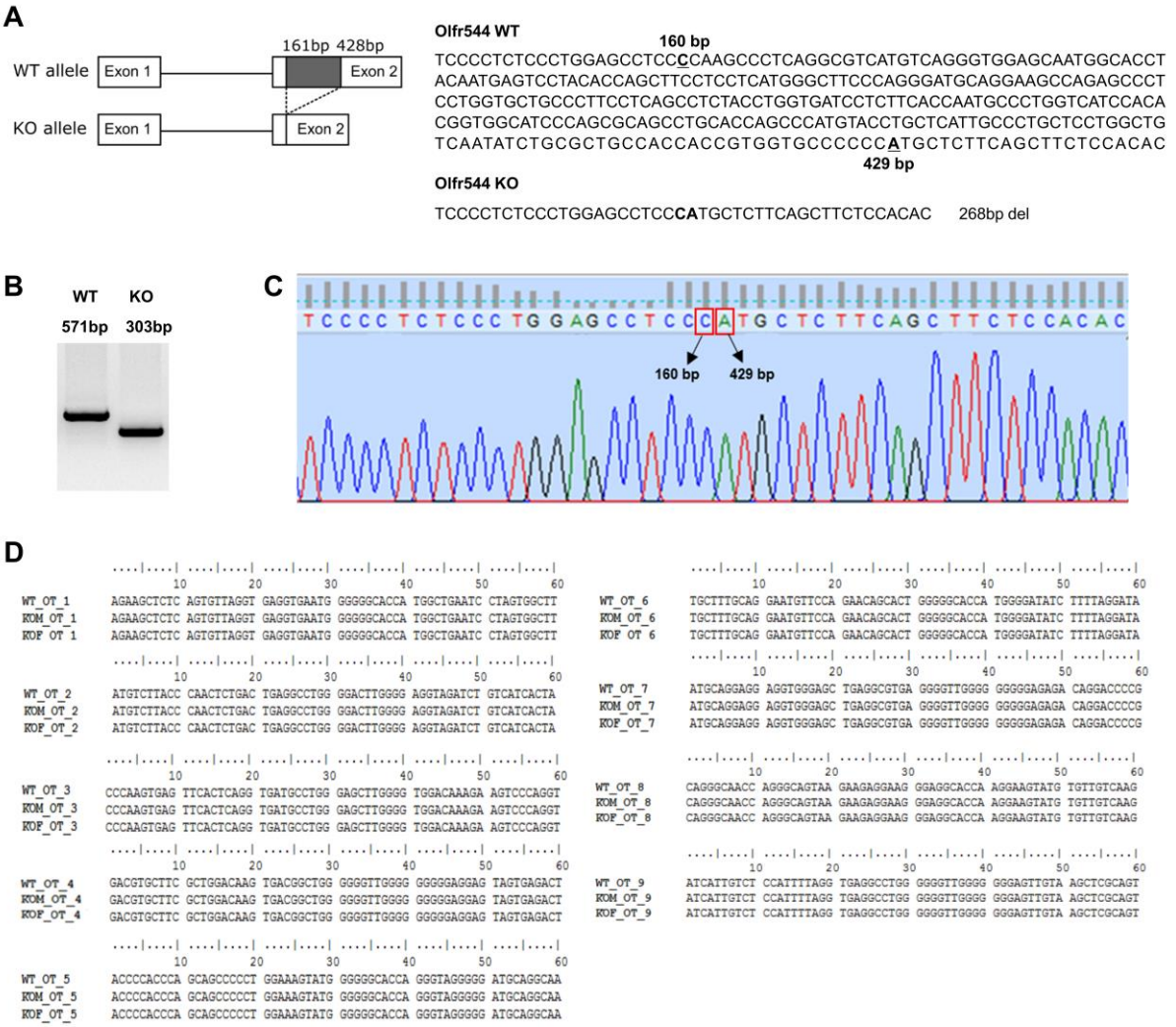

Figure S7. Confirmation of *Olfr544 gene knockout in* mice. (**A**) *Olfr544* gene knockout scheme with CRISPR/Cas9 system. Base pairs 161–428 in exon 2 were deleted. The deleted sequences were shown on right side. (**B**) Genotyping RT-PCR results of WT *Olfr544* and partially deleted *Olfr544*$^{-/-}$ (KO) mice. The PCR product was 571 bp for WT mice, and 303 bp for *Olfr544*$^{-/-}$ mice with 268 bp deletion. (**C**) DNA sequencing results confirmed that 268 bp sequences were deleted in *Olfr544*$^{-/-}$ mice (161-428 bp). (**D**) Analysis of CRISPR/Cas9 off target effects in WT, *Olfr544*$^{-/-}$ male (KOM) and female (KOF) mice. Nine potential off target sites for RNA guided endonuclease were identified using Cas-OFFinder online tool (prediction parameters and the detail information were shown in Supplemental Method). Primers were designed to amplify the sequences which flank the potential off target sites. The amplicons were sequenced by Sanger sequencing. As shown in the sequencing results, positions from 21 to 40 were the off target site, and 20 nucleotides up stream and 20 downstream to the off target site were shown together. No off target effects were found in the corresponding regions of these nine potential off target sites. KOM, *Olfr544*$^{-/-}$ male mouse; KOF, *Olfr544*$^{-/-}$ female mice; OT, off target.

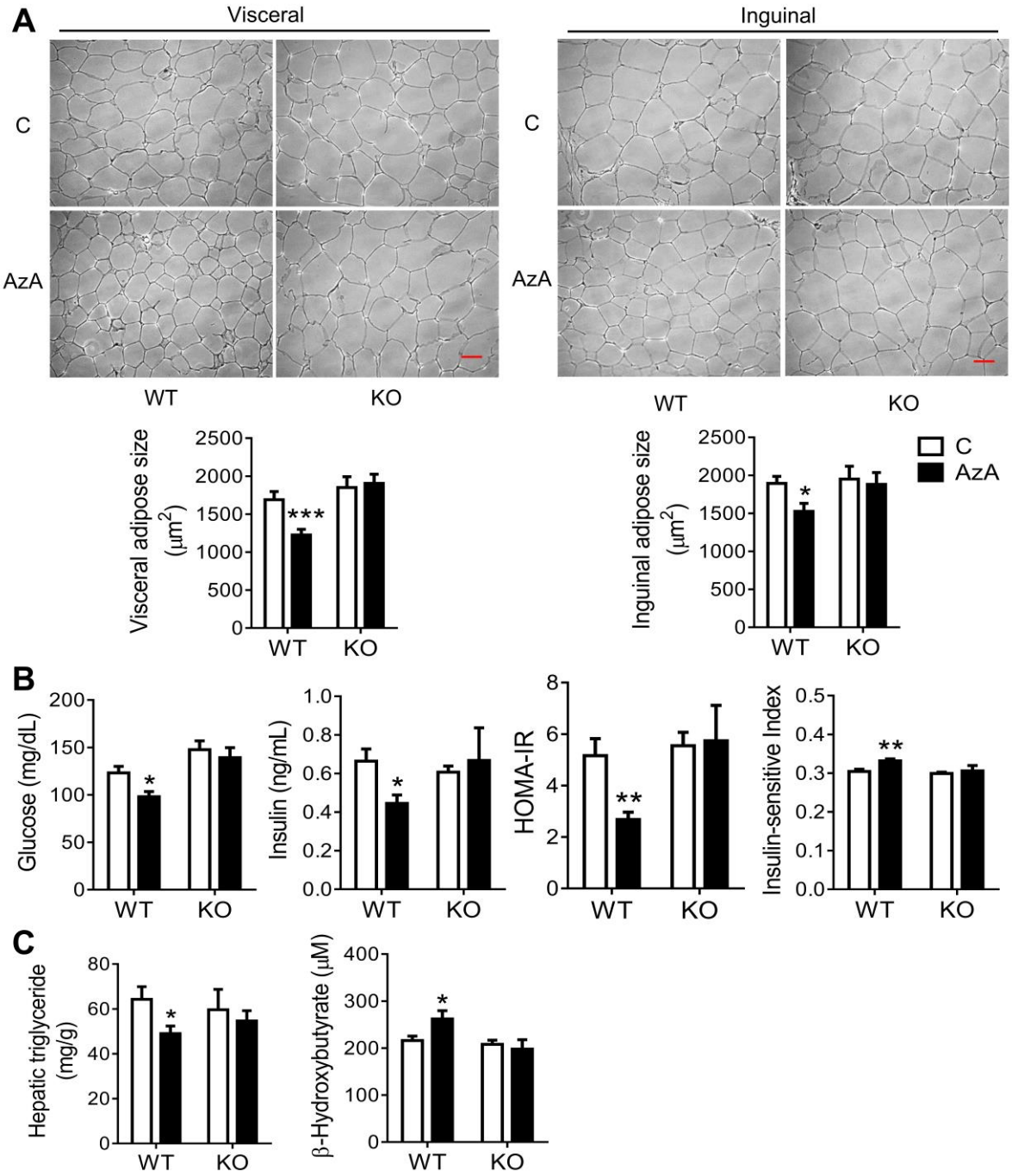

Figure S8. Administration of AzA reduces adiposity and improves insulin sensitivity in HFD-fed WT mice but not in HFD-fed Olfr544 knockout (KO) mice. (**A**) H&E staining and the cell size of visceral and inguinal adipocytes in HFD-fed WT and KO mice (n=3). Scale bar: 50 μm. (**B**) Plasma glucose, insulin, and insulin sensitivity indices, and (**C**) hepatic triglyceride and β-hydroxybutyrate concentrations in HFD-fed WT (n=7–8) and KO (n=5) mice after overnight fasting. C, vehicle control group mice; AzA, mice administering 50 mg/kg body weight AzA; HOMA-IR, homeostatic model assessment-insulin resistance. Data are presented as mean ± SEM. Student's *t*-test or one-way ANOVA followed by Tukey's HSD test was used for comparing two or multiple groups. **P* < 0.05; ***P* < 0.01; ****P* < 0.001 vs. respective vehicle control (Student's *t*-test).

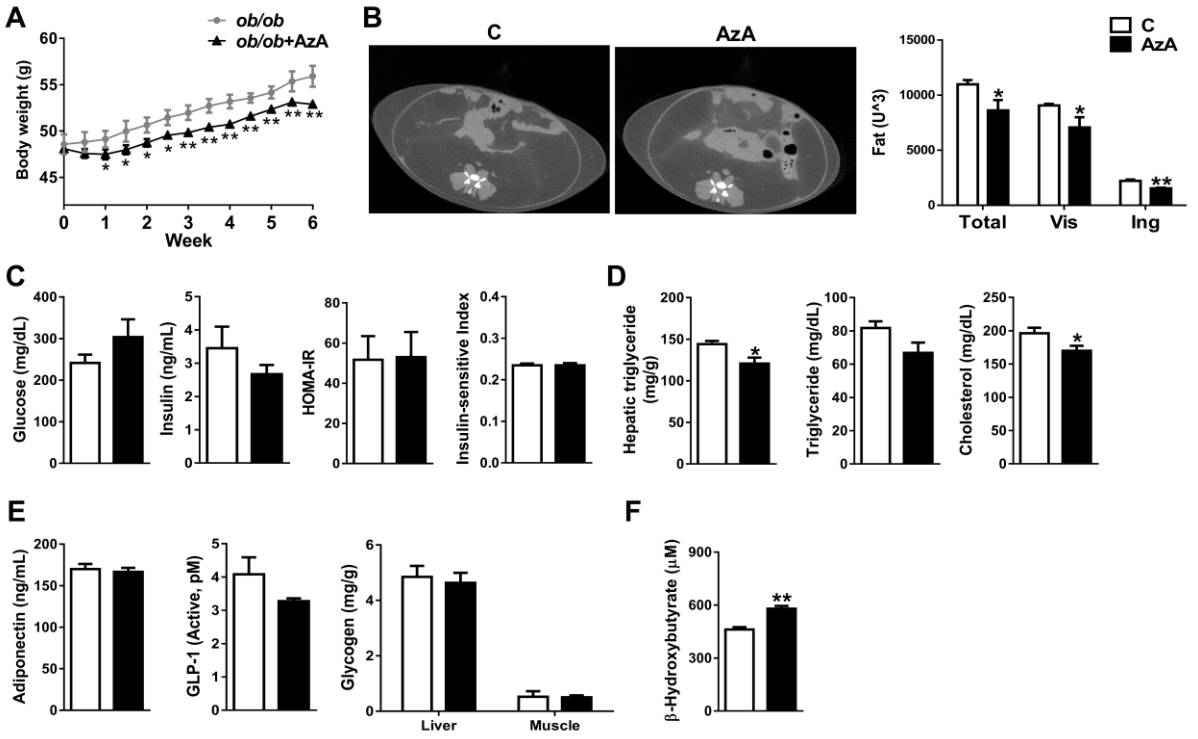

Figure S9. Administration of AzA reduces adiposity in HFD-fed *ob/ob* mice. (**A**) Body weight and (**B**) adipocyte tissue mass assessed by micro-computed tomography (CT, adipose tissues are in dark gray) of HFD-fed *ob/ob* mice (n = 6–7) after AzA administration for 6 weeks (50 mg/kg of body weight). (**C**) Plasma glucose, insulin, and insulin sensitivity indices in HFD-fed *ob/ob* mice (n = 6–7). (**D**) Hepatic triglyceride, plasma triglyceride, and cholesterol levels in HFD-fed *ob/ob* mice (n = 6–7). (**E**) Adiponectin, glucagon-like peptide-1, and glycogen in liver and muscle in HFD-fed *ob/ob* mice (n = 6–7). (**F**) β-hydroxybutyrate concentration in HFD-fed *ob/ob* mice (n = 6–7). C, vehicle control; Vis, visceral fat; Ing, inguinal fat; HOMA-IR, homeostatic model assessment-insulin resistance. Data are presented as mean ± SEM. **P* < 0.05; ***P* < 0.01 vs. controls (Student's t-test).

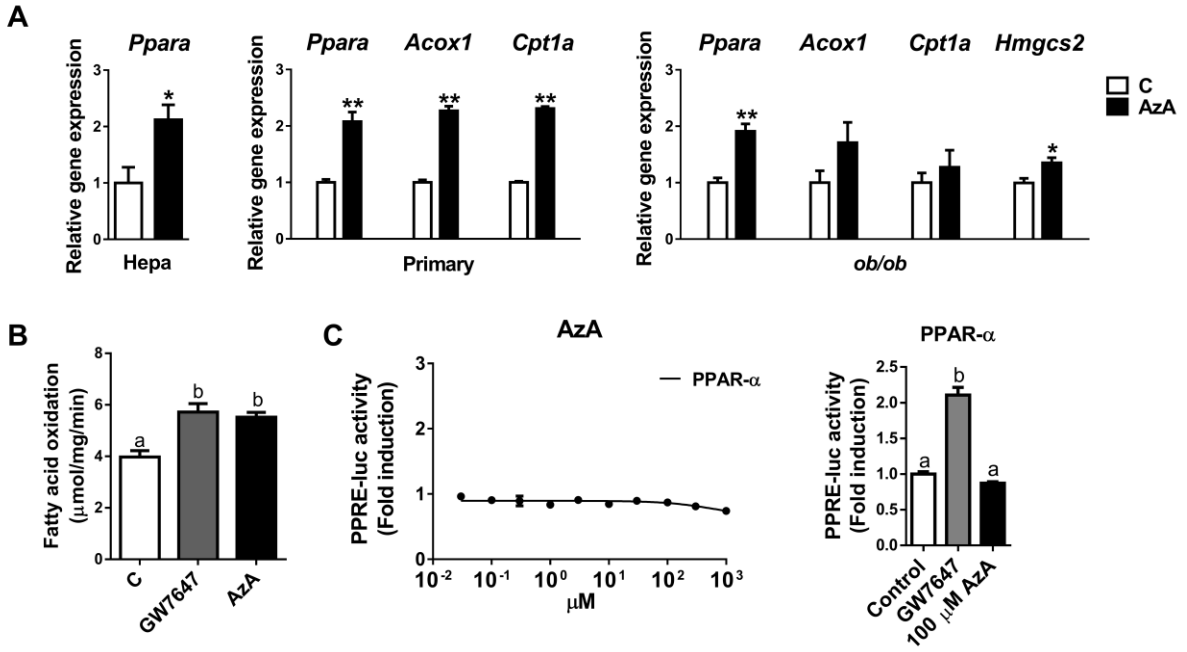

Figure S10. Olfr544 activation by AzA induces hepatic PPAR-α expression and fatty acid oxidation. (**A**) The expression levels of PPAR-α and its target genes in cultured Hepa1c1c-7 hepatocytes (50 μM AzA treated for 24 h), primary hepatocytes (400 μM AzA treated for 6 h), and HFD-fed *ob/ob* mouse livers (50 mg/kg of body weight AzA for 6 weeks) (n=3). (**B**) Fatty acid oxidation in cultured Hepa1c1c-7 hepatocytes (n=4). (**C**) PPRE-luciferase assay of AzA in HEK293T cells (n=3). HEK293T cells were seeded and transfected with pSG5-PPAR-α, pCMV-3xPPRE-Luc and Renilla expression vectors using Lipofectamine 2000. At 24 h post-transfection, cells were stimulated with various concentrations of AzA or 1 μM GW7647 for 24 h, and then luciferase activity was quantified. C, vehicle control; AzA, azelaic acid; GW7647, a PPAR-α agonist (1 μM). One-way ANOVA followed by Tukey's HSD test or Student's t-test was used for multiple- or two-group comparisons. *$P < 0.05$; **$P < 0.01$ vs. controls (Student's t-test). Different letters indicate a significant difference at $P < 0.05$ (ANOVA).

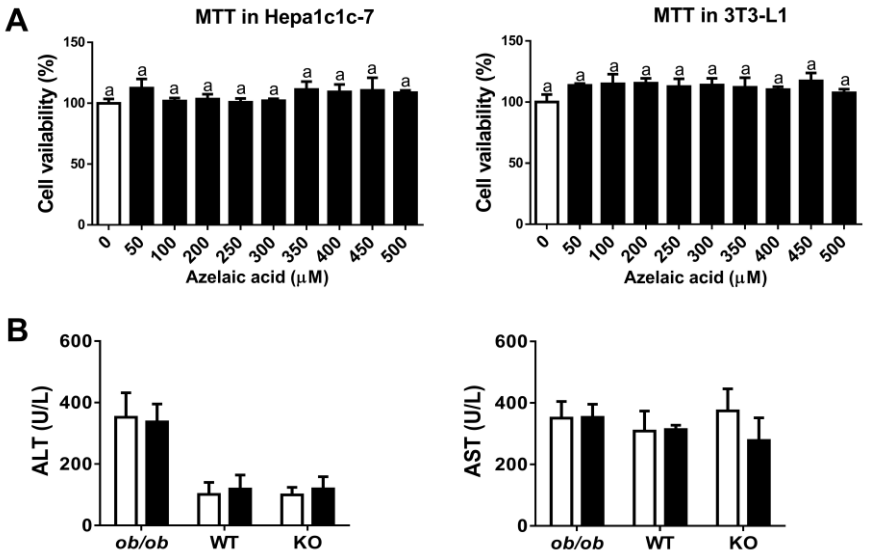

Figure S11. Cytotoxicity and liver enzyme levels. (**A**) Cytotoxicity test of AzA in cultured hepatocyte Hepa1c1c-7 cells, and cultured adipocyte 3T3-L1 cells (n=3). (**B**) Plasma levels of aspartate transaminase (AST) and alanine transaminase (ALT) in HFD-fed *ob/ob* (n=6-7), HFD-fed WT (n=7-8) and HFD-fed Olfr544 knockout (n=5) mice orally administered with AzA (50 mg/kg body weight) for 6 weeks. One-way ANOVA followed by Tukey's HSD test or Student's t-test was used for multiple- or two groups comparisons. Same letter or absence of annotation indicated no significant difference in multiple- or two-group comparisons.

Supplemental Methods

Antibodies and Reagents

Azelaic acid, suberic acid and forskolin were purchased from Sigma (St.-Louis, MO, USA). Primary antibodies against β-actin (SC-47778, 1:500), α-tubulin (SC-5286, 1:500), HSL (SC-74489, 1:1000), CREB (SC-186, 1:500), p-CREB (Ser133; SC-101663, 1:500), and FLAG (SC-807, 1:1000) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-HSL (4107S, 1:1000) and anti-p-HSL (Ser-660; 4126S, 1:1000) were purchased from Cell Signaling (Danvers, MA, USA). Anti-mouse (31430) and anti-rabbit (31460) immunoglobulin G secondary antibodies (1:5000), anti-rabbit secondary antibody (Alexa Fluor 488; A11008, 1:1000), and DAPI were from Invitrogen (Carlsbad, CA, USA). Antibodies were used in immunoblot and immunocytochemistry analyses with a referred dilution in the parenthesis.

Cell Culture

Hana3A cells (a gift from Dr. Hiroaki Matsunami's laboratory) were cultured in Eagle's minimal essential medium with Earle's balanced salt solution (Hyclone, Logan, UT, USA) with 10% fetal bovine serum (FBS, Hyclone) and 1% penicillin/streptomycin (PEST, Welgene Inc., Korea). Hepa1c1c-7 and 3T3-L1 cells are cultured mouse hepatocytes and adipocytes, which were obtained from the Korean Cell Line Bank (Seoul, Korea). Hepa1c1c-7 cells were cultured in minimum essential medium Eagle alpha modification medium (MEM-α, Hyclone) containing 10% FBS and 1% PEST. 3T3-L1 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Hyclone, Logan, UT, USA) containing 10% heat-inactivated bovine calf serum (Gibco, Grand Island, NY, USA) and 1% PEST. To induce adipogenic differentiation, cells were stimulated with insulin (10 μg/mL), dexamethasone (1 μM), and 3-isobutyl-1-

methyl-xanthine (0.5 mM) for 48 h. Then, the cells were cultured in DMEM with 10% FBS and insulin (10 µg/mL) for an additional 9 days. Cells were maintained in a humidified atmosphere of 5% CO_2 at 37°C.

Lipid Analysis and Hormones Measurements in 3T3-L1 cells and mice, Measurements of Second Messengers and PKA Activity Assay in 3T3-L1 cells and Hepa1c1c-7 cells

Triglyceride concentrations were enzymatically determined using the Triglyceride Quantification Colorimetric/Fluorometric Kit (BioVision, Milpitas, CA, USA) in mouse plasma and 3T3-L1 cells. Cholesterol levels were measured using an Amplex Red cholesterol assay (Invitrogen) in mouse plasma and 3T3-L1 cells. Glucose concentrations, plasma alanine transaminase and aspartate transaminase levels were analyzed with a Cobas C111 autoanalyzer (Roche, Basel, Switzerland) in mouse plasma samples. cAMP concentrations were assayed using a fluorimetric ELISA kit (AAT Bioquest, Sunnyvale, CA, USA), inositol phosphates were measured with a HTRF IP-one Tb kit (Cisbio, Bedford, MA, USA), intracellular calcium was quantified with Fluo-4 Direct Ca^{2+} reagent (Invitrogen) and PKA kinase activity was measured using a commercial kit from Enzo Life Science (Farmingdale, NY, USA) in Hepa-1c1c1 and 3T3-L1 cells. PKA kinase activity was measured according to the manufacturer's instructions. Briefly, cells were seeded overnight and treated with 50 µM AzA or 1 µM forskolin for 30 min. Then, PKA kinase activity was assayed based on a solid-phase enzyme-linked immuno-absorbent assay that utilizes a specific synthetic peptide as a substrate for PKA and a polyclonal antibody that recognizes the phosphorylated form of the substrate. Color develops in proportion to PKA phosphotransferase activity. Color development is stopped with an acid stop solution and color intensity is measured in a microplate reader at 450 nm. Relative PKA kinase activity was normalized to protein levels in the cell lysates. Plasma insulin

(Millipore, Bedford, MA, USA), adiponectin (Abcam, Seoul, Korea), β-hydroxybutyrate (Cayman, Ann Arbor, MI, USA), GLP-1 (Millipore), and glycogen (Cayman) in liver and muscle of mice were quantified by ELISA kits according to the manufacturer's instructions. The homeostatic model assessment - insulin resistance (HOMA-IR) index was calculated according to this formula: $\mathrm{HOMA-IR} = \frac{\text{Glucose x Insulin}}{405}$ (Glucose in mass unit of mg/dL). The insulin-sensitive index was derived using the inverse of the sum of the logarithms of the fasting insulin and fasting glucose following this formula: 1 / (log(fasting insulin μU/mL) + log(fasting glucose mg/dL)).

Immunoblot

Total cellular proteins were extracted using radioimmunoprecipitation assay (RIPA) buffer supplemented with Halt protease and phosphatase inhibitor reagent (Thermo Fisher Scientific, Carlsbad, CA, USA) at 4°C. The soluble protein fraction was obtained after centrifugation for 10 min at 13,000 rpm. Protein concentration was determined with a bicinchoninic acid protein assay kit (Pierce Biotechnology, Rockford, IL, USA). The denatured proteins were run on a sodium dodecyl sulfate polyacrylamide gel (10 %), and the separated proteins were transferred to the nitrocellulose membranes (Daeillab, Seoul, Korea). The membranes were blocked in TBS with 5% (w/v) non-fat dried milk and probed with specific antibodies in TBS with 0.1% Tween-20 and 5% non-fat dried milk. Immunoblot images were obtained with a ChemiDoc touch imaging system and analyzed with the Image Lab 5.2 software (Bio-Rad, Redmond, WA, USA). Protein expression levels were normalized to β-actin or α-tubulin expression.

Immunocytochemistry

Hana3A cells were seeded on glass coverslips for 24 h and transfected with Lucy-Flag-tagged Olfr544 or Olfr545 vector using Lipofectamine 2000 (Invitrogen) for 24 h. The cells were washed twice with PBS and fixed with 4% paraformaldehyde for 10 min. Fixed cells were incubated with 5% bovine serum albumin (BSA) in PBS for 45 min, and then incubated with anti-Flag antibody (1:200; Santa Cruz) overnight at 4°C. Alexa Fluor 488-tagged anti-IgG secondary antibody (1:1000; Invitrogen) was incubated for 1 h in the dark. After washing, the nuclei were stained with DAPI (Invitrogen, CA, USA) for 5 min. Then, the coverslips were embedded in anti-fade mounting solution (Thermo Fisher Scientific). Image analysis was performed using a LSM510 META confocal microscope and the LSM700 version 3.2 software (Carl Zeiss, Jena, Germany). Lucy-Flag-Olfr544 or Olfr545 was stained in green and nuclei were stained in blue.

RT-PCR and qPCR Analyses

Total RNA from cells and tissues were isolated using RNAiso Plus reagent (Takara Bio, Otsu, Japan) and then 1 μg RNA was used to synthesize cDNA by using a ReverTra Ace® RT Master Mix kit (Toyobo, Osaka, Japan) according to the manufacturer's instructions. RT-PCR was performed with Emerald Amp® GT PCR Master Mix reagent according to the manufacturer's instructions. The RT-PCR products were analyzed by running on agarose gel electrophoresis (2%–4%) for 25 min and Image Lab 5.2 software (Bio-Rad, PA, USA) was used for image analysis of the separated bands. qPCR was performed with the Thunderbird SYBR® qPCR Mix reagent (Takara Bio) on an iQ5 Cycler System (Bio-Rad) according to the manufacturer's instructions. Amplification was performed using an initial denaturation step at 95 °C for 30 s, followed by 50 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 20 s, and extension at 68 °C for 20 s. The fluorescent signal was quantified automatically at the end of

each PCR cycle. Primer sequences used to amplify the genes of interest are presented in Supplemental Table 2. The expression levels were calculated with the threshold cycle (CT) method according to the manufacturer's guidelines and normalized by *Gapdh* expression level.

Cloning of Lucy-Flag-tagged Olfr544 and Olfr545 and CRE-luciferase Assay

Lucy-Flag-tagged pME18s vector was obtained from Dr. Jeniffer Pluznick (Johns Hopkins University, Baltimore, MD, USA). The full-length cDNA sequence of Olfr544 or Olfr545 (Origene, Rockville, MD, USA) was cloned into the vector using *EcoR*I and *Xho*I restriction enzymes (New England Biolabs, Ipswich, MA, USA) followed by ligation. The Lucy-Flag-tagged Olfr544 or Olfr545 pME18s vector was co-transfected with CRE-luciferase reporter, and Renilla expression vectors into Hana3A cells (a gift from Dr. Hiroaki Matsunami from Duke University, Durham, NC, USA) with Lipofectamine 2000 (Invitrogen). At 24 h post-transfection post transfection, the cells were stimulated with various concentrations of AzA for 18 h. For experiment of adenylyl cyclase inhibitor, cells were pretreated with 200 μM SQ22536 for 18 h, and then treated with various concentrations of AzA for 18 h in the presence of SQ22536. Luciferase activity was assayed with a dual luciferase assay kit (Promega, Fitchburg, WI, USA). The luminescence of the samples was quantified with Victor X2 (PerkinElmer, Santa Clara, CA, USA). The firefly luminescence signal was normalized to that of *Renilla*.

PPRE-luciferase assay of AzA in HEK293 cells

HEK293 cells were cultured in DMEM (Hyclone) medium containing 10% FBS and 1% PEST. Cells were seeded in 24-well plates at a density of 2×10^5/well. pSG5-PPAR alpha (Addgene, MA, USA) was co-transfected with pCMV-3xPPRE-Luc, Renilla expression vectors into HEK293T cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's

instructions. At 24 h post-transfection, cells were stimulated with various concentrations of AzA for 24 h, and luciferase activity was quantified with a dual luciferase assay kit (Promega, Fitchburg, WI, USA) with Victor X2 (PerkinElmer, Santa Clara, CA, USA). The firefly luminescence signal was normalized to that of *Renilla*.

shRNA-mediated *Olfr544* Knockdown

Cells were maintained in a humidified atmosphere of 5% CO_2 at 37°C. Oligonucleotides encoding shRNA hairpin sequences targeting Olfr544 (top strand: 5′-CACCGCTCACTGTTCGCATCTTCATTCGAAAATGAAGATGCGA-ACAGTGAG-3′) or encoding non-targeting scrambled shRNA hairpins (top strand: 5′-CACCGTAAGGCT-ATGAAGAGATACCGAAGTATCTCTTCATAGCCTTA-3′) were inserted into the shRNA cloning site of the pENTR/U6 vector using the Block-iT U6 RNAi entry vector kit (Invitrogen). Hepa1c1c-7 and 3T3-L1 cells were seeded in 6-well plate and then transfected with 2.5 μg Olfr544 shRNA or scrambled shRNA using 10 μL Lipofectamin2000 (Invitrogen) for 48 h. Experiments were carried out with or without 50 μM azelaic acid or 1 μM forskolin, respectively.

Mouse Experiments

Eight weeks old C57BL/6J WT and *ob/ob* male mice were purchased from Samtako (Gyeonggi-do, Korea) and *Olfr544*$^{-/-}$ mice generated with CRISPR/Cas9 system in the genetic background of C57BL/6J mice (Supplemental Figure 6) were from Macrogen Company (Seoul, Korea). All experiments were conducted according to protocols approved by the Animal Experiment Committee of Korea University (Protocol No. KUIACUC-20090420-4). Mice were maintained under a 12-h photoperiod at 21–25°C and a relative humidity of 50%–60%.

Mice were fed on 60 % HFD for 6 weeks with oral feeding of 50 mg/kg body weight AzA or distilled water as vehicle control. HFD used in this study is rodent diet containing 60% of calories from fat (D12492, Research Diets, Inc). After 6 weeks, mice were sacrificed after overnight fasting. Food intake was measured following a conventional method (1) with careful consideration of accuracy and reproducibility (2). Each mouse was caged individually and food intake was measured during a 24-h period. Weighed portion of food was given in hoppers soon after lights-on after normal nocturnal feeding and intake measured during a 24-h period. Food intake (g/kg body weight) was measured as dry mass content and was calculated as [(food given*dry mass content) – (dry food uneaten)]/kg mouse. Since we were unable to collect the finely crumbled orts, our food consumption values may be slightly overestimated. Day-time food intake detects a strong stimulus to see an effect, thus marginal effect of AzA on food intake could be slightly underestimated. Water was provided ad libitum.

Blood was collected in EDTA tubes (BD Vacutainer) retroorbitally or by cardiac puncture, centrifuged at 300*g for 20 min at 4°C to collect plasma samples, and stored at -80°C. Total cholesterol, triglyceride, glucose insulin, adiponectin, GLP-1, and β-hydroxybutyrate levels were quantified from mouse plasma. Liver and adipose tissues were collected, frozen in liquid nitrogen, and stored at -80°C. The interscapular brown adipose tissue was isolated from mice as previously described (3).

Measurement of Lipolysis

In vitro lipolysis was determined in 3T3-L1 adipocytes after 2-h treatment with azelaic acid (50 μM) or forskolin (1 μM). Glycerol concentrations were measured in the culture media. In vivo lipolysis was measured after a 16-h fast and following i.p. injection of 100 mg/kg body weight AzA or PBS vehicle. Plasma glycerol concentrations were quantified at 45 min after

injection. Glycerol concentrations were enzymatically determined using the PicoProbe free glycerol fluorometric assay kit (BioVision).

Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT)

OGTT was performed in HFD-fed mice after overnight fasting. The mice were orally administered glucose (1.5 g/kg body weight) or distilled water as vehicle control. For ITT, mice were fasted for 4 h and i.p. injected with insulin (0.35 unit/kg body weight) or PBS as vehicle control. The blood glucose concentrations were measured at 0, 15, 30, 60, 90, and 120 min after feeding of glucose or injection of insulin using a portable glucometer (Accu-Check Go, Roche).

Micro-computed Tomography (CT)

After a 6-week administration of 50 mg/kg body weight azelaic acid, the adipocyte tissue masses of HFD-fed WT, *Olfr544*$^{-/-}$ and *ob/ob* mice were assessed by CT using the SkyScan 1176 (SkyScan, Kontich, Belgium) set at 40 kV and 600 µA. Three-dimensional reconstruction resulted in data sets with 3-µm pixel resolution that were analyzed using the CTAn software (Skyscan) using post-threshold-based segmentation. The thresholds for fat, soft tissue, and bone were 65-97, 98-182, and 183-255, respectively. Visceral and inguinal fat tissues were separated on the basis of region-of-interest (ROI): the fats inside the ROI were identified as visceral fats and the fats outside the ROI were inguinal fats. Visceral fat mass was calculated from the bone of spines from 15 to 20, while inguinal fat mass was calculated from the whole body.

Histological analysis

After overnight fasting, mice were sacrificed and the mice visceral, inguinal and interscapular brown adipose tissues were fixed with 4% paraformaldehyde and then stained by hematoxylin and eosin (H&E) at the Histopathology Department of Anam Korea University Hospital (Seoul, Korea). The images were obtained with Carl-Zeiss microscope (Oberkochen, Germany) and software (Axio Imager M1, Carl-Zeiss, Germany). The adipocyte sizes were assessed using an Axio Imager M1 microscope (Carl-Zeiss, Oberkochen, Germany).

Quantification of hepatic triglyceride

Hepatic triglycerides were extracted from HFD-fed WT, *Olfr544*$^{-/-}$ and *ob/ob* mice livers. Briefly, fifteen mg of mice livers were weighted and homogenized in 600 μL acetone (Deajung, Seoul, Korea), then the samples were agitated and incubated at 4°C overnight. Samples were centrifuged at 12000*g for 10 min to collect the lipid fractions. The lipid fractions were dried and dissolved in 95% ethanol. Hepatic triglyceride concentrations were quantified with a Cobas C111 autoanalyzer (Roche, Basel, Switzerland) and normalized with the liver weight.

Generation of *Olfr544*$^{-/-}$ mice

Olfr544$^{-/-}$ mice were produced using the CRISPR-Cas9 system. First, four single-guided (sg) RNA sequences specific to exon 2 of the *Olfr544* gene were designed: sgRNA RG1 targeted to the sequence of exon 2, 5′-GTCATGTCAGGGTGGAGCAATGG-3′; sgRNA RG2 targeted to the sequence of exon 2, 5′-TGACGCCTGAGGGCTTGGGGAGG-3′; sgRNA RG3 targeted to the sequence of exon 2, 5′- TATCTGCGCTGCCACCACCGTGG-3′; and sgRNA RG4 targeted to the sequence of exon 2, 5′- GAAGAGCATGGGGGGCACCACGG-3′ (Supplemental Figure 6A). The DNA-cleavage activities of the sgRNAs complexed with recombinant Cas9 protein were first evaluated in vitro with a digestion assay. Briefly, PCR-amplified Olfr544 was

incubated with 300 ng of Cas9 protein and 150 ng of sgRNA in NEB buffer 3 at 37°C for 90 min. Then, 1 μL of RNase was added and incubated at 37°C for 20 min. The reaction was stopped by adding stop solution containing 30% glycerol, 1.2% SDS, and 100 mM EDTA and incubated at 37°C for 20 min. The cleaved products by each sgRNA were analyzed by agarose gel electrophoresis. The sizes of the cleaved products are listed in the Supplemental Figure 6B. The gel electrophoresis results revealed that all of these four sgRNA worked well cutting the double-stranded *Olfr544* DNA helix into two fragments (Supplemental Figure 6B). RG1 and RG4 were associated with more cleavage than RG2 and RG3 (larger cleavage, shown in Figure S6A right side) in *Olfr544* gene. Subsequently, the Cas9 protein and sgRNAs (RG1 and RG4 with large cleavage) were injected into one-cell embryos and transferred into pseudopregnant recipient mice (C57BL/6J). To screen the 26 F_0 mice, T7 endonuclease 1 (T7E1) assays were performed with mouse tail DNA samples as described previously (4). Briefly, the target genomic region was amplified by PCR. The PCR products were denatured and reannealed to form a heteroduplex DNA between WT DNA and CRISPR-Cas9-mutated DNA (10 μL), which was treated with T7E1 (0.2 μL; Toolgen, Seoul, South Korea) in NEB buffer 2 at 37°C for 20 min. T7E1 was used to digest the heteroduplexes to recognize and cleave the mismatched DNA. Then, the PCR products were analyzed by agarose gel electrophoresis (Supplemental Figure 6C). *Olfr544* mutant alleles were further confirmed by sequencing and most featured small deletions. Among the 26 F_0 mice, the *Olfr544* mutant male mouse with a large 161–428-bp deletion in exon 2 (Line 22) was selected for further experiments after backcrossing five times with C57BL6J mice (Supplemental Figure 7). The following primers were used for genotyping: forward primer 5′-AAAAAGAAGGGGGAAAATGG-3′ and reverse primer 5′-GACCAGGAGGATGTTGCAGT-3′.

Genomic DNA (gDNA) extraction from WT and *Olfr544*$^{-/-}$ mice

The gDNA was isolated from liver tissues of WT male mice and *Olfr544*$^{-/-}$ female and male mice using the RNAiso Plus reagent (Takara Bio, Otsu, Japan), in accordance with the manufacturer's instructions as described previously (5-6). The method using RNAiso Plus solution provided high-purity gDNA for DNA sequencing. *Olfr544*$^{-/-}$ female and male were F5 mice after backcrossing with C57BL/6J mice. Briefly, 100 mg of liver tissue was homogenized in 1 mL of RNAiso Plus, and the homogenized sample was incubated for 5 min at room temperature. Then, 0.2 mL of chloroform was added to each sample, and the samples were centrifuged at 12,000 × g for 15 min at 4°C. The interphase and organic phenol-chloroform phase were used to isolate the gDNA. A 0.3-mL aliquot of ethanol was added to each sample, and the samples were centrifuged at 2,000 × g for 5 min at 4°C to precipitate the gDNA. The gDNA pellet was washed twice with 1 mL of sodium citrate/ethanol solution (0.1 M sodium citrate in 10% ethanol, pH 8.5) and washed once with 1.5 mL of 75% ethanol. The gDNA pellet was air-dried for 10 min and resuspended in 100 µL of autoclaved distilled water. gDNA concentration and purity were analyzed with the Multiskan GO instrument (Thermo Scientific, Waltham, MA, USA) for analysis of CRISPR/Cas9 off target effects in WT, *Olfr544*$^{-/-}$ male and female mice.

Analysis of CRISPR/Cas9 off-target effects in *Olfr544*$^{-/-}$ mice

Potential off-target sites for RNA-guided endonuclease were identified using the Cas-OFFinder (http://www.rgenome.net/cas-offinder/) online tool. The off-target prediction parameters are as follows: RGEN–SpCas9 from *Streptococcus pyogenes*: 5′-NGG-; maximum mismatch number = 3, DNA or RNA bulge size = 0; target genome = *Mus musculus* (10 mm). Total output comprised 34 potential off-target sites. Among them, sites in which the last six bases in the 3′-

region of the protospacer were conserved and 5′-regions with up to three mismatches were filtered out. Nine potential off-target sites were summarized as follows. Lower-case letters indicate mismatches in the off-target sites, as shown in Supplemental Table 3.

The primers were designed to amplify the sequences flanking the potential off-target sites. Each primer was included with an adapter sequence used in the sequencing step. Upper-case letters correspond to the adapter sequence and lower-case letters correspond to the annealing sequence, as shown in Supplemental Table 4.

Each off-target site was amplified using gDNA extracted from WT, *Olfr544*$^{-/-}$ male and female mice. The PCR amplification reaction contained 1.0 unit of Taq polymerase, 1× Taq polymerase buffer, 200 μM dNTP (all from Takara Bio), and forward and reverse primers (0.25 μM each) in a 25-μl reaction volume. Amplification was performed using an initial denaturation step at 95°C for 30 s, followed by 25 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 45 s, and a final extension step at 72°C for 5 min. The amplicons were sequenced by Sanger sequencing (Cosmogenetech, Seoul, South Korea). Sequence positions 21–40 were the off-target site; 20 nucleotides upstream and 20 downstream from the off-target site are shown together in the sequencing results (Supplemental Figure 7D).

Liver fatty acid oxidation

Fatty acid oxidation in HFD-fed mouse liver was assessed by homogenizing 100 mg livers in 9 volume of cold 0.25 M sucrose, and centrifuged at 600*g for 10 min at 4°C to collect the supernatant. Then 50 μL of 10% Triton X-100 was added to 450 μL supernatants, and then 5 μL of sample was mixed with 950 μL of 50 mM Tris-HCl (pH8.0), 10 μL of 20 mM NAD, 3

µL of 0.33 M Dithothreitol, 5 µL of 1.5% BSA, 5 µL 2% Triton X-100, 10 µL of 10 mM CoA, and 10 µL of 1 mM FAD. Reaction was initiated by adding 2 µL of 5 mM palmitoyl-CoA at 37°C, and the signal was determined by a spectrophotometer at wave length of 340 nm for 5 min. The rate of palmitoyl-CoA oxidation was directly related to the rate of fatty acid oxidation in the liver.

Cell Viability Test

Viability was assayed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Hepa1c1c-7 and 3T3-L1 cells were seeded in 96-well plates and cultured for 24 h. The cells were then treated with increasing concentrations of azelaic acid up to 500 µM. After 24 h, the culture medium was removed, and 1 ml of culture medium with 10% MTT solution was added and incubated for 3 h. The MTT solution was removed and the cells were dried. Then, 1 ml of dimethyl sulfoxide was added and incubated for 2 h with mild shaking. The absorbance at 570 nm was measured in a Multiskan GO microplate spectrophotometer (Thermo Fisher Scientific, Carlsbad, CA, USA).

Indirect Calorimetry

C57BL/6J mice and *Olfr544*$^{-/-}$ were fed on 60% high-fat diet and were orally administered 50 mg/kg body weight/day azelaic acid, the same volume of distilled water was given to the vehicle control group for 6 weeks. Indirect calorimetry was performed for 3 days in the metabolic cages and the data on the 3rd day was used for analysis. Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured using the Oxylet Physiocage System (Panlab/Harvard apparatus, Cornella, Spain) and the software suite METABOLISM (V2.2.01, Panlab). The respiratory quotient (RQ) was calculated as VCO_2/VO_2 and energy expenditure

was calculated according to the following formula: EE = VO_2*1.44*(3.815 + 1.232*RQ). Fatty acid oxidation was calculated according to the following formula: (1.6946.VO_2)-(1.7012*VCO_2).

Microarray

C57BL/6J mice were fed on normal or 60% high-fat diet for 4 weeks prior to isolate tissues. Total RNA from liver and adipose tissues was isolated using RNAiso Plus reagent (Takara Bio, Otsu, Japan). The RNA quality was evaluated using a NanoDrop spectrophotometer (Thermo Scientific, USA) and Experion Automated Electrophoresis Station (Bio-Rad, PA, USA) using Experion RNA Stdsens chips (Bio-Rad, PA, USA). From the NanoDrop results, concentration, 260/280 ratio of the RNA samples were determined and the electropherograms of the Experion data were compared visually with the control samples assessing the ratio of 28S:18S and RNA integrity number. Samples were labeled with Cy3 (ND) and Cy5 (HFD) during reverse transcription and hybridized on a Agilent Mouse GE (V2) 4 X 44K microarray (39,473 probes). Data were normalized with LOWESS method and corrected with backgroud to select 24,040 significantly expressed genes. Microarray hybridization and data analysis were performed by Genomic Works (Daejeon, Korea). The expression of olfactory receptors was analyzed in the dataset. Expression values were summarized after background correction and normalization steps.

Supplemental Table 1. Food intake of mouse feeding experiments

Week	WT		Olfr544 KO		*ob/ob*	
	Vehicle	AzA	Vehicle	AzA	Vehicle	AzA
1	1.91±0.13	1.90±0.16	2.19±0.20	2.06±0.09	5.22±0.2	4.68±0.27
2	2.02±0.12	1.92±0.16	1.89±0.16	2.13±0.09	4.92±0.21	4.65±0.17
3	2.22±0.12	2.07±0.16	1.97±0.16	2.15±0.03	4.95±0.21	4.42±0.16
4	2.03±0.13	2.04±0.16	2.06±0.16	2.17±0.09	4.89±0.22	4.46±0.19
5	2.27±0.12	2.33±0.16	2.02±0.16	2.19±0.31	4.70±0.33	4.21±0.30
6	2.31±0.17	2.12±0.16	2.12±0.16	1.91±0.10	4.82±0.21	4.68±0.25

Data are shown as mean ± standard error. Food intake in WT, Olfr544 KO, and *ob/ob* mice did not differ significantly between vehicle-fed control and azelaic acid (AzA) groups by Student's *t*-test. $P < 0.05$ was considered significant.

Supplemental Table 2. Primers used in this study for RT- and qPCR analyses

Gene	Forward Primer	Reverse Primer
Olfr544	CCTTATTGTCTTTGACTGCAACAT	TCGGTTGAAGATGCGAACAG
Olfr545	TCTGGTTTCTATGGACTGCAAC	TCTATTAAAGGTTCGAATAATGAGTC
Olfr1500	CCACACCCCAATGTACTTCC	GAGCCAAGAGGTAGCAATCG
Olfr1466	TTCTCCTGCTGGGACTCACT	GACCAGCCATGACTTTTGGT
Olfr373	CCAAGTGTGTCTGGTGATGG	GTGGCCGTAGGAAATGAAGA
Olfr1368	ACGGAGGGTGTGTACTCCAG	CACACCTTGGCAACTTCAAA
Gnal	GCTGCAGGGTAAGTCTCAGG	ACGCTCATTGCTCATCCTCT
Adcy3	CCACATAGGAAGGGGGATTT	CTGCTTTGGGGAAGCTACAG
Ppara	ACCTTGTGTATGGCCGAGAA	AAGGAGGACAGCATCGTGAA
Hmgcs2	GGCTGTCAAAACAGTGCTCA	GCAATGTCACCACAGACCAC
Acox1	TGCCTTTGTTGTCCCTATCCGTGA	TTACATACGTGCCGTCAGGCTTCA
Cpt1a	CATGTCAAGCCAGACGAAGA	TGGTAGGAGAGCAGCACCTT
Ppargc1a	ACCCACAGGATCAGAACAAACCCT	TTGGTGTGAGGAGGGTCATCGTTT
Ucp1	TCTTCTCAGCCGGAGTTTCAGCTT	ACCTTGGATCTGAAGGCGGACTTT
Prdm16	CCGCTGTGATGAGTGTGATG	GGACGATCATGTGTTGCTCC
Gapdh	AACTTTGGCATTGTGGAAGG	ACACATTGGGGGTAGGAACA

Supplemental Table 3. Nine potential off-target sites

	DNA	Chrome	Position	Direction	Mismatches
OT_1	GAgGtGaATGGGGG GCACCATGG	chr8	116769972	+	3
OT_2	TGAgGCCTGgGGaC TTGGGGAGG	chr7	75981858	+	3
OT_3	TGAtGCCTGgGaGC TTGGGGTGG	chr10	75511864	-	3
OT_4	TGACGgCTGgGGGg TTGGGGGGG	chr5	90891011	-	3
OT_5	GgAaAGtATGGGGG GCACCAGGG	chr5	142408068	-	3
OT_6	GAAcAGCActGGGG GCACCATGG	chr2	179881069	+	3
OT_7	TGAgGCgTGAGGGg TTGGGGGGG	chr15	78905183	+	3
OT_8	GAAGAGgAaGGGa GGCACCAAGG	chr15	77340789	-	3
OT_9	TGAgGCCTGgGGGg TTGGGGGGG	chr17	50057352	+	3

Supplemental Table 4. Primers used to amplify the sequences flanking the potential off-target sites

Gene	Forward Primer	Reverse Primer
OT_1	ACACTCTTTCCCTACACGACGCTC TTCCGATCTGAggctctctataggaacaagat gat	GTGACTGGAGTTCAGACGTGTG CTCTTCCGATCTACtagggctccacgatc tagca
OT_2	ACACTCTTTCCCTACACGACGCTC TTCCGATCTGAtttccatgaagacatggtagta aag	GTGACTGGAGTTCAGACGTGTG CTCTTCCGATCTACtcccttaaccaaattg cccca
OT_3	ACACTCTTTCCCTACACGACGCTC TTCCGATCTGAcaggcacgagacactctgtt	GTGACTGGAGTTCAGACGTGTG CTCTTCCGATCTACgtaaccaggatggg agccag
OT_4	ACACTCTTTCCCTACACGACGCTC TTCCGATCTGAtgcgttaatctgccacactc	GTGACTGGAGTTCAGACGTGTG CTCTTCCGATCTACgctctgagtcctgttg tggag
OT_5	ACACTCTTTCCCTACACGACGCTC TTCCGATCTGAccatatgtcatggtggctttgct	GTGACTGGAGTTCAGACGTGTG CTCTTCCGATCTACccttctccatgatgcc agact
OT_6	ACACTCTTTCCCTACACGACGCTC TTCCGATCTGAcaggagcccagtaaggaagc	GTGACTGGAGTTCAGACGTGTG CTCTTCCGATCTACctgccaactgtggtc cgtta
OT_7	ACACTCTTTCCCTACACGACGCTC TTCCGATCTGAtctgccctaaccttactgcc	GTGACTGGAGTTCAGACGTGTG CTCTTCCGATCTACctggattcctcgggt

		gattct
OT_8	ACACTCTTTCCCTACACGACGCTC TTCCGATCTGAtcatctccagggttctgtttacc	GTGACTGGAGTTCAGACGTGTG CTCTTCCGATCTACgcccatcatgctattc agtctc
OT_9	ACACTCTTTCCCTACACGACGCTC TTCCGATCTGAtgtgtcatcttggttccacac	GTGACTGGAGTTCAGACGTGTG CTCTTCCGATCTACcactctacactcacc ctgtctg

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