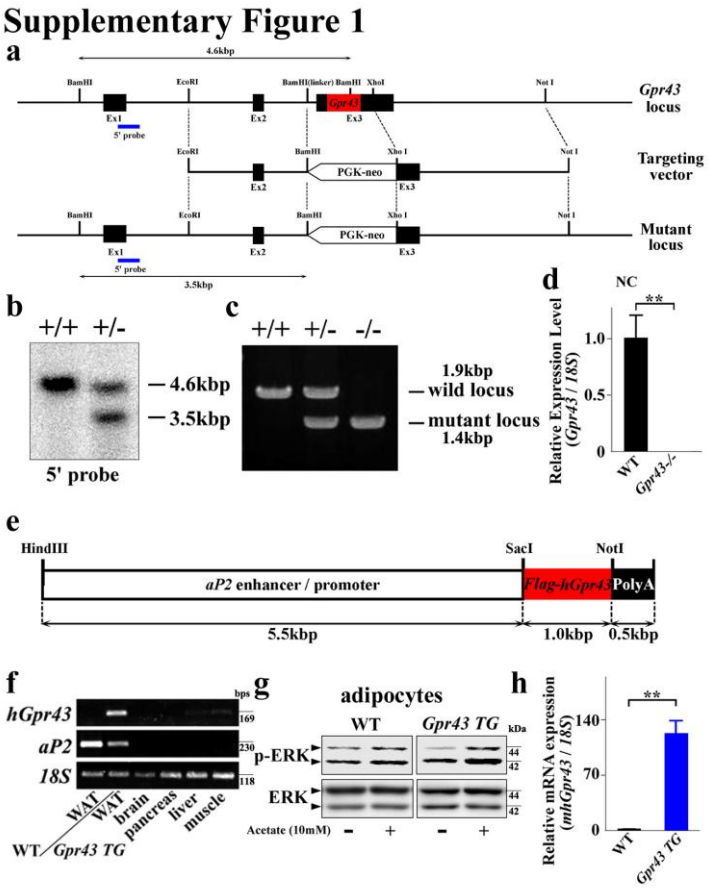
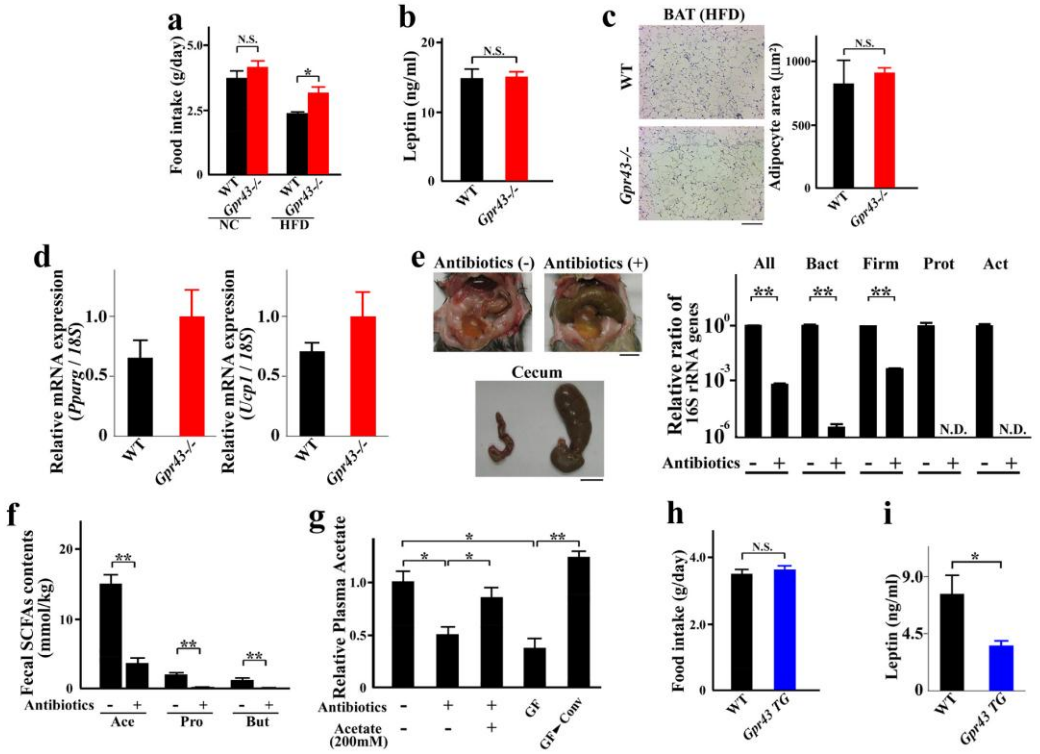


Supplementary Figure S1 | Targeted disruption and overexpression of *Gpr43* in mice.

(**a**) A targeting vector was constructed by ligation of 3 fragments: the 5' and 3' homology recombination arms and a fragment of the LacZ-PGK-neo cassette. A 1.6-kb fragment of mouse DNA containing the exon coding for *Gpr43* was replaced with the LacZ-PGK-neo cassette. The linearized targeting vector was then electroporated into 129/Sv ES cells. (**b**) The 5' probe used for Southern blotting is indicated below the map of the target allele. The 4.6-kb and 3.5-kb fragments, which corresponded to the wild-type and mutant alleles, respectively, were detected by Southern blotting of genomic DNA digested with *Bam* HI. (**c**) Genotypes of mice were determined by PCR using the 3 primers: P1, P2, and P3 (wild-type allele: 1.9-kb, P1/P3; mutant allele, 1.3-kb, P1/P2). P1: 5'-AAGGGAACCGATCACAGCTA-3', P2: 5'-CGGCATAACAGTGGAGACAA-3', P3: 5'-GGCTATTCGGCTATGACTGG-3' (**d**) Expression of *Gpr43* mRNA in mouse adipose tissues of wild-type and *Gpr43-/-* mice. *18S* rRNA expression was used as an internal control. (**e**) Schematic representation of the *aP2* promoter fused with human *Gpr43* cDNA. (**f**) Expression of the transgene *hGpr43* mRNA in the WAT of *aP2-Gpr43TG* mice. (**g**) GPR43 signaling in the adipocytes of *aP2-Gpr43TG* mice. (**h**) Total *Gpr43* mRNA expression in the adipose tissue of wild-type and *aP2-Gpr43TG* mice. *18S* rRNA expression was used as an internal control. (**d, h**) Data are presented as mean±s. e. m. Student's *t*-test; $^{**}p < 0.005$.

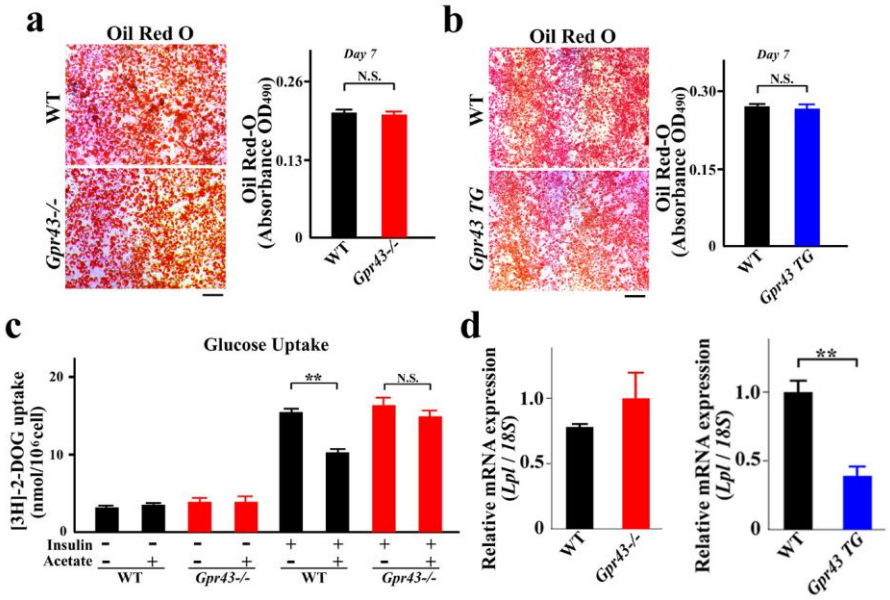
Supplementary Figure 2



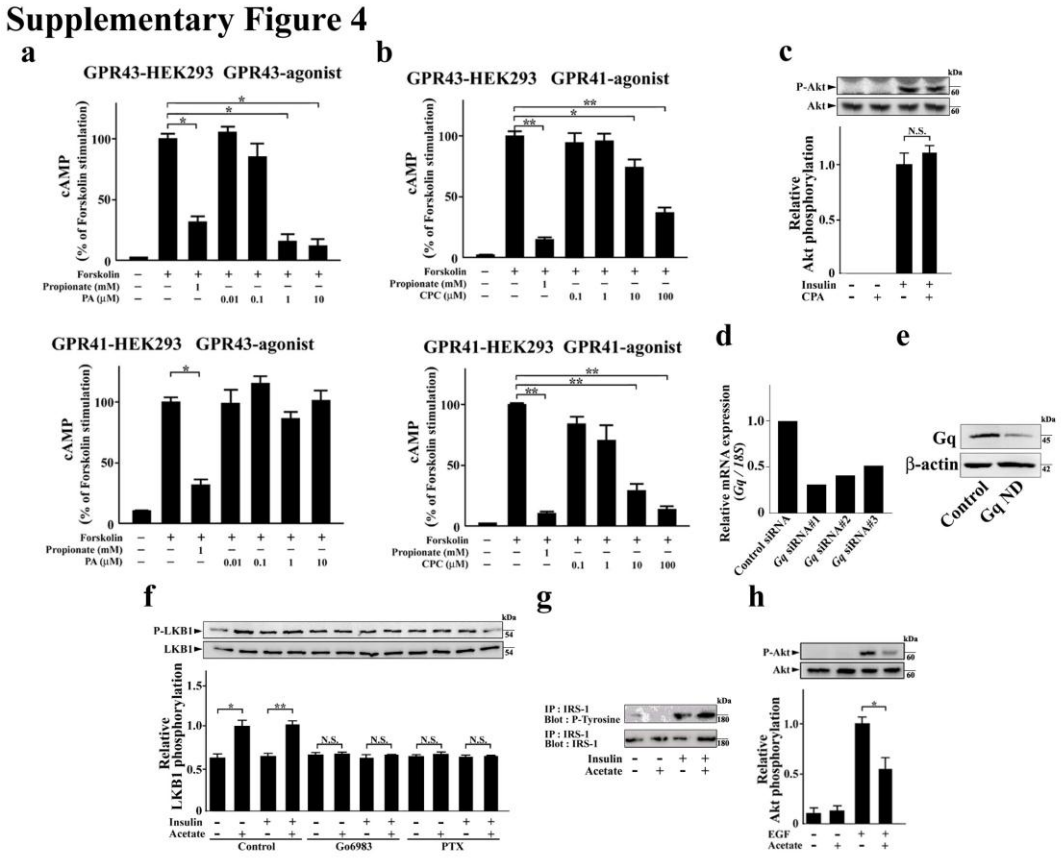
Supplementary Figure S2 | Phenotype of *Gpr43-/-* and *aP2-Gpr43TG* mice, and under antibiotic treatment and germ-Free (GF) conditions.

(**a**) Food intake of NC- and HFD-fed *Gpr43-/-* mice (n = 7-8). (**b**) Plasma leptin concentration in HFD-fed *Gpr43-/-* mice (n = 5,7). (**c**) H&E stained brown adipocyte tissue (BAT) and mean area of adipocytes of *Gpr43-/-* mice fed an HFD (n = 4,3). Scale bar = 100 μm. (**d**) Expression of *Pparg* and *Ucp1* mRNA in the BAT of *Gpr43-/-* mice fed an HFD (n = 3). (**e**) Gross cecal appearance of mice under antibiotic treatment, and comparison of microbial communities under antibiotic treatment, as assessed by qPCR (n = 4). All, all bacteria; Bact, Bacteroidetes; Firm, Firmicutes; Prot, gamma-proteobacteria; Act, actinobacteria. (n = 8). Scale bar = 1 cm. (**f**) Fecal major short-chain fatty acid contents in HFD-fed mice under antibiotic treatment (n = 8). (**g**) Plasma acetate level with or without acetate, under antibiotic treatment, under GF conditions, or under CONV conditions after GF conditions (n = 3-8). (**h**) Food intake of *aP2-Gpr43TG* mice (n = 6). (**i**) Plasma leptin level in *aP2-Gpr43TG* mice (n = 5). All mice were analyzed at 16 weeks of age. All data are presented as mean±s. e. m. Student's *t*-test (**a-f, h,** and **i**) and ANOVA followed by Tukey-Kramer's *post hoc* test (**g**); **p* < 0.05; ***p* < 0.005.

Supplementary Figure 3



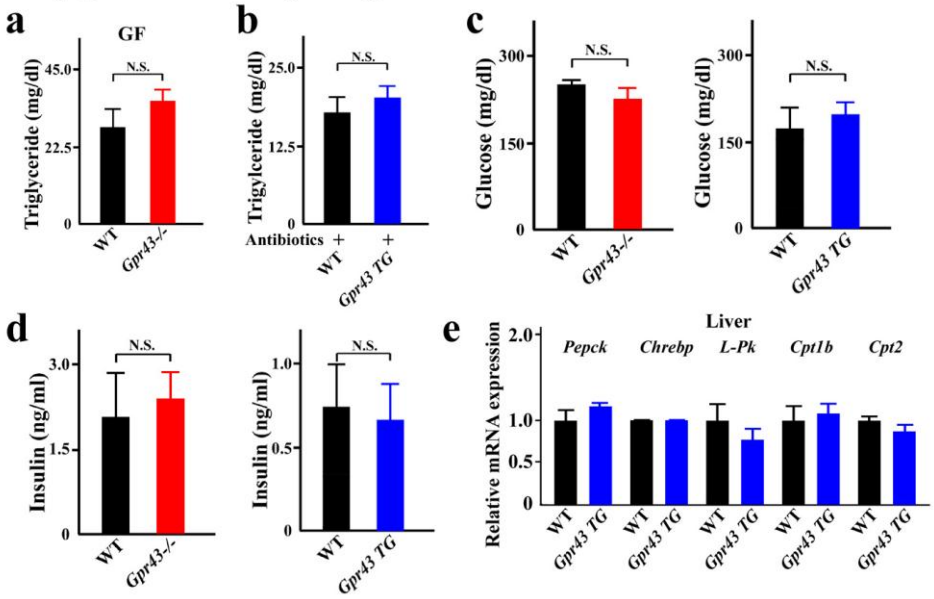
Supplementary Figure S3 | Adipocytes and adipose tissues of *Gpr43-/-* and *aP2-Gpr43TG* mice. (**a, b**) Oil Red O staining of mouse embryonic fibroblast (MEF)-derived adipocytes of *Gpr43-/-* and *aP2-Gpr43TG* mice with treatment of pioglitazone (10 μM) and inducer (n = 4). Scale bar = 100 μm. (**c**) Effect of acetate on glucose uptake in adipose tissues from wild-type and *Gpr43-/-* mice (n = 3). (**d**) Expression of *Lpl* mRNA in the WAT in *Gpr43-/-* mice fed an HFD (n = 4) and *aP2-Gpr43TG* (n = 4) mice. Mice were analyzed at 16 weeks of age. All data are presented as mean±s. e. m. Student's *t*-test; $^{**}p < 0.005$.



Supplementary Figure S4 | GPR43 cell signaling.

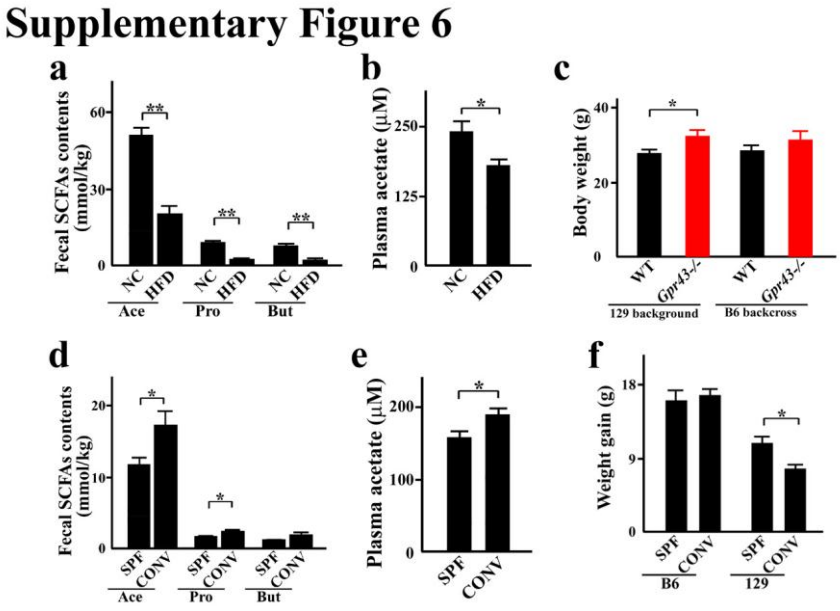
(**a, b**) Reduction in the cAMP level in response to GPR43 agonist (phenylacetamide; PA) (**a**) and GPR41 agonist (cyclopropanecarboxylic acid; CPC) (**b**) treatment in GPR43- and GPR41-expressing HEK293 cells (n = 3). (**c**) Effects of CPA (N^6-cyclopentyladenosine) on insulin-induced Akt phosphorylation in 3T3-L1-derived adipocytes (n = 4). Cells were stimulated with insulin (3 μg/ml) in the presence of CPA (100 nM) for 5 min after pretreatment with CPA for 2 h. (**d**) Efficiencies of siRNA knockdown in 3T3-L1 cells. (**e**) Effects of siRNA knockdown on protein expression in 3T3-L1 cells. (**f**) Effects of GPR43 stimulation on LKB1 phosphorylation in 3T3-L1-derived adipocytes (n = 3). Cells were stimulated with or without insulin (3 μg/ml) in the presence of acetate (10mM), Go6983 (10 μM), or PTX (1 μg/ml) for 5 min after pretreatment with acetate for 2 h, Go6983 or PTX for 4 h. (**g**) Effects of GPR43 stimulation on IRS1 phosphorylation in 3T3-L1-derived adipocytes (n = 3). Cells were stimulated with or without insulin (3 μg/ml) in the presence of acetate (10mM), for 5 min after pretreatment with acetate for 2 h. IP; immunoprecipitation. (**h**) Effects of acetate on EGF-induced Akt phosphorylation in 3T3-L1-derived adipocytes (n = 3). Cells were stimulated with EGF (10 ng/ml) in the presence of acetate (10 mM) for 5 min after pretreatment with acetate for 2 h. All data are presented as mean±s. e. m. ANOVA followed by Tukey-Kramer's *post hoc* test; **p* < 0.05; ***p* < 0.005.

Supplementary Figure 5



Supplementary Figure S5 | Biochemical parameters of *Gpr43-/-* and *aP2-Gpr43TG* mice and qRT-PCR analysis of the liver of *aP2-Gpr43TG* mice.

(**a**) Plasma triglycerides in *Gpr43-/-* mice fed an HFD under GF conditions (n = 4,6). (**b**) Plasma triglycerides in *aP2-Gpr43TG* mice treated with antibiotics (n = 7,6). (**c**) Plasma glucose concentration in *Gpr43-/-* mice fed an HFD (n = 9,8) and *aP2-Gpr43TG* mice (n = 5). (**d**) Plasma insulin concentration in *Gpr43-/-* mice (n = 11,6) fed an HFD and *aP2-Gpr43TG* mice (n = 9, 8). (**e**) mRNA levels of genes involved in gluconeogenesis (*Pepck*), glycolysis (*Chrebp* and *L-pk*), and beta-oxidation (*Cpt1b* and *Cpt2*) in the liver of *aP2-Gpr43TG* mice fed an HFD (n = 4). All mice were analyzed at 15-16 weeks of age. All data were statistically analyzed by Student's *t*-test and are presented as mean±s. e. m.



Supplementary Figure S6 | The impact of differences in diet, genetic backgrounds and gut microbiota on the phenotype of the mice.

(**a**) Fecal major short-chain fatty acid contents in NC- and HFD-fed mice (n=4). Ace, acetate; Pro, propionate; But, butyrate. (**b**) Plasma acetate concentration in NC- and HFD-fed mice (n = 4). Mice were analyzed at 13 weeks of age (**a, b**). (**c**) Body weight of wild-type and *Gpr43-/-* mice fed an HFD in 129/SvEv (129) background or C57BL/6 (B6) backcross (n = 5-8) mice at the age of 8 weeks. (**d**) Fecal major short-chain fatty acid content in specific pathogen-free (SPF) or conventional (CONV) mice fed an HFD (n = 8). Ace, acetate; Pro, propionate; But, butyrate. CONV, a colony housed under conventional condition; SPF, a colony housed under SPF conditions. (**e**) Plasma acetate concentration in SPF or CONV mice fed an HFD (n = 8). (**f**) Weight gain (between 5-13 weeks of age) in B6 and 129 mice. All data are presented as mean±s. e. m. Student's *t*-test; *$p < 0.05$; **$p < 0.005$.

Supplementary Table S1 | Composition of diets.

	NC (MF)	HFD (58Y1)
Energy (kcal/g)	**3.6**	**5.1**
Fat (% of energy)	**12.8**	**61.6**
Carbohydrate (% of energy)	**61.5**	**20.3**
Protein (% of energy)	**25.7**	**18.1**
Carbohydrate (% by weight)	**54.4**	**25.9**
Fiber (% by weight)	**2.8**	**6.5**

Supplementary Table S2 | Primers used for PCR.

Primer	Forward	Reverse
Gpr43 **(mouse)**	**5’-GGCTTCTACAGCAGCATCTA-3’**	**5’-AAGCACACCAGGAAATTAAG-3’**
Gpr41 **(mouse)**	**5’-GTGACCATGGGGACAAGCTTC-3’**	**5’-CCCTGGCTGTAGGTTGCATT-3’**
Pparg2 **(mouse)**	**5’-GCTGTTATGGGTGAAACTCTGG-3’**	**5’-TTCTTGTGAAGTGCTCATAGGC-3’**
C/ebpa **(mouse)**	**5’-AAGAAGTCGGTGGACAAGAACAG-3’**	**5’-GTTGCGTTGTTTGGCTTTATCTC-3’**
C/ebpb **(mouse)**	**5’-GACTACGCAACACACGTGTAACT-3’**	**5’-CAAAACCAAAAACATCAACAACCC-3’**
aP2 **(mouse)**	**5’-GATGCCTTTGTGGGAACCTGG-3’**	**5’-CTGTCGTCTGCGGTGATTTC-3’**
Leptin **(mouse)**	**5'-TGCTCCAGCAGCTGCAAGGT-3'**	**5'-GAAGAATGTCCTGCAGAGAG-3'**
Adipoq **(mouse)**	**5'-TCCTGCTTTGGTCCCTCCAC-3'**	**5'-TCTCCAGCCCCACACTGAAC-3'**
F4/80 **(mouse)**	**5’-GCCTGGACGAATCCTGTGAA-3’**	**5’-GCTAGATGCAAAGCCAGGGT-3’**
TNFa **(mouse)**	**5’-GGCAGGTCTACTTTGGAGTC-3’**	**5’-TCGAGGCTCCAGTGAATTCG-3’**
18S **(mouse)**	**5’-CTCAACACGGGAAACCTCAC-3’**	**5’-AGACAAATCGCTCCACCAAC-3’**
Pgc1a **(mouse)**	**5’-GAGAATGAGGCAAACTTGCTAGCG -3’**	**5’-TGCATGGTTCTGAGTGCTAAGACC -3’**
Pepck **(mouse)**	**5’-CCACAGCTGGTGCAGAACA-3’**	**5’-GAAGGGTCGATGGCAAA-3’**
Hk1 **(mouse)**	**5’-AGGAAGAACCAACCCACAAAAC-3’**	**5’-ACCCCAAGGAAACACCACTC-3’**
Pkm2 **(mouse)**	**5’-GGAGATGTGGTCATTGTGCTG-3’**	**5’-AAAGGATAGGGGAGGGGAAG-3’**
Hk2 **(mouse)**	**5’-GGAGGAGATGCGTAATGTGG-3’**	**5’-TGCCAGGGTTGAGAGAGAG-3’**
Cpt1b **(mouse)**	**5’-ACCACAAAGGTCGCTTCTTC-3’**	**5’-TCTTCATCCAGGGTCACAAAG-3’**
Cpt2 **(mouse)**	**5'-CTCATCCGCTTTGTTCCTTC-3'**	**5'-AGTTCATCACGACTGGGTTTG-3'**
Gpr43 **(human)**	**5'-TCTGCTACTGGCGTTTTGTG-3'**	**5'-CAGGGGCTTTTTCTCTGGTG-3'**
Lpl **(mouse)**	**5’-CTGCTGGCGTAGCAGGAAGT-3’**	**5’-GCTGGAAAGTGCCTCCATTG-3’**

***Chrebp* (mouse)**	**5'-CTGGGGACCTAAACAGGAGC-3'**	**5'-GAAGCCACCCTATAGCTCCC-3'**
***L-Pk* (mouse)**	**5'-GAGTCGGAGGTGGAAATTGTG-3'**	**5'-GTCCACCCACACTGTCTTTG-3'**
***Gq* (mouse)**	**5'-TGGAGAAGGTGTCTGCTTTTG-3'**	**5'-ATTCCCGTCGTCTGTCGTAG-3'**

Supplementary Table S3 | Primers used for fecal microbiota qRT-PCR.

Primer	Forward	Reverse
Universal	**5'-ACTCCTACGGGAGGCAGCAGT-3'**	**5'-ATTACCGCGGCTGCTGGC-3'**
Bacteroidetes	**5'-CRAACAGGATTAGATACCCT-3'**	**5'-GGTAAGGTTCCTCGCGTAT-3'**
Firmicutes	**5'-GGAGYATGTGGTTTAATTCGAAGCA-3'**	**5'-AGCTGACGACAACCATGCAC-3'**
g-Proteobacteria	**5'-TCGTCAGCTCGTGTYGTGA-3'**	**5'-CGTAAGGGCCATGATG-3'**
Actinobacteria	**5'-TACGGCCGCAAGGCTA-3'**	**5'-TCRTCCCCACCTTCCTCCG-3'**

Supplementary Table S4 | siRNA used for RNAi.

siRNA	Sequence
Gq #1	**5'-UUGUUGUGUAGGCAGAUAGGAAGGG-3'**
Gq #2	**5'-AAAUGACACUUUGUAAGUCAAAGGG-3'**
Gq #3	**5'-AGAACUUGAUCAUAUUCGCUAAGCG-3'**

Supplementary Methods

Biochemical analyses The plasma leptin concentrations were measured using the Leptin Mouse ELISA Kit (R&D Systems). Plasma insulin concentrations were measured using the Insulin ELISA Kit (Shibayagi, Japan).

Oil red O staining of adipocytes Cultured cells were fixed with 4% paraformaldehyde for 30 min at 4°C and washed with 60% isopropyl alcohol. Fixed cells were stained with oil red O staining solution (0.3% oil red O in 60% isopropyl alcohol) for 20 min at room temperature, washed with 60% isopropyl alcohol, and then washed several times with distilled water. Finally, the stained cells were destained with isopropanol and the optical density (OD) at 490 nm was measured by spectrophotometry.

Generation of HEK293 cells expressing mouse GPR43 or GPR41 Flp-In T-REx HEK293 cells were transfected with a mixture of mouse FLAG-GPR43 or FLAG-GPR41 cDNA in pcDNA5/FRT/TO and pOG44, respectively, using the Lipofectamine reagent (Invitrogen). After 48 h, the medium was exchanged to medium supplemented with 200 μg/ml hygromycin B to initiate selection of stably transfected cells. Following isolation of resistant cells, expression of GPR43 or GPR41 from the Flp-In locus was induced by treatment with 1 μg/ml doxycycline for 48 h as described previously[14]. Expression of GPR43 or GPR41 was confirmed by RT-PCR and FACS Calibur (BD Biosciences) using the FLAG-tag.

cAMP determination The cells were lysed with 0.1N HCl. After acetylation, the cAMP level was determined in duplicate using an Enzyme Immunoassay Kit (Cayman).

Detection of phosphorylation of IRS1 Cells were lysed with 1 mL lysis buffer containing 50 mM Hepes (pH 7.5), 150 mM NaCl, 1 mM EGTA, 1% Nonidet P-40, and 0.25% sodium deoxycholate, Protease and Phosphatase Inhibitor Cocktail (Nacalai Tesque). The cell lysates were centrifuged at 20,400 x g for 20 min. Anti-IRS-1 antibody (Cell Signaling Technology; rabbit, 1:1000) was pre-bound to Protein A Dynabeads (Life Technologies) according to manufacturer's protocol. The supernatant was incubated with the Protein A Dynabeads with gentle rocking for 2h at 4°C. The beads were collected using a magnetic stand and were washed 3 times with lysis buffer. To release the IRS-1 protein, the beads were boiled in 30 ul premixed NuPAGE LDS Sample Buffer and Reducing Agent (Life Technologies) at 70°C for 10 min. Proteins were separated by NuPAGE Novex 4-12% Bis-Tris Gel in MES-SDS Running Buffe (Life Technologies) and immunoblotted with an anti-IRS- 1 and anti-Phospho-Tyrosine (Cell Signaling Technology; rabbit, 1:2000).