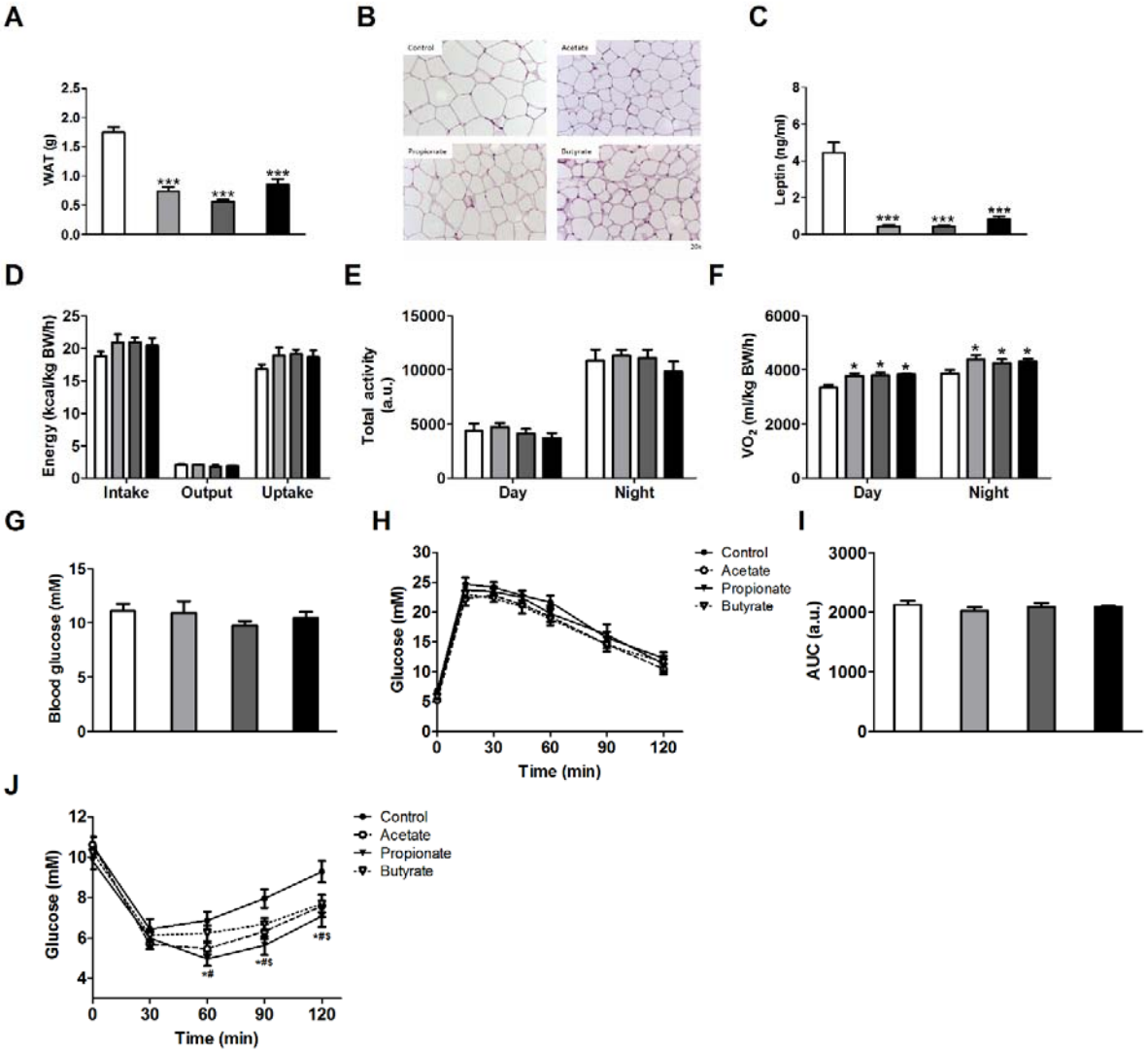
SUPPLEMENTARY DATA

Supplementary Table 1. List of oligonucleotide primer pairs used in qPCR analysis.

Gene	Forward (5`-3`)	Reverse (5`-3`)
PPARγ	CACAATGCCATCAGGTTTGG	GCTGGTCGATATCACTGGAGATC
Cd36	GATCGGAACTGTGGGCTCAT	GGTTCCTTCTTCAAGGACAACTTCT
Lpl	AAGGTCAGAGCCAAGAGAAGCA	CCAGAAAAGTGAATCTTGACTTGGT
Fabp4	CCAGACAAGGGTTTTACAGATAAGCT	ACCTGCTGTGCACCACAATG
Pltp	TTCCTCCTCAACCAGCAGATCT	CAGGAGGGAGTTGAGCAACAC
FASN	GGCATCATTGGGCACTCCTT	GCTGCAAGCACAGCCTCTCT
Elovl5	TGGCTGTTCTTCCAGATTGGA	CCCTTTCTTGTTGTAAGTCTGAATGTA
Elovl6	ACACGTAGCGACTCCGAAGAT	AGCGCAGAAAACAGGAAAGACT
Acc1	GCCATTGGTATTGGGGCTTAC	CCCGACCAAGGACTTTGTTG
Acc2	CATACACAGAGCTGGTGTTGGACT	CACCATGCCCACCTCGTTAC
Scd1	ATGCTCCAAGAGATCTCCAGTTCT	CTTCACCTTCTCTCGTTCATTTCC
Srebp-1c	GGAGCCATGGATTGCACATT	CCTGTCTCACCCCCAGCATA
Cpt1a	CTCAGTGGGAGCGACTCTTCA	GGCCTCTGTGGTACACGACAA
Mcad	GCAGCCAATGATGTGTGCTTAC	CACCCTTCTTCTCTGCTTTGGT
Lcad	TACGGCACAAAAGAACAGATCG	CAGGCTCTGTCATGGCTATGGT
PPARα	TATTCGGCTGAAGCTGGTGTAC	CTGGCATTTGTTCCGGTTCT
Acox1	GGAGAAGTTGGGAAGACCACTG	CAATGGCCGTCATGTGAGTT
Pdk4	GCATTTCTACTCGGATGCTCATG	CCAATGTGGCTTGGGTTTCC
Fgf21	CCGCAGTCCAGAAAGTCTCC	TGACACCCAGGATTTGAATGAC
Ucp1	AGCCATCTGCATGGGATCAAA	GGGTCGTCCCTTTCCAAAGT
36b4	GCTTCATTGTGGGAGCAGACA	CATGGTGTTCTTGCCCATCAG

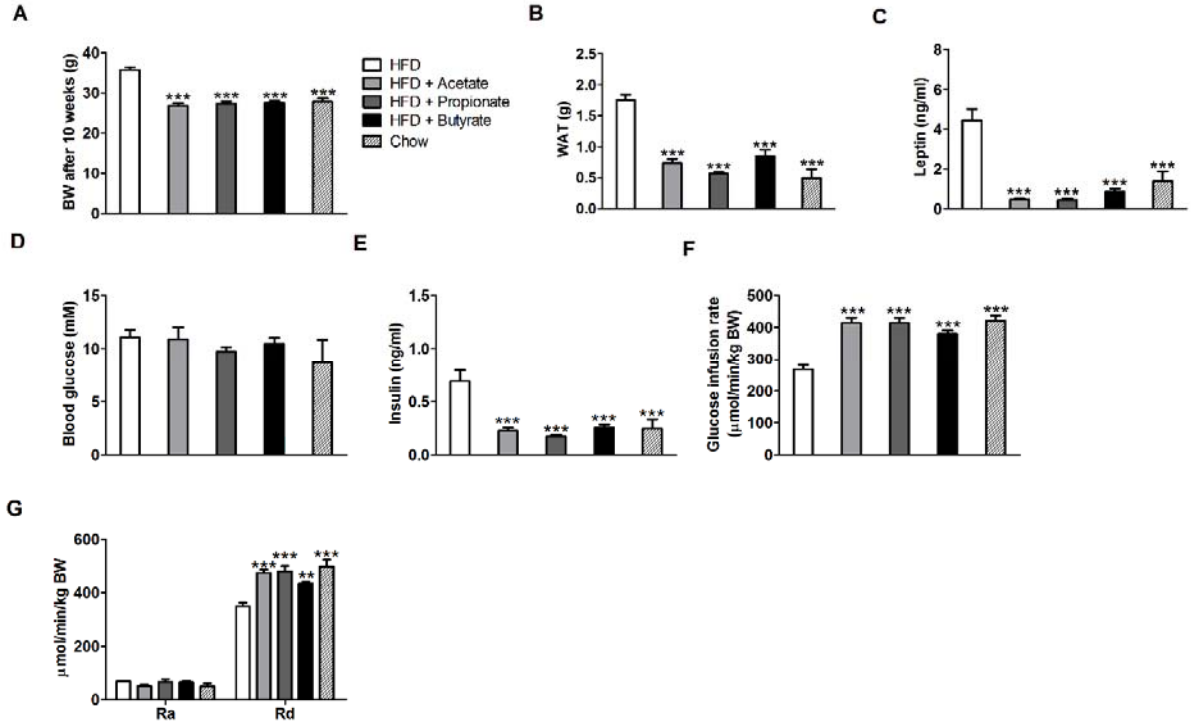
SUPPLEMENTARY DATA

Supplementary Figure 1. SCFA treatment increases energy expenditure and insulin sensitivity. Eight-week-old male C57Bl/6J mice were fed a high-fat diet mixed either with acetate, propionate or butyrate (5% w/w). (A-B) White adipose tissue mass and morphology after 12 weeks on diet. (C) Plasma leptin levels after 12 weeks on diet. (D) Energy balance was determined by measuring the energy content of the diet and dried homogenized feces. Uptake is defined as the difference between intake and output. (E) Locomotor activity was evaluated by beam breaks using indirect calorimetry cages after 10 weeks on high-fat diet. (F) VO_2 was evaluated using indirect calorimetry data after 10 weeks on high-fat diet. (G) Blood glucose levels measured in animals with their respective diets for 12 weeks after a 4h fast. (H) After 11 weeks on their respective diets an intraperitoneal glucose tolerance test was performed in mice that were fasted overnight. *p<0.05 acetate vs control, $^{\#}$p<0.05 propionate vs control, $^{\$}$p<0.05 butyrate versus control. (I) Area under the curve of the intraperitoneal glucose tolerance test. (J) Insulin tolerance tests were performed on mice for 11 weeks on their respective diets after a 4h fast. Values are means ± SEM for n=7-8; *p<0.05, ***p<0.001 vs control.



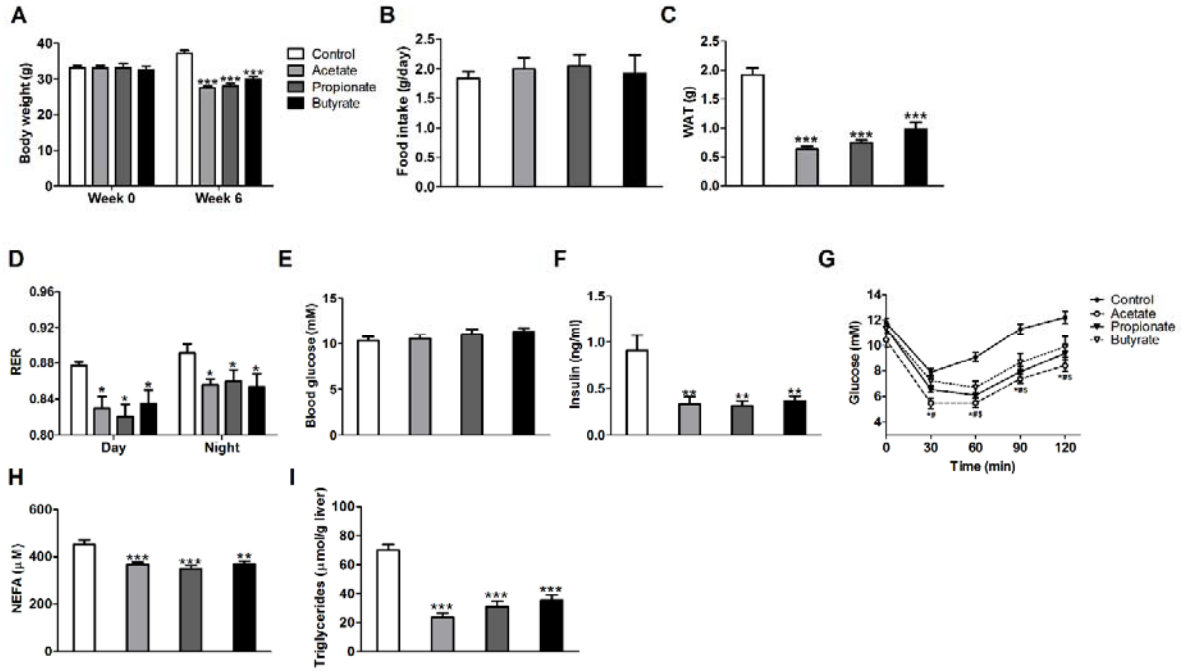
SUPPLEMENTARY DATA

Supplementary Figure 2. SCFA treatment reduces HFD-induced obesity and insulin resistance to normal chow diet levels. Eight-week-old male C57Bl/6J mice were fed a normal chow diet or a high-fat diet mixed either with acetate, propionate or butyrate (5% w/w) for 10 weeks. (A-B) Body weight and white adipose mass after 10 weeks of diet. (C-E) Plasma leptin, glucose and insulin levels after 10 weeks of diet. (F-G) Hyperinsulinemic-euglycemic clamp studies were performed in animals fed the indicated diets for 10 weeks. Glucose infusion rate (GIR), glucose production rate (Ra) and glucose uptake rate (Rd) were calculated after the test. Values are means ± SEM for n=7-8; ***p<0.001 vs HFD.



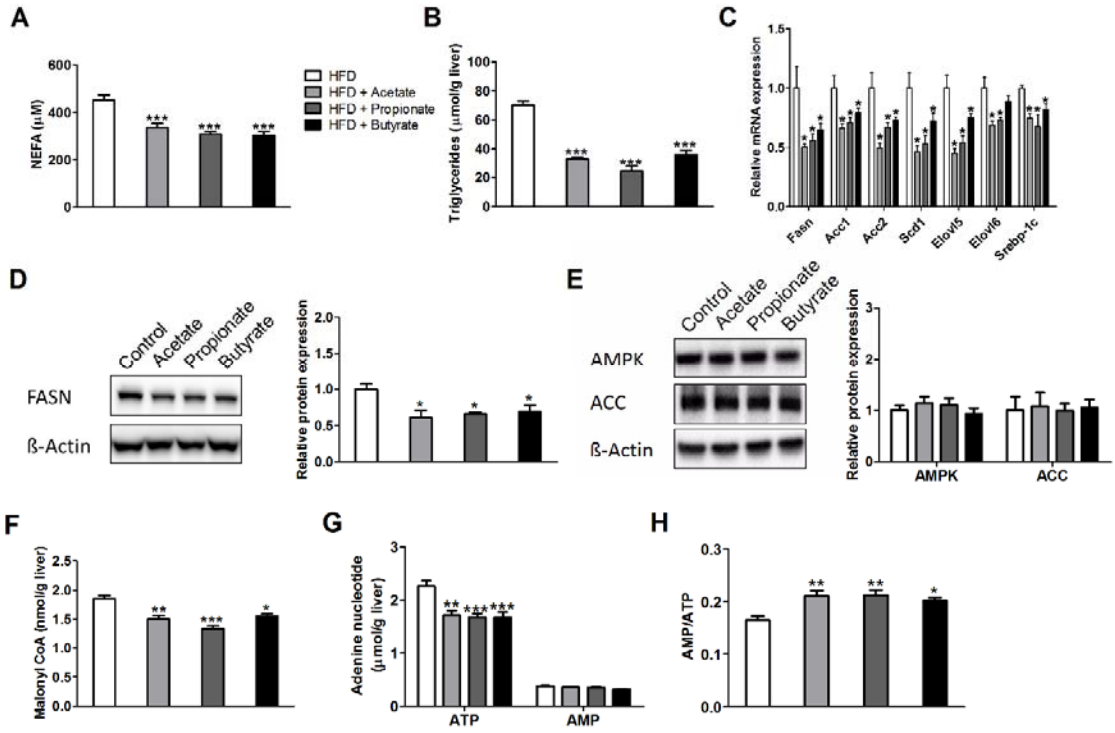
SUPPLEMENTARY DATA

Supplementary Figure 3. SCFAs reverse HFD-induced obesity and insulin resistance. Eight-week-old male C57Bl/6J mice were fed a high-fat diet for 12 weeks and switch to a high-fat diet supplemented with acetate, propionate or butyrate (5% w/w) for 6 weeks. The start of the SCFA treatment is indicated as time point zero. (A) Body weight was measured before and after the 6-week intervention with the indicated diets. (B) Food intake was measured after 5 weeks' intervention with the indicated diets. (C) White adipose tissue mass of mice after the 6-week intervention with the indicated diets. (D) RER was evaluated using indirect calorimetry data in mice after 5 weeks' intervention with the indicated diets. (E-F) Blood glucose and insulin levels measured in animals after the 6-week intervention with the indicated diets and after a 4h fast. (G) Insulin tolerance tests were done in mice after 5 weeks' intervention with the indicated diets and after a 4h fast. *p<0.05 acetate vs control, $^{\#}$p<0.05 propionate vs control, $^{\$}$p<0.05 butyrate vs control. (H-I) Plasma NEFA concentrations and liver triglycerides in mice after the 6-week intervention with the indicated diets. Values are means ± SEM for n=7-8; *p<0.05, **p<0.01, ***p<0.001 vs control.



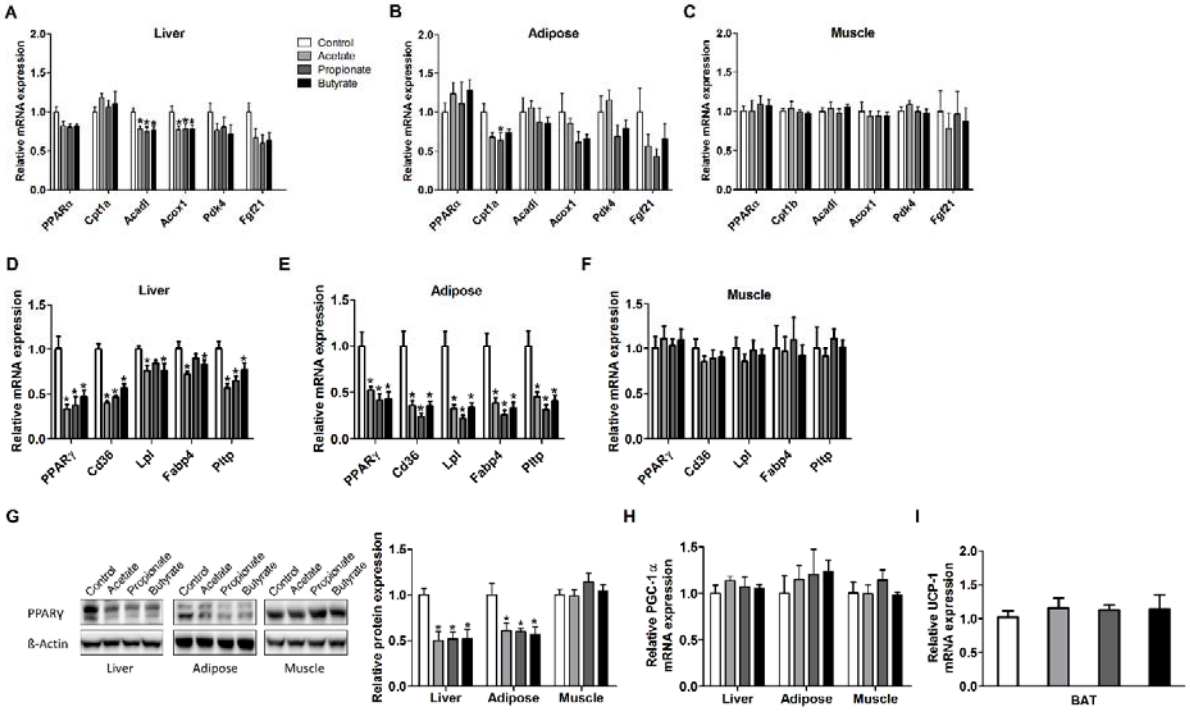
SUPPLEMENTARY DATA

Supplementary Figure 4. SCFA treatment switch hepatic metabolism from lipogenesis to fat oxidation. Eight-week-old male C57Bl/6J mice were fed a high-fat diet mixed either with acetate, propionate or butyrate (5% w/w). (A-B) Plasma NEFA concentrations and liver triglycerides in mice after 12 weeks on diet. (C) Hepatic mRNA expression of lipogenic genes was assessed via qPCR. (D) Hepatic FAS protein expression was assessed by western blot. (E) Hepatic total AMPK and ACC protein expression was assessed by western blot. (F-G) Hepatic malonyl CoA and adenine nucleotides were determined by HPLC. (G) Hepatic AMP/ATP ratio triglycerides in mice after 12 weeks on diet. Values are means ± SEM for n=7-8; *p<0.05, **p<0.01, ***p<0.001 vs control.



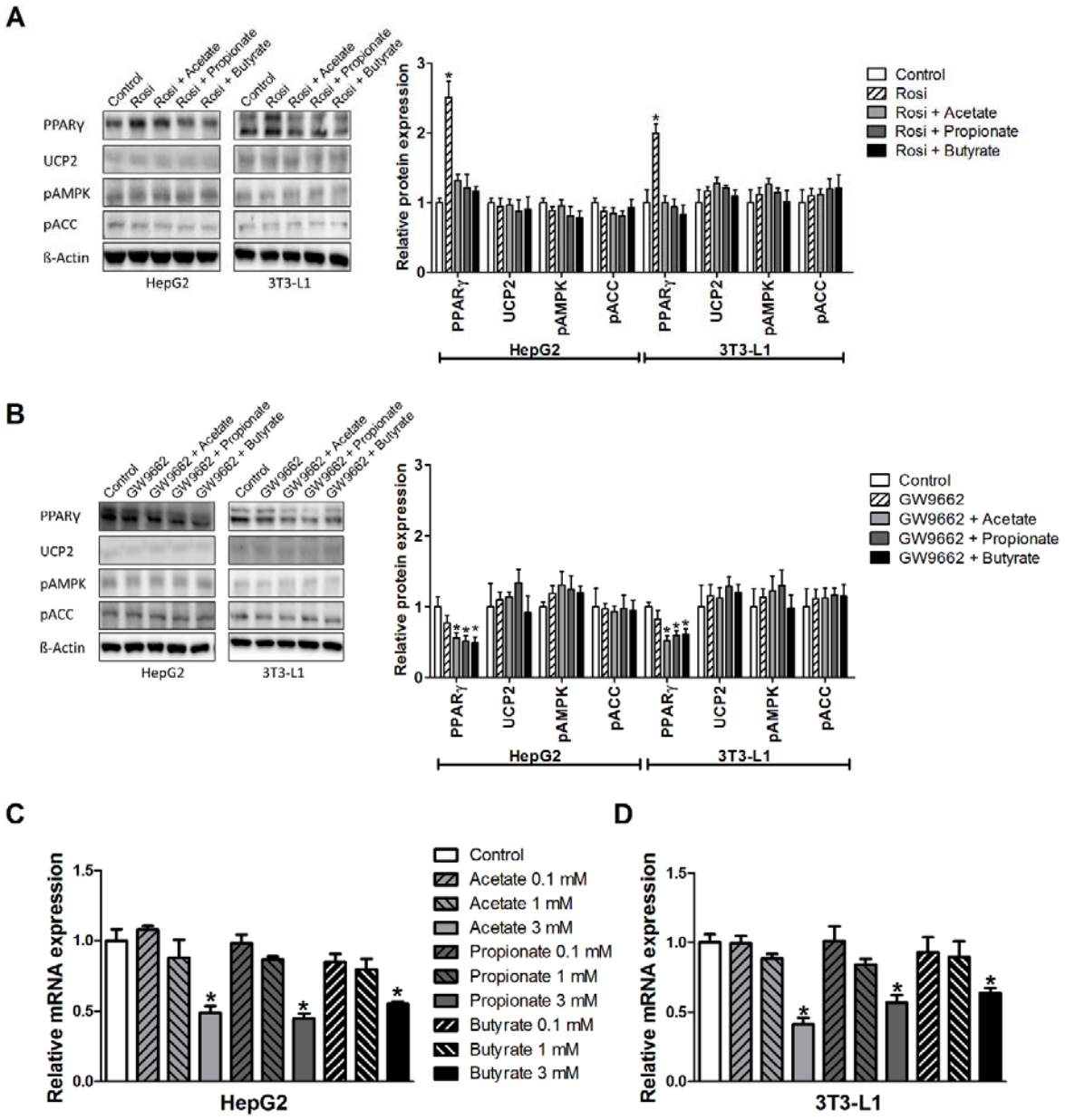
SUPPLEMENTARY DATA

Supplementary Figure 5. SCFA treatment affects PPARγ in liver and adipose tissue. Eight-week-old male C57Bl/6J mice were fed a high-fat diet mixed either with acetate, propionate or butyrate (5% w/w). (A-C) mRNA expression of PPARα and its target genes was assessed via qPCR in liver, adipose and muscle tissue of mice after 12 weeks on the diet. (D-F) mRNA expression of PPARγ and target genes was assessed via qPCR in liver, adipose and muscle tissue of mice after 12 weeks on the diet. (G) Protein expression of PPARγ was analyzed by western blot in liver, adipose and muscle tissue of mice after 12 weeks on the diet. (H) mRNA expression of PGC-1α was assessed via qPCR in liver, adipose and muscle tissue. (I) mRNA expression of UCP-1 was assessed via qPCR in brown adipose tissue. Values are means ± SEM for n=6-8; *p<0.05 vs control.



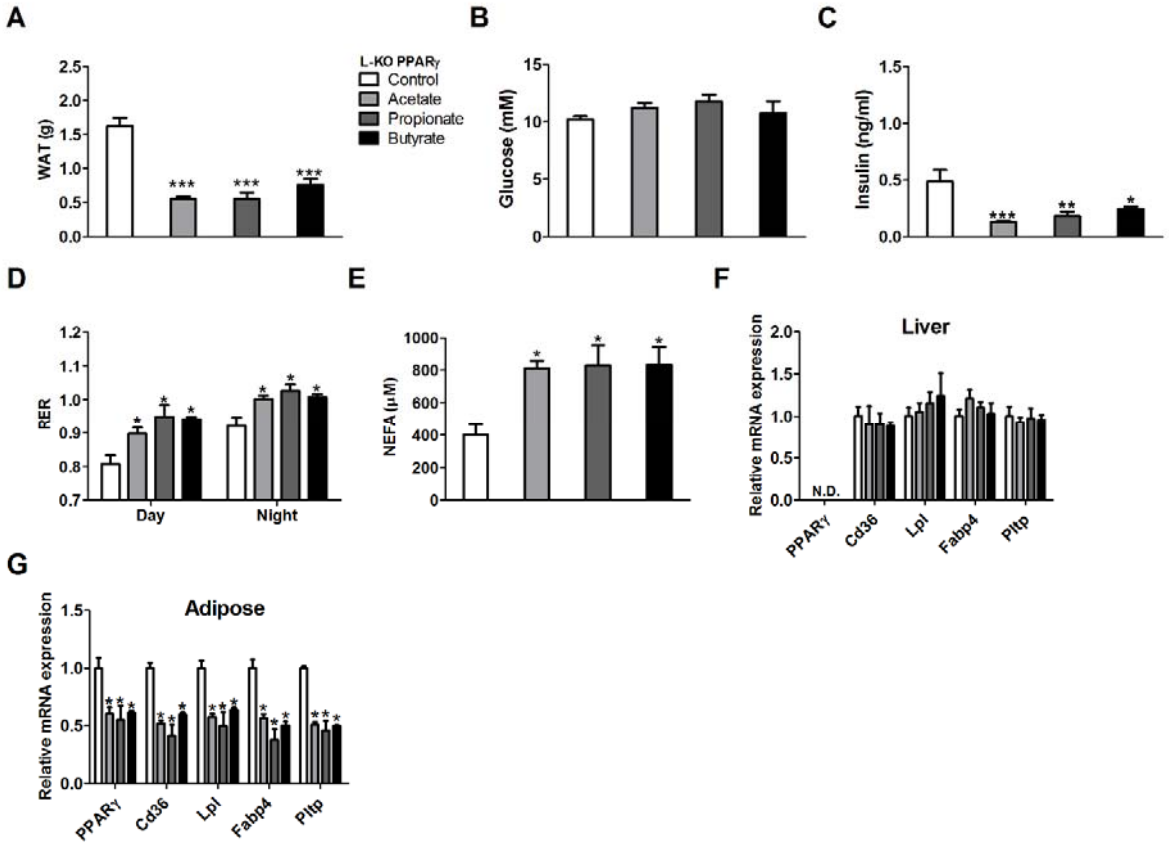
SUPPLEMENTARY DATA

Supplementary Figure 6. Activation of the UCP2-AMPK-ACC pathway by SCFAs depends on PPARγ activity. HepG2 cells and differentiated 3T3-L1 cells were incubated with 3 mM SCFAs for 24 hours in the presence of 100 nM rosiglitazone or 10 µM GW9662 as indicated. (A) PPARγ, UCP2, pAMPK and pACC protein levels were assessed by western blot in HepG2 and differentiated 3T3-L1 cells after incubation with SCFAs and the PPARγ agonist rosiglitazone (Rosi). (B) PPARγ, UCP2, pAMPK and pACC protein levels were assessed by western blot in HepG2 and differentiated 3T3-L1 cells after incubation with SCFAs and the PPARγ antagonist GW9662. (C-D) mRNA expression of PPARγ was assessed via qPCR in HepG2 and differentiated 3T3-L1 cells after 24h incubation with 0.1, 1 and 3 mM SCFAs. Values are means ± SEM for n=6; *p<0.05 vs control.



SUPPLEMENTARY DATA

Supplementary Figure 7. SCFA treatment affects WAT metabolism in L-KO PPARγ mice. Eight-week-old male liver-specific PPARγ knock-out mice were fed a high-fat diet mixed either with acetate, propionate or butyrate (5% w/w). (A) White adipose tissue mass after 10 weeks on diet. (B-C) Blood glucose and insulin levels measured in animals after 10 weeks on their respective diets and after a 4h fast. (D) RER was evaluated using indirect calorimetry data in mice after 9 weeks on diet. (E) Plasma NEFA concentrations in mice after 10 weeks on their respective diets. (F-G) mRNA expression of PPARγ and target genes was assessed via qPCR in liver and adipose tissue of mice after 10 weeks on diet. Values are means ± SEM for n=7-8; *p<0.05, **p<0.01, ***p<0.001 vs control.



SUPPLEMENTARY DATA

Supplementary Figure 8. SCFA treatment affects liver metabolism in A-KO PPARγ mice. Eight-week-old male adipose-specific PPARγ knock-out mice were fed a high-fat diet mixed either with acetate, propionate or butyrate (5% w/w). (A) White adipose tissue mass after 10 weeks on diet. (B-C) Blood glucose and insulin levels measured in animals after 10 weeks on their respective diets and after a 4h fast. (D) RER was evaluated using indirect calorimetry data in mice after 9 weeks on diet. (E) Plasma NEFA concentrations in mice after 10 weeks on their respective diets. (F-G) mRNA expression of PPARγ and target genes was assessed via qPCR in adipose and liver tissue of mice after 10 weeks on diet. Values are means ± SEM for n=7-8; *p<0.05 vs control.