Supplementary information

Microbiome remodeling leads to inhibition of intestinal farnesoid X receptor signaling and decreased obesity

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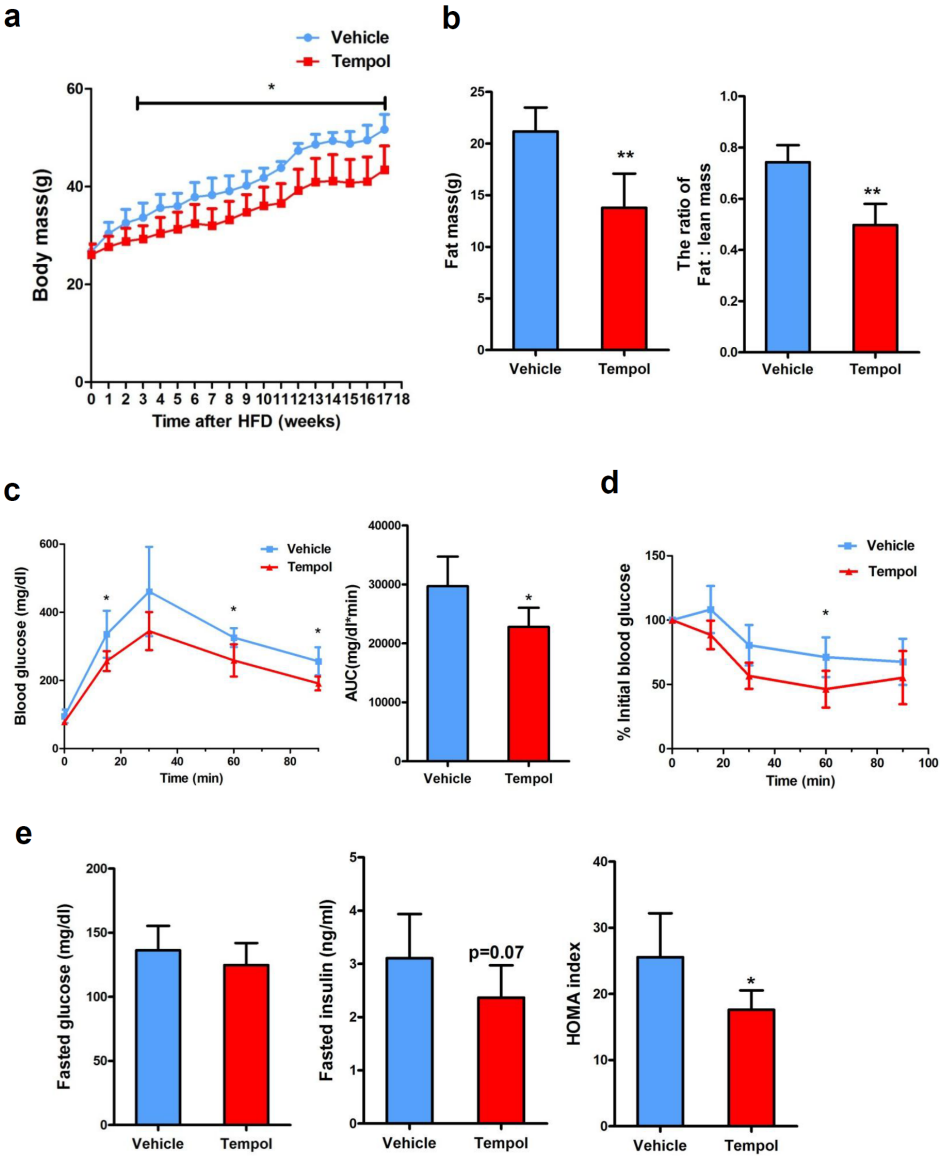
[1]These authors contributed equally to this work.

Supplementary Figures



Supplementary Figure S1. Tempol ameliorates HFD-induced obesity and glucose intolerance.

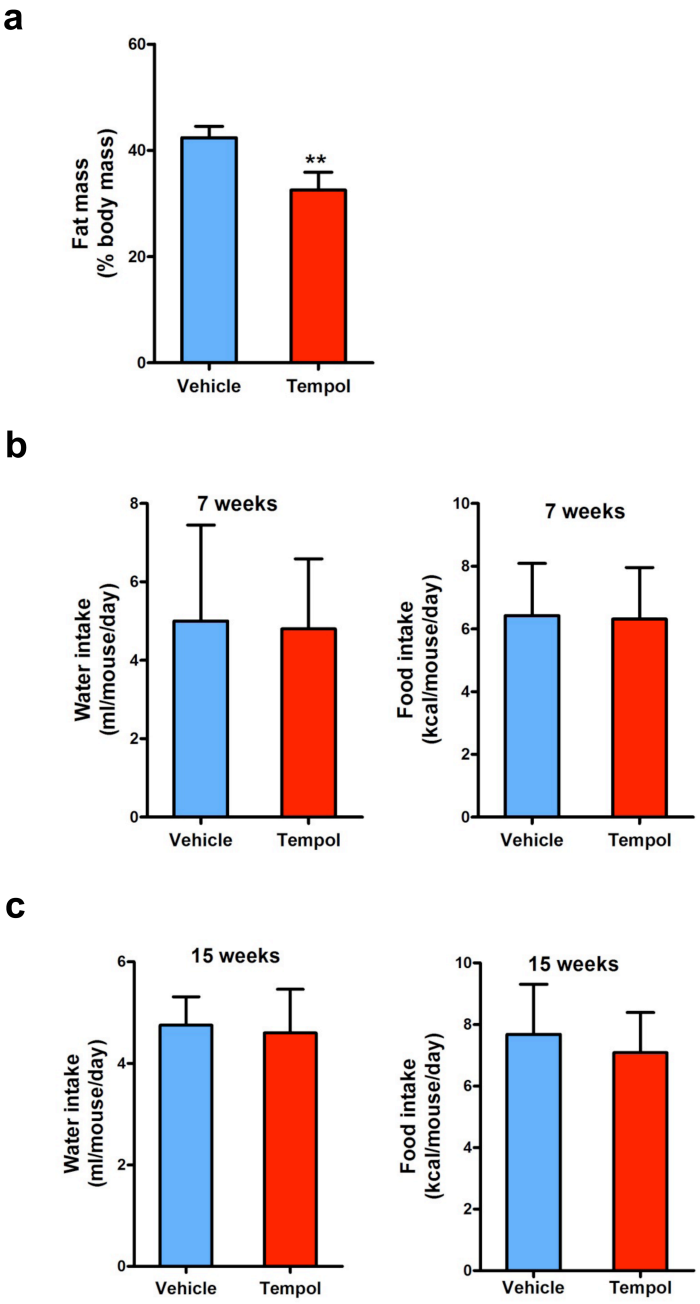
(a) Growth curves of vehicle and tempol-treated mice on a HFD. n=5 mice per group.

(b) Body composition by NMR to show the fat mass (left) and fat mass to lean mass ratio (right) in vehicle and tempol-treated mice after 16 weeks of HFD. n=5 mice per group.

(c) Glucose tolerance test (GTT) and the area under the curve (AUC) after 11 weeks of HFD. n=5 mice per group.

(d) Insulin tolerance test (ITT) after 14 weeks of HFD. n=5 mice per group.

(e) Fasted glucose, fasted serum insulin levels, and HOMA index after tempol treatment on a HFD for 17 weeks. n=5 mice per group. All data are presented as mean ± SD. Analysis of variance followed by two-tailed Student's t-test. **P*<0.05, ***P*<0.01 compared to vehicle treated mice.

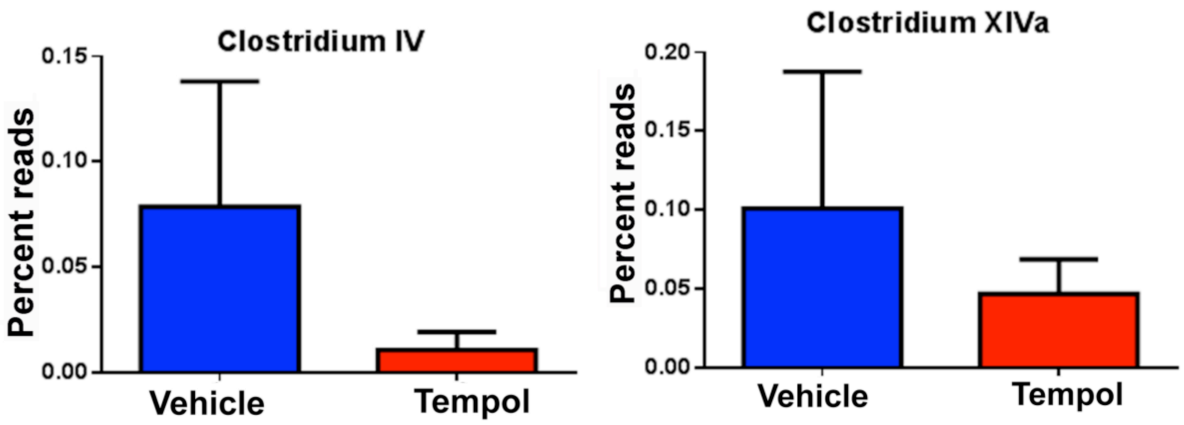


Supplementary Figure S2. Tempol protected mice from HFD-induced obesity and glucose intolerance.

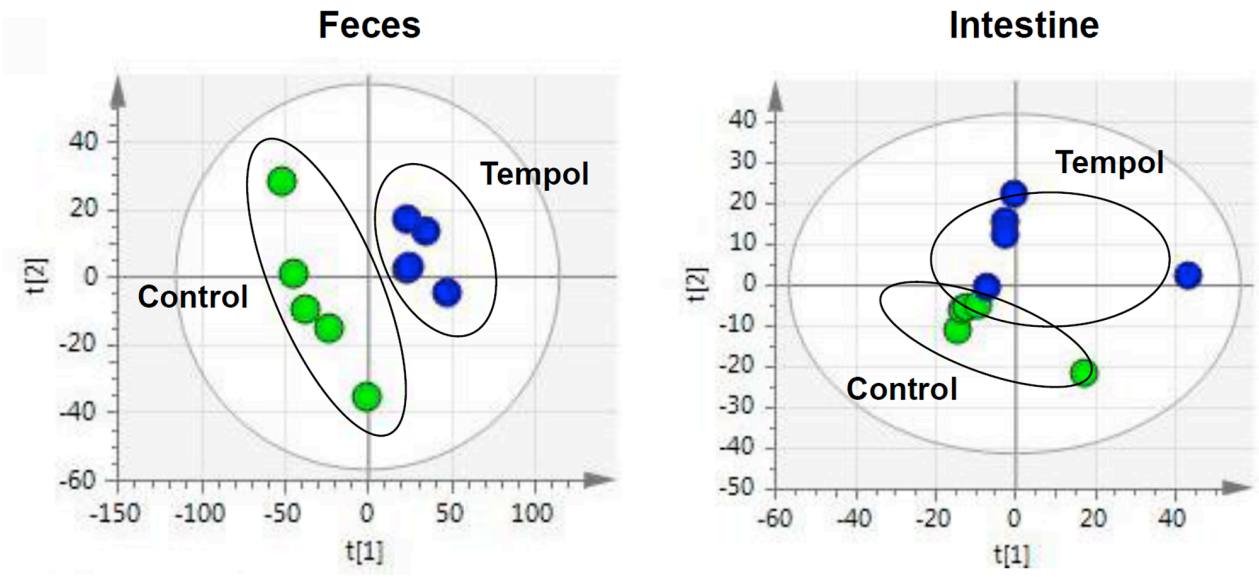
(a) Fat mass to body mass ratio in vehicle and tempol-treated mice after 16 weeks of HFD. n=5 mice per group.

(b) Water and food intake in vehicle and tempol treated mice on an HFD measured after 7 weeks of treatment. n=5 mice per group.

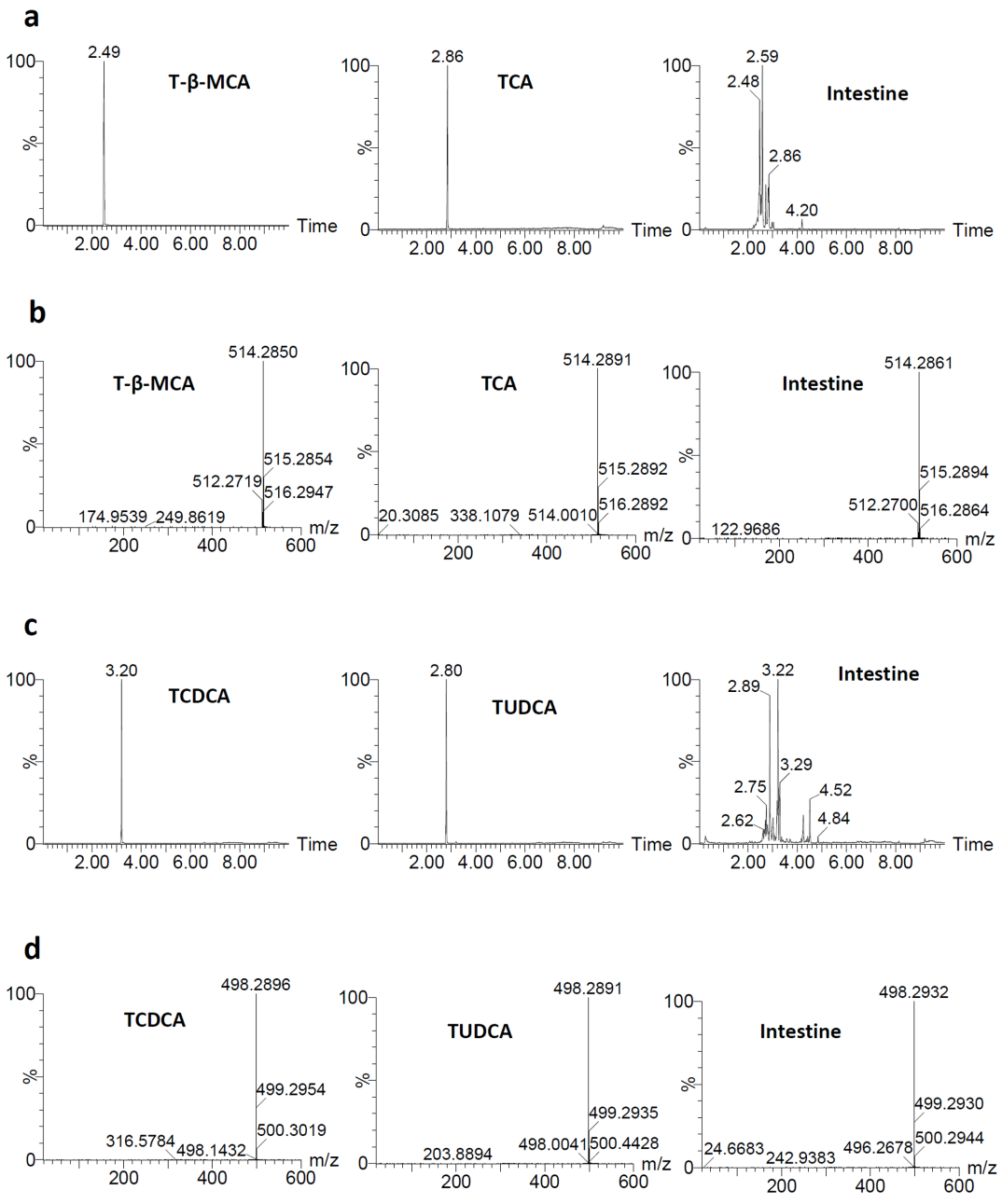
(c) Water and food intake in vehicle and tempol treated mice on an HFD measured after 15 weeks of treatment. n=5 mice per group. All data are presented as mean ± SD. Analysis of variance followed by two-tailed Student's t-test. **P*<0.05 compared to vehicle treated mice.



Supplementary Figure S3. Tempol treatment induces gut microbiome robust shifts.
(a) 16S rRNA gene sequencing analysis of genus *Clostridium* cluster IV and *Clostridium* cluster XIVa of cecum content after 5 days tempol treatment by gavage (250 mg/kg). n=3/vehicle group, n=4/tempol group. Data are presented as mean ± SD.

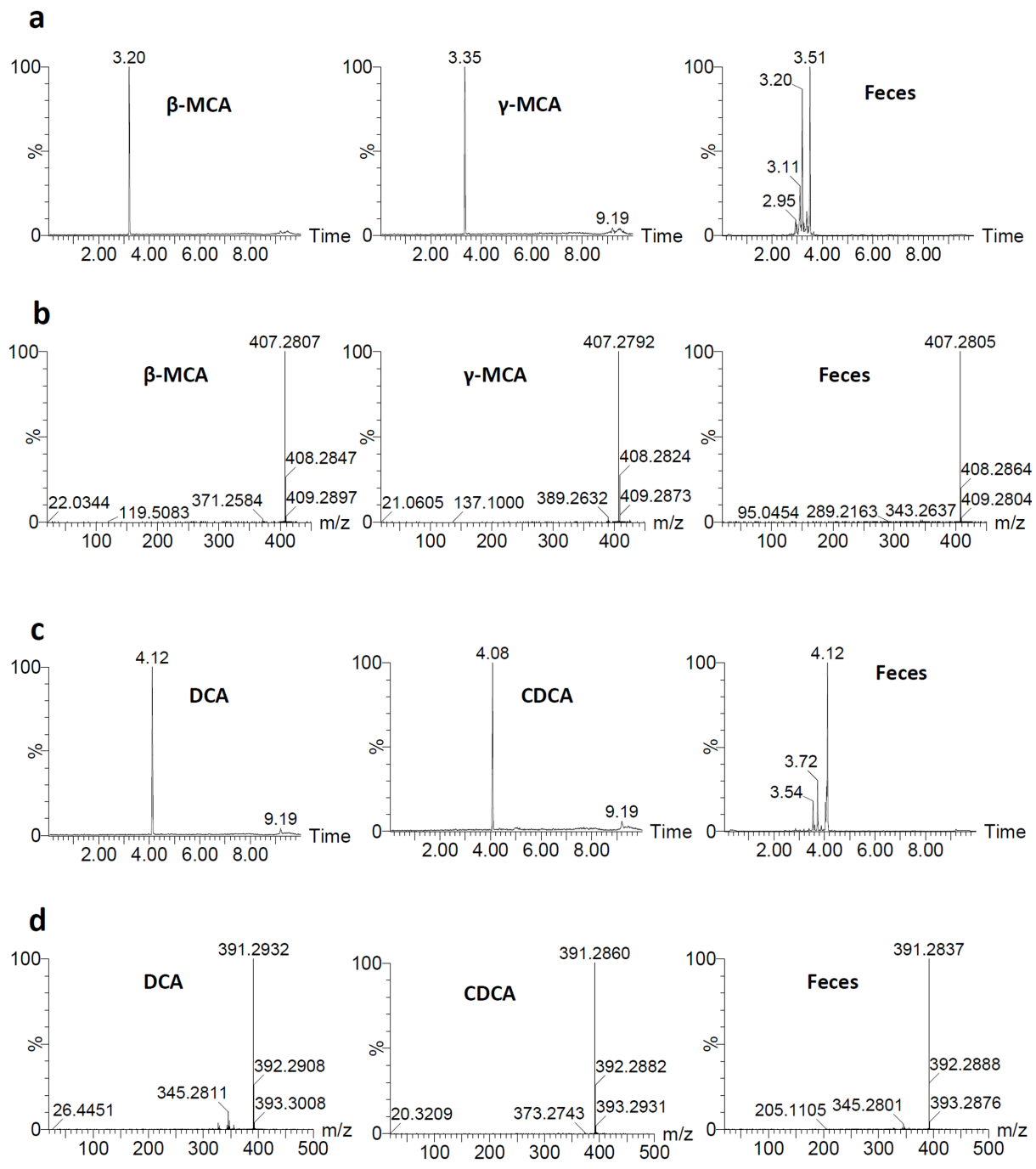


Supplementary Figure S4. Metabolomics analysis of feces and intestine.
PLS-DA of feces and intestine metabolites between control control (green closed circle) and tempol group (blue closed circle) in HFD. Each point represents an individual mouse sample. n=4-5 mice per group.

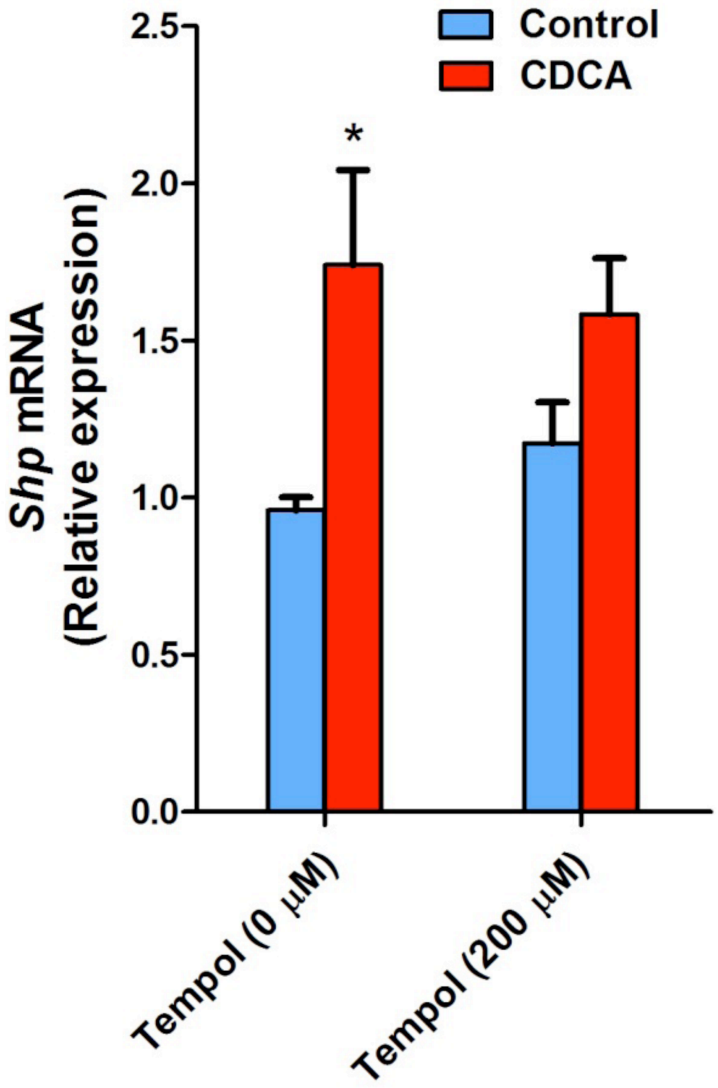


Supplementary Figure S5. Validation of taurine-conjugated bile acids increased in the intestine by tempol.

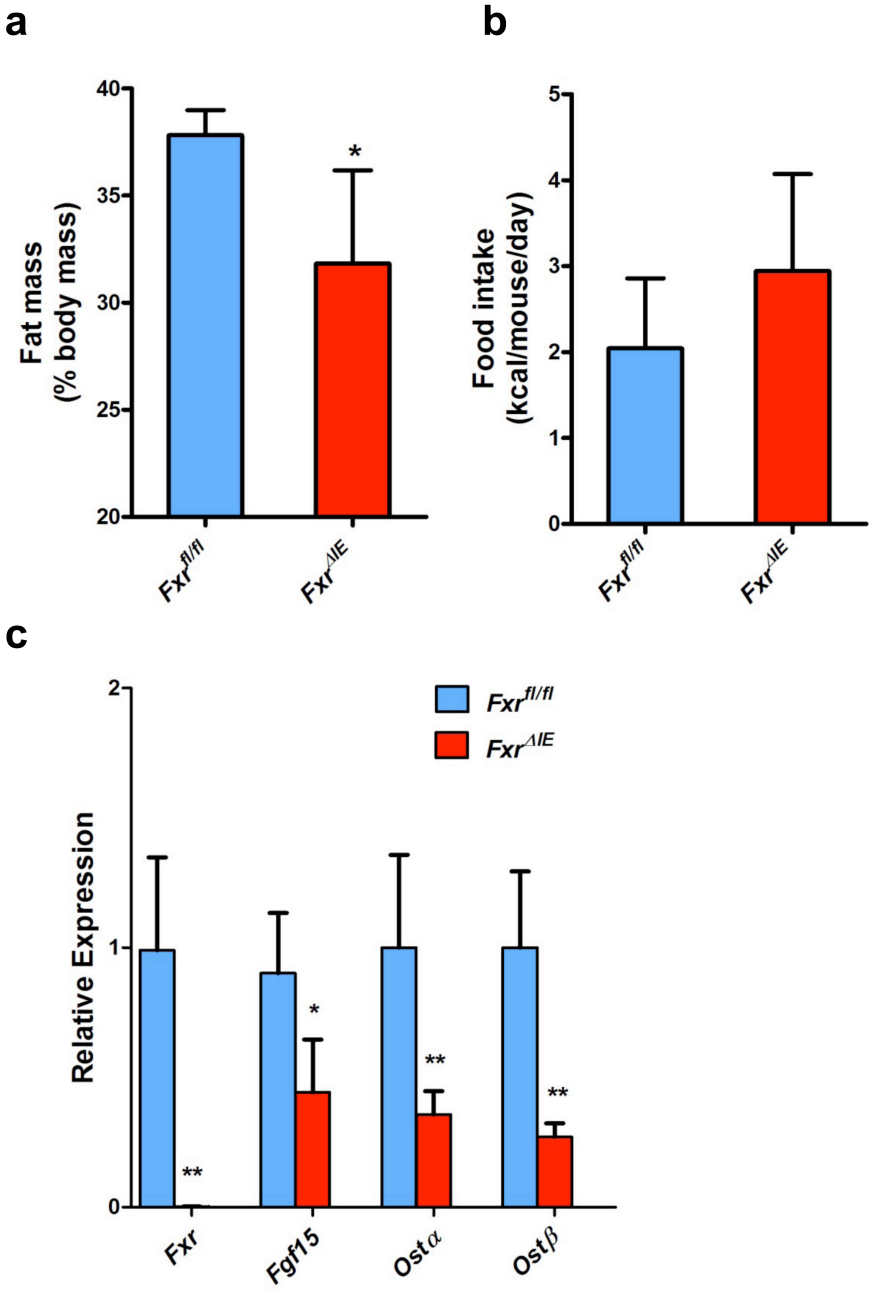
(a-d) Ion identification was performed by tandem mass spectrometry MS/MS fragmentation and retention time comparisons with authentic standards.



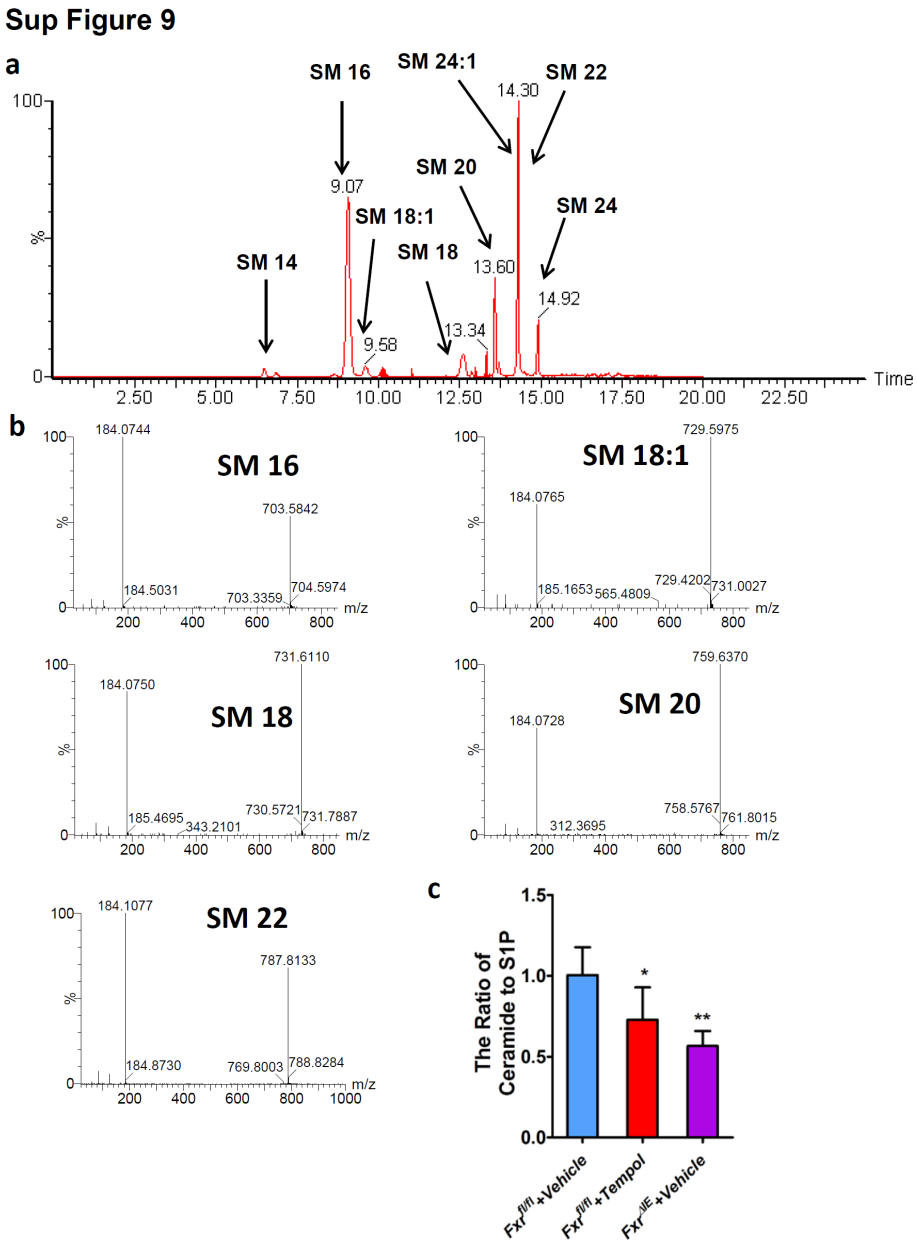
Supplementary Figure S6. Validation of free bile acids increased in the feces by tempol. **(a-d)** Ion identification was performed by tandem mass spectrometry MS/MS fragmentation and retention time comparisons with authentic standards.



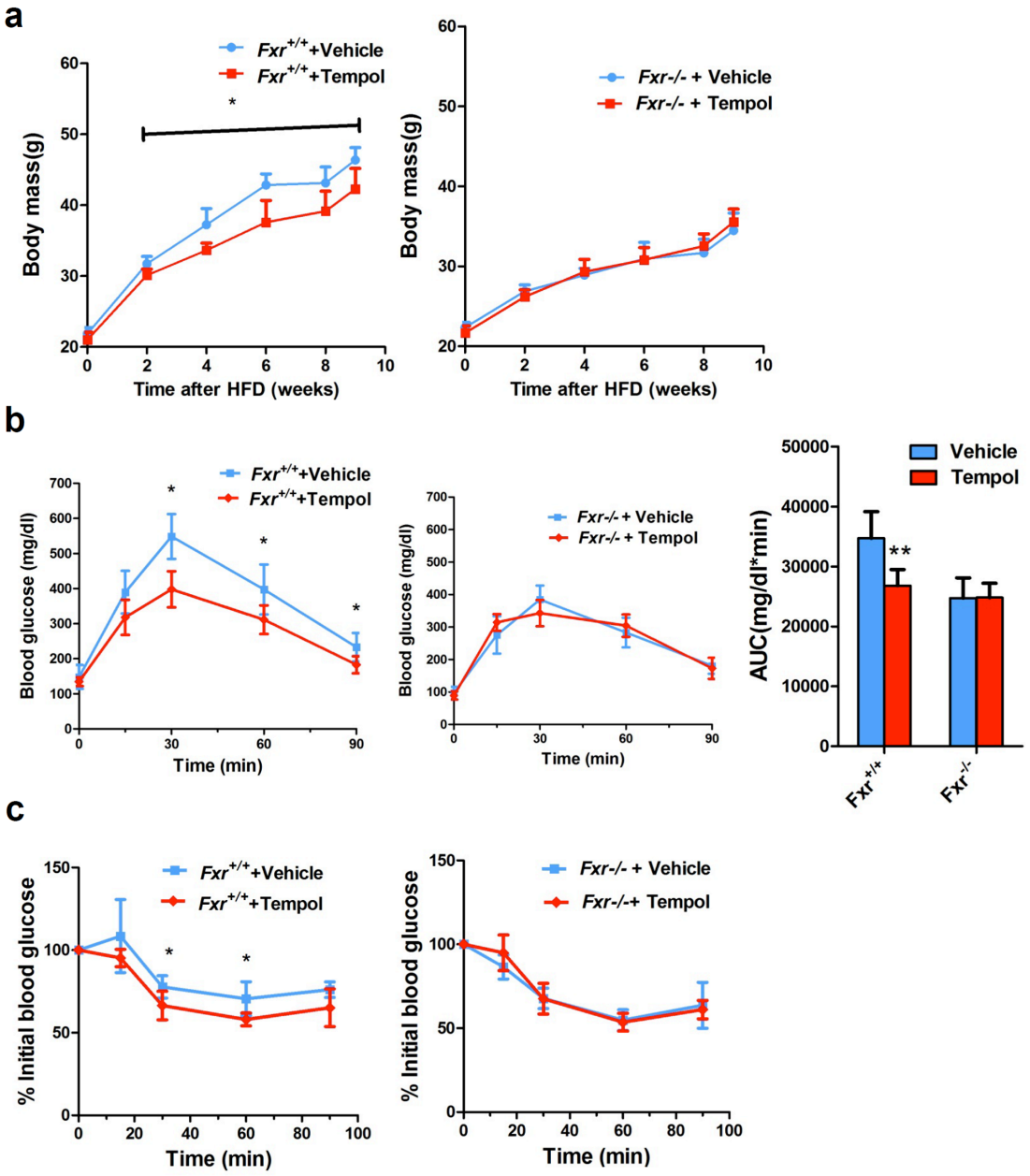
Supplementary Figure S7. The influence of tempol on FXR signaling in HepG2 cells. *Shp* mRNA expression was determined in HepG2 cells after treatment with 200 μM tempol and co-treatment of tempol with 100 μM chenodeoxycholic acid (CDCA) an FXR agonist. The expression was normalized to 18S RNA. n=3. All data are presented as mean ± SD. Analysis of variance followed by one-way ANOVA with Dunnett's test.*P<0.01 compared to control.



Supplementary Figure S8 Intestine-specific FXR knockout mice are resistant to HFD-induced obesity and insulin resistance.
(a) The fat mass to body mass ratio of the mice on 6 weeks of HFD. n =5/group.
(b) Food intake in $Fxr^{fl/fl}$ and $Fxr^{\Delta IE}$ mice on an HFD measured after 14 weeks of treatment. Data are mean ± SD. n=5/group.
(c) qPCR analysis of *Fxr* and *Fxr* target genes in the intestinal mucosa on a HFD. Expression was normalized to 18S. n =5/group. All data are presented as mean ± SD. Analysis of variance followed by two-tailed Student's t-test. $*P<0.05$, $**P<0.01$ compared to $Fxr^{fl/fl}$ mice.



Supplementary Figure S9. Lipidomics analysis of serum sphingolipid.
(a) Chromatogram of target analysis of serum sphingomyelin (SM).
(b) Tandem mass spectrometry MS/MS fragmentation of several typical SM, including SM 16, SM 18, SM 18:1, SM 20, and SM 22.
(c) The ratio of Ceramide to S1P in $Fxr^{fl/fl}$ (wild type)+vehicle, $Fxr^{fl/fl}$ (wild type) +tempol, and $Fxr^{\Delta IE}$ +vehicle**.** Serum Ceramide is quantified by an ACQUITY UPLC system coupled with a XEVO triplequadrupole tandem mass spectrometer (Waters). Serum S1P is detected by the S1P ELISA kit. n =4-5/group. All data are presented as mean ± SD. Analysis of variance followed by two-tailed Student's t-test. $^{**}P<0.01$ compared to $Fxr^{fl/fl}$ + vehicle mice.

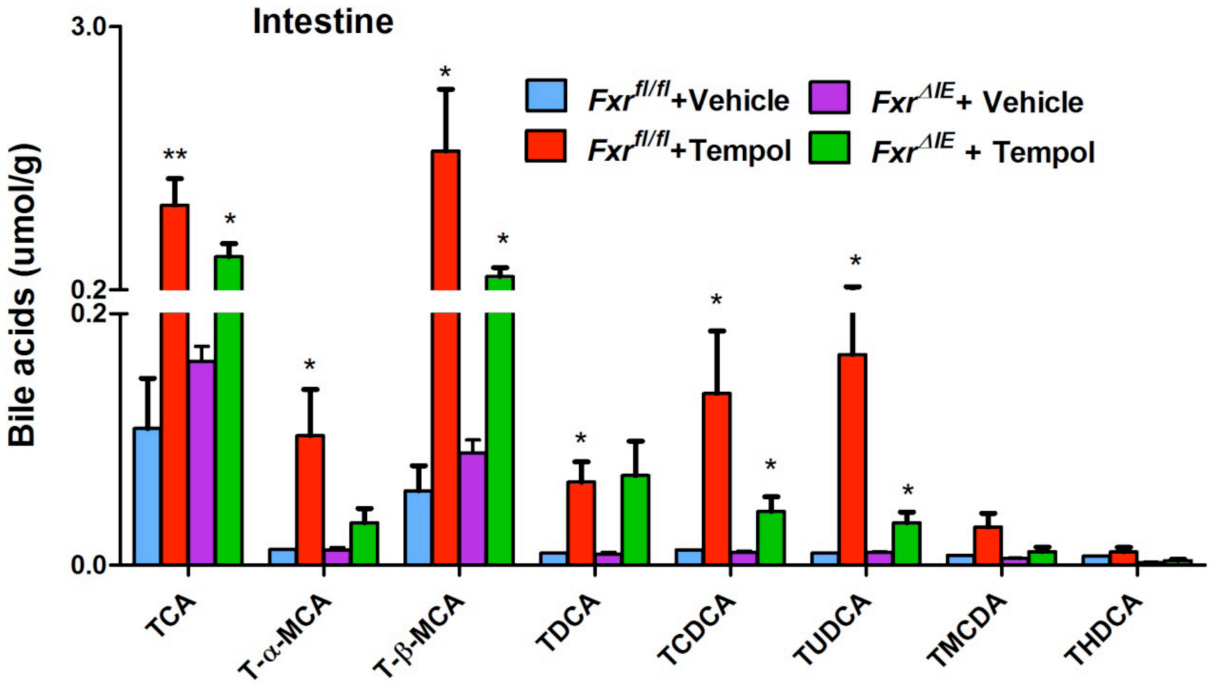


Supplementary Figure S10. The inhibition of FXR is essential for tempol to ameliorate metabolic homeostasis.

(a) Growth curves of vehicle and tempol-treated *Fxr*$^{+/+}$ and *Fxr*$^{-/-}$ mice on a HFD. n =5/group.

(b) Blood glucose levels in GTT and the area under the curve (AUC) of 8 weeks tempol-treated *Fxr*$^{+/+}$ and *Fxr*$^{-/-}$ mice maintained on a HFD. n =5/group.

(c) Insulin tolerance test (ITT) of 9 weeks after HFD. n =4-5/group.. All data are presented as mean ± SD. Analysis of variance followed by two-tailed Student's t-test. **P*<0.05, ***P*<0.01 compared to vehicle-treated mice at the same genotyping mice.



Supplementary Figure S11. Metabolomics analysis identified the alteration of bile acid composition by tempol.

Bile acid composition in intestine of *Fxr*$^{fl/fl}$+Vehicle, *Fxr*$^{fl/fl}$ +Tempol, *Fxr*$^{\Delta IE}$ + Vehicle, and *Fxr*$^{\Delta IE}$ +Tempol. Bile acid composition was determined using UPLC-ESI-QTOFMS. n=5 mice per group. All data are presented as mean ± SD. Analysis of variance followed by one-way ANOVA with Tukey's test. **P*<0.05, ***P*<0.01 compared to vehicle treated mice of the same genotype.

Supplementary Table S1. Primers used for qPCR.

Bacterial Primers	Sequence
All groups FWD	5'- ACT CCT ACG GGA GGC AGC AG -3'
All groups REV	5'- ATT ACC GCG GCT GCT GG -3'
Firmicutes FWD	5'- GCA GTA GGG AAT CTT CCG -3'
Firmicutes REV	5'- ATT ACC GCG GCT GCT GG -3'
Bacteroidetes FWD	5'- GTA CTG AGA CAC GGA CCA -3'
Bacteroidetes REV	5'- ATT ACC GCG GCT GCT GG -3'
Actinobacteria FWD	5'- CGC GGC CTA TCA GCT TGT TG -3'
Actinobacteria REV	5'- ATT ACC GCG GCT GCT GG -3'
α-Proteobacteria FWD	5'- ACT CCT ACG GGA GGC AGC AG -3'
α-Proteobacteria REV	5'- TCT ACG RAT TTC ACC YCT AC -3'
β-Proteobacteria FWD	5'- ACT CCT ACG GGA GGC AGC AG -3'
β-Proteobacteria REV	5'- TCA CTG CTA CAC GYG -3'
Mouse primers	Sequence
18S FWD	5'- ATTGGAGCTGGAATTACCGC -3'
18S REV	5'- CGGCTACCACATCCAAGGAA -3'
Fxr FWD	5'- TGGGCTCCGAATCCTCTTAGA -3'
Fxr REV	5'- TGGTCCTCAAATAAGATCCTTGG -3'
Shp FWD	5'- TCTGCAGGTCGTCCGACTATTC -3'

Shp REV	5'- AGGCAGTGGCTGTGAGATGC -3'
Cyp7a1 FWD	5'- AACAACCTGCCAGTACTAGATAGC -3'
Cyp7a1 REV	5'- GTGTAGAGTGAAGTCCTCCTTAGC -3'
Bsep FWD	5'- TCTGACTCAGTGATTCTTCGCA -3'
Bsep REV	5'- GTGTAGAGTGAAGTCCTCCTTAGC -3'
Fgf15 FWD	5'- GCCATCAAGGACGTCAGCA -3'
Fgf15 REV	5'- CTTCCTCCGAGTAGCGAATCAG -3'
Ostα FWD	5'- TACAAGAACACCCTTTGCCC -3'
*Ostα*REV	5'- CGAGGAATCCAGAGACCAAA -3'
Ostβ FWD	5'- GTATTTTCGTGCAGAAGATGCG -3'
*Ostβ*REV	5'- TTTCTGTTTGCCAGGATGCTC -3'
Fabp1 FWD	5'- GGAATTGGGAGTAGGAAGAGCC -3'
Fabp1 REV	5'- TGGACTTGAACCAAGGAGTCAT -3'
Fabp2 FWD	5'- GTGGAAAGTAGACCGGAACGA -3'
Fabp2 REV	5'- CCATCCTGTGTGATTGTCAGTT -3'
Fabp3 FWD	5'- ACCTGGAAGCTAGTGGACAG -3'
Fabp3 REV	5'- TGGACTTGAACCAAGGAGTCAT -3'
Fabp4 FWD	5'- AAGGTGAAGAGCATCATAACCCT -3'
Fabp4 REV	5'- TCACGCCTTTCATAACACATTCC -3'
Fabp6 FWD	5'- CTTCCAGGAGACGTGATTGAAA -3'
Fabp6 REV	5'- AACTTGTTGCTCATAATGTTGCC -3'
Pparα FWD	5'- CCCAAGGGAGGAATAGCTTCT -3'
*Pparα*REV	5'- CTCTGCGATGCGGTTCCAA -3'
*Cpt1*FWD	5'- TCTTCACTGAGTTCCGATGGG -3'

Cpt1 REV	5'- ACGCCAGAGATGCCTTTTCC -3'
Cpt2 FWD	5'- CAGCACAGCATCGTACCCA -3'
Cpt2 REV	5'- TCCCAATGCCGTTCTCAAAAT-3'
*Acox1*FWD	5'- CCGCCACCTTCAATCCAGAG -3'
*Acox1*REV	5'- CAAGTTCTCGATTTCTCGACGG -3'
Acox2 FWD	5'- AACCCAGGGGATCGAGTGT -3'
Acox2 REV	5'- CGCAGCTCAGTGTTTGGGAT -3'
Cd36 FWD	5'- GCGACATGATTAATGGCACA -3'
Cd36 REV	5'- CCTGCAAATGTCAGAGGAAA -3'
Acsl FWD	5'- TGCCAGAGCTGATTGACATTC -3'
Acsl REV	5'- GGCATACCAGAAGGTGGTGAG -3'
Acsm FWD	5'- CTTTGGCCCCAGCAGTAGATG -3'
*Acsm*REV	5'- GGCTGTCACTGGCATATTTCAT-3'
Human primers	Sequence
18S FWD	5'- GATATGCTCATGTGGTGTTG -3'
18S REV	5'- AATCTTCTTCAGTCGCTCCA -3'
Shp FWD	5'- AGGGACCATCCTCTTCAACC -3'
Shp REV	5'- ACTTCACACAGCACCCAGTG -3'