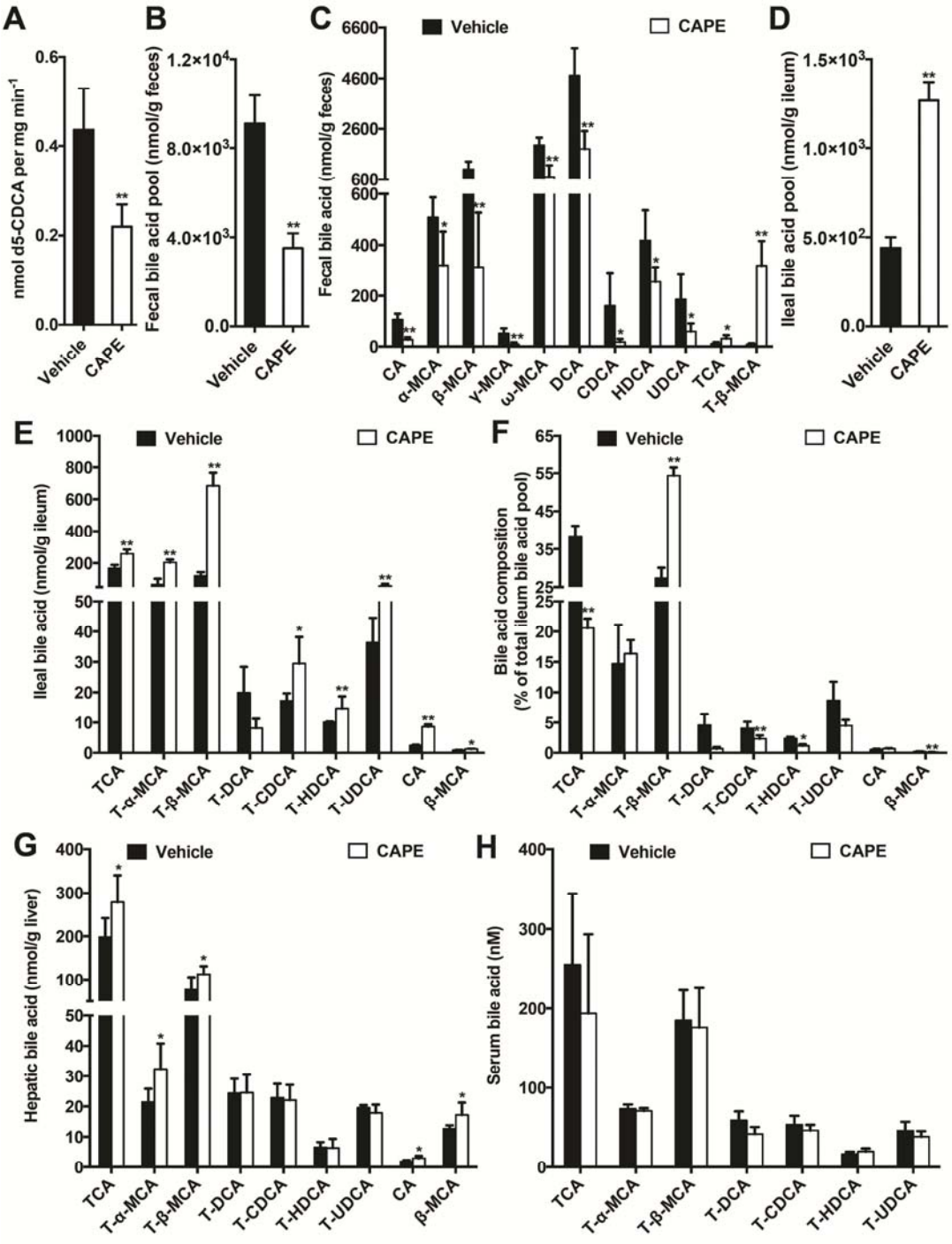
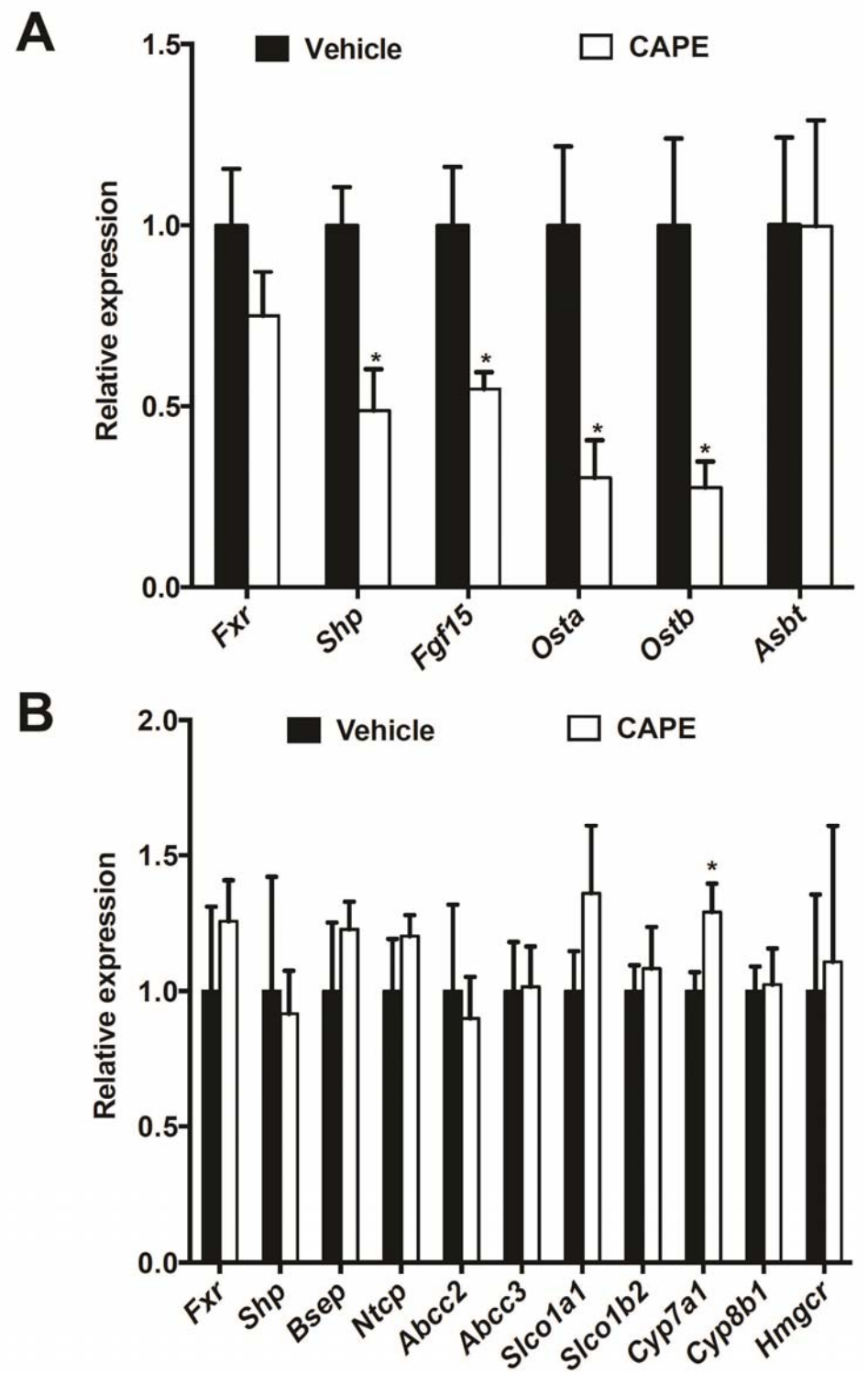
SUPPLEMENTARY DATA

Supplementary Figure 1. Metabolomics analysis identifies an alteration of bile-acid composition by CAPE. Mice on a HFD were orally treated with or without CAPE for 4 weeks (n = 5). (A) Bile salt hydrolase (BSH) enzyme activity was determined from the cecal contents. (B,D) Total levels of bile acids in the feces (B) and the ileums (D). (C) Individual bile acid levels in the feces. (E) Individual bile acid levels in the ileum. (F) The percentage of the individual taurine-conjugated bile acids in the ileum. (G) Individual bile acid levels in the liver. (H) Individual bile acid levels in serum. Data are presented as the means ± sd. Two-tailed Student's t-test: $^{**}P<0.01$ compared to the vehicle.



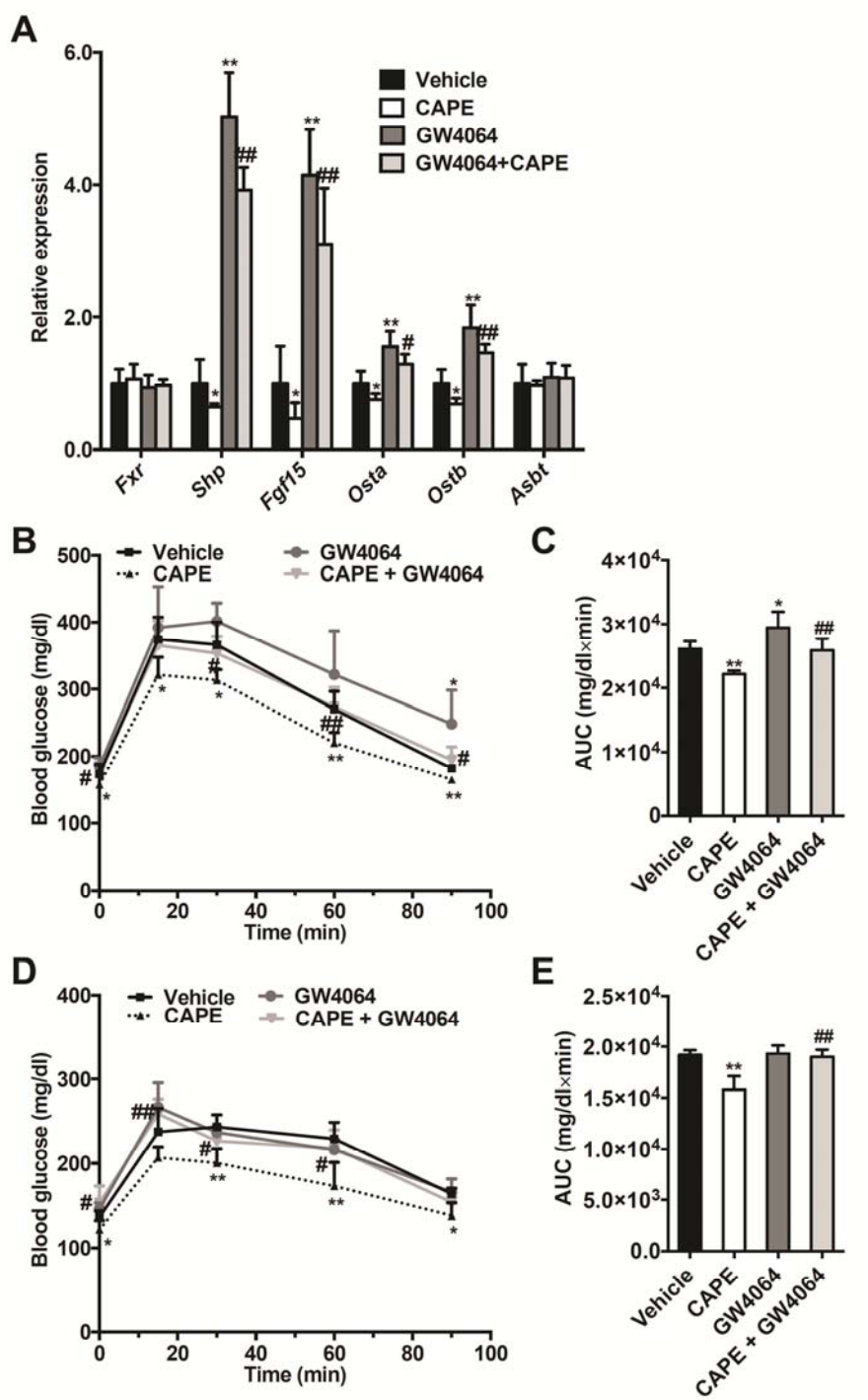
SUPPLEMENTARY DATA

Supplementary Figure 2. CAPE inhibits intestinal FXR signaling. Mice were orally treated with or without CAPE o.p.d. for 4 weeks on a HFD (n = 5). qPCR analysis of the mRNA levels of FXR target genes in the ileum (A) and the liver (B). Data are presented as the means ± sd. Two-tailed Student's t-test: *P < 0.05 compared to the vehicle.



SUPPLEMENTARY DATA

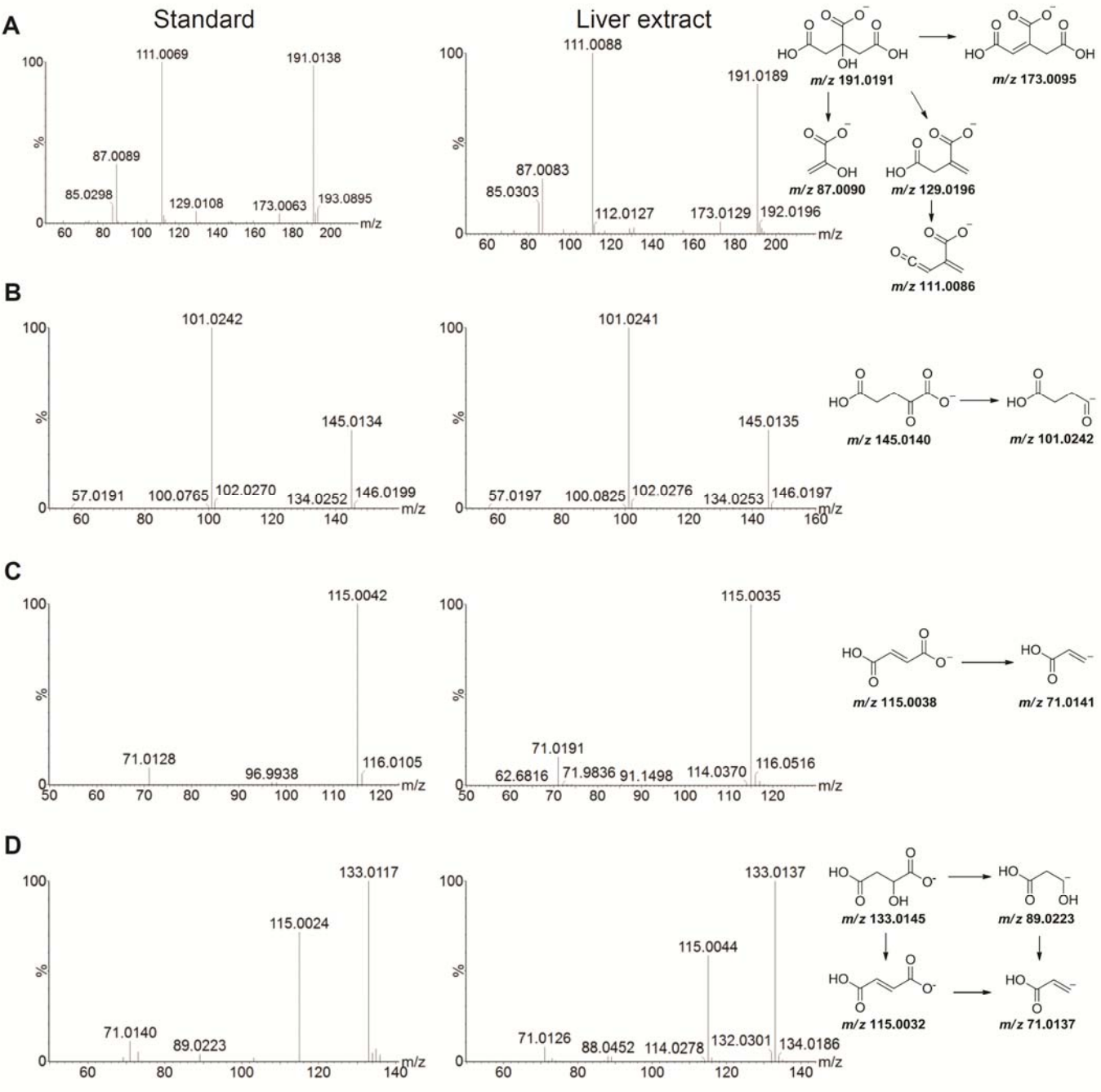
Supplementary Figure 3. CAPE suppresses the HFD-induced increases in hepatic gluconeogenesis through inhibition of the intestinal FXR. Vehicle-, CAPE-, GW4064-, and CAPE+GW4064-treated mice fed a HFD for 4 weeks (n = 5). (A) qPCR analysis of the mRNA levels of FXR target genes in the ileum. (B,C) GTT (B) and AUC (C). (D,E) PTT (D) and AUC (E). Data are presented as the means ± sd. One-way ANOVA with Tukey's correction: *P < 0.05, **P < 0.01 compared to the vehicle. #P < 0.05, ## P < 0.01 compared to the CAPE treatment.



SUPPLEMENTARY DATA

Supplementary Figure 4. Comparison of the ions identified in the PLS-DA model with authentic standards.

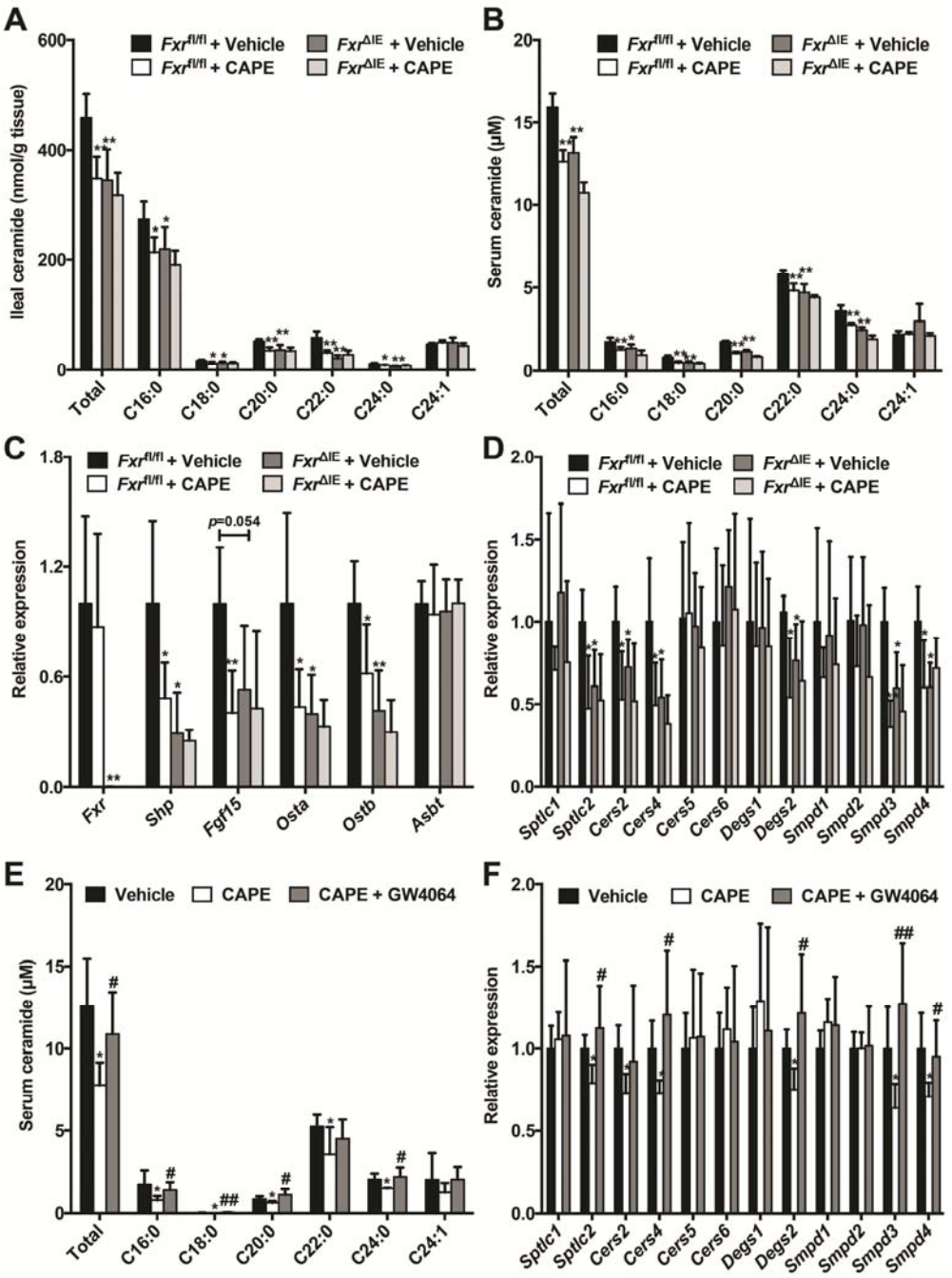
The fragmentation patterns of authentic standards for (A) citrate, (B) α-ketoglutarate, (C) fumarate, and (D) malate are compared to the fragmentation patterns of molecules in the liver extracts.



SUPPLEMENTARY DATA

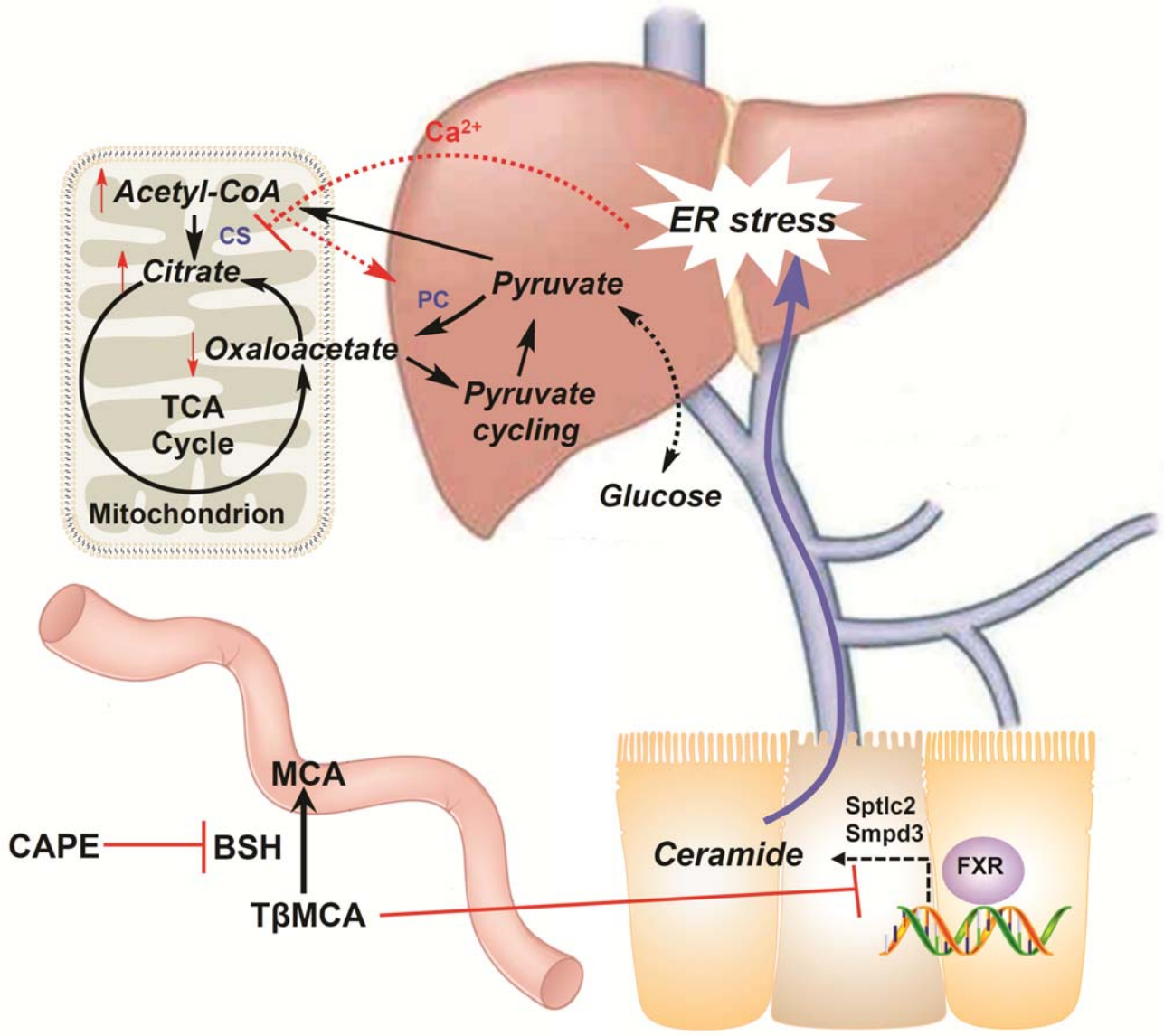
Supplementary Figure 5. CAPE treatment decreases ileum and serum ceramide levels through inhibition of intestinal FXR. (A, B) The total and individual ceramide levels in the ileum (A) and serum (B) from $Fxr^{fl/fl}$ mice and $Fxr^{\Delta IE}$ mice on a HFD and treated with or without CAPE for 4 weeks (n = 5). (C) The mRNA levels of FXR target genes in the ileum from $Fxr^{fl/fl}$ mice and $Fxr^{\Delta IE}$ mice on a HFD and treated with or without CAPE for 4 weeks (n = 5). (D) The expression of mRNAs encoding ceramide synthesis-related genes in the ileum from $Fxr^{fl/fl}$ mice and $Fxr^{\Delta IE}$ mice on a HFD and treated with or without CAPE for 4 weeks (n = 5). (A-D) Data are presented as the means ± sd. One-way ANOVA with Tukey's correction: $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the vehicle-treated $Fxr^{fl/fl}$ mice. (E) The total and individual ceramide levels in the serum from vehicle-, CAPE-, and CAPE+GW4064-treated mice on a HFD for 4 weeks (n = 5). (F) The expression of mRNAs encoding ceramide synthesis-related genes in the ileum from vehicle-, CAPE-, and CAPE+GW4064-treated mice on a HFD for 4 weeks (n = 5). Data are presented as the means ± sd. One-way ANOVA with Tukey's correction: $^{*}P < 0.05$ compared to the vehicle. $\#P < 0.05$, $\#\#\ P < 0.01$ compared to the CAPE treatment.

SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

Supplementary Figure 6. Summary of CAPE-improved HFD-disrupted hepatic glucose homeostasis through BSH-T-β-MCA-intestinal FXR-ceramide pathway, leading to decreased hepatic mitochondrial acetyl-CoA levels and PC activities without affecting hepatic insulin signaling. Ceramides substantially attenuate mitochondrial citrate synthase activity primarily through the induction of ER stress, excessive ER-mitochondria coupling, and calcium overload, primarily triggering increased hepatic mitochondrial acetyl-CoA levels and PC activities. Administration of CAPE, an inhibitor of BSH, increases intestinal T-β-MCA that selectively suppresses intestinal FXR signaling, thus repressing intestinal ceramide synthesis genes *Smpd3* and *Sptlc2,* which are novel intestinal FXR target genes. Decreased ceramides decrease hepatic mitochondrial acetyl-CoA levels and PC activities, and further attenuated hepatic gluconeogenesis, independent of body weight and hepatic insulin signaling changes.



SUPPLEMENTARY DATA

Supplementary Table 1. Primers used for qPCR

Mouse primers	Sequence
β-Actin FWD	5'- TTCTTTGCAGCTCCTTCGTT -3’
β-Actin REV	5'- ATGGAGGGGAATACAGCCC -3’
Fxr FWD	5'- TGGGCTCCGAATCCTCTTAGA -3'
Fxr REV	5'- TGGTCCTCAAATAAGATCCTTGG -3'
Shp FWD	5'- TCTGCAGGTCGTCCGACTATTC -3'
Shp REV	5'- AGGCAGTGGCTGTGAGATGC -3'
Fgf15 FWD	5'- GCCATCAAGGACGTCAGCA -3'
Fgf15 REV	5'- CTTCCTCCGAGTAGCGAATCAG -3'
Osta FWD	5'- AATTACAGCATCTCCCCTGC -3’
Osta REV	5'- GGTCAAGATGATGGTGAGGG -3’
Ostb FWD	5'- AGAGAAAGCTGCAGCCAATG -3’
Ostb REV	5'- CCAGGACCAGGATGGAATAA -3’
Bsep FWD	5'- TCTGACTCAGTGATTCTTCGCA -3’
Bsep REV	5'- GTGTAGAGTGAAGTCCTCCTTAGC -3’
Ntcp FWD	5'- CAAACCTCAGAAGGACCAAACA -3’
Ntcp REV	5'- GTAGGAGGATTATTCCCGTTGTG -3’
Abcc2 FWD	5'- GTGTGGATTCCCTTGGGCTTT -3’
Abcc2 REV	5'- CACAACGAACACCTGCTTGG -3’
Abcc3 FWD	5'- GGGCTCCAAGTTCTGGGAC -3’
Abcc3 REV	5'- CCGTCTTGAGCCTGGATAAC -3’
Slco1a1 FWD	5'- ACTCCCATAATGCCCTTGG -3’
Slco1a1 REV	5'- TAATCGGGCCAACAATCTTC -3’
Slco1b2 FWD	5'- ACCAAACTCAGCATCCAAGC -3’
Slco1b2 REV	5'- TAGCTGAATGAGAGGGCTGC -3’
Sptlc1 FWD	5'- CGAGGGTTCTATGGCACATT-3'
Sptlc1 REV	5'- GGTGGAGAAGCCATACGAGT -3'
Sptlc2 FWD	5'- TCACCTCCATGAAGTGCATC -3'
Sptlc2 REV	5'- CAGGCGTCTCCTGAAATACC -3’
Degs1 FWD	5'- AATGGGTCTACACGGACCAG -3’
Degs1 REV	5'- TGGTCAGGTTTCATCAAGGAC -3’
Degs2 FWD	5'- AAGCCAATGGACCACAAACT -3’
Degs2 REV	5'- TGCTTGGAGAGCCCTTCTAAT -3’
Cers2 FWD	5'- AAGTGGGAAACGGAGTAGCG-3’
Cers2 REV	5'- ACAGGCAGCCATAGTCGTTC -3’
Cers4 FWD	5'- GGATTAGCTGATCTCCGCAC -3’
Cers4 REV	5'- CCAGTATGTCTCCTGCCACA -3’
Cers5 FWD	5'- CTTCTCCGTGAGGATGCTGT-3’
Cers5 REV	5'- GTGTCATTGGGTTCCACCTT -3’
Cers6 FWD	5'- AAGCCAATGGACCACAAACT -3’
Cers6 REV	5'- TGCTTGGAGAGCCCTTCTAAT -3’
Smpd1 FWD	5'- GTTACCAGCTGATGCCCTTC -3’
Smpd1 REV	5'- AGCAGGATCTGTGGAGTTG -3’
Smpd2 FWD	5'- AGCAGGATCTGTGGAGTTG -3’
Smpd2 REV	5'- CTCCAGCCATGAAGCTCAAC -3’
Smpd3 FWD	5'- CCTGACCAGTGCCATTCTTT -3’
Smpd3 REV	5'- AGAAACCCGGTCCTCGTACT -3’
Smpd4 FWD	5'- ACCTGGCCCTCAATCCATTTG -3’
Smpd4 REV	5'- ATAGGCACAGTCCGAAGTACG -3’

SUPPLEMENTARY DATA

Cs FWD	5'- GGACAATTTTCCAACCAATCTGC -3’
Cs REV	5'- TCGGTTCATTCCCTCTGCATA -3’
Idh3a FWD	5'- TGGGTGTCCAAGGTCTCTC -3’
Idh3a REV	5'- TGGGTGTCCAAGGTCTCTC -3’
Idh3b FWD	5'- TGGAGAGGTCTCGGAACATCT -3’
Idh3b REV	5'- TGGAGAGGTCTCGGAACATCT -3’
Idh3g FWD	5'- GGTGCTGCAAAGGCAATGC -3’
Idh3g REV	5'- TATGCCGCCCACCATACTTAG -3’
Pc FWD	5'- CTGAAGTTCCAAACAGTTCGAGG -3’
Pc REV	5'- CGCACGAAACACTCGGATG -3’
Pepck FWD	5'- CTGCATAACGGTCTGGACTTC -3’
Pepck REV	5'- CAGCAACTGCCCGTACTCC -3’
G6pc FWD	5'- CGACTCGCTATCTCCAAGTGA -3’
G6pc REV	5'- GTTGAACCAGTCTCCGACCA -3’
Pdh FWD	5'- GAAATGTGACCTTCATCGGCT -3’
Pdh REV	5'- TGATCCGCCTTTAGCTCCATC -3’
Atf4 FWD	5'- ATGGCGCTCTTCACGAAATC -3’
Aft4 REV	5'- ACTGGTCGAAGGGGTCATCAA -3’
Atf6 FWD	5'- TCGCCTTTTAGTCCGGTTCTT -3’
Aft6 REV	5'- GGCTCCATAGGTCTGACTCC -3’
Bip FWD	5'- ACTTGGGGACCACCTATTCCT -3’
Bip REV	5'- ATCGCCAATCAGACGCTCC -3’
Chop FWD	5'- ACCTTCACTACTCTTGACCCTG -3’
Chop REV	5'- GATGTGCGTGTGACCTCTGT -3’
Vdac1 FWD	5'- CCCACATACGCCGATCTTGG -3’
Vdac1 REV	5'- GTGGTTTCCGTGTTGGCAGA -3’
Pacs2 FWD	5'- GCAGGAAGCGGTACAAGAACA -3’
Pacs2 REV	5'- CTGGACAGGGAGACGATCC -3’
Mfn2 FWD	5'- CCAACTCCAAGTGTCCGCTC -3’
Mfn2 REV	5'- GTCCAGCTCCGTGGTAACATC -3’
Ip3r1 FWD	5'- CGTTTTGAGTTTGAAGGCGTTT -3’
Ip3r1 REV	5'- CATCTTGCGCCAATTCCCG -3’
Ip3r2 FWD	5'- TTCAGTTCCTATCGAGAGGATGT -3’
Ip3r2 REV	5'- GCTGATTGACGCAAGGTCG -3’