Supplementary materials

TGFβ-SMAD3 signal is involved in hepatic phospholipid and bile acid metabolism following lithocholic acid-induced liver injury.

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Synthesis of bile salts

1. Tauromurideoxycholate: C-6 alcohol's selective oxidation of hyodeoxycholic acid methyl ester was converted to 6-oxo-lithocholic acid methyl ester with potassium chromate in acetic acid. 6-Oxo-lithocholic acid methyl ester was reduced to 6α-alcohol with sodium borohydride and palladium chloride in methanol. Continuously, this 6α-hydroxy-lithocholic acid methyl ester was hydrolyzed with 5% methanolic potassium hydroxide to murideoxycholic acid.[1] The taurine conjugate was prepared via the pentafluorophenyl ester intermediate. Murideoxycholic acid was treated with pentafluorophenol, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimde hydrochloride, and catalytic amount of N,N-dimethylformamide in methanol to obtain its pentafluorophenyl ester intermediate which was reacted with taurine and 1,8-diazabicyclo[5,4,0]undec-7-ene in methylene chloride to afford tauromurideoxycholate (TMDC). The synthesized chemical was confirmed with ^{1}H-nuclear magnetic resonance (^{1}H-NMR, Supplementary figure 4A) and high-resolution mass spectrometry (MS, Supplementary figure 4B).

2. Tauro-5β-cholanic acid-3-one: touro-5β-cholanic acid-3-one (T3KL) was prepared from 5β-cholanic acid-3-one (3-ketolithocholic acid, Sigma-Aldrich) using the same method as tauromurideoxycholate synthesis from murideoxycholic acid. The synthesized chemical was confirmed by ^{1}H-NMR and MS (Supplementary figure 4C and 4D).

Microsomal assay

To measure bile acid hydroxylase activities, reaction mixtures consisting of 50 μg of liver microsomal protein 100 mM Tris buffer (pH 7.4) and bile acids (substrate), were made in a total volume of 75 μL. The reaction was started by the addition of NADPH to a final concentration of 1 mM, incubated at 37°C for 30 min, and terminated by the addition of 75 μL of acetonitrile

including 1 μM dehydrocholic acid as an internal standard. After vortexing, the reaction mix was centrifuged at 18,000g for 15 min and the supernatant subjected to UPLC-ESI-QTOFMS.

Supplementary Table 1. Sequences of qPCR primers.

This table shows oligonucleotides for qPCR used in this study.

(A) Mouse primer sets

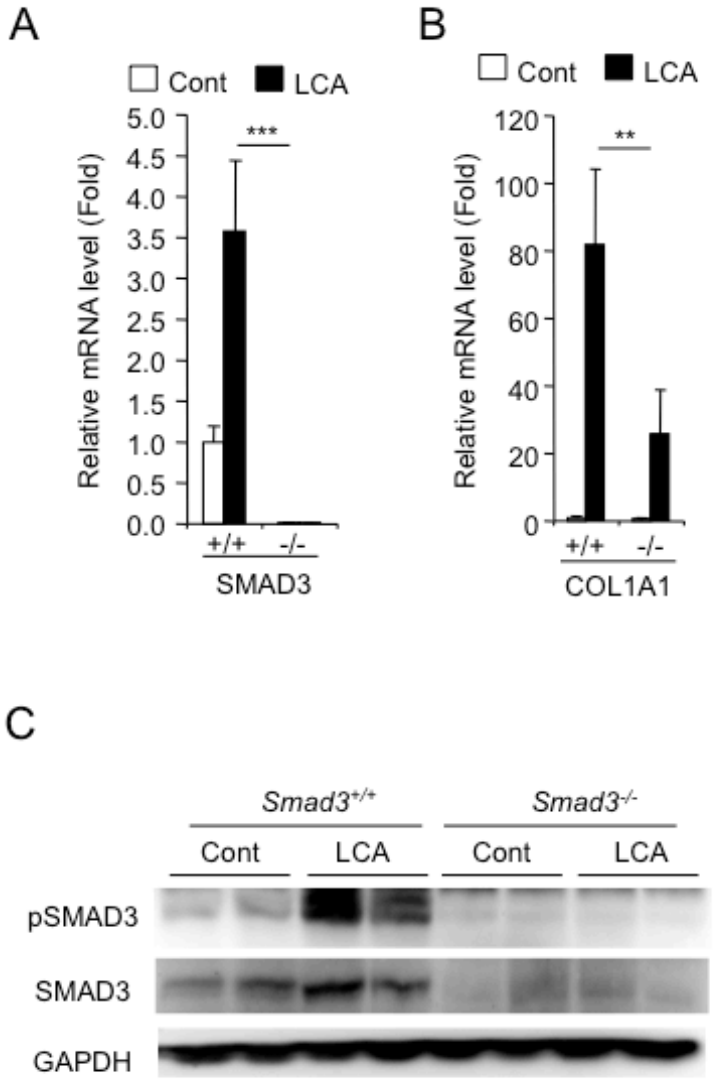
Gene	Forward (5' to 3')	Reverse (5' to 3')
18S	ATTGGAGCTGGAATTACCGC	CGGCTACCACATCCAAGGAA
Tgfb1	TCGAGGGCGAGAGAAGTTTA	AAAAGAATGTCCCGGCTCTC
Tgfbr1	CTCCTCATCGTGTTGGTGG	GCAAAGACCATCTGTCTCACA
Tgfbr2	GTCGGATGTGGAAATGGAAG	CTGGCCATGACATCACTGTT
Tgfbr3	AGGAGGTGAAAGTCCCCG	AGTAGCCCAGACGAGTCCC
Cyp3a11	TTCTGTCTTCACAAACCGGC	GGGGGACAGCAAAGCTCTAT
Sult2a*	GAAGAATCCAGGGTCACTCG	CATTCTCTCATGGACAGCCA
Slco1a1	ACTCCCATAATGCCCTTGG	TAATCGGGCCAACAATCTTC
Slco1a4	CCCAGAGCTCTCCAGTTTTG	TCCCATGTTGTTCTTCTGATTG
Slco1b2	ACCAAACTCAGCATCCAAGC	TAGCTGAATGAGAGGGCTGC
Slc10a1	CTTGCGCCATAGGGATCTTC	GACAGCCACAGAGAGGGAGAA
Abcc2	TCCAGGACCAAGAGATTTGC	TCTGTGAGTGCAAGAGACAGGT
Abcb11	ACAGAAGCAAAGGGTAGCCATC	GGTAGCCATGTCCAGAAGCAG
Ostα	AATTACAGCATCTCCCCTGC	GGTCAAGATGATGGTGAGGG
Ostβ	AGAGAAAGCTGCAGCCAATG	CCAGGACCAGGATGGAATAA
Abcc1	GATGGCTCCGATCCACTCT	AGGTAGAAACAAGGCACCCA
Abcc3	GGGCTCCAAGTTCTGGGAC	CCGTCTTGAGCCTGGATAAC
Abcc4	AGCTTCAACGGTACTGGGATA	TCGTCGGGGTCATACTTCTC
Abcc5	GCCCTGGGTACAGAAGTGAC	TCTTGGCATTCCAACGATCT
Lpcat1	CACGAGCTGCGACTGAGC	ATGAAAGCAGCGAACAGGAG
Lpcat2	ACCTGTTTCCGATGTCCTGA	CCAGGCCGATCACATACTCT
Lpcat3	AGCCTTAACAAGTTGGCGAC	ATGCCGGTAAAACAGAGCC
Lpcat4	GAGTTACACCTCTCCGGCCT	GGCCAGAGGAGAAAGAGGAC
Lypla1	CCTTCACGGATTGGGAGATA	GGGGCATGTGGACAGATGTA
Enpp2	TCGAGGGCGAGAGAAGTTTA	AAAAGAATGTCCCGGCTCTC
Pld1	CTGCATCCTCAAACGGAAAG	GCTTGCTGTACTCGCTGTTG
Pld2	CAGCTACATCAGCATGACAGC	CTGCCACAGCAGCAAAGTAA
Chkα	AAAGTGCTCTTGCGGCTCTA	GACCTCTCTGCAAGAATGGC
Chkβ	GCAGAGGTTCAGAAGGGTGA	CCCCAGAAAAAGTGAGATGC
Pcyt1α	AGCCCTATGTCAGGGTGACT	GGCATGACCAGAGTGAAACA
Pcyt1β	ATAGAGCACACATGCCCACA	GGCAACGGTCAGTTTTTCAT

*Sult2a primer set should detect both Sult2a1 and Sult2a2.

(B) Human primer sets

Gene	Forward (5’ to 3’)	Reverse (5’ to 3’)
18S	CAGCCACCCGAGATTGAGCA	TAGTAGCGACGGGCGGTGTG
OSTβ	GAGCTGCTGGAAGAGATGCT	TGCTTATAATGACCACCACAGC
LPCAT4	CCCTTCGTGCATGAGTTACA	ATAAAGGCCAGAAGCACTCG

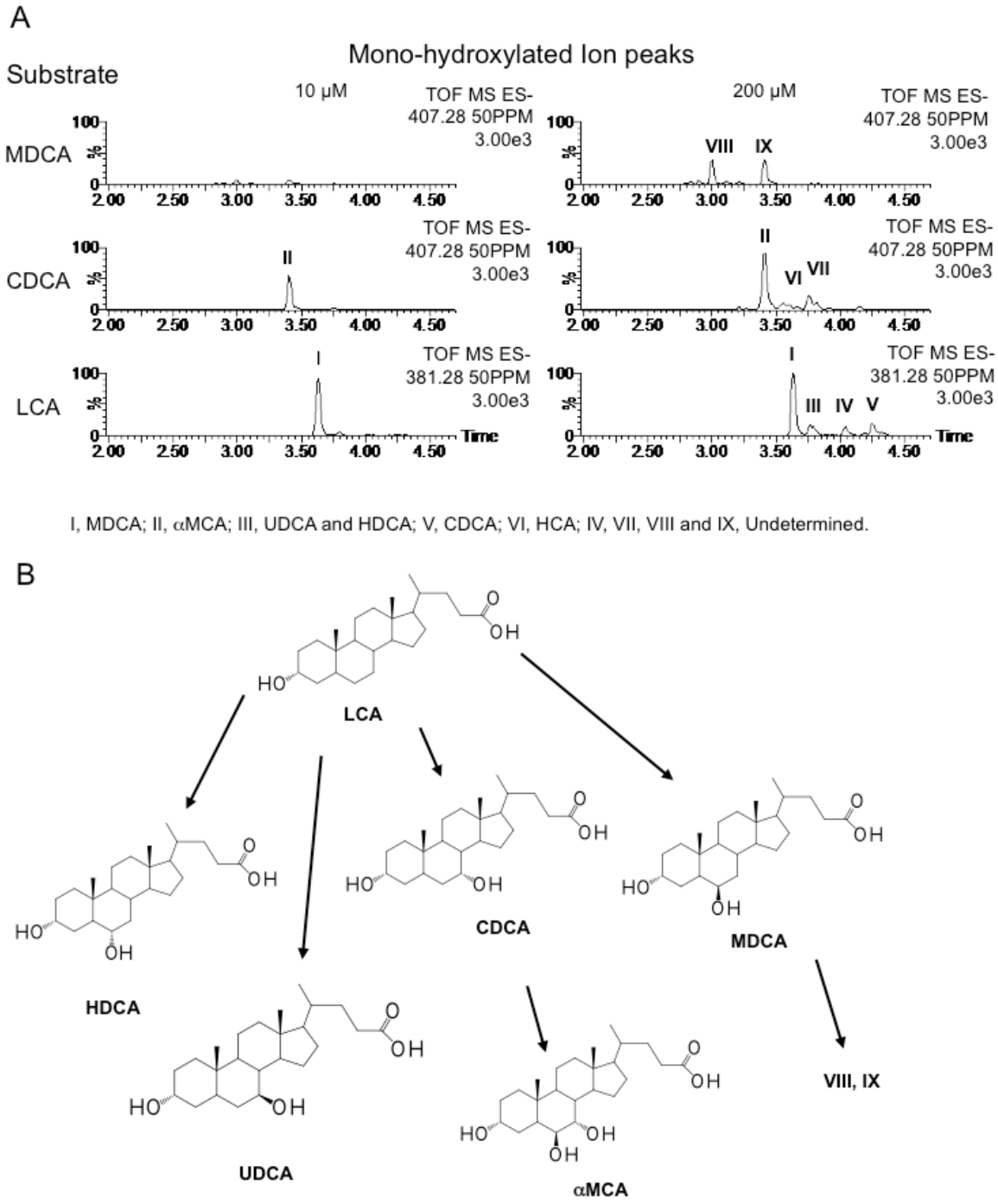
Supplementary figure 1



Supplementary figure 1. TGF-β-SMAD3 signal in *Smad3*-null mice after LCA exposure.

qPCR analysis of hepatic SMAD3 (A) and COL1A1 (B) expression. qPCR analysis was performed with primers; SMAD3 sense 5'-GCT GCC CTC CTA GCT CAG TC-3', SMAD3 antisense 5'-GGT GCT GGT CAC TGT CTG TC-3', COL1A1 sense5'-ACA TGT TCA GCT TTG TGG ACC-3', and COL1A1 antisense 5'-TAG GCC ATT GTG TAT GCA GC-3'. Samples were normalized to 18S ribosomal RNA. Significance was determined by one way-ANOVA with Bonferroni's test (**, $P<0.01$; ***, $P<0.001$). (C) Western blotting of hepatic SMAD3 phosphorylation. Proteins were detected with antibody against SMAD3 (1:1000, ab28379, Abcam) and phospho-SMAD3 (1:1000, Epitomics Cat. No. 1880-1). The signals were normalized to GAPDH (1:10,000, MAB374, Millipore, Billerica, MA).

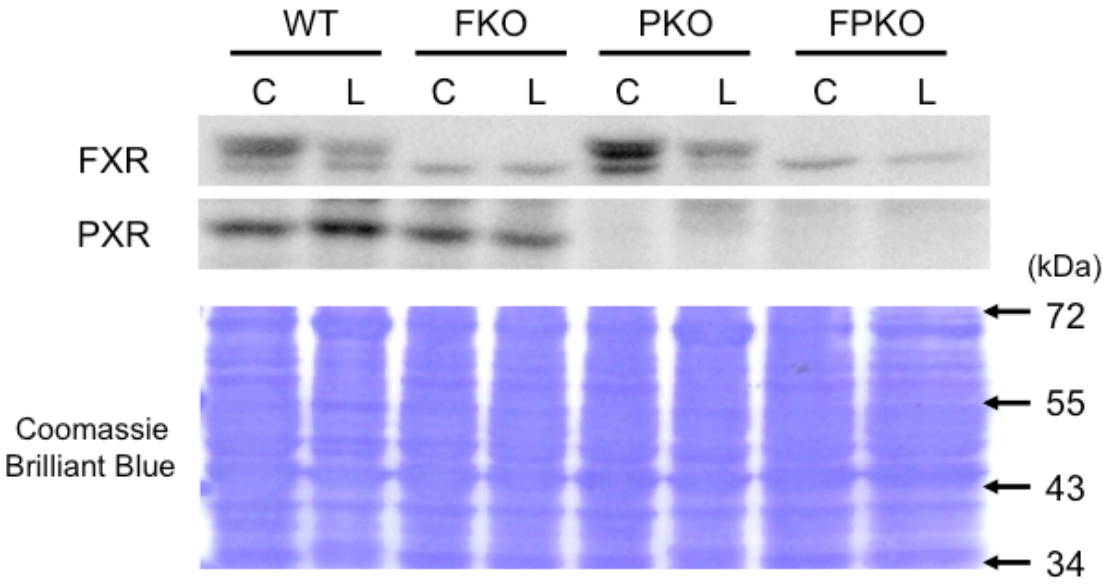
Supplementary figure 2



Supplementary figure 2. Microsomal hydroxylation of bile acid in mouse livers.

(A) UPLC-ESI-QTOFMS of bile acid metabolites after hepatic microsomal mono-hydroxylation assays. (B) Putative metabolic pathway of LCA in mouse liver.

Supplementary figure 3

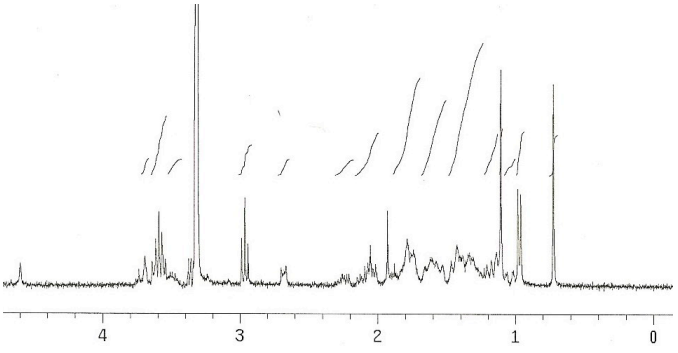


Supplementary figure 3. Nuclear FXR level is attenuated after LCA exposure, but the pregnane X receptor is not.

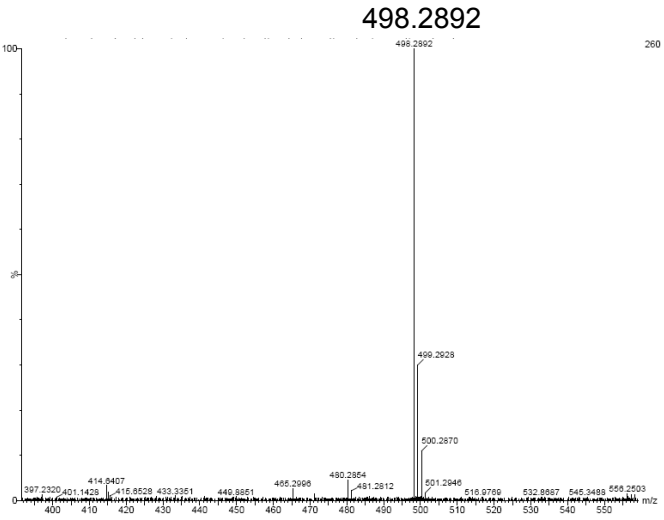
A nuclear fraction was prepared with NE-PER® Nuclear Protein Extraction Kit (Thermo Fisher Scientific, Waltham, MA), and subjected to western blotting. The samples were boiled for 5 min and then separated and transferred to PVDF membranes using standard western blotting techniques. The membranes were incubated with an antibody against FXR at a dilution of 1:1,000 (sc13063 H-130, Santa Cruz Biology, Inc) or PXR at a dilution of 1:1,000 (generously provided by Toshiya Tanaka and Tatsuhiko Kodama, University of Tokyo). WT, wild-type; FKO, *Fxr*-null; PKO, *Pxr*-null; FPKO, *Fxr*- and *Pxr*-null; C, control diet (AIN93G); L, LCA-supplemented diet.

Supplementary figure 4

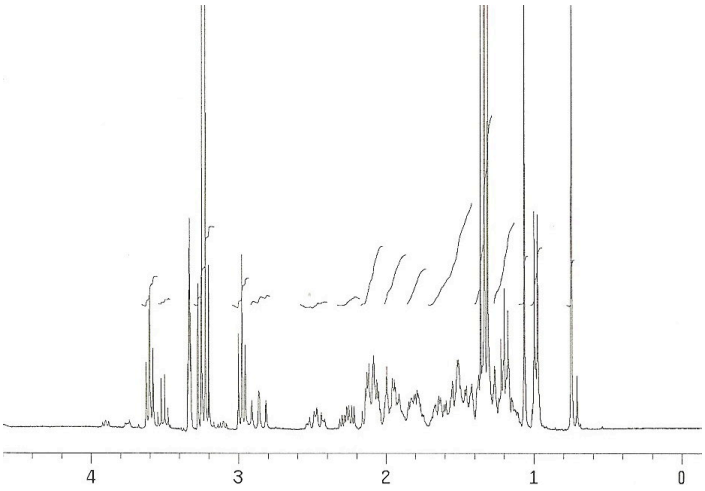
A. TMDC-^{1}H-NMR



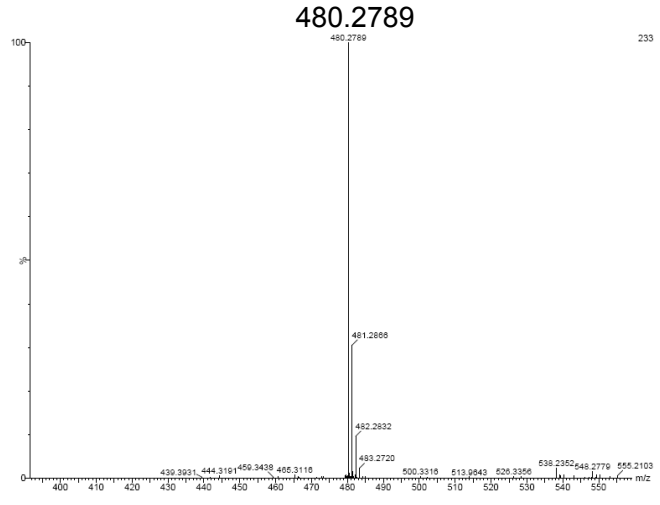
B. TMDC-MS



C. T3KL-^{1}H-NMR



D. T3KL-MS



Supplementary figure 4. ^{1}H-nuclear magnetic resonance (^{1}H-NMR) and high-resolution mass spectrometry (MS) of the synthesized chemicals

^{1}H-NMR (A) and MS (B) of tauromurideoxycholate (TMDC) and ^{1}H-NMR (C) and MS (D) of touro-5β-cholanic acid-3-one (T3KL).