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Supplementary Information for

# Hepatic JNK-mediated bile acid homeostasis regulates cholangiocarcinoma through PPARα

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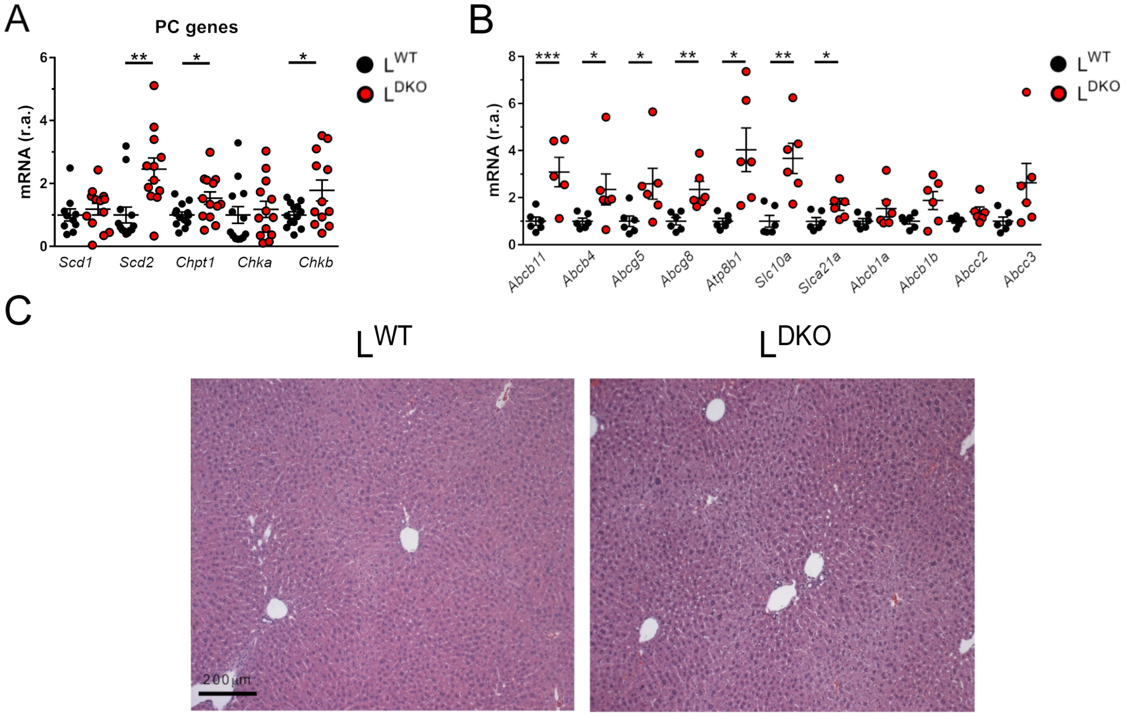

**Fig. S1. Effects of Hepatic JNK-deficiency liver.** $L^{DKO}$ and $L^{WT}$ mice (age 4 months) were fasted overnight prior to removal of the liver. (**A**), (**B**) The expression of genes related to phosphatidylcholine (PC) synthesis, hepatocyte-mediated transport of PC and BA was measured by quantitative RT-PCR. (mean ± SEM; n = 6-12). Gene expression was normalized to the amount of *18S* RNA in each sample. Student's t test differences between $L^{DKO}$ and $L^{WT}$ are indicated (*, $P < 0.05$; **, $P < 0.01$). (**C**) Representative liver sections stained with hematoxylin and eosin (H&E). Scale bar = 200 µm.

**A**

| **Ingenuity Canonical Pathways** | **-log(B-H p-value)** |
|---|---|
| FXR/RXR Activation | 3.63 |
| LXR/RXR Activation | 2.34 |
| PXR/RXR Activation | 1.9 |

| **Upstream Regulator** | **Expr Log Ratio** | **Z-score** | **p-value of overlap** |
|---|---|---|---|
| PPARA | 0.393 | 1.576 | 0.000000001 |
| RXR | 0.04 | 2.328 | 0.000022 |
| PPARGC1A | 0.299 | 1.986 | 0.0000231 |
| HNF4A | 0.02 | 2.134 | 0.000142 |
| NCOR-LXR-Oxysterol-RXR-9 cis RA | | 1.633 | 0.000623 |
| PPARG1B | 0.298 | 1.245 | 0.00492 |
| RAR ligand-RARα-Retinoic acid-RXRα | | | 0.00921 |
| FXR ligand-FXR-Retinoic acid-RXRα | 0.045 | | 0.0406 |

**B**

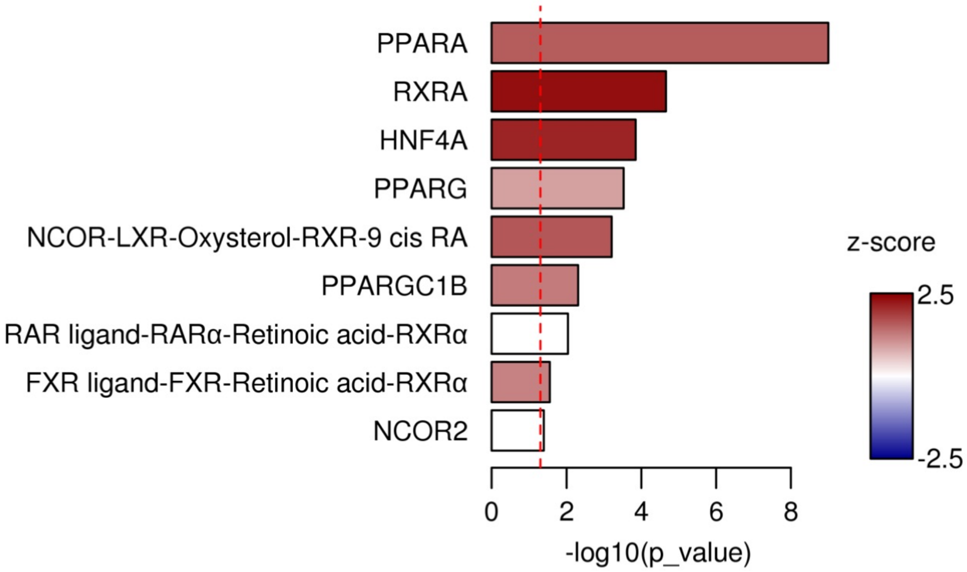

**Fig. S2. Pathway analysis of hepatic RNA-seq data obtained from $L^{WT}$ and $L^{DKO}$ mice.** RNA-Seq data (GEO: GSE55190) were examined using Ingenuity pathway analysis software (http://ingenuity.com) to identify canonical pathways and upstream regulators enriched in the set of hepatic genes differentially expressed between $L^{WT}$ and $L^{DKO}$ mice. (**A**) Enriched canonical pathways and upstream regulators related with the BA synthesis and homeostasis are presented. (**B**) Enriched upstream regulators are presented.

**TABLE S1. Taqman$^{©}$ assays probes – Related to RT-qPCR**

| Gene ID | Gene Name | Probe ID (Applied Biosystems) |
|---|---|---|
| *Abcb11* | ATP-binding cassette, sub-family B (MDR/TAP) , member 11 | Mm00445168_m1 |
| *Abcb1a* | ATP-binding cassette, sub-family B (MDR/TAP) | member 1A Mm00440761_m1 |
| *Abcb1b* | ATP-binding cassette, sub-family B (MDR/TAP) member 1A | Mm01324120_m1 |
| *Abcc2* | ATP-binding cassette, sub-family C (CFTR/MRP), member 2 | Mm00496899_m1 |
| *Abcc3* | ATP-binding cassette, sub-family C (CFTR/MRP), member 3 | Mm00551550_m1 |
| *Abcc4* | binding cassette, sub-family C (CFTR/MRP), member 4 | Mm01226380_m1 |
| *Atp8b1* | ATPase, class I, type 8B, member 1 | Mm01257688_m1 |
| *Baat* | bile acid-Coenzyme A: amino acid N-acyltransferase | Mm00476075_m1 |
| *Bmp4* | bone morphogenetic protein 4 | Mm00432087_m1 |
| *Cd68* | CD68 | Mm00839636_g1 |
| *Chka* | choline kinase alpha | Mm00442760_m1 |
| *Chkb* | choline kinase beta | Mm00432498_m1 |
| *Chpt1* | choline phosphotransferase 1 | Mm00522694_m1 |
| *Cpt1* | choline-phosphotransferase | Mm00550438_m1 |
| *Cyp27a* | cytochrome P450, family 27, subfamily a, polypeptide 1 | Mm00470430_m1 |
| *Cyp7a1* | cytochrome P450, family 7, subfamily a, polypeptide 1 | Mm00484152_m1 |
| *Cyp7b1* | cytochrome P450, family 7, subfamily b, polypeptide 1 | Mm00484157_m1 |
| *Cyp8b1* | cytochrome P450, family 8, subfamily b, polypeptide 1 | Mm00501637_s1 |
| *Gpbar1* | G protein-coupled bile acid receptor 1 (TGR5) | Mm00558112_s1 |

| | | |
|---|---|---|
| *Hmgcr* | 3-hydroxy-3-methylglutaryl-Coenzyme A reductase | Mm01282501_m1 |
| *Hmgcs1* | 3-hydroxy-3-methylglutaryl-Coenzyme A synthase | Mm00524111_m1 |
| *Ifng* | Interferon gamma | Mm00801778_m1 |
| *Scd1* | stearoyl-Coenzyme A desaturase 1 | Mm00772290_m1 |
| *Scd2* | stearoyl-Coenzyme A desaturase 2 | Mm01208542_m1 |
| *Shh* | sonic hedgehog | Mm00436528_m1 |
| *Slc10a1* | solute carrier family 10 (sodium/bile acid cotransporter family) member 1 | Mm00441421_m1 |
| *Slc10a2* | solute carrier family 10, member 2 | Mm00488258_m1 |
| *Smo* | smoothened homolog | Mm01162710_m1 |
| *Tnf* | Tumor necrosis factor | Mm00443258_m1 |

**TABLE S2. qPCR primers - Related to RT-qPCR**

| GENE | Forward Primer | Reverse Primer |
|---|---|---|
| *Abcb11* | TCTGACTCAGTGATTCTTCGCA | CCCATAAACATCAGCCAGTTGT |
| *Abcb4* | CAGCGAGAAACGGAACAGCA | TCAGAGTATCGGAACAGTGTCA |
| *Abcg5* | AGGGCCTCACATCAACAGAG | GCTGACGCTGTAGGACACAT |
| *Abgc8* | GTAGCTGATGCCGATGACAA | GGGGCTGATGCAGATTCA |
| *Actb* | GGCTGTATTCCCCTCCATCG | CCAGTTGGTAACAATGCCATGT |
| *Baat* | AGGTAAAGGAAAGCCGCATC | AGTCAATGACCCCTGGAAAA |
| *Cyp7a* | GTCCGGATATTCAAGGATGCA | AGCAACTAAACAACCTGCCAGTACTA |
| *Cyp7b1* | AATTGGACAGCTTGGTCTGC | TTCTCGGATGATGCTGGAGT |
| *Cyp8b1* | CAGGAAGTTCCGTCGATTTG | GGCCCCAGTAGGGAGTAGAC |
| *Cyp27a1* | CCTCACCTATGGGATCTTCATC | TTTAAGGCATCCGTGTAGAGC |
| *Elane* | AGCAGTCCATTGTGTGAACGG | CACAGCCTCCTCGGATGAAG |
| *f4/80* | CCCCAGTGTCCTTACAGAGTG | GTGCCCAGAGTGGATGTCT |
| *Fgfr4* | TTGGCCCTGTTGAGCATCTTT | GCCCTCTTTGTACCAGTGACG |
| *Foxm1* | CTGATTCTCAAAAGACGGAGGC | TTGATAATCTTGATTCCGGCTGG |
| *FxR* | GCTTGATGTGCTACAAAAGCTG | CGTGGTGATGGTTGAATGTCC |
| *FxRb* | ACTCTCAGAGGTATCAGTCCTGC | CAGAGGTTGAGTCTTTCCCAC |
| *Gapdh* | TGAAGCAGGCATCTGAGGG | CGAAGGTGGAAGAGTGGGA |
| *Hmgcr* | AGCTTGCCCGAATTGTATGTG | TCTGTTGTGAACCATGTGACTTC |
| *Hmgcs1* | CAGGGTCTGATCCCCTTTG | GCAACGATTCCCACATCTTT |
| *Il10* | GCTCTTACTGACTGGCATGAG | CGCAGCTCTAGGAGCATGTG |
| *Lyz2* | ATGGAATGGCTGGCTACTATGG | ACCAGTATCGGCTATTGATCTGA |
| *Nr5a2/LRH* | TGAGGAACAACTCCGGGAAAA | CAGACACTTTATCGCCACACA |

| | | |
|---|---|---|
| *Shp* | TGGGTCCCAAGGAGTATGC | GCTCCAAGACTTCACACAGTG |
| *Gpbar1/TGR5* | GCTAGGGCTCTCACCTGGA | CCCCAACACAGCAAGAAGAG |
| *Tnf* | CCCTCACACTCAGATCATCTTCT | GCTACGACGTGGGCTACAG |

Figure 3D

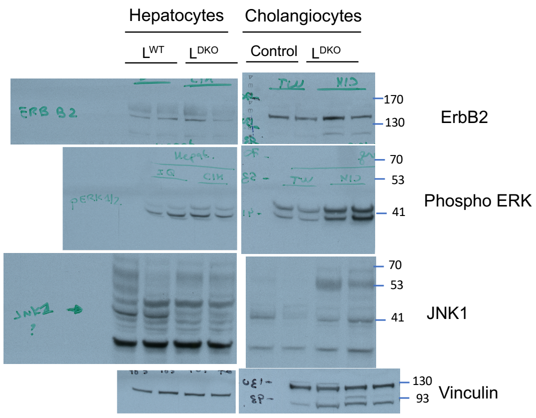

Figure 5E