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Supplementary Materials for

Farnesoid X receptor antagonizes macrophage-dependent licensing of effector T lymphocytes and progression of sclerosing cholangitis

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Supplementary Materials

MATERIALS AND METHODS:

HUMAN STUDIES

Diagnoses of cholestatic liver diseases: Subjects #1-2 were diagnosed with EHBA based on histopathological review of the biliary remnant at the time of Kasai portoenterostomy exhibiting fibroinflammatory obstruction. The 7 subjects with PSC met the AASLD criteria for diagnosis of large duct PSC, including elevation of cholestatic serum biochemistries at diagnosis (GGT and/or ALP) and presence of cholangiopathy on MRCP, i.e. focal stricturing of bile duct(s), dominant stricture of the common bile duct, saccular dilatation of bile duct(s), beaded appearance of bile duct(s), or pruning appearance of the distal bile ducts (*47*). Overlap with autoimmune hepatitis was diagnosed in those patients who scored ≥6 on the simplified IAIHG score, including serology at diagnosis (IgG concentrations and titer of ANA antibodies), presence of histomorpholigical features of AIH on liver biopsy, and absence of viral hepatitis (*48*). The liver histopathology revealed interface hepatitis and was compatible with AIH in all patients with overlap syndrome. Clinical characteristics are summarized in Table S1. Serum biochemsitries obtained at the time of PBMC collection are listed next to experimentally determined IFNγ expression of CD4+ and CD8+ lymphocytes following stimulation with LPS or TCDCA in prsenece or absence of sFXR agonists in Table S2. Experimental procedures are described below.

Intracellular flow cytometry on human PBMCs: Cryopreserved PBMC were thawed and re-suspended in DMEM with 10% FBS. PBMCs (2.5 × 10^5 cells) were seeded in 96-well plates in 100µl of medium supplemented with IL-2 (1,000U/mL, Sigma-Aldrichand treated with either 0.1 or 1.0µM sFXR, M044, or vehicle (DMSO) for 3 hours. The PBMCs were then stimulated with either 100 ng/mL of LPS (Sigma-Aldrich), 100µM of TCDCA (Cambridge Isotope Laboratories)

or vehicle (1X PBS) and cultured for 16 hours at 37°C. Cells were then centrifuged (1000 rpm for 5 minutes) and supernatant was collected to measure cytokine release. IL1β concentration in supernatant was measured by IL1β TR-FRET assay (Cisbio) as detailed below. Cells were then washed and stimulated with PMA (20 ng/mL, Sigma-Aldrich) and ionomycin (1µg/mL, Sigma-Aldrich) for 4 hours in the presence of brefeldin A (BD Pharmingen) to measure intracellular cytokine production (*49*). Cell surface marker staining was performed on unstimulated or stimulated PBMCs using the antibodies listed in Table S3. Cell suspensions were incubated for 30 minutes on ice. For intracellular staining, the cells were fixed and permeabilized with Fixation/Permeabilization Buffer (BD Biosciences) and stained for IFNγ AF700 (details in Table S3) for 30 minutes in the dark. After washing with the staining buffer, the cell pellets were resuspended in 300µl cold staining buffer, followed by analysis with Fortessa1 flow cytometer (Becton Dickinson). Data analysis was performed using FlowJo version 10 (TreeStar).

Intracellular flow cytometry on human LMNCs: LMNCs were isolated from transplanted tissue from patients with fibrosing cholangitis using the enzymatic digestion method in DMEM with 10% FBS (*50*). 2.0-3.0 × 10^6 cells were seeded in 48-well plate in 300-400µl of medium supplemented with IL-2 (1,000U/mL, Sigma-Aldrich) and treated with 1.0µM sFXR, M044, or vehicle (DMSO) for 3 hours. The LMNCs were then stimulated with 100 ng/mL of LPS (Sigma-Aldrich) or vehicle (1X PBS) and cultured for 16 hours at 37°C. Intracellular flow cytometry was performed as described above for human PBMCs.

MURINE STUDIES

Serum and plasma biochemistries: Mice were euthanized via Isoflurane and blood was aspirated by cardiac puncture. Serum samples were assayed for alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin (TB) using Discretpak reagents (C164-0A;

Catachem, Inc.; Bridgeport, CT), VETSPEC (ALP) Kit C (C174-0C; Catachem, Inc.), and the Total Bilirubin Reagent Set (B7576-120; Pointe Scientific, Inc.) with Chemistry Calibrator Set (C7506-50; Pointe Scientific, Inc.), respectively, as described before (*20, 51*).

Quantitative PCR: Snap frozen pieces of whole liver or terminal ileum tissue were disrupted using BeadRuptor4 (Omni International) followed by isolation of total RNA with the Omni Tissue RNA PurificationKit (Omni International), according to the manufacturer's instructions. cDNA was transcribed using Revert Aid RT kit (ThermoFisher Scientific) and 7900HT Fast Real Time PCR System (Applied Biosystems), as described by our group before (*46*). For purification of total RNA from liver mononuclear cells (LMNC), cells were stored in RNAlater Stabilization Solution (ThermoFisher Scientific) overnight at 4ºC. The pellet was then spun down at 6000g for 5 minutes and RNA was extracted using the RNeasy Mini Kit (Qiagen), according to the manufacturer's instructions. For gene expression studies on cDNA from hepatic, ileal, and mononuclear cells, qPCR protocols were employed as describe before andTaqMan probes were used are detailed in Table S2 (*46*). ΔΔCt method was used for computation of relative mRNA concentrations, and data were normalized to mouse 18S expression.

Bile Acids (BA) and plasma C4 analysis: Serum was diluted 1:10 before assaying it for concentrations of total bile acids using the mouse Total Bile Acids Assay Kit (CrystalChem). Liver tissue was weighed and disrupted with the BeadRuptor4 (Omni International) before centrifugation. 75% EtOH was added to the supernatant in equal volume and incubated at 55ºC for 2 hours, followed by centrifugation at 6000g for 10 minutes at 4ºC, and subsequent measurement of bile acid concentration in the supernatant. Plasma C4 concentrations were assayed by mass spectrometry as described in detail before (*46*).

Immunohistochemistry: In studies of xenobiotic sclerosing cholangitis, sections from formalin fixed paraffin embedded (FFPE) liver issue were subjected to a citrate-based antigen retrieval (pH 6), followed by blocking with goat serum, incubation with anti-PanCK primary antibody

(Agilent Dako), and subsequently biotinylated anti-rabbit secondary antibody (Vector Laboratories). Binding of antibody complexes was detected using Avidin/Biotin (Vectastain ABC reagent PK-4001; Vector Laboratories) and 3,3' diaminobenzidine (DAB) substrate (Vector Kit, SK-4100; Vector Laboratories). In studies of MDR2$^{-/-}$ mice, CK-19 immunohistochemistry was performed on liver sections from FFPE liver tissue. Staining and image analysis were performed as described previously (*20*).

Hepatic Mononuclear Cell Isolation: At the time of harvest, livers were perfused with 10mL of Hyclone RPMI-1640 (GE Healthcare Life Sciences) with 1µg/mL of collagenase D (Roche Diagnostics) before mincing with a scalpel, incubation for 30 minutes in collagenase/RPMI at 37ºC, dissociation through a 70µm strainer, and resupension in MACS isolation buffer (1X PBS, 0.5% bovine serum albumin (BSA), and 2 mM EDTA). Mononuclear cells were further purified using RPMI/ 33% Percoll (GE Healthcaregradient) and centrifugation at room temperature at 2200 rpm for 25 minutes. The pellet was resuspended in 1X RBC lysis buffer (Biolegend) for 5 minutes at room temperature to lyse red blood cells, as described previously (*51*).

FACS sorting of immune cells: Mice were fed either 0.1% DDC chow or control diet for 7 days and single cell suspension was prepared from mouse livers according to published protocols (*52*). For 10 minutes, cells were blocked with a Mouse BD Fc Block (BD Biosciences) and incubated on ice with cell surface markers for 30 minutes (Table S5). Cells were then washed and resuspended in 500µl cold staining buffer. Sorting was performed using FACS Aria II (Becton Dickinson). Total live, CD45^{+} CD3^{-} CD11b^{+}F480^{+}: Macrophages, CD45^{+}CD3^{+}CD4^{+} and CD45^{+}CD3^{+}CD8^{+}: T cells were collected and used for *in vitro* FXR agonist studies.

Intracellular flow cytometry on murine LMNCs and FACS sorted immune cells: 0.5X10^6 LMNCs or 0.25X10^5 isolated immune cells from 0.1% DDC fed mice were plated in 48 or 96 well dishes in DMEM/F12 supplemented with 10% FBS, 1% penicillin and streptomycin (Thermo Fisher Scientific) and 100IU/mL IL2 (Sigma Aldrich), respectively. Similar to the treatment of

human PBMCs, LMNCs and isolated immune cells ($CD45^+$ $CD3^-$ $CD11b^+F480^+$: Macrophages; $CD45^+CD3^+CD4^+$ and $CD45^+CD3^+CD8^+$: T cells) were treated with either vehicle (DMSO) or 1.0μM of FXR agonist M044 for 3 hours and then stimulated with 100ng/mL LPS for 16 hours. To study the effect of IL1 neutralization, LMNCs were treated for 1 hour with IL1Ra (Sigma Aldrich) before 16 hours of LPS (100ng/mL) stimulation. The cells were washed and stimulated PMA/Ionomycin/brefeldin A and flow cytometry was performed to determine intracellular cytokine production. Antibodies used for surface and intracellular staining are listed in Table S4.

Primary hepatocyte cultures: Cells were isolated from WT, FXRΔMC and $FXR^{-/-}$ mice using the previously published protocol (*53*). Cultured cells were plated in William's medium (Sigma Aldrich) supplemented with 2 mM l-glutamine (Sigma Aldrich), 6.25μg/ml transferrin (Sigma Aldrich), 0.1μM dexamethasone (Sigma Aldrich), 1μM insulin (Sigma Aldrich). After 2 days, cells were pretreated with media containing 1.0μM of FXR agonist (M044 in DMSO) or vehicle (DMSO) for 3 hours prior to stimulation with 100ng/mL of LPS for 16hrs and subsequent RNA isolation and qPCR analysis was performed. For immunofluorescence (IF), cells were cultured on glass bottom Mattek dishes (MatTek Corporation).

Isolation and culture of primary neutrophils: Cells were isolated from bone marrow of WT or FXRΔMC mice and cultured in RPMI 1640 medium supplemented with 10% FBS and 10 ng/mL G-CSF (Peprotech) according to a previously published protocol (*54*). Similar to hepatocyte cultures, these cells were subjected to FXR agonist treatment, LPS stimulation, and purification of total RNA for qPCRs. Cells were also used for confocal microscopy as described below.

Primary hepatic macrophage cultures: Macrophages were isolated from livers of WT, FXRΔMC and $FXR^{-/-}$ using enzymatic dissociation, and were separated from hepatocytes and other sinusoidal cells by gradient centrifugation and selective adherence, as previously described (*55*). Hepatic macrophages were plated at a density of 1-3X10^7 cells/well in DMEM supplemented with 10% FBS and incubated for 2hrs in the incubator. Non-adherent cells were

then removed by gently washing with PBS and the remaining cells were cultured in DMEM media supplemented with 10 ng/ml M-CSF (Peprotech) for 6 days. Cells were then pretreated with 0.1 or 1 μM of FXR agonist (M044) or vehicle (DMSO) for three hours prior to stimulation with either 100ng/mL LPS or 100 μM of TCDCA for 16hrs, and subsequent RNA isolation for qPCR assays. Cells labeled as “untreated” served as controls; they were cultured under identical conditions, but not stimulated with LPS or TCDCA or exposed to FXR agoinst. Concentrations of IL1β and TNFα in supernatants were measured by TR-FRET and ELISA, respectively, as described below. For IF, MP were plated on glass bottom Mattek dishes (MatTek Corporation).

Bone Marrow Derived Macrophage Cultures: Bones were harvested from WT and $FXR^{-/-}$ mice, marrow was isolated and further processed as described previously (*56*). Bone marrow derived cells were cultured in DMEM supplemented with 10% FBS, 1% P/S and 10ng/mL M-CSF (Peprotech). Media was changed every other day for 6-7 days and the cells were harvested on 7^{th} day of culture. Cultures containing 90-95% pure $CD11b^{+}$ $F4/80^{+}$ cells were used for in vitro assays.

Immunofluorescence: IF was performed as previously described (*57*). Briefly, primary hepatocytes, neutrophils or MP were plated on Mattek glass bottom dishes and were fixed in ice cold 100% methanol for 10 minutes and standard immunocytochemistry was performed. Primary antibodies were used at the following dilutions: mouse anti-FXR(ThermoFisher Scientific) 1:500, rabbit anti-HNF4α (Abcam) 1:1000, rat anti-Gr-1 (ThermoFisher Scientific) 1:1000, rabbit anti-CD11b (Abcam) 1:1000. Samples were rinsed after overnight incubation at 4°C, and were incubated for 2 hours with appropriate Alexa488 and 647-conjugated secondary antibody (Jackson Immunoresearch). Confocal microscopy was performed using Nikon A1RSi Inverted Confocal Microscope at the CCHMC confocal imaging core.

ELISA: Both mouse TNFα (ThermoFisher Scientific) and mouse NLRP3 (Abcam) ELISAs were performed using manufacturers' protocols. Briefly, for the TNFα ELISA, supernatants from BMDM and MP cultures were collected following LPS or TCDCA stimulation and concentrations of TNFα was determined using a colorimetric microplate reader. For the NLRP3 ELISA, BMDM were lysed following LPS treatment in the lysis buffer provided in the kit to measure NLRP3 protein concentration in cell extracts.

IL1β TR-FRET Assay: BMDM were cultured as described above and serum deprived for 12 hours on 7th day of culture. The cells were treated with vehicle or 1.0μM of M044 for 3 hours prior to stimulation with either LPS (100 ng/mL), TCDCA (100μM) for 16 hours. IL1β concentrations were measured by TR-FRET (CisBio Bioassays), according to manufacturer's instructions. Briefly, this sandwich assay employed two specific antibodies, one labeled with Europium Cryptate (donor) and the second with d2 (acceptor). The two antibodies bind to the IL1β protein present in the sample, thereby generating FRET. Signal intensity is directly proportional to the number of antigen-antibody complexes formed and therefore to the IL1β concentration in the supernatant.

SUPPLEMENTARY FIGURES

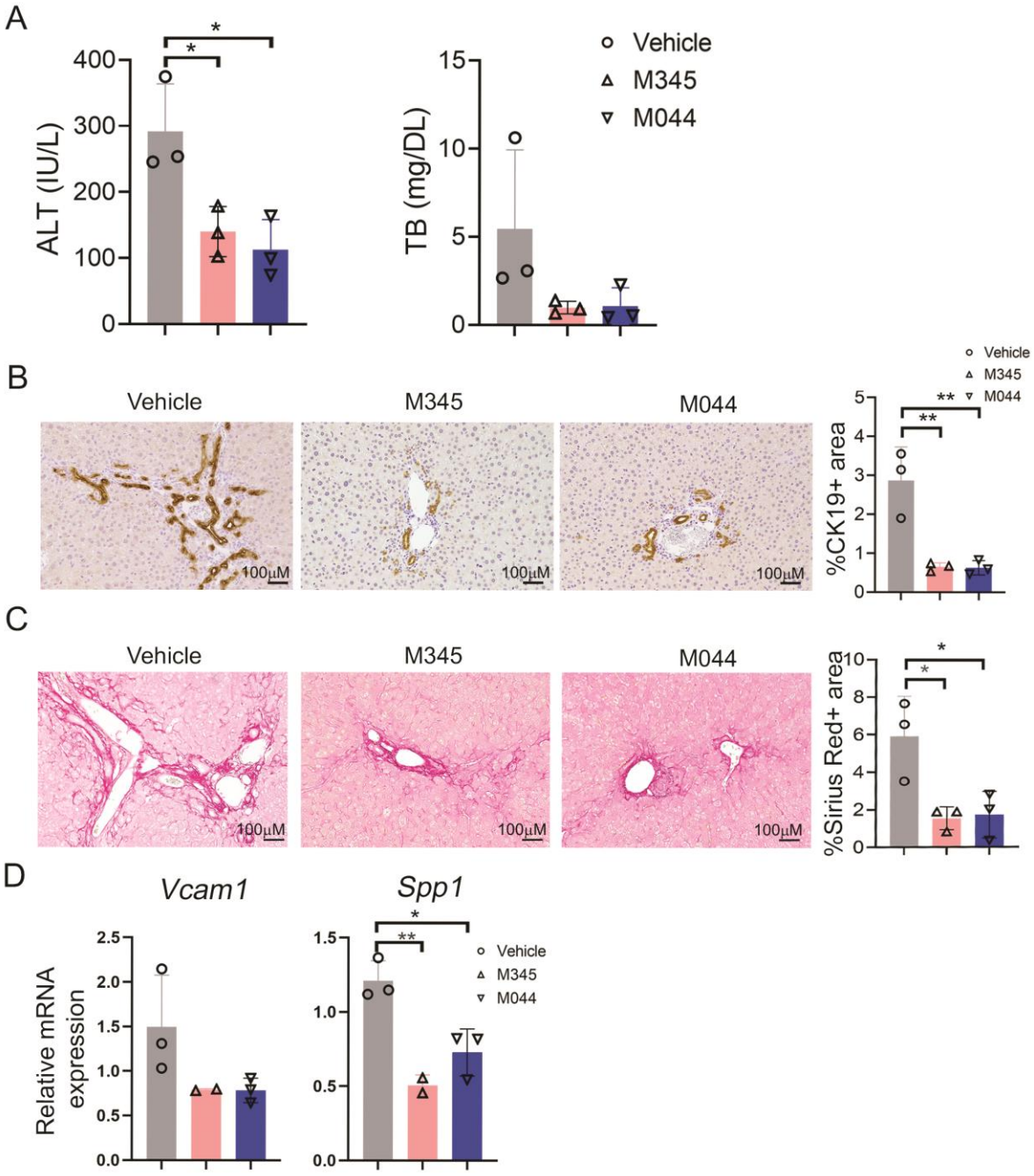


Fig. S1. Systemic FXR agonists M345 and M044 have similar hepatoprotective effects on murine sclerosing cholangitis. (A) 45-day-old, female MDR2$^{-/-}$ mice were treated with either vehicle (corn oil), 30 mg/kg of M345, or 10 mg/kg of M044 by once daily oral gavage for 7 days. Serum was collected at the end of treatment for colorimetric assays. (B/C) Sections from livers collected at the end of treatment were subjected to CK19 immunohistochemistry or Sirius Red staining with subsequent image analysis. D) Total RNA was purified from liver tissue samples obtained at the end of treatment and gene expression as quatitaed by TaqMan based PCR. Results are mean± SD; dots represent results from individual mice. Multiplicity adjusted *P* values were determined using one-way ANOVA with Dunnett's post hoc test compared to mean of the vehicle. **P*<0.05; ***P*<0.01.

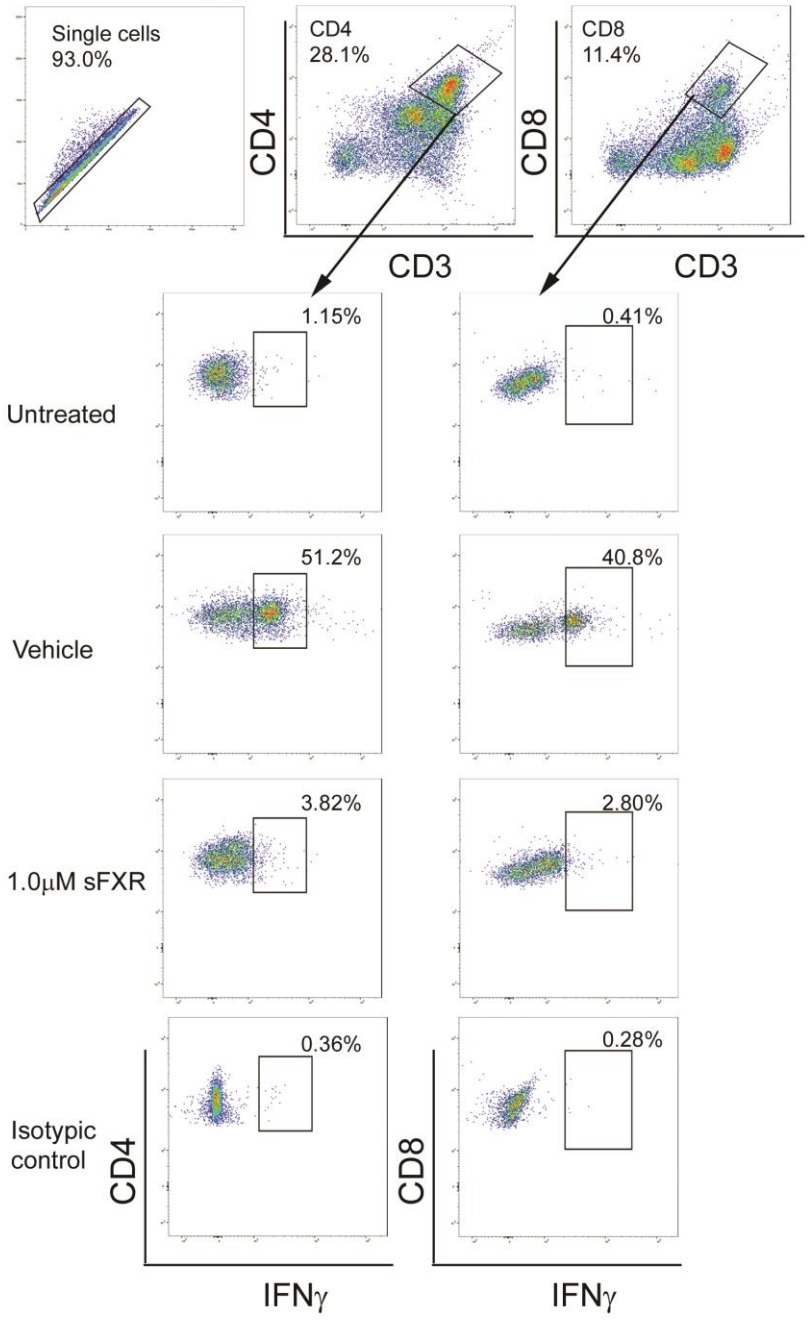

Single cells
93.0%
CD4
28.1%
CD8
11.4%
CD4
CD8
CD3
CD3
1.15%
0.41%
Untreated
51.2%
40.8%
Vehicle
3.82%
2.80%
1.0μM sFXR
0.36%
0.28%
Isotypic
control
CD4
CD8
IFNγ
IFNγ

Fig. S2. Representative gating strategy to measure intracellular IFNγ expression by liver mononuclear cells (LMNC) from MDR2$^{-/-}$ mice.

LMNC isolated form MDR2$^{-/-}$ mice were stimulated with 100 ng/ml of LPS for 16 hours in presence of FXR agonist (M044) or vehicle (DMSO) prior to determination of expression of IFNγ on CD4^{+} and CD8^{+} cells by intracellular flow cytometry (IFC) analysis. Cultured LMNC without exposure to LPS or FXR agonist served as untreated controls. 1st row: Doublets were excluded on a SCS-A vs. SCS-H plot. Live cells were gated as live/dead UV negative. Leukocytes were then gated as to exclude debris, red blood cells, and platelets. Subpopulations were gated as: CD4^{+}T cells (CD45^{+}CD3^{+}CD4^{+}) or CD8^{+} T cells (CD45^{+}CD3^{+}CD8^{+}). 2nd , 3rd, 4th row: Representative panels and images showing percentage of immune cells positive for IFNγ without stimulation (untreated) and after stimulation with 100 ng/mL LPS for 16 hours in media conditioned with M044 or vehicle and re-stimulation with PMA/Ionomycin. 5th row: Control for IFNγ using an isotypic IgG control antibody after LPS stimulation to confirm antibody binding specificity.

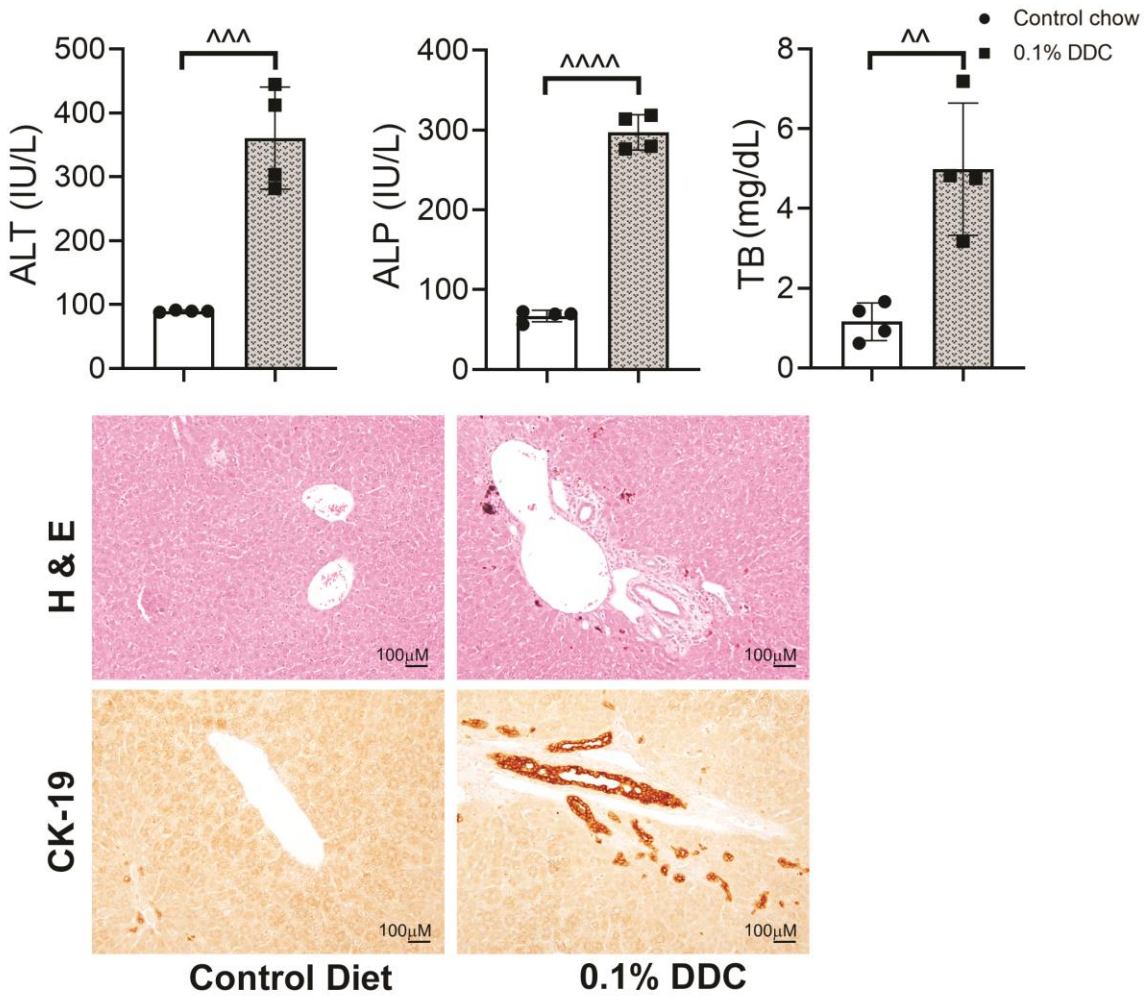


Fig S3. Feeding of 0.1% DDC diet induces sclerosing cholangitis.

3-month-old, female WT mice were exposed to 0.1% DDC admixed to the chow for 7 days. (A) Serum liver biochemistries were measured in colorimetric assays. (B). Section from livers collected at the end of treatments were subjected to H&E staining and CK19 immunohistomistry. Representative photomicrographs denote the key histological features of DDC-induced sclerosing cholangitis, including precipitation of porphyrin microcrystals, periportal inflammation, bile duct proliferation, and periductal sclerosis. Results are mean± SD; dots represent results from individual mice. *P* values were calculated using unpaired, two-tailed *t*-tests with ^^*P*<0.01; ^^^*P*<0.001; ^^^^*P*<0.0001.

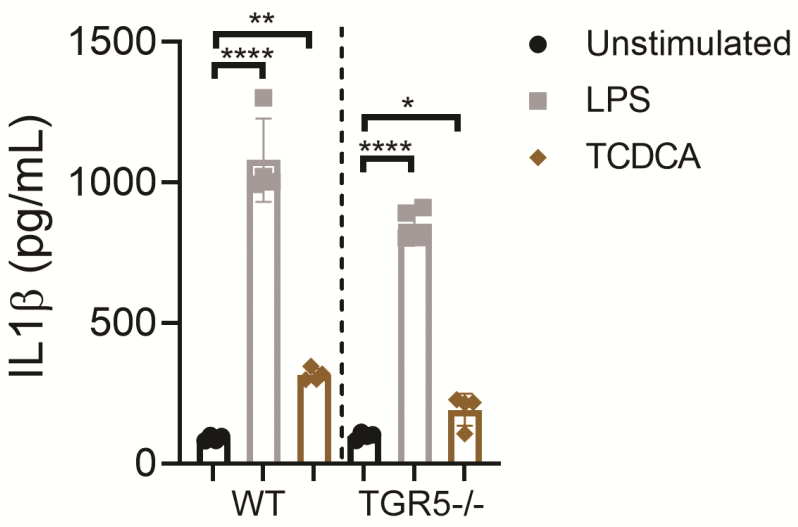


Fig S4. Impact of deletion of TGR5 on LPS- and TCDCA-induced IL1β production by bone marrow-derived mecrophages.

Bone marrow-derived macrophages from WT or TGR5$^{-/-}$ mice were cultured and then stimulated with 100 ng/mL of LPS or 100 µM of TCDCA for 16 hours. IL1β concentration was measured in supernatant by TR-FRET. Unstimulated cells served as controls. Results are mean± SD; dots represent results from individual wells. Multiplicity adjusted *P* values were determined using one-way ANOVA with Dunnett's post hoc test compared to mean of unstimulated cells. *P<0.05; **P<0.01; ****P<0.0001.

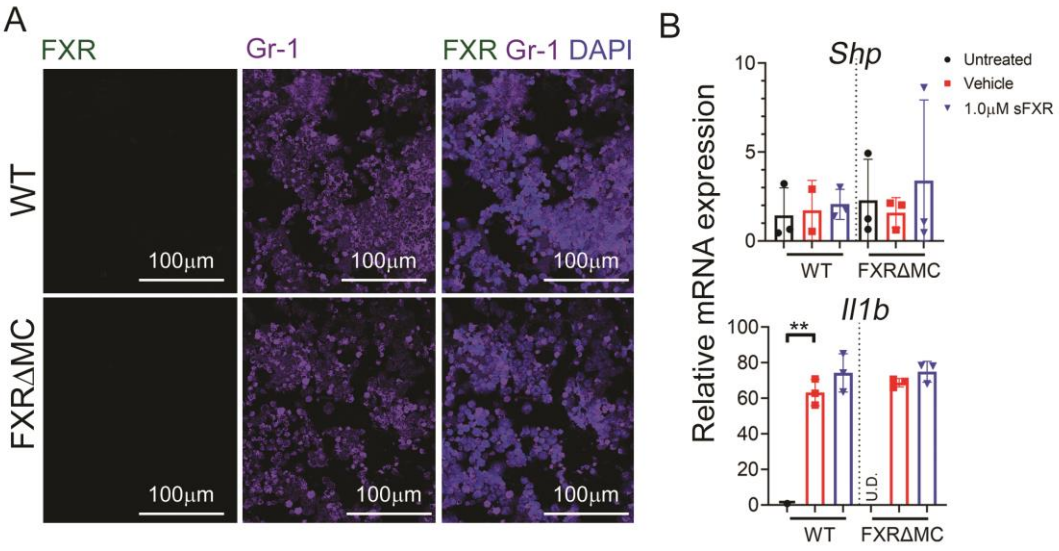


Fig. S5. Neutrophils lack FXR expression and do not respond to treatment with synthetic FXR agonists.

Primary neutrophils were isolated from bone marros of WT and FXRΔMC mice. (A) Cells were cultured and subjected to immunofluorescence with antibodies against FXR and the neutrophil marker Gr-1. DAPI was used to counter stain the nuclei. Representative images were obtained by confocal microscopy. (B) Neutrophils were cultured in presence of LPS and FXR agonist (M044) or vehicle (DMSO) prior to RNA isolation for qPCR assays. Results are mean± SD; dots represent results from individual wells. Multiplicity adjusted *P* values were determined using one-way ANOVA with Dunnett's post hoc test compared to mean of the vehicle. ***P*<0.01. U.D. undetected

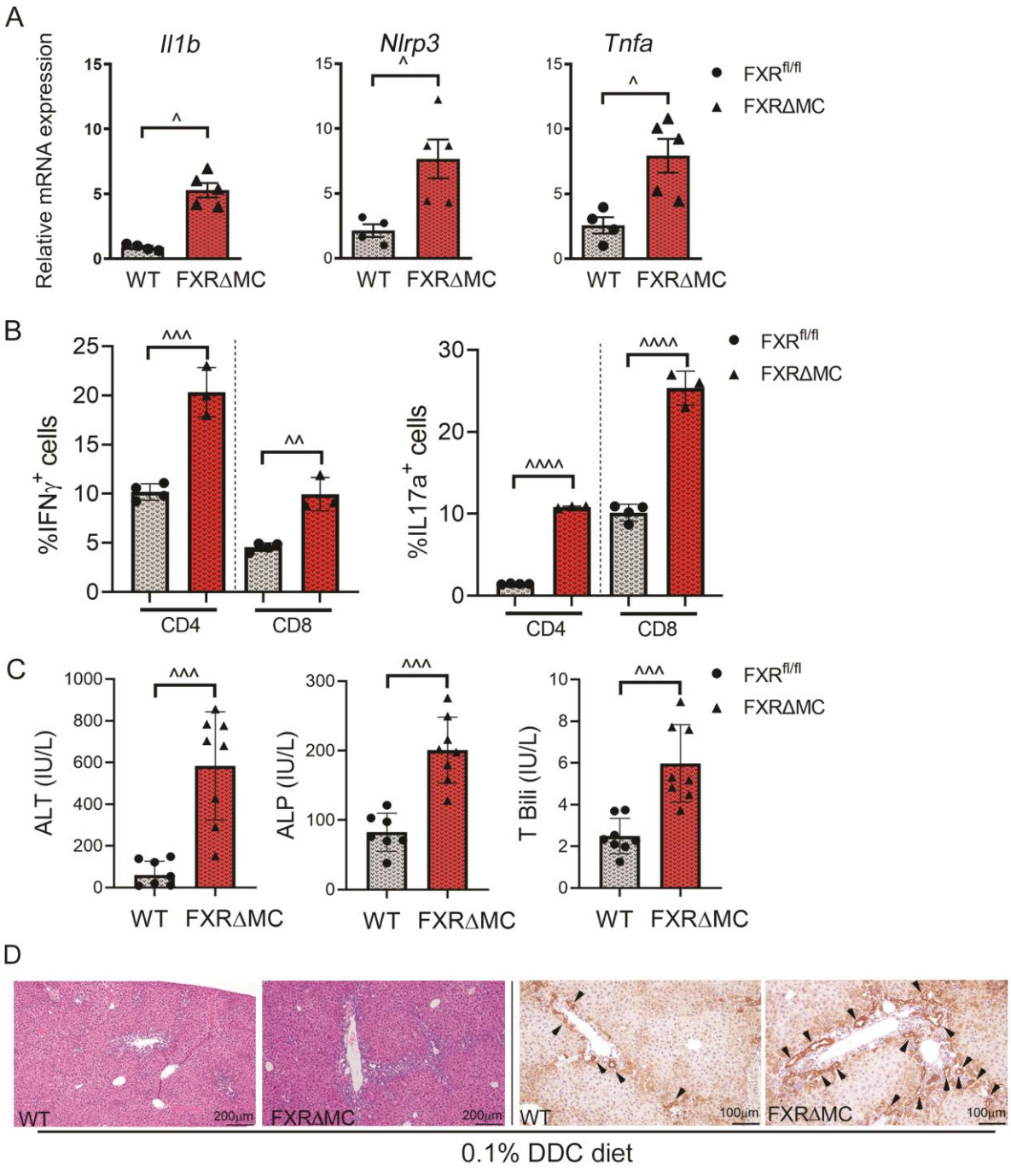

A
Il1b
Nlrp3
Tnfa
Relative mRNA expression
FXRfl/fl
FXRΔMC
WT
FXRΔMC
B
%IFNγ+ cells
%IL17a+ cells
CD4
CD8
C
ALT (IU/L)
ALP (IU/L)
T Bili (IU/L)
D
WT
FXRΔMC
200μm
100μm
0.1% DDC diet

Fig. S6. Loss of FXR in myeloid cells confers susceptibility to xenobiotic sclerosing cholangitis.

2-month-old, female WT and FXRΔMC mice were exposed to 0.1%DDC admixed to the chow for 7 days prior to collection of serum and liver samples for downstream assays. (A) Total RNA was isolated from liver tissues and relative mRNA expression of proinflammatory genes was quantitated by TaqMan-based qPCR. (B) LMNCs from both groups of mice were stimulated with LPS followed by IFC to determine production of IFNγ and IL17A by $CD4^+$ and $CD8^+$ lymphocyte populations. (C) Serum samples were collected after 7 days of DDC treatment and assayed for alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin (TB) by colorimetry. (D) Periportal inflammation and ductal proliferation were assessed by H&E staining and CK19 immunohistochemistry of liver sections from both groups following DDC challenge, respectively. Representative photomicrographs show aggravated inflammation and ductal proliferation in FXRΔMC mice. Arrow heads denote bile duct profiles. Results are mean± SD; dots represent results from individual mice. *P* values were calculated using unpaired, two-tailed *t*-tests with ^$P<0.05$; ^^$P<0.01$; ^^^$P<0.001$; ^^^^$P<0.0001$.

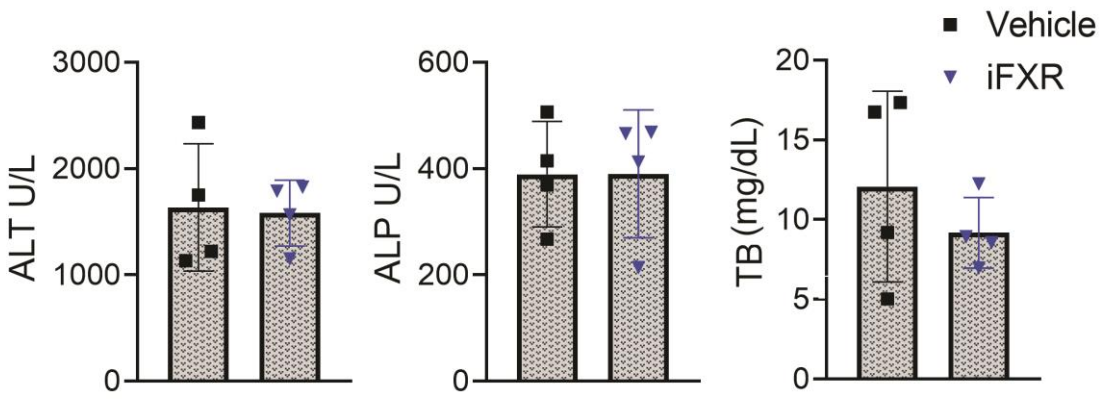


Fig. S7. Intestinally restricted FXR agonist fails to improve xenobiotic sclerosing cholangitis.

2-month-old, female WT mice were exposed to 0.1% DDC admixed to the chow for 7 days. Treatment with vehicle (corn oil) or 100 mg/kg/day of the intestinal FXR agonist M379 was started 24 hours after begin of DDC challenge and continued for six days. Serum liver biochemistries were measured in colorimetric assays. Results are shown as mean± SD. Dots represent results from individual mice. No differences between groups were found when unpaired, two-tailed *t*-tests were applied.

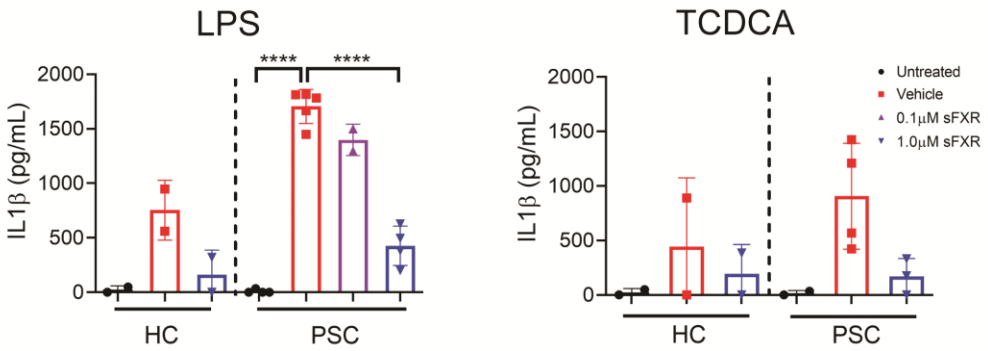
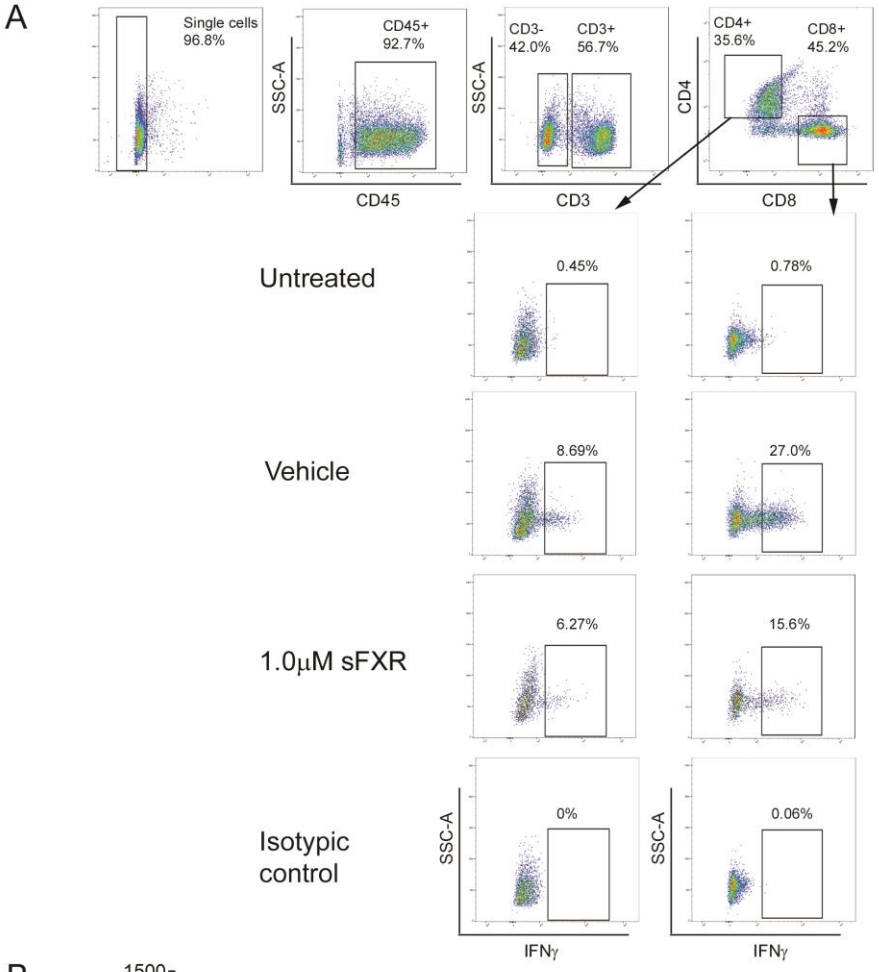


Fig. S8. FXR agonists repress innate cytokine production by circulating leukocytes from patients with PSC.

PBMCs from patients with PSC and from healthy controls (HC) were treated with various concentrations of FXR agonist M044 and stimulated with either 100 ng/mL of LPS or with 100μM of TCDCA. Supernatants were collected after 16 hours of culture and IL1β concentrations were determined by TR-FRET. Results are shown as mean± SD. Dots represent results from individual subjects. Multiplicity adjusted *P* values were determined using one-way ANOVA with Dunnett's post hoc test compared to mean of the vehicle. ****$P<0.0001$.


A
Single cells
96.8%
SSC-A
CD45+
92.7%
CD45
CD3-
42.0%
CD3+
56.7%
CD3
CD4+
35.6%
CD8+
45.2%
CD4
CD8
Untreated
0.45%
0.78%
Vehicle
8.69%
27.0%
1.0μM sFXR
6.27%
15.6%
Isotypic
control
0%
0.06%
IFNγ

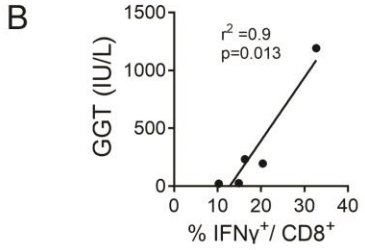

B
GGT (IU/L)
1500
1000
500
0
r2 =0.9
p=0.013
0
10
20
30
40
% IFNγ+/ CD8+

Fig. S9. Representative gating strategy to measure intracellular IFNγ expression in peripheral blood mononuclear cells (PBMCs).

(A) PBMCs from subject #4 were cultured in media conditioned with vehicle (DMSO) or 1.0 μM of FXR agonist (M044) and stimulated with LPS for 16 hours prior to IFC. 1st row: Doublets were excluded on a SSC-A vs. SSC-H plot. Live cells were gated as live/dead UV negative. Leukocytes were then gated as to exclude debris, red blood cells, and platelets. Subpopulations were gated as: $CD4^+$ T cells ($CD3^+CD4^+$) or $CD8^+$ T cells ($CD3^+CD8^+$). The position of each of the gated subpopulation was confirmed by back gating analyses (not shown). 2nd, 3rd and 4th row: Representative panels and images showing percentage of immune cells positive for IFNγ after ex vivo stimulation of PBMCs with 100ng/mL LPS in presence/absence of FXR agonist and from untreated control samples. 5^{th} row: Specificity for IFNγ was determined by using the isotypic IgG control antibody. (B) Frequencies of IFNγ+/ CD8+ lymphocytes observed following TCDCA stimulation for PBMC from 5 patients with PSC that were plotted against the serum GGT concentrations of these patients at the time of blood collection for research. Simple linear regression was used to test for association.

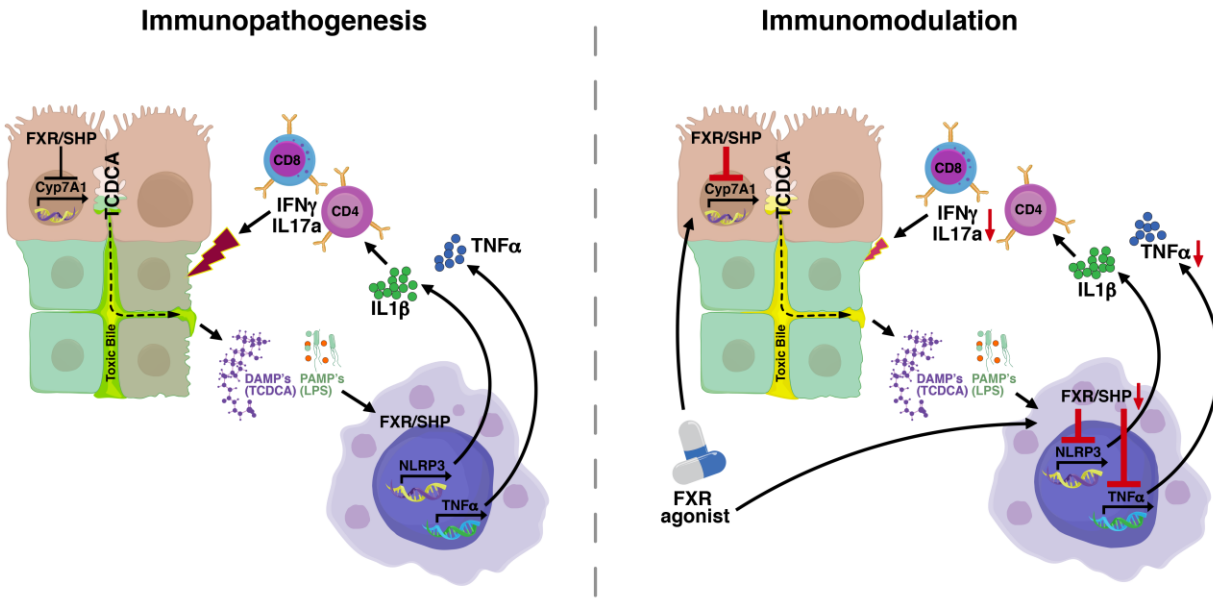


Fig. S10. Graphical summary of findings.

FXR controls innate cytokine production by hepatic macrophages in response to intrinsic and extrinsic danger signals, TCDCA and LPS, respectively. Treatment with systemic, fexaramine-derived FXR agonist reduces bile acid de novo synthesis, directly inhibits innate cytokine production by hepatic macrophages, blocks IL1β and TNFα-dependent licensing of T lymphcoytes, and protects from progression of sclerosing cholangitis in preclinical models of fibrosing cholangiopathies.

SUPPLEMENTARY TABLES:

Table S1. Characteristics of human subjects enrolled into the study for in vitro studies on liver mononuclear cells (LMNCs; subject #1-2) or peripheral blood mononuclear cells (PBMCs; subjects #3-16)

Subject	Age (years)	Sex	Disease	IBD (0=no;1 =yes)	Type of IBD	overlap with AIH (0=no; 1=yes)	Treatment at the time of sample collection: (1=UDCA, 2=OV, 3= infliximab, 4= vedolizumab, 0= none of above)
#1	14.8	F	EHBA	0		0	1
#2	1.6	F	EHBA	0		0	1
#3	16.6	M	PSC	1	UC	1	0
#4	22.5	M	PSC	1	UC	0	2, 4
#5	8.9	M	PSC	1	UC	0	0
#6	19	F	PSC	0		1	0
#7	16.8	F	PSC	1	CD	0	3
#8	21.1	F	PSC	0		1	1
#9	17.3	F	PSC	1	IC	1	0
#10	11.6	M	HC	0			
#11	15.7	F	HC	0			
#12	13.5	M	HC	0			
#14	18.7	F	HC	0			
#15	21.3	F	HC	0			
#16	14.5	F	HC	0			

M: male, F: female, EHBA: extrahepatic biliary atresia, PSC: Primary Sclerosing Cholangitis, IBD: Inflammatory Bowel Disease, IC: Indeterminate Colitis, UC: Ulcerative Colitis, CD: Crohn's disease; UDCA: ursodeoxycholic acid; OV: oral vancomycin

Table S2. Liver biochemistries at the time of PBMC collection in patients with PSC and percent IFNγ expressing CD4+ and CD8+ following stimulation with LPS or TCDCA in presence or absence of 1.0 μM of sFXR agonist (M044).

ID	Disease	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	ALP (IU/L)	TB (mg/dL)	%IFNg+/CD8 1) LPS +veh 2) LPS+FXRa 3) TCDCA + veh 4) TCDCA+FXRa	%IFNg+/CD4 1) LPS +veh 2) LPS+FXR 3) TCDCA + veh 4) TCDCA+FXR
#3	PSC	146	76	1192	493	0.7	41.0 4.3 32.7 1.0	18.4 3.3 11.1 2.5
#4	PSC	38	20	22	92	0.5	16.9 1.5 10.3 1.6	6.2 1.6 5.6 8.50
#5	PSC	82	70	233	846	1.4	11.9 0.5 16.3 9.8	4.9 0.3 12.3 1.3
#6	PSC	89	54	30	45	0.5	16 12.5 NA NA	12.3 11.8 NA NA
#7	PSC	37	20	27	77	0.2	36 6.5 14.9 2.6	38.6 1.7 20.0 1.1
#8	PSC	40	18	20	50	0.9	16.4 1.1 NA NA	18.8 0.9 NA NA
#9	PSC	294	111	196	140	1.1	NA NA 20.4 1.3	NA NA 18.5 1.04

Veh: vehicle; NA: not available

Table S3. List of TaqMan probes used to quantify and measure relative gene expression.

TaqMan probes	
Shp	(Mm00442278_m1)
Cyp7a1	(Mm00484150_m1)
Fgf15	(Mm00433278_m1)
Cyp8b1	Mm00501637_s1)
Ifng	(Mm01168134_m1
Il1b	(Mm00434228_m1)
Il6	(Mm00446190_m1)
Tnfa	(Mm00443258_m1)
Nlrp3	(Mm00840904_m1)
18s	(Mm04277571_s1)

Table S4. Flow antibody panel for immunophenotyping human PBMCs and intracellular cytokine detection. All antibodies were used at 1:200 dilution.

Name	Fluorophore	Clone	Company	Catalog
CD3	PB	HIT3a	Biolegend	300330
CD56	PE	NCAM16.2	BD Biosciences	340363
IgG	AF700	MHM-88	Biolegend	314537
IFNγ	AF700	B27	BD Pharmingen	557995
CD14	BV711	M5E2	Biolegend	301838
CD16	FITC	3G8	BD Pharmingen	555406
CD4	PerCP Cyanine5.5	RPA-T4	eBioscience	45-0049-42
CD8	APC	RPA-T8	BD Pharmingen	555369

Table S5. Flow antibody panel for immunophenotyping of murine hepatic mononuclear cells and intracellular cytokine detection. All antibodies were used at 1:200 dilution.

Name	Fluorophore	Clone	Company	Catalog
CD45	PB	30-F-11	Biolegend	103126
CD3	BV510	145-2C11	Biolegend	100352
CD4	APCCy7	RM4-5	Biolegend	100526
CD8	PerCp Cyanine 5.5	53-6.7	Ebiosciences	45-0081-82
CD11b	AF700	M1/70	Biolegend	10122
F4/80	AF647	BM8	Biolegend	123122
IFNγ	PECy7	XMG1.2	Invitrogen	4332567
IL17a	PE	TC11-18H10.1	Biolegend	506904
IgG	PECy7	Poly4053	Biolegend	405315