Cell Metabolism, Volume *25*

Supplemental Information

Farnesoid X Receptor Regulation of the NLRP3 Inflammasome Underlies Cholestasis-Associated Sepsis

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Table S1. Murine primer sequences for qPCR.

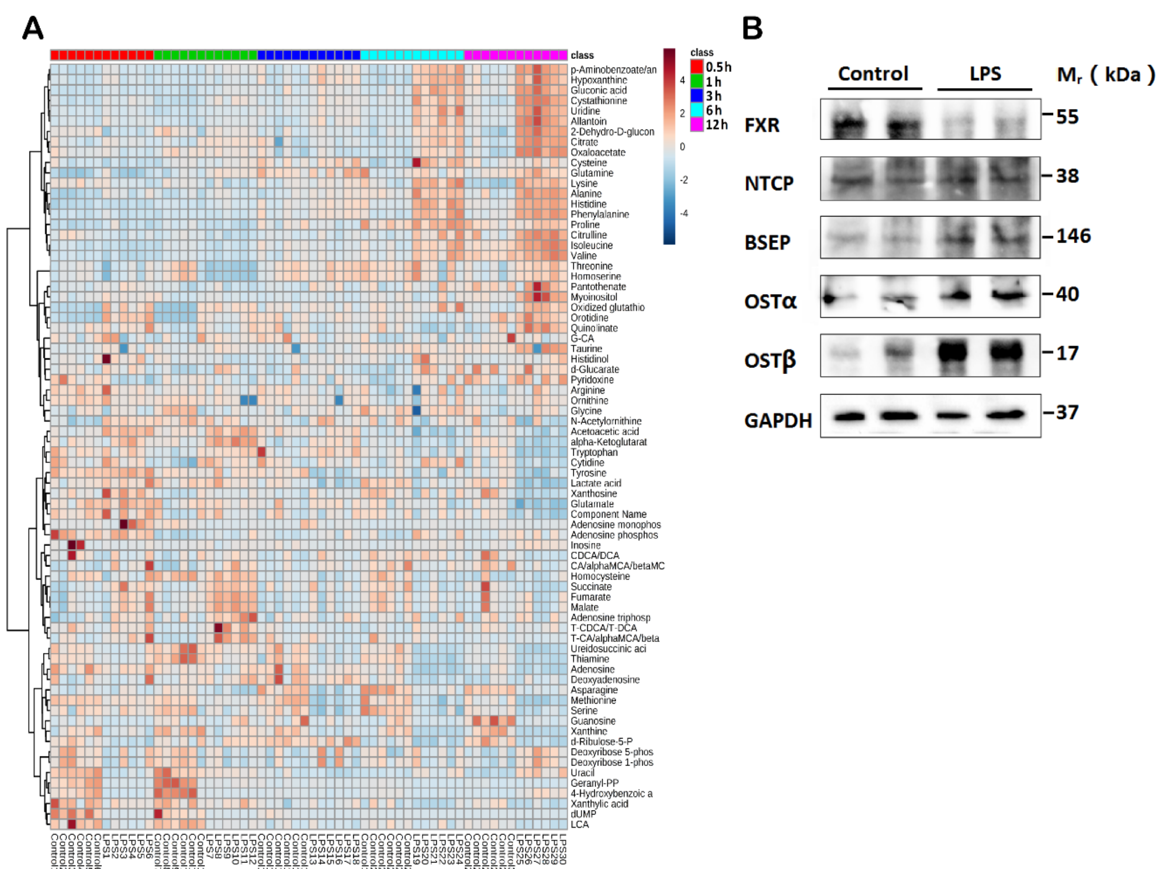

Figure S1. Time-course of the metabolomic alterations in LPS-induced septic mice. (Related to Figure 1)

(A) Heatmap illustrating a time-course of overall serum metabolic profiles of LPS-induced sepsis in mice. Distance was measured is by Pearson correlation and clustering was determined using the Ward algorithm. Mice were intraperitoneally injected with LPS at a dose of 30 mg/kg for the indicated times.

(B) Protein levels of FXR and BAs transporters in the liver of mice after 6 hours of LPS treatment; GAPDH was used as internal loading control in immunoblot analyses.

Data are representative of two independent experiments and shown as mean ±SEM of six mice.

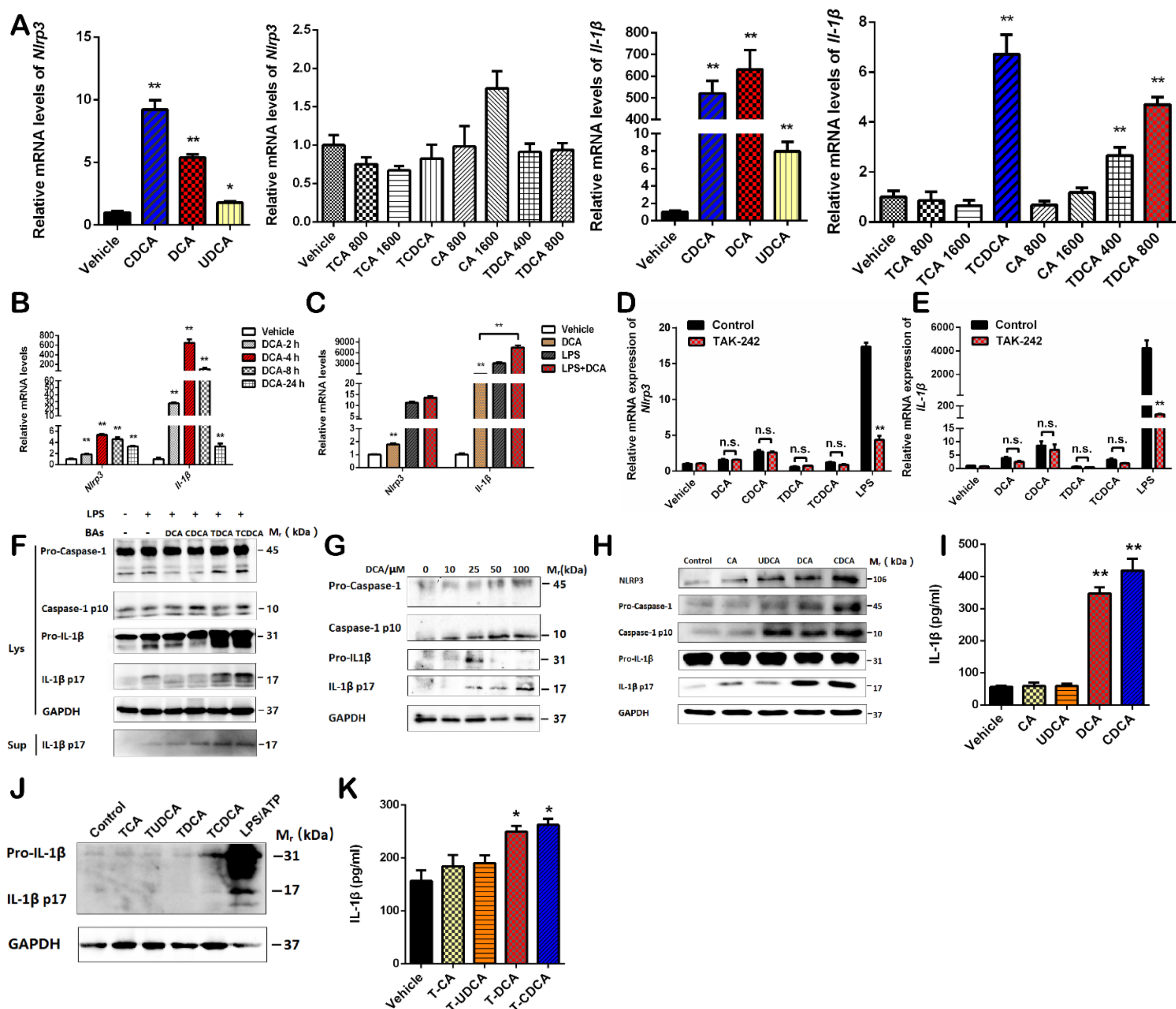

Figure S2. Bile acids activate signal 1 and 2 of the NLRP3 inflammasome (Related to Figure 2)

(**A-C**) Relative mRNA expression levels of *Nlrp3* and *Il-1β*. RAW264.7 cells were treated with bile acids for 4 hours at the indicated concentrations (A), or treated with 200 μM DCA for the indicated hours (B), or treated with 200μM DCA for 4 hours with or without 50 ng/ml LPS priming for 2 hours. *Gapdh* mRNA was used as the internal standard in the qPCR analyses.

(**D-E**) Relative mRNA expression levels of *Nlrp3* and *Il-1β*. Mouse peritoneal macrophages were pretreated with 1 μM TAK-242 for 1 h prior to 50 μM bile acids or 100 ng/ml LPS for 4 hours. *Gapdh* mRNA was used as the internal standard in the qPCR analyses.

(**F-H, J**) Representative western blot analysis of Caspase-1 and IL-1β in THP-1 cells. Cells were primed with 50 ng/ml LPS for 2 hours before treating with 100 μM bile acids for 4 hours (**F**), or treated with DCA for 4 hours at the indicated concentrations (**G**), or treated with various unconjugated bile acids (**H**) or tauro-bile acids (**J**) at 200 μM for 4 hours. GAPDH was used as a loading control in the immunoblot analyses.

(**I, K**) ELISA analysis of IL-1β concentrations in the supernatants.

One representative experiment of three independent experiments is shown; for qPCR and ELISA assays, each was done in triplicate. Data are presented as mean ±SEM. **P < 0.01, *P < 0.05 versus vehicle unless indicated otherwise; by Student's *t*-test (two-tailed) (**A**, **D**, **E**, **I, K**) or two-way ANOVA (**B-C**).

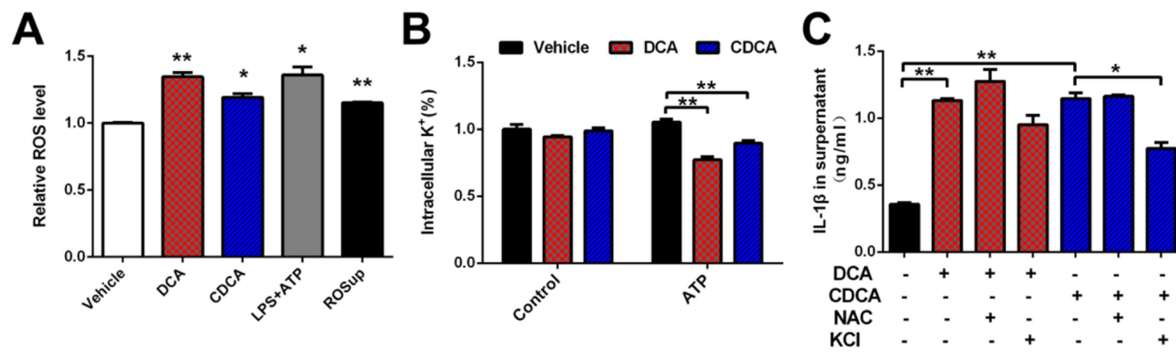

Figure S3. Role of ROS and potassium efflux in bile acids activation of NLRP3 inflammasome in THP-1 cells (Related to Figure 3)

(**A**) ROS levels in THP-1 cells. THP-1 cells were stimulated with 200 μM bile acids for 4 hours or 50 ng/ml LPS for 2 hours followed by 2 mM ATP for 30 min; ROSup was used as a positive control.

(**B**) Intracellular potassium levels. Fluorescence detection was conducted at 4 hours after 200 μM bile acid treatment with or without spiking of 2mM ATP at last 30 min.

(**C**) IL-1β levels in supernatant of THP-1 cells. THP-1 cells were stimulated with 200 μM bile acids for 4 hours; ROS was scavenged by pretreatment with 3mM NAC for 30 min or K^+ efflux was blocked by high K^+ (136 mM KCl) in medium.

One representative experiment of three independent experiments is shown and each was done in triplicates; data are presented as mean ± SEM (n=5, **A, B**; n=3, **C**). **P < 0.01, *P < 0.05 versus vehicle unless indicated otherwise; by Student's *t*-test, two-tailed.

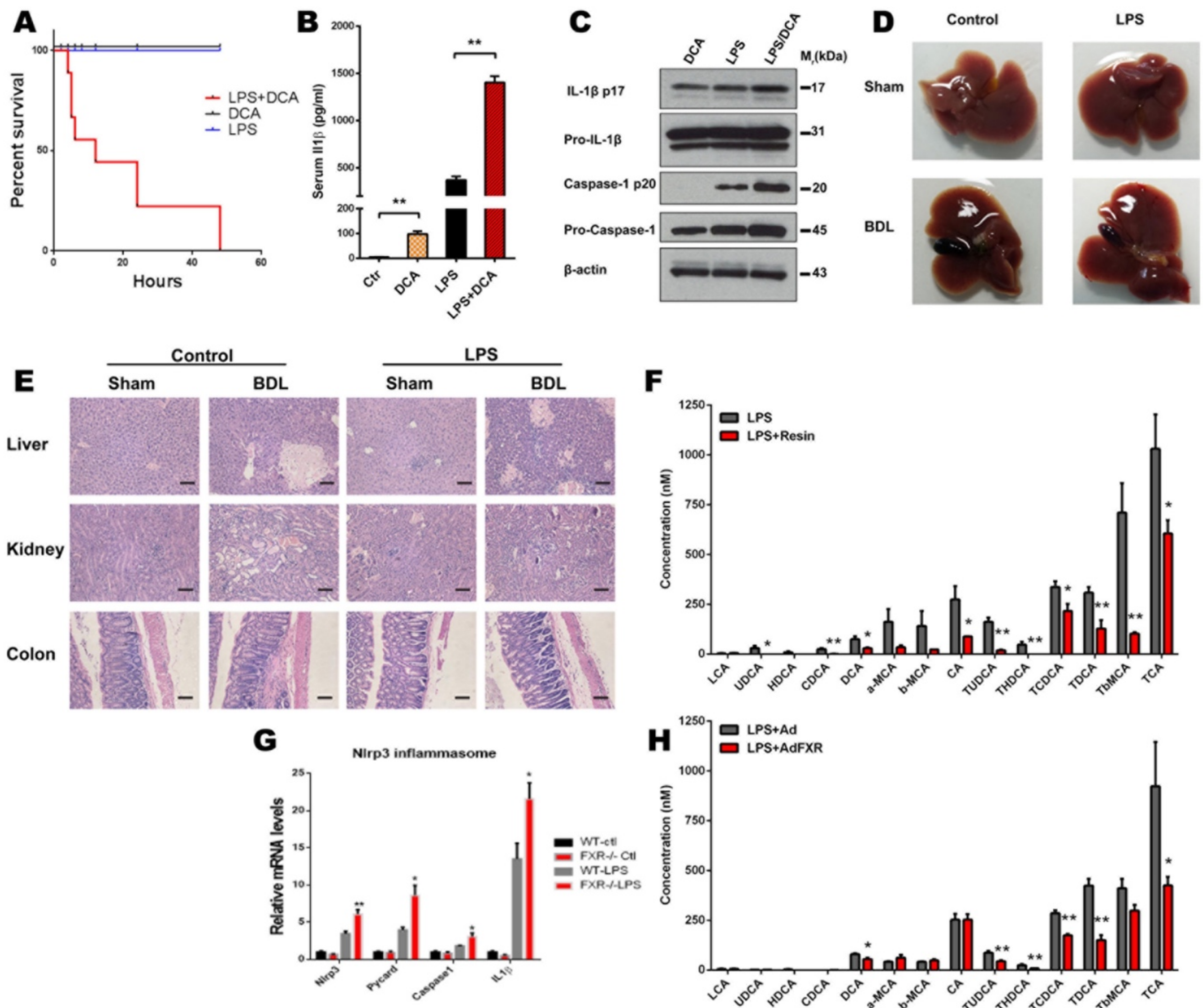

Figure S4. Cholestasis aggravates septic shock associated with Nlrp3 inflammasome activation (Related to Figure 4)

(**A**) Survival analysis (n=10).

(**B**) Serum levels of IL-1β.

(**C**) Western blot analysis of caspase 1 and IL-1β in PMs ex-vivo. β-actin was used as loading control.

Mice were intraperitoneally injected with 30 mg/kg LPS for 2 hours prior to 50 mg/kg DCA-Na, and were killed 4 hours after DCA-Na injection, unless for survival analysis.

(**D**) Photograph of gallbladder from mice that were challenged by LPS at a dose of 20 mg/kg for 6 hours three days after BDL operation.

(**E**) Histopathology of liver, kidney and colon analyzed by H&E staining; scale bars=50 μm.

(**F**, **H**) Individual bile acids in serum.

(**G**) Relative mRNA levels of *Nlrp3*, *Pycard*, *Caspase-1* and *Il-1β* in PMs.

One representative experiment of two independent experiments is shown; for the qPCR and ELISA assays, each was done in triplicate. Data are shown as mean ± SEM of six mice; **P < 0.01, *P < 0.05 compared to LPS group unless indicated otherwise, by two-way ANOVA (**B**) or Student's *t*-tests, two-tailed (**F, G, H**).

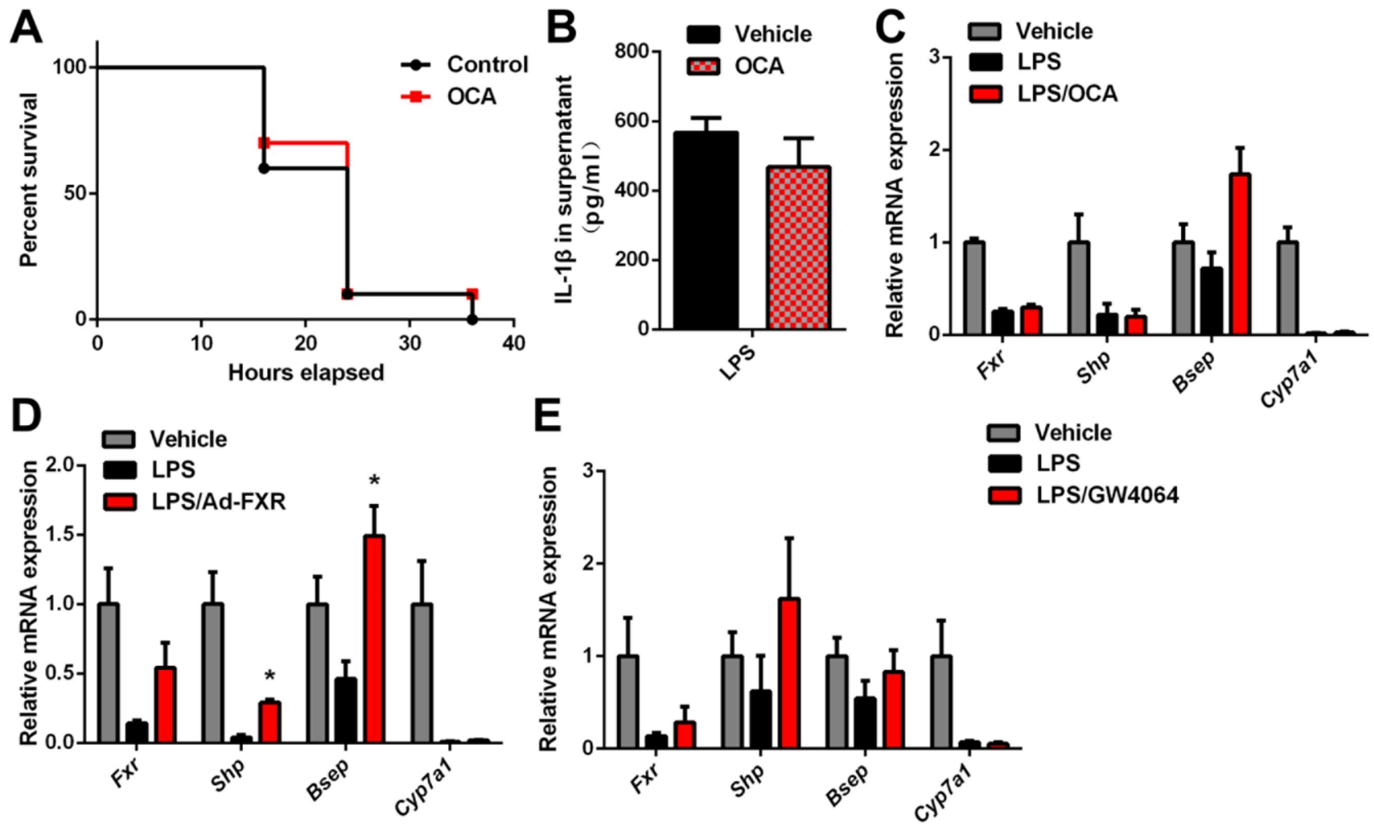

Figure S5. FXR agonist do not inhibit septic shock in mice (Related to Figure 5)

(**A**) Survival analysis (n=10).

(**B**) Serum levels of IL-1β.

(**C-E**) Relative mRNA levels of *Fxr*, *Shp*, *Bsep* and *Cyp7a1* mRNAs in liver of mice after 6 hours of LPS treatment. Mice were intragastrically administered 15 mg/kg OCA 1 hour prior to LPS challenge (30 mg/kg, i.p.), and killed 6 hours after LPS injection unless for survival analysis. One representative experiment of two independent experiments is shown; for the qPCR and ELISA assays, each was done in triplicate. Data are shown as mean ± SEM of six mice; *P < 0.05 compared to LPS group, by Student's *t*-tests, two-tailed.

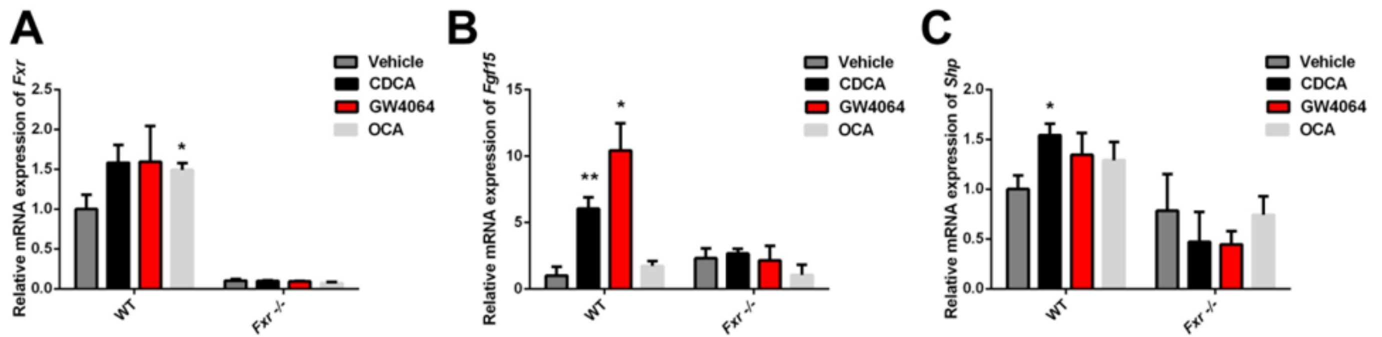

Figure S6. FXR is positively expressed and functional in macrophages (Related to Figure 6)

(**A-C**) Relative mRNA levels of *Fxr* mRNA (**A**), and its target gene *Fgf15* (**B**) and *Shp* (**C**) mRNAs in PMs from WT and *Fxr*$^{-/-}$ mice. Cells were treated with 50 μM CDCA, 2 μM GW4064 or 2 μM OCA for 12 hours; *Gapdh* mRNA was used as the internal standard.

One representative experiment of three independent experiments is shown, and each was done in triplicate. Data are shown as mean ±SEM (n=3); *P < 0.05, ** P < 0.01 compared to vehicle control in each group, by Student's *t*-tests, two-tailed.

Table S1. Murine primer sequences for qPCR.

	Forward (5'to 3')	Reverse (5'to 3')
Nlrp3	ATTACCCGCCCGAGAAAGG	TCGCAGCAAAGATCCACACAG
Il-1β	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
Pycard	CTTGTCAGGGGATGAACTCAAAA	GCCATACGACTCCAGATAGTAGC
Caspase-1	ACAAGGCACGGGACCTATG	TCCCAGTCAGTCCTGGAAATG
Pannexin-1	CCACCGAGCCCAAGTTCAA	GGAGAAGCAGCTTATCTGGGT
Gapdh	TTGAGGTCAATGAAGGGGTC	TCGTCCCGTAGACAAAATGG
Fxr	TGGGCTCCGAATCCTCTTAGA	TGGTCCTCAAATAAGATCCTTGG
Shp	CGATCCTCTTCAACCCAGATG	AGGGCTCCAAGACTTCACACA
Fgf15	ATGGCGAGAAAGTGGAACGG	CTGACACAGACTGGGATTGCT
Ostα	AGGCAGGACTCATATCAAACTTG	TGAGGGCTATGTCCACTGGG
Ostβ	AGATGCGGCTCCTTGGAATTA	TGGCTGCTTCTTTCGATTTCTG
Bsep	TCTGACTCAGTGATTCTTCGCA	CCCATAAACATCAGCCAGTTGT
Ntcp	CAAACCTCAGAAGGACCAAACA	GTAGGAGGATTATTCCCGTTGTG
Cyp7a1	GCTGTGGTAGTGAGCTGTTG	GTTGTCCAAAGGAGGTTCACC