Supplemental Materials

Cholesterol 7α-hydroxylase protects the liver from inflammation and fibrosis by maintaining cholesterol homeostasis

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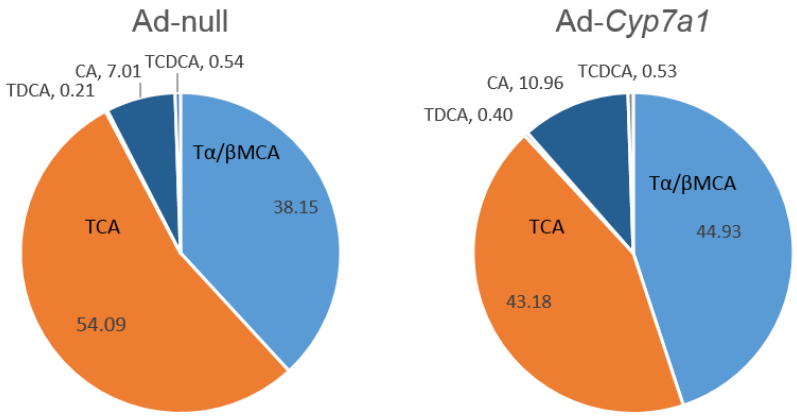
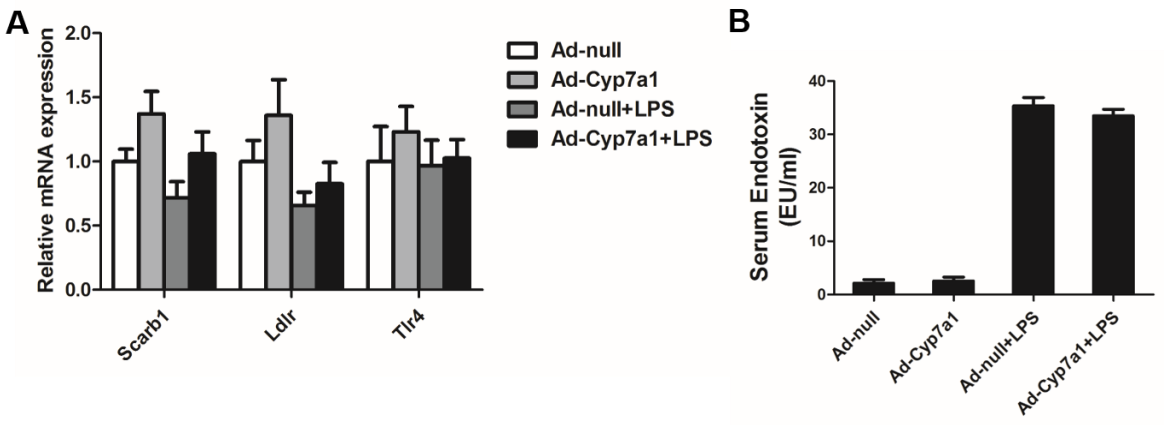
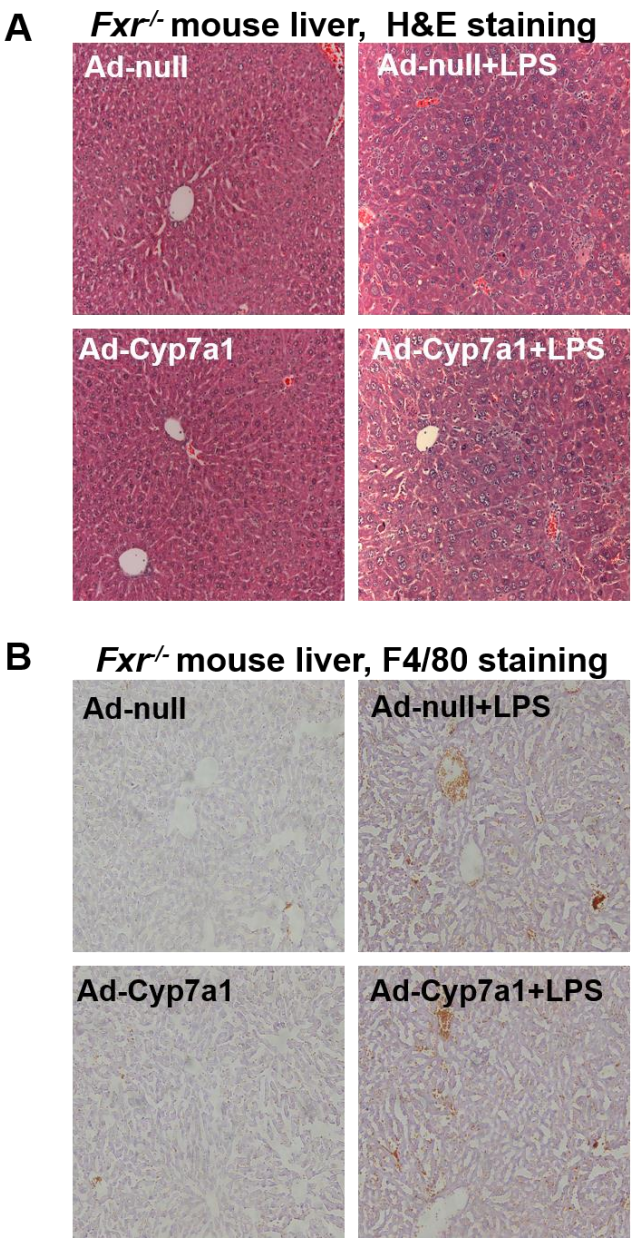


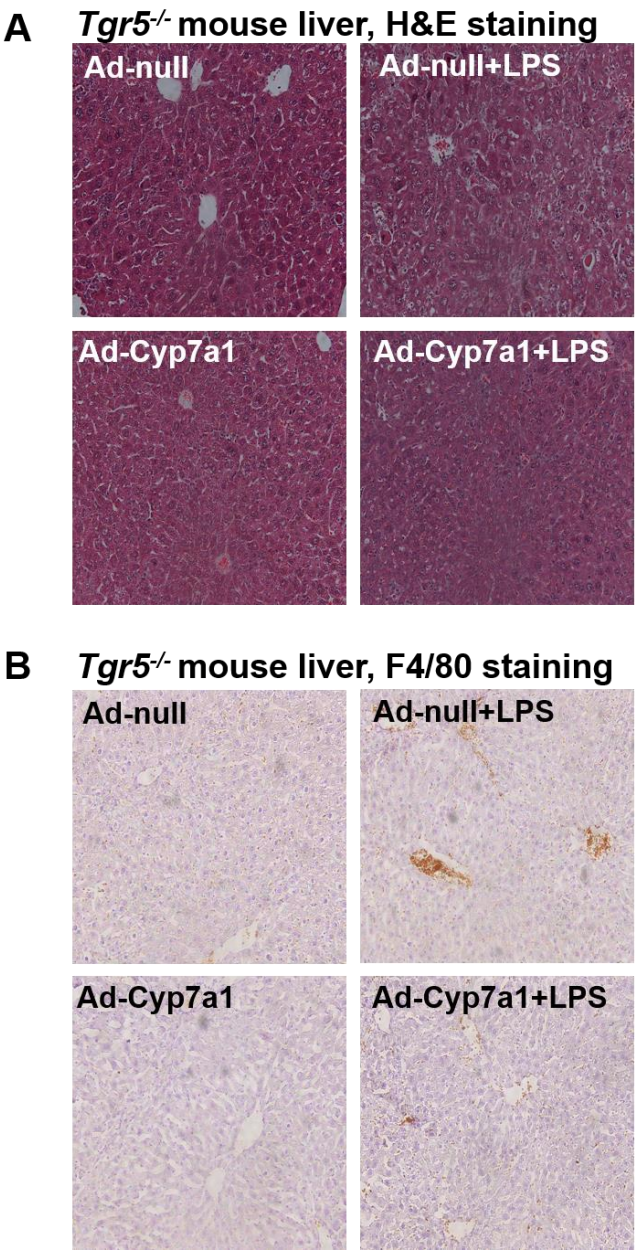
Supplemental Figure S1: Bile acid composition in gallbladder bile of Ad-Cyp7a1 mice. Mice were injected via tail vein with $1x10^9$ plaque-forming units per mouse of either adenovirus-null or adenovirus-Cyp7a1. After 7 days, gallbladder bile was collected and incubated in 500 µl isopropanol overnight in a 55°C water bath. After centrifuging at 15,000 g for 15 min, the supernatant (20 µl) was diluted with 980 µl of water: methanol (1:1), re-centrifuged at 14,000 g for 20 min, and the supernatant was transferred to a sample vial for analysis. The bile sample (5 µl) was injected to a UHPLC-QTOFMS for analysis. Numbers are averaging % of each bile acid in total gallbladder bile from five Ad-null (n=5) (left) and five Ad-Cyp7a1 mice (n=5) (right).



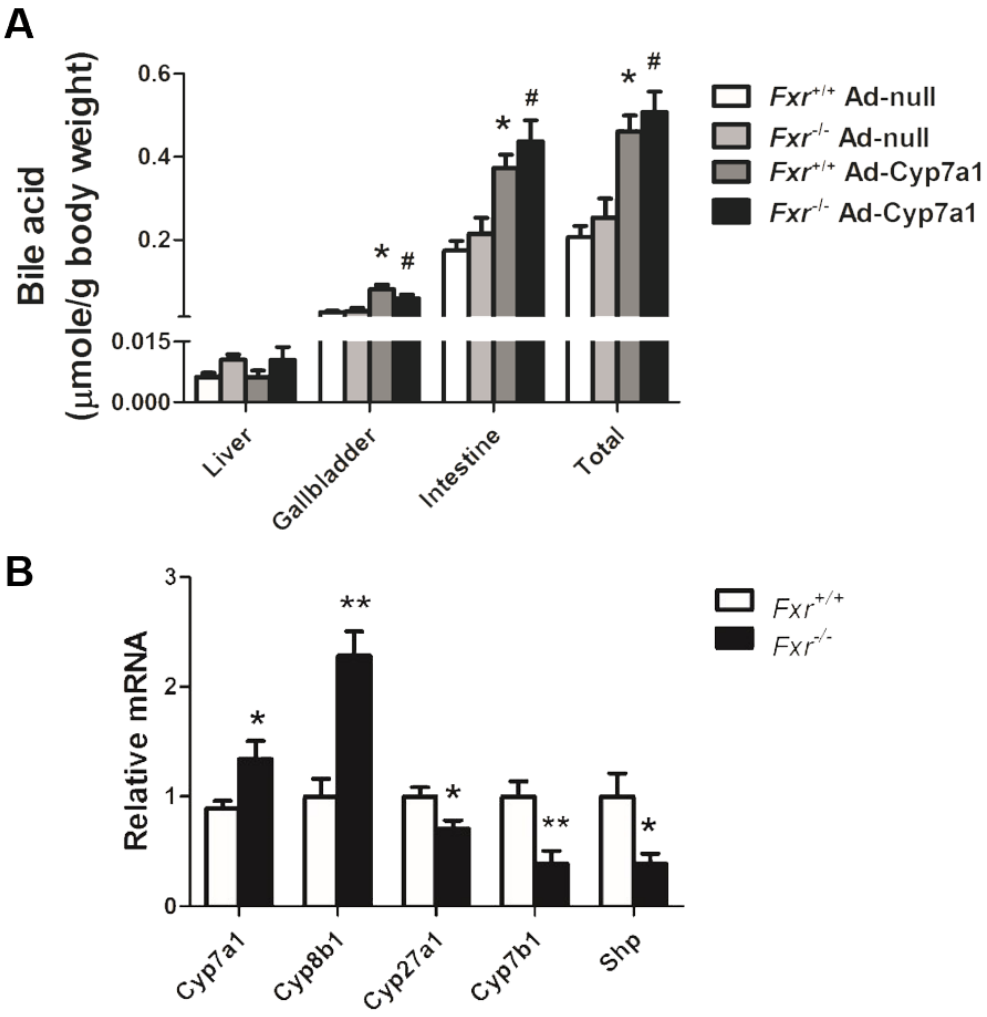
Supplemental Figure S2: The effect of Adenovirus mediated overexpression of Cyp7a1 on endotoxin clearance in LPS-treated mice. Wild type mice (C57BL/6J) were injected in the tail vein with $1x10^9$ pfu per mouse of either Ad-null or Ad-Cyp7a1. After 7 days, mice were injected i.p. with 20 mg/kg LPS or PBS (control). After 6 h, liver tissues and sera were collected for analysis. (A) QPCR analysis of liver mRNA levels of *Scarb1, Ldlr* and *Toll-like receptor 4 (Tlr4)*. *Gapdh* mRNA was used as a control for analysis. (B) Serum endotoxin levels. The levels of endotoxin in the serum were measured by a Pierce LAL chromogenic endotoxin quantitation Kit (#88282).



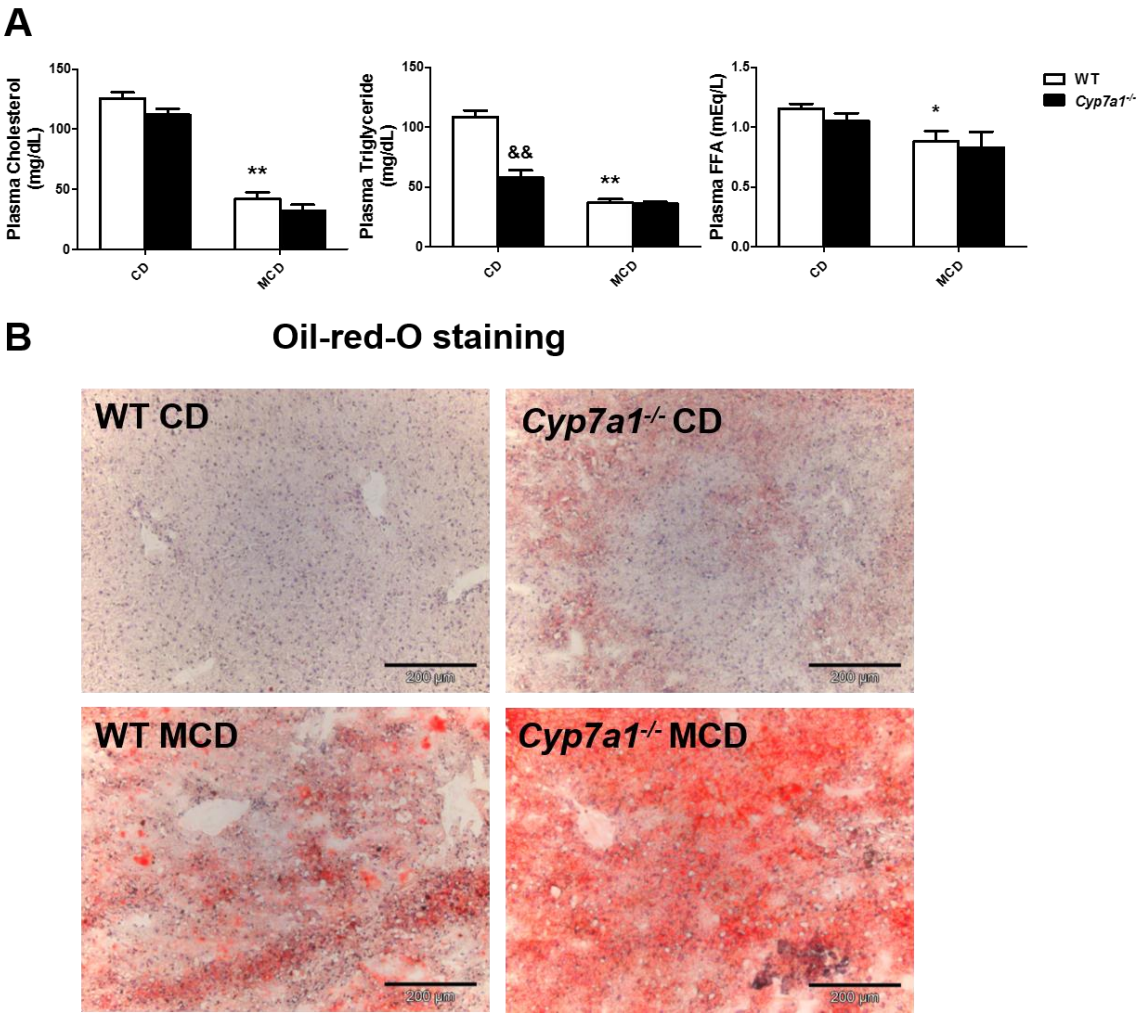
Supplemental Figure S3: Ad-Cyp7a1 failed to ameliorate LPS-induced hepatic inflammation in *Fxr*$^{-/-}$ mice. *Fxr*$^{-/-}$ mice were injected via the tail vein with 1x10^{9} plaque-forming units per mouse of either Ad-null or Ad-*Cyp7a1*. After 7 days, mice were injected i.p. with 20 mg/kg LPS or PBS (control). After 6 h, liver tissues were collected for analysis. (A) Representative H&E staining of mouse liver sections. Fresh liver samples were kept in 10% formalin solution and 5 µM sections were stained with H &E. (B) Representative immunohistochemistry staining for F4/80 of mouse liver sections.



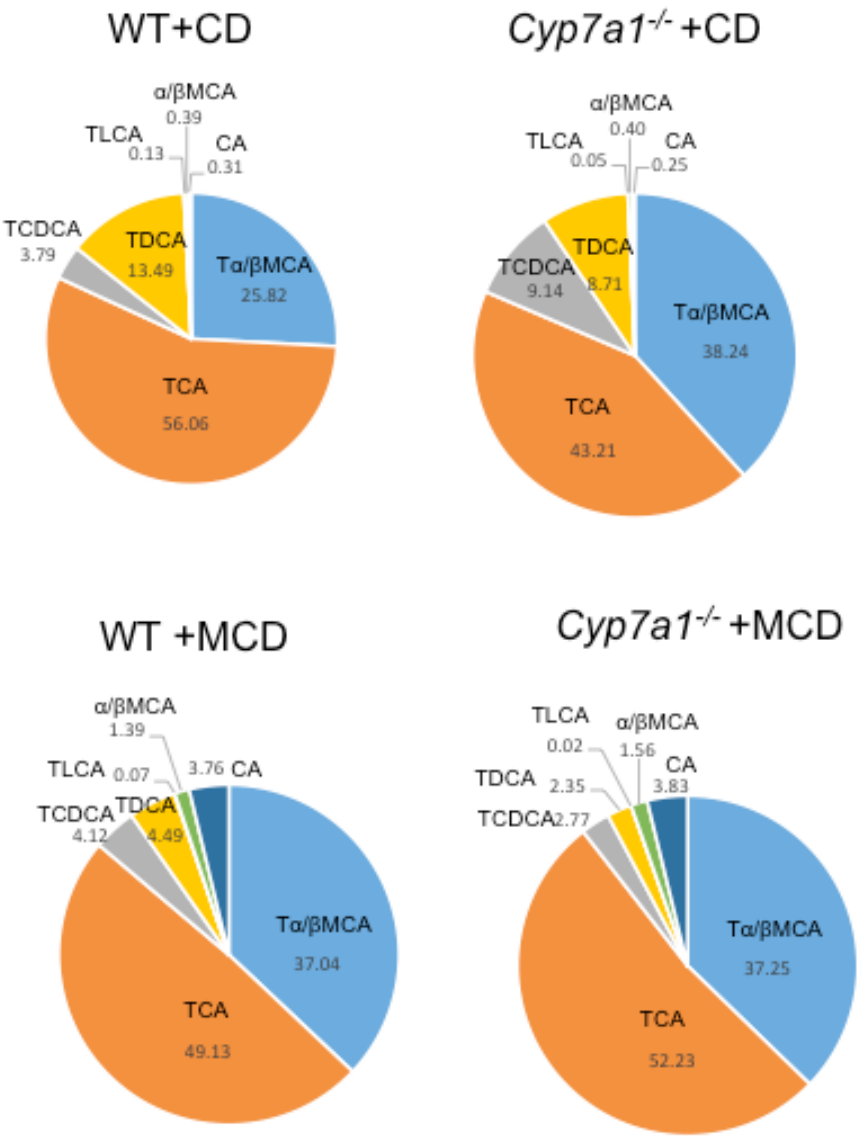
Supplemental Figure S4: Ad-Cyp7a1 ameliorated LPS-induced hepatic inflammation in *Tgr5*$^{-/-}$ mice. *Tgr5*$^{-/-}$ mice were injected via the tail vein with 1x10^{9} plaque-forming units per mouse of either Ad-null or Ad-*Cyp7a1*. After 7 days, mice were injected i.p. with 20 mg/kg LPS or PBS (control). After 6 h, liver tissues were collected for analysis. (A) Representative H&E staining of mouse liver sections. Fresh liver samples were kept in 10% formalin solution and 5 µM sections were stained with H&E. (B) Representative immunohistochemistry staining for F4/80 of mouse liver sections.



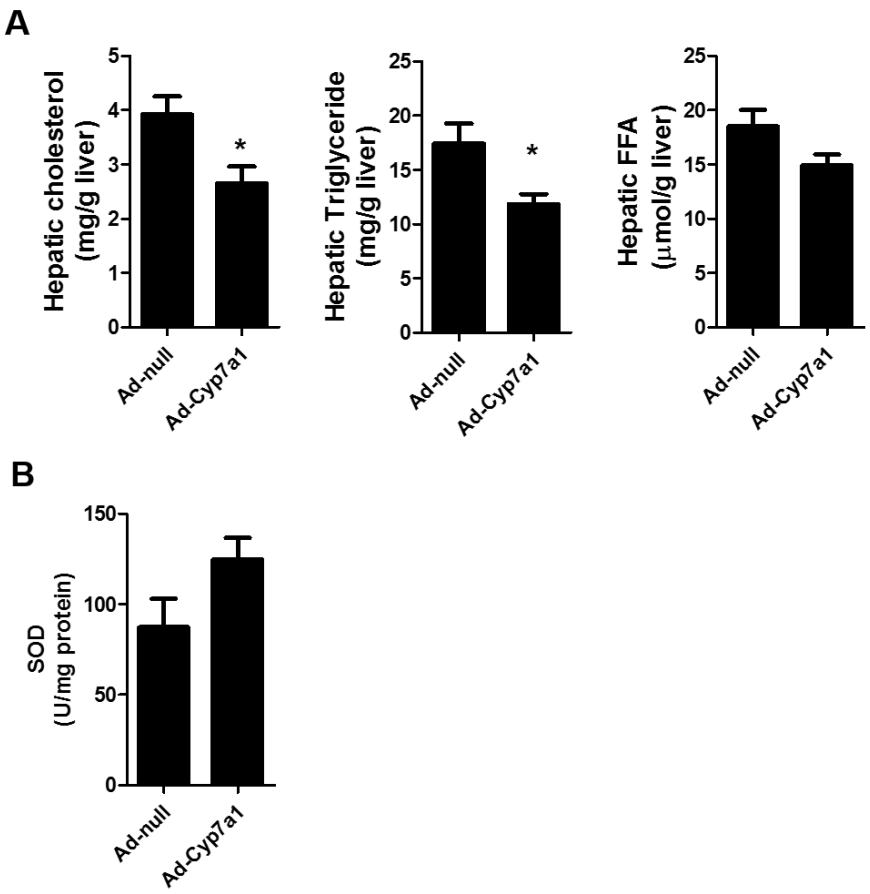
Supplemental Figure S5: Adenovirus-mediated *Cyp7a1* gene transduction increased bile acid pool size in *Fxr*$^{-/-}$ mice. (A) Bile acid pool sizes in wild type (*Fxr*$^{+/+}$) and *Fxr*$^{-/-}$ mice transfected with Ad-Cyp7a1 and Ad-null (control). Mice were injected in the tail vein with 1x10^{9} pfu per mouse of either adenovirus-null (Ad-null, control) or adenovirus-*Cyp7a1* (Ad-*Cyp7a1*) for 7 days. Bile acid contents in liver, intestine and gallbladder of Ad-null and Ad-Cyp7a1 mice were determined, and added up to total bile acid pool sizes. Results shown are means ± S.E. **p* <0.05, *Fxr*$^{+/+}$ Ad-*Cyp7a1* vs *Fxr*$^{+/+}$ Ad-null mice, #*p* <0.05, *Fxr*$^{-/-}$ *Ad-Cyp7a1* vs *Fxr*$^{-/-}$ Ad-null mice. ANOVA was used for statistical analysis. (B) QPCR assay of liver bile acid synthesis gene mRNA levels in wild type (*Fxr*$^{+/+}$) and *Fxr*$^{-/-}$ mice. Amplification of *Gapdh* was used as control. Results shown are means ± S.E. * *p* <0.05, Student's t-test was used for statistical analysis.



Supplemental Figure S6: MCD diet increased hepatic steatosis in C*yp7a1*$^{-/-}$ mice. Wild-type and C*yp7a1*$^{-/-}$ mice were fed with chow diet or MCD diet for 3 weeks. (A) Serum cholesterol, triglyceride and free fatty acid levels. (B) Representative Oil-red-O staining of mouse liver sections. Fresh liver samples were embedded with O.T.C. and 5 μM sections were stained with Oil-red-O. Results are shown as means ±S.E. *p <0.05, **p <0.01 vs WT fed with chow diet. $^{\#}p$ <0.05, $^{\#\#}p$ <0.01 vs WT fed with MCD diet. $^{\&}p$ <0.05, $^{\&\&}p$ <0.01 vs WT fed with chow diet. ANOVA was used for statistical analysis. (n=7-9 male mice per group)



Supplemental Figure S7: MCD diet altered bile acid composition in the bile. Bile acid compositions were analyzed by UHPLC-QTOFMS (detail under Supplemental Figure S1). Numbers are averaging percentage of each bile acid in total gallbladder bile of five chow diet and five MCD-fed wild type (n=5 each, Left panels) and *Cyp7a1*$^{-/-}$ mice (n=5 each, Right).



Supplemental Figure S8: Ad-Cyp7a1 reduced MCD diet-induced hepatic steatosis in *Cyp7a1*$^{-/-}$ mice. *Cyp7a1*$^{-/-}$ mice were fed with MCD diet for 3 weeks. On week 2, mice were injected in the tail vein with 1x10^{9} pfu per mouse of either Ad-null (control) or Ad-Cyp7a1. One week later, mice were sacrificed and liver tissues were collected for analysis. (A) Cholesterol, triglyceride and free fatty acid levels in mouse liver. (B) Superoxide dismutase (SOD) in mouse livers. Liver tissues were homogenized and (SOD) activity in the liver was determined by a SOD Kit. Results are shown as means ±S.E. *p <0.05, Ad-*Cyp7a1* vs Ad-null in C*yp7a1*$^{-/-}$ mice fed with MCD diet. Student's t-test was used for statistical analysis. (n=5-7 male mice per group).