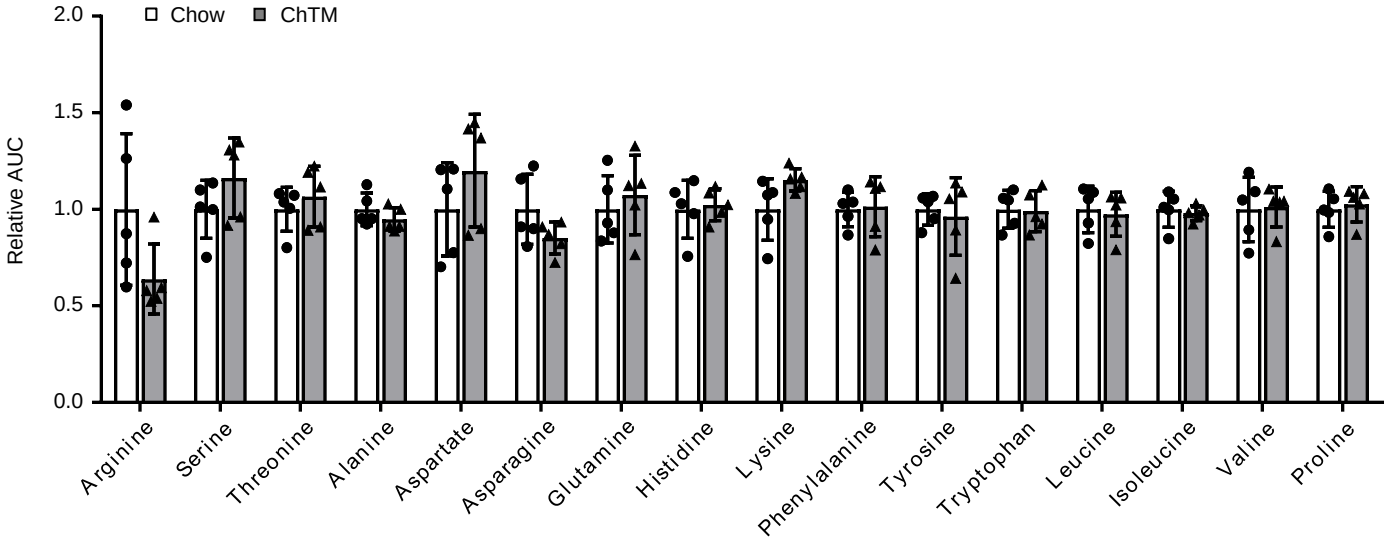
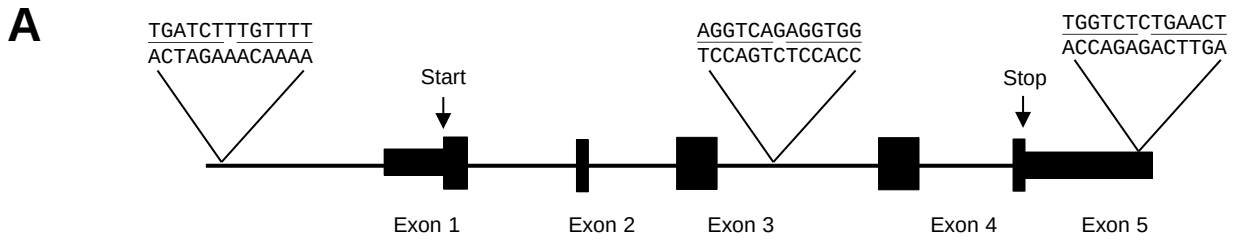
Supplemental Figure S1



Supplemental figure S1. Disrupting the enterohepatic bile acid recycling did not have a general effect on hepatic amino acid concentration in mice. Male C57BL/6J mice at 12 week of age were fed a chow diet or 2% (cholestyramine) ChTM-containing chow diet for 6 days. Livers were collected after ~ 6 h fasting. Liver metabolites were measured by metabolomics analysis. All results are expressed as mean ± SD. n=5. AUC: peak area under the curve.

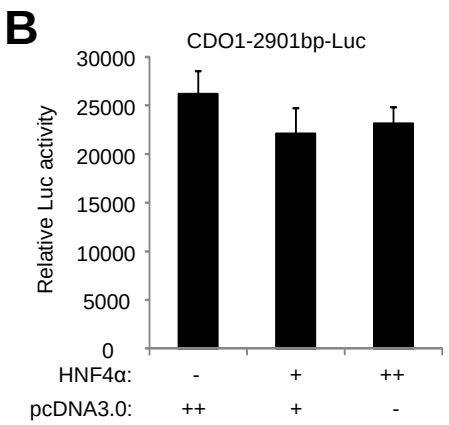
Supplemental Figure S2

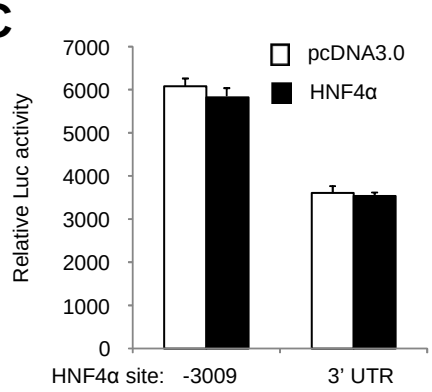


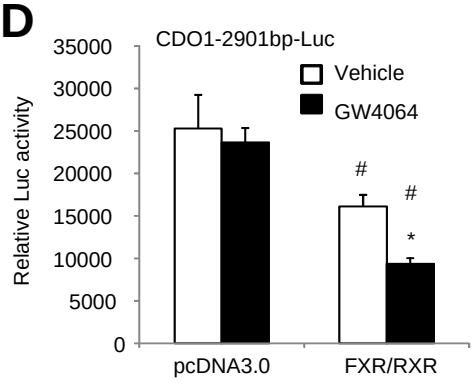
CDO1 promoter: $^{-3001}$GAAGCCTTAAAACAAAGATCAACAGCAAACCAGGGAGCGACTAAT

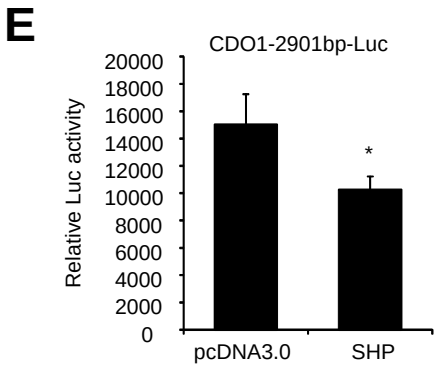
WT CDO1-Intron 3: CAAGTTTCCGACCTCTGGTTTCTACAGGTCAGAGGTGGGCATGGG
Mut CDO1-Intron 3: CAAGTTTCCGACCTCTGGTTTCTACAGGGGGGGGGGGGGCATGGG

CDO1 3'UTR: TTGATTATTAGAGTTCAGAGACCAGAGTGTTTCCTAAGGCTGAAA



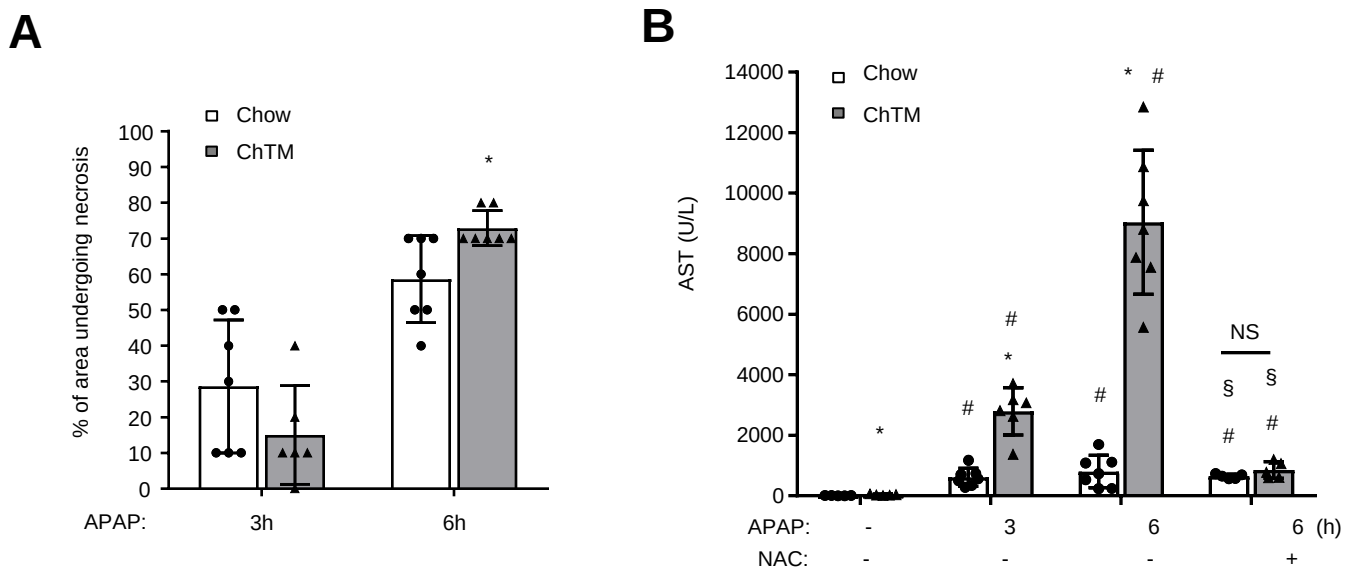






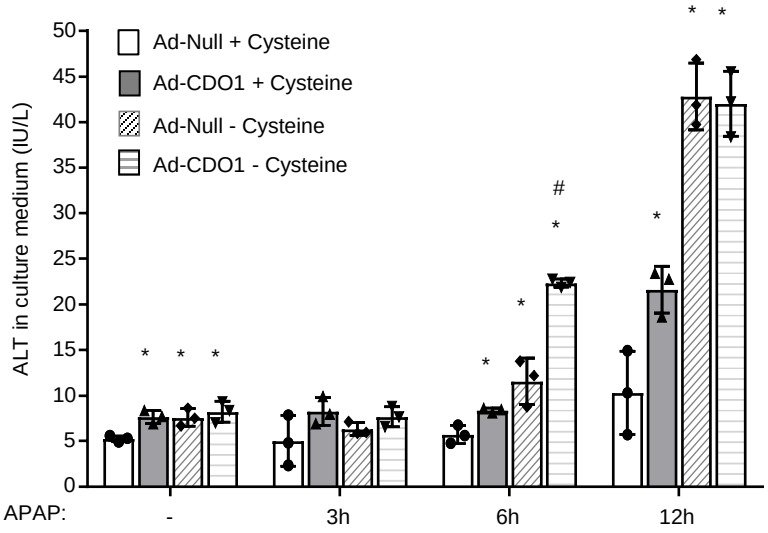
Supplemental figure S2. HNF4α did not induce the reporter activity driven by the 2901 bp CDO1 promoter or the putative HNF4 binding site located at 3009 bp upstream of the CDO1 promoter or at the CDO1 3' UTR. **A**. Putative HNF4α binding sites in the CDO1 chromatin are illustrated. Sequence of the DNA fragments used to construct the CDO1 reporter constructs are shown. Putative HNF4α binding sites are underlined. Start: start codon. Stop: stop codon. **B**. CDO1-2901bp-luc construct (0.2 μg), β-gal expression construct (0.05 μg) and 0.05 μg (+) or 0.1 μg (+) pcDNA3.0 or HNF4α expression plasmid were co-transfected into AML12 cells. Luciferase and β-gal activities were measured 48 h later. **C**. CDO1-Luc constructs (0.2 μg), β-gal expression construct (0.05 μg) and 0.1 μg pcDNA3.0 or HNF4α expression plasmid were co-transfected into AML12 cells. Luciferase and β-gal activities were measured 48 h later. **D**. CDO1-2901bp-luc construct (0.2 μg), β-gal expression construct (0.05 μg) and 0.2 μg pcDNA3.0 or 0.1 μg FXR and 0.1 μg RXR expression plasmids were co-transfected into AML12 cells. After 24 h, cells were treated with vehicle (DMSO) or GW4064 (1 μM) for 24 h. "*", vs. FXR/RXR + vehicle. "#", vs. corresponding pcDNA3.0. **E**. CDO1-2901bp-luc construct (0.2 μg), β-gal expression construct (0.05 μg) and 0.1 μg pcDNA3.0 or SHP expression plasmids were co-transfected into AML12 cells. Luciferase and β-gal activities were measured 48 h later. "*", vs. pcDNA3.0. All results are shown as mean of triplicate assays ± SD (technical repeats). In D, two-way ANOVA and Tukey post-hoc test were used. In E, 2-tailed Student's *t* test was used in statistical analysis of reporter assay results ($p<0.05$).

Supplemental Figure S3



Supplemental figure S3. Cholestyramine sensitized mice to APAP hepatotoxicity. Chow or 6-day ChTM-fed mice without APAP injection or with 400 mg/kg APAP injection for 3 h or 6 h. In some mice, 500 mg/kg N-acetylcysteine (NAC) was administered 15 min after APAP injection. **A**. Liver necrotic area in H&E liver sections. **B**. Plasma AST. All results are expressed as mean ± SD. A "*" indicates statistical significance, vs. chow at the same time point and experimental condition. A "#" indicates statistical significance, vs. none APAP-injected controls on the same diet. A "§" indicates statistical significance, vs. 6 h APAP-injected mice on the same diet. In "A", 2-tailed Student's t test was used. In "B", two-way ANOVA and Tukey post-hoc test were used for statistical analysis.

Supplemental Figure S4



Supplemental Figure S4. **Overexpression of CDO1 in primary mouse hepatocytes increased APAP-induced cytotoxicity**. On the same day of isolation, mouse hepatocytes were infected with Ad-Null or Ad-CDO1 (MOI =10) in regular DMEM medium containing 100 μM cysteine. After 16 h, cells were switched to DMEM without cysteine/cystine or with 100 uM cysteine and treated with 2.5 mM APAP for 3, 6 and 12 h. ALT levels in the culture medium was measured. The results from a representative triplicate assay (technical repeats) were plotted as mean ± SD. A "*" indicates statistical significance, vs. Ad-Null + Cysteine at the same time point. A "#" indicates statistical significance, vs. Ad-Null - Cysteine at the same time point. Two-way ANOVA and Tukey post-hoc test were used for statistical analysis.