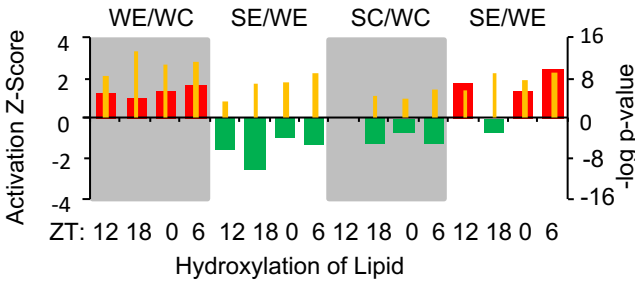
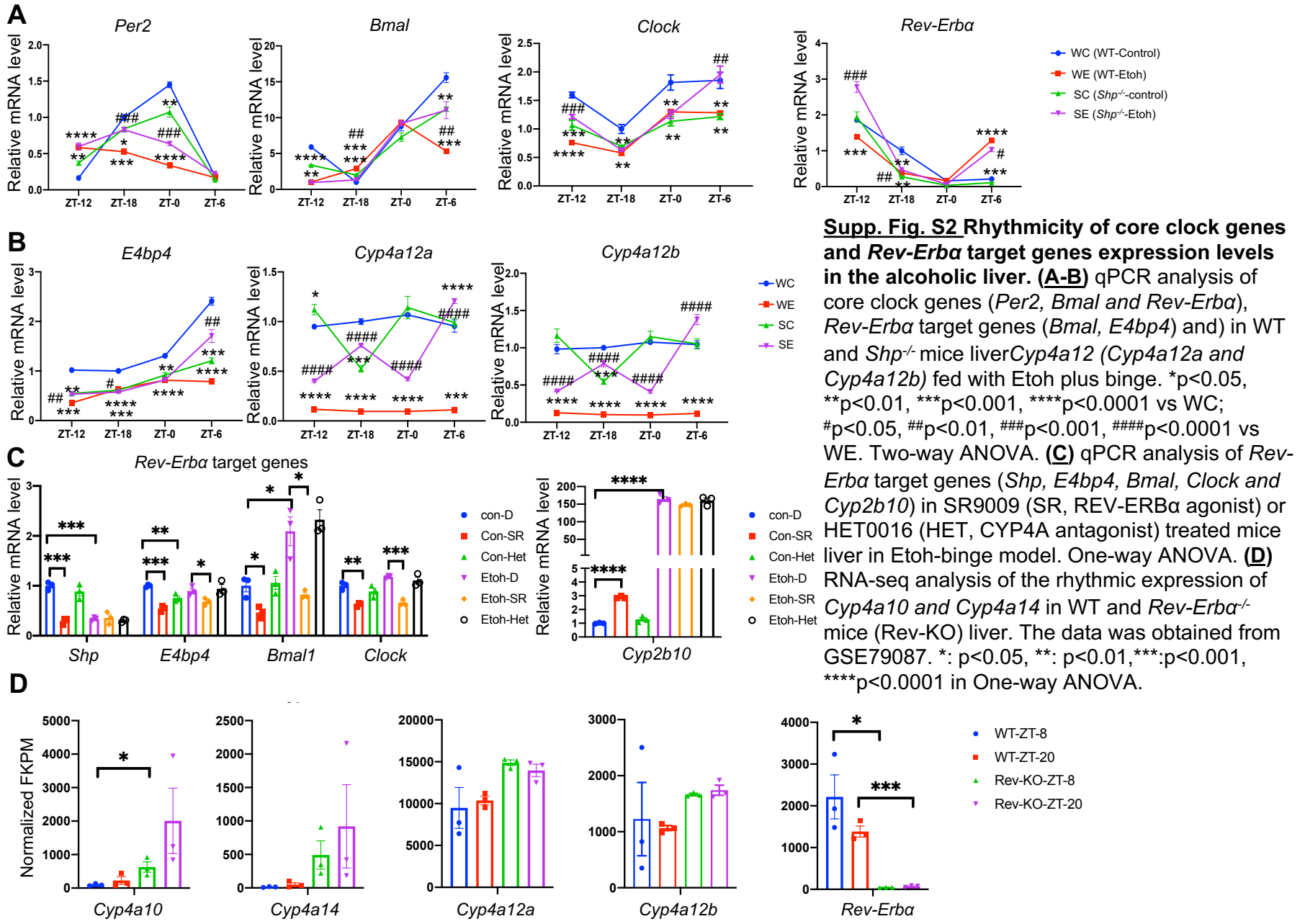


Etoh Rev-Cyp, Supp. Fig. S1

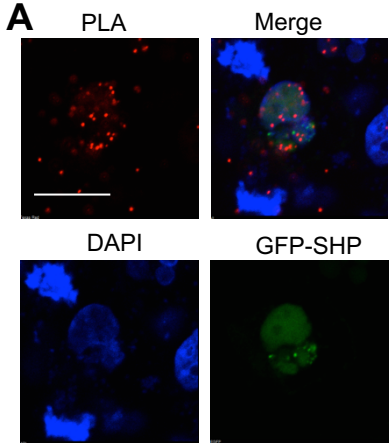


Supp. Fig. S1 IPA activation scores at all ZT using Ingenuity Pathway Analysis (IPA). Red: up-regulated in indicated comparisons; Green: down-regulated in indicated comparisons; Yellow: p-value for enrichment of the lipid hydroxylation pathway at each ZT (Fisher's exact test).

Etoh Rev-Cyp, Supp. Fig. S2

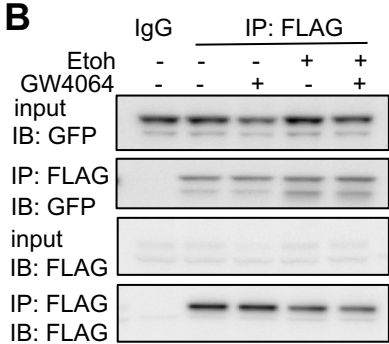


Etoh Rev-Cyp, Supp. Fig. S3

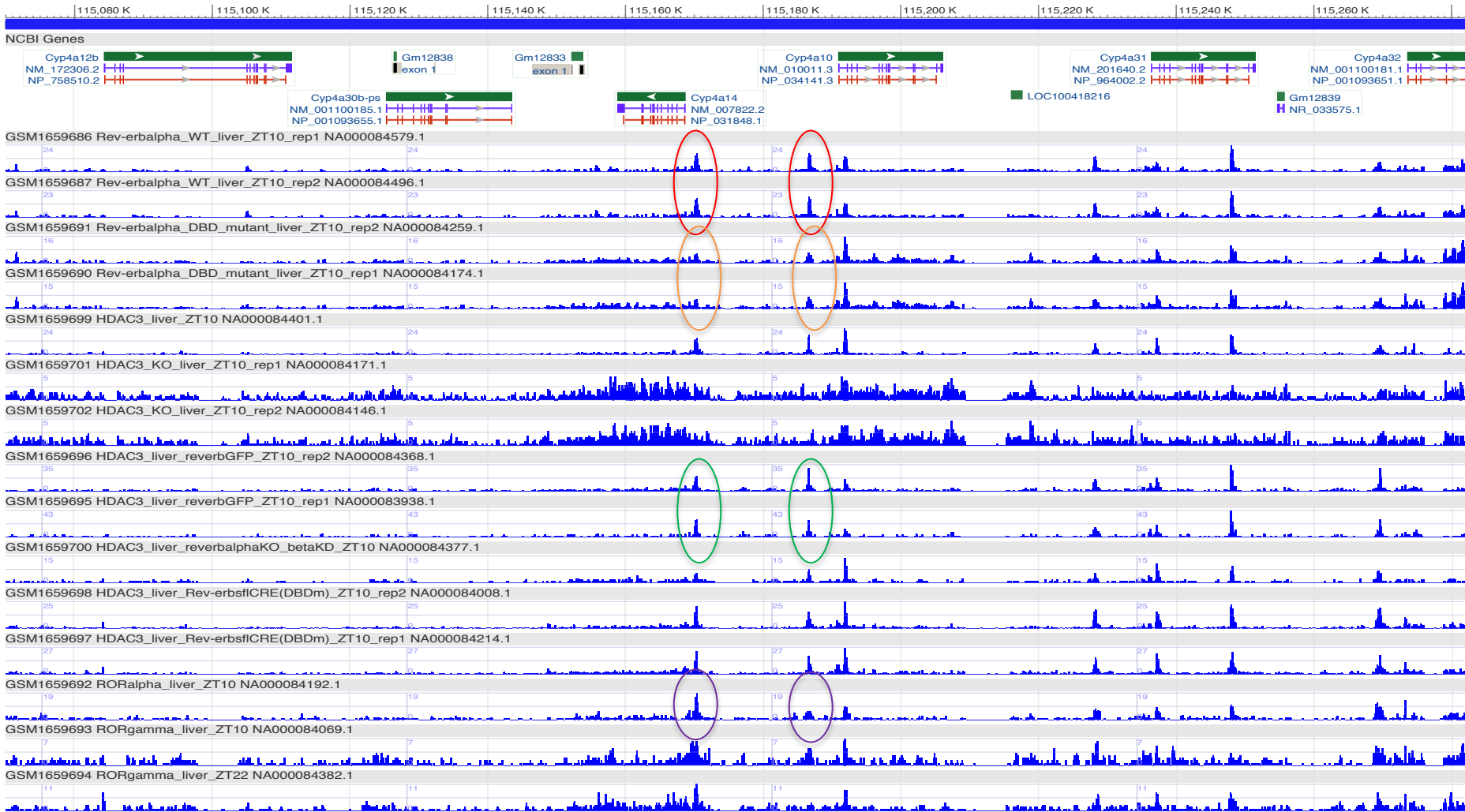


PLA: FLAG-REV-ERB α and GFP-SHP

Supp. Fig. S3 Interaction between GFP-SHP and FLAG-REV-ERB α proteins located in the nucleus of HEK293T cells. (A) Proximity ligation assay (PLA) were performed using rabbit anti-GFP and mouse anti-FLAG antibodies. Red indicated the interacted SHP and REV-ERB α ; Green indicated the expression of GFP-SHP. Scale bar: 50 μ m. **(B)** Immunoprecipitation (IP) between SHP and REV-ERB α in condition of ethanol and/or FXR agonist (GW4064) treatment. HEK 293T cells were transfected with FLAG-REV-ERB α and GFP-SHP for 24 hours and fasted for another 24 hours. Then the cells were pre-treated with 5nM GW4064 for 1 hours before treated 50mM Etoh overnight. The cell lysis were immunoprecipitated with anti-FLAG antibody. The anti-FLAG and anti-GFP antibodies were used for WB.



Etoh Rev-Cyp, Supp. Fig. S4



Supp. Fig. S4 REV-ERBA binding on both Cyp4a10 and Cyp4a14 promoter from CHIP-seq analysis (GSE67962). The bindings (Red circles) were diminished when REV-ERBA DNA-binding domain was mutant (Orange circles). HDAC3 (Green circles) and RORAlpha (Purple circles) bind to both promoter at same location.

Etoh Rev-Cyp, Supp. Fig. S5

>NC_000070.5:115186263-115186950 Mus musculus strain C57BL/6J chromosome 4, MGSCv37 C57BL/6J

Cyp4a10 pro

CAACCCCTGACAGGTTGACTATGTTCCAGTGAATGGCCACATAGGAATATATGTATTACACTAACTGGACTTGA
TAGGTTTtagggTCACAAGAAGAAGACAAGGAATTCAGTCTAAAGGGAAATGGAGTAGAGATCTAGGAGGAAT
TGTGAGGGCATGAATATAGACAAAACATGTTGTATAACGTTTTTAAAAGATTTCGTAACAGTAACTGACAAGCAG
ATTTAAATTCTAAAATTCTTTACAACATGTTCCCTTTATTTTAAAGGGTGACAAATGGGTTCTTGGATATAGAAGGA
AAAGGCCACCGTCTAAAAGTTCT **AGGTCA** GAGGAGAAGAGTACAATGTGGATAGAAAAAGTAGTCATGAAAC
TAGGGTAAAGTTCAGTGAGATGGAGTGTAAAGTTATTCCATTGCCCTTTGCTCTAGTTTTTGACAGAGCATATG
ATTTGGGAGGAAAAAAAAGACCTTTGGCCCTAGGAGTTGAGAAATAAATCCCTTGTTATTAGAAAAGCAGA
GGAGAAGCCTTTGAGGGCATGTCAGCAGATTCTGGGGGAGGCAGCCTTCTAGTCCAATAGGGAAGGTTACC
AATACCACCATGGACTCTAAATGATTGTTTTCTTTGAACAATATCACTTGGACTCTCTGTTATCAGTTTCTTAT
CACTGAAATAAGGGCAAGGGTAAAACCAA

mC4a10-pro-F: GGGGTACCCAGTGAATGGCCACATAGGA (Kpn1)

mC4a10-pro-R: CCAAGCTTCTCCCCAGAATCTGCTGAC (Hind3)

mC4a10-CHIP-F: GAAGGAAAAGGCCACCGTCT

mC4a10-CHIP-R: ACTCCTAGGGCCAAAGGTCT

>NC_000070.5:115170123-115170466 Mus musculus strain C57BL/6J chromosome 4, MGSCv37 C57BL/6J

CYP4a14 pro

CCCACTACTTTTTCTTTCTTTCCACAATTCTGAGCTTT GGTCAAAAACCAAAGACCGAGAGGGACATGCTCCAT
ACGTCTGAACTTTTAGCCCACTTTGT GGTCAATTTTACAACCTTCAAATATTTGTTGGATGAGTAAATGATTGAAA
GATCCAGTCCATTTCTTGGGAC **AGGTCA** TTTTGTACCCTTTCTCCTTCCCTACTTGGATCCTCTCCCACTTTC
AGAGTGGAATTCAGTAGTGGAAGAGCTTTGGCGTCCAAGACTACTGCTGAATTTTAGTTTTGACTGTTTCTT
TCTCTGAATCTGAGTTCTTTTTGTTTGACCCATATTATGGTAAGCATGAAC

mC4a14-pro-F: GGGGTACCCACAATTCTGAGCTTTGGTCA (Kpn1)

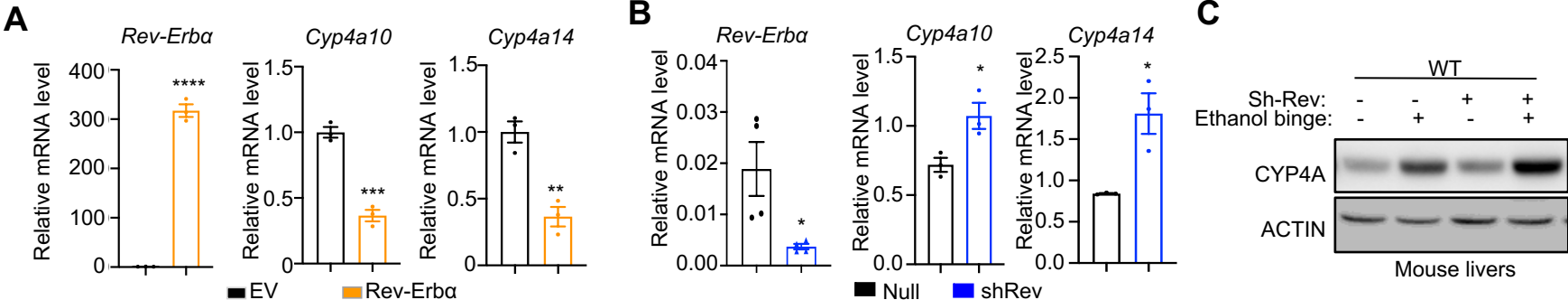
mC4a14-pro-R: CCAAGCTTTGGGTCAAACAAAAGAACTCAGA (Hind3)

mC4a14-CHIP-F: GGGACATGCTCCATACGTCT

mC4a14-CHIP-R: ACGCCAAAGCTCTTTCCACT

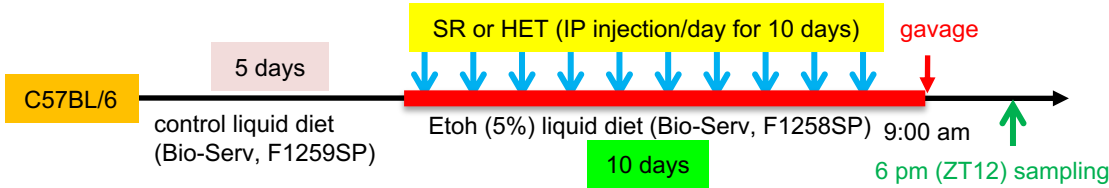
Supp. Fig. S5 Sequences of cyp4a10 and cyp4a14 promoter around REV-ERB α binding peaks. Underlined sequences indicated the REV-ERB α binding sites. Primers for cloning those promoters and for CHIP assay were provided as labeled.

Etoh Rev-Cyp, Supp. Fig. S6



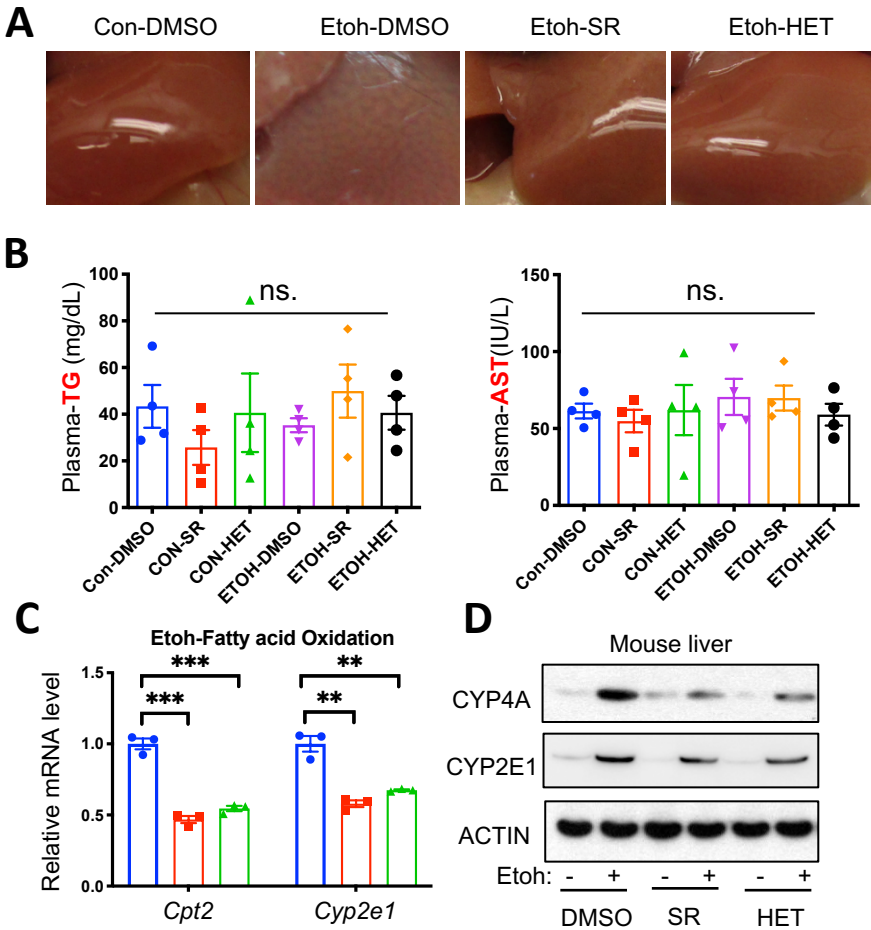
Supp. Fig. S6 REV-ERBa regulated CYP4A in vivo. (A-B) qPCR analysis of Rev-Erba, *Cyp4a10* and *Cyp4a14* mRNAs in Rev-Erba overexpressed mice liver (**A**) or Rev-Erba knock down mice liver. T-test. (**B**). EV: Empty vector; shRev: shRNA for Rev-Erba. *: p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs EV or Null. T-test. (**C**) Western blot analysis of CYP4A protein level in indicated group.

Etoh Rev-Cyp, Supp. Fig. S7



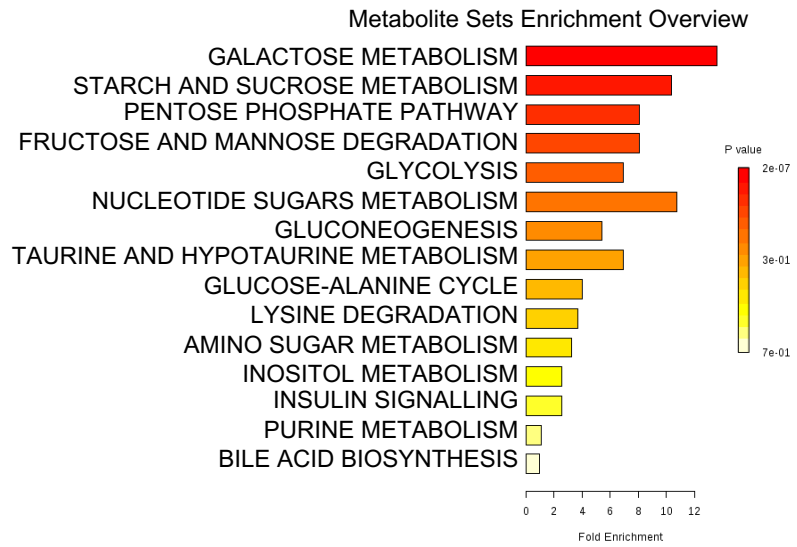
Supp. Fig. S7 Schemes for agonist or antagonist application in Etoh-binge model. Rev-Erba agonist (SR9009, 100mg/kg/day) or Cyp4a antagonist (HET0016, 5mg/kg/day) was injected through IP daily in ethanol feeding period in Etoh-binge model.

Etoh Rev-Cyp, Supp. Fig. S8



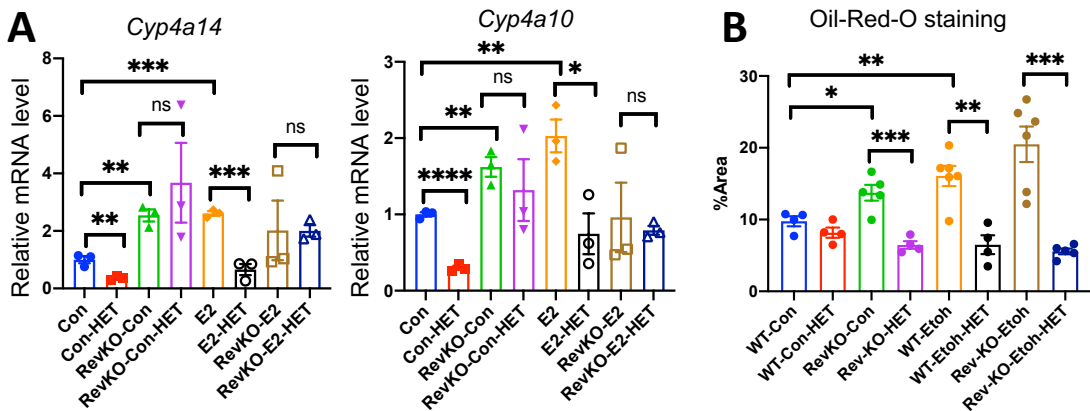
Supp. Fig. S8 Treatment of REV-ERB α agonist SR9009 or CYP4A antagonist HET0016 significantly improved alcoholic steatosis and alcohol-induced liver injury. (A) Gross appearance of the liver surface. (n=4/group). **(B)** Serum TG and AST levels in indicated groups. One-way ANOVA. **(C)** qPCR analysis of genes involved in fatty acid oxidation. **p<0.01, ***p<0.001 vs Etoh-DMSO. One-way ANOVA. **(D)** Western blot analysis with CYP2E1 and CYP4A antibodies in each group. Con: control; Etoh: ethanol; SR: REV-ERB α agonist-SR9009; HET: CYP4A antagonist-HET0016.

Etoh Rev-Cyp, Supp. Fig. S9



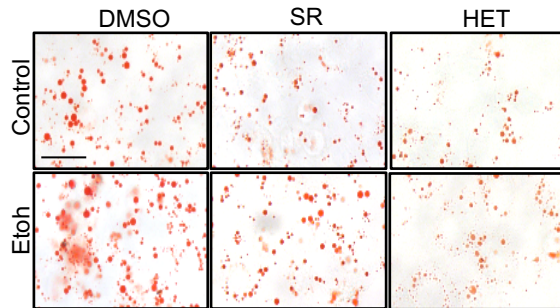
Supp. Fig. S9 The overview of Metabolite Sets Enrichment (MESA) in ethanol fed mice treated with SR9009 (Etoh-SR) vs ethanol fed mice (Etoh-DMSO).

Etoh Rev-Cyp, Supp. Fig. S10



Supp. Fig. S10 Deficiency of Rev-Erb α blocked HET reduced *Cyp4a14* and *Cyp4a10*. **(A)** qPCR analysis of Rev-Erb α , *Cyp4a10* and *Cyp4a14* mRNAs in WT or Rev-Erb α ^{-/-} (RevKO) mouse primary hepatocyte treated with E2 (50 mM) or HET (4nM) for 24 hours. One-way ANOVA. **(B)** Quantification of the positive area in Oil-Red-O staining for **Fig. 7D**. *: p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs indicated group. One-way ANOVA.

Etoh Rev-Cyp, Supp. Fig. S11



HC04 (human hepatocyte, (10X))

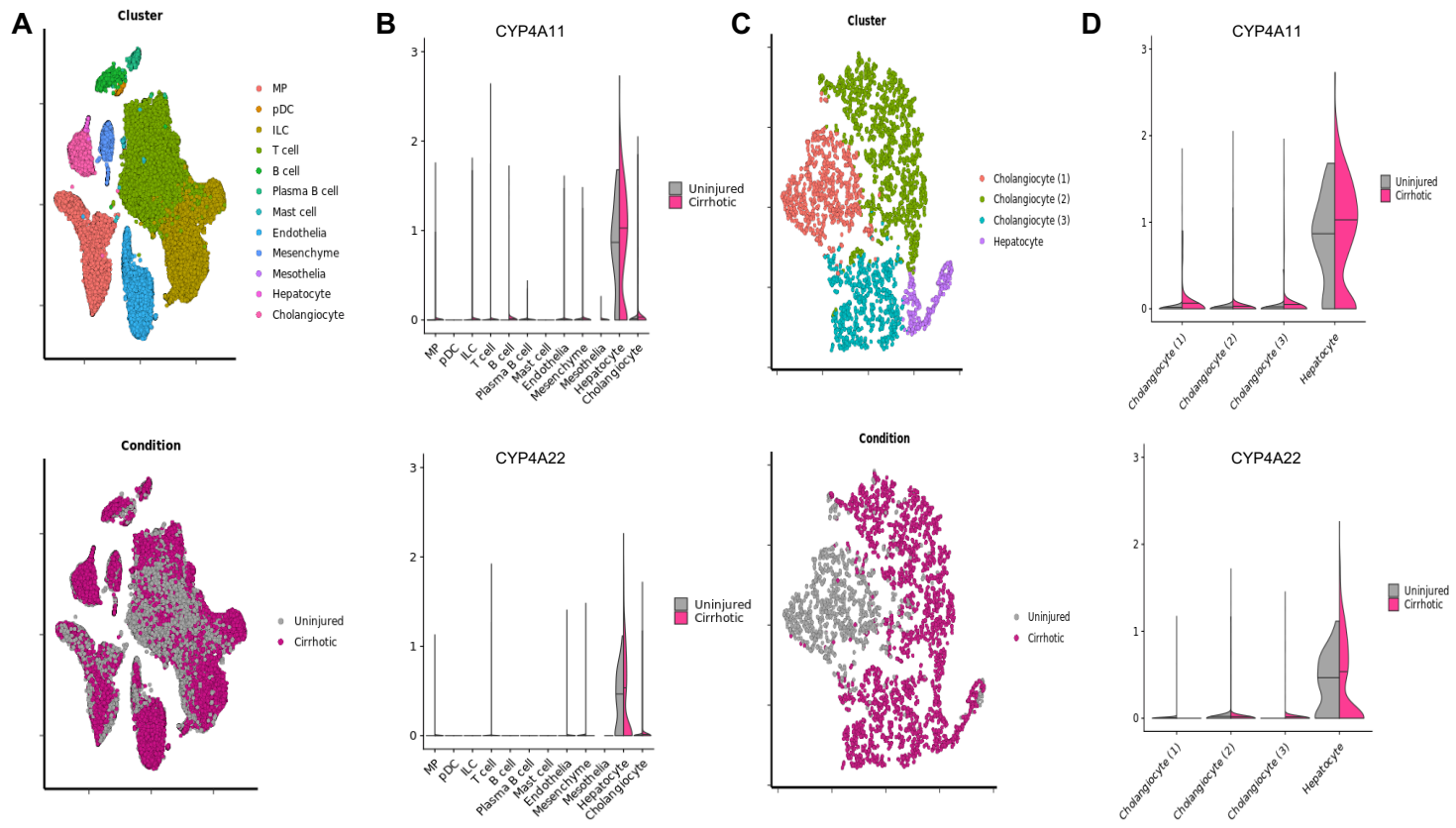
Supp. Fig. S11 Oil-red O staining in

human hepatocyte cell line HC04.

HC04 cells were pre-treated with SR9009 (5 μ M) or HET0016 (4 μ M) for 6 hours followed by ethanol 50 mM (Etoh) for another 24 hours.

Scale bar: 100 μ m.

Etoh Rev-Cyp, Supp. Fig. S12



Supp. Fig. S12 CYP4A11 and CYP4A22 expression in human cirrhotic livers at single cell level . (A) human liver cells from 5 healthy and 5 cirrhosis were isolated using FACS sorting and subjected for single cell sequencing. The cells were clustered and the color indicated the cell types or samples as shown in figure. **(B)** The expressions of CYP4A11 and CYP4A22 were high only in hepatocyte compared to other cell types. **(C)** The clustering of epithelia cells (upper) and distribution of samples (bottom). **(D)** The expression levels of both CYP4A11 and CYP4A22 were increased in cirrhotic liver hepatocytes. The plot were downloaded from and open-access gene browser (<http://www.livercellatlas.mvm.ed.ac.uk>) and the raw data is available in GSE136103.

Fig. 1

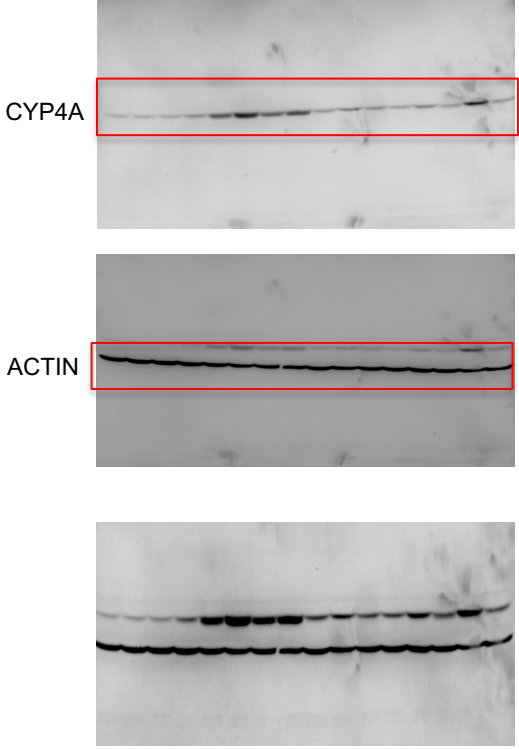


Fig. 3

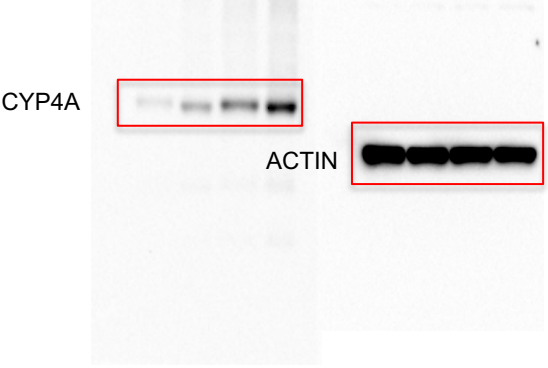
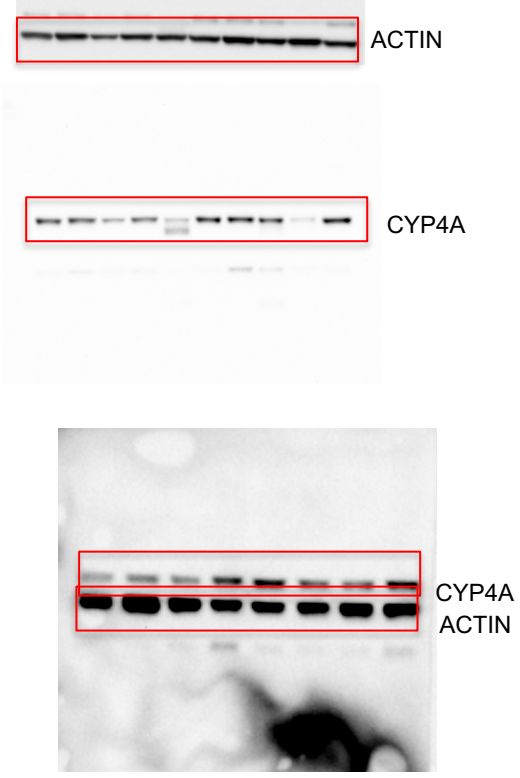


Fig. 8



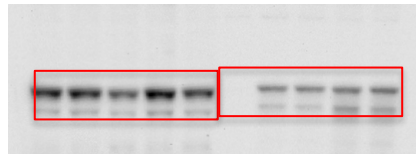
Supp. Fig. S13 Original blot for the Western blot.

Etoh Rev-Cyp, Supp. Fig. S13 continue

Supp. Fig. S3

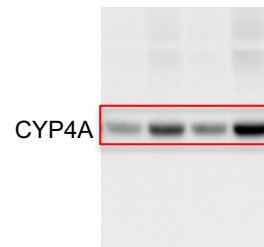


Flag-HRP

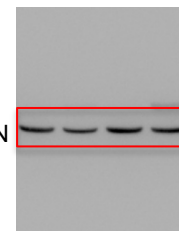


GFP-SHP

Supp. Fig. S6

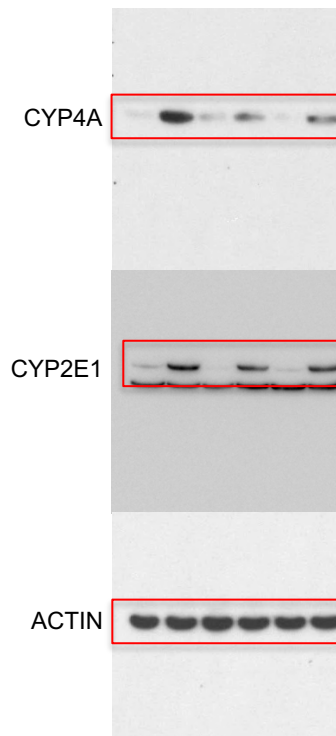


CYP4A



ACTIN

Supp. Fig. S8



CYP4A

CYP2E1

ACTIN