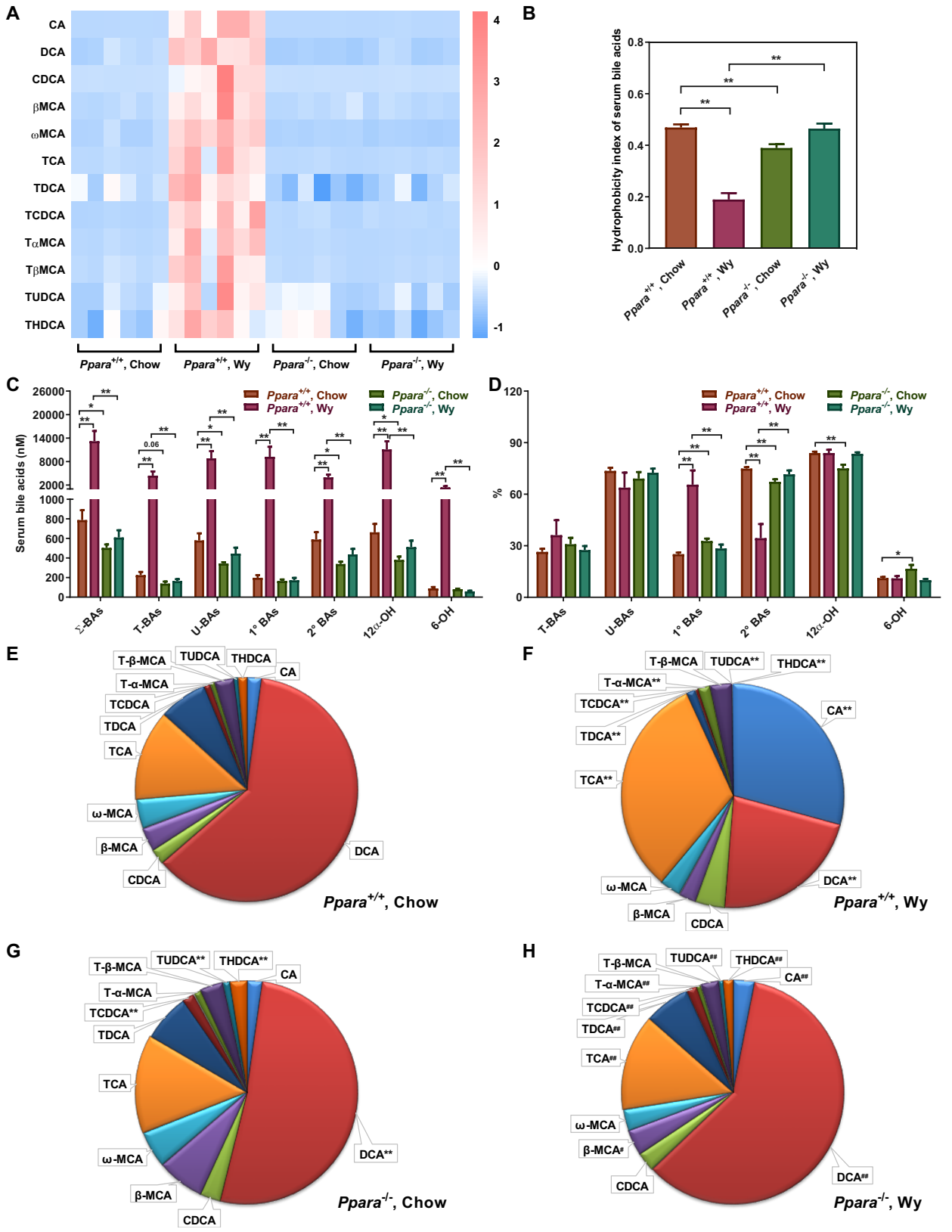
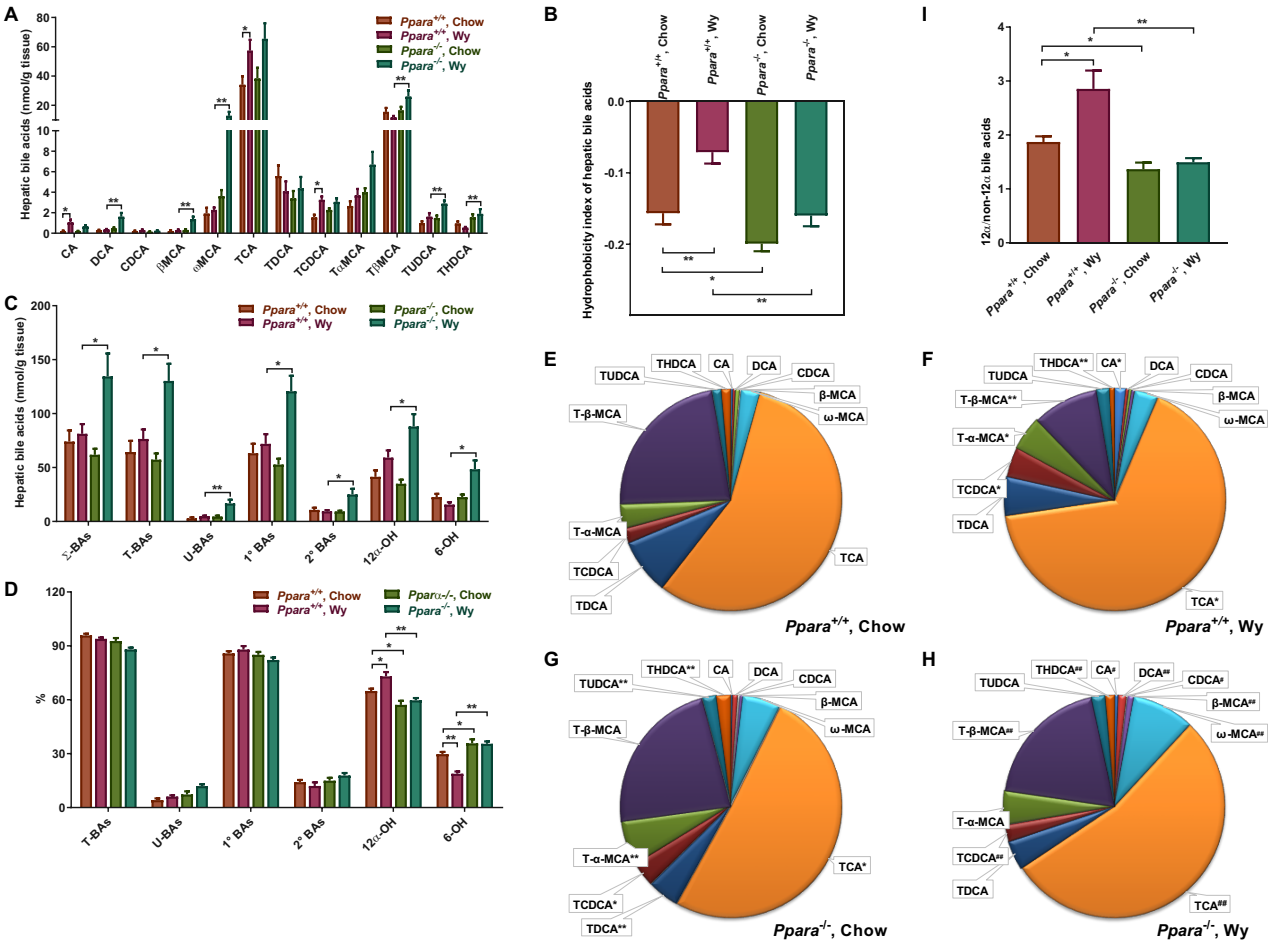


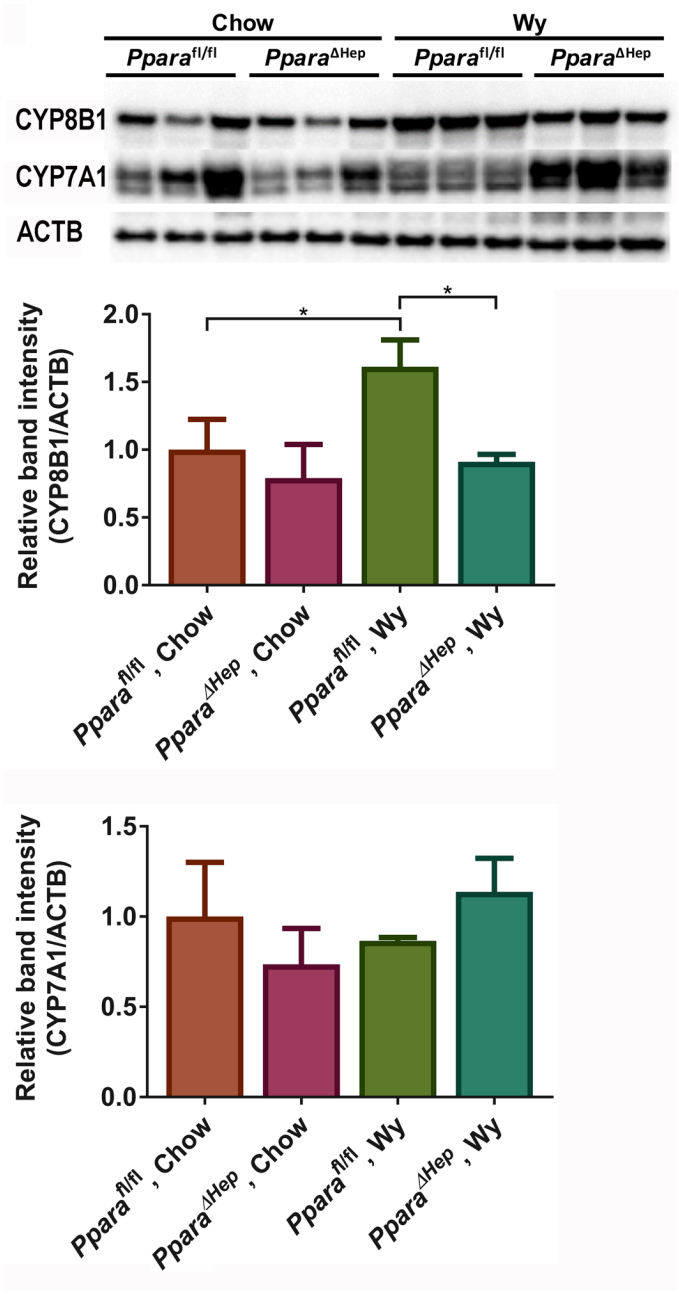
Supplementary Figure 1. Effect of Wy on serum BA composition in *Ppara*$^{+/+}$ and *Ppara*$^{-/-}$ mice.

A. Heat map of individual BA levels. B. Hydrophobicity index of serum bile acids. C. Total

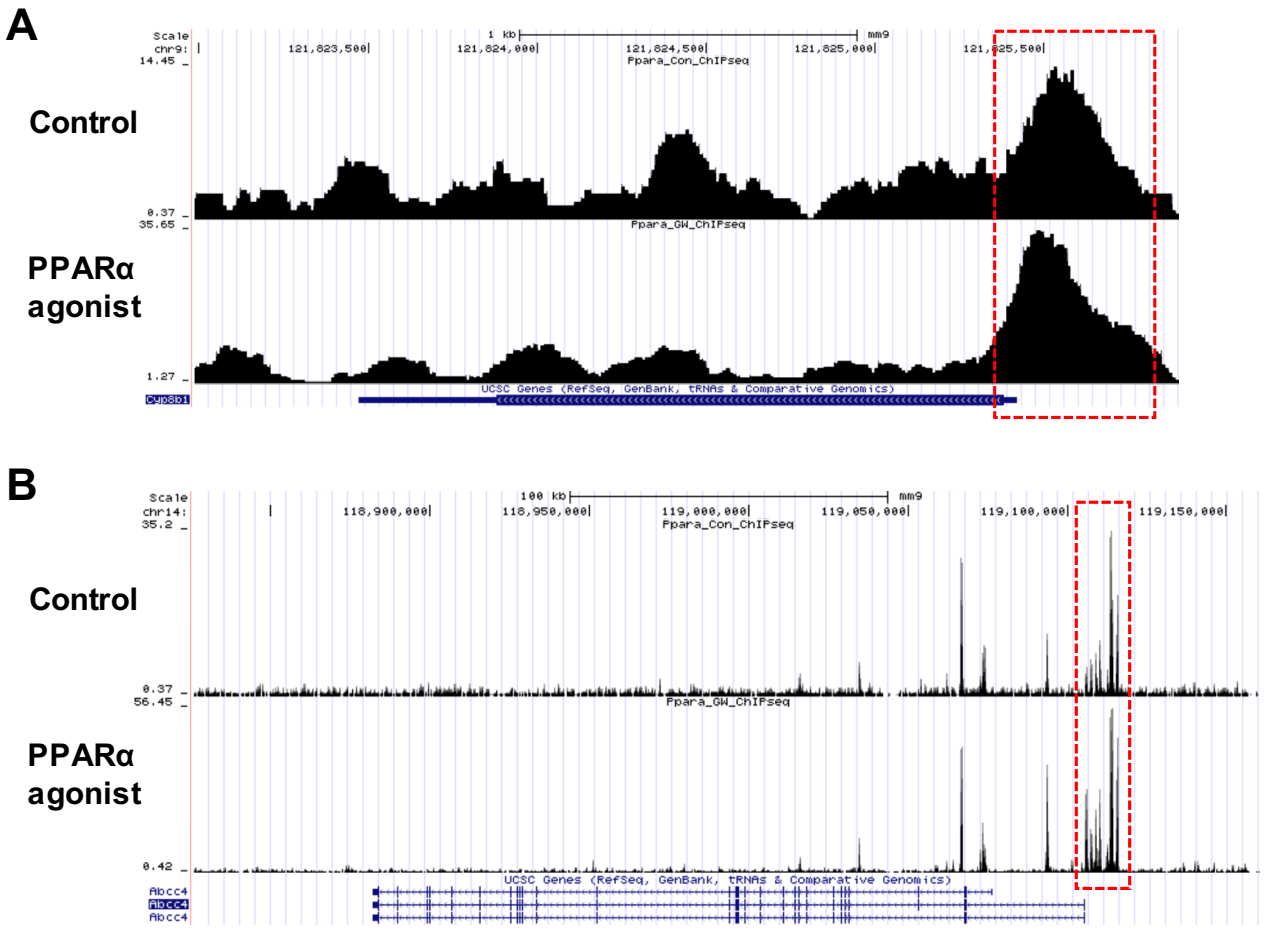
concentration of different BA classes. D. Relative percentage of different BA classes to total BAs. E. Relative fraction of individual BAs in serum of chow-treated *Ppara*$^{+/+}$ mice. F. Relative fraction of individual BAs in serum of Wy-treated *Ppara*$^{+/+}$ mice. G. Relative fraction of individual BAs in serum of chow-treated *Ppara*$^{-/-}$ mice. H. Relative fraction of individual BAs in serum of Wy-treated *Ppara*$^{-/-}$ mice. n = 6/group. ∑-BAs, total BAs. T-BAs, taurine-conjugated BAs. U-BAs, unconjugated BAs. 1° BAs, primary BAs. 2° BAs, secondary BAs. 12α-OH, 12α-hydroxylated BAs. 6-OH, 6-hydroxylated BAs. Data are presented as mean ± SEM; n = 6/group. *P < 0.05 or **P < 0.01, by one-way ANOVA followed by Tukey's *post-hoc* correction.



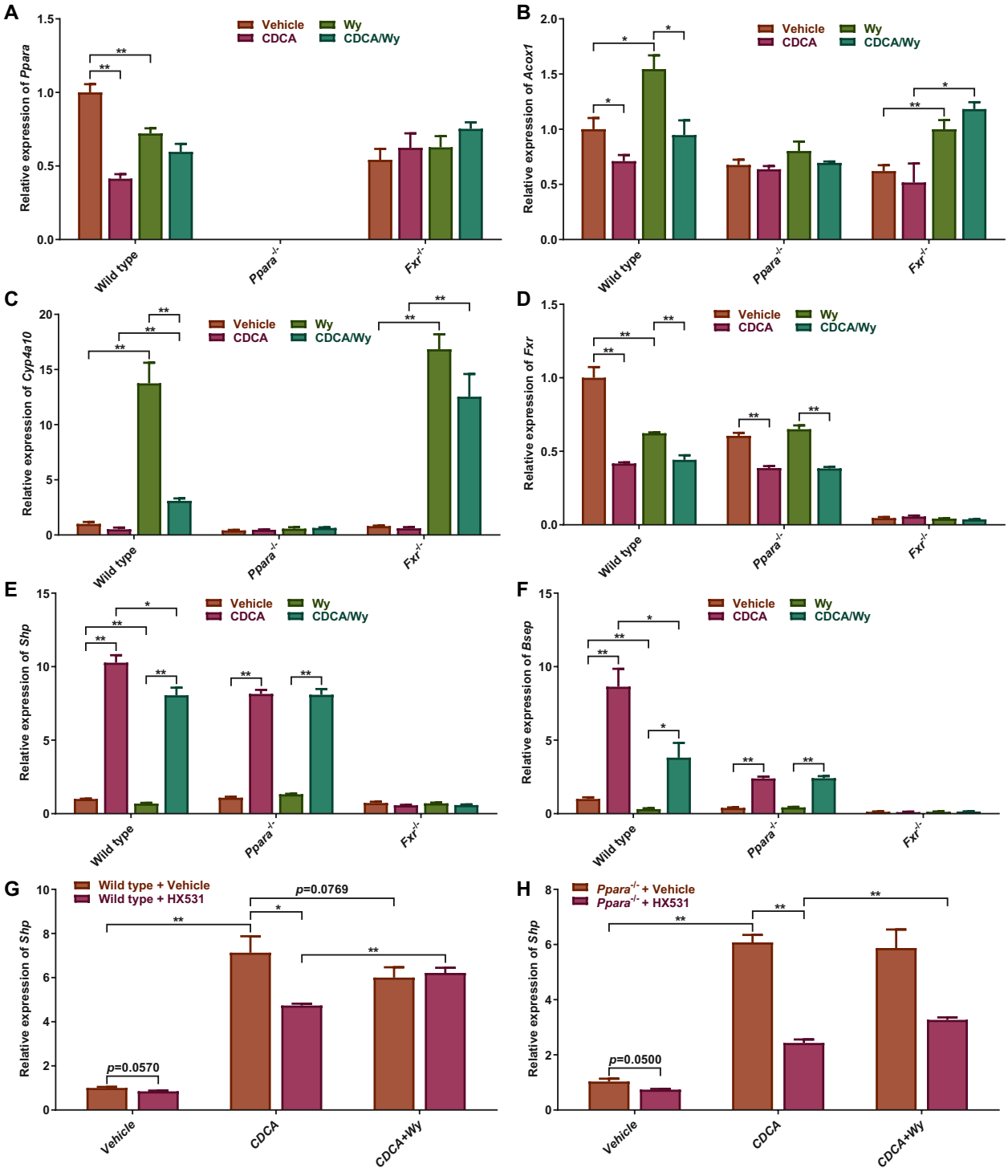
Supplementary Figure 2. Determination of BA concentrations in the liver of chow- and Wy-treated *Ppara*$^{+/+}$ and *Ppara*$^{-/-}$ mice. A. Heat map of individual BA levels. B. Hydrophobicity index of hepatic BAs. C. Total concentration of different BA classes. D. Relative percentage of different BA classes to total BAs. E. Relative fraction of individual BAs in livers of chow-treated *Ppara*$^{+/+}$ mice. F. Relative fraction of individual BAs in livers of Wy-treated *Ppara*$^{fl/fl}$ mice. G. Relative fraction of individual BAs in livers of chow-fed *Ppara*$^{-/-}$ mice. H. Relative fraction of individual BAs in livers of Wy-treated *Ppara*$^{-/-}$ mice. I. 12α-OH/non-12α-OH BAs ratio. ∑-BAs, total BAs. T-BAs, taurine-conjugated BAs. U-BAs, unconjugated BAs. 1° BAs, primary BAs. 2° BAs, secondary BAs. 12α-OH, 12α-hydroxylated BAs. 6-OH, 6-hydroxylated BAs. Data are presented as mean ± SEM; *n* = 6/group. **P* < 0.05 or ***P* < 0.01, by one-way ANOVA followed by Tukey's *post-hoc* correction.



Supplementary Figure 3. The effects of Wy treatment on CYP8B1 and CYP7A1 proteins in *Ppara*$^{fl/fl}$ and *Ppara*$^{\Delta Hep}$ mice. Western blot analysis of CYP8B1 and CYP7A1 was carried out. Data are presented as mean ± SEM; n = 3/group. *$P < 0.05$ by one-way ANOVA followed by Tukey's *post-hoc* correction.

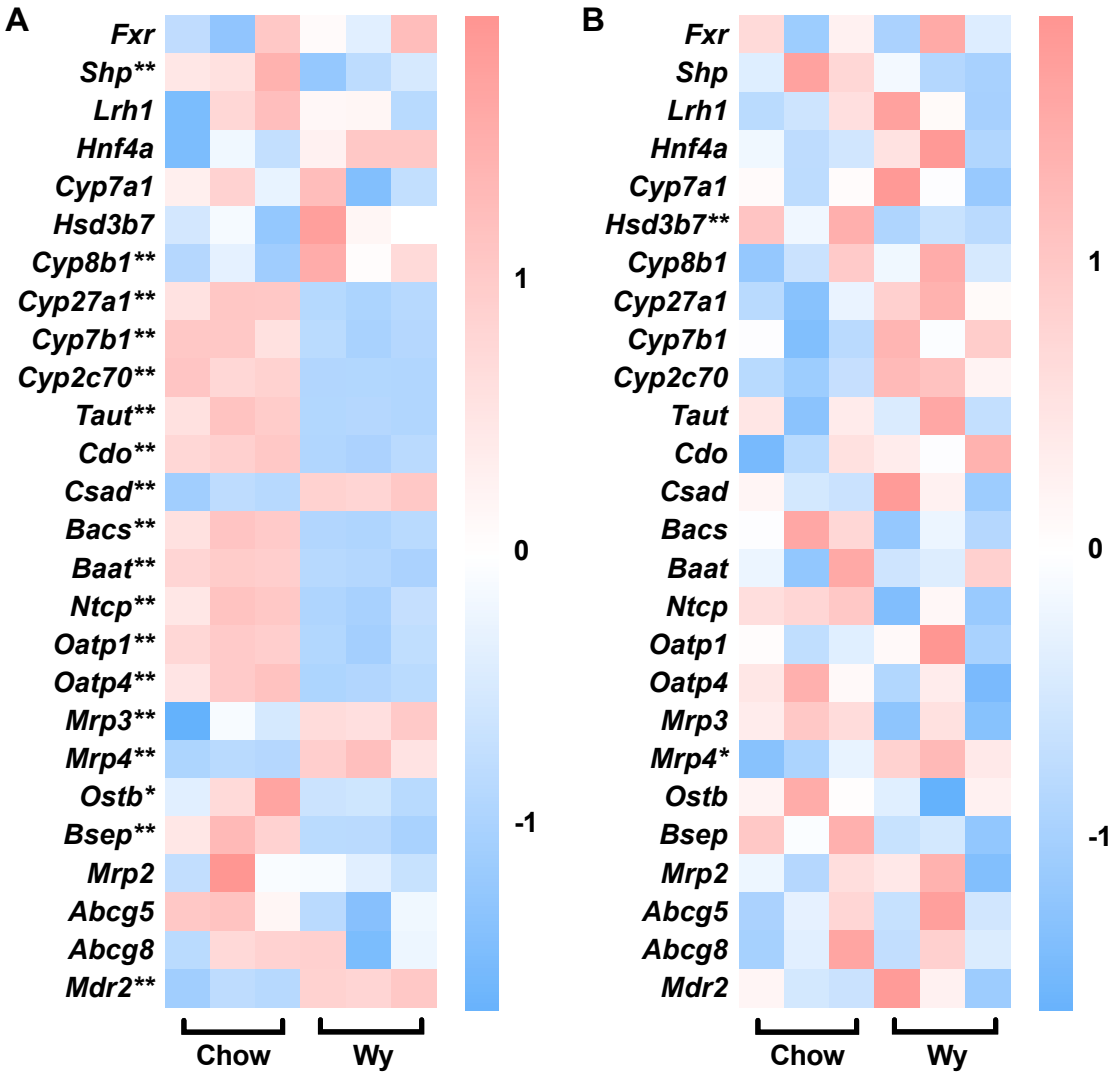


Supplementary Figure 4. PPARα binds to promoters of genes involved in BA homeostasis. A. Potential PPARα binding site in the *Cyp8b1* promoter. B. Potential PPARα binding site in *Abcc4* promoter. ChIP-seq data were obtained from the NIH GEO database (GSE61817). PPARα ChIP-seq data was analyzed and raw reads mapped to the genome. Mice were treated with control or the PPARα agonist Wy. The gene transcription direction is indicated by the arrow, and the beginning of the arrow denotes the transcription start site. The red box indicates the binding peaks found within the promoter region containing the transcription start site (TSS).

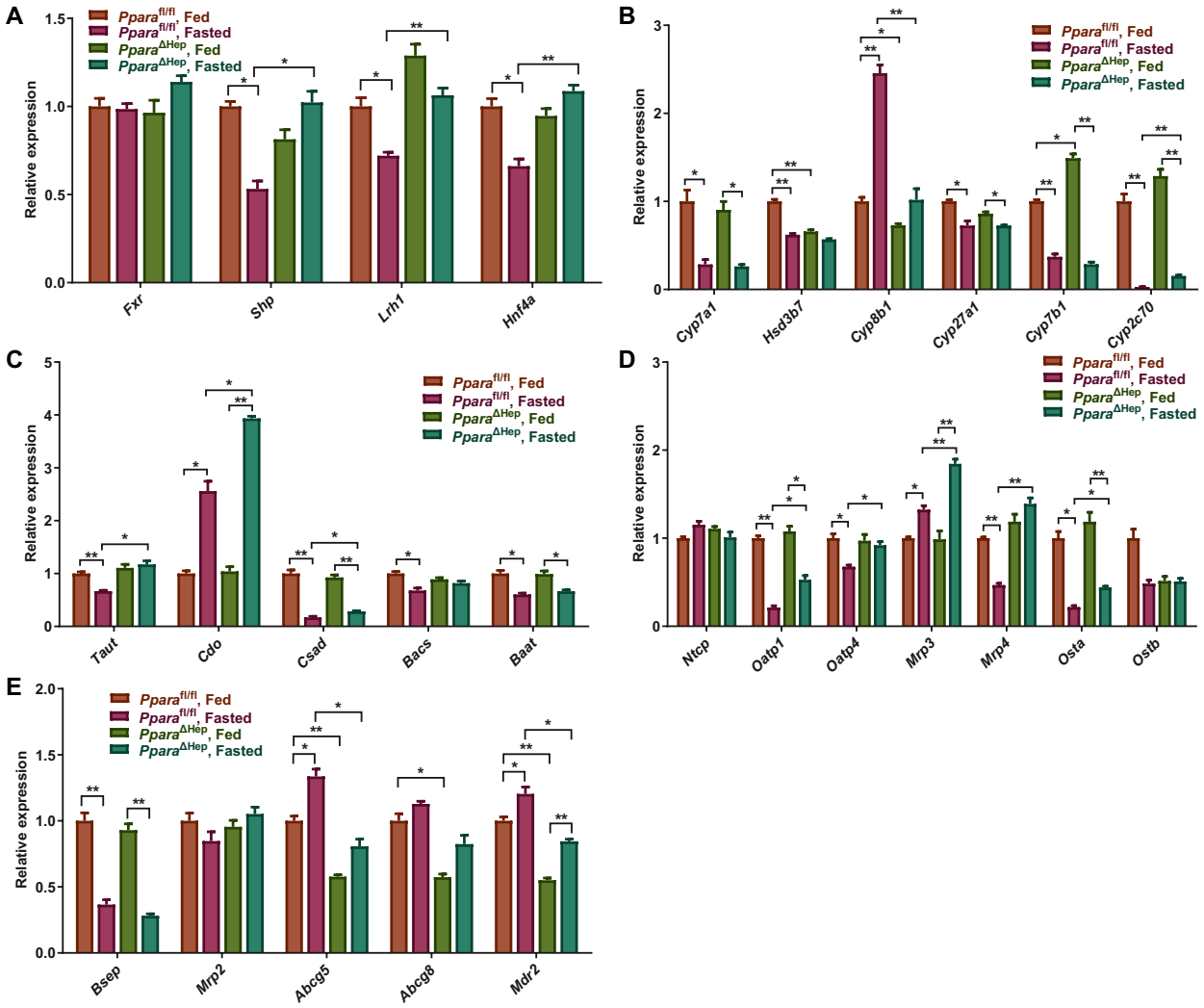


Supplementary Figure 5. The crosstalk between PPARα and FXR in primary hepatocytes. A. mRNA expression of *Ppara.* B. mRNA expression of PPARα target gene *Acox1.* C. mRNA expression of PPARα target gene *Cyp4a10.* D. mRNA expression of *Fxr.* E. mRNA expression of FXR target gene *Shp.* F. mRNA expression of FXR target gene *Bsep.* A-F. The primary hepatocytes were isolated from wild type, *Ppara*$^{-/-}$ and *Fxr*$^{-/-}$ mice treated with vehicle, CDCA, Wy, and

CDCA/Wy. Data are presented as mean ± SEM; n = 4/group. G. mRNA expression of FXR target gene *Shp* in wild type primary hepatocytes treated with vehicle, CDCA, and CDCA/Wy. H. mRNA expression of FXR target gene *Bsep* in *Ppara*$^{-/-}$ primary hepatocytes treated with vehicle, CDCA, and CDCA/Wy. Data are presented as mean ± SEM; n = 3/group. *$P < 0.05$ or **$P < 0.01$, by one-way ANOVA followed by Tukey's *post-hoc* correction.



Supplementary Figure 6. Heat map of RNA-seq analysis showed 48-hour Wy treatment alters hepatic expression profiles of genes involved in BA homeostasis. A. chow- and Wy-treated *Ppara*$^{+/+}$ mice. B. chow- and Wy-treated *Ppara*$^{-/-}$ mice. **Q* (FDR-adjusted *P* value) < 0.05 or ***Q* < 0.01, was determined by two-tailed Wilcoxon signed rank test.



Supplementary Figure 7. Fasting alters hepatic expression of genes involved in BA synthesis, conjugation, and transport. A. mRNA expression of PPARα and its target genes. B. mRNA levels of genes involved in BA synthesis. C. mRNA levels of genes involved in taurine conjugation. D. mRNA levels of genes related to the BA sinusoidal transporters. E. mRNA levels of genes related to the BA canalicular transporters. Data are presented as mean ± SEM; $n = 6$/group. *$P < 0.05$ or **$P < 0.01$, by one-way ANOVA followed by Tukey's *post-hoc* correction.

Supplementary Table 1. Primer list

Gene	Forward primer sequence (5'→3')	Reverse primer sequence (5'→3')
Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Abcg5	AGGGCCTCACATCAACAGAG	GCTGACGCTGTAGGACACAT
Abcg8	CTGTGGAATGGGACTGTACTTC	GTTGGACTGACCACTGTAGGT
Bacs	TCTATGGCCTAAAGTTCAGGCG	CTTGCCGCTCTAAAGCATCC
Baat	GGAAACCTGTTAGTTCTCAGGC	GTGGACCCCCATATAGTCTCC
Bsep	TCTGACTCAGTGATTCTTCGCA	GTGTAGAGTGAAGTCCTCCTTAGC
Cdo	GGGGACGAAGTCAACGTGG	ACCCCAGCACAGAATCATCAG
Csd	CCAGGACGTGTTTGGGATTGT	ACCAGTCTTGACACTGTAGTGA
Cyp2c70	TGGCTTTCTCAGCAGGAAGAA	AACTGGCTTGGTGTCGATGT
Cyp27a1	CTGCACTTCCTGCTGACCAAT	TGTCAGTGTGTTGGATGTCGTG
Cyp7a1	AACAACCTGCCAGTACTAGATAGC	GTGTAGAGTGAAGTCCTCCTTAGC
Cyp7b1	GGAGCCACGACCCTAGATG	TGCCAAGATAAGGAAGCCAAC
Cyp8b1	CTAGGGCCTAAAGGTTCGAGT	GTAGCCGAATAAGCTCAGGAAG
Fxr	TGGGCTCCGAATCCTCTTAGA	TGGTCCTCAAATAAGATCCTTGG
Hnf4	GGTTTAGCCGACAATGTGTGG	TCCCGCTCATTTTGGACAGC
Hsd3b7	CGCCCTGGGACTTACTACAG	CGAACAATATGTTCCCCCAG
Lrh1	TGAGGAACAACTCCGGGAAAA	CAGACACTTTATCGCCACACA
Mdr1	CCCCCGAGATTGACAGCTAC	ACTCCACTAAATTGCACATTTCCTTC
Mdr2	CTACGACCCCACAGAGGGTA	ACGGGCTCTTGACTTACCAC
Mrp2	GTGTGGATTCCCTTGGGCTTT	CACAACGAACACCTGCTTGG
Mrp3	GGGCTCCAAGTTCTGGGAC	CCGTCTTGAGCCTGGATAAC
Mrp4	AGCTTCAACGGTACTGGGATA	TCGTCGGGGTCATACTTCTC
Ntcp	CAAACCTCAGAAGGACCAAACA	GTAGGAGGATTATTCCCGTTGTG
Oapt1	ACTCCCATAATGCCCTTGG	TAATCGGGCCAACAATCTTC
Oatp4	ACCAAACTCAGCATCCAAGC	TAGCTGAATGAGAGGGCTGC
Osta	GGCATCTATGACCCAGGAGA	TGGATCCCATGTTCTGTTCA
Ostb	GACCACAGTGCAGAGAAAGC	ATTCCAAGGAGCCGCATCT
Shp	TCTGCAGGTCGTCCGACTATTC	AGGCAGTGGCTGTGAGATGC
Taut	GCACACGGCCTGAAGATGA	ATTTTTGTAGCAGAGGTACGGG

Supplementary Table 2. Summary of serum concentration and composition of grouped BAs in *Ppara*$^{fl/fl}$ and *Ppara*$^{\Delta Hep}$ mice

	Concentration, nM				Percentage, %			
	***Ppara*$^{fl/fl}$**	***Ppara*$^{fl/fl}$**	***Ppara*$^{\Delta Hep}$**	***Ppara*$^{\Delta Hep}$**	***Ppara*$^{fl/fl}$**	***Ppara*$^{fl/fl}$**	***Ppara*$^{\Delta Hep}$**	***Ppara*$^{\Delta Hep}$**
	Chow	**Wy**	**Chow**	**Wy**	**Chow**	**Wy**	**Chow**	**Wy**
Total BAs	817±122	10100±2050**	630±71	628±94##	100	100	100	100
Total conjugated BAs	206±27	2270±664*	201±66	183±17##	24.5±4.8	22.9±6.7	30.3±7.3	30.4±2.4
Total unconjugated BAs	649±99	7850±1980**	428±61	445±80##	75.5±4.8	77.1±6.7	69.7±7.3	69.6±2.4
Total primary BAs	196±30	6200±3060**	183±43	183±59.2#	32.2±4.7	60.1±4.2**	28.3±4.3	29.9±1.8##
Total secondary BAs	575±73	3920±897*	446±51	444±72##	67.8±4.7	39.9±4.2**	71.7±4.3	70.1±1.8##
Total 12α-OH BAs	664±93	7320±1340**	516±59	501±82##	81.8±1.2	80.8±1.6	82.0±0.8	79.0±1.3
Total 6-OH BAs	150±62	1240±246*	80.6±9.0	72.5±8.4##	12.6±1.3	12.7±1.9	11.5±0.6	11.9±0.8

Data are presented as mean ± SEM; n = 6/group. *P < 0.05 or **P < 0.01, versus chow-treated *Ppara*$^{fl/fl}$ group, #P < 0.05 or ##P < 0.01, versus Wy-treated *Ppara*$^{fl/fl}$ group, by one-way ANOVA followed by Tukey's *post-hoc* correction.

Supplementary Table 3. Summary of serum concentration and composition of grouped BAs in *Ppara*$^{+/+}$ and *Ppara*$^{-/-}$ mice

	Concentration, nM				Percentage, %			
	***Ppara*$^{+/+}$**	***Ppara*$^{+/+}$**	***Ppara*$^{-/-}$**	***Ppara*$^{-/-}$**	***Ppara*$^{+/+}$**	***Ppara*$^{+/+}$**	***Ppara*$^{-/-}$**	***Ppara*$^{-/-}$**
	Chow	**Wy**	**Chow**	**Wy**	**Chow**	**Wy**	**Chow**	**Wy**
Total BAs	788±100	13200±2600**	503±35*	610±74##	100	100	100	100
Total conjugated BAs	225±30	4410±1070**	138±20	165±20##	26.4±1.8	36.2±8.8	30.9±3.8	27.5±2.4
Total unconjugated BAs	579±72	8810±1860**	342±14**	445±61##	73.6±1.8	63.8±8.8	69.1±3.8	72.5±2.4
Total primary BAs	198±27	9240±2540**	165±15	173±24##	25.1±0.9	65.6±8.2**	32.7±1.4**	28.4±2.3##
Total secondary BAs	589±75	3980±694**	338±23**	437±56##	74.9±0.9	34.4±8.2**	67.3±1.4**	71.6±2.3##
Total 12α-OH BAs	662±85	11100±2100**	380±34*	511±66##	84.0±0.8	83.9±2.0	75.0±2.1**	83.5±0.9
Total 6-OH BAs	89.3±14.1	1400±317**	80.8±6.2	59.6±6.6##	11.2±0.6	11.0±1.4	16.6±2.2*	9.97±0.7

Data are presented as mean ± SEM; n = 6/group. *P < 0.05 or **P < 0.01, versus chow-treated *Ppara*$^{+/+}$ group, #P < 0.05 or ##P < 0.01, versus Wy-treated *Ppara*$^{+/+}$ group, by one-way ANOVA followed by Tukey's *post-hoc* correction.

Supplementary Table 4. Summary of hepatic concentration and composition of grouped BAs in *Ppara*$^{fl/fl}$ and *Ppara*$^{\Delta Hep}$ mice

	Concentration, nM				Percentage, %			
	***Ppara*$^{fl/fl}$**	***Ppara*$^{fl/fl}$**	***Ppara*$^{\Delta Hep}$**	***Ppara*$^{\Delta Hep}$**	***Ppara*$^{fl/fl}$**	***Ppara*$^{fl/fl}$**	***Ppara*$^{\Delta Hep}$**	***Ppara*$^{\Delta Hep}$**
	Chow	**Wy**	**Chow**	**Wy**	**Chow**	**Wy**	**Chow**	**Wy**
Total BAs	78.1±7.6	72.6±10.5	83.9±13.0	127±16.7#	100	100	100	100
Total conjugated BAs	71.6±7.4	69.2±10.3	76.3±11.3	115±14.2#	91.4±1.1	94.8±1.1	91.7±2.5	85.7±2.1##
Total unconjugated BAs	6.52±0.79	4.18±0.94	7.59±3.06	18.3±4.5#	8.56±1.1	5.23±21.1	8.31±2.5	14.2±2.1##
Total primary BAs	65.1±6.7	62.8±9.2	68.0±10.6	107±13.4#	83.2±1.0	86.7±2.3	81.0±2.5	78.9±1.4#
Total secondary BAs	13.0±1.1	10.5±1.9	15.9±3.5	26.4±4.0##	16.8±1.0	13.3±2.3	19.0±2.5	21.1±1.4#
Total 12α-OH BAs	49.7±4.4	50.1±6.7	48.8±6.1	78.7±11.3	64.0±1.5	70.6±2.5*	60.2±3.4	57.0±2.5##
Total 6-OH BAs	24.3±3.3	15.5±3.7	30.0±7.0	47.1±6.1##	30.5±1.8	17.8±1.4**	33.5±3.7	37.7±2.6##

Data are presented as mean ± SEM; n = 6/group. *P < 0.05 or **P < 0.01, versus chow-treated *Ppara*$^{fl/fl}$ group, #P < 0.05 or ##P < 0.01, versus Wy-treated *Ppara*$^{fl/fl}$ group, by one-way ANOVA followed by Tukey's *post-hoc* correction.

Supplementary Table 5. Summary of hepatic concentration and composition of grouped BAs in *Ppara*$^{+/+}$ and *Ppara*$^{-/-}$ mice

	Concentration, nM				Percentage, %			
	***Ppara*$^{+/+}$**	***Ppara*$^{+/+}$**	***Ppara*$^{-/-}$**	***Ppara*$^{-/-}$**	***Ppara*$^{+/+}$**	***Ppara*$^{+/+}$**	***Ppara*$^{-/-}$**	***Ppara*$^{-/-}$**
	Chow	**Wy**	**Chow**	**Wy**	**Chow**	**Wy**	**Chow**	**Wy**
Total BAs	74.0±9.5	81.3±9.0	61.8±5.0	134±21#	100	100	100	100
Total conjugated BAs	64.3±10.4	76.4±8.8	57.3±5.3	120±15#	95.8±0.9	93.9±0.7	92.7±1.7	88.0±1.0$^{\#\#}$
Total unconjugated BAs	2.88±0.87	4.84±0.59	4.54±0.75	16.7±3.4##	4.18±0.93	6.13±0.7	7.30±1.70	12.0±1.0$^{\#\#}$
Total primary BAs	63.3±7.99	71.9±9.0	52.7±4.9	120±13#	85.8±1.2	87.9±2.0	85.0±1.6	82.1±1.4$^{\#}$
Total secondary BAs	10.7±1.8	9.33±1.14	9.12±0.72	25.1±5.2#	14.2±1.2	12.1±2.0	15.0±1.6	17.9±1.4$^{\#}$
Total 12α-OH BAs	41.3±6.1	59.1±6.7	34.9±3.5	88.1±10.4#	64.9±1.3	73.0±2.4*	57.2±2.3*	59.7±1.3$^{\#\#}$
Total 6-OH BAs	20.2±3.4	15.6±2.2	22.6±2.2	48.4±8.2##	29.8±1.2	18.9±1.2**	35.8±2.3*	35.5±1.3$^{\#\#}$

Data are presented as mean ± SEM; n = 6/group. *P < 0.05 or **P < 0.01, versus chow-treated *Ppara*$^{+/+}$ group, $^{\#}P$ < 0.05 or $^{\#\#}P$ < 0.01, versus Wy-treated *Ppara*$^{+/+}$ group, by one-way ANOVA followed by Tukey's *post-hoc* correction.