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Supplemental information

Integrative systems analysis identifies genetic and dietary modulators of bile acid homeostasis

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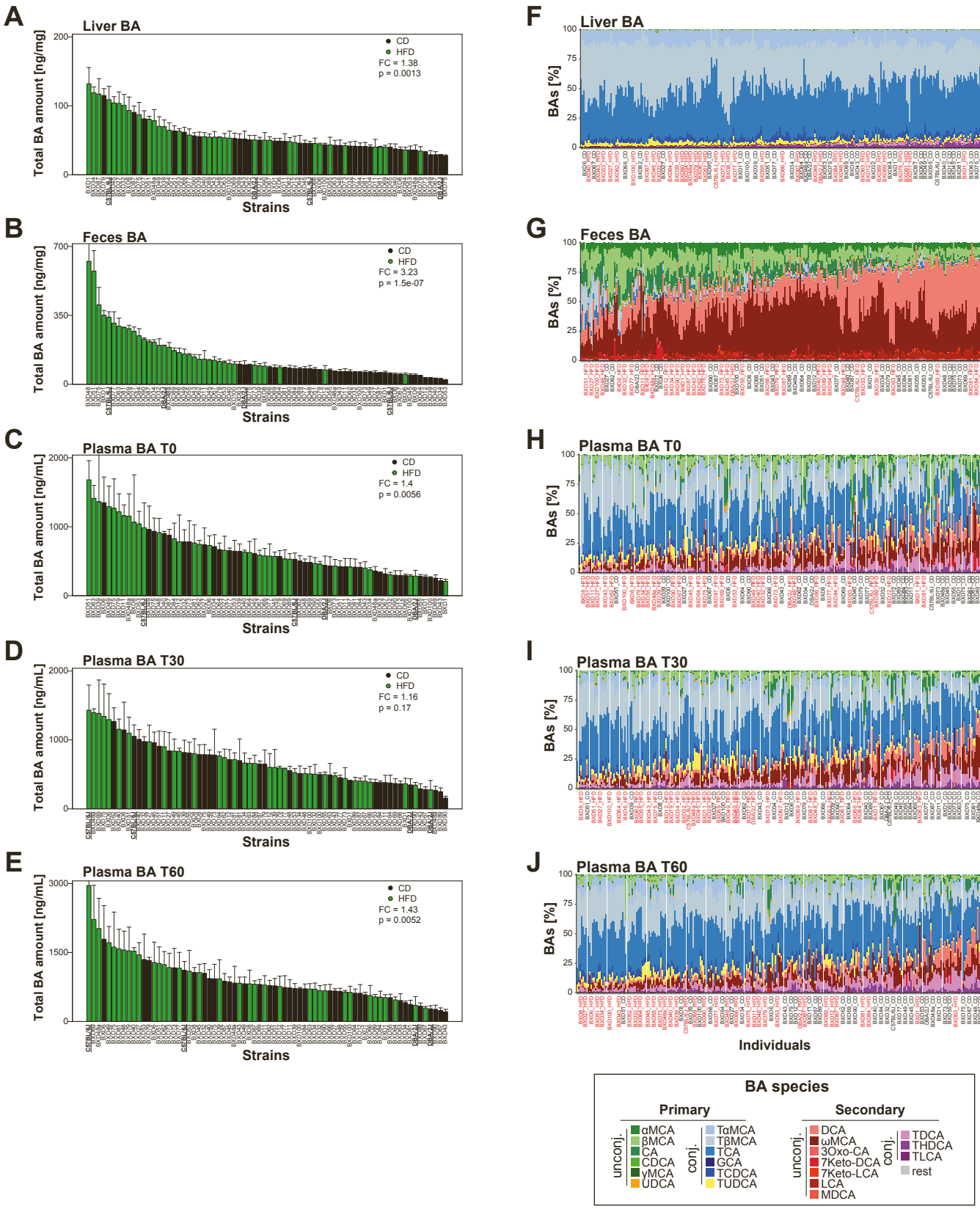

Figure S1. Related to Figure 2. Diversity of the BA composition and amount across strains and diets. (**A-E**) Barplots showing the BA amount in liver (**A**), feces (**B**), and plasma (**C-E**) of each BXD strain fed with either CD or HFD. Strains are ordered by the BA amount. Barplots are expressed as mean + SEM. P-values comparing strains fed with different diets were calculated using paired samples t-tests. Parental strains (C57BL/6J and DBA/2J) are indicated in bold and underlined. (**F-J**) Stacked barplots showing the BA composition (% of the BA amount in the various compartments at a given time) in liver, feces, and plasma (before and 30 or 60 minutes after gavage of a test meal) of each individual animal from different BXD strains. Animals were grouped by strain and diet, and ranked ordered by strain average of the proportion of total primary BAs. Strains fed a HFD are depicted in red. BA species are categorized and colored to highlight primary unconjugated (green and UDCA in orange), primary conjugated (blue and TUDCA in yellow), secondary unconjugated (red), and secondary conjugated (purple) BAs. The BA abbreviations are included in Table S1.

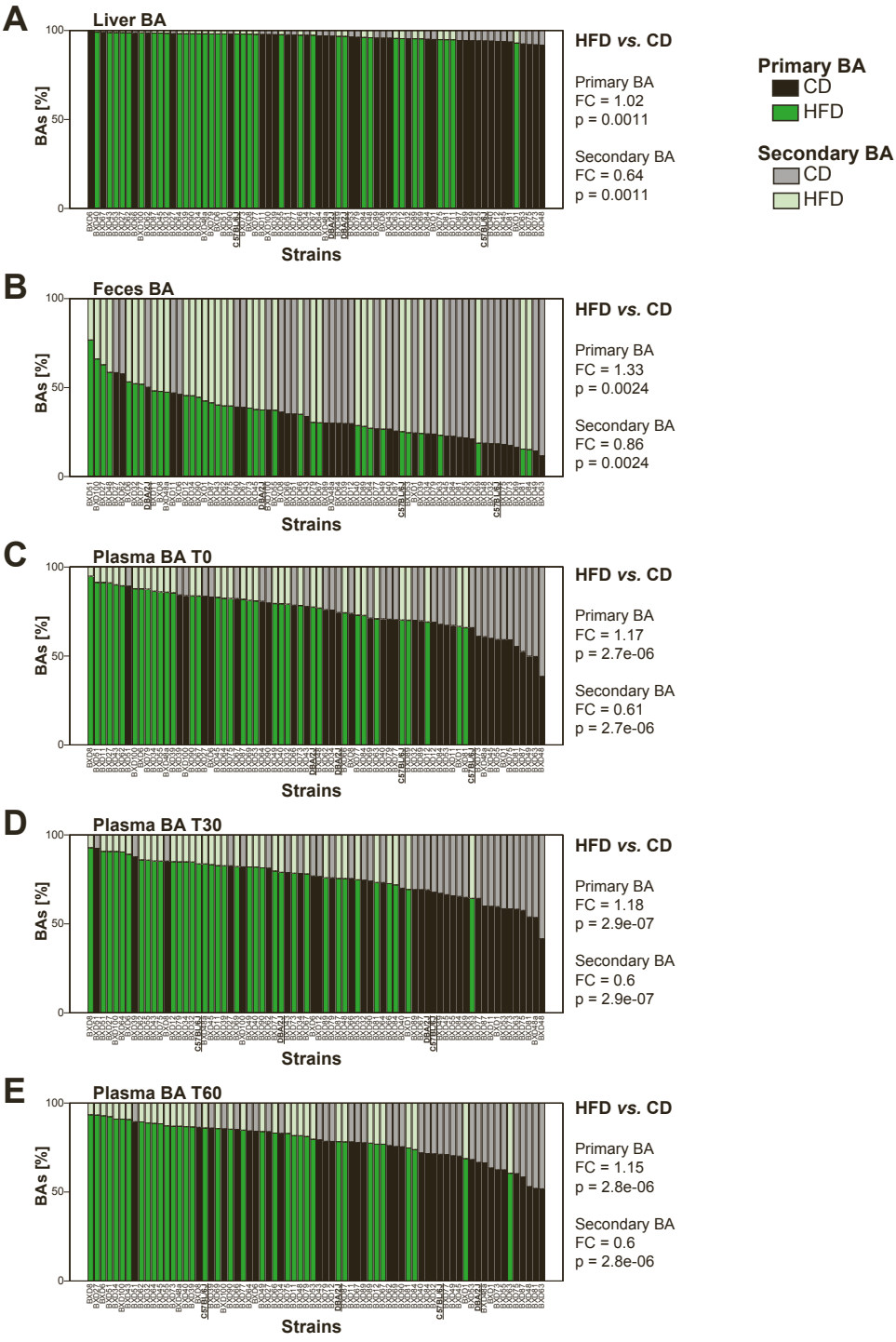

Figure S2. Related to Figure 2. The effects of diet on BA levels are influenced by the genetic background of the animals, the BA species, and the biological compartment. Barplots showing the percentage of primary (dark colors) and secondary (light colors) BAs in liver (**A**), feces (**B**), and plasma (**C-E**) of each BXD strain fed with either CD or HFD. Strains are ordered by the percentage of primary BA abundance over the total BA amount in the indicated biological compartment. Barplots are expressed as the averages of the animals in each strain. Fold changes (FC) were calculated between animals fed with HFD against those fed with CD. P-values comparing strains fed with different diets were calculated using paired samples t-tests.

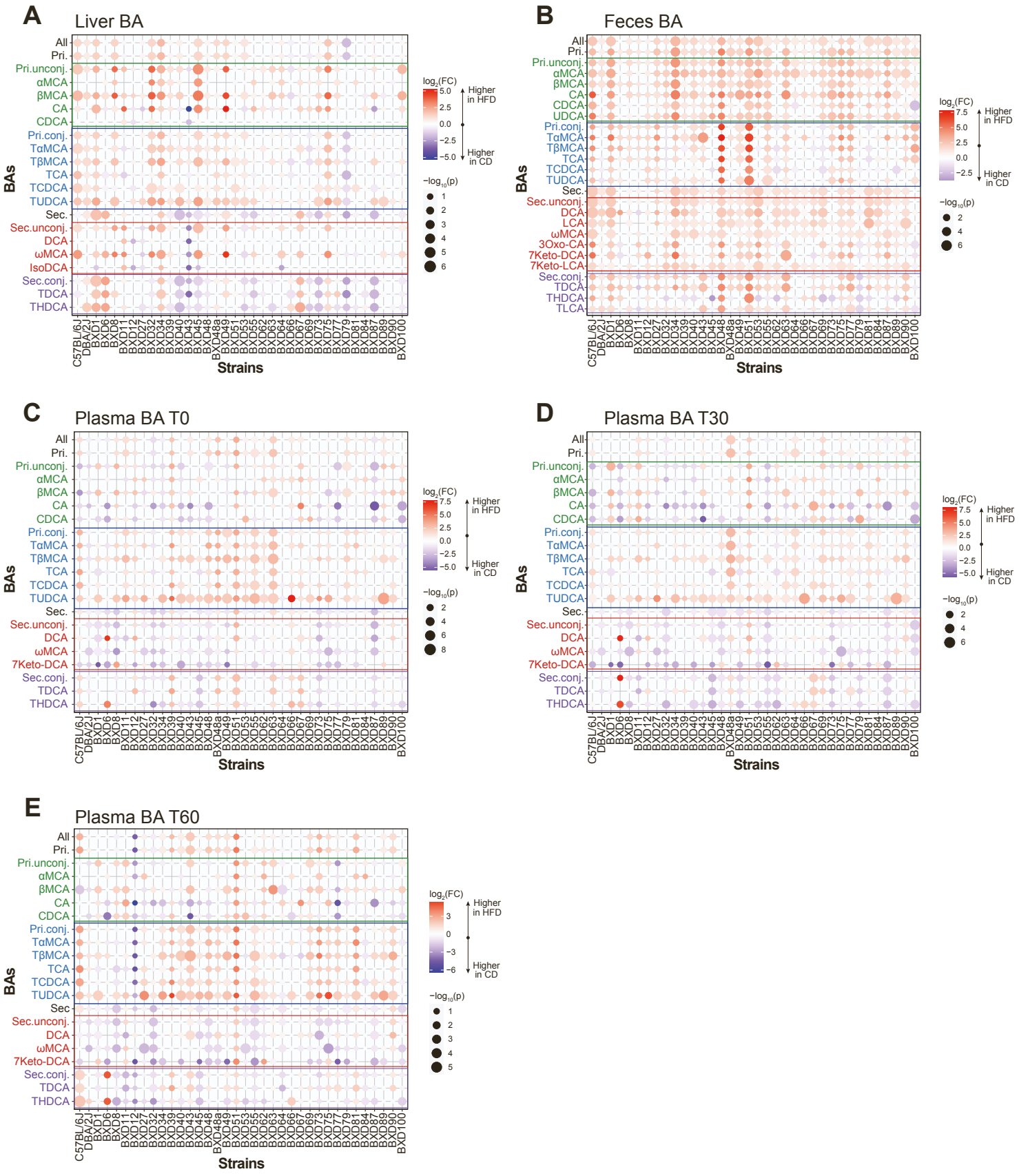

Figure S3. Related to Figure 2. The effects of diet on BA levels are influenced by the genetic background of the animals, the BA species, and the biological compartment. (**A-E**) Dot plots showing the BA level changes after HFD challenge in the liver (**A**), feces (**B**), and plasma (**C-E**) of each BXD strain. The dot color represents the fold change (FC) between the indicated BA levels in HFD versus CD, with red indicating higher in HFD and blue indicating higher in CD, and dot size refers to the significance of the changes. P-value was calculated using a two-tailed Student's t-test.

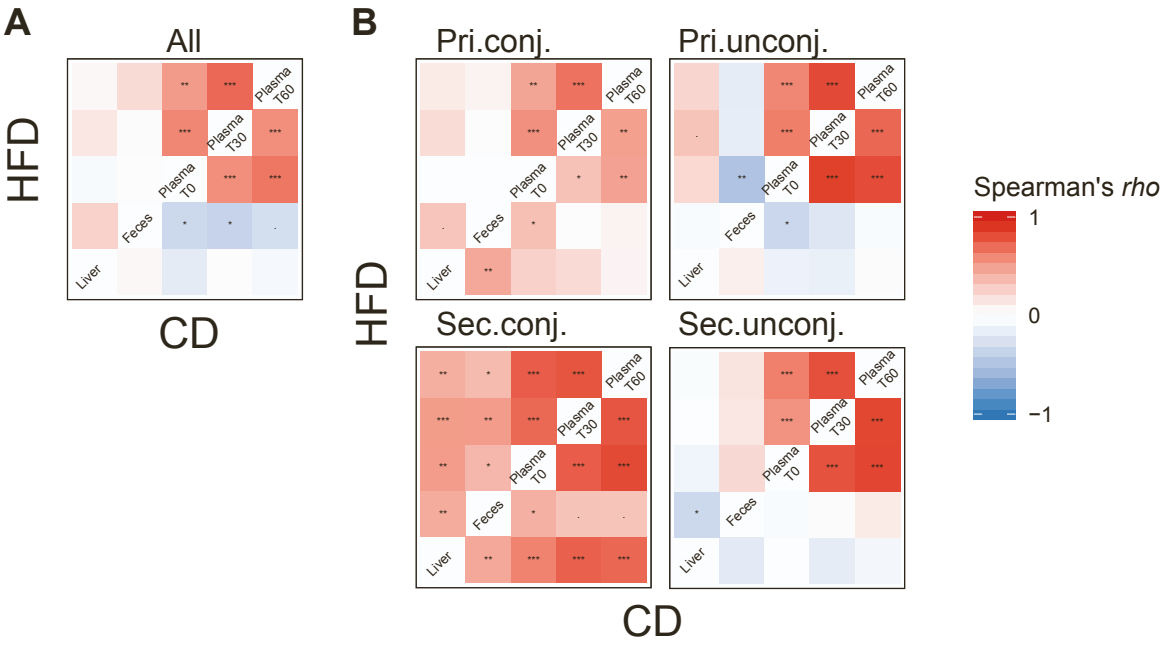

Figure S4. Related to Figure 2. Correlations between BA levels across different biological compartments and diets. Heatmap showing the correlations of the total BA amount (All) (**A**) or the sum of respective BA categories (**B**) between different compartments. Bottom right triangle of each heatmap is data from CD and top left triangle is data from HFD. Pri.conj., primary conjugated; Pri.unconj., primary unconjugated; Sec.conj., secondary conjugated; Sec.unconj., secondary unconjugated.

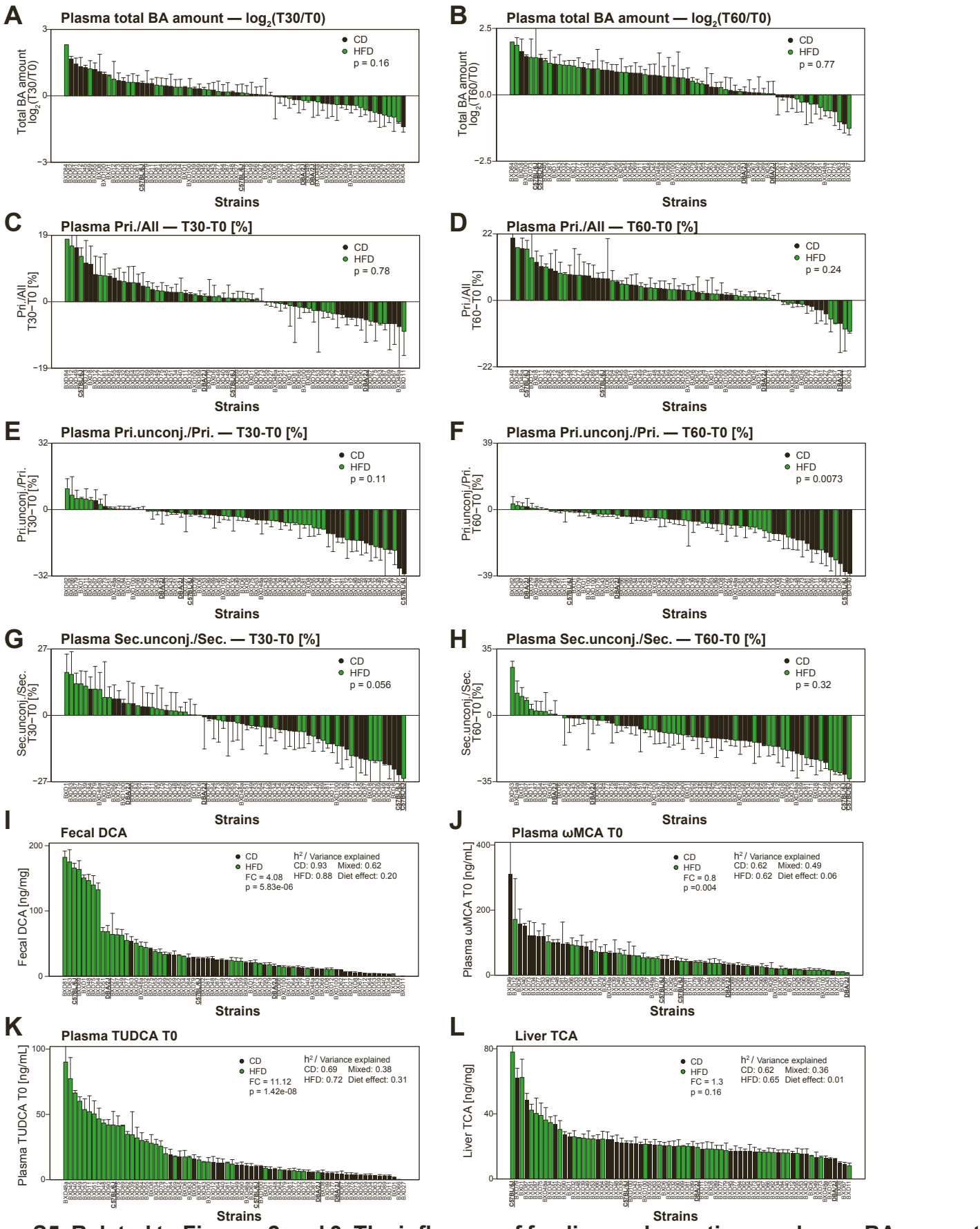

Figure S5. Related to Figures 2 and 3. The influence of feeding and genetics on plasma BA amount and conjugation. (**A-B**) The changes of plasma BA amount at 30 min (T30, **A**) or 60 min (T60, **B**) after test meal gavage compared to fasting levels (T0). (**C-D**) The changes of the ratio of plasma primary BAs to total BAs (Pri./All) at 30 min (T30, **C**) or 60 min (T60, **D**) after test meal gavage compared to fasting levels (T0). (**E-H**) The changes of the ratio of primary unconjugated BAs to primary BAs (Pri.unconj./Pri.) (**E-F**) or the ratio of secondary unconjugated BAs to secondary BAs (Sec.unconj./Sec.) (**G-H**) at 30 min (T30, **E**,**G**) or 60 min (T60, **F**,**H**) after test meal gavage compared to fasting levels (T0). (**I-L**) Barplots showing the levels of the same BAs (fecal DCA, plasma ωMCA T0, plasma TUDCA T0, hepatic TCA) represented in Figure 3B for each BXD strain. Strains are ordered by the respective BA levels. Barplots are expressed as mean + SEM. P-values were calculated using paired samples t-tests between CD and HFD. Fold change (FC) were calculated based on the average of BA changes in HFD compared to CD.

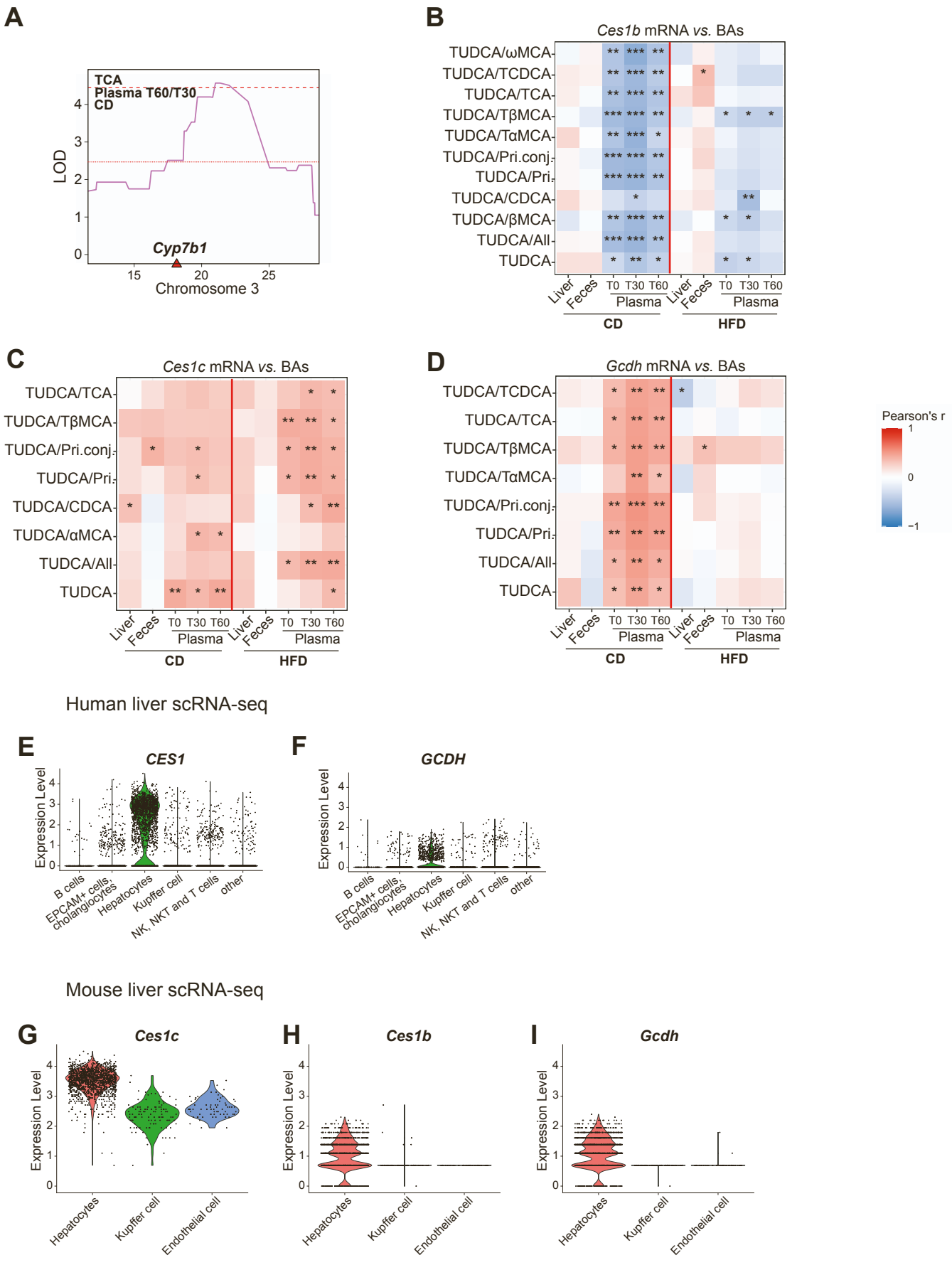

Figure S6. Related to Figures 5 and 6. Additional analysis of known and potential BA modulators. (**A**) Genetic position of the BA synthesis enzyme *Cyp7b1* on Chromosome 3 under the TCA QTL in plasma T60/plasma T30 in CD. (**B-D**) Heatmap representing the correlation between *Ces1b* (**B**), *Ces1c* (**C**), and *Gcdh* (**D**) hepatic mRNA expression and TUDCA levels in the indicated biological compartments. *p<0.05, **p<0.01, ***p<0.001. (**E-I**) Liver single-cell RNA-seq (scRNA-seq) data revealing the specific expression of *Ces1b*, *Ces1c* and *Gcdh* in hepatocytes. (**E-F**) Violin plots show the expression of for CES1 (**E**) and GCDH (**F**) across different cell types in human liver. (**G-I**) The expression of *Ces1c* (**G**), *Ces1b* (**H**), and *Gcdh* (**I**) across different cell types in mouse liver using violin plots.

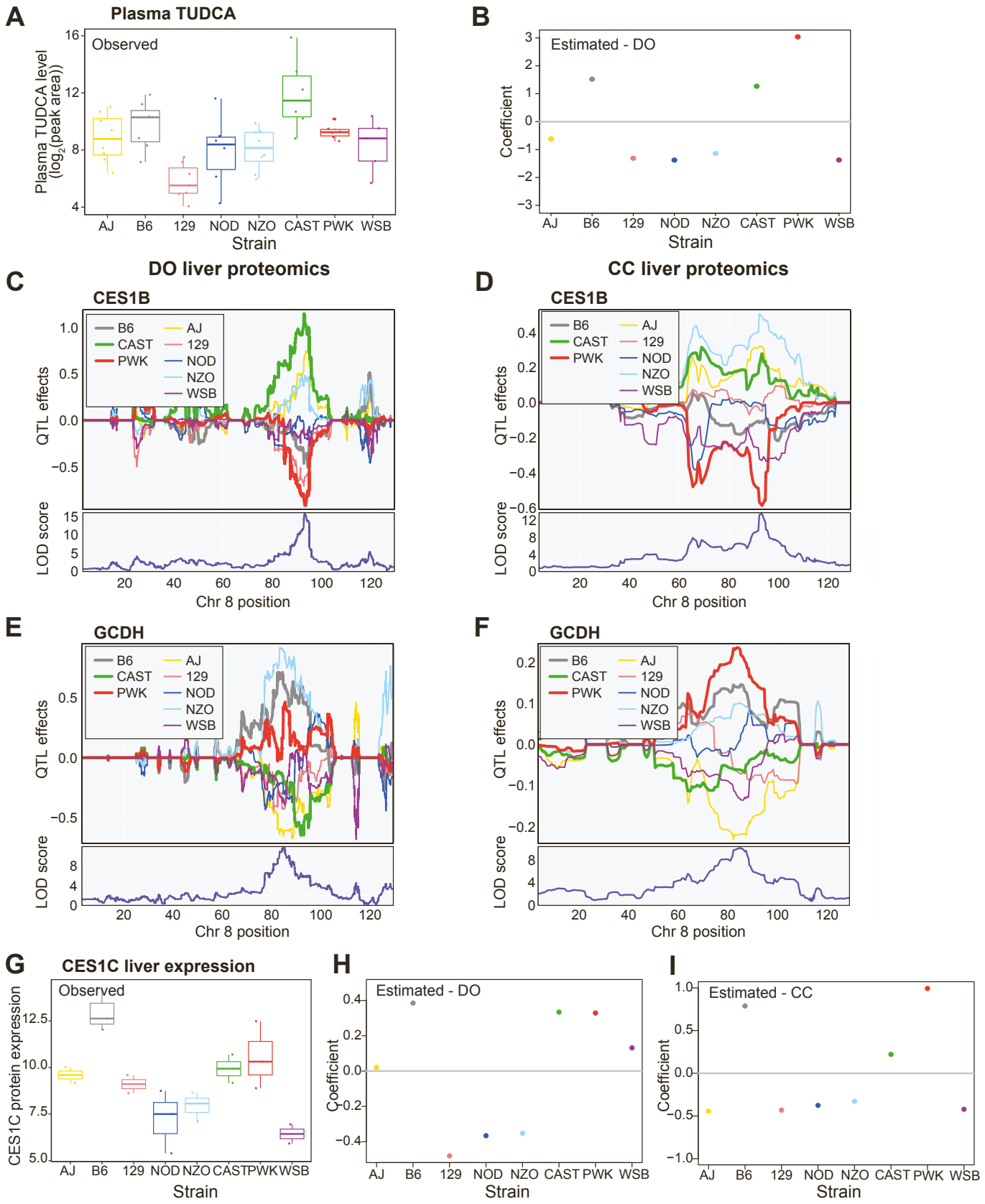

Figure S7. Related to Figure 7. Genetic effects of the QTL on plasma TUDCA levels in the DO animals and CC cohorts. (**A**) TUDCA levels (log_2 data) measured in the fasting plasma samples from male mice of the founder strains at 22 weeks of age. (**B**) Estimated coefficients of the founder strains haplotypes on plasma TUDCA levels for the TUDCA QTL on chromosome 8 in the DO mice, revealing the genetic effects of genotype alleles from each founder strain. (**C-I**) Haplotype effects and QTL results of CES1B (**C-D**) or GCDH (**E-F**) liver protein levels in DO (**C** and **E**) and CC (**D** and **F**) cohorts. For each plot, the x-axis indicates the genetic position on chromosome (Chr) 8. The y-axis for the top panel is the haplotype effects for the 8 founder strains, and the y-axis in the bottom panel is the LOD score. (**G**) CES1C liver protein levels measured from male mice of the founder strains at 26 weeks of age. (**H-I**) Estimated coefficients of the founder strains haplotypes on CES1C liver protein levels for the CES1C liver cis-pQTL on chromosome 8 in the DO (**H**) mice of 26 weeks of age and CC (**I**) mice of 8 weeks of age.

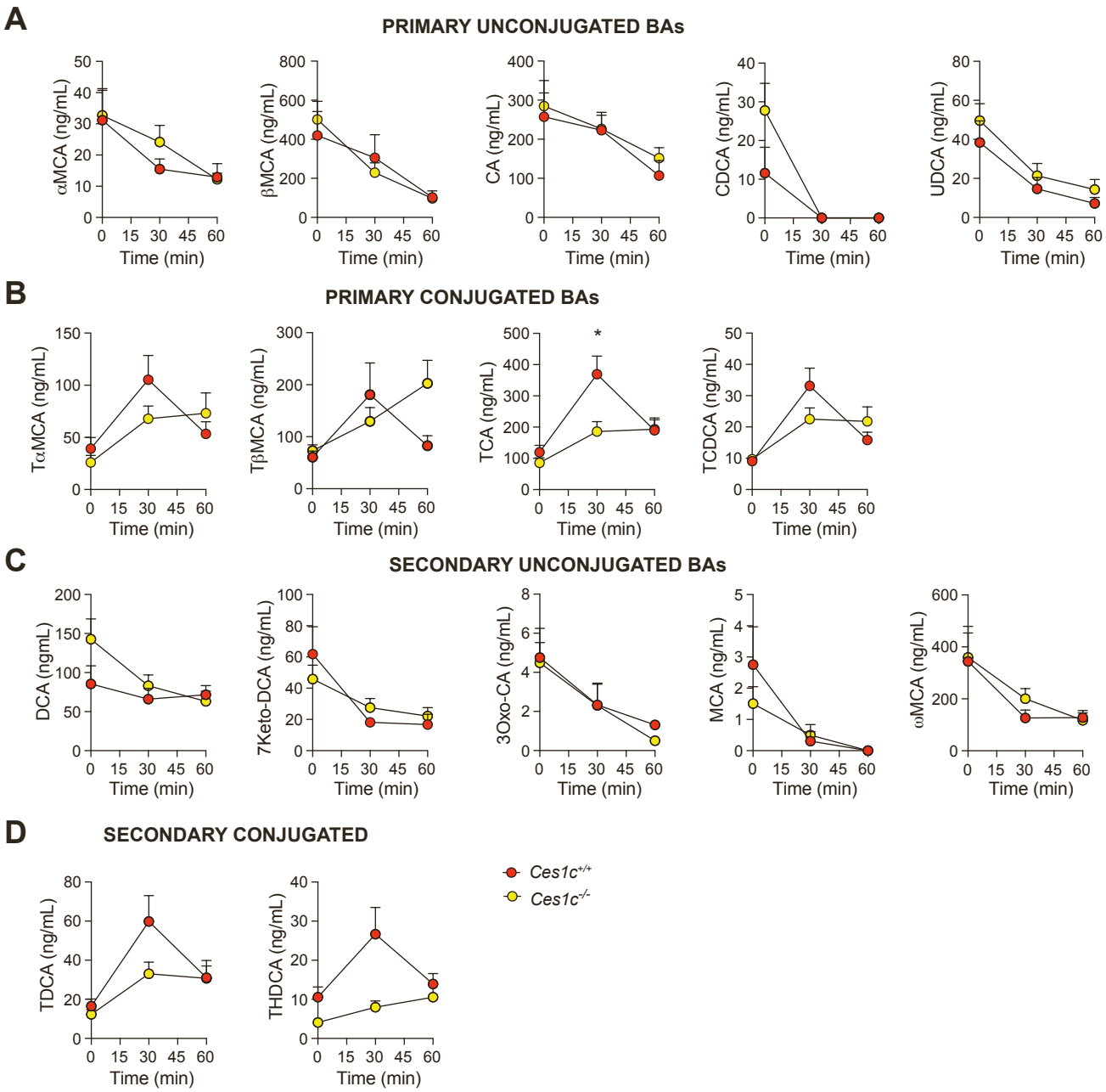

Figure S8. Related to Figure 7. Plasma BA levels in *Ces1c* wild-type and knock-out mice. (**A-D**) Primary unconjugated (**A**), primary conjugated (**B**), secondary unconjugated (**C**), secondary conjugated (**D**) BA levels in plasma in *Ces1c*$^{+/+}$ (n=9) and *Ces1c*$^{-/-}$ (n=11) male mice (20-week-old, fed CD – following the timeline in Figure 1A) before and 30 or 60 minutes after oral administration of a test meal. *p<0.05 vs. *Ces1c*$^{+/+}$ by two-way ANOVA followed by Bonferroni post-hoc test. Results are expressed as mean +SEM.

Table S1. List of the bile acids measured, their abbreviations, accurate m/z ratio, corresponding internal standards (used for quantification) and retention times (RT). Chromatographic resolution was essential for the separation of isobaric species.

Bile Acid	Abbreviation	H^- (m/z)	Internal Standard	RT min
Lithocholic acid	LCA	375.29047	d_4-LCA	20.6
7-Ketolithocholic acid	7Keto-LCA	389.26973	d_4-GDCA	15.5
Murocholic acid	MDCA	391.28538	d_4-CA	12.4
Ursodeoxycholic acid	UDCA	391.28538	d_4-GDCA	13.3
Chenodeoxycholic acid	CDCA	391.28538	d_4-CDCA	18.3
Deoxycholic acid	DCA	391.28538	d_4-DCA	18.5
Isodeoxycholic acid	isoDCA	391.28538	d_4-GDCA	19.7
3-Oxocholic acid	3Oxo-CA	405.26465	d_4-TCDCA	12.6
7-Ketodeoxycholic acid	7Keto-DCA	405.26465	d_4-GCA	11.0
α-Muricholic acid	α-MCA	407.28030	d_4-GCA	10.8
β-Muricholic acid	β-MCA	407.28030	d_4-GCA	11.2
ω-Muricholic acid	ω-MCA	407.28030	d_4-GCA	10.4
γ-Muricholic acid	MCA	407.28030	d_4-GCA	11.8
Cholic acid	CA	407.28030	d_4-CA	12.6
Glycolithocholic acid	GLCA	432.31193	d_4-GDCA	13.5
Glycoursodeoxycholic acid	GUDCA	448.30685	d_4-GCA	10.2
Glycohyodeoxycholic acid	GHDCA	448.30685	d_4-CA	10.6
Glycochenodeoxycholic acid	GCDCA	448.30685	d_4-GCDCA	12.9
Glycocholic acid	GCA	464.30176	d_4-GCA	10.3
Taurolithocholic acid	TLCA	482.29457	d_5-TLCA	13.0
Tauroursodeoxycholic acid	TUDCA	498.28948	d_4-TCA	5.1
Taurohyodeoxycholic acid	THDCA	498.28948	d_4-TCDCA	5.5
Taurochenodeoxycholic acid	TCDCA	498.28948	d_4-TCDCA	10.6
Taurodeoxycholic acid	TDCA	498.28948	d_5-TDCA	10.9
Tauro-α-muricholic acid	TαMCA	514.28440	d_4-TαMCA	2.1
Tauro-β-muricholic acid	TβMCA	514.28440	d_4-TβMCA	2.3
Taurocholic acid	TCA	514.28440	d_4-TCA	5.8