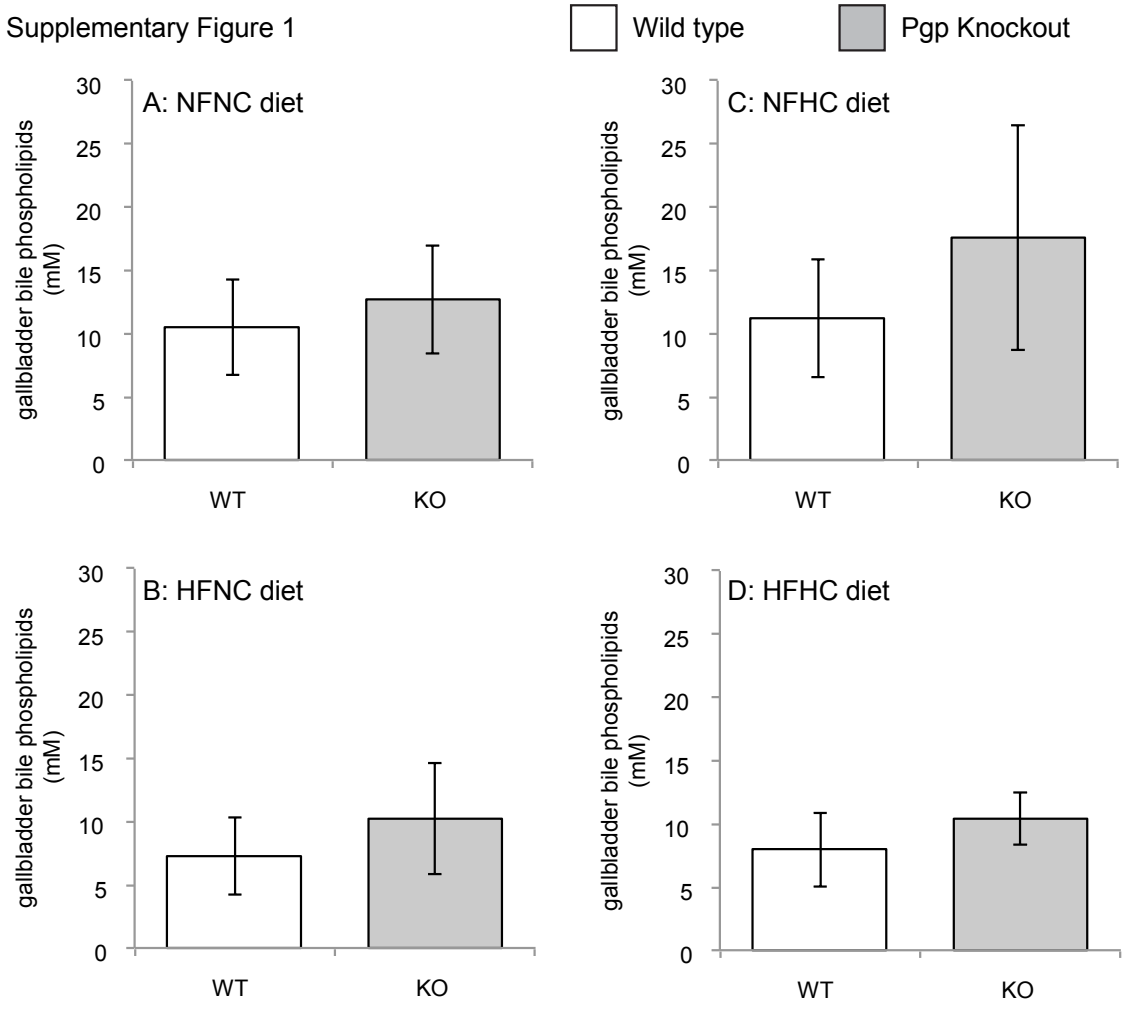
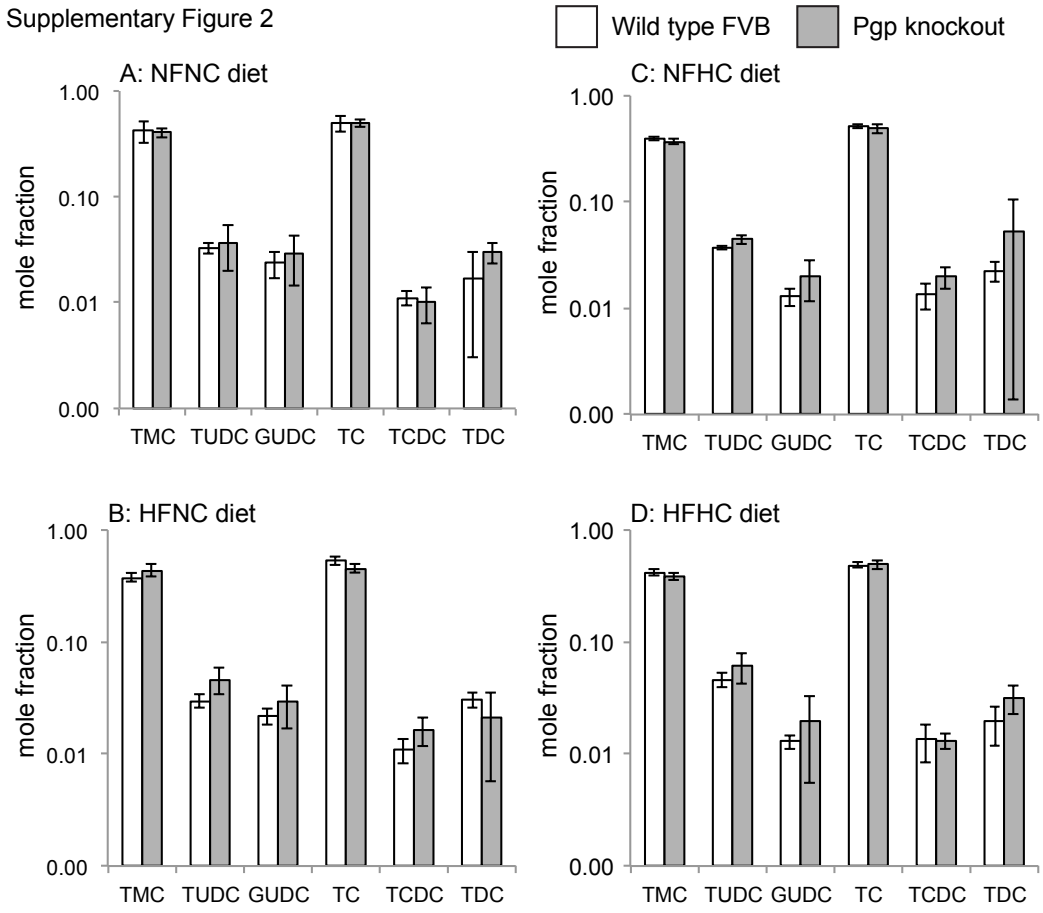
SUPPLEMENTARY INFORMATION

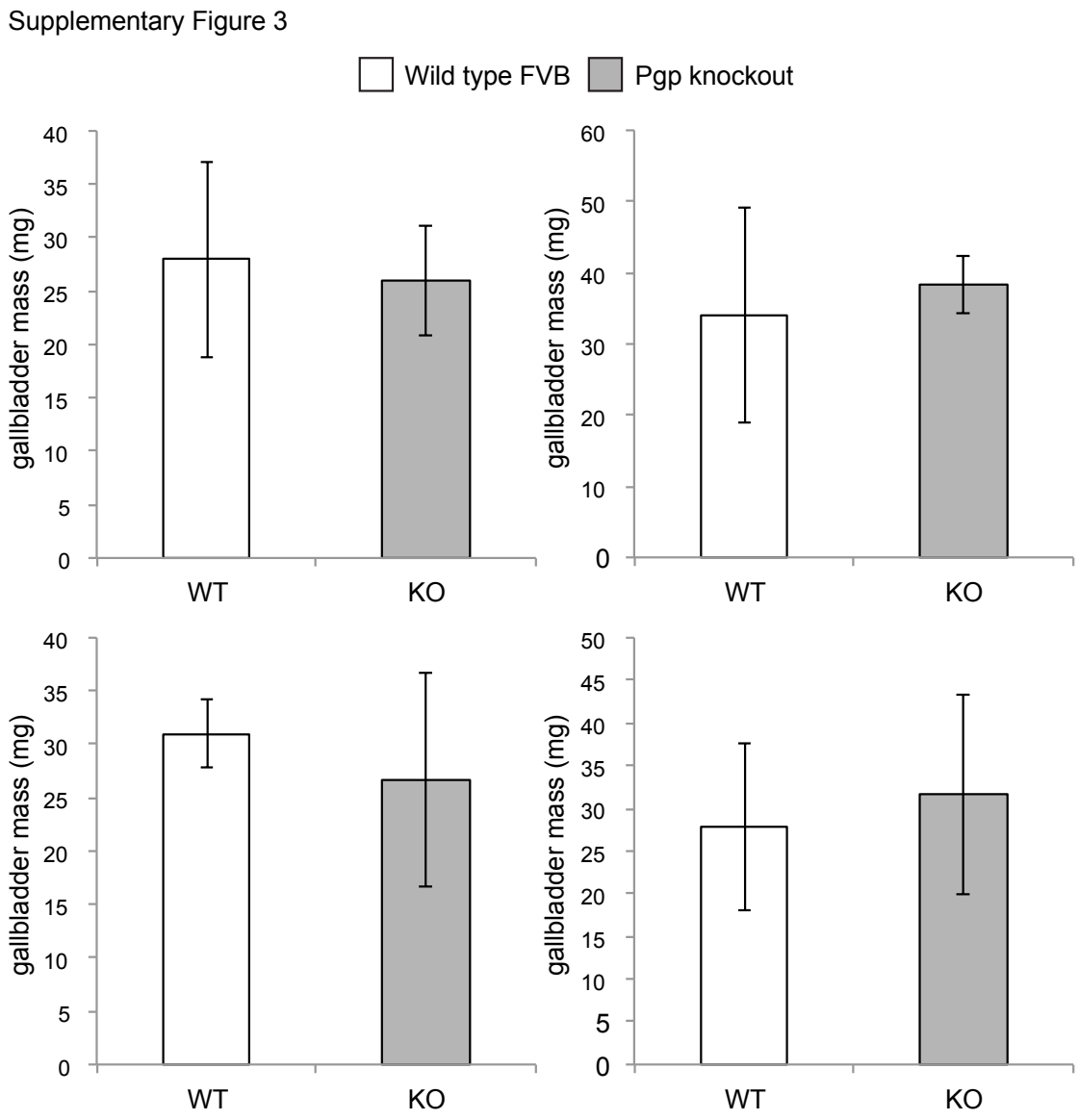
Supplementary Figure 1: Gallbladder phospholipid concentrations in wild type and knockout mice on each of the four test diets. The gallbladder phospholipid content was determined for each mouse after a 60min ligation of the common bile duct. The mean values are shown ± SD for each of the diets: NFNC (25%kcal fat and 0.02% w/w cholesterol) HFNC (45%kcal fat and 0.02% w/w cholesterol), NFHC (25%kcal fat and 0.20% w/w cholesterol), HFHC (45%kcal fat and 0.20% w/w cholesterol). No differences ($p>0.05$ by unpaired t-test) were seen between the wild type and *Abcb1a*$^{-/-}$/*1b*$^{-/-}$ Pgp knockout mice, n=6-10.



Supplementary Figure 2: Effect of diet and Pgp deletion on the bile salt composition of gallbladder bile. Individual bile acids in the gallbladder were quantified by HPLC and the mean mole fractions of the individual bile acid species are shown on a log scale ± SD for Wild Type FVB mice (WT: hollow bars) and *Abcb1a*$^{-/-}$/*1b*$^{-/-}$ Pgp knockout mice (KO: grey bars); n=5, except for HFNC knockout mice where n=4. TMC: Tauromuricholate, TUDC: Tauroursodeoxycholate, GUDC: Glycoursodeoxycholate, TC: Taurocholate, TCDC: Taurochenodeoxycholate, TDC: Taurodeoxycholate. **(**A**)** NFNC diet, 25%kcal fat and 0.02% (w/w) cholesterol; **(**B**)** HFNC diet, 45%kcal fat and 0.02% (w/w) cholesterol; **(**C**)** NFHC diet, 25%kcal fat and 0.20% (w/w) cholesterol; **(**D**)** HFHC diet, 45%kcal fat and 0.20% (w/w) cholesterol.



Supplementary Figure 3: Effects of Pgp deletion on gallbladder mass. The potential effect of Pgp deletion on gallbladder size was tested by measuring the mass of each gallbladder after a 60min ligation of the common bile duct. The mean values are shown ± SD. No statistically significant differences were seen between wild type and *Abcb1a*$^{-/-}$/*1b*$^{-/-}$ Pgp knockout mice; n=6-10.



Supplementary Table 1: Accumulation of cholesterol in the livers of wild type and *Abcb1a*$^{-/-}$*/1b*$^{-/-}$ knockout mice.

Group	n	Hepatic cholesterol content (mg/g)			Ratio of free to total cholesterol		
		mean	±	SD	mean	±	SD
WT-NFNC	9	7.10	±	1.06	0.86	±	0.04
WT-HFNC	10	7.24	±	0.75	0.85	±	0.04
WT-NFHC	10	**#13.40**	±	2.58	**#0.73**	±	0.07
WT-HFHC	10	**#9.31**	±	1.29	**#0.78**	±	0.04
KO-NFNC	10	6.96	±	1.39	0.87	±	0.05
KO-HFNC	9	6.49	±	1.09	0.89	±	0.02
KO-NFHC	10	***#10.84**	±	1.63	**#0.72**	±	0.06
KO-HFHC	10	**#9.20**	±	1.04	**#0.74**	±	0.06

The cholesterol content in total lipid extracts obtained from liver samples was measured using cholesterol oxidase-based assays. The mean total cholesterol content per gram of liver is shown along with the ratio of unesterified cholesterol to total cholesterol ± SD. Samples were analyzed from wild type FVB mice (WT) and Pgp knockout mice (KO) fed each of the test diets: NFNC diet (25%kcal fat and 0.02% w/w cholesterol) HFNC diet (45%kcal fat and 0.02% w/w cholesterol), NFHC diet (25%kcal fat and 0.20% w/w cholesterol), HFHC diet (45%kcal fat and 0.20% w/w cholesterol). #$p<0.05$ vs. NFNC diet by two way ANOVA with Tukey *post-hoc* tests. *$p<0.05$ vs. WT by two-way ANOVA with Tukey *post-hoc* tests.

Supplementary Table 2: Accumulation of phospholipid and triacylglycerol in the livers of wild type FVB and *Abcb1a*$^{-/-}$/*1b*$^{-/-}$ knockout mice.

Group	Hepatic phospholipid content (mg/g)				Hepatic triacylglycerol content (mg/g)			
	n	mean	±	SD	n	mean	±	SD
WT-NFNC	10	6.87	±	2.93	10	5.59	±	3.39
WT-HFNC	10	9.07	±	1.52	9	10.22	±	2.61
WT-NFHC	10	7.12	±	0.86	10	9.58	±	3.68
WT-HFHC	10	7.54	±	3.63	10	14.75	±	5.96
KO-NFNC	8	14.49	±	1.35	10	18.08	±	7.69
KO-HFNC	10	16.36	±	3.12	10	34.62	±	8.72
KO-NFHC	10	5.09	±	0.80	10	15.99	±	5.72
KO-HFHC	10	4.70	±	0.89	10	17.67	±	3.90

Total lipids were extracted from frozen liver samples taken from wild type FVB and Abcb1a$^{-/-}$/1b$^{-/-}$ knockout mice using the Folch method. The triacylglycerol and phospholipid contents in the resulting lipid films were measured using enzymatic assays. The mean mass of lipid per mg of liver are shown ± SD. Samples were analyzed from wild type FVB mice (WT) and Pgp knockout mice (KO) fed each of the test diets: NFNC diet (25%kcal fat and 0.02% w/w cholesterol) HFNC diet (45%kcal fat and 0.02% w/w cholesterol), NFHC diet (25%kcal fat and 0.20% w/w cholesterol), HFHC diet (45%kcal fat and 0.20% w/w cholesterol). There were no statistically significant differences by two-way ANOVA with Tukey *post-hoc* tests.

Supplementary Table 3: Accumulation of cholesterol in the jejunum of wild type and *Abcb1a*$^{-/-}$/*1b*$^{-/-}$ knockout mice fed the NFNC diet.

Group	n	Jejunum cholesterol content (mg/g)			Ratio of free to total cholesterol		
		mean		SD	mean		SD
WT	9	3.88	±	1.16	0.93	±	0.026
KO	9	3.76	±	0.79	0.92	±	0.022

Lipids were extracted from frozen jejunum samples taken from wild type FVB mice (WT) and *Abcb1a*$^{-/-}$/*1b*$^{-/-}$ knockout mice (KO) using the Folch method. The cholesterol content in the resulting lipid films was determined using the Amplex Red cholesterol assay and is represented as the mean cholesterol content per gram of tissue ± SD.

Supplementary Table 4: Summary of the TaqMan gene expression assays used.

Gene	RefSeq	TaqMan Assay ID	Probe Sequence	Exon-Exon Boundary
Abca1	NM_013454.3	Mm01350760_m1	AGGAGACAAACATGTCAGCTGTTAC	2-3
Abcb1a	NM_011076.2	Mm00440761_m1	TCAAGTGAAAGGGGCTACAGGGTCT	20-21
Abcb4	NM_008830.2	Mm00435630_m1	GCAGCATCAGCAACCAAGGCAGAGA	2-3
Abcb11	NM_021022.3	Mm00445168_m1	GCTATGTTTTCAGGGTGGTCTCTTC	23-24
Abcg5	NM_031884.1	Mm00446249_m1	TGTGTGTTATTGGACTCTGGGCTTG	10-11
Abcg8	NM_026180.2	Mm00445980_m1	TGGATAGTGCCTGCATGGATCTCCA	11-12
Actb	NM_007393.3	Mm00607939_s1	ACTGAGCTGCGTTTTACACCCTTTC	
Cyp7a1	NM_007824.2	Mm00484152_m1	ACAACCTGCCAGTACTAGATAGCAT	4-5
Hmgcr	NM_008255.2	Mm01282499_m1	CCTGCCTGCAGATGCTAGGTGTTCA	18-19
Ldlr	NM_010700.2	Mm00440169_m1	GCGGAGCTGCCTCACAGAAGTCGAC	14-15
Npc1l1	NM_207242.2	Mm01191973_m1	CTCTACTGTGCCAATGCCCCTCTCA	2-3
Scarb1	NM_016741.1	Mm00450234_m1	CTGTCAAGGGCATCGGGCAAACAGG	9-10

Supplementary Table 5: Summary of controls for the RT-qPCR experiments. This is intended to provide the reviewers with all information required to assess the validity of the qPCR experiments according to the MIQE guidelines and can be included in the final manuscript at the discretion of the editorial staff.

RNA quality	Quantification of the RNA was performed using the RiboGreen assay, which has been demonstrated to recognize high quality RNA. Purity of the isolation was also assessed qualitatively using the ratio of UV absorption at 260nm and 280nm
Consistency of the RT reaction	The cDNA produced by the reverse transcription reaction was quantified using the OliGreen ssDNA assay. All samples were subsequently diluted to 2ng/µl prior to the PCR reaction.
PCR amplification efficiency	Dilution curves were run for each gene to determine the minimum input of cDNA that would result in amplification efficiency of >90% for all genes. This was found to be 0.2-1.0ng. To ensure that the reactions were above this threshold 10ng of template cDNA was used for all qPCR reactions.
Reproducibility	Each sample was analyzed for each gene in triplicate, additionally; the coefficient of determination was used to demonstrate that the Ct value correlates with cDNA input. The reference gene, encoding murine β-Actin, was assayed for each sample on each plate in triplicate to ensure that there was no day to day variability
No Template Control	Duplicate no template control wells were assayed on each PCR plate
Genomic DNA control	The TaqMan assays for all genes except *Actb* were designed to flank an intron. Additionally, each RNA sample was assayed for *Actb* without a reverse transcription step – there was no evidence of interference by genomic DNA
Equal loading of wells	The equal loading of each well was confirmed using the passive dye ROX, which was incorporated into the iTaq SuperMix
Reference Gene	The reference gene (*Actb*) demonstrated consistent expression in all treatment groups. The slight plate-to-plate variability in Ct value for any given sample was greater than any differences between treatment groups