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Supplemental Material

Exposure of *Ldlr*$^{-/-}$ Mice to a PFAS Mixture and Outcomes Related to Circulating Lipids, Bile Acid Excretion, and the Intestinal Transporter ASBT

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Figure S3. Additional hepatic bile acid transporters after exposure to the PFAS mixture. Total hepatic RNA from n=10 mice from each treatment group was isolated and expression levels of the transporters (A) *Abcc2*, (B) *Slc51β*, (C) *Slc10a2*, and (D) *Ephx1* were determined by RT-PCR. GAPDH was used as a housekeeping gene. Hepatic NTCP protein levels were analyzed via western blot for: female$^+$vehicle (n=10), female$^+$PFAS (n=10), male$^+$vehicle (n=10), and male$^+$PFAS (n=9). E) Western blot analysis of NTCP protein in the liver. F) Quantification of band intensity for NTCP protein relative to β-actin. Non-normally distributed data was Log10 transformed prior to statistical analysis. Two-Way ANOVA was utilized to analyze both main effects (sex, PFAS) as well as the interaction between sex and PFAS ($p<0.05$). The Holm-Sidak post-hoc test was used for multiple comparisons. With past consultation from biostatisticians, a significant interaction term supersedes the main effects and can make their meaning unclear. We therefore have not included the main effects p-values for any result with a significant interaction. Box plots represent the median values with upper and lower quartiles; whiskers extend to the 1st and 99th percentiles. Bold p-values represent $p<0.05$; italicized p-values represent $p<0.10$. Data reported in Excel Table S20 and S21.

Figure S4. Sex hormones in female mice after exposure to the PFAS mixture. Circulating hormone levels for (A) FSH, (B) progesterone, and (C) LH were measured from plasma collected at euthanasia from female control mice (n=8) and female PFAS-exposed mice (n=8). Statistical significance for FSH was determined by t-test ($p<0.05$). Statistical significance for progesterone and LH was determined by Mann-Whitney Rank Sum Test ($p<0.05$). Box plots represent the median values with upper and lower quartiles; whiskers extend to the 1st and 99th percentiles. Data reported in Excel Table S22.

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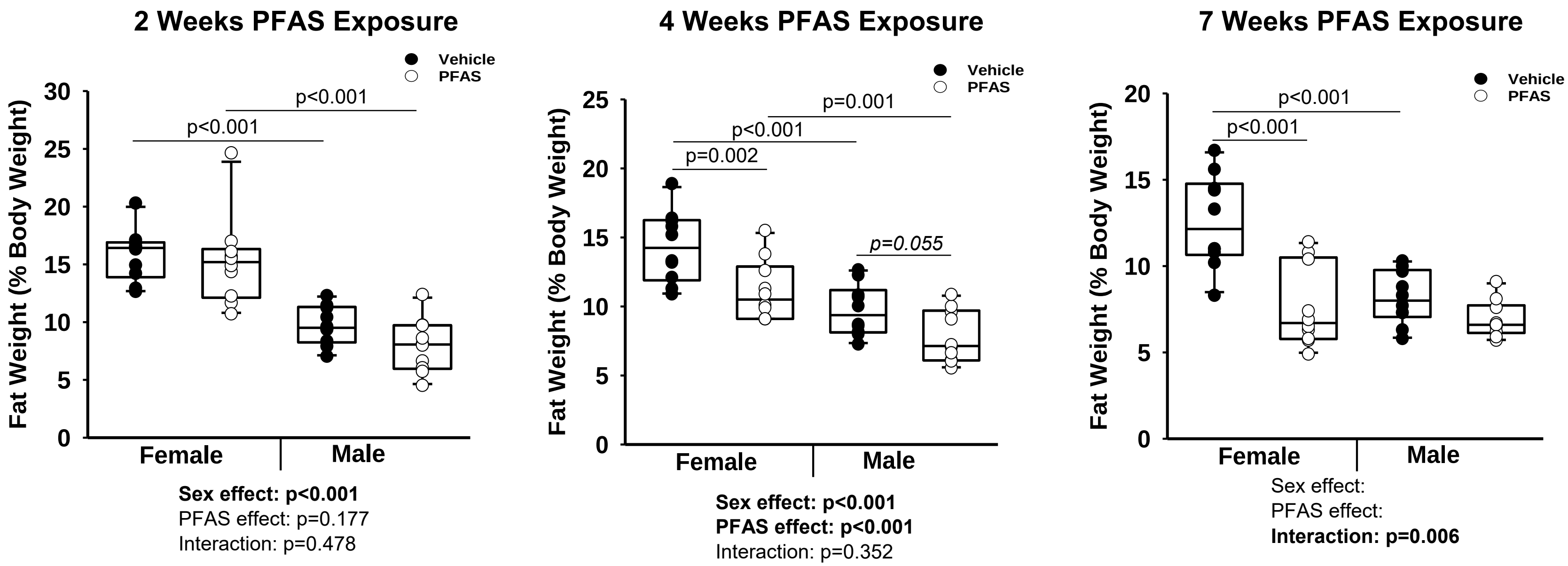


Figure S1. Fat weight in $Ldlr^{-/-}$ mice exposed to the PFAS mixture. Male and female $Ldlr^{-/-}$ mice were exposed to vehicle water or the PFAS mixture for 7 weeks. Fat weight was measured (n=10 mice per treatment group) via EchoMRI after 2, 4, and 7 weeks of PFAS exposure. Data given as a percentage of total body weight. Two-Way ANOVA was utilized to analyze both main effects (sex, PFAS) as well as the interaction between sex and PFAS ($p<0.05$). The Holm-Sidak post-hoc test was used for multiple comparisons. With past consultation from biostatisticians, a significant interaction term supersedes the main effects and can make their meaning unclear. We therefore have not included the main effects p-values for any result with a significant interaction. Box plots represent the median values with upper and lower quartiles; whiskers extend to the 1^{st} and 99^{th} percentiles. Bold p-values represent $p<0.05$; italicized p-values represent $p<0.10$. Data is reported in Excel Table S18.

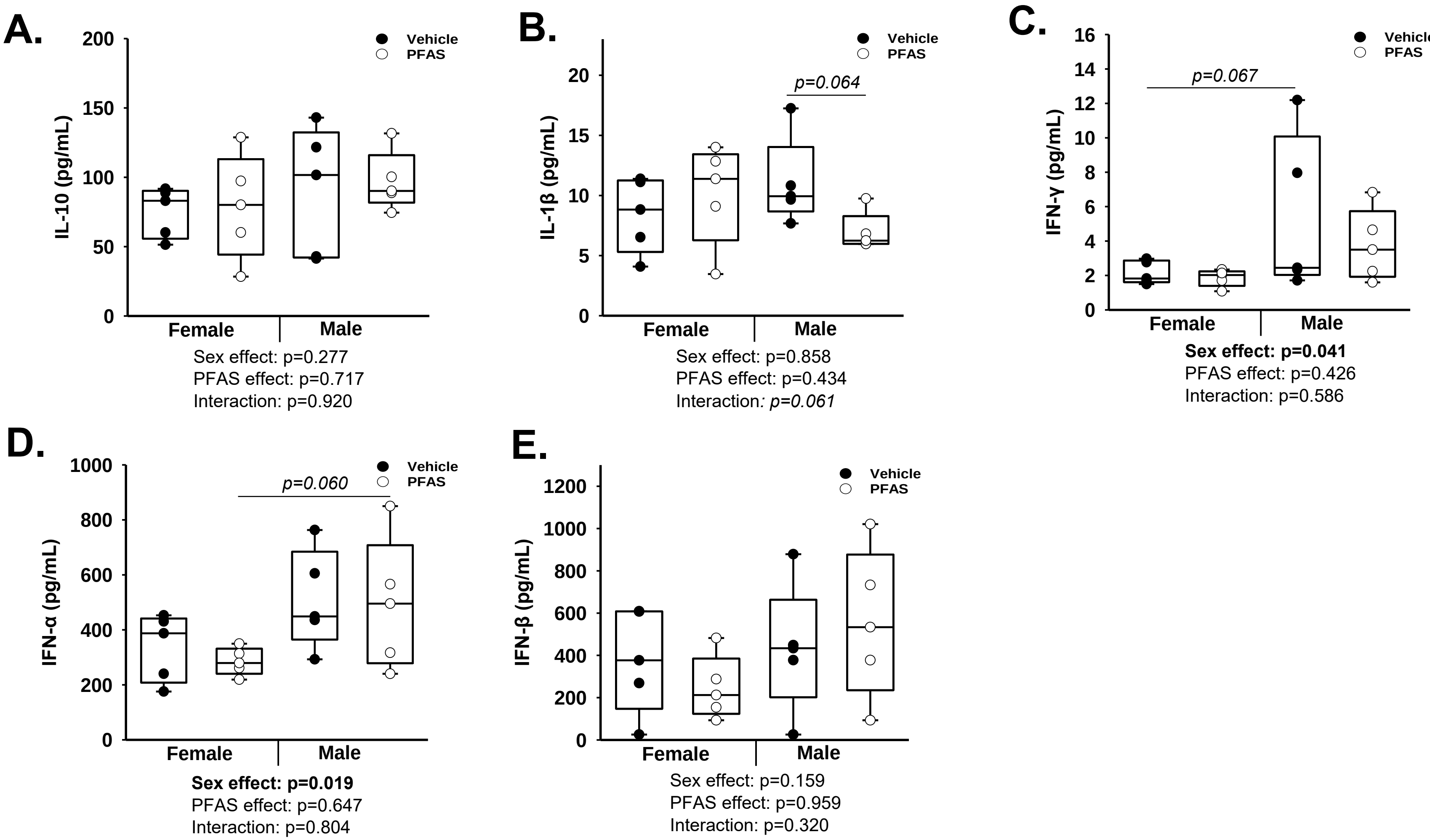
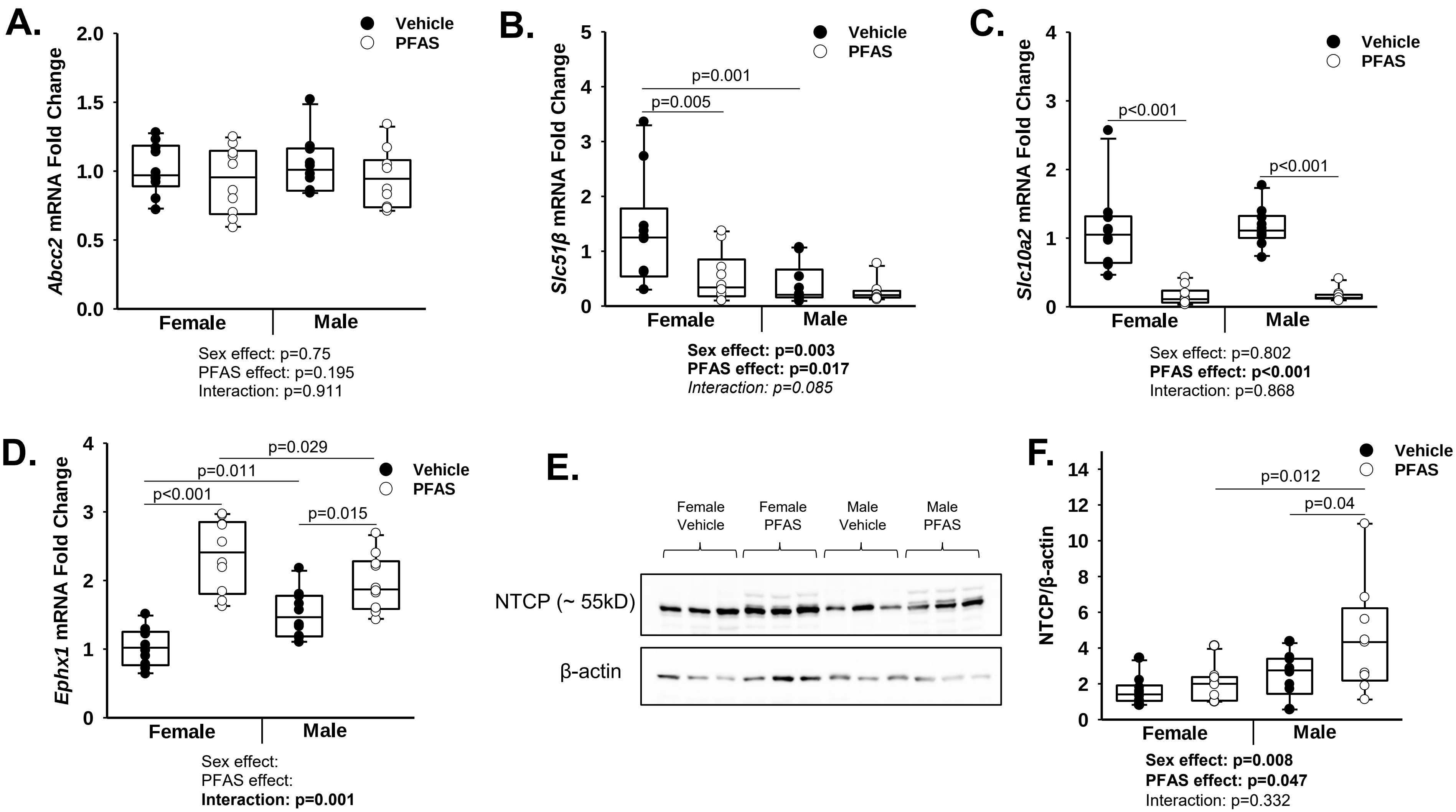


Figure S2. Additional circulating cytokines in Ldlr$^{-/-}$ mice exposed to the PFAS mixture . Circulating cytokine protein levels for (A) IL-10, (B) IL-1β, (C) IFN-γ, (D) IFN-α, and (E) IFN-β were measured from plasma collected at euthanasia from mice of each treatment group (n=5). Two-Way ANOVA was utilized to analyze both main effects (sex, PFAS) as well as the interaction between sex and PFAS ($p<0.05$). The Holm-Sidak post-hoc test was used for multiple comparisons. Box plots represent the median values with upper and lower quartiles; whiskers extend to the 1st and 99th percentiles. Bold p-values represent $p<0.05$; italicized p-values represent $p<0.10$. Data is reported in Excel Table S19.



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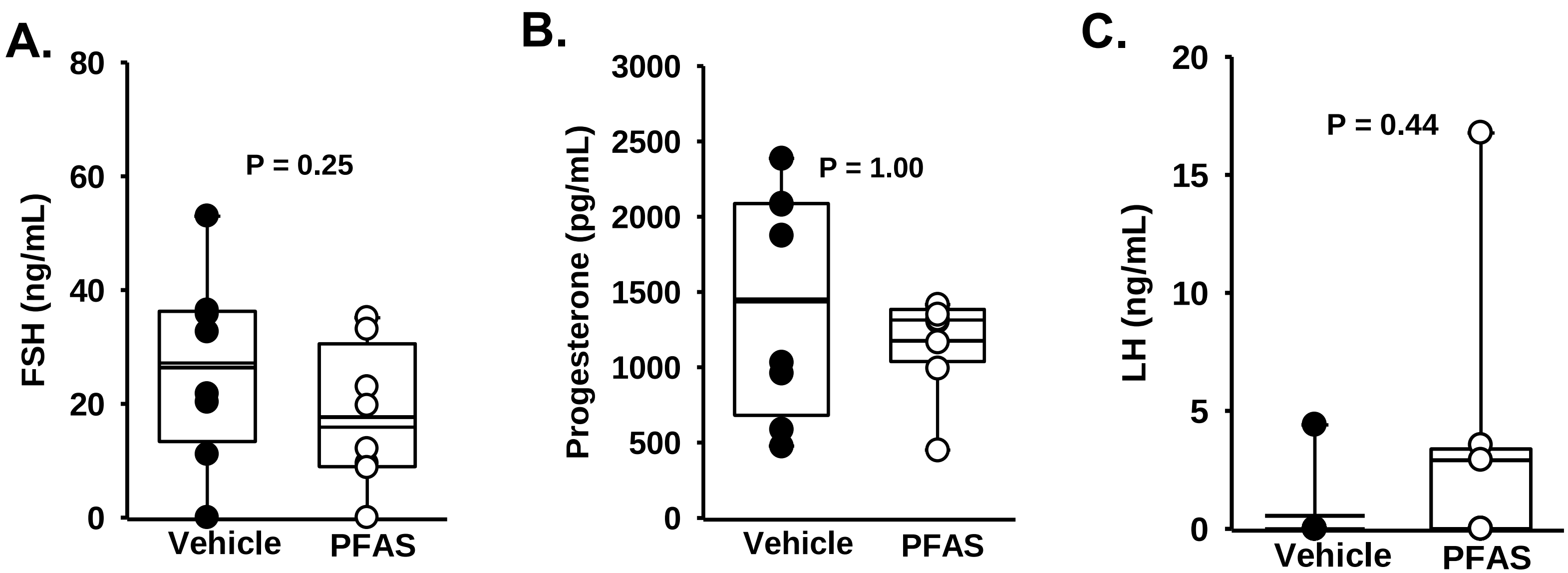

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Table S1: Clinton-Cybulsky Diet (Research Diets; New Brunswick, NJ, USA)

Product #	**D01061401C**	
%	**gm**	***kcal***
Protein	19.2	*20.0*
Carbohydrate	67.2	*70.0*
Fat	4.3	*10.0*
Total		*100.0*
Kcal/gm	3.84	
Ingredient	**gm**	***kcal***
Casein, Lactic	200	*800*
L-Cystine	3	*12*
Corn Starch	375	*1500*
Maltodextrin 10	125	*500*
Sucrose	200	*800*
Cellulose, BW200	50	*0*
Soybean Oil	25	*225*
Cocoa Butter	20	*180*
Mineral Mix S10021	10	*0*
Dicalcium Phosphate	13	*0*
Calcium Carbonate	5.5	*0*
Potassium Citrate	16.5	*0*
Vitamin Mix V10001	10	*40*
Choline Bitartrate	2	*0*
Cholesterol	1.6	*0*
Red Dye, FD&C #40	0	*0*
Blue Dye, FD&C #1	0	*0*
Yellow Dye, FD&C #5	0	*0*
Total	**1056.60**	**4057**

Table S2. Primer sequences used in RT-PCR.

SYBR Green Primers		
Target Gene	**Forward Primer Sequence (5'-3')**	**Reverse Primer Sequence (5'-3')**
Hmgcr	GCAGCTTGGGTCCAAGTACA	CAAGGCATTCCACAAGAGCG
Abcc2	GCAACTCTACTTTTTGGAATCTCT	CCAAGAGCCAAAGAAAGCCC
Abcc3	GGACTCTCATGTGGCGAAGC	TTACCAGCACCCGAGTCTTGC
Abcc4	ACACCGAGGTGAAACCCAACC	GCGGGTTGAGCCACCAGAA
Slc10a1	GGACATGAACCTCAGCATTGTG	GCCTTTGTAGGGCACCTTGT
Slc10a2	TGGGTTTCTTCCTGGCTAGACT	TGTTCTGCATTCCAGTTTCCAA
Abcb11	TTCGAATCAGATGGTTTCCATAAC	GCGCACACACTTCCCATAAA
Ephx1	GGGTCAAAGCCATCAGCCA	CCTCCAGAAGGACACCACTTT
Slc51b	CAGGAACTGCTGGAAGAAATGC	GCAGGTCTTCTGGTGTTTCTTTGT
Nr1h4	TGTGAGGGCTGCAAAGGTTT	ACATCCCCATCTCTCTGCAC
mFgf15	TCGCTACTCGGAGGAAGACTGTAC	TCTGGTCCTGGAGCTGTTCTCTG
Nr0b2	AAGGGCACGATCCTCTTCAA	CTGTTGCAGGTGTGCGATGT
Nr1i2	GATGGAGGTCTTCAAATCTGCC	CAGCCGGACATTGCGTTTC
Cyp3a11	CTGGAGGTGATGTTGAGTGTT	TGCTAGCCTAAGCATTGGAC
Nr1i3	GATCAGCTACAAGAGGAGATGG	TTGCATACAGAAACCGACTTTG
Cyp2b10	TTTCTGCCCTTCTCAACAGGAA	TGGACGTGAAGAAAAGGAACAAC
Keap1	AGCGTGGAGAGAGATATGAGCC	ATCATCCGCCACTCATTCCT
Gstm1	ATACTGGGATACTGGAACGTCC	AGTCAGGGTTGTAACAGAGCAT
Pparα	GAGGCAGCCGCTTACG	GCCACAAACGTCAGTTCACAG
Pparδ/β	GCCACAACGCACCCTTTG	CCACACCAGGCCCTTCTCT
Gapdh	CAAGGAGTAAGAAACCCTGGACC	CGAGTTGGGATAGGGCCTCT
TaqMan Probes		
Target Gene	**TaqMan Probe**	
Cyp7a1	Mm00484152_m1	
Cyp27a1	Mm00470430_m1	
Gapdh	Mm99999915_g1	

Table S3. Correlations between circulating PFAS and cholesterol subfractions after exposure of Ldlr$^{-/-}$ mice to the PFAS mixture

PFAS[a]	Spearman Correlation Coefficient	P value[b]	Spearman Correlation Coefficient	P value[b]	Spearman Correlation Coefficient	P value[b]	Spearman Correlation Coefficient	P value[b]
	Total VLDL & LDL Cholesterol		Free VLDL & LDL Cholesterol		Esterified VLDL & LDL Cholesterol		Total HDL	
PFOS	0.515	p=0.128	*0.685	*****p=0.029	*0.552*	*p=0.098*	*-0.636	*****p=0.048
PFOA	*0.600*	*0.067*	*0.624*	*0.054*	*0.649	*****0.043	*-0.624*	*0.054*
PFHxS	0.321	0.366	0.249	0.489	0.406	0.244	-0.321	0.366
PFNA	0.455	0.187	0.467	0.174	0.527	0.117	-0.418	0.229
HFPO-DA	-0.1636	0.652	-0.261	0.467	-0.079	0.829	0.091	0.803

PFOS= Perfluorooctanesulfonic acid; PFOA= Perfluorooctanoic acid; PFHxS= Perfluorohexane sulfonic acid. PFNA= Perfluorononanoic acid; HFPO-DA (GenX)= Hexafluoropropylene oxide dimer acid.
* represents statistical significance ($p<0.05$); italicized p-values represent $p< 0.10$.
[a]n=10 total; data from 5 PFAS exposed females and 5 PFAS exposed males were used.
[b] Spearman correlations between PFAS and cholesterol variables were determined using JMP 15 software.

Table S4. Differentially expressed genes in the ileum after exposure of male Ldlr$^{-/-}$ mice to the PFAS mixture.

Gene Name[a]	Overall PFAS Exposure Log2Fold Change[b]	Overall PFAS Exposure FDR-adjusted q-value
Marco	5.33	q=0.018
Gm16596	4.36	q=0.006
5930412G12Rik	4.29	q=0.026
Igfals	3.90	q=0.012
Rnase2b	3.59	q=0.046
Cpn2	3.37	q=0.007
Tmem255a	2.41	q=0.011
Gm39556	2.33	q=0.019
Bmpr1b	2.33	q=0.046
Gm5415	2.26	q=0.008
Gm34828	2.15	q=0.011
Macc1	2.15	q=0.040
Cyp3a44	2.02	q<0.001
Prss27	1.82	q<0.001
Sprr2b	1.75	q=0.015
Tlr7	1.72	q=0.028
Steap1	1.63	q=0.006
Ifit3b	1.61	q=0.050
Saa1	1.52	q=0.001
Reg3g	1.40	q<0.001
Gm15658	1.39	q=0.008
Pla2g5	1.38	q=0.008
Thpo	1.37	q<0.001
B3galt5	1.34	q=0.008
Acta1	1.32	q=0.007
Adora1	1.31	q=0.006
Nfkbie	1.24	q=0.046
Reg3b	1.23	q<0.001
Socs3	1.16	q<0.001
Alpk1	1.13	q=0.004
Tifa	1.13	q<0.001
Pfkfb3	1.11	q<0.001
Duoxa2	1.07	q=0.025
Duox2	1.06	q=0.004
Tctex1d2	1.05	q=0.041
Scd2	1.04	q<0.001
Npr3	-1.01	q=0.013
Ehhadh	-1.01	q=0.007
Gm40078	-1.04	q=0.011
Snord89	-1.07	q=0.027
G6pc	-1.14	q<0.001
Retsat	-1.15	q<0.001
Hist1h1b	-1.16	q=0.002

Edn1	-1.16	q=0.013
Arg2	-1.17	q=0.038
Tcf23	-1.19	q=0.002
Rn7sk	-1.24	q=0.014
Acot2	-1.31	q<0.001
Gm35166	-1.33	q=0.026
Grin3b	-1.36	q=0.005
LOC108168346	-1.37	q=0.022
Gm30810	-1.53	q=0.011
Gstm3	-1.56	q<0.001
Ccdc168	-1.57	q=0.015
LOC102636299	-1.68	q=0.028
Dct	-1.75	q=0.025
Acot1	-1.87	q<0.001
Snora75	-2.07	q=0.023
Hmgcs2	-2.08	q<0.001
Ugt1a1	-2.15	q<0.001
4930415O20Rik	-2.33	q=0.044
Col17a1	-2.35	q=0.032
Gjb5	-2.53	q=0.037
Gm38851	-2.61	q=0.029
Gm30757	-2.68	q=0.021
LOC108168164	-2.72	q=0.003
Cyp4a10	-2.76	q=0.006
Uprt	-2.83	q=0.009
Neurod2	-4.39	q=0.031
Zpbp2	-4.62	q=0.003

[a] n=20 total; n=10 male vehicle, n=10 male PFAS
[b] Data presented as log2 fold change based on RNA-sequencing. The genes included are based on |log2 fold change| ≥ 1; q-value < 0.05)
Statistical significance was determined by t-test using the R statistical software package.

Table S5: Expression of genes related to the acute inflammatory response and lipid metabolism after exposure of male Ldlr$^{-/-}$ mice to the PFAS mixture.

Gene Name	Male Vehicle (Average ± SEM) RNA-seq Counts	Male PFAS (Average ± SEM) RNA-seq Counts	Overall PFAS Exposure Fold Change	Overall PFAS Exposure p-value	Overall PFAS Exposure FDR-adjusted q-value
GO:0002526~acute inflammatory response[a]					
C2cd4b	5.3 ± 1.3	10.8 ± 1.8	2.21	p=0.013	q=0.276
Mylk3	34.7 ± 5.0	16 ± 5.4	0.45	p=0.003	q=0.119
Oprm1	1.5 ± 0.6	0.1 ± 0.1	0.15	p=0.009	q=0.231
Tnf	2 ± 0.7	6 ± 1.2	3.17	p=0.005	q=0.153
F3	10.8 ± 2.5	22.5 ± 6.6	2.04	p=0.034	q=0.447
F8	6.5 ± 1.1	14.1 ± 3.9	2.30	p=0.005	q=0.153
Reg3g	16160.6 ± 1617.1	40838.5 ± 5913.6	2.64 *	p<0.001	*q<0.001
Defb1	12.6 ± 2.7	5.2 ± 1.4	0.41	p=0.007	q=0.193
Il31ra	0.3 ± 0.2	2.7 ± 1.1	6.12	p=0.020	q=0.344
Saa1	0.6 ± 0.3	3 ± 1.1	2.87 *	p<0.001	*q=0.001
Adora1	15.8 ± 2.7	38.6 ± 6.5	2.47 *	p<0.001	*q=0.006
Saa2	724.5 ± 119.1	2002.3 ± 399.2	4.34	p=0.011	q=0.246
Reg3b	39598.6 ± 3754.0	88972 ± 9567.8	2.34 *	p<0.001	*q<0.001
Ugt1a1	18.8 ± 2.7	4 ± 0.9	0.23 *	p<0.001	*q<0.001
Masp1	5.7 ± 1.4	11 ± 3.2	2.15	p=0.031	q=0.431
Cd5l	1.6 ± 0.7	7.7 ± 6.0	5.28	p=0.016	q=0.301
GO:0006629~lipid metabolic process[a]					
Cyp11b1	1.1 ± 0.6	0 ± 0	0.12	p=0.025	q=0.390
4833423E24Rik	0.7 ± 0.4	0 ± 0	0.14	p=0.044	q=0.507
Fabp1	1116.1 ± 300.1	397.4 ± 77.7	*0.39*	p=0.001	*q=0.062*
Enpp6	2.8 ± 0.5	6.3 ± 1.6	2.30	p=0.030	q=0.419
Acss3	2.4 ± 0.7	0.4 ± 0.2	0.23	p=0.017	q=0.312
Pla2g5	10.4 ± 1.1	26.4 ± 6.4	2.59 *	p<0.001	*q=0.008
Plppr5	6.2 ± 1.6	1.8 ± 0.7	0.31	p=0.007	q=0.202
Cyp2d12	0.4 ± 0.2	3.4 ± 1.0	*6.38*	p=0.001	*q=0.068*
Cyb5r2	2.9 ± 0.8	0.8 ± 0.3	0.31	p=0.025	q=0.384
Tnf	2 ± 0.7	6 ± 1.2	3.17	p=0.005	q=0.153
Cyp4a14	0 ± 0	3.6 ± 2.5	*26.24*	p=0.001	*q=0.053*
Bmpr1b	0.8 ± 0.4	4.5 ± 0.9	5.02 *	p=0.001	*q=0.046
Acsm5	4.3 ± 1.1	9.8 ± 1.3	2.30	p=0.003	q=0.108
Cyp4a12a	0 ± 0.0	0.8 ± 0.5	6.64	p=0.040	q=0.486
B3galt5	12.1 ± 1.4	28.9 ± 5.1	2.54 *	p<0.001	*q=0.008
Socs3	177 ± 21.9	367.2 ± 39.8	2.23 *	p<0.001	*q<0.001
Ehhadh	28.8 ± 2.7	14.2 ± 2.6	0.50 *	p<0.001	*q=0.007
Zpbp2	3 ± 0.8	0 ± 0.0	0.04 *	p<0.001	*q=0.003
Pdk4	18.9 ± 3.5	8.5 ± 2.6	0.45	p=0.013	q=0.270
Cyp4a10	21.4 ± 5.4	3.1 ± 1.3	0.15 *	p<0.001	*q=0.006
Cyp1a1	0 ± 0.0	1.3 ± 0.8	12.16	p=0.018	q=0.324

Saa1	724.5 ± 119.1	2002.3 ± 399.2	2.87 *	p<0.001	*q=0.001
Acot2	485.7 ± 32.3	193.8 ± 24.7	0.40 *	p<0.001	*q<0.001
G6pc	353.2 ± 37.4	157.2 ± 20.2	0.45 *	p<0.001	*q<0.001
Fam132b	0.1 ± 0.1	2.7 ± 1.1	12.18	p=0.003	q=0.127
Cyp2c66	1.1 ± 0.6	0 ± 0	0.11	p=0.027	q=0.397
Serpina12	0 ± 0	1.2 ± 0.6	11.98	p=0.018	q=0.326
Retsat	632.6 ± 70.9	279.4 ± 33.2	0.45 *	p<0.001	*q<0.001
Ces1b	0.1 ± 0.1	1.9 ± 0.6	9.49	p=0.002	q=0.101
Acsm1	1.5 ± 0.6	0.2 ± 0.1	0.22	p=0.027	q=0.399
Acsl6	6.5 ± 0.8	3.1 ± 0.9	0.49	p=0.024	q=0.378
Pla2g4c	28.2 ± 5.8	12.5 ± 2.5	0.48	p=0.004	q=0.144
Cers3	1.4 ± 0.5	0.3 ± 0.2	0.28	p=0.045	q=0.515
Ugt2b1	0.8 ± 0.4	3.2 ± 0.6	3.75	p=0.006	q=0.177
Hmgcs2	1383.6 ± 121.8	323.20 ± 49.3	0.24 *	p<0.001	*q<0.001
Cyp2d9	4.8 ± 2.0	11.6 ± 3.2	2.60	p=0.025	q=0.386
Acot1	127 ± 13.0	33.7 ± 5.2	0.27 *	p<0.001	*q<0.001
Edn1	45.4 ± 6.5	21 ± 5.5	0.45 *	p<0.001	*q=0.013
Adora1	15.8 ± 2.7	38.6 ± 6.5	2.47 *	p<0.001	*q=0.006
Scd2	175 ± 11.0	361.9 ± 60.4	2.06 *	p<0.001	*q<0.001
Acot8	1.3 ± 0.6	3.9 ± 0.9	2.86	p=0.012	q=0.259
Ugt1a1	18.8 ± 2.7	4 ± 0.9	0.23 *	p<0.001	*q<0.001
Cyp3a44	8.8 ± 2.2	34.9 ± 5.2	4.05 *	p<0.001	*q<0.001
Acat3	1.8 ± 0.7	0.3 ± 0.2	0.21	p=0.035	q=0.455
Pigb	2.7 ± 0.6	0.3 ± 0.2	*0.16*	p=0.001	*q=0.055*

Data presented as RNA-sequencing counts (average ± SEM).
Statistical significance was determined by t-test using the R statistical software package.
* Represents significant effect relative to vehicle control (q<0.05); italicized q-values represent q<0.10.
[a] n=20 total; n=10 male vehicle, n=10 male PFAS.

Table S6: Modulation of hepatic nuclear receptor signaling after exposure of Ldlr$^{-/-}$ mice to the PFAS mixture

Gene Name	Female Vehicle (Average Fold Change ($2^{-\Delta\Delta Ct}$) ± SEM)	Female PFAS (Average Fold Change ($2^{-\Delta\Delta Ct}$) ± SEM)	Male Vehicle (Average Fold Change ($2^{-\Delta\Delta Ct}$) ± SEM)	Male PFAS (Average Fold Change ($2^{-\Delta\Delta Ct}$) ± SEM)	Overall PFAS Exposure Fold Change	Overall PFAS Exposure p-value[b]	Fold Change PFAS Male vs. PFAS Female	PFAS-Sex Interaction p-value[c]
Nuclear Receptor Signaling[a]								
Nr1h4 (FXR)	1.14 ± 0.19	0.81 ± 0.18	1.37 ± 0.10	0.76 ± 0.07	0.63 *	*p=0.002	0.94	p=0.35
mFgf15	1.23 ± 0.27	0.46 ± 0.15	0.50 ± 0.08	1.46 ± 1.25	1.08	p=0.89	3.18	p=0.20
Nr0b2 (SHP)	1.43 ± 0.41	0.28 ± 0.07	0.58 ± 0.14	0.29 ± 0.03	0.28 #	Sex Interaction	1.06	#p=0.002
Nr1i2 (PXR)	1.04 ± 0.09	0.97 ± 0.07	0.73 ± 0.05	0.79 ± 0.08	0.99	p=0.952	0.81	p=0.384
Cyp3a11	1.40 ± 0.23	1.43 ± 0.38	0.80 ± 0.26	1.43 ± 0.61	1.30	p=0.412	1.00	p=0.456
Nr1i3 (CAR)	1.11 ± 0.14	0.70 ± 0.10	0.50 ± 0.09	0.52 ± 0.09	0.75 #	Sex Interaction	0.74	#p=0.047
Cyp2b10	4.17 ± 2.42	10.41 ± 4.73	9.47 ± 3.00	8.33 ± 1.66	1.37	p=0.425	0.80	p=0.251
Nfe2l2 (NRF2)	1.16 ± 0.14	0.81 ± 0.09	0.61 ± 0.11	0.66 ± 0.06	0.83	p=0.156	0.82	p=0.054
Keap1	1.03 ± 0.08	0.87 ± 0.03	0.74 ± 0.07	0.83 ± 0.14	0.96	p=0.717	0.95	p=0.277
Gstm1	1.02 ± 0.07	2.11 ± 0.18	0.92 ± 0.16	1.21 ± 0.05	1.71 #	Sex Interaction	0.57	#p=0.004
Pparα	1.02 ± 0.06	0.82 ± 0.07	0.77 ± 0.08	1.24 ± 0.58	1.16	p=0.637	1.51	p=0.264
Pparδ/β	1.03 ± 0.08	0.85 ± 0.06	0.60 ± 0.05	0.54 ± 0.10	0.86	p=0.079	0.64	p=0.971

Statistical significance for the interaction and main effects of PFAS exposure and sex between all treatment groups (male vehicle, male PFAS, female vehicle, female PFAS) was determined using Two-Way ANOVA.
* Represents significant overall PFAS exposure fold change (p<0.05).
# Represents significant PFAS-Sex Interaction (p<0.05).
[a] n=40 total; n=10 male vehicle, n=10 male PFAS, n=10 female vehicle, n=10 female PFAS.
[b] Overall PFAS exposure p-value; Sex Interaction indicates that there was a significant interaction between PFAS exposure and sex.
[c] PFAS-Sex Interaction p-values determined by Two-Way ANOVA using SigmaPlot. With past consultation from biostatisticians, a significant interaction term supersedes the main effects and can make their meaning unclear. We therefore have not included the main effects p-values for any result with a significant interaction.