Supporting Information

Disturbances in microbial and metabolic communication across the gut-liver axis induced by a dioxin-like pollutant: An integrated metagenomics and metabolomics analysis

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Number of pages: 20
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FIGURE CAPTIONS

Figure S1. Changes in the bacterial phylum composition (A) and Firmicutes/Bacteroidetes (F/B) ratio (B) in zebrafish feces after exposure to PCB126. Values are presented as mean ± SEM of three replicates ($n = 3$). *P <0.05 and **P <0.01 indicate significant difference between the PCB126 exposure group and the corresponding control group.

Figure S2. (A) Hierarchical clustering based on representative genera (relative abundance >1%) showing the fecal microbial community at genus level; Intensity of red color is proportional to the genus abundance. (B) Co-occurrence network based on representative genera (relative abundance >1%) showing the dynamic interaction; Positive interactions among bacteria are indicated by blue lines, and negative interactions are indicated by red lines; Genus abundance is represented by the circular area and width of lines is proportional to the magnitude of correlation. Values are presented as means of three replicates ($n = 3$).

Figure S3. Significant alterations in the relative abundance of representative bacterial genera (relative abundance >1%) in (A) male and (B) female zebrafish after exposure to PCB126. Values are presented as mean ± SEM of three replicates ($n = 3$). *P <0.05, **P <0.01 and ***P <0.001 indicate significant difference between the PCB126 exposure group and the corresponding control group.

Figure S4. Principal component analysis (PCA) of genus composition in male and female zebrafish after exposure to PCB126. The arrows indicate the major contribution of each genus to the variances among samples.

Figure S5. Significantly enriched biological processes of fecal bacteria according to metagenomic analysis in (A) male and (B) female zebrafish after exposure to PCB126. *P <0.05, **P <0.01 and ***P <0.001 indicate significant enrichment in PCB126 exposure group relative to the control group.

Figure S6. Volcano plots of intestinal metabolomic features in male and female zebrafish after exposure to PCB126. (A) Male C18; (B) Male HILIC; (C) Female C18; (D) Female HILIC. A P-value <0.05 and VIP >1 were selected as the filtering criteria

to identify the significantly changed metabolites. Significantly up-regulated metabolites are labeled by red dots, and significantly down-regulated metabolites are labeled by green dots. Values are presented as means of three replicates ($n = 3$).

Figure S7. Summary of differential metabolites (A, Male; B, Female) based on C18 or HILIC separation modes in zebrafish intestines after exposure to PCB126. Venn diagrams show the unique and common metabolites between males and females (C, C18; D, HILIC).

Figure S8. Volcano plots of blood metabolomic features in male and female zebrafish after exposure to PCB126. (A) Male C18; (B) Male HILIC; (C) Female C18; (D) Female HILIC. A P-value <0.05 and VIP >1 were selected as the filtering criteria to identify the significantly changed metabolites. Significantly up-regulated metabolites are labeled by red dots, and significantly down-regulated metabolites are labeled by green dots. Values are presented as means of three replicates ($n = 3$).

Figure S9. Summary of differential metabolites (A, Male; B, Female) based on C18 or HILIC separation modes in zebrafish blood after exposure to PCB126. Venn diagrams show the unique and common metabolites between males and females (C, C18; D, HILIC).

Figure S10. OPLS-DA analysis with the input of intestinal metabolomic features. (A) Male C18; (B) Male HILIC; (C) Female C18; (D) Female HILIC.

Figure S11. OPLS-DA analysis with the input of blood metabolomic features. (A) Male C18; (B) Male HILIC; (C) Female C18; (D) Female HILIC

Figure S12. Significant enrichment of metabolic pathways under C18 mode (A) and HILIC mode (B) in male and female zebrafish intestines after exposure to PCB126. *P <0.05 and **P <0.01 indicate significant enrichment in PCB126 exposure group relative to the control group.
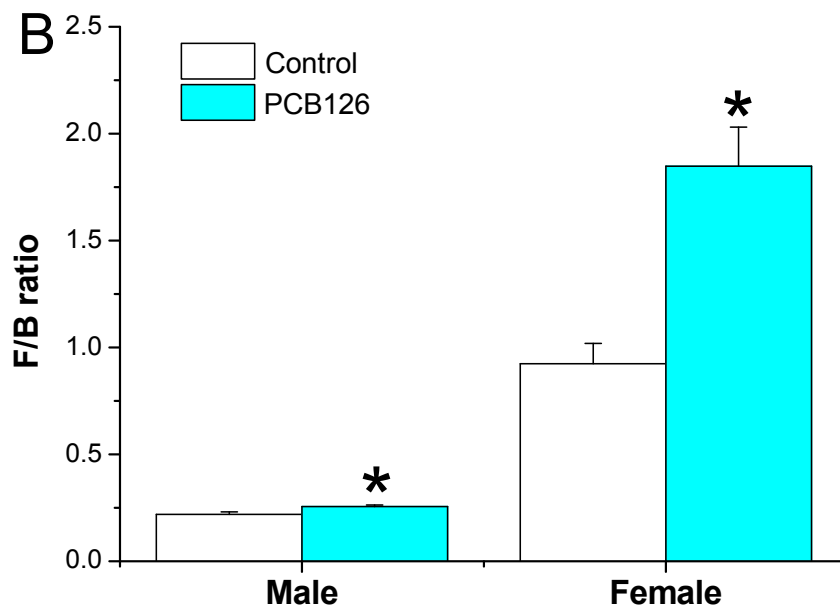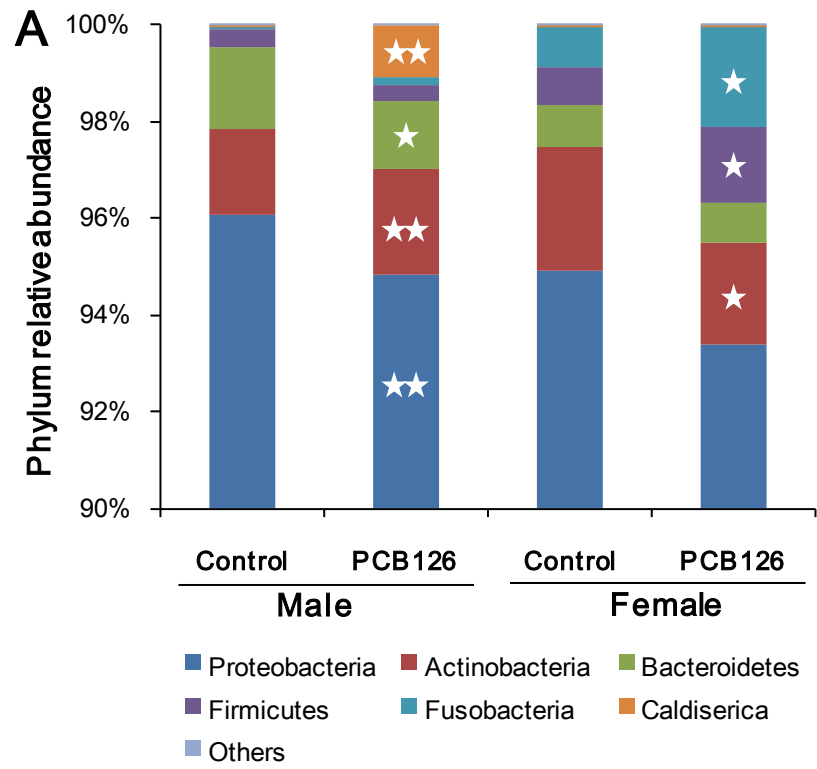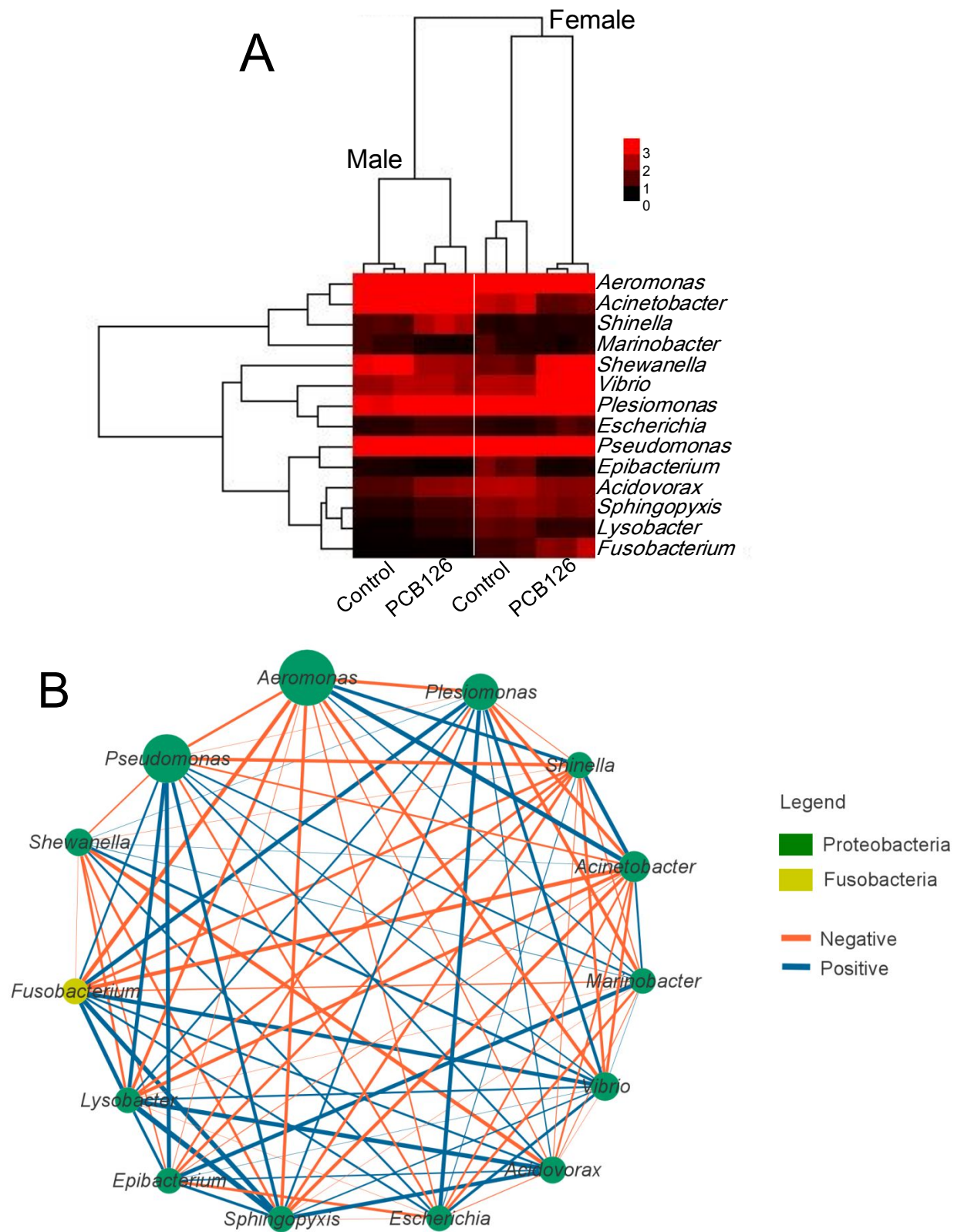
Figure S13. Significant enrichment of metabolic pathways under C18 mode (A) and HILIC mode (B) in male and female zebrafish blood samples after exposure to PCB126. *P <0.05 and **P <0.01 indicate significant enrichment in PCB126 exposure group relative to the control group.
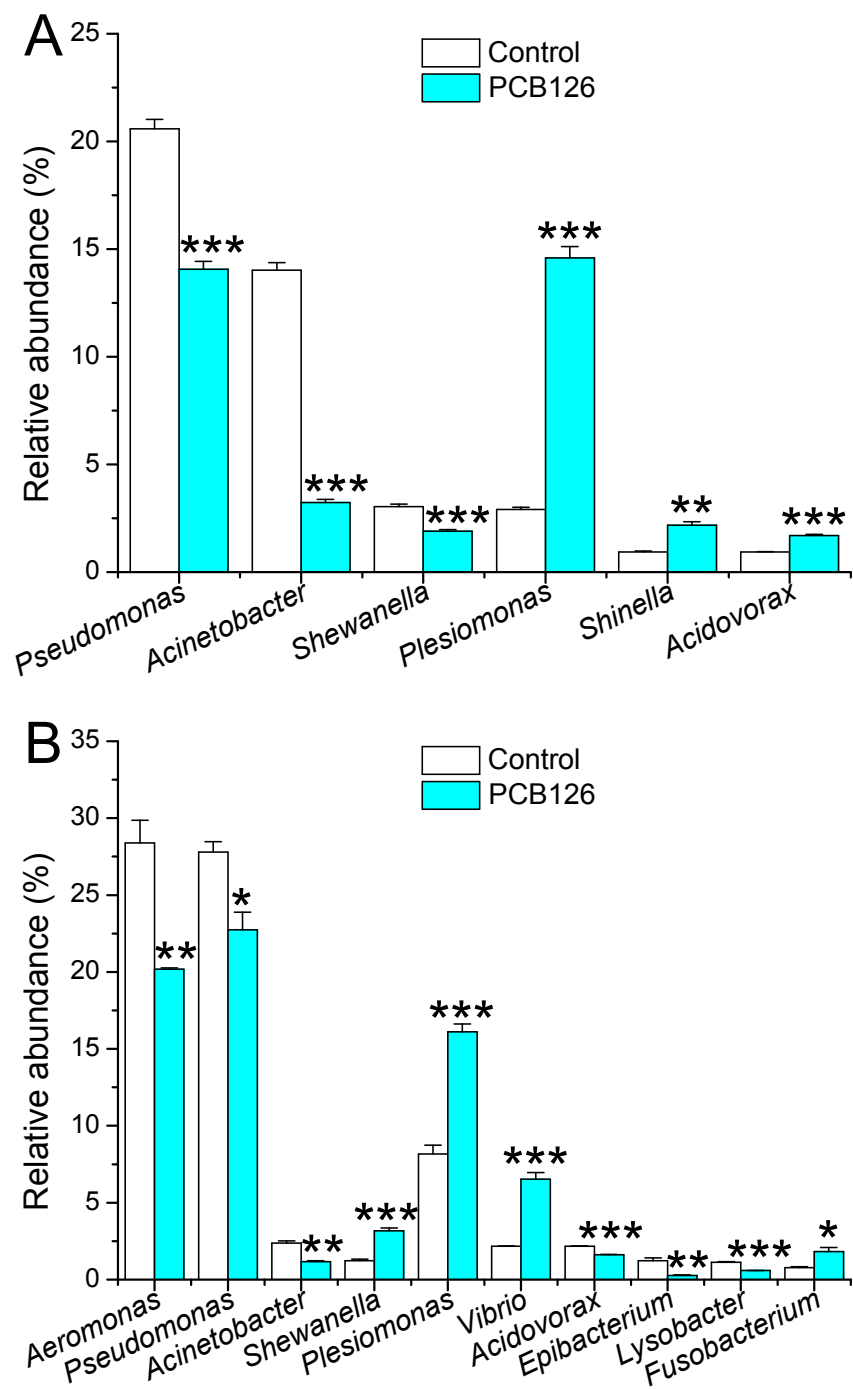
Figure S1

A

B

Figure S2

Figure S3
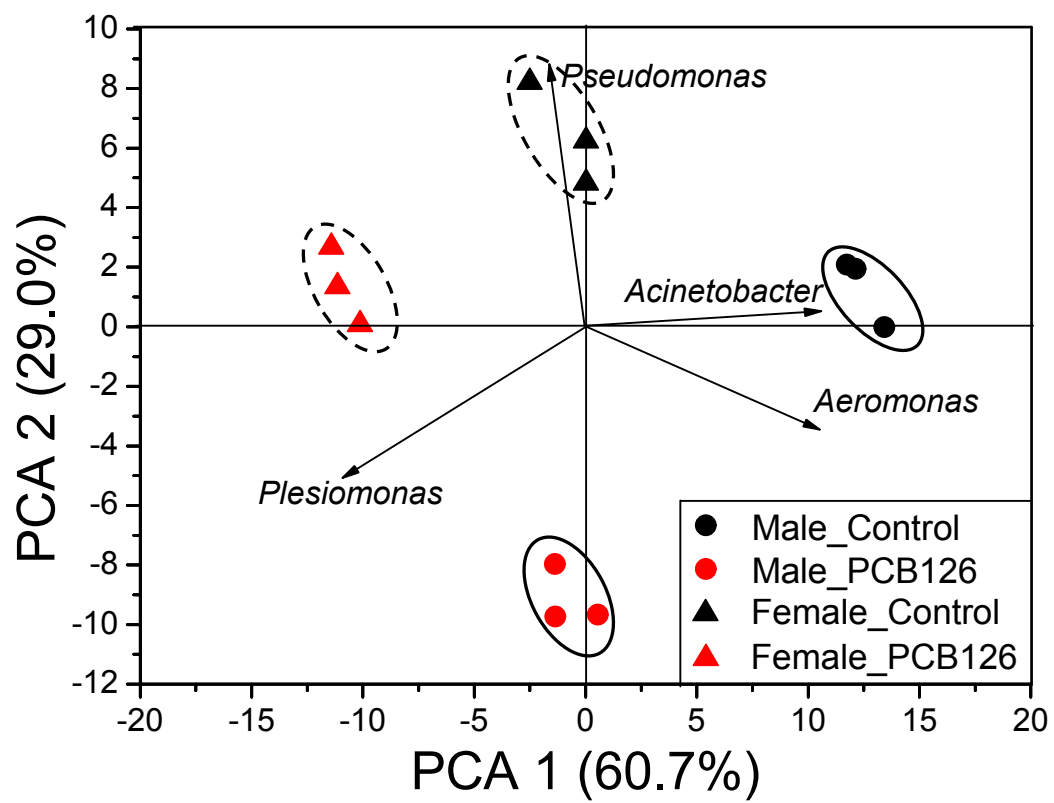
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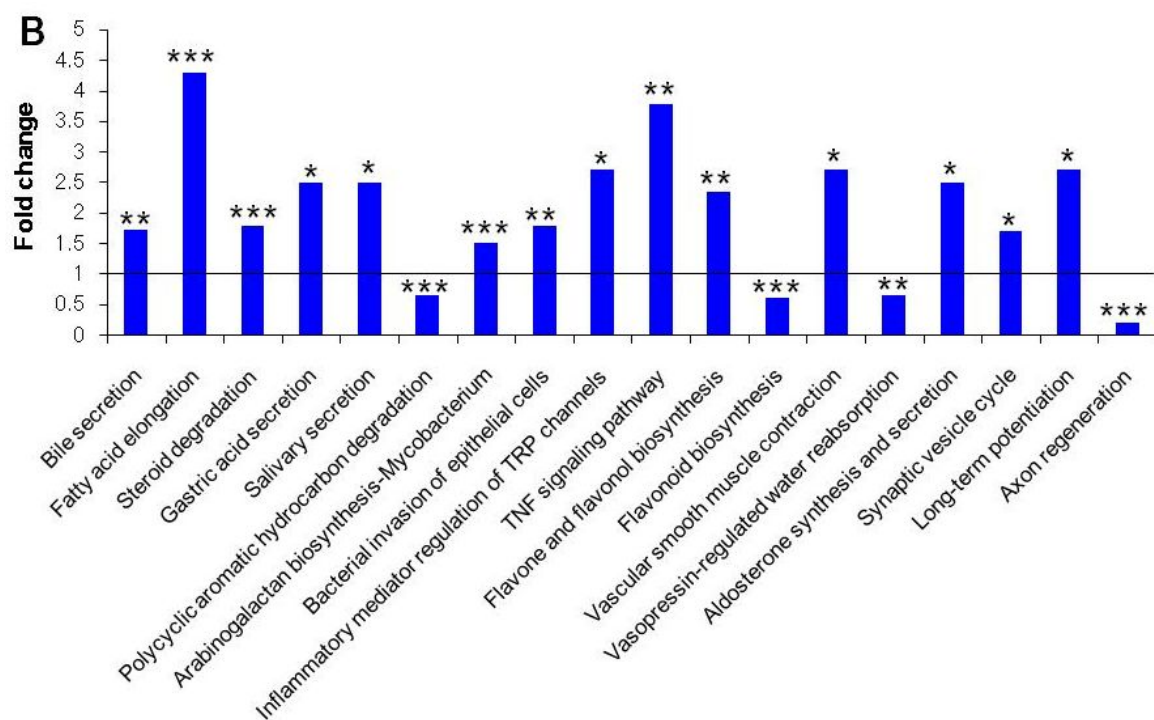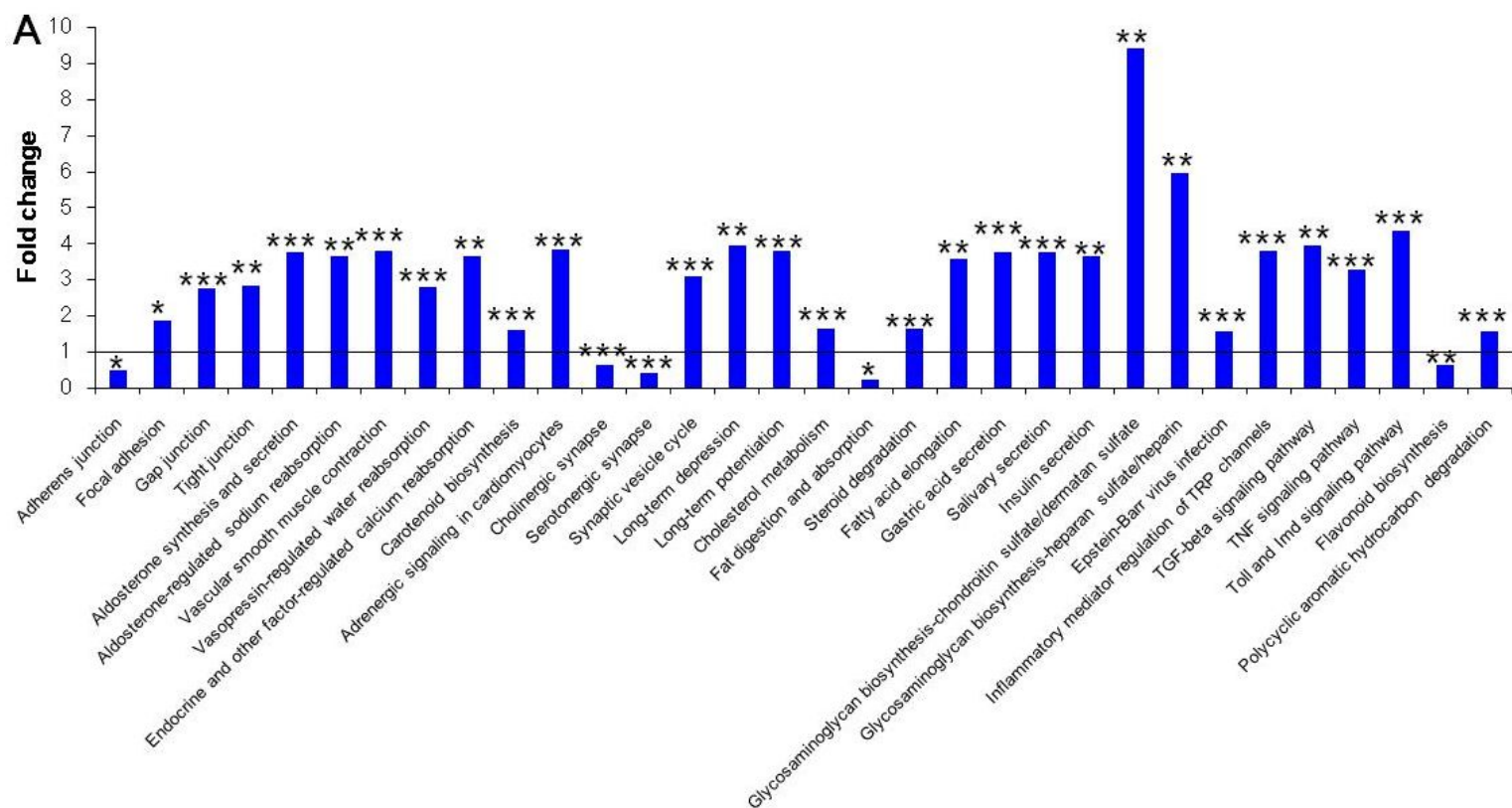
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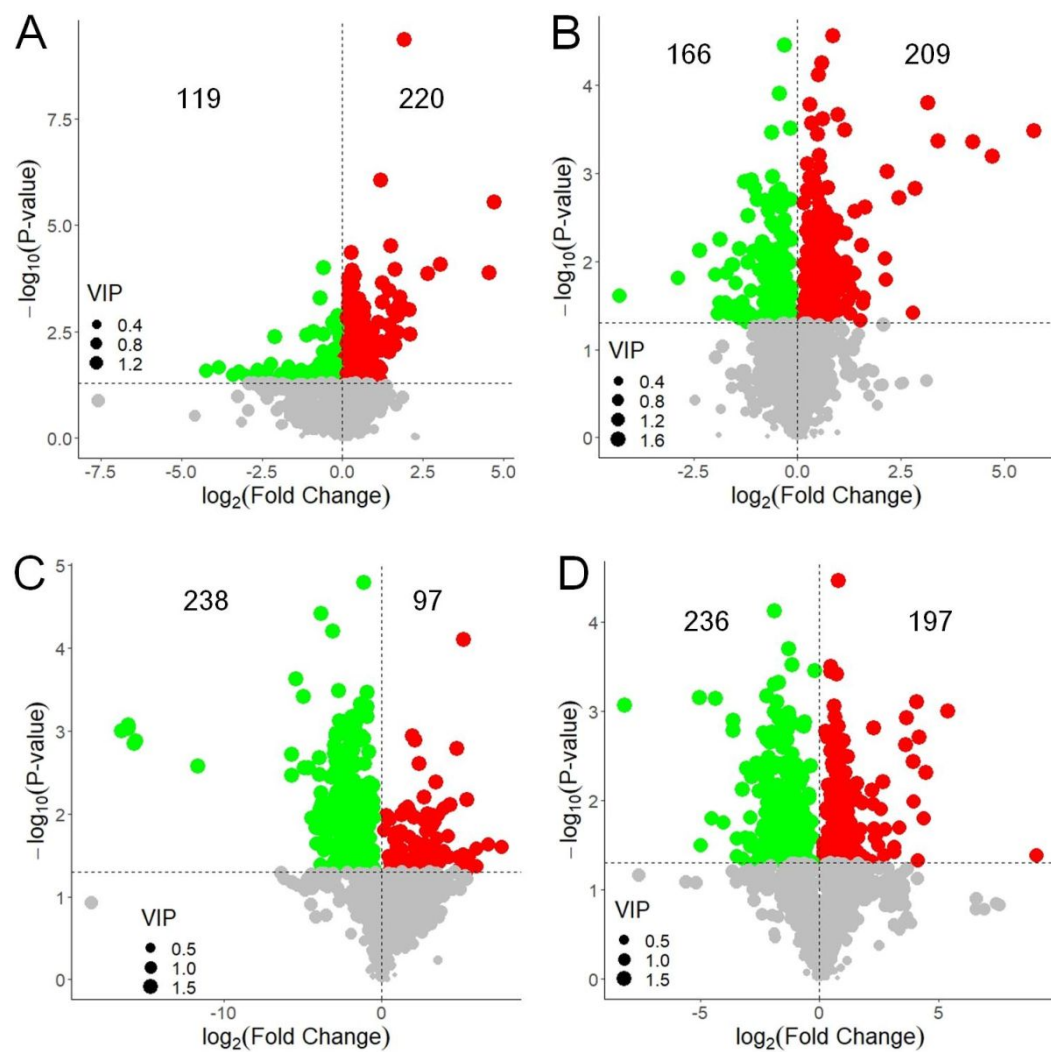
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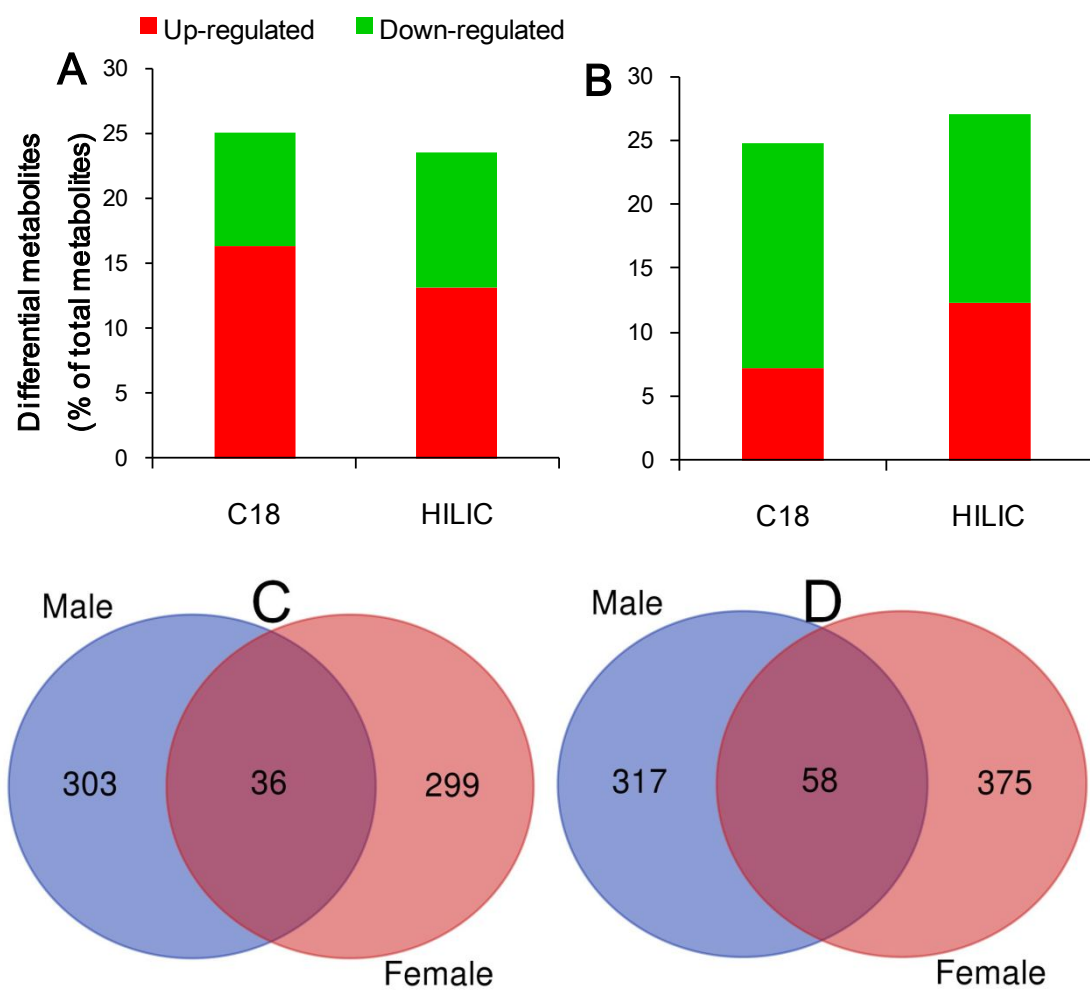
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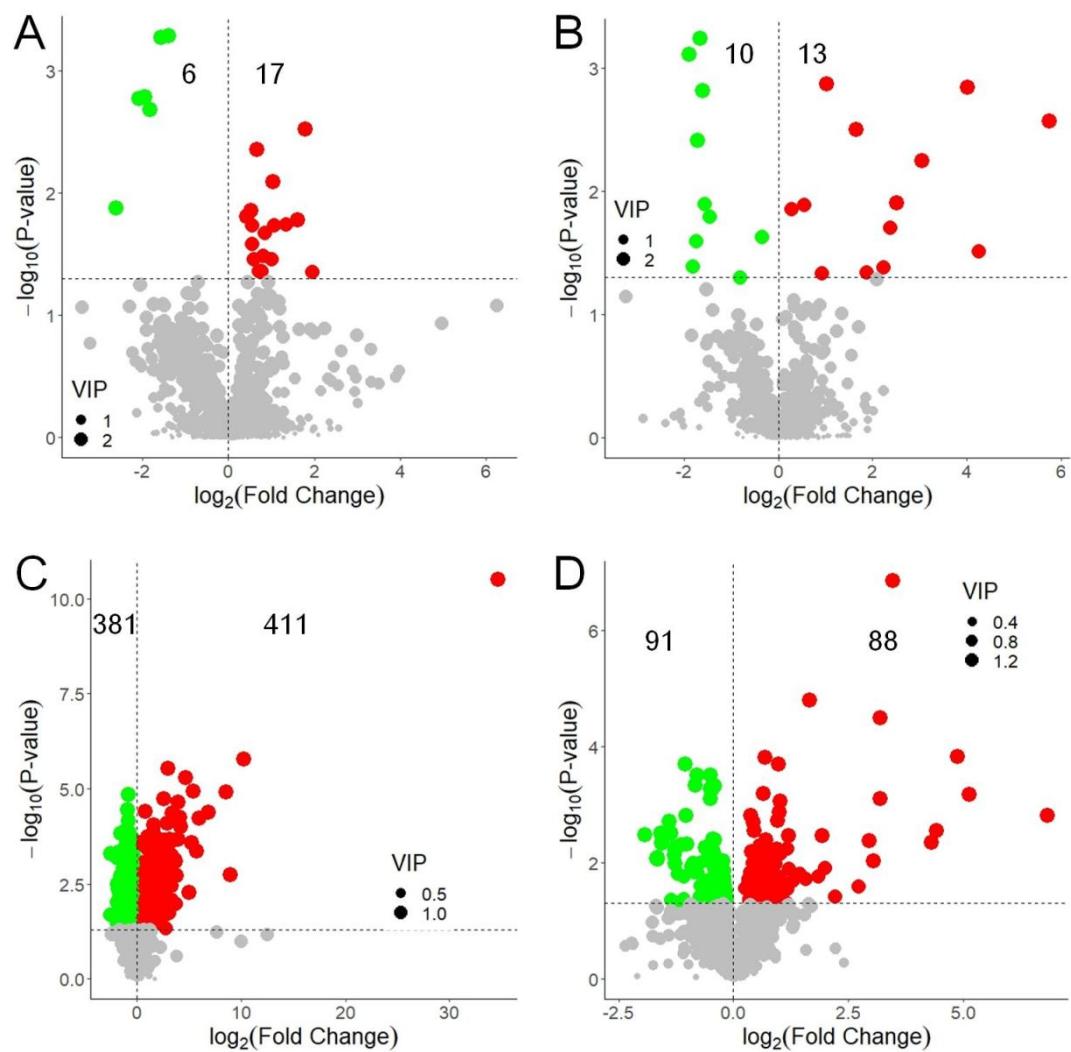
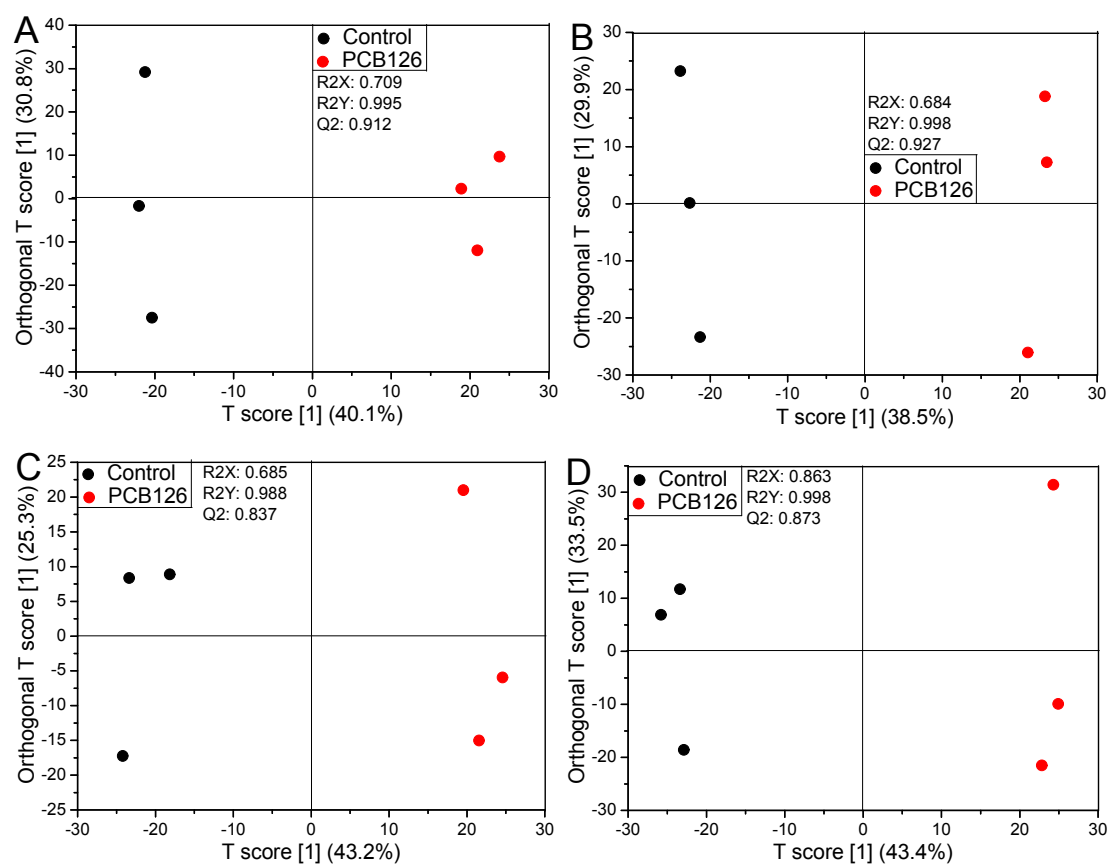**Figure S8**
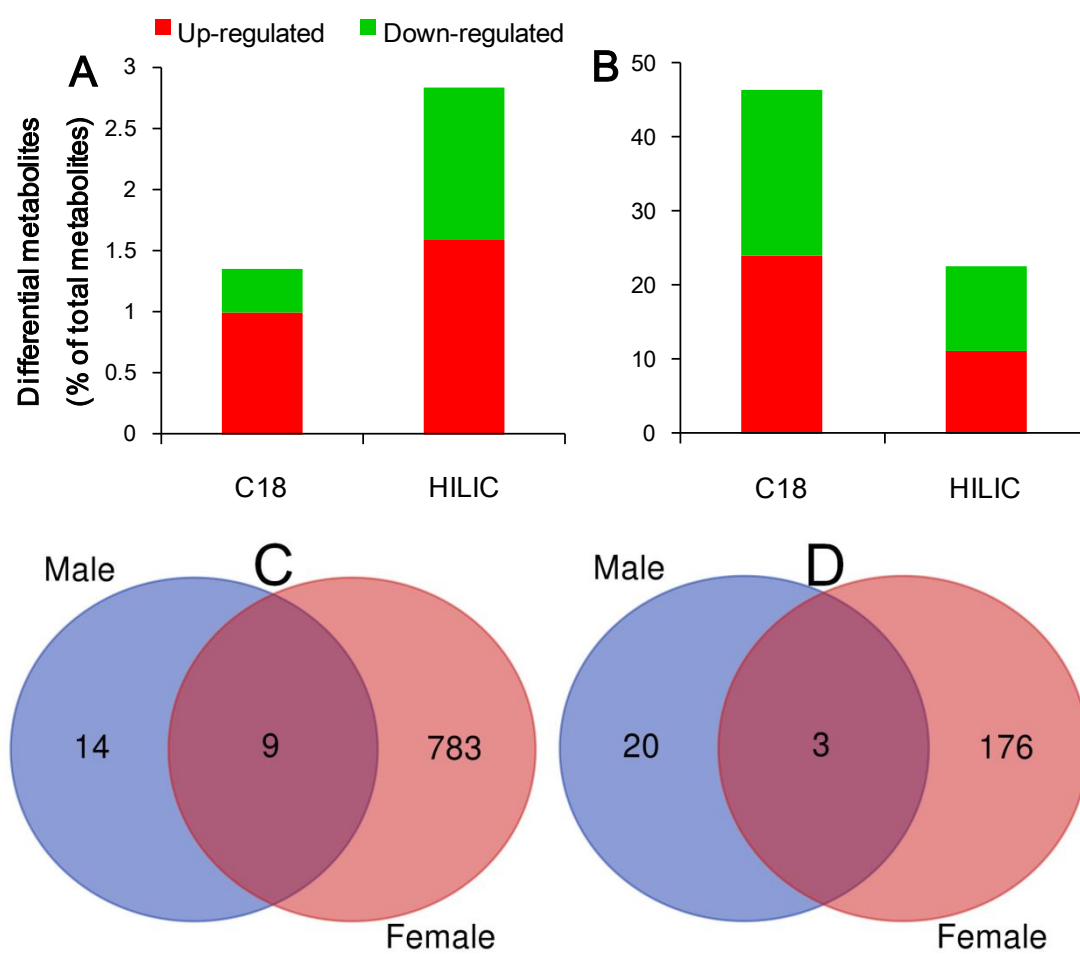
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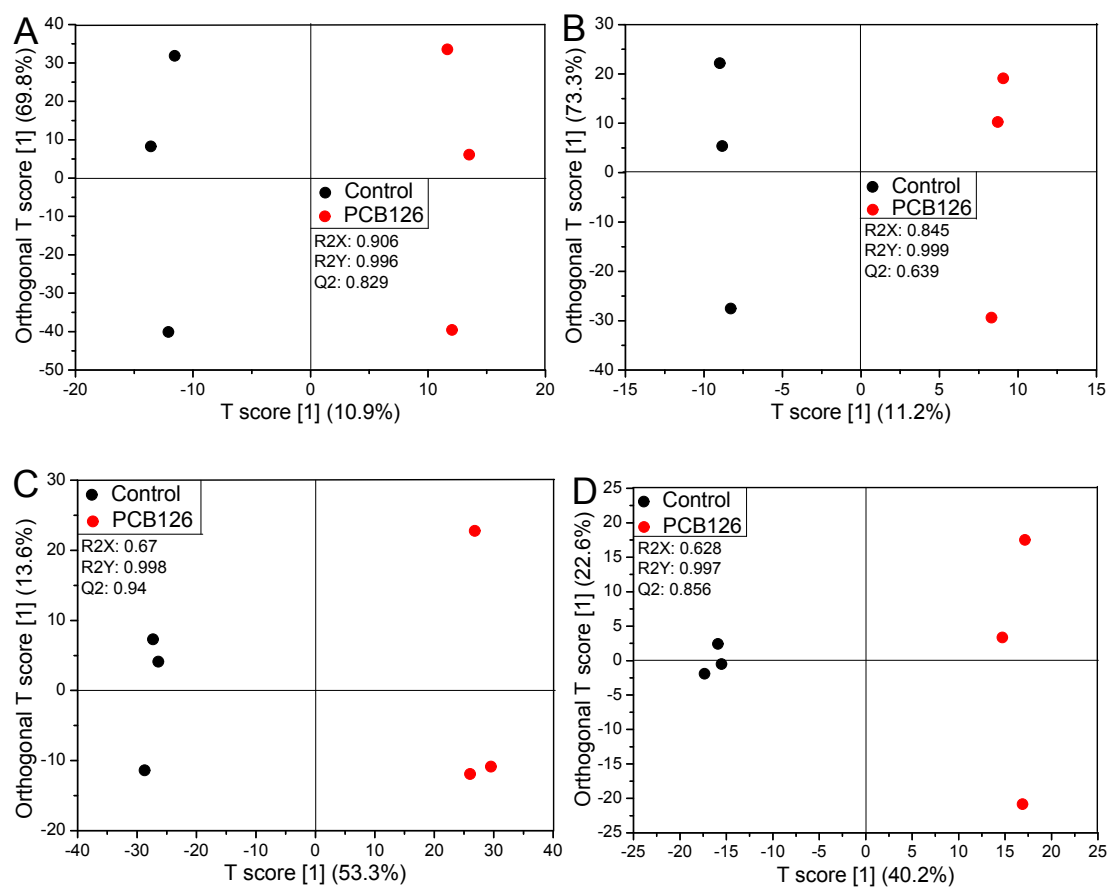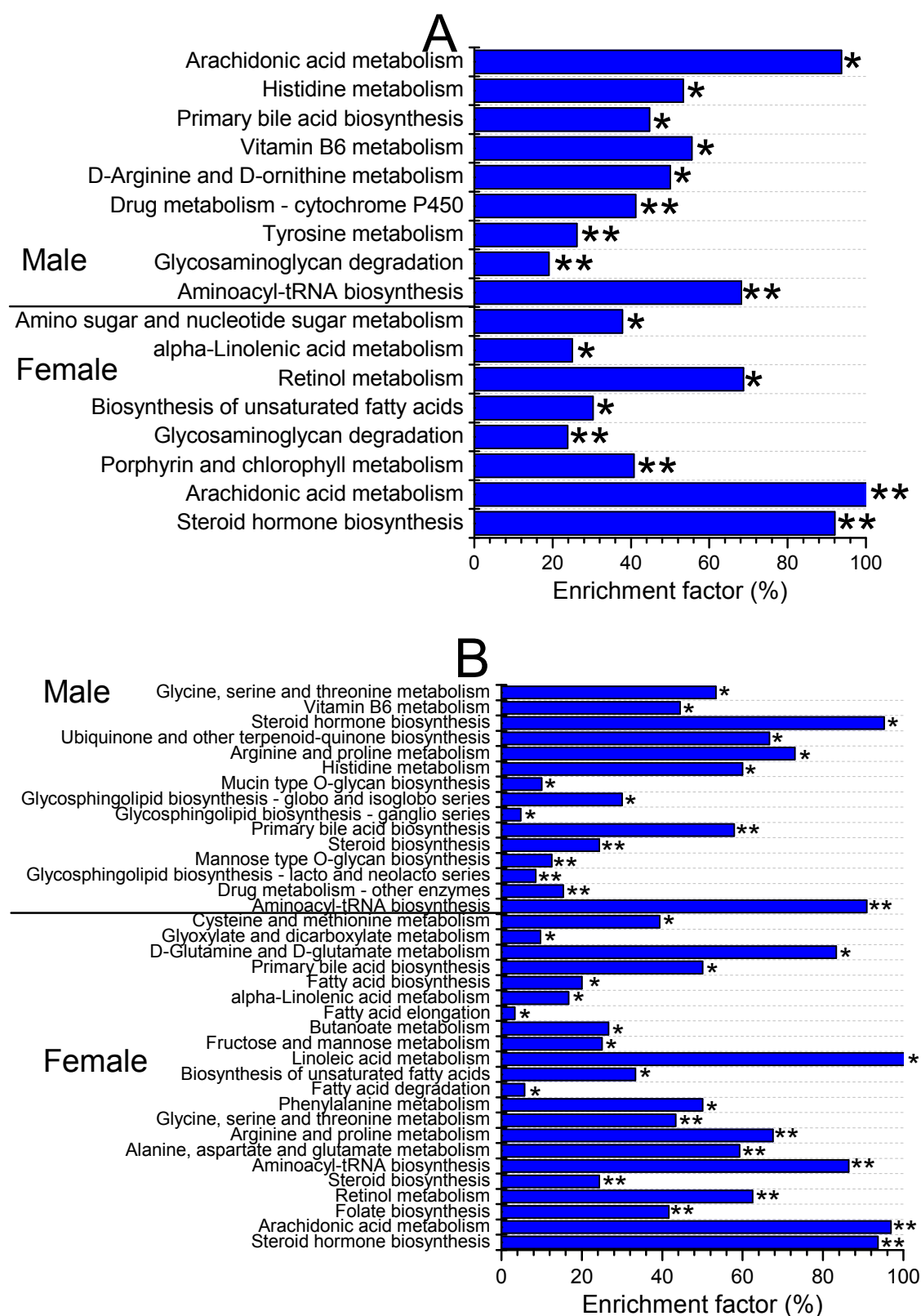
Figure S10

Figure S11

Figure S12

Figure S13

Table S1. Primer pairs of the selected genes examined in this study.[a]

Gene name	Sequence of the primer (5'–3')	Accession number
cyp7a1	F: GATCTTCCCAGCTCTGATCG	NM_201173 [1]
	R: GCAGAGTGTTGGCTTGTGAA	
cyp7b1	F: CCGAACAGCTCTTTTTCAGG	XM_693936 [1]
	R: TTTTTCTCTCCGAGCACGTT	
cyp8b1	F: GTGGTCAAAGAGGCAAGAGC	NM_001003736 [1]
	R: ACACTTTGCCATTCCCAGAC	
hsd3b7	F: CTCTGCAGGAACATCCCAAT	NM_199809 [1]
	R: TGATCCACAGCATCCACACT	
cyp27a1	F: TCCAAAGGACTCGCTTCAGT	NM_001328513 [1]
	R: GCGTCTCGAAGAGAATGGAC	
nr1h4	F: CACAACAAACATCGCATTCC	NM_001002574 [1]
	R: GCTGAAGACTTGGGCTGAAC	
rpl8	F: TTGTTGGTGTTGTTGCTGGT	NM_200713 [1]
	R: GGATGCTCAACAGGGTTCAT	

[a] Abbreviations: **cyp7a1**, cholesterol 7-alpha-monooxygenase; **cyp7b1**, oxysterol 7α-hydroxylase; **cyp8b1**, sterol 12α-hydroxylase; **hsd3b7**, 3β-hydroxy-Δ5-C27-steroid oxidoreductase; **cyp27a1**, sterol 27-hydroxylase; **nr1h4**, nuclear receptor subfamily 1, group H, member 4 (farnesoid X receptor); **rpl8**, ribosomal protein L8.

Reference

(1) Chen, L.; Lam, J. C. W.; Tang, L.; Hu, C.; Liu, M.; Lam, P. K. S.; Zhou, B. Probiotic modulation of lipid metabolism disorders caused by perfluorobutanesulfonate pollution in zebrafish. *Environ. Sci. Technol.* **2020**, *54*, 7494−7503.

Table S2. Information of the metagenomic sequencing and assembled contigs.

Metagenomes		Total raw reads	Total clean reads	Q30%	Assembled contigs	N50 of contigs (bp)	Predicted ORFs
Male	Control-1	87780808	84774084	94.07	282209	1868	565064
	Control-2	88998548	85993166	93.97	301811	1863	604907
	Control-3	81514398	79364820	95.04	254026	2125	528038
	PCB126-1	1.05E+08	1.02E+08	94.38	230320	3391	564413
	PCB126-2	52200881	50769638	95.04	173749	2813	410126
	PCB126-3	1.14E+08	1.1E+08	93.71	242616	3503	601370
Female	Control-1	91292840	88481070	94.31	251234	1980	520262
	Control-2	1.19E+08	1.15E+08	94.12	276534	1965	573100
	Control-3	1.06E+08	1.03E+08	93.84	270791	2127	572020
	PCB126-1	1.1E+08	1.06E+08	93.93	184460	3032	422390
	PCB126-2	77762530	75299952	94.27	153184	2972	353740
	PCB126-3	91314034	87775040	92.81	175420	2874	395711

Table S3. Biochemical alterations in intestine, blood and liver tissues of male and female zebrafish after exposure to 1.0 μg/L PCB126.[a,b]

Tissues	Indices	Male		Female	
		Control	PCB126	Control	PCB126
Intestine	Bile acids (nmol/mg protein)	0.6 ± 0.2	0.5 ± 0.2	0.4 ± 0.0	0.3 ± 0.0
	Total cholesterol (μmol/mg protein)	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1	0.1 ± 0.0
	Free fatty acid (μmol/mg protein)	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
	Glycerol (nmol/mg protein)	0.4 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	0.3 ± 0.1
Blood	Bile acids (nmol/mg protein)	5.6 ± 1.8	4.8 ± 0.7	3.5 ± 0.2	1.9 ± 0.4*
	Total cholesterol (μmol/mg protein)	2.4 ± 0.8	2.2 ± 0.4	3.1 ± 0.2	2.4 ± 0.3
	HDL-C (μmol/mg protein)	20.9 ± 7.4	6.6 ± 1.4	9.3 ± 0.4	7.5 ± 0.6
	LDL-C (μmol/mg protein)	0.1 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	0.3 ± 0.1
Liver	Bile acids (nmol/mg protein)	6.9 ± 0.4	5.1 ± 0.4*	8.7 ± 0.6	8.0 ± 1.8
	Total cholesterol (μmol/mg protein)	0.4 ± 0.2	0.2 ± 0.0	0.7 ± 0.1	0.8 ± 0.2
	Cholecystokinin (pg/mg protein)	108.2 ± 28.0	121.2 ± 13.2	182.6 ± 32.9	140.1 ± 50.9

[a] Abbreviations: LDL-C, Low-density lipoprotein cholesterol; HDL-C, High-density lipoprotein cholesterol;

[b] Values represent the mean ± SEM of three replicates ($n = 3$);

*$P < 0.05$ indicates significant difference between the PCB126 exposure group and the corresponding control group.

Table S4. Gene transcriptional changes involved in hepatic bile acid metabolisms of male and female zebrafish after exposure to 1.0 μg/L PCB126.[a,b]

Genes	Male		Female	
	Control	PCB126	Control	PCB126
cyp7a1	1.0 ± 0.0	0.1 ± 0.0***	1.1 ± 0.4	1.5 ± 0.4
cyp7b1	1.0 ± 0.3	4.8 ± 0.7*	1.1 ± 0.3	1.4 ± 0.5
cyp8b1	1.0 ± 0.1	0.8 ± 0.0	1.0 ± 0.1	2.4 ± 0.3**
hsd3b7	1.0 ± 0.0	2.0 ± 0.2**	1.0 ± 0.2	2.7 ± 0.4*
cyp27a1	1.0 ± 0.2	0.8 ± 0.1	1.0 ± 0.2	1.3 ± 0.1
nr1h4	1.0 ± 0.1	1.2 ± 0.1	1.0 ± 0.0	1.7 ± 0.2*

[a] Abbreviations: **cyp7a1**, cholesterol 7-alpha-monooxygenase; **cyp7b1**, oxysterol 7α-hydroxylase; **cyp8b1**, sterol 12α-hydroxylase; **hsd3b7**, 3β-hydroxy-Δ5-C27-steroid oxidoreductase; **cyp27a1**, sterol 27-hydroxylase; **nr1h4**, nuclear receptor subfamily 1, group H, member 4 (farnesoid X receptor).
[b] Values represent the mean ± SEM of three replicates ($n = 3$);
*P <0.05, **P <0.01 and ***P <0.001 indicate significant difference between the PCB126 exposure group and the corresponding control group.