Supporting Information

Perfluorobutanesulfonate exposure skews sex ratio in fish and transgenerationally impairs reproduction

Lianguo Chen [†,*], James C. W. Lam [§,‡], Chenyan Hu [#], Mirabelle M. P. Tsui [§], Paul K. S. Lam [§], Bingsheng Zhou [†]

[†] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China

[§] State Key Laboratory in Marine Pollution, City University of Hong Kong, Kowloon, Hong Kong SAR, China

[‡] Department of Science and Environmental Studies, The Education University of Hong Kong, Hong Kong SAR, China

[#] School of Chemistry and Environmental Engineering, Wuhan Institute of Technology, Wuhan 430072, China

*** Corresponding author:**

Dr. Lianguo CHEN

Tel: +86 27 68780042

Fax: +86 27 68780123

E-mail: lchenam@ihb.ac.cn

Number of pages: 9

Number of figures: 4

Number of tables: 2

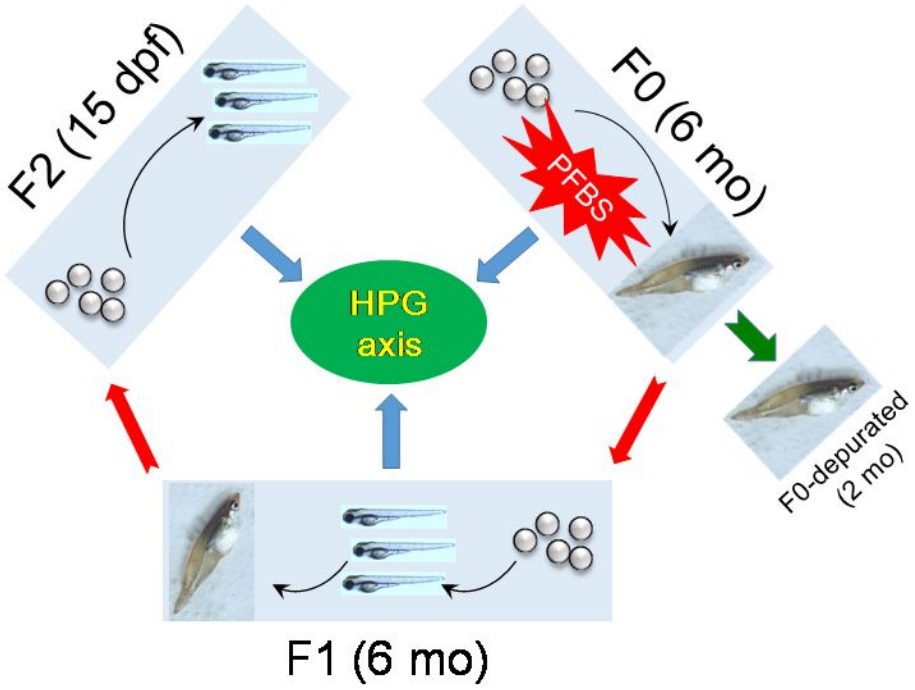

Figure S1. Experimental design. A life-cycle exposure of F0 marine medaka to 0, 1.0, 2.9 and 9.5 μg/L PFBS is conducted from embryos until 6 mo. In order to examine elimination rate of PFBS, some F0 adults are further depurated in clean seawater for 2 mo without PFBS exposure. During the final 2 weeks of exposure of F0 generation, F1 embryos are collected and cultured in clean seawater without further chemical exposure until 6 mo age. F2 embryos are then collected and reared in clean seawater until 15 dpf.

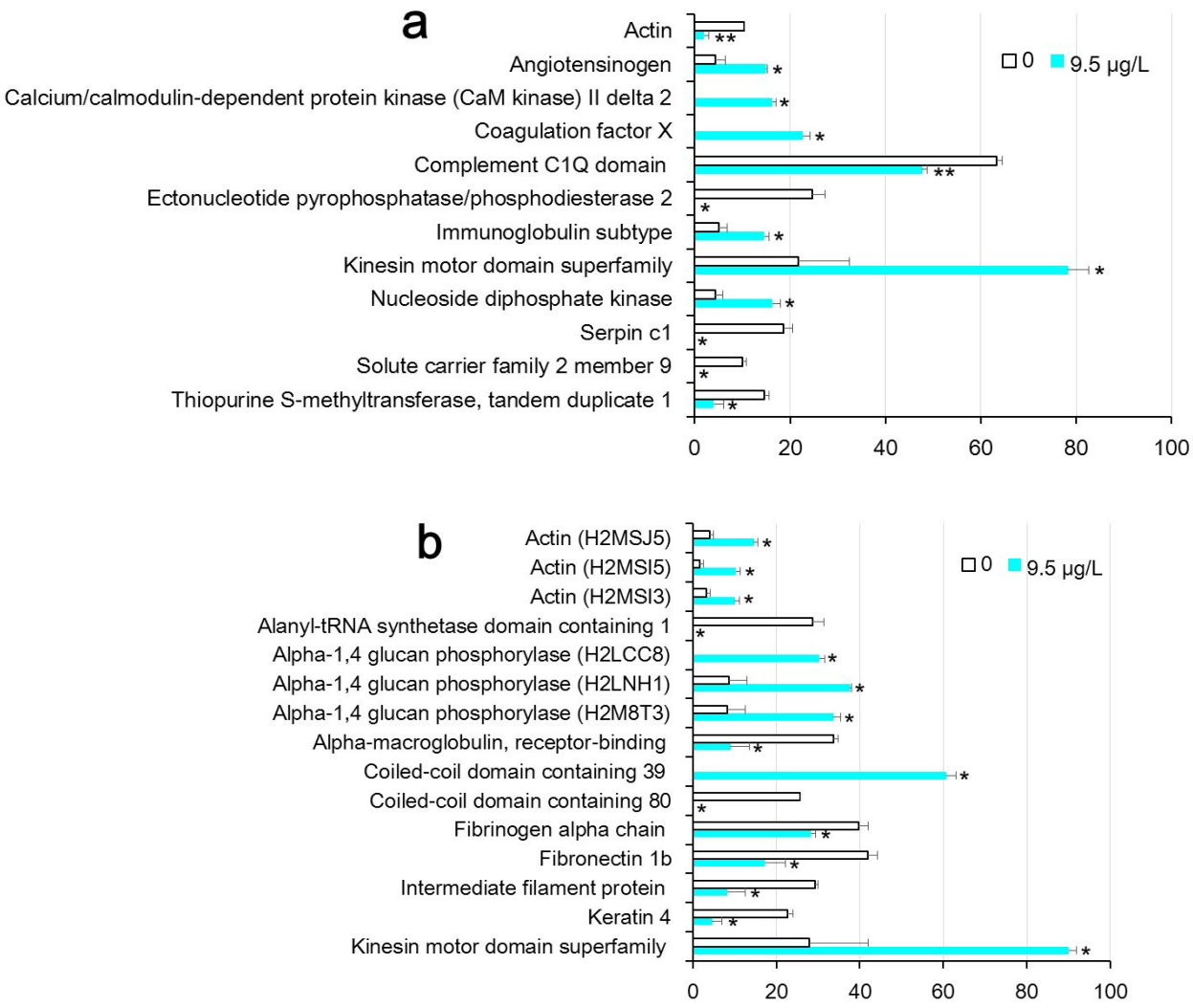

Figure S2. Proteomics identify differential proteins in plasma. Changes in proteomic profiles of F0 adult plasma (**a**, Male; **b**, Female) are shown after a life-cycle exposure to 0 and 9.5 µg/L PFBS. Data are presented as the means ± SEM. Significant difference between control and exposure group is indicated by *$P < 0.05$, and **$P < 0.01$.

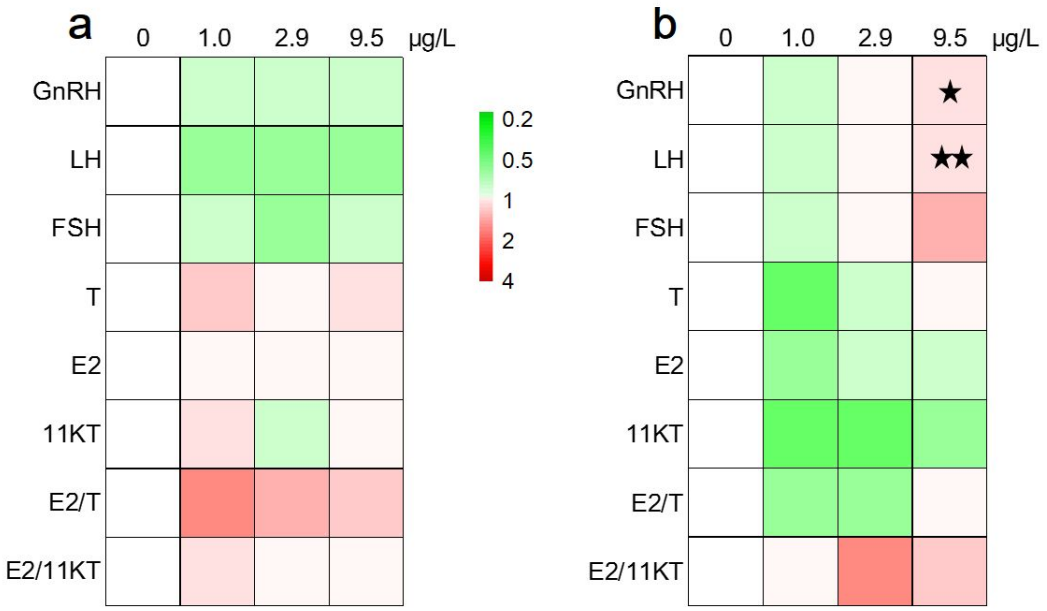

Figure S3. Alterations in HPG hormonal levels of F1 adult medaka by parental exposure. (**a**) Male; (**b**) Female. Green colors represent down-regulation and red colors represent up-regulation relative to control. Significant difference between control and exposure groups (1.0, 2.9 and 9.5 µg/L) is indicated by $*P < 0.05$, and $**P < 0.01$. Abbreviations: Gonadotropin releasing hormone (GnRH); luteinizing hormone (LH); follicle stimulating hormone (FSH); testosterone (T); estradiol (E2); 11-keto-testosterone (11-KT).

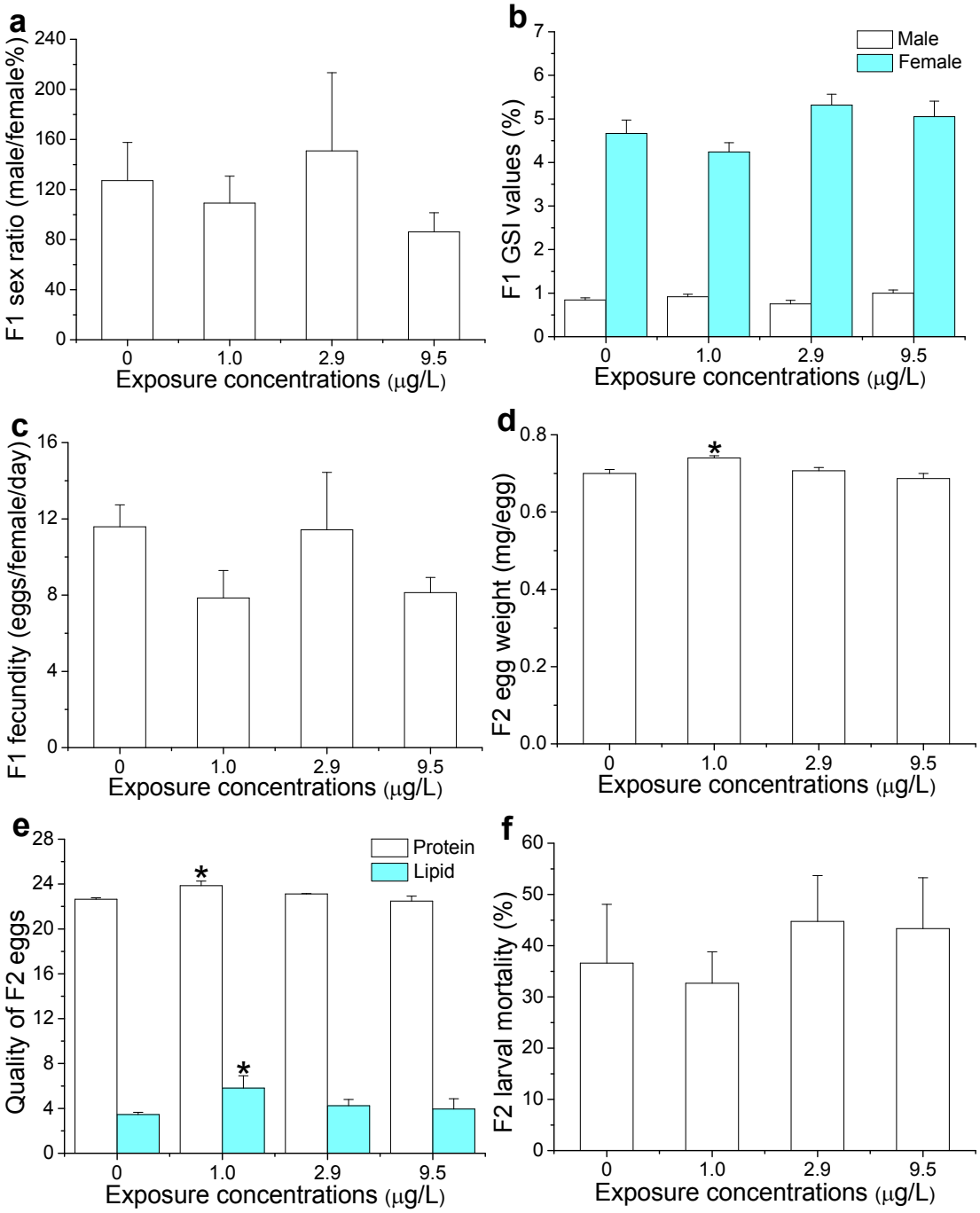

Figure S4. Fitness of F2 generation is transgenerationally impaired by ancestral exposure. Reproductive fitness of F1 adult (**a**, Sex ratio; **b**, GSI values; **c**, Egg production) and condition of F2 generation (**d**, Egg weight; **e**, Protein and lipid storage; **f**, F2 larval mortality) after ancestral exposure to environmentally realistic concentrations of PFBS (0, 1.0, 2.9 and 9.5 μg/L). GSI refers to the gonadosomatic index (GSI = gonad weight/body weight × 100). Data are presented as the means ± SEM. Significant difference between control and exposure group is indicated by $*P < 0.05$.

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

Gene	Sequence of primers (5'–3')
ERα [1]	F: TCGCCGCTGTTGTGCTGTGATGTT
	R: TCCTGGATCTGAGTGCGGGTCCGA
ERβ [1]	F: TGATCCAGGAGGCTGAGCTCCACGA
	R: ACCGCTGACGGAGGCTGTTGTGAT
ERγ [1]	F: TGCGTGTTGTGTATCGCTCACTGTCA
	R: TTTAGGTCCAACAGTCCGGCCAGCTT
ARα [2]	F: TTTGATGAACTGCGGACCTCCTAC
	R: AACTGGTGCAATTTCCTCACAACC
ChgH [1, 3]	F: ATGCCGACTATCCTGTGACC
	R: AGCGTCCAAGAGTCAGAACC
ChgL [1, 3]	F: CAAAGTTCGTGTCTCGACCA
	R: TCAGCATCAAGGGGATAAGC
VTG1 [1]	F: TTGGCAGAGATGCAGCAGCGGT
	R: GGAAATGCAGGACACCCCAGTAGCC
VTG2 [1]	F: AAGCCCTCAACCACTGGCTCCCTAAT
	R: TGGGCAGCGCCGTTCAAGATGTTGAT
CYP17a1 [4]	F: CAGACTACAGCGACCACGTT
	R: CTGGATAATGGATCAGGTAGGT
17βHSD [2]	F: CGCTACCTCCACAAAGTTGTTGTC
	R: AGTTCTGCCTCAACAGTTTCACCT
CYP19a [5]	F: GACACCGTTGTTGGTGACAG
	R: CATCAAAAAGGGCTCTACGC
CYP19b [1]	F: AGGCACAAAGATCAGGAGGGGAACCA
	R: GGTCCACAGCCGAAGGGTTGAAAGAA
FSHR [5]	F: GCGTGTGCGGCTGCTACC
	R: CGAGATGGCGAAGAAGGAGATGG
LHR [5]	F: CCTGGTGGTGTGCTACTGCTAC
	R: CGGCGGAGATGGCGAAGAAG
3βHSD [5]	F: TGGTCAACCCCGTCTATGTG
	R: GGTGTCGTCAGTGGCGAAGT
StAR [2]	F: AGAAGGCTATCAGCATCCTCAGTG
	R: AGGCAGTACCTTACTCAGGACCTT
CYP11a [5]	F: CGTTTGGGTTTACTGCTGGACTAC
	R: GCGGTCAGCTTGGTTGAATATCC
GTHα [5]	F: TGACACCTGCTGTTCCTCTG
	R: GTTGGGTACGCTCTGGAGAA
FSHβ [5]	F: ATATGCGAAGGACGGTGCTAC
	R: AGTACGTGGTTCTGGTGTTGC
LHβ [5]	F: CGGGTTGGCAGAGGGATGTTTC
	R: GGGTCCTTGGTGAAGCAGTGG
sGnRH [6]	F: CAGAGTGACGGTGCGGGTGTT
	R: TATGGTCTAAGTCTCTCTTGGGT

mGnRH [7]	F: ATGAAAACGTGGATGCTGTG
	R: CAGGGTGCATTGCTGTTAAG
GnRHR [2]	F: GAAGGCTCGGATGAAGACACTGAA
	R: CCAGATCCCAAGCAGGTAGTATGG
CYP11b [2]	F: GCATTGGCCTGTTCTCCTCATCT
	R: CGGAGGGGTGGTTGTTAGCATT
VTGR [7]	F: GACAACCCGGTCTACCTGAA
	R: CTGGATTGAAGGAGGGATGA
18s	F: GACAAATCGCTCCACCAACT
(control) [3, 5]	R: CCTGCGGCTTAATTTGACCC
	R: ACTGGCCAAAGTCTTCCAACGGGA

[a] Abbreviations: ER, Estrogen receptor; AR, Androgen receptor; Chg, Choriogenin; VTG, Vitellogenin; CYP17a1, Cytochrome P450 17a1; 17βHSD, 17β-Hydroxysteroid dehydrogenase; CYP19, Cytochrome P450 19; FSHR, Follicle stimulating hormone receptor; LHR, Luteinizing hormone receptor; 3βHSD, 3β-Hydroxysteroid dehydrogenase; StAR, Steroidogenic acute regulatory protein; CYP11a, 20,22-Desmolase; GTHα, Gonadotropin α; FSHβ, Follicle stimulating hormone β; LHβ, Luteinizing hormone β; sGnRH, salmon-type Gonadotropin releasing hormone (GnRH); mGnRH, medaka-type GnRH; GnRHR, GnRH receptor; CYP11b, Cytochrome P450 11b; VTGR, VTG receptor.

References

(1) Xu, E. G. B.; Liu, S.; Ying, G. G.; Zheng, G. J. S.; Lee, J. H. W.; Leung, K. M. Y. The occurrence and ecological risks of endocrine disrupting chemicals in sewage effluents from three sewage treatment plants, and in natural seawater from a marine reserve of Hong Kong. *Mar. Pollut. Bull.* **2014**, *85* (2), 352–362.

(2) Ye, T.; Kang, M.; Huang, Q.; Fang, C.; Chen, Y.; Shen, H.; Dong, S. Exposure to DEHP and MEHP from hatching to adulthood causes reproductive dysfunction and endocrine disruption in marine medaka (*Oryzias melastigma*). *Aquat. Toxicol.* 2**014**, *146*, 115– 126.

(3) Chen, X.; Li, V. W. T.; Yu, R. M. K.; Cheng, S. H. Choriogenin mRNA as a sensitive molecular biomarker for estrogenic chemicals in developing brackish medaka (*Oryzias melastigma*). *Ecotoxicol. Environ. Saf.* **2008**, *71* (1), 200–208.

(4) Rhee, J. S.; Kim, B. M.; Choi, B. S.; Choi, I. Y.; Wu, R. S. S.; Nelson, D. R.; Lee, J. S. Whole spectrum of cytochrome P450 genes and molecular responses to water-accommodated fractions exposure in the marine medaka. *Environ. Sci. Technol.* **2013**, *47* (9), 4804–4812.

(5) Tse, A. C. K.; Lau, K. Y.; Ge, W.; Wu, R. S. A rapid screening test for endocrine disrupting chemicals using primary cell culture of the marine medaka. *Aquat. Toxicol.* **2013**, *144-145*, 50–58.

(6) Sun, L.; Zuo, Z.; Chen, M.; Chen, Y.; Wang, C. Reproductive and transgenerational toxicities of phenanthrene on female marine medaka (*Oryzias melastigma*). *Aquat. Toxicol.* **2015**, *162*, 109–116.

(7) Chen, L.; Zhang, W.; Ye, R.; Hu, C.; Wang, Q.; Seemann, F.; Au, D. W. T.; Zhou, B.; Giesy, J. P.; Qian, P. Y. Chronic exposure of marine medaka (*Oryzias melastigma*) to 4,5-dichloro-2-n-octyl-4-isothiazolin-3-one (DCOIT) reveals its mechanism of action in endocrine disruption via the hypothalamus-pituitary-gonadal-liver (HPGL) axis. *Environ. Sci. Technol.* **2016**, *50*, 4492–4501.

Table S2. Gene transcriptional changes along HPG axis of adult F0 medaka after a life-cycle exposure to environmentally realistic concentrations of PFBS (0, 1.0, 2.9 and 9.5 μg/L).[a]

Tissues	Genes	Male				Female			
		0 μg/L	1.0 μg/L	2.9 μg/L	9.5 μg/L	0 μg/L	1.0 μg/L	2.9 μg/L	9.5 μg/L
Brain	*sGnRH*	1.1±0.3	1.2±0.2	1.8±0.4	1.1±0.4	1.1±0.4	2.1±1.2	6.7±1.7**	1.8±0.6
	mGnRH	1.1±0.2	1.8±0.2	1.6±0.5	1.4±0.5	1.2±0.6	3.4±0.7	13.3±6.5*	2.7±0.8
	GnRHR	1.1±0.3	1.1±0.2	1.0±0.1	0.6±0.2	1.2±0.4	1.4±0.2	2.2±0.9	0.7±0.2
	GTHα	1.1±0.4	0.6±0.2	0.5±0.1	0.7±0.2	1.2±0.5	1.1±0.2	2.5±0.7	0.5±0.1
	LHβ	1.1±0.4	0.9±0.1	0.4±0.0*	0.8±0.2	1.0±0.1	1.0±0.1	1.3±0.4	0.5±0.1
	FSHβ	1.1±0.4	1.0±0.3	0.4±0.1	0.4±0.1	1.2±0.6	0.8±0.2	3.8±1.8	0.4±0.2
	CYP19b	1.1±0.3	0.8±0.1	0.9±0.3	0.8±0.1	1.1±0.4	1.6±0.3	2.1±0.2	1.0±0.3
	ERα	1.1±0.3	1.1±0.2	1.2±0.3	1.1±0.2	1.3±0.6	1.0±0.2	2.3±0.5	1.0±0.1
	ERβ	1.2±0.4	0.9±0.1	1.2±0.3	1.0±0.3	1.2±0.6	0.9±0.2	1.9±0.3	1.0±0.1
	ERγ	1.1±0.4	1.1±0.3	1.3±0.3	0.8±0.2	1.3±0.6	1.4±0.1	2.6±0.9	0.8±0.1
	ARα	1.0±0.1	1.3±0.3	1.6±0.0*	1.2±0.2	1.0±0.2	1.1±0.1	1.7±0.4*	0.9±0.1
Gonad	*LHR*	1.0±0.2	0.5±0.1**	0.5±0.1*	0.3±0.0**	1.0±0.0	2.0±0.5	0.8±0.3	0.8±0.1
	FSHR	1.0±0.1	0.5±0.1**	0.7±0.1*	0.5±0.0**	1.0±0.1	1.3±0.4	0.3±0.2	1.0±0.3
	VTGR	1.0±0.1	0.4±0.0*	0.5±0.2*	0.3±0.0**	1.1±0.4	1.1±0.2	0.8±0.3	1.1±0.3
	Star	1.0±0.2	0.5±0.2	0.4±0.1*	0.4±0.1*	1.1±0.2	0.8±0.4	1.1±0.2	0.2±0.1*
	CYP11a	1.0±0.0	0.6±0.1*	0.5±0.1**	0.3±0.1**	1.0±0.2	1.6±0.6	0.8±0.4	0.5±0.1
	3βHSD	1.0±0.1	0.7±0.1	0.6±0.1*	0.4±0.1**	1.0±0.1	1.2±0.5	0.4±0.2	0.4±0.2
	CYP17a1	1.0±0.1	0.5±0.1**	0.4±0.1**	0.2±0.1**	1.0±0.2	1.1±0.3	0.8±0.4	0.4±0.2
	17βHSD	1.0±0.1	0.4±0.1**	0.4±0.1**	0.3±0.0**	1.0±0.2	1.4±0.7	1.0±0.5	2.1±0.8
	CYP19a	1.0±0.2	0.3±0.0**	0.8±0.1	0.3±0.0**	1.0±0.1	1.6±1.0	0.7±0.3	0.4±0.3

	CYP11b	1.0±0.0	0.5±0.1**	0.5±0.0**	0.4±0.1**	1.0±0.1	0.9±0.3	0.7±0.1	2.0±0.9
Liver	*ERα*	1.0±0.1	0.4±0.1*	1.1±0.2	0.4±0.1*	1.3±0.7	1.1±0.2	1.1±0.3	0.2±0.1**
	ERβ	1.0±0.2	0.8±0.1	0.6±0.3	0.4±0.1*	1.1±0.3	0.8±0.1	0.8±0.2	0.8±0.2
	ERγ	1.1±0.4	0.4±0.1	0.5±0.3	0.8±0.3	1.4±0.8	2.5±1.5	2.6±1.2	2.6±2.2
	ARα	1.0±0.2	1.0±0.3	0.8±0.5	0.6±0.2	1.1±0.4	1.4±0.4	1.0±0.1	1.2±0.4
	VTG-1	1.1±0.3	0.6±0.1	0.4±0.3	0.2±0.1*	1.2±0.5	1.1±0.4	0.9±0.3	0.1±0.0**
	VTG-2	1.1±0.3	0.6±0.1	0.6±0.3	0.2±0.1*	1.1±0.5	1.2±0.8	0.8±0.4	0.0±0.0**
	ChGH	1.1±0.3	0.3±0.1*	0.7±0.5	0.3±0.1*	1.3±0.8	1.4±0.5	1.2±0.4	0.1±0.0**
	ChGL	1.2±0.4	0.5±0.1	0.8±0.6	0.3±0.1	1.2±0.6	1.7±0.5	1.9±0.8	0.2±0.0

[a] Values represent the mean ± SEM of three replicates.

*$P < 0.05$, and **$P < 0.01$ indicate significant difference between exposure groups and the corresponding control group.