SUPPLEMENTAL MATERIALS

Promotion of Hepatocarcinogenesis by Perfluoroalkyl Acids in Rainbow Trout

Abby D. Benninghoff, Gayle A. Orner, Clarissa H. Buchner, Jerry D. Hendricks,
Aaron M. Duffy and David E. Williams

TABLE OF CONTENTS

Page

Supplemental Tables

Supplemental Figures

SUPPLEMENTAL TABLES

Supplemental Table 1. Primer set sequences for real-time RT-PCR validation of gene expression

Gene name (symbol)	Array feature	Forward primer (5’ to 3’)	Reverse primer (5’ to 3’)	Size (bp)	OAT (°C)[f]
Genes of interest					
Alpha-3-macroglobulin (*a2m*)	OmyOSU8	ACAAGGCTCGGGGAATACTT	CTCCAGCATTGAAGCAGTGA	235	60
Cathepsin D (*ctsd*) [a]	OmyOSU139	TCCACTATCCATCATCTACC	AGATCAGTGCATTTCAACTC	272	56-58
Cytochrome P450 1A1 (*cyp1a1*) [b,c]	OmyOSU396	TCAACTTACCTCTGCTGGAAGC	GGTGAACGGCAGGAAGGA	85	60
Cytochrome P450 2K5 (*cyp2k5*) [a]	OmyOSU1389	GTGTCAACTCTAATCTAGTGCCC	CCGTCCCTGATTGAAGTGAC	368	58-60
Hemopexin (*hpx*)	OmyOSU699	GCAGCAGAAGCAAAACATCA	CAGCACATTCAGAGGGACAA	161	55
Prostaglandin D synthase (*pgds*) [a]	OmyOSU1395	CATAATGGGAGTTCTGCTGTG	TGGGATGTCAGTCTTCTTGG	293	57
Trout c-polysaccharide binding protein (*tcpbp*) [e]	OmyOSU1478	GGCCAAAGGAGACATCGTTT	TCCCAACCTACACCCTGACC	155	62-64
Thioredoxin (*thx*)	OmyOSU1422	TCCCAACAGCATTGCTCTAA	CCATGCCTCTAAATCCTCCA	122	55
Vitellogenin (*vtg1*) [e]	OmyOSU203	TTGCCTTTGCCAACATCGAC	CGGACATTGACGTATGCTTT	238	54
Genes for normalization					
β-actin (*actb*) [a]	OmyOSU205	GTGCGGGATTATATCATTTACCCT	CCACGTAGCTGTCTTTCTGG	221	58-60
Glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) [a]	OmyOSU229	CCAACCAAACGCTACCGAAC	CCAGATTCCATCTCACCTT	173	60
DNA topoisomerase 2 (*top2a*)	OmyOSU1644	CTGCAGCAGTCCCTCACTT	CAAAGAAATCCCTCAGCACA	100	55
ATP synthase subunit beta (*atp5b*)	OmyOSU1585	GCCCATGGTGGTTACTCTGT	AGGTGTCGTCCTTCAGGTTG	112	55

[a] Benninghoff and Williams (2008).
[b] Primer pair does not surround the sequence for the corresponding 70mer oligonucleotide on the OSUrbt array.
[c] Rees and Li (2004).
[d] Mortensen *et al.* (2006).
[e] Tilton *et al.* (2006).
[f] Measured optimal annealing temperature (OAT).

Supplemental Table 2. Logistic regression analyses for experimental factors diet, sex, body weight, replicate tank and symptoms of liver disease in AFB_1-initiated trout

		All subjects [a]		Final subjects [a]	
Treatment	**Factor**	***p*-Value**	**OR (95% CI)**	***p*-Value**	***OR* (95% CI)**
AFB_1/E2	Diet	**<0.0001**	**34.0 (10.3 - 112)**	**<0.0001**	**26.4 (7.61 - 91.6)**
	Sex	0.4478	0.82 (0.49 - 1.38)	0.4407	0.08 (0.44 - 1.44)
	Body weight	**0.0027**	**1.02 (1.01 - 1.03)**	**0.0046**	**1.02 (1.01 - 1.03)**
	Replicate tank	0.7710	1.08 (0.63 - 1.87)	0.2238	1.47 (0.79 - 2.72)
	Liver disease symptoms	0.0923	0.52 (0.24 - 1.11)		
AFB_1/PFOA	Diet	**<0.0001**	**25.1 (7.94 - 79.5)**	**0.0014**	**27. 3 (3.57 - 209)**
	Sex	0.2148	0.73 (0.44 - 1.20)	0.5655	0.78 (0.33 - 1.84)
	Body weight	**0.0010**	**1.02 (1.01 - 1.03)**	**0.0384**	**1.01 (1.00 - 1.02)**
	Replicate tank	0.5498	0.86 (0.53 - 1.41)	0.3298	0.66 (0.29 - 1.52)
	Liver disease symptoms	0.4167	1.45 (0.59 - 3.54)		
AFB_1/PFNA	Diet	**<0.0001**	**26.0 (7.09 - 95.1)**	**<0.0001**	**24.2 (5.96 - 98.2)**
	Sex	0.6675	0.88 (0.50 - 1.57)	0.4457	0.78 (0.14 - 1.49)
	Body weight	**0.0003**	**1.02 (1.01 - 1.03)**	**0.0002**	**1.02 (1.01 - 1.03)**
	Replicate tank	0.8392	1.06 (0.60 - 1.87)	0.4507	1.29 (0.67 - 2.46)
	Liver disease symptoms	0.7215	1.15 (0.54 - 2.47)		
AFB_1/PFDA	Diet	**<0.0001**	**41.7 (9.52 - 182)**	**<0.0001**	**34.0 (6.89 - 168)**
	Sex	0.7178	0.88 (0.44 - 1.75)	0.9424	0.97 (0.45 - 2.10)
	Body weight	**0.0102**	**1.01 (1.00 - 1.02)**	**0.0186**	**1.01 (1.00 - 1.03)**
	Replicate tank	0.7750	1.11 (0.56 - 2.20)	0.3806	1.45 (0.63 - 3.34)
	Liver disease symptoms	0.9061	1.06 (0.42 - 2.69)		
AFB_1/8:2FtOH	Diet	0.1652	2.34 (0.70 - 7.78)	0.5649	1.51 (0.37 - 6.11)
	Sex	0.9379	1.02 (0.62 - 1.69)	0.5693	1.18 (0.66 - 2.12)
	Body weight	**<0.0001**	**1.02 (1.01 - 1.03)**	**<0.0001**	**1.02 (1.01 - 1.03)**
	Replicate tank	0.8213	1.06 (0.62 - 1.82)	0.4806	1.25 (0.67 - 2.31)
	Liver disease symptoms	**0.0064**	**2.43 (1.28 - 4.61)**		
AFB_1/CLOF	Diet	0.8866	0.92 (0.27 - 3.11)	0.3397	0.51 (0.13 - 2.05)
	Sex	0.0428	0.55 (0.31 - 0.98)	0.2793	0.71 (0.38 - 1.32)
	Body weight	**0.0003**	**1.01 (1.01 - 1.02)**	**0.0016**	**1.01 (1.01 - 1.02)**
	Replicate tank	0.3261	1.32 (0.76 - 2.31)	0.1199	1.65 (0.88 - 3.12)
	Liver disease symptoms	**0.0195**	**2.27 (1.14 - 4.53)**		
AFB_1/PFOS (15 wks)	Diet	**0.0025**	**19.2 (2.83 - 131)**	**0.0014**	**27.3 (3.57 - 209)**
	Sex	0.9021	0.95 (0.42 - 2.12)	0.5655	0.78 (0.33 - 1.84)
	Body weight	0.0559	1.01 (1.00 - 1.02)	**0.0384**	**1.01 (1.00 - 1.02)**
	Replicate tank	0.3349	0.68 (0.31 - 1.50)	0.3298	0.66 (0.29 - 1.52)
	Liver disease symptoms	0.9054	1.10 (0.23 - 5.17)		

[a] Logistic regression analysis was performed including all experimental subjects to determine the impact of five experimental factors on liver tumor outcome in AFB_1-initiated trout; all comparisons were made compared to the AFB_1/CON treatment group, except for the PFOS treatment which was compared to AFB_1(15wk)/CON. Firth's bias correction was used as the likelihood penalty when a maximum likelihood estimate was not obtained. Chi-square *P*-values and odds ratios (OR) with 95% confidence intervals (CI) are shown. A significant effect of the indicated experimental factor was inferred when $p<0.05$.

[b] Logistic regression analyses were performed as before, but subjects with symptoms of idiopathic liver disease were excluded; thus, only four experimental factors were evaluated.

Supplemental Table 3. Logistic regression analyses for experimental factors diet, sex, body weight, replicate tank and symptoms of liver disease in MNNG-initiated trout

		Liver tumors [c]		**Kidney tumors** [c]		**Stomach tumors** [c]		**Swim bladder tumors** [c]	
Treatment	**Factor**	***p*-Value**	***OR* (95% CI)**	***p*-Value**	***OR* (95% CI)**	***p*-Value**	***OR* (95% CI)**	***p*-Value**	***OR* (95% CI)**
Including all subjects [a]									
MNNG/E2	Diet	**<0.0001**	**17.7 (4.98 – 62.6)**	0.1946	2.00 (0.70 - 5.70)	0.0512	190 (0.97 - ∞)	**0.0134**	**3.76 (1.32 - 10.7)**
	Sex	0.0861	1.58 (0.94 – 2.68)	0.7165	1.09 (0.70 - 1.70)	0.5341	1.74 (0.30 - 9.93)	**0.0079**	**1.84 (1.17 - 2.88)**
	Body weight	0.1702	1.01 (1.00 - 1.01)	0.3607	1.00 (1.00 - 1.01)	0.4075	0.99 (0.97 - 1.01)	**0.0044**	**1.01 (1.00 - 1.02)**
	Tank	0.6588	0.88 (0.50 – 1.56)	0.3450	0.80 (0.49 - 1.28)	0.0523	0.07 (0.01 - 1.03)	**0.0139**	**0.55 (0.34 - 0.89)**
	Liver disease symptoms	0.1007	0.58 (0.30 – 1.11)	0.0201	0.51 (0.29 - 0.90)	0.2097	0.25 (0.03 - 2.21)	0.3785	1.28 (0.74 - 2.22)
MNNG/PFOA	Diet	**<0.0001**	**17.1 (5.04 – 57.9)**	0.8457	0.90 (0.30 - 2.68)	0.3915	0.13 (0.00 - 14.4)	**<0.0001**	**12.3 (3.92 -38.4)**
	Sex	0.5145	1.18 (0.71 - 1.97)	0.8797	1.04 (0.65 - 1.66)	0.2572	3.65 (0.39 - 34.4)	0.5430	1.42 (0.88 - 2.31)
	Body weight	0.1001	1.01 (1.00 - 1.01)	0.1867	1.01 (1.00 - 1.01)	0.5701	0.99 (0.97 - 1.02)	**0.0005**	**1.01 (1.01 - 1.02)**
	Tank	0.2723	0.75 (0.44 - 1.26)	0.8432	0.95 (0.59 - 1.55)	0.8590	1.18 (0.18 - 7.60)	**<0.0001**	**0.29 (0.17 - 0.47)**
	Liver disease symptoms	0.3296	0.70 (0.34 - 1.44)	0.7962	0.92 (0.48 - 1.75)	0.1657	0.18 (0.02 - 2.04)	0.1813	1.58 (0.81 - 3.06)
Final subjects [b]									
MNNG/E2	Diet	**<0.0001**	**18.9 (4.37 – 82.2)**	0.1127	2.58 (0.80 – 8.36)	0.3497	14.0 (0.06 - ∞)	**0.0083**	**5.05 (1.52 – 16.8)**
	Sex	0.2360	1.47 (0.78 – 2.77)	0.7898	1.07 (0.64 - 1.79)	0.7729	1.33 (0.20 – 8.94)	0.0857	1.58 (0.94 – 2.67)
	Body weight	0.1525	1.01 (1.00 – 1.02)	0.7820	1.00 (0.99 – 1.01)	0.5054	0.99 (0.97 - 1.02)	0.0677	1.01 (1.00 - 1.02)
	Tank	0.9725	0.99 (0.52 – 1.88)	0.3355	0.78 (0.46 – 1.30)	0.3316	0.28 (0.02 – 3.64)	**0.0230**	**0.55 (0.33 – 0.92)**
MNNG/PFOA	Diet	**<0.0001**	**17.0 (4.47 – 64.4)**	0.4424	0.63 (0.19 – 2.07)	0.3163	0.05 (0.00 – 18.5)	**<0.0001**	**12.47 (3.59 – 43.4)**
	Sex	0.8812	0.96 (0.54 – 1.69)	0.8380	0.95 (0.56 – 1.61)	0.5522	2.11 (0.18 – 24.7)	0.5034	1.20 (0.70 – 2.07)
	Body weight	**0.0213**	**1.01 (1.00 – 1.02)**	0.7580	1.00 (0.99 - 1.01)	0.2675	0.98 (0.95 - 1.01)	**0.0074**	**1.01 (1.00 – 1.02)**
	Tank	0.5656	0.85 (0.48 – 1.50)	0.7980	1.07 (0.63 – 1.83)	0.4622	2.56 (0.21 – 31.4)	**<0.0001**	**0.28 (0.17 – 0.50)**

[a] Logistic regression analysis was performed including all experimental subjects to determine the impact of five experimental factors on liver tumor outcome in MNNG-initiated trout; all comparisons were made compared to the MNNG/CON treatment group. Firth's bias correction was used as the likelihood penalty when a maximum likelihood estimate was not obtained.

[b] Logistic regression analyses were performed as before, but subjects with symptoms of idiopathic liver disease were excluded; thus, only four experimental factors were evaluated.

[c] Chi-square *P*-values and odds ratios (OR) with 95% confidence intervals (CI) are shown. A significant effect of the indicated experimental factor was inferred when $p<0.05$.

Supplemental Table 4. Summary of array data following application of selection filters for significance, level of response and feature consistency

	Number of features passing indicated criterion				
Treatment	**Welch's *t*-test** [a]	**Mean 2-fold change** [b]	**Spot consistency** [c]	**All criteria** [d]	**Portion of array differentially regulated (%)**
CON	N/A	3	0	0	0
E2	266	103	107	60	3.8
PFOA	205	109	118	88	5.3
PFNA	342	266	241	175	10.8
PFDA	230	139	132	91	5.6
PFOS	204	115	73	44	2.8
8:2FtOH	99	35	33	26	1.7
CLOF	101	21	21	5	0.30

[a] Number of array features that were identified as significantly regulated by the Welch's *t*-test ($p < 0.05$) when comparing each experimental treatment to CON ($n = 3$).

[b] Number of array features for which a minimum 2-fold change in the geometric mean of expression values was observed.

[c] Number of array features for which 9 out of 10 spots (including all technical and biological replicates) were differentially regulated >1.5-fold.

[d] Number of array features that passed all filtering criteria.

Supplemental Table 5. Select genes differentially regulated by experimental diets in trout liver.

				Mean $\log_2$ fold-change in gene expression by treatment [b]							
Array ID	**DFCI ID [a]**	**Gene name (accession number; species)**	**Symbol**	**CON**	**E2**	**PFOA**	**PFNA**	**PFDA**	**PFOS**	**FTOH**	**CLOF**
Liver-specific proteins (vitellogenesis)											
OmyOSU208	TC132491	Vitellogenin (Q92093; *Oncorhynchus mykiss*) [c]	*vtg1*	-0.27	**6.07***	**6.61***	**6.22***	**6.07***	**5.62***	**5.83***	-0.12
OmyOSU1552	BX306977	Vitelline Envelope Protein gamma (Q9I9M6; *O. mykiss*)	*veg*	-0.26	**5.79***	**5.78***	**5.22***	**5.67***	**4.87***	**4.40***	0.11
OmyOSU1540	TC133595	Vitelline Envelope Protein alpha (Q9I9M8; *O. mykiss*)	*vepa*	-0.21	**5.76***	**5.84***	**5.27***	**5.74***	**5.39***	**5.11***	0.49
OmyOSU1542	TC169120	Zona radiata structural protein (Q90XC3; *O. mykiss*)	*zrp*	-0.02	**5.73***	**5.38***	**5.41***	**5.75***	**5.38***	**4.92***	0.70
Cell proliferation (cell signaling, regulation of transcription, cell growth and apoptosis)											
OmyOSU244	NP543968	Estrogen receptor beta (P57782; *O. mykiss*)	*esr2*	-0.27	**5.53***	**6.41***	**5.49**	**5.66***	**3.75***	**4.79***	0.01
OmyOSU1015	RTL00033	Inhibitor of *NFκB* subunit alpha (Q4G3H4; *Danio rerio*) [c]	*ikk1*	-0.01	**4.72***	**5.15***	**4.67**	**4.22***	**3.67***	**2.79***	0.30
OmyOSU127	TC146408	Nuclear factor NF-kappa-B p105 subunit (A3FJ60; *Siniperca chuatsi*)	*nfkb1*	-0.50	**3.78***	**3.75***	**4.20***	**2.78***	**2.77***	**2.27***	0.07
OmyOSU212	TC138144	TATA box binding protein (C0HA61; *Salmo salar*)	*tbp*	-0.39	**2.97***	**2.81***	**3.48***	**3.10***	1.16	1.45	-0.07
OmyOSU151	TC162795	Estrogen receptor alpha (P16058; *O. mykiss*) [c]	*esr1*	0.20	**2.46***	**3.62***	**2.94***	**3.04***	**2.18***	**1.85***	0.85
OmyOSU1667	TC169305	Poly A binding protein, cytoplasmic 1 b (Q6P3L1; *D. rerio*)	*pabpc1b*	0.04	**1.59***	**1.73***	**2.01***	**1.43**	0.93	0.38	0.08
OmyOSU1191	TC146689	NF-kappa-B inhibitor epsilon (B5X3Y7; *S. salar*)	*ikbe*	0.09	**1.42***	0.83	**1.31***	1.06	0.16	0.41	0.11
OmyOSU1484	TC150787	Tryptophanyl-tRNA synthetase (Q28BU4; *Xenopus tropicalis*)	*wars*	-0.07	0.81	0.38	**2.04***	0.85	0.22	0.24	0.41
OmyOSU1615	TC141666	Transmembrane 4 superfamily member protein (Q9DFD3; *O. mykiss*)	*tm4sf*	0.09	0.73	0.50	**1.58***	0.98	0.12	-0.05	0.22
OmyOSU803	BX079929	Growth arrest and DNA-damage-inducible, beta (C1BER7; *O. mykiss*)	*ga45b*	-0.12	0.43	0.39	**1.92***	**1.04**	-0.33	-0.11	-0.18
OmyOSU1428	TC143767	Reticulon RTN9-A1 (Q6IEJ0; *O. mykiss*)	*rtn9-a1*	-0.01	0.03	**1.71**	**1.21**	**1.00**	0.47	0.53	0.42
OmyOSU1427	TC140351	Reticulon RTN9-A2 (Q6IEI9; *O. mykiss*)	*rtn9-a2*	-0.06	0.42	**1.12**	0.68	0.79	0.06	0.18	0.18
OmyOSU1669	NP814796	Tumor necrosis factor receptor associated factor 2 (Q7T2X2; *O. mykiss*)	*traf2*	0.08	-0.37	**-1.57***	**-1.61***	**-1.67***	-0.65	-0.06	-0.05
OmyOSU387	TC156633	Allograft inflammatory factor 1 (B5XGK1; *S. salar*)	*aif1*	0.23	-0.70	-1.07	**-2.72***	**-1.59***	-0.12	-0.39	-0.28
OmyOSU725	CA379375	Putative hepatocyte growth factor activator (Q9DFD4; *O. mykiss*)	*hgfac*	-0.01	-1.29	-0.80	**-1.58***	**-1.19***	0.17	-0.39	-0.11
OmyOSU313	TC132515	Bone morphogenetic protein-7 (Q5BN41; *O. mykiss*)	*bmp7*	0.07	**-1.35***	**-1.34***	**-1.84***	-0.98	-0.54	0.16	0.27
OmyOSU916	NP544392	Mixed lineage leukemia-like protein (Q9PT21; *O. mykiss*)	*mll*	0.01	**-1.61***	**-1.46***	**-2.52***	**-1.84***	-0.34	-0.45	-0.16
Protein stability and transport											
OmyOSU893	TC145126	Heat shock protein 90 (P87397; *Oncorhynchus tshawytscha*)	*hsp90*	0.19	**2.58***	**3.61***	**3.37***	2.37	1.20	**1.17***	0.64
OmyOSU139	TC150271	Cathepsin D (P87370; *O. mykiss*)	*ctsd*	-0.17	**1.99***	**1.44***	**1.88***	**1.29***	0.98	0.28	0.05
OmyOSU1308	TC164544	Protein transport protein Sec61 α subunit isoform B (Q98SN8; *O. mykiss*) [c]	*sec61ab*	0.16	**1.89***	**1.14***	**1.59***	**1.20***	0.50	0.42	0.82
OmyOSU859	TC145413	Heat shock protein 47 (Q5DW60; *O. mykiss*)	*hsp47*	0.22	**1.11***	0.28	**1.91***	0.38	0.39	0.34	0.31
OmyOSU1602	TC160913	Coatomer subunit epsilon (C1BG73; *O. mykiss*)	*cope*	0.06	0.97	0.39	**1.17***	0.71	0.02	0.34	0.26

Supplemental Table 5. Select genes differentially regulated by experimental diets in trout liver.

				Mean $\log_2$ fold-change in gene expression by treatment [b]							
Array ID	**DFCI ID** [a]	**Gene name (accession number; species)**	**Symbol**	**CON**	**E2**	**PFOA**	**PFNA**	**PFDA**	**PFOS**	**FTOH**	**CLOF**
OmyOSU85	TC132785	Proteasome subunit beta type (B5XAY5; *S. salar*)	*psb7*	0.32	0.52	0.74	**1.43***	**1.03***	0.61	0.49	0.40
OmyOSU861	TC161889	60 kDa Heat shock protein, mitochondrial (C0HBF1; *S. salar*)	*ch60*	0.16	0.32	0.20	**1.78***	0.52	**1.14***	0.62	0.17
OmyOSU216	BX315865	40S Ribosomal protein S11 (Q9DF27; *S. salar*)	*rs11*	-0.28	0.21	0.71	**1.09***	0.35	-0.10	-0.15	-0.01
OmyOSU910	TC132590	Proteasome subunit beta type 9 precursor (Q9PT26; *O. mykiss*)	*psmb9*	-0.24	-0.11	0.51	**1.37***	0.16	-0.20	-0.09	0.37
OmyOSU1042	BX879214	Ubiquitin B (B9EQM0; *S. salar*)	*ubb*	-0.17	-0.13	0.13	0.13	0.34	**1.09***	0.16	**1.02***
Phase I and II metabolism											
OmyOSU1380	TC150232	Cytochrome P450 2k1 (Q92090; *O. mykiss*) [c]	*cyp2k1*	0.15	1.41	**3.68***	**3.27***	**3.04**	**3.11**	0.25	0.81
OmyOSU754	TC146311	Microsomal glutathione S-transferase (C1BFV1; *O. mykiss*)	*mgst*	0.16	-0.10	0.23	1.39	0.58	**1.01***	**1.89***	**1.27**
OmyOSU396	TC158463	Cytochrome P450 1a1 (Q92110; *O. mykiss*) [c]	*cyp1a1*	0.43	-0.26	**-1.58***	**-2.44***	-0.59	-0.07	-0.42	-0.35
OmyOSU993	TC147595	Glutathione S-transferase Mu 3 (C1BZU6; *Esox lucius*)	*gstm3*	0.22	-0.27	-0.48	**-2.47***	-0.89	-0.66	0.10	-0.28
OmyOSU1134	TC143007	Cytosolic sulfotransferase 2 (B5X695; *S. salar*)	*st2s2*	0.04	-0.31	0.48	-0.15	0.39	**1.19***	0.23	0.48
OmyOSU1203	BX306987	Microsomal glutathione S-transferase 1 (C1BM45; *Osmerus mordax*)	*gst1*	0.25	-0.34	0.01	**-1.62***	-0.46	0.14	0.59	0.40
OmyOSU1507	BX085479	Glutathione S-transferase (Q9W647; *Oncorhynchus nerka*)	*gstp1*	0.25	-0.35	-0.32	0.05	-0.45	0.07	**1.19***	0.65
OmyOSU829	BX085279	Carbonyl reductase (Q9PT38; *O. mykiss*)	*cbr1*	-0.06	-0.35	0.53	0.31	0.24	**1.26***	**1.20***	0.94
OmyOSU115	BX310129	Glutathione S-transferase P (B5XC10; *S. salar*)	*gstp1*	0.21	-0.36	-0.12	-0.63	-0.29	-0.25	**3.03***	0.20
OmyOSU398	TC139996	Cytochrome P450 3a27 (O42563; *O. mykiss*) [c]	*cyp3a27*	0.06	-0.96	0.58	0.58	-0.27	**2.07***	-0.05	0.69
OmyOSU356	TC150068	Cytochrome P450 2j24 (Q5TZ75; *D. rerio*)	*cyp2j24*	-0.08	**-1.24***	-0.55	**-2.32***	**-1.07***	0.59	-0.04	0.04
OmyOSU460	TC134882	3-Oxo-5-beta-steroid 4-dehydrogenase (B9EMZ1; *S. salar*)	*ark1d1*	-0.15	-1.41	**-1.94***	**-3.74***	**-1.75***	-0.23	-0.18	-0.47
OmyOSU1389	TC133687	Cytochrome P450 2k5 (Q9IAT1; *O. mykiss*) [c]	*cyp2k5*	-0.10	**-1.58***	**1.39***	0.15	0.76	**2.82***	-0.08	**1.67***
OmyOSU392	CB491885	Cytochrome P450 2m1 (Q92088; *O. mykiss*) [c]	*cyp2m1*	0.19	**-1.77***	**-2.27***	**-2.61***	**-1.42***	-0.08	0.16	0.10
Redox regulation											
OmyOSU245	TC152739	Succinate dehydrogenase complex subunit A flavoprotein (B5DFZ8; *S. salar*)	*sdha*	0.22	1.98	1.88	**1.88**	**2.62**	**1.26**	**1.15**	0.36
OmyOSU238	TC147716	Glutathione peroxidase (D2CKK9; *D. rerio*)	*gpx4a*	0.08	**1.21**	**1.61**	**2.29**	**2.22**	0.03	0.57	0.14
OmyOSU1490	TC135270	Cytochrome c oxidase subunit 3 (P69218; *O. nerka*)	*cox3*	-0.18	1.20	**1.49**	**1.75**	1.26	0.40	0.16	0.02
OmyOSU32	TC132824	Glutathione peroxidase (B5RI90; *S. salar*)	*gpx4b*	-0.07	0.32	0.40	**1.08**	0.36	0.07	-0.11	0.16
OmyOSU55	TC143718	Peroxiredoxin-5, mitochondrial (B5X5Q6; *S. salar*)	*prdx5*	0.42	0.26	0.69	**1.56**	0.89	0.36	0.60	0.92
OmyOSU1633	CA373161	Cytochrome C-1 (Q3B7R0; *D. rerio*)	*cyc1*	0.10	0.23	0.48	**1.72**	0.84	0.45	0.08	0.20
OmyOSU92	BX074038	Cu/Zn-superoxide dismutase (C1BFL3; *O. mykiss*)	*sod1*	0.29	0.21	0.50	**1.01**	0.46	0.07	-0.03	0.33
OmyOSU572	TC156955	Cytochrome c oxidase polypeptide Via (O13085; *O. mykiss*)	*coxa*	0.12	0.05	0.03	**-1.86**	-0.46	0.16	-0.09	0.20
OmyOSU566	CU068081	Peroxisomal carnitine O-octanoyltransferase (Q503F8; *D. rerio*)	*crot*	-0.14	-0.02	0.20	-0.29	-0.24	0.31	-0.18	-0.11

Supplemental Table 5. Select genes differentially regulated by experimental diets in trout liver.

				Mean $\log_2$ fold-change in gene expression by treatment [b]							
Array ID	**DFCI ID [a]**	**Gene name (accession number; species)**	**Symbol**	**CON**	**E2**	**PFOA**	**PFNA**	**PFDA**	**PFOS**	**FTOH**	**CLOF**
OmyOSU325	TC146929	Acetyl-CoA acetyltransferase, mitochondrial (Q6AZA0; *D. rerio*)	*acat1*	-0.22	-0.03	-0.10	-0.20	-0.04	0.22	-0.25	-0.13
OmyOSU1422	TC147703	Thioredoxin (C1BH85; *O. mykiss*)	*trx*	-0.68	-0.41	-0.07	**2.48**	0.27	0.59	**1.54**	0.34
OmyOSU37	TC141467	Catalase (C0HAV1; *S. salar*)	*cat*	0.04	-0.75	-0.76	**-1.38**	-1.00	0.02	-0.07	-0.23
Extracellular matrix and vascularization factors											
OmyOSU1557	TC135220	Secreted protein acidic and rich in cysteine protein (Q9YGD9; *O. mykiss*)	*sparc*	-0.03	-0.79	-0.68	**-1.21***	-0.18	-0.52	-0.31	-0.28
OmyOSU419	CA356156	Angiogenin (B5XAZ0; *S. salar*)	*ang1*	-0.23	**-1.44***	**-1.70***	**-1.95***	**-1.50***	0.03	-0.28	0.11
Immune response											
OmyOSU1564	TC140147	VHSV4 (Q8QGB4; *O. mykiss*)	*vhsv4*	0.04	**5.62***	**6.32***	**5.57***	**6.11***	**4.90***	**4.26***	-0.13
OmyOSU1566	TC132651	VHSV6 (Q8QGB2; *O. mykiss*)	*vhsv6*	0.15	**3.88***	**3.90***	**3.36***	**3.78***	**1.92***	1.93	0.02
OmyOSU582	TC167667	P-selectin (B5X3V6 ; *S. salar*)	*lyam3*	0.18	**1.65***	0.99	0.99	0.62	0.55	-0.13	0.54
OmyOSU1590	TC132541	VHSV-induced protein 2 (Q9DD73; *O. mykiss*)	*vhsv2*	-0.16	0.83	0.58	**1.08***	0.30	0.13	-0.17	0.10
OmyOSU148	TC161751	Differentially regulated trout protein 1 (Q9DFD5; *O. mykiss*)[c]	*drtp1*	-0.04	0.71	0.16	**4.02***	2.55	-0.56	0.33	0.40
OmyOSU634	TC162804	CD209-like protein (Q64HY2; *O. mykiss*)	*cd209*	0.09	0.67	-0.12	**1.12***	0.67	0.46	0.34	0.07
OmyOSU15	BX311693	Complement component C3-3 (Q98977; *O. mykiss*)	*c3-3*	0.20	-0.06	**-1.24***	**-2.39***	-0.94	-0.15	-0.07	-0.81
OmyOSU44	BX306395	Complement component C3-4 (Q9DDV9; *O. mykiss*)	*c3-4*	0.08	-0.10	**-1.39***	**-2.60***	-0.92	0.08	-0.18	-0.84
OmyOSU411	BX882784	Complement C9 (Q4QZ25; *O. mykiss*)	*c9*	0.14	-0.13	-0.39	**-1.18***	-0.20	-0.08	0.09	-0.10
OmyOSU348	TC149604	CD59-like protein (B5X604; *S. salar*)	*cd59*	0.41	-0.14	**-1.52***	**-1.61***	**-1.31***	**-2.19***	-0.11	-0.94
OmyOSU371	TC159535	C1q-like adipose specific protein (Q8JI26; *Salvelinus fontinalis*)	*c1q*	-0.18	-0.29	-1.54	**-2.67***	**-1.84***	-0.04	-0.25	-0.97
OmyOSU1147	TC152823	Pentraxin (P79899; *O. mykiss*) [c]	*ptx*	-0.08	-0.41	**-2.97***	**-2.60***	**-1.51***	-0.32	0.01	-0.13
OmyOSU638	TC137183	C-type mannose-binding lectin (Q8JJ68; *O. mykiss*)	*mbl-1*	-0.17	-0.51	**-1.21***	**-1.49***	**-1.56***	-0.58	-0.36	-0.41
OmyOSU636	TC138355	C-type MBL-2 protein (Q4LAN6; *O. mykiss*)	*mbl*	0.23	-0.54	**-1.35***	**-1.49***	-1.68	-0.88	-0.21	-0.16
OmyOSU34	TC141865	C1 inhibitor (Q70W32; *O. mykiss*)	*c1 inh*	0.03	-0.58	-0.55	**-1.33***	-0.42	0.34	-0.13	0.11
OmyOSU76	TC132971	Complement receptor-like protein 1 (Q2PDG0; *O. mykiss*)	*crlp1*	-0.05	-0.67	-0.86	**-1.21***	-0.75	-0.14	-0.12	0.34
OmyOSU1426	TC142005	Complement factor H protein (Q4QZ18; *O. mykiss*)	*cfh*	0.11	-0.74	-0.26	**-1.09***	-0.76	0.23	-0.10	0.26
OmyOSU1469	TC167824	Cathepsin S (C0HDJ6; *S. salar*)	*cats*	-0.09	-0.96	-1.87	**-4.59***	**-1.69***	-0.08	-0.48	-0.03
OmyOSU878	CA383049	Chemotaxin (Q9DFJ1; *O. mykiss*)	*ctx*	-0.08	**-1.16***	**-2.04***	**4.00***	-0.13	-0.71	0.48	0.76
OmyOSU866	TC148069	Hemagglutinin/amebocyte aggregation factor (B5XF94; *S. salar*)	*haaf*	0.06	**-1.31***	**-2.00***	**-1.87***	-1.80	-0.89	-0.41	-1.62
OmyOSU1	CA347121	CD80-like protein (A1IMH7; *O. mykiss*)	*cd80*	-0.18	-1.95	**-1.73***	**-3.75***	**-2.37***	-0.35	-0.45	-0.71
OmyOSU1477	TC139517	Trout C-polysaccharide binding protein 1 (Q9DFE5; *O. mykiss*) [c]	*tcpbp*	0.55	**-2.11***	**-2.60***	**-3.66***	**-2.48***	0.36	-0.69	0.21

Supplemental Table 5. Select genes differentially regulated by experimental diets in trout liver.

				Mean $\log_2$ fold-change in gene expression by treatment [b]							
Array ID	**DFCI ID [a]**	**Gene name (accession number; species)**	**Symbol**	**CON**	**E2**	**PFOA**	**PFNA**	**PFDA**	**PFOS**	**FTOH**	**CLOF**
Blood factors and coagulation											
OmyOSU723	TC138894	Plasminogen (Q5DVP8; *O. mykiss*)	*plg*	0.04	-0.34	-0.16	**-1.05***	-0.50	0.44	-0.23	-0.06
OmyOSU90	TC157215	Beta-2-glycoprotein 1 (C0H7U1; *S. salar*)	*apoh*	0.03	-0.57	-0.70	**-1.11***	-0.66	-0.09	-0.18	-0.30
OmyOSU649	TC145770	Coagulation factor VIII (Q804W6; *Takifugu rubripes*) [c]	*f8*	0.11	-0.62	-0.44	**-1.71***	-0.93	-0.02	-0.25	0.17
OmyOSU665	TC149137	Alpha-globin I (Q98973; *O. mykiss*)	*hbaa1*	0.07	-0.65	-0.86	**-1.72***	-0.59	-0.94	-0.20	-0.27
OmyOSU775	TC139629	Protein C (Q7T3B6; *D. rerio*)	*proc*	-0.25	-0.68	-0.23	**-1.07***	-0.51	0.41	-0.31	-0.19
OmyOSU1338	TC143725	Antithrombin (Q9PTA8; *S. salar*) [c]	*at*	0.12	-0.70	-0.52	**-1.25***	-0.78	0.37	-0.29	-0.09
OmyOSU667	CU064560	Hemoglobin subunit alpha-1 (Q98974; *O. mykiss*)	*hba1*	0.26	-0.72	**-1.32***	**-2.74***	**-1.72***	-1.08	0.09	-0.23
OmyOSU219	TC135277	Complement factor Bf-1 (Q9YGE7; *O. mykiss*)	*cfb1*	-0.15	-0.72	**-1.21***	**-1.85***	**-1.24***	0.20	-0.22	-0.03
OmyOSU355	TC172297	Serum albumin 1 (P21848; *S. salar*) [c]	*alb1*	0.06	-0.76	-0.71	**-3.48***	**-1.86***	-0.07	-0.24	0.00
OmyOSU677	TC151435	Fibrinogen (Q9DFD8; *O. mykiss*)	*fgg*	0.16	**-1.07***	**-1.08***	-0.49	**-1.10***	-0.14	-0.26	-0.16
OmyOSU8	TC164674	Alpha 2 macroglobulin (C1K6P9; *Perca flavescens*) [c]	*a2m*	0.13	-1.19	**-1.10***	**-2.67***	**-1.30***	0.05	-0.22	-0.42
OmyOSU1502	TC132862	Tissue factor (Q90W13; *O. mykiss*)	*f3a*	0.68	-1.49	-1.14	**-2.26***	**-1.75***	0.17	-0.59	0.03
Lipid and cholesterol, metabolism and transport											
OmyOSU449	TC151125	Apolipoprotein B (C3UZW7; *P. flavescens*)	*apob*	0.03	**1.17***	-0.21	0.27	0.60	0.55	0.33	-0.26
OmyOSU721	TC145590	Fatty acid binding protein, heart (O13008; *O. mykiss*)	*fabp3*	0.09	**1.07***	**1.50***	0.40	**1.21***	0.45	0.23	0.88
OmyOSU153	TC162807	Fatty acid binding protein (Q9DFE6; *O. mykiss*)	*fabp*	0.27	0.88	**2.53***	**4.83***	**3.57***	-1.02	-0.48	0.35
OmyOSU904	TC132457	Lipoprotein lipase (Q9W6Y2; *O. mykiss*)	*lpl*	0.00	-0.77	-0.57	**-1.24***	-0.87	0.00	-0.26	-0.27
OmyOSU908	TC148996	Epidermis-type lipoxygenase 3 (B5X0R4 ; *S. salar*)	*loxe3*	0.04	-0.82	**-1.08***	**-2.11***	**-1.36***	-0.12	-0.29	-0.40
OmyOSU453	BX073289	Apolipoprotein A-I-1 (O57523; *O. mykiss*)	*apo-AI-1*	-0.09	**-1.05***	**-1.37***	-0.83	-1.01	-0.02	-0.42	-0.05
OmyOSU1652	TC161762	Apolipoprotein E (Q9PT02; *O. mykiss*) [c]	*apoE*	-0.17	-1.36	**-1.61***	0.00	**-1.43***	0.11	-0.46	-0.40
Ion binding and transport											
OmyOSU1020	TC165296	Metallothionein B (P68501; *O. mykiss*) [c]	*mt-b*	-0.32	**1.18***	**1.23***	**3.29***	**1.34***	0.88	**1.33***	0.82
OmyOSU846	TC147024	Transferrin (Q9PT13; *O. mykiss*) [c]	*tf*	0.05	-0.23	-0.98	**-2.72***	-0.61	0.23	0.09	0.25
OmyOSU657	TC169785	Haptoglobin 1 (Q9DFG1; *O. mykiss*) [c]	*hp1*	-0.15	-0.41	-0.82	**-1.60***	-0.04	-0.04	0.06	-0.20
OmyOSU685	BX073517	Ferritin (P79823; *O. mykiss*) [c]	*ft*	-0.29	**-1.20***	**-1.78***	**-1.11***	**-1.56***	-0.96	-0.22	-0.31
OmyOSU699	TC169206	Hemopexin (P79825; *O. mykiss*)	*hpx*	-0.24	**-1.99***	**-2.08***	**-3.28***	**-2.50***	-0.57	-0.78	-0.90
Glycolysis and carbohydrate metabolism											
OmyOSU1241	TC152596	6-Phosphofructokinase type C (C0HAA0; *S. salar*)	*k6pp*	-0.09	**2.43***	0.91	**1.72***	1.69	-0.25	-0.05	-0.02
OmyOSU875	CU072064	Glucokinase (O93314; *O. mykiss*)	*gk*	-0.27	**2.32***	1.10	0.98	-0.81	**2.30***	-0.30	-0.10
OmyOSU1149	TC132998	Phosphoenolpyruvate carboxykinase (Q98T97; *O. mykiss*)	*pck*	0.04	-0.12	-0.68	**-1.30***	-0.63	0.33	0.18	0.05
OmyOSU116	TC150193	Phosphorylase (C0PUK4; *S. salar*)	*pygm*	0.31	-0.75	-0.75	**-1.69***	-0.76	0.35	-0.04	-0.05

Supplemental Table 5. Select genes differentially regulated by experimental diets in trout liver.

Array ID	DFCI ID [a]	Gene name (accession number; species)	Symbol	Mean $\log_2$ fold-change in gene expression by treatment [b]							
				CON	E2	PFOA	PFNA	PFDA	PFOS	FTOH	CLOF
Nucleoside Metabolism											
OmyOSU252	TC152774	Hypoxanthine phosphoribosyltransferase 1 (Q7ZV49; *D. rerio*)	*hprt1*	0.28	**2.50**	2.04	**2.22**	**2.45**	**1.16**	0.43	0.20
OmyOSU1518	TC171662	Uridine phophorylase (B5X227; *S. salar*)	*upp1*	-0.04	**2.53**	**2.32**	**2.23**	**2.08**	**1.01**	**1.08**	0.03
Miscellaneous											
OmyOSU343	TC141079	Biotinidase (Q8AV84; *T. rubripes*)	*btd*	-0.13	**-2.41**	**-2.57**	**-3.58**	**-2.44**	-0.72	-0.24	-0.04

[a] Manual array feature annotation was performed by querying the DFCI R.trout Gene Index (http://compbio.dfci.harvard.edu/tgi/) for the closest EST match to the array 70-mer sequence. Matching EST sequences were then BLASTX queried in the NCBI genome database. The top hit (lowest E-score) was selected as the matching gene. If an EST had no significant (E-value <10-6) BLASTX hit, then the most significant BLASTN hit is shown.

[b] Log_2 geometric mean fold change values are shown (N = 3) and represent background corrected, ratio-centered and Lowess-normalized signal ratios. Values in bold and marked with an asterisk are considered statistically significant ($P \leq 0.05$ by Welch's t-test) and passed all stringency criteria. Color scales are provided to indicate visually the similarities or differences in gene expression among treatment groups (red, induced; green, repressed; white, no change).

[c] Unique array features targeted the same gene in some cases, though a single representative array feature is shown in this table.

Supplemental Table 6. Pearson correlation coefficients (*r*) for pair-wise comparisons by treatment group.

	PFOA	**PFNA**	**PFDA**	**PFOS**	**FtOH**	**CLOF**
Differentially regulated genes [a]						
E2	0.93	0.84	0.93	0.78	0.83	0.30
PFOA		0.86	0.95	0.82	0.82	0.39
PFNA			0.91	0.66	0.73	0.45
PFDA				0.78	0.83	0.41
PFOS					0.83	0.42
8:2FtOH						0.30
All array features						
E2	0.85	0.75	0.83	0.61	0.50	0.25
PFOA		0.73	0.83	0.62	0.68	0.27
PFNA			0.83	0.53	0.61	0.40
PFDA				0.63	0.74	0.38
PFOS					0.70	0.46
8:2FtOH						0.35

[a] Correlation analysis were performed using a data subset including OSUrbt array features that were determined to be significantly differentially regulated in any one of the experimental treatments.

Suppl. Table 7. Over-represented Gene Ontology biological process annotations associated with genes induced by the indicated dietary treatments[a]

			E2		**PFOA**		**PFNA**		**PFDA**		**PFOS**		**8:2FtOH**		**CLOF**	
# [b]	**Accession**	**Biological Process**	**E**	**FDR**	**E**	**FDR**	**E**	**FDR**	**E**	**FDR**	**E**	**FDR**	**E**	**FDR**	**E**	**FDR**
1	GO:0032355	response to estradiol stimulus	178.6	**0.030**	202.4	**<0.001**	86.7	**0.040**	151.8	**<0.001**	189.8	**0.040**	303.6	**<0.001**	1518.0	0.210
2	GO:0030520	estrogen receptor signaling pathway	259.8	**0.020**	294.4	**<0.001**	126.2	**0.030**	220.8	**<0.001**	276.0	**<0.001**	441.6	**<0.001**	2208.0	0.220
3	GO:0006914	autophagy	75.2	0.080	85.2	**0.027**	36.5	0.073	63.9	**0.048**	39.9	0.165	0.0	0.996	0.0	0.982
4	GO:0046651	lymphocyte proliferation	14.9	0.352	0.0	0.976	21.7	**0.040**	12.7	0.360	31.6	0.071	0.0	0.905	0.0	0.584
5	GO:0070661	leukocyte proliferation	14.4	0.352	0.0	0.997	21.0	**0.031**	12.3	0.358	30.7	0.065	0.0	1.000	0.0	0.999
6	GO:0032943	mononuclear cell proliferation	14.4	0.352	0.0	0.997	21.0	**0.031**	12.3	0.358	30.7	0.065	0.0	1.000	0.0	0.999
7	GO:0032602	chemokine production	0.0	0.965	0.0	0.974	40.8	0.306	0.0	0.958	178.6	**0.027**	0.0	0.988	0.0	0.985
8	GO:0009719	response to endogenous stimulus	18.2	0.073	13.7	0.308	8.8	0.250	15.4	0.056	19.3	**0.025**	20.6	0.455	102.9	0.503
9	GO:0010033	response to organic substance	11.5	0.060	6.5	0.386	5.6	0.248	7.4	0.206	12.3	**0.015**	9.8	0.656	49.1	0.528
10	GO:0001816	cytokine production	7.9	0.379	8.9	0.481	7.7	0.309	6.7	0.399	25.2	**0.020**	0.0	0.999	0.0	0.994
11	GO:0001818	negative regulation of cytokine production	47.6	0.325	54.0	0.377	23.1	0.338	40.5	0.291	101.2	**0.020**	0.0	0.995	0.0	0.978
12	GO:0030522	intracellular receptor-mediated signaling pathway	32.8	0.151	37.2	0.193	16.0	0.261	27.9	0.095	34.9	0.071	55.8	**0.031**	279.2	0.362
13	GO:0048545	response to steroid hormone stimulus	37.6	0.068	42.6	0.069	18.3	0.254	32.0	0.105	39.9	**0.039**	63.9	**0.030**	319.6	0.382
14	GO:0030518	steroid hormone receptor signaling pathway	43.3	0.051	49.1	**0.050**	21.0	0.188	36.8	0.051	46.0	**0.033**	73.6	**0.032**	368.0	0.375
15	GO:0043627	response to estrogen stimulus	64.9	0.068	73.6	**0.030**	31.5	0.090	55.2	0.051	69.0	**0.027**	110.4	**0.013**	552.0	0.390
16	GO:0009410	response to xenobiotic stimulus	55.0	0.063	62.3	**0.028**	26.7	0.110	46.7	0.063	58.4	**0.026**	93.4	**0.035**	467.1	0.363

[a] Gene ontology enrichment analysis was performed using High Throughput GoMiner. A significant enrichment of the GO term category was inferred when $p<0.05$ as determined by a one-sided Fisher's exact test after false discovery rate (FDR) correction. Significantly enriched GO terms are indicated in bold with yellow or orange highlight.

[b] Number (#) corresponds to position in Cluster Image Map presented in Figure 4.

Abbreviations: E, enrichment value; FDR, false discovery rate-corrected *p*-value.

Suppl. Table 8. Over-represented Gene Ontology biological process annotations associated with genes repressed by the indicated dietary treatments[a]

			E2		PFOA		PFNA		PFDA		PFOS		8:2FtOH		CLOF	
#[b]	Accession	Biological Process	E	FDR	E	FDR	E	FDR	E	FDR	E	FDR	E	FDR	E	FDR
1	GO:0002440	production of molecular mediator of immune response	32	0.236	55	**0.007**	25	**0.020**	55	**0.008**	0	1.000	0	0.998	0	1.000
2	GO:0009967	positive regulation of signal transduction	12	0.172	16	**0.008**	10	**0.010**	16	**0.008**	0	0.999	9	0.557	0	1.000
3	GO:0010647	positive regulation of cell communication	12	0.173	15	**0.009**	9	**0.012**	15	**0.010**	0	1.000	8	0.548	0	1.000
4	GO:0031347	regulation of defense response	30	0.056	40	**0.002**	18	**0.008**	40	**0.002**	0	0.999	0	0.995	0	1.000
5	GO:0002700	regulation of production of molecular mediator of immune response	51	0.205	89	**0.006**	41	**0.012**	89	**0.004**	0	1.001	0	0.981	0	1.000
6	GO:0032623	interleukin-2 production	58	0.199	101	**0.005**	46	**0.012**	101	**0.004**	0	0.996	0	0.992	0	1.000
7	GO:0010033	response to organic substance	7	0.239	12	**0.006**	7	**0.009**	12	**0.005**	0	0.999	10	0.456	0	1.000
8	GO:0032663	regulation of interleukin-2 production	64	0.186	112	**0.004**	51	**0.009**	112	**0.003**	0	1.000	0	0.994	0	1.000
9	GO:0048583	regulation of response to stimulus	13	**0.047**	23	**0.000**	16	**0.000**	23	**0.000**	0	0.999	0	0.992	0	1.000
10	GO:0002526	acute inflammatory response	36	**0.046**	47	**0.001**	43	**0.000**	47	**0.002**	0	0.965	0	0.769	0	1.000
11	GO:0002250	adaptive immune response	32	**0.045**	43	**0.002**	26	**0.000**	43	**0.002**	0	1.000	0	0.995	0	1.000
12	GO:0002460	adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	33	**0.046**	43	**0.002**	26	**0.000**	43	**0.002**	0	1.000	0	1.000	0	1.000
13	GO:0002702	positive regulation of production of molecular mediator of immune response	173	0.130	304	**0.001**	139	**0.002**	304	**0.003**	0	0.985	0	0.971	0	1.000
14	GO:0048584	positive regulation of response to stimulus	16	0.132	29	**0.001**	20	**0.000**	29	**0.003**	0	1.000	0	0.973	0	1.000
15	GO:0002253	activation of immune response	18	0.296	48	**0.001**	37	**0.000**	48	**0.002**	0	1.000	0	0.997	0	1.000
16	GO:0050778	positive regulation of immune response	28	0.070	48	**0.000**	33	**0.000**	48	**0.002**	0	0.976	0	0.834	0	1.000
17	GO:0002252	immune effector process	19	0.112	33	**0.000**	22	**0.000**	33	**0.002**	0	1.000	0	0.980	0	1.000
18	GO:0045087	innate immune response	18	0.112	24	**0.006**	18	**0.000**	24	**0.004**	0	1.000	0	0.998	0	1.000
19	GO:0002699	positive regulation of immune effector process	53	0.205	92	**0.006**	63	**0.000**	92	**0.004**	0	1.000	0	0.995	0	1.000
20	GO:0002824	positive regulation of adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	75	0.176	132	**0.004**	91	**0.000**	132	**0.004**	0	1.000	0	0.990	0	1.000
21	GO:0002705	positive regulation of leukocyte mediated immunity	67	0.185	117	**0.004**	80	**0.000**	117	**0.003**	0	1.000	0	1.000	0	1.000
22	GO:0002708	positive regulation of lymphocyte mediated immunity	67	0.185	117	**0.004**	80	**0.000**	117	**0.003**	0	1.000	0	1.000	0	1.000
23	GO:0006954	inflammatory response	10	0.198	17	**0.003**	13	**0.000**	21	**0.003**	0	1.000	0	0.998	0	1.000
24	GO:0002821	positive regulation of adaptive immune response	72	0.180	127	**0.004**	87	**0.000**	127	**0.004**	0	0.995	0	0.990	0	1.000
25	GO:0051605	protein maturation by peptide bond cleavage	23	0.273	40	**0.010**	37	**0.000**	40	**0.011**	0	1.000	0	0.999	0	1.000
26	GO:0002703	regulation of leukocyte mediated immunity	36	0.223	63	**0.007**	43	**0.001**	63	**0.007**	0	1.000	0	0.998	0	1.000

Suppl. Table 8. Over-represented Gene Ontology biological process annotations associated with genes repressed by the indicated dietary treatments[a]

			E2		PFOA		PFNA		PFDA		PFOS		8:2FtOH		CLOF	
#[b]	Accession	Biological Process	E	FDR	E	FDR	E	FDR	E	FDR	E	FDR	E	FDR	E	FDR
27	GO:0006956	complement activation	39	0.219	69	**0.006**	63	**0.000**	69	**0.007**	0	1.000	0	0.973	0	1.000
28	GO:0002706	regulation of lymphocyte mediated immunity	39	0.219	69	**0.006**	47	**0.000**	69	**0.007**	0	1.000	0	0.973	0	1.000
29	GO:0002541	activation of plasma proteins involved in acute inflammatory response	36	0.223	63	**0.007**	58	**0.000**	63	**0.007**	0	1.000	0	0.998	0	1.000
30	GO:0080134	regulation of response to stress	0	0.969	20	**0.007**	15	**0.000**	20	**0.007**	0	0.999	0	0.936	0	1.000
31	GO:0006826	iron ion transport	72	**0.009**	63	**0.007**	29	**0.018**	63	**0.007**	0	1.000	0	0.998	0	1.000
32	GO:0045088	regulation of innate immune response	89	**0.007**	78	**0.006**	36	**0.015**	78	**0.006**	0	1.000	0	1.000	0	1.000
33	GO:0050731	positive regulation of peptidyl-tyrosine phosphorylation	81	**0.009**	71	**0.006**	32	**0.015**	71	**0.007**	0	0.999	0	0.997	0	1.000
34	GO:0002682	regulation of immune system process	15	**0.018**	21	**0.000**	16	**0.000**	21	**0.004**	0	0.999	0	0.986	0	1.000
35	GO:0002697	regulation of immune effector process	42	**0.018**	56	**0.001**	34	**0.000**	56	**0.002**	0	1.000	0	1.000	0	1.000
36	GO:0006959	humoral immune response	40	**0.018**	53	**0.001**	40	**0.000**	53	**0.002**	0	0.999	0	0.992	0	1.000
37	GO:0000041	transition metal ion transport	55	**0.000**	48	**0.001**	22	**0.005**	48	**0.002**	258	0.773	0	0.997	0	1.000
38	GO:0030005	cellular di- tri-valent inorganic cation homeostasis	29	**0.000**	32	**0.000**	15	**0.000**	32	**0.000**	103	0.811	0	0.990	0	1.000
39	GO:0006879	cellular iron ion homeostasis	122	**0.000**	107	**0.000**	49	**0.000**	107	**0.000**	426	1.127	0	0.922	0	1.000
40	GO:0055072	iron ion homeostasis	0	0.976	100	**0.000**	46	**0.000**	100	**0.000**	398	0.880	0	0.995	0	1.000
41	GO:0002920	regulation of humoral immune response	434	**0.000**	380	**0.001**	173	**0.001**	380	**0.003**	0	1.000	0	0.980	0	1.000
42	GO:0055080	cation homeostasis	0	0.965	25	**0.000**	12	**0.001**	25	**0.002**	81	0.637	0	0.974	0	1.000
43	GO:0055066	di- tri-valent inorganic cation homeostasis	0	0.997	31	**0.000**	14	**0.000**	31	**0.003**	99	0.732	0	0.993	0	1.000
44	GO:0030003	cellular cation homeostasis	27	**0.000**	29	**0.000**	13	**0.000**	29	**0.003**	93	0.676	0	0.986	0	1.000
45	GO:0050801	ion homeostasis	17	**0.005**	18	**0.000**	8	**0.004**	18	**0.002**	58	0.591	0	0.995	0	1.000
46	GO:0006873	cellular ion homeostasis	19	**0.007**	20	**0.000**	9	**0.003**	20	**0.003**	65	0.666	0	0.986	0	1.000
47	GO:0055082	cellular chemical homeostasis	0	0.701	20	**0.000**	9	**0.003**	20	**0.003**	63	0.624	0	0.968	0	1.000
48	GO:0002684	positive regulation of immune system process	25	**0.008**	37	**0.000**	24	**0.000**	37	**0.000**	0	0.972	0	0.810	0	1.000
49	GO:0050776	regulation of immune response	27	**0.008**	40	**0.000**	26	**0.000**	40	**0.000**	0	0.999	0	0.970	0	1.000
50	GO:0002819	regulation of adaptive immune response	77	**0.009**	101	**0.000**	62	**0.000**	101	**0.003**	0	0.995	0	0.783	0	1.000
51	GO:0002822	regulation of adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	79	**0.009**	104	**0.000**	63	**0.000**	104	**0.003**	0	1.000	0	0.973	0	1.000
52	GO:0001934	positive regulation of protein amino acid phosphorylation	43	**0.018**	37	**0.011**	17	0.062	37	**0.012**	0	0.987	0	0.990	0	1.000
53	GO:0050730	regulation of peptidyl-tyrosine phosphorylation	57	**0.016**	50	**0.008**	23	**0.037**	50	**0.009**	0	1.000	0	0.995	0	1.000
54	GO:0031399	regulation of protein modification process	25	**0.008**	22	**0.006**	10	**0.037**	22	**0.006**	0	0.976	0	0.992	0	1.000

Suppl. Table 8. Over-represented Gene Ontology biological process annotations associated with genes repressed by the indicated dietary treatments[a]

			E2		PFOA		PFNA		PFDA		PFOS		8:2FtOH		CLOF	
#[b]	**Accession**	**Biological Process**	**E**	**FDR**	**E**	**FDR**	**E**	**FDR**	**E**	**FDR**	**E**	**FDR**	**E**	**FDR**	**E**	**FDR**
55	GO:0010627	regulation of protein kinase cascade	7	0.368	13	0.066	9	**0.049**	13	0.070	0	0.973	10	0.525	0	1.000
56	GO:0002888	positive regulation of myeloid leukocyte mediated immunity	0	0.992	0	0.981	347	**0.036**	0	0.983	0	1.000	0	0.993	0	1.000
57	GO:0001796	regulation of type IIa hypersensitivity	0	0.992	0	0.981	347	**0.036**	0	0.983	0	1.000	0	0.993	0	1.000
58	GO:0008065	establishment of blood-nerve barrier	0	0.992	0	0.981	347	**0.036**	0	0.983	0	1.000	0	0.993	0	1.000
59	GO:0007597	blood coagulation intrinsic pathway	0	0.992	0	0.981	347	**0.036**	0	0.983	0	1.000	0	0.993	0	1.000
60	GO:0002445	type II hypersensitivity	0	0.992	0	0.981	347	**0.036**	0	0.983	0	1.000	0	0.993	0	1.000
61	GO:0034392	negative regulation of smooth muscle cell apoptosis	0	0.992	0	0.981	347	**0.036**	0	0.983	0	1.000	0	0.993	0	1.000
62	GO:0002892	regulation of type II hypersensitivity	0	0.992	0	0.981	347	**0.036**	0	0.983	0	1.000	0	0.993	0	1.000
63	GO:0002894	positive regulation of type II hypersensitivity	0	0.992	0	0.981	347	**0.036**	0	0.983	0	1.000	0	0.993	0	1.000
64	GO:0001794	type IIa hypersensitivity	0	0.992	0	0.981	347	**0.036**	0	0.983	0	1.000	0	0.993	0	1.000
65	GO:0001798	positive regulation of type IIa hypersensitivity	0	0.992	0	0.981	347	**0.036**	0	0.983	0	1.000	0	0.993	0	1.000
66	GO:0016064	immunoglobulin mediated immune response	26	0.257	23	0.112	21	**0.038**	23	0.118	0	0.999	0	0.997	0	1.000
67	GO:0045765	regulation of angiogenesis	0	0.947	0	0.789	22	**0.037**	0	0.797	0	1.000	0	0.997	0	1.000
68	GO:0051271	negative regulation of cell motion	0	0.994	25	0.108	23	**0.037**	25	0.113	0	1.000	0	1.000	0	1.000
69	GO:0051240	positive regulation of multicellular organismal process	10	0.341	17	0.052	12	**0.017**	17	0.055	0	1.000	0	0.998	0	1.000
70	GO:0010740	positive regulation of protein kinase cascade	11	0.336	19	0.050	13	**0.015**	19	0.052	0	0.997	0	0.834	0	1.000
71	GO:0046486	glycerolipid metabolic process	0	0.988	9	0.154	12	**0.015**	9	0.171	0	1.000	0	0.995	0	1.000
72	GO:0050818	regulation of coagulation	0	0.630	38	0.094	35	**0.015**	38	0.099	0	0.990	0	0.833	0	1.000
73	GO:0043542	endothelial cell migration	0	0.748	37	0.095	34	**0.015**	37	0.100	0	1.000	0	0.971	0	1.000
74	GO:0006953	acute-phase response	48	0.208	42	0.092	39	**0.013**	42	0.097	0	0.998	0	0.977	0	1.000
75	GO:0050819	negative regulation of coagulation	0	0.968	47	0.088	43	**0.012**	47	0.094	0	0.998	0	0.990	0	1.000
76	GO:0030193	regulation of blood coagulation	0	0.994	46	0.090	42	**0.012**	46	0.095	0	1.000	0	0.995	0	1.000
77	GO:0002455	humoral immune response mediated by circulating immunoglobulin	53	0.205	46	0.090	42	**0.012**	46	0.095	0	1.000	0	0.995	0	1.000
78	GO:0030336	negative regulation of cell migration	0	0.861	28	0.104	25	**0.020**	28	0.109	0	1.000	0	0.998	0	1.000
79	GO:0042440	pigment metabolic process	36	0.223	32	0.100	29	**0.018**	32	0.105	0	1.000	0	0.998	0	1.000
80	GO:0016525	negative regulation of angiogenesis	0	0.920	0	0.986	56	**0.009**	0	0.987	0	0.996	0	0.998	0	1.000
81	GO:0030195	negative regulation of blood coagulation	0	0.993	58	0.080	53	**0.009**	58	0.086	0	1.000	0	1.000	0	1.000
82	GO:0010594	regulation of endothelial cell migration	0	0.973	69	0.074	63	**0.007**	69	0.080	0	0.995	0	0.983	0	1.000
83	GO:0002889	regulation of immunoglobulin mediated immune response	83	0.173	72	0.073	66	**0.007**	72	0.079	0	1.000	0	0.995	0	1.000

Suppl. Table 8. Over-represented Gene Ontology biological process annotations associated with genes repressed by the indicated dietary treatments[a]

			E2		PFOA		PFNA		PFDA		PFOS		8:2FtOH		CLOF	
#[b]	Accession	Biological Process	E	FDR	E	FDR	E	FDR	E	FDR	E	FDR	E	FDR	E	FDR
84	GO:0002712	regulation of B cell mediated immunity	83	0.173	72	0.073	66	**0.007**	72	0.079	0	1.000	0	0.995	0	1.000
85	GO:0010596	negative regulation of endothelial cell migration	0	0.972	117	0.063	107	**0.003**	117	0.067	0	1.000	0	0.989	0	1.000
86	GO:0042730	fibrinolysis	0	0.970	0	0.953	77	**0.004**	0	0.955	0	1.000	0	0.981	0	1.000
87	GO:0042060	wound healing	8	0.359	14	0.061	13	**0.003**	14	0.065	0	1.000	0	0.973	0	1.000
88	GO:0002673	regulation of acute inflammatory response	108	0.159	95	0.067	87	**0.003**	95	0.072	0	1.000	0	0.919	0	1.000
89	GO:0020027	hemoglobin metabolic process	108	0.159	95	0.067	87	**0.003**	95	0.072	0	1.000	0	0.919	0	1.000
90	GO:0006957	complement activation alternative pathway	0	0.956	101	0.066	139	**0.000**	101	0.070	0	1.000	0	0.990	0	1.000
91	GO:0051918	negative regulation of fibrinolysis	0	0.979	0	0.945	278	**0.000**	0	0.948	0	0.999	0	0.986	0	1.000
92	GO:0006641	triglyceride metabolic process	0	0.943	36	0.097	50	**0.000**	36	0.101	0	1.000	0	0.997	0	1.000
93	GO:0051917	regulation of fibrinolysis	0	0.733	0	0.547	154	**0.002**	0	0.563	0	0.999	0	0.903	0	1.000
94	GO:0006639	acylglycerol metabolic process	0	0.880	32	0.100	44	**0.001**	32	0.104	0	0.994	0	0.997	0	1.000
95	GO:0018904	organic ether metabolic process	0	0.963	30	0.102	41	**0.001**	30	0.106	0	1.000	0	0.984	0	1.000
96	GO:0006638	neutral lipid metabolic process	0	0.990	32	0.100	43	**0.001**	32	0.105	0	1.000	0	0.998	0	1.000
97	GO:0006662	glycerol ether metabolic process	0	0.975	30	0.102	42	**0.001**	30	0.106	0	0.999	0	0.971	0	1.000
98	GO:0050878	regulation of body fluid levels	11	0.335	19	0.050	17	**0.002**	19	0.052	0	0.996	0	0.837	0	1.000
99	GO:0002714	positive regulation of B cell mediated immunity	217	0.114	190	0.052	173	**0.001**	190	0.055	0	1.000	0	0.980	0	1.000
100	GO:0002891	positive regulation of immunoglobulin mediated immune response	217	0.114	190	0.052	173	**0.001**	190	0.055	0	1.000	0	0.980	0	1.000
101	GO:0007599	hemostasis	13	0.325	23	**0.042**	21	**0.001**	23	**0.044**	0	1.000	0	0.972	0	1.000
102	GO:0032101	regulation of response to external stimulus	13	0.325	22	**0.042**	20	**0.001**	22	**0.044**	0	1.000	0	0.936	0	1.000
103	GO:0050817	coagulation	13	0.325	22	**0.042**	20	**0.001**	22	**0.044**	0	1.000	0	0.936	0	1.000
104	GO:0007596	blood coagulation	13	0.325	23	**0.038**	21	**0.000**	23	**0.040**	0	1.000	0	1.000	0	1.000
105	GO:0051604	protein maturation	14	0.326	24	**0.039**	28	**0.000**	24	**0.041**	0	0.999	0	0.934	0	1.000
106	GO:0002449	lymphocyte mediated immunity	17	0.304	30	**0.026**	21	**0.006**	30	**0.025**	0	1.000	0	0.984	0	1.000
107	GO:0016485	protein processing	15	0.316	27	**0.032**	31	**0.000**	27	**0.034**	0	0.999	0	0.992	0	1.000
108	GO:0002443	leukocyte mediated immunity	15	0.318	26	**0.032**	18	**0.009**	26	**0.034**	0	0.951	0	0.937	0	1.000
109	GO:0050727	regulation of inflammatory response	28	0.252	48	**0.008**	22	**0.037**	48	**0.009**	0	1.000	0	0.984	0	1.000
110	GO:0030162	regulation of proteolysis	35	0.225	62	**0.007**	14	0.213	62	**0.007**	0	0.989	0	0.994	0	1.000
111	GO:0019220	regulation of phosphate metabolic process	8	0.224	14	**0.005**	5	0.142	14	**0.004**	0	0.998	0	0.991	0	1.000
112	GO:0051174	regulation of phosphorus metabolic process	8	0.224	14	**0.005**	5	0.142	14	**0.004**	0	0.998	0	0.991	0	1.000

Suppl. Table 8. Over-represented Gene Ontology biological process annotations associated with genes repressed by the indicated dietary treatments[a]

			E2		PFOA		PFNA		PFDA		PFOS		8:2FtOH		CLOF	
#[b]	Accession	Biological Process	E	FDR	E	FDR	E	FDR	E	FDR	E	FDR	E	FDR	E	FDR
113	GO:0051247	positive regulation of protein metabolic process	18	0.125	24	**0.006**	7	0.151	24	**0.004**	0	0.946	0	0.984	0	1.000
114	GO:0032270	positive regulation of cellular protein metabolic process	19	0.113	25	**0.005**	8	0.147	25	**0.004**	0	1.000	0	0.996	0	1.000
115	GO:0042325	regulation of phosphorylation	8	0.220	14	**0.004**	5	0.135	14	**0.003**	0	0.998	0	0.986	0	1.000
116	GO:0050865	regulation of cell activation	11	0.330	30	**0.003**	9	0.123	30	**0.003**	0	1.000	0	0.999	0	1.000
117	GO:0031348	negative regulation of defense response	72	0.180	127	**0.004**	29	0.158	127	**0.004**	0	0.995	0	0.990	0	1.000
118	GO:0050870	positive regulation of T cell activation	26	0.259	45	**0.009**	20	0.050	45	**0.010**	0	1.000	0	0.986	0	1.000
119	GO:0051251	positive regulation of lymphocyte activation	21	0.281	37	**0.010**	17	0.062	37	**0.013**	0	1.000	0	1.000	0	1.000
120	GO:0042176	regulation of protein catabolic process	24	0.265	43	**0.009**	10	0.243	43	**0.011**	0	0.999	0	0.992	0	1.000
121	GO:0048585	negative regulation of response to stimulus	24	0.265	43	**0.009**	10	0.243	43	**0.011**	0	0.999	0	0.992	0	1.000
122	GO:0001869	negative regulation of complement activation lectin pathway	867	0.055	759	**0.027**	347	**0.036**	759	**0.027**	0	1.000	0	0.993	0	1.000
123	GO:0015886	heme transport	867	0.055	759	**0.027**	347	**0.036**	759	**0.027**	0	1.000	0	0.993	0	1.000
124	GO:0045627	positive regulation of T-helper 1 cell differentiation	867	0.055	759	**0.027**	347	**0.036**	759	**0.027**	0	1.000	0	0.993	0	1.000
125	GO:0060760	positive regulation of response to cytokine stimulus	0	0.997	759	**0.027**	347	**0.036**	759	**0.027**	0	1.000	0	0.993	0	1.000
126	GO:0001868	regulation of complement activation lectin pathway	867	0.055	759	**0.027**	347	**0.036**	759	**0.027**	0	1.000	0	0.993	0	1.000
127	GO:0002369	T cell cytokine production	0	0.993	253	**0.048**	116	0.082	253	**0.049**	0	1.000	0	0.992	0	1.000
128	GO:0032743	positive regulation of interleukin-2 production	0	0.993	253	**0.048**	116	0.082	253	**0.049**	0	1.000	0	0.992	0	1.000
129	GO:0010875	positive regulation of cholesterol efflux	0	0.993	253	**0.048**	0	0.967	253	**0.049**	0	1.000	0	0.992	0	1.000
130	GO:0032604	granulocyte macrophage colony-stimulating factor production	289	0.097	253	**0.048**	116	0.082	253	**0.049**	0	1.000	0	0.992	0	1.000
131	GO:0042253	granulocyte macrophage colony-stimulating factor biosynthetic process	289	0.097	253	**0.048**	116	0.082	253	**0.049**	0	1.000	0	0.992	0	1.000
132	GO:0002724	regulation of T cell cytokine production	0	0.993	253	**0.048**	116	0.082	253	**0.049**	0	1.000	0	0.992	0	1.000
133	GO:0010953	regulation of protein maturation by peptide bond cleavage	289	0.097	253	**0.048**	116	0.082	253	**0.049**	0	1.000	0	0.992	0	1.000
134	GO:0034447	very-low-density lipoprotein particle clearance	0	0.993	253	**0.048**	0	0.967	253	**0.049**	0	1.000	0	0.992	0	1.000
135	GO:0002694	regulation of leukocyte activation	12	0.328	21	**0.048**	10	0.120	21	**0.048**	0	1.000	0	0.983	0	1.000
136	GO:0009991	response to extracellular stimulus	12	0.329	21	**0.048**	10	0.123	21	**0.048**	166	0.728	0	1.000	0	1.000
137	GO:0032645	regulation of granulocyte macrophage colony-stimulating factor production	347	0.083	304	**0.042**	139	0.072	304	**0.044**	0	0.999	0	0.986	0	1.000
138	GO:0002726	positive regulation of T cell cytokine production	0	0.979	304	**0.042**	139	0.072	304	**0.044**	0	0.999	0	0.986	0	1.000
139	GO:0045939	negative regulation of steroid metabolic process	0	0.979	304	**0.042**	0	0.997	304	**0.044**	0	0.999	0	0.986	0	1.000
140	GO:0030240	muscle thin filament assembly	0	0.979	304	**0.042**	139	0.072	304	**0.044**	0	0.999	0	0.986	0	1.000

Suppl. Table 8. Over-represented Gene Ontology biological process annotations associated with genes repressed by the indicated dietary treatments[a]

			E2		PFOA		PFNA		PFDA		PFOS		8:2FtOH		CLOF	
#[b]	**Accession**	**Biological Process**	**E**	**FDR**	**E**	**FDR**	**E**	**FDR**	**E**	**FDR**	**E**	**FDR**	**E**	**FDR**	**E**	**FDR**
141	GO:0031641	regulation of myelination	347	0.083	304	**0.042**	139	0.072	304	**0.044**	0	0.999	0	0.986	0	1.000
142	GO:0045423	regulation of granulocyte macrophage colony-stimulating factor biosynthetic process	347	0.083	304	**0.042**	139	0.072	304	**0.044**	0	0.999	0	0.986	0	1.000
143	GO:0010894	negative regulation of steroid biosynthetic process	0	0.979	304	**0.042**	0	0.997	304	**0.044**	0	0.999	0	0.986	0	1.000
144	GO:0034382	chylomicron remnant clearance	0	0.979	304	**0.042**	0	0.997	304	**0.044**	0	0.999	0	0.986	0	1.000
145	GO:0034380	high-density lipoprotein particle assembly	0	0.979	304	**0.042**	0	0.997	304	**0.044**	0	0.999	0	0.986	0	1.000
146	GO:0042159	lipoprotein catabolic process	0	0.979	304	**0.042**	0	0.997	304	**0.044**	0	0.999	0	0.986	0	1.000
147	GO:0060333	interferon-gamma-mediated signaling pathway	0	0.986	304	**0.042**	139	0.072	304	**0.044**	0	0.999	0	0.986	0	1.000
148	GO:0051249	regulation of lymphocyte activation	14	0.326	24	**0.039**	11	0.114	24	**0.041**	0	1.000	0	0.991	0	1.000
149	GO:0031667	response to nutrient levels	14	0.326	24	**0.038**	11	0.114	24	**0.040**	190	0.797	0	0.799	0	1.000
150	GO:0002921	negative regulation of humoral immune response	434	0.071	380	**0.038**	173	0.062	380	**0.040**	0	0.995	0	0.968	0	1.000
151	GO:0045063	T-helper 1 cell differentiation	434	0.071	380	**0.038**	173	0.062	380	**0.040**	0	0.995	0	0.968	0	1.000
152	GO:0042508	tyrosine phosphorylation of Stat1 protein	434	0.071	380	**0.038**	173	0.062	380	**0.040**	0	0.995	0	0.968	0	1.000
153	GO:0060334	regulation of interferon-gamma-mediated signaling pathway	0	0.926	380	**0.038**	173	0.062	380	**0.040**	0	0.995	0	0.968	0	1.000
154	GO:0002825	regulation of T-helper 1 type immune response	434	0.071	380	**0.038**	173	0.062	380	**0.040**	0	0.995	0	0.968	0	1.000
155	GO:0030825	positive regulation of cGMP metabolic process	0	0.917	380	**0.038**	0	0.706	380	**0.040**	0	0.995	0	0.968	0	1.000
156	GO:0045624	positive regulation of T-helper cell differentiation	434	0.071	380	**0.038**	173	0.062	380	**0.040**	0	0.995	0	0.968	0	1.000
157	GO:0010544	negative regulation of platelet activation	0	0.917	380	**0.038**	0	0.706	380	**0.040**	0	0.995	0	0.968	0	1.000
158	GO:0045824	negative regulation of innate immune response	434	0.071	380	**0.038**	173	0.062	380	**0.040**	0	0.995	0	0.968	0	1.000
159	GO:0002923	regulation of humoral immune response mediated by circulating immunoglobulin	434	0.071	380	**0.038**	173	0.062	380	**0.040**	0	0.995	0	0.968	0	1.000
160	GO:0030828	positive regulation of cGMP biosynthetic process	0	0.917	380	**0.038**	0	0.706	380	**0.040**	0	0.995	0	0.968	0	1.000
161	GO:0002922	positive regulation of humoral immune response	578	0.057	506	**0.032**	231	0.050	506	**0.034**	0	1.000	0	0.989	0	1.000
162	GO:0045425	positive regulation of granulocyte macrophage colony-stimulating factor biosynthetic process	578	0.057	506	**0.032**	231	0.050	506	**0.034**	0	1.000	0	0.989	0	1.000
163	GO:0042511	positive regulation of tyrosine phosphorylation of Stat1 protein	578	0.057	506	**0.032**	231	0.050	506	**0.034**	0	1.000	0	0.989	0	1.000
164	GO:0010955	negative regulation of protein maturation by peptide bond cleavage	578	0.057	506	**0.032**	231	0.050	506	**0.034**	0	1.000	0	0.989	0	1.000
165	GO:0002925	positive regulation of humoral immune response mediated by circulating immunoglobulin	578	0.057	506	**0.032**	231	0.050	506	**0.034**	0	1.000	0	0.989	0	1.000
166	GO:0002639	positive regulation of immunoglobulin production	578	0.057	506	**0.032**	231	0.050	506	**0.034**	0	1.000	0	0.989	0	1.000
167	GO:0045916	negative regulation of complement activation	578	0.057	506	**0.032**	231	0.050	506	**0.034**	0	1.000	0	0.989	0	1.000

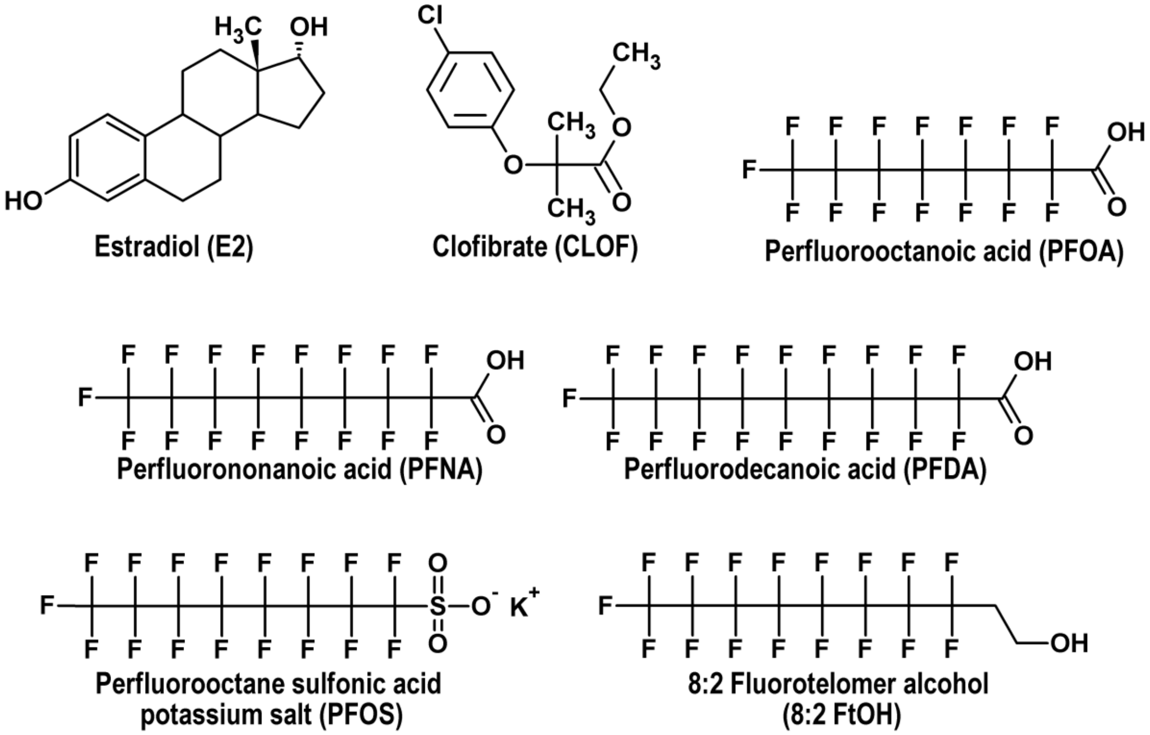
Suppl. Table 8. Over-represented Gene Ontology biological process annotations associated with genes repressed by the indicated dietary treatments[a]

			E2		PFOA		PFNA		PFDA		PFOS		8:2FtOH		CLOF	
#[b]	**Accession**	**Biological Process**	**E**	**FDR**	**E**	**FDR**	**E**	**FDR**	**E**	**FDR**	**E**	**FDR**	**E**	**FDR**	**E**	**FDR**
168	GO:0030449	regulation of complement activation	578	0.057	506	**0.032**	231	0.050	506	**0.034**	0	1.000	0	0.989	0	1.000
169	GO:0045625	regulation of T-helper 1 cell differentiation	578	0.057	506	**0.032**	231	0.050	506	**0.034**	0	1.000	0	0.989	0	1.000
170	GO:0042510	regulation of tyrosine phosphorylation of Stat1 protein	578	0.057	506	**0.032**	231	0.050	506	**0.034**	0	1.000	0	0.989	0	1.000
171	GO:0001961	positive regulation of cytokine-mediated signaling pathway	578	0.057	506	**0.032**	231	0.050	506	**0.034**	0	1.000	0	0.989	0	1.000
172	GO:0060759	regulation of response to cytokine stimulus	0	0.990	506	**0.032**	231	0.050	506	**0.034**	0	1.000	0	0.989	0	1.000
173	GO:0031401	positive regulation of protein modification process	31	0.057	27	**0.032**	12	0.091	27	**0.034**	0	1.001	0	0.922	0	1.000
174	GO:0050863	regulation of T cell activation	17	0.305	29	**0.032**	13	0.083	29	**0.034**	0	0.999	0	0.990	0	1.000
175	GO:0009894	regulation of catabolic process	17	0.307	29	**0.032**	7	0.290	29	**0.034**	0	0.972	0	0.981	0	1.000
176	GO:0045541	negative regulation of cholesterol biosynthetic process	0	0.986	506	**0.032**	0	0.899	506	**0.034**	0	1.000	0	0.989	0	1.000
177	GO:0032803	regulation of low-density lipoprotein receptor catabolic process	0	0.986	506	**0.032**	0	0.899	506	**0.034**	0	1.000	0	0.989	0	1.000
178	GO:0032802	low-density lipoprotein receptor catabolic process	0	0.986	506	**0.032**	0	0.899	506	**0.034**	0	1.000	0	0.989	0	1.000
179	GO:0018212	peptidyl-tyrosine modification	37	**0.046**	33	**0.026**	15	0.071	33	**0.026**	0	0.994	0	0.989	0	1.000
180	GO:0045937	positive regulation of phosphate metabolic process	38	**0.048**	33	**0.026**	15	0.071	33	**0.026**	0	1.000	0	0.998	0	1.000
181	GO:0010562	positive regulation of phosphorus metabolic process	38	**0.048**	33	**0.026**	15	0.071	33	**0.026**	0	1.000	0	0.998	0	1.000
182	GO:0018108	peptidyl-tyrosine phosphorylation	38	**0.048**	33	**0.026**	15	0.071	33	**0.026**	0	1.000	0	0.998	0	1.000
183	GO:0002696	positive regulation of leukocyte activation	19	0.294	33	**0.026**	15	0.071	33	**0.026**	0	1.000	0	0.998	0	1.000
184	GO:0031644	regulation of neurological system process	18	0.301	31	**0.026**	7	0.282	31	**0.026**	0	0.974	0	0.986	0	1.000
185	GO:0050867	positive regulation of cell activation	18	0.296	32	**0.026**	15	0.071	32	**0.026**	0	1.000	0	0.997	0	1.000
186	GO:0051969	regulation of transmission of nerve impulse	0	0.999	32	**0.026**	7	0.278	32	**0.026**	0	1.000	0	0.997	0	1.000
187	GO:0042327	positive regulation of phosphorylation	39	0.054	34	**0.027**	16	0.072	34	**0.027**	0	1.000	0	0.971	0	1.000
188	GO:0001775	cell activation	5	0.411	13	**0.027**	4	0.234	13	**0.027**	0	1.000	0	0.995	0	1.000
189	GO:0007584	response to nutrient	21	0.281	37	**0.027**	17	0.062	37	**0.027**	293	0.876	0	0.995	0	1.000

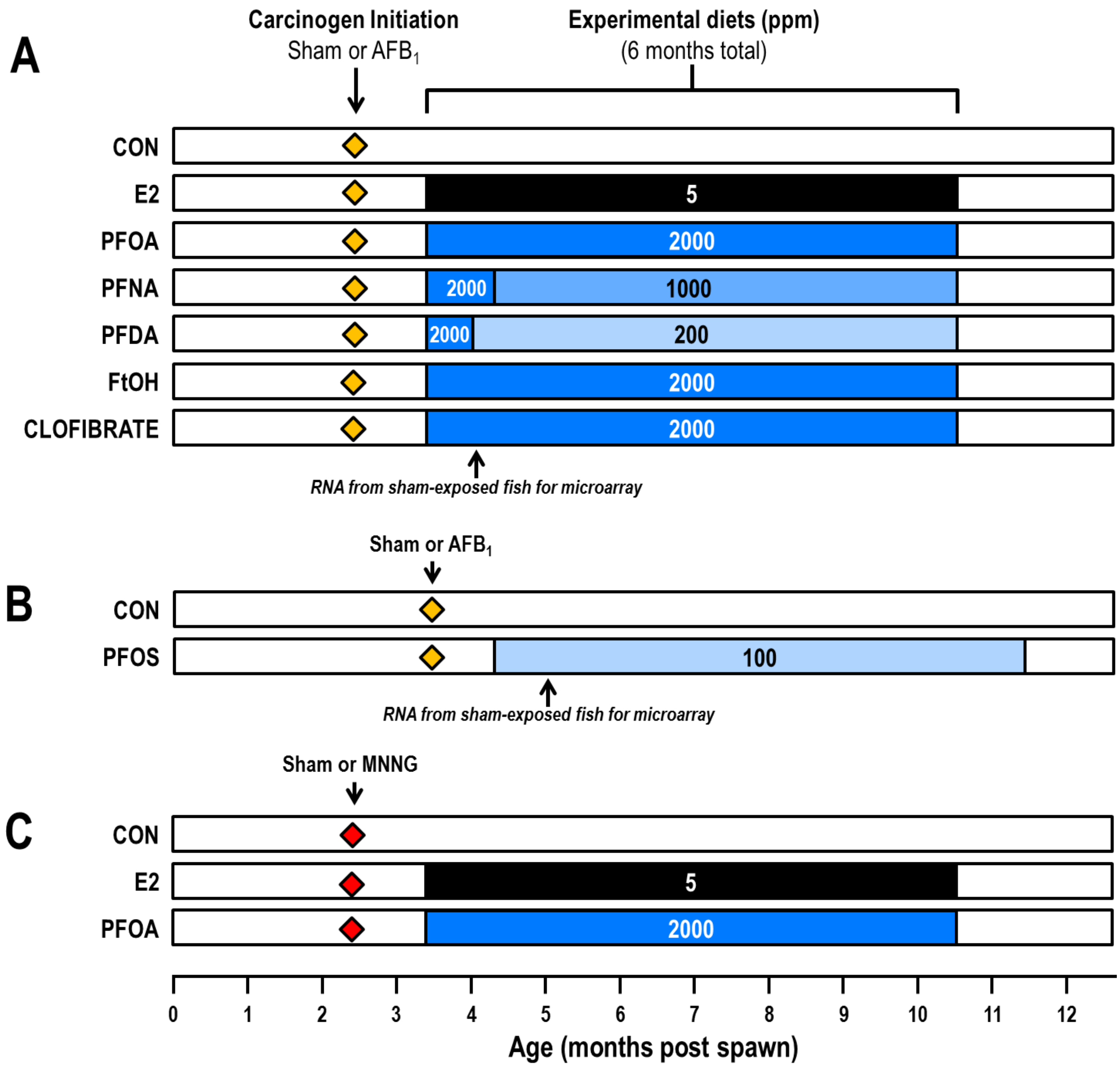
[a] Gene ontology enrichment analysis was performed using High Throughput GoMiner. A significant enrichment of the GO term category was inferred when $p<0.05$ as determined by a one-sided Fisher's exact test after false discovery rate (FDR) correction. Significantly enriched GO terms are indicated in bold with yellow or orange highlight.

[b] Number (#) corresponds to position in Cluster Image Map presented in Figure 4.

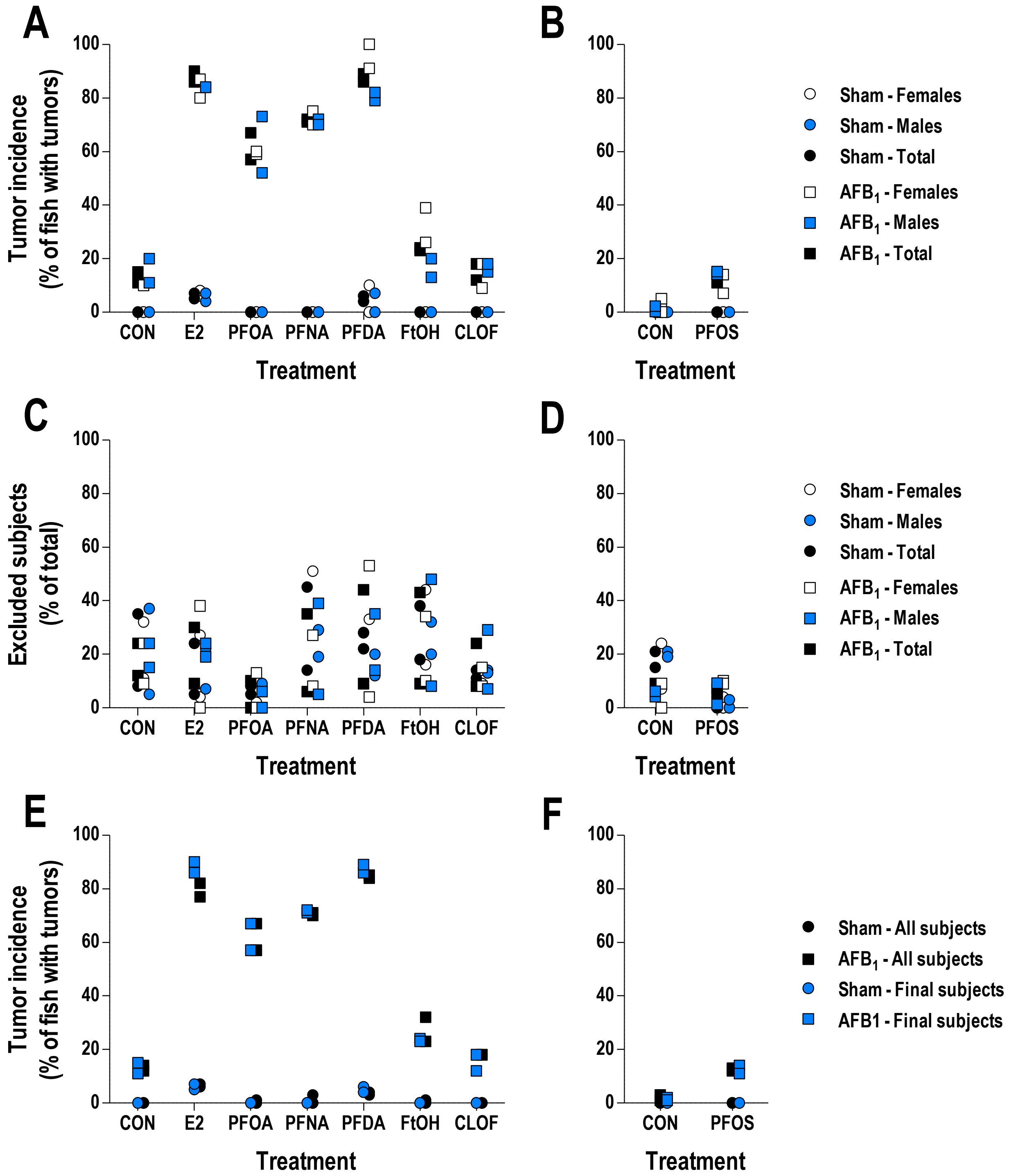
Abbreviations: E, enrichment value; FDR, false discovery rate-corrected *p*-value.



Supplemental Figure 1. Chemical structures of compounds tested for promotion of hepatocarcinogenesis in rainbow trout.



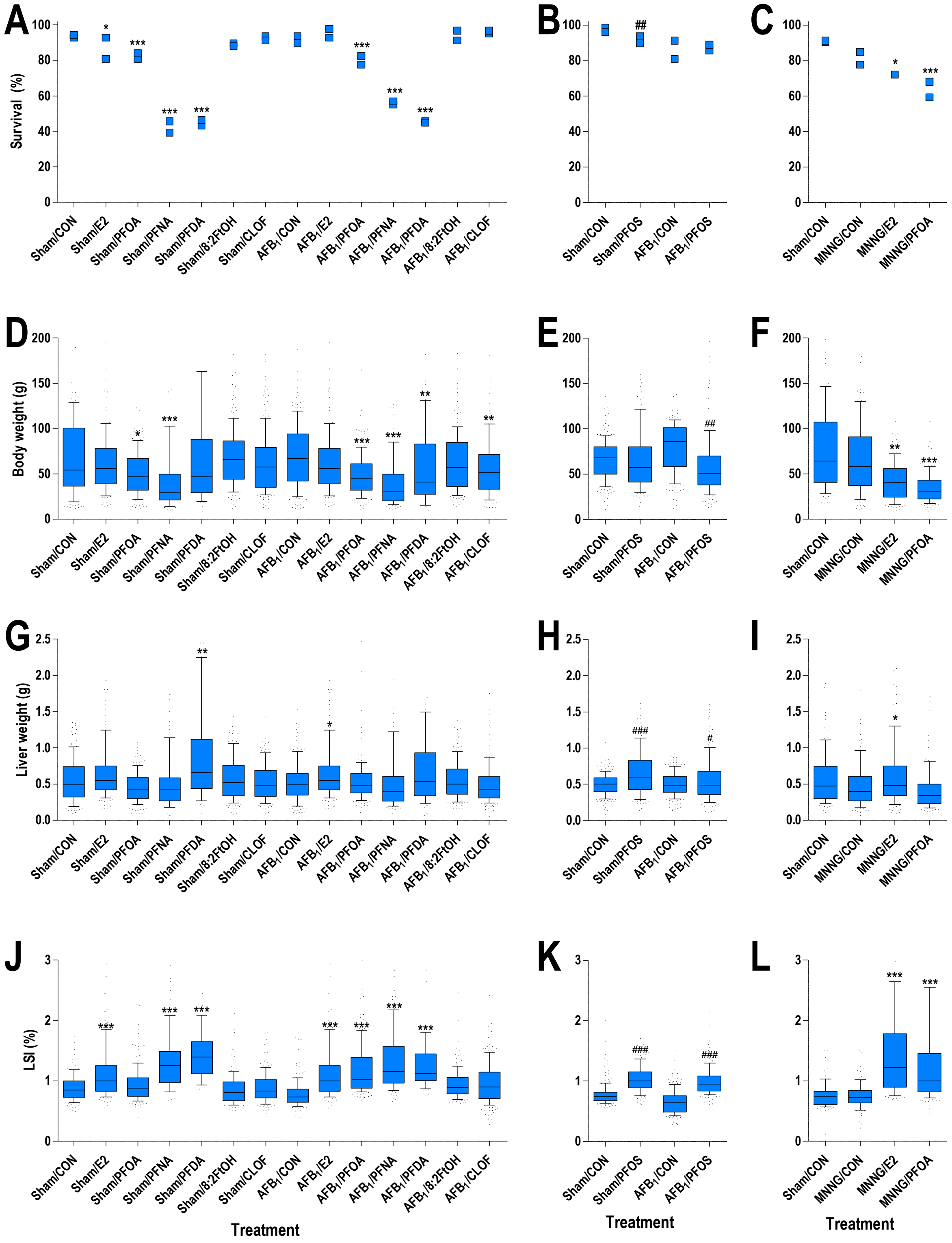
Supplemental Figure 2. Outline of AFB_1 and MNNG tumor studies in trout with dietary polyfluorinated chemicals. Fry were initiated at 10 weeks of age post spawn with 10 ppb AFB_1 (A) or 35 ppm MNNG (C), though one cohort of animals were initiated with AFB_1 at a slightly delayed age of 14 weeks (B). Four weeks after initiation, animals were fed the indicated experimental diets *ad libitum* (2.8-5.6% of body weight ration) for 6 months. PFOS was also initially tested at a diet concentration of 2000 ppm, though this exposure level was lethal in trout (data not shown). Consequently, a second cohort of trout fry (B) was initiated with a month delay compared to the other initiated groups, and a much lower diet concentration of 100 ppm PFOS was tested; as in the other treatment groups, the length of diet exposure was 6 months for PFOS-exposed trout. Necropsies for all treatment groups were performed at 12.5 months of age and were completed within one week.



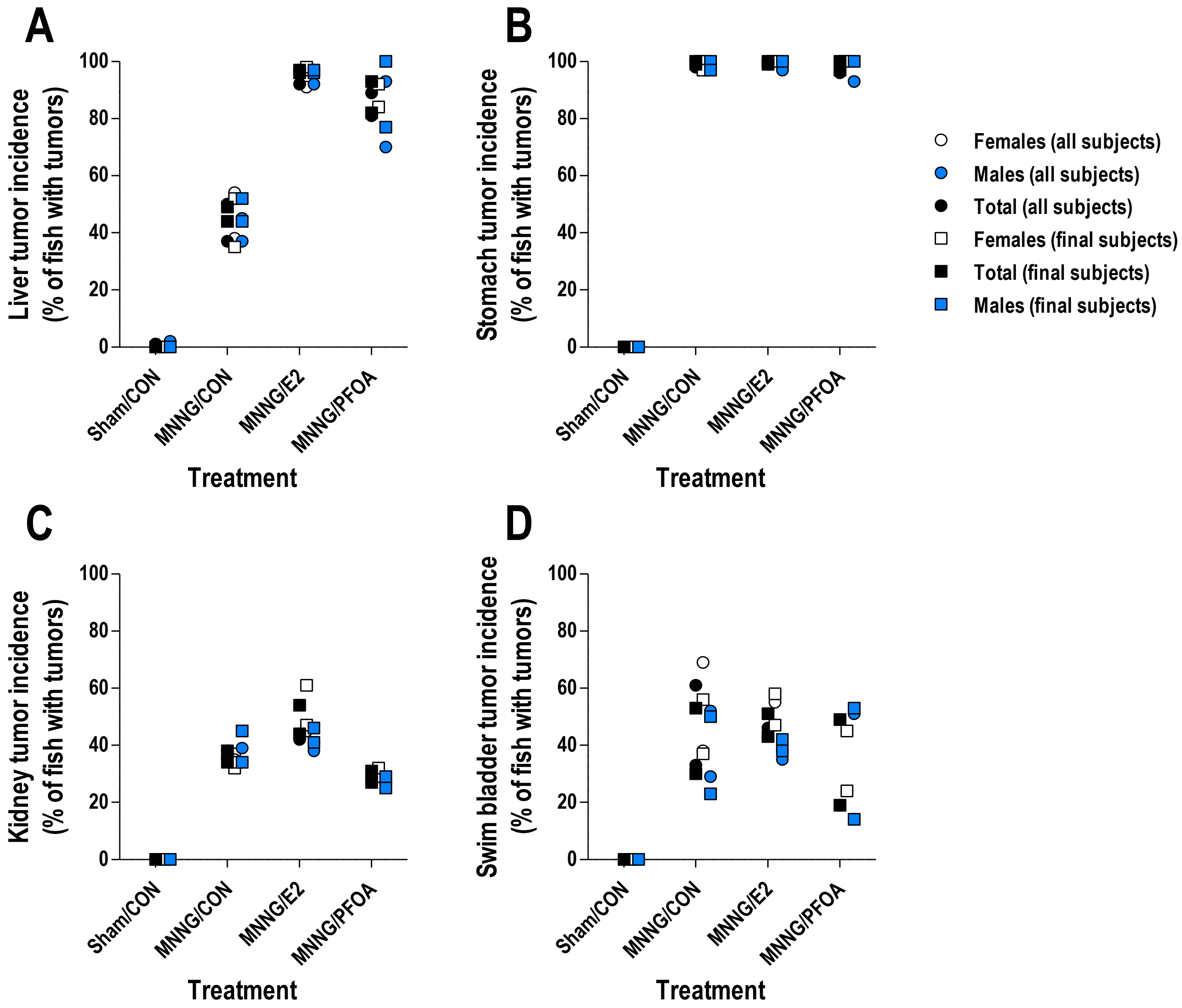
Supplemental Figure 3. Legend on following page.

Supplemental Figure 3. Lack of effect of sex, experimental tank or idiopathic liver disease on AFB_1-induced liver carcinogenesis. (previous page) (A, B) Comparison of liver tumor incidence data between male and female fish and between duplicate experimental tanks. Values are tumor incidence in animals initiated by 10 ppm AFB_1 (at 10 weeks of age for panel A, 15 weeks for panel B) following a 6-month dietary exposure to the indicated treatments. Tumor outcome was not significantly different between male and female fish or between the duplicate tanks as determined by logistic regression analysis. (C, D) Frequency of observed idiopathic liver disease unrelated to experimental condition. Values shown are incidence of fish with symptoms of liver disease from unknown origin, characterized by pale or jaundiced livers, which were excluded from the final experimental analyses. With the exception of PFOA treatment ($p < 0.05$), no effect of either carcinogen or dietary treatment on the frequency of idiopathic liver disease was observed in this study as determined by logistic regression analyses. (E, F) Liver tumor incidence in all experimental subjects compared to those with no symptoms of idiopathic liver disease (final subjects). No differences in tumor outcome were observed between the *all subject* and *final subject* data sets, as determined by logistic regression analyses. For all panels, symbols represent duplicate experimental tanks.

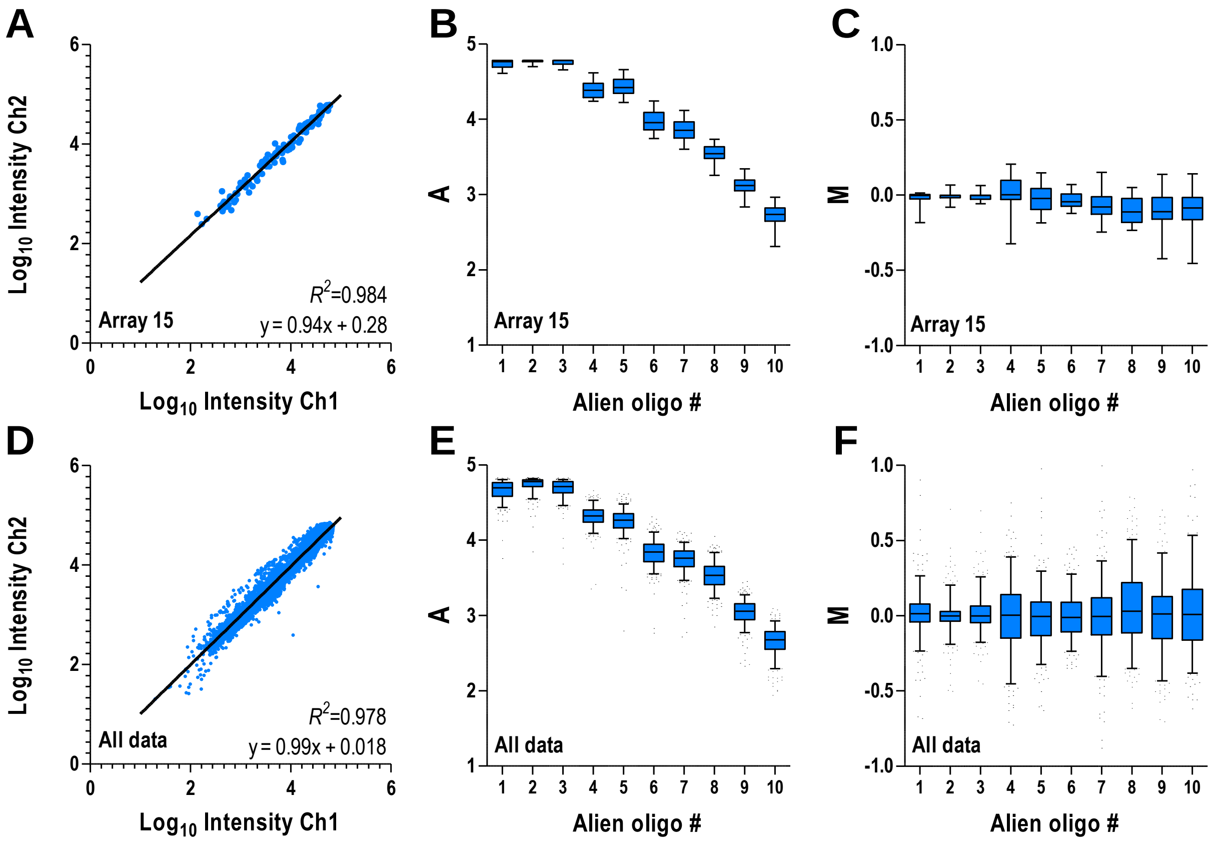
Supplemental Figure 4. Impact of promotion diets on morphological parameters in trout. (next page) Animals were initiated with 10 ppb AFB_1 or ethanol sham treatment at 10 weeks of age (A, D, G and J) or at 15 weeks (B, E, H and K); some animals were initiated with 35 ppm MNNG or DMSO sham treatment (C, F, I and L). See *Methods* for details on experimental diets. (A-C) Survival at termination of the study (12.5 months post spawn) for all treatment groups; in each figure, symbols represent duplicate tanks. (D-L) Average body weight, liver weight and liver somatic index (LSI = [liver weight/body weight]*100) values are shown as box-whisker plots with 10-90 percentile whisker bars. *, $p < 0.05$; **, $p < 0.01$, ***, $p < 0.001$ compared to control diet (within initiation group, Sham/CON, AFB_1/CON or MNNG/CON) as determined by the Kuskal-Wallis test with Dunn's multiple comparison test. ##, $p<0.05$; ###, $p<0.001$ compared to control diet (15wk initiation) as determined by unpaired Welch's *t*-test.



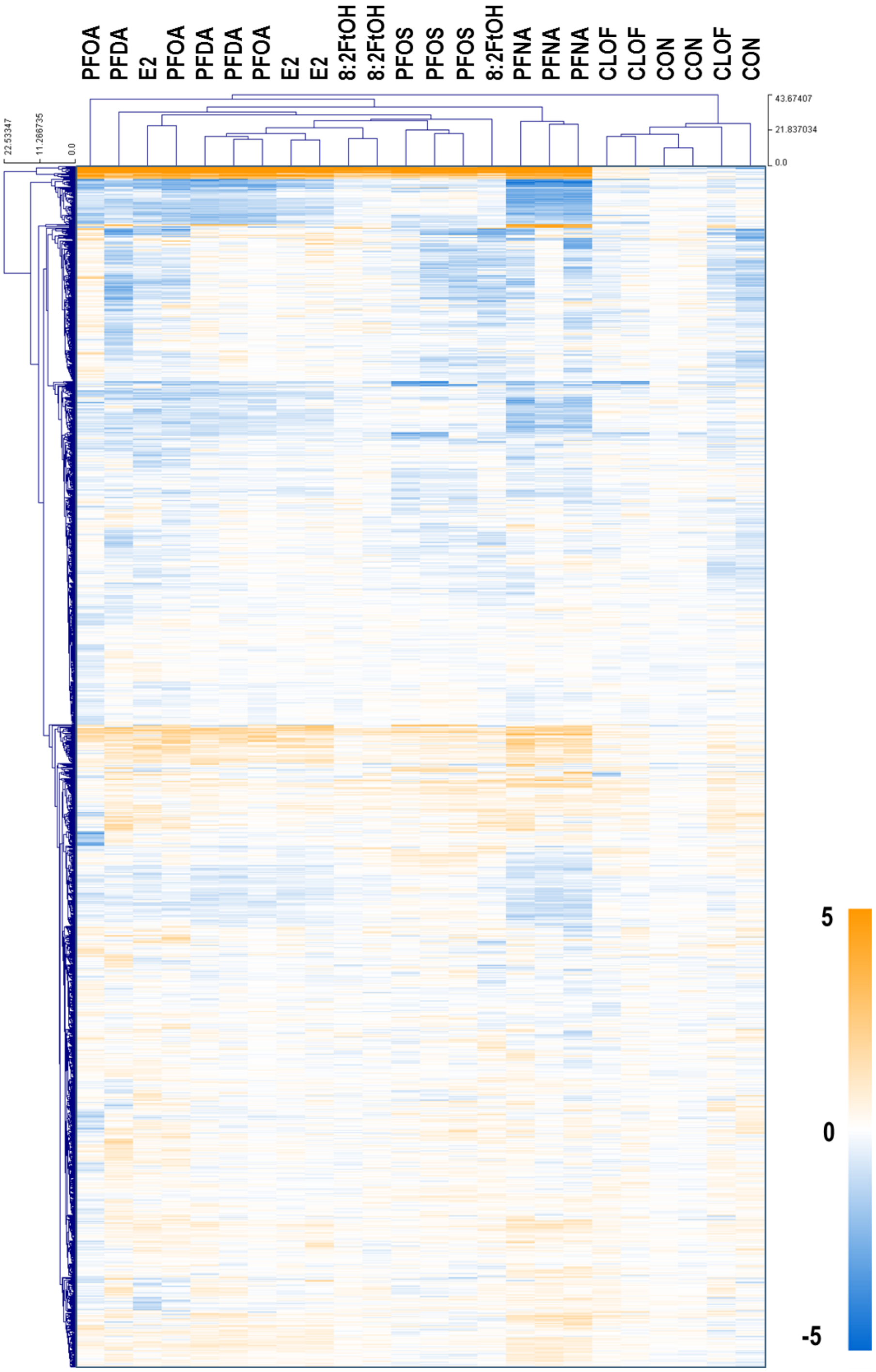
Supplemental Figure 4. Legend on previous page.



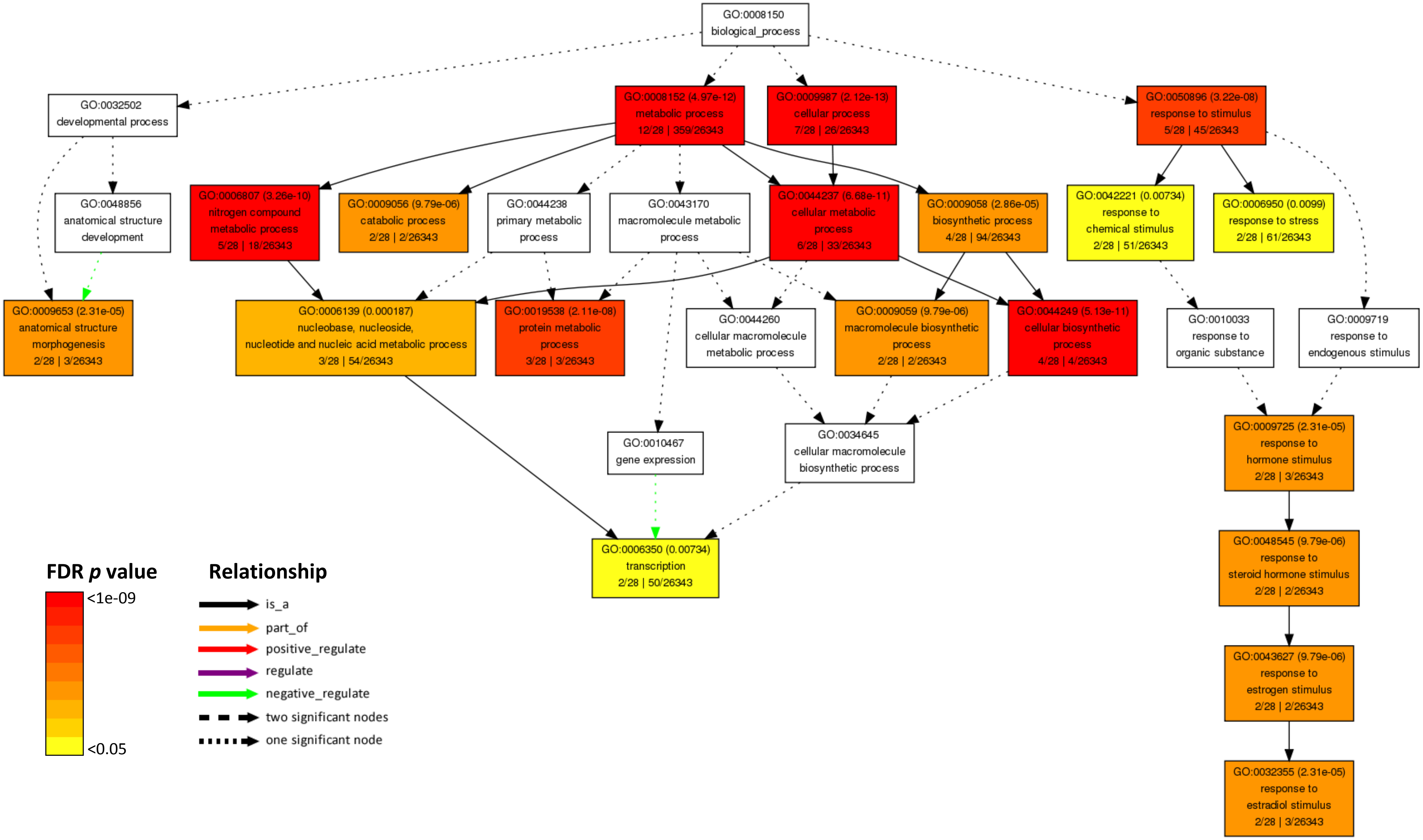
Supplemental Figure 5. Lack of effect of sex, experimental tank or idiopathic liver disease on MNNG-induced carcinogenesis. Comparisons of tumor incidence data for liver (A), stomach (B), kidney (C) and swim bladder (D) tumors between male and female fish and between duplicate experimental tanks. Values are tumor incidence in animals initiated by 35 ppm MNNG following a 6-month dietary exposure to the indicated treatments (see *Methods*). In each figure, symbols represent duplicate experimental tanks, and values for all experimental subjects and final subjects (no symptoms of idiopathic liver disease) are shown. For all tumor types, tumor outcome was not significantly different between male and female fish, between the duplicate tanks or between the *all subjects* and *final subjects* data groups; the single exception to this observation was for swim bladder tumors in MNNG/PFOA treatment group, where significant over-dispersion between experimental tanks was observed (*p* = 0.003).



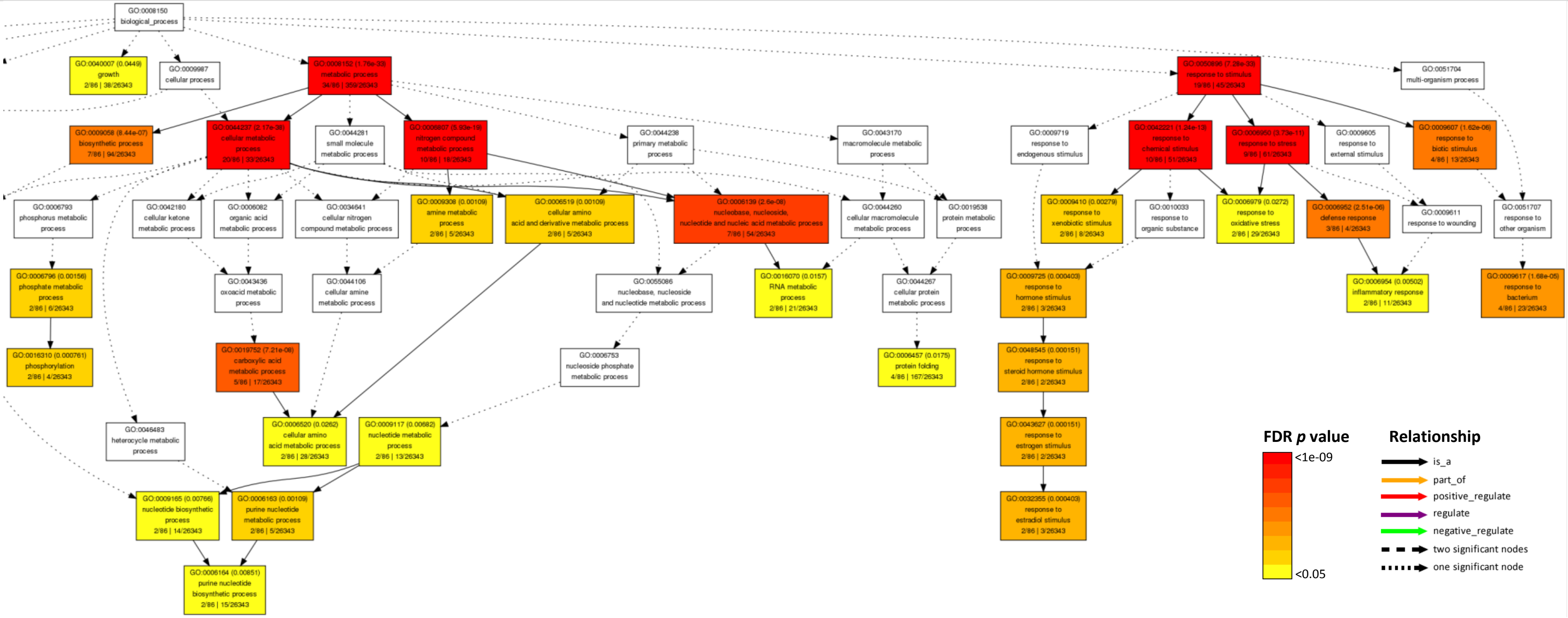
Supplemental Figure 6. Quality control analysis of array hybridization. Data for each SpotReport Alien Oligo feature for a representative array (A-C; *N* = 16 spots per olio per array) or for all arrays hybridized in this study (D-F; *N* = 640) are shown. Pairwise correlation analyses of Ch1 and Ch2 intensities are shown for all SpotReport Alien features (A) or those for a representative array (D). Box and whiskers (10-90 confidence interval) plots of values for mean intensity (panels B and E; A = $\log_{10}$(sqrt(Ch1*Ch2)) and the ratio of intensities (panels C and F; M = $\log_2$(Ch1/Ch2) are also shown for the entire array and for array 15. In summary, non-specific hybridization to buffer spots was not detected, background fluorescence was consistently low across the array and there was no apparent spatial bias on these arrays. As expected, a very strong correlation between Ch1 and Ch2 intensities was observed with slopes of the linear regression trend lines of 0.94 and 0.99 for array 15 or all arrays, respectively, indicating that the potential problem of dye bias was eliminated by ratio-centering and Lowess-normalization of the raw data. The quality control analysis shows that these arrays can detect changes in gene expression across a broad range of signal intensities (three orders of magnitude) and that hybridization to the OSUrbt array was consistent and reproducible.



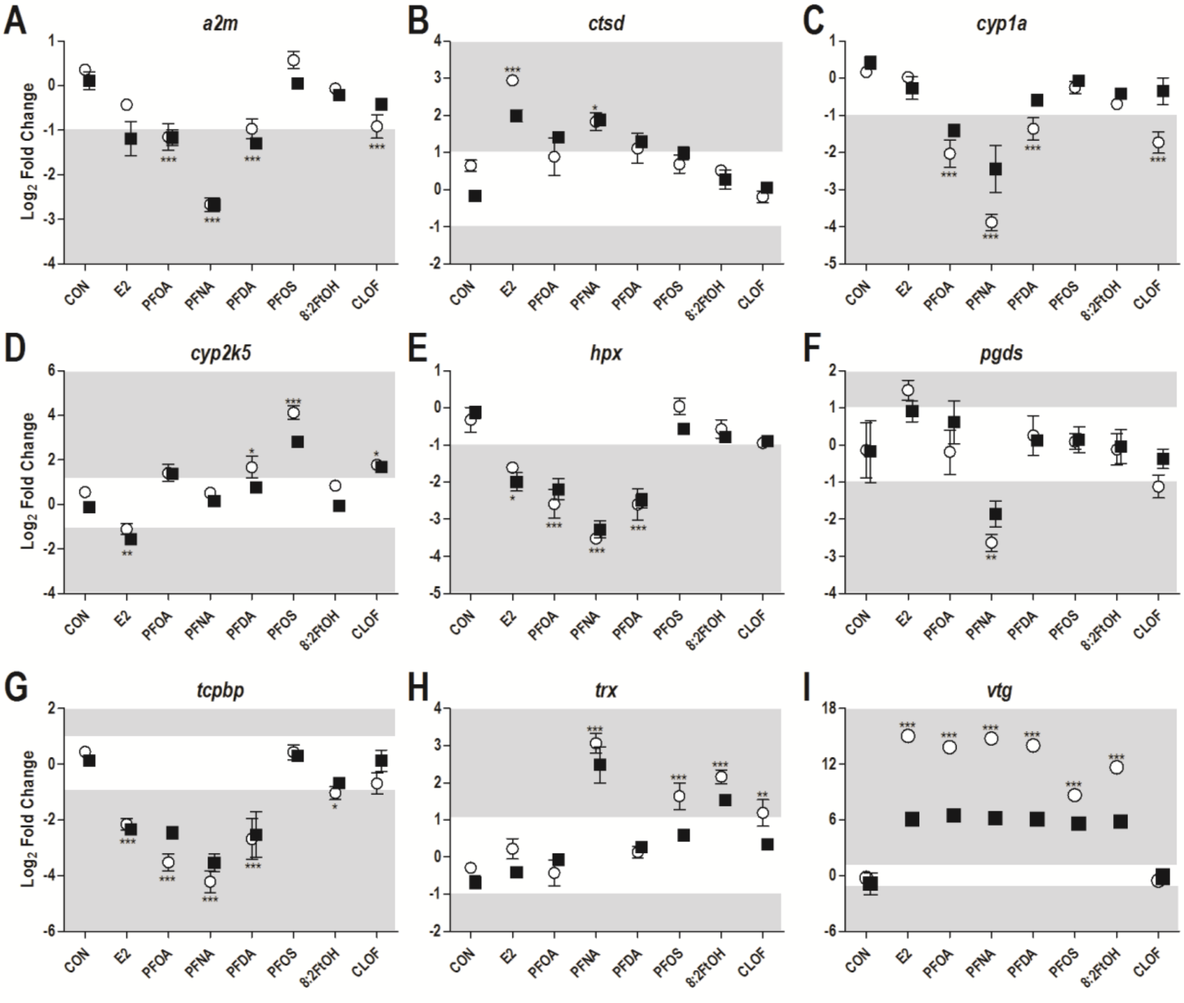
Supplemental Figure 7. Unsupervised bidirectional hierarchical cluster analysis of hepatic gene expression profiles. Bidirectional hierarchical clustering analysis was performed using sample data for either all array features; $\log_2$ fold change expression values are shown for each biological replicate ($n = 3$). Patterns of gene expression were clustered in two directions, by gene (left tree) and treatment (top tree), using the Euclidean distance method with average linkage.



Supplemental Figure 8. Directed acyclic graph for selected enriched GO terms associated with dietary E2. GO term enrichment was performed using agriGO singular enrichment analysis tool against the zebrafish gene ontology database. The scale bar indicates level of significance (FDR-corrected *p*-value) for enriched GO terms, and the inset legend indicates the type of relationship between terms as depicted by different arrow types.



Supplemental Figure 9. Directed acyclic graph (DAG) for selected enriched GO terms associated with dietary PFNA. GO term enrichment was performed using agriGO singular enrichment analysis tool against the zebrafish gene ontology database. Because of the extensive network of GO terms associated with PFNA exposure, only a portion of the DAG is shown for comparison to the E2 DAG shown in Suppl. Fig. 8. The scale bar indicates level of significance (FDR-corrected *p*-value) for enriched GO terms, and the inset legend indicates the type of relationship between terms as depicted by different arrow types. Best viewed using Adobe PDF viewer.



Supplemental Figure 10. Validation of treatment-induced changes in hepatic gene expression determined by microarray analysis using qRT-PCR. Values from qRT-PCR (open circles) are expressed as mean fold change (log$_2$) normalized to expression of housekeeping genes *actb*, *atp5b*, *gapdh* and *top2a* in a reference pool of cDNA from control treatments; values from the OSUrbt microarray (black squares) are expressed as mean fold change (log$_2$) compared to the control reference pool + SEM ($N = 3$) for select genes including (A) *a2m*, (B) *ctsd*, (C) *cyp1a*, (D) *cyp2k5*, (E) *hpx*, (F) *pgds*, (G) *tcpbp*, (H) *trx* and (I) *vtg*. qRT-PCR values for the PFOS treatment were normalized to the appropriate time-matched reference pool to correctly reflect array hybridization conditions for this treatment group. Light gray shading in the plot area indicates regions of two-fold induction or repression of gene expression. Asterisks indicate that the qRT-PCR expression value is significantly different (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$) from control treatment (CON) as determined by the Kruskal-Wallis test with Dunnett's test for multiple comparisons. Results of statistical analyses of microarray data are provided in Supplementary Table 6.

Supplemental References

Mortensen, A. S., Tolfsen, C. C. and Arukwe, A. (2006). Gene expression patterns in estrogen (nonylphenol) and aryl hydrocarbon receptor agonists (PCB-77) interaction using rainbow trout (*Oncorhynchus mykiss*) primary hepatocyte culture. *J. Toxicol. Environ. Health A* **69**, 1-19.

Rees, C. B. and Li, W. (2004). Development and application of a real-time quantitative PCR assay for determining CYP1A transcripts in three genera of salmonids. *Aquat. Toxicol.* **66**, 357-68.

Tilton, S. C., Givan, S. A., Pereira, C. B., Bailey, G. S. and Williams, D. E. (2006). Toxicogenomic profiling of the hepatic tumor promoters indole-3-carbinol, 17β-estradiol and β-naphthoflavone in rainbow trout. *Toxicol. Sci.* **90**, 61-72.