Supplementary Data:

Ancestral TCDD exposure induces multigenerational histologic and transcriptomic alterations in gonads of male zebrafish

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Supplemental Figure 1: Diagram of germ cell classification stages and example image of germ cell quantification within seminiferous tubules.

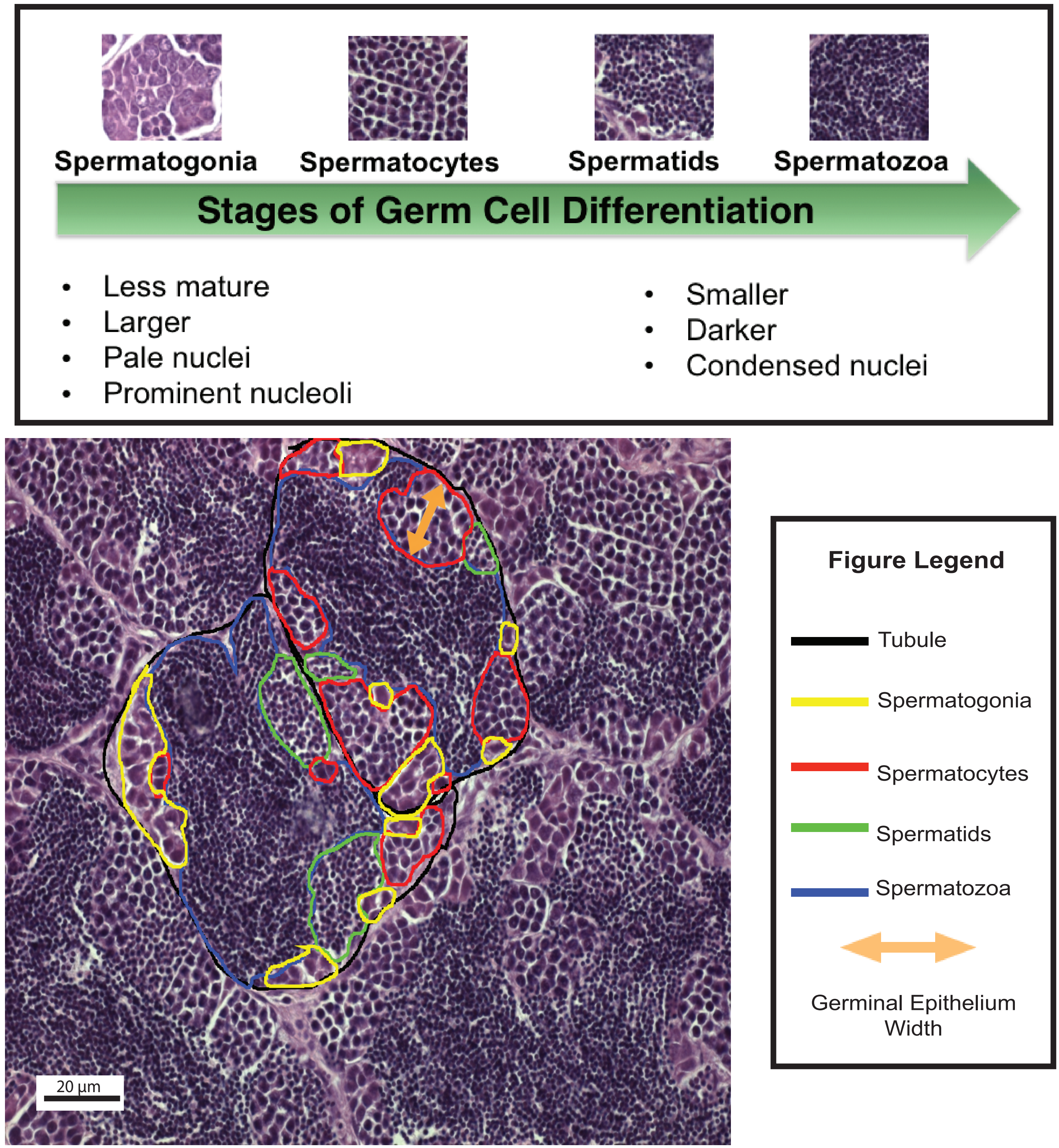
Supplemental Table 1: RIN and Quality Control values for RNA extracted from F_1 and F_2 zebrafish testes.

Supplemental Table 2: Top 100 differentially regulated microarray transcripts in both directions for F_1 and F_2 generations.

Supplemental Table 3: Microarray transcripts from classical hormone synthesis and response pathways for F_0-F_2 generations

Supplemental Table 4: Top five enriched pathways in TCDD-lineage F_1 and F_2 zebrafish testes (IPA).

Supplemental Table 5: qRT-PCR validation of select microarray genes in F_1 and F_2 testes



Supplemental Figure 1: Germ cell classification and quantification within seminiferous tubules. *Above* diagram indicates the progression of germ cell differentiation in the testes, as divided into 4 main stages of maturation. The 4 stages, from least mature to most mature, are as follows: spermatogonia, spermatocytes, spermatids, and spermatozoa. *Below left* is an example image of H&E-stained testicular tissue taken at 40X, with seminiferous tubule and germ cell areas marked as denoted in the figure legend (*bottom right*); H&E, Hematoxylin and Eosin.

Supplemental Table 1: RIN and Quality Control values for RNA extracted from F_1 and F_2 zebrafish testes.

	#	Sample ID	Date	RIN	Nucleic Acid Conc (ng/ul)	260/280	260/230
F1	**1**	**F1-CM1**	7/16/2015	7.20	77.3	1.99	1.01
	2	**F1-CM2**	7/16/2015	7.50	112.6	2.1	0.89
	3	**F1-CM3**	7/16/2015	7.90	105.3	2.06	1.65
	4	**F1-CM4**	7/16/2015	7.60	106.1	1.99	1.49
	5	**F1-DM1**	7/16/2015	7.10	101.9	2.04	1.61
	6	**F1-DM2**	7/16/2015	7.60	104.9	2.06	1.69
	7	**F1-DM3**	7/16/2015	7.40	102.6	2.04	1.74
	8	**F1-DM4**	7/16/2015	8.10	108.7	2.08	1.57
	9	**F1-CM5**	10/15/2015	8.20	95	2.01	2.34
	10	**F1-DM5**	10/15/2015	7.50	67.7	2.03	1.92
F2	**1**	**F2-CM1**	10/15/2015	8.60	79.3	2.03	1.86
	2	**F2-CM2**	10/15/2015	8.70	79	2	1.93
	3	**F2-CM3**	10/15/2015	7.50	108.6	1.98	2.21
	4	**F2-CM4**	10/15/2015	7.60	95.4	2.01	2.27
	5	**F2-CM5**	10/15/2015	7.20	120.3	2	2.21
	6	**F2-DM2**	10/15/2015	8.00	38	2.05	1.67
	7	**F2-DM3**	10/15/2015	8.20	61.4	2.02	1.4
	8	**F2-DM4**	10/15/2015	7.70	89.5	2.01	1.67
	9	**F2-DM5**	10/15/2015	7.90	105.3	2.02	1.8
	10	**F2-DM6**	10/15/2015	8.00	100.6	2.04	1.3

Samples are identified by generation (F_1 or F_2), condition (C=TCDD, D=DMSO control), sex (M=male), and number. RIN values, RNA concentrations (in ng/μl), 260/280 and 260/230 ratios are included for quality control purposes. RIN, RNA Integrity Number; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; DMSO, dimethyl sulfoxide.

Supplemental Table 2: Top 100 differentially regulated microarray transcripts in TCDD-lineage F1 and F2 zebrafish testes.

F1

Gene Symbol	Name	FC	P-value
acox1	*acyl-Coenzyme A oxidase 1, palmitoyl*	10.65	0.007122
ela2l	*elastase 2 like*	8.09	0.043571
gcga	*glucagon a*	7.96	0.033595
gusb	*glucuronidase, beta*	7.02	0.014511
LOC569427	*olfactomedin-4-like*	6.21	0.044714
nos1apa	*nitric oxide synthase 1 (neuronal) adaptor protein a*	4.49	0.019202
cpa2	*carboxypeptidase A2 (pancreatic)*	4.22	0.013748
CABZ01056052.1		4.01	0.014195
ins	*preproinsulin*	3.86	0.037729
si:dkey-65l23.2; si:ch211-233b23.4; LOC100150789	*si:dkey-65l23.2; si:ch211-233b23.4; uncharacterized LOC100150789*	3.51	0.03613
ush1c	*Usher syndrome 1C (autosomal recessive, severe)*	3.43	0.034805
atp1a1a.4	*ATPase, Na+/K+ transporting, alpha 1a.4 polypeptide*	3.39	0.049122
gcga	*glucagon a*	3.22	0.015751
slc20a1a	*solute carrier family 20, member 1a*	3.07	0.01682
slc20a1a	*solute carrier family 20, member 1a*	3.05	0.017882
LOC100537487	*endonuclease domain-containing 1 protein-like*	2.96	0.033988
SI:CH73-213J4.4		2.9	0.038319
cdx1a	*caudal type homeo box transcription factor 1 a*	2.84	0.046777
slc20a1a	*solute carrier family 20, member 1a*	2.84	0.009027
ndufa4	*NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4*	2.82	0.009371
klf4a	*Kruppel-like factor 4a*	2.79	0.037474
LOC100330987	*uncharacterized protein C11orf96-like*	2.79	0.003261
cygb1	*cytoglobin 1*	2.41	0.042789
CU638714.1; CR381686.1		2.38	0.048911
ptger4b	*prostaglandin E receptor 4 (subtype EP4) b*	2.38	0.047819
LOC100536370	*uncharacterized LOC100536370*	2.37	0.009538
LOC566587	*ERBB receptor feedback inhibitor 1-like*	2.33	0.038645
irf1b	*interferon regulatory factor 1b*	2.27	0.035662
loxl2a; LOC100535557	*lysyl oxidase-like 2a; lysyl oxidase homolog 2-like*	2.26	0.01103
csrnp1b	*cysteine-serine-rich nuclear protein 1b*	2.24	0.043279
rhag	*Rhesus blood group-associated glycoprotein*	2.24	0.032828
sgk1	*serum/glucocorticoid regulated kinase 1*	2.22	0.039467
junbb	*jun B proto-oncogene b*	2.2	0.034422
zgc:113317	*zgc:113317*	2.15	0.007183
gpr81-4	*GPR81-like protein 4*	2.12	0.049067
LOC100535673	*uncharacterized LOC100535673*	2.11	0.021724

LOC565181; TNS1 (2 of 2)	*tensin-1-like; tensin 1*	2.09	0.049091
si:ch211-195h23.3	*si:ch211-195h23.3*	2.09	0.047065
gcgb	*glucagon b*	2.08	0.030273
syne2b	*spectrin repeat containing, nuclear envelope 2b*	2.08	0.017059
cxcr7b	*chemokine (C-X-C motif) receptor 7b*	2.06	0.01365
LOC100331765	*serine/threonine-protein kinase WNK2-like*	2.06	0.046246
serpine1	*serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1*	2.06	0.041486
lrrc15	*leucine rich repeat containing 15*	2.05	0.018957
acta2	*actin, alpha 2, smooth muscle, aorta*	2.03	0.049748
dab1a; DAB1A	*disabled homolog 1a (Drosophila)*	2.03	0.016919
hivep2	*human immunodeficiency virus type I enhancer binding protein 2*	2	0.039192
slco1f1	*solute carrier organic anion transporter family, member 1F1*	2	0.040159
acta1a	*actin, alpha 1a, skeletal muscle*	1.99	0.011515
LOC100536370	*uncharacterized LOC100536370*	1.99	0.005428

c1galt1a	*core 1 synthase, glycoprotein-N-acetylgalactosamine 3-beta-galactosyltransferase, 1a*	-2.18	0.04205
LOC100537663	*KH domain-containing, RNA-binding, signal transduction-associated protein 3-like*	-2.18	0.03649
zgc:64116	*zgc:64116*	-2.19	0.045147
zgc:174320; LOC795264	*zgc:174320; uncharacterized LOC795264*	-2.22	0.030928
or137-2	*odorant receptor, family H, subfamily 137, member 2*	-2.23	0.002726
spra	*sepiapterin reductase a*	-2.27	0.009448
LOC100005785	*uncharacterized LOC100005785*	-2.28	0.048225
arf3b	*ADP-ribosylation factor 3b*	-2.29	0.003067
homez	*homeodomain leucine zipper gene*	-2.3	0.045257
si:dkey-253d23.11	*si:dkey-253d23.11*	-2.3	0.040684
si:ch211-106h4.6	*si:ch211-106h4.6*	-2.35	0.008779
si:ch211-106h4.6	*si:ch211-106h4.6*	-2.35	0.008779
si:ch211-106h4.6	*si:ch211-106h4.6*	-2.36	0.010954
cldna	*claudin a*	-2.38	0.026446
LOC100002266; SI:CH211-236P5.3	*NACHT, LRR and PYD domains-containing protein 1-like*	-2.39	0.002994
si:dkey-222p3.1	*si:dkey-222p3.1*	-2.43	0.023267
SI:DKEY-78K22.2; ZNF519 (2 of 4)	*zinc finger protein 519*	-2.45	0.019099
LOC799595	*immunoglobulin superfamily DCC subclass member 3-like*	-2.46	0.018036
or137-7	*odorant receptor, family H, subfamily 137, member 7*	-2.49	0.00389
carm1l	*coactivator-associated arginine methyltransferase 1, like*	-2.5	0.040069
LOC568123	*cyclic nucleotide-gated cation channel alpha-4-like*	-2.5	0.009544
or137-4	*odorant receptor, family H, subfamily 137, member 4*	-2.52	0.008726
gna15.2	*guanine nucleotide binding protein (G protein), alpha 15.2 (Gq class)*	-2.54	0.009415
adcy8	*adenylate cyclase 8 (brain)*	-2.56	0.008292
rlbp1b	*retinaldehyde binding protein 1b*	-2.56	0.046991
LOC564083	*uncharacterized LOC564083*	-2.62	0.018925

murcb	*muscle-related coiled-coil protein b*	-2.68	0.048577
zymnd12	*zinc finger, MYND-type containing 12*	-2.77	0.013232
slc4a8	*solute carrier family 4, sodium bicarbonate cotransporter, member 8*	-2.78	0.027951
LOC573741; LOC100537537	*uncharacterized LOC573741; myocardin-like*	-2.79	0.026416
pcdh8	*protocadherin 8*	-2.81	0.007844
sgms2	*sphingomyelin synthase 2*	-2.83	0.001406
zgc:173614; SI:DKEY-78K22.2; SI:DKEY-82I20.3	*zgc:173614*	-2.85	0.044021
clrn1	*clarin 1*	-2.9	0.027305
LOC100537130; LOC560161	*protein NLRC3-like; neoverrucotoxin subunit beta-like*	-2.91	0.0179
LOC100537832	*uncharacterized protein C1orf222-like*	-2.91	0.018541
LOC100151470	*olfactory receptor 4F6-like*	-3	0.001323
zgc:113886	*zgc:113886*	-3.01	0.018457
si:dkeyp-72h1.1	*si:dkeyp-72h1.1*	-3.08	0.021971
LOC100151470	*olfactory receptor 4F6-like*	-3.41	0.0029
CU929354.3; zgc:100951	*zgc:100951*	-3.43	0.013361
smyd1a	*SET and MYND domain containing 1a*	-3.6	0.006411
or137-5; or137-9	*odorant receptor, family H, subfamily 137, member 5; odorant receptor, family H, subfamily 137, member 9*	-3.86	0.003423
or137-4	*odorant receptor, family H, subfamily 137, member 4*	-3.88	0.001813
or137-5	*odorant receptor, family H, subfamily 137, member 5*	-4.17	0.004994
zgc:113969	*zgc:113969*	-4.49	0.030852
LOC100004625	*c-C chemokine receptor type 2-like*	-5.78	0.013501
or137-3	*odorant receptor, family H, subfamily 137, member 3*	-6.01	0.001026
LOC100005908	*apolipoprotein A-IV-like*	-7.65	0.03141
LOC100331852	*uncharacterized LOC100331852*	-8.88	0.047343

F2

Gene Symbol	Name	FC	P-value
CELA1 (3 of 8)	*chymotrypsin-like elastase family, member 1*	29.62	0.004719
LOC100536200	*uncharacterized LOC100536200*	22.39	0.031717
ptprc	*protein tyrosine phosphatase, receptor type, C*	18.35	0.004682
LOC100330158	*novel NACHT domain containing protein-like*	15.16	0.016091
LOC100536612; LOC100536895	*uncharacterized LOC100536612; protein NLRC3-like*	13.68	0.000022
LOC100149909	*NACHT, LRR and PYD domains-containing protein 3-like*	11.13	0.000006
LOC100330158	*novel NACHT domain containing protein-like*	10.94	0.007158
SC:D217		10.54	2.34E-09
zgc:113098	*zgc:113098*	10.48	0.001195
btf3	*basic transcription factor 3*	9.67	0.002795
LOC100001465	*NACHT, LRR and PYD domains-containing protein 12-like*	9.22	0.023902
LOC564346; LOC100330158	*NACHT, LRR and PYD domains-containing protein 3-like; novel NACHT domain containing protein-like*	9.09	0.009308

LOC100538282	*v-set domain-containing T-cell activation inhibitor 1-like*	9.06	0.006737
LOC100330140	*oocyte zinc finger protein XlCOF6-like*	8.97	0.006213
ptprc	*protein tyrosine phosphatase, receptor type, C*	8.79	0.009325
LOC100149909	*NACHT, LRR and PYD domains-containing protein 3-like*	8.59	0.000003
LOC100333695; LOC100333766	*protein NLRC3-like; stonustoxin subunit alpha-like*	8.32	0.01293
spata4; zgc:112531	*spermatogenesis associated 4; zgc:112531*	8.22	0.000078
LOC560036; LOC100537841; LOC100332192; SI:CH211-145H19.5	*zinc finger protein 271-like*	7.89	0.002452
LOC100536726	*uncharacterized LOC100536726*	7.48	0.027077
oc90; LOC100537480	*otoconin 90; uncharacterized LOC100537480*	7.28	0.0015
LOC100534835	*uncharacterized LOC100534835*	7.09	0.014181
LOC799802	*stonustoxin subunit beta-like*	6.93	0.008803
MDFIC (1 of 2)	*MyoD family inhibitor domain containing*	6.39	0.000651
LOC100329764; LOC100330473	*uncharacterized LOC100329764; uncharacterized LOC100330473*	6.36	0.000193
acox1	*acyl-Coenzyme A oxidase 1, palmitoyl*	6.12	0.000522
nos1apa	*nitric oxide synthase 1 (neuronal) adaptor protein a*	6.02	0.000003
si:ch73-190m4.1	*si:ch73-190m4.1*	5.99	0.014795
LOC568664	*NACHT, LRR and PYD domains-containing protein 3-like*	5.95	0.017124
LOC100333766; LOC100333695	*stonustoxin subunit alpha-like; protein NLRC3-like*	5.87	0.007413
LOC100333695; LOC100333766	*protein NLRC3-like; stonustoxin subunit alpha-like*	5.52	0.014855
sell	*selenoprotein L*	5.48	0.021391
zgc:136302	*zgc:136302*	5.31	0.011571
LOC100333818	*coiled-coil domain-containing protein 15-like*	5.28	0.004599
zgc:113119; LOC100332485	*zgc:113119; zinc finger protein 585B-like*	5.27	0.000006
LOC100535078; pttg1; LOC100535199	*securin-like; pituitary tumor-transforming 1; uncharacterized LOC100535199*	5.03	0.00073
SC:D217		4.63	6.91E-07
zgc:91850; LOC561730	*zgc:91850; e3 ubiquitin-protein ligase RBBP6-like*	4.63	0.015358
im:7141269	*im:7141269*	4.42	0.006418
CABZ01056052.1		4.28	0.000608
LOC100537304; mrps22	*28S ribosomal protein S22, mitochondrial-like; mitochondrial ribosomal protein S22*	4.27	0.000024
mlphb	*melanophilin b*	4.18	0.014774
LOC100536484	*gastrula zinc finger protein XlCGF57.1-like*	4.16	0.027618
SI:DKEY-237G15.5; SI:DKEY-16P6.1		4.15	0.002425
LOC568525	*uncharacterized LOC568525*	4	0.020579
zgc:173703; SI:DKEY-81I14.3	*zgc:173703*	3.84	0.022208
LOC100149903	*GTPase IMAP family member 7-like*	3.82	0.008797
cyb5d1	*cytochrome b5 domain containing 1*	3.68	0.028146
nudt15	*nudix (nucleoside diphosphate linked moiety X)-type motif 15*	3.68	0.002318
mdh1a	*malate dehydrogenase 1a, NAD (soluble)*	3.57	0.00175
si:ch211-149a19.3; SI:CH211-149A19.4; SI:CH211-149A19.3	*si:ch211-149a19.3*	-2.94	0.039149

LOC794617	*uncharacterized LOC794617*	-2.96	0.022567
zgc:153440	*zgc:153440*	-2.99	0.045296
LOC100006875	*uncharacterized LOC100006875*	-3	0.000011
zgc:136254	*zgc:136254*	-3.03	0.021341
LOC569848; SI:CH211-59H6.1	*uncharacterized LOC569848*	-3.25	0.000022
pde6h	*phosphodiesterase 6H, cGMP-specific, cone, gamma*	-3.25	0.001533
LOC100148709	*protein NLRC3-like*	-3.38	0.024599
LOC100006805	*signaling lymphocytic activation molecule-like*	-3.39	0.000101
SI:CH211-178J18.2		-3.54	0.000309
rasa1b	*RAS p21 protein activator 1b*	-3.56	0.000721
si:dkey-65b12.12	*si:dkey-65b12.12*	-3.58	0.001313
LOC568902	*NACHT, LRR and PYD domains-containing protein 8-like*	-3.63	0.019847
LOC100005785	*uncharacterized LOC100005785*	-3.79	0.016891
CABZ01081294.1		-3.96	0.011416
SI:DKEY-65B12.10		-4.01	0.000954
SI:DKEY-256I11.3		-4.09	0.002904
LOC100537269	*uncharacterized LOC100537269*	-4.18	0.002509
SI:DKEY-78K22.2		-4.45	0.000019
LOC100333560	*protein NLRC3-like*	-4.5	0.000234
LOC100333474	*uncharacterized LOC100333474*	-4.51	0.000156
SI:DKEY-211G8.8		-4.61	0.005055
si:dkey-211g8.5	*si:dkey-211g8.5*	-4.88	0.007155
LOC100151195	*hereditary hemochromatosis protein homolog*	-4.92	0.040714
adam28	*a disintegrin and metallopeptidase domain 28*	-5.13	0.035483
LOC100535646	*uncharacterized LOC100535646*	-5.15	0.009627
LOC100006875	*uncharacterized LOC100006875*	-5.19	0.000003
LOC100002043	*uncharacterized LOC100002043*	-5.27	0.000098
si:ch211-149a19.3; SI:CH211-149A19.4; SI:CH211-149A19.3	*si:ch211-149a19.3*	-5.35	0.017917
LOC568902	*NACHT, LRR and PYD domains-containing protein 8-like*	-6.23	0.007425
ftr19	*finTRIM family, member 19*	-6.37	0.006521
SI:CH73-213J4.4		-6.46	0.004166
si:dkey-65b12.12; SI:DKEY-65B12.10	*si:dkey-65b12.12*	-6.58	0.001345
si:ch211-149a19.3; SI:CH211-149A19.4; SI:CH211-149A19.3	*si:ch211-149a19.3*	-6.86	0.029359
LOC568902	*NACHT, LRR and PYD domains-containing protein 8-like*	-7.03	0.003139
LOC568944	*NACHT, LRR and PYD domains-containing protein 1-like*	-7.17	0.020758
si:dkey-29d5.1	*si:dkey-29d5.1*	-7.3	0.000008
grn1	*granulin 1*	-7.92	0.040113
grn1	*granulin 1*	-8.63	0.049484
LOC100536768	*uncharacterized LOC100536768*	-9.93	0.007219
trim35-3; LOC100003935	*tripartite motif containing 35-3; ret finger protein-like*	-11.06	0.038682
si:dkey-65l23.2; si:ch211-233b23.4; LOC100150789	*si:dkey-65l23.2; si:ch211-233b23.4; uncharacterized LOC100150789*	-11.17	0.016002

LOC571714; PPP1R32	*uncharacterized protein C11orf66 homolog; protein phosphatase 1, regulatory subunit 32*	-15.58	0.023156
cmtm6; LOC100535165	*CKLF-like MARVEL transmembrane domain containing 6; CKLF-like MARVEL transmembrane domain-containing protein 6-like*	-19.67	0.003737
aste1	*asteroid homolog 1 (Drosophila)*	-19.84	0.005095
grn2	*granulin 2*	-22.33	0.013033
LOC100006764	*uncharacterized LOC100006764*	-23.14	1.49E-07
mhc1uba	*major histocompatibility complex class I UBA*	-26.44	0.004289
LOC100004625	*c-C chemokine receptor type 2-like*	-42.39	5.37E-07
ftr50; LOC100330813	*finTRIM family, member 50; e3 ubiquitin/ISG15 ligase TRIM25-like*	-131.88	0.03826

Gene symbols and names are listed for the 50 most up- and down-regulated transcripts in TCDD-lineage F_1 and F_2 fish. TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; FC, fold change.

Supplemental Table 3: Microarray transcripts from classical hormone synthesis and response pathways for F_0-F_2 generations

		F0		**F1**		**F2**	
Symbol	**Name**	*FC*	*P-value*	*FC*	*P-value*	*FC*	*P-value*
amh	*anti-Mullerian hormone*	1.62	0.15	1.31	0.55	-1.5	0.43
ar	*androgen receptor*	1.08	0.07	1.06	0.21	1.2	0.07
cyp1a	*cytochrome P450, family 1, subfamily A*	2.2	0.06	1.7	0.39	-1.25	0.89
cyp1b1	*cytochrome P450, family 1, subfamily B, polypeptide 1*	-1.02	0.1	1.09	0.31	-1.02	0.91
cyp19a1a	*cytochrome P450, family 19, subfamily A, polypeptide 1a*	-1.12	0.24	1.23	0.42	-1.07	0.78
cyp19a1b	*cytochrome P450, family 19, subfamily A, polypeptide 1b*	-1.03	0.8	1.14	0.93	-1.1	0.51
dmrt1	*doublesex and mab-3 related transcription factor 1*	1.07	0.73	-1.15	0.36	-1.08	0.33
esr1	*estrogen receptor 1*	1.08	0.59	-1.06	0.85	1.24	0.028
esr2a	*estrogen receptor 2a*	1.17	0.12	1.06	0.29	-1.04	0.89
esr2b	*estrogen receptor 2b*	1.55	0.06	1.3	0.18	1.29	0.75
foxl2	*forkhead box L2*	-1.01	0.98	1.02	0.8	1.08	0.36
fshb	*follicle-stimulating hormone, beta polypeptide*	-1.13	0.1	-1.35	0.14	1.06	0.30
fshr	*follicle-stimulating hormone receptor*	1.28	0.2	1.16	0.4	-1.16	0.97
gstal	*glutathione S-transferase, alpha-like*	-1.15	0.69	1.4	0.13	-1.48	0.57
hsd3b7	*hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase*	-1.17	0.57	-1.01	0.78	1.3	0.3
hsd11b2	*hydroxysteroid 11-beta dehydrogenase 2*	1.3	0.1	1.06	0.59	1.27	0.12
hsd17b3	*hydroxysteroid (17-beta) dehydrogenase 3*	-1.03	0.52	1.5	0.54	1.17	0.69
lhb	*luteinizing hormone, beta polypeptide*	1.05	0.85	1.03	0.8	-1.04	0.66
nqo1	*NAD(P)H dehydrogenase, quinone 1*	1.16	0.1	1.16	0.25	1.04	0.38
star	*steroidogenic acute regulatory protein*	**1.42**	**0.03**	1.06	0.64	1.22	0.56
ugt1a2	*UDP glucuronosyltransferase 1 family, polypeptide A2*	1.09	0.56	1.82	0.05	-1.24	0.91
vtg1	*vitellogenin 1*	1.89	0.28	-1.47	0.17	1.14	0.54
vtg3	*vitellogenin 3*	1.06	0.87	1.19	0.65	-1.08	0.7
vtg5	*vitellogenin 5*	-1.01	0.96	1.26	0.23	-1.11	0.97

Fold changes and p-values for microarray data are listed per gene; p-value is determined using ANOVA. Bolded text indicates significant difference from control (FC >1.5, p-value < 0.05). FC, fold change; ANOVA, analysis of variance.

Supplemental Table 4: Ingenuity Pathway Analysis-generated list of the top 5 enriched pathways in each category of disease and biological functions in TCDD-lineage F_1 and F_2 fish.

F1 IPA Top Diseases and Biological Functions		
Category	**P-value**	**# of genes**
Diseases and Disorders		
Cancer	1.15E-02-5.76E-06	126
Organismal Injury and Abnormalities	1.17E-02-5.76E-06	128
Endocrine System Disorders	1.07E-02-7.27E-06	26
Gastrointestinal Disease	1.11E-02-7.27E-06	101
Metabolic Disease	1.07E-02-7.27E-06	29
Molecular and Cellular Functions		
Protein Synthesis	9.55E-03-4.76E-06	18
Cellular Movement	1.15E-02-3.12E-05	36
Cell Death and Survival	9.94E-03-3.91E-05	47
Cell Morphology	1.22E-02-8.50E-05	52
Cellular Assembly and Organization	9.94E-03-8.50E-05	39
Physiological System Development and Function		
Behavior	1.10E-02-1.76E-05	30
Organismal Development	1.15E-02-2.26E-05	57
Hematological System Development and Function	1.17E-02-3.12E-05	43
Immune cell Trafficking	1.15E-02-3.12E-05	17
Nervous System Development and Function	1.05E-02-8.50E-05	45

F2 IPA Top Diseases and Biological Functions

Category	P-value	# of genes
Diseases and Disorders		
Inflammatory Disease	2.40E-02-4.45E-05	34
Dermatological Diseases and Conditions	2.40E-02-3.83E-04	15
Hematological Disease	2.27E-02-3.83E-04	16
Immunological Disease	2.40E-02-3.83E-04	30
Organismal Injury and Abnormalities	2.40E-02-3.83E-04	150
Molecular and Cellular Functions		
Molecular Transport	2.40E-02-3.88E-05	41
Cell Death and Survival	2.40E-02-6.35E-04	12
Cell Morphology	2.40E-02-6.35E-04	15
Antigen Presentation	2.40E-02-9.47E-04	5
Protein Synthesis	1.60E-02-9.47E-04	5
Physiological System Development and Function		
Embryonic Development	2.40E-02-6.35E-04	14
Cardiovascular System Development and Function	1.60E-02-7.00E-04	17
Organ Morphology	2.40E-02-7.00E-04	20
Organismal Development	2.40E-02-7.00E-04	24
Skeletal and Muscular System Development and Function	2.40E-02-7.69E-04	20

TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

Supplemental Table 5: qRT-PCR validation of select microarray genes in F_1 and F_2 testes.

			Microarray		**qRT-PCR**		
	Gene Symbols	**Gene Names**	*Fold Change*	*P-value (ANOVA)*	*Fold Change ($2^{-\Delta\Delta Ct}$)*	*SEM*	*P-value*
F1	*si:dkeyp-72h1.1*	*si:dkeyp-72h1.1*	-3.08	0.022	-2.52	0.0963	0.015
	smyd1a	*SET and MYND domain containing 1a*	-3.6	0.0064	-3.21	0.1495	0.005
	socs1a	*suppressor of cytokine signaling 1a*	1.28	0.0782	2.07	0.4494	0.026
	spata4	*spermatogenesis associated 4*	-19.12	0.0506	-4.71	0.1981	0.031
F2	*aste1*	*asteroid homolog 1 (Drosophila)*	-19.84	0.005	-8.62	0.1107	0.019
	mcf2la	*mcf.2 cell line derived transforming sequence-like a*	-1.62	0.072	-1.96	0.1768	0.022
	mhc1uba	*major histocompatibility complex class I UBA*	-26.44	0.004	-489.76	0.0018	0.004
	spata4	*spermatogenesis associated 4*	8.22	0.0001	80.4	27.8188	0.011
	zgc:158731	*zgc:158731*	2.02	0.0001	3.96	0.8674	0.005

Fold changes and p-values for microarray and qRT-PCR are listed per gene, with SEM indicated for qRT-PCR data. P-value is determined using ANOVA for microarray and Student's t-test for qRT-PCR. P-values below 0.05 are considered significant. qRT-PCR, quantitative reverse transcription polymerase chain reaction; SEM, standard error of the mean; ANOVA, analysis of variance.