Benzo[a]pyrene-Induced Multigenerational Changes in Gene Expression, Behavior, and DNA Methylation are Primarily Influenced by Paternal Exposure

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Running head: Paternal exposure to BaP causes multigenerational adverse impacts

Table S1: Adult zebrafish length (mm), wet weight (g), liver somatic index (LSI), gonad somatic index (GSI) displayed as mean ± standard error, n = 10 fish per sex. *Indicates a significant difference in F0 fish between controls and BaP treatment (t-test, p = 0.002). There were no significant differences in F1 fish (One-way ANOVA, p > 0.05).

Gen	Treatment	Sex	Length (mm)	Wet Weight (g)	LSI	GSI
F0	Control	M	31.9 ± 0.57	0.27 ± 0.011	0.95 ± 0.14	1.11 ± 0.09
F0	BaP	M	32.8 ± 0.44	0.28 ± 0.011	0.68 ± 0.07	**0.68 ± 0.08***
F0	Control	F	32.1 ± 0.35	0.30 ± 0.010	2.82 ± 0.14	5.42 ± 0.42
F0	BaP	F	33.7 ± 0.42	0.31 ± 0.009	2.52 ± 0.14	5.67 ± 0.43
F1	Control	M	36.3 ± 0.46	0.42 ± 0.029	0.73 ± 0.06	0.71 ± 0.19
F1	Cm x Bf	M	35.5 ± 0.96	0.38 ± 0.025	0.59 ± 0.09	0.75 ± 0.10
F1	Bm x Cf	M	35.4 ± 0.19	0.36 ± 0.014	0.65 ± 0.09	0.75 ± 0.06
F1	BaP	M	34.8 ± 0.66	0.37 ± 0.020	0.64 ± 0.05	0.71 ± 0.09
F1	Control	F	37.1 ± 0.84	0.45 ± 0.038	2.54 ± 0.19	4.92 ± 0.48
F1	Cm x Bf	F	37.5 ± 0.42	0.45 ± 0.017	2.13 ± 0.09	4.32 ± 0.33
F1	Bm x Cf	F	36.7 ± 0.60	0.43 ± 0.014	2.46 ± 0.11	4.54 ± 0.31
F1	BaP	F	37.5 ± 0.55	0.51 ± 0.052	2.55 ± 0.28	4.79 ± 0.56

Table S2: Adult zebrafish fecundity displayed as mean ± standard error, n = 5 breeding groups per treatment. Eggs were collected on two consecutive days from 5 tanks (2 males and 2 females per tank). There were no significant differences in F0 or F1 fish (One-way ANOVA, p > 0.05).

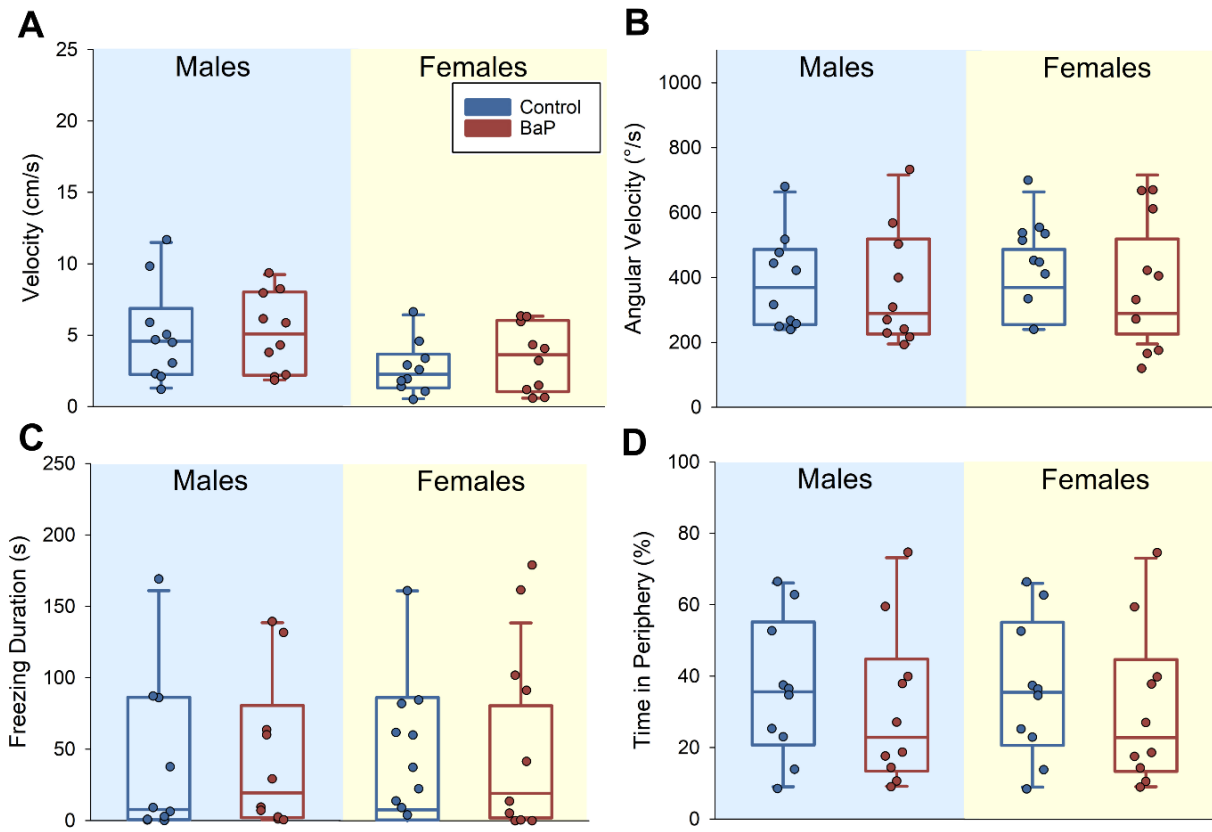| Gen	Treatment	Avg # Eggs / week	% Survival (24 hpf)	% Hatch (96 hpf)	% Survival (96 hpf)
F0	Control	191.0 ± 17.2	88.5 ± 5.0	100 ± 0	88.5 ± 5.0
F0	Cm x Bf	203.4 ± 20.6	77.5 ± 13.5	99.5 ± 0.5	77.0 ± 13.4
F0	Bm x Cf	169.6 ± 27.4	91.3 ± 1.2	100 ± 0	88.2 ± 2.8
F0	BaP	286.6 ± 48.4	75.0 ± 15.4	99.5 ± 0.5	75.0 ± 15.4
F1	Control	171.6 ± 20.8	97.5 ± 0.8	100 ± 0	95.9 ± 2.0
F1	Cm x Bf	218.8 ± 27.8	94.0 ± 2.3	100 ± 0	94.0 ± 2.3
F1	Bm x Cf	167.4 ± 24.3	93.8 ± 5.4	100 ± 0	93.8 ± 5.4
F1	BaP	277.0 ± 55.4	99.0 ± 1	100 ± 0	99 ± 1.0

Figure S1: Adult F0 open field behavior, n = 10 fish per sex. There were no significant differences between control and exposed fish (t-test, p > 0.05).
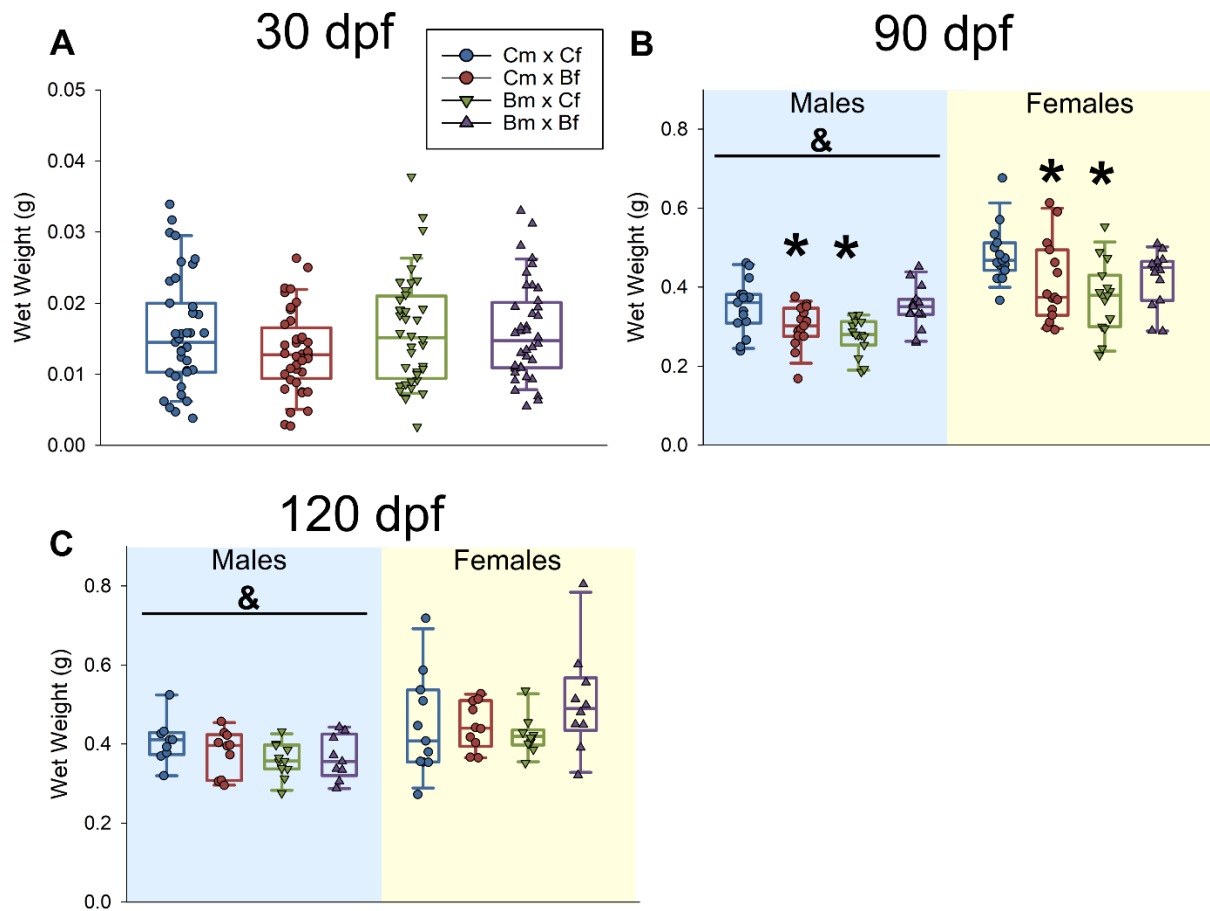
Figure S2: Wet weight of F1 zebrafish produced by parents exposed to BaP measured at 30 dpf (n=40), 90 dpf (n=15/sex), 120 dpf (n=10/sex). Letters that are not in common are significantly different (ANOVA, SNK post-hoc $p \leq 0.05$), *Indicates a significant difference from the control cross, &indicates a significant difference between males and females (two-way-ANOVA, SNK post-hoc $p \leq 0.05$).

Figure S3: Total length of F1 zebrafish produced by parents exposed to BaP measured at 96 hpf (n=48), 30 dpf (n=40), 90 dpf (n=15/sex), 120 dpf (n=10/sex). Letters that are not in common are significantly different (ANOVA, SNK post-hoc $p \leq 0.05$), *Indicates a significant difference from the control cross, &indicates a significant difference between males and females (two-way-ANOVA, SNK post-hoc $p \leq 0.05$).
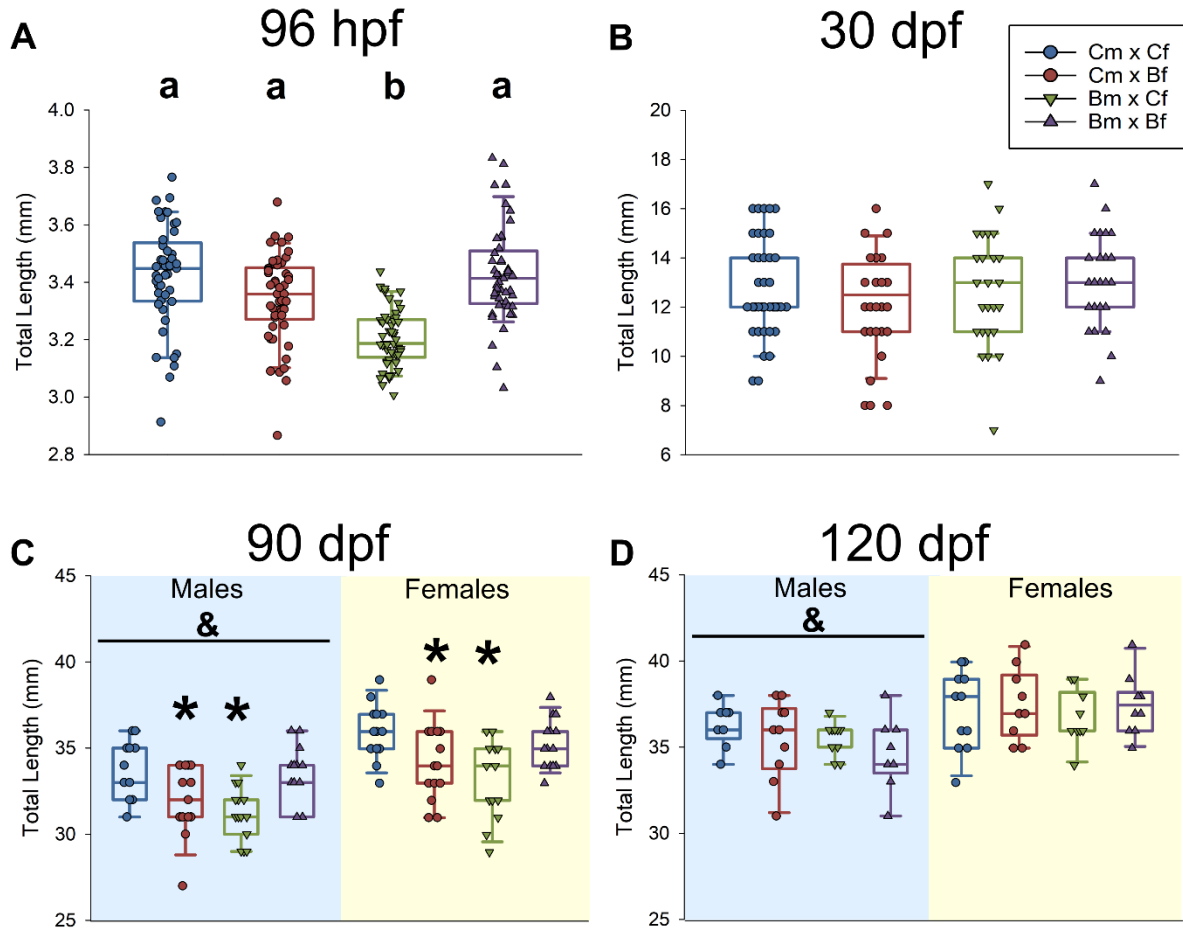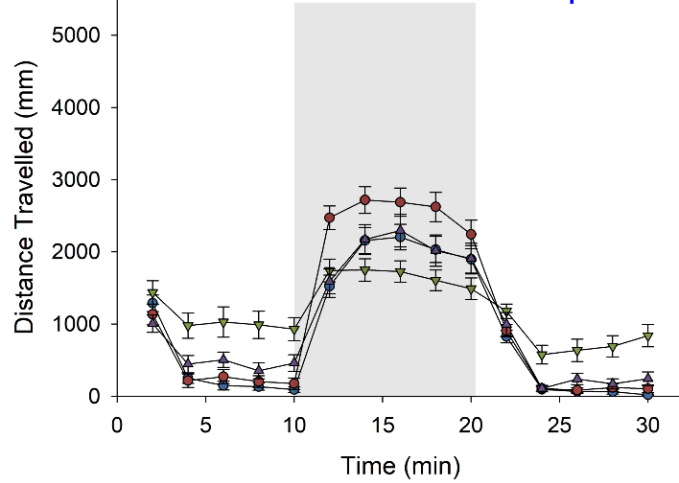
A F2 Larval Photomotor Response

B F2
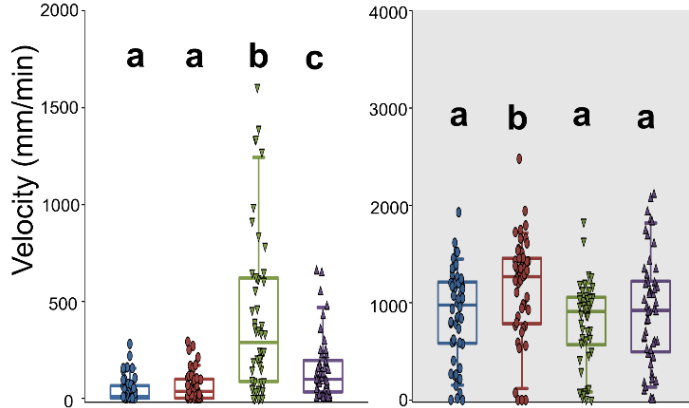
Figure S4: Impact of parental BaP exposure on offspring behavior in F2 generations. 96hpf F2 generation embryos from all four F0 crosses were assessed for photomotor behavioral responses. Distance traveled over 30 min larval photomotor response test (A, B) 10 min light acclimation, 10 min dark, 10 min light) of 96 hpf F2 zebrafish (n=50). Within light phase, hyperactivity was measured in Bm x Cf larval F1 zebrafish while in the dark phase Cm x Bf were hyperactive. Letters that are not in common are significantly different (ANOVA, SNK post-hoc $p \leq 0.05$).

A Bm x Cf

Name and Proportion of the Molecular Function (Inner Ring)
- GO1 ephrin receptor activity, 68.3%
- GO2 cation channel activity, 16.9%
- retinoic acid 4-hydroxylase activity, 8.9%
- deacetylase activity, 5.9%

retinoic acid 4-hydroxylase activity
deacetylase activity
phosphatidylinositol transfer activity
ephrin receptor activity
acetylgalactosaminyltransferase activity
cation channel activity
GO 2
GO 1
histone deacetylase activity
cysteine-type endopeptidase activity involved in apoptotic process
nicolinate-nucleotide diphosphorylase (carboxylating) activity
glycerol kinase activity
peptide receptor activity
G protein-coupled peptide receptor activity
peptidyl-dipeptidase activity

B Bf x Cm

Name and Proportion of the Molecular Function (Inner Ring)
- GO1 [heparan sulfate]-glucosamine N-sulfotransferase activity, 39.8%
- GO2 peptide transmembrane transporter activity, 22.8%
- GO3 GTPase regulator activity, 21.3%
- cysteine-type endopeptidase activity involved in apoptotic process, 10.3%
- phosphatidylinositol binding, 5.8%

cysteine-type endopeptidase activity involved in apoptotic process
phosphatidylinositol binding
[heparan sulfate]-glucosamine N-sulfotransferase activity
aralkylamine N-acetyltransferase activity
rRNA binding
O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase activity
translation release factor activity
GO 3
GO 1
heparan sulfate sulfotransferase activity
GTPase regulator activity
GO 2
hexosyltransferase activity
voltage-gated cation channel activity
sulfotransferase activity
amino acid:sodium symporter activity
peptide transmembrane transporter activity

C Sperm

Name and Proportion of the Molecular Function (Inner Ring)
- GO1 ion transmembrane transporter activity, 64.6%
- nuclear receptor activity, 13.4%
- glycerol kinase activity, 11.7%
- transporter activity, 10.3%

ion transmembrane transporter activity
transporter activity
sodium ion transmembrane transporter activity
glycerol kinase activity
secondary active transmembrane transporter activity
GO 1
nuclear receptor activity
ammonium transmembrane transporter activity
neurotransmitter:sodium symporter activity
symporter activity
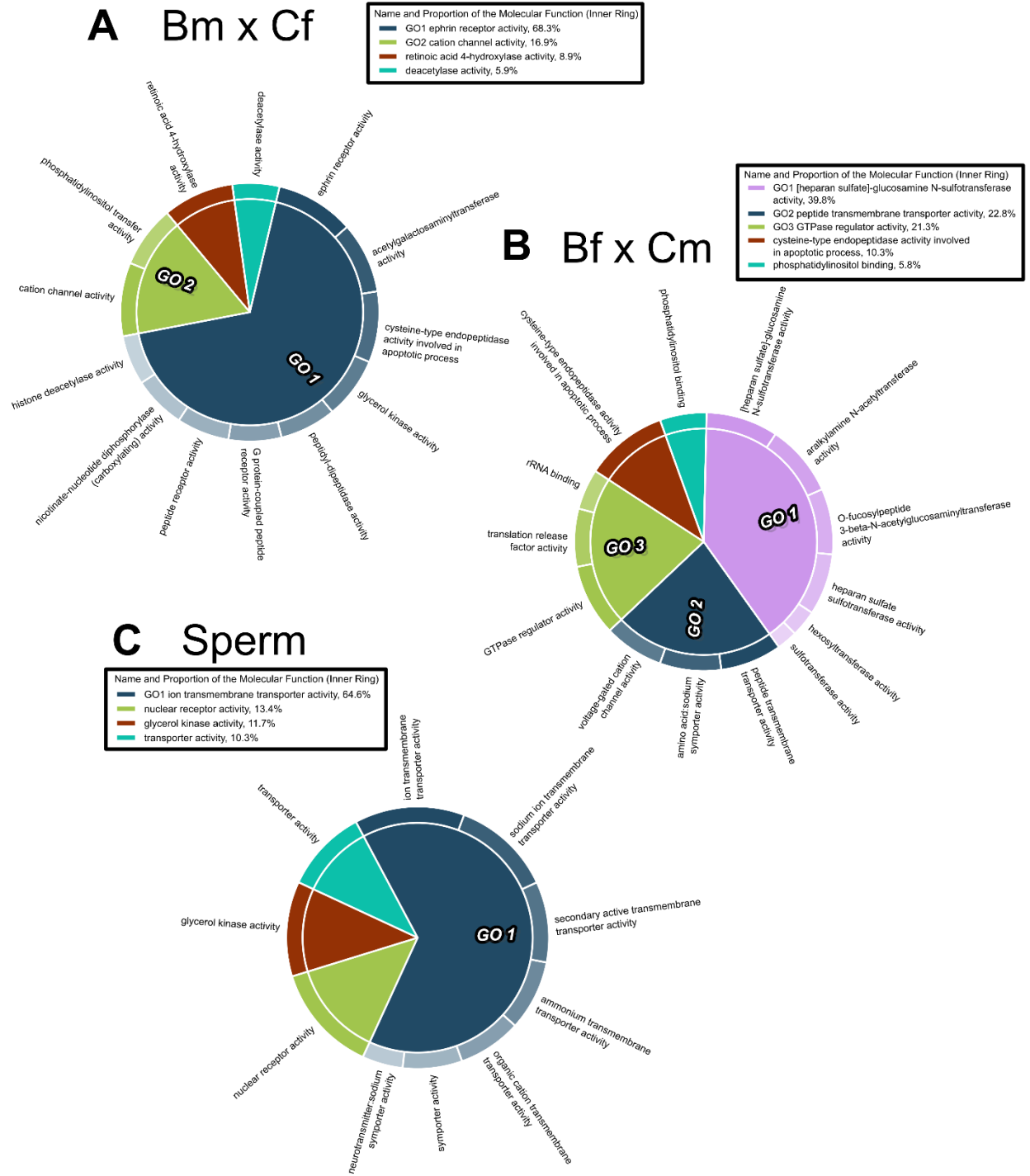organic cation transmembrane transporter activity

Figure S5: Gene Ontology analysis (molecular function pathways) of genes associated with DMRs in the F1 generation 10 hpf embryos from the BaP exposed and control parental crosses (Bm x Cf and Cm x Bf) and sperm from exposed males. The size of each parental GO term is determined by its statistical significance (p-value) and the different child terms under each parental GO term are represented by color gradient along the circumference of the plot.
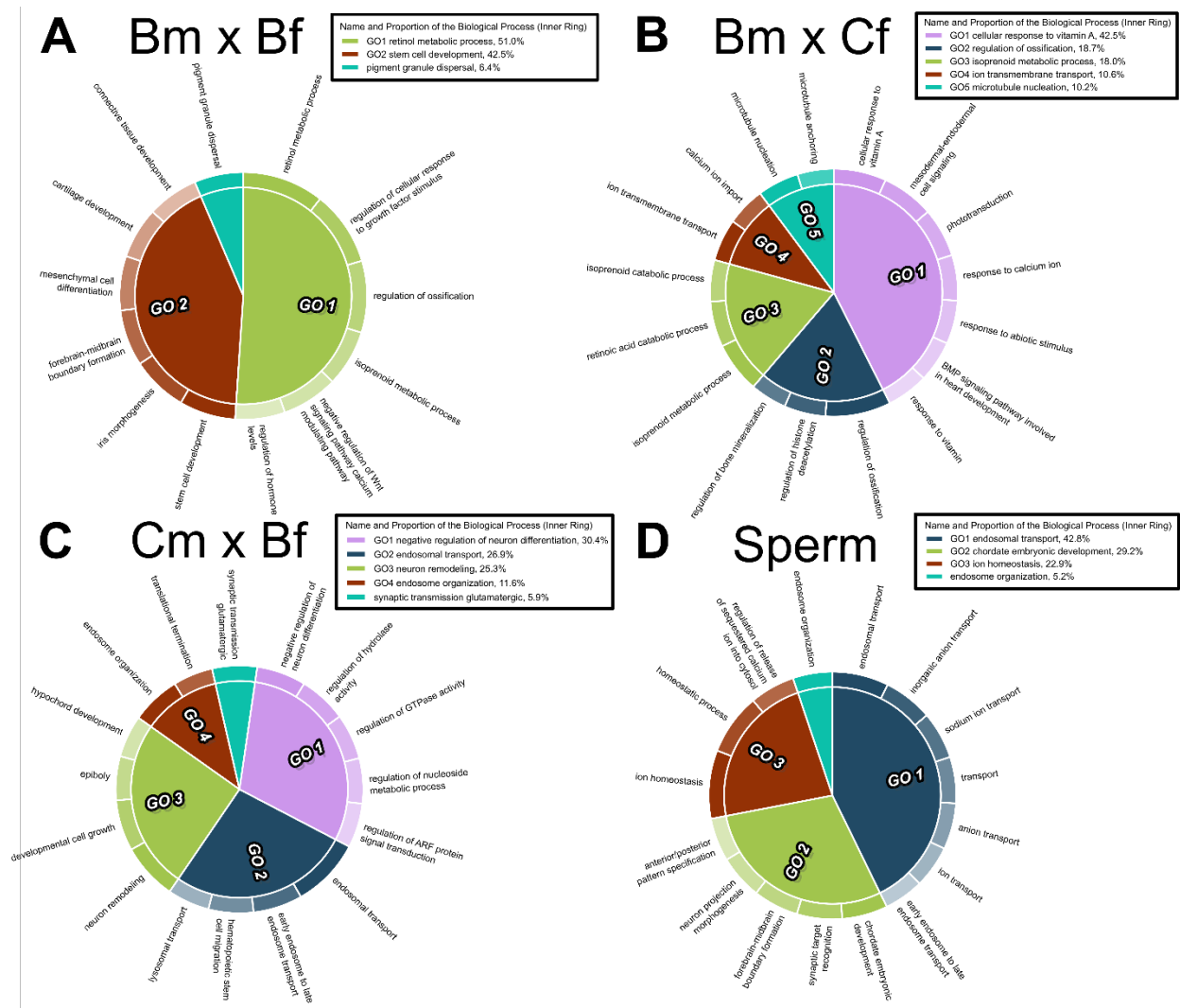
Figure S6: Gene Ontology analysis (biological function pathways) of genes associated with DMRs in the F1 generation 10 hpf embryos from the BaP exposed and control parental crosses (Bm x Cf and Cm x Bf) and sperm from exposed males. The size of each parental GO term is determined by its statistical significance (p-value) and the different child terms under each parental GO term are represented by color gradient along the circumference of the plot.

Figure S7: Protein-protein interaction network Cluster 1 from (STRING). Gene descriptions and gene stable IDs are provided in BaP_SIxlsx. The nodes are proteins, and the links are interactions between them.
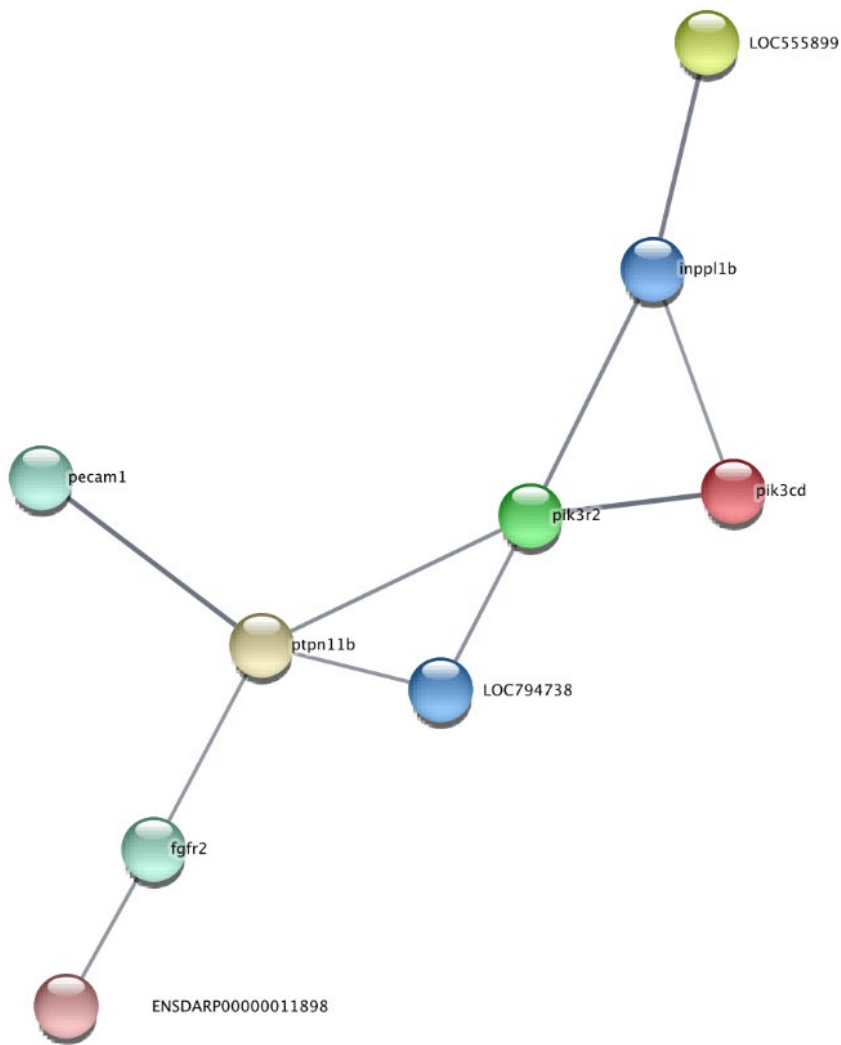
Figure S8: Protein-protein interaction network Cluster 2 from (STRING). Gene descriptions and gene stable IDs are provided in BaP_SIxlsx. The nodes are proteins, and the links are interactions between them.
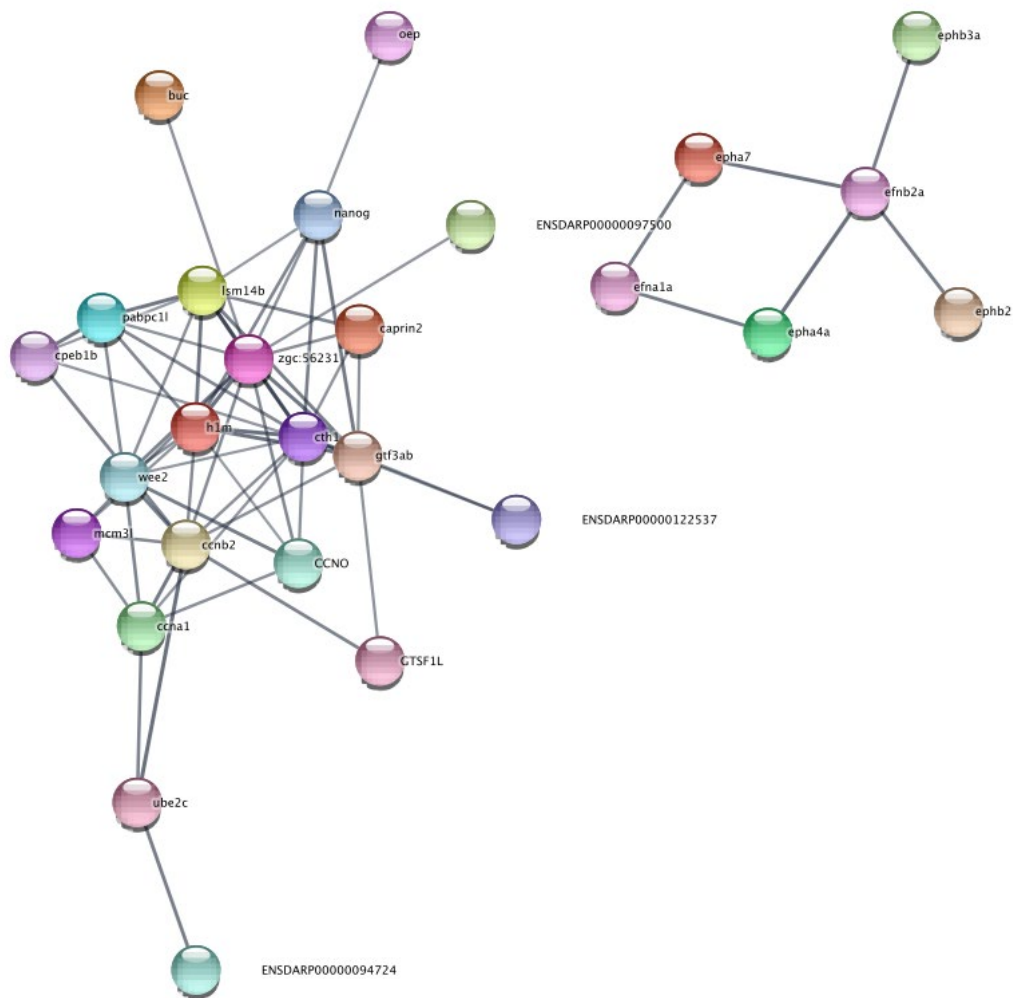
Figure S9: Protein-protein interaction network Cluster 3 from (STRING). Gene descriptions and gene stable IDs are provided in BaP_SIxlsx. The nodes are proteins, and the links are interactions between them.