

($p < 0.05$) from both the 24 and 48 hpf time points were combined to create the regulatory network.

Table 1. Mean percentage of embryos (95 percentile) with malformations observed at 120 hpf following exposure to 25 μ M BAA, DBT, PYR or DMSO control from 6-48 hpf.

Table 2. Comparison of malformations induced by individual PAH treatments. p values of Tukey's all pairwise post hoc test are displayed for each comparison, malformations that were significantly different between treatment groups are shaded ($p < 0.05$).

Table 3. Mean log₂ fold change values of differentially regulated transcripts from the microarray, compared with log₂ fold change (mean \pm SD) detected with QPCR. ^a Significantly different from vehicle control (One-way ANOVA with Tukey's post hoc test and 5% FDR, $p < 0.05$) ^b Significantly different from vehicle control (One-way ANOVA with Tukey's post hoc test, $p < 0.05$).

Table 4. Significantly enriched biological functions identified by DAVID analysis of all transcripts differentially regulated ($p < 0.05$) by BAA exposure or by DBT and PYR at 24 hpf. E score: overall cluster enrichment score, %: percentage of total gene list involved in functional cluster, p -values determined by modified Fischer's Exact test (EASE score).

Table 5. Significantly enriched biological functions identified by DAVID analysis of all transcripts differentially regulated ($p < 0.05$) in BAA exposed embryos, and common transcripts disrupted by DBT and PYR at 48 hpf. E score: overall cluster enrichment score, %: percentage of total gene list involved in functional cluster, p -values determined by modified Fischer's Exact test (EASE score).

Table S1. Primer sequences used for qRT-PCR

Table S2. Percent recovery GC-MS method of PAH detection in zebrafish embryos, calculated from laboratory control samples spiked with BAA, DBT or PYR in DMSO.

Table S3. Log₂ fold change and significance values of microarray transcripts significantly differentially expressed (One-way ANOVA with Tukey's post hoc test and 5% FDR, $p < 0.05$) by at least one PAH exposure group compared to control.

Table S4. Transcription factors identified as significantly over-connected ($p < 0.05$) to genes differentially expressed ($p < 0.05$) in response to PAH exposure at 24 and 48 hpf. Significance

was calculated by hypergeometric distribution in MetaCore. Actual: Number of genes in the dataset that interact with the transcription factor, Expected: Number of genes in the dataset predicted to interact with the transcription factor based on total number of interactions on the Agilent platform calculated as mean value for hypergeometric distribution, Ratio: Connectivity ratio (Actual/Expected), p-value: Probability to have the given value of Actual or higher (FDR adjusted p-value <0.05)

References

- Aguilar-Alonso, P., Martinez-Fong, D., Pazos-Salazar, N. G., Brambila, E., Gonzalez-Barrios, J. A., Mejorada, A., Flores, G., Millan-Perezpena, L., Rubio, H., and Leon-Chavez, B. A. (2008). The increase in zinc levels and upregulation of zinc transporters are mediated by nitric oxide in the cerebral cortex after transient ischemia in the rat. *Brain research* **1200**, 89-98.
- Alexeyenko, A., Wassenberg, D. M., Lobenhofer, E. K., Yen, J., Linney, E., Sonnhammer, E. L., and Meyer, J. N. (2010). Dynamic zebrafish interactome reveals transcriptional mechanisms of dioxin toxicity. *PLoS One* **5**, e10465.
- Andreasen, E. A., Spitsbergen, J. M., Tanguay, R. L., Stegeman, J. J., Heideman, W., and Peterson, R. E. (2002). Tissue-specific expression of AHR2, ARNT2, and CYP1A in zebrafish embryos and larvae: effects of developmental stage and 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure. *Toxicol Sci* **68**, 403-419.
- Archuleta, M. M., Schieven, G. L., Ledbetter, J. A., Deanin, G. G., and Burchiel, S. W. (1993). 7,12-Dimethylbenz[a]anthracene activates protein-tyrosine kinases Fyn and Lck in the HPB-ALL human T-cell line and increases tyrosine phosphorylation of phospholipase C-gamma 1, formation of inositol 1,4,5-trisphosphate, and mobilization of intracellular calcium. *Proc Natl Acad Sci U S A* **90**, 6105-6109.
- Barron, M. G., Heintz, R., and Rice, S. D. (2004). Relative potency of PAHs and heterocycles as aryl hydrocarbon receptor agonists in fish. *Mar Environ Res* **58**, 95-100.
- Benjamini, Y., and Hochberg, Y. (1995). Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. *J Roy Stat Soc B Met* **57**, 289-300.
- Binder, R. L., and Stegeman, J. J. (1984). Microsomal electron transport and xenobiotic monooxygenase activities during the embryonic period of development in the killifish, *Fundulus heteroclitus*. *Toxicol Appl Pharmacol* **73**, 432-443.
- Bostrom, C. E., Gerde, P., Hanberg, A., Jernstrom, B., Johansson, C., Kyrklund, T., Rannug, A., Tornqvist, M., Victorin, K., and Westerholm, R. (2002). Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. *Environ Health Perspect* **110 Suppl 3**, 451-488.
- Brasier, A. R., Jamaluddin, M., Han, Y., Patterson, C., and Runge, M. S. (2000). Angiotensin II induces gene transcription through cell-type-dependent effects on the nuclear factor-kappaB (NF-kappaB) transcription factor. *Mol Cell Biochem* **212**, 155-169.

Table S1

Ensembl Transcript ID	Zebrafish Gene Symbol	Forward primer	Reverse Primer
ENSDART00000010918	<i>agt</i>	TGACGGACACACAGTTTAC	GTTGCTCAGGTTGAAATGC
ENSDART000000105896	<i>atp2a1l</i>	AGCAGTTCATTCGTTACCTG	AGAACAACCAGCCAGAAATC
ENSDART00000077511	<i>ccr9a</i>	GCATGTTGGTATTTGAAGCC	CTGTGTCCGACATAACAGAG
ENSDART00000066439	<i>ch25h</i>	ACCACAAATACACATCCACC	TCATTCAAAGTGCAAGTGTCC
ENSDART00000017756	<i>ctsl.1</i>	GGACTCCTACCCCTATGAAG	ATAACCAACAGCCAGAACAC
ENSDART00000038200	<i>cyp1a</i>	TGCCGATTTTCATCCCTTTCC	AGAGCCGTGCTGATAGTGTC
ENSDART00000099870	<i>cyp1b1</i>	CTGCATTGATTTCCGAGACGTG	CACACTCCGTGTTGACAGC
ENSDART00000019953	<i>cyp1c1</i>	AGTGGCACAGTCTACTTTGAGAG	TCGTCCATCAGCACTCAG
ENSDART00000016487	<i>cyp1c2</i>	GTGGTGGAGCACAGACTAAG	TTCAGTATGAGCCTCAGTCAAAC
ENSDART000000103784	<i>edn2</i>	CCAGGATCAGCTAGAGAGAG	ATTTCACTGGTGTGGAAGAG
ENSDART000000109464	<i>g0s2</i>	ATAACCACCGACAAACAAGG	AGCATGTCAAAGTCTGGTTC
ENSDART000000100386	<i>mstnb</i>	AAGAGGACGATGAACATGC	GATCGTATTCGGTGTCTTCC
ENSDART00000025669	<i>slc16a9b</i>	TCCCTGTCAACCAAGAACTAC	TGAAGTAAACGCCAGATCG
ENSDART000000130131	<i>sult6b1</i>	GTGGGTTTAACTGGATGGTG	GAGACCACTGTGTCTTTCG
ENSDART00000017569	<i>tnfb</i>	GTCCTACAGCACCATTTACC	ATTCAGTGCACAACCTCTCAC

Table S2

Spike (nmoles)	Average Detected (nmoles)	Percent Recovery	Standard Deviation
BAA 24 hpf			
5	4.45	89.07	3.74
25	22.14	88.57	5.75
50	47.19	94.39	3.83
125	118.55	94.84	4.86
DBT 24 hpf			
5	5.07	101.37	2.47
25	25.03	100.14	1.68
50	49.94	99.88	3.78
125	134.50	107.60	2.55
PYR 24 hpf			
5	5.48	109.68	3.98
25	29.11	116.43	10.33
50	61.55	123.11	5.15
125	156.69	125.35	10.56
BAA 48 hpf			
5	4.55	91.05	1.05
25	20.07	80.27	1.53
50	44.09	88.18	3.03
125	111.25	89.00	2.45
DBT 48 hpf			
5	5.29	105.72	3.09
25	25.96	103.84	3.32
50	51.19	102.39	4.70
125	136.17	108.94	2.94
PYR 48 hpf			
5	5.12	102.31	6.14
25	25.84	103.35	3.70
50	55.96	111.93	4.23
125	144.70	115.76	5.23

Table S4. Transcription factors predicted to regulate transcripts significantly differentially expressed in response to PAH exposure

Network Object Name	24 hpf								48 hpf							
	BAA Actual	BAA Expected	BAA Ratio	BAA p-value	DBT-PYR Actual	DBT-PYR Expected	DBT-PYR Ratio	DBT-PYR p-value	BAA Actual	BAA Expected	BAA Ratio	BAA p-value	DBT-PYR Actual	DBT-PYR Expected	DBT-PYR Ratio	DBT-PYR p-value
AHR	4.00	0.51	7.78	1.53E-03												
TFAP2A									10.00	2.65	3.77	2.81E-04				
ARNT	4.00	0.13	30.03	8.38E-06					5.00	0.69	7.29	6.08E-04				
CEBPD	3.00	0.19	15.68	8.74E-04					6.00	0.95	6.31	3.66E-04				
JUN					19.00	9.07	2.09	1.95E-03					25.00	13.87	1.80	3.29E-03
CREB1									14.00	5.14	2.73	4.69E-04	55.00	30.53	1.80	1.31E-05
CUX1									6.00	0.60	9.97	2.95E-05				
EGR2													9.00	3.10	2.90	3.81E-03
EN1					4.00	0.33	12.21	2.58E-04								
ERG													8.00	1.38	5.81	5.67E-05
ESR1					37.00	19.46	1.90	1.12E-04								
ESR2					11.00	2.89	3.81	1.51E-04								
FOXO1													15.00	3.91	3.83	8.20E-06
FOSL2													6.00	1.13	5.32	7.97E-04
NR3C1					26.00	12.96	2.01	5.73E-04	10.00	3.09	3.24	9.31E-04				
GLI1													7.00	1.50	4.66	6.80E-04
HBP1													3.00	0.19	15.97	5.68E-04
HES1					6.00	1.19	5.05	1.16E-03								
HOXB1													3.00	0.19	15.97	5.68E-04
IRF4													6.00	1.44	4.17	2.95E-03
JUND													12.00	3.82	3.14	4.38E-04
KLF4													14.00	5.54	2.53	1.35E-03
KLF5													6.00	0.81	7.37	1.23E-04
NR1H3													9.00	2.69	3.34	1.45E-03
MYEF2													2.00	0.06	31.94	9.78E-04
NFATC4					4.00	0.45	8.88	9.40E-04								
NFE2L2									7.00	1.19	5.89	1.77E-04				
REST													20.00	9.99	2.00	2.55E-03
NR4A2					5.00	0.76	6.60	8.84E-04								
OTX2													6.00	1.38	4.36	2.34E-03
TP53													42.00	27.11	1.55	3.05E-03
PITX2													8.00	1.66	4.82	2.22E-04
PPARA					13.00	2.50	5.20	1.25E-06					13.00	3.82	3.40	1.14E-04
PKNOX1													3.00	0.28	10.65	2.22E-03

Table S4. Transcription factors predicted to regulate transcripts significantly differentially expressed in response to PAH exposure

PGR										17.00	6.07	2.80	1.28E-04
RARA										10.00	3.69	2.71	3.90E-03
RELA	24.00	11.39	2.11	4.65E-04	16.00	3.81	4.20	7.49E-07	35.00	17.41	2.01	6.65E-05	
RORA	10.00	2.46	4.07	1.76E-04									
RXRA	10.00	2.68	3.73	3.59E-04					11.00	4.10	2.68	2.71E-03	
SF1									9.00	2.38	3.78	5.89E-04	
SIX1	5.00	0.53	9.39	1.60E-04									
SIX4	3.00	0.20	14.65	9.16E-04									
SOX5									11.00	3.29	3.35	4.39E-04	
SOX6									5.00	0.72	6.94	6.19E-04	
SP1	74.00	48.31	1.53	5.79E-05	24.00	11.95	2.01	3.48E-04	112.00	73.86	1.52	1.38E-06	
SP3					9.00	1.97	4.56	1.45E-04					
STAT5A									13.00	4.10	3.17	2.34E-04	
TBP									27.00	15.03	1.80	2.40E-03	
TCF7L2									17.00	6.67	2.55	3.88E-04	
TWIST2									2.00	0.06	31.94	9.78E-04	
UBTF									2.00	0.06	31.94	9.78E-04	
YY1									30.00	15.56	1.93	4.52E-04	