Electronic Supplementary Material

15 pages, 3 tables, 11 figures

Metabolomic analysis to define and compare the effects of PAHs and oxygenated PAHs in developing zebrafish

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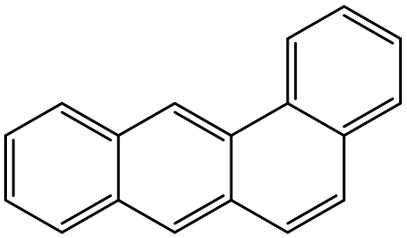
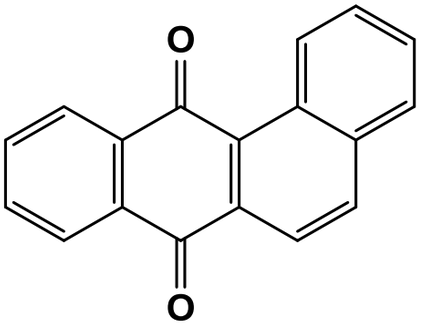


Benz[a]anthracene (BAA)



Benz[a]anthracene-7,12-dione (BAQ)

Figure S1. Structures of selected PAH and oxy-PAH compounds.

Table S1. Selected physicochemical properties of selected compounds.

Compound	**CAS #**	**Molecular Weight (g mol^{-1})**	**Solubility in Water @ 25°C (mg L^{-1})**	**Log K_{ow}**
Benz[a]anthracene	56-55-3	228.28	0.0094	5.76
Benz[a]anthracene-7,12-dione	2498-66-0	258.27	0.29	4.40

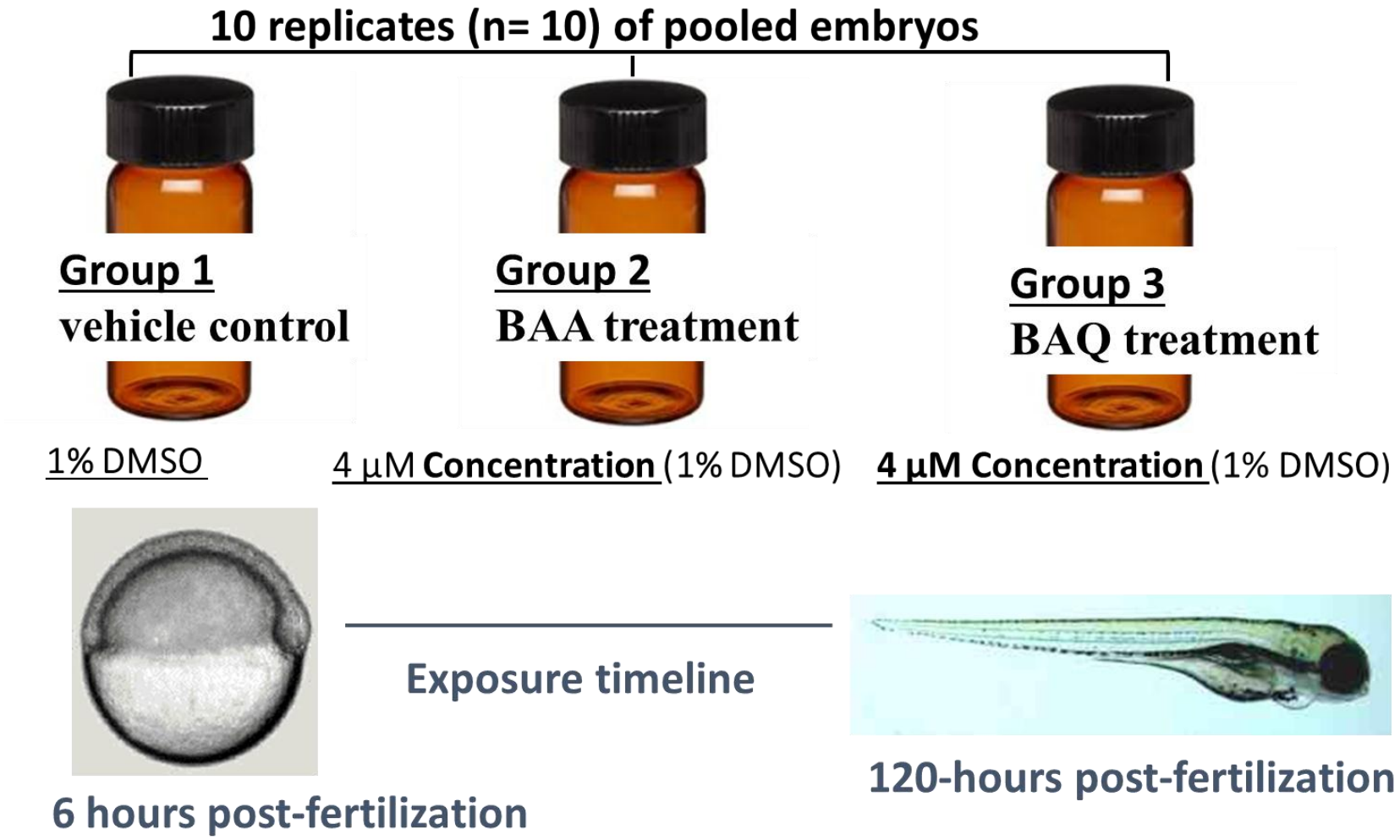


Figure S2. Schematic representation of experimental set up

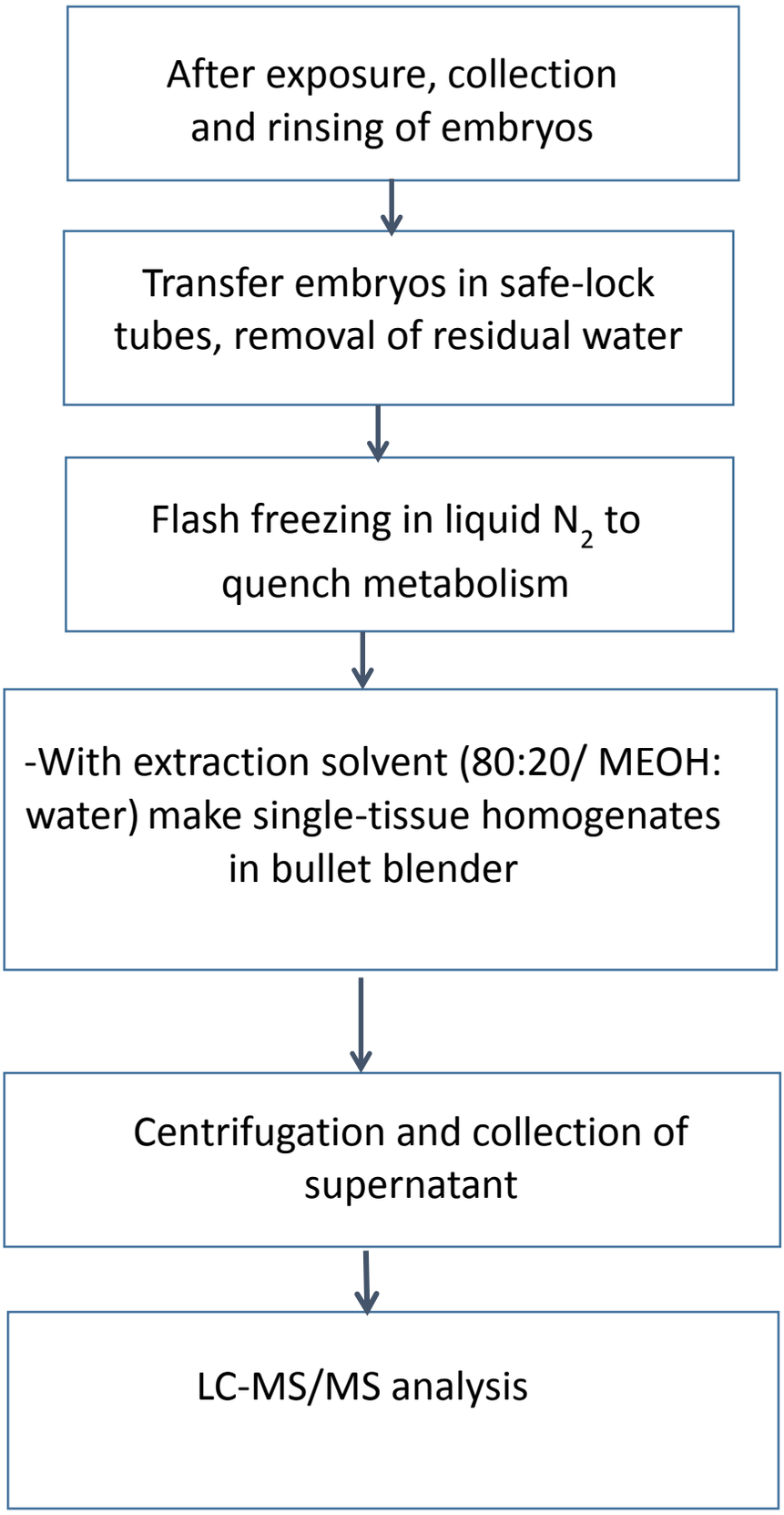


Figure S3. Flowchart of the procedure followed for sample preparation, metabolite extractions and analyses.

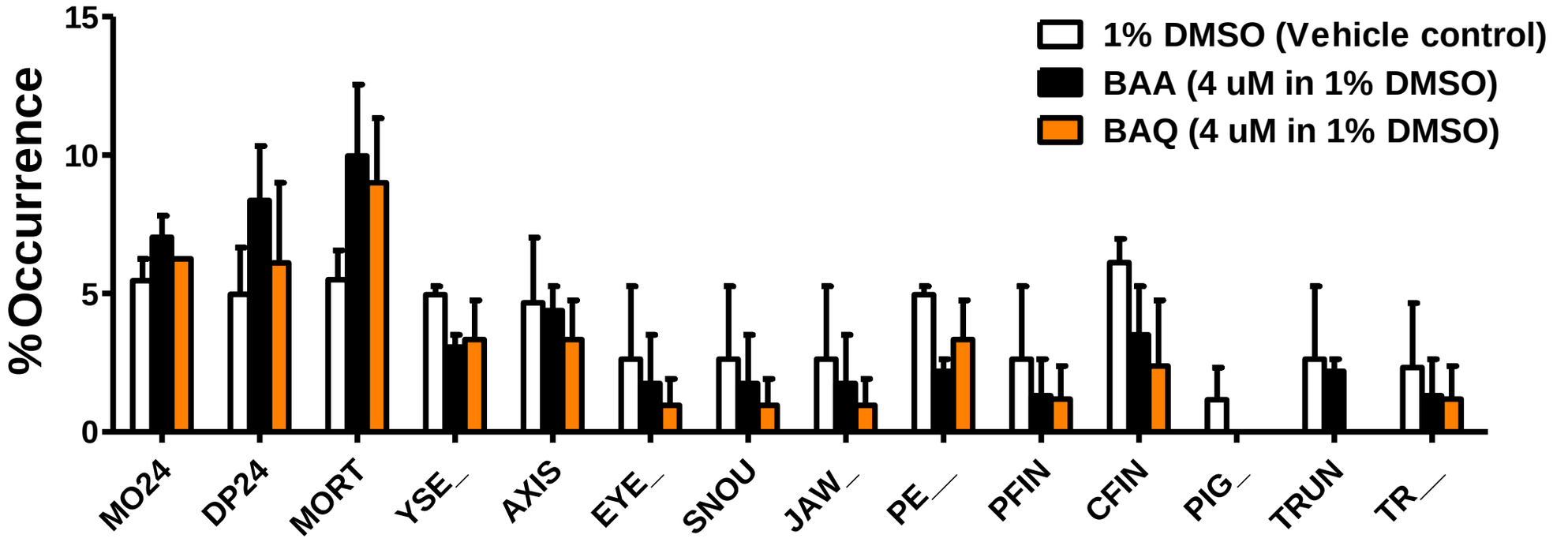


Figure S4. Morphological malformations observed in embryos exposed to the vehicle control, 4 µM BAA and 4 µM BAQ. The following toxicological endpoints were assessed: 24 hpf and 120 hpf total mortality (MO24, MORT), developmental progress (DP24), yolk sac edema (YSE), pericardial edema (PE), body axis (AXIS), snout (SNOU), jaw, trunk (TRUN), fin malformations (PFIN, CFIN), pigment (PIG), and touch response (TR). For each exposure treatment, the mean percent occurrences (± standard error) were based on a total of 128 embryos. Embryos were individually exposed to 100 µL of solution in 340 µL wells (96 wells/plate) (BD Biosciences, San Jose, CA). No significant differences ($p < 0.05$, two-way ANOVA) were observed among the groups.

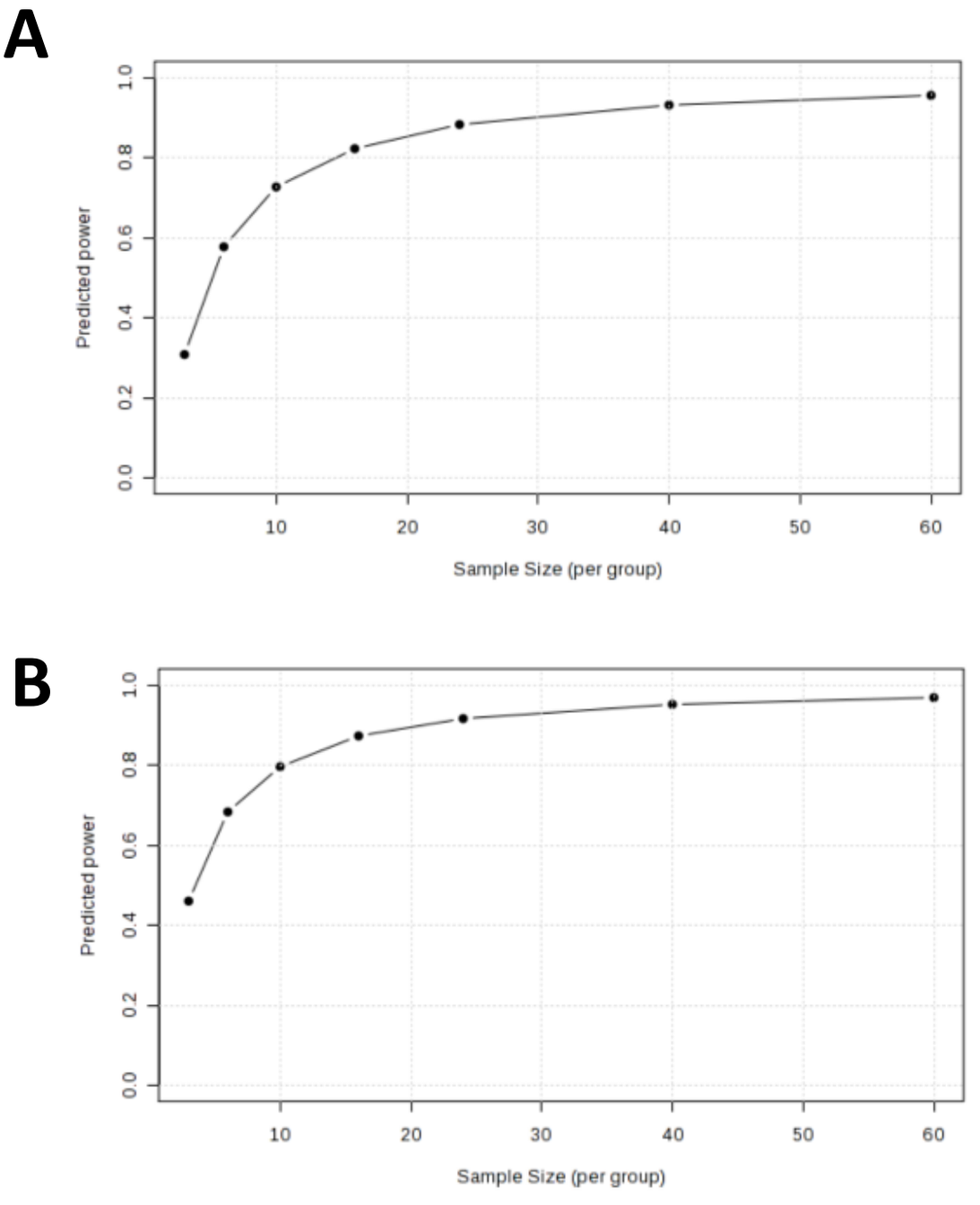
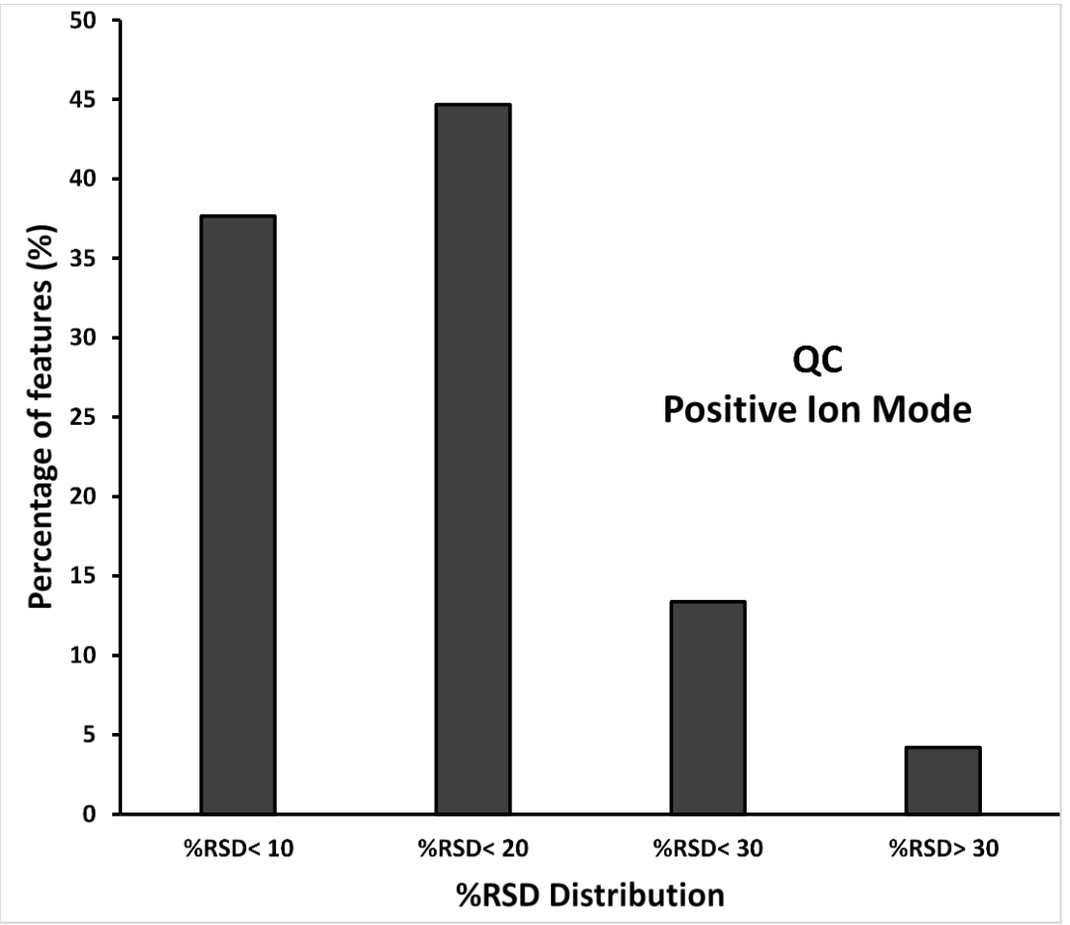


Figure S5. Power analysis curves for (A) BAA and (B) BAQ treatments with sample size on x-axis and estimated power using 5% FDR on the y-axis.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Percentage of features (%)
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Positive Ion Mode
%RSD< 10
%RSD< 20
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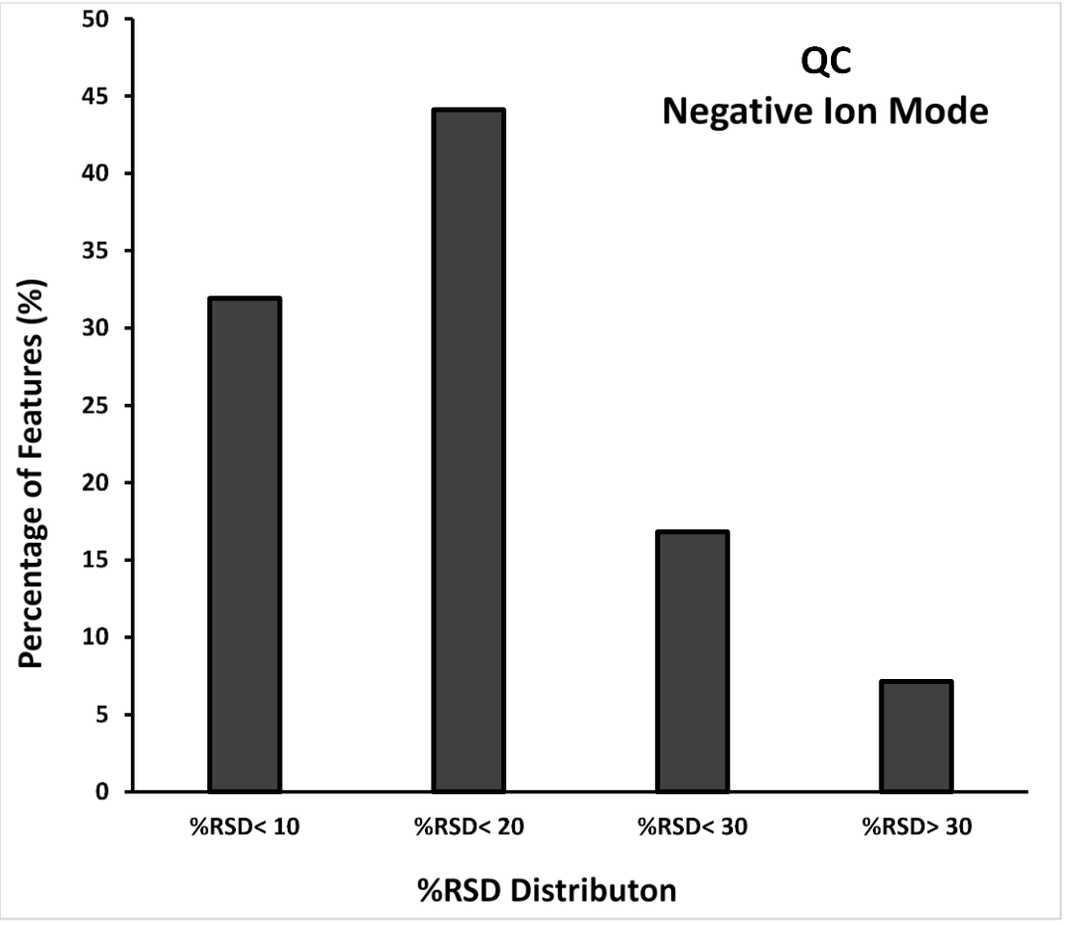
%RSD> 30
%RSD Distribution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Percentage of Features (%)
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Negative Ion Mode
%RSD< 10
%RSD< 20
%RSD< 30
%RSD> 30
%RSD Distributon

Figure S6.

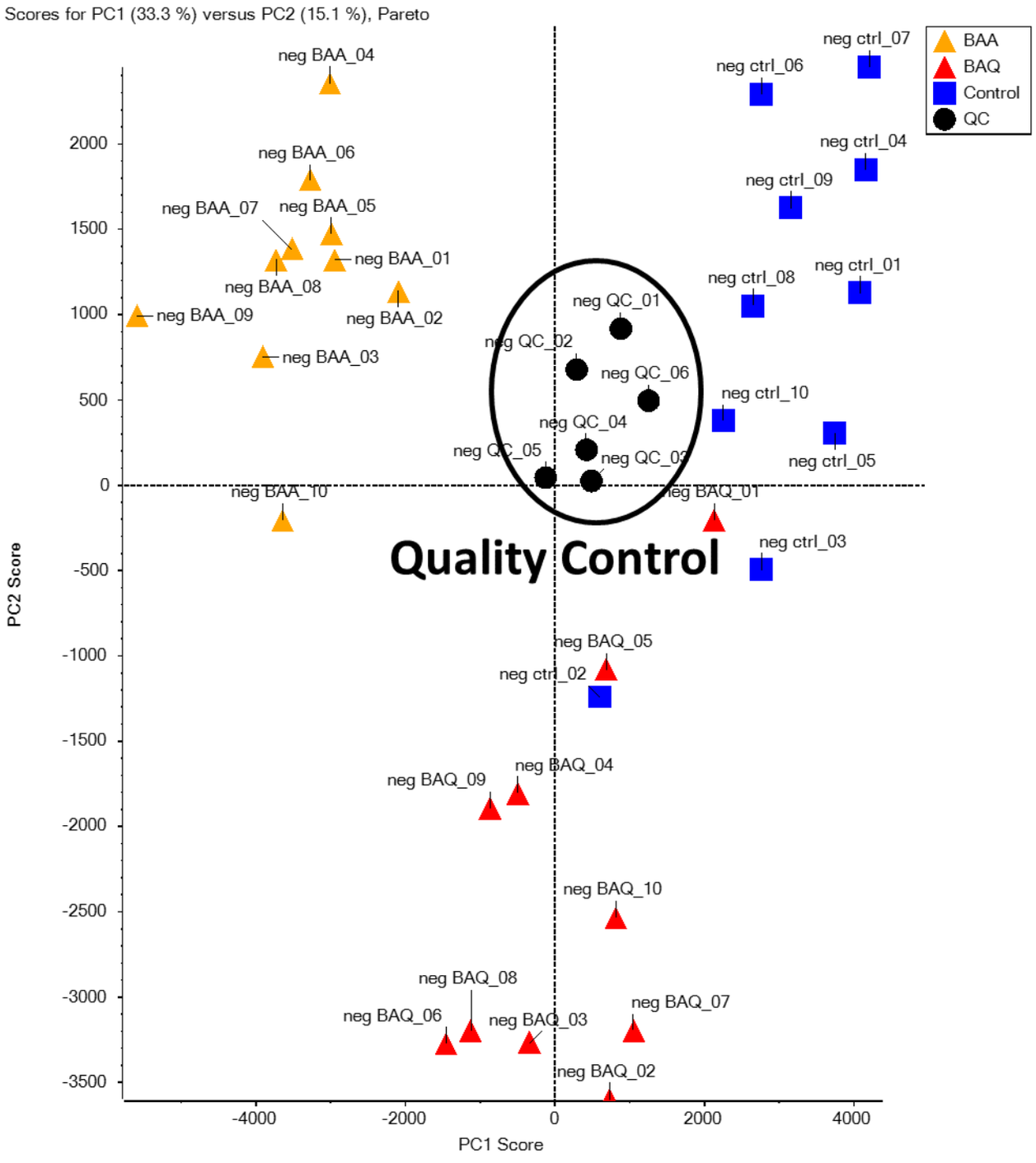

Figure S7. PCA scores plot showing tight clustering of quality control (QC) samples.

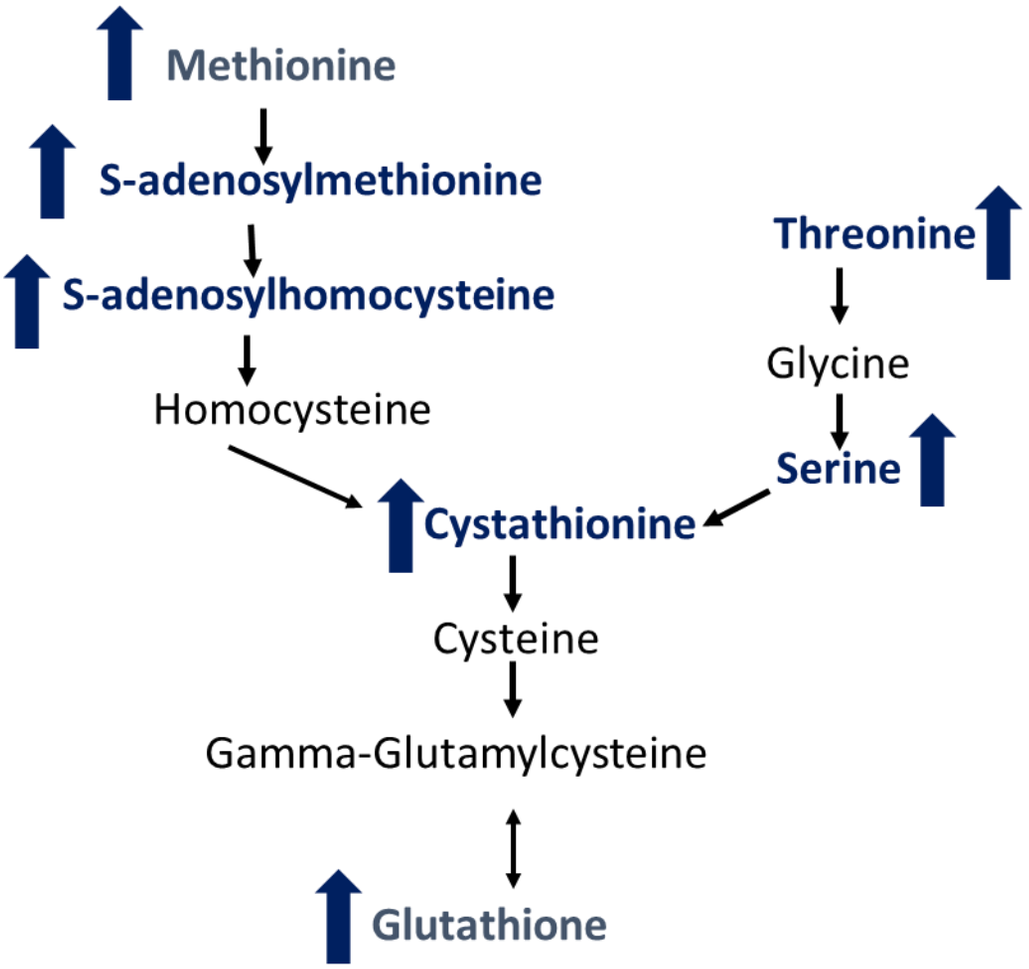

Figure S8. Metabolic scheme related to BAQ influence on glutathione metabolism. Compounds marked in blue represent significant up-regulation.

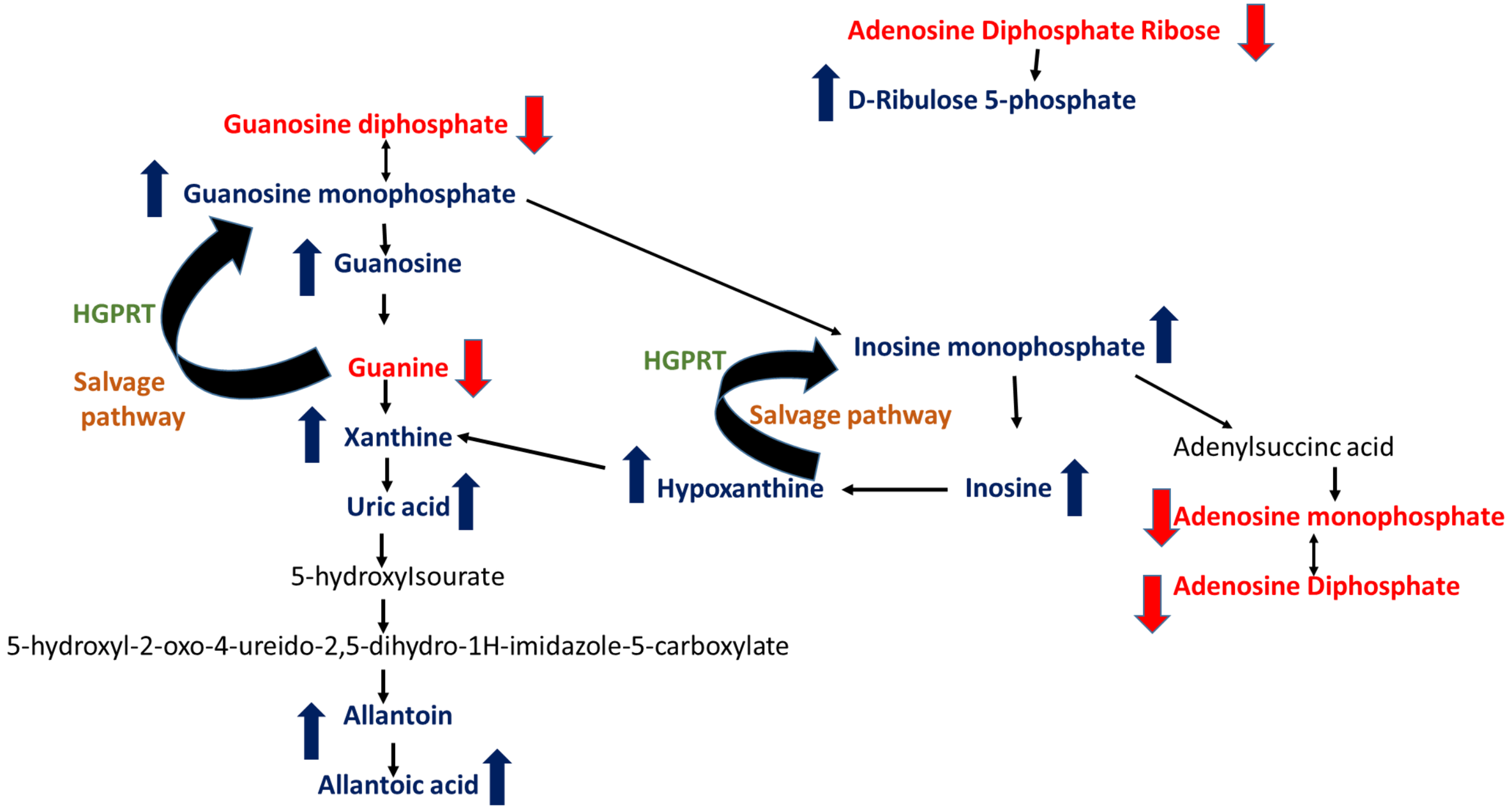

Figure S9. Metabolic diagram related to BAA influence on the purine metabolism. Compounds marked in red and blue represent significant down-regulation and up-regulation, respectively. Reactions shown with curved arrows represent the salvage pathways in which purine bases can be reutilized. HGPRT, hypoxanthine-guanine phosphoribosyltransferase enzymes.

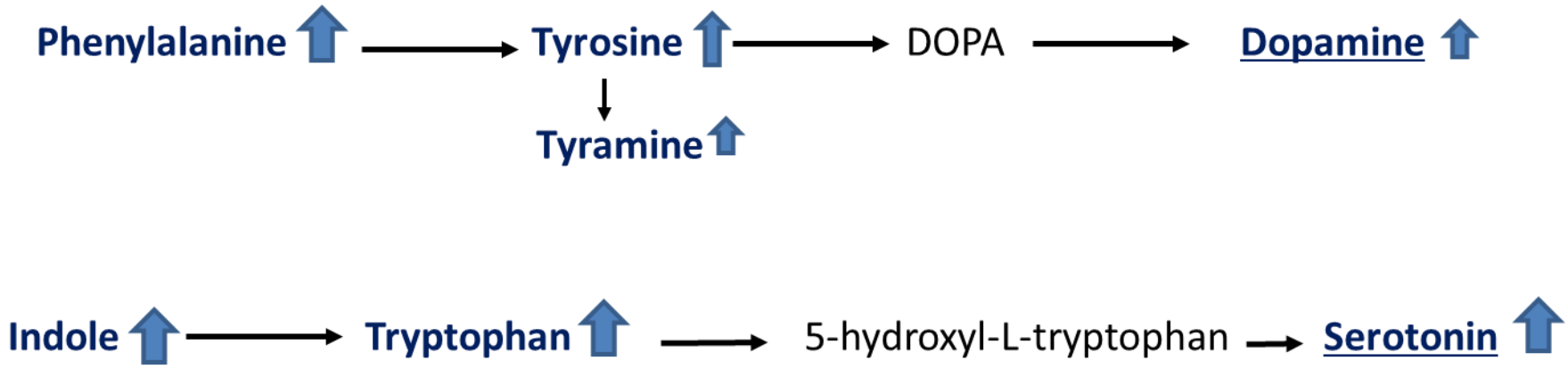

Figure S10. Metabolic diagram illustrating the influence of BAA and BAQ exposures on the phenylalanine, tyrosine and tryptophan metabolism. Compounds marked in blue represent significant up-regulation.

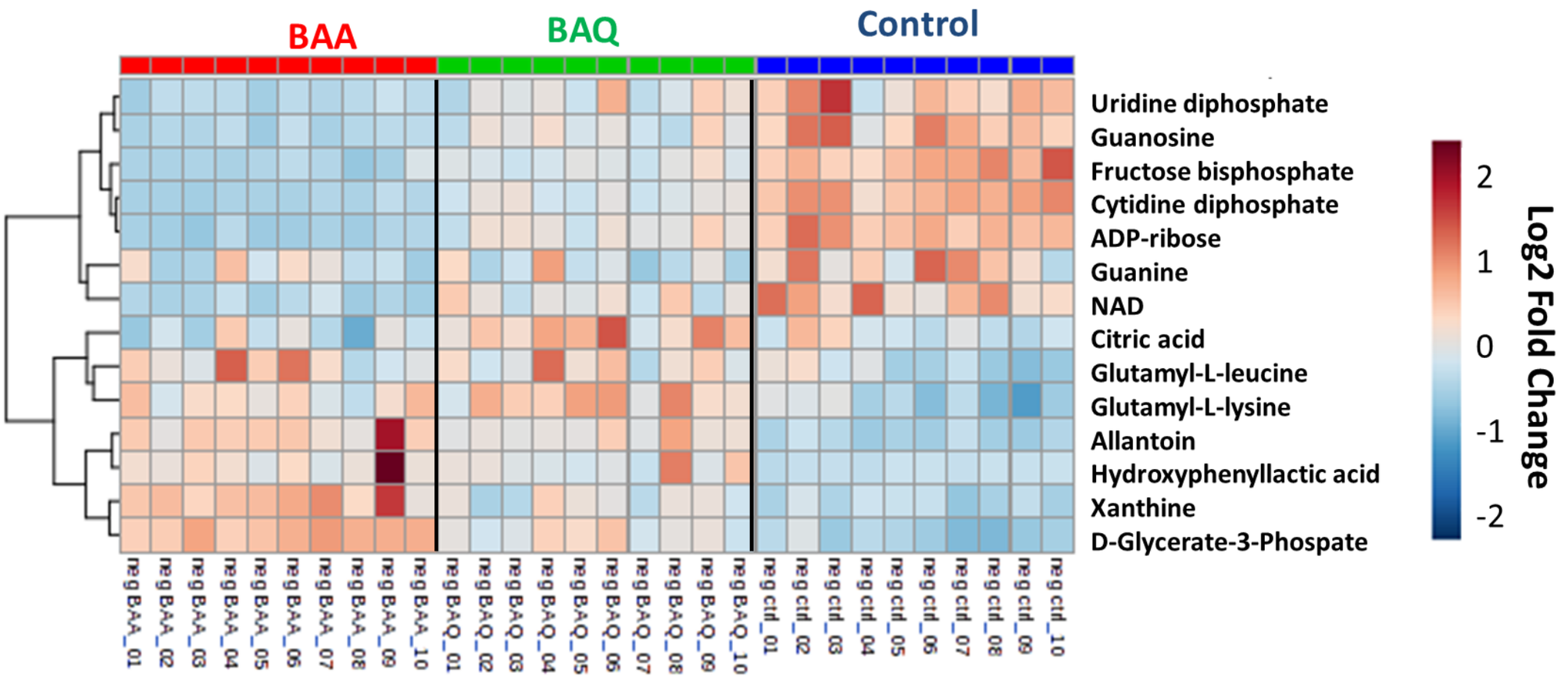

Figure S11. Heat map produced by hierarchical clustering of the most significantly different metabolites obtained from the negative ion mode. The log2 fold change in metabolite levels is color-coded: red pixels denote up regulation; blue pixels denote down regulation. Fold changes were based on peak intensities and relative to a pooled average sample from the control group.

Table S2. Percentage of features in which normality, homogeneity of variance or both assumptions are met [a].

Mode	Number of Features	Groups	Normality (Shapiro-Wilk's test)[b]	Homogeneity of Variance (Levene's test)[c]	Normality & Homogeneity
Positive	1285	BAA	87%	80%	69%
		Control	91%		72%
Positive	1285	BAQ	85%	79%	67%
		Control	91%		72%
Negative	1832	BAA	87%	78%	67%
		Control	83%		64%
Negative	1832	BAQ	89%	85%	76%
		Control	83%		71%

[a] Percentages represent those features, out of the total, in which there was not enough evidence to reject the null hypothesis (H_0) at conventional $\alpha = 0.05$.

[b] H_0 = Data are sampled from a Gaussian or normal distribution (Shapiro-Wilk's test).

[c] H_0 = Within-group variances are equal (Levene's test).

Table S3. List of identified and significant metabolites ($p < 0.05$)[a]

Mode	*m/z*	*RT (min)*	*Compound*	***BAA vs Control***			***BAQ vs Control***			*Trend*
				P-value	*Ratio*[b]	*FDR*	*P-value*	*Ratio*[b]	*FDR*	
POS	104.1068	5.32	Choline	6.43×10^{-4}	1.5	1.78×10^{-3}	--	--[c]	--	↑
POS	112.0504	5.07	Cytosine [e]	2.61×10^{-6}	2.1	3.59×10^{-5}	--	--[c]	--	↑
POS	116.0705	5.27	Proline [e]	2.16×10^{-4}	1.8	9.34×10^{-4}	2.33×10^{-3}	1.51	7.83×10^{-3}	↑
POS	118.0641	11.99	Indole	1.81×10^{-5}	1.9	1.5×10^{-4}	9.83×10^{-4}	1.62	4.28×10^{-3}	↑
POS	118.0864	5.85	Betaine [e]	2.07×10^{-3}	1.5	4.6×10^{-3}	--	--[c]	--	↑
POS	120.0646	4.5	Threonine [e]	4.50×10^{-7}	1.9	1.39×10^{-5}	--	--[c]	--	↑
POS	120.0799	9.65	Tyramine	7.35×10^{-5}	2.2	4.13×10^{-3}	9.30×10^{-4}	1.51	4.17×10^{-3}	↑
POS	123.0555	10.05	Niacinamide [e]	1.51×10^{-6}	2.1	2.99×10^{-5}	1.08×10^{-2}	1.53	2.68×10^{-2}	↑
POS	130.0493	8.24	5-Oxoproline [e]	7.57×10^{-3}	1.42	1.47×10^{-2}	--	--[c]	--	↑
POS	130.0851	4.03	Pipecolic acid	3.60×10^{-4}	2.33	1.27×10^{-3}	8.35×10^{-4}	1.74	3.94×10^{-3}	↑
POS	132.1014	6.70	Isoleucine [e]	3.50×10^{-4}	1.6	1.24×10^{-3}	--	--[c]	--	↑
POS	136.0752[d]	5.85	Dopamine	1.64×10^{-7}	2.12	7.02×10^{-6}	6.73×10^{-3}	1.70	9.80×10^{-3}	↑
POS	137.0454		Hypoxanthine [e]	1.10×10^{-6}	2.92	2.40×10^{-5}	9.50×10^{-4}	1.70	4.22×10^{-3}	↑
POS	147.043[d]	5.87	p-Coumaric acid	2.80×10^{-7}	2.2	9.40×10^{-7}	1.01×10^{-2}	1.70	2.51×10^{-2}	↑
POS	147.1131	4.00	Lysine [e]	6.20×10^{-4}	2.4	1.74×10^{-3}	6.43×10^{-4}	1.80	3.43×10^{-3}	↑
POS	150.0587	5.73	Methionine [e]	3.82×10^{-5}	2.3	2.50×10^{-4}	5.72×10^{-4}	1.80	3.21×10^{-3}	↑
POS	159.0913[d]	12.07	Serotonin	6.70×10^{-3}	1.97	7.48×10^{-5}	9.60×10^{-4}	1.70	4.24×10^{-3}	↑
POS	165.0540	5.85	2-Hydroxycinnamic acid	5.27×10^{-8}	2.24	3.67×10^{-6}	7.29×10^{-4}	1.73	1.91×10^{-3}	↑
POS	166.0869	9.70	Phenylalanine [e]	4.30×10^{-5}	2.2	2.74×10^{-3}	4.08×10^{-4}	1.53	2.76×10^{-3}	↑
POS	175.119	4.26	Arginine [e]	1.47×10^{-6}	2.1	2.97×10^{-5}	1.56×10^{-4}	1.70	1.46×10^{-3}	↑
POS	177.0619	4.82	Allantoic acid	2.34×10^{-4}	1.5	9.71×10^{-4}	2.90×10^{-4}	1.50	2.16×10^{-3}	↑
POS	182.0811	5.87	Tyrosine [e]	1.52×10^{-8}	2.2	1.67×10^{-6}	4.13×10^{-3}	1.70	8.21×10^{-3}	↑
POS	184.0726	25.26	Phosphocholine [e]	7.92×10^{-3}	0.695	151×10^{-2}	--	--[c]	--	↓
POS	188.0709	11.96	Indolelactic acid	2.65×10^{-6}	2.04	3.59×10^{-5}	5.04×10^{-4}	1.71	3.03×10^{-3}	↑
POS	203.1519	5.13	Dimethyl-L-arginine	1.60×10^{-3}	2.02	3.70×10^{-3}	--	--[c]	--	↑
POS	205.0980	12.01	Tryptophan [e]	1.79×10^{-6}	2.11	3.15×10^{-5}	2.55×10^{-4}	1.76	2.06×10^{-3}	↑
POS	212.0433	4.9	Phosphocreatine [e]	7.10×10^{-5}	0.35	4.02×10^{-4}	2.41×10^{-4}	0.46	1.98×10^{-3}	↓
POS	218.140	11.18	Propionylcarnitine	2.33×10^{-5}	1.90	1.80×10^{-4}	2.70×10^{-5}	2.03	4.16×10^{-4}	↑
POS	219.0984	4.77	5-L-glutamyl-L-alanine	1.83×10^{-3}	2.3	4.20×10^{-3}	6.34×10^{-7}	2.60	3.05×10^{-5}	↑
POS	223.0749	4.25	Cystathionine [e]	3.02×10^{-5}	1.64	2.13×10^{-4}	3.00×10^{-4}	1.50	2.23×10^{-3}	↑
POS	244.0941	5.10	Cytidine [e]	2.65×10^{-6}	2.43	3.59×10^{-5}	--	--[c]	--	↑
POS	249.1082		L-Threoninyl-L-Glutamate	5.16×10^{-4}	2.39	1.52×10^{-3}	7.38×10^{-8}	2.57	6.15×10^{-6}	↑
POS	269.0873	8.36	Inosine [e]	3.14×10^{-6}	3.80	4.09×10^{-5}	5.08×10^{-4}	1.82	3.03×10^{-3}	↑
POS	308.0910	5.35	Glutathione [e]	2.02×10^{-2}	1.46	3.43×10^{-2}	1.34×10^{-2}	1.50	3.21×10^{-2}	↑
POS	329.2458	26.32	Docosahexaenoic acid [e]	1.25×10^{-3}	0.52	3.12×10^{-3}	2.81×10^{-3}	0.58	5.94×10^{-3}	↓
POS	349.0534	5.76	Inosine monophosphate[e]	4.10×10^{-3}	2.56	8.38×10^{-3}	--	--[c]	--	↑
POS	364.0674	5.24	Guanosine monophosphate [e]	7.02×10^{-5}	1.76	4.01×10^{-4}	1.39×10^{-3}	1.53	5.25×10^{-3}	↑
POS	385.1298	8.56	S-adenosylhomocysteine [e]	1.46×10^{-5}	2.3	1.26×10^{-4}	2.46×10^{-6}	1.92	8.24×10^{-5}	↑
POS	399.1437	5.00	S-adenosymethionine	--	--[c]	--	3.23×10^{-3}	1.63	7.01×10^{-3}	↑
POS	428.0376	4.85	Adenosine diphosphate [e]	6.60×10^{-4}	0.48	1.82×10^{-3}	3.41×10^{-2}	0.72	4.69×10^{-2}	↓
NEG	104.377	4.38	Serine [e]	6.40×10^{-7}	1.7	3.33×10^{-6}	6.08×10^{-3}	1.33	9.60×10^{-3}	↑
NEG	150.0423	6.74	Guanine [e]	2.10×10^{-2}	0.71	2.90×10^{-2}	2.32×10^{-2}	0.70	4.83×10^{-2}	↓
NEG	151.0266	8.73	Xanthine [e]	1.39×10^{-6}	2.3	6.62×10^{-6}	7.90×10^{-3}	1.4	1.98×10^{-2}	↑
NEG	157.0384	4.98	Allantoin [e]	3.46×10^{-5}	2.3	1.10×10^{-4}	3.77×10^{-6}	1.87	3.54×10^{-5}	↑
NEG	166.9761	4.62	Phosphoenol pyruvate [e]	4.21×10^{-11}	2.04	9.85×10^{-10}	--	--[c]	--	↑
NEG	167.022	6.34	Uric acid [e]	3.53×10^{-2}	1.30	4.55×10^{-2}	--	--[c]	--	↑

NEG	181.05191	12.42	Hydroxyphenyllactic acid [e]	1.11×10^{-2}	3.12	1.78×10^{-2}	7.17×10^{-3}	2.32	1.84×10^{-2}	↑
NEG	184.9865	4.39	D-glycerate-3-phosphate	1.56×10^{-10}	2.03	2.67×10^{-9}	2.78×10^{-5}	1.52	1.96×10^{-4}	↑
NEG	191.0208	5.51	Citric acid [e]	--	--[c]	--	3.00×10^{-3}	1.43	8.76×10^{-3}	↑
NEG	229.011	4.34	D-ribose-5-phosphate [e]	2.23×10^{-11}	2.04	6.80×10^{-10}	--	--[c]	--	↑
NEG	243.0616	6.89	Uridine [e]	6.18×10^{-3}	1.47	9.93×10^{-3}	--	--[c]	--	↑
NEG	259.1320	6.64	L-gamma-glutamyl-L-leucine	7.70×10^{-3}	2.12	1.20×10^{-2}	9.36×10^{-3}	1.95	2.24×10^{-2}	↑
NEG	274.1413	3.98	E-(gamma-glutamyl)-lysine	5.10×10^{-4}	1.63	1.07×10^{-3}	4.43×10^{-5}	1.90	2.77×10^{-4}	↑
NEG	275.0169	4.31	6-phosphogluconic acid	3.40×10^{-3}	1.55	5.81×10^{-3}	--	--[c]	--	↑
NEG	282.0856	8.12	Guanosine [e]	3.50×10^{-3}	1.53	5.92×10^{-3}	--	--[c]	--	↑
NEG	338.9882	4.6	D-fructose 1,6-bisphosphate [e]	4.44×10^{-6}	0.34	1.76×10^{-5}	9.85×10^{-4}	0.50	3.47×10^{-3}	↓
NEG	346.053	5.29	Adonosine monophosphate [e]	4.26×10^{-7}	0.28	2.40×10^{-6}	--	--[c]	--	↓
NEG	402.0071	4.39	Cytidine diphosphate [e]	4.90×10^{-11}	0.26	1.10×10^{-9}	1.43×10^{-7}	0.51	1.96×10^{-6}	↓
NEG	402.9948	4.40	Uridine diphosphate [e]	2.70×10^{-5}	0.47	8.56×10^{-5}	1.40×10^{-2}	0.69	3.16×10^{-2}	↓
NEG	410.2226	23.87	N-arachidonoyl taurine	--	--[c]	--	2.71×10^{-5}	2.10	1.93×10^{-4}	↑
NEG	442.0132	5.11	Guanosine 5’diphosphate	6.98×10^{-7}	0.41	3.60×10^{-6}	5.30×10^{-4}	0.63	2.06×10^{-3}	↓
NEG	558.0631	4.79	Adenosine diphosphate ribose [e]	6.32×10^{-11}	0.21	1.26×10^{-9}	1.15×10^{-5}	0.58	8.92×10^{-5}	↓
NEG	558.0631	4.79	Nucleotide adenine dinucleotide [e]	3.12×10^{-6}	0.48	1.31×10^{-5}	5.10×10^{-3}	0.72	1.36×10^{-2}	↓

[a] *p* values were obtained from comparing either BAA or BAQ group to the same control group using the Welch *t*-test.

[b] Fold-change value obtained from comparing the average of metabolites in either the BAA or BAQ treatment with the control group.

[c] No significant change when compared to respective controls.

[d] m/z value of molecular ion adduct $[M+H-H_2O]^+$ in positive ion mode. Metabolite identified by spectral fragmentation pattern.

[e] Metabolite identified by database search and confirmed by authentic standards.