

# Supplementary Materials: Effects of the Toxic Non-Protein Amino Acid $\beta$ -Methylamino-L-Alanine (BMAA) on Intracellular Amino Acid Levels in Neuroblastoma Cells

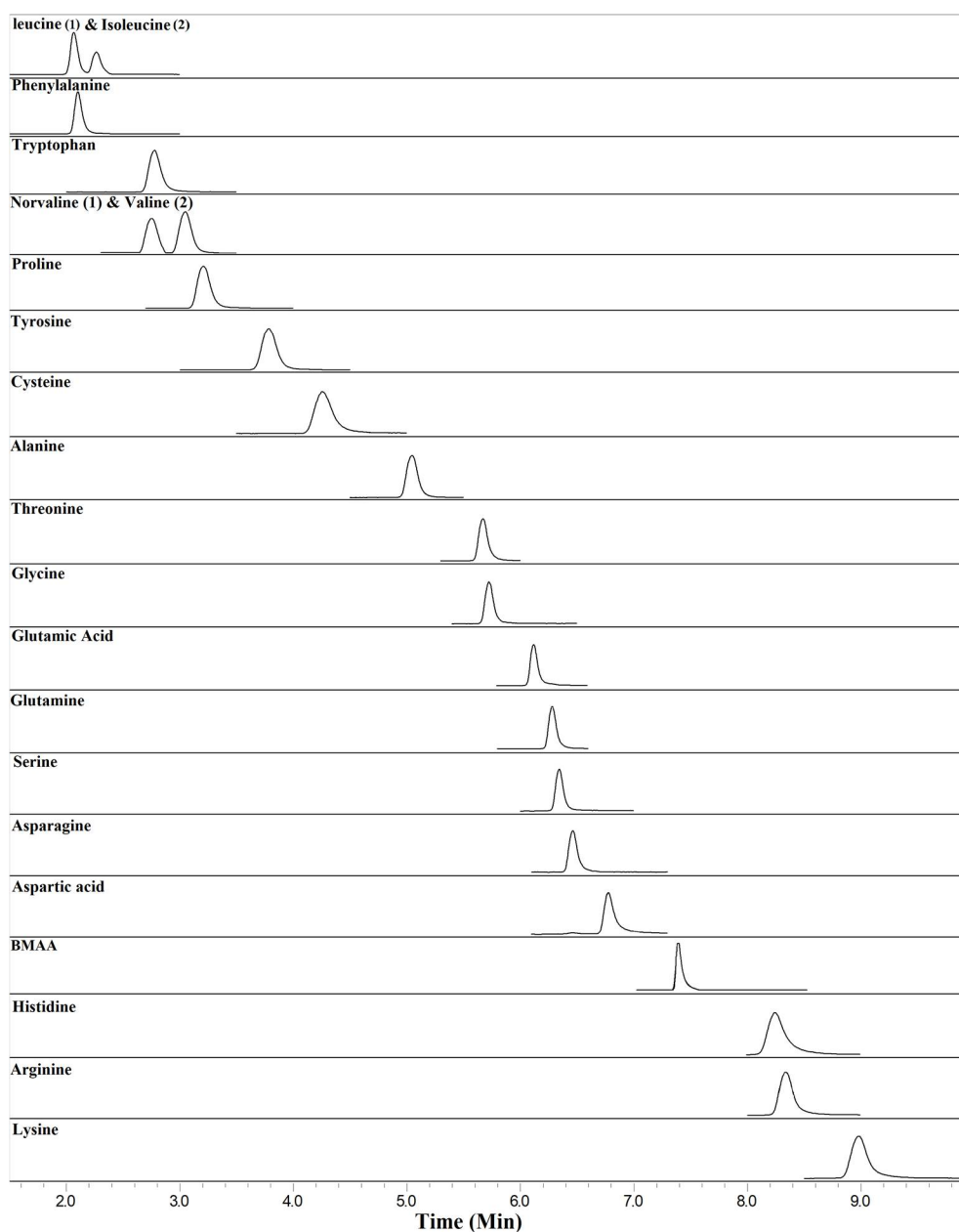
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**Table S1.** Limit of Detection (LOD), Limit of Quantification (LOQ), and Multiple Reaction Monitoring (MRM) transitions for all amino acids analysed in this study. \*denotes the internal standard.

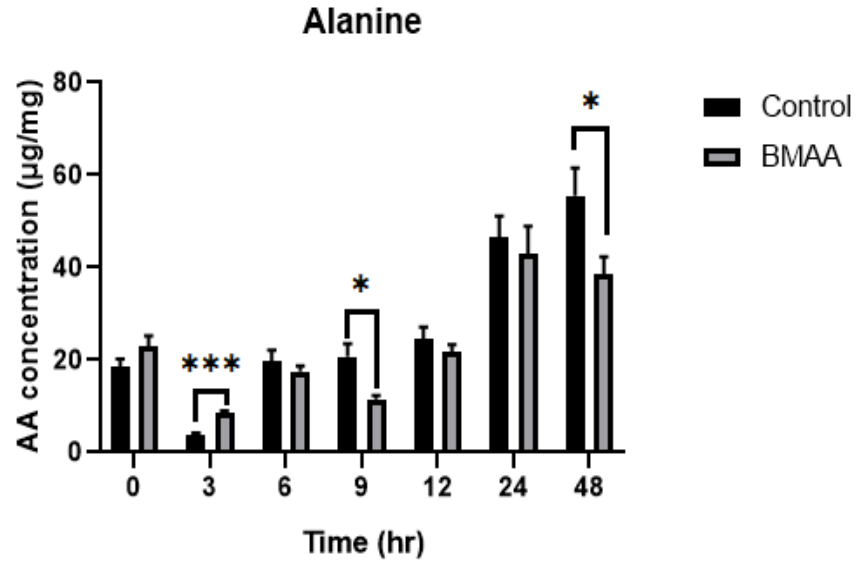
Amino acid	Collision energy (eV)	MRM transitions (m/z)	LOD (ng/ mL)	LOQ (ng/mL)
Alanine	-29.40	131.10 $\rightarrow$ 44.15	0.02	0.05
	-8.80	$\rightarrow$ 90.15		
Arginine	-22.00	175.20 $\rightarrow$ 70.15	1.89	5.72
	-12.00	$\rightarrow$ 60.15		
Asparagine	-13.80	174.10 $\rightarrow$ 87.15	0.73	2.19
	-9.80	$\rightarrow$ 133.10		
Aspartic acid	-22.10	175.10 $\rightarrow$ 74.10	0.98	2.99
	-9.20	$\rightarrow$ 134.20		
BMAA	-17.00	119.10 $\rightarrow$ 44.10	0.38	1.17
	-15.00	$\rightarrow$ 88.10		
Cysteine	-29.40	163.10 $\rightarrow$ 59.25	0.58	1.77
	-8.80	$\rightarrow$ 122.15		
Glutamic acid	-16.00	148.10 $\rightarrow$ 84.10	1.49	4.51
	-21.90	189.10 $\rightarrow$ 84.10		
Glutamine	-20.00	147.10 $\rightarrow$ 84.10	0.47	1.44
	-14.00	$\rightarrow$ 130.10		
Glycine	-17.90	117.20 $\rightarrow$ 76.20	3.14	9.52
	-7.20	$\rightarrow$ 30.15		
Histidine	-15.00	156.15 $\rightarrow$ 110.15	4.80	14.55
	-28.00	$\rightarrow$ 82.15		
Isoleucine	-15.40	173.20 $\rightarrow$ 86.25	0.03	0.09
	-8.80	$\rightarrow$ 132.20		
Leucine	-15.40	173.20 $\rightarrow$ 86.25	0.02	0.06
	-8.80	$\rightarrow$ 132.20		
Lysine	-14.00	147.20 $\rightarrow$ 67.10	2.86	8.67
	-30.00	$\rightarrow$ 84.15		
Methionine	-30.30	191.10 $\rightarrow$ 61.20	0.19	0.58
	-7.80	$\rightarrow$ 150.15		
Norvaline*	-16.90	159.10 $\rightarrow$ 72.05	0.02	0.06
	-8.20	$\rightarrow$ 118.20		

Phenylalanine	-14.00	207.10 → 120.15	0.03	0.10
	-8.70	→ 166.20		
Proline	-24.10	157.00 → 70.10	0.02	0.05
	-9.80	→ 116.20		
Serine	-28.30	147.15 → 60.15	0.11	0.32
	-17.60	→ 42.15		
Threonine	-16.90	161.10 → 74.20	0.19	0.58
	-9.20	→ 120.20		
Tryptophan	-6.70	246.10 → 72.05	0.18	0.56
	-22.90	→ 118.20		
Tyrosine	-35.10	223.10 → 182.20	0.07	0.21
	-6.70	→ 91.05		
Valine	-16.90	159.10 → 72.05	0.05	0.16
	-27.30	→ 118.20		

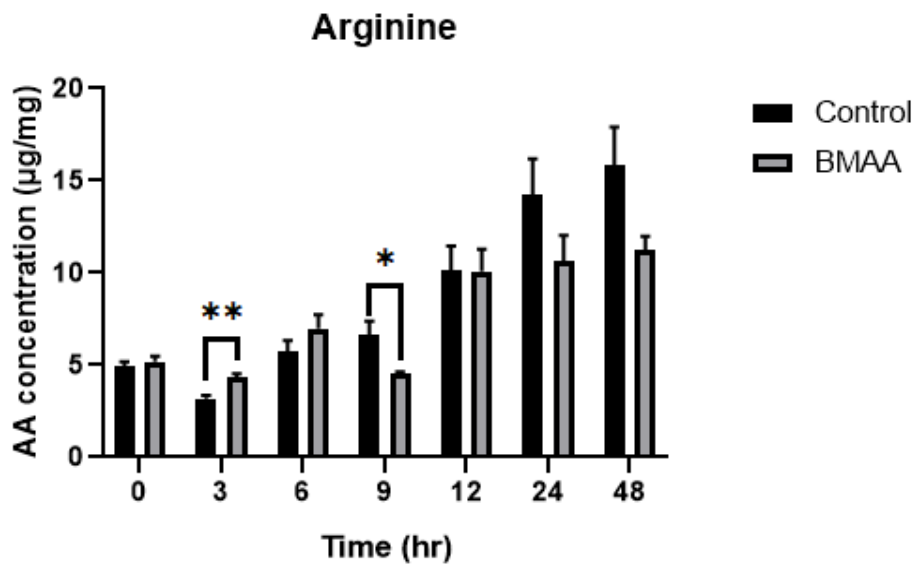
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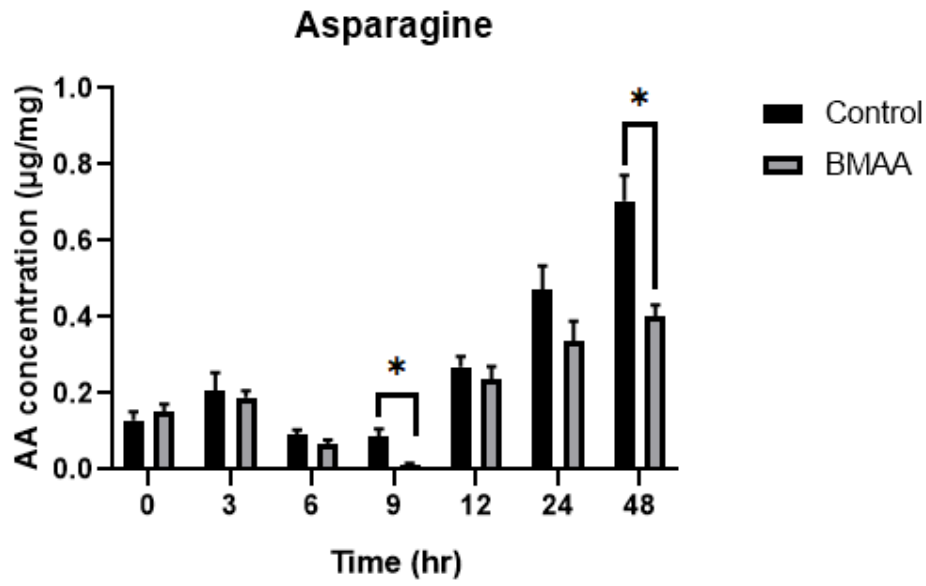
**Figure S1.** Total ion chromatograms (TICs) for each amino acid analysed.



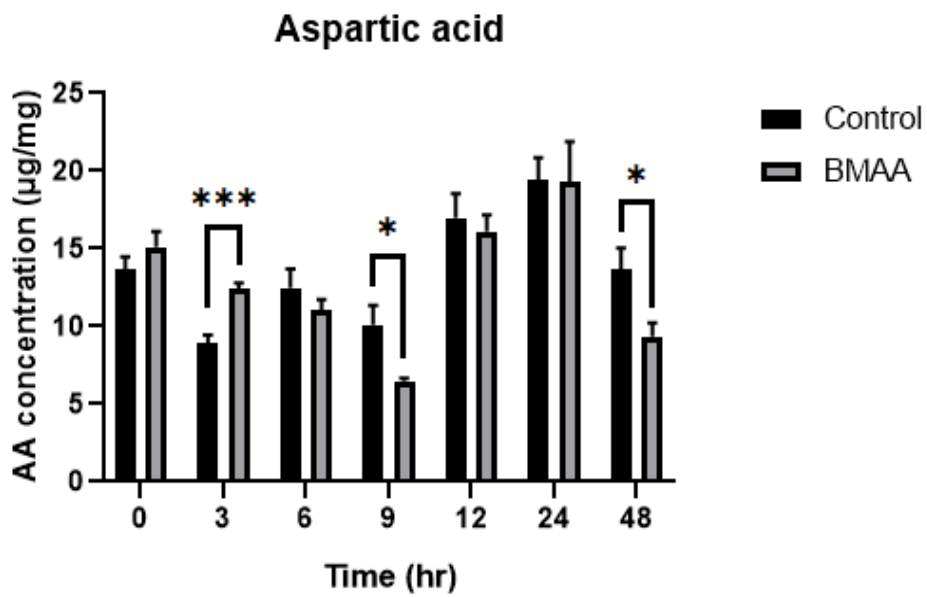
**Figure S2.** Intracellular concentrations of alanine for control and BMAA-treated cells (n = 6). Error bars denote the standard error of the mean (SEM). \* Denotes a *p*-value of < 0.05, \*\* denotes a *p*-value of <0.005, and \*\*\* denotes a *p*-value of <0.0005.



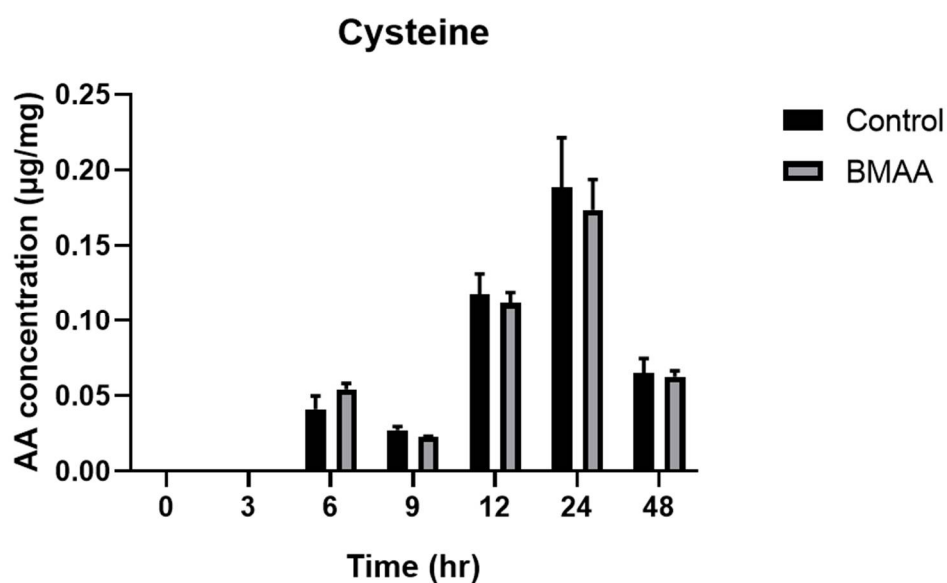
**Figure S3.** Intracellular concentrations of arginine for control and BMAA-treated cells (n = 6). Error bars denote the standard error of the mean (SEM). \* Denotes a *p*-value of < 0.05 and \*\* denotes a *p*-value of <0.005.



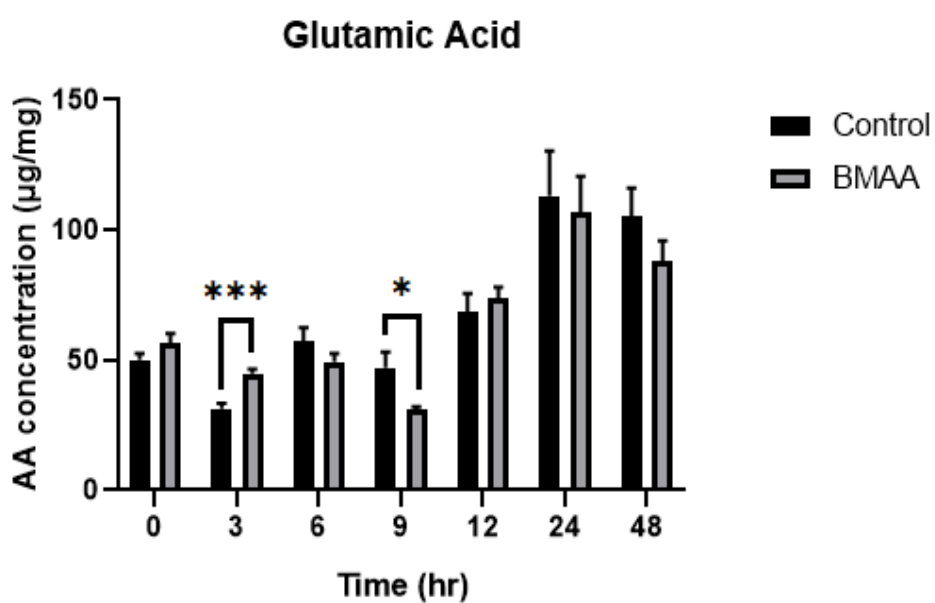
**Figure S4.** Intracellular concentrations of asparagine for control and BMAA-treated cells (n = 6). Error bars denote the standard error of the mean (SEM). \* Denotes a *p*-value of < 0.05.



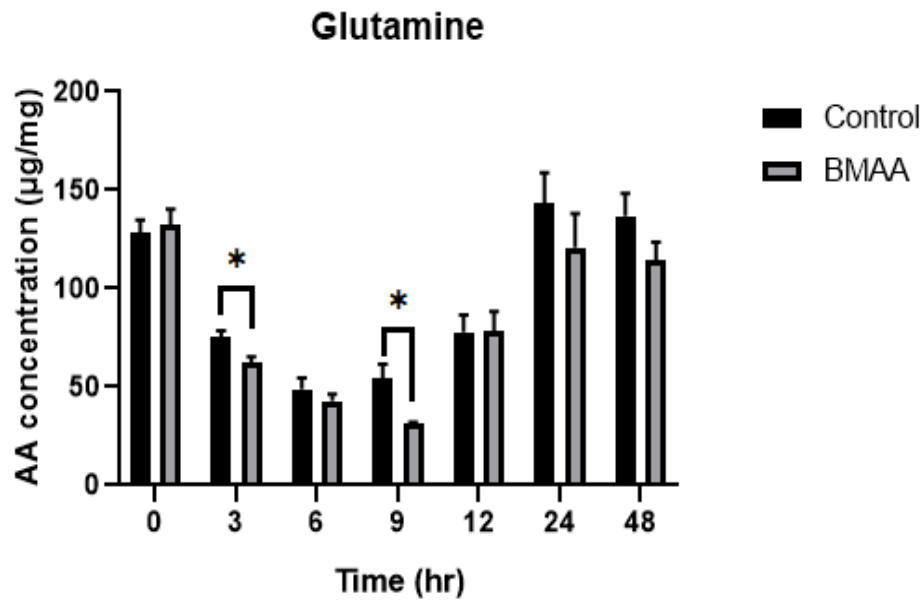
**Figure S5.** Intracellular concentrations of aspartic acid for control and BMAA-treated cells (n = 6). Error bars denote the standard error of the mean (SEM). \* Denotes a *p*-value of < 0.05, \*\* denotes a *p*-value of < 0.005, and \*\*\* denotes a *p*-value of < 0.0005.



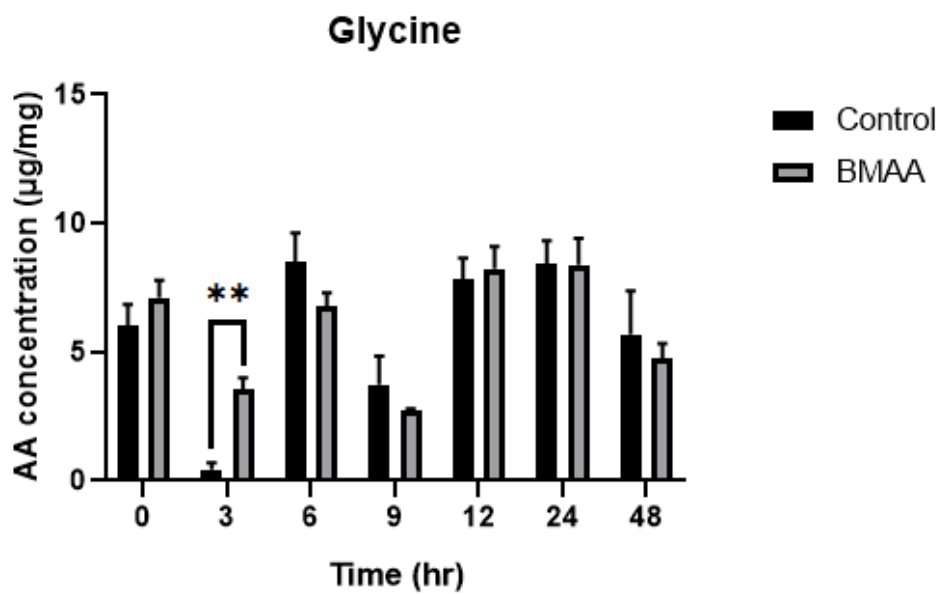
**Figure S6.** Intracellular concentrations of cysteine for control and BMAA-treated cells ( $n = 6$ ). Error bars denote the standard error of the mean (SEM).



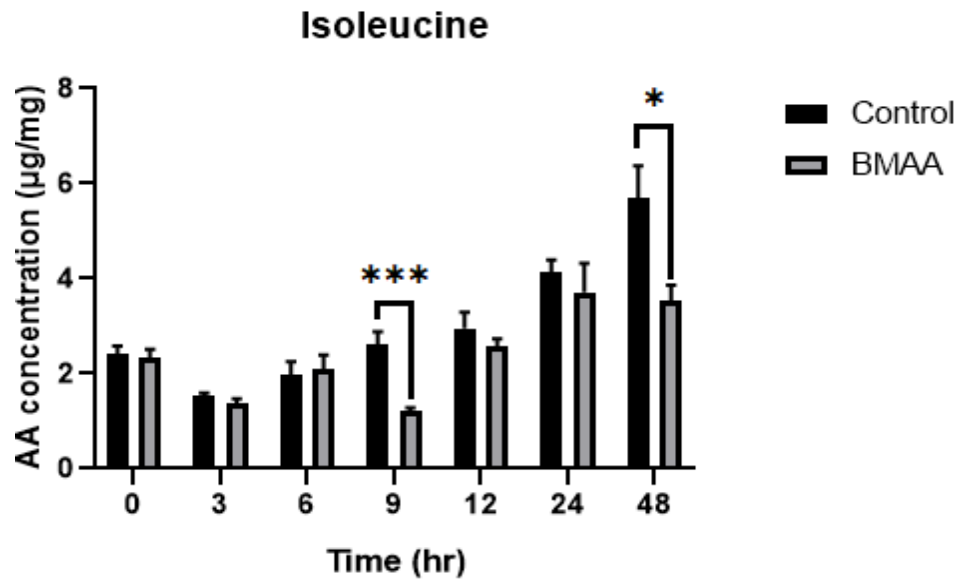
**Figure S7.** Intracellular concentrations of glutamic acid for control and BMAA-treated cells ( $n = 6$ ). Error bars denote the standard error of the mean (SEM). \* Denotes a  $p$ -value of  $< 0.05$ , \*\* denotes a  $p$ -value of  $< 0.005$ , and \*\*\* denotes a  $p$ -value of  $< 0.0005$ .



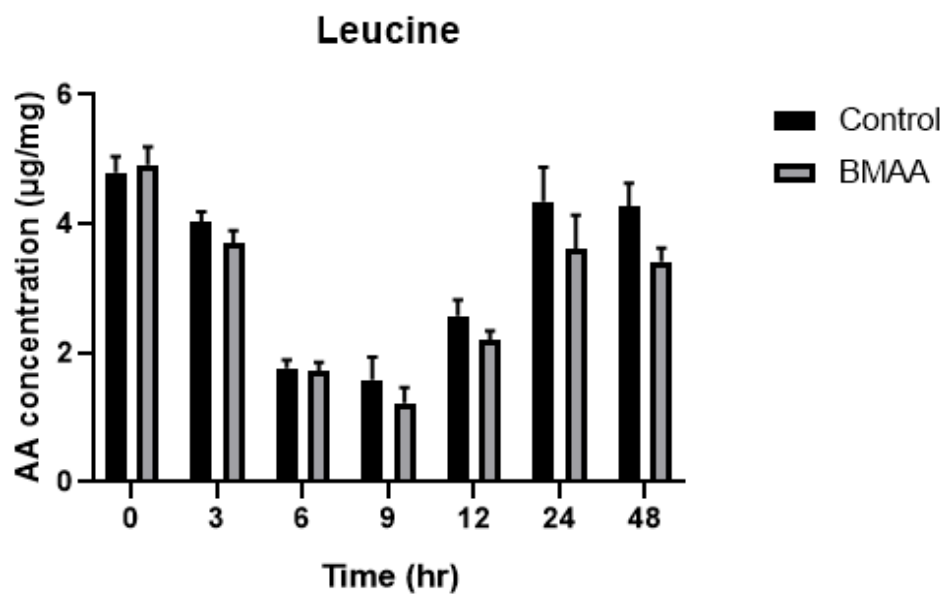
**Figure S8.** Intracellular concentrations of glutamine for control and BMAA-treated cells (n = 6). Error bars denote the standard error of the mean (SEM). \* Denotes a *p*-value of < 0.05.



**Figure S9.** Intracellular concentrations of glycine for control and BMAA-treated cells (n = 6). Error bars denote the standard error of the mean (SEM). \* Denotes a *p*-value of < 0.05 and \*\* denotes a *p*-value of < 0.005.

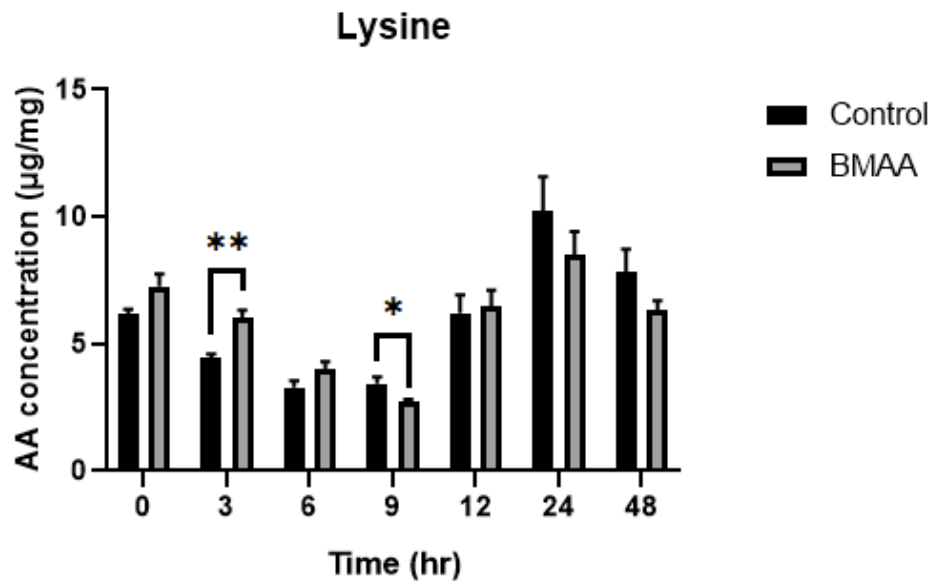


**Figure S10.** Intracellular concentrations of isoleucine for control and BMAA-treated cells (n = 6). Error bars denote the standard error of the mean (SEM). \* Denotes a *p*-value of < 0.05, \*\* denotes a *p*-value of < 0.005, and \*\*\* denotes a *p*-value of < 0.0005.

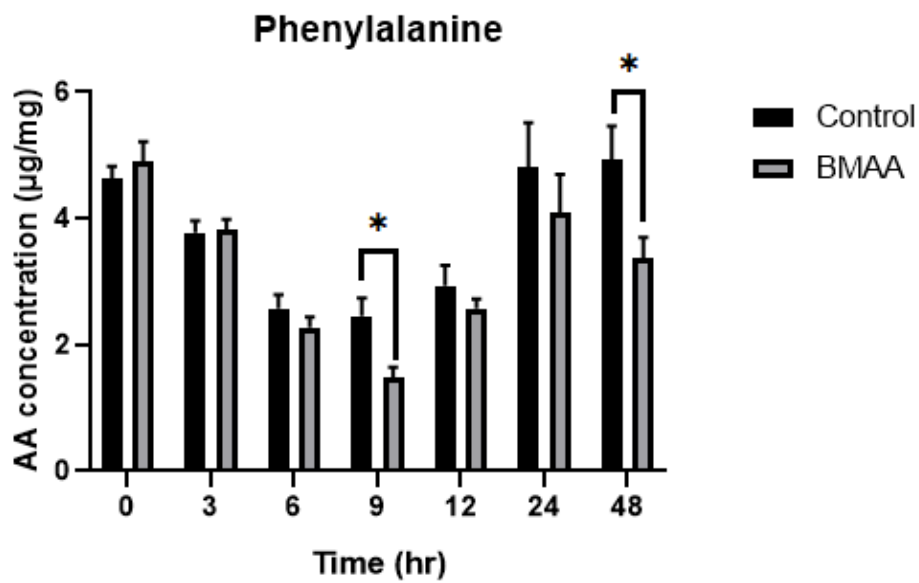


**Figure S11.** Intracellular concentrations of leucine for control and BMAA-treated cells (n = 6). Error bars denote the standard error of the mean (SEM).

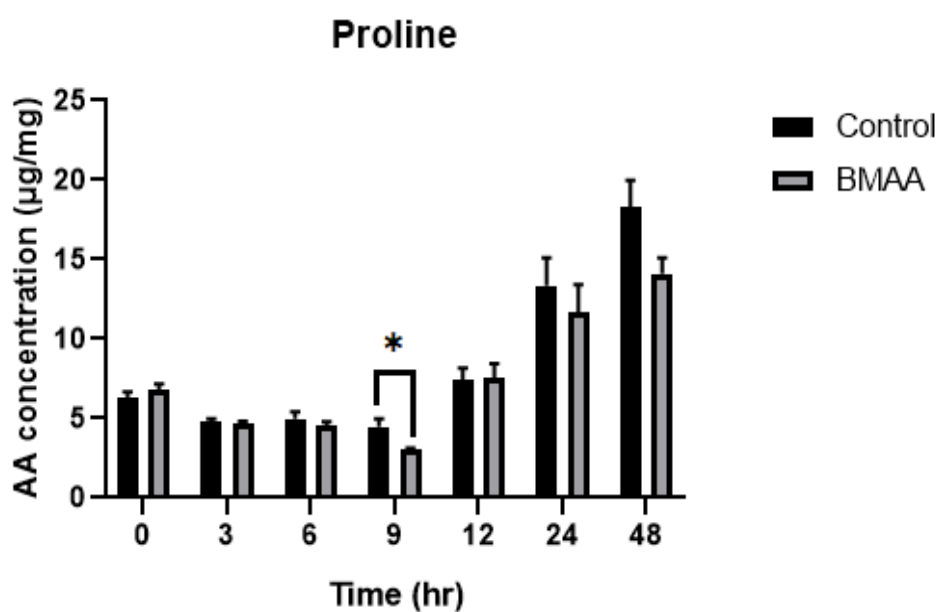




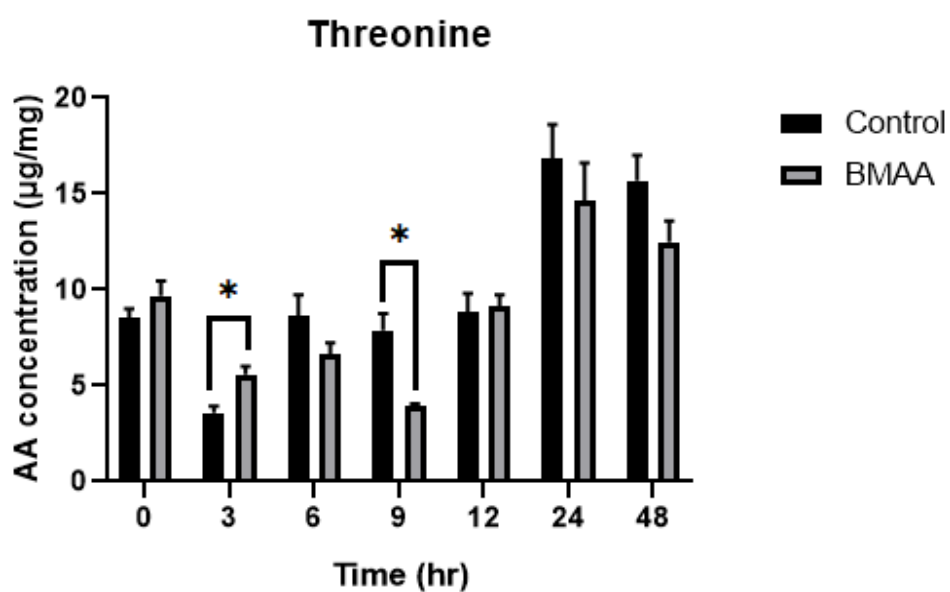
**Figure S12.** Intracellular concentrations of lysine for control and BMAA-treated cells (n = 6). Error bars denote the standard error of the mean (SEM). \* Denotes a *p*-value of < 0.05 and \*\* denotes a *p*-value of < 0.005.



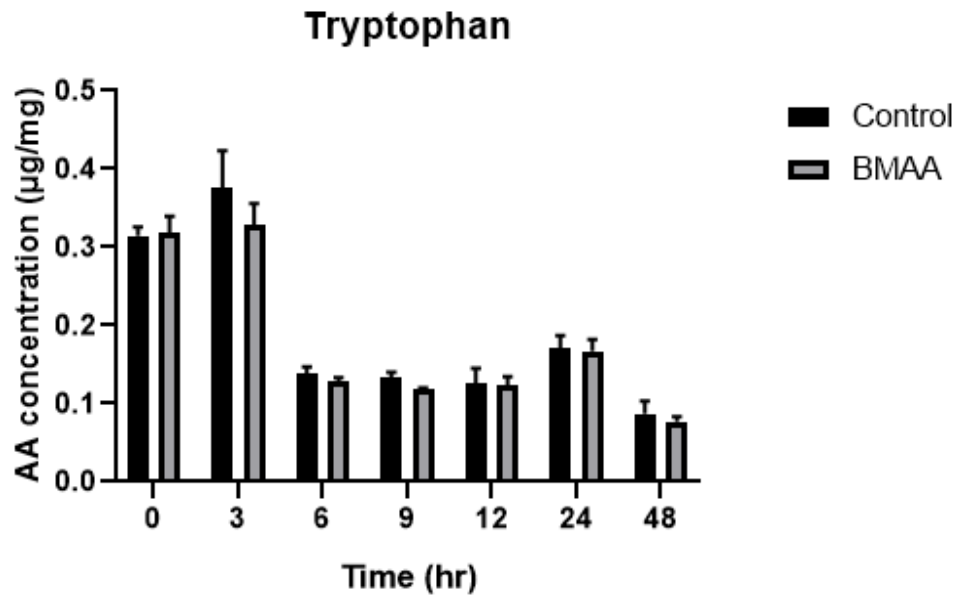
**Figure S13.** Intracellular concentrations of phenylalanine for control and BMAA-treated cells (n = 6). Error bars denote the standard error of the mean (SEM). \* Denotes a *p*-value of < 0.05.



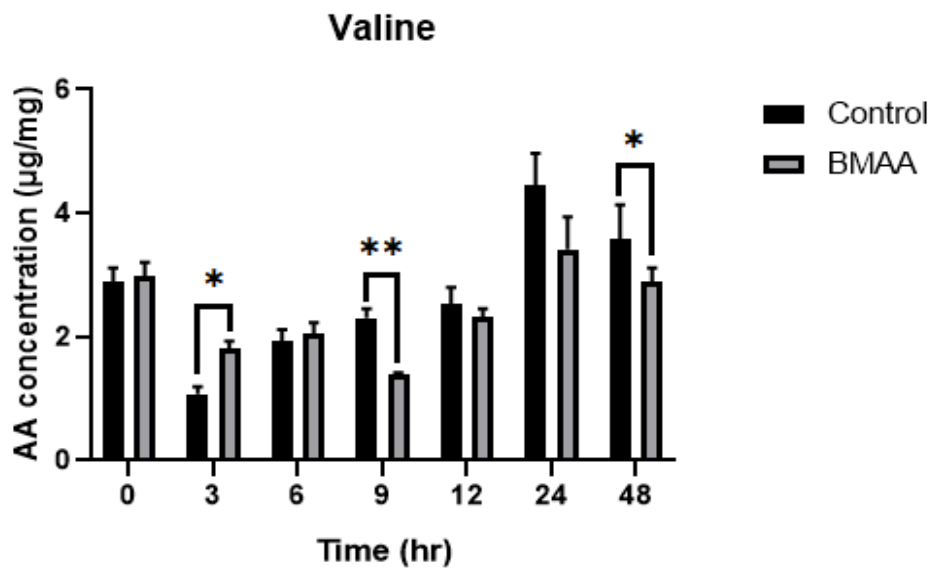
**Figure S14.** Intracellular concentrations of proline for control and BMAA-treated cells (n = 6). Error bars denote the standard error of the mean (SEM). \* Denotes a *p*-value of < 0.05.



**Figure S15.** Intracellular concentrations of threonine for control and BMAA-treated cells (n = 6). Error bars denote the standard error of the mean (SEM). \* Denotes a *p*-value of < 0.05.



**Figure S16.** Intracellular concentrations of tryptophan for control and BMAA-treated cells (n = 6). Error bars denote the standard error of the mean (SEM).



**Figure S17.** Intracellular concentrations of valine for control and BMAA-treated cells (n = 6). Error bars denote the standard error of the mean (SEM). \* Denotes a *p*-value of < 0.05 and \*\* denotes a *p*-value of < 0.005.

**Table S2.** Amino acid content of the culture medium used in the studies (ThermoFisher 11090081). \*L-glutamine was freshly added before use from a 200 mM solution diluted 1 in 100.

Amino acid	mg/L	mM
L-Arginine hydrochloride	126	0.597
L-Cystine-2HCl	31	0.099
*L-Glutamine		2.0
L-Histidine hydrochloride-H <sub>2</sub> O	42	0.2
L-Isoleucine	52	0.397
L-Leucine	52	0.397
L-Lysine hydrochloride	73	0.397
L-Methionine	15	0.101
L-Phenylalanine	32	0.194
L-Threonine	48	0.403
L-Tryptophan	10	0.049
L-Tyrosine disodium salt dihydrate	52	0.199
L-Valine	46	0.393