

Supplementary Material A

Contents

Additional File Table 1: Characteristics of individuals in the PDD Metabolomics cohort	3
Additional File Table 2: Summary of PDD Metabolomics cohort	6
Additional File Figure 1A – Significant Altered Metabolites in PDD Brain: Amino Acids 1.....	7
Additional File Figure 2B – Significant Altered Metabolites in PDD Brain: Amino Acids 2.....	8
Additional File Figure 1C – Significant Altered Metabolites in PDD Brain: Glucose Metabolism Pathways	9
Additional File Figure 1D – Significant Altered Metabolites in PDD Brain: Purine Metabolism Pathways	10
Additional File Figure 1F – Significant Altered Metabolites in PDD Brain: Other Metabolites	11
Additional File Figure 2A – Heat Map: CG	13
Additional File Figure 2B – Heat Map: CB	14
Additional File Figure 2C – Heat Map: MCX.....	15
Additional File Figure 2D – Heat Map: MED	16
Additional File Figure 2E – Heat Map: MTG.....	17
Additional File Figure 2F – Heat Map: Pons.....	18
Additional File Figure 2G – Heat Map: SN	19
Additional File Figure 2H – Heat Map: PVC	20

Additional File Figure 3 – PCA Plots	21
Additional File Figure 4A – Correlation Matrix: CG.....	22
Additional File Figure 4B – Correlation Matrix: CB	23
Additional File Figure 4C – Correlation Matrix: MCX.....	24
Additional File Figure 4D – Correlation Matrix: MED	25
Additional File Figure 4E – Correlation Matrix: MTG.....	26
Additional File Figure 4F – Correlation Matrix: Pons.....	27
Additional File Figure 4G – Correlation Matrix: SN.....	28
Additional File Figure 4H – Correlation Matrix: PVC.....	29

Additional File Table 1: Characteristics of individuals in the PDD Metabolomics cohort

Code	Sex	Age at death	Clinical diagnosis	Neuropathological Findings	Post-mortem Delay (hours)	Whole-brain weight (g)	Cause of Death
C1 (no SN tissue)	Male	61	No dementia present	Normal adult brain	12.5	1182	Respiratory failure; heart failure; coronary artery disease
C2	Male	71	No dementia present	Normal adult brain	23.9	1371	Acute myocardial infarction; severe coronary artery disease; pulmonary oedema; diabetes mellitus; cardiopulmonary arrest
C3	Male	74	No dementia present	Unaffected control with incidental Lewy bodies	25.5	1300	Aortic dissection
C4 (no SN tissue)	Male	70	No dementia present	Low level Alzheimer's disease neuropathological change (B0, C1)	12.7	1350	Atherosclerotic and hypertensive heart disease
C5	Female	68	No dementia present	Normal adult brain	19.1	1270	Acute myocardial infarction; coronary artery disease
C6	Female	65	No dementia present	Normal adult brain with minimal small vessel ischaemic changes	19.4	1372	Hypertensive arteriosclerotic cardiovascular disease; morbid obesity; respiratory arrest; suspected embolus
C7	Female	77	No dementia present	Normal adult brain	21.4	1135	Pending death certificate

C8	Female	79	No dementia present	Normal adult brain	17.8	1300	Acute myocardial infarction; coronary artery disease; atrial fibrillation; COPD
C9	Female	67	No dementia present	Alzheimer's disease neuropathological changes (A1, B1, C0), low level normal aging, cerebrovascular disease, focal minimal atherosclerosis, congestion, oedema, perivascular haemorrhage	25.9	1382	Pending death certificate
C10 (SN ONLY)	Male	68	No dementia present	Normal adult brain	10.8	1550	Pending death certificate
C11 (SN ONLY)	Male	62	No dementia present	Normal adult brain; capillary haemangioma in caudate nucleus	21.3	Not available	Diabetes; triple vessel disease of the heart; hyperlipidaemia
PDD1	Male	61	Parkinson's disease dementia	Limbic (transitional) LBD	13.8	1382	Cardiopulmonary arrest; probable acute myocardial infarction; Parkinson's disease
PDD2	Male	79	Parkinson's disease dementia	Limbic (transitional) LBD	16.2	1188	End stage Parkinson's disease
PDD3	Male	71	Parkinson's disease	Brainstem predominant LBD; a-synuclein Braak	16.2	1250	Pending death certificate

			dementia	stage III-IV			
PDD4	Male	78	Parkinson's disease dementia	Diffuse neocortical LBD; a-synuclein Braak stage VI; low level of AD neuropathic change (B1, C1)	20.4	1520	Respiratory failure; aspiration pneumonia; dysphagia; Parkinson's disease
PDD5	Male	70	Parkinson's disease dementia	Diffuse neocortical LBD	4.3	1218	Aspiration pneumonia; Parkinson's disease
PDD6	Female	69	Parkinson's disease dementia	Brain with morphological findings most compatible with PD with dementia; a-synuclein Braak stage IV-V	17.5	1218	Pending death certificate
PDD7	Female	81	Parkinson's disease dementia	Diffuse neocortical LBD; low level Alzheimer's neuropathological change in limbic system (B1, C1)	7.0	1187	End stage Parkinson's disease
PDD8	Female	79	Parkinson's disease dementia	Limbic (transitional) LBD; mild small vessel ischaemic disease	21.9	1415	Respiratory failure; Parkinson's disease
PDD9	Female	67	Parkinson's disease dementia	Diffuse neocortical LBD; low level Alzheimer's neuropathological change (A3, B1, C1, Braak II)	14.5	1200	Lewy body disease; Parkinson's disease

Additional File Table 2: Summary of PDD Metabolomics cohort

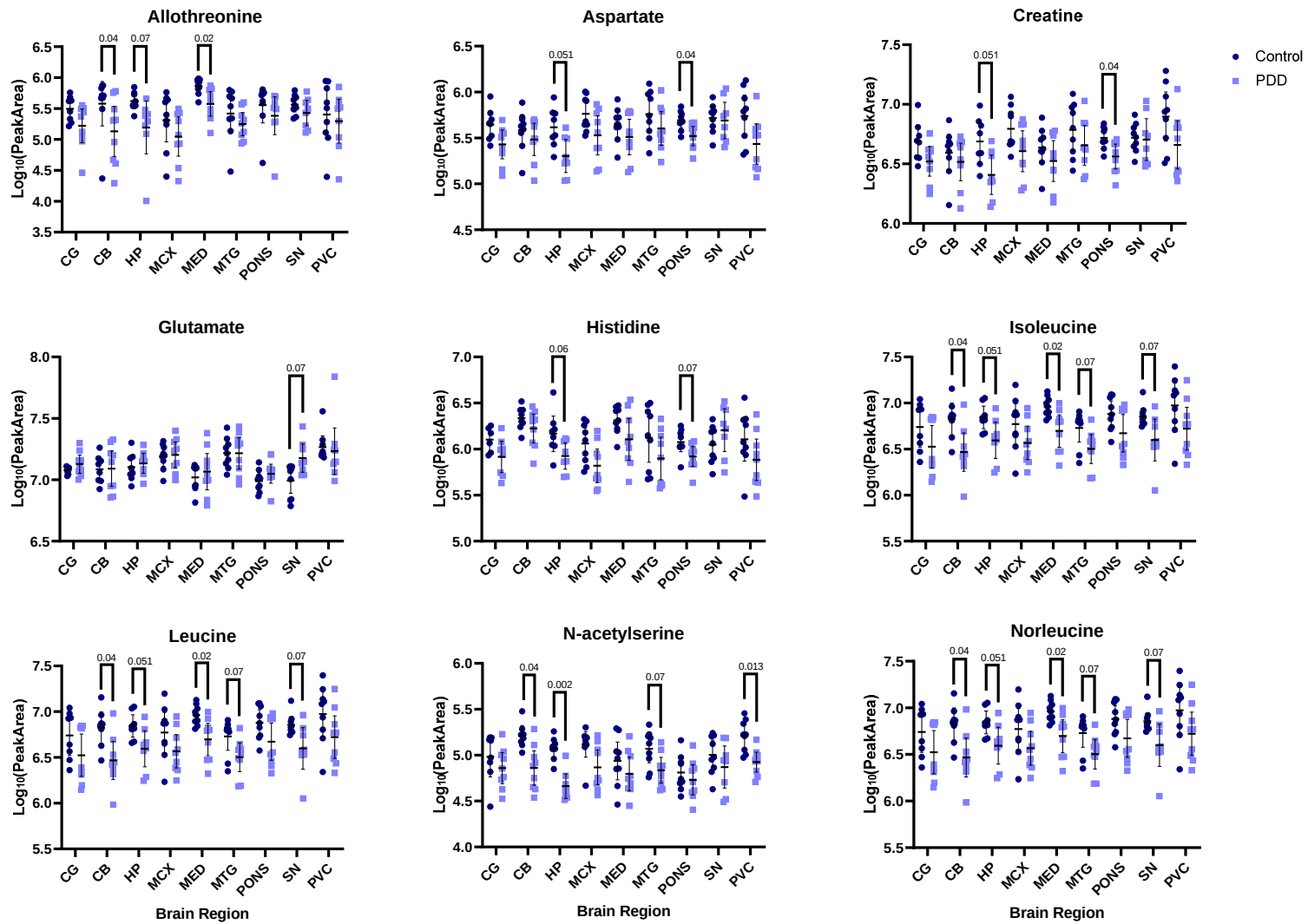
	Gender (% male)	Age at death (years)	PMD (hours)	Brain Weight (g)
Controls		70	19.8	1296
(n = 9)	38	(65–79)	(12.7–25.9)	(1135–1382)
SN Controls		70	20.6	1335
(n = 9)	44	(62–79)	(10.8–25.9)*	(1135–1550) [†]
Cases		73	14.6	1286
(n = 9)	66	(61–81)	(4.3–21.9)	(1187–1520)

Table shows mean (range) age, PMD, brain weight and Braak stage. [†]Brain weight unavailable for

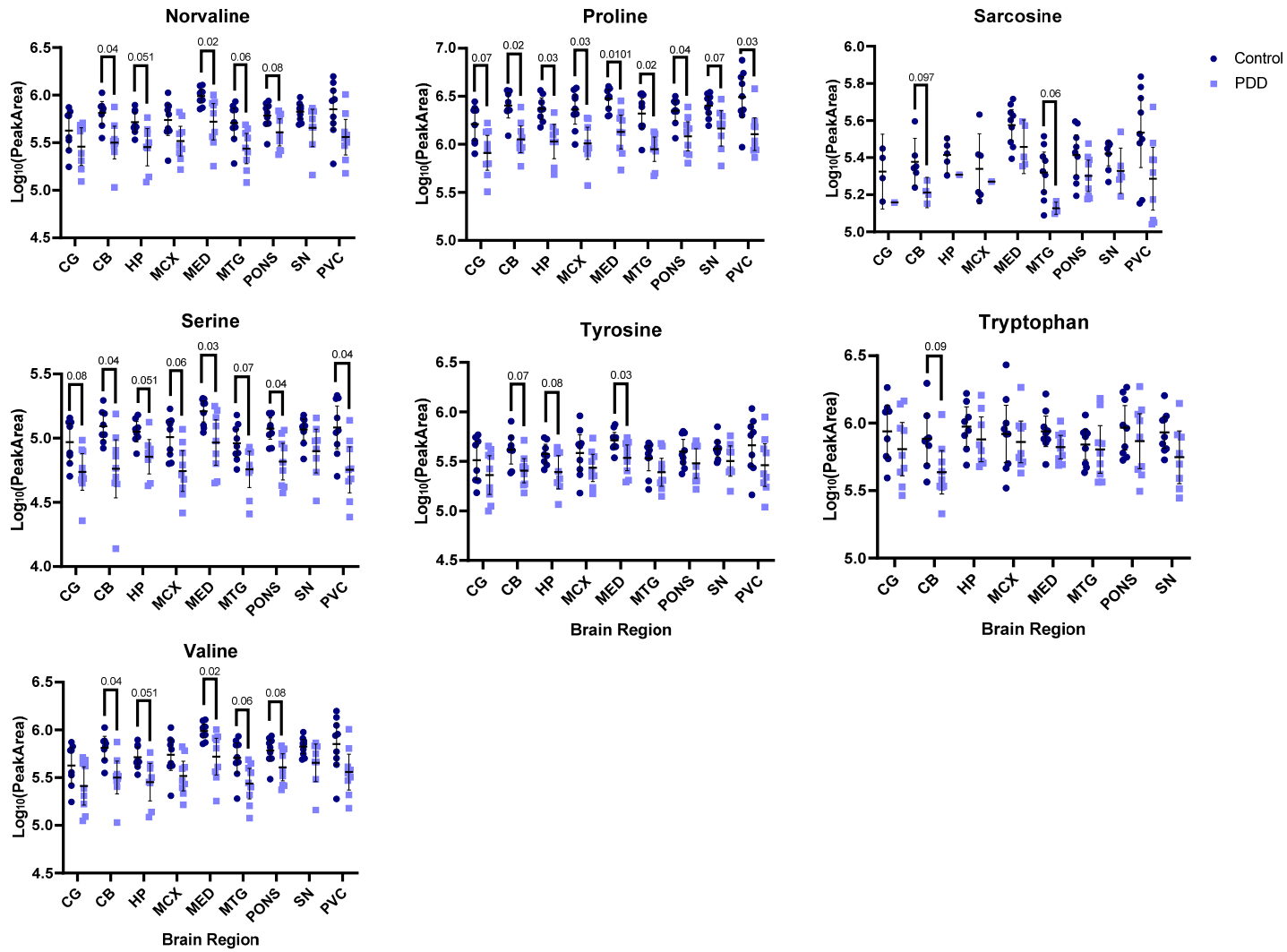
*C10. * SN controls had higher PMD than SN cases; $p = 0.03$.*

Brain tissue for all nine investigated regions was obtained from the same case and control donors, with the exception of the SN, for which two control samples had to be substituted due to a lack of available tissue in the standard cohort donors (controls C10 and C11 substituted for C1 and C4; see Table 1 for individual data). PDD cases and controls for all regions except the SN showed no significant difference in age, gender distribution, or brain weight; PMD was lower in cases (mean 14.6 hours) compared to controls (mean 20.6 hours; $p = 0.03$) in the SN, but matched in all other regions. Due to an unavailability of tissue or high blank values, the C1 CG, C5 CB, C2 HP, and PDD3 HP could not be analysed; these regions remained age, gender, brain weight, and PMD-matched. The PMD was kept as low as possible, at a maximum of 25.9 hours in any region and at an average of 17.1 hours.

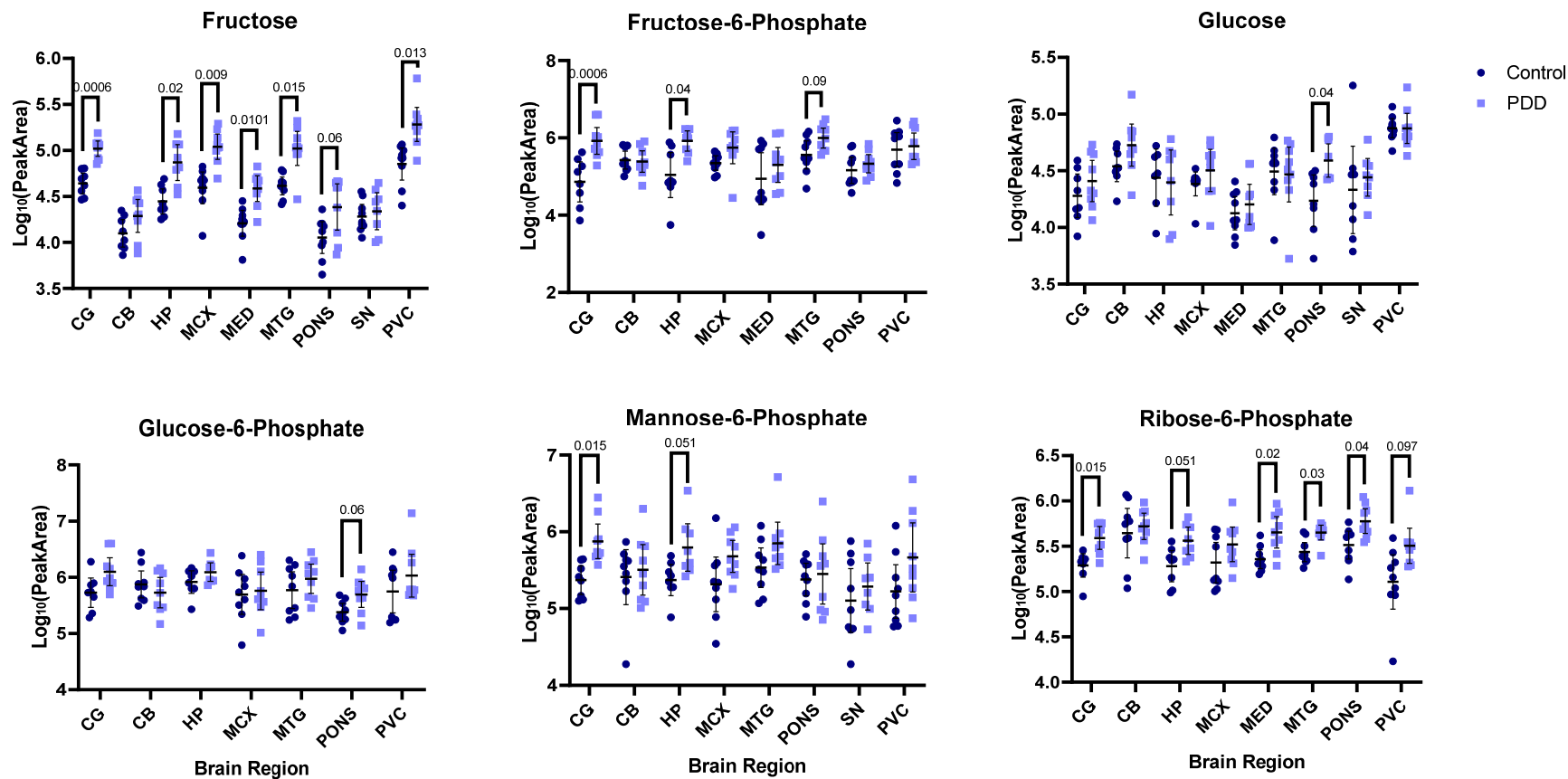
Additional File Figure 1A – Significant Altered Metabolites in PDD Brain: Amino Acids 1



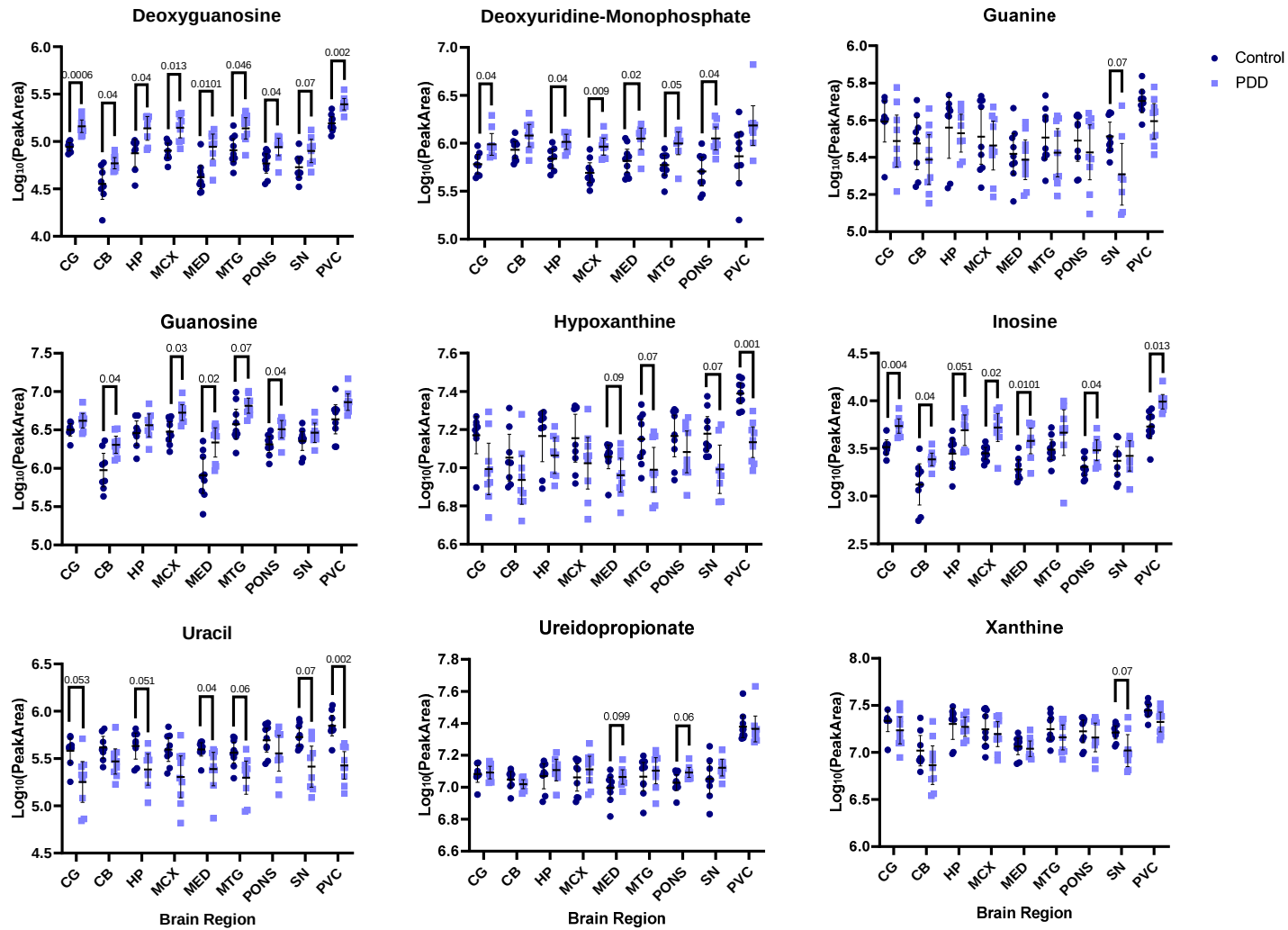
Additional File Figure 2B – Significant Altered Metabolites in PDD Brain: Amino Acids 2



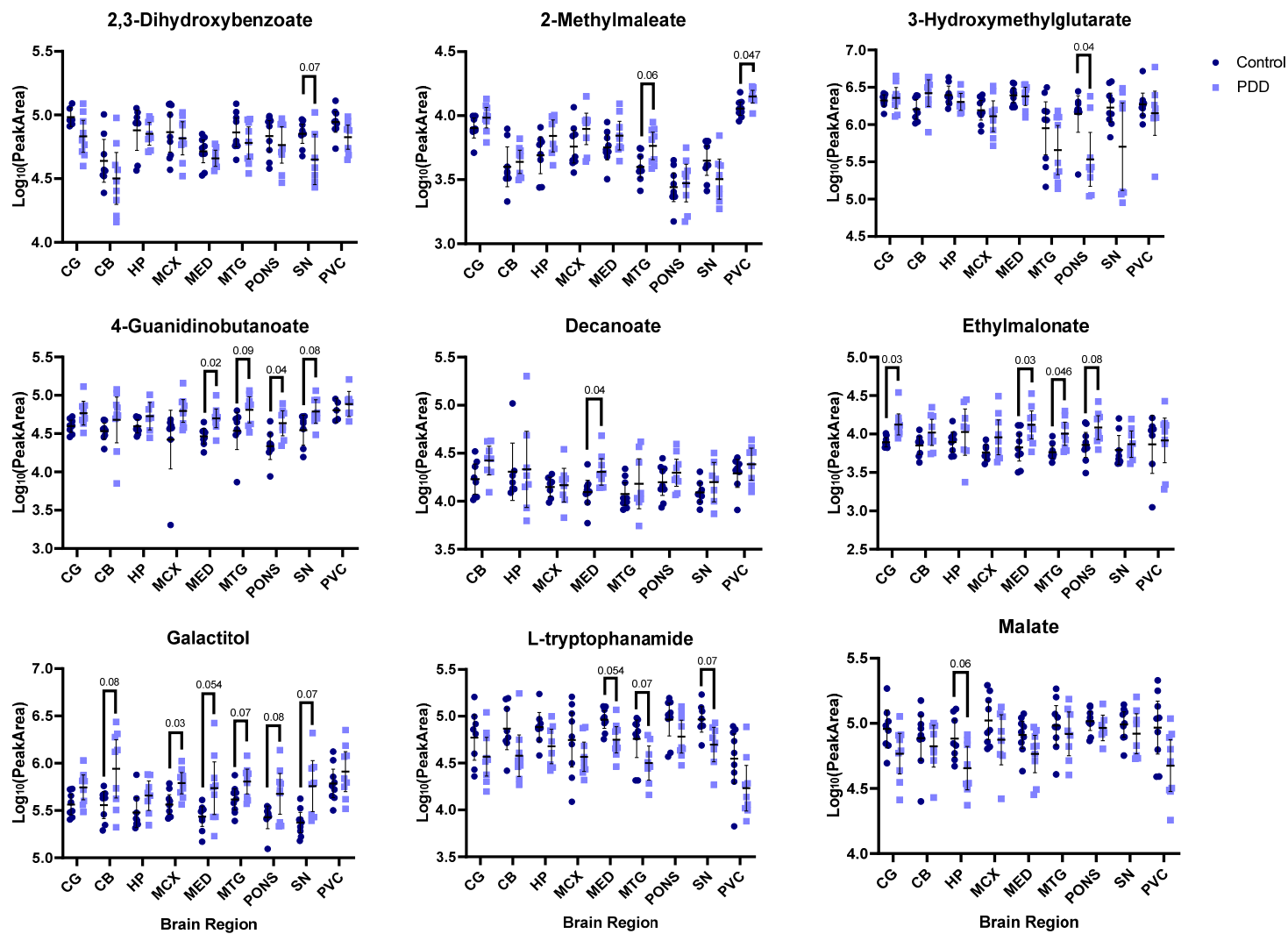
Additional File Figure 3C – Significant Altered Metabolites in PDD Brain: Glucose Metabolism Pathways



Additional File Figure 4D – Significant Altered Metabolites in PDD Brain: Purine Metabolism Pathways



Additional File Figure 5F – Significant Altered Metabolites in PDD Brain: Other Metabolites 1



Additional File Figure 6G – Significant Altered Metabolites in PDD Brain: Other Metabolites 2

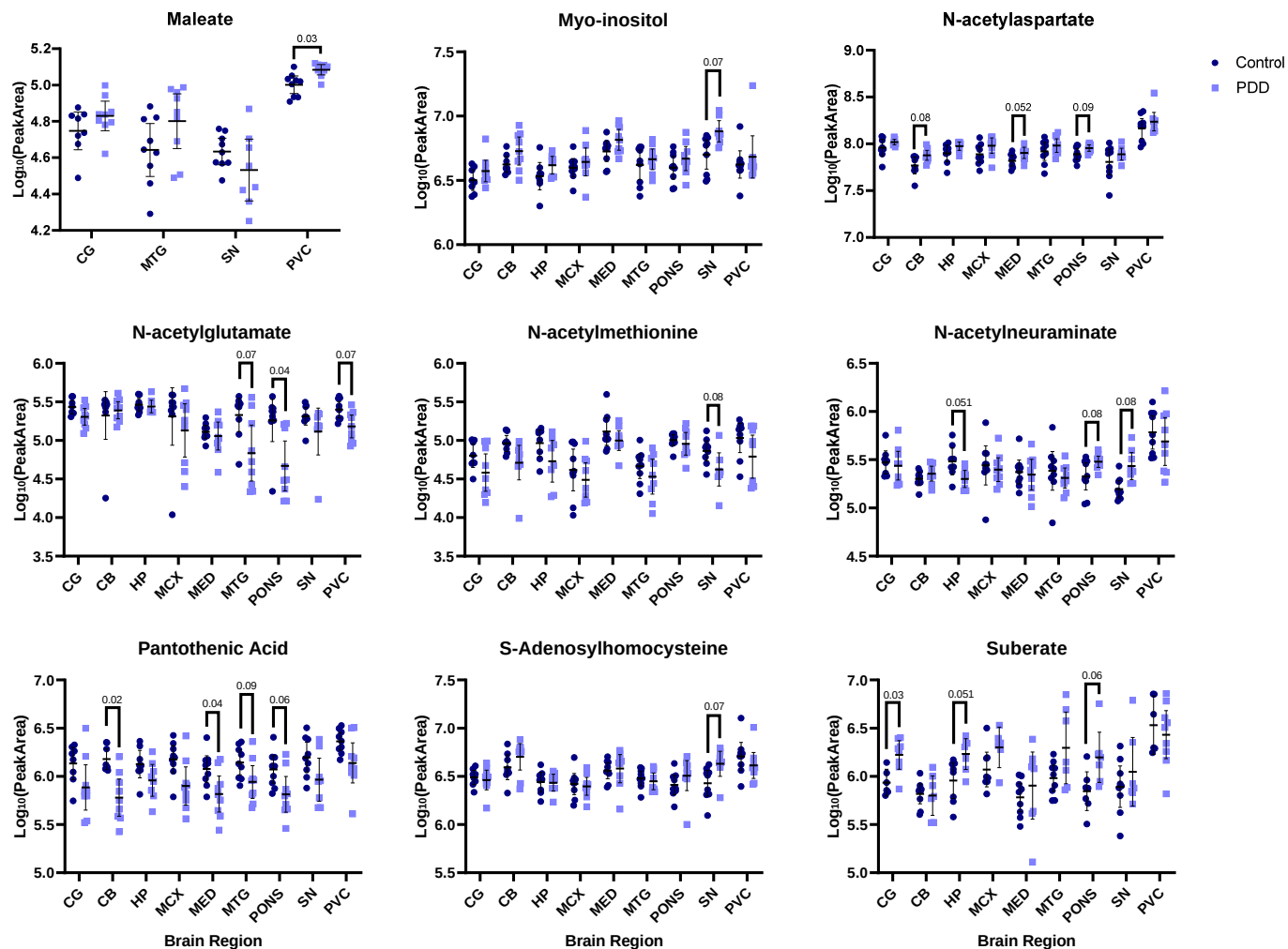
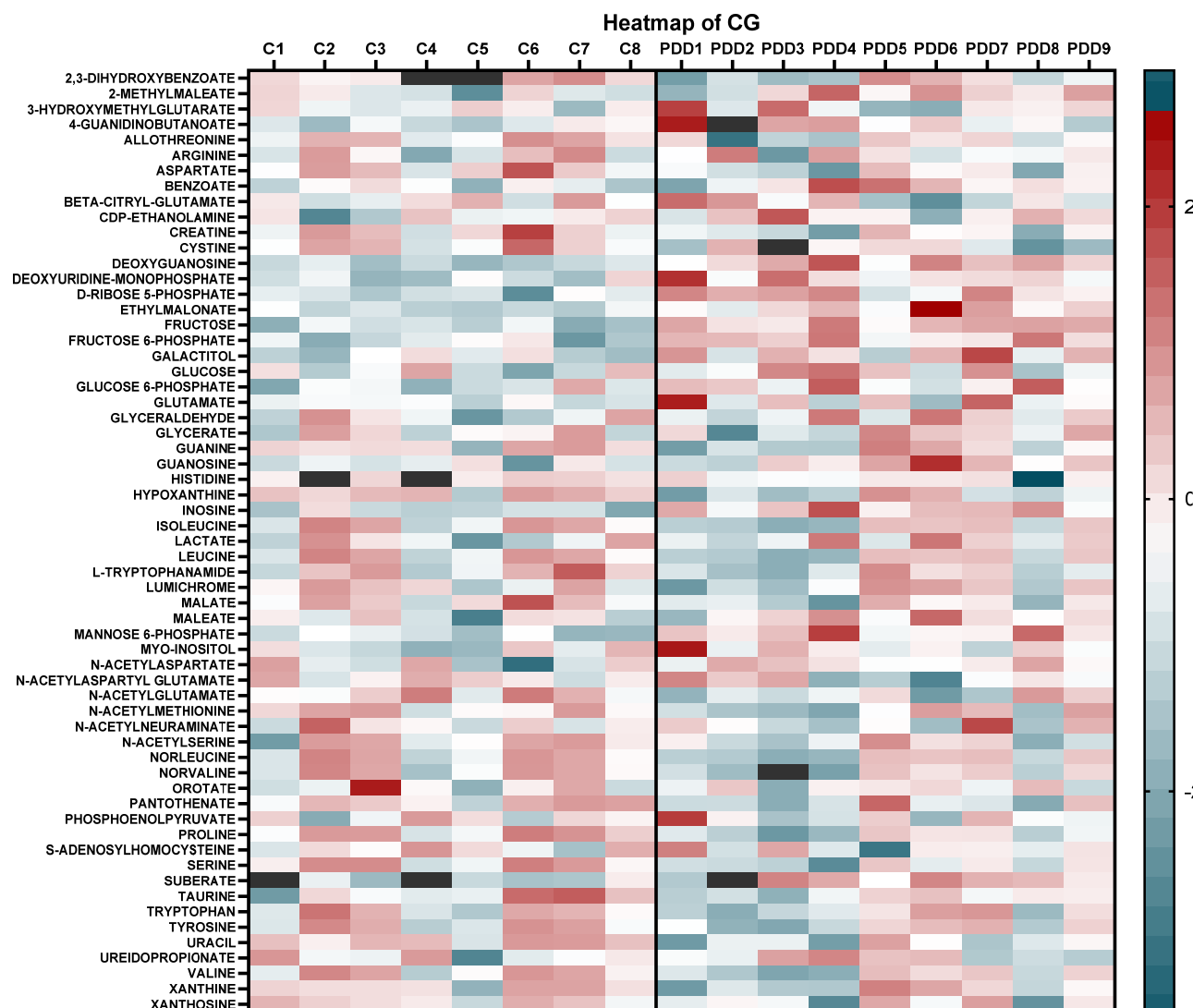


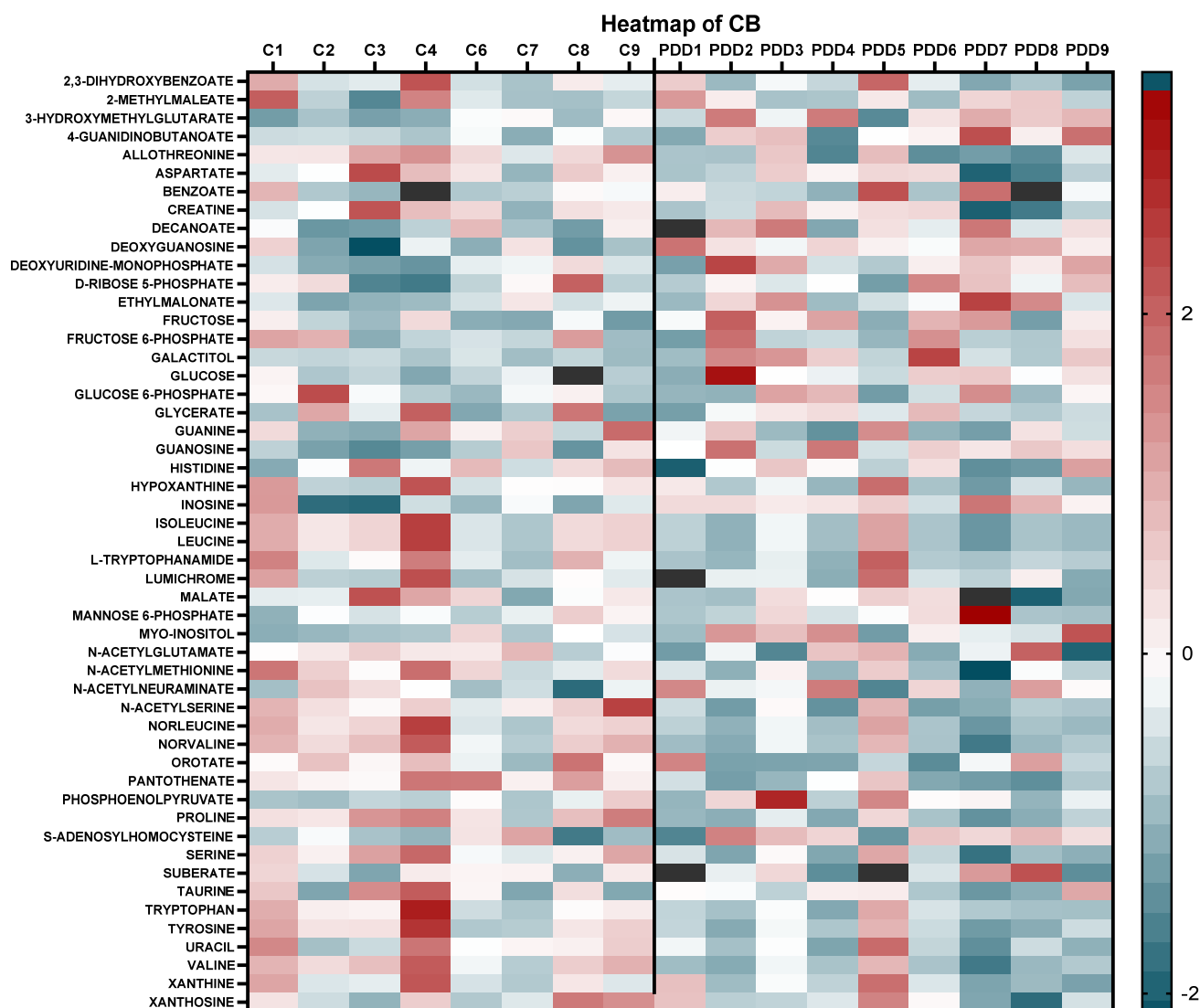
Figure 5A-G. Graphs show $\log_{10}$ peak area data $\pm$ 95% confidence intervals. Q-values are shown for discoveries following multiple t-tests with 10% FDR correction.

Additional File Figure 2A – Heat Map: CG



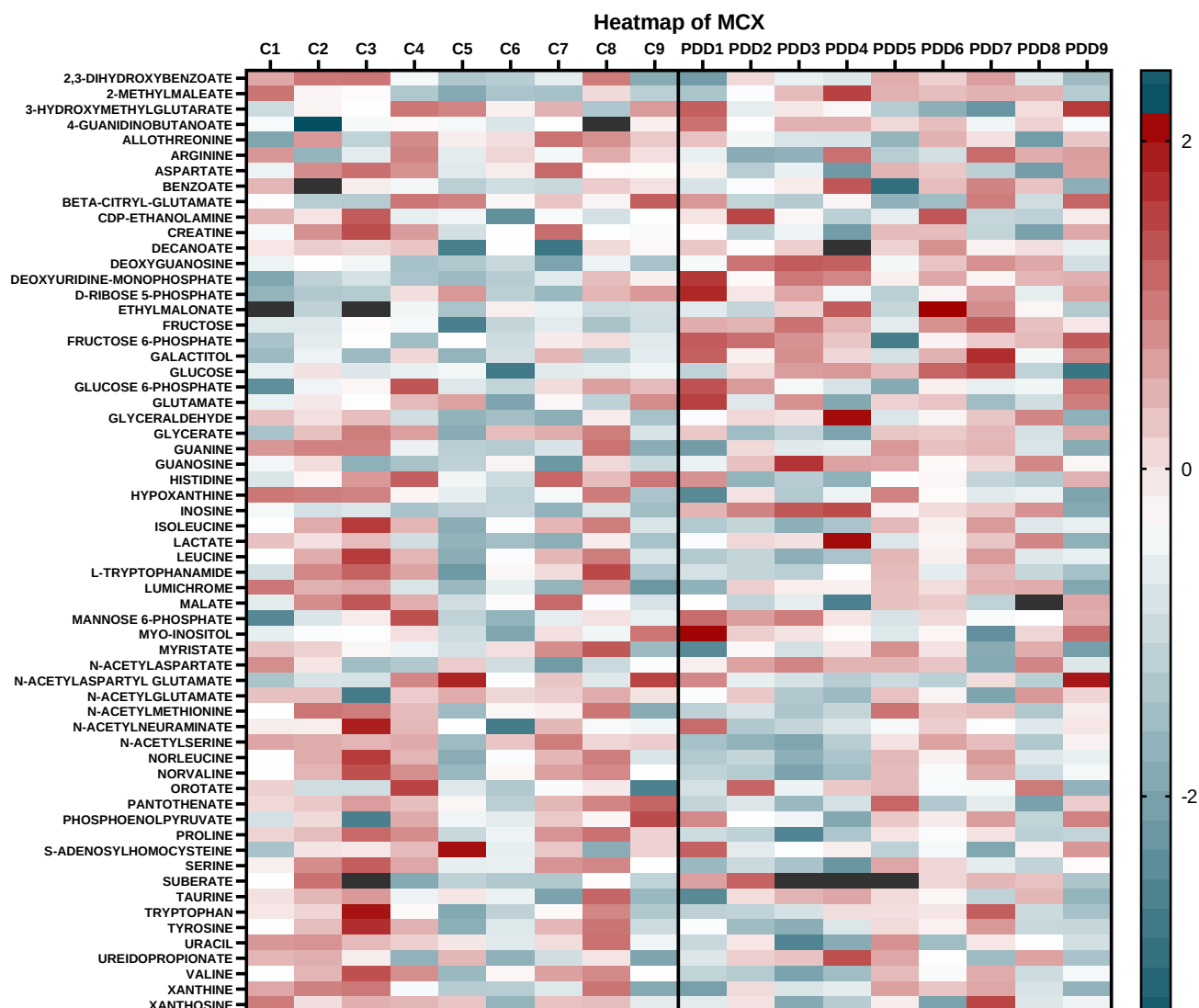
Heat map created using z-score normalised data in GraphPad Prism v9.1.2. All identified metabolites are shown. The darker the colour, the greater the difference in concentration relative to the mean; the red colour denotes increased relative concentrations; the blue colour denotes decreased relative concentrations. Black rectangles denote excluded values. C = control; PDD = Parkinson's disease dementia case.

Additional File Figure 2B – Heat Map: CB



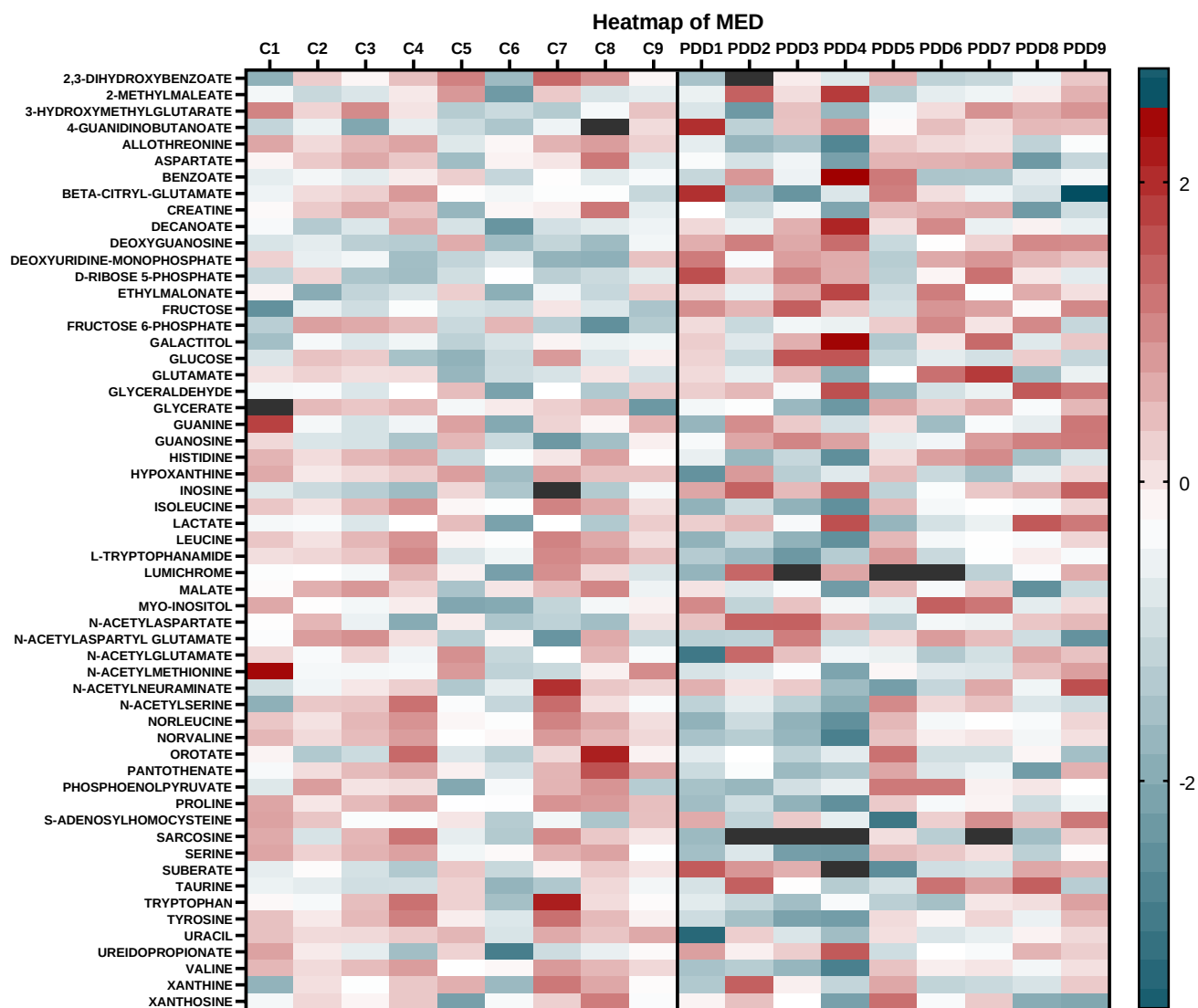
Heat map created using z-score normalised data in GraphPad Prism v9.1.2. All identified metabolites are shown. The darker the colour, the greater the difference in concentration relative to the mean; the red colour denotes increased relative concentrations; the blue colour denotes decreased relative concentrations. Black rectangles denote excluded values. C = control; PDD = Parkinson's disease dementia case.

Additional File Figure 2C – Heat Map: MCX



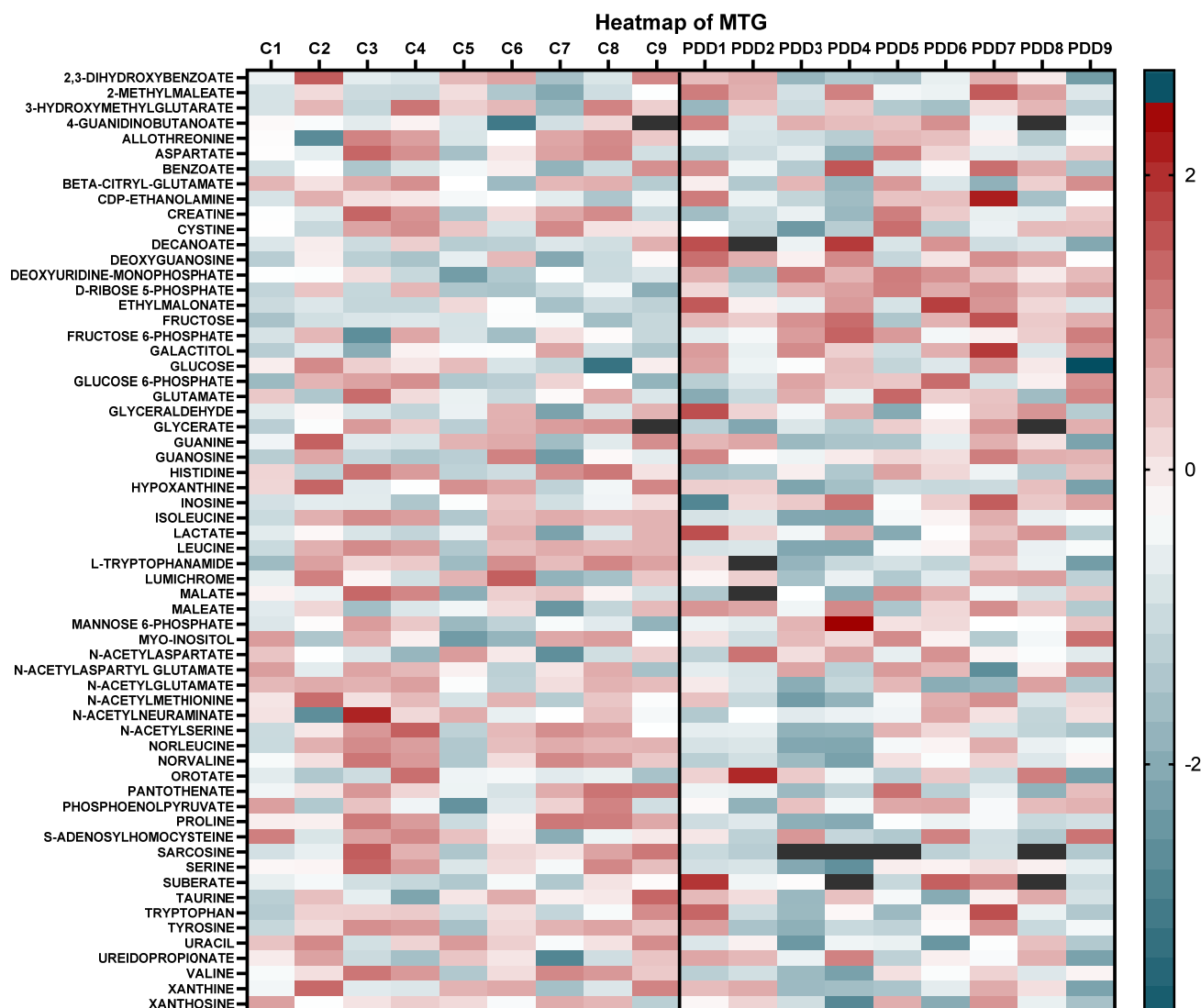
Heat map created using z-score normalised data in GraphPad Prism v9.1.2. All identified metabolites are shown. The darker the colour, the greater the difference in concentration relative to the mean; the red colour denotes increased relative concentrations; the blue colour denotes decreased relative concentrations. Black rectangles denote excluded values. C = control; PDD = Parkinson's disease dementia case.

Additional File Figure 2D – Heat Map: MED



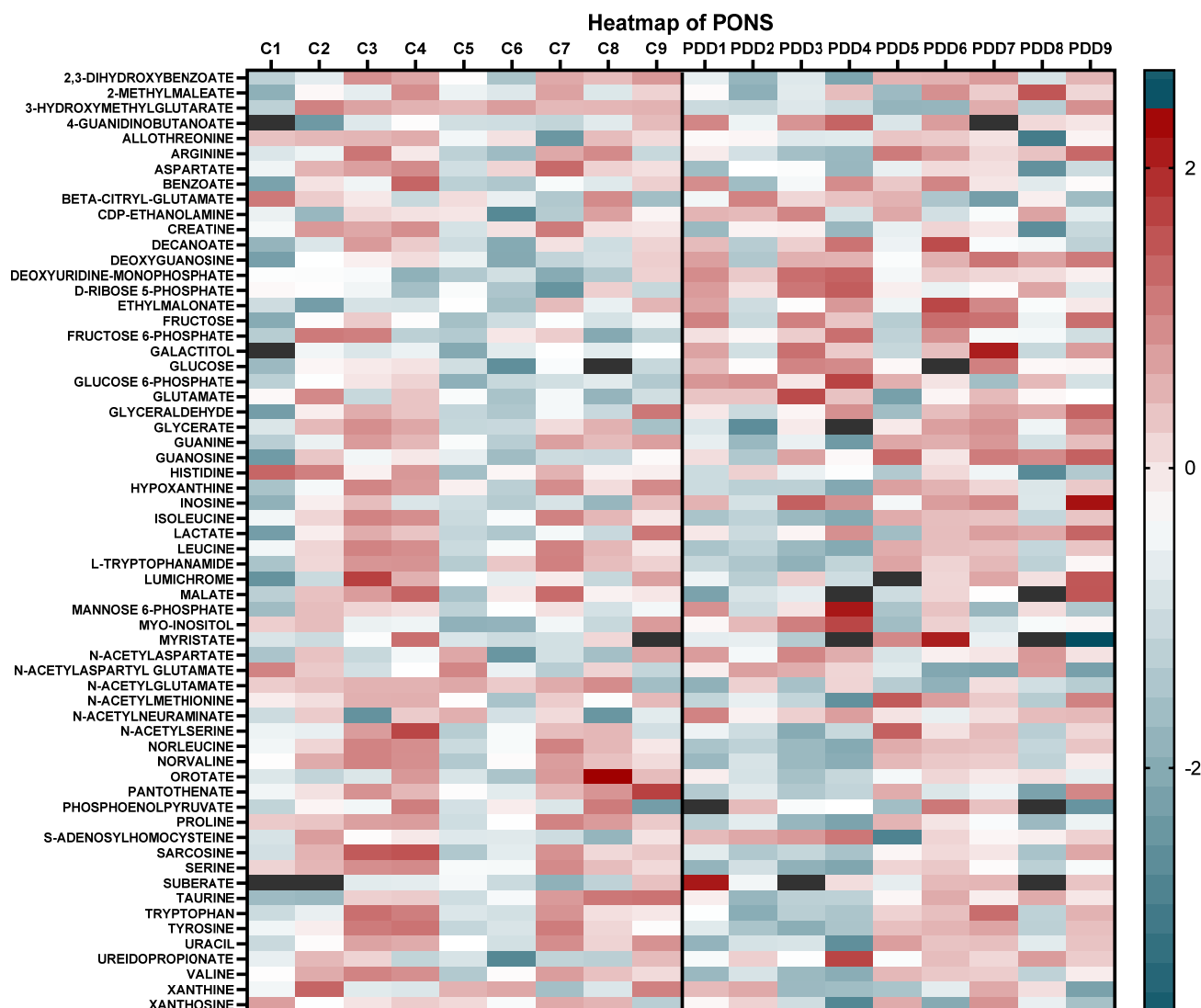
Heat map created using z-score normalised data in GraphPad Prism v9.1.2. All identified metabolites are shown. The darker the colour, the greater the difference in concentration relative to the mean; the red colour denotes increased relative concentrations; the blue colour denotes decreased relative concentrations. Black rectangles denote excluded values. C = control; PDD = Parkinson's disease dementia case.

Additional File Figure 2E – Heat Map: MTG



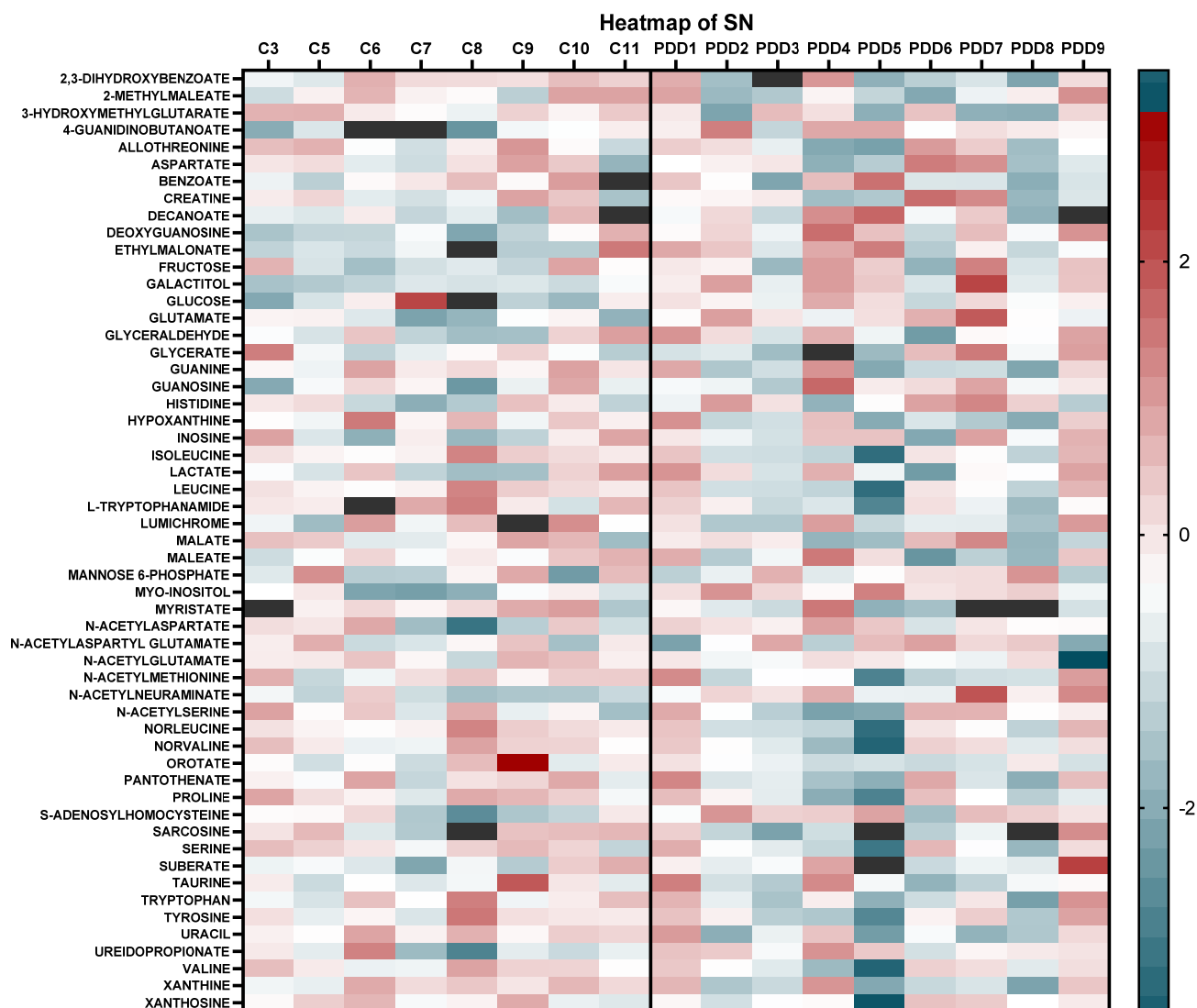
Heat map created using z-score normalised data in GraphPad Prism v9.1.2. All identified metabolites are shown. The darker the colour, the greater the difference in concentration relative to the mean; the red colour denotes increased relative concentrations; the blue colour denotes decreased relative concentrations. Black rectangles denote excluded values. C = control; PDD = Parkinson's disease dementia case.

Additional File Figure 2F – Heat Map: Pons



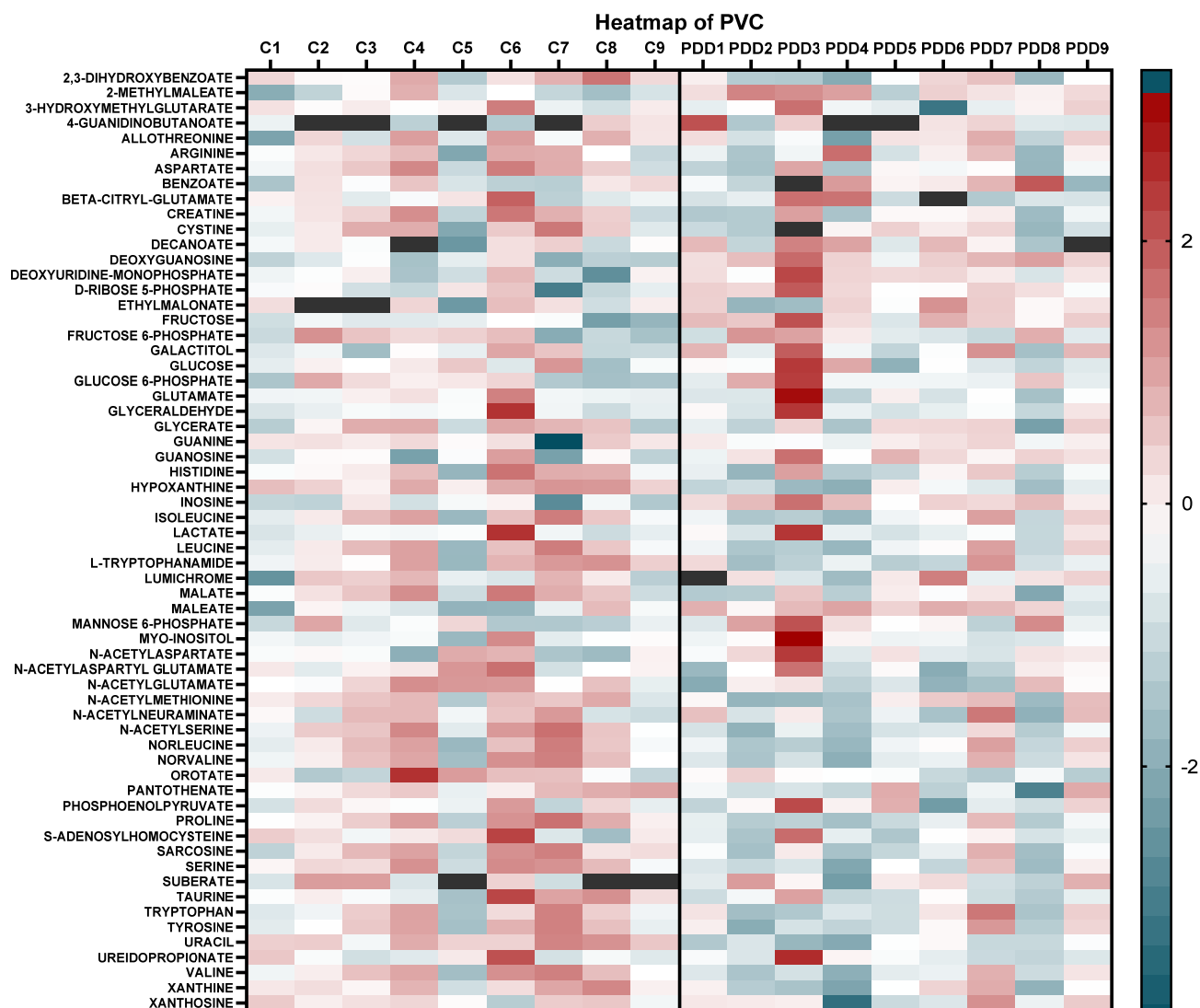
Heat map created using z-score normalised data in GraphPad Prism v9.1.2. All identified metabolites are shown. The darker the colour, the greater the difference in concentration relative to the mean; the red colour denotes increased relative concentrations; the blue colour denotes decreased relative concentrations. Black rectangles denote excluded values. C = control; PDD = Parkinson's disease dementia case.

Additional File Figure 2G – Heat Map: SN



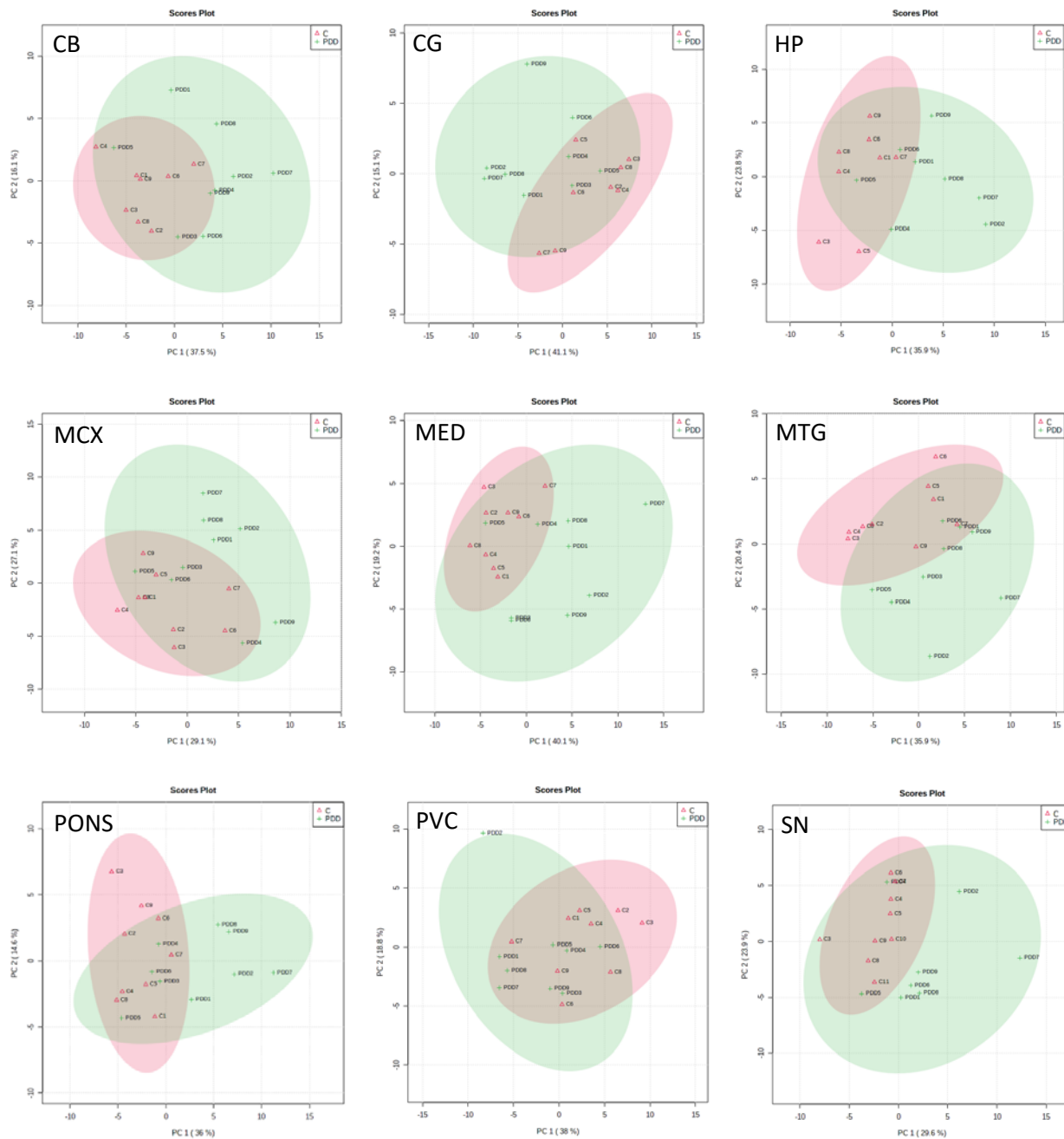
Heat map created using z-score normalised data in GraphPad Prism v9.1.2. All identified metabolites are shown. The darker the colour, the greater the difference in concentration relative to the mean; the red colour denotes increased relative concentrations; the blue colour denotes decreased relative concentrations. Black rectangles denote excluded values. C = control; PDD = Parkinson's disease dementia case.

Additional File Figure 2H – Heat Map: PVC



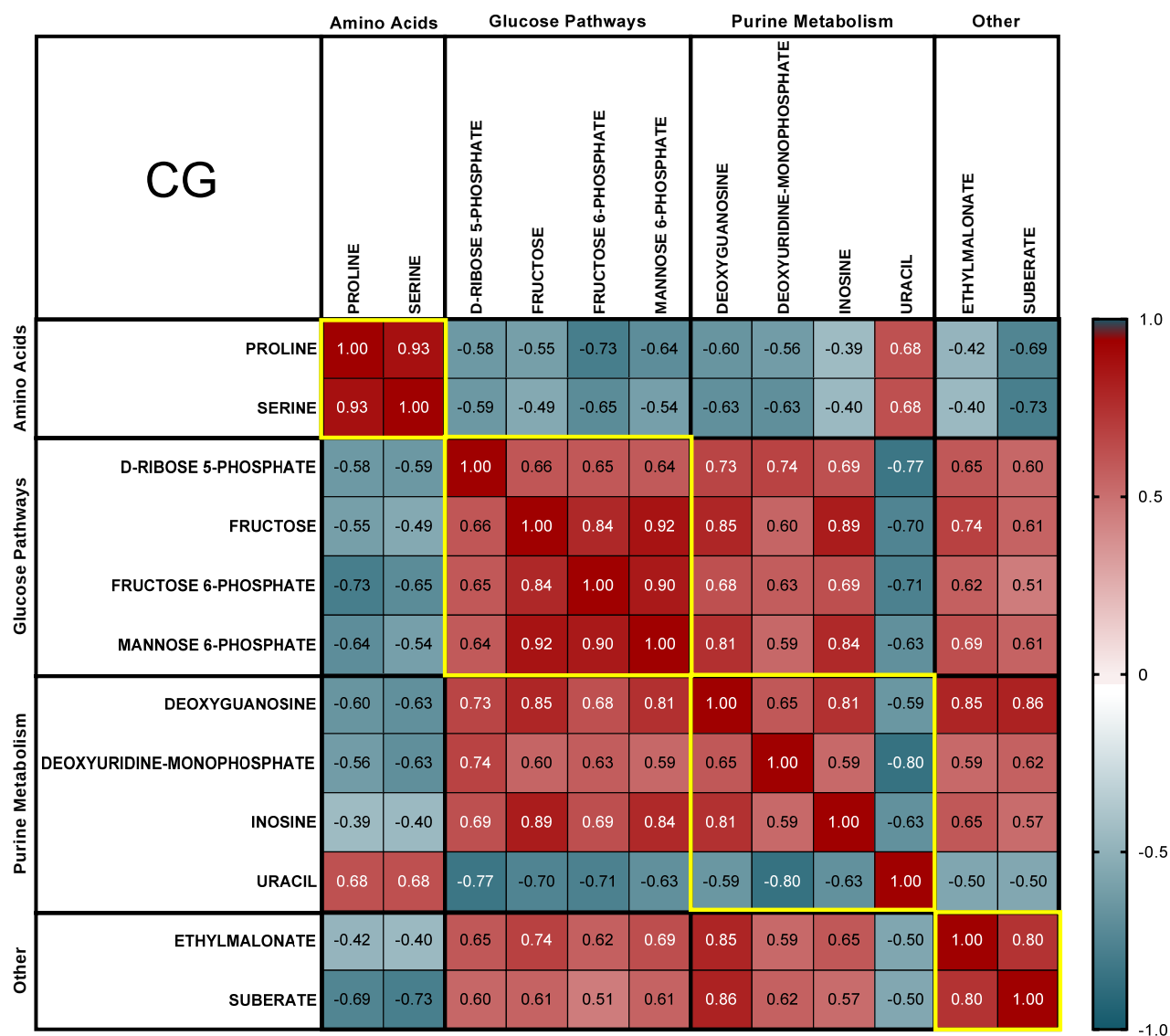
Heat map created using z-score normalised data in GraphPad Prism v9.1.2. All identified metabolites are shown. The darker the colour, the greater the difference in concentration relative to the mean; the red colour denotes increased relative concentrations; the blue colour denotes decreased relative concentrations. Black rectangles denote excluded values. C = control; PDD = Parkinson's disease dementia case.

Additional File Figure 3 – PCA Plots



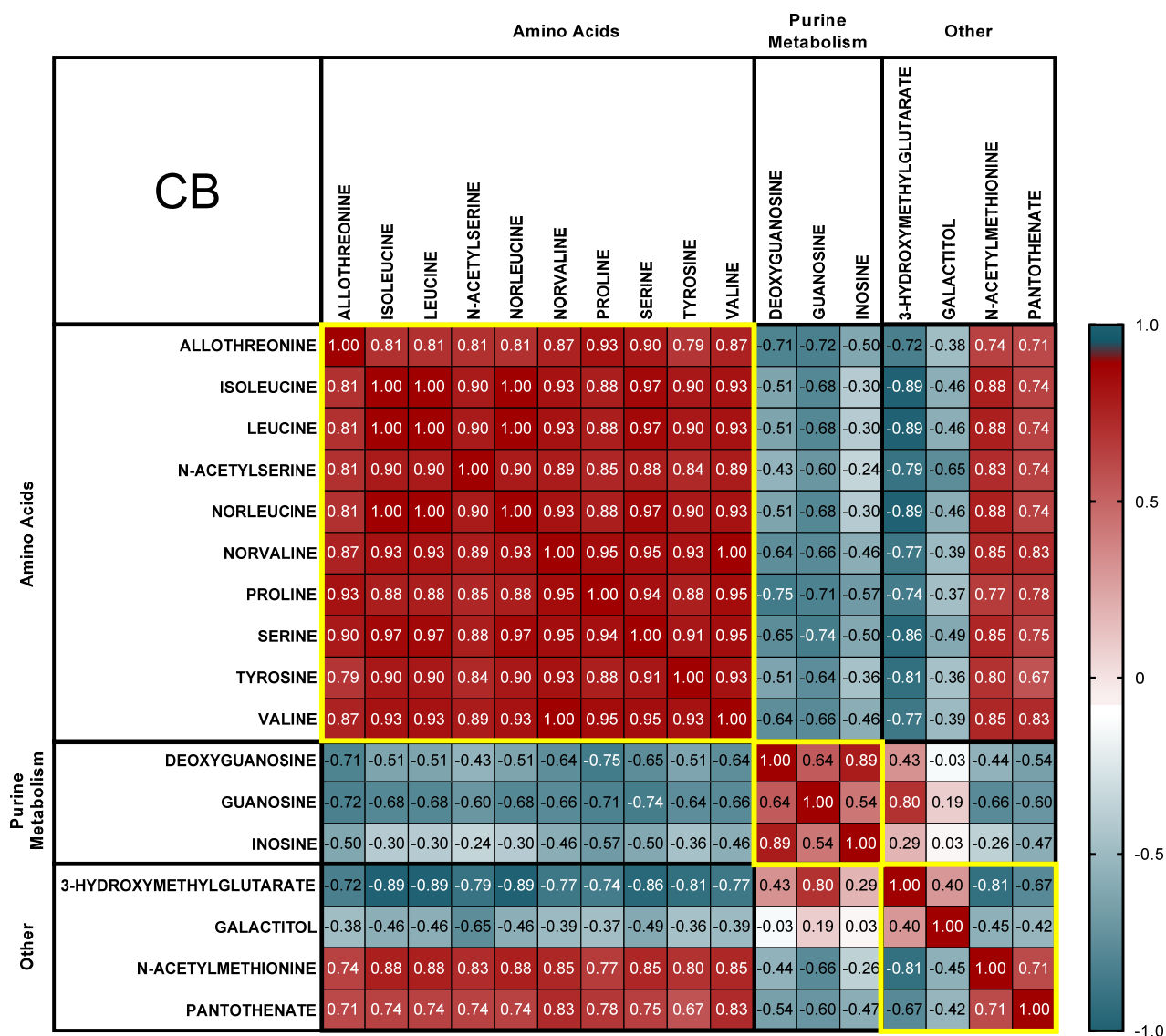
Two-dimensional PCA plots for human dry-weight post-mortem tissues from nine regions of PDD and control brains. Plots show 95% confidence intervals. No visible separation in apparent between cases and controls in any region. C = Control; PDD = Parkinson's disease dementia case.

Additional File Figure 4A – Correlation Matrix: CG



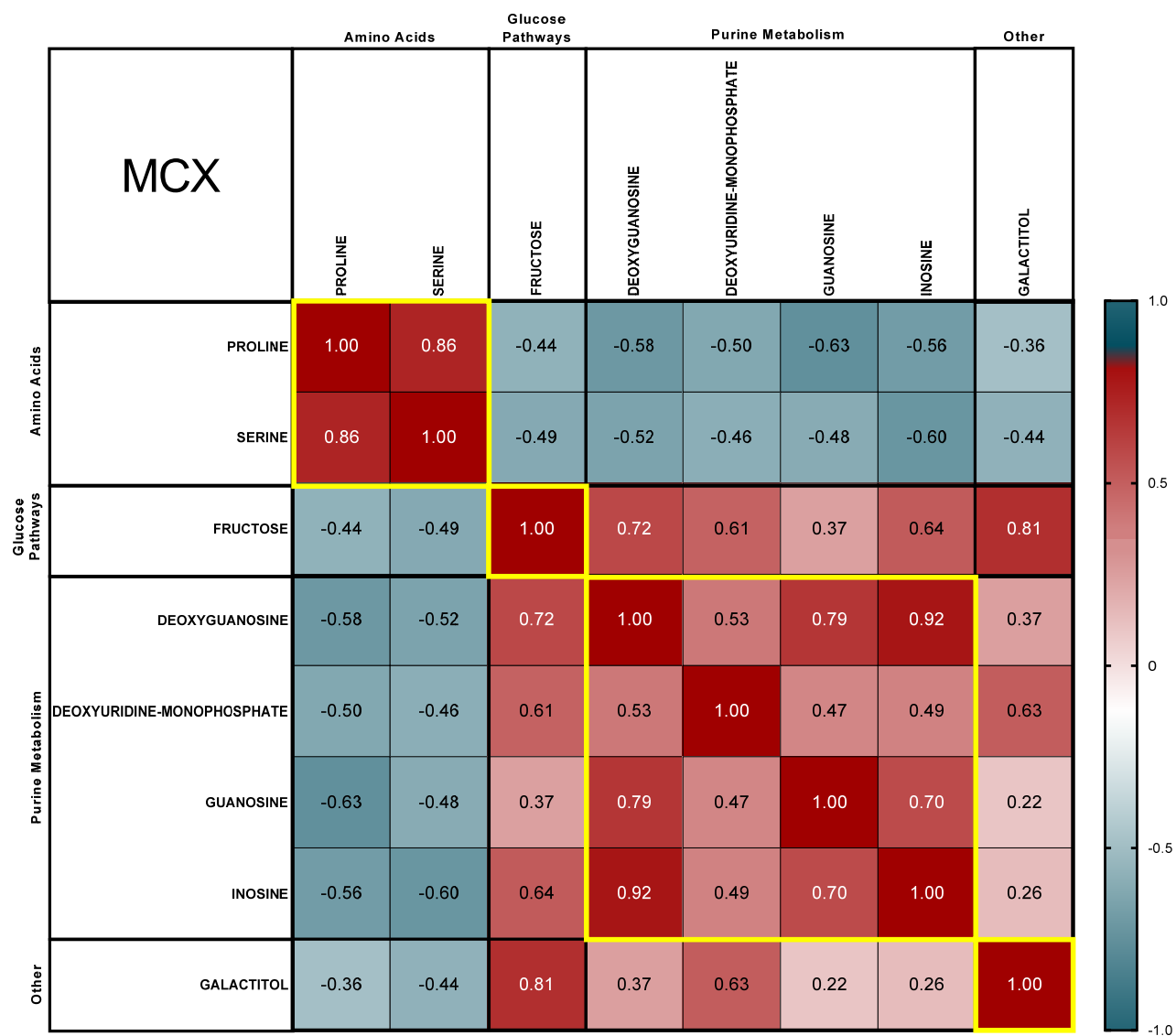
Matrix shows non-parametric Pearson correlation coefficient r values. Correlations were classed as non-significant (r -0.5–0.5; $p > 0.05$), weak ($r = 0.5–0.6$ or -0.5 to -0.6), moderate ($r = 0.6–0.7$ or -0.6 to -0.7), strong ($r = 0.7–0.9$ or -0.7 to -0.9), or very strong ($r > 0.9$ or < -0.9). All correlations of $r = -0.5–0.5$ had a p -value ≥ 0.05 . The red colour denotes positive correlations while blue denotes negative correlations; the darker the colour, the stronger the correlation.

Additional File Figure 4B – Correlation Matrix: CB



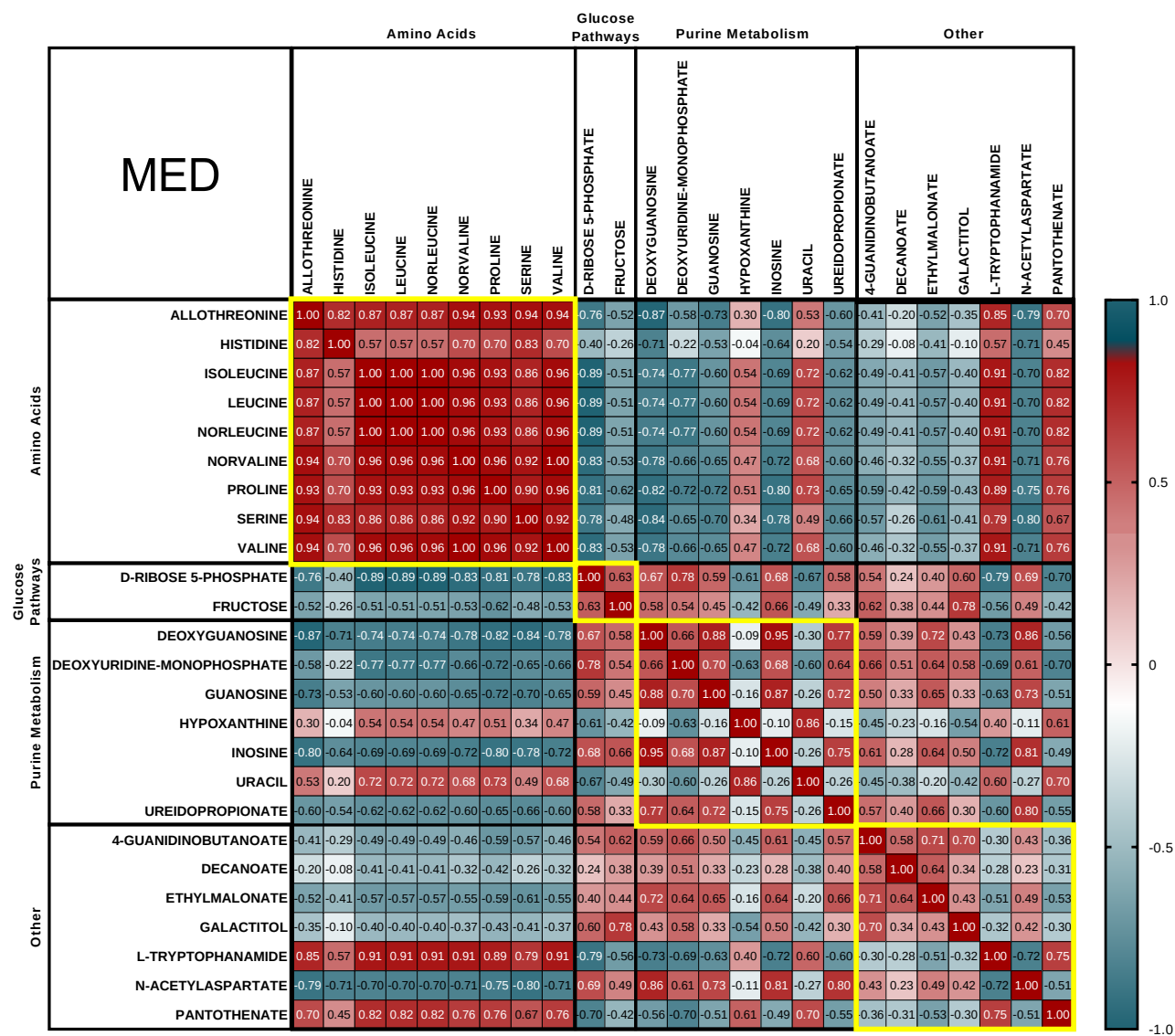
Matrix shows non-parametric Pearson correlation coefficient r values. Correlations were classed as non-significant (r -0.5–0.5; $p > 0.05$), weak ($r = 0.5–0.6$ or -0.5 to -0.6), moderate ($r = 0.6–0.7$ or -0.6 to -0.7), strong ($r = 0.7–0.9$ or -0.7 to -0.9), or very strong ($r > 0.9$ or < -0.9). All correlations of $r = -0.5–0.5$ had a p -value ≥ 0.05 . The red colour denotes positive correlations while blue denotes negative correlations; the darker the colour, the stronger the correlation.

Additional File Figure 4C – Correlation Matrix: MCX



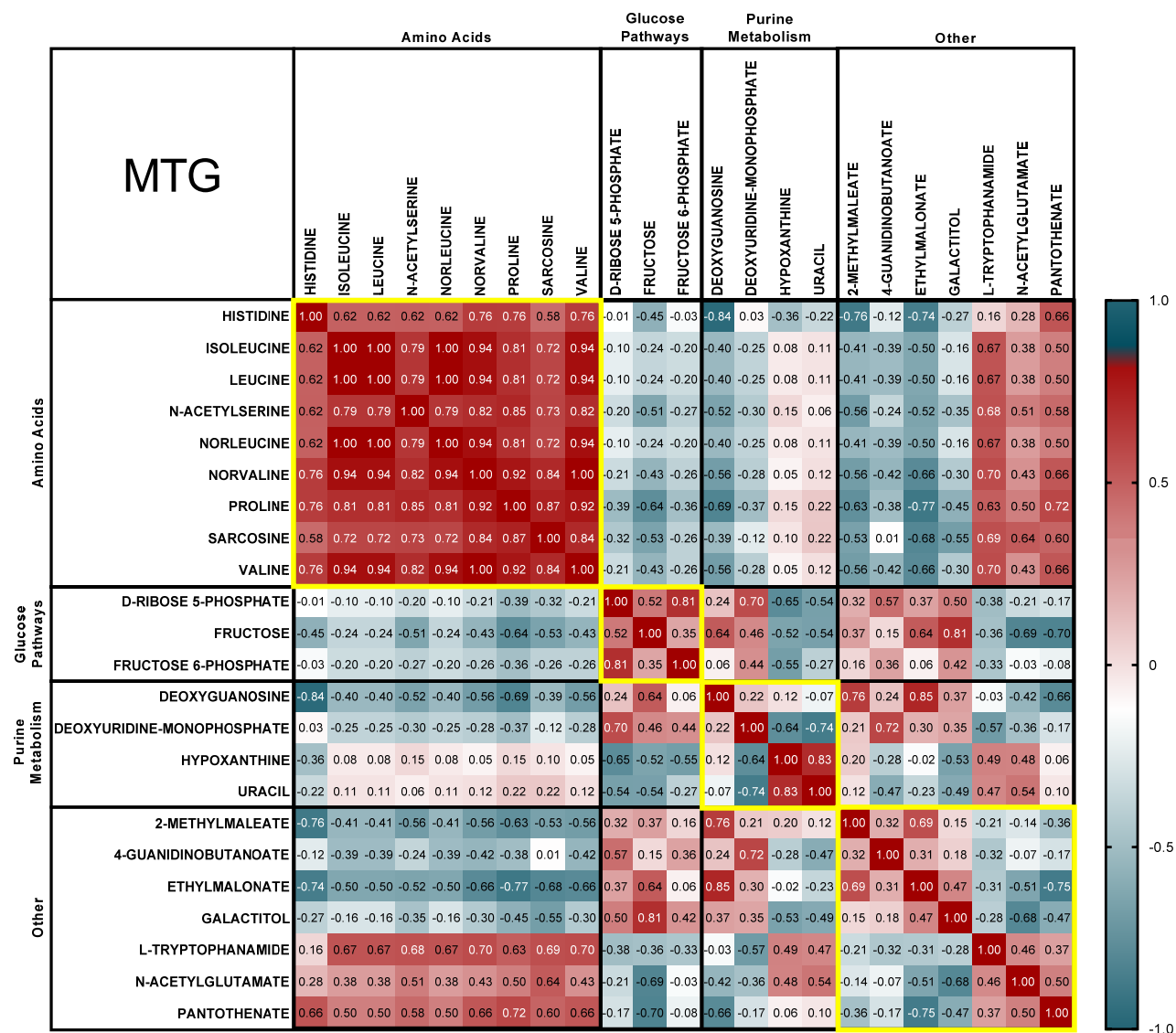
Matrix shows non-parametric Pearson correlation coefficient r values. Correlations were classed as non-significant (r -0.5–0.5; $p > 0.05$), weak ($r = 0.5–0.6$ or -0.5 to -0.6), moderate ($r = 0.6–0.7$ or -0.6 to -0.7), strong ($r = 0.7–0.9$ or -0.7 to -0.9), or very strong ($r > 0.9$ or < -0.9). All correlations of $r = -0.5–0.5$ had a p -value ≥ 0.05 . The red colour denotes positive correlations while blue denotes negative correlations; the darker the colour, the stronger the correlation.

Additional File Figure 4D – Correlation Matrix: MED



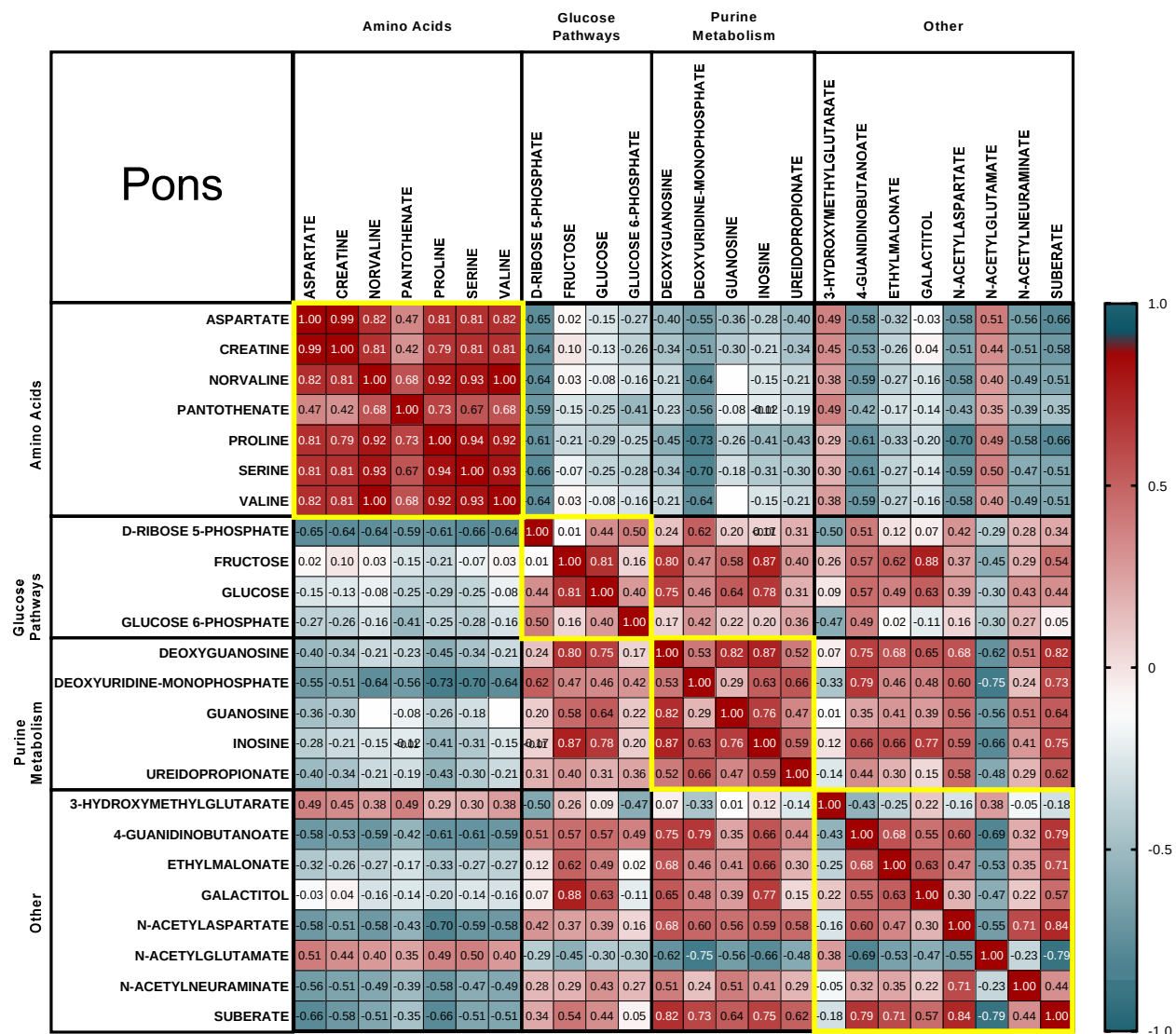
Matrix shows non-parametric Pearson correlation coefficient r values. Correlations were classed as non-significant (r -0.5–0.5; $p > 0.05$), weak ($r = 0.5–0.6$ or -0.5 to -0.6), moderate ($r = 0.6–0.7$ or -0.6 to -0.7), strong ($r = 0.7–0.9$ or -0.7 to -0.9), or very strong ($r > 0.9$ or < -0.9). All correlations of $r = -0.5–0.5$ had a p -value ≥ 0.05 . The red colour denotes positive correlations while blue denotes negative correlations; the darker the colour, the stronger the correlation.

Additional File Figure 4E – Correlation Matrix: MTG



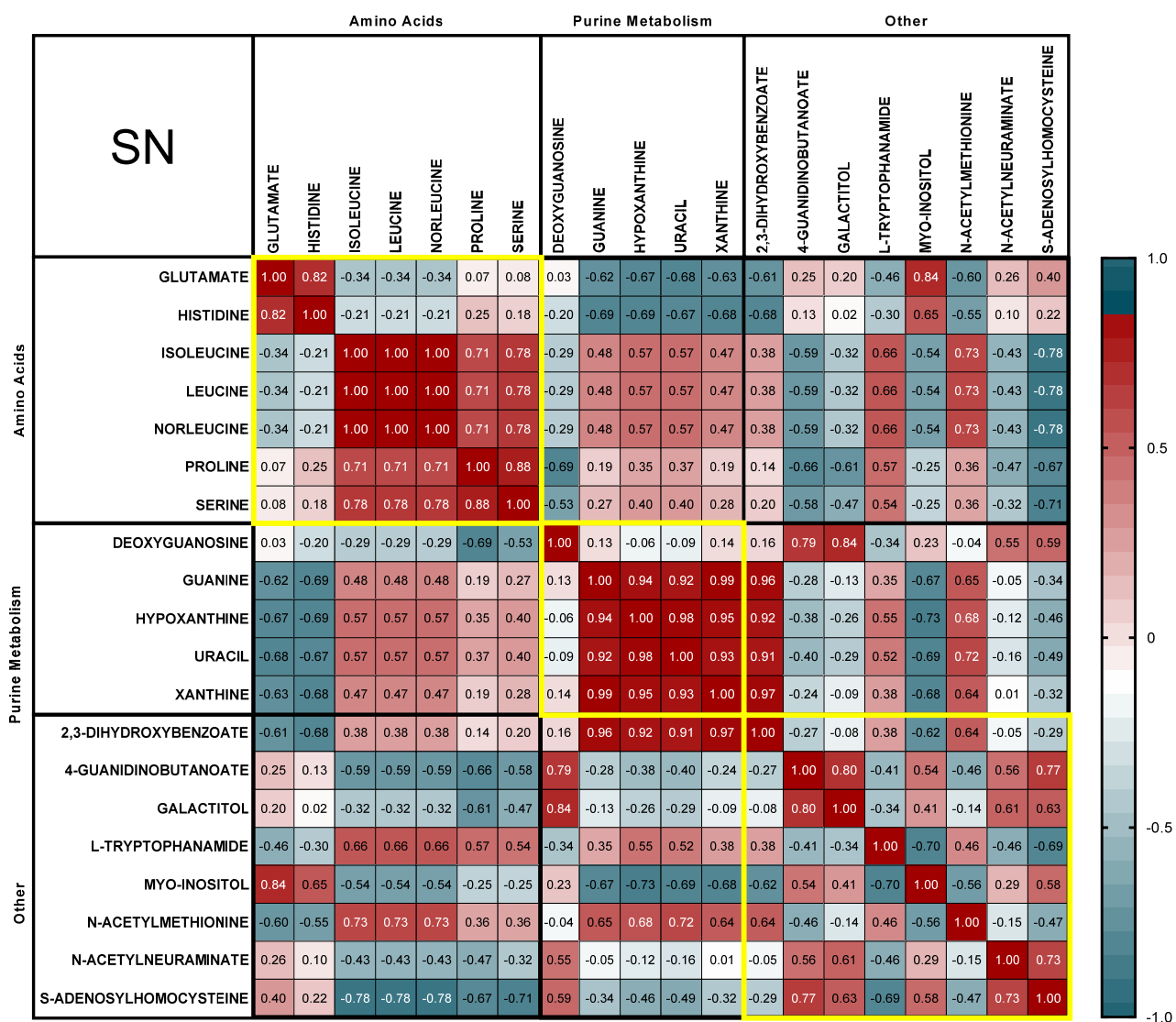
Matrix shows non-parametric Pearson correlation coefficient r values. Correlations were classed as non-significant (r -0.5–0.5; $p > 0.05$), weak ($r = 0.5–0.6$ or -0.5 to -0.6), moderate ($r = 0.6–0.7$ or -0.6 to -0.7), strong ($r = 0.7–0.9$ or -0.7 to -0.9), or very strong ($r > 0.9$ or < -0.9). All correlations of $r = -0.5–0.5$ had a p -value ≥ 0.05 . The red colour denotes positive correlations while blue denotes negative correlations; the darker the colour, the stronger the correlation.

Additional File Figure 4F – Correlation Matrix: Pons



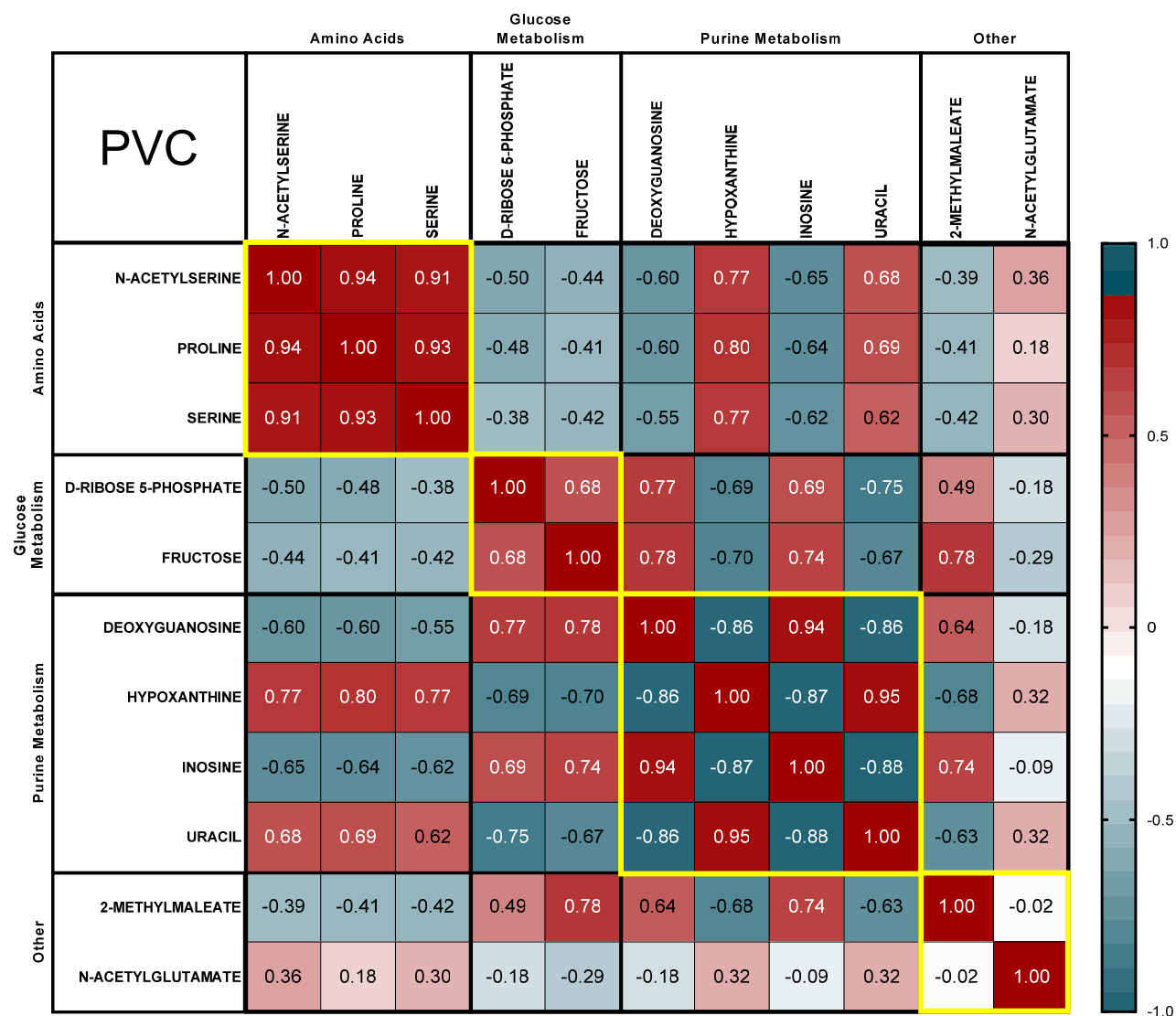
Matrix shows non-parametric Pearson correlation coefficient r values. Correlations were classed as non-significant (r -0.5–0.5; $p > 0.05$), weak ($r = 0.5–0.6$ or -0.5 to -0.6), moderate ($r = 0.6–0.7$ or -0.6 to -0.7), strong ($r = 0.7–0.9$ or -0.7 to -0.9), or very strong ($r > 0.9$ or < -0.9). All correlations of $r = -0.5–0.5$ had a p -value ≥ 0.05 . The red colour denotes positive correlations while blue denotes negative correlations; the darker the colour, the stronger the correlation.

Additional File Figure 4G – Correlation Matrix: SN



Matrix shows non-parametric Pearson correlation coefficient r values. Correlations were classed as non-significant (r -0.5–0.5; $p > 0.05$), weak ($r = 0.5–0.6$ or -0.5 to -0.6), moderate ($r = 0.6–0.7$ or -0.6 to -0.7), strong ($r = 0.7–0.9$ or -0.7 to -0.9), or very strong ($r > 0.9$ or < -0.9). All correlations of $r = -0.5–0.5$ had a p -value ≥ 0.05 . The red colour denotes positive correlations while blue denotes negative correlations; the darker the colour, the stronger the correlation.

Additional File Figure 4H – Correlation Matrix: PVC



Matrix shows non-parametric Pearson correlation coefficient r values. Correlations were classed as non-significant (r -0.5–0.5; $p > 0.05$), weak ($r = 0.5–0.6$ or -0.5 to -0.6), moderate ($r = 0.6–0.7$ or -0.6 to -0.7), strong ($r = 0.7–0.9$ or -0.7 to -0.9), or very strong ($r > 0.9$ or < -0.9). All correlations of $r = -0.5–0.5$ had a p -value ≥ 0.05 . The red colour denotes positive correlations while blue denotes negative correlations; the darker the colour, the stronger the correlation.