Single-cell metabolomics: changes in the metabolome of freshly isolated and cultured neurons

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Supporting Information

Neuron identification

In this study, single cells of the *Aplysia californica* CNS were identified based on earlier work[1] describing the location and physical appearance of neurons. For example, a metacerebral cell (MCC) was located in the cerebral ganglion, had a cell body of ~150–200 µm in diameter, and was dark yellow. Likewise, 100–150 µm diameter B1 and B2 neurons were readily identifiable in the buccal ganglion. Figure S1 presents a representative microscopy image of the appropriate ganglion, with arrows pointing out each cell type.

Multivariate data analysis

Unsupervised PCA helped to appreciate chemical differences among neurons. Loading plots corresponding to the score plots of Figure 2 are presented in Figure S2.

Statistical analysis

Variations in signal ion abundances reached statistical significance on the basis of 35 identified small molecules (see Table 1). Peak areas were determined in *m/z*-selected electropherograms and evaluated pairwise for ASW- and glycerol-treated MCC cells and between freshly isolated and cultured B1 and B2 neurons. Calculated statistical medians, errors, and confidence intervals are noted in Figures S3 and S4.

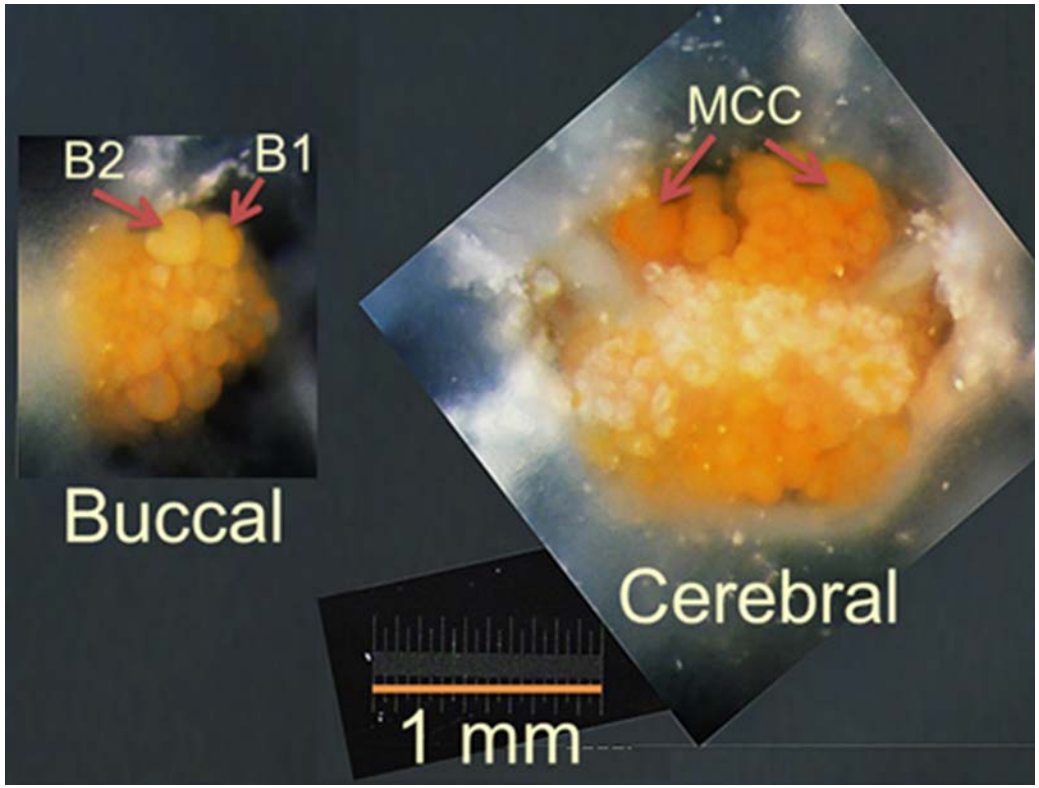


Figure S1. Optical microscopy-aided neuron identification in the *A. californica* CNS. Arrows highlight MCC neurons in the left and right hemiganglia as well as B1 and B2 neurons in the left buccal ganglion. The right buccal ganglion containing other B1 and B2 neurons is not shown. Scale bar = 1 mm.

[1] Kupfermann, I., Carew, T. J., and Kandel, E. R. (1974) Local, reflex, and central commands controlling gill and siphon movements in Aplysia. *J. Neurophysiol.* 37, 996-1019.

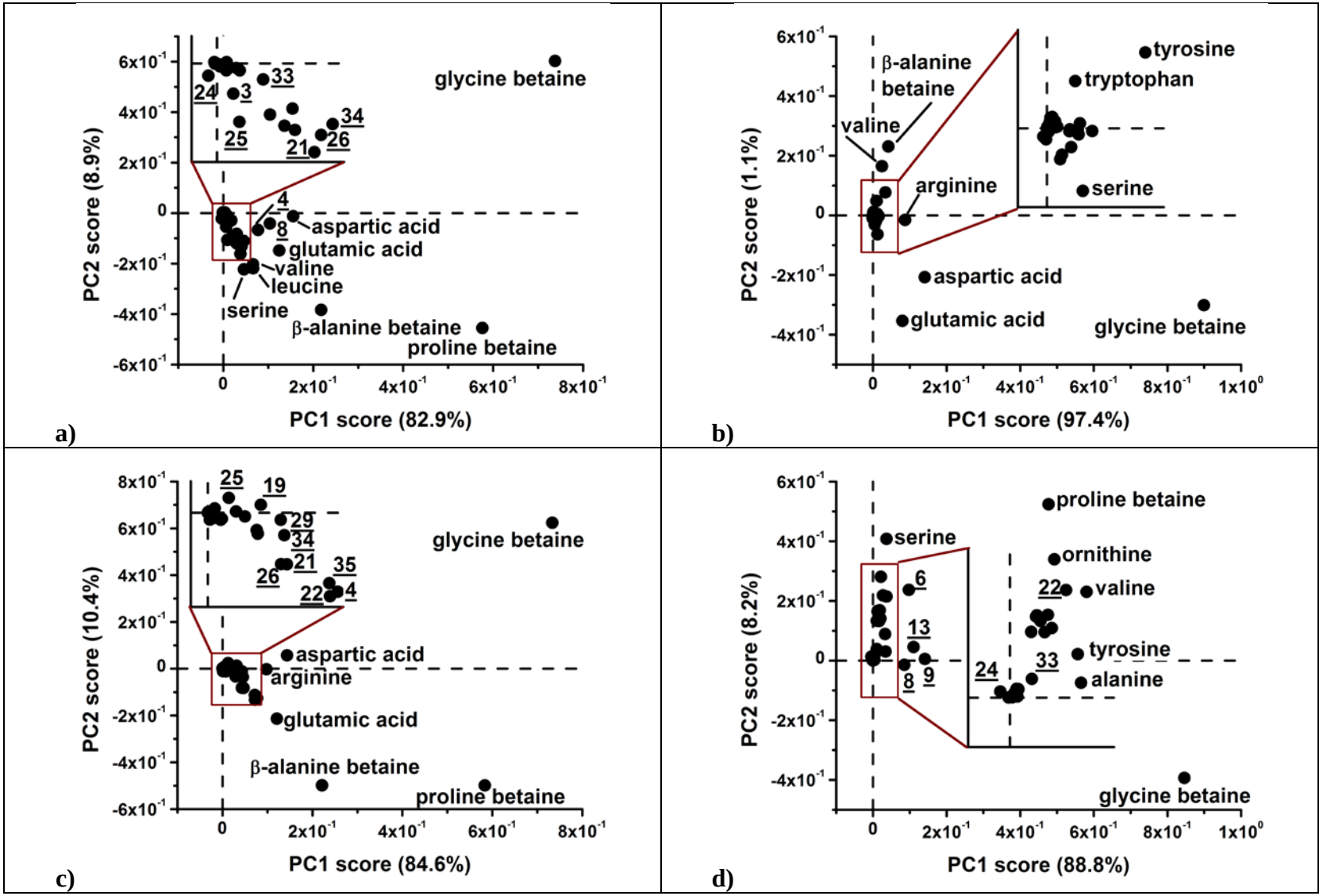


Figure S2. PCA loading plots helped to gauge the individual metabolite contributions in explaining the differences among extracts of **(a)** freshly isolated B1 and B2 neurons, **(b)** cultured B1 and cultured B2 neurons, **(c)** freshly isolated B1 and cultured B1 neurons, and **(d)** freshly isolated B2 and cultured B2 neurons. The corresponding PCA score plots are presented in Figure 2. Underlined numbers correspond to metabolites identified in Table 1.

Figure S3. Statistical comparison of 35 metabolite ion abundances between freshly isolated B1 (B1) and cultured B1 (cB1) neuron extracts. Key: square, box, and whisker represent calculated median, standard error, and 1.96 × standard error, respectively. Independent axis marks the compared neuron types. Dependent axis shows the ion signal area integrated in the *m/z*-selected electropherogram corresponding to the compound of interest.

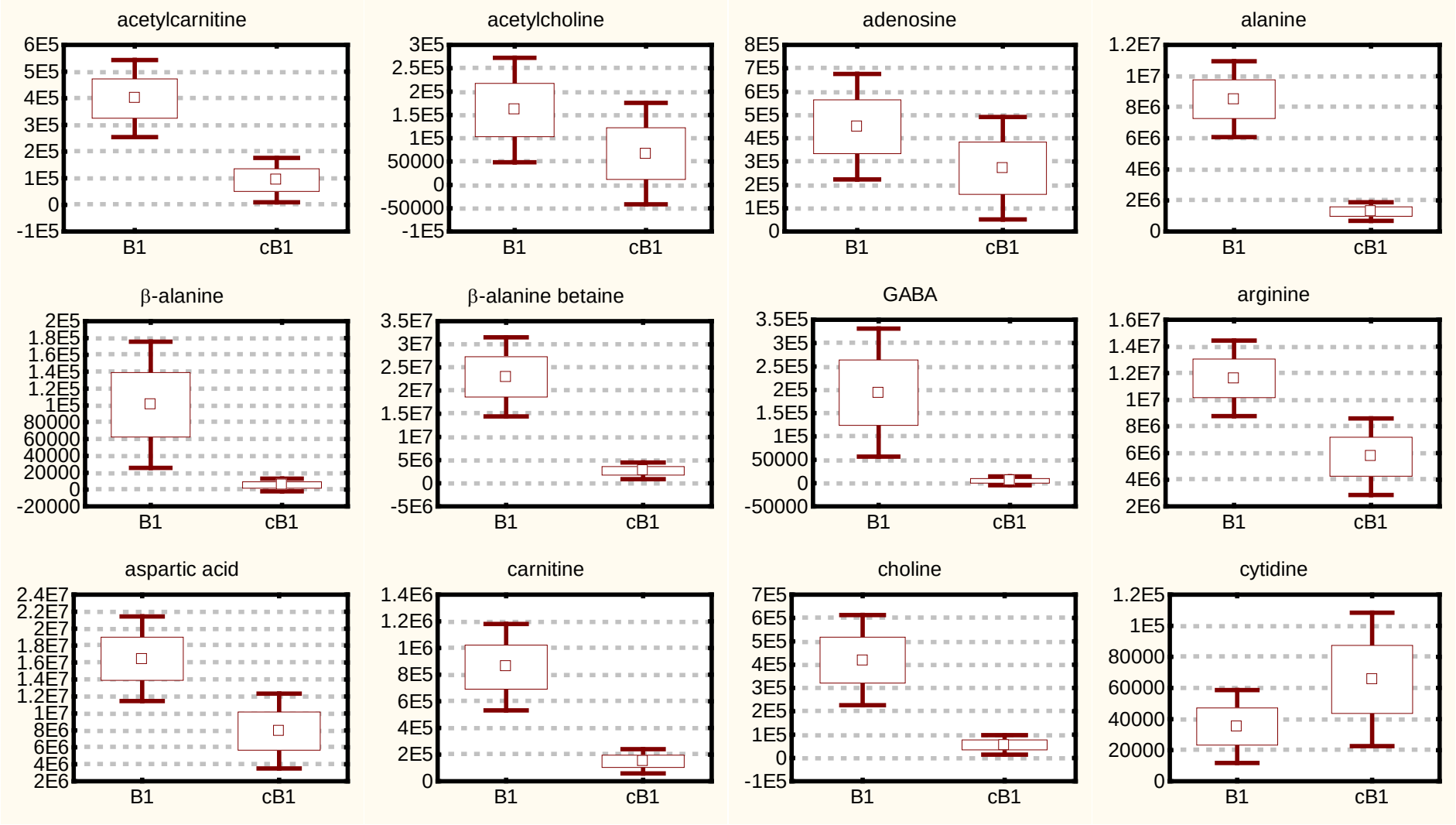


Figure S3 continued…

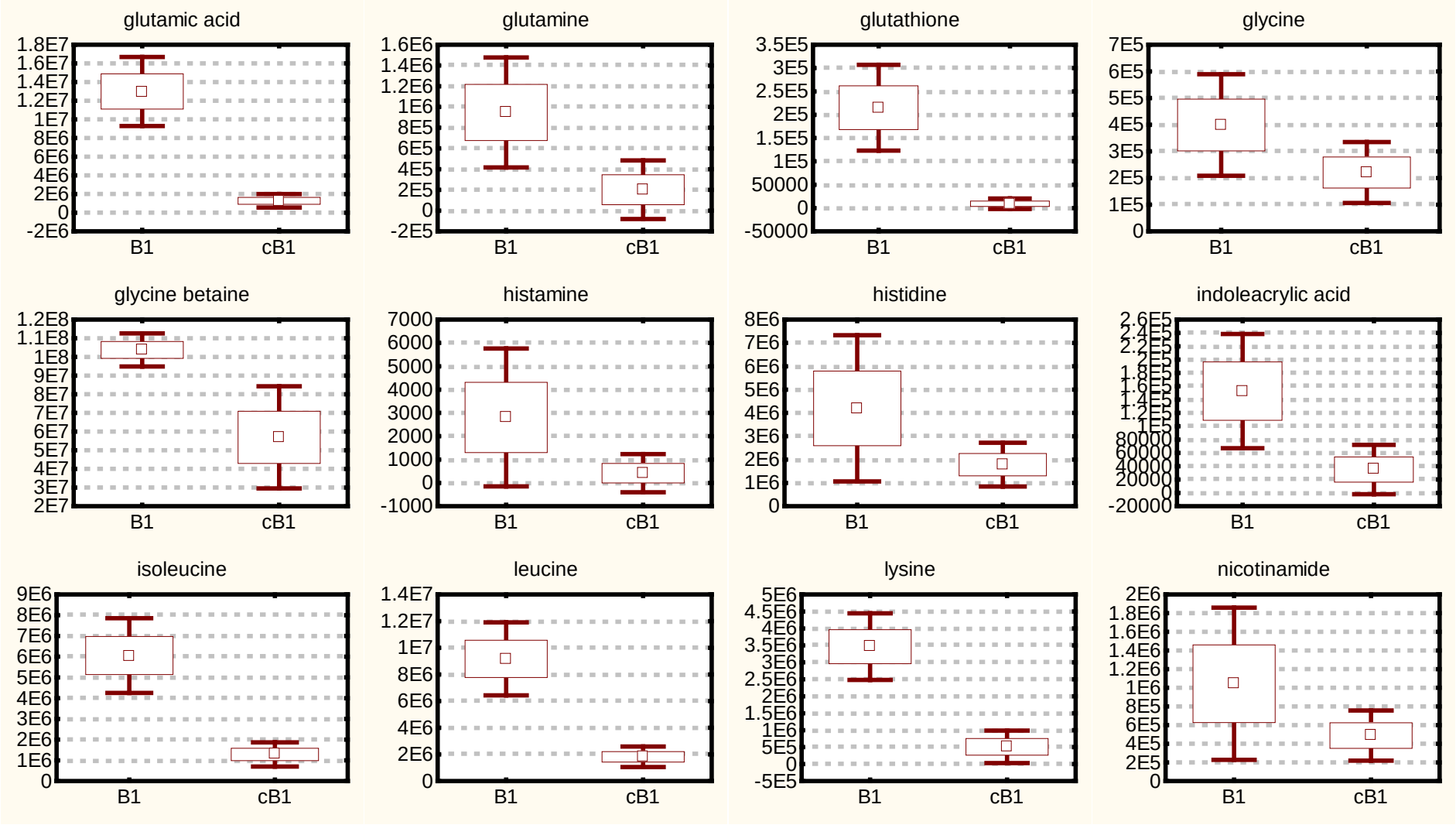
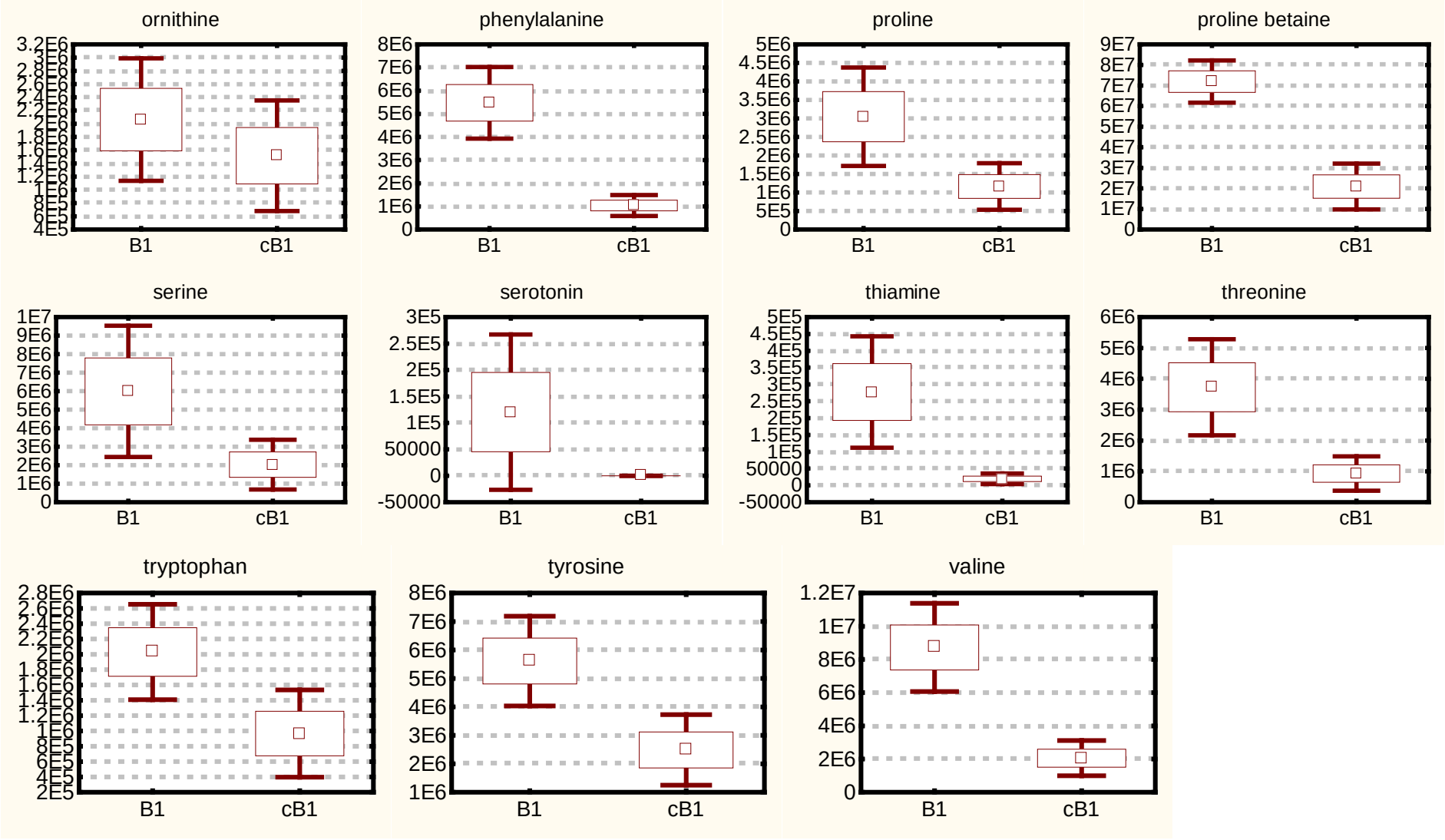


Figure S3 continued…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2.6E6
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2.2E6
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1E6
8E5
6E5
4E5
B1
cB1
phenylalanine
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7E6
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5E6
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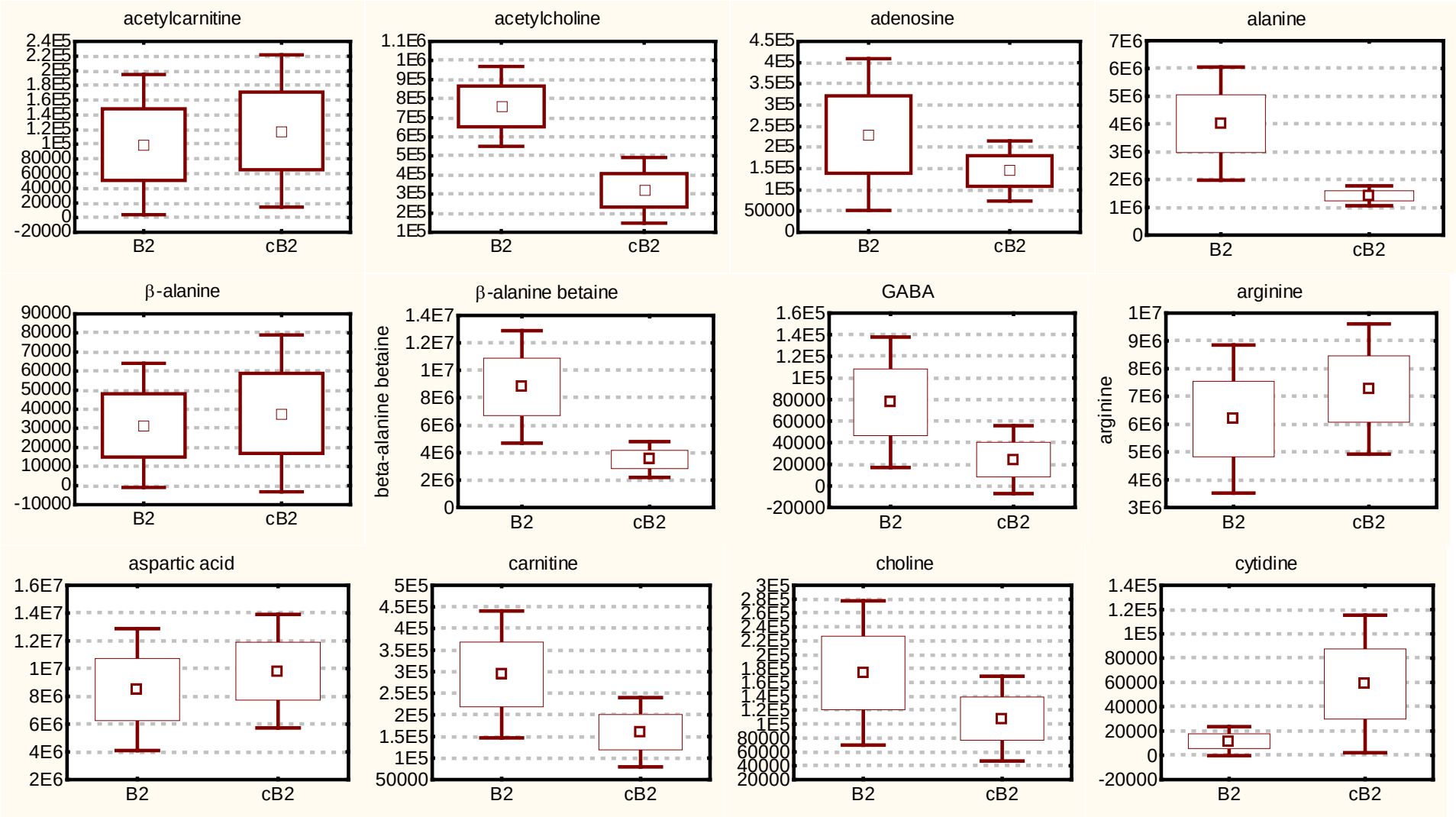
Figure S4. Statistical comparison of 35 metabolite ion abundances between freshly isolated B2 (B2) and cultured B2 (cB2) neuron extracts. Key: square, box, and whisker represent calculated median, standard error, and 1.96 × standard error, respectively. Independent axis marks the compared neuron types. Dependent axis shows the ion signal area integrated in the *m/z*-selected electropherogram corresponding to the compound of interest.



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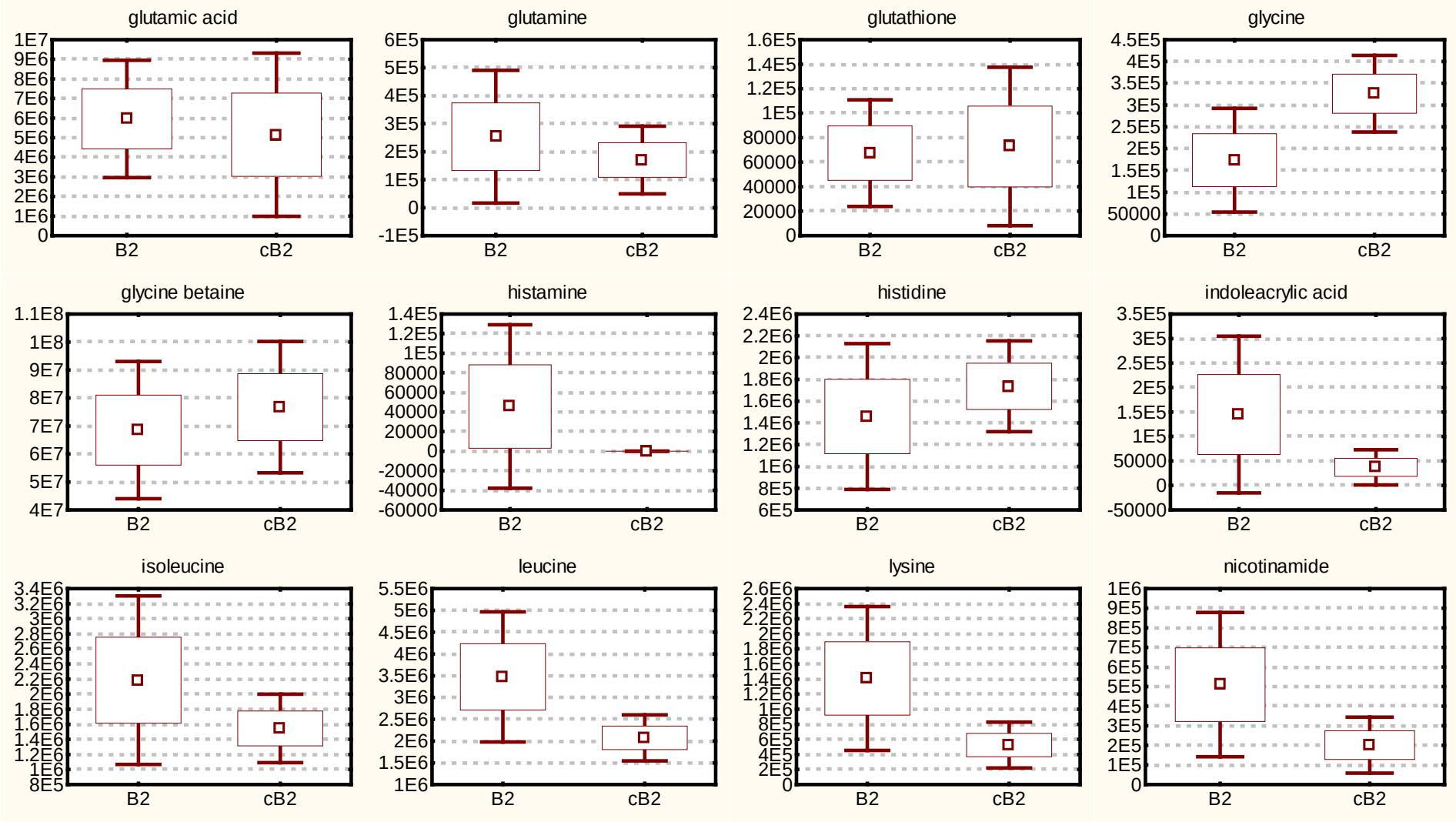

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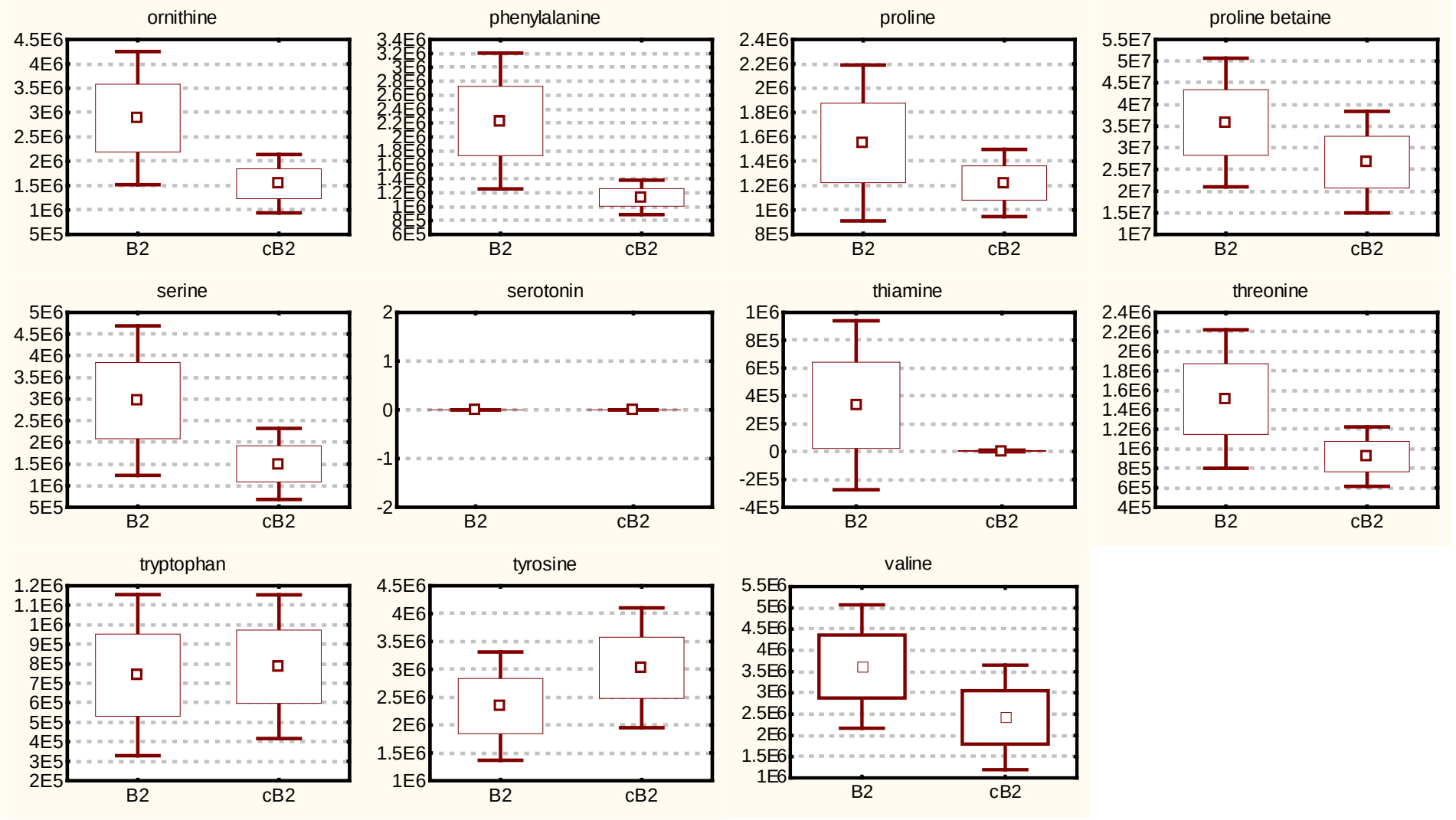
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