Supplementary Material for

Identifying Euglena gracilis Metabolic and Transcriptomic Adaptations in Response to Mercury Stress

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This file contains:

3 Supporting Tables (Attached as Excel Files)

5 Figures

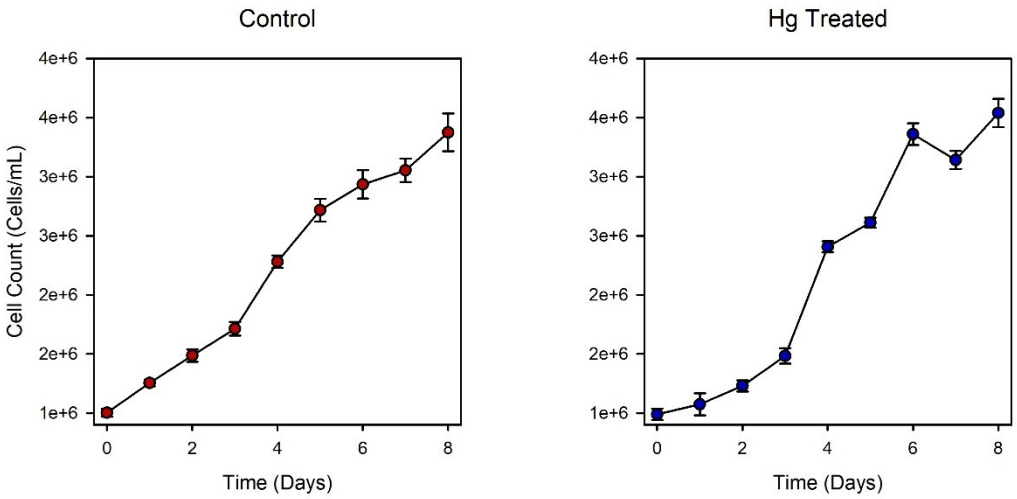

Figure S1: Growth curves of *E. gracilis* cultivated (A) in the absence of Hg (control) and (B) in the presence of Hg (Hg Treated) determined by automatic cell counts (Countess II FI, ThermoFisher Scientific). Cultures were harvested after 5 days of cultivation, consistent with mid-exponential growth.

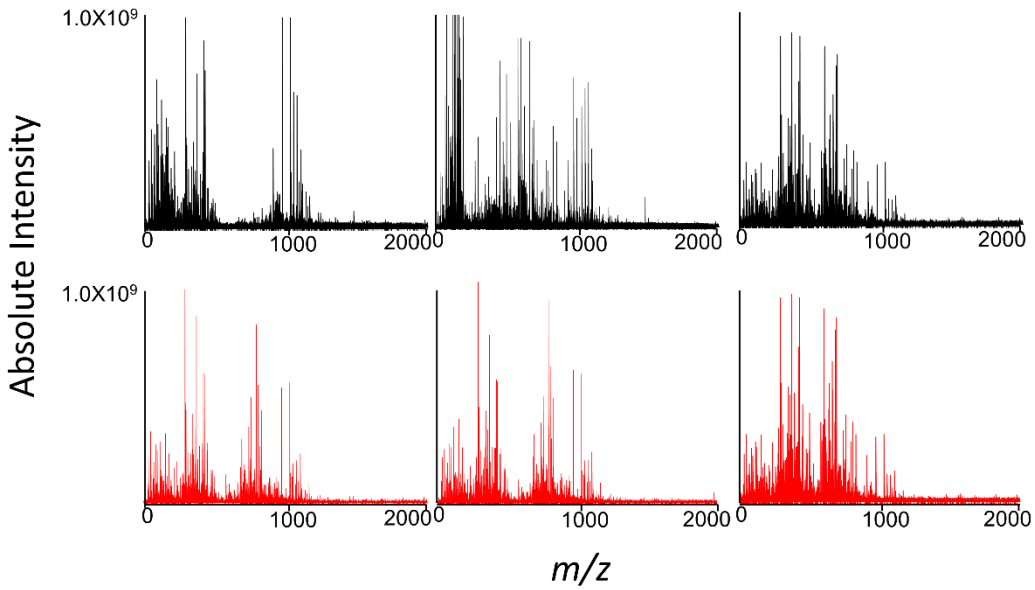

Figure S2: High resolution mass spectrometry spectra of released exudates found in control (black) and Hg (II) (red) cultures (n=3). Mass spectra were acquired from *m/z 150-2000* to capture a broad representation of lower molecular weight metabolites, metabolite classes, and chemical processes. In total, 5203 ± 411 *m/z* molecules were assigned formulae in released exudates.

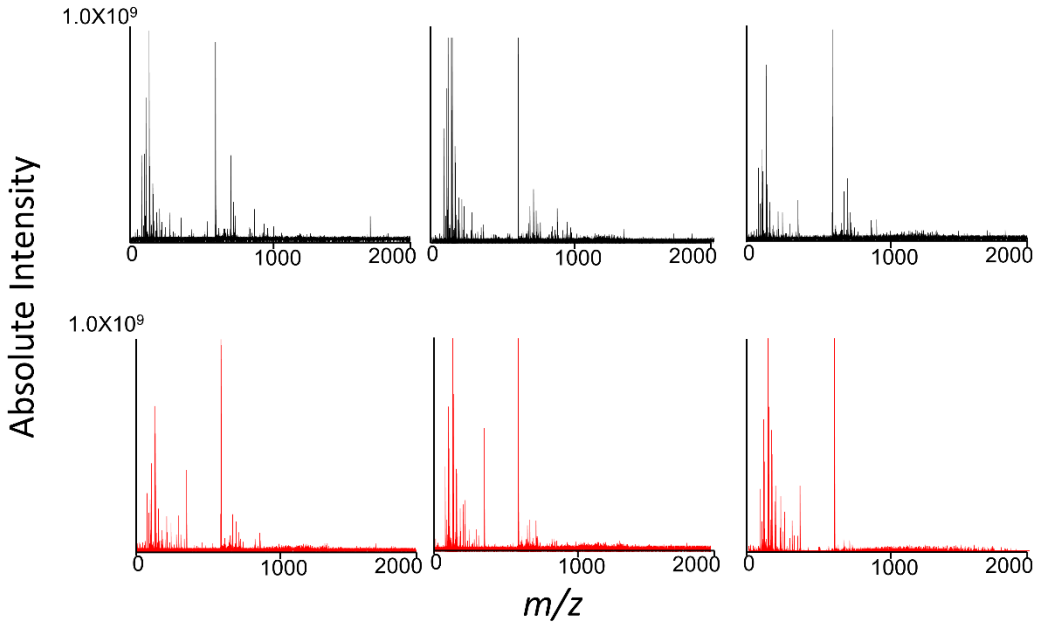

Figure S3: High resolution mass spectrometry spectra of cellular biomolecules found in control (black) and Hg (II) (red) cultures (*n*=3). In total 7940 ± 523 *m/z* molecules were assigned formulae in cellular biomolecules in spectra acquired from *m/z 150-2000.*

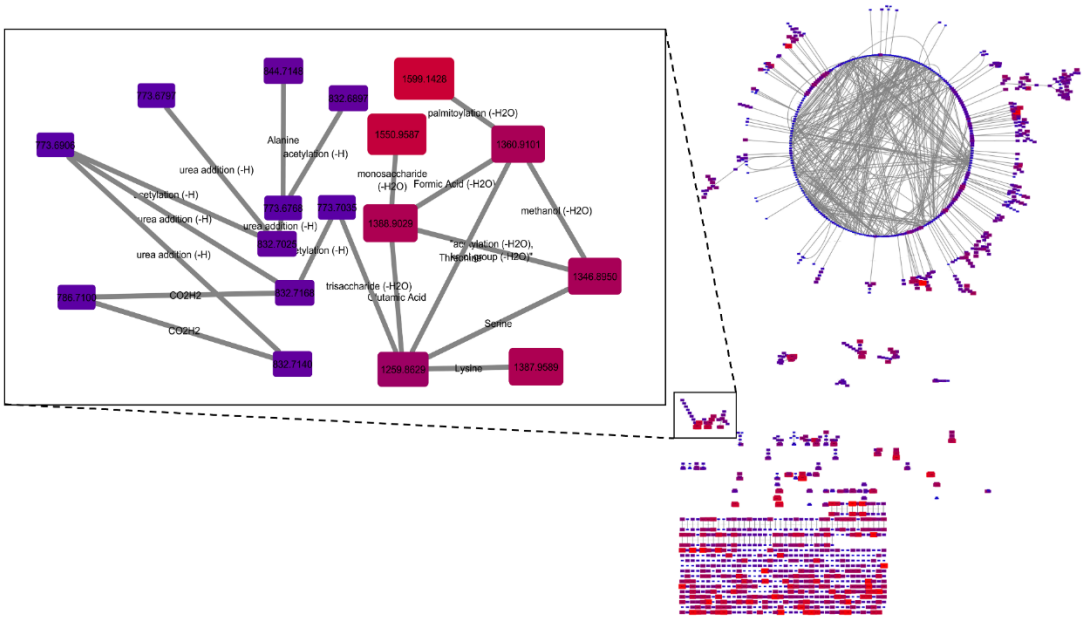

Figure S4: Cytoscape framework of 75 different amino acids, metabolite classes, and metabolomic processes acquired from FT-ICR high resolution mass spectrometry in released exudates. Nodes are represented by *m/z* derived from FT-ICR spectra and grey lines represent transformations between 2 nodes to account for metabolic shifts. These shifts were quantified using MetaNetter2 in Cytoscape and the relative abundance of these different shifts were used in subsequent analyses in MetaboAnalyst.

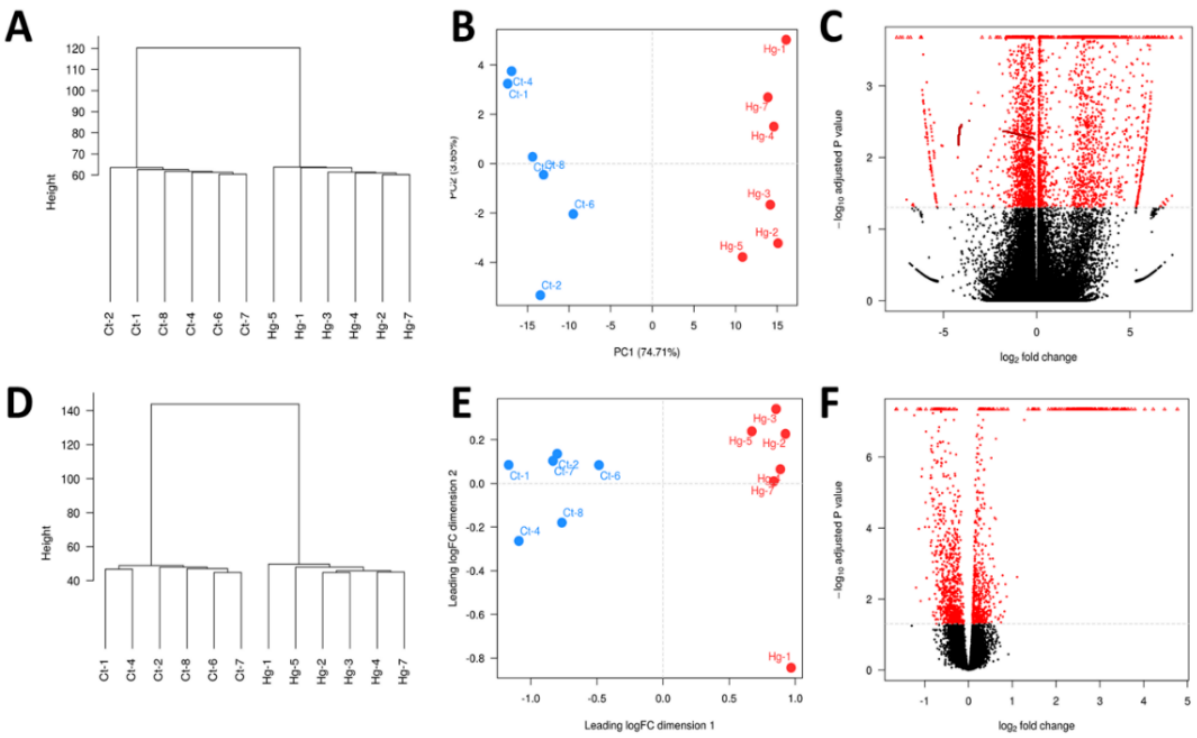

Figure S5: Analysis of *Euglena gracilis* cellular biomolecules grown in the absence or presence of mercury reveals a consistent difference in transcriptomic response. We used DESeq2 (A-C) and edgeR (D-F) to test for significant (FDR < 0.05) differential (> 2-fold change) transcript expression between *E. gracilis* cultures grown in the absence (Ct) or presence of mercury (Hg). Sample correlation depicted by dendrogram (A, D), principal components analysis (B), and multi-dimensional scaling plots reveals that *E. gracilis* samples are more similar in gene expression profiles based on the treatment group (Ct = blue circles; Hg = red circles). Differential gene expression was detected based on comparisons between growth conditions. For each analysis, a volcano plot (C, F), was produced by plotting the negative log of the adjusted P value versus the log of the fold change between the two groups. Data points were colored based on fold change and FDR threshold, where red dots indicate statistically significant differentially expressed genes, and black dots indicate genes that were not statistically significant differentially expressed.