Contrasting Metabolic Patterns Associated with Acute and Chronic Restraint Stress in Sprague Dawley Rats: A ^{1}H-NMR-Based Metabolomics Study

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ABBREVIATIONS: NMR-Nuclear Magnetic Resonance; CPMG-Carr–Purcell–Meiboom–Gill; AUROC-Area under the ROC curve; PCA-Principal Component Analysis; PLS-DA-Partial Least Squares Discriminant Analysis; RS-Restraint stress; AS-Acute stress; CS-Chronic stress.
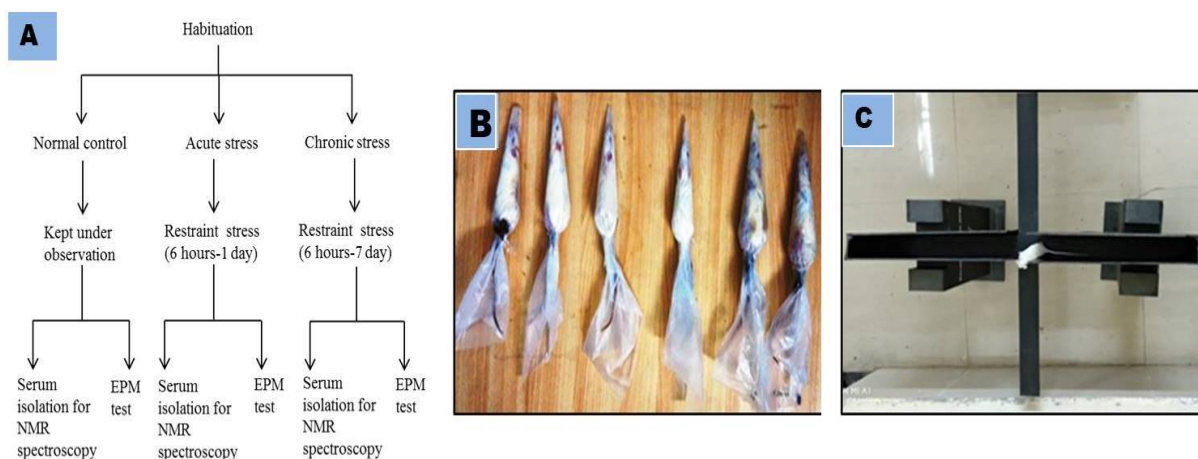
Figure S1: (**A**) represents the protocol used in the study. (B) represents the restraint stress given in plastic cone. (**C**) animals were tested on the EPM to know the effect of stress duration on the anxiety.
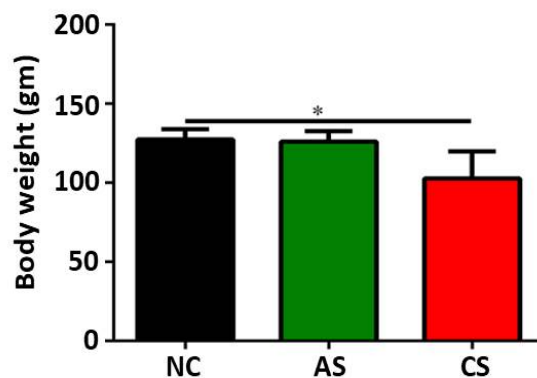

Figure S2: represents the effect of AS and CS on the body weight of animals. CS grouped animals showed significant decreased body weight ($p<0.05$) as compared to the NC and AS group while no change was observed in between the NC and AS grouped animals ($p>0.05$).
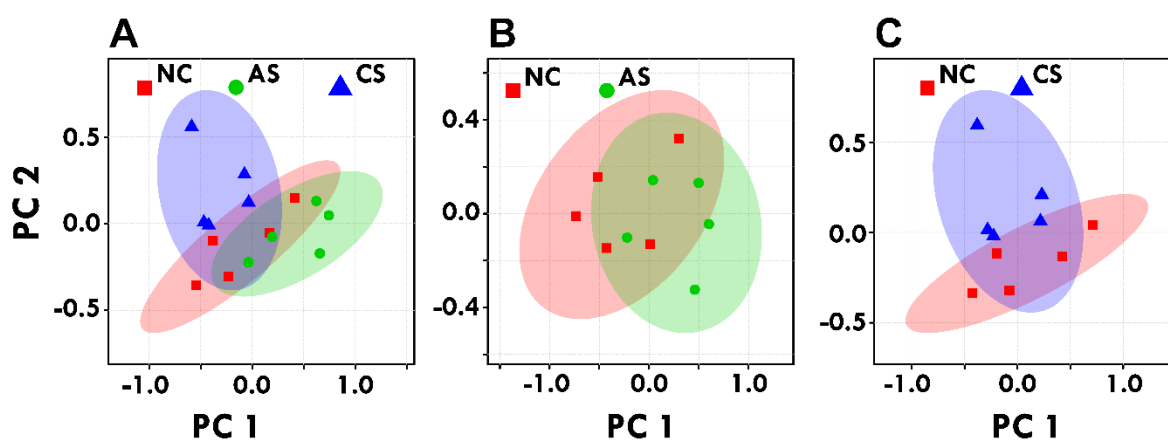

Figure S3: (A) Combined 2D PCA score plot comprising of all 3 groups NC, AS and CS. (B) and (C) Pairwise 2D PCA score plot of AS and CS with the NC respectively. Derived from 1D ^{1}H NMR CPMG spectra of serum samples.
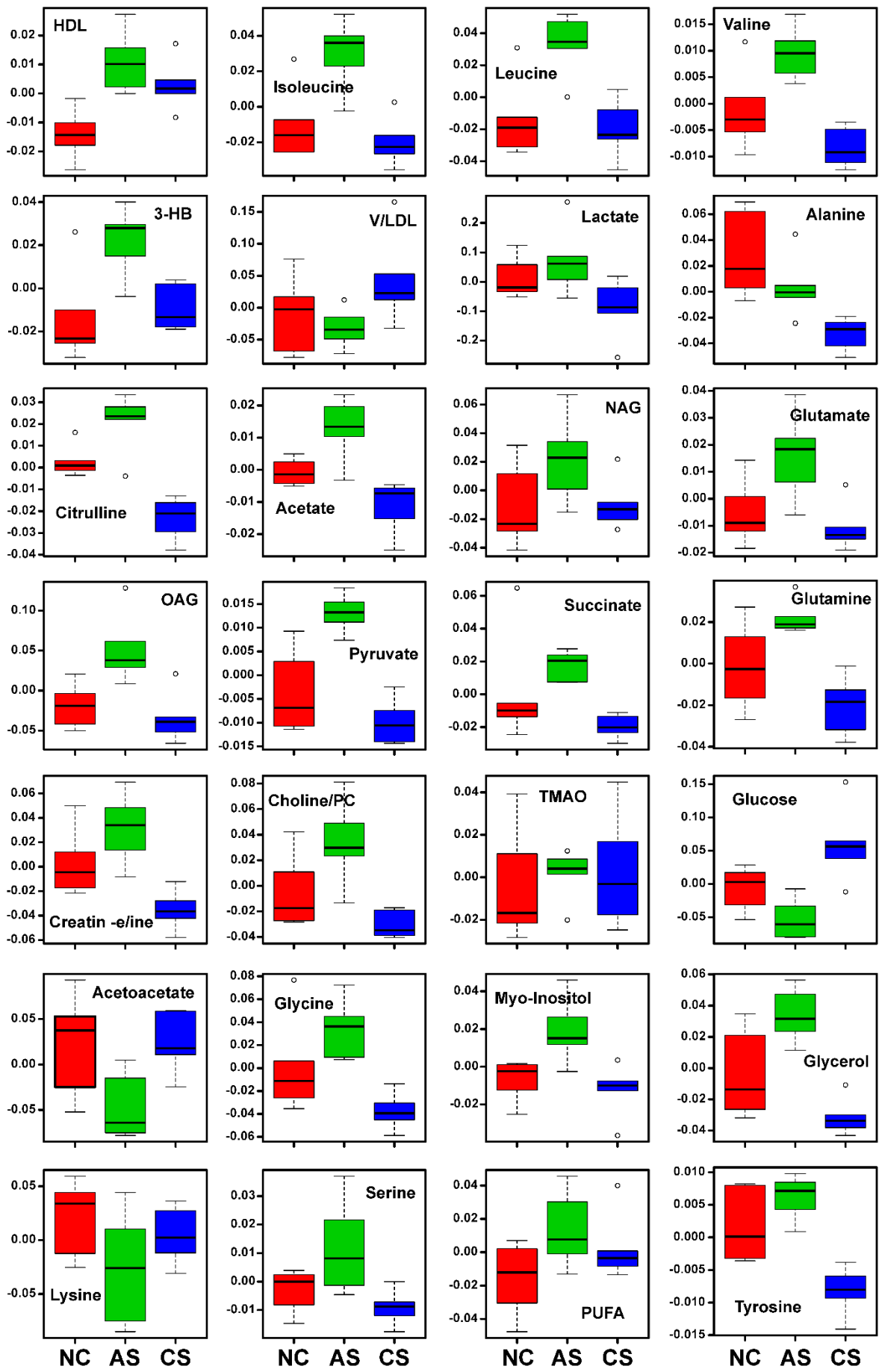
Figure S4: Box-whisker plots of metabolites that were significantly perturbed in AS and CS with respect to the Control group derived from 1H NMR CPMG spectra.
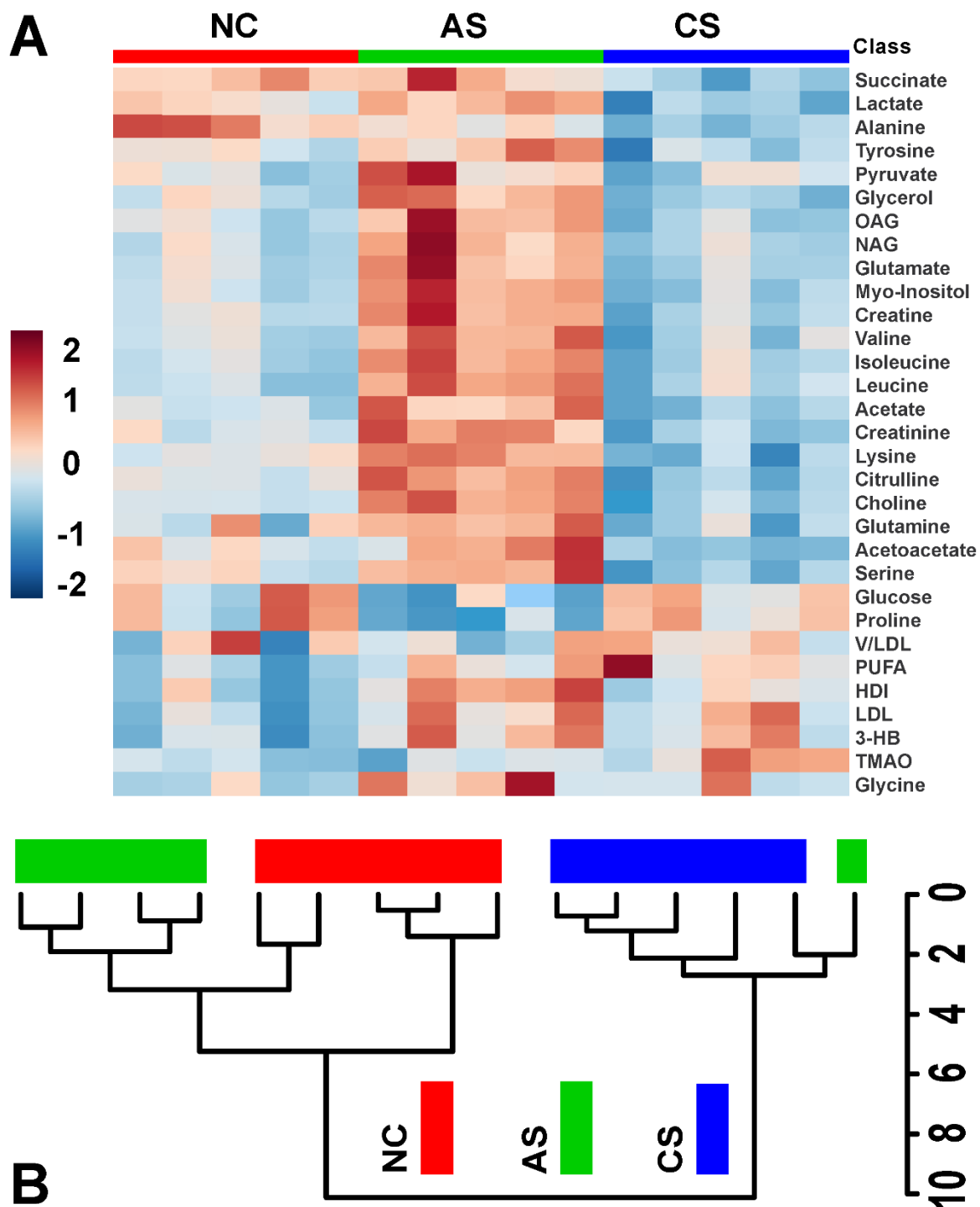
Figure S5: (A) Heat map of statistically significant metabolites in between Acute and Chronic -stress groups with respect to Control group as derived from CPMG 1H NMR Spectra. Red=elevated; Cyan=reduced. Each column represents a sample, and each row represents a metabolite. (B) Hierarchical Clustering Analysis The dendrogram of control, acute and chronic –stress groups.