



Metabolic Implications of Using BioOrthogonal Non-Canonical Amino Acid Tagging (BONCAT) for Tracking Protein Synthesis

Katherine F. Steward¹, Brian Eilers¹, Brian Tripet¹, Amanda Fuchs¹, Michael Dorle¹, Rachel Rawle¹, Berliza Soriano¹, Narayanaganesh Balasubramanian¹, Valérie Copié^{1,2}, Brian Bothner^{1,2*} and Roland Hatzenpichler^{1,2,3*}

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*Correspondence:

Brian Bothner
bbothner@montana.edu
Roland Hatzenpichler
roland.hatzenpichler@montana.edu

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¹ Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT, United States, ² Thermal Biology Institute, Montana State University, Bozeman, MT, United States, ³ Center for Biofilm Engineering, Montana State University, Bozeman, MT, United States

BioOrthogonal Non-Canonical Amino acid Tagging (BONCAT) is a powerful tool for tracking protein synthesis on the level of single cells within communities and whole organisms. A basic premise of BONCAT is that the non-canonical amino acids (NCAA) used to track translational activity do not significantly alter cellular physiology. If the NCAA would induce changes in the metabolic state of cells, interpretation of BONCAT studies could be challenging. To address this knowledge-gap, we have used a global metabolomics analyses to assess the intracellular effects of NCAA incorporation. Two NCAA were tested: L-azidohomoalanine (AHA) and L-homopropargylglycine (HPG); L-methionine (MET) was used as a minimal stress baseline control. Liquid Chromatography Mass Spectrometry (LC-MS) and Nuclear Magnetic Resonance (NMR) were used to characterize intracellular metabolite profiles of *Escherichia coli* cultures, with multivariate statistical analysis using XCMS and MetaboAnalyst. Results show that doping with NCAA induces metabolic changes, however, the metabolic impact was not dramatic. A second set of experiments in which cultures were placed under mild stress to simulate real-world environmental conditions showed a more consistent and more robust perturbation. Pathways that changed include amino acid and protein synthesis, choline and betaine, and the TCA cycle. Globally, these changes were statistically minor, indicating that NCAA are unlikely to exert a significant impact on cells during incorporation. Our results are consistent with previous reports of NCAA doping under replete conditions and extend these results to bacterial growth under environmentally relevant conditions. Our work highlights the power of metabolomics studies in detecting cellular response to growth conditions and the complementarity of NMR and LCMS as omics tools.

Keywords: metabolomics, BONCAT, non-canonical amino acids, L-azidohomoalanine, L-homopropargylglycine

INTRODUCTION

Dieterich et al. (2006) introduced a method for visualizing newly synthesized proteins in mammalian cells termed BioOrthogonal Non-Canonical Amino acid Tagging (BONCAT). BONCAT facilitates the tracking and localization of protein translation in single cells following a short incubation with a synthetic amino acid that later can be detected via azide-alkyne click-chemistry, a sensitive and precise biocompatible reaction (Kolb et al., 2001). BONCAT has proven to be particularly useful for monitoring cellular activity in complex microbial communities (Hatzenpichler et al., 2014, 2016; Samo et al., 2014; Leizeaga et al., 2017; Sebastián et al., 2019), and adds a convenient approach to the molecular tool box available for analyzing microbial community function (Hatzenpichler et al., 2020) because it avoids the use of radioactive substrates and is understood to only minimally impact protein structure and cell physiology. Currently, the two most widely used non-canonical amino acids (NCAA) are L-azidohomoalanine (AHA) and L-homopropargylglycine (HPG), which both replace L-methionine (MET) during translation (Küick et al., 2002). These amino acids contain either an azide (AHA) or an alkyne functional group (HPG) which are amenable to azide-alkyne click chemistry (Kolb et al., 2001). Experimental protocols for performing BONCAT studies and click-labeling newly made proteins are well established in microbiology and microbial ecology (Bagert et al., 2014; Hatzenpichler et al., 2014, 2016; Mahdavi et al., 2014; Hatzenpichler and Orphan, 2015; Babin M. B. et al., 2016; Bagert et al., 2016).

Phenotypic markers of optical density, behavioral tests, and responses to visual cues have been utilized to assess the impact of cell treatments with NCAA. Studies on HeLa cells (Bagert et al., 2014), a range of bacterial and archaeal pure cultures (Bagert et al., 2014; Hatzenpichler et al., 2014; Hatzenpichler and Orphan, 2015), and environmental samples (Hatzenpichler et al., 2014, 2016) have demonstrated that the addition of low concentrations (nM–mM range) of AHA or HPG to a sample over short periods of time (typically 1–2 cell generations) has only minimal effects on the physiology, growth rate, or protein expression patterns of organisms. Hinz et al. studied zebrafish and investigated the potential effects of NCAA labeling *in vivo*, which revealed that AHA was successfully incorporated into proteins in a ratio consistent with time and concentration, that AHA was non-toxic and had no detrimental effect on animal behavior (Hinz et al., 2012). A recent proteomic study investigating the effect of AHA and HPG on protein expression and the ability to incorporate these reagents into mice showed that a small percentage (~10%) of proteins change their expression patterns in response to AHA doping (Calve et al., 2016). Lastly, a recent study indicated that the incorporation of AHA into a model protein only minimally affected the protein tertiary structure (Lehner et al., 2017). The recent application of proteomics investigating the cell machinery have also shown that AHA and HPG have little impact on the overall fitness of the organism (Dieterich et al., 2006; Landgraf et al., 2015). However, a deeper look into the metabolism of NCAA doped organisms has, to our knowledge, never been carried out.

This study aimed to characterize the metabolome of *Escherichia coli* when grown in the presence of NCAA and identify potentially differentiated metabolite patterns that might inform us on the metabolic impact of NCAA on cell homeostasis and organismal health. In order to investigate how NCAA exposure affects intracellular metabolism, *E. coli* cell cultures were grown with and without AHA or HPG. One sample group included additional MET as a minimal perturbation to be used as a control experiment for baseline stress due to media supplementation. Control cultures were also grown in media without amino acid amendment. “Real world” experimental and cell culturing conditions were utilized in our analysis to best evaluate the potential effects of NCAA, and to mimic current field work in the environment that attempts to find suitable growth conditions for otherwise unculturable microorganisms. Comprehensive metabolite mapping techniques using Liquid Chromatography Mass Spectrometry (LC-MS) and Nuclear Magnetic Resonance (NMR) spectroscopy were employed to assess potential metabolome differences between different *E. coli* cell cultures and growth conditions.

MATERIALS AND METHODS

Reagents

HPLC grade solvents: water, methanol and acetonitrile were purchased from Fisher (Waltham, MA, United States). AHA and HPG were purchased from Click Chemistry Tools (Scottsdale, AZ, United States). All other chemicals were purchased from Millipore Sigma (St. Louis, MO, United States) and were used as provided, with no additional purification steps.

Cell Culturing

An overnight culture of *E. coli* K12 DH10B, which had been grown on M9 minimal medium (200 mg/L thiamine, 0.2% glucose), was inoculated 1:20 into 6 L of M9 medium (200 mg/L thiamine, 0.2% glucose) to yield a fresh culture of optical density, measured at a wavelength λ of 600 nm, i.e., OD₆₀₀ of 0.041. 150 mL aliquots of this culture were then aliquoted into 36 Erlenmeyer flasks, which were incubated at 37°C on rotary shakers run at 200 rpm. Temperatures were independently checked with a thermometer at regular intervals to validate that temperatures were consistent across incubators throughout the experiments. Immediately following inoculation, the following incubations were started: five flasks each for (1) 50 μ M MET; (2) 50 μ M AHA; and (3) 50 μ M HPG. One additional control flask without amendment was used to monitor growth of the cultures via optical density (OD₆₀₀). This was done to avoid disturbing the experimental cultures given the large number of flasks. Growth experiments with 50 μ M amino acid addition (MET, AHA, or HPG) were stopped after 85 min of incubation when the control flask had reached an OD₆₀₀ of 0.072, corresponding to ~0.74 cell generations (Supplementary Figure S1). The 2 $\times$ 50 mL cell cultures were then decanted into two 50 mL tubes. Tubes were centrifuged for 5 min at 4,700 g at room temperature. Resulting supernatants were decanted and the cell pellets flash-frozen in liquid N₂.

and stored at -80°C until further processing. After these samples had been stored at -80°C , the remaining 20 flasks were processed the following way: 1 mM of (1) MET, (2) AHA, and (3) HPG were added to 5 flasks; Five additional culture flasks served as no-amendment control, which were used to track cell growth. The incubation was continued as described, with a starting OD_{600} of control cell cultures of 0.27, and stopped after 5 min of amino acid pulse labeling whereby the control cultures had reached an OD_{600} of 0.31, corresponding to ~ 0.04 cell generations (**Supplementary Table S1**). Cells were pelleted, pellets flash frozen, and samples stored as described above. Cultures for the heat stress experiments were conducted as described above except that cell cultures were grown and maintained at 42°C .

Metabolite Extraction

Escherichia coli intracellular metabolites were extracted using published protocols (Hamerly et al., 2015). Briefly, frozen cell pellets were re-suspended with water, then sonicated using a Biologics Ultrasonic Homogenizer model 3000 for 10 pulses of 3 s each. Resulting supernatant was centrifuged and transferred to 10 mL scintillation vials to which four volumes of ice cold acetone were added, followed by storage of the samples at -80°C overnight for protein precipitation. Protein concentration in the samples was determined using a Bradford assay (Bradford, 1976) (**Supplementary Table S2**). Samples were vortexed, centrifuged, and split into two fractions for concurrent analysis by LC-MS and NMR: 1 mL for LC-MS analysis and 4 mL for NMR metabolomics analysis. Both fractions were dried completely using vacuum speed concentration with no heat, and subsequently frozen at -80°C until further use.

LCMS Instrumentation and Metabolite Analysis

The dried metabolite fraction used for liquid chromatography mass spectrometry (LC-MS) was re-suspended with 20 μL of 50:50 MeOH/ H_2O before injection into the mass spectrometer. MS-based analysis of polar metabolites was accomplished using an Agilent 1290 ultra-high performance liquid chromatography (UPLC) system coupled to an Agilent 6538 Accurate-Mass quadrupole Time of Flight (TOF) mass spectrometer. A Cogent diamond hydride HILIC chromatography column (2.2 μM , 120 A, 150 mm $\times$ 2.1 mm Microsolv, Leland, NC, United States) was used for metabolite separation. The gradient began with solvent B (0.1% formic acid in acetonitrile) for 2 min at 50%, followed by a gradient ramp of 50–100% B over 14 min. This step was followed by a hold at 100% solvent B for 1 min, and then return to initial conditions. Mass analysis was conducted in positive mode with a capillary voltage of 3500 V, dry gas temperature of 350°C at a flow of 8 L/min and the nebulizer was set at 60 psi, injecting 2 μL sample volumes, with blanks run intermittently between samples. Data acquisition parameters were as follows: 50–1,000 mass range at 1 Hz scan rate with a resolution of 18,000. Accuracy based on calibration standards was approximately 5 ppm.

Statistical Analysis of MS Data

Extracted ion chromatograms, peak detection, peak annotation, chromatogram alignment, gap filling and relative quantitation of identified features was completed using MZmine (Pluskal et al., 2010), MetaboAnalyst (Chong and Xia, 2018), and XCMS (Tautenhahn et al., 2012). Metabolite identifications were made based on exact mass and retention time matches to authentic standards using an in house library of ~ 500 compounds. Statistical analysis of the MZmine output was done using Microsoft Excel version 2016 and MetaboAnalyst v4.0. XCMS utilizes an all-inclusive processing package with a similar workflow, in which it extracts chromatograms, identifies peaks, matches peaks across samples, gap fills, performs statistical analyses and *in silico* compound identification, and graphical visualization of the data. Identifications of unknown features were made using the MetLin Metabolite Database, which provided a list of possible metabolites based on exact mass, species, and likelihood (Myers et al., 2017).

Sample Preparation and NMR Analysis

Dried metabolite mixtures were re-suspended in 600 μL of NMR buffer (containing 0.25 mM 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) in 90% H_2O /10% D_2O , 25 mM sodium phosphate, pH 7), and transferred into 5 mm NMR tubes. All one dimensional (1D) ^1H NMR spectra were recorded at 298 K using a Bruker AVANCE III solution NMR spectrometer operating at 600.13 MHz ^1H Larmor frequency and equipped with a 5 mm liquid-helium-cooled TCI cryoprobe with Z-gradient and a SampleJetTM automatic sample loading system. 1D ^1H NMR data were acquired using the Bruker supplied 1D excitation sculpting water suppression pulse sequence ‘zgesgp’ with 256 scans, a ^1H spectral window of 9,600 Hz, 32K data points, a dwell time interval of 52 μsec , and a recycle delay of 5 s between scan acquisitions. The data were first processed with the Bruker TOPSPIN 3.5 software¹ using standard parameters for chemical shift referencing using the DSS signal and line broadening (0.3 Hz). Spectral phases were manually adjusted, and a polynomial function was applied (qfl, 0.2 ppm width) on the residual water peak to remove its signal. Metabolite identification and quantification were carried out using the ChenomxTM NMR suite software (version 8.3)² and its associated 600 MHz small molecule reference spectral database. DSS was used as an internal standard for metabolite quantification, while imidazole NMR signals were used to correct for small chemical shift changes arising from slight pH variations between samples. The metabolite concentration tables (mM) generated with Chenomx were exported to a.csv file and converted to μM , and normalized to sample protein concentration as established from Bradford protein assays.

Validation of metabolite IDs, which were annotated in Chenomx³, was accomplished using 2D ^1H - ^1H and 2D ^1H - ^{13}C total correlation spectroscopy (TOCSY) NMR or by spiking,

¹<https://www.bruker.com/service/support-upgrades/software-downloads/nmr.html>

²<https://www.chenomx.com/products/>

³<https://www.chenomx.com/products/>

when available, pure metabolite standards into the samples and monitoring resulting spectral changes in the 1D ^1H NMR spectra. 2D ^1H - ^1H TOCSY spectra were acquired for representative samples using the Bruker-supplied 'mlevphpr.2/mlevgpph19' pulse sequences (256 $\times$ 2048 data points, 2 s relaxation delay, 32 transients per FID, ^1H spectral window of 6602.11 Hz, 80 ms TOCSY spin lock mixing period). 2D ^1H - ^1H TOCSY spectra were processed using Topspin software (Bruker version 3.2)⁴.

Statistical Analysis of NMR Data

The NMR-based metabolite data were uploaded to the MetaboAnalyst v4.0 web server for multivariate statistical analysis. Metabolite concentrations were normalized by log-transformation and auto-scaling (mean centered divided by the standard deviation of each variable) prior to univariate and multivariate statistical analysis. Student *t*-test, principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were performed to identify potentially distinct metabolite patterns between the *E. coli* sample groups grown under the different conditions. Variable importance in projection (VIP) plots generated from the PLS-DA data were employed to assess the importance of each variable (i.e., metabolite) in the projection used in PLS-DA model building; statistics were calculated for the data shown in Figure 5, using 3 components, yielding Q2 and R2 values of 0.646 and 0.913, respectively. PLS-DA model validity was further assessed using the (B/W) permutation test function of MetaboAnalyst which, using 2,000 permutation steps yielded a *p*-value of $<5 \times 10^{-4}$, as a measure of the significance of the PLS-DA model. For hierarchical clustering analysis (HCA), distances were measured using a Euclidean correlation and clustering by the Ward algorithm.

RESULTS AND DISCUSSION

Mass Spectrometry-Based Metabolomics of Non-canonical Amino Acids

An initial set of experiments was conducted to determine the physiological impact of NCAA additions to *E. coli* cell cultures under otherwise normal growth conditions. This study established which NCAA concentrations are needed to evaluate changes in the metabolome of *E. coli* that may be relevant to normal growth conditions (i.e., cells grown at 37°C). Cultures were spiked with either 1 mM or 50 μM concentrations of AHA, HPG, or MET. MET was added as a baseline perturbation control experiment to account for the impact additional amino acid would have on the metabolism of *E. coli*, as opposed to the control group, which had no amendment to the minimal growth medium. Metabolite extracts from the six conditions and control groups were prepared and analyzed by LC-MS using a high-resolution Q-TOF instrument. A total of 4,036 mass features were detected across all sample groups using the MZmine data reduction approach, as described above. Statistical

analysis was done in Excel (2016) using the MZmine output, with additional statistical analysis performed using MetaboAnalyst (Chong and Xia, 2018) and XCMS (Tautenhahn et al., 2012). PCA was used to gather information about the variation between sample treatments and replicates. 2D-PCA plots indicated no clear separation among the different experimental groups when all *m/z* features were analyzed as a single input (Figure 1A). The 1 mM MET and both HPG groups displayed the largest separation from the other treatment groups. While principal component 1 (PC1) accounted for 44.5% of the variance, it primarily separated the 1 mM HPG samples from the other experimental conditions. The second principal component (PC2) accounted for 14.7% of the variance but did little to differentiate between the different sample groups. Overall, there was greater variation between sample treatment replicates than between the different sample treatment groups.

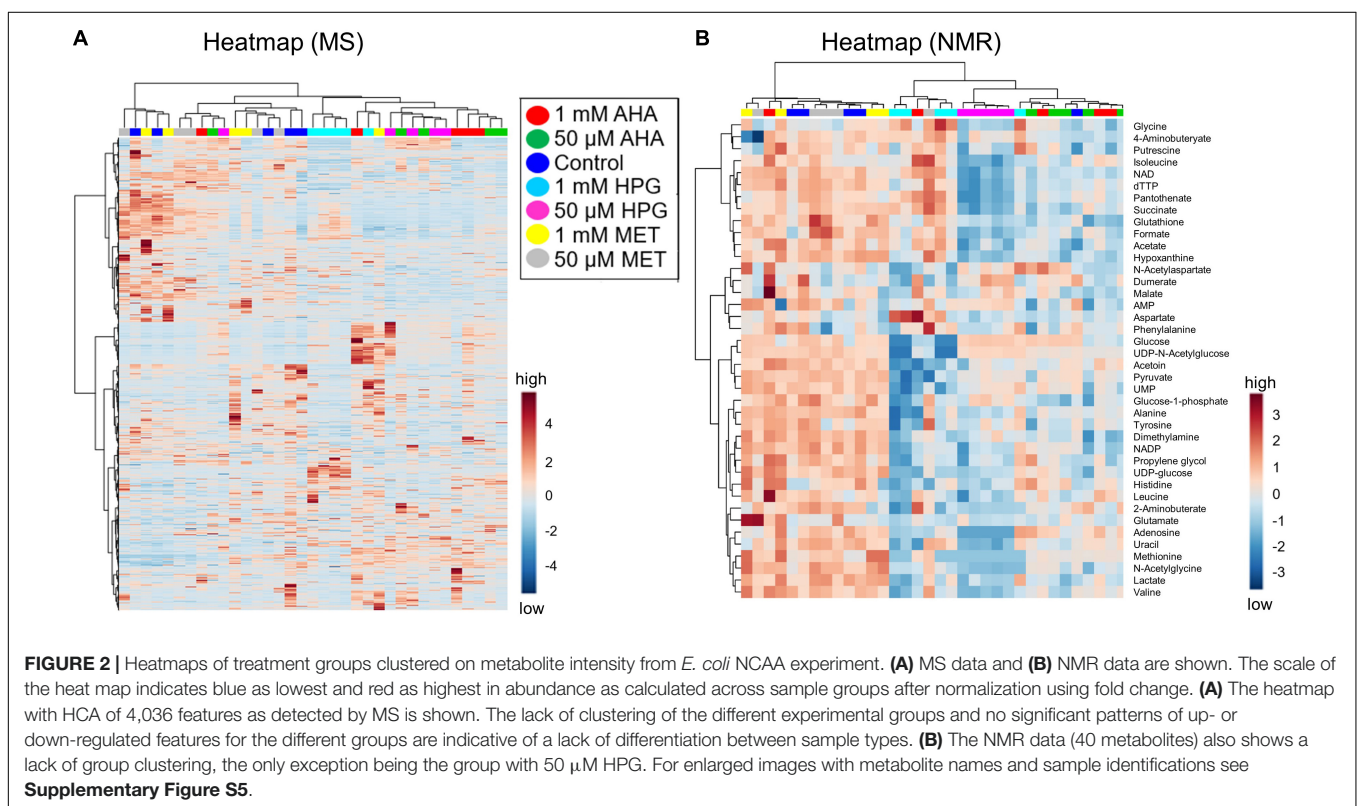
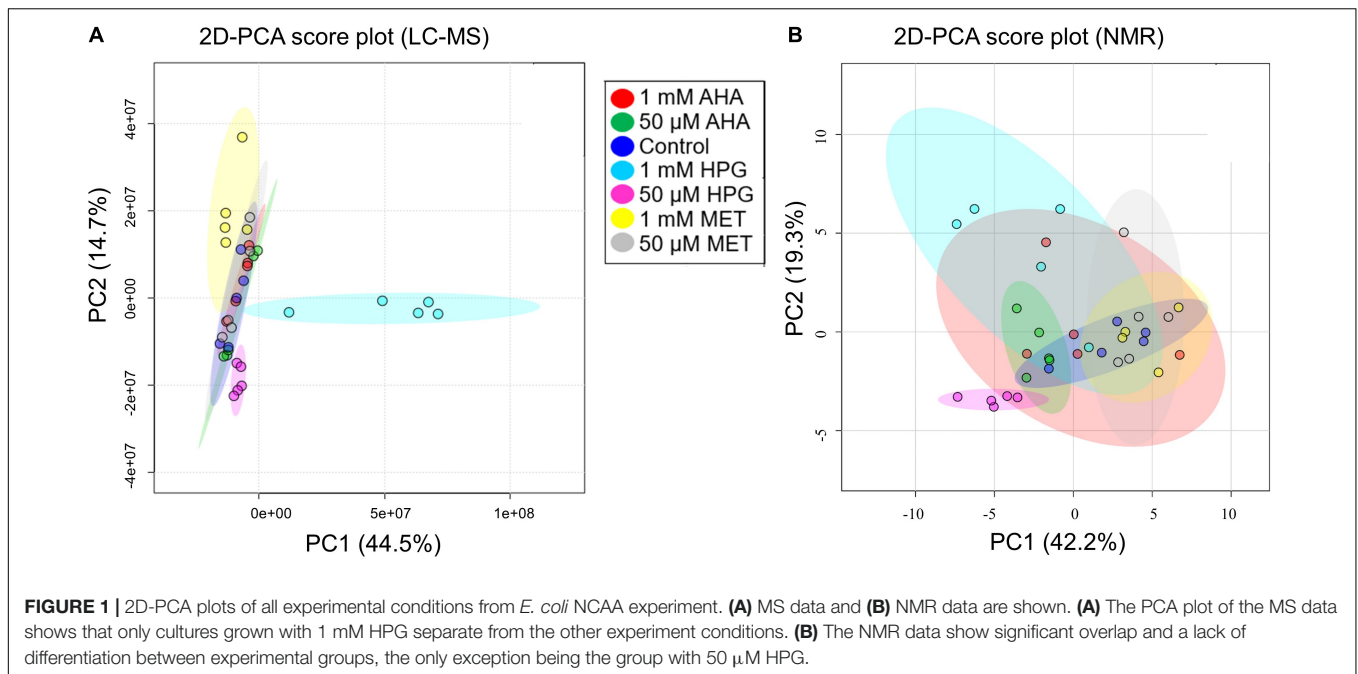
A heatmap was constructed to visualize differentiated MS features between treatment groups. Heatmaps are a powerful tool for visualizing trends and correlated changes via hierarchical clustering across all samples and all features. The control and baseline-MET samples intermingled, while AHA and the HPG sample treatments mixed on the hierarchical cluster but were generally clustered apart from the MET and control samples (Figure 2A). The heatmap patterns indicated that not all replicates from each treatment clustered with each other; however, a general grouping by type and concentration of NCAA was discernable. The relatively few "hot zones," or regions of high (dark red) or low (dark blue) abundance features on the heatmap suggested that only a small number of MS features exhibited log2fold changes > 2 , indicating that generally minor metabolic differences existed between the different *E. coli* treatment groups. Based on 2D-PCA, ANOVA (Supplementary Table S3), *t*-test and HCA, we thus concluded, from the global MS data, that only small metabolic changes are occurring in *E. coli* grown in the presence of NCAA.

NMR Metabolite Profiles of *E. coli* Grown in the Presence of Non-canonical Amino Acids

Intracellular metabolite extracts from the same *E. coli* cell cultures were analyzed using 1D ^1H NMR spectroscopy. As with the MS studies, the NMR metabolomics data readily detected some changes in the metabolome of *E. coli* as a function of NCAA incorporation. However, these metabolite pattern changes were found to be relatively small and insufficient to unambiguously distinguish the different *E. coli* cell cultures based on 2D-PCA and HCA analyses (Figures 1A, 2B) of the different NMR-based metabolite profiles. 54 metabolites were annotated by analysis of the 1D ^1H NMR spectra of the *E. coli* intracellular metabolite extracts using Chenomx (Supplementary Table S4). These metabolite IDs were further validated using spiking of standards and 2D ^1H - ^1H and natural abundance ^1H - ^{13}C TOCSY experiments (Supplementary Table S5).

Metabolite patterns between the different *E. coli* sample groups, i.e., *E. coli* grown with MET, HPG and AHA at 1 mM and 50 μM conditions, were investigated by PCA analysis of resulting

⁴<https://www.bruker.com/service/support-upgrades/software-downloads/nmr.html>



NMR-based metabolite profiles and concentrations (**Figure 1B**). As with the MS data, no clear clustering pattern or group separation were observable under these experimental growth conditions. The lack of separation along principal components 1 and 2 was particularly noticeable, leading us to conclude that while there exist metabolome differences between the different

E. coli treatment groups, the NMR-based metabolite profile differences are not sufficiently pronounced to allow for clear treatment group separation.

A heatmap of all NMR features, based on HCA, did not result in group clustering by NCAA treatment type, rather sample types were intermingled showing no significant changes

between groups (**Figure 2B**). The NMR metabolomics results are consistent with the MS spectral data. Only the 50 μ M HPG samples separated as a unique cluster, similar to what is observed in the heatmap of the LC-MS spectral features (**Figure 2A**). Taken together, our results suggest that overall, the variability between biological replicates is comparable in magnitude to potential metabolic changes arising from the addition of NCAA in the *E. coli* cell cultures.

This combined MS and NMR metabolomics analysis of NCAA-treated *E. coli* cell cultures demonstrated that these analytical platforms can detect subtle changes in the metabolomes of *E. coli* grown under different culturing conditions, but that additional studies were needed to parse out how these small but potentially significant metabolic changes may impact cellular phenotypes.

***E. coli* Grown With Non-canonical Amino Acid Under Heat Stress**

While planning additional experiments, we evaluated how NCAA are typically used in field work and “real-world” research applications to evaluate how an organism regulates its translational activity in response to environmental (Samo et al., 2014; Hatzenpichler et al., 2016; Leizeaga et al., 2017; Sebastián et al., 2019) or (co)cultivation conditions (Mahdavi et al., 2014; Babin S. A. et al., 2016; Bagert et al., 2016). For such purposes, cellular organisms are often grown for short periods of time under environmental perturbations or cellular stress. We concluded that a more real-world evaluation of a NCAA treatment would include an environmental stressor. Because of the extensive literature available on heat stress response in *E. coli* (Jozefczuk et al., 2010; Ye et al., 2012), we chose temperature increase as an appropriate stressor. Assessing metabolome changes under BONCAT treatment during heat stress would thus not only help clarify how *E. coli* cells are adapting to the incorporation of NCAA, it would also recreate a stress condition that may best reflect “real world” research applications. This rationale thus led to a second set of metabolomics investigations, which utilized high temperature as a stress condition during *E. coli* cell growth with or without a NCAA or MET present.

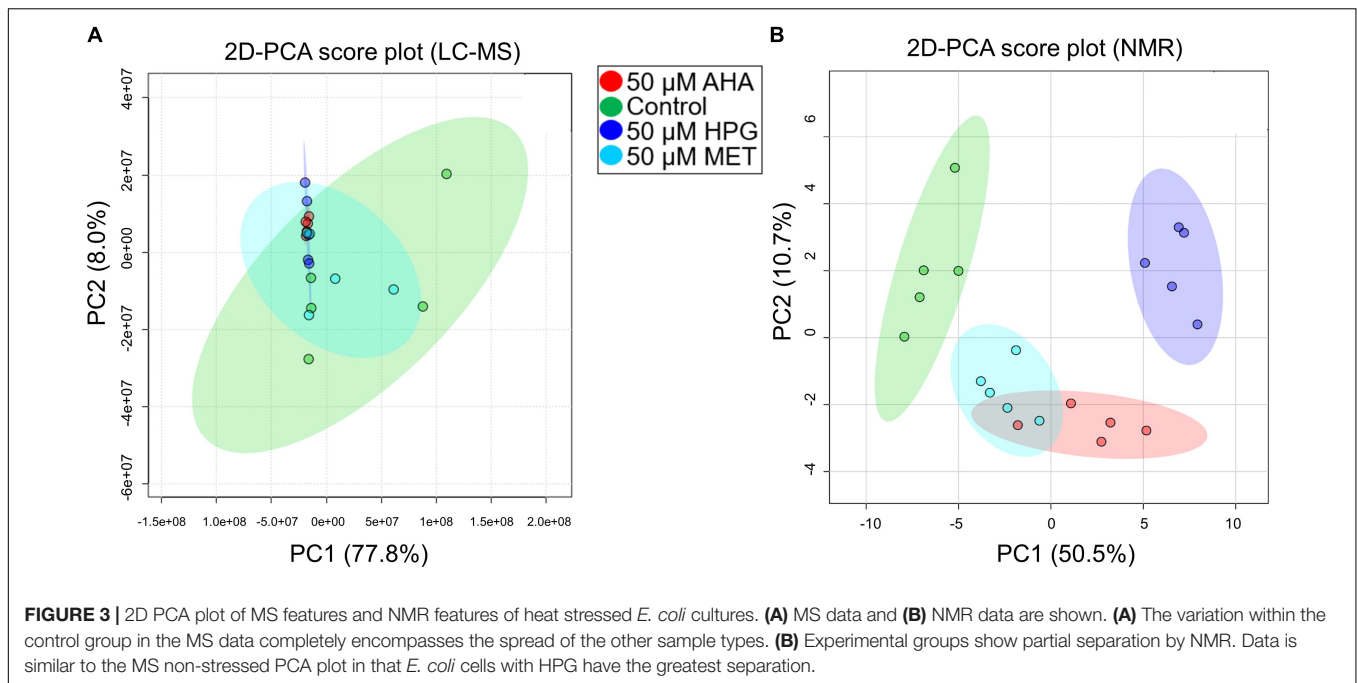
The heat-treated experiment of *E. coli* consisted of four groups grown at 42°C. Based on the first set of experiments, we narrowed the experimental conditions to 50 μ M AHA, HPG or MET. A MET-supplemented culture was again used as a baseline comparison for BONCAT addition, while the control samples contained minimal media. Metabolomics studies and resulting multivariate statistical analysis of LC-MS and NMR metabolite profiles were conducted on heat stressed *E. coli* cell cultures, using the same approach described above for the initial study. As with our initial NCAA addition experiments, physiological data was recorded throughout the growth of the *E. coli* to monitor phenotypic changes and to assess microbial health. Optical density measurements, averaged over biological replicates, were recorded throughout the *E. coli* incubation and growth periods, and indicated that all of the cultures were within 8% OD₆₀₀ of each other, with an average OD₆₀₀ of 1.3 after 210 min of cell growth. Bradford protein assays were

utilized to assess protein content and translational activity, prior to intracellular metabolite extraction, and indicated an average protein concentration of 2.1 mg/mL with all samples within 15% of the average concentration (**Supplementary Table S2**). The range in protein concentration revealed that *E. coli* grown in the presence of AHA, HPG or MET resulted in greater intracellular amounts of proteins than the control cell cultures.

MS Metabolomics of Cultures Grown With Non-canonical Amino Acids Under Heat Stress

NMR and MS analyses of the intracellular metabolomes of *E. coli* cell cultures grown under heat stress were undertaken utilizing the same analytical approaches described for our first set of experiments. LC-MS analysis identified 5,960 features across all samples. To assess variation and replication trends in the data, PCA analysis was undertaken using the MS metabolite profile data recorded on the heat stressed *E. coli* cell cultures and grown in the presence of AHA, HPG, MET or the no addition control conditions. Resulting 2D-PCA plots did not reveal significant separations between these different groups (**Figure 3A**), with PC1 accounting for 77.8% of the variance between AHA, HPG or MET treated groups (red, blue, and cyan circles) compared to control (green circle). Principal component 2 accounted for an additional 8% of the variance, reinforcing that similarities rather than differences in metabolic profiles between the treatment groups were most prominent. Variability between the control and the MET-treated cell cultures was as great as the difference between these two groups and the AHA and HPG treated groups. The MET, AHA, and HPG groups clustered more tightly, as illustrated by the shaded 95% confidence intervals of the different groups in the 2D PCA scores plot shown in **Figure 3A**, compared to that of the control group. The NCAA-treated samples clustered with each other, as did the control and MET-treated *E. coli* samples. This trend was present in the initial set of experiments conducted without heat stress and became more apparent in the PCA analysis of the stressed *E. coli* sample groups (**Figure 3A**).

An analysis of variance (ANOVA) was also undertaken for the MS-based metabolite profiles of the heat stressed and amino acid treated *E. coli* cell cultures (**Supplementary Figure S2**). A comparison of the treatment groups to each other, resulted in an *F* value (St Hle and Wold, 1989) of 0.274, and an *F* critical value of 2.61, indicating that the means of the metabolite profiles, i.e., means of the intensities of the MS spectral features, for all the sample groups were not significantly different, and no treatment group differed significantly from the others (**Supplementary Table S5**). A *post hoc* analysis using Tukey's honestly significant difference test (Tukey's HSD test) was conducted with MetaboAnalyst on individual MS spectral features to identify which features accounted most significantly for group differences between the different *E. coli* growth conditions (**Supplementary Table S6**). The analysis resulted in the identification of 907 features that changed in abundance, **Supplementary Figure S2**. This is less than 15% of all observed mass features. Each significantly changed feature was subjected to



the *post hoc* Tukey HSD test. Features that fell outside the means of other treatment groups is listed (**Supplementary Table S6**).

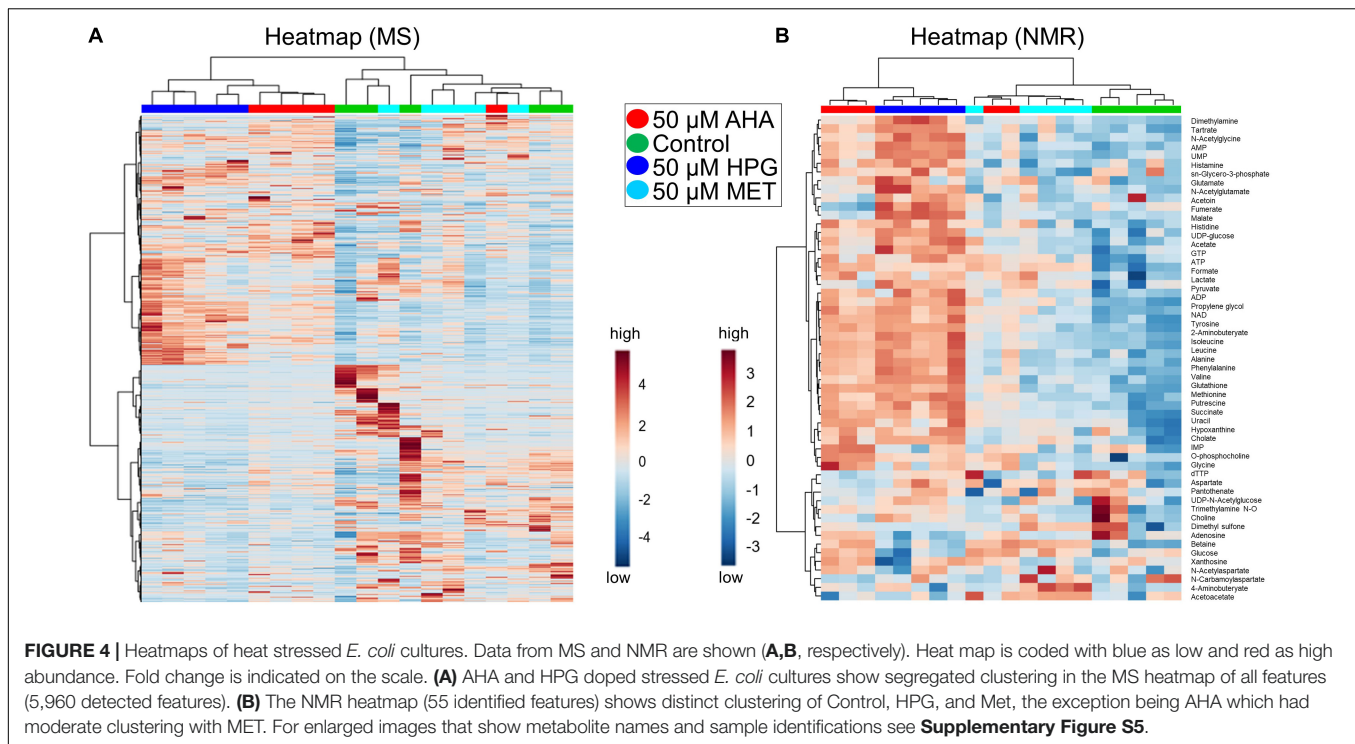
A second method employed to assess the data and the impact of AHA and HPG on the intracellular metabolome of *E. coli* was to analyze differentially regulated mass features in the heat stressed and AHA or HPG cell cultures compared to the *E. coli* heat stressed control groups and MET-doped cell cultures. Pairwise comparisons of AHA or HPG treated groups against the MET-treated *E. coli* cell cultures were conducted, as well as comparisons of control group and MET-treated cultures, which found that only 7% of the mass features were significantly different (fold change > 2, $p < 0.10$). Using the same criteria, the AHA and HPG samples were found to contain differentially expressed features at levels of 8 and 19% respectively compared to the MET-doped cultures. These analyses indicate that, while *E. coli* adapts metabolically to the presence of NCAA in its growth medium in the presence of heat stress, each NCAA supplementation impacts the intracellular metabolomes of the *E. coli* cultures in different ways. It also appears that HPG has a greater impact on the metabolome of *E. coli* than AHA, based on pairwise *t*-tests.

HCA results were plotted on a heatmap to visualize changes in the patterns of individual MS features identified between the NCAA supplemented heat stressed *E. coli* cell cultures. When taking into account all of the MS features, the AHA and HPG supplemented *E. coli* samples separated to a greater extent from the control and MET-supplemented samples, compared to the same groups analyzed in our initial study in the absence of heat stress (**Figures 2A, 4A**). Although the boundaries between groups were clearer, the HCA did not separate all replicates of a group into unique clusters, nor did the heatmap reveal the presence of a large number of features with significant fold changes (**Figure 4A**). A heatmap of the top 250 differentiated MS

features, as assessed by Tukey's HSD test, segregated into distinct sample groups best described by growth condition. The heatmap contained blocks of upregulated features that were characteristic of each of the treatment group (**Supplementary Figure S3**). In this HCA analysis, the AHA and HPG-supplemented groups clustered next to each other while the MET-supplemented and *E. coli* control groups were more similar. The differences between the AHA and HPG treated *E. coli* cell cultures compared to the control and MET-treated groups again showed that MS metabolomics can easily distinguish between the different growth conditions, even when the differentiated features amount to a relatively small proportion of the intracellular metabolome mass spectral features. The color changes on the heatmap indicate fold change, and although not large does reveal that a metabolic adaptation takes place upon addition of the NCAA to the growth medium. To complement the MS-based metabolomics analysis, NMR was utilized to expand metabolite identification and coverage, and to help with the assessment of the potential biological impact of those metabolic adaptations on the cellular phenotypes of *E. coli*.

NMR Metabolomics Analysis of Cultures Grown With Non-canonical Amino Acids Under Heat Stress

From analysis of 1D ^1H NMR spectra and spectral profiling using the Chenomx software, 55 metabolites were identified and quantified from intracellular metabolite extracts of the heat-stressed, NCAA-supplemented *E. coli* cultures (**Supplementary Table S7**). While the MS metabolomics data demonstrated the presence of a certain degree of metabolic adaptation occurring in these cell cultures, the 55 metabolites annotated and validated by NMR provided some clues as to which



metabolic pathways may be involved in these metabolic adaptations. The NMR metabolomics studies of the heat stressed, AHA, HPG, and MET supplemented *E. coli* cell cultures employed the same experimental workflow used for examining the intracellular metabolomes of the *E. coli* cell cultures in absence of heat stress.

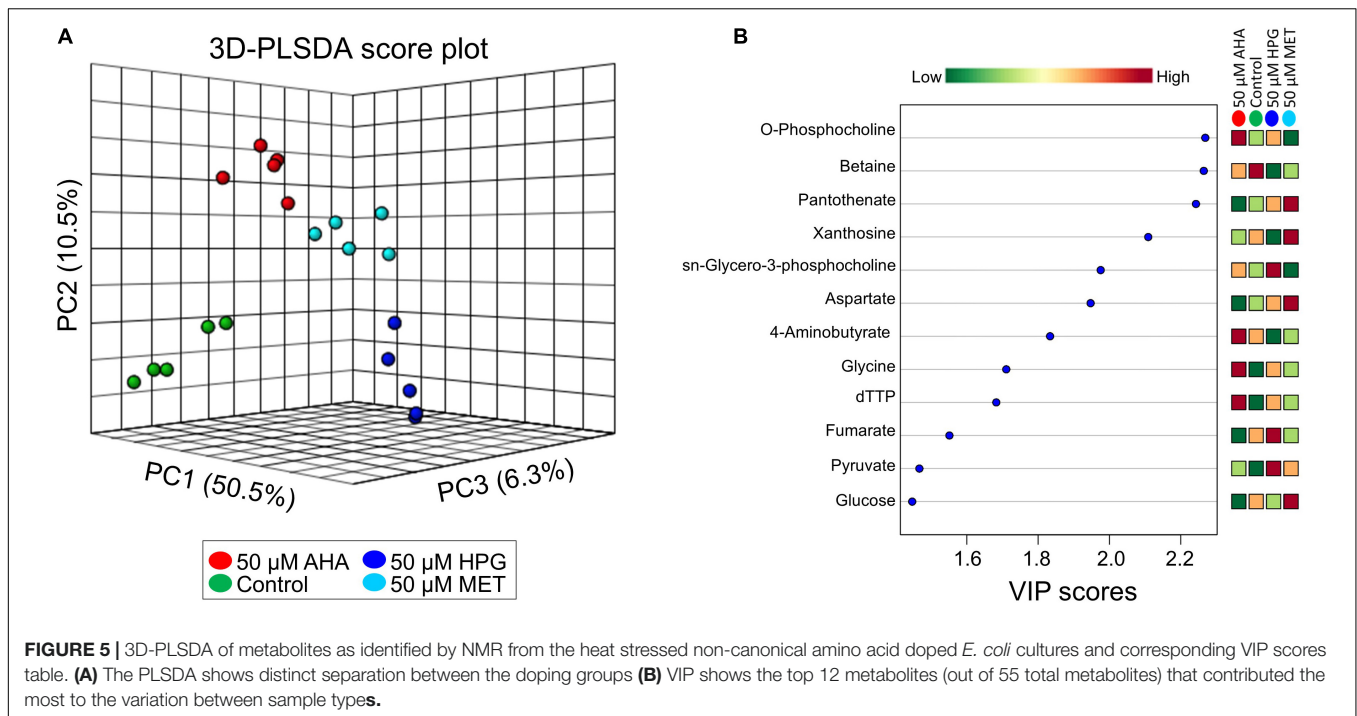
Group separations were assessed using PCA. The resulting 2D-PCA scores plots (**Figure 3B**) revealed that HPG, AHA, and MET-supplemented *E. coli* groups could be separated based on their distinct NMR-based metabolome profiles from the control group. Furthermore, the AHA and HPG-treated groups also separated from each other based on distinct metabolite patterns (**Figure 3B**, red and purple 95% confidence interval circles), while the metabolic profile of the MET supplemented group overlapped with that of the AHA-treated group (**Figure 3B**, red and cyan 95% confidence intervals). Analysis of loading factors (**Supplementary Table S8**) contributing to PC1 and PC2 of the 2D PCA-score plots revealed that betaine, xanthosine, *N*-carbamoyl-aspartate, glucose, 4-aminobutyrate, adenosine contributed significantly to PC1, which accounted for 50.5% of the variance, while PC2 accounted for an additional 10.7%.

Although the samples separated from each other primarily along the PC1 axis, as with the MS metabolomics findings, the variation between sample replicates was rather large and resulted in minimal separation by treatment type. In other words, although metabolic adaptations occur within the cell in the presence of NCAA under heat stress, those metabolic responses appear to be rather limited and do not seem to suggest that a significant overhaul of the metabolic machinery of *E. coli* is taking place.

HCA, schematically represented as a heatmap of relative NMR metabolite abundance, was employed to further evaluate differences among the metabolite profiles of each *E. coli* treatment group (**Figure 4B**). This heatmap was generated from changes in metabolite concentrations observed for the 54 metabolites that were identified by NMR. The control and MET-supplemented groups clustered more closely, while the NCAA treated groups formed a second cluster. The one exception was a replicate from the *E. coli* cell cultures supplemented with MET (**Figure 4B**). Consistent with the HCA analysis of the MS spectral features, the heatmap representation of the NMR-based metabolite profiles suggest that although AHA or HPG supplementation does induce changes in intracellular metabolome of *E. coli*, no dramatic metabolic alterations appeared to have taken place within the cells.

Combined Pathway Analysis of Heat Stressed *E. coli* Cultures

The NMR metabolomics data provided important information about potential changes in metabolic pathway usage based on a metabolic pathway impact analysis that was conducted using MetaboAnalyst. Partial least squares discriminate analysis (PLS-DA) (**Figure 5A**), with resulting variable importance in projection (VIP) scores for metabolites that have the highest discriminatory power among the treatment groups was used (Cho et al., 2008). Metabolites that contributed most to the separation of the different sample groups are listed in the VIP scores plot and revealed interesting trends between the different cell culture treatment groups (**Figure 5B**). This analysis indicated that NCAA addition impacted



amino acid, protein, and lipid metabolism. Intermediates in central carbon metabolism via lipid and amino acid synthesis and TCA cycle related metabolites, including aspartate, glycine, fumarate, glucose, pyruvate, malate, and 4-aminobutyrate were altered as a result of AHA or HPG supplementation in the growth medium, resulting in high differentiation between sample treatments for these molecules (**Supplementary Tables S9, S10**). Several metabolites related to pyruvate metabolism demonstrated a statistically significant differentiation between the NCAA-treated *E. coli* groups, supporting the idea that TCA cycle activity was altered in the AHA and HPG supplemented *E. coli* cell cultures. Intracellular levels of pyruvate, succinate, formate, and acetate were found to be higher in the *E. coli* cell cultures grown under heat stress and supplemented with NCAA. Metabolites associated with purine and amino acid metabolism, like xanthosine, dTTP, glycine and adenosine, also pointed to metabolic networks related to energy production as being altered in the NCAA treated cells. In addition to amino acid biosynthesis, glycerophospholipid metabolism was dysregulated as a result of NCAA incorporation with O-phosphocholine, and sn-glycero-3-phosphocholine are present at higher concentrations in the HPG and AHA supplemented cultures. Metabolites associated with lipid, amino acid and purine metabolism were consistently higher in abundance in the NCAA-treated *E. coli* groups than the control and methionine treated *E. coli*.

While NCAA addition impacted TCA cycle activity within the cell and potentially energy production via amino acid, purine and lipid metabolism, the implications of such metabolic changes remain unclear. Amino acid biosynthesis and

degradation were altered, as leucine, MET, and tyrosine were present at higher concentrations in the AHA and HPG doped samples than in the control and the MET doped samples (**Supplementary Figure S4**). These results indicate that amino acid metabolism in *E. coli* is altered upon addition of a NCAA suggesting that leucine, MET, and tyrosine catabolism may be suppressed in the AHA and HPG-supplemented cell cultures, or that other metabolites serve as metabolic precursors for energy production under these conditions, sparing the utilization of leucine, MET, and tyrosine for such purpose.

In the heat exposed, AHA or HPG doped growth conditions, intracellular levels of amino acids were found to be higher than in control or MET-treated groups (**Supplementary Tables S9, S10**), including higher abundance of acetylated amino acids like N-acetylglutamine, N-acetylglycine, and N-acetylserine. Acetylated amino acids could represent breakdown products of proteins that have been acetylated (Arnesen, 2011) and have been reported to be used for metabolic adaptations of microorganisms. Protein acetylation is a common post- and co-translational modification process for metabolic enzymes involved in central metabolism (Christensen et al., 2019). This modification is usually found on the side chains of amino acids, not on protein backbone residues, and could explain why free acetylated amino acids were detected in high abundances in the AHA and HPG-treated *E. coli* cell cultures. Amino acid acetylation could also be indicative of a higher rate of post translational modifications in the NCAA doped samples (Elf and Ehrenberg, 2005), which would suggest changes in the accuracy of protein translation, protein signaling, and protein-protein interactions. There are differing hypotheses on

the implications of acetylation on protein degradation. One school of thought indicates that it is protective (Carabetta and Cristea, 2017), while more recent studies have reported the opposite (Arnesen, 2011). Additional studies are needed to fully elucidate the impact of AHA or HPG supplementation on protein acetylation.

Screening for Potential Degradation Products of AHA and HPG

A question remaining to be addressed on the use of BONCAT relates to whether protein synthesis indeed serves as the only sink for the incorporation of NCAA or whether some organisms could be capable of metabolizing NCAA for their energetic needs. In an attempt to provide answers to this issue, our LC-MS data was searched for potential breakdown and conversion products of AHA and HPG as predicted from KEGG pathways and assuming that AHA or HPG could serve as substrates for enzymatic conversions. Potential compounds of interest included *N*-formyl-AHA and *N*-formyl-HPG as well as AHA/HPG versions of 4-(methylsulfanyl)-2-oxobutanoate. Other degradation products were ruled out because they all required activation of the MET-sulfur functional group, which is absent from both HPG and AHA. No features that matched these suspected products were detected. This implies that breakdown of AHA and HPG is not a major metabolic activity of *E. coli*, and that the main sink for AHA and HPG is, indeed, protein synthesis.

Summary

Utilizing NMR and LC-MS approaches, we were able to establish that NCAA addition can cause metabolic perturbation and adaptation in *E. coli*, especially when the bacteria are subjected to heat stress. MS analyses indicated that the presence of NCAA altered the concentration of approximately 15% of the global mass features identified based on ANOVA. To put this into perspective, the addition of MET altered the abundance of 7% of the all the mass spectral features detected in the *E. coli* cells. This mild perturbation is consistent with previous studies that have investigated the impact of AHA or HPG replacement of MET (Dieterich et al., 2006; Bagert et al., 2014; Hatzenpichler et al., 2014, 2016; Hatzenpichler and Orphan, 2015; Landgraf et al., 2015; Calve et al., 2016; Lehner et al., 2017). Although the observed metabolic changes were mild, the heatmaps and 2D-PCA score plots highlighted trends between the different *E. coli* treatment groups. HCA also showed that while AHA and HPG addition impacts the global metabolism of *E. coli* to some extent, the lack of group separation based on distinct metabolite profiles suggests that these metabolic changes are minimal under regular growth conditions and become more pronounced when cells are subjected to heat stress.

The global NMR and MS data are consistent in revealing the absence of significant group separation between the different *E. coli* cell cultures. The largest difference between groups was observed for the HPG-treated cells. Along this same trend, the AHA- and MET-doped cultures were more similar in metabolite

profiles, group clustering, and metabolic change at the individual metabolite level. HPG seemed to perturb *E. coli* to a larger extent than AHA based on paired *t*-tests which had 19 and 8% of metabolites changing, respectively. This was not expected because the differential impact of AHA and HPG on *E. coli* had not been reported previously.

The NMR data lent power to our analysis in the form of metabolite annotation and validation. Changes in specific metabolite levels indicated that pyruvate metabolism and intermediates of the TCA cycle were affected. Changes in central carbon metabolism is a common stress response in *E. coli*, so the perturbations we observed as a result of NCAA additions are consistent with this archetypical stress response (Jozefczuk et al., 2010). Along with TCA metabolites, glycerophospholipids, amino acids and acetylated amino acids were detected at higher concentrations in the AHA and HPG supplemented *E. coli* samples.

In-depth NMR and MS metabolomic analyses show that supplementing *E. coli* cultures with NCAA has an impact on the concentration of specific metabolites leading to a metabolic adjustment. This should serve as a cautionary note to scientists about how and when NCAA can be used. Our data implies that the common practices of using optical density for cells in culture or behavioral analyses for multicellular species to assess the impact of NCAA supplementation are not telling a complete story. Metabolic profiles do change, but our overall assessment is that under normal or even moderately stressful growth conditions, NCAA doping causes minor perturbations to the overall metabolic homeostasis of microbial cells.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the Metabolomics Work Bench, https://www.metabolomicsworkbench.org/data/MWTABMetadata4.php?F=kfsteward_20191206_111451_mwtab_analysis_1.txt&Mode=Study&DataMode=AllData&StudyType=MS#DataTabs.

AUTHOR CONTRIBUTIONS

RH, BB, VC, and KS conceptualized and designed the study. RH, BS, MD, BE, and NB worked on the experimental setup and manipulations. All authors analyzed and interpreted the data, critically revised the manuscript for important intellectual content. KS, BB, RH, and VC drafted the manuscript.

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SUPPLEMENTARY MATERIAL

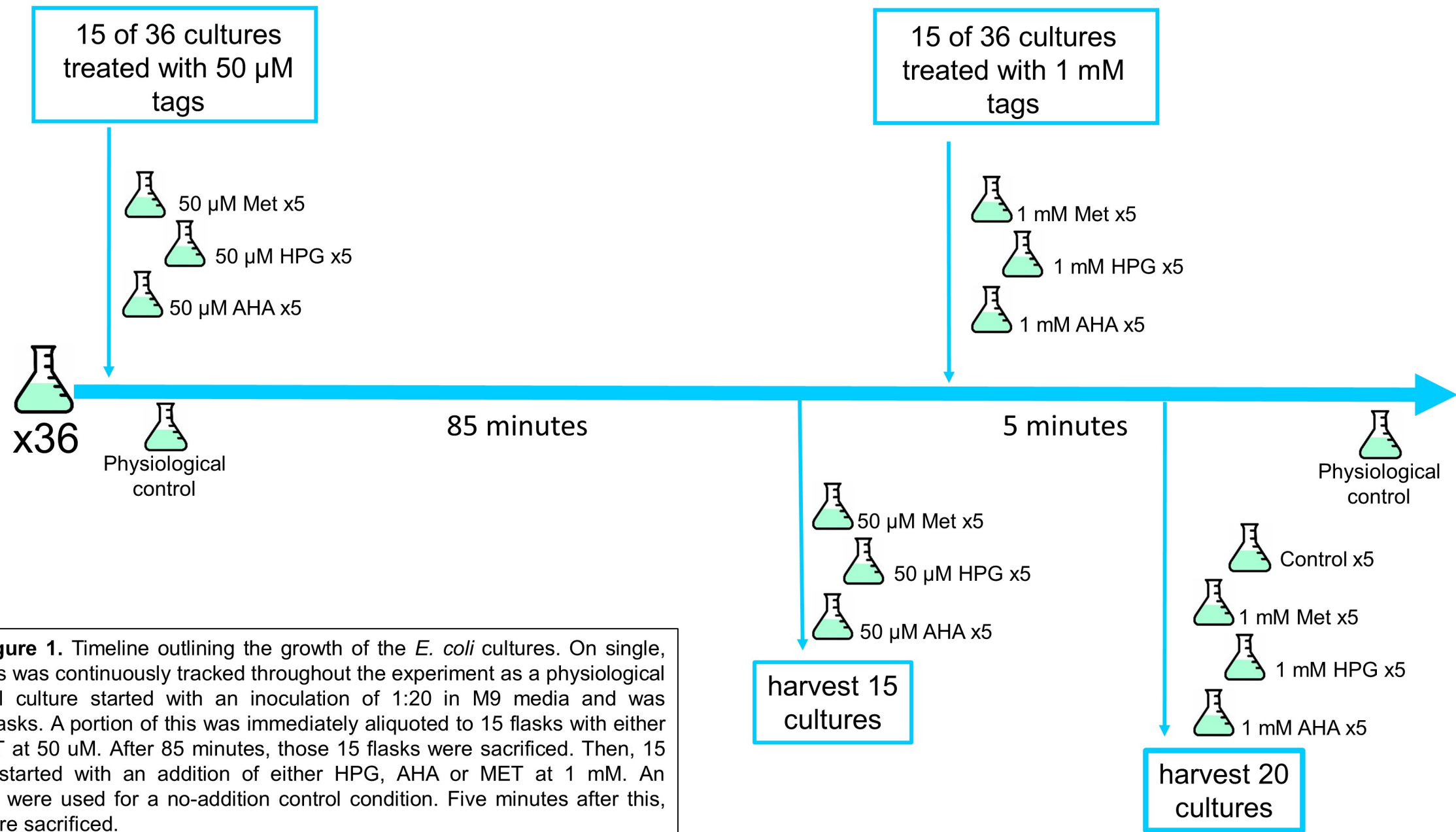
The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2020.00197/full#supplementary-material>

- opportunistic Heterotrophic bacteria. *Front. Microbiol.* 10:760. doi: 10.3389/fmicb.2019.00760
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplemental figure 1



Supplemental Figure 1. Timeline outlining the growth of the *E. coli* cultures. On single, no-amendment flask was continuously tracked throughout the experiment as a physiological control. The initial culture started with an inoculation of 1:20 in M9 media and was distributed to 36 flasks. A portion of this was immediately aliquoted to 15 flasks with either HPG, AHA or MET at 50 μ M. After 85 minutes, those 15 flasks were sacrificed. Then, 15 new flasks were started with an addition of either HPG, AHA or MET at 1 mM. An additional 5 flasks were used for a no-addition control condition. Five minutes after this, those 20 flasks were sacrificed.

Supplemental Figure 2

A. Data summary from heat stressed experiment

Anova: Single Factor

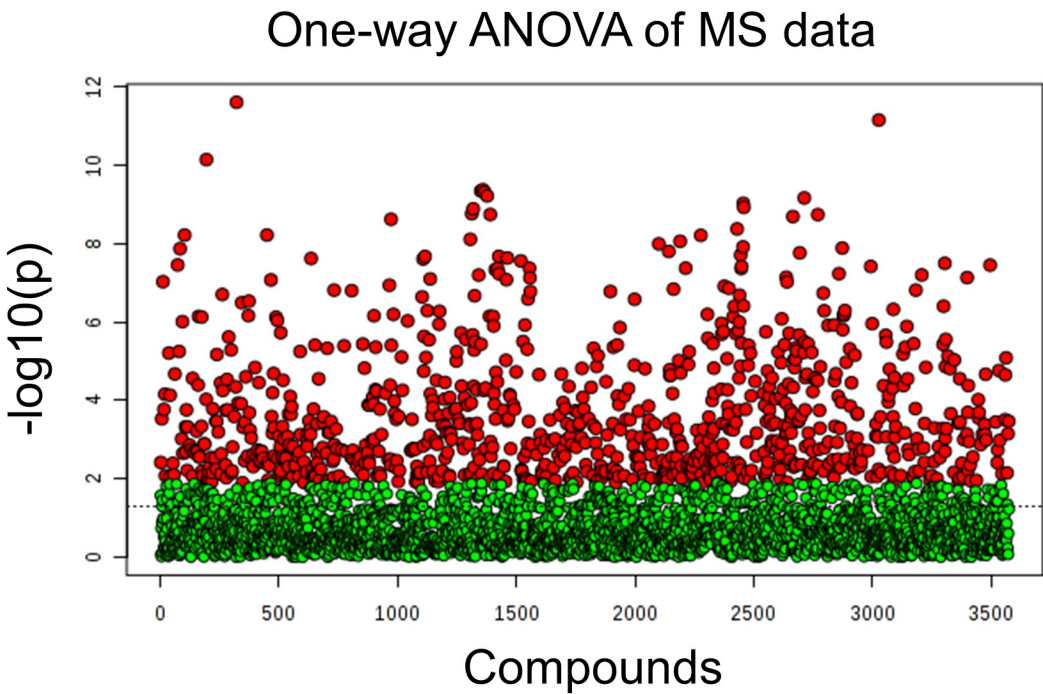
SUMMARY

Groups	Count	Sum	Average	Variance
CTRL avg	5960	9.80E+08	164503	1.53E+12
MET avg	5960	1.10E+09	184611	2.22E+12
AHA avg	5960	9.93E+08	166573	1.65E+12
HPG avg	5960	1.01E+09	169402	1.83E+12

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.49E+12	3	4.95E+11	0.27394	0.84424	2.60528
Within Groups	4.31E+16	23836	1.81E+12			
Total	4.31E+16	23839				

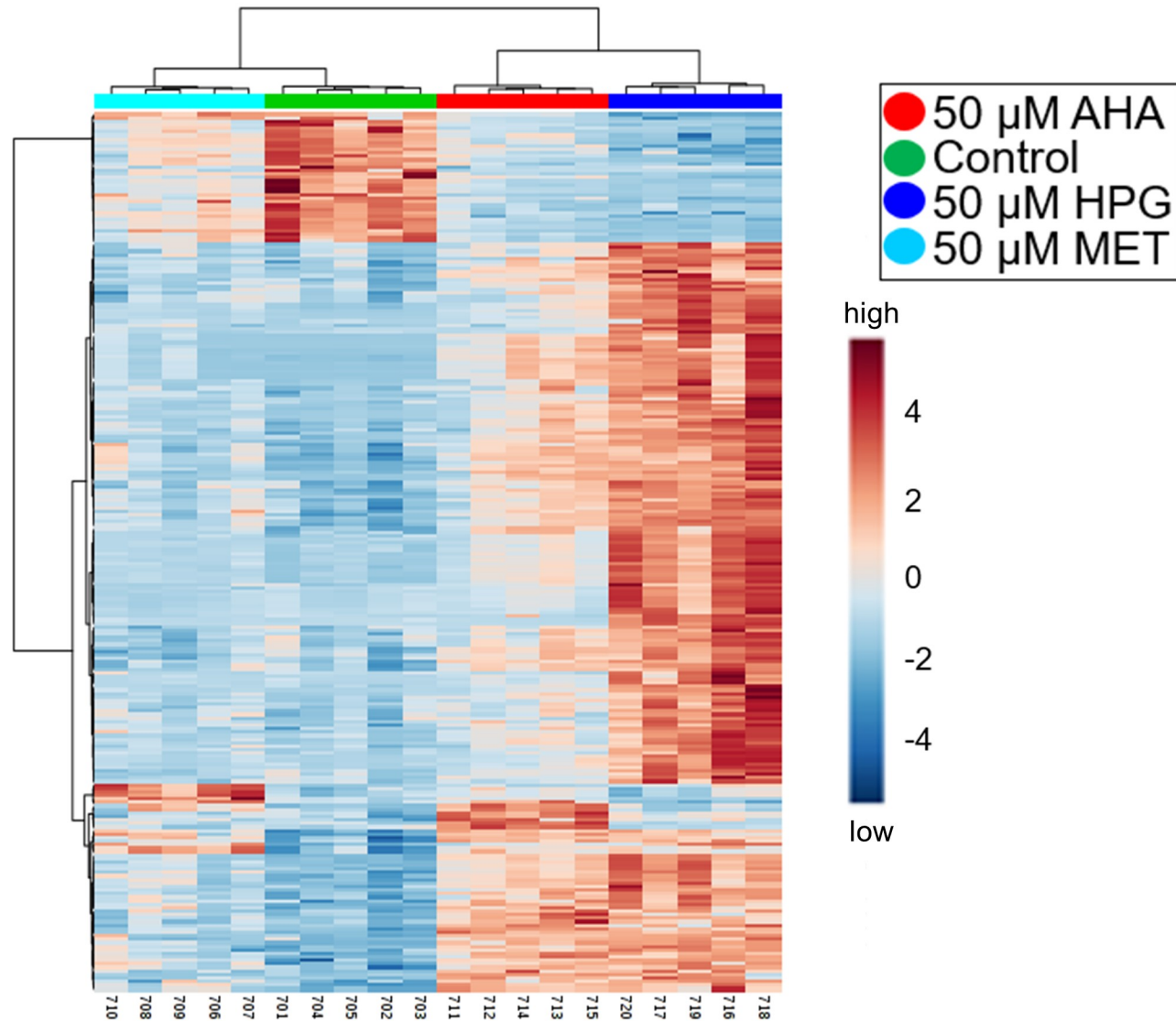
B.



907 features differentiated by ANOVA

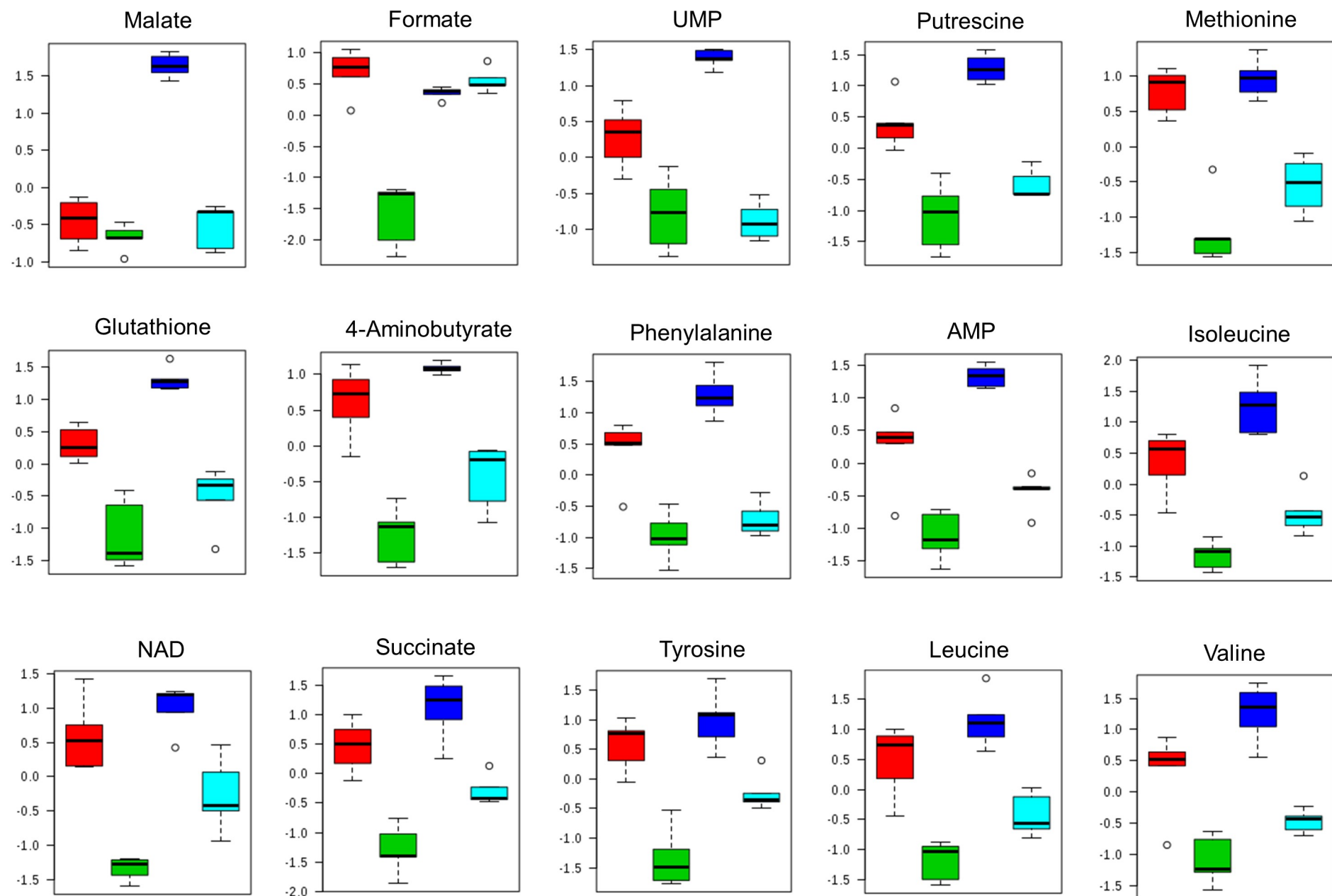
Supplemental Figure 2. (A) Table and (B) graphical ANOVA representation of heat stressed *E. coli* including all MS features. (B) Red circles represent features that were considered significant with a $-\log_{10}(P)$ value ≥ 1.5 . The graph indicates that 907 features (red circles) of the 5,960 total mass featured detected by LC-MS are differentially regulated. In contrast, mass features that are considered not significant ($-\log_{10}(P)$ value ≤ 1.5) are indicated as green circles.

Supplemental Figure 3



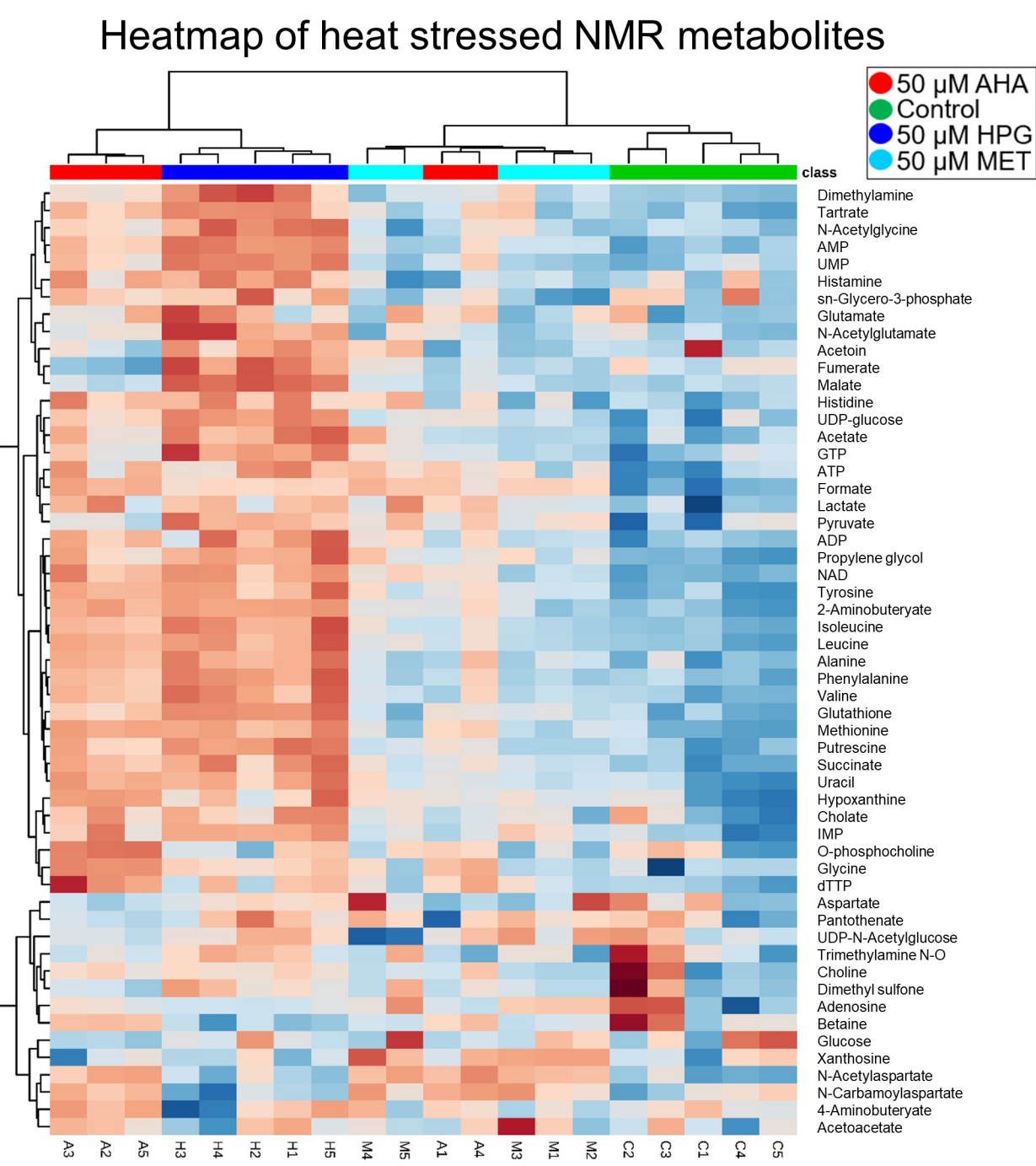
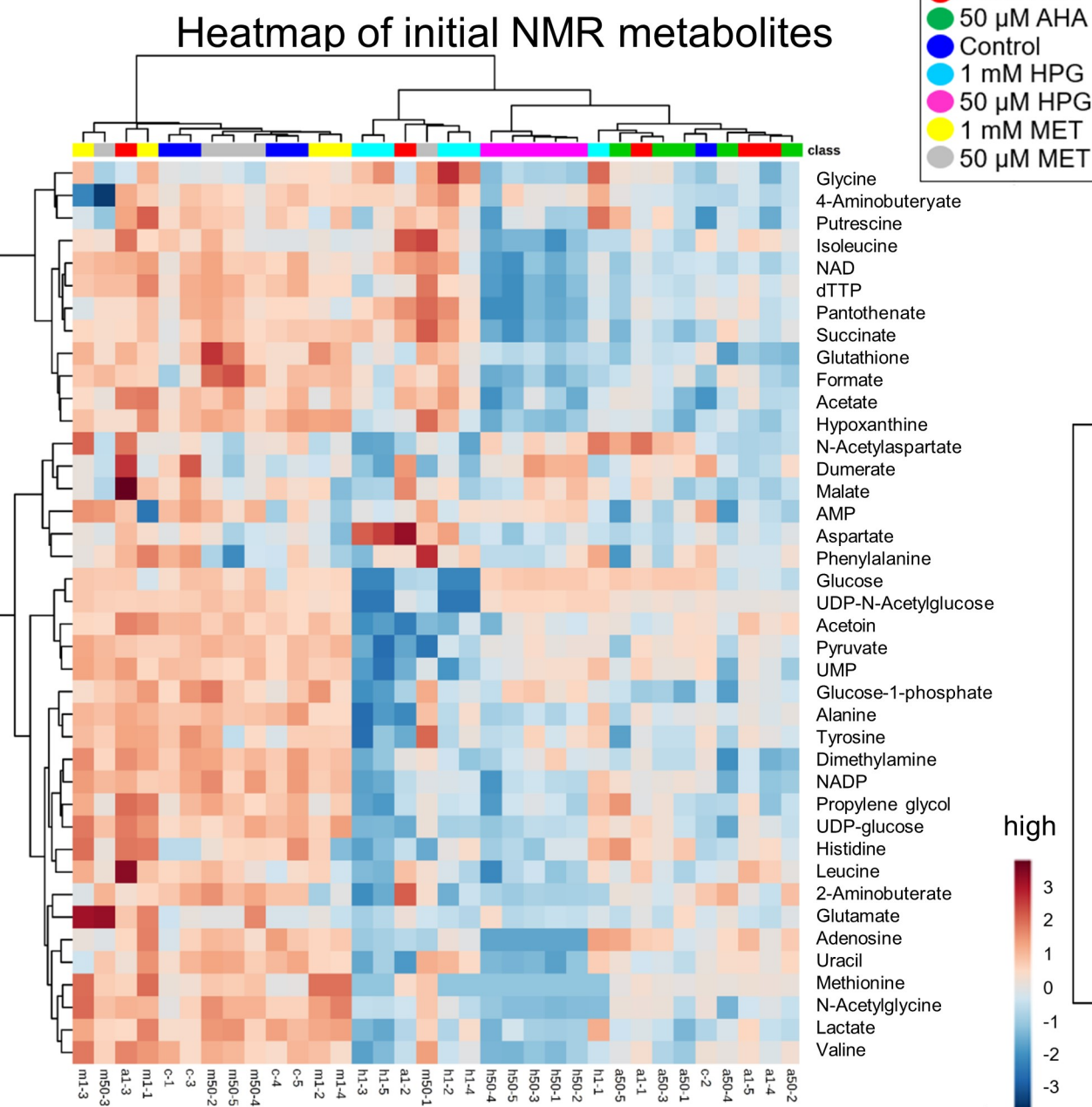
Supplemental figure 3. Heatmap of the top 250 features based on ANOVA significance for the heat stressed *E. coli* NCAA doped experiment.

Supplemental Figure 4



Supplemental Figure 4. Box and Whisker plots of the top 15 metabolites from the heat stressed *E. coli* NCAA doped experiment considered significant ($-\log_{10}(P)$ value ≥ 1.5) by ANOVA

Supplemental figure 5



Supplemental Figure 5. Enlarged images of NMR heatmaps from both initial and heat stressed conditions.

Table S1. OD growth curves. All within 8.0% of the overall average at 1.3.

Sample	OD		Average OD
	0 min	210 min	
C1	0.871	1.231	1.217
C2	0.871	1.264	
C3	0.871	1.224	
C4	0.871	1.180	
C5	0.871	1.184	
A1	0.870	1.290	1.335
A2	0.870	1.349	
A3	0.870	1.412	
A4	0.870	1.276	
A5	0.870	1.347	
H1	0.872	1.493	1.418
H2	0.872	1.311	
H3	0.872	1.354	
H4	0.872	1.456	
H5	0.872	1.474	
M1	0.871	1.283	1.265
M2	0.871	1.230	
M3	0.871	1.258	
M4	0.871	1.334	
M5	0.871	1.222	

Table S2. Bradford assay protein concentrations. All within 15.0% of the overall average at 2.1.

Sample #	mg/mL	Average mg/mL
1	1.94	1.83
2	1.90	
3	1.87	
4	1.74	
5	1.69	
6	1.91	1.94
7	1.91	
8	1.91	
9	2.16	
10	1.82	
11	2.15	2.36
12	2.37	
13	2.45	
14	2.25	
15	2.56	
16	2.37	2.43
17	2.35	
18	2.40	
19	2.40	
20	2.63	

Table S3. ANOVA single factor MS data in initial study

SUMMARY						
Groups	Count	Sum	Average	Variance		
CTRL avg	4036	9.57E+08	237029	5.66E+11		
M1 avg	4036	9.99E+08	247604	7.78E+11		
M50 avg	4036	9.43E+08	233555	6.93E+11		
A1 avg	4036	9.11E+08	225683	6.16E+11		
A50 avg	4036	8.59E+08	212862	5.88E+11		
H1 avg	4036	9.24E+08	228978	1.44E+12		
H50 avg	4036	8.56E+08	212201	7.09E+11		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	3.96E+12	6	6.60E+11	0.85787	0.52509	2.09892
Within Groups	2.17E+16	28245	7.69E+11			
Total	2.17E+16	28251				

ANOVA test on non-stressed data show that all are the same, because p -value is greater than the alpha value at 0.05, and the F value is much smaller than the F critical value.

Table S4. NMR metabolites in initial study.

Metabolite	Control		Met-1mM		Met-50µM		AHA-1mM		AHA-50µM		HPG-1mM		HPG-50µM	
	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
2-Aminobutyrate	3.0	0.3	1.8	0.5	3.3	0.7	2.7	1.8	2.5	0.8	1.0	0.4	1.2	0.0
4-Aminobutyrate	159.7	44.6	138.3	63.4	143.8	74.5	138.3	53.8	106.3	15.1	198.6	45.0	130.2	37.1
Acetate	613.4	225.4	747.9	245.0	691.1	134.6	675.0	255.0	429.4	85.3	604.8	184.3	345.5	105.8
Acetoin	2.6	0.5	3.0	1.0	2.1	0.9	2.2	1.7	1.8	0.4	1.0	0.6	1.4	0.5
Adenosine	1.6	1.1	2.3	1.9	1.3	0.9	1.3	0.7	1.6	0.9	1.1	1.2	0.0	0.0
Alanine	123.6	28.8	119.1	19.4	125.7	6.2	85.3	43.9	71.0	10.9	65.7	43.2	57.7	7.4
AMP	10.3	4.4	6.6	6.6	11.9	2.7	6.4	2.2	3.4	1.9	5.8	1.2	8.3	0.9
Aspartate	5.0	0.8	4.3	1.5	4.7	1.7	9.6	10.7	3.5	0.6	10.2	6.8	3.6	0.8
Dimethylamine	1.9	0.3	2.0	0.3	1.8	0.2	1.5	0.5	1.0	0.2	1.1	0.2	1.4	0.3
dTTP	28.4	6.2	30.9	7.6	34.6	6.6	23.2	5.0	18.2	2.5	21.5	3.9	11.4	1.5
Formate	274.8	86.8	369.7	24.8	470.7	136.5	295.6	62.4	249.4	46.8	286.9	88.0	170.7	20.1
Fumarate	5.6	2.0	4.2	0.3	3.5	0.7	5.7	2.8	4.5	0.5	3.1	0.6	5.6	1.0
Glucose	288.5	243.0	250.0	196.8	348.6	257.8	167.9	201.8	281.0	246.7	49.5	110.7	445.6	88.4
Glucose-1-phosphate	6.2	1.3	7.0	1.4	7.1	1.3	4.9	1.3	3.8	0.8	3.9	1.1	5.7	0.9
Glutamate	17.1	2.2	66.1	70.9	58.7	70.0	20.3	7.9	18.6	5.8	13.0	2.6	15.7	5.7
Glutathione	36.1	4.6	47.8	7.5	54.4	21.2	28.4	11.3	21.0	4.7	32.6	6.6	23.7	2.4
Glycine	15.3	3.7	21.4	3.6	15.9	7.0	13.0	2.9	14.6	2.7	35.5	11.1	10.4	1.1
Histidine	1.1	0.5	1.5	0.7	1.1	0.3	1.3	0.5	1.2	0.4	1.0	0.4	0.8	0.1
Hypoxanthine	9.3	3.1	12.2	1.7	11.2	3.7	5.5	1.6	4.7	1.1	6.5	2.2	3.4	0.5
Isoleucine	2.6	0.5	2.5	0.3	3.6	2.0	4.0	1.8	1.8	0.3	2.3	0.5	1.1	0.2
Lactate	118.1	62.0	210.3	38.9	158.5	61.8	73.9	31.2	46.5	13.8	71.5	65.7	37.2	12.2
Leucine	4.0	0.4	4.3	0.6	4.2	0.4	5.3	1.7	3.6	0.3	3.3	0.6	3.0	0.5
Malate	59.8	9.3	55.4	11.5	52.3	4.7	72.2	35.4	47.1	6.0	50.1	8.5	56.6	8.9
Methionine	3.2	1.0	361.1	34.5	16.9	2.9	5.9	2.6	2.7	0.4	0.0	0.0	0.0	0.0
N-Acetylaspargate	15.5	3.4	20.2	16.1	11.8	2.8	21.0	17.2	17.9	8.1	12.9	14.1	16.8	3.5
N-Acetylglutamine	1.1	0.2	2.1	0.5	1.7	0.3	1.0	0.4	0.7	0.2	0.7	0.2	0.5	0.0
NAD ⁺	22.2	5.0	24.1	4.9	27.5	3.9	20.8	7.6	14.3	1.4	21.4	5.4	10.3	1.5
NADP ⁺	3.4	0.8	3.5	0.5	3.6	1.0	2.2	1.0	2.0	0.7	1.9	0.9	1.6	0.4
Pantothenate	1.2	0.3	1.1	0.3	1.6	0.6	1.2	0.5	0.8	0.1	1.4	0.5	0.4	0.1
Phenylalanine	2.5	0.6	2.2	1.0	2.2	1.7	2.0	0.4	1.7	0.5	2.1	0.7	1.7	0.3
Propylene glycol	1.7	0.4	1.9	0.5	1.7	0.3	1.6	0.7	1.5	0.5	1.2	0.5	1.1	0.2
Putrescine	53.5	18.5	64.6	30.8	52.2	13.7	50.8	21.4	49.7	13.7	59.3	25.7	36.0	9.7
Pyruvate	95.0	28.0	129.4	42.8	105.9	59.5	54.8	35.7	34.7	23.8	20.3	19.5	47.3	10.5
Succinate	97.3	27.2	119.1	8.7	143.4	57.3	92.8	32.3	67.7	27.9	114.9	30.4	39.9	5.7
Tyrosine	9.6	2.5	10.4	1.2	10.4	3.9	7.7	3.0	5.4	1.1	5.9	2.7	7.1	1.5
UDP-glucose	15.9	4.6	22.3	4.7	16.9	3.1	15.0	7.6	10.7	3.1	9.4	3.9	9.1	1.0
UDP-N-Acetylglucosamine	3.7	0.6	3.9	0.8	4.2	1.8	2.5	0.9	2.4	0.3	0.8	1.8	3.6	0.4
UMP	10.4	1.3	10.8	2.2	9.8	2.3	8.4	4.3	5.7	2.0	4.4	3.9	7.5	1.1
Uracil	11.6	3.2	10.9	5.1	13.5	2.2	6.7	2.7	7.7	1.9	8.1	4.4	3.1	0.4
Valine	14.3	2.6	17.8	4.6	16.1	2.4	13.0	5.2	9.7	2.2	8.0	2.2	6.4	0.6

*Assignment with best-matched signals, all others validated.

Table S5. ANOVA single factor MS heat-stressed data.

SUMMARY						
Groups	Count	Sum	Average	Variance		
CTRL avg	5960	9.80E+08	164503	1.53E+12		
MET avg	5960	1.10E+09	184611	2.22E+12		
AHA avg	5960	9.93E+08	166573	1.65E+12		
HPG avg	5960	1.01E+09	169402	1.83E+12		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1.49E+12	3	4.95E+11	0.27394	0.84424	2.60528
Within Groups	4.31E+16	23836	1.81E+12			
Total	4.31E+16	23839				

Table S6. Tukey's results from MS ANOVA heat-stressed analysis. FC and t-test at 0.1 M v. C = 438 features, M v. H = 1119 features, M v. A = 465 features.

MZ feature	f.value	p.value	RT	FDR	Tukey's HSD
128.0671	169.6	2.46E-12	11.609	8.79E-09	HPG-AHA; HPG-CTRL; MET-HPG
334.9927	148.12	7.00E-12	11.155	1.25E-08	HPG-AHA; HPG-CTRL; MET-HPG
82.06306	109.35	7.15E-11	10.146	8.52E-08	HPG-AHA; HPG-CTRL; MET-HPG
614.1522	86.512	4.20E-10	9.3764	2.90E-07	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
613.1495	85.707	4.51E-10	9.346	2.90E-07	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
615.1487	84.844	4.86E-10	9.313	2.90E-07	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
635.1304	82.439	6.03E-10	9.2194	3.04E-07	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
219.076	81.135	6.80E-10	9.1676	3.04E-07	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-CTRL; MET-HPG
997.2834	77.759	9.34E-10	9.0297	3.71E-07	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
999.2831	75.321	1.18E-09	8.9267	4.19E-07	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
425.0357	74.471	1.29E-09	8.89	4.19E-07	CTRL-AHA; MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
613.1494	71.637	1.72E-09	8.7648	4.63E-07	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
651.1023	71.206	1.80E-09	8.7454	4.63E-07	HPG-AHA; HPG-CTRL; MET-HPG
356.986	71.127	1.81E-09	8.7419	4.63E-07	HPG-AHA; HPG-CTRL; MET-HPG
84.95764	70.038	2.03E-09	8.6922	4.84E-07	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
255.9729	68.546	2.38E-09	8.6231	5.32E-07	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
622.0188	63.458	4.20E-09	8.3762	8.84E-07	CTRL-AHA; MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
159.1455	60.438	6.01E-09	8.221	1.09E-06	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
614.1521	60.405	6.04E-09	8.2193	1.09E-06	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-CTRL; MET-HPG
140.0778	60.285	6.12E-09	8.2129	1.09E-06	CTRL-AHA; MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
823.4129	58.394	7.73E-09	8.1119	1.32E-06	HPG-AHA; HPG-CTRL; MET-HPG
173.1247	57.464	8.69E-09	8.0611	1.41E-06	CTRL-AHA; HPG-AHA; MET-AHA
168.072	56.271	1.01E-08	7.9947	1.57E-06	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
921.9953	54.912	1.21E-08	7.9176	1.80E-06	CTRL-AHA; MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
125.9832	54.396	1.29E-08	7.8879	1.85E-06	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
384.9744	54.105	1.35E-08	7.8711	1.85E-06	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
824.416	52.933	1.58E-08	7.8023	2.09E-06	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
939.3285	52.321	1.71E-08	7.7658	2.19E-06	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
767.4632	51.419	1.94E-08	7.7113	2.40E-06	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
881.3702	50.845	2.11E-08	7.6762	2.45E-06	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
317.1124	50.803	2.12E-08	7.6736	2.45E-06	CTRL-AHA; HPG-AHA; MET-AHA
663.4688	50.168	2.32E-08	7.6343	2.59E-06	HPG-AHA; HPG-CTRL; MET-HPG
261.9587	49.961	2.39E-08	7.6214	2.59E-06	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
300.2842	49.76	2.46E-08	7.6088	2.59E-06	CTRL-AHA; MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
134.0423	48.977	2.76E-08	7.5594	2.82E-06	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
283.2581	48.025	3.17E-08	7.4983	3.15E-06	CTRL-AHA; MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
384.1085	47.391	3.49E-08	7.457	3.31E-06	HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
765.4545	47.336	3.52E-08	7.4534	3.31E-06	HPG-AHA; HPG-CTRL; MET-HPG
158.0435	46.817	3.81E-08	7.4192	3.42E-06	CTRL-AHA; HPG-CTRL; MET-CTRL
118.1198	46.785	3.83E-08	7.4171	3.42E-06	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
825.4134	46.165	4.21E-08	7.3758	3.48E-06	HPG-AHA; HPG-CTRL; MET-HPG
941.3278	46.164	4.21E-08	7.3757	3.48E-06	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
690.4968	46.136	4.23E-08	7.3739	3.48E-06	HPG-AHA; HPG-CTRL; MET-HPG
882.3721	46.048	4.29E-08	7.3679	3.48E-06	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
226.1151	45.591	4.60E-08	7.3371	3.66E-06	HPG-AHA; HPG-CTRL; MET-HPG
132.0004	44.09	5.84E-08	7.2338	4.46E-06	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
883.3709	44.063	5.86E-08	7.2319	4.46E-06	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
168.0735	43.637	6.28E-08	7.2021	4.62E-06	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
622.0184	43.59	6.33E-08	7.1988	4.62E-06	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-CTRL; MET-HPG
59.07153	42.815	7.18E-08	7.1436	5.11E-06	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-CTRL; MET-HPG
395.6704	42.686	7.34E-08	7.1344	5.11E-06	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
691.5001	42.608	7.43E-08	7.1288	5.11E-06	HPG-AHA; HPG-CTRL; MET-HPG
693.5094	42.211	7.94E-08	7.1001	5.36E-06	HPG-AHA; HPG-CTRL; MET-HPG
662.4658	41.942	8.31E-08	7.0805	5.46E-06	HPG-AHA; HPG-CTRL; MET-HPG
837.6148	41.878	8.40E-08	7.0758	5.46E-06	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
377.1388	41.248	9.34E-08	7.0295	5.92E-06	HPG-AHA; HPG-CTRL; MET-HPG
391.2777	41.184	9.45E-08	7.0247	5.92E-06	CTRL-AHA; HPG-CTRL; MET-CTRL; MET-HPG
470.2531	40.06	1.15E-07	6.9404	7.07E-06	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
921.9952	39.715	1.22E-07	6.914	7.39E-06	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-CTRL; MET-HPG
178.9888	39.05	1.37E-07	6.8627	8.17E-06	HPG-AHA; HPG-CTRL; MET-HPG
766.4573	38.761	1.44E-07	6.8403	8.47E-06	HPG-AHA; HPG-CTRL; MET-HPG
144.0445	38.441	1.53E-07	6.8151	8.71E-06	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
202.1014	38.428	1.53E-07	6.8141	8.71E-06	HPG-AHA; HPG-CTRL; MET-HPG
523.1978	38.265	1.58E-07	6.8012	8.83E-06	CTRL-AHA; HPG-CTRL; MET-CTRL
522.1944	38.001	1.66E-07	6.7803	9.01E-06	CTRL-AHA; MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
692.5048	37.983	1.66E-07	6.7788	9.01E-06	HPG-AHA; HPG-CTRL; MET-HPG
117.1357	37.478	1.83E-07	6.7384	9.74E-06	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
408.0006	37.041	1.98E-07	6.703	1.04E-05	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
718.0465	36.827	2.06E-07	6.6856	1.07E-05	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
614.152	36.646	2.13E-07	6.6708	1.09E-05	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-CTRL; MET-HPG

283.258	36.304	2.28E-07	6.6426	1.15E-05	CTRL-AHA; MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
688.4808	35.659	2.58E-07	6.5888	1.28E-05	HPG-AHA; HPG-CTRL; MET-HPG
462.0506	35.564	2.63E-07	6.5808	1.29E-05	HPG-AHA; HPG-CTRL; MET-HPG
424.0967	35.002	2.93E-07	6.5331	1.42E-05	HPG-AHA; HPG-CTRL; MET-HPG
219.13	34.528	3.22E-07	6.4924	1.53E-05	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
122.0933	33.615	3.87E-07	6.4125	1.81E-05	CTRL-AHA; HPG-CTRL; MET-CTRL; MET-HPG
261.1229	33.518	3.95E-07	6.404	1.81E-05	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
598.17	33.508	3.95E-07	6.4031	1.81E-05	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
605.0016	32.626	4.74E-07	6.324	2.15E-05	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
609.6501	32.297	5.08E-07	6.2941	2.25E-05	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
303.136	32.251	5.13E-07	6.2898	2.25E-05	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
172.0386	32.225	5.16E-07	6.2874	2.25E-05	CTRL-AHA; MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
395.6703	32.074	5.33E-07	6.2736	2.29E-05	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
608.6577	31.325	6.25E-07	6.2039	2.65E-05	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
631.1122	31.244	6.36E-07	6.1963	2.65E-05	HPG-AHA; HPG-CTRL; MET-HPG
535.8933	31.238	6.37E-07	6.1957	2.65E-05	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
423.9749	30.892	6.87E-07	6.163	2.78E-05	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
425.0983	30.864	6.91E-07	6.1604	2.78E-05	HPG-AHA; HPG-CTRL; MET-HPG
598.6707	30.855	6.93E-07	6.1595	2.78E-05	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
622.0182	30.722	7.13E-07	6.1469	2.80E-05	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-CTRL; MET-HPG
702.0789	30.687	7.19E-07	6.1435	2.80E-05	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
394.9512	30.673	7.21E-07	6.1421	2.80E-05	HPG-AHA; HPG-CTRL; MET-HPG
597.6689	30.551	7.40E-07	6.1305	2.82E-05	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
689.4839	30.513	7.47E-07	6.1268	2.82E-05	HPG-AHA; HPG-CTRL; MET-HPG
437.2066	30.495	7.50E-07	6.1251	2.82E-05	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
718.0466	30.033	8.31E-07	6.0804	3.09E-05	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
859.2791	29.633	9.09E-07	6.0414	3.35E-05	HPG-AHA; HPG-CTRL; MET-HPG
239.1244	29.511	9.35E-07	6.0293	3.41E-05	MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
386.0198	29.319	9.76E-07	6.0103	3.53E-05	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
717.4595	29.207	1.00E-06	5.9992	3.58E-05	HPG-AHA; HPG-CTRL; MET-HPG
425.0079	28.886	1.08E-06	5.967	3.82E-05	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
307.5795	28.804	1.10E-06	5.9588	3.85E-05	HPG-AHA; HPG-CTRL; MET-HPG
146.0365	28.661	1.14E-06	5.9443	3.95E-05	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
445.9557	28.488	1.18E-06	5.9268	4.05E-05	HPG-AHA; HPG-CTRL; MET-HPG
144.0455	28.47	1.19E-06	5.925	4.05E-05	CTRL-AHA; MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
198.0808	28.36	1.22E-06	5.9137	4.11E-05	CTRL-AHA; MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
220.1319	28.232	1.26E-06	5.9007	4.20E-05	HPG-AHA; HPG-CTRL; MET-HPG
597.6687	28.108	1.29E-06	5.8879	4.28E-05	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
153.0373	27.831	1.38E-06	5.8593	4.53E-05	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
571.2017	27.302	1.57E-06	5.8041	5.10E-05	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
688.1455	27.088	1.65E-06	5.7815	5.33E-05	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
469.0623	26.878	1.74E-06	5.7591	5.56E-05	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
158.0435	26.781	1.78E-06	5.7487	5.64E-05	CTRL-AHA; HPG-CTRL; MET-CTRL
311.0875	26.578	1.88E-06	5.7269	5.87E-05	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
605.0014	26.55	1.89E-06	5.7239	5.87E-05	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
572.9464	26.481	1.92E-06	5.7166	5.92E-05	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
616.6419	26.389	1.97E-06	5.7066	6.00E-05	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
429.1327	26.226	2.05E-06	5.6888	6.20E-05	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
276.044	26.07	2.13E-06	5.6718	6.37E-05	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
845.2762	26.055	2.14E-06	5.6702	6.37E-05	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
600.1662	25.993	2.17E-06	5.6633	6.41E-05	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
300.283	25.722	2.33E-06	5.6335	6.81E-05	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-CTRL; MET-HPG
416.082	25.58	2.41E-06	5.6178	7.01E-05	HPG-AHA; HPG-CTRL; MET-HPG
632.115	25.311	2.58E-06	5.5878	7.45E-05	HPG-AHA; HPG-CTRL; MET-HPG
275.041	25.055	2.76E-06	5.559	7.84E-05	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
307.0895	25.053	2.76E-06	5.5587	7.84E-05	HPG-AHA; HPG-CTRL; MET-HPG
370.0449	24.941	2.84E-06	5.5461	8.01E-05	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
686.0868	24.574	3.13E-06	5.5041	8.71E-05	CTRL-AHA; HPG-CTRL; MET-CTRL; MET-HPG
824.9636	24.561	3.14E-06	5.5027	8.71E-05	CTRL-AHA; HPG-CTRL; MET-CTRL; MET-HPG
810.6257	24.527	3.17E-06	5.4988	8.72E-05	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
128.1403	24.432	3.25E-06	5.4878	8.88E-05	CTRL-AHA; MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
909.3967	24.244	3.42E-06	5.466	9.26E-05	CTRL-AHA; HPG-CTRL; MET-HPG
420.0487	24.103	3.55E-06	5.4497	9.50E-05	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
168.0689	24.093	3.56E-06	5.4486	9.50E-05	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
149.0774	24.058	3.59E-06	5.4444	9.52E-05	CTRL-AHA; HPG-CTRL; MET-HPG
225.8532	24.007	3.64E-06	5.4385	9.58E-05	CTRL-AHA; HPG-CTRL; MET-CTRL; MET-HPG
597.6691	23.978	3.67E-06	5.4351	9.58E-05	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
247.9936	23.916	3.73E-06	5.4278	9.67E-05	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
760.5372	23.869	3.78E-06	5.4222	9.73E-05	HPG-AHA; HPG-CTRL; MET-HPG
533.2459	23.801	3.85E-06	5.4143	9.84E-05	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
237.0858	23.758	3.90E-06	5.4092	9.84E-05	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
500.0049	23.744	3.91E-06	5.4076	9.84E-05	HPG-AHA; HPG-CTRL; MET-HPG
188.1718	23.721	3.94E-06	5.4049	9.84E-05	MET-AHA; MET-CTRL; MET-HPG
307.5796	23.615	4.05E-06	5.3923	0.000101	HPG-AHA; HPG-CTRL; MET-HPG

256.0985	23.339	4.37E-06	5.3594	0.000107	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
103.9531	23.336	4.37E-06	5.359	0.000107	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-CTRL; MET-HPG
421.2288	23.318	4.40E-06	5.3569	0.000107	CTRL-AHA; HPG-AHA; MET-AHA; MET-CTRL
164.9909	23.203	4.54E-06	5.3431	0.00011	CTRL-AHA; HPG-CTRL; MET-CTRL; MET-HPG
293.1112	23.098	4.67E-06	5.3305	0.000111	HPG-AHA; HPG-CTRL; MET-HPG
307.1264	23.098	4.67E-06	5.3305	0.000111	HPG-AHA; HPG-CTRL; MET-HPG
811.2938	22.976	4.83E-06	5.3157	0.000114	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
687.0901	22.914	4.92E-06	5.3082	0.000116	CTRL-AHA; HPG-CTRL; MET-CTRL; MET-HPG
418.9942	22.751	5.15E-06	5.2884	0.00012	HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
598.1698	22.728	5.18E-06	5.2855	0.00012	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
832.9592	22.551	5.45E-06	5.2639	0.000126	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
402.9468	22.433	5.63E-06	5.2492	0.000129	HPG-AHA; HPG-CTRL; MET-HPG
233.0604	22.424	5.65E-06	5.2481	0.000129	CTRL-AHA; HPG-CTRL; MET-CTRL
116.0675	22.386	5.71E-06	5.2435	0.000129	CTRL-AHA; HPG-CTRL; MET-CTRL
592.1349	22.365	5.74E-06	5.2409	0.000129	CTRL-AHA; HPG-AHA; MET-AHA
268.1231	22.334	5.79E-06	5.2371	0.000129	CTRL-AHA; HPG-CTRL; MET-CTRL; MET-HPG
175.0187	22.239	5.95E-06	5.2253	0.000132	HPG-AHA; HPG-CTRL; MET-HPG
831.9568	22.229	5.97E-06	5.224	0.000132	CTRL-AHA; HPG-CTRL; MET-CTRL
377.0975	22.102	6.19E-06	5.2082	0.000136	HPG-AHA; HPG-CTRL; MET-HPG
188.876	22.056	6.27E-06	5.2024	0.000137	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
259.1555	21.894	6.57E-06	5.1821	0.000142	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
402.2102	21.812	6.73E-06	5.1718	0.000145	MET-AHA; MET-CTRL; MET-HPG
216.954	21.681	7.00E-06	5.1552	0.00015	HPG-AHA; HPG-CTRL; MET-HPG
235.0712	21.592	7.18E-06	5.1439	0.000153	HPG-AHA; HPG-CTRL; MET-HPG
144.045	21.5	7.38E-06	5.1322	0.000156	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
259.0674	21.302	7.82E-06	5.1068	0.000164	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
319.1255	21.228	7.99E-06	5.0972	0.000167	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
283.258	21.139	8.21E-06	5.0856	0.000171	CTRL-AHA; HPG-CTRL; MET-CTRL; MET-HPG
192.0281	20.985	8.60E-06	5.0656	0.000178	CTRL-AHA; MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
624.6259	20.922	8.76E-06	5.0575	0.00018	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
320.7104	20.825	9.02E-06	5.0447	0.000184	CTRL-AHA; HPG-CTRL; MET-CTRL
241.1794	20.734	9.27E-06	5.0328	0.000188	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
656.8367	20.669	9.46E-06	5.0243	0.000191	HPG-AHA; HPG-CTRL; MET-HPG
173.039	20.621	9.59E-06	5.018	0.000193	HPG-AHA; HPG-CTRL; MET-HPG
591.1312	20.523	9.89E-06	5.005	0.000197	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL
708.0687	20.301	1.06E-05	4.9754	0.00021	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
704.0622	20.194	1.09E-05	4.9611	0.000216	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
794.486	19.874	1.21E-05	4.9179	0.000237	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
457.2414	19.733	1.26E-05	4.8987	0.000247	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
310.0818	19.707	1.27E-05	4.8951	0.000247	CTRL-AHA; HPG-CTRL; MET-CTRL; MET-HPG
766.9187	19.566	1.33E-05	4.8759	0.000257	HPG-AHA; HPG-CTRL; MET-HPG
307.5797	19.491	1.36E-05	4.8655	0.000262	HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
306.1902	19.469	1.37E-05	4.8626	0.000262	CTRL-AHA; HPG-AHA; MET-CTRL; MET-HPG
851.9494	19.432	1.39E-05	4.8573	0.000264	CTRL-AHA; HPG-CTRL; MET-CTRL
147.1094	19.337	1.43E-05	4.8443	0.000271	HPG-AHA; HPG-CTRL; MET-HPG
96.04388	19.317	1.44E-05	4.8415	0.000271	HPG-AHA; HPG-CTRL; MET-HPG
823.6105	19.266	1.46E-05	4.8343	0.000274	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
385.1121	19.196	1.50E-05	4.8247	0.000278	HPG-AHA; HPG-CTRL; MET-HPG
189.1746	19.193	1.50E-05	4.8242	0.000278	MET-AHA; MET-CTRL; MET-HPG
480.9936	19.142	1.52E-05	4.8171	0.000281	HPG-AHA; HPG-CTRL; MET-HPG
221.0254	19.105	1.54E-05	4.8119	0.000282	CTRL-AHA; HPG-CTRL; MET-HPG
162.0182	19.095	1.55E-05	4.8104	0.000282	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
402.0125	19.013	1.59E-05	4.7989	0.000288	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
171.1452	18.894	1.65E-05	4.7822	0.000298	MET-AHA; MET-CTRL; MET-HPG
439.0625	18.751	1.73E-05	4.762	0.000311	CTRL-AHA; HPG-CTRL; MET-CTRL
606.1243	18.684	1.77E-05	4.7524	0.000316	HPG-AHA; HPG-CTRL; MET-HPG
367.0533	18.626	1.80E-05	4.7441	0.000321	HPG-AHA; HPG-CTRL; MET-HPG
921.9952	18.503	1.88E-05	4.7266	0.000332	CTRL-AHA; HPG-CTRL; MET-CTRL; MET-HPG
283.258	18.439	1.92E-05	4.7174	0.000338	CTRL-AHA; HPG-CTRL; MET-CTRL
173.5405	18.301	2.01E-05	4.6975	0.000352	HPG-AHA; HPG-CTRL; MET-HPG
180.0476	18.242	2.05E-05	4.6889	0.000357	CTRL-AHA; HPG-CTRL; MET-CTRL; MET-HPG
758.2555	18.198	2.08E-05	4.6826	0.00036	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
382.9945	18.147	2.11E-05	4.6751	0.000365	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
456.2377	18.126	2.13E-05	4.6721	0.000365	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
102.1253	18.118	2.13E-05	4.6708	0.000365	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
369.1177	18.095	2.15E-05	4.6676	0.000366	HPG-AHA; HPG-CTRL; MET-HPG
145.047	18.021	2.20E-05	4.6567	0.000373	CTRL-AHA; MET-AHA; HPG-CTRL; MET-CTRL; MET-HPG
520.9049	17.986	2.23E-05	4.6516	0.000376	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
832.2904	17.952	2.26E-05	4.6466	0.000379	HPG-AHA; HPG-CTRL; MET-HPG
171.1817	17.856	2.33E-05	4.6325	0.000389	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
132.099	17.662	2.49E-05	4.6037	0.000414	CTRL-AHA; HPG-CTRL; MET-CTRL
616.6419	17.517	2.62E-05	4.5821	0.000432	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-HPG
100.1096	17.509	2.62E-05	4.5809	0.000432	CTRL-AHA; HPG-AHA; MET-AHA
655.1169	17.474	2.66E-05	4.5758	0.000436	HPG-AHA; HPG-CTRL; MET-HPG

272.1374	17.46	2.67E-05	4.5736	0.000436	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
118.0835	17.435	2.69E-05	4.5699	0.000437	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-CTRL
482.9484	17.388	2.74E-05	4.5627	0.000443	HPG-AHA; HPG-CTRL; MET-HPG
242.0615	17.332	2.79E-05	4.5543	0.000449	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
141.0013	17.312	2.81E-05	4.5513	0.000449	CTRL-AHA; HPG-CTRL; MET-HPG
289.0438	17.311	2.81E-05	4.5511	0.000449	HPG-AHA; HPG-CTRL; MET-HPG
845.9586	17.29	2.83E-05	4.5481	0.00045	CTRL-AHA; HPG-CTRL; MET-CTRL
615.1509	17.2	2.92E-05	4.5344	0.000462	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
873.2821	17.069	3.06E-05	4.5145	0.00048	HPG-AHA; HPG-CTRL; MET-HPG
373.0805	17.064	3.06E-05	4.5138	0.00048	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
846.2929	17.046	3.08E-05	4.511	0.000481	CTRL-AHA; HPG-CTRL; MET-CTRL
473.1627	16.994	3.14E-05	4.503	0.000488	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
132.099	16.882	3.27E-05	4.4858	0.000506	CTRL-AHA; HPG-CTRL; MET-CTRL
394.9969	16.829	3.33E-05	4.4777	0.000513	HPG-AHA; HPG-CTRL; MET-HPG
825.2973	16.783	3.38E-05	4.4706	0.000519	HPG-AHA; HPG-CTRL; MET-HPG
764.6054	16.71	3.47E-05	4.4594	0.000531	CTRL-AHA; HPG-CTRL; MET-CTRL
402.8166	16.675	3.52E-05	4.454	0.000535	CTRL-AHA; HPG-CTRL; MET-CTRL; MET-HPG
169.0786	16.651	3.55E-05	4.4501	0.000537	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
405.0025	16.621	3.59E-05	4.4455	0.000541	CTRL-AHA; HPG-CTRL; MET-HPG
72.0791	16.486	3.76E-05	4.4245	0.000565	CTRL-AHA; HPG-CTRL; MET-HPG
383.0236	16.29	4.04E-05	4.3936	0.000604	HPG-AHA; HPG-CTRL; MET-HPG
479.0816	16.262	4.08E-05	4.3892	0.000608	HPG-AHA; HPG-CTRL; MET-HPG
405.0024	16.197	4.18E-05	4.3789	0.000617	CTRL-AHA; HPG-CTRL; MET-HPG
260.0701	16.19	4.19E-05	4.3778	0.000617	HPG-AHA; HPG-CTRL; MET-HPG
372.9554	16.187	4.19E-05	4.3774	0.000617	HPG-AHA; HPG-CTRL; MET-HPG
607.1046	16.119	4.30E-05	4.3665	0.00063	CTRL-AHA; MET-AHA; HPG-CTRL
824.6286	16.077	4.37E-05	4.3599	0.000636	CTRL-AHA; HPG-CTRL; MET-CTRL
636.4502	16.066	4.38E-05	4.3581	0.000636	HPG-AHA; HPG-CTRL; MET-HPG
304.1808	16.059	4.40E-05	4.357	0.000636	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-CTRL
420.2265	15.999	4.49E-05	4.3474	0.000648	CTRL-AHA; HPG-AHA; MET-CTRL; MET-HPG
823.2758	15.921	4.62E-05	4.3349	0.000664	HPG-AHA; HPG-CTRL; MET-HPG
489.9245	15.911	4.64E-05	4.3332	0.000664	CTRL-AHA; HPG-CTRL; MET-HPG
648.2625	15.802	4.83E-05	4.3158	0.000688	CTRL-AHA; HPG-CTRL; MET-HPG
307.0785	15.779	4.87E-05	4.3121	0.000691	HPG-AHA; HPG-CTRL; MET-HPG
157.0418	15.709	5.00E-05	4.3007	0.000707	CTRL-AHA; MET-AHA; HPG-CTRL
750.9434	15.631	5.15E-05	4.288	0.000725	HPG-AHA; HPG-CTRL; MET-HPG
771.5548	15.606	5.20E-05	4.284	0.000729	CTRL-AHA; HPG-CTRL; MET-CTRL
433.1229	15.561	5.29E-05	4.2766	0.000739	HPG-AHA; HPG-CTRL; MET-HPG
192.0272	15.512	5.39E-05	4.2687	0.000749	CTRL-AHA; MET-AHA; HPG-CTRL; MET-CTRL
617.1651	15.499	5.41E-05	4.2665	0.00075	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
352.2203	15.453	5.51E-05	4.2589	0.00076	CTRL-AHA; MET-AHA; HPG-CTRL
277.0402	15.431	5.55E-05	4.2554	0.000764	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
411.1067	15.42	5.58E-05	4.2535	0.000764	CTRL-AHA; HPG-CTRL; MET-HPG
446.0681	15.323	5.79E-05	4.2375	0.00079	HPG-AHA; HPG-CTRL; MET-HPG
832.2893	15.255	5.94E-05	4.2262	0.000807	CTRL-AHA; HPG-CTRL; MET-CTRL
174.0385	15.066	6.39E-05	4.1948	0.000865	HPG-AHA; HPG-CTRL; MET-HPG
859.9611	15.017	6.51E-05	4.1867	0.000878	CTRL-AHA; HPG-CTRL; MET-CTRL
449.7817	14.993	6.57E-05	4.1827	0.000882	CTRL-AHA; HPG-CTRL; MET-CTRL
129.9289	14.98	6.60E-05	4.1805	0.000884	CTRL-AHA; HPG-CTRL; MET-CTRL
724.9749	14.97	6.62E-05	4.1789	0.000884	HPG-AHA; HPG-CTRL; MET-HPG
832.6236	14.961	6.65E-05	4.1772	0.000884	HPG-AHA; HPG-CTRL; MET-HPG
370.2523	14.922	6.75E-05	4.1707	0.000894	CTRL-AHA; HPG-CTRL; MET-CTRL
402.0676	14.845	6.95E-05	4.1577	0.000917	CTRL-AHA; HPG-CTRL; MET-HPG
665.1087	14.79	7.10E-05	4.1485	0.000934	CTRL-AHA; HPG-CTRL; MET-CTRL
378.2164	14.702	7.35E-05	4.1335	0.000963	CTRL-AHA; HPG-AHA; MET-AHA; HPG-CTRL; MET-CTRL
397.0897	14.687	7.40E-05	4.131	0.000965	HPG-AHA; HPG-CTRL; MET-HPG
189.1746	14.59	7.68E-05	4.1145	0.000997	MET-AHA; MET-CTRL; MET-HPG
534.8978	14.586	7.70E-05	4.1138	0.000997	CTRL-AHA; HPG-CTRL; MET-HPG
636.1335	14.571	7.74E-05	4.1112	0.000999	HPG-AHA; HPG-CTRL; MET-HPG
156.0386	14.549	7.81E-05	4.1075	0.001004	CTRL-AHA; MET-AHA; HPG-CTRL
116.0676	14.536	7.85E-05	4.1051	0.001006	CTRL-AHA; HPG-CTRL; MET-CTRL
665.4842	14.515	7.92E-05	4.1015	0.001008	HPG-AHA; HPG-CTRL; MET-HPG
979.5301	14.512	7.92E-05	4.1011	0.001008	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
692.1309	14.471	8.05E-05	4.0941	0.001021	HPG-AHA; HPG-CTRL; MET-HPG
418.0747	14.45	8.12E-05	4.0904	0.001026	HPG-AHA; HPG-CTRL; MET-HPG
481.2328	14.393	8.31E-05	4.0806	0.001046	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
360.1707	14.362	8.41E-05	4.0752	0.001055	CTRL-AHA; HPG-CTRL; MET-CTRL
438.1669	14.342	8.48E-05	4.0718	0.00106	HPG-AHA; HPG-CTRL; MET-HPG
417.082	14.21	8.94E-05	4.0489	0.001113	HPG-AHA; HPG-CTRL; MET-HPG
200.9836	14.123	9.25E-05	4.0337	0.001149	HPG-AHA; HPG-CTRL; MET-HPG
564.0406	14.104	9.33E-05	4.0303	0.001154	CTRL-AHA; HPG-CTRL; MET-CTRL
73.04166	14.076	9.43E-05	4.0255	0.001163	HPG-AHA; HPG-CTRL; MET-HPG
321.1064	14.037	9.58E-05	4.0185	0.001177	HPG-AHA; HPG-CTRL; MET-HPG
372.077	13.951	9.92E-05	4.0034	0.001215	HPG-AHA; HPG-CTRL; MET-HPG

678.4901	13.925	0.0001	3.9988	0.001224	HPG-AHA; HPG-CTRL; MET-HPG
559.9045	13.829	0.000104	3.9819	0.001268	CTRL-AHA; HPG-CTRL; MET-HPG
394.9508	13.694	0.00011	3.9577	0.001336	HPG-AHA; HPG-CTRL; MET-HPG
466.1084	13.68	0.000111	3.9553	0.001339	HPG-AHA; HPG-CTRL; MET-HPG
462.1224	13.622	0.000114	3.9449	0.001367	CTRL-AHA; HPG-AHA; MET-AHA; MET-CTRL
508.1504	13.554	0.000117	3.9326	0.0014	CTRL-AHA; HPG-CTRL; MET-HPG
784.0531	13.547	0.000117	3.9315	0.0014	CTRL-AHA; HPG-CTRL; MET-HPG
421.935	13.535	0.000118	3.9292	0.001403	HPG-AHA; HPG-CTRL; MET-HPG
219.0755	13.474	0.000121	3.9183	0.001434	CTRL-AHA; HPG-CTRL; MET-CTRL
423.1836	13.375	0.000126	3.9002	0.00149	MET-AHA; HPG-CTRL; MET-HPG
757.7545	13.365	0.000126	3.8984	0.001491	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
536.8797	13.352	0.000127	3.8961	0.001494	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
399.9712	13.298	0.00013	3.8863	0.001523	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
491.8094	13.273	0.000131	3.8817	0.001534	CTRL-AHA; HPG-CTRL; MET-CTRL
676.4811	13.258	0.000132	3.8789	0.001539	HPG-AHA; HPG-CTRL; MET-HPG
339.1708	13.247	0.000133	3.877	0.001541	CTRL-AHA; HPG-AHA; MET-HPG
143.1508	13.217	0.000134	3.8715	0.001555	CTRL-AHA; HPG-CTRL; MET-CTRL
163.057	13.122	0.00014	3.8539	0.001614	CTRL-AHA; HPG-CTRL; MET-CTRL; MET-HPG
818.6205	13.113	0.000141	3.8523	0.001615	HPG-AHA; HPG-CTRL; MET-HPG
587.027	13.097	0.000141	3.8494	0.001621	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
312.1085	13.061	0.000144	3.8427	0.001641	CTRL-AHA; HPG-AHA; MET-CTRL
702.4954	12.924	0.000152	3.8174	0.001734	HPG-AHA; HPG-CTRL; MET-HPG
465.0275	12.81	0.00016	3.796	0.001816	HPG-AHA; HPG-CTRL; MET-HPG
143.1508	12.785	0.000162	3.7914	0.001829	CTRL-AHA; HPG-CTRL; MET-HPG
589.0333	12.776	0.000162	3.7897	0.00183	HPG-AHA; HPG-CTRL; MET-HPG
400.9488	12.752	0.000164	3.7851	0.001844	HPG-AHA; HPG-CTRL; MET-HPG
307.0789	12.731	0.000166	3.7812	0.001853	HPG-AHA; HPG-CTRL; MET-HPG
455.2523	12.726	0.000166	3.7803	0.001853	HPG-AHA; HPG-CTRL; MET-HPG
345.0401	12.706	0.000167	3.7765	0.001863	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
677.4844	12.684	0.000169	3.7724	0.001873	HPG-AHA; HPG-CTRL; MET-HPG
186.2175	12.679	0.000169	3.7715	0.001873	HPG-AHA; HPG-CTRL; MET-HPG
319.1116	12.67	0.00017	3.7696	0.001873	HPG-AHA; HPG-CTRL; MET-HPG
420.0489	12.665	0.00017	3.7688	0.001873	CTRL-AHA; MET-AHA; HPG-CTRL
143.0782	12.615	0.000174	3.7593	0.001903	CTRL-AHA; MET-AHA; HPG-CTRL
387.0203	12.614	0.000174	3.7592	0.001903	HPG-AHA; HPG-CTRL; MET-HPG
618.8803	12.606	0.000175	3.7576	0.001905	HPG-AHA; HPG-CTRL; MET-HPG
368.005	12.592	0.000176	3.7549	0.001905	HPG-AHA; HPG-CTRL; MET-HPG
76.0197	12.585	0.000176	3.7536	0.001905	CTRL-AHA; HPG-CTRL; MET-HPG
702.0786	12.585	0.000176	3.7536	0.001905	CTRL-AHA; HPG-CTRL; MET-HPG
285.9626	12.565	0.000178	3.7499	0.001916	CTRL-AHA; HPG-AHA; HPG-CTRL; MET-HPG
176.0993	12.543	0.00018	3.7457	0.001925	HPG-AHA; HPG-CTRL; MET-HPG
846.6265	12.54	0.00018	3.7452	0.001925	CTRL-AHA; HPG-CTRL; MET-CTRL
472.2145	12.492	0.000184	3.7359	0.00196	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
572.9469	12.438	0.000188	3.7256	0.002001	HPG-AHA; HPG-CTRL; MET-HPG
477.2414	12.327	0.000198	3.7044	0.002095	MET-AHA; MET-CTRL; MET-HPG
403.0226	12.282	0.000201	3.6958	0.002131	CTRL-AHA; MET-AHA; HPG-CTRL
326.0417	12.266	0.000203	3.6926	0.00214	HPG-AHA; HPG-CTRL; MET-HPG
378.9783	12.236	0.000206	3.6868	0.002163	HPG-AHA; HPG-CTRL; MET-HPG
569.1498	12.174	0.000211	3.6749	0.002217	CTRL-AHA; MET-AHA; HPG-CTRL
134.0416	12.163	0.000212	3.6727	0.002221	MET-AHA; HPG-CTRL; MET-HPG
588.9191	12.142	0.000214	3.6686	0.002235	HPG-AHA; HPG-CTRL; MET-HPG
560.1328	12.132	0.000215	3.6667	0.002239	HPG-AHA; HPG-CTRL; MET-HPG
232.02	12.107	0.000218	3.6618	0.002258	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
200.9833	12.068	0.000222	3.6542	0.002291	HPG-AHA; HPG-CTRL; MET-HPG
241.2174	12.049	0.000224	3.6505	0.002304	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
890.7436	12.026	0.000226	3.6461	0.002321	CTRL-AHA; HPG-CTRL; MET-HPG
980.0314	12.018	0.000227	3.6446	0.002322	CTRL-AHA; HPG-CTRL; MET-HPG
258.1457	11.928	0.000236	3.6269	0.002412	CTRL-AHA; HPG-AHA; MET-AHA
372.0315	11.887	0.000241	3.6189	0.00245	HPG-AHA; HPG-CTRL; MET-HPG
268.1477	11.832	0.000247	3.6079	0.002505	CTRL-AHA; HPG-CTRL; MET-CTRL
766.916	11.795	0.000251	3.6006	0.00254	HPG-AHA; HPG-CTRL; MET-HPG
632.8737	11.745	0.000257	3.5908	0.002583	CTRL-AHA; HPG-CTRL; MET-HPG
258.6563	11.741	0.000257	3.59	0.002583	CTRL-AHA; HPG-CTRL; MET-HPG
161.0984	11.74	0.000257	3.5897	0.002583	CTRL-AHA; HPG-CTRL; MET-HPG
242.0613	11.711	0.000261	3.5839	0.00261	CTRL-AHA; HPG-CTRL; MET-HPG
672.8103	11.7	0.000262	3.5817	0.002616	HPG-AHA; HPG-CTRL; MET-HPG
240.1545	11.689	0.000263	3.5796	0.002622	CTRL-AHA; HPG-CTRL; MET-HPG
846.961	11.669	0.000266	3.5757	0.002638	CTRL-AHA; HPG-CTRL; MET-HPG
818.2865	11.631	0.00027	3.568	0.002678	HPG-AHA; HPG-CTRL; MET-HPG
239.0871	11.546	0.000281	3.551	0.002777	MET-AHA; MET-CTRL; MET-HPG
664.4804	11.512	0.000286	3.5441	0.002814	HPG-AHA; HPG-CTRL; MET-HPG
486.0867	11.49	0.000289	3.5398	0.002834	CTRL-AHA; MET-AHA; HPG-CTRL
363.9966	11.441	0.000295	3.5299	0.002891	HPG-AHA; HPG-CTRL; MET-HPG
653.1198	11.418	0.000298	3.5251	0.002915	CTRL-AHA; HPG-CTRL; MET-HPG

767.5631	11.386	0.000303	3.5188	0.002934	CTRL-AHA; HPG-CTRL; MET-CTRL
272.0926	11.384	0.000303	3.5183	0.002934	CTRL-AHA; HPG-AHA; MET-HPG
257.1434	11.38	0.000304	3.5174	0.002934	CTRL-AHA; HPG-AHA; MET-AHA
549.4793	11.376	0.000304	3.5167	0.002934	HPG-AHA; HPG-CTRL; MET-HPG
262.7527	11.375	0.000304	3.5165	0.002934	CTRL-AHA; HPG-CTRL; MET-CTRL
519.2645	11.334	0.00031	3.5083	0.002976	HPG-AHA; HPG-CTRL; MET-HPG
652.1088	11.327	0.000311	3.5068	0.002976	HPG-AHA; HPG-CTRL; MET-HPG
158.0433	11.327	0.000311	3.5068	0.002976	CTRL-AHA; HPG-CTRL; MET-CTRL
654.8548	11.319	0.000313	3.5051	0.002979	CTRL-AHA; HPG-CTRL; MET-CTRL
297.0226	11.312	0.000314	3.5036	0.002982	HPG-CTRL; MET-HPG
844.6069	11.24	0.000324	3.4891	0.003075	HPG-AHA; HPG-CTRL; MET-HPG
235.1606	11.198	0.000331	3.4805	0.003128	HPG-AHA; HPG-CTRL; MET-HPG
664.1059	11.191	0.000332	3.4789	0.003131	CTRL-AHA; HPG-CTRL
386.2023	11.148	0.000339	3.4702	0.003186	CTRL-AHA; HPG-CTRL; MET-HPG
584.9771	11.136	0.000341	3.4677	0.003194	CTRL-AHA; HPG-CTRL; MET-HPG
242.0614	11.125	0.000342	3.4655	0.003194	HPG-AHA; HPG-CTRL; MET-HPG
689.1392	11.123	0.000343	3.4651	0.003194	HPG-AHA; HPG-CTRL; MET-HPG
173.0168	11.121	0.000343	3.4646	0.003194	CTRL-AHA; HPG-CTRL; MET-CTRL
600.9523	11.11	0.000345	3.4623	0.003203	CTRL-AHA; HPG-CTRL; MET-HPG
838.281	11.09	0.000348	3.4582	0.003224	CTRL-AHA; HPG-CTRL; MET-HPG
402.2099	11.045	0.000356	3.4489	0.003283	MET-AHA; MET-CTRL; MET-HPG
348.8508	11.041	0.000356	3.4482	0.003283	CTRL-AHA; HPG-CTRL; MET-CTRL
240.1761	11.025	0.000359	3.4448	0.003299	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
703.0635	11.02	0.00036	3.4438	0.003299	CTRL-AHA; HPG-CTRL; MET-HPG
73.08239	10.995	0.000364	3.4385	0.003331	CTRL-AHA; HPG-CTRL
822.015	10.951	0.000372	3.4295	0.003392	CTRL-AHA; HPG-CTRL; MET-HPG
172.039	10.919	0.000378	3.4228	0.003436	CTRL-AHA; HPG-CTRL; MET-HPG
144.0621	10.903	0.000381	3.4194	0.003455	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
825.2962	10.882	0.000385	3.4151	0.003464	CTRL-AHA; HPG-CTRL; MET-HPG
462.7409	10.877	0.000385	3.4141	0.003464	CTRL-AHA; MET-AHA; HPG-CTRL
479.136	10.874	0.000386	3.4135	0.003464	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
403.0227	10.874	0.000386	3.4133	0.003464	CTRL-AHA; MET-AHA; HPG-CTRL
184.1483	10.871	0.000387	3.4128	0.003464	CTRL-AHA; MET-AHA; MET-HPG
219.076	10.866	0.000388	3.4116	0.003464	CTRL-AHA; HPG-CTRL; MET-CTRL
301.9367	10.846	0.000391	3.4076	0.003487	HPG-AHA; HPG-CTRL; MET-HPG
238.089	10.824	0.000395	3.4029	0.003517	HPG-AHA; HPG-CTRL
329.1077	10.813	0.000397	3.4007	0.003526	HPG-CTRL; MET-HPG
469.0622	10.8	0.0004	3.3979	0.00354	HPG-AHA; HPG-CTRL; MET-HPG
312.1117	10.788	0.000402	3.3954	0.003551	CTRL-AHA; HPG-AHA; MET-CTRL; MET-HPG
973.3348	10.755	0.000409	3.3886	0.003599	CTRL-AHA; MET-AHA; HPG-CTRL
244.0579	10.728	0.000414	3.3828	0.003638	CTRL-AHA; HPG-CTRL; MET-CTRL
667.1098	10.692	0.000421	3.3752	0.003693	CTRL-AHA; HPG-CTRL
556.2098	10.687	0.000423	3.3741	0.003694	CTRL-AHA; MET-AHA; HPG-CTRL
478.2439	10.681	0.000424	3.3728	0.003696	MET-AHA; MET-CTRL; MET-HPG
307.0801	10.667	0.000427	3.37	0.003711	HPG-AHA; HPG-CTRL; MET-HPG
615.1638	10.63	0.000434	3.3622	0.003769	CTRL-AHA; HPG-CTRL; MET-HPG
410.924	10.598	0.000441	3.3552	0.003821	HPG-AHA; HPG-CTRL; MET-HPG
497.1464	10.537	0.000455	3.3422	0.003918	HPG-CTRL; MET-HPG
312.9957	10.535	0.000455	3.3419	0.003918	HPG-AHA; HPG-CTRL; MET-HPG
396.1521	10.528	0.000457	3.3404	0.003918	HPG-AHA; HPG-CTRL; MET-HPG
532.9632	10.527	0.000457	3.3401	0.003918	HPG-AHA; HPG-CTRL; MET-HPG
196.0934	10.514	0.00046	3.3374	0.003925	HPG-CTRL; MET-HPG
175.1397	10.513	0.00046	3.3372	0.003925	CTRL-AHA; HPG-CTRL; MET-HPG
174.8918	10.499	0.000463	3.3341	0.003944	CTRL-AHA; HPG-CTRL; MET-CTRL
72.93538	10.477	0.000468	3.3295	0.003976	CTRL-AHA; HPG-CTRL; MET-HPG
160.0841	10.469	0.00047	3.3277	0.003983	CTRL-AHA; HPG-CTRL; MET-CTRL
634.8554	10.46	0.000472	3.3258	0.003991	HPG-CTRL; MET-HPG
388.0505	10.448	0.000475	3.3233	0.004005	HPG-CTRL; MET-HPG
670.8278	10.402	0.000486	3.3134	0.004087	HPG-CTRL; MET-HPG
852.2834	10.374	0.000493	3.3075	0.004134	HPG-AHA; HPG-CTRL; MET-HPG
193.0313	10.322	0.000506	3.2962	0.004233	CTRL-AHA; HPG-AHA; MET-AHA
162.0547	10.302	0.000511	3.2918	0.004257	HPG-AHA; HPG-CTRL
124.084	10.298	0.000512	3.291	0.004257	CTRL-AHA; HPG-CTRL; MET-HPG
464.9768	10.297	0.000512	3.2907	0.004257	HPG-AHA; HPG-CTRL; MET-HPG
498.9219	10.29	0.000514	3.2893	0.004261	HPG-AHA; HPG-CTRL; MET-HPG
302.077	10.277	0.000517	3.2864	0.004279	CTRL-AHA; HPG-AHA; MET-AHA
214.0721	10.248	0.000524	3.2803	0.00433	HPG-AHA; MET-CTRL; MET-HPG
180.0481	10.225	0.000531	3.2752	0.004371	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
487.158	10.214	0.000534	3.2727	0.00438	CTRL-AHA; HPG-CTRL
770.5178	10.212	0.000534	3.2724	0.00438	CTRL-AHA; HPG-CTRL
858.9457	10.204	0.000536	3.2708	0.004386	CTRL-AHA; HPG-CTRL; MET-HPG
837.4282	10.185	0.000541	3.2666	0.004418	CTRL-AHA; HPG-AHA
618.1666	10.152	0.00055	3.2594	0.004482	CTRL-AHA; HPG-CTRL; MET-HPG
541.9353	10.13	0.000556	3.2546	0.004521	CTRL-AHA; HPG-CTRL; MET-HPG

112.073	10.111	0.000562	3.2503	0.004556	CTRL-AHA; MET-AHA
222.1034	10.073	0.000573	3.242	0.004633	HPG-CTRL; MET-HPG
362.0105	10.056	0.000578	3.2384	0.004661	HPG-AHA; HPG-CTRL; MET-HPG
393.1449	9.9636	0.000605	3.218	0.004874	CTRL-AHA; HPG-CTRL; MET-HPG
403.0227	9.8487	0.000642	3.1925	0.005149	CTRL-AHA; MET-AHA; HPG-CTRL
326.1106	9.8475	0.000642	3.1923	0.005149	CTRL-AHA; HPG-CTRL; MET-CTRL
768.5247	9.8376	0.000646	3.1901	0.005163	CTRL-AHA; HPG-CTRL; MET-CTRL
570.1556	9.804	0.000657	3.1826	0.005241	CTRL-AHA; MET-AHA
769.5601	9.795	0.00066	3.1806	0.005254	CTRL-AHA; HPG-CTRL
215.0021	9.7886	0.000662	3.1791	0.005255	CTRL-AHA; HPG-CTRL; MET-HPG
325.1071	9.7858	0.000663	3.1785	0.005255	CTRL-AHA; HPG-CTRL
413.9823	9.7518	0.000675	3.1709	0.005325	HPG-AHA; HPG-CTRL; MET-HPG
820.5198	9.7517	0.000675	3.1709	0.005325	HPG-AHA; HPG-CTRL; MET-HPG
853.9633	9.7434	0.000678	3.169	0.005336	CTRL-AHA; HPG-CTRL; MET-HPG
146.0351	9.731	0.000682	3.1662	0.005343	CTRL-AHA; MET-AHA; HPG-CTRL
847.295	9.7301	0.000682	3.166	0.005343	HPG-CTRL; MET-HPG
144.0447	9.728	0.000683	3.1656	0.005343	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
160.0492	9.7095	0.00069	3.1614	0.005383	CTRL-AHA; HPG-CTRL
776.5119	9.6648	0.000706	3.1514	0.005496	CTRL-AHA; HPG-CTRL
550.2891	9.6603	0.000707	3.1504	0.005497	CTRL-AHA; HPG-CTRL; MET-HPG
391.2776	9.6297	0.000719	3.1435	0.005573	CTRL-AHA; HPG-CTRL; MET-CTRL
58.06367	9.6235	0.000721	3.1421	0.005579	CTRL-AHA; HPG-CTRL; MET-CTRL
259.0171	9.5905	0.000733	3.1346	0.005645	HPG-AHA; HPG-CTRL; MET-HPG
364.9927	9.5833	0.000736	3.133	0.005645	HPG-AHA; HPG-CTRL; MET-HPG
716.5119	9.5808	0.000737	3.1325	0.005645	HPG-AHA; HPG-CTRL; MET-HPG
227.0281	9.5806	0.000737	3.1324	0.005645	CTRL-AHA; HPG-CTRL; MET-CTRL
703.4991	9.5801	0.000737	3.1323	0.005645	HPG-CTRL; MET-HPG
234.0572	9.5598	0.000745	3.1277	0.005693	HPG-CTRL; MET-HPG
851.4414	9.5447	0.000751	3.1243	0.005726	CTRL-AHA; HPG-CTRL; MET-HPG
516.9894	9.5295	0.000757	3.1208	0.005759	CTRL-AHA; HPG-CTRL
353.1575	9.4481	0.00079	3.1023	0.005997	HPG-AHA; HPG-CTRL; MET-HPG
864.6026	9.4204	0.000802	3.096	0.006059	CTRL-AHA; HPG-CTRL; MET-HPG
158.9182	9.4204	0.000802	3.096	0.006059	CTRL-AHA; HPG-CTRL; MET-CTRL
310.0832	9.3981	0.000811	3.0909	0.006118	HPG-CTRL; MET-HPG
306.1902	9.3842	0.000817	3.0877	0.00615	HPG-AHA; MET-HPG
400.8204	9.3432	0.000835	3.0783	0.006271	CTRL-AHA; HPG-CTRL; MET-HPG
609.9229	9.2968	0.000856	3.0676	0.006414	CTRL-AHA; HPG-CTRL; MET-HPG
244.0742	9.2746	0.000866	3.0625	0.006476	CTRL-AHA; HPG-CTRL
131.1148	9.2639	0.000871	3.06	0.0065	CTRL-AHA; HPG-AHA; MET-AHA
673.087	9.2541	0.000875	3.0578	0.006515	HPG-AHA; HPG-CTRL; MET-HPG
305.1595	9.2516	0.000877	3.0572	0.006515	CTRL-AHA; HPG-CTRL; MET-HPG
284.3249	9.2419	0.000881	3.055	0.006535	HPG-CTRL; MET-HPG
224.0875	9.2	0.000901	3.0453	0.006669	MET-AHA; MET-HPG
211.0492	9.1508	0.000925	3.0338	0.006833	CTRL-AHA; HPG-AHA; MET-HPG
879.9437	9.1417	0.00093	3.0317	0.006852	HPG-CTRL; MET-HPG
145.1665	9.1053	0.000948	3.0232	0.006973	CTRL-AHA; HPG-CTRL
163.0755	9.097	0.000952	3.0213	0.006989	HPG-AHA; HPG-CTRL; MET-HPG
449.0029	9.088	0.000957	3.0192	0.006998	CTRL-AHA; HPG-CTRL; MET-HPG
385.6999	9.0871	0.000957	3.019	0.006998	CTRL-AHA; HPG-CTRL; MET-HPG
873.6133	9.0676	0.000967	3.0144	0.007057	CTRL-AHA; HPG-CTRL; MET-HPG
425.973	9.0512	0.000976	3.0106	0.007105	HPG-CTRL; MET-HPG
413.157	9.0269	0.000989	3.0049	0.007184	HPG-AHA; HPG-CTRL; MET-HPG
617.054	9.0122	0.000997	3.0015	0.007227	CTRL-AHA; HPG-CTRL; MET-CTRL
745.4803	8.9494	0.001031	2.9867	0.007462	HPG-AHA; HPG-CTRL; MET-HPG
301.0027	8.9393	0.001037	2.9843	0.007488	HPG-AHA; HPG-CTRL; MET-HPG
439.0972	8.9284	0.001043	2.9817	0.007516	CTRL-AHA; HPG-CTRL; MET-HPG
235.0703	8.9246	0.001045	2.9808	0.007516	HPG-AHA; MET-HPG
455.2181	8.9212	0.001047	2.98	0.007516	HPG-CTRL; MET-HPG
252.9821	8.8973	0.001061	2.9744	0.007599	HPG-AHA; HPG-CTRL; MET-HPG
846.6278	8.8772	0.001072	2.9696	0.007645	HPG-CTRL; MET-HPG
774.5159	8.8768	0.001073	2.9696	0.007645	CTRL-AHA; HPG-CTRL; MET-CTRL
653.1026	8.8753	0.001074	2.9692	0.007645	HPG-AHA; HPG-CTRL; MET-HPG
824.6287	8.8683	0.001078	2.9675	0.007651	CTRL-AHA; HPG-AHA; MET-HPG
201.2283	8.8639	0.00108	2.9665	0.007651	CTRL-AHA; HPG-CTRL
550.4827	8.8629	0.001081	2.9663	0.007651	HPG-AHA; HPG-CTRL; MET-HPG
652.9641	8.8022	0.001117	2.9518	0.007894	CTRL-AHA; HPG-CTRL
179.9998	8.7849	0.001128	2.9477	0.007953	CTRL-AHA; HPG-CTRL; MET-HPG
289.0837	8.7759	0.001134	2.9456	0.007977	CTRL-AHA; HPG-CTRL
221.1447	8.7494	0.00115	2.9392	0.008078	CTRL-AHA; MET-AHA
187.0497	8.7293	0.001163	2.9344	0.008152	HPG-CTRL; MET-CTRL
123.0525	8.7121	0.001174	2.9303	0.008213	CTRL-AHA; HPG-CTRL; MET-CTRL
368.1138	8.7041	0.001179	2.9284	0.008234	HPG-AHA; HPG-CTRL; MET-HPG
717.5152	8.6849	0.001192	2.9238	0.008305	HPG-AHA; HPG-CTRL; MET-HPG
421.1276	8.6783	0.001196	2.9222	0.008319	HPG-AHA; HPG-CTRL; MET-HPG

273.116	8.671	0.001201	2.9205	0.008337	CTRL-AHA; MET-AHA; HPG-CTRL
322.9843	8.6607	0.001208	2.918	0.008368	HPG-AHA; HPG-CTRL; MET-HPG
330.0629	8.6462	0.001218	2.9145	0.008419	HPG-CTRL; MET-HPG
319.1122	8.6224	0.001234	2.9088	0.008515	HPG-CTRL; MET-HPG
551.4893	8.5941	0.001253	2.902	0.008619	HPG-AHA; HPG-CTRL; MET-HPG
629.4765	8.5936	0.001254	2.9018	0.008619	CTRL-AHA; HPG-CTRL; MET-HPG
205.0602	8.5482	0.001286	2.8909	0.008822	CTRL-AHA; HPG-CTRL; MET-HPG
163.0573	8.528	0.0013	2.886	0.008888	CTRL-AHA; HPG-CTRL; MET-CTRL
906.7182	8.525	0.001303	2.8852	0.008888	HPG-CTRL; MET-HPG
165.963	8.5247	0.001303	2.8852	0.008888	HPG-CTRL
110.0061	8.5158	0.001309	2.883	0.008906	CTRL-AHA; HPG-CTRL
146.0353	8.5137	0.001311	2.8825	0.008906	CTRL-AHA; MET-AHA; HPG-CTRL
251.0591	8.5095	0.001314	2.8815	0.008906	HPG-AHA; HPG-CTRL; MET-HPG
177.0467	8.5075	0.001315	2.881	0.008906	MET-AHA; HPG-CTRL; MET-HPG
466.1048	8.488	0.00133	2.8763	0.008969	CTRL-AHA; HPG-CTRL; MET-HPG
168.9857	8.4879	0.00133	2.8762	0.008969	CTRL-AHA; HPG-CTRL
702.0606	8.4838	0.001333	2.8752	0.008973	CTRL-AHA; HPG-CTRL; MET-HPG
310.1434	8.4734	0.001341	2.8727	0.009009	HPG-AHA; HPG-CTRL
244.0539	8.4627	0.001349	2.8701	0.009039	CTRL-AHA; HPG-CTRL; MET-CTRL
400.9482	8.4607	0.00135	2.8696	0.009039	CTRL-AHA; HPG-CTRL; MET-CTRL
388.0186	8.4192	0.001382	2.8595	0.009235	CTRL-AHA; HPG-CTRL; MET-HPG
391.1765	8.4074	0.001391	2.8566	0.009279	HPG-CTRL; MET-HPG
132.9808	8.3998	0.001397	2.8547	0.009302	CTRL-AHA; HPG-CTRL; MET-CTRL
159.0727	8.3676	0.001423	2.8469	0.009454	CTRL-AHA; HPG-CTRL
173.1975	8.3461	0.00144	2.8416	0.009552	CTRL-AHA; HPG-CTRL
244.0542	8.3403	0.001445	2.8402	0.009566	CTRL-AHA; HPG-CTRL; MET-CTRL
430.3803	8.2973	0.001481	2.8296	0.009784	CTRL-AHA; HPG-CTRL
202.1758	8.2828	0.001493	2.826	0.009834	CTRL-AHA; HPG-CTRL; MET-CTRL
199.0285	8.2815	0.001494	2.8257	0.009834	HPG-CTRL; MET-HPG
866.2809	8.2786	0.001496	2.825	0.009834	CTRL-AHA; HPG-CTRL; MET-HPG
325.0439	8.2618	0.001511	2.8208	0.009909	CTRL-AHA; HPG-CTRL; MET-CTRL
859.2768	8.2587	0.001513	2.8201	0.009909	CTRL-AHA; HPG-CTRL; MET-CTRL
779.4702	8.248	0.001523	2.8174	0.009951	CTRL-AHA; HPG-AHA
361.0966	8.2179	0.001549	2.81	0.010105	CTRL-AHA; HPG-CTRL; MET-CTRL
206.0442	8.2092	0.001557	2.8078	0.010137	CTRL-AHA; MET-AHA; HPG-CTRL
831.051	8.1978	0.001567	2.805	0.010172	MET-CTRL; MET-HPG
473.9481	8.1967	0.001568	2.8047	0.010172	CTRL-AHA; HPG-CTRL
240.1496	8.1697	0.001592	2.798	0.010311	CTRL-AHA; HPG-CTRL
860.9605	8.1616	0.0016	2.796	0.010341	CTRL-AHA; HPG-CTRL
376.9129	8.1445	0.001615	2.7917	0.010424	HPG-AHA; HPG-CTRL; MET-HPG
146.1617	8.141	0.001619	2.7909	0.010426	CTRL-AHA; HPG-CTRL; MET-CTRL
461.1197	8.111	0.001647	2.7834	0.010588	CTRL-AHA; HPG-AHA
274.119	8.106	0.001651	2.7822	0.010599	CTRL-AHA; MET-AHA; HPG-CTRL
690.141	8.0919	0.001665	2.7786	0.010666	HPG-AHA; HPG-CTRL; MET-HPG
629.226	8.0775	0.001679	2.775	0.010736	HPG-CTRL
124.0843	8.0717	0.001684	2.7736	0.010753	MET-AHA; MET-HPG
437.1762	8.0522	0.001703	2.7687	0.010855	HPG-CTRL; MET-HPG
527.8406	8.0457	0.00171	2.7671	0.010876	CTRL-AHA; HPG-CTRL; MET-CTRL
329.1277	8.0271	0.001728	2.7624	0.010974	CTRL-AHA; HPG-AHA; MET-AHA
361.1471	8.0103	0.001745	2.7582	0.011049	CTRL-AHA; HPG-CTRL
127.0115	8.0093	0.001746	2.7579	0.011049	HPG-CTRL; MET-HPG
116.0316	7.9943	0.001761	2.7541	0.011125	HPG-CTRL; MET-HPG
158.9182	7.9802	0.001776	2.7506	0.011197	CTRL-AHA; HPG-CTRL; MET-CTRL
466.1411	7.9747	0.001782	2.7492	0.011213	HPG-AHA; HPG-CTRL; MET-HPG
232.9253	7.9323	0.001826	2.7385	0.011472	HPG-AHA; HPG-CTRL; MET-HPG
490.808	7.9264	0.001832	2.737	0.011482	CTRL-AHA; HPG-CTRL; MET-CTRL
434.0106	7.9248	0.001834	2.7366	0.011482	HPG-AHA; MET-CTRL; MET-HPG
247.1262	7.9206	0.001838	2.7355	0.01149	HPG-AHA; HPG-CTRL; MET-HPG
429.1474	7.9073	0.001853	2.7322	0.011559	CTRL-AHA; MET-AHA
588.9201	7.8859	0.001876	2.7268	0.011672	HPG-AHA; HPG-CTRL; MET-HPG
262.1708	7.8827	0.00188	2.726	0.011672	CTRL-AHA; HPG-CTRL
356.9835	7.8817	0.001881	2.7257	0.011672	HPG-AHA; HPG-CTRL; MET-HPG
360.1437	7.8623	0.001902	2.7208	0.011785	CTRL-AHA; HPG-CTRL
487.0758	7.8512	0.001915	2.7179	0.011842	HPG-AHA; HPG-CTRL; MET-HPG
518.7544	7.8339	0.001934	2.7136	0.011941	CTRL-AHA; HPG-AHA; MET-HPG
430.1504	7.8215	0.001948	2.7104	0.012007	CTRL-AHA; MET-AHA
74.09463	7.8121	0.001959	2.708	0.012045	CTRL-AHA; HPG-CTRL
431.3857	7.8103	0.001961	2.7075	0.012045	CTRL-AHA; HPG-CTRL; MET-CTRL
343.9376	7.7958	0.001978	2.7038	0.012128	CTRL-AHA; HPG-CTRL
853.0327	7.7852	0.00199	2.7011	0.012159	MET-CTRL; MET-HPG
158.0433	7.7836	0.001992	2.7007	0.012159	CTRL-AHA; HPG-CTRL; MET-CTRL
296.0037	7.779	0.001997	2.6996	0.012159	CTRL-AHA; HPG-CTRL; MET-HPG
394.2301	7.7782	0.001998	2.6993	0.012159	CTRL-AHA; HPG-AHA
214.9126	7.7765	0.002	2.6989	0.012159	HPG-AHA; HPG-CTRL; MET-HPG

456.9886	7.774	0.002003	2.6983	0.012159	MET-AHA; MET-CTRL; MET-HPG
430.3806	7.7629	0.002016	2.6954	0.012202	CTRL-AHA; HPG-CTRL
293.0748	7.7622	0.002017	2.6952	0.012202	HPG-AHA; HPG-CTRL; MET-HPG
860.6283	7.7255	0.002061	2.6859	0.012448	CTRL-AHA; HPG-CTRL
204.0384	7.7145	0.002075	2.683	0.012505	CTRL-AHA; HPG-CTRL; MET-CTRL
209.0168	7.7121	0.002078	2.6824	0.012505	CTRL-AHA; HPG-CTRL; MET-CTRL
194.9619	7.6857	0.002111	2.6756	0.012667	HPG-AHA; HPG-CTRL; MET-HPG
566.7092	7.683	0.002114	2.6749	0.012667	CTRL-AHA; HPG-CTRL; MET-CTRL
425.1171	7.6818	0.002115	2.6746	0.012667	HPG-CTRL
134.9776	7.6617	0.002141	2.6695	0.012789	CTRL-AHA; HPG-CTRL; MET-CTRL
239.081	7.66	0.002143	2.669	0.012789	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
824.9636	7.6163	0.002199	2.6577	0.013092	HPG-AHA; MET-HPG
391.2776	7.615	0.002201	2.6574	0.013092	CTRL-AHA; HPG-CTRL; MET-HPG
323.1479	7.61	0.002208	2.6561	0.013109	CTRL-AHA; HPG-CTRL
412.0284	7.582	0.002245	2.6489	0.013308	CTRL-AHA; HPG-AHA; MET-CTRL
133.9249	7.554	0.002283	2.6416	0.01351	HPG-AHA; HPG-CTRL; MET-HPG
405.9614	7.5435	0.002297	2.6389	0.013573	CTRL-AHA; HPG-CTRL
59.05887	7.5298	0.002316	2.6353	0.013662	CTRL-AHA; HPG-CTRL
308.0853	7.5263	0.002321	2.6344	0.013668	HPG-CTRL; MET-HPG
194.0271	7.5045	0.002351	2.6287	0.013825	CTRL-AHA; MET-AHA
225.1553	7.4953	0.002364	2.6263	0.013879	CTRL-AHA; HPG-AHA; MET-AHA
104.0507	7.4657	0.002407	2.6186	0.014096	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
724.0422	7.4618	0.002412	2.6175	0.014096	HPG-CTRL; MET-HPG
142.0287	7.4614	0.002413	2.6174	0.014096	HPG-CTRL; MET-HPG
683.0647	7.4522	0.002426	2.615	0.014151	HPG-CTRL; MET-HPG
216.9555	7.4481	0.002432	2.614	0.014162	HPG-CTRL; MET-HPG
134.0414	7.433	0.002455	2.61	0.014269	MET-HPG
854.295	7.3411	0.002595	2.5859	0.015061	CTRL-AHA; HPG-CTRL
200.0322	7.3327	0.002608	2.5836	0.015113	CTRL-AHA; HPG-CTRL
724.9814	7.322	0.002625	2.5808	0.015187	HPG-AHA; HPG-CTRL; MET-HPG
314.1171	7.313	0.00264	2.5784	0.015234	CTRL-AHA; HPG-AHA; MET-AHA
513.0579	7.3117	0.002642	2.5781	0.015234	CTRL-AHA; HPG-AHA
300.2834	7.3074	0.002649	2.577	0.015249	CTRL-AHA; HPG-CTRL; MET-CTRL
871.2655	7.3043	0.002654	2.5761	0.015253	CTRL-AHA; HPG-CTRL
260.7554	7.2806	0.002692	2.5699	0.01545	CTRL-AHA; HPG-CTRL; MET-CTRL
428.3645	7.275	0.002702	2.5684	0.015479	CTRL-AHA; HPG-CTRL
395.152	7.2477	0.002747	2.5611	0.015692	HPG-AHA; HPG-CTRL; MET-HPG
543.9308	7.2473	0.002748	2.561	0.015692	HPG-AHA; HPG-CTRL; MET-HPG
290.9377	7.2383	0.002763	2.5586	0.015753	HPG-AHA; HPG-CTRL; MET-HPG
395.2331	7.231	0.002775	2.5567	0.015799	CTRL-AHA; HPG-AHA
291.9004	7.2199	0.002794	2.5537	0.015882	CTRL-AHA; HPG-CTRL
362.9962	7.2122	0.002808	2.5517	0.015897	CTRL-AHA; HPG-CTRL; MET-CTRL
866.9587	7.2114	0.002809	2.5515	0.015897	CTRL-AHA; HPG-CTRL
432.0961	7.2088	0.002813	2.5508	0.015897	CTRL-AHA; HPG-AHA; MET-AHA
531.7691	7.2052	0.00282	2.5498	0.015897	HPG-CTRL; MET-HPG
974.3374	7.2048	0.00282	2.5497	0.015897	CTRL-AHA; MET-AHA; HPG-CTRL
764.4486	7.2029	0.002824	2.5492	0.015897	CTRL-AHA; HPG-CTRL; MET-CTRL
573.8006	7.1893	0.002847	2.5456	0.016004	HPG-AHA; MET-HPG
206.0948	7.1613	0.002897	2.5381	0.016249	CTRL-AHA; HPG-CTRL
229.9019	7.1595	0.0029	2.5376	0.016249	CTRL-AHA; HPG-CTRL
172.9523	7.1376	0.002939	2.5318	0.01643	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
177.1199	7.1365	0.002941	2.5314	0.01643	MET-AHA; MET-HPG
867.957	7.1339	0.002946	2.5308	0.016431	CTRL-AHA; HPG-CTRL
765.5662	7.1218	0.002968	2.5275	0.016527	CTRL-AHA; HPG-CTRL
860.2959	7.1187	0.002974	2.5267	0.016527	CTRL-AHA; HPG-CTRL; MET-CTRL
309.128	7.1146	0.002981	2.5256	0.016527	HPG-AHA; HPG-CTRL; MET-HPG
230.1819	7.1144	0.002982	2.5255	0.016527	CTRL-AHA; HPG-AHA
160.0896	7.1065	0.002996	2.5234	0.016576	CTRL-AHA; HPG-CTRL
431.1484	7.1045	0.003	2.5229	0.016576	CTRL-AHA; MET-AHA; MET-HPG
715.4408	7.0583	0.003087	2.5104	0.017032	HPG-AHA; HPG-CTRL; MET-HPG
128.1403	7.0427	0.003117	2.5062	0.017171	CTRL-AHA; HPG-CTRL
807.2878	7.0365	0.003129	2.5046	0.01721	CTRL-AHA; MET-AHA; HPG-CTRL
619.0516	7.0231	0.003155	2.5009	0.01731	CTRL-AHA; HPG-CTRL; MET-CTRL
229.9018	7.0223	0.003157	2.5007	0.01731	CTRL-AHA; HPG-CTRL
867.6254	7.0167	0.003168	2.4992	0.017344	CTRL-AHA; HPG-CTRL; MET-CTRL
225.0264	7.0129	0.003176	2.4982	0.017358	MET-AHA; HPG-CTRL; MET-HPG
466.105	7.0025	0.003196	2.4954	0.017445	HPG-CTRL; MET-HPG
74.92914	6.9736	0.003254	2.4875	0.017735	CTRL-AHA; HPG-CTRL
358.0612	6.9681	0.003265	2.4861	0.017768	HPG-CTRL; MET-HPG
625.2765	6.9547	0.003293	2.4824	0.017873	CTRL-AHA; HPG-AHA; MET-HPG
858.6098	6.9539	0.003295	2.4822	0.017873	CTRL-AHA; HPG-CTRL; MET-HPG
429.9569	6.9459	0.003311	2.48	0.017935	CTRL-AHA; HPG-CTRL; MET-HPG
170.0081	6.9145	0.003377	2.4715	0.018263	CTRL-AHA; MET-AHA
430.0974	6.9109	0.003385	2.4705	0.018277	CTRL-AHA; HPG-AHA; MET-AHA

105.9511	6.8796	0.003452	2.462	0.01859	CTRL-AHA; HPG-CTRL
188.8759	6.8767	0.003458	2.4612	0.01859	CTRL-AHA; HPG-CTRL
687.0257	6.8767	0.003458	2.4612	0.01859	HPG-AHA; HPG-CTRL; MET-HPG
263.1549	6.8698	0.003473	2.4593	0.018634	CTRL-AHA; MET-AHA
175.1152	6.8681	0.003477	2.4588	0.018634	MET-HPG
172.1847	6.8655	0.003482	2.4581	0.018637	HPG-CTRL
338.9579	6.8398	0.003539	2.4511	0.018911	HPG-AHA; HPG-CTRL; MET-HPG
336.6841	6.8357	0.003549	2.45	0.018911	CTRL-AHA; HPG-CTRL; MET-CTRL
135.9737	6.8352	0.00355	2.4498	0.018911	CTRL-AHA; MET-AHA
243.0486	6.8062	0.003615	2.4419	0.019232	HPG-AHA; MET-HPG
589.5096	6.7923	0.003647	2.4381	0.019373	HPG-CTRL; MET-HPG
150.9762	6.7839	0.003667	2.4357	0.019448	CTRL-AHA; HPG-CTRL
176.0365	6.7742	0.003689	2.4331	0.019539	CTRL-AHA
851.2839	6.7667	0.003707	2.431	0.019603	CTRL-AHA; HPG-CTRL; MET-HPG
811.2944	6.7605	0.003721	2.4293	0.019651	HPG-AHA; HPG-CTRL; MET-HPG
608.078	6.7466	0.003754	2.4255	0.019797	CTRL-AHA; HPG-CTRL
132.115	6.7437	0.003761	2.4247	0.019802	HPG-AHA; HPG-CTRL
434.9663	6.74	0.00377	2.4236	0.019802	MET-CTRL; MET-HPG
146.044	6.7392	0.003772	2.4234	0.019802	CTRL-AHA; HPG-CTRL; MET-HPG
429.2329	6.733	0.003787	2.4217	0.019852	CTRL-AHA; MET-AHA; HPG-CTRL; MET-HPG
420.9916	6.7271	0.003801	2.4201	0.019897	HPG-CTRL; MET-HPG
671.06	6.7193	0.00382	2.4179	0.019942	HPG-CTRL; MET-HPG
454.9601	6.719	0.003821	2.4178	0.019942	HPG-CTRL
418.2052	6.6971	0.003875	2.4118	0.020192	MET-AHA; MET-CTRL; MET-HPG
134.1122	6.6858	0.003903	2.4086	0.020294	MET-AHA; MET-HPG
252.0098	6.6846	0.003906	2.4083	0.020294	CTRL-AHA; HPG-AHA
58.06368	6.6513	0.00399	2.3991	0.020701	CTRL-AHA; HPG-CTRL; MET-CTRL
429.233	6.6463	0.004002	2.3977	0.020737	CTRL-AHA; HPG-CTRL
253.0887	6.6434	0.00401	2.3969	0.020745	HPG-CTRL; MET-HPG
207.0727	6.6347	0.004032	2.3944	0.020832	CTRL-AHA; HPG-CTRL
168.071	6.6213	0.004067	2.3907	0.020982	HPG-CTRL; MET-HPG
177.0464	6.6161	0.004081	2.3893	0.020997	HPG-CTRL; MET-HPG
337.9747	6.6157	0.004082	2.3891	0.020997	CTRL-AHA; HPG-CTRL
825.9633	6.6079	0.004102	2.387	0.021071	HPG-CTRL; MET-HPG
225.1213	6.5839	0.004166	2.3803	0.021367	CTRL-AHA; HPG-CTRL
844.9418	6.5756	0.004188	2.378	0.021452	HPG-CTRL; MET-HPG
294.8486	6.5242	0.00433	2.3636	0.022143	CTRL-AHA; HPG-CTRL; MET-CTRL
323.0838	6.5126	0.004362	2.3603	0.022277	CTRL-AHA
119.0858	6.5077	0.004376	2.3589	0.02229	CTRL-AHA; HPG-CTRL; MET-CTRL
98.98167	6.5074	0.004377	2.3588	0.02229	HPG-CTRL
237.9869	6.4964	0.004408	2.3558	0.022417	CTRL-AHA
206.0625	6.4934	0.004417	2.3549	0.022428	CTRL-AHA; HPG-CTRL; MET-HPG
414.8051	6.4898	0.004427	2.3539	0.022449	CTRL-AHA; MET-CTRL
255.1402	6.4651	0.004499	2.3469	0.02278	HPG-AHA; MET-HPG
88.03718	6.4557	0.004526	2.3443	0.022886	MET-HPG
410.8482	6.4498	0.004543	2.3426	0.022942	CTRL-AHA; HPG-CTRL
538.7737	6.4131	0.004653	2.3322	0.023444	HPG-AHA; MET-HPG
825.6314	6.4122	0.004656	2.332	0.023444	HPG-AHA; HPG-CTRL; MET-HPG
124.0029	6.408	0.004669	2.3308	0.023475	CTRL-AHA; HPG-CTRL
379.1769	6.4037	0.004682	2.3296	0.023488	HPG-AHA; HPG-CTRL; MET-HPG
115.0916	6.3995	0.004695	2.3284	0.023488	HPG-AHA; HPG-CTRL
135.0445	6.3992	0.004696	2.3283	0.023488	HPG-CTRL; MET-HPG
334.1182	6.3986	0.004698	2.3281	0.023488	HPG-AHA; HPG-CTRL; MET-HPG
286.0806	6.3892	0.004727	2.3255	0.023599	CTRL-AHA; MET-AHA
871.5969	6.3862	0.004736	2.3246	0.023613	HPG-CTRL
74.09462	6.3814	0.004751	2.3233	0.023653	CTRL-AHA; HPG-CTRL
538.8754	6.3772	0.004764	2.322	0.023686	HPG-CTRL; MET-HPG
285.1212	6.3642	0.004805	2.3184	0.023856	CTRL-AHA; HPG-AHA; MET-AHA
286.1484	6.3367	0.004892	2.3105	0.024256	CTRL-AHA; HPG-AHA
808.2883	6.3344	0.004899	2.3099	0.024258	CTRL-AHA; HPG-CTRL
225.1195	6.3304	0.004912	2.3087	0.024288	CTRL-AHA; HPG-CTRL
550.2711	6.3247	0.00493	2.3071	0.024346	HPG-CTRL; MET-HPG
231.1849	6.3224	0.004938	2.3065	0.024349	CTRL-AHA; HPG-AHA; MET-AHA
269.1049	6.3126	0.00497	2.3037	0.024473	CTRL-AHA
85.0264	6.2957	0.005025	2.2988	0.024712	CTRL-AHA; HPG-CTRL
132.099	6.2884	0.00505	2.2967	0.024798	HPG-CTRL; MET-HPG
587.0269	6.2735	0.0051	2.2925	0.025008	CTRL-AHA; MET-AHA
304.136	6.2709	0.005108	2.2917	0.025015	HPG-CTRL; MET-HPG
578.9677	6.2672	0.005121	2.2907	0.025042	HPG-CTRL; MET-HPG
124.9612	6.2639	0.005132	2.2897	0.025063	HPG-CTRL; MET-HPG
448.1318	6.2571	0.005155	2.2878	0.025141	CTRL-AHA
105.9511	6.2415	0.005208	2.2833	0.025367	HPG-CTRL; MET-HPG
404.1343	6.2015	0.005348	2.2718	0.026013	CTRL-AHA; MET-AHA
236.0361	6.1694	0.005463	2.2625	0.026537	HPG-AHA; HPG-CTRL; MET-HPG

133.5581	6.1606	0.005495	2.26	0.026656	HPG-AHA; HPG-CTRL
845.959	6.1405	0.005569	2.2542	0.026979	HPG-AHA; MET-HPG
319.1924	6.1059	0.005699	2.2442	0.02754	HPG-AHA; HPG-CTRL; MET-HPG
331.0301	6.105	0.005703	2.2439	0.02754	HPG-CTRL; MET-HPG
402.1262	6.1036	0.005708	2.2435	0.02754	HPG-CTRL; MET-HPG
691.1376	6.085	0.00578	2.2381	0.027847	HPG-CTRL; MET-HPG
254.0082	6.0664	0.005852	2.2327	0.028157	CTRL-AHA; HPG-AHA
196.9568	6.062	0.005869	2.2314	0.028203	HPG-CTRL
808.7886	6.0434	0.005943	2.226	0.028518	CTRL-AHA; HPG-CTRL
173.0171	6.0409	0.005953	2.2253	0.028529	CTRL-AHA; HPG-CTRL
807.7887	6.0319	0.005989	2.2226	0.028662	CTRL-AHA; HPG-CTRL
132.099	6.021	0.006033	2.2195	0.028835	HPG-CTRL; MET-HPG
190.0086	6.0008	0.006116	2.2136	0.02919	CTRL-AHA; HPG-CTRL
424.1181	5.997	0.006131	2.2124	0.029203	HPG-AHA; MET-HPG
258.8941	5.9963	0.006135	2.2122	0.029203	HPG-CTRL
176.099	5.9929	0.006149	2.2112	0.02923	HPG-AHA; HPG-CTRL; MET-HPG
730.527	5.9873	0.006172	2.2096	0.029277	HPG-AHA; HPG-CTRL; MET-HPG
719.5306	5.9863	0.006176	2.2093	0.029277	HPG-CTRL; MET-HPG
401.952	5.9846	0.006183	2.2088	0.029277	HPG-AHA; HPG-CTRL; MET-HPG
222.1034	5.975	0.006223	2.206	0.029388	HPG-CTRL; MET-HPG
112.0188	5.9745	0.006225	2.2058	0.029388	HPG-AHA; HPG-CTRL
219.0232	5.9731	0.006231	2.2054	0.029388	CTRL-AHA; HPG-CTRL; MET-CTRL
866.6183	5.9618	0.006279	2.2021	0.029575	CTRL-AHA; HPG-CTRL
176.8903	5.9594	0.006289	2.2014	0.029584	CTRL-AHA; HPG-CTRL
290.1314	5.955	0.006308	2.2001	0.029633	MET-CTRL; MET-HPG
528.754	5.9517	0.006322	2.1991	0.02966	CTRL-AHA; HPG-CTRL
515.1381	5.9412	0.006367	2.1961	0.029833	CTRL-AHA; HPG-CTRL
338.6813	5.9277	0.006426	2.1921	0.030068	CTRL-AHA; HPG-CTRL
338.1318	5.9222	0.00645	2.1905	0.03012	HPG-AHA; HPG-CTRL; MET-HPG
712.2757	5.9213	0.006454	2.1902	0.03012	CTRL-AHA; HPG-CTRL
150.0708	5.9171	0.006472	2.189	0.030165	MET-AHA; MET-HPG
731.5305	5.9095	0.006505	2.1867	0.030262	HPG-AHA; MET-HPG
268.1257	5.9086	0.00651	2.1865	0.030262	CTRL-AHA; MET-AHA
168.0694	5.8985	0.006554	2.1835	0.03043	HPG-CTRL; MET-HPG
277.0398	5.8855	0.006613	2.1796	0.030661	CTRL-AHA; HPG-CTRL; MET-CTRL
143.0364	5.8834	0.006622	2.179	0.030665	HPG-AHA; HPG-CTRL
132.0992	5.8718	0.006675	2.1756	0.030869	CTRL-AHA; HPG-AHA
268.1029	5.8621	0.006719	2.1727	0.031032	CTRL-AHA; HPG-CTRL
428.3649	5.858	0.006737	2.1715	0.031078	CTRL-AHA; HPG-CTRL
187.1765	5.8493	0.006777	2.1689	0.031223	CTRL-AHA; MET-AHA
235.9886	5.8368	0.006836	2.1652	0.031451	CTRL-AHA; HPG-CTRL; MET-CTRL
362.9939	5.8336	0.006851	2.1643	0.031479	HPG-CTRL; MET-HPG
500.0828	5.8142	0.006942	2.1585	0.031828	HPG-CTRL
170.9538	5.8137	0.006944	2.1584	0.031828	HPG-CTRL; MET-HPG
272.0958	5.8033	0.006994	2.1553	0.032016	CTRL-AHA; HPG-AHA; MET-AHA
345.0416	5.7982	0.007019	2.1537	0.032087	HPG-CTRL; MET-HPG
344.7354	5.7954	0.007032	2.1529	0.032106	HPG-CTRL; MET-HPG
538.1633	5.7778	0.007117	2.1477	0.032454	CTRL-AHA
311.1792	5.769	0.00716	2.1451	0.03261	HPG-CTRL; MET-HPG
111.089	5.7651	0.007179	2.1439	0.032655	HPG-AHA; HPG-CTRL
771.5029	5.7595	0.007207	2.1422	0.032739	HPG-CTRL; MET-HPG
625.7776	5.7558	0.007226	2.1411	0.032756	HPG-AHA
734.8668	5.7551	0.007229	2.1409	0.032756	CTRL-AHA
514.135	5.7439	0.007285	2.1376	0.032966	CTRL-AHA; HPG-CTRL
865.2857	5.7367	0.007321	2.1354	0.033089	CTRL-AHA; HPG-CTRL
213.0117	5.733	0.00734	2.1343	0.033108	HPG-CTRL; MET-HPG
810.6258	5.7322	0.007344	2.1341	0.033108	HPG-AHA; MET-HPG
362.1484	5.7298	0.007356	2.1333	0.033122	HPG-CTRL
311.0342	5.7271	0.00737	2.1326	0.03314	CTRL-AHA; HPG-CTRL
482.2554	5.7246	0.007383	2.1318	0.033156	MET-HPG
638.1492	5.7056	0.00748	2.1261	0.033552	HPG-CTRL
405.1192	5.6865	0.007579	2.1204	0.033954	CTRL-AHA; HPG-CTRL
170.0774	5.6778	0.007625	2.1178	0.034117	CTRL-AHA; MET-AHA
720.5337	5.6597	0.007721	2.1123	0.034504	HPG-CTRL; MET-HPG
615.459	5.6502	0.007772	2.1095	0.034688	CTRL-AHA; HPG-CTRL
492.1201	5.6347	0.007857	2.1048	0.035022	CTRL-AHA; HPG-CTRL; MET-CTRL
381.1375	5.6258	0.007905	2.1021	0.035192	HPG-AHA; HPG-CTRL; MET-HPG
766.4085	5.6198	0.007938	2.1003	0.035271	HPG-AHA; MET-HPG
381.0165	5.619	0.007942	2.1001	0.035271	CTRL-AHA; HPG-CTRL
274.1349	5.6116	0.007984	2.0978	0.035412	HPG-AHA; HPG-CTRL; MET-HPG
140.0496	5.5969	0.008066	2.0934	0.03573	MET-HPG
524.049	5.5886	0.008113	2.0908	0.035858	CTRL-AHA; MET-CTRL
276.103	5.5882	0.008114	2.0907	0.035858	CTRL-AHA; HPG-CTRL
236.0651	5.5844	0.008136	2.0896	0.035885	CTRL-AHA; HPG-AHA

362.0085	5.5836	0.008141	2.0893	0.035885	HPG-CTRL; MET-HPG
342.2057	5.5768	0.00818	2.0873	0.036012	CTRL-AHA; MET-AHA
338.096	5.5705	0.008215	2.0854	0.036125	HPG-CTRL; MET-HPG
149.9895	5.5688	0.008225	2.0848	0.036125	HPG-CTRL
495.8513	5.566	0.008241	2.084	0.036151	CTRL-AHA; HPG-CTRL
410.9241	5.5629	0.00826	2.083	0.036186	HPG-CTRL; MET-HPG
375.0693	5.5601	0.008275	2.0822	0.036211	HPG-CTRL; MET-HPG
156.023	5.5567	0.008295	2.0812	0.036211	HPG-CTRL; MET-HPG
851.6175	5.5566	0.008296	2.0812	0.036211	HPG-CTRL
864.269	5.5541	0.00831	2.0804	0.03623	HPG-AHA; HPG-CTRL
701.4238	5.5453	0.008362	2.0777	0.036388	HPG-AHA; MET-HPG
347.1615	5.5444	0.008367	2.0774	0.036388	CTRL-AHA; HPG-CTRL
211.1435	5.5387	0.0084	2.0757	0.036489	MET-HPG
245.1557	5.5301	0.008451	2.0731	0.036665	HPG-AHA; HPG-CTRL
342.1334	5.5275	0.008466	2.0723	0.036687	CTRL-AHA; HPG-CTRL
586.3255	5.525	0.008481	2.0715	0.036708	MET-CTRL; MET-HPG
182.0407	5.5103	0.008569	2.0671	0.037043	HPG-CTRL; MET-HPG
318.1954	5.5071	0.008588	2.0661	0.037077	MET-AHA; MET-CTRL
145.0714	5.5055	0.008598	2.0656	0.037077	HPG-CTRL; MET-CTRL
718.527	5.497	0.008649	2.063	0.037254	HPG-CTRL; MET-HPG
536.264	5.4936	0.00867	2.062	0.0373	HPG-CTRL
210.1431	5.4867	0.008713	2.0599	0.037436	HPG-CTRL; MET-HPG
267.0563	5.4762	0.008777	2.0567	0.037608	HPG-AHA; MET-HPG
413.9823	5.4744	0.008788	2.0561	0.037608	CTRL-AHA; HPG-CTRL
136.9279	5.4739	0.008791	2.056	0.037608	CTRL-AHA; HPG-CTRL
231.8991	5.4734	0.008794	2.0558	0.037608	CTRL-AHA; HPG-CTRL
589.0331	5.4705	0.008812	2.0549	0.037637	CTRL-AHA; HPG-CTRL
439.0626	5.4673	0.008832	2.0539	0.037649	CTRL-AHA; HPG-CTRL
269.1444	5.4667	0.008836	2.0538	0.037649	HPG-AHA; MET-AHA
378.1742	5.4629	0.008859	2.0526	0.037705	HPG-CTRL; MET-CTRL
859.6124	5.4607	0.008873	2.0519	0.037719	HPG-CTRL
156.059	5.4503	0.008939	2.0487	0.037951	CTRL-AHA; MET-CTRL
743.3573	5.4472	0.008958	2.0478	0.037989	CTRL-AHA
393.1975	5.4322	0.009054	2.0432	0.038349	HPG-AHA; MET-HPG
402.3499	5.4256	0.009095	2.0412	0.03848	CTRL-AHA; HPG-CTRL
729.4556	5.4077	0.009211	2.0357	0.038924	HPG-AHA; MET-HPG
243.1	5.4004	0.009259	2.0334	0.039081	HPG-AHA; MET-AHA
113.1043	5.3781	0.009407	2.0266	0.039656	CTRL-AHA; HPG-CTRL
122.9214	5.3722	0.009446	2.0247	0.039733	HPG-CTRL
218.0622	5.3721	0.009447	2.0247	0.039733	HPG-CTRL
103.9531	5.3545	0.009566	2.0193	0.040175	HPG-CTRL
503.1652	5.3532	0.009575	2.0189	0.040175	CTRL-AHA; HPG-AHA
164.9262	5.3465	0.00962	2.0168	0.04032	CTRL-AHA
345.0149	5.3401	0.009664	2.0148	0.040457	CTRL-AHA; HPG-CTRL
777.4742	5.3291	0.009741	2.0114	0.040728	HPG-CTRL
282.1068	5.3238	0.009777	2.0098	0.040832	CTRL-AHA; HPG-AHA; MET-AHA
685.0449	5.3148	0.00984	2.007	0.041047	HPG-CTRL; MET-HPG
724.3783	5.3037	0.009918	2.0036	0.041325	CTRL-AHA; HPG-AHA
825.4211	5.2983	0.009957	2.0019	0.041438	HPG-AHA; MET-HPG
686.2203	5.2889	0.010024	1.999	0.041626	HPG-AHA; HPG-CTRL; MET-HPG
260.092	5.2887	0.010025	1.9989	0.041626	HPG-AHA; MET-AHA
292.1449	5.2828	0.010068	1.9971	0.041754	HPG-AHA; HPG-CTRL; MET-HPG
265.1069	5.2785	0.010099	1.9957	0.041833	CTRL-AHA; HPG-CTRL
169.0707	5.2723	0.010144	1.9938	0.041972	CTRL-AHA; MET-AHA
851.9508	5.2551	0.010269	1.9885	0.042443	HPG-CTRL; MET-HPG
214.0543	5.2461	0.010335	1.9857	0.042666	HPG-CTRL
161.1248	5.2372	0.010402	1.9829	0.042893	CTRL-AHA
260.0489	5.2308	0.01045	1.9809	0.043038	CTRL-AHA; HPG-CTRL; MET-CTRL
617.0556	5.2149	0.01057	1.9759	0.043485	CTRL-AHA; HPG-CTRL
159.0358	5.2091	0.010614	1.9741	0.043599	MET-HPG
290.1637	5.208	0.010622	1.9738	0.043599	HPG-AHA; HPG-CTRL
112.0479	5.1922	0.010745	1.9688	0.04405	CTRL-AHA; HPG-CTRL
435.0064	5.1904	0.010758	1.9683	0.044056	MET-HPG
278.8746	5.1862	0.010791	1.9669	0.044139	CTRL-AHA; MET-CTRL
449.1345	5.1769	0.010863	1.964	0.044385	CTRL-AHA
688.1451	5.1714	0.010907	1.9623	0.04448	HPG-CTRL
308.0426	5.1708	0.010912	1.9621	0.04448	HPG-CTRL
307.0599	5.1663	0.010947	1.9607	0.044574	CTRL-AHA
296.2165	5.1627	0.010976	1.9596	0.04464	CTRL-AHA; HPG-CTRL
649.2657	5.1573	0.011019	1.9579	0.044763	HPG-CTRL
744.2565	5.1462	0.011107	1.9544	0.045059	CTRL-AHA
88.03713	5.145	0.011117	1.954	0.045059	MET-AHA; MET-HPG
666.1204	5.141	0.011149	1.9528	0.045138	HPG-CTRL
834.5365	5.128	0.011254	1.9487	0.0455	HPG-CTRL

550.2256	5.1269	0.011264	1.9483	0.0455	CTRL-AHA; HPG-CTRL
182.9575	5.1203	0.011318	1.9462	0.045667	CTRL-AHA; HPG-CTRL; MET-CTRL
294.0544	5.0976	0.011506	1.9391	0.046372	HPG-CTRL; MET-HPG
151.9877	5.0869	0.011596	1.9357	0.046683	HPG-CTRL
244.8948	5.0784	0.011667	1.933	0.046919	HPG-CTRL
117.0516	5.0739	0.011705	1.9316	0.047019	HPG-CTRL
292.8122	5.0649	0.011782	1.9288	0.047274	HPG-CTRL
399.9637	5.0626	0.011802	1.928	0.047301	HPG-CTRL; MET-HPG
392.1779	5.06	0.011825	1.9272	0.047339	HPG-CTRL; MET-HPG
432.7755	5.042	0.011981	1.9215	0.047911	CTRL-AHA; HPG-CTRL; MET-CTRL
275.078	5.0397	0.012002	1.9208	0.04794	HPG-CTRL
280.1333	5.0272	0.012111	1.9168	0.048323	CTRL-AHA; HPG-CTRL
396.9908	5.0158	0.012213	1.9132	0.048674	HPG-CTRL
320.1855	5.005	0.01231	1.9097	0.048987	HPG-CTRL; MET-HPG
231.1291	5.004	0.012319	1.9094	0.048987	HPG-CTRL; MET-HPG
266.1098	5.0007	0.012349	1.9084	0.049053	CTRL-AHA
592.5292	4.9964	0.012387	1.907	0.049131	HPG-CTRL; MET-HPG
344.1288	4.9932	0.012416	1.906	0.049131	CTRL-AHA; HPG-CTRL
527.8404	4.9926	0.012422	1.9058	0.049131	CTRL-AHA; HPG-CTRL
323.0582	4.9924	0.012424	1.9058	0.049131	HPG-CTRL
872.948	4.9895	0.01245	1.9048	0.049181	CTRL-AHA; HPG-CTRL
178.1301	4.9735	0.012598	1.8997	0.049684	CTRL-AHA
499.1641	4.9727	0.012605	1.8995	0.049684	CTRL-AHA

Table S7. NMR metabolites.

Metabolite	Control		Met-50µM		AHA-50µM		HPG-50µM	
	Average	SD	Average	SD	Average	SD	Average	SD
2-Aminobutyrate	6.1	0.8	7.9	1.2	11.2	1.8	13	0.3
4-Aminobutyrate *	136.5	24.4	118.2	43.4	177.7	30.6	122.3	73.5
Acetate *	656.4	82.9	763.9	109.5	795.4	92.9	1001	90.7
Acetoacetate *	2.8	0.7	3.4	1.3	3.2	0.5	2.9	0.8
Acetoin *	3.8	3.4	3	1.2	2.5	0.8	5.1	1
Adenosine *	1.8	1.3	1.9	0.3	1.4	0.2	1.3	0.1
ADP	4.7	0.5	5.8	0.5	6.7	0.9	7.6	1.3
Alanine	178.7	36.8	208.9	22.4	288.3	52.9	344.5	39.6
AMP	25.9	3.5	33	3.2	43	8.7	62.7	3.9
Aspartate	18.3	7.2	23.2	12.3	13.6	1.6	18.5	1.6
ATP	2.6	0.9	4.7	1.2	5.7	1.4	6.2	1.7
Betaine	4.7	2.8	2.8	0.1	4	0.2	2.2	0.5
Cholate	2.4	1	2.6	0.5	3.1	0.7	3.7	0.7
Choline	1.5	1	1	0.1	1.3	0.2	1.3	0.1
Dimethyl sulfone *	1.2	1.3	0.8	0.3	0.7	0.1	1	0.2
Dimethylamine	0.5	0	0.7	0.2	0.8	0.1	1.3	0.3
dTTP *	23.4	1.9	26.6	2.5	35.5	5	29.6	3.9
Formate	382.5	50.2	678.9	36.6	705.6	69.4	642.7	16.6
Fumarate	6.5	1	5.8	0.9	4.8	0.9	11.2	2
Glucose	49	38.6	48.6	40.6	19.8	3.4	33.6	17.6
Glutamate	63.2	38.7	79.3	41.5	103.3	23.4	138.6	71.1
Glutathione	51.8	10.3	63.8	10.2	85.2	8.4	122.3	8.6
Glycine	50.4	15.8	57.8	11.1	104.2	10.4	81	5.5
GTP *	3.7	0.9	4.4	0.6	4.9	0.6	7.5	1.2
Histamine	2.4	0.7	2.1	0.5	3	1.1	3.9	0.6
Histidine	2.2	0.3	2.6	0.7	3.1	0.6	3.4	0.5
Hypoxanthine	17.2	5	23.1	1.4	27.4	4.5	26.8	5.8
IMP	3.4	1	5	0.8	5.6	1.7	7.1	0.3
Isoleucine	7.4	0.5	9.1	1	11.7	1.7	15.4	2.2
Lactate	53.9	21.1	104.5	43.4	130	40.1	123.3	24.3
Leucine	14.4	1.3	17.8	1.8	23	3.6	27.5	3.6
Malate	58.1	4.1	61.9	7.1	63.4	7.5	143.6	8.8
Methionine	6.7	1.3	8.3	1.2	13.3	1.5	14.2	1.4
N-Acetylaspartate *	54.2	12.7	96.9	5.6	101	10.9	61.2	12.9
N-Acetylglutamate *	5.9	1.1	6.1	1.3	7.2	0.5	11.7	2.5
N-Acetyl glycine	3.5	0.4	3.6	0.8	4.4	0.5	6.6	0.7
NAD ⁺	15.1	0.7	21	3.4	27.2	4.5	30.4	3
N-Carbamoylaspartate *	83.3	25	117.7	25.4	128.4	10.2	61.9	17.9
O-Phosphocholine *	0.7	0.2	0.7	0.1	1	0.1	0.7	0.1
Pantothenate	3.6	1.8	4.6	0.7	2.9	1.1	4.8	1.6
Phenylalanine	5.5	0.6	5.9	0.5	8.1	1.1	10.3	1
Putrescine	5.9	1.4	7.2	0.7	11	2.1	16	1.6
Pyruvate	22.8	13.2	42.3	9.8	36.2	9.4	64.1	16.6
sn-Glycero-3-phosphocholine *	2	0.8	1.2	0.2	2.1	0.3	2.5	0.6
Succinate	152.9	19.3	205.9	16.2	259.5	34.6	316.5	50
Tartrate	6.6	0.9	8.2	1.8	10.4	1.3	12.8	1.4
Trimethylamine N-oxide	0.6	0.7	0.2	0.2	0.1	0.1	0.3	0.1
Tyrosine	12.7	2.1	17.6	1.8	22.4	2.8	25.5	3.8
UDP-glucose *	8.1	2.9	10.1	1.1	13.5	1.4	19.8	1.3
UDP-N-Acetylglucosamine *	3.1	1.1	2.8	1.8	2.8	0.6	3.5	0.6
UMP	20.4	2.8	19.7	1.4	26.8	3	35.7	1.2
Uracil	27.6	5.2	34.2	3	41.2	5.8	45.3	4.7
Valine	18.7	2.6	23.3	1.5	31.5	6.6	43.7	7.1
Xanthosine	1.4	0.3	1.9	0.2	1.4	0.3	1.3	0.2

*Assignment with best-matched signals, all others validated.

Table S8. Loading factors for 2D-PCA scores plot of heat-stressed *E. coli* cultures from Figure 3B.

Metabolite	PC1	PC2
2-Aminobutyrate	0.18327	-0.042002
4-Aminobutyrate	-0.012652	-0.18683
ADP	0.16284	-0.087807
AMP	0.17848	0.0011266
ATP	0.13204	-0.18101
Acetate	0.16316	-0.010254
Acetoacetate	0.015504	-0.14884
Acetoin	0.069363	0.17966
Adenosine	-0.0073455	0.018199
Alanine	0.17416	-0.029211
Aspartate	0.0020064	0.10747
Betaine	-0.069893	-0.0070998
Cholate	0.1413	0.059908
Choline	0.046082	0.16315
Dimethyl sulfone	0.029407	0.2601
Dimethylamine	0.15715	0.091441
Formate	0.12576	-0.26046
Fumarate	0.083996	0.29072
GTP	0.15447	0.014695
Glucose	-0.051666	0.053545
Glutamate	0.10733	0.089474
Glutathione	0.17402	0.030719
Glycine	0.12451	-0.14154
Histamine	0.13025	0.076048
Histidine	0.1491	0.0034941
Hypoxanthine	0.14492	-0.10708
IMP	0.16094	0.0042664
Isoleucine	0.18581	0.00074766
Lactate	0.13004	-0.124
Leucine	0.18495	-0.021526
Malate	0.15156	0.18264
Methionine	0.17547	-0.054267
N-Acetylaspartate	0.021105	-0.29746
N-Acetylglutamate	0.14634	0.18789
N-Acetylglycine	0.16222	0.079985
N-Carbamoylaspartate	-0.055414	-0.35668
NAD ⁺	0.17684	-0.083403
O-Phosphocholine	0.07488	-0.089337
Pantothenate	0.046712	0.11346
Phenylalanine	0.18293	0.04043
Propylene glycol	0.17549	-0.065088
Putrescine	0.18109	0.029436
Pyruvate	0.11508	-0.057185
Succinate	0.18301	-0.05308
Tartrate	0.16883	-0.0016467
Trimethylamine N-oxide	0.021261	0.27334
Tyrosine	0.17679	-0.090745

UDP-N-Acetylglucosamine	0.020713	0.12983
UDP-glucose	0.16733	-0.010497
UMP	0.16571	0.062401
Uracil	0.17514	-0.047694
Valine	0.18301	0.029837
Xanthosine	-0.059567	-0.14554
dTTP	0.12974	-0.18943
sn-Glycero-3-phosphocholine	0.093846	0.14058

Table S9. ANOVA of heat-stressed NMR metabolites.

#	Compound	f.value	p.value	-log10(p)
1	Malate	100.3	1.38E-10	9.9
2	Formate	51.6	1.91E-08	7.7
3	UMP	41.8	8.46E-08	7.1
4	Putrescine	37.7	1.74E-07	6.8
5	Methionine	36.8	2.07E-07	6.7
6	Glutathione	35.2	2.83E-07	6.5
7	2-Aminobutyrate	34.7	3.10E-07	6.5
8	Phenylalanine	34.6	3.14E-07	6.5
9	AMP	34.5	3.24E-07	6.5
10	Isoleucine	32.2	5.18E-07	6.3
11	NAD ⁺	30	8.28E-07	6.1
12	Succinate	28.5	1.17E-06	5.9
13	Tyrosine	26.4	1.96E-06	5.7
14	Leucine	25.8	2.25E-06	5.6
15	Valine	24.1	3.53E-06	5.5
16	Fumarate	24	3.66E-06	5.4
17	Dimethylamine	22.4	5.64E-06	5.2
18	UDP-glucose	21.2	8.12E-06	5.1
19	N-Acetylaspartate	20.9	8.91E-06	5
20	N-Acetylglycine	19.9	1.18E-05	4.9
21	Tartrate	17.3	2.80E-05	4.6
22	Alanine	16.9	3.19E-05	4.5
23	N-Acetylglutamate	15.2	6.09E-05	4.2
24	GTP	15	6.58E-05	4.2
25	Uracil	13.7	1.08E-04	4
26	dTTP	11.5	2.82E-04	3.5
27	Glycine	11.5	2.84E-04	3.5
28	Acetate	11.2	3.25E-04	3.5
29	ADP	11.1	3.48E-04	3.5
30	IMP	10.4	4.90E-04	3.3
31	ATP	9.9	6.37E-04	3.2
32	N-Carbamoylaspartate	9.7	7.02E-04	3.2
33	sn-Glycero-3-phosphocholine	7.9	1.85E-03	2.7
34	Pyruvate	7.3	2.60E-03	2.6
35	Lactate	7.2	2.75E-03	2.6
36	Hypoxanthine	6.4	4.57E-03	2.3
37	Betaine	5	1.22E-02	1.9
38	Histidine	4.9	1.33E-02	1.9
39	Xanthosine	4.6	1.65E-02	1.8
40	Histamine	4.5	1.76E-02	1.8
41	O-Phosphocholine	3.7	3.38E-02	1.5

Table S10. Fold changes from NMR heat-stressed data.

Metabolite	Fold change		
	Met/Control	AHA/Control	HPG/Control
2-Aminobutyrate	1.3	1.9	2.2
4-Aminobutyrate	0.9	1.3	0.9
Acetate	1.2	1.2	1.5
Acetoacetate	1.2	1.1	1.0
Acetoin	0.8	0.6	1.3
Adenosine	1.1	0.8	0.7
ADP	1.2	1.4	1.6
Alanine	1.2	1.6	1.9
AMP	1.3	1.7	2.4
Aspartate	1.3	0.7	1.0
ATP	1.8	2.2	2.4
Betaine	0.6	0.9	0.5
Cholate	1.1	1.3	1.6
Choline	0.7	0.9	0.9
Dimethyl sulfone	0.6	0.6	0.8
Dimethylamine	1.2	1.4	2.3
dTTP	1.1	1.5	1.3
Formate	1.8	1.8	1.7
Fumarate	0.9	0.7	1.7
Glucose	1.0	0.4	0.7
Glutamate	1.3	1.6	2.2
Glutathione	1.2	1.6	2.4
Glycine	1.1	2.1	1.6
GTP	1.2	1.3	2.0
Histamine	0.9	1.2	1.6
Histidine	1.2	1.4	1.6
Hypoxanthine	1.3	1.6	1.6
IMP	1.5	1.6	2.1
Isoleucine	1.2	1.6	2.1
Lactate	1.9	2.4	2.3
Leucine	1.2	1.6	1.9
Malate	1.1	1.1	2.5
Methionine	1.3	2.0	2.1
N-Acetylaspartate	1.8	1.9	1.1
N-Acetylglutamate	1.0	1.2	2.0
N-Acetylglycine	1.0	1.3	1.9
NAD ⁺	1.4	1.8	2.0
N-Carbamoylaspartate	1.4	1.5	0.7
O-Phosphocholine	0.9	1.4	1.1
Pantothenate	1.3	0.8	1.3
Phenylalanine	1.1	1.5	1.9
Putrescine	1.2	1.9	2.7
Pyruvate	1.9	1.6	2.8
sn-Glycero-3-phosphocholine	0.6	1.0	1.3
Succinate	1.3	1.7	2.1
Tartrate	1.2	1.6	1.9
Trimethylamine N-oxide	0.4	0.2	0.6

Tyrosine	1.4	1.8	2.0
UDP-glucose	1.3	1.7	2.5
UDP-N-Acetylglucosamine	0.9	0.9	1.1
UMP	1.0	1.3	1.8
Uracil	1.2	1.5	1.6
Valine	1.2	1.7	2.3
Xanthosine	1.4	1.0	0.9