Supplementary Information for:

Combining DI-ESI-MS and NMR Datasets for Metabolic Profiling

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SUPPLEMENTARY METHODS

Preparation of standard bacterial metabolomics samples

Escherichia coli Mach1 starter culture was grown at 37 °C in LB media. The culture was shaken at 200 rpm for 12 hours and grown to an approximate $O.D._{600}$ of 12.0. Then 1 mL of the starter culture was added to three 250 mL flasks containing 25 mL of LB media at 37 °C. The bacteria were allowed to grow to a stationary growth phase (6 h) with an $O.D._{600}$ of 10.75. *E .coli* cells were then harvested by transferring 5 mL from each 250 mL flask to three corresponding 15 mL falcon tubes, centrifuged for 10 min at 2057 g at 25 °C. The supernatant media was discarded. After two washing steps with PBS, the supernatant was discarded and the cell pellets were washed with 10 mL of cold distilled water three times. The cell pellets were re-suspended with 1 mL of distilled water and transferred to 1.6 mL microcentrifuge tubes and centrifuged at 15,294 g for 5 minutes at 25 °C. The cell pellets were then lysed with a Sonic Dismembrator model 100 (Fisher Scientific) for 5 minutes, then centrifuged for 10 minutes at 15,294 g at 25 °C. 700 µL of the metabolite extract was transferred to a second 1.6 mL microcentrifuge tube. Another extraction was performed on the remaining cell debris by adding 1.0 mL of 50/50 ddH_2O/methanol to the first microcentrifuge tube, which was then centrifuged for 10 minutes at 15,294 g at 25 °C. 800 µL of the supernatant was extracted and combined with the initial 700 µL extract. The resulting 1.5 mL cell lysate was frozen, lyophilized (Freezone 4.5, Kansas City, MO, USA), and then resuspended in 1.0 mL of ddH_2O/methanol/FA (49.75:49.75:0.5). To generate analytical replicates, each of the three metabolomics samples was separated into 3 aliquots of 100 µL and then diluted ten-fold with ddH_2O/methanol/FA (49.75:49.75:0.5) containing 10 µM caffeine as an internal mass reference.

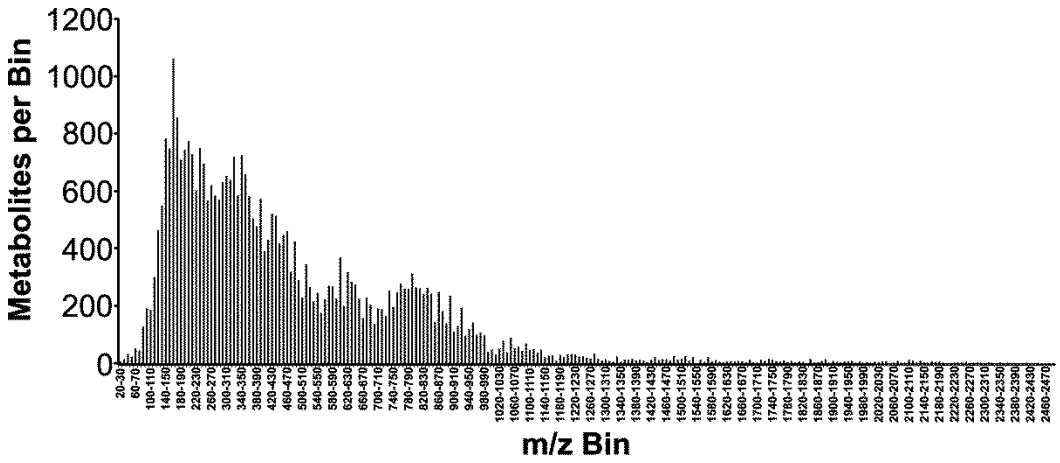

Figure S1. Mass distribution of known metabolites from the Human Metabolome Database (HMDB, http://www.hmdb.ca/).

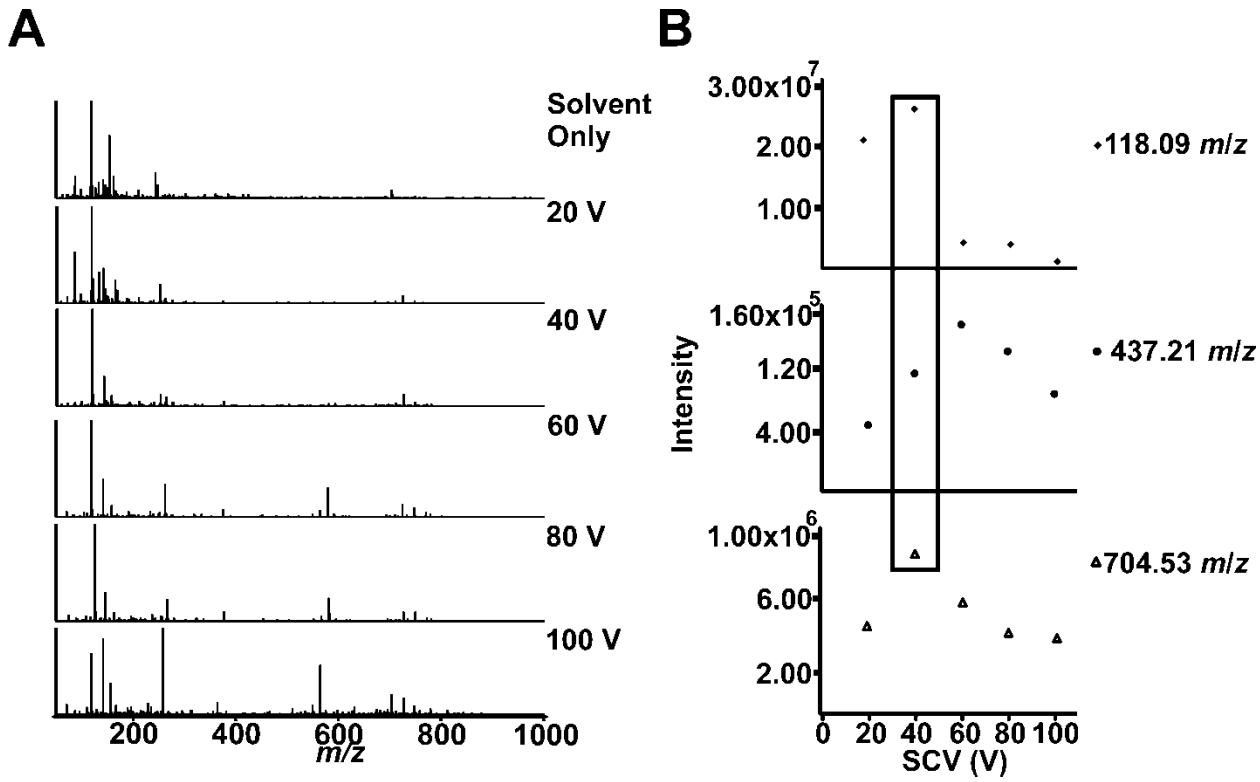

Figure S2. The effects of sampling cone voltage on DI-ESI-MS on total ion count for *E. coli* Mach1 metabolite extract. (A) Mass spectra at varying SCVs (B) Intensity change plotted against SCV for three spectrometric peaks at *m/z* 118.09, 437.21, and 704.53.

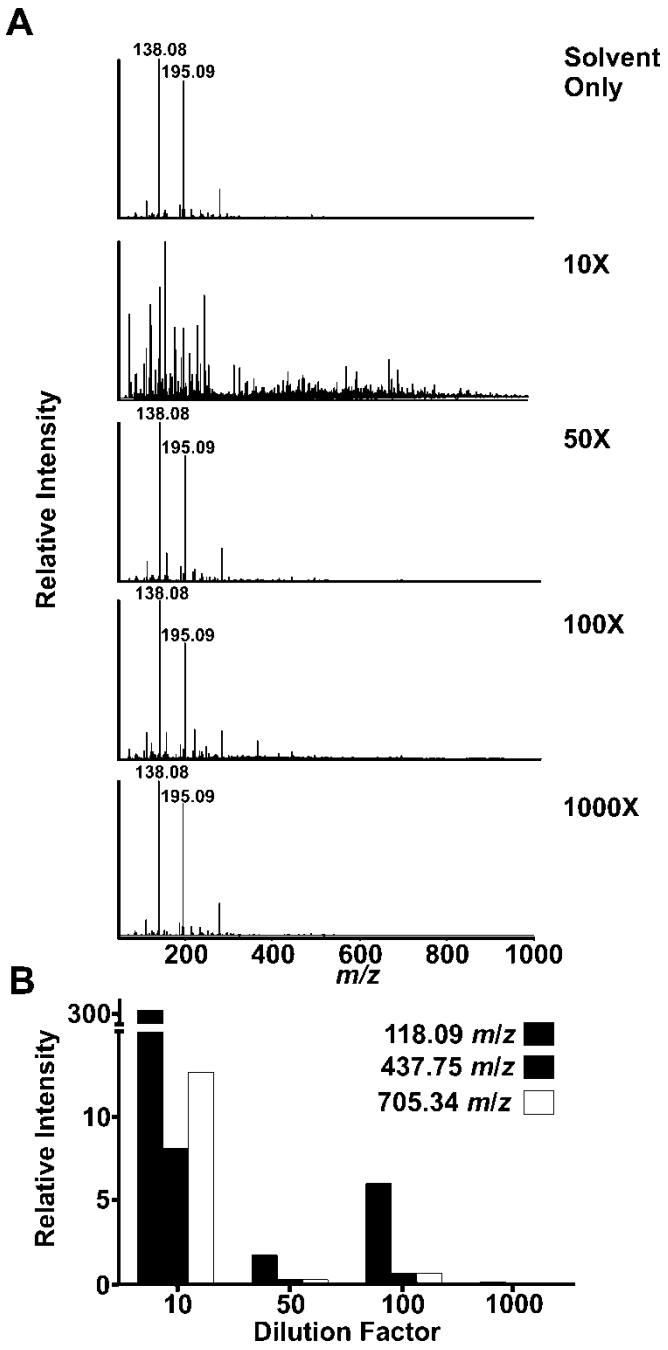

Figure S3. (A) DI-ESI-MS spectra of *E*. coli Mach1 metabolite extract spiked with $[caffeine+H]^+$ *m/z* 195.09 and its primary fragment *m/z* 138.07 (internal mass reference) at various dilutions; H_2O/methanol/FA (49.75:49.75:0.5) and 10 µM caffeine (solvent blank), 10 fold dilution, 50 fold dilution, 100 fold dilution, and 1000 fold dilution. (B) Bar graph of relative intensities for 10, 50, 100, 1000 times dilution for three spectrometric peaks at *m/z* 118.09 (red), 437.21 (blue), and 704.53 (green) normalized to $[caffeine+H]^+$.

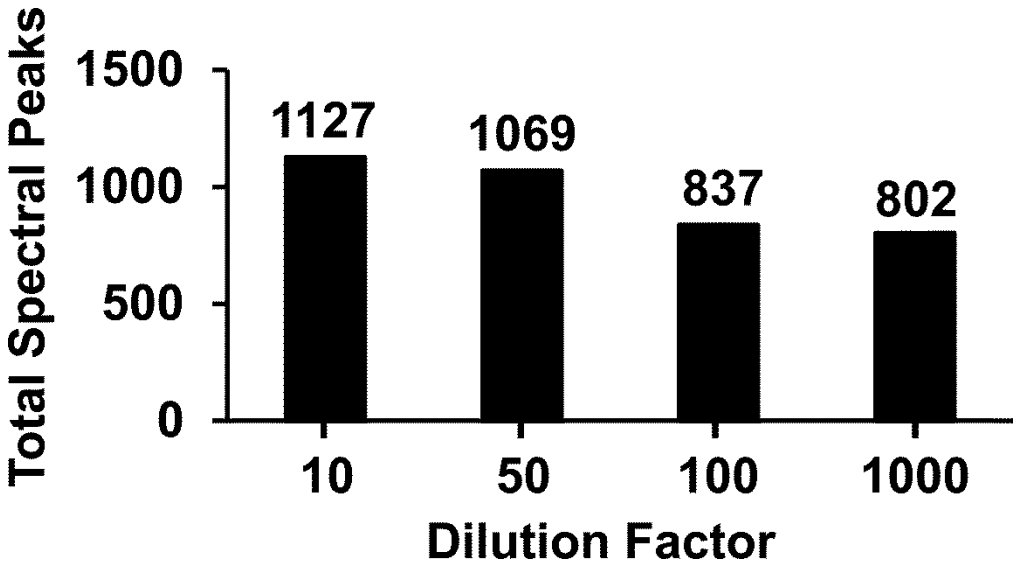

Figure S4. Total number of peaks in the MS spectrum above the noise threshold is plotted as a function of dilution factor.

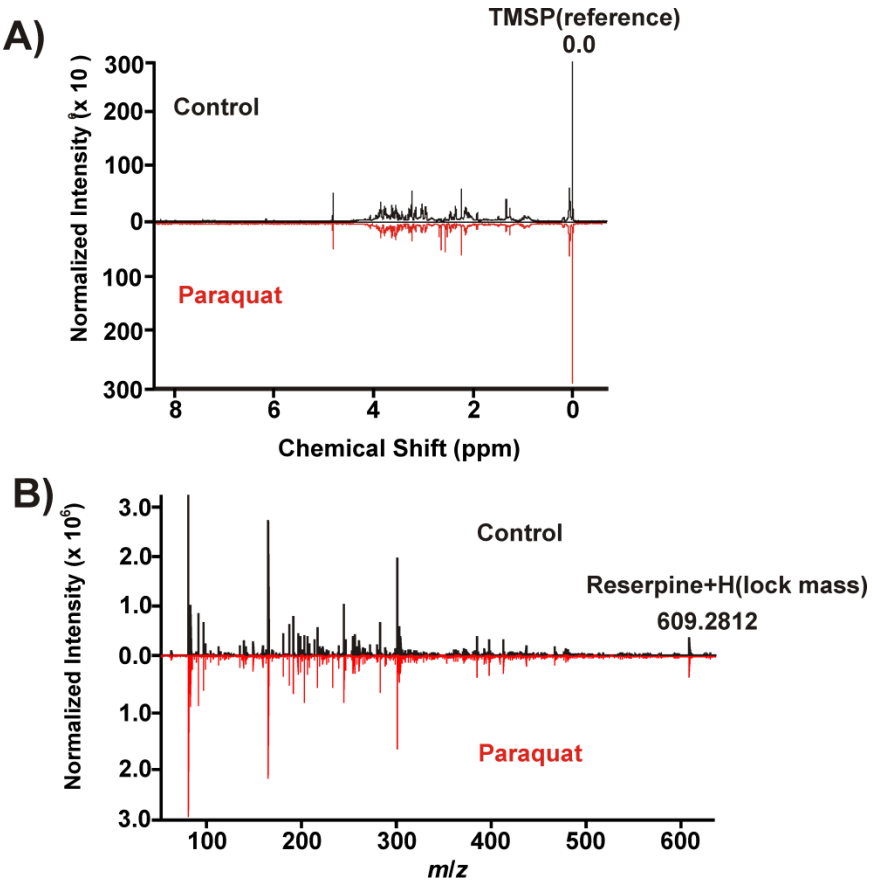

Figure S5. nano-DI-ESI-MS and 1D ^{1}H NMR spectra of control and paraquat treated samples.

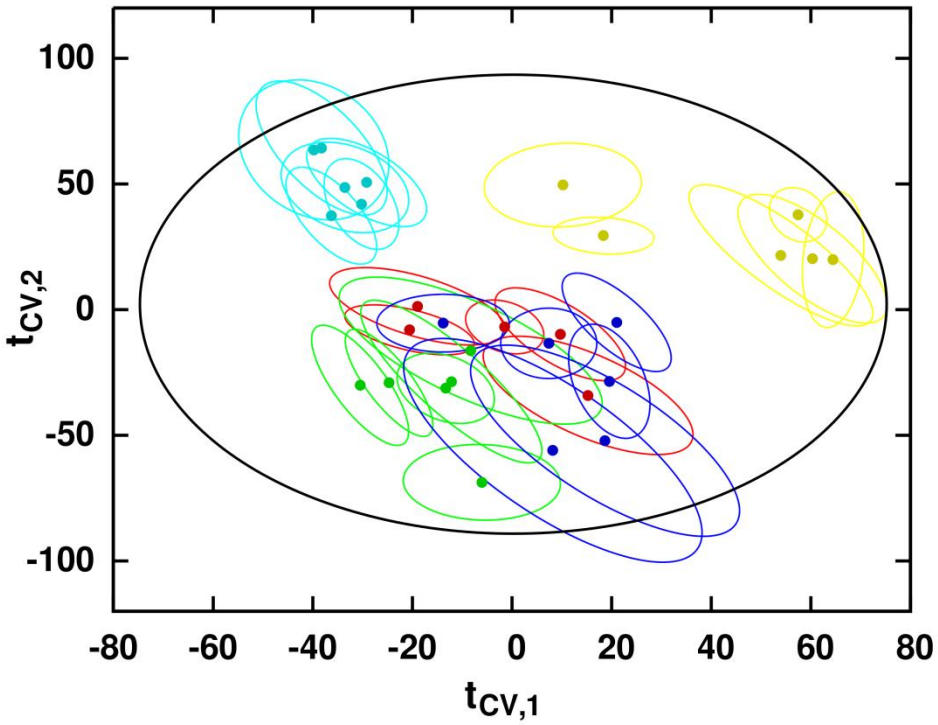

Figure S6. PLS-DA scores computed from single Monte Carlo leave-*n*-out cross-validation of the *in vacuo* ^{1}H NMR dataset. Symbol colors designate the following classes: Control (●), Rotenone (●), 6-OHDA (●), MPP+ (●), and Paraquat (●). Symbols designate the mean value of an observation from all iterations of a Monte Carlo cross-validation, and ellipses designate the 95% confidence ellipse for the respective observation.

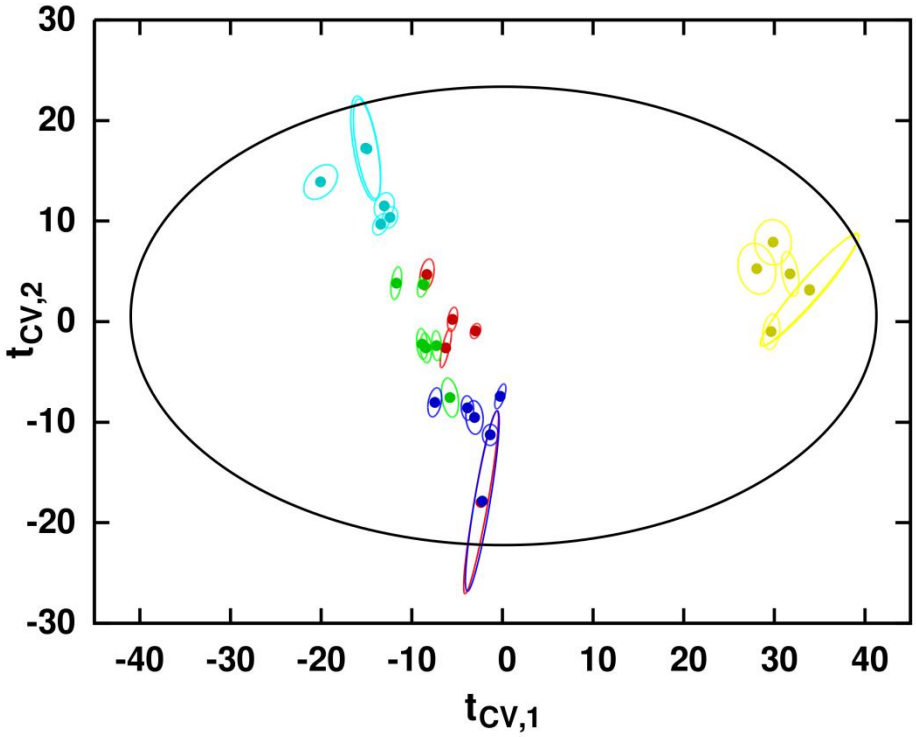

Figure S7. PLS-DA scores computed from a single Monte Carlo leave-*n*-out cross-validation of the *in vacuo* DI-ESI-MS dataset. See Figure S6 for color, symbol and ellipse definitions.

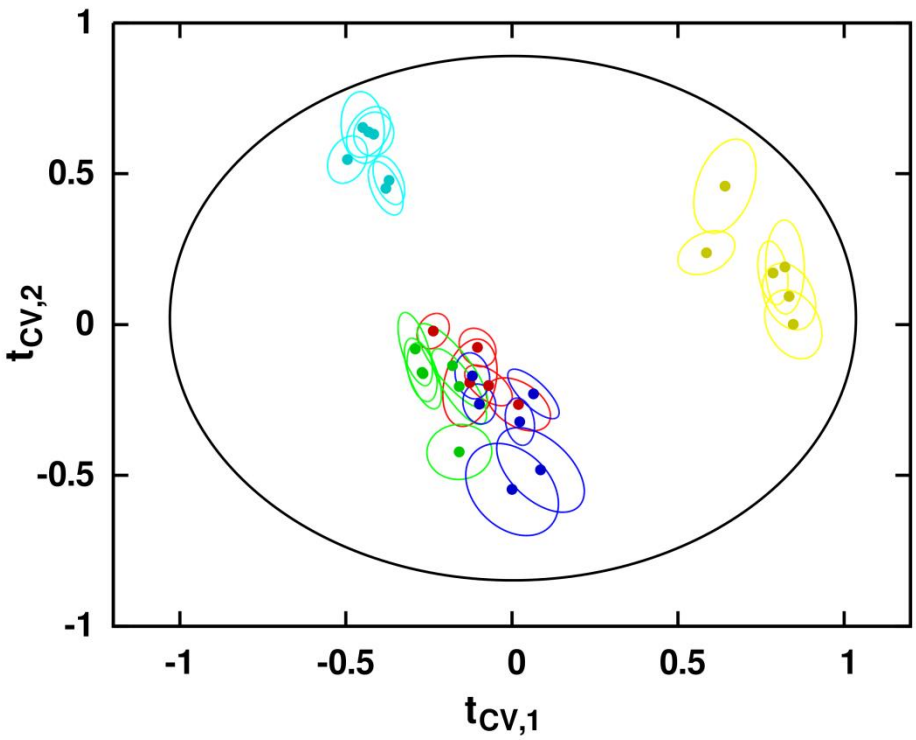

Figure S8. MB-PLS-DA super scores computed from a single Monte Carlo leave-*n*-out cross-validation of the combined MS and NMR datasets. See Figure S6 for color, symbol and ellipse definitions.

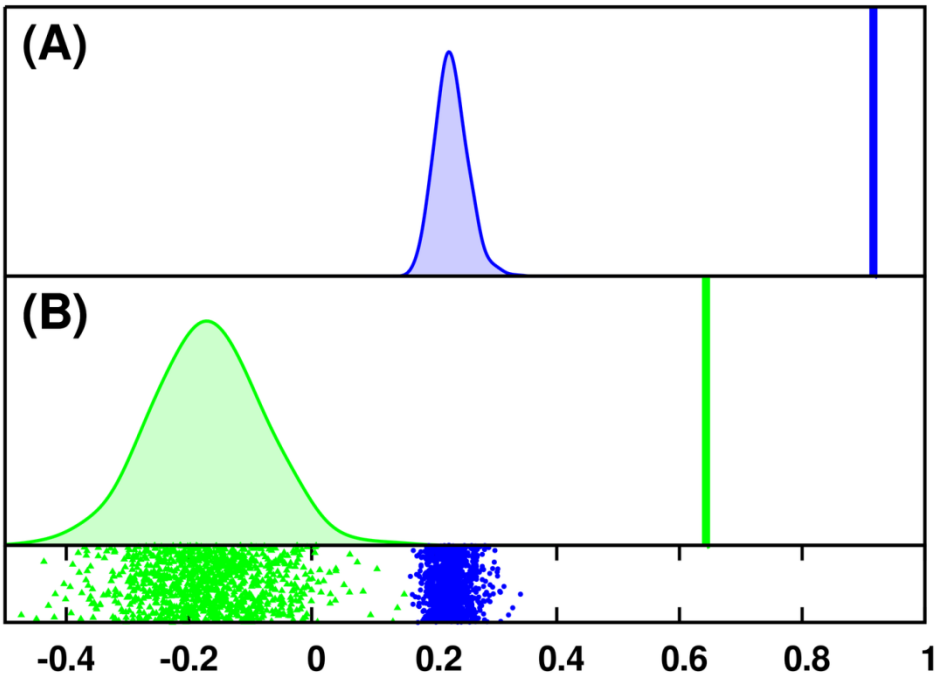

Figure S9. Response permutation test results for the PLS-DA model of the *in vacuo* ^{1}H NMR dataset. Model fit (R^2) statistics (A) are shown in blue, and model predictive ability (Q^2) statistics are shown in green. True values of R^2 and Q^2 are represented by vertical bars, and null distributions are computed through kernel density estimation of the values from permutation. Scatter plots of the permutation R^2 and Q^2 statistics are shown in the lower pane.

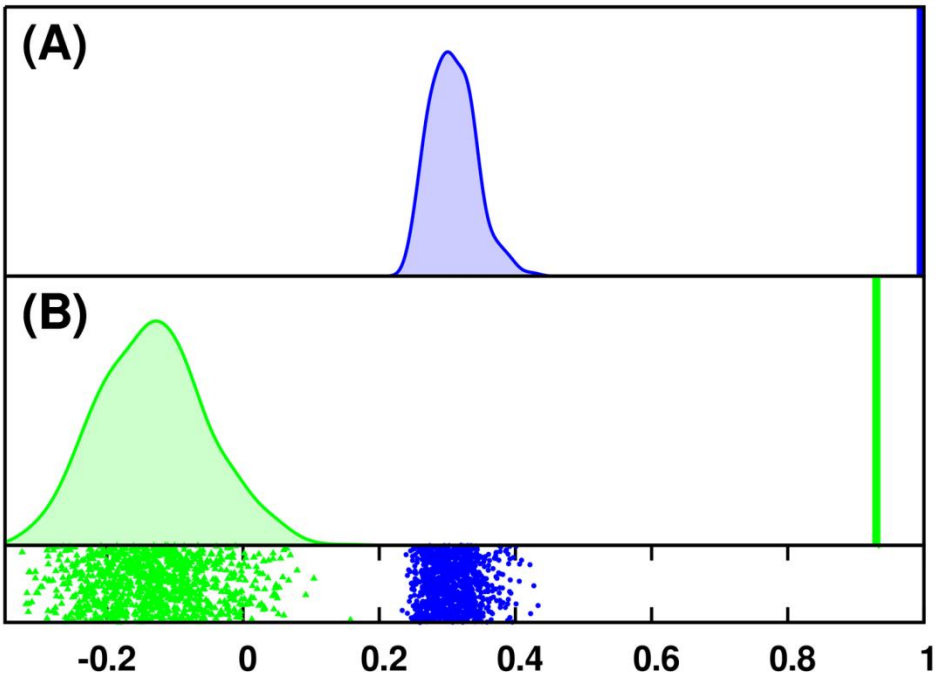

Figure S10. Response permutation test results for the PLS-DA model of the *in vacuo* DI-ESI-MS dataset. Model fit (R^2) statistics (A) are shown in blue, and model predictive ability (Q^2) statistics are shown in green. True values of R^2 and Q^2 are represented by vertical bars, and null distributions are computed through kernel density estimation of the values from permutation. Scatter plots of the permutation R^2 and Q^2 statistics are shown in the lower pane.

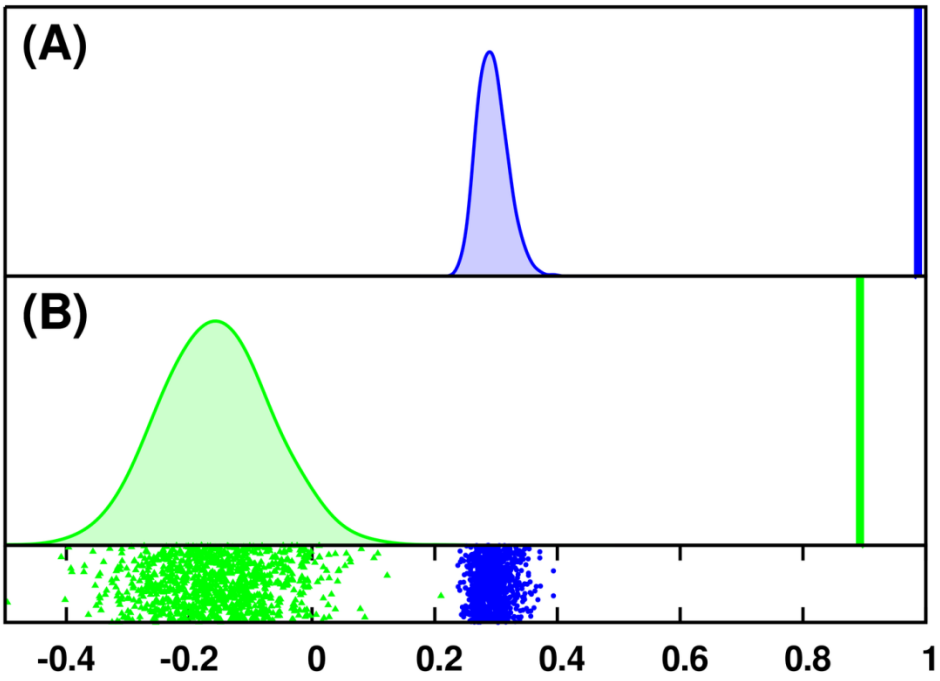

Figure S11. Response permutation test results for the MB-PLS-DA model of combined MS and NMR datasets. Model fit (R^2) statistics (A) are shown in blue, and model predictive ability (Q^2) statistics are shown in green. True values of R^2 and Q^2 are represented by vertical bars, and null distributions are computed through kernel density estimation of the values from permutation. Scatter plots of the permutation R^2 and Q^2 statistics are shown in the lower pane.