Metabolomic Data collection:

Prepared samples were analyzed via chromatographic separation, in-line with mass spectrometry. Ultra-high-performance liquid chromatography (UHPLC) was performed using the Thermo Scientific Ultimate 3000 RS UHPLC platform. Specific settings and flow regimes are in Supplemental Methods. For reversed-phase analysis the instrument was fitted with a Waters Acquity UPLC BEH, 1.7 µm, 2.1 x 100 mm, C-18 column heated at 40oC with a flow rate of 0.4 ml/min. For positive mode C18 runs buffer A was water, 0.1% formic acid and buffer B was 100% acetonitrile, 0.1% formic acid. The gradient started at 2% B and went up to 25% B in 1 min., then 80% B in 7 min., then 100% B in 6 min, and was held at 100% B for 2 min. For negative mode C18 the gradient was the same except the buffer A was 10 mM ammonium acetate, pH 9 and buffer B was 100% acetonitrile. For hydrophobic interactions chromatography (HILIC) separations samples were run in negative polarity using a Millipore Sequant® Zic®-pHILIC, 5 µm, 4.6 x 150 mm column at 0.4 ml/min. For HILIC, buffer A was 15 mM ammonium acetate pH 7 in 20% percent acetonitrile, 80% water and buffer B was 100% acetonitrile. The column was heated at 40oC and the flow rate was 0.4 ml/min starting at 81% B for 1 min, 81%-25% B over 18 min, and then held at 25% B for 2 min. For all separations, the column was in-line with a heated electrospray ionization source mounted to a Q Exactive™ Quadrupole-Orbitrap mass spectrometer (Thermo Scientific, Waltham, MA). The spray voltage was 3.4 kV in positive and negative mode, sheath gas flow at 50, auxiliary gas flow at 8, Slens RF at 55 V, probe heater at 400 oC, and the heated capillary was maintained at 320 °C. The orbitrap was set to acquire MS1 mass spectra from 70–1050 m/z with a resolution of 70,000 at 200 m/z, an automatic gain control (AGC) of 1.0E6, and a max injection time of 200 ms. In order to obtain a thorough profile, the instrument was initially operated in full scan mode (MS1 only). After data analysis and identification of metabolites of interest fragmentation (MS2) studies were performed to assist in identification and quantification of metabolites of interest. For MS2 tandem mass spectra runs a target inclusion list of m/z values of interest was imported. Fragmentation by higher-energy collisional dissociation with stepped normalized collision energy of 30 and 60, a detector setting of 17.5 k resolution, AGC 1e5 ions, 100 ms maximum injection time was used. Dynamic exclusion was set to 1.5 s with a 10 ppm mass tolerance.

Metabolomic Data Analysis:

Raw data were processed using the Progenesis QI (Nonlinear Dynamics, Waters Corporation) software package. This software package generates and aligns chromatographic peaks, then infers relative metabolite abundances between sample condition groups. QC samples generated from a pooled sample stock were run at regular intervals. Comparison of metabolite abundances from pooled samples interspersed across the entire experimental run were used to filter the raw data20. Peaks of insufficient width to allow accurate quantitation were removed. Features displaying high variation (i.e., coefficient of variation score above 20%) across the pooled samples were removed. Features were retained if abundance estimates were at least 5-fold higher in QC samples above blank samples. This software also attempts to identify metabolites. Accurate mass and retention time comparisons to an in-house generated small molecule database and the Metlin (25) small molecule MS/MS database (Scripps Research Institute, La Jolla, California) were used to assign identifications to compounds presenting unique mz/retention time. Briefly, a precursor match within 10 ppm mass accuracy, a retention time within 0.1 min of our in-house library, and a MS/MS forward search dot-product score of at least 700. Agreement of these three pieces of evidence lead to a high confident identification match in the absence of internal standards. In addition, isotope distribution information and MS/MS fragmentation spectral comparisons were employed for metabolite identification.

Proteomic Data Collection:

Samples were prepared as previously described (26,27). The Tandem Mass Tag™ isobaric labeled sample was resuspended in Buffer A (20 mM ammonium formate pH 10 in 98:2 water:acetonitrile) and fractionated offline by high pH C18 reversed-phase (RP) chromatography as previously described(28) with the following changes. A Shimadzu Prominence HPLC (Shimadzu, Columbia, MD) with a Hot Sleeve-25L Column Heater (Analytical Sales & Products, Inc., Pompton Plains, NJ) was used with a Security Guard precolumn housing a Gemini NX C18 cartridge (Phenomemex, Torrance, CA) attached to a C18 XBridge column, 150 mm x 2.1 mm internal diameter, 5 µm particle size (Waters Corporation, Milford, MA). Buffer A was 20 mM ammonium formate, pH 10 in 98:2 water:acetonitrile and Buffer B was 20 mM ammonium formate, pH 10 in 10:90 water:acetonitrile. The flow rate was 200 µl/min with a gradient from 2-7% buffer B over 0.5 min, 7-15% buffer B over 7.5 min, 15-35% buffer B over 45 min, and 35-60% buffer B over 15 min. Fractions were collected every 2 min and UV absorbances were monitored at 215 nm and 280 nm. Peptide containing fractions were divided into two equal numbered groups, “early” and “late”. A volume equal to 15 milli-absorbance unit of the first “early” fraction was concatenated with the first “late” fraction, and so on. Concatenated samples were dried in vacuo, resuspended in load solvent (98:2:0.01, water:acetonitrile:formic acid) and run on the Thermo Fusion. LC-MS data was acquired for each concatenated fraction using an Easy-nLC 1000 HPLC (Thermo Scientific Inc., Waltham, MA) in tandem with a Thermo Fisher Orbitrap Fusion (Thermo Scientific Inc., Waltham, MA). Peptides were loaded directly onto a 75 cm x 100-µm internal diameter fused silica PicoTip Emitter (New Objective, Woburn, MA) packed in-house with ReproSil-Pur C18-AQ (1.9 µm particle, 120 Å pore; Dr. Maish GmbH Ammerbuch, Germany). The column was heated to 55 °C and a flow rate of 300 µL/minute was applied during the gradient. The gradient was as follows: 5-22% Buffer B (A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile) for 45 min, 22-35% B for 25 min, and 35-95% B over 10 min. The column was mounted in a nanospray source directly in line with an Orbitrap Fusion mass spectrometer (Thermo Scientific). Spray voltage was 2.1 kV in positive mode, and the heated capillary was maintained at 275 °C. The orbital trap was set to acquire survey mass spectra (380–1580 m/z) with a resolution of 60,000 at 100 m/z with automatic gain control (AGC) 1.0E6, 250-ms min injection. EASY-IC was selected for internal mass calibration. The 12 most intense ions (2-7 charged state) from the full scan were selected for fragmentation by higher-energy collisional dissociation with normalized collision energy 35%, and detector settings of 60k resolution, AGC 5E4 ions, 250 ms maximum injection time and FT first mass mode fixed at 110 m/z. Dynamic exclusion was set to 40s with a 10-ppm high and low mass tolerance.