Sample preparation

Samples were analyzed via Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS and LC-HRMS/MS)-based metabolomics using previously described methods. Briefly, cell pellets were normalized by total protein (200 µg) and the corresponding cell supernatants were normalized by volume (200 µL). Metabolites were extracted with methanol/water 80:20. Heavy labeled phenylalanine-D8 and biotin-D2 were added to individual samples prior to protein precipitation. Following overnight incubation at -80°C, precipitated proteins were pelleted by centrifugation at 10,000 rpm for 10 min and metabolite extracts were transferred into two Eppendorf tubes in equal amounts and dried down *in vacuo* and stored at -80°C.

For the RPLC separation, individual extracts were reconstituted in 60 µL of acetonitrile/water (3:97, v/v) with 0.1% formic acid containing heavy-labeled carnitine-D9, tryptophan-D3, valine-D8, and inosine-4N15, and centrifuged for 5 min at 10,000 rpm to remove insoluble material. Similarly, for the HILIC separation, individual extracts were reconstituted in 60 µL of acetonitrile/water (80:20, v/v) containing the same heavy labeled standards. A pooled quality control sample (QC) was prepared by pooling equal volumes of individual samples for each LC separation mode. The pooled QC samples was used for column conditioning (8 injections prior to sample analysis), retention time alignment and to assess mass spectrometry instrument reproducibility (1 injection every 5 sample injections) throughout the sample set and to determine sample acceptance.

Mass Spectrometry Analysis

Global, untargeted mass spectrometry analyses were performed on a high-resolution Q-Exactive HF hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a Vanquish UHPLC binary system (Thermo Fisher Scientific, Bremen, Germany). Extracts (6 µL injection volume) were separated by RPLC on a Hypersil Gold, 1.9 µm, 2.1 mm × 100 mm column (Thermo Fisher) held at 40 °C. LC was performed at 250 µL/min using solvent A (0.1% FA in water) and solvent B (0.1% FA in acetonitrile/water 80:20) with a gradient length of 30 min as previously described. HILIC separation was performed by injecting 6 µL extracts on an ACQUITY UPLC BEH Amide HILIC 1.7µm, 2.1 × 100 mm column (Waters Corporation, Milford, MA) held at 30°C. LC was performed at 200 µL/min using solvent A (5 mM ammonium formate in 90% water, 10% acetonitrile and 0.1% formic acid) and solvent B (5 mM ammonium formate in 90% acetonitrile, 10% water and 0.1% formic acid) with a gradient length of 30 min. Full MS analyses were acquired over the mass-to-charge ratio (*m/z*) range of 70-1,050 in positive ion mode for RPLC and negative ion mode for HILIC separations. Full mass scan was acquired at 120,000 resolution with a scan rate of 3.5 Hz, automatic gain control (AGC) target of $1x10^6$, and maximum ion injection time of 100 ms, and MS/MS spectra were collected at 15,000 resolution, AGC target of $2x10^5$ ions, with a maximum ion injection time of 100 ms. Both RPLC and HILIC LC-MS workflows were used to enhance separation selectivity for both hydrophobic and hydrophilic metabolites.

Metabolomics Data Processing, and Pathway Analysis

Mass spectrometry raw data was imported, processed, normalized, and reviewed using Progenesis QI v.3.0 (Non-linear Dynamics, Newcastle, UK). All MS and MS/MS sample runs were aligned against a pooled QC reference run. Unique ions (retention time and m/z pairs) were de-adducted and de-isotoped to generate unique "features" (retention time and m/z pairs). Data were normalized to all features and significance was assessed using p-values generated using ANOVA (analysis of variance) from normalized compound abundance data. Tentative and putative annotations were determined by using accurate mass measurements (< 5 ppm error), isotope distribution similarity, and fragmentation spectrum matching (when applicable) by searching the Human Metabolome Database (HMDB), METLIN, and the CIT's in-house library. Annotations (Confidence Level 1-3) were determined for all compounds with a match to any of the searched libraries or databases. Metabolites annotated Confidence Level 1-3 were used for pathway analysis.

Metaboanalyst 5.0 (www.metaboanalyst.ca/) was used to perform pathway and metabolite enrichment analyses from annotated compounds with statistical significance (p-value ≤ 0.05). The heat maps were generated for Pareto scaled, log transformed data, using Pearson distance and average clustering on the top 500 most significant metabolites when comparing all groups. Within Progenesis QI, a one-way analysis of variance (ANOVA) test was used to assess significance between groups and returned a *p*-value for each feature (retention time_m/z descriptor), with a nominal *p*-value≤0.05 required for significance. Pathway impact values, representing a combination of centrality and pathway enrichment results, from pathway topology analysis were determined based on the downstream effect of affected species in the pathway. Higher impact values represent higher relative importance of the pathway. A hypergeometric test was then used to determine pathway enrichment.