**Metabolomics Sample Preparation, Mass Spectrometry and Data Analysis**

Steady-State Metabolite Extraction: cells were seeded in 6-cm plates at densities so that cells would reach ~70% confluence after 24 hours based on their calculated doubling times in each condition (Supplemental Table S1). Samples were plated in triplicate for metabolite extraction. The following day, basal medium was removed and replaced with isotope labeled medium or control and treated with experimental compounds for a time course (0-12 hours) or standard 6-hour incubation (**Supplementary Figure 3**).  Post-treatment, medium was removed, plates were immediately placed on dry ice and polar metabolites were extracted directly on the dish using 1 mL ice-cold 80% methanol containing applicable internal standards.  Plates were then transferred to the -80°C freezer and extracted for 15 minutes. Cells were scraped into the solvent on dry ice, transferred to eppendorf tubes, and centrifuged at 20,000xg for 10 min at 4°C. The supernatant was then split into two new eppendorf tubes for technical replicates, dried for four hours at room temp using a savant speedvac (Thermo Scientific) and stored at -80°C until analysis. Prior to mass spectrometry, samples were diluted in 10 µL of HPLC grade H20, followed by 10 µL 50:50 HPLC grade Methanol/Acetonitrile, samples were quickly centrifuged, and supernatant was diluted into 80 µL methanol/acetonitrile/water. 5 µL of this final solution was injected to LC-MS for analysis.

Metabolites were analyzed using either a hydrophilic interaction liquid chromatography tandem mass spectrometry (HILIC-MS) method using an Xbridge amide column (100 × 2.1 mm i.d., 3.5 µm; Waters), a polar end-capped reverse phase method using a Synergi Hydro-RP column, or reverse phase method using a C18 column coupled to an Agilent 6460 QQQ.  For the HILIC method, the mobile phase A contained 20 mM ammonium acetate and 15 mM ammonium hydroxide in water with 3% acetonitrile, pH 9.0 and mobile phase B was acetonitrile. Linear gradients were: 0 min, 85% B; 1.5 min, 85% B, 5.5 min, 35% B; 10min, 35% B, 10.5 min, 35% B, 14.5 min, 35% B, 15 min, 85% B, and 20 min, 85% B. The flow rate was 0.15 ml/min from 0 to 10 min and 15 to 20 min, and 0.3 ml/min from 10.5 to 14.5 min. For the polar end-capped reverse phase method, extracts (10 µL, prepared as above) were injected onto a Synergi 4 µm Hydro-RP 80 Å LC Column (150 x 4.6 mm, Phenomenex, 00F-4375-E0). The column was eluted at a flow rate of 1.5 mL/min using a mobile phase A (10% MeOH in Water) and mobile phase B (10% Water in MeOH). Linear gradients were 5 minutes, 100% mobile phase A, followed by a 20 min linear gradient to 100% mobile phase B and held 10 minutes at 100% mobile phase B, returned to 100% mobile phase A over 10 minutes and held for an additional 10 minutes. All solvents were LC-MS grade and purchased from Fisher Scientific.

For the reverse phase method analyzing NAD/NADH metabolome, mammalian cell pellets were washed once in ice-cold potassium buffered saline. Cells were resuspended in 300 μL of a 75% ethanol/25% 10 mM HEPES, pH 7.1 v/v (buffered ethanol) solution, preheated to 80 °C. Samples were shaken at 1000 rpm in an 80 °C block for three minutes. Soluble metabolites were separated from particulate by centrifugation (10 min, 16,000xg). Both the particulate and soluble metabolites were dried by speed vacuum at 40 °C. Samples (10µL) were injected onto an onto a Agilent Poroshell 120 EC-C18 pre-column (2.1 x 5 mm, 2.7 μm, Chrom Tech, Apple Valley, MN) attached to an Agilent Poroshell 120 EC-C18 analytical column (2.1 x 100 mm, 2.7 μm, Chrom Tech, Apple Valley, MN) and the column was eluted at a flow rate of 250 µL/min starting with 5% mobile phase B (10% Water in Acetonitrile and 0.1% formic acid) and 95% mobile phase A (10% Acetonitrile in 90% Water and 0.1% formic acid) for 2 minutes followed by a linear gradient to 40% mobile phase B (acetonitrile with 0.1% formic acid) over 10 minutes.  Solvent B was then increased to 90% mobile phase B over 1 minute and held for 2 minutes, and returned to 5% mobile phase B over 1 minute and held for an additional 10 minutes.

All MS analyses are carried out using AJS electrospray ionization, with sheath gas flow of 12 L/min, Nebulizer pressure of 50 psi, sheath gas temp of 375°C, Capillary voltage 3.5 kV, nozzle voltage 1 kV, auxiliary gas 9 L/min, capillary temperature 100°C, and heater temperature 325°C. Peak identities were confirmed matching masses and retention times to authenticate reference standards. Less than ~100 MRM transitions were optimized per dynamic MRM method. Peak widths were filtered using 0.1-minute thresholds.  For tracing studies, masses for 15N-isotopologues were assessed on every targeted metabolite containing nitrogen atom(s). To confirm isotopologue peaks, mass spectrum from control samples (not treated with metabolite isotopes) were scanned for the same 15N-isotopologues to subtract out the natural isotope abundance of 15N-metabolites. LC-MS/MS method was validated using pure compounds alone and in a complex mixture to ascertain retention time. Peaks meeting S/N and retention time thresholds were integrated. Integrated signal intensities were normalized to isotopically labeled standards included in the extraction buffer, and then to total protein content from the extract measured by resuspension in RIPA buffer and analysis by DC assay. For quality control a standard metabolite mix was run at the end of each run. For Urea cycle metabolites, isotopically labelled internal standards were Serine, Proline, Arginine, Aspartate, Fumarate, Nicotinamide, dCTP, and Uridine. For pyrimidine focused metabolomics method, isotopically labelled internal standards were Uridine, Cytidine, UMP, UTP, CTP, dCTP, ATP, and GTP.