Tissue samples

Tissue samples were lyophilized overnight prior to bead mill homogenization in 18:1 (µl:mg wet tissue weight) ice-cold 2:2:1 methanol:acetonitrile:water extraction buffer containing a mixture of internal standards (D4-citric acid, D4-succinic acid, D8-valine, and U13C-labeled glutamine, glutamic acid, lysine, methionine, serine, and tryptophan; Cambridge Isotope Laboratories). Homogenates were rotated for 1 hour at -20°C. Homogenates were centrifuged for 10 minutes at 21,000 x g, and 150 µl of the cleared metabolite extracts were transferred to autosampler vials and dried using a SpeedVac vacuum concentrator (Thermo). Dried metabolite extracts were reconstituted in 30 µl of 11.4 mg/ml methoxyamine (MOX) in anhydrous pyridine, vortexed for 5 minutes, and heated for 1 hour at 60°C. Next, to each sample 20 µl of N,O-Bis(trimethylsilyl)trifluoroacetamide (TMS) was added, samples were vortexed for 1 minute, and heated for 30 minutes at 60°C.

GC-MS method

Derivatized samples were analyzed by GC-MS. 1 µl of derivatized sample was injected into a Trace 1300 GC (Thermo) fitted with a TraceGold TG-5SilMS column (Thermo) operating under the following conditions: split ratio = 20-1, split flow = 24 µl/minute, purge flow = 5 ml/minute, carrier mode = Constant Flow, and carrier flow rate = 1.2 ml/minute. The GC oven temperature gradient was as follows: 80°C for 3 minutes, increasing at a rate of 20°C/minute to 280°C, and holding at a temperature at 280°C for 8 minutes. Ion detection was performed by an ISQ 7000 mass spectrometer (Thermo) operated from 3.90 to 21.00 minutes in EI mode (-70eV) using select ion monitoring (SIM).

Data analysis

Raw data were analyzed using TraceFinder 4.1 (Thermo). Metabolite identification and annotation required at least two ions (target + confirming) and a unique retention time that corresponded to the ions and retention time of a reference standard previously determined in-house. A pooled-sample generated

prior to derivatization was analyzed at the beginning, at a set interval during, and the end the analytical run to correct peak intensities using the NOREVA tool (PMID: 28525573). NOREVA corrected data were then normalized to the D4-succinate signal per sample to control for extraction, derivatization, and/or loading effects.