GAT1_2.1 assay with plant extracts:

Fifty mg of root tissue was excised from 10 day old seedlings of Arabidopsis grown with 5 mM KNO3, collected in 2 mL Eppendorf tubes and flash frozen in liquid N_2. Frozen tissue was homogenized using a tissue lyser and metabolites were isolated using 1 mL of methanol:water (4:1) with incubation in an ultra-sonication bath for 20 min followed by shaking for 30 min at 4 ˚C. The mixture was centrifuged at 12,000 × *g* for 10 min at 4 ˚C and 700 µl of the supernatant was transferred into fresh tubes and evaporated to dryness using a Vacufuge at ambient temperature. Dried metabolite extracts were re-suspended in HEPES buffer pH 7.5 instead of 1:1 methanol:water. Samples were spiked with a final concentration of 1 µM ^{15}N Gln and 5 µg of the full length or glutaminase domain versions of recombinant GAT1_2.1 protein along with 2 mM DTT and 5 mM $MgCl_2$. Following this, samples were incubated at 37 ˚C for 2 hours and then filtered through a 3K micro centrifuge filter (Sigma-Aldrich) to remove the protein. Samples were then evaporated to dryness using a vacufuge at ambient temperature and the residue was re-dissolved in 1:1 methanol:water, filtered with a 0.2 µm PTFE microfuge filters (Whatman) and subjected to LC-MS analysis and ammonium quantification.

Data acquisition:

LC-MS data were obtained on a Q-Exactive Quadrupole Orbitrap mass spectrometer (Thermo Fisher Scientific) coupled to an Agilent 1290 high performance liquid chromatography (HPLC) system. Compounds were resolved using a SeQuant® ZIC®-HILIC column; 3.5µm, 100 Å, 100 × 2.1 mm (Sigma-Aldrich) with mobile phase 5 mM ammonium acetate, pH = 4.00 (A); 90% acetonitrile, 0.1% formic acid (B) and the following gradient: 87% B for 5 min, decreased to 55% over 8 min and held for 4 min before returning to 87% over 3 min. The following heated electrospray ionization (HESI) conditions were optimized for the analysis of amino and organic acids: spray voltage, 3.9 kV (ESI+), 3.5 kV (ESI-); capillary temperature, 250 °C; probe heater temperature, 450 °C; sheath gas, 30 arbitrary units; auxiliary gas, 8 arbitrary units; and S-Lens RF level, 60%. Injections of 5 µl were used with a flow rate of 0.3 mL min^{-1}. Full MS measurements were collected from mass ranges of 75-1100 m/z and 65-900 m/z in positive and negative ionization modes respectively at 140,000 resolutions. The AGC target and maximum IT was set to 3 e6 and 524 ms respectively.