NMR metabolomics

Plasma from Ctrl and hM3Dq^{GFRAL} mice collected 6 h post CNO injection was thawed at room temperature for 15 min before transferring 150 µl to pre-washed ultrafiltration tubes (Amicon Ultra 0.5 ml, 3k MWCO, Merck Millipore) which were spun at 14000 x g, at 4°C for 90 min. 90 µl of each filtrate was mixed with 90 µl buffer (75 mM sodium phosphate pH 7.4, 0.1% w/v sodium azide, 3.66 mM 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid (TSPd4), in 20% v/v D2O) and transferred to 3 mm SampleJet NMR-tubes. An Oxford magnet operating at 599.76 MHz equipped with a Bruker NEO console, a 5mm TCI cryoprobe and cooled SampleJet sample changer was used to acquire 1D 1H data for each sample. The standard Bruker pulse sequence 'noesygppr1d' encompassed a 1D NOESY sequence using pre-saturation at 4.7 ppm for water suppression. Data was collected into 64k data points at 298 K, using 64 scans and 8 dummy scans, with a spectral width of 20 ppm, an acquisition time of 2.75 s, a relaxation delay of 4 s and a receiver gain of 101. Data was zero-filled twice and an exponential line broadening of 0.3 Hz applied before Fourier transformation. Acquisition and spectral processing were performed in TopSpin 4.1.4 (Bruker BioSpin). Processed data were imported into Chenomx 8.6 (Chenomx Inc.) and metabolite quantification done using the TSP-d4 as internal standard.