Metabolomics analysis

Peritoneal fluid samples were spiked with stable isotope internal standards and metabolites were enriched with solid phase extraction cartridges (Hypersep C18-500 mg, 3 mL, Thermo Scientific-Bellefonte, PA, USA) and methanol/water as solvents. After metabolite extraction, samples were transferred to autosampler vials for LC-MS analysis using TripleTOF5600+ Mass Spectrometer (Sciex-Foster, CA, USA) coupled to an Ultra-High-Performance Liquid Chromatography (UHPLC) system (Nexera X2, Shimadzu-Kyoto, HO, Japan). Reversed-phase chromatography was accomplished with a 100 × 4.6 mm, 2.7 µm Ascentis Express C18 column (Supelco - St. Louis, MO, USA). The gradient consisted of Phase A, H2O/ACN/acetic acid (69.98:30:0.02, v/v/v) at pH 5.8 (adjusted with NH4OH), and Phase B, an ACN/isopropanol (70:30, v/v). Gradient elution was carried out for 25 min at a flow rate of 0.6 mL. / min. Gradient conditions were as follows: 0 to 2.0 min, 0% B; 2.0 to 5.0 min, 15% B; 5.0 to 8.0 min, 20% B; 8.0 to 11.0 min, 35% B; 11.0 to 15.0 min, 70% B; and 15.0 to 19 min, 100% B. At 19.0 min, the gradient returned to the initial condition of 0% B, and the column was re-equilibrated until 25.0 min. Mass spectral data was acquired with negative electrospray ionization and the full scan of mass-to-charge ratio (m/z) ranged from 100 to 1500. Proteowizard software was used to convert wiff files into mzXML files. Peak peaking, noise filtering, retention time and *m/z* alignment, and feature quantification were performed with apLCMS. Three parameters define a metabolite feature: mass-to-charge ratio (*m/z*), retention time and intensity values. Data were log2 transformed and only features detected in at least 80% of samples from one group (5439 *m/z* features) were used in further analysis. Missing values were imputed using half mean of the feature across all samples. The mummichog software (version 2) was used for metabolic pathway enrichment analysis (mass accuracy under 10 ppm).