## Sample Prep

Aqueous metabolites for targeted LC-MS analysis were extracted using a protein precipitation method similar to the one described elsewhere (1,2). Samples were first homogenized in 200 µL purified deionized water at 4 ˚C, and then 800 µL of methanol containing $6C_{13}$-glucose and $2C_{13}$-glutamate (reference internal standards) was added. Afterwards samples were vortexed, stored for 30 minutes at -20 ˚C, sonicated in an ice bath for 10 minutes, centrifuged for 15 min at 14,000 rpm and 4 ˚C, and then 600 µL of supernatant was collected from each sample. Lastly, recovered supernatants were dried on a SpeedVac and reconstituted in 1.0 mL of LC-matching solvent containing $2C_{13}$-tyrosine and $3C_{13}$-lactate (reference internal standards). Protein pallets that were left over from the sample prep were saved for BCA protein assay

## LC-MS Assay

Targeted LC-MS metabolite analysis was performed on a duplex-LC-MS system composed of two Shimadzu UPLC pumps, CTC Analytics PAL HTC-xt temperature-controlled auto-sampler and AB Sciex 6500+ Triple Quadrupole MS equipped with ESI ionization source (2). UPLC pumps were connected to the auto-sampler in parallel and were able to perform two chromatography separations independently from each other. Each sample was injected twice on two identical analytical columns (Waters XBridge BEH Amide XP) performing separations in hydrophilic interaction liquid chromatography (HILIC) mode. While one column was performing separation and MS data acquisition in ESI+ ionization mode, the other column was getting equilibrated for sample injection, chromatography separation and MS data acquisition in ESI- mode. Each chromatography separation was 18 minutes (total analysis time per sample was 36 minutes). MS data acquisition was performed in multiple-reaction-monitoring (MRM) mode. LC-MS system was controlled using AB Sciex Analyst 1.6.3 software. Measured MS peaks were integrated using AB Sciex MultiQuant 3.0.3 software. The LC-MS assay was targeting 361 metabolites (plus 4 spiked reference internal standards). Up to X metabolites and 4 reference standards were measured in the study samples. In the addition to the study samples, two sets of quality control (QC) samples were used to monitor the assay performance as well as data reproducibility. One QC [QC(I)] was a pooled human serum sample used to monitor system performance and the other QC [QC(S)] was pooled study samples and this QC was used to monitor data reproducibility. Each QC sample was injected per every 10 study samples. The data were well reproducible with a median CV of X %. Generated MS data were normalized vs. BCA total protein count.

(1) Mathon C, Bovard D, Dutertre Q, Sendyk S, Bentley M, Hoeng J, Knorr A. Impact of sample preparation upon intracellular metabolite measurements in 3D cell culture systems. *Metabolomics* 15:92, **2019**.

(2) Meador JP, Bettcher LF, Ellenberger MC, Senn TD. Metabolomic profiling for juvenile Chinook salmon exposed to contaminants of emerging concern. Science *Total Environ*. 747:141097, **2020**.