**Microfluidic Gut Model (Cell Media Samples)**

Cell media was collected from 13 transwell plates (5 controls, 8 infected) and 19 microfluidic devices (6 controls, 13 infected) 7 days after the Caco-2 culture were initiated. The media was then stored at -80° C until metabolomic analysis. Each cell media sample was thawed on ice for 30–60 minutes. Aliquots of 25 µL were transferred for an All Pooled QC sample. To each of the media and pooled QC samples, 1 mL of 50% Acetonitrile:Water to each tube, vortex for 5 min on the multi-tube vortexer at speed 5,000 rpm, and centrifuged at room temperature and at 16,000 rcf for 5 min. A 800 µl aliquot of the supernatant was transferred into pre-labeled 2 mL Lo-Bind tubes and topped with a rubber stopper, making sure hole is open. The samples are frozen at -80 °C for 1 hr in a long, skinny plastic tube rack and lyophilize to dryness overnight. The dried samples were solubilized with 700 µL D2O+Chenomx ISTD, which contains 5mM 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS, Chemical Shift Indicator), 6 mM Imidazole (pH indicator), and 0.2% NaN3 (inhibit bacterial growth), vortexed for 2 min on the multi-tube vortexed at speed 5,000 rpm, centrifuged samples at room temperature and at 16,000 rcffor 5 min, and 600 µl was transferred to the supernatant into pre-labeled 7mm (4”) NMR tubes (Bruker-Biospin, Switzerland).

1H NMR spectra of cell-media extracted samples were acquired on a Bruker Avance III 700 MHz NMR spectrometer (located at the NC State Biochemistry department in Raleigh, NC, USA) using a 5 mm cryogenically cooled ATMA inverse probe and ambient temperature of 25℃. A pulse sequence with a 1D NOESY presaturation pulse sequence (noesypr1d) was used for data acquisition. For each sample 32 transients were collected into 64k data points using a spectral width of 8 kHz (12.0 ppm), 2 s relaxation delay, 100 ms mixing time, and an acquisition time of 3.89 s per FID. The water resonance was suppressed using resonance irradiation during the relaxation delay and mixing time. Spectra were zero filled, and Fourier transformed after exponential multiplication with line broadening factor of 0.5. Phase and baseline of the spectra were manually corrected for each spectrum. Spectra were referenced internally to the DSS signal. The quality of each NMR spectrum was assessed for the level of noise and alignment of identified markers. Spectra were assessed for missing data and underwent quality checks.

NMR spectra were pre-processed using ACD 1D NMR Processor 12.0 (ACD Labs, Toronto, CA) for metabolomics analysis. NMR bins (-0.10 – 9.00 ppm) were made after excluding DSS (-0.03 – 0.03 ppm) and water (4.83 – 4.85 ppm) using intelligent binning width of 0.04 ppm and 50% looseness factor. In a subsequent analysis, the bins associated with lactate (1.28 – 1.33 ppm, 4.83 – 4.85 ppm) were also removed. Integrals of each of the bins were normalized to total integral of each of the spectrum.