LC-MS Metabolomics Methods

**Sample Preparation:**

Lung samples were homogenized in 50:50 acetonitrile: water (10uL to every 1mg of tissue) with washed ceramic beads on a bead beater, using three 30s pulses at 1750rpm. Samples were vortexed on a multi-tube vortexer for 2 min. at 5000rpm and centrifuged for 4 min. at 16000rcf. Pooled samples were created by combining aliquots from the appropriate study samples to form all-pooled, control-pooled, day-4-infected-pooled, and day-8-infected pooled samples and were prepared using the same methods as the study samples. 50uL of L-tryptophan-d5 (used as an internal standard) and 300uL of acetonitrile was then added to 300uL aliquots of lung homogenate supernatant or pooled samples. Samples were again vortexed on a multi-tube vortexer for 2 min. at 5000rpm and centrifuged for 4 min. at 16000rcf. 600uL of the supernatant was then transferred to new tubes and dried on a SpeedVac for 2 hours at 30C. Samples were reconstituted in 100uL of 95:5 water: methanol, vortexed on a multi-tube vortexer for 2 min. at 5000rpm, and centrifuged for 4 min. at 16000rcf. The supernatant was transferred to autosampler vials.

**UPLC-MS Methods:**

UPLC-MS spectra were collected for all samples. UPLC was performed on a Waters Acquity UPLC with an Acquity BEH HSS T3 column (2.1x 100mm x 1.8 um) at 50C using the reversed phase method. Water with 0.1% formic acid (mobile phase A) and methanol with 0.1% formic acid (mobile phase B) were injected following the Dunn 22 minute method (see the 3. BECK-LUNG\_DIET MetaData and Analytical Metadata.xlsx file for the flow gradient). Mass spectroscopy analysis was performed using a Synapt G2 Q-TOF. 10uL of each sample was injected into the instrument, and MS data was collected between 70-1000m/z in both positive and negative modes.