LC-MS Metabolomics Methods

**Sample Preparation:**

Urine 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 study samples to form all-pooled sample. Phenotypic pools were not created due to low sample volume. The pooled sample was prepared using the same methods as the study samples. 50uL of L-tryptophan-d5 (used as an internal standard) and 25uL of acetonitrile was then added to 25uL 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. 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 Amie column (2.1x 100mm x 1.8 um) at 40 ˚C using the HILIC method and 10mM ammonium acetate in 95/5 acetonitrile/water with 0.1% formic acid (mobile phase A) and 10mM ammonium acetate in 50/50 acetonitrile/water with 0.1% formic acid (mobile phase B) as mobile phases (see the 3. BECK-Urine-Genetic\_HILIC MetaData and Analytical Metadata.xlsx file for the flow gradient). Mass spectroscopy analysis was performed using a Synapt G2 Q-TOF. A 2uL of each extract was injected into the instrument, and MS data was collected between 50-1000m/z in both positive and negative modes.