**Transpulmonary metabolomics in pulmonary arterial hypertension**

De-identified samples were shipped to the NIH RTI-RCMRC on dry ice and immediately stored at -80 °C after being logged in for metabolomics analysis. Study samples were thawed on ice for sample preparation. A 150 µL aliquot of plasma was transferred to new labeled tubes for each study sample. A total study pool was generated by transferring 15 µL of plasma from each sample into a new 10 mL Falcon tube. The total pool sample was vortexed and 150 µL aliquots were transferred into 10 total pool-labeled tubes. Plasma and pooled samples were extracted with 450 µL methanol, vortexed for 2 min on a multi-tube vortexer, and centrifuged at 16,000 rcf for 5 min. A 400 µl aliquot of the supernatant was transferred into pre-labeled 2.0mL LoBind Eppendorf tubes, and the supernatant was lyophilized to complete dryness overnight. Samples were reconstituted with 250 µL of NMR master mix solution containing Chenomx ISTD: DSS-d6 and 0.20 M phosphate buffer at 7.4 pH. The tubes were vortexed for 2 min on a multi-tube vortexer and centrifuged at 16,000 rcf for 5 min. A 200uL aliquot of supernatants were transferred into a pre-labeled 3mm 4" NMR tubes for data acquisition on a 700 MHz spectrometer.

1H NMR spectra of cellular extracts were acquired on a Bruker Avance III 700 MHz spectrometer (located at the David H. Murdock Research Institute at Kannapolis, NC, USA) using a 5 mm cryogenically cooled ATMA inverse probe and ambient temperature of 25 °C. A noesypr-1d pulse sequence was used for data acquisition. For each sample 64 transients were collected into 65 k data points using a spectral width of 12.0227 ppm, 2s 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. 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.