Sample Preparation

Extraction of metabolites and lipids from red cell pellets was as follows: 36 µL of cold MeOH:MeCN:H2O (5:3:2, v:v:v) was added to all pellets (pellet size pre-determined to be 10 million prior to extraction). Samples were vortexed at 4°C for 30 minutes. Insoluble material was pelleted by centrifugation (18000*g*, 10 min) and supernatants were isolated for analysis by UHPLC-MS for metabolomics and lipidomics.

Ultra-High Pressure Liquid Chromatography-Mass Spectrometry Metabolomics Analysis

Metabolomics analysis employed a Vanquish UHPLC (Thermo Fisher Scientific) coupled to an Orbitrap Exploris 120 mass spectrometer (Thermo Fisher Scientific). 10 µL injections of the samples were resolved across a 2.1 x 150 mm, 1.7 um particle size Kinetex SB-C18 column (Phenomenex) using a 5 minute, reversed-phase gradient as previously described [1]. The Exploris 120 was run independently in negative and positive ion mode, scanning in full MS mode from 65-975 m/z at 120,000 resolution, with 50 Arb sheath gas, 10 Arb auxiliary gas, and 3 kV and 3.4 kV spray voltage for negative and positive modes, respectively. Calibration was performed prior to the run using the Easy-IC internal standard (Thermo Fisher Scientific). Run order of samples was randomized and technical replicates were injected after every 4 samples to assess quality control. Raw files were converted to .mzXML using RawConverter. The resultant files were processed with El-Maven (Elucidata) alongside the KEGG database for metabolite assignment and peak integration as previously described [2].

Ultra-High Pressure Liquid Chromatography-Mass Spectrometry Lipidomics Analysis

Lipidomics analysis employed a Vanquish UHPLC system (Thermo Fisher Scientific) coupled to a Q Exactive mass spectrometer (Thermo Fisher Scientific). 5 µL injections of the samples were resolved across a 2.1 x 30 mm, 1.7 um particle size Kinetex C18 column (Phenomenex) using a 5 minute, reversed-phase gradient adapted from a previous method [3]. The Q Exactive was run independently in positive and negative ion mode, scanning using data dependent MS^2 (top 10) from 125-1500 m/z at 17,500 resolution, with 45 Arb sheath gas, 25 Arb auxiliary gas, and 4 kV spray voltage. Calibration was performed prior to the run using the PierceTM Positive and Negative Ion Calibration Solutions (Thermo Fisher Scientific). Run order of samples was randomized and technical replicates were injected after every 4 samples to assess quality control. Lipid assignments and peak integration were performed using LipidSearch v 5.0 (Thermo Fisher Scientific).

Please include in the acknowledgment: “The University of Colorado School of Medicine Metabolomics Core is supported in part by the University of Colorado Cancer Center award from the National Cancer Institute P30CA046934.”

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