Untargeted and targeted polar metabolomic profiling

15 mg of cardiac tissue was homogenized in 80% methanol (Sigma) using Tissue Tearer (BioSpec) on dry ice. Samples were incubated at -80ºC for 4 hours. Homogenates were then centrifuged at 14,000 rfc for 20 min at 4ºC. The supernatant was extracted and stored at -80ºC. The Weill Cornell Medicine Meyer Cancer Center Proteomics & Metabolomics Core Facility performed hydrophilic interaction liquid chromatography-mass spectrometry (LC-MS) for relative quantification of polar metabolite profiles. Metabolites were measured on a Q Exactive Orbitrap mass spectrometer, coupled to a Vanquish UPLC system by an Ion Max ion source with a HESI II probe (Thermo Scientific). A Sequant ZIC-pHILIC column (2.1 mm i.d. × 150 mm, particle size of 5 µm, Millipore Sigma) was used for separation. The MS data was processed using XCalibur 4.1 (Thermo Scientific) to obtain the metabolite signal intensity for relative quantitation. Targeted identification was available for 205 metabolites based on an in-house library established using known chemical standards. Identification required exact mass (within 5ppm) and standard retention times. For untargeted metabolomics, metabolites were identified by mass matching of the MS signal to metabolites in the HMDB database. If multiple metabolites in the database were matched to a certain MS signal, all matched metabolites were grouped into a single identification, and ordered based on the number of references included in the HMDB database (high to low). We used the first ranked metabolite in the downstream analyses. When multiple values with the same metabolite attribution occurred (different metabolites with same mass and retention time), we opted to use all values in the analyses to avoid biases on which intensities/attributions to consider. Peak intensities for metabolites were screened for missing values and relative metabolite abundance data was analyzed by using MetaboAnalyst software version 5.0 (Pang *et al*, 2021). Metabolite significance was determined with one-way ANOVA with post-hoc t-tests, with the cutoff being a raw p value < 0.05, and the pathway significance cutoff was FDR corrected p value < 0.05.