

## Methods for Untargeted metabolomics in Fu et al. 2020 and Fu et al. 2021

*Cell treatment.* Human islets were maintained in PIM(S) media (Prodo Labs) with 5% human A/B serum (Gemini BioProducts) 5.8 mM glucose at a density of 1000 islets per 10 ml as previously described (Fu et al., 2020). Islets were treated for 5 h with 5  $\mu$ M of BAD SAHB<sub>A</sub> SD in 0.5% DMSO in uptake medium, which consisted of PIM(S) for human islets at pH 6.2, which is the optimal pH for cellular uptake of BAD stapled peptides. Control islets were treated with vehicle composed of 0.5% DMSO in uptake medium. Islets were then washed and left to recover in complete physiological medium (pH 7.4) prior to cytokine treatment. The cocktail of inflammatory cytokines (R&D Systems) used to treat human islets consisted of 10 ng/ml TNF- $\alpha$ , 10 ng/ml IL-1 $\beta$ , and 100 ng/ml IFN $\gamma$ . Peptide synthesis were performed as previously described (Danial et al., 2008, Ljubcic et al., 2015). SAHBs corresponding to human and mouse BAD BH3 domain variants were used for treatment of human and mouse islets, respectively. SAHB<sub>A</sub> SD is modeled after phosphomimic S118 and S155 in the human and mouse BAD sequence, respectively (Danial et al., 2008, Ljubcic et al., 2015, Szlyk et al., 2014).

*Untargeted Metabolomics.* Human islets from 5 independent donors were treated as indicated, rinsed in 150 mM ammonium formate and stored at -80°C. 150 human islets per replicate were extracted in hot 70% ethanol at 75°C for 3 min, placed on ice, and clarified by centrifugation. Extracts from all donors were pooled and analyzed in 8 replicates by flow-injection, non-targeted metabolomics in negative ionization mode on an Agilent 6550 Quadrupole Time-of-flight mass spectrometer (Agilent Technologies) as previously described (Fuhrer et al., 2011). These analyses were performed using the services provided by General Metabolics, LLC. For sample extracts, 1.5  $\mu$ l was injected using a MPS3 autosampler (Gerstel). The mobile phase contained isopropanol/water (60:40, v/v) 1 mM ammonium fluoride with a flow rate of 150  $\mu$ l per min. Mass spectra were recorded in profile mode from m/z 50 to 1000 with a frequency of 1.4 spectra/s for 0.48 min using maximum resolving power (4 GHz HiRes). For online mass axis correction, homotaurine and hexakis (1H, 1H, 3H tetrafluoropropoxy) phosphazine (HP-0921, Agilent Technologies) were spiked in the mobile phase. Source temperature was 325°C, with 5 L per min drying gas and a nebulizer pressure of 30 psig. Ions were putatively annotated by matching their measured mass with compounds in the KEGG database for *Homo sapiens*, allowing a tolerance of 0.001 Da, only deprotonated ions (without adducts) were included and duplicate matches were retained.