

Data Acquisition with Data Dependent Decision Tree An Orbitrap Fusion Tribrid Mass Spectrometer system (Thermo Fisher Scientific, San Jose, CA, USA) was interfaced with an ultra-performance Easy-nLC 1200 system (Thermo Fisher Scientific, Bremen, Germany). Each desalted sample was loaded onto a Acclaim Pepmap 100 pre-column (20 mm × 75 μm; 3 μm-C18) and eluted using a PepMap RSLC analytical column (500 mm × 75 μm; 2 μm-C18). The flow rate was set at 250 nl/min with solvent A (0.1% formic acid, 99.9% water (v/v)) and solvent B (0.1% formic acid, 80% acetonitrile, 19.9% water (v/v)) as the mobile phases followed by a linear gradient: 1 - 2 % of solvent B in 5 min, 2 - 40 % of solvent B in 95 min, ramping up to 98% solvent B in 2 min, and isocratic at 98 % in 13 min. The mass spectrometer acquired the data under the collision ion dissociation (CID) mode in each MS and MS/MS cycle scanning from 350 to 1800 m/z. The maximum ion injection times for the survey scan and the MS/MS scans were 35 ms. MS1 spectra were recorded at resolution at 120,000 FWHM from 350– 1800 m/z with quadrupole isolation was followed by one MS/MS scans of the most intense precursor ions in the linear ion trap. The automated gain control (AGC) target was set to 2×10^5 , with a max. injection time of 50 ms. The quadrupole was used for precursor isolation with an isolation window of 1.3 m/z. Only precursors with charge states 2–7 with an intensity higher than 1×10^4 were selected for fragmentation. The monoisotopic precursor selection (MIPS) filter was activated. The option to inject ions for all available parallelizable time was selected. Targeted MS2 spectra were acquired and were performed in the ion trap with CID fragmentation (Rapid; NCE 35%; maximum injection time 35 ms; AGC 1×10^4). The normalized collision energy (NCE) was set to 35% for each fragmentation method and one microscan was acquired for each spectrum