The hybrid trapped ion mobility-quadrupole time-of-flight mass spectrometer (timsTOF fleX, Bruker Daltonics, Bremen, Germany) was interfaced with an ultra-performance EvoSep One system (Evosep Biosystem, Odense, Denmark). The digested peptides were desalted and loaded using Evotip (Evosep Biosystem) according to the manufacturer's instructions. Briefly, Evotip was wetted with 100 µl of 100% iso-propanol, rinsed with 20 µl of Solvent B (99.9% acetonitrile and 0.1% formic acid (v/v)), equilibrated with 20 ul of 0.1% formic acid (v/v), loaded 200 ng of digested peptides, and subsequently washed with 20 ul of 0.1% formic acid (v/v) using centrifugal force at 700 x g for 1 min. 100 µL of 0.1% formic acid was added to Evotip to prevent drying. Samples were injected into the Bruker timsTOF fleX MS coupled with the Evosep One instrument (Evosep Biosystems). The standard preset method of 30 samples per day was used with the EV1106 Analytical column NT (Evosep Biosystem) of the run. The LC-MSMS spectra were produced in the data-dependent mode with Parallel Accumulation Serial Fragmentation (PASEF) to improve ion utilization efficiency and data acquisition speed. The dual TIMS was operated the system at 100% duty cycle and recorded the MS/MS mode scanning from 100 to 1700 m/z. The ion mobility was scanned from 0.6 to 1.6 Vs/cm^2, and TIMS ion charge control was set to 5e6. The TIMS dimension was calibrated linearly using four selected ions from the Agilent ESI LC/MS tuning mix [*m/z*, 1/K_0: (322.0481, 0.7363 Vs cm^{-2}), (622.0289, 0.9915 Vs cm^{-2}), (922.0097, 1.1996 Vs cm^{-2}), (1221,9906, 1.3934 Vs cm^{-2})] in positive mode.