Nano LC separation was performed with a nanoACQUITY system (Waters, Milford, MA, USA) interfaced to an LTQ Orbitrap XL mass spectrometers from Thermo Scientific (San Jose, CA, USA). Peptides were trapped on a homemade 100 μm i.d. × 20 mm long precolumn packed with 200 Å (5 μm) Magic C18 particles (C18AQ; Michrom), separated on a 75 μm i.d. × 350 mm long analytical column with a laser pulled emitter tip packed with 100 Å (5μm) Magic C18 particles (C18AQ; Michrom) and analyzed in positive ion mode. For each LC-MS/MS analysis, an estimated amount of 0.5 μg of peptides was loaded on the precolumn at 2 μL/min in water/acetonitrile (98/2) with 0.1% (v/v) formic acid. Peptides were eluted using an acetonitrile gradient flowing at 250 nL/min using mobile phase consisting of the following: A, water, 0.1% formic acid; B, acetonitrile, 0.1% formic acid. The gradient program was as follows: 0 - 1 min, A (98%), B (2%); 1 min, A (90%), B (10%); 90 min, A (65%), B (35%); 91 - 101 min, A (20%), B (80%); 102- 120 min, A (98%), B (2%).

Experiments were performed with a Thermo LTQ OrbitrapXL in positive ion mode. High resolution full precursor scans were acquired in the Orbitrap at 400–2000 m/z range and 60000 resolution followed by 6 data dependant MS/MS scans in the Linear ion trap (LTQ). Ion selection threshold was 5000 counts for MS/MS, and the maximum allowed ion accumulation times were 500 ms for full scans and 100 ms for MS/MS measurements. The number of ions accumulated was set to 1 million for Orbitrap scans, and 10000 for linear ion trap MS/MS scans. All samples were analyzed in triplicates.

Experiments were performed with a Thermo Q Exactive in positive ion mode. High resolution full precursor scans were acquired at 400–2000 m/z range and 70000 resolution followed by 20 data dependant MS/MS scans at 17500 resolution with 25 normalized collision energy. Ion selection threshold was 10000 counts for MS/MS and the number of ions accumulated was set to 1 million for both the full scan and for the MS/MS scans, with a maximum allowed ion accumulation times were 50 ms for both full and MS/MS measurements. All samples were analyzed in triplicates.