Electronic Supplementary Material (ESI) for Molecular Omics.

Supplementary information

a. Samples preparation of proteomics analysis

TMT-based quantitative proteomic profiling was performed according to the manufacturer's instructions (Thermo Fisher Scientific, MA, USA). Before the mass spectrometer analysis, the samples were prepared as follow: (**i**) Protein extraction: Protein was extracted from every four samples in the same group and randomly pooled into one tube; each group was divided into two pooled replicates and similarly pre-processed. Samples were treated with a lysis buffer (1% w/v sodium dodecyl sulfate (SDS), 7 M urea, 1× protease inhibitor cocktail (Roche Ltd. Basel, Switzerland)), vibrated, and milled for 120 s three times. Samples were then lysed on ice for 30 min and centrifuged at 18,000 ×*g* for 15 min at 4°C. The supernatant was collected and transferred to a new Eppendorf tube. (**ii**) Protein purification, trypsin digestion, and desalting: The protein concentration of the supernatant was determined using bicinchoninic acid (BCA) protein assay, and 100 μg of protein per condition was transferred into a new Eppendorf tube; the final volume was adjusted to 100 μL with 8 M urea. Then, 2 μL of 0.5 M TCEP tris(2-carboxyethyl) phosphine hydrochloride) was added to the sample, which was allowed to reduce at 37°C for 1 h and then incubated with 4 μL of 1 M iodoacetamide in the dark at 25°C for 40 min. Later, samples were treated with five volumes of −20°C pre-chilled acetone for overnight (12 h in the night) at −20°C to precipitate the proteins. The precipitates were washed twice with 1 mL of a pre-chilled 90% (v/v) acetone aqueous solution and then re-dissolved in 100 μL of 100 mM Triethylammonium bicarbonate (TEAB) buffer. Sequence-grade modified trypsin (Promega, Madison, WI) was added to the sample at a ratio of 1:50 (enzyme:protein, weight:weight) to digest the proteins at 37°C for

12 h. The peptide mixture was desalted using C18 ZipTip, quantified by Pierce™ Quantitative Colorimetric Peptide Assay (23275), and then lyophilized using SpeedVac. (**iii**) TMT-isobaric mass tag labeling: The resultant peptide mixture was labeled with TMT-10plex Isobaric Mass Tag Labeling Kit (Thermo Fisher Scientific, MA, USA) following the manufacturer's instructions as follows: 130 and 131 tags for the control group, 126 and 127 tags for the CUMS group. The labeled peptide samples were pooled and lyophilized in a vacuum concentrator. (iv) High pH reverse-phase fractionation: The peptide mixture was re-dissolved in buffer A (20 mM ammonium formate in water, pH 10.0, adjusted with ammonium hydroxide), and then fractionated by high pH separation using an Ultimate 3000 system (Thermo Fisher Scientific, MA, USA) connected to a reverse phase column (X Bridge C18 column, 4.6 mm × 250 mm, 5 μm, Waters Corporation, MA, USA). High pH separation was performed using a linear gradient starting from 5% B to 45% B in 40 min (B: 20 mM ammonium formate in 80% (v/v) acetonitrile (ACN), pH 10.0, adjusted with ammonium hydroxide). The column was re-equilibrated for 15 min prior to the subsequent separation. The flow rate and column temperature were maintained at 1 mL/min and 30°C, respectively. Twelve fractions were collected, and each fraction was dried in a vacuum concentrator for the next step. (v) Nano UHPLC-MS/MS analysis: The dried samples were re-dissolved in a 5% (v/v) ACN aqueous solution containing 0.5% (v/v) formic acid and analyzed by on-line Nanospray liquid chromatography tandem mass spectrometry (LC-MS/MS) on Q Exactive™ (Thermo Fisher Scientific, MA, USA) coupled to a Nano ACQUITY UPLC system (Waters Corporation, Milford, MA).

b. Evaluation of the quality control of proteomics method

Figure S1A shows that the results of identification were credible and that the quantity was ideal when the FDR was close to 1%, as the curve rose smoothly and the value of identified peptide

spectrum match (PSM) was reasonable. The distribution of peptide score and the corresponding PSM number (**Figure 3B**) showed that the greater the distribution of the blue histogram (target peptide) carrying more than 20 points, the higher was the proportion of high-quality spectrum results. **Figure 3C** shows the distribution of peptide score and instrument accuracy; the score was distributed in the range of ± 5 ppm for most of the blue spots, indicating that the mass spectrometer has high detection accuracy and stability (Note: brown dots are the scores for the wrong results). The closer the distribution is to point 0 on the x-axis, the better is the quality of the results. The instrument's mass accuracy was evaluated from the distribution of the number of PSM and mass error (**Figure 3D**), and the yellow and blue areas indicate the distribution maps before and after correction, respectively. After correction, the mass errors fell within ± 5 ppm, indicating high reliability and quality accuracy of the identification results. **Figure 3E** shows the mass error distribution of the detected peptides at different m/z values. An increase in the m/z of the peptide led to a slight decrease in the mass accuracy, but the value was still within the ultra-high precision range. These results suggest that the quality accuracy of this experiment was very high and stable.