Supplementary methods: shotgun proteomics

The gel plugs were washed three times with 50 µL of 25 mM ammonium hydrogen carbonate (NH_4HCO_3) and 50 µL of acetonitrile. The cysteine residues were reduced by 50 µL of 10 mM dithiothreitol at 57°C and alkylated by 50 µL of 55 mM iodoacetamide. After two washes with NH_4HCO_3 and acetonitrile, the gel plugs were dehydrated by acetonitrile. The digestion of proteins was done in gel with 20 µL of 12 ng/µL of modified porcine trypsin (Promega, Madison, WI, USA) in 25 mM NH4HCO3. The digestion was performed overnight at room temperature. The generated peptides were extracted with 40 µL of 60% acetonitrile in 0.1% formic acid. Acetonitrile was evaporated under vacuum and samples were resuspended with 40 µL of a solution of 1% acetonitrile and 0.1% formic acid in order to obtain a final concentration of 500 ng/µL.

NanoLC-MS/MS analysis was performed using a nanoACQUITY Ultra-Performance-LC (Waters Corporation, Milford, USA) coupled to a Q-Exactive HF-X mass spectrometer (Thermo Fisher Scientific, Bremen, Germany).

Samples (200 ng) were first concentrated/desalted onto a NanoEaseTM M/Z Symmetry C18 precolumn (100Å, 5 µm, 180 µm × 20 mm, Waters Corporation, Milford, USA) using 99% of solvent A (0.1% formic acid in water) and 1% of solvent B (0.1% formic acid in acetonitrile) at a flow rate of 5 µl/min for 3 min. A solvent gradient from 1 to 6% of B in 0.5 min then from 6 to 35% of B in 58 min was used for peptide elution, which was performed at a flow rate of 350 nl/min using a NanoEaseTM M/Z BEH C18 column (130Å, 1.7 µm, 75 µm × 250 mm, Waters Corporation, Milford, USA) maintained at 60 °C.

The Q-Exactive Plus was operated in positive ion mode with source temperature set to 250 °C and spray voltage to 1.8 kV. Full-scan MS spectra (300–1800 m/z) were acquired at a resolution of 60,000 at m/z 200. MS parameters were set as follows: maximum injection time of 50 ms, AGC target value of 3e6 ions, lock-mass option enabled (polysiloxane, 445.12002 m/z), selection of up to 10 most intense precursor ions (doubly charged or more) per full scan for subsequent isolation using a 2 m/z window, fragmentation using higher energy collisional dissociation (HCD, normalised collision energy of 27), dynamic exclusion of already fragmented precursors set to 30 s. MS/MS spectra (200–2000 m/z) were acquired with a resolution of 15,000 at m/z 200. MS/MS parameters were set as follows: maximum injection time of 50 ms, AGC target value of 1e5 ions, peptide match selection option turned on. Raw data were converted into mgf files using the MSConvert tool from ProteomeWizard (v3.0.6090; http://proteowizard.sourceforge.net/).

For protein identification, the MS/MS data were interpreted using a local Mascot server with MASCOT 2.6.2 algorithm (Matrix Science, London, UK) against a database containing all *Mus musculus* and *Rattus norvegicus* entries from UniProtKB/SwissProt (version 2019_10, 25,156 sequences) and the corresponding 25,156 reverse entries. The database was generated using MSDA software (Carapito et al., 2014, DOI: 10.1002/pmic.201300415). Spectra were searched with a mass tolerance of 10 ppm for MS and 0.05 Da for MS/MS data, allowing a maximum of one trypsin missed cleavage. Trypsin was specified as enzyme. Acetylation of protein N-termini, carbamidomethylation of cysteine residues and oxidation of methionine residues were specified as variable modifications. Identification results were imported into Proline software version 2.2 (http:// profiproteomics.fr/proline/) for validation. Peptide Spectrum Matches (PSM) with pretty rank equal to one and length greater than 7 amino acids were retained. False Discovery Rate was then optimized to be below 1% at PSM level using

Mascot Adjusted E-value and below 1% at Protein Level using Mascot Standard score.

-Peptides

Peptides abundances were extracted thanks to Proline software version 2.2 (http://profiproteomics.fr/proline/) using a m/z tolerance of 10 ppm. Alignment of the LC-MS runs was performed using Loess smoothing. Cross Assignment was performed within groups only. Protein Abundances were computed by sum of peptides abundances (normalized using the median).

Proteins were considered as significantly different if their U value in the Mann-Whitney U test was ≤2 in the control vs. sample comparison.