*LC-MS/MS*

Ten microliters of serum was diluted with 190 μl of 100 mM ammonium bicarbonate, and filtered on with a 0.22 μm. The filtrate was ultrafiltrated with an Amicon ultrafiltration unit (Amicon Ultra, 3 kDa, Millipore, MA, USA). Concentrated samples were denatured with an equivalent volume of trifluoroethanol (25 μl) and reduced with 1 μl of 200 mM dithiothreitol (DTT). The samples were incubated at 90 °C for 30 min, and cooled to room temperature. Free cysteine residues were alkylated with 4 μl of 200 mM iodoacetamide for 60 min at room temperature in the dark and the remaining iodoacetamide was quenched by adding 1 μl of 200 mM DTT. The samples were then mixed with 300 μl of 100 mM ammonium bicarbonate. Fifteen microliters of the sample was diluted with 85 μl of 100 mM ammonium bicarbonate and incubated with 1 μg trypsin (TPCK treated, AB Sciex, Framingham, MA, USA) at 37 °C for 18 h. The samples were desalted with C18 ZipTip (Millipore, Bedford, MA, USA) and eluted with H2O/acetonitrile (5/5; v/v). The ZipTip eluates were dried in a vacuum centrifuge. Desalted samples were rehydrated in 0.1% formic acid (FA) and were analyzed by LC-MS using a nanoLC Eksigent 400 system (Eksigent, AB Sciex), coupled online to an TripleTOF6600 mass spectrometer (AB Sciex). Peptide separation was performed using liquid chromatography with a trap and elution conﬁguration using a nano trap column (350 μm × 0.5 mm, 3 μm, 120 Å, AB Sciex) and a nano ChromXP C18 reverse phase column (75 μm × 15 cm, 3 μm, 120 Å, AB Sciex) at 300 nl/min with a 90 min linear gradient of 8-30% acetonitrile in 0.1% FA, and then, with a 10 min linear gradient of 30% to 40% acetonitrile in 0.1% FA. The mass spectrometer was operated in information-dependent acquisition (IDA) mode, scanning full spectra (400–1500 *m/z*) for 250 ms, followed by up to 30 MS/MS scans (100–1800 *m/z* for 50 ms each), for a cycle time of 1.8 s. Candidate ions with a charge state between +2 and + 5 and counts above a minimum threshold of 125 counts per second were isolated for fragmentation, and one MS/MS spectrum was collected before adding those ions to the exclusion list for 12 s. Rolling collision energy was used with a collision energy spread of 15. The mass spectrometer was operated using the Analyst TF 1.7.1 software program (AB Sciex). For data dependent acquisition (DDA, SWATH acquisition), the parameters were set as follows: 100 ms TOF MS scan, followed by 200 variable SWATH windows each at 50 ms accumulation time for *m/z* 400–1250. MS/MS SWATH scans were set at 5 Da window overlapping by 1 Da for *m/z* 400–1250 and varied on each side of the mass range. The total cycle time was 9.6 s. A rolling collision energy (CE) parameters script was used to automatically control the CE.

*Data Analysis*

Acquired spectra were searched against the UniProt reviewed database using the Paragon algorithm embedded in the ProteinPilot 5.0.1 software program (AB Sciex), with the following search parameters: (i) sample type: identiﬁcation, (ii) Cys alkylation: iodoacetamide, (iii) digestion: trypsin, (iv) instrument: TripleTOF 6600, (v) species: *Mus musculus*, (vi) ID focus: biological modiﬁcations, (vii) detected protein threshold: > 0.05 (10% conﬁdence). The detected protein threshold was set to the minimum level to enhance the number of wrong answers to enable the curve ﬁtting by an independent FDR analysis [33]. This was carried out by the target-decoy approach provided with the ProteinPilot software program, which was used to assess the quality of the identiﬁcations. Positive identiﬁcations were considered when identiﬁed proteins and peptides reached a 1% local FDR [34]. The resulting group file was loaded into Peakview (v2.2.0, AB Sciex) and peaks from SWATH runs were extracted with a peptide confidence threshold of 99% and a false discovery rate <1%. The SWATH ﬁles were then exported to the MarkerView software program (version 1.3.0.1; AB Sciex) and the peak areas of individual peptides were normalized to the sum of the peak areas of all detected peptides. A principal component analysis (PCA) and orthogonal partial least square-discriminant analysis (OPLS-DA) were performed as multivariate analyses using the Simca software program (Infocom Corp., Tokyo, Japan). Pareto scaling was applied to the peak area values acquired by SWATH prior to the analyses, which represents a compromise between the extremes of no scaling and unit variance scaling and involves dividing each spectral variable by the square root of its standard deviation after first centering. Gene ontology enrichment analyses of the differentially expressed proteins between group A and group B were performed using the PANTHER classification system in Gene Ontology Consortium (http://www.geneontology.org/). The Mouse Swiss-prot database was used as a reference set for the gene ontology analyses. Bonferroni correction was used for multiple testing.