Supplementary Methods 1

Proteomic assay performance

Samples of serum and ICV were analysed by Data-Independent Acquisition (DIA) with quantitative Sequential Window Acquisition of all Theoretical Fragment Ion Spectra (SWATH)-MS proteomics to identify differential expressed proteins (DEP) between groups as previously described (Del Pilar Chantada et al., 2019; Alvarez et al., 2020).

First of all, 30µL of serum and ICV extracts were depleted of high-abundant serum proteins using dithiothreitol (DTT) following the protocol described by Warder et al. (2009) and Fernández et al. (2011), This step enriched the low-abundance protein fraction, enhancing the resolution of low expressed proteins. The samples were prepared to have an equal amount of protein and were analysed on a 10 % SDS-PAGE gel. Protein bands were detected using Sypro-Ruby fluorescent staining (Lonza, Basel, Switzerland), manually excised, and processed for in-gel tryptic digestion, as previously described (Shevchenko et al., 1996).

Briefly, for trypsin digestion, protein bands were first washed with a solution 50% (v/v) of 50 mM ammonium bicarbonate (Sigma-Aldrich, Saint Louis, USA) / methanol (HPLC grade, Scharlau, Barcelona, Spain) and dehydrated in acetonitrile (ACN) (HPLC grade, Scharlau, Barcelona, Spain). These bands were reduced in 10 mM dithiothreitol (Sigma-Aldrich, Saint Louis, USA) in 50 mM ammonium bicarbonate at 56◦C for 30 min, followed by an alkylation step using 55 mM iodoacetamide (Sigma-Aldrich, Saint Louis, MO) in 50 mM ammonium bicarbonate for 20 min at room temperature (RT) in darkness. Protein bands were rinsed with 50% (v/v) of 50 mM ammonium bicarbonate/methanol and dehydrated with acetonitrile. Finally, tryptic digestion was carried out by adding modified porcine trypsin (Promega, Madison, WI, USA) at a final concentration of 20 ng/µL in 20 mM ammonium bicarbonate and incubating for 16 h at 37 ◦C. Tryptic peptides were extracted generated through three consecutive 20-minute incubations in 40 µL of 60% acetonitrile containing 0.5% formic acid. The resulting extracts were pooled, vacuum-dried, and stored at -20°C for subsequent analysis.

For Data-Dependent Acquisition (DDA) , digested peptides (> 4 µg of each sample) from all samples were analyzed with a reverse phase liquid chromatography coupled to Triple-TOF mass spectrometry (nano LC-ESI-MS/MS) analysis using a nano liquid chromatography system (Eksigent Technologies nanoLC 400, SCIEX Foster City, CA,

USA) coupled to a high-speed Triple TOF 6600 mass spectrometer (SCIEX, Foster City, CA, USA) as described previously (Alvarez et al., 2020).

Digested peptides separation was performed on a silica-based reversed phase column C18 150 × 0.30 mm, with a 3 mm particle size and 120 Å pore size (Eksigent, SCIEX, Foster City, CA, USA) with a trap column YMC-TRIART C18 (YMC Technologies, Teknokroma). Peptides were fractionated at a flow rate of 5 µL/min in a 90 min gradient with increasing concentrations of ACN (2% to 90%). Triple-TOF 6600 system was operated with the following settings: ion spray voltage floating (ISVF) 5500 V, curtain gas (CUR) 25, collision energy (CE), 10 and, ion source gas 1 (GS1) 25. The instrument was operated with Analyst TF 1.7.1 software (SCIEX, Foster City, CA, USA). MS/MS fragmentation was performed in 350−1400 m/z range considering a charge state of 2−5. Dynamic exclusion was set to 15 s. The mass spectrometry data obtained were processed using ProteinPilot TM 5.0.1 software (SCIEX, Foster City, CA, USA). Data were searched using the Bos taurus protein database (UniProt knowledgebase) in order to identify cysteine alkylation as a fixed modification and methionine oxidation as a variable modification and determine relative abundance at a >95% confidence interval. A non-lineal false discovery rate (FDR) was applied for peptide identification. only peptides with FDR < 1% cutoff were considered.

For quantitative analysis, a SWATH-MS analysis was performed. First of all, a mass spectrometry (MS/MS) spectral library is generated. Equal volumes from the original samples were combined to create pooled vials for each group, ensuring the representation of all peptides and proteins across all samples. These pools of peptide solutions per condition were analysed using a shotgun DDA approach by micro-LC-MS/MS (SCIEX, Foster City, CA, USA), as described above and previously described (Alvarez et al., 2020). The spectral library for SWATH peak extraction was constructed from the MS2 spectra (MS/MS spectra) of the identified peptides using an add-on for PeakView Software (version 2.2, SCIEX): MS/MS^{ALL} with SWATH Acquisition MicroApp (version 2.0, SCIEX). Only peptides with a confidence score >99% were included.

SWATH–MS acquisition was performed on a TripleTOF® 6600 LC-MS/MS system (SCIEX, Foster City, CA, USA). The analysis is carried out in technical triplicate on each sample studied using a DIA method. Each 4-µL sample was analyzed with the LC-MS equipment, applying the aforementioned LC gradient used to build the spectral library,

but using the SWATH-MS acquisition method instead described previously (Del Pilar Chantada et al., 2019; Alvarez et al., 2020).

The SWATH-MS method consisted of the cyclic acquisition of 100 TOF MS2 (MS/MS) scans. The Triple-TOF 6600 system was configured with MS/MS fragmentation in the range 400-1500 m/z, operating in high sensitivity mode, with an acquisition time of 50 ms. The cycle included overlapping sequential precursor isolation windows of variable width (1 m/z overlap), covering the range 400-1250 m/z. Each cycle was preceded by a TOF MS1 scan (400-1500 m/z, 50 ms acquisition time). The total cycle time was 6.3 seconds. The widths of the 100 variable windows were optimised based on the ion density data from the DDA runs, using a SWATH variable window calculator (SCIEX, Foster City, CA, USA).

Data analysis for proteomic assay performance

The targeted data extraction of the fragment ion chromatogram traces from the SWATH runs was performed by PeakView (version 2.2) using the SWATH Acquisition MicroApp (version 2.0). This application processed the data using the spectral library created from the shotgun data. Up to **ten peptides per protein and seven fragments per peptide** were selected, based on signal intensity; any shared and modified peptides were excluded from the processing. Five-minute windows and 30 ppm widths were used to extract the ion chromatograms; SWATH quantization was attempted for all proteins in the ion library that were identified by ProteinPilot with an FDR below 1%. The retention times from the peptides that were selected for each protein were realigned in each run according to the iRT peptides spiked in each sample and eluted along the whole time axis. The extracted ion chromatograms were then generated for each selected fragment ion; the peak areas for the peptides were obtained by summing the peak areas from the corresponding fragment ions. PeakView computed an FDR and a score for each assigned peptide according to the chromatographic and spectra components; only peptides with an FDR below 1% were used for protein quantization. Protein quantization was calculated by adding the peak areas of the corresponding peptides.

The integrated peak areas (processed. mrkvw files from PeakView) were directly exported to the MarkerView software (Sciex) for relative quantitative analysis. The export will generate three files containing quantitative information about individual ions, the

summed intensity of different ions for a particular peptide and the summed intensity of different peptides for a particular protein. MarkerView has been used for analysis of SWATH-MS data reported in other proteomics studies because of its data-independent method of quantization. MarkerView uses processing algorithms that accurately find chromatographic and spectral peaks direct from the raw SWATH data. The software's alignment of the data compensates for small variations in the mass and RT values, ensuring precise comparisons of identical compounds in different samples. To homogenise the data obtained, a most likely ratio (MLR) normalisation was performed (Lambert et al., 2013). Unsupervised multivariate statistical analysis using principal component analysis (PCA) was performed to compare the data across the samples. The average MS peak area of each protein was derived from the replicates of the SWATH-MS of each sample, followed by Student's *t*-test analysis using the MarkerView software for comparison among the samples based on the averaged area sums of all the transitions derived for each protein. Statistical significance differences between groups were evaluated using the Mann-Whitney U-test or Student's t-test. Proteins were considered as DEPs those with a FDR-corrected p-values <0.05 and a FC > 1.5 fold in- or <0.6.

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