Gel Informatics:

Digital gel images were created using the UVP Bioimaging System and further processed by LabWorks 4.6 (UVP LLC, Upland, CA). Gel images were analyzed with Delta 2D v3.4 image analysis software (Decodon GmbH, Greifswald, Germany). Image wrapping and spot matching was performed automatically and manually validated afterward. After background subtraction, a fused image was created by adding all spots from all images as a collective universal proteome map. A unique-spots ID list was created based on the proteome map and reassigned back to each individual gel to assure complete spot matching. To prevent identification of false spots, spots were removed from consideration if the spot quality was < 0.25 and if the spot percent volume in the densitometry intensity analysis was < 0.002. Spot quantification was based on fold-changes based on percent volume (% vol_i = 100 x $vol_i/\sum vol_n$) of all spots resolved in the gel, where vol_i is the volume of spot *i* in a gel containing *n* spots. Statistical analysis was carried out with the Student's *t*-test included in the Delta 2D package, using a cut-off value of $p < 0.05$. Three biological replicates each with two technical replicate gels were performed for each sample. Spots with significantly densitometric value changes were further checked visually to exclude false spots, mismatches, and spots showing evidence of co-migration.

MS Identification:

Gel spots were cut with a spot picker with 1.5 mm or 3.0 mm diameter PDM tips (Gel Company, San Francisco, CA) depending on spot size. Excised spots were destained with 80 µL 1:1 solution of 30 mM potassium ferricyanide and 100 mM sodium thiosulfate for 15 min, and washed twice for 15 min in 100 µL 100 mM ammonium bicarbonate [42], and dehydrated with 100 µL 100% ACN. The supernatant was removed and gel plugs were dried in a SPD SpeedVac (Thermo Electron, Waltham, MA). Tryptic digestion was accomplished by incubation of the gel spots with 0.6 µg sequencing-grade trypsin (Promega, Madison, WI, USA) in 40 µL of 100 mM ABC for 12 h at 37 ^{o}C. After the incubation, the supernatant was collected. Peptides were further extracted by sequentially incubating gel plugs with 80 µL of 0.1% formic acid (FA) in 50% ACN, 0.1% FA in 70% ACN, 40 mM ABC, and 100% ACN each for 15 min. The pooled supernatants from these steps were dried in the SpeedVac to a final volume of 10 µL. Peptides were desalted using ZipTips C18 pipette tips (Millipore, Billerica, MA, USA) and reconstituted in 10 µL of 0.1% FA with 3% ACN prior to analysis on a ESI-Q-TOF (Agilent 6150, Santa

Clara CA).

Desalted protein digests were injected onto a G4240-62001 C-18 HPLC-Chip (40 nL enrichment column, 75 µm x 43 mm analytical column, 5 µm C-18SB-ZX, 300 Å, Agilent) interfaced with the ESI. Peptides were separated during a 30-min gradient of 16–90% ACN in 0.1% FA at 4 µL/min. Peptides were analyzed using the data-dependent MS/MS mode over the m/z range of 59–3000. Three precursor ions were selected for MS/MS following each survey scan and only ions exhibiting a detection intensity exceeding 1000 counts were selected for MS/MS fragmentation by collision-induced dissociation (CID). MS/MS data were acquired in centroid mode.

MS/MS spectra were converted into Mascot generic format (.mgf) and uploaded to Mascot v2.3.01 (Matrix Science, London, UK) for database searching against a target-reverse concatenated International Protein Index (IPI, European Bioinformatics Institute, http://www.ebi.ac.uk/IPI) mouse database (v3.74, 113868 sequences). The search parameters were set to allow for up to two missed cleavages, carboxyamidomethylation on cysteine and variable modification of oxidation on methionine, a peptide tolerance of 0.2 Da, and an MS/MS tolerance of 0.1 Da. All identifications from Mascot were transferred into Scaffold v3.00.03 (Proteome Software, Inc., Portland, OR) for validation, where peptides that met the following two criteria, whichever was stricter, were used for protein identifications: (1) ion score must be equal to or higher than the identity score from each individual Mascot search result, and (2) the score of a +2, +3, or +4 ion must be equal or higher than 34, 37 or 40, respectively. For protein identification, the match with the highest protein score (must be $p < 0.05$) and with at least with two unique peptide matches screened by the aforementioned peptide criteria was considered as the protein identification. Common contaminants such as keratins and trypsin were excluded from the results.