**Protein analysis by mass spectrometry**

For lysis, cell pellets were reconstituted in 100mM HEPES pH 7.3 containing 4% SDS, 10mM dithiothreitol, and 0.5X cOmplete protease inhibitor (Sigma) and transferred to lysing matrix Y tubes (MP Biomedicals). Samples were disrupted on a FastPrep 5G instrument (6M/s, 45-seconds, 2 cycles). Lysed samples were incubated at +60°C for 30-minutes and then chloroacetamide was added to a final concentration of 40mM. After a 30-minute incubation in the dark at room temperature (+24°C), samples were quenched by addition of dithiothreitol to a final concentration of 40mM. For clean-up prior to digestion, samples were prepared using SP3 in standard conditions as described previously (PMID: 25358341, 30464214). Cleaned proteins were subject to proteolysis using trypsin and rLysC (Promega) and digested at +37°C for 18-hours.

After tryptic digestion, samples were centrifuged at 10,000g for 30-seconds and peptide-containing supernatant recovered using a magnetic rack. Samples were then tandem mass tag (TMT) labeled using 6-plex reagents (Thermo Scientific) as described previously (PMID: 27713570). Briefly, reconstituted TMT label (10µg/µL) was added to each peptide sample at a concentration ratio of 2:1 (µg:µg TMT label to peptide) and incubated at room temperature for 30-minutes. An additional aliquot of the TMT was then added (4:1 final ratio) and incubated for a further 30-minutes. For TMT layout, wild-type sample replicates were in channels 126 – 128, with mutant samples in 129 – 131. Individually labeled samples were combined and concentrated in a SpeedVac centrifuge and then desalted using SepPak cartridges (50mg t-C18 material, Waters). Briefly, columns were rinsed twice with acetonitrile (+0.1% TFA) and twice with water (+0.1% TFA). After sample loading, cartridges were rinsed twice with water (+0.1% formic acid, FA) and then eluted with 80% acetonitrile (+0.1% FA). After clean-up, peptides were fractionated using high-pH reversed phase chromatography with fraction concatenation as described previously (PMID: 27713570). A final set of 12 fractions was desalted prior to MS analysis using StageTips (3-disc plug, C18 Empore material, 3M). Eluted peptides were concentrated to dryness using a SpeedVac centrifuge (Thermo Scientific) and subsequently reconstituted in 1% formic acid.

Analysis of peptide samples was carried out on an Orbitrap Fusion MS system (Thermo Scientific). Specifically, peptide samples were initially injected and chromatographically separated using an Easy nLC 1000 system (Thermo Scientific) with a trapping-analytical column setup. Trapping columns were packed in 75µm internal diameter capillaries to a length of 3cm with 1.9µm Reprosil-Pur C18 beads (Dr. Maisch). Trap columns were packed in-house in fritted capillaries prepared with a combination of formamide and Kasil (1:3 ratio). Gradient elution of peptides was performed on a C18 (Reprosil-Pur, 1.9µm C18, Dr. Maisch) analytical column packed to a length of 25cm in a 75µm internal diameter capillary with a nanospray tip (New Objective). The analytical column was heated to 50°C using an AgileSLEEVE oven (Analytical Sales & Service) and eluted across a 120-minute gradient of acetonitrile (+0.1% FA) (20-minute column rinsing and 20-minute injection overhead for a total run time per injection of 160-minutes) at a flow rate of 350nL/min.

Data acquisition on the Orbitrap Fusion was carried out using a data-dependent tandem MS/MS (MS2) method with synchronous precursor selection MS/MS/MS (SPS-MS3) detection of TMT reporter ions. Survey scans (MS1) covering the mass range of 380 – 1500m/z were acquired at a resolution of 120,000 (at m/z 200), with quadrupole isolation enabled, an S-Lens RF level of 60%, a maximum fill time of 50 milliseconds, and an automatic gain control (AGC) target value of 4e5. For MS2 identification scan triggering, monoisotopic precursor selection was enabled, charge state filtering of 2 – 5, and dynamic exclusion of previously selected masses for 30 seconds (10ppm mass threshold). MS2 scans were acquired in the ion trap with the Turbo scan mode after CID fragmentation, a maximum fill time of 50ms, an isolation window of 2m/z, collision energy of 35%, and an AGC target of 1e4. Fragments for MS3 scans were selected based on a range of 400 – 1200m/z, precursor ion exclusion (20ppm high, 5ppm low), and isobaric tag exclusion set to TMT. MS3 scans covering the mass range of 110 – 750m/z were acquired at a resolution of 60,000 in the Orbitrap after HCD fragmentation, a maximum fill time of 120ms, an isolation window of 2m/z, collision energy of 65%, and an AGC target of 1e5. The total allowable cycle time was set to 3-seconds. MS2 and MS3 scans were acquired in centroid format, with MS1 in profile mode.

Acquired MS data were processed using Proteome Discoverer (version 2.1.0.62) as described previously (PMID: 27713570). Search engine parameters were specified as: trypsin enzyme, 2 missed cleavages allowed, precursor mass tolerance of 20ppm, and a fragment mass tolerance of 0.8 Daltons. Carbamidomethylation of cysteine and TMT-6plex of the peptide N-terminus were set as fixed modifications. Oxidation of methionine, TMT-6plex of lysine, and TMT 6-plex of lysine + 8 (for SILAC) were set as variable modifications. MS2 spectra were searched against the UniProt human proteome database (version 2016Jan) appended to a list of common contaminants. Peptide spectral match error rates were determined using the target-decoy strategy coupled to Percolator modeling of positive and false matches. Reporter ions were quantified from MS3 scans using an integration tolerance of 20ppm with the most confident centroid setting. Output quantification values represented the signal-to-noise of the TMT value relative to the Orbitrap preamplifier. Data were filtered at the peptide spectral match-level to control for false discoveries using a q-value cutoff of 0.05 as determined by Percolator. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (PMID: 30395289) partner repository with the dataset identifier PXD021551.