Supplementary methods

LC-MS analysis

SILAC labelled-samples were pooled before being run on a 10% SDS-PAGE gel. The gel lanes were then cut into 10 equal slices and using a DigestPro automated digestion unit (Intavis), each gel underwent in-gel tryptic digestion. An UltiMate3000 nano-LC system was used to fractionate the resulting peptides in line with an LTQ Orbitrap Velos mass spectrometer (Thermo). Peptides in 1% (vol/vol) formic acid were injected on an Acclaim PepMap C18 nano-trap column (Thermo) and then washed with 0.5% (vol/vol) acetonitrile before 0.1% (vol/vol) formic acid peptides were resolved on a 250 mm × 75 lm Acclaim PepMap C18 reverse phase analytical column (Thermo) over a 150 min organic gradient, using seven gradient segments (1–6% solvent B over 1 min, 6–15% B over 58 min, 15–32% B over 58 min, 32–40% B over 5 min, 40–90% B over 1 min, held at 90% B for 6 min and then reduced to 1% B over 1 min) with a flow rate of 300 nl/min. The solvents were: A, 0.1% formic acid and B, aqueous 80% acetonitrile in 0.1% formic acid. Nano-electrospray ionisation was used to ionised the peptides at 2.1 kB using a stainless-steel emitter that has an internal diameter of 30 µm (Thermo) and a 250°C capillary temperature. An LTQ Orbitrap Velos mass spectrometer controlled by Xcalibur 2.0 software (Thermo) was used to obtain mass spectra, and operated in data-dependent acquisition mode. Survey scans were analysed using the Orbitrap set at 60,000 resolution (at m/z 400) in the mass range m/z 300–2,000 and the top ten multiply charged ions in each duty cycle selected for MS/MS in the LTQ linear ion trap. Charge stage filtering (unassigned precursor ions were not selected for fragmentation) and dynamic exclusion (repeat count, 1; repeat duration, 30 s; exclusion list size, 500) were used. LTQ Conditions for fragmentation were: normalised collision energy, 40%; activation q, 0.25; activation time, 10 ms; and minimum ion selection intensity, 500 counts.

Proteomics data analysis

Proteome Discoverer software v1.4 (Thermo) was used to process and quantify the raw data files and the SEQUEST algorithm was used to search against the UniProt Human database (122,604 entries). Peptide precursor mass tolerance was set at 10 ppm and MS/MS tolerance at 0.8 Da. Search criteria included oxidation of methionine (+15.9949) and appropriate SILAC labels as variable modifications and carbamidomethylation of cysteine (+57.0214) as a fixed modification. Searches were carried out with full tryptic digestion, allowing only one missed cleavage. The reverse database search option was enabled, and all peptide data were filtered to a false discovery rate (FDR) of 5%.