SUPPLEMENTAL MATERIALS AND METHODS

Cultured mouse lymphoma cells were lysed with a buffer containing 50 mM Tris-HCl (pH 8.0) and 8 M Urea. The samples were kept on ice during protein extraction. In some experiments, for comparative analyses, a protease inhibitor cocktail (Sigma) was added to the lysis buffer according to the manufacturer's instruction (1:100). The protein inhibitor cocktail contains 6 components including 4-(2-aminoethyl)benzenesulfonyl fluoride (MW=239.69), pepstatin A (MW=685.89), E-64 (MW=357.41), bestatin (MW=308.4), leupeptin (475.59), and aprotinin (MW=6512), which together possess a broad specificity for the inhibition of serine, cysteine, aspartic proteases and aminopeptidases. The lysed cells were sonicated five times (15% amplitude, 10 seconds each) on ice using a Digital Sonifier cell disruptor with 1/8" microtip (Branson Ultrasonics Corporation, Model 250). The lysate was centrifuged at 15,000 × g for 15 minutes at 4°C. The supernatant was collected and desalted into 50 mM NH_4HCO_3, pH 8.3, using D-Salt Excellulose Desalting columns (5 mL bed volume, 5000 Da cutoff, ThermoFisher Scientific).

Protein concentration was determined using a bicinchonic acid (BCA) assay. In some cases, Bradford and Lowry methods were also used. All protein concentration assays were performed using commercial kits obtained from Thermo Scientific, and followed the manufacturer instructions. In brief, a standard series with different protein concentrations (C = 0, 25, 125, 250, 500 and 750 μg/ml, respectively) was prepared for all assays using 2.0 mg/ml bovine serum albumin diluted with desalting buffer (50 mM NH_4HCO_3). For the BCA method, 2 ml Reagent A and 40 μl Reagent B (ratio 50:1) were mixed to generate a working reagent (WR). Each protein sample (10 μl) was mixed with WR (200 μl), and incubated at 37^{o}C for 30 min. For the Bradford method, each protein sample (10 μl) was mixed with Coomassie Plus (300 μl), and

incubated at room temperature for 10 min. For the Lowry method, each protein sample (10 µl) was mixed with the Lowry reagent (50 µl), and incubated at room temperature for 10 min. Then, 5 µl 1N Phenol was added to each sample. The samples were further incubated at room temperature for 30 min. Optical absorptions of all samples were measured using a NanoDrop spectrophotometer (ND-1000, NanoDrop Technologies, Inc.) with the manufacturer preset optical wavelengths for the BCA, Bradford, and Lowry, respectively. All standard curves and interpolated protein concentrations were generated by the NanoDrop 1000 software.

An aliquot of lysate containing 100 µg of solubilized protein was digested overnight at 37°C with sequencing-grade modified trypsin (Promega) at a ratio of 50:1 (w/w, protein-to-trypsin). The digestion reaction was terminated by boiling the sample in a water bath for 10 minutes. The sample was acidified by 0.1% formic acid. The digestate was aliquoted (5 µg each), lyophilized and stored at −80°C.

The digestate aliquot was dissolved in 0.1% formic acid, vortexed, and centrifuged at 12,000 × g for 7 minutes at room temperature. The supernatant was transferred to a glass sample vial (Waters). Nanoflow RPLC separation of peptides was conducted using a 9 cm long × 75 µm inner diameter (i.d.) fused silica capillary electrospray ionization (ESI) column which was coupled online to a linear ion trap mass spectrometer (LTQ XL, Thermo Electron, San Jose, CA) for MS/MS analysis. The ESI column was slurry packed with 5 µm, 300 Å pore size Jupiter C18 RP particles (Phenomenex, Torrence, CA) against a 9 cm × 75 µm i.d. fused-silica capillary (Polymicro Technologies, Phoenix, AZ) with a flame-pulled fine i.d. (i.e., 5-7 µm) tip. Mobile phases A (0.1% formic acid in water) and B (0.1% formic acid in CH_3CN) were delivered by an Agilent 1200 Nanoflow LC system (Agilent Technologies). Peptides were loaded in 30 min while the column was maintained with 2% solvent B at a flow rate of 0.5 µL/min, and then

separated using a step gradient of 2%-40% solvent B for 85 min, 40%-98% solvent B for 20 min, and maintained with 98% solvent B for 5 min at a flow rate of ~250 nL/min. Following the MS survey scan with *m/z* 350-1800, data-dependent MS/MS scans were acquired in which the 7 most intense peptide molecular ions in the MS scan were sequentially and dynamically selected for subsequent collision-induced dissociation (CID) using a normalized collision energy of 35%. Dynamic exclusion was enabled with duration of 1 min to prevent repeated acquisition of MS/MS spectra of the same peptide for which the MS/MS spectrum had been acquired in the previous scan. Electrospray voltage was 1.6 kV, and the voltage and temperature for the ion source capillary were 45 V and 160 °C, respectively.

The raw MS/MS data were searched using SEQUEST running under BioWorks (Rev. 3.3.1 SP1) (Thermo Electron, San Jose, CA) against a mouse IPI proteome database (Version 3.62) downloaded from the European Bioinformatics Institute (EBI) (http://www.ebi.ac.uk). For the SEQUEST analysis, the peptide mass tolerance was set as 2.0 Da and the fragment ion tolerance was 1.0 Da. A tryptic enzyme restriction with a maximum of two internal missed cleavage sites was used. Methionine oxidation was set by addition of 15.9949 Da. Based on the reversed sequence database search, the SEQUEST criteria such as cross correlation (Xcorr) and delta cross correlation score ($\Delta Cn \geq 0.08$) for confident peptide identification was set and applied to the global mouse peptide and protein identification with false discovery rate <0.1%. The Xcorr versus charge state values were filtered at Xcorr ≥ 1.7 for $[M+H]^{1+}$ ions, ≥ 2.5 for $[M+2H]^{2+}$ ions, ≥ 3.2 for $[M+3H]^{3+}$ ions and $P \leq 0.01$ for identification of fully tryptic peptides. To measure the abundance or intensity of each identified peptide, the peptide ion chromatogram was extracted using a minimum intensity threshold of 100, mass tolerance of 2.0 amu and smoothing point of 5, and the area of the extracted ion chromatographic (XIC) peak was integrated and calculated

using the PepQuan module in Bioworks (Rev. 3.3.1 SP1, Thermo Electron, San Jose, CA). The filtered results were further analyzed using ArrayTrack™ (http://www.fda.gov/ScienceResearch/BioinformaticsTools/Arraytrack/default.htm).