Supplemental Data 1

LC/MS/MS experiments — LTQ-FT (Thermo) and nano AUQUITY UPLC (Waters) systems were used for these experiments. A nano AUQUITY UPLC system was modified to equip an in-house capillary column (75 μm ID × 360 μm OD × 75 cm) and online SPE column. The column was packed with C18-bonded particles (3 μm diameter, 300 Å pore size, Phenomenex). The four-peptide and enolase peptide mixtures were loaded onto the online SPE column for 6 min in solvent A (0.1% formic acid in water) and eluted from the column with a 60-min gradient of 10–50% solvent B (0.1% formic acid in acetonitrile) and a flow rate of 0.35 μL/min. For the LC/MS/MS analysis of the human serum peptides, we used a longer gradient (120 min from 5 to 50% solvent B).

A 7-tesla Fourier transform ion cyclotron resonance mass spectrometer (FTICR, LTQ-FT, Thermo Electron, San Jose, CA) was used to collect the mass spectra. MS precursor ion scans (m/z 450–1800) were acquired with an AGC target value of 1×10^6, a mass resolution of 1×10^5, and a maximum ion accumulation time of 1000 ms. The mass spectrometer was operated in data-dependent tandem MS mode; the three most abundant ions detected in a precursor MS scan were dynamically selected for MS/MS experiments simultaneously incorporating a dynamic exclusion option (exclusion mass

width low: 1.10 Th; exclusion mass width high: 2.10 Th; exclusion list size: 120; exclusion duration: 30 s) to prevent reacquisition of MS/MS spectra of the same peptides. Collision-induced dissociations of the precursor ions were performed in an ion trap (LTQ) with the collisional energy and isolation width set to 35% and 3 Th, respectively. The Xcalibur software package (v. 2.0 SR1, Thermo Electron) was used to construct the experimental methods.

Database search — All tandem mass spectrometric data (i.e. DTA files) were extracted using the ExtractMSn (v. 3; creation date: 2006.7.18) of Bioworks™ software (v. 3.2, Thermo Electron) and subsequently were subjected to filtration and mass refinement process (PE-MMR). A detailed description of PE-MMR is provided elsewhere[1]. Charge states from +1 to +8 were considered, and the precursor peptide mass range was set to 400–10,000 Da. The MS/MS data were then searched against a composite database—containing either a human international protein index (IPI; v. 3.14) database and its reversed complements using SEQUEST (Version 27). The tolerance was set to 10 ppm for precursor ions and 1 Da for fragment ions. The maximum number of internal cleavage sites was set to 3, and variable modification options were used for the carbamidomethylation of cysteine (57.021460 Da), the oxidation of methionine (15.994920 Da). The search results were filtered using an estimated FP rate. The FP

rate of peptide assignment was estimated through a composite target/decoy database search. The values of Xcorr and the ΔCn threshold for the 1% FP rate were used to obtain peptide IDs.

REFERENCES

(1) "Post-Experiment Monoisotopic Mass Filtering and Refinement (PE-MMR) of Tandem Mass Spectrometric Data Increases Accuracy of Peptide Identification in LC/MS/MS" Shin, B.; Jung, H.-J.; Hyung, S.-W.; Kim, H.; Lee, D.; Lee, C.; Yu, M.-H.; Lee, S.-W. *Mol. Cell. Proteomics* in press.