

Supporting Information

An Optimization of LC-MS/MS Workflow for Deep Proteome

Profiling on Orbitrap Fusion

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Supplementary Table 1 Comparison of protein quantitative accuracy with different resolutions with SILAC method.

Supplementary Table 2 Protein identifications across 20 fractions. Tryptic peptides from HeLa whole cell lysate digest were pooled into 20 fractions and each fraction were analyzed by a 60 min gradient nano-HPLC/MS/MS analysis.

Supplementary Table 3 Protein identifications across 10 fractions. Tryptic peptides from HeLa whole cell lysate digest were pooled into 10 fractions and each fraction were analyzed by a 60 min gradient nano-HPLC/MS/MS analysis.

Supplementary Table 4 Protein identifications across 10 fractions. Tryptic peptides from HeLa whole cell lysate digest were pooled into 10 fractions and each fraction were analyzed by a 90 min gradient nano-HPLC/MS/MS analysis.

Supplementary Table 5 Protein identifications across 5 fractions. Tryptic peptides from HeLa whole cell lysate digest were pooled into 5 fractions and each fraction was analyzed by a 60 min gradient nano-HPLC/MS/MS analysis.

Supplementary Table 6 Protein identifications across 5 fractions. Tryptic peptides from HeLa whole cell lysate digest were pooled into 5 fractions and each fraction were analyzed by a 120 min gradient nano-HPLC/MS/MS analysis.

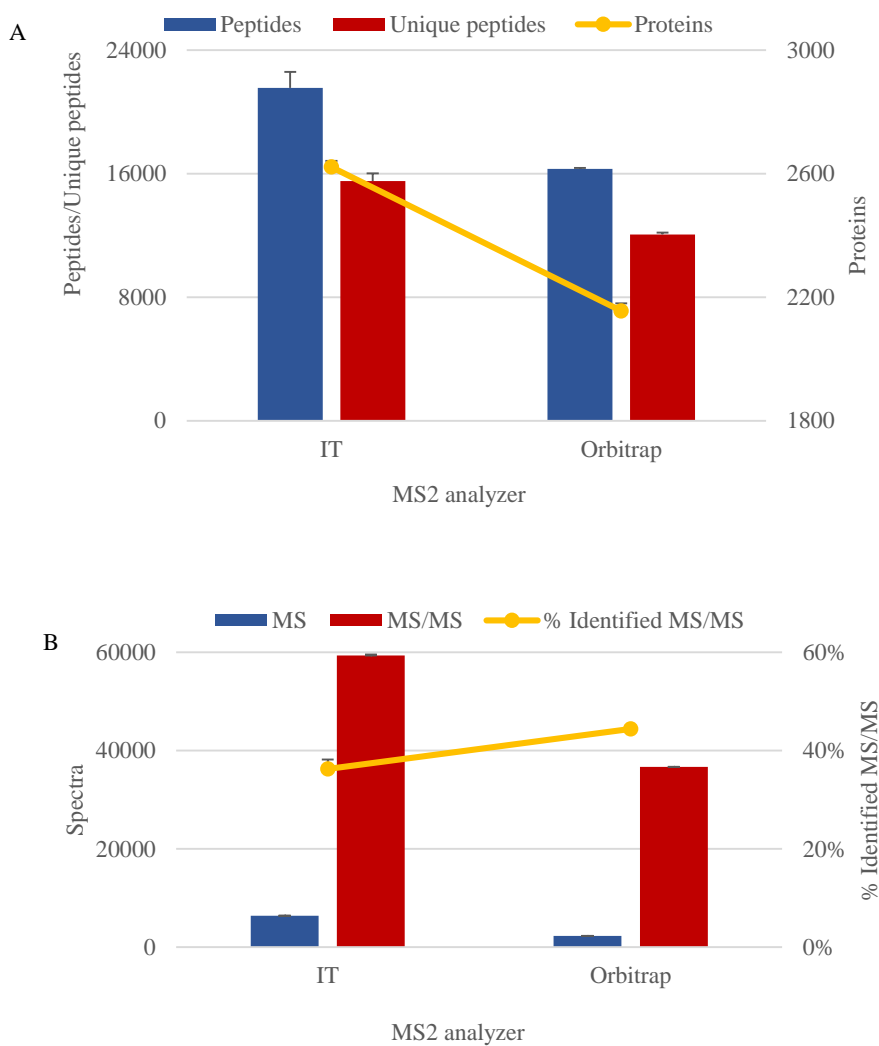
Supplementary Table 7 At 1% FDR at the protein level, 8493 proteins were identified across 10 fractions. Tryptic peptides from HeLa whole cell lysate digest were pooled into 10 fractions and each fraction were analyzed by a 60 min gradient nano-HPLC/MS/MS analysis.

Supplementary Table 8 KEGG pathways analysis of the proteins identified across all the 10 fractions over a 60 min gradient.

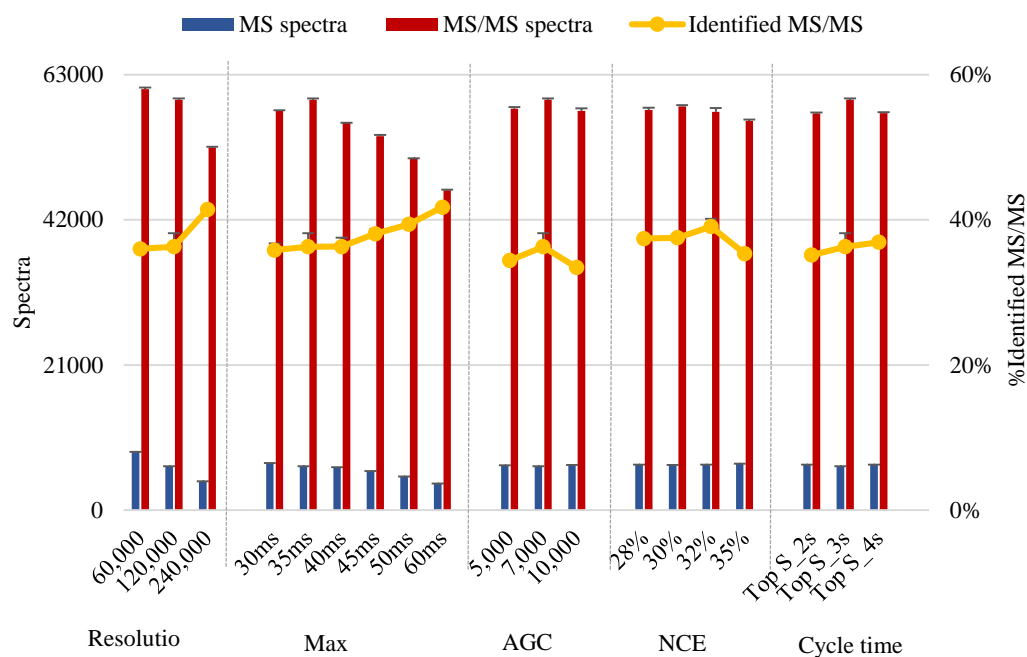
Supplementary Table 9 Phosphopeptides table with protein group accessions

identified in mouse liver tissue.

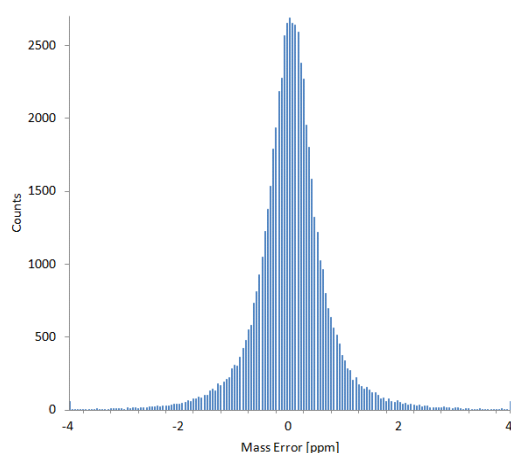
Supplementary Table 10 KEGG pathways analysis of the phosphoproteins from mouse liver tissue.



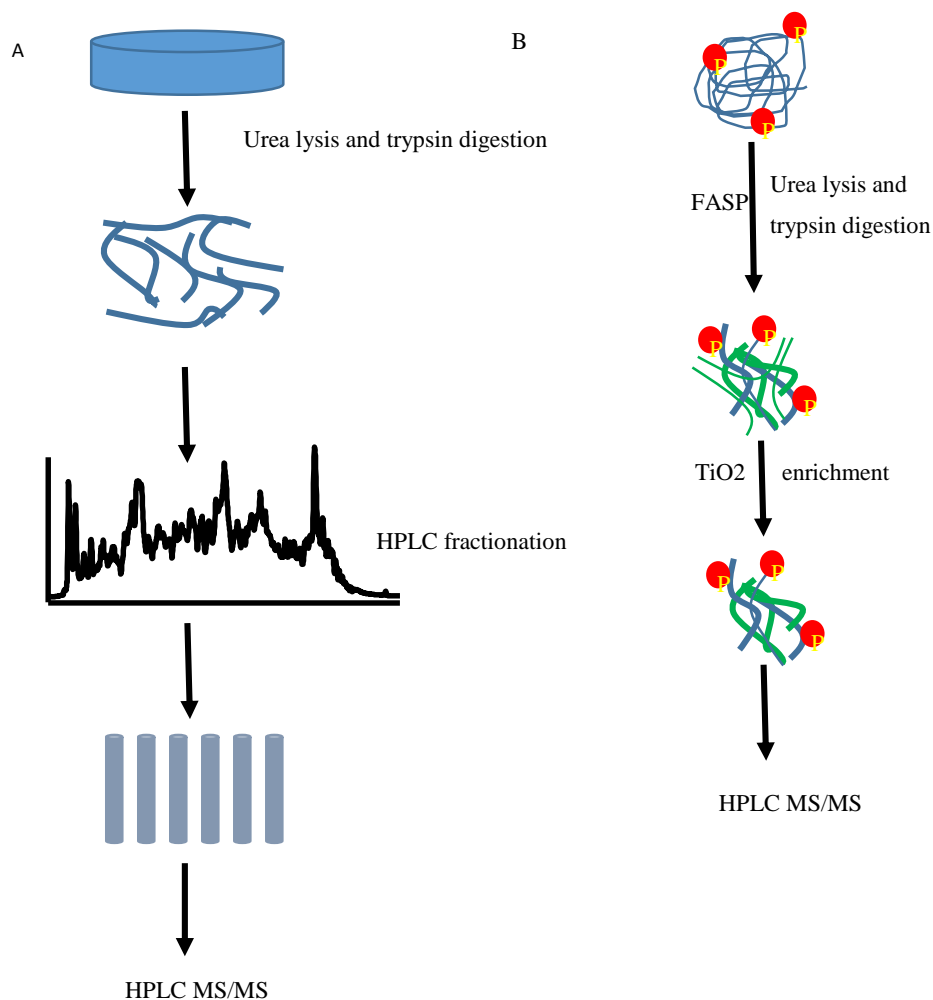
Supplementary Figure 1 Comparison of the performance of Orbitrap Fusion with different MS2 mass analyzers. (A) Number of proteins, peptides and unique peptides identified at different MS2 analyzers: ion trap (IT) or Orbitrap. (B) Number of MS and MS/MS identified, percentage of identified MS/MS at different MS2 analyzers: ion trap (IT) or Orbitrap.



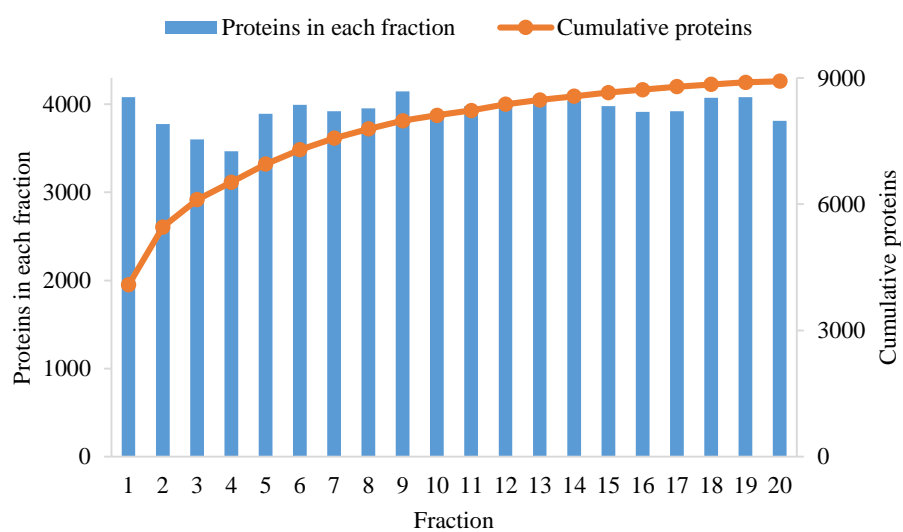
Supplementary Figure 2 Optimization of instrument parameters in Orbitrap-HCD-IT mode by a replicate measurement. Resolution, isolation window, maximum injection time (Max IT), AGC target, HCD normalized collision energy (NCE) and cycle time of Top speed were optimized. General parameters were as described in method of nano-HPLC-MS/MS analysis while optimizing each single parameter. Number of MS and MS/MS identified, percentage of identified MS/MS at different parameters were displayed.



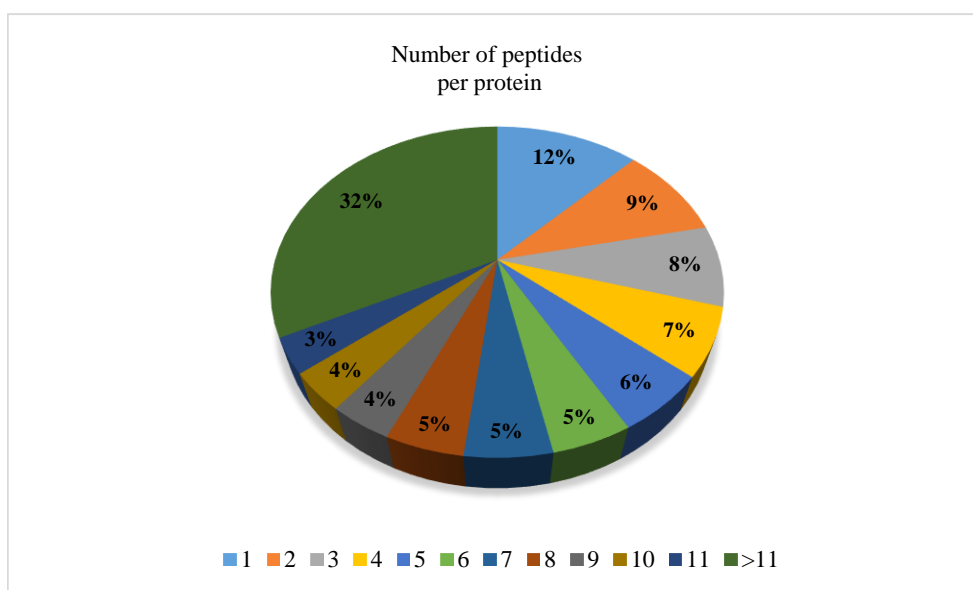
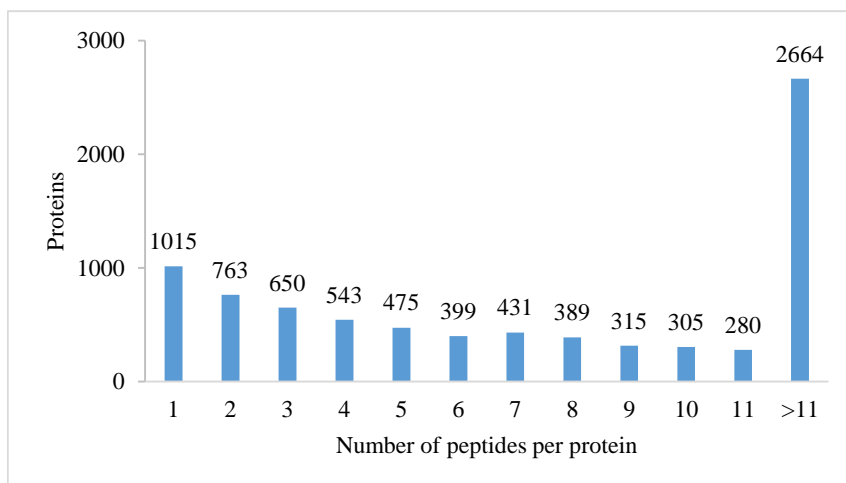
Supplementary Figure 3 Mass accuracy for precursor ions from 200 ng whole cell lysate.



Supplementary Figure 4 Streamlined workflow (A) Streamlined workflow of depth proteome analysis. Total proteins were extracted in 8M urea solution and then digested by trypsin. Reverse phase chromatography in pH 10.0 condition was employed to pre-fractionate peptides. Then 2nd dimension reverse phase chromatography in acid condition was applied. (B) Streamlined workflow of phosphorylation analysis. Total proteins were extracted and digested by FASP. The phosphopeptides was enriched by TiO₂ micro-columns. Then reverse phase chromatography in acid condition was applied.



Supplementary Figure 5 Deep proteome coverage in two-dimension RP method. Individual proteins identified in each fractions and cumulative proteins identified over a 60-minute LC-MS/MS gradient across 20 fractions.



Supplementary Figure 6 The distribution of number of peptides per protein identified across all the 10 fractions with a 60 min gradient.