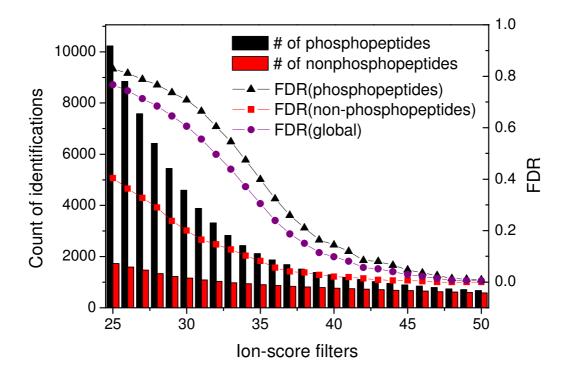
Supplemental figure S1:



Numbers of identifications and FDRs of phosphopeptides and nonphosphopeptides. The black bars and red bars were the numbers of phosphopeptide and non-phosphopeptides identifications by setting different ion-score filters. The corresponding FDRs of all the peptide identifications, phosphopeptide identifications and non-phosphopeptide identifications by using these filter cutoffs were also shown.

This data set was collected from enriched phosphopeptides of Hela cells using ZrO₂ as described in our previous work ¹. Only MS2 mass spectra was extracted from raw files and used for database search using Mascot database search algorithm against a composite database with the following parameters: peptide mass tolerance,

2 Da; MS2 and MS3 fragment ion mass tolerance, 1Da; enzyme set as trypsin and allowance up to two missed cleavages; static modification was cysteine carbamidomethylation (+57 Da); dynamic modifications were methionine oxidation (+16 Da), phosphorylation on serine, threonine, and tyrosine (+80 Da). The original database was a human proteome sequence database (v3.17) from European Bioinformatics Institute (http://www.ebi.ac.uk/IPI/IPIhuman.html).

References

1. Jiang, X. N.; Han, G. H.; Feng, S.; Jiang, X. G.; Ye, M. L.; Yao, X. B.; Zou, H. F., Automatic Validation of Phosphopeptide Identifications by the MS2/MS3 Target-Decoy Search Strategy. *J. Proteome Res.* **2008**, 7, (4), 1640-1649.