Supporting Information

A rapid, multiplexed kinase activity assay using 8-plex iTRAQ labeling, SPE, and MALDI-TOF/TOF MS

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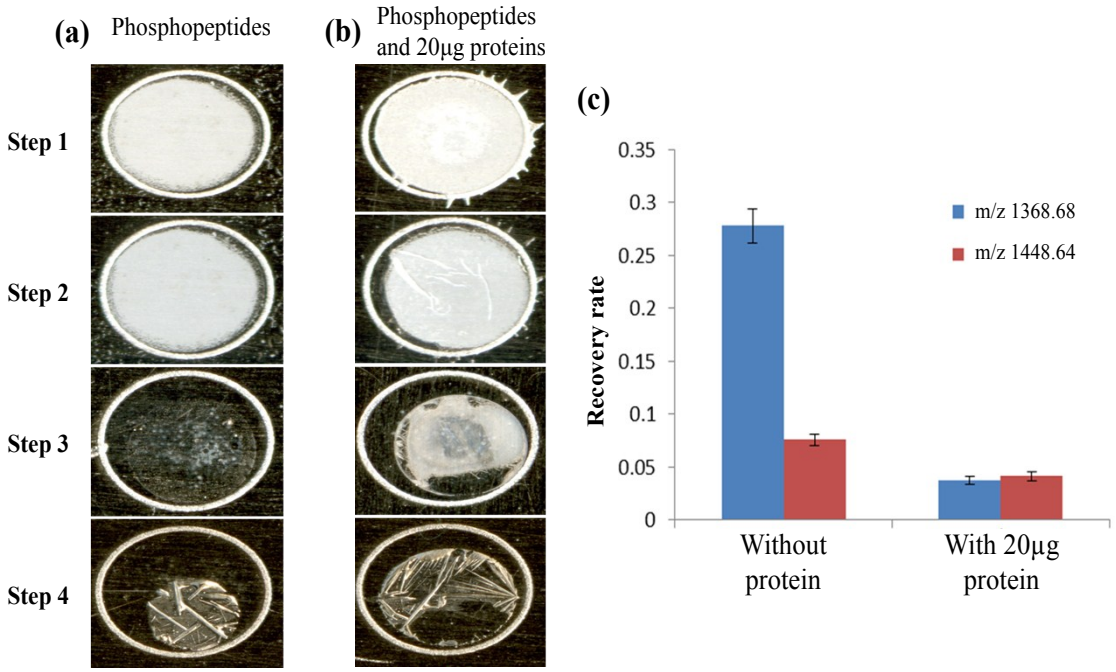
Supplementary Figure 1. Evaluation of protein effect for phosphopeptides enrichment by TiO_2 plate.

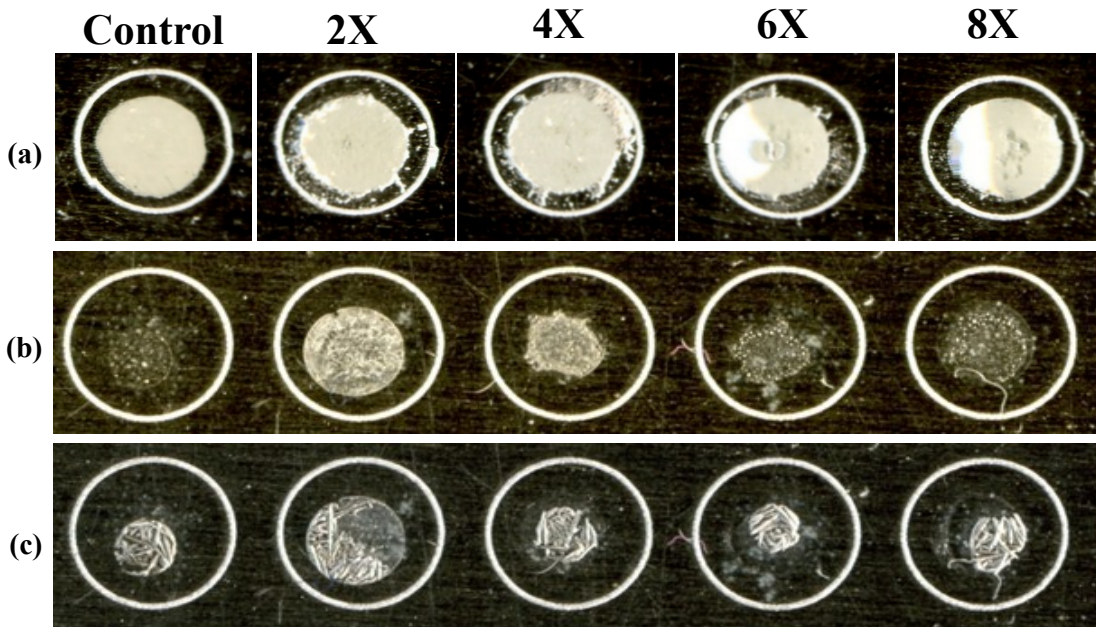
Supplementary Figure 2. Solvent precipitation effect on phosphopeptide purification.

Supplementary Figure 3. MALDI-TOF MS spectra of PS1-PS6 after multikinase reaction.

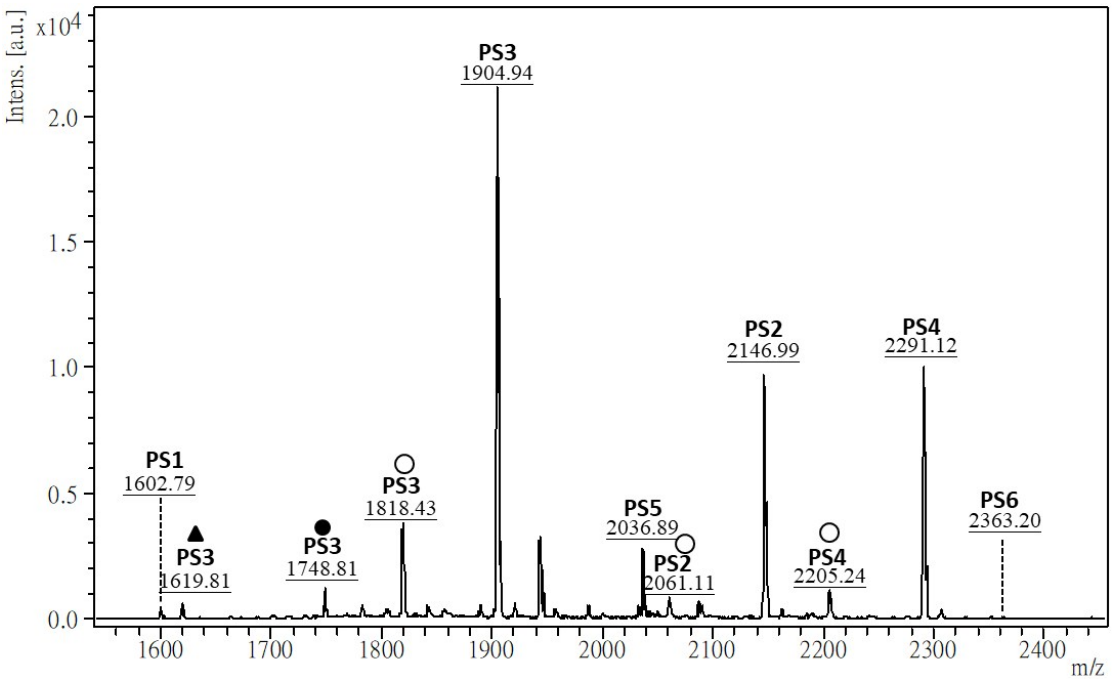
Supplementary Figure 4. MALDI-TOF/TOF MS spectra of mass tag signals of PS1-PS6 after multikinase reaction with different protein amount and incubation times.



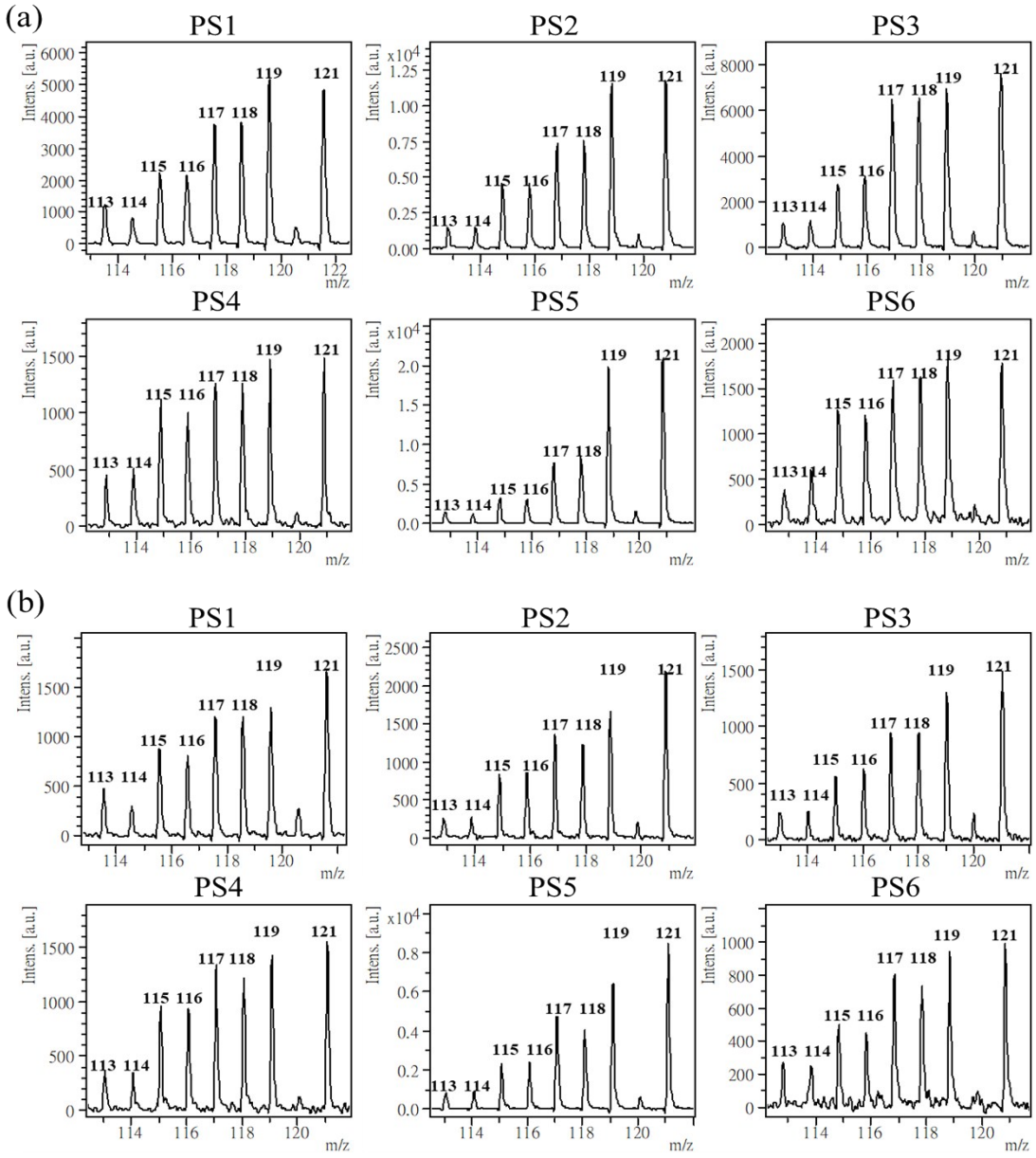
Supplementary Figure 1. Evaluation of protein effect for phosphopeptides enrichment by TiO_2 plate. (a) 2 pmol phosphopeptides and (b) the mixture of phosphopeptides (2 pmol) and proteins (20 μg) were applied to TiO2 plate. Step 1: samples were dissolved in loading buffer (80% ACN/ 2% TFA/ 20 mg mL^{-1} of DHB) were applied to TiO_2 plate. Step 2: the spots were washed with the 80% ACN/2% TFA solution. Step 3: phosphopeptides were eluted with 0.05% NH_4OH and deposited on PDMS plate. Step 4: DHB/PA matrix solution (2 mg/mL DHB in 25% ACN and 1% PA) were applied to analysts. (c) MALDI-TOF MS analysis of the two phosphopeptides VNQIG(pT)LSESIK (1368.68 m/z) and VNQIGTL(pS)E(pS)IK (1448.64 m/z) without/with protein after TiO_2 plate enrichment. The phosphopeptide signals were normalized to the internal standard of KFTRQTPVDSPFR (1578.84 m/z, 300 fmole).



Supplementary Figure 2. Solvent precipitation effect on phosphopeptide purification. The solvent (ACN/MeOH, 50%/50%)/sample volume ratio of 2, 4, 6 and 8 folds were used for precipitating proteins in the samples followed by TiO_2 plate purification and MALDI-TOF MS analysis. (a) White protein aggregates on TiO_2 plate were significantly observed using solvent/sample volume ratio from 2-8 folds. (b) The eluent analyte from the TiO_2 plate were deposited on a PDMS-coated MALDI target. (c) The sample/DHB matrix co-crystals on a PDMS-coated MALDI target. The control sample had phosphopeptides without adding proteins and solvent precipitation.



Supplementary Figure 3. MALDI-TOF MS spectra of PS1-PS6 after multikinase reaction. The phosphorylated iTRAQ labeled peptide substrates are the major peaks with some minor signals of truncated and metastable peaks. ▲: truncated form of PS3 missing ER. ●: truncated form of PS3 missing R. ○: metastable ion after loss of 86 Da of the phosphopeptide peak.



Supplementary Figure 4. MALDI-TOF/TOF MS spectra of mass tag signals of PS1-PS6 after multikinase reaction with different protein amount and incubation times. The reporter ion signals of phosphorylated peptide substrates from (a) different protein amounts (113 and 114: 5 μg ; 115 and 116:10 μg; 117 and 118: 20 μg; 119 and 121: 40 μg) and (b) incubation times (113 and 114: 30 min; 115 and 116: 60 min; 117 and 118: 90 min, 119 and 121: 120 min).