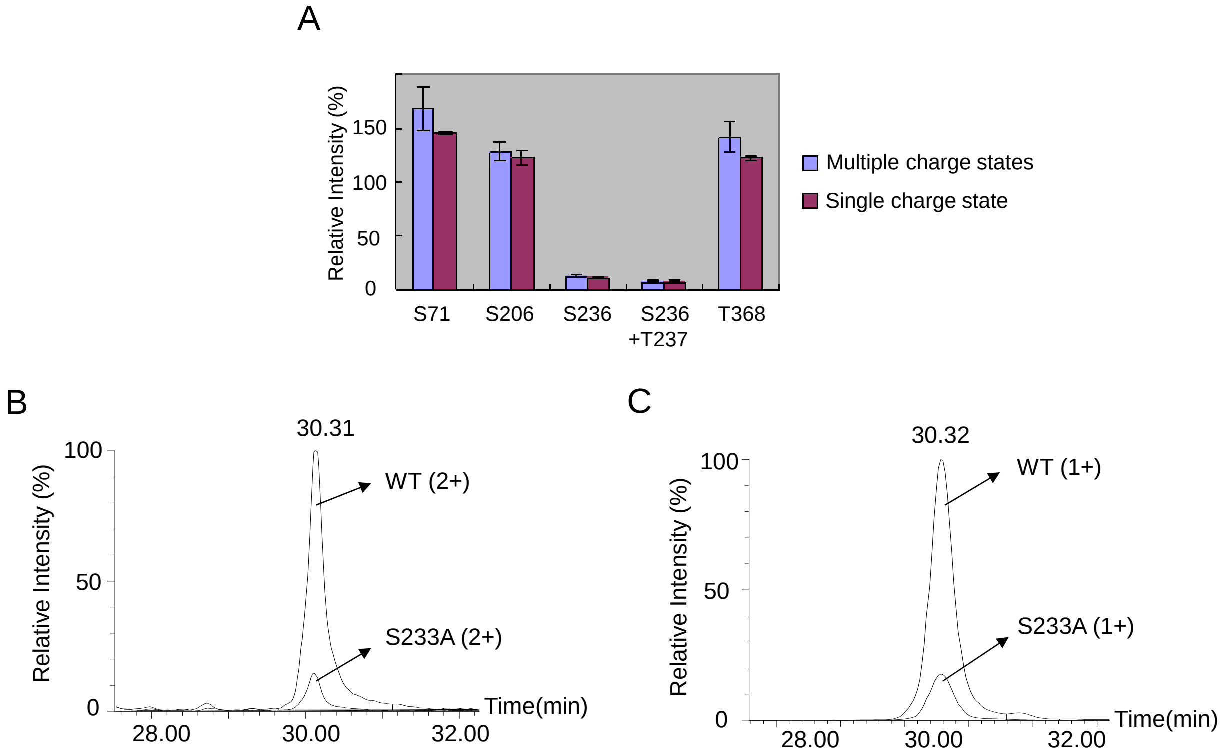
Supporting Information

Figure S1. Comparison of phosphopeptide quantitation using one-charge-state *vs* multi-charge-state method



A, MS-based relative quantitation of phosphopeptides from BIK1 S233A. The phosphorylation level of a peptide from the mutant was relative to that from the wide-type (defined as 100%). Quantitation was based on peptide MS signals from either multiple charge states or a single charge state of the highest abundance. The phosphosite(s) of each peptide is indicated below individual columns. Error bars are STD from three independent experiments. B, extracted ion chromatograms (XICs) of the doubly charged peptide phosphorylated on S236 + T237 derived from WT and the mutant. C, XICs of the singly charged peptide with the same phosphosites as B derived from WT and the mutant. The relative ratio of this phosphopeptide was independent of the charge state used for quantitation.

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Figure S2. MS/MS spectra of identified BIK1 Ser/Thr-phosphorylated peptides

Figure S2A. peptide sequence: NFRPD**pS**VIGEGGFGCVFK phosphosite: S71

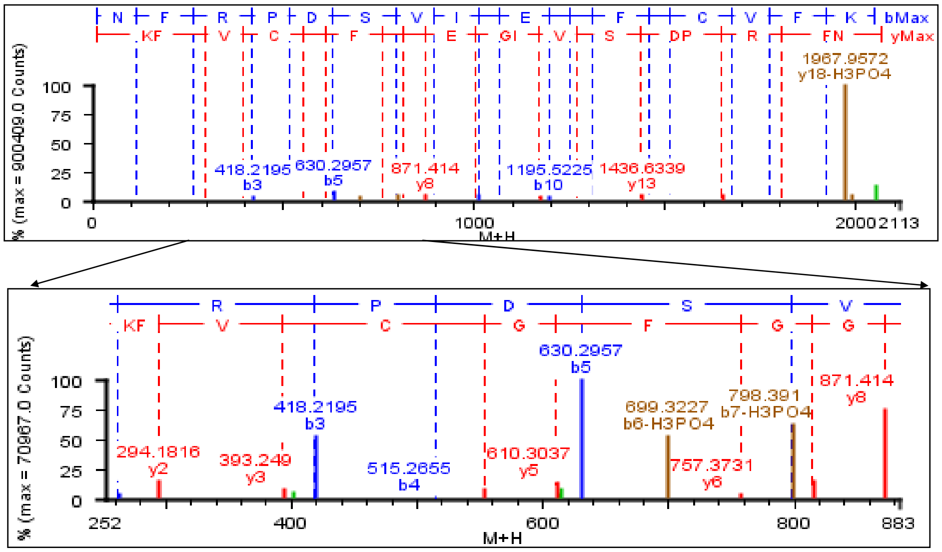

Figure S2B. peptide sequence: ALQQLQDNLGKP**pS**QTNPVKD**pT**K phosphosite: S360 and T368

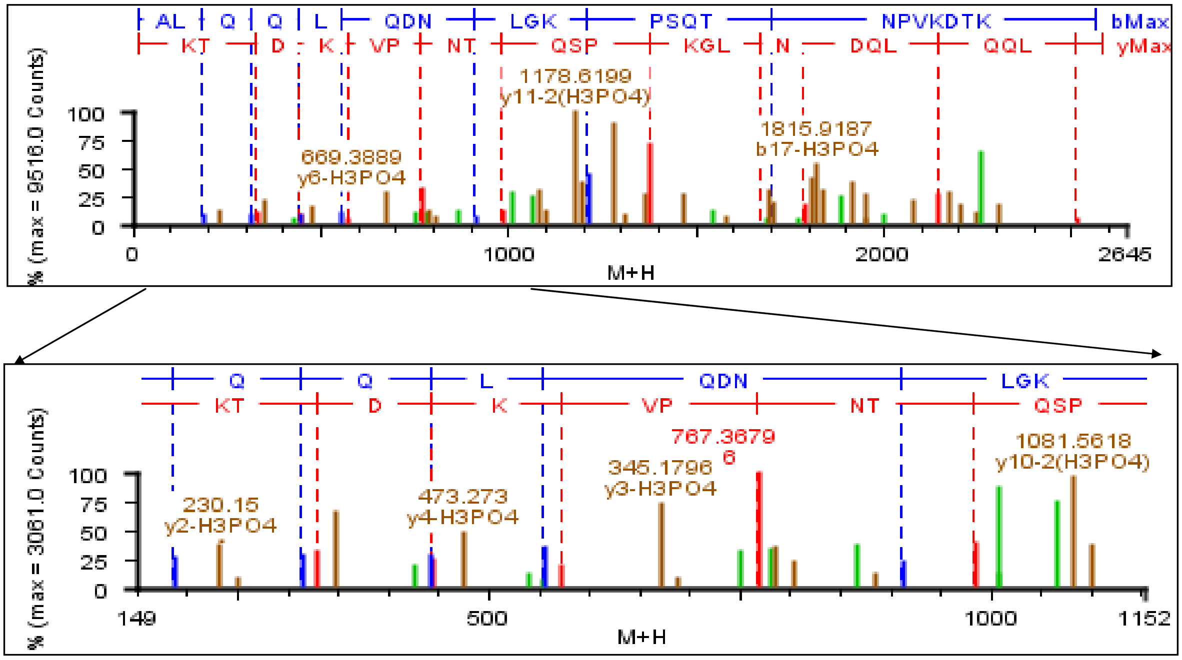

Figure S2C. peptide sequence: ALQQLQDNLGKPSQ**pT**NPVKD**pT**K phosphosite: T362 and T368

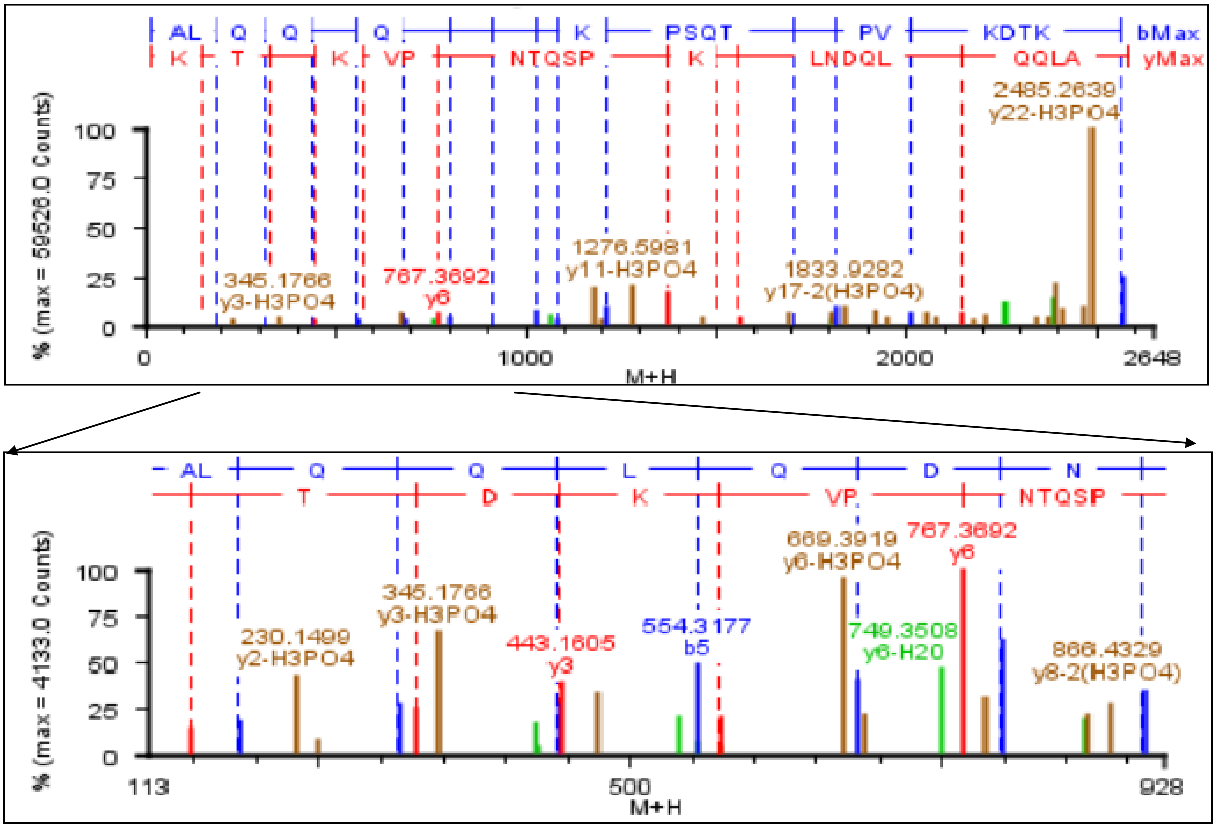

Figure S2D. peptide sequence: DGPMGDLSYV**pS**TR phosphosite: S236

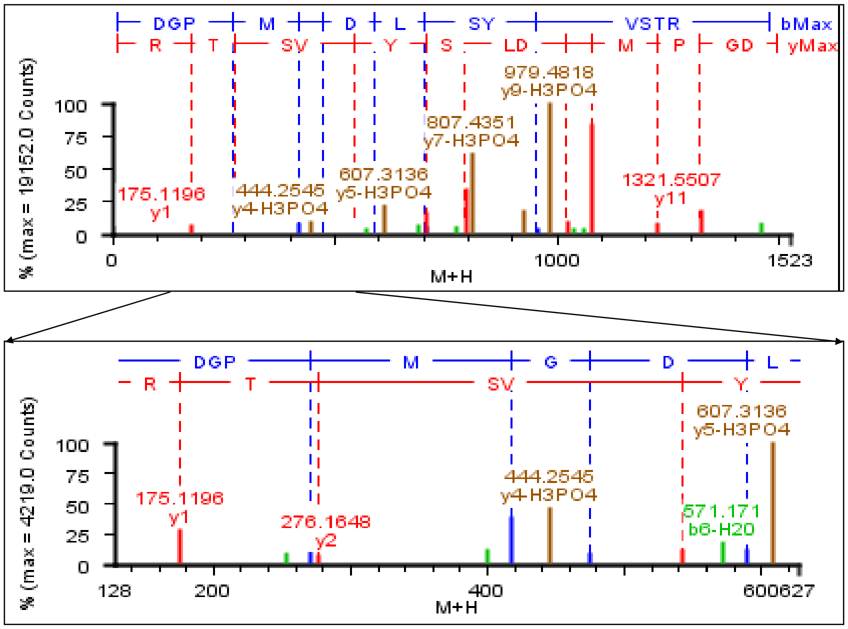

Figure S2E. peptide sequence: DGPMGDL**pS**YV**pS**TR phosphosite: S233+S236

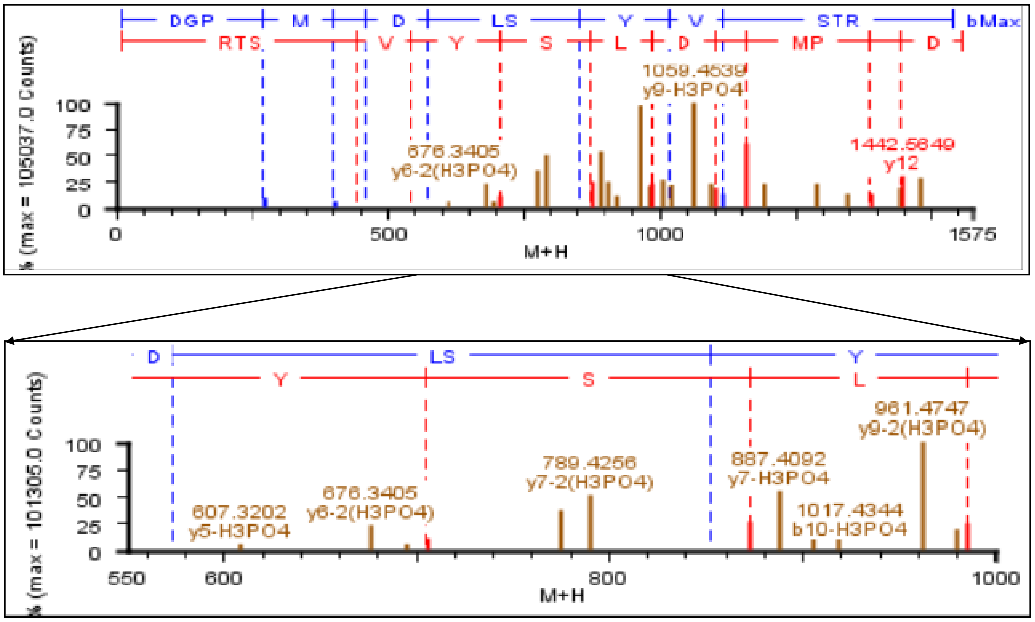

Figure S2F. peptide sequence: DGPMGDLSYV**pSpT**R phosphosite: S236+T237

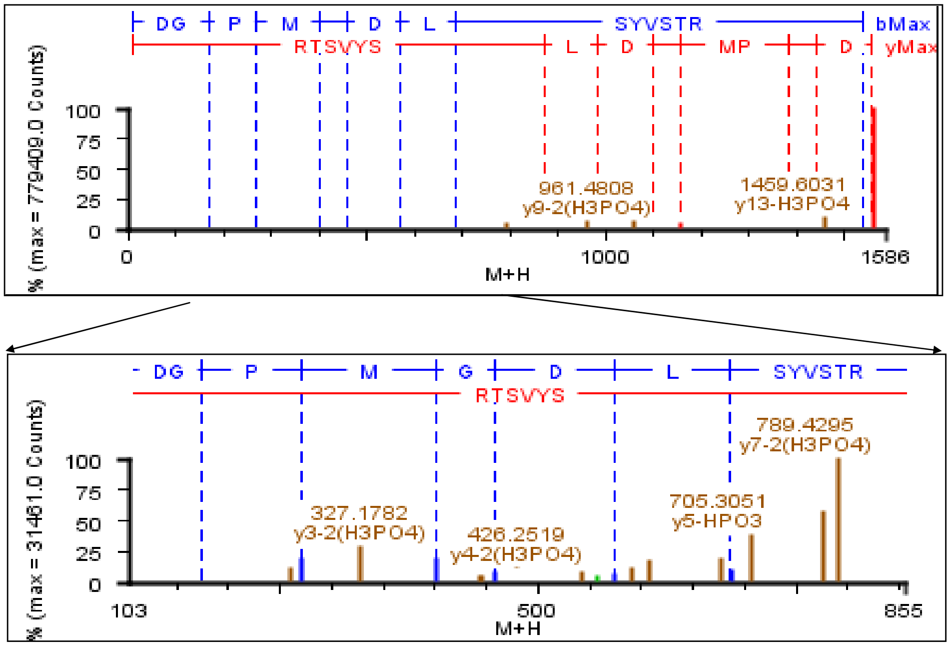

Figure S2G. peptide sequence: A**pS**NILLDADYNAK phosphosite: S206

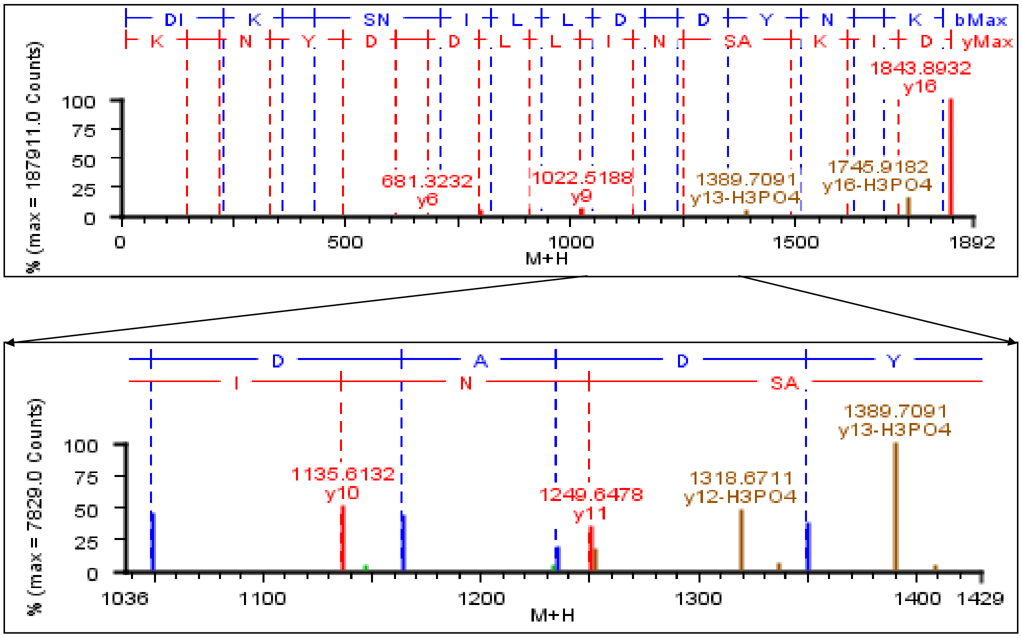

Figure S2H. peptide sequence: **pS**F**pT**FNELK   phosphosite: S54+T56

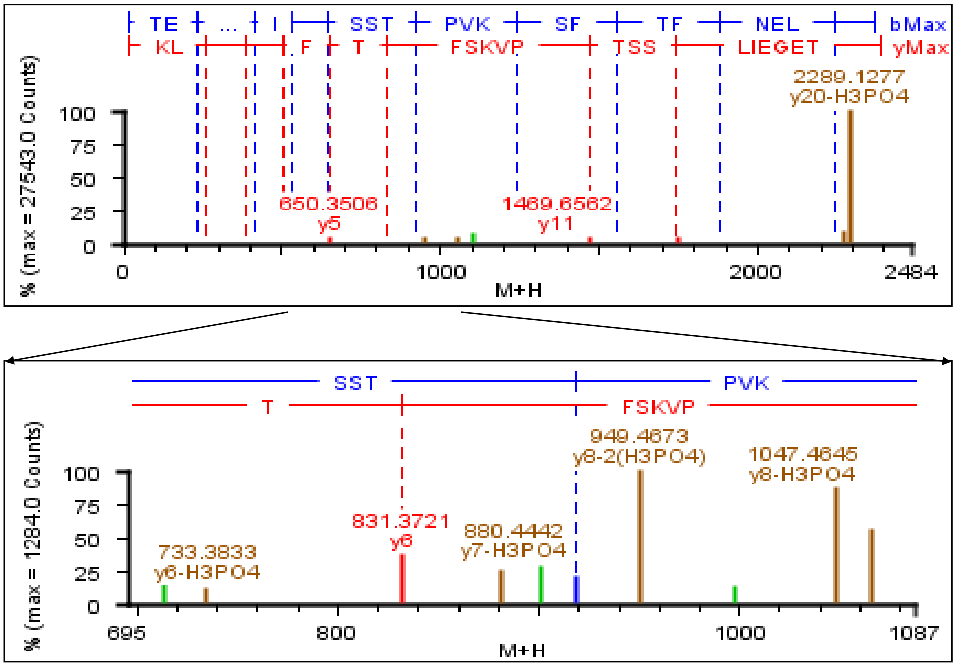

Figure S2I. peptide sequence: VMGTYGYAAPEYMS**pS**GHLNAR phosphosite :S253

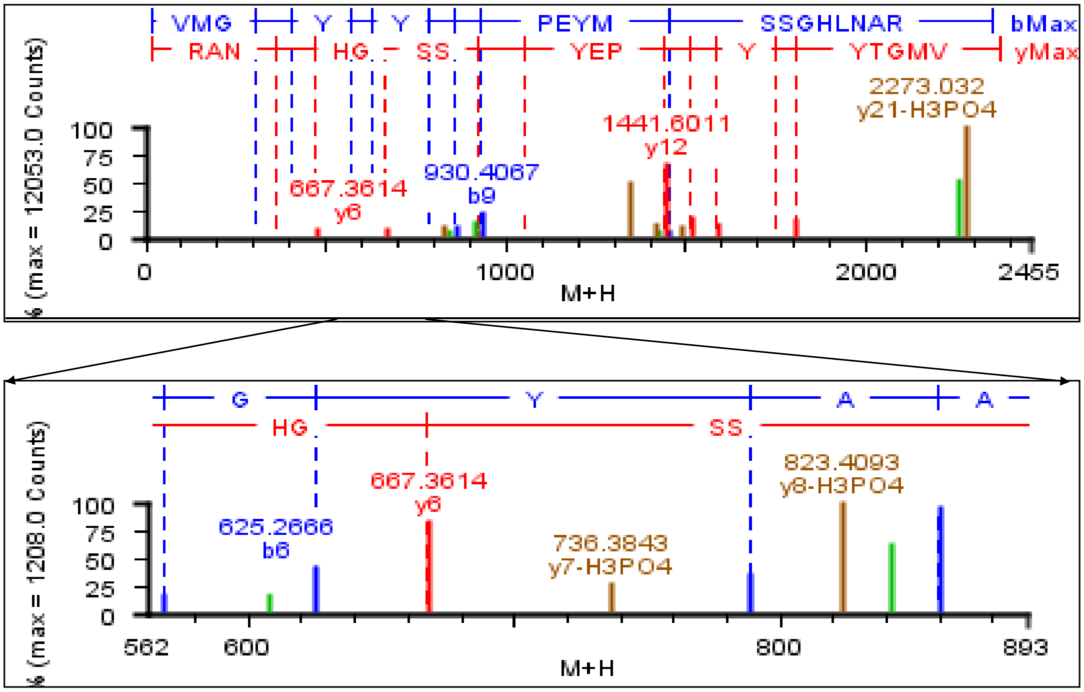

Figure S2J. peptide sequence: VMGTYGYAAPEYM**pSpS**GHLNAR phosphosite :S252+S253

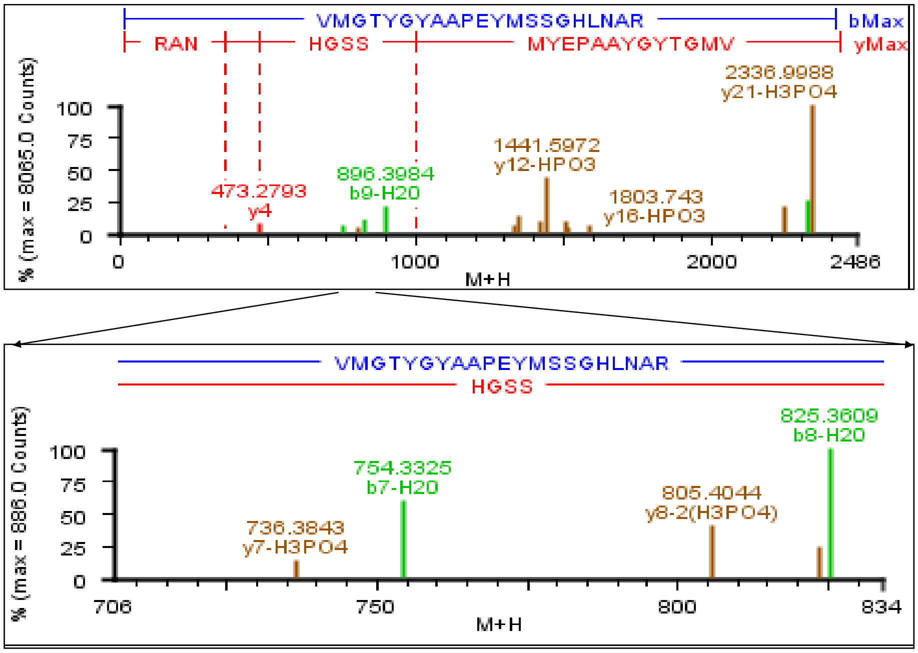

Figure S2K. peptide sequence: VMG**pT**YGYAAPEYMSSGHLNAR phosphosite: T242

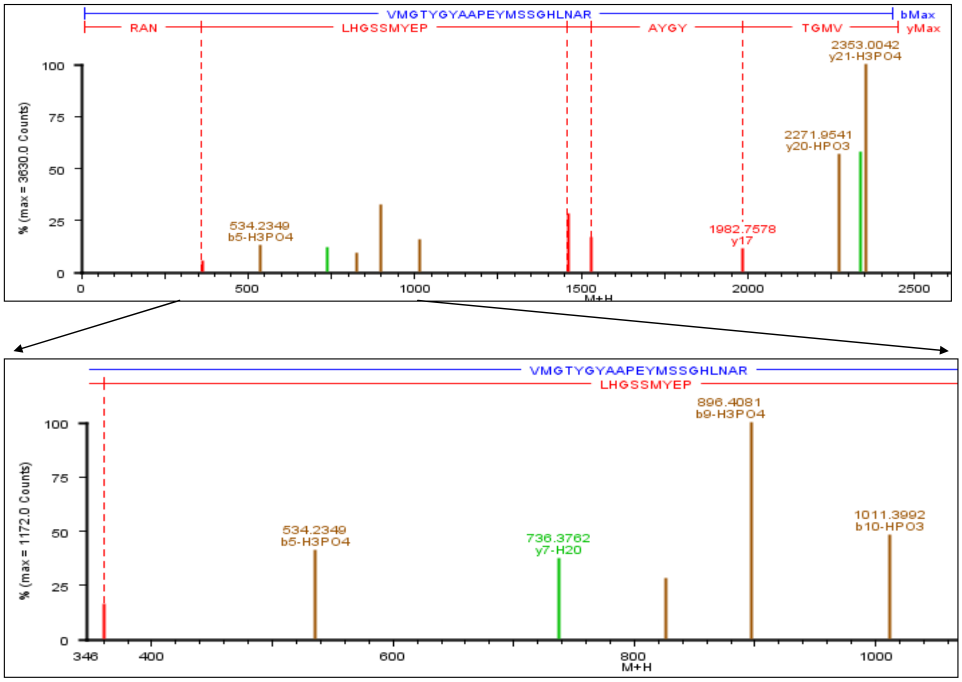

Figure S2L. peptide sequence:EWLTEINYLGQL**pS**HPNLVK phosphosite:S129

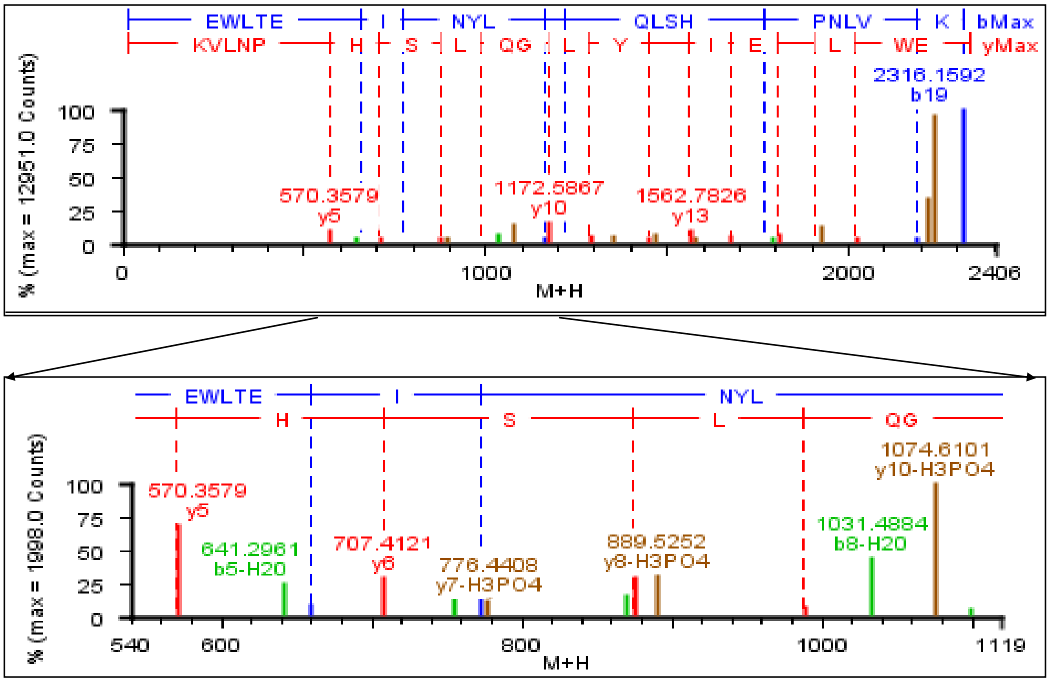

Figure S2M. peptide sequence:LD**pT**QYLPEEAVR phosphosite:T314

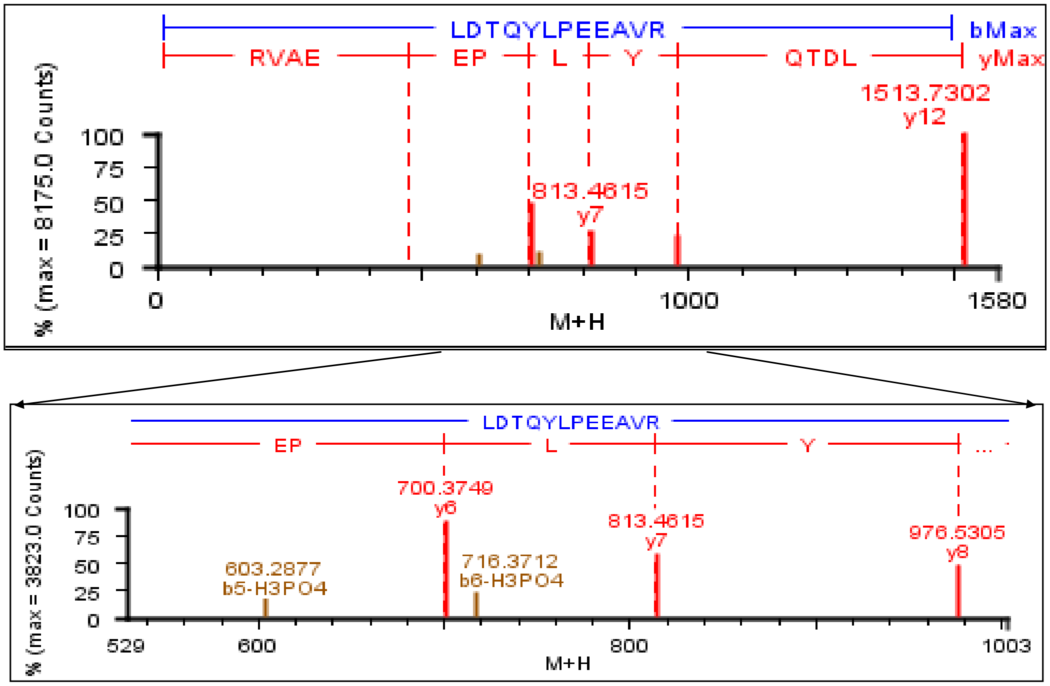

Figure S2N. peptide sequence:TEGEIL**pS**STPVK phosphosite: S48

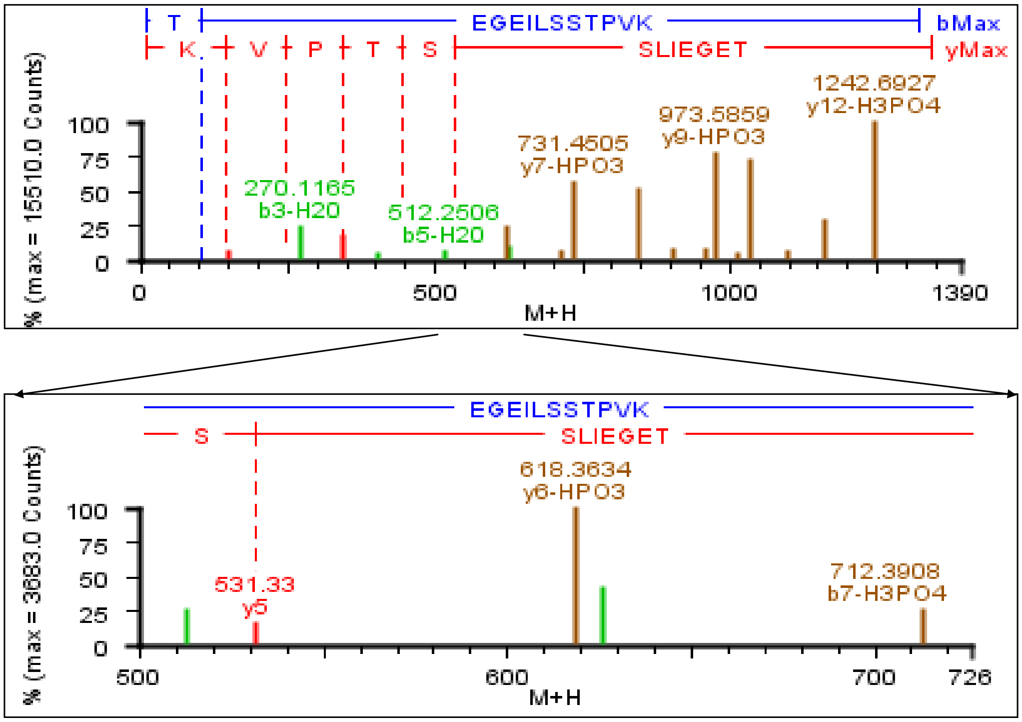

Figure S2O. peptide sequence:TEGEILS**pS**TPVK phosphosite:S49

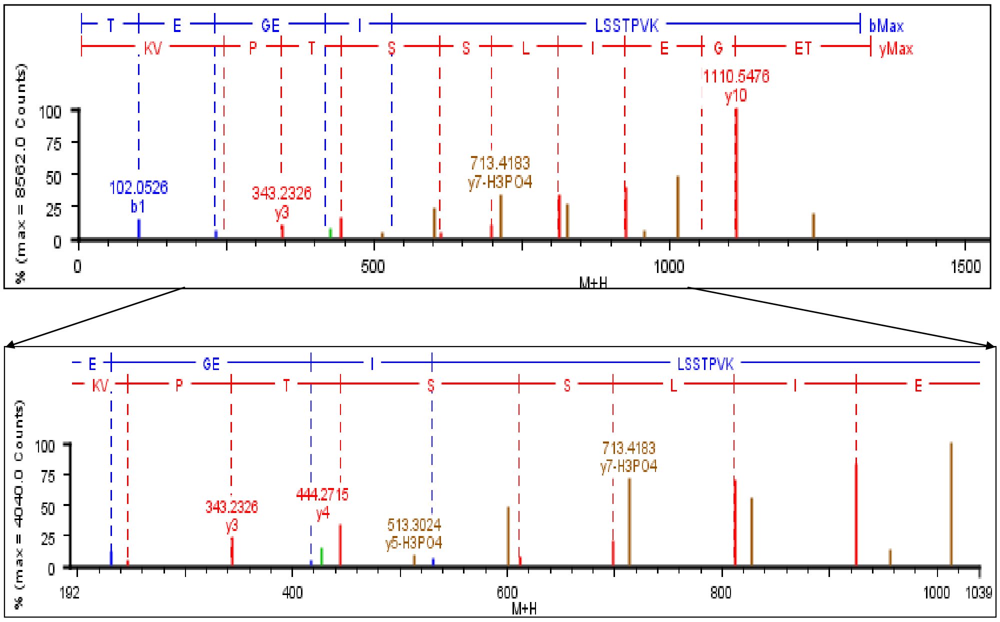

Figure S3. MS/MS spectra of phosphopeptides identified from FLS2-CD treated by BIK1

Figure S3A. peptide sequence: ILGFAWESGK**pT**K phosphosite: T941

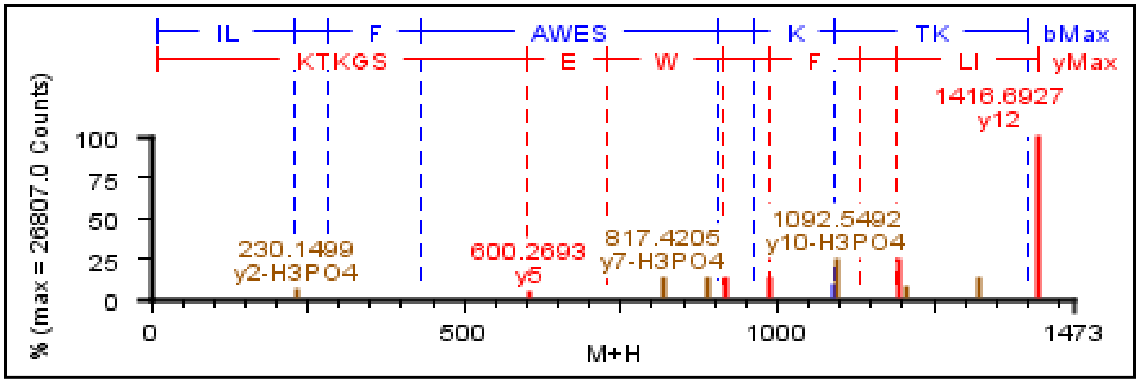

Figure S3B. peptide sequence: ILGFAWE**pS**GKpTK phosphosite:S938

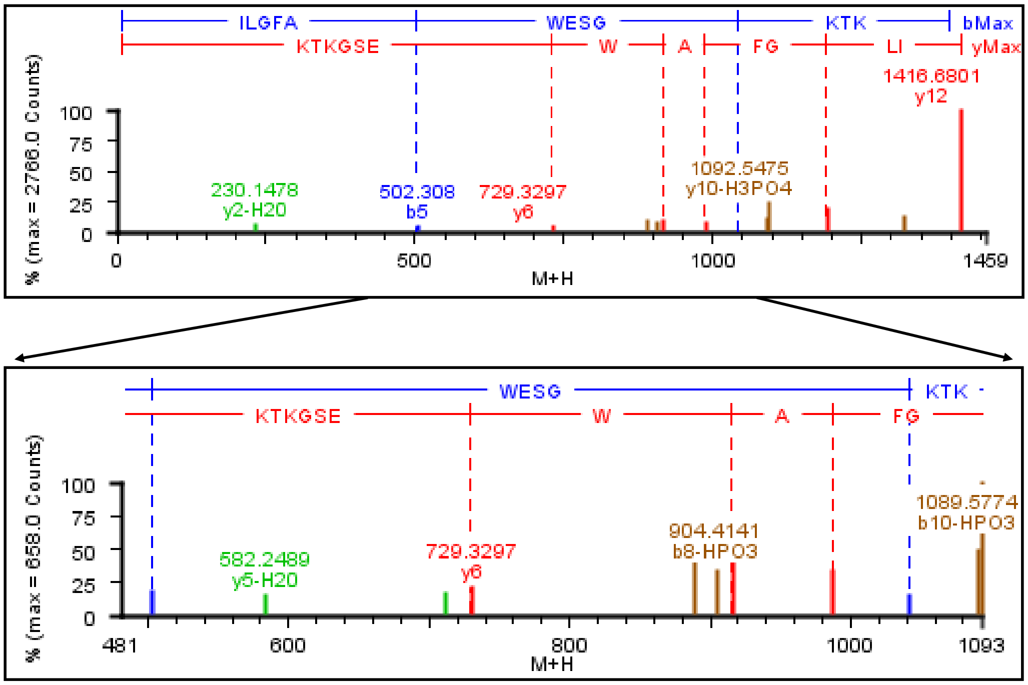

Figure S3C. peptide sequence: QRPTSLNDED**pS**QDMTLR   phosphosite: S1084

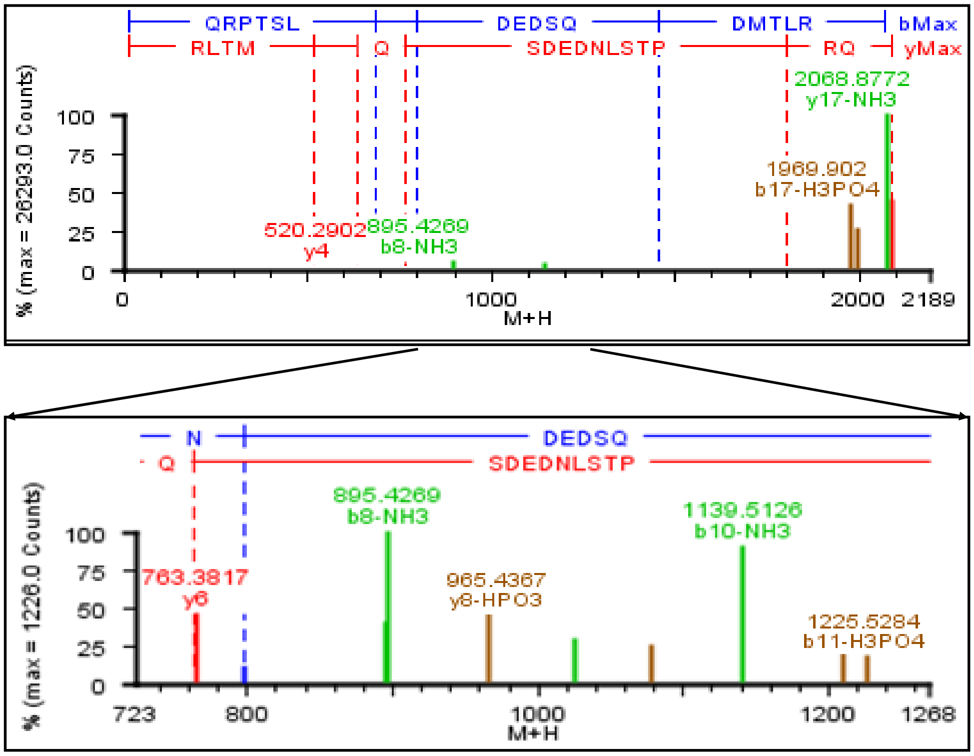

Figure S3D. peptide sequence : EF**pS**AESDKWFYTEAK phosphosite: S906

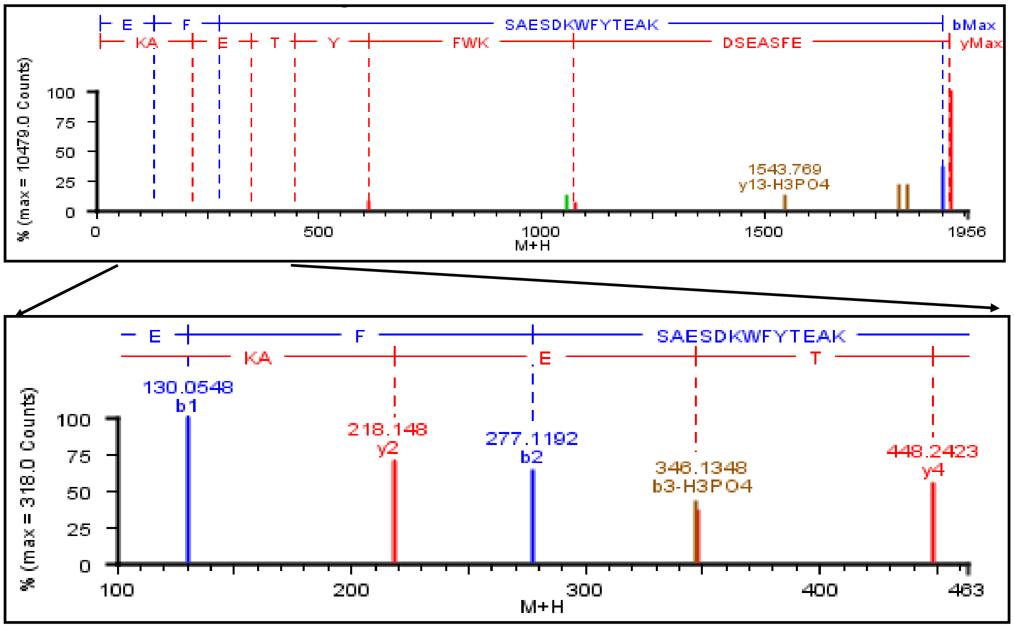

Figure S3E. peptide sequence : VLDMELGD**pS**IVSLK phosphosite: S1115

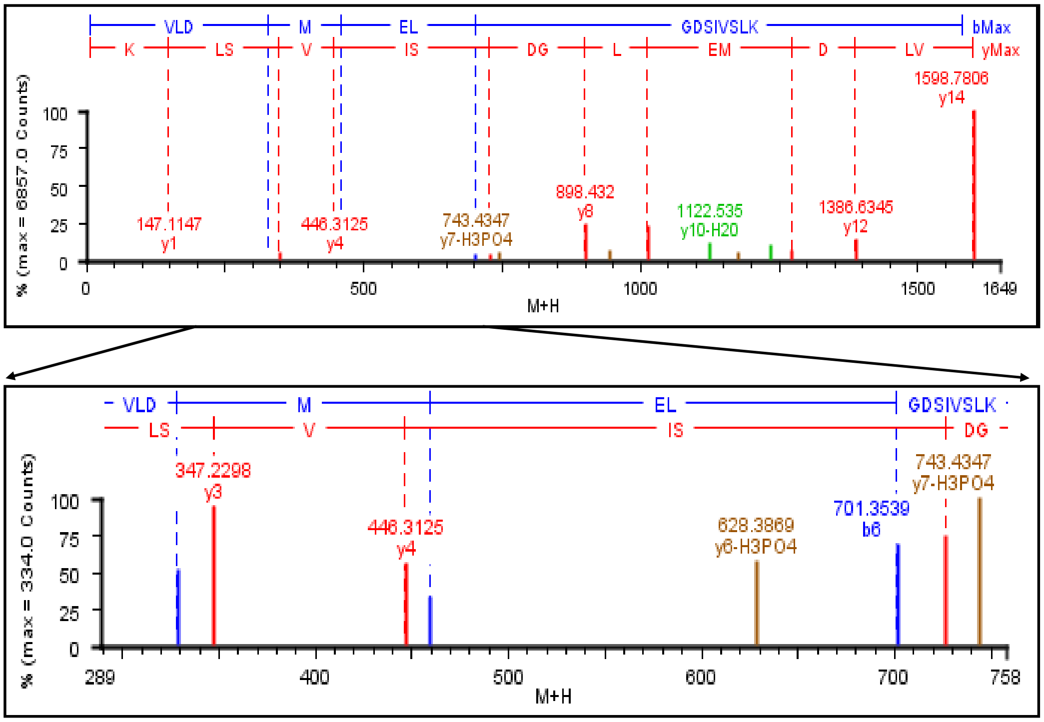

Figure S4. MS/MS spectra of Tyr-phosphorylated peptides identified from BIK1

Figure S4A. peptide sequence: GA**pY**FKPLPWFLR phosphosite: Y168

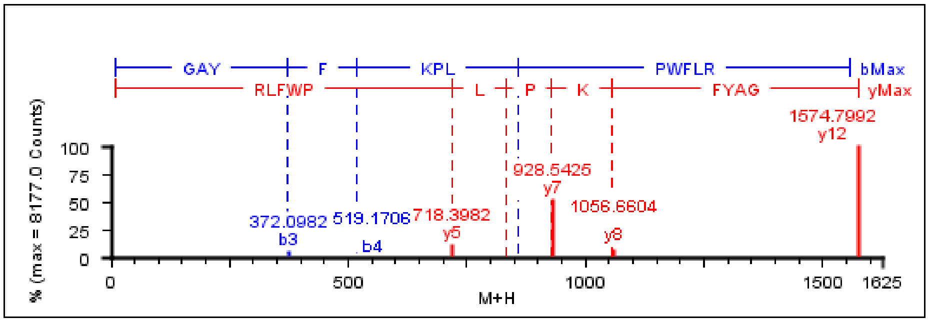

Figure S4B. peptide sequence: ASNILLDAD**pY**NAK phosphosite: pY214

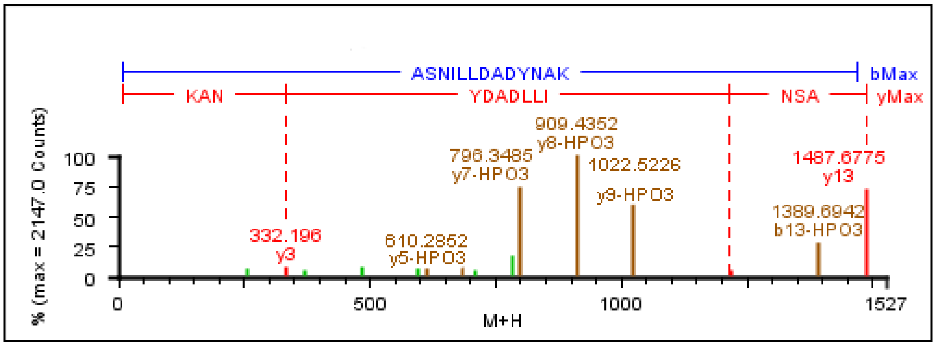

Figure S4C. peptide sequence: VMGTYGYAAPE**pY**MSSGHLNAR phosphosite: pY250