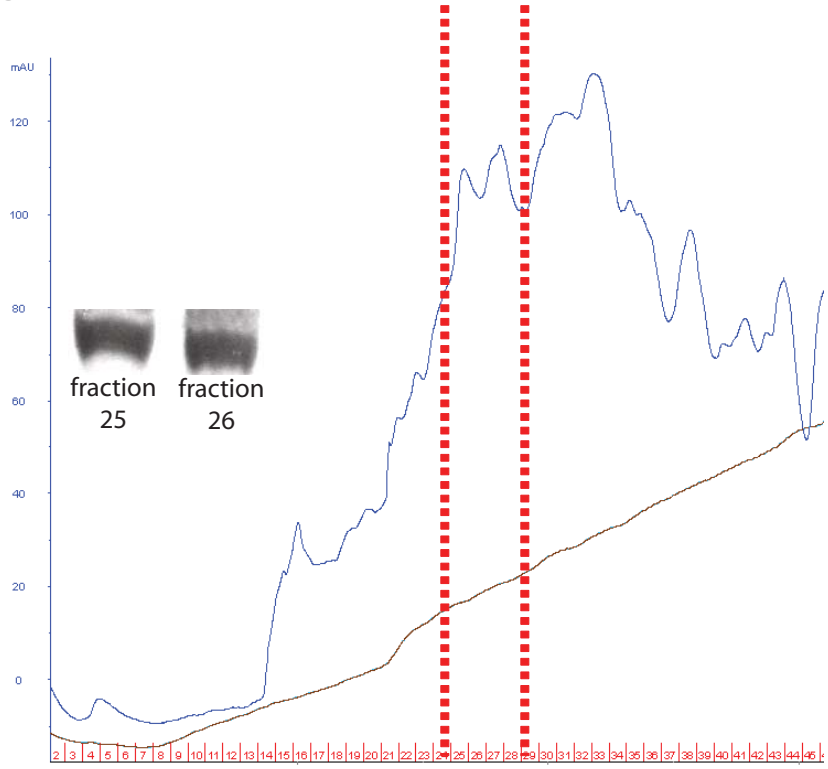
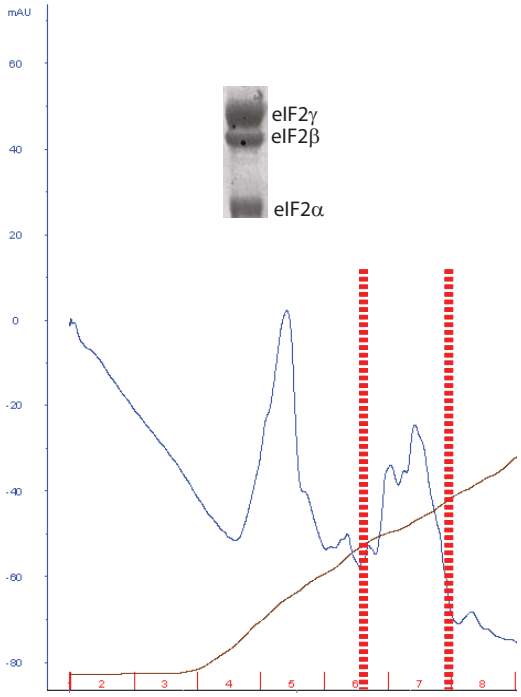
Supplemental Figure 1

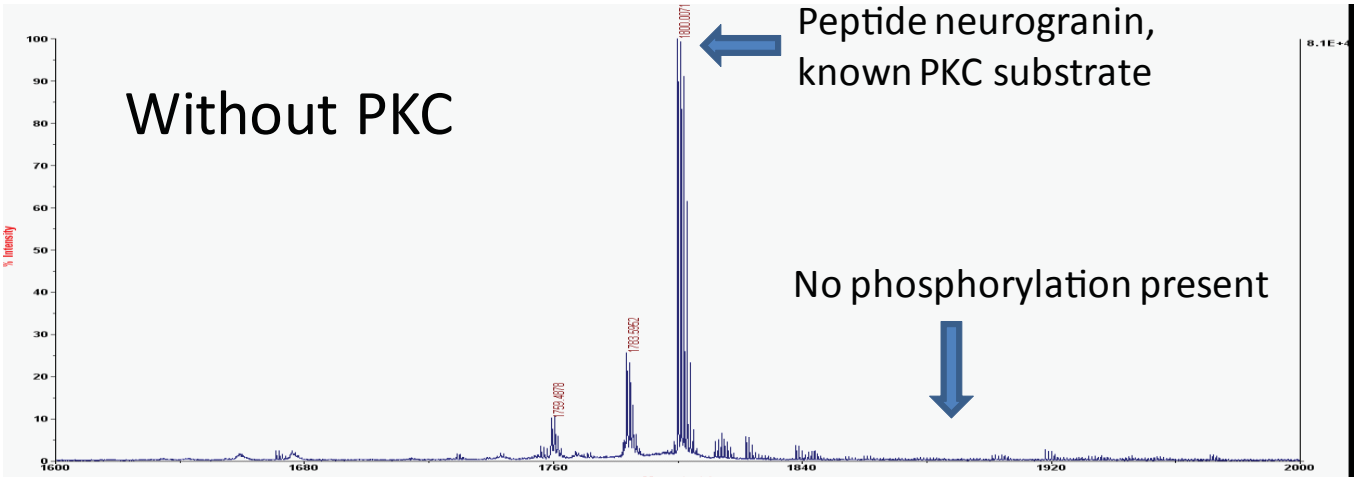
A

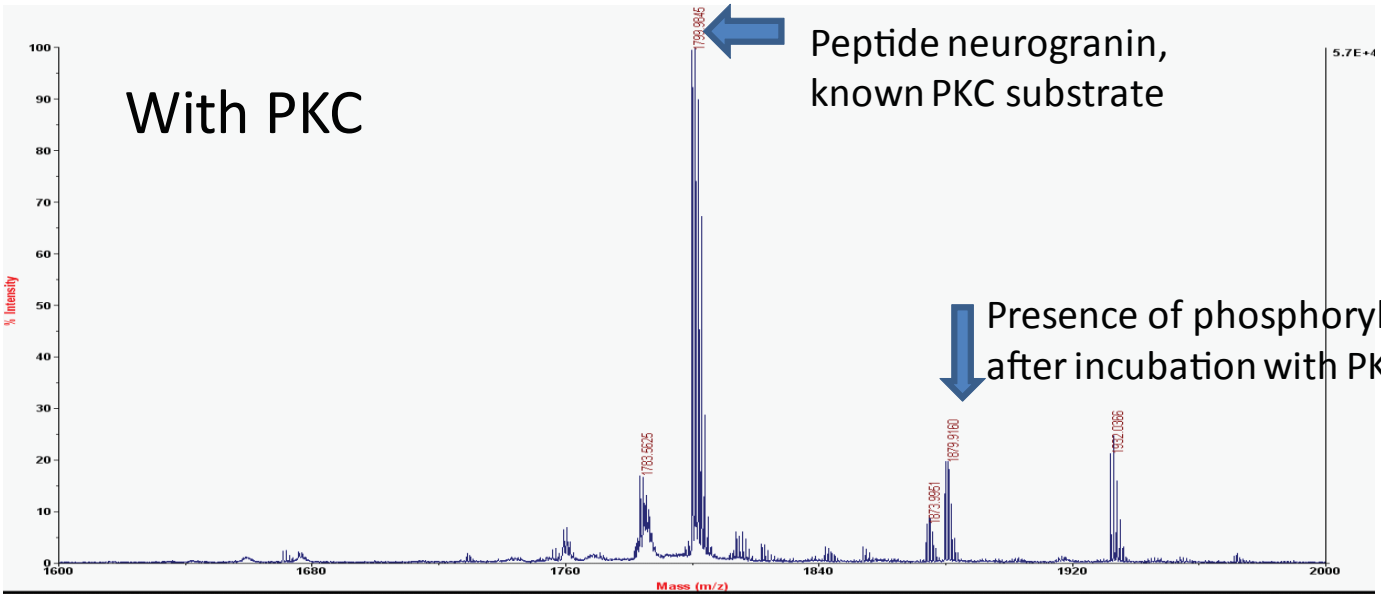


B



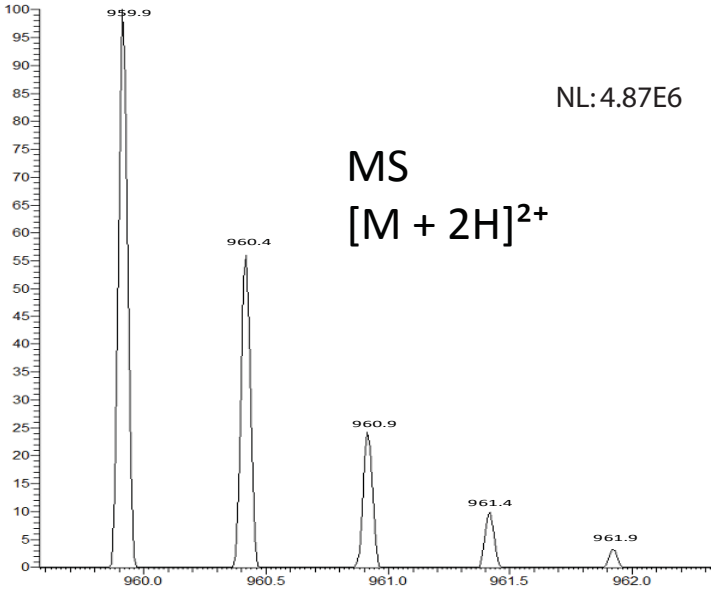
Supplemental Figure 2





Supplemental Figure 3

A

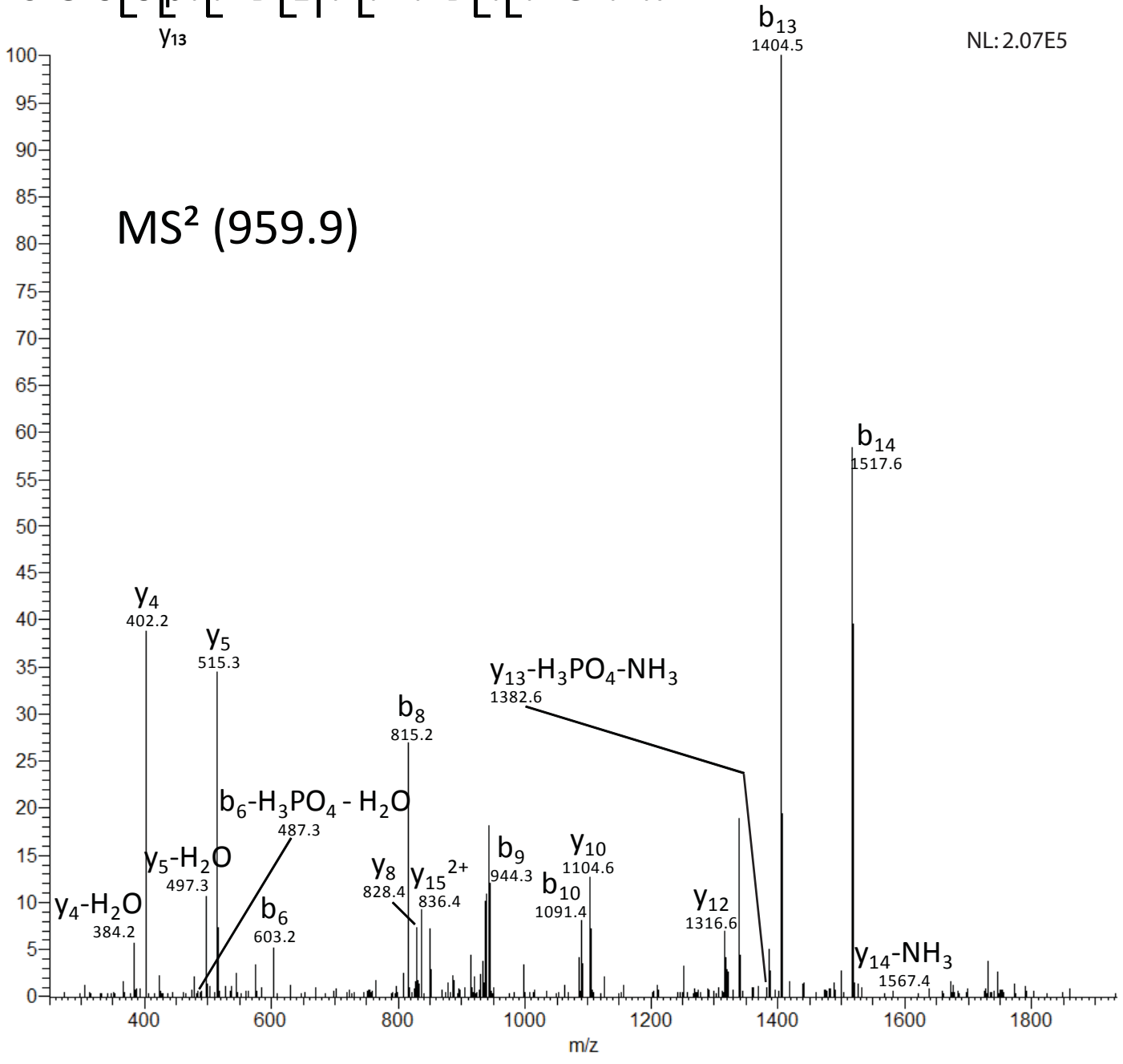


104 S-C-G-S-S-pT-P-D-E-F-P-T-D-I-P-G-T-K 121

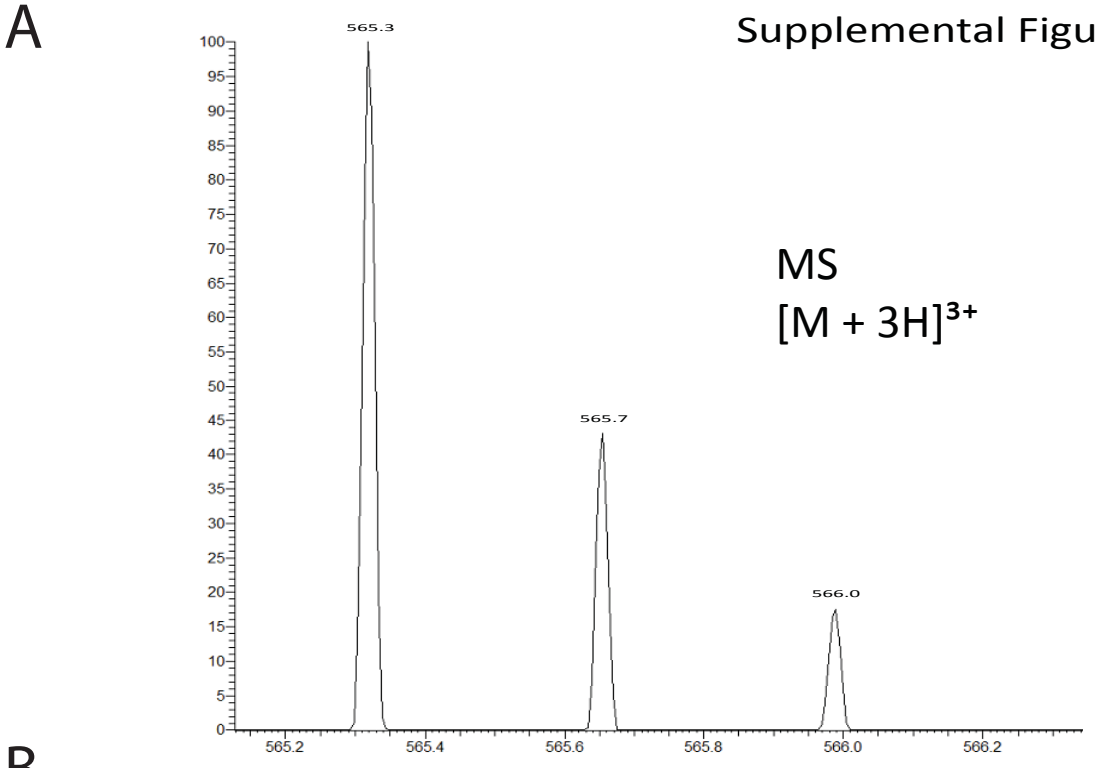
b_6

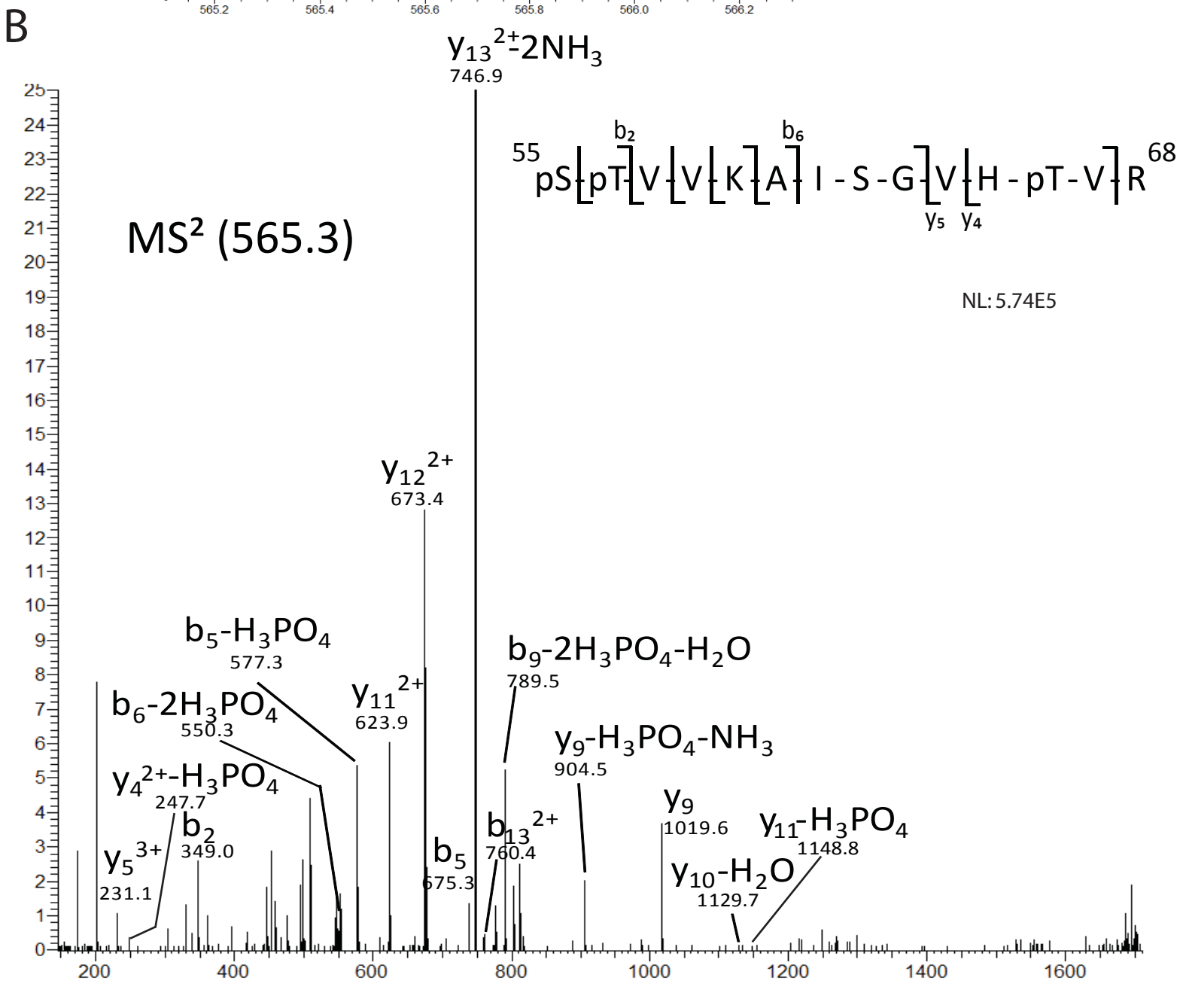
y_{13}

B



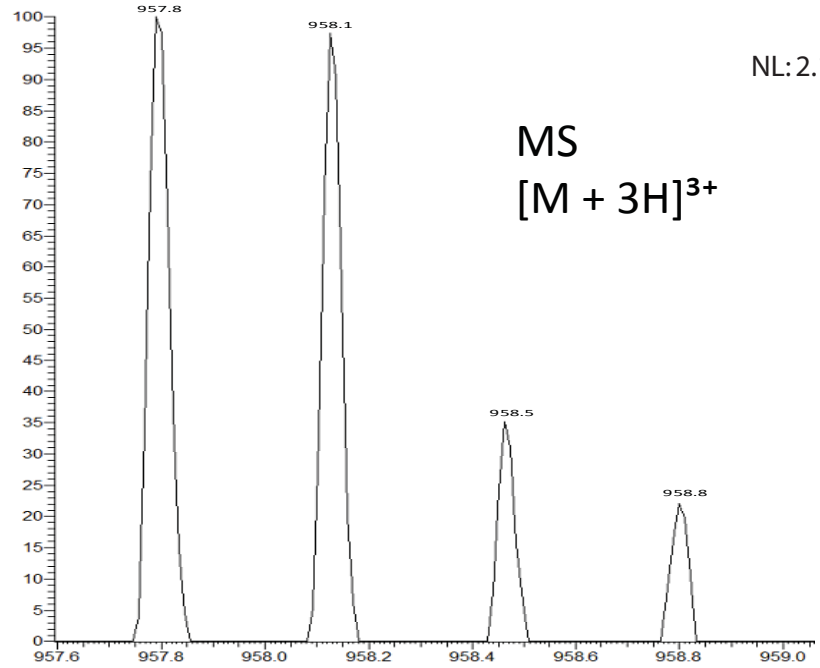
Supplemental Figure 4





Supplemental Figure 5

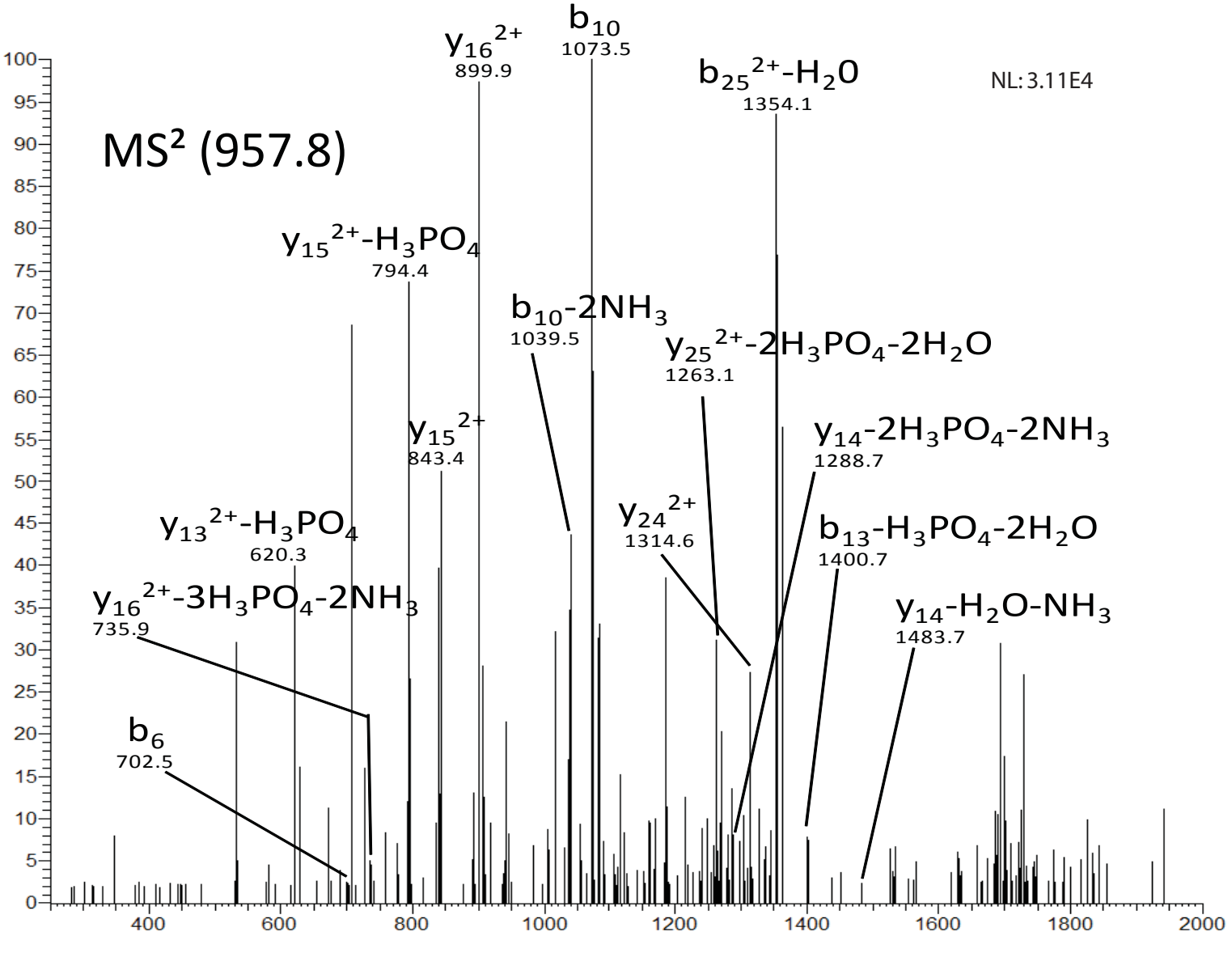
A



401 N-E-V-L-M(ox)-V-N-I-G-S-L-pS-pT-G-G-R-V-pS-A-V-K-A-D-L-G-K 426

y_{15}

B



Supplemental Table 1

Sequence	protein derived from	phosphorylated residue(s)	start-end	[M + H] (Da)	z	Xcorr	Delta Cn
IVLTNPVCTEVGEK	eukaryotic initiation factor 2γ	T435	427-440	1581.76	2	3.14	0.236
SCGSSTPDEFPTDIPGTK	eukaryotic initiation factor 2γ	T109	104-121	1918.78	2	3.209	0.522
STVVKAISGVHTVR	eukaryotic initiation factor 2γ	S55, T56, T66	55-68	1693.75	3	3.757	0.266
NEVLMVNIGSLSTGGRVSAVKADLGK	eukaryotic initiation factor 2γ	S412, T413, S418	401-426	2871.31	3	3.823	0.225
STVVKAISGVHTVR	eukaryotic initiation factor 2γ	T66	55-68	1693.75	3	3.007	0.192
TCVADESAENCDK	human serum albumin	S82	76-88	1971.83	2	3.124	0.453

Supplemental Figure 1. Shown are UV traces of fractions eluting off a strong anion exchange column (Mono Q 10 10 column). UV reading in mAu is plotted against fraction number. (A) Fractions corresponding to eIF2 are encircled in red. Inset shows western blot of fractions 25 & 26 using an antibody against eIF2©. Fractions 25-28 are collected for subsequent monoS 5 5 separation. (B) Fractions corresponding to eIF2 are encircled in red and further validated using imperial staining of the pooled fraction (shown in inset).

Supplemental Figure 2. MALDI-ToF of a peptide from the protein neurogranin, a known substrate of PKC. Without PKC, there is no presence of a phosphorylated peptide (upper panel). When incubated with PKC, a phosphorylated peptide appears with an increase of mass of 80 Da compared to the nonphosphorylated peptide.

Supplemental Figure 3. Identification of the phosphorylation site thr-109 from eIF2© derived from HeLa cell lysate. (A) Precursor mass scan of the [M+2H]2+ ion is shown. (B) MS/MS spectra of m/z ion 959.9 illustrating phosphorylation of threonine 109 on human eIF2©. Diagnostic ions are labeled that indicate the phosphosite. An asterisk is used to demarcate location of TMT label.

Supplemental Figure 4. Identification of the phosphorylation sites ser-55, thr-56, and thr-66 from eIF2© derived from HeLa cell lysate. (A) Precursor mass scan of the [M+3H]3+ ion is shown. (B) MS/MS spectra of m/z ion 565.3 illustrating phosphorylation of ser-55, thr-56, and thr-66 on human eIF2©. Diagnostic ions are labeled that indicate the phosphosites. An asterisk is used to demarcate location of TMT label.

Supplemental Figure 5. Identification of the phosphorylation sites ser-412, thr-413, and ser-418 from eIF2© derived from HeLa cell lysate. (A) Precursor mass scan of the [M+3H]3+ ion is shown. (B) MS/MS spectra of m/z ion 957.8 illustrating phosphorylation of ser-412, thr-413, and ser-418 on human eIF2©. Diagnostic ions are labeled that indicate the phosphosites. An asterisk is used to demarcate location of TMT label.

Supplemental Table 1. Shown are the phosphorylation sites identified via tandem mass spectrometry with the SEQUEST scores. The corresponding mass of the peptide along with the charge at which the peptide was observed is also shown in the table.