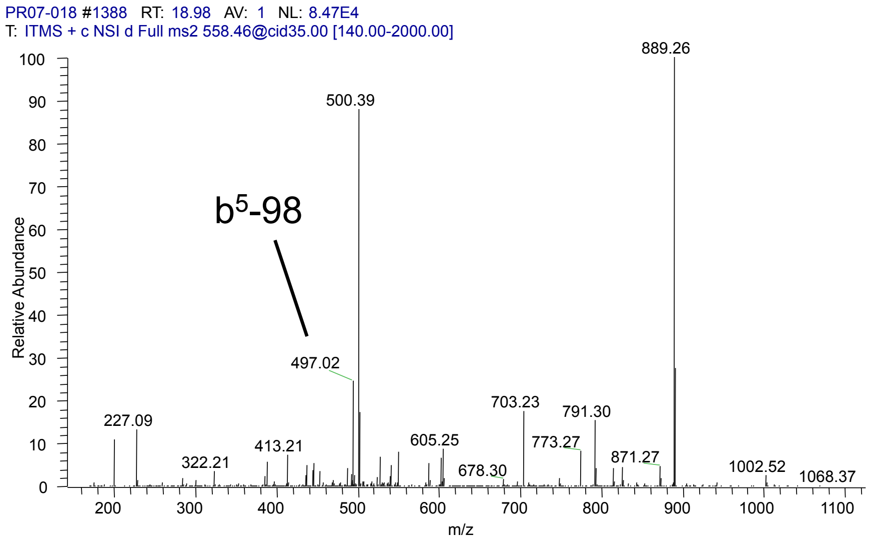
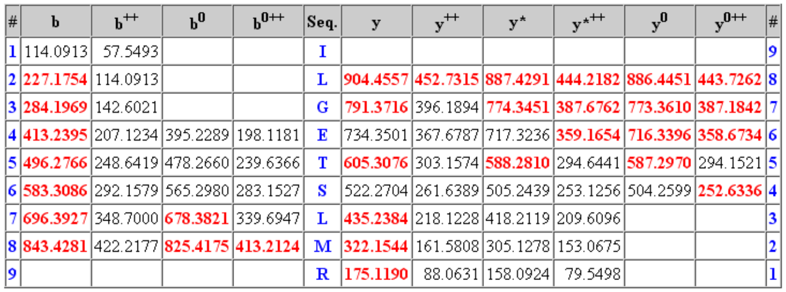
Supplimental Figure 1.

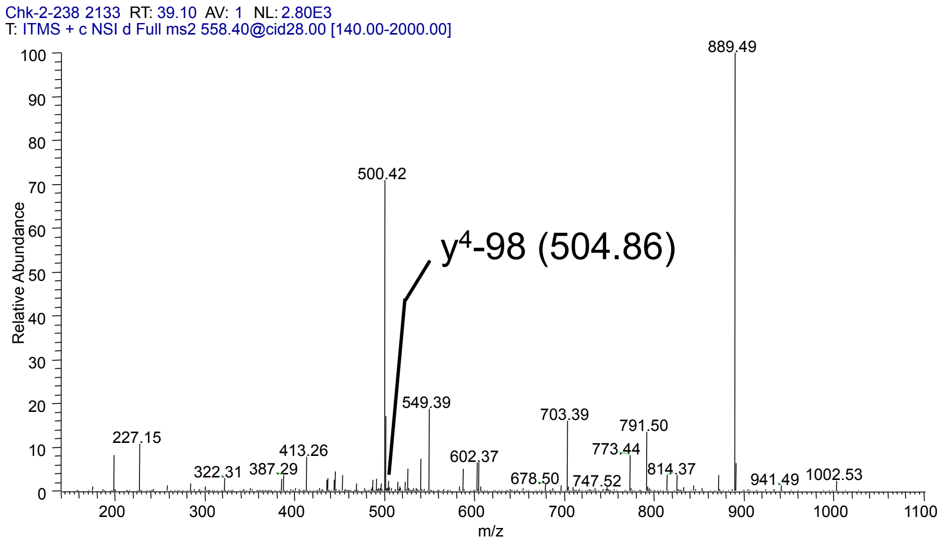
A. ILGEpTSL*MR

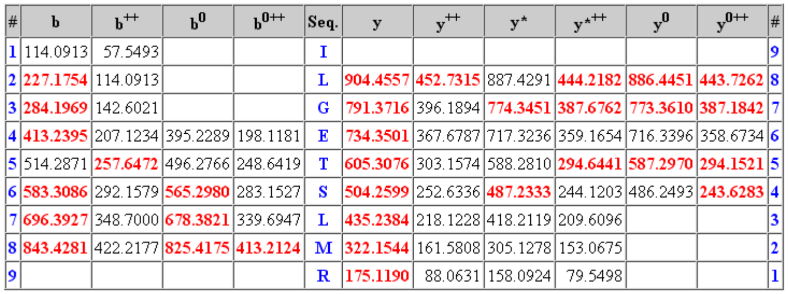




#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y^{*}	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493			I							9
2	**227.1754**	114.0913			L	**904.4557**	**452.7315**	**887.4291**	**444.2182**	**886.4451**	**443.7262**	8
3	**284.1969**	142.6021			G	**791.3716**	396.1894	**774.3451**	**387.6762**	**773.3610**	**387.1842**	7
4	**413.2395**	207.1234	395.2289	198.1181	E	734.3501	367.6787	717.3236	**359.1654**	**716.3396**	**358.6734**	6
5	**496.2766**	248.6419	478.2660	239.6366	T	**605.3076**	303.1574	**588.2810**	294.6441	**587.2970**	294.1521	5
6	**583.3086**	292.1579	565.2980	283.1527	S	522.2704	261.6389	505.2439	253.1256	504.2599	**252.6336**	4
7	**696.3927**	348.7000	**678.3821**	339.6947	L	**435.2384**	218.1228	418.2119	209.6096			3
8	**843.4281**	422.2177	**825.4175**	**413.2124**	M	**322.1544**	161.5808	305.1278	153.0675			2
9					R	**175.1190**	88.0631	158.0924	79.5498			1

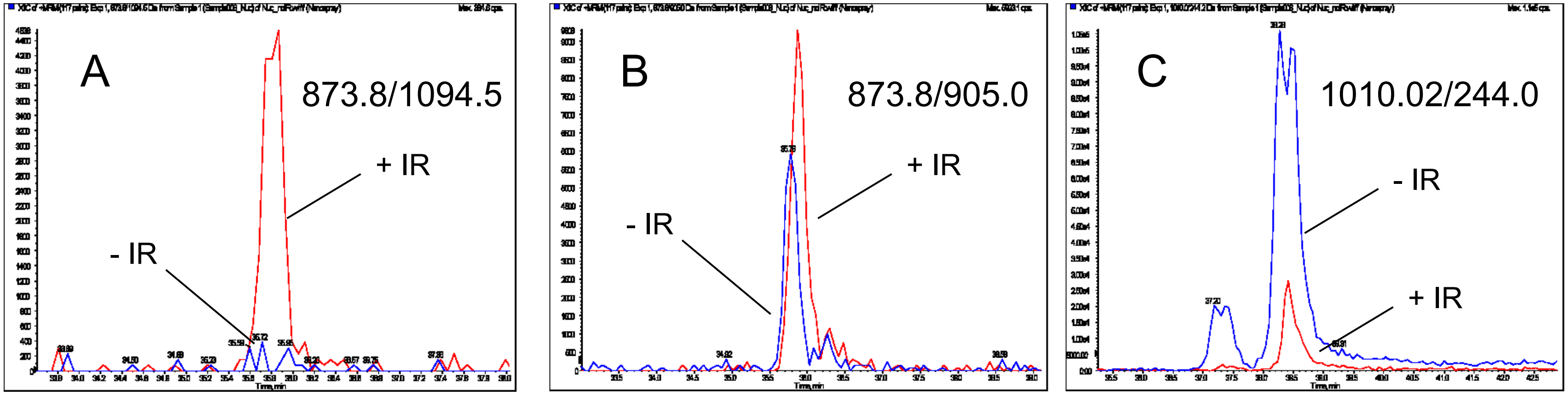
B. ILGETpSL*MR





#	b	b^{++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y^{*}	y^{*++}	y^{0}	y^{0++}	#
1	114.0913	57.5493			I							9
2	**227.1754**	114.0913			L	**904.4557**	**452.7315**	887.4291	**444.2182**	**886.4451**	**443.7262**	8
3	**284.1969**	142.6021			G	**791.3716**	396.1894	**774.3451**	**387.6762**	**773.3610**	**387.1842**	7
4	**413.2395**	207.1234	395.2289	198.1181	E	**734.3501**	367.6787	717.3236	359.1654	716.3396	358.6734	6
5	514.2871	**257.6472**	496.2766	248.6419	T	**605.3076**	303.1574	588.2810	**294.6441**	**587.2970**	**294.1521**	5
6	**583.3086**	292.1579	**565.2980**	283.1527	S	**504.2599**	252.6336	**487.2333**	244.1203	486.2493	**243.6283**	4
7	**696.3927**	348.7000	**678.3821**	339.6947	L	**435.2384**	218.1228	418.2119	209.6096			3
8	**843.4281**	422.2177	**825.4175**	**413.2124**	M	**322.1544**	161.5808	305.1278	153.0675			2
9					R	**175.1190**	88.0631	158.0924	79.5498			1

Supplemental Figure 2



Supplemental Table 1

Peptide	Q1a	Q1b	Q3a	Q3b
ITDFGHSK	452.7		575.3	234.1
ITDFGHpSK	492.7		655.3	314.1
ILGETSLMR	510.3		607.3	306.2
ILGETSL*MR	518.3		623.3	322.1
ILGEpTSLMR	550.3		506.27	254.13
ILGETpSLMR	550.3		586.24	514.28
ILGEpTSL*MR	558.3		496.27	506.27
ILGETpSL*MR	558.3		586.24	504.25
ILGEpTpSLMR	590.2		767.3	306.2
ILGEpTpSL*MR	598.2		783.2	322.1
TLCGTPTYLAPEVLVSVGTAGYNR	847.1	1270.8	1461.8	289.2
pTLCGTPTYLAPEVLVSVGTAGYNR	873.76	1310.13	512.15	1055.05
TLCGpTPTYLAPEVLVSVGTAGYNR	873.76	1310.13	613.21	1094.54
TLCGTPpTYLAPEVLVSVGTAGYNR	873.76	1310.13	1044.01	630.29
TLCGTPTpYLAPEVLVSVGTAGYNR	873.76	1310.13	316.14	1044.51
pTLCGpTPTYLAPEVLVSVGTAGYNR	900.41	1350.11	455.13	904.98
pTLCGTPpTYLAPEVLVSVGTAGYNR	900.41	1350.11	891.27	1054.35
pTLCGTPTpYLAPEVLVSVGTAGYNR	900.41	1350.11	811.3	1095.04
TLCGpTPpTYLAPEVLVSVGTAGYNR	900.41	1350.11	432.19	710.26
TLCGpTPTpYLAPEVLVSVGTAGYNR	900.41	1350.11	1054.33	1134.52
TLCGTPpTpYLAPEVLVSVGTAGYNR	900.41	1350.11	1083.99	630.3
pTLCGpTPpTYLAPEVLVSVGTAGYNR	927.07	1390.09	790.22	1134.3
pTLCGpTPTpYLAPEVLVSVGTAGYNR	927.07	1390.09	693.17	995.49
pTLCGTPpTpYLAPEVLVSVGTAGYNR	927.07	1390.09	891.27	944.96
TLCGpTPpTpYLAPEVLVSVGTAGYNR	927.07	1390.09	710.26	1214.27
pTLCGpTPpTpYLAPEVLVSVGTAGYNR	953.72	1430.08	790.22	731.88
ILGEpTpSLMRTLCGTPTYLAPEVLVSVGTAGYNR	1233.92	1850.38	1470.25	874.34
ILGETpSLMRpTLCGpTPTYLAPEVLVSVGTAGYNR	1260.57	1890.36	681.29	887.34
ILGEpTpSLMRTLCGTPTpYLAPEVLVSVGTAGYNR	1260.57	1890.36	594.25	1684.25
ILGETpSLMRpTLCGpTPTpYLAPEVLVSVGTAGYNR	1287.23	1930.34	944.96	681.29
ILGEpTpSLMRpTLCGpTPpTpYLAPEVLVSVGTAGYNR	1340.54		1804.19	1244.41
EADPALNVETEIEILK (IS-1)	595.3		1468.8	260.2
VFVFFDLTVDDQSVYPK (IS-2)	673.7		836.4	244.2

MRM transition pair list for the Chk-2 activation loop region.
Two Q1 m/z (Q1a, Q1b) were selected that would ionize with multiple charge states. Those ions with only a single Q1 mass were either not observed to have higher charge states or the mass was out of the instrument mass range. Multiple diagnostic Q3 m/z (Q3a, Q3b) were selected for each peptide. Carbamidomethylcysteine was a fixed modification. A total of 110 MRM transition pairs were incorporated into the analysis.

Supplemental Data

Supplemental Figure 1. Example MS/MS Spectra used for Quantitation. MS/MS spectra and annotated ion tables of the Chk-2 peptide K-ILGETSLMR-T illustrating the ability to distinguish adjacent phosphorylation sites by MRM. Panel A represents the fragmentation spectra for phosphorylation at T-378 indicated by the presence of the neutral loss ion at b5. Panel B shows the fragmentation spectra for the S-379 phosphorylation by the presence of the neutral loss ion at y4. Both peptides had oxidated Methionine as an additional modification indicated by (*).

Supplemental Figure 2. Example of Quantitative Change in the T389 Phosphopeptide. A-B) Extracted ion overlay chromatograms (XIC) of two transistion masses unique to the tryptic phosphopeptide R-TLCGTPpTYLAPEVLVSVGTAGYNR-I. C). The XIC of the 2 internal standards from nuclear lysate. Each trace is representative of the total, non-normalized area under the curve for each condition. Shown are the extrapolated peaks for before (-IR) and after (IR+) ionizing radiation.