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Identification of CaMKII Phosphorylation Sites in Connexin43 by High-Resolution Mass Spectrometry

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Supporting Information

Figure S1. Number of casein phosphorylation sites identified after in vitro phosphorylation by CaMKII as a function of the amount of casein substrate used in the reaction. The higher the casein concentration, the more phosphorylated residues were identified.

Figure S2. MALDI spectra of autocamtide. (A) Positive control shows the phosphorylated peptide (KKALHRQEpTVDAL) of *m/z* 1588.6028. (B) Negative control shows the non-phosphorylated peptide (KKALHRQETVDAL) of *m/z* 1508.7915. (C) In vitro phosphorylation of autocamtide by CaMKII resulted in the phosphorylated peptide (KKALHRQEpTVDAL) of *m/z* 1588.8795 (arrow).

Figure S3. CaMKII phosphorylation of Cx43-CT at S325 and S328. (A) Extracted chromatogram of *m/z* 953.0603-953.0699. (B) MS spectrum of *m/z* 659.0708 represents triply charged peptide MGQAGSTISNSHAQPFDFPDDNQNAK (one phosphorylation site). (C) CID MS2 spectrum indicates peptide phosphorylated on S325. (D) ETD MS2 spectrum indicates peptide phosphorylated on S325. (E) ETD MS2 spectrum indicates peptide phosphorylated on S328. See Supplemental Table S3 for complete list of theoretical (ProteinProspector) and observed fragment ions.

Figure S4. CK1 phosphorylation of Cx43-CT at S306. (A) Extracted chromatogram of *m/z* 988.3947-988.4007. (B) MS spectrum of 988.3977 represents doubly charged peptide QASEQNWANYSAEQNR (one phosphorylation site). (C) CID MS2 spectrum indicates peptide phosphorylated on S306.

Figure S5. CaMKII phosphorylation of Cx43-CT at S255 and S257. (A) Extracted chromatogram of *m/z* 617.6179-617.6203. (B) MS spectrum of *m/z* 617.6193 represents triply charged peptide GRSDPYHATTGPLSPSK (one phosphorylation site). (C) CID MS2 spectrum indicates peptide phosphorylated on S255. (D) ETD MS2 spectrum indicates peptide phosphorylated on S255. (E) CID MS2 spectrum indicates peptide phosphorylated on S257. (F) ETD MS2 spectrum indicates peptide phosphorylated on S257. See Supplemental Table S4 for complete list of theoretical (ProteinProspector) and observed fragment ions.

Figure S6. CaMKII phosphorylation of Cx43-CT at S296 and S297. (A) Extracted chromatogram of *m/z* 668.7571-668.7611. (B) MS spectrum of *m/z* 668.7591 represents doubly charged peptide NNSSCRNYNK (one phosphorylation site, one carbamidomethylation site). (C) CID MS2 spectrum indicates peptide phosphorylated on S296. (D) Extracted chromatogram of *m/z* 659.9552-659.9692. (E) MS spectrum of *m/z* 659.9572 represents triply charged peptide LVTGDRNNSSCRNYNK (one phosphorylation site, one carbamidomethylation site). (F) CID MS2 spectrum indicates peptide phosphorylated on S297. (G) Extracted chromatogram of *m/z* 487.2102-487.2132. (H) MS spectrum of *m/z* 487.2094 represents triply charged peptide LVTGDRNNSSCR (one phosphorylation site, one carbamidomethylation site). (I) ETD MS2

spectrum indicates peptide phosphorylated on S296. (J) Extracted chromatogram of *m/z* 730.8048-730.8092. (K) MS spectrum of *m/z* 730.8062 represents doubly charged peptide LVTGDRNNSSCR (one phosphorylation site, one deamidation site, one carbamidomethylation site). (L) ETD MS2 spectrum indicates peptide phosphorylated on S297. See Supplemental Table S6 for complete list of theoretical (ProteinProspector) and observed fragment ions.

Figure S7. CaMKII phosphorylation of Cx43-CT at S364 and S365. (A) Extracted chromatogram of *m/z* 742.0376-742.0420. (B) MS spectrum of *m/z* 742.0398 represents triply charged peptide VAAGHELQPLAIVDQRPSSR (one phosphorylation site). (C) CID MS2 spectrum indicates peptide phosphorylated on S364. (D) CID MS2 spectrum indicates peptide phosphorylated on S365. (E) ETD MS2 spectrum indicates peptide phosphorylated on S364. See Supplemental Table S7 for complete list of theoretical (ProteinProspector) and observed fragment ions.

Figure S8. CaMKII phosphorylation of Cx43-CT at S369, S372 and S373. (A) Extracted chromatogram of *m/z* 718.3220-718.3292. (B) MS spectrum of *m/z* 718.3282 represents doubly charged peptide ASSRPRPDDLEI (one phosphorylation site). (C) CID MS2 spectrum indicates peptide phosphorylated on S372. (D) CID MS2 spectrum indicates peptide phosphorylated on S373. (E) Extracted chromatogram of *m/z* 612.9569-612.9631. (F) MS spectrum of *m/z* 612.9589 represents triply charged peptide ASSRASSRPRPDDLEI (one phosphorylation site). (G) ETD MS2 spectrum indicates peptide phosphorylated on S372. (H) Extracted chromatogram of *m/z* 639.6068-639.6132. (I) MS spectrum of *m/z* 639.6113 represents triply charged peptide ASSRASSRPRPDDLEI (two phosphorylation sites). (J) ETD MS2 spectrum indicates peptide

phosphorylated on S369 and S373. See Supplemental Table S8 for complete list of theoretical (ProteinProspector) and observed fragment ions.

Figure S9. Baseline phosphorylation of native ventricular Cx43 at S306. (A) Extracted chromatogram of *m/z* 988.4000-988.4020. (B) MS spectrum of *m/z* 988.4009 represents doubly charged peptide QASEQNWANYSAEQNR (one phosphorylation site). (C) CID MS2 spectrum indicates peptide phosphorylated on S306. See Supplemental Table S9 for complete list of theoretical (ProteinProspector) and observed fragment ions.

Figure S10. Baseline phosphorylation of native ventricular Cx43 at S372 and S373. (A) Extracted chromatogram of *m/z* 639.6127-639.6153. (B) MS spectrum of *m/z* 639.6140 represents triply charged peptide ASSRASSRPRPDDLEI (two phosphorylation sites). (C) ETD MS2 spectrum indicates peptide phosphorylated on S372 and S373. See Supplemental Table S10 for complete list of theoretical (ProteinProspector) and observed fragment ions.

Figure S1

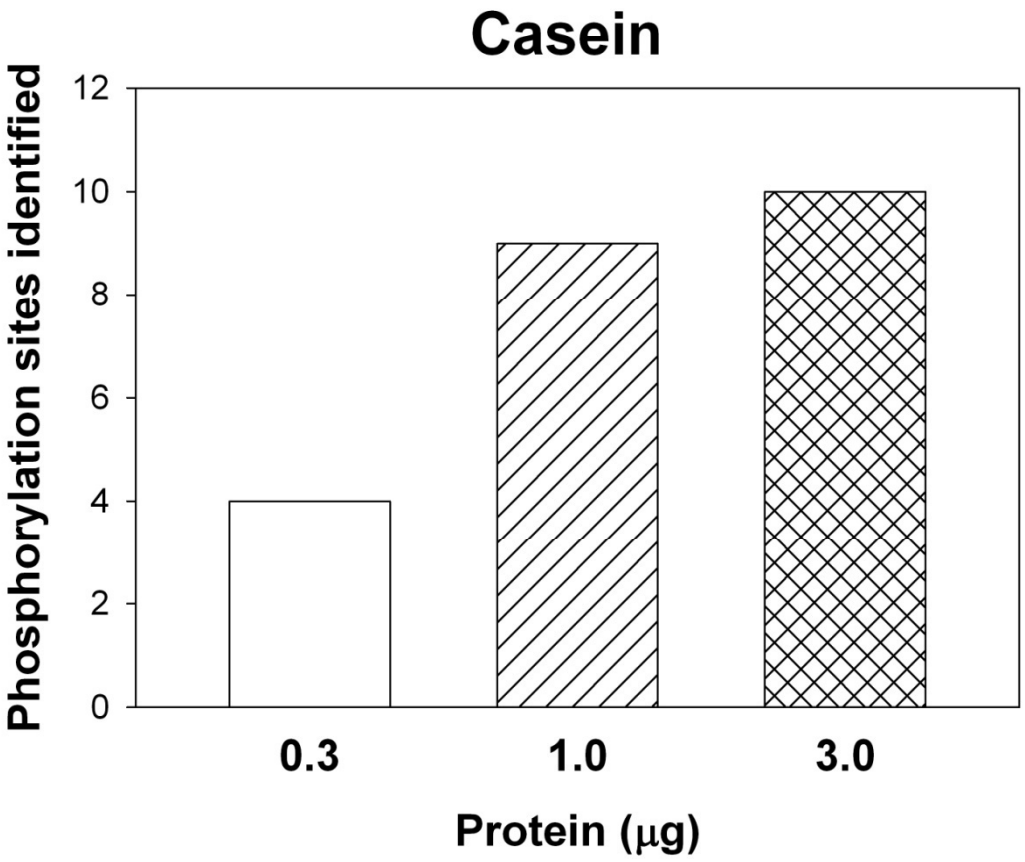
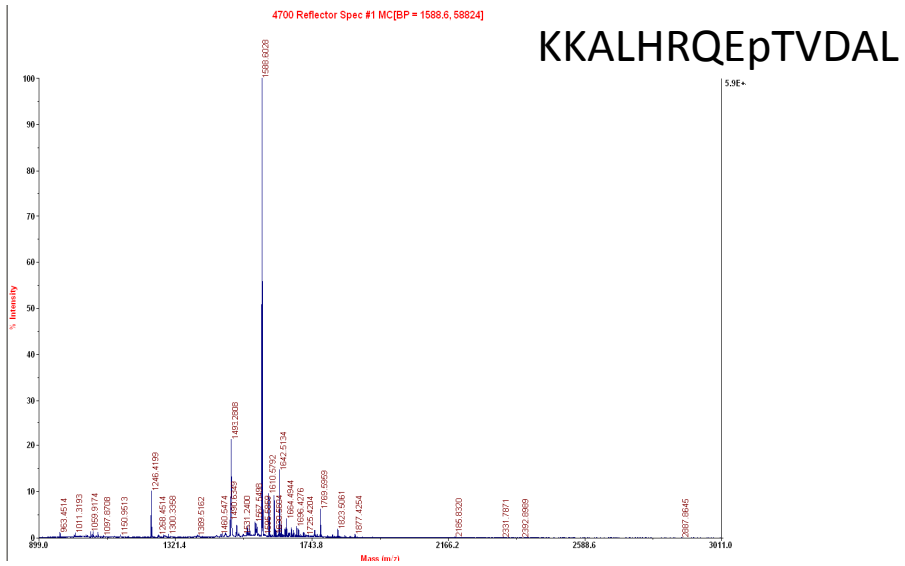

Casein
Phosphorylation sites identified
12
10
8
6
4
2
0
0.3
1.0
3.0
Protein (μg)

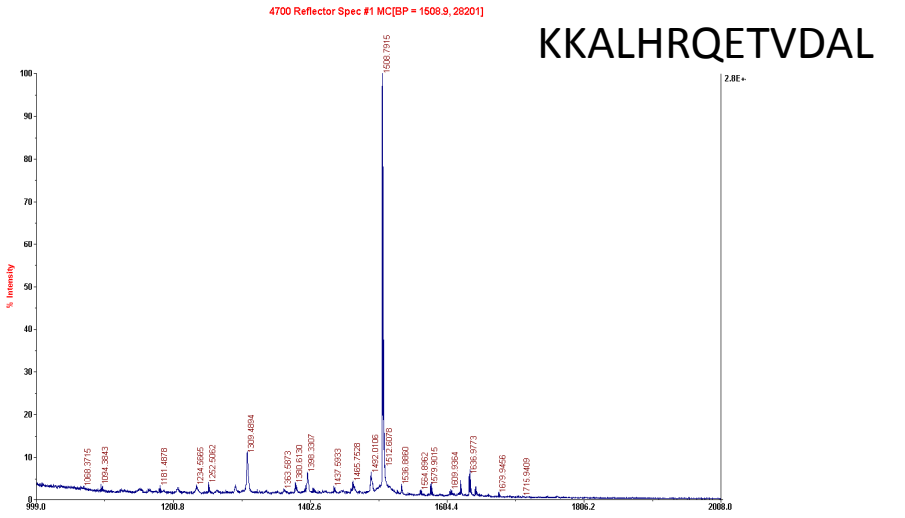
Figure S2

A



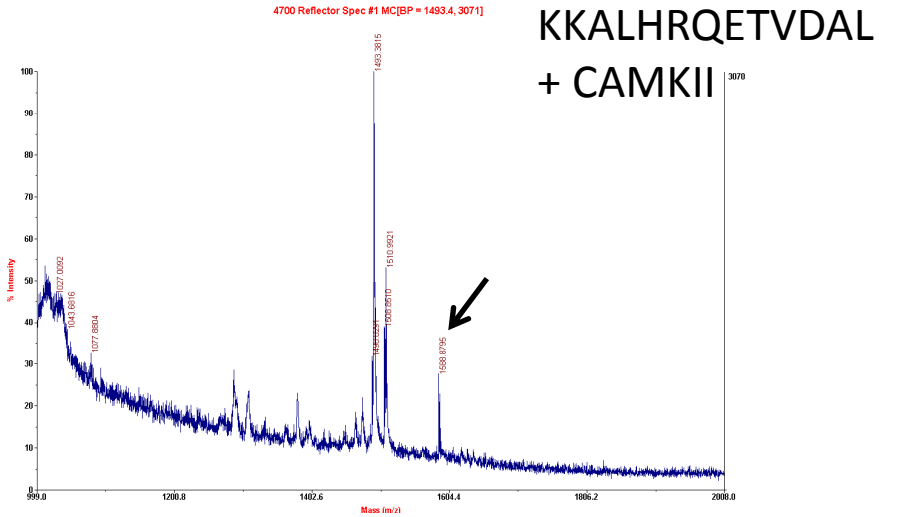
KKALHRQEpTVDAL

B



KKALHRQETVDAL

C



KKALHRQETVDAL
+ CAMKII

Figure S3

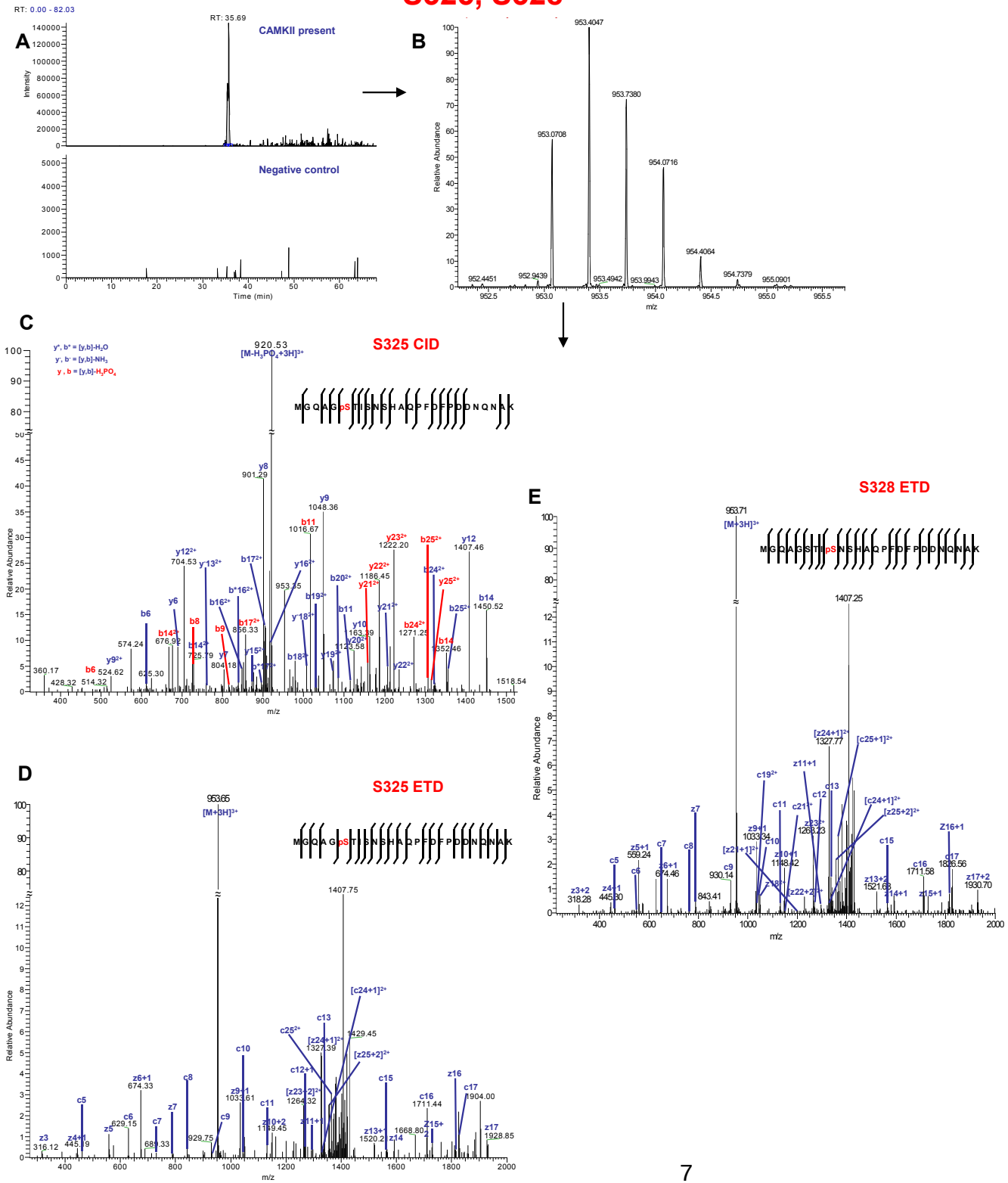

S325, S328
A
RT: 0.00 - 82.03
RT: 35.69
CAMKII present
Negative control
Intensity
Time (min)
B
953.4047
953.7380
953.0708
954.0716
954.4064
954.7379
955.0901
952.4451
952.9439
953.4942
953.9943
Relative Abundance
m/z
C
S325 CID
y*, b* = [y,b]-H2O
y, b = [y,b]-NH3
y , b = [y,b]-H3PO4
920.53
[M-H3PO4+3H]3+
MGQAGpSTTSNSHAQPFDFPDDNQNAK
Relative Abundance
m/z
D
S325 ETD
963.65
[M+3H]3+
MGQAGpSTTSNSHAQPFDFPDDNQNAK
1407.75
Relative Abundance
m/z
E
S328 ETD
963.71
[M+3H]3+
MGQAGSTTpSNSHAQPFDFPDDNQNAK
1407.25
Relative Abundance
m/z

Figure S4

S306 phosphorylation by CK1

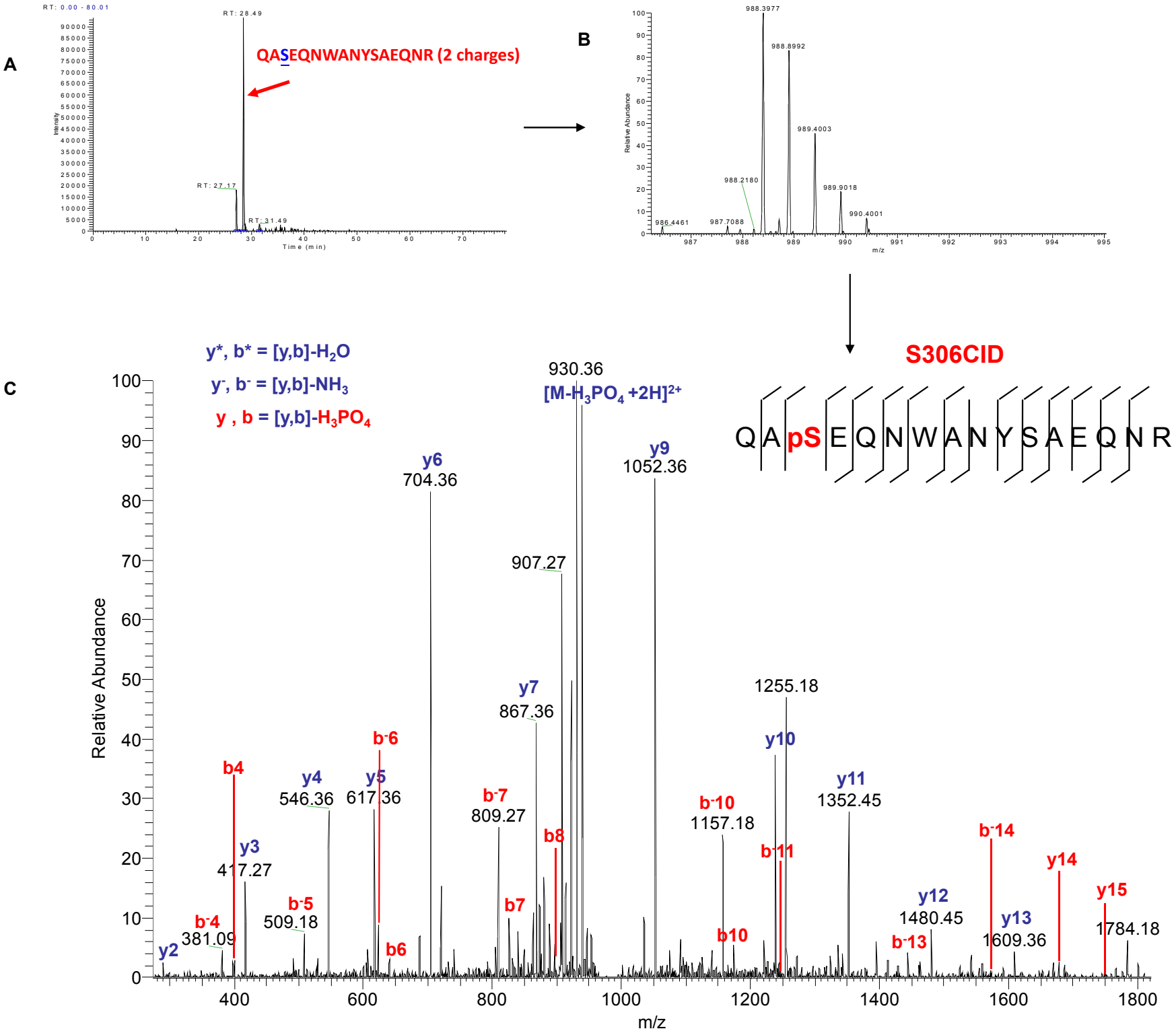


Figure S5

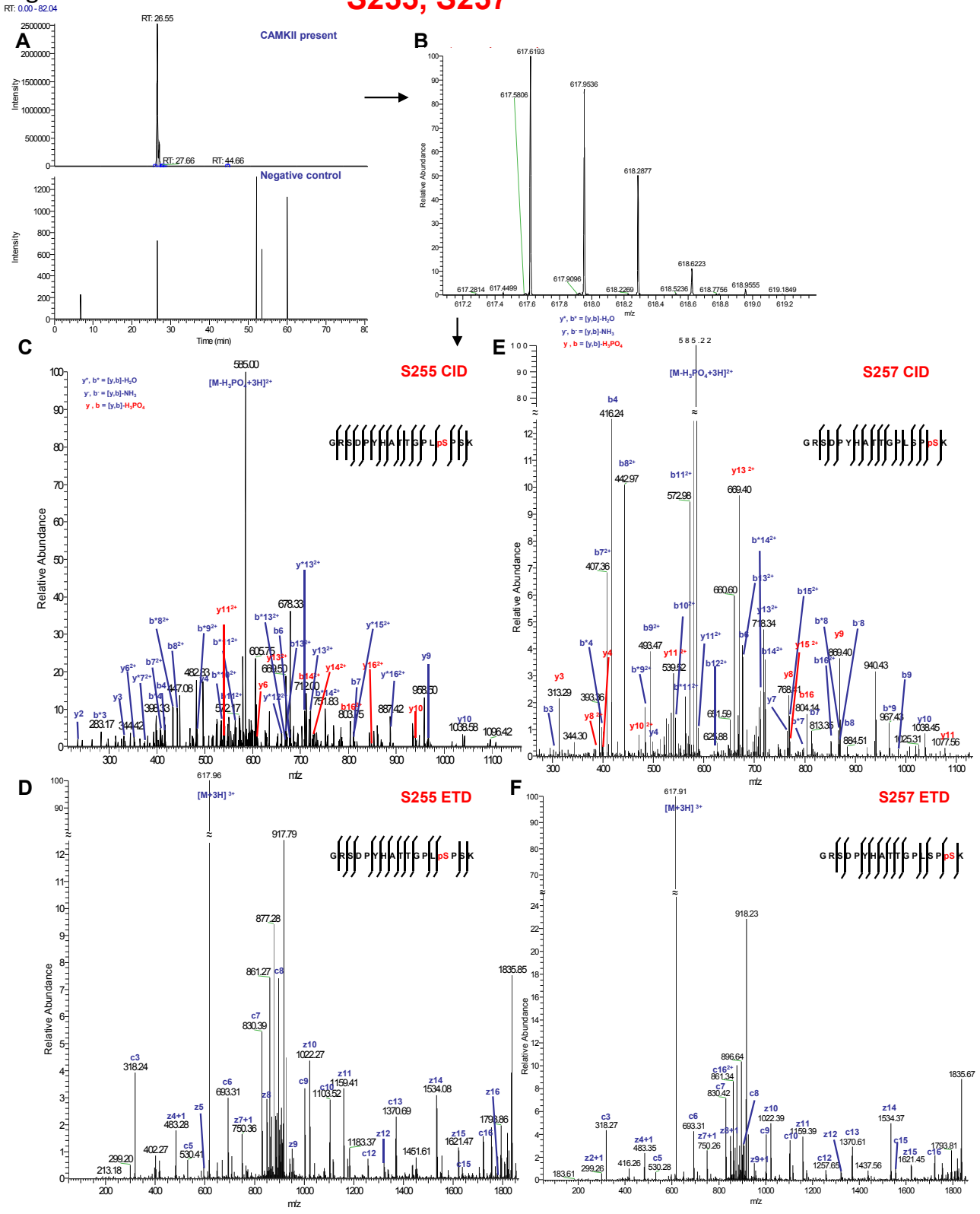

S255, S257
A
CAMKII present
Negative control
RT: 26.55
RT: 27.66
RT: 44.66
Intensity
Time (min)
B
617.6193
617.5806
617.9536
618.2877
618.6223
617.9096
Relative Abundance
m/z
C
S255 CID
585.00
[M-H3PO4+3H]2+
GRSDPYHATTGPLpSPSK
Relative Abundance
m/z
D
S255 ETD
617.96
[M+3H] 3+
917.79
877.28
861.27
830.39
1835.85
GRSDPYHATTGPLpSPSK
E
S257 CID
585.22
[M-H3PO4+3H]2+
GRSDPYHATTGPLSPpSK
F
S257 ETD
617.91
[M+3H] 3+
918.23
1835.67
GRSDPYHATTGPLSPpSK

Figure S6

S296, S297

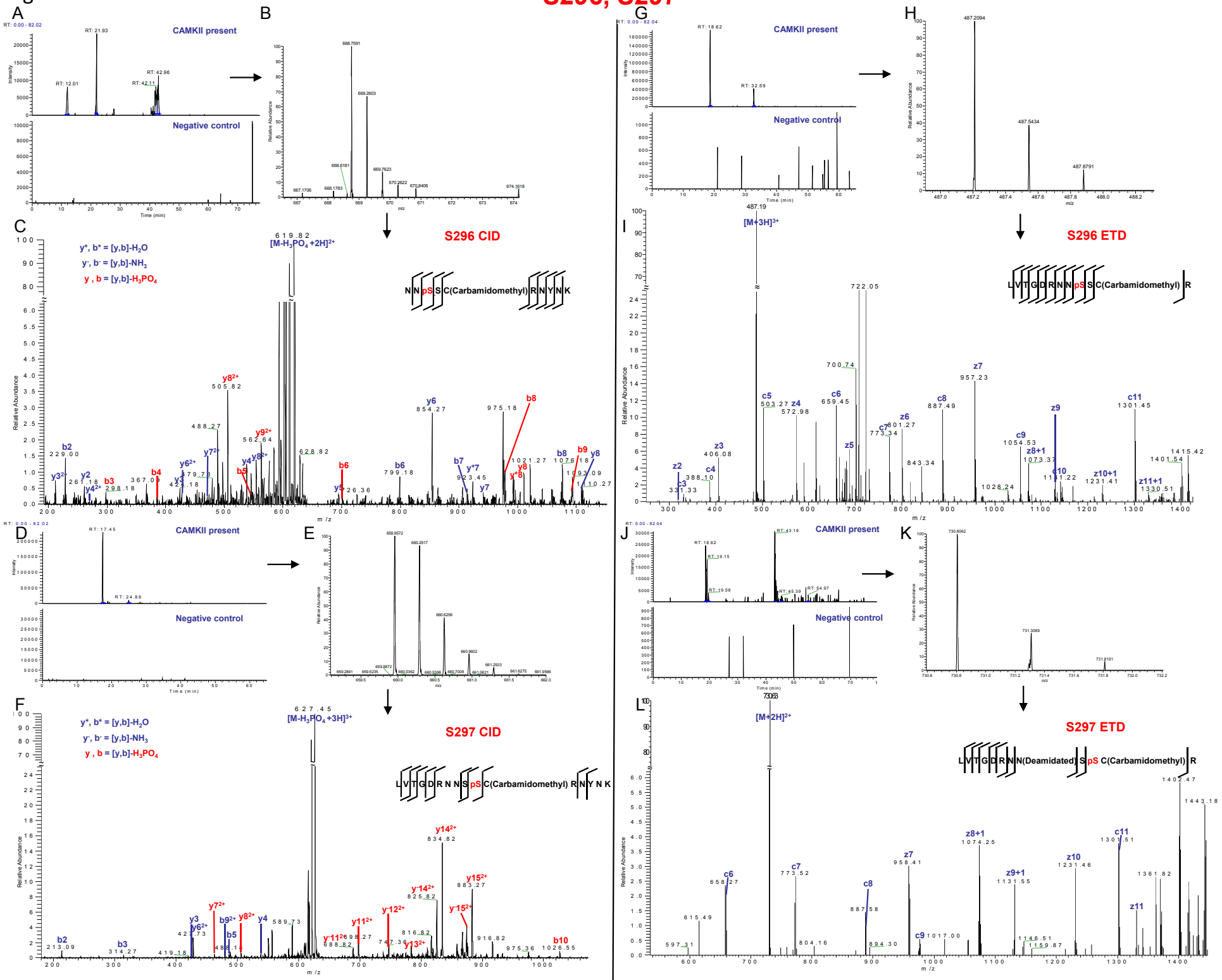


Figure S7

S364, S365

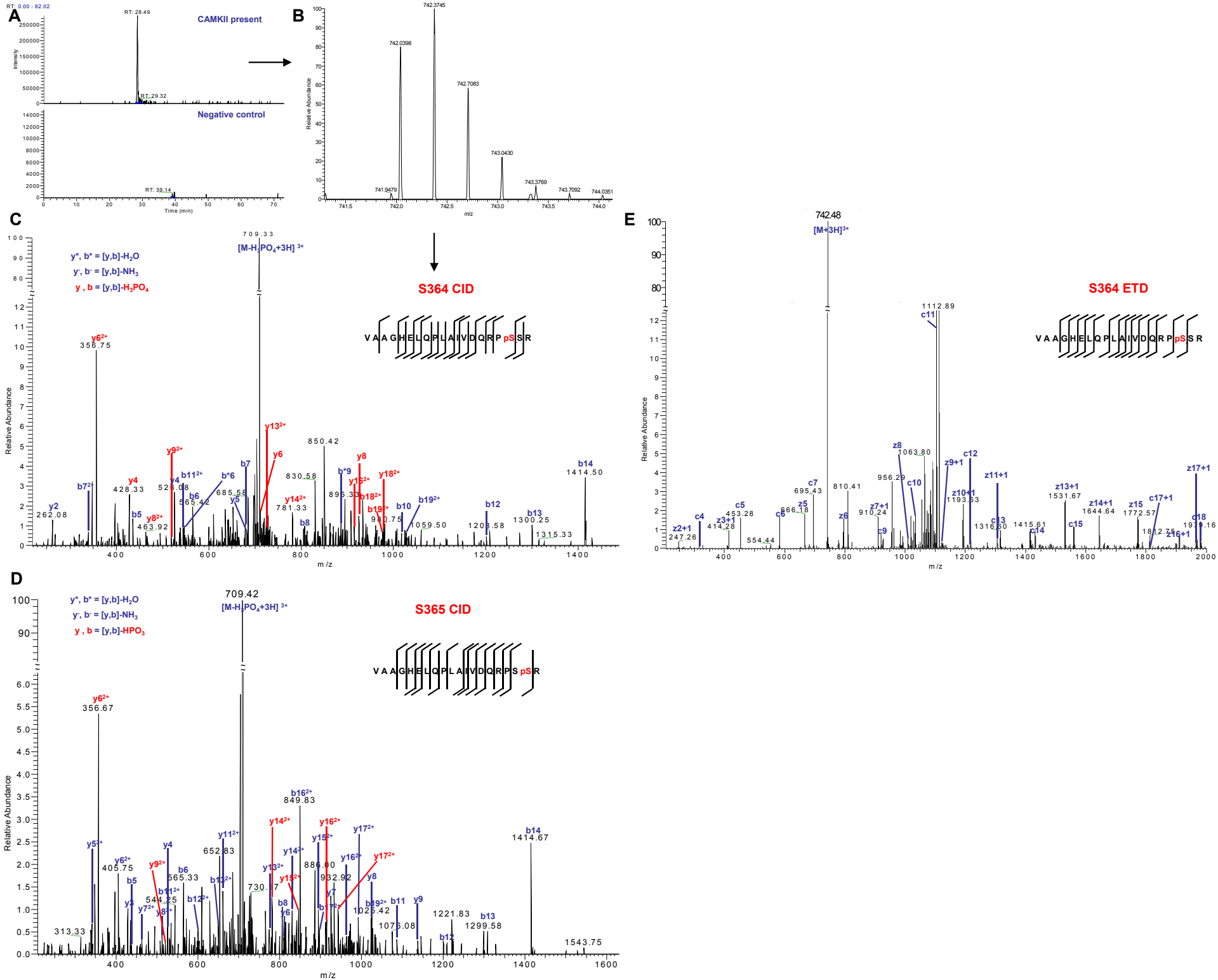


Figure S8

S369, S372, S373

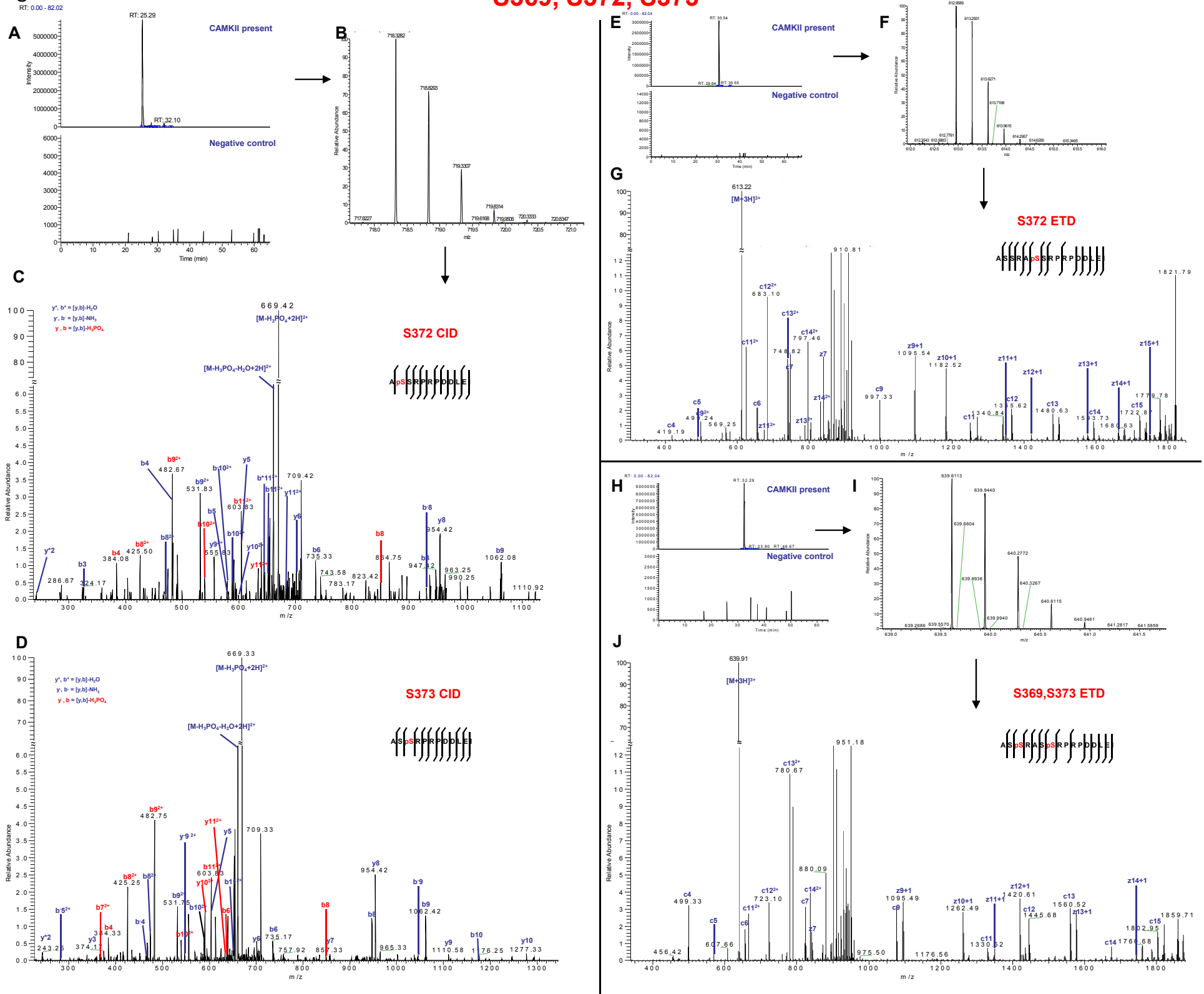


Figure S9

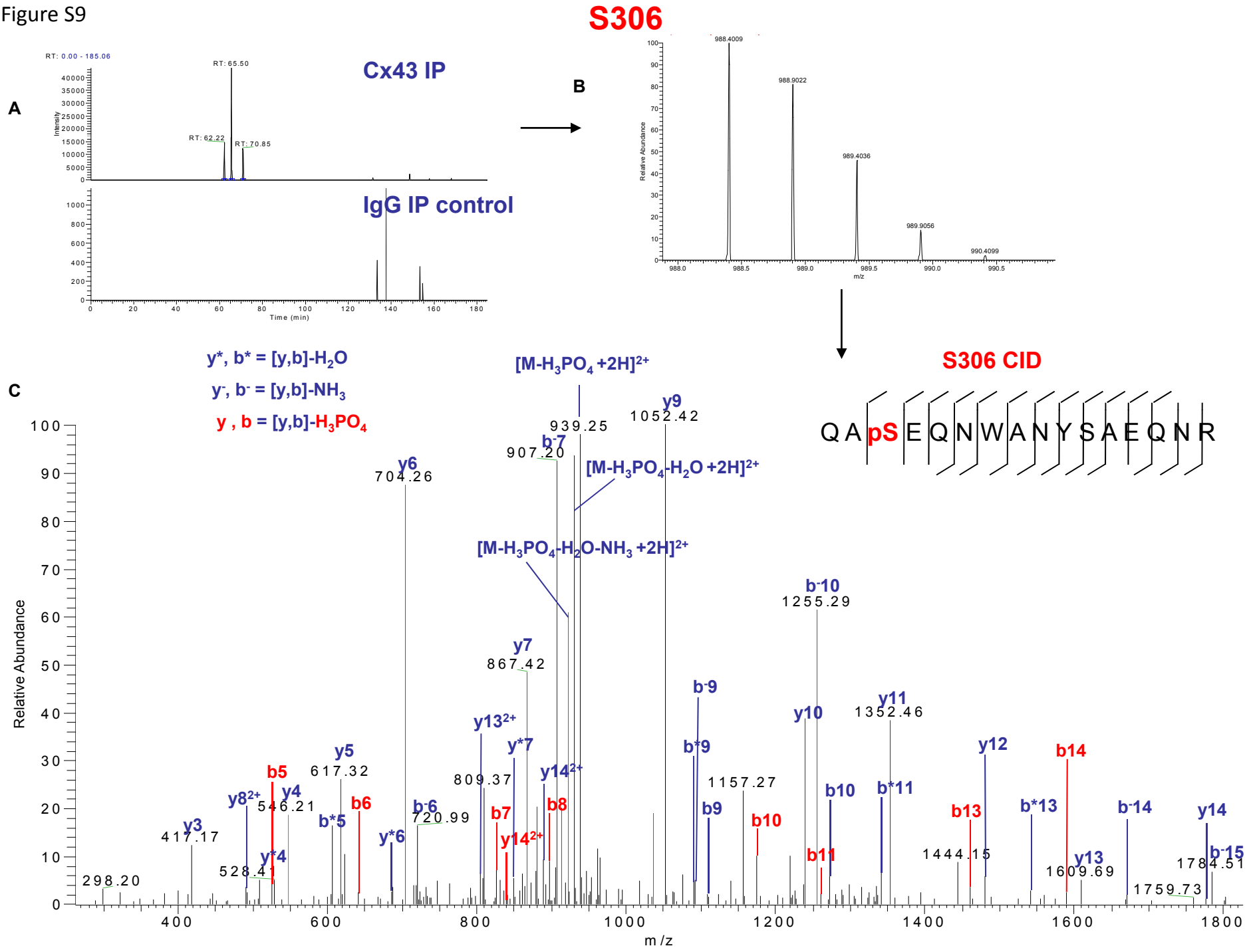
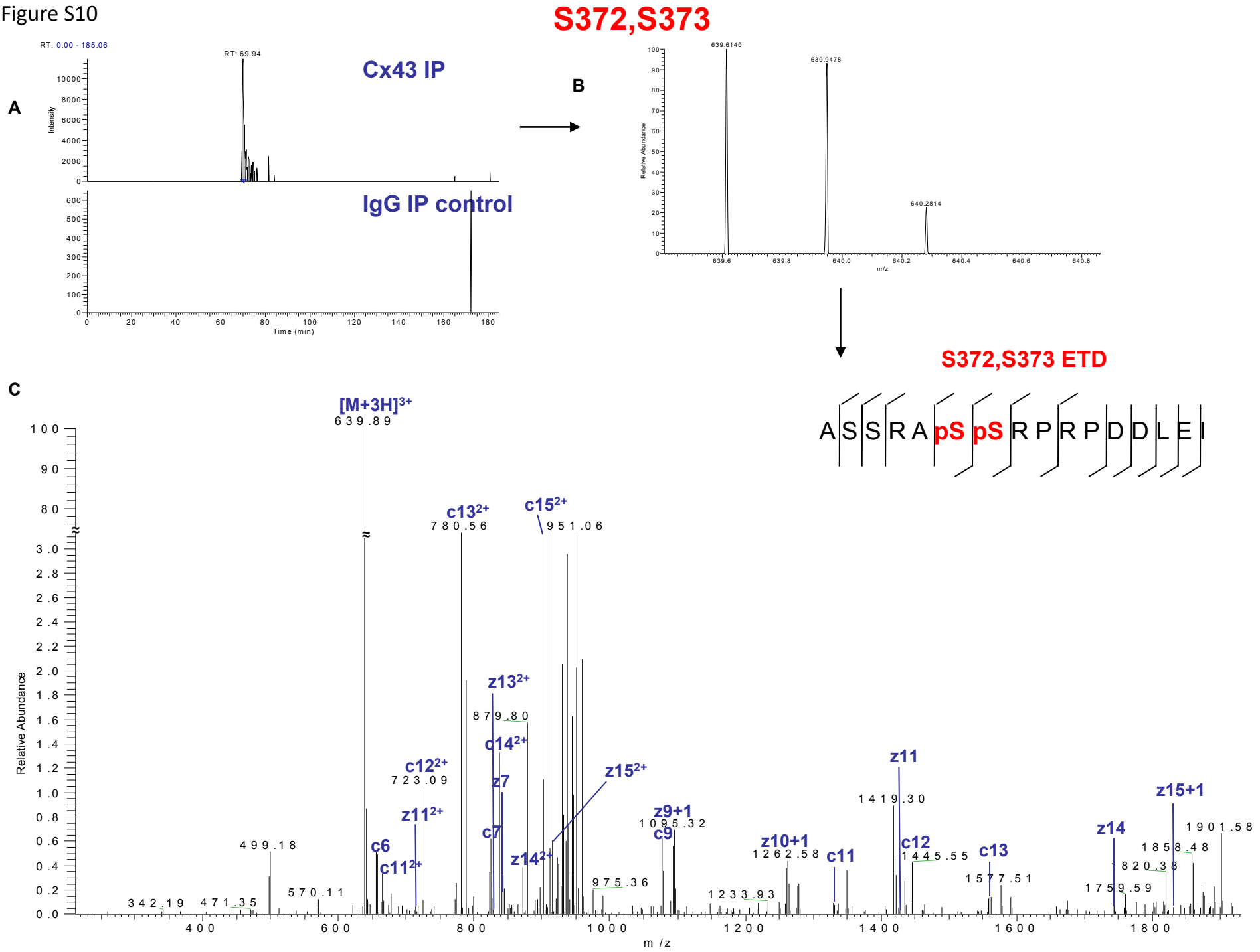

S306
A
Cx43 IP
RT: 0.00 - 185.06
RT: 65.50
RT: 62.22
RT: 70.85
IgG IP control
Time (min)
B
988.4009
988.9022
989.4036
989.9056
990.4099
m/z
S306 CID
Q A pS E Q N W A N Y S A E Q N R
C
y*, b* = [y,b]-H2O
y-, b- = [y,b]-NH3
y , b = [y,b]-H3PO4
[M-H3PO4 +2H]2+
[M-H3PO4-H2O +2H]2+
[M-H3PO4-H2O-NH3 +2H]2+
Relative Abundance
m/z

Figure S10


S372,S373
A
RT: 0.00 - 185.06
RT: 69.94
Cx43 IP
IgG IP control
Intensity
Time (min)
B
639.6140
639.9478
640.2814
Relative Abundance
m/z
S372,S373 ETD
A S S R A pS pS R P R P D D L E I
C
[M+3H]3+
639.89
c13 2+
780.56
c15 2+
951.06
z13 2+
879.80
c14 2+
c12 2+
723.09
z7
z15 2+
z11 2+
c7
c6
c11 2+
z14 2+
z9+1
1095.32
c9
z11
1419.30
z10+1
1262.58
c11
c12
1445.55
c13
1577.51
z14
z15+1
1901.58
1858.48
1820.38
1759.59
499.18
570.11
342.19
471.35
975.36
1233.93
Relative Abundance
m/z

Supplemental Table S1. Complete list of phosphorylated serine residues in each of four in vitro phosphorylation experiments.

Experiment	Condition	Serine residues phosphorylated														
		S244 (novel)	S255 (MAPK)	S257 (no K)	S296 (no K) (*CAMKII?)	S297 (no K)	S306 (no K)	S314 (no K)	S325 (CK1)	S328 (CK1)	S330 (CK1)	S364 (PKA)	S365 (PKA?) (*CAMKII?)	S369 (PKA?) (*CAMKII?)	S372 (PKC)	S373 (PKA,Akt) (*CAMKII?)
1 (CID only)	CAMKII	**a**	**a**	**a**	✓	✓	✓	**b**	**a**	**a**	**a**	×	×	**a**	**a**	**a**
	control	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
2 (CID only)	CAMKII	✓	✓	✓	×	×	✓	**b**	✓***	**b**	**b**	✓	✓	×	✓	✓
	control	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
3 (CID and ETD)	CAMKII	✓	✓	✓	✓**	**b**	✓	✓	✓	✓**	✓*	✓**	**b**	✓**	✓***	✓**
	control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4 (CID and ETD)	CAMKII	✓	✓**	✓	✓**	✓**	✓	✓	✓	✓**	✓	✓	**b**	✓**	✓**	✓**
	control	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×

(novel)	Novel phosphorylation site identified
(no K)	No kinase previously reported as being responsible for phosphorylating this residue
(*CAMKII?)	CAMKII consensus sequence RXX**S**[47-49]
a	MS spectrum was found, but no MS2 triggered
b	MS spectrum was found, but MS2 spectrum indicates other phosphorylated site
✓	Both MS and MS2 spectra were confirmed
✓*	Unique to CID
✓**	Unique to ETD
✓***	Not identified by MASCOT
×	Neither MS nor MS2 spectra were found
n.d.	Not done

Supplemental Table S2. Fragmentation ions for peptide containing S306.

Cx43-36 #3118 @988.39 CID

S306 CID	b	[b-NH3]	[b-H3PO4]	y	y 2+	[y-H3PO4]2+	[y-NH3]	[y-NH3]2+	[y-H2O]2+
in figure as:	b	b-	b	y	y2+	y2+	y-	y-2+	
Q									
A	200.10	183.08		1847.73	924.37	875.38	1830.71	915.86	915.37
S	367.10	350.07	269.12	1776.70 (1776.43)	888.85 (888.67)	839.86 (839.86)	1759.67	880.34 (880.31)	879.85 (879.44)
E	496.14	479.12	398.17	1609.70 (1609.47)	805.35		1592.67	796.84	796.35
Q	624.20	607.18 (607.12)	526.22	1480.66 (1480.52)	740.83 (740.77)		1463.63 (1463.62)	732.32	731.83
N	738.25	721.22 (721.19)	640.27	1352.60 (1352.47)	676.80		1335.57 (1335.54)	668.29	667.80
W	924.32 (924.11)	907.30 (907.09)	826.35 (826.26)	1238.55 (1238.44)	619.78		1221.53 (1221.29)	611.27	610.78
A	995.36	978.34	897.39 (897.40)	1052.48 (1052.42)	526.74		1035.45 (1035.37)	518.23	517.74
N	1109.40 (1109.40)	1092.38	1011.43	981.44	491.22		964.41 (964.33)	482.71	482.22
Y	1272.47 (1272.35)	1255.44 (1255.28)	1174.49 (1174.47)	867.40 (867.29)	434.20		850.37	425.69	425.20
S	1359.50 (1359.97)	1342.47	1261.52 (1261.35)	704.33 (704.31)	352.67		687.31 (687.49)	344.16	343.66
A	1430.54	1413.51	1332.56 (1332.21)	617.30 (617.37)	309.15		600.27 (600.35)	300.64	300.15
E	1559.58	1542.55	1461.60	546.26 (546.28)	273.64		529.24	265.12	264.63
Q	1687.64	1670.61	1589.66	417.22 (417.32)	209.11		400.19	200.60	
N	1801.68 (1801.61)	1784.65 (1784.32)	1703.70	289.16	145.08		272.14	136.57	
R				175.12	88.06		158.10	79.55	

[M+2H]2+	988.19
[M-H3PO4+2H]2+	939.36

Yellow:	Low&High mass cut off
Red:	Relative intensity >1.0
Theoretical m/z (Observed m/z)	

Cx43-36 (11102009) #3941 @659.27 ETD MASCOT score: 33

S306 ETD	c	[c+1]	z	[z+1]	[z+2]
in figure as:	c	c+1	z	z+1	
Q					
A	217.13 (217.22)	218.13	1831.72 (1831.79)	1832.72 (1832.74)	1833.73 (1833.57)
S	384.13 (384.21)	385.13	1760.68 (1760.55)	1761.69 (1761.57)	1762.69 (1762.48)
E	513.17 (513.35)	514.17 (514.39)	1593.68 (1593.57)	1594.69 (1594.60)	1595.69 (1595.73)
Q	641.23 (641.17)	642.23 (642.31)	1464.64 (1464.40)	1465.65 (1465.57)	1466.65 (1466.74)
N	755.27 (755.31)	756.28 (756.16)	1336.58 (1336.38)	1337.59 (1337.49)	1338.59 (1338.65)
W	941.35 (941.40)	942.35 (942.40)	1222.54 (1222.39)	1223.54 (1223.80)	1224.55
A	1012.39 (1012.32)	1013.39 (1013.37)	1036.46 (1036.37)	1037.46 (1037.23)	1038.47
N	1126.43 (1126.33)	1127.43 (1127.40)	965.42	966.43 (966.26)	967.43
Y	1289.49 (1289.31)	1290.5 (1290.39)	851.38 (851.29)	852.38 (852.33)	853.39
S	1376.53 (1376.52)	1377.53 (1377.44)	688.31 (688.29)	689.32 (689.35)	690.32 (690.42)
A	1447.56 (1447.41)	1448.57 (1448.41)	601.28 (601.21)	602.29	603.29
E	1576.61 (1576.45)	1577.61 (1577.46)	530.24 (530.28)	531.25	532.26
Q	1704.67 (1704.63)	1705.67 (1705.53)	401.2 (401.17)	402.21 (402.36)	403.21
N	1818.71 (1818.56)	1819.71 (1819.54)	273.14	274.15	275.15
R			159.1	160.11	161.11

[M+3H]3+		659.5

Yellow:	Low&High mass cut off
Red:	Relative intensity >0.4
Theoretical m/z (Observed m/z)	

Supplemental Table S3. Fragmentation ions for peptide containing S325, S328 and S330.

Cx43-36 #3600 @953.07 CID

S325 CID	b	b 2+	[b-H2O]2+	[b-H3PO4]	[b-H3PO4]2+	y	y 2+	[y-H3PO4]2+	[y-NH3]	[y-NH3]2+	[y-H2O]
M											
G	189.07					2726.15	1363.58	1314.59 (1314.79)	2709.12	1355.06	2708.14
Q	317.13					2669.13	1335.07	1286.08	2652.10	1326.55	2651.12
A	388.16					2541.07	1271.04	1222.05 (1222.20)	2524.04	1262.52	2523.06
G	445.19					2470.03	1235.52 (1235.39)	1186.53 (1186.45)	2453.00	1227.01	2452.02
S	612.18 (612.02)			514.21 (514.32)		2413.01	1207.01 (1207.05)	1158.02 (1158.29)	2395.98	1198.50	2395.00
T	713.23			615.26		2246.01	1123.51 (1123.58)		2228.98	1115.00	2228.00
I	826.32			728.34 (728.23)		2144.96	1072.99 (1072.74)		2127.94	1064.47	2126.95
S	913.35			815.37 (815.30)		2031.88	1016.44		2014.85	1007.93 (1007.89)	2013.87
N	1027.39			929.41		1944.85	972.93		1927.82	964.41	1926.84
S	1114.42 (1114.98)			1016.45 (1016.67)		1830.80	915.91 (915.96)		1813.78	907.39 (907.15)	1812.79
H	1251.48	626.24	617.24	1153.51	577.26	1743.77	872.39 (872.18)		1726.75	863.88	1725.76
A	1322.52	661.76	652.76	1224.54	612.77	1606.71	803.86		1589.69	795.35	1588.70
Q	1450.58 (1450.52)	725.79 (725.79)	716.79	1352.60 (1352.46)	676.80 (676.92)	1535.68	768.34		1518.65	759.83 (759.73)	1517.67
P	1547.63	774.32	765.31	1449.65	725.33	1407.62 (1407.46)	704.31 (704.53)		1390.59	695.80	1389.61 (1389.45)
F	1694.70	847.85 (847.32)	838.85 (838.45)	1596.72	798.86	1310.56	655.79		1293.54	647.27	1292.55
D	1809.73	905.37 (905.21)	896.36 (896.25)	1711.75	856.38 (856.33)	1163.50 (1163.39)	582.25		1146.47	573.74	1145.49
F	1956.79	978.90 (978.71)	969.90	1858.82	929.91	1048.47 (1048.36)	524.74 (524.62)		1031.44 (1031.45)	516.23	1030.46
P	2053.85	1027.43 (1027.05)	1018.42	1955.87	978.44	901.40 (901.29)	451.20		884.37 (884.52)	442.69	883.39 (883.42)
D	2168.87	1084.94 (1084.64)	1075.94	2070.90	1035.95	804.35 (804.18)	402.68		787.32	394.16	786.34
D	2283.90	1142.45	1133.45	2185.92	1093.47	689.32 (689.37)	345.16		672.29	336.65	671.31
N	2397.94	1199.48	1190.47	2299.97	1150.49	574.29	287.65		557.27	279.14	
Q	2526.00	1263.51	1254.50	2428.03	1214.52	460.25	230.63		443.22	222.12	
N	2640.05	1320.53 (1320.74)	1311.52	2542.07	1271.54 (1271.25)	332.19	166.60		315.17	158.09	
A	2711.08	1356.05 (1356.32)	1347.04	2613.11	1307.06 (1307.50)	218.15	109.58		201.12	101.07	
K						147.11	74.06		130.09	65.55	

[M-H3PO4+3H]3+	920.53
[M-H3PO4-NH3+3H]3+	914.76
[M-H3PO4-NH3-H2O+3H]3+	908.63

Yellow:	Low&High mass cut off
Red:	Relative intensity >1.5
Theoretical m/z (Observed m/z)	

Cx43-36 (11102009) #4586 @953.07 ETD — MASCOT score: 55

S325 ETD	c	c 2+	[c+1]	[c+1]2+	[c+2]2+	z	[z+1]	[z+1]2+	[z+2]	[z+2]2+
M										
G	206.10		207.10			2710.13	2711.14	1356.07	2712.14	1356.57 (1356.79)
Q	334.15		335.16			2653.11	2654.12	1327.56 (1327.39)	2655.12	1328.06 (1328.65)
A	405.19		406.19			2525.05	2526.06	1263.53 (1263.58)	2527.06	1264.03 (1264.32)
G	462.21 (462.17)		463.22			2454.01	2455.02	1228.01	2456.02	1228.52
S	629.21 (629.15)		630.21 (630.07)			2396.99	2398.00	1199.50	2399.00	1200.00
T	730.26 (730.23)		731.26			2229.99	2231.00	1116.00	2232.00	1116.51
I	843.34 (843.05)		844.35			2128.94	2129.95	1065.48	2130.96	1065.98
S	930.38 (930.60)		931.38 (931.41)			2015.86	2016.87	1008.94	2017.87	1009.44
N	1044.42 (1044.39)		1045.42 (1045.54)			1928.83 (1928.85)	1929.84 (1929.89)	965.42	1930.84	965.92
S	1131.45 (1131.21)		1132.45 (1132.19)			1814.79 (1814.41)	1815.79 (1815.86)	908.40	1816.80 (1816.79)	908.90
H	1268.51	634.76	1269.51 (1269.34)	635.26	635.76	1727.75	1728.76 (1728.83)	864.88	1729.76 (1729.64)	865.39
A	1339.55 (1339.62)	670.28	1340.55 (1340.56)	670.78	671.28	1590.69 (1590.23)	1591.70 (1591.76)		1592.71	
Q						1519.66 (1519.17)	1520.67 (1520.21)		1521.67 (1521.97)	
P	1564.66 (1564.27)	782.83	1565.66 (1565.73)	783.33	783.84					
F	1711.73 (1711.44)	856.37	1712.73 (1712.43)	856.87	857.37	1294.55	1295.55 (1295.71)		1296.56 (1296.53)	
D	1826.75 (1826.63)	913.88	1827.76 (1827.72)	914.38	914.88	1147.48 (1147.38)	1148.49 (1148.28)		1149.49 (1149.45)	
F						1032.45 (1032.32)	1033.46 (1033.61)		1034.46 (1034.48)	
P	2070.87	1035.94	2071.88	1036.44	1036.94					
D	2185.90	1093.45	2186.90	1093.96	1094.46	788.33 (788.25)	789.34		790.34	
D	2300.93	1150.97	2301.93	1151.47	1151.97	673.30	674.31 (674.33)		675.31 (675.45)	
N	2414.97	1207.99	2415.97	1208.49	1208.99	558.28 (558.15)	559.28 (559.39)		560.29 (560.39)	
Q	2543.03	1272.02	2544.03	1272.52	1273.02	444.23	445.24 (445.19)		446.24	
N	2657.07	1329.04	2658.08	1329.54 (1329.55)	1330.04 (1330.43)	316.17 (316.12)	317.18		318.19	
A	2728.11	1364.56 (1364.27)	2729.11	1365.06 (1364.99)	1365.56 (1365.73)	202.13	203.14		204.14	
K						131.09	132.10		133.11	

[M+3H]3+	953.65

Yellow:	Low&High mass cut off
Red:	Relative intensity >0.2
Theoretical m/z (Observed m/z)	

Cx43-37 (11102009) #4643 @953.40 ETD — MASCOT score: 59

S328 ETD	c	c 2+	[c+1]	[c+1]2+	z	z 2+	[z+1]	[z+1]2+	[z+2]	[z+2]2+
M										
G	206.10		207.10		2710.13	1355.57 (1355.68)	2711.14	1356.07	2712.14	1356.57 (1356.59)
Q	334.15		335.16		2653.11	1327.06 (1326.87)	2654.12	1327.56 (1327.77)	2655.12	1328.06 (1328.53)
A	405.19		406.19		2525.05	1263.03 (1263.23)	2526.06	1263.53 (1263.97)	2527.06	1264.03 (1264.60)
G	462.21 (462.08)		463.22 (463.37)		2454.01	1227.51	2455.02	1228.01	2456.02	1228.52 (1228.73)
S	549.25 (549.36)		550.25 (550.25)		2396.99	1199.00	2398.00	1199.50 (1199.51)	2399.00	1200.00
T	650.29 (650.15)		651.30		2309.96	1155.48	2310.97	1155.99	2311.97	1156.49
I	763.38 (763.20)		764.38		2208.91	1104.96	2209.92	1105.46	2210.92	1105.96
S	930.38 (930.14)		931.38 (931.83)		2095.83	1048.42 (1048.33)	2096.83	1048.92	2097.84	1049.42 (1049.34)
N	1044.42 (1044.21)		1045.42 (1045.35)		1928.83 (1928.65)	964.92	1929.84 (1929.78)	965.42	1930.84 (1930.70)	965.92
S	1131.45 (1131.47)		1132.45 (1132.37)		1814.79	907.90	1815.79 (1815.44)	908.40	1816.80 (1816.71)	908.90
H	1268.51 (1268.47)	634.76	1269.51 (1269.36)	635.26	1727.75 (1727.83)	864.38	1728.76 (1729.00)	864.88	1729.76 (1729.76)	865.39
A	1339.55 (1339.41)	670.28	1340.55 (1340.26)	670.78	1590.69 (1590.35)		1591.70 (1591.61)		1592.71 (1592.69)	
Q					1519.66 (1519.77)		1520.67 (1520.66)		1521.67 (1521.68)	
P	1564.66 (1564.38)	782.83	1565.66 (1565.21)	783.33						
F	1711.73 (1711.58)	856.37	1712.73 (1712.56)	856.87	1294.55		1295.55 (1295.55)		1296.56 (1296.32)	
D	1826.75 (1826.56)	913.88	1827.76 (1827.64)	914.38	1147.48 (1147.35)		1148.49 (1148.42)		1149.49 (1149.43)	
F					1032.45 (1032.41)		1033.46 (1033.34)		1034.46 (1034.36)	
P	2070.87	1035.94 (1035.55)	2071.88	1036.44						
D	2185.90	1093.45	2186.90	1093.96	788.33 (788.23)		789.34 (789.35)		790.34 (790.60)	
D	2300.93	1150.97 (1150.85)	2301.93	1151.47	673.30 (673.48)		674.31 (674.46)		675.31 (675.32)	
N	2414.97	1207.99	2415.97	1208.49	558.28		559.28 (559.24)		560.29 (560.24)	
Q	2543.03	1272.02	2544.03	1272.52	444.23 (444.36)		445.24 (445.30)		446.24 (446.39)	
N	2657.07	1329.04	2658.08	1329.54 (1329.22)	316.17 (316.51)		317.18 (317.22)		318.19 (318.28)	
A	2728.11	1364.56 (1364.43)	2729.11	1365.06 (1365.28)	202.13		203.14		204.14	
K					131.09		132.10		133.11	

[M+3H]3+	953.71

Yellow:	Low&High mass cut off
Red:	Relative intensity >0.1
Theoretical m/z (Observed m/z)	

Cx43-37 #4342-4832 @953.73 CID

S330 CID	b	b 2+	[b-H2O]	[b-H2O]2+	[b-NH3]	[b-NH3]2+	[b-H3PO4]	[b-H3PO4]2+	y	y 2+	y-H3PO4]2:	[y-NH3]	[y-NH3]2+	[y-H2O]
M														
G	189.07								2728.12	1364.56	1315.57	2711.09	1356.05 (1356.49)	2710.11
Q	317.13				300.10				2671.09	1336.05	1287.06	2654.07	1327.54	2653.08
A	388.16				371.14				2543.04	1272.02	1223.03 (1223.34)	2526.01	1263.51 (1263.85)	2525.03
G	445.19 (445.07)				428.16				2472.00	1236.50 (1236.65)	1187.51 (1187.70)	2454.97	1227.99	2453.99
S	532.22		514.21 (514.19)		515.19				2414.98	1207.99	1159.00 (1159.53)	2397.95	1199.48	2396.97
T	633.27		615.26		616.24 (616.09)				2327.95	1164.48	1115.49	2310.92	1155.96	2309.93
I	746.35		728.34 (728.35)		729.32 (729.14)				2226.90	1113.95 (1113.88)	1064.96 (1064.79)	2209.87	1105.44	2208.89
S	833.38		815.37		816.36				2113.81	1057.41 (1057.72)	1008.42 (1008.40)	2096.79	1048.90 (1048.30)	2095.80
N(Deamidated)	948.41		930.40		931.38				2026.78	1013.89 (1013.34)	964.91	2009.75	1005.38	2008.77
S	1115.41 (1115.29)		1097.40 (1097.47)		1098.38		1017.43 (1017.31)		1911.75	956.38 (956.58)	907.39 (907.26)	1894.73	947.87	1893.74
H	1252.47	626.74	1234.46	617.73	1235.44 (1235.41)	618.22	1154.49	577.75	1744.76	872.88 (872.54)		1727.73	864.37	1726.75
A	1323.50	662.26	1305.49	653.25	1306.48	653.74	1225.53	613.27	1607.70	804.35		1590.67	795.84	1589.69
Q(Deamidated)	1452.55 (1452.31)	726.78	1434.54 (1434.35)	717.77	1435.52	718.26	1354.57 (1354.14)	677.79 (677.92)	1536.66	768.83		1519.63 (1519.37)	760.32	1518.65 (1518.20)
P	1549.60	775.30	1531.59	766.30	1532.57	766.79	1451.62 (1451.38)	726.31	1407.62 (1407.52)	704.31 (704.25)		1390.59 (1390.52)	695.80	1389.61
F	1696.67	848.84	1678.66	839.83	1679.64	840.32	1598.69	799.85	1310.56	655.79		1293.54	647.27	1292.55
D	1811.69	906.35 (906.33)	1793.68	897.35 (897.37)	1794.67	897.84	1713.72	857.36	1163.50 (1163.39)	582.25		1146.47	573.74	1145.49
F	1958.76	979.89 (979.44)	1940.75	970.88 (970.73)	1941.74	971.37 (971.58)	1860.79	930.90	1048.47 (1048.30)	524.74		1031.44	516.23	1030.46 (1030.42)
P	2055.82	1028.41 (1028.31)	2037.80	1019.41	2038.79	1019.95	1957.84	979.42 (979.44)	901.40 (901.26)	451.20 (451.25)		884.37 (884.28)	442.69	883.39 (883.33)
D	2170.84	1085.92	2152.83	1076.92	2153.82	1077.41	2027.87	1036.94 (1036.43)	804.35 (804.26)	402.68		787.32	394.16	786.34
D	2285.87	1143.44 (1143.07)	2267.86	1134.43 (1134.27)	2268.84	1134.93	2187.89	1094.45	689.32 (689.25)	345.16		672.29	336.65	671.31 (671.75)
N	2399.91	1200.46 (1200.52)	2381.90	1191.45	2382.89	1191.95	2301.94	1151.47	574.29 (574.22)	287.65		557.27	279.14	
Q	2527.97	1264.49	2509.96	1255.48	2510.94	1255.98	2429.99	1215.50	460.25	230.63		443.22	222.12	
N	2642.01	1321.51	2624.00	1312.51 (1312.78)	2624.99	1313.00	2544.04	1272.52 (1272.33)	332.19	166.60		315.17	158.09	
A	2713.05	1357.03 (1357.01)	2695.04	1348.02	2696.02	1348.52	2615.07	1308.04	218.15	109.58		201.12	101.07	
K									147.11	74.06		130.09	65.55	

[M-H3PO4+3H]3+	921.39
[M+3H]3+	953.47
[M-H3PO4-NH3-H2O+3H]3+	909.44

Yellow:	Low&High mass cut off
Red:	Relative intensity >0.03
Theoretical m/z (Observed m/z)	

Cx43-37 (11102009) #4585-4708 @953.73 ETD — MASCOT score: 60

S330 ETD	c	c 2+	[c+1]	[c+1]2+	z	z 2+	[z+1]	[z+1]2+
M								
G	206.10		207.10		2711.14	1356.06 (1355.90)	2712.12	1356.56 (1356.77)
Q	334.15		335.16		2654.09	1327.55 (1327.46)	2655.10	1328.05 (1327.97)
A	405.19 (405.25)		406.19		2526.03	1263.52 (1263.24)	2527.04	1264.02 (1264.01)
G	462.21 (462.11)		463.22		2455.00	1228.00 (1228.09)	2456.00	1228.51
S	549.25 (549.23)		550.25 (550.16)		2397.97	1199.49	2398.98	1199.99
T	650.29 (650.54)		651.30		2310.94	1155.97	2311.95	1156.48
I	763.38 (763.37)		764.38 (764.52)		2209.89	1105.45	2210.90	1105.96
S	850.41 (850.31)		851.41		2096.81	1048.91 (1048.97)	2097.82	1049.41 (1049.53)
N	964.45 (964.47)		965.46		2009.78	1005.39	2010.79	1005.90
S	1131.45 (1131.74)		1132.45 (1132.53)		1895.74 (1895.85)	948.37	1896.74 (1896.81)	948.88
H	1268.51 (1268.30)	634.76	1269.51 (1269.33)	635.26	1728.74 (1728.67)	864.87	1729.75 (1729.63)	865.38
A	1339.55 (1339.40)	670.28	1340.55 (1340.43)	670.78	1591.68 (1591.62)		1592.69 (1592.67)	
Q					1520.64 (1520.75)		1521.65 (1521.66)	
P	1564.66 (1564.83)	782.83	1565.66 (1565.62)	783.33				
F	1711.73 (1711.56)	856.37	1712.73 (1712.60)	856.87	1295.53 (1295.47)		1296.54 (1296.71)	
D	1826.75 (1826.65)	913.88	1827.76 (1827.64)	914.38	1148.46 (1148.40)		1149.47 (1149.48)	
F					1033.43 (1033.48)		1034.44 (1034.44)	
P	2070.87	1035.94	2071.88	1036.44				
D	2185.90	1093.45	2186.90	1093.96	789.31 (789.30)		790.32 (790.44)	
D	2300.93	1150.97 (1150.51)	2301.93	1151.47	674.29 (674.36)		675.29 (675.40)	
N(Deamidated)	2415.95	1208.48	2416.96	1208.98	559.26 (559.36)		560.27 (560.39)	
Q	2544.01	1272.51	2545.02	1273.01	444.23 (444.34)		445.24 (445.38)	
N	2658.06	1329.53 (1329.82)	2659.06	1330.03 (1330.53)	316.17		317.18 (317.28)	
A	2729.09	1365.05 (1364.70)	2730.10	1365.55 (1365.61)	202.13		203.14	
K					131.09		132.10	

[M+3H]3+	953.77

Yellow:	Low&High mass cut off
Red:	Relative intensity >0.1
Theoretical m/z (Observed m/z)	

Supplemental Table S4. Fragmentation ions for peptide containing S244, S255 and S257.

Cx43-37(11102009) #3484 @617.62 CID

S244 CID	b	b 2+	[b-H2O]	[b-H2O]2+	[b-NH3]2+	[b-H3PO4]	[b-H3PO4]2+	y	y 2+	[y-NH3]	[y-NH3]2+	[y-H2O]
G												
R	214.12 (214.30)	107.57			99.06			1793.82	897.41	1776.80	888.90 (888.50)	1775.81
S	381.12 (381.32)	191.07			182.55	283.15 (283.20)	142.08	1637.72	819.36	1620.69	810.85	1619.71
D	496.16 (496.14)	248.58	478.14	239.58	240.07	398.18 (398.25)	199.59	1470.72	735.86	1453.70	727.35	1452.71
P	593.21	297.11	575.20	288.10	288.59	495.23	248.12	1355.70	678.35 (678.51)	1338.67	669.84 (669.61)	1337.68
Y	756.27 (756.26)	378.64	738.26 (738.32)	369.63	370.13	658.29 (658.28)	329.65	1258.64	629.83	1241.62	621.31	1240.63
H	893.33 (893.43)	447.17 (447.39)	875.32	438.16	438.66 (438.46)	795.35 (795.30)	398.18	1095.58 (1095.45)	548.29 (548.54)	1078.55	539.78 (539.53)	1077.57 (1077.72)
A	964.37	482.69 (482.90)	946.36	473.68	474.17	866.39 (866.57)	433.70 (433.66)	958.52 (958.43)	479.76	941.49 (941.40)	471.25	940.51 (940.51)
T	1065.41	533.21 (533.35)	1047.40	524.21 (524.19)	524.70	967.44	484.22 (484.45)	887.48 (887.46)	444.25	870.46	435.73	869.47 (869.56)
T	1166.46	583.74 (583.75)	1148.45	574.73 (574.87)	575.22	1068.49	534.75 (534.71)	786.44 (786.45)	393.72	769.41	385.21	768.42 (768.54)
G	1223.48	612.25	1205.47	603.24	603.73	1125.51	563.26 (563.32)	685.39 (685.37)	343.20	668.36	334.68	667.38
P	1320.54	660.77 (660.97)	1302.53	651.77 (651.26)	652.26	1222.56	611.78	628.37 (628.38)	314.69	611.34	306.17	610.36
L	1433.62	717.31	1415.61	708.31 (708.52)	708.80	1335.64	668.33 (668.48)	531.31	266.16	514.29	257.65	513.30
S	1520.65	760.83 (760.63)	1502.64	751.82 (751.78)	752.32	1422.68	711.84	418.23 (418.26)	209.62	401.20	201.11	400.22
P	1617.71	809.36	1599.70	800.35	800.84	1519.73	760.37	331.20 (331.28)	166.10	314.17	157.59	313.19 (313.22)
S	1704.74	852.87 (852.87)	1686.73	843.87 (843.56)	844.36 (844.29)	1606.76	803.88 (803.81)	234.15	117.58	217.12	109.06	216.13 (216.33)
K								147.11	74.06	130.09	65.55	

[M-H3PO4+3H]3+	585.19
[M-H3PO4-H2O+3H]3+	579.22
[M-H3PO4-NH3-H2O+3H]3+	573.06

Yellow:	Low&High mass cut off
Red:	Relative intensity >1.0
Theoretical m/z (Observed m/z)	

Cx43-37(11102009) #3508 @617.62 ETD MASCOT score: 37

S244 ETD	c	c 2+	[c+1]	[c+1]2+	[c+2]2+	z	z 2+	[z+1]	[z+2]	[z+2]2+
G										
R	231.16 (231.23)	116.08	232.16	116.58	117.09	1777.8	889.41 (889.47)	1778.81 (1778.65)	1779.81 (1779.80)	890.41 (890.12)
S	398.15 (398.07)	199.58	399.16	200.08	200.58	1621.7 (1621.53)	811.35	1622.71 (1622.75)	1623.71	812.36
D						1454.7 (1454.64)	727.86	1455.71 (1455.56)	1456.71 (1456.75)	728.86
P	610.23 (610.38)	305.62	611.24	306.12	306.62					
Y	773.3 (773.43)	387.15	774.3 (774.37)	387.65	388.16	1242.62 (1242.53)	621.82	1243.63	1244.64	622.82
H	910.36 (910.42)	455.68	911.36 (911.54)	456.18	456.69	1079.56 (1079.36)	540.28	1080.57 (1080.57)	1081.57 (1081.54)	541.29
A	981.39 (981.47)	491.2	982.4 (982.41)	491.7	492.2	942.5 (942.25)		943.51 (943.40)	944.51	
T	1082.44 (1082.51)	541.72	1083.44 (1083.46)	542.23	542.73	871.46		872.47	873.48	
T	1183.49 (1183.50)	592.25	1184.49 (1184.34)	592.75	593.25	770.42 (770.35)		771.42 (771.34)	772.43 (772.42)	
G						669.37		670.38	671.38	
P	1337.56 (1337.29)	669.29 (669.28)	1338.57	669.79	670.29 (670.25)					
L	1450.65 (1450.59)	725.83	1451.65 (1451.63)	726.33	726.83	515.3 (515.22)		516.3	517.31	
S						402.21 (402.16)		403.22 (403.34)	404.22	
P	1634.73	817.87	1635.74	818.37	818.87					
S	1721.76 (1721.71)	861.39 (861.31)	1722.78 (1722.79)	861.89 (862.04)	862.39 (862.66)	218.13		219.13	220.14	
K						131.09		132.1	133.11	

[M+3H]3+	617.94

Yellow:	Low&High mass cut off
Red:	Relative intensity >1.0
Theoretical m/z (Observed m/z)	

Cx43-35 #2265 @617.61 CID

S255 CID	b	b 2+	[b-H2O]	[b-H2O]2+	[b-H3PO4]2+	y	y 2+	[y-H3PO4]	[y-H3PO4]2+	[y-H2O]2+
G										
R	214.13	107.57				1793.82	897.41	1695.84	848.42 (848.33)	888.41 (888.50)
S	301.16	151.08	283.15 (283.17)	142.08		1637.72	819.36	1539.74	770.38	810.36 (810.25)
D	416.19 (416.42)	208.60	398.18 (398.33)	199.59		1550.69	775.85	1452.71	726.85 (726.75)	766.84
P	513.24	257.12	495.23	248.12		1435.66	718.33 (718.42)	1337.68	669.35 (669.50)	709.33 (709.08)
Y	676.30 (676.25)	338.66	658.29	329.65		1338.61	669.81	1240.63	620.82	660.80 (660.50)
H	813.36 (813.25)	407.19 (407.17)	795.35	398.18		1175.55	588.28	1077.57	539.29 (539.17)	579.27
A	884.40	442.70 (442.58)	866.39	433.70 (433.33)		1038.49 (1038.58)	519.75	940.50 (940.50)	470.76	510.74
T	985.45	493.23	967.44 (967.42)	484.22 (484.83)		967.45 (967.42)	484.23	869.47	435.24	475.22
T	1086.50	543.75	1068.49	534.75 (534.75)		866.40	433.70	768.43	384.72	424.70
G	1143.52	572.26 (572.17)	1125.51	563.26 (563.08)		765.35	383.18	667.38	334.19	374.18 (374.83)
P	1240.57	620.79	1222.56	611.78		708.33	354.67 (354.25)	610.35 (610.17)	305.68	345.66
L	1353.65	677.33 (677.67)	1335.64	668.33 (668.50)		611.28	306.14	513.30	257.16	297.14
S	1520.65	760.83	1502.64	751.82 (751.83)	711.84 (712.00)	498.20 (498.25)	249.60	400.22	200.61	240.60
P	1617.71	809.36	1599.70	800.35	760.37	331.20 (331.25)	166.10			157.10
S	1704.74	852.87	1686.73	843.87	803.88 (803.75)	234.14 (234.33)	117.57			108.57
K						147.11	74.06			

[M-H3PO4+3H]3+	585
[M-H3PO4-H2O+3H]3+	578.92

Yellow:	Low&High mass cut off
Red:	Relative intensity >2.5
Theoretical m/z (Observed m/z)	

Cx43-37(11102009) #3445-3487 @617.62 ETD MASCOT score: 42

S255 ETD	c	c 2+	[c+1]	[c+1]2+	[c+2]	[c+2]2+	z	z 2+	[z+1]	[z+1]2+	[z+2]	[z+2]2+
G												
R	231.16	116.08	232.16	116.58	233.16	117.09	1777.8 (1777.96)	889.41 (889.47)	1778.81 (1778.72)	889.91 (890.13)	1779.81 (1779.72)	890.41
S	318.19 (318.24)	159.6	319.2 (319.33)	160.1	320.2	160.6	1621.7 (1621.47)	811.35	1622.71 (1622.64)	811.86	1623.71 (1623.63)	812.36
D							1534.67 (1534.83)	767.84	1535.68 (1535.52)	768.34	1536.68 (1536.54)	768.84
P	530.27 (530.41)	265.64	531.27	266.14	532.27	266.64						
Y	693.33 (693.31)	347.17	694.33 (694.40)	347.67	695.34	348.17	1322.59 (1322.37)	661.8	1323.6 (1323.52)	662.3	1324.6	662.8
H	830.39 (830.39)	415.7	831.39 (831.45)	416.2	832.4 (832.44)	416.7	1159.53 (1159.41)	580.27	1160.53 (1160.43)	580.77	1161.54 (1161.43)	581.27
A	901.43 (901.46)	451.22	902.43 (902.55)	451.72	903.43 (903.54)	452.22	1022.47 (1022.27)		1023.48 (1023.35)		1024.48 (1024.36)	
T	1002.48 (1002.48)	501.74	1003.48 (1003.59)	502.24	1004.48	502.74	951.43 (951.36)		952.44 (952.38)		953.44 (953.49)	
T	1103.52 (1103.52)	552.27	1104.53 (1104.56)	552.77	1105.53	553.27	850.38 (850.25)		851.39 (851.38)		852.39 (852.53)	
G							749.33 (749.22)		750.34 (750.36)		751.35 (751.40)	
P	1257.6 (1257.53)	629.3	1258.6 (1258.64)	629.8	1259.6	630.31						
L	1370.68 (1370.70)	685.84	1371.68 (1371.76)	686.35	1372.69 (1372.69)	686.85	595.26 (595.22)		596.27 (596.24)		597.27	
S							482.18 (482.22)		483.19 (483.28)		484.19 (484.20)	
P	1634.73 (1634.72)	817.87	1635.74	818.37	1636.74	818.87						
S	1721.76 (1721.84)	861.39 (861.27)	1722.77 (1722.74)	861.89 (862.09)	1723.77 (1723.67)	862.39 (862.66)	218.13		219.13		220.14	
K							131.09		132.1		133.11	

[M+3H]3+	617.95

Yellow:	Low&High mass cut off
Red:	Relative intensity >0.2
Theoretical m/z (Observed m/z)	

Cx43-37(11102009) #3442 @617.62 CID

S257 CID	b	b 2+	[b-H2O]	[b-H2O]2+	[b-NH3]	[b-H3PO4]2+	y	y 2+	[y-H3PO4]	[y-H3PO4]2+	[y-H2O]
G											
R	214.13	107.57			197.10		1793.82	897.41	1695.84	848.43	1775.81
S	301.16 (301.39)	151.08	283.15	142.08	284.14		1637.72	819.36	1539.74	770.38 (770.42)	1619.71
D	416.19 (416.24)	208.60	398.18 (398.27)	199.59	399.16		1550.69	775.85	1452.71	726.86	1532.68
P	513.24	257.12	495.23	248.11	496.22		1435.66	718.33 (718.34)	1337.68	669.35 (669.40)	1417.65
Y	676.30 (676.30)	338.66	658.29	329.65	659.28		1338.61	669.81	1240.63	620.82	1320.60
H	813.36 (813.35)	407.19 (407.36)	795.35 (795.44)	398.18	796.34		1175.55	588.28 (588.48)	1077.57 (1077.56)	539.29 (539.52)	1157.54
A	884.4 (884.51)	442.70 (442.97)	866.39 (866.43)	433.70	867.37 (867.66)		1038.49 (1038.45)	519.75	940.51 (940.43)	470.76 (470.94)	1020.48 (1020.26)
T	985.45 (985.49)	493.23 (493.47)	967.44 (967.43)	484.22 (484.23)	968.42 (968.36)		967.45	484.23	869.47 (869.40)	435.24	949.44
T	1086.50	543.75 (543.78)	1068.49	534.75	1069.47		866.40	433.70	768.43 (768.41)	384.72 (384.88)	848.39
G	1143.52	572.26 (572.98)	1125.51	563.26 (563.49)	1126.49		765.35 (765.34)	383.18	667.38	334.19	747.34 (747.29)
P	1240.57	620.79 (620.58)	1222.56	611.78	1223.54		708.33	354.67	610.36	305.68	690.32
L	1353.65	677.33 (677.44)	1335.64	668.33	1336.63		611.28	306.14	513.30	257.16	593.27
S	1440.69	720.85 (720.89)	1422.68	711.84 (711.93)	1423.66		498.2 (498.11)	249.60	400.22 (400.32)	200.61	480.19
P	1537.74	769.37 (769.34)	1519.73	760.37	1520.71		411.16	206.09	313.19 (313.29)	157.10	
S	1704.74	852.87 (852.62)	1686.73	843.87	1687.71	803.88 (804.14)	314.11	157.56	216.13	108.57	
K							147.11	74.06			

[M-H3PO4+3H]3+	585.22
[M-H3PO4-H2O+3H]3+	579.22

Yellow:	Low&High mass cut off
Red:	Relative intensity >0.2
Theoretical m/z (Observed m/z)	

Cx43-37(11102009) #3424 @617.62 ETD MASCOT score: 45

S257 ETD	c	c 2+	[c+1]	[c+1]2+	[c+2]	[c+2]2+	z	[z+1]	[z+2]
G									
R	231.16	116.08	232.16	116.58	233.16	117.09	1777.8	1778.81	1779.81
S	318.19 (318.27)	159.6	319.2 (319.22)	160.1	320.2	160.6	1621.7 (1621.45)	1622.71 (1622.55)	1623.71
D							1534.67 (1534.37)	1535.68 (1535.45)	1536.68 (1536.51)
P	530.27 (530.28)	265.64	531.27	266.14	532.27	266.64			
Y	693.33 (693.31)	347.17	694.33 (694.48)	347.67	695.34	348.17	1322.59 (1322.52)	1323.6 (1323.51)	1324.6
H	830.39 (830.42)	415.7	831.39 (831.44)	416.2 (416.26)	832.4	416.7	1159.53 (1159.39)	1160.53 (1160.40)	1161.54
A	901.43 (901.54)	451.22	902.43	451.72	903.43	452.22	1022.47 (1022.39)	1023.48 (1023.31)	1024.48 (1024.31)
T	1002.48 (1002.37)	501.74	1003.48 (1003.45)	502.24	1004.48 (1004.56)	502.74	951.43 (951.52)	952.44 (952.45)	953.44
T	1103.52 (1103.46)	552.27	1104.53 (1104.48)	552.77	1105.53	553.27	850.38 (850.30)	851.39 (851.29)	852.39 (852.37)
G							749.34 (749.20)	750.34 (750.26)	751.35 (751.46)
P	1257.6 (1257.65)	629.3	1258.6 (1258.53)	629.8	1259.6	630.31			
L	1370.68 (1370.61)	685.84	1371.68 (1371.60)	686.35	1372.69	686.85	595.26	596.27	597.27
S							482.18 (482.27)	483.19 (483.35)	484.19
P	1554.77 (1554.72)	777.89	1555.77 (1555.71)	778.39	1556.77	778.89			
S	1721.76 (1721.72)	861.39 (861.34)	1722.77 (1722.53)	861.89	1723.77 (1723.49)	862.39 (862.14)	298.09	299.1 (299.26)	300.1
K							131.09	132.1	133.11

[M+3H]3+	617.91

Yellow:	Low&High mass cut off
Red:	Relative intensity >0.5
Theoretical m/z (Observed m/z)	

Supplemental Table S5. Fragmentation ions for peptide containing S314.

Cx43-36 #3004 @659.26 CID

S314 CID	b	[b-H2O]	[b-NH3]	y	y 2+	[y-H3PO4]	[y-H3PO4]2+	[y-NH3]	[y-NH3]2+
in figure as:	**b**	**b***	**b-**	**y**	**y^{2+}**	**y**	**y^{2+}**	**y-**	
Q									
A	200.10		183.08	1847.73	924.37	1749.76	875.38	1830.71	915.86
S	287.14	269.12	270.11	1776.70	888.85 (888.91)	1678.72	839.86	1759.67	880.34
E	416.18	398.17	399.15	1689.67	845.34 (845.35)	1591.69	796.35	1672.64	836.82
Q	544.24 (544.19)	526.23	527.21 (527.08)	1560.62	780.82	1462.65	731.83	1543.60	772.30
N	658.28 (658.07)	640.27	641.25	1432.56	716.79 (716.85)	1334.59	667.80 (667.98)	1415.54	708.27 (708.42)
W	844.36 (844.27)	826.35 (826.18)	827.33 (827.30)	1318.52	659.76	1220.54	610.78 (610.79)	1301.49	651.25
A	915.40 (915.32)	897.39 (897.55)	898.37 (898.29)	1132.44 (1132.22)	566.72 (566.79)	1034.47	517.74 (517.80)	1115.42 (1115.26)	558.21
N	1029.45 (1029.24)	1011.43	1012.41 (1012.12)	1061.40 (1061.20)	531.21 (531.18)	963.43 (963.49)	482.22 (482.30)	1044.38 (1044.32)	522.69
Y	1192.50 (1192.60)	1174.49 (1174.28)	1175.48	947.36 (947.26)	474.18 (474.17)	849.39	425.20 (425.72)	930.34 (930.12)	465.67 (465.53)
S	1359.50	1341.49	1342.47	784.30 (784.20)	392.65	686.32 (686.45)	343.66	767.27	384.14
A	1430.54	1412.53	1413.51	617.30 (617.28)	309.15			600.27 (600.48)	300.64
E	1559.58	1541.57	1542.55	546.26 (546.29)	273.64			529.24 (529.29)	265.12
Q	1687.64	1669.63	1670.61	417.22 (417.17)	209.11			400.19	200.60
N	1801.68	1783.67	1784.65	289.16 (289.20)	145.08			272.14	136.57
R				175.12	88.06			158.09	79.55

[M-H3PO4+3H]3+	626.66
[M-H3PO4-NH3+3H]3+	620.9
[M-H3PO4-NH3-H2O+3H	615.18

Yellow:	Low&High mass cut off	
Red:	Relative intensity >2.0	
Theoretical m/z (Observed m/z)		

Cx43-36 (11102009) #3787 @659.27 ETD **MASCOT score: 85**

S314 ETD	c	[c+1]	z	[z+1]
in figure as:	**c**	**c+1**	**z**	**z+1**
Q				
A	217.13 (217.22)	218.13	1831.72	1832.72 (1832.45)
S	304.16	305.16	1760.69 (1760.44)	1761.69 (1761.49)
E	433.2 (433.45)	434.21 (434.28)	1673.64 (1673.48)	1674.65 (1674.59)
Q	561.26 (561.49)	562.27 (562.15)	1544.6 (1544.52)	1545.61 (1545.52)
N	675.31 (675.31)	676.31 (676.60)	1416.55 (1416.34)	1417.55 (1417.47)
W	861.39 (861.50)	862.39 (862.36)	1302.5 (1302.33)	1303.51
A	932.42 (932.52)	933.43 (933.43)	1116.42 (1116.25)	1117.43 (1117.31)
N	1046.47 (1046.56)	1047.47 (1047.47)	1045.39 (1045.05)	1046.39
Y	1209.53 (1209.36)	1210.53 (1210.26)	931.34 (931.63)	932.35
S	1376.53 (1376.40)	1377.53 (1377.56)	768.28	769.29
A	1447.56	1448.57 (1448.51)	601.28 (601.45)	602.29
E	1576.61 (1576.56)	1577.61 (1577.51)	530.24 (530.55)	531.25
Q	1704.67 (1704.66)	1705.67 (1705.67)	401.2	402.21
N	1818.71 (1818.74)	1819.71 (1819.63)	273.14	274.15
R			159.1	160.11

[M+3H]3+		659.42

Yellow:	Low&High mass cut off
Red:	Relative intensity >1.0
Theoretical m/z (Observed m/z)	

Supplemental Table S6. Fragmentation ions for peptides containing S296 and S297.

Cx43-29(CAMKII) #521 @668.76 CID

S296 CID	b	[b-H3PO4]	y	y 2+	[y-H3PO4]	[y-H3PO4]2+	[y-H2O]	[y-H2O-H3PO4]
N								
N	229.09 (229.00)		1222.47	611.74	1124.49	562.75 (562.64)	1204.46	1106.48
S	396.09	298.11 (298.18)	1108.42 (1108.36)	554.72 (554.64)	1010.45 (1010.45)	505.73 (505.82)	1090.41	992.44 (992.45)
S	483.12	385.15 (385.00)	941.43 (941.00)	471.22 (471.18)			923.42 (923.45)	
C (Carbamidomethyl)	643.15	545.18 (545.27)	854.39 (854.27)	427.70 (427.91)				
R	799.26 (799.18)	701.28 (701.18)	694.36 (694.36)	347.69				
N	913.30 (913.36)	815.32	538.26 (538.45)	269.63 (269.82)				
Y	1076.36 (1076.18)	978.38 (978.18)	424.22 (424.18)	212.61 (212.00)				
N	1190.40	1092.43 (1092.27)	261.16 (261.18)	131.08				
K			147.11	74.06				

[M-H3PO4+2H]2+	619.82
[M-H3PO4-NH3+2H]2+	611.27
[M-H3PO4-NH3-H2O+2H]2+	602.27

Yellow:	Low&High mass cut off
Red:	Relative intensity >0.5
Theoretical m/z (Observed m/z)	

Cx43-37(11102009) #2444 @487.21 ETD **MASCOT score: 61**

S296 ETD	c	[c+1]	z	[z+1]
L				
V	230.19	231.19	1329.51	1330.52 (1330.51)
T	331.23 (331.33)	332.24 (332.26)	1230.44 (1230.03)	1231.45 (1231.41)
G	388.26 (388.10)	389.26 (389.30)	1129.40 (1129.02)	1130.40 (1130.31)
D	503.28 (503.27)	504.29 (504.21)	1072.38 (1072.24)	1073.38 (1073.37)
R	659.38 (659.45)	660.39 (660.56)	957.35 (957.23)	958.36 (958.22)
N	773.43 (773.34)	774.43 (774.48)	801.25 (801.27)	802.25 (802.14)
N	887.47 (887.49)	888.47 (888.56)	687.20 (687.09)	688.21 (688.16)
S	1054.47 (1054.53)	1055.47 (1055.50)	573.16 (572.98)	574.17 (574.10)
S	1141.50 (1141.22)	1142.50 (1142.41)	406.16 (406.08)	407.17 (407.28)
C (Carbamidomethyl)	1301.53 (1301.45)	1302.53 (1302.53)	319.13 (319.64)	320.14
R			159.10	160.11

[M+3H]3+	487.19

Yellow:	Low&High mass cut off
Red:	Relative intensity >0.5
Theoretical m/z (Observed m/z)	

Cx43-29(CAMKII) #863 @659.96 CID

S297 CID	b	b 2+	[b-H3PO4]	y	y 2+	[y-H3PO4]2+	[y-H2O-H3PO4]2+
L							
V	213.16 (213.09)			1864.78	932.89	883.90 (883.27)	874.90 (874.82)
T	314.21 (314.27)			1765.71	883.36	834.37 (834.82)	825.36 (825.82)
G	371.23			1664.66	832.83	783.84 (783.91)	774.84
D	486.26 (486.27)			1607.64	804.32	755.33	746.33 (746.64)
R	642.36	321.68		1492.61	746.81	697.82 (697.82)	688.82 (688.82)
N	756.40	378.70		1336.51	668.76	619.77	610.76
N	870.45	435.73		1222.47	611.74	562.75	553.74
S	957.47	479.24 (479.27)		1108.42	554.72	505.73 (505.82)	496.72
S	1124.47	562.74	1026.5 (1026.55)	1021.39	511.20	462.21 (462.27)	
C (Carbamidomethryl)	1284.50	642.76	1186.53	854.39	427.70 (427.73)		
R	1440.61	720.81	1342.63	694.36	347.69		
N	1554.65	777.83	1456.67	538.26 (538.36)	269.63		
Y	1717.71	859.36	1619.73	424.22 (424.18)	212.61		
N	1831.75	916.38	1733.78	261.16	131.08		
K				147.11	74.06		

[M-H3PO4+3H]3+	627.45
[M-H3PO4-NH3+3H]3+	621.55
[M-H3PO4-NH3-H2O+3H]3+	615.64

Yellow:	Low&High mass cut off
Red:	Relative intensity >0.2
Theoretical m/z (Observed m/z)	

Cx43-37(11102009) #2502 @730.81 ETD **MASCOT score: 30**

S297 ETD	c	[c+1]	z	[z+1]
L				
V	230.19	231.19	1330.50 (1330.98)	1331.50 (1331.71)
T	331.23	332.24	1231.43 (1231.46)	1232.44 (1232.63)
G	388.26	389.26	1130.38 (1130.49)	1131.39 (1131.55)
D	503.28	504.29	1073.36 (1073.10)	1074.37 (1074.25)
R	659.38 (659.37)	660.39 (660.20)	958.33 (958.41)	959.34 (959.36)
N	773.43 (773.52)	774.43	802.23	803.24
N (Deamidated)	888.45 (888.51)	889.46	688.19	689.20
S	975.49 (975.35)	976.49 (976.36)	573.16	574.17
S	1142.48	1143.49	486.13	487.14
C (Carbamidomethyl)	1302.51 (1302.56)	1303.52 (1303.51)	319.13	320.14
R			159.10	160.11

[M+2H]2+	730.63

Yellow:	Low&High mass cut off
Red:	Relative intensity >0.5
Theoretical m/z (Observed m/z)	

Supplemental Table S7. Fragmentation ions for peptide containing S364 and S365.

Cx43-35 #3763 @742.04 CID

S364 CID	b	b 2+	[b-H2O]	[b-NH3]	[b-NH3]2+	[b-H3PO4]2+	y	[y-H3PO4]	[y-H3PO4]2+
V									
A	171.11						2125.06	2027.08	1014.04
A	242.15						2054.02	1956.04	978.52 (978.75)
G	299.17						1982.98	1885.00	943.01
H	436.23 (436.08)	218.62					1925.96	1827.98	914.49 (914.33)
E	565.27 (565.42)	283.14	547.26 (547.25)				1788.90	1690.92	845.97
L	678.36 (678.42)	339.68 (339.75)	660.35				1659.86	1561.88	781.44 (781.33)
Q	807.40 (807.33)	404.20	789.39	790.37	395.69		1546.77	1448.80	724.90 (724.75)
P	904.45	452.73	886.44 (886.33)	887.43 (887.08)	444.22		1417.73	1319.75	660.38
L	1017.54 (1017.67)	509.27	999.53	1000.51	500.76		1320.68	1222.70	611.85
A	1088.57	544.79 (544.75)	1070.56	1071.55	536.28 (536.83)		1207.59	1109.62	555.31
I	1201.66 (1201.50)	601.33	1183.65	1184.63	592.82		1136.56	1038.58	519.79 (519.50)
V	1300.73 (1300.25)	650.87	1282.72	1283.70	642.35 (642.50)		1023.47	925.50 (925.00)	463.25 (463.92)
D	1414.77 (1414.50)	707.89	1396.76	1397.74	699.37 (699.33)		924.40	826.43	413.72
Q	1542.83	771.92	1524.82	1525.80	763.40		810.36	712.38 (712.08)	356.70 (356.75)
R	1698.93	849.97	1680.92	1681.90	841.45		682.30 (682.42)	584.33	292.67
P	1795.98	898.49	1777.97	1778.95	889.98		526.20 (526.08)	428.23 (428.33)	214.62
S	1962.98	981.99	1944.97	1945.95	973.48	933.01 (933.08)	429.15	331.17	166.09
S	2050.01	1025.51 (1025.25)	2032.00	2032.99	1017.00	976.52 (976.58)	262.15 (262.08)		
R							175.12		

[M-H3PO4+3H]3+	709.33
[M-H3PO4-H2O+3H]3+	703.58
[M-H3PO4-NH3-H2O+3H]3	697.67

Yellow:	Low&High mass cut off
Red:	Relative intensity >0.4
Theoretical m/z (Observed m/z)	

Cx43-35 #3757 @742.04 CID

S365 CID	b	b 2+	[b-H2O]2+	[b-NH3]2+	y	y 2+	[y-H3PO4]2+
V							
A	171.11				2125.06	1063.03	1014.04
A	242.15				2054.02	1027.51	978.52
G	299.17				1982.98	991.99 (991.83)	943.01 (943.00)
H	436.23 (436.00)	218.62			1925.96	963.48 (963.58)	914.49 (914.50)
E	565.27 (565.33)	283.14	274.13		1788.90	894.95 (894.92)	845.97 (845.92)
L	678.36 (678.00)	339.68	330.68		1659.86	830.43 (830.17)	781.44 (781.42)
Q	806.42 (806.17)	403.71	394.71	395.20	1546.77	773.89 (774.08)	724.90
P	903.47	452.24	443.23	443.72	1418.72	709.86	660.87
L	1016.55	508.78	499.77	500.27	1321.66	661.33 (661.33)	612.35
A	1087.59 (1087.33)	544.30 (544.25)	535.29	535.79	1208.58	604.79	555.80
I	1200.67 (1200.42)	600.84 (600.92)	591.84	592.33 (592.42)	1137.54 (1137.33)	569.27 (569.08)	520.29 (520.17)
V	1299.74 (1299.58)	650.37 (650.58)	641.37	641.86	1024.46 (1024.58)	512.73 (512.67)	463.74
D	1414.77 (1414.67)	707.89	698.88 (698.67)	699.37 (699.50)	925.39 (925.17)	463.20 (463.42)	414.21
Q	1542.83	771.92	762.91	763.40	810.36 (810.58)	405.68 (405.75)	356.70 (356.67)
R	1698.93	849.97 (849.83)	840.96	841.45 (841.33)	682.30	341.66 (341.67)	292.67
P	1795.98	898.49 (898.42)	889.49	889.98	526.20 (526.33)	263.60	214.62
S	1883.01	942.01	933.01	933.50	429.15 (429.25)	215.08	166.09
S	2050.01	1025.51 (1025.42)	1016.50	1017.00	342.12	171.56	122.57
R					175.12	88.06	

[M-H3PO4+3H]3+	709.42
[M-H3PO4-H2O+3H]3+	703.75
[M-H3PO4-NH3-H2O+3H]3+	697.67

Yellow:	Low&High mass cut off
Red:	Relative intensity >0.3
Theoretical m/z (Observed m/z)	

Cx43-36(11102009) #4619 @742.38 ETD **MASCOT score: 35**

S364 ETD	c	[c+1]	z	[z+1]
V				
A	188.14	189.14	2109.04	2110.04
A	259.18	260.18	2038.00	2039.01
G	316.20 (316.29)	317.20	1966.96 (1966.80)	1967.97 (1967.90)
H	453.26 (453.28)	454.26 (454.35)	1909.94 (1910.09)	1910.95 (1910.82)
E	582.30 (582.44)	583.30 (583.42)	1772.88 (1772.57)	1773.89 (1773.72)
L	695.38 (695.43)	696.39 (696.30)	1643.84 (1643.66)	1644.85 (1644.64)
Q			1530.76 (1530.53)	1531.76 (1531.67)
P	920.49 (920.48)	921.50 (921.70)		
L	1033.58 (1033.51)	1034.58 (1034.49)	1305.64 (1305.39)	1306.65 (1306.36)
A	1104.62 (1104.83)	1105.62 (1105.67)	1192.56 (1192.25)	1193.57 (1193.53)
I	1217.70 (!217.75)	1218.70 (1218.66)	1121.52	1122.53 (1122.38)
V	1316.77 (1316.60)	1317.77 (1317.26)	1008.44 (1008.40)	1009.45 (1009.52)
D	1431.80 (1431.71)	1432.80 (1432.71)	909.37	910.38 (910.24)
Q	1559.85 (1559.88)	1560.86 (1560.62)	794.34 (794.34)	795.35 (795.38)
R			666.28 (666.18)	667.29 (667.37)
P	1813.01	1814.01 (1814.22)		
S	1980.01 (1980.14)	1981.01 (1980.95)	413.13	414.14 (414.28)
S	2067.04	2068.04	246.13	247.14 (247.26)
R			159.10	160.11

[M+3H]3+	742.48

Yellow:	Low&High mass cut off
Red:	Relative intensity >0.4
Theoretical m/z (Observed m/z)	

Supplemental Table S8. Fragmentation ions for peptides containing S369, S372 and S373.

Cx43-35 #3294 @718.33 CID

S372 CID	b	b 2+	[b-H2O]2+	[b-NH3]	[b-NH3]2+	[b-H3PO4]	[b-H3PO4]2+	y	y 2+	[y-H3PO4]2+	[y-NH3]2+	[y-H2O]	[y-H2O]2+
A													
S	239.04					141.07		1364.62	682.81 (682.83)	633.83 (634.00)	674.30	1346.61	673.81 (673.67)
S	326.07 (326.25)					228.10		1197.62	599.31 (599.25)		590.80 (590.67)	1179.61	590.31 (590.25)
R	482.18 (482.25)	241.59	232.59	465.15	233.08	384.20 (384.08)	192.60	1110.59	555.80 (555.83)		547.29	1092.58	546.79
P	579.23 (579.17)	290.12	281.11	562.20	281.60	481.25	241.13	954.49 (954.42)	477.75		469.23	936.48 (936.67)	468.74
R	735.33 (735.33)	368.17	359.16	718.30	359.66	637.35	319.18	857.44	429.22		420.71	839.43	420.22
P	832.38	416.69	407.69	815.36	408.18	734.41	367.71	701.34 (701.67)				683.32 (683.25)	
D	947.41 (947.42)	474.21 (474.33)	465.20	930.38 (930.75)	465.70	849.43 (849.75)	425.22 (425.50)	604.28 (604.42)				586.27	
D	1062.44 (1062.58)	531.72 (531.83)	522.72	1045.41	523.21	964.46	482.73 (482.67)	489.26				471.24	
L	1175.52	588.26 (588.33)	579.26	1158.49	579.75 (579.75)	1077.54	539.28 (539.25)	374.23				356.22	
E	1304.56	652.79 (652.75)	643.78 (643.67)	1287.54	644.27	1206.59	603.80 (603.83)	261.14				243.13 (243.17)	
I								132.10					

[M-H3PO4+2H]2+	669.42
[M-H3PO4-H2O+2H]2+	660.33
[M-H3PO4-NH3-H2O+2H]2+	651.42

Yellow:	Low&High mass cut off
Red:	Relative intensity >0.3
Theoretical m/z (Observed m/z)	

Cx43-35 #3198 @718.33 CID

S373 CID	b	b 2+	[b-H2O]	[b-H2O]2+	[b-NH3]	[b-NH3]2+	[b-H3PO4]	[b-H3PO4]2+	y	y 2+	[y-H3PO4]2+	[y-NH3]2+	[y-H2O]
A													
S	158.08		141.07						1364.62	682.81 (682.92)	633.83 (633.75)	674.30	1346.61
S	326.07		308.06				228.10		1277.59 (1277.33)	639.30 (639.42)	590.31 (590.33)	630.78	1259.58
R	482.18	241.59	464.17	232.59	465.15 (465.08)	233.08	384.20 (384.33)	192.60	1110.59 (1110.58)	555.80 (555.75)		547.29 (547.08)	1092.58
P	579.23	290.12	561.22	281.11	562.20	281.60 (281.75)	481.25	241.13	954.49 (954.42)	477.75		469.23	936.48 (936.42)
R	735.33 (735.17)	368.17	717.32	359.16	718.30	359.66	637.35 (637.50)	319.18	857.44 (857.33)	429.22		420.71	839.43
P	832.38	416.69	814.37	407.69	815.36	408.18	734.41	367.71 (367.58)	701.34 (701.42)				683.32 (683.25)
D	947.41 (947.25)	474.21 (474.33)	929.40	465.20	930.38	465.70 (465.83)	849.43 (849.33)	425.22 (425.25)	604.28 (604.33)				586.27 (586.25)
D	1062.44 (1062.42)	531.72 (531.75)	1044.43 (1044.50)	522.72	1045.41 (1045.67)	523.21	964.46	482.73 (482.75)	489.26				471.24
L	1175.52 (1175.42)	588.26 (588.33)	1157.51	579.26	1158.49	579.75	1077.54	539.28 (539.33)	374.23 (374.17)				356.22
E	1304.56	652.79 (652.83)	1286.55	643.78 (643.83)	1287.54	644.27	1206.59	603.80 (603.83)	261.14				243.13 (243.25)
I									132.10				

[M-H3PO4+2H]2+	669.33
[M-H3PO4-H2O+2H]2+	660.33
[M-H3PO4-NH3-H2O+2H]2+	651.50

Yellow:	Low&High mass cut off
Red:	Relative intensity >0.1
Theoretical m/z (Observed m/z)	

Cx43-37 (11102009) #3921-3965 @612.96 ETD **MASCOT score: 36**

S372 ETD	c	c 2+	[c+1]	[c+1]2+	z	z 2+	[z+1]	[z+1]2+
A								
S	176.10		177.11		1749.80 (1749.78)	875.41 (875.13)	1750.81 (1750.72)	875.91 (875.64)
S	263.14		264.14		1662.77 (1662.39)	831.89 (831.89)	1663.78 (1663.71)	832.39 (832.67)
R	419.24 (419.19)	210.12	420.24	210.62	1575.74 (1575.88)	788.37 (788.65)	1576.75 (1576.82)	788.88 (789.65)
A	490.27 (490.40)	245.64	491.28	246.14	1419.64 (1419.45)	710.32	1420.65 (1420.55)	710.83
S	657.27 (657.32)	329.14	658.27 (658.29)	329.64	1348.60 (1348.29)	674.80 (674.74)	1349.61 (1349.30)	675.31 (675.53)
S	744.30 (744.33)	372.66	745.31 (745.34)	373.16	1181.60 (1181.40)	591.31	1182.61 (1182.52)	591.81
R					1094.57 (1094.31)	547.79	1095.58 (1095.54)	548.29
P	997.46 (997.33)	499.23 (499.24)	998.46 (998.48)	499.73				
R					841.42 (841.16)		842.43 (842.40)	
P	1250.61 (1250.63)	625.81 (625.54)	1251.61 (1251.64)	626.31 (626.24)				
D	1365.64 (1365.62)	683.32 (683.10)	1366.64 (1366.64)	683.82 (684.03)				
D	1480.67 (1480.63)	740.84 (740.53)	1481.67 (1481.60)	741.34 (741.11)				
L	1593.75 (1593.73)	797.38 (797.46)	1594.75 (1594.64)	797.88 (798.17)				
E	1722.79 (1722.87)	861.90	1723.80 (1723.75)	862.40 (862.10)				
I								

[M+3H]3+		613.22

Yellow:	Low&High mass cut off
Red:	Relative intensity >0.1
Theoretical m/z (Observed m/z)	

Cx43-37 (11102009) #4145-4196 @639.61 ETD **MASCOT score: 30**

S369,S373 ETD	c	c 2+	[c+1]	[c+1]2+	z	z 2+	[z+1]
A							
S	176.10		177.11		1829.77	915.39	1830.78
S	343.10		344.10		1742.74 (1742.79)	871.87 (871.86)	1743.75 (1743.74)
R	499.20 (499.33)	250.10	500.21 (500.35)	250.61	1575.74	788.37	1576.75 (1576.71)
A	570.24 (570.29)	285.62	571.24 (571.57)	286.13	1419.64 (1419.46)	710.32	1420.65 (1420.61)
S	657.27 (657.35)	329.14	658.27 (658.31)	329.64	1348.60	674.80	1349.61 (1349.45)
S	824.27 (824.29)	412.64	825.27 (825.29)	413.14	1261.57	631.29	1262.58 (1262.49)
R					1094.57 (1094.42)	547.79	1095.58 (1095.49)
P	1077.42 (1077.42)	539.22	1078.43 (1078.45)	539.72			
R					841.42 (841.34)		842.43 (842.45)
P	1330.58 (1330.52)	665.79 (665.57)	1331.58 (1331.52)	666.29 (666.40)			
D	1445.60 (1445.68)	723.31 (723.10)	1446.61 (1446.72)	723.81 (723.87)			
D	1560.63 (1560.52)	780.82 (780.67)	1561.63 (1561.63)	781.32 (781.39)			
L	1673.72 (1673.77)	837.36 (837.52)	1674.72 (1674.82)	837.86			
E	1802.76 (1802.95)	901.88	1803.76 (1803.78)	902.38			
I							

[M+3H]3+		639.91

Yellow:	Low&High mass cut off
Red:	Relative intensity >0.2
Theoretical m/z (Observed m/z)	

Supplemental Table S9. Fragmentation ions for native Cx43 peptide containing S306.

Cx43-IP #3093 @988.39 CID

S306 CID	b	[b-H2O]	[b-NH3]	[b-H3PO4]	y	y 2+	[y-H3PO4]2+	[y-H2O]
Q								
A	200.10		183.08		1847.73	924.37	875.38	1829.72
S	367.10		350.07	269.12	1776.70 (1776.46)	888.85 (888.50)	839.86 (839.81)	1758.69
E	496.14	478.13	479.12	398.17	1609.70 (1609.69)	805.35 (804.95)		1591.69
Q	624.20	606.19 (606.08)	607.18	526.23 (526.16)	1480.66 (1480.45)	740.83		1462.65
N	738.24	720.23	721.27 (720.99)	640.27 (640.22)	1352.60 (1352.46)	676.80		1334.59
W	924.32	906.31	907.30 (907.20)	826.35 (826.40)	1238.55 (1238.51)	619.78		1220.54
A	995.36	977.35	978.34	897.39 (897.42)	1052.48 (1052.42)	526.74		1034.46
N	1109.40 (1108.81)	1091.39 (1091.28)	1092.38 (1092.20)	1011.43	981.44	491.22 (491.11)		963.43
Y	1272.47 (1272.33)	1254.46	1255.44 (1255.29)	1174.49 (1174.32)	867.40 (867.42)	434.20		849.39 (849.49)
S	1359.50	1341.49 (1341.32)	1342.47 (1342.08)	1261.52 (1261.46)	704.33 (704.26)	352.67		686.32 (686.36)
A	1430.54	1412.53	1413.51	1332.56	617.30 (617.32)	309.15		599.29
E	1559.58	1541.57 (1541.45)	1542.55	1461.60 (1461.47)	546.26 (546.21)	273.64		528.25 (528.41)
Q	1687.64	1669.63	1670.61 (1670.13)	1589.66 (1589.23)	417.22 (417.17)	209.11		
N	1801.68	1783.67	1784.65 (1784.51)	1703.70	289.16	145.08		
R					175.12	88.06		

[M-H3PO4+2H]2+	939.25
[M-H3PO4-H2O+2H]2+	930.53
[M-H3PO4-NH3-H2O+2H]2+	922.16

Yellow:	Low&High mass cut off
Red:	Relative intensity >2
Theoretical m/z (Observed m/z)	

Supplemental Table S10. Fragmentation ions for native Cx43 peptide containing S372 and S373.

Cx43-IP #3307-3419 @639.61 ETD **MASCOT score: 14**

S372,S373 ETD	c	c 2+	[c+1]	[c+1]2+	z	z 2+	[z+1]
A							
S	176.10		177.11		1829.77	915.39 (915.20)	1830.78 (1830.56)
S	263.14		264.14		1742.74 (1742.64)	871.87 (871.61)	1743.75 (1743.74)
R	419.24	210.12	420.24	210.62	1655.71	828.36 (828.04)	1656.71
A	490.27	245.64	491.28	246.14	1499.61	750.31	1500.61
S	657.27 (657.41)	329.14	658.27 (658.31)	329.64	1428.57 (1428.28)	714.79 (714.66)	1429.58
S	824.27 (824.26)	412.64	825.27 (825.31)	413.14	1261.57 (1261.26)	631.29	1262.58 (1262.58)
R					1094.57 (1094.29)	547.79	1095.58 (1095.32)
P	1077.42 (1077.34)	539.22	1078.43 (107837)	539.72			
R					841.42 (841.29)	421.21	842.43 (842.45)
P	1330.58 (1330.35)	665.79 (665.45)	1331.58 (1331.15)	666.29 (666.07)			
D	1445.60 (1445.55)	723.31 (723.09)	1446.61 (1446.75)	723.81 (723.88)	588.26		589.27
D	1560.63 (1560.66)	780.82 (780.56)	1561.63 (1561.73)	781.32 (781.41)	473.24		474.24
L	1673.72	837.36 (837.36)	1674.72	837.86	358.21		359.22
E	1802.76	901.88 (901.89)	1803.76	902.38 (902.63)	245.13		246.13
I					116.08		117.09

[M+3H]3+	639.89

Yellow:	Low&High mass cut off
Red:	Relative intensity >0.1
Theoretical m/z (Observed m/z)	

Supplemental Table S11. Phosphoserine-containing peptides from native (in vivo) Cx43.

Sequence	Start-end	Charge	Theoretical mass	Observed mass	Phosphorylation site localized	MASCOT score (CID)	MASCOT score (ETD)	Diagnostic ions (CID)	Diagnostic ions (ETD)
QASEQNWANYSAEQNR	304-319	2	1974.786	1974.787	S306	52	N/A	y_{13}, y_{14}	N/A
ASSRASSRPRPDDLEI	367-382	3	1915.819	1915.809	S372, S373	N/A	14	N/A	z_9, z_{10}, z_{11}