SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Optimal reaction conditions for WT-hCTPS1 and WT-hCTPS2 activity. (A) Protein expression corresponding to CTPS activity assays. HEK293 cells expressing the designated hCTPS construct were lysed, immunoprecipitated with FLAG antibody conjugated to agarose beads, separated by SDS-PAGE and blotted for FLAG expression. Total protein immunoprecipitated for immunoblots corresponds to 10% of total protein immunoprecipitated for use in CTPS activity assays (0.5 mg for WT-CTPS1, 1.2 mg for ΔC-CTPS1, and 0.1 mg for WT-CPTS2, CTPS2-S568A, and CTPS2-S571A). Data is represented as SEM of total FLAG expression from samples corresponding to each day of CTPS activity assays. Activity conditions for hCTPS. HEK293 cells expressing WT-hCTPS1-FLAG (solid squares, solid line) and WT-hCTPS2-FLAG (open triangles, dotted line) were processed as in (A) and assayed for CTPS activity. (B) Samples were incubated at pH 8.1 at the indicated time intervals at 37ºC from 1 min to 90 min. Data are expressed as nmol CTP formed and are from n=2 (CTPS1) or n=6 (CTPS2) separate experiments. *Note: The y-axis is expressed as nmol CTP synthesized, not pmol/min CTP as on the other graphs.* (C) Samples were incubated in buffers with the indicated pH values for 1 hr (CTPS1) or 15 min (CTPS2) at 37ºC. Data are expressed as pmol/min CTP formed (n=3).

Supplemental Figure 2. Analysis of hCTPS oligomerization by gel filtration experiments. (A) Gel filtration standards were separated over a Superose 12 column in an ÄKTA FPLC. Data show a representative experiment and are expressed in mAU detected by the FPLC for each fraction. Peaks represent protein standards. (B) Gel filtration standards from panel A were plotted as a log of the standard molecular weight vs. fraction number, providing a linear standard curve. (C) Lysate from HEK293 cells expressing WT-hCTPS2-FLAG were separated over a Superose 12 column. Data show a representative experiment and are expressed in mAU detected by the FPLC for each fraction in buffer containing no nucleotides. Lysates from HEK293 cells expressing WT-hCTPS2-FLAG were separated over a Superose 12 column in the absence of nucleotides (blue lines, n=5 (CTPS1) and n=4 (CTPS2)), in the presence of 1 mM ATP (red lines, n=4 (CTPS1) and n=3 (CTPS2)), in the presence of 1 mM UTP (green lines, n=5 (CTPS1) and n=4 (CTPS2)), or in the presence of 1 mM ATP and 1 mM UTP (black lines, n=4 (CTPS1) and n=3 (CTPS2)). Fractions were then immunoblotted for (D) endogenous CTPS1 expression or (E) CTPS2-FLAG expression. CTPS monomers elute in fractions 24 and higher, CTPS dimers elute in fractions 22/23, and CTPS tetramers elute in fractions 21 and lower. Data are expressed as percent of total CTPS1 or CTPS2 from all fractions.

Supplemental Figure 3. Effect of carboxyl-terminal regulatory domain (CRD) deletion on hCTPS1 activity. HEK293 cells expressing WT-hCTPS1-FLAG (solid squares, solid line) or ΔC-hCTPS1-FLAG (open triangles, dotted line) were processed as in Supp Fig.1 and assayed for CTPS activity. (A) Samples were incubated for the indicated time intervals at 37ºC from 1 min to 90 min. Data are expressed as nmol CTP formed and are from 3 separate experiments. (B) Samples were incubated with the indicated UTP concentrations for 1 hr at 37ºC. (C) Samples were incubated with the indicated CTP concentrations for 1 hr at 37ºC. Data in panels B and C are expressed as pmol/min CTP formed and are from n=3 experiments. *Note: The WT-hCTPS1 data is the same data as presented in Supp. Fig. 1 and is shown here for comparison purposes.*

Supplemental Figure 4. MS/MS spectral evidence for hCTPS2 phosphorylation. HEK293 cells expressing WT-hCTPS2-FLAG were prepared for mass spectrometry analysis as detailed in Experimental Procedures after treatment with low serum (A-D) or high serum (E-G). Phosphorylation sites identified by LTQ-OrbiTrap were (A) S564, (B) S571, (C) S574, (D) S568 and S571, (E) Y567, (F) S571, and (G) S568 and S571.

Supplemental Figure 5. Total tryptic peptide sequence coverage of WT-hCTPS2 mass spectral analysis after (A) low serum and (B) high serum treatment.

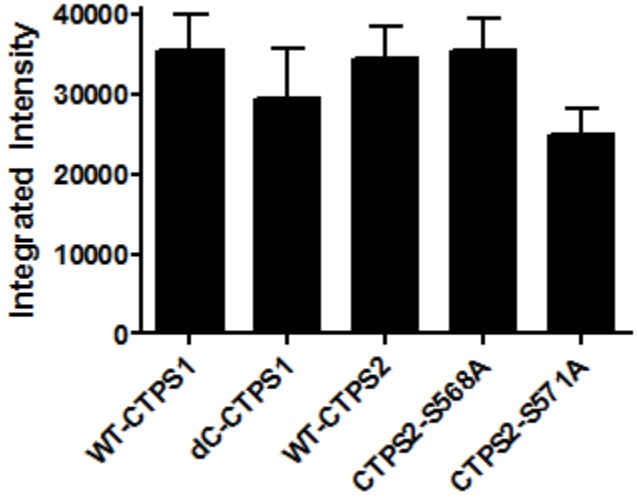
SUPPLEMENTAL TABLE

Supplemental Table 1. Primers used to make CTPS2 mutations. Mutated sequence is bolded and underlined.

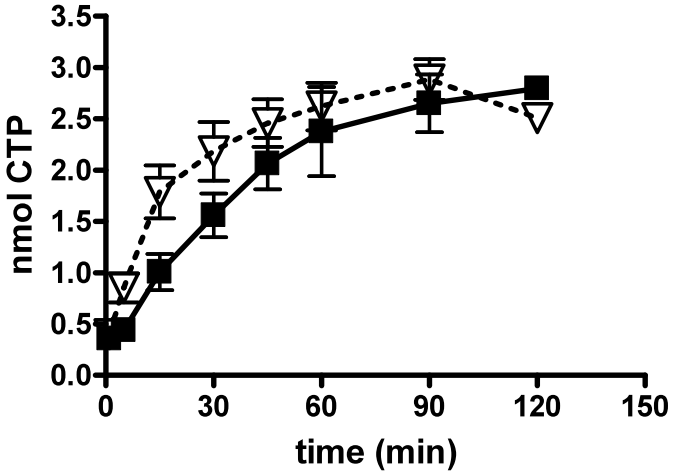
Mutation	Direction	Primer sequence
CTPS2-S564A	Forward	5'-GCAAACTGTCTTCC**GC**TGATAGATACAGTGATGCC-3'
CTPS2-S564A	Reverse	5'-GGCATCACTGTATCTATCA**GC**GGAAGACAGTTTGC-3'
CTPS2-Y567A	Forward	5'-TCTTCCAGTGATAGA**GC**CAGTGATGCCAGTGAT-3'
CTPS2-Y567A	Reverse	5'-ATCACTGGCATCACTG**GC**TCTATCACTGGAAGA-3'
CTPS2-S568A	Forward	5'-TCCAGTGATAGATAC**GC**TGATGCCAGTGATGAC-3'
CTPS2-S568A	Reverse	5'-GTCATCACTGGCATCA**GC**GTATCTATCACTGGA-3'
CTPS2-S571A	Forward	5'-AGATACAGTGATGCC**GC**TGATGACAGCTTTTCA-3'
CTPS2-S571A	Reverse	5'-TGAAAAGCTGTCATCA**GC**GGCATCACTGTATCT-3'
CTPS2-S574A	Forward	5'-GATGCCAGTGATGAC**GC**CTTTTCAGAGCCAAGG-3'
CTPS2-S574A	Reverse	5'-CCTTGGCTCTGAAAAG**GC**GTCATCACTGGCATC-3'

Supplemental Figure 1

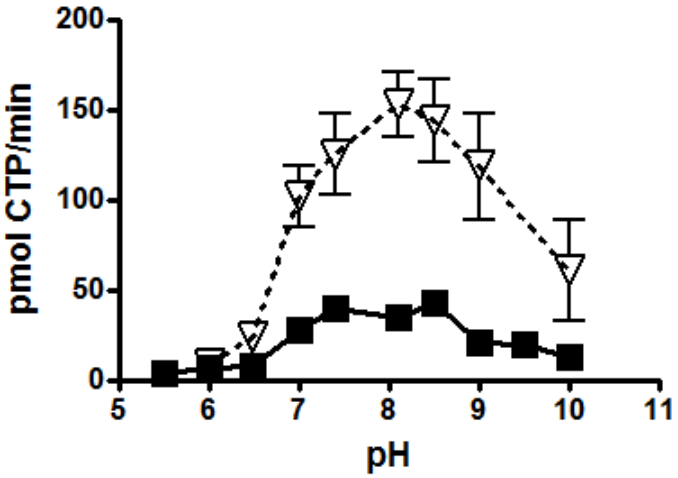
A



B

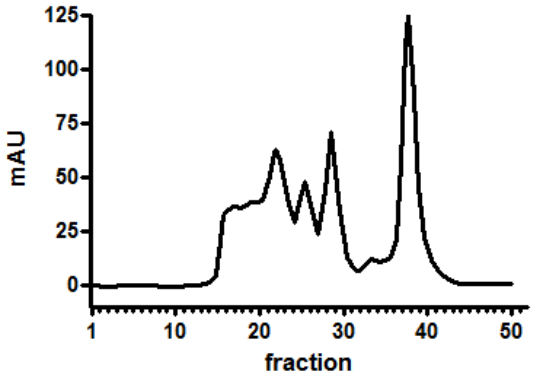


C



Supplemental Figure 2

A



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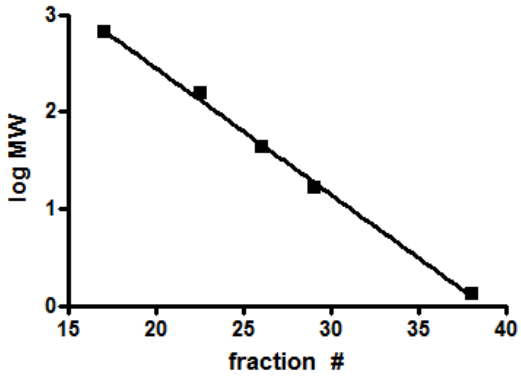

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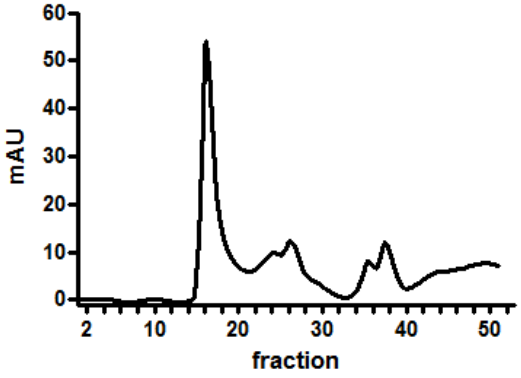

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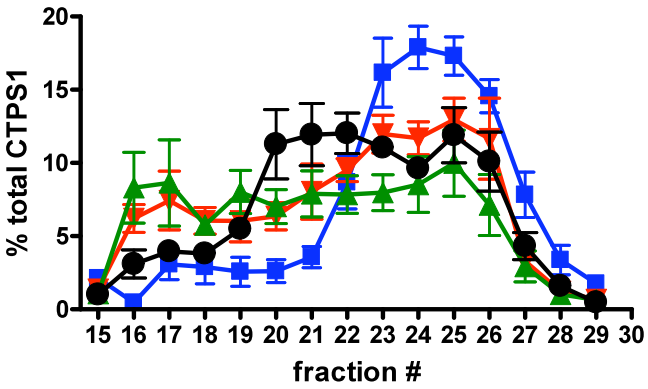

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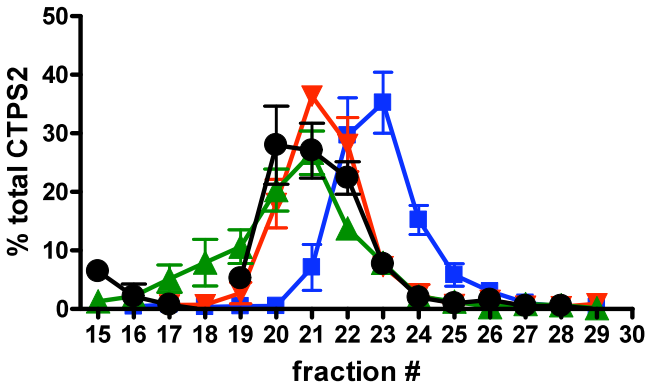
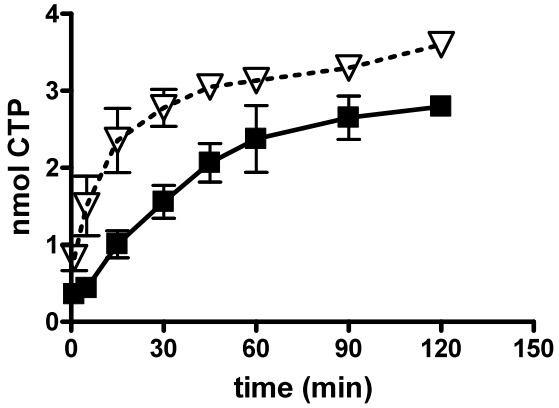
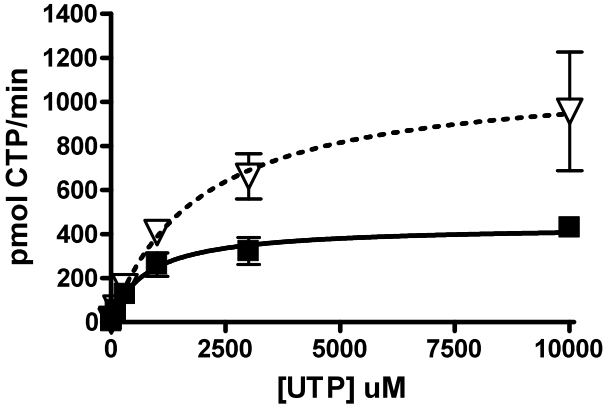


Supplemental Figure 3

A



B



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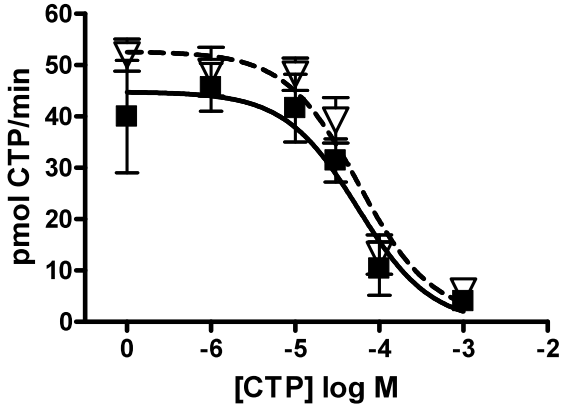
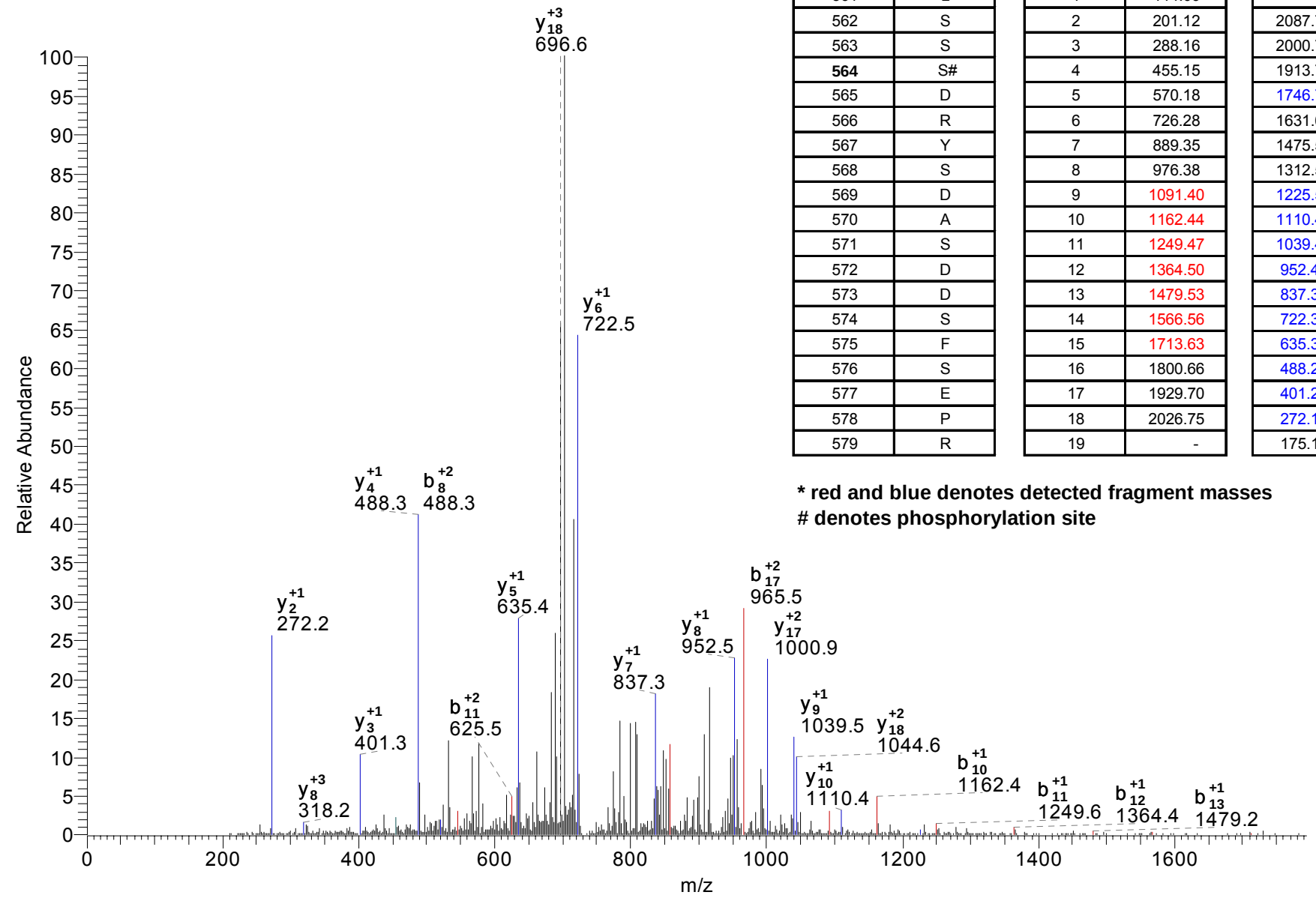


Supplemental Figure 4A



Residue	
561	L
562	S
563	S
564	S#
565	D
566	R
567	Y
568	S
569	D
570	A
571	S
572	D
573	D
574	S
575	F
576	S
577	E
578	P
579	R

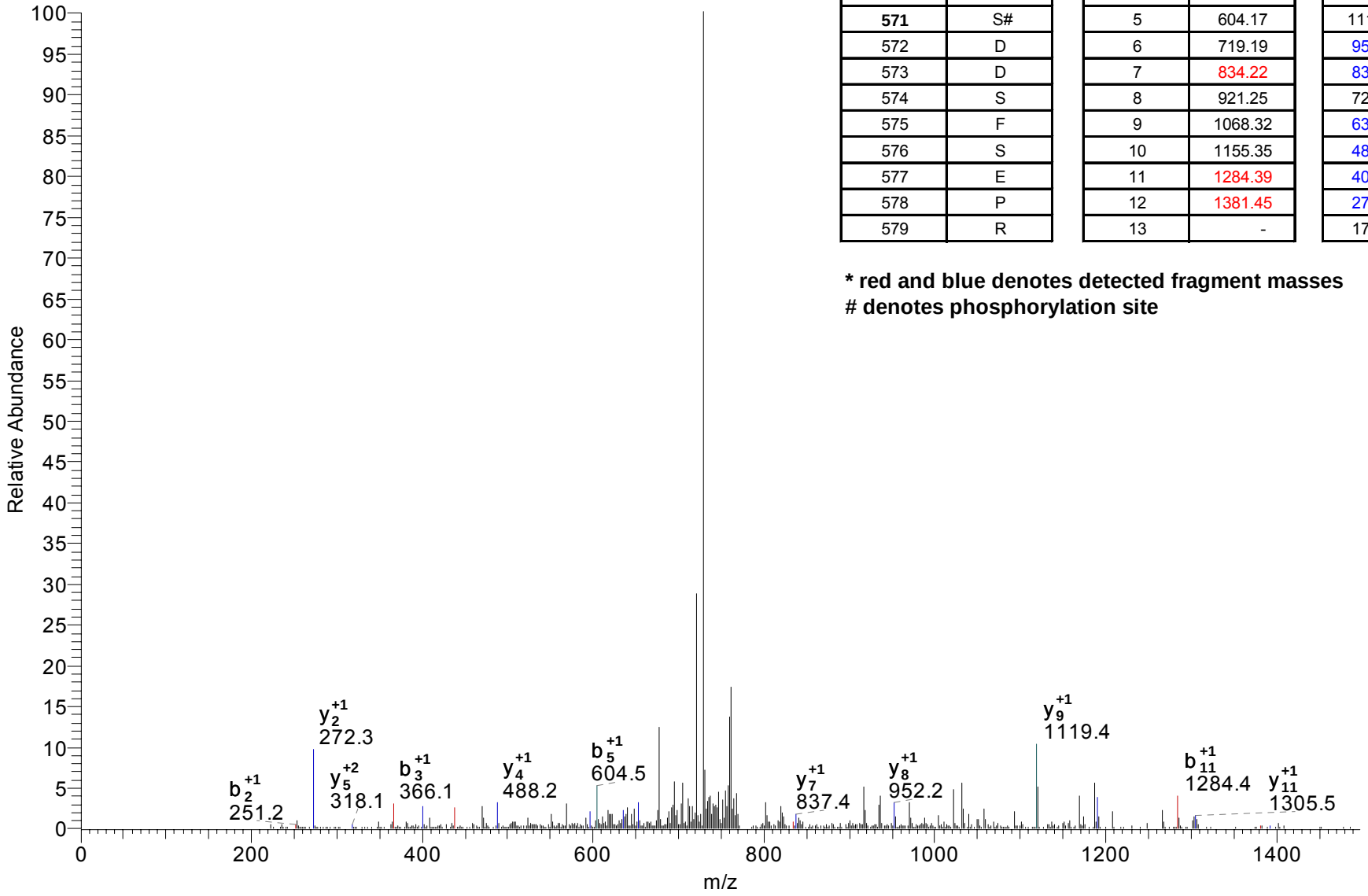
b-fragment ions	
1	114.09
2	201.12
3	288.16
4	455.15
5	570.18
6	726.28
7	889.35
8	976.38
9	1091.40
10	1162.44
11	1249.47
12	1364.50
13	1479.53
14	1566.56
15	1713.63
16	1800.66
17	1929.70
18	2026.75
19	-

y-fragment ions	
-	19
2087.78	18
2000.75	17
1913.72	16
1746.72	15
1631.69	14
1475.59	13
1312.53	12
1225.50	11
1110.47	10
1039.43	9
952.40	8
837.37	7
722.35	6
635.31	5
488.25	4
401.21	3
272.17	2
175.12	1

*** red and blue denotes detected fragment masses**
 # denotes phosphorylation site

Supplemental Figure 4B

#1768-1768 RT:32.07-32.07 NL: 1.01E3



Residue		b-fragment ions		y-fragment ions	
567	Y	1	164.07	-	13
568	S	2	251.10	1392.50	12
569	D	3	366.13	1305.46	11
570	A	4	437.17	1190.44	10
571	S#	5	604.17	1119.40	9
572	D	6	719.19	952.40	8
573	D	7	834.22	837.37	7
574	S	8	921.25	722.35	6
575	F	9	1068.32	635.31	5
576	S	10	1155.35	488.25	4
577	E	11	1284.39	401.21	3
578	P	12	1381.45	272.17	2
579	R	13	-	175.12	1

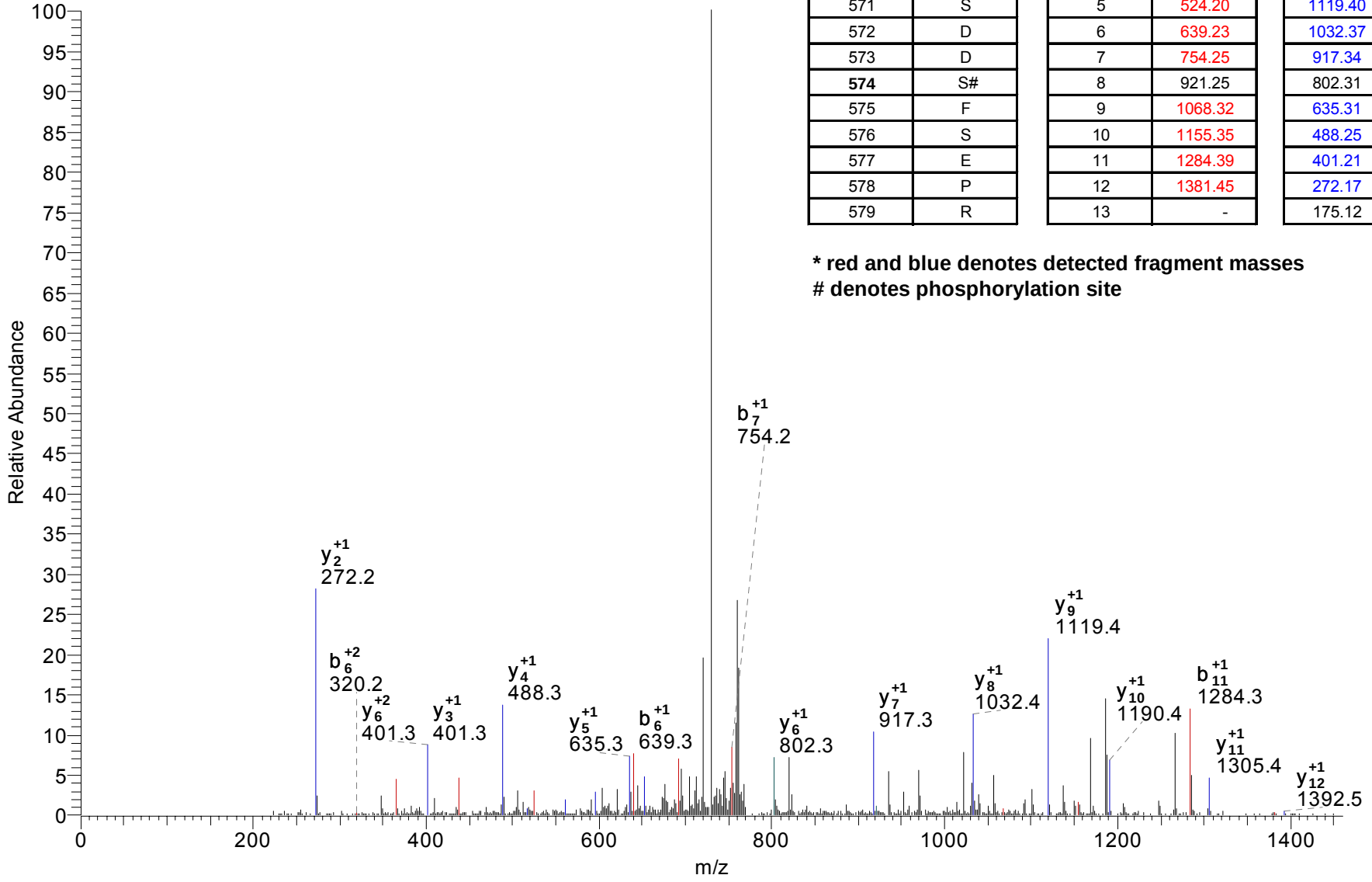
*** red and blue denotes detected fragment masses**
 # denotes phosphorylation site

Supplemental Figure 4C

#1734-1734 RT:31.48-31.48 NL: 9.74E2

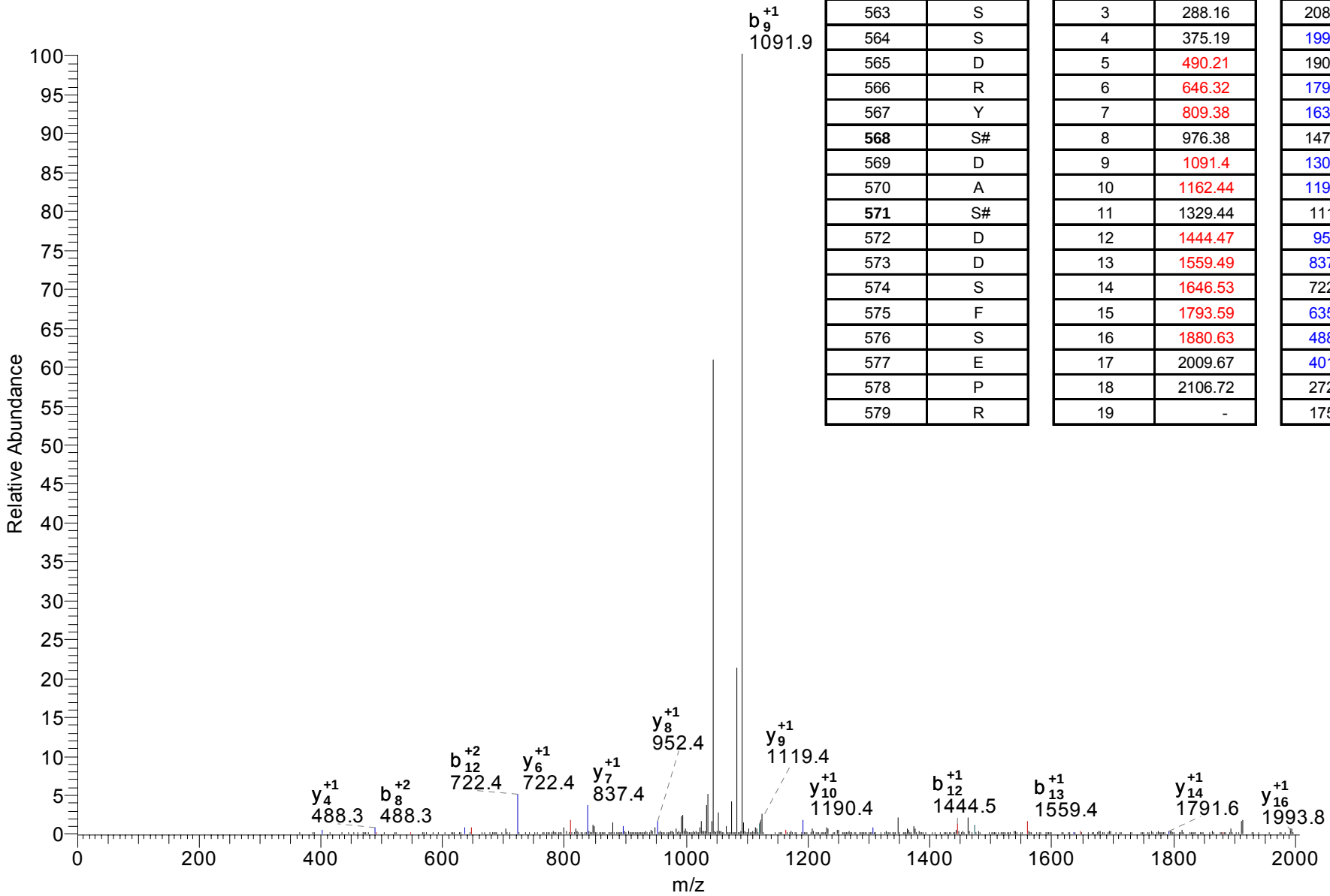
Residue		b-fragment ions		y-fragment ions	
567	Y	1	164.07	-	13
568	S	2	251.10	1392.50	12
569	D	3	366.13	1305.46	11
570	A	4	437.17	1190.44	10
571	S	5	524.20	1119.40	9
572	D	6	639.23	1032.37	8
573	D	7	754.25	917.34	7
574	S#	8	921.25	802.31	6
575	F	9	1068.32	635.31	5
576	S	10	1155.35	488.25	4
577	E	11	1284.39	401.21	3
578	P	12	1381.45	272.17	2
579	R	13	-	175.12	1

*** red and blue denotes detected fragment masses**
 # denotes phosphorylation site



Supplemental Figure 4D

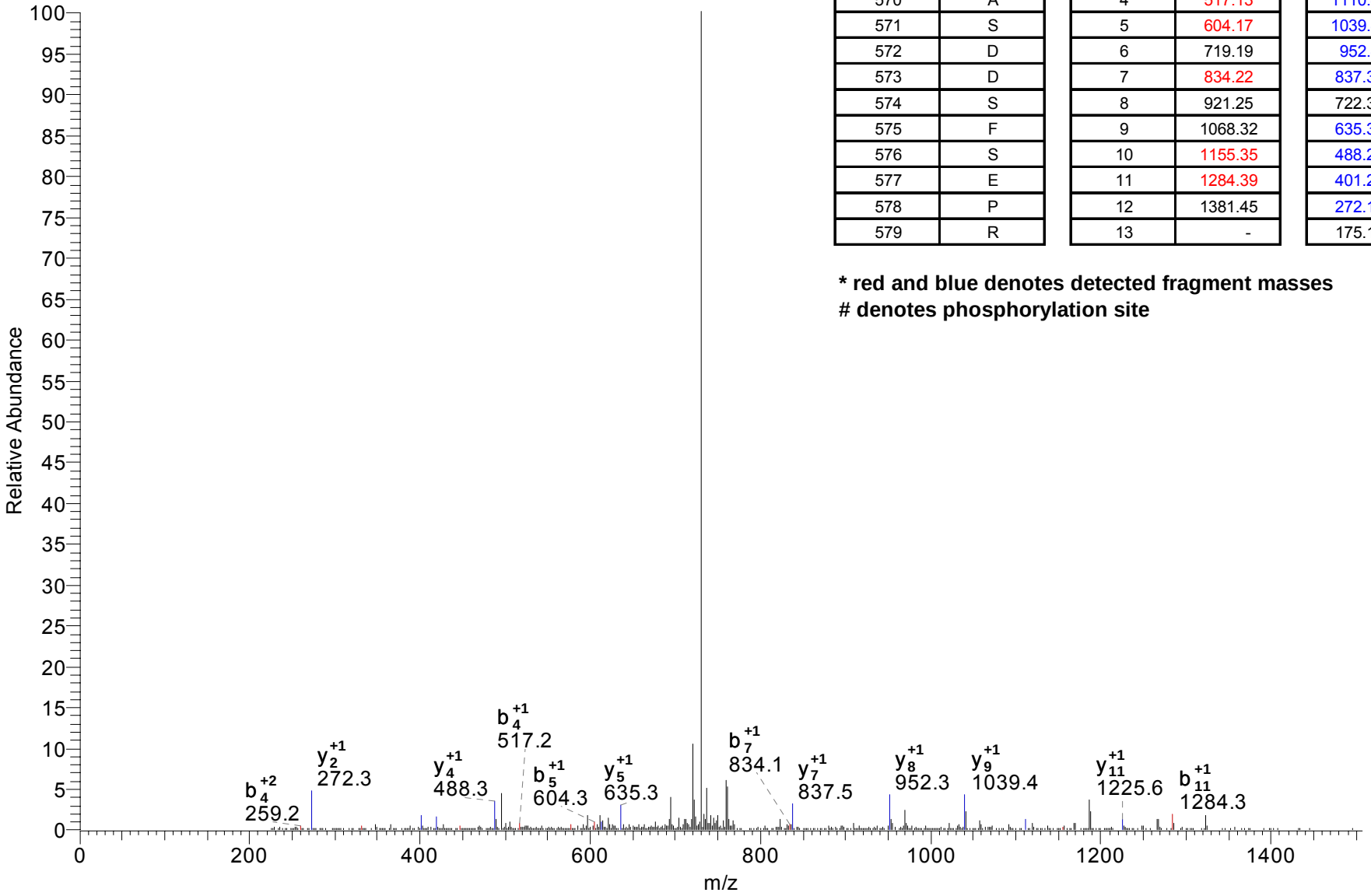
#1888-1888 RT:34.11-34.11 NL: 8.75E3



Residue		b-fragment ions		y-fragment ions	
561	L	1	114.09	-	19
562	S	2	201.12	2167.75	18
563	S	3	288.16	2080.72	17
564	S	4	375.19	1993.68	16
565	D	5	490.21	1906.65	15
566	R	6	646.32	1791.63	14
567	Y	7	809.38	1635.52	13
568	S#	8	976.38	1472.46	12
569	D	9	1091.4	1305.46	11
570	A	10	1162.44	1190.44	10
571	S#	11	1329.44	1119.4	9
572	D	12	1444.47	952.4	8
573	D	13	1559.49	837.37	7
574	S	14	1646.53	722.35	6
575	F	15	1793.59	635.31	5
576	S	16	1880.63	488.25	4
577	E	17	2009.67	401.21	3
578	P	18	2106.72	272.17	2
579	R	19	-	175.12	1

Supplemental Figure 4E

#2142-2142 RT:38.51-38.51 NL: 5.00E3



Residue	
567	Y#
568	S
569	D
570	A
571	S
572	D
573	D
574	S
575	F
576	S
577	E
578	P
579	R

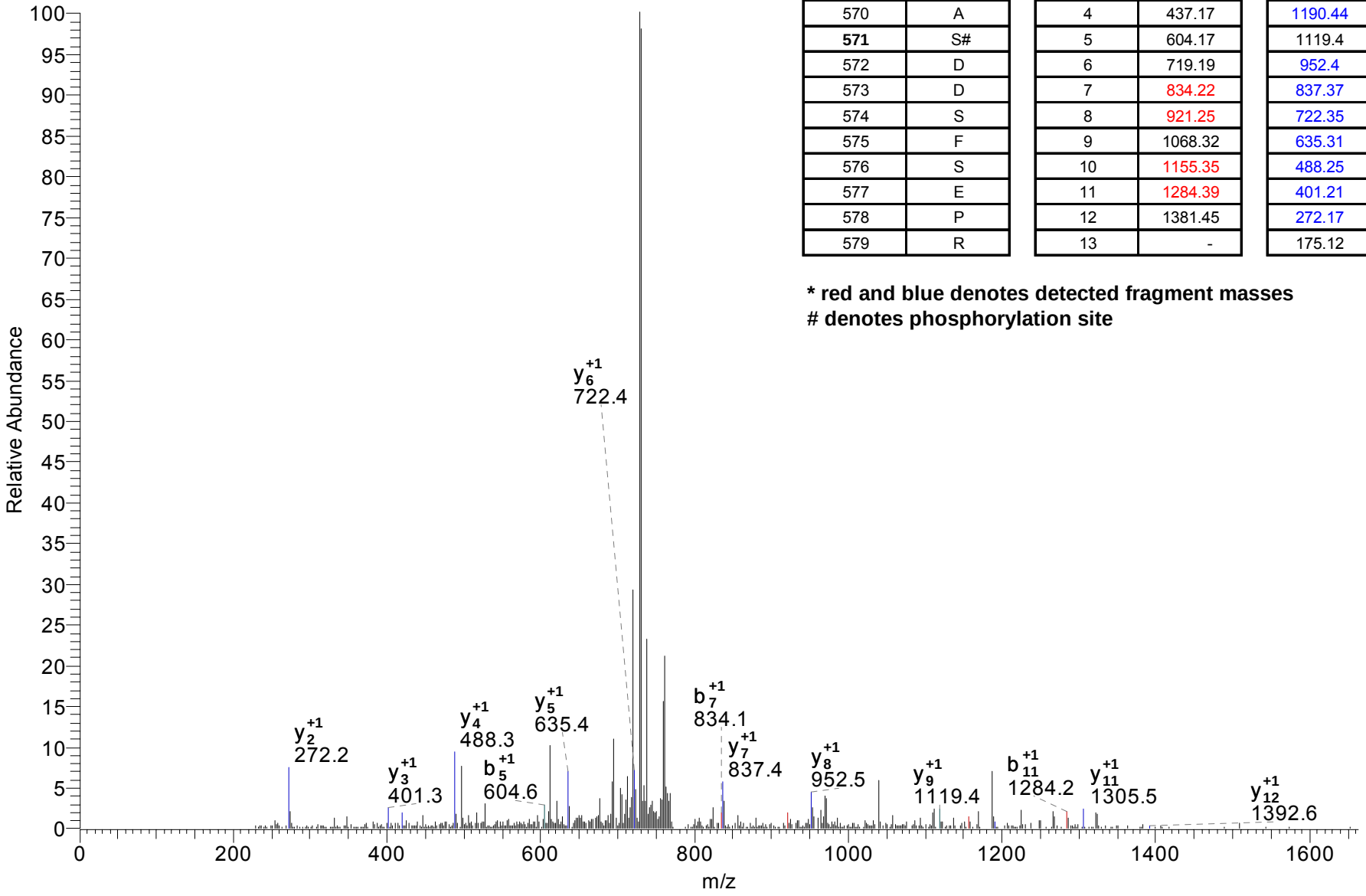
b-fragment ions	
1	244.04
2	331.07
3	446.1
4	517.13
5	604.17
6	719.19
7	834.22
8	921.25
9	1068.32
10	1155.35
11	1284.39
12	1381.45
13	-

y-fragment ions	
-	13
1312.53	12
1225.5	11
1110.47	10
1039.43	9
952.4	8
837.37	7
722.35	6
635.31	5
488.25	4
401.21	3
272.17	2
175.12	1

*** red and blue denotes detected fragment masses**
 # denotes phosphorylation site

Supplemental Figure 4F

#2129-2129 RT:38.29-38.29 NL: 1.47E3

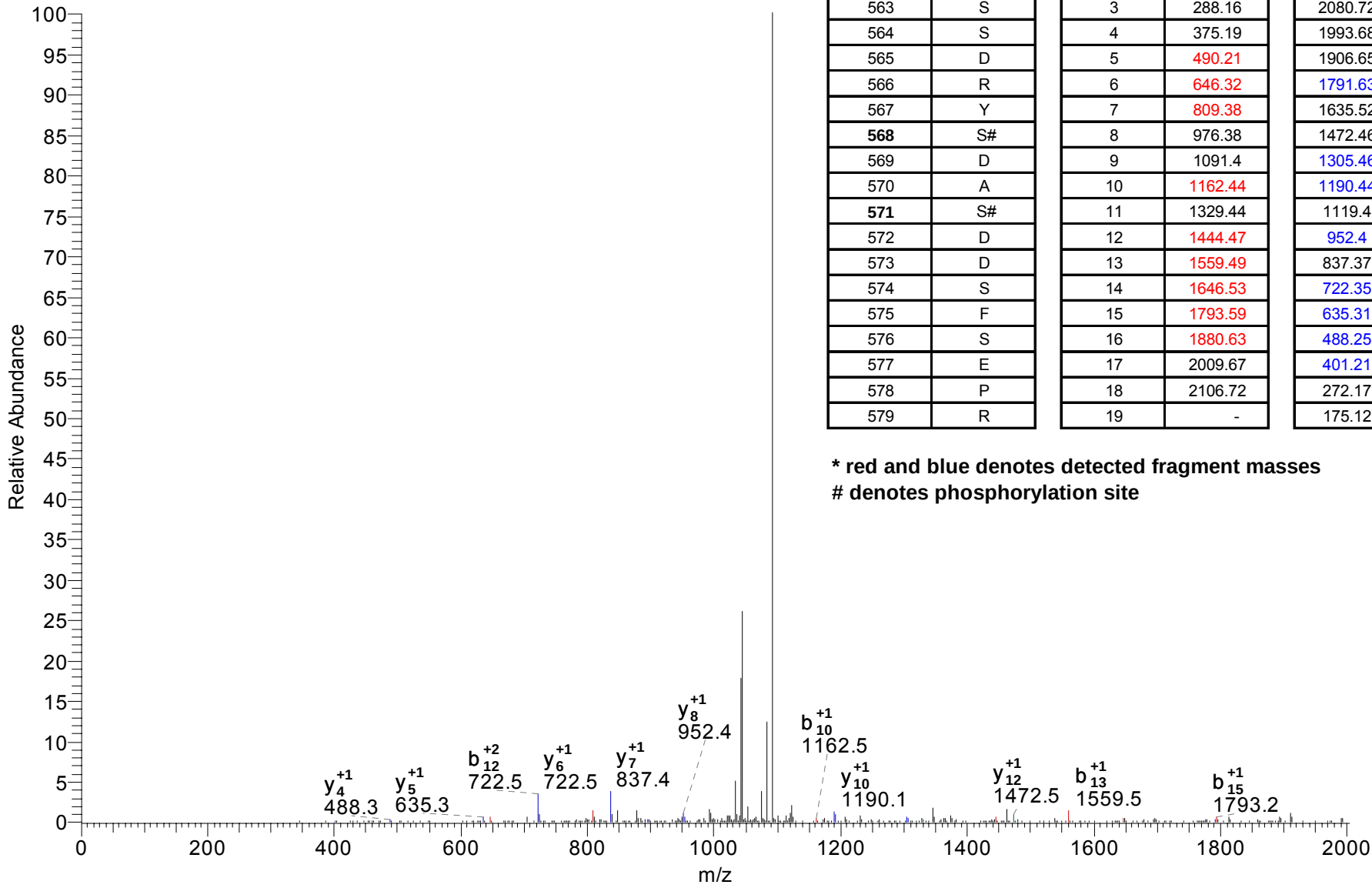


Residue		b-fragment ions		y-fragment ions	
567	Y	1	164.07	-	13
568	S	2	251.1	1392.5	12
569	D	3	366.13	1305.46	11
570	A	4	437.17	1190.44	10
571	S#	5	604.17	1119.4	9
572	D	6	719.19	952.4	8
573	D	7	834.22	837.37	7
574	S	8	921.25	722.35	6
575	F	9	1068.32	635.31	5
576	S	10	1155.35	488.25	4
577	E	11	1284.39	401.21	3
578	P	12	1381.45	272.17	2
579	R	13	-	175.12	1

*** red and blue denotes detected fragment masses**
 # denotes phosphorylation site

Supplemental Figure 4G

#2239-2239 RT:40.10-40.10 NL: 5.30E3



Residue	
561	L
562	S
563	S
564	S
565	D
566	R
567	Y
568	S#
569	D
570	A
571	S#
572	D
573	D
574	S
575	F
576	S
577	E
578	P
579	R

b-fragment ions	
1	114.09
2	201.12
3	288.16
4	375.19
5	490.21
6	646.32
7	809.38
8	976.38
9	1091.4
10	1162.44
11	1329.44
12	1444.47
13	1559.49
14	1646.53
15	1793.59
16	1880.63
17	2009.67
18	2106.72
19	-

y-fragment ions	
-	19
2167.75	18
2080.72	17
1993.68	16
1906.65	15
1791.63	14
1635.52	13
1472.46	12
1305.46	11
1190.44	10
1119.4	9
952.4	8
837.37	7
722.35	6
635.31	5
488.25	4
401.21	3
272.17	2
175.12	1

*** red and blue denotes detected fragment masses**
 # denotes phosphorylation site

Supplemental Figure 5

A LS Sequence Coverage (39.25% by amino acids)

MKYILVTGGVISGIGKGIIASSIGTILKSCGLRVTAIKIDPYINIDAGTFSPYEHGEVFVLNDGGEVDLDLGNYERFLD
INLYKDNNITTGKIYQHVINKERRGDYLGKTVQVVPHITDAVQEWVMNQAKVPVDGNKEEPQICVIELGGTIGDIEGMP
FVEAFRQFQFKAKRENFCNIHVSLVPQLSATGEQKTKPTQNSVRALRGLGLSPDLIVCRSSTPIEMAVKEKISMFCHVN
PEQVICIHDVSSTYRVPVLLEEQSIVKYFKERLHLPIGDSASNLLFKWRNMADRYERLQKICSIALVGKYTKLRDCYAS
VFKALEHSALAINHKLNLMYIDSIDLEKITETEDPVKFHEAWQKLCKADGILVPGGFGIRGTLGKLQAISWARTKKIPF
LGVCLGMQLAVIEFARNCLNLKDADSTEFRPNAPVPLVIDMPEHNPGNLGGTMRLGIRRTVFKTENSILRKLYGDVPFI
EERHRHRFEVNPNLIKQFEQNDLSFVGQDVDGDRMEIIELANHPYFVGVQFHPEFSSRPMKPSPPYLGLLLAATGNLNA
YLQQGCKLSSSDRYSDASDDSFSEPRIAELEIS _

B HS Sequence Coverage (37.54% by amino acids)

MKYILVTGGVISGIGKGIIASSIGTILKSCGLRVTAIKIDPYINIDAGTFSPYEHGEVFVLNDGGEVDLDLGNYERFLD
INLYKDNNITTGKIYQHVINKERRGDYLGKTVQVVPHITDAVQEWVMNQAKVPVDGNKEEPQICVIELGGTIGDIEGMP
FVEAFRQFQFKAKRENFCNIHVSLVPQLSATGEQKTKPTQNSVRALRGLGLSPDLIVCRSSTPIEMAVKEKISMFCHVN
PEQVICIHDVSSTYRVPVLLEEQSIVKYFKERLHLPIGDSASNLLFKWRNMADRYERLQKICSIALVGKYTKLRDCYAS
VFKALEHSALAINHKLNLMYIDSIDLEKITETEDPVKFHEAWQKLCKADGILVPGGFGIRGTLGKLQAISWARTKKIPF
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YLQQGCKLSSSDRYSDASDDSFSEPRIAELEIS _