

# ProPhosSI MS/MS report

Mass: 480.5522 Charge: 3+

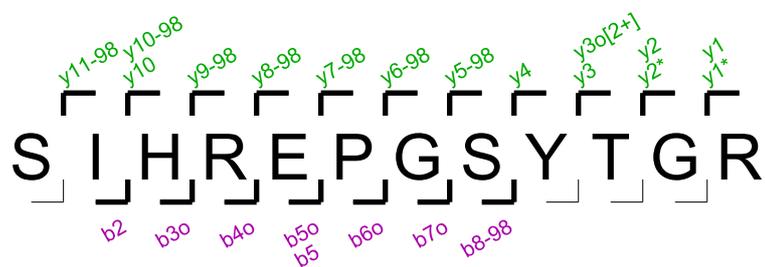
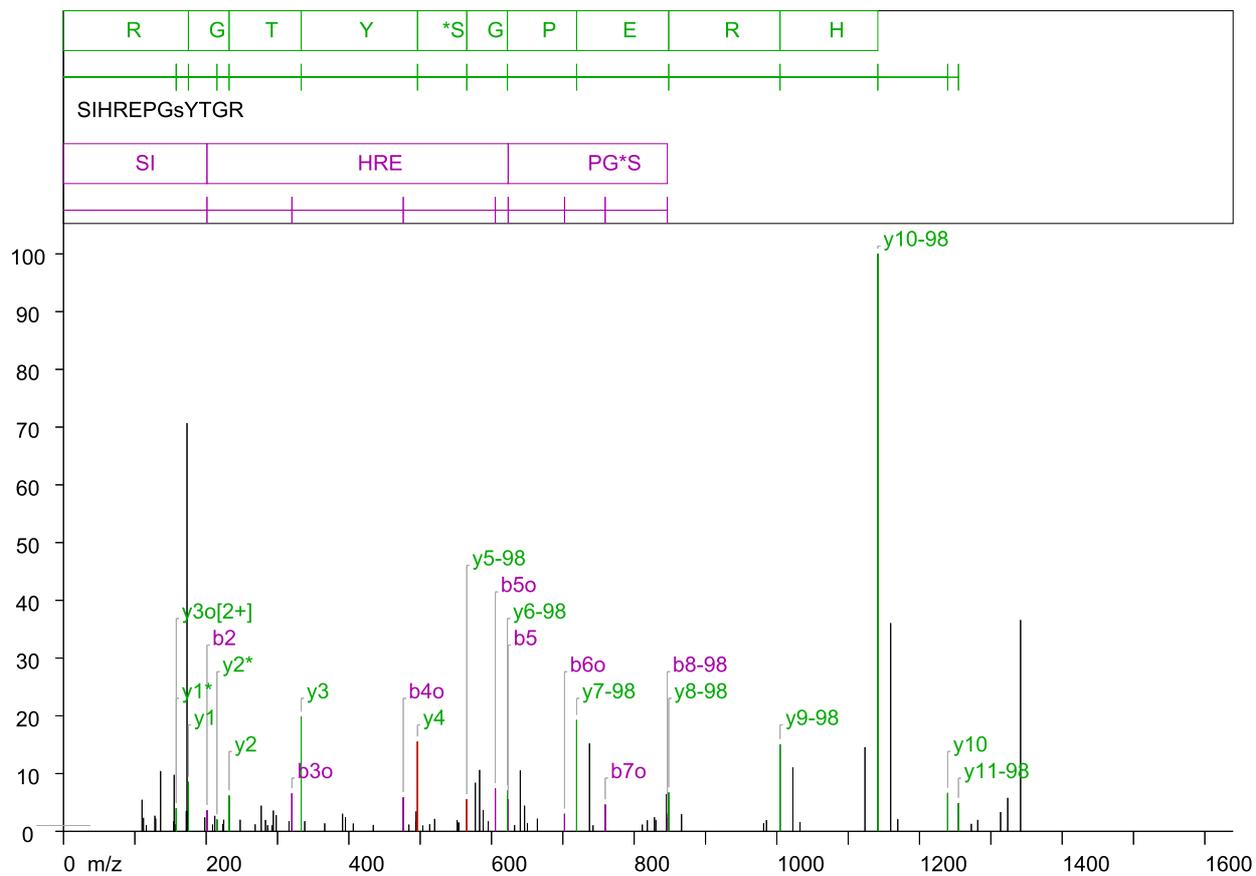


Figure S3

## 5-HT2A

(19) 298 SIHREPGsYTGR 309 1438.635 (-0.0023) Da

Parent Ion	m/z
No parent ions observed	

### Modifications and supporting evidence

Position	Residue ID	Modification	Evidence
8	(305)	Phospho (ST)	y4 => y5-98

### Spectrum interpretation

Rule	passed/tests	Description
Parent ions present	0/1	Parent ions corresponding to the parent fragment - 1 phosphate ions were not found
Three -98 Ions present	1/1	8 des-phospho fragment ions were found.
Unique -98 transitions present	1/1	transition b5 to b8-98, transition y4 to y5-98 support unique phosphorylation at position 8  
Four Sequential b or y ions	1/1	Sequence of four y ions found from y1 to y4.
Five of six sequential ions present	1/1	Five of Six ions found between y0 and y5 Five of Six ions found between y1 and y6 Five of Six ions found between y2 and y7 Five of Six ions found between y3 and y8 Five of Six ions found between y4 and y9 Five of Six ions found between y5 and y10 Five of Six ions found between y6 and y11 Five of Six ions found between y7 and y12
Proline directed fragmentation pattern	2/2	PASS: y7-98> y6-98 with ratio 2.73  PASS: b6< b5 
PhosphoTyrosine transition present	0/0	0 of 0 phosphotyrosine transitions were found.
Six of top ten ions identified	6/6	ion 1 (mass: 1141.54739802914: intensity: 44986.46) assigned 1 times ion 2 (mass: 173.128267363354: intensity: 31804.3) assigned 1 times ion 3 (mass: 1341.66350384628: intensity: 16458.98) assigned 0 times ion 4 (mass: 1159.55771029917: intensity: 16222.57) assigned 0 times ion 5 (mass: 333.187740955122: intensity: 8918.521) assigned 1 times ion 6 (mass: 719.345574992868: intensity: 8661.293) assigned 1 times ion 7 (mass: 496.250112167794: intensity: 6978.598) assigned 1 times ion 8 (mass: 737.357172637009: intensity: 6848.646) assigned 0 times ion 9 (mass: 1004.48869263796: intensity: 6775.796) assigned 1 times ion 10 (mass: 1123.52751604662: intensity: 6555.282) assigned 0 times

### Ion Table

27 ions assigned of 58 ions above threshold (46%).

#### N-terminal ions

AA	N-ion	b	b*	b-98	bo
S	1	88.039	71.013	-	70.029
I	2	201.123 201.122769713569 (3)	184.097	-	183.113

Figure S3

H	3	338.182	321.156	-	320.172 320.171716192964 (6)
R	4	494.283	477.257	-	476.273 476.27210229895 (5)
E	5	623.326 623.327106517243 (5)	606.300	-	605.315 605.315428579618 (7)
P	6	720.379	703.352	-	702.368 702.365826177584 (3)
G	7	777.400	760.374	-	759.390 759.389875865003 (4)
s	8	944.399	927.372	846.413 846.416022543775 (2)	926.388
Y	9	1107.462	1090.435	1009.476	1089.451
T	10	1208.510	1191.483	1110.524	1190.499
G	11	1265.531	1248.505	1167.545	1247.521
R	12	-	-	-	-

### C-terminal ions

AA	C-ion	y	y*	y-98	yo
S	12	-	-	-	-
I	11	1352.611	1335.584	1254.625 1254.62997686966 (4)	1334.600
H	10	1239.527 1239.52446492728 (6)	1222.500	1141.541 1141.54739802914 (100)	1221.516
R	9	1102.468	1085.441	1004.482 1004.48869263796 (15)	1084.457
E	8	946.367	929.340	848.381 848.389900903719 (6)	928.356
P	7	817.324	800.298	719.338 719.345574992868 (19)	799.313
G	6	720.271	703.245	622.285 622.292991198655 (7)	702.261
s	5	663.250	646.223	565.264 565.271975898375 (5)	645.239
Y	4	496.251 496.250112167794 (15)	479.225	-	478.241
T	3	333.188 333.187740955122 (19)	316.162	-	315.178 *158.092181421098 [2+] (3)
G	2	232.140 232.139823910178 (6)	215.114 *215.114104417971 (2)	-	214.130
R	1	175.119 175.1188112221 (8)	158.092 *158.092181421098 (3)	-	157.108

### Ion distribution

Threshold	Ion count	Matches	% matched
0	167	43	25
0.5	131	42	32
1	91	36	39
2	57	26	45
3	43	23	53
4	33	20	60
5	29	18	62

Figure S3

10	14	6	42
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**Observed ions > 1%**

m/z	Intensity	% max	Assignment (delta)
110.071076830779	2454.305 <sub>2</sub>	5.45	
112.086742942952	1050.391 <sub>2</sub>	2.33	
116.070168040569	489.2661 <sub>2</sub>	1.08	
128.106678287448	1210.183 <sub>2</sub>	2.69	
129.102281820922	996.1835 <sub>2</sub>	2.21	
136.075537451598	4694.133 <sub>2</sub>	10.43	
154.086152299264	766.0502 <sub>2</sub>	1.70	
155.117711920818	4397.351 <sub>2</sub>	9.77	a2o (-0.00)
157.107434236106	543.4645 <sub>2</sub>	1.20	y1o (-0.00)
158.092181421098	1798.877 <sub>2</sub>	3.99	y1* (-0.00) : z1 (-0.00) : y3o[2+] (-0.00)
172.097676354675	1578.232 <sub>2</sub>	3.50	
173.128267363354	31804.3 <sub>2</sub>	70.69	a2 (-0.00)
174.131935646885	1609.095 <sub>2</sub>	3.57	
175.1188112221	3847.437 <sub>2</sub>	8.55	y1 (-0.00)
198.110720465684	1084.236 <sub>2</sub>	2.41	
201.122769713569	1641.54 <sub>2</sub>	3.64	b2 (-0.00)
209.093127220799	525.1947 <sub>2</sub>	1.16	
212.107887020317	1203.554 <sub>2</sub>	2.67	
215.114104417971	917.289 <sub>2</sub>	2.03	y2* (-0.00) : z2 (-0.00)
223.107291724338	574.8371 <sub>2</sub>	1.27	
224.642198621442	901.2441 <sub>2</sub>	2.00	a4o[2+] (-0.00)
232.139823910178	2780.697 <sub>2</sub>	6.18	y2 (-0.00)
247.645068826104	897.6563 <sub>2</sub>	1.99	b4[2+] (-0.00)
268.650235767384	543.7061 <sub>2</sub>	1.20	
277.140888243323	1994.866 <sub>2</sub>	4.43	
283.140609162195	886.0125 <sub>2</sub>	1.96	y5-98[2+] (0.00)
286.152345542359	475.0163 <sub>2</sub>	1.05	
292.174451389299	467.0362 <sub>2</sub>	1.03	a3o (-0.00)
294.167178001733	1616.965 <sub>2</sub>	3.59	
298.169941088898	1258.481 <sub>2</sub>	2.79	a5[2+] (0.00)
316.16079132642	783.9474 <sub>2</sub>	1.74	y3* (-0.00) : z3 (-0.00)
320.171716192964	2947.234 <sub>2</sub>	6.55	b3o (-0.00)
333.187740955122	8918.521 <sub>2</sub>	19.82	y3 (-0.00)
338.182145398699	785.4046 <sub>2</sub>	1.74	b3 (-0.00) : a6*[2+] (-0.00)
366.20048450588	620.6774 <sub>2</sub>	1.37	a7o[2+] (-0.00)
391.190164483198	1367.319 <sub>2</sub>	3.03	
395.214813943905	1098.155 <sub>2</sub>	2.44	
406.182324393038	604.7844 <sub>2</sub>	1.34	

Figure S3

434.255563645439	497.3898 <sub>2</sub>	1.10	
476.27210229895	2641.643 <sub>2</sub>	5.87	b4o (-0.00)
484.220848757801	522.1862 <sub>2</sub>	1.16	
493.741901545571	799.8482 <sub>2</sub>	1.77	
494.237456448707	1548.247 <sub>2</sub>	3.44	
496.250112167794	6978.598 <sub>2</sub>	15.51	y4 (-0.00)
513.313764564873	554.2643 <sub>2</sub>	1.23	
520.262459462494	966.4487 <sub>2</sub>	2.14	
551.73855844142	883.6255 <sub>2</sub>	1.96	y9[2+] (0.00)
553.76437739151	698.5909 <sub>2</sub>	1.55	
554.256369829851	654.6957 <sub>2</sub>	1.45	
565.271975898375	2487.078 <sub>2</sub>	5.52	y5-98 (0.00)
577.321064695588	3784.517 <sub>2</sub>	8.41	a5o (-2.03)
583.282146582763	4782.396 <sub>2</sub>	10.63	
588.284787843726	1658.776 <sub>2</sub>	3.68	
595.33330447741	783.0561 <sub>2</sub>	1.74	a5 (0.00)
605.315428579618	3332.373 <sub>2</sub>	7.40	b5o (-0.00)
622.292991198655	3168.379 <sub>2</sub>	7.04	y6-98 (0.00)
623.327106517243	2486.213 <sub>2</sub>	5.52	b5 (0.00)
632.279768050137	492.2994 <sub>2</sub>	1.09	
640.302910738211	4740.84 <sub>2</sub>	10.53	
646.302397227137	1994.46 <sub>2</sub>	4.43	
650.28781272197	624.7202 <sub>2</sub>	1.38	
664.292443229736	982.4763 <sub>2</sub>	2.18	
702.365826177584	1360.006 <sub>2</sub>	3.02	b6o (-0.00)
719.345574992868	8661.293 <sub>2</sub>	19.25	y7-98 (0.00)
737.357172637009	6848.646 <sub>2</sub>	15.22	
742.356767068762	468.9181 <sub>2</sub>	1.04	
759.389875865003	2081.157 <sub>2</sub>	4.62	b7o (-0.00)
811.377117946756	522.7847 <sub>2</sub>	1.16	
818.42739371774	859.532 <sub>2</sub>	1.91	
828.411541627073	1096.676 <sub>2</sub>	2.43	
830.37852526995	884.2842 <sub>2</sub>	1.96	
845.415563251431	2900.609 <sub>2</sub>	6.44	
846.416022543775	981.6791 <sub>2</sub>	2.18	b8-98 (0.00)
848.389900903719	3015.715 <sub>2</sub>	6.70	y8-98 (0.00)
866.395009921475	1343.407 <sub>2</sub>	2.98	
981.489141769933	633.0308 <sub>2</sub>	1.40	
985.447603657645	874.1708 <sub>2</sub>	1.94	
1004.48869263796	6775.796 <sub>2</sub>	15.06	y9-98 (0.00)
1022.50039036938	4975.601 <sub>2</sub>	11.06	
1032.48010537916	714.6226 <sub>2</sub>	1.58	
1123.52751604662	6555.282 <sub>2</sub>	14.57	

Figure S3

1141.54739802914	44986.46 <sub>2</sub>	100	y10-98 (0.00)
1159.55771029917	16222.57 <sub>2</sub>	36.06	
1169.53917620416	944.6916 <sub>2</sub>	2.09	
1239.52446492728	2985.584 <sub>2</sub>	6.63	y10 (-0.00)
1254.62997686966	2199.473 <sub>2</sub>	4.88	y11-98 (0.00)
1272.64146974132	580.8317 <sub>2</sub>	1.29	
1281.60705586191	888.5591 <sub>2</sub>	1.97	
1313.67024780943	1500.13 <sub>2</sub>	3.33	
1323.65136649451	2605.259 <sub>2</sub>	5.79	
1341.66350384628	16458.98 <sub>2</sub>	36.58	

Figure S3

# ProPhosSI MS/MS report

Mass: 480.5517 Charge: 3+

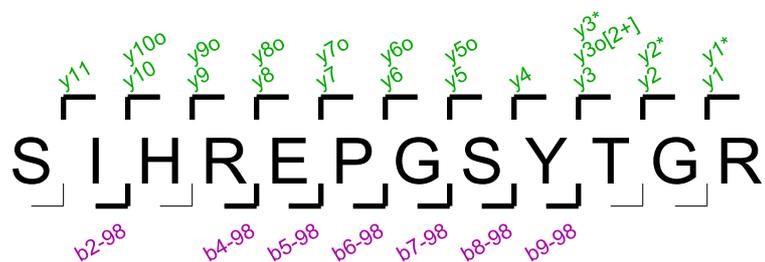
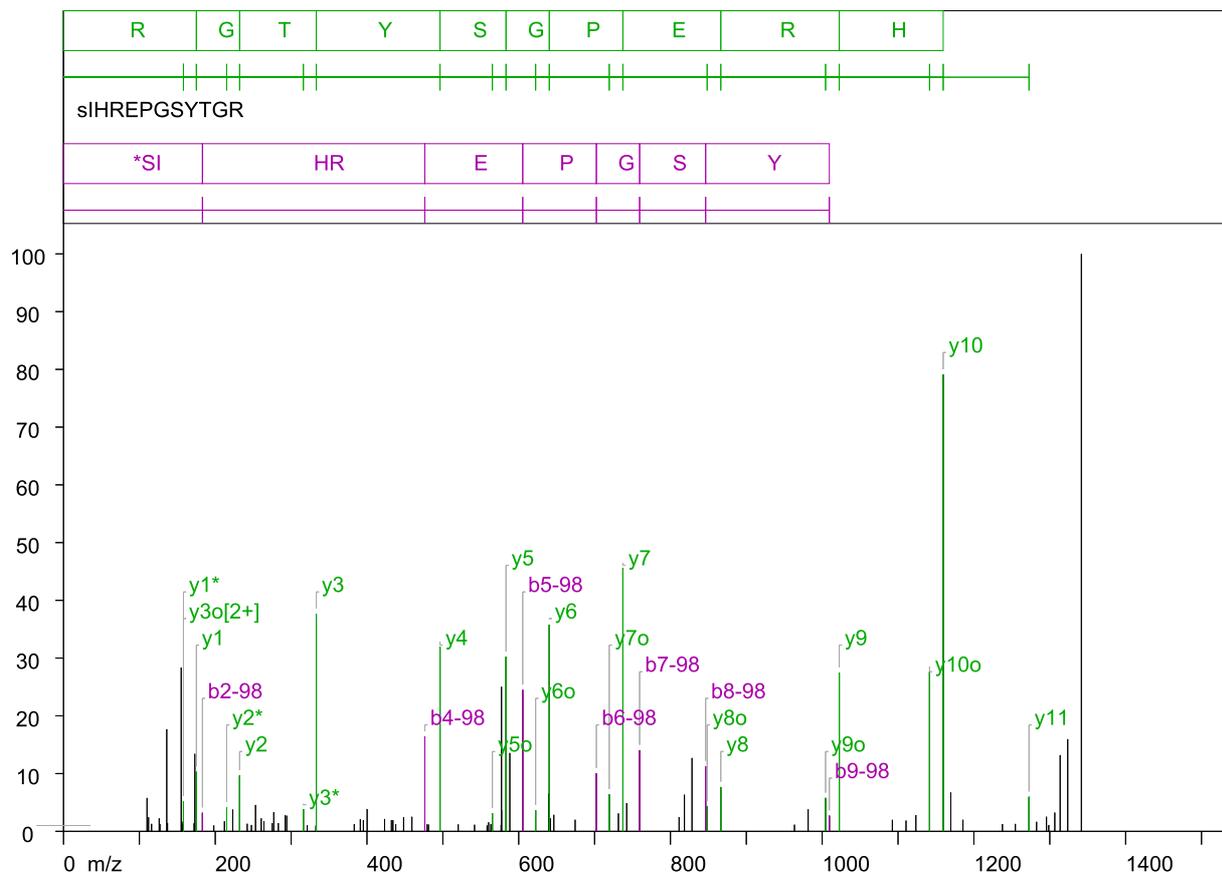


Figure S3

## 5-HT2A

(80) 298 SIHREPGSYTGR 309 1438.635 (-0.0038) Da

Parent Ion	m/z
No parent ions observed	

### Modifications and supporting evidence

Position	Residue ID	Modification	Evidence
1	(298)	Phospho (ST)	

### Spectrum interpretation

Rule	passed/tests	Description
Parent ions present	0/1	Parent ions corresponding to the parent fragment - 1 phosphate ions were not found
Three -98 Ions present	1/1	7 des-phospho fragment ions were found.
Unique -98 transitions present	0/1	No transitions found to support unique phosphorylation at position 1  
Four Sequential b or y ions	1/1	Sequence of four b ions found from b4-98 to b7-98. Sequence of four y ions found from y1 to y4.
Five of six sequential ions present	1/1	Five of Six ions found between b2 and b7 Five of Six ions found between b3 and b8 Five of Six ions found between b4 and b9 Five of Six ions found between b5 and b10 Five of Six ions found between y0 and y5 Five of Six ions found between y1 and y6 Five of Six ions found between y2 and y7 Five of Six ions found between y3 and y8 Five of Six ions found between y4 and y9 Five of Six ions found between y5 and y10 Five of Six ions found between y6 and y11 Five of Six ions found between y7 and y12
Proline directed fragmentation pattern	2/2	PASS: y7> y6 with ratio 1.27  PASS: b6-98< b5-98 with ratio 2.43 
PhosphoTyrosine transition present	0/0	0 of 0 phosphotyrosine transitions were found.
Six of top ten ions identified	8/6	ion 1 (mass: 1341.66428190574: intensity: 54190.61) assigned 0 times ion 2 (mass: 1159.55888638911: intensity: 42862.47) assigned 1 times ion 3 (mass: 737.356789040024: intensity: 24702.68) assigned 1 times ion 4 (mass: 333.187958487745: intensity: 20414.28) assigned 1 times ion 5 (mass: 640.3044195413: intensity: 19398.64) assigned 1 times ion 6 (mass: 496.250786913584: intensity: 17297.91) assigned 1 times ion 7 (mass: 583.281865540999: intensity: 16375.15) assigned 1 times ion 8 (mass: 155.117804860757: intensity: 15375.14) assigned 0 times ion 9 (mass: 1141.54436301008: intensity: 14976.99) assigned 1 times ion 10 (mass: 1022.50002958932: intensity: 14881.49) assigned 1 times

Figure S3

## Ion Table

29 ions assigned of 65 ions above threshold (44%).

N-terminal ions

AA	N-ion	b	b*	b-98	bo
s	1	168.006	150.979	70.020	149.995
I	2	281.090	264.063	183.104 183.112610422417 (3)	263.079
H	3	418.149	401.122	320.163	400.138
R	4	574.250	557.223	476.264 476.272991194634 (16)	556.239
E	5	703.292	686.266	605.306 605.315713430744 (24)	685.282
P	6	800.345	783.319	702.359 702.366877547714 (10)	782.335
G	7	857.367	840.340	759.381 759.387920543106 (14)	839.356
S	8	944.399	927.372	846.413 846.420647314435 (11)	926.388
Y	9	1107.462	1090.435	1009.476 1009.47975410937 (2)	1089.451
T	10	1208.510	1191.483	1110.524	1190.499
G	11	1265.531	1248.505	1167.545	1247.521
R	12	-	-	-	-

C-terminal ions

AA	C-ion	y	y*	yo
s	12	-	-	-
I	11	1272.644 1272.64291718875 (5)	1255.618	1254.634
H	10	1159.560 1159.55888638911 (79)	1142.534	1141.550 1141.54436301008 (27)
R	9	1022.501 1022.50002958932 (27)	1005.475	1004.491 1004.48476856643 (5)
E	8	866.400 866.398150269719 (7)	849.374	848.390 848.386937011057 (4)
P	7	737.358 737.356789040024 (45)	720.331	719.347 719.344578842031 (6)
G	6	640.305 640.3044195413 (35)	623.278	622.294 622.291625623833 (3)
S	5	583.284 583.281865540999 (30)	566.257	565.273 565.272424698873 (3)
Y	4	496.251 496.250786913584 (31)	479.225	478.241
T	3	333.188 333.187958487745 (37)	316.162 *316.160901892697 (3)	315.178 *158.092201751113 [2+] (5)
G	2	232.140 232.14030575892 (9)	215.114 *215.113745536328 (4)	214.130
R	1	175.119 175.118844494869 (10)	158.092 *158.092201751113 (5)	157.108

Ion distribution

Threshold	Ion count	Matches	% matched
0	167	41	24
0.5	134	40	29

Figure S3

1	99	34	34
2	64	29	45
3	48	27	56
4	37	23	62
5	33	20	60
10	23	14	60

### Observed ions > 1%

m/z	Intensity	% max	Assignment (delta)
110.071145568154	3131.183 <sub>2</sub>	5.77	
112.08700662787	1321.062 <sub>2</sub>	2.43	
116.070545059378	700.5259 <sub>2</sub>	1.29	
126.091042523963	1225.568 <sub>2</sub>	2.26	
127.122635599504	681.2238 <sub>2</sub>	1.25	
136.075587119945	9581.771 <sub>2</sub>	17.68	
137.079216568136	783.1667 <sub>2</sub>	1.44	
155.117804860757	15375.14 <sub>2</sub>	28.37	
156.121537171056	909.0309 <sub>2</sub>	1.67	
157.108491179957	652.6622 <sub>2</sub>	1.20	y1o (-0.00)
158.092201751113	2829.971 <sub>2</sub>	5.22	y1* (-0.00) : z1 (-0.00) : y3o[2+] (-0.00)
172.106243091294	757.1894 <sub>2</sub>	1.39	
173.128310988403	7273.57 <sub>2</sub>	13.42	
175.118844494869	5579.059 <sub>2</sub>	10.29	y1 (-0.00)
183.112610422417	1737.389 <sub>2</sub>	3.20	b2-98 (0.00)
198.112044138983	552.2602 <sub>2</sub>	1.01	
212.108612988614	946.1813 <sub>2</sub>	1.74	
215.113745536328	2254.97 <sub>2</sub>	4.16	y2* (-0.00) : z2 (-0.00)
223.10740228001	2044.852 <sub>2</sub>	3.77	
232.14030575892	5236.721 <sub>2</sub>	9.66	y2 (-0.00)
242.113287737645	713.8466 <sub>2</sub>	1.31	
247.644484094883	596.7056 <sub>2</sub>	1.10	
253.094787040832	2472.114 <sub>2</sub>	4.56	a2 (-0.00)
260.634838127631	1221.267 <sub>2</sub>	2.25	
264.182071630994	951.2807 <sub>2</sub>	1.75	
275.167600423796	769.3176 <sub>2</sub>	1.41	
277.140907862277	1800.585 <sub>2</sub>	3.32	
283.139178292044	770.4491 <sub>2</sub>	1.42	y5o[2+] (-0.00)
292.176663270949	1519.757 <sub>2</sub>	2.80	
294.168020248633	1466.152 <sub>2</sub>	2.70	
316.160901892697	2079.863 <sub>2</sub>	3.83	y3* (-0.00) : z3 (-0.00)
321.17543418855	573.3276 <sub>2</sub>	1.05	
333.187958487745	20414.28 <sub>2</sub>	37.67	y3 (-0.00)

Figure S3

383.203515546083	679.0351,	1.25	
391.192931346075	1132.898,	2.09	
395.214175397315	1052.432,	1.94	
400.197851780108	2092.563,	3.86	
423.210589153696	1158.745,	2.13	
432.215056061196	1048.389,	1.93	
434.254204645363	1048.766,	1.93	
437.891508497632	670.83,	1.23	
448.277634061733	1325.867,	2.44	
459.244297747327	1364.922,	2.51	
476.272991194634	8884.699,	16.39	b4-98 (0.00)
479.225460892356	663.2619,	1.22	y4* (2.08) : z4 (2.08)
481.115190142967	639.5734,	1.18	
496.250786913584	17297.91,	31.92	y4 (-0.00)
520.262076975617	656.251,	1.21	
541.771395779703	621.3683,	1.14	
558.269527518834	552.6188,	1.01	
560.297944738835	851.821,	1.57	
563.260136651842	677.9745,	1.25	
565.272424698873	1690.396,	3.11	y5o (-0.00)
576.77223720154	604.8056,	1.11	
577.32054799328	13570.14,	25.04	
577.327079840984	1976.361,	3.64	
583.281865540999	16375.15,	30.21	y5 (-0.00)
588.288305817865	7352.627,	13.56	
605.315713430744	13271.13,	24.48	b5-98 (0.00)
622.291625623833	1981.545,	3.65	y6o (-0.00)
639.335866347165	3535.485,	6.52	
640.3044195413	19398.64,	35.79	y6 (-0.00)
641.815642108035	1212.874,	2.23	
646.303258857081	1549.799,	2.85	
674.370532663164	1090.435,	2.01	
702.366877547714	5451.421,	10.05	b6-98 (0.00)
719.344578842031	3470.693,	6.40	y7o (-0.00)
731.395421622825	1682.974,	3.10	
737.356789040024	24702.68,	45.58	y7 (-0.00)
742.363910283723	2649.273,	4.88	
759.387920543106	7614.098,	14.05	b7-98 (0.00)
811.375439993199	1334.089,	2.46	a7o (0.01)
818.425751211907	3437.215,	6.34	
828.406202408152	6879.253,	12.69	
846.420647314435	6113.353,	11.28	b8-98 (0.00)
848.386937011057	2356.613,	4.34	y8o (-0.00)

Figure S3

866.398150269719	4152.22 <sub>,</sub>	7.66	y8 (-0.00)
963.475573721226	623.5931 <sub>,</sub>	1.15	
981.488700599117	2068.481 <sub>,</sub>	3.81	
1004.48476856643	3130.875 <sub>,</sub>	5.77	y9o (-0.00)
1009.47975410937	1465.932 <sub>,</sub>	2.70	b9-98 (0.00)
1022.50002958932	14881.49 <sub>,</sub>	27.46	y9 (-0.00)
1092.52186524243	1079.839 <sub>,</sub>	1.99	
1110.53151302606	1008.215 <sub>,</sub>	1.86	b10-98 (0.00)
1123.5300596897	1521.007 <sub>,</sub>	2.80	
1141.54436301008	14976.99 <sub>,</sub>	27.63	y10o (-0.00)
1159.55888638911	42862.47 <sub>,</sub>	79.09	y10 (-0.00)
1169.54410902066	3646.388 <sub>,</sub>	6.72	
1185.5681545011	1084.941 <sub>,</sub>	2.00	
1237.60320338312	685.0226 <sub>,</sub>	1.26	
1254.63099356743	696.7817 <sub>,</sub>	1.28	y11o (-0.00)
1272.64291718875	3235.923 <sub>,</sub>	5.97	y11 (-0.00)
1282.62189198887	893.0173 <sub>,</sub>	1.64	
1295.64500857914	1371.536 <sub>,</sub>	2.53	
1298.64028094885	592.4398 <sub>,</sub>	1.09	
1306.63082572143	1765.065 <sub>,</sub>	3.25	
1313.6700493599	7152.337 <sub>,</sub>	13.19	
1323.6539086902	8646.836 <sub>,</sub>	15.95	
1341.66428190574	54190.61 <sub>,</sub>	100	

Figure S3

# ProPhosSI MS/MS report

Mass: 559.2409 Charge: 3+

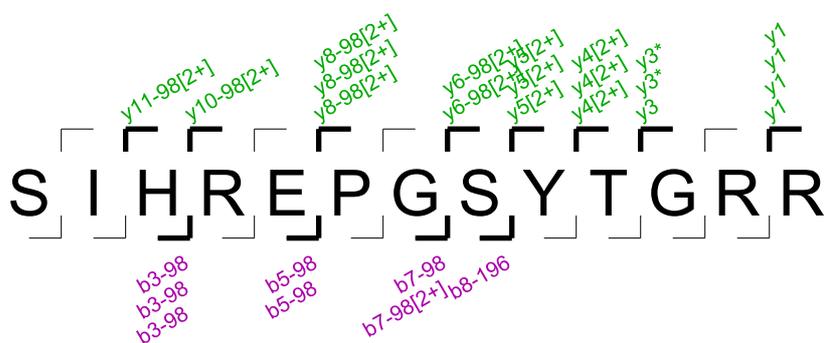
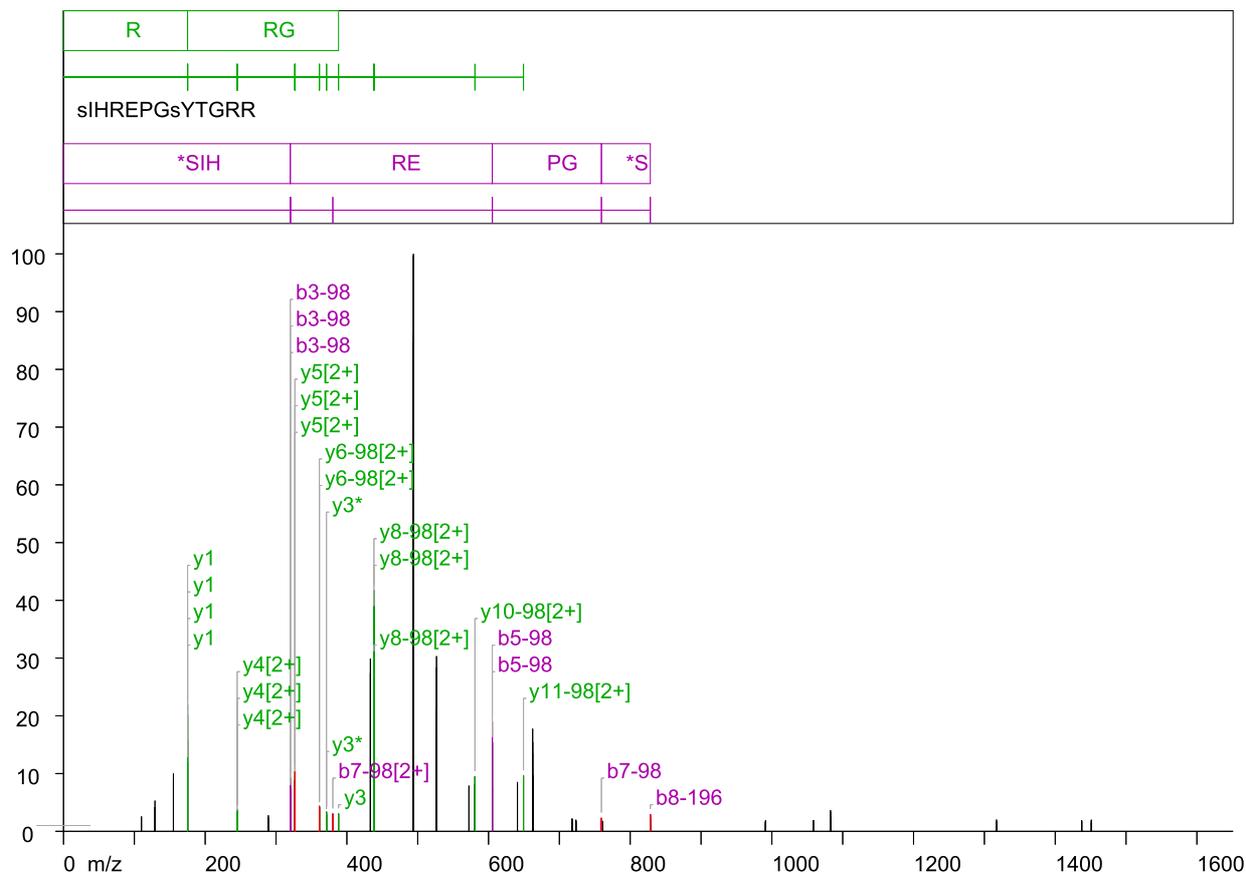


Figure S3

## 5-HT2A

(20) 298 SIHREPGsYTGRR 309 1674.702 (-0.0036) Da

Parent Ion	m/z
No parent ions observed	

### Modifications and supporting evidence

Position	Residue ID	Modification	Evidence
8	(305)	Phospho (ST)	b7-98 => b8-196 : y5 => y6-98
1	(298)	Phospho (ST)	

### Spectrum interpretation

Rule	passed/tests	Description
Parent ions present	0/1	Parent ions corresponding to the parent fragment - 2 phosphate ions were not found
Three -98 Ions present	1/1	8 des-phospho fragment ions were found.
Unique -98 transitions present	1/2	transition b7-98 to b8-196, transition y5 to y6-98 support unique phosphorylation at position 8  No transitions found to support unique phosphorylation at position 1  
Four Sequential b or y ions	1/1	Sequence of four y ions found from y3 to y6-98.
Five of six sequential ions present	1/1	Five of Six ions found between y1 and y6 Five of Six ions found between y3 and y8
Proline directed fragmentation pattern	2/2	PASS: y8-98> y7-98  PASS: b6-98< b5-98 
PhosphoTyrosine transition present	0/0	0 of 0 phosphotyrosine transitions were found.
Six of top ten ions identified	3/6	ion 1 (mass: 493.949212: intensity: 2973.545898) assigned 0 times ion 2 (mass: 493.89942: intensity: 2963.411133) assigned 0 times ion 3 (mass: 494.2647363: intensity: 2566.40723) assigned 0 times ion 4 (mass: 493.9160165: intensity: 2520.39063) assigned 0 times ion 5 (mass: 494.2481222: intensity: 2501.13281) assigned 0 times ion 6 (mass: 493.9326139: intensity: 2097.53125) assigned 0 times ion 7 (mass: 438.2230308: intensity: 1240.870117) assigned 1 times ion 8 (mass: 438.2369016: intensity: 1133.389526) assigned 1 times ion 9 (mass: 526.5882446: intensity: 902.3140869) assigned 0 times ion 10 (mass: 438.2091607: intensity: 899.5979004) assigned 1 times

### Ion Table

28 ions assigned of 70 ions above threshold (40%).

#### N-terminal ions

AA	N-ion	b	b*	b-196	b-98	bo
s	1	168.006	150.979	-	70.020	149.995
l	2	281.090	264.063	-	183.104	263.079
H	3	418.149	401.122	-	320.163 320.1709319 (9) 320.16227 (7) 320.1795941 (7)	400.138

Figure S3

R	4	574.250	557.223	-	476.264	556.239
E	5	703.292	686.266	-	605.306 605.2920684 (15) 605.3145852 (18)	685.282
P	6	800.345	783.319	-	702.359	782.335
G	7	857.367	840.340	-	759.381 759.3878605 (2) 380.1943357 [2+] (3)	839.356
s	8	1024.365	1007.338	828.393 828.4035732 (2)	926.379	1006.354
Y	9	1187.428	1170.402	991.456	1089.442	1169.418
T	10	1288.476	1271.449	1092.504	1190.490	1270.465
G	11	1345.497	1328.471	1149.526	1247.512	1327.487
R	12	1501.599	1484.572	1305.627	1403.613	1483.588
R	13	-	-	-	-	-

### C-terminal ions

AA	C-ion	y	y*	y-98	y0
s	13	-	-	-	-
I	12	1508.712	1491.685	1410.726	1490.701
H	11	1395.628	1378.601	1297.642 649.3251225 [2+] (9)	1377.617
R	10	1258.569	1241.542	1160.583 580.7982526 [2+] (9)	1240.558
E	9	1102.468	1085.441	1004.482	1084.457
P	8	973.425	956.399	875.439 438.2230308 [2+] (41) 438.2369016 [2+] (38) 438.2091607 [2+] (30)	955.415
G	7	876.372	859.346	778.387	858.362
s	6	819.351	802.324	721.365 361.1882224 [2+] (4) 361.1986015 [2+] (4)	801.340
Y	5	652.353 326.6694658 [2+] (7) 326.6873207 [2+] (8) 326.6783931 [2+] (10)	635.326	-	634.342
T	4	489.289 245.1466714 [2+] (3) 245.140868 [2+] (2) 245.1524749 [2+] (3)	472.263	-	471.279
G	3	388.242 388.2445281 (3)	371.215 *371.2104351 (3) *371.2212491 (2)	-	370.231
R	2	331.220	314.194	-	313.210
R	1	175.119 175.1175357 (21) 175.1140319 (15) 175.1245436 (11) 175.1210396 (20)	158.092	-	157.108

### Ion distribution

Threshold	Ion count	Matches	% matched
0	100	31	31
0.5	100	31	31
1	94	29	30

Figure S3

2	70	28	40
3	54	24	44
4	47	19	40
5	44	17	38
10	30	10	33

### Observed ions > 1%

m/z	Intensity	% max	Assignment (delta)
110.0726142	76.84295654,	2.58	
129.099488	124.7159424,	4.19	
129.1017059	158.1997528,	5.32	
155.1166758	299.0040894,	10.05	
155.1195969	270.3562927,	9.09	
175.1140319	470.1944275,	15.81	y1 (-0.00)
175.1175357	651.4014893,	21.90	y1 (-0.00)
175.1210396	599.161438,	20.14	y1 (0.00)
175.1245436	355.9265747,	11.96	y1 (0.00)
245.140868	78.25357056,	2.63	y4[2+] (-0.00)
245.1466714	114.6404724,	3.85	y4[2+] (-0.00)
245.1524749	103.7242126,	3.48	y4[2+] (0.00)
245.1582787	57.135746,	1.92	y4[2+] (0.00)
289.1533527	62.05736923,	2.08	
289.1607872	82.65490723,	2.77	
289.1682219	72.44944763,	2.43	
320.16227	210.6694946,	7.08	b3-98 (-0.00)
320.1709319	275.2634888,	9.25	b3-98 (0.00)
320.1795941	222.7120972,	7.48	b3-98 (0.01)
326.6694658	236.5240021,	7.95	y5[2+] (-0.01)
326.6783931	307.4874268,	10.34	y5[2+] (-0.00)
326.6873207	263.0691528,	8.84	y5[2+] (0.00)
361.1882224	130.5734253,	4.39	y6-98[2+] (0.00)
361.1986015	122.6739655,	4.12	y6-98[2+] (0.01)
371.2104351	100.7621002,	3.38	y3* (-0.00) : z3 (-0.00)
371.2212491	86.91004944,	2.92	y3* (0.00) : z3 (0.00)
380.1943357	90.4498291,	3.04	b7-98[2+] (-0.00)
388.2445281	91.05412292,	3.06	y3 (0.00)
433.2174815	889.463501,	29.91	
433.2311153	813.2438965,	27.34	
438.2091607	899.5979004,	30.25	y8-98[2+] (-0.01)
438.2230308	1240.870117,	41.73	y8-98[2+] (-0.00)
438.2369016	1133.389526,	38.11	y8-98[2+] (0.01)
438.250773	670.756958,	22.55	

Figure S3

493.89942	2963.411133,	99.65	
493.9160165	2520.39063,	84.76	
493.9326139	2097.53125,	70.53	
493.949212	2973.545898,	100	
494.2481222	2501.13281,	84.11	
494.2647363	2566.40723,	86.30	
526.5517052	512.7440796,	17.24	
526.5699744	844.5879517,	28.40	
526.5882446	902.3140869,	30.34	
526.6065158	634.8270264,	21.34	
526.8989835	515.4677124,	17.33	
526.9172708	603.3769531,	20.29	
572.2796124	235.4198151,	7.91	
580.7982526	283.2157898,	9.52	y10-98[2+] (0.00)
580.8194166	241.5894318,	8.12	
605.2695528	231.6360626,	7.78	
605.2920684	459.3583069,	15.44	b5-98 (-0.01)
605.3145852	564.5996704,	18.98	b5-98 (0.00)
605.3371033	456.4438171,	15.35	
640.8284828	254.0968781,	8.54	
649.3251225	286.9752197,	9.65	y11-98[2+] (7.49)
662.3002543	402.8986511,	13.54	
662.326026	528.7164307,	17.78	
662.3517992	459.2440186,	15.44	
662.8417757	288.0767517,	9.68	
717.886156	47.35419083,	1.59	
717.9152393	65.20471191,	2.19	
717.9443244	64.6086731,	2.17	
723.326217	48.87817001,	1.64	
723.3556316	59.82880783,	2.01	
723.3850479	55.20959091,	1.85	
759.356221	45.51073074,	1.53	
759.3878605	68.92932129,	2.31	b7-98 (0.00)
759.419502	62.04956436,	2.08	
760.9406217	44.62907028,	1.50	
760.9723603	53.5132103,	1.79	
761.0041009	52.21364975,	1.75	
828.331477	30.37163162,	1.02	
828.3675239	70.32487488,	2.36	
828.4035732	88.03901672,	2.96	b8-196 (0.00)
828.4396248	65.00915527,	2.18	
990.9978802	40.59950256,	1.36	
991.0450509	51.90354538,	1.74	

Figure S3

991.0922249	56.95181656,	1.91	
991.1394024	48.40657425,	1.62	
1058.610518	56.72603226,	1.90	
1058.662598	57.70243454,	1.94	
1082.709669	55.91621017,	1.88	
1082.763537	100.2455444,	3.37	
1082.817409	109.0979156,	3.66	
1082.871285	75.09506226,	2.52	
1317.0936	46.05416489,	1.54	
1317.165875	60.63010788,	2.03	
1317.238156	52.68044662,	1.77	
1437.26618	54.32481766,	1.82	
1437.348569	57.98490524,	1.95	
1437.430966	52.94816208,	1.78	
1450.95691	49.26804733,	1.65	
1451.040479	59.09872818,	1.98	
1451.124056	58.75348282,	1.97	

Figure S3

# ProPhosSI MS/MS report

Mass: 904.4283 Charge: 2+

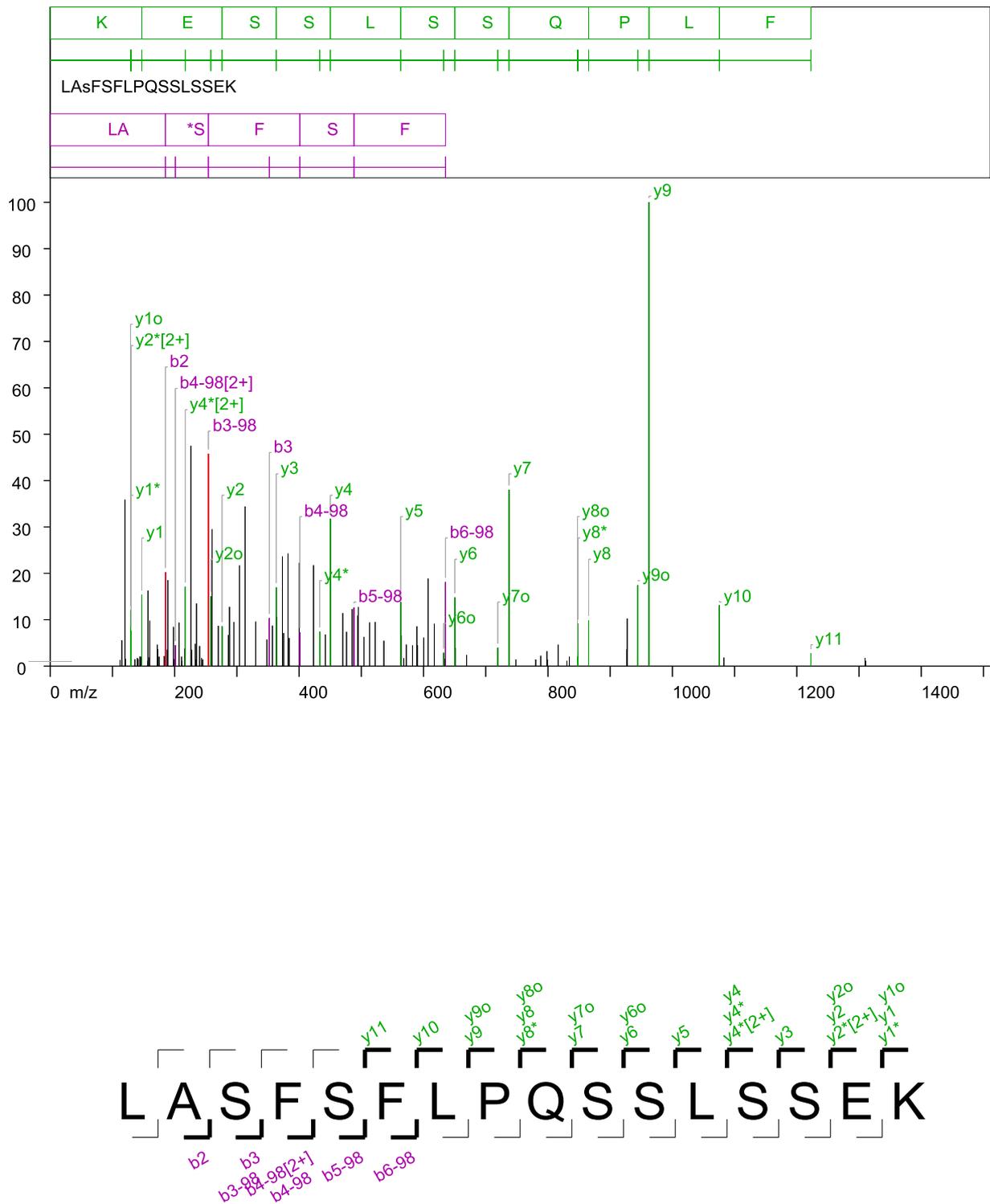


Figure S3

## 5-HT2A

(44) 278 LAsFSFLPQSSLSSEK 293 1806.844 (-0.0031) Da

Parent Ion	m/z
No parent ions observed	

### Modifications and supporting evidence

Position	Residue ID	Modification	Evidence
3	(280)	Phospho (ST)	b2 => b3-98

### Spectrum interpretation

Rule	passed/tests	Description
Parent ions present	0/1	Parent ions corresponding to the parent fragment - 1 phosphate ions were not found
Three -98 Ions present	1/1	4 des-phospho fragment ions were found.
Unique -98 transitions present	1/1	transition b2 to b3-98 support unique phosphorylation at position 3  
Four Sequential b or y ions	1/1	Sequence of four b ions found from b2 to b5-98. Sequence of four y ions found from y1 to y4.
Five of six sequential ions present	1/1	Five of Six ions found between b1 and b6 Five of Six ions found between b2 and b7 Five of Six ions found between y0 and y5 Five of Six ions found between y1 and y6 Five of Six ions found between y2 and y7 Five of Six ions found between y3 and y8 Five of Six ions found between y4 and y9 Five of Six ions found between y5 and y10 Five of Six ions found between y6 and y11 Five of Six ions found between y7 and y12
Proline directed fragmentation pattern	1/1	PASS: y9> y8 with ratio 10.1  No proline ions at b8-98 
PhosphoTyrosine transition present	0/0	0 of 0 phosphotyrosine transitions were found.
Six of top ten ions identified	4/6	ion 1 (mass: 962.479236133074: intensity: 41396.28) assigned 1 times ion 2 (mass: 226.118906761395: intensity: 19660.8) assigned 0 times ion 3 (mass: 254.150357835796: intensity: 18965.04) assigned 1 times ion 4 (mass: 737.368075744833: intensity: 15750.32) assigned 1 times ion 5 (mass: 120.080898579207: intensity: 14860.93) assigned 0 times ion 6 (mass: 313.15124289743: intensity: 14249.74) assigned 0 times ion 7 (mass: 450.218905106955: intensity: 13146.76) assigned 1 times ion 8 (mass: 260.139768464907: intensity: 12207.13) assigned 0 times ion 9 (mass: 382.173439588294: intensity: 10051.92) assigned 0 times ion 10 (mass: 373.223858383509: intensity: 9780.26) assigned 0 times

### Ion Table

34 ions assigned of 92 ions above threshold (36%).

Figure S3

### N-terminal ions

AA	N-ion	b	b*	b-98	bo
L	1	114.091	97.065	-	96.081
A	2	185.129 185.128732413761 (20)	168.102	-	167.118
s	3	352.127 352.127247512411 (10)	335.100	254.141 254.150357835796 (45)	334.116
F	4	499.195	482.169	401.209 201.123786059486 [2+] (4) 401.21802852119 (7)	481.185
S	5	586.227	569.201	488.241 488.250423464514 (12)	568.217
F	6	733.296	716.269	635.310 635.320227763599 (18)	715.285
L	7	846.380	829.353	748.394	828.369
P	8	943.433	926.406	845.447	925.422
Q	9	1071.491	1054.465	973.505	1053.481
S	10	1158.523	1141.497	1060.537	1140.513
S	11	1245.555	1228.529	1147.569	1227.545
L	12	1358.639	1341.613	1260.653	1340.629
S	13	1445.671	1428.645	1347.685	1427.661
S	14	1532.703	1515.677	1434.717	1514.693
E	15	1661.746	1644.719	1563.760	1643.735
K	16	-	-	-	-

### C-terminal ions

AA	C-ion	y	y*	y-98	yo
L	16	-	-	-	-
A	15	1694.767	1677.741	1596.781	1676.757
s	14	1623.730	1606.704	1525.744	1605.720
F	13	1456.732	1439.705	-	1438.721
S	12	1309.664	1292.637	-	1291.653
F	11	1222.631 1222.63148437197 (2)	1205.605	-	1204.621
L	10	1075.563 1075.56115253088 (13)	1058.537	-	1057.553
P	9	962.479 962.479236133074 (100)	945.452	-	944.468 944.46700169931 (17)
Q	8	865.426 865.427795988115 (9)	848.400 *848.402679647264 (9)	-	847.416 847.413299677502 (2)
S	7	737.368 737.368075744833 (38)	720.341	-	719.357 719.360607764844 (3)
S	6	650.336 650.336620591607 (14)	633.309	-	632.325 632.325871241278 (2)
L	5	563.304 563.304327461494 (13)	546.277	-	545.293
S	4	450.220 450.218905106955 (31)	433.193 *217.097138629192 [2+] (17) *433.186051802783 (7)	-	432.209
S	3	363.187 363.187643261989 (16)	346.161	-	345.177
E	2	276.155 276.154367521989 (8)	259.129 *130.086280806094 [2+] (7)	-	258.145 258.143165424161 (15)
K	1	147.113	130.086	-	129.102

Figure S3

-	-	147.112957223734 (15)	*130.086280806094 (7)	-	129.102435870568 (12)
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### Ion distribution

Threshold	Ion count	Matches	% matched
0	136	37	27
0.5	136	37	27
1	116	36	31
2	92	34	36
3	81	30	37
4	73	29	39
5	65	28	43
10	37	19	51

### Observed ions > 1%

m/z	Intensity	% max	Assignment (delta)
112.110808362555	541.58 <sub>3</sub>	1.30	
115.086712554077	2306.057 <sub>3</sub>	5.57	
120.080898579207	14860.93 <sub>3</sub>	35.89	
121.084623422422	629.936 <sub>3</sub>	1.52	
129.066510039428	444.5616 <sub>3</sub>	1.07	
129.102435870568	4978.855 <sub>3</sub>	12.02	y1o (-0.00)
130.086280806094	3168.582 <sub>3</sub>	7.65	y2*[2+] (0.01) : z2[2+] (0.01) : y1* (-0.00) : z1 (-0.00)
136.076156566285	611.2229 <sub>3</sub>	1.47	
140.010370053276	720.9639 <sub>3</sub>	1.74	
141.065567135597	621.9658 <sub>3</sub>	1.50	
144.080913518304	855.5474 <sub>3</sub>	2.06	
146.09638580999	834.8638 <sub>3</sub>	2.01	
147.112957223734	6355.815 <sub>3</sub>	15.35	y1 (-0.00)
157.060872712379	479.7785 <sub>3</sub>	1.15	
157.133768772855	6742.691 <sub>3</sub>	16.28	a2 (-0.00)
158.092110397658	780.9307 <sub>3</sub>	1.88	
160.075831980548	4051.067 <sub>3</sub>	9.78	
172.076216744924	1909.849 <sub>3</sub>	4.61	
173.128726921804	1488.069 <sub>3</sub>	3.59	
175.070830781504	844.8469 <sub>3</sub>	2.04	
183.149854566158	895.7849 <sub>3</sub>	2.16	
185.128732413761	8384.55 <sub>3</sub>	20.25	b2 (-0.00)
188.143612345075	1472.827 <sub>3</sub>	3.55	
189.102507707806	7677.758 <sub>3</sub>	18.54	
198.088442792378	538.8403 <sub>3</sub>	1.30	
198.124028764484	3493.08 <sub>3</sub>	8.43	
199.072219204723	610.2178 <sub>3</sub>	1.47	

Figure S3

201.123786059486	1850.89,	4.47	b4-98[2+] (0.01)
207.113277642125	3889.098,	9.39	
211.10805171564	478.2105,	1.15	
211.14469316499	844.5006,	2.04	
216.098635163389	1558.116,	3.76	
217.097138629192	7088.592,	17.12	y4*[2+] (-0.00) : z4[2+] (-0.00)
226.118906761395	19660.8,	47.49	
227.124530800424	1464.621,	3.53	
227.172883839368	645.4805,	1.55	
233.164766399286	1986.596,	4.79	
234.124702086647	583.6202,	1.40	
235.107954215908	5590.154,	13.50	
240.134933276472	1786.425,	4.31	
243.148074226397	714.5485,	1.72	
245.093267958584	590.4103,	1.42	
254.150357835796	18965.04,	45.81	b3-98 (0.00)
258.143165424161	6241.207,	15.07	y2o (-0.00)
260.139768464907	12207.13,	29.48	
270.14462141072	3594.362,	8.68	
276.154367521989	3546.055,	8.56	y2 (-0.00)
286.120673241642	2767.427,	6.68	
288.136441174643	5280.99,	12.75	
295.140833086247	3928.224,	9.48	
304.131834731903	8983.082,	21.70	x2 (-0.01)
313.15124289743	14249.74,	34.42	
330.182833919203	3974.479,	9.60	
348.194215721447	2368.065,	5.72	
352.127247512411	4275.81,	10.32	b3 (-9.74)
357.15416143806	3614.713,	8.73	
363.187643261989	7032.785,	16.98	y3 (-0.00)
364.163304208729	4389.09,	10.60	
373.223858383509	9780.26,	23.62	
375.16249594876	2947.318,	7.11	
382.173439588294	10051.92,	24.28	
384.096383075743	2508.972,	6.06	
400.183223205291	9218.771,	22.26	
401.21802852119	3021.007,	7.29	b4-98 (0.00)
423.203610080163	8997.795,	21.73	
433.186051802783	3072.227,	7.42	y4* (-0.00) : z4 (-0.00)
442.245156095451	2819.474,	6.81	
450.218905106955	13146.76,	31.75	y4 (-0.00)
470.239424666205	4731.826,	11.43	
476.231238906203	3058.477,	7.38	

Figure S3

485.274548856679	5085.367,	12.28	
488.250423464514	5218.591,	12.60	b5-98 (0.00)
494.23986990931	4516.383,	10.91	
495.257154657915	5266.945,	12.72	
504.224660917366	2604.886,	6.29	
513.266709928954	3901.369,	9.42	a9o[2+] (0.01)
522.233981440114	3935.494,	9.50	a9[2+] (-0.01)
536.287814920022	2265.333,	5.47	
563.304327461494	5713.876,	13.80	y5 (0.00)
564.271376100074	2732.356,	6.60	
568.216919058501	704.8585,	1.70	b5o (-0.00)
572.292189974785	1922.463,	4.64	
582.289720061945	1846.304,	4.46	
589.311796282809	3550.618,	8.57	
590.302385353407	1861.027,	4.49	
600.298214182075	2544.852,	6.14	a11o[2+] (0.01)
607.325291949869	7822.735,	18.89	
617.309379222844	3775.55,	9.12	
632.325871241278	1184.104,	2.86	y6o (0.00)
634.29101345958	637.8583,	1.54	
635.320227763599	7497.377,	18.11	b6-98 (0.00)
650.336620591607	6120.709,	14.78	y6 (0.00)
651.304649632059	1607.844,	3.88	
669.322766628333	986.433,	2.38	x12[2+] (-0.01)
719.360607764844	1625.92,	3.92	y7o (0.00)
737.368075744833	15750.32,	38.04	y7 (-6.92)
748.410003894712	594.2305,	1.43	b7-98 (0.01)
780.355596319013	592.1029,	1.43	
788.385255082495	920.7234,	2.22	
798.364969282095	1333.587,	3.22	
799.35576493776	580.4562,	1.40	
816.373928614576	1913.229,	4.62	
830.393392541607	468.3553,	1.13	
834.396268679503	841.9946,	2.03	
847.413299677502	862.1363,	2.08	y8o (-0.00)
848.402679647264	3774.62,	9.11	y8* (0.00) : z8 (0.00)
865.427795988115	4095.613,	9.89	y8 (0.00)
926.452747792427	1479.787,	3.57	
927.446267731064	4247.418,	10.26	
944.46700169931	7215.603,	17.43	y9o (-0.00)
962.479236133074	41396.28,	100	y9 (-0.00)
1075.56115253088	5434.923,	13.12	y10 (-0.00)
1082.80616182065	762.7844,	1.84	

Figure S3

1222.63148437197	1134.231,	2.73	y11 (-0.00)
1309.64238422237	732.9919,	1.77	
1310.66326868999	471.8918,	1.13	

Figure S3

# ProPhosSI MS/MS report

Mass: 811.3813 Charge: 3+

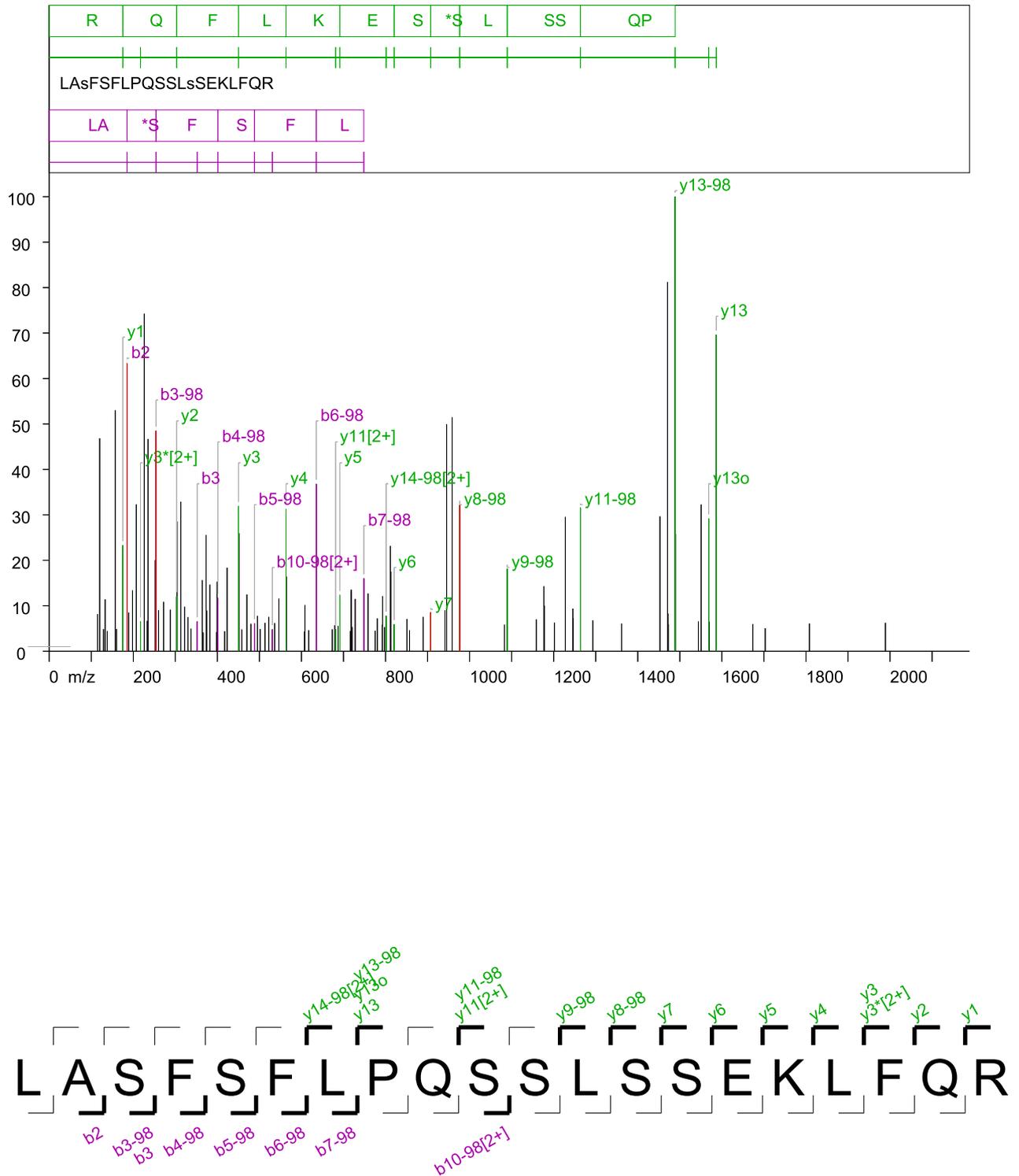


Figure S3

## 5-HT2A

(25) 278 LAsFSFLPQSSLsSEKLFQR 297 2431.122 (-0.0021) Da

Parent Ion	m/z
No parent ions observed	

### Modifications and supporting evidence

Position	Residue ID	Modification	Evidence
3	(280)	Phospho (ST)	b2 => b3-98
13	(290)	Phospho (ST)	y7 => y8-98

### Spectrum interpretation

Rule	passed/tests	Description
Parent ions present	0/1	Parent ions corresponding to the parent fragment - 2 phosphate ions were not found
Three -98 Ions present	1/1	11 des-phospho fragment ions were found.
Unique -98 transitions present	2/2	transition b2 to b3-98 support unique phosphorylation at position 3  transition y7 to y8-98 support unique phosphorylation at position 13  
Four Sequential b or y ions	1/1	Sequence of four b ions found from b2 to b5-98. Sequence of four y ions found from y1 to y4.
Five of six sequential ions present	1/1	Five of Six ions found between b1 and b6 Five of Six ions found between b2 and b7 Five of Six ions found between b3 and b8 Five of Six ions found between y0 and y5 Five of Six ions found between y1 and y6 Five of Six ions found between y2 and y7 Five of Six ions found between y3 and y8 Five of Six ions found between y4 and y9 Five of Six ions found between y5 and y10 Five of Six ions found between y6 and y11
Proline directed fragmentation pattern	2/2	PASS: y13-98> y12-98  PASS: b8-98< b7-98 
PhosphoTyrosine transition present	0/0	0 of 0 phosphotyrosine transitions were found.
Six of top ten ions identified	5/6	ion 1 (mass: 1488.77389764326: intensity: 2989.639) assigned 1 times ion 2 (mass: 1470.76517456316: intensity: 2428.688) assigned 0 times ion 3 (mass: 226.118445106373: intensity: 2219.913) assigned 0 times ion 4 (mass: 1586.7561430589: intensity: 2082.125) assigned 1 times ion 5 (mass: 185.128330521536: intensity: 1892.978) assigned 1 times ion 6 (mass: 157.133575744062: intensity: 1584.816) assigned 1 times ion 7 (mass: 958.508435569047: intensity: 1537.952) assigned 0 times ion 8 (mass: 945.509676448229: intensity: 1492.796) assigned 0 times ion 9 (mass: 254.149773843174: intensity: 1449.208) assigned 1 times ion 10 (mass: 120.080668708344: intensity: 1400.47) assigned 0 times

Figure S3

## Ion Table

27 ions assigned of 114 ions above threshold (23%).

### N-terminal ions

AA	N-ion	b	b*	b-196	b-98	bo
L	1	114.091	97.065	-	-	96.081
A	2	185.129 185.128330521536 (63)	168.102	-	-	167.118
s	3	352.127 352.127391867676 (6)	335.100	-	254.141 254.149773843174 (48)	334.116
F	4	499.195	482.169	-	401.209 401.216305119661 (11)	481.185
S	5	586.227	569.201	-	488.241 488.255690256077 (6)	568.217
F	6	733.296	716.269	-	635.310 635.318824691056 (36)	715.285
L	7	846.380	829.353	-	748.394 748.399418606985 (16)	828.369
P	8	943.433	926.406	-	845.447	925.422
Q	9	1071.491	1054.465	-	973.505	1053.481
S	10	1158.523	1141.497	-	1060.537 530.76110534525 [2+] (4)	1140.513
S	11	1245.555	1228.529	-	1147.569	1227.545
L	12	1358.639	1341.613	-	1260.653	1340.629
s	13	1525.638	1508.611	1329.666	1427.652	1507.627
S	14	1612.670	1595.643	1416.698	1514.684	1594.659
E	15	1741.712	1724.686	1545.740	1643.726	1723.702
K	16	1869.807	1852.781	1673.835	1771.821	1851.797
L	17	1982.891	1965.865	1786.919	1884.905	1964.881
F	18	2129.960	2112.933	1933.988	2031.974	2111.949
Q	19	2258.018	2240.992	2062.046	2160.032	2240.008
R	20	-	-	-	-	-

### C-terminal ions

AA	C-ion	y	y*	y-196	y-98	yo
L	20	-	-	-	-	-
A	19	2319.046	2302.019	2123.074	2221.060	2301.035
s	18	2248.009	2230.982	2052.037	2150.023	2229.998
F	17	2081.010	2063.984	-	1983.025	2063.000
S	16	1933.942	1916.915	-	1835.956	1915.931
F	15	1846.910	1829.883	-	1748.924	1828.899
L	14	1699.842	1682.815	-	1601.856 801.426194157124 [2+] (7)	1681.831
P	13	1586.757 1586.7561430589 (69)	1569.731	-	1488.772 1488.77389764326 (100)	1568.747 1568.73886882621 (29)
Q	12	1489.705	1472.678	-	1391.719	1471.694
S	11	1361.646 681.309080577769 [2+] (4)	1344.620	-	1263.660 1263.67087190437 (31)	1343.636
S	10	1274.614	1257.588	-	1176.628	1256.604
L	9	1187.582	1170.556	-	1089.596 1089.58419755801	1169.572

Figure S3

-	-	-	-	-	(18)	-
s	8	1074.498	1057.471	-	976.512 976.523419150955 (32)	1056.487
S	7	907.500 907.5038370039 (8)	890.473	-	-	889.489
E	6	820.468 820.463717645503 (5)	803.441	-	-	802.457
K	5	691.425 691.421961972494 (12)	674.399	-	-	673.414
L	4	563.330 563.331964890352 (31)	546.304	-	-	545.320
F	3	450.246 450.245545428816 (31)	433.219 *217.095389985263 [2+] (6)	-	-	432.235
Q	2	303.178 303.176176635407 (12)	286.151	-	-	285.167
R	1	175.119 175.118734586684 (23)	158.092	-	-	157.108

### Ion distribution

Threshold	Ion count	Matches	% matched
0	114	27	23
0.5	114	27	23
1	114	27	23
2	114	27	23
3	114	27	23
4	114	27	23
5	97	25	25
10	53	17	32

### Observed ions > 1%

m/z	Intensity	% max	Assignment (delta)
115.086475812849	244.6062 <sub>s</sub>	8.18	
120.080668708344	1400.47 <sub>s</sub>	46.84	
129.100698571422	144.9496 <sub>s</sub>	4.84	
133.074874476376	340.933 <sub>s</sub>	11.40	
137.914739560717	132.2962 <sub>s</sub>	4.42	
157.133575744062	1584.816 <sub>s</sub>	53.01	a2 (-0.00)
160.077432357752	146.1855 <sub>s</sub>	4.88	
175.118734586684	696.2208 <sub>s</sub>	23.28	y1 (-0.00)
185.128330521536	1892.978 <sub>s</sub>	63.31	b2 (-0.00)
189.102032292474	254.6457 <sub>s</sub>	8.51	
198.125090265951	400.3843 <sub>s</sub>	13.39	
207.112369876585	965.6066 <sub>s</sub>	32.29	
217.095389985263	195.0688 <sub>s</sub>	6.52	y3*[2+] (-0.01) : z3[2+] (-0.01)

Figure S3

226.118445106373	2219.913,	74.25	
233.161412782682	199.8186,	6.68	
235.107242740976	1395.886,	46.69	
252.134829494968	599.0894,	20.03	
254.149773843174	1449.208,	48.47	b3-98 (0.00)
260.139474300509	270.0221,	9.03	
272.160675060668	324.5262,	10.85	
288.134667700049	273.1284,	9.13	
303.176176635407	360.7036,	12.06	y2 (-0.00)
304.128796244548	852.9989,	28.53	
313.149358401612	983.3651,	32.89	
322.138965028784	292.356,	9.77	
330.179425439348	224.4186,	7.50	
337.257873463128	148.3311,	4.96	
352.127391867676	195.5966,	6.54	b3 (4.68)
364.164215111735	467.5916,	15.64	
366.22232586477	122.9942,	4.11	
373.225342600265	763.7272,	25.54	
375.166578982087	266.4028,	8.91	
382.174580569964	437.7142,	14.64	
397.809225410496	125.7324,	4.20	
399.20119785153	457.2064,	15.29	
401.216305119661	348.9869,	11.67	b4-98 (0.00)
417.360264183244	131.7106,	4.40	
423.200056609128	549.5289,	18.38	
450.245545428816	953.6787,	31.89	y3 (-0.00)
451.196983650786	776.7166,	25.98	
458.198034194686	142.9333,	4.78	
470.238287092367	372.8445,	12.47	
479.75640320603	179.9287,	6.01	
488.255690256077	182.9139,	6.11	b5-98 (0.01)
495.263689898459	232.1086,	7.76	
501.796876402038	145.7105,	4.87	
513.266786368295	186.4981,	6.23	a9o[2+] (0.01)
522.233539564453	225.5887,	7.54	a9[2+] (-0.01)
530.76110534525	143.4934,	4.79	b10-98[2+] (-0.01)
536.291881801932	184.5802,	6.17	
546.272328880725	345.8319,	11.56	
563.331964890352	936.213,	31.31	y4 (0.00)
564.278627371613	490.7664,	16.41	
607.334281421138	129.8538,	4.34	
608.317947031845	303.4037,	10.14	
617.301535459411	138.1768,	4.62	

Figure S3

635.318824691056	1099.768,	36.78	b6-98 (0.00)
673.33309459599	143.9009,	4.81	
679.348398504103	170.5854,	5.70	
681.309080577769	145.7011,	4.87	y11[2+] (-0.01)
687.36746109763	166.6562,	5.57	
691.421961972494	369.2962,	12.35	y5 (-0.00)
716.420303382814	132.7292,	4.43	
717.867275501356	395.429,	13.22	
718.377737859127	405.3716,	13.55	
720.395728873642	159.2284,	5.32	
727.872943151538	343.8822,	11.50	
748.399418606985	478.5472,	16.00	b7-98 (0.00)
758.400939284111	379.8635,	12.70	
775.424990108099	134.3638,	4.49	
780.410737784568	215.7874,	7.21	
792.429513325155	175.1945,	5.86	
792.940345202008	361.6625,	12.09	
797.338309971147	158.5562,	5.30	
801.426194157124	232.5715,	7.77	y14-98[2+] (-0.00)
811.393808880328	692.2307,	23.15	
812.509549427392	523.9662,	17.52	
820.463717645503	177.4988,	5.93	y6 (-0.00)
850.91871539137	211.5274,	7.07	
857.06735232585	137.4559,	4.59	
889.464423671115	225.528,	7.54	
907.5038370039	255.6985,	8.55	y7 (0.00)
941.496323260819	268.9549,	8.99	
945.509676448229	1492.796,	49.93	
958.508435569047	1537.952,	51.44	
976.523419150955	962.0775,	32.18	y8-98 (0.01)
1082.80681154668	174.272,	5.82	
1089.58419755801	541.5483,	18.11	y9-98 (-0.01)
1158.61948938424	209.3681,	7.00	
1176.60576093931	427.1814,	14.28	
1177.64814926174	300.1272,	10.03	
1201.68888091753	188.1896,	6.29	
1227.63938781833	883.517,	29.55	
1245.66128208397	280.2534,	9.37	
1246.63774488712	218.1376,	7.29	
1263.67087190437	943.4734,	31.55	y11-98 (0.01)
1293.15318440604	203.1151,	6.79	
1361.6693602814	181.3989,	6.06	
1452.75551771001	886.8753,	29.66	

Figure S3

1470.76517456316	2428.688,	81.23	
1470.79890440855	195.3391,	6.53	
1471.74689396861	247.293,	8.27	
1472.78159857269	177.0214,	5.92	
1488.77389764326	2989.639,	100	y13-98 (0.00)
1489.78454999192	772.5894,	25.84	
1544.05693321753	195.805,	6.54	
1550.72670352451	964.5644,	32.26	
1568.73886882621	872.6167,	29.18	y13o (-0.00)
1569.75646225908	193.6003,	6.47	
1586.7561430589	2082.125,	69.64	y13 (-0.00)
1673.6528527171	178.2573,	5.96	
1703.14601197146	150.9439,	5.04	
1808.26361864817	181.9556,	6.08	
1989.07881921523	186.5828,	6.24	

Figure S3

# ProPhosSI MS/MS report

Mass: 811.3806 Charge: 3+

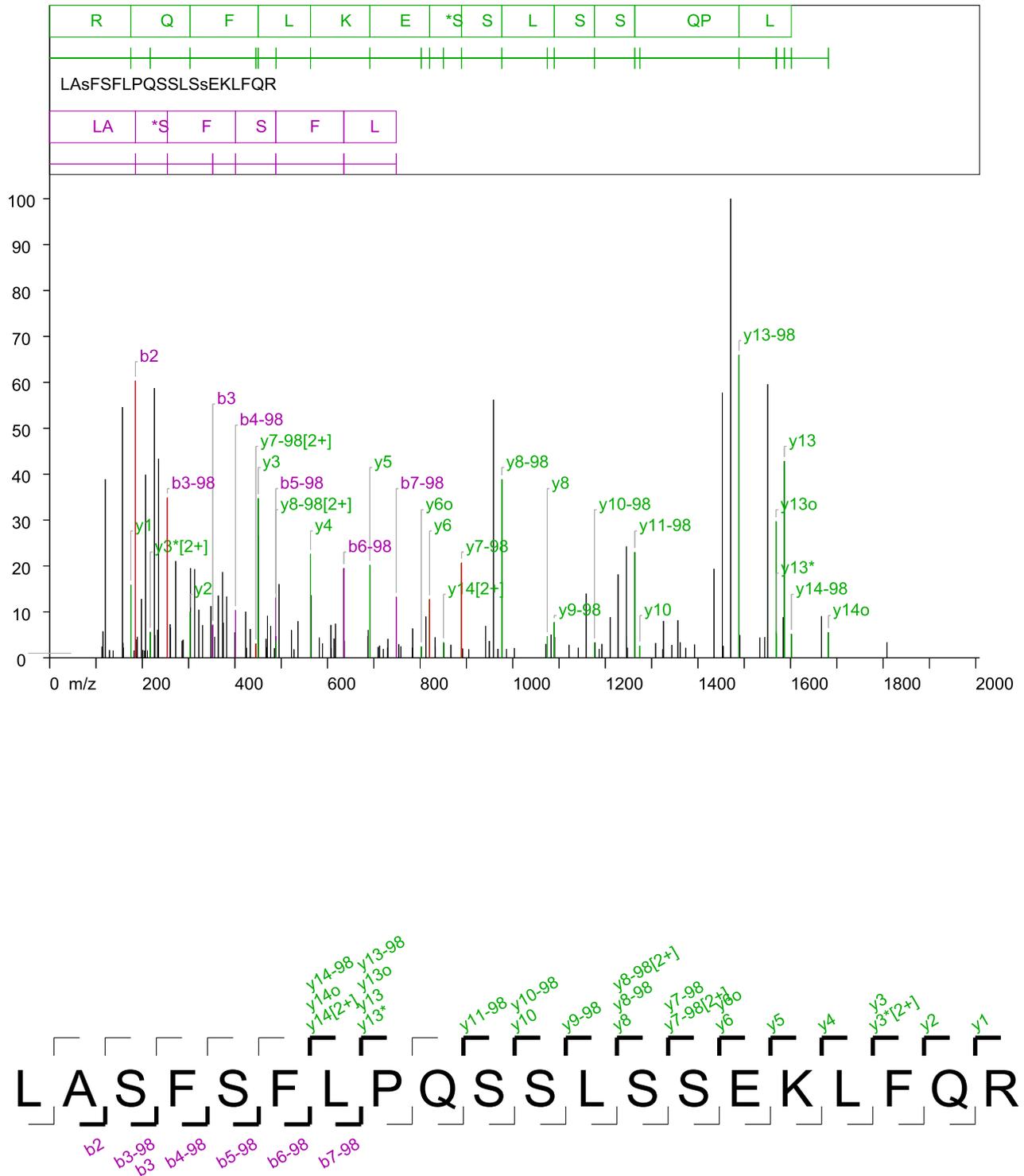


Figure S3

## 5-HT2A

(40) 278 LAsFSFLPQSSLSsEKLFR 297 2431.122 (-0.0042) Da

Parent Ion	m/z
No parent ions observed	

### Modifications and supporting evidence

Position	Residue ID	Modification	Evidence
3	(280)	Phospho (ST)	b2 => b3-98
14	(291)	Phospho (ST)	y6 => y7-98

### Spectrum interpretation

Rule	passed/tests	Description
Parent ions present	0/1	Parent ions corresponding to the parent fragment - 2 phosphate ions were not found
Three -98 Ions present	1/1	12 des-phospho fragment ions were found.
Unique -98 transitions present	2/2	transition b2 to b3-98 support unique phosphorylation at position 3  transition y6 to y7-98 support unique phosphorylation at position 14  
Four Sequential b or y ions	1/1	Sequence of four b ions found from b2 to b5-98. Sequence of four y ions found from y1 to y4.
Five of six sequential ions present	1/1	Five of Six ions found between b1 and b6 Five of Six ions found between b2 and b7 Five of Six ions found between b3 and b8 Five of Six ions found between y0 and y5 Five of Six ions found between y1 and y6 Five of Six ions found between y2 and y7 Five of Six ions found between y3 and y8 Five of Six ions found between y4 and y9 Five of Six ions found between y5 and y10 Five of Six ions found between y6 and y11 Five of Six ions found between y7 and y12 Five of Six ions found between y8 and y13 Five of Six ions found between y9 and y14
Proline directed fragmentation pattern	2/2	PASS: y13-98> y12-98  PASS: b8-98< b7-98 
PhosphoTyrosine transition present	0/0	0 of 0 phosphotyrosine transitions were found.
Six of top ten ions identified	4/6	ion 1 (mass: 1470.76163348332: intensity: 14058.48) assigned 0 times ion 2 (mass: 1488.77368212528: intensity: 9271.057) assigned 1 times ion 3 (mass: 185.127930453159: intensity: 8483.474) assigned 1 times ion 4 (mass: 1550.72672251521: intensity: 8377.197) assigned 0 times ion 5 (mass: 226.118077260304: intensity: 8259.873) assigned 0 times ion 6 (mass: 1452.75525480323: intensity: 8120.36) assigned 0 times ion 7 (mass: 958.509129623671: intensity: 7904.004) assigned 0 times ion 8 (mass: 157.133107477626: intensity: 7678.937) assigned 1 times ion 9 (mass: 235.107092285844: intensity: 6094.658) assigned 0 times ion 10 (mass: 1586.74881196566: intensity: 6021.309) assigned 1 times

Figure S3

## Ion Table

33 ions assigned of 133 ions above threshold (24%).

### N-terminal ions

AA	N-ion	b	b*	b-196	b-98	bo
L	1	114.091	97.065	-	-	96.081
A	2	185.129 185.127930453159 (60)	168.102	-	-	167.118
s	3	352.127 352.1270259368 (7)	335.100	-	254.141 254.149336220203 (34)	334.116
F	4	499.195	482.169	-	401.209 401.215271569225 (10)	481.185
S	5	586.227	569.201	-	488.241 488.250691595505 (13)	568.217
F	6	733.296	716.269	-	635.310 635.315208568773 (19)	715.285
L	7	846.380	829.353	-	748.394 748.404633472677 (13)	828.369
P	8	943.433	926.406	-	845.447	925.422
Q	9	1071.491	1054.465	-	973.505	1053.481
S	10	1158.523	1141.497	-	1060.537	1140.513
S	11	1245.555	1228.529	-	1147.569	1227.545
L	12	1358.639	1341.613	-	1260.653	1340.629
S	13	1445.671	1428.645	-	1347.685	1427.661
s	14	1612.670	1595.643	1416.698	1514.684	1594.659
E	15	1741.712	1724.686	1545.740	1643.726	1723.702
K	16	1869.807	1852.781	1673.835	1771.821	1851.797
L	17	1982.891	1965.865	1786.919	1884.905	1964.881
F	18	2129.960	2112.933	1933.988	2031.974	2111.949
Q	19	2258.018	2240.992	2062.046	2160.032	2240.008
R	20	-	-	-	-	-

### C-terminal ions

AA	C-ion	y	y*	y-196	y-98	yo
L	20	-	-	-	-	-
A	19	2319.046	2302.019	2123.074	2221.060	2301.035
s	18	2248.009	2230.982	2052.037	2150.023	2229.998
F	17	2081.010	2063.984	-	1983.025	2063.000
S	16	1933.942	1916.915	-	1835.956	1915.931
F	15	1846.910	1829.883	-	1748.924	1828.899
L	14	1699.842 850.41549439817 [2+] (3)	1682.815	-	1601.856 1601.86266460894 (5)	1681.831 1681.81315529978 (5)
P	13	1586.757 1586.74881196566 (42)	1569.731 *1569.74846759354 (4)	-	1488.772 1488.77368212528 (65)	1568.747 1568.74044423296 (29)
Q	12	1489.705	1472.678	-	1391.719	1471.694
S	11	1361.646	1344.620	-	1263.660 1263.66620099434 (23)	1343.636
S	10	1274.614 1274.60468775584 (2)	1257.588	-	1176.628 1176.63722089752 (3)	1256.604
L	9	1187.582	1170.556	-	1089.596 1089.6033394263 (7)	1169.572

Figure S3

S	8	1074.498 1074.49434922973 (4)	1057.471	-	976.512 976.515837348386 (38) 488.761862141534 [2+] (3)	1056.487
s	7	987.466	970.439	-	889.480 445.246729161158 [2+] (3) 889.490767651596 (20)	969.455
E	6	820.468 820.464599924209 (12)	803.441	-	-	802.457 802.458061915472 (2)
K	5	691.425 691.422549632987 (20)	674.399	-	-	673.414
L	4	563.330 563.328881184491 (22)	546.304	-	-	545.320
F	3	450.246 450.243174369696 (34)	433.219 *217.096677224044 [2+] (5)	-	-	432.235
Q	2	303.178 303.176913504457 (10)	286.151	-	-	285.167
R	1	175.119 175.118617339375 (15)	158.092	-	-	157.108

### Ion distribution

Threshold	Ion count	Matches	% matched
0	146	34	23
0.5	146	34	23
1	146	34	23
2	133	33	24
3	108	30	27
4	90	26	28
5	74	24	32
10	44	19	43

### Observed ions > 1%

m/z	Intensity	% max	Assignment (delta)
112.650561704402	343.8671 <sub>s</sub>	2.44	
115.086004524778	812.8741 <sub>s</sub>	5.78	
120.080296160141	5466.93 <sub>s</sub>	38.88	
129.103513479681	236.8871 <sub>s</sub>	1.68	
136.884157104203	225.0041 <sub>s</sub>	1.60	
157.133107477626	7678.937 <sub>s</sub>	54.62	a2 (-0.00)
158.13592222714	461.1295 <sub>s</sub>	3.28	
159.075557546639	310.3123 <sub>s</sub>	2.20	
175.118617339375	2236.423 <sub>s</sub>	15.90	y1 (-0.00)
182.174006504626	226.0673 <sub>s</sub>	1.60	
185.127930453159	8483.474 <sub>s</sub>	60.34	b2 (-0.00)

Figure S3

186.131772909014	562.3774,	4.00	
189.102126798161	643.1686,	4.57	
198.123134657274	1803.747,	12.83	
201.122176790028	238.8865,	1.69	b4-98[2+] (0.01)
205.024880759745	221.4104,	1.57	
207.112063864184	5606.69,	39.88	
211.140466818875	222.2124,	1.58	
217.096677224044	789.4785,	5.61	y3*[2+] (-0.01) : z3[2+] (-0.01)
226.118077260304	8259.873,	58.75	
227.122308298695	695.4792,	4.94	
233.164926222617	860.9671,	6.12	
235.107092285844	6094.658,	43.35	
254.149336220203	4900.375,	34.85	b3-98 (0.00)
260.13824156774	1029.558,	7.32	
261.157695224926	919.1305,	6.53	
272.159647818619	2961.461,	21.06	
286.117129799716	521.2686,	3.70	
288.132980452366	554.0073,	3.94	
303.176913504457	1409.1,	10.02	y2 (-0.00)
304.128532442337	2744.249,	19.52	
313.15005368221	2713.158,	19.29	
322.139015224226	1471.461,	10.46	
330.180008203185	1001.264,	7.12	
348.19079482348	1582.574,	11.25	
352.1270259368	1007.959,	7.16	b3 (-0.00)
356.197415048707	642.4418,	4.56	
364.163078839505	1906.082,	13.55	
373.222433671109	2624.351,	18.66	
375.165511023253	1076.39,	7.65	
382.172653680704	1878.478,	13.36	
400.180962797082	783.3706,	5.57	
401.215271569225	1452.29,	10.33	b4-98 (0.00)
423.201582919969	1414.322,	10.06	
425.220043245679	304.0442,	2.16	
433.18538190622	882.4131,	6.27	
445.246729161158	429.89,	3.05	y7-98[2+] (0.00)
450.243174369696	4880.013,	34.71	y3 (-0.00)
451.19813208846	3744.778,	26.63	
467.265009869109	581.7351,	4.13	
469.204628695936	328.0904,	2.33	
470.236728188091	1284.61,	9.13	
477.24561502676	976.1146,	6.94	
485.270071049158	295.1492,	2.09	

Figure S3

488.250691595505	1837.385,	13.06	b5-98 (0.00)
488.761862141534	539.3345,	3.83	y8-98[2+] (0.00)
495.255828787344	2255.698,	16.04	
522.229011078452	847.3423,	6.02	
527.63232028096	259.5875,	1.84	
536.283124141096	1122.323,	7.98	
563.328881184491	3176.474,	22.59	y4 (-0.00)
564.277402541035	1916.86,	13.63	
582.289329235562	619.9152,	4.40	
589.314036132004	438.3084,	3.11	
607.324159961669	1005.97,	7.15	
608.315981009447	287.2435,	2.04	
614.326431215456	591.5825,	4.20	
617.309262276776	1044.226,	7.42	
635.315208568773	2739.549,	19.48	b6-98 (0.00)
636.330793176896	524.0677,	3.72	
687.363963628419	672.401,	4.78	
687.855335716055	849.4122,	6.04	
691.422549632987	2839.987,	20.20	y5 (-0.00)
709.359792075255	332.6599,	2.36	a13[2+] (0.01)
712.39008267781	374.526,	2.66	
720.420552085708	268.7951,	1.91	
729.899762691378	307.4103,	2.18	
730.387208414242	578.7772,	4.11	
748.404633472677	1861.163,	13.23	b7-98 (0.01)
753.88831889407	415.5214,	2.95	
758.355225156759	351.3311,	2.49	
783.409751314286	307.8015,	2.18	
783.921216732234	898.6155,	6.39	
802.458061915472	347.4336,	2.47	y6o (0.00)
812.502206175493	1265.406,	9.00	
820.464599924209	1788.022,	12.71	y6 (-0.00)
832.413823865856	628.1226,	4.46	
832.902580685863	327.6867,	2.33	
850.41549439817	472.0758,	3.35	y14[2+] (-0.00)
850.93836138324	452.1765,	3.21	
866.459226157088	400.112,	2.84	
889.490767651596	2906.054,	20.67	y7-98 (0.01)
891.964382445441	284.574,	2.02	
905.176554801675	260.7159,	1.85	
941.481127778634	975.5826,	6.93	
949.467270226536	514.9477,	3.66	
958.509129623671	7904.004,	56.22	

Figure S3

968.00691283512	273.9839 <sub>1</sub>	1.94	
976.515837348386	5459.811 <sub>1</sub>	38.83	y8-98 (0.00)
986.553875457366	268.2345 <sub>1</sub>	1.90	
1003.48246092062	299.6903 <sub>1</sub>	2.13	
1071.59137153271	425.4649 <sub>1</sub>	3.02	
1074.49434922973	651.7751 <sub>1</sub>	4.63	y8 (-0.00)
1082.79269419486	710.7668 <sub>1</sub>	5.05	
1089.6033394263	1085.764 <sub>1</sub>	7.72	y9-98 (0.00)
1090.59484674019	641.1929 <sub>1</sub>	4.56	
1121.60283480062	399.0441 <sub>1</sub>	2.83	
1141.61608710246	308.3931 <sub>1</sub>	2.19	
1158.63037854916	1965.693 <sub>1</sub>	13.98	
1176.63722089752	468.5769 <sub>1</sub>	3.33	y10-98 (0.00)
1177.59696516822	472.8737 <sub>1</sub>	3.36	
1186.70975472891	270.214 <sub>1</sub>	1.92	
1192.62039087947	424.9232 <sub>1</sub>	3.02	
1210.63441196721	1246.278 <sub>1</sub>	8.86	
1227.64390343266	2553.451 <sub>1</sub>	18.16	
1245.64871773718	3411.422 <sub>1</sub>	24.26	
1245.66332316627	659.2913 <sub>1</sub>	4.68	
1247.67920293057	307.9808 <sub>1</sub>	2.19	
1263.66620099434	3236.759 <sub>1</sub>	23.02	y11-98 (0.00)
1274.60468775584	371.0995 <sub>1</sub>	2.63	y10 (-0.00)
1308.60580343289	452.6648 <sub>1</sub>	3.21	
1324.21415091289	263.7643 <sub>1</sub>	1.87	
1325.62517946075	1127.813 <sub>1</sub>	8.02	
1343.61406401428	394.5172 <sub>1</sub>	2.80	
1356.69455035672	1151.534 <sub>1</sub>	8.19	
1361.62480132484	475.5161 <sub>1</sub>	3.38	
1373.69160381894	305.6039 <sub>1</sub>	2.17	
1392.75329906264	408.2734 <sub>1</sub>	2.90	
1434.7414618845	2724.375 <sub>1</sub>	19.37	
1452.75525480323	8120.36 <sub>1</sub>	57.76	
1454.72564478204	363.8668 <sub>1</sub>	2.58	
1470.76163348332	14058.48 <sub>1</sub>	100	
1488.77368212528	9271.057 <sub>1</sub>	65.94	y13-98 (0.00)
1490.7973479686	693.8813 <sub>1</sub>	4.93	
1533.69816845886	619.4782 <sub>1</sub>	4.40	
1544.32138738546	632.3989 <sub>1</sub>	4.49	
1550.72672251521	8377.197 <sub>1</sub>	59.58	
1568.72055538327	732.8612 <sub>1</sub>	5.21	
1568.74044423296	4172.783 <sub>1</sub>	29.68	y13o (-0.00)
1569.74846759354	685.3838 <sub>1</sub>	4.87	y13* (0.01) : z13 (0.01)

Figure S3

1583.8499999924	1244.955 <sub>2</sub>	8.85	
1586.74881196566	6021.309 <sub>2</sub>	42.83	y13 (-0.00)
1601.86266460894	725.6587 <sub>2</sub>	5.16	y14-98 (0.00)
1666.70826454442	1274.329 <sub>2</sub>	9.06	
1681.81315529978	781.3789 <sub>2</sub>	5.55	y14o (-0.01)
1808.28095750175	474.6764 <sub>2</sub>	3.37	

Figure S3

# ProPhosSI MS/MS report

Mass: 838.035 Charge: 3+

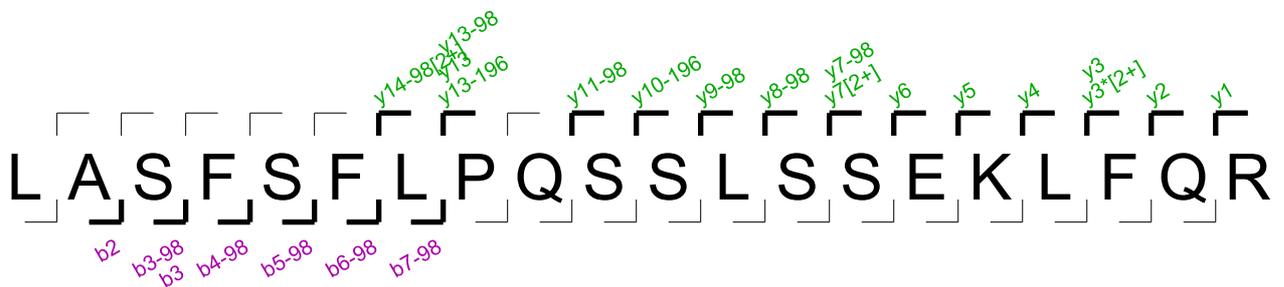
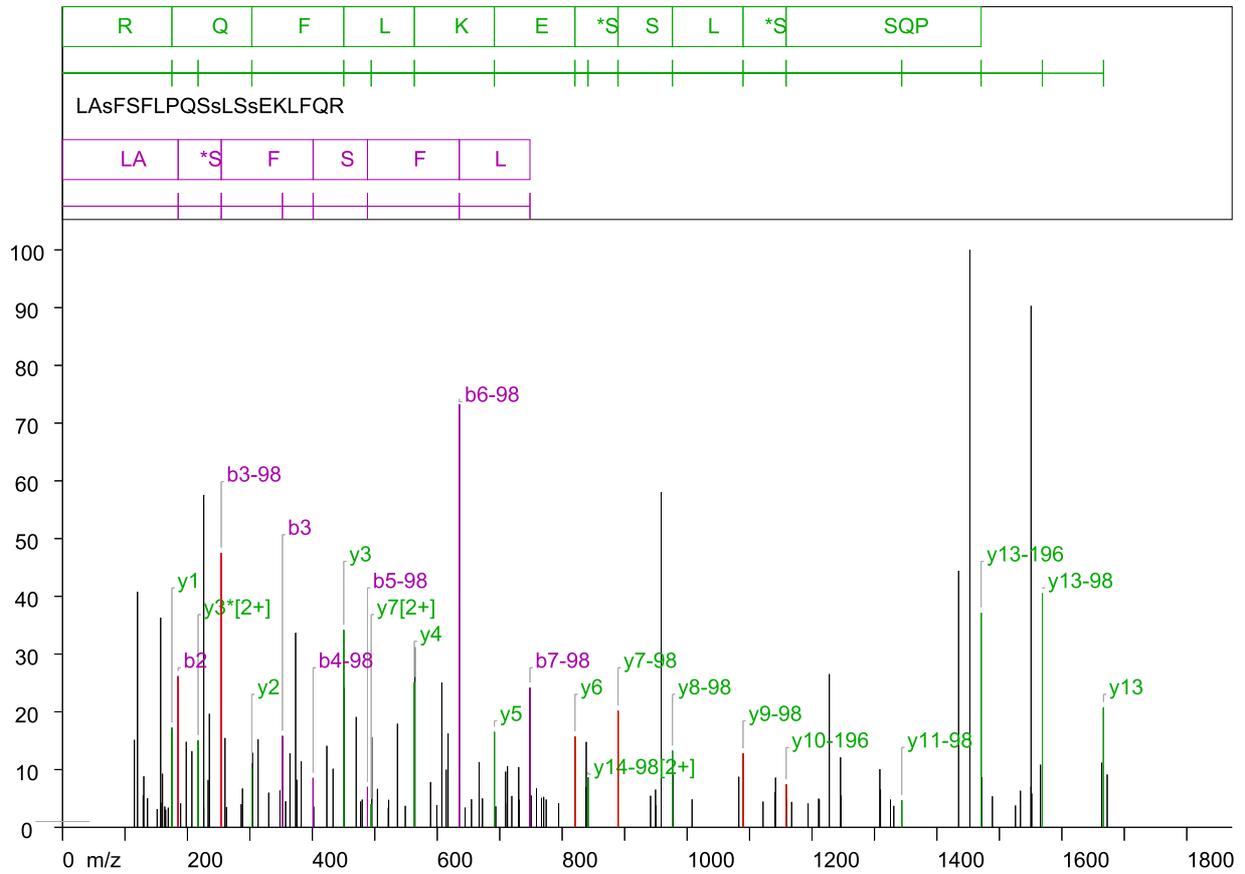


Figure S3

## 5-HT2A

(21) 278 LAsFSFLPQSSLSsEKLFQR 297 2511.088 (-0.0073) Da

Parent Ion	m/z
No parent ions observed	

### Modifications and supporting evidence

Position	Residue ID	Modification	Evidence
3	(280)	Phospho (ST)	b2 => b3-98
14	(291)	Phospho (ST)	y6 => y7-98
11	(288)	Phospho (ST)	y9-98 => y10-196

### Spectrum interpretation

Rule	passed/tests	Description
Parent ions present	0/1	Parent ions corresponding to the parent fragment - 3 phosphate ions were not found
Three -98 Ions present	1/1	10 des-phospho fragment ions were found.
Unique -98 transitions present	3/3	transition b2 to b3-98 support unique phosphorylation at position 3  transition y6 to y7-98 support unique phosphorylation at position 14  transition y9-98 to y10-196 support unique phosphorylation at position 11  
Four Sequential b or y ions	1/1	Sequence of four b ions found from b2 to b5-98. Sequence of four y ions found from y1 to y4.
Five of six sequential ions present	1/1	Five of Six ions found between b1 and b6 Five of Six ions found between b2 and b7 Five of Six ions found between b3 and b8 Five of Six ions found between y0 and y5 Five of Six ions found between y1 and y6 Five of Six ions found between y2 and y7 Five of Six ions found between y3 and y8 Five of Six ions found between y4 and y9 Five of Six ions found between y5 and y10 Five of Six ions found between y6 and y11
Proline directed fragmentation pattern	2/2	PASS: y13-196> y12-196  PASS: b8-98< b7-98 
PhosphoTyrosine transition present	0/0	0 of 0 phosphotyrosine transitions were found.
Six of top ten ions identified	4/6	ion 1 (mass: 1452.76047263653: intensity: 4105.77) assigned 0 times ion 2 (mass: 1550.73199749264: intensity: 3707.406) assigned 0 times ion 3 (mass: 635.31681567314: intensity: 3009.225) assigned 1 times ion 4 (mass: 958.510632082482: intensity: 2383.28) assigned 0 times ion 5 (mass: 226.118333902931: intensity: 2363.376) assigned 0 times ion 6 (mass: 254.149968050718: intensity: 1950.642) assigned 1 times ion 7 (mass: 1434.74552317685: intensity: 1823.894) assigned 0 times ion 8 (mass: 120.080402043637: intensity: 1675.335) assigned 0 times ion 9 (mass: 1568.74683510991: intensity: 1662.965) assigned 1 times ion 10 (mass: 1470.77166105214: intensity: 1526.216) assigned 1 times

Figure S3

## Ion Table

27 ions assigned of 129 ions above threshold (20%).

### N-terminal ions

AA	N-ion	b	b*	b-196	b-294	b-98	bo
L	1	114.091	97.065	-	-	-	96.081
A	2	185.129 185.127985070283 (26)	168.102	-	-	-	167.118
s	3	352.127 352.126095233316 (15)	335.100	-	-	254.141 254.149968050718 (47)	334.116
F	4	499.195	482.169	-	-	401.209 401.218031937405 (8)	481.185
S	5	586.227	569.201	-	-	488.241 488.24951760832 (6)	568.217
F	6	733.296	716.269	-	-	635.310 635.31681567314 (73)	715.285
L	7	846.380	829.353	-	-	748.394 748.401767107408 (24)	828.369
P	8	943.433	926.406	-	-	845.447	925.422
Q	9	1071.491	1054.465	-	-	973.505	1053.481
S	10	1158.523	1141.497	-	-	1060.537	1140.513
s	11	1325.521	1308.495	1129.550	-	1227.536	1307.511
L	12	1438.606	1421.579	1242.634	-	1340.620	1420.595
S	13	1525.638	1508.611	1329.666	-	1427.652	1507.627
s	14	1692.636	1675.609	1496.664	1398.678	1594.650	1674.625
E	15	1821.679	1804.652	1625.707	1527.721	1723.693	1803.668
K	16	1949.773	1932.747	1753.802	1655.816	1851.788	1931.763
L	17	2062.858	2045.831	1866.886	1768.900	1964.872	2044.847
F	18	2209.926	2192.899	2013.954	1915.968	2111.940	2191.915
Q	19	2337.985	2320.958	2142.013	2044.027	2239.999	2319.974
R	20	-	-	-	-	-	-

### C-terminal ions

AA	C-ion	y	y*	y-196	y-294	y-98	yo
L	20	-	-	-	-	-	-
A	19	2399.012	2381.986	2203.040	2105.054	2301.026	2381.002
s	18	2327.975	2310.949	2132.003	2034.017	2229.989	2309.964
F	17	2160.977	2143.950	1965.005	-	2062.991	2142.966
S	16	2013.908	1996.882	1817.937	-	1915.922	1995.898
F	15	1926.876	1909.850	1730.904	-	1828.890	1908.866
L	14	1779.808	1762.781	1583.836	-	1681.822 841.415044669496 [2+] (8)	1761.797
P	13	1666.724 1666.72813552082 (20)	1649.697	1470.752 1470.77166105214 (37)	-	1568.738 1568.74683510991 (40)	1648.713
Q	12	1569.671	1552.644	1373.699	-	1471.685	1551.660
S	11	1441.612	1424.586	1245.641	-	1343.627 1343.6351505328 (4)	1423.602
s	10	1354.580	1337.554	1158.609 1158.61028713913 (7)	-	1256.595	1336.570
L	9	1187.582	1170.556	-	-	1089.596	1169.572

Figure S3

-	-	-	-	-	-	1089.60761154859 (12)	-
S	8	1074.498	1057.471	-	-	976.512 976.516367001958 (13)	1056.487
s	7	987.466 494.239412262855 [2+] (4)	970.439	-	-	889.480 889.488540482363 (20)	969.455
E	6	820.468 820.468253809435 (15)	803.441	-	-	-	802.457
K	5	691.425 691.424339792412 (16)	674.399	-	-	-	673.414
L	4	563.330 563.328552340466 (25)	546.304	-	-	-	545.320
F	3	450.246 450.244266503683 (34)	433.219 *217.096688080591 [2+] (15)	-	-	-	432.235
Q	2	303.178 303.178683154425 (10)	286.151	-	-	-	285.167
R	1	175.119 175.118636663601 (17)	158.092	-	-	-	157.108

### Ion distribution

Threshold	Ion count	Matches	% matched
0	129	27	20
0.5	129	27	20
1	129	27	20
2	129	27	20
3	129	27	20
4	113	27	23
5	91	24	26
10	54	19	35

### Observed ions > 1%

m/z	Intensity	% max	Assignment (delta)
115.086480820804	622.1433 <sub>s</sub>	15.15	
120.080402043637	1675.335 <sub>s</sub>	40.80	
129.101949042445	228.6353 <sub>s</sub>	5.56	
130.141086798261	362.6477 <sub>s</sub>	8.83	
136.074481044153	207.2301 <sub>s</sub>	5.04	
151.520162050041	129.2537 <sub>s</sub>	3.14	
157.133287185323	1490.943 <sub>s</sub>	36.31	a2 (-0.00)
159.090740399527	174.754 <sub>s</sub>	4.25	
160.07533092322	380.9922 <sub>s</sub>	9.27	
163.729546137367	147.9486 <sub>s</sub>	3.60	
165.10598256474	125.799 <sub>s</sub>	3.06	

Figure S3

169.260319328271	139.5669,	3.39	
175.118636663601	708.5631,	17.25	y1 (-0.00)
185.127985070283	1073.942,	26.15	b2 (-0.00)
189.10000453798	171.2428,	4.17	
198.123619882197	607.6479,	14.79	
207.112777669348	540.7701,	13.17	
217.096688080591	618.7856,	15.07	y3*[2+] (-0.01) : z3[2+] (-0.01)
226.011897460721	140.6161,	3.42	
226.118333902931	2363.376,	57.56	
233.164664291274	336.6304,	8.19	
235.107120314373	807.2631,	19.66	
254.149968050718	1950.642,	47.50	b3-98 (0.00)
260.139409760326	634.9448,	15.46	
262.49897390195	144.5072,	3.51	
286.121514659249	164.1084,	3.99	
288.134831351107	275.8215,	6.71	
303.178683154425	420.4379,	10.24	y2 (0.00)
304.128904048143	530.0134,	12.90	
313.150480693503	625.2482,	15.22	
330.176320973064	246.2573,	5.99	
348.19195343307	262.1448,	6.38	
352.126095233316	649.5034,	15.81	b3 (-0.00)
357.151064140371	185.1748,	4.51	
364.162281887819	526.2432,	12.81	
373.223224157046	1383.174,	33.68	
375.169632801367	338.524,	8.24	
382.17148213732	469.9827,	11.44	
401.218031937405	350.7733,	8.54	b4-98 (0.00)
402.114234471859	146.7309,	3.57	
423.201761341213	579.367,	14.11	
433.186365565489	416.4528,	10.14	
450.244266503683	1402.18,	34.15	y3 (-0.00)
451.199843915376	991.3551,	24.14	
470.239617368218	784.5166,	19.10	
477.242462240826	183.048,	4.45	
479.759934439468	197.1762,	4.80	
488.24951760832	286.7546,	6.98	b5-98 (0.00)
494.239412262855	165.5757,	4.03	y7[2+] (0.00)
495.258526799137	641.1547,	15.61	
504.222713669824	273.2846,	6.65	
521.668963857833	139.8686,	3.40	
522.237478382682	195.3264,	4.75	a9[2+] (-0.01)
536.28664110993	736.7483,	17.94	

Figure S3

548.832134545499	152.5863,	3.71	
563.328552340466	1031.771,	25.12	y4 (-0.00)
564.280732774695	1281.054,	31.20	
589.308727646618	322.1092,	7.84	
599.326350390134	158.4441,	3.85	
607.323210689886	1030.289,	25.09	
614.326082970576	409.9028,	9.98	
617.308267853442	667.5524,	16.25	
635.31681567314	3009.225,	73.29	b6-98 (0.00)
644.590245487522	139.8018,	3.40	
654.796693368156	199.0289,	4.84	
667.012454898479	463.8052,	11.29	
672.344779430786	205.669,	5.00	
691.424339792412	677.8307,	16.50	y5 (-0.00)
693.655635364186	149.1974,	3.63	
709.366475360465	395.8629,	9.64	
711.877051175271	170.435,	4.15	
712.368755445476	435.3008,	10.60	
719.333964983733	222.5768,	5.42	
730.395318604563	428.6717,	10.44	
731.260655565435	197.0375,	4.79	
748.401767107408	992.6113,	24.17	b7-98 (0.00)
750.310149905696	226.7974,	5.52	c6 (-0.01)
758.849765029345	276.5911,	6.73	
766.872784561531	209.1769,	5.09	
770.380570848054	216.3179,	5.26	
774.417262288684	197.9304,	4.82	
794.371509581576	171.2828,	4.17	
820.468253809435	644.6912,	15.70	y6 (0.00)
838.003622141148	286.8811,	6.98	
838.490706448835	607.1793,	14.78	
841.415044669496	355.8518,	8.66	y14-98[2+] (-9.53)
889.488540482363	827.569,	20.15	y7-98 (0.00)
941.484423265577	224.3257,	5.46	
949.457881446895	268.056,	6.52	
949.921483641343	156.1527,	3.80	
958.510632082482	2383.28,	58.04	
976.516367001958	544.449,	13.26	y8-98 (0.00)
977.536048177668	373.4191,	9.09	
1007.9828788406	198.6952,	4.83	
1082.7972228078	359.4011,	8.75	
1089.60761154859	524.6043,	12.77	y9-98 (0.01)
1121.60644080603	183.1289,	4.46	

Figure S3

1140.66469731777	249.3321,	6.07	
1141.60550735998	353.3557,	8.60	
1158.61028713913	305.033,	7.42	y10-196 (0.00)
1159.64316702849	198.7959,	4.84	
1167.42921090871	180.5409,	4.39	
1193.67427759038	169.1491,	4.11	
1210.63168520998	204.3067,	4.97	
1211.60899919745	198.0866,	4.82	
1227.64355635021	1089.793,	26.54	
1245.66856117944	497.6163,	12.11	
1246.69067834453	225.2937,	5.48	
1308.6032807395	414.0063,	10.08	
1309.58396580644	268.5123,	6.53	
1325.60327900402	197.2018,	4.80	
1330.94984335853	152.1884,	3.70	
1343.6351505328	192.6155,	4.69	y11-98 (0.00)
1434.74552317685	1823.894,	44.42	
1452.76047263653	4105.77,	100	
1470.77166105214	1526.216,	37.17	y13-196 (0.01)
1471.76558743869	356.5122,	8.68	
1488.76909949956	220.3363,	5.36	
1525.74884394765	154.8456,	3.77	
1533.71406226352	260.1852,	6.33	
1550.70614943227	288.1833,	7.01	
1550.73199749264	3707.406,	90.29	
1551.74720401062	240.8931,	5.86	
1565.84586921423	445.4182,	10.84	
1568.71830089248	411.129,	10.01	
1568.74683510991	1662.965,	40.50	y13-98 (0.00)
1663.81437523561	459.253,	11.18	
1666.72813552082	853.3339,	20.78	y13 (0.00)
1672.59290642457	375.5474,	9.14	

Figure S3

**Figure S3. Interpretation of MS2 spectra matching phosphorylated peptides of 5-HT<sub>2A</sub> receptor.** MS2 spectra matching phosphorylated peptides were inspected using the Prohossi software (17) for automatic annotation (in red) of unique transitions that pinpoint the position of phosphorylated sites. For each phosphorylated peptide an annotated MS/MS spectrum is illustrated along with a table of fragment ion matches and a table summarizing the data for phosphorylation site assignment.