SUPPLEMENTAL DATA

SUPPLEMENTAL FIGURES

Supplemental Figure 1. BMMSCs were cultured in osteogenic medium containing 5% serum for 7 days. Transcript expression of the osteogenic and adipogenic markers ALP and PPARγ, respectively, was analyzed by qPCR at Days 3 and 7 to confirm progression of osteogenesis. Values are expressed as a percentage of the Day 3 mean. Error bars are ± S.E.M: $p < 0.01$ (**).

Supplemental Figure 2. BMMSCs were cultured for 6 days (Days 1-7) in osteogenic medium containing 5% serum and Thiamet G (0-20 μM) or its DMSO vehicle. OGT and OGA transcript levels were analyzed by qPCR. Transcript expression was normalized against GAPDH as an endogenous reference. Error bars are ± S.E.M: $p < 0.05$ (*).

Supplemental Figure 3. BMMSCs were cultured in osteogenic medium with 10% serum and rhBMP2/7 (50 ng/mL) or its vehicle. On the left, immunoblot analysis of total protein (25 μg) at Day 8. On the right, densitometric analysis of OGA protein levels normalized against GAPDH as a loading control. OGA protein expression was averaged across three independent replications and is expressed as a percentage of the vehicle control mean. The effect of BMP2/7 on elevation of global O-GlcNAc levels observed under osteogenic conditions with 5% serum (Fig. 5A) was masked under 10% serum, possibly due to enhanced baseline O-GlcNAcylation levels resulting from increased concentrations of growth factors and nutrients.

The following supplemental data (pages 4-26) are HCD or ETD MS/MS spectra which support peptide sequencing and assignment of unambiguous HexNAc, phosphorylation, or methylation events (bold). The theoretical fragment ions are in tabular format with the observed peaks underlined. The listed ETD *m/z* values are given for *c* and *z* ions only and not for c-1, c+1, or z+1 fragment ions which may be present in the scan. The gene symbol is given at the top of the page for each unique peptide, along with peptide residue numbers, the assigned site of modification, and the peptide charge state.

Gene: RUNX2

Acc#: Q13950

26-38 +Phospho

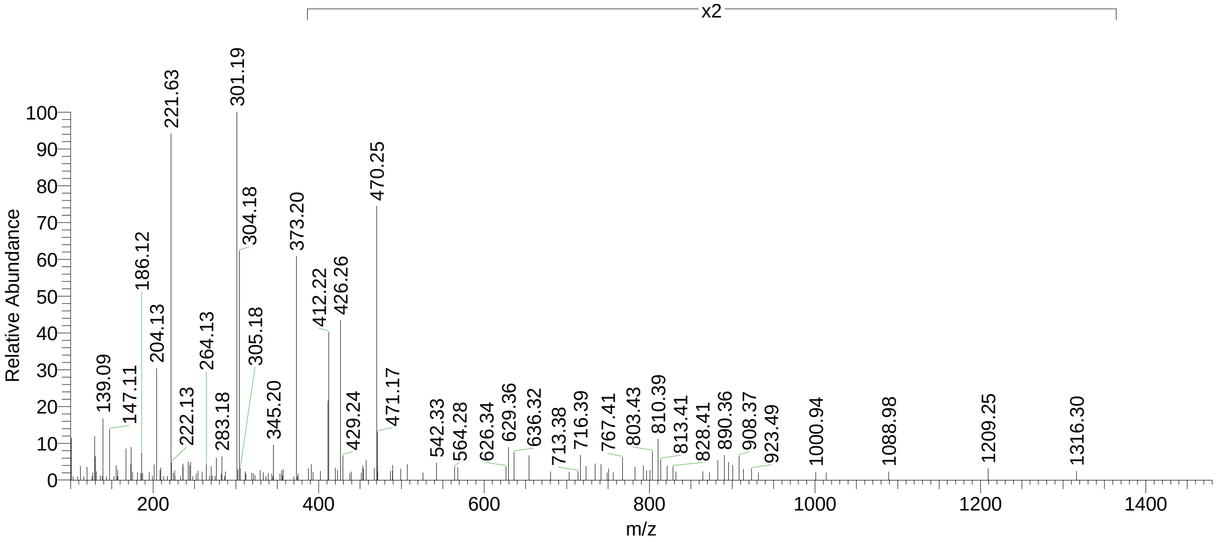
+3 charge state

The HCD fragmentation spectrum below contains both modified and unmodified species due to neutral loss of H_3PO_4.

b	b^{+2}				y	y^{+2}
---	---	1	R	13	---	---
304.18	152.59	2	F	12	1231.63	616.32
391.21	196.11	3	S	11	1084.56	542.79
488.26	244.63	4	P	10	997.53	499.27
585.31	293.16	5	P	9	900.48	450.74
672.35	336.68	6	S	8	803.43	402.22
759.38	380.19	7	S	7	716.39	358.70
846.41	423.71	8	S	6	629.36	315.18
959.49	480.25	9	L	5	542.33	271.67
1087.55	544.28	10	Q	4	429.25	215.13
1184.61	592.81	11	P	3	301.19	151.10
1241.63	621.32	12	G	2	204.13	102.57
---	---	13	K	1	147.11	74.06

b_3+Phospho 471.18

082813_SCW_DrBall_RNX2 #2411 RT: 68.21 AV: 1 NL: 1.76E4
T: FTMS + c NSI d Full ms2 489.90@hcd35.00 [100.00-1480.00]



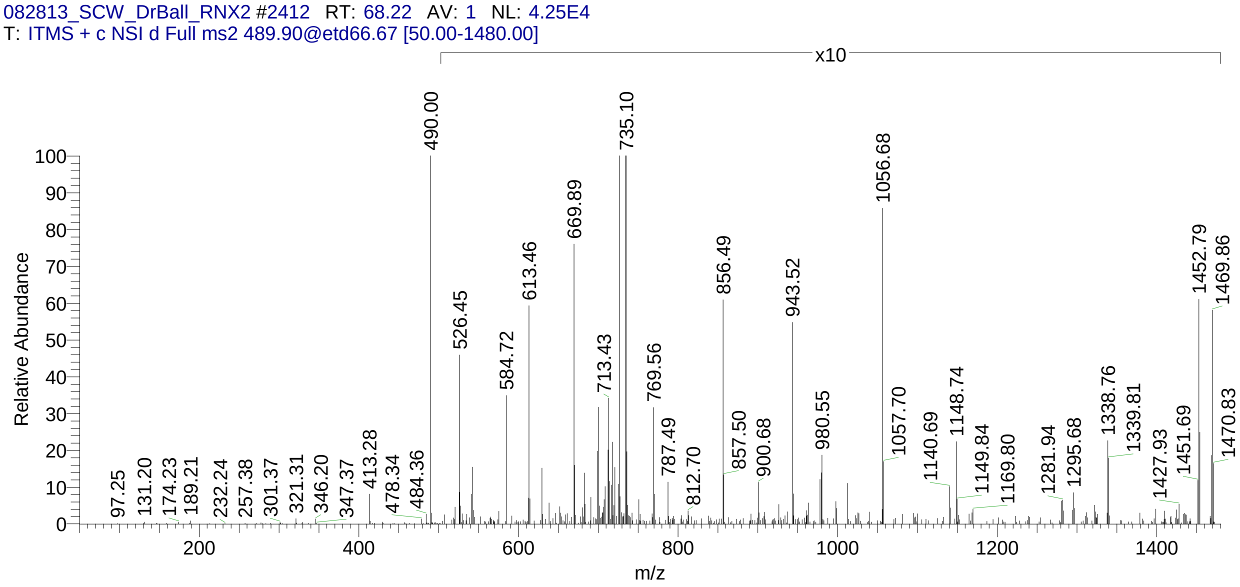
26-38 +Phospho

+3 charge state

The ETD fragmentation spectrum localizes the modification to Ser28

c	**c^{+2}**				**z**	**z^{+2}**
174.13	87.57	1	R	13	---	---
321.20	161.11	2	F	12	1295.58	648.29
---	---	3	**S(Phospho)**	11	1148.51	574.76
---	---	4	P	10	---	---
682.31	341.66	5	P	9	---	---
769.34	385.17	6	S	8	787.41	394.21
856.37	428.69	7	S	7	700.38	350.69
943.40	472.21	8	S	6	613.34	307.18
1056.49	528.75	9	L	5	526.31	263.66
---	---	10	Q	4	413.23	207.12
1281.60	641.30	11	P	3	---	---
1338.62	669.81	12	G	2	188.12	94.56
---	---	13	K	1	131.09	66.05

MH+2	734.35
MH+3	489.90


082813_SCW_DrBall_RNX2 #2412 RT: 68.22 AV: 1 NL: 4.25E4
T: ITMS + c NSI d Full ms2 489.90@etd66.67 [50.00-1480.00]

Gene: RUNX2

Acc#: Q13950

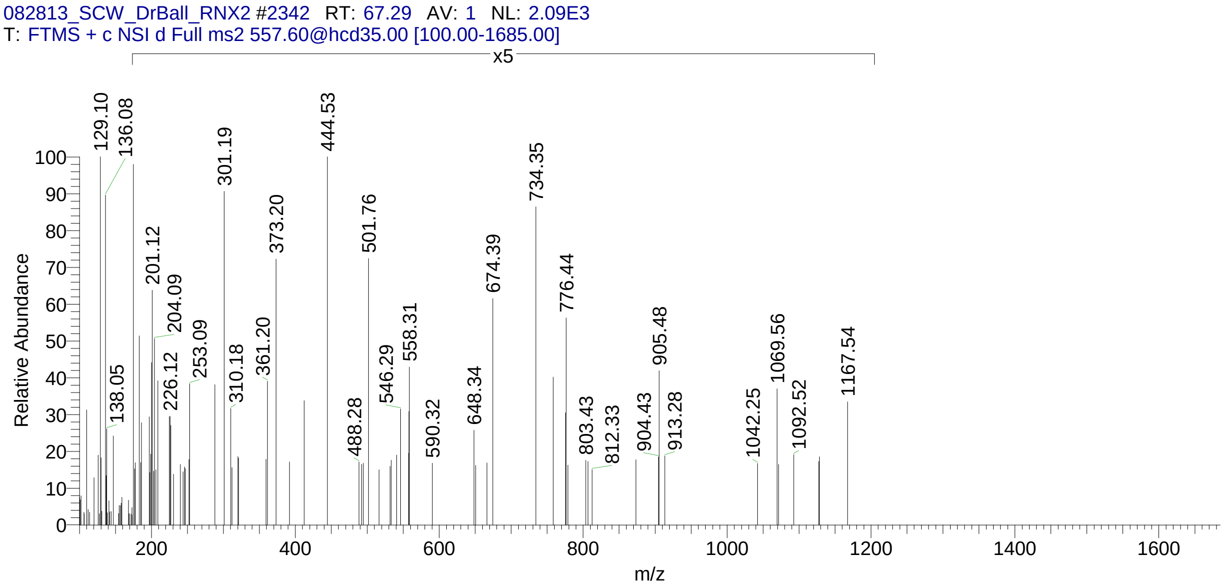
26-38 +Phospho, HexNAc

+3 charge state

The HCD fragmentation spectrum contains both modified and unmodified species due to neutral loss of H_3PO_4 and HexNAc. The ETD fragmentation spectrum localizing modification sites to Ser28 (phospho) and Ser32 (HexNAc) is represented in Fig. 2A.

b	**b^{+2}**				**y**	**y^{+2}**
---	---	1	R	13	---	---
304.18	152.59	2	F	12	1231.63	616.32
391.21	196.11	3	S	11	1084.56	542.79
488.26	244.63	4	P	10	997.53	499.27
585.31	293.16	5	P	9	900.48	450.74
672.35	336.68	6	S	8	803.43	402.22
759.38	380.19	7	S	7	716.39	358.70
846.41	423.71	8	S	6	629.36	315.18
959.49	480.25	9	L	5	542.33	271.67
1087.55	544.28	10	Q	4	429.25	215.13
1184.61	592.81	11	P	3	301.19	151.10
1241.63	621.32	12	G	2	204.13	102.57
---	---	13	K	1	147.11	74.06

b_3-H_2O	373.2
b_{10}-H_2O	1069.54
b_{11}-NH_3	1167.58
b_7+Phospho+HexNAc	1042.42


082813_SCW_DrBall_RNX2 #2342 RT: 67.29 AV: 1 NL: 2.09E3
T: FTMS + c NSI d Full ms2 557.60@hcd35.00 [100.00-1685.00]
x5
Relative Abundance
m/z
0
10
20
30
40
50
60
70
80
90
100
200
400
600
800
1000
1200
1400
1600
129.10
136.08
138.05
201.12
204.09
226.12
253.09
301.19
310.18
361.20
373.20
444.53
488.28
501.76
546.29
558.31
590.32
648.34
674.39
734.35
776.44
803.43
812.33
904.43
905.48
913.28
1042.25
1069.56
1092.52
1167.54

Gene: RUNX2

Acc#: Q13950

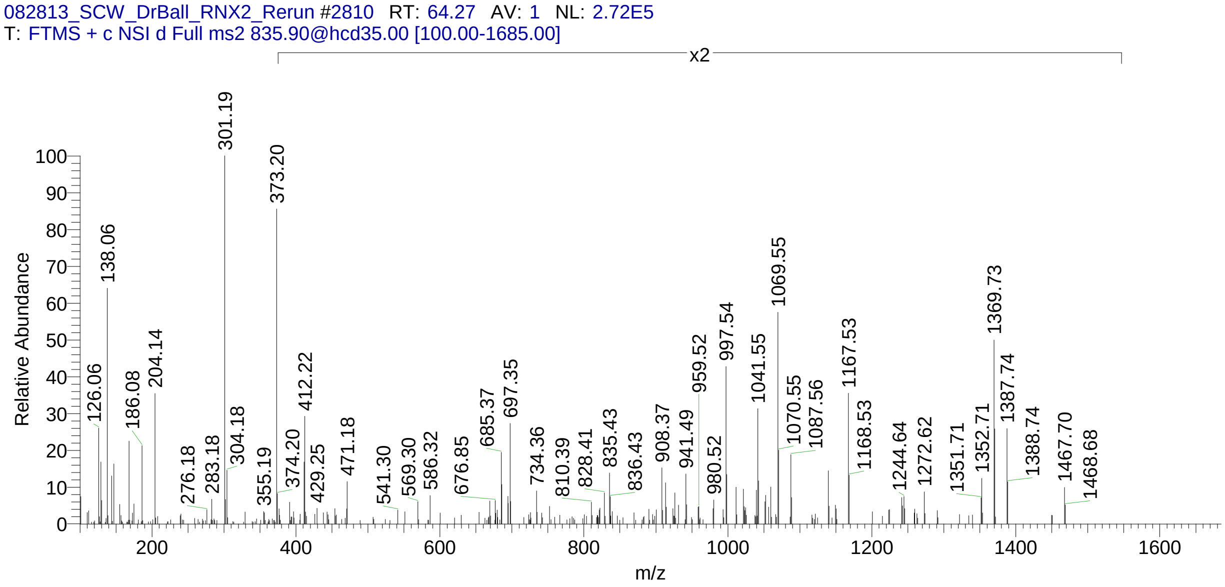
26-38 +Phospho, HexNAc

+2 charge state

The HCD fragmentation spectrum contains both modified and unmodified species due to neutral loss of H_3PO_4 and HexNAc.

b	**b^{+2}**				**y**	**y^{+2}**
---	---	1	R	13	---	---
304.18	152.59	2	F	12	1231.63	616.32
391.21	196.11	3	S	11	1084.56	542.79
488.26	244.63	4	P	10	997.53	499.27
585.31	293.16	5	P	9	900.48	450.74
672.35	336.68	6	S	8	803.43	402.22
759.38	380.19	7	S	7	716.39	358.70
846.41	423.71	8	S	6	629.36	315.18
959.49	480.25	9	L	5	542.33	271.67
1087.55	544.28	10	Q	4	429.25	215.13
1184.61	592.81	11	P	3	301.19	151.10
1241.63	621.32	12	G	2	204.13	102.57
---	---	13	K	1	147.11	74.06

b_3-H_20	373.2
b_{10}-H_20	1069.54
b_{11}-NH_3	1167.58
b_3+Phospho	471.18



082813_SCW_DrBall_RNX2_Rerun #2810 RT: 64.27 AV: 1 NL: 2.72E5
T: FTMS + c NSI d Full ms2 835.90@hcd35.00 [100.00-1685.00]

Gene: RUNX2

Acc#: Q13950

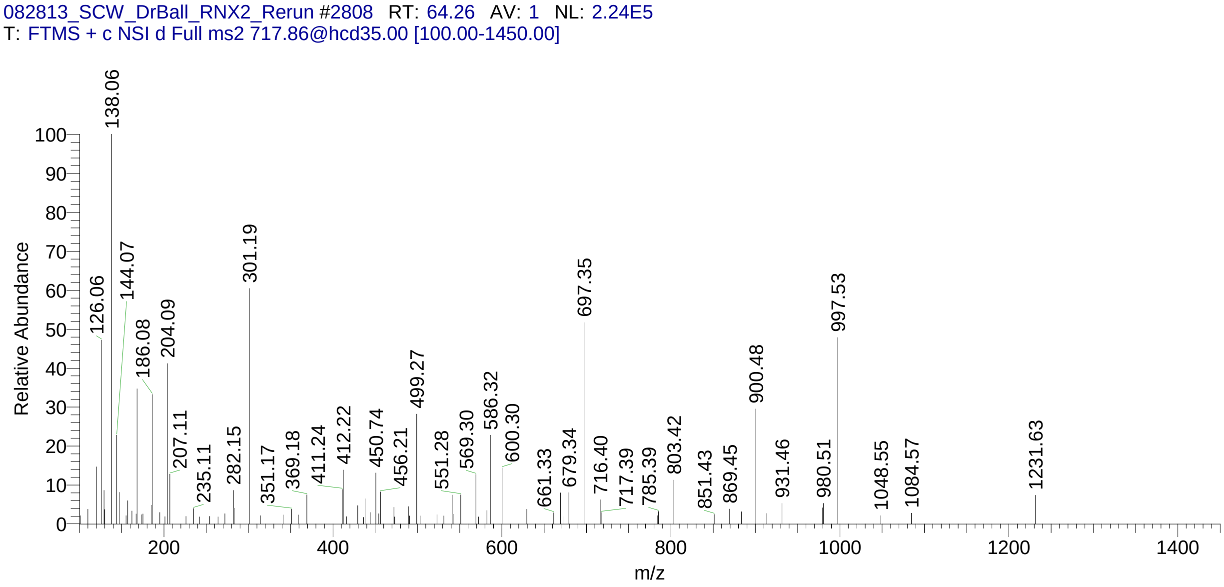
27-38 +HexNAc

+2 charge state

The HCD fragmentation spectrum below contains unmodified species due to neutral loss of HexNAc. The ETD fragmentation spectrum localizing modification sites to Ser32 or Ser33 is represented in Fig. 2B.

b				**y**	**y^{+2}**
---	1	F	12	---	---
235.11	2	S	11	1084.56	542.79
332.16	3	P	10	997.53	499.27
429.21	4	P	9	900.48	450.74
516.25	5	S	8	803.43	402.22
603.28	6	S	7	716.39	358.70
690.31	7	S	6	629.36	315.18
803.39	8	L	5	542.33	271.67
931.45	9	Q	4	429.25	215.13
1028.50	10	P	3	301.19	151.10
1085.53	11	G	2	204.13	102.57
---	12	K	1	147.11	74.06

y_8-H_20	785.38
y_{10}-H_20	979.53
y_4-NH_3	412.22
y_9-NH_3	883.45
y_{10}-NH_3	980.51


082813_SCW_DrBall_RNX2_Rerun #2808 RT: 64.26 AV: 1 NL: 2.24E5
T: FTMS + c NSI d Full ms2 717.86@hcd35.00 [100.00-1450.00]
Relative Abundance
m/z
0
10
20
30
40
50
60
70
80
90
100
200
400
600
800
1000
1200
1400
138.06
126.06
144.07
186.08
204.09
207.11
235.11
282.15
301.19
351.17
369.18
411.24
412.22
450.74
456.21
499.27
551.28
569.30
586.32
600.30
661.33
679.34
697.35
716.40
717.39
785.39
803.42
851.43
869.45
900.48
931.46
980.51
997.53
1048.55
1084.57
1231.63

Gene: RUNX2

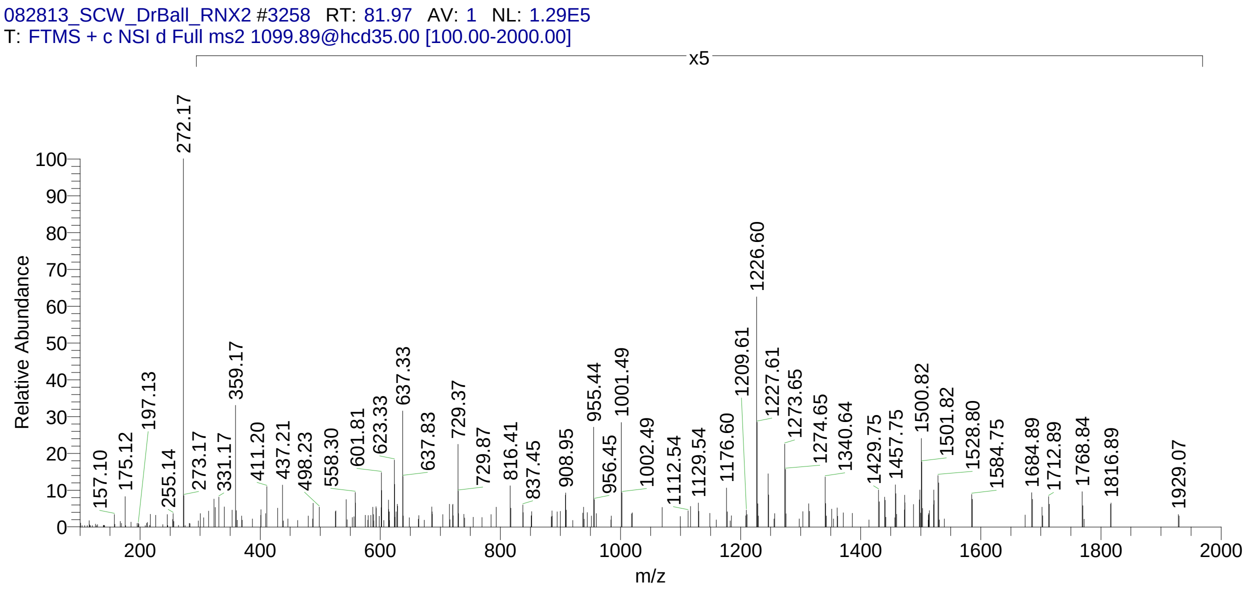
Acc#: Q13950

259-289 +Methyl at 267

+3 charge state

b	**b^{+2}**				**y**	**y^{+2}**
---	---	1	V	31	---	---
157.10	---	2	G	30	3197.61	1599.31
256.17	---	3	V	29	3140.59	1570.80
353.22	---	4	P	28	3041.52	1521.26
450.27	---	5	P	27	2944.47	1472.74
578.33	---	6	Q	26	2847.41	1424.21
692.37	---	7	N	25	2719.35	1360.18
789.43	---	8	P	24	2605.31	1303.16
959.54	480.27	9	**R(Methyl)**	23	2508.26	1254.63
1056.59	528.80	10	P	22	2338.14	1169.57
1143.63	572.32	11	S	21	2241.09	1121.05
1256.71	628.86	12	L	20	2154.06	1077.53
1370.75	685.88	13	N	19	2040.97	1020.99
1457.79	729.40	14	S	18	1926.93	963.97
1528.82	764.92	15	A	17	1839.90	920.45
1625.88	813.44	16	P	16	1768.86	884.93
1712.91	856.96	17	S	15	1671.81	836.41
1809.96	905.48	18	P	14	1584.78	792.89
1957.03	979.02	19	F	13	1487.72	744.37
2071.07	1036.04	20	N	12	1340.66	670.83
2168.12	1084.57	21	P	11	1226.61	613.81
2296.18	1148.60	22	Q	10	1129.56	565.28
2353.20	1177.11	23	G	9	1001.50	501.25
2481.26	1241.14	24	Q	8	944.48	472.74
2568.30	1284.65	25	S	7	816.42	408.71
2696.35	1348.68	26	Q	6	729.39	365.20
2809.44	1405.22	27	I	5	601.33	301.17
2910.49	1455.75	28	T	4	488.25	244.63
3025.51	1513.26	29	D	3	387.20	194.10
3122.57	1561.79	30	P	2	272.17	136.59
---	---	31	R	1	175.12	88.06

y_2-NH_3	255.15
y_{18}-NH_3^{+2}	955.46


082813_SCW_DrBall_RNX2 #3258 RT: 81.97 AV: 1 NL: 1.29E5
T: FTMS + c NSI d Full ms2 1099.89@hcd35.00 [100.00-2000.00]
x5
Relative Abundance
0
10
20
30
40
50
60
70
80
90
100
m/z
200
400
600
800
1000
1200
1400
1600
1800
2000
157.10
175.12
197.13
255.14
272.17
273.17
331.17
359.17
411.20
437.21
498.23
558.30
601.81
623.33
637.33
637.83
729.37
729.87
816.41
837.45
908.95
955.44
956.45
1001.49
1002.49
1112.54
1129.54
1176.60
1209.61
1226.60
1227.61
1273.65
1274.65
1340.64
1429.75
1457.75
1500.82
1501.82
1528.80
1584.75
1684.89
1712.89
1768.84
1816.89
1929.07

Gene: RUNX2

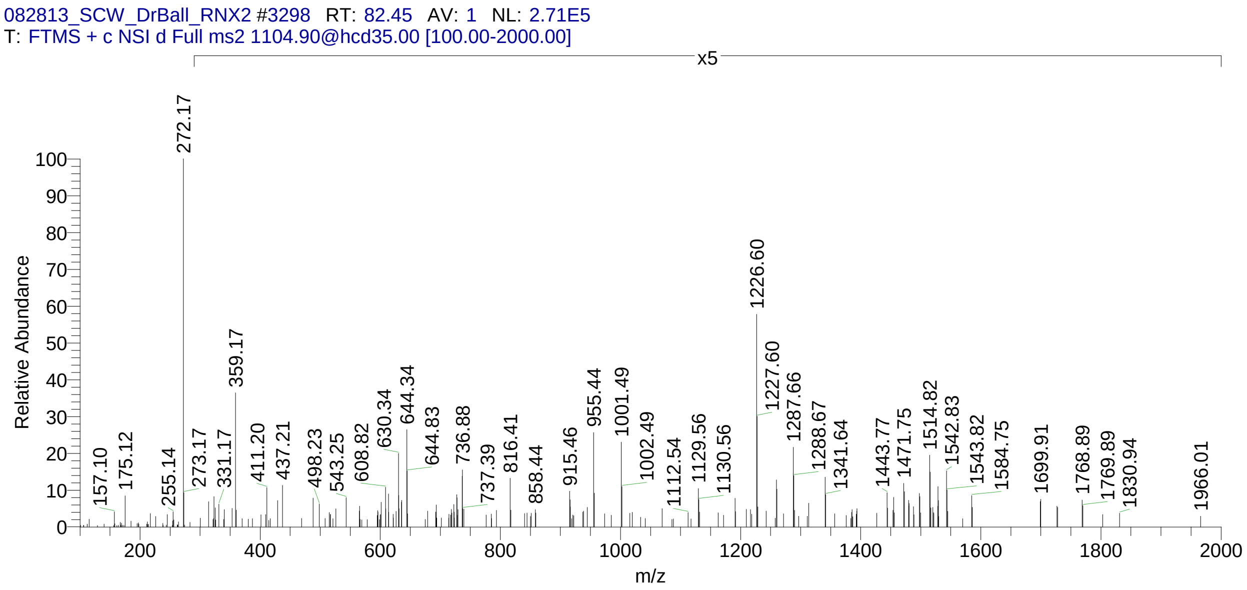
Acc#: Q13950

259-289 +Dimethyl at 267

+3 charge state

b	**b^{+2}**				**y**	**y^{+2}**
---	---	1	V	31	---	---
157.10	---	2	G	30	3211.62	1606.32
256.17	---	3	V	29	3154.60	1577.81
353.22	---	4	P	28	3055.53	1528.27
450.27	---	5	P	27	2958.48	1479.74
578.33	---	6	Q	26	2861.43	1431.22
692.37	---	7	N	25	2733.37	1367.19
789.43	---	8	P	24	2619.33	1310.17
973.56	487.28	9	**R(Dimethyl)**	23	2522.27	1261.64
1070.61	535.81	10	P	22	2338.14	1169.57
1157.64	579.32	11	S	21	2241.09	1121.05
1270.73	635.87	12	L	20	2154.06	1077.53
1384.77	692.89	13	N	19	2040.97	1020.99
1471.80	736.40	14	S	18	1926.93	963.97
1542.84	771.92	15	A	17	1839.90	920.45
1639.89	820.45	16	P	16	1768.86	884.93
1726.92	863.97	17	S	15	1671.81	836.41
1823.98	912.49	18	P	14	1584.78	792.89
1971.04	986.03	19	F	13	1487.72	744.37
2085.09	1043.05	20	N	12	1340.66	670.83
2182.14	1091.57	21	P	11	1226.61	613.81
2310.20	1155.60	22	Q	10	1129.56	565.28
2367.22	1184.11	23	G	9	1001.50	501.25
2495.28	1248.14	24	Q	8	944.48	472.74
2582.31	1291.66	25	S	7	816.42	408.71
2710.37	1355.69	26	Q	6	729.39	365.20
2823.45	1412.23	27	I	5	601.33	301.17
2924.50	1462.75	28	T	4	488.25	244.63
3039.53	1520.27	29	D	3	387.20	194.10
3136.58	1568.79	30	P	2	272.17	136.59
---	---	31	R	1	175.12	88.06

y_2-NH_3	255.14
y_{10}-NH_3	1112.53
y_{18}-NH_3^{+2}	955.46


082813_SCW_DrBall_RNX2 #3298 RT: 82.45 AV: 1 NL: 2.71E5
T: FTMS + c NSI d Full ms2 1104.90@hcd35.00 [100.00-2000.00]
x5
Relative Abundance
m/z
0
10
20
30
40
50
60
70
80
90
100
200
400
600
800
1000
1200
1400
1600
1800
2000
157.10
175.12
255.14
272.17
273.17
331.17
359.17
411.20
437.21
498.23
543.25
608.82
630.34
644.34
644.83
736.88
737.39
816.41
858.44
915.46
955.44
1001.49
1002.49
1112.54
1129.56
1130.56
1226.60
1227.60
1287.66
1288.67
1341.64
1443.77
1471.75
1514.82
1542.83
1543.82
1584.75
1699.91
1768.89
1769.89
1830.94
1966.01

Gene: RUNX2

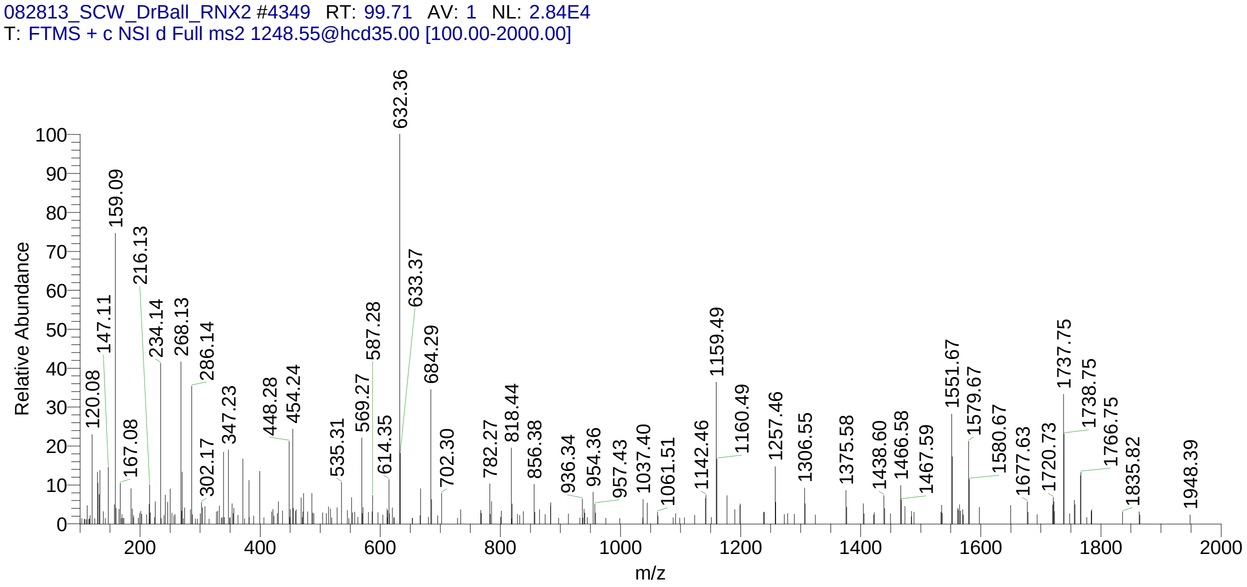
Acc#: Q13950

338-358 +Phospho at 340

+2 charge state

b	b^{+2}				y	y^{+2}
---	---	1	R	21	---	---
270.19	135.60	2	I	20	2338.98	1169.99
437.19	219.10	3	**S(Phospho)**	19	2225.89	1113.45
552.22	276.61	4	D	18	2058.90	1029.95
667.24	334.13	5	D	17	1943.87	972.44
782.27	391.64	6	D	16	1828.84	914.92
883.32	442.16	7	T	15	1713.82	857.41
954.36	477.68	8	A	14	1612.77	806.89
1055.40	528.21	9	T	13	1541.73	771.37
1142.44	571.72	10	S	12	1440.68	720.85
1257.46	629.24	11	D	11	1353.65	677.33
1404.53	702.77	12	F	10	1238.62	619.82
1564.56	782.78	13	**C(Carbamidomethyl)**	9	1091.56	546.28
1677.65	839.33	14	L	8	931.52	466.27
1863.73	932.37	15	W	7	818.44	409.72
1960.78	980.89	16	P	6	632.36	316.68
2047.81	1024.41	17	S	5	535.31	268.16
2148.86	1074.93	18	T	4	448.28	224.64
2261.94	1131.47	19	L	3	347.23	174.12
2348.97	1174.99	20	S	2	234.14	117.58
---	---	21	K	1	147.11	74.06

b_5 neutral loss of H_3PO_4	587.28
b_6 neutral loss of H_3PO_4	702.31


082813_SCW_DrBall_RNX2 #4349 RT: 99.71 AV: 1 NL: 2.84E4
T: FTMS + c NSI d Full ms2 1248.55@hcd35.00 [100.00-2000.00]
Relative Abundance
m/z
0
10
20
30
40
50
60
70
80
90
100
200
400
600
800
1000
1200
1400
1600
1800
2000
120.08
147.11
159.09
167.08
216.13
234.14
268.13
286.14
302.17
347.23
448.28
454.24
535.31
569.27
587.28
614.35
632.36
633.37
684.29
702.30
782.27
818.44
856.38
936.34
954.36
957.43
1037.40
1061.51
1142.46
1159.49
1160.49
1257.46
1306.55
1375.58
1438.60
1466.58
1467.59
1551.67
1579.67
1580.67
1677.63
1720.73
1737.75
1738.75
1766.75
1835.82
1948.39

Gene: RUNX2

Acc#: Q13950

359-374 +HexNAc

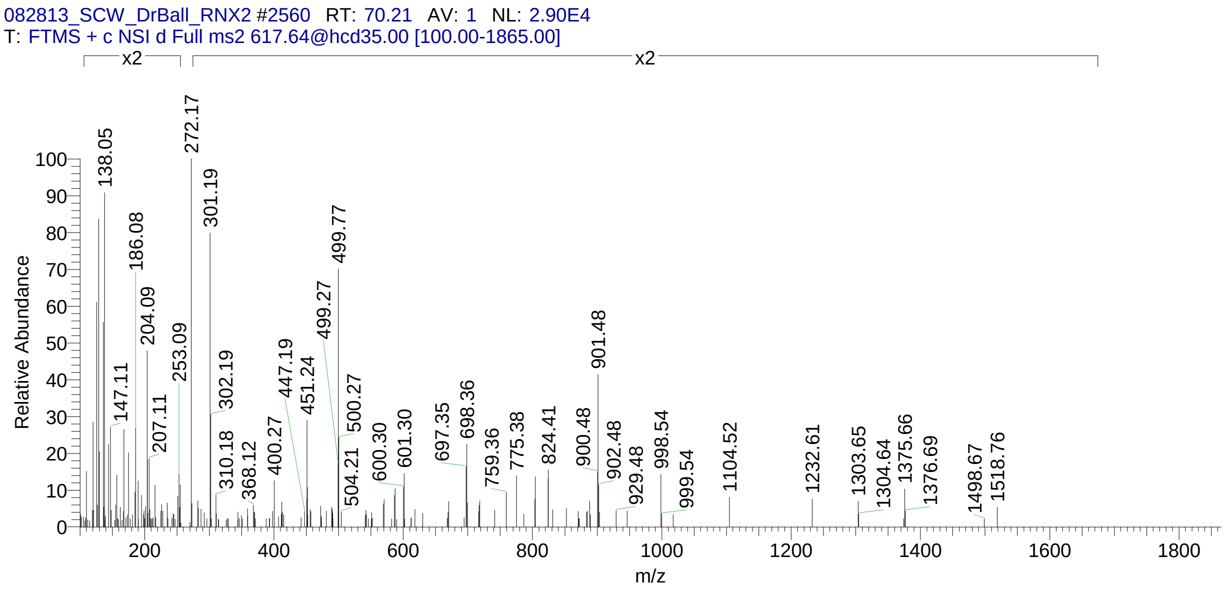
+3 charge state

The HCD fragmentation spectrum contains both modified and unmodified species due to neutral loss of HexNAc. The ETD fragmentation spectrum localizing the site of modification to Ser371 is represented in Fig 2C.

b				**y**
---	1	K	16	---
216.13	2	S	15	1518.72
344.19	3	Q	14	1431.69
415.23	4	A	13	1303.63
472.25	5	G	12	1232.59
543.29	6	A	11	1175.57
630.32	7	S	10	1104.53
759.36	8	E	9	1017.50
872.45	9	L	8	888.46
929.47	10	G	7	775.37
1026.52	11	P	6	718.35
1173.59	12	F	5	621.30
1260.62	13	S	4	474.23
1375.65	14	D	3	387.20
1472.70	15	P	2	272.17
---	16	R	1	175.12

y_5+HexNAc	824.38

y_5-H_2O^{+2}	302.15
y_9-H_2O^{+2}	500.25
y_9-H_2O	999.49


082813_SCW_DrBall_RNX2 #2560 RT: 70.21 AV: 1 NL: 2.90E4
T: FTMS + c NSI d Full ms2 617.64@hcd35.00 [100.00-1865.00]
x2
x2
Relative Abundance
m/z
0
10
20
30
40
50
60
70
80
90
100
200
400
600
800
1000
1200
1400
1600
1800
138.05
147.11
186.08
204.09
207.11
253.09
272.17
301.19
302.19
310.18
368.12
400.27
447.19
451.24
499.27
499.77
500.27
504.21
600.30
601.30
697.35
698.36
759.36
775.38
824.41
900.48
901.48
902.48
929.48
998.54
999.54
1104.52
1232.61
1303.65
1304.64
1375.66
1376.69
1498.67
1518.76

Gene: RUNX2

Acc#: Q13950

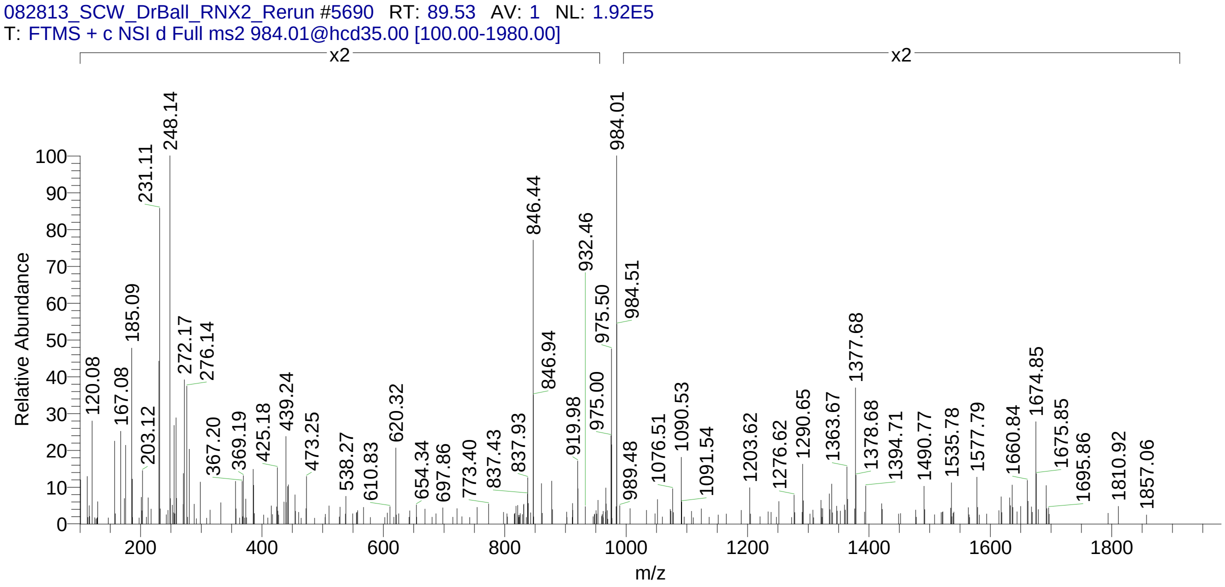
375-391 +Methyl at 386

+2 charge state

b	**b^{+2}**				**y**	**y^{+2}**
---	---	1	Q	17	---	---
276.13	---	2	F	16	1838.94	919.97
373.19	---	3	P	15	1691.87	846.44
460.22	---	4	S	14	1594.82	797.91
573.30	---	5	I	13	1507.79	754.40
660.34	---	6	S	12	1394.70	697.85
747.37	---	7	S	11	1307.67	654.34
860.45	---	8	L	10	1220.64	610.82
961.50	---	9	T	9	1107.55	554.28
1090.54	---	10	E	8	1006.51	503.76
1177.57	---	11	S	7	877.46	439.24
1347.69	674.35	12	**R(Methyl)**	6	790.43	395.72
1494.76	747.88	13	F	5	620.32	310.66
1581.79	791.40	14	S	4	473.25	237.13
1695.83	848.42	15	N	3	386.21	193.61
1792.89	896.95	16	P	2	272.17	136.59
---	---	17	R	1	175.12	88.06

y_3-NH$_3$	369.19
y_6-NH$_3$	773.41
y_{15}-NH$_3$$^{+2}$	837.93
MH^{+2}	984.00

y_8-NH$_3$	989.48
y_{10}-NH$_3$	1203.61
y_{11}-NH$_3$	1290.64
y_{12}-NH$_3$	1377.68
y_{13}-NH$_3$	1490.76
y_{14}-NH$_3$	1577.79
y_{15}-NH$_3$	1674.84


082813_SCW_DrBall_RNX2_Rerun #5690 RT: 89.53 AV: 1 NL: 1.92E5
T: FTMS + c NSI d Full ms2 984.01@hcd35.00 [100.00-1980.00]
x2
x2
Relative Abundance
m/z
100
90
80
70
60
50
40
30
20
10
0
200
400
600
800
1000
1200
1400
1600
1800
120.08
167.08
185.09
203.12
231.11
248.14
272.17
276.14
367.20
369.19
425.18
439.24
473.25
538.27
610.83
620.32
654.34
697.86
773.40
837.43
837.93
846.44
846.94
919.98
932.46
975.00
975.50
984.01
984.51
989.48
1076.51
1090.53
1091.54
1203.62
1276.62
1290.65
1363.67
1377.68
1378.68
1394.71
1490.77
1535.78
1577.79
1660.84
1674.85
1675.85
1695.86
1810.92
1857.06

Gene: RUNX2

Acc#: Q13950

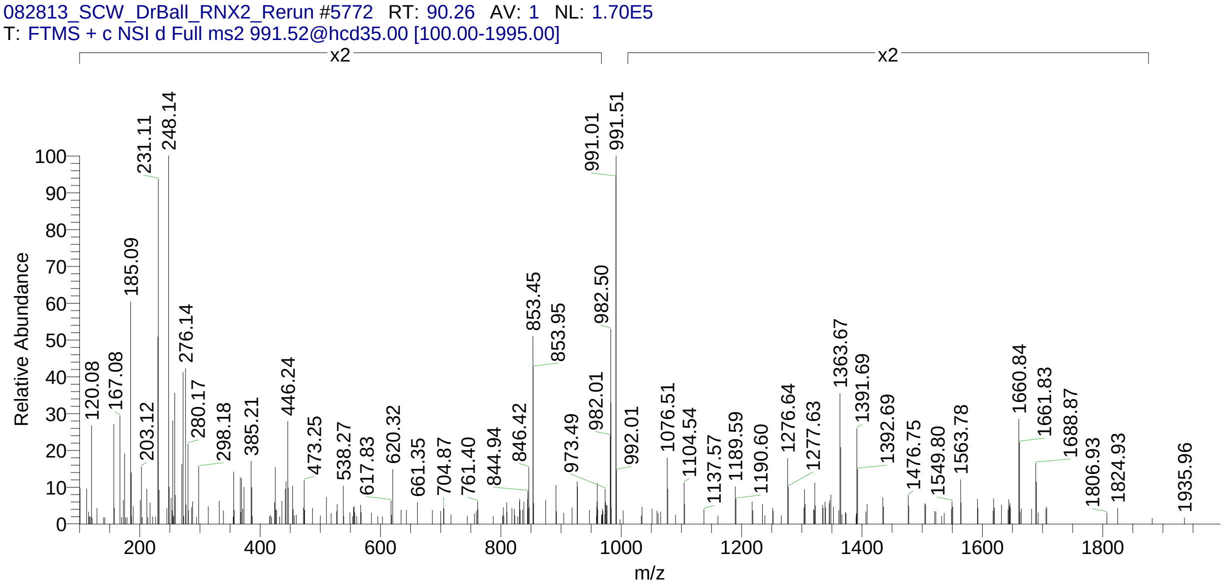
375-386 +Dimethyl at 386

+2 charge state

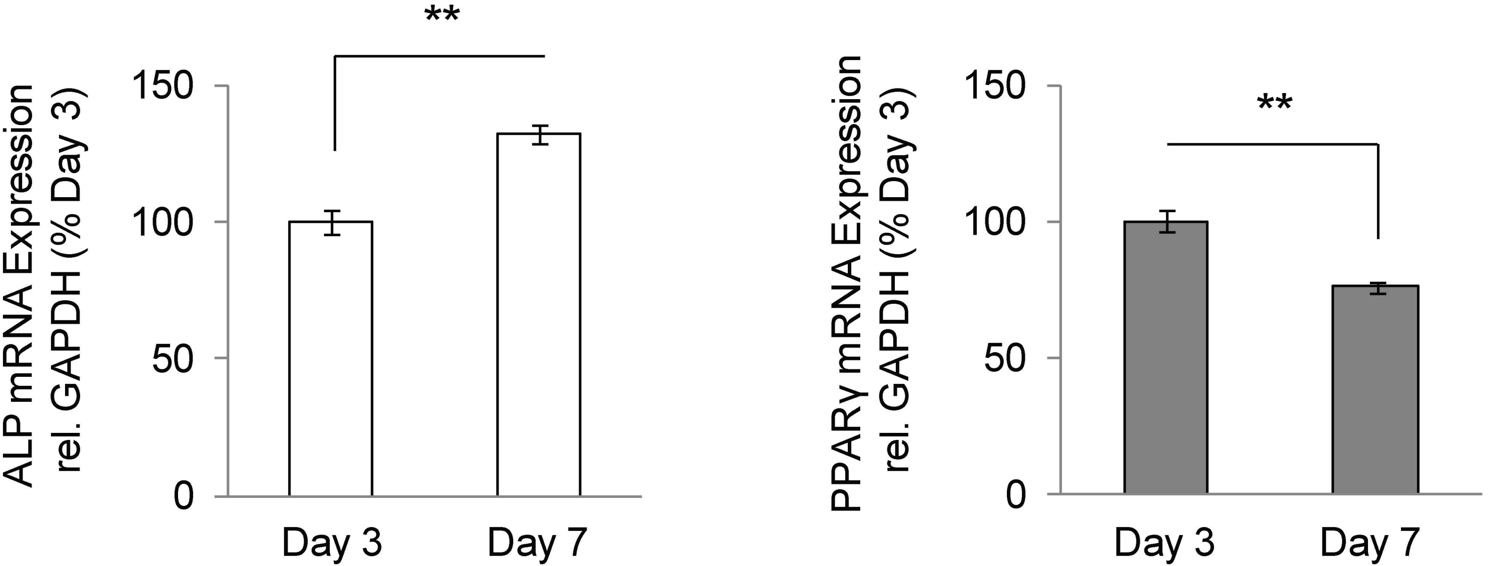
b	**b^{+2}**				**y**	**y^{+2}**
---	---	1	Q	17	---	---
276.13	---	2	F	16	1852.96	926.98
373.19	---	3	P	15	1705.89	853.45
460.22	---	4	S	14	1608.83	804.92
573.30	---	5	I	13	1521.80	761.40
660.34	---	6	S	12	1408.72	704.86
747.37	---	7	S	11	1321.69	661.35
860.45	---	8	L	10	1234.65	617.83
961.50	---	9	T	9	1121.57	561.29
1090.54	---	10	E	8	1020.52	510.76
1177.57	---	11	S	7	891.48	446.24
1361.71	681.36	12	**R(Dimethyl)**	6	804.45	402.73
1508.77	754.89	13	F	5	620.32	310.66
1595.81	798.41	14	S	4	473.25	237.13
1709.85	855.43	15	N	3	386.21	193.61
1806.90	903.95	16	P	2	272.17	136.59
---	---	17	R	1	175.12	88.06

y_9-NH_3	1104.54
y_{12}-NH_3	1391.69

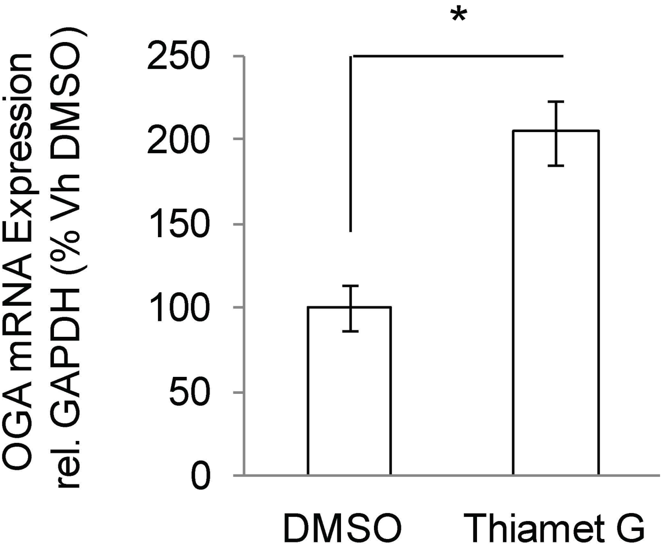
MH^{+2}	991.01

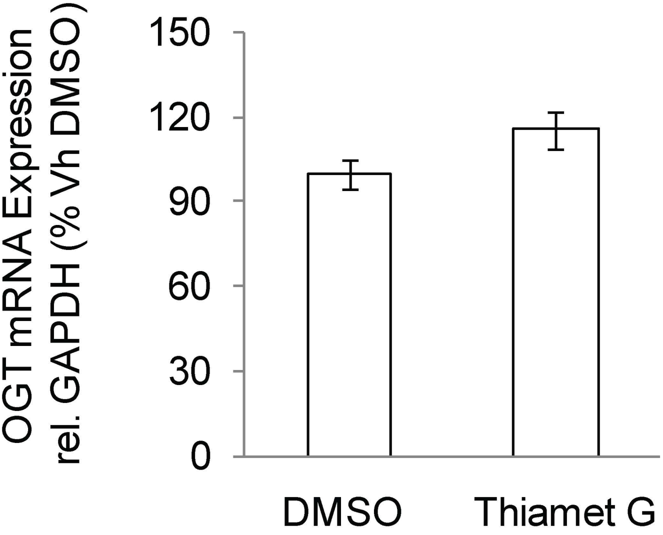

082813_SCW_DrBall_RNX2_Rerun #5772 RT: 90.26 AV: 1 NL: 1.70E5
T: FTMS + c NSI d Full ms2 991.52@hcd35.00 [100.00-1995.00]
x2
x2
Relative Abundance
m/z
0
10
20
30
40
50
60
70
80
90
100
200
400
600
800
1000
1200
1400
1600
1800
120.08
167.08
185.09
203.12
231.11
248.14
276.14
280.17
298.18
385.21
446.24
473.25
538.27
617.83
620.32
661.35
704.87
761.40
844.94
846.42
853.45
853.95
973.49
982.01
982.50
991.01
991.51
992.01
1076.51
1104.54
1137.57
1189.59
1190.60
1276.64
1277.63
1363.67
1391.69
1392.69
1476.75
1549.80
1563.78
1660.84
1661.83
1688.87
1806.93
1824.93
1935.96

Supplemental Figure 1



Supplemental Figure 2





Supplemental Figure 3