

**Supporting Information for:**

***O*-Fucosylation of ADAMTSL2 is required for secretion and is impacted by geleophysic dysplasia-causing mutations**

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**Supporting Information Tables:**

**Table S1. Site-directed mutagenesis primers for mouse ADAMTSL2 construct.**

Clone	Primer Name	Sequence	T <sub>m</sub>
N813Q	ADAMTSL2_N813Q	5' - GGCTCAAGACTGGGAAAGGTGCCAAACCACCTGTGGGCGTGGTG - 3'	74.77°C
	ADAMTSL2_N813Q-r	5' - CACCAGGCCACAGGTGGTTTGGCACCTTCCAGTCTGAGCC - 3'	74.77°C
T815V	ADAMTSL2_T815V	5' - GGCTCAAGACTGGGAAAGGTGCAATACCGTGTGTGGGCGTGGTG - 3'	73.85°C
	ADAMTSL2_T815V-r	5' - CACCACGCCACACACGGTATTGCACCTTCCAGTCTGAGCC - 3'	73.85°C
N813Q_T815V	ADAMTSL2_N813Q_T815V	5' - GGCTCAAGACTGGGAAAGGTGCCAAACCAGTGTGTGGGCGTGGTG - 3'	74.77°C
	ADAMTSL2_N813Q_T815V-r	5' - CACCACGCCACACACGGTATTGCACCTTCCAGTCTGAGCC - 3'	74.77°C
G817R	TSL2_G817R_s2	5' - CAATACCACCTGTAGGCGTGGTGTGAAAAAGCGGTTAGTTCTCTG - 3'	70°C
	TSL2_G817R_s2-r	5' - GTTATGGTGGACATCCGCACCACACTTTTCGCCAATCAAGAGAC - 3'	70°C
S641L	TSL2_S641L	5' - GACCAGCAGCTGGAGTGAGTGCTTACGTACCTGTGGTGAGGGCC - 3'	74.77°C
	TSL2_S641L-r	5' - CTGGTCGTCGACCTCACTCACGAATGCATGGACACCCTCCCGG - 3'	74.77°C

**Table S2. Sequencing primers for mouse ADAMTSL2 construct.**

Primer Name	Sequence	T <sub>m</sub>
<i>Adamtsl2</i> seq 1 F	5' - GTGGGAGCTGTGGACAGGGC - 3'	68.6
<i>Adamtsl2</i> seq 1 R	5' - GCCCTGTCCACAGCTCCCAC - 3'	68.6
<i>Adamtsl2</i> seq 2 F	5' - CGGTACCAGCTCTGCAGAGT - 3'	62.4
<i>Adamtsl2</i> seq 2 R	5' - ACTCTGCAGAGCTGGTACCG - 3'	62.4
<i>Adamtsl2</i> seq 3 F	5' - TCAACACCTCCTCTGAGGC - 3'	58.9
<i>Adamtsl2</i> seq 3 R	5' - GCCTCAGAGGAGGTGTTGA - 3'	58.9
<i>Adamtsl2</i> seq 4 F	5' - CAGTGGACTGTCTCGGACTG - 3'	59.4
<i>Adamtsl2</i> seq 4 R	5' - CAGTCCGAGACAGTCCACTG - 3'	59.4
<i>Adamtsl2</i> seq 5 F	5' - GCAGCCCCTGAGATATAAGC - 3'	58.91
<i>Adamtsl2</i> seq 5 R	5' - GCTTATATCTCAGGGGCTGC - 3'	58.91

**Table S3: Trypsin Digestion of WT ADAMTSL2**

**Table S4: Chymotrypsin digestion of WT ADAMTSL2**

**Table S5: Trypsin digestion of S641L mutant of ADAMTSL2**

**Table S6: Chymotrypsin digestion of S641L mutant of ADAMTSL2**

**Table S7: Trypsin glycopeptides from WT ADAMTSL2 analyzed in Figure S2**

**Table S8: Chymotryptic glycopeptides from WT ADAMTSL2 analyzed in Figure S2**

**Table S9: Tryptic glycopeptides from S641L ADAMTSL2 used to generate Figure S4**

**Table S10: Chymotryptic glycopeptides from S641L ADAMTSL2 used to generate Figures S3 and S4**

**Supporting Information Figure Legends:**

**Figure S1. Sequence comparison of TSR3 and TSR6 from human and mouse ADAMTSL2.** Color coding as in Figure 1. Identity, colon; similarity, period.

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TSR3 (Sequence identity: 95%)
Human:      CQPRWETSSWSECSRTCGEGYQFRVVRWCWKMLSPGFDSSVYSDLCEAAEAVRPEERKTCRNPACG
Mouse:      CQPRWETSSWSECSRTCGEGHQFRIVRCWKMLSPGFDSSVYSDLCEATEAVRPEERKTCRNPACG
Similarity: ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::

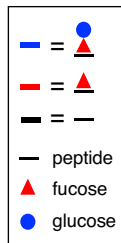
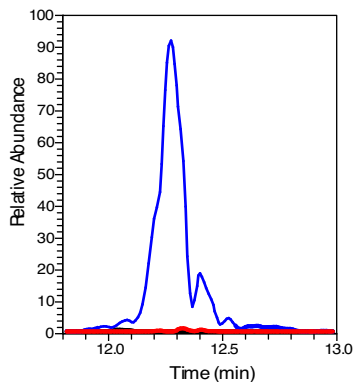
TSR6 (Sequence identity: 96%)
Human:      HWLAQDWERCNTTCGRGVKKRLVLCMELANGKPQTRSGPECGLAKKPPEESTCFE
Mouse:      HWLAQDWERCNTTCGRGVKKRLVLCMELANGKPQIRSGPECGLARKPPEESTCFE
Similarity: ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
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**Figure S2. Mass spectral mapping of TSRs from ADAMTSL2 modified with *O*-fucosylation and *C*-mannosylation.** mADAMTSL2 was expressed in WT HEK293T cells, purified from medium, reduced/alkylated, digested with proteases, and the resulting peptides were analyzed by nano-LC-MS/MS as described in Experimental Procedures. Glycopeptides were identified using Byonic. Spectra for the major glycoform of peptides from different TSRs are shown, and Extracted Ion Chromatograms (EICs) for each glycoform were generated using Xcaliber. Peptides are shown from TSR1 (A, B), TSR2 (C), TSR3 (D), TSR4 (E), TSR5 (F), TSR6 (G), and TSR7 (H, I). Data can be found in Supporting Information Tables S3, S4, S7 and S8.

**Figure S3. EIC and MS/MS of peptides from TSR3 in S641L ADAMTSL2.** Spectra and EIC of the peptide containing the S641L mutation from TSR3 is shown as in Figure S2. Data can be found in Supporting Information Tables S6 and S10.

**Figure S4. Mouse ADAMTSL2 GPHYSD1 S641L mutation reduced *O*-fucosylation of TSR3 but did not alter glycosylation of other TSRs.** (A) Relative abundance of GlcFuc disaccharide, *O*-fuc monosaccharide, and unmodified glycoforms on peptides from TSRs of mADAMTSL2 S641L containing the C-X-X-(S/T)-C *O*-fucose consensus site analyzed by mass spectral analyses as in Figure 2A. (B) Relative abundance of *C*-mannosylated forms of peptides from TSRs of mADAMTSL2 S641L containing W-X-X-(W/C) consensus sequence analyzed by mass spectral analyses as in Figure 2C. Data can be found in Supporting Information Tables S5, S6, S9, and S10.

Figure S2A



#1	b*	b <sup>2*</sup>	b <sup>3*</sup>	b <sup>4*</sup>	Seq.	y*	y <sup>2*</sup>	y <sup>3*</sup>	y <sup>4*</sup>	#2
1	102.05496	51.53112	34.68984	26.26920	T					19
2	173.09207	87.04967	58.36887	44.02847	A	2329.98057	1165.49392	777.33171	583.26060	18
3	333.12272	167.06500	111.71242	84.03614	C-Carbami...	2258.94346	1129.97537	753.65267	565.49132	17
4	420.15475	210.58101	140.72310	105.79414	S	2098.91281	1049.96004	700.30912	525.48366	16
5	576.25586	288.63157	192.75680	144.81942	R	2011.88078	1006.44403	671.29844	503.72565	15
6	971.39862	486.20295	324.47106	243.60511	S-dHex(1)...	1855.77967	928.39347	619.26474	464.70037	14
7	1131.42927	566.21827	377.81461	283.61277	C-Carbami...	1460.63691	730.82209	487.55049	365.91468	13
8	1188.45073	594.72900	396.82176	297.86814	G	1300.60626	650.80677	434.20694	325.90702	12
9	1245.47219	623.23973	415.82892	312.12351	G	1243.58479	622.29604	415.19978	311.65166	11
10	1302.49366	651.75047	434.83607	326.37887	G	1186.56333	593.78530	396.19263	297.39629	10
11	1401.56207	701.28467	467.85887	351.14598	V	1129.54187	565.27457	377.18547	283.14092	9
12	1502.60975	751.80851	501.54143	376.40789	T	1030.47345	515.74037	344.16267	258.37382	8
13	1589.64178	795.32453	530.55211	398.16590	S	929.42578	465.21653	310.48011	233.11190	7
14	1717.70036	859.35382	573.23830	430.18066	Q	842.39375	421.70051	281.46843	211.35389	6
15	1846.74286	923.87511	616.25250	462.44119	E	714.33517	357.67122	238.78324	179.33925	5
16	2002.84406	1001.92567	668.28620	501.46647	R	585.29258	293.14993	195.76904	147.07860	4
17	2139.90297	1070.45512	713.97251	535.73120	H	429.19147	215.09937	143.73534	108.05332	3
18	2299.93362	1150.47045	767.31606	575.73886	C-Carbami...	292.13255	146.56992	98.04904	73.78860	2
19					L	132.10191	66.55459	44.70549	33.78093	1

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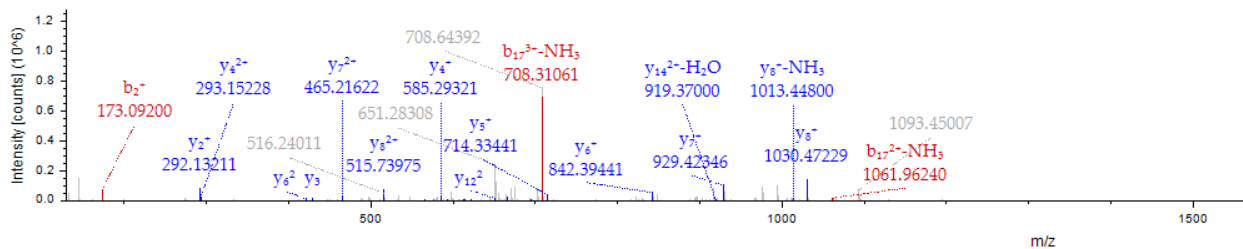
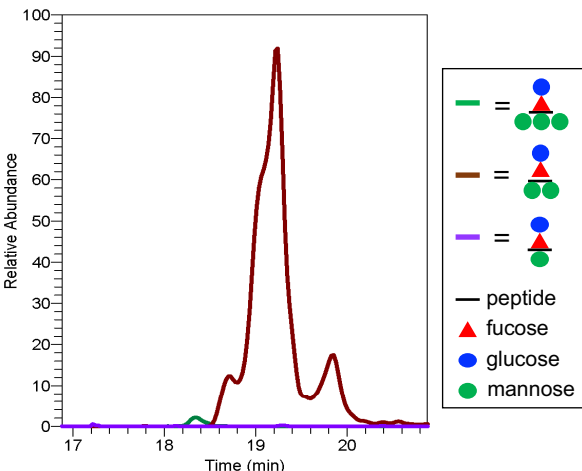


Figure S2B

TSR1 <sup>49</sup>WWGEWTKWTACSR<sup>5</sup>SCGGGVTSQERHCL<sup>75</sup>

#1	b <sup>+</sup>	b <sup>2+</sup>	b <sup>3+</sup>	b <sup>4+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	y <sup>3+</sup>	y <sup>4+</sup>	#2
1	187.08659	94.04693	63.03371	47.52710	W					27
2	373.16590	187.08659	125.06015	94.04693	W	3728.57853	1864.79290	1243.53103	932.90009	26
3	430.18737	215.59732	144.06731	108.30230	G	3542.49922	1771.75325	1181.50459	886.38026	25
4	559.22996	280.11862	187.08150	140.56295	E	3485.47776	1743.24252	1162.49474	872.12490	24
5	907.36210	454.18469	303.12555	227.59598	W-Hex	3356.43516	1678.72122	1119.48324	839.86425	23
6	1008.40977	504.70853	336.80811	252.85790	T	3008.30303	1504.65515	1003.43919	752.83121	22
7	1136.50474	568.75601	379.50643	284.88164	K	2907.25535	1454.13131	969.75663	727.56929	21
8	1484.63687	742.82208	495.55048	371.91468	W-Hex	2779.16038	1390.08383	927.05831	695.54555	20
9	1893.79528	947.40128	631.93661	474.20428	T-dHex(1)	2431.02825	1216.01776	811.01427	608.51252	19
10	1964.83240	982.91984	655.61565	491.96356	A	2021.86984	1011.43896	674.62813	506.22292	18
11	2124.86305	1062.93516	708.95920	531.97122	C-Carbami...	1950.83272	975.92000	650.94909	488.46364	17
12	2211.89508	1106.45118	737.96988	553.72923	S	1790.80207	895.90468	597.60554	448.45598	16
13	2367.99619	1184.50173	790.00358	592.75450	R	1703.77005	852.38866	568.59487	426.63975	15
14	2455.02821	1228.01775	819.01426	614.51251	S	1547.66894	774.33811	516.56116	387.67269	14
15	2615.05886	1308.03307	872.35781	654.52017	C-Carbami...	1460.63691	730.82209	487.55049	365.91468	13
16	2672.08033	1336.54380	891.36496	668.77554	G	1300.60626	650.80677	434.20694	325.90702	12
17	2729.10179	1365.05453	910.37211	683.03091	G	1243.58479	622.29604	415.19978	311.65166	11
18	2786.12325	1393.56527	929.37927	697.28627	G	1186.56333	593.78530	396.19263	297.39629	10
19	2885.19167	1443.09947	962.40207	722.05337	V	1129.54187	565.27457	377.18547	283.14092	9
20	2986.23935	1493.62331	996.08463	747.31529	T	1030.47345	515.74037	344.16267	258.37382	8
21	3073.27137	1537.13933	1025.09531	769.07330	S	929.42578	465.21653	310.48011	233.11190	7
22	3201.32995	1601.16861	1067.78150	801.08795	Q	842.39375	421.70051	281.46943	211.35389	6
23	3330.37255	1665.68991	1110.79570	833.34859	E	714.33517	357.67122	238.78324	179.33925	5
24	3486.47366	1743.74047	1162.82940	872.37387	R	585.29258	293.14993	195.76904	147.07860	4
25	3623.53257	1812.26992	1208.51571	906.63860	H	429.19147	215.09937	143.73534	108.05332	3
26	3783.56322	1892.28525	1261.85926	946.64626	C-Carbami...	292.13255	146.56992	98.04904	73.78860	2
27					L	132.10191	66.55459	44.70549	33.78093	1

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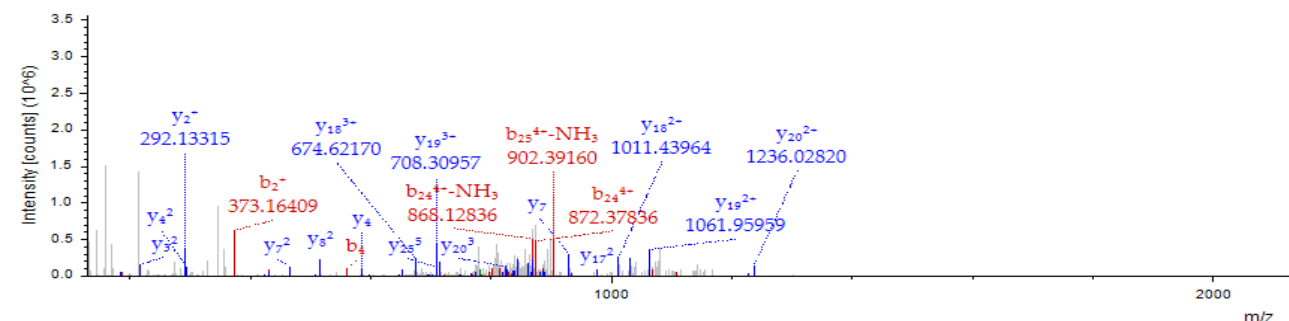
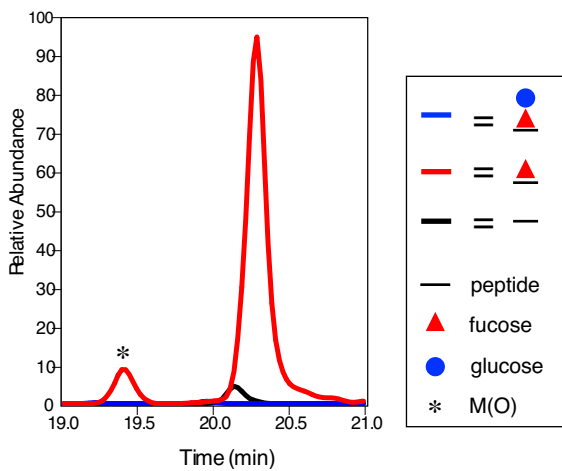


Figure S2C

TSR2<sup>576</sup>LSSHEPCSATICTTGVMSTYAMCVR<sup>599</sup>



#1	b <sup>+</sup>	b <sup>2+</sup>	b <sup>3+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	y <sup>3+</sup>	#2
1	114.09134	57.54931	38.70196	L				24
2	201.12337	101.06532	67.71264	S	2739.11872	1370.06300	913.71109	23
3	288.15540	144.58134	96.72332	S	2652.08669	1326.54698	884.70041	21
4	425.21431	213.11079	142.40962	H	2565.05466	1283.03097	855.68974	22
5	554.25690	277.63209	185.42382	E	2427.99575	1214.50151	810.00343	20
6	651.30967	326.15847	217.77474	P	2298.95316	1149.98022	766.98924	19
7	811.34031	406.17380	271.11829	C-Carbami...	2201.90039	1101.45384	734.63832	18
8	898.37234	449.68981	300.12897	S	2041.86975	1021.43851	681.29477	17
9	969.40946	485.20837	323.80800	A	1954.83772	977.92250	652.28409	16
10	1216.51504	608.76116	406.17653	T-dHex	1883.80060	942.40394	628.60505	15
11	1376.54569	688.77648	459.52008	C-Carbami...	1636.69502	818.85115	546.23652	14
12	1477.59337	739.30032	493.20264	T	1476.66437	738.83582	492.89297	13
13	1578.64105	789.82416	526.88520	T	1375.61669	688.31198	459.21041	12
14	1635.66251	818.33489	545.89236	G	1274.56901	637.78814	425.52785	11
15	1734.73093	867.86910	578.91516	V	1217.54755	609.27741	406.52070	10
16	1865.77141	933.38934	622.59532	M	1118.47913	559.74320	373.49790	9
17	1952.80344	976.90536	651.60600	S	987.43865	494.22296	329.81773	8
18	2053.85112	1027.42920	685.28856	T	900.40662	450.70695	300.80706	7
19	2216.91445	1108.96086	739.64300	Y	799.35894	400.18311	267.12450	6
20	2287.95156	1144.47942	763.32204	A	636.29561	318.65144	212.77006	5
21	2418.99204	1209.99966	807.00220	M	565.25850	283.13289	189.09102	4
22	2579.02269	1290.01498	860.34575	C-Carbami...	434.21801	217.61265	145.41086	3
23	2678.09111	1339.54919	893.36855	V	274.18737	137.59732	92.06731	2
24				R	175.11895	88.06311	59.04450	1

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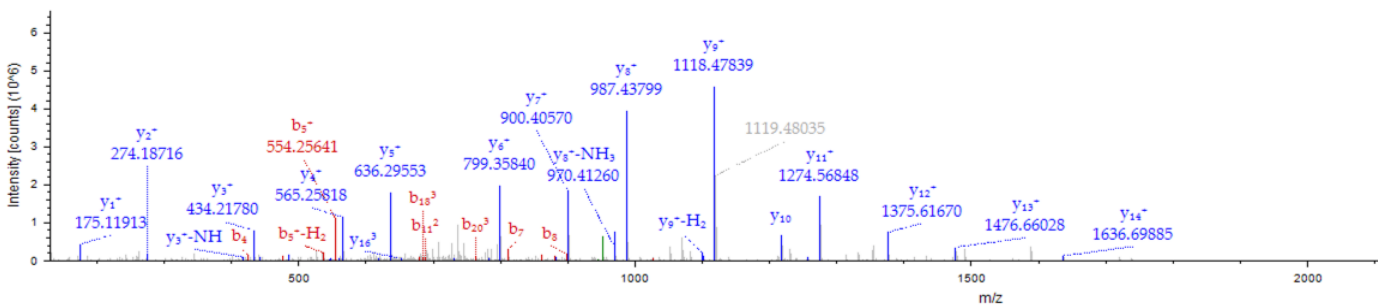
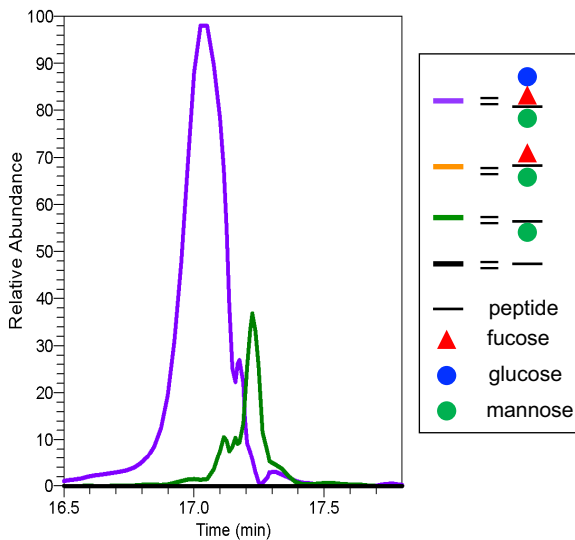


Figure S2D

TSR3 WT <sup>623</sup>CAGRECQPRWETSSWSECSRTC<sup>650</sup>GE<sup>650</sup>HQF



#1	b <sup>+</sup>	b <sup>2+</sup>	b <sup>3+</sup>	b <sup>4+</sup>	b <sup>5+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	y <sup>3+</sup>	y <sup>4+</sup>	y <sup>5+</sup>	#2
1	161.03793	81.02260	54.35083	41.01494	33.01341	C-Carbami...						28
2	232.07504	116.54116	78.02886	58.74222	47.22083	A	3755.52789	1878.26788	1252.51415	939.63743	751.91140	27
3	289.09650	143.05189	97.03702	73.02968	58.62512	G	3684.49078	1842.74903	1228.83511	921.87015	737.70398	26
4	445.19761	223.10245	149.07072	112.05486	89.84534	R	3627.46392	1814.23830	1209.82796	907.62279	726.29968	25
5	<b>574.24021</b>	287.62374	192.08492	144.31551	115.65386	E	3471.36820	1736.18774	1157.79425	<b>868.59751</b>	695.07946	24
6	734.27085	367.63907	245.42847	184.32317	147.65999	C-Carbami...	3342.32561	1671.69644	1114.78005	836.33286	669.27094	23
7	862.32943	431.66835	288.11466	216.33782	173.27171	Q	3182.29496	1591.65112	1061.43651	796.32920	637.26481	22
8	959.38220	480.19474	320.46558	240.60101	192.68226	P	3054.23639	1527.62183	1018.75031	764.31455	611.65310	21
9	1115.48331	<b>558.24529</b>	372.49629	279.62628	223.90248	R	2957.16362	1479.09545	986.39939	740.05136	582.24255	20
10	1301.56262	<b>651.28495</b>	434.52572	326.14611	261.11835	W	2801.08251	1401.04489	934.36569	701.02609	561.02232	19
11	1430.60521	<b>715.80624</b>	<b>477.53992</b>	358.40676	286.92686	E	2615.00320	1308.00524	872.33925	654.50626	523.80846	18
12	1839.76362	920.38545	613.92806	460.69636	368.75855	T-dHex(1)	2489.96060	1243.48394	<b>829.32505</b>	622.24561	497.99794	17
13	1926.79565	963.90146	642.93674	482.45437	386.16495	S	2076.80219	1038.90474	692.93892	519.95601	416.16626	16
14	2013.82768	1007.41748	671.94741	504.21238	403.57135	S	1989.77017	995.38272	663.92824	498.19800	388.75985	15
15	2361.95882	1181.48355	787.99146	591.24541	473.19778	W-Hex	1902.73814	951.87271	634.91756	476.43999	381.35345	14
16	2448.99185	1224.99896	817.00213	613.00342	490.60419	S	<b>1554.60900</b>	<b>777.80264</b>	518.87352	389.40696	311.72702	13
17	2578.03444	1289.52086	850.01633	645.26407	516.41721	E	1467.57397	<b>734.29062</b>	498.86284	367.64895	294.32062	12
18	2738.06309	1369.53818	913.35888	685.27173	548.41884	C-Carbami...	1338.53138	<b>689.78933</b>	446.84864	335.38830	268.51210	11
19	2825.09712	1413.06220	942.37066	707.02974	565.82524	S	1178.50073	<b>588.75400</b>	393.50609	295.38064	236.50597	10
20	2981.19823	1491.10275	994.40426	746.05501	597.04547	R	1091.46870	546.23799	364.49442	273.62263	219.08956	9
21	3082.24590	1547.62659	1028.08682	771.31693	617.25500	T	<b>935.36759</b>	468.18743	312.46071	234.59736	187.87934	8
22	3242.27655	1621.64191	1081.43037	811.32460	649.26113	C-Carbami...	<b>834.31991</b>	417.66359	278.77816	209.33544	167.66880	7
23	3299.29802	1650.15265	<b>1100.43752</b>	<b>825.57996</b>	660.66542	E	<b>674.28926</b>	337.64827	226.43461	169.32777	135.66367	6
24	3428.34051	1714.67394	1143.45172	<b>857.84051</b>	686.47394	G	617.26780	309.13754	206.42745	155.07241	124.26938	5
25	3485.36207	1743.18468	1162.45888	872.09588	697.87824	G	<b>488.22521</b>	244.51624	163.41325	122.81176	98.5086	4
26	3622.42099	1811.71413	1208.14518	906.36070	<b>725.29002</b>	H	<b>431.20374</b>	216.10551	144.40610	108.55639	87.04657	3
27	3750.47956	1875.74342	1250.83137	938.37535	750.90173	Q	<b>294.14483</b>	147.57605	99.71890	74.29167	59.63479	2
28						F	166.08626	83.54677	56.03360	42.27702	34.02307	1

KZ051018\_5\_raw #4739 RT: 16.9557 min

FTMS, 784.3149@hcd27.00, z=+5, Mono m/z=783.91443 Da, MH+=3915.54304 Da, Match Tol=20 ppm

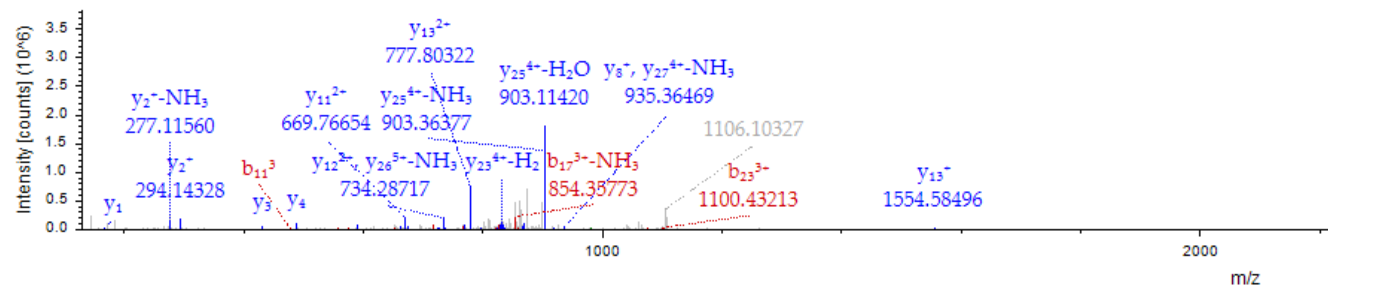
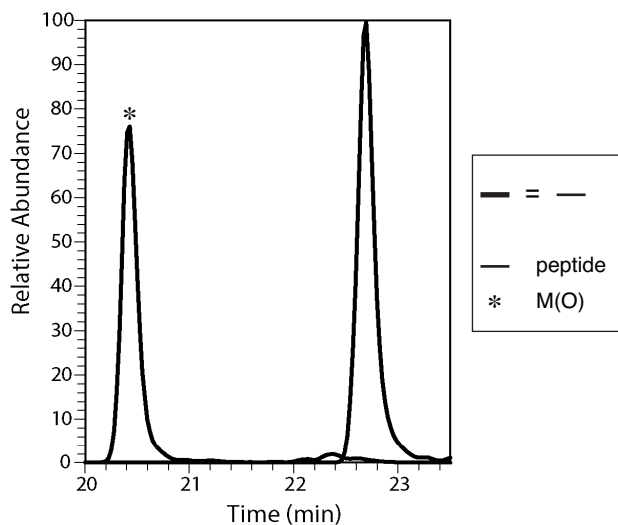




Figure S2E

TSR4<sup>688</sup>NPACGPQWEM<sup>\*</sup>SEWSECTAK<sup>706</sup>

#1	b <sup>+</sup>	b <sup>2+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	#2
1	115.05020	58.02874	N			19
2	212.10297	106.55512	P	2169.86744	1085.43736	18
3	283.14008	142.07368	A	2072.81467	1036.91097	17
4	443.17073	222.08900	C-Carbami...	2001.77756	1001.39242	16
5	500.19219	250.59974	G	1841.74691	921.37709	15
6	597.24496	299.12612	P	1784.72545	892.86636	14
7	725.30353	363.15541	Q	1687.67268	844.33998	13
8	911.38285	456.19506	W	1559.61411	780.31069	12
9	1040.42544	520.71636	E	1373.53479	687.27103	11
10	1187.46084	594.23406	M-Oxidation	1244.49220	622.74974	10
11	1274.49287	637.75007	S	1097.45680	549.23204	9
12	1403.53546	702.27137	E	1010.42477	505.71602	8
13	1589.61477	795.31103	W	881.38218	441.19473	7
14	1676.64680	838.82704	S	695.30287	348.15507	6
15	1805.68940	903.34834	E	608.27084	304.63906	5
16	1965.72004	983.36366	C-Carbami...	479.22824	240.11776	4
17	2066.76772	1033.88750	T	319.19760	160.10244	3
18	2137.80484	1069.40606	A	218.14992	109.57860	2
19			K	147.11280	74.06004	1

KZ040518\_3\_raw #6827 RT: 20.4463 min  
 FTMS, 1142.9596@hcd27.00, z=+2, Mono m/z=1142.45837 Da, MH+=2283.90947 Da, Match Tol =20 ppm

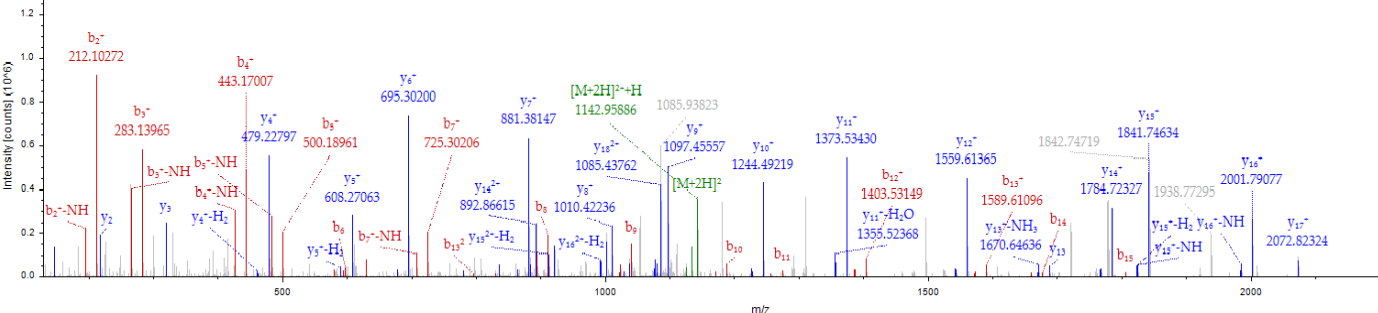
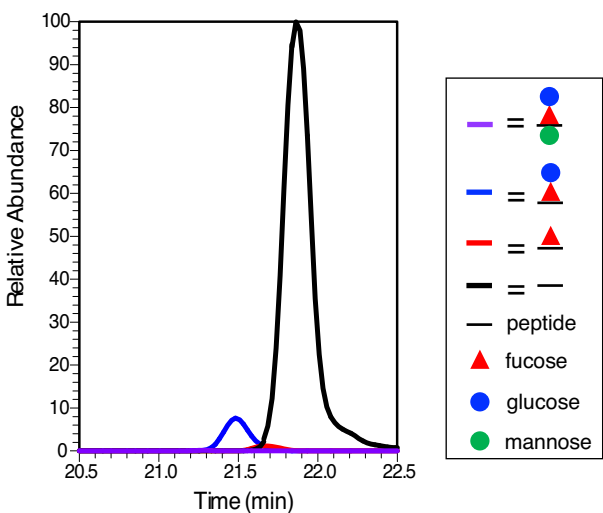


Figure S2F

TSR5<sup>746</sup>QWTVSDWGPCSGSCGQGR<sup>763</sup>

#1	b <sup>+</sup>	b <sup>2+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	#2
1	129.06585	65.03657	Q			18
2	315.14517	158.07622	W	1896.77519	948.89123	17
3	416.19285	208.60006	T	1710.69588	855.85158	16
4	515.26126	258.13427	V	1609.64820	805.32774	15
5	602.29329	301.65028	S	1510.57979	755.79353	14
6	717.32023	359.16375	D	1423.54776	712.27752	13
7	903.39954	452.20341	W	1308.52081	654.76405	12
8	960.42101	480.71414	G	1122.44150	561.72439	11
9	1057.47377	529.24052	P	1065.42004	533.21366	10
10	1217.50442	609.25585	C-Carbami...	968.36727	484.68728	9
11	1304.53645	652.77186	S	808.33663	404.67195	8
12	1361.55791	681.28259	S	721.30460	361.15594	7
13	1448.58994	724.79861	G	664.28313	332.64521	6
14	1608.62059	804.81393	C-Carbami...	577.25111	289.12919	5
15	1665.64205	833.32466	G	417.22046	209.11387	4
16	1793.70063	897.35395	Q	360.19899	180.60314	3
17	1850.72209	925.86468	G	232.14042	116.57385	2
18			R	175.11895	88.06311	1

KZ040518\_2.raw #7774 RT: 22.1356 min

FTMS, 1012.9209@hcd27.00, z=+2, Mono m/z=1012.92078 Da, MH+=2024.83428 Da, Match Tol.=20 ppm

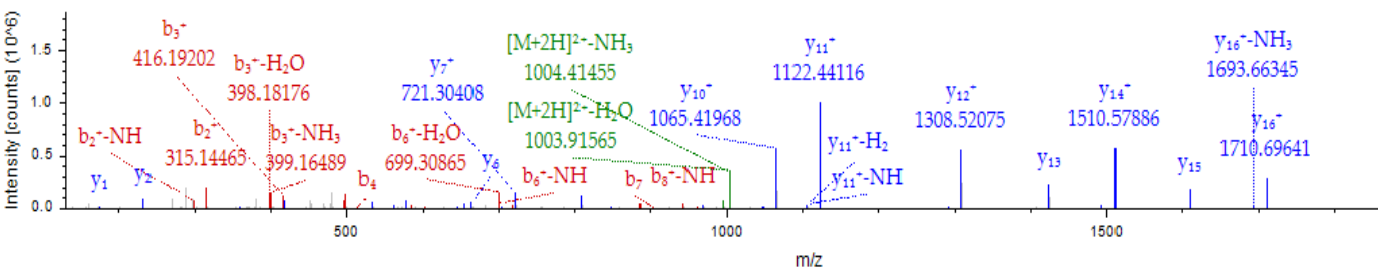
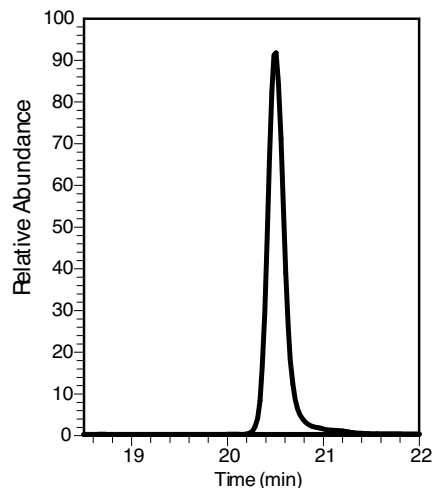


Figure S2G

TSR6<sup>799</sup>NCPAHLWAQDWER<sup>811</sup>

#1	b <sup>+</sup>	b <sup>2+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	#2
1	115.05020	58.02874	N			13
2	275.08085	138.04406	C-Carbami...	1568.70631	784.85679	12
3	372.13362	186.57045	P	1408.67566	704.84147	11
4	443.17073	222.08900	A	1311.62289	656.31509	10
5	580.22964	290.61846	H	1240.58578	620.79653	9
6	766.30895	383.65812	W	1103.52687	552.26707	8
7	879.39302	440.20015	L	917.44756	459.22742	7
8	950.43013	475.71870	A	804.36349	402.68538	6
9	1078.48871	539.74799	Q	733.32638	367.16683	5
10	1193.51565	597.26146	D	605.26780	303.13754	4
11	1379.59497	690.30112	W	490.24086	245.62407	3
12	1508.63756	754.82242	E	304.16155	152.58441	2
13			R	175.11895	88.06311	1

KZ040518\_3\_raw #6796 RT: 20.3756 min  
 FTMS, 841.8777@hcd27.00, z=+2, Mono m/z=841.87775 Da, MH+=1682.74822 Da, Match Tol =±20 ppm

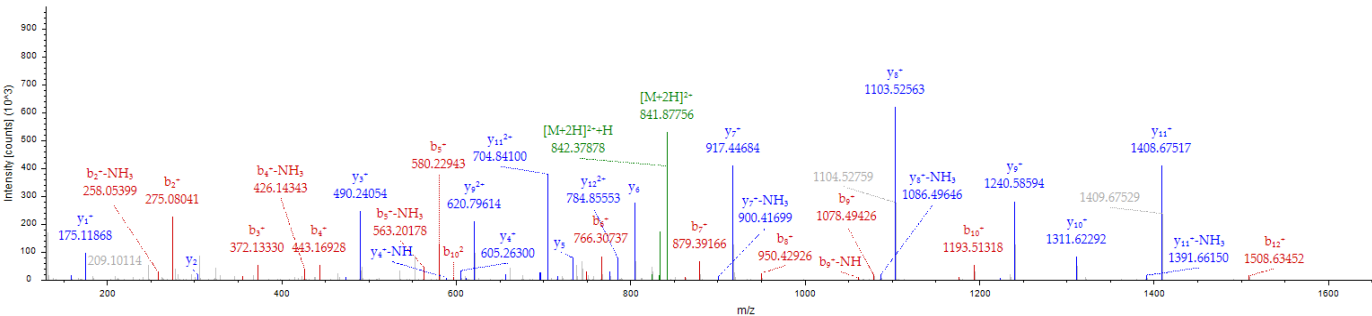
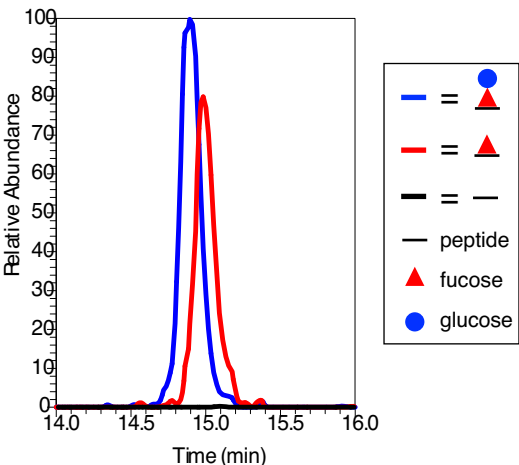


Figure S2H

TSR7<sup>869</sup>SECTKICGVGVRMRDVKCY<sup>887</sup>

#1	b <sup>+</sup>	b <sup>2+</sup>	b <sup>3+</sup>	b <sup>4+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	y <sup>3+</sup>	y <sup>4+</sup>	#2
1	88.03930	44.52329	30.01795	22.76528	S					19
2	217.08190	109.04459	73.03215	55.02593	E	2527.12954	1264.06841	843.04803	632.53784	18
3	377.11255	189.05991	126.37570	95.03359	C-Carbami	2398.08695	1199.54711	800.03383	600.27719	17
4	478.16022	239.58375	160.05826	120.29551	T	2238.05630	1119.53179	746.69028	560.26953	16
5	606.25519	303.63123	202.75658	152.31925	K	2137.00862	1069.00795	713.00772	535.00761	15
6	1015.41360	508.21044	339.14272	254.60886	T-dHex(1)	2008.91366	1004.96047	670.30940	502.98387	14
7	1175.44425	588.22576	392.48627	294.61652	C-Carbami	1599.75525	800.38126	533.92327	400.69427	13
8	1232.46571	616.73649	411.49342	308.87189	G	1439.72460	720.36594	480.57972	360.68661	12
9	1331.53412	666.27070	444.51623	333.63899	V	1382.70314	691.85521	461.57256	346.43124	11
10	1388.55559	694.78143	463.52338	347.89435	G	1283.63472	642.32100	428.54976	321.66414	10
11	1487.62400	744.31564	496.54619	372.66146	V	1226.61326	613.81027	409.54260	307.40877	9
12	1643.72511	822.36619	548.57989	411.68674	R	1127.54484	564.27606	376.51980	282.64167	8
13	1774.76560	887.88644	592.26005	444.44686	M	971.44373	486.22551	324.48610	243.61639	7
14	1930.86671	965.93699	644.29375	483.47213	R	840.40325	420.70526	280.80593	210.85627	6
15	2045.89365	1023.45046	682.63607	512.22887	D	694.30214	342.65471	228.77223	171.83099	5
16	2144.96207	1072.98467	715.65887	536.99597	V	569.27520	285.14124	190.42992	143.07426	4
17	2273.05703	1137.03215	758.35719	569.01971	K	470.20678	235.60703	157.40711	118.30715	3
18	2433.08768	1217.04748	811.70074	609.02738	C-Carbami	342.11182	171.55955	114.70879	86.28341	2
19					Y	182.08117	91.54422	61.38524	46.27575	1

KZ040518\_5\_raw #4608 RT: 14.7532 min

FTMS, 654.5459@hcd27.00, z=+4, Mono m/z=654.29620 Da, MH+=2614.16298 Da, Match Tol.=20 ppm

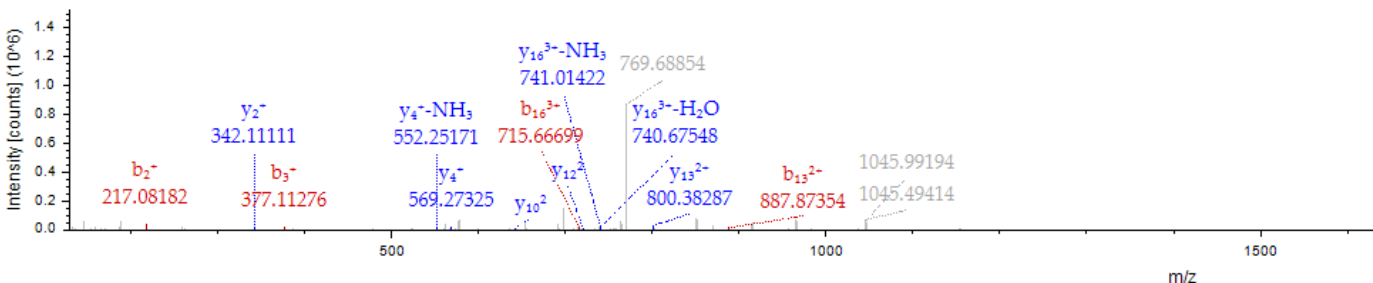
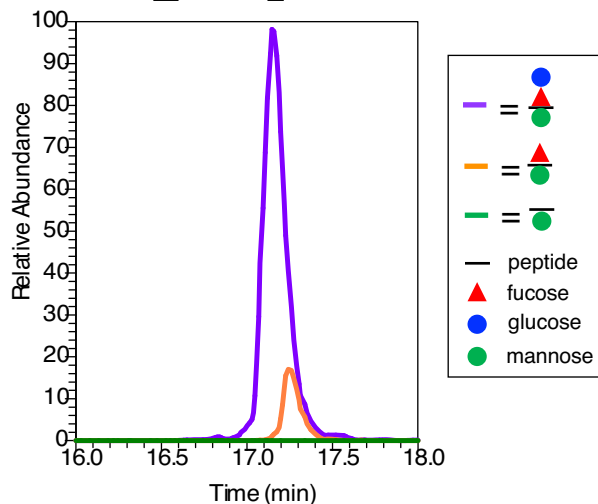


Figure S21

TSR7<sup>865</sup>TSPWSECTK**T**CGVGVRMRDVKCY<sup>887</sup>

#1	b <sup>+</sup>	b <sup>2+</sup>	b <sup>3+</sup>	b <sup>4+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	y <sup>3+</sup>	y <sup>4+</sup>	#2
1	102.06496	51.53112	34.68984	26.26920	T					23
2	189.08698	95.04713	63.70051	48.02720	S	3146.37850	1573.69289	1049.46435	787.35008	22
3	286.13975	143.57351	96.05143	72.29039	P	3059.34647	1530.17687	1020.45367	765.59208	21
4	634.27188	317.63958	212.09548	159.32343	W-Hex	2962.29371	1481.65049	988.10275	741.32888	19
5	721.30391	361.15559	241.10616	181.08144	S	2614.16157	1307.58442	872.05871	654.29585	20
6	850.34651	425.67689	284.12035	213.34208	E	2527.12954	1264.06841	843.04803	632.53784	18
7	1010.37715	505.69222	337.46390	253.34975	C-Carbami...	2398.08695	1199.54711	800.03383	600.27719	17
8	1111.42483	556.21605	371.14646	278.61167	T	2238.09630	1119.53179	746.69028	560.26953	16
9	1239.51980	620.26354	413.84478	310.63541	K	2137.00862	1069.00795	713.00772	535.00761	15
10	1648.67821	824.34274	550.23092	412.92501	T-dHex(1)	2008.91366	1004.96047	670.30940	502.98387	14
11	1808.70885	904.85807	603.57447	452.93267	C-Carbami...	1599.75525	800.38126	533.92327	400.69427	13
12	1865.73032	933.36880	622.58162	467.18804	G	1439.72460	720.36594	480.57972	360.68661	12
13	1964.79873	982.30300	655.60443	491.95514	V	1382.70314	691.85521	461.57256	346.43124	11
14	2021.82020	1011.41374	674.61158	506.21051	G	1283.63472	642.32100	428.54976	321.66414	10
15	2120.88861	1060.94794	707.63439	530.97761	V	1226.61326	613.81027	409.54260	307.40877	9
16	2276.98972	1138.99850	759.66809	570.00289	R	1127.54484	564.27606	376.51980	282.64167	8
17	2408.03020	1204.51874	803.34825	602.76301	M	971.44373	486.22551	324.48610	243.61639	7
18	2564.13132	1282.56930	855.38196	641.78829	R	840.40325	420.70526	280.80993	210.89627	6
19	2679.15826	1340.08277	893.72427	670.54502	D	684.30214	342.65471	228.77223	171.83099	5
20	2778.22667	1389.61697	926.74708	695.31213	V	569.27520	285.14124	190.42992	143.07426	4
21	2906.32164	1453.66446	969.44540	727.33587	K	470.20678	235.60703	157.40711	118.30715	3
22	3066.35228	1533.67978	1022.78895	767.34353	C-Carbami...	342.11182	171.56965	114.70879	86.28341	2
23					Y	182.08117	91.54422	61.36524	46.27575	1

KZ051018\_4\_raw #4806 RT: 17.1246 min

FTMS, 813.1097@hcd27.00, z=+4, Mono m/z=812.60834 Da, MH+=3247.41152 Da, Match Tol.=20 ppm

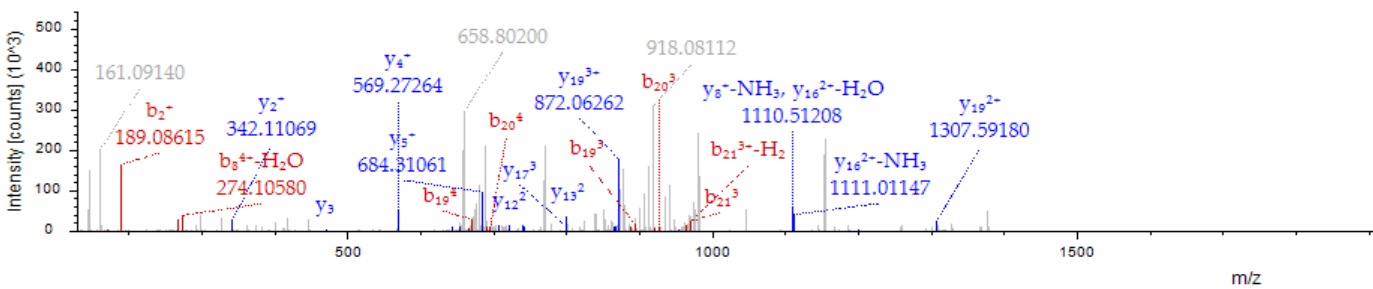
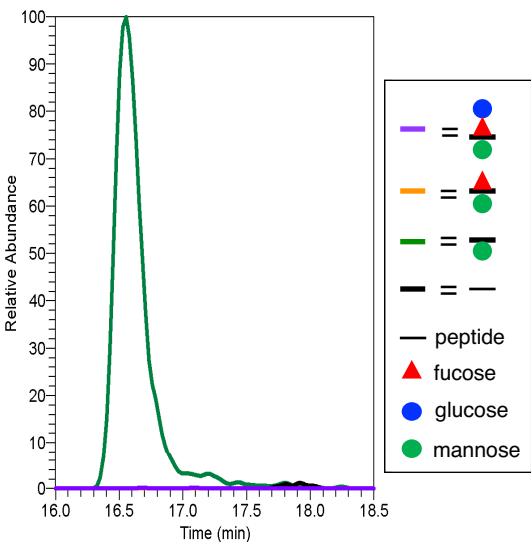


Figure S3

TSR3 S641L <sup>623</sup>CAGRECQPRWETSSWSECLRICGEGHQF<sup>650</sup>



#1	b <sup>+</sup>	b <sup>2+</sup>	b <sup>3+</sup>	b <sup>4+</sup>	b <sup>5+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	y <sup>3+</sup>	y <sup>4+</sup>	y <sup>5+</sup>	#2
1	161.03793	81.02260	54.35083	41.01494	33.01341	C-Carbami...						28
2	232.07504	116.54116	78.02986	58.77422	47.22083	A	3473.46920	1737.23824	1158.49458	869.12276	696.49966	27
3	289.09650	145.05189	97.03702	73.02958	58.62512	G	3402.43208	1701.71968	1134.81555	851.36348	681.29224	26
4	445.19761	223.10245	149.07072	112.05496	89.84634	R	3345.41062	1673.20895	1115.80039	837.10811	669.89795	25
5	574.24021	287.62374	192.08492	144.31551	115.65306	E	3189.30951	1595.15039	1063.77469	798.08283	638.66772	24
6	734.27085	367.63907	245.42847	184.32317	147.65999	C-Carbami...	3060.26691	1530.63710	1020.76049	765.82219	612.85920	23
7	862.32943	431.66835	288.11466	216.33782	173.27171	Q	2900.23627	1450.62177	967.41694	725.81452	580.85307	22
8	969.38220	480.19474	320.46558	240.60101	192.68226	P	2772.17769	1386.59248	924.73075	693.79988	585.24136	21
9	1115.48331	558.24529	372.49929	279.62628	223.90248	R	2675.12493	1338.06610	892.37983	669.53669	535.83081	20
10	1301.56262	651.28495	434.52572	326.14611	261.11835	W	2519.02381	1260.01595	840.34612	630.51141	504.61058	19
11	1430.60521	715.80624	477.53992	358.40676	286.92686	E	2332.94450	1166.97589	778.31968	583.99158	467.39472	18
12	1531.65289	766.33008	511.22248	383.66688	307.13640	T	2203.90191	1102.45459	735.30549	551.73093	441.58620	17
13	1618.68492	809.84610	540.23316	405.42669	324.54281	S	2102.85423	1051.93075	701.62293	526.46901	421.37667	16
14	1705.71695	853.36211	569.24383	427.18469	341.94921	S	2015.82220	1008.41474	672.61225	504.71101	403.97026	15
15	2053.84909	1027.42818	685.28788	514.21773	411.57564	W-Hex	1928.79017	964.89872	643.60158	482.95300	386.56386	14
16	2140.88111	1070.94420	714.28956	538.97574	428.98204	S	1580.65804	790.83266	527.55763	395.91997	316.93743	13
17	2289.92371	1135.46549	757.31275	568.23638	454.79056	E	1493.62601	747.31664	498.54685	374.16196	299.53102	12
18	2429.95435	1215.48082	810.65630	608.24405	486.79669	C-Carbami...	1364.68342	682.79535	455.53266	341.90131	273.72250	11
19	2543.03842	1272.02285	848.35059	636.51506	509.41351	L	1204.55277	602.78002	402.18911	301.89365	241.71637	10
20	2639.13953	1350.07340	900.38469	675.54034	540.63373	R	1091.46870	546.23739	364.49442	273.62263	219.09666	9
21	2800.18721	1400.59724	934.06725	700.80226	560.84326	T	935.36759	468.18743	312.46071	234.59736	187.87934	8
22	2960.21786	1480.61257	987.41080	740.80992	592.84930	C-Carbami...	834.31991	417.66359	278.78716	209.33544	167.68980	7
23	3017.23932	1508.12330	1006.41796	756.06529	604.25369	G	674.28926	337.64827	225.43461	169.32777	135.66367	6
24	3146.28191	1573.64459	1049.43216	787.32594	630.06220	E	617.26780	309.13754	206.42745	155.07241	124.25938	5
25	3203.30388	1602.15533	1068.43991	801.58130	641.46650	G	488.22521	244.61624	163.41325	122.81176	96.45086	4
26	3340.36229	1670.68478	1114.12561	835.84603	668.87828	H	431.20374	216.10551	144.40610	108.55699	87.04657	3
27	3468.42087	1734.71407	1156.81181	867.86067	694.48999	Q	294.14483	147.57805	98.71980	74.29167	59.63479	2
28						F	166.08826	83.54677	56.03360	42.27702	34.02307	1

KZ090619\_3.raw #5342 RT: 16.6369 min

FTMS, 727.9048@hcd27.00, z=+5, Mono m/z=727.50470 Da, MH+=3633.49439 Da, Match Tol.=20 ppm

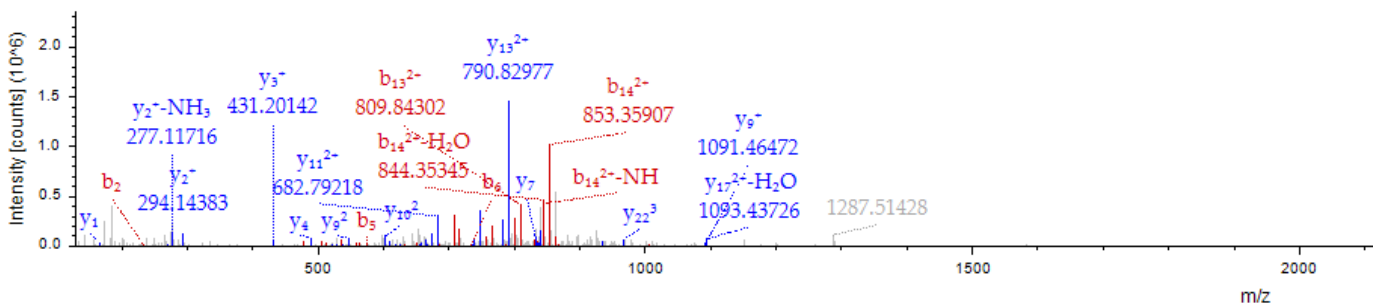
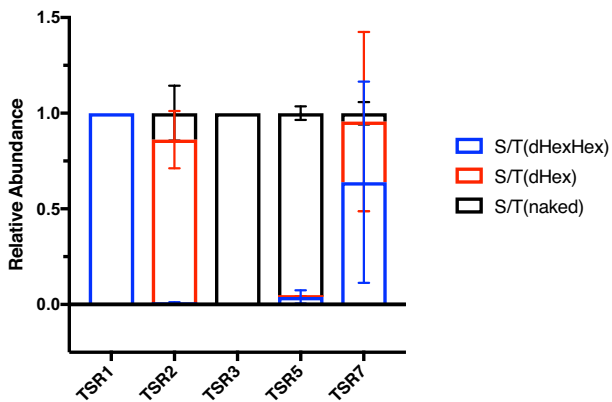


Figure S4

A



B

