

**Supplementary Materials for:**

**Hydrocephalus in mouse *B3glct* mutants is likely caused by defects in multiple B3GLCT substrates in ependymal cells and subcommissural organ**

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Supplemental Table I

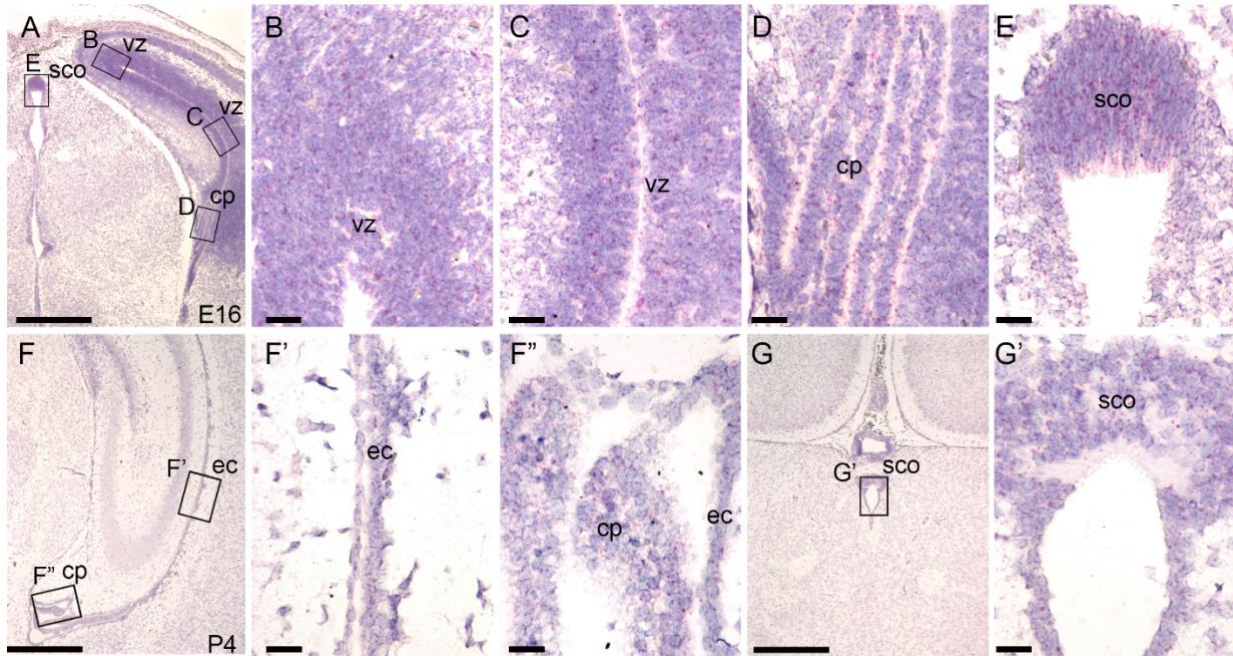
Supplemental Figures 1-4

**Supplemental Table I: Primers used for qRT-PCR**

<b>Gene</b>	<b>Forward Primer (5'-&gt;3')</b>	<b>Reverse Primer (5'-&gt;3')</b>	<b>References</b>
<i>Chop</i>	CCT AGC TTG GCT GAC AGA GG	CTG CTC CTT CTC CTT CAT GC	(Deldicque, L., Bertrand, L., et al. 2011)
<i>Atf4</i>	GAG CTT CCT GAA CAG CGA AGT G	TGG CCA CCT CCA GAT AGT CAT C	
<i>Xbp1-Spliced</i>	GAG TCC GCA GCA GGT G	GTG TCA GAG TCC ATG GGA	
<i>Xbp1-Unspliced</i>	AAG AAC ACG CTT GGG AAT GG	ACT CCC CTT GGC CTC CAC	
<i>BiP</i>	TTC AGC CAA TTA TCA GCA AAC TCT	TTT TCT GAT GTA TCC TCT TCA CCA GT	
<i>Total XbP1</i>	TGG CCG GGT CTG CTG AGT CCG	GTC CAT GGG AAG ATG TTC TGG	
<i>Hprt</i>	CCT AAG ATG AGC GCA AGT TG	CAC AGG ACT AGA ACA CCT GCT AA	(Bang, S.P., Yeon, C.Y., et al. 2019)
<i>B3glct</i>	TCC GCG GCT TTT GCT TTG AT	TCT GCC TCA AAT CCT GAG AAG G	This study
<i>Pofut2</i>	CTGTACTCGCAGGACAAGCA	TCAGACCCCGTGTTTCTTCG	This study

**Supplemental Figure 1. *Pofut2* mRNA localization in brain at E16 and P4**




**(A-G)** *Pofut2* mRNA localization at E16 **(A-E)** and P4 **(F-G')**. Areas indicated by black rectangles in panel A, F and G are expanded in panels B-E, F'-F'' and G', respectively. Scale bars: panels A, F and G 500  $\mu$ m; panels B-E, F'-F'' and G' 50  $\mu$ m. Abbreviations: vz, ventricular zone of cortex; cp, choroid plexus; ec, ependymal cells of lateral ventricle; sco, subcommissural organ.



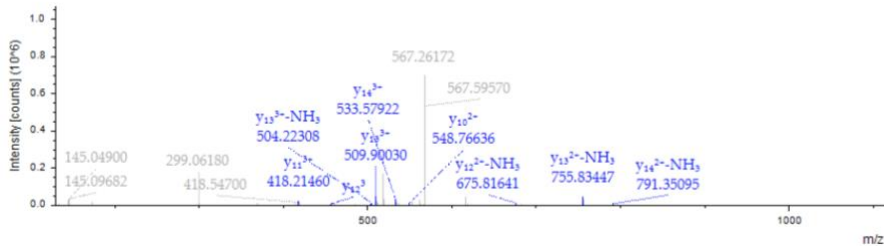
**Supplemental Figure 2: MS2 spectra of ions corresponding to O-fucosylated peptides from SSPO TSRs 6, 7, 8 and 9.** MS2 spectra of the disaccharide form of peptides containing the POFUT2 consensus sequence from each TSR are shown. The parent ion masses (shown on the right, charge state in parentheses) were used to generate the EICs in Fig. 4B. The masses for the other glycoforms were calculated. Due to the lability of the fucose-peptide bond in high energy collision dissociation experiments, Byonic is frequently unable to correctly assign the O-fucosylated Ser/Thr residue in a peptide. All assignments are based on the well-documented consensus sequence for O-fucosylation of TSRs: C-X-X-(S/T)-C.

**TSR6 [W].TACDRSCGSGVRRARF.[R]**

#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	410.16569	205.58648	137.39341	103.29688	T-Hex(1)F...					15
2	481.20280	241.10504	161.07245	121.05616	A	1598.72745	799.86736	533.58067	400.43732	14
3	641.23345	321.12036	214.41600	161.06382	C-Carbami...	1527.69034	764.34881	509.90163	382.67804	13
4	756.26039	378.63383	252.75832	189.82056	D	1367.65969	684.33348	456.55808	342.67038	12
5	912.36150	456.68439	304.79202	228.84583	R	1252.63275	626.82001	418.21577	313.91364	11
6	999.39353	500.20040	333.80270	250.60384	S	1096.53164	548.76946	366.18206	274.88837	10
7	1159.42418	580.21573	387.14624	290.61150	C-Carbami...	1009.49961	505.25344	337.17139	253.13036	9
8	1216.44564	608.72646	406.15340	304.86687	G	849.46896	425.23812	283.82784	213.12270	8
9	1303.47767	652.24247	435.16408	326.62488	S	792.44750	396.72739	264.82068	198.86733	7
10	1360.49914	680.75321	454.17123	340.88024	G	705.41547	353.21137	235.81001	177.10932	6
11	1459.56755	730.28741	487.19403	365.64734	V	648.39400	324.70064	216.80285	162.85396	5
12	1615.66866	808.33797	539.22774	404.67262	R	549.32559	275.16643	183.78005	138.08686	4
13	1686.70577	843.85653	562.90678	422.43190	A	393.22448	197.11588	131.74634	99.06158	3
14	1842.80689	921.90708	614.94048	461.45718	R	322.18737	161.59732	108.06731	81.30230	2
15					F	166.08626	83.54677	56.03360	42.27702	1




-  502.72620 (4)
-  462.21300 (4)
-  425.69852 (4)

AI091919\_2.raw #3202 RT: 12.3860 min  
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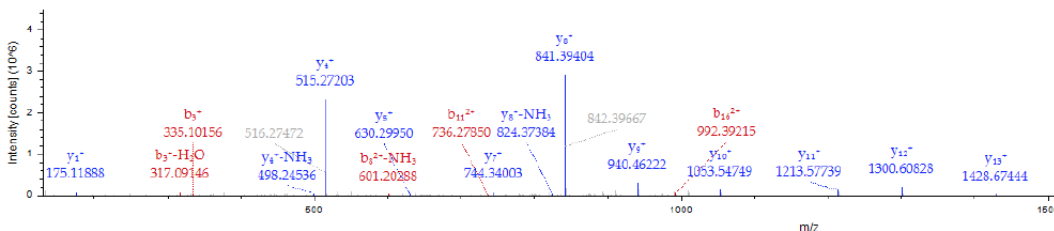


**TSR7 [W].SSCSQSLVPGGDPGWR.[Q]**

#1	b <sup>+</sup>	b <sup>2+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	#2
1	88.03930	44.52329	S			17
2	175.07133	88.03930	S	2070.87430	1035.94079	16
3	335.10198	168.05463	C-Carbami...	1983.84227	992.42477	15
4	730.24474	365.62601	S-Hex(1)F...	1823.81162	912.40945	14
5	858.30332	429.65530	Q	1428.66886	714.83807	13
6	945.33535	473.17131	S	1300.61028	650.80878	12
7	1105.36600	553.18664	C-Carbami...	1213.57825	607.29276	11
8	1218.45006	609.72867	L	1053.54760	527.27744	10
9	1317.51847	659.26288	V	940.46354	470.73541	9
10	1414.57124	707.78926	P	841.39513	421.20120	8
11	1471.59270	736.29999	G	744.34236	372.67482	7
12	1528.61417	764.81072	G	687.32090	344.16409	6
13	1643.64111	822.32419	D	630.29944	315.65336	5
14	1740.69387	870.85057	P	515.27249	258.13988	4
15	1797.71534	899.36131	G	418.21973	209.61350	3
16	1983.79465	992.40096	W	361.19827	181.10277	2
17			R	175.11895	88.06311	1




-  1079.4574 (2), 719.9741 (3)
-  998.43095 (2), 665.95647 (3)
-  925.40200 (2), 617.27050 (3)

AI061920\_3.raw #6422 RT: 19.2394 min  
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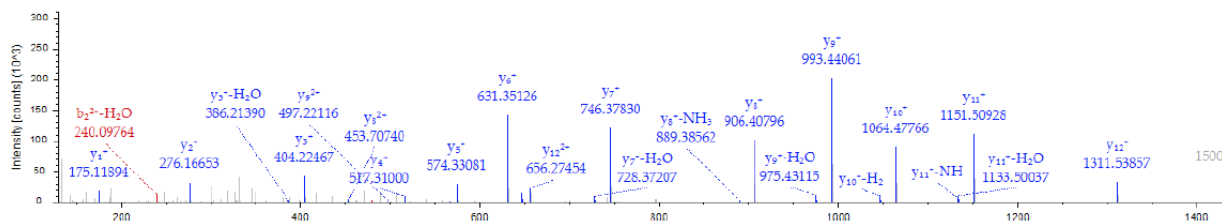


TSR8 [W].STCSA\_S\_CNGGIQTR.[G]

#1	b <sup>+</sup>	b <sup>2+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	#2
1	396.15004	198.57866	S-Hex(1)F...			14
2	497.19772	249.10250	T	1412.58929	706.79828	13
3	657.22836	329.11782	C-Carbami...	1311.54161	656.27444	12
4	744.26039	372.63383	S	1151.51096	576.25912	11
5	815.29751	408.15239	A	1064.47893	532.74310	10
6	902.32953	451.66841	S	993.44182	497.22455	9
7	1062.36018	531.68373	C-Carbami...	906.40979	453.70853	8
8	1177.38713	589.19720	N-Deamid...	746.37914	373.69321	7
9	1234.40859	617.70793	G	631.35220	316.17974	6
10	1291.43005	646.21867	G	574.33074	287.66901	5
11	1404.51412	702.76070	I	517.30927	259.15827	4
12	1532.57270	766.78999	Q	404.22521	202.61624	3
13	1633.62037	817.31383	T	276.16663	138.58695	2
14			R	175.11895	88.06311	1




-  904.36847 (2), 603.24695 (3)
-  823.34202 (2), 549.22932 (3)
-  750.31307 (2), 500.54335 (3)

AI061920\_3.raw #3290 RT: 12.0196 min  
FTMS, 904.3685@hcd27.00, z=+2, Mono m/z=904.36847 Da, MH+=1807.72966 Da, Match Tol=30 ppm

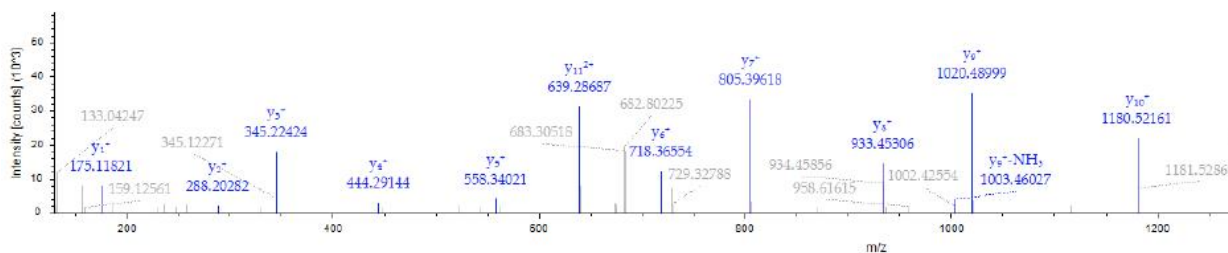


TSR9 [W].SPCSQSCNVGIR.[R]

#1	b <sup>+</sup>	b <sup>2+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	#2
1	396.15004	198.57866	S-Hex(1)F...			12
2	493.20280	247.10504	P	1277.57252	639.28990	11
3	653.23345	327.12036	C-Carbami...	1180.51975	590.76351	10
4	740.26548	370.63638	S	1020.48910	510.74819	9
5	868.32406	434.66567	Q	933.45708	467.23218	8
6	955.35608	478.18168	S	805.39850	403.20289	7
7	1115.38673	558.19700	C-Carbami...	718.36647	359.68687	6
8	1229.42966	615.21847	N	558.33582	279.67155	5
9	1328.49807	664.75268	V	444.29289	222.65009	4
10	1385.51954	693.26341	G	345.22448	173.11588	3
11	1498.60360	749.80544	I	288.20302	144.60515	2
12			R	175.11895	88.06311	1

-  836.86108 (2)
-  823.34202 (2)
-  750.31307 (2)

AI061920\_3.raw #3799 RT: 13.2194 min  
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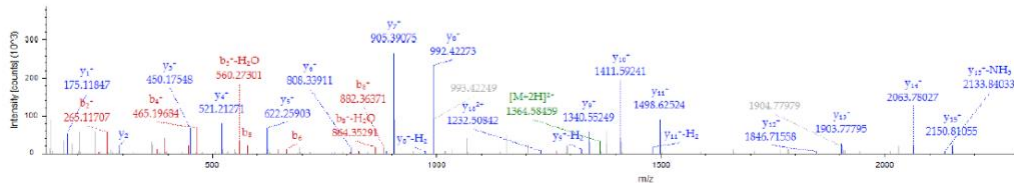
**Supplemental Figure 3: MS2 spectra of ions corresponding to C-mannosylated peptides from SSPO TSRs 6, 7, 8 and 9.** MS2 spectra of the most abundant form of peptides containing the W-x-x-W/C consensus sequence from each TSR are shown. The parent ion masses (shown on the right, charge state in parentheses) were used to generate the EICs in Fig. 4C. The masses for the other glycoforms were calculated. Since the C-mannose linkage to tryptophan is stable, the presence of specific b- and/or y-ions reveals the location of the C-mannose in the peptides from TSRs 6, 7 and 8. There are not sufficient b- and/or y-ions to determine the location of the C-mannose on the peptide from TSR9.

**TSR6 [R].TYEALSCGWSAWSPWTACDR.[S]**

#1	b <sup>+</sup>	b <sup>2+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	#2
1	102.05496	51.53112	T			20
2	265.11828	133.06278	Y	2627.07010	1314.03869	19
3	394.16088	197.58408	E	2464.00677	1232.50702	18
4	465.19799	233.10263	A	2334.96417	1167.98573	17
5	578.28205	289.64467	L	2263.92706	1132.46717	16
6	665.31408	333.16068	S	2150.84300	1075.92514	15
7	825.34473	413.17600	C-Carbami...	2063.81097	1032.40912	14
8	882.36619	441.68674	G	1903.78032	952.39380	13
9	1230.49833	615.75280	W-Hex(1)	1846.75886	923.88307	12
10	1317.53036	659.26882	S	1498.62672	749.81700	11
11	1388.56747	694.78737	A	1411.59469	706.30098	10
12	1736.69961	868.85344	W-Hex(1)	1340.55758	670.78243	9
13	1823.73164	912.36946	S	992.42544	496.71636	8
14	1920.78440	960.89584	P	905.39341	453.20034	7
15	2106.86371	1053.93550	W	808.34065	404.67396	6
16	2207.91139	1104.45933	T	622.26134	311.63431	5
17	2278.94851	1139.97789	A	521.21366	261.11047	4
18	2438.97916	1219.99322	C-Carbami...	450.17654	225.59191	3
19	2554.00610	1277.50669	D	290.14590	145.57659	2
20			R	175.11895	88.06311	1

- 3 1445.58914 (2), 964.06142 (3)
- 2 1364.56274 (2), 910.04382 (3)
- 1 1283.53634 (2), 856.02622 (3)
- 1202.50994 (2), 802.00663 (3)

A00919\_1.raw #8142 RT: 22.8907 min  
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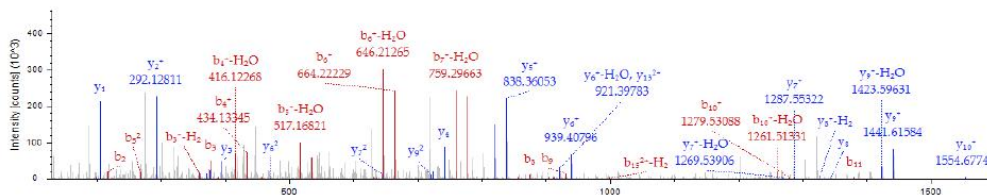


**TSR7 [Y].TDCGTEIPGWTPWTSW.[S]**

#1	b <sup>+</sup>	b <sup>2+</sup>	Seq.	y <sup>+</sup>	y <sup>2+</sup>	#2
1	102.05496	51.53112	T			16
2	217.08190	109.04459	D	2116.86919	1058.93824	15
3	377.11255	189.05991	C-Carbami...	2001.84225	1001.42476	14
4	434.13401	217.57064	G	1841.81160	921.40944	13
5	535.18169	268.09448	T	1784.79014	892.89871	12
6	664.22428	332.61578	E	1683.74246	842.37487	11
7	777.30835	389.15781	I	1554.69987	777.85357	10
8	874.36111	437.68419	P	1441.61580	721.31154	9
9	931.38257	466.19492	G	1344.56304	672.78516	8
10	1279.51471	640.26099	W-Hex(1)	1287.54158	644.27443	7
11	1380.56239	690.78483	T	939.40944	470.20836	6
12	1477.61515	739.31121	P	838.36176	419.68452	5
13	1825.74729	913.37728	W-Hex(1)	741.30900	371.15814	4
14	1926.79497	963.90112	T	393.17686	197.09207	3
15	2013.82699	1007.41714	S	292.12918	146.56823	2
16			W	205.09715	103.05222	1

- 3 1190.48941 (2)
- 2 1109.46301 (2)
- 1 1028.43661 (2)
- 947.41021 (2)

A051820\_3.raw #10679 RT: 27.6720 min  
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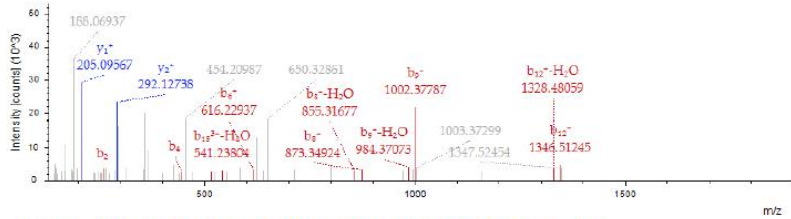


TSR8 [F].CPGEATQEEPCSPVPVPSAWGLWASW.[S]

#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	161.03793	81.02260	54.35083	C-Carbami...				28
2	258.09069	129.54898	86.70175	P	3319.44459	1660.22593	1107.15305	27
3	315.11215	158.08971	105.70890	G	3222.39182	1611.69955	1074.80213	26
4	444.15475	222.58101	148.72310	E	3165.37036	1583.18882	1055.79497	25
5	515.19186	258.09957	172.40214	A	3036.32777	1518.66752	1012.78077	24
6	616.23954	308.62341	206.08470	T	2965.29065	1483.14896	989.10174	23
7	744.29812	372.65270	248.77089	Q	2864.24297	1432.62513	955.41918	22
8	873.34071	437.17399	291.78509	E	2736.18440	1368.59584	912.73298	21
9	1002.38330	501.69529	334.79928	E	2607.14180	1304.07454	869.71879	20
10	1099.43607	550.22167	367.15021	P	2478.09921	1239.55324	826.70459	19
11	1259.46671	630.23700	420.49376	C-Carbami...	2381.04645	1191.02686	794.35367	18
12	1346.49874	673.75301	449.50443	S	2221.01580	1111.01154	741.01012	17
13	1443.55151	722.27939	481.85535	P	2133.98377	1067.49552	711.99944	16
14	1540.60427	770.80577	514.20627	P	2036.93101	1018.96914	679.64852	15
15	1639.67268	820.33998	547.22908	V	1939.87824	970.44276	647.29760	14
16	1799.70333	900.35530	600.57263	C-Carbami...	1840.80983	920.90855	614.27479	13
17	1896.75610	948.88169	632.92355	P	1680.77918	840.89323	560.93124	12
18	1995.82451	998.41589	665.94635	V	1583.72642	792.36685	528.58032	11
19	2092.87727	1046.94227	698.29728	P	1484.65800	742.83264	495.55752	10
20	2179.90930	1090.45829	727.30795	S	1387.60524	694.30626	463.20660	9
21	2250.94642	1125.97685	750.98699	A	1300.57321	650.79024	434.19592	8
22	2599.07855	1300.04291	867.03104	W-Hex(1)	1229.53610	615.27169	410.51688	7
23	2656.10002	1328.55365	886.03819	G	881.40396	441.20562	294.47284	6
24	2769.18408	1385.09568	923.73288	L	824.38250	412.69489	275.46568	5
25	3117.31622	1559.16175	1039.77692	W-Hex(1)	711.29843	356.15285	237.77100	4
26	3188.35333	1594.68030	1063.45596	A	363.16630	182.08679	121.72695	3
27	3275.38536	1638.19632	1092.46664	S	292.12918	146.56823	98.04791	2
28				W	205.09715	103.05222	69.03724	1

- 3 1214.51015 (3)
- 2 1160.49255 (3)
- 1 1106.47495 (3)
- 1052.45735 (3)

AI101820\_1.raw #6237 RT: 17.1238 min  
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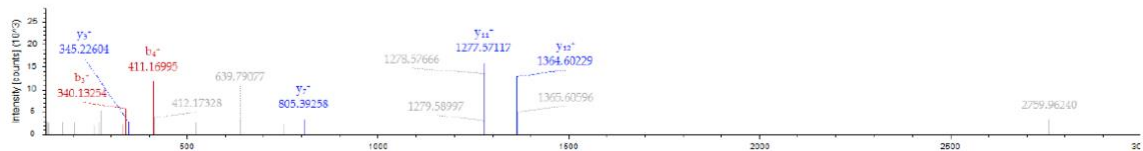


TSR9 [R].CPVACGWSPWTPWSPCSQSCNVGIR.[R]

#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	144.01138	72.50933	48.67531	C-Carbami...				25
2	241.06414	121.03571	81.02623	P	3259.40168	1630.20448	1087.13874	24
3	340.13255	170.56992	114.04904	V	3162.34891	1581.67809	1054.78782	23
4	411.16967	206.08847	137.72807	A	3063.28050	1532.14389	1021.76502	22
5	571.20032	286.10380	191.07162	C-Carbami...	2992.24339	1496.62533	998.08598	21
6	628.22178	314.61453	210.07878	G	2832.21274	1416.61001	944.74243	20
7	814.30109	407.65418	272.10522	W	2775.19127	1388.09927	925.73528	19
8	1209.44385	605.22556	403.81947	S-Hex(1)F...	2589.11196	1295.05962	863.70884	18
9	1306.49662	653.75195	436.17039	P	2193.96920	1097.48824	731.99458	17
10	1492.57593	746.79160	498.19683	W	2096.91644	1048.96186	699.64366	16
11	1593.62361	797.31544	531.87939	T	1910.83712	955.92220	637.61723	15
12	1690.67637	845.84182	564.23031	P	1809.78944	905.39836	603.93467	14
13	2038.80851	1019.90789	680.27435	W-Hex(1)	1712.73668	856.87198	571.58374	13
14	2125.84054	1063.42391	709.28503	S	1364.60454	682.80591	455.53970	12
15	2222.89330	1111.95029	741.63595	P	1277.57252	639.28990	426.52902	11
16	2382.92395	1191.96561	794.97950	C-Carbami...	1180.51975	590.76351	394.17810	10
17	2469.95598	1235.48163	823.99018	S	1020.48910	510.74819	340.83455	9
18	2598.01456	1299.51092	866.67637	Q	933.45708	467.23218	311.82388	8
19	2685.04658	1343.02693	895.68705	S	805.39850	403.20289	269.13768	7
20	2845.07723	1423.04225	949.03060	C-Carbami...	718.36647	359.68687	240.12701	6
21	2959.12016	1480.06372	987.04490	N	558.33582	279.67155	186.78346	5
22	3058.18857	1529.59792	1020.06771	V	444.29289	222.65009	148.76915	4
23	3115.21004	1558.10866	1039.07486	G	345.22448	173.11588	115.74634	3
24	3228.29410	1614.65069	1076.76955	I	288.20302	144.60515	96.73919	2
25				R	175.11895	88.06311	59.04450	1

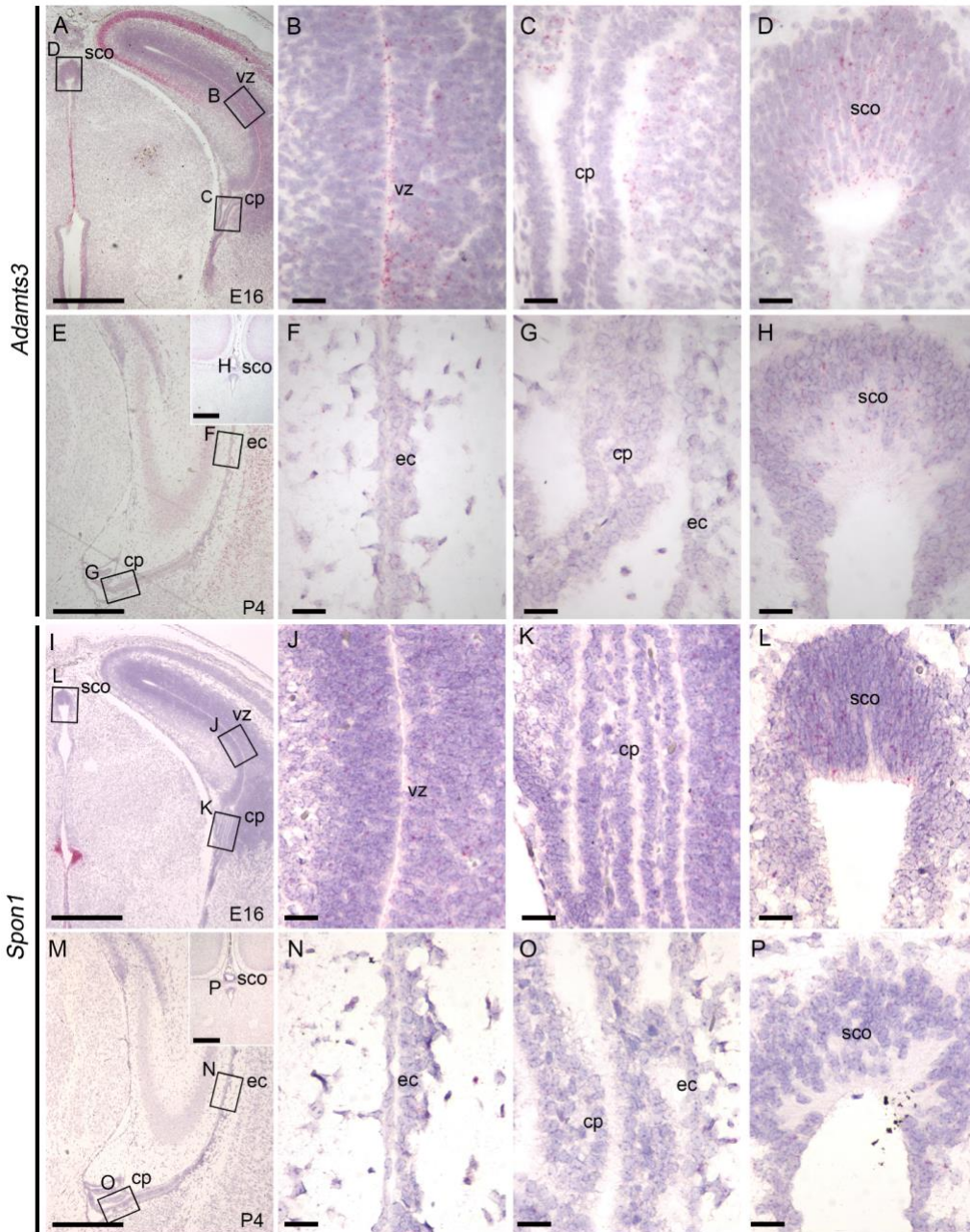
- 3 1242.89157 (3)
- 2 1188.81997 (3)
- 1 1134.80237 (3)
- 1080.78477 (3)

AI061920\_2.raw #6629 RT: 27.0424 min  
FTMS, 1134.8024@hcd27.00, z=+3, Mono m/z=1134.80237 Da, MH+=3402.39255 Da, Match Tol=30 ppm



**Supplemental Figure 4. *Adamts3* and *Spon1* mRNA localization in brain at E16 and P4**

**(A-H) *Adamts3* mRNA localization at E16 (A-D) and P4 (E-H).** Areas indicated by black rectangles in panel A and E are expanded in panels B-D and F-H, respectively. **(I-P) *Spon1* mRNA localization at E16 (I-L) and P4 (M-P).** Areas indicated by black rectangles in I and M are expanded in J-L and N-P, respectively. Scale bars: panels A, E, I, M and insets 500  $\mu$ m; panels B-D, F-H, J-L, and N-P 50  $\mu$ m. Abbreviations: vz, ventricular zone of cortex; cp, choroid plexus; ec, ependymal cells of lateral ventricle; sco, subcommissural organ





### ***Supplementary References***

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