SUPPORTING INFORMATION

Site-specific characterization of threonine, serine and tyrosine glycosylations of amyloid precursor protein/amyloid β-peptides in human cerebrospinal fluid

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Content

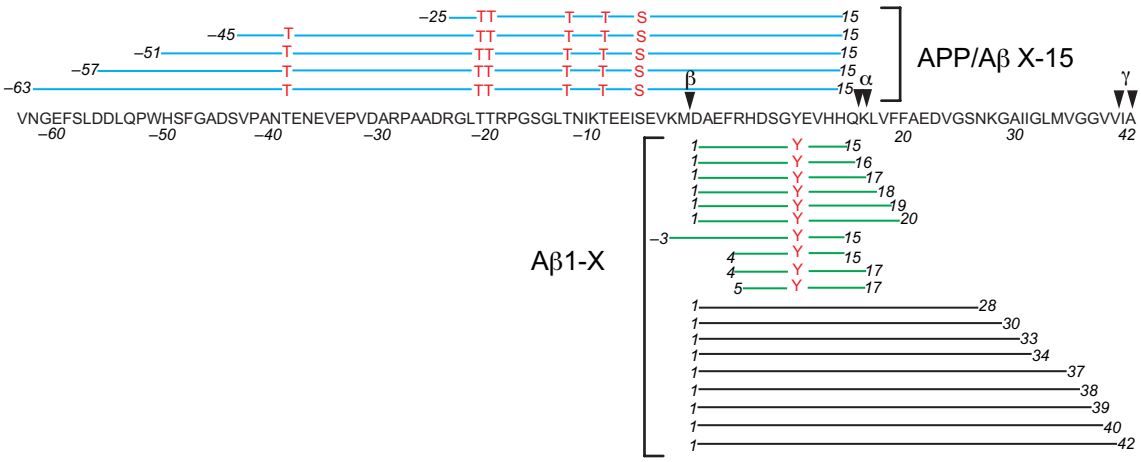


Fig. S1. Amyloid Precursor Protein (APP) amino acid sequence containing the Aβ1-X and APP/AβX-15 glycopeptides identified in human CSF. The positions of the α–, β– and γ-secretase cleavage sites are shown as arrowheads above the APP amino acid sequence. The APP/AβX-15 glycopeptides, all ending at Asn15, are shown above the sequence (blue series). The Aβ1-X glycopeptides are shown below the sequence (green series). The glycosylation sites presented in this study are indicated as red S/T/Y residues. Longer Aβ1-X peptides could not be detected in glycosylated form (black series).

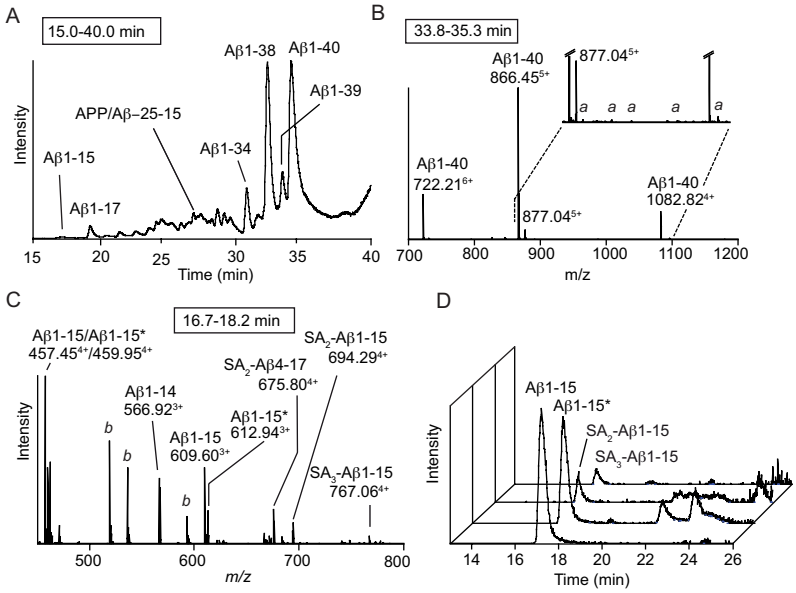
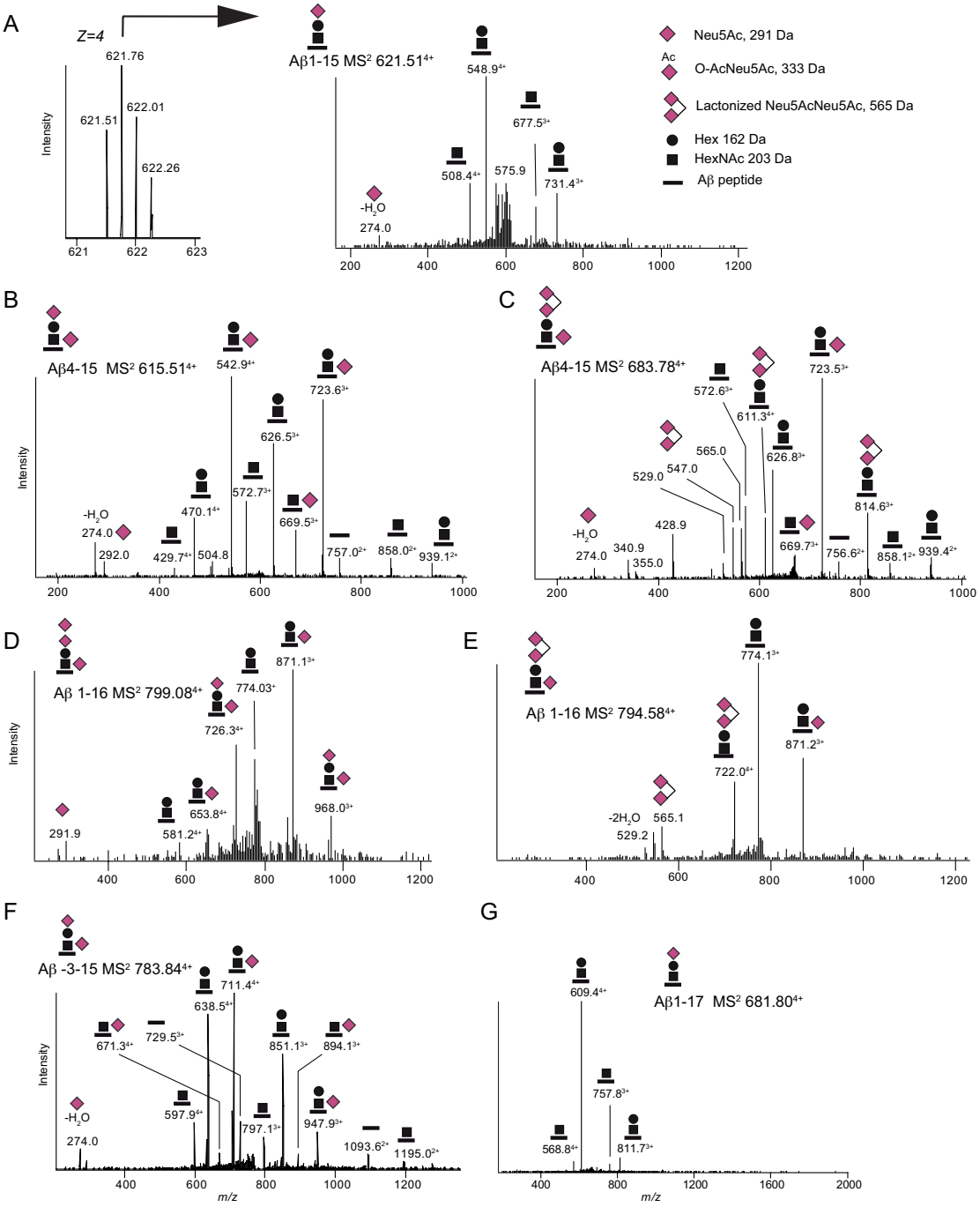
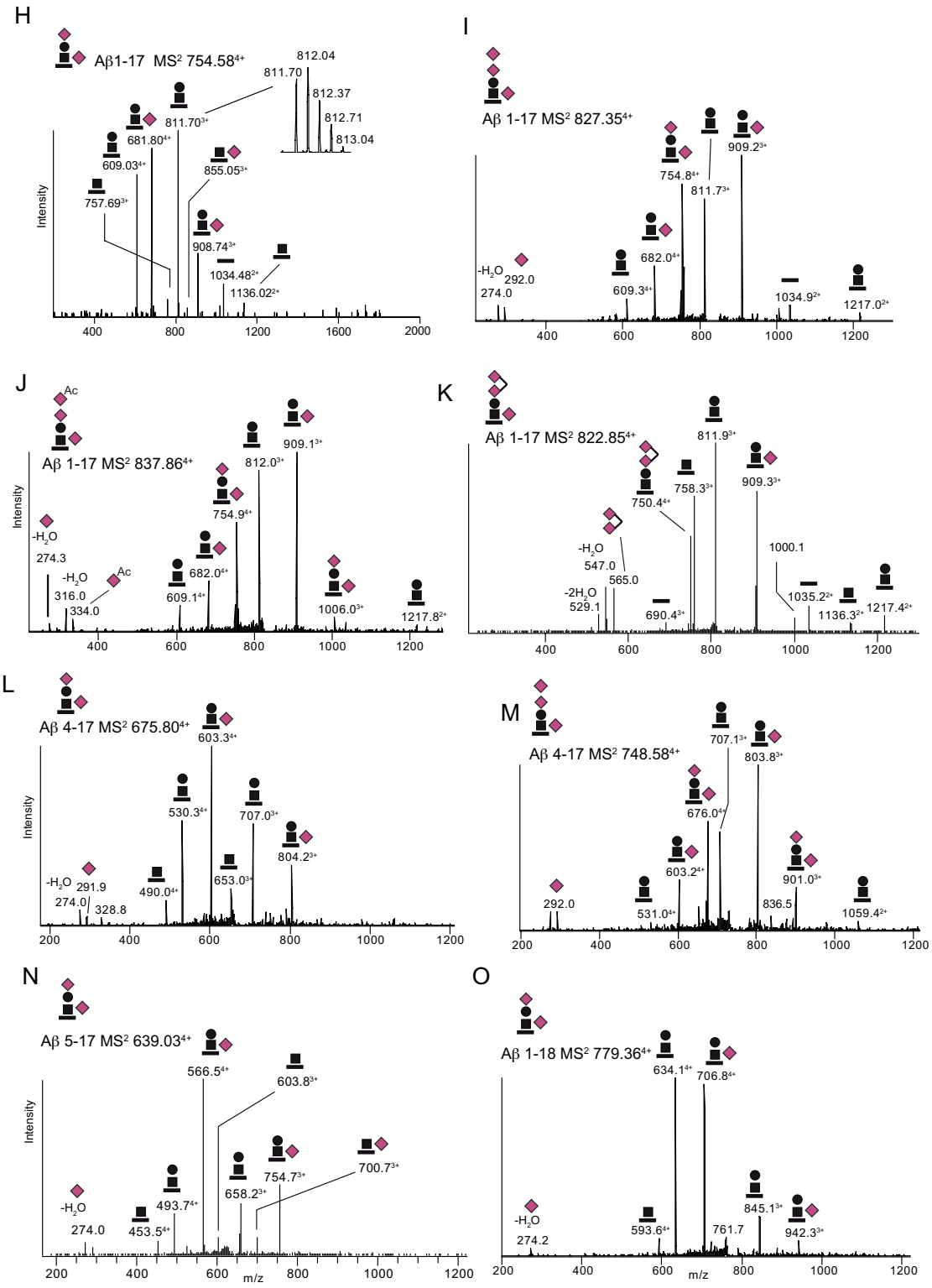


Fig. S2. Immunopurified Aβ peptides and glycopeptides in human CSF. (**A**) Total-ion current chromatogram at 15.0-40.0 min of immunopurified Aβ peptides and glycopeptides. (**B**) Combined full mass spectra at 33.8-35.3 min showing Aβ1-40 and an apparent lack of Aβ1-40 glycopeptides. (**C**) Combined full mass spectra at 16.7-18.2 min showing Aβ1-15, SA_2-Aβ1-15 and SA_3-Aβ1-15, as well as additional Aβ peptides and glycopeptides. Minor ions[(a)] and singly charged ions[(b)] are of unknown origin. (**D**) Extracted ion chromatograms for the *m/z* values corresponding to $[M + 4H]^{4+}$ of the Aβ1-15 peptide, Aβ1-15-Arg-$^{13}C^{15}N$ standard (Aβ1-15*), SA_2-Aβ1-15, and SA_3-Aβ1-15. Parent ions are in the $[M + zH]^{z+}$ form and charges are shown in superscript when z >1.


A
Z=4
621.76
621.51
622.01
622.26
Intensity
621
622
623
Aβ1-15 MS² 621.51⁴⁺
548.9⁴⁺
677.5³⁺
508.4⁴⁺
575.9
731.4³⁺
-H2O
274.0
200
400
600
800
1000
1200
Neu5Ac, 291 Da
Ac
O-AcNeu5Ac, 333 Da
Lactonized Neu5AcNeu5Ac, 565 Da
Hex 162 Da
HexNAc 203 Da
Aβ peptide
B
Aβ4-15 MS² 615.51⁴⁺
542.9⁴⁺
723.6³⁺
626.5³⁺
572.7³⁺
470.1⁴⁺
669.5³⁺
-H2O
274.0
292.0
429.7⁴⁺
504.8
757.0²⁺
858.0²⁺
939.1²⁺
Intensity
200
400
600
800
1000
C
Aβ4-15 MS² 683.78⁴⁺
723.5³⁺
572.6³⁺
611.3⁴⁺
626.8³⁺
565.0
529.0
547.0
814.6³⁺
-H2O
274.0
428.9
340.9
355.0
669.7³⁺
756.6²⁺
858.1²⁺
939.4²⁺
200
400
600
800
1000
D
Aβ 1-16 MS² 799.08⁴⁺
871.1³⁺
774.03⁺
726.3³⁺
653.8⁴⁺
968.0³⁺
291.9
581.2⁴⁺
Intensity
400
600
800
1000
1200
E
Aβ 1-16 MS² 794.58⁴⁺
774.1³⁺
871.2³⁺
722.0⁴⁺
-2H2O
565.1
529.2
400
600
800
1000
1200
F
Aβ -3-15 MS² 783.84⁴⁺
711.4⁴⁺
638.5⁴⁺
729.5³⁺
671.3⁴⁺
851.1³⁺
894.1³⁺
597.9⁴⁺
797.1³⁺
947.9³⁺
-H2O
274.0
1093.6²⁺
1195.0²⁺
Intensity
400
600
800
1000
1200
m/z
G
609.4⁴⁺
Aβ1-17 MS² 681.80⁴⁺
757.8³⁺
568.8⁴⁺
811.7³⁺
400
800
1200
1600
2000
m/z


H
Aβ1-17 MS² 754.58⁴⁺
812.04
811.70
812.37
812.71
813.04
811.70³⁺
681.80⁴⁺
609.03⁴⁺
855.05³⁺
757.69³⁺
908.74³⁺
1034.48²⁺
1136.02²⁺
Intensity
400
800
1200
1600
2000
I
Aβ 1-17 MS² 827.35⁴⁺
909.2³⁺
754.8⁴⁺
811.7³⁺
682.0⁴⁺
-H2O
292.0
274.0
609.3⁴⁺
1034.9²⁺
1217.0²⁺
400
600
800
1000
1200
J
Ac
Aβ 1-17 MS² 837.86⁴⁺
909.1³⁺
812.0³⁺
754.9⁴⁺
Intensity
-H2O
274.3
-H2O
316.0
Ac
334.0
682.0⁴⁺
609.1⁴⁺
1006.0³⁺
1217.8²⁺
400
600
800
1000
1200
K
Aβ 1-17 MS² 822.85⁴⁺
811.9³⁺
758.3³⁺
909.3³⁺
750.4⁴⁺
-H2O
547.0
565.0
1000.1
-2H2O
529.1
690.4³⁺
1035.2²⁺
1136.3²⁺
1217.4²⁺
400
600
800
1000
1200
L
Aβ 4-17 MS² 675.80⁴⁺
603.3⁴⁺
530.3⁴⁺
707.0³⁺
804.2³⁺
Intensity
-H2O
291.9
274.0
328.8
490.0⁴⁺
653.0³⁺
200
400
600
800
1000
1200
M
Aβ 4-17 MS² 748.58⁴⁺
707.1³⁺
803.8³⁺
676.0⁴⁺
603.2⁴⁺
901.0³⁺
292.0
531.0⁴⁺
836.5
1059.4²⁺
200
400
600
800
1000
1200
N
Aβ 5-17 MS² 639.03⁴⁺
566.5⁴⁺
603.8³⁺
700.7³⁺
754.7³⁺
658.2³⁺
Intensity
-H2O
274.0
493.7⁴⁺
453.5⁴⁺
200
400
600
m/z
800
1000
1200
O
Aβ 1-18 MS² 779.36⁴⁺
634.1⁴⁺
706.8⁴⁺
-H2O
274.2
593.6⁴⁺
761.7
845.1³⁺
942.3³⁺
200
400
600
m/z
800
1000
1200

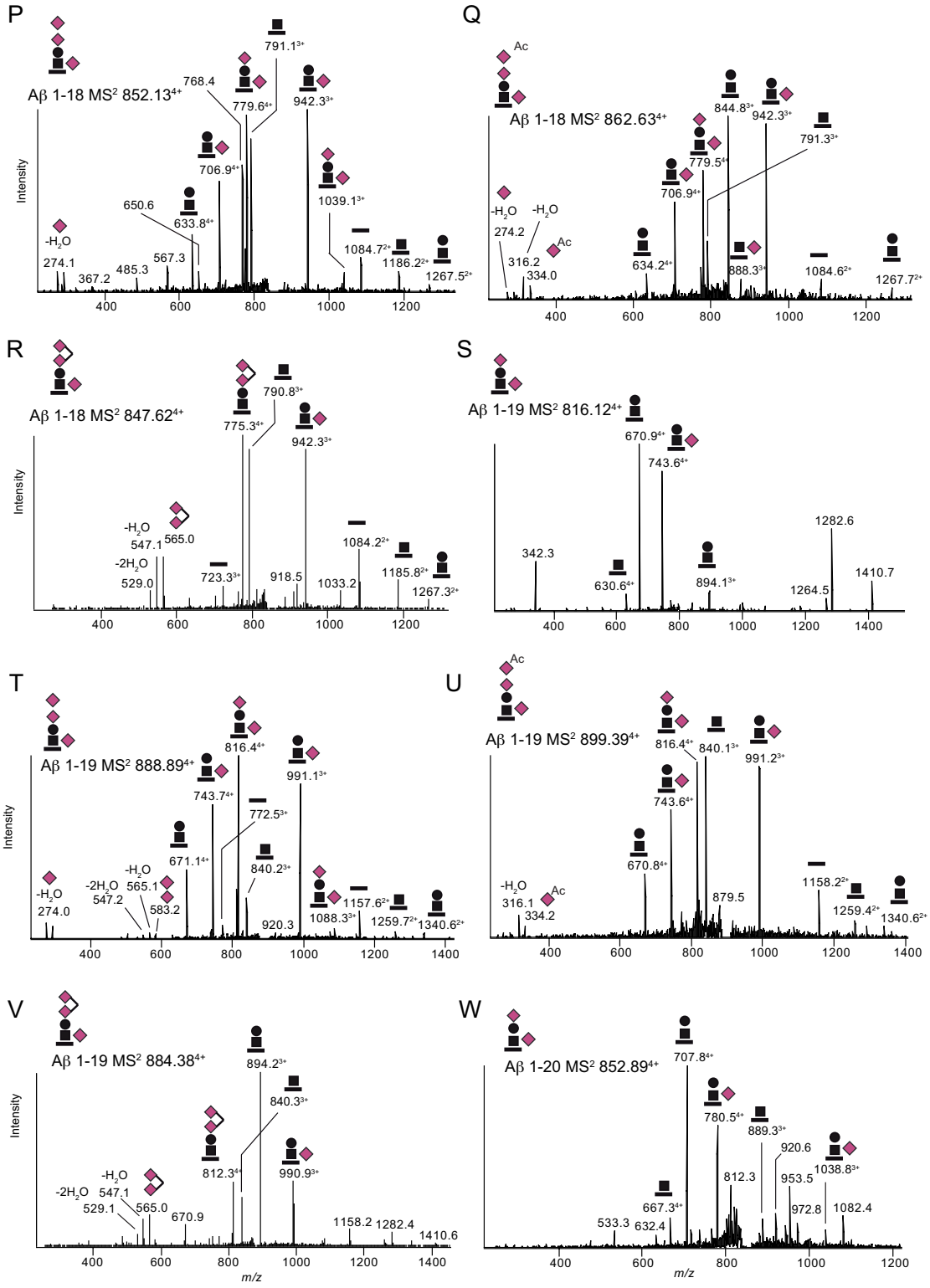
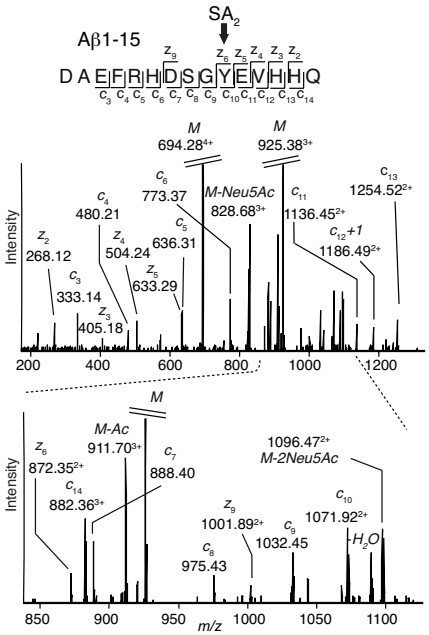


Fig. S3. CID MS^2 spectra of Aβ1-*X* glycopeptides. (**A**) MS^1 (left) and CID MS^2 (right) of SA-Aβ1-15. (**B**) CID MS^2 of SA_2-Aβ4-15. (**C**) CID MS^2 of lactonized SA_3-Aβ4-15. The presence of the peptide+Neu5AcHexNAc fragment ion at *m/*z 669.7 demonstrates that the disialosyl group was attached to the Hex rather than to the

HexNAc. (**D**) CID MS^2 spectrum of SA_3-Aβ1-16. (**E**) CID MS^2 spectrum of lactonized SA_3-Aβ 1-16. (**F**) CID MS^2 spectrum of SA_2-Aβ –3-15. (**G**) MS^2 spectrum of SA-Aβ1-17 (**H**) MS^2 spectrum of SA_2-Aβ1-17. This MS^2 spectrum was acquired in the ion cyclotron resonance (ICR) mode. The isotopic resolution of the fragment ions is demonstrated in the insert. The sensitivity in ICR mode is lower than for the linear quadrupole ion trap (LQIT) mode, which was used for the collection of most CID MS^2 spectra. (**I**) - (**W**) Additional CID MS^2 spectra of Aβ1-*X* glycopeptides. Ions are in the $[M + zH]^{z+}$ form and the charges are shown in superscript when z>1. The mass accuracy was <5 ppm for all precursor ions and their monoisotopic *m/z* values and charge (4+) are shown. The annotated *m/z* values of fragment ions are those picked by the Xcalibur program. The Neu5Ac oxonium ion and its loss of H_2O are present at *m/z* 292 and 274, respectively. The O-AcNeu5Ac oxonium ion and its loss of H_2O are present at *m/z* 334 and 316, respectively. The lactonized Neu5AcNeu5Ac oxonium ion and its loss of one and two H_2O are present at *m/z* 565, 547 and 529, respectively.

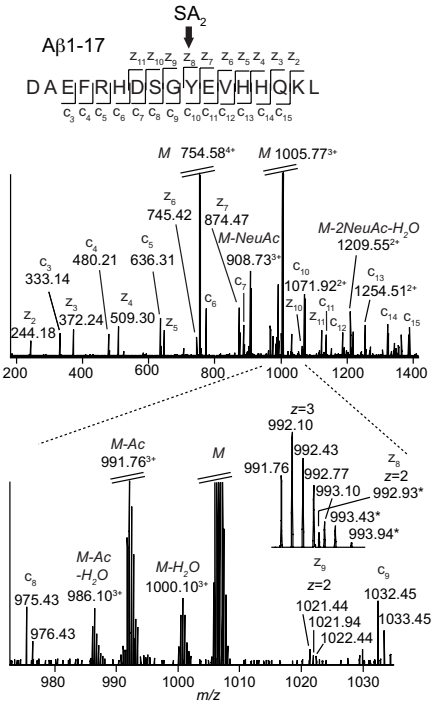
A



DAEFRHDSGY(947.3230)EVHHQ

c	c^{+2}				z	z^{+2}
---	---	1	**D**	15	---	---
204.0979	102.5526	2	**A**	14	2643.0614	1322.0344
333.1405	167.0739	3	**E**	13	2572.0243	1286.5158
480.2089	240.6081	4	**F**	12	2442.9817	1221.9945
636.3100	318.6586	5	**R**	11	2295.9133	1148.4603
773.3689	387.1881	6	**H**	10	2139.8122	1070.4097
888.3959	444.7016	7	**D**	9	2002.7533	1001.8803
975.4279	488.2176	8	**S**	8	1887.7263	944.3668
1032.4493	516.7283	9	**G**	7	1800.6943	900.8508
2142.8357	1071.9215	10	**Y(947.3230)**	6	1743.6729	872.3401
2271.8783	1136.4428	11	**E**	5	633.2865	317.1469
2370.9467	1185.9770	12	**V**	4	504.2439	252.6256
2508.0056	1254.5064	13	**H**	3	405.1755	203.0914
2645.0645	1323.0359	14	**H**	2	268.1166	134.5619
---	---	15	**Q**	1	131.0577	66.0325

B



DAEFRHDSGY(947.3230)EVHHQKL

c	c^{+2}				z	z^{+2}
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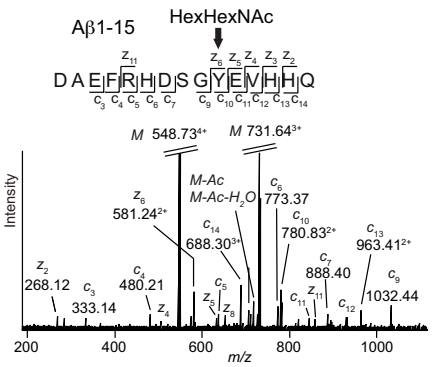
333.1405	167.0739	3	**E**	15	2813.2033	1407.1053
480.2089	240.6081	4	**F**	14	2684.1608	1342.5840
636.3100	318.6586	5	**R**	13	2537.0923	1269.0498
773.3689	387.1881	6	**H**	12	2380.9912	1190.9993
888.3959	444.7016	7	**D**	11	2243.9323	1122.4698
975.4279	488.2176	8	**S**	10	2128.9054	1064.9563
1032.4493	516.7283	9	**G**	9	2041.8733	1021.4403
2142.8357	1071.9215	10	**Y(947.3230)**	8	1984.8519	992.9296
2271.8783	1136.4428	11	**E**	7	874.4656	437.7364
2370.9467	1185.9770	12	**V**	6	745.4230	373.2151
2508.0056	1254.5064	13	**H**	5	646.3545	323.6809
2645.0645	1323.0359	14	**H**	4	509.2956	255.1515
2773.1231	1387.0652	15	**Q**	3	372.2367	186.6220
2901.2180	1451.1127	16	**K**	2	244.1781	122.5927
---	---	17	**L**	1	116.0832	58.5452

C



DAEFRHDSGY(365.1322)EVHHQ

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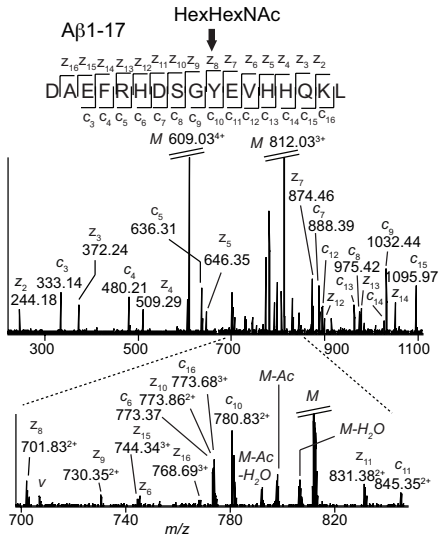
133.0608	67.0340	1	**D**	15	---	---
204.0979	102.5526	2	**A**	14	2060.8706	1030.9390
333.1405	167.0739	3	**E**	13	1989.8335	995.4204
480.2089	240.6081	4	**F**	12	1860.7909	930.8991
636.3100	318.6586	5	**R**	11	1713.7225	857.3649
773.3689	387.1881	6	**H**	10	1557.6214	779.3143
888.3959	444.7016	7	**D**	9	1420.5625	710.7849
975.4279	488.2176	8	**S**	8	1305.5355	653.2714
1032.4493	516.7283	9	**G**	7	1218.5035	609.7554
1560.6449	780.8261	10	**Y(365.1322)**	6	1161.4821	581.2447
1689.6875	845.3474	11	**E**	5	633.2865	317.1469
1788.7559	894.8816	12	**V**	4	504.2439	252.6256
1925.8148	963.4110	13	**H**	3	405.1755	203.0914
2062.8737	1031.9405	14	**H**	2	268.1166	134.5619
---	---	15	**Q**	1	131.0577	66.0325

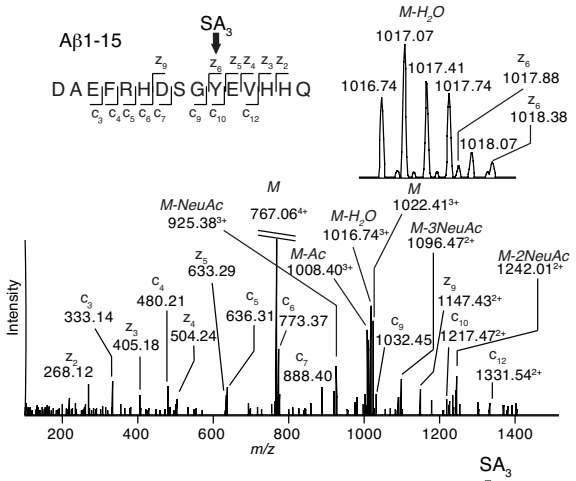
D



DAEFRHDSGY(365.1322)EVHHQKL

c	c^{+2}				z	z^{+2}
133.0608	67.0340	1	**D**	17	---	---
204.0979	102.5526	2	**A**	16	2302.0497	1151.5285
333.1405	167.0739	3	**E**	15	2231.0125	1116.0099
480.2089	240.6081	4	**F**	14	2101.9700	1051.4886
636.3100	318.6586	5	**R**	13	1954.9015	977.9544
773.3689	387.1881	6	**H**	12	1798.8004	899.9039
888.3959	444.7016	7	**D**	11	1661.7415	831.3744
975.4279	488.2176	8	**S**	10	1546.7146	773.8609
1032.4493	516.7283	9	**G**	9	1459.6825	730.3449
1560.6449	780.8261	10	**Y(365.1322)**	8	1402.6611	701.8342
1689.6875	845.3474	11	**E**	7	874.4656	437.7364
1788.7559	894.8816	12	**V**	6	745.4230	373.2151
1925.8148	963.4110	13	**H**	5	646.3545	323.6809
2062.8737	1031.9405	14	**H**	4	509.2956	255.1515
2190.9323	1095.9698	15	**Q**	3	372.2367	186.6220
2319.0272	1160.0173	16	**K**	2	244.1781	122.5927
---	---	17	**L**	1	116.0832	58.5452

E



DAEFRHDSGY(1238.4184)EVHHQ

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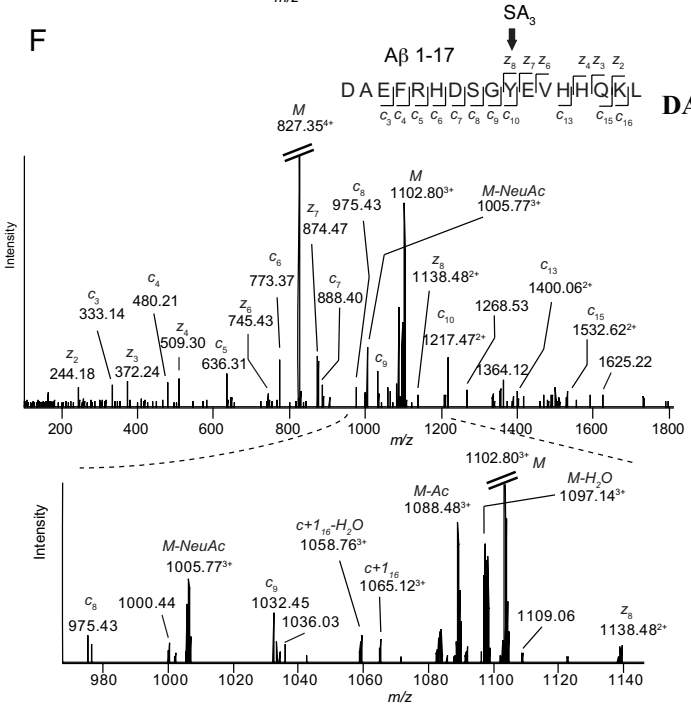
204.0979	102.5526	2	**A**	14	2934.1568	1467.5821
333.1405	167.0739	3	**E**	13	2863.1197	1432.0635
480.2089	240.6081	4	**F**	12	2734.0771	1367.5422
636.3100	318.6586	5	**R**	11	2587.0087	1294.0080
773.3689	387.1881	6	**H**	10	2430.9076	1215.9574
888.3959	444.7016	7	**D**	9	2293.8487	1147.4280
975.4279	488.2176	8	**S**	8	2178.8217	1089.9145
1032.4493	516.7283	9	**G**	7	2091.7897	1046.3985
2433.9311	1217.4692	10	**Y(1238.4184)**	6	2034.7683	1017.8878
2562.9737	1281.9905	11	**E**	5	633.2865	317.1469
2662.0421	1331.5247	12	**V**	4	504.2439	252.6256
2799.1010	1400.0541	13	**H**	3	405.1755	203.0914
2936.1599	1468.5836	14	**H**	2	268.1166	134.5619
---	---	15	**Q**	1	131.0577	66.0325

F



DAEFRHDSGY(1238.4184)EVHHQKL

c	c^{+2}				z	z^{+2}
---	---	1	**D**	17	---	---
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333.1405	167.0739	3	**E**	15	3104.2987	1552.6530
480.2089	240.6081	4	**F**	14	2975.2562	1488.1317
636.3100	318.6586	5	**R**	13	2828.1877	1414.5975
773.3689	387.1881	6	**H**	12	2672.0866	1336.5470
888.3959	444.7016	7	**D**	11	2535.0277	1268.0175
975.4279	488.2176	8	**S**	10	2420.0008	1210.5040
1032.4493	516.7283	9	**G**	9	2332.9687	1166.9880
2433.9311	1217.4692	10	**Y(1238.4184)**	8	2275.9473	1138.4773
2562.9737	1281.9905	11	**E**	7	874.4656	437.7364
2662.0421	1331.5247	12	**V**	6	745.4230	373.2151
2799.1010	1400.0541	13	**H**	5	646.3545	323.6809
2936.1599	1468.5836	14	**H**	4	509.2956	255.1515
3064.2185	1532.6129	15	**Q**	3	372.2367	186.6220
3192.3134	1596.6604	16	**K**	2	244.1781	122.5927
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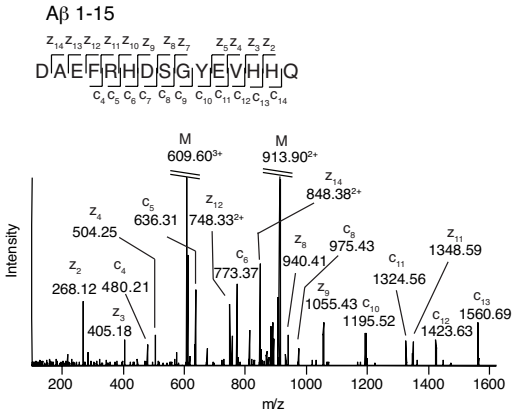
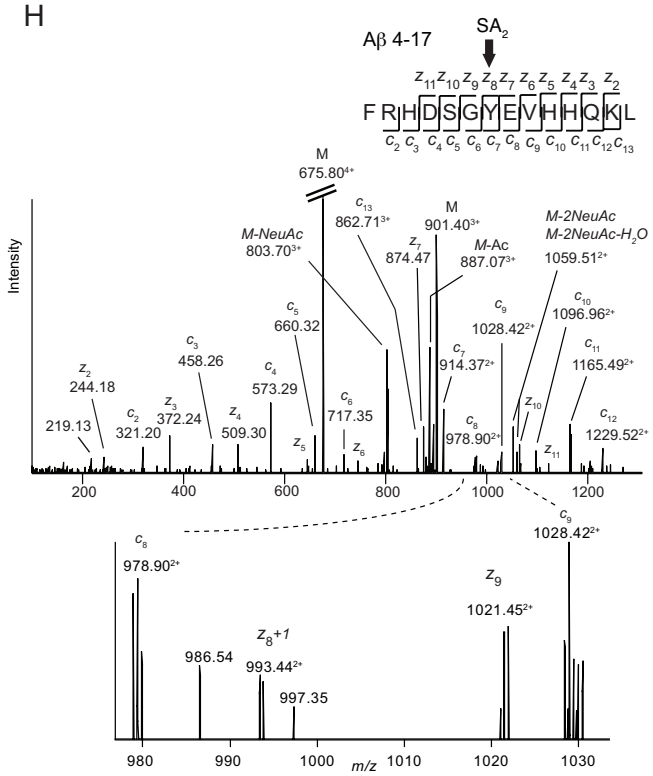
G



DAEFRHDSGYEVHHQ

c	c^{+2}				z	z^{+2}
---	---	1	**D**	15	---	---
204.0979	102.5526	2	**A**	14	1695.7384	848.3729
333.1405	167.0739	3	**E**	13	1624.7013	812.8543
480.2089	240.6081	4	**F**	12	1495.6587	748.3330
636.3100	318.6586	5	**R**	11	1348.5903	674.7988
773.3689	387.1881	6	**H**	10	1192.4892	596.7482
888.3959	444.7016	7	**D**	9	1055.4303	528.2188
975.4279	488.2176	8	**S**	8	940.4033	470.7053
1032.4493	516.7283	9	**G**	7	853.3713	427.1893
1195.5127	598.2600	10	**Y**	6	796.3499	398.6786
1324.5553	662.7813	11	**E**	5	633.2865	317.1469
1423.6237	712.3155	12	**V**	4	504.2439	252.6256
1560.6826	780.8449	13	**H**	3	405.1755	203.0914
1697.7415	849.3744	14	**H**	2	268.1166	134.5619
---	---	15	**Q**	1	131.0577	66.0325

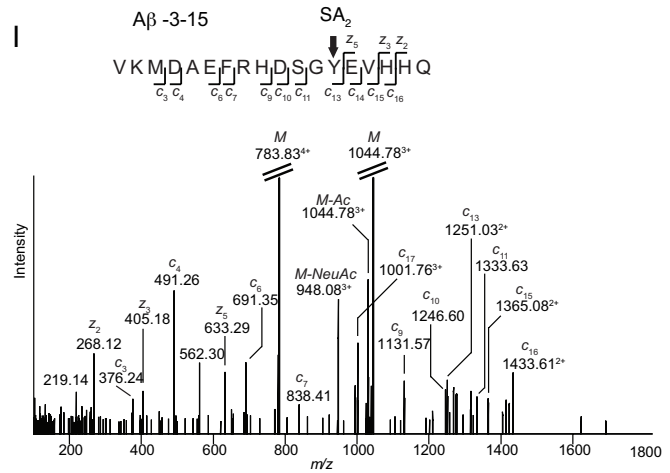
H



FRHDSGY(947.3230)EVHHQKL

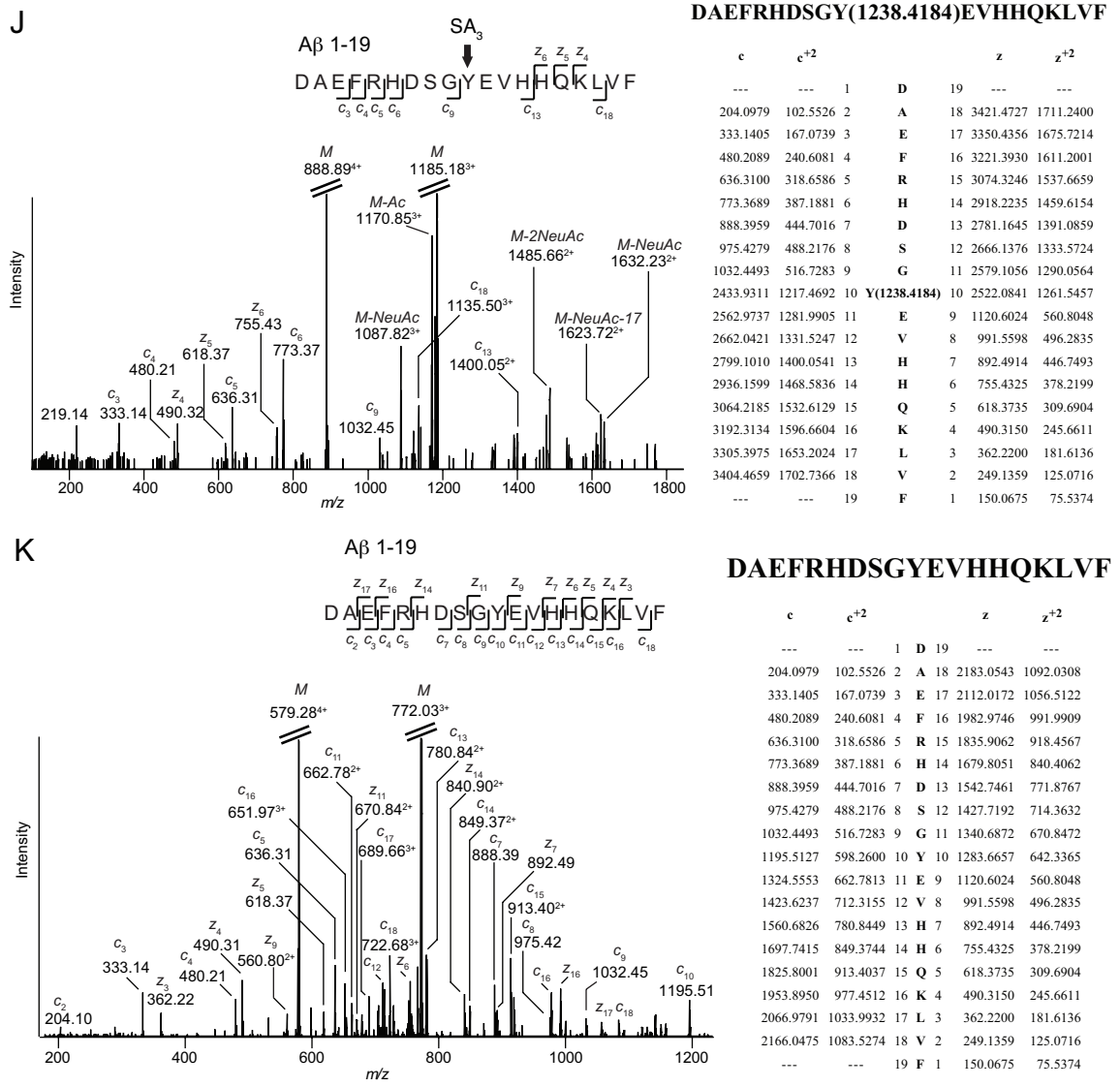
c	c^{+2}				z	z^{+2}
---	---	1	**F**	14	---	---
321.2034	161.1053	2	**R**	13	2537.0923	1269.0498
458.2623	229.6348	3	**H**	12	2380.9912	1190.9993
573.2892	287.1482	4	**D**	11	2243.9323	1122.4698
660.3212	330.6643	5	**S**	10	2128.9054	1064.9563
717.3427	359.1750	6	**G**	9	2041.8733	1021.4403
1827.7290	914.3682	7	**Y(947.3230)**	8	1984.8519	992.9296
1956.7716	978.8894	8	**E**	7	874.4656	437.7364
2055.8400	1028.4237	9	**V**	6	745.4230	373.2151
2192.8989	1096.9531	10	**H**	5	646.3545	323.6809
2329.9579	1165.4826	11	**H**	4	509.2956	255.1515
2458.0164	1229.5119	12	**Q**	3	372.2367	186.6220
2586.1114	1293.5593	13	**K**	2	244.1781	122.5927
---	---	14	**L**	1	116.0832	58.5452

I



VKMDAEFRHDSGY(947.3230)EVHHQ

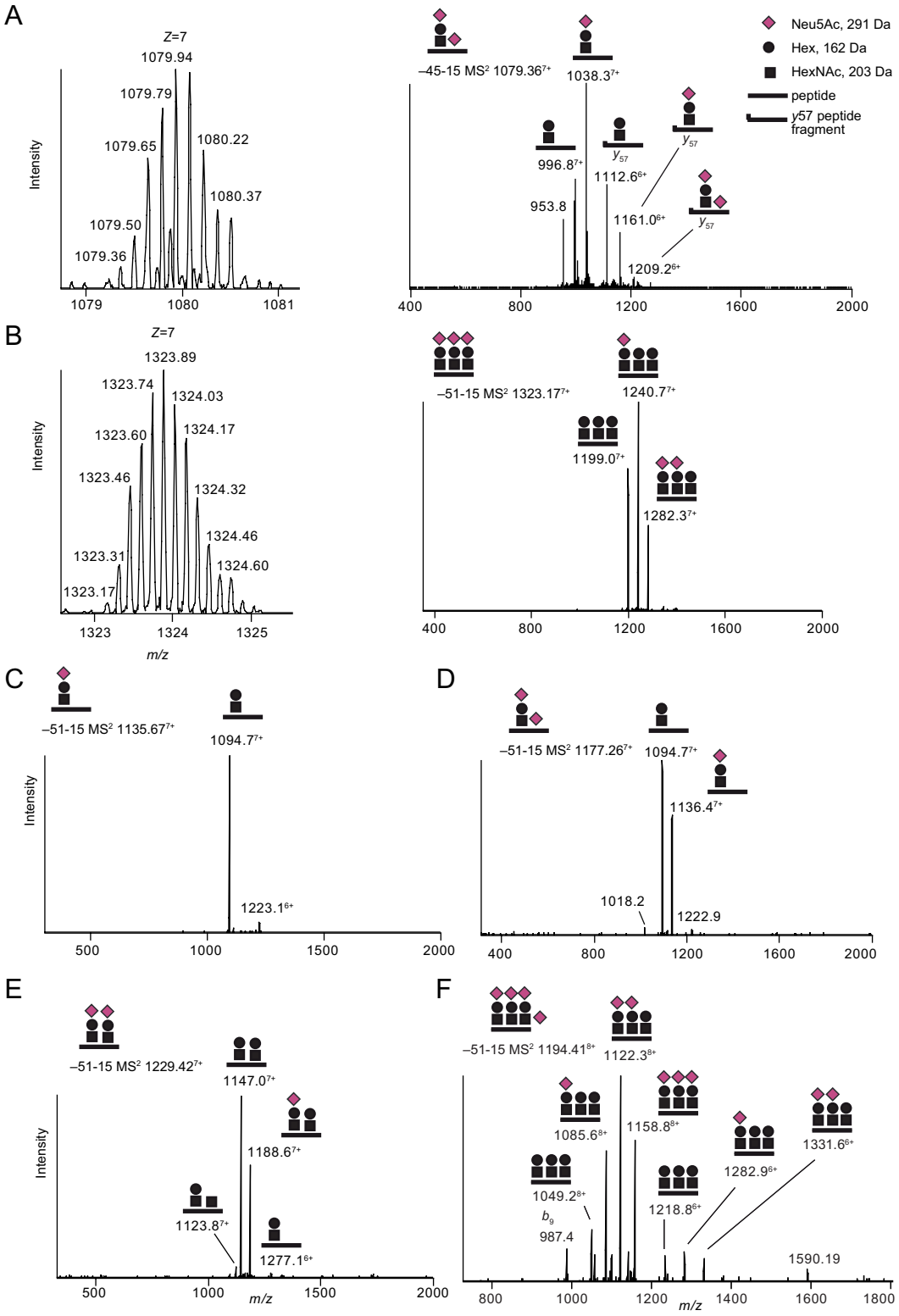
c	c^{+2}				z	z^{+2}
---	---	1	**V**	18	---	---
245.1972	123.1022	2	**K**	17	3017.2238	1509.1155
376.2377	188.6225	3	**M**	16	2889.1289	1445.0681
491.2646	246.1360	4	**D**	15	2758.0884	1379.5478
562.3017	281.6545	5	**A**	14	2643.0614	1322.0344
691.3443	346.1758	6	**E**	13	2572.0243	1286.5158
838.4128	419.7100	7	**F**	12	2442.9817	1221.9945
994.5139	497.7606	8	**R**	11	2295.9133	1148.4603
1131.5728	566.2900	9	**H**	10	2139.8122	1070.4097
1246.5997	623.8035	10	**D**	9	2002.7533	1001.8803
1333.6317	667.3195	11	**S**	8	1887.7263	944.3668
1390.6532	695.8302	12	**G**	7	1800.6943	900.8508
2501.0395	1251.0234	13	**Y(947.3230)**	6	1743.6729	872.3401
2630.0821	1315.5447	14	**E**	5	633.2865	317.1469
2729.1505	1365.0789	15	**V**	4	504.2439	252.6256
2866.2095	1433.6084	16	**H**	3	405.1755	203.0914
3003.2684	1502.1378	17	**H**	2	268.1166	134.5619
---	---	18	**Q**	1	131.0577	66.0325



c	c^{+2}				z	z^{+2}
---	---	1	**D**	19	---	---
204.0979	102.5526	2	**A**	18	3421.4727	1711.2400
333.1405	167.0739	3	**E**	17	3350.4356	1675.7214
480.2089	240.6081	4	**F**	16	3221.3930	1611.2001
636.3100	318.6586	5	**R**	15	3074.3246	1537.6659
773.3689	387.1881	6	**H**	14	2918.2235	1459.6154
888.3959	444.7016	7	**D**	13	2781.1645	1391.0859
975.4279	488.2176	8	**S**	12	2666.1376	1333.5724
1032.4493	516.7283	9	**G**	11	2579.1056	1290.0564
2433.9311	1217.4692	10	**Y(1238.4184)**	10	2522.0841	1261.5457
2562.9737	1281.9905	11	**E**	9	1120.6024	560.8048
2662.0421	1331.5247	12	**V**	8	991.5598	496.2835
2799.1010	1400.0541	13	**H**	7	892.4914	446.7493
2936.1599	1468.5836	14	**H**	6	755.4325	378.2199
3064.2185	1532.6129	15	**Q**	5	618.3735	309.6904
3192.3134	1596.6604	16	**K**	4	490.3150	245.6611
3305.3975	1653.2024	17	**L**	3	362.2200	181.6136
3404.4659	1702.7366	18	**V**	2	249.1359	125.0716
---	---	19	**F**	1	150.0675	75.5374

c	c^{+2}				z	z^{+2}
---	---	1	**D**	19	---	---
204.0979	102.5526	2	**A**	18	2183.0543	1092.0308
333.1405	167.0739	3	**E**	17	2112.0172	1056.5122
480.2089	240.6081	4	**F**	16	1982.9746	991.9909
636.3100	318.6586	5	**R**	15	1835.9062	918.4567
773.3689	387.1881	6	**H**	14	1679.8051	840.4062
888.3959	444.7016	7	**D**	13	1542.7461	771.8767
975.4279	488.2176	8	**S**	12	1427.7192	714.3632
1032.4493	516.7283	9	**G**	11	1340.6872	670.8472
1195.5127	598.2600	10	**Y**	10	1283.6657	642.3365
1324.5553	662.7813	11	**E**	9	1120.6024	560.8048
1423.6237	712.3155	12	**V**	8	991.5598	496.2835
1560.6826	780.8449	13	**H**	7	892.4914	446.7493
1697.7415	849.3744	14	**H**	6	755.4325	378.2199
1825.8001	913.4037	15	**Q**	5	618.3735	309.6904
1953.8950	977.4512	16	**K**	4	490.3150	245.6611
2066.9791	1033.9932	17	**L**	3	362.2200	181.6136
2166.0475	1083.5274	18	**V**	2	249.1359	125.0716
---	---	19	**F**	1	150.0675	75.5374

Fig. S4. ECD MS^2 spectra of Aβ1-X glycopeptides (**A**)-(**D**) Fully *m/z* annotated ECD spectra and lists of *c*- and *z*-ions accompanying **Fig. 2** of the main text. (**E**) ECD MS^2 spectrum of SA_3-Aβ1-15. The presence of a glycosylated *z*6 peak is shown in the insert. (**F**) ECD MS^2 spectrum of SA_3-Aβ1-17. The presence of a glycosylated *z*8 peak is shown in the expansion. (**G**) ECD MS^2 spectrum of Aβ1-15 peptide. (**H**) ECD MS^2 spectrum of SA_2-Aβ4-17. The presence of glycosylated *z*8 and *z*9 peaks is shown in the expansion (**I**) ECD MS^2 spectrum of SA_2-Aβ–3-15. (**J**) ECD MS^2 spectrum of SA_3-Aβ1-19. (**K**) ECD MS^2 spectrum of Aβ1-19 peptide. The lists of *c*- and *z*-series of peptide backbone fragments were prepared by the use of the MS product utility at the Protein Prospector homepage (http://prospector.ucsf.edu/prospector/cgi-bin/msform.cgi?form=msproduct). The identified fragment ions are annotated in the spectra, and shown in relation to the peptide sequences. The molecular ion and its charged reduced forms (*M*) are present in the spectra. *M-Ac*, loss of acetyl from *M*. The peaks were expanded and the *M* peaks were cropped.


A
Z=7
1079.94
1079.79
1079.65
1080.22
1080.37
1079.50
1079.36
Intensity
1079
1080
1081
–45-15 MS² 1079.36⁷⁺
1038.3⁷⁺
996.8⁷⁺
953.8
1112.6⁶⁺
1161.0⁶⁺
1209.2⁶⁺
y57
400
800
1200
1600
2000
Neu5Ac, 291 Da
Hex, 162 Da
HexNAc, 203 Da
peptide
y57 peptide fragment
B
Z=7
1323.89
1323.74
1324.03
1323.60
1324.17
1323.46
1324.32
1324.46
1323.31
1324.60
1323.17
Intensity
1323
1324
1325
m/z
–51-15 MS² 1323.17⁷⁺
1240.7⁷⁺
1199.0⁷⁺
1282.3⁷⁺
C
–51-15 MS² 1135.67⁷⁺
1094.7⁷⁺
Intensity
1223.1⁶⁺
500
1000
1500
2000
D
–51-15 MS² 1177.26⁷⁺
1094.7⁷⁺
1136.4⁷⁺
1018.2
1222.9
E
–51-15 MS² 1229.42⁷⁺
1147.0⁷⁺
1188.6⁷⁺
1123.8⁷⁺
1277.1⁶⁺
Intensity
m/z
F
–51-15 MS² 1194.41⁸⁺
1122.3⁸⁺
1085.6⁸⁺
1158.8⁸⁺
1331.6⁶⁺
1282.9⁶⁺
1049.2⁸⁺
b9
987.4
1218.8⁶⁺
1590.19
800
1000
1200
1400
1600
1800
m/z

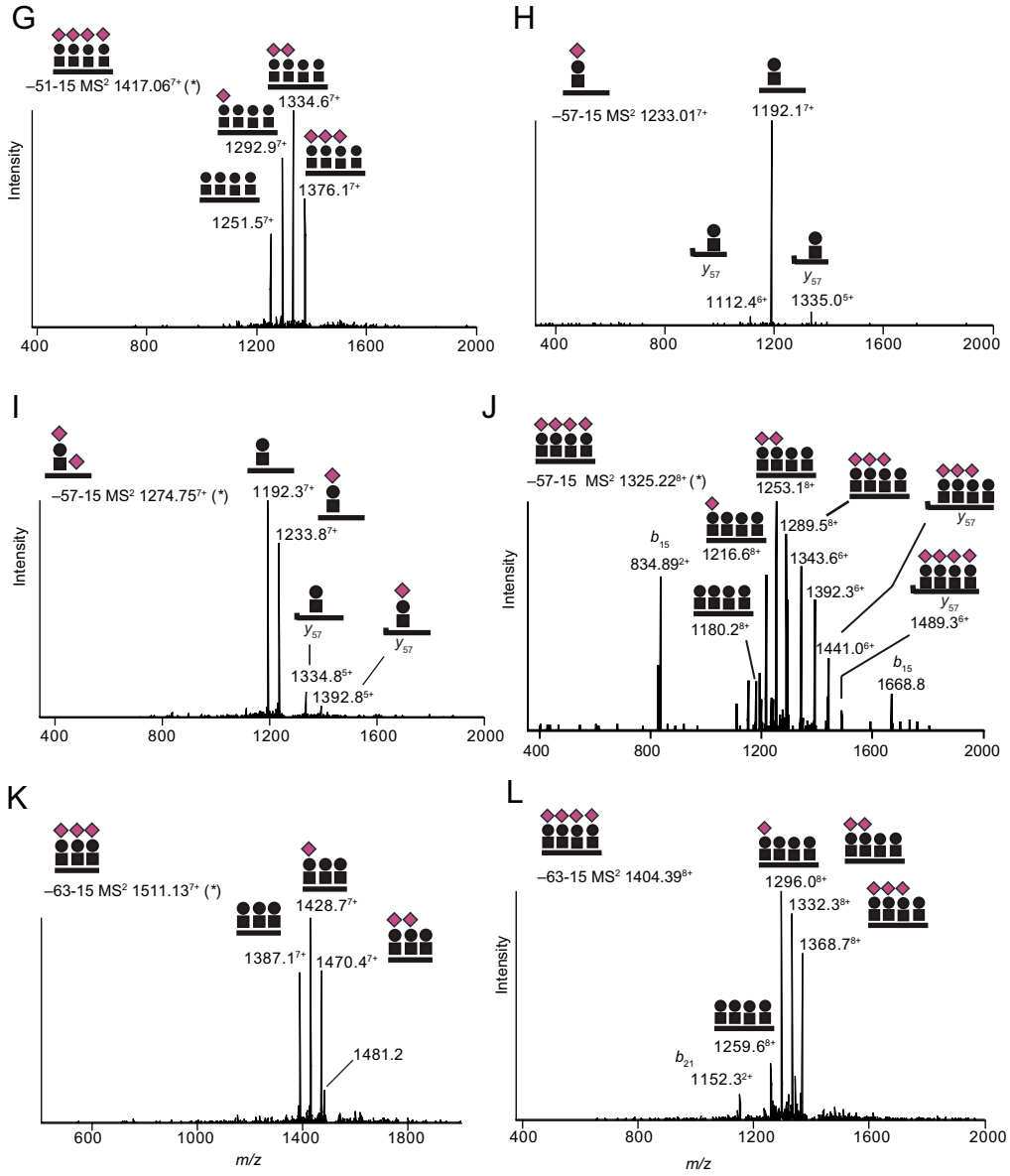


Fig. S5. CID MS^2 of additional APP/Aβ*X*-15 glycopeptides. (**A**) MS^1 and CID MS^2 of Neu5AcHex(Neu5Ac)HexNAc APP/Aβ–45-15 showing the diagnostic *y*57 peptide fragment peaks. (**B**) MS^1 and CID MS^2 of $(Neu5AcHexHexNAc)_3$ APP/Aβ–51-15. The same glycopeptide possessing different charges, are fragmented here **(**z = 7) and in **Fig. 4C** of the main text (z = 8). The diagnostic *b*9 and *y57* ion peaks were only visible for z = 8, and were generally more pronounced at the higher charge state of the APP/Aβ*X*-15 glycopeptides. (**C**) - (**L**) CID MS^2 spectra of additional APP/Aβ*X*-15 glycopeptides. *The MS^1 peaks for the monoisotopic *m/z* value were not visible and the measured *m/z* values for the second isotopes are given instead.

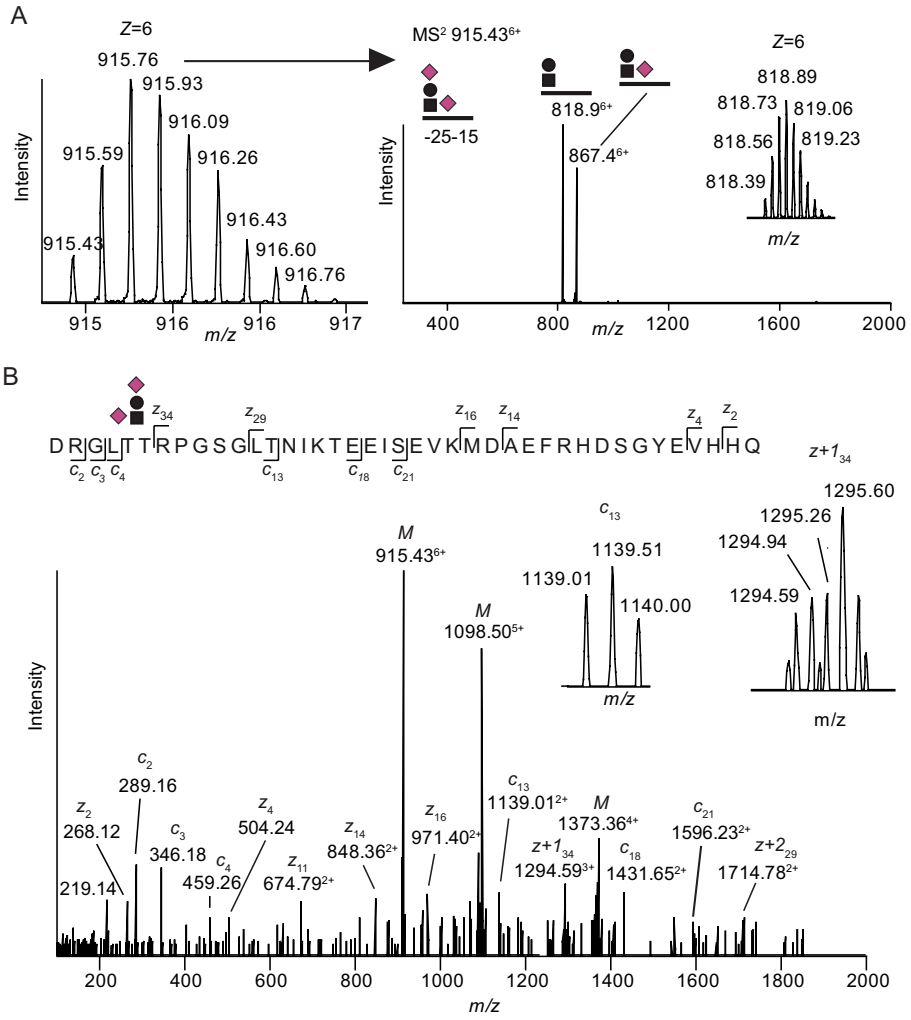


Fig. S6. CID and ECD MS^2 of Neu5AcHex(Neu5Ac)HexNAc-APP/Aβ–25-15 (**A**) MS^1 (left) and CID MS^2 (right) and (**B**) ECD MS^2 spectrum of Neu5AcHex(Neu5Ac)HexNAc - APP/Aβ–25-15. CID MS^2 was acquired in ICR mode. Isotopic distributions of fragment ions are demonstrated in the inserts supporting the evidence of significant fragments. Selected *c*- and *z*-fragments are annotated and shown in relation to the peptide sequence. The attachment site for the glycan was either Thr(–21) or Thr(–20).

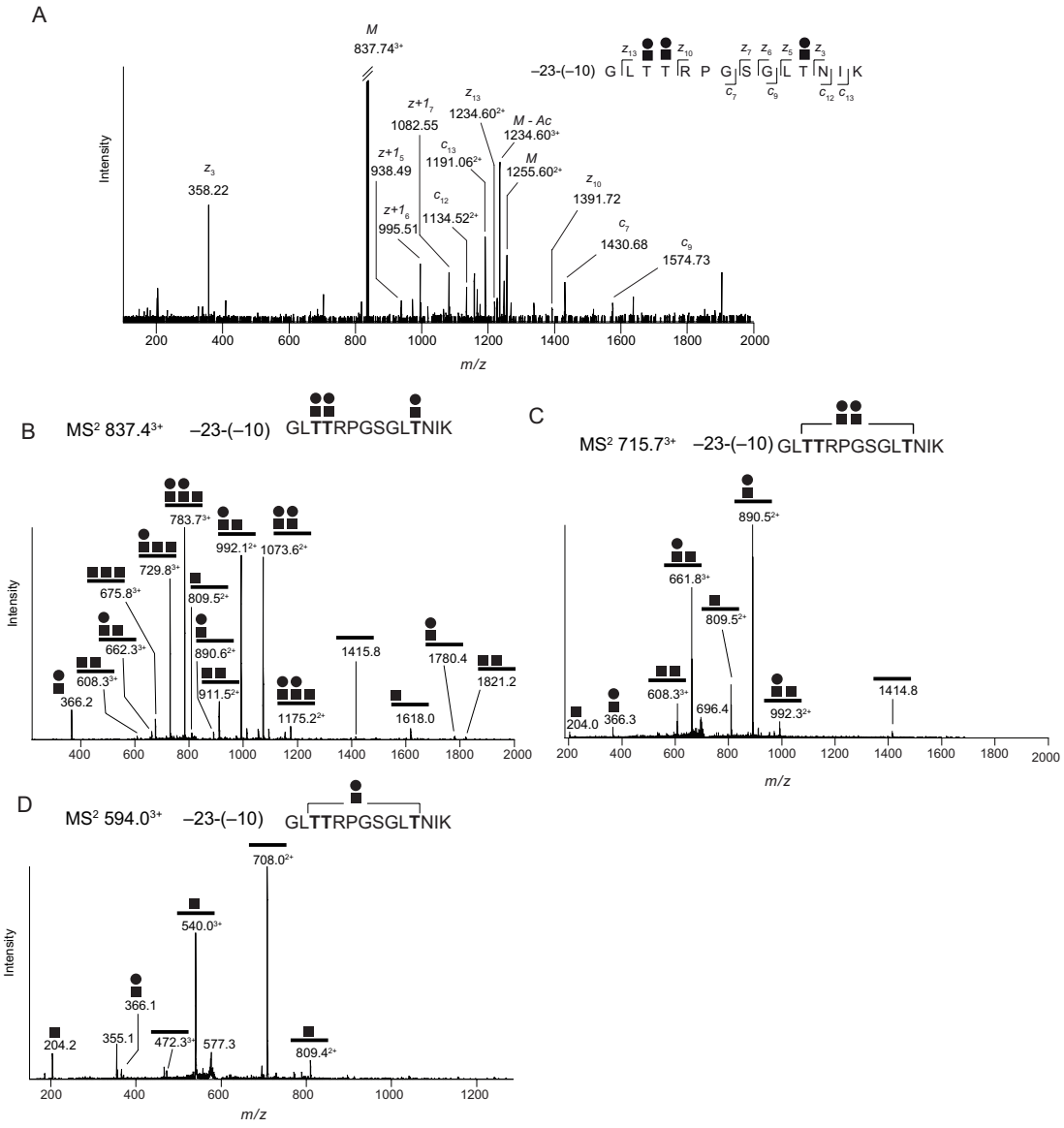


Fig. S7. **Identification of Thr (–21, –20 and –13) glycosylation sites.** (**A)** ECD spectrum of the –23-(–10) glycopeptide with three HexHexNAc glycans at Thr (–21, –20 and –13). The amino acid numbering is according to the Aβ sequence. Ser(–16) was excluded as a glycosylation site in this glycopeptide. Sialic acids were originally present on the glycopeptides but were removed as a part of the enrichment protocol. (**B**) CID MS^2 spectrum of the same –23-(–10) glycopeptide as in (**A)**. (**C**) CID MS^2 of the –23-(–10) glycopeptide with two HexHexNAc glycans. (**D**) CID MS^2 of the –23-(–10) glycopeptide with one HexHexNAc glycan. The peptide ion peak in the MS^2 spectra (*m/z* 708.0) gave peptide fragmentation in the MS^3 spectrum, which was used to determine the peptide identity by Mascot database searching (**See Table S2**).

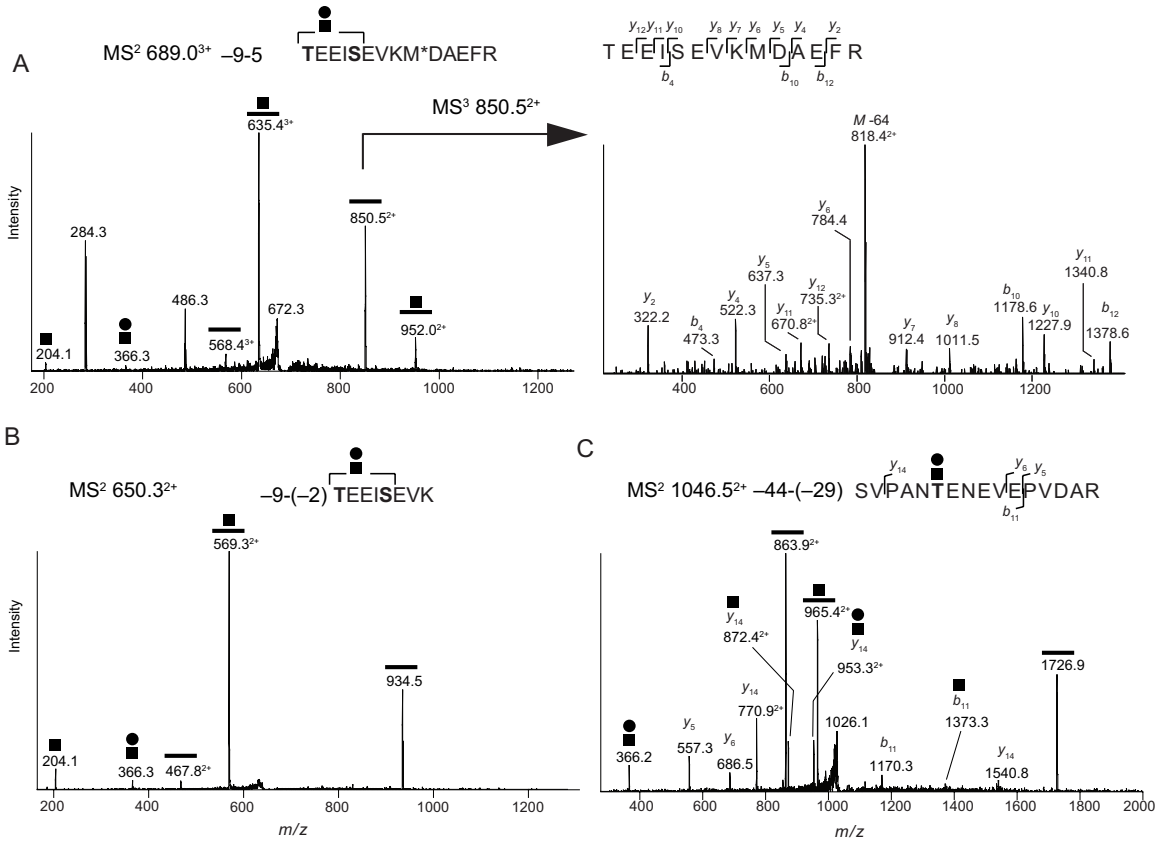


Fig. S8. **Identification of Thr (–39) and Ser(–5) / Thr(–9) glycosylation sites.** (**A**) CID MS^2 of the –9-5 glycopeptide with one HexHexNAc glycan (left). The glycosylation site was either Ser(–5) or Thr(–9). The deglycosylated peptide ion (*m/z* 850.5) present in the MS^2 spectrum gave peptide fragmentation in the MS^3 spectrum (right), which was subjected to Mascot database searching to determine the glycopeptide identity (**see Table S2**). *The methionine was oxidized (+16 Da) resulting in a characteristic neutral loss of 64 Da from the oxidized Met in the CID MS^3 spectrum (*M-64*). (**B**) CID MS^2 of the –9-(–2) glycopeptide carrying one HexHexNAc glycan on either Ser(–5) or Thr(–9). (**C**) CID MS^2 of the –44-(–29) glycopeptide carrying one HexHexNAc glycan. The presence of glycosylated *y*14 peptide fragments in the MS^2 spectrum pinpointed the glycosylation site to Thr(–39). The *y*14 fragments represent the same fragmentation site as for the prominent *y*57 fragment ions for the APP/Aβ*X*-15 glycopeptides (see **Fig. 4** and **Fig. S5**).

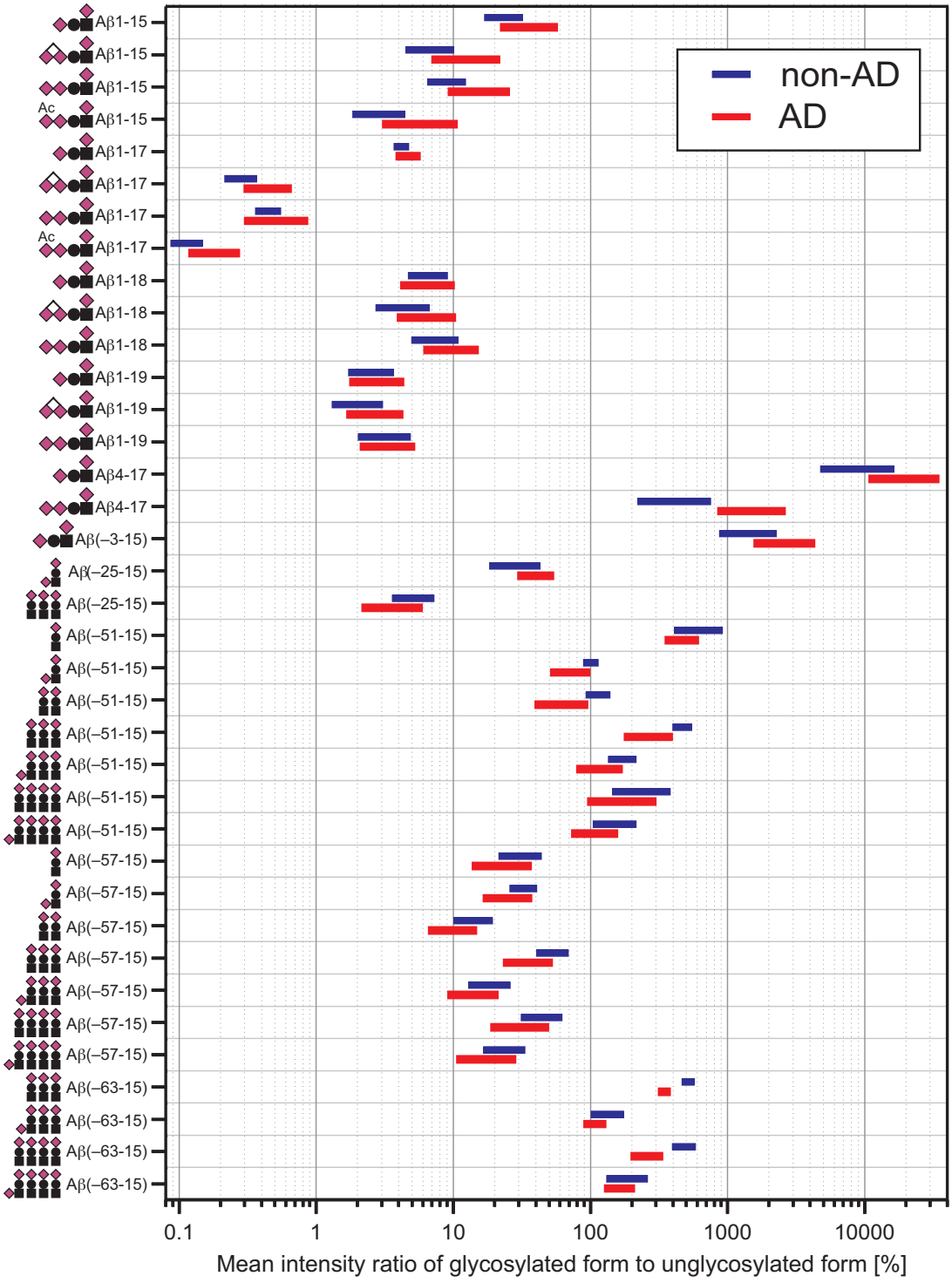


Fig. S9. **Mean intensity ratios of APP/Aβ glycopeptides versus the corresponding unglycosylated peptides for AD and non-AD patients**. A mean ratio of 1 was set to 100% and the extensions of the bars represent one standard deviation around the mean. The ratios were generally higher for AD compared to non-AD for most of the Aβ1-*X* peptides, while the opposite was observed for most of the APP/Aβ *X*-15 peptides.

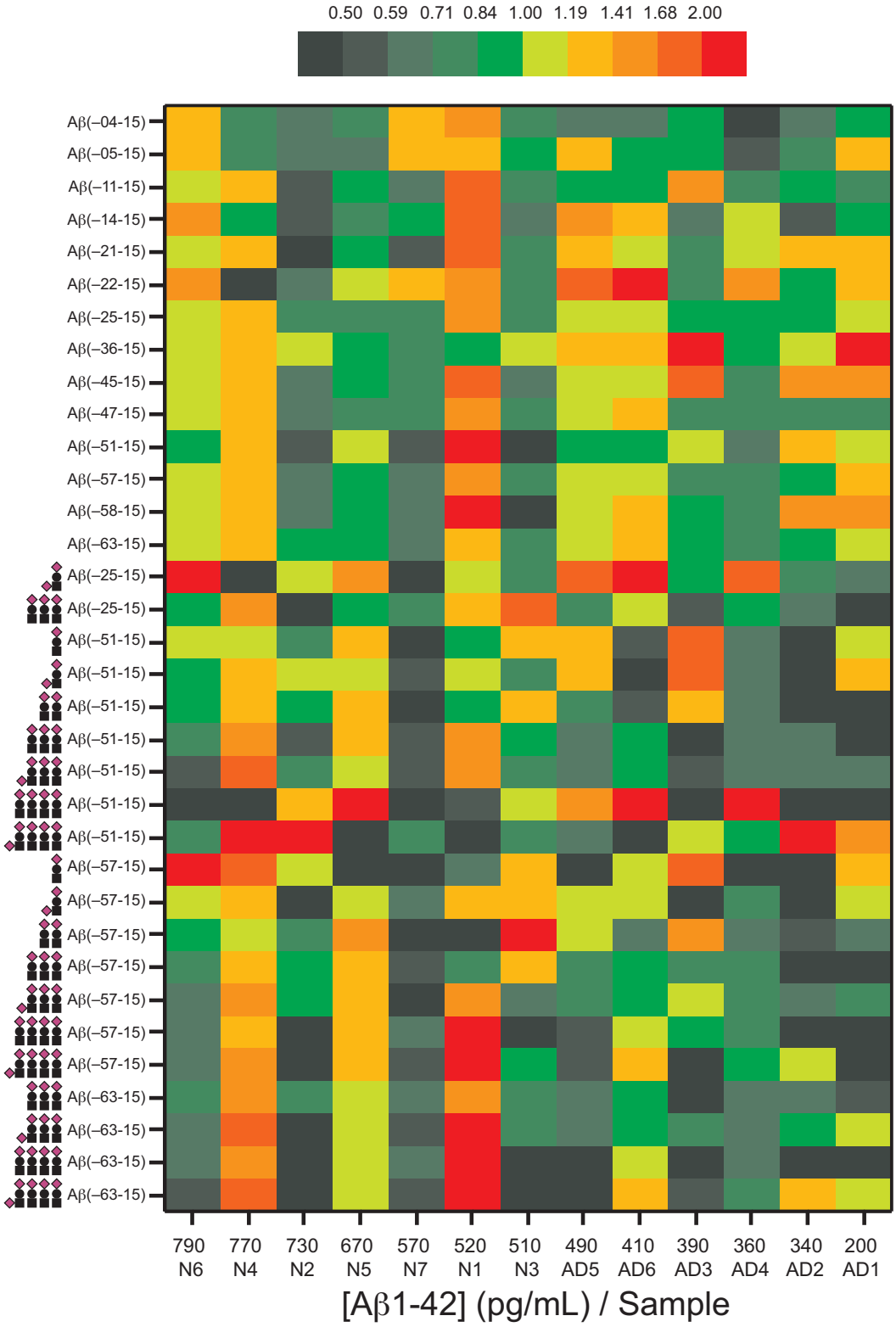


Fig. S10. **Heat map showing the relative signal intensities of individual APP/Aβ*X*-15 peptides and glycopeptides for AD and non-AD patients.** Data were normalized in the same way as in **Fig. 3** of the main text (see also **Fig. S9** and **Table S1**).

Table S1. Structures and relative intensities of 6E10 immunopurified APP/Aβ peptides and glycopeptides of human CSF samples. The glycosylated/unglycosylated spectral intensity ratios for Aβ peptides and glycopeptides from AD versus non-AD patients (nonAD) are shown.

Peptide/Glycopeptide[a]	Calculated monoisotopic mass (Da)	MS^2 data[b]	AD vs. C (mean C set to 1)[c]				Mean intensity (log_{10})[d]
			Mean nonAD	Std dev. non-AD	Mean AD	Std dev. AD	
Aβ1-13	1560.6593	Y	1.0	0.2	0.7	0.2	5.3
Aβ1-14	1697.7182	Y	1.0	0.1	0.9	0.1	5.9
Aβ1-15	1825.7768	S4G	1.0	0.2	0.9	0.2	6.0
SaHHn-Aβ1-15	2482.0044	S3A	1.0	0.3	1.1	0.5	3.6
Sa_2HHn-Aβ1-15	2773.0999	F1A, F2A	1.0	0.1	1.4	0.1	5.1
$LaSa_2$SaHHn-Aβ1-15	3046.1847	F1D	1.0	0.2	1.9	0.1	4.4
Sa_3HHn-Aβ1-15	3064.1953	F1B, S4E	1.0	0.1	1.6	0.0	4.7
Ac-Sa_3HHn-Aβ1-15	3106.2058	F1C	1.0	0.3	2.3	0.3	4.0
Aβ1-16	1953.8718	Y	1.0	0.2	1.1	0.2	5.3
$LaSa_2$SaHHn-Aβ1-16	3174.2797	S3E	Below threshold				<3.0
Sa_3HHn-Aβ1-16	3192.2902	S3D	Below threshold				<3.0
Aβ1-17	2066.9559	Y	1.0	0.1	1.0	0.1	7.3
SaHHn-Aβ1-17	2723.1835	S3G	1.0	0.4	1.1	0.3	4.6
Sa_2HHn-Aβ1-17	3014.2789	F2B, S3H	1.0	0.1	1.2	0.1	5.8
$LaSa_2$SaHHn-Aβ1-17	3287.3637	S3K	1.0	0.2	1.6	0.1	4.5
Sa_3HHn-Aβ1-17	3305.3743	S3I, S4F	1.0	0.1	1.2	0.3	4.7
Ac-Sa_3HHn-Aβ1-17	3347.3849	S3J	1.0	0.2	1.5	0.1	4.1
Aβ1-18	2166.0243	Y	1.0	0.2	1.0	0.1	6.0
Sa_2HHn-Aβ1-18	3113.3473	S3O	1.0	0.1	1.1	0.1	4.4
$LaSa_2$SaHHn-Aβ1-18	3386.4321	S3R	1.0	0.3	2.5	0.5	4.1
Sa_3HHn-Aβ1-18	3404.4427	S3P	1.0	0.2	1.4	0.4	4.5
Ac-Sa_3HHn-Aβ1-18	3446.4533	S3Q	1.0	0.2	1.3	0.5	3.2
Aβ1-19	2313.0927	S4K	1.0	0.1	1.2	0.1	7.3
Sa_2HHn-Aβ1-19	3260.4157	S3S	1.0	0.1	1.4	0.2	5.3
$LaSa_2$SaHHn-Aβ1-19	3533.5006	S3V	1.0	0.4	2.5	0.6	5.2
Sa_3HHn-Aβ1-19	3551.5111	S3T, S4J	1.0	0.2	1.9	0.4	5.4
Ac-Sa_3HHn-Aβ1-19	3593.5217	S3U	1.0	0.2	1.4	0.3	4.2
Aβ1-20	2460.1611	Y	1.0	0.2	1.0	0.2	5.9
Sa_2HHn-Aβ1-20	3407.4841	S3W	1.0	0.5	0.8	0.4	3.9
Aβ1-28	3260.5275	Y	1.0	0.3	0.5	0.2	6.3
Aβ1-30	3388.5861	Y	1.0	0.1	1.1	0.1	6.7
Aβ1-33	3671.7757	Y	1.0	0.1	1.0	0.1	7.5
Aβ1-34	3784.8598	Y	1.0	0.1	0.8	0.1	7.6
Aβ1-37	4071.9901	Y	1.0	0.0	1.0	0.0	7.9
Aβ1-38	4129.0116	Y	1.0	0.0	1.0	0.0	8.6
Aβ1-39	4228.0800	Y	1.0	0.0	1.2	0.1	7.8
Aβ1-40	4327.1484	Y	1.0	0.0	1.0	0.0	8.8

Aβ1-42	4511.2696	Y	1.0	0.2	0.5	0.1	6.6
Aβ4-15	1510.6702	N	Below threshold				<3.0
Sa_2HHn-Aβ4-15	2457.9932	S3B	1.0	0.4	2.2	0.9	4.0
LaSa_2SaHHn-Aβ4-15	2731.0781	S3C	Below threshold				<3.0
Aβ4-17	1751.8492	N	1.0	0.2	0.9	0.1	4.3
Sa_2HHn-Aβ4-17	2699.1722	S3L, S4H	1.0	0.1	1.4	0.1	5.3
Sa_3HHn-Aβ4-17	2990.2676	S3M	1.0	0.1	2.1	0.1	4.1
Aβ5-17	1604.7808	N	1.0	0.2	0.6	0.1	4.3
Sa_2HHn-Aβ5-17	2552.1038	S3N	Below threshold				<3.0
Aβ(–3-15)	2183.9812	N	1.0	0.4	0.4	0.2	3.6
Sa_2HHn-Aβ(–3-15)	3131.3043	S3F, S4I	1.0	0.1	1.1	0.2	4.4
Aβ(–4-15)	2313.0238	Y	1.0	0.2	0.7	0.1	5.2
Aβ(–5-15)	2400.0558	Y	1.0	0.1	1.0	0.1	5.5
Aβ(–11-15)	3113.4518	Y	1.0	0.2	1.0	0.1	5.9
Aβ(–14-15)	3441.6264	Y	1.0	0.2	1.0	0.2	5.5
Aβ(–21-15)	4097.9506	Y	1.0	0.2	1.1	0.1	6.0
Aβ(–22-15)	4211.0347	Y	1.0	0.2	1.4	0.2	6.4
Aβ(–25-15)	4539.1842	Y	1.0	0.1	1.0	0.1	7.3
SaHHn-Aβ(–25-15)	5195.4118	N	1.0	0.1	1.0	0.2	6.3
Sa_2HHn-Aβ(–25-15)	5486.5073	S6	1.0	0.3	1.4	0.3	6.8
$(SaHHn)_3$-Aβ(–25-15)	6507.8671	F4B	1.0	0.2	0.7	0.2	5.8
Aβ(–45-15)	6601.1445	Y	1.0	0.2	1.3	0.2	7.0
Sa_2HHn-Aβ(–45-15)	7548.4676	S5A	1.0	0.1	0.9	0.1	6.6
Aβ(–51-15)	7286.4418	Y	1.0	0.2	1.0	0.1	6.4
SaHHn-Aβ(–51-15)	7942.6694	S5C	1.0	0.1	0.9	0.2	7.1
Sa_2HHn-Aβ(–51-15)	8233.7649	S5D	1.0	0.1	0.9	0.3	6.4
$(SaHHn)_2$-Aβ(–51-15)	8598.8970	S5E	1.0	0.1	0.5	0.2	6.5
Sa_2HHnSaHHn-Aβ(–51-15)	8889.9925	N	1.0	0.4	1.0	0.5	6.0
$(SaHHn)_3$-Aβ(–51-15)	9255.1247	F4C, S5B	1.0	0.2	0.5	0.1	7.1
Sa_2HHn$(SaHHn)_2$-Aβ(–51-15)	9546.2201	S5F	1.0	0.2	0.7	0.0	6.7
$(SaHHn)_4$-Aβ(–51-15)	9911.3523	S5G	1.0	0.5	1.1	0.6	6.8
Sa_2HHn$(SaHHn)_3$-Aβ(–51-15)	10202.4477	Y	1.0	0.4	1.1	0.3	6.6
$(Sa_2HHn)_2(SaHHn)_2$-Aβ(–51-15)	10493.5431	N	1.0	0.3	0.8	0.2	6.0
$(SaHHn)_5$-Aβ(–51-15)	10567.5799	N	1.0	0.4	0.7	0.3	5.8
Sa_2HHn$(SaHHn)_4$-Aβ(–51-15)	10858.6753	N	1.0	0.3	0.6	0.1	5.3
Aβ(–57-15)	7967.7751	Y	1.0	0.1	1.0	0.1	7.2
SaHHn-Aβ(–57-15)	8624.0027	S5H	1.0	0.3	0.8	0.3	6.6
Sa_2HHn-Aβ(–57-15)	8915.0982	S5I	1.0	0.1	0.8	0.2	6.8
$(SaHHn)_2$-Aβ(–57-15)	9280.2303	N	1.0	0.3	0.9	0.2	6.4
Sa_2HHnSaHHn-Aβ(–57-15)	9571.3258	N	1.0	0.2	0.8	0.1	5.8
$(SaHHn)_3$-Aβ(–57-15)	9936.4580	F4D	1.0	0.1	0.7	0.1	6.9
Sa_2HHn$(SaHHn)_2$-Aβ(–57-15)	10227.5534	N	1.0	0.2	0.8	0.1	6.5
$(SaHHn)_4$-Aβ(–57-15)	10592.6856	S5J	1.0	0.3	0.6	0.2	6.9
Sa_2HHn$(SaHHn)_3$-Aβ(–57-15)	10883.7810	N	1.0	0.3	0.7	0.2	6.6
$(Sa_2HHn)_2(SaHHn)_2$-Aβ(–57-15)	11174.8764	N	1.0	0.5	0.5	0.3	5.6
$(SaHHn)_5$-Aβ(–57-15)	11248.9132	N	1.0	0.2	75.7	40.0	6.2

$Sa_2HHn(SaHHn)_4$-Aβ(–57-15)	11540.0086	N	1.0	0.3	0.9	0.5	4.8
Aβ(–58-15)	8054.8072	Y	1.0	0.2	1.2	0.1	6.7
Aβ(–63-15)	8601.0510	N	1.0	0.1	1.0	0.1	6.4
SaHHn-Aβ(–63-15)	9257.2786	N	1.0	0.3	1.0	0.3	6.7
Sa_2HHn-Aβ(–63-15)	9548.3741	N	1.0	0.5	1.3	0.6	6.2
$(SaHHn)_2$-Aβ(–63-15)	9913.5062	N	1.0	0.6	36.2	17.5	6.3
$Sa_2HHnSaHHn$-Aβ(–63-15)	10204.6017	N	1.0	0.3	0.9	0.1	5.9
$(SaHHn)_3$-Aβ(–63-15)	10569.7339	S5K	1.0	0.1	0.7	0.1	7.0
$Sa_2HHn(SaHHn)_2$-Aβ(–63-15)	10860.8293	N	1.0	0.3	0.8	0.1	6.6
$(SaHHn)_4$-Aβ(–63-15)	11225.9615	S5L	1.0	0.3	0.5	0.1	7.0
$Sa_2HHn(SaHHn)_3$-Aβ(–63-15)	11517.0569	F4E	1.0	0.4	0.9	0.2	6.8
$(Sa_2HHn)_2(SaHHn)_2$-Aβ(–63-15)	11808.1523	N	1.0	0.3	1.0	0.4	6.1
$(SaHHn)_5$-Aβ(–63-15)	11882.1891	N	1.0	0.4	0.4	0.3	6.0
$Sa_2HHn(SaHHn)_4$-Aβ(–63-15)	12173.2845	N	1.0	0.3	0.8	0.2	5.7

[a] Sa = Neu5Ac, H = Hex, Hn = HexNAc, $LaSa_2$ = two Neu5Ac linked with lactone formation, Ac = O-acetyl

[b] Y = CID MS^2 data obtained but not shown in this publication; N = MS^2 data not obtained; F# = MS^2 data obtained and shown in Fig. #; S# = MS^2 data obtained and shown in Fig. S#

[c] Values obtained as described in the *Materials and Methods* section. Below threshold = no signal detected in the AD vs. non-AD study, but detected in other experiments.

[d] $\log_{10}$ values of mean peak intensity of the 13 spectra used in the quantitative AD vs. non-AD

study. Signal threshold was subjectively set to 1000 ($\log_{10}1000 = 3$). Peptides with $\log_{10}$ peak intensity <3.0 in this set were thus below the detection threshold.

Table S2. Sequence and structures of APP/Aβ glycopeptides obtained after sialic acid capture-and-release of peptides immunopurified from CSF with the 6E10 antibody. CID MS^2 spectra of these glycopeptides are shown in **Figs. S7 and S8**.

Peptide	APP/Aβ region	Glycan	Mascot score	Threshold (p<0.05)
K.TEEISEVK.M	–9-(–2)	HexHexNAc	34	>25
K.TEEISEVKMDAEFR.H	–9 - 5	HexHexNAc	62	>21
R.GLTTRPGSGLTNIK.T	–23-(–10)	$(HexHexNAc)_3$	-	-
R.GLTTRPGSGLTNIK.T	–23-(–10)	$(HexHexNAc)_2$	19	>18
R.GLTTRPGSGLTNIK.T	–23-(–10)	HexHexNAc	39	>19
D.SVPANTENEVEPVDAR.P	–44-(–29)	HexHexNAc	43	>25