Electronic Supplementary Material (ESI) for Molecular Omics.

Supplementary Data

Differential *N*-glycosylation Profiling of Formalin-Fixed Paraffin-Embedded (FFPE) Invasive Ductal Carcinoma Tissues Using MALDI-TOF-MS

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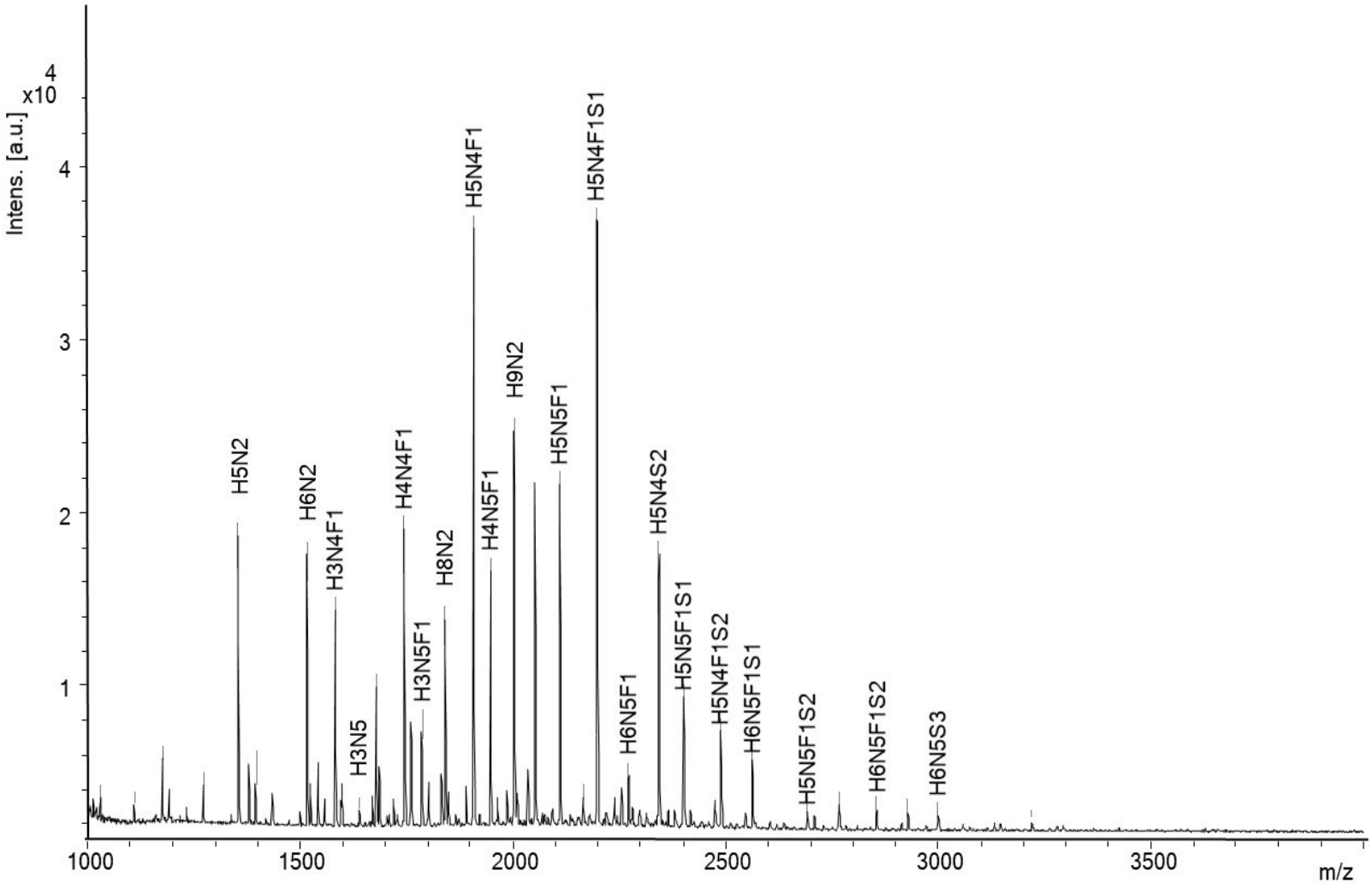


Figure S1. MALDI-TOF-MS spectrum of 2-AA labelled *N*-glycans performed in negative ionization and reflectron mode.

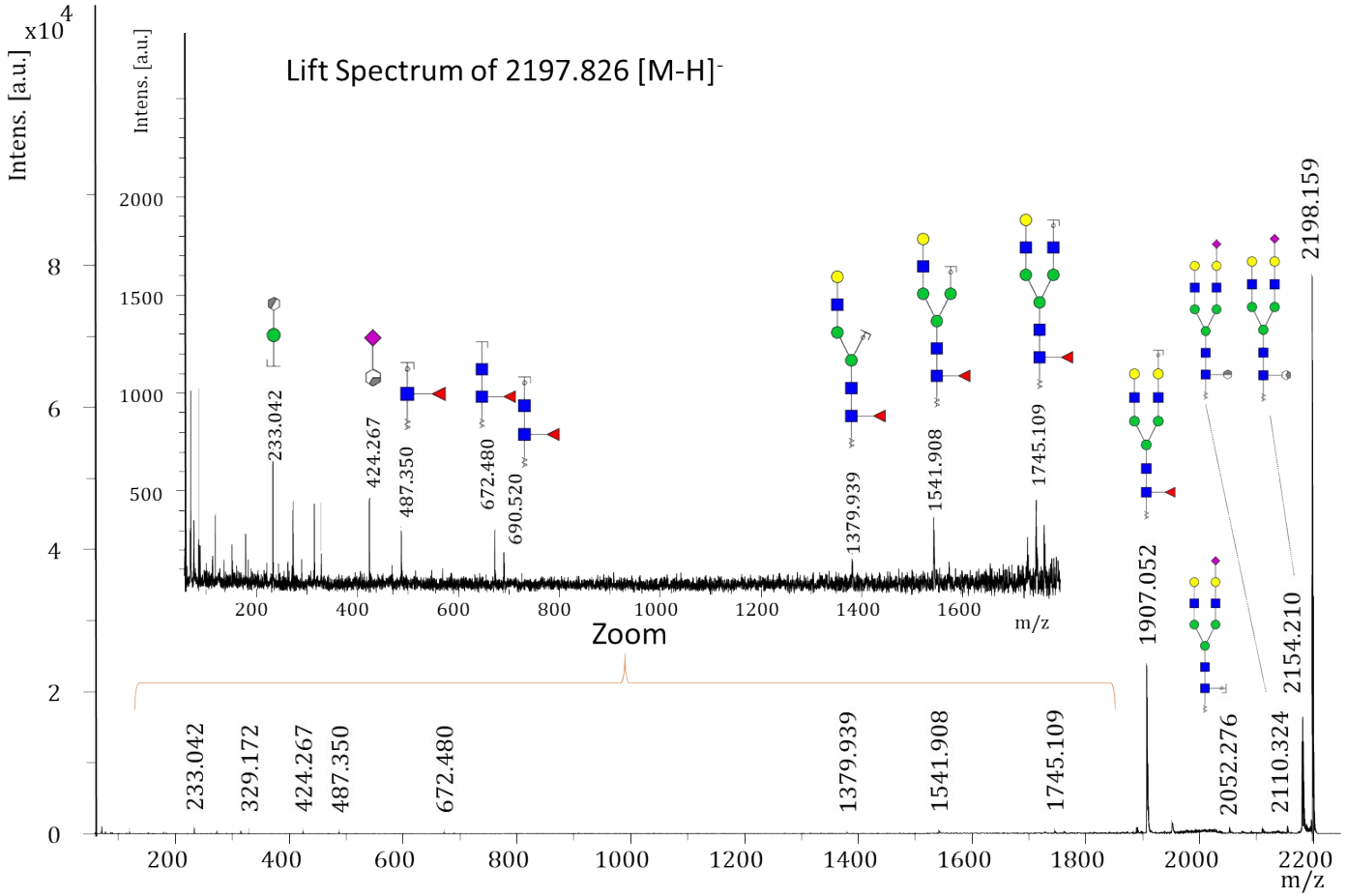


Figure S2. An example of MALDI-TOF/TOF-MS/MS spectrum of 2-AA labelled *N*-glycans. Annotation of *N*-glycans regarding to H5N4F1S1 structure was achieved using Protein Scape Software Version 4 (Bruker Daltonik, Bremen, Germany).

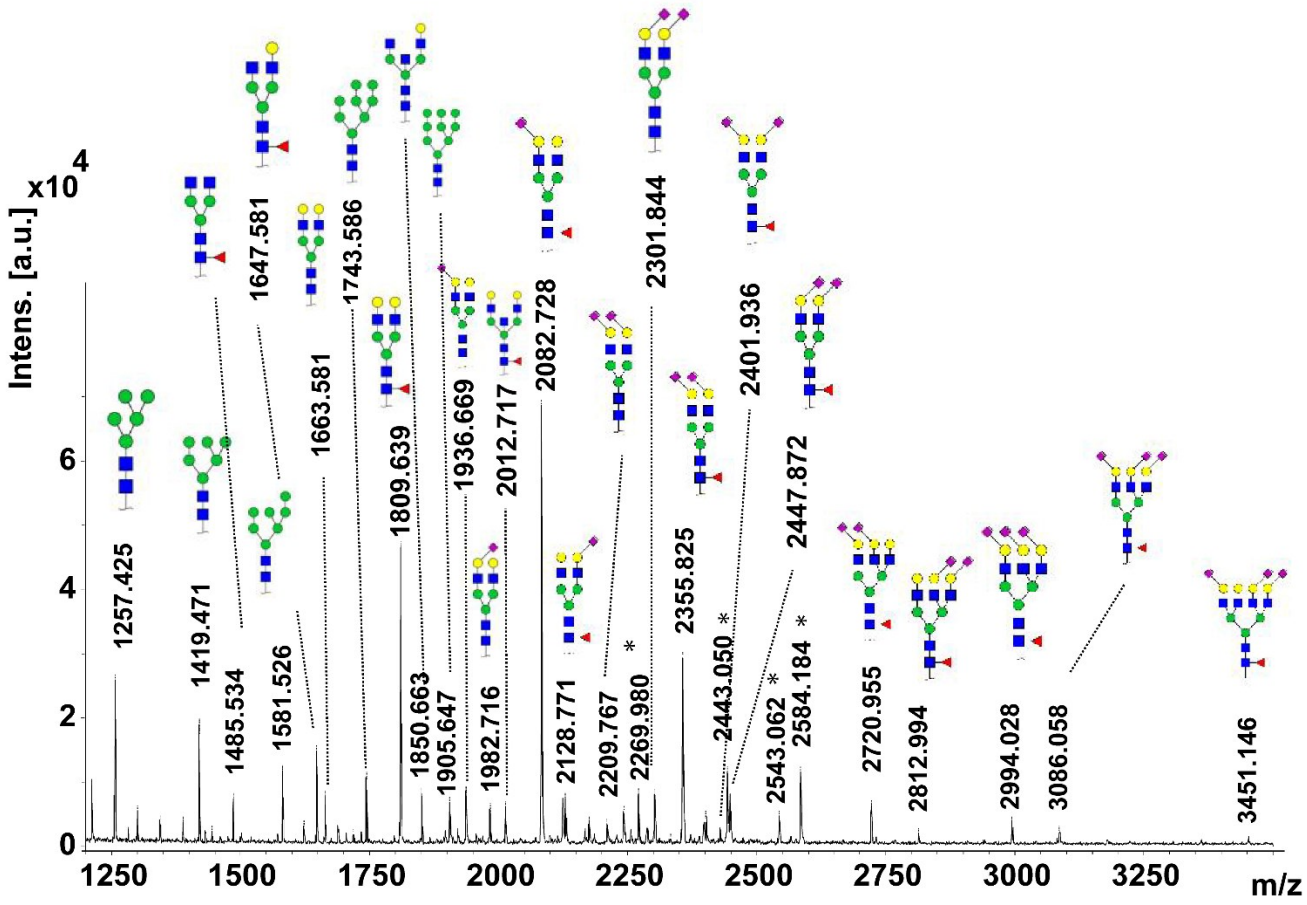


Figure S3. An example of MALDI-TOF-MS spectrum of ethyl-esterified *N*-glycans. *: unassigned peaks.

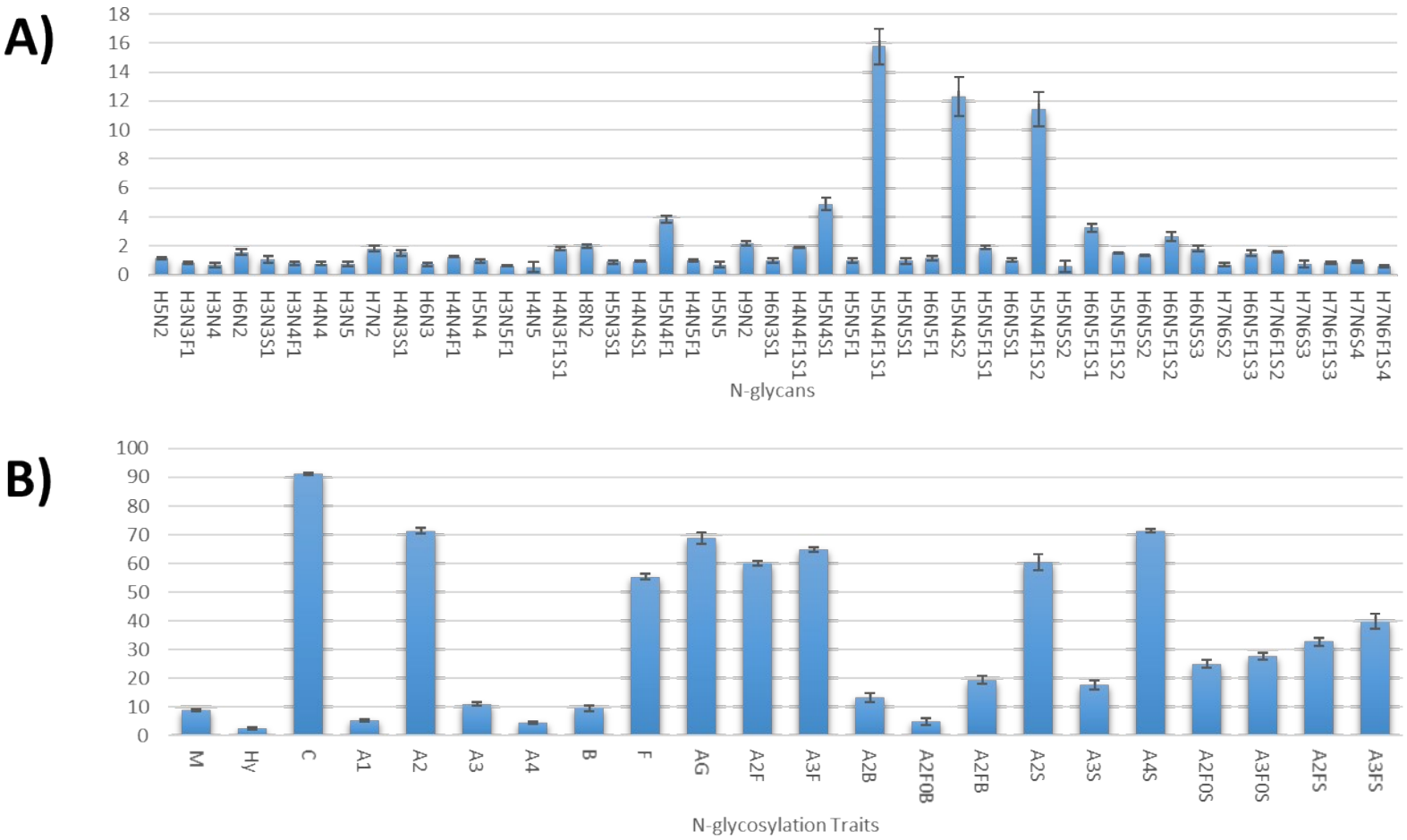


Figure S4. An example of relative quantification data obtained from MALDI-TOF-MS analysis of 2-AA labelled *N*-glycans. Analysis was achieved in negative ionization and linear mode. Standard deviation of each *N*-glycan is based on technical quadruplicate analysis. Relative abundances of (A) *N*-glycans, and (B) *N*-glycan traits.

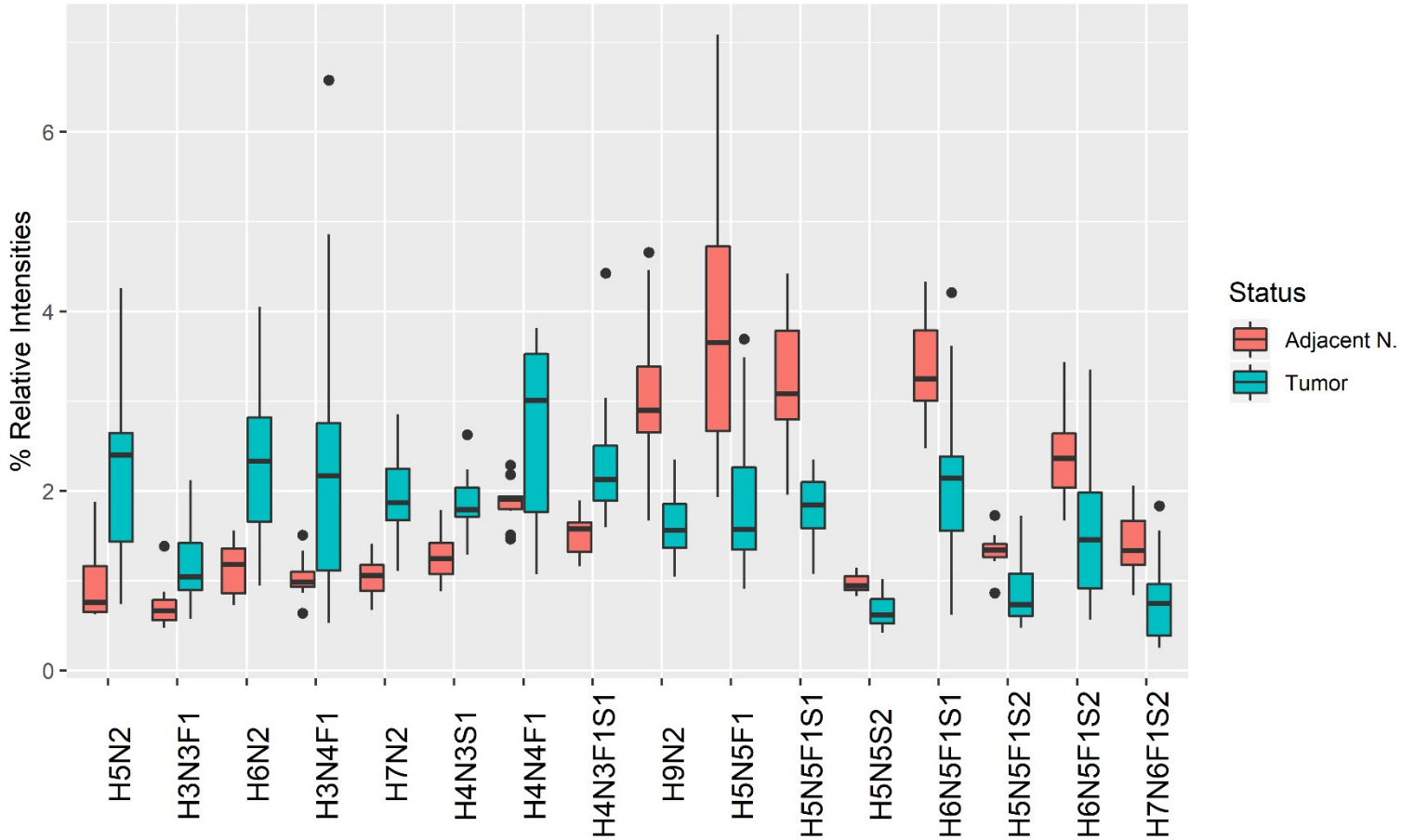


Figure S5. Box graphs of the variables showing significant differences in $p<0.001$ between tumor and control groups for 2-AA labelled *N*-glycan values**.**

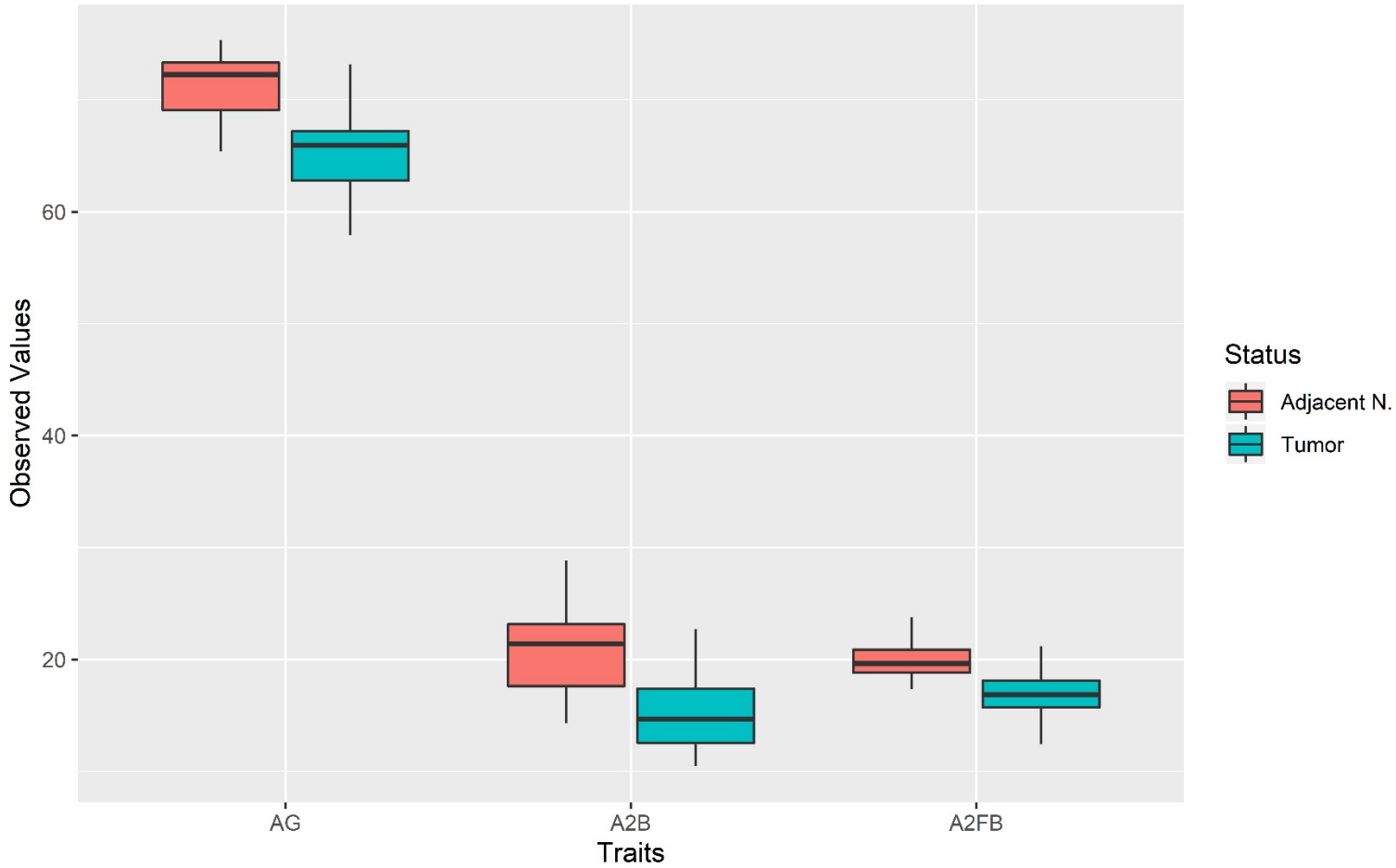


Figure S6. Box graphs of the variables showing significant differences in $p<0.001$ between tumor and control groups for 2-AA labelled *N*-glycan trait values.

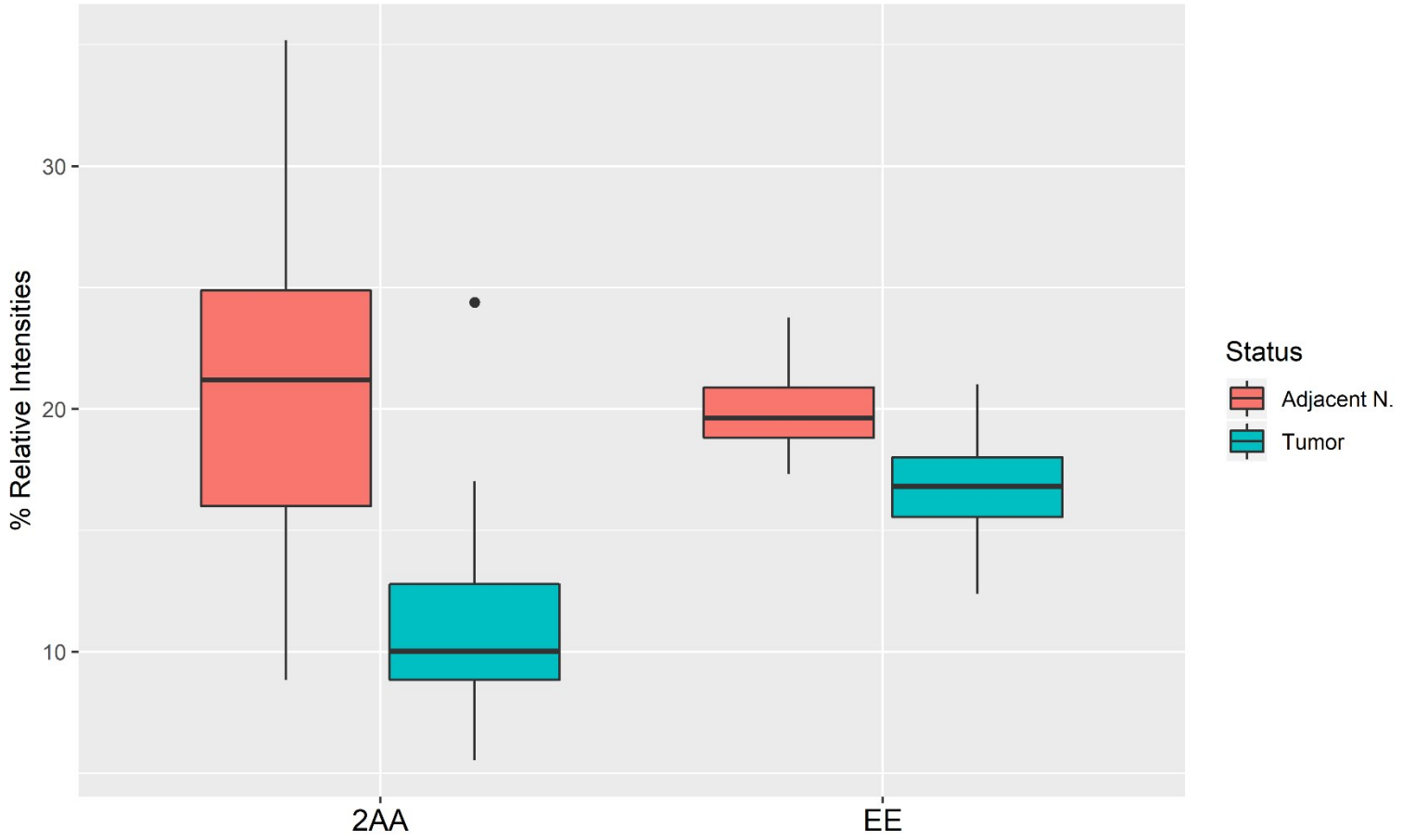


Figure S7. Box graphs of the variables showing significant differences in $p<0.001$ between tumor and control groups for *N*-glycan trait A2FB (bisection of fucosylated diantennary compositions). 2AA: 2-AA labelled *N*-glycans; EE: Etyhl-esterified *N*-glycans.

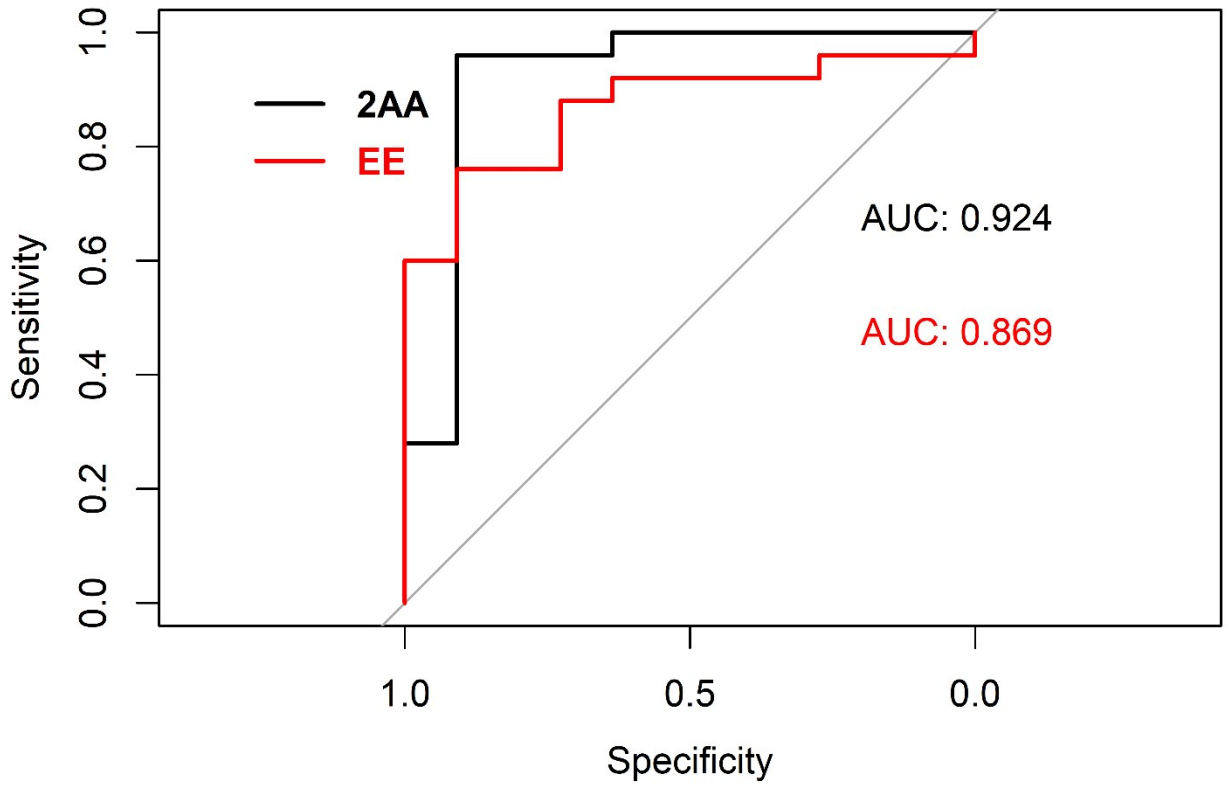


Figure S8. ROC analysis result of A2FB. 2AA:2-AA labelled *N*-glycans; EE: Ethyl-esterified *N*-glycans.

Table S1. 2-aminobenzoic acid Labelled N-glycan compositions detected and extracted from MALDI-TOF-MS and MALDI TOF/TOF-MS in both reflectron and linear mode. The mass peaks used for the internal calibration are indicated in red and bold. H = hexose; N = N-acetylhexosamine; F = deoxyhexose; S = N-acetylneuraminic acid.

										Literature		
No	**N-glycan Composition**	**m/z meas. (mono)**	**z**	**m/z calc. (mono)**	**Δ MH [Da] (mono)**	**m/z meas. (avg)**	**z**	**m/z calc. (avg)**	**Δ MH [Da] (avg)**	***Reiding et.al.*[1]**	***MS/MS***	***MS/MS Method***
1	H5N2-AA	1354,4720	-1	**1354,4789**	-0,0069	1354,6	-1,0	1355,2	-0,6	x	x	QTOF-MS/MS
2	H3N3F1-AA	1379,4987	-1	1379,5105	-0,0119	1380,7	-1,0	1380,3	0,4	x	x	QTOF-MS/MS
3	H3N4-AA	ND	-1	1436,5320	/	1436,6	-1,0	1437,3	-0,7	x	x	QTOF-MS/MS
4	H6N2-AA	1516,5090	-1	**1516,4888**	0,0202	1517,4	-1,0	1517,4	0,0	x	x	QTOF-MS/MS
5	H3N3S1-AA	1524,5660	-1	1524,5481	0,0179	1524,8	-1,0	1525,4	-0,6	x		
6	H3N4F1-AA	1582,5651	-1	1582,5899	-0,0248	1583,6	-1,0	1583,5	0,1	x	x	QTOF-MS/MS
7	H4N4-AA	1598,5544	-1	1598,5848	-0,0305	1598,9	-1,0	1599,5	-0,6	x	x	QTOF-MS/MS
8	H3N5-AA	1639,6090	-1	1639,6114	-0,0024	1638,9	-1,0	1640,5	-1,6	x	x	QTOF-MS/MS
9	H7N2-AA	1678,5636	-1	1678,5846	-0,0210	1678,4	-1,0	1679,5	-1,1	x	x	QTOF-MS/MS
10	H4N3S1-AA	1686,5779	-1	1686,6009	-0,0230	1687,4	-1,0	1687,5	-0,1	x	x	QTOF-MS/MS
11	H6N3-AA	1719,5925	-1	1719,6111	-0,0186	1721,0	-1,0	1720,6	0,4	x	x	QTOF-MS/MS
12	H4N4F1-AA	1744,6258	-1	**1744,6427**	-0,0169	1746,5	-1,0	**1745,6**	0,9	x	x	QTOF-MS/MS MALDI-LIFT
13	H5N4-AA	1760,6222	-1	1760,6377	-0,0155	1760,9	-1,0	1761,6	-0,7	x	x	QTOF-MS/MS
14	H3N5F1-AA	1785,6537	-1	1785,6693	-0,0156	1786,8	-1,0	1786,7	0,2	x	x	QTOF-MS/MS
15	H4N5-AA	1801,6464	-1	1801,6642	-0,0179	1802,5	-1,0	1802,7	-0,2	x	x	QTOF-MS/MS
16	H4N3F1S1-AA	1832,6408	-1	1832,6588	-0,0180	1833,7	-1,0	1833,7	0,1	x	x	QTOF-MS/MS
17	H8N2-AA	1840,6276	-1	1840,6374	-0,0098	1841,4	-1,0	**1841,7**	-0,3	x	x	QTOF-MS/MS
18	H5N3S1-AA	1848,6426	-1	1848,6537	-0,0111	1848,4	-1,0	1849,7	-1,3	x	x	QTOF-MS/MS
19	H4N4S1-AA	1889,6760	-1	1889,6803	-0,0042	1889,2	-1,0	1890,7	-1,6	x	x	QTOF-MS/MS
20	H5N4F1-AA	1906,6889	-1	**1906,6956**	-0,0067	1908,0	-1,0	**1907,8**	0,2	x	x	QTOF-MS/MS MALDI-LIFT
21	H4N5F1-AA	1947,7214	-1	1947,7221	-0,0007	1948,9	-1,0	1948,8	0,1	x	x	QTOF-MS/MS
22	H5N5-AA	1963,7116	-1	1963,7170	-0,0054	1965,1	-1,0	1964,8	0,3	x	x	QTOF-MS/MS
23	H9N2-AA	2002,6899	-1	2002,6902	-0,0003	2003,8	-1,0	2003,8	0,0	x	x	QTOF-MS/MS

Table S1 continued…

No	N-glycan Composition	m/z meas. (mono)	z	m/z calc. (mono)	Δ MH [Da] (mono)	m/z meas. (avg)	z	m/z calc. (avg)	Δ MH [Da] (avg)	*Reiding et.al.*[1]	*MS/MS*	*MS/MS Method*
24	H6N3S1-AA	2010,7067	-1	2010,7065	0,0002	2011,1	-1,0	2011,8	-0,7	x	x	
25	H4N4F1S1-AA	2035,7348	-1	2035,7382	-0,0034	2036,7	-1,0	2036,9	-0,2	x	x	QTOF-MS/MS
26	H5N4S1-AA	2051,7337	-1	**2051,7331**	0,0006	2053,1	-1,0	2052,9	0,2	x	x	QTOF-MS/MS MALDI-LIFT
27	H5N5F1-AA	2109,7810	-1	2109,7749	0,0061	2111,0	-1,0	2111,0	0,1	x	x	QTOF-MS/MS
28	H5N4F1S1-AA	2197,7963	-1	**2197,7910**	0,0053	2199,2	-1,0	**2199,0**	0,2	x	x	MALDI-LIFT QTOF-MS/MS
29	H5N5S1-AA	2254,8324	-1	2254,8124	0,0199	2257,0	-1,0	2256,1	0,9	x	x	QTOF-MS/MS
30	H6N5F1-AA	2271,8409	-1	2271,8278	0,0131	2273,6	-1,0	2273,1	0,5		x	QTOF-MS/MS
31	H5N4S2-AA	2342,8360	-1	**2342,8285**	0,0075	2344,0	-1,0	**2344,1**	-0,1	x	x	MALDI-LIFT
32	H5N5F1S1-AA	2400,8833	-1	2400,8704	0,0129	2402,6	-1,0	2402,2	0,4	x	x	QTOF-MS/MS
33	H6N5S1-AA	2416,8757	-1	2416,8653	0,0104	2419,0	-1,0	2418,2	0,8	x	x	QTOF-MS/MS
34	H5N4F1S2-AA	2488,8916	-1	2488,8864	0,0052	2490,2	-1,0	**2490,3**	-0,1	x	x	QTOF-MS/MS
35	H5N5S2-AA	2545,9254	-1	2545,9079	0,0176	2547,5	-1,0	2547,3	0,1	x	x	QTOF-MS/MS
36	H6N5F1S1-AA	2562,9291	-1	**2562,9232**	0,0059	2565,1	-1,0	2564,3	0,7	x		
37	H5N5F1S2-AA	2691,9642	-1	2692,9736	-1,0094	2694,1	-1,0	2693,5	0,6	x	x	QTOF-MS/MS
38	H6N5S2-AA	2707,9478	-1	2707,9607	-0,0129	2710,0	-1,0	2709,5	0,6	x	x	QTOF-MS/MS
39	H6N5F1S2-AA	2854,0280	-1	2854,0186	0,0094	2855,7	-1,0	2855,6	0,1	x	x	QTOF-MS/MS
40	H6N5S3-AA	2999,0297	-1	**2999,0561**	-0,0264	3000,2	-1,0	3000,7	-0,5	x	x	QTOF-MS/MS
41	H7N6S2-AA	ND	-1	3073,0929	/	3075,1	-1,0	3074,8	0,3	x		
42	H6N5F1S3-AA	3145,1314	-1	3145,1140	0,0174	3146,7	-1,0	3146,9	-0,2	x	x	QTOF-MS/MS
43	H7N6F1S2-AA	3219,2540	-1	3219,1508	0,1032	3221,8	-1,0	3220,9	0,9	x		
44	H7N6S3-AA	3364,3220	-1	3364,1883	0,1337	3363,8	-1,0	3366,0	-2,3	x		
45	H7N6F1S3-AA	ND	-1	3510,2462	/	3511,7	-1,0	3512,2	-0,4	x		
46	H7N6S4	ND	-1	3655,2837	/	3656,8	-1,0	3657,3	-0,5	x		
47	H7N6F1S4	ND	-1	3801,3416	/	3801,7	-1,0	3803,4	-1,8	x		

[1]REIDING, K.R.., 2017. Molecular & Cellular Proteomics 16, 228-242.

Table S2. Ethyl-esterified *N*-glycan compositions detected and extracted from MALDI-MS. The mass peaks used for the internal calibration are indicated in red and bold. H = hexose; N = N-acetylhexosamine; F = deoxyhexose; E = α2,6-linked Sialic Acid; L = α2,3-linked Sialic Acid.

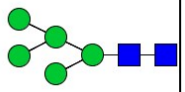
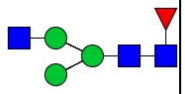
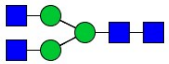
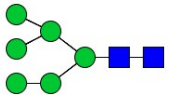
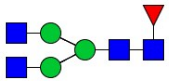
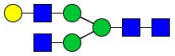
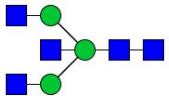
					Literature		
No	***N*-glycan Composition**	**m/z meas. (mono)**	**m/z calc. (mono)**	**Δ MH+ [Da] (mono)**	***Reiding et.al.*[1]**	***MS/MS***	***MS/MS Method***
1	H5N2	1257,425	**1257,423**	0,0024	x		
2	H3N3F1	1282,460	1282,454	0,0057	x		
3	H3N4	1339,476	1339,476	0,0003	x		
4	H6N2	1419,471	**1419,475**	-0,0045	x		
5	H4N3F1	1444,494	1444,507	-0,0131	x		
6	H5N3	1460,499	1460,502	-0,0030	x		
7	H3N4F1	1485,534	1485,534	0,0003	x		
8	H4N4	1501,526	1501,529	-0,0026	x		
9	H3N5	1542,549	1542,555	-0,0061	x		
10	H4N3L1	1571,533	1571,534	-0,0010	x		
11	H7N2	1581,526	1581,528	-0,0023	x		
12	H6N3	1622,556	1622,555	0,0012	x		
13	H4N4F1	1647,581	1647,586	-0,0055	x		
14	H5N4	1663,581	1663,581	-0,0004	x		
15	H3N5F1	1688,611	1688,613	-0,0020	x		
16	H4N5	1704,600	1704,608	-0,0079	x		
17	H5N3L1	1733,596	1733,587	0,0091	x		
18	H8N2	1743,586	1743,581	0,0049	x		
19	H5N4F1	1809,639	**1809,639**	-0,0003	x	x	MALDI-LIFT
20	H4N4E1	1820,656	1820,655	0,0007	x		
21	H6N4	1825,620	1825,634	-0,0142	x		
22	H4N5F1	1850,663	1850,666	-0,0029	x		
23	H5N5	1866,651	1866,661	-0,0098	x		
24	H9N2	1905,647	1905,634	0,0131	x		
25	H5N4L1	1936,669	1936,666	0,0028	x		
26	H5N4E1	1982,716	1982,708	0,0079	x		
27	H5N5F1	2012,717	2012,719	-0,0017	x		
28	H5N4F1L1	2082,728	**2082,724**	0,0038	x	x	MALDI-LIFT
29	H6N4L1	2098,725	2098,719	0,0059	x		
30	H5N4F1E1	2128,771	2128,766	0,0050	x	x	MALDI-LIFT
31	H5N4L2	2209,767	2209,751	0,0159	x		
32	H5N4E1L1	2255,800	2255,793	0,0070	x	x	MALDI-LIFT
33	H5N4E2	2301,844	2301,835	0,0092	x	x	MALDI-LIFT
34	H5N5F1E1	2331,859	2331,845	0,0136	x		
35	H6N5E1	2347,854	2347,840	0,0137	x		
36	H5N4F1L2	2355,825	**2355,809**	0,0160	x	x	MALDI-LIFT
37	H5N4F1E1L1	2401,861	2401,851	0,0101	x		
38	H5N4F1E2	2447,872	**2447,893**	-0,0207	x	x	MALDI-LIFT
39	H6N5F1E1	2493,906	2493,898	0,0078	x		

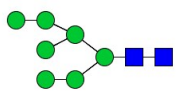
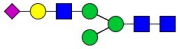
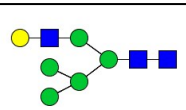
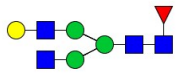
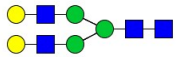
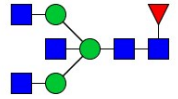
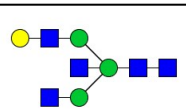
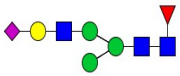
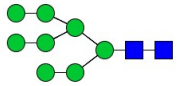
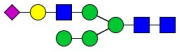
Table S2 continued…

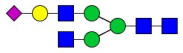
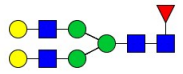
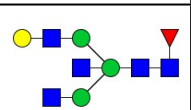
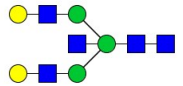
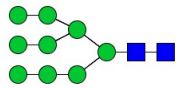
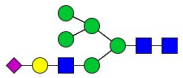
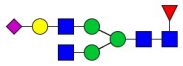
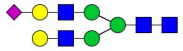
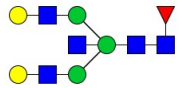
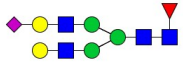
No	*N*-glycan Composition	m/z meas. (mono)	m/z calc. (mono)	Δ MH+ [Da] (mono)	*Reiding et.al.*[1]		
40	H6N5E1L1	2620,944	2620,925	0,0188	x		
41	H5N5F1E2	2650,954	2650,972	-0,0181	x		
42	H6N5E2	2666,957	2666,967	-0,0100	x		
43	H6N5F1L2	2720,955	2720,941	0,0138	x		
44	H6N5F1E1L1	2766,982	2766,983	-0,0011	x		
45	H6N5F1E2	2812,994	2813,025	-0,0309	x		
46	H6N5E2L1	2940,031	2940,052	-0,0209	x	x	MALDI-LIFT
47	H6N5F1L3	2994,028	**2994,026**	0,0019	x		
48	H6N5F1E1L2	3040,100	3040,068	0,0321	x		
49	H6N5F1E2L1	3086,058	3086,110	-0,0518	x		
50	H7N6F1E2L1	3451,146	3451,242	-0,0960	x		

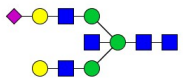
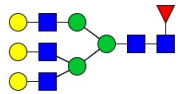
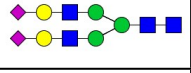
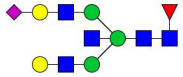
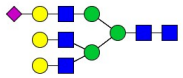
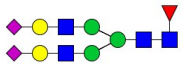
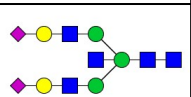
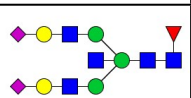
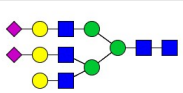
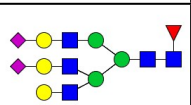
[1]REIDING, K.R., 2014. Analytical chemistry 86, 5784-5793.

Table S3. 2-AA Labelled *N*-glycan structures detected from QTOF-MS/MS analysis. The data were analyzed by Protein Scape Software with GlycoQuest search engine at the MS/MS level. Diagnostic fragments used for the recognition of the detected *N*-glycan structures were given at the bottom of the table. hexose; N = N-acetylhexosamine; F = deoxyhexose; S = N-acetylneuraminic acid.

Row	*Composition*	*Detected Structure*	*m/z meas.*	*z*	*m/z calc.*	*Mr calc.*	*Δ MH+ [Da]*	*Rt [min]*	*Score*	*Frag Cov. [%]*	*MH+ meas.*	*MH+ calc.*	*Δ m/z [Da]*	*Glycan Type & Diagnostic Fragments*
1	H5N2-AA		678,7303	2	678,7504	1355,4862	-0,0402	23,84	101,1	152	1356,4532	1356,4935	-0,0201	High Mannose
2	H3N3F1-AA		691,2479	2	691,2662	1380,5178	-0,0366	29,35	75,7	102	1381,4885	1381,5251	-0,0183	Complex Core fucose [489.189^{+}]
3	H3N4-AA		719,7578	2	719,7769	1437,5393	-0,0382	24,20	68,2	109	1438,5084	1438,5466	-0,0191	Complex
4	H6N2-AA		759,7546	2	759,7768	1517,5390	-0,0443	23,29	91,6	100	1518,5019	1518,5463	-0,0222	High Mannose
5	H3N4F1-AA		792,7849	2	792,8059	1583,5972	-0,0420	28,83	102,2	120	1584,5625	1584,6045	-0,0210	Complex Core fucose [489.195]
6	H4N4-AA		800,7799	2	800,8033	1599,5921	-0,0468	25,64	77,0	78	1600,5525	1600,5994	-0,0234	Complex
7	H3N5-AA		821,2933	2	821,3166	1640,6187	-0,0466	26,45	100,8	123	1641,5793	1641,6259	-0,0233	Bisecting [911.334^{+}]

8	H7N2-AA		840,7790	2	840,8032	1679,5918	-0,0483	21,81	80,8	89	1680,5508	1680,5991	-0,0242	High Mannose
9	H4N3S1-AA		844,7902	2	844,8114	1687,6082	-0,0422	34,74	76,3	70	1688,5732	1688,6154	-0,0211	Complex
10	H6N3-AA		861,2911	2	861,3165	1720,6184	-0,0508	24,40	79,3	79	1721,5749	1721,6257	-0,0254	Hybrid
11	H4N4F1-AA		873,8089	2	873,8323	1745,6500	-0,0468	28,10	89,4	85	1746,6105	1746,6573	-0,0234	Complex Core fucose [489.195^{+}]
12	H5N4-AA		881,8042	2	881,8297	1761,6449	-0,0511	24,76	109,3	135	1762,6011	1762,6522	-0,0255	Complex
13	H3N5F1-AA		894,3220	2	894,3456	1786,6766	-0,0472	29,17	103,1	123	1787,6366	1787,6838	-0,0236	Bisecting [911.334; 1057.389^{+}]
14	H4N5-AA		902,3174	2	902,3430	1802,6715	-0,0513	27,24	82,5	96	1803,6275	1803,6788	-0,0256	Bisecting [911.334^{+}]
15	H4N3F1S1-AA		917,8180	2	917,8403	1833,6661	-0,0447	37,42	73,0	64	1834,6286	1834,6733	-0,0224	Complex Core fucose [489.194^{+}]
16	H8N2-AA		921,8033	2	921,8296	1841,6447	-0,0525	22,82	96,6	131	1842,5994	1842,6519	-0,0263	Complex
17	H5N3S1-AA		925,8155	2	925,8378	1849,6610	-0,0445	34,04	76,3	66	1850,6238	1850,6683	-0,0222	Hybrid

18	H4N4S1-AA		946,3267	2	946,3510	1890,6875	-0,0487	33,17	67,9	62	1891,6461	1891,6948	-0,0244	Complex
19	H5N4F1-AA		954,8320	2	954,8587	1907,7028	-0,0534	26,59	106,0	129	1908,6568	1908,7101	-0,0267	Complex Core fucose [489.196^{+}]
20	H4N5F1-AA		975,3460	2	975,3720	1948,7294	-0,0520	29,07	94,1	99	1949,6847	1949,7367	-0,0260	Bisecting (911.340^{+}; 1057.386) Core fucose [489.194^{+}]
21	H5N5-AA		983,3411	2	983,3694	1964,7243	-0,0566	25,44	62,3	76	1965,6750	1965,7316	-0,0283	Bisecting [911.340^{+}]
22	H9N2-AA		1002,8270	2	1002,8560	2003,6975	-0,0580	21,86	98,9	122	2004,6467	2004,7048	-0,0290	High Mannose
23	H6N3S1-AA		1006,8381	2	1006,8642	2011,7138	-0,0522	31,75	75,8	72	2012,6689	2012,7211	-0,0261	Hybrid
24	H4N4F1S1-AA		1019,3547	2	1019,3800	2036,7454	-0,0507	37,46	72,7	60	2037,7020	2037,7527	-0,0253	Complex Core fucose [489.200^{+}]
25	H5N4S1-AA		1027,3512	2	1027,3775	2052,7404	-0,0526	33,94	82,4	72	2053,6951	2053,7476	-0,0263	Complex
26	H5N5F1-AA		1056,3696	2	1056,3984	2110,7822	-0,0575	28,00	79,3	80	2111,7320	2111,7895	-0,0288	Bisecting [911.338^{+}; 1057.444^{+}] Core fucose [489.194^{+}]
27	H5N4F1S1-AA		1100,3798	2	1100,4064	2198,7983	-0,0532	35,95	84,7	74	2199,7524	2199,8055	-0,0266	Complex Core fucose [489.196^{+}]

28	H5N5S1-AA		1128,8879	2	1128,9171	2255,8197	-0,0586	34,69	55,2	51	2256,7684	2256,8270	-0,0293	Bisecting [911.350^{+}]
29	H6N5F1-AA		1137,3925	2	1137,4248	2272,8350	-0,0646	27,95	56,2	54	2273,7778	2273,8423	-0,0323	Complex Core fucose [489.203^{+}]
30	H5N4S2-AA		782,2650	3	782,2859	2343,8358	-0,0626	45,78	93,3	94	2344,7805	2344,8430	-0,0209	Complex
31	H5N5F1S1-AA		1201,9164	2	1201,9461	2401,8776	-0,0595	37,67	73,5	63	2402,8254	2402,8849	-0,0297	Bisecting [911.344^{+}; 1057.400^{+}] Core fucose [489.196^{+}]
32	H6N5S1-AA		1209,9127	2	1209,9436	2417,8725	-0,0617	32,26	55,2	45	2418,8181	2418,8798	-0,0309	Complex
33	H5N4F1S2-AA		830,9498	3	830,9718	2489,8937	-0,0662	56,54	50,2	44	2490,8348	2490,9010	-0,0221	Complex Core fucose [489.196^{+}]
34	H5N5S2-AA		1274,4282	2	1274,4648	2546,9151	-0,0734	45,73	76,5	75	2547,8491	2547,9224	-0,0367	Bisecting [911.018^{+}]
35	H5N5F1S2-AA		898,6407	3	898,6650	2692,9731	-0,0728	48,09	68,5	58	2693,9075	2693,9803	-0,0243	Bisecting [911.330^{+}; 1057.388^{+}] Core fucose [489.195^{+}]
36	H6N5S2-AA		903,9722	3	903,9966	2708,9680	-0,0732	43,05	58,7	48	2709,9020	2709,9752	-0,0244	Complex
37	H6N5F1S2-AA		1428,4796	2	1428,5202	2855,0259	-0,0813	49,46	62,9	49	2855,9518	2856,0332	-0,0407	Complex Core fucose [489.197^{+}]

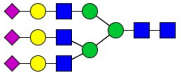
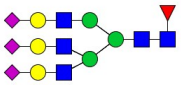
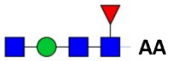
38	H6N5S3-AA		1000,9974	3	1001,0284	3000,0634	-0,0931	38,41	47,0	43	3000,9776	3001,0707	-0,0310	Complex
39	H6N5F1S3-AA		1574,0330	2	1574,0679	3146,1213	-0,0698	34,91	58,0	54	3147,0587	3147,1286	-0,0349	Complex Core fucose [489.196^{+}]
*	N1F1-AA (Core Fucose)					489,2079								
*	N3H1-AA (Bisecting)					911,3615								
*	N3H1F1-AA (Bisecting Core Fucosylated)					1057,4195								

Table S4. Calculation formulas of described 2-AA labelled *N*-glycan traits in the study. H = hexose; N = N-acetylhexosamine; F = deoxyhexose; S = N-acetylneuraminic acid.

Derived Glycan Code	Description of Trait	Calculation
M	Percentage of highmannose glycans	(1 * (H5N2 + H6N2 + H7N2 + H8N2 + H9N2)).
Hy	Percentage of hybrid glycans	(1 * (H6N3 + H5N3S1 + H6N3S1)).
C	Percentage of complex glycans	(1* (H3N4 + H3N3F1 + H3N3S1 + H3N4F1 + H4N4 + H3N5 + H4N3S1+ H4N4F1 + H5N4 + H3N5F1 + H4N5 + H4N3F1S1 + H5N3S1 + H4N4S1 + H5N4F1 + H4N5F1 + H5N5 + H4N4F1S1 + H5N4S1 + H5N5F1 + H5N4F1S1 + H5N5S1 + H6N5F1 + H5N4S2 + H5N5F1S1 + H6N5S1 + H5N4F1S2 + H5N5S2 + H6N5F1S1 + H5N5F1S2 + H6N5S2 + H6N5F1S2 + H6N5S3 + H7N6S2 + H6N5F1S3 + H7N6F1S2 + H7N6S3 + H7N6F1S3 + H7N6S4 + H7N6F1S4)).
A1	Percentage of monoantennary glycans	1* (H3N3F1 + H3N3S1 + H4N3S1 + H4N3F1S1).
A2	Percentage of diantennary glycans	(1 * (H3N4 + H3N3F1 + H3N3S1 + H3N4F1 + H4N4 + H3N5 + H4N3S1+ H4N4F1 + H5N4 + H3N5F1 + H4N5 + H4N3F1S1 + H5N3S1 + H4N4S1 + H5N4F1 + H4N5F1 + H5N5 + H4N4F1S1 + H5N4S1 + H5N5F1 + H5N4F1S1 + H5N5S1 + H5N4S2 + H5N5F1S1 + H5N4F1S2 + H5N5S2 + H5N5F1S2)).
A3	Percentage of triantennary glycans	(1 * (H6N5F1 + H6N5S1 + H6N5F1S1 + H6N5S2 + H6N5F1S2 + H6N5S3 + H6N5F1S3)).
A4	Percentage of tetraantennary glycans	(1 * (H7N6S2 + H7N6F1S2 + H7N6S3+ H7N6F1S3 + H7N6S4 + H7N6F1S4)).
B	Percentage of bisection	(1 *(H3N5+ H3N5F1 + H4N5+ H4N5F1 + H5N5+ H5N5F1 + H5N5S1 + H5N5F1S1 + H5N5S2 + H5N5F1S2)).

F	Percentage of fucosylation	(1 * (H3N3F1 + H3N4F1 + H4N4F1 + H3N5F1 + H4N3F1S1 + H5N4F1 + H4N5F1 + H4N4F1S1 + H5N5F1 + H5N4F1S1 + H6N5F1 + H5N5F1S1 + H5N4F1S2 + H6N5F1S1 + H5N5F1S2 + H6N5F1S2 + H6N5F1S3 + H7N6F1S2 + H7N6F1S3 + H7N6F1S4)).
AG	Percentage of galactosylation of antennary glycans	(1/2 * (H4N4 + H4N4F1 + H4N5 + H4N4S1 + H4N5F1 + H4N4F1S1) + 2/2 * (H5N4 + H5N4F1 + H5N5 + H5N4S1 + H5N5F1 + H5N4F1S1 + H5N5S1 + H5N4S2 + H5N5F1S1 + H5N4F1S2 + H5N5S2 + H5N5F1S2) + 3/3 * (H6N5F1 + H6N5S1 + H6N5F1S1 + H6N5S2 + H6N5F1S2 + H6N5F1S2 + H6N5S3 + H6N5F1S3) + 4/4 * (H7N6S3 + H7N6F1S2 + H7N6F1S3 + H7N6S4 + H7N6F1S4)) / (1 *(H3N4 + H3N4F1 + H4N4 + H3N5 + H4N4F1 + H5N4 + H3N5F1 + H4N5 + H4N4S1 + H5N4F1 + H4N4S1 + H4N5F1 + H5N5 + H5N4S1 + H4N4F1S1 + H5N5F1 + H5N4F1S1 + H5N5S1 + H5N4S2 + H5N5F1S1 + H5N4F1S2 + H5N5S2 + H5N5F1S2 + H6N5S1 + H6N5F1S1 + H6N5S2 + H6N5F1S2 + H6N5S3 + H6N5F1S3 + H10N6 + H7N6F1S2 + H7N6S3 + H7N6F1S3 + H7N6S4 + H7N6F1S4)).
A2F	Percentage of Fucosylation of diantennary compositions	(1 * (H3N3F1 + H3N4F1 + H4N4F1 + H3N5F1 + H4N3F1S1 + H5N4F1 + H4N5F1 + H4N4F1S1 + H5N5F1 + H5N4F1S1 + H5N5F1S1 + H5N4F1S2 + H5N5F1S2))/ (1 * H3N4 + H3N3F1 + H3N3S1 + H3N4F1 + H4N4 + H3N5 + H4N3S1+ H4N4F1 + H5N4 + H3N5F1 + H4N5 + H4N3F1S1 + H5N3S1 + H4N4S1 + H5N4F1 + H4N5F1 + H5N5 + H4N4F1S1 + H5N4S1 + H5N5F1 + H5N4F1S1 + H5N5S1 + H5N4S2 + H5N5F1S1 + H5N4F1S2 + H5N5S2 + H5N5F1S2)).
A3F	Percentage of Fucosylation of triantennary compositions	(1 *(H6N5F1 + H6N5F1S1 + H6N5F1S2 + H6N5F1S3)) / (1 * (H6N5F1 + H6N5S1 + H6N5F1S1 + H6N5S2 + H6N5F1S2 + H6N5S3 + H6N5F1S3)).
A2B	Percentage of Bisection of diantennary compositions	(1 * (H3N5+ H3N5F1 + H4N5+ H4N5F1 + H5N5+ H5N5F1 + H5N5S1 + H5N5F1S1 + H5N5S2 + H5N5F1S2)) / 1* (H3N4 + H3N3F1 + H3N3S1 + H3N4F1 + H4N4 + H3N5 + H4N3S1+ H4N4F1 + H5N4 + H3N5F1 + H4N5 + H4N3F1S1 + H5N3S1 + H4N4S1 + H5N4F1 + H4N5F1 + H5N5 + H4N4F1S1 + H5N4S1 + H5N5F1 + H5N4F1S1 + H5N5S1 + H5N4S2 + H5N5F1S1 + H5N4F1S2 + H5N5S2 + H5N5F1S2))
A2F0B	Percentage of Bisection of non-fucosylated diantennary compositions	(1 * (H3N5 + H4N5+ H5N5 + H5N5S1 + H5N5S2)) / (1* (H3N4 + H3N3F1 + H3N3S1 + H3N4F1 + H4N4 + H3N5 + H4N3S1+ H4N4F1 + H5N4 + H3N5F1 + H4N5 + H4N3F1S1 + H5N3S1 + H4N4S1 + H5N4F1 + H4N5F1 + H5N5 + H4N4F1S1 + H5N4S1 + H5N5F1 + H5N4F1S1 + H5N5S1 + H5N4S2 + H5N5F1S1 + H5N4F1S2 + H5N5S2 + H5N5F1S2))
A2FB	Percentage of Bisection of fucosylated diantennary compositions	(1 * (H3N5F1 + H4N5F1 + H5N5F1 + H5N5F1S1 + H5N5F1S2)) / (1 *(H3N4 + H3N3F1 + H3N3S1 + H3N4F1 + H4N4 + H3N5 + H4N3S1+ H4N4F1 + H5N4 + H3N5F1 + H4N5 + H4N3F1S1 + H5N3S1 + H4N4S1 + H5N4F1 + H4N5F1 + H5N5 + H4N4F1S1 + H5N4S1 + H5N5F1 + H5N4F1S1 + H5N5S1 + H5N4S2 + H5N5F1S1 + H5N4F1S2 + H5N5S2 + H5N5F1S2)).
A2S	Percentage of Sialylation of diantennary compositions	(1/2* (H3N3S1 + H4N3S1 + H4N3F1S1 + H5N3S1 + H4N4S1 + H4N4F1S1 + H5N4S1 + H5N4F1S1 + H5N5S1 + H5N5F1S1) + 2/2 * (H5N4S2 + H5N4F1S2 + H5N5S2 + H5N5F1S2)) / (1 * (H3N4 + H3N3F1 + H3N3S1 + H3N4F1 + H4N4 + H3N5 + H4N3S1+ H4N4F1 + H5N4 + H3N5F1 + H4N5 + H4N3F1S1 + H5N3S1 + H4N4S1 + H5N4F1 + H4N5F1 + H5N5 + H4N4F1S1 + H5N4S1 + H5N5F1 + H5N4F1S1 + H5N5S1 + H5N4S2 + H5N5F1S1 + H5N4F1S2 + H5N5S2 + H5N5F1S2)).

A3S	Percentage of Sialylation of triantennary compositions	(1/3* (H6N5S1 + H6N5F1S1) + 2/3* (H6N5S2 + H6N5F1S2) + 3/3* (H6N5S3 + H6N5F1S3)) / (1 *(H6N5F1 + H6N5S1 + H6N5F1S1 + H6N5S2 + H6N5F1S2 + H6N5S3 + H6N5F1S3)).
A4S	Percentage of Sialylation of tetraantennary compositions	(2/4 * (H7N6S2 + H7N6F1S2) + 3/4 * (H7N6S3+ H7N6F1S3) + 4/4 * (H7N6S4 + H7N6F1S4) / (H7N6S2 + H7N6F1S2 + H7N6S3+ H7N6F1S3 + H7N6S4 + H7N6F1S4)).
A2F0S	Percentage of Sialylation of non-fucosylated diantennary compositions	(1/3* (H6N5S1) + 2/3* (H6N5S2) + 3/3* (H6N5S3) / (1 *(H6N5F1 + H6N5S1 + H6N5F1S1 + H6N5S2 + H6N5F1S2 + H6N5S3 + H6N5F1S3))
A3F0S	Percentage of Sialylation of non-fucosylated triantennary compositions	(1/3* (H6N5S1) + 2/3* (H6N5S2) + 3/3* (H6N5S3)) / (1 *(H6N5F1 + H6N5S1 + H6N5F1S1 + H6N5S2 + H6N5F1S2 + H6N5S3 + H6N5F1S3))
A2FS	Percentage of Sialylation of fucosylated diantennary compositions	(1/2* (H4N3F1S1 + H4N4F1S1 + H5N4F1S1 + H5N5F1S1) + 2/2 * (H5N4F1S2 + H5N5F1S2)) / (1 *(H3N4 + H3N3F1 + H3N3S1 + H3N4F1 + H4N4 + H3N5 + H4N3S1+ H4N4F1 + H5N4 + H3N5F1 + H4N5 + H4N3F1S1 + H5N3S1 + H4N4S1 + H5N4F1 + H4N5F1 + H5N5 + H4N4F1S1 + H5N4S1 + H5N5F1 + H5N4F1S1 + H5N5S1 + H5N4S2 + H5N5F1S1 + H5N4F1S2 + H5N5S2 + H5N5F1S2))
A3FS	Percentage of Sialylation of fucosylated triantennary compositions	(1/3* (H6N5F1S1) + 2/3* (H6N5F1S2) + 3/3* (H6N5F1S3)) /(1 *(H6N5F1 + H6N5S1 + H6N5F1S1 + H6N5S2 + H6N5F1S2 + H6N5S3 + H6N5F1S3)).

Table S5. Calculation formulas of described ethyl-esterified N-glycan traits in the study. H = hexose; N = N-acetylhexosamine; F = deoxyhexose; E = α2,6-linked Sialic Acid; L = α2,3-linked Sialic Acid.

Derived Glycan Code	Description of Trait	Calculation
M	Percentage of highmannose glycans	H4N2 + H5N2 + H6N2 + H7N2 + H8N2 + H9N2 + H10N2
Hy	Percentage of hybrid glycans	H5N3 +H6N3 + H6N3F1 + H5N3E1 + H6N4 + H5N3L1F1 + H6N3F2 + H6N3E1 + H6N4F1 + H6N3L1F1 + H6N3E1F1 + H6N4F2 + H6N4E1F1
C	Percentage of complex glycans	H3N4 + H3N4F1 + H4N4 + H4N3E1 + H4N4F1 + H5N4 + H3N5F1 + H4N5 + H5N4F1 + H4N5F1 + H5N5 + H5N4L1 + H5N4E1 + H5N5F1 + H5N4L1F1 + H5N4E1F1 + H6N5F1 + H5N4L2 + H5N4L1E1 + H5N4E2 + H5N5E1F1 + H6N5E1 + H5N4L2F1 + H5N4L1E1F1 + H6N5E1F1 + H6N5L2F1 + H6N5L1E1F1 + H6N5L1E2 + H6N5L3F1 + H6N5L2E1F1 + H6N5E3F1 + H7N6L1E2F1
A1	Percentage of monoantennary glycans	H4N3L1
A2	Percentage of diantennary glycans	H3N4F1 + H4N4F1 + H5N4 + H5N4F1 + H4N4E1 + H6N4 + H5N4L1 + H4N4E1F1 + H5N4E1 + H5N4L1F1 +H5N4E1F1 + H5N4L2+ H5N4E2 + H5N4L2F1 + H5N4L1E1F1 + H5N4E2F1
A3	Percentage of triantennary glycans	H3N5 + H3N5F1 + H4N5 +H4N5F1 + H5N5 + H5N5F1 + H5N5E1 + H5N5E1F1 + H6N5E1 + H6N5E1F1 + H6N5E2 + H6N5L1E1F1 + H6N5L3F1 + H6N5L2E1F1 + H6N5L1E2F1
A4	Percentage of tetraantennary glycans	H7N6L1E2F1
B	Percentage of bisection	H3N5F1 + H4N5 + H4N5F1 + H5N5 + H5N5F1 +H5N5F1E1 + H5N5F1E2

F	Percentage of fucosylation	H3N3F1+H4N3F1+ H3N4F1+H4N4F1+ H3N5F1+ H5N4F1+ H4N4E1+H4N5F1+H5N5F1+ H5N4F1L1+ H5N4F1E1+ H5N5F1E1+ H5N4F1L2+ H5N4F1E1L1+ H5N4F1E2+ H6N5F1E1+ H5N5F1E2+ H6N5F1L2+ H6N5F1E1L1+ H6N5F1E2+ H6N5F1L3+ H6N5F1E1L2+ H6N5F1E2L1+ H6N5F1E3+ H7N6F1E2L1+H3N5F1 + H4N5F1 + H5N5F1 + H4N5F1E1 + H5N5F1E1 + H5N5F1E1L1 + H5N5F1E2
AG	Percentage of galactosylation of antennary glycans	H4N4 + H4N4F1 + H5N4 + H4N5 + H5N4F1 + H4N4E1 + H4N5F1 + H5N5 + H5N4L1 + H5N4E1 + H5N5F1 + H5N4F1L1 + H5N4F1E1 + H5N5F1E1 + H6N5E1 + H5N4F1L2 + H5N4F1E1L1 + H5N4F1E2 + H6N5F1E1 + H6N5E1L1 + H5N5F1E2 + H6N5E2 + H6N5F1L2 + H6N5F1E1L1 + H6N5F1E2 + H6N5E2L1 + H6N5F1E1L2 + H6N5F1E2L1 + H6N5F1E3
A2F	Percentage of Fucosylation of diantennary compositions	(1 * (H3N4F1 + H4N4F1 + H5N4F1 + H5N4L1F1 + H5N4E1F1 + H5N4L2F1 + H5N4L1E1F1 + H5N4E2F1 +)) / (1 * (H3N4F1 + H4N4F1 + H5N4 + H5N4F1 + H4N4E1 + H6N4 + H5N4L1 + H5N4E1 + H5N4L1F1 + H5N4E1F1 + H5N4L2 + H5N4L1E1 + H5N4E2 + H5N4L2F1 + H5N4L1E1F1 + H5N4E2F1))
A3F	Percentage of Fucosylation of triantennary compositions	(1 * (H3N5F1 + H4N5F1 + H5N5F1 + H5N5E1F1 + H6N5E1F1 + H6N5L1E1F1 + H6N5L2E1F1 H6N5L1E2F1)) / (1 * (H3N5 + H3N5F1 + H4N5 + H4N5F1 + H5N5 + H5N5F1 + H5N5E1F1 + H6N5E1 + H6N5E1F1 + H6N5L1E1 + H6N5E2 + H6N5L1E1F1 + H6N5L1E2 + H6N5L3F1 + H6N5L2E1F1 + H6N5L1E2F1))
A2B	Percentage of Bisection of diantennary compositions	(H3N5F1 + H4N5 + H4N5F1 + H5N5 + H5N5F1 + H5N5F1E1 + H5N5E2 + H5N5F1E2) / (H3N4F1 + H4N4 + H4N4F1 + H5N4 + H3N5F1 + H4N5 + H5N4F1 + H4N4E1 + H4N5F1 + H5N5 + H5N4L1 + H5N4E1 + H5N5F1 + H4N5E1 + H5N4F1L1 + H5N4F1E1 + H5N4L2 + H5N4E1L1 + H5N4E2 + H5N5F1E1 + H5N4F1L2 + H5N4F1E1L1 + H5N4F1E2 + H5N5F1E2)
A2F0B	Percentage of Bisection of non-fucosylated diantennary compositions	(H3N5F1 + H4N5 + H4N5F1 + H5N5 + H5N5F1 + H5N5F1E1 + H5N5F1E2) / (H3N4F1 + H4N4 + H4N4F1 + H5N4 + H3N5F1 + H4N5 + H5N4F1 + H4N4E1 + H4N5F1 + H5N5 + H5N4L1 + H5N4E1 + H5N5F1 + H5N4F1L1 + H5N4F1E1 + H5N4L2 + H5N4E1L1 + H5N4E2 + H5N5F1E1 + H5N4F1L2 + H5N4F1E1L1 + H5N4F1E2 + H5N5F1E2)
A2FB	Percentage of Bisection of fucosylated diantennary compositions	(H3N5F1 + H4N5F1 + H5N5F1 + H5N5F1E1 + H5N5F1E2) / (H3N4F1 + H4N4F1 + H3N5F1 + H5N4F1 + H4N5F1 + H5N5F1 + H5N4F1L1 + H5N4F1E1 + H5N5F1E1 + H5N4F1L2 + H5N4F1E1L1 + H5N4F1E2 + H5N5F1E2)
A2E	Percentage of (α2,6) Sialylation of diantennary compositions	(0/2 * (H3N4F1 + H4N4 + H4N4F1 + H5N4 + H3N5F1 + H4N5 + H5N4F1 + H4N5F1 + H5N5 + H5N4L1 + H5N5F1 + H5N4F1L1 + H5N4L2 + H5N4F1L2) + 1/2 * (H4N4E1 + H5N4E1 + H5N4F1E1 + H5N4E1L1 + H5N5F1E1 + H5N4F1E1L1) + 2/2 * (H5N4E2 + H5N4F1E2 + H5N5F1E2)) / (H3N4F1 + H4N4 + H4N4F1 + H5N4 + H3N5F1 + H4N5 + H5N4F1 + H4N4E1 + H4N5F1 + H5N5 + H5N4L1 + H5N4E1 + H5N5F1 + H5N4F1L1 + H5N4F1E1 + H5N4L2 + H5N4E1L1 + H5N5F1E1 + H5N4F1L2 + H5N4F1E1L1 + H5N4F1E2 + H5N5F1E2)
A2L	Percentage of Sialylation of diantennary compositions	(0/2 * (H3N4F1 + H4N4 + H4N4F1 + H5N4 + H3N5F1 + H4N5 + H5N4F1 + H4N4E1 + H4N5F1 + H5N5 + H5N4E1 + H5N5F1 + H5N4F1E1 + H5N4E2 + H5N5F1E1 + H5N4F1E2 + H5N5F1E2) + 1/2 * (H5N4L1 + H5N4F1L1 + H5N4E1L1 + H5N4F1E1L1) + 2/2 * (H5N4L2 + H5N4F1L2)) / (H3N4F1 + H4N4 + H4N4F1 + H5N4 + H3N5F1 + H4N5 + H5N4F1 + H4N4E1 + H4N5F1 + H5N5 + H5N4L1 + H5N4E1 + H5N5F1 + H5N4F1L1 + H5N4F1E1 +H5N4L2 + H5N4E1L1 +

		H5N4E2 + H5N5F1E1 + H5N4F1L2 + H5N4F1E1L1 + H5N4F1E2 + H5N5F1E2)
A3E	Percentage of Sialylation of triantennary compositions	(1/3 * (H5N5E1F1 + H6N5E1 + + H6N5E1F1 + H6N5L1E1 + H6N5L1E1F1 + H6N5L2E1F1) + 2/3 * (H6N5E2 + H6N5L1E2 + H6N5L1E2F1)) / (1 * (H3N5 + H3N5F1 + H4N5 + H4N5F1 + H5N5 + H5N5F1 + H5N5E1F1 + H6N5E1 + H6N5E1F1 + H6N5L1E1 + H6N5E2 + H6N5L1E1F1 +H6N5L1E2 + H6N5L3F1 + H6N5L2E1F1 + H6N5L1E2F1))
A3L	Percentage of Sialylation of triantennary compositions	(H6N5F1L2 + H6N5F1L1E1 + H6N5F1L3 + H6N5F1L2E1 + H6N5F1L1E2 + H6N5F2L2E1 + H6N5F2L1E2) / (H6N5L2 + H6N5L1E1 + H6N5F1L2 + H6N5F1L1E1 + H6N5L3 + H6N5L2E1 + H6N5L1E2 + H6N5F1L3 + H6N5F1L2E1 + H6N5F1L1E2 + H6N5F2L2E1 + H6N5F2L1E2)
A4E	Percentage of Sialylation of tetraantennary compositions	(1/4 * (0) + 2/4 * (H7N6L1E2F1) + 3/4 * (0) + 4/4 * (0)) / (1 * (H7N6L1E2F1))
A4L	Percentage of Sialylation of tetraantennary compositions	(1/4 * (H7N6L1E2F1) + 2/4 * (0) + 3/4 * (0) + 4/4 * (0)) / (1 * (H7N6L2E2F1))
A2F0E	Percentage of (α2,6) Sialylation of non-fucosylated diantennary compositions	(0/2 * (H4N4 + H5N4 + H4N5 + H5N5 + H5N4L1 + H5N4L2) + 1/2 * (H4N4E1 + H5N4E1 + H5N4E1L1) + 2/2 * (H5N4E2)) / (H4N4 + H5N4 + H4N5 + H4N4E1 + H5N5 + H5N4L1 + H5N4E1 + H5N4L2 + H5N4E1L1 + H5N4E2)
A2F0L	Percentage of (α2,3) Sialylation of non-fucosylated diantennary compositions	(0/2 * (H4N4 + H5N4 + H4N5 + H4N4E1 + H5N5 + H5N4E1 + H5N4E2) + 1/2 * (H5N4L1) + 2/2 * (H5N4L2)) / (H4N4 + H5N4 + H4N5 + H4N4E1 + H5N5 + H5N4L1 + H5N4E1 + H5N4L2 + H5N4E1L1 + H5N4E2)
A3F0E	Percentage of (α2,6)Sialylation of non-fucosylated triantennary compositions	(1/3 * (H6N5E1 + H6N5L1E1) + 2/3 * (H6N5E2 + H6N5L1E2 + 3/3 * (0)) / (1 * (H3N5 + H4N5 + H5N5 + H6N5E1 + H6N5L1E1 +H6N5E2 + H6N5L1E2))
A3F0L	Percentage of (α2,3) Sialylation of non-fucosylated triantennary compositions	(1/3 * (H6N5L1E1 + H6N5L1E2) + 2/3 * (0) + 3/3 * (0)) / (1 * (H3N5 + H4N5 + H5N5 + H6N5E1 + H6N5L1E1 + H6N5L1E2))
A2FE	Percentage of (α2,6) Sialylation of fucosylated diantennary compositions	(0/2 * (H3N4F1 + H4N4F1 + H3N5F1 + H5N4F1 + H4N5F1 + H5N5F1 + H5N4F1L1 + H5N4F1L2) + 1/2 * (H5N4F1E1 + H5N4F2E1 + H5N5F1E1+ H5N4F1E1L1) + 2/2 * (H5N4F1E2 + H5N5F1E2)) / (H3N4F1 + H4N4F1 + H3N5F1 + H5N4F1 + H4N5F1 + H5N5F1 + H5N4F1L1 + H5N4F1E1 + H5N4F2E1 + H5N5F1E1 + H5N4F1L2 + H5N4F1E1L1 + H5N4F1E2 + H5N5F1E2)
A2FL	Percentage of (α2,3) Sialylation of fucosylated diantennary compositions	(0/2 * (H3N4F1 + H4N4F1 + H3N5F1 + H5N4F1 + H4N5F1 + H5N5F1 + H5N4F1E1 + H5N5F1E1 + H5N4F1E2 + H5N5F1E2) + 1/2 * (H5N4F1L1 + H5N4F1E1L1) + 2/2 * (H5N4F1L2)) / (H3N4F1 + H4N4F1 + H3N5F1 + H5N4F1 + H4N5F1 + H5N5F1 + H5N4F1L1 + H5N4F1E1 + H5N5F1E1 + H5N4F1L2 + H5N4F1E1L1 + H5N4F1E2 + H5N5F1E2)
A3FE	Percentage of (α2,6) Sialylation of fucosylated triantennary compositions	(1/3 * (H5N5E1F1 + H6N5E1F1 + H6N5L1E1F1 + H6N5L2E1F1) + 2/3 * (H6N5L1E2F1) + 3/3 * (0)) / (1 * (H3N5F1 + H4N5F1 + H5N5F1 + H5N5E1F1 + H6N5E1F1 + H6N5L1E1F1 + H6N5L3F1 + H6N5L2E1F1 + H6N5L1E2F1))

A3FL	Percentage of (α2,3) Sialylation of fucosylated triantennary compositions	(1/3 * (H6N5L1E1F1 + H6N5L1E2F1) + 2/3 * (H6N5L2E1F1) + 3/3 * (H6N5L3F1)) / (1 * (H3N5F1 + H4N5F1 + H5N5F1 + H4N5E1F1 + H5N5E1F1 + H6N5E1F1 + H6N5L1E1F1 + H6N5L3F1 + H6N5L2E1F1 + H6N5L1E2F1))

Table S6 (A). t-test and AUC results of *N*-glycan compositions between cancer cases and controls **for 2AA derivatization method.** p values were classified based on their significant degrees. Green Filled Lines: p<0.001; Blue Filled Lines: p<0.01; Yellow Filled Lines: p<0.05

***N*-glycan Composition**	**Tumor**		**Adjacent Normal**		**p Value**	**AUC**	**Direction**	**Fold Ratio**
	Mean	**SD**	**Mean**	**SD**				
H5N2	2,23	± 0,93	0,94	± 0,39	1,46E-03	0.913	↑	**2,37**

H3N3F1	1,16	± 0,38	0,72	± 0,25	0,00034	0.880	↑	**1,61**
H3N4	0,65	± 0,17	0,59	± 0,30	0,5242	0.676	↑	**1,11**
H6N2	2,40	± 0,89	1,13	± 0,29	3,01E-04	0,935	↑	**2,12**
H3N3S1	1,02	± 0,22	0,83	± 0,21	0,0264	0.760	↑	**1,22**
H3N4F1	2,31	± 1,38	1,03	± 0,23	0,0001	0.840	↑	**2,24**
H4N4	0,89	± 0,23	0,75	± 0,23	0,119	0.702	↑	**1,19**
H3N5	0,88	± 0,48	0,55	± 0,28	0,0141	0.753	↑	**1,60**
H7N2	1,92	± 0,50	1,06	± 0,23	3,33E-05	0.960	↑	**1,82**
H4N3S1	1,84	± 0,32	1,26	± 0,26	5,94E-03	0.970	↑	**1,46**
H6N3	0,75	± 0,17	0,64	± 0,15	0,0867	0.691	↑	**1,16**
H4N4F1	2,69	± 0,94	1,87	± 0,24	0,00034	0.686	↑	**1,44**
H5N4	1,73	± 0,54	1,38	± 0,18	0,006	0.673	↑	**1,26**
H3N5F1	1,40	± 0,74	0,85	± 0,24	0,0021	0.749	↑	**1,64**
H4N5	0,82	± 0,24	0,71	± 0,28	0,2505	0.676	↑	**1,17**
H4N3F1S1	2,26	± 0,61	1,52	± 0,24	9,64E-03	0.962	↑	**1,49**
H8N2	1,85	± 0,42	1,59	± 0,37	0,0786	0.662	↑	**1,16**
H5N3S1	1,00	± 0,18	0,84	± 0,17	0,0143	0.764	↑	**1,20**
H4N4S1	1,11	± 0,20	0,92	± 0,17	0,0065	0.807	↑	**1,21**
H5N4F1	7,46	± 2,57	6,56	± 1,00	0,1392	0.615	↑	**1,14**
H4N5F1	2,09	± 1,03	2,21	± 0,54	0,6671	0.629	↓	**0,95**
H5N5	0,67	± 0,17	0,76	± 0,16	0,1805	0.633	↓	**0,89**
H9N2	1,64	± 0,37	3,10	± 0,90	0,0002	0.894	↓	**0,53**

Table S6(A) continued…

***N*-glycan Composition**	**Tumor**		**Adjacent Normal**		**p Value**	**AUC**	**Direction**	**Fold Ratio**
	Mean	**SD**	**Mean**	**SD**				
H6N3S1	0,97	± 0,13	1,09	± 0,21	0,0952	0.647	↓	**0,89**
H4N4F1S1	1,93	± 0,36	1,66	± 0,18	0,0056	0.705	↑	**1,16**

H5N4S1	6,26	± 1,08	5,19	± 0,97	0,0083	0.778	↑	**1,20**
H5N5F1	1,88	± 0,78	3,89	± 1,53	0,001	0.902	↓	**0,48**
H5N4F1S1	15,21	± 2,02	15,72	± 2,32	0,5358	0.589	↓	**0,97**
H5N5S1	0,89	± 0,18	1,12	± 0,18	0,0026	0.822	↑	**0,80**
H6N5F1	1,15	± 0,24	1,41	± 0,20	0,0025	0.778	↓	**0,82**
H5N4S2	9,57	± 3,90	9,33	± 2,06	0,8124	0.491	↑	**1,03**
H5N5F1S1	1,82	± 0,35	3,23	± 0,77	8,30E-02	0.928	↓	**0,56**
H6N5S1	0,85	± 0,19	1,07	± 0,16	0,0016	0.789	↓	**0,79**
H5N4F1S2	7,62	± 2,62	8,10	± 1,46	0,4887	0.604	↓	**0,94**
H5N5S2	0,65	± 0,16	0,97	± 0,11	1,73E-07	0.985	↓	**0,67**
H6N5F1S1	2,22	± 0,85	3,38	± 0,58	5,85E-02	0.799	↓	**0,66**
H5N5F1S2	0,89	± 0,38	1,34	± 0,21	7,22E-02	0.815	↓	**0,66**
H6N5S2	0,93	± 0,38	1,08	± 0,26	0,182	0.633	↓	**0,86**
H6N5F1S2	1,52	± 0,74	2,42	± 0,55	0,0003	0.836	↓	**0,63**
H6N5S3	1,17	± 0,73	1,35	± 0,34	0,3436	0.618	↓	**0,87**
H7N6S2	0,45	± 0,16	0,59	± 0,16	0,0256	0.736	↓	**0,77**
H6N5F1S3	0,87	± 0,49	1,45	± 0,42	0,0015	0.811	↓	**0,60**
H7N6F1S2	0,78	± 0,44	1,42	± 0,41	0,0003	0.858	↓	**0,54**
H7N6S3	0,40	± 0,18	0,535	± 0,23	0,1075	0.705	↓	**0,75**
H7N6F1S3	0,41	± 0,20	0,66	± 0,22	0,0057	0.796	↓	**0,63**

Table S6 (B). t-test and AUC results of *N*-glycan compositions between cancer cases and controls for **ethyl-esterified derivatization method.** p values were classified based on their significant degrees. Green Filled Lines: p<0.001; Blue Filled Lines: p<0.01; Yellow Filled Lines: p<0.05

H7N6S4	0,46	± 0,27	0,71	± 0,35	0,0487	0.742	↓	**0,65**
H7N6F1S4	0,33	± 0,14	0,49	± 0,22	0,0458	0.764	↓	**0,68**

***N*-glycan Composition**	**Tumor**	**Adjacent Normal**	**p value**	**AUC**	**Direction**	**Fold Ratio**

	Mean	SD	Mean	SD				
H5N2	2,93	± 0,77	1,33	± 0,27	2.818e-10	0.989	↑	2,20
H3N3F1	0,50	± 0,29	0,15	± 0,05	5.065e-06	0.975	↑	3,23
H3N4	0,86	± 0,37	0,35	± 0,38	1.071e-03	0.884	↑	2,45
H6N2	2,55	± 0,76	1,33	± 0,43	8.146e-07	0.942	↑	1,91
H4N3F1	0,58	± 0,34	0,26	± 0,08	1.348e-04	0.942	↑	2,21
H5N3	0,29	± 0,10	0,27	± 0,07	0.534	0.505	↑	1,07
H3N4F1	1,76	± 1,12	1,03	± 0,46	0.00989	0.720	↑	1,70
H4N4	0,48	± 0,18	0,32	± 0,14	0.0102	0.756	↑	1,50
H3N5	0,35	± 0,32	0,19	± 0,11	0.0260	0.651	↑	1,89
H4N3L1	0,20	± 0,10	0,14	± 0,10	0.1518	0.669	↑	1,39
H7N2	1,71	± 0,65	0,72	± 0,27	1.904e-07	0.920	↑	2,38
H6N3	0,34	± 0,11	0,33	± 0,08	0.8167	0.502	↑	1,02
H4N4F1	2,93	± 0,91	2,37	± 0,63	0.0501	0.647	↑	1,24
H5N4	2,44	± 0,77	1,82	± 0,48	0.0098	0.720	↑	1,34
H3N5F1	1,07	± 0,50	0,82	± 0,27	0.0636	0.640	↑	1,31
H4N5	0,43	± 0,22	0,60	± 0,22	0.0464	0.724	↓	0,72
H5N3L1	0,26	± 0,10	0,23	± 0,14	0.5041	0.531	↑	1,15
H8N2	1,65	± 0,77	1,05	± 0,39	0.0031	0.727	↑	1,57
H5N4F1	13,51	± 3,47	11,91	± 3,18	0.2163	0.615	↑	1,13
H4N4E1	0,33	± 0,33	0,14	± 0,14	0.0149	0.724	↑	2,46
H6N4	0,17	± 0,16	0,29	± 0,21	0.0847	0.727	↓	0,57
H4N5F1	2,18	± 0,61	3,69	± 1,19	0.0017	0.898	↓	0,59
H5N5	0,35	± 0,14	0,56	± 0,24	0.0188	0.782	↓	0,63
H9N2	1,20	± 0,63	1,86	± 0,88	0.0335	0.738	↓	0,64
H5N4L1	2,39	± 0,55	1,99	± 0,55	0.0784	0.695	↑	1,20
H5N4E1	2,14	± 0,57	3,02	± 1,22	0.0742	0.673	↓	0,71
H5N5F1	2,47	± 0,78	6,60	± 3,09	9.520e-04	0.989	↓	0,37

Table S6(B) continued…

***N*-glycan Composition**	**Tumor**		**Adjacent Normal**		**p value**	**AUC**	**Direction**	**Fold Ratio**
	Mean	**SD**	**Mean**	**SD**				
H5N4F1L1	16,93	± 4,36	16,94	± 5,17	0.9921	0.418	↓	1,00

H6N4L1	0,57	± 0,19	0,56	± 0,23	0.8844	0.549	↑	1,02
H5N4F1E1	4,08	± 1,36	2,51	± 0,62	2.749e-05	0.887	↑	1,63
H5N4L2	0,95	± 0,33	0,67	± 0,20	0.0048	0.764	↑	1,41
H5N4E1L1	1,03	± 0,42	0,91	± 0,38	0.4319	0.571	↑	1,14
H5N4E2	3,47	± 1,84	10,16	± 5,56	0.0066	0.782	↓	0,34
H5N5F1E1	0,62	± 0,29	0,53	± 0,41	0.5086	0.629	↑	1,17
H6N5E1	0,73	± 0,62	0,48	± 0,44	0.1702	0.644	↑	1,53
H5N4F1L2	11,28	± 3,47	7,92	± 2,49	0.0026	0.789	↑	1,42
H5N4F1E1L1	2,56	± 1,13	1,11	± 0,29	1.46e-06	0.931	↑	2,30
H5N4F1E2	3,25	± 0,94	3,80	± 0,51	0.0578	0.662	↓	0,86
H6N5F1E1	0,46	± 0,16	0,38	± 0,12	0.1355	0.618	↑	1,20
H6N5E1L1	0,12	± 0,15	0,23	± 0,16	0.0996	0.673	↓	0,53
H5N5F1E2	0,33	± 0,23	1,31	± 0,72	0.0019	0.865	↓	0,25
H6N5E2	0,11	± 0,16	0,25	± 0,19	0.1054	0.687	↓	0,45
H6N5F1L2	2,36	± 0,64	2,42	± 0,76	0.7985	0.527	↓	0,97
H6N5F1E1L1	0,46	± 0,20	0,27	± 0,14	0.0034	0.787	↑	1,68
H6N5F1E2	0,66	± 0,22	0,77	± 0,21	0.1783	0.640	↓	0,86
H6N5E2L1	0,34	± 0,30	1,30	± 0,77	0.0293	0.813	↓	0,26
H6N5F1L3	1,53	± 0,63	1,13	± 0,55	0.0698	0.676	↑	1,35
H6N5F1E1L2	0,51	± 0,50	0,20	± 0,19	0.0122	0.753	↑	2,51
H6N5F1E2L1	1,13	± 0,48	2,00	± 0,85	0.0098	0.807	↓	0,57
H7N6F1E2L1	0,38	± 0,27	0,63	± 0,42	0.0759	0.680	↓	0,59

Table S7 (A). t-test and AUC results of N-glycan traits between cancer cases and controls **for 2-AA derivatization method**. p values were classified based on their significant degrees. Green Filled Lines: p<0.001; Blue Filled Lines: p<0.01; Yellow Filled Lines: p<0.05

***N*-Glycan Traits**	**Tumor**		**Adjacent Normal**		**p value**	**AUC**	**Direction**	**Fold Ratio**
	Mean	**SD**	**Mean**	**SD**				

M	10.042	± 2.21	7.801	± 1.75	0,0034	0.789	↑	**1,29**
Hy	2.715	± 0.41	2.571	± 0.44	0,3712	0.598	↑	**1,06**
C	90.612	± 2.20	92.409	± 1.67	0,0126	0.745	↓	**0,98**
A1	6.276	± 1.03	4.331	± 0.89	0,008947	0.920	↑	**1,45**
A2	79.940	± 4.22	74.358	± 2.39	0,01904	0.865	↑	**1,08**
A3	16	± 2.43	10.796	± 1.55	0,04043	0.862	↓	**0,00**
A4	2.367	± 1.05	3.691	± 1.11	0,0037	0.818	↓	**0,64**
B	11.998	± 3.08	15.627	± 3.12	0,0045	0.811	↓	**0,77**
F	55.996	± 3.99	59.938	± 3.17	0,0042	0.782	↓	**0,93**
AG	66.096	± 4.03	71.173	± 3.25	0,00054	0.829	↓	**0,93**
A2F	62.975	± 5.58	65.030	± 3.15	0,1709	0.614	↓	**0,97**
A3F	65.214	± 4.59	66.647	± 3.03	0,2785	0.589	↓	**0,98**
A2B	15.359	± 3.44	20.834	± 4.10	0,00013	0.851	↓	**0,74**
A2F0B	5.031	± 0.97	5.473	± 1.02	0,2415	0.614	↓	**0,92**
A2FB	16.733	± 2.43	20.087	± 1.96	0,00021	0.880	↓	**0,83**
A2S	50.576	± 7.47	52.256	± 3.54	0,3672	0.611	↓	**0,97**
A3S	13.445	± 4.13	17.097	± 2.67	0,0036	0.760	↓	**0,79**
A4S	1.497	± 0.56	2.167	± 0.57	0,0044	0.814	↓	**0,69**
A2F0S	21.074	± 5.71	20.533	± 2.97	0,7122	0.513	↑	**1,03**
A3F0S	26.789	± 7.49	22.641	± 4.64	0,0522	0.716	↑	**1,18**
A2FS	24.731	± 4.59	27.358	± 2.83	0,0448	0.712	↓	**0,90**
A3FS	33.448	± 5.30	38.529	± 3.45	0,0019	0.793	↓	**0,87**

Table S7 (B). t-test and AUC results of N-glycan traits between cancer cases and controls **for ethyl-esterified derivatization method**. p values were classified based on their significant degrees. Green Filled Lines: p<0.001; Blue Filled Lines: p<0.01; Yellow Filled Lines: p<0.05

N-Glycan Traits	Tumor		Adjacent Normal					
	Mean	SD	Mean	SD	p value	AUC	Direction	Fold Ratio
M	10,04	± 3.03	6,27	± 1.78	5.795e-05	0.862	↑	**1,60**
Hy	4,88	± 1.37	3,40	± 0.54	5.339e-05	0.862	↑	**1,43**
C	80,76	± 3.53	82,81	± 2.29	0.0470	0.694	↓	**0,98**
A1	0,20	± 0.10	0,14	± 0.10	0.1518	0.669	↑	**1,39**
A2	68,18	± 3.49	65,71	± 6.38	0.2490	0.629	↑	**1,04**
A3	12,41	± 2.47	17,72	± 4.70	0.0038	0.869	↓	**0,70**
A4	0,38	± 0.27	0,63	± 0.41	0.0757	0.680	↓	**0,59**
B	6,87	± 1.67	13,45	± 5.09	0.0015	0.949	↓	**0,51**
F	54,66	± 2.37	51,97	± 4.67	0.0943	0.774	↑	**1,05**
AG	75,03	± 4.04	75,05	± 6.25	0.9913	0.530	↓	**1,00**
A2F	81,27	± 3.47	71,27	± 11.13	0.0141	0.753	↑	**1,14**
A3F	69,58	± 6.60	77,42	± 7.96	0.0111	0.792	↓	**0,90**
A2B	9,97	± 2.83	18,47	± 7.44	0.0035	0.909	↓	**0,54**
A2F0B	9,67	± 2.68	17,53	± 6.71	0.0029	0.909	↓	**0,55**
A2FB	10,59	± 2.86	21,71	± 7.63	0.0006	0.923	↓	**0,49**
A2E	16,95	± 5.28	30,23	± 16.98	0.0277	0.716	↓	**0,56**
A2L	24,51	± 3.97	19,20	± 4.71	0.0047	0.821	↑	**1,28**
A3E	15,93	± 4.10	15,99	± 7.29	0.9794	0.534	↓	**1,00**
A3L	20,13	± 7.87	13,13	± 5.62	0.0054	0.785	↑	**1,53**
A2F0E	2,48	± 0.964	1,99	± 0.469	0.1537	0.909	↑	**1,24**
A2F0L	15,40	± 3.30	9,88	± 5.76	0.0108	0.818	↑	**1,56**
A3F0E	24,09	± 9.01	30,89	± 12.75	0.1307	0.654	↓	**0,78**
A3F0L	6,97	± 6.57	13,06	± 7.74	0.0366	0.721	↓	**0,53**
A2FE	11,53	± 3.17	12,41	± 4.36	0.5523	0.567	↓	**0,93**
A2FL	33,28	± 5.67	27,79	± 5.71	0.0153	0.756	↑	**1,20**
A3FE	13,97	± 3.66	12,04	± 4.96	0.2641	0.607	↑	**1,16**
A3FL	23,12	± 8.30	13,31	± 5.98	4.54e-04	0.814	↑	**1,74**