Supporting Information

Experimental section:

Materials and Chemicals. Ovalbumin from chicken egg white (OVA), myoglobin (MYO), ammonium bicarbonate (ABC), dithiothreitol (DTT), iodoacetamide (IAA), sodium cyanoborohydrate ($NaCNBH_3$), dimethyl sulfoxide (DMSO), sodium deoxycholate (SDC), sodium lauroyl sarcosinate (SLS), 2, 5-dihydroxybenzoinic acid (DHB), trifluoro-acetic acid (TFA) were purchased from Sigma. Ethyl acetate (EtOAc) and sodium chloride (NaCl) were purchased from Sinopharm. PNGase F was obtained from New England Biolabs. Phosphate-buffered saline (PBS) and AminoLink Coupling Resin were purchased from Thermo Fisher Scientific. Acetonitrile (ACN) was obtained from Merck. Protease inhibitors (Complete tablets) was purchased from Roche Diagnostics. HILIC Martial was purchased from Welch. Sep-Pak C18 column was purchased from Waters. Exosome Purification Filter and Blood PureExo Solution were purchased from Umibio. Deionized water used for all experiments was obtained from a Milli-Q system (Millipore, Bedford, MA).

Isolation of Serum exosome. The Institutional Review Board of Zhongshan Hospital approved the use of human plasma samples. Exosome were separated from 0.5 mL of pooled serum from 6 HCC patient or healthy control by differential centrifugation. In brief, an initial spin was performed at 3,000 g at 4°C for 10 min and 10,000 g at 4 °C for 20 min to remove cells and debris, then the 0.5 mL of Blood PureExo Solution (BPS) were added to the sample, according to the manufacturer's instructions. Mixtures were vortexed and incubated at 4 °C for 2 h and then centrifuged at 10, 000 g at 4 °C for 60 min to precipitate the exosome pellets. Pellets were resuspended with 400 μL PBS and purified with Exosome Purification Filter. All exosomes were stored at -80 °C immediately after isolation until further analysis.

Characterization of exosome. The exosome was measured using transmission electron microscope (TEM). First, exosomes were fixed in 2% formaldehyde in PBS overnight at 4 °C for negative staining. Exosomes were added to mesh grids coated with Formvar in chloroform and carbon and were further fixed with 50 μL 1% glutaraldehyde in PBS for 5 min. Then the grids were transferred to eight successive drops of distilled water (2 min in total) to remove salts, with the following step that transferred to a drop of 1% uranyl acetate in 1% methyl cellulose for 5 min. In a second drop of negative stain solution, the grids were placed for 5 min. Excess stain was blotted off and grids were air dried (about 5-10 min). Finally, the grids were observed at 80 kV on a transmission electron microscope and photographed. The exosome particle size and concentration were measured using nanoparticle tracking analysis (NTA) with ZetaView PMX 110 (Particle Metrix, Meerbusch, Germany) and corresponding software ZetaView 8.04.02. First, isolated exosomes were diluted with PBS buffer. Then, NTA measurement was recorded and analyzed at 11 positions. For immunoblotting, proteins from exosomes were analyzed by SDS-PAGE and transferred to polyvinyledene fluoride membrane that were then blocked for 30 min with 5 % non-fat powered milk in Tris-buffered saline (TBS) with 0.1% Tween-20 (TBST). The following antibody was used: rabbit anti-CD9 (1:1000) (Abcam). Secondary antibody was goat antirabbit immunoglobulin G (IgG) coupled to HRP (1:10000). The membrane was washed with TBST and CD9 was analyzed in nonreducing conditions.

Exosome Lysis and Protein Digestion. The exosome lysis and digestion were performed according to phase transfer surfactant aided (PTS) digestion with modification.[1] The exosomes were

solubilized by lysis buffer that containing 12 mM SDC, 12 mM SLS, and protease inhibitors in 100 mM Tris-HCl at pH 8.5. After being measured the concentration by BCA protein determination method, the obtained proteins were reduced with 10 mM DTT for 45 min at 37 °C, and subsequently alkylated by 20 mM IAA for 30 min at 25 °C in the dark. The solution was then diluted to five-folds with 50 mM ABC and digested with Lys-C in a 1:50 (w/w) enzyme-to-protein ratio at 37 °C for 3 h. Then trypsin was added to a final 1:50 (w/w) enzyme-to-protein ratio for overnight digestion. After digestion, trifluoroacetic acid was added to a final concentration of 0.5% to acidify the sample and followed by adding of ethyl acetate with the same volume of the sample. The mixture was vortexed for 2 min, and then centrifuged at 15,000 g for 2 min to separate the sample to two phases. Upper layer was removed, and the lower aqueous phase was dried in speed vacuum. After dried, the sample was desalted with Sep-Pak C18 column and lyophilized in vacuum.

Reverse capture of N-glycans. AminoLink aldehyde-functionalized resin (50% slurry, Pierce) was pre-washed three times using PBS. Peptides were incubated overnight with resin in the presence of 50 mM $NaCNBH_3$ at 37 °C to immobilize all the peptides on the resin. Then the resin was washed three times with PBS and 1 M Tris-HCl solution (pH 7.4), respectively. Finally, the N-glycans were released by 1 μL PNGase F at 37 °C overnight with shaking. The supernatant that contained N-glycans was collected and dried by vacuum for further use.

Protect the sialic acid. Methylamidation of sialyated N-glycans was performed according to the reported protocol.[2, 3] Briefly, 45 μL of 5 M methylamide chroloride in DMSO and 45 μL of 1 M PyAOP in 30% N-methylmorpholine/DMSO were added to the N-glycans. The solution was incubated for 1.5 h in room temperature. After reaction, 2 mL 80% ACN, 1% TFA was added to quench the reaction.

Investigation the ratio of peptides to resin and the capacity of resin. 100 μg of tryptic OVA peptides were incubated with different amounts of resin and proceeded with the reverse phase capture procedure, then the supernatants containing the released N-glycans were analyzed by MS. According to the number of glycans detected and the intensity of base peak, the appropriate ratio of glycoprotein to resin was estimated as 100 μg peptides using 200 μL resin (Figure S2). Next, the binding capacity was checked. 200 μL of resin was incubated with 100 μL of OVA tryptic peptides solutions at different concentration and the supernatants were analyzed. After the samples were loaded, the flow-through fractions were analyzed by MS. When the total amount of peptides was lower than the capacity of the resin, the peptides could not be detected. Once the signal of peptides was detected, it indicated that the material could not capture all the peptides at the concentration in question.

MALDI Sample Preparation and Analysis. DHB was dissolved in 50% ACN containing 0.1% TFA at a concentration of 10 mg/mL. 1 μL of sample was loaded onto a MALDI target and mixed with 1 μL of DHB solution, then the mixture was dried before MS analysis. The MALDI-TOF MS analysis was performed on a 5800 Proteomics Analyzer (Applied Biosystems, Framingham, MA, USA) equipped with a Nd:YAG laser (355 nm), an acceleration voltage of 20 kV, and a repetition rate of 400 Hz. The mass spectrometer was operated in positive mode. In addition, external mass calibration was performed using peptides from myoglobin digests.

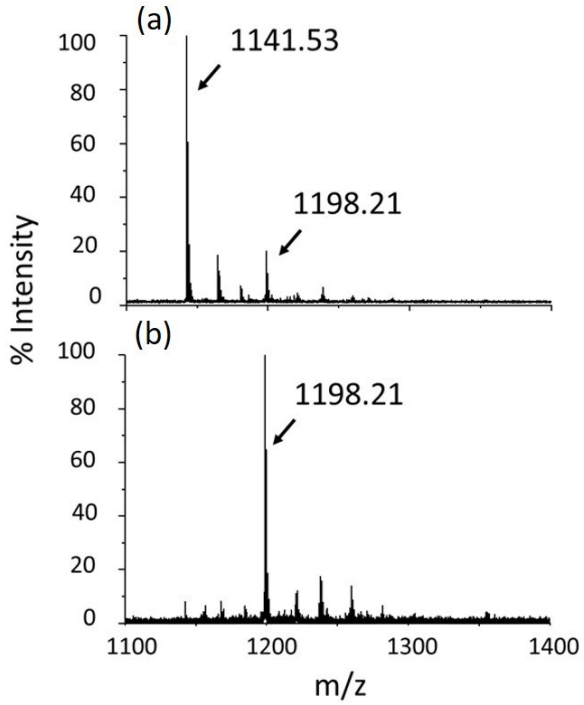


Fig. S1. MALDI mass spectra of the mixture of standard peptide GGYTLVSGYPK and its dimethylated counterparts (with a molar ratio 10:1) (a) before and (b) after captured by resin.

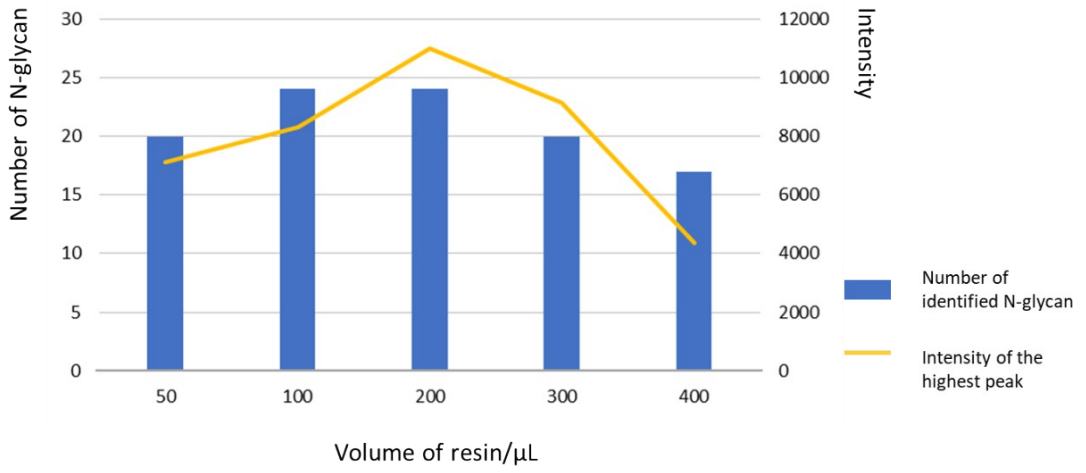


Fig. S2 The number of detected N-glycans and the highest intensity of base peak from the same amount OVA enriched by a series of different volume of serum.

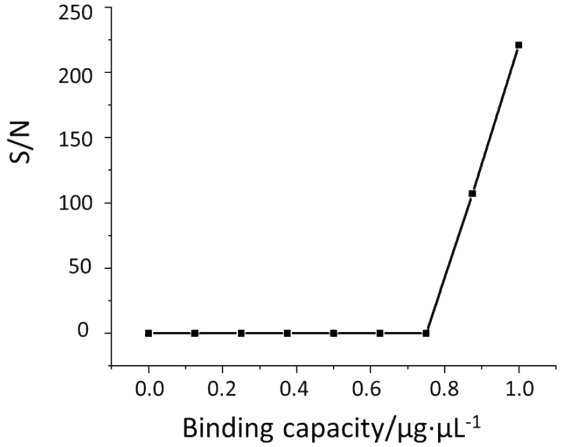


Fig. S3 Binding capacity analysis of the reverse capture method.

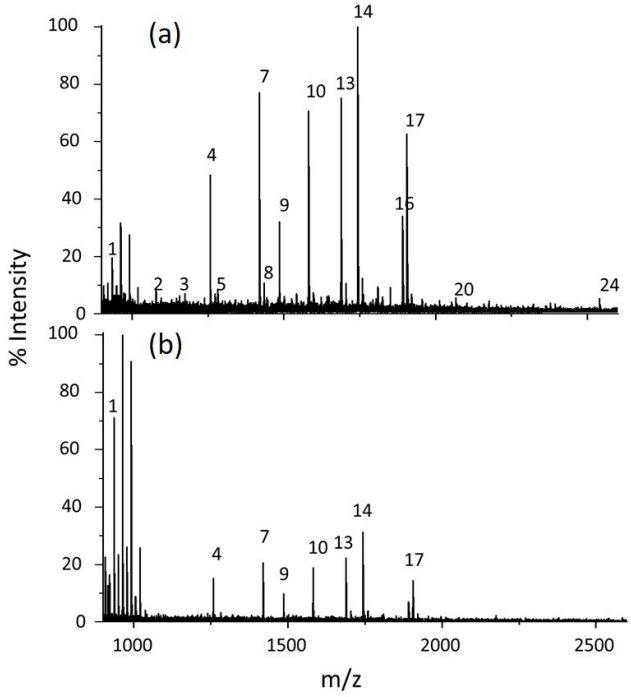


Fig. S4 MALDI mass spectra of the tryptic digest of 0.5 ng/μL OVA (a) after enrichment by the reverse phase capture protocol, and (b) after enrichment by HILIC tips (glycan peaks are labeled with Arabic numbers).

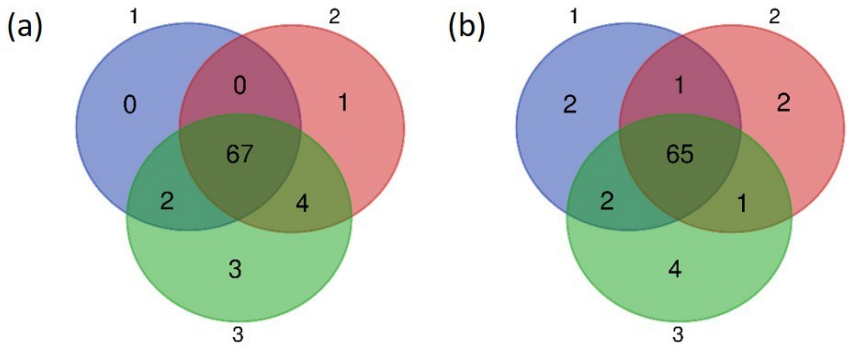


Fig. S5 Venn diagram of the number of N-glycans identified from serum exosomes of (a) HCC patients and (b) normal controls.

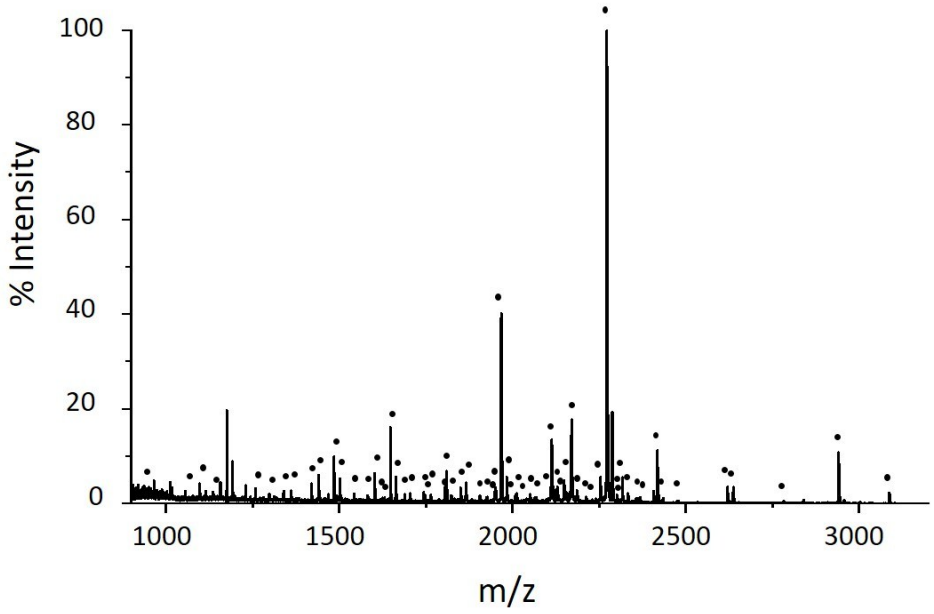


Fig. S6 MALDI mass spectrum of the N-glycans from serum of normal controls.

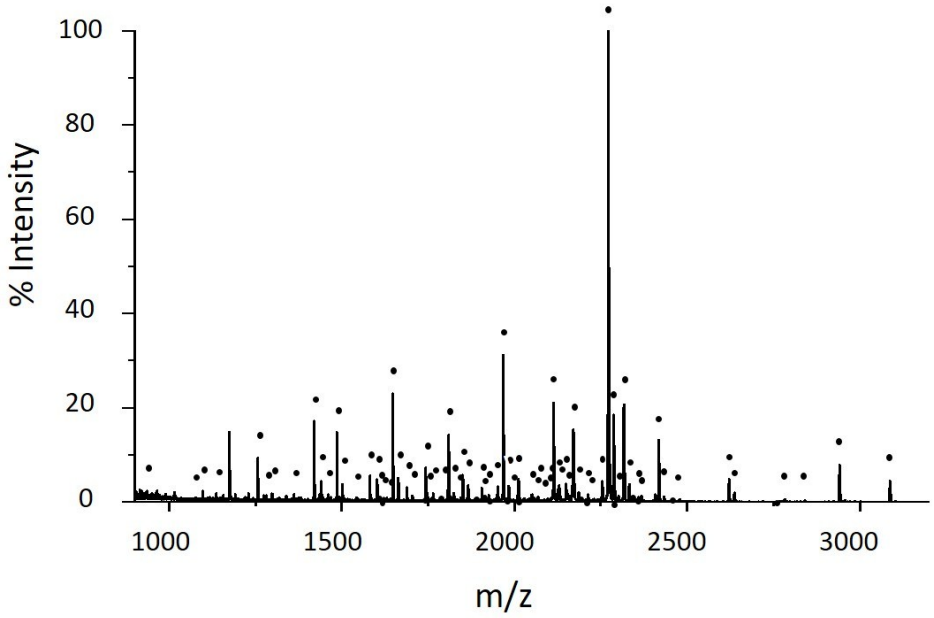


Fig. S7 MALDI mass spectrum of the N-glycans from serum of HCC patients.

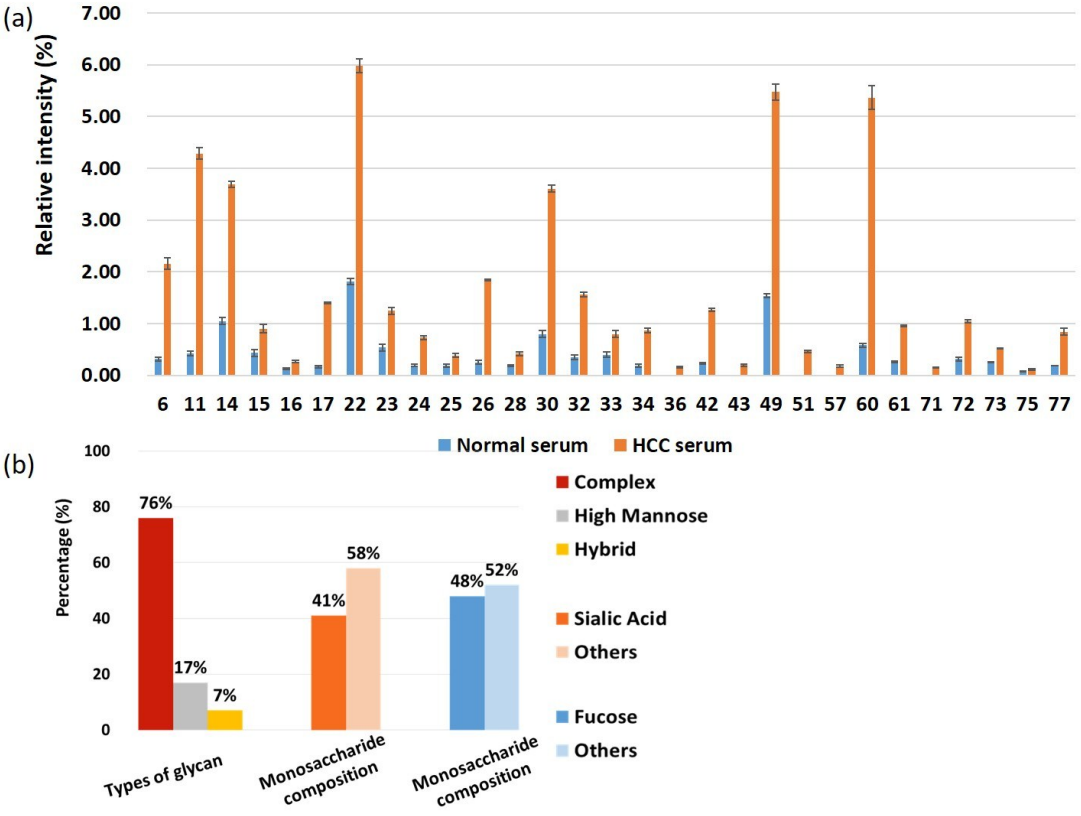
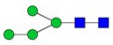
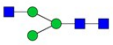
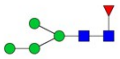
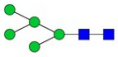
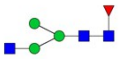
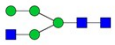
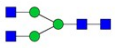
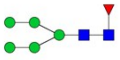
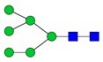
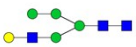
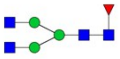
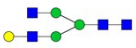
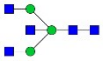
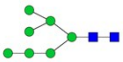
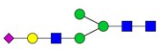
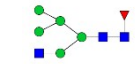
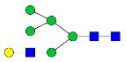
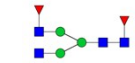
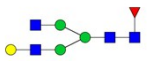
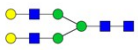
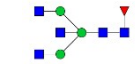
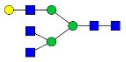
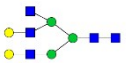
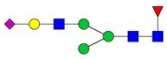
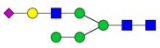
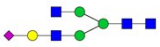
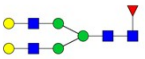
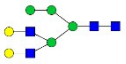
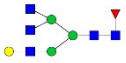
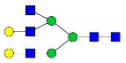
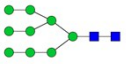
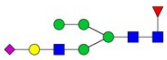
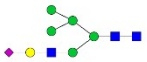
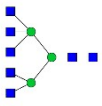
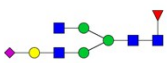
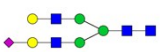
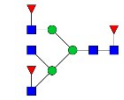
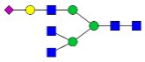
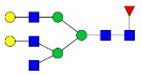
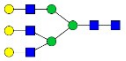
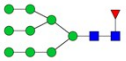
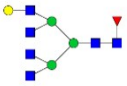
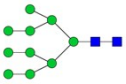
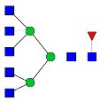
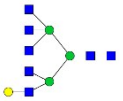
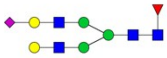
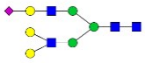
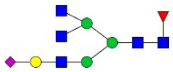
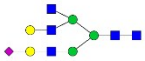
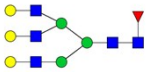
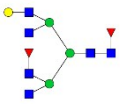
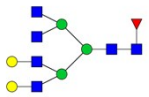


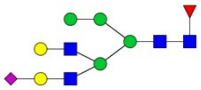
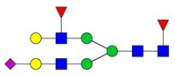
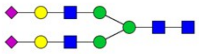
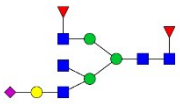
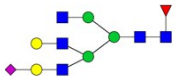
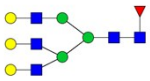
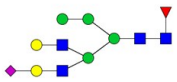
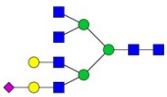
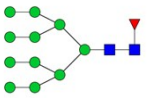
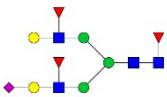
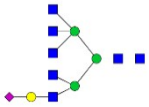
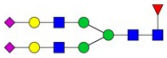
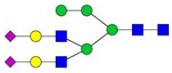
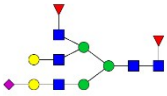
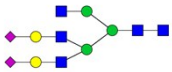
Fig. S8. (a) Relative intensity of N-glycans from serums of normal controls and HCC patients, (b) Type distribution and monosaccharide composition distributions of the increased glycans of serum.

Table S1 N-glycan compositions identified from exosomes in serum of HCC patients.

No.	Potential Structure*	Native mass** /Da	Detection		Relative Intensity (%)	CV
			Peak***	m/z Da		
1		910.33	$[M+Na]^+$	933.22	0.25	0.42
2		1056.39	$[M+Na]^+$	1079.26	0.23	0.62
3		1072.38	$[M+Na]^+$	1095.26	0.29	0.52
4		1113.41	$[M+Na]^+$	1136.29	0.43	0.55
5		1218.44	$[M+Na]^+$	1241.25	0.18	0.46
6		1234.43	$[M+Na]^+$	1257.30	3.22	0.21
7		1259.47	$[M+Na]^+$	1282.32	0.60	0.48
8		1275.46	$[M+Na]^+$	1298.30	0.52	0.54
9		1316.49	$[M+Na]^+$	1339.33	0.24	0.46
10		1380.49	$[M+Na]^+$	1403.25	0.19	0.21
11		1396.49	$[M+Na]^+$	1419.32	4.58	0.12
12		1421.52	$[M+Na]^+$	1444.33	0.52	0.34
13		1437.51	$[M+Na]^+$	1460.33	0.55	0.23
14		1462.54	$[M+Na]^+$	1485.36	3.51	0.07
15		1478.54	$[M+Na]^+$	1501.34	1.14	0.23
16		1519.57	$[M+Na]^+$	1542.35	0.22	0.36
17		1558.54	$[M+Na]^+$	1581.33	1.74	0.09
18		1566.56	$[M+ma+Na]^+$	1602.37	0.95	0.36

19		1583.57	$[M+Na]^+$	1606.37	0.30	0.15
20		1599.57	$[M+Na]^+$	1622.37	0.25	0.18
21		1608.60	$[M+Na]^+$	1631.38	0.21	0.24
22		1624.60	$[M+Na]^+$	1647.38	6.16	0.02
23		1640.59	$[M+Na]^+$	1663.36	1.77	0.11
24		1665.62	$[M+Na]^+$	1688.40	0.62	0.09
25		1681.62	$[M+Na]^+$	1704.38	0.37	0.19
26		1720.59	$[M+Na]^+$	1743.34	2.48	0.01
27		1712.61	$[M+ma+Na]^+$	1748.40	0.65	0.24
28		1728.61	$[M+ma+Na]^+$	1764.40	0.63	0.14
29		1769.63	$[M+ma+Na]^+$	1805.41	1.16	0.09
30		1786.65	$[M+Na]^+$	1809.40	4.59	0.07
31		1802.64	$[M+Na]^+$	1825.38	0.70	0.03
32		1827.68	$[M+Na]^+$	1850.42	1.94	0.04
33		1843.67	$[M+Na]^+$	1866.39	1.12	0.07
34		1882.64	$[M+Na]^+$	1905.36	2.33	0.06
35		1874.67	$[M+ma+Na]^+$	1910.41	0.25	0.58
36		1890.66	$[M+ma+Na]^+$	1926.40	0.34	0.05
37		1925.72	$[M+Na]^+$	1948.44	0.38	0.33
38		1915.69	$[M+ma+Na]^+$	1951.45	0.98	0.07
39		1931.69	$[M+ma+Na]^+$	1967.43	9.74	0.07

40		1957.74	$[M+Na]^+$	1980.41	0.69	0.02
41		1972.71	$[M+ma+Na]^+$	2008.43	0.24	0.18
42		1989.73	$[M+Na]^+$	2012.44	1.42	0.10
43		2005.72	$[M+Na]^+$	2028.44	0.52	0.10
44		2028.70	$[M+Na]^+$	2051.47	0.22	0.91
45		2030.76	$[M+Na]^+$	2053.42	0.34	0.13
46		2044.70	$[M+Na]^+$	2067.47	0.24	0.15
47		2071.78	$[M+Na]^+$	2094.42	0.26	0.25
48		2087.78	$[M+Na]^+$	2110.41	0.44	0.18
49		2077.75	$[M+ma+Na]^+$	2113.47	7.62	0.13
50		2093.74	$[M+ma+Na]^+$	2129.44	1.22	0.03
51		2118.77	$[M+ma+Na]^+$	2154.40	0.68	0.07
52		2134.77	$[M+ma+Na]^+$	2170.45	2.24	0.07
53		2151.78	$[M+Na]^+$	2174.44	0.92	0.24
54		2176.81	$[M+Na]^+$	2199.45	0.21	0.25
55		2192.81	$[M+Na]^+$	2215.44	0.25	0.58

56		2239.80	$[M+ma+Na]^+$	2256.00	0.50	0.21
57		2223.80	$[M+ma+Na]^+$	2259.52	0.23	0.12
58		2222.78	$[M+2ma+Na]^+$	2271.42	13.78	0.13
59		2264.83	$[M+ma+Na]^+$	2300.00	0.29	0.23
60		2280.82	$[M+ma+Na]^+$	2316.50	5.27	0.14
61		2296.82	$[M+ma+Na]^+$	2332.40	1.70	0.10
62		2321.85	$[M+ma+Na]^+$	2357.49	0.38	0.25
63		2337.85	$[M+ma+Na]^+$	2373.46	0.15	0.22
64		2352.81	$[M+Na]^+$	2375.47	0.19	0.39
65		2369.86	$[M+ma+Na]^+$	2405.54	0.17	0.18
66		2378.87	$[M+ma+Na]^+$	2414.62	0.17	0.58
67		2368.84	$[M+2ma+Na]^+$	2417.55	2.07	0.15
68		2411.88	$[M+2ma+Na]^+$	2433.54	0.21	0.02
69		2426.88	$[M+ma+Na]^+$	2462.53	0.14	0.24
70		2425.86	$[M+2ma+Na]^+$	2474.30	0.06	0.25

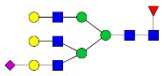
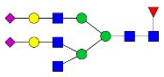
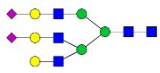
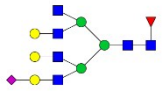
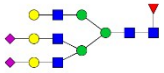
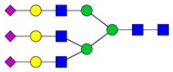
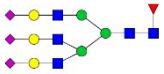
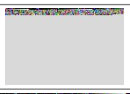
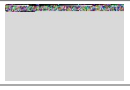
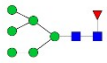
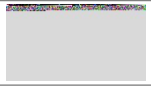
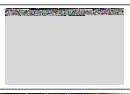
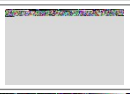
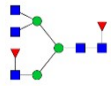
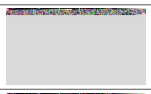
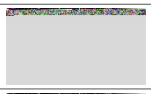
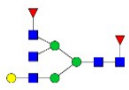
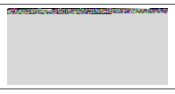
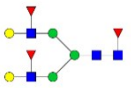
71		2442.88	$[M+ma+Na]^+$	2478.53	0.29	0.14
72		2571.92	$[M+2ma+Na]^+$	2620.59	0.87	0.22
73		2587.92	$[M+2ma+Na]^+$	2636.59	0.20	0.10
74		2645.96	$[M+ma+Na]^+$	2681.55	0.03	0.31
75		2733.97	$[M+2ma+Na]^+$	2782.51	0.04	0.07
76		2879.00	$[M+3ma+Na]^+$	2941.62	0.13	0.25
77		3025.07	$[M+3ma+Na]^+$	3087.62	0.05	0.13

Table S2 N-glycan compositions identified from exosomes in serum of normal controls.

No.	Potential Structure*	Native mass** /Da	Detection		Relative Intensity (%)	CV
			Peak***	m/z Da		
1		910.33	$[M+Na]^+$	933.25	0.28	0.33
2		1056.39	$[M+Na]^+$	1079.29	0.33	0.28
3		1072.38	$[M+Na]^+$	1095.28	0.43	0.30
4		1113.41	$[M+Na]^+$	1136.30	0.88	0.24
5		1218.44	$[M+Na]^+$	1241.30	0.30	0.26

6		1234.43	$[M+Na]^+$	1257.31	5.01	0.13
7		1259.47	$[M+Na]^+$	1282.34	0.49	0.22
8		1275.46	$[M+Na]^+$	1298.33	0.97	0.20
9		1316.49	$[M+Na]^+$	1339.34	0.36	0.19
10		1380.49	$[M+Na]^+$	1403.34	0.28	0.23
11		1396.49	$[M+Na]^+$	1419.34	6.25	0.07
12		1421.52	$[M+Na]^+$	1444.36	0.51	0.12
13		1437.51	$[M+Na]^+$	1460.35	1.09	0.07
14		1462.54	$[M+Na]^+$	1485.38	1.67	0.07
15		1478.54	$[M+Na]^+$	1501.36	0.95	0.13
16		1519.57	$[M+Na]^+$	1542.40	0.24	0.35
17		1542.54	$[M+Na]^+$	1565.00	0.23	0.15
18		1558.54	$[M+Na]^+$	1581.36	3.03	0.05
19		1566.56	$[M+ma+Na]^+$	1602.41	1.09	0.10
20		1583.57	$[M+Na]^+$	1606.39	0.56	0.02
21		1599.57	$[M+Na]^+$	1622.37	0.39	0.04
22		1608.60	$[M+Na]^+$	1631.40	0.20	0.58
23		1624.60	$[M+Na]^+$	1647.41	2.33	0.04
24		1640.59	$[M+Na]^+$	1663.39	1.50	0.06
25		1665.62	$[M+Na]^+$	1688.44	0.30	0.10
26		1681.62	$[M+Na]^+$	1704.42	0.33	0.05
27		1720.59	$[M+Na]^+$	1743.38	3.72	0.03

28		1712.61	$[M+ma+Na]^+$	1748.44	1.07	0.06
29		1728.61	$[M+ma+Na]^+$	1764.43	0.70	0.02
30		1769.63	$[M+ma+Na]^+$	1805.45	1.62	0.03
31		1786.65	$[M+Na]^+$	1809.44	1.42	0.01
32		1802.64	$[M+Na]^+$	1825.42	0.52	0.06
33		1811.68	$[M+Na]^+$	1834.43	0.27	0.58
34		1827.68	$[M+Na]^+$	1850.46	2.47	0.03
35		1843.67	$[M+Na]^+$	1866.43	1.85	0.06
36		1882.64	$[M+Na]^+$	1905.41	1.37	0.05
37		1874.67	$[M+ma+Na]^+$	1910.47	0.38	0.01
38		1890.66	$[M+ma+Na]^+$	1926.45	0.38	0.02
39		1925.72	$[M+Na]^+$	1948.49	0.40	0.25
40		1915.69	$[M+ma+Na]^+$	1951.48	1.10	0.58
41		1931.69	$[M+ma+Na]^+$	1967.48	14.50	0.04
42		1957.74	$[M+Na]^+$	1980.42	0.87	0.25
43		1973.73	$[M+Na]^+$	1996.50	0.27	0.58
44		1972.71	$[M+ma+Na]^+$	2008.53	0.27	0.03
45		1989.73	$[M+Na]^+$	2012.48	2.06	0.07
46		2005.72	$[M+Na]^+$	2028.49	1.14	0.03

47		2028.70	$[M+Na]^+$	2051.50	0.34	0.06
48		2030.76	$[M+Na]^+$	2053.45	0.52	0.10
49		2044.70	$[M+Na]^+$	2067.44	0.30	0.03
50		2078.77	$[M+Na]^+$	2101.51	0.37	0.07
51		2087.78	$[M+Na]^+$	2110.48	0.43	0.06
52		2077.75	$[M+ma+Na]^+$	2113.51	4.64	0.09
53		2093.74	$[M+ma+Na]^+$	2129.49	0.97	0.08
54		2118.77	$[M+ma+Na]^+$	2154.54	1.53	0.24
55		2134.77	$[M+ma+Na]^+$	2170.52	1.76	0.01
56		2151.78	$[M+Na]^+$	2174.52	0.56	0.02
57		2192.81	$[M+Na]^+$	2215.50	0.34	0.07
58		2239.80	$[M+ma+Na]^+$	2256.00	0.47	0.02
59		2222.78	$[M+2ma+Na]^+$	2271.57	9.25	0.09
60		2280.82	$[M+ma+Na]^+$	2316.56	7.53	0.10
61		2296.82	$[M+ma+Na]^+$	2332.55	2.01	0.07
62		2321.85	$[M+ma+Na]^+$	2357.54	0.21	0.18

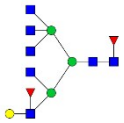
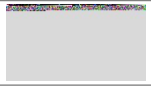
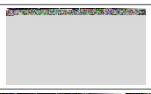
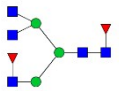
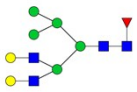
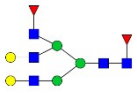
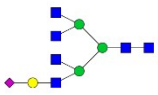
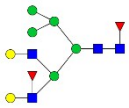
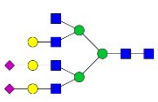
63		2352.81	$[M+Na]^+$	2376.53	0.29	0.19
64		2379.89	$[M+Na]^+$	2402.53	0.16	0.58
65		2369.86	$[M+ma+Na]^+$	2405.57	0.14	0.03
66		2378.87	$[M+ma+Na]^+$	2414.61	0.16	0.58
67		2368.84	$[M+2ma+Na]^+$	2417.60	0.94	0.20
68		2411.88	$[M+2ma+Na]^+$	2434.56	0.13	0.58
69		2426.88	$[M+ma+Na]^+$	2462.59	0.11	0.58
70		2442.88	$[M+ma+Na]^+$	2478.58	0.19	0.18
71		2571.92	$[M+2ma+Na]^+$	2620.63	0.81	0.14
72		2587.92	$[M+2ma+Na]^+$	2636.61	0.15	0.10
73		2645.96	$[M+ma+Na]^+$	2681.67	0.03	0.59
74		2879.00	$[M+3ma+Na]^+$	2941.62	0.06	0.12

Table S3 N-glycan compositions identified from serum of HCC patients.

No.	Potential Structure*	Native mass** /Da	Detection		Relative Intensity (%)	CV
			Peak***	m/z Da		
1		910.33	$[M+Na]^+$	933.49	0.45	0.49
2		1056.39	$[M+Na]^+$	1079.22	0.26	0.58
3		1072.38	$[M+Na]^+$	1095.25	0.43	0.69
4		1113.41	$[M+Na]^+$	1136.24	0.31	0.57
5		1234.43	$[M+Na]^+$	1257.24	2.16	0.10
6		1259.47	$[M+Na]^+$	1282.28	0.26	0.46
7		1275.46	$[M+Na]^+$	1298.25	0.34	0.39
8		1316.49	$[M+Na]^+$	1339.29	0.23	0.37
9		1396.49	$[M+Na]^+$	1419.27	4.29	0.05
10		1421.52	$[M+Na]^+$	1444.30	0.23	0.41
11		1437.51	$[M+Na]^+$	1460.29	0.39	0.15
12		1462.54	$[M+Na]^+$	1485.32	3.69	0.03
13		1478.54	$[M+Na]^+$	1501.30	0.89	0.18
14		1519.57	$[M+Na]^+$	1542.32	0.23	0.19
15		1558.54	$[M+Na]^+$	1581.29	1.40	0.02
16		1566.56	$[M+ma+Na]^+$	1602.33	0.99	0.28
17		1583.57	$[M+Na]^+$	1606.32	0.18	0.59
18		1599.57	$[M+Na]^+$	1622.29	0.22	0.16

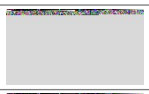
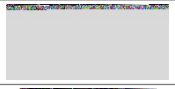
19		1608.60	$[M+Na]^+$	1631.33	0.20	0.19
20		1624.60	$[M+Na]^+$	1647.34	5.98	0.04
21		1640.59	$[M+Na]^+$	1663.32	1.24	0.11
22		1665.62	$[M+Na]^+$	1688.36	0.73	0.09
23		1681.62	$[M+Na]^+$	1704.34	0.31	0.17
24		1720.59	$[M+Na]^+$	1743.32	1.84	0.01
25		1712.61	$[M+ma+Na]^+$	1748.37	0.36	0.21
26		1728.61	$[M+ma+Na]^+$	1764.37	0.42	0.19
27		1769.63	$[M+ma+Na]^+$	1805.37	0.51	0.15
28		1786.65	$[M+Na]^+$	1809.37	3.61	0.04
29		1802.64	$[M+Na]^+$	1825.34	0.47	0.10
30		1811.68	$[M+Na]^+$	1834.35	0.15	0.63
31		1827.68	$[M+Na]^+$	1850.39	1.56	0.06
32		1843.67	$[M+Na]^+$	1866.36	0.80	0.16
33		1882.64	$[M+Na]^+$	1905.34	0.86	0.10
34		1874.67	$[M+ma+Na]^+$	1910.39	0.16	0.24
35		1890.66	$[M+ma+Na]^+$	1926.40	0.34	0.10
36		1915.69	$[M+ma+Na]^+$	1951.41	0.77	0.14
37		1931.69	$[M+ma+Na]^+$	1967.40	7.57	0.03
38		1957.74	$[M+Na]^+$	1980.46	0.19	0.08
39		1972.71	$[M+ma+Na]^+$	2008.46	0.32	0.06

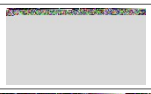
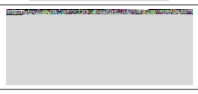
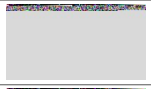
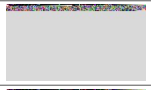
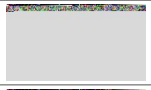
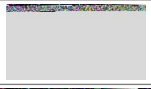
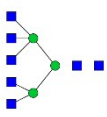
40		1989.73	$[M+Na]^+$	2012.41	1.27	0.06
41		2005.72	$[M+Na]^+$	2028.40	0.20	0.23
42		2028.70	$[M+Na]^+$	2051.43	0.34	0.21
43		2030.76	$[M+Na]^+$	2053.44	0.18	0.58
44		2044.70	$[M+Na]^+$	2067.35	0.19	0.27
45		2071.78	$[M+Na]^+$	2094.45	0.18	0.58
46		2087.78	$[M+Na]^+$	2110.50	0.58	0.58
47		2077.75	$[M+ma+Na]^+$	2113.44	5.47	0.06
48		2093.74	$[M+ma+Na]^+$	2129.42	0.96	0.05
49		2110.76	$[M+Na]^+$	2133.46	0.17	0.58
50		2118.77	$[M+ma+Na]^+$	2154.45	0.46	0.09
51		2135.79	$[M+Na]^+$	2158.41	0.17	0.58
52		2134.77	$[M+ma+Na]^+$	2170.44	3.87	0.04
53		2151.78	$[M+Na]^+$	2174.46	0.28	0.58
54		2175.79	$[M+ma+Na]^+$	2211.48	0.32	0.46
55		2192.81	$[M+Na]^+$	2215.47	0.25	0.58

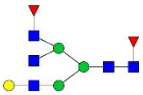
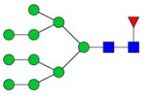
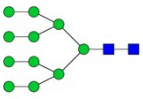
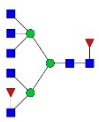
56		2223.80	$[M+ma+Na]^+$	2259.41	0.18	0.24
57		2222.78	$[M+2ma+Na]^+$	2271.48	26.55	0.09
58		2256.81	$[M+Na]^+$	2279.56	0.55	0.58
59		2264.83	$[M+ma+Na]^+$	2300.36	0.25	0.18
60		2280.82	$[M+ma+Na]^+$	2316.49	5.37	0.09
61		2296.82	$[M+ma+Na]^+$	2332.46	0.95	0.04
62		2321.85	$[M+ma+Na]^+$	2357.44	0.20	0.11
63		2352.81	$[M+Na]^+$	2375.46	0.12	0.58
64		2368.84	$[M+2ma+Na]^+$	2417.33	3.15	0.08
65		2411.88	$[M+2ma+Na]^+$	2433.21	0.26	0.11
66		2442.88	$[M+ma+Na]^+$	2478.40	0.15	0.11
67		2571.92	$[M+2ma+Na]^+$	2620.38	1.04	0.06
68		2587.92	$[M+2ma+Na]^+$	2636.34	0.42	0.03
69		2733.97	$[M+2ma+Na]^+$	2782.39	0.11	0.17
70		2790.99	$[M+2ma+Na]^+$	2839.40	0.08	0.61

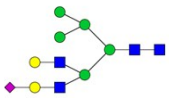
71		2879.00	$[M+3ma+Na]^+$	2941.35	1.48	0.12
72		3025.07	$[M+3ma+Na]^+$	3087.39	0.84	0.17

Table S4 N-glycan compositions identified from serum of normal controls.

No.	Potential Structure*	Native mass** /Da	Detection		Relative Intensity (%)	CV
			Peak***	m/z Da		
1		910.33	$[M+Na]^+$	933.85	0.95	0.18
2		1056.39	$[M+Na]^+$	1079.24	0.42	0.62
3		1072.38	$[M+Na]^+$	1095.13	1.26	0.58
4		1113.41	$[M+Na]^+$	1136.26	0.54	0.37
5		1234.43	$[M+Na]^+$	1257.23	0.78	0.25
6		1259.47	$[M+Na]^+$	1282.28	0.39	0.58
7		1275.46	$[M+Na]^+$	1298.24	0.45	0.31
8		1316.49	$[M+Na]^+$	1339.28	0.62	0.25
9		1396.49	$[M+Na]^+$	1419.28	1.06	0.19
10		1421.52	$[M+Na]^+$	1444.32	0.40	0.58
11		1462.54	$[M+Na]^+$	1485.32	2.63	0.14
12		1478.54	$[M+Na]^+$	1501.31	1.18	0.29
13		1519.57	$[M+Na]^+$	1542.32	0.37	0.29

14		1558.54	$[M+Na]^+$	1581.30	0.42	0.20
15		1566.56	$[M+ma+Na]^+$	1602.34	1.85	0.08
16		1599.57	$[M+Na]^+$	1622.35	0.33	0.58
17		1608.60	$[M+Na]^+$	1631.32	0.34	0.61
18		1624.60	$[M+Na]^+$	1647.35	4.53	0.07
19		1640.59	$[M+Na]^+$	1663.33	1.34	0.24
20		1665.62	$[M+Na]^+$	1688.36	0.47	0.23
21		1681.62	$[M+Na]^+$	1704.35	0.50	0.28
22		1720.59	$[M+Na]^+$	1743.33	0.62	0.31
23		1712.61	$[M+ma+Na]^+$	1748.38	0.36	0.58
24		1728.61	$[M+ma+Na]^+$	1764.37	0.47	0.16
25		1769.63	$[M+ma+Na]^+$	1805.39	0.91	0.12
26		1786.65	$[M+Na]^+$	1809.38	1.99	0.16
27		1802.64	$[M+Na]^+$	1825.35	0.45	0.07
28		1827.68	$[M+Na]^+$	1850.40	0.87	0.24
29		1843.67	$[M+Na]^+$	1866.38	0.99	0.27
30		1882.64	$[M+Na]^+$	1905.36	0.47	0.26
31		1890.66	$[M+ma+Na]^+$	1926.39	0.40	0.08
32		1925.72	$[M+Na]^+$	1948.39	0.51	0.58
33		1915.69	$[M+ma+Na]^+$	1951.44	0.83	0.24
34		1931.69	$[M+ma+Na]^+$	1967.41	12.24	0.03

35		1957.74	$[M+Na]^+$	1980.47	0.38	0.10
36		1973.73	$[M+Na]^+$	1996.41	0.25	0.58
37		1972.71	$[M+ma+Na]^+$	2008.57	0.49	0.58
38		1989.73	$[M+Na]^+$	2012.41	0.59	0.15
39		2028.70	$[M+Na]^+$	2051.42	0.57	0.14
40		2044.70	$[M+Na]^+$	2067.46	0.33	0.25
41		2087.78	$[M+Na]^+$	2110.44	0.58	0.60
42		2077.75	$[M+ma+Na]^+$	2113.45	3.84	0.05
43		2093.74	$[M+ma+Na]^+$	2129.44	1.06	0.58
44		2110.76	$[M+Na]^+$	2133.47	0.55	0.15
45		2135.79	$[M+Na]^+$	2158.58	0.45	0.58
46		2134.77	$[M+ma+Na]^+$	2170.45	4.88	0.07
47		2190.76	$[M+ma+Na]^+$	2213.48	0.36	0.23
48		2206.75	$[M+Na]^+$	2229.45	0.21	0.58
49		2217.84	$[M+Na]^+$	2240.47	0.24	0.29
50		2223.80	$[M+Na]^+$	2256.43	0.57	0.58

51		2222.78	$[M+2ma+Na]^+$	2271.49	34.88	0.17
52		2255.79	$[M+ma+Na]^+$	2291.73	0.41	0.58
53		2264.83	$[M+ma+Na]^+$	2300.52	0.57	0.03
54		2280.82	$[M+ma+Na]^+$	2316.58	1.46	0.13
55		2296.82	$[M+ma+Na]^+$	2332.47	0.65	0.15
56		2321.85	$[M+ma+Na]^+$	2357.46	0.28	0.25
57		2352.81	$[M+Na]^+$	2375.45	0.21	0.58
58		2368.84	$[M+2ma+Na]^+$	2417.53	3.21	0.10
59		2411.88	$[M+2ma+Na]^+$	2433.58	0.34	0.18
60		2425.86	$[M+2ma+Na]^+$	2474.53	0.16	0.58
61		2571.92	$[M+2ma+Na]^+$	2620.58	0.79	0.25
62		2587.92	$[M+2ma+Na]^+$	2636.57	0.77	0.06
63		2733.97	$[M+2ma+Na]^+$	2782.63	0.17	0.09
64		2879.00	$[M+3ma+Na]^+$	2941.67	2.49	0.06
65		3025.07	$[M+3ma+Na]^+$	3087.69	0.47	0.05

*: One potential structure corresponding to the glycan mass was displayed.

**: The monoisotopic molecular mass of the glycan with free reducing end.

***: “M” refers to the native molecule and “+(n) ma” means that (n) sialic acid units were converted into methylamidated ones, with mass shift of 13.03*n Da.

1. I. H. Chen, L. Xue, C.-C. Hsu, J. S. P. Paez, L. Pan, H. Andaluz, M. K. Wendt, A. B. Iliuk, J.-K. Zhu and W. A. Tao, *PNAS*, 2017, **114**, 3175-3180.
2. X. Liu, H. Qiu, R. K. Lee, W. Chen and J. Li, *Anal. Chem.*, 2010, **82**, 8300-8306.
3. L. Wei, Y. Cai, L. Yang, Y. Zhang and H. Lu, *Anal. Chem.*, 2018, **90**, 10442-10449.