SUPPLEMENTAL FIGURE LEGENDS

Supplemental Fig. 1. MALDI-TOF Spectra of Permethylated N- and O-glycans Derived from Untreated and 4-F-GlcNAc-Treated T cells. N-glycomic profile of **(a)** untreated and **(b)** 4-F-GlcNAc-treated T cells; O-glycomic profile of **(c)** untreated and **(d)** 4-F-GlcNAc-treated T cells. N- and O-glycomic profiles were obtained from the 50% MeCN fraction from a C_{18} Sep-Pak column ("Materials and Methods"). Cartoon structures are according to the Consortium for Functional Glycomics (http://www.functionalglycomics.org) guidelines. All molecular ions are $[M+Na]^+$. Putative structures based on composition, tandem MS and biosynthetic knowledge. Structures that show sugars outside a bracket have not been unequivocally defined.

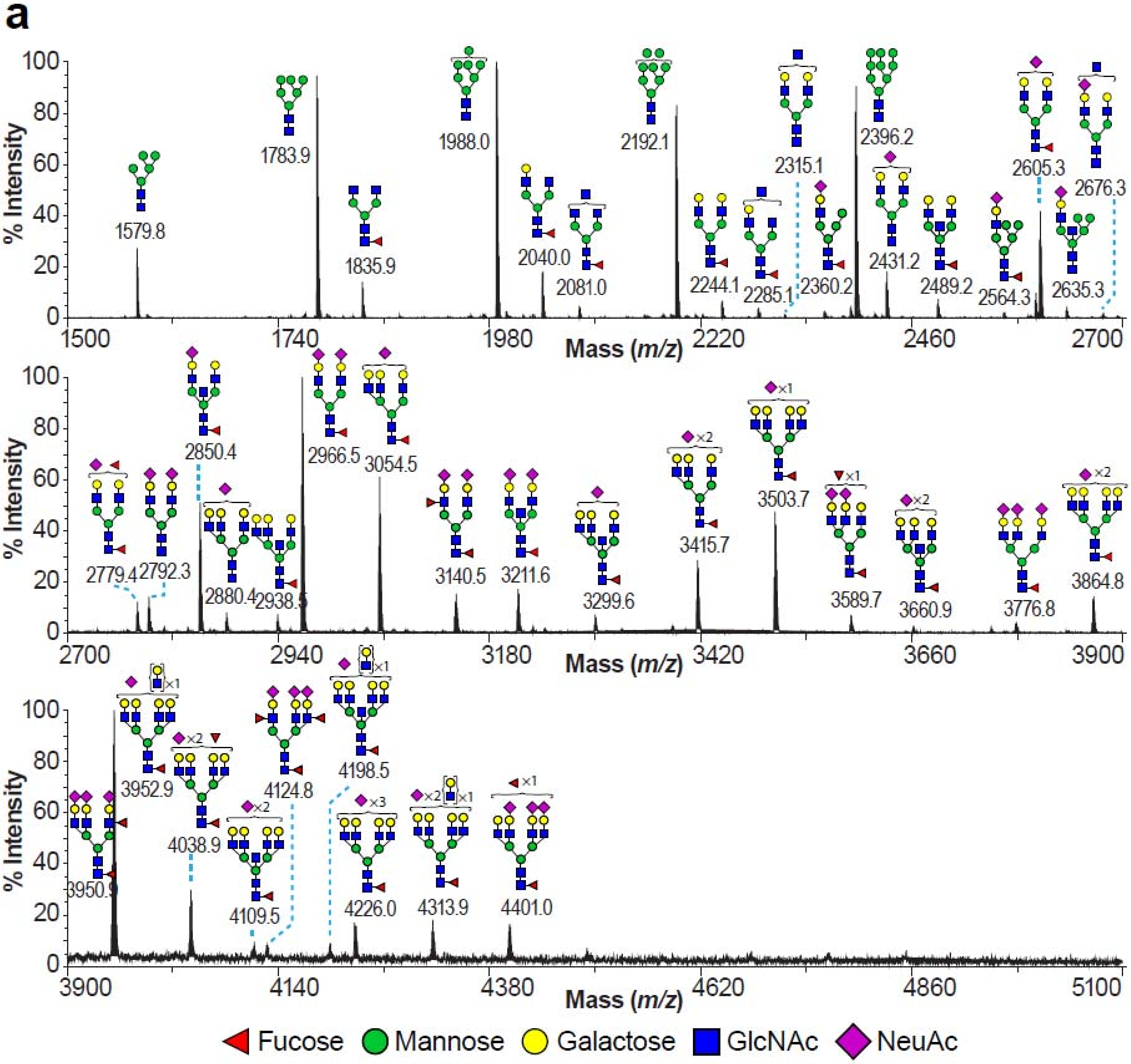
Supplemental Fig. 2. MALDI-TOF Mass Spectra of Permethylated N-glycans from Endo-β-Galactosidase Digestion of KG1a and T cells. Profile of N-glycans of **(a)** untreated and **(b)** 4-F-GlcNAc-treated KG1a cells; profiles of N-glycans of **(c)** untreated and **(d)** 4-F-GlcNAc treated T cells. All profiles are from the 50% MeCN fraction from a C_{18} Sep-Pak column ("Materials and Methods"). All molecular ions are $[M+Na]^+$. Putative structures based on composition, tandem MS and biosynthetic knowledge. Cartoon structures are according to the Consortium for Functional Glycomics (http://www.functionalglycomics.org) guidelines.

Supplemental Fig. 3. Low Mass MALDI-TOF Mass Spectra of Permethylated N-glycans from Endo-β-Galactosidase Digestion of T cells. Profile of N-glycans of **(a)** untreated and **(b)** 4-F-GlcNAc-treated T cells. Profiles are from the 35% MeCN fraction from a C_{18} Sep-Pak column ("Materials and Methods"). All molecular ions are $[M+Na]^+$. Putative structures based on composition, tandem MS and biosynthetic knowledge. Cartoon structures are according to the Consortium for Functional Glycomics (http://www.functionalglycomics.org) guidelines.

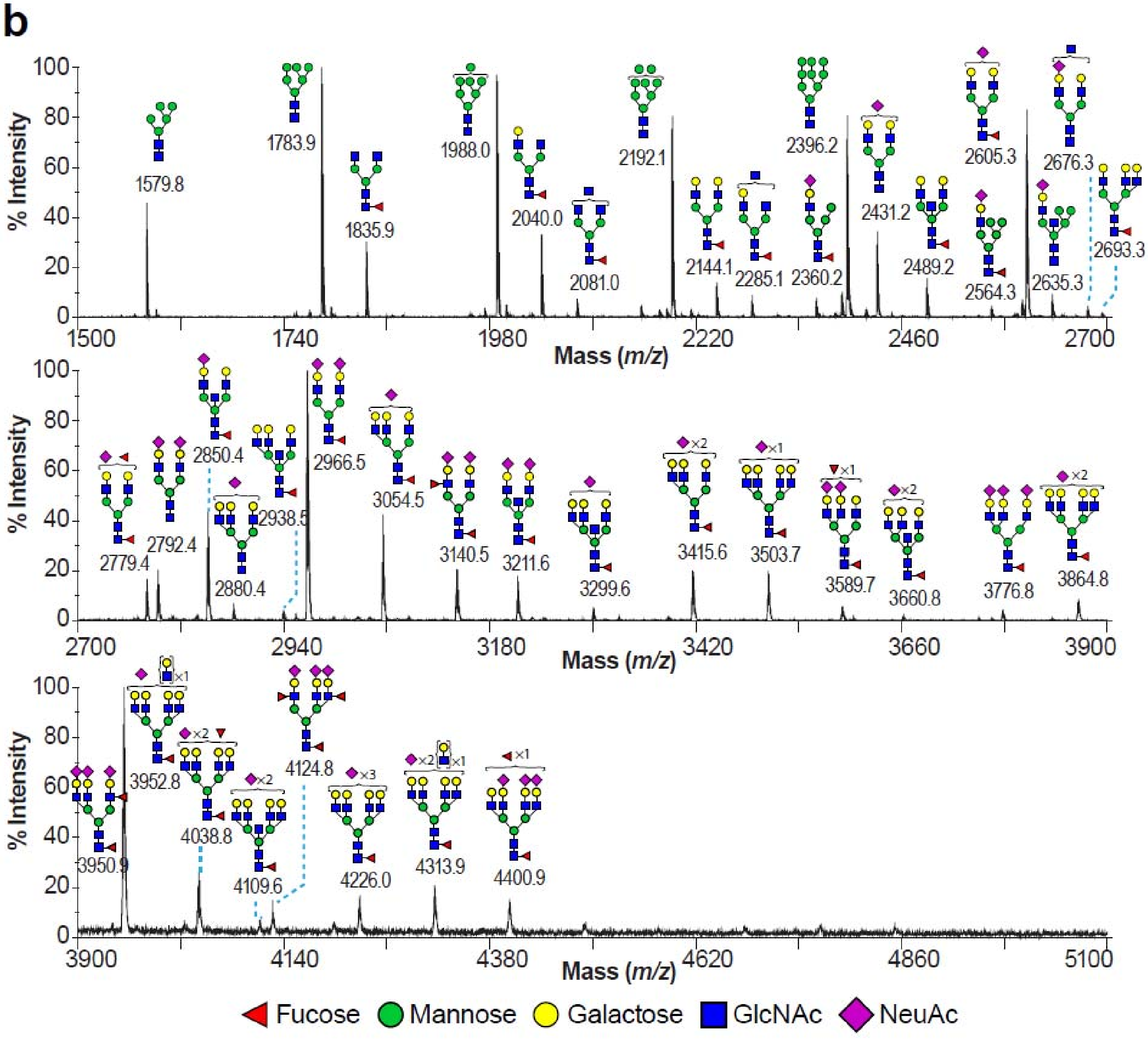
Supplemental Fig. 4. Scan Zoom MALDI-TOF Spectra of Permethylated N- and O-glycans Derived from Untreated and 4-F-GlcNAc-Treated T cells. Zoom scan signals present at *m/z* 1835 (N-glycans) and 779 (O-glycans). N-glycomic profiles were obtained from the 35% MeCN fraction, while O-glycomic profiles were obtained from the 50% MeCN fraction (Supplementary Figure 2), both from a C_{18} Sep-Pak column ("Materials and Methods"). All molecular ions are $[M+Na]^+$. Putative structures based on composition, tandem MS and the literature shown. Cartoon structures are according to the Consortium for Functional Glycomics (http://www.functionalglycomics.org) guidelines. Signals at *m/z* 1823 in **(a)** and **(b)** and at *m/z* 767 in **(c)** and **(d)** correspond to the theoretical incorporation of 4-F-GlcNAc.

Supplemental Fig. 5. Effect of GlcNAc Variants on Galectin-1 Ligands on sLe^X (+) KG1a and T cells. (a and c) Representative flow cytometric histograms of KG1a and T cells treated with the indicated GlcNAc analogs. After treatment, cells were stained with Gal-1hFc (10 ug/ml) along with APC-goat anti-human IgG secondary (gray histogram= staining with hFc control; black histogram = staining of untreated cells with Gal-1hFc; dotted histogram= staining of sugar-treated cells with Gal-1hFc). KG1a cells were incubated with or without GlcNAc variants (0.05mM) for 48hrs before being harvested and stained. T cells were incubated with GlcNAc variants (0.05mM) for 48hrs, except for peracetylated 4-F-GlcNAc, which was incubated at 0.015mM for 38 hrs. **(b and d)** Quantitation of Gal-1hFc binding based on the flow cytometric results shown in (**a and c**). The mean fluorescent intensity of Gal-1hFc binding to treated cells was normalized to untreated cells, which was set at 100%. Graphed are data from experiments performed in triplicate on two different occasions with the standard error of the mean (SEM) indicated. **, $p<0.01$, statistically significant compared with untreated control, one-way ANOVA with Dunnett's post hoc test.

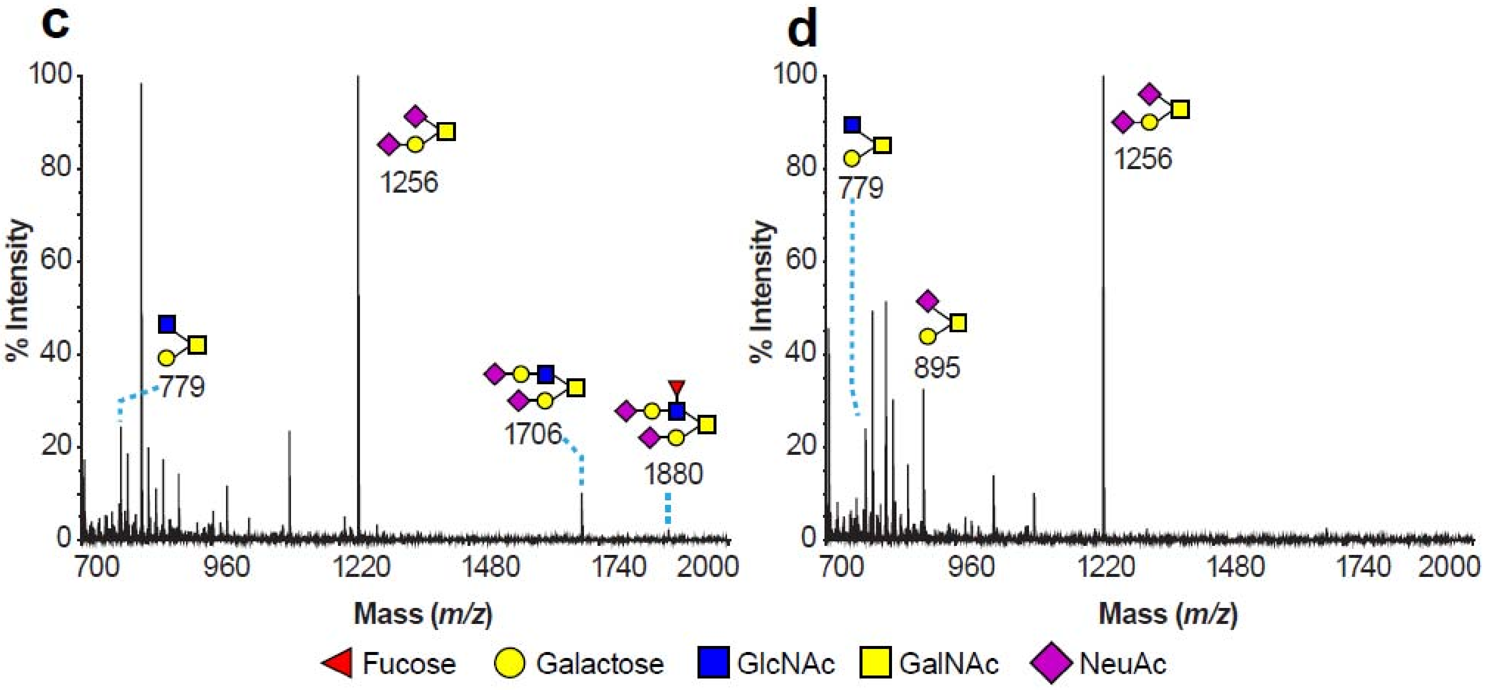
Supplemental Figure 1



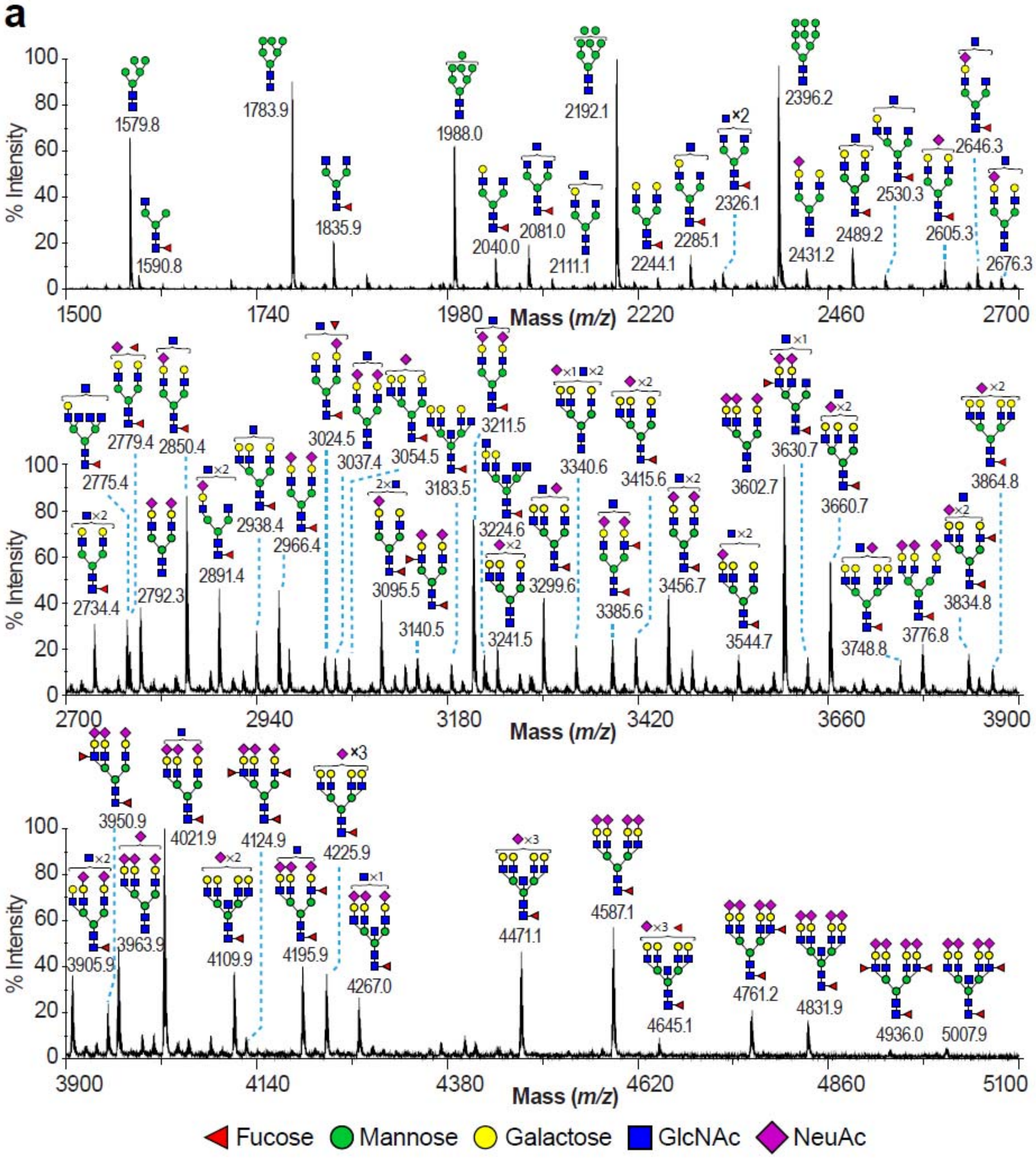
Supplemental Figure 1



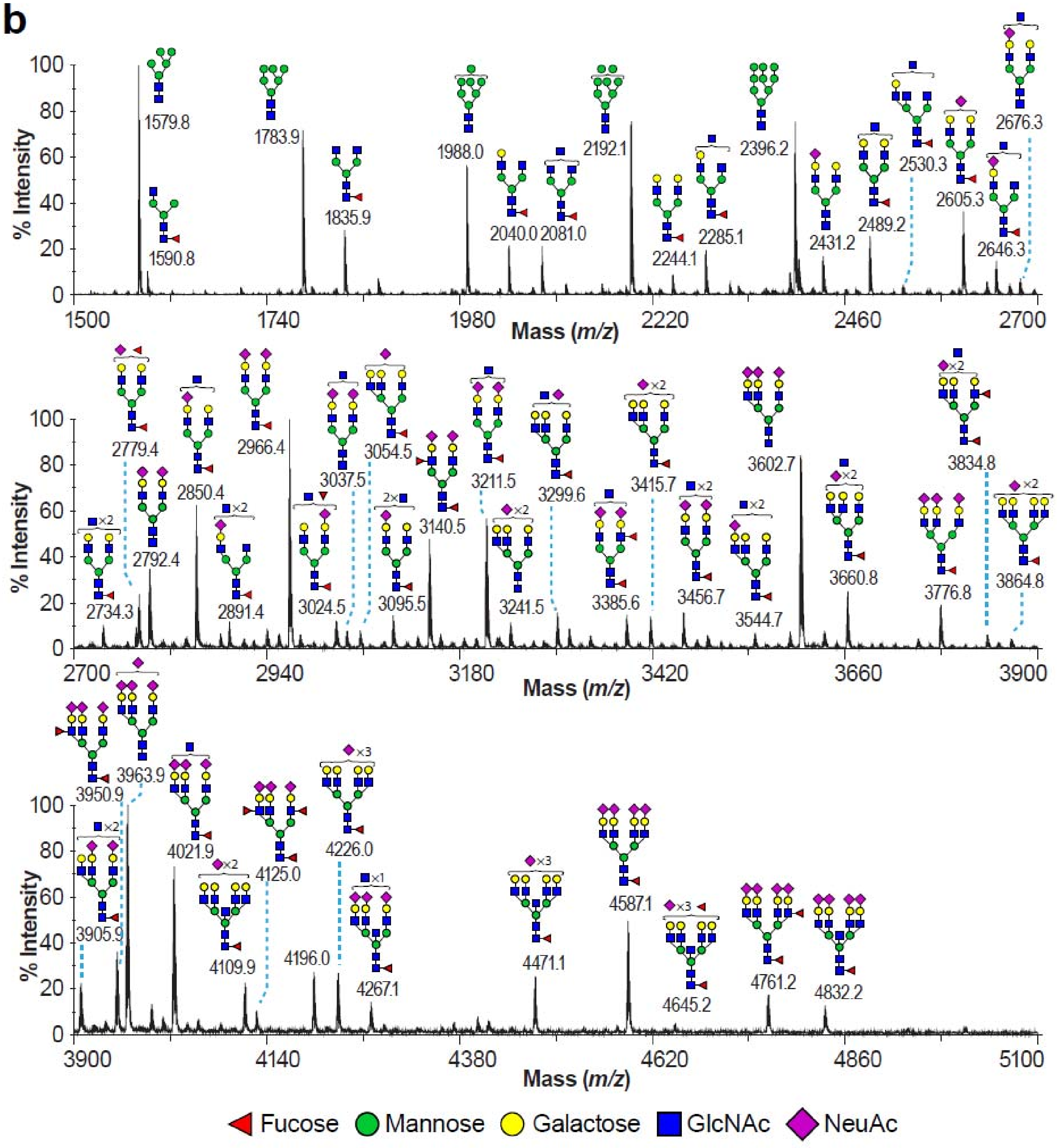
Supplemental Figure 1



Supplemental Figure 2

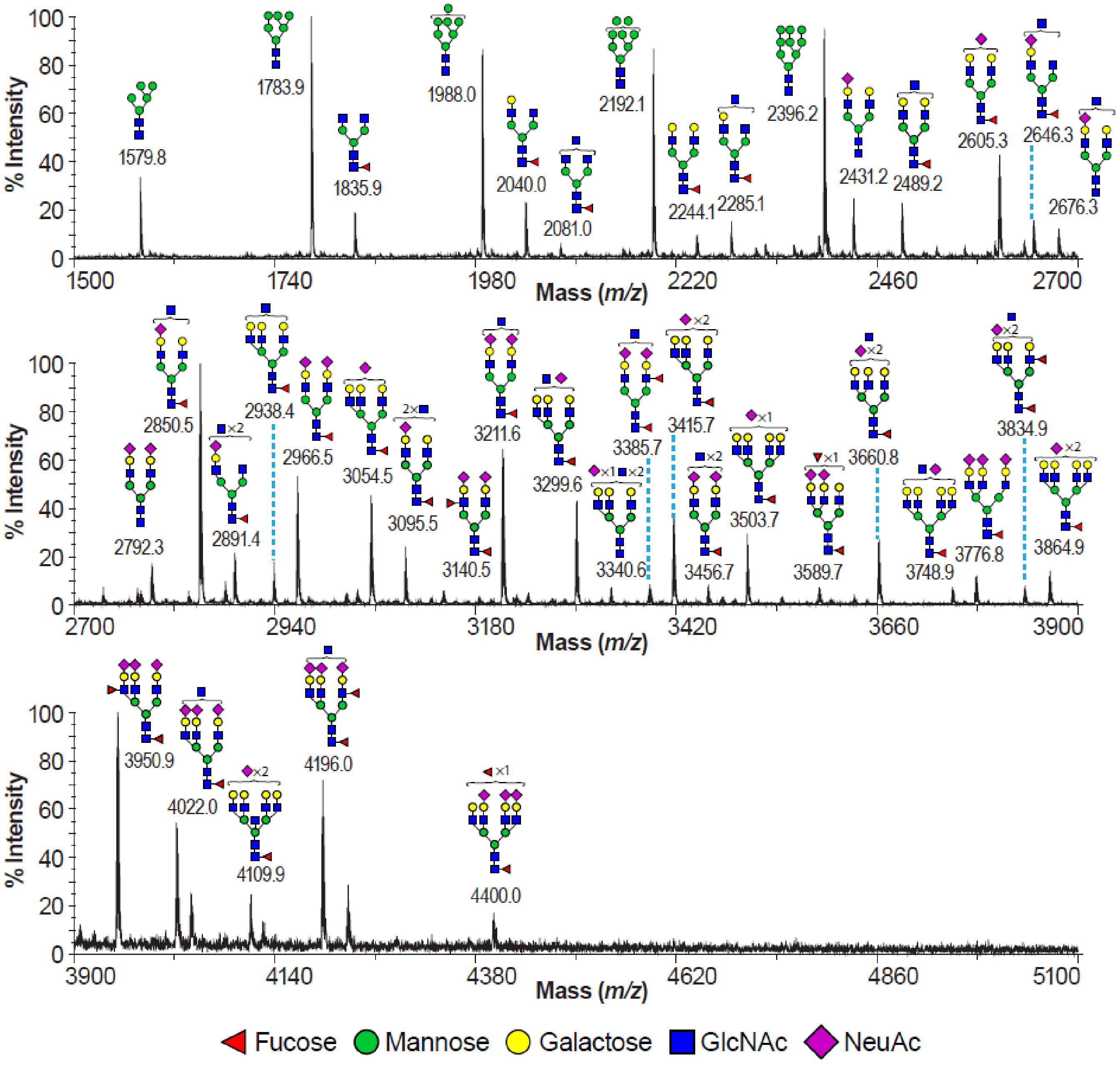


Supplemental Figure 2



Supplemental Figure 2

c



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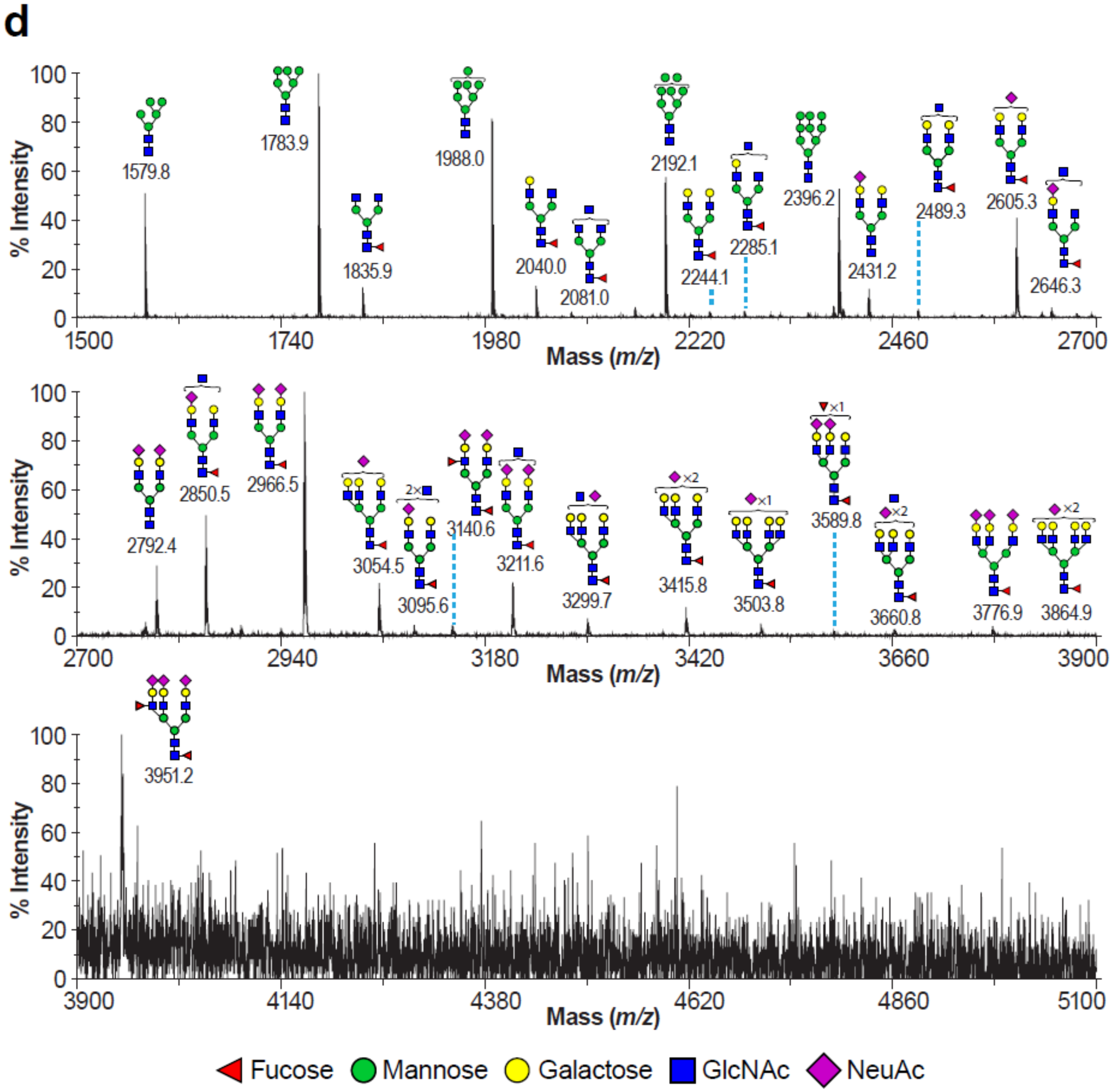

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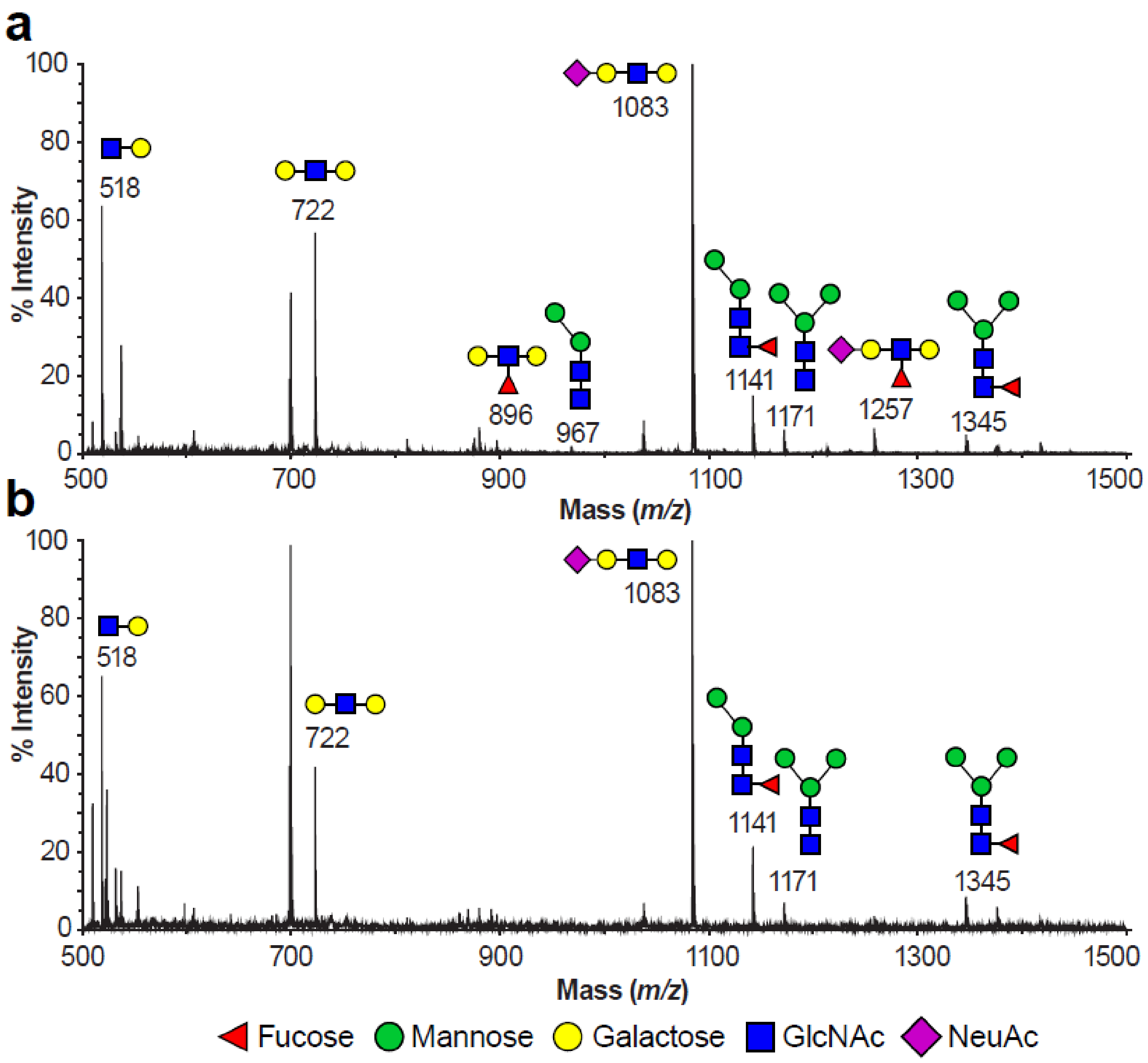
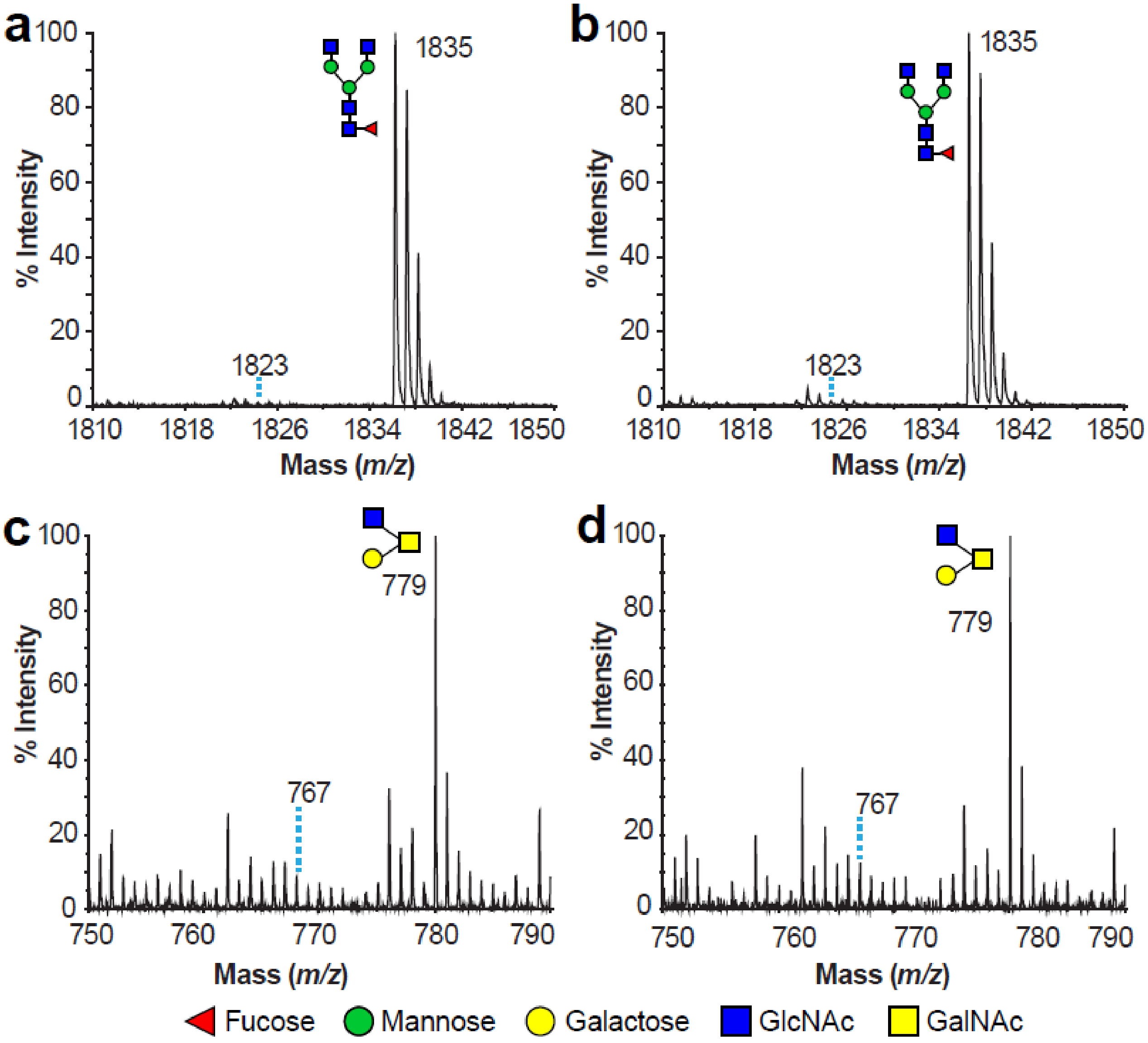
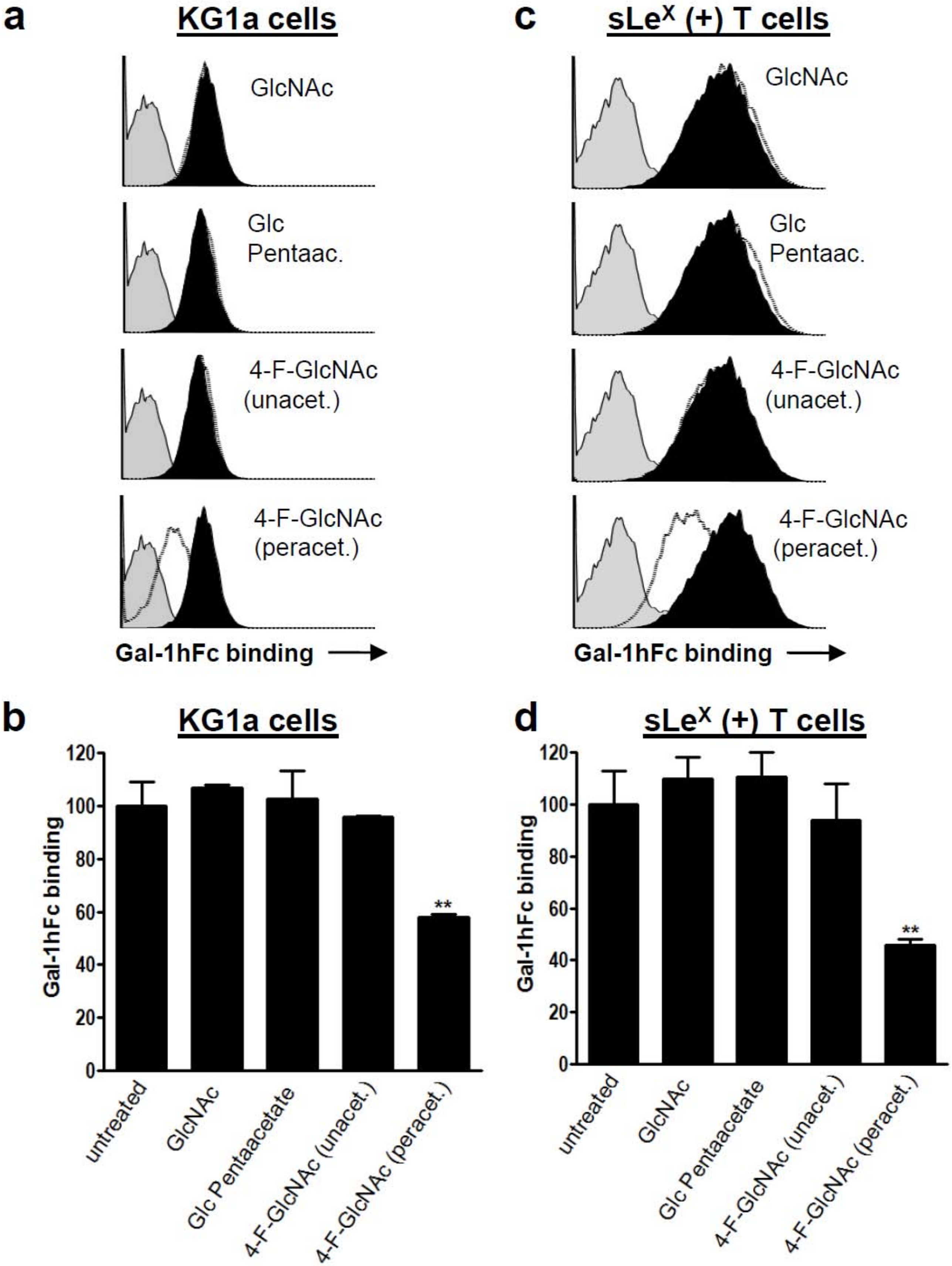


Supplemental Figure 4



Supplemental Figure 5



Supplemental Table 1.

Compositional assignments and masses of singly charged sodiated molecular ions $[M+Na]^+$, observed in MALDI-TOF MS spectra of permethylated *N*-glycans derived from the untreated and 4-F-GlcNAc treated KG1a cells.

Composition	**Calculated mass (*m/z*)**	**Detected Mass (*m/z*)** **Untreated KG1a**	 **4-F-GlcNAc KG1a**
$Hex_5GlcNAc_2$	1579.8	1579.8	1579.8
$Fuc_1Hex_3HexNAc_3$	1590.8	1590.8	1590.8
$Hex_6GlcNAc_2$	1783.9	1783.9	1783.9
$Fuc_1Hex_3HexNAc_4$	1835.9	1835.9	1835.9
$Hex_7GlcNAc_2$	1988.0	1988.0	1988.0
$Fuc_1Hex_4HexNAc_4$	2040.0	2040.0	2040.0
$Fuc_1Hex_3HexNAc_5$	2081.1	2081.0	2081.0
$Hex_8GlcNAc_2$	2192.1	2192.1	2192.1
$Fuc_1Hex_5HexNAc_4$	2244.1	2244.1	2244.1
$Fuc_1Hex_4HexNAc_5$	2285.2	2285.1	2285.1
$Hex_5HexNAc_5$	2315.2	2315.2	2315.2
$Hex_9GlcNAc_2$	2396.2	2396.2	2396.2
$Hex_5HexNAc_4NeuAc_1$	2431.2	2431.2	2431.2
$Fuc_1Hex_5HexNAc_5$	2489.3	2489.2	2489.2
$Hex_{10}GlcNAc_2$	2600.3	2600.3	2600.3
$Fuc_1Hex_5HexNAc_4NeuAc_1$	2605.3	2605.3	2605.3
$Hex_6HexNAc_4NeuAc_1$	2635.3	2635.3	2635.3
$Fuc_2Hex_5HexNAc_5$	2663.3	2663.5	2663.3
$Hex_5HexNAc_5NeuAc_1$	2676.3	2676.5	2676.3
$Fuc_1Hex_5HexNAc_6$	2734.4	2734.3	-
$Fuc_2Hex_5HexNAc_4NeuAc_1$	2779.4	2779.3	2779.3
$Hex_5HexNAc_4NeuAc_2$	2792.4	2792.3	2792.3
$Fuc_1Hex_5HexNAc_5NeuAc_1$	2850.4	2850.4	2850.4
$Hex_6HexNAc_5NeuAc_1$	2880.4	2880.4	2880.4
$Fuc_1Hex_6HexNAc_6$	2938.5	2938.4	2938.4
$Fuc_1Hex_5HexNAc_4NeuAc_2$	2966.5	2966.4	2966.4
$Fuc_2Hex_5HexNAc_5NeuAc_1$	3024.5	3024.5	3024.5

Hex$_5$HexNAc$_5$NeuAc$_2$	3037.5	3037.6	3037.6
Fuc$_1$Hex$_6$HexNAc$_5$NeuAc$_1$	3054.5	3054.5	3054.5
Fuc$_2$Hex$_6$HexNAc$_6$	3112.6	3112.7	-
Hex$_6$HexNAc$_6$NeuAc$_1$	3125.6	3125.7	-
Fuc$_2$Hex$_5$HexNAc$_4$NeuAc$_2$	3140.6	3140.5	3140.5
Fuc$_1$Hex$_5$HexNAc$_5$NeuAc$_2$	3211.6	3211.5	3211.6
Hex$_6$HexNAc$_5$NeuAc$_2$	3241.6	3241.5	3241.6
Fuc$_1$Hex$_6$HexNAc$_6$NeuAc$_1$	3299.7	3299.6	3299.6
Fuc$_3$Hex$_5$HexNAc$_4$NeuAc$_2$	3314.7	-	3314.6
Fuc$_2$Hex$_5$HexNAc$_5$NeuAc$_2$	3385.7	3385.6	3385.6
Fuc$_1$Hex$_7$HexNAc$_7$	3387.7	3387.6	3387.6
Fuc$_1$Hex$_6$HexNAc$_5$NeuAc$_2$	3415.7	3415.6	3415.6
Fuc$_2$Hex$_6$HexNAc$_6$NeuAc$_1$	3473.7	3473.7	-
Hex$_6$HexNAc$_6$NeuAc$_2$	3486.7	3486.6	-
Fuc$_1$Hex$_7$HexNAc$_6$NeuAc$_1$	3503.8	3503.7	3503.7
Hex$_6$HexNAc$_5$NeuAc$_3$	3602.8	3602.7	3602.7
Fuc$_1$Hex$_6$HexNAc$_6$NeuAc$_2$	3660.8	3660.7	3660.7
Fuc$_1$Hex$_7$HexNAc$_7$NeuAc$_1$	3748.9	3748.8	3748.8
Fuc$_1$Hex$_6$HexNAc$_5$NeuAc$_3$	3776.9	3776.7	3776.7
Fuc$_2$Hex$_6$HexNAc$_6$NeuAc$_2$	3834.9	3834.8	3834.8
Fuc$_1$Hex$_7$HexNAc$_6$NeuAc$_2$	3864.9	3864.8	3864.8
Fuc$_2$Hex$_6$HexNAc$_5$NeuAc$_3$	3951.0	3950.8	3950.9
Fuc$_1$Hex$_6$HexNAc$_6$NeuAc$_3$	4022.0	4021.8	4021.9
Fuc$_1$Hex$_7$HexNAc$_7$NeuAc$_2$	4110.1	4109.9	4109.9
Fuc$_2$Hex$_6$HexNAc$_6$NeuAc$_3$	4196.1	4195.9	4195.9
Fuc$_1$Hex$_8$HexNAc$_8$NeuAc$_1$	4198.1	4197.9	4197.9
Fuc$_1$Hex$_7$HexNAc$_6$NeuAc$_3$	4226.1	4225.9	4225.9
Fuc$_2$Hex$_7$HexNAc$_7$NeuAc$_2$	4284.1	4283.9	4283.9
Fuc$_1$Hex$_8$HexNAc$_7$NeuAc$_2$	4314.2	4313.9	4314.0
Fuc$_2$Hex$_7$HexNAc$_6$NeuAc$_3$	4400.2	4400.6	4400.0
Fuc$_1$Hex$_7$HexNAc$_7$NeuAc$_3$	4471.2	4471.0	4471.0
Fuc$_1$Hex$_8$HexNAc$_8$NeuAc$_2$	4559.3	4559.0	4559.1
Fuc$_2$Hex$_7$HexNAc$_7$NeuAc$_3$	4645.3	4645.8	4645.1
Fuc$_1$Hex$_8$HexNAc$_7$NeuAc$_3$	4675.3	4674.8	4675.2

$Fuc_1Hex_9HexNAc_8NeuAc_2$	4763.4	4763.0	4763.0
$Fuc_1Hex_8HexNAc_8NeuAc_3$	4920.5	4920.7	4921.0

Supplemental Table 2.

Compositional assignments and masses of singly charged sodiated molecular ions $[M+Na]^+$, observed in MALDI-TOF MS spectra of permethylated N-glycans derived from the untreated and 4-F-GlcNAc treated T cells.

		Detected Mass (*m/z*)	
Composition	**Calculated mass (*m/z*)**	**Untreated T Cells**	**4-F-GlcNAc T Cells**
Hex$_5$GlcNAc$_2$	1579.8	1579.8	1579.8
Hex$_6$GlcNAc$_2$	1783.9	1783.9	1783.9
Fuc$_1$Hex$_3$HexNAc$_4$	1835.9	1835.9	1835.9
Hex$_7$GlcNAc$_2$	1988.0	1988.0	1988.0
Fuc$_1$Hex$_4$HexNAc$_4$	2040.0	2040.0	2040.0
Fuc$_1$Hex$_3$HexNAc$_5$	2081.1	2081.0	2081.0
Hex$_8$GlcNAc$_2$	2192.1	2192.1	2192.1
Fuc$_1$Hex$_5$HexNAc$_4$	2244.1	2244.1	2244.1
Fuc$_1$Hex$_4$HexNAc$_5$	2285.2	2285.1	2285.1
Hex$_5$HexNAc$_5$	2315.2	2315.1	2315.1
Hex$_9$GlcNAc$_2$	2396.2	2396.2	2396.2
Hex$_5$HexNAc$_4$NeuAc$_1$	2431.2	2431.2	2431.2
Fuc$_1$Hex$_5$HexNAc$_5$	2489.3	2489.2	2489.2
Fuc$_1$Hex$_6$HexNAc$_3$NeuAc$_1$	2564.3	2564.3	2564.3
Fuc$_1$Hex$_5$HexNAc$_4$NeuAc$_1$	2605.3	2605.3	2605.3
Hex$_6$HexNAc$_4$NeuAc$_1$	2635.3	2635.3	2635.2
Hex$_5$HexNAc$_5$NeuAc$_1$	2676.3	2676.3	2676.3
Fuc$_2$Hex$_5$HexNAc$_4$NeuAc$_1$	2779.4	2779.4	2779.4
Hex$_5$HexNAc$_4$NeuAc$_2$	2792.4	2792.4	2792.4
Fuc$_1$Hex$_5$HexNAc$_5$NeuAc$_1$	2850.4	2850.4	2850.4
Hex$_6$HexNAc$_5$NeuAc$_1$	2880.4	2880.4	2880.4
Fuc$_1$Hex$_6$HexNAc$_6$	2938.5	2938.5	2938.5
Fuc$_1$Hex$_5$HexNAc$_4$NeuAc$_2$	2966.5	2966.5	2966.5
Fuc$_1$Hex$_6$HexNAc$_5$NeuAc$_1$	3054.5	3054.5	3054.5
Fuc$_2$Hex$_5$HexNAc$_4$NeuAc$_2$	3140.6	3140.5	3140.5
Fuc$_1$Hex$_5$HexNAc$_5$NeuAc$_2$	3211.6	3211.6	3211.6
Fuc$_1$Hex$_6$HexNAc$_6$NeuAc$_1$	3299.7	3299.6	3299.6

Fuc$_1$Hex$_6$HexNAc$_5$NeuAc$_2$	3415.7	3415.7	3415.6
Fuc$_1$Hex$_7$HexNAc$_6$NeuAc$_1$	3503.8	3503.7	3503.7
Fuc$_2$Hex$_6$HexNAc$_5$NeuAc$_2$	3589.8	3589.7	3589.7
Fuc$_1$Hex$_6$HexNAc$_6$NeuAc$_2$	3660.8	3660.9	3660.8
Fuc$_1$Hex$_6$HexNAc$_5$NeuAc$_3$	3776.9	3776.8	3776.8
Fuc$_1$Hex$_7$HexNAc$_6$NeuAc$_2$	3864.9	3864.8	3864.8
Fuc$_2$Hex$_6$HexNAc$_5$NeuAc$_3$	3951.0	3950.9	3950.9
Fuc$_1$Hex$_8$HexNAc$_7$NeuAc$_1$	3953.0	3952.9	3952.9
Fuc$_2$Hex$_7$HexNAc$_6$NeuAc$_2$	4039.0	4038.8	4038.8
Fuc$_1$Hex$_7$HexNAc$_7$NeuAc$_2$	4110.1	4109.5	4109.6
Fuc$_2$Hex$_6$HexNAc$_5$NeuAc$_3$	4125.1	4124.8	4124.8
Fuc$_1$Hex$_8$HexNAc$_8$NeuAc$_1$	4198.1	4198.5	-
Fuc$_1$Hex$_7$HexNAc$_6$NeuAc$_3$	4226.1	4226.0	4226.0
Fuc$_1$Hex$_8$HexNAc$_7$NeuAc$_2$	4314.2	4313.9	4313.9
Fuc$_2$Hex$_7$HexNAc$_6$NeuAc$_3$	4400.2	4401.0	4400.9

Supplemental Table 3.

GC-MS Linkage Analyses of Partially Methylated Alditol Acetates (PMAA) of KG1a and T cells Obtained after Peptide *N*-glycosidase F Digestion on Non-desialylated and Desialylated Samples.

Characteristic Fragment Ion [a,b]	Assignment	Relative Abundance[c], %			
		Non-Desialylated		Desialylated	
		KG1a Untreated 4-F-GlcNAc	**T cells** Untreated 4-F-GlcNAc	**KG1a** Untreated 4-F-GlcNAc	**T cells** Untreated 4-F-GlcNAc
102, 115, 118, 131, 162, 175	t-Fuc	27.9 50.3	54.5 48.5	61.3 64.3	92.0 36.3
102, 118, 129, 145, 161, 162, 205	t-Man	115.3 135.3	84.4 82.1	103.9 77.1	163.1 75.9
102, 118, 129, 145, 161, 162, 205	t-Gal	25.6 25.4	41.5 31.1	130.3 62.6	211.6 88.5
100, 101, 129, 130, 161, 190, 205	2-Man	100.0 100.0	100.0 100.0	100.0 100.0	100.0 100.0
118, 129, 143, 161, 174, 190, 203, 217, 234, 245, 277	3-Gal	40.6 32.9	29.3 21.4	29.6 6.0	30.4 19.4
99, 102, 118, 129, 159, 162, 173, 189, 233	6-Gal	9.8 11.5	23.7 21.9	0.0 0.0	0.0 0.0
113, 130, 173[b], 190[b], 233[b]	2,4-Man	10.8 7.8	10.4 6.6	17.5 2.8	19.1 9.4
99, 100, 129, 130[b], 189, 190[b]	2,6-Man	8.3 6.3	10.2 6.5	15.7 4.0	13.8 10.1
118[b], 129, 139, 143, 174, 189, 202, 234[b], 305	3,6-Man	64.8 61.2	43.6 39.9	77.2 35.4	67.0 42.0
118[b], 129, 139[b], 160, 202, 259, 333	3,4,6-Man	8.4 7.4	3.8 3.1	2.0 0.6	5.0 3.4
117, 129, 143, 145, 159, 203, 205	t-GlcNAc	3.8 1.3	0.4 0.5	0.7 0.1	0.5 0.3
117, 129, 143, 159, 173, 203, 233	4-GlcNac	32.5 13.7	5.7 6.3	19.1 2.2	6.6 6.0
117, 142, 159, 244, 301	3,4-GlcNAc	1.3 0.7	0.6 0.6	3.4 0.5	0.4 0.6
117, 143, 159, 261	4,6-GlcNAc	2.2 1.0	0.2 0.3	0.4 0.0	0.2 0.2

Permethylated N-glycans (non-desialylated and desialylated) were hydrolyzed, reduced, acetylated and analyzed by GC-MS ("Materials and Methods"). Upper panel values correspond to untreated PMAA linkage data (Untreated); lower panel values correspond to 4-F-GlcNAc-treated PMAA linkage data (4-F-GlcNAc). Values correspond to the relative abundance of the residue normalized to the abundance of 2-linked mannose.

a. Electron impact fragment ions used for identification of the PMAA residues.

b. For relative abundance, in order to minimize interferences from the baseline and/or contamination, all fragment ions greater than 100 (>100) were used for the extracted ion current (XIC) chromatogram, as indicated above, expect for the following residues: 2,4-linked mannose, *m/z* 173 + *m/z* 190 + *m/z* 233; 2,6-linked mannose, *m/z* 130 + *m/z* 190; 3,6-linked mannose, *m/z* 118 + *m/z* 234; 3,4,6-linked mannose, *m/z* 118 + *m/z* 139

c. Relative abundances are expressed as follows: the integrated area peak of the extracted ion current (XIC) chromatogram of a specific residue was divided with the integrated area peak of the XIC chromatogram of 2-linked mannose.