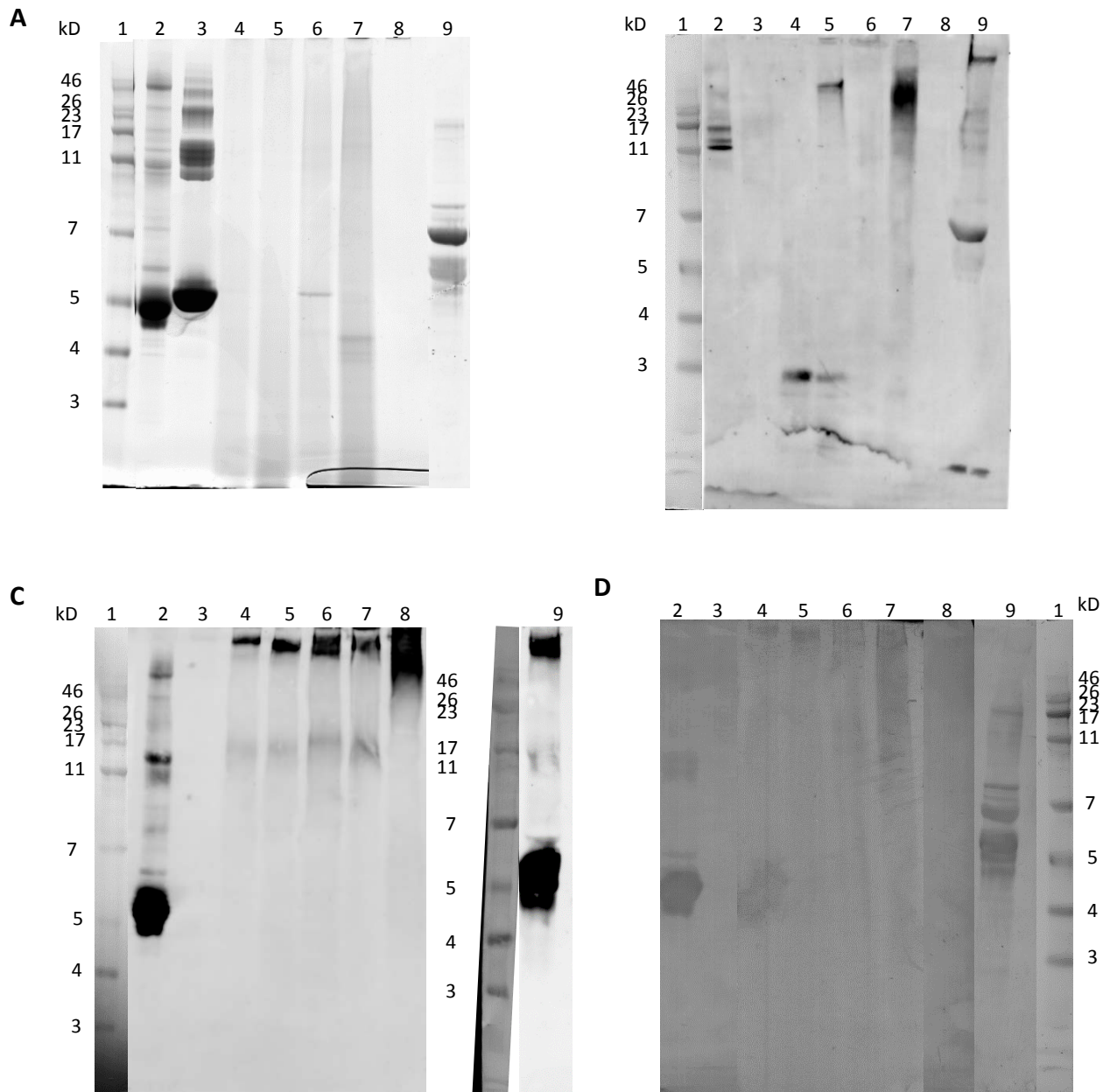


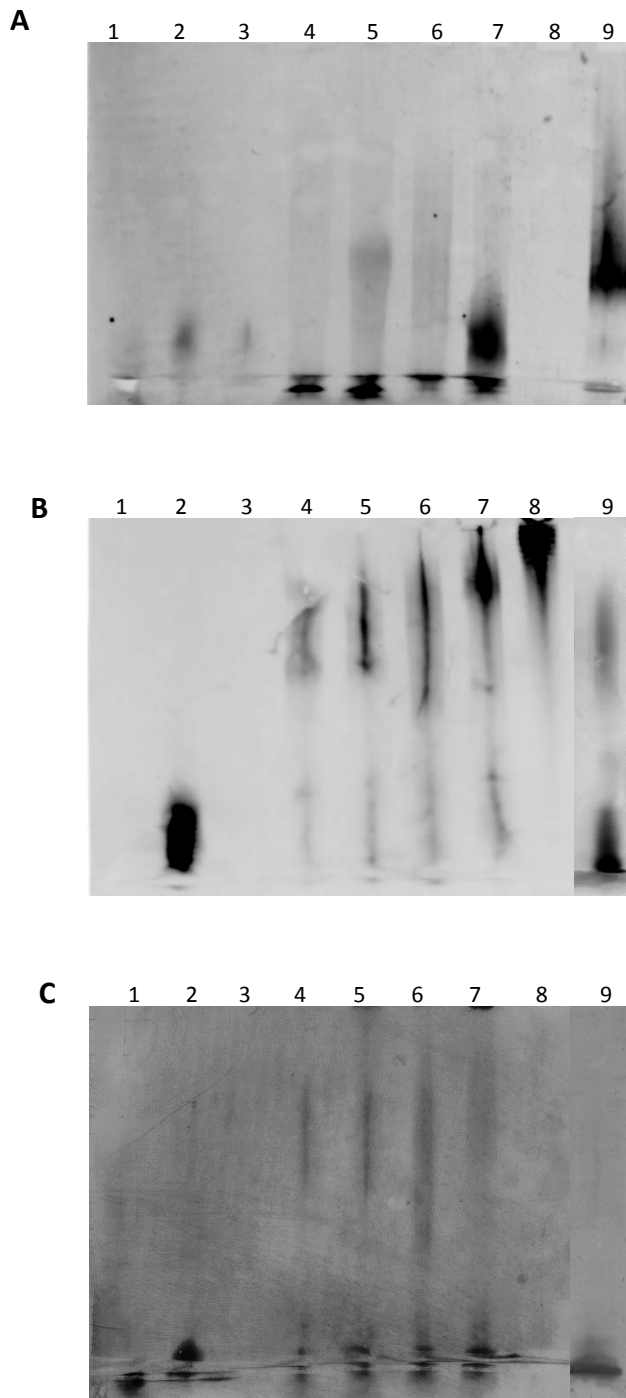
## Supplemental data

**Figure S1 Separation of the guanidinium chloride insoluble mucins by SDS-PAGE (small proteins).**

Protein were detected by (A) Coomassie blue, (B) anti-MUC2, (C) WGA, (D) Alcian blue staining. The Coomassie blue staining shows the total proteins in the samples. 1- HiMark protein standard (Invitrogen, Thermo Fisher Scientific), 2-Fetuin (glycoprotein control), 3- BSA (glycosylation negative control), 4-WT SI Muc2, 5-*C3GnT*<sup>-/-</sup> SI Muc2, 6- WT colon Muc2, 7- *C3GnT*<sup>-/-</sup> colon Muc2, 8-pPGM, 9- LS174T MUC2

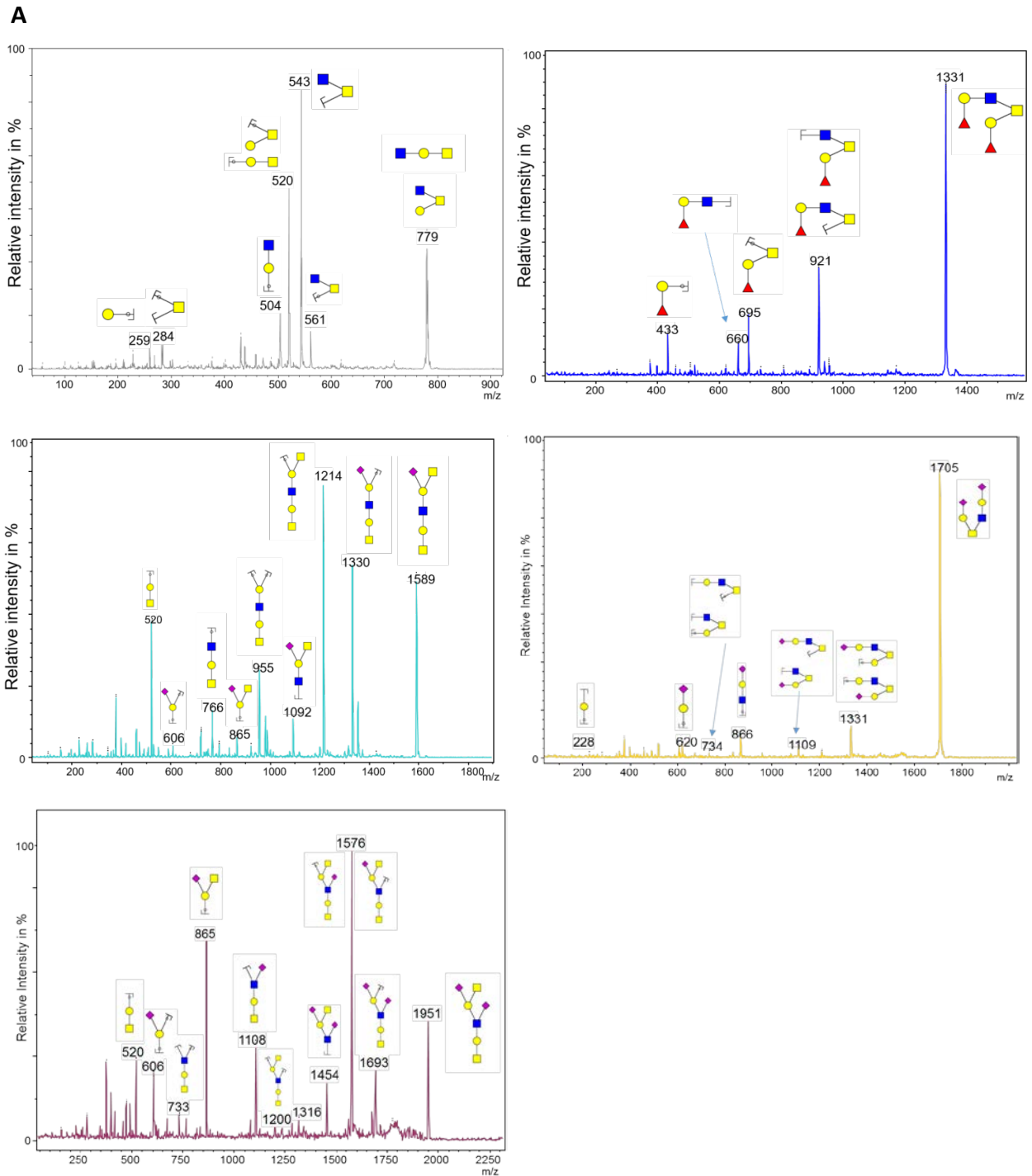


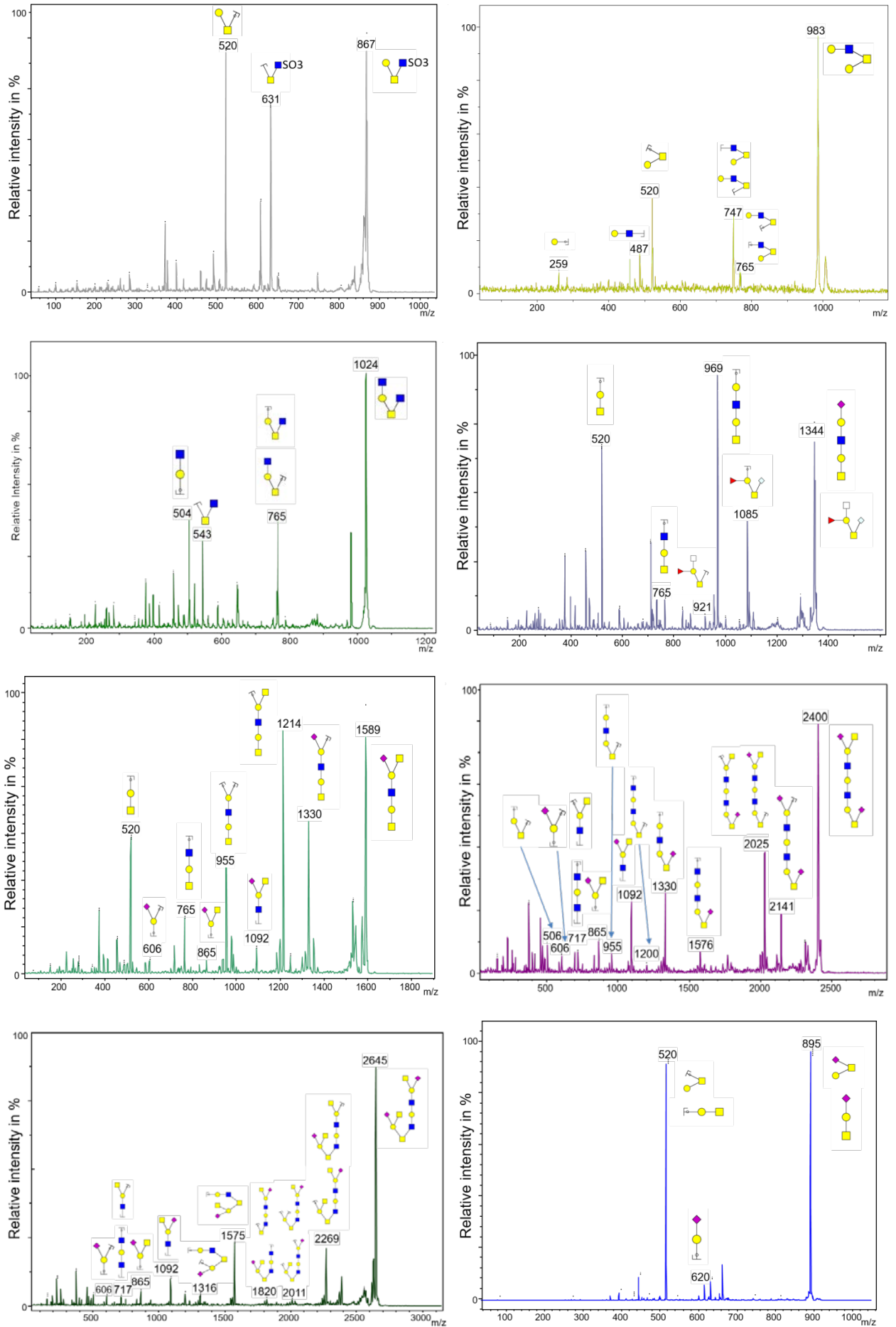
**Figure S2 Separation of the guanidinium chloride insoluble mucins by VAGE** (high molecular weight proteins). Proteins were detected by (A) anti-MUC2, (B) WGA, (C) Alcian blue staining. 1- HiMark protein standard, 2-Fetuin (glycoprotein control), 3- BSA (glycosylation negative control), 4-WT SI Muc2, 5-*C3GnT*<sup>-/-</sup> SI Muc2, 6- WT colon Muc2, 7- *C3GnT*<sup>-/-</sup> colon Muc2, 8-pPGM, 9- LS174T MUC2



**Figure S3 MS/MS spectra of *O*-glycans from mice *Muc2*.**

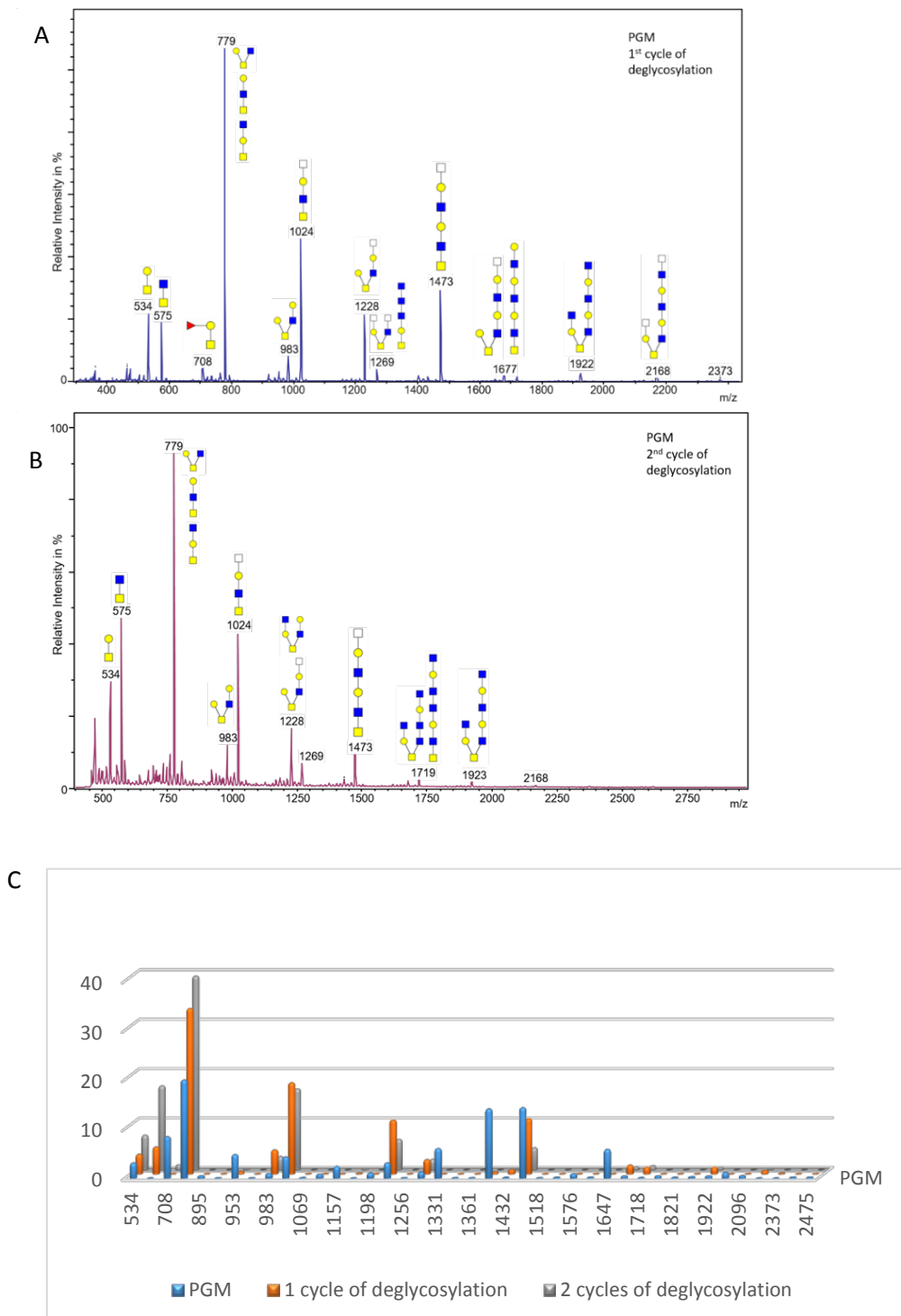
*O*-glycans were fragmented in positive mode from (A) colon WT and *C3GnT*<sup>-/-</sup> mice and (B) from small intestine WT and *C3GnT*<sup>-/-</sup> mice. Monosaccharide symbols follow the Symbol Nomenclature for Glycans (SNFG) system (1). Key: fucose (red triangle), GlcNAc (blue square), sialic acid (purple diamond), galactose (yellow circle) and HexNAc (off-white square).



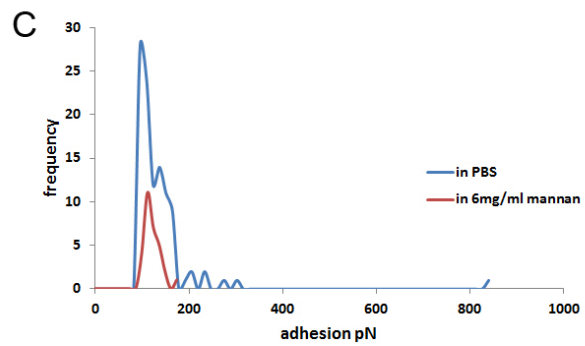
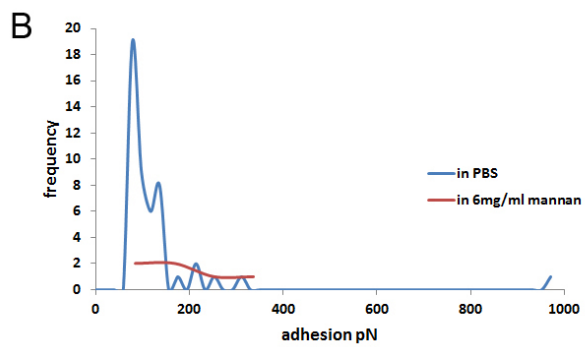
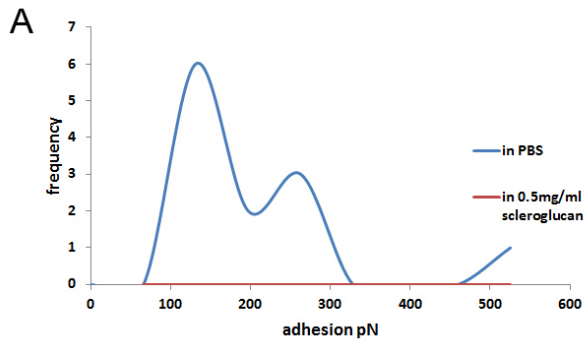
**B**

**Figure S4 Structural characterisation of pPGM *O*-glycan structures after deglycosylation cycles**

MALDI-MS spectra, acquired in the positive ion mode  $[M + Na]^+$  of permethylated *O*-glycans from pPGM after (A) one cycle of deglycosylation and (B) two cycles of deglycosylation. (C) shows a histogram of the different *O*-glycans found on pPGM after several cycles of deglycosylation. The proportion of each *O*-glycan is relative to other species in a sample Monosaccharide symbols follow the Symbol Nomenclature for Glycans (SNFG) system (1). Key: fucose (red triangle), GlcNAc (blue square), sialic acid (purple diamond), N-glycolyl sialic acid (light blue diamond), galactose (yellow circle), and HexNAc (off-white square).

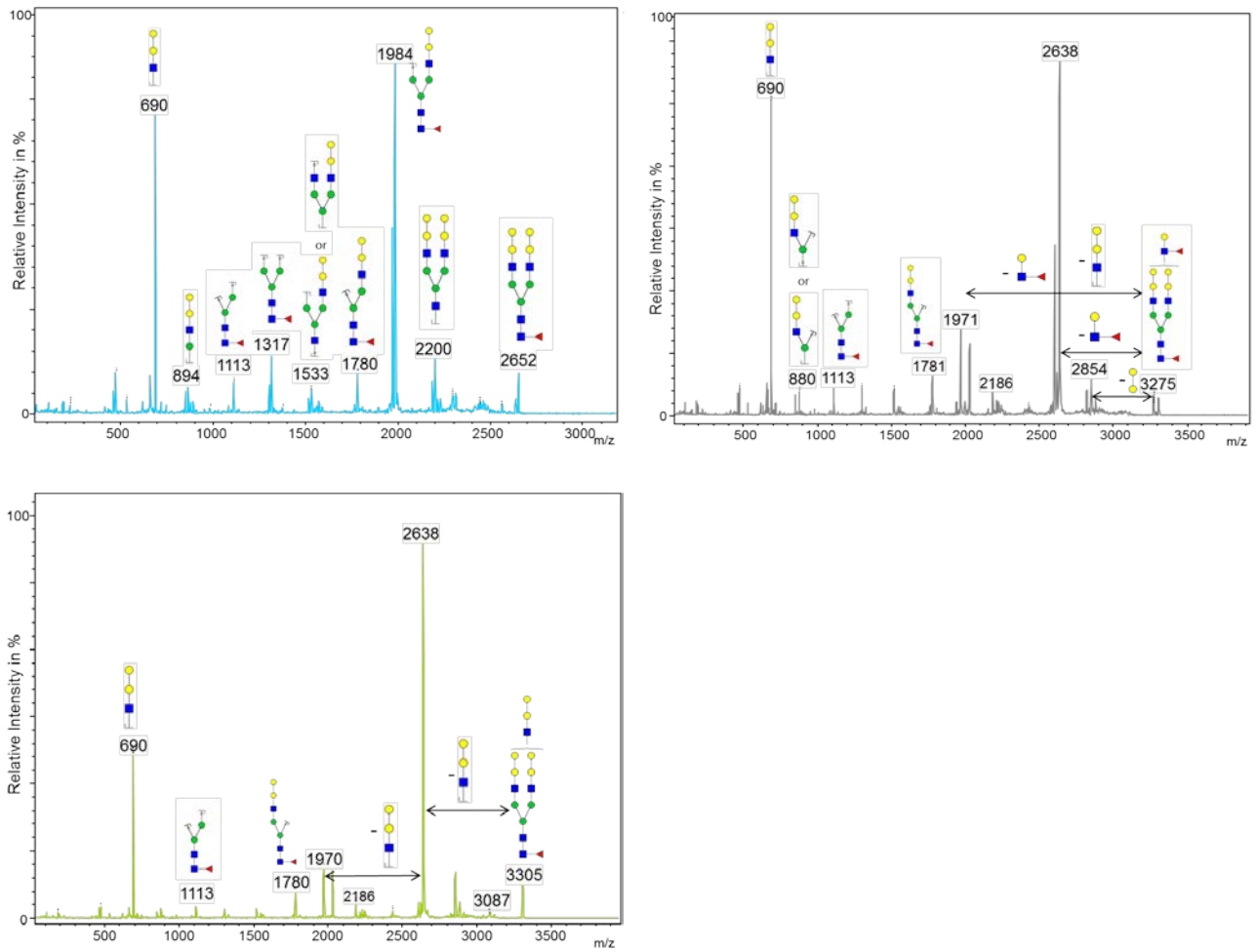


**Figure S5 Force spectra histograms of adhesion between human C-type lectins and mucin.** Interaction between WT colonic Muc2 and (A) hDectin-1, (B) hDectin-2, (C) DC-SIGN in presence or absence of potential inhibitors.



**Figure S6: MS/MS spectra of *N*-glycans from mDectin-2.**

MS/MS spectra of parent ion at  $m/z=2652$ ; 3275 and 3305 fragmented in positive mode. Monosaccharide symbols follow the Symbol Nomenclature for Glycans (SNFG) system (1). Key: fucose (red triangle), GlcNAc (blue square), sialic acid (purple diamond), galactose (yellow circle) and HexNAc (off-white square).


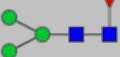

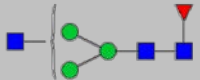
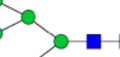





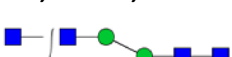




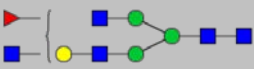
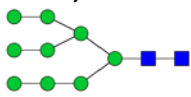
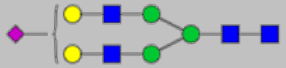
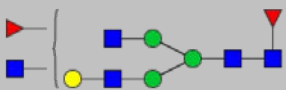
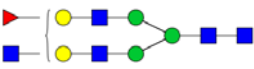

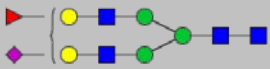
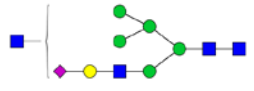
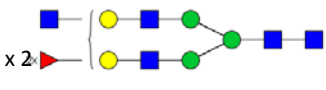
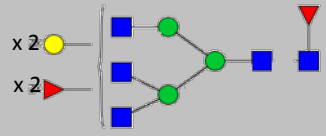
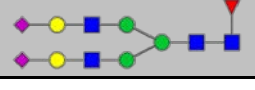
**Table S1 Proposed pPGM O-glycan structures after deglycosylation cycles.** Quantification through the relative percentage of each oligosaccharide was calculated based on integration of peaks on MS spectra.

<i>Sequence of oligosaccharides</i>	<b>mass</b>	<b>pPGM</b>	<b>pPGM 1st cycle</b>	<b>pPGM 2nd cycle</b>
		<b>relative %</b>	<b>relative %</b>	<b>relative %</b>
1 Gal, GalNAcol	534	3.18 +/-1.29	4.06 +/-3.25	7.03 +/-1.93
HexNAc, GalNAcol	575		5.53 +/-5.66	17.02 +/-8.48
1 Gal, 1 Fuc, GalNAcol	708	8.51 +/-2.12		1.04 +/- 0.74
1 Gal, 1 HexNAc, GalNAcol	779	19.99 +/-6.69	33.61 +/-2.67	39.30 +/-5.74
1 Gal, 1 NeuAc, GalNAcol	895	0.55 +/-0.19		
2 Gal, 1 Fuc, GalNAcol	912	0.08 +/-0.12		
1 Gal, 1 HexNAc, 1 Fuc, GalNAcol	953	4.87 +/-0.81	0.57 +/-0.24	0.09 +/-0.12
NeuGc,HexNAc,GalNAcol	967	0.09 +/-0.13		
2 Gal, 1 HexNAc, GalNAcol	983	1.06 +/-0.33	4.95 +/-1.52	2.70 +/-0.58
1 Gal, 2 HexNAc, GalNAcol	1024	4.42 +/-0.34	18.55 +/-1.86	16.43 +/-5.44
1 Gal, 1 Fuc, 1 NeuAc, GalNAcol	1069	0.14 +/-0.20		
1 Gal,1 HexNAc, 1 NeuAc, GalNAcol	1140	0.91 +/- 0.34		
2 Gal, 1 HexNAc, 1 Fuc, GalNAcol	1157	2.49 +/-0.84		
3 Gal, 1 HexNAc, GalNAcol	1187		0.15 +/-0.22	0.20 +/-0.28
1 Gal, 2 HexNAc, 1 Fuc, GalNAcol	1198	1.14 +/-0.31		
2 Gal, 2 HexNAc, GalNAcol	1228	3.19 +/-0.75	11.00 +/-1.22	6.18 +/-3.54
1 Gal, 2 NeuAc, GalNAcol	1256	0.10 +/-0.14		
1 Gal, 3 HexNAc, GalNAcol	1269	1.36 +/-0.22	2.98 +/-1.08	
2 Gal, 1 HexNAc, 2 Fuc, GalNAcol	1331	6.11 +/-2.18		2.13 +/-1.30
2 Gal, 1 HexNAc, 1 NeuAc, GalNAcol	1344	0.12 +/-0.14		
3 Gal, 1 HexNAc, 1 Fuc, GalNAcol	1361	0.11 +/-0.15		
2 Gal, 2 HexNAc, 1 Fuc, GalNAcol	1402	14.14 +/-0.73	0.44 +/-0.62	
3 Gal, 2 HexNAc, GalNAcol	1432	0.19 +/-0.27	0.96 +/-0.88	0.17 +/-0.24
2 Gal, 3 HexNAc, GalNAcol	1473	14.38 +/-2.84	11.30 +/-6.20	4.47 +/-3.06
2 Gal, 1 HexNAc, 1 Fuc, 1 NeuAc, GalNAcol	1518	0.11 +/-0.15		
3 Gal, 1 HexNAc, 2 Fuc, GalNAcol	1535	0.21 +/-0.30		
2 Gal, 2 HexNAc, 2 Fuc, GalNAcol	1576	1.00 +/-0.72		
3 Gal, 2 HexNAc, 1 Fuc, GalNAcol	1606	0.24 +/-0.34		
2 Gal, 3 HexNAc, 1 Fuc, GalNAcol	1647	5.94 +/-0.96		
3 Gal, 3 HexNAc, GalNAcol	1677	0.49 +/-0.38	1.89 +/-0.72	0.65 +/-0.49
2 Gal, 4 HexNAc, GalNAcol	1718	0.14 +/-0.20	1.46 +/-0.73	0.83 +/-0.63
3 Gal, 2 HexNAc, 2 Fuc, GalNAcol	1780	0.54 +/-0.43		
2 Gal, 3 HexNAc, 2 Fuc, GalNAcol	1821	0.22 +/-0.31		
3 Gal, 3 HexNAc, 1 Fuc, GalNAcol	1851	0.35 +/-0.49		
3 Gal, 4 HexNAc, GalNAcol	1922	0.51 +/-0.37	1.45 +/-0.05	0.52 +/-0.38
3 Gal, 3 HexNAc, 2 Fuc, GalNAcol	2025	1.26 +/-0.28		
3 Gal, 4 HexNAc, 1 Fuc, GalNAcol	2096	0.58 +/-0.31		
3 Gal, 5 HexNAc, GalNAcol	2168		0.75 +/-0.57	0.18 +/-0.25
4 Gal, 3 HexNAc, 3 Fuc, GalNAcol	2404	0.27 +/-0.27		
4 Gal, 5 HexNAc, GalNAcol	2373		0.15 +/-0.22	
4 Gal, 4 HexNAc, 2 Fuc, GalNAcol	2475	0.26 +/-0.19		



**Table S2 Proposed oligosaccharide structures of colonic WT and *C3GnT*<sup>-/-</sup> Muc2 N-glycans.** Reduced and alkylated mucins were digested by trypsin and then treated by PNGase F to release N-glycans. Quantification through the relative percentage of each oligosaccharide was calculated based on the integration of peaks from the MS spectra.

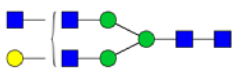
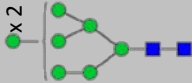
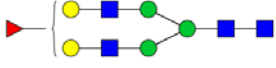
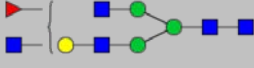

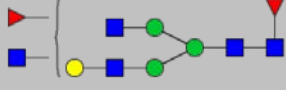
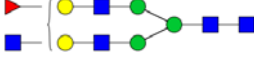

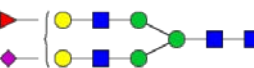
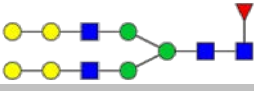

<i>Proposed structures or sequence of oligosaccharides</i>	<i>[M+Na]<sup>+</sup></i>	<i>Relative %</i>	
		<i>MUC2 Colon C3GnT<sup>-/-</sup></i>	<i>MUC2 Colon WT</i>
3 Man, 2 GlcNAc 	1171	0,92	-
3 Man, 1 Fuc, 2 GlcNAc 	1345	1,10	-
5 Man, 2 GlcNAc 	1579	10,12	5,57
3 Man, 1 Fuc, 3 GlcNAc 	1590	-	1,11
6 Man, 2 GlcNAc 	1783	6,38	3,83
3 Man, 1 Fuc, 4 GlcNAc 	1835	2,25	3,20
3 Man, 5 GlcNAc 	1906	-	1,53
7 Man, 2 GlcNAc 	1987	3,87	3,05
1 Gal, 3 Man, 1 Fuc, 4 GlcNAc 	2040	2,09	2,12
2 Gal, 3 Man, 4 GlcNAc 	2070	1,80	-
1 Gal, 3 Man, 5 GlcNAc 	2111	1,49	1,61
8 Man, 2 GlcNAc 	2192	5,59	4,17
2 Gal, 3 Man, 1 Fuc, 4 GlcNAc 	2244	4,01	3,10

1 Gal, 3 Man, 1 Fuc, 5 GlcNAc	2285	5,13	4,28
			
9 Man, 2 GlcNAc	2396	6,91	7,16
			
2 Gal, 3 Man, 1 NeuAc, 4 GlcNAc	2431	1,30	-
			
1 Gal, 5 Man, 1 Fuc, 4 GlcNAc ( <i>hybrid</i> )	2448	1,46	-
1 Gal, 3 Man, 2 Fuc, 5 GlcNAc	2459	1,87	-
			
2 Gal, 3 Man, 1 Fuc, 5 GlcNAc	2489	3,06	3,13
			
1 Gal, 3 Man, 1 Fuc, 6 GlcNAc	2530	-	2,69
			
3 Man, 1 Fuc, 5 HexNAc, 2 GlcNAc	2571	-	2,34
2 Gal, 3 Man, 1 NeuAc, 1 Fuc, 4 GlcNAc ( <i>biantennary monosialylated and fucosylated</i> )	2605	2,45	2,07
			
5 Hex, 1 Fuc, 1 NeuGc, 4 GlcNAc/ 6 Hex, 1 NeuAc, 4 GlcNAc ( <i>hybrid</i> )	2635	2,53	-
			
1 Gal, 3 Man, 1 NeuAc, 1 Fuc, 5 HexNAc	2646	2,11	2,38
2 Gal, 3 Man, 2 Fuc, 5 GlcNAc	2663	1,69	2,25
			
2 Gal, 3 Man, 1 Fuc, 6 GlcNAc	2734	1,62	3,13
1 Gal, 3 Man, 1 Fuc, 5 HexNAc, 2 GlcNAc	2775	-	2,07
2 Gal, 3 Man, 3 Fuc, 6 GlcNAc	2837	2,00	-
			
2 Gal, 3 Man, 1 Fuc, 1 NeuAc, 5 GlcNAc	2850	-	3,88
2 Gal, 3 Man, 2 NeuAc, 1 Fuc, 4 GlcNAc ( <i>biantennary disialylated and fucosylated</i> )	2966	2,62	3,49
			

2 Gal, 3 Man, 1 Fuc, 5 HexNAc, 2 GlcNAc	2979	-	2,34
2 Gal, 3 Man, 2 Fuc, 1 NeuAc, 3 HexNAc, 2 GlcNAc	3024	-	4,41
3 Gal, 3 Man, 1 Fuc, 5 HexNAc, 2 GlcNAc	3183	-	2,22
2 Gal, 3 Man, 1 Fuc, 2 NeuAc, 5 GlcNAc	3211	8,27	10,31
2 Gal, 3 Man, 1 Fuc, 6 HexNAc, 2 GlcNAc	3224	-	2,79
2 Gal, 3 Man, 1 Fuc, 2 NeuAc, 6 GlcNAc	3456	5,23	3,57
3 Gal, 3 Man, 1 Fuc, 1 NeuAc, 7 GlcNAc	3544	2,95	-
2 Gal, 3 Man, 1 Fuc, 3 NeuAc, 4 HexNAc, 2 GlcNAc	3817	-	3,19
1 Gal, 3 Man, 3 Fuc, 2 NeuAc, 7 GlcNAc / 3 Gal, 3 Man, 1 Fuc, 2 NeuAc, 7 GlcNAc	3905	3,52	-
3 Gal, 3 Man, 1 Fuc, 3 NeuAc, 7 GlcNAc	4267	5,70	2,99

**Table S3 Proposed oligosaccharide structures of C-type lectins N-glycans.** Quantification through the relative percentage of each oligosaccharide

<i>Proposed structures or sequence of oligosaccharides</i>	<i>[M+Na]<sup>+</sup></i>	<i>Relative %</i>		
		<i>mDectin-1</i>	<i>mDectin-2</i>	<i>SIGN-R1</i>
5 Man, 2 GlcNAc 	1579	-	-	14,94
3 Man, 1 Fuc, 3 GlcNAc 	1590	-	-	1,70
3 Man, 4 GlcNAc 	1661	-	-	1,37
6 Man, 2 GlcNAc 	1783	-	-	2,23
5 Man, 3 GlcNAc	1824	-	-	4,09
3 Man, 1 Fuc, 4 GlcNAc 	1835	-	-	2,30
7 Man, 2 GlcNAc 	1987	-	-	1,56
1 Gal, 3 Man, 1 Fuc, 4 GlcNAc 	2040	1,46	-	2,78

1 Gal, 3 Man, 5 GlcNAc	2110	-	-	2,83
				
8 Man, 2 GlcNAc	2192	-	-	1,41
				
2 Gal, 3 Man, 1 Fuc, 4 GlcNAc	2244	1,42	-	5,05
				
1 Gal, 3 Man, 1 Fuc, 5 GlcNAc	2285	-	-	7,54
				
9 Man, 2 GlcNAc	2396	-	-	0,86
				
1 Gal, 3 Man, 2 Fuc, 5 GlcNAc	2459	-	-	2,56
				
2 Gal, 3 Man, 1 Fuc, 5 GlcNAc	2489	5,16	-	4,89
				
3 Man, 2 Fuc, 2 HexNAc, 4 GlcNAc	2499	-	-	1,36
1 Gal, 3 Man, 1 Fuc, 6 GlcNAc	2530	-	-	1,14
				
3 Man, 1 Fuc, 5 HexNAc, 2 GlcNAc	2571	-	-	0,56
2 Gal, 3 Man, 1 NeuAc, 1 Fuc, 4 GlcNAc (biantennary monosialylated and fucosylated)	2605	0,93	-	11,28
				
1 Gal, 3 Man, 3 Fuc, 5 GlcNAc	2633	-	-	1,37
3 Gal, 3 Man, 1 NeuAc, 4 GlcNAc/ 2 Gal, 3 Man, 1 NeuGc, 1 Fuc, 4 GlcNAc	2636	5,96	-	
1 Gal, 3 Man, 1 NeuAc, 1 Fuc, 5 HexNAc	2646	-	-	1,80
4 Gal, 3 Man, 4 GlcNAc, 1 Fuc	2652	15,48	17,50	-
				
2 Gal, 3 Man, 2 Fuc, 5 GlcNAc	2663	-	-	1,54
				
2 Gal, 3 Man, 1 Fuc, 6 GlcNAc	2734	-	-	0,75
2 Gal, 3 Man, 2 Fuc, 1 NeuAc, 4 GlcNAc	2778	-	-	1,38

2 Gal, 3 Man, 1 Fuc, 1 NeuAc, 5 GlcNAc	2850	1,67	-	1,92
4 Hex, 2 NeuAc, 2 Fuc, 3 GlcNAc	2895	2,97	-	-
2 Gal, 3 Man, 2 NeuAc, 1 Fuc, 4 GlcNAc (biantennary disialylated and fucosylated)	2966	-	-	1,95
3 Man, 2 Fuc, 8 HexNAc	2990	1,51	-	-
3 Gal, 3 Man, 1 NeuAc, 1 Fuc, 5 GlcNAc	3054	-	-	4,95
5 Gal, 3 Man, 5 GlcNAc, 1 Fuc	3101	5,93	2,64	-
4 Gal, 3 Man, 1 Fuc, 6 GlcNAc	3142	4,83	-	1,27
4 Gal, 3 Man, 5 GlcNAc, 3 Fuc	3245	-	1,83	-
5 Gal, 3 Man, 5 GlcNAc, 2 Fuc	3275	10,26	11,93	-
3 Gal, 3 Man, 1 Fuc, 1 NeuAc, 6 GlcNAc	3298	-	-	0,80
6 Gal, 3 Man, 5 GlcNAc, 1 Fuc	3305	22,90	54,22	-
2 Gal, 3 Man, 3 Fuc, 2 NeuAc, 4 GlcNAc	3314	-	-	0,62
2 Gal, 3 Man, 2 NeuGc, 3 Fuc, 4 GlcNAc	3374	4,47	-	-
2 Gal, 3 Man, 1 Fuc, 2 NeuAc, 6 GlcNAc	3414	-	-	1,67
4 Hex, 3 Man, 1 NeuAc, 2 Fuc, 3 HexNAc, 2 GlcNAc/ 3 Hex, 3 Man, 3 Fuc, 1 NeuGc, 3 HexNAc, 2 GlcNAc	3432	1,57	-	-
5 Gal, 3 Man, 5 GlcNAc, 1 Fuc, 1 NeuAc/4 Gal, 3 Man, 5 GlcNAc, 2 Fuc, , 1 NeuGc	3462	6,07	4,27	-
5 Gal, 3 Man, 5 GlcNAc, 1 Fuc, 1 NeuGc/6 Gal, 3 Man, 2 GlcNAc, 3 HexNAc, 1 NeuAc	3492	3,13	4,11	-
2 Gal, 3 Man, 4 Fuc, 5 HexNAc, 2 GlcNAc	3501	-	-	1,20
3 Gal, 3 Man, 1 Fuc, 2 NeuAc, 6 GlcNAc	3660	-	-	0,63
4 Gal, 3 Man, 2 Fuc, 1 NeuAc, 6 GlcNAc	3677	-	-	1,28
7 Gal, 3 Man, 6 GlcNAc, 1 Fuc	3754	1,71	3,50	-
4 Gal, 3 Man, 1 Fuc, 2 NeuAc, 6 GlcNAc	3863	-	-	1,68
4 Gal, 3 Man, 1 Fuc, 3 NeuAc, 6 GlcNAc	4226	-	-	0,92