Supporting Information

Examination of differential glycoprotein preferences of *N*-acetylglucosaminyltransferase-IV isozymes a and b

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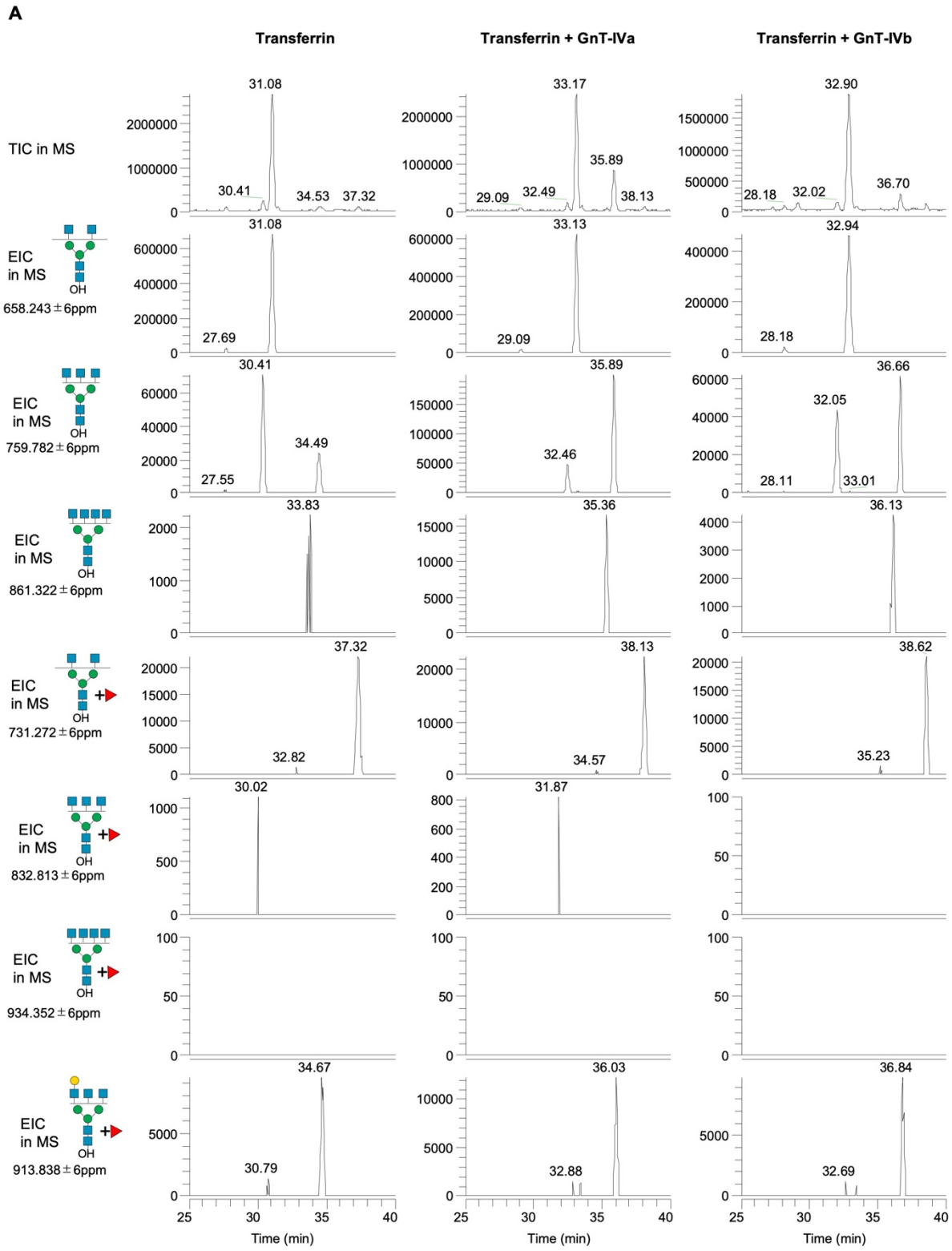
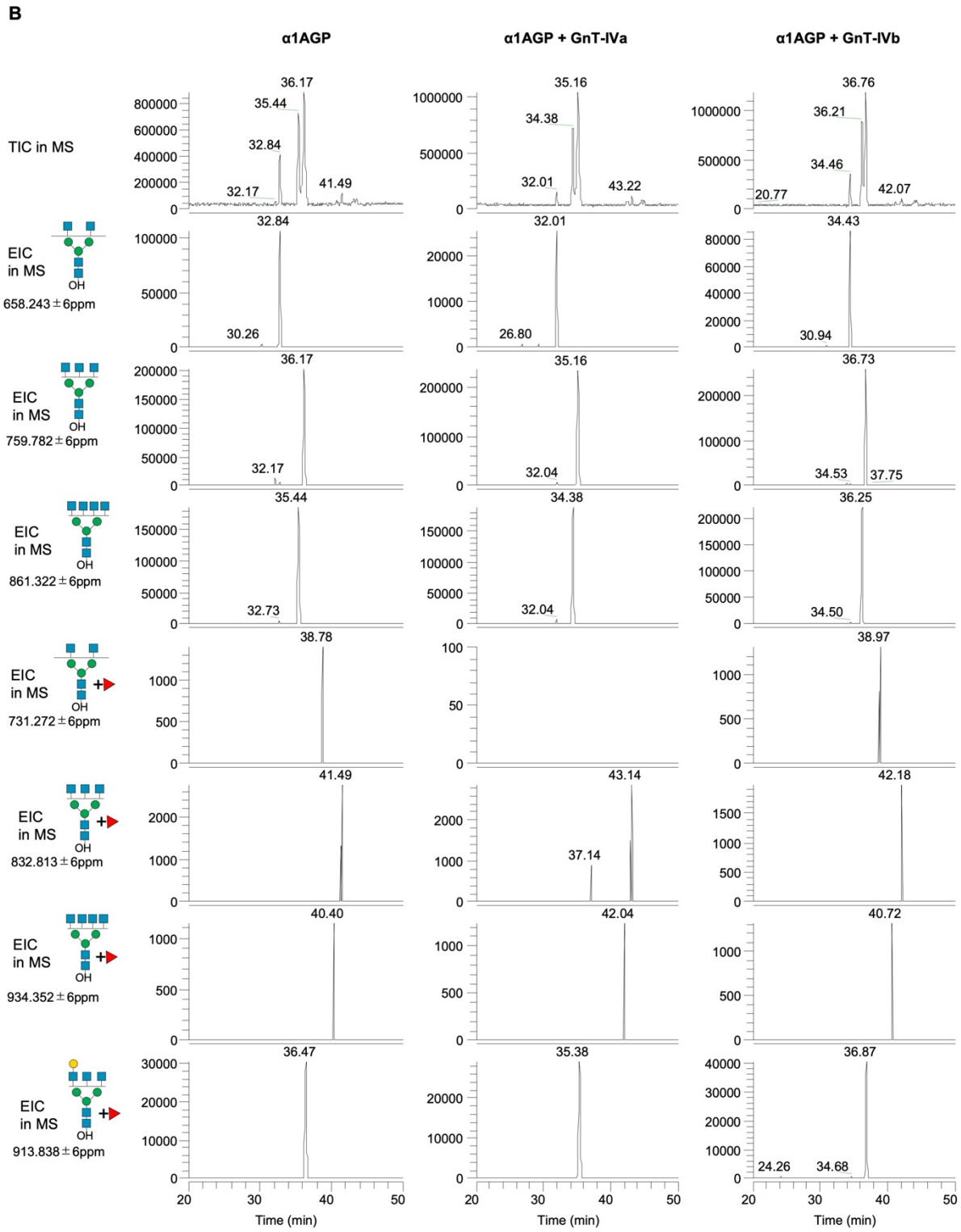
This Supporting information includes:

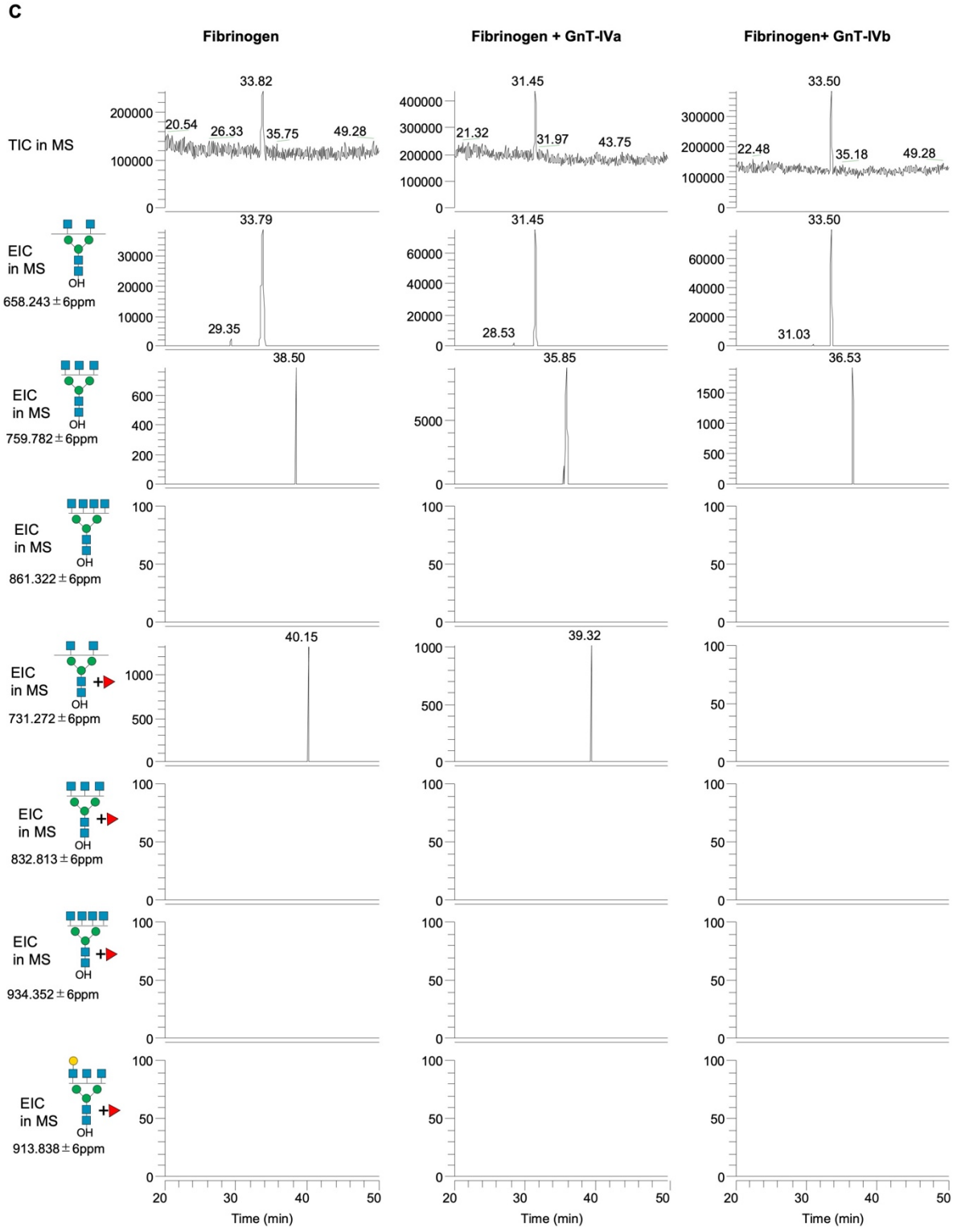
Figs. S1–S7 (included in this PDF)

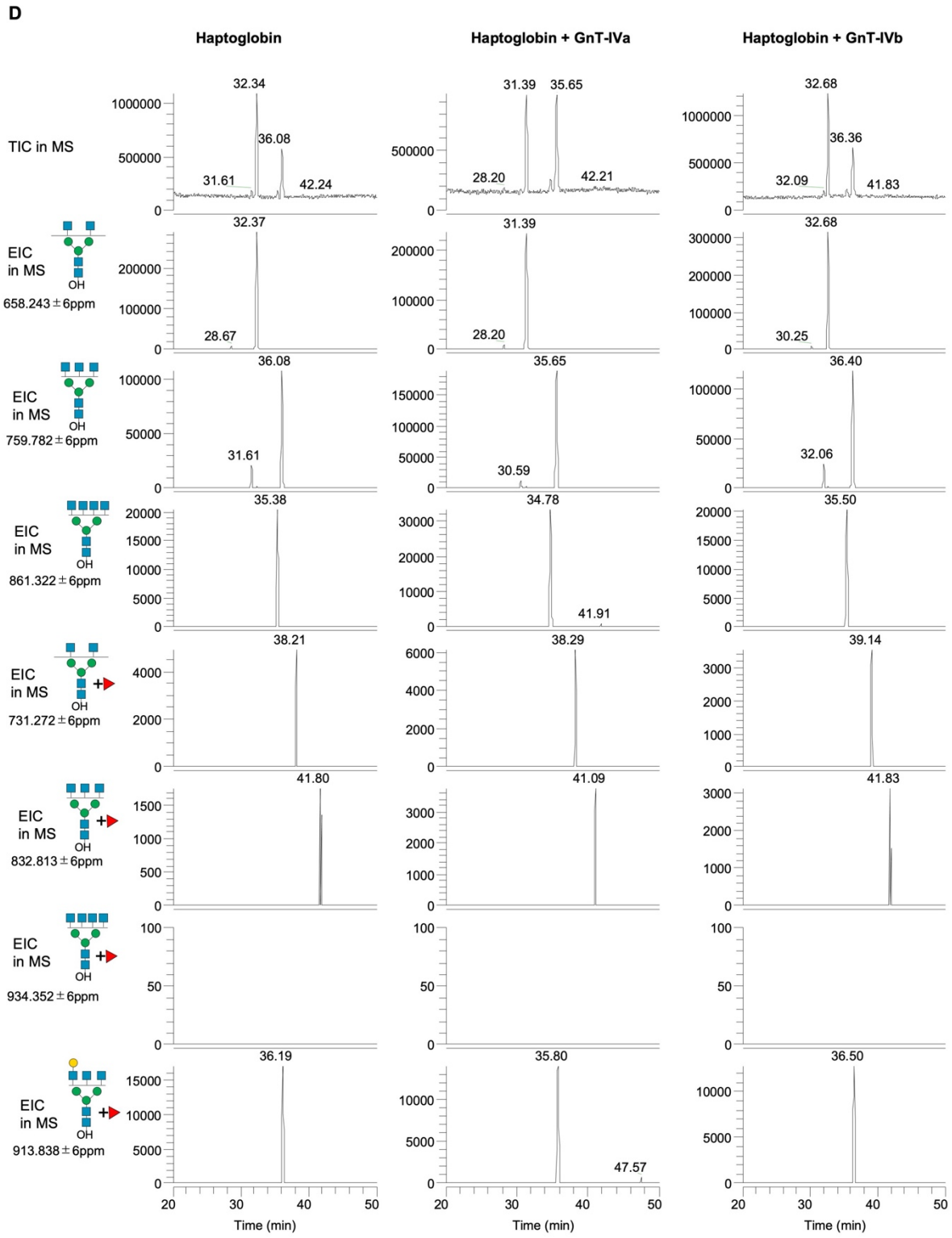
Table S1-S4 (S1 and S4 are included in this PDF. S2 and S3 are separate files)

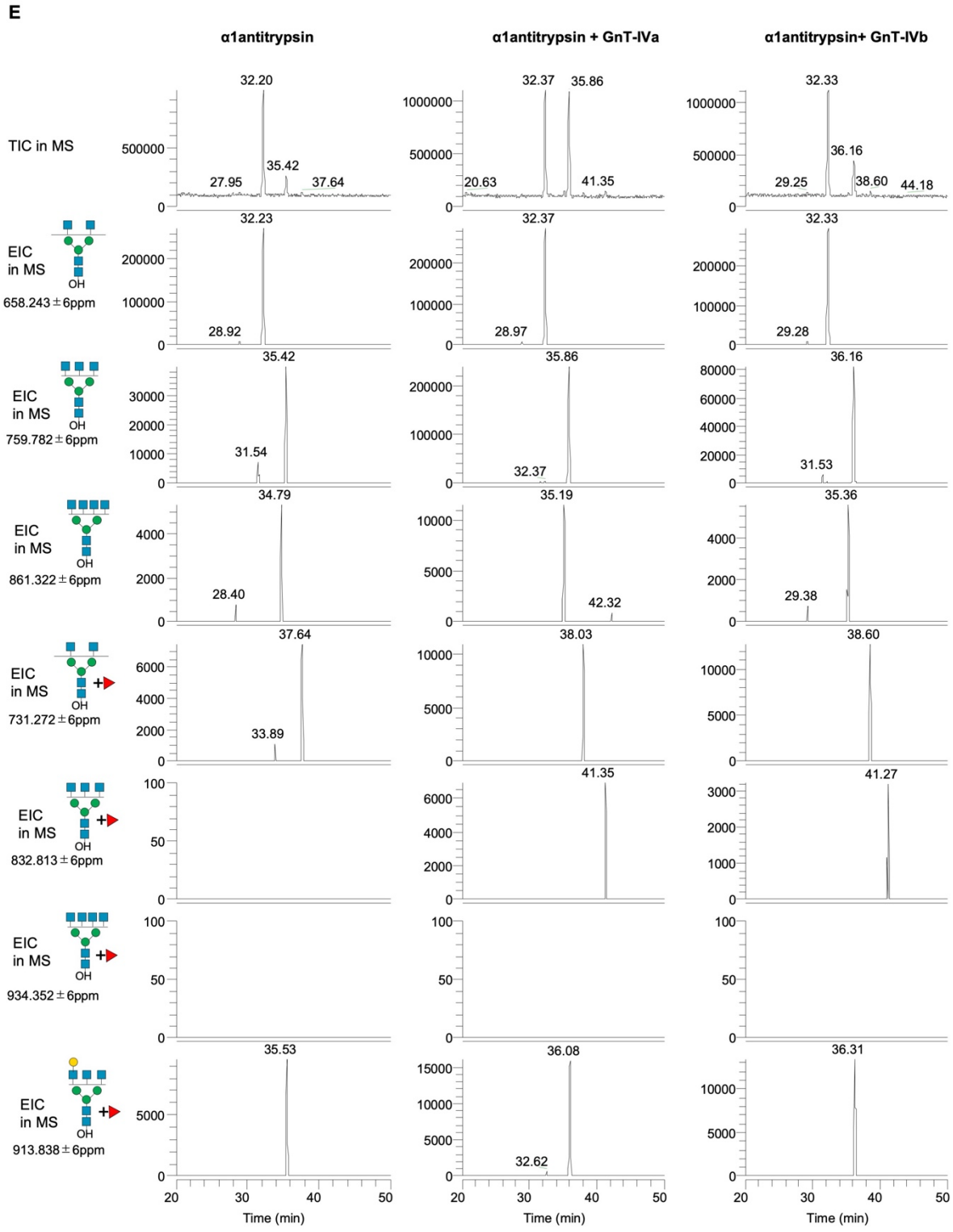
Figure S1


A
Transferrin
Transferrin + GnT-IVa
Transferrin + GnT-IVb
TIC in MS
EIC in MS
658.243 ± 6ppm
759.782 ± 6ppm
861.322 ± 6ppm
731.272 ± 6ppm
832.813 ± 6ppm
934.352 ± 6ppm
913.838 ± 6ppm
OH
Time (min)


B
α1AGP
α1AGP + GnT-IVa
α1AGP + GnT-IVb
TIC in MS
EIC in MS
658.243 ± 6ppm
759.782 ± 6ppm
861.322 ± 6ppm
731.272 ± 6ppm
832.813 ± 6ppm
934.352 ± 6ppm
913.838 ± 6ppm
Time (min)


C
Fibrinogen
Fibrinogen + GnT-IVa
Fibrinogen+ GnT-IVb
TIC in MS
EIC in MS
658.243 ± 6ppm
759.782 ± 6ppm
861.322 ± 6ppm
731.272 ± 6ppm
832.813 ± 6ppm
934.352 ± 6ppm
913.838 ± 6ppm
OH
33.82
20.54
26.33
35.75
49.28
31.45
21.32
31.97
43.75
33.50
22.48
35.18
49.28
33.79
29.35
31.45
28.53
33.50
31.03
38.50
35.85
36.53
40.15
39.32
Time (min)


D
Haptoglobin
Haptoglobin + GnT-IVa
Haptoglobin + GnT-IVb
TIC in MS
EIC in MS
658.243 ± 6ppm
759.782 ± 6ppm
861.322 ± 6ppm
731.272 ± 6ppm
832.813 ± 6ppm
934.352 ± 6ppm
913.838 ± 6ppm
OH
32.34
36.08
31.61
42.24
31.39
35.65
28.20
42.21
32.68
36.36
32.09
41.83
32.37
28.67
31.39
28.20
32.68
30.25
36.08
31.61
35.65
30.59
36.40
32.06
35.38
34.78
41.91
35.50
38.21
38.29
39.14
41.80
41.09
41.83
36.19
35.80
47.57
36.50
Time (min)


E
α1antitrypsin
α1antitrypsin + GnT-IVa
α1antitrypsin+ GnT-IVb
TIC in MS
EIC in MS
658.243 ± 6ppm
759.782 ± 6ppm
861.322 ± 6ppm
731.272 ± 6ppm
832.813 ± 6ppm
934.352 ± 6ppm
913.838 ± 6ppm
OH
Time (min)

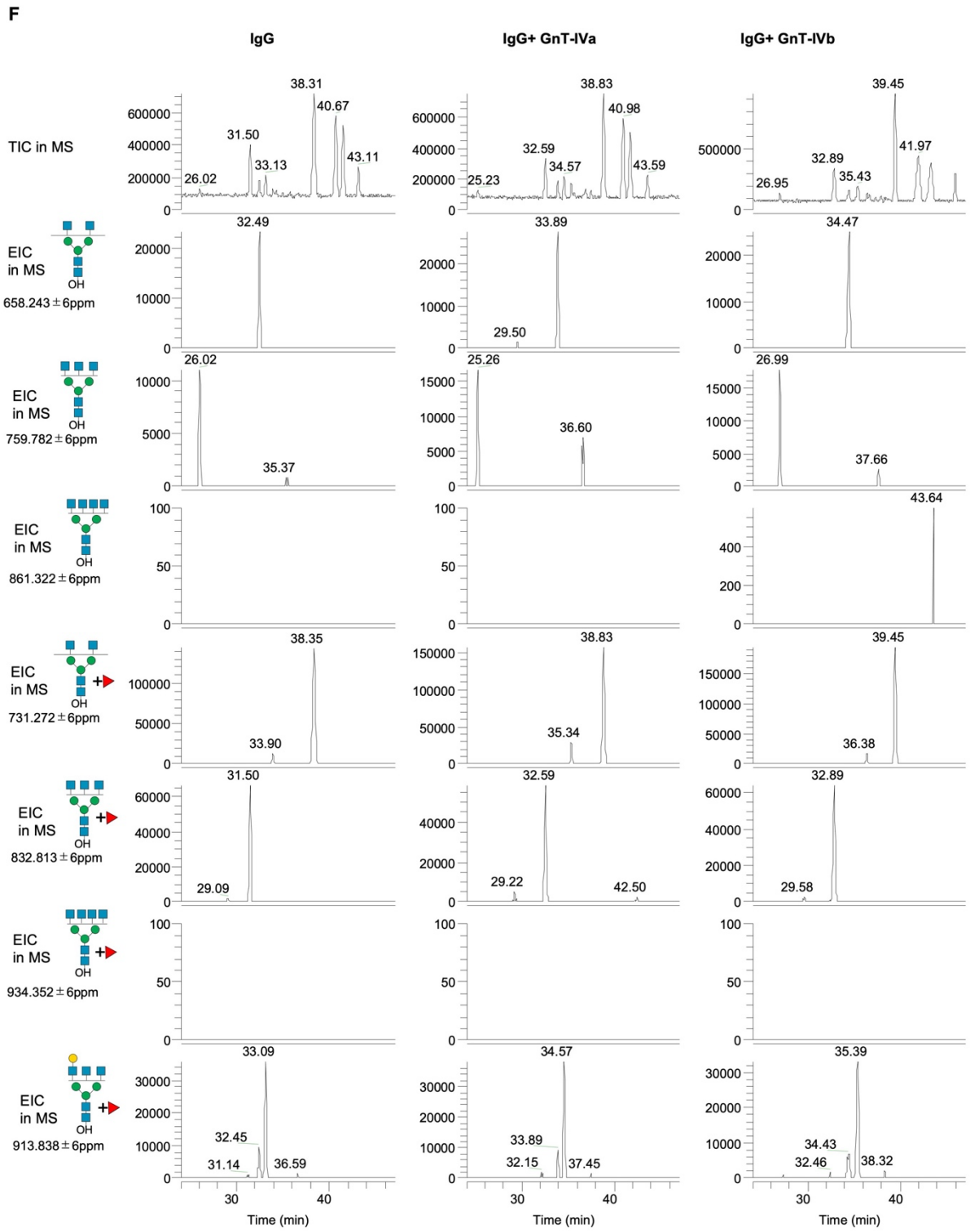


Figure S1. LC-MS analysis of *N*-glycans derived from six glycoproteins. Total ion chromatogram (TIC) and extracted ion chromatograms (EICs) of the major *N*-glycans from glycoproteins used in UDP-Glo assay which had been desialylated and degalactosylated. *A*. Transferrin. *B*. α1AGP. *C*. Fibrinogen. *D*. Haptoglobin. *E*. α1antitrypsin. *F*. IgG.

Figure S2

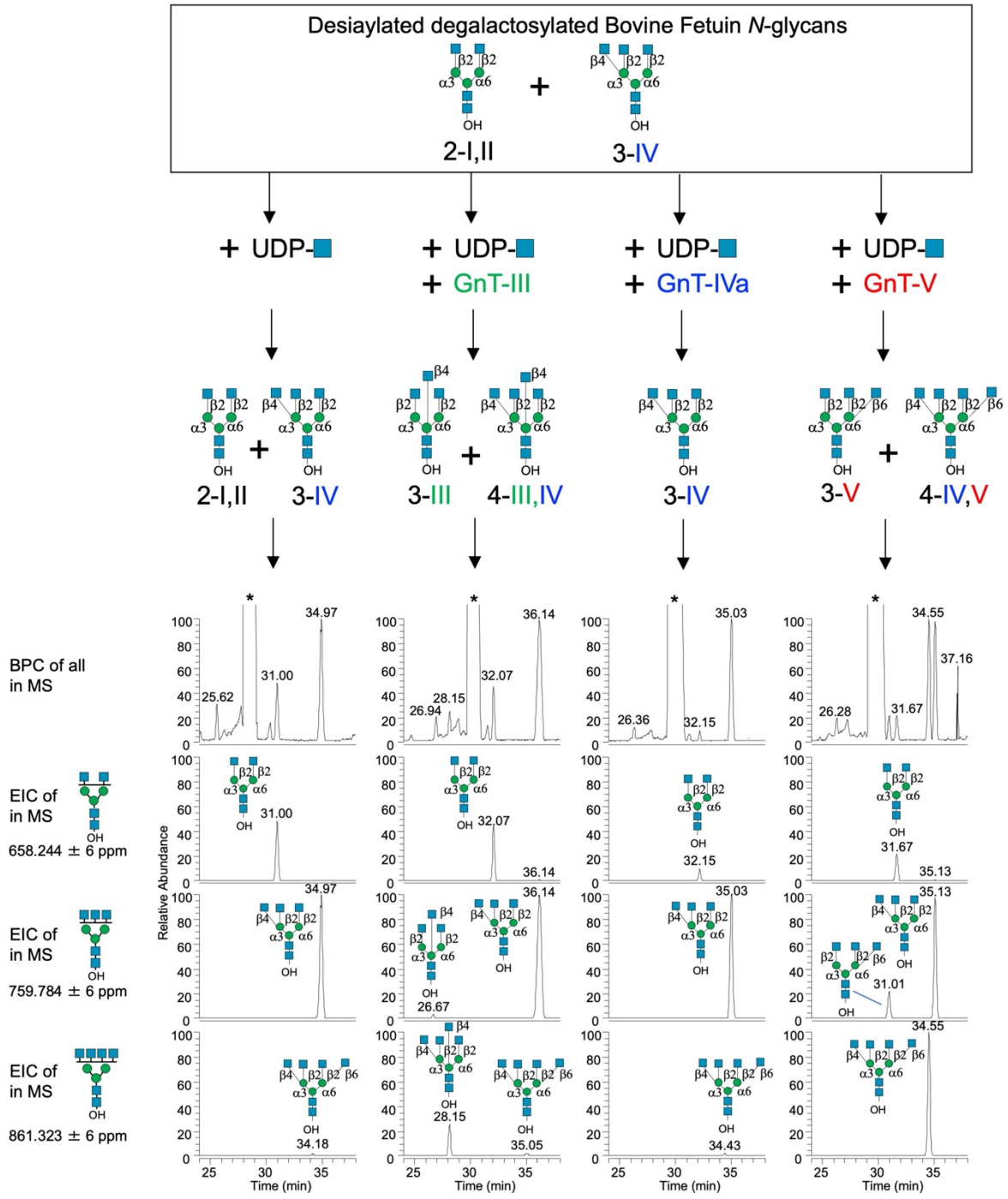
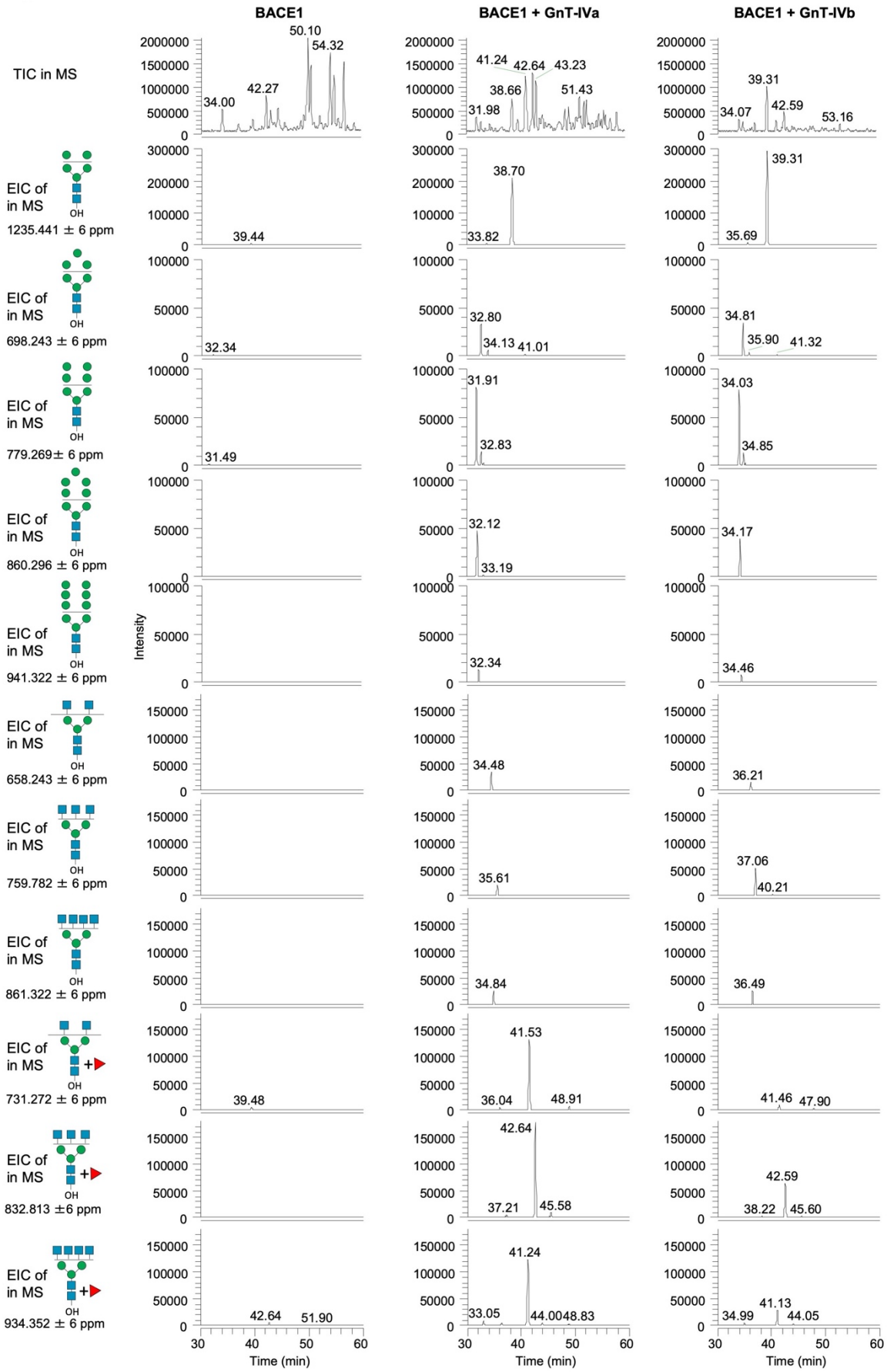


Figure S2. LC-MS analysis of the standard *N*-glycans having defined GlcNAc branches derived from bovine fetuin. The standard tri- and tetra-antennary *N*-glycans having different GlcNAc branches were enzymatically formed from the bi- and tri-antennary glycans of bovine fetuin using purified GnT-III, -IVa, and -V. EICs of the standard glycans with 2, 3, and 4 HexNAc residues (HexNAc in chitobiose is excluded) from LC-MS analysis are shown. Asterisks indicate UDP-GlcNAc used for the enzyme reactions.

Figure S3


BACE1
BACE1 + GnT-IVa
BACE1 + GnT-IVb
TIC in MS
EIC of in MS
1235.441 ± 6 ppm
698.243 ± 6 ppm
779.269± 6 ppm
860.296 ± 6 ppm
941.322 ± 6 ppm
658.243 ± 6 ppm
759.782 ± 6 ppm
861.322 ± 6 ppm
731.272 ± 6 ppm
832.813 ±6 ppm
934.352 ± 6 ppm
Intensity
Time (min)

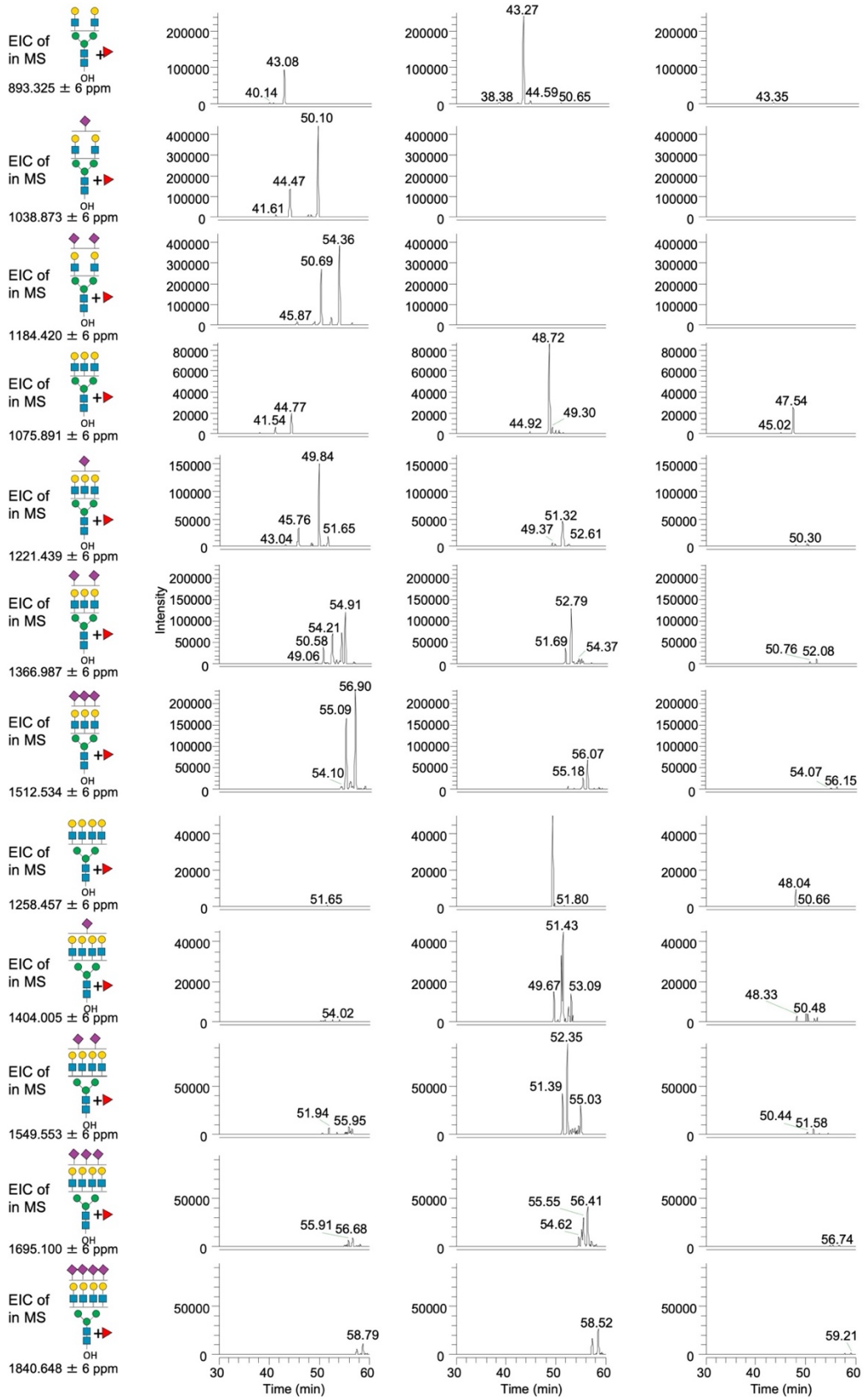


Figure S3. LC-MS analysis of *N*-glycans derived from BACE1 co-expressed with GnT-IVa or GnT-IVb. extracted ion chromatograms (EICs) of the major *N*-glycans.

Figure S4

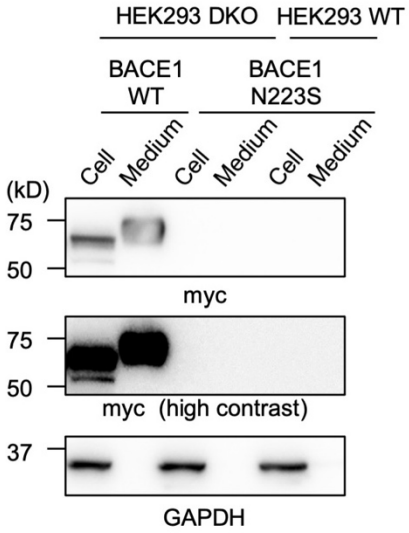


Figure S4. Expression of BACE1ΔTM WT and N223S mutant. HEK293 DKO or WT cells were transfected with the plasmids for expression of BACE1ΔTM WT or N223S mutant. Cell lysates (Cell) and secreted proteins in culture media purified with Ni^{2+}-beads (Medium) were subjected to SDS-PAGE and blotted with the anti-myc antibody (upper) and anti-GAPDH antibody (lower).

Figure S5

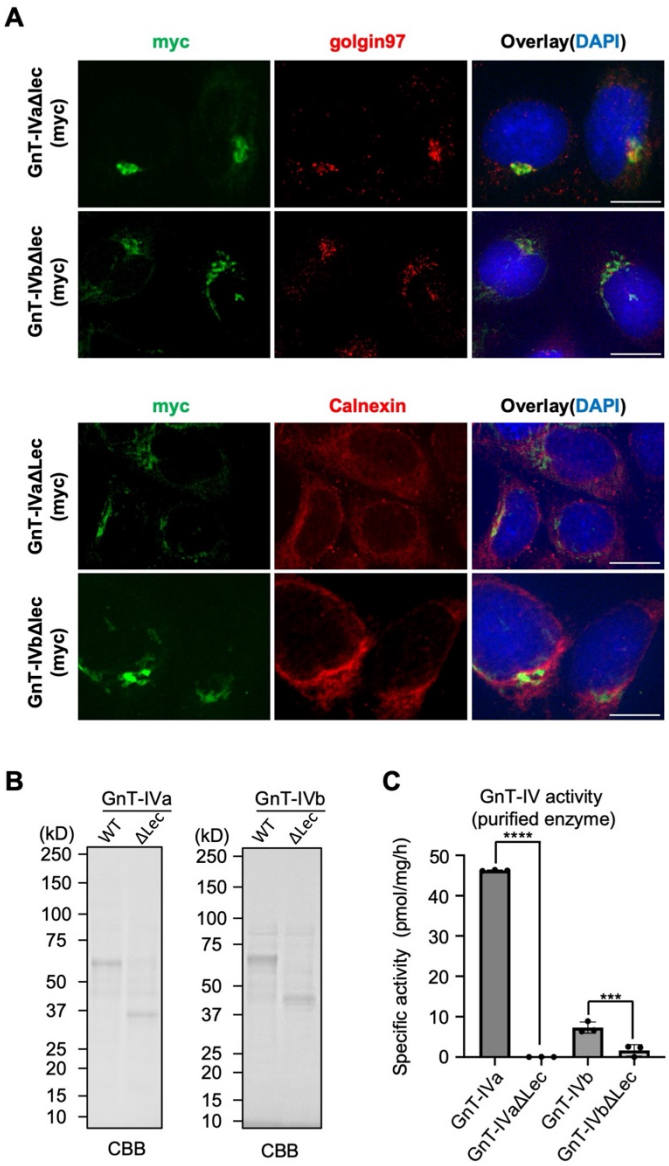


Figure S5. Localization, expression and activity of Δlec mutants. *A*, DKO cells transfected with a plasmid for expression of GnT-IVaΔlec or GnT-IVbΔlec were stained with anti-myc (green), antibodies for marker proteins (golgin-97 for the Golgi or Calnexin for the ER) (red), and DAPI (blue). Scale bar, 10 μm. *B*, soluble GnT-IVaΔlec and -IVbΔlec were expressed in COS7 cells and purified from the media using a Ni^{2+}-column. Purified GnT-IVaΔlec and -IVbΔlec were separated by SDS-PAGE and visualized by CBB staining. *C*, The specific activity of the purified GnT-IVaΔlec and -IVbΔlec ($n = 3$, means ± S.D., ***$p < 0.001$, ****$p < 0.0001$, Tukey's multiple comparisons test).

Figure S6

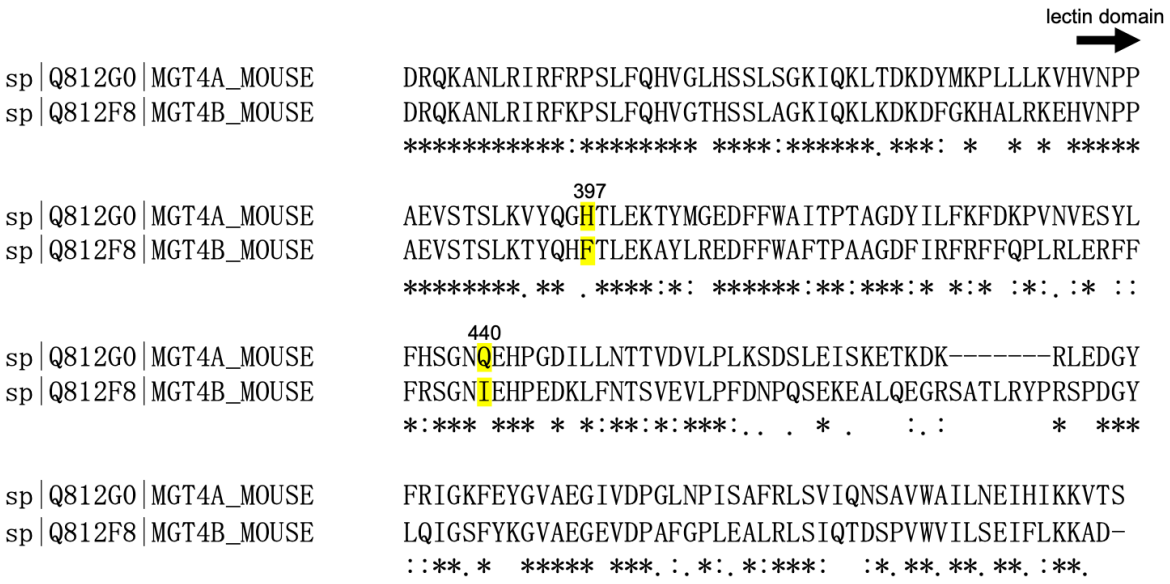


Figure S6. Sequence comparison of mouse GnT-IVa and GnT-IVb lectin domains. Sequence alignment of murine GnT-IVa and -IVb lectin domains. Amino acid residues mutated in Fig. 5 are highlighted in yellow.

Figure S7

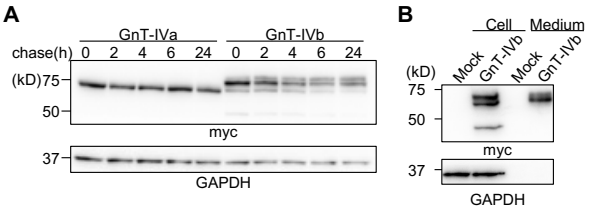


Figure S7. Degradation rate and secretion of GnT-IVb. *A*, DKO cells transfected with GnT-IVa or -IVb were chased for the indicated times in the presence of cycloheximide. Cell lysates were blotted with anti-myc (upper) and anti-GAPDH (lower) antibodies. *B*, proteins from mock-treated DKO cells transfected with an empty vector (Mock) or a plasmid for expression of GnT-IVb (Cell) and secreted GnT-IVb purified with Ni^{2+}-beads (Medium) were subjected to SDS-PAGE and blotted with anti-myc antibody (upper), anti-GAPDH antibody (lower).

Table S1.

Glycoprotein	Uniprot number	Function	N-glycosylation sites	Site occupancy	Glycan species
Alpha-1-acid glycoprotein	P02763; P19652	Transport of lipophilic compounds	Overall		A3G3S3 (33.5 %); A4G4S4 (19.5 %); A3FG3S3 (9 %); A4G4S3 (8.2 %); A3G3S2 (5 %); A2G2S2 (4.5 %); A4G4S2 (4 %); A4FG4S4 (2.5 %)
			Asn33		A3G3S3 (60 %); A3FG3S3 (20 %); A3G3S2 (12.5 %)
			Asn56		A3G3S3 (55 %); A2G2S2 (22.5 %); A3FG3S3 (12.5 %); A3G3S2 (10 %)
			Asn72		A4G4S4 (30 %); A4G4S3 (15 %); A3G3S3 (15 %); A4G4S2 (10 %); A4FG4S3 (5 %); A4FG4S4 (5 %)
			Asn93		A4G4S4 (22.5 %); A4G4S3 (20 %); A3G3S3 (17.5 %); A4FG4S4 (7.5 %); A4FG3S3 (7.5 %); A3FG3S3 (7.5 %); A4G4S2 (7.5 %)
			Asn103		A4G4S4 (45 %); A3G3S3 (20 %); A4G4S2 (10 %); A4G4S3 (7.5 %); A3FG3S3 (5 %)
Alpha-1-antitripsin	P01009	Serine protease inhibitor	Overall		A2G2S2 (81 %); A3G3S3 (9.8 %); A3FG3S3 (5.6 %); FA2G2S2 (3.6 %)
			Asn70		A2G2S2 (91.3 %); FA2G2S2 (8.6 %)
			Asn107		A2G2S2 (52.5 %); A3G3S3 (29.5 %); A3FG3S3 (16.7 %); FA2G2S2 (1.5 %)
			Asn271		A2G2S2 (99.3 %); FA2G2S2 (0.7 %)
Fibrinogen		Coagulation (platelet aggregation)	Overall		A2G2S1(6) (53 %); A2G2S2(6) (33 %)
Fibrinogen alpha-chain	P02671		Asn453	0%	—
			Asn686	0%	—
Fibrinogen beta-chain	P02675		Asn394		**A2G2S1(6)**; A2G2S2(6)
Fibrinogen gamma-chain	P02679		Asn78		**A2G2S1(6)**; A2G2S2(6)
			Asn334		**A2G2S1(6)**; A2G2S2(6)
Haptoglobin	P00738	Scavenger of hemoglobin	Overall		A2G2S2 (45 %); A2G2S1 (26 %); A3G3S3 (9 %); A3FG3S3 (6 %); A3G3S2 (5 %); A3G3S1 (5 %); A2FG2S1 (2 %); A2FG2S2 (1 %)
			Asn184	97.70%	A2G2S2 (46 %); A2G2S1 (38 %); A3G3S3 (4 %); A3G3S2 (3 %); A3G3S1 (2 %); A2FG2S2 (3 %); A2FG2S1 (3 %); A3FG3S3 (1 %)
			Asn207	97.40%	A2G2S2 (47 %); A2G2S1 (39 %); A3G3S1 (7 %); A4G4S1 (2 %); A3FG3S1 (2 %); A4G4S2 (1 %); A2FG2S1 (1 %); A2FG2S2 (1 %)
			Asn211	98.50%	A2G2S2 (40 %); A3G3S3 (29 %), A3FG3S3 (21 %); A3G3S2 (10 %)
			Asn241	95.80%	A2G2S2 (47 %); A2G2S1 (26 %); A3G3S1 (10 %); A3G3S2 (8 %); A3G3S3 (4 %); A2FG2S1 (2 %); A2FG2S2 (1 %); A3FG3S2 (1 %); A4G4S1 (1 %)
Serotransferrin	P02787	Iron transport	Overall		A2G2S2 (96.5 %); FA2G2S2 (2.5 %); A3G3S2 (1 %)
			Asn432		A2G2S2 (93.5 %); A3G3S2 (2.5 %); A2G2S1 (2.4 %); A2FG2S2 (1.6 %)
		Minor Asn-X-Cys site	Asn491	2%	A2G2S2 (100 %)
			Asn630		A2G2S2 (85.9 %); FA2G2S2 (6.9 %); A2FG2S2 (2.8 %); A2G2S1 (2.2 %); A3G3S2 (1.0 %); FA3G3S2 (0.9 %); FA2FG2S2 (0.3 %)
Immunoglobulin G		Immunity (primary; secondary; complement system)	Overall		FA2G1 (31 %); FA2G2 (23 %); FA2G2S1 (13 %); FA2 (10 %); FA2BG1 (5 %)
Immunoglobulin G1	P01857		Asn180		
Immunoglobulin G2	P01859		Asn176		
Immunoglobulin G3	P01860		Asn227		
			Asn322		
Immunoglobulin G4	P01861		Asn177		

Summary of the *N*-glycan structures of the 6 human serum glycoproteins used for the enzyme assay. This table was modified from a previous paper (Clerc et al., Glycoconj. J., 2016, 33, 309-343).

Table S4.

Plasmid name	Forward primer	Reverse primer
mGnT-IVb	ggggagATGAGGCTCCGCAATGGCA	acccttggaagccttCTAGTCGGCC
mGnT-IVb-myc	TTTGAATTCgccaccATGAGGCTCCGCAATGGCAC	TTTCTCGAGGTCGGCCTTTTTCAGAAAGA
mGnT-IVb-A1	-	TGGGTCAGTCTCGGCGATCA
mGnT-IVb-S1	TGATCGCCGAGACTGACCCA	-
mGnT-IVb-S2	CCAGCATGTGGGCACTCACT	-
GnT-IVa ΔLec	TGTGCGGCCGCgccaccATGAGGCTCCGAA	TTTCTCGAGCACGTGGACCTTGAGAAGCA
GnT-IVb ΔLec	TTTGAATTCgccaccATGAGGCTCCGCAATGGCAC	TTTCTCGAGCACGTGCTCCTTCCGGAGAG
GnT-IVa-Lec(b)	TCAAGGTCCACGTGCTCGAGAACCCACCGGCAGAGGTGAG	AAGGGCCCTCTAGACTCGAGGTCGGCCTTTTTCAGAAAGA
GnT-IVb-Lec(a)	GGAAGGAGCACGTGCTCGAGAACCCGCCTGCAGAGGTCTC	AAGGGCCCTCTAGACTCGAGACTGGTGACTTTTTTAATAT
pcDNA-IH/GnT-IVb	GCTCCAAGGAGCTAAACCTG	TTTCTCGAGCTAGTCGGCCTTTTTCAGAA
pcDNA-IH/GnT-IVb Phe413His	CCTCAAGACGTACCAGCATcacACCCTGGAGAAGGCCTACT	Complementary
pcDNA-IH/GnT-IVb Ile456Glu	CTTCTTCCGAAGCGGGAACcaaGAGCACCCGGAAGATAAGC	Complementary
pcDNA-IH/GnT-IVb Δlec	GCTCCAAGGAGCTAAACCTG	TTTCTCGAGGCCCGCCAGTGATGAGTGAG
BACE1 Asn153Ser	TAAGCATCCCCCATGGCCCCagcGTCACTGTGCGTGCCAACAT	Complementary
BACE1 Asn172Ser	AATCAGACAAGTTCTTCATCagcGGCTCCAACTGGGAAGGCATC	Complementary
BACE1 Asn223Ser	TTGTGGTGCTGGCTTCCCCCTCagcCAGTCTGAAGTGCTGGCCTCTG	Complementary
BACE1 Asn354Ser	ACCTAATGGGTGAGGTTACCagcCAGTCCTTCCGCATCACCAT	Complementary

Primers used in this study.