

COMPARISONS OF *CAENORHABDITIS* FUCOSYLTRANSFERASE MUTANTS REVEAL A MULTIPLICITY OF ISOBARIC N-GLYCAN STRUCTURES

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Supplementary Information

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Supplementary Figure S-6: 2D-HPLC analyses of *fut-1;fut-6* N-glycans.

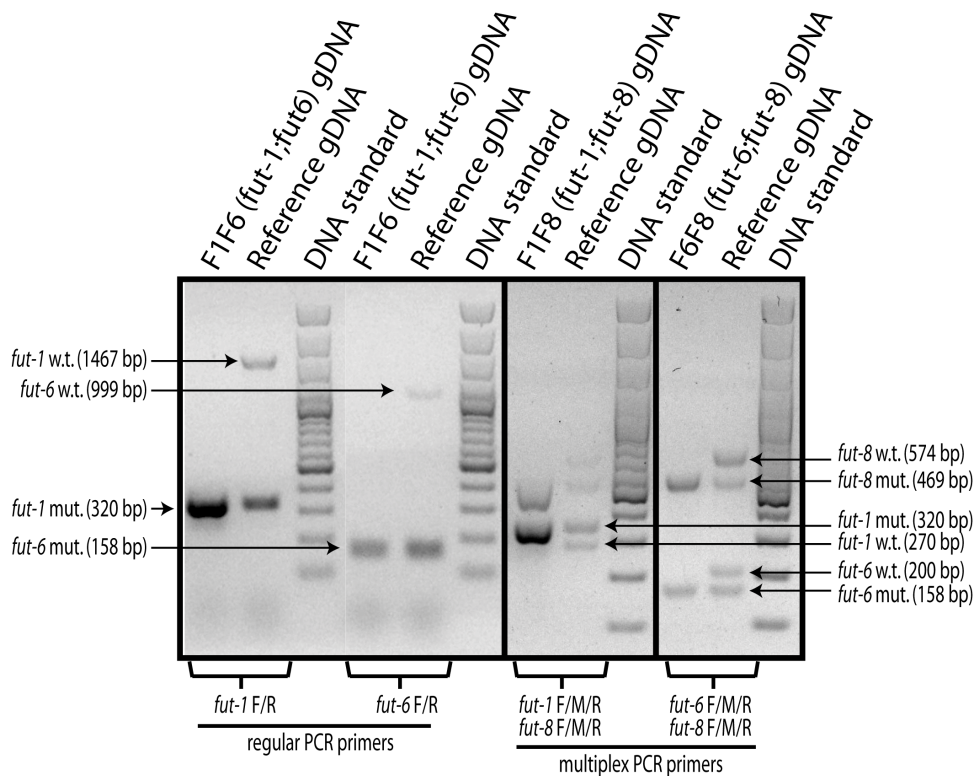
Supplementary Figure S-7: Sequential digestions of a predominant N-glycan from the *fut-1;fut-6* strain.

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Supplementary Table S-1. Summary of evidence for the proposed structures of N-glycans from *Caenorhabditis* double fucosyltransferase deletion mutants.
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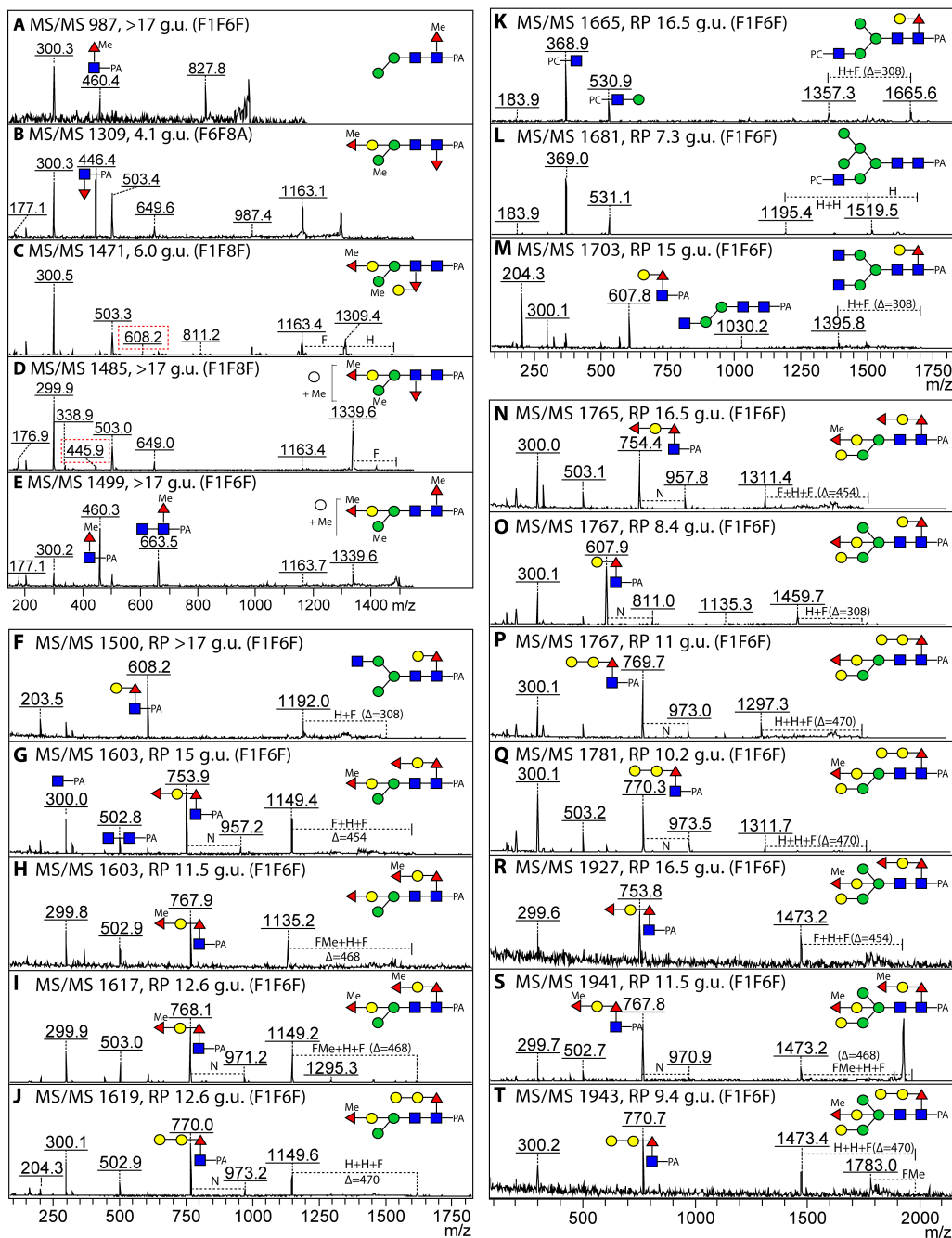
Supplementary Figure S-1: Genotypes of the obtained double fucosyltransferase mutants. The homozygous genotype of the *fut-1;fut-6* (F1F6) double mutant, with deletions in both the *fut-1* and *fut-6* genes, was verified by genomic PCR using *fut-1* and *fut-6*-specific forward and reverse primers (left panel). The *fut-1;fut-8* (F1F8) and *fut-6;fut-8* (F6F8) double mutants were examined by multiplex PCR, using relevant forward (F), internal (M) and reverse (R) primers specific for two genes; as judged from the size of the amplicons (listed below), the deletions in both genes were verified in both double mutants (middle and right panels).



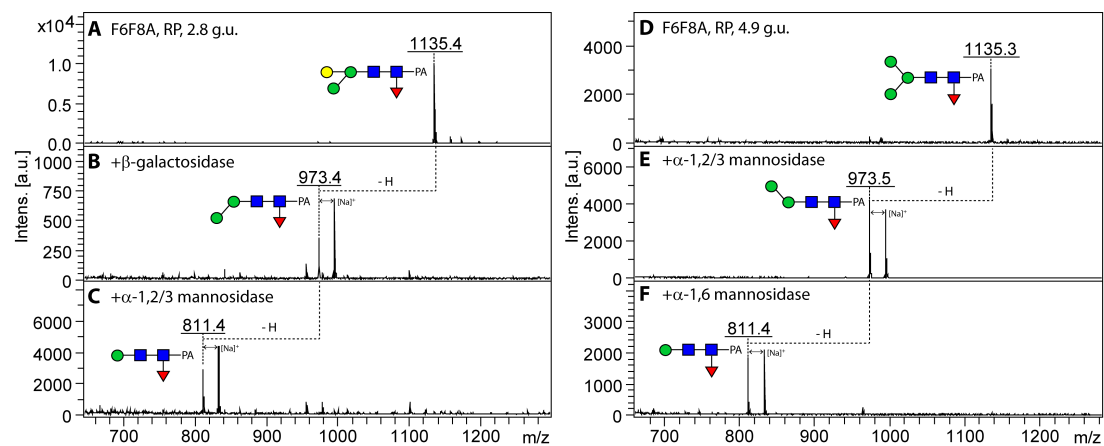
Fucosyltransferase mutations and primer sets used in genotyping PCRs

Gene	Allele	Strain	Nucleotide change	Primers	Product size
<i>fut-1</i>	ok892	VC585	1147 bp deletion with insertion of AAAGAA	F+R	1467(w.t.) 320(deletion)
				F+M+R	270(w.t.) 320(deletion)
<i>fut-6</i>	ok475	RB706	841 bp deletion	F+R	999(w.t.) 158(deletion)
				F+M+R	200(w.t.) 158(deletion)
<i>fut-8</i>	ok2558	RB1945	2239 bp deletion	F+M+R	574(w.t.) 469(deletion)

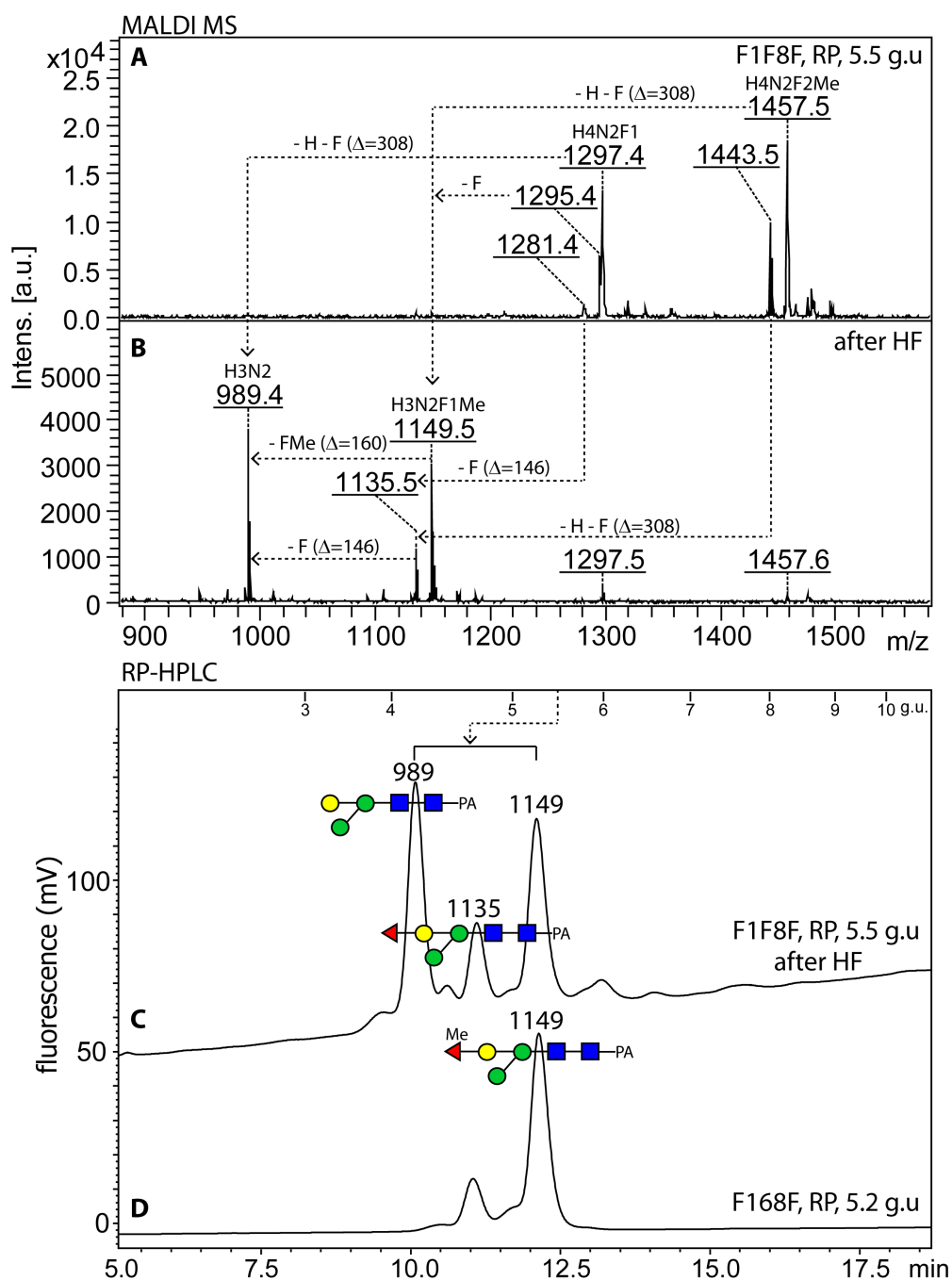
Supplementary Figure S-2: MS/MS spectra of selected N-glycans from the double fucosyltransferase mutants. Methylated structures ($\text{Hex}_{2-4}\text{HexNAc}_2\text{Fuc}_{1-2}\text{Me}_{1-4}\text{-PA}$) in the three mutants (*fut-1;fut-6* F digest, F1F6F; *fut-1;fut-8* F digest, F1F8F; *fut-6;fut-8* A digest, F6F8A) are shown in panels A-E, while selected structures identified in the *fut-1;fut-6* mutant are arranged according to mass in panels F-T. Key fragments are annotated according to the nomenclature of the Consortium for Functional Glycomics; in the general, either the core fragment Y-ions (e.g., 446, 460 through to 770) or ions resulting from loss of the substituted core fucose (indicated by Δ m/z values) are observed. In case of distal GlcNAc modifications, diagnostic fragments resulting from rearrangements only observed in the *fut-1;fut-8* mutant are indicated in red dashed boxes.



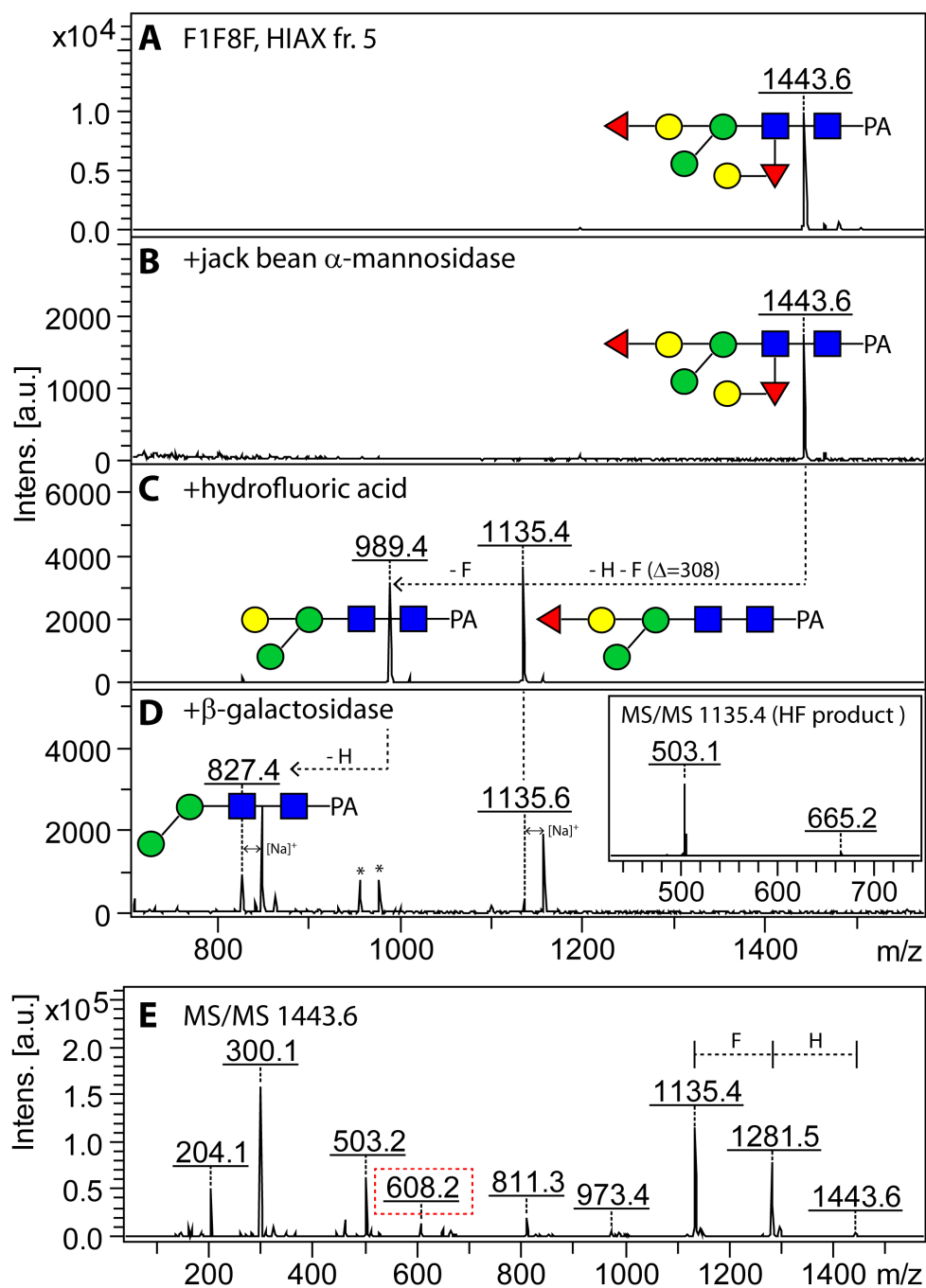
Supplementary Figure S-3: Exoglycosidase digestion of two forms of Hex₃HexNAc₂Fuc₁ of the *fut-6;fut-8* mutant. Two RP-HPLC fractions (2.8 g.u., A-C, and 4.9 g.u., D-F) containing glycans of m/z 1135 ($[M+H]^+$) were subject to different sequential exoglycosidase treatments (*Aspergillus* β -galactosidase followed by α 1,2/3-mannosidase or α 1,2/3-mannosidase followed by α 1,6-specific mannosidase) to yield products of m/z 811; the degree of sodiation depends on the components in the enzyme preparations used. MS/MS indicated the presence of m/z 446 core fragment ions (see **Supplementary Table**). The two isomers can thereby be distinguished as having either a bisecting galactose or an α 1,6-mannose on a core fucosylated form of Man₂GlcNAc₂.



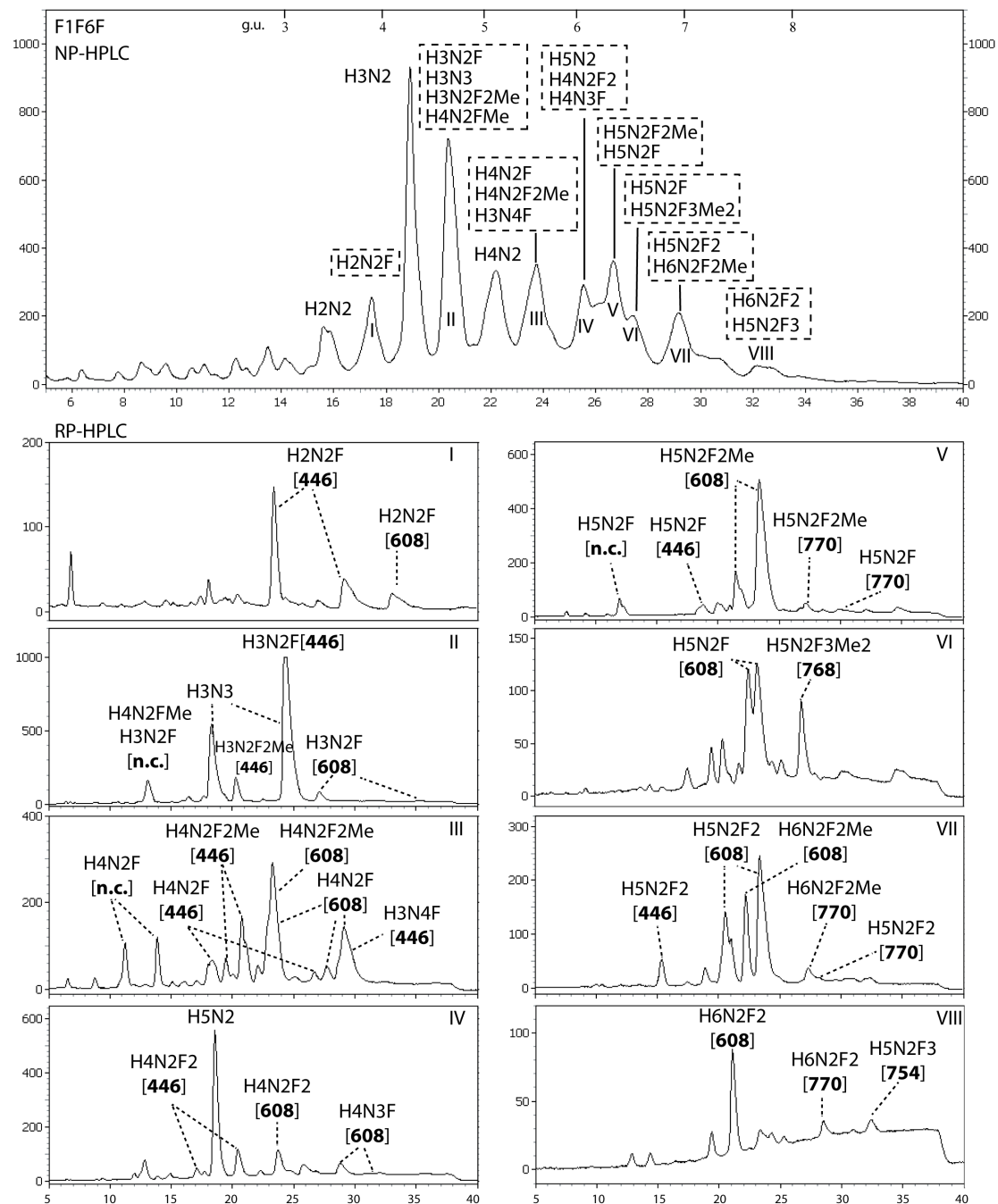
Supplementary Figure S-4. Structural analyses of HF-treated glycans from the *fut-1;fut-8* strain. The RP-HPLC fraction eluting at 5.5 g.u. (see Figure 4A in the main text) was treated with hydrofluoric acid which resulted in a degradation of glycans down to Hex₃HexNAC₂Fuc₀₋₁Me₀₋₁-PA (compare panels A and B) due to loss of distal fucosylation and partial loss of α 1,2-fucose on the bisecting galactose. An aliquot of the HF-treated fraction was re-injected onto the RP-HPLC column to compare with the elution properties of a previously-studied 5.2 g.u. N-glycan structure (Hex₃HexNAC₂Fuc₁Me₁-PA, *m/z* 1149), isolated from the triple fucosyltransferase mutant (*fut-1;fut-6;fut-8* PNGase F release; compare C and D). The dashed arrow in C indicates the original elution position at 5.5 g.u.



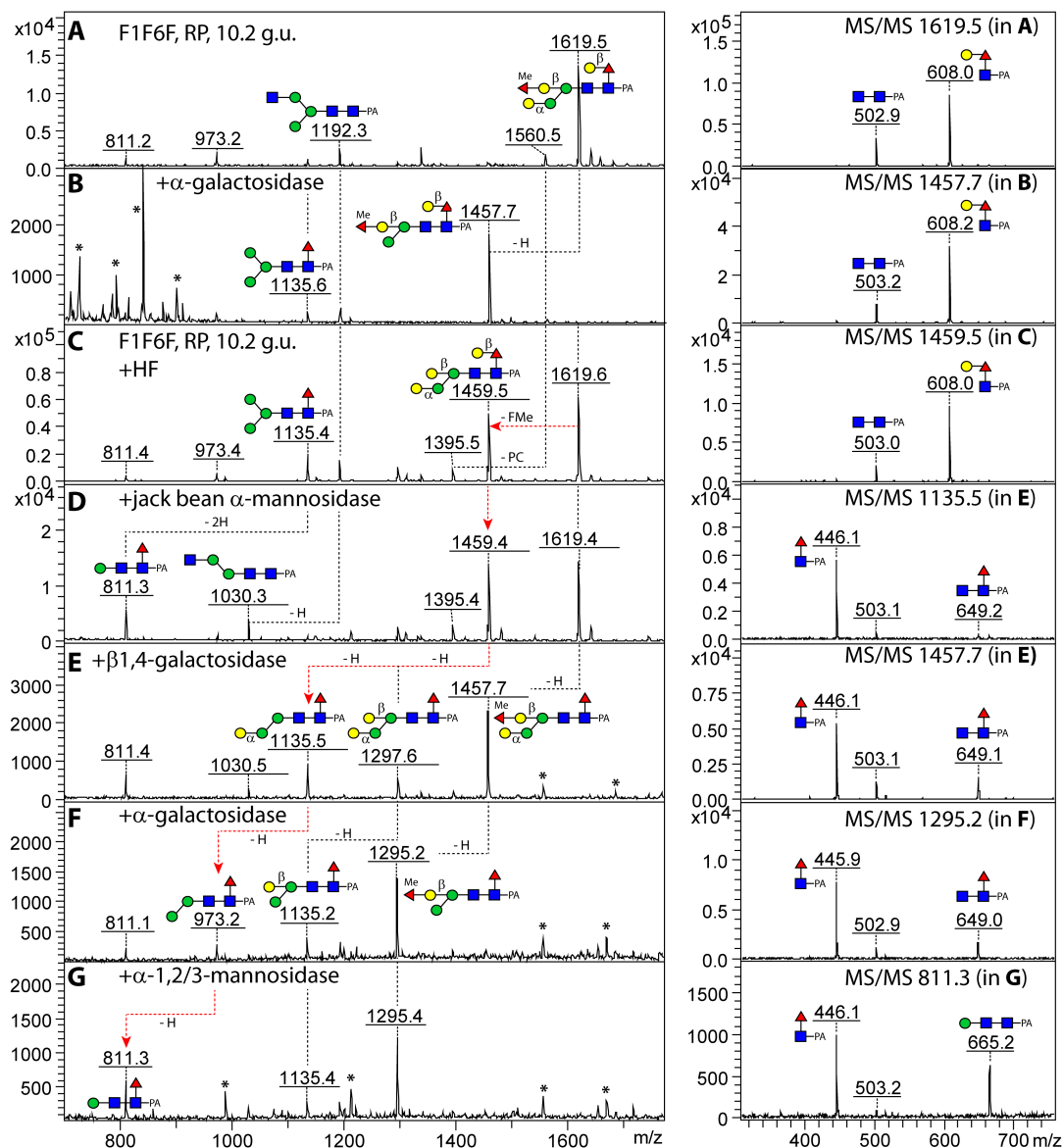
Supplement Figure S-5: Sequential treatments of a Hex₄HexNAc₂Fuc₂ glycan from the *fut-1;fut-8* strain. The 2D-HPLC fraction (HIAX fraction 5 of the 5.5 g.u. RP-HPLC fraction; A with MS/MS in E) was treated sequentially with (B) jack bean α -mannosidase, (C) hydrofluoric acid and then (D) *Aspergillus* β -galactosidase. The major product of m/z 827 was observed alongside an intermediate one of m/z 1135 (for MS/MS, see insets) resulting from incomplete release of the fucose by the chemical treatment. The rearranged fragment ion at m/z 608 (highlighted in a red dashed box; absent after hydrofluoric acid treatment) and the serial loss of hexose and fucose from the parent ion are diagnostic of the distal modification.



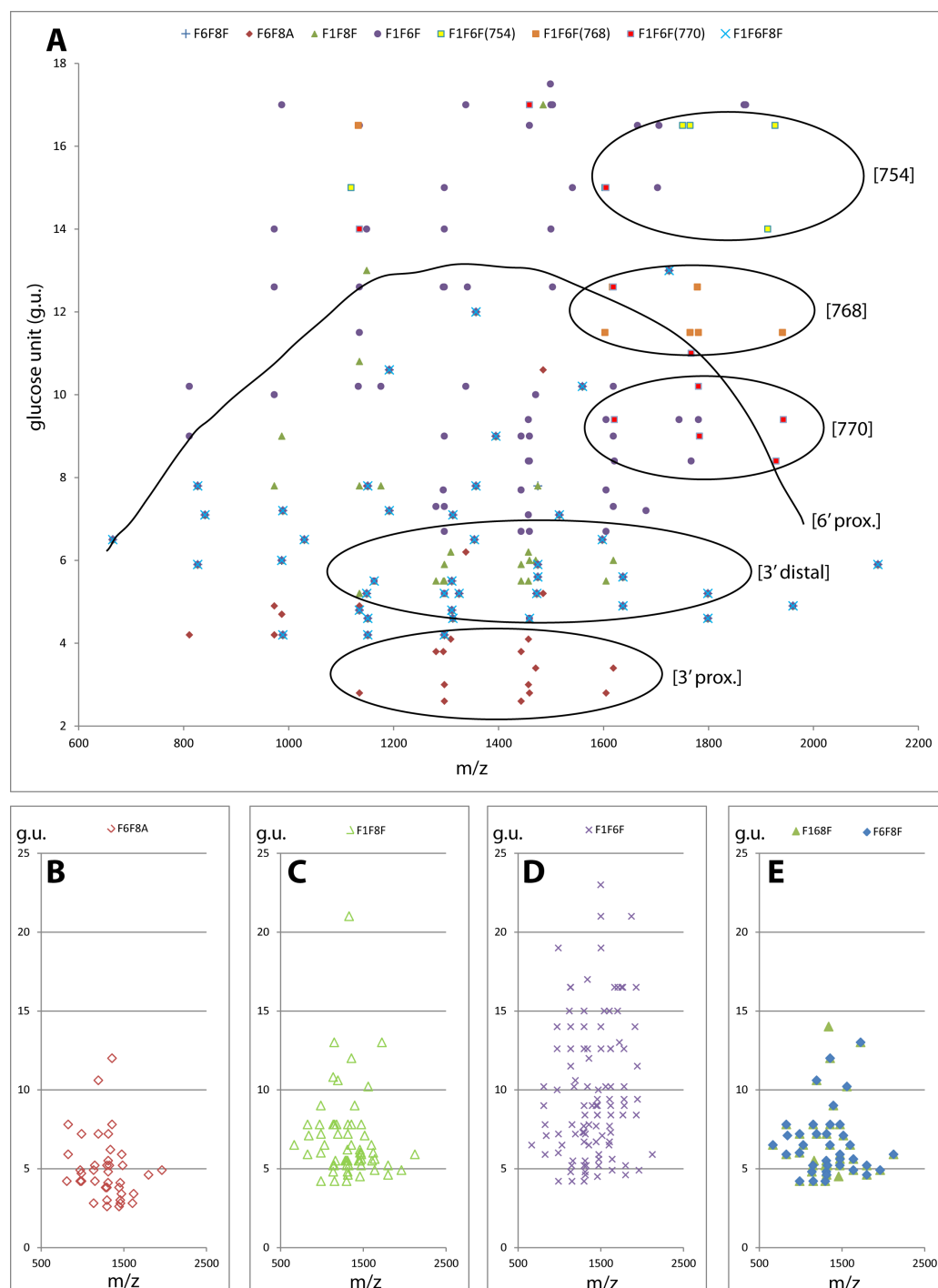
Supplementary Figure S-6: 2D-HPLC analyses of *fut-1*;*fut-6* N-glycans. Selected NP-HPLC fractions (I to VIII) were collected, dried and re-fractionated by RP-HPLC. The compositions of major identified glycans were indicated as well as the key MS/MS fragment ions (bold, in brackets). H: hexose; N: *N*-acetylhexosamine; F: fucose; Me: methyl; *m/z* 446: Fuc₁HexNAC₁-PA; *m/z* 608: Hex₁Fuc₁HexNAC₁-PA; *m/z* 754: Hex₁Fuc₂HexNAC₁-PA; *m/z* 768: Hex₁Fuc₂HexNAC₁Me₁-PA; *m/z* 770: Hex₂Fuc₁HexNAC₁-PA; n.c.: no core fucose residues.



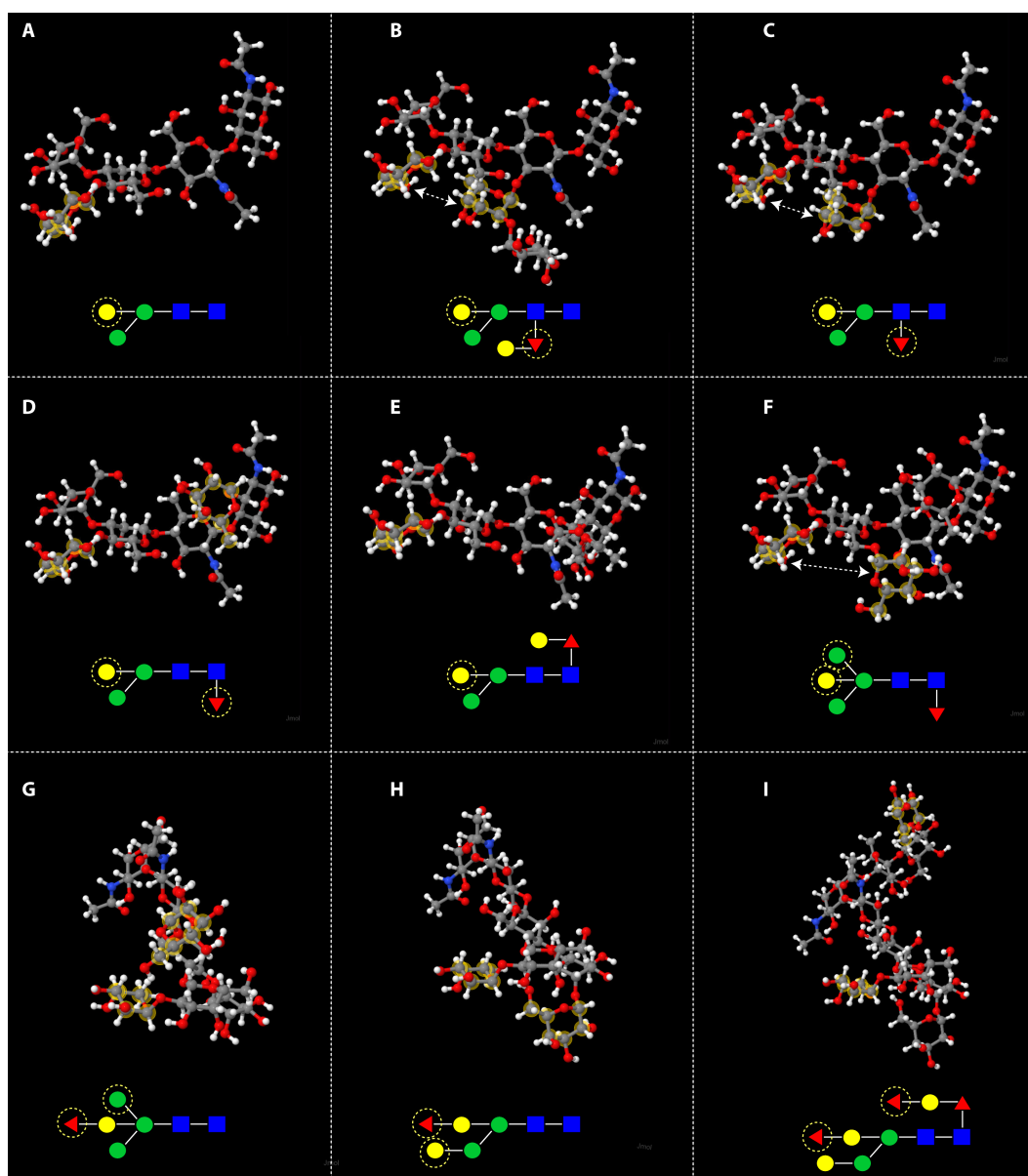
Supplementary Figure S-7: Sequential digestions of a predominant N-glycan from the *fut-1;fut-6* strain. The fraction eluting at 10.2 g.u., containing Hex₅HexNAC₂Fuc₂Me₁-PA (*m/z* 1619) was sensitive to α -galactosidase digestion (A and B). Sequential treatments of this glycan with HF, jack bean α -mannosidase, *Aspergillus* β -galactosidase, α -galactosidase and α 1,2/3-mannosidase were carried out and resulted in a final product with *m/z* 811 (Hex₁HexNAC₂Fuc₁-PA, C-G). The sequential degradation of *m/z* 1619 glycan is indicated with arrows in red; key MS/MS fragments of the products are displayed in the right panels. Asterisks indicate non-glycan contaminants in the enzyme preparations. Note that, in contrast to the distal GalFuc found in the *fut-1;fut-8* mutant, hydrofluoric acid does not release the proximal GalFuc modification.



Supplementary Figure S-8: Relationship between retention time and mass of N-glycans from double and triple fucosyltransferase deletion mutants. (A) Overall plot of RP-HPLC (C18) retention time (in terms of glucose units; y-axis) and m/z of the individual glycans (x-axis). Ovals indicate glycans with different core modifications (α 1,3-fucose on either the proximal or distal GlcNAc, α 1,6-fucose on the proximal GlcNAc or Gal β 1,4Gal β 1,4Fuc α 1,6 (770 core fragment), Fuc α 1,2Gal β 1,4Fuc α 1,6 (754 core fragment), MeFuc α 1,2Gal β 1,4Fuc α 1,6 (768 core fragment) modifications of the reducing terminal GlcNAc. (B-E) Individual scatter plots with a comparison of PNGase F released glycans of the triple mutant and the *fut-6;fut-8* strain in panel E highlighting the similarity of the glycan pools in these two preparations.

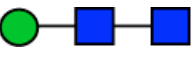

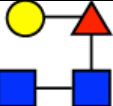
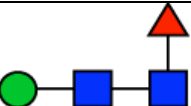
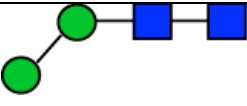
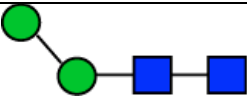
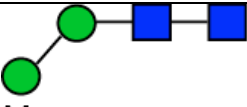
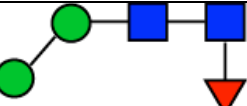
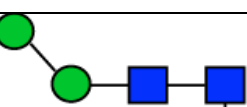
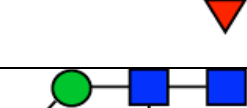
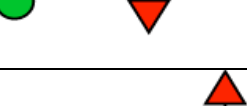
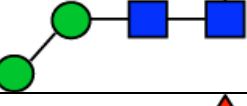
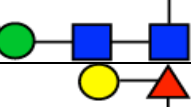


Supplementary Figure S-9. Predicted models of selected bisected N-glycans. 3D structures of glycans with compositions of $\text{Hex}_{3-5}\text{HexNAc}_2\text{Fuc}_{0-3}$ were simulated using Carbohydrate Builder (<http://glycam.org/tools/molecular-dynamics/oligosaccharide-builder/build-glycan?id=1>) which was developed by Dr. Robert Woods' group and exported as PDB files; the 3D structures were visualised using Jmol 13.0 software. Colour codes of atoms are: grey as carbon, red as oxygen, white as proton and blue as nitrogen. The ringed monosaccharides on the CFG structures are marked in yellow on the 3D models. Close proximity of the bisecting galactose to the distal fucose, leading to reduced β -galactosidase sensitivity (see, *e.g.*, Figure 5C in the main text), is highlighted in panels B and C with an arrow. The space between the bisecting galactose and α 1,6-mannose (F, also highlighted with an arrow) is larger and so the latter has less of an effect on the action of the β -galactosidase. On the other hand, the α 1,6-mannose is close to the α 1,2-fucose linked to the bisect (G), which may correlate with the reduced hydrolysis by bovine α -fucosidase as compared to the structures in panels H and I.



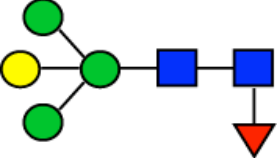
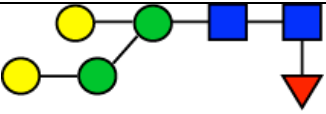
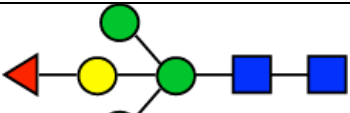
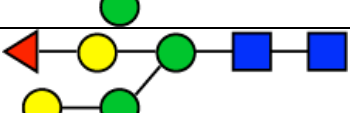
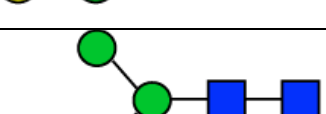
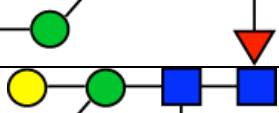
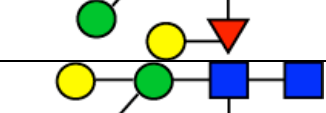
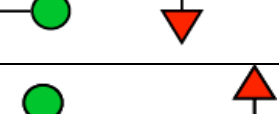
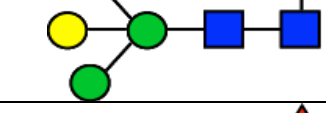
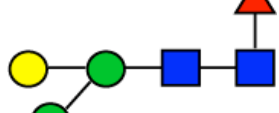


Supplementary Table S-1. Summary of evidence for the proposed structures of N-glycans from *Caenorhabditis* double fucosyltransferase deletion mutants. This extended Table presents brief summaries of the evidence for the proposed structures (including calculated m/z of the pyridylaminated glycans, retention on the RP C18 column in terms of glucose units, observed key fragments and sensitivities to enzymes) referring also to relevant figures, supplementary figures and comparisons with the triple fucosyltransferase mutant, the *pmk-1* strain and *Pristionchus* (Yan *et al*, 2015; Refs. 13 and 25). The qualitative indication of abundance (trace through to +++) is judged by the fluorescence intensity.

Table I: List of predicted N-glycans for *C. elegans* double *FUT* knockouts

Structures	<i>m/z</i> , PA, [M+H] ⁺ ; Composition; g.u.	F6F8 F	F6F8 A	F1F8 F	F1F6 F	Evidence
	665.28 H1N2 6.5	trace		trace	trace	Key fragments: 300, 503 Co-elution with <i>Pristionchus</i> and F168 strain
	811.35 H1N2F1 4.2		+			Key fragment: 446 Only found in PNGase A released pool. Co-elution with <i>Pristionchus</i>
	811.35 H1N2F1 9.0				+	Key fragments: 608, 503 (loss of 308) sensitive to β-galactosidase (-1H), then MS/MS frag. 446
	811.35 H1N2F1 10.2				+	Key fragment: 446 Co-elution with <i>Pristionchus</i>
	827.34 H2N2 (0M) 5.9	++	trace	+++	++	Key fragments: 300, 665 Co-elution with <i>Pristionchus</i> , F168 and <i>pmk-1</i> strains
	827.34 H2N2 (M0) 7.8	++	+	++	+	Key fragments: 300, 665 Co-elution with <i>Pristionchus</i> , F168 and <i>pmk-1</i> strains
	841.36 H2N2Me1 7.1	trace		trace	+	Key fragments: 177, 665 Co-elution with F168 strain retarded elution compared to 827 at 5.9 g.u.
	973.40 H2N2F1 (0MF ³) 4.2		++			Key fragments: 446, 827 Only found in PNGase A released pool. Co-elution with <i>Pristionchus</i> sensitive to α1,2/3 mannosidase (-1H)
	973.40 H2N2F1 (M0F ³) 4.9		+			Key fragments: 446, 827 Only found in PNGase A released pool. Co-elution with <i>Pristionchus</i> sensitive to α1,6 mannosidase (-1H)
	973.40 H2N2F1 7.8			+		Key fragments: trace amount of 446 fragment due to rearrangement, 827 (loss of 146) Co-elution with <i>Pristionchus</i> sensitive to α1,2/3 mannosidase (-1H)
	973.40 H2N2F1 (0MF ⁶) 10.0				+++	Key fragments: 446, 827 Co-elution with <i>Pristionchus</i> and <i>pmk-1</i> strain resistant to α1,6 mannosidase; sensitive to α1,2/3 mannosidase (-1H)
	973.40 H2N2F1 (M0F ⁶) 12.6				++	Key fragments: 446, 827 Co-elution with <i>Pristionchus</i> and <i>pmk-1</i> strain resistant to α1,2/3 mannosidase; sensitive to α1,6 mannosidase (-1H)
	973.40 H2N2F1 14.0				+	Key fragments: 608, 665 (loss of 308) sensitive to β-galactosidase (-1H), then MS/MS frag. 446

	1135.45 H3N2F1 5.2			+++		Key fragments: trace 446 due to re-arrangement, 989 (loss of 146) sensitive to Bov. α -fucosidase (-1F); sensitive to β -galactosidase (-1H)
	1135.45 H3N2F1 7.8			+		Key fragments: trace 446 due to re-arrangement, 989 (loss of 146) resistant to β -galactosidase; sensitive to α -galactosidase (-1H)
	1135.45 H3N2F1 10.8			++		Key fragments: trace 608, due to re-arrangement, 827 (loss of 308) sensitive to J.B. mannosidase (-1H); sensitive to α 1,2/3 mannosidase (-1H)
	1135.45 H3N2F1 (MMF ⁶) 11.5			++++		Key fragments: 446, 989 Co-elution with <i>Pristionchus</i> and <i>pmk-1</i> strain sensitive to α 1,2/3 mannosidase (-1H)
	1135.45 H3N2F1 12.6			++		Key fragments: 608, 827 (loss of 308) resistant to α 1,6 mannosidase; sensitive to β -galactosidase (-1H), then MS/MS frag. 446; sensitive to α 1,2/3 mannosidase (-1H)
	1135.45 H3N2F1 14.0			trace		Key fragments: 770, 665 (loss of 470)
	1135.45 H3N2F1 16.5			+		Key fragments: 608, 827 (loss of 308) sensitive to α 1,6 mannosidase (-1H)
	1149.47 H3N2F1Me1 5.2	trace	trace	+	+	Key fragments: 300, 503 (no core Fuc), 987 Co-elution with F168 strain resistant to J.B. mannosidase, Bov. α -fucosidase and β -galactosidase; sensitive to HF (-160), then sensitive to β -galactosidase (-1H), then sensitive to α 1,2/3 mannosidase (-1H)
	1149.47 H3N2F1Me1 7.8		trace			Key fragments: 446, 827, 1003 Only found in PNGase A released pool.
	1149.47 H3N2F1Me1 13.0			+		Key fragments: trace 608 due to re-arrangement, 841 (loss of 308)
	1149.47 H3N2F1Me1 14.0				+	Key fragments: 446, 1003 resistant to α -galactosidase and β -galactosidase; sensitive to J.B. mannosidase (-1H)
	1151.45 H4N2 4.2	++	+	+++	++	Key fragments: 300, 503 Co-elution with F168 and <i>pmk-1</i> strains resistant to J.B. mannosidase; sensitive to β -galactosidase (-1H), then sensitive to α 1,2/3 mannosidase (-1H)
	1151.45 H4N2 4.6	trace		trace	trace	Key fragments: 300, 503 Co-elution with F168 and <i>pmk-1</i> strains sensitive to α -galactosidase (-1H); sensitive to β -galactosidase (-1H),

	1151.45 H4N2 7.8	+		+	trace	Key fragment: 827 Co-elution with F168 strain and <i>pmk-1</i> strains resistant to α 1,6 mannosidase; sensitive to J.B. mannosidase (-3H) or α 1,2/3 mannosidase (-1H)
	1163.48 H3N2F1Me2 5.5			trace	trace	Key fragments: 177, 503 (no core Fuc), 987, 1003 Co-elution with F168 strain
	1176.48 H2N3F1 7.8			+		Key fragments: trace 446 due to re-arrangement, 1030 (loss of 146) Co-elution with <i>hex-2;hex-3</i> strain
	1176.48 H2N3F1 10.2				trace	Key fragments: 446, 1030 Co-elution with <i>Pristionchus</i>
	1192.47 H3N3 (MGn) 7.2	+++	trace	+++	++	Key fragments: 365, 1030 Co-elution with <i>Pristionchus</i> , F168 and <i>pmk-1</i> strains resistant to α 1,2/3 mannosidase; sensitive to FDL (-1N)
	1192.47 H3N3 (GnM) 10.6	++	+	++	++	Key fragments: 365, 989 Co-elution with <i>Pristionchus</i> , F168 and <i>pmk-1</i> strains sensitive to α 1,2/3 mannosidase (-1H)
	1281.51 H3N2F2 3.8		++			Key fragments: 446, 973, 1135 Only found in PNGase A released pool. sensitive to α 1,2 fucosidase (-1F) MS/MS then still 446; sensitive to HF (-2x146), then sensitive to β -galactosidase (-1H), then sensitive to α 1,2/3 mannosidase (-1H); Fig. 3F-J
	1281.51 H3N2F2 5.5			+		Key fragments: trace 446 due to re-arrangement, 1135 (loss of 146)
	1281.51 H3N2F2 7.3				+	Key fragments: 446, 1135 resistant to J.B. mannosidase; sensitive to Bov. α -fucosidase (-2F)
	1295.52 H3N2F2Me1 3.8		++			Key fragments: 446, 1149 Only found in PNGase A released pool. Co-elution with <i>pmk-1</i> strain PNGase A released sensitive to HF (-160 and -146), then sensitive to β -galactosidase (-1H), then sensitive to α 1,2/3 mannosidase (-1H); Fig. 3F-J
	1295.52 H3N2F2Me1 5.5			+++		Key fragments: trace 446 due to re-arrangement, 1149 (loss of 146) sensitive to HF (-160 and -146), then sensitive to β -galactosidase (-1H)
	1295.52 H3N2F2Me1 7.7				++	Key fragments: 446, 1135, 1149 resistant to α -galactosidase and β -galactosidase
	1295.52 H3N2F2Me1 12.6				trace	Key fragments: 608, 987 (loss of 308)

	1297.50 H4N2F1 2.6		++++			Key fragments: 446, 1151; MS/MS Fig. 2A Only found in PNGase A released pool. Co-elution with <i>pmk-1</i> strain PNGase A released resistant to J.B. mannosidase and α -galactosidase; sensitive to β -galactosidase (-1H), then sensitive to α 1,2/3 mannosidase (-1H) and then to α 1,6 mannosidase (-1H); Fig. 3A-E
	1297.50 H4N2F1 3.0		+			Key fragments: 446, 1151 Only found in PNGase A released pool. Co-elution with <i>pmk-1</i> strain PNGase A released sensitive to α -galactosidase (-1H); sensitive to β -galactosidase (-1H)
	1297.50 H4N2F1 4.2	+		+++	++	Key fragments: 300, 503 (no core Fuc); MS/MS Fig. 2B Co-elution with F168 and <i>pmk-1</i> strains resistant to α -galactosidase; sensitive to HF (-146), then sensitive to β -galactosidase (-1H), then sensitive to α 1,2/3 mannosidase (-1H)
	1297.50 H4N2F1 5.2			+	++	Key fragments: 300, 503 (no core Fuc), 973 Co-elution with F168 strain sensitive to α -galactosidase (-1H); sensitive to HF (-146) or to Bov. α -fucosidase (-1F) then sensitive to β -galactosidase (-1H)
	1297.50 H4N2F1 5.2		+			Key fragments: 446, 1151 Only found in PNGase A released pool. sensitive to α -galactosidase (-1H); sensitive to J.B. mannosidase (-1H)
	1297.50 H4N2F1 5.5			+++		Key fragments: trace 608 due to re-arrangement, 989 (loss of 308); MS/MS Fig. 2C sensitive to β -galactosidase (-1H); sensitive to HF (-308); Fig. 5A-F
	1297.50 H4N2F1 5.9			+		Key fragments: trace 446 due to re-arrangement, 1151 (loss of 146) Co-elution with <i>pmk-1</i> strain sensitive to β -galactosidase (-1H) sensitive to α -galactosidase (-1H)
	1297.50 H4N2F1 6.7				++	Key fragments: 446, 1151; MS/MS Fig. 2D Co-elution with <i>pmk-1</i> strain resistant to α -galactosidase and J.B. mannosidase; sensitive to β -galactosidase (-1H)
	1297.50 H4N2F1 7.3				+	Key fragments: 446, 1151 sensitive to α -galactosidase (-1H); sensitive to β -galactosidase (-1H)
	1297.50 H4N2F1 9.2				++	Key fragments: 608, 989 (loss of 308) resistant to α -galactosidase, Bov. α -fucosidase and HF; sensitive to β -galactosidase (-2H), then MS/MS frag. 446 Fig. 7D-F
	1297.50 H4N2F1 12.6				+	Key fragments: 446, 1151 resistant to β -galactosidase; sensitive to J.B. mannosidase (-1H); sensitive to α -galactosidase (-1H), then sensitive to J.B. mannosidase (-2H)
	1297.50 H4N2F1 14.0				+	Key fragments: 608, 989 (loss of 308) sensitive to α -galactosidase (-1H), then sensitive to α 1,2/3 mannosidase (-1H); sensitive to β -galactosidase (-1H), then MS/MS frag. 446 Fig. 7A-C

	1341.53 H2N3F1PC1 12.6				trace	Key fragments: 369, 531, 895 (loss of 446) Co-elution with <i>Pristionchus</i>
	1354.53 H4N3 6.5	trace		trace	trace	Key fragment: 1030 Co-elution with F168 and <i>pmk-1</i> strain resistant to α -galactosidase and β -galactosidase; sensitive to J.B. mannosidase (-2H)
	1357.53 H3N3PC1 7.8	++	+	++	++	Key fragments: 369, 531 Co-elution with <i>Pristionchus</i> , F168 strain and <i>pmk-1</i> strains sensitive to J.B. mannosidase (-1H)
	1357.53 H3N3PC1 12.0	+	trace	+	+	Key fragments: 369, 531 Co-elution with <i>Pristionchus</i> , F168 strain and <i>pmk-1</i> strains sensitive to J.B. mannosidase (-1H)
	1395.55 H3N4 (GnGn) 9.0	++		++	++	Key fragments: 365, 1030, 1192 Co-elution with <i>Pristionchus</i> , F168 strain and <i>pmk-1</i> strains
	1443.56 H4N2F2 2.6		+++			Key fragments: 446, 1297 Only found in PNGase A released pool. Co-elution with <i>pmk-1</i> strain PNGase A released resistant to J.B. mannosidase, β -galactosidase and α -galactosidase; Fig. 3A-E
	1443.56 H4N2F2 3.8		+			Key fragments: 446, 973 Only found in PNGase A released pool. sensitive to α -galactosidase (-1H); sensitive to HF (-2x146)
	1443.56 H4N2F2 5.5			+++		Key fragments: trace 608 due to re-arrangement, 1135 (loss of 308); MS/MS Suppl. 5E sensitive to HF (-308 and -146), then sensitive to β -galactosidase (-1H); Suppl. 5A-D
	1443.56 H4N2F2 5.9			+		Key fragments: trace 446 due to re-arrangement, 1297 (loss of 146) sensitive to α -galactosidase (-1H);
	1443.56 H4N2F2 6.7				+	Key fragments: 446, 1297 Co-elution with <i>pmk-1</i> strain resistant to α -galactosidase and β -galactosidase; sensitive to Bov. α -fucosidase (-1F), then MS/MS frag. 300, 503 (no core Fuc)
	1443.56 H4N2F2 7.7				++	Key fragments: 446, 1297 resistant to β -galactosidase; sensitive to α -galactosidase (-1H); sensitive to Bov. α -fucosidase (-2F), then MS/MS frag. 300, 503 (no core Fuc)
	1443.56 H4N2F2 9.0				++	Key fragments: 608, 1135 (loss of 308) resistant to J.B. mannosidase and α -galactosidase; sensitive to β -galactosidase (-1H), then MS/MS frag. 446

	1459.56 H5N2F1 8.4				+++	Key fragments: 608, 1151 (loss of 308) Co-elution with <i>pmk-1</i> resistant to α -galactosidase and to J.B. mannosidase; sensitive to β -galactosidase (-2H), then MS/MS frag. 446, then sensitive to α 1,2/3 mannosidase (-1H) Fig. 7K-M
	1459.56 H5N2F1 9.0				++++	Key fragments: 608, 1151 (loss of 308) sensitive to α -galactosidase (-1H); sensitive to β -galactosidase (-2H), then MS/MS frag. 446 Fig. 7N-P
	1459.56 H5N2F1 16.5				+	Key fragments: 608, 1151 (loss of 308) Co-elution with <i>pmk-1</i> sensitive to J.B. mannosidase (-1H)
	1459.56 H5N2F1 17				trace	Key fragments: 770, 989 (loss of 470)
	1471.59 H4N2F2Me2 3.4		+			Key fragments: 446, 1325 Only found in PNGase A released pool. Co-elution with <i>pmk-1</i> strain PNGase A released resistant to J.B. mannosidase and α -galactosidase
	1471.59 H4N2F2Me2 6.0			+		Key fragments: trace 608 due to re-arrangement, 1163 (loss of 308), 987 ; MS/MS Suppl. 2C
	1471.59 H4N2F2Me2 10.0				trace	Key fragments: 608, 1163 (loss of 308), resistant to Bov. α -fucosidase; sensitive to β -galactosidase (-1H), then MS/MS frag. 446;
	1473.57 H5N2F1Me1 5.2	trace		+	++	Key fragments: 300, 503 (no core F), 987 Co-elution with F168 and <i>pmk-1</i> strains resistant to Bov. α -fucosidase and to J.B. mannosidase sensitive to α -galactosidase (-1H); sensitive to HF (-160), then sensitive to β -galactosidase (-1H)
	1475.55 H6N2 5.6	+		+	+	Key fragment: 827 Co-elution with <i>Pristionchus</i> , F168 and <i>pmk-1</i> strains sensitive to J.B. mannosidase (-5H)
	1475.55 H6N2 5.9	++	trace	++	++	Key fragment: 989 Co-elution with <i>Pristionchus</i> , F168 and <i>pmk-1</i> strains sensitive to J.B. mannosidase (-5H)

	1475.55 H6N2 7.8	+		+		Key fragment: 827 Co-elution with <i>pmk-1</i> strain
	1485.61 H4N2F2Me3 5.2			+		Key fragments: 177, 446, 1339 Only found in PNGase A released pool. sensitive to HF (-160 and -146)
	1485.61 H4N2F2Me3 10.6			+		Key fragments: 177, 446, 1339 Only found in PNGase A released pool.
	1485.61 H4N2F2Me3 >17				+	Key fragments: 177, 339, trace 446 due to re-arrangement, 1017, 1339 (loss of 146); MS/MS Suppl. 2D
	1499.62 H4N2F2Me4 >17				+	Key fragments: 177, 339, 460, 663, 1339; MS/MS Suppl. 2E
	1500.58 H4N3F1 14.0				+	Key fragments: 608, 1192 (loss of 308) Co-elution with <i>hex-2;hex-3</i> and <i>pmk-1</i> strains resistant to α -galactosidase resistant to α 1,2/3 mannosidase; sensitive to β -galactosidase (-1H), then MS/MS frag. 446; sensitive to J.B. mannosidase (-1H)
	1500.58 H4N3F1 >17				trace	Key fragment: 608, 1192 (loss of 308) MS/MS Suppl. 2F Co-elution with <i>pmk-1</i> strain sensitive to J.B. mannosidase (-1H)
	1503.59 H3N3F1PC1 12.6				++	Key fragments: 369, 531, 1058 (loss of 445) Co-elution with <i>Pristionchus</i> and <i>pmk-1</i> strain
	1503.59 H3N3F1PC1 >17				+	Key fragments: 369, 531, 1058 (loss of 445) Co-elution with <i>Pristionchus</i> and <i>pmk-1</i> strain sensitive to α 1,2/3 mannosidase (-1H)
	1516.58 H5N3 7.1	+		+	+	Key fragments: 365, 1030 Co-elution with <i>hex-2;hex-3</i> , <i>pmk-1</i> and F168 strains resistant to α -galactosidase, β -galactosidase and Bov. α -fucosidase sensitive to J.B. mannosidase (-3H)
	1541.61 H3N4F1 (GnGnF ⁶) 15.0				+++	Key fragments: 365, 446, 1030, 1192 Co-elution with <i>Pristionchus</i> and <i>pmk-1</i> strain

	1560.61 H3N4PC1 10.2	++		++	+	Key fragments: 369, 531 Co-elution with <i>Pristionchus</i> and F168 strain
	1598.63 H3N5 6.5	+		++	trace	Key fragments: 569, 1030 Co-elution with <i>Pristionchus</i> , F168, <i>pmk-1</i> and <i>hex-2;hex-3</i> strains
	1603.64 H4N2F3Me 11.5				trace	Key fragments: 768, 1135 (loss of 468) MS/MS Suppl. 2H
	1603.64 H4N2F3Me 15.0				trace	Key fragments: 754, 1149 (loss of 454); MS/MS Suppl. 2G
	1605.61 H5N2F2 2.8		+			Key fragments: 446, 1459 Only found in PNGase A released pool. Co-elution with <i>pmk-1</i> PNGase A released sensitive to α -galactosidase (-1H)
	1605.61 H5N2F2 5.5			++		Key fragments: trace 608 due to re-arrangement, 1297 (loss of 308) Co-elution with <i>pmk-1</i> strain resistant to β -galactosidase and J.B. mannosidase; sensitive to α -galactosidase (-1H); sensitive to Bov. α -fucosidase (-1F)
	1605.61 H5N2F2 6.7				++	Key fragments: 446, 1459 Co-elution with <i>pmk-1</i> strain sensitive to α -galactosidase (-1H); sensitive to Bov. α -fucosidase (-1F), then MS/MS frag. 300, 503 (no core Fuc)
	1605.61 H5N2F2 7.7				+++	Key fragments: 608, 1297 (loss of 308) Co-elution with <i>pmk-1</i> strain resistant to α -galactosidase; sensitive to β -galactosidase (-1H), then MS/MS frag. 446;
	1605.61 H5N2F2 9.4				++++	Key fragments: 608, 1297 (loss of 308) Co-elution with <i>pmk-1</i> strain resistant to J.B. mannosidase; sensitive to α -galactosidase (-1H) Fig. 9A-C
	1605.61 H5N2F2 15.0				trace	Key fragments: 770, 1135 (loss of 470)
	1617.65 H4N2F3Me2 12.6				trace	Key fragments: 768, 1149 (loss of 468); MS/MS Suppl. 2I resistant to J.B. mannosidase, α -galactosidase, β -galactosidase and α 1,2 fucosidase

	1779.70 H5N2F3Me2 12.6				+++	Key fragments: 768, 987, 1311 (loss of 468) resistant to J.B. mannosidase and α 1,6 mannosidase; sensitive to α -galactosidase (-1H); sensitive to HF (-2x160) MS/MS then 608, then resistant to J.B. mannosidase, but sensitive to β -galactosidase (-2H), MS/MS then 446, then resistant to α 1,2/3 mannosidase Fig. 9G-I
	1781.68 H6N2F2Me1 9.4				+++	Key fragments: 608, 1473 (loss of 308) Co-elution with <i>pmk-1</i> strain resistant to Bov. α -fucosidase; sensitive to β -galactosidase (-1H), then MS/MS frag. 446; sensitive to α -galactosidase (-1H) sensitive to HF (-160), then sensitive to β -galactosidase (-2H) then MS/MS frag. 446; Fig. 9A-C
	1781.68 H6N2F2Me1 10.2				+	Key fragments: 770, 1311 (loss of 470); MS/MS Suppl. 2Q sensitive to β -galactosidase (-2H), then MS/MS frag. 446; sensitive to α -galactosidase (-1H)
	1781.68 H6N2F2Me1 11.5				trace	Key fragments: 768, 1313 (loss of 468)
	1783.66 H7N2F1 9.0				+	Key fragments: 770, 1313 (loss of 470) sensitive to α -galactosidase (-1H)
	1799.66 H8N2 (Man8B) 4.6	+++	+	++	+++	Key fragment: 1151 Co-elution with <i>Pristionchus</i> , <i>pmk-1</i> and F168 strains
	1799.66 H8N2 (Man8A) 5.2	+		+	+	Key fragment: 989 Co-elution with <i>Pristionchus</i> , <i>pmk-1</i> and F168 strains
	1868.72 H4N4F1PC1 17				trace	Key fragments: 369; 531, 1261 (loss of 607) Co-elution with <i>pmk-1</i> strain sensitive to HF (-165), MS/MS then 608
	1871.72 H3N4F1PC2 >17				++	Key fragments: 369, 531, 1426 (loss of 445) Co-elution with <i>Pristionchus</i> and <i>pmk-1</i>
	1913 H6N2F3 14.0				+	Key fragments: 754, 1459 (loss of 454) sensitive to α -galactosidase (-1H)

	1927.74 H6N2F3Me 16.5				+	Key fragments: 754, 1473 (loss of 454); MS/MS Suppl. 2R sensitive to α -galactosidase (-1H)
	1929.72 H7N2F2 8.4				+	Key fragments: 770, 1459 (loss of 470) sensitive to β -galactosidase (-2H), then MS/MS frag. 446; sensitive to α -galactosidase (-1H)
	1941.76 H6N2F3Me2 11.5				+	Key fragments: 768, 1473 (loss of 468); MS/MS Suppl. 2S
	1943.74 H7N2F2Me1 9.4				trace	Key fragments: 770, 1473 (loss of 470) MS/MS Suppl. 2T sensitive to β -galactosidase (-2H) then MS/MS 446; sensitive to α -galactosidase (-1H)
	1961.71 H9N2 (Man9) 4.9	+++	+	++	+++	Key fragment: 1151 Co-elution with <i>Pristionchus</i> , <i>pmk-1</i> and F168 strains
	2123.76 H10N2 5.9	trace		+	+	Key fragment: 1313 Co-elution with <i>Pristionchus</i> , <i>pmk-1</i> and F168 strains