THE N-GLYCANS OF *TRICHOMONAS VAGINALIS* CONTAIN VARIABLE CORE AND ANTENNAL MODIFICATIONS

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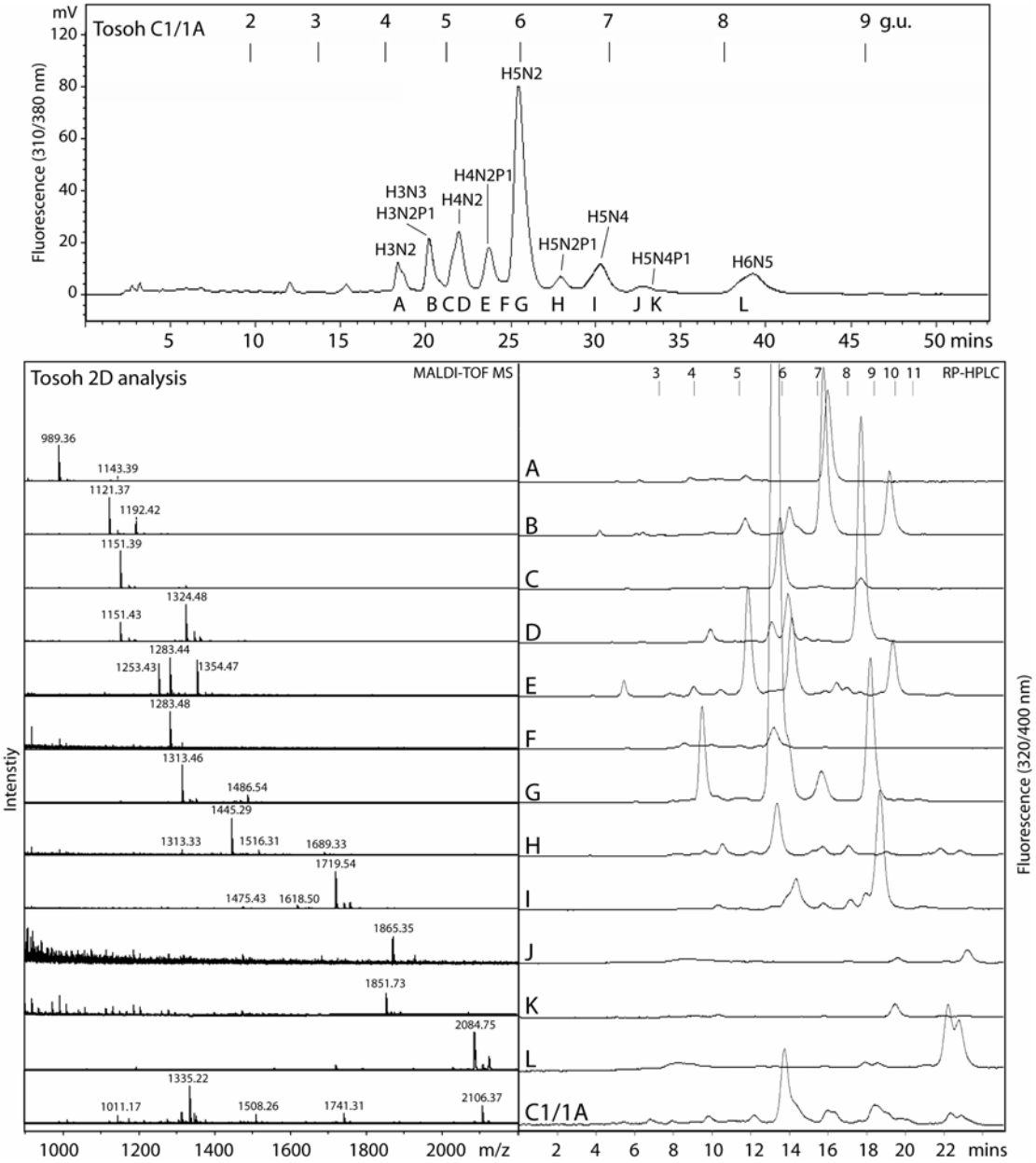
Supplementary Data

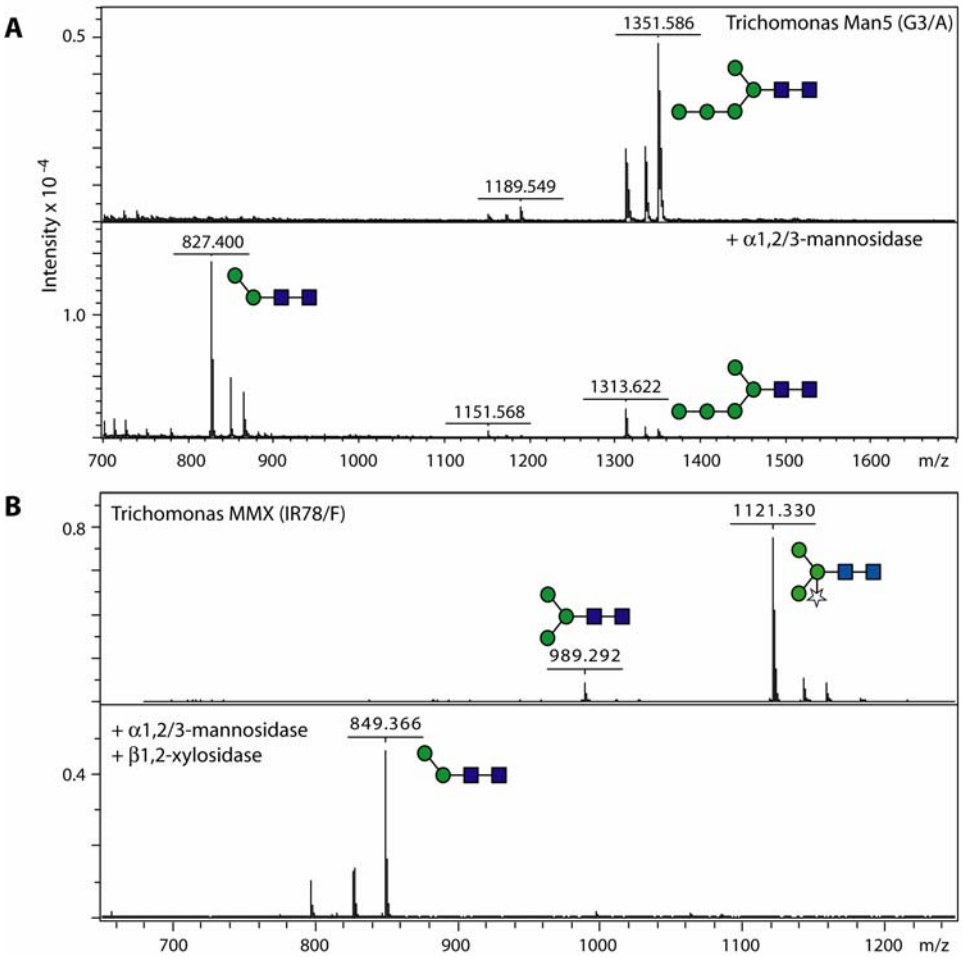
Supplementary Table: Summary of sample nomenclature, release and fractionation. As described in the main text, seven glycan samples derived from four independent cultivations in three different laboratories were compared. The C1/4, G3, TV2 and IR-78 samples were split into two prior to enzymatic glycan release.

Culture	***Release method***	***Glycan sample***	***Normal phase fractions***	***Reversed phase fractions***
C1/1 (bovine serum)	PNGase A	C1/1A	A, B ….	A1, A2, B1, B2 … etc. ('2D')
C1/2 (serum-free)	PNGase F	C1/2F	none	C1/2F/1, C1/2F/2, etc.
C1/3 (serum-free)	Sequential A then F	C1/3FA	none	C1/3FA/1, C1/3FA/2, etc.
C1/4 (horse serum)	PNGase A	C1/4A	none	C1/4A/1, etc.
	PNGase F	C1/4F	none	C1/4F/1, etc.
G3 (horse serum)	PNGase A	G3/A	none	G3/A /1, etc.
	PNGase F	G3/F	none	G3/F /1, etc.
TV2 (horse serum)	PNGase A	TV2/A	none	TV2/A/1, etc.
	PNGase F	TV2/F	none	TV2/F/1, etc.
IR78 (horse serum)	PNGase A	IR78/A	none	IR78/A/1, etc.
	PNGase F	IR78/F	none	IR78/F/1, etc.

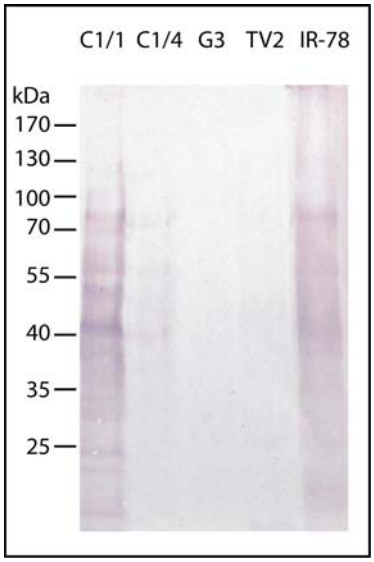
Supplementary Figure 1: 2D-analysis of *T. vaginalis* C1 glycans by NP-HPLC and RP-HPLC. In the upper panel, the chromatogram for Tosoh Amide 80 fractionation of N-glycans from the C1/1A sample is annotated with the fraction names, glucose units and abridged compositions of the dominant components of the major peaks. In the lower panel, MALDI-TOF MS spectra and RP-HPLC chromatograms (Hypersil ODS, MZ Analytik) for each of the Tosoh Amide 80 fractions A-L as well as of the original unfractionated sample (C1/1A) are presented. The data are summarised in Table 1 of the main text.



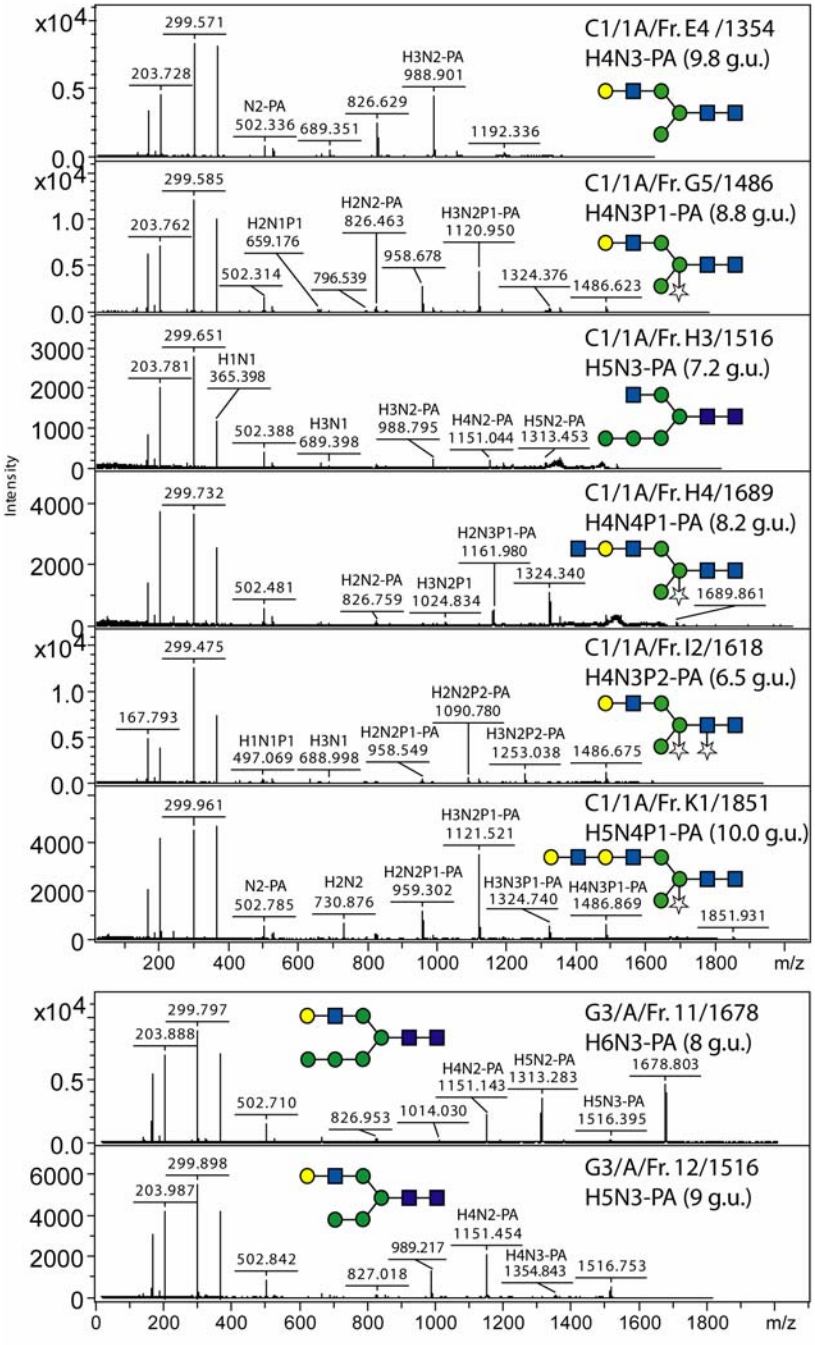
Supplementary Figure 2: α1,2/3-Mannosidase sensitivity of *Trichomonas* glycans. (A) α1,2/3-Mannosidase treatment of $Man_5GlcNAc_2$. MALDI-TOF MS analysis of the major fraction of sample G3/A before and after treatment with *Xanthomonas* α1,2/3-mannosidase with annotations of the relevant *m/z* values ($[M+H]^+$ or $[M+K]^+$). This mannosidase treatment results in the loss of two α1,2- and one α1,3-mannose residues. **(B) Combined α1,2/3-mannosidase and β1,2-xylosidase treatment of $Man_3GlcNAc_2Xyl_1$.** MALDI-TOF MS analysis of fraction 7 (7.5 g.u.; presumed MMX) from sample IR-78/F before and after combined treatment with *Xanthomonas* α1,2/3-mannosidase and β1,2-xylosidase, which results in the loss of one α1,3-mannose and one xylose residue to *m/z* 849 ($[M+Na]^+$). The glycans are drawn according to the nomenclature of the Consortium for Functional Glycomics (green circles, mannose; blue squares, GlcNAc).



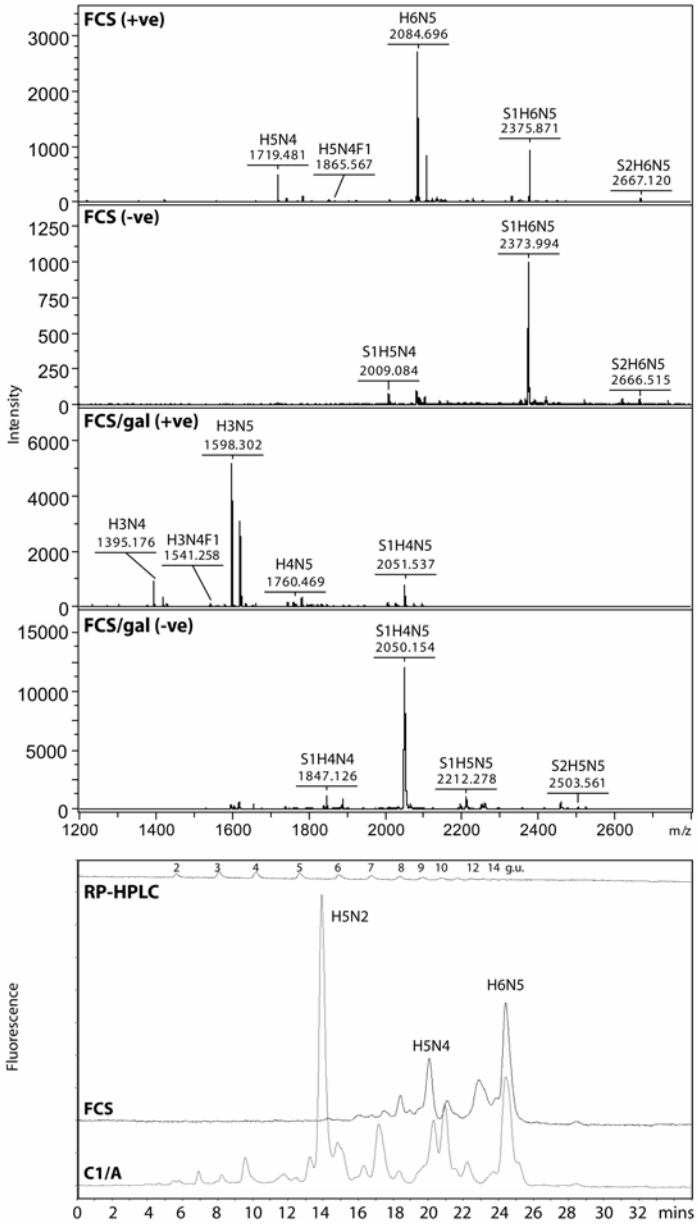
Supplementary Figure 3: Anti-horseradish peroxidase reactivity of four *T. vaginalis* strains. Aliquots of cellular material were subject to SDS-PAGE followed by Western blotting; the blots were incubated with polyclonal rabbit anti-horseradish peroxidase (1:10000; Sigma-Aldrich) followed by alkaline phosphatase-conjugated goat anti-rabbit IgG (1:10000; Vector Laboratories) and use of a chromogenic substrate (SigmaFAST NBT/BCIP). The approximately equal protein loading was estimated by Coomassie staining. The results suggest a far higher presence of xylose β1,2-linked to the core β1,4-mannose residue in the C1/1 and the IR-78 samples; faint staining is apparent in the C1/4 sample, whereas no apparent staining is seen with G3 or TV2, compatible with the MS/MS data.



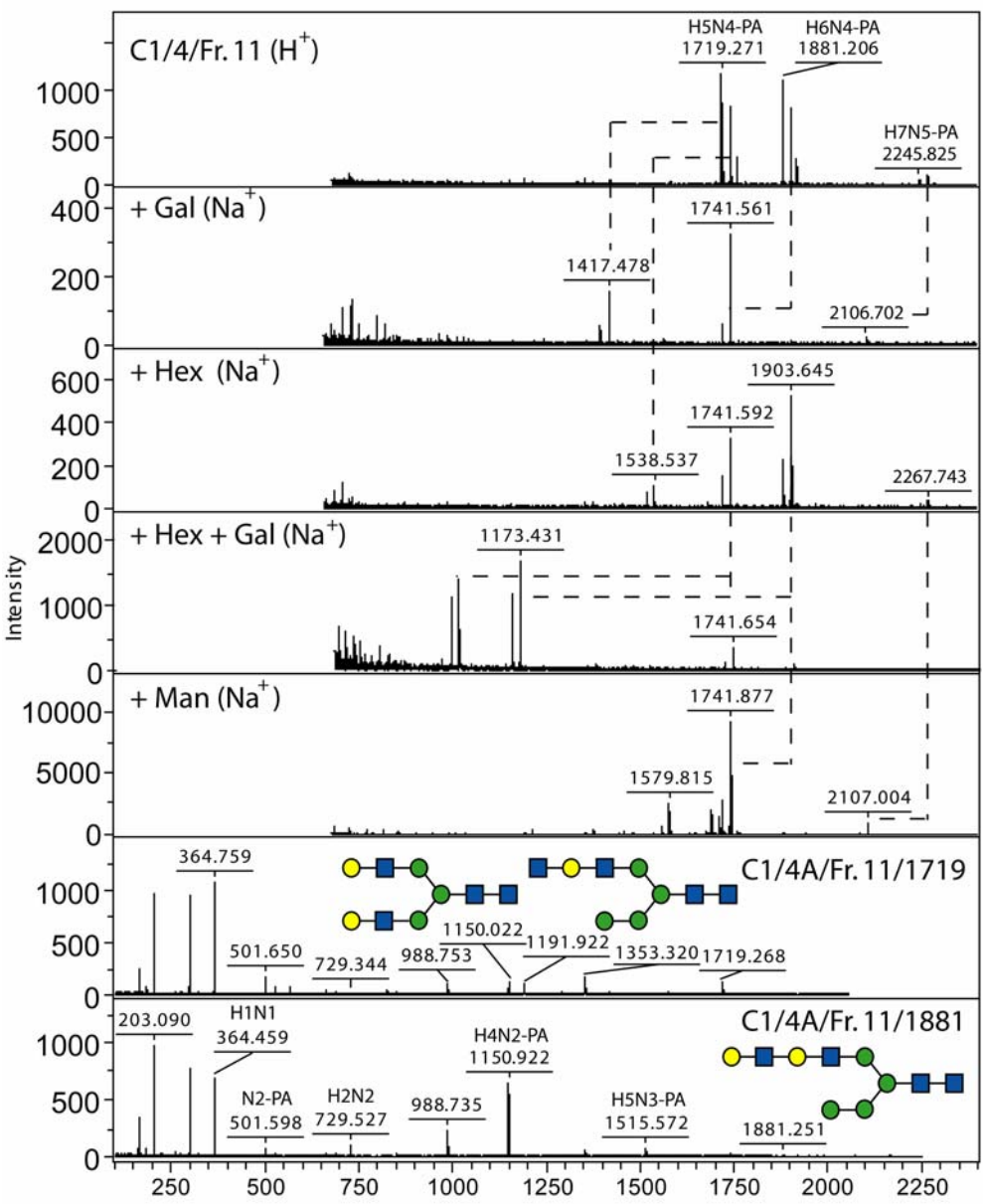
Supplementary Figure 4: MS/MS analysis of selected N-glycans from the C1 and G3 strains putatively modified by *N*-acetyllactosamine. 2D-HPLC-purified fractions of the C1/1A sample and RP-HPLC fractions of the G3/A sample were subject to MS/MS; the approximate RP-HPLC glucose units are also indicated. Note, for instance, the contrasting fragmentation pattern for the *m/z* 1516 species ($Hex_5HexNAc_3$; C1/1A/H3 and G3/A/12) eluting at either 7.2 or 9 glucose units (either non-reducing terminal HexNAc or Hex). The $Hex_4HexNAc_3Pent_{0-2}$ (E4, G5 and I2), $Hex_5HexNAc_4Pent_1$ (K1), and $Hex_{5-6}HexNAc_3$ glycans (G3/A/11 and G3/A/12) have terminal galactose as demonstrated by their galactosidase sensitivity (see Figure 4 for the digests of E4 and G5). Proposed structures are shown according to the nomenclature of the Consortium for Functional Glycomics (green circles, mannose; yellow circles, galactose; blue squares, GlcNAc; stars, pentose).



Supplementary Figure 5: MALDI-TOF MS and HPLC analysis of N-glycans isolated from foetal calf serum. N-glycans were prepared after pepsinisation of 0.5 ml foetal calf serum (Gibco, Invitrogen) and released with PNGase F. The free N-glycans were analysed in both positive (+ve) and negative (-ve) modes before and after *Aspergillus* β-1,4-galactosidase digestion using ATT as matrix as well as by RP-HPLC. In the latter case, the chromatograms of the dextran standard (2-14 g.u.) and N-glycans from *T. vaginalis* sample C1/1A (red), run on the same day, are also presented; the data indicate that the major triantennary $Hex_6HexNAc_5$ (H6N5) glycan from serum co-elutes with fraction L1 from the C1/1A sample, which is proposed to originate from the serum in which the parasites were grown (see also discussion in the main text and Table 1). The negative mode spectrum indicates the presence of sialylated species in the foetal calf serum sample ($Sia_{1-2}Hex_{5-6}HexNAc_{4-5}$; S1H5N4, S1H6N5 and S2H6N5); furthermore, galactosidase digestion results in removal of multiple galactose residues from serum glycans, e.g. three from H6N5 to yield H3N5, indicative of a triantennary structure as previously found on fetuin.



Supplementary Figure 6: MS/MS and exoglycosidase digestion analysis of selected N-glycans from the C1 strain putatively modified by one or more *N*-acetyllactosamine units. RP-HPLC-purified glycans from the C1/4A sample (fraction 11; ~10 g.u.) were subject to exoglycosidase digestions (jack bean β-hexosaminidase, recombinant β1,4-galactosidase or *A. saitoi* α1,2-mannosidase) and to MS/MS analysis. The putative shifts upon exoglycosidase treatment are indicated as dashed lines. The interpretation, as shown, is that the *m/z* 1719 species is a mixture of a typical biantennary N-glycan derived from the cultivation medium and a glycan with a single α1,2-mannose residue on the α1,3-arm (see main text). The *m/z* 1881 species also carries a single α1,2-mannose residue as well as two LacNAc units. The glycans are present as a mixture of $[M+H]^+$ and $[M+Na]^+$ species with the latter dominant in the exoglycosidase treated samples.



Supplementary Figure 7: MALDI-TOF MS of *Trichomonas vaginalis* N-glycans with or without hydrofluoric acid treatment. (A) Pyridylaminated PNGase F-released N-glycans prepared from the G3 and TV2 strains, were subject to negative-ion mode MALDI-TOF MS before and after treatment with hydrofluoric acid (HF). Annotations include two shifts indicative of the loss of phosphodiesters from N-glycans in their $[M-H]^-$ forms: loss of 123 mass units from both the *m/z* 1637 species (putatively $Hex_5HexNAc_3[EtNP]_1$) in the G3 sample and from the *m/z* 1434 species (putatively $Hex_5HexNAc_2[EtNP]_1$) in TV2 sample. The molecular nature of the asterisked *m/z* 1595 species is unknown; however, in comparison to that of $Hex_5HexNAc_2$ (*m/z* 1311 as an $[M-H]^-$ ion), its intensity appears to be unaffected by hydrofluoric acid. **(B)** The same samples as shown in (A) were analysed in positive-ion mode; the shifts of two glycans upon hydrofluoric acid treatment are again indicated as a loss of 123 mass units. Glycans which are not postulated to carry phosphodiesters are resistant to this treatment.