Molecular and Cellular Proteomics Supplementary information for:

Asparagine-Linked Glycans of *Cryptosporidium parvum* Contain a Single Long Arm, Are Barely Processed in the ER or Golgi, and Show a Strong Bias for Sites with Threonine

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Fig. S1. Topology of the second most abundant glycoform of deutero-rreduced and permethylated $Hex_5HexNAc_2$ released from *C. parvum* glycoproteins, determined by EED FT-ICR MS/MS at 14 eV.

Fig. S2. Determination of linkage positions based on cross-ring cleavages observed in the 14-eV EED FT-ICR MS/MS spectrum of $Hex_5HexNAc_2$ glycans released from *C. parvum* glycoproteins, after deutero-reduction and permethylation, $[M + Na]^+$ *m/z* 1596.8199.

Fig. S3. The predicted lipid-linked *N*-glycan precursors of *C. parvum* and *T. gondii*.

Table S1. Glycotransferase enzymes predicted from the genomes of *C. parvum*, and a related organism, *T. gondii*.

Excel S1. (separate file). Fragment ion assignments for FT-ICR EED MS/MS spectra of deutero-reduced and permethylated *N*-glycans released from *C. parvum* glycoproteins.

Excel S2 (separate file). NxT vs. NxS (x ≠P) Occupancy on C. parvum glycopeptides

Excel S3 (separate file). Complete list of glycopeptides, proteins, and related bioinformatics data.

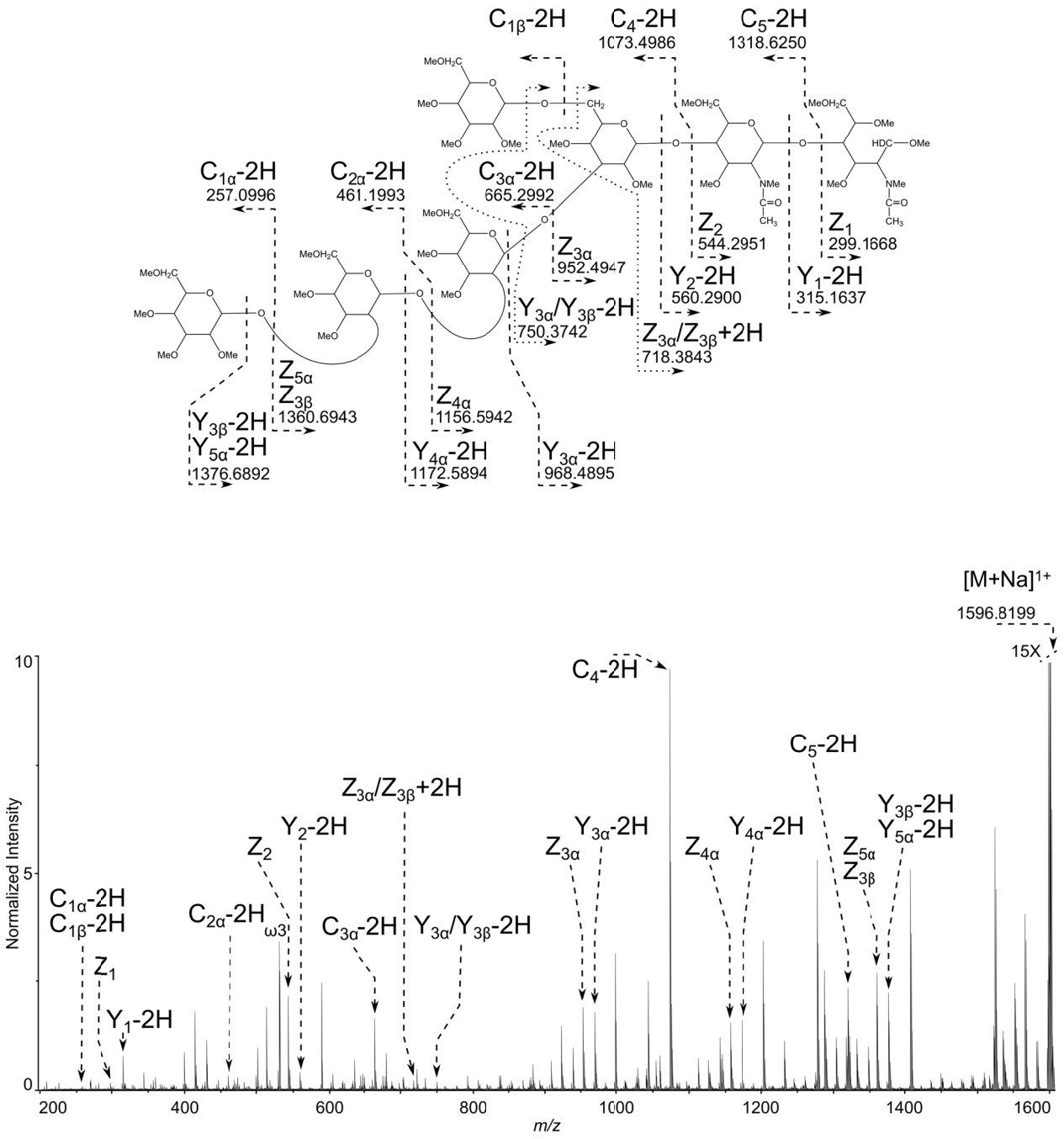

Fig. S1. Assignments of glycan sequence and topology for the second most abundant glycoform $HexNAc_2Hex_5$ released from *C. parvum*, glycoproteins. Glycans were reduced with $NaBD_4$ and permethylated. The EED spectrum was determined at 14 eV for $[M + Na]^+$ *m/z* 1596.8199. Glycosidic fragments provide information on residue masses and connectivity. The assignments for glycosidic bond fragments are shown in this figure. Cross-ring cleavages are assigned in Figure S2.

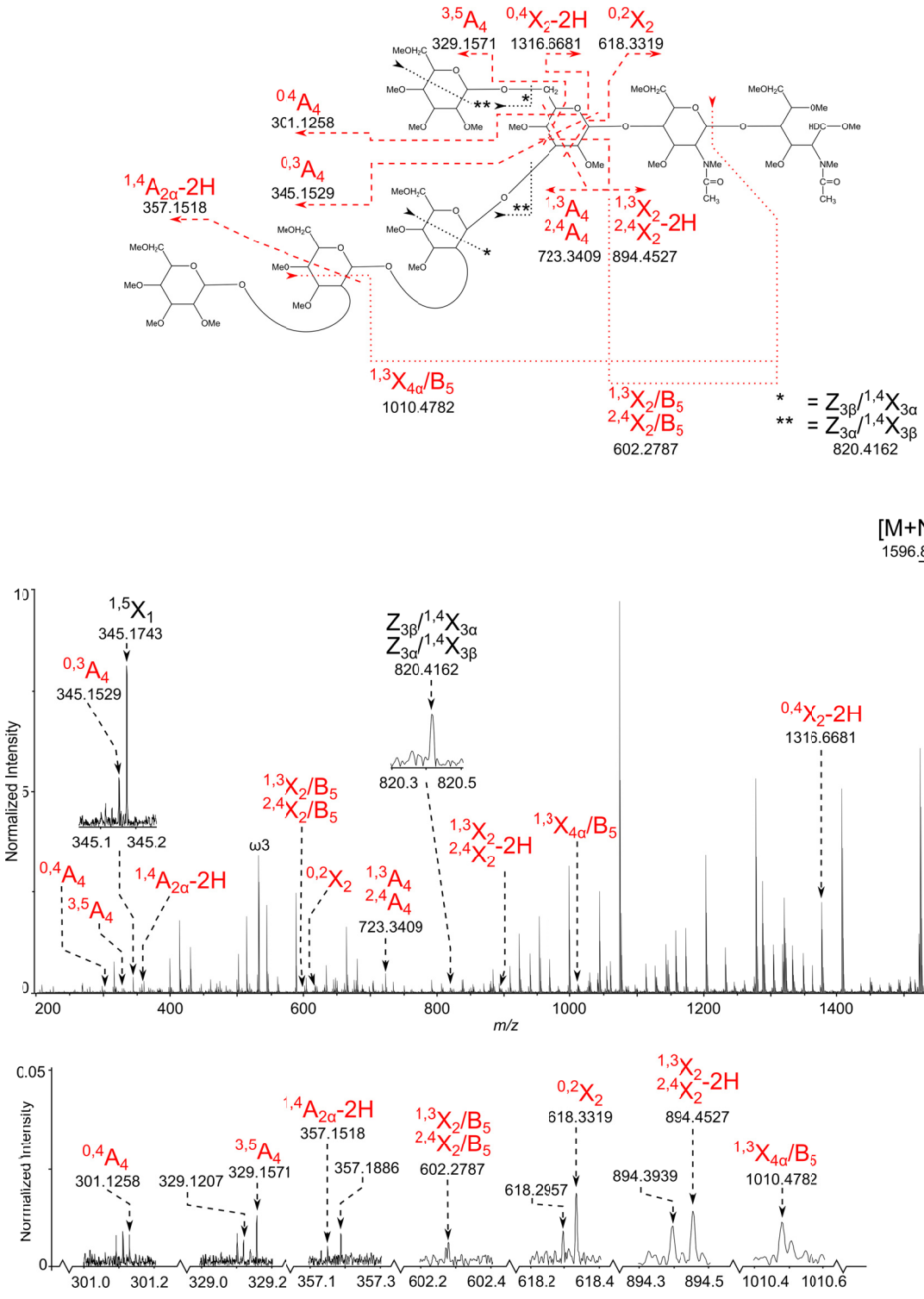

Figure S2. Assignments of cross-ring fragments in the 14-eV EED mass spectrum of deutero-reduced and permethylated $Hex_5HexNAc_2$ released from *C. parvum* glycoproteins, $[M + Na]^+$ m/z 1596.8199. Cross-ring cleavages provide information on linkage positions.

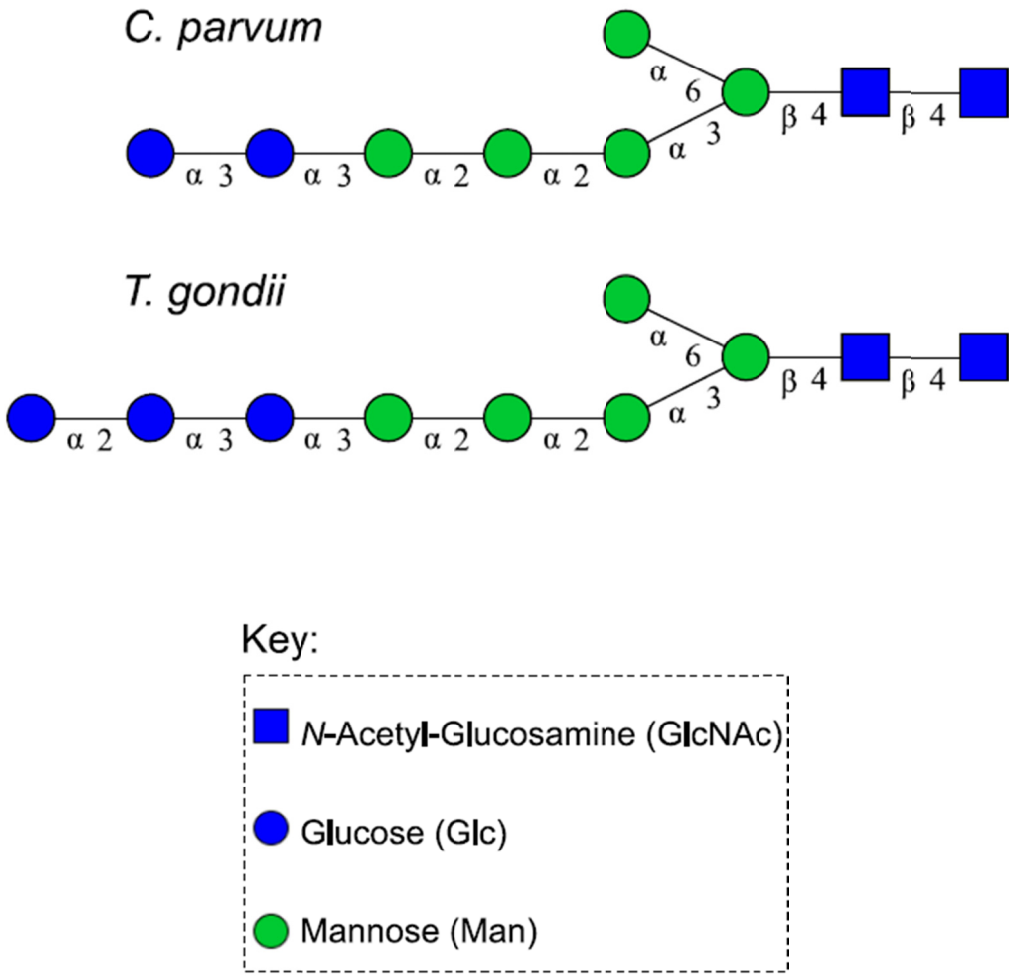

Figure S3. The predicted Lliid-linked N-glycan precursors of *C. parvum* and *T. gondii*.

Table S1. Predicted Alg enzymes, glucosidases, and OST peptides of *C. parvum* and *T. gondii*.

Protein	*C. parvum*	*T. gondii*
Alg7	cgd5_2240	TGGT1_244520
Alg13	absent*	TGGT1_268340
Alg14	cgd7_4930	TGGT1_207070
Alg1	cgd7_1810	TGGT1_230590
Alg2	cgd1_230	TGGT1_227790
Alg11	cgd4_2990	TGGT1_246982
DPM1	cgd5_2040	TGGT1_277970
Alg5	cgd5_2590	TGGT1_216540
Alg6	cgd4_3120	TGGT1_262030
Alg8	cgd1_2100	TGGT1_314730
Alg10	absent	TGGT1_321660
Gls 1	absent	TGGT1_242020
Gls2-α	cgd8_1420	TGGT1_253030
ER MNS1	absent**	absent
Golgi MNS2	absent	absent
UGGT	absent	absent
Calnexin	absent	TGGT1_310320
ERGIC53	cgd6_5140	TGGT1_258950
STT3	cgd6_2040	TGGT1_231430
WBP1	cgd2_1650	TGGT1_203970
Ribophorin1	cgd6_5070	TGGT1_202572
DAD1	gd5_2300	TGGT1_305870

*Alg13 is absent in *C. parvum* and *C. hominis* but is present in *C. muris* (CMU-005550).

** probes for MNS1, MNS2, and UGGT derive from Saccharomyces cerevisiae (Bannerjee 2007).