***O*-fucosylation of thrombospondin-like repeats is required for processing of MIC2 and for efficient host cell invasion by *Toxoplasma gondii* tachyzoites**

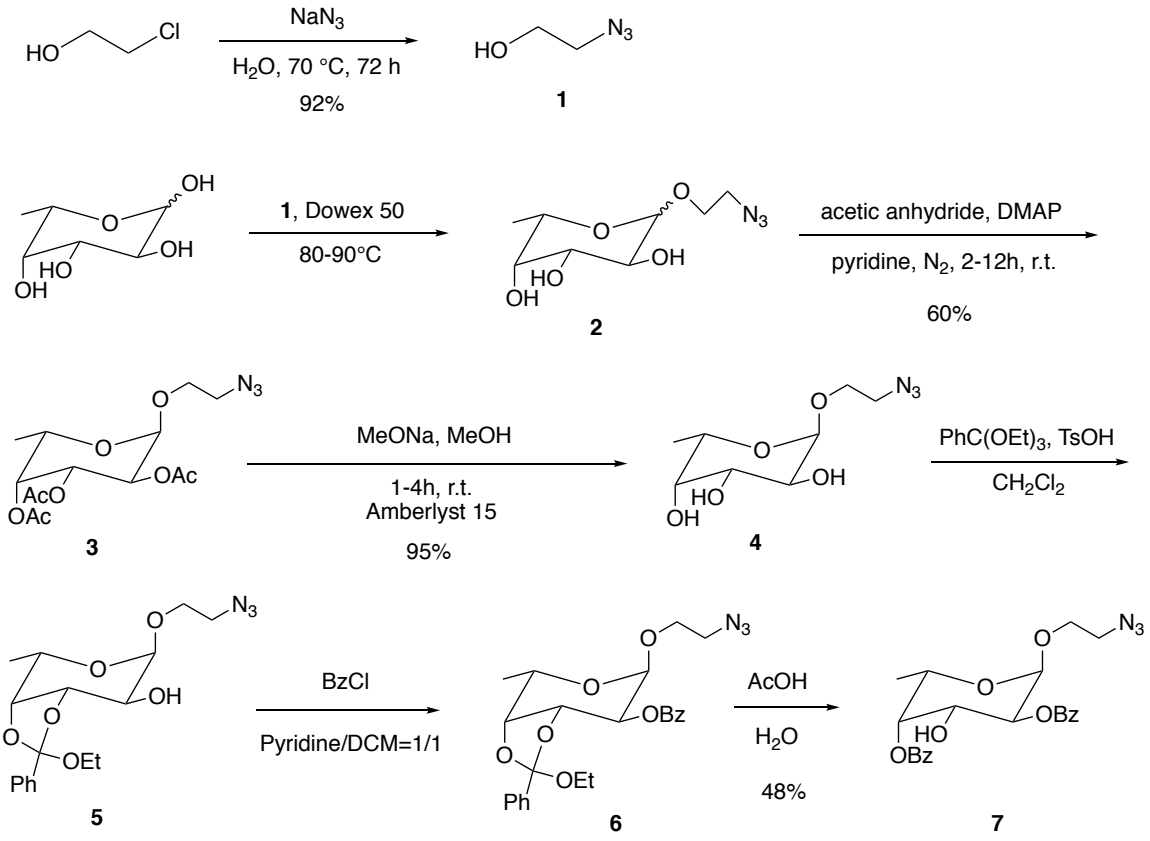
Giulia Bandini[‡], Deborah R. Leon[§], Carolin M. Hoppe[¶,1], Yue Zhang[ʃ], Carolina Agop-Nersesian[‡], Melanie J. Shears[◊], Lara K. Mahal[ʃ], Françoise H. Routier[¶], Catherine E. Costello[§], and John Samuelson[‡,2]

Supporting Information

Supporting Information

Supporting Experimental Procedures

Synthesis of Glc-β-1,3-Fuc-α-KLH (1-4).



Scheme 1. Preparation of fucoside acceptor (compound **7**)

2-Azidoethanol (1). Sodium azide (1.4 eq.) was dissolved in water (5 ml/1 g of sodium azide) at RT and 2-chloroethanol was added to the solution. The reaction mixture was stirred at 30 °C for 1 h and then at 70 °C for 72 h. The resulting solution was cooled to room temperature, saturated with Na_2SO_4, and extracted with dichloromethane. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated on a rotary evaporator to give 2-azidoethanol **(1)** (92% yield).

α/β-Azidoethyl fucoside (2). **α/β** L-fucose mixture, Dowex-50 (120 mg/mmol) and 4 Å molecular sieves were taken together in a two-neck round bottom flask and followed by addition of **(1)** (4 ml/mmol). The reaction mixture was stirred at 80-90 °C for 2 h. After completion of the reaction, the reaction mixture was filtered through cotton funnel and resin was washed thoroughly with distilled MeOH. The filtrate was concentrated under reduced pressure to get the crude residue. The mixture contained both α/β-azidoethyl fucoside **(2)** and residue 2-azidoethanol. It was not further purified and used in the next step for peracetylation.

Peracetylated α-azidoethyl fucoside (3). DMAP (catalytic amount) and acetic anhydride (12 eq.) were added dropwise under N_2 to **(2)**. The reaction was continued with stirring for 12 h at RT. After completion of the reaction (TLC: hexane/ethyl acetate = 2/1), two to three cubes of ice were added and stirred. The reaction mixture was diluted with ethyl acetate and washed with 1 M HCl, saturated $NaHCO_3$, and brine. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated to dryness under vacuum. Column chromatography (230-400 mesh) was carried out using solvent hexane/ethyl acetate = 4/1 to separate the α- and β- isomers, yield for α-isomer **(3)** in the two steps 60%. For α-isomer, ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.16 (d, 3H, H-6, J = 6.4 Hz), 2.00 (s, 3H, -$COCH_3$), 2.08 (s, 3H, -$COCH_3$), 2.17 (s, 3H, -$COCH_3$), 3.43 (m, 2H, -CH_2), 3.62 (m, 1H, -CH_a), 3.86 (m, 1H, -CH_b), 4.18 (q, 1H, H-5, J = 6.4 Hz), 5.12 (d, 1H, H-4, J = 4.0 Hz), 5.14 (dd, 1H, H-3, J = 4.0, 10.0 Hz), 5.32 (d, 1H, H-1, J = 3.2 Hz), 5.38 (dd, 1H, H-2, J = 3.2, 10.0 Hz).

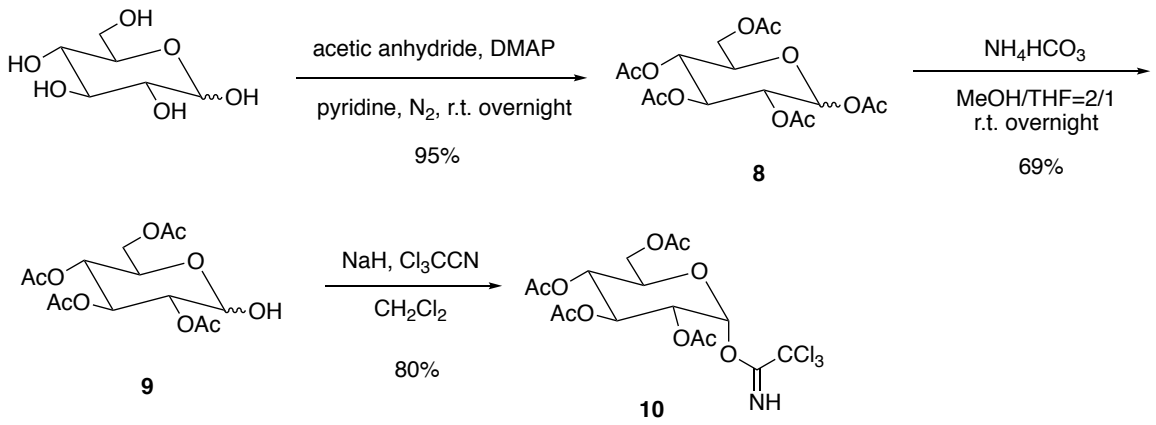
α-Azidoethyl fucoside (4). To a well-stirred solution of peracetylated α-azidoethyl fucoside in MeOH (4 ml/mmol) was added catalytic amount of MeONa and stirring was continued for 2 h at room temperature. After TLC (hexane/ethyl acetate = 3/2) showed no starting material, reaction mixture was diluted with MeOH and neutralized with Amberlyst 15 until pH 7. Resin was filtered off and the filtrate was concentrated to yield the compound quantitatively.

3,4-protected α-azidoethyl fucoside (5). Compound **4** was dissolved in dry dichloromethane (5

ml/mmol) with 4 Å molecular sieves and a speck of TsOH under Ar. Triethyl orthobenzoate (4 eq.) was slowly added to the mixture and stirred at RT overnight. After completion of the reaction (TLC: hexane/ethyl acetate = 1/1), a small amount of NaOH was added and the mixture was filtered. The filtrate was co-evaporate with toluene to yield brown liquid as the product.

2,3,4-protected α-azidoethyl fucoside (6). Compound **5** was dissolved in dry dichloromethane (DCM) (5 ml/mmol) and was dropwise added to the pre-mixed benzoyl chloride (3 eq.) in dry pyridine (equivalent amount to DCM) under Ar at 0 °C with ice bath. The mixture was stirred at RT overnight until reaction was complete (TLC: hexane/ethyl acetate = 3/1). The product was opaque brownish orange color.

2,4-protected α-azidoethyl fucoside (7). Acetic acid (1.7 ml/mmol) and water (1.1 ml/mmol) were added dropwise to the above reaction mixture. The mixture was stirred at RT overnight to achieve full conversion (TLC: hexane/ethyl acetate = 3/1), diluted with DCM, filtered, and poured into sat. $NaHCO_3$. The aqueous phase was extracted with DCM and the combined organic layer was washed with $NaHCO_3$ and brine, dried over Na_2SO_4, concentrated to yield orange liquid. Column chromatography (230-400 mesh) was carried out using solvent hexane/ethyl acetate = 4/1 to get compound **7** as yellow syrup. The yield for the three steps is 48%. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 1.26 (d, 3H, H-6, J = 6.5 Hz), 3.40 (m, 2H, -CH_2), 3.66 (ddd, 1H, -CH_a, J = 3.5, 7.0, 10.5 Hz), 3.93 (ddd, 1H, -CH_b, J = 3.5, 7.0, 10.5 Hz), 4.32 (q, 1H, H-5, J = 6.5 Hz), 4.54 (dd, 1H, H-3, J = 3.5, 10 Hz), 5.28 (d, 1H, H-1, J = 3.5 Hz), 5.38 (dd, 1H, H-2, J = 3.5, 10 Hz), 5.60 (d, 1H, H-4, J = 3.5 Hz), 7.45 (t, 2H, -Ar, J = 7.5 Hz), 7.49 (t, 2H, -Ar, J = 7.5 Hz), 7.58 (t, 1H, -Ar, J = 7.5 Hz), 7.60 (t, 1H, -Ar, J = 7.5 Hz), 8.10 (d, 2H, -Ar, J = 7.5 Hz), 8.15 (d, 2H, -Ar, J = 7.5 Hz), 8.62 (s, 1H, -OH).



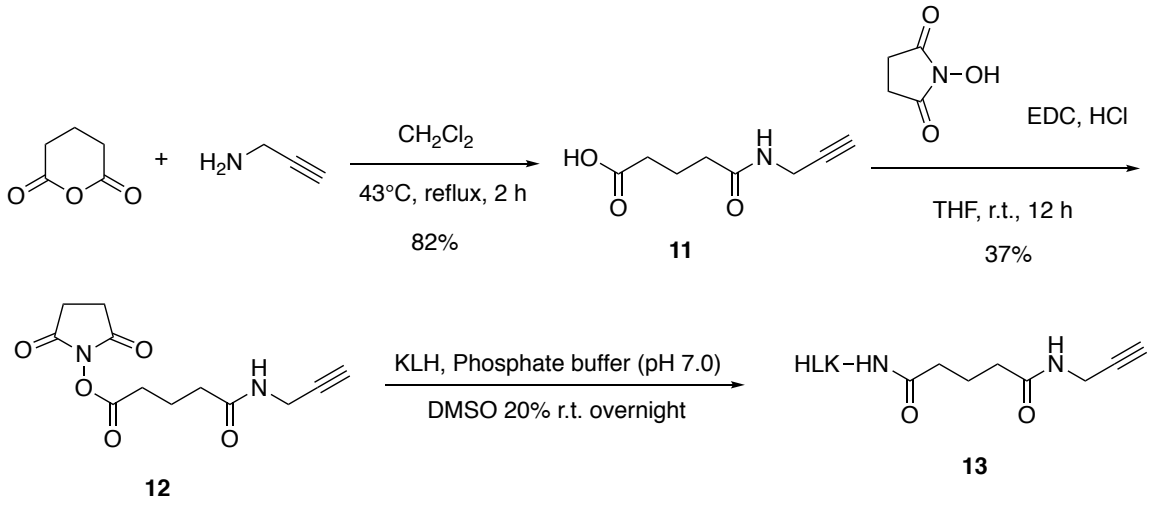
Scheme 2. Preparation of glucoside donor (compound **10**)

Peracetylated-α/β-glucose (8). D-glucose and DMAP (0.1 eq.) was dissolved in dry pyridine (1.5 ml/mmol) followed by dropwise addition of acetic anhydride (10 eq.) over 20 min at 0 °C under N_2. The reaction was stirred at RT overnight to completion. The mixture was diluted with ethyl acetate, the organic layer washed with 1 M HCl, sat. $NaHCO_3$ and brine, and dried over $MgSO_4$. After filtration, the solvent was evaporated under reduced pressure, affording the product in quantitative yield.

2,3,4,6-Tetra-acetyl-α/β-glucose (9). Compound **8** and ammonium acetate (4 eq.) in MeOH/THF (2/1, 4 mL/mmol) were stirred at RT with 4-Å molecular sieves overnight under N_2. Upon completion (TLC: hexane/ethyl acetate = 1/1), the molecular sieves were filtered off and the solvent was evaporated *in vacuo*. The residue was re-dissolved in ethyl acetate, washed with 1 M HCl, sat. $NaHCO_3$ and brine, dried over $MgSO_4$, concentrated and purified by silica gel column chromatography (hexane/ethyl acetate = 1/1) to give product **9** as a white solid, yield 69%.

2,3,4,6-Tetra-acetyl-a-glucosyl trichloroacetimidate (10). Compound **9** was dissolved in dry DCM (5 ml/mmol) under N_2 and trichloroacetonitrile (4.5 eq.) was added. The solution was put in an ice bath and sodium hydride (1.5 eq.) added in three portions. The reaction was stirred at RT

for 12 h and achieved full conversion (TLC: hexane/ethyl acetate = 3/2). The mixture was filtered over a celite bed and concentrated under vacuo. Column chromatography (230-400 mesh) was carried out using solvent hexane/ethyl acetate = 3/1 to get compound **10** as colorless oil, yield 80%. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 2.02 (s, 3H, -CH_3), 2.04 (s, 3H, -CH_3), 2.05 (s, 3H, -CH_3), 2.08 (s, 3H, -CH_3), 4.14 (dd, 1H, H-6, J = 2.2, 12.0 Hz), 4.22 (m, 1H, H-5, J = 2.2, 4.0 Hz), 4.28 (dd, 1H, H-6', J = 4.0, 12.0 Hz), 5.14 (dd, 1H, H-2, J = 3.5, 10 Hz), 5.18 (t, 1H, H-3, J = 10.0 Hz), 5.57 (t, 1H, H-4, J = 10.0 Hz), 6.57 (d, 1H, H-1, J = 3.5 Hz), 8.70 (s, 1H, NH).



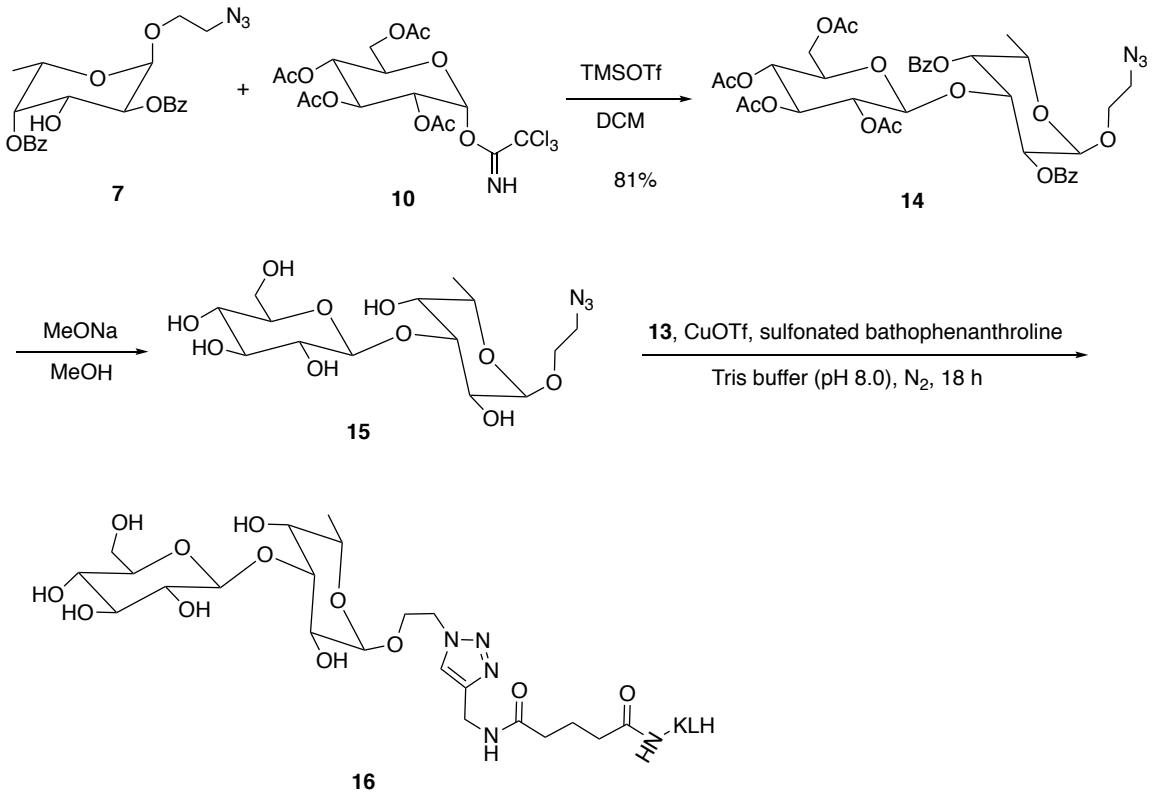
Scheme 3. Preparation of NHS-linker (compound **13**)

5-oxo-5-(prop-2-yn-1-ylamino)pentanoic acid (11). Glutaric anhydride was dissolved in dry DCM. Propargylamine (1 eq.) was added and the solution was refluxed at 43 °C for 2 h until full conversion (TLC: dichloromethane/MeOH = 9/1). The solvent was evaporated and the mixture was purified by column chromatography (dichloromethane/MeOH = 9/1) to get compound **11** as yellow liquid, yield 82%. ^{1}H NMR (500 MHz, MeOD) δ ppm: 1.92 (p, 2H, -CH_2, J = 7.5 Hz), 2.29 (t, 2H, -CH_2, J = 7.5 Hz), 2.37 (p, 2H, -CH_2, J = 7.5 Hz), 2.59 (t, 1H, NH, J = 2.5 Hz), 3.38 (s, 1H, alkyne-H), 3.97(d, 2H, -CH_2, J = 2.5 Hz), 5.00 (br, 1H, COOH).

2,5-dioxopyrrolidin-1-yl 5-oxo-5-(prop-2-yn-1-ylamino)pentanoate (12). Compound **11**, *N*-

hydroxy succinimide (1.4 eq.) and EDC·HCl (1 eq.) was dissolved in dry THF (10 ml/mmol). The mixture was degassed with argon and stirred at RT overnight until completion (TLC: dichloromethane/MeOH = 19/1). The solvent was evaporated and the mixture was re-dissolved in DCM, washed with H_2O, $NaHCO_3$ and brine. The organic layer was collected, dried over Na_2SO_4 and concentrated *in vacuo*. Column chromatography (230-400 mesh) was carried out using solvent DCM/MeOH = 98/2 to get compound **12** as clear liquid, yield 37%. ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 1.93 (p, 2H, -CH_2, J = 7.5 Hz), 2.20 (m, 2H, -CH_2, J = 7.5 Hz), 2.56 (t, 2H, -CH_2, J = 7.5 Hz), 2.68 (s, 1H, alkyne-H), 2.72 (s, 2H, NHS-H), 3.88(d, 2H, -CH_2, J = 2.5 Hz), 6.75 (t, 1H, NH, J = 2.5 Hz).

NHS linker-KLH (13). KLH was dissolved in PBS (pH 7.4) to make a 4 mg/ml stock solution and the NHS linker (compound **12**) was dissolved in DMSO. NHS linker (340 equivalent) was added to KLH solution to make a final 20% DMSO in water (for 8 mg of KLH, 1.85 mg of NHS linker was used). The solution was agitated under room temperature overnight and dialyzed in 2 L of distilled water for 24 h twice.



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131 ***Scheme 4.*** Synthesis of Glc-β-1,3-Fuc-α-KLH antigen (compound **16**)

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133 **Peracetylated glucosyl-β-1,3-(2,4-benzoyl)α-azidoethyl fucoside (14)**. Compound **7** (1 eq) and
134 **10** (2.5 eq) was dissolved in dry DCM (5 ml/mmol) with 4 Å molecular sieves under argon. After
135 stirring at RT for 30 min, pre-diluted TMSOTf (0.35 eq) in DCM (0.03 M) was added dropwise
136 to the solution. The reaction was stirred at RT overnight until completion (TLC: hexane/ethyl
137 acetate = 1/1). The mixture was diluted with DCM and solid $NaHCO_3$ was added and stirred for
138 10 min. The solution was filtered and the filtrate was concentrated and purified by column
139 chromatography (hexane/ethyl acetate = 2/1) to get compound **14** as yellow oil, yield 81%. ^1H
140 NMR (400 MHz, $CDCl_3$) δ ppm: 1.22 (d, 3H, H-6, J = 6.4 Hz), 1.53 (s, 3H, -$COCH_3$), 1.88 (s,
141 3H, -$COCH_3$), 1.98 (s, 3H, -$COCH_3$), 2.01 (s, 3H, -$COCH_3$), 3.32 (ddd, 1H, -CH_a, J = 3.5, 7.0,
142 10.5 Hz), 3.45 (ddd, 1H, -CH_b, J = 3.5, 7.0, 10.5 Hz), 3.66 (m, 2H, -CH_2), 3.88 (m, 1H, H-5'),
143 3.93 (m, 1H, H-6'), 4.22 (m, 1H, H-6''), 4.25 (m, 1H, H-2'), 4.28 (m, 1H, H-5), 4.63 (dd, 1H, H-

3, J = 3.2, 10.4 Hz), 4.78 (d, 1H, H-1', J = 8.8 Hz), 4.98 (t, 1H, H-4', J = 9.6 Hz), 5.13 (t, 1H, H-3', J = 9.6 Hz), 5.31 (d, 1H, H-1, J =3.6 Hz), 5.44 (dd, 1H, H-2, J = 3.6, 10.4 Hz), 5.64 (d, 1H, H-4, J = 3.2 Hz), 7.43 (t, 2H, -Ar, J = 7.6 Hz), 7.47 (t, 2H, -Ar, J = 7.6 Hz), 7.59 (t, 1H, -Ar, J = 7.6 Hz), 7.60 (t, 1H, -Ar, J = 7.6 Hz), 8.08 (d, 2H, -Ar, J = 7.6 Hz), 8.11 (d, 2H, -Ar, J = 7.6 Hz).

Glucosyl-β-1,3-α-azidoethyl fucoside (15). Compound **14** was dissolved in dry MeOH and sodium methoxide (2 eq) was added. The reaction was stirred at RT overnight until completion (TLC: ethyl acetate/MeOH = 5/1). The solution was neutralized with Amberlyst 15 until pH 7, filtered and concentrated *vacuo*. Prep-TLC was performed (ethyl acetate/MeOH = 5/2) to get compound **15** as colorless syrup with quantitative yield. ^{1}H NMR (600 MHz, MeOD) δ ppm: 1.28 (d, 3H, H-6, J = 6.6 Hz), 3.30 (m, 1H, H-2'), 3.37 (m, 2H, H-6'), 3.43 (m, 1H, -CH_a), 3.55 (m, 1H, H-5'), 3.57 (m, 1H, -CH_b), 3.66 (m, 2H, -CH_2), 3.69 (m, 3H, -CH_2, H-3'), 3.86 (m, 1H, H-4'), 3.89 (m, 1H, H-4), 3.92 (m, 1H, H-2), 4.01 (dd, 1H, H-3, J = 3.0, 10.2 Hz), 4.05 (q, 1H, H-5, J = 6.6 Hz), 4.45 (d, 1H, H-1', J = 7.8 Hz), 4.87 (s, 1H, H-1).

Glc-β-1,3-Fuc-α-KLH antigen (16). All the reagents were dissolved in argon degassed double distilled water and the reaction was carried out under argon. In the NHS linker-KLH solution, Tris buffer (pH 8.0), compound **15**, and the ligand bathophenanthroline sulfonated sodium salt, CuOTf ware added sequentially to make a final solution containing 2 mg/ml NHS-KLH, 0.1 M Tris, 0.3 mM disaccharide, 2 mM ligand, and 1 mM CuOTf. The mixture was degassed with argon for 1 min and then agitated at RT overnight followed by dialyzing in 2 L of distilled water for 24 h twice. After dialysis, the solution was snap-frozen in liquid N_2 and lyophilized to yield blue powder.

Disaccharide agarose beads preparation. A slurry of agarose-alkyne beads in DMF was treated with compound **15** (4 eq.), 2,6-lutidine (8 eq.), 2,2'-bipyridine (8 eq.), cuprous bromide (4 eq.), and sodium ascorbate (8 eq.). The solution was degassed with argon for 1 min and agitated at RT overnight. The resulting mixture was washed sequentially with DMF, H_2O, MeOH, 0.1 M

aqueous EDTA, H_2O, and DMF.

References

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Supporting Tables

Table S1. *In silico* identification putative *T. gondii* POFut2 protein acceptors.

Gene ID	Protein Name	POFut2 consensus sequence(s)
TGGT1_201780	microneme protein MIC2 (MIC2)	see Fig. 1A
TGGT1_209060	thrombospondin type 1 domain-containing protein	[460]WSPCTA**S**CEGGE[473] [586]WSACSA**T**CGEGW[599]
TGGT1_218310	microneme protein MIC14 (MIC14)	[639]WSECSR**T**CRSGG[652]
TGGT1_223480	sushi domain (scr repeat) domain-containing protein	[538]WSDCST**T**CGEGQ[551] [593]WGPCGA**S**CGGGE[606] [756]WSVCTA**S**CGFGT[769] [872]WGPCSA**T**CGGGQ[885] [934]FRECDA**T**CGVGR[947] [999]WSSCSV**T**CGGGQ[1012] [1113]WTGCSQ**S**CGLGG[1126] [1282]WTPCSV**T**CGVGK[1295] [1338]WSTCSA**S**CGGGV[1351]

188 **TableS2. Primers and homologous recombination sequences (ROs) used in this study**

Primer	Sequence (5'-3')
P20	AGCGCTTCTGTXGCTGTCGTTATTTCCAGCAGTTAACGCGGGTTCGAAGGCTGCT AGTACTG
P21	GAGCGGATCGCGACCAAACGTTACAGTGTCGAACTGGGGTGCTTAACACCATTG CATTCC
P22	AGGCCTGGAGATTCTGGCCGCCGCTTTTCCCCCAGATCACCTCGAAGGCTGCTAG TACTG
P23	TACGTAAACGGTATCTCCCTTTTGAAGTGGGTTTCGGCTTAACACCATTGCATTCC
P24	AAGTTGAGTGCATGCAGGCAGCACCGG
P25	AAAACCGGTGCTGCCTGCATGCACTCA
P26	AAGTTGCAGTGTCGAACTGGGGTCCAG
P27	AAAACTGGACCCCAGTTCGACACTGCA
P28	AAGTTGCAGGTATCGACCTACCAGGG
P29	AAAACCCTGGTAGGTCGATACCTGCA
P30	AAGTTGTGTCTGGCAAAGTTGCAGATG
P31	AAAACATCTGCAACTTTGCCAGACACA
P32	GAGTTTACACTGGACGCAG
P33	CAAACAGACCGGCAGGTGCT
P34	GCTTAATTAACTATTTGTATAGTTCATCCATG
P35	GTATACGCTCGCGACTCTC
P36	CATGCATTGTCAACTAGG
P37	CTTCCGTTTCCTCGTGTAC
P38	CATAAGGAGACTCCTGACC
P39	GTAGTAACAGTGTCTTACACG
P40	CACCGTTCAAGTCTTCCTCGG
P41	CCATGGATGAGGCGCCACGTCTCAGTT
P42	CAGATCTATGCATTGTCAACTAGGAGG
P43	CCTAGGCAGTGTCGAACTGGGGT
RO1	TGG CCA GAA GGA CGC AGT CGA AGT GAG CTC AGG AGG AAA TAC TGG CCT TCa TTG GAC CCC AGT TCG ACA CTG GAA CAA AAG TTG ATT TCT GAA GAA GAT TTG AAC GGT GAA CAA AAG CTA ATC TCC GAG GAA GAC TTG AAC GGT GCT AGG GCC GAG GAG CAG AAG CTG ATC TCC GAG GAG GAC CTG TAA CGT TTG GTC GCG ATC CGA CTC CCC ATT TTC GTT TCG GGC ATC TTG GAG ACG TCA CGC TGC TGC CAG CTG

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Table S3. Statistical analysis of the attachment/ invasion assay comparing parental strain and mutants.

Comparison	*p* value
Δku80 vs. *pofut2*KO	0.0004
Δku80 vs. *nst2*KO	0.03
Δku80 vs. *mic2*KO	0.0002
*pofut2*KO vs. *nst2*KO	n.s. (0.28)
*pofut2*KO vs. *mic2*KO	n.s. (0.43)
*nst2*KO vs. *mic2*KO	n.s. (0.1)
Δku80 vs. *pofut2*KO	0.0005
*pofut2*KO vs. *pofut2*KO+ POFUT2	1.8xE-06
Δku80 vs. *pofut2*KO+ POFUT2	n.s. (0.87)

n.s.: not significant

Supporting Figures

Figure S1.

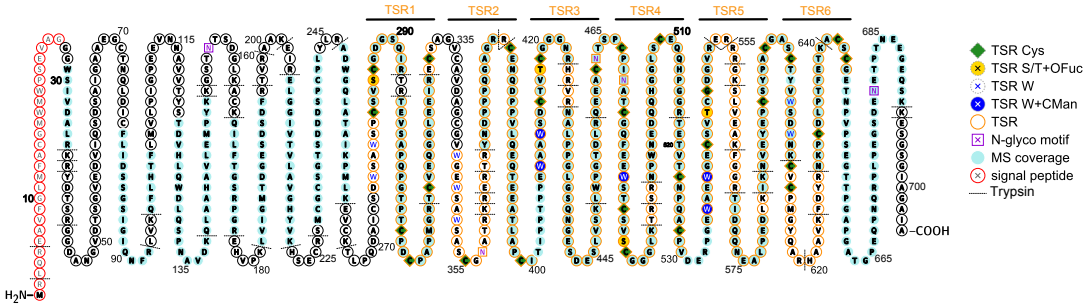

Figure S1. Mass spectrometry analysis of MIC2 performed in this study. Protter was used to build a graphic representation of the MIC2 peptides detected in this study. The sequence of secreted MIC2 (plus signal peptide) was used. A legend is shown above.

Figure S2.

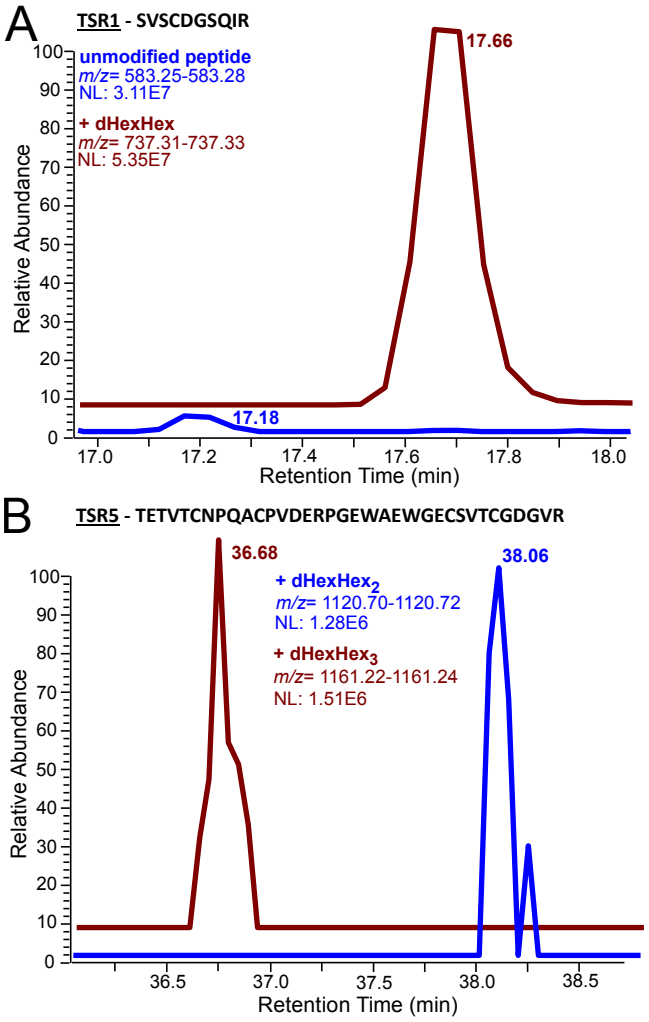

Figure S2. Extracted Ion Chromatograms (XIC) comparing the relative abundances of MIC2 TSR1 and TSR5 observed glycoforms. *A*. The semi-tryptic peptide [282]SVSCDGSQIR[294] from TSR1 was observed as unglycosylated (*m/z* 583.2670) and modified by dHexHex on S284 (*m/z* 737.3221), however the unglycosylated species is a very minor isoform. *B*. Two glycoforms were consistently observed for peptides from TSR5 differentiated by the presence of a dHex or dHexHex on the POFUT2 site. The tryptic peptide (one missed cleavage) [517]TETVTCNPQACPVDERPGEWAEWGECSVTCGDGVR[553] was used to assess the relative abundance of these two glycoforms. Comparison of the XIC for *m/z* 1120.7179 ($dHexHex_2$) and 1161.2313 ($dHexHex_3$) suggests they have similar abundances.

Figure S3

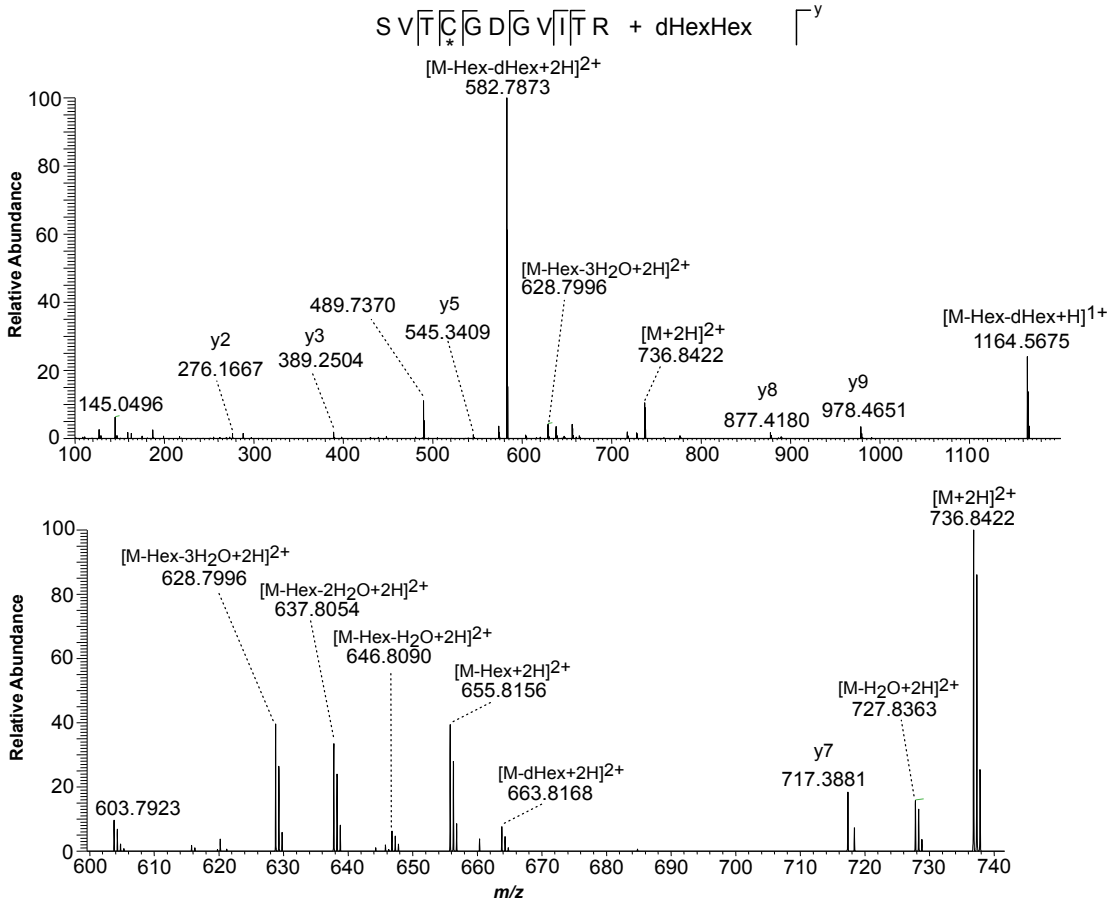

Figure S3. Low energy HCD of a semi-tryptic glycopeptide from the TSR2 of thrombospondin 1. A collision energy of 15 eV was used: the *top panel* shows the full MS/MS spectrum and an enlargement of the *m/z* range 600-740 is shown at the *bottom*. Together with product ions corresponding to the precursor minus a Hex and a dHexHex, with or without the additional loss of 1-3 H_2O molecules, we observed a low abundance product ion corresponding to the precursor minus dHex ($[M+2H]^{2+}$ *m/z* 663.8168). The detection of this product ions originating from a TSP1 glycopeptide suggest that, both in this case and for the MIC2 glycopeptides, a very minor but nevertheless detectable glycan rearrangement took place during the mass spectrometry analysis.

Figure S4.

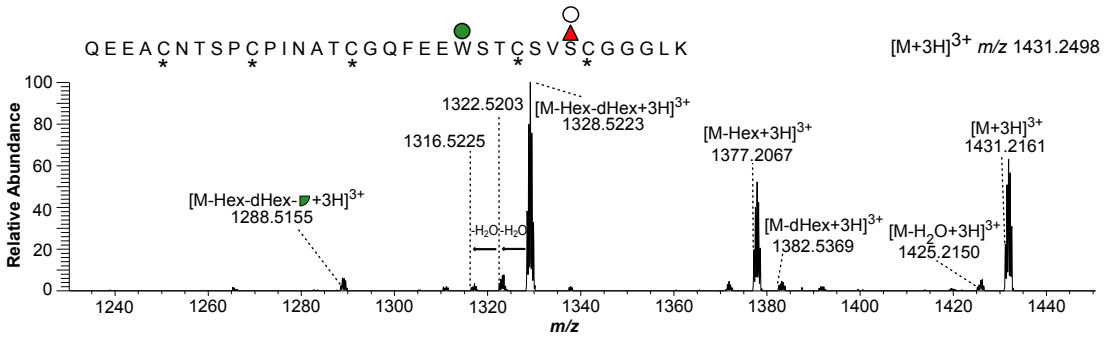

Figure S4. Low collision energy HCD MS/MS of an *O*-fucosylated and *C*-mannosylated glycopeptide from the TSR4 of MIC2. A collision energy of 10 eV was used. The MS/MS spectrum shows product ions corresponding to the precursor minus a Hex, a dHexHex, or minus dHexHex and 120.04 Da (broken green circle). As observed for the TSR3, a minor product ion arising from the precursor minus a dHex is also observed and it is likely due to rearrangement of the glycan. Red triangle: fucose; Green circle: mannose; White circle: Hex; Asterisk: cysteine carbamidomethylation.

Figure S5.

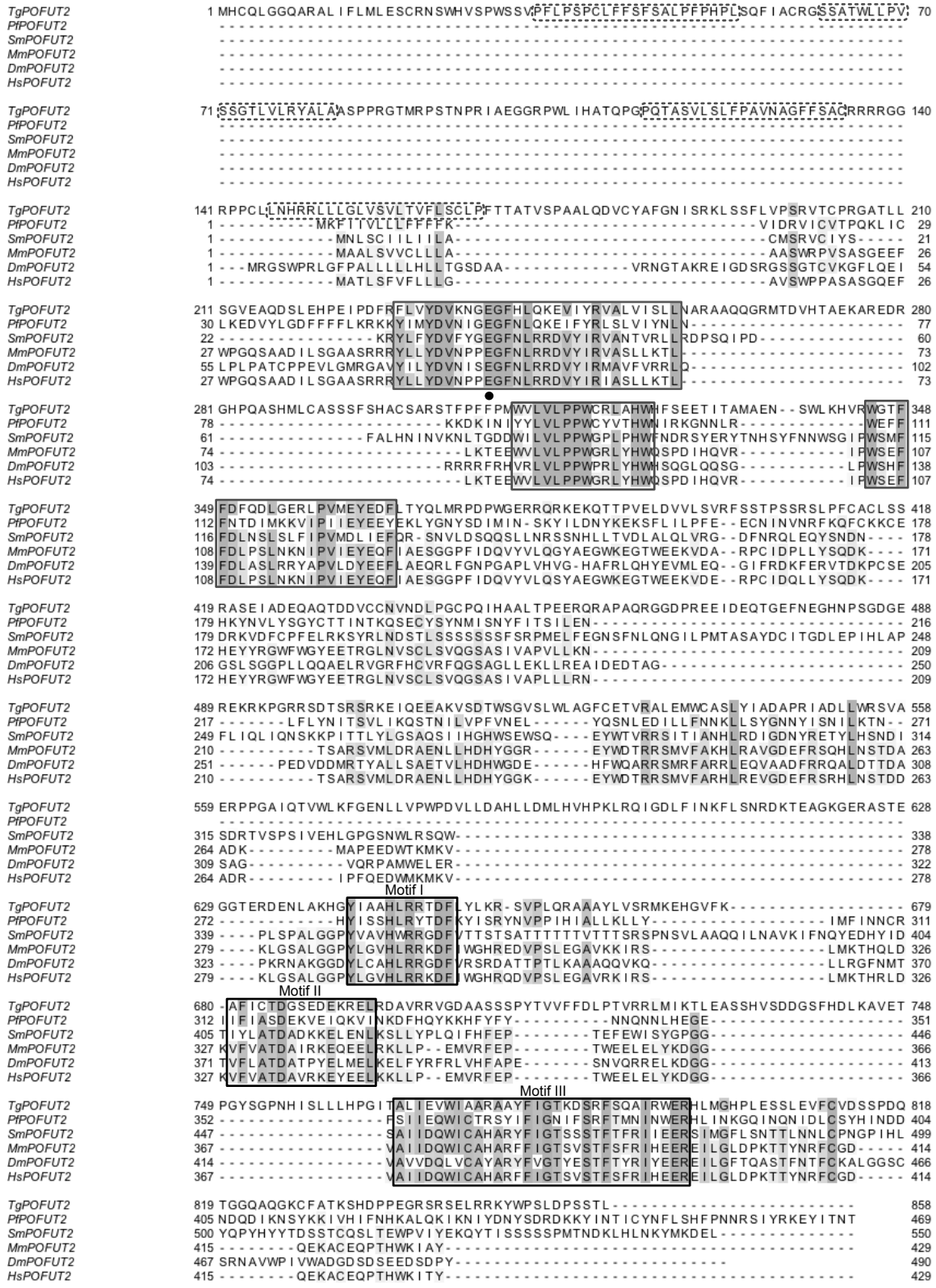

Figure S5. Sequence alignment of *T. gondii* POFUT2 with other eukaryotic POFUT2 highlights conserved motifs. Conserved sequences are boxed in black (FTs superfamily conserved motifs I-III) or gray (POFUT2-specific) (35, 37). The catalytic Glu residue is marked by a black dot. Gray dashed boxes mark transmembrane helices. *Pf: Plasmodium falciparum; Dm: Drosophila melanogaster; Hs: Homo sapiens; Mm: Mus musculus;Sm: Schistosoma mansoni.*

Figure S6.

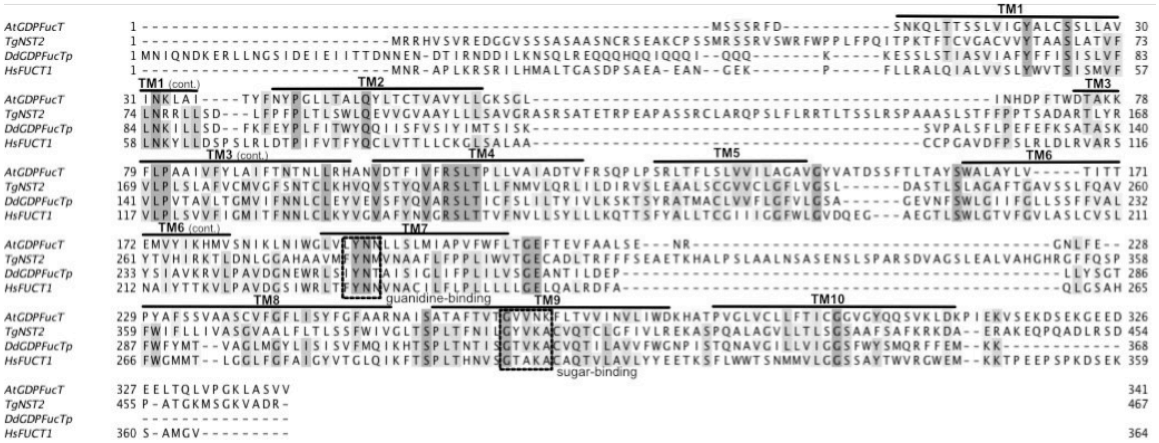

Figure S6. Sequence alignment of *T. gondii* NST2 with other GDP-Fuc transporters. The predicted topology and conserved motifs for the *Hs*FUCT1 (42) was used to annotate the alignment. TM1-10 mark the transmembrane domains. The two dashed boxes highlight the motif involved in guanidine and sugar binding, as recently described for the GDP-Mannose transporter (42). *Dd: Dictyostelium discoideum; Hs: Homo sapiens; At: Arabidopsis thaliana.*

Figure S7.

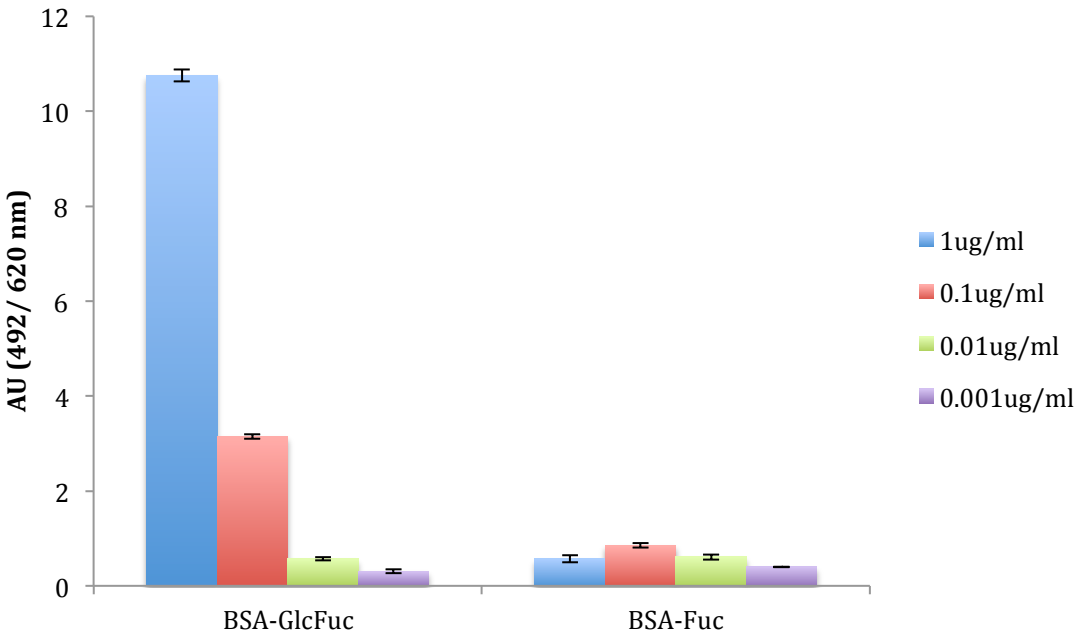

Figure S7. Anti-GlcFuc Antibody Titration. Different concentrations of anti-GlcFuc polyclonal IgY were tested by ELISA to identify the concentration that provided the best signal against BSA-GlcFuc.

Figure S8.

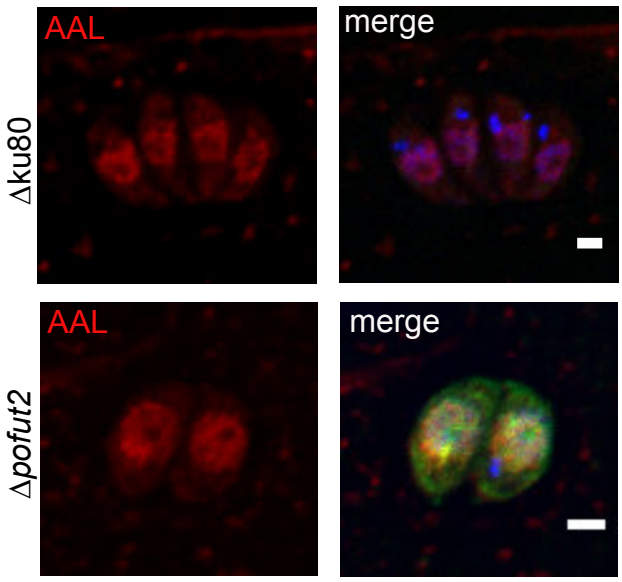

Figure S8. **Knockout of *pofut2* does not affect nuclear *O*-fucosylation**. IFA staining with *Aleuria aurantia* lectin (AAL) of wild type and Δ*pofut2* tachyzoites shows that nuclear *O*-fucosylated proteins are still present in the KO.

Figure S9.

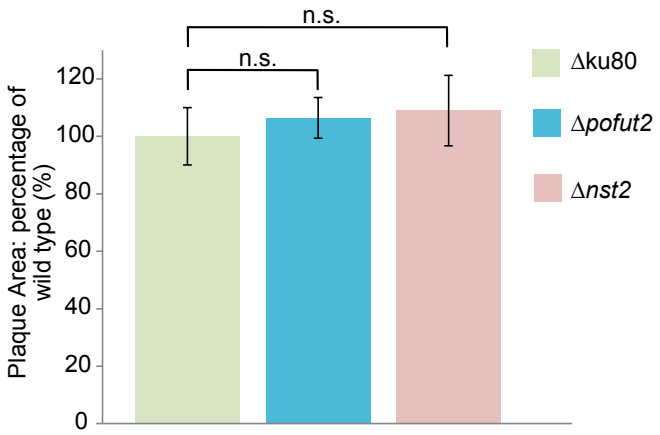

Figure S9. **No difference in plaque area is observed between wild type and *pofut2* and *nst2* deficient parasites.** The lack of statistical difference in the size of plaques, suggest the *O*-fucosylation deficient parasites are replicating as efficiently as the parental strain. Plaque areas were determined as described in material and methods. The average of two biological repeats is shown. n.s.: not significant.