Protein *O*-fucosyltransferase 2–mediated *O*-glycosylation of the adhesin MIC2 is dispensable for *Toxoplasma gondii* tachyzoite infection

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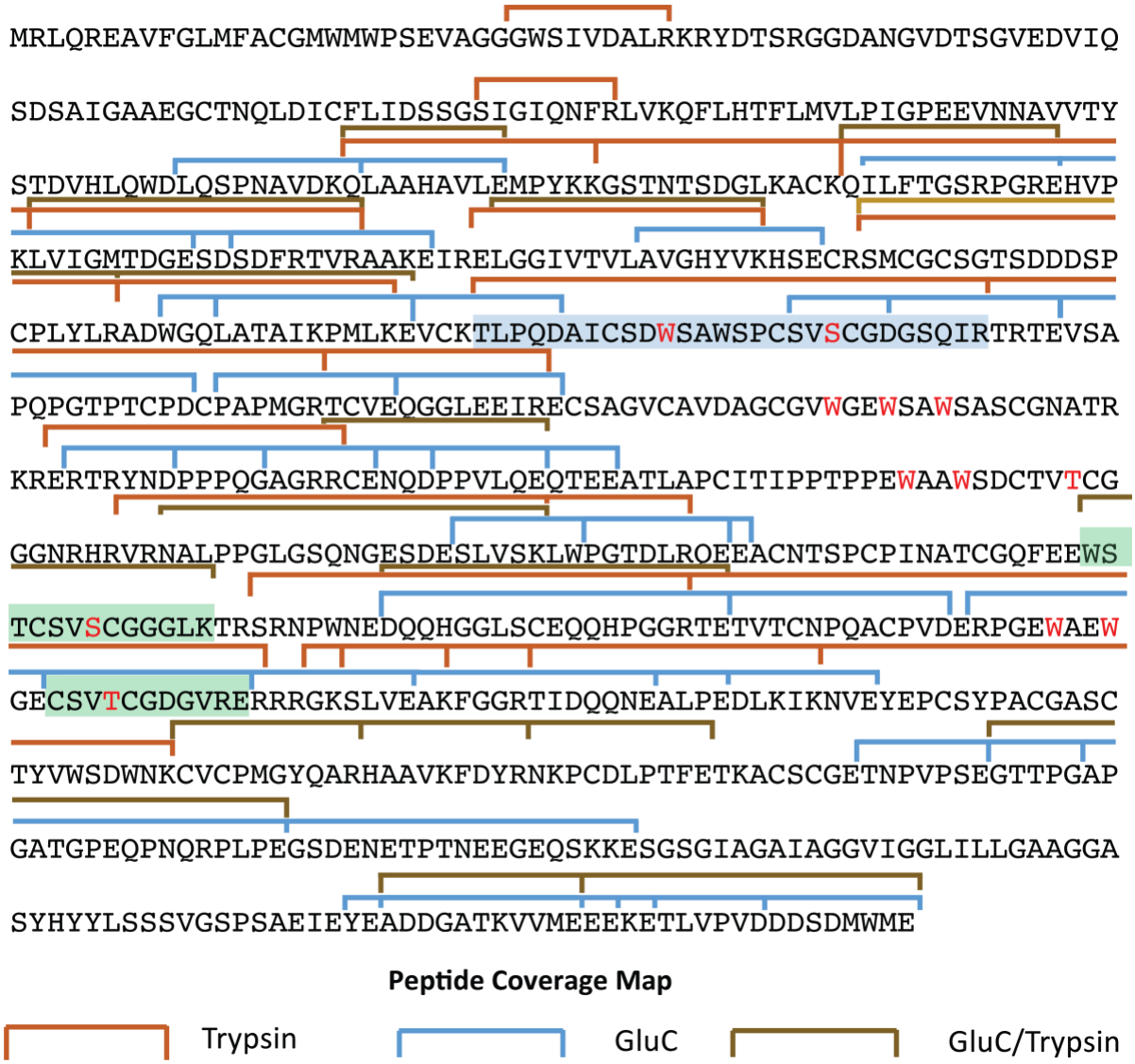
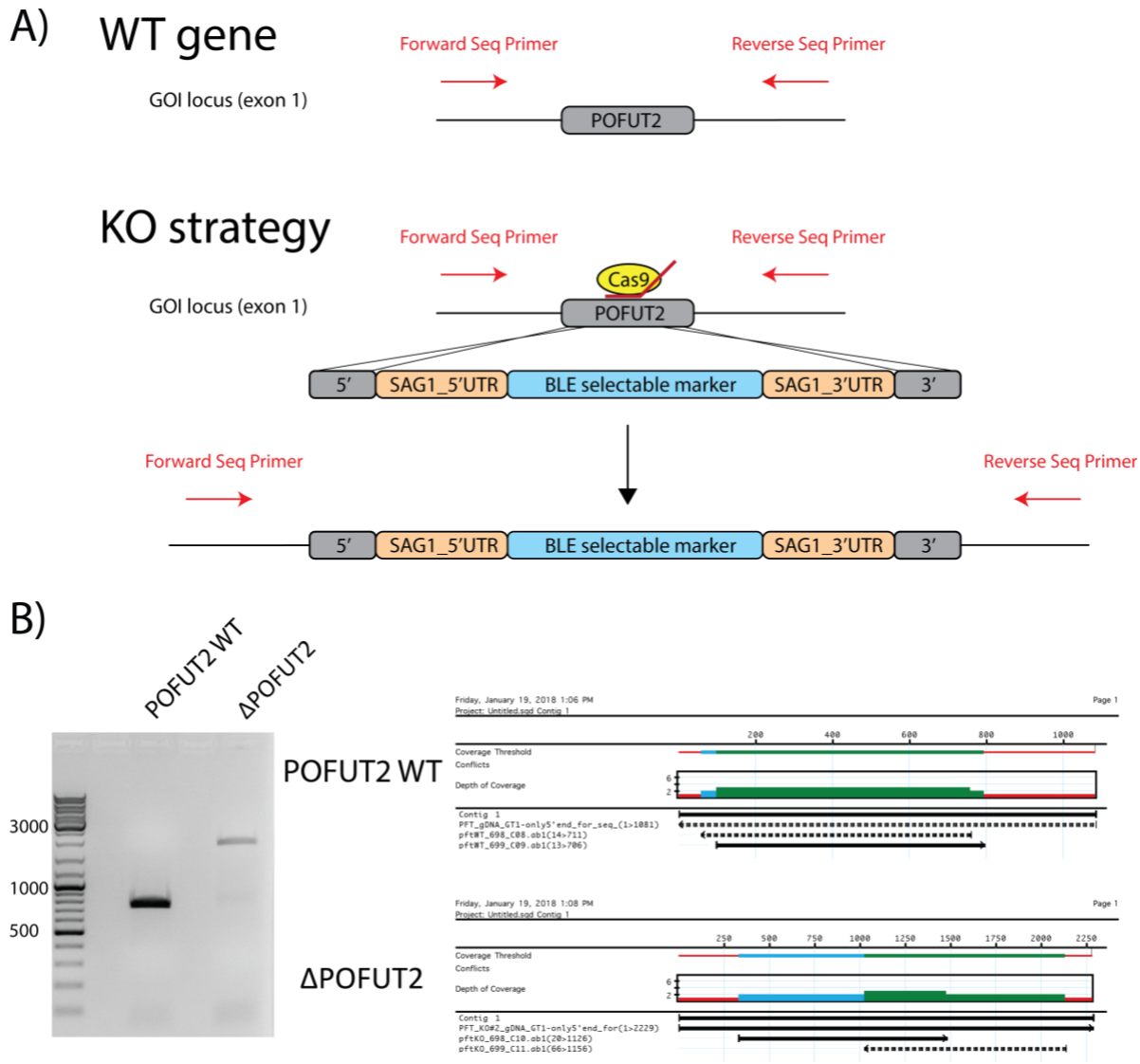
Supplementary Document

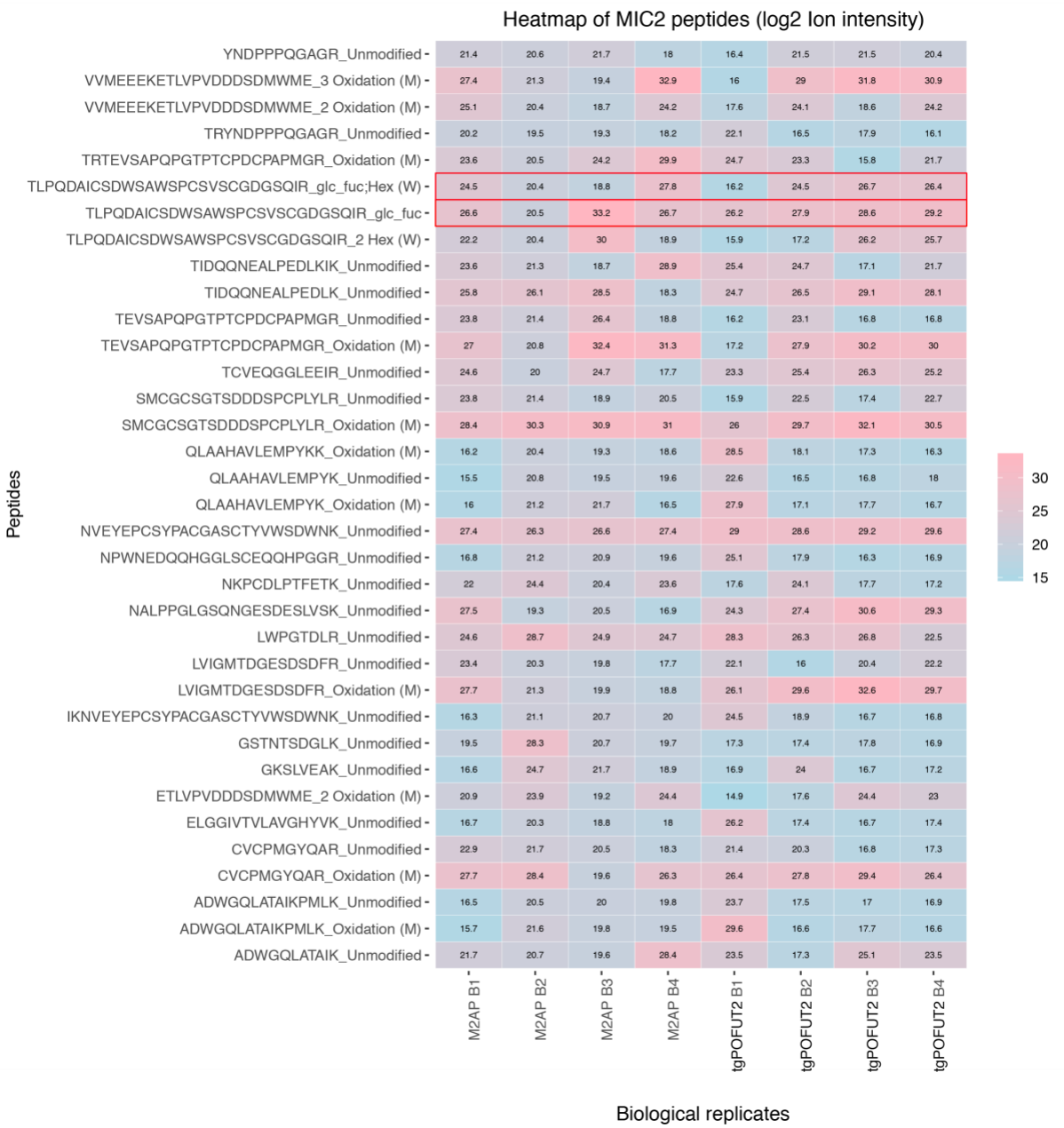
Supplementary Figures



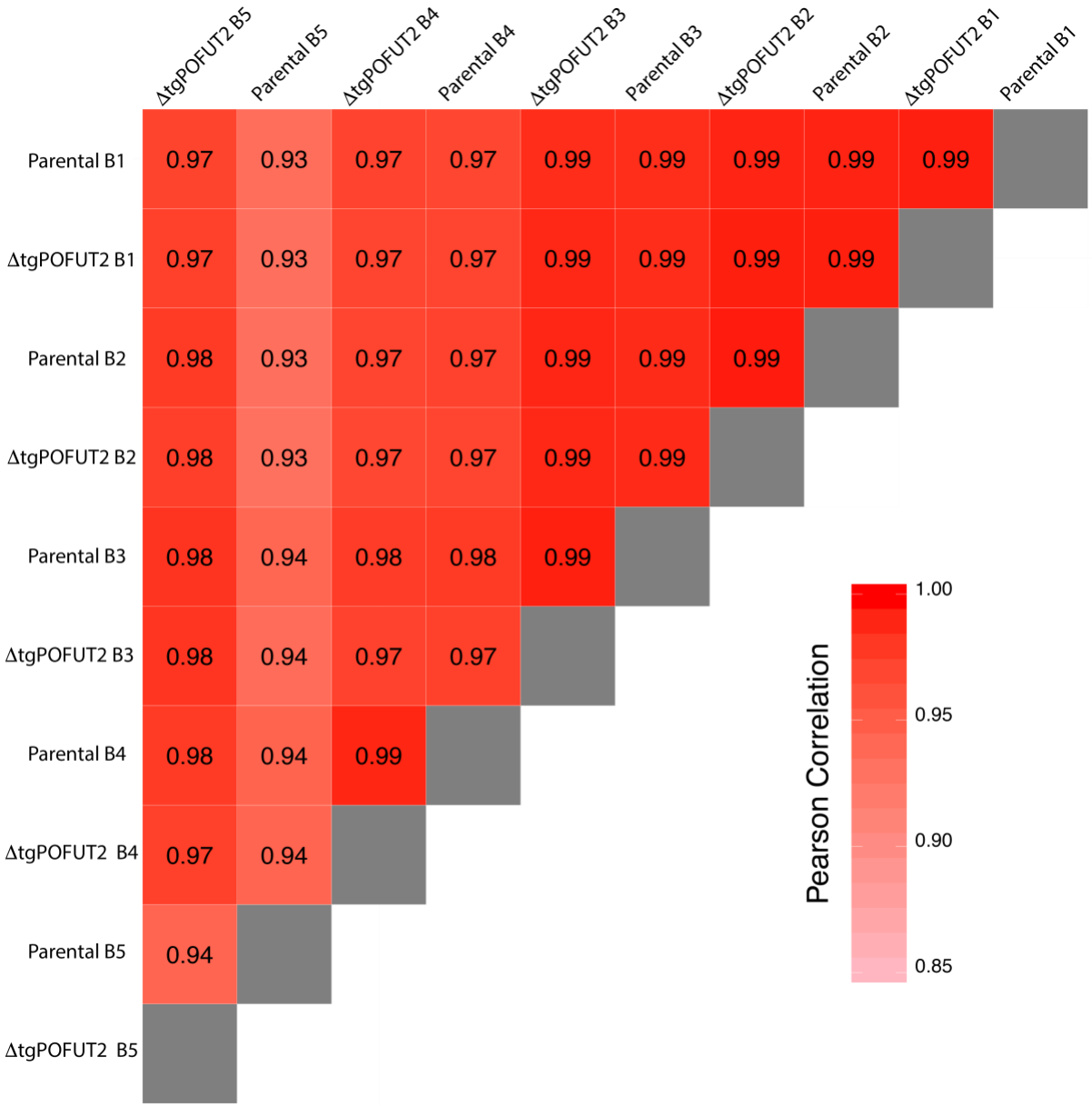
Supplementary Figure 1. Peptide coverage of MIC2 using our multiple enzymes approach. Putative glycosylation sites are shown in red. Peptide containing O-fucosylated sites are highlighted in green.



Supplementary Figure 2. Construct and validation of TgPOFUT2 within *T. gondii* GT1using cas9 driven mutagenesis. Exon 1 of tgPOFUT2 was targeted for disruption using Cas9 driven mutagenesis. Introduction of a double strain break by guide enable the introduction of the BLE selectable marker



Supplementary Figure 3. MIC2 derived peptides observed from TgPOFUT2-HA$_3$ line. To assess if the tagging of TgPOFUT2 with the HA$_3$ epitope interfered with *O*-glycosylation MIC2 enriched via affinity enrichment of M2AP-SF-TAP was digested and subjected to LC-MS. Normalized ion intensities for peptide derived from MIC2 are shown for four biological replicate pulldowns from TgPOFUT2-HA$_3$ and four M2AP-SF-TAP pulldowns prepared in parallel as controls. *O*-glycosylation of MIC2 peptides, lighted in red lines were identified within TgPOFUT2-HA$_3$ lines at comparable levels to the M2AP-SF-TAP line supporting the functionality of the tagged TgPOFUT2.



Supplementary Figure 4. Pearson correlation of LFQ proteome experiments. To assess the reproducibility of proteome analysis heat maps of observed LFQ proteome are provided. The mean Pearson correlation is >0.95

Supplementary Tables

Supplementary Table 1. Peptides observed from Trypsin, GluC and a combination of Trypsin followed by GluC digestion of parental MIC2. MIC2 derived peptides from four digestions conditions revealed the presence of multiple glycosylation events, both *C* and *O*-glycosylation in MIC2. A total of four conditions were used to map glycosylation in MIC2; Tab 1) Trypsin reduced with DTT, Tab 1) Trypsin reduced with TCEP, Tab 2) GluC reduced with TCEP and Tab 3) Trypsin digestion followed by GluC digestion reduced with TCEP. For each condition the assigned Peptide sequence, Modification status, Mass, Protein and Maxquant assignment associated information (Score, Protein group, Scan number and data file name) are provided.

Modification status	XIC m/z (charge state)	Area under the curve	%
unmodified	1048.10-1048.15 (+3)	3583734	1%
O-fucosylated (+308.11 Da)	1150.13-1150.18 (+3)	138860237	44%
O-fucosylated (+308.11 Da) and C-Hexose (+162.05 Da)	1204.15-1204.20 (+3)	169260407	53%
O-fucosylated (+308.11 Da) and 2x C-Hexose (+162.05 Da)	1258.17-1258.22 (+3)	3040118	1%
C-Hexose (+162.05 Da)	1101.45-1101.50 (+3)	3552376	1%
	total observed ion current	318296872	

Supplementary Table 2. Relative Quantitation of ^{266}TLPQDAICSDWSA WSPCSVSCGDGSQIR^{293}: Multiple glycoforms of the peptide ^{266}TLPQDAICSDWSA WSPCSVSCGDGSQIR^{293} are observable within MIC2. The most abundant of these forms all bear of *O*-fucosylation events support that S^{285} is modified at a high occupancy.

Supplementary Table 3. Peptides observed from Trypsin of Δtgpofut2 MIC2. MIC2 derived peptides from a Tryptic digested reduced with TCEP. For this digest the assigned Peptide sequence, Modification status, Mass, Protein and Maxquant assignment associated information (Score, Protein group, Scan number and data file name) are provided.

Modification status	XIC m/z (charge state)	Area under the curve	%
unmodified	1048.10-1048.15 (+3)	293671856	25%
O-fucosylated (+308.11 Da)	1150.13-1150.18 (+3)	0	0%
O-fucosylated (+308.11 Da) and C-Hexose (+162.05 Da)	1204.15-1204.20 (+3)	0	0%
O-fucosylated (+308.11 Da) and 2x C-Hexose (+162.05 Da)	1258.17-1258.22 (+3)	0	0%
C-Hexose (+162.05 Da)	1101.45-1101.50 (+3)	425515277	36%
C-Hexose (+324.10 Da)	1155.47-1155.52 (+3)	463078203	39%
	total observed ion current	1182265336	

Supplementary Table 4. Relative Quantitation of ^{266}TLPQDAICSDWSA WSPCSVSCGDGSQIR^{293} within Δ*tgpofut2* strains: Multiple glycoforms of the peptide ^{266}TLPQDAICSDWSAWSPCSVSCGDGSQIR^{293} are observable within MIC2 yet no *O*-fucosylation were detectible.

Supplementary Table 5. LFQ based analysis of ΔTgPOFUT2 vs parental strain. A total of the 3839 protein groups observed across *T. gondii* proteome samples. For each protein group the observed LFQ values for all biological replicates, identification type, t-test significance, score, number of MS/MS events, iBAQ values and protein name gene generated using Maxquant are provided