Steric constraints control processing of glycosylphosphatidylinositol anchors in *Trypanosoma brucei*

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Supporting Information: Figure S1

Figure S2

Figure S3

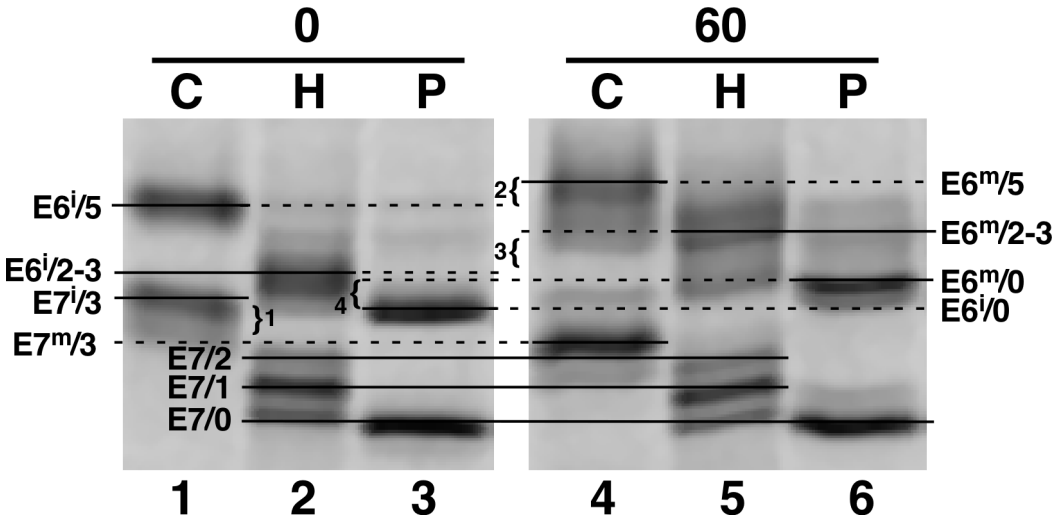

Figure S1. Identification of glycoforms in de-N-glycosylated TfR. Phosphorimages from Fig. 2 are presented. Individual species are identified as E6 or E7 followed by immature (i) or mature (m) / # of N-glycans (0-5). Major shifts are: 1) Apparent decrease in size of fully glycosylated E7 from immature (lane 1, $E7^{i}/3$) to mature (lane 4, $E7^{m}/3$). We attribute this to trimming of N-glycans during cell transit (see also Fig. 3, lanes 4 & 10; Fig. 6, lanes 9 & 10). 2) Increase in size of fully glycosylated E6 from immature (lane 1, $E6^{i}/5$) to mature (lane 4, $E6^{m}/5$) glycoforms. This represents combined processing of paucimannose N-glycans and the GPI glycan core. 3) Increase in size of partially de-N-glycosylated (Endo H treatment) E6 from immature (lane 2, $E6^{i}/2$-3) to mature (lane 5, $E6^{m}/2$-3) glycoforms. This represents combined processing of paucimannose N-glycans and the GPI glycan. 4) Increase in size of fully de-N-glycosylated (PNGase F treatment) E6 from immature (lane 3, $E6^{i}/0$) to mature (lane 6, $E6^{m}/0$). This represents processing of the GPI glycan core.

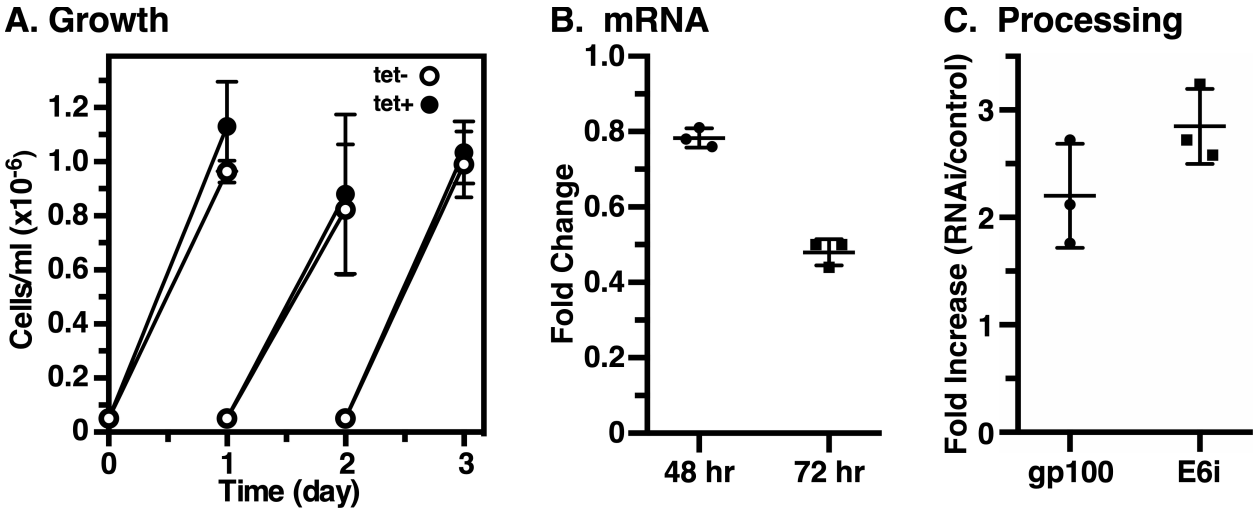

Figure S2. TbSTT3A knock down. **A.** The TbSTT3A RNAi cell line was cultured without (tet-) or with (tet+) tetracycline to initiate specific dsRNA synthesis. Cell density was monitored by microscopic counting, and cultures were adjusted to starting densities every day. Data presented as means ± std. dev. (n=3). **B.** TbSTT3A mRNA was quantified by qRT-PCR at the indicated times post induction (means ± std. dev., n=3). Signals are normalized to uninduced cells at each time point. **C.** The signals for gp100 and E6i in Fig. 4 and two identical biological replicates were quantified by phosphorimaging (means ± std. dev., n=3). In each case the signals of control (tet-, lanes 1 & 4) and silenced (tet+, lanes 7 & 10) were averaged as technical replicates within each experiment. The data are graphed as fold increase (tet+/tet-).

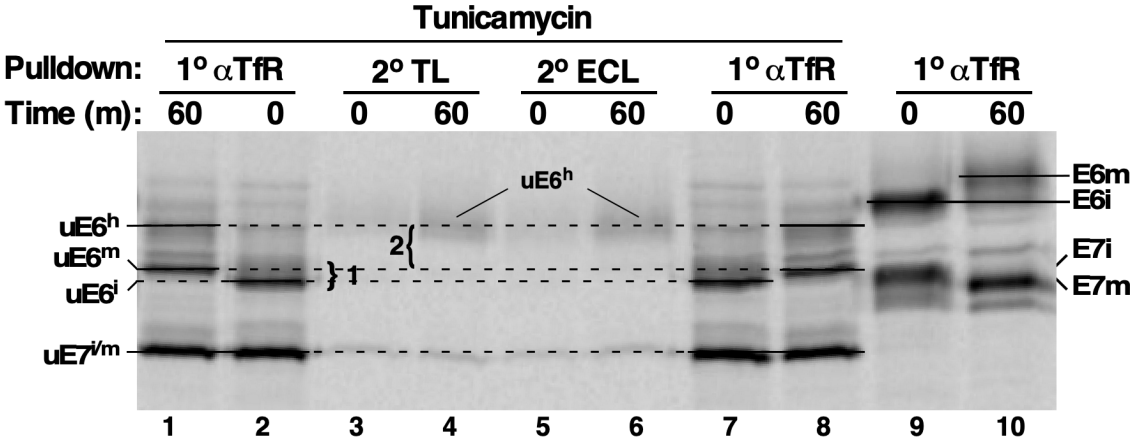

Figure S3. Identification of glycoforms in un-N-glycosylated TfR. The phosphorimage from Fig. 7 is presented. Individual species are identified as E6 or E7 followed by immature (i) or mature (m). Un-N-glycosylated species are indicated by the prefix 'u'. Major shifts are: 1) Increase in size of un-N-glycosylated E6 from immature ($uE6^{i}$, lanes 2 &7) to mature ($uE6^{m}$, lanes 1 & 8) glycoforms. This represents processing of the GPI glycan in the absence of N-glycans. 2) Increase in size of un-N-glycosylated E6 from mature ($uE6^{m}$, lanes 1 & 8) to the TL- and ELC-reactive hypermodified ($uE6^{h}$, lanes 4 & 6) glycoform. We presume that this represents extensive modification of a subset of mature un-N-glycosylated E6 by addition of poly-N-acetyllactosamine to the GPI glycan.