Supplementary Data

The fate of GPI-less procyclin and characterisation of sialylated non-GPI anchored surface coat molecules of procyclic form *Trypanosoma brucei*

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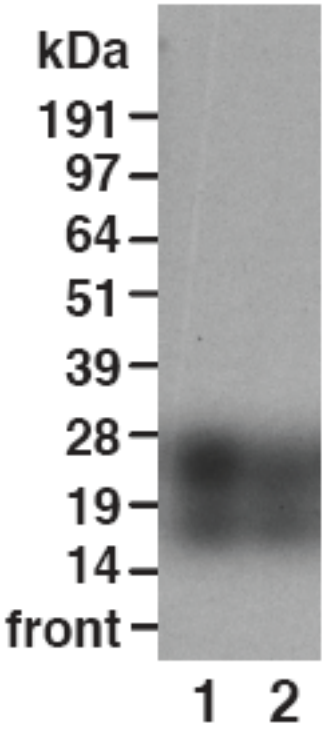

Figure S1: Procyclin precursor degradation is partially inhibited by FMK24. *TbGPI12* null cells ($5x10^6$) were pre-treated with 20 μM FMK024 (lane 1) or with DMSO control (lane 2) for 30 min at 28°C and metabolically labelled with 10 μCi/ml [^{14}C]proline for 20 h at 28 °C. The culture supernatants were immunoprecipitated with 5 μg anti-EP procyclin and 20 μg protein G-agarose (Pierce) for 2 h at 4°C, washed and subsequently analysed by SDS-PAGE and fluorography. Molecular weight standards are shown on the left.

Note the accumulation of the 26-28 kDa procyclin fragments (upper band) in the FMK24 treated cells (lane 1) relative to the control cells (lane 2).

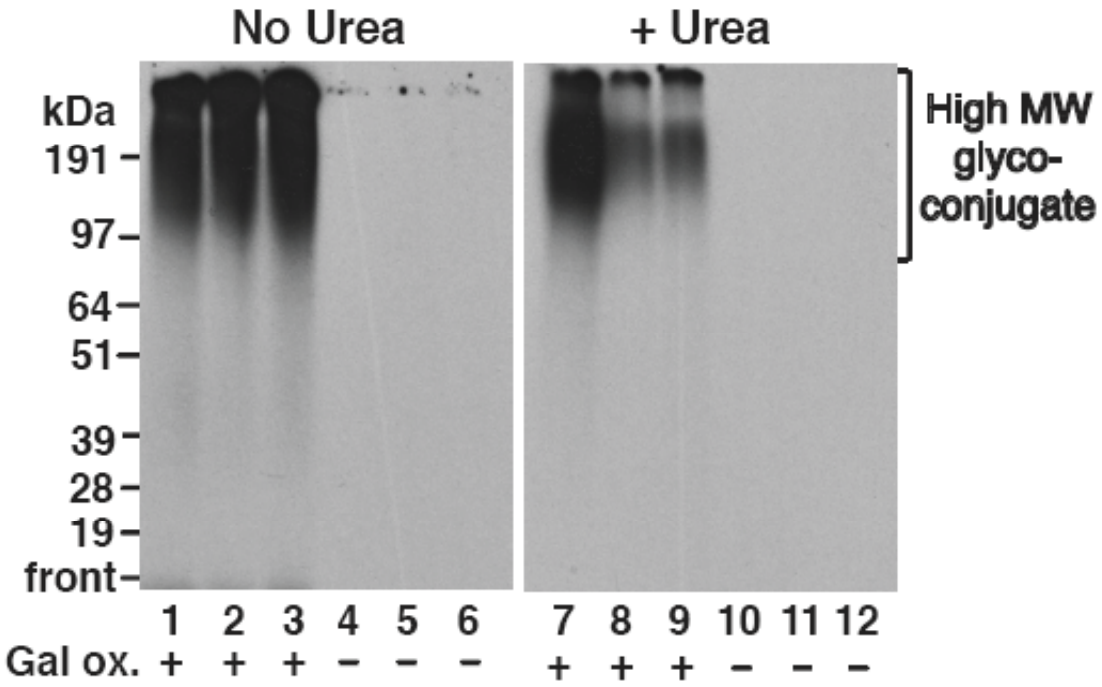

Figure S2: The appearance of the high MW glycoconjugate under different extraction conditions. *TbGPI12* null cells were labelled *in vivo* with NaB^3H_4 before (-) and after (+) treatment with galactose oxidase (Gal ox.), as described in Materials and Methods, lysed in SDS-sample buffer with 0.1M DTT with (lanes 7 to 12) or without (lanes 1 to 6) 4 M urea and heated to 100 ºC (lanes 1, 4, 7 and 10), 50 ºC (lanes 2, 5, 8 and 11) or 37ºC (lanes 3, 6, 9 and 12). The samples were analysed by SDS-PAGE and fluorography. Molecular weight standards are shown on the left.

Note (a) the absence of temperature effect for the extractions without urea (lanes 1-3), (b) the higher efficiency of extraction at 100ºC, versus 59ºC and 37ºC, in the presence of 4 M urea and (c) the greater dissociation of very high molecular weight material in the presence of 4 M urea (compare lanes 1-3 with lanes 7-9).