SUPPLEMENATARY FIGURES

FIGURE S1 Southern blot analysis of *T. brucei* genomic DNA, using *TbINO1* ORF as a probe. The genomic DNA was digested with several restriction enzymes and analysed by Southern blotting using a fluorescein labelled *INO1* ORF as the probe, as described in Experimental Procedures. Lane 1. *Nco*I, Lane 2. *Spe*I, Lane 3. *Afl*III/*Sac*II, Lane 4. *Eco*RV. Shown below the autoradiogram is a schematic of the predicted restriction enzyme cut sites within the *INO1* ORF and adjacent UTR's. NB. There was no restriction site predicted within the ORF or adjacent UTR's for *Spe*I.

FIGURE S2 Deamination of lipid samples from *in vivo* labelling. Cells were labelled with [^{3}H]-glucose (Lanes 1 and 2) or [^{3}H]-mannose (Lane 3), lipids extracted and an aliquot of these lipids analysed by HPTLC (Lane 1 and 3 respectively) as detailed in Experimental Procedures. Lipids from each labelling were also subjected to deamination and butanol water partitioning, the resulting lipid products from the [^{3}H]-glucose labelling sample were analysed by HPTLC (Lane 2). NB. As scintillation counting of the butanol and aqueous fractions from the deamination of lipids from the [^{3}H]-mannose labelling (see Supplementary Data Table 1) showed that there was no [^{3}H]-lipid reaction products present, therefore HPTLC of the butanol phase was not performed.

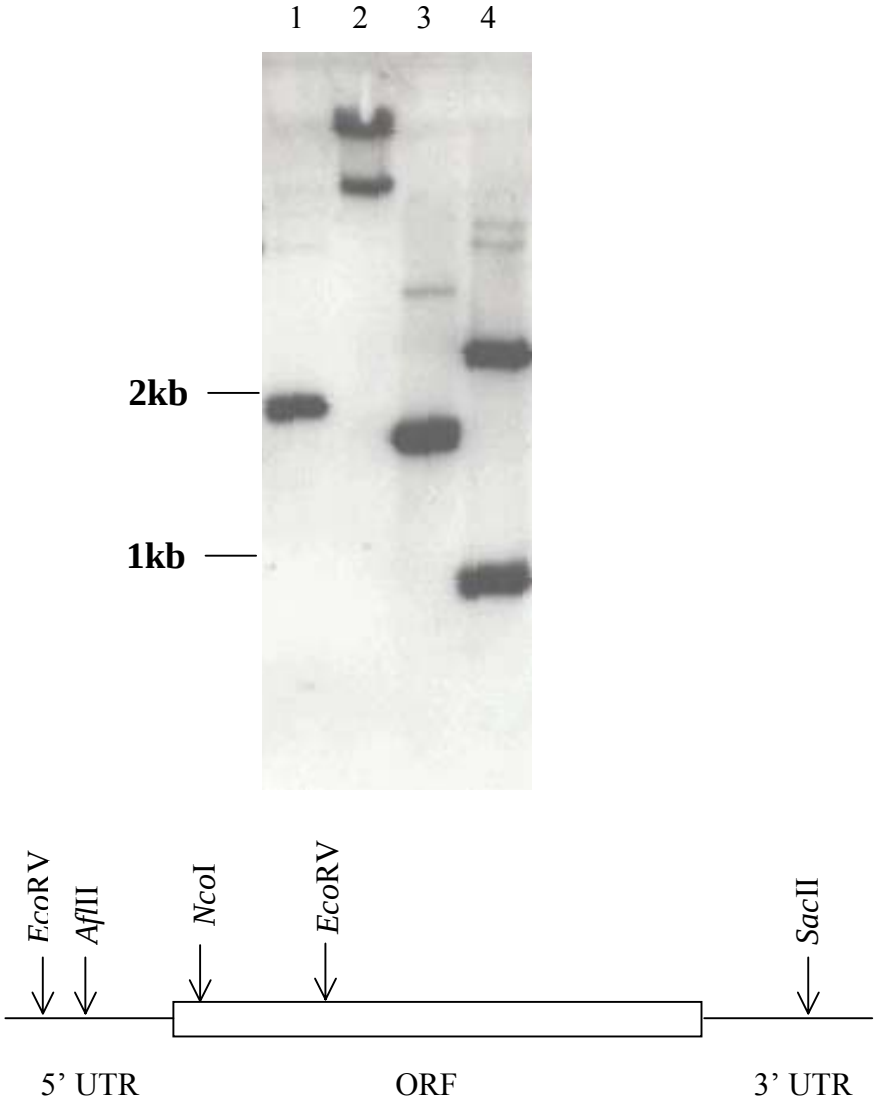
FIGURE S3 sVSG and deglycosylation analysis of samples from *in vivo* labelling. Panel A, cells were labelled with [^{3}H]-glucose and sVSG isolated. This sVSG was deglycosylated with PNGaseF, proteins were separated on SDS-PAGE and detected

by coomassie staining (Lanes 1 and 2) or fluorography (Lanes 3 and 4). Lanes 1 and 3 show undeglycosylated sVSG, Lanes 2 and 4 show deglycosylated sVSG. Panel B, wild type cells were labelled in the presence of tunicamycin and the proteins separated by SDS-PAGE. Detection was either by coomassie blue staining (Panel B) or by fluorography (Panel C). Lane 1, [^{3}H]-mannose pulse labelling, Lane 2 [^{3}H]-mannose pulse-chase labelling, Lane 3, [^{3}H]-glucose pulse labelling, Lane 4, [^{3}H]-glucose pulse-chase labelling

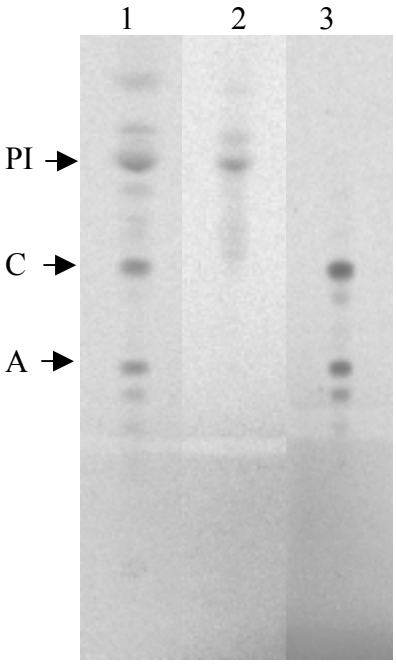
FIGURE S4 Analysis of exchange reaction with GPI-VSG as substrate. Cells were labelled with either [^{3}H]-mannose, [^{3}H]-glucose or [^{3}H]-myristate in the presence or absence of cycloheximide. Proteins were separated by SDS-PAGE, proteins were detected by coomassie blue staining (Lanes 1 to 6), labelled proteins detected by fluorography (Lanes 7 to 12). Lane 1 and 7, [^{3}H]-mannose labelling without cycloheximide; Lane 2 and 8, [^{3}H]-mannose labelling with cycloheximide; Lane 3 and 9, [^{3}H]-glucose labelling without cycloheximide; Lane 4 and 10, [^{3}H]-glucose labelling with cycloheximide; Lane 5 and 11 [^{3}H]-myristate labelling without cycloheximide; Lane 6 and 12, [^{3}H]-myristate labelling with cycloheximide.

	Deamination		Negative	
	Butanol	Aqueous	Butanol	aqueous
Mannose	7	93	94	6
Glucose	96	4	92	8

Table S1 Deamination of lipid samples from *in vivo* labelling. Cells were labelled with [^{3}H]-glucose or [^{3}H]-mannose, lipids extracted, deaminated and subjected to butanol water partitioning. [^{3}H]-Levels in the butanol and water phases were determined by scintillation spectrometry and are expressed as a percentage of the total cpm.

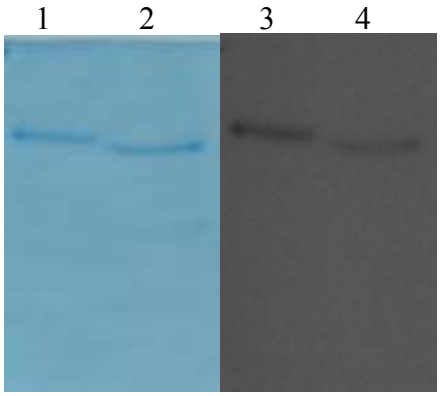


Supplementary Figure 1

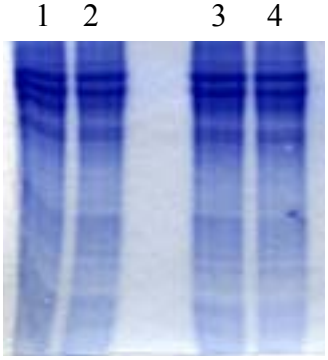


Supplementary Figure 2

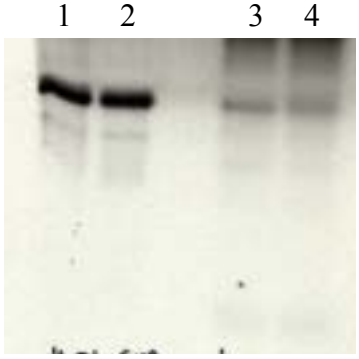
A)



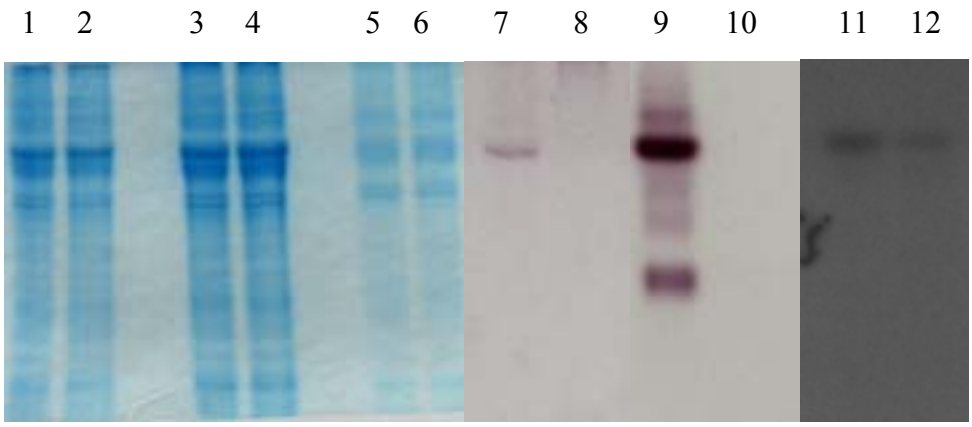
B)



C)



Supplementary Figure 3



Supplementary Figure 4