Figure S1

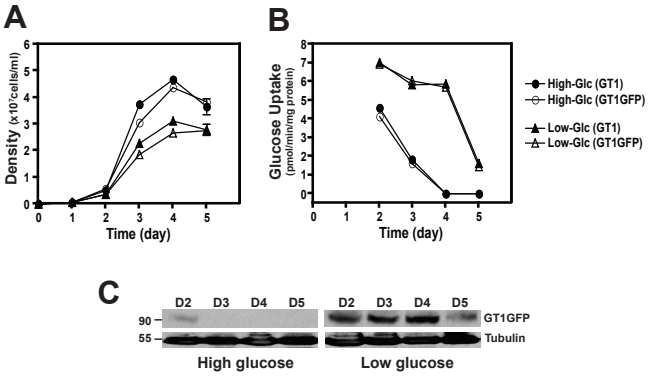

Figure S2

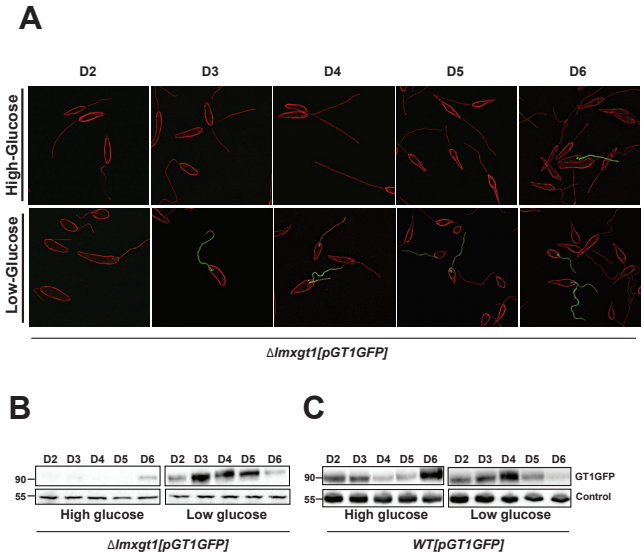

Figure S3

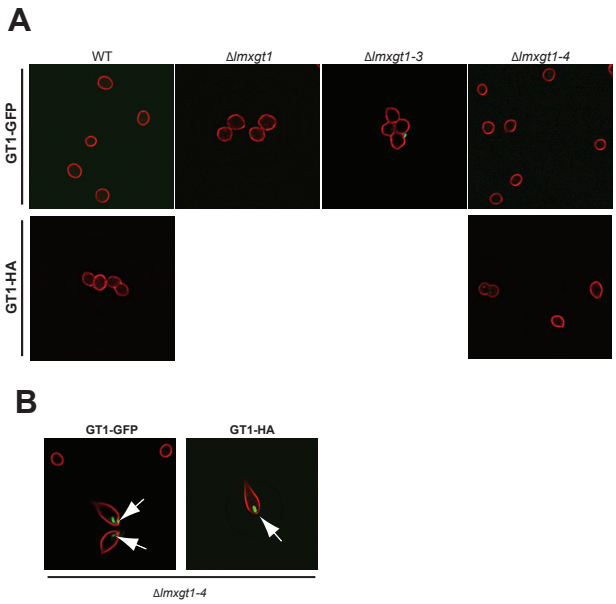

Figure S4

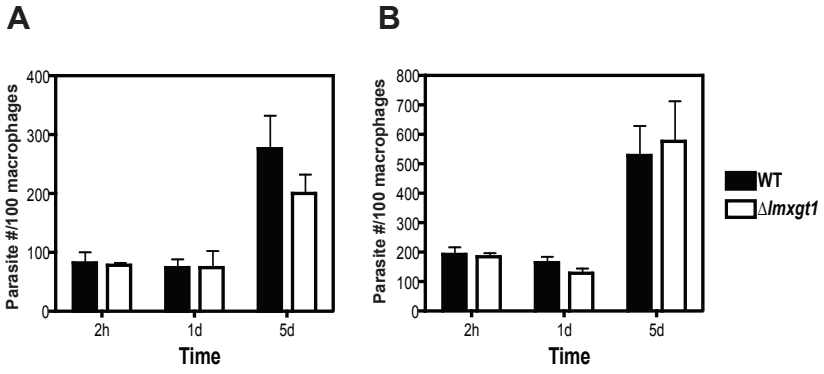

Fig. S1. Expression of untagged LmxGT1 and LmxGT1-GFP as a function of days in culture and in the presence of high or low glucose. Each *LmxGT1* gene was expressed in the Δ*lmxgt1-3* null mutant background. A) Cell density for each transgenic null mutant was quantified in triplicate samples with values plotted representing the average and standard deviations (error bars) for each sample. Parasites were inoculated at a density of 2 x 10^5 cells/ml and grown for 5 days in RPMI medium containing high (10 mM) or low (0.5 mM) initial glucose. B) The rate of uptake of 100 μM [^{3}H]D-glucose by each transgenic line as a function of days of growth and high or low glucose. C) Western blot of total lysates from Δ*lmxgt1-3*[p*GT1-GFP*] parasites probed with anti-GFP antibody. Samples were taken from day 2 to day 5 (D2-D5). The control band represents α-tubulin (Tubulin).

Fig. S2. Episomal expression of LmxGT1-GFP in Δ*lmxgt1* and wild type background. A) Immunofluorescence images of Δ*lmxgt1* null mutant parasites expressing LmxGT1-GFP. Parasites were inoculated at a density of 1 x 10^5 cells/ml and grown for 6 days in RPMI medium containing high (10 mM) or low (0.5 mM) initial glucose. Samples were withdrawn at days 2, 3, 4, 5, and 6 (D2-D6) for fixation and imaging by deconvolution microscopy. Promastigotes were stained with anti-α-tubulin and Alexa-594 conjugated antibodies (red) to visualize the subpellicular microtubule network. Green represents GFP fluorescence. B, C) Western blot of total cell lysates from Δ*lmxgt1[pGT1-GFP]* (B) or WT[*pGT1-GFP*] (C) promastigotes growing in high and low glucose. Western blots were probed with anti-GFP antibody. The Control bands in the lower panel represent α-tubulin.

Fig. S3. Expression of LmxGT1-GFP and LmxGT1-HA is not detected by immunofluorescence miscroscopy in axenic amastigotes. A) Immunofluorescence images of wild type (WT) and several null mutant parasites, expressing LmxGT1-GFP (WT, *Δlmxgt1, Δlmxgt1-3* and *Δlmxgt1-4*) or LmxGT1-HA (WT and *Δlmxgt1-4*) from an episomal expression vector. Images were taken by deconvolution microscopy. Axenic amastigotes were stained with anti-α-tubulin and Alexa-594 conjugated antibodies (red) to visualize the subpellicular microtubule network. Green represents GFP fluorescence in cell lines expressing LmxGT1-GFP. Cell lines expressing LmxGT1-HA were stained with anti-HA and Alexa-488 conjugated antibodies (green) to visualize the HA-tagged protein. B) Detection of LmxGT1-GFP and LmxGT1-HA in parasites that had not transformed into amastigotes. Cultures of axenic amastigotes typically contain a small percentage of parasites that have a body shape similar to promastigotes but do not have an extended flagellum. These parasites likely have not transformed completely into axenic amastigotes and often exhibit fluroescence from LmxGT1-GFP or LmxGT1-HA in the region of the flagellar pocket. Fusion proteins were expressed in the *Δlmxgt1-4* background

Fig. S4. Macrophage infection by wild type and *Δlmxgt1* promastigotes that had been pasaged through mice. Primary peritoneal macrophages from BALB/c mice were infected with stationary-phase promastigotes at a multiplicity of 2 (A) or 5 (B) and incubated in Dulbecco's modified Eagle Medium at 37°C for 2 h, 1 day or 5 days, as described in the Material and Methods section. Data represent the average and range of two independent experiments. Data are plotted as number of parasites per 100 macrophages. Wild type (WT) is shown as black bars and *Δlmxgt1* parasites as white bars.