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**Supporting Information to**  
**The *de novo* and salvage pathways of GDP-mannose**  
**biosynthesis are both sufficient for the growth of**  
**bloodstream form *Trypanosoma brucei***

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16 **Supplementary figures:**

17 **Figure legends**

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19 **Fig. S1.** Sequence alignment of PMI shows the conserved amino acids between the  
20 different organisms. The *pmi* sequence of *Trypanosoma brucei* is compared with other  
21 protozoans *Trypanosoma cruzi* and *Leishmania donovani*, with yeasts (*Saccharomyces*  
22 *cerevisiae* and *Candida albicans*) and with mammals (*Homo sapiens*).

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24 **Fig. S2.** RT-PCR. *TbPMI* is transcribed in both the procyclic and bloodstream form life  
25 cycle stages of the *T.brucei*. The RT-PCR was performed using *TbPMI* primers, DPMS  
26 (Dolichyl-phosphate-mannose synthetase) primers as a positive control and DPMS  
27 primers without reverse transcriptase as a negative control.

28 (A) RT-PCR product of procyclic RNA, used in different amounts. (B) RT-PCR product  
29 of bloodstream RNA, used in different amounts. (1) Procyclic negative control. (2)  
30 Procyclic positive control. (3) Bloodstream negative control. (4) Bloodstream positive  
31 control.

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33 **Fig. S3.** The optimum pH for *TbPMI* activity was determined by performing reactions  
34 over a range of pH values from pH 5.0 to pH 10. The reactions were performed in  
35 triplicate for each pH value. *TbPMI* was found to have a pH optimum between pH 7.3  
36 and 8.5.

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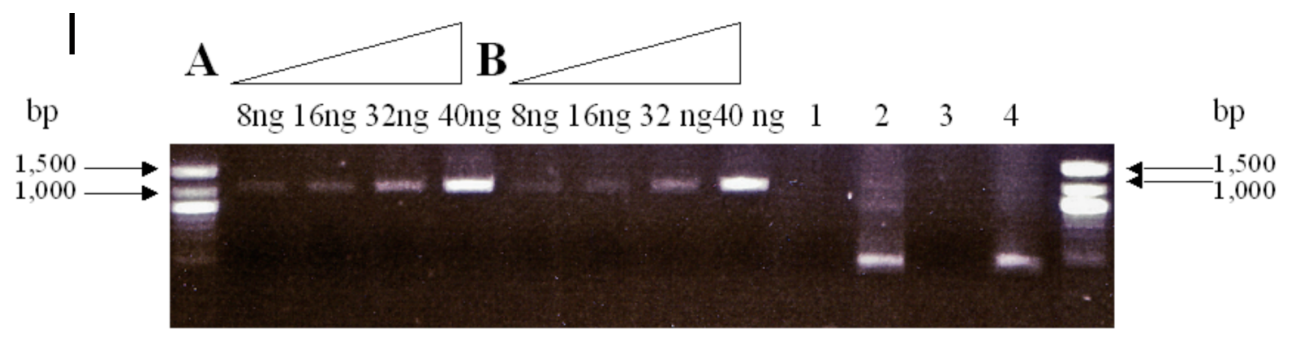
39 **Fig. S4.** Gas chromatography-mass spectrometry (GC-MS). Monosaccharides released  
40 from VSG-glycoconjugates by acid hydrolysis (4 M TFA, 100°C, 4 hours) were TMS-  
41 derivatized and then analyzed by GC-MS. The ions 204 and 206 corresponding to <sup>12</sup>C-  
42 and <sup>13</sup>C-hexoses, respectively, were extracted from the GC-MS total ion chromatogram of  
43 acid hydrolyzed, TMS-derivatized (A) mannose and galactose standards (1 nmol each) or  
44 VSG (10 µg) isolated from wild type *T.brucei* bloodstream cells grown in (B) 5 mM <sup>13</sup>C-  
45 glucose, (C) 5 mM <sup>13</sup>C-glucose and 55 µM <sup>12</sup>C-mannose, or (D) 5 mM <sup>13</sup>C-glucose and  
46 1.5 mM <sup>12</sup>C-mannose.

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48 **Fig. S1:**

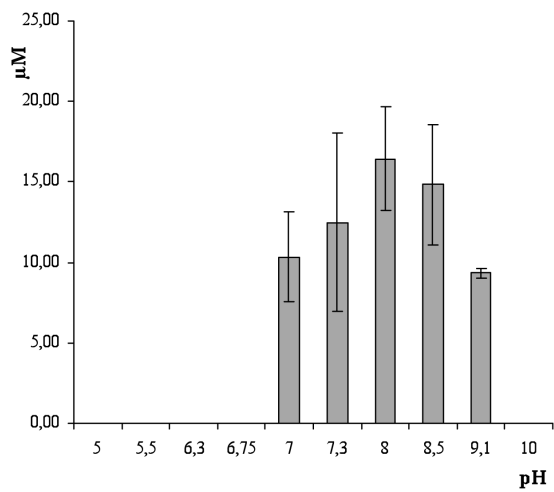
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51 **Fig. S2:**



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53 Fig. S3:

T.brucei	1	--MSKELIKLDCGVQHYANGKEAAEYVAKMKGEGNKE-----GKYAELWGWTHMPCSKTFSG--QNLDDFLKNDNMMSRFVHPKQADPRFRDTPVFLKLLSVQTALSIOAHPNK109	
T.cruzi	1	--MVGKLVKLDGCVQHYDNGKVPAESFVAKMKHETBGT-----RKYAEWGWTHMPCSKRLLSG--QLLGEYLRDPGIANSFFSPEHQSNVELRNNVPFLKLVSVQTALSIOAHPNKK109	
L.donovani	1	--MSELVKLEVBHDIYANGKDAASSFVAKMKGLTNDKS---GKMFRELWGWTHMPCSRDIADGDAQLLEEFLKOPENKMKKYVFEAHOATT--FRDTPVYLLKILSIRITALSIOAHPCKK112	
C.albicans	1	MSSEKLFRIQCGYQNDYDNGKIGSSSAVAQFVHNSDPSITIDEKPYAELWGWTHMPCSKAIDLNN--QTLRDLVTAKPOEYLGESIIITKFGSSKELPFLFKVLSIEKVLVLSIOAHPDKK117	
S.cerevisiae	1	MSN-KLFRIDAGYQYDNGKIGSSSAVAQFAAHSDPVQIQEQDKPYAELWGWTHMPCSKMPSYNHESK---ESLRDIISKNPASMLGKDIIDKFHATNELPFLFKVLSIEKVLVLSIOAHPDKA115	
H.sapiens	1	MAAPRVFPLSGAVQQYANGKMGNSSEVARLLASSDPLAQIAEDKPYAELWGWTHMPCSKAIDLNNRISOKTLSOWIAEN-QDSLGSVKYDFTNGN--LPFLFKVLSVETPLSIOAHPNKE116	
T.brucei	110	LAEKLRHREPEKYKDPNHHKPELVVALTPFEALCCFRPLKDI LEFL ESASP LKTL LGPAADV-----LPGEVEDSEAIKHMDIIVYNTDAKKHAEIQEHAEELRSRGGEMT215	
T.cruzi	110	LAEKLRHRENSKYKDPNHHKPELIVALTPFEALCCFRPLQEI LSLVSTKPLKTL LGNIAVV-----SANES-DGDLIEGMMRVLYSLNPEVHTKALREHAAAVSAKGEAAS214	
L.donovani	113	LAEKLHAARPDKYKDPNHHKPELICALTPFEALCCFRPLGAI IAYLKRIPELAEVLGADAVLGQYM-MAPESALPATDSDEEKQSLKAMITNYVAAASDDIVTKALRHLQRIEETG-AQC229	
C.albicans	118	LGAQLHAADPKNYDDNHHKPEMAIAVTD FEGFCGFKPLDQAKTLATVPELNEIIGOE LVDEFISGIKLPAEVGSODDVNNRKL LQKVFGLMNTDDVIVKQATAKLLERTDREPQVFK236	
S.cerevisiae	116	LGIKILHAQDPKNYPDDNHHKPEMAIAVTD FEGFCGFKPLQEI LAELKRIPELRNIIVGEE TSRNF IENIQPSAQKGSPEDEQNKLLQAVSRVMNASDDKIKIQARS LVERSKNPSDFN234	
H.sapiens	117	LAEKLHLQAPQHYPDANHHKPEMAIALTPFQGLCGFRVVEIIVTF LKKVPEFQFLIGDEATHLKTQMSHDSQAVSS-----LQSCFSHLMKSEKVVVEQLNLLVKRISQAAAAGN228	
T.brucei	216	KED---SVFLRVLYQYDDDMGQWM-VYFLNYVQLAPGEGFLADSEPHAYLFGDSVEIMACSDNVVRAGLTPKWKDVPTLLRMLRYGTDGLERAKFERYRAPEGSEWELQHYSPREF329	
T.cruzi	215	TED---RFLRLMSQYRDDIGQWM-LYFLNYVQMPGQGLFLSDSEPHAYLFGDVEIMANSNDNVVRAGLTPKWKDVPTLLEMLHYNTGLERAKFERYRAAEAGEAWEVQYRSPRPF328	
L.donovani	230	AED---ELFVRIYROYPPDDVGGWM-VYFLNYVQMPGQGLFLSDSEPHAYIIGDVEIMACSDNVVRAGLTPKWKDVPTLISMLKYDTGLASARHEKKSSEDAQWQVQYRPPAQF343	
C.albicans	237	DISRLPEL IORLNKGFNDI GLFCGCLLNHVGLNKGEMFLQAKDPHAYISDIIIECMAASDNVVRAGFTPKFKDVKNLVEMLTYSYSEVQKQMPLOEFPRSSKGDVAVKSVLYDPP1355	
S.cerevisiae	235	KPD--LPEL IORLNKGFDDVGLFCGCLLNHCRLNAGEAIFLRAKDPHAYISDIMECMAASDNVVRAGFTPKFKDVKNLVSMLTYTDPVEKQKMQPLKFDRSSGNG-KSVLYNPP1350	
H.sapiens	229	NMEDI FGE LLLQLHOGYFGDIGGFA-IYFLNLLTLKPGEMFL EANVPHAYLKGDCVECMACSDNTVRAGLTPKFI DVP T LCEMLSYTPSSSKDRLFLP---TRSQEDP-YLSIYDPPV342	
T.brucei	330	QDFSLYRIEHRVER-OGQTHIKLPTVGLGFCVECCGIVNGERVRLGECFLVPYGD LKIEAFG-DFOIFVASMNYSLHSASHM-----	409
T.cruzi	329	TEFSLYRLEHVAQK-ASETCVNLPI NLGLGFC LGEGVVNGTHVAEGDCFAVPHGLKCESNG-RCVLVFASTNDGLPEOAHI-----	408
L.donovani	344	PDFSLYRMOYEHASGKGTTSVTPTI GLGFCLESAKVNQTTVNAQDCFAVPYGVYTCOAE GAKALVFASTND-LSDR-----	421
C.albicans	356	AEFSVLQTI FDKSKGKQVIEELNGPSIVIAITNGKGTIOITGDDSTKQKIDTGYVFFVAPGSSIELTADSDNODDFTTYRAFVEA-	441
S.cerevisiae	351	EEFVLETTTFDEKLG-QRHFEQVDPGSI LIITTKNGYIKADGQ---KLLAEFGVFFIAP---HLVPDLEAEDEAF TTYRAFVEPN	429
H.sapiens	343	PDFTIMKTEVPGSVT-EYKVLAD SASILLMVGQ-TVIASPTTQTPIPLQRGGVLFIGANESVSLKLT---EPKDLLIFRACCLL-	423

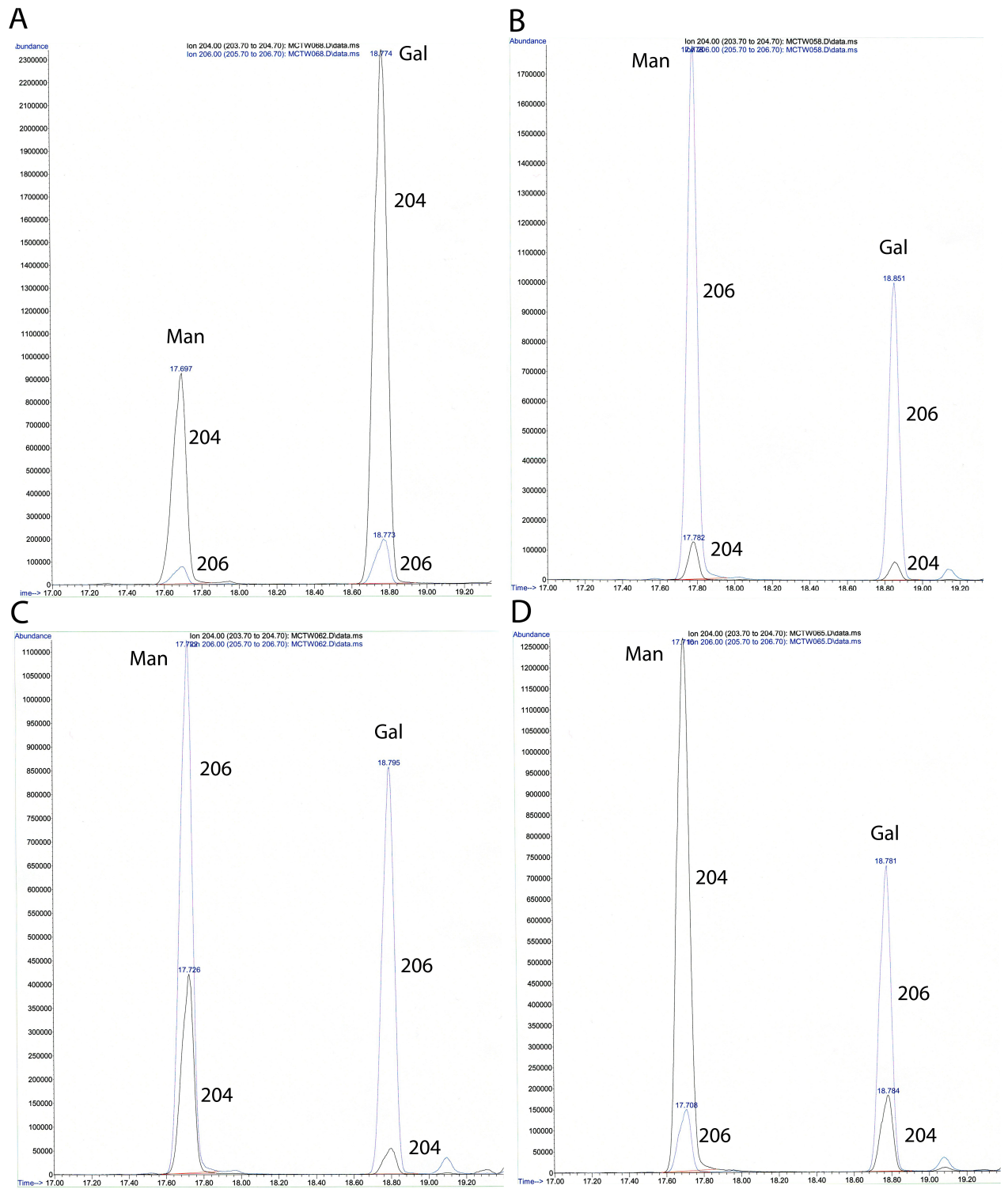
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56 Fig. S4:

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