April 20 2017

Supplemental information for **"Genetic Metabolic Complementation Establishes a Requirement for GDP-Fucose in *Leishmania*",** by Hongjie Guo, Natalia M. Novozhilova, Giulia Bandini, Salvatore J. Turco, Michael A. J. Ferguson, and Stephen M. Beverley

SUPPLEMENTAL FIGURES

Figure S1. PCR confirmation drug marker replacements within *A/FKP80* single gene knockouts. Panel A shows the map of the planned *AFKP80::HYG* and *AFKP80::PAC* replacements, and **Panel C** shows the map of the planned *FKP40::BSD* and *FKP40::SAT* replacements. The large grey boxes depict the *HYG*, *PAC*, *BSD* and *SAT* ORFs, the narrow flanking grey and white bars depict regions conserved between *A/FKP*s on the 5' and 3' sides respectively, and the narrow black bar depicts the extent of the targeting fragments. The analysis of the *A/FKP* coding regions is shown in Figs. 2B, C. **Panel B**. PCR confirmation of planned integration of drug resistant markers in a representative Δ*afkp80*$^-$ mutant. Primers outside the 5'- and 3'-flanking regions used for gene replacement and primers inside drug marker ORFs were used for PCR to confirm the integration of the drug marker ORFs into the chromosome. Primers a/e and d/f establish the left (5') and right side (3') of *PAC* replacement (***ℓ*** *PAC*/*r PAC*), and primers a/b and d/c establish the left (5') and right side (3') of *HYG* replacement (***ℓ*** *HYG*/*r HYG*). Primer a, SMB2796; e, SMB 2889; f, SMB2888; b, SMB2566; c, SMB2565; d, SMB2793. **Panel D**. PCR confirmation of planned integration of drug resistant markers in a representative Δ*fkp40*$^-$ mutant. Primers g/l and k/j establish the left (5') and right side (3') of *SAT* replacement (***ℓ*** *SAT/r SAT*), and primers g/h and i/j establish the left (5') and right side (3') of *BSD* replacement (***ℓ*** *BSD/r BSD*). Primer g, SMB2783; l, SMB 2768; k, SMB2769; h, SMB2555; i, SMB2556; j, SMB2784.

Figure S2. The size of LPG in logarithmic and stationary phase is unchanged in the arabinose-deficient Δ*afkp80*$^-$ mutant. A western blot with antibody WIC79.3 of LPG purified

from logarithmic or stationary growth phases from both WT and Δ*afkp80*$^{-}$ is shown. WIC79.3 recognizes Gal-modified LPG (66).

Figure S3. Attempts to generate *A/FKPs* double mutants by successive rounds of replacement yielded 4 successful replacements but retention of *FKP40*. Panel A. Planned disruption of *FKP40* by homologous gene replacement by methods depicted in Fig. 2, S1. In a third round of gene replacement, the Δ*afkp80*$^{-}$ mutant was transfected with *FKP40::BSD*. PCR tests (not shown) confirmed this was successful, yielding a line lacking 3 of the 4 *A/FKP* alleles (Δ*afkp80*$^{-}$/*FKP40::BSD*/*FKP40*). In a fourth round, this line was transfected with the *FKP40::SAT* targeting fragment, with the expectation of yielding homozygous *A/FKP* double mutants. **Panel B**. PCR tests established that this line lacked *AFKP80* (lanes labeled *r AFKP*), but retained *FKP40* (*r FKP* lanes*)* despite the presence of the planned *FKP40::BSD* and *FKP40::SA*T replacements (lanes labeled ***ℓ*** *BSD*, *r BSD*, ***ℓ*** *SAT* and *r SAT*). See Fig. S1 for location of primers used in PCR tests and not shown are data for the *AFKP80* replacements (Figs. 2, S1).

Supplemental Table.

Table S1. Oligonucleotides used in this work.

Primer	Sequence
SMB2446	AGCTGCGCGACGCTGAGTTCCTGCACTA
SMB2450	GATTGAGCAGATGCTGACTGCTGGT
SMB2451	TACATGTACATGTGCGCGAAGGACGAG
SMB2453	GACAGGAACGACCTCTTC
SMB2555	**GGTAACGGTGCGGGCTGACG**CCACCATGGGCCAAGCCTTTGTCTCA
SMB2556	**CGAGATCCCACGTAAGGTGC**TTAGCCCTCCCACACATAACCAGAG
SMB2557	**GGTAACGGTGCGGGCTGACG**CCACCATGACCGAGTACAAGCCC
SMB2558	**CGAGATCCCACGTAAGGTGC**TCAGGCACCGGGCTTGCG
SMB2561	**GGTAACGGTGCGGGCTGACG**CCACCATGAAAAGCCTGAACTC
SMB2562	**CGAGATCCCACGTAAGGTGC**CTATTCCTTTGCCCTCG
SMB2565	AATACGAGGTCGCCAACATC
SMB2566	GAAAGCACGAGATTCTTCGC
SMB2630	**GGTAACGGTGCGGGCTGACG**CCACCATGAAGATTTCGGTGATCC
SMB2631	**CGAGATCCCACGTAAGGTGC**TTAGGCGTCATCCTGTGCTCC
SMB2664	CATGCCATGGGGCGAAACAGCGACCACA
SMB2665	**CGTCAGCCCGCACCGTTACC**CGTGGCACACGGCTCTGTG
SMB2666	**GCACCTTACGTGGGATCTCG**AGAGGCGTGCGGCGGTGTG
SMB2667	GGGAATTCCATATGCGCCTCAAGCTCTACCCACC
SMB2668	CATGCCATGGCATCCCTGCCGAACGCCGAACC
SMB2669	**CGTCAGCCCGCACCGTTACC**GGTTGCAGGGGAGGCTGAAGA
SMB2670	**GCACCTTACGTGGGATCTCG**TCTTAGAGGCGTGCGGCGGT
SMB2671	GGGAATTCCATATGGAGGTGCGATGCGGTGTTCGGGGTT
SMB2768	CTTGGTCGGATAGGTGCAC
SMB2769	AGCGATGTACTGGTACTGG
SMB2783	CATAGCAGCTGAGCAGCAG
SMB2784	TGGTGCGACGCAGCCGTAG
SMB2793	AGATGAGGAACCTAGAGAG
SMB2796	CCCGTATTCCTTGTCCTTTCATGGTACC
SMB2828	TCCCCCCGGGCCACCATGAACGTGAGCTTTCTCTTCAGCC
SMB2829	TCCCCCCGGGCTAAGACCGGCTGATCTGGAGGCCGGACG
SMB2830	GGAGCTGATGCGATGCTCTCC
SMB2889	ACCGTGGGCTTGTACTCGG
SMB2890	ATTGTTCAGGCTTCAAGCGACC
SMB3176	CAGCAGCCCTCTATATACCCGC
SMB3448	TCCCCCCGGGCCACCATGGAACAAAAACTCATCTCAGAAGAGGATCT GTCAGCACGTCGACTGGC
SMB3449	TCCCCCCGGGTATTGCCCCGCTGCTCAACAC
SMB3450	GGAAGATCTCCACCATGTACCCATACGATGTTCCAGATTACGCTTTAG GGTCCCTACCGAGT
SMB3451	GGAAGATCTTCACTTCCGTGCGACATCGTAGTT

SMB3522	CCAAGAGGTGTCTAATCGCTGC
SMB3523	GACTGGACAGTCAACGATCCGC
SMB3524	GCGGATCGTTGACTGTCCAGTC
SMB3525	GAAGAAGCAGCTCATCAAGTGG
SMB3526	CCACTTGATGAGCTGCTTCTTC
SMB3527	ACGCACCCTCTCTAGGTTCC
SMB3752	AACTGCTGCTGCAGCAGCGCAACC

Restriction sites are underlined and linker sequences are in bold.