SUPPLEMENTARY INFORMATION

TABLE S1. Strains and plasmids used in this study

Name	Description	Reference/source
Strains		
H37Rv	Virulent reference laboratory strain; ATCC 25618	London School of Hygiene and Tropical Medicine
Δ*mutAB*	*mutAB* deletion mutant of H37Rv, lacking 2342 bp internal *Asc*I / *Bgl*II fragment	This study
Δ*prpDC*	*prpDC* deletion mutant of H37Rv, lacking the 2660 bp region from the start codon of *prpD* to the stop codon of *prpC* (2)	This study
Δ*mutAB*::*mutAB*	Reversion mutant of Δ*mutAB* in which the wild type *mutAB* allele was restored by knock-in mutagenesis	This study
Δ*prpDC*::*prpDC*	*prpDC* mutant carrying a 3115 bp gene region containing the *prpDC* locus in pPRPDC (2) integrated at the *attB* locus; Km^R	This study
Plasmids		
p2NIL	Cloning vector; Km^R	(3)
pGOAL17	Plasmid carrying *lacZ* and *sacB* genes as a *Pac*I cassette; Ap^R	(3)
p2*mutAB*	p2NIL carrying 7760 bp *Eco*RI fragment excised from the H37Rv cosmid Rv58 (1) and containing the *mutAB* genes plus 1431 bp of 3'- and 2228 bp of 5'-flanking chromosomal sequence; Km^R	This study
p2Δ*mutAB*	p2NIL carrying Δ*mutAB* allele generated by digestion of p2*mutAB* with *AscI* and *Bgl*II and re-ligation to eliminate 2342 bp of sequence within the *mutAB* region; Km^R	This study
p2Δ*mutAB*17	Δ*mutAB* knockout vector – p2Δ*mutAB* containing *Pac*I cassette from pGOAL17; Km^R Suc^S	This study
p2*mutAB*17	*mutAB* knock-in vector – p2*mutAB* containing *Pac*I cassette from pGOAL17; Km^R Suc^S	This study
pAU100	pJG1111 carrying Δ*prpDC* allele – fusion of 1kb PCR products upstream and downstream of *prpDC* eliminating 2660bp *prpDC* coding sequence; Km^R Hyg^R	(2)
pPRPDC	pMV306 (4) carrying a 3115 bp *Eco*RI / *Nco*I fragment containing *prpDC*; Km^R	(2)

TABLE S2. Oligonucleotides used in this study

Name	Sequence (5'-3')[a]	Application	Properties
MutA-F	*GGGGTAC*CGAAGCAAGCCCAAGCA	Forward primer used for PCR genotyping of *mutAB* and Δ*mutAB* alleles	2084 bp amplicon generated from the wild type *mutAB* allele using MutA-F and MutA-R primers
MutA-R	*GGACTA*GTCAATGCCTTCCGGCGT	Reverse primer used for PCR-based genotyping of the *mutAB* allele	
MutB-R	*GGAAG*CTTGCAGGCGCTGGCGA	Reverse primer used for PCR-based genotyping of the Δ*mutAB* allele	1933 bp amplicon generated from the Δ*mutAB* allele using MutA-F and MutB-R primers
MutAB-F	AGA TCCGTACCATTTCCGGG	Forward and reverse primer pair used to generate the DNA probe used for genotypic confirmation of the Δ*mutAB* mutant by Southern blot analysis	904 bp amplicon spanning from the 3'-end of *mutB* to within Rv1495 (see Fig. S1 in the Supplementary Information)
MutAB-R	TTG TTGGAGACGATGAGCCA		
PrpDC-F	*GGGGG*CTGCTCTGCGGCACGGTG	Forward primer used for PCR-based genotyping of the *prpDC* and Δ*prpDC* alleles	1073 bp amplicon generated from wild type *prpDC* allele using PrpDC-F and PrpDC-R
PrpDC-R	*GGGGG*ATCTTGTAGGCCATGTGCTC	Reverse primer used for PCR genotyping of *prpDC* allele	
PrpDC-R2	*GGGGG*TACAACAGGATCTTGGCGAC	Reverse primer used for PCR genotyping of Δ*prpDC* allele.	1195bp amplicon from Δ*prpDC* allele using PrpDC-F and PrpDC-R2

a. GC-clamp sequences (non-H37Rv) are italicized.

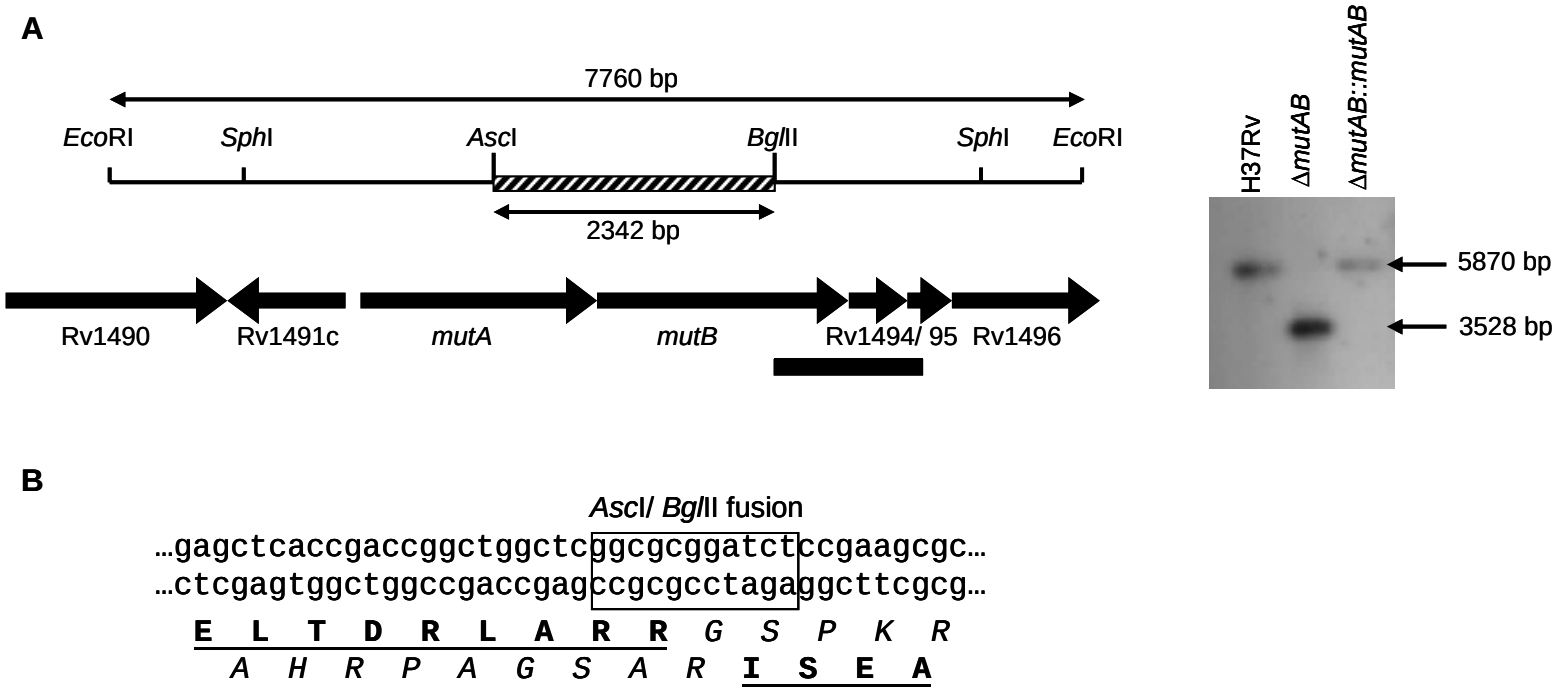

FIG. S1. Construction and genotypic characterization of the Δ*mutAB* mutant of MTB H37Rv and its reverted (knock-in) derivative (Δ*mutAB*::*mutAB*). **A.** The *mutAB* and flanking genes are shown as solid arrows (not to scale). The line drawing above shows the 2342-bp segment internal to *mutAB* that was deleted in the Δ*mutAB* mutant strain (hatched box). For the Southern blot analysis (right), genomic DNA from the wild type, Δ*mutAB* and reverted (Δ*mutAB*::*mutAB*) strains was digested with *Sph*I which cuts on either side of the deleted region to produce a 5870-bp fragment from H37Rv and Δ*mutAB*::*mutAB* and a 3528-bp fragment from Δ*mutAB* which were detected using the PCR-generated probe denoted by a grey box. **B.** Sequence at the junction between the truncated *mutA* and *mutB* genes in the Δ*mutAB* mutation. The DNA sequence is shown in lowercase and the fusion between the *Asc*I and *Bgl*II sites is boxed. The encoded amino acid sequences are shown in uppercase. The first (top) reading frame shows the sequence of MutA (bold underlined), which terminates within the junction site at Arg402. The third reading frame (bottom) shows the sequence of MutB (bold underlined) which starts from Ile567 within the junction site. Spurious fused sequences are shown in uppercase italics.

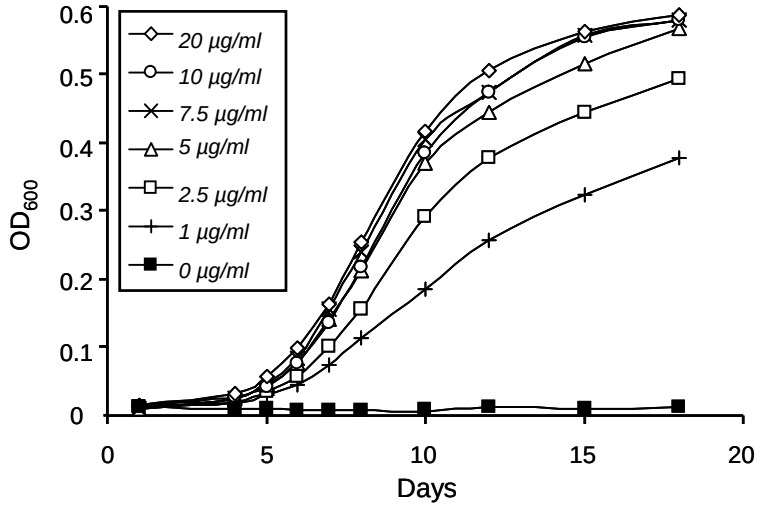

FIG. S2. Effect of vitamin B_{12} (cyanocobalamin) concentration on growth of the Δ*prpDC* mutant on propionate

References

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3. **Parish, T., and N. G. Stoker.** 2000. Use of a flexible cassette method to generate a double unmarked *Mycobacterium tuberculosis tlyA plcABC* mutant by gene replacement. Microbiology **146:**1969-1975.
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