Supplementary Information

bis-Molybdopterin guanine dinucleotide is required for persistence of *Mycobacterium tuberculosis* in guinea pigs

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Running Head: Molybdenum cofactor in *M. tuberculosis*

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Table S1: Strains, plasmids and primers used in this study

Name	Description	Source/ reference
Strains		
M. tuberculosis		
H37Rv	ATCC 25618; virulent laboratory strain	Laboratory stock
Δ*mobA*	Derivative of H37Rv carrying an unmarked deletion of the *mobA* gene;	This work
Δ*mobA attB*::pMobA	Derivative of Δ*mobA* carrying the *mobA* gene integrated at the *attB* locus; Hyg^r	This work
Plasmids		
p2NIL	Cloning vector; Km^r	[1]
pGOAL17	Plasmid carrying *lacZ* and *sacB* genes as a *Pac*I cassette; Ap^r	[1]
p2mobA	Knockout vector for creating unmarked deletion in *mobA* constructed by cloning PCR-amplified upstream and downstream flanks of *mobA* in p2NIL and insertion of *lacZ-sacB* cassette from pGOAL17; Km^r	This work
pMV306H	*E. coli-Mycobacterium* integrating shuttle vector. Derivative of pMV306 [2] carrying a *hyg* gene; Hyg^r	H. Boshoff
pMobA	Derivative of pMV306H containing the *mobA* gene fused to the 300bp region upstream of *Rv2455c*	This work
Oligonucleotides		
TmobUF: gcaagcttCGACCATATGCTCCACCAG	For amplification of the 2212 bp of upstream homologous sequence and 136 bp of the 5''-end of *mobA*	This work
TmobUR: cgaattcCGAACTGTCCAAGCACAAGA		This work
TmobDF: cggtaccTCGTTAGAAGCGGTGCTC	For amplification of the 59 bp of the 3'-end of *mobA* and 2040 bp of downstream sequence	This work
TmobDR: gcaagcttCGCTGACCAACGTCAAT		This work
TmobF:cggtaccTATCTCGAAAGGCCACCAG	For amplification of the 10 bp upstream *mobA* to 39 bases downstream of *mobA*	This work
TmobR: GTCGACTGACGTGGCTGAG		This work
promF: ctcagccacgtcagtcgacCTCCGCCTTCCATGTGTTAT	For amplification of the 285bp region upstream of *Rv2455c*	This work
promR: cggtaccAAACCGTGGATTCGGATGT		This work

Hyg^r – Hygromycin resistant; Km^r – Kanamycin resistant; Ap^r – Ampicillin resistant

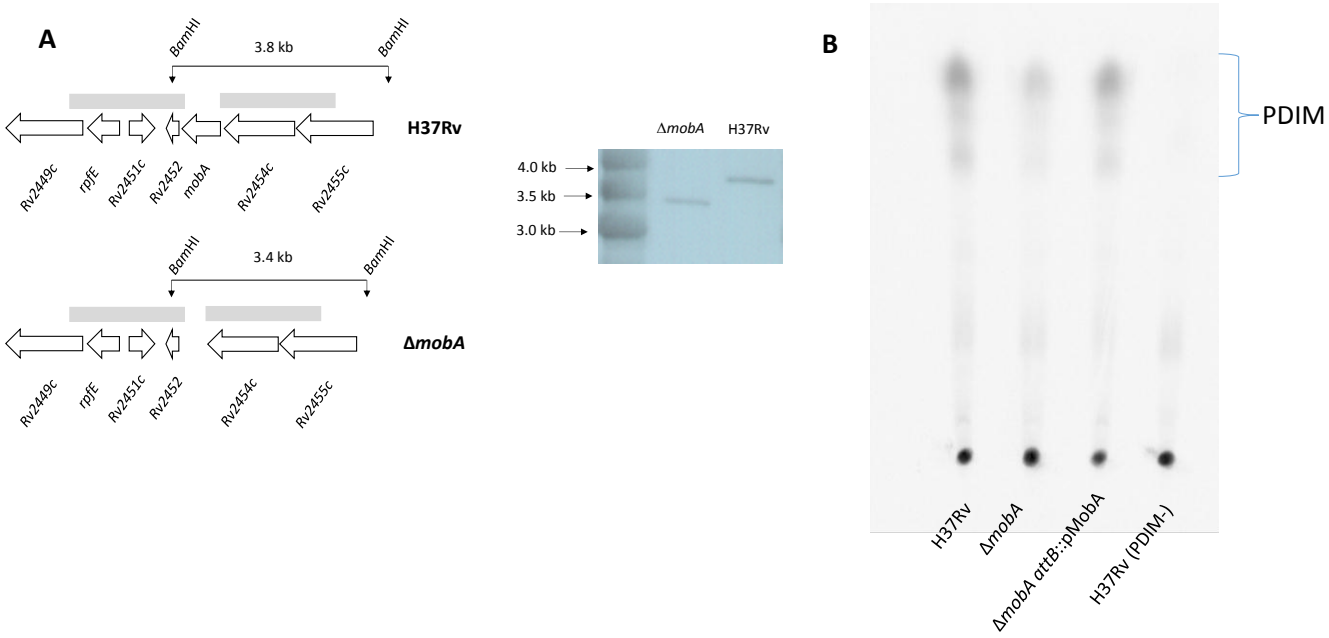

Figure S1. (A) Genotypic confirmation of the Δ*mobA* mutant of *M. tuberculosis*. Left panel shows the restriction maps of the wild type (top) and the Δ*mobA* mutant strains (bottom). Regions of homology that were cloned in the suicide vector used for allelic exchange mutagenesis are denoted by grey boxes. The upstream region of homology was used as a probe for Southern blotting. Right panel shows the hybridization pattern obtained by probing genomic DNA digested with *Bam*HI, Lane 1: Marker, Lane 2: Δ*mobA*, Lane 3: H37Rv. Maps are not drawn to scale. **(B)** PDIM production by *M. tuberculosis* strains. PDIM production was measured by incorporation of ^{14}C propionate. A strain known to be deficient in PDIM synthesis (lane 4) was used as a negative control.

REFERENCES

1. Parish T, Stoker NG. Use of a flexible cassette method to generate a double unmarked *Mycobacterium tuberculosis tlyA plcABC* mutant by gene replacement. Microbiology (Reading, Engl.) **2000**; 146:1969-1975

2. Stover CK, de la Cruz VF, Fuerst TR, et al. New use of BCG for recombinant vaccines. Nature **1991**; 351:456-460