SUPPLEMENTARY INFORMATION

Table S1. Bacterial strains and plasmids used in this study

Name	Description	Source/ reference
Strains		
Yeast		
AH109	*HIS3*, *ADE2*, *lacZ*, *MEL1* containing reporter strain for screening protein interactions, *MATa*, *trp1-901*, *leu2-3*, *112*, *ura3-52*, *his3-200*, *gal4*Δ, *gal80*Δ, *LYS2*::*GAL1*$_{UAS}$-*GAL1*$_{TATA}$-*HIS3*, *MEL1 GAL2*$_{UAS}$-*GAL2*$_{TATA}$-*ADE2*, *URA3*::*MEL1*$_{UAS}$-*MEL1*$_{TATA}$-*lacZ*	Clontech
Y187	*lacZ*, MEL1 containing reporter strain, *MATα*, *ura3-52*, *his3-200*, *ade 2-101*, *trp 1-901*, *leu 2-3*, *112*, *gal4*Δ, *met*$^-$, *gal80*Δ, *URA3*::*GAL1*$_{UAS}$-*GAL1*$_{TATA}$-*lacZ*, *MEL1*	Clontech
M. smegmatis		
mc^{2}155	Laboratory stock of *ept* mutant of mc^{2}6	(11)
HIS5T	Derivative of mc^{2}155 carrying a +1 (4T→5T) frameshift mutation at codon 205 in the *hisD* gene resulting in histidine auxotrophy	This work
HIS5T (pOLYGaa)	Derivative of HIS5T carrying pOLYGaa; Hyg^r	This work
HIS5T(pGaaDinB1)	Derivative of HIS5T carrying pGaaDinB1; Hyg^r	This work
HIS5T(pGaaDinB2)	Derivative of HIS5T carrying pGaaDinB2; Hyg^r	This work
M. tuberculosis		
H37Rv	Laboratory strain (ATCC 25618)	Laboratory stock
dinB1::*hyg*	Derivative of H37Rv harboring *hyg*-marked insertion mutation in *dinB1*; Hyg^r	This work
dinB1::*hyg attB*::*dinB1*	Derivative of *dinB1*::*hyg* carrying pMDINB1 integrated at the *attB* site; Hyg^r, Km^r	This work
Δ*dinB2*	Derivative of H37Rv harboring an out-of-frame, unmarked deletion mutation in *dinB2* encoding the first 13 amino acids of DinB2	This work
Δ*dinB2 attB*::*dinB2*	Derivative of Δ*dinB2* carrying pMDINB2 integrated at the *attB* site; Hyg^r	This work
Δ*dinB1*::*hyg* Δ*dinB2*	Derivative of *dinB1*::*hyg* harboring an unmarked deletion mutation in *dinB2*; Hyg^r	This work
Plasmids		
pMV306K	*E. coli*-Mycobacterium integrating shuttle vector; Km^r	(6)
pMV306H	*E. coli*-Mycobacterium integrating shuttle vector; Hyg^r	H. Boshoff
p2NIL	*E. coli* cloning vector; Km^r	(10)

pGOAL17	Plasmid carrying *lacZ-sacB* markers as a *Pac*I cassette; Ap^r	(10)
pOLYG	Multicopy *E. coli-Mycobacterium* shuttle vector; Hyg^r	(8)
pAGAN11	Plasmid carrying a 4152-bp *Bam*HI acetamidase fragment from *M. smegmatis*	(9)
pOLYGaa	Derivative of pOLYG carrying the *M. smegmatis* acetamidase fragment from pAGAN11; Hyg^r	This work
pGaaDinB1	Derivative of pOLYGaa carrying the MTB *dinB1* ORF, cloned as a 1472-bp fragment amplified with primers dinB1F and dinB1R (Table S2), and inserted downstream of the acetamidase fragment; Hyg^r	This work
pGaaDinB2	Derivative of pOLYGaa carrying the MTB *dinB2* ORF, cloned as a 1091 bp fragment amplified with primers dinB2F and dinB2R (Table S2), and inserted downstream of the acetamidase fragment; Hyg^r	This work
pDINB1KO	Knockout vector for creating insertion mutation in *dinB1* – p2NIL subclone carrying *dinB1*::*hyg* allele (*hyg* gene insertion at *PvuII* site in *dinB1* with 2546 bp of 5'-flanking and 1509 bp of 3'-flanking homologous sequence) and *lacZ-sacB* marker cassette from pGOAL17; Km^r, Hyg^r	This work
pDINB2KO	Knockout vector for creating out-of-frame deletion mutation in *dinB2* – p2NIL subclone carrying the Δ*dinB2* allele (Table S2) and *lacZ-sacB* cassette from pGOAL17; Km^r	This work
pMDINB1	*dinB1* complementation vector – pMV306K carrying a 2717 bp *Kpn*I fragment containing *dinB1,* 1266 bp of upstream sequence (212 bp upstream of *dinB1* and 1054 bp of the 3'-end of *ileS*) and 96 bp of downstream sequence (3'-end of *ansA*); Km^r	This work
pMDINB2	*dinB2* complementation vector – pMV306H carrying a 1835 bp *Eco*RV fragment containing *dinB2* and792 bp of 5'-flanking sequence (Rv3055 and a further 167 bp) and 3 bp of 3'-flanking sequence; Hyg^r	This work
pGHISD5T	Derivative of pGEM3Z(+)f carrying a 1132-bp fragment amplified with HISF and HISR into which the *hisD* frameshift mutation was introduced using the mutagenic primer, HISM (Table S2)	This work
pHISD5T	Suicide vector for transferring the +1 frameshift mutation (*hisD*5T) into *M. smegmatis hisD* at codon 205 – p2NIL subclone derived from pGHISD5T and carrying the *hisD*5T allele on a 796-bp *Pst*I-*Bam*HI fragment and the *lacZ-sacB* cassette from pGOAL17; Km^r	This work
pGADT7	Y2H vector to produce AD fusions, $GAL4_{(768\text{-}881)}$AD, *LEU2*, HA epitope tag; Ap^r	Clontech
pGBKT7	Y2H vector to produce BD fusions, $GAL4_{(1\text{-}147)}$DNA-BD, *TRP1*, c-MYC epitope tag; Km^r	Clontech
pGADDinB1	pGADT7 containing DinB1 ORF fused to *GAL4* AD	This work
pGADDinB2	pGADT7 containing DinB2 ORF fused to *GAL4* AD	This work
pGADDnaE1	pGADT7 containing DnaE1 ORF fused to *GAL4* AD	This work
pGADDnaE2	pGADT7 containing DnaE2 ORF fused to *GAL4* AD	This work
pGADDnaN	pGADT7 containing DnaN ORF fused to *GAL4* AD	This work
pGBDDinB1	pGBKT7 containing DinB1 ORF fused to *GAL4* BD	This work
pGBDDinB2	pGBKT7 containing DinB2 ORF fused to *GAL4* BD	This work
pGBDDnaE1	pGBKT7 containing DnaE1 ORF fused to *GAL4* BD	This work
pGBDDnaE2	pGBKT7 containing DnaE2 ORF fused to *GAL4* BD	This work
pGBDDnaN	pGBKT7 containing DnaN ORF fused to *GAL4* BD	This work

Table S2. PCR primers, others oligonucleotides and amplicons used in this study

Name	Sequence (5'-3')[a]	Application	Amplicon properties/ region targeted/ references
PPF1	GGCCCTGCAGCGCCTTGATGCGATCGGGGCG	Forward primer used to amplify 5'-flanking homologous segment used for construction of pDINB2KO	2014 bp amplicon extending from the 3'-end of MTB *nrdH* (Rv3053c) and containing 35 bp of the 5'-end of *dinB2* (Rv3056)
PPR1	GCGCAGATCTACGTGCAGGATCCATCGCGG	Reverse primer used to amplify 5'-flanking homologous segment used for construction of pDINB2KO	
PPF3	CCGGGGATCCGGTTGCTGGGAGTGCGG	Forward primer used to amplify 3'-flanking homologous segment used for construction of pDINB2KO	1961 bp amplicon containing 32 bp of the 3' end of MTB *dinB2* (Rv3056) and extending to the 5'-end of Rv3059 (*cyp136)*
PPR3	GCGCCAAGCTTGAGCCCGCTGCCTGGCG	Reverse primer used to amplify 3'-flanking homologous segment used for construction of pDINB2KO	
HISF	CGCGCCGCGCTGCAGGTCTCCATCGACCG	Forward primer used to amplify internal segment of *M. smegmatis hisD* gene	1132 bp amplicon extending from base position 271 the *M. smegmatis hisD* gene to 78 bp downstream of the stop codon. Used to construct the vector pGHISD5T (Table S1)
HISR	CTGCAGTACGGCGACTTGCCCCGC	Reverse primer used to amplify internal segment of *M. smegmatis hisD* gene	
HISM	GGCCGTGGCGCTTTTTGGCCTACGGCGGC	Mutagenic primer for the introduction of a +1 frameshift mutation (4T→5T) at codon 205 of the *M. smegmatis hisD* gene	Position 600-627 in *M smegmatis hisD*
B1F2	AGCGCTTCTCAGCGGATT	PCR primers for quantitative detection of MTB *dinB1* gene expression	Position 504-648 in MTB *dinB1*
B1R2	GTCGCGCCTAGGATGTTG		
B2F2	CGTACCGTCGAAGCACTGT	PCR primers for quantitative detection of MTB *dinB2* gene expression	Position 544-780 in MTB *dinB2*
B2R	GAGGTCTCGTGGAAAGGTGA		
E1HF	CGTGTGACTCCACCTTGTTGA	PCR primers for quantitative detection of	Position 845-903 in MTB *dnaE1* (1)

E1HR	TGTCCACACGTCGGCG	MTB *dnaE1* gene expression	
E2F2	GGCGGTGTATGAGATGCTG	PCR primers for quantitative detection of MTB *dnaE2* gene expression	Position 1842-2060 in MTB *dnaE2*
E2R2	GACGGGTGCTCGTAGATGAC		
SigAF	TGCAGTCGGTGCTGGACAC	PCR primers for quantitative detection of MTB *sigA* gene expression	Position 1379-1573 in MTB *sigA* (3)
SigAR	CGCGCAGGACCTGTGAGCGG		
SMB1F	GACGTCGAGGCGTTCTGT	PCR primers for detection of *M. smegmatis dinB1* gene expression	Position 331-430 in *M. smegmatis dinB1*
SMB1R	TCTTGGCGATCTGTTTACCC		
SMB2F	GGGATCAGTGACAACAAGCA	PCR primers for detection of *M. smegmatis dinB2* gene expression	Position 427-526 in *M. smegmatis dinB2*
SMB2R	CCATCACCGTCATCCAGTTC		
SMSigF	GGGCGTGATGTCCATCTCCT	PCR primers for detection of *M. smegmatis sigA* gene expression	Position 367-488 in *M. smegmatis sigA* (7)
SMSigR	GTATCCCGGTGCATGGTC		
B1RT2	TAGCTTCACCGTGATGGTG	Reverse primer used for cDNA synthesis	Position 844-861in MTB *dinB1*
B2RT2	GGGTGACGGTTCGCGACG	Reverse primer used for cDNA synthesis	Position 851-868 in MTB *dinB2*
E1RT	GGCGACGATCAGAAAGTACG	Reverse primer used for cDNA synthesis	Position 1076-1095 in MTB *dnaE1*
E2RT2	AAAGCCGGCGCAGTCGAC	Reverse primer used for cDNA synthesis	Position 2128-2145 in MTB *dnaE2*
SigART	CTGACATGGGGGCCCGCTACGTTG	Reverse primer used for cDNA synthesis	Position 20-43 downstream of MTB *sigA*
RpoBF2	GAGGGTCAGACCACGATGAC	Forward primer for amplification of RRDR in *rpoB*	Position 1030-1478 in MTB *rpoB*
RpoBR2	GAGCCGATCAGACCGATGT	Reverse primer for amplification of RRDR in *rpoB*	Position 1460-1478 in MTB *rpoB*
RpoBS	GCAGACGTTGATCAACATCC	Internal primer for sequencing RRDR in *rpoB*	Position 1224-1243 in MTB *rpoB*

dinB1F	GGGGGGATATCGCCATAGCGAAAACTAGCTCG	Forward primer used to amplify the MTB *dinB1* ORF for construction of pGaaDinB1	1472 bp amplicon extending from 65 bp upstream of the 5'-end of MTB *dinB1* to the stop codon
dinB1R	CCCCCCTATAGTGGCCAGCGGCTGCAGTAG	Reverse primer used to amplify the MTB *dinB1* ORF for construction of pGaaDinB1	
dinB2F	GGGGGGATATCGAAAACTGTGCGGACGATGAT	Forward primer used to amplify the MTB *dinB2* ORF for construction of pGaaDinB2	1091 bp amplicon extending from 30 bp upstream of the 5'-end of MTB *dinB2* to the stop codon
dinB2R	CCCCCCTATAGATCCGGTCAAGATTGGCGTGA	Reverse primer used to amplify the MTB *dinB2* ORF for construction of pGaaDinB2	

a. Restriction sites or additional bases engineered into primer sequences are underlined

Figure S1. Multiple sequence alignment of DinB homologs from bacteria. The DinB homologs from *E. coli* (Q47155), *P. aeruginosa* (AAG04312), *P. putida* (Q88NK4), *B. subtilis* PolY1 (P54545) and PolY2(NP_390252), MTB DinB1 (Rv1537) and MTB DinB2 (Rv3055) were aligned using the ClustalW2 multiple sequence alignment tool at the European Bioinformatics Institute website, http://www.ebi.ac.uk/Tools/clustalw2/index.html. The residues in pink represent acidic amino acids that are important for catalysis and the sequences in red denote the putative β-clamp binding motifs. The two residues in the MTB DinB1 sequence shown in purple (Q200 and L201) denote the site of inactivation of

DinB1 in the *dinB1*::*hyg* mutant strain in which the thumb and little finger domains that are required for binding to the minor and major grooves of DNA, respectively, are disrupted. The annotation of these domains is derived from the crystal structure of the DinB homologue in *Sulfolobus solfataricus*, Dpo4 (5), and the little finger domain of *E. coli* PolIV (2). NCBI accession or genome annotation numbers are given in parentheses.

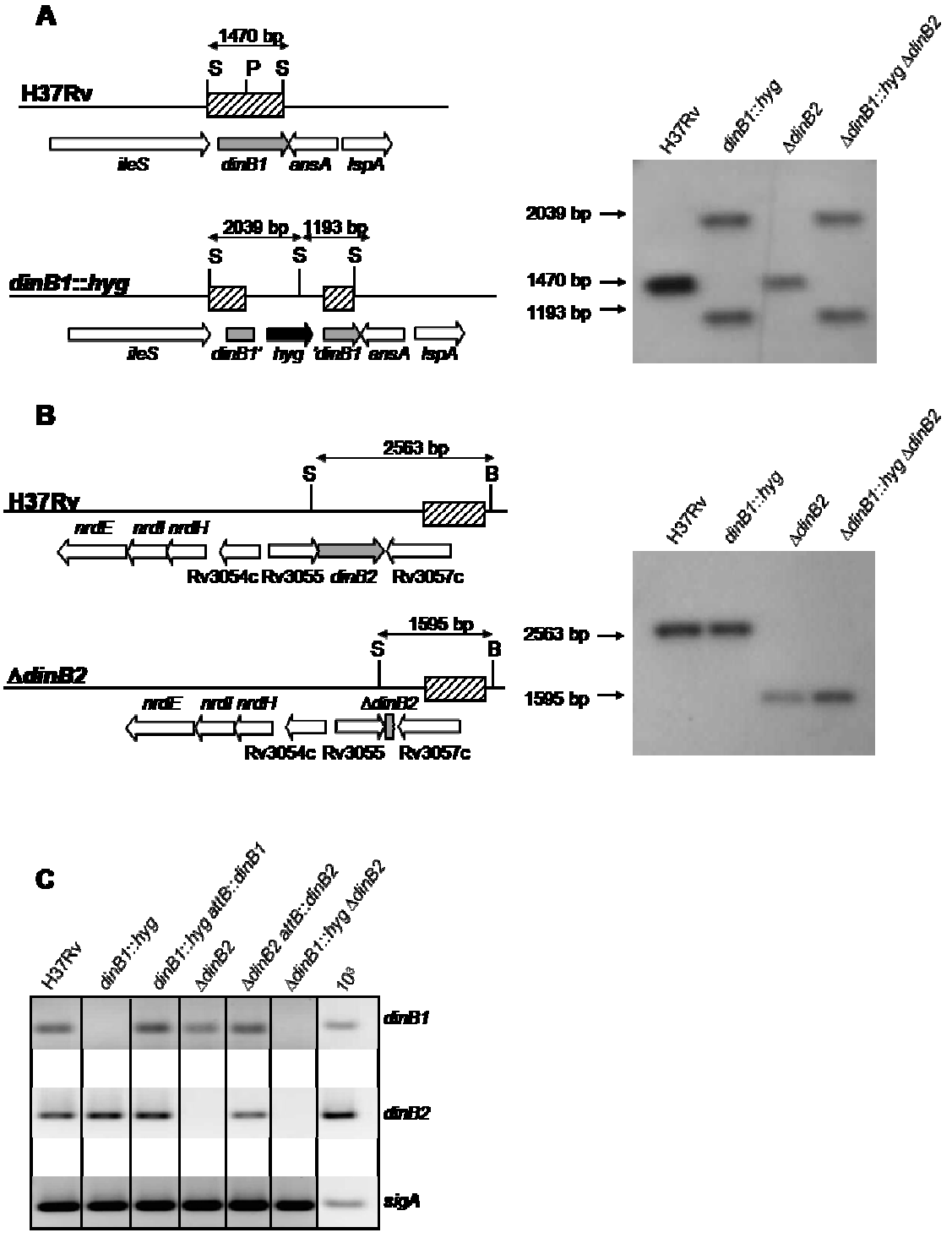

Figure S2. Construction and genotypic characterization of allelic exchange mutants of MTB. Restriction maps of regions carrying *dinB1* and *dinB1*::*hyg* (A) and *dinB2* and Δ*dinB2* (B) alleles in MTB H37Rv (not to scale). The *dinB1* gene was inactivated by insertion of a *hyg* marker at a *Pvu*II site located 598 bp downstream of the *dinB1* start codon, thus disrupting the *dinB1* ORF between the Gln200 and Leu201 codons (Fig. S1). The *dinB2* gene was inactivated by deletion of an internal 974-bp segment of this gene, resulting in the formation of an out-of-frame deletion mutation, Δ*dinB2*, encoding only the first 13 amino

acids of DinB2 (Table S1). The Δ*dinB2* mutation was introduced into the *dinB1*::*hyg* mutant to produce a double mutant strain. Genomic DNA was digested with *Sal*I (A) or *Bgl*II/*Sal*I (B) and Southern blotting carried out as described previously (4) probing with fragments denoted by hatched boxes. P, *Pvu*II; S, *Sal*I.; B, *Bgl*II. (C) RT-PCR anaysis of *dinB1 and dinB2* expression in MTB using mRNA isolated from cells grown to mid-logarithmic phase.

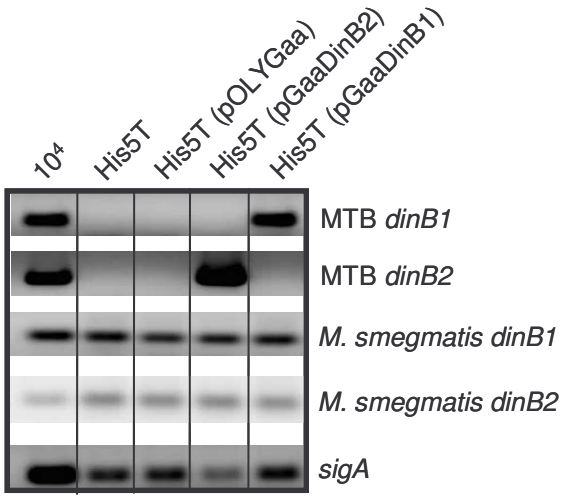

Figure S3. RT-PCR analysis of MTB *dinB1* and *dinB2* expressed ectopically in *M. smegmatis*. Expression analysis was conducted using mRNA isolated from bacteria grown to mid-logarithmic phase in Middlebrook 7H9 media supplemented with 0.2% glycerol, 0.085% NaCl, 0.2% glucose and 0.05% Tween 80. For strains carrying vectors, hygromycin was added to a final concentration of 50 µg/ml. In this experiment, no acetamide inducer was added to the cultures. RT-PCR was carried our using the primers described in Table S2. For comparison, *M. smegmatis dinB1*, *dinB2* and *sigA* transcripts were analyzed in the same samples alongside a genomic DNA control (first lane).

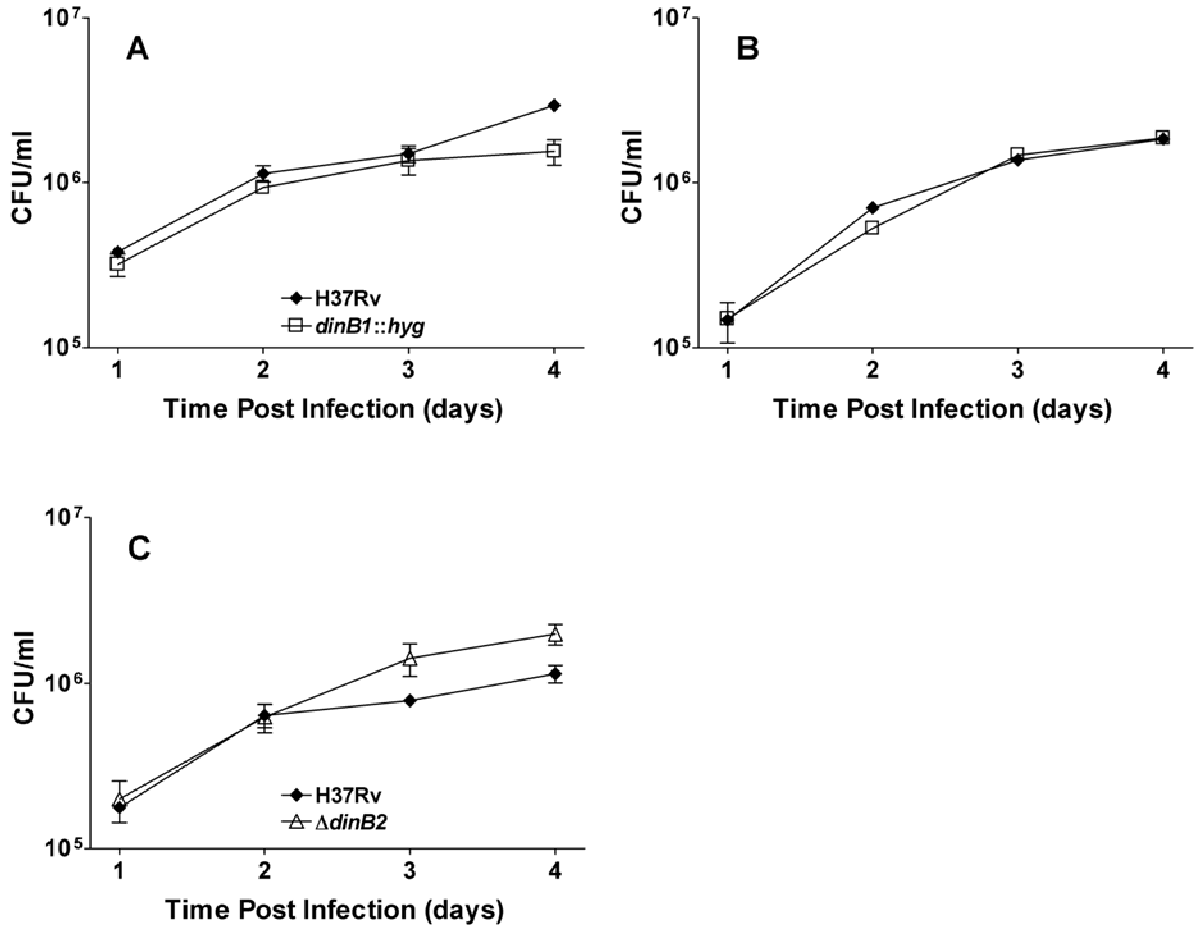

Figure S4. Intracellular growth of the DinB1 and DinB2 mutant strains of MTB. (A) Growth of the *dinB1*::*hyg* mutant in resting human peripheral blood-derived monocytes (PBMCs) PBMCs. (B) Growth of the *dinB1*::*hyg* mutant in LPS activated PBMCs. (C) Growth of the Δ*dinB2* mutant in LPS activated PBMCs from a different donor. ♦, Wild-type H37Rv; □, *dinB1*::*hyg*; Δ, Δ*dinB2*.

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