SUPPLEMENTARY INFORMATION

A vitamin B_{12} transporter in *Mycobacterium tuberculosis*

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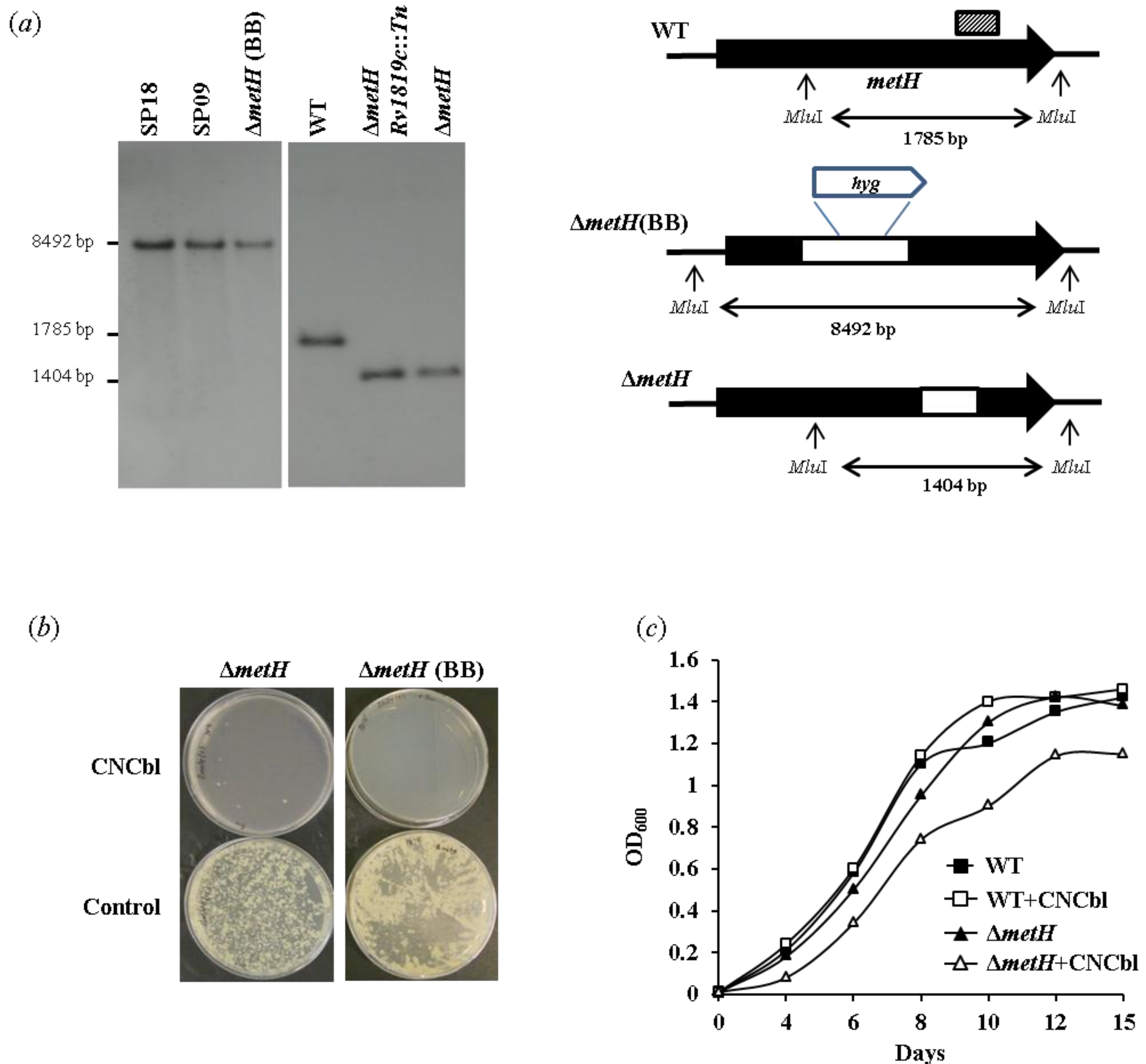
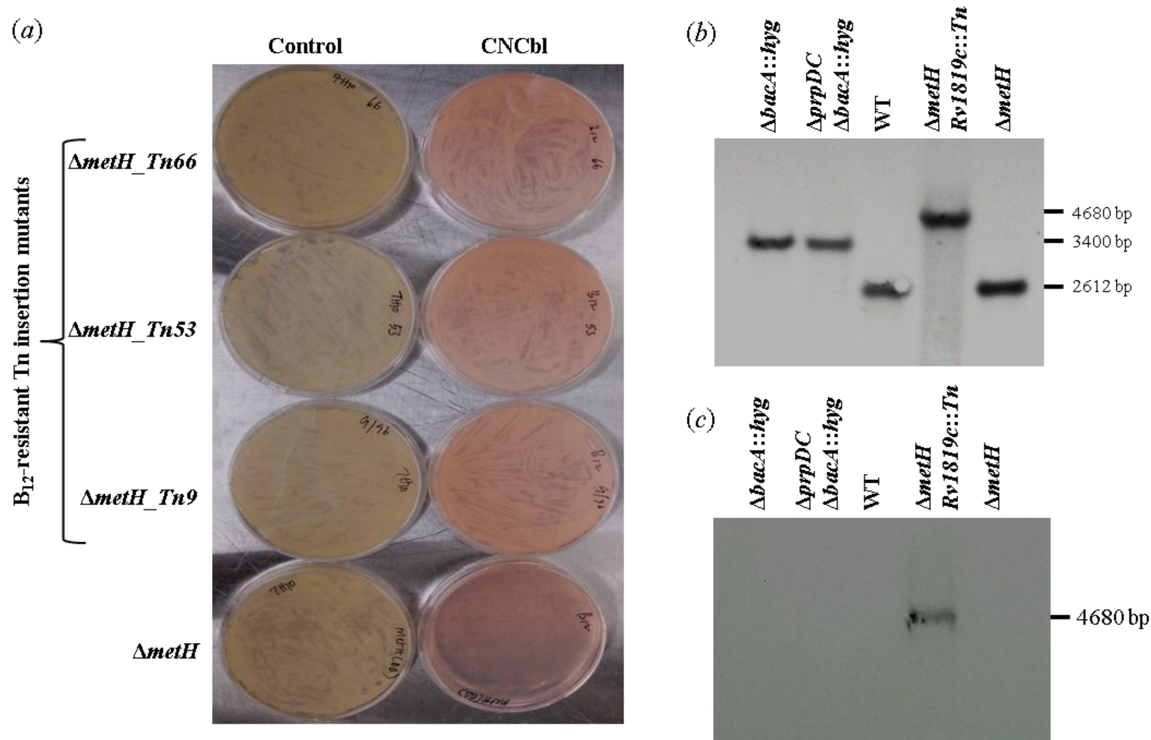


Figure S1. Disruption of *metH* renders *M. tuberculosis* sensitive to exogenous vitamin B_{12}. (*a*) Genotypic characterization of Δ*metH* parental strains and derivative mutants. Genomic DNA was digested with *Mlu*I and probed with a 644 bp *Mlu*I-*Bgl*II fragment internal to wildtype *metH* (metHp, represented by a hatched box). Restriction maps are illustrated schematically in the line drawings adjacent to the Southern blot (not to scale). The Δ*metH* (BB) allele eliminates 1417 bp *metH* coding sequence including an *Mlu*I site (WT, wildtype H37Rv). The strains analyzed here are the *hyg*-marked *metH* deletion mutant, Δ*metH* (BB), which was described previously (1); spontaneous B_{12}-resistant mutants of Δ*metH* (BB), SP09 and SP18 (Table S1); the unmarked Δ*metH* mutant used to construct the Tn library; a representative Δ*metH Rv1819c*::Tn insertion mutant; and wildtype H37RvJO (2). (*b*) Effect of exogenous vitamin B_{12} on growth of Δ*metH* mutants. Cells were incubated on solid 7H10 medium containing 10 µg/ml CNCbl. Representative plates are shown from two independent experiments performed in duplicate. (*c*) The Δ*metH* mutant exhibits a minor growth defect relative to WT *M. tuberculosis* H37Rv in standard liquid medium supplemented with 10 µg/ml CNCbl.



(d)

Gene	Length (bp)	Number of -TA- sites	Total insertions	Insertions mapped*
Rv1819c (bacA)	1920	34	72	1 3 1 2 1 1 2 3 3 1 1 2 1 2 1 173 261 471 580 863 931 971 980 1163 1306 1436 1502 1538 1732 1919
Rv1314c (pduO)	581	16	6	1 1 106 286
Rv3083 (mymA)	1488	26	2	2 380
Rv2927c	738	13	1	1 216
Rv1009 (rpfB)	1089	12	1	1 187
Rv1492 (mutA)	1848	17	1	1 177

*For *Rv1819c* and *Rv1314c*, Tn insertion sites were mapped by sequencing 25/72 and 2/6 clones, respectively; the remaining insertions were confirmed by PCR. Values in the red arrows denote the number of insertion mutants mapping to a specific –TA– dinucleotide whose position within the gene is indicated in black text.

Figure S2. (*a*) Effect of exogenous vitamin B_{12} on growth of Δ*metH* Tn insertion mutants. Cells were incubated on solid 7H10 medium containing 10 µg/ml CNCbl. Representative plates are shown from independent experiments performed in duplicate. (*b*) Genotypic analysis of *Rv1819c* mutants. Genomic DNA was digested with *Sph*I and hybridized with a *Rv1819c*-specific probe. (*c*) The blot in (*b*) was washed and re-hybridized with a MycoMar-1-specific probe to confirm a representative *metH Rv1819c*::Tn mutant. (*d*) Tn insertion sites identified in 83 of 84 putative "B12 uptake" defective mutants of Δ*metH*.

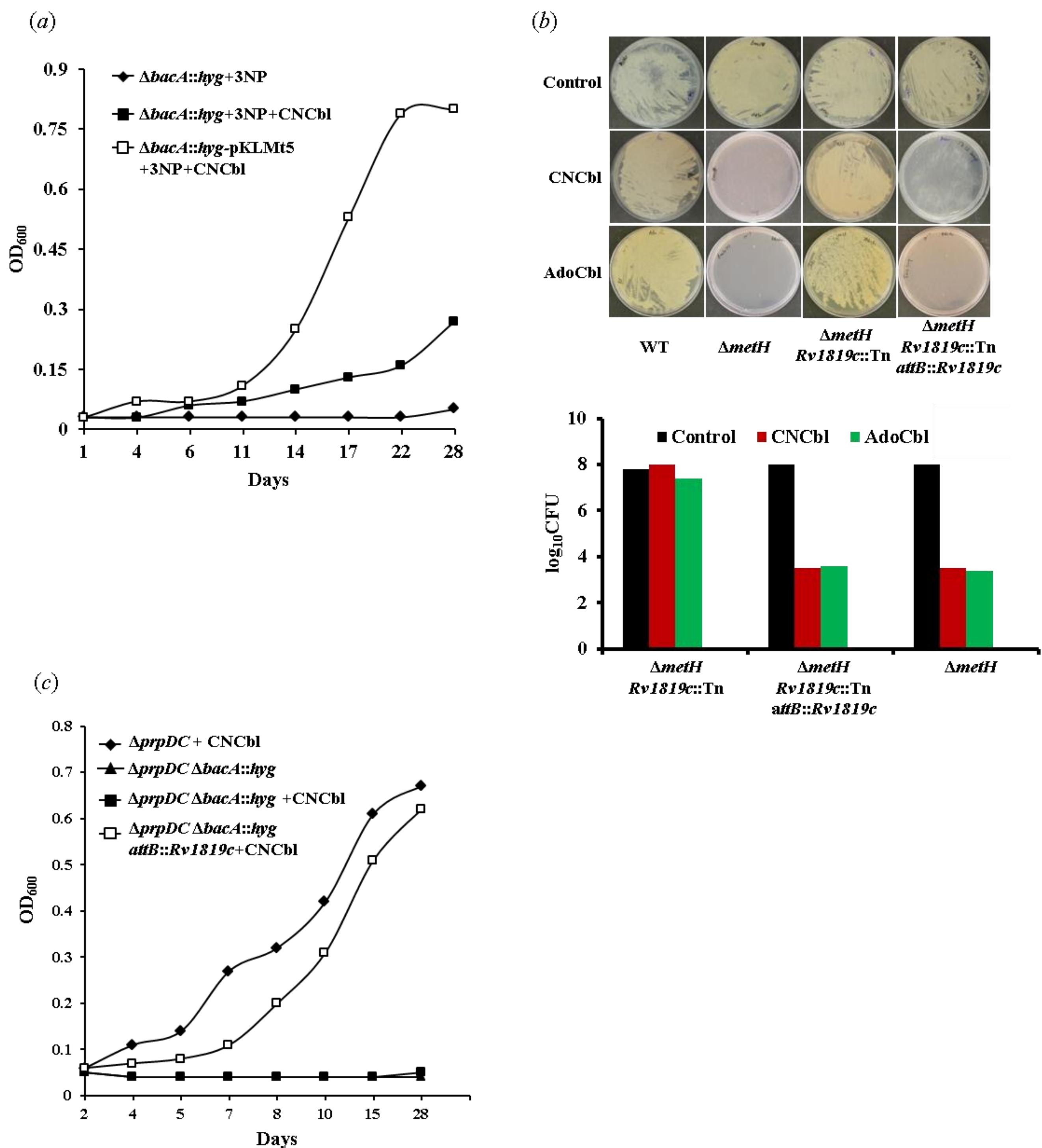
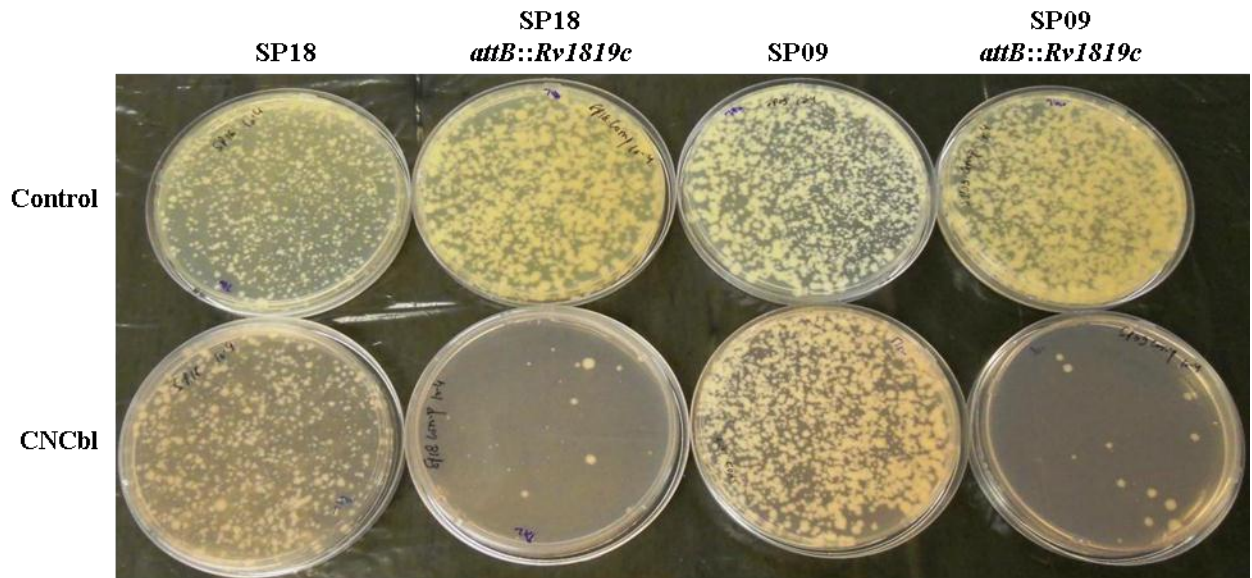


Figure S3. Disruption of *Rv1819c* eliminates uptake of vitamin B_{12} in *M. tuberculosis*. (*a*) The Δ*bacA*::*hyg* deletion mutant (3) is unable to utilize B_{12} for growth in 0.1% propionate containing 3NP. Data are from a representative experiment performed in duplicate. (*b*) Effect of exogenous vitamin B_{12} on growth of Δ*metH* parental strain and a representative Δ*metH Rv1819c*::Tn mutant. Cells were incubated on solid 7H10 medium containing CNCbl or AdoCbl at 10 µg/ml, and CFUs scored after 4 weeks. Representative plates are shown from two independent experiments performed in duplicate, and CFU counts plotted in the adjacent bar graph. (*c*) Deletion of *Rv1819c* eliminates the ability of a methylcitrate cycle-deficient Δ*prpDC* mutant to utilize B_{12} for growth in propionate. Data are from a representative experiment performed in duplicate.



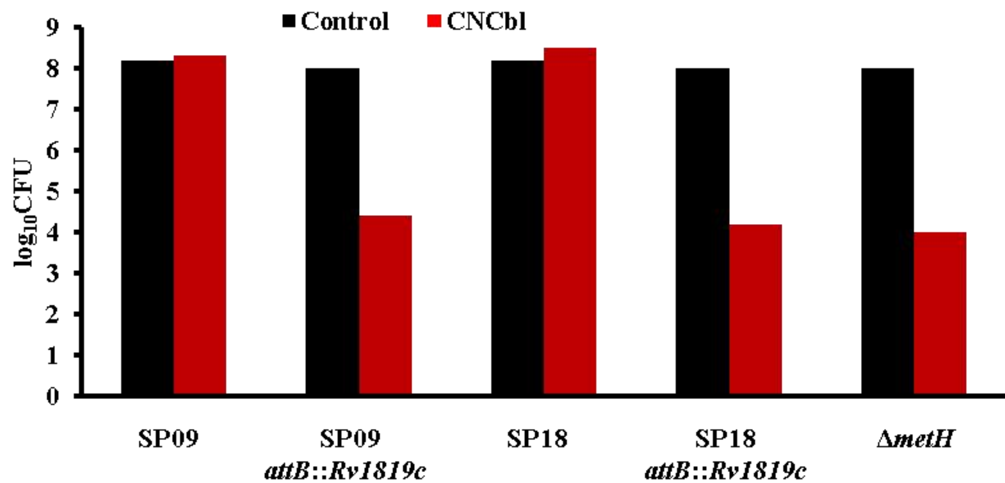


Figure S4. SNPs in *Rv1819c* alleviate B_{12} sensitivity in spontaneous B_{12}-resistant mutants of Δ*metH*. Cells were incubated on solid 7H10 medium containing 10 µg/ml CNCbl and CFUs were scored after 4 weeks. Representative plates are shown from two independent experiments performed in duplicate, with CFU counts plotted below.

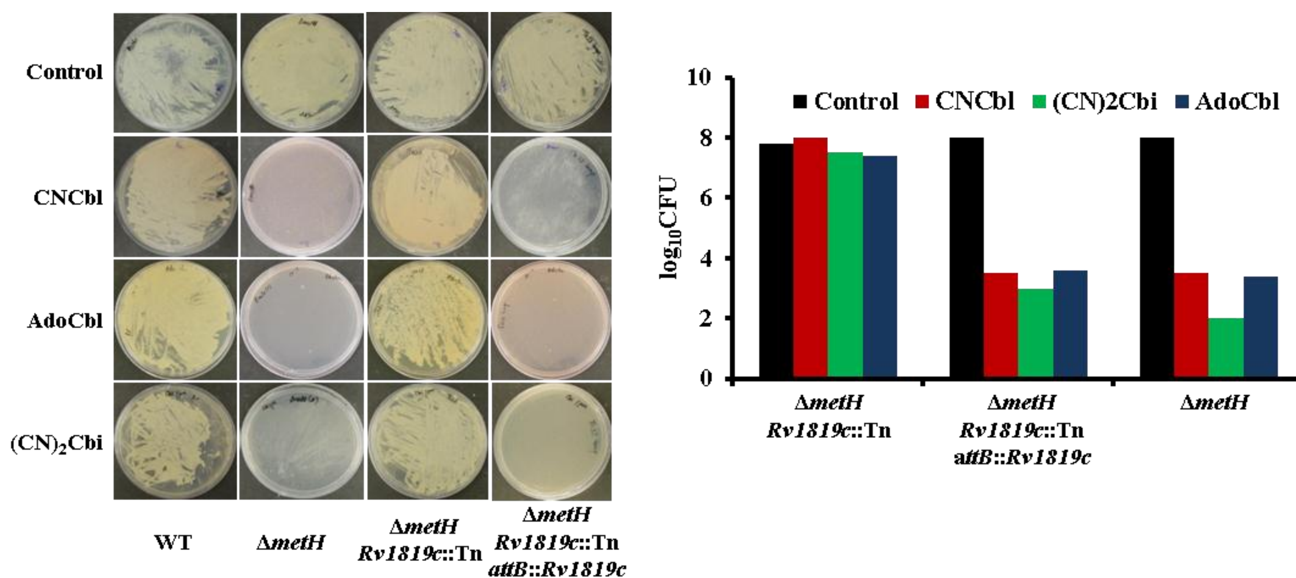
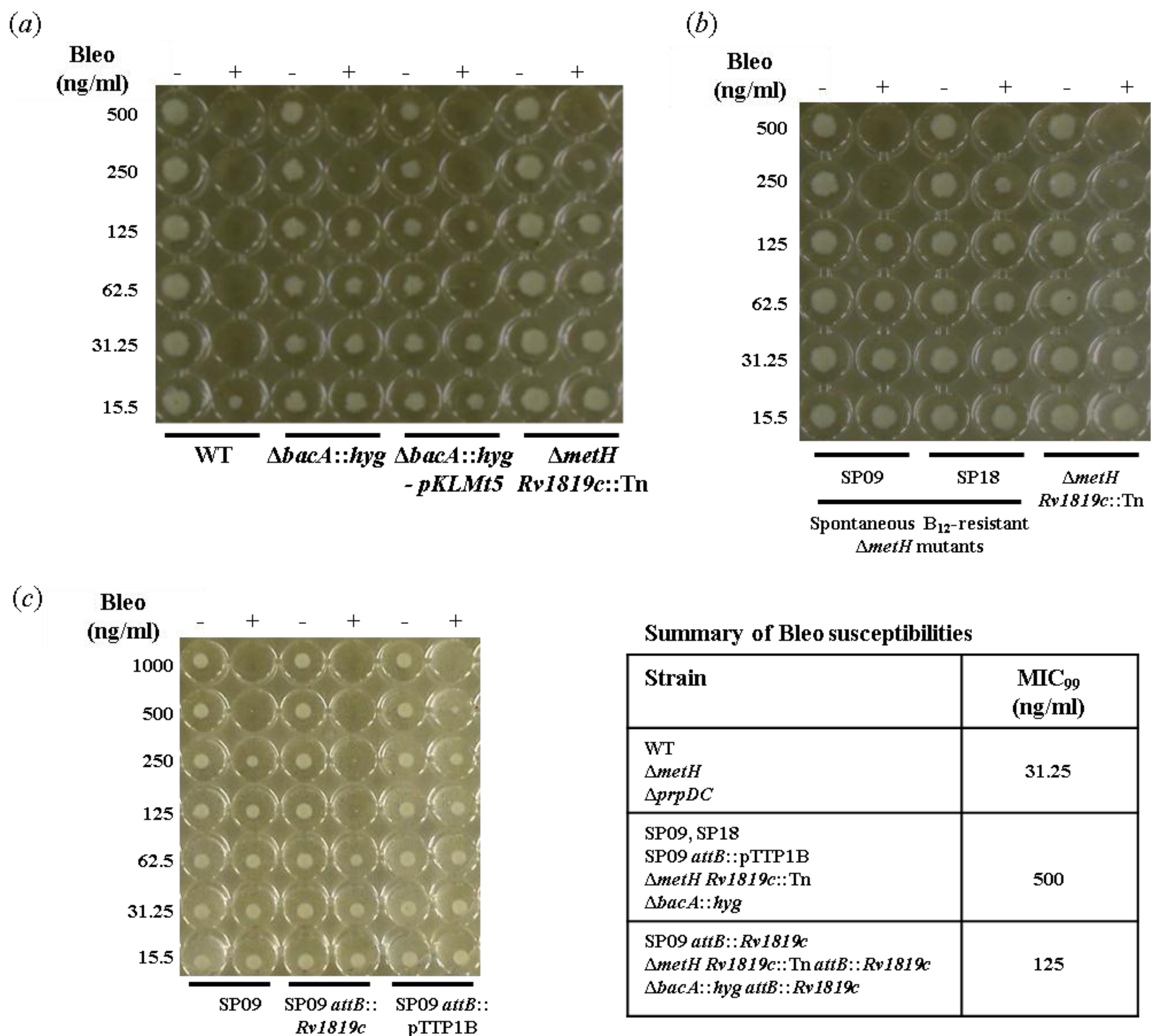


Figure S5. Disruption of *Rv1819c* alleviates sensitivity to corrinoids in *M. tuberculosis* Δ*metH*. Cells were incubated on solid 7H10 medium containing CNCbl or AdoCbl at 10 µg/ml, or $(CN)_2$Cbi at 1 µM, and CFUs scored after 4 weeks. Representative plates are shown from two independent experiments performed in duplicate, and CFU counts plotted in the adjacent bar graph.



Summary of Bleo susceptibilities

Strain	MIC_{99} (ng/ml)
WT *ΔmetH* *ΔprpDC*	31.25
SP09, SP18 SP09 *attB*::pTTP1B *ΔmetH Rv1819c*::Tn *ΔbacA*::*hyg*	500
SP09 *attB*::*Rv1819c* *ΔmetH Rv1819c*::Tn *attB*::*Rv1819c* *ΔbacA*::*hyg attB*::*Rv1819c*	125

Figure S6. Inactivation of *Rv1819c* decreases the susceptibility of *M. tuberculosis* to bleomycin (Bleo). The broth microdilution method (4) was used to determine Bleo MIC values for (*a*) targeted (Δ*bacA*::*hyg*) and Tn insertion (Δ*metH Rv1819c*::Tn) mutants of *Rv1819c* and a complemented derivative (Δ*bacA*::*hyg pKLMt5)* (3) and (*b*) for spontaneous B_{12}-resistant mutants, SP09 and SP18 (Table S2), which contain SNPs in *Rv1819c*. (*c*) Complementation with full-length *Rv1819c* restores Bleo susceptibility in a spontaneous B_{12}-resistant mutant of Δ*metH*. The broth microdilution method (4) was used to determine Bleo MIC values for SP09, its complemented derivative (SP09 *attB*::*Rv1819c*), and a strain carrying the empty vector (SP09 *attB*::pTTP1B). The table summarizes the Bleo MIC_{99} values for the *M. tuberculosis* strains tested in panels (*a*)-(*c*).

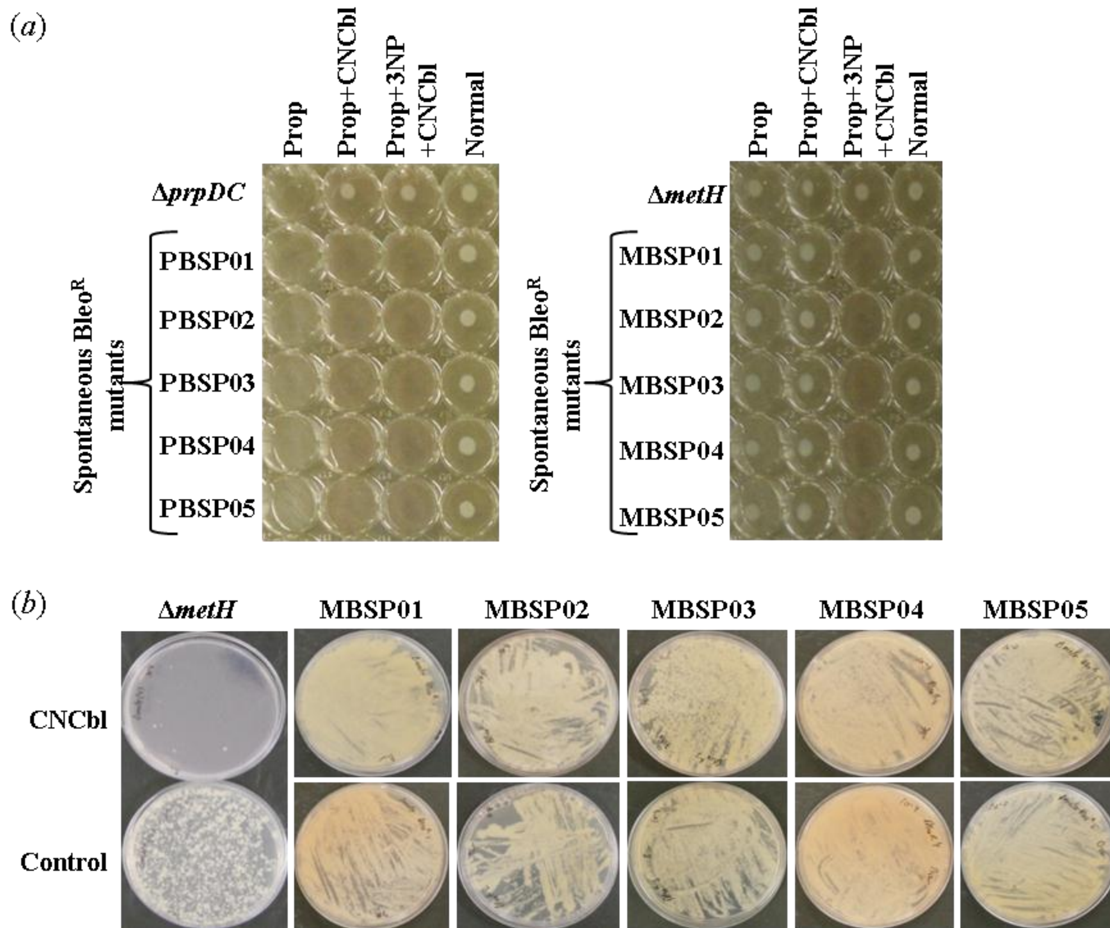


Figure S7. Overlapping functions of Rv1819c in B_{12} transport and Bleo sensitivity. (*a*) Spontaneous $Bleo^R$ mutants containing SNPs in *Rv1819c* are impaired in their ability to utilize B_{12} for propionate metabolism. (*b*) Cells were incubated on solid 7H10 medium containing 10 µg/ml CNCbl growth evaluated after 4 weeks. Representative plates are shown from two independent experiments performed in duplicate.

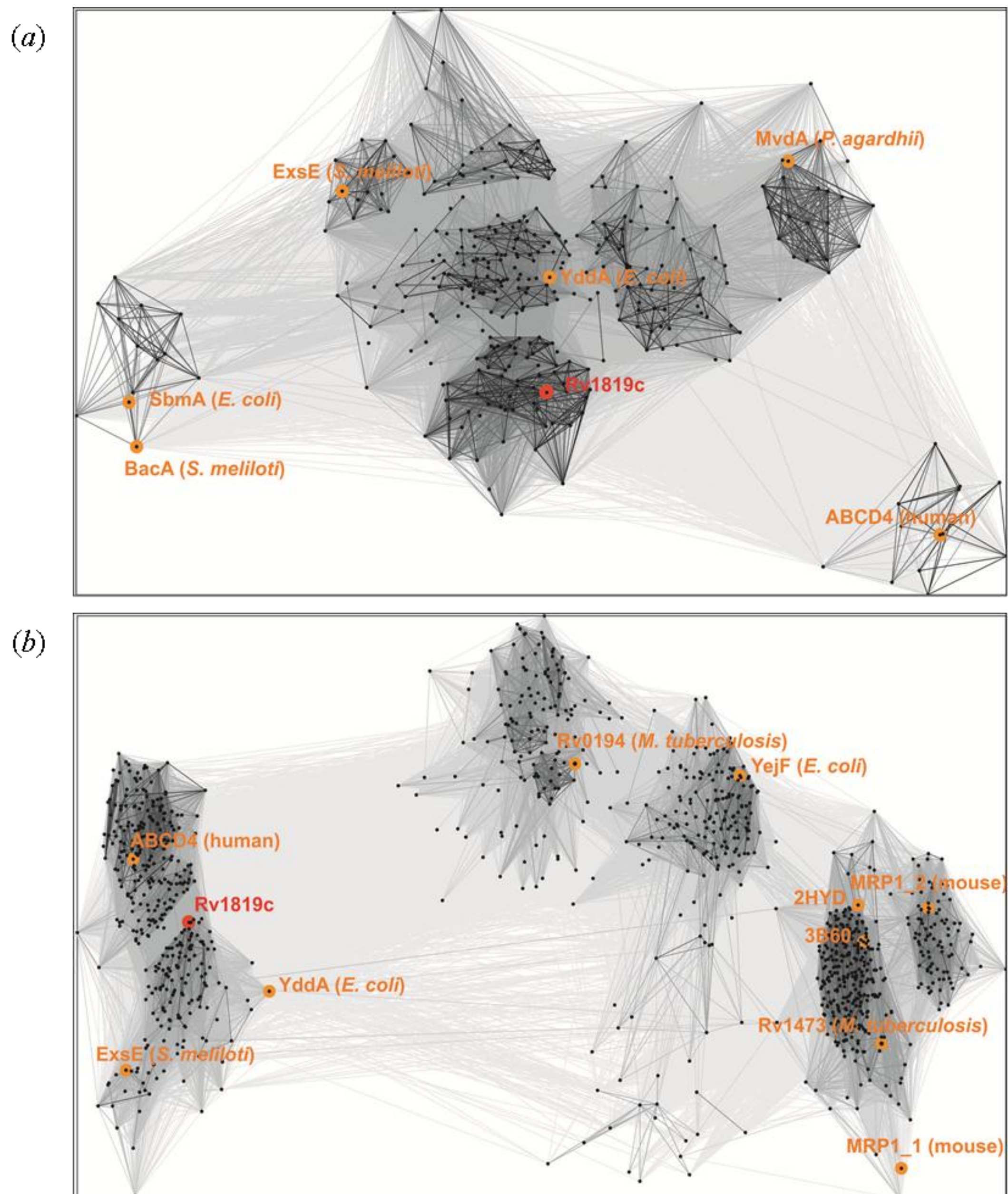


Figure S8. (*a*) The Rv1819c transmembrane domain (TMD) and its homologs were clustered using CLANS (Cluster Analysis of Sequences) (5). The analysis included 343 sequences, and each dot represents a single sequence. Only connections that correspond to P-values < 1e-10 are shown, with thicker lines indicating higher similarity. The Rv1819c TMD is denoted in red, with selected homologs (shown in orange) corresponding to the following Uniprot entries: Ydda (YDDA_ECOLI), ExsE (O54061_RHIML), SbmA (SBMA_ECOLI), BacA (BACA_RHIME), ABCD4 (ABCD4_HUMAN), and MvdA (B7SMU1_OSCAG). In (*b*), the Rv1819c nucleotide binding domain (NBD) and its homologs (total 1070 sequences) were clustered. Only connections corresponding to P-values < 1e-15 are shown. The red label indicates the Rv1819c NBD with selected homologs (orange) corresponding to the following Uniprot entries: ABCD4 (ABCD4_HUMAN), Ydda (YDDA_ECOLI), ExsE (O54061_RHIML), Rv0194 (O53645_MYCTU), Rv1473 (O53164_MYCTU), YejF (YEJF_ECOLI), and MRP1_1 and MRP1_2 – the NBD domains of MRP1 (MRP1_MOUSE). 2HYD and 3B60 denote the PDB codes of the crystal structures of *Staphylococcus aureus* Sav1866 and *Salmonella typhimurium* MsbA, respectively.

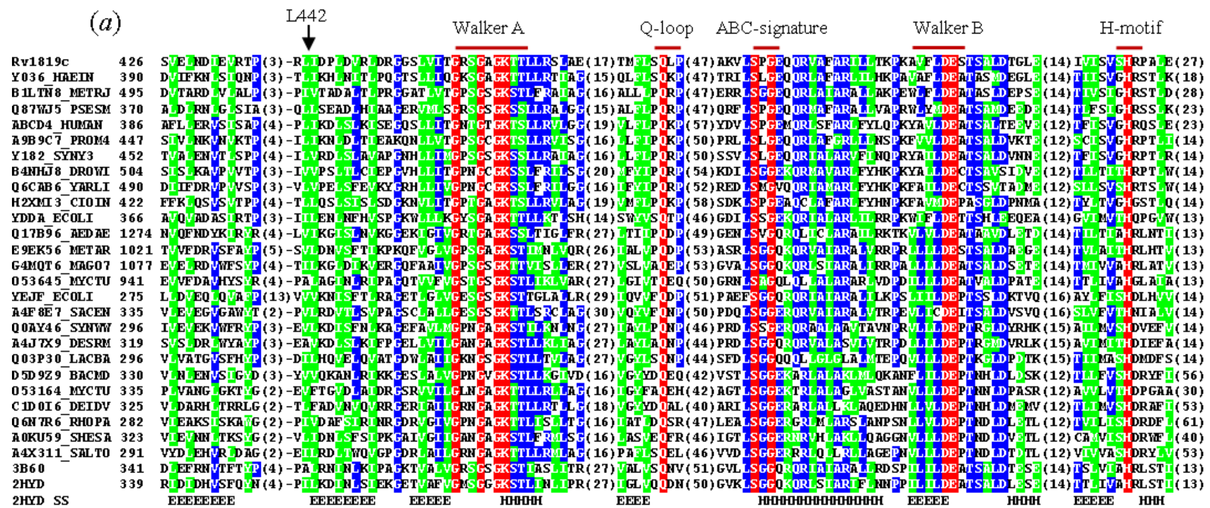


Figure S9. (*a*) Alignment of Rv1819c NBD with selected homologous sequences. Homologous sequences are denoted by their Uniprot accession numbers and structures used for modeling Rv1819c are indicated by their respective PDB codes. The secondary structure of 2HYD is shown below the alignment, where “E” indicates a β-strand and “H” indicates an α-helix. Conserved sequence motifs are shown above the alignment, and residues excluded from the alignment to make it more compact are indicated in parentheses. The arrow indicates the position of the L442 residue which is mutated in SP09 (Table S1). (*b*) Rv1819c topology diagram. Rv1819c is predicted to possess seven transmembrane helices. Six of these (colored green) correspond to transmembrane helices identified in *Staphylococcus aureus* Sav1866 (PDB ID: 2HYD) and *Salmonella typhimurium* MbsA (PDB ID: 3B60) and are included in the Rv1819c model.

Table. S1. Strains, plasmids, PCR primers, oligonucleotides used in the study

Strain	Description	Source
<u>*E. coli*</u>		
DH5α	F- *φ80lacZΔM15* Δ(*lacZYA-argF*)U169 *recA1 endA1 hsdR17*(rk-, mk+) *phoAsupE44 thi*-1 *gyrA96 relA1* λ-	Invitrogen
DH5α λ(Pir)	λ pir lysogen of DH5α	Eric J. Rubin
<u>*Mtb*</u>		
H37RvJO	Virulent reference laboratory strain; ATCC 25618	Laboratory Stock
H37RvMA	Virulent reference laboratory strain from the laboratory of Dr. C. Sassetti; ATCC 27294	Ref. 2
Δ*metH*(BB)	*metH* deletion mutant of H37Rv lacking 1417 bp internal *Bgl*II-*Bcl*I fragment; Hyg^R	Ref. 1
Δ*metH*	*metH* deletion mutant of H37Rv; unmarked	This study
Δ*bacA*::*hyg*	*bacA* deletion mutant of H37Rv (Pasteur); lacking 541 bp internal *Eco*RV-*Xba*I fragment; Hyg^R	Ref. 3
Δ*bacA::hyg pKLMt5*	Complemened *bacA*::*hyg* mutant carrying 2283 bp region of the *M. tuberculosis* H37Rv chromosome containing the *bacA* gene flanked by 203 bp upstream and 161 bp downstream in the integrative vector pMV306K; Hyg^R Kan^R	Ref. 3
Δ*bacA*::*hyg* *attB*::*Rv1819c*	Δ*bacA*::*hyg* complemented with full-length *Rv1819c* carried on pTTP1B vector integrated at *attB* locus; Hyg^RGm^R	This study
Δ*metH Rv1819c*::Tn	Representative transposon mutants with disrupted *Rv1819c* identified in the B_{12} uptake screen; Kan^R	This study
Δ*metH Rv1314c*::Tn	Representative transposon mutants with disrupted *Rv1314c* (*pduO*) identified in the B_{12} uptake screen; Km^R	This study
Δ*metH Rv1819c*::Tn *attB*::*Rv1819c*	Δ*metH Rv1819c*::*Tn* complemented with full-length *Rv1819c* carried on pTTP1B vector integrated at *attB* locus; Km^RGent^R	This study
Δ*prpDC*	*prpDC* deletion mutant of H37RvMA lacking the 2660 bp region from the start codon of *prpD* to the stop codon of *prpC*	This study
Δ*bacA*::*hyg* Δ*prpDC*	*prpDC* deletion mutant of Δ*bacA*::*hyg*; Hyg^R	This study
Δ*bacA*::*hyg* Δ*prpDC* *attB::bacA*	Δ*bacA*::*hyg* Δ*prpDC* complemented with full-length *Rv1819c* carried on pTTP1B vector integrated at *attB* locus*;* Hyg^R $Gent^R$	This study
SP09	Spontaneous vitamin B_{12}-resistant mutant of Δ*metH*(BB)	This study
SP18	Spontaneous vitamin B_{12}-resistant mutant of Δ*metH*(BB)	This study
SP09 *attB*::*Rv1819c*	SP09 strain complemented with full-length *Rv1819c* carried on pTTP1B vector integrated at *attB* locus; Hyg^RGent^R	This study
SP18 *attB*::*Rv1819c*	SP18 strain complemented with full-length *Rv1819c* carried on pTTP1B vector integrated at *attB* locus; Hyg^RGent^R	This study
MBSP01, MBSP02 MBSP03, MBSP04 MBSP05	Spontaneous bleomycin-resistant mutants of Δ*metH*	This study
MBSP01 *attB*::*Rv1819c*	MBSP01 complemented with full-length *Rv1819c* carried on pTTP1B vector integrated at *attB* locus; $Gent^R$	This study
PBSP01, PBSP02 PBSP03, PBSP04 PBSP05	Spontaneous bleomycin-resistant mutants of Δ*prpDC*	This study
PBSP04 *attB*::*Rv1819c*	PBSP04 complemented with full-length *Rv1819c* carried on pTTP1B vector integrated at *attB* locus; $Gent^R$	This study

Plasmids	Description	Source
pAU100	pJG1111 carrying Δ*prpDC* allele – fusion of 1kb PCR products upstream and downstream of *prpDC* eliminating 2660 bp *prpDC* coding sequence; Km^R Hyg^R	Ref. 6
pTTP1B(Gm)	Gentamicin-resistant derivative of pTTP1B; Gm^R, Amp^R	Ref. 7
p*bacA*-Comp	pTTP1B(Gm) carrying 2274 bp region of the *M. tuberculosis* H37Rv chromosome containing the *Rv1819c* gene flanked by 203 bp upstream and 151 bp downstream sequence, Gm^R	This study

Oligonucleotides	Sequence (5´-3´)[a]	Application	Amplicon properties/ region targeted/ reference
Linker 1	TTTCTGCTCGAATTCAAGCTTCTAACGATGTACG GGGACACATG	Used to identify the Tn insertion site. PCR products obtained were sequenced using TnSeqF and TnSeqR	Ref. 8
Linker 2	TGTCCCCGTACATCGTTAGAACTACTCGTACCAT CCACAT		
Y linker	CTGCTCGAATTCAAGCTTCT		
TnSeqF	CGAGATAGGGTTGAGTGT	Sequencing primer	Sequencing primers used to identify Tn insertion sites from the plasmids obtained by rescue cloning
TnSeqR	GTTGGCTACCCGTGATATTG	Sequencing primer	
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* alleles	
prpDC-R2	*GGGGG*TACAACAGGATCTTGGCGAC	Reverse primer used for PCR genotyping of Δ*prpDC* allele	1195bp amplicon from Δ*prpDC* using prpDC-F and PrpDC-R2
Tn_Mar_In	CGCACTGAGAAGCCCTTA	Forward and reverse primer used for PCR-based screen to confirm the presence of MycoMar-1 sequence in Tn mutants	838-bp amplicon generated from Tn mutants
Tn_Mar_Out	GCAAGGTGAGATGACAGGA		
*met*B12seqF1	GGCCCAGTAGCCTTCGGT	Sequencing primer	*met*B12seqF1& R2 were used to amplify the genomic region of the B_{12} riboswitch located upstream of *metE.* The resulting 500 bp amplicon was sequenced using the same primers
*met*B12seqR1	GGCCAGTAGGAGCACCCA	Sequencing primer	
*met*B12seqF2	GGCTGGCAGGTCTTCGGA	Sequencing primer	
*met*B12seqR2	GGCCGATGTCACCGGAGT	Sequencing primer	
bacA F1	CGTGACCACAAATGACAT	Sequencing primer	bacA F1& R5 were used to amply the genomic region of *Rv1819c* with 200 bp upstream and downstream flaking sequences. The resulting 2264 bp amplicon was sequenced using the indicated primers.
bacA F2	TGGCTGATGCTCGGCGTG	Sequencing primer	
bacA F3	ATCGGGCGGCCCCTGATC	Sequencing primer	
bacA F4	ATCATCCGATTGCATGGG	Sequencing primer	
bacA F5	TGACCGGCTGGACGAGGA	Sequencing primer	
bacA R1	AGATGGAAAACAGGTGGC	Sequencing primer	
bacA R2	ATCCTCGCACGCCTGAGC	Sequencing primer	
bacA R3	ACGGTCCAGAACATTGCG	Sequencing primer	
bacA R4	GAGTCGTGAATGTTGCCG	Sequencing primer	
bacA R5	CGCCACCTTGGTCAGCGT	Sequencing primer	
bacA Comp_F	G*GGCG***GAATTC**GGCAGCCGTC	Primers used to amplify *Rv1819c* with flanking region for construction of p*bacA*-Comp	bacA Comp_F and bacA Comp_R were used to generate the 2274 bp complementing allele containing *Eco*RI restriction sites
bacA Comp_R	*GGCGG***GAATTC**GTGACCACAA	Primers used to amplify *Rv1819c* with flanking region for construction of p*bacA*-Comp	
TTP1b	GTCACCGAAAGGCGTGCCCTTGTC	TTP1b and bacA Compl_Forward primer used for PCR screen to confirm the transformants	

a. GC-clamp sequences (non-H37Rv) are italicized; Restriction sites are shown in bold

Table S2: ***Rv1819c*** **polymorphisms in spontaneous mutants of Δ*metH* and Δ*prpDC***

Parent	Strain ID	Selected on	SNP	Amino acid change[§]	Complemented by full-length *Rv1819c*[†]	Whole-genome sequence data[‡]
Δ*metH* (BB)	**Parental**	**-**	**-**	**-**	**-**	**Coverage: 83.5x Additional polymorphisms: none**
Δ*metH* (BB)	**SP09**	**B_{12}**	**T – C (1325)**	**L442S**	**Yes**	**Coverage: 121.4x Additional polymorphisms: none**
Δ*metH* (BB)	**SP18**	**B_{12}**	**C – T (1045)**	**P349T**	**Yes**	**-**
Δ*metH* (BB)	**SP06**	**B_{12}**	**G – A (459)**	**W153***	**ND**	**-**
Δ*metH* (BB)	**SP03**	**B_{12}**	**C – T (1027)**	**Q343***	**ND**	**-**
Δ*metH*	**Parental**	**-**	**-**	**-**	**-**	**-**
Δ*metH*	**MBSP01**	**Bleomycin**	**G – A (1232)**	**G411D**	**ND**	**-**
Δ*metH*	**MBSP04**	**Bleomycin**	**G – A (35)**	**W12***	**ND**	**-**
Δ*metH*	**MBSP05**	**Bleomycin**			**ND**	**-**
Δ*prpDC*	**Parental**	**-**	**-**	**-**	**-**	**-**
Δ*prpDC*	**PBSP01**	**Bleomycin**	**G – A (347)**	**W52***	**ND**	**-**
Δ*prpDC*	**PBSP02**	**Bleomycin**			**ND**	**-**
Δ*prpDC*	**PBSP03**	**Bleomycin**			**ND**	**-**
Δ*prpDC*	**PBSP04**	**Bleomycin**			**Yes**	**-**

[§] An asterisk(*) denotes a nonsense mutation

[†] ND, not done

[‡] Polymorphisms were determined relative to the parental strain, H37RvJO (Ref. 2)

Supplementary References

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