Supplementary information

Table S1 List of strains

Strain	Genotype	Source
E. coli DH5α	Cloning strain (the genotype: F^- Φ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F) U169 *rec*A1 *end*A1 *hsd*R17(r_k^-, m_k^+) *pho*A *sup*E44 *thi*-1 *gyr*A96 *rel*A1 λ^-	Invitrogen
E. coli BL21(DE3)pLysS	Protein expression strain F-ompT hsdSB (rB-, mB-) galdcmrne131 (DE3) carrying pLysS (Cam^r)	Invitrogen
M. smegmatis mc^2155	*ept-1*; high-frequency transformation mutant of *M. smegmatis* ATCC607	(1)
M. tuberculosis mc^26230	Δ*RD1* Δ*panCD*	(2)
M. smegmatis Δ*rnhA*	Derivative of mc^2155 carrying inactivated allele of *rnhA* (*MSMEG_5562)* marked with Kan^r	This study .
M. smegmatis Δ*rnhC*	Derivative of mc^2155 carrying inactivated allele of *rnhC* (*MSMEG_4305*) marked with Hyg^r	This study
M. smegmatis Δ*rnhA* p*rnhA*	Ms Δ*rnhA* complemented with p*rnhA*; Kanr, Hygr	This study
M. smegmatis Δ*rnhC* p*rnhC*	Ms ΔrnhC complemented with p*Rv2228c*	This study
M. smegmatis Δ*rnhC* pMV306G13+ LuxABCDE	*Ms* Δ*rnhC* containing pMV306G13+ LuxABCDE chromosomally integrated; Kan^r	This study
M. smegmatis mc^2155 pMV306G13+ LuxABCDE	*M. smegmatis* mc^2155 containing pMV306G13+ LuxABCDE chromosomally integrated; Kan^r	This study

Table S2 List of plasmids

	Characteristic	Reference
pMAL-C2	Expression vector with IPTG inducible expression of the maltose binding protein as affinity tag and Factor Xa cleavage site upstream of the multiple cloning site; Amp^r	NEB
pAAZ-Rv2228c	pMAL-C2 plasmid modified by replacement of factor Xa site by His6-tag and 3C protease cleavage site; Rv2228c cloned in-frame for expression.	This study
pMV306G13+ LuxABCDE	Mycobacterial integrating vector containing the *luxABCDE* operon from *Photorhabdus luminescens*, Kan^r	(3)
pOLYG	pAL5000-based multicopy *E. coli–Mycobacterium* shuttle vector; Hyg^r	(4)
pEJ414	Integrating plasmid containing promoterless *lacZ* gene, Km^r	(5)
pBBK	p32ΔL with promoter and start codon of *M. tuberculosis fbpA* cloned as a 241 bp *Xba*I–*Bam*HI fragment with *kmr* gene replacing *hyg* to give Kanamycin resistance.	(6)
p*rnhA*	pOLYG containing *rnhA* with 350 bp promoter region cloned as a *Bgl*II/*Hin*dIII fragment.	This study
p32GoriM	p32ΔL-*nuc* with 0.8 kb *aacC1* gene replacing *hyg* to give gentamicin resistance.	This study
p*Rv2228c*	Rv2228c cloned in-frame as a *Bam*HI/*Eco*R1 fragment to the p32ΔL promoter in p32GoriM	This study
pAAZ-*TopA*	pEJ414 containing 530bp upstream and 30bp coding sequence of *MSMEI_6157* fused in frame to *lacZ*	This study
pAAZ-*GyrBA*	pEJ414 containing 231bp upstream and 1000bp coding sequence of *MSMEI_0007* fused in frame to *lacZ*	This study

Table S3. List of HIV RNase HI inhibitors and their activity against *M. tuberculosis* RNase HI at 100 µM (Active: compounds that show a reduction in the activity *M. tuberculosis* RNase HI at 100 µM. Inactive: compounds that show no reduction in the activity of *M. tuberculosis* at 100 µM.)

compound	**Activity against *M. tuberculosis* RNase HI**
NSC353720	Active
NSC600285	Active
NSC600286	Active
NSC353681	Active
NSC14543	Inactive
NSC20410	Inactive
NSC143101	Active
NSC35676	Inactive
NSC45382	Inactive
NSC56351	Inactive
NSC73300	Inactive
NSC18806	Active
NSC112200	Inactive
NSC130796	Inactive
NSC133457	Inactive
NSC228148	Inactive
NSC605657	Inactive
NSC610984	Inactive
NSC99726	Active
NSC80693	Active
NSC668394	Inactive
NSC31892	Inactive
NSC727447	Inactive
NSC51535	Active
NSC117949	Active
NSC128437	Inactive

NSC204474	Inactive
NSC203867	Inactive
NSC99722	Inactive
NSC657715	Inactive
NSC613575	Inactive
NSC205497	Inactive
NSC99727	Inactive

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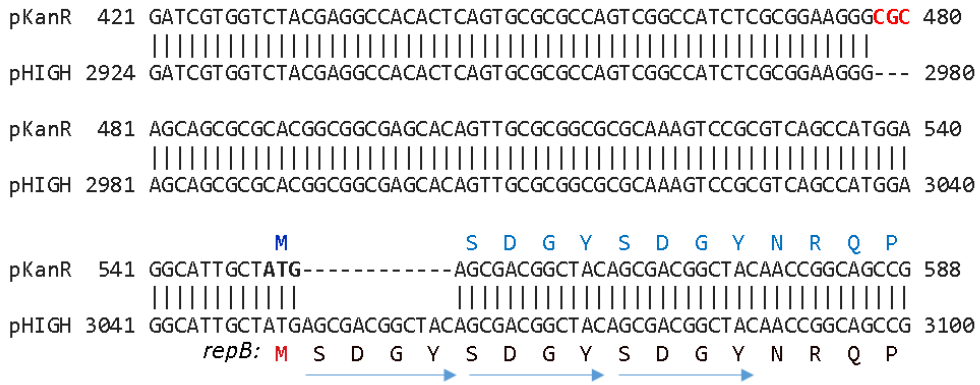

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14 **FIG S1** Nucleotide sequence of the C-terminus of *repA* of pHIGH101 (pHIGH) showing position of the 3 bp
15 deletion reported by Bourn et al which confers 8-fold higher copy number, compared to the 12 bp deletion
16 detected in this study in *repB* on the kanamycin-resistant vector backbone (pKanR). The stop codon of *repA*
17 overlaps with the start codon of *repB.* Arrows indicate three identical direct repeats that could favour a
18 polymerase slippage mechanism of deletion. The amino acid sequence of RepB is indicated next to the coding
19 sequence in each case.

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References

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