

Supplementary Table S2.**Oligonucleotides used in the study**

Name	Sequence, 5'-3'
Oligonucleotides used for F6 deletion mutant construction	
LHA_F6_R	TCTCTCGAATTCACGCCTCCGTTACACGTCCCGAAAA
RHA_F6_F	TCTCTCGAATTCGGTTCGGGAGTGGCTTGATCCAAAGA
RHA_F6_R	CTCTCTGGATCCCGTGAGGCCGCCGACACCAT
LHA-F6_F	TCTCTCAAGCTTGCCTTTCGCTCGCGGTACTACCT
RHA_F6_seq_R	ATCCCGGAGATGCAGGAGGCCA
RHA_F6_seq_F	CCTGATCAGCGGCGCACACAGATCTT
LHA_F6_seq_F	GCATCGCCGCGGGTCGCTA
LHA_F6_seq_R	GAACGCCTCGTGGATCTCGTAGAAGTCG
F6-KO-check_for	TCGCGGAGAAGAAGAAATCCA
F6-KO-check_rev	TGGGGGCGTCACTACTCGT
Oligonucleotides used for Northern blotting	
NB_F6	GTCGGGTCGGGGGGTCAGACGGCAACACGGAGCTA
NB-5S	GTGGTATCCAACGCAGAGT
Oligonucleotides used for quantitative RT-PCR	
Msm0149-for	ACACCGAAACCGACACCCC
Msm0149-rev	TTGGGGCGCAGACTTTCCC
Msm0150-for	ATCTCTCTGCTGAACGCCTTGAC
Msm0150-rev	AACACGATGGCCGGAATGGA
Msm0157-rev	TTGGTGGTGGCATTGGCAAGAG
Msm0157-for	TTGCGCTACATCGGATTCCGG
Msm0162-for	GCGCACTTCGGTTACCTGC
Msm0162-rev	CGGCGTAGATCAGGCTGAACA

msm4640-for1	GTCAACTGGGACGCCATC
msm4640-rev1	ACGTGGACTGCTTGAAGTGC
16S-for	ATGTCGGTTCCTTGTGGC
16S-rev	CAAGGGTTGCGCTCGTTG
Oligonucleotides used for constructions of plasmids	
UTR4640-for	ACCTCTAGATAGTGATCGCGCGGTTG
UTR4640-rev	CCAGGATCCTGCCGTACAAACGTTC
UTR4640-mut-rev	GACTTCCGACACGAGGACCG
UTR4640_mut-for	TGTGAAGGGCCACGATGATGATG
F6-mut-rev	TGTGGGTCAGACGGCAACACG
F6-mut-for	GACTCGACGGCAACACGGG
4640-for_BamH1	GGATCCGTGAAGAGGGCCTTCG
4640-rev_EcoR1	GAATTCCTAGCGACCGAAGGCG
pAMYC-For	AGCAAGAGATTACGCGCAGAC
pAMYC-Rev	GACAGTCATAAGTGCGGCGA
pMV306-For	TATGGAAAAACGCCAGCAACGC
pMV306-Rev	ATGCCTGGCAGTCGATCGTA

Plasmids used and generated in this study

Plasmid	Description	Reference or source
p2NIL	Cloning vector; Km ^r	[1]
pGOAL19	Plasmid carrying <i>hyg</i> , <i>lacZ</i> , and <i>sacB</i> genes in a PacI cassette; Amp ^r , Hyg ^r	[1]
pF6_new_Knockout	Suicide vector to delete F6 in <i>M. smegmatis</i> ; Km ^r , Hyg ^r	This study
pMV306hsp	Integrative shuttle vector, Km ^r	gift from Brian Robertson & Siouxsie Wiles (Addgene plasmid # 26155 ;

		http://n2t.net/addgene:26155 ; RRID:Addgene_26155)
pMV306_rrnB	<p>pMV306hsp derivative bearing <i>Msm rrnB</i> promoter (-79) and <i>rrnB</i> terminator, Km^r</p> <p>The fragment <i>Msm rrnB</i> promoter-<i>rrnB</i> terminator was taken from pMV261_<i>rrnB</i> [2] and cloned into pMV306hsp at the XbaI/SalI restriction sites</p>	This study
pMV306_F6	<p>pMV306_rrnB derivative expressing F6 under <i>Msm rrnB</i> promoter, Km^r</p> <p>F6 gene was amplified from the <i>M. smegmatis</i> genomic DNA and cloned into pMV306_rrnB at the BamHI/HindIII restriction sites</p>	This study
pMV306_MSMEG4640 ^{5'utr} GFP	<p>pMV306_rrnB derivative expressing GFP fused with MSMEG4640 5'UTR under <i>Msm rrnB</i> promoter, Km^r</p> <p>GFP (a kind gift of Dr. Irina Boni, Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russia) was cloned into pMV306_rrnB at the BamHI/HindIII restriction sites. 5'UTR MSMEG-4640 was amplified with UTR-4640-for/UTR-4640-rev primers and cloned upstream GFP at XbaI/BamHI restriction sites.</p>	This study
pMV306_MSMEG4640 ^{5'utr} mut_GFP	<p>pMV306_rrnB derivative expressing GFP fused with mutant MSMEG4640 5'UTR, Km^r</p> <p>Mutations in 5'UTR4640 were introduced into pMV306_MSMEG4640^{5'utr}GFP using a Q5 Site-Directed Mutagenesis Kit (NEB) and primers UTR4640-mut-for/UTR4640_mut-rev</p>	This study

pAMYC	Replicative shuttle vector, Tet ^r , Cm ^r (pACYC184 derivative carrying <i>oriM</i>)	[3]
pAMYC_rrnB	pAMYC bearing <i>Msm rrnB</i> promoter and <i>rrnB</i> terminator, Tet ^r , Cm ^r	This study
pAMYC_F6	pAMYC_rrnB derivative expressing F6 under <i>Msm rrnB</i> promoter, Tet ^r , Cm ^r	This study
pAMYC_F6mut	pAMYC_rrnB derivative expressing mutant F6 gene under <i>Msm rrnB</i> promoter, Tet ^r , Cm ^r Mutations in the F6 seed were introduced into pAMYC_F6 using a Q5 Site-Directed Mutagenesis Kit (NEB) and primers F6-mut-for/F6-mut-rev	This study

***Mycobacterium smegmatis* strains generated in this study**

Strain	Description
ΔF6	<i>M. smegmatis</i> mc ² 155 lacking F6 gene
ΔF6:pMV306	<i>M. smegmatis</i> ΔF6 with integrated plasmid pMV306 rrnB (Km ^r)
ΔF6:F6	<i>M. smegmatis</i> ΔF6 with integrated plasmid pMV306 F6 (Km ^r)
Msm_GFP	<i>M. smegmatis</i> bearing <i>Msm rrnB</i> '- ' <i>gfp</i> reporter in the chromosome (Km ^r)
Msm_MSMEG4640 _{5'utr} ::GFP	<i>M. smegmatis</i> bearing <i>Msm rrnB</i> '- ' <i>MSMEG4640</i> _{5'utr} '- ' <i>gfp</i> reporter in the chromosome (Km ^r)
Msm_MSMEG4640 _{5'utr} mut::GFP	<i>M. smegmatis</i> bearing <i>Msm rrnB</i> '- ' <i>MSMEG4640</i> _{5'utr} mut'- ' <i>gfp</i> reporter
MsmΔF6_MSMEG4640 _{5'utr} ::GFP	<i>M. smegmatis</i> ΔF6 bearing <i>Msm rrnB</i> '- ' <i>MSMEG4640</i> _{5'utr} '- ' <i>gfp</i> reporter
MsmΔF6__F6_MSMEG4640 _{5'utr} ::GFP	<i>M. smegmatis</i> ΔF6 bearing F6 gene in the replicative shuttle vector (Cm ^r) and <i>Msm rrnB</i> '- ' <i>MSMEG4640</i> _{5'utr} '- ' <i>gfp</i> reporter in the chromosome (Km ^r)
MsmΔF6__F6mut_MSMEG4640 _{5'utr} ::GFP	<i>M. smegmatis</i> ΔF6 bearing mutant F6 gene in the replicative shuttle vector (Cm ^r) and <i>Msm rrnB</i> '- ' <i>MSMEG4640</i> _{5'utr} '- ' <i>gfp</i> reporter in the chromosome (Km ^r)
MsmΔF6__F6mut_MSMEG_4640 _{5'utr} mut::GFP	<i>M. smegmatis</i> ΔF6 bearing mutant F6 gene and <i>Msm rrnB</i> '- ' <i>MSMEG4640</i> _{5'utr} mut'- ' <i>gfp</i> reporter (Km ^r)

Amp^r, ampicillin resistant; Km^r, kanamycin resistant, Tet^r, tetracycline resistant; Cm^r, chloramphenicol resistant

References:

1. Parish, T.; Stoker, N.G. Use of a flexible cassette method to generate a double unmarked *Mycobacterium tuberculosis* tlyA plcABC mutant by gene replacement. *Microbiology* **2000**, *146*, 1969-1975.
2. Ignatov, D.V.; Salina, E.G.; Fursov, M.V.; Skvortsov, T.A.; Azhikina, T.L.; Kaprelyants, A.S. Dormant non-culturable *Mycobacterium tuberculosis* retains stable low-abundant mRNA. *BMC genomics* **2015**, *16*, 1-13.
3. Aseev, L.V., Koledinskaya, L.S., Bychenko, O.S. and Boni, I.V., Regulation of Ribosomal Protein Synthesis in *Mycobacteria*: The Autogenous Control of rpsO. *International Journal of Molecular Sciences*, **2021**. 22(18), p.9679.