Table S1. Plasmids used in this work.

Plasmids	Relevant genotype and/or information	Ref.
pET24a(+)	Phagemid vector (Km ^r <i>lacZ</i> ') for expression of recombinant proteins under control of strong transcription and translation signals	Novagen
pFR3	pET24a(+) with $Rv3208$ (o $fasR_{MT}$) His tag fusion gene, under T7 promoter control, Km ^r	This study
pMV306	Single copy integrative vector; inserts into the phage L5 chromosomal integrating site <i>attB</i> in many mycobacteria, Km ^r	(Stover, C. K. et al., 1991)
pJAM2	<i>Mycobacterium</i> expression vector, carrying inducible acetamidase promoter, <i>oriM</i> , Km ^r	(Triccas, J. A. et al., 1998)
pFR9	$fasR_{MT}$ His tag fusion gene under acetamidase promoter control in pJAM2, Km ^r	This study
pPR27	<i>E. coli-Mycobacterium</i> shuttle vector, <i>oriM</i> temp ^s , <i>sacB</i> , <i>xylE</i> , Gm ^r	(Pelicic, V. <i>et al.</i> , 1997)
pFR20	pPR27 derivative carrying P _{ptr} - 5'fasR _{MS}	This study
pFRA42B	pFRA40 derivative; P_{smyc} -tet $R(\rightarrow)$; $P_{furA102}$ tet O - pip (\rightarrow) ; P_{ptr} -lacZ; int; Str ^r	(Boldrin, F. <i>et al.</i> , 2010)
pSM128	<i>Mycobacterium</i> integrative promoter-probe vector, carrying the mycobacteriophage L5 integrase gene, attachment sites, Str ^r	(Dussurget, O. et al., 1999)
pFR47	pSM128 derivative carrying a transcriptional fusion of $P_{fas_{MT}}$ to a promoterless <i>lacZ</i> , Str ^r	This study
pFR48	pSM128 derivative carrying a transcriptional fusion of mutated $P_{fas_{MT}}$ (Mut1) to a promoterless <i>lacZ</i> , Str ^r	This study
pFR49	pSM128 derivative carrying a transcriptional fusion of mutated $P_{fas_{MT}}$ (Mut2) to a promoterless <i>lacZ</i> , Str ^r	This study
pFR50	pSM128 derivative carrying a transcriptional fusion of mutated $P_{fas_{MT}}$ (Mut3) to a promoterless <i>lacZ</i> , Str ^r	This study

Gm^r, gentamicin resistance; Km^r, kanamycin resistance; Str^r, streptomycin/ spectinomycin resistance; Apra^r, apramycin resistance

Table S2. Bacterial strains used in this work.

Strain	Comments	
DH5a	E. coli K12 F- ΔlacU169 (φ80lacZΔM15) endA1 recA1 hsdR17 deoR supE44 thi-1λ- gyrA96 relA1	(Hanahan, D., 1983)
BL21λ(DE3) Codon Plus	E. coli B F- ompt hsdS (rB- mB-) dcm+ TetR gal (DE3)endA Hte [argU ileY leuW], CmR]	Stratagene
<i>M. smegmatis</i> mc ² 155	Electroporation-proficient <i>ept</i> mutant of <i>M. smegmatis</i> strain mc26	(Snapper, S. B. <i>et al.</i> , 1990)
<i>M. tuberculosis</i> H37Rv	Wild type, virulent strain	(Cole, S. T. <i>et al.</i> , 1998)
MSpFR47	<i>M. smegmatis</i> mc ² 155 harboring pFR47, Str ^r	This study
MSpSM128	<i>M. smegmatis</i> mc ² 155 harboring pSM128, Str ^r	This study
MSpFR9	<i>M. smegmatis</i> mc ² 155 harboring pFR9, Km ^r	This study
MSpSM128 pFR9	<i>M. smegmatis</i> mc ² 155 harboring pSM128 and pFR9, Str ^r Km ^r	This study
MSpFR47 pFR9	<i>M. smegmatis</i> mc ² 155 harboring pFR47 and pFR9, Str ^r Km ^r	This study
MSpFR48 pFR9	<i>M. smegmatis</i> mc ² 155 harboring pMR48 and pFR9, Str ^r Km ^r	This study
MSpFR49 pFR9	<i>M. smegmatis</i> mc ² 155 harboring pMR49 and pFR9, Str ^r Km ^r	This study
MSpFR50 pFR9	<i>M. smegmatis</i> mc ² 155 harboring pMR50 and pFR9, Str ^r Km ^r	This study
MSP <i>tr:fasR</i> _{MS}	<i>M. smegmatis fasR</i> conditional mutant strain harboring pFR20 and pFRA42B, Apra ^r Str ^r	This study

Gm^r, gentamicin resistance; Km^r, kanamycin resistance; Apra^r, apramycin resistance; Str^r, streptomycin/ spectinomycin resistance.

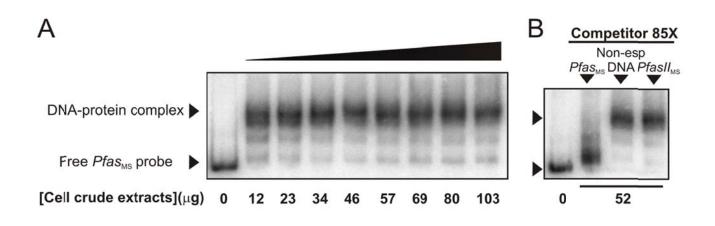


Figure S1. Detection of DNA-binding activity to *Pfas*_{MS} in extracts of *M. smegmatis* wild type strain mc²155.

A. Gel shift assay was performed by incubating the ³²P-labeled 448 bp probe with increasing concentrations (from 0 to 103 μ g) of *M. smegmatis wild type* strain mc²155 crude extracts from exponential phase cultures, in the presence of poly-dIdC.

B. The specificity of the binding was confirmed by competing labelled $Pfas_{MS}$ probe with a 85-fold excess of unlabelled $Pfas_{MS}$ probe or a 85-fold excess of non-related DNA or $PfasII_{MS}$ probe.

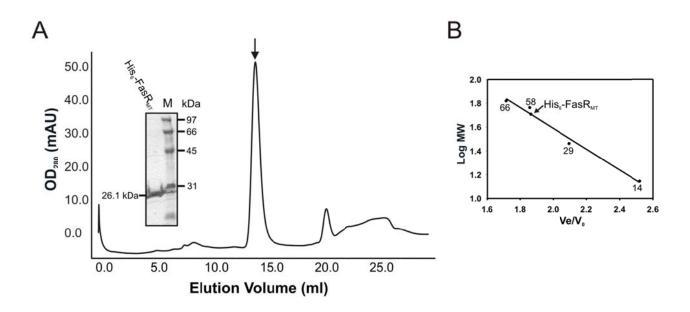


Figure S2. FasR is a dimer in solution

A. Gel exclusion chromatographic profile of recombinant $FasR_{MT}$ -His₆ run on a Superdex 200 GL column (GE Healthcare). The expected peak of purified $FasR_{MT}$ -His₆ was eluted at the position of 14.24 ml (indicated with an arrow). The inset gel is the SDS-PAGE analysis of pure $FasR_{MT}$ -His₆. The apparent molecular weight of $FasR_{MT}$ -His₆ is about 26.1 kDa. OD₂₈₀, optical density at 280 nm; mAU, milli-absorbance units. B. Determination of $FasR_{MT}$ -His₆ solution structure according to elution patterns of a series of standard proteins. The standard proteins were lysozyme (~14 kDa), carbonic anhydrase (monomer ~29 kDa; dimer ~58 kDa) and bovine serum albumin (66 kDa). The elution position of $FasR_{MT}$ -His₆ is indicated with an arrow. Ve/V₀, relative elution volume.

Mtb	
BCG	AACCCCGGG
Msmeg	
Msp.	
Mmar	AGCGCCG
Mul	TGGGCAGCGCCG
Mav Mabs	
Mabs	CATGGGTT6CAACGGCGCGGCAGTGTTAGCAAACATTCGGCACACGTTCTGATTTCTTGC
Mtb	
BCG	CCAACGCCGCCAATGGCGCGGCCTTTGGGTCGGCGCGTCGGCACGCGCCCGGCGTAGAGC
Msmeg	
Msp.	GCCTGCGCTGTCGACCCATTTGCGTCCCTGAACTCGTCATTCGGATGCT
Mmar	TCCGCGCCGATGCCCGCGCGCTGAAACGGCTGAGACGATGTCGCACGGTGGCCTGGGTGC
Mul	TCCGCGCCGATGCCCACGCGCTGAAACGGCTGAGACGATGTCGCACGGTGGCCTTGGCGC
Mav	GGCGATCGCCGCACGGCGCCGCCGACGCCGGCTCGACGCGCCTCGCGCGCG
Mabs	CCTGCTGGCATCCTTGGTAGAGCCGCCCACGCCGACACGCGCGAATGTGGTCGGCGCCAC
Mtb	CATAACGATTTGATAACAAAACTGCCTTGATCAGCGCAGTTATAGCTACT
BCG	GAATTCCCAGCATAACGATTTGATAACAAAACTGCCTTGATCAGCGCAGTTATAGCTACT
Msmeg	GATAACGATTAGATAACAATGCTGCCTTGATCTGCGGTGTTGTCCCTACT
Msp.	GAAAAATTTA GATAACGATTTGGTAACGATAATGCTTTGATCAGCGTAGTTATAGCTACT
Mmar	CAACTTTTAACATAACGATTTGGTAACAATACTGCCTTGATCAGCGCAGTTATAGCTACT
Mul	CAACTTTTAACATAACGATTTGGTAACAATACTGCCTTGATCAGCGCAGTTATAGCTACT
Mav	AAAATTTTTACATAACGATTCGATAACAAAACTGCTTTGATCTGCGCAGTTATAGCTACT
Mabs	CCAAACGCAACATAACGATTCGATAACAATCGATGTCTGATCTGCGATGTTATACCTACT
Mtb	CCACCGTAACCACCTGCACGCAGGACGTTTGGTCAACACATACGCGCCGGCCG
BCG	CCACCGTAAC CACCTGCACGCAGGACGTTTGGTCAACACATACGCGCCGGCCG
Msmeg	CGGCCGTAACCACCTGCCAACAGGACGTTTGTCCTGAACGCCTCGGGCCGCCCGTCGGCG
Msp.	CGACCGTAACCACCCGCCAGCAGGACGTTTGTCCCGGAAGCCGTGCGCGCCGTCCGAACA
Mmar	CAGGCGTAAC CACCTGCATGCAGGACGTTTGTTCGGCATGCCTGTGCCGTCGGTTAAACG
Mul	CAGGCGTAACCACCTGCATGCAGGACGTTTGTTCGGCATGCCTGTGCCGTCGGTTAAACG
Mav	CGACCGTAACCACCTGCACGCAGCAGGTTTGTTCGCCGCGTCCGAGCGGCCGGC
Mabs	CGACCGTAACCACCTGCATGCAGCACGTTTGATTCGGCGGTTAGTTGCCAGCGCCTGGCC
Mtb	CGGCGTTACCCACGACACGGT <u>TACCCGTACGTA</u> G <u>AACTCGCCAGTA</u> ACCGATCTG-TGTA
BCG	CGGCGTTACCCACGACACGGTTACCCGTACGTAGAACTCGCCAGTAACCGATCTG-TGTA GCGCGTAACGTTACCGACGGTTACCCGTGCGTAGAACTCATCAGTAACCGATCCG-CGCC
Msmeg	GCGCGTAACGTTACCGACGGTTACCCGTGCGTAGAACTCATCAGTAACCGATCCG-CGCC GGGTGTTACCCAAGCGATGGTTACCCGAGCGTAGAACTCGCCAGTAACCGATTCGGCGAC
Msp. Mmar	CGGTGTTACCCATGACACGGTTACCCGTGCGTAGAACTCGCCAGTAACCGATTCGGCGAC CGGTGTTACCCATGACACGGTTACCCGTGCGTAGAACTCGCCAGTAACTTATTGG=CGTC
Mul	CGGTGTTACCCATGACACGGTTACCCGTGCGTAGAACTCGCCAGTAACTTATTGG-CGTC
Mav	CGGTGTTACCCACAACACGGTTACCCATGCGTAGAACTCACCAGTAACCGATCAG-CACG
Mabs	TGGTGTTACGTAAGCGGTGGTTACTCCCGCGTAGAACTCGGCAGTAACTATTACG-CCAG
	* ** ** ** ****** * ******** ****** * *
	3 3 ~ 3 2 ~ ~ ~ 2 ~ ~ 2 ~ ~ ~ ~ ~ ~ ~ ~
Mtb	AACAAGGTGGACCGATCCTTATGAGACTCTTATAA-GGCCGATGCAGCCCGTCGCGCTGC
BCG	AACAAGGTGGACCGATCCTTATGAGACTCTTATAA-GGCCGATGCAGCCCGTCGCGCTGC
Msmeg	GAAAAGGCCGCTCTGTTCTTATGCCACTCTTATTA-GGGCGCCGCCGCGAGCAGACCCAC CCGGGAGCCCCTCC-TTCTTATGCCACTCTTAGTG-GGGCTGGGTTGTCCGCAGGCGGAC
Msp. Mmar	AAAAGAGTGGACGGATCCTTATGACACTCTTATAT-GGCCGATGCAGTCCGTCGCGCCGC
Mul	AAAAGAGTGGACGGATCCTTATGAGACACTCTTATAT-GGCCGATGCAGTCCGTCGCGCCGC
Mav	CGAAACGAGGGCCGATCCTTATGACACTCTTAGTGCAGCCGATGAAGTCCGCCGCCGC
Mabs	AAGAAATCGCAACCCCTCTTATGACACTCTTAAGTCCGGACCCTGCGGTGA
	****** ****** * * * * *
Mtb	TCGACCCACGGGCCGGCCCACCGACAACACAC
BCG	TCGACCCACGGGCCGGCCCACCGACAACACAC
Msmeg	-CCGGGACCGCTCCGCACGCCCCAGGATTCGACGTCGAATCGCCGTTGGCCTGACGCAC
Msp.	TCCACGAGAGGTCCGCTCGCGACAGCGACCAGCCGGATTCGACGC
Mmar	CCGATCCATGGGCCGGCCCGCCGACAACACAC
Mul	CCGATCCATGGGCCGGCCCGCCGACAACACAC
Mav	
	GCGG-CCGCCGACGTCACAC
Mabs	Tregeneeaceaceaceaceaceaceaceaceaceaceaceaceac
Mabs	
Mabs Mtb	
	7GGTGCAGATGAACGTGC
Mtb BCG Msmeg	TGGTGCAGATGAACGTGC * * CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGGGATGCCC CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGGGATGCCC CGAGCGCCCAGGTCGCCCCCAGGACCCCCCAGAGCCCCCCAGGACTCCGCCAAGACC
Mtb BCG Msmeg Msp.	TGGTGCAGATGAACGTGC * * * CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGGGATGCCC CG-GTCAGAGGATTTCGGCTGGGACTCGGTGCCGCAGTCGGGGATGCCC CGAGGCGCCCAGGGCCCCCAGGACCCGCCACAACACCCACGAGATCCCCGCAAGACC CGAATGCCGTCGGCCGCCCCCAGGGCACGGCCCGGCCGCCAGAAGACC
Mtb BCG Msmeg	TGGTGCAGATGAACGTGC CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGGGATGCCC CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGGGATGCCC CGAGTCGCCCASGTCGCGCCGCAGAGACCGCCACAGAGCACCACCAGAGATCCCCC CGATTGCCGTCGGCCGCCGCGGCGCCC
Mtb BCG Msmeg Msp. Mmar Mul	TGGTGCAGATGAACGTGC CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGGGATGCCC CG-GTCAGAGGATTTCGGCTGGGAGCGGGGGGGGGGGG
Mtb BCG Msmeg Msp. Mmar Mul Mav	TGGTGCAGATGAACGTGC CG-GTCAGAGGATTTCGGCTGGGAGTCGGGAGCGGAGTCGGGGATGCCC CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGGGATGCCC CGAGCGCCC-AGTGCGCCCCACGAGCGCCACCAGCACCACCACGAGATCCCGCCAGAGCC CG-GTTACAGGATTTTTCGGCTGCGATTCGACGCGGGAATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC
Mtb BCG Msmeg Msp. Mmar Mul	TGGTGCAGATGAACGTGC CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGGGATGCCC CG-GTCAGAGGATTTCGGCTGGGAGCGGGGGGGGGGGG
Mtb BCG Msmeg Msp. Mmar Mul Mav	TGGTGCAGATGAACGTGC * * * CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGGGATGCCC CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCCGAGTCGGCGGATGCCC CGAGCGGCCCAGGTCGCCGCCGCGGGCGCGCGCCGTCGGCGCAGAAGACC CG-GTTACGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC CG-GCCGCC-GTTTCCCGGCCGCGATCGACGGGGAATCGACGGCGCACC CG-GCCGCC-GTTTCCCGCCGCGATCGACGGGGAATCGACGGGCGCC
Mtb BCG Msmeg Msp. Mmar Mul Mav Mabs	TGGTGCAGATGAACGTGC CG-GTCAGAGGATTTCGGCTGGGAGTCGGGAGAGTCGGGGATGCCC CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGGGATGCCC CGAGGCGCC-AGTGCGCCCCACGAGCGCCACGACCACCACGAGATCCCGCCAGAGACC CGATTGCCGTCGCGCGCGCGGCAGCGGGGACATCGAGCAACCCC CG-GTTACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC CG-GCCCGC-GTTTCCGACGCGGAGACGACACCGGGAC CACAACACGCCCCCGATCGACAGAGACGACGACACCGGGTGC
Mtb BCG Msmeg Msp. Mmar Mul Mav	TGGTGCAGATGAACGTGC * * * CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGGGATGCCC CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCCGAGTCGGCGGAGAGCC CGAGCGGCCCAGGTCGCCGCCGCGGGCGCGCGCCGTCGGCGCAGAAGAC CG-GTTACGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCCGCGATCGACGCGGAATCGAGCAACCCC CG-GCCGCC-GTTTCCCGGCCGCGATCGACGGGGAATCGACGGCGCAC CCCACACACGCCCCCCGATCGACGGGAATCGACGGGGTGC
Mtb BCG Mameg Mmar Mul Mav Mabs Mtb	TGGTGCAGATGAACGTGC CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGGGATGCCC CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGCGGATGCCC CGAGTCGCCGTCGCCGCCGCAGGACACCGCCACAGACACCACGAGATCCCCCAGAGACC CG-GTTACAGGATTTTCGGCTGGCATTCGACGCGGAATCGAGCAGACCCC CG-GTCACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCCGCGATTCGACGCGGAATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCCGCGATTCGACGCGGAATCGAGCAGCCC CG-GTCACAGGATTTTTCGGCCGCGACATCGAGCAGCACCC CACAACACGCCCCCGATCGACAGAGACGACGACACGGGTGC * * CGCACGGCCGCCCGGCCCGCTGTACGAACTGACACGTACCGATGCACATAG
Mtb BCG Msmeg Msp. Mmar Mul Mav Mabs Mtb BCG	TGGTGCAGATGAACGTGC CG-GTCAGAGGATTTCGGCTGGGAGTCGGGGAGTCGGGGATGCCC CG-GTCAGAGGATTTCGGCTGGGACTCGGTGCGGAGTCGGGGATGCCC CGAGCGCCCGTCGGCCCCCCGCAGCGCACCGCCACGACCACCACGCAGATCCCCGCAAGACC CG-GTTACAGGATTTTTCGGCTGGGTTCGACGCGGAATCGACGACCCC CG-GTCACAGGATTTTTCGGCTGGGTTCGACGCGGAATCGACGACCCC CG-GTCACGGCTGTTCCGCCGCGATTCGACGCGGAATCGACGACCCC CG-GCCGGCC-GTTTCCCCGGCCGCATCGACGGGAATCGACGGCACC CC-GCCGGCC-GTTTCCCCGCCCGATCGACGGGAATCGACGGCCCC CACAACACGCCCCCGATCGACGAGCGGAATCGACGGGCACC CACAACACGCCCCCGATCGACGGGAATCGACGACGGGCC CACAACACGCCCCCGATCGACGGGGA
Mtb BCG Msmeg Mmar Mul Mav Mabs Mtb BCG Mtb BCG Msmeg	TGGTGCAGATGAACGTGC CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGGGATGCCC CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGGGATGCCC CGAGTCGCCGTCGGCGCGCCGCGGGGCGCCCCCACGAGCCCCCCCGCAGAGCC CGATTGCCGTCGGCCGCCGCGGCGGCGGCGCCATCGAGCAACCCC CG-GTTACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGCGCATCGACGGGAATCGAGCAACCCC CG-GCCCGCC-GTTTCCGCCGCCGCATCGACAGGACCGCGACAGCGGGCAC CACAACACGCCCCCCGATCGACAGAGACGACGACACGGGCAC * *
Mtb BCG Mameg Mmar Mul Nav Mabs Mtb BCG BCG Mameg Msp. Mmar Mul	TGGTGCAGATGAACGTGC CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGGGATGCCC CGAGTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGCGGATGCCC CGAGTCGCCGTCGCCGCGCGCGCGGCGGCGCGTCGGCGGCAGAAGACC CG-GTTACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAGCCC CG-GCCCGCC
Mtb BCG Msmeg Msp. Mmar Mul Mav Mabs Mtb BCG Msmeg Msp. Mmar Mul Mav	ACGTGC CG-GTCAGAGGATTTCGGCTGGGAGTCGGGGACGTCGGGGATGCCC CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGGGATGCCC CGAGCGCCGTCGCGCCCACGACCGCCACAGACCACCACGAGATCCCC CGAGTGCCGTCGCGCCCCCGCGGCGCAGCGGCGCACGGCGCCATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGGCATTCGACGGGGAATCGAGGCAACCCC CG-GTCACAGGATTTTTCCGCTGCGATTCGACGGGGAATCGAGGCAACCCC CG-GCCGGCC-GTTTCCCGGCCGCGATTCGACGGGGAATCGAGGCAACCCC CC-GCCGGCC-GTTTCCCGGCCCGCATCGACGAGAGCGACGACACGGGCAC CC-GCCGGCC-GTTTCCCGGCCGGATTCGACGGGGAATCGACGGCACC CC-GCCGGCC-GTTTCCCGGCCGGATTCGACGGGGAATCGACGGCACC CC-GCCGGCC-GTTTCCCGGCCGGATTCGACGGGGAGACGACACGGGGCC CC-GCCGGCC-GTTTCCCGGCCGGATCGACCGTACCGATGCACATAG
Mtb BCG Mameg Mmar Mul Nav Mabs Mtb BCG BCG Mameg Msp. Mmar Mul	TGGTGCAGATGAACGTGC CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGGGATGCCC CGAGTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGCGGATGCCC CGAGTCGCCGTCGCCGCGCGCGCGGCGGCGCGTCGGCGGCAGAAGACC CG-GTTACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGCGATTCGACGCGGAATCGAGCAGCCC CG-GCCCGCC
Mtb BCG Msmeg Msp. Mmar Mul Mav Mabs Mtb BCG Msmeg Msp. Mmar Mul Mav	ACGTGC CG-GTCAGAGGATTTCGGCTGGGAGTCGGGGACGTCGGGGATGCCC CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGGGATGCCC CGAGCGCCGTCGCGCCCACGACCGCCACAGACCACCACGAGATCCCC CGAGTGCCGTCGCGCCCCCGCGGCGCAGCGGCGCACGGCGCCATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGGCATTCGACGGGGAATCGAGGCAACCCC CG-GTCACAGGATTTTTCCGCTGCGATTCGACGGGGAATCGAGGCAACCCC CG-GCCGGCC-GTTTCCCGGCCGCGATTCGACGGGGAATCGAGGCAACCCC CC-GCCGGCC-GTTTCCCGGCCCGCATCGACGAGAGCGACGACACGGGCAC CC-GCCGGCC-GTTTCCCGGCCGGATTCGACGGGGAATCGACGGCACC CC-GCCGGCC-GTTTCCCGGCCGGATTCGACGGGGAATCGACGGCACC CC-GCCGGCC-GTTTCCCGGCCGGATTCGACGGGGAGACGACACGGGGCC CC-GCCGGCC-GTTTCCCGGCCGGATCGACCGTACCGATGCACATAG
Mtb BCG Msmeg Msp. Mmar Mul Mav Mabs Mtb BCG Msmeg Msp. Mmar Mul Mav	ACGTGC CG-GTCAGAGGATTTCGGCTGGGAGTCGGGGACGTCGGGGATGCCC CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGGGATGCCC CGAGCGCCGTCGCGCCCACGACCGCCACAGACCACCACGAGATCCCC CGAGTGCCGTCGCGCCCCCGCGGCGCAGCGGCGCACGGCGCCATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGGCATTCGACGGGGAATCGAGGCAACCCC CG-GTCACAGGATTTTTCCGCTGCGATTCGACGGGGAATCGAGGCAACCCC CG-GCCGGCC-GTTTCCCGGCCGCGATTCGACGGGGAATCGAGGCAACCCC CC-GCCGGCC-GTTTCCCGGCCCGCATCGACGAGAGCGACGACACGGGCAC CC-GCCGGCC-GTTTCCCGGCCGGATTCGACGGGGAATCGACGGCACC CC-GCCGGCC-GTTTCCCGGCCGGATTCGACGGGGAATCGACGGCACC CC-GCCGGCC-GTTTCCCGGCCGGATTCGACGGGGAGACGACACGGGGCC CC-GCCGGCC-GTTTCCCGGCCGGATCGACCGTACCGATGCACATAG
Mtb BCG Msmeg Msp. Mmar Mul Mav Mabs Mtb BCG Msmeg Msp. Mmar Mul Mav	ACGTGC CG-GTCAGAGGATTTCGGCTGGGAGTCGGGGACGTCGGGGATGCCC CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGTCGGGGATGCCC CGAGCGCCGTCGCGCCCACGACCGCCACAGACCACCACGAGATCCCC CGAGTGCCGTCGCGCCCCCGCGGCGCAGCGGCGCACGGCGCCATCGAGCAACCCC CG-GTCACAGGATTTTTCGGCTGGCATTCGACGGGGAATCGAGGCAACCCC CG-GTCACAGGATTTTTCCGCTGCGATTCGACGGGGAATCGAGGCAACCCC CG-GCCGGCC-GTTTCCCGGCCGCGATTCGACGGGGAATCGAGGCAACCCC CC-GCCGGCC-GTTTCCCGGCCCGCATCGACGAGAGCGACGACACGGGCAC CC-GCCGGCC-GTTTCCCGGCCGGATTCGACGGGGAATCGACGGCACC CC-GCCGGCC-GTTTCCCGGCCGGATTCGACGGGGAATCGACGGCACC CC-GCCGGCC-GTTTCCCGGCCGGATTCGACGGGGAGACGACACGGGGCC CC-GCCGGCC-GTTTCCCGGCCGGATCGACCGTACCGATGCACATAG
Mtb BCG Mameg Map. Mul Mav Mabs Mtb BCG Msmeg Msp. Mmar Mul Mav Mabs	CG-GTCAGA-GGATTCGGCGGGGGGGGGCGCCCCAGGGGGGGGGG
Mtb BCG Mameg Map. Mmar Mul Mav Mabs Mtb BCG Msmeg Msp. Mmar Mul Mav Mabs Mtb BCG Msmeg Mtb BCG Msmeg	GAGGCGCGGCGGCGGAACGACGGACGGCGGCGGCGGCGGC
Mtb BCG Map. Mmar Mul Mav Mabs Mtb BCG Map. Mmar Mul Mav Mabs Mtb BCG Mmar Mul May Mabs	GGTGCAGATGAACGTGC CG-GTCAGAGGATTTCGGCTGGGAGTCGGTGCGGAGGCGGGGATGCCC CGAGTGCAGGATTTCGGCTGGGAGTCGGTGCGGAGGCGGGGATGCCC CGAGTGCCGTGGCGGCGCCAGAGCGCCACAGACCACCACGAGATCCCGCCAGAGCC CG-GTCACAGGATTTTCGGCTGGGATTCGACGGGGAATCGAGGCACCC CG-GTCACAGGATTTTCGGCTGGGATTCGACGGCGAATCGAGGCACCC CG-GTCACAGGATTTTCGGCTGGGATTCGACGGCGAATCGAGGCACCC CG-GTCACAGGATTTTTCGGCTGGCGATTCGACGGCGAATCGAGGCACCC CG-GCCCGGCCGCGTTCGACGGCGAATCGAGGCAGCC CACAACACG
Mtb BCG Mameg Map. Mmar Mul Mav Mabs Mtb BCG Msp. Mul Mav Mabs Mtb BCG Msmeg Msp. Msmeg Msp.	GAGGCCCAGATCACGGCGCGCGACGGCGGCGGCGGCGGCGGCGGCGGCGCGCGCGCGCGCGCG
Mtb BCG Msmeg Mmar Mul Mabs Mtb BCG Msmeg Msp. Mmar Mul Mav Mabs Mtb BCG Msp. Mtb BCG Msp. Mtb BCG Msp. Mul	GGAGACATAGACC-GTGACGATCCACGAGCGCGCGGCGTGTCCGCTGATCG CGC-GTAGACATAGACC-GTGACGATCCACGACCGCCGCGACGCCCCCCGCAGACCCCCCGCACGACCACC
Mtb BCG Mameg Mmar Mul Mav Mabs Mtb BCG Mameg Mar Mul Mav Mabs Mtb BCG Mameg Map. Mmar Mul May May	GAGGGCGCGAGATCGACGGGGGGGGCGCGAGGGGGGGGGG
Mtb BCG Msmeg Mmar Mul Mabs Mtb BCG Msmeg Msp. Mmar Mul Mav Mabs Mtb BCG Msp. Mtb BCG Msp. Mtb BCG Msp. Mul	GGAGACATAGACC-GTGACGATCCACGAGCGCGCGGCGTGTCCGCTGATCG CGC-GTAGACATAGACC-GTGACGATCCACGACCGCCGCGACGCCCCCCGCAGACCCCCCGCACGACCACC

Figure S3. Sequence alignment of the promoter region of the *fas* gene of several mycobacteria.

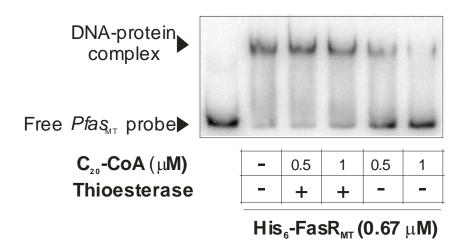


Figure S4. Long chain acyl-CoA inhibition of FasR binding is reversible.

Gel shift assays were performed by incubating the ³²P-labeled 398 bp probe with 0.67 μ M of His₆-FasR_{MT} in the absence and in the presence of C₂₀-CoA at a final concentration of 0.5 and 1 μ M. When indicated, the reaction mix was supplemented with 4 U of *E. coli* thioesterase I. A unit of thioesterase activity is the amount of enzyme required to hydrolyze 1 nmol of acyl-CoA per min at 25 °C. The plasmid for the expression and purification of *E. coli* thioesterase I was the generous gift of Dr. Ana Arabolaza (IBR, Rosario).

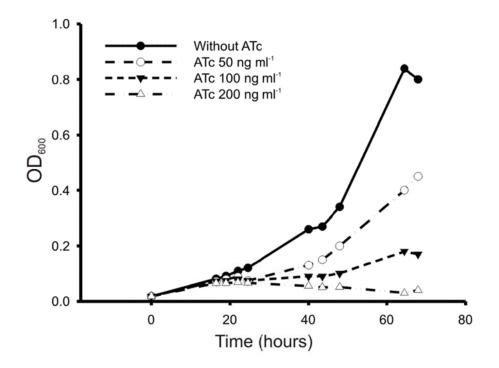


Figure S5. FasR is essential for *M. smegmatis* viability

A. Growth curve of strain MSP*tr:fasR*_{MS} incubated at 42 °C in 7H9 medium containing 200 ng ml⁻¹ ATc (empty triangles), 100 ng ml⁻¹ ATc (filled triangles), 50 ng ml⁻¹ ATc (empty circles) or No ATc (filled circles).

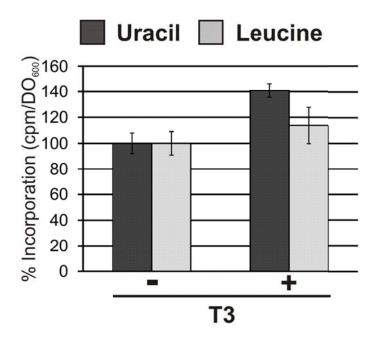


Figure S6. Determination of metabolic activity by incorporation of radioactive precursors

Aliquots of 5 ml of MSP*tr:fasR*_{MS} cultures with (+) and without (-) ATc 200 ng ml⁻¹ were labeled for 1 h at T3 with L-[4,5 - 3 H(N)] leucine (60 Ci/mmol) or [5,6 - 3 H] uridine (36 Ci/mmol) (New England Nuclear), at concentrations of 0.5 µCi/ml. Cells were then pelleted, washed three times with Tris-HCl buffer, and resuspended in 1 ml scintillation liquid. Radioactivity was determined in a Beckman scintillation liquid counter. The results were normalized by OD_{600nm} and are the average of three independent experiments.

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