Table S1. Plasmids used in this work.

Plasmids	Relevant genotype and/or information	Ref.
pET24a(+)	Phagemid vector (Km^r *lacZ´*) 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 *attB* in many mycobacteria, Km^r	(Stover, C. K. *et al.*, 1991)
pJAM2	*Mycobacterium* expression vector, carrying inducible acetamidase promoter, *oriM*, 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	*E. coli-Mycobacterium* shuttle vector, *oriM* $temp^s$, *sacB, xylE,* Gm^r	(Pelicic, V. *et al.*, 1997)
pFR20	pPR27 derivative carrying P_{ptr}- 5'$fasR_{MS}$	This study
pFRA42B	pFRA40 derivative; P_{smyc}-*tetR*(→); $P_{furA102}$*tetO*-*pip*(→); P_{ptr}-*lacZ*; *int*; Str^r	(Boldrin, F. *et al.*, 2010)
pSM128	*Mycobacterium* 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 Pfas_{MT} to a promoterless *lacZ*, Str^r	This study
pFR48	pSM128 derivative carrying a transcriptional fusion of mutated Pfas_{MT} (Mut1) to a promoterless *lacZ*, Str^r	This study
pFR49	pSM128 derivative carrying a transcriptional fusion of mutated Pfas_{MT} (Mut2) to a promoterless *lacZ*, Str^r	This study
pFR50	pSM128 derivative carrying a transcriptional fusion of mutated Pfas_{MT} (Mut3) to a promoterless *lacZ*, 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	
DH5α	*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
M. smegmatis mc^2155	Electroporation-proficient *ept* mutant of *M. smegmatis* strain mc26	(Snapper, S. B. *et al.*, 1990)
M. tuberculosis H37Rv	Wild type, virulent strain	(Cole, S. T. *et al.*, 1998)
MSpFR47	*M. smegmatis* mc^2155 harboring pFR47, Str^r	This study
MSpSM128	*M. smegmatis* mc^2155 harboring pSM128, Str^r	This study
MSpFR9	*M. smegmatis* mc^2155 harboring pFR9, Km^r	This study
MSpSM128 pFR9	*M. smegmatis* mc^2155 harboring pSM128 and pFR9, Str^r Km^r	This study
MSpFR47 pFR9	*M. smegmatis* mc^2155 harboring pFR47 and pFR9, Str^r Km^r	This study
MSpFR48 pFR9	*M. smegmatis* mc^2155 harboring pMR48 and pFR9, Str^r Km^r	This study
MSpFR49 pFR9	*M. smegmatis* mc^2155 harboring pMR49 and pFR9, Str^r Km^r	This study
MSpFR50 pFR9	*M. smegmatis* mc^2155 harboring pMR50 and pFR9, Str^r Km^r	This study
MSP*tr*:*fasR*$_{MS}$	*M. smegmatis fasR* 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.

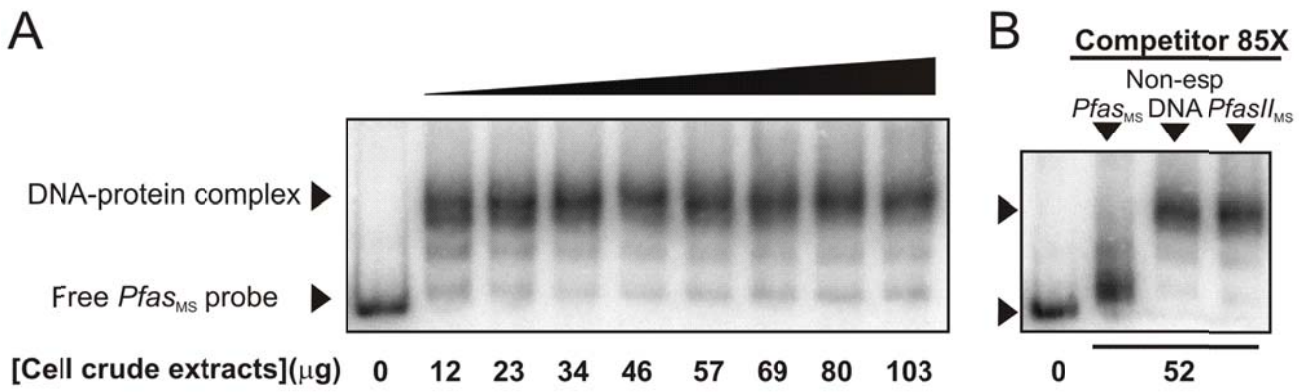

Figure S1. Detection of DNA-binding activity to *Pfas*$_{MS}$ in extracts of *M. smegmatis wild type* strain mc^{2}155.

A. Gel shift assay was performed by incubating the ^{32}P-labeled 448 bp probe with increasing concentrations (from 0 to 103 μg) of *M. smegmatis wild type* strain mc^{2}155 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 P*fas*$_{MS}$ probe or a 85-fold excess of non-related DNA or *PfasII*$_{MS}$ probe.

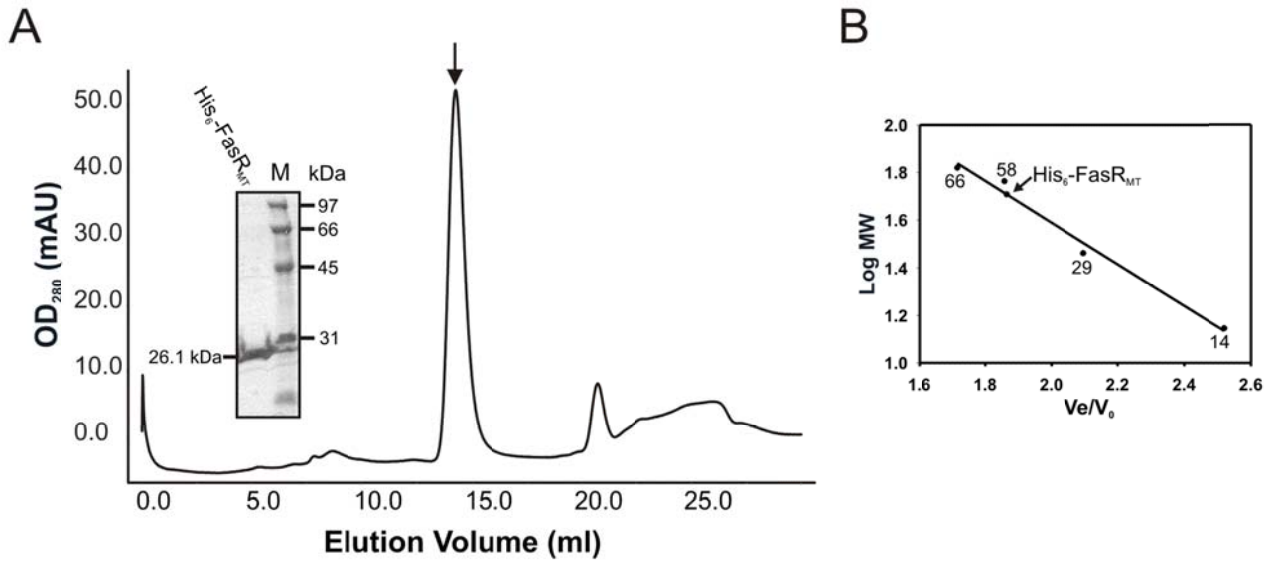

Figure S2. FasR is a dimer in solution

A. Gel exclusion chromatographic profile of recombinant $FasR_{MT}$-His_6 run on a Superdex 200 GL column (GE Healthcare). The expected peak of purified $FasR_{MT}$-His_6 was eluted at the position of 14.24 ml (indicated with an arrow). The inset gel is the SDS-PAGE analysis of pure $FasR_{MT}$-His_6. The apparent molecular weight of $FasR_{MT}$-His_6 is about 26.1 kDa. OD_{280}, optical density at 280 nm; mAU, milli-absorbance units.

B. Determination of $FasR_{MT}$-His_6 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 $FasR_{MT}$-His_6 is indicated with an arrow. Ve/V_0, relative elution volume.

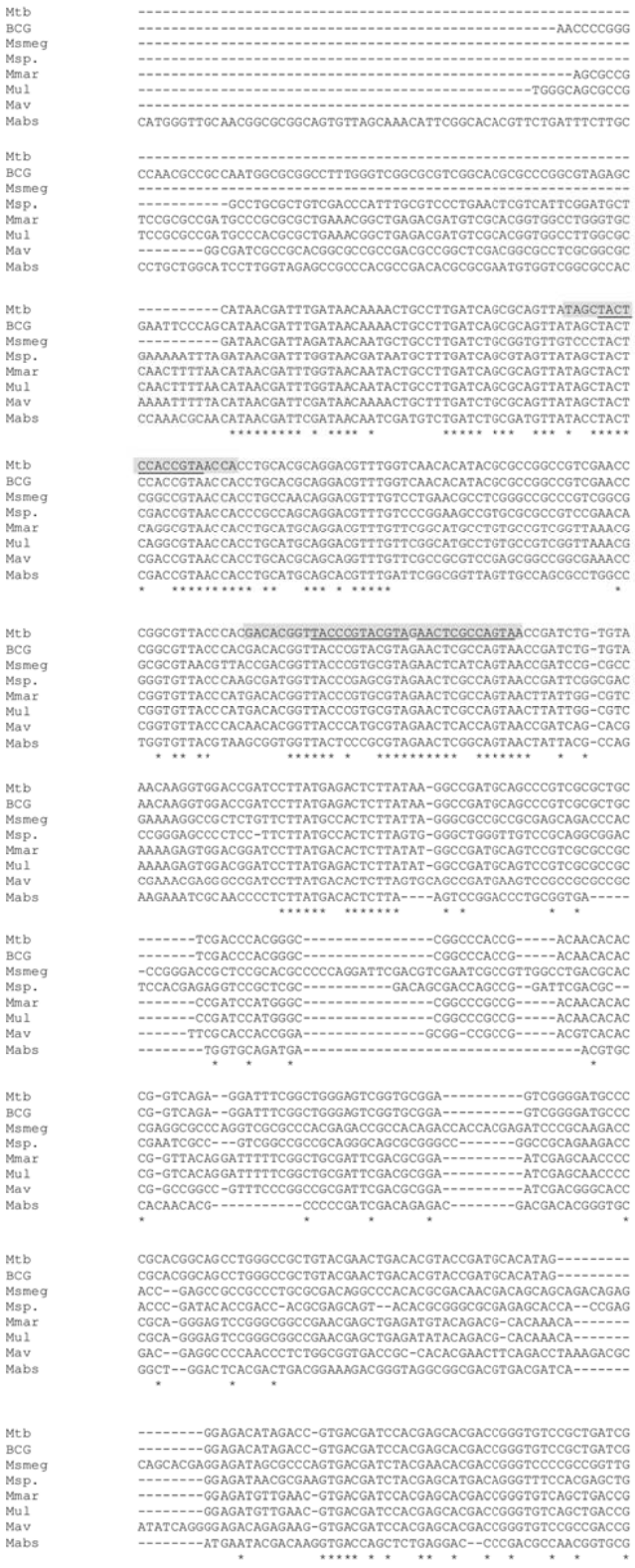

Figure S3. Sequence alignment of the promoter region of the *fas* gene of several mycobacteria.

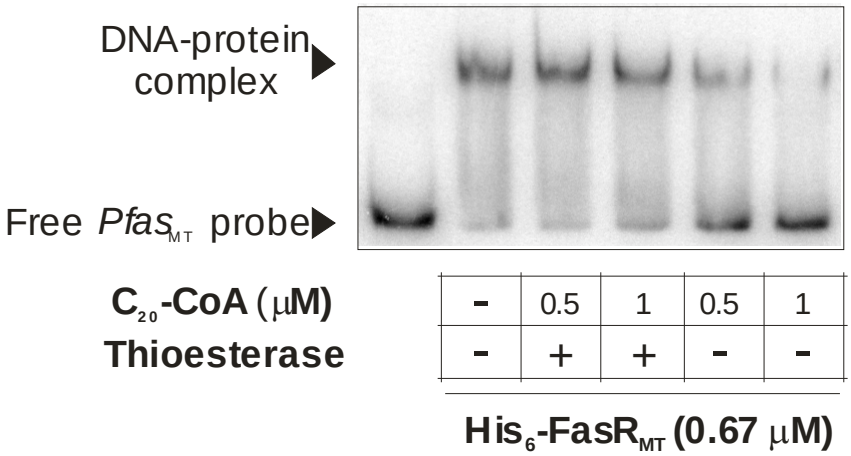

Figure S4. Long chain acyl-CoA inhibition of FasR binding is reversible.

Gel shift assays were performed by incubating the ^{32}P-labeled 398 bp probe with 0.67 μM of His$_6$-FasR$_{MT}$ in the absence and in the presence of C_{20}-CoA at a final concentration of 0.5 and 1 μM. 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 ºC. The plasmid for the expression and purification of *E. coli* thioesterase I was the generous gift of Dr. Ana Arabolaza (IBR, Rosario).

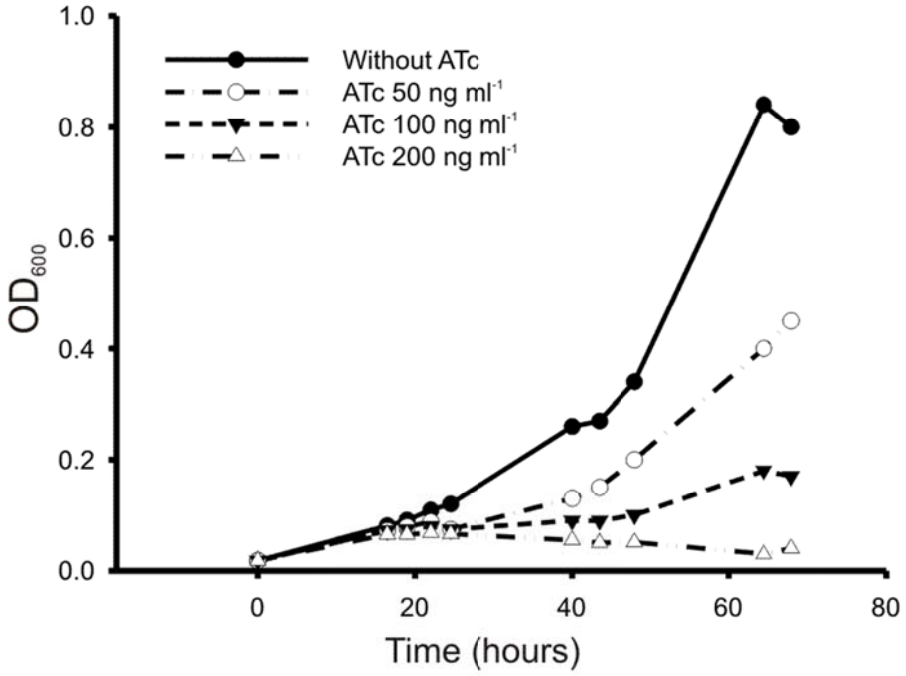

Figure S5. FasR is essential for *M. smegmatis* viability

A. Growth curve of strain MSP*tr:fasR*$_{MS}$ incubated at 42 ºC 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).

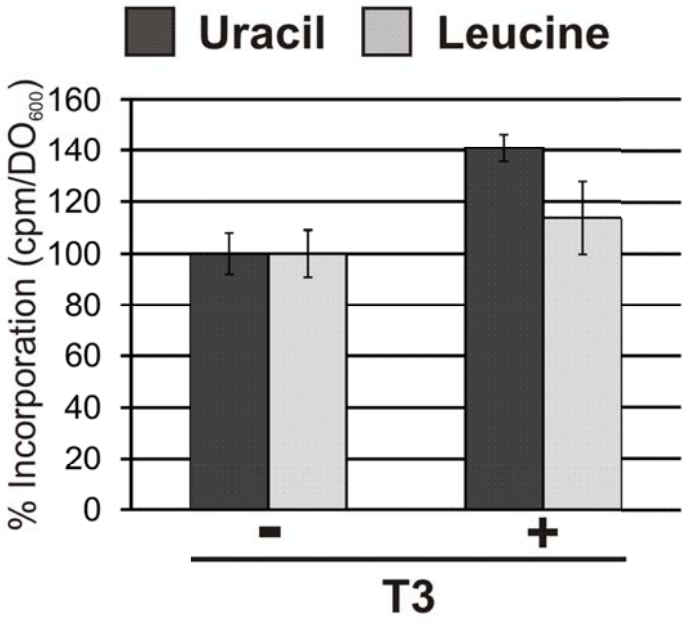

Figure S6. Determination of metabolic activity by incorporation of radioactive precursors

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.

References

Boldrin, F., Casonato, S., Dainese, E., Sala, C., Dhar, N., Palu, G.*, et al.* (2010) Development of a repressible mycobacterial promoter system based on two transcriptional repressors. *Nucleic Acids Res* **38**: e134.

Cole, S. T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D.*, et al.* (1998) Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* **393**: 537-544.

Dussurget, O., Timm, J., Gomez, M., Gold, B., Yu, S., Sabol, S. Z.*, et al.* (1999) Transcriptional control of the iron-responsive *fxbA* gene by the mycobacterial regulator IdeR. *J Bacteriol* **181**: 3402-3408.

Hanahan, D. (1983) Studies on transformation of *Escherichia coli* with plasmids. *J Mol Biol* **166**: 557-580.

Pelicic, V., Jackson, M., Reyrat, J. M., Jacobs, W. R., Jr., Gicquel, B. and Guilhot, C. (1997) Efficient allelic exchange and transposon mutagenesis in *Mycobacterium tuberculosis*. *Proc Natl Acad Sci.U.S.A* **94**: 10955-10960.

Snapper, S. B., Melton, R. E., Mustafa, S., Kieser, T. and Jacobs, W. R., Jr. (1990) Isolation and characterization of efficient plasmid transformation mutants of *Mycobacterium smegmatis*. *Mol Microbiol* **4**: 1911-1919.

Stover, C. K., de la Cruz, V. F., Fuerst, T. R., Burlein, J. E., Benson, L. A., Bennett, L. T. *et al.*, (1991) New use of BCG for recombinant vaccines. *Nature* **351**: 456-460.

Triccas, J. A., Parish, T., Britton, W. J. and Gicquel, B., (1998) An inducible expression system permitting the efficient purification of a recombinant antigen from *Mycobacterium smegmatis*. *FEMS Microbiol.Lett.* **167**: 151-156.