Cholesterol-dependent transcriptome remodeling reveals new insight into the contribution of cholesterol to *Mycobacterium tuberculosis* pathogenesis

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Supplementary Information:

Supplementary Figure S1. - Southern blot confirmation of the *Mtb* knockout mutants genotype.

Original images for Figure S1. - Southern blot photographic films that were used to crop the final images for preparing the Supplementary Figure S1. Cropped areas are marked with white frames. The blots were not cut prior to hybridisation. Cropping helped only to present the data from lines that were not adjacent to each other and to minimize the size of the whole figure.

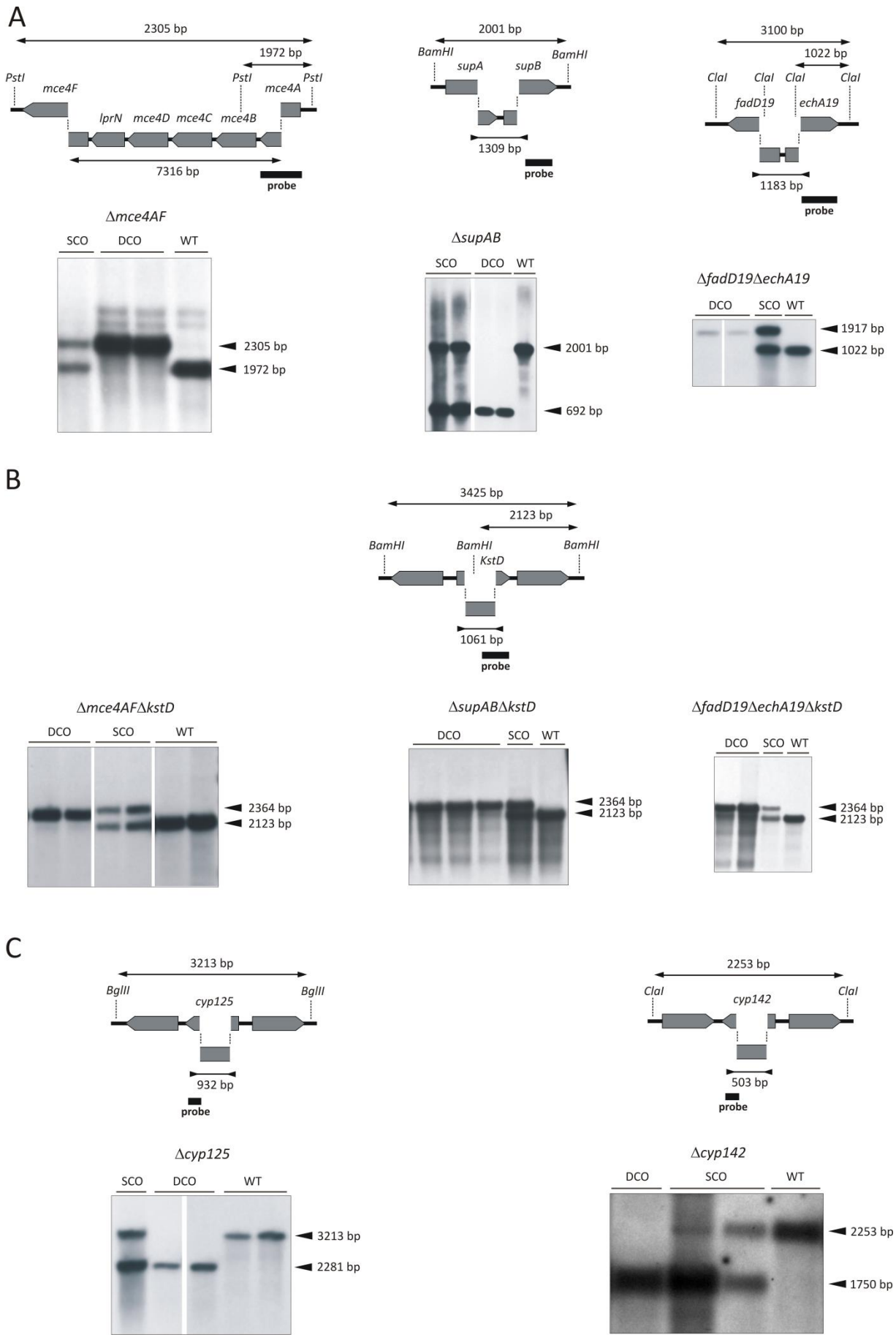
Supplementary Figure S2. - *DosR/S/T* regulon genes upregulated in the transcriptome of *Mtb* growing on cholesterol as the sole carbon source. See the color key for log2FC values.

Supplementary Dataset S1. - see the Excel file

Supplementary Dataset S2. - see the Excel file

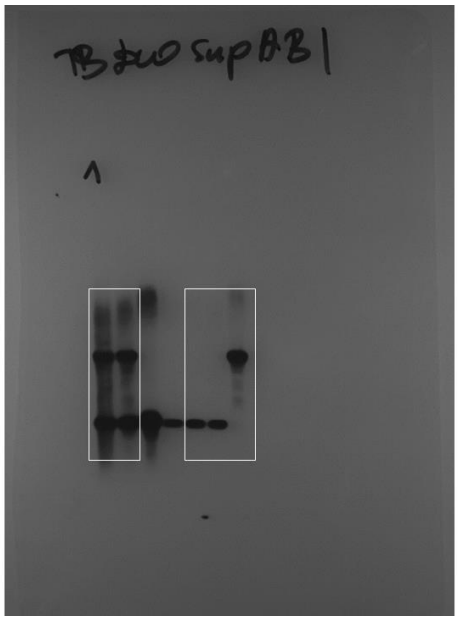
Supplementary Table S1. - Oligonucleotides used for PCR amplification in this study.

Supplementary Table S2. - Plasmid vectors and bacterial strains used in this study.

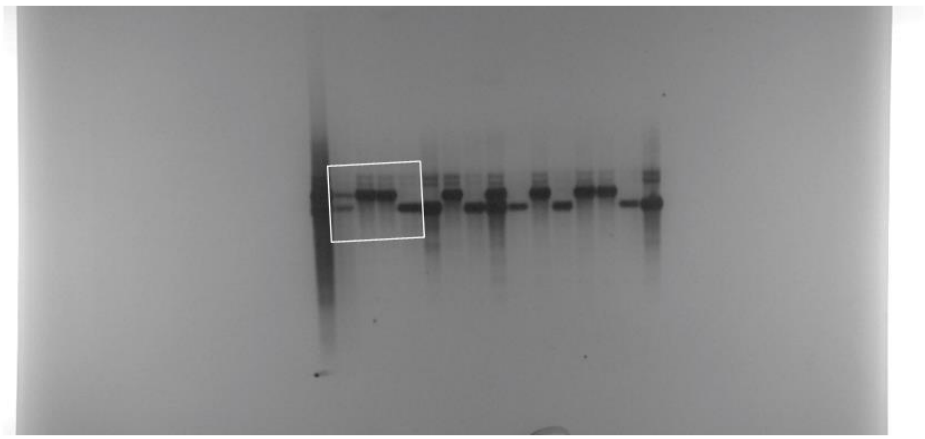


Supplementary Figure S1. Southern blot confirmation of the *Mtb* knockout mutants genotype. Diagrams show the size of deleted regions and restriction fragments after digestion with appropriate enzymes (PstI, BamHI, ClaI, BglII). The site of DNA probe hybridization is presented as a black bar. The size of the

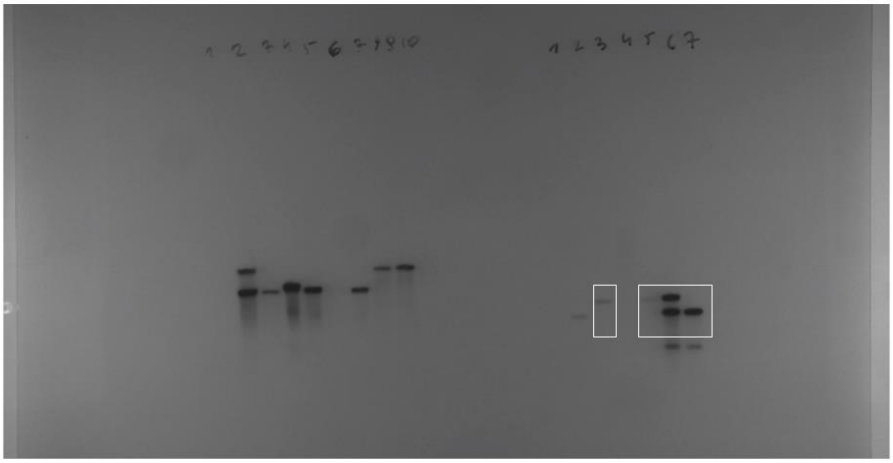
restriction fragments hybridized to a DNA probe is marked next to the blots. Lines represent genomic DNA of WT – *Mtb* wild-type strain, SCO – single-crossover mutant carrying both wild-type and mutated copy of a gene, DCO – double-crossover mutant carrying a mutated copy of a gene. **A.** Southern blot confirmation of the Δ*mce4AF*, Δ*supAB* and Δ*fadD19*Δ*echA19* mutants genotype. **B.** Southern blot confirmation of the *kstD* gene deletion in Δ*mce4AF*Δ*kstD*, Δ*supAB*Δ*kstD* and Δ*fadD19*Δ*echA19*Δ*kstD* mutants. **C.** Southern blot confirmation of the Δ*cyp125* and Δ*cyp142* mutants genotype.



Refers to **Supplementary Figure S1 A (center)** – *supAB* mutant



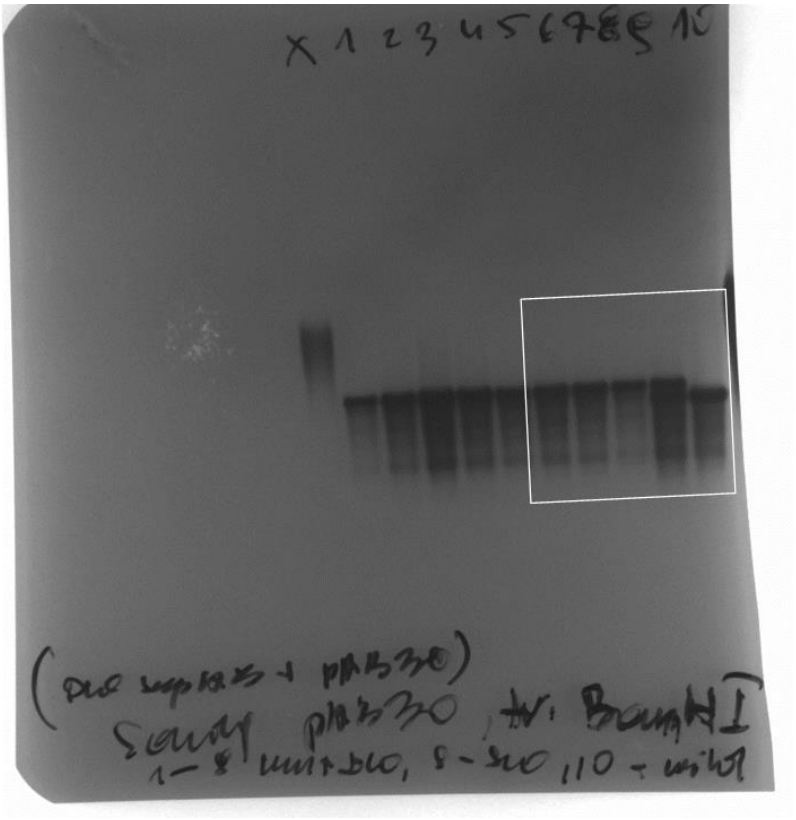
Refers to **Supplementary Figure S1 A (left)** – *mce4AF* mutant

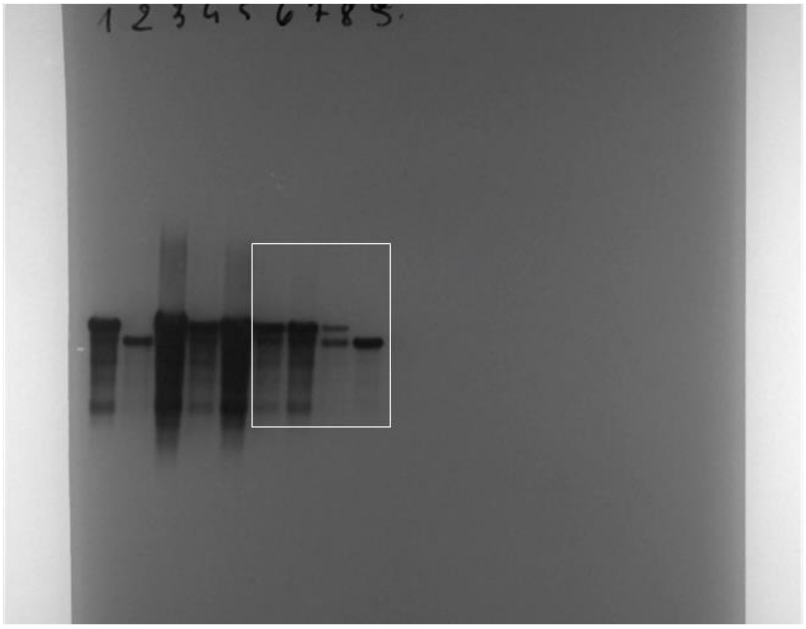


Refers to **Supplementary Figure S1 A (right)** – *fadD19echA19* mutant

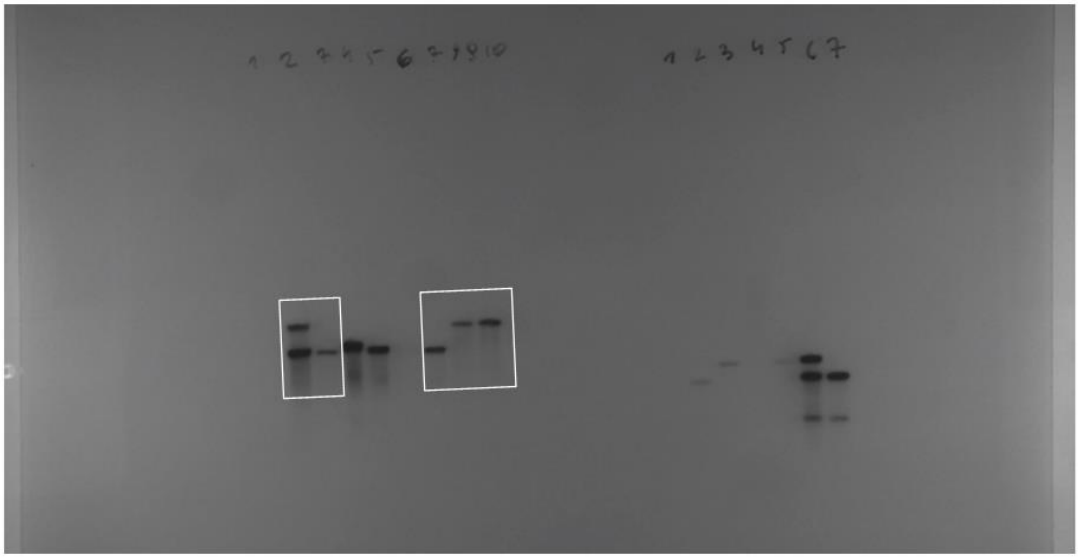
Refers to **Supplementary Figure S1 B (left)** – *mce4AFkstD* mutant

Refers to **Supplementary Figure S1 B (center)** – *supABkstD* mutant

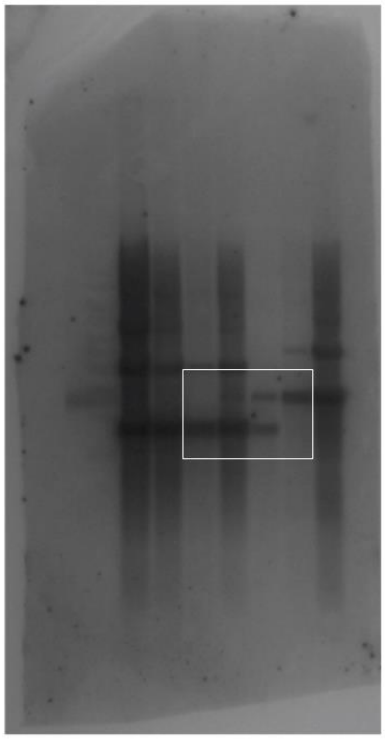




Refers to **Supplementary Figure S1 B (right)** – *fadD19echA19kstD* mutant



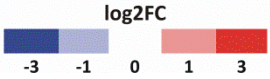
Refers to **Supplementary Figure S1 C (left)** – *cyp125* mutant



Refers to **Supplementary Figure S1 C (right)** – *cyp142* mutant

Original images for Figure S1. - Southern blot photographic films that were used to crop the final images for preparing the Supplementary Figure S1. Cropped areas are marked with white frames. The blots were not cut prior to hybridisation. Cropping helped only to present the data from lines that were not adjacent to each other and to minimize the size of the whole figure.

log2FC	Gene number	Gene name	Probable protein function	log2FC	Gene number	Gene name	Probable protein function
	Rv0079		HP		Rv2028c		Universal stress protein
	Rv0080		CHP		Rv2029c	*pfkB*	Phosphofructokinase
	Rv0081		Transcriptional factor		Rv2030c		CHP
	Rv0082		Oxidoreductase		Rv2031c	*hspX*	Heat shock protein
	Rv0083		Oxidoreductase		Rv2032	*Acg*	CHP
	Rv0569		CHP		Rv2623	*TB31.7*	Universal stress protein
	Rv0570	*nrdZ*	Ribonucleotide red.		Rv2624c		Universal stress protein
	Rv0571c		CHP		Rv2625c		Leucine rich protein
	Rv0572c		HP		Rv2626c	*hrp1*	Hypoxic response protein
	Rv0574c		CHP		Rv2627c		CHP
	Mt0639		HP		Rv2628		HP
	Rv1733c		Transmembrane protein		Rv2629		CHP
	Rv1734c		CHP		Rv2630		HP
	Rv1736c	*narX*	Nitrate reductase		Rv2631		CHP
	Rv1737c	*narK2*	Nitrite transporter		Rv2830c	*vapB22*	Antitoxin
	Rv1738		CHP		Rv3126c		HP
	Rv1812c		Dehydrogenase		Rv3127		CHP
	Rv1813c		CHP		Rv3128c		CHP
	Rv1996		Universal stress protein		Rv3129		CHP
	Rv1997	*ctpF*	Cation trans. ATPase		Rv3130c	*tgs1*	Triacylglycerol synthase
	Rv2003c		CHP		Rv3131		CHP
	Rv2004c		HP		Rv3132c	*dosS*	Sensor hist. kinase
	Rv2005c		Universal stress protein		Rv3133c	*dosR*	Two-comp. resp. reg.
	Rv2006	*otsB1*	Trehalose phosphatize		Rv3134c		Universal stress protein
	Rv2007c	*fdxA*	Ferredoxin		Rv3841	*bfrB*	Bacterioferritin
	Rv2027c	*dosT*	Sensor hist. kinase				



Supplementary Figure S2. *DosR/S/T* regulon genes upregulated in the transcriptome of *Mtb* growing on cholesterol as the sole carbon source. See the color key for log2FC values.

Primer	Sequence 5' → 3'	Description
Construction of gene replacement vectors		
Rvmce-GR1 **Rvmce-GR2**	F: GCTGCAGTACGGACCGGCTGCAGAACTTCAG R: CAAGCTTCGGTCCGGCCTACACCATCTATGA	3' fragment of *mce4F* gene (145 bp) together with downstream sequence (1504 bp)
Rvmce-GR3 **Rvmce-GR4**	F: CAAGCTTGGATCCCACCATCAGCCCGG R: CGGTACCGCAACGTGCGGGCCCTAAGC	5' fragment of *mce4A* gene (75 bp) together with upstream sequence (1734 bp)
RvsupAB-GR1 **RvsupAB-GR2**	F: AACTGCAGACCGTTTCCTCGGATGTAGCAGG R: GCAAGCTTCATTTCGAAGAACCCGCCGAC	5' fragment of *supA* (*yrbE4A*) gene (54 bp) together with upstream sequence (1473 bp)
RvsupAB-GR3 **RvsupAB-GR4**	F: GCAAGCTTTTCGTCAATGGGCAGTCCGC R: GGGGTACCCGTCTGCTTGGTCGGGATATCG	3' fragment of *supB* (*yrbE4B*) gene (279 bp) together with downstream sequence (1078 bp)
Rvcyp142-GR1 **Rvcyp142-GR2**	F: CAGTACTGTCGTGGAGGCAGGGATGAAGC R: CAAGCTTGTTGGTTGGCCCGCATCCAC	5' fragment of *cyp142* gene (94 bp) together with upstream sequence (1850 bp)

Rvcyp142-GR3 **Rvcyp142-GR4**	F: CAAGCTTGACCTGGTCAGCGTGCTGGTGA R: CGCGGCCGCAGGCGCTGACCAAGGCTCTAGA	3' fragment of *cyp142* gene (600 bp) together with downstream sequence (1214 bp)
Rvcyp125-GR1 **Rvcyp125-GR2**	F: GTTGAGGTACCACATCGGGTTC R: GCAAGCTTAGCCGTTCGGCGTAGATTG	5' fragment of *cyp125* gene (113 bp) together with upstream sequence (1671 bp)
Rvcyp125-GR3 **Rvcyp125-GR4**	F: GCAAGCTTAGGTTTTCCAGGATCCGTTCAC R: CTTCTTCTTCTGCAGCTCGCTG	3' fragment of *cyp125* gene (257 bp) together with downstream sequence (1330 bp)
Rvfadech-GR1 **Rvfadech-GR2**	F: AACTGCAGCTGAAGCACCACCTGCCACC R: GCAAGCTTTGATCCGCTACCCGATCCC	3' fragment of *fadD19* gene (915 bp) together with downstream sequence (562 bp)
Rvfadech-GR3 **Rvfadech-GR4**	F: GCAAGCTTCCGGTGAAAGTGCGAAGTTC R: ATGAGTAGCAGACGCAGCCAC	3' fragment of *echA19* gene (414 bp) together with downstream sequence (1490 bp)
qRT-PCR primers		
mce4A-s **mce4A-r**	F: ACCGATTTGGCGCCGAAGAT R: AAGGGCGCCAAGGTCAAATACC	*mce4A* (Rv3499c) expression analysis
supA-s **supA-r**	F: ACTTAGCGGCTTGGGCGACG R: GGAGAAGGGCGCCAAGGTCA	*supA* (*yrbE4A*) (Rv3501c) expression analysis
kstD-s **kstD-r**	F: ACGAGGTCCTCAAGCGCCGC R: CGGAGCCTCGGGGTAGTAGTCGG	*kstD* (Rv3537) expression analysis
fadE30-s **fadE30-r**	F: TCGCCGAAGTGGTTGACCTTGT R: CGAATTCGCGGCACTTAAGGG	*fadE30* (Rv3560c) expression analysis
prpR-s **prpR-r**	F: TCTTCGATCTGGGCGCCGCT R: GCTGGCCAAGGCCCTGGACTT	*prpR* (Rv1129) expression analysis
icl1-s **icl1-r**	F: GCACCTACTCCGCCGAGGACGT R: CTGCCAGCCCGACAGGTAGATGG	*icl1* (Rv0467) expression analysis
fadD26-s **fadD26-r**	F: GTTTTAGCGCCACAAGGACTGGAA R: GGCTGGCTGCGTATTTCGTTACAT	*fadD26* (Rv2930) expression analysis
papA5-s **papA5-r**	F: CTGCGCGGTGTTATCGATGTCG R: GCAATAGGGACACGCTCTGGTCG	*papA5* (Rv2939) expression analysis
mutA-s **mutA-r**	F: CGTGCATTCCGGCTGGAAGG R: AGATACACCCCGGACAGCAGCG	*mutA* (Rv1492) expression analysis
mutB-s **mutB-r**	F: GATGTATGTGAACCAGCCGTGGACC R: TGGAATCGATTGCCACACCGG	*mutB* (Rv1493) expression analysis
tgs1-s **tgs1-r**	F: GGCGATCAGCTCGAATAACTGGTCT R: TTCTTATCGTCGCTCGCTCAACG	*tgs1* (Rv3130c) expression analysis
prpD-s **prpD-r**	F: GCCTACAAGATCGCCCAGGTGG R: ATACCTTCGCCCCGTGTCGC	*prpD* (Rv1130) expression analysis
metE-s **metE-r**	F: CGTCGACGGCCTTGCTCAGC R: GGTTTCCGACGGGCTGGACC	*metE* (Rv1133c) expression analysis
espA-s **espA-r**	F: TGGCGCCCTCCAGGATGTC R: GCAGCAGCGTTTCCGGGTG	*espA* (Rv3616c) expression analysis
sigA-s **sigA-r**	F: CCGATGACGACGAGGAGATC R: CGGAGGCCTTGTCCTTTTC	*sigA* (Rv2703) expression analysis

Supplementary Table S1. Oligonucleotides used for PCR amplification in this study. Underlined regions indicate the restriction sites used during the cloning steps (PstI, HindIII, KpnI, ScaI, NotI, BglII). In some cases, natural restriction sites (KpnI, PstI, BamHI) were used.

Plasmid	Description	Source or reference
Cloning vectors		
pJET1.2/blunt	PCR product cloning vector, Amp^R	Thermo Scientific
p2NIL	Recombination vector, nonreplicating in mycobacteria, Kan^R	[1]
pGOAL17	Source of PacI marker cassette (*sucB*, *lacZ*), Amp^R	[1]
Vectors used for gene replacement		
p2N*mce4*$_{Tb}$	p2NIL-based recombination vector carrying the 3' end of *mce4F* and its downstream flanking sequence (GR1-GR2) (1649 bp) cloned next to the 5' end of *mce4A* and its upstream flanking sequence (GR3-GR4) (1809 bp),	This study

	enriched with the PacI cassette from pGOAL17, Kan^R	
p2N*sup*$_{Tb}$	p2NIL-based recombination vector carrying the 5' end of *supA* and its upstream flanking sequence (GR1-GR2) (1527 bp) cloned next to the 3' end of *supB* and its downstream flanking sequence (GR3-GR4) (1357 bp), enriched with the PacI cassette from pGOAL17, Kan^R	This study
p2N*fadech*$_{Tb}$	p2NIL-based recombination vector carrying the 3' end of *fadD19* and its downstream flanking sequence (GR1-GR2) (1477 bp) cloned next to the 3' end of *echA19* and its downstream flanking sequence (GR3-GR4) 1904 bp), enriched with the PacI cassette from pGOAL17, Kan^R	This study
p2N*cyp125*$_{Tb}$	p2NIL-based recombination vector carrying the 5' end of *cyp125* and its upstream flanking sequence (GR1-GR2) (1784 bp) cloned next to the 3' end of *cyp125* and its downstream flanking sequence (GR3-GR4) (1587 bp), enriched with the PacI cassette from pGOAL17, Kan^R	This study
p2N*cyp142*$_{Tb}$	p2NIL-based recombination vector carrying the 5' end of *cyp142* and its upstream flanking sequence (GR1-GR2) (1944 bp) cloned next to the 3' end of *cyp142* and its downstream flanking sequence (GR3-GR4) (1814 bp), enriched with the PacI cassette from pGOAL17, Kan^R	This study
Bacterial strains used in this study		
***M. tuberculosis* H37Rv**	*Mycobacterium tuberculosis* wild-type strain	Lab stock
Δ*mce4AF*	*Mtb* H37Rv-based knockout mutant carrying an unmarked deletion (7316 bp) encompassing six genes of *mce4* operon (*mce4A*, *mce4B*, *mce4C*, *mce4D*, *lprN* and *mce4F*)	This study
Δ*mce4AF*Δ*kstD*	Δ*mce4AF*-derived mutant containing additional unmarked deletion (1061 bp) within *kstD* gene	This study
Δ*supAB*	*Mtb* H37Rv-based knockout mutant carrying an unmarked deletion (1309 bp) encompassing the 3' end of *supA* and 5' end of *supB* gene	This study
Δ*supAB*Δ*kstD*	Δ*supAB*-derived mutant containing additional unmarked deletion (1061 bp) within *kstD* gene	This study
Δ*fadD19*Δ*echA19*	*Mtb* H37Rv-based knockout mutant carrying an unmarked deletion (1183 bp) encompassing the 5' end of *fadD19* end *echA19* gene	This study
Δ*fadD19*Δ*echA19*Δ*kstD*	Δ*fadD19*Δ*echA19*-derived mutant containing additional unmarked deletion (1061 bp) within *kstD* gene	This study
Δ*cyp125*	*Mtb* H37Rv-based knockout mutant carrying an unmarked deletion (932 bp) within *cyp125* gene	This study
Δ*cyp142*	*Mtb* H37Rv-based knockout mutant carrying an unmarked deletion (503 bp) within *cyp142* gene	This study
Δ*prpR*	*Mtb* H37Rv-based knockout mutant carrying an unmarked deletion (1051 bp) within *prpR* (Rv1129c) gene	[2]

Supplementary Table S2. Plasmid vectors and bacterial strains used in this study.

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* **146**, 1969-75 (2000).

2. Masiewicz, P., Brzostek, A., Wolański, M., Dziadek, J., Zakrzewska-Czerwińska, J. A novel role of the PrpR as a transcription factor involved in the regulation of methylcitrate pathway in Mycobacterium tuberculosis. *PLoS One* **7**, e43651 (2012).