Table S2 Primers used for PCR amplification. Underlined regions indicate the restriction sites used during the cloning steps (BamHI GGATCC, ClaI ATCGAT, EcoRI GAATTC, HindIII AAGCTT, KpnI - GGTACC, PstI CTGCAG, XbaI TCTAGA).

Primer	Sequence	Description
Construction of the gene replacement vector for *M. tuberculosis*:		
TBaccGR1	F: 5' GGGGTACCGCTGCGCGACGGCGTCATC	GR1-GR2 PCR fragment of $accD6_{Mtb}$
TBaccGR2	R: 5' CCCAAGCTTCGCGGGGGTCGAGCGACTCG	
TBaccGR3	F: 5' CCCAAGCTTCCGCTACGCCTGGGCGGCTGC	GR3-GR4 PCR fragment of $accD6_{Mtb}$
TBaccGR4	R: 5' AACTGCAGTCGGCGGCCTCGCTGCTCAG	
Construction of the gene replacement vector for *M. smegmatis*:		
MSaccGR1	F: 5' GGGGTACCAACGCCCTCCGGGTTGCCG	GR1-GR2 PCR fragment of $accD6_{Msm}$
MSaccGR2	R: 5' GCAAGCTTCAGCACACCGGAGCGGTCACG	
MSaccGR3	F: 5' GCAAGCTTCGCGTCGCGCCTACGATGTG	GR3-GR4 PCR fragment of $accD6_{Msm}$
MSaccGR4	R: 5' AACTGCAGCCGATCGCCGAAACCACCG	
Construction of the complementation vectors for *M. tuberculosis*:		
TbP-fas2-Not-nat	F: 5' CCGATGCGGCCGAGGCAC	pFASTb2 and pFD6Tb1 vector construction
TbP-fas2-Xba-nat	R: 5' ATGGCTGGGCGACCGCGG	
Tb-fas2-sense-Xb	F: 5' CGGCGCAGCGGACCAGATC	pFASTb2 vector construction
Tb-fas2-reve-Ec	R: 5' CCGCGTCAGCTGGTGGTCG	
Tb-fas2-senEcoRI	F: 5' GCCAAAGCGGGATCCGTACG	pFASTb2 and pPD6Tb vector construction
Tb-fas2-revEcoRI	R: 5' GGAATTCGCGTGCGATTCTGCGTCTGC	
TBaccD6-XbaIs	F: 5' GCTCTAGACTAAACCCCAGCGTTACGCGAC	pFD6Tb1 vector construction, SCO/DCO mutant screening, Southern blot probe synthesis
TBaccD6-HindIIIrev	R: 5' GAAGCTTGTGCGATTCTGCGTCTGCTCG	
Construction of the complementation vectors for *M. smegmatis*:		
MsaccD6Xs	F: 5' GCTCTAGAATGACGATCATGGCCCCCG	pAceD6Ms vector construction, SCO/DCO mutant screening, Southern blot probe synthesis
MsaccD6Xr	R: 5' GCTCTAGATTACAGCGGGATGTTCTTGTGGC	pAceD6Ms vector construction
MsaccD6HXr	R: 5' GCTCTAGAAAGCTTACAGCGGGATGTTCTTGTGG	Δ*accD6* mutant confirmation, Southern blot probe synthesis
TBaccD6B	F: 5' CGGGATCCATGACAATCATGGCCCCCG	pAceD6Tb vector construction
TBaccD6X	R: 5' GCTCTAGATTACAGCGGGATGTTCTTGTGGCG	
MsD6Cns	F: 5' CGCGGTCGAATCCATCCTGA	pPD6Ms1 vector construction
MsD6prEr	R: 5' GGAATTCGGAGCAGTTTTGCGTCTGTTCGC	
MsD6PCls	F: 5' CCATCGATCGTCTCATGGTGTCGATGGGCA	pPD6Ms1 vector construction
MsD6nCr	R: 5' GCTGACGGCCTCGAAGAAACATTC	
Construction of the vector for $AccD6_{Mtb}$ overexpression:		
TBaccD6s	F: 5' CGGGATCCGATGACAATCATGGCCCCCG	pHD6Tb vector construction
TBaccD6r	R: 5' CCCAAGCTTCTACAGCGGGATGTTCTTGTGGCG	
qRT-PCR primers for *M. smegmatis*:		
accD1 (*MSMEG_4717*)	F: 5' GGTGAAAGGCGGGACGTACTACCC	R: 5' GAGTCCACGAGATAGATGCACGG
accD2 (*MSMEG_5492*)	F: 5' ATCCCTGGACGCTCAAGAAGATCCT	R: 5' TGAAGATCTCTTTCTGCGTCGGCA
accD3 (*MSMEG_5642*)	F: 5' AGCACGGTTGGCTGTCCCCTCTTC	R: 5' AGGTCTGTCGAGCGCACGCCTTG
accD4 (*MSMEG_6391*)	F: 5' GCACTCGGAATGCCCTTCTTCTC	R: 5' ACGAACAAGACCACCGCTGAACTC
accD5 (*MSMEG_1813*)	F: 5' GAAGTTGGTGCTGCGATGCTTGGC	R: 5' GGTGCACGCCAAAGGCAAACTCAC
accD6 (*MSMEG_4329*)	F: 5' CCTGCTGGCAGAACAATCCGACCA	R: 5' GGAGGCTCACCACAAGAAGTCCGG
sigA	F: 5' AGGGCTACAAGTTCTCGACCTACGCC	R: 5' CCGAGCTTGTTGATCACCTCGACC
qRT-PCR primers for *M.. tuberculosis*: Daniel *et al.*, 2006 (18)		
PCR analysis of the FAS-II gene cluster in *M. smegmatis*:		
Ms-fabD-s-pol	F: 5' GCCACGCTGCGTGATCGCTTC	amplification of *fabD-acpM* intergenic region - 1
Ms-acpM-r-pol	R: 5' CGACTTCTCCGGGGTGACCTCG	
Ms-acpM-s-pol	F: 5' GGACGAGGATCTGGCCGGTC	amplification of *acpM-kasA* intergenic region - 2
Ms-kasA-r-pol	R: 5' CGGCAGGTCCCACTTGGTG	
Ms-kasA-s-pol	F: 5' CTCTCCGCGACGGCGTCATC	amplification of kasA-*kasB* intergenic region - 3
Ms-kasB-r-pol	R: 5' GGCCGTCGAGCAGCTTCTTCC	
Ms-kasB-s-pol	F: 5' CGATCTGGATGTTGTCGCCG	amplification of *kasB-accD6* intergenic region - 4, confirmation of $accD6_{Msm}$ deletion (Fig. S3B)
Ms-accD6-r-pol	R: 5' TCGAAGAACGTGCTCAGGCG	
MsD6pr2	R: 5' GGCTCTGCTCCTCGATCGCG	confirmation of $accD6_{Msm}$ deletion (Fig. S3B)
PCR analysis of the FAS-II gene cluster in *M. tuberculosis*:		
Tb-fabD-s-pol	5' ACAGTCACGGCGATCGTGGAGTTC	amplification of *fabD-acpM* intergenic region - 1
Tb-acpM-r-pol	5' CGAACGACTTCTCCGGGGTGATCT	
Tb-acpM-s-pol	5' GGACAAGTACGGCGTCAAGATCCC	amplification of *acpM-kasA* intergenic region - 2
Tb-kasA-r-pol	5' TGAGGTGACCGCCGATCTTGAC	
Tb-kasA-s-pol	5' CCCTGAACTACGAGACACCCGATCC	amplification of kasA-*kasB* intergenic region - 3
Tb-kasB-r-pol	5' GCCGGTGACGACTACGTAGGGAAAG	
Tb-kasB-s-pol	5' TCGATTTGGACGTGGTGGCG	amplification of *kasB-accD6* intergenic region - 4
Tb-accD6-r-pol	5' CACGCTCGTGCAGCAATTCCAC	