Supplemental Figures:

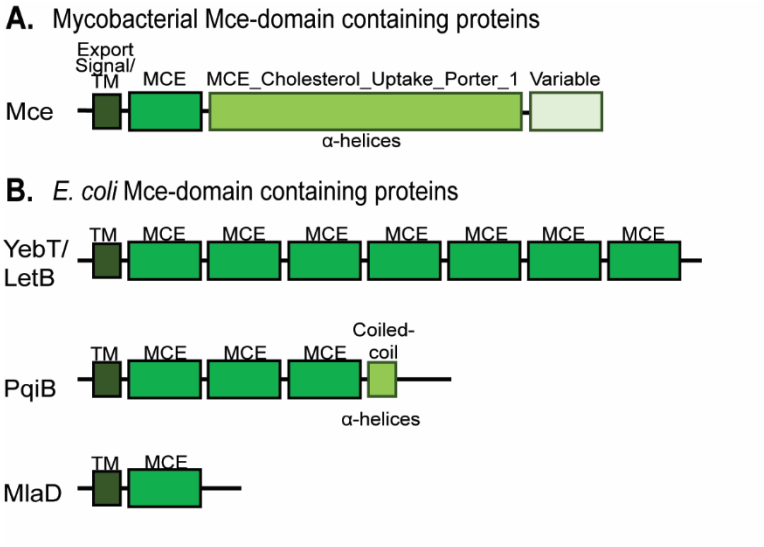

Figure S1:

Domains of Mce-domain containing proteins of **A.** mycobacterial Mce proteins and **B.** *E. coli* Mla, YebT/LetB and PqiB proteins. The Mce domain was identified by PFam02470 and the Mce_CUP1 domain was identified PFam11887 (1).

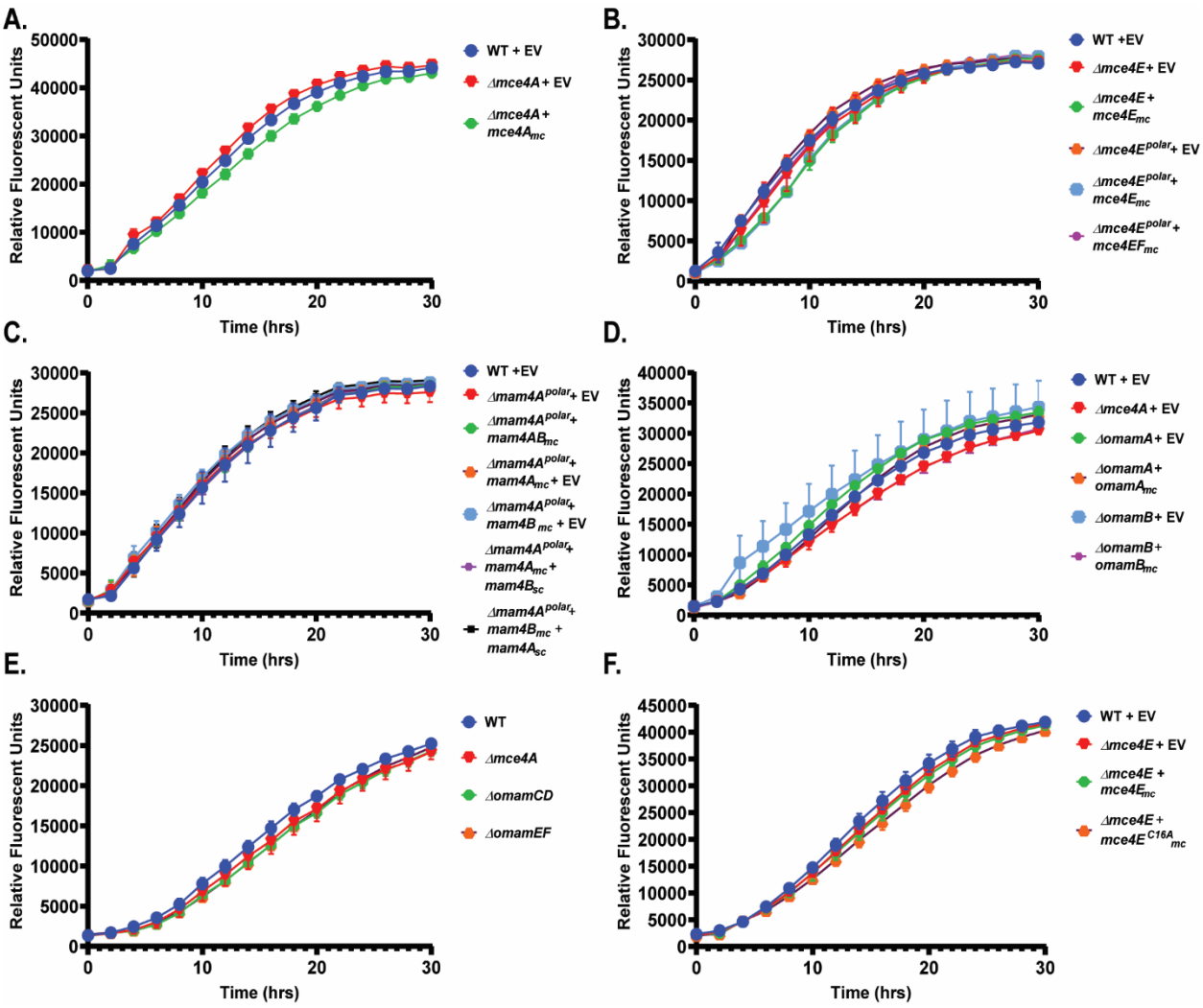

Figure S2:

Individual *mce4*, *mam4*, and *omamA* and *omamB M. smegmatis* mutants and complemented strains do not have a growth defect compared to WT when growing on Glucose and Glycerol carbon sources. **A-E.** Growth of 10^4 colony forming units (cfu) of *M. smegmatis* strains on glucose and glycerol as the sole carbon sources was measured over time using resazurin reduction as a fluorescent readout of metabolic activity (2, 3). Glucose and Glycerol growth curves measured by resazurin reduction of **A.** *Δmce4A* and complemented strains **B.** *Δmce4E*, *Δmce4E*^{polar} and complemented strains **C.** *Δmam4A*^{polar} and complemented strains **D.** *ΔomamA*,

ΔomamB, and complemented strains **E.** Δ*omamCD* and *ΔomamEF* mutants and **F.** *Δmce4E* with Mce4E^{C16A} complementation strains of *M. smegmatis* strains are depicted. Mutant strains contain empty vectors (EV) as indicated.

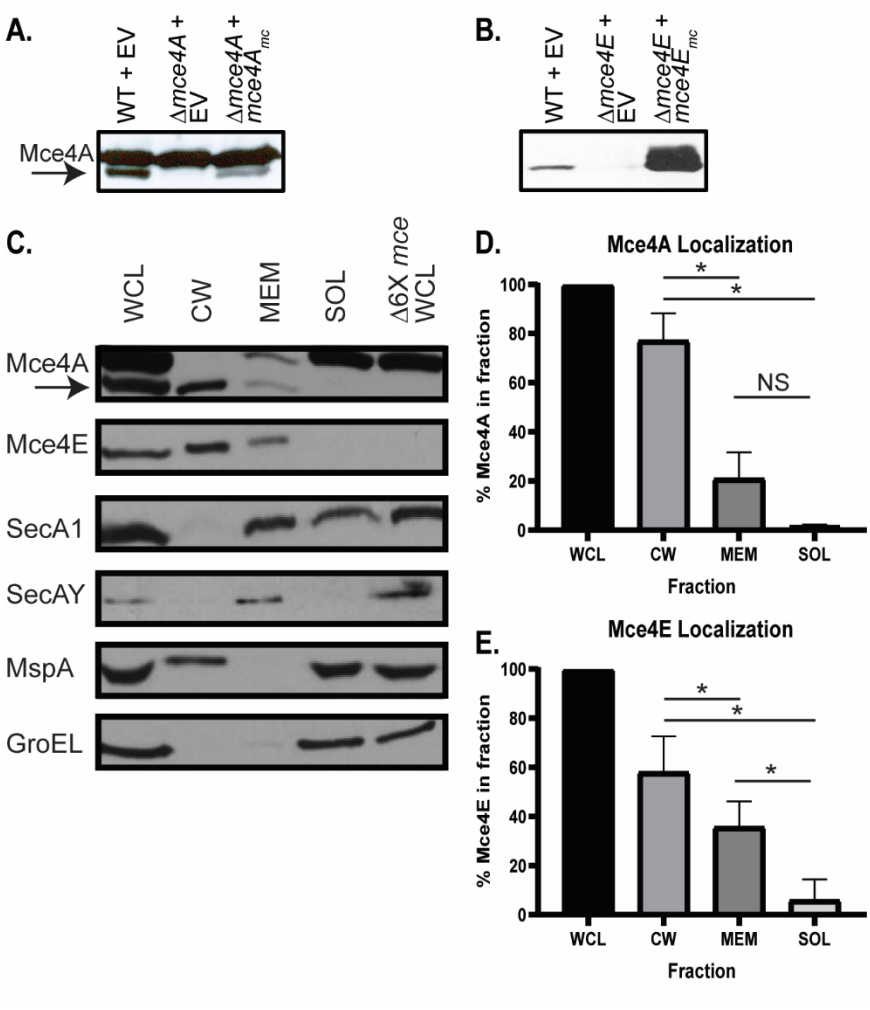

Figure S3:

Localization of Mce4A and Mce4E proteins. **A.** Immunoblots of Mce4A and **B.** Mce4E proteins show that Mce4A and Mce4E antibodies are specific and do not cross react with other Mce proteins. Arrow indicates Mce4A band to distinguish it from the higher species cross-reacting band. Mutant strains contain empty vectors (EV) as indicated. **C.** Localization of Mce4A and Mce4E protein in WT *M. smegmatis*. Subcellular fractions were isolated using ultracentrifugation, during which clarified whole cell lysate (WCL) was separated into cell wall (CW), membrane (MEM), and soluble (SOL) fractions. Fractions were separated by SDS-PAGE and were immunoblotted for Mce4A and Mce4E proteins. The total amount of CW, MEM, and

SOL fractions shown is equivalent to the amount of WCL loaded. WCL from a *M. smegmatis* strain lacking all six Mce systems (Δ6X *mce*) acts as a control for Mce4A and Mce4E antibody specificity (5). Loading controls for the various fractions include the SecA1 ATPase (MEM and SOL) (6), the SecY translocation permease (MEM) (7), the MspA porin protein (CW and SOL) (8), and the GroEL chaperone protein (SOL) (8). Quantification of **D.** Mce4A and **E.** Mce4E proteins in subcellular fraction western blots using ImageJ software (9). * indicates $p<0.001$ and error bars indicate the standard error of the mean of at least three independent experiments.

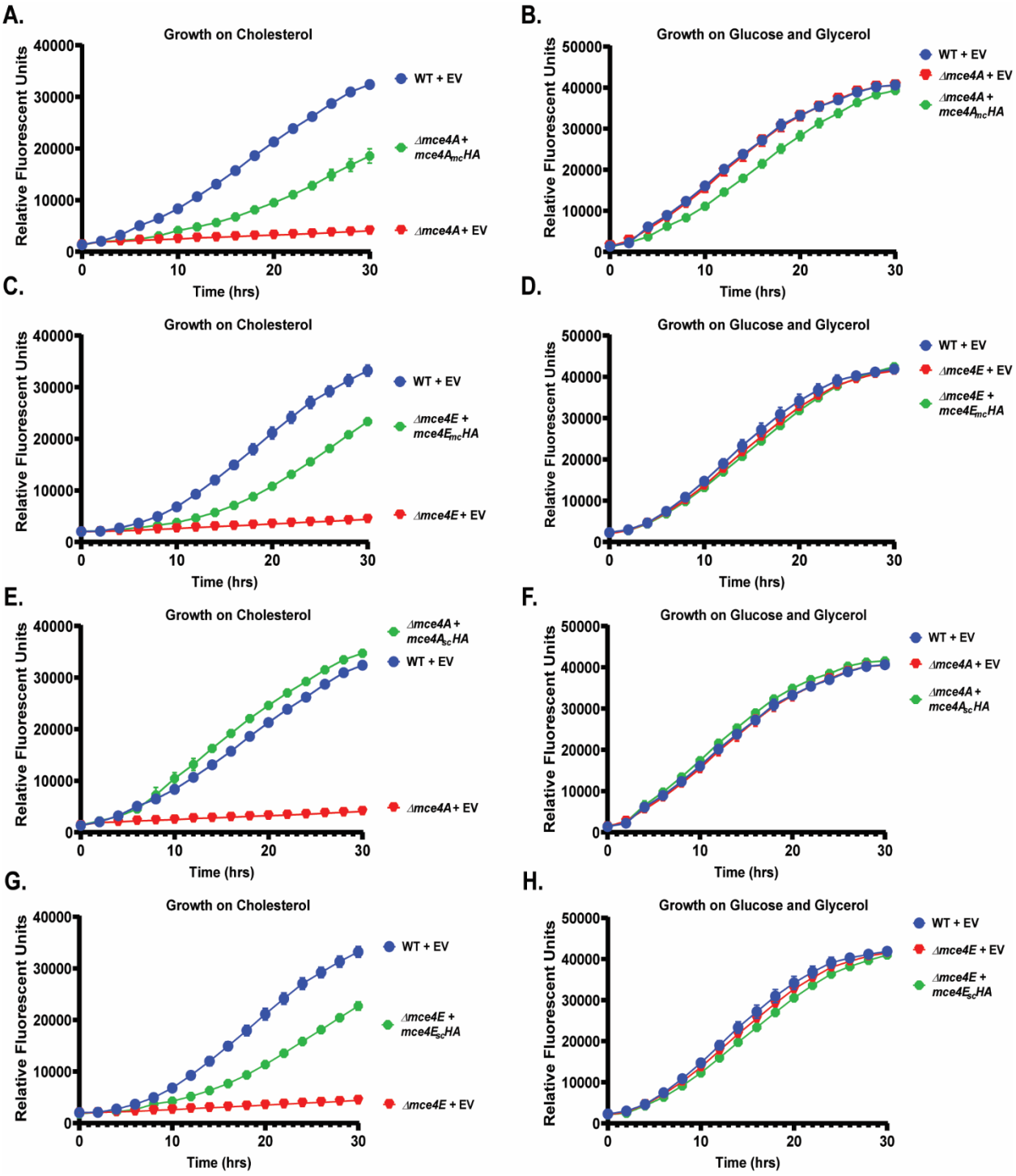

Figure S4:

Mce4A-HA and Mce4E-HA expression plasmids complement the mutant phenotypes of *Δmce4A* and *Δmce4E* strains. **A-E.** Growth of 10^4 colony forming units (cfu) of *M. smegmatis* strains with

cholesterol or glucose and glycerol as the sole carbon source(s) was measured over time using resazurin reduction as a fluorescent readout of metabolic activity (2). Cholesterol growth curves measured by resazurin reduction of **A.** *Δmce4A* and *mce4A-HA* multicopy expression strains **C.** *Δmce4E* and *mce4E-HA* multicopy expression strains **E.** *Δmce4A* and *mce4A-HA* single copy expression strains **G.** *Δmce4E* and *mce4E-HA* single copy expression strains of *M. smegmatis* strains are depicted. Glucose and Glycerol growth curves (cfu) of **B.** *Δmce4A* and *mce4A-HA* multicopy expression strains **D.** *Δmce4E* and *mce4E-HA* multicopy expression strains **F.** *Δmce4A* and *mce4A-HA* single copy expression strains **H.** *Δmce4E* and *mce4E-HA* single copy expression strains of *M. smegmatis* strains are depicted. Mutant strains contain empty vectors (EV) as indicated.

Supplemental Tables

Table S1: Percent identity between *M. smegmatis* Mce4 proteins determined with the NCBI BLAST Two-sequence Alignment function, using the BLOSUM62 matrix (10)

	Mce4A	Mce4B	Mce4C	Mce4D	Mce4E	Mce4F
Mce4A	100	22.87	26.04	22.14	24.71	21.26
Mce4B	22.87	100	21.68	23.02	18.97	24.18
Mce4C	26.04	21.68	100	23.19	25.00	21.76
Mce4D	22.14	23.02	23.19	100	21.13	22.73
Mce4E	24.71	18.97	25.00	21.13	100	24.39
Mce4F	21.26	24.18	21.76	22.73	24.39	100

Table S2: *Mycobacterium smegmatis* strains

***Mycobacterium smegmatis* Strain**	**Genotype and/or description**	**Source or reference**
mc^2155	Wildtype (WT)	Snapper et al., 1990 (11)
Δmce4A	ΔMSMEG_5900	This study
Δmce4E	ΔMSMEG_5896	This study
$\Delta mce4E^{polar}$	ΔMSMEG_5896 with polar effects that disrupt MSMEG_5895	This study
$\Delta mam4A^{polar}$	ΔMSMEG_5894 with polar effects that disrupt MSMEG_5893	This study
ΔomamA	ΔMSMEG_0235	Perkowski et al., 2016 (3)
ΔomamB	ΔMSMEG_0236	This study
ΔomamCD	ΔMSMEG_4771-4770	This study
ΔomamEF	ΔMSMEG_2864-2865	This study
Δ6X mce	*Δmce1, Δmce3, Δmce4, Δmce5, Δmce5b,* and *Δmce7*	Klepp et al., 2012 (5)

121 **Table S3:** Plasmids

Plasmids	Genotype and/or description	Source or reference
pMV261	Mycobacterial multicopy plasmid with *hsp60* promoter; Kan^R	Stover et al., 1991 (12)
pLL2	Mycobacterial single copy plasmid that integrates at the tweety *attB* site; Hyg^R	Ligon et al., 2013 (7)
pJSC77	pMV261 with a cloning site for generating HA tagged fusions; Kan^R	Glickman et al., 2000 (13)
pUC19	E. coli cloning vector; $Carb^R$	New England Biolabs
pCR2.1 - TOPO	Cloning vector for topo cloning; Amp^R and Kan^R	Invitrogen
pMP62 (also referred to as pYUB657)	Allelic exchange suicide vector containing *sacB* counterselection marker; Hyg^R	Pavelka et al., 1999 (14)
pMP1064	pBluescript KS res1-hyg-res1 cassette flanked by SmaI sites	Martinelli and Pavelka, 2016 (15)
pJV53	Mycobacterial multicopy plasmid encoding recombineering proteins gp60 and gp61; Kan^R	Van Kessel and Hatful, 2007 (16)
pMP854	Mycobacterial multicopy plasmid encoding *resolvase*; Kan^R	Sanders AN et al., 2014 (17)
pLR62; *Δmce4A* allelic exchange suicide vector	pMP62 containing upstream and downstream fragments of *mce4A* (MSMEG_5900); Hyg^R	This study
pBM44; P_{hsp60}-$mce4A_{ms}$-HA multicopy	pJSC77 containing *mce4A*	Miller et al., 2019 (18)
pLR101; P_{hsp60}-$mce4A_{ms}$ multicopy	pBM44 with HindIII site filled in by Klenow and self-ligated at the HpaI site to remove the HA tag from *mce4A*.	This study
pLR111; P_{hsp60}-$mce4A_{ms}$-HA singlecopy	*mce4A-HA* from pBM44 cloned into pLL2 cut with NotI and EcoRV	This study
pLR112; *Δmce4E* precursor recombineering plasmid	Upstream and downstream fragments of *mce4E*	This study

	(MSMEG_5896) with an EcoRV site at the junction cloned into pUC19 cut with EcoRI and HindIII	
pLR113; *Δmce4E* recombineering plasmid	Hygromycin resistance cassette from pMP1064 was cloned into pLR112 cutwith EcoRV	This study
pLR142; *mce4E* TOPO intermediate	*mce4E* in PCR-2.1 TOPO vector	This study
pLR106; P $_{hsp60}$-*mce4E*$_{ms}$ multicopy	*mce4E* from pLR142 (MSMEG_5896) cloned into pMV261 cut with EcoRI and HindIII restriction sites	This study
pLR93; P $_{hsp60}$-*mce4E*$_{ms}$-HA multicopy	pJSC77 containing *mce4E* (MSMEG_5896) cloned into MscI and HindIII restriction sites	This study
pLR116; P $_{hsp60}$-*mce4E*$_{ms}$-HA singlecopy	*mce4E-HA* from pLR93 cloned into pLL2 cut with NotI and EcoRV	This study
pLR114; P $_{hsp60}$-*mce4E*$^{C16A}_{ms}$ multicopy	Site Directed Mutagenesis of pLR106 to construct *mce4E C16A*	This study
pMB250; *Δmce4EF* precursor recombineering plasmid	Upstream and Downstream fragments of *mce4E* (MSMEG_5896) with an EcoRV site at the junction cloned into pUC19 cut with EcoRI and HindIII	This study
pLR97*; Δmce4EF* recombineering plasmid	Hygromycin resistance cassette from pMP1064 cloned into pMB250 cut with EcoRV	This study
pLR110; P $_{hsp60}$-*mce4EF*$_{ms}$ multicopy	pMV261 containing *mce4EF* (MSMEG_5896 and MSMEG_5895) cloned into MscI and HindIII restriction sites	This study
pLR96; *Δmam4AB* precursor recombineering plasmid	Upstream and Downstream fragments of *mam4A* (MSMEG_5894) with an EcoRV site at the junction cloned into pUC19 cut with EcoRI and HindIII	This study

pLR98; *Δmam4AB* recombineering plasmid	Hygromycin resistance cassette from pMP1064 cloned into pLR96 cut with EcoRV	This study
pLR143; *mam4A* TOPO intermediate	*mam4A* in PCR2.1 TOPO vector	This study
pLR107; P $_{hsp60}$-*mam4A*$_{ms}$ multicopy	pMV261 containing *mam4A* (MSMEG_5894) from pLR143 cloned into pmv261 cut with EcoRI and HindIII restriction sites	This study
pLR130; P $_{hsp60}$-*mam4A*$_{ms}$ singlecopy	*mam4A* from pLR107 cloned into pLL2 cut with NotI and EcoRV	This study
pLR126; P $_{hsp60}$-*mam4B*$_{ms}$ multicopy	pMV261 containing *mam4B* (MSMEG_5893) cloned into MscI and HindIII restriction sites	This study
pLR132; P $_{hsp60}$-*mam4B*$_{ms}$ singlecopy	*mam4B* from pLR126 cloned into pLL2 cut with NotI and EcoRV	This study
pLR124; P $_{hsp60}$-*mam4AB*$_{ms}$ multicopy	pMV261 containing *mam4AB* (MSMEG_5894 and MSMEG_5893) cloned into MscI and HindIII restriction sites	This study
pEP139; P $_{hsp60}$-*omamA*$_{ms}$ multicopy	pMV261 containing *omamA* (MSMEG_0235)	Perkowski et al., 2016 (3)
pLR128; *ΔomamB* precursor recombineering plasmid	Upstream and Downstream fragments of *omamB* (MSMEG_0236) with an EcoRV site at the junction cloned into pUC19 cut with EcoRI and HindIII	This study
pLR129; *ΔomamB* recombineering plasmid	Hygromycin resistance cassette from pMP1064 cloned into pLR128 digested with EcoRV	This study
pLR141; P $_{hsp60}$-*omamB*$_{ms}$ multicopy	pMV261 containing *omamB* (MSMEG_0236) cloned into MscI and HindIII restriction sites	This study
pLR134; *ΔomamCD* precursor recombineering plasmid	Upstream and Downstream fragments of *omamCD* (MSMEG_4771-4770) with an EcoRV site at the junction	This study

	cloned into pUC19 cut with EcoRI and HindIII	
pLR135; *ΔomamCD* recombineering plasmid	Hygromycin resistance cassette from pMP1064 cloned into pLR134 cut with EcoRV	This study
pLR138; *ΔomamEF* precursor recombineering plasmid	Upstream and Downstream fragments of *omamEF* (MSMEG_2864-2865) with an hpaI site at the junction cloned into pUC19 cut with EcoRI and HindIII	This study
pLR140; *ΔomamEF* recombineering plasmid	Hygromycin resistance cassette from pMP1064 cloned into pLR138 digested with HpaI	This study

Table S4: Primers

Primer	plasmid the primer was used to make	Sequence (5'-3')	Description
Mce4A_UPflank_forward	pLR62	ACTAGTATGATCGGCGGCACTGTCG	Amplification of flank Upstream of *mce4A* (MSMEG_5900) for cloning into pMP62 (ApaI)
Mce4A_UPflank_reverse	pLR62	GCCCGGGTTTGGCGTTTCCGTTCGAC	
Mce4A_DWNflank_forward	pLR62	GATATCGCGTTCGCGGAAAGGGACG	Amplification of flank Downstream of *mce4A* (MSMEG_5900) for cloning into pMP62 (ApaI)
Mce4A_DWNflank_reverse	pLR62	ATAGGGCCCGACGCGAGCGGTTCGATGG	
Mce4E_UPflank_forward	pLR112	CAGCTATGACCATGATTACGCCACTAGTGTGGGCAAGCTCGCCGACTTCAC	Amplification of flank Upstream of *mce4E* (MSMEG_5896) for cloning into pUC19 (EcoRI, HindIII)
Mce4E_UPflank_reverse	pLR112	CGGCGGCCTGATATCCGCCGCTGCCGATCGCCAGGGTG	
Mce4E_DWNflank_forward	pLR112	AGCGGCGGATATCAGGCCGCCGACCCGTTCAAGATC	Amplification of flank Downstream of *mce4E* (MSMEG_5896) for cloning into pUC19 (EcoRI, HindIII)
Mce4E_DWNflank_reverse	pLR112	GTAAAACGACGGCCAGTGTCTAGAGGGACTTGCTGTAGATGAC	
Mce4E_pmv261_forward	pLR142	CGGAATTCTGACCTAAGGAGGTGAACGGGTGCGCACCCTGGC	Amplification of *mce4E* (MSMEG_5896) for cloning into PCR2.1 TOPO
Mce4E_pmv261_reverse	pLR142	CGAAGCTTCTACGGCCCCTCGTGCTG	
Mce4E_pJSC77_forward	pLR93	CGGAGGAATCACTTCGCAATGGCCACCGTGCGCACCCTGGCGATC	Amplification of *mce4E* (MSMEG_5896) for cloning into pJSC77 (MscI-HindIII)
Mce4E_pJSC77_reverse	pLR93	CGTAGTCCGGCACGTCGTACGGGTAAGCTTGCGGCC	

		CCTCGTGCTGCGTCG	
Mce4E^{C16A}_SDM_forward	pLR114	GACCACCGAACTGAGCGCCCGCAAGCAGCA	Site Directed Mutagenesis of *mce4E*^{C16A}
Mce4E^{C16A}_SDM_reverse	pLR114	TGCTGCTTGCGGGCGCTCAGTTCGGTGGTC	
Mce4EF_UPflank_forward	pMB250	CAGCTATGACCATGATTACGCCACTAGTGTGGGCAAGCTCGCCGACTTCAC	Amplification of flank Upstream of *mce4E* (MSMEG_5896) for cloning into pUC19 (EcoRI, HindIII)
Mce4EF_UPflank_reverse	pMB250	GATCATCTACGGGATATCCATGTTGAGCGAGTTGAGAC	
Mce4EF_DWNflank_forward	pMB250	CAACATGGATATCCCGTAGATGATCGACCGGCTGAC	Amplification of flank Downstream of *mce4E* (MSMEG_5896) for cloning into pUC19 (EcoRI, HindIII)
Mce4EF_DWNflank_reverse	pMB250	GTAAAACGACGGCCAGTGTCTAGAGGGACTTGCTGTAGATGAC	
Mce4EF_pmv261_forward	pLR110	CGGAGGAATCACTTCGCAATGGCCACCGTGCGCACCCTGGCGATC	Amplification of *mce4EF* (MSMEG_5896 and MSMEG_5895) for cloning into pmv261 (MscI-HindIII)
Mce4EF_pmv261_reverse	pLR110	ACTACGTCGACATCGATATCAAGCCTGCCTTGGATCCA	
Mam4AB_UPflank_forward	pLR96	GTTGTAAAACGACGGCCAGTGACTAGTTGCCGCCCAACAAGTTCCCGATG	Amplification of flank Upstream of *mam4A* (MSMEG_5894) for cloning into pUC19 (EcoRI, HindIII)
Mam4AB_UPflank_reverse	pLR96	TCAGTTGATATCGATGGTGTCCAGCGGCTCACTGCT	
Mam4AB_DWNflank_forward	pLR96	ACCATCGATATCAACTGATGCGCTCCAAGCTCGTC	Amplification of flank Downstream of *mam4A* (MSMEG_5894) for cloning into pUC19 (EcoRI, HindIII)
Mam4AB_DWNflank_reverse	pLR96	GCTATGACCATGATTACGCCATCTAGAGCACGATCCGCGCCCGCTCAACAC	

Mam4A_pmv261_forward	pLR143	CGGAATTCTGACCTAAGGAGGTGAATAGTGAGCAGTGAGCCGCTGG	Amplification of *mam4A* (MSMEG_5894) for cloning into PCR2.1 TOPO
Mam4A_pmv261_reverse	pLR143	CGAAGCTTAGCTTGGAGCGCATCAGTTACC	
Mam4B_pmv261_forward	pLR126	CGGAGGAATCACTTCGCAATGGCCACCATGCGCTCCAAGCTCGTC	Amplification of *mam4B* (MSMEG_5893) for cloning into pmv261 (MscI-HindIII)
Mam4B_pmv261_reverse	pLR126	ACTACGTCGACATCGATACTAGATCGGCTTGATGGCGTCGATCAG	
Mam4AB_pmv261_forward	pLR124	CGGAATTCTGACCTAAGGAGGTGAATAGTGAGCAGTGAGCCGCTGG	Amplification of *mam4AB* (MSMEG_5894 and MSMEG_5893) for cloning into pmv261 (MscI-HindIII)
Mam4AB_pmv261_reverse	pLR124	ACTACGTCGACATCGATACTAGATCGGCTTGATGGCGTCGATCAG	
OmamB_UPflank_forward	pLR128	AAACGACGGCCAGTGTCTAGACCCGACATTCTTCCAGAC	Amplification of flank Upstream of *omamB* (MSMEG_0236) for cloning into pUC19 (EcoRI-HindIII)
OmamB_UPflank_reverse	pLR128	CCGGGCGTACGTCGTCGGATATCAAGGCCAGCACGCGCAACCGG	
OmamB_DWNflank_forward	pLR128	GCGTGCTGGCCTTGATATCCGACGACGTACGCCCGGCCCACTC	Amplification of flank Downstream of *omamB* (MSMEG_0236) for cloning into pUC19 (EcoRI-HindIII)
OmamB_DWNflank_reverse	pLR128	GACCATGATTACGCCAACTAGTGGTCGCTGGCGGTGTTGGAG	
OmamB_pmv261_forward	pLR141	CGGAGGAATCACTTCGCAATGGCCACCTGGAGCCCATCCGATGAGGAAC	Amplification of *omamB* (MSMEG_0236) for cloning into pmv261 (MscI-HindIII)
OmamB_pmv261_reverse	pLR141	ACTACGTCGACATCGATAGCTCAGC	

		GGGAGTGGGCCG GGCGTAC	
OmamCD_UPflank_forward	pLR134	AAACGACGGCCA GTGTCTAGAAACT CATCCGGCGCATC CAC	Amplification of flank Upstream of *omamC* (MSMEG_4771) for cloning into pUC19 (EcoRI-HindIII)
OmamCD_UPflank_reverse	pLR134	CGTCGATCTTGGG ATATCCGTTGATC ACGGGCAACGTA GG	
OmamCD_DWNflank_forward	pLR134	CCGTGATCAACG GATATCCCAAGA TCGACGGCAACT G	Amplification of flank Downstream of *omamD* (MSMEG_4770) for cloning into pUC19 (EcoRI-HindIII)
OmamCD_DWNflank_reverse	pLR134	GACCATGATTAC GCCAACTAGTGG GTGAGTTCGTCGT TTAC	
OmamEF_UPflank_forward	pLR138	AAACGACGGCCA GTGTCTAGAGAC GAGCTGTGCGGG CTAC	Amplification of flank Upstream of *omamE* (MSMEG_2864) for cloning into pUC19 (EcoRI-HindIII)
OmamEF_UPflank_reverse	pLR138	AGTTCCCATCGGT TAACTCGGTACCC ACTCGTTCAGTCG TGCTCAG	
OmamEF_DWNflank_forward	pLR138	GTGGGTACCGAG TTAACCGATGGG AACTGGCTGATCT C	Amplification of flank Downstream of *omamF* (MSMEG_2865) for cloning into pUC19 (EcoRI-HindIII)
OmamEF_DWNflank_reverse	pLR138	GACCATGATTAC GCCAACTAGTAG GCATACGACCGG GTGTTC	

References:

1. El-Gebali S, Mistry J, Bateman A, Eddy SR, Luciani A, Potter SC, Qureshi M, Richardson LJ, Salazar GA, Smart A, Sonnhammer ELL, Hirsh L, Paladin L, Piovesan D, Tosatto SCE, Finn RD. 2019. The Pfam protein families database in 2019. Nucleic Acids Res 47:D427–D432.

2. Hayden JD, Brown LR, Gunawardena HP, Perkowski EF, Chen X, Braunstein M. 2013. Reversible acetylation regulates acetate and propionate metabolism in *Mycobacterium smegmatis*. Microbiol Read Engl 159:1986–1999.

3. Perkowski EF, Miller BK, McCann JR, Sullivan JT, Malik S, Allen IC, Godfrey V, Hayden JD, Braunstein M. 2016. An orphaned Mce-associated membrane protein of *Mycobacterium tuberculosis* is a virulence factor that stabilizes Mce transporters. Mol Microbiol 100:90–107.

4. Feltcher ME, Gibbons HS, Ligon LS, Braunstein M. 2013. Protein export by the mycobacterial SecA2 system Is determined by the preprotein mature domain. J Bacteriol 195:672–681.

5. Klepp LI, Forrellad MA, Osella AV, Blanco FC, Stella EJ, Bianco MV, Santangelo M de la P, Sassetti C, Jackson M, Cataldi AA, Bigi F, Morbidoni HR. 2012. Impact of the deletion of the six mce operons in *Mycobacterium smegmatis*. Microbes Infect 14:590–599.

6. Guo XV, Monteleone M, Klotzsche M, Kamionka A, Hillen W, Braunstein M, Ehrt S, Schnappinger D. 2007. Silencing essential protein secretion in *Mycobacterium smegmatis* by using tetracycline repressors. J Bacteriol 189:4614–4623.

7. Ligon LS, Rigel NW, Romanchuk A, Jones CD, Braunstein M. 2013. Suppressor analysis reveals a role for SecY in the secA2-dependent protein export pathway of mycobacteria. J Bacteriol 195:4456–4465.

8. Rengarajan J, Murphy E, Park A, Krone CL, Hett EC, Bloom BR, Glimcher LH, Rubin EJ. 2008. *Mycobacterium tuberculosis* Rv2224c modulates innate immune responses. Proc Natl Acad Sci 105:264–269.

9. Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997-2018.

10. Alstchul SF, Gish W, Miller Q, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Bio. 215:403-41.

11. Snapper SB, Melton RE, Mustafa S, Kieser T, Jacobs WR. 1990. Isolation and characterization of efficient plasmid transformation mutants of *Mycobacterium smegmatis*. Mol Microbiol 4:1911–1919.

12. Stover CK, de la Cruz VF, Fuerst TR, Burlein JE, Benson LA, Bennett LT, Bansal GP, Young JF, Lee MH, Hatfull GF. 1991. New use of *BCG* for recombinant vaccines. Nature 351:456–460.

180 13. Glickman MS, Cox JS, Jacobs WR. 2000. A novel mycolic acid cyclopropane synthetase is
181 required for cording, persistence, and virulence of *Mycobacterium tuberculosis*. Mol Cell
182 5:717–727.

183 14. Pavelka MS, Jacobs WR. 1999. Comparison of the construction of unmarked deletion
184 mutations in *Mycobacterium smegmatis, Mycobacterium bovis bacillus Calmette-Guérin*,
185 and *Mycobacterium tuberculosis* H37Rv by allelic exchange. J Bacteriol 181:4780–4789.

186 15. Martinelli DJ, Pavelka MS. 2016. The RipA and RipB Peptidoglycan endopeptidases are
187 individually nonessential to *Mycobacterium smegmatis*. J Bacteriol 198:1464–1475.

188 16. van Kessel JC, Hatfull GF. 2007. Recombineering in *Mycobacterium tuberculosis*. Nat
189 Methods 4:147–152.

190 17. Sanders AN, Wright LF, Pavelka MS. 2014. Genetic characterization of mycobacterial L,D-
191 transpeptidases. Microbiol Read Engl 160:1795–1806.

192 18. Miller BK, Hughes R, Ligon LS, Rigel NW, Malik S, Anjuwon-Foster BR, Sacchettini JC,
193 Braunstein M. 2019. *Mycobacterium tuberculosis* SatS is a chaperone for the SecA2 protein
194 export pathway. eLife 8:e40063.

195