Supplementary Information

An orphaned Mce-associated membrane protein of *Mycobacterium tuberculosis* is a virulence factor that stabilizes Mce transporters

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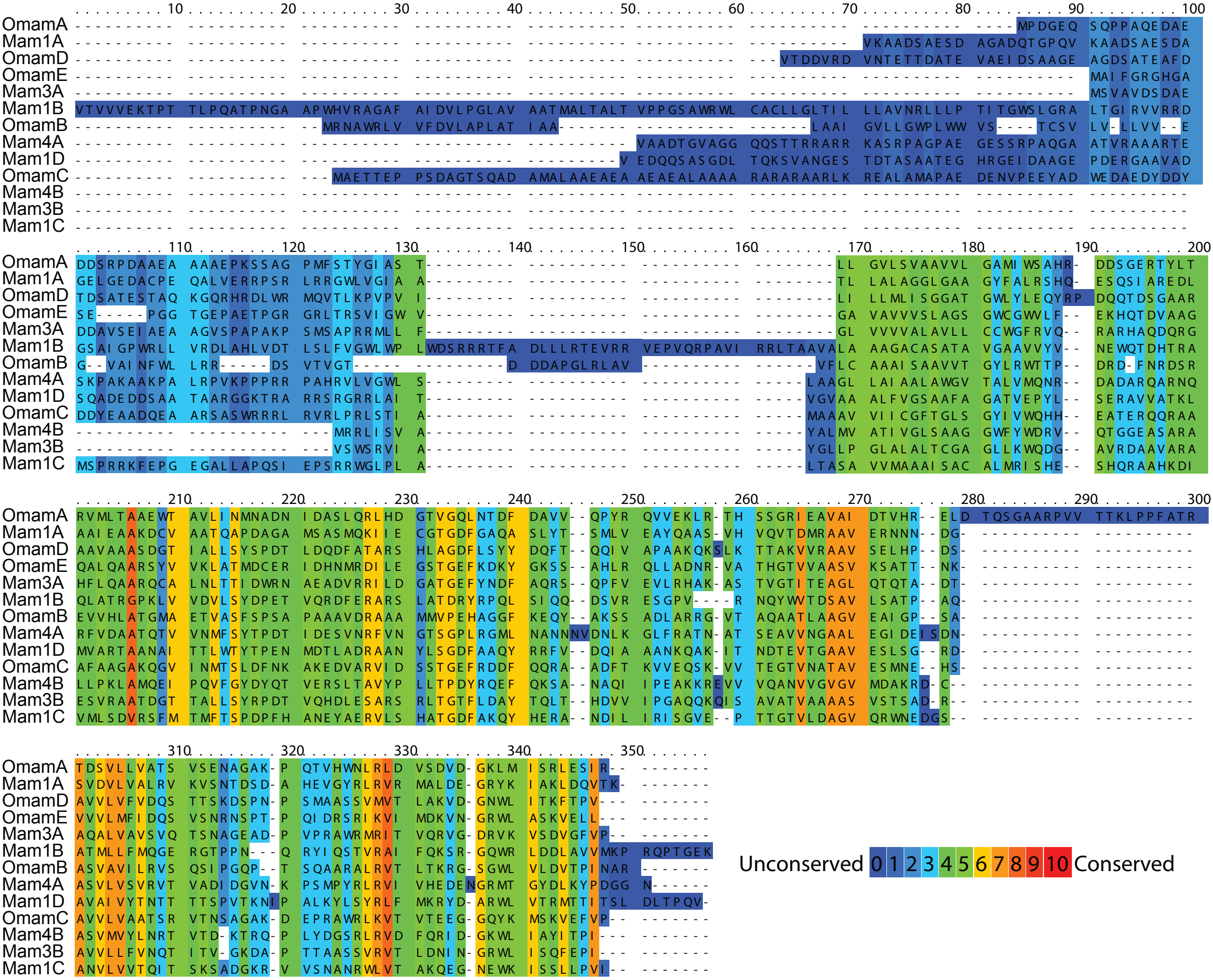
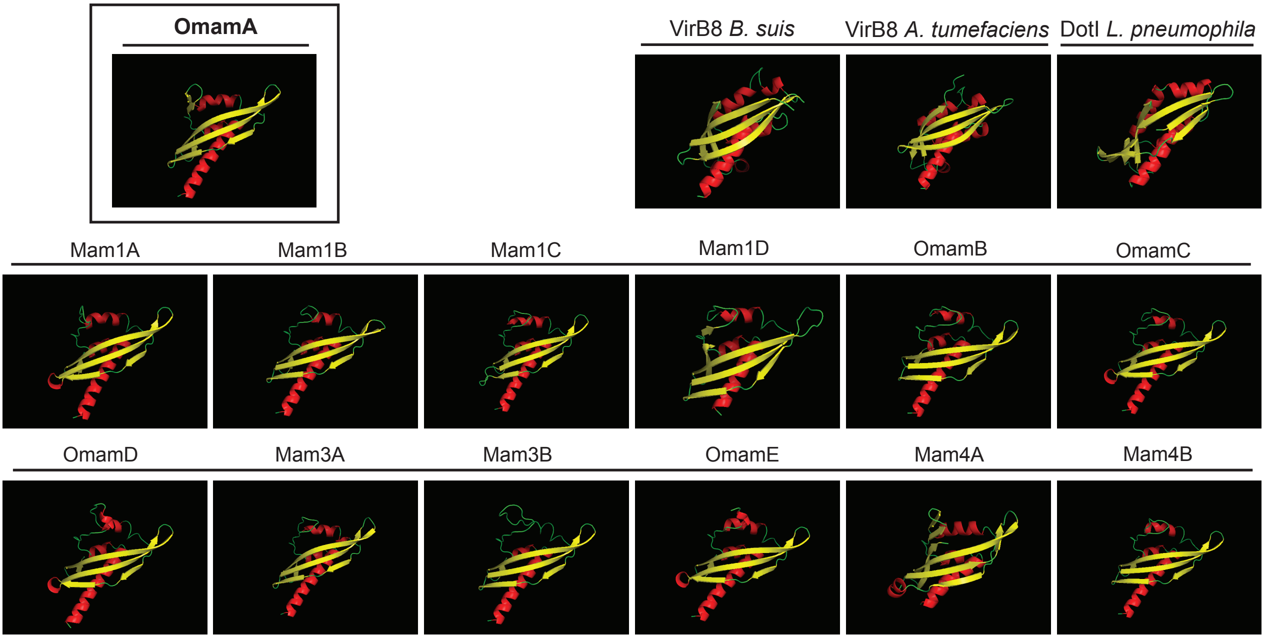
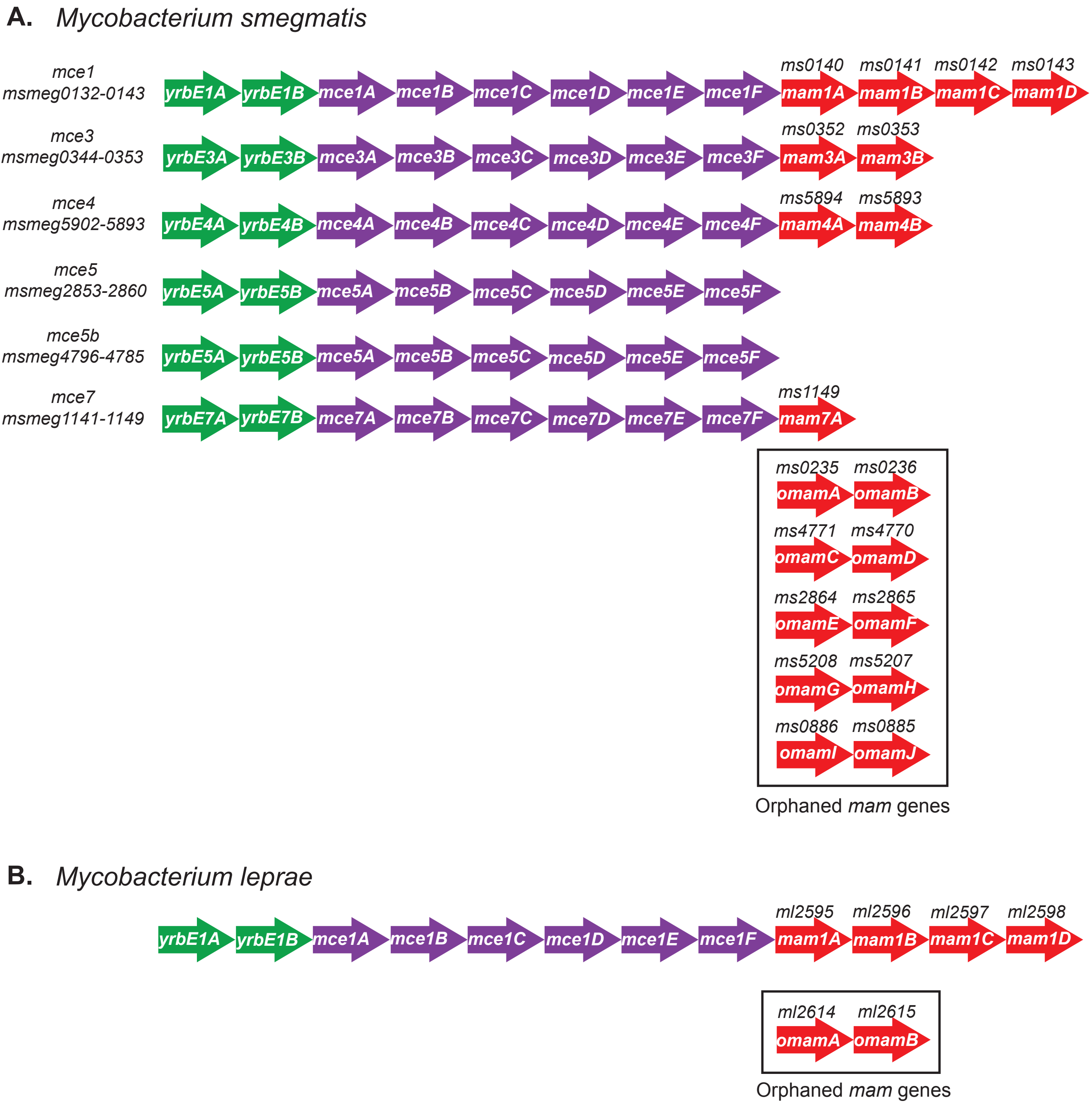


Supplemental Figure 1. Alignment of all Mam proteins. Alignment between all predicted *M. tuberculosis* Mam and Omam proteins in PRALINE (Bawono & Heringa, 2014). Colors represent amino acid conservation with blue representing no/low conservation and red indicating high level conservation.



Supplemental Figure 2. Phyre 2, an online structural prediction program, predicts with high confidence that all Mam and Omam proteins form a NTF2-like fold. Ribbon diagrams shown represent Phyre 2 (Kelley & Sternberg, 2009) predicted structures of OmamA, Mam and Omam proteins colored by secondary structure in Pymol. Ribbon diagrams representing the solved crystal structures of VirB8 from *Brucella suis* and *Agrobacterium tumefaciens* and DotI from *Legionella pneumophila* are shown for comparison (Smith *et al.*, 2012; Bailey *et al.*, 2006; Terradot *et al.*, 2005; Kuroda *et al.*, 2015). Alpha helices are colored in red, Beta-strands in yellow, and turns in green.



Supplemental Figure 3. A. *M. smegmatis* has six *mce* operons organized as shown. Mce operons are organized by two *yrbE* genes upstream (green), six *mce* genes (purple) and many have *mam* genes (red) downstream. Several genes, including *msmeg0235*, are predicted to encode Mam proteins but are not located in *mce* operons and thus are labeled *orphaned mce-associated membrane* genes (*omam*) (Casali & Riley, 2007). **B.** The *mce* operon structure in *Mycobacterium leprae* which only contains one *mce* operon and two *orphaned mam* genes (Casali & Riley, 2007).

Supplemental Table 1. Bacterial strains used in this study

***M. tuberculosis* strains**	**Description**	**Source**
PM638	*M. tuberculosis* H37Rv *ΔblaC*	Flores et al., 2005
MBTB126	PM638 carrying integrating vector pJES137 (expressing *'blaTEM-1*). Strain is hyg^R	McCann et al., 2011
MBTB156	PM638, *mce2F::tn* (with a *'blaTEM-1* transposon inserted into *mce2F* at amino acid position 476). Strain is hyg^R	McCann et al., 2011
MBTB178	MBTB126 + pMV261.kan. Strain is hyg^R and kan^R	McCann et al., 2011
MBTB183	PM638, *omamA(rv0199)::tn* (with a '*blaTEM-1* transposon inserted in *rv0199* at amino acid position 74). Strain is hyg^R	McCann et al., 2011
MBTB204	PM638, *mce1A::tn* (with a '*blaTEM-1* transposon inserted in *mce1A* at amino acid position 449). Strain is hyg^R	McCann et al., 2011
MBTB288	PM638, *mce4F::tn* (with a '*blaTEM-1* transposon inserted in *mce4F* at amino acid position 539). Strain is hyg^R	McCann et al., 2011
MBTB319	MBTB183 + pMV261. Strain is hyg^R and kan^R	McCann et al., 2011
MBTB320	MBTB183 + pJES178 (expressing *omamA* $_{mtb}$). Strain is hyg^R and kan^R	McCann et al., 2011
MBTB329	PM638, *mce4B::tn* (with a '*blaTEM-1* transposon inserted in *mce4B* at amino acid position 350). Strain is hyg^R	McCann et al., 2011

***M. smegmatis* strains**	**Description**	**Source**
mc^{2}155	*M. smegmatis* wild-type (WT)	Snapper et. al, 1990
Δmce4 mutant	*Δmce4*	Klepp et al., 2012
Δmce6X mutant	*Δmce1, Δmce2, Δmce3, Δmce4, Δmce5, Δmce6*	Klepp et al., 2012
EP1178	*ΔomamA, Δmce4*	This study
EP1179	*ΔomamA*	This study
EP1180	*ΔomamA, Δmce6X*	This study
EP1182	mc^{2}155 + pMV261. Strain is kan^R	This study
EP1193	EP1179 + pMV261. Strain is kan^R	This study
EP1194	EP1179 + pEP139 (expressing *omamA* $_{ms}$*)* . Strain is kan^R	This study
EP1195	EP1179 + pEP166 (expressing *omamA* $_{mtb}$ HA). Strain is kan^R	This study
EP1203	EP1179 + pJES178 (expressing *omamA* $_{mtb}$). Strain is kan^R	This study
EP1204	*Δmce4* + pMV261. Strain is kan^R	This study
EP1206	EP1178 + pMV261. Strain is kan^R	This study
EP1208	*Δmce6X* + pMV261. Strain is kan^R	This study
EP1210	EP1180 + pMV261. Strain is kan^R	This study

Supplemental Table 2. Plasmids used in this study			
Plasmid	**Antibiotic resistance**	**Notes**	**Source**
pMV261	kan	Multicopy mycobacterial vector with *hsp60* promoter	Stover et al., 1991
pJSC77	kan	Multicopy mycobacterial vector, HA tag cloned into pMV261	Glickman et al., 2000
pJV53	kan	Multicopy vector for recombineering, phage recombinase genes expressed from acetamide inducible promoter	van Kessel & Hatfull, 2008; van Kessel & Hatfull, 2007
pMP614	hyg	*res-hyg-res* vector for constructing recombineering vectors	Kind gift from Martin Pavelka
pMP854	kan	Resolvase plasmid to remove *hyg* resistance marker	Kind gift from Martin Pavelka
pJES137	hyg	Integrating mycobacterial vector with consitituitively expressed *'blaTEM-1*	McCann et al., 2011
pJES178	kan	P_{hsp60}-$omamA_{mtb}$ cloned into pMV261	McCann et al., 2011
pEP139	kan	P_{hsp60}-$omamA_{ms}$ cloned into pMV261	This study
pEP166	kan	P_{hsp60}-$omamA_{mtb}$ cloned into pJSC77	This study

Supplemental Table 3. Primers used in this study

Gene	Forward primer	Reverse primer
sigA	GAGATCGGCCAGGTCTACGGCGTG	CTGACATGGGGGCCCGCTACGTTG
mce1A	GTCTTCGCGCTCGTAGTTG	ACGTGTGACCTCCGAGATG
mce1F	TCGTCTCCGTAGTCGCAATC	ACATTGGCCGTCGGATACAG
mce4A	ATCCGCAGTGCTGACTTATC	TGCCACGGTATTTGACCTTG
mce4F	ATCACCGTCATCACCCTGAG	CGTTGGCGTTCTTGTACAGG
msmeg0235	GAGAATTCCATGCCTGACGCGCCCAGCAAG	AGGAATTCGGTTCCTCATCGGATGGGCTCCAGAC
rv0199HA	AAGCTTGTCGAATCGACTCCAACCGGGAGATCA	TTGGCCATGCCTGACGGGGAGCAGAGC

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