

Table S2: *M. marinum* strains, plasmids and primers used in this study.

Strains		
Name	Genotype	Reference
<i>M. marinum</i> M strain	Wild type; parent for all strains	ATCC BAA-535
Δ <i>whiB6</i>	M with a deletion in the <i>whiB6</i> (MMAR_5437) gene	R. E. Bosserman <i>et al. Proc Natl Acad Sci U S A</i> 114(50):E10772-E10781, 2017 doi: 10.1073/pnas.1710167114.
Δ <i>whiB6::lacZ+</i>	M with the <i>whiB6</i> gene replaced with the <i>lacZ</i> gene	This study
Δ <i>whiB6::lacZ</i> + Δ <i>eccCb₁</i>	Δ <i>whiB6::lacZ+</i> strain with a deletion of the <i>eccCb₁</i> (MMAR_5446) gene.	This study
<i>whiB6FI</i>	M with <i>whiB6</i> allele tagged with 3X-FLAG at C-terminus, at the <i>whiB6</i> locus; parental strain for the following strains	R. E. Bosserman <i>et al. Proc Natl Acad Sci U S A</i> 114(50):E10772-E10781, 2017 doi: 10.1073/pnas.1710167114.
Δ <i>whiB6</i> Δ <i>espM</i>	M with deletions in the <i>whiB6</i> (MMAR_5437) and <i>espM</i> (MMAR_5438) genes.	This study
<i>whiB6FI</i> Δ <i>espM</i>	<i>whiB6FI</i> with deletion of the <i>espM</i> (MMAR_5438) gene	This study
<i>whiB6FI</i> Δ <i>eccA₁</i>	<i>whiB6FI</i> with deletion of the <i>eccA₁</i> (MMAR_5443) gene	This study
<i>whiB6FI</i> Δ <i>espM</i> Δ <i>eccA₁</i>	<i>whiB6FI</i> Δ <i>espM</i> with deletion of the <i>eccA₁</i> (MMAR_5443) gene	This study
<i>whiB6FI</i> Δ <i>eccB₁</i>	<i>whiB6FI</i> with deletion of the <i>eccB₁</i> (MMAR_5444) gene	R. E. Bosserman <i>et al. Proc Natl Acad Sci U S A</i> 114(50):E10772-E10781, 2017 doi: 10.1073/pnas.1710167114.
<i>whiB6FI</i> Δ <i>espM</i> Δ <i>eccB₁</i>	<i>whiB6FI</i> Δ <i>espM</i> with deletion of the <i>eccB₁</i> (MMAR_5444) gene	This study
<i>whiB6FI</i> Δ <i>eccCa₁</i>	<i>whiB6FI</i> with deletion of the <i>eccCa₁</i> (MMAR_5445) gene	This study
<i>whiB6FI</i> Δ <i>espM</i> Δ <i>eccCa₁</i>	<i>whiB6FI</i> Δ <i>espM</i> with deletion of the <i>eccCa₁</i> (MMAR_5445) gene	This study

<i>whiB6FIΔeccCb₁</i>	<i>whiB6FL</i> with deletion of deletion of the <i>eccCb₁</i> (MMAR_5446) gene	R. E. Bosserman <i>et al. Proc Natl Acad Sci U S A</i> 114(50):E10772-E10781, 2017 doi: 10.1073/pnas.1710167114.
<i>whiB6FIΔespMΔeccCb₁</i>	<i>whiB6FIΔespM</i> with deletion of the <i>eccCb₁</i> (MMAR_5446) gene	This study
<i>whiB6FIΔeccD₁</i>	<i>whiB6FI</i> with deletion of the <i>eccD₁</i> (MMAR_5452) gene	R. E. Bosserman <i>et al. Proc Natl Acad Sci U S A</i> 114(50):E10772-E10781, 2017 doi: 10.1073/pnas.1710167114.
<i>whiB6FIΔespMΔeccD₁</i>	<i>whiB6FIΔespM</i> with deletion of the <i>eccD₁</i> (MMAR_5452) gene	This study
<i>whiB6FIΔeccE₁</i>	<i>whiB6FI</i> with deletion of the <i>eccE₁</i> (MMAR_5458) gene	R. E. Bosserman <i>et al. Proc Natl Acad Sci U S A</i> 114(50):E10772-E10781, 2017 doi: 10.1073/pnas.1710167114.
<i>whiB6FIΔespMΔeccE₁</i>	<i>whiB6FIΔespM</i> with deletion of the <i>eccE₁</i> (MMAR_5458) gene	This study
Plasmids		
Name	Genotype/ Phenotype	Reference
p2NIL	<i>kan^R, amp^R, oriE</i> ; Parental vector for allelic exchange	T. Parish and N.G.Stoker, <i>Microbiology</i> , 146 (Pt 8):1969-75,2000, https://doi.org/10.1099/00221287-146-8-1969
pGOAL19	<i>amp^R</i> , GOAL cassette includes <i>hyg^R, lacZ, sacB, oriE</i> ; Parental vector for allelic exchange	T. Parish and N.G.Stoker, <i>Microbiology</i> , 146 (Pt 8):1969-75,2000, https://doi.org/10.1099/00221287-146-8-1969
p2NILΔespMGOAL	<i>espM_{MM}</i> flanking regions, <i>kan^R, hyg^R, sacB, lacZ</i>	This study
p2NILΔeccA ₁ GOAL	<i>eccA₁</i> flanking regions, <i>kan^R, hyg^R, sacB, lacZ</i>	This study
p2NILΔeccB ₁ GOAL	<i>eccB₁</i> flanking regions, <i>kan^R, hyg^R, sacB, lacZ</i>	R. E. Bosserman <i>et al. Proc Natl Acad Sci U S A</i> 114(50):E10772-E10781, 2017

		doi: 10.1073/pnas.1710167114.
p2NIL Δ <i>eccCa</i> ₁ G OAL	<i>eccCa</i> ₁ flanking regions, <i>kan</i> ^R , <i>hyg</i> ^R , <i>sacB</i> , <i>lacZ</i>	R. E. Bosserman <i>et al. Proc Natl Acad Sci U S A</i> 114(50):E10772-E10781, 2017 doi: 10.1073/pnas.1710167114.
p2NIL Δ <i>eccCb</i> ₁ G OAL	<i>eccCb</i> ₁ flanking regions, <i>kan</i> ^R , <i>hyg</i> ^R , <i>sacB</i> , <i>lacZ</i>	E.A. Williams et al. <i>Infect Immun.</i> , 85(2). pii: e00653-16, 2017. doi: 10.1128/IAI.00653-16
p2NIL Δ <i>eccD</i> ₁ GOAL	<i>eccD</i> ₁ flanking regions, <i>kan</i> ^R , <i>hyg</i> ^R , <i>sacB</i> , <i>lacZ</i>	R. E. Bosserman <i>et al. Proc Natl Acad Sci U S A</i> 114(50):E10772-E10781, 2017 doi: 10.1073/pnas.1710167114.
p2NIL Δ <i>eccE</i> ₁ GOAL	<i>eccE</i> ₁ flanking regions, <i>kan</i> ^R , <i>hyg</i> ^R , <i>sacB</i> , <i>lacZ</i>	R. E. Bosserman <i>et al. Proc Natl Acad Sci U S A</i> 114(50):E10772-E10781, 2017 doi: 10.1073/pnas.1710167114.
p2NIL Δ <i>whiB6</i> GOAL	<i>whiB6</i> flanking regions, <i>kan</i> ^R , <i>hyg</i> ^R , <i>sacB</i> , <i>lacZ</i>	R. E. Bosserman <i>et al. Proc Natl Acad Sci U S A</i> 114(50):E10772-E10781, 2017 doi: 10.1073/pnas.1710167114.
p2NIL Δ <i>whiB6</i> Δ <i>espM</i> GOAL	<i>whiB6</i> and <i>espM</i> flanking regions, <i>kan</i> ^R , <i>hyg</i> ^R , <i>sacB</i> , <i>lacZ</i>	This study
p2NIL Δ <i>whiB6::lacZ</i> GOAL	<i>lacZ</i> with <i>whiB6</i> flanking regions, <i>kan</i> ^R , <i>hyg</i> ^R , <i>sacB</i> , <i>lacZ</i> ; used to generate a transcriptional reporter at the <i>whiB6</i> locus.	This study
pMH406Hyg	<i>M. tuberculosis</i> <i>esxBA</i> behind mycobacterial optimal promoter (MOP), <i>oriE</i> , L5 integrase, <i>hyg</i> ^R	Original plasmid from (K.M. Guinn et al, <i>Mol Microbiol</i> 51(2):359-70, 2004. https://doi.org/10.1046/j.1365-2958.2003.03844.x); <i>hyg</i> ^R derivative was a gift from the Jeffery S. Cox laboratory
pMV306Hyg	Promoterless shuttle vector, <i>oriE</i> , L5 integrase, <i>hyg</i> ^R	C. K. Stover et al, <i>Dev Biol Stand</i> (82): 163-170, 1994
p _{MOP} <i>lacZ</i>	<i>lacZ</i> behind the pMOP promoter, <i>hyg</i> ^R	This study
p _{MOP} <i>eccA</i> ₁	<i>eccA</i> ₁ from <i>M. marinum</i> behind MOP promoter, L5	This study

	integrase, <i>hygR</i> .	
pR2Hyg-dsRed	<i>dsRed</i> expressed behind the <i>msp12</i> promoter	C.L. Cosma, O. Humbert, and L. Ramakrishnan, Nat Immunol 5: 828-835, 2004.DOI: 10.1038/ni1091
p _{MOP} mOrange	mOrange (from Addgene #29770) behind mycobacterial optimal promoter (MOP), <i>oriE</i> , L5 integrase, <i>hyg</i> ^R	This study
p _{msp} mOrange	mOrange behind the <i>msp</i> promoter, <i>oriE</i> , L5 integrase, <i>hyg</i> ^R	This study
p _{msp} <i>espM</i> _{Mm}	<i>espM</i> (<i>MMAR_5438</i>) behind <i>msp</i> promoter, L5 integrase, <i>hyg</i> ^R	This study
p _{msp} <i>espM</i> _{Mt}	<i>espM</i> (<i>ERDMAN_4236</i>) from <i>M. tuberculosis</i> str. Erdman behind <i>msp</i> promoter, L5 integrase, <i>hyg</i> ^R	This study
p _{msp} <i>espM</i> _{Ms}	<i>espM</i> (<i>MSMEG_0052</i>) from <i>M. smegmatis</i> str. mc ² 155 behind <i>msp</i> promoter, L5 integrase, <i>hyg</i> ^R	This study
pKT25	Bacterial 2-hybrid prey vector. T25 fragment of <i>Bordatella pertussis cyaA</i> gene, P15A origin, <i>kan</i> ^R	Euromedex
pKT25 <i>espM</i> _{Mt}	<i>espM</i> (<i>ERDMAN_4236</i>) from <i>M. tuberculosis</i> str. Erdman fused to T25 fragment of <i>cyaA</i> , <i>kan</i> ^R	This study.
pET15b	T7 expression vector with N-terminal 6xHis tag, <i>amp</i> ^R	Novagen
pET15 <i>mCherry</i>	<i>mCherry</i> (from Addgene #29769) flanked by NdeI and SpeI restriction sites in the pET15 vector, <i>amp</i> ^R	This study
pET15 <i>espM</i> _{Mm}	<i>espM</i> _{MM} with an N-terminal His6 affinity tag, <i>amp</i> ^R	This study
pET15 <i>espM</i> _{Mm} N T	N-terminal half of <i>espM</i> _{MM} (aa 1-133) with N-terminal His6 affinity tag, <i>amp</i> ^R	This study
pET15 <i>espM</i> _{Mm} C T	C-terminal half of <i>espM</i> _{MM} (aa 127-363) with N-terminal His6 affinity tag, <i>amp</i> ^R	This study.
pET15 <i>espM</i> _{Ms} full	Heterologous expression of full-length <i>M. smegmatis</i> <i>espM</i> ORF with N-terminal His6 affinity tag. <i>amp</i> ^R	This study.
pET15 <i>espM</i> _{Mt}	<i>espM</i> _{Mt} with an N-terminal His6 affinity tag, <i>amp</i> ^R	This study.
pET29	T7 expression vector with C-terminal 6xHis tag, <i>kan</i> ^R	Novagen
pET29 <i>whiB6</i>	<i>whiB6</i> _{MM} with an C-terminal His6 affinity tag, <i>kan</i> ^R	This study.

pMV306 <i>whiB6::lacZ+</i>	Upstream sequence of <i>whiB6</i> fused to <i>lacZ</i> . L5 integrase, <i>hyg^R</i>	This study.
pMV306 <i>espM::lacZ+</i>	Upstream sequence of <i>espM</i> fused to <i>lacZ</i> . L5 integrase, <i>hyg^R</i>	This study.
Primers		
Name	Sequence (5' → 3')	Application
orb14	TCAATAGCCTCGGCGGCTTC	Amplification of 1.5 kb upstream of <i>whiB6</i> , R. E. Bosserman <i>et al. Proc Natl Acad Sci U S A</i> 114(50):E10772-E10781, 2017 doi: 10.1073/pnas.1710167114.
orb72	ATCCACGACTGCCCCGAAAC	Used for verification of <i>eccCb₁</i> deletion.
orb73	CGAGCGTTGCCGCTCAATAG	
ors221	TGATGCCCGGCCGCGTCC	Amplification of sequence downstream of <i>whiB6</i> , R. E. Bosserman <i>et al. Proc Natl Acad Sci U S A</i> 114(50):E10772-E10781, 2017 doi: 10.1073/pnas.1710167114.
OKS18	/BiotinTEG/AAACGCCAGCGGAATCT	Amplification of 979 bp fragment containing the <i>whiB6</i> promoter used as ~1 kb probe in promoter pulldown assays.
OKS19	/BiotinTEG/TTGATGATCCCGTCGGCT	
RpoA Fwd	/BiotinTEG/ATGCTGATCTCTCAGCGGC	Amplification of 1044 bp fragment containing the <i>whiB6</i> promoter used as ~1 kb probe in promoter pulldown and EMSA assays.
RpoA Rev	/BiotinTEG/TTAGAGCTGTTCCGGTTTCGGC	
OKS77	GATAACACGGGATCATCCAG	Amplification of 555 bp fragment containing the <i>whiB6</i> promoter used as ~500 bp probe in EMSA assays.
OKS88	GTTGCAGGTTTCGTAACCTC	
OKS94	ATGGAATCGACCGGGATGC	Amplification of 499 bp fragment of <i>rpoA</i> gene used as ~500 bp probe in EMSA assays.
5438FqRT	CGTCACCAACAGCCCAAACG	

5438RqRT	CTGCGCTGACTGATGTCGAG	Primers for qRT-PCR expression of the interior of <i>espM</i> .
SigA F	TCGAGGTGATCAACAAGCTG	Primers for qRT-PCR expression of the interior of <i>sigA</i> .
SigA R	TGGATCTCCAGCACCTTCTC	
ORS225	AGATTCCGCTGGGCGTTTGC	Primers for qRT-PCR expression of the interior of <i>whiB6</i> .
ORS226	TCTGCCAGCGACCGAAGTTG	
OKS109	CCTCTGCCTGCCTAGC	Amplification of 515 bp fragment of <i>whiB6</i> Erdman promoter used as ~500 bp probe in EMSA assays.
OKS110	CTCGCAGATGTGTCTGAGAG	
OMF004	GAGCCACCCGCAGTTCGAAAAATGACTAGTCGGGA CCGCTCAGGCGT	Amplify pMOP for mOrange cloning
OMF042	CATGCTGGACTCCTGAATTCTGCAGCT	
OMF047	GAATTCAGGAGTCCAGCATGGTGAGCAAGGGCGA GGAG	Clone mOrange into pMOP
OMF048	CGAACTGCGGGTGGCTCCAATTTCCCTTGTACAGC TCGTCCATGCC	
OMF049	GTTGGACTCAAGACGATAGTTACCGGATAAG	Plasmid amplification from pBR322 origin
OMF050	CTTATCCGGTAACTATCGTCTTGAGTCCAAC	
OMF072	GATCTTTAAATCTAGAGGATCTGACCCGCTCCACAA C	Amplification of MSP promoter
OMF074	TCTAGATTTAAAGATCTGGTACCGCGG	Amplification of pMV306 vector; MSP promoter swap
OMF082	CATATGTATATCTCCTTCTTAAATCTAGATTTAAAGA TCTGGTACCGG	Amplification of MSP promoter
OMF083	CAGATCTTTAAATCTAGATTTAAGAAGGAGATATAC ATATGGTGAGCAAGGGCGAGGAG	Amplification of pMOP-mOrange for MSP promoter swap.
OMF096	ACGAGCGTGACACCACGATGCC	Amplification of p2NIL vector
OMF097	ACGGTGCCTGACTGCGTTAGCAATTTAACTG	

OMF173	GCTGATTAATATGATGAAAACGGCAACC	Amplification from <i>lacZ</i> , for confirmation of reporter location and orientation
OMF174	GGTTGCCGTTTTTCATCATATTTAATCAGC	
OMF180	CGTGGTGTACGCTCGTTGGCCAACAATCTGATCTCGG	Amplification of upstream region flanking the <i>eccA₁</i> gene; Used for construction of p2NILΔ <i>eccA₁</i> GOAL suicide plasmid
OMF181	CATATTGAGGTGCTTAAGTGCACTTTCGAACAAACGGC	
OMF182	CGAAAGTGCACTTAAGCACCTCAATATGAGAGAGTGAAACATGG	Amplification of downstream region flanking the <i>eccA₁</i> gene; Used for construction of p2NILΔ <i>eccA₁</i> GOAL suicide plasmid
OMF183	ACGCAGTCAGGCACCGTGTAGGGCAGGATCTTCTGCACG	
OMF184	CTGCGAACATGTCGGAATCC	Used for verification of <i>eccA₁</i> deletion
OMF185	GCCACCAAGTTTTCCAGCTGG	
OMF190	ACTCCTGCAGGCAGCCAAAACG	Used for verification of <i>eccB₁</i> deletion
OMF191	GGCATCATCAGCATGTATGGCG	
OMF196	GGAAATTTGTTTCAGTTGCAGTCACC	Used for verification of <i>eccCa₁</i> deletion
OMF197	AGGTCGATGCAATAGAACTGGACC	
OMF202	GTGTCGGAACAGCAGTTGGTCC	Used for verification of <i>eccD₁</i> deletion
OMF203	CTCCAGTTGCTGCTATCGGCG	
OMF208	GGTGGTCAATGCTGTCGCGG	Used for verification of <i>eccE₁</i> deletion
OMF209	CTGTTCAAGATTTCTGCTGATCCACC	
OMF313	CATATGTATATCTCCTTCTTAAAGTTAAACAAAATTA TTTCTAGAGG	Amplification of pET29 vector.
OMF351	ACGTCGTACGGGTACATATCTAAGCGTTCCTCCATA AAGCAAAGCGTTGC	Amplification of sequence immediately upstream of <i>whiB6</i> gene to introduce linker for HA epitope and fusion to <i>lacZ</i> .
OMF352	ACGTCGTACGGGTACATATCTAAGCGTTCCTCCATA AAGCAAAGCGTTGC	Reverse primer for amplification of <i>lacZ</i> with linker for sequence downstream of <i>whiB6</i> .

OMF353	ATGTACCCGTACGACGTGCCCGACTACGCGGATCC CGTCGTTTTACAACG	Forward primer for amplification of <i>lacZ</i> with linker encoding HA epitope for fusion to sequence immediately before <i>whiB6</i> .
OMF355	GCGGTACCAGATCTTTAGTACGTCGATTCTCGCT CGCGGC	Amplification of <i>whiB6</i> and flanking sequence for cloning into pMV306Hyg.
OMF356	ATCGTACGCTAGTTAACGGCTTCTGGTCCGCTGGA TGCTCG	
OMF512	ACTAGTAACTAGCATAACCCCTTGGGGC	Amplification of pET15 vector
OMF513	CATATGATGATGATGATGATGGCTGC	
OMF514	ATCATCATCATCATATGAGCAAGGGCGAGGAGGAT	Clone mCherry into pET15 with flanking NdeI and SpeI restriction sites.
OMF515	TTATGCTAGTTACTAGTCTACTTGTACAGCTCGTCC ATGCC	
OMF606	CACCACCACCACCACCACTG	Amplification of pET29 vector.
OMF607	AGGAGATATACATATGACTGCAACTGCTCTGTACGA GATTCCG	Amplification of <i>whiB6</i> for cloning into pET29.
OMF608	GGTGGTGGTGGTGGTGTGCCGATTGGGCGGTGAT CC	
OMF615	GTGGTGTCACGCTCGTCCGGTTGAGCAGCAGGATC GATCCC	Amplification of upstream region flanking the <i>espM</i> gene. Used for construction of p2NILΔ <i>espM</i> GOAL suicide plasmid.
OMF616	CCTCGGCCTTAAGCGATTCCGGCGTCGGGCATGTAC G	
OMF617	CGAATCGCTTAAGCCGAGGCTATTGAAGCGCTCA TCC	Amplification of downstream region flanking the <i>espM</i> gene. Used for construction of p2NILΔ <i>espM</i> GOAL suicide plasmid.
OMF618	CGCAGTCAGGCACCGTGGTTTGGCTCGCTATGGCT TTGGTAGG	
OMF619	GCTGGATGTCGTTCAAGAAACGTAGCG	Used for verification of <i>espM</i> deletion
OMF620	GTGGTCGACGCACGAATTTCTTGG	
OMF621	TGGCAGTCGACGCCGATACC	Used for verification of p2NILΔ <i>espM</i> GOAL suicide plasmid integration (merodiploid).
OMF622	CATGGGGGTAGACCTTCTCTACG	
OMF624	TCATCATCATCATATGCCCGACGCCGAATCGACTG	

OMF625	TATGCTAGTTACTAGTTCAATTGATTTGACGGATGAGCGC	Amplification of <i>espM_{MM}</i> for cloning into pET15
OMF626	ATGCTAGTTACTAGTTCAACCAGGATCGGCTTCGTTGGTCTGAG	Amplification of N-terminal half of <i>espM_{MM}</i> ORF for cloning into pET15
OMF627	CATCATCATCATATGACCAACGAAGCCGATCCTGGTG	Amplification of C-terminal half of <i>espM_{MM}</i> ORF for cloning into pET15
OMF630	ACTAGTCGGGACCGCTCAGGCGTCC	Amplification of pMOP vector.
OMF631	TCATCATCATCATATGACCGACGCGGAGTTCGACC	Amplification of <i>M. smegmatis espM</i> for cloning into pET15 expression vector.
OMF632	ATGCTAGTTACTAGTTCAGCGCAGCGAGTCGATGAGC	Amplification of <i>M. smegmatis espM</i> for cloning into pET15 expression vector.
OMF645	GGAGTCCAGCCATATGACTGATCGCCTGGCCGG	Amplification of <i>eccA₁</i> ORF for cloning into pMOP.
OMF646	AGCGGTCCCGACTAGTCACTCTCTCATATTGAGGTGTGCATGCAC	
OMF701	GATCTTTAAATCTAGAGACACAGCGTCTTGGCCTCCTCG	Amplify upstream of <i>espM</i> ORF for fusion to <i>lacZ</i> and cloning into pMV306Hyg.
OMF709	CCAAGCCTTAAGACCGAAAACGTAGCGTCTGAGACCTAGG	Used for verification of <i>whiB6 espM</i> double deletion.
OMF733	GTCGTACGGGTACATGTACGTCGATTCCCTCGCTCGCGGC	Amplify upstream of <i>espM</i> ORF for fusion to <i>lacZ</i> and cloning into pMV306Hyg.
OMF734	TCGTACGCTAGTTAACTCATTTCGACACCAGACCAACTGG	Amplify <i>lacZ</i> with linker for cloning into pMV306Hyg.
RpoA-Fwd	ATGCTGATCTCTCAGCGGC	Amplification of <i>M. marinum rpoA</i> ORF
RpoA-Rev	TTAGAGCTGTTCCGGTTTCGGC	
V-KT25-F	ACTAGTGTCGACTCTAGAGGATCCCCGGGTACCTAAGTAACTAAGAATTCGGCCG	Amplification of pKT25 vector.
V-KT25-R	CATATGTCTAGAGAATTCAGCCCGCCGCGTGCGGCCAGGTAATCG	

ERD-4236-F	GAATTCTCTAGACATATGATTGAGCCCCGTCGCGG	Amplify <i>ERDMAN_4236</i> with linkers for cloning into pKT25.
ERD-4236-R	CTCTAGAGTCGACACTAGTTCAATTGATCTGACGGA TCAGGGC	