

**Table S1. Strains, plasmids and oligonucleotides used in this study**

| Strain                             | Genotype  | Reference or source   |
|------------------------------------|---|-----------------------|
| <b><i>M. marinum</i> strain</b>    |   |                       |
| <b>M</b>                           | wild-type <i>M. marinum</i>   | ATCC                  |
| <b>120A3</b>                       | M strain bearing a Tn insertion in the <i>MMAR_0039</i> gene  | This study            |
| <b>120A3/ P<sub>MOP</sub>0039</b>  | Complemented; 120A3 strain bearing an integrating plasmid expressing <i>MMAR_0039</i> from the constitutive Mycobacterial Optimal Promoter (MOP)  | This study            |
| <b><i>eccD</i><sub>1</sub>::Tn</b> | M strain bearing a Tn insertion in the <i>eccD</i> <sub>1</sub> gene; insertion site between bases 6594673/6594674 in <i>M. marinum</i> genome; Isolated from Library with smooth colony morphology | Laboratory collection |
| <b><i>eccCb</i>::Tn</b>            | M strain bearing a Tn insertion in the <i>eccCb</i> gene  | (1)                   |
| <b>Δ<i>esxBA</i></b>               | M strain bearing a genomic deletion of the <i>esxBA</i> genes   | (2)                   |
| <b>ΔRD1</b>                        | M strain bearing a genomic deletion including ' <i>eccCb</i> '- <i>espK</i>   | (3)                   |

| Plasmid               | Genotype  | Reference or source  |
|-----------------------|---|--|
| <b>pMH406 Hyg</b>     | an integrating plasmid expressing <i>esxBA</i> from <i>M. tuberculosis</i> from the constitutive Mycobacterial Optimal Promoter (MOP); Parent for complementation plasmid | Original Plasmid from (4); Hygromycin resistant derivative was a gift from the Jeffery S. Cox laboratory |
| <b>pMB272B</b>        | suicide plasmid which carries the Mariner Tn  | (5)  |
| <b>pBluescriptSK+</b> |   | Agilent Technologies, La Jolla CA  |

| Name          | Sequence (5'-3')                                 | Use  | Reference or source |
|---------------|--|--|---------------------|
| <b>opc242</b> | GCGTAAACGGGTGTCTATGGGCATCAG                      | Primer pair for construction of PMOP0039 complementation plasmid. Restriction sites underlined | This study          |
| <b>opc244</b> | GCTACGTACAGAATTCAGGAGTCCAGCATGA<br>CTGCCGACAGGAC |  |                     |
| <b>821A</b>   | CGCATCTTCCCGACAACGCAGACCGTTCC                    | Primer pair for mapping the Mariner Transposon insertion site in the <i>M. marinum</i> genome  | (5)                 |
| <b>822A</b>   | TAATCGCGGCCTCGAGCAAGACGTTTCCCG                   |  |                     |

|                                 |                          |   |            |
|---------------------------------|--------------------------|---|------------|
| <b>ofm23</b>                    | ACCTTCACACCGCTACTTGG     | Primer pair for amplifying <i>MMAR_0040</i> for RT-PCR  | This study |
| <b>ofm24</b>                    | ATCACCAATTCTTCGGCAAA     |   |            |
| <b>ofm19</b>                    | AGGCGGGCAAGTACACGAT      | Primer pair for amplifying <i>MMAR_0039</i> for RT-PCR; flanks the Tn insertion in the 120A3 strain | This study |
| <b>ofm20</b>                    | TTGCAGGGCTTCTCCAC        |   |            |
| <b>opc235</b>                   | GGCAGCATCCAGCGCAATTC     | Primer pair for amplifying <i>esxA</i> for RT-PCR   | This study |
| <b>opc236</b>                   | CCTTCGTCGGAGTTCAGATA     |   |            |
| <b>IFN-<math>\beta</math>-F</b> | CTGGAGCAGCTGAATGGAAAG    | Primer pair for amplifying IFN- $\beta$ for qRT-PCR   | (6)        |
| <b>IFN-<math>\beta</math>-R</b> | CTTGAAGTCCGCCCTGTAGGT    |   |            |
| <b>GAPDH-F</b>                  | TCTCCCTCACAATTTCCATCCAG  | Primer pair for amplifying GAPDH for qRT-PCR  | This study |
| <b>GAPDH-R</b>                  | GGGTGCAGCGAACTTTATTGATGG |   |            |

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