

**Table S1: Bacterial strains and plasmids used in this study****Bacterial strains**

|  |  |                      |
|--|--|----------------------|
| <i>Escherichia coli</i> DH5αF'                 | F' endA1 hsdR17 (rκ- mx- supE44 thi-1 recA1 gyrA (Nalr) relA1 Δ(lacZYA-argF)U169 deoR [φ80dlacΔ(lacZ)M15]                    | Raleigh et al., 1989 |
| <i>M. smegmatis</i> mc <sup>2</sup> 155        | Transformable strain of the isolate <i>M. smegmatis</i> ATCC 607   | Snapper et al., 1990 |
| <i>M. smegmatis</i> Δzur (MSMEGΔzur)           | markerless zur ( <i>msmeg</i> 4487) deletion mutant of <i>M. smegmatis</i> mc <sup>2</sup> 155                               | Eckelt et al., 2014  |
| <i>M. smegmatis</i> ΔsmtB (MSMEGΔsmtB)         | markerless <i>smtB</i> ( <i>msmeg</i> 4486) deletion mutant of <i>M. smegmatis</i> mc <sup>2</sup> 155                       | this work            |
| <i>M. smegmatis</i> ΔsmtBΔzur (MSMEGΔsmtBΔzur) | markerless <i>smtB</i> -zur ( <i>msmeg</i> 4486) doublemutant of <i>M. smegmatis</i> mc <sup>2</sup> 155                     | this work            |
| MSMEGΔzur <sup>C</sup>                         | MSMEGΔzur complemented with pMV306hyg containing <i>msmeg</i> 4487 under control of the <i>msmeg</i> 4486 promotor           | this work            |
| MSMEGΔsmtB <sup>C</sup>                        | MSMEGΔsmtB complemented with pMV306hyg containing <i>msmeg</i> 4486 under control of its own promotor                        | this work            |
| MSMEGΔsmtBΔzur <sup>C</sup>                    | MSMEGΔsmtB-zur complemented with pMV306hyg containing <i>msmeg</i> 4486-4487 under control of the <i>msmeg</i> 4486 promotor | this work            |
| MSMEGΔsmtB <sup>Flag</sup>                     | MSMEGΔsmtB complemented with pMV306hyg containing FLAG-tagged <i>msmeg</i> 4486 under control of its own promotor            | this work            |
| MSMEGΔzur <sup>HA</sup>                        | MSMEGΔzur complemented with pMV306hyg containing HA-tagged <i>msmeg</i> 4487 under control of the <i>msmeg</i> 4486 promotor | this work            |

**Plasmids**

|                            |  |   |
|----------------------------|--|---|
| pJET™4.2                   | subcloning blunt end suicide vector, <i>amp</i>  | ThermoFisher Scientific, Waltham, MA, USA |
| p2NIL                      | <i>E. coli</i> - mycobacterium shuttle vector for gene manipulation, <i>kan</i>  | Parish & Stoker 2000                      |
| pGOAL19                    | <i>hyg</i> , <i>P<sub>AG85</sub>-lacZ</i> , <i>P<sub>hspe0</sub>-sacB</i> , <i>PacI</i> cassette vector, <i>amp</i>                                | Parish & Stoker 2000                      |
| pMV306hyg                  | integrative <i>E.coli</i> - mycobacterium shuttle vector, <i>hyg</i>   | Stover et al. 1991                        |
| pMV-MSsmtB                 | pMV306hyg containing <i>msmeg</i> 4486 under its own promotor  | this work                                 |
| pMV-MSsmtB <sup>Flag</sup> | pMV306hyg containing FLAG-tagged <i>msmeg</i> 4486 under its own promotor  | this work                                 |
| pMV-MSzur                  | pMV306hyg containing <i>msmeg</i> 4487 under control of the <i>msmeg</i> 4486 promotor   | this work                                 |
| pMV-MSzur <sup>HA</sup>    | pMV306hyg containing HA-tagged <i>msmeg</i> 4487 under control of the <i>msmeg</i> 4486 promotor   | this work                                 |
| pMV-MSsmtBzur              | pMV306hyg containing <i>msmeg</i> 4486 and <i>msmeg</i> 4487 under its own promotor  | this work                                 |
| p2NIL_MSMEG4486_AB         | p2NIL containing 1500 bp up- and downstream fragments of <i>msmeg</i> 4486   | this work                                 |
| p2NIL_MSMEG4486_Del        | p2NIL containing <i>PacI</i> restricted selection marker gene cassette of pGOAL19 and 1500 bp up- and downstream fragments of <i>msmeg</i> 4486    | this work                                 |
| p2NIL_MSMEG4486-87_AB      | p2NIL containing 1500 bp up- and downstream fragments of <i>msmeg</i> 4486-87  | this work                                 |
| p2NIL_MSMEG4486-87_Del     | p2NIL containing <i>PacI</i> restricted selection marker gene cassette of pGOAL19 and 1500 bp up- and downstream fragments of <i>msmeg</i> 4486-87 | this work                                 |
| pJEM15                     | promoterless <i>E. coli</i> - mycobacterium shuttle vector for <i>lacZ</i> -fusion, <i>kan</i>   | Timm et al. 1994                          |
| pJEM-MSsmtB1               | pJEM15 containing 471 bp 5'UTR of <i>msmeg</i> 4486 (pos. 4568740-4569210) <sup>a</sup>  | this work                                 |
| pJEM-MS0755-1              | pJEM15 containing 150 bp 5'UTR of <i>msmeg</i> 0755 (pos. 842101-842250) <sup>a</sup>  | this work                                 |

<sup>a</sup> The position numbers given related to GenBank accession no. NC\_008596

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