

**Table S2: Vectors and bacterial strains used in this study**

Plasmid/strain	Genotype/description	Source/reference
<b>Plasmids</b>		
pBCSK+	Phagemid, cloning vector	Stratagene
pBCSKrho1A	pBCSK+MSMEG_5036 (Rhomboid protease 1 from <i>M. smegmatis</i> Mc2155 SMR5)	This study
pBCSKrho2B	pBCSK+MSMEG_4904 (Rhomboid protease 2 from <i>M. smegmatis</i> Mc2155 SMR5)	This study
pBCSKrho1C	pBCSK+Rv0110 (Rhomboid protease 1 from <i>MTB</i> H <sub>37</sub> Rv <i>rpsL</i> +) )	This study
pBCSKrho2D	pBCSK+Rv1337 (Rhomboid protease 2 from <i>MTB</i> H37Rv <i>rpsL</i> +) )	This study
pBCSKrho1E	pBCSK+Rv0110 (Rhomboid protease 1 from <i>MTB</i> strain BN44)	This study
pBCSKrho2F	pBCSK+Rv1337 (Rhomboid protease 2 from <i>MTB</i> strain BN44)	This study
pBCSKrho1G	pBCSK+BCG_0143 (Rhomboid protease 1 from <i>M. bovis</i> BCG)	This study
pBCSKrho2H	pBCSK+BCG_1399 (Rhomboid protease 2 from <i>M. bovis</i> BCG)	This study
pBCSKrho1I	pBCSK+Mb0114 (Rhomboid protease 1 from <i>M. bovis</i> , cattle strain JN55)	This study
pBCSKrho2J	pBCSK+Mb1372 (Rhomboid protease 2 from <i>M. bovis</i> cattle strain JN55)	This study
pBCSKrho2K	pBCSK+MAV1554 (Rhomboid protease 2 from <i>M. avium</i> , clinical strain SU-36800)	This study
pBCSKrho2L	pBCSK+MAP2426c (hypothetical protein without rhomboid domains; similar to N-terminus of MAV_1554)	This study
pBCSKrho2M	pBCSK+MAP2425c (hypothetical protein with rhomboid domains; similar to C-terminus of MAV_1554)	This study
pBCSKrho2N	pBCSK+MAP2425c/MAP2426c (in-frame clone with MAP2426c and MAP2425c)	This study
pMN252	Suicide vector for <i>rpsL</i> + cells; ColE1 origin, <i>FRT-hyg-FRT</i> , <i>rpsL</i> ; Amp <sup>R</sup> ; Hyg <sup>R</sup> ; 4642bp	[1]
pMN49	Suicide delivery vector for deleting MSMEG_4904; pMN252 derivative; MSMEG_4904up and MSMEG_4904down; 6416bp	This study
pMN50	Suicide delivery vector for deleting MSMEG_5036; pMN252 derivative; MSMEG_5036up and MSMEG_5036down; 6635bp	This study
pML597	Unmarking vector for <i>rpsL</i> + cells; pMN210 derivative; <i>flp<sub>m</sub></i> ; 6627bp	[2]
pMV361	Mycobacterial integrating vector	[3]
pMV261	Mycobacterial replicating vector	[3]
<b>Bacterial strains</b>		
XL1 Blue MRF'	<i>E. coli</i> , <i>recA1 endA1 gyrA96 thi-1 hsdR17 supE44</i>	Stratagene

	<i>relA1 D(lac-pro) [F9 proAB lacIq lacZDM15 Tn10]</i>	
XD37	<i>P. stuartii</i> , PR50 <i>cma37::mini-Tn5 lacZ1</i> ; wild type for <i>aarA</i>	[4]
XD37.A	XD37 derivative, $\Delta$ <i>aarA</i>	[4]
SMR5	<i>M. smegmatis</i> , mc <sup>2</sup> 155 derivative; Sm <sup>R</sup>	JCRC
DPK1	SMR5 derivative; $\Delta$ MSMEG_5036- <i>hyg-FRT</i> ; Hyg <sup>R</sup> ; single rhomboid mutant (marked)	This study
DPK2	SMR5 derivative; $\Delta$ MSMEG_5036- <i>FRT</i> , Hyg <sup>S</sup> ; single rhomboid mutant (unmarked)	This study
DPK3	SMR5 derivative; $\Delta$ MSMEG_4904- <i>hyg-FRT</i> ; Hyg <sup>R</sup> , single rhomboid mutant (marked)	This study
DPK4	SMR5 derivative; $\Delta$ MSMEG_4904- <i>FRT</i> , Hyg <sup>S</sup> , single rhomboid mutant (unmarked)	This study
DPK5	SMR5 derivative; $\Delta$ MSMEG_4904- $\Delta$ MSMEG_5036- <i>hyg-FRT</i> ; Hyg <sup>R</sup> , double rhomboid mutant (marked)	This study
DPK6	SMR5 derivative; $\Delta$ MSMEG_4904- $\Delta$ MSMEG_5036- <i>FRT</i> , Hyg <sup>S</sup> , double rhomboid mutant (unmarked)	This study
H37Rv+rpsL	<i>MTB H<sub>37</sub>Rv</i> , Sm <sup>R</sup> derivative	[5]
BN44	<i>MTB Uganda</i> genotype strain	[6]
BCG+rpsL	<i>M. bovis</i> BCG, Sm <sup>R</sup>	[5]
JN55	<i>M. bovis</i> cattle isolate	[7]
<i>M. avium</i>	<i>M. avium</i> , strain SU-36800	JCRC
MAP7	MAP cattle isolate	[8]

MAP, *Mycobacterium avium* subsp. *paratuberculosis*; SM<sup>R</sup>, streptomycin resistant; Hyg<sup>R</sup>, hygromycin resistant; Hyg<sup>S</sup>, Hygromycin susceptible; Amp<sup>R</sup>, ampicillin resistant

### Additional references

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