

[illegible]

Figure S1. Multiple alignments of the Mrx proteins from different Actinobacteria. Cg, *Corynebacterium glutamicum*; Cd, *Corynebacterium diphtheriae*; Mt, *Mycobacterium tuberculosis*; Re, *Rhodococcus equi*; Rery, *Rhodococcus erythropolis*; Nf, *Nocardia farcinica*. Most and least conserved regions are highlighted in red and blue, respectively. The CxxC active site of each Mrx is highlighted within black rectangles. The alignment was done with T-coffee (<http://tcoffee.crg.cat>).

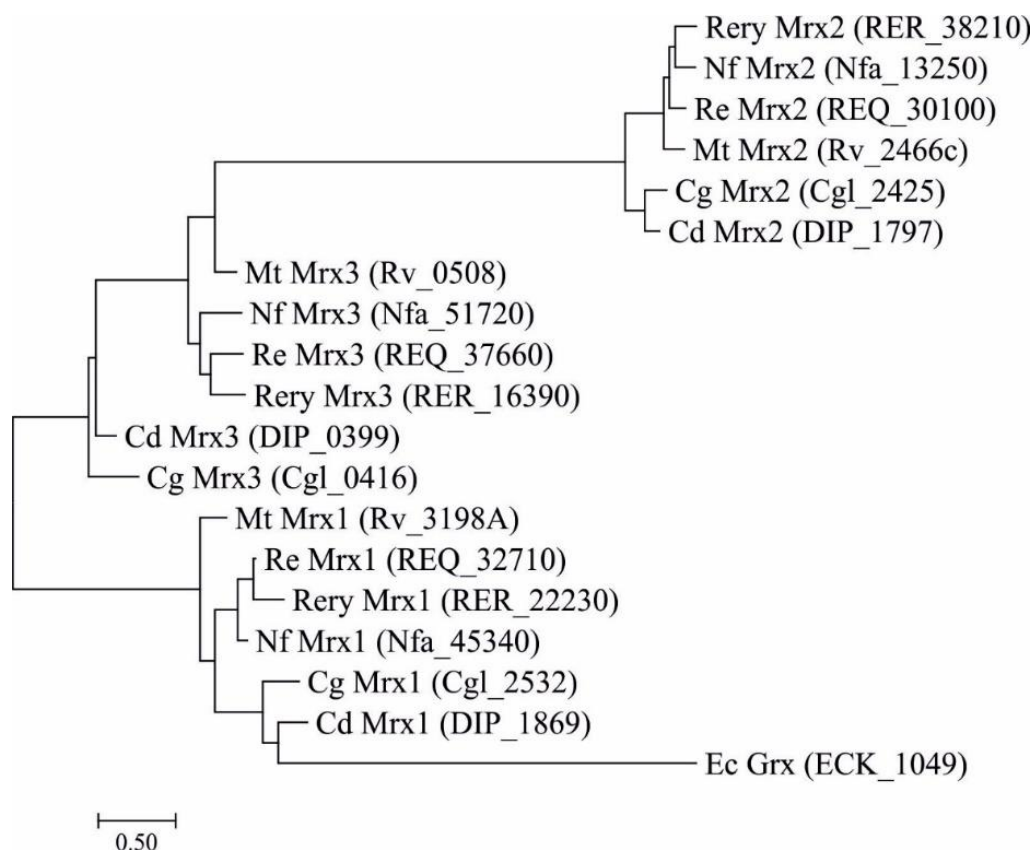


Figure S2. Unrooted evolutionary distance tree based on amino-acid identity of putative mycoredoxins from different actinobacteria. The tree was constructed by maximum likelihood method using 18 Mrx homologues; the *E. coli* Grx was included as outgroup. Rery: *Rhodococcus erythropolis*; Nf: *Nocardia farcinica*; Mt: *Mycobacterium tuberculosis*; Re *Rhodococcus equi*; Cg: *Corynebacterium glutamicum*; Cd: *Corynebacterium diphtheriae*; Ec: *Escherichia coli*; Mrx: mycoredoxin; Grx: Glutaredoxin. The GenBank access numbers are in brackets. Scale represents amino acidic changes.

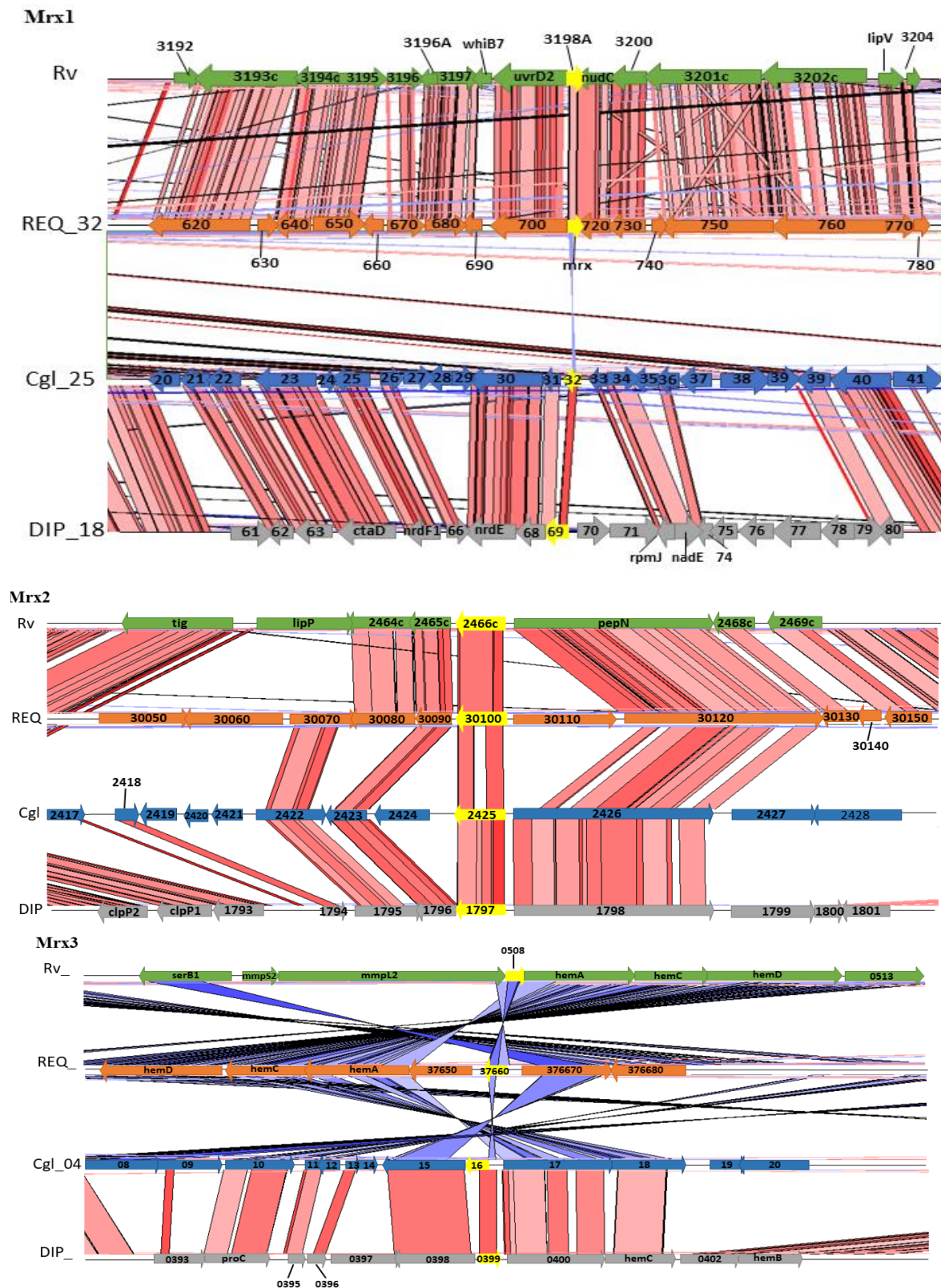


Figure S3. Artemis Comparison Tool (ACT) pairwise chromosome tBLASTx alignment of mycoredoxins (in yellow) in different Actinobacteria: *M. tuberculosis* (Rv), *Rhodococcus equi* (REQ), *Corynebacterium glutamicum* (Cgl) and *Corynebacterium diphtheriae* (DIP). Similarity between chromosome regions is depicted by coloured lines: in red, sequences in direct orientation; in blue, inverted sequences. Colour intensity represents sequence homology percentage, being pink/light blue the lowest and red/deep blue the highest.

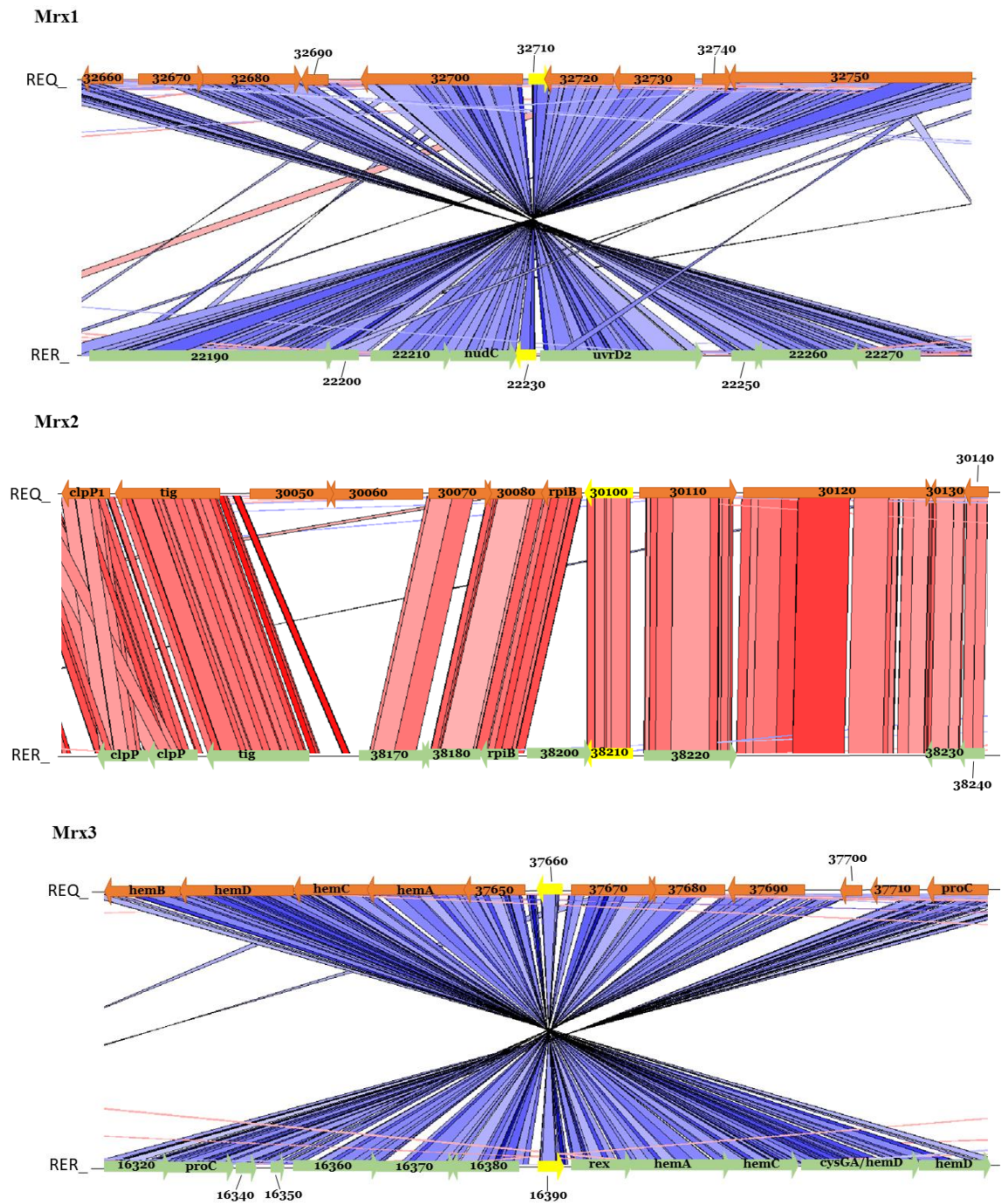


Figure S4. Artemis Comparison Tool (ACT) pairwise chromosome tBLASTx alignment of mycoredoxins (in yellow) in *R. equi* (REQ) and *Rhodococcus erythropolis* (RER). Similarity between chromosome regions is depicted by coloured lines: in red, sequences in direct orientation; in blue, inverted sequences. Colour intensity represents sequence homology percentage, being pink/light blue the lowest and red/deep blue the highest.

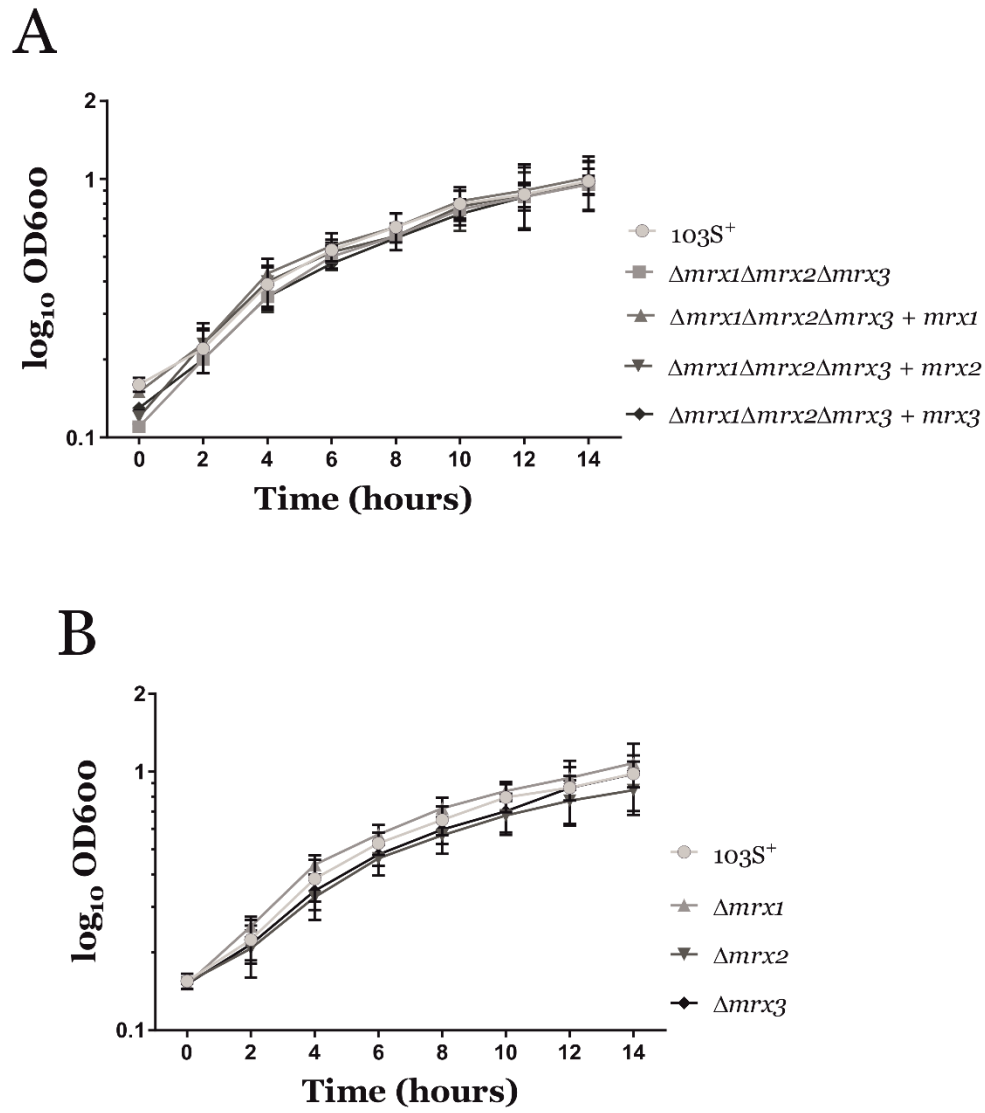


Figure S5: Growth curves of *R. equi* 103S⁺ and *mrx*-null mutants strains cultured in TSB (means $\pm$ SD of three replicates). (a) Triple *mrx* deletion mutant and its *mrx*-complemented derivative strains. (b) Single deletion *mrx* mutants.

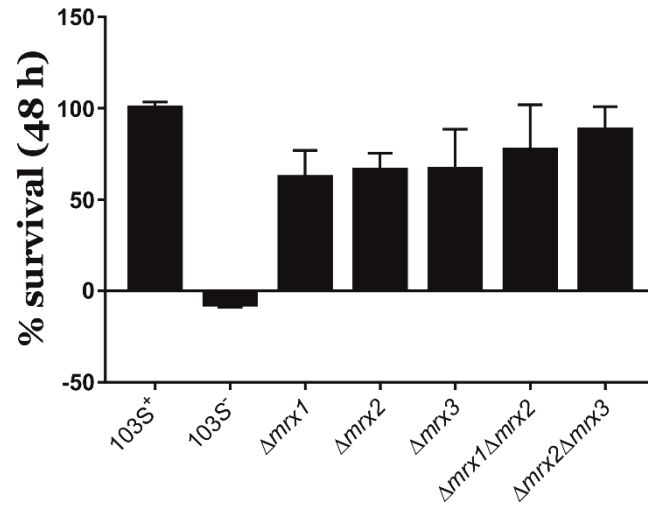


Figure S6. Macrophage infection assays with single and double *mrx*-null mutants. Intracellular survival in J774.A macrophages of the wild type type *R. equi* 103S⁺ strain, the virulence plasmid cured *R. equi* 103S⁻ and single or double deletion mutants: $\Delta mrx1$, $\Delta mrx2$, $\Delta mrx3$, $\Delta mrx1\Delta mrx2$ and $\Delta mrx2\Delta mrx3$. Bar charts of the means $\pm$ SD of three independent experiments after 48 h post-infection. One-way ANOVA and post hoc Tukey's multiple comparison tests were performed to assess for statistical significance across conditions. **P-value < 0,01.

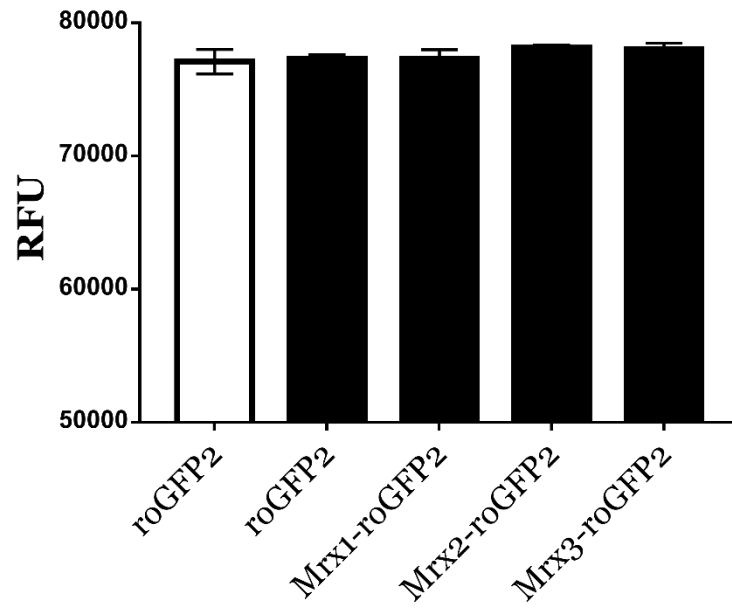


Figure S7. Total fluorescence emitted by *R. equi* derivative strains expressing *roGFP2*. RFU: Relative Fluorescence Units. One-way ANOVA and *post hoc* Tukey's multiple comparison tests were performed to assess for statistical significance across conditions.

SUPPLEMENTARY TABLES

Table S1: Bacterial strains, cell lines and plasmids used in this study

Name	Genotype or characteristics	Reference
<i>Escherichia coli</i> DH5 α	F- ϕ 80d <i>lacZ</i> Δ M15 Δ (<i>lacZYA-argF</i>) U169 <i>thi-1 recA1 relA1 endA1 hsdR17</i> (rk-,mk+) <i>gyrA96 supE44</i> λ	Strategene
<i>Rhodococcus equi</i> 103S ⁺	Virulent strain containing pVAPA plasmid	Ladrón <i>et al.</i> , 2003
<i>R. equi</i> 103S ⁻	Virulence plasmid cured strain - <i>R. equi</i> 103S ⁺ derivative	Ladrón <i>et al.</i> , 2003
<i>R. equi</i> Δ <i>mrx1</i>	Δ <i>mrx1</i> deletion mutant - <i>R. equi</i> 103S ⁺ derivative	This study
<i>R. equi</i> Δ <i>mrx2</i>	Δ <i>mrx2</i> deletion mutant - <i>R. equi</i> 103S ⁺ derivative	This study
<i>R. equi</i> Δ <i>mrx3</i>	Δ <i>mrx3</i> deletion mutant - <i>R. equi</i> 103S ⁺ derivative	This study
<i>R. equi</i> Δ <i>mrx1</i> Δ <i>mrx2</i>	Δ <i>mrx1</i> Δ <i>mrx2</i> deletion mutant - Δ <i>mrx1</i> derivative	This study
<i>R. equi</i> Δ <i>mrx2</i> Δ <i>mrx3</i>	Δ <i>mrx2</i> Δ <i>mrx3</i> deletion mutant – Δ <i>mrx2</i> derivative	This study
<i>R. equi</i> Δ <i>mrx1</i> Δ <i>mrx2</i> Δ <i>mrx3</i>	Δ <i>mrx1</i> Δ <i>mrx2</i> Δ <i>mrx3</i> deletion mutant - Δ <i>mrx1</i> Δ <i>mrx2</i> derivative	This study
<i>R. equi</i> Δ <i>mrx1</i> Δ <i>mrx2</i> Δ <i>mrx3</i> pSET152- <i>mrx1</i>	Δ <i>mrx1</i> Δ <i>mrx2</i> Δ <i>mrx3</i> derivative, complemented with pSET152- <i>mrx1</i>	This study
<i>R. equi</i> Δ <i>mrx1</i> Δ <i>mrx2</i> Δ <i>mrx3</i> pSET152- <i>mrx2</i>	Δ <i>mrx1</i> Δ <i>mrx2</i> Δ <i>mrx3</i> derivative, complemented with pSET152- <i>mrx2</i>	This study
<i>R. equi</i> Δ <i>mrx1</i> Δ <i>mrx2</i> Δ <i>mrx3</i> pSET152- <i>mrx3</i>	Δ <i>mrx1</i> Δ <i>mrx2</i> Δ <i>mrx3</i> derivative, complemented with pSET152- <i>mrx3</i>	This study
<i>R. equi</i> Δ <i>mrx1</i> Δ <i>mrx2</i> Δ <i>mrx3</i> pSET152- <i>mrx1-roGFP2</i>	Δ <i>mrx1</i> Δ <i>mrx2</i> Δ <i>mrx3</i> derivative, complemented with pSET152- <i>mrx1-roGFP2</i>	This study
<i>R. equi</i> Δ <i>mrx1</i> Δ <i>mrx2</i> Δ <i>mrx3</i> pSET152- <i>mrx2-roGFP2</i>	Δ <i>mrx1</i> Δ <i>mrx2</i> Δ <i>mrx3</i> derivative, complemented with pSET152- <i>mrx2-roGFP2</i>	This study
<i>R. equi</i> Δ <i>mrx1</i> Δ <i>mrx2</i> Δ <i>mrx3</i> pSET152- <i>mrx3-roGFP2</i>	Δ <i>mrx1</i> Δ <i>mrx2</i> Δ <i>mrx3</i> derivative, complemented with pSET152- <i>mrx3-roGFP2</i>	This study
<i>R. equi</i> Δ <i>mrx1</i> Δ <i>mrx2</i> Δ <i>mrx3</i> pSET152- <i>roGFP2</i>	Δ <i>mrx1</i> Δ <i>mrx2</i> Δ <i>mrx3</i> derivative, complemented with pSET152- <i>roGFP2</i>	This study
<i>R. equi</i> 103S ⁺ pSET152- <i>roGFP2</i>	103S ⁺ derivative, complemented with pSET152- <i>roGFP2</i>	This study

J774A.1	Mouse BALB/c monocyte macrophages	Sigma-Aldrich
pSET152	ϕ C31 integrase <i>attP</i> Apr ^R	Bierman <i>et al.</i> , 1992
pSelAct	Apr ^R , <i>lacZ</i> , <i>codA:upp</i>	van der Geize <i>et al.</i> , 2008
pSET152- <i>mrx1</i>	pSET152 containing <i>mrx1</i> and 500 bp upstream	This study
pSET152- <i>mrx2</i>	pSET152 containing <i>mrx2</i> and 500 bp upstream	This study
pSET152- <i>mrx3</i>	pSET152 containing <i>mrx3</i> and 500 bp upstream	This study
pSET152- <i>mrx1-roGFP2</i>	pSET152 containing <i>mrx1</i> fused to <i>roGFP2</i> under the control of P _{kan}	This study
pSET152- <i>mrx2-roGFP2</i>	pSET152 containing <i>mrx2</i> fused to <i>roGFP2</i> under the control of P _{kan}	This study
pSET152- <i>mrx3-roGFP2</i>	pSET152 containing <i>mrx3</i> fused to <i>roGFP2</i> under the control of P _{kan}	This study
pSET152- <i>roGFP2</i>	pSET containing <i>roGFP2</i> under the control of P _{kan}	This study
pSelAct Δ <i>mrx1</i>	pSelAct containing 1500 bp upstream and downstream of <i>mrx1</i>	This study
pSelAct Δ <i>mrx2</i>	pSelAct containing 1500 bp upstream and downstream of <i>mrx2</i>	This study
pSelAct Δ <i>mrx3</i>	pSelAct containing 1500 bp upstream and downstream of <i>mrx3</i>	This study

Table S2: Primers used in this study

Name	Sequence	Target
Mrx1 Del F	CTAGTCTAGACGGTCGCGTCCAGCAAC	1,500 bp upstream of <i>mrx1</i>
Mrx1 Mix R	CTAGAGGCCCCAGGGCAGCTTCGGTAGTCAC	1,500 bp upstream of <i>mrx1</i>
Mrx1 Mix F	GTGACTACCGAAGCTGCCCTGGGCCTCTAG	1,500 bp downstream of <i>mrx1</i>
Mrx1 Del R	CTAGTCTAGATGACGGATCGGCCGGAC	1,500 bp downstream of <i>mrx1</i>
Mrx2 Del F	ATCTAGTATAGACCATCACGCACCCCGCATCT	1,500 bp upstream of <i>mrx2</i>
Mrx2 Mix R	TCAGTCGAACTCGGGGGTCTGTGCTTGCAAT	1,500 bp upstream of <i>mrx2</i>
Mrx2 Mix F	ATGCAAGCACAGACCCCGAGTTCGACTGA	1,500 bp downstream of <i>mrx2</i>
Mrx2 Del R	ATCTAGTCTAGACCGGTGACGGCGACTTCGGC	1,500 bp downstream of <i>mrx2</i>
Mrx3 Del F	CTAGTCTAGAGGGTTCGAGGTCCGCGGTG	1,500 bp upstream of <i>mrx3</i>
Mrx3 Mix R	ATCAGCCTGCCGAGAGTCGCGACTGATCCAT	1,500 bp upstream of <i>mrx3</i>
Mrx3 Mix F	ATGGATCAGTCGCGACTCTCGGCAGGCTGAT	1,500 bp downstream of <i>mrx3</i>
Mrx3 Del R	CTAGTCTAGAGCGTCCGGCGTCGGATGCG	1,500 bp downstream of <i>mrx3</i>
Mrx1 F	CTAGTCTAGAGCCCGACGAGCGCGAAC	Complementation of <i>mrx1</i>
Mrx1 R	CTAGTCTAGACTAGAGGCCAGGGCCTG	Complementation of <i>mrx1</i>
Mrx2 F	CTAGTCTAGAAGAATGCCGCGCGGTGAG	Complementation of <i>mrx2</i>
Mrx2 R	CTAGTCTAGATCAGCCTGCCGAGAGGTC	Complementation of <i>mrx2</i>
Mrx3 F	ATCTAGTCTAGACGAGCTTGTCGTAACCGA	Complementation of <i>mrx3</i>
Mrx3 R	ATCTAGGTCTAGAGACCTCAGTCGAACTCGG	Complementation of <i>mrx3</i>
Mrx1 F roGFP2	ATCTAGCATATGACTACCGAAGCTCCCG	<i>mrx1</i> fusion to roGFP2

Mrx1 R Mix ro	TGAACCACCACTAGTGAGGCCAGGGCCTG	<i>mrx1</i> fusion to roGFP2
Mrx1 F Mix ro	CAGGCCCTGGGCCTCACTAGTGGTGGTTCA	<i>mrx1</i> fusion to roGFP2
Mrx2F roGFP2	ATCTAGCATATGCAAGCACAGACCCCAGG	<i>mrx2</i> fusion to roGFP2
Mrx2 R Mix ro	TGAACCACCACTAGTGTCGAACTCGGGGTC	<i>mrx2</i> fusion to roGFP2
Mrx2 F Mix ro	GACCCCGAGTTCGACACTAGTGGTGGTTCA	<i>mrx2</i> fusion to roGFP2
Mrx3 F roGFP2	ATCGAGCATATGGATCAGTCGCGACATCGG	<i>mrx3</i> fusion to roGFP2
Mrx3 R Mix ro	TGAACCACCACTAGTGCCTGCCGAGAGGTC	<i>mrx3</i> fusion to roGFP2
Mrx3 F Mix ro	GACCTCTCGGCAGGCACTAGTGGTGGTTCA	<i>mrx3</i> fusion to roGFP2
roGFP2 R	CCCCTCGAGTTACTTGTACAGCTCGTC	<i>mrxs</i> fusion to roGFP2
roGFP2 F	CATTACATATGGGTGGTCAGGGTGGT	Unfused <i>roGFP2</i>
VapA F	AGATGAAGACTCTTCACAAGACGG	Amplification of <i>vapA</i>
VapA R	CTAGGCGTTGTGCCAGCTACCA	Amplification of <i>vapA</i>
Ori pVAP F	TAGCGTATCGATTTAAACAAGCCCGAGCGTCTCGC	Amplification of <i>Ori</i> pVAPA
Ori pVAP R	GCATCGTTTAAATCGATGCTGCGGGTAACGCAGCTG	Amplification of <i>Ori</i> pVAPA