

1 **Supplementary data**

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3 **Supplementary Table S1.** Antibiotic susceptibility of *mptC* mutant

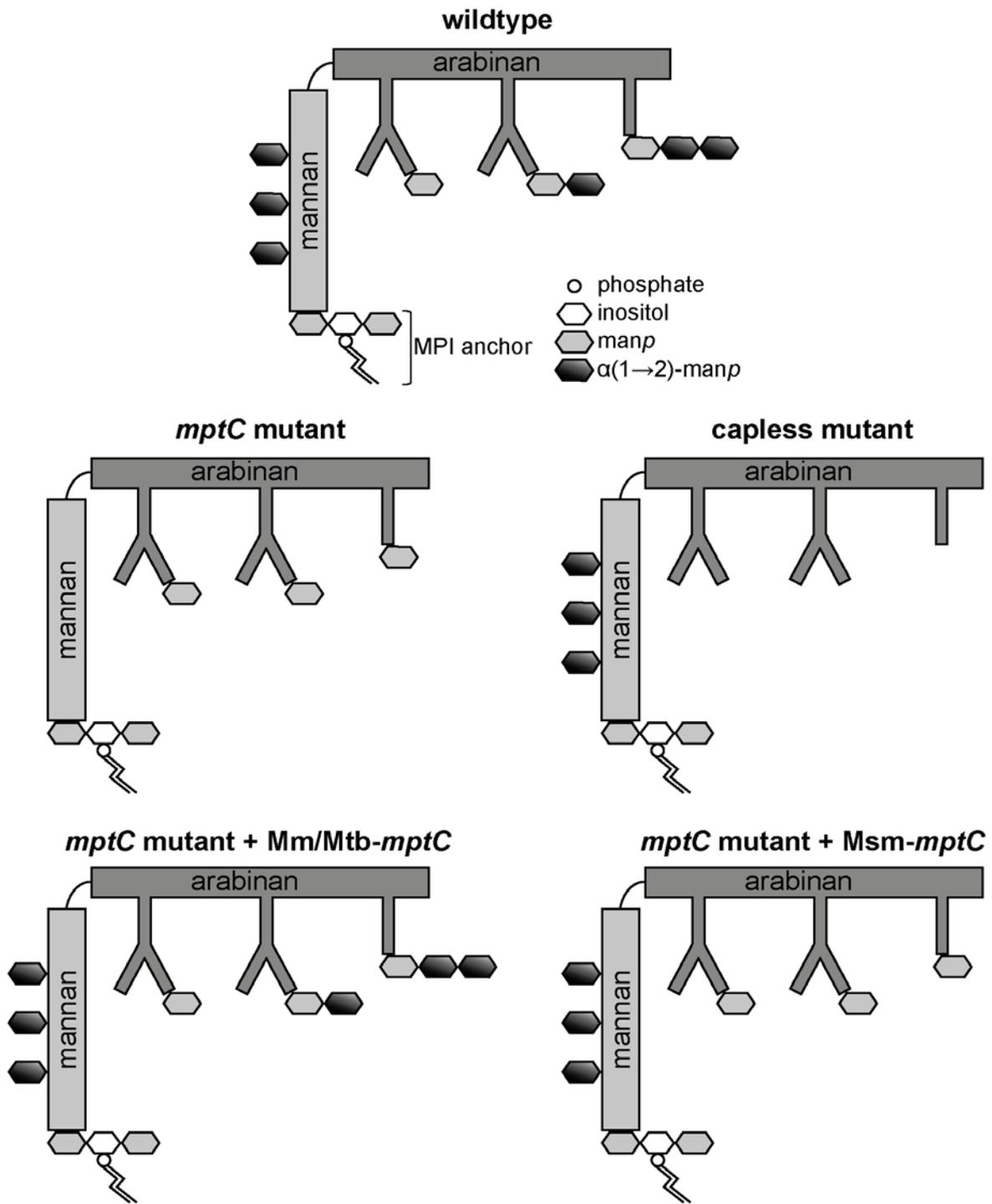
antibiotic	MIC ($\mu\text{g/ml}$)		
	wildtype	<i>mptC</i> mutant	<i>mptC</i> + Mm- <i>mptC</i>
streptomycin	4	4	4
erythromycin	8	8	8
isoniazid	>32	>32	>32
rifampicin	0.04	0.04	0.04
polymyxin B	>1600	>1600	>1600
chloramphenicol	<16	<16	256*

4 *The vector used to complement the *mptC* mutant contains a chloramphenicol resistance

5 cassette.

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1 **Supplementary Figures & Figure legends**

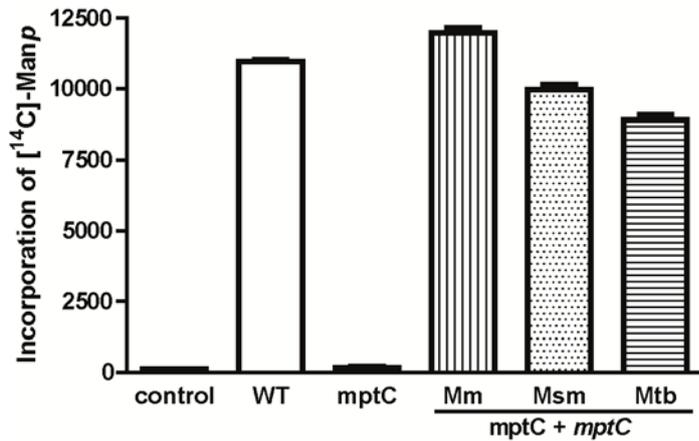


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3 **Figure S1. Schematic structure of lipoarabinomannan (LAM) of the different strains**
4 **used in this study.** Schematic representations of LAM of wildtype *M. marinum*, the *mptC*
5 mutant, the capless mutant, the *mptC* mutant complemented with the *M. marinum* or *M.*
6 *tuberculosis mptC* (*mptC* mutant + Mm/*Mtb-mptC*), or the *mptC* mutant complemented with

1 the *M. smegmatis* *mptC* (*mptC* mutant + *Msm-mptC*). ManLAM is anchored into the
2 mycobacterial cell envelope by its mannosylphosphatidyl-*myo*-inositol (MPI) moiety. The MPI
3 anchor is linked to a mannan core consisting of $\alpha(1\rightarrow6)$ -linked mannopyranose (*manp*)
4 residues branched with $\alpha(1\rightarrow2)$ -linked *manp* units. The structure is further glycosylated with
5 an arabinan domain consisting of a linear $\alpha(1\rightarrow5)$ -linked arabinofuranosyl (*araf*) polymer
6 branched with linear or bifurcating *araf* side chains. The non-reducing termini of the arabinan
7 domain can be substituted with one to three *manp* residues; the mannose caps. The first
8 *manp* unit is $\alpha(1\rightarrow5)$ -linked and the following *manp* residues are $\alpha(1\rightarrow2)$ -linked.

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2 **Figure S2. $\alpha(1\rightarrow2)$ -Mannosyltransferase activity of *mptC* mutant and related strains.** *In*

3 *vitro* $\alpha(1\rightarrow2)$ -mannosyltransferase assay with synthetic nonasaccharide acceptor and

4 membrane fractions from the blank (control), wildtype *M. marinum* (WT), *mptC* mutant

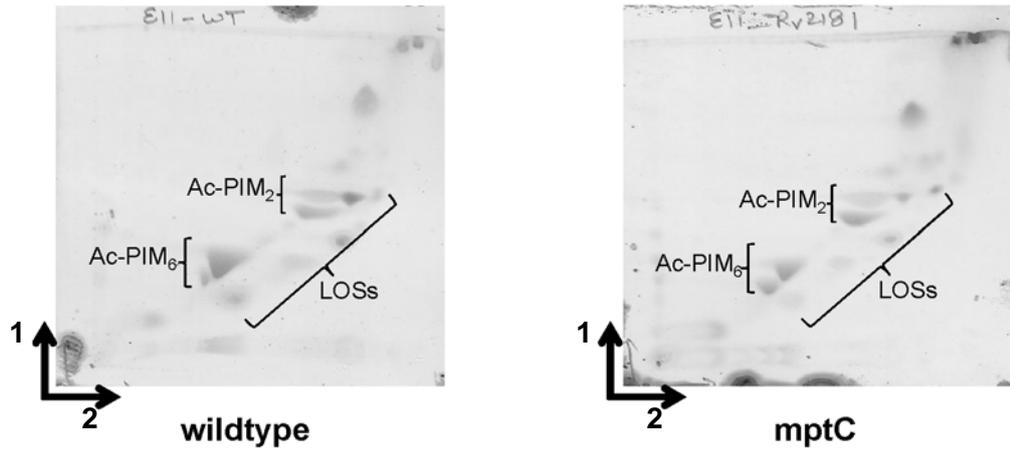
5 (*mptC*), *mptC* mutant complemented with *mmar_3225* (*mptC* + *mptC* Mm), *mptC* mutant

6 complemented with *msmeg4247* (*mptC* + *mptC* Msm) and *mptC* mutant complemented with

7 *Rv2181* (*mptC* + *mptC* Mtb). The results represent mean + SEM of three independent

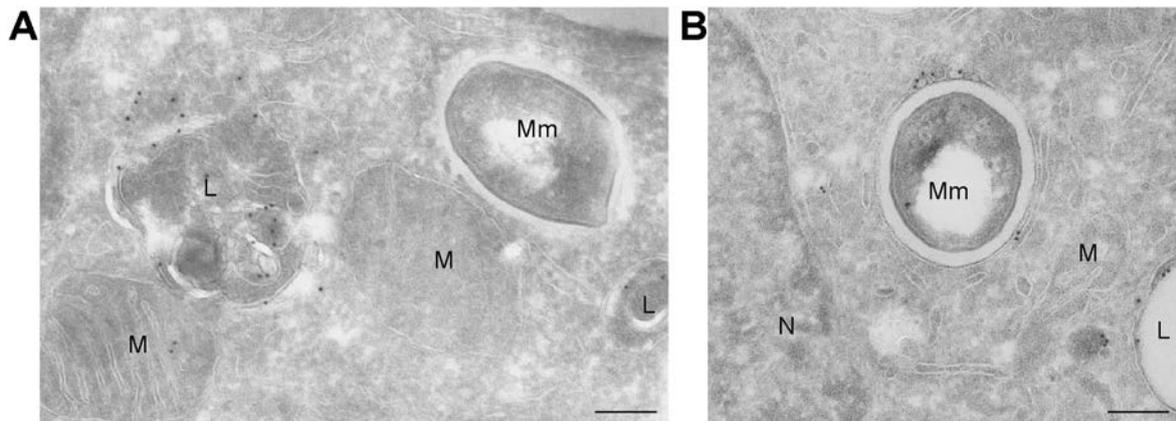
8 experiments.

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Figure S3. Analysis of PIM biosynthesis in wildtype *M. marinum* (wildtype) and the *mptC* mutant (*mptC*). The polar lipids were extracted and examined by 2D-TLC. First and second dimensions are indicated by arrows and numbers.



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2 **Figure S4. EM images of sections of THP-1 cells infected for 24 hours with the *M.***
3 ***marinum mptC* mutant.** Representative images of sections of cells with (A) cytosolic and
4 (B) phagosomal *mptC* mutant bacterium (Mm), and immunogold labeled for CD63 with 10 nm
5 gold particles, indicating the presence of phagosomal membranes. L= lysosome, M=
6 mitochondria, N= nucleus and the bars represent 200 nm.