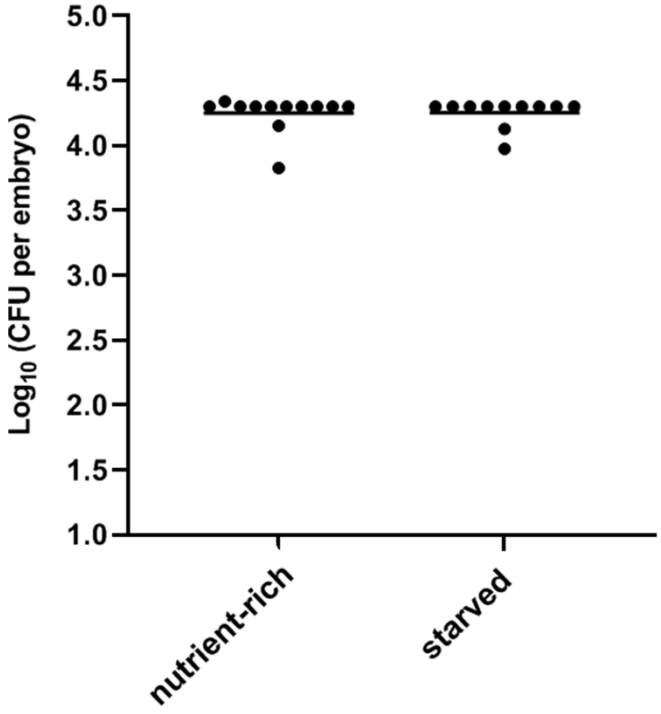
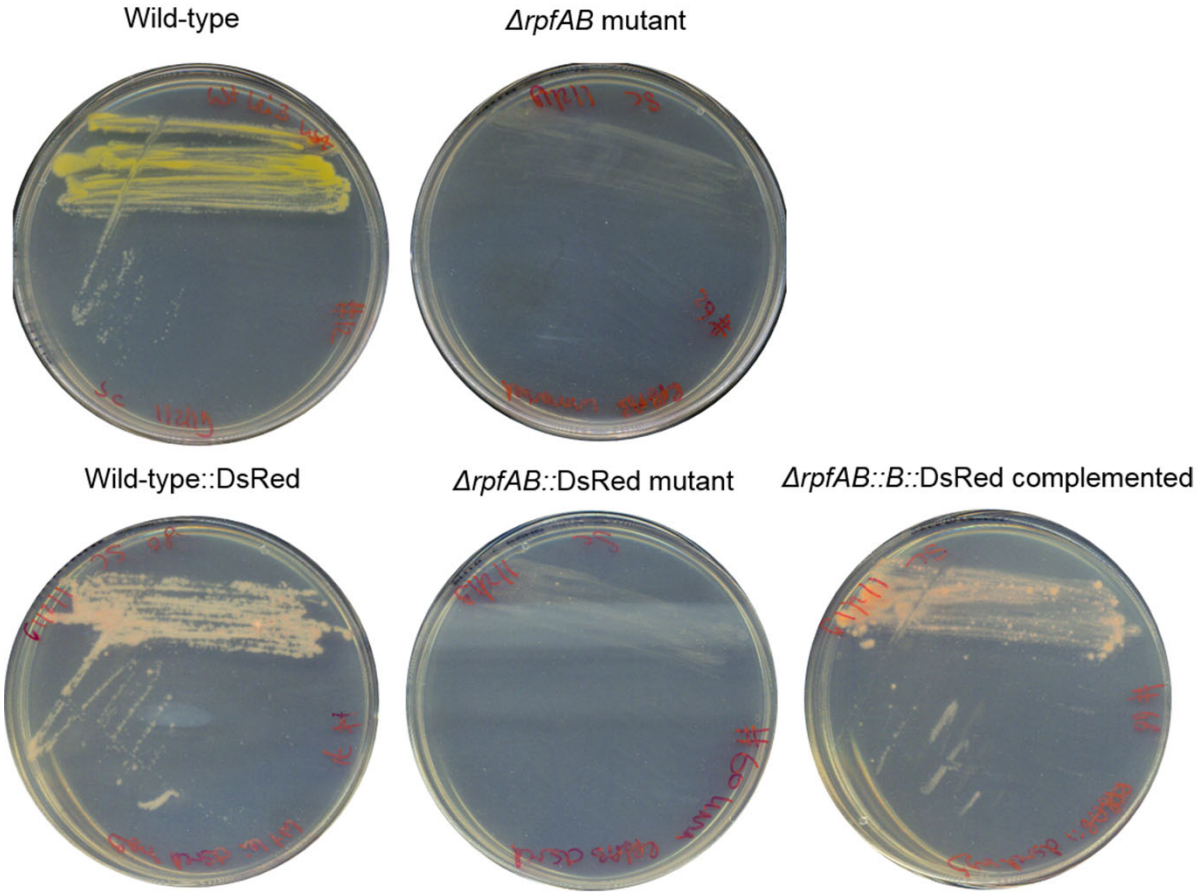
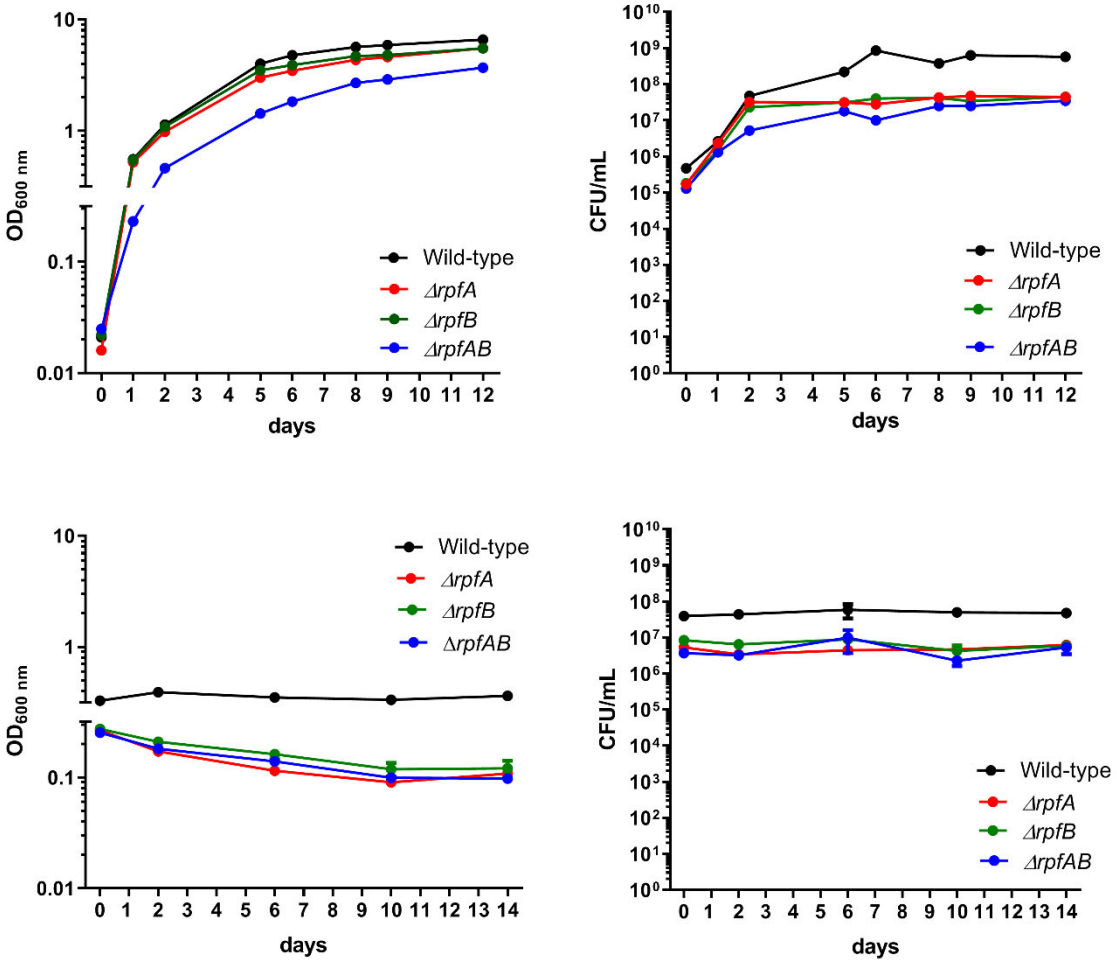


Supplementary Figure 1. Loss of starved M. marinum *persister phenotype* in vivo.
One dpf zebrafish embryos were infected with either nutrient-rich *M. marinum* or starved *M. marinum* for 5 days. Bacterial load (CFU) was determined per embryo and log-transformed. Inoculum of nutrient-rich *M. marinum* ranged between 55-137 CFU and inoculum of starved *M. marinum* ranged between 66-236 CFU.



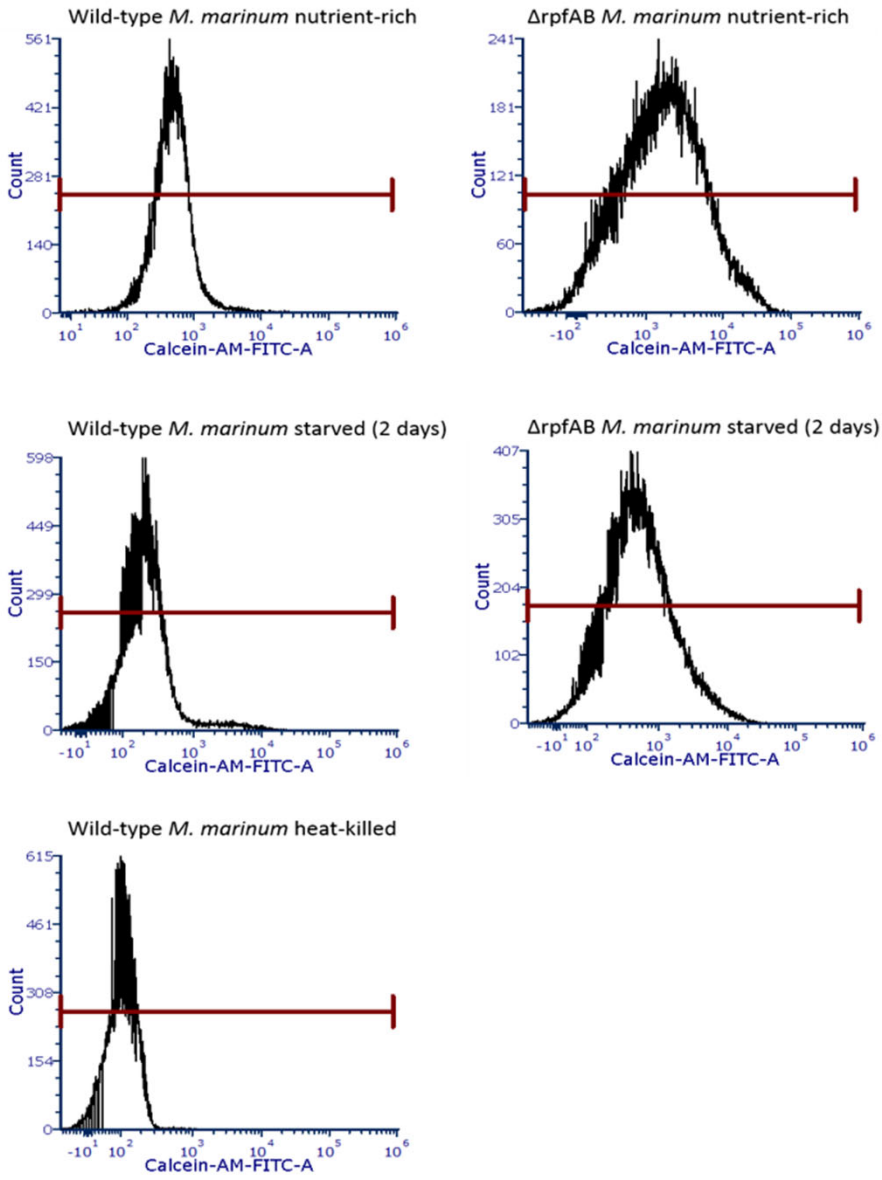
Supplementary Figure 2. Growth delay of ΔrpfAB mutant.

Plates inoculated from -80 ºC stocks show delayed growth of *ΔrpfAB* mutant.



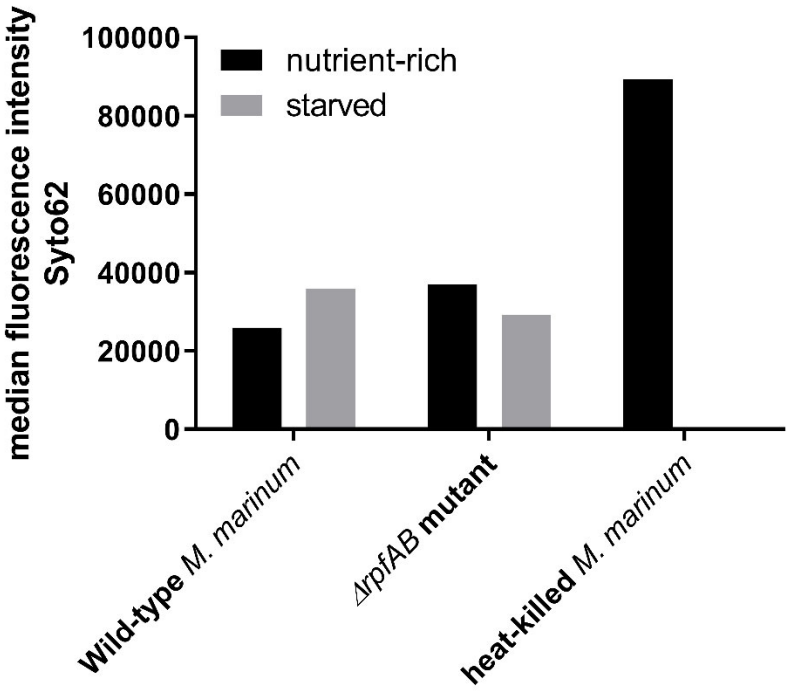
Supplementary Figure 3. Survival of starved rpf deletion mutants

Growth curve of wild-type *M. marinum, ΔrpfA*, *ΔrpfB* and *ΔrpfAB* mutants inoculated in nutrient-rich (A-B) or starved (C-D) conditions. Both optical density (A) and colony forming units (CFU) (B) were determined per mL. Representative growth curve and CFU for nutrient-rich conditions (n=three independent experiments) and mean of three replicates from one experiment with standard deviation plotted for starved conditions (representative of three independent experiments).

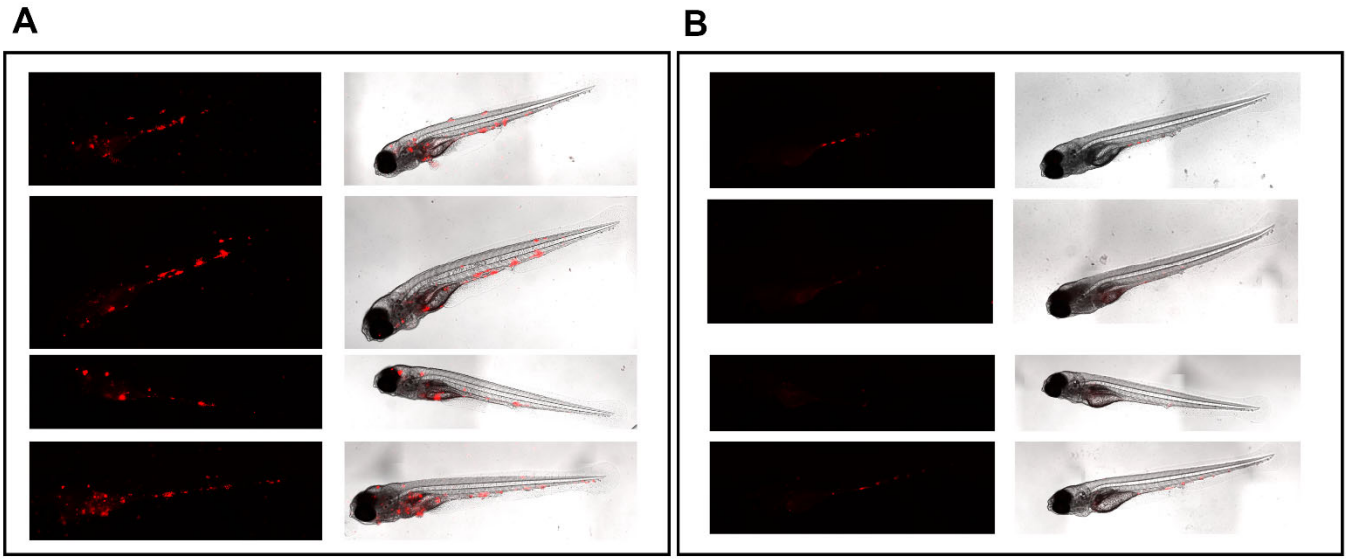


Supplementary Figure 4. Histogram plots of Calcein-AM staining.

Calcein-AM intensity plotted on *x*-axis and event counts plotted on *y*-axis. Red bar indicates the range used for calculating median fluorescence intensity.



Supplementary Figure 5. Median fluorescence intensity of Syto62.
Nutrient-rich and starved wild-type and *ΔrpfAB* mutant were stained with Syto62. Median fluorescence intensity was determined by flow cytometry. Representative of two independent experiments.



Supplementary Figure 6. Zebrafish embryos infected with starved wild-type and *ΔrpfAB M. marinum.*

At five dpi zebrafish embryos were imaged to determine the bacterial load by quantification of DsRed2 fluorescence per embryo. Representative DsRed2 fluorescence and overlay images of zebrafish embryos infected with starved wild-type *M. marinum* (A) and *ΔrpfAB M. marinum* (B).

Supplementary Table 1. Primers used in this study

Primer name	Primer Sequence (5'-3')	Restriction site	Purpose
Δ*rpfA FR1F*	gcc *AAG CTT* ccc tgt tta tcc ggc act cgg tag	HindIII	Generation of Δ*rpfA*
Δ*rpfA FR1R*	gat *GGT ACC* tcc act cat acg ttc agt aat tcc	KpnI	Generation of Δ*rpfA*
Δ*rpfA FR2F*	tga *GGT ACC* acc gtc ttc gcc tga gac agg gtc	KpnI	Generation of Δ*rpfA*
Δ*rpA F21R*	gcg *GCG GCC GC*a ccg gtg acc ttg cgc cgc gga	NotI	Generation of Δ*rpfA*
Δ*rpfB FR1F*	ccg *AAG CTT* act atc gct gca acc tgc gaa	HindIII	Generation of Δ*rpfB*
Δ*rpfB FR1R*	tga *GGT ACC* ggc gcc tgg ccc gtg tgt agt	KpnI	Generation of Δ*rpfB*
Δ*rpfB FR2F*	ttt *GGT ACC* cag att caa cgt ccg tct atc	KpnI	Generation of Δ*rpfB*
Δ*rpfB FR2R*	aga *GCG GCC* GCt att ggt tag tcc tat tgt	NotI	Generation of Δ*rpfB*
M*rpfB*pMV306F	Atc *GGT ACC* gcc aac agg cga ttc ccg gtt cg	KpnI	Generation of Δ*rpfAB::rpfB*
M*rpfB*pMV306R	Cat *GAA TTC* ggt acg ccc gag cag ccg gat g	EcoRI	Generation of Δ*rpfAB::rpfB*
Δ*rpfA* Test F	ctc acg taa cgg aac gat aac	N/A	Confirmation of Δ*rpfA*
Δ*rpfA* Test R	tgg ctg ccg atc atc gcg agg	N/A	Confirmation of Δ*rpfA*
Δ*rpfB* Test F	cgt cgt gga cga agt tct gt	N/A	Confirmation of Δ*rpfB*
Δ*rpfB* Test R	gct agg cca aca ggc gat tc	N/A	Confirmation of Δ*rpfB*
pMV306F	TGGTATCTTTATAGTCCTGTC	N/A	Sequencing
pMV306R	TAGTTAACTACGTCGACATCGA	N/A	Sequencing