

Figure S1

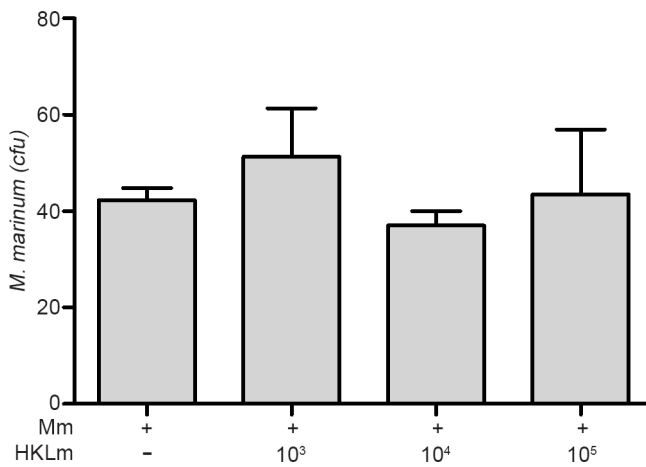
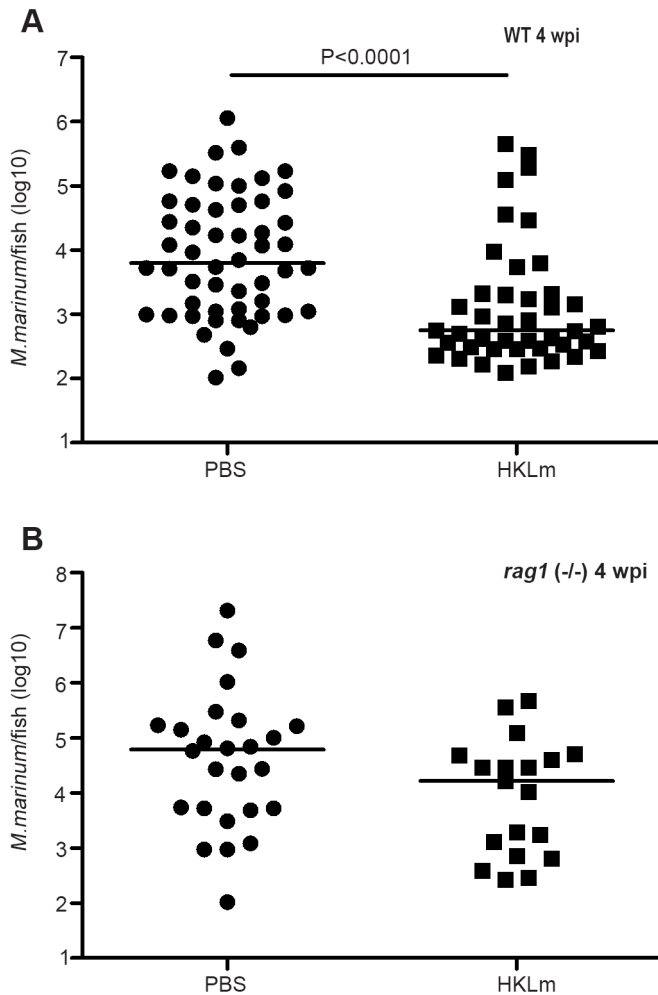


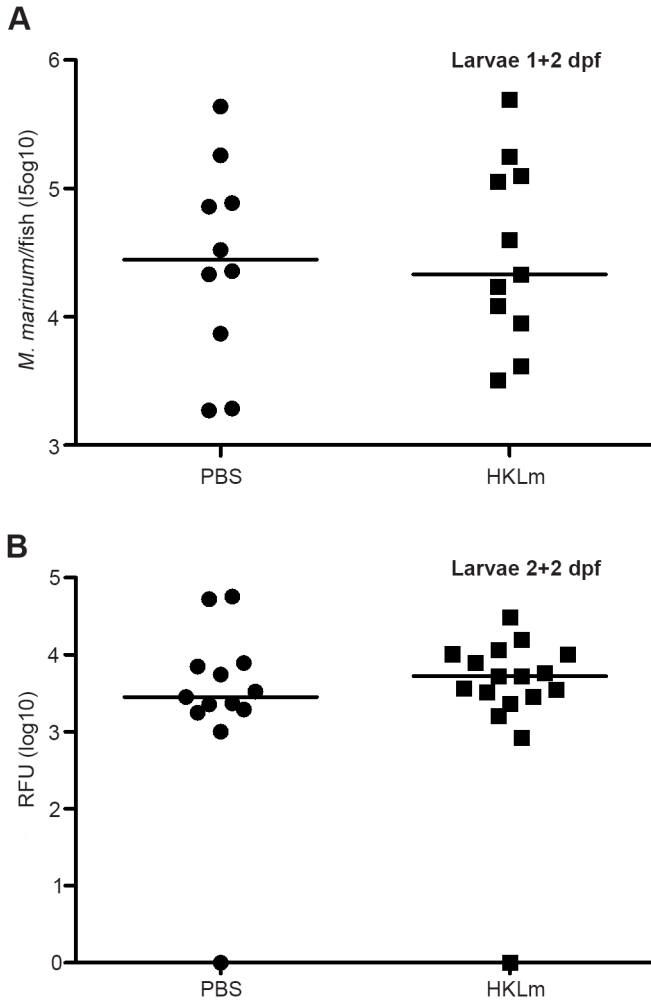
Figure S1. HKLm does not exhibit bactericidal effects on *Mycobacterium marinum* in vitro. *M. marinum* was incubated for 12 days in 7H9 medium together with different concentrations of HKLm. The bacterial cultures were plated on 7H10 plates and the colony forming units (cfu) of *M. marinum* were determined. 10<sup>3</sup>, 10<sup>4</sup>, and 10<sup>5</sup> denote the ratio of HKLm compared to the initial cfu in the *M. marinum* culture. Mm= *Mycobacterium marinum*, HKLm= Heat-killed *Listeria monocytogenes*. n=3 for each sample.

## Figure S2



**Figure S2. HKLm priming decreases the mycobacterial loads also in non-cleared populations.** The data represented in A and B exclude those individuals that had bacterial counts below the detection limit of the *M. marinum*-specific qPCR method i.e. had cleared the infection. Also the wild-type individuals that were unable to clear the infection benefit from HKLm by showing significantly lowered mycobacterial loads (A). *Rag1* (-/-) fish show a similar trend (B). P-values were calculated with a two-tailed non-parametric Mann-Whitney test with GraphPad Prism. Medians for the experiments are shown in the figures. Data are pooled from 4 individual experiments for A (representative experiment shown in Fig 2A, n=52 for PBS and n=42 for HKLm) and from 2 individual experiments in Fig. 2D for B (n=26 for PBS and n=19 for HKLm).

## Figure S3



**Figure S3. HKLm priming does not protect zebrafish larvae from mycobacterial infection.** Zebrafish larvae were primed with 240 cfu of HKLm 1 day post-fertilization (dpf) (A) or 490 cfu of HKLm 2 dpf (B). *M. marinum* infections ( $39 \pm 13$  cfu for A and  $39 \pm 16$  cfu for B) were carried out 2 dpf. Mycobacterial loads were determined with an *M. marinum*-specific qPCR (A) or by measuring the fluorescent signal of a wasabi-*M. marinum* strain (B) with a plate reader.  $n=10$  for PBS and  $n=11$  for HKLm in A and  $n=13$  for PBS and  $n=17$  for HKLm in B.