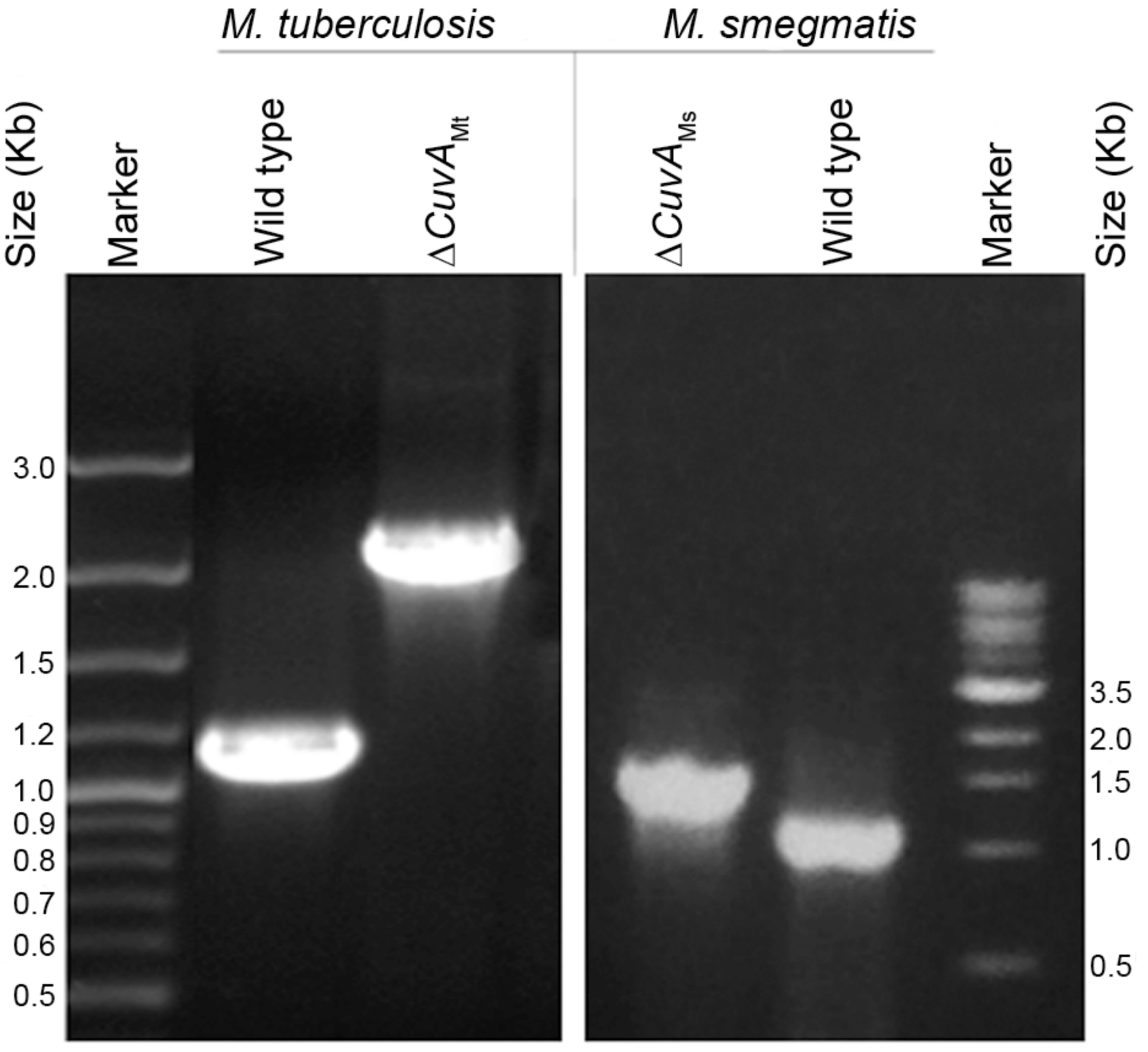

Fig. S1. PCR analysis of Δ*cuvA*$_{Mt}$ and Δ*cuvA*$_{Ms}$. Genomic DNA isolated from wild type *M. tuberculosis* H37Rv, *M. tuberculosis* Δ*cuvA*$_{Mt}$, wild type *M. smegmatis* and *M. smegmatis* Δ*cuvA*$_{Ms}$ were amplified with primers flanking the *Rv1422* and *MSMEG_3080* genes. Expected sizes of PCR products are: wild type *M. tuberculosis*, 1.091 kb; Δ*cuvA*$_{Mt}$, 2.091 kb; wild type *M. smegmatis*, 1.065 kb; Δ*cuvA*$_{Ms}$, 1.5 kb.

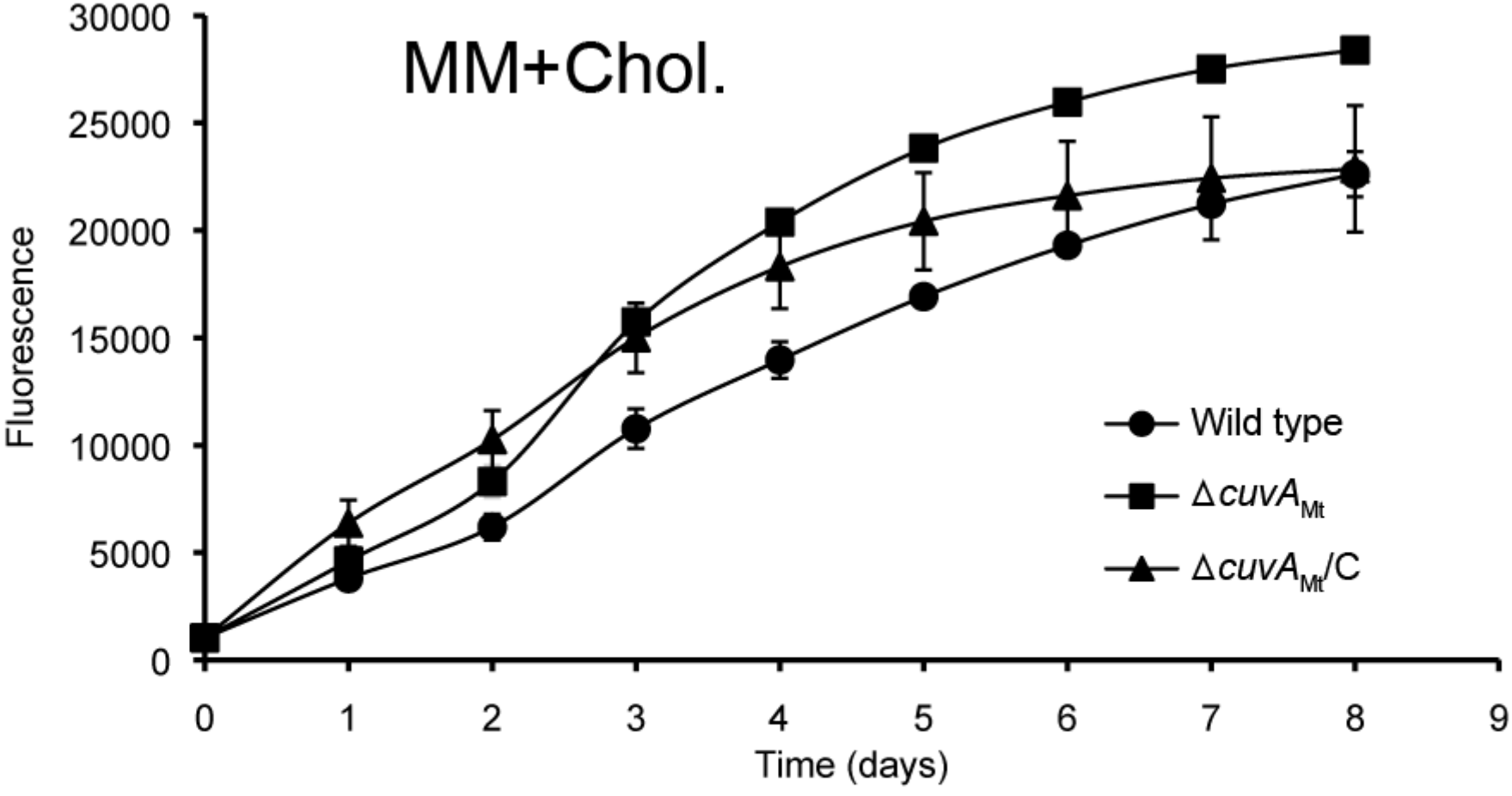

Fig. S2. . Wild type *M. tuberculosis* H37Rv, Δ*cuvA*$_{Mt}$ and complemented Δ*cuvA* (Δ*cuvA*$_{Mt}$/C) strains were diluted to OD_{600}=0.005 and analyzed using the MABA in MM containing 0.01% cholesterol. Data from one of 2 biological replicates, with measurements obtained from duplicate wells.
Error bars are +/- 1 S.D.

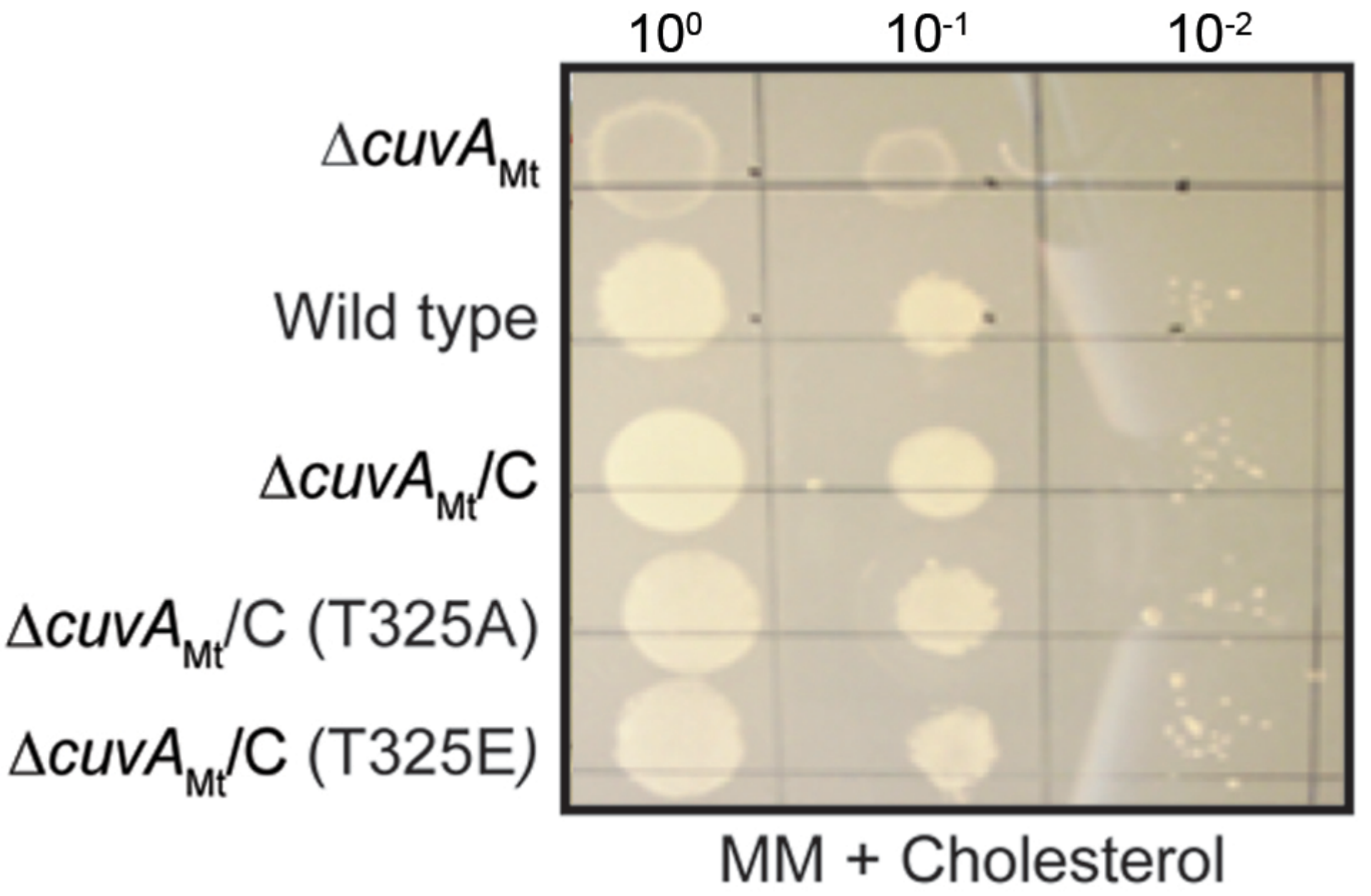

Fig. S3. *M. tuberculosis* wild type H37Rv, Δ*cuvA*$_{Mt}$ and Δ*cuvA*$_{Mt}$ complemented with the wild type *cuvA*$_{Mt}$ allele or with alleles encoding *cuvA*$_{Mt}$ with non-phosphorylatable (T325A) or phosphomimetic (T325E) substitutions grown on MM agar plus cholesterol.

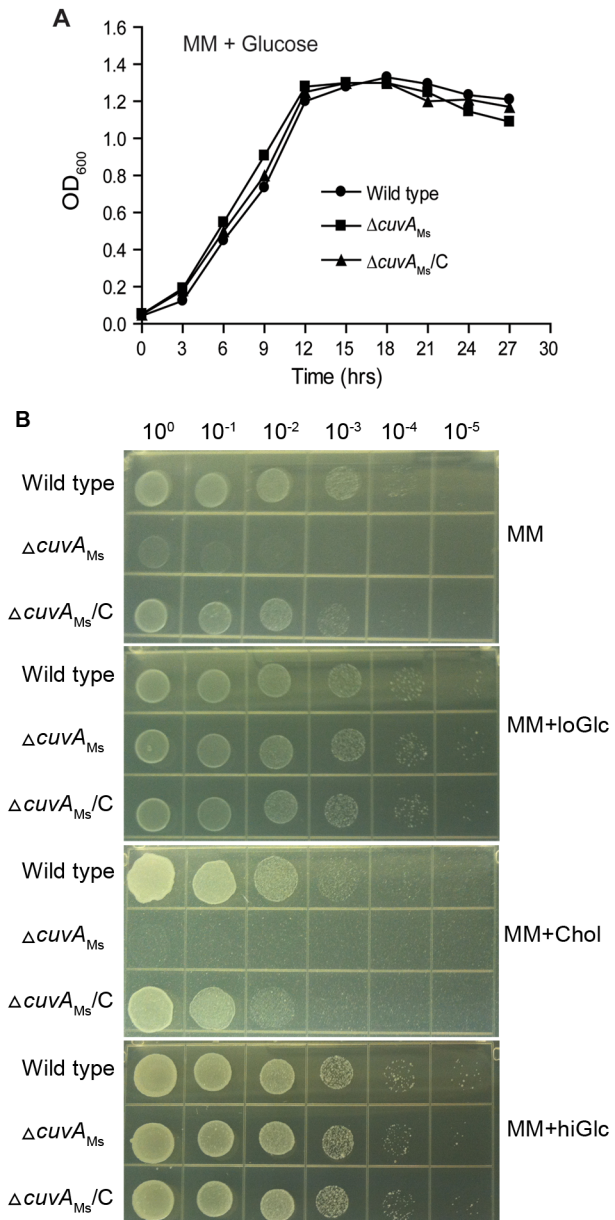

Fig. S4. **A**. Growth of *M. smegmatis* wild type, Δ*cuvA*$_{Ms}$ and complemented Δ*cuvA*$_{Ms}$ strains in Middlebrook 7H9-ADC-Tw medium containing 0.2% glucose, measured by OD_{600}. Data shown are from a single experiment that was repeated once with similar results. **B**. Growth of *M. smegmatis* wild type, Δ*cuvA*$_{Ms}$ and complemented Δ*cuvA*$_{Ms}$ strains on MM agar alone or MM agar plus 0.01% glucose, 0.01% cholesterol or 0.2% glucose.

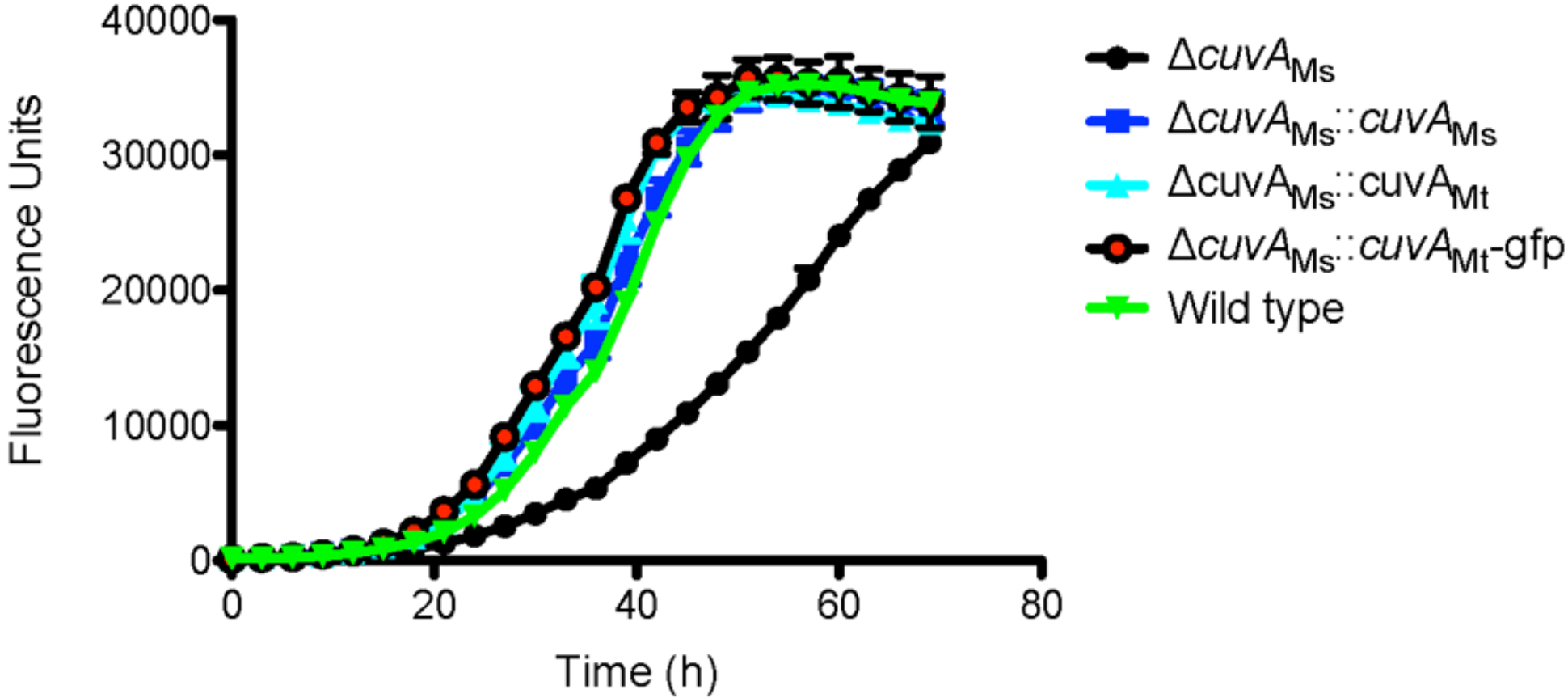

Fig. S5. MABA analysis of *M. smegmatis* wild type, Δ*cuvA*$_{Ms}$ and Δ*cuvA*$_{Ms}$ complemented with *cuvA*$_{Ms}$, *cuvA*$_{Mt}$ or *cuvA*$_{Mt}$-gfp grown in MM plus 0.01% cholesterol. Growth of Δ*cuvA*$_{Ms}$ strain is significantly different from all other strains ($P<0.0001$). There are no significant differences between the growth of wild type and Δ*cuvA*$_{Ms}$ complemented with *cuvA*$_{Ms}$, *cuvA*$_{Mt}$ or *cuvA*$_{Mt}$-gfp ($P>0.05$ for all comparisons).

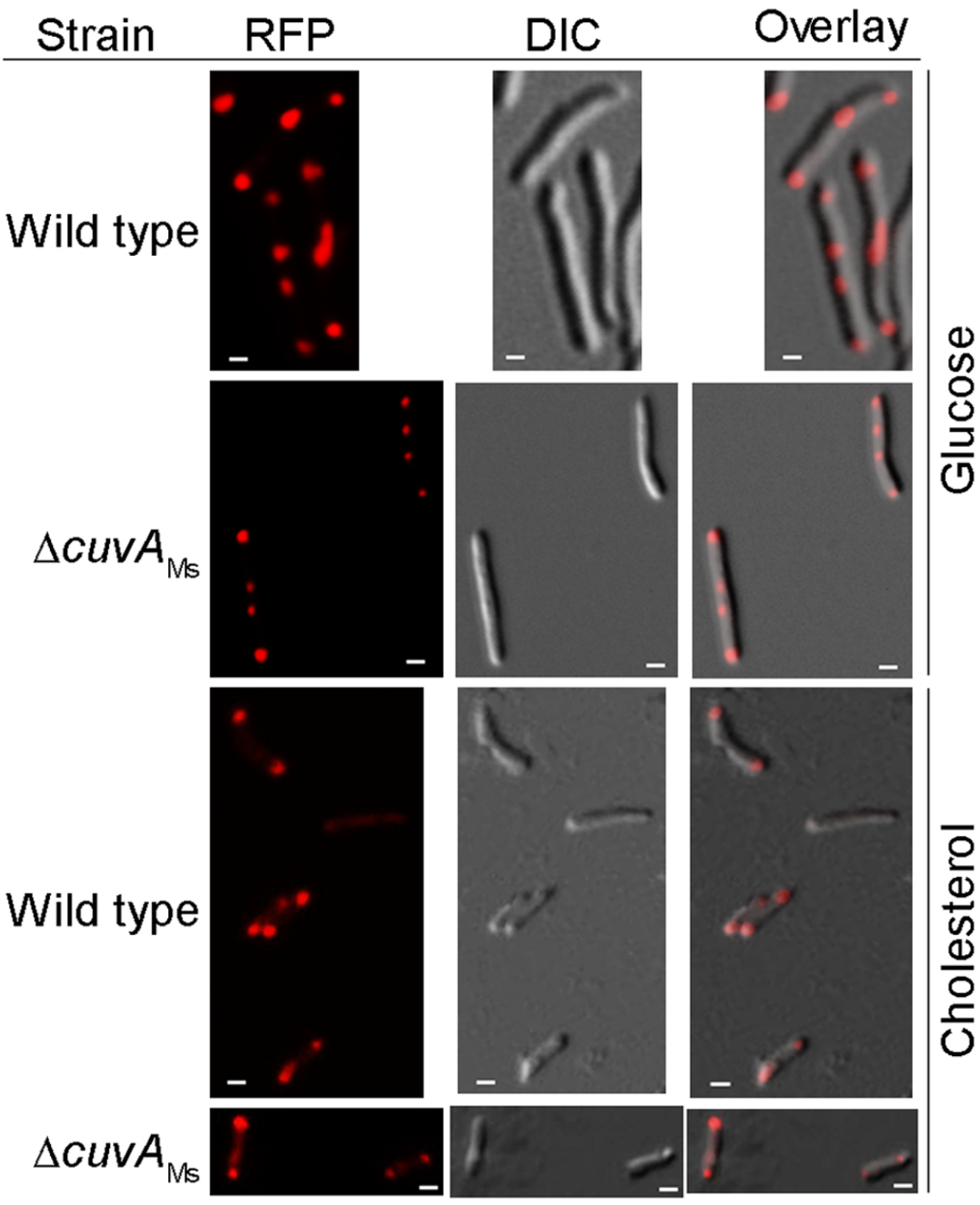

Fig. S6. Localization of PBP1-RFP. Wild type *M. smegmatis* cells expressing PBP1-RFP or RFP alone were grown in MM containing 0.2% glucose or 0.01% cholesterol as the carbon source, and examined by fluorescence and DIC microscopy after 16 hrs. Bar = 1 μm.

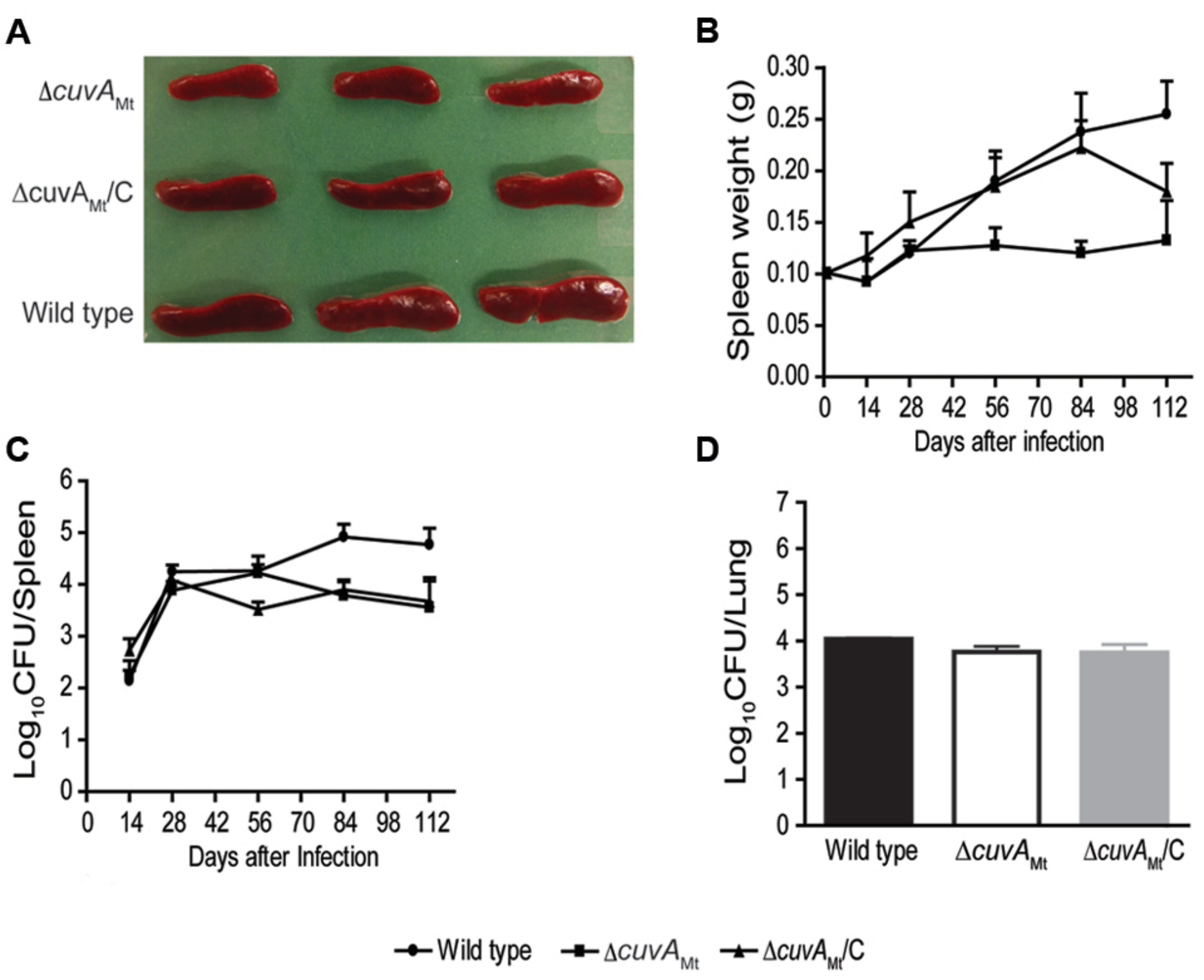

Fig. S7. Mouse infection with wild type H37Rv, Δ*cuvA*$_{Mt}$ or the Δ*cuvA*$_{Mt}$ complemented strain. For all graphs, error bars are +/- 1 S.D for the mean data from four mice at each time point. **A**. Gross pathology of spleens from day 84 following infection. **B**. Spleen weight of mice infected with each strain from 0 to 112 days following infection. **C**. CFU in spleen from day 14 to day 112 following infection. **D**. Lung inocula for the survival experiment (Fig. 7 in main text) for each strain on day 1 after infection. Data are the mean CFU from 3 mice. Error bars = 1 SD.

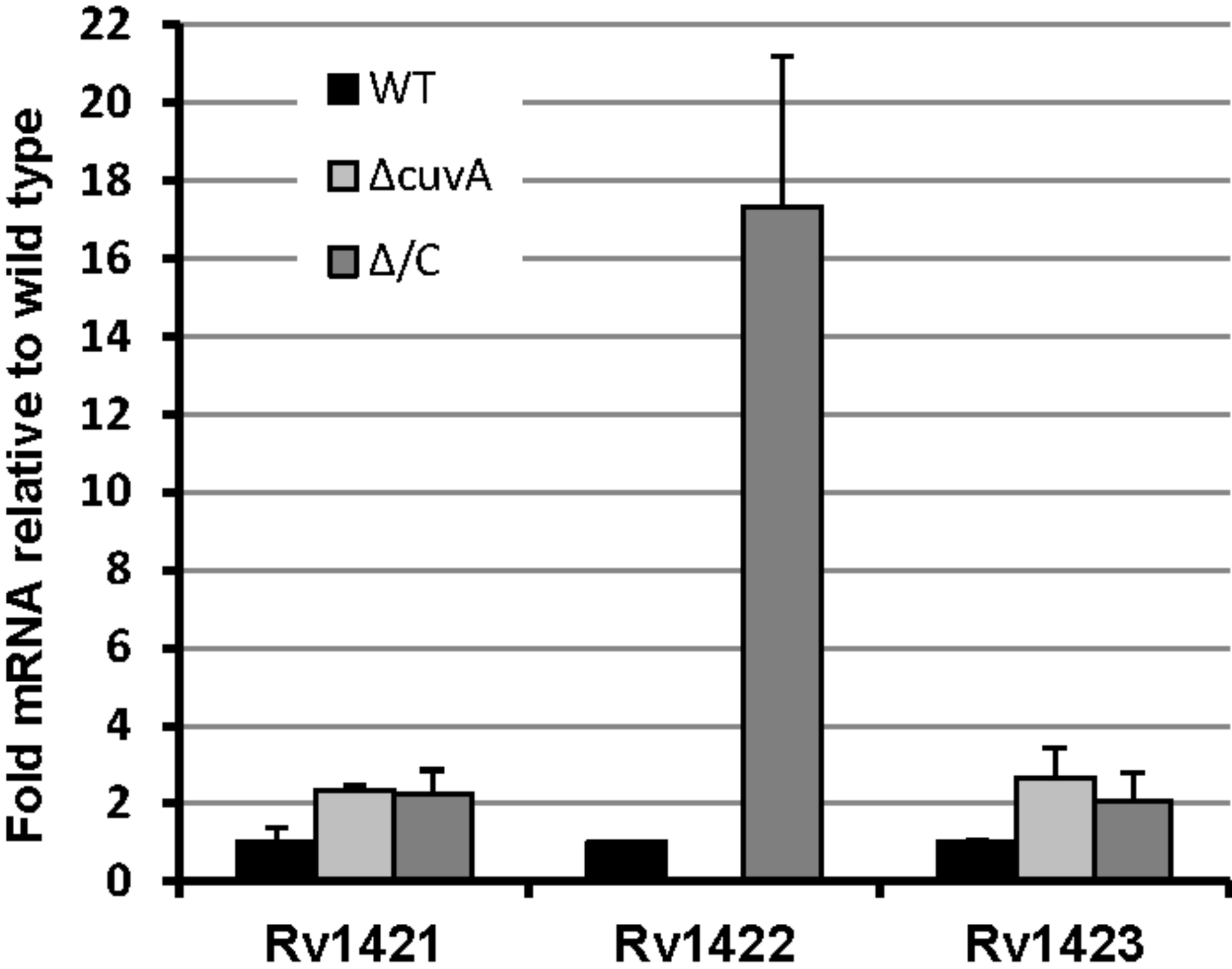

Fig. S8. Relative expression of genes in the *Rv1421-Rv1423* operon in wild type H37Rv, Δ*cuvA*$_{Mt}$ and Δ*cuvA*$_{Mt}$ complemented strains.