## Table S1. Bacterial strains, plasmids and primers used in this study

| Strains | Relevant features | Reference |
|---|---|---|
| ***Mycobacterium tuberculosis*** | | |
| H37Ra | Slow-growing virulent strain, $Amp^R$, $Chx^R$, $Cb^R$ | ATCC 25177 |
| H37Rv | Slow-growing attenuated strain, $Amp^R$, $Chx^R$, $Cb^R$ | ATCC 25618 |
| H37Rv:pJV53 | Recombineering strain (with pJV53), $Amp^R$, $Chx^R$, $Cb^R$, $Km^R$ | This study |
| H37RvΔ*ctpF* | Δ*ctpF*, gene replaced by a $Hyg^R$ cassette | This study |
| **Plasmids** | **Relevant features** | **Reference** |
| pJV53 | Derivative of pLAM12 with Che9c 60–61 genes under control of the acetamidase promoter | Gift from Unizar |
| pYUB854 | $Hyg^R$ cassette is flanked by the γδ-res sites and by two MCSs for directional cloning of the homologous recombination substrates | Gift from Unizar |
| pLNA22 | 607 bp upstream and 520 bp downstream of *Mtb Rv1997* (*ctpF*) in pYUB854 | This study |
| **Primer** | **Sequence (5'-3')** | |
| F-RT Dir | CAGTGATCTTCGGTGTGGTG | |
| F-RT Rev | TGACTCGTTCACGCTCAATC | |
| 16SrRNAdir | GAGATAGGCGTTCCCTTGTG | |
| 16SrRNArev | CTGGACATAAGGGGCATGAT | |
| RTctpEdir | ACAACGAGCGGGCCTATCCG | |
| RTctpErev | GCCTGTTCCTGCTCCTGCCA | |
| RTctpHdir | TTGCTGCCCGCAATCCTGGA | |
| RTctpHrev | GGCGAGGTCCCGGTGATAGC | |
| A-RT-Dir | GACCACCTCGACGTTGTACC | |
| A-RT-Rev | CAAGCTGTTTGAGACCACGA | |
| I-RT-Dir | CTGTCCTACGAACCGGTGAT | |
| I-RT-Rev | AGTAGCGCGTCGATATTGCT | |
| pJV53dir | GTCAGTCACCAACCCTCCAC | |
| pJV53rev | GAATCCTGCTTGGTGACAGC | |
| ctpF_interno_dir | CTATGCACCCGACGTCCT | |
| cpF_interno_rev | GAACCTGGTATCACGTTTTCG | |
| Comp_Up-ctpF | TCGTCGAACACTCGTACCTG | |

| | |
|---|---|
| Comp_Down_ctpF | CGTCCGCAACCTAGTTGAAT |
| primerpYUB854 | GTGGCTCCCTCACTTTCTGG |
| Hyg_dir_out | ACTTCGAGGTGTTCGAGGAG |
| Bdir2013 | TTTTTCTAGATATCGGGGTGTGGGTGC |
| Brev2013 | TTTTTCATGATACCACCAGCACGATCCAG |
| Adir2013 | TTTTCTCGAGCGGATGGCAAGACC |
| Arev2013 | TTTTGCTAGCGCGCGTTACCACC |
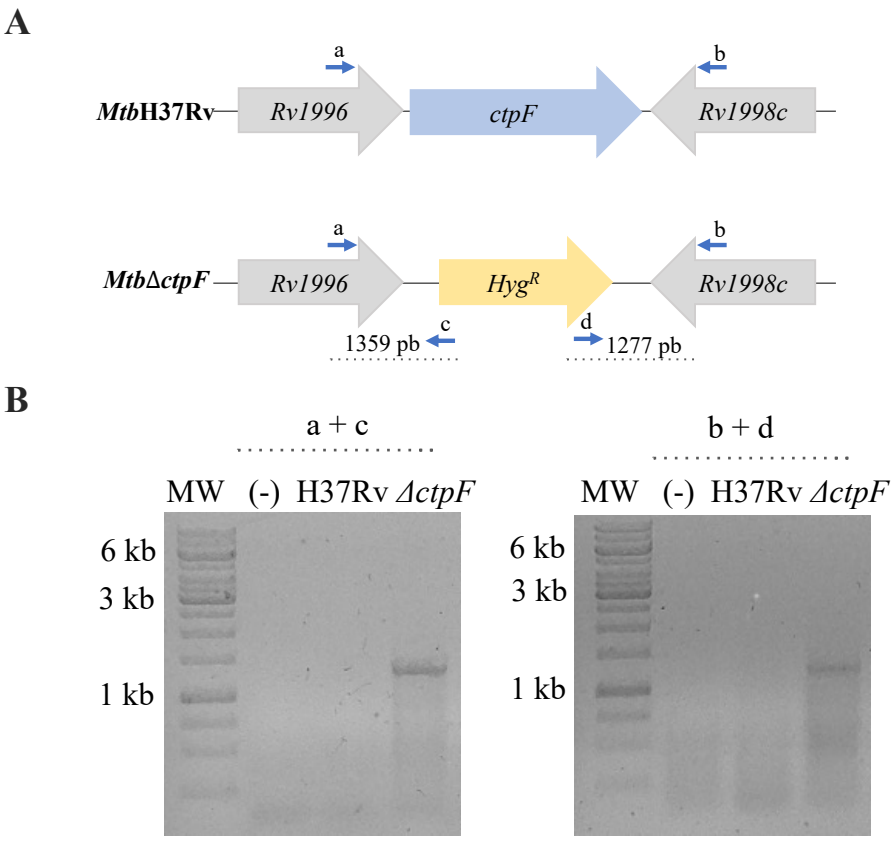
**Figure S1.** Construction of the *Mtb*H37Rv *ctpF* mutant.