



**Figure S1, related to Figure 1 and supplemental experimental procedures. Genetic manipulation of *Mtb* to generate *LprG-Rv1410c* double knockout.** The cosmid 2\_33 containing the *lprG-rv1410c* genes was used as a template to amplify a linear PCR product (ajm2F, ajm2R) with 50 basepair (bp) homology upstream and downstream of the operon flanking a chloramphenicol/hygromycin (Chlor/Hyg) resistance cassette. Step (1) Recombineering was performed in the *E. coli* pkD119 strain containing a lambda-red recombinase plasmid to exchange the Chlor/Hyg cassette with the *lprG-rv1410c* genetic locus on the 2\_33 cosmid. Step (2) the recombinant cosmid is used as a template to generate a linear PCR product (ajm3F, ajm3R) with 500 bp chromosomal homology upstream and downstream of the *lprG-rv1410c* operon (replaced by Chlor/Hyg). Step (3) the 3kb linear PCR product is transformed into confirmed PDIM positive *Mtb* (*H37Rv*) previously transformed with pRecET, expressing the mycobacterial che9 phage recombinase under control of an isovaleronitrile inducible promoter (pNIT). Step (4), successful recombination results in a chromosomal deletion of *lprG-rv1410c* with replacement by the chloramphenicol/hygromycin cassette. The recombinase plasmid is cured by counter-selection on 10% sucrose.