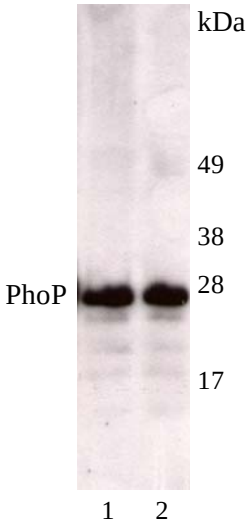
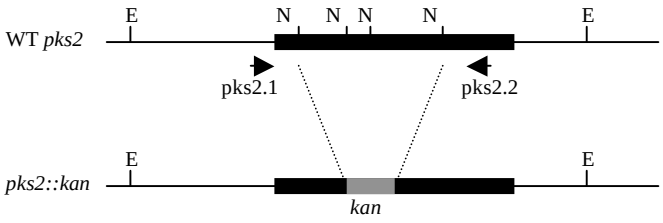
<u>Supplemental Figure 1:</u> Production of recombinant forms of PhoP-Rv and PhoP-Ra in 1237Δ*phoPR::hyg*.

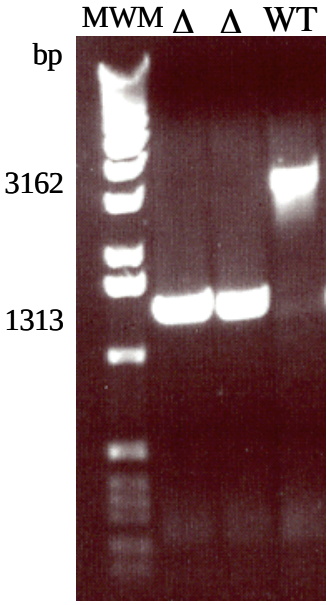
The production of PhoP-Rv and PhoP-Ra from pVVphoP-Rv and pVVphoP-Ra in 1237Δ*phoPR::hyg* was analyzed by immunoblotting with the monoclonal Penta-His antibody from Qiagen. 30 μg of protein were loaded per lane. 1, 1237Δ*phoPR::hyg*/pVVphoP-Rv; 2, 1237Δ*phoPR::hyg*/pVVphoP-Ra.



<u>Supplemental Figure 2:</u> Generation of a *pks2-pk3/4* mutant of *M. tb* H37Rv.

The *ts-sacB* method (30) was used to achieve allelic replacement at the *pks2* locus of the *pks3/4 M. tb* mutant, msl3 (12). The *M. tb* H37Rv *pks2* gene and flanking regions was extracted from cosmid MTCY409 on a 6576-bp EcoRI restriction fragment and a disrupted allele, *pks2::kan*, was obtained by replacing 3049-bp of the coding sequence of this gene bracketed between two NruI sites with the kanamycin resistance cassette from pUC4K (Amersham Pharmacia Biotech). Allelic replacement at the *pks2* locus of the msl3 strain was confirmed by PCR using primers pks2.1 and pks2.2 (see Materials and Methods). In the mutants (Δ), the wild-type 3162-bp pks2.1/pks2.2 PCR fragment (WT) is replaced by a 1313-bp PCR fragment due to the 3049-bp NruI deletion in their *pks2* gene and insertion of a 1.2 kb-kanamycin resistance cassette. MWM, molecular weight marker (Invitrogen). N, NruI restriction site.





<u>Supplemental Figure 3:</u> Acyltrehalose composition of the msl3Δ*pks2* mutant.

Autoradiograms of thin-layer chromatograms of lipids derived from [1-^{14}C]propionate (30,000 cpm per lane) are shown. The TLC was developed in chloroform: methanol: water (90:10:1, vol.:vol.:vol.). msl3 was reported to be deficient in DAT and PAT synthesis (12,33). The disruption of *pks2* in this mutant further leads to the abolition of SL synthesis in this strain. Lane 1, *M. tb* H37Rv; lane 2, msl3Δ*pks2*.