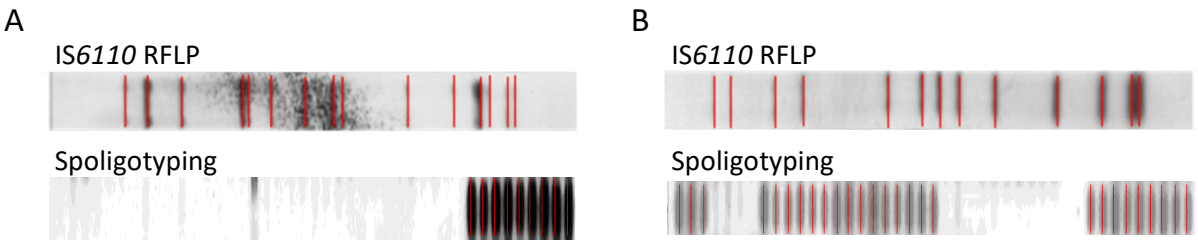

Figure S1. Genotyping of Lineage 2 and lineage 3 clinical isolates used as parental strains for the genetic construction in the present study. IS*6110*-RFLP and spoligotyping of (A) GC1237 (lineage 2 Beijing strain) and (B) HMS13037 (lineage 3 strain).

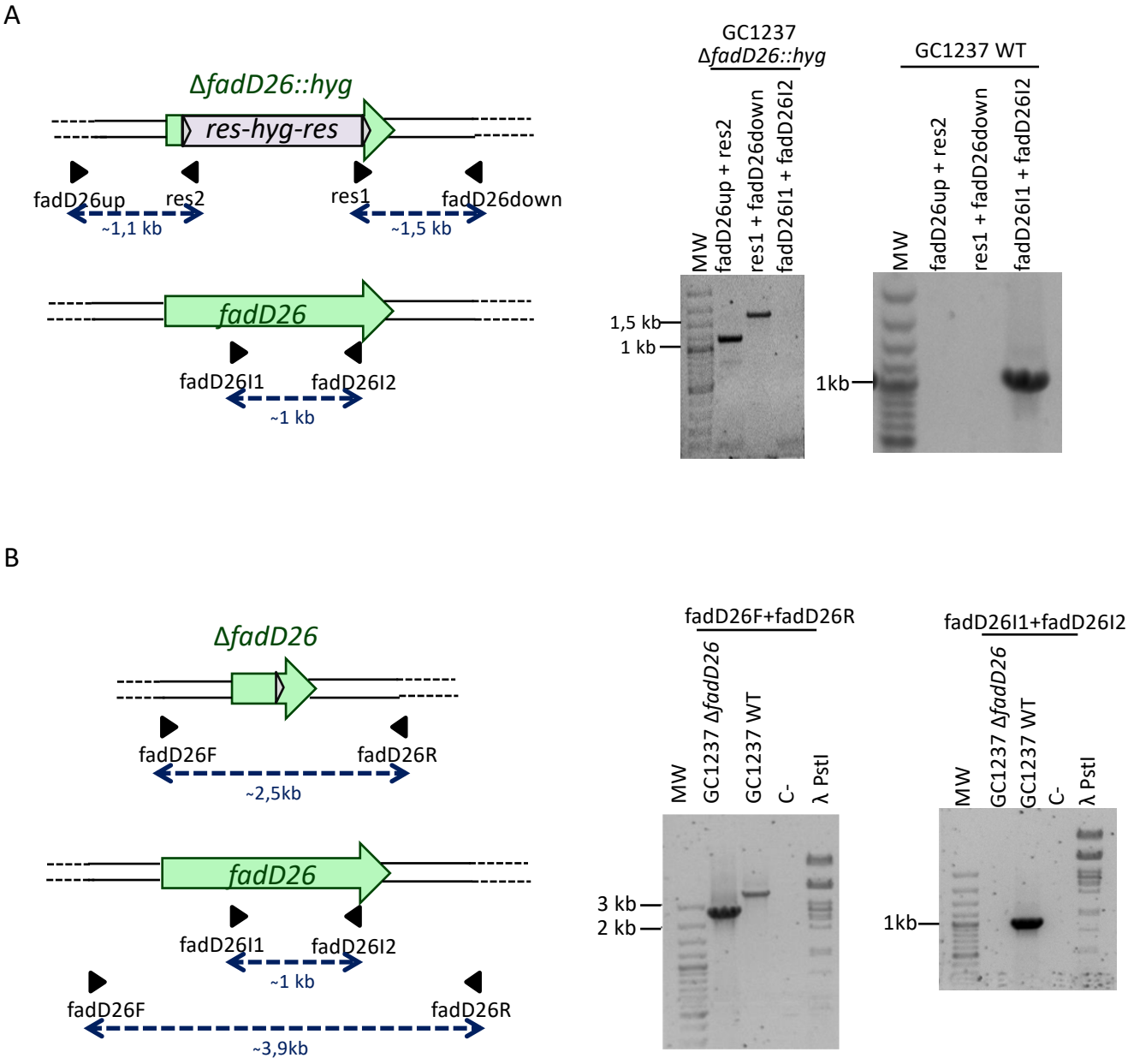

Figure S2. PCR analysis of *fadD26* deletion in GC1237 using the pAZ5 suicide plasmid. Representation of genetic arrangements and PCR amplification products obtained in the mutant and the wild type using the primers (depicted by filled triangles) indicated in the diagrams. (A) PCR analysis of GC1237 disrupted with the *res-hyg-res* cassette. (B) PCR analysis of the unmarked GC1237 Δ*fadD26* deletion.

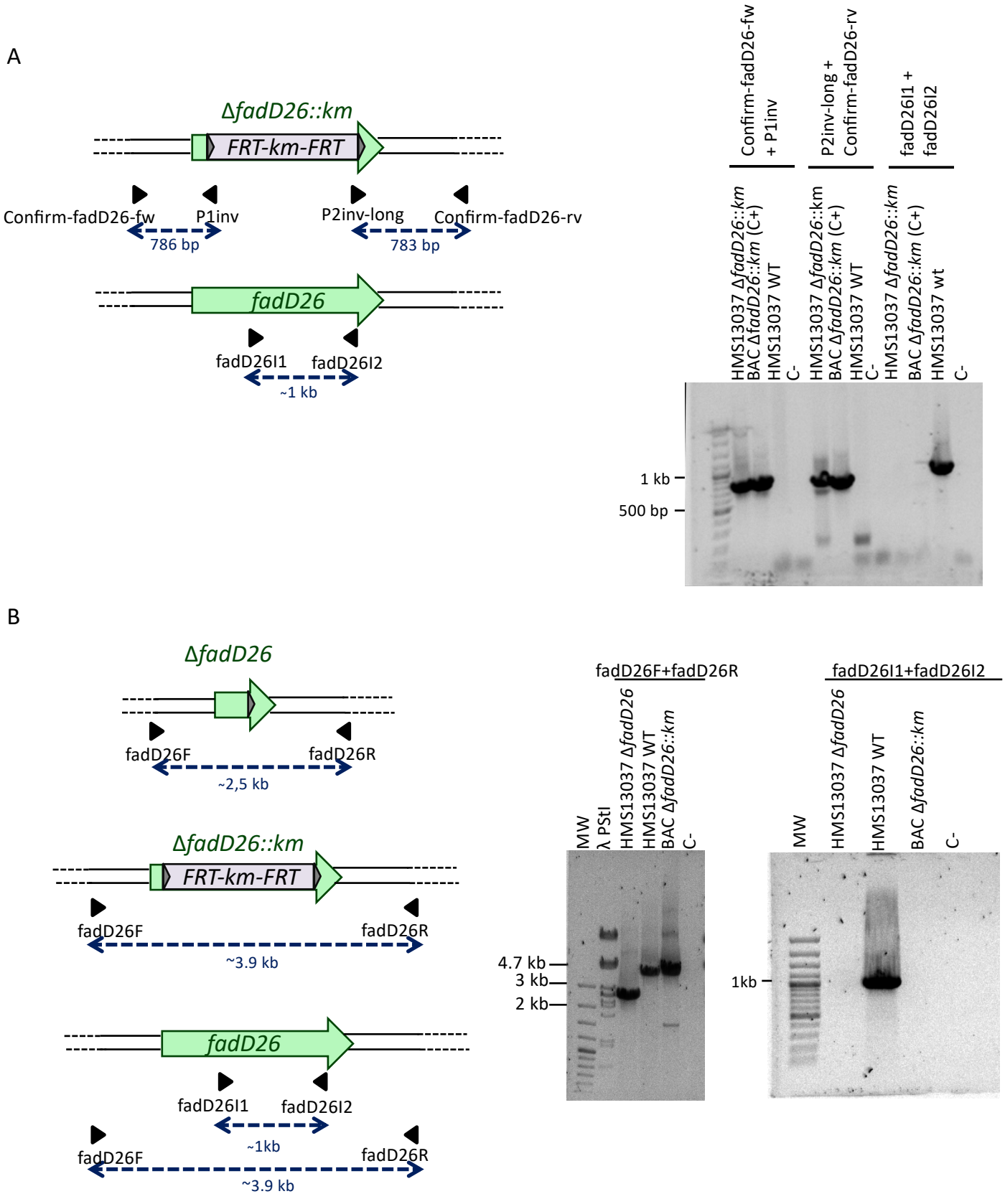

Figure S3. Analysis of the *fadD26* deletion in HMS13037 using the the BAC-rec strategy. Representation of genetic organization and PCR amplification products obtained in the mutant and the parental strain using the primers (depicted by filled triangles) indicated in the diagrams. (A) PCR results of the *fadD26* gene deletion disrupted by *FRT-Km-FRT* cassette. (B) PCR results of the unmarked HMS13037 Δ*fadD6* deletion.

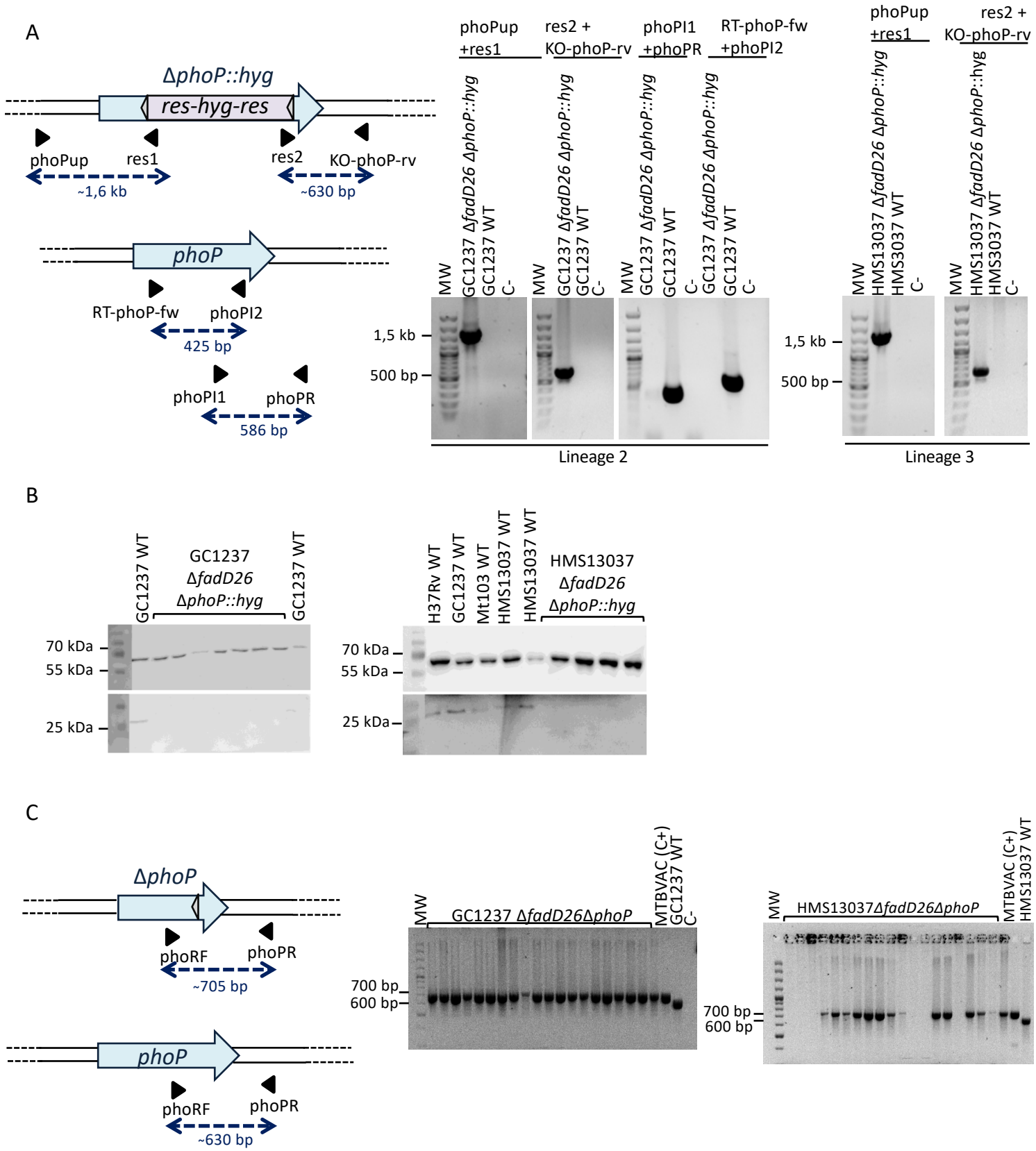

Figure S4. Analysis of *phoP* deletion in GC1237 and HMS13037. (A) Representation of genetic organization and PCR analyses using the primers (depicted by filled triangles) indicated in the diagrams of *phoP* disrupted with *res-hyg-res* cassette. (B) Western-blot analysis of PhoP. The upper panels show GroEL2 used as loading control and the lower panels show hybridization with anti-PhoP antiserum. (C) Genetic organization and PCR verification of antibiotic removal in the *phoP* gene. MTBVAC was used as control of unmarked *phoP* deletion.

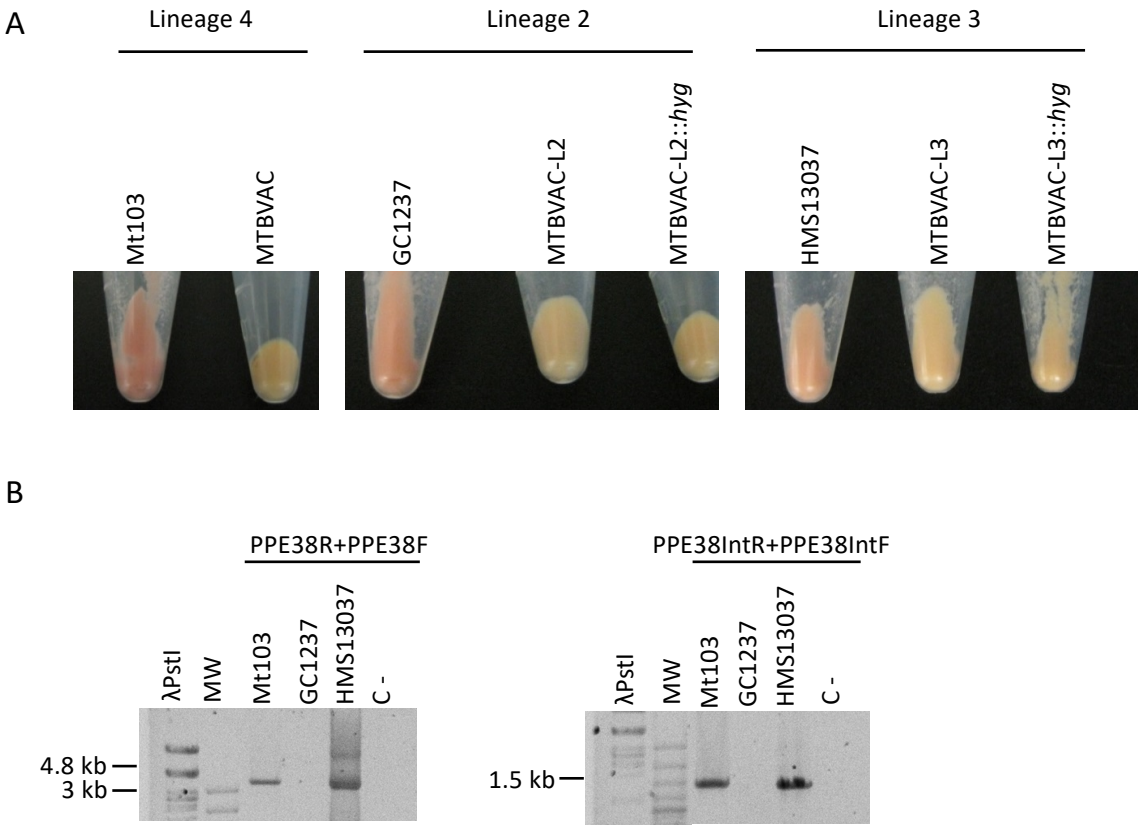

Figure S5. Phenotypic and genetic characterization of the new MTBVAC-L2 and MTBVAC-L3 vaccine candidates. (A) Neutral red staining of parental and double marked /unmarked *phoP-fadD26* mutants. (B) Study of *ppe38* locus organization using primers and conditions described in McEvoy and colleagues.

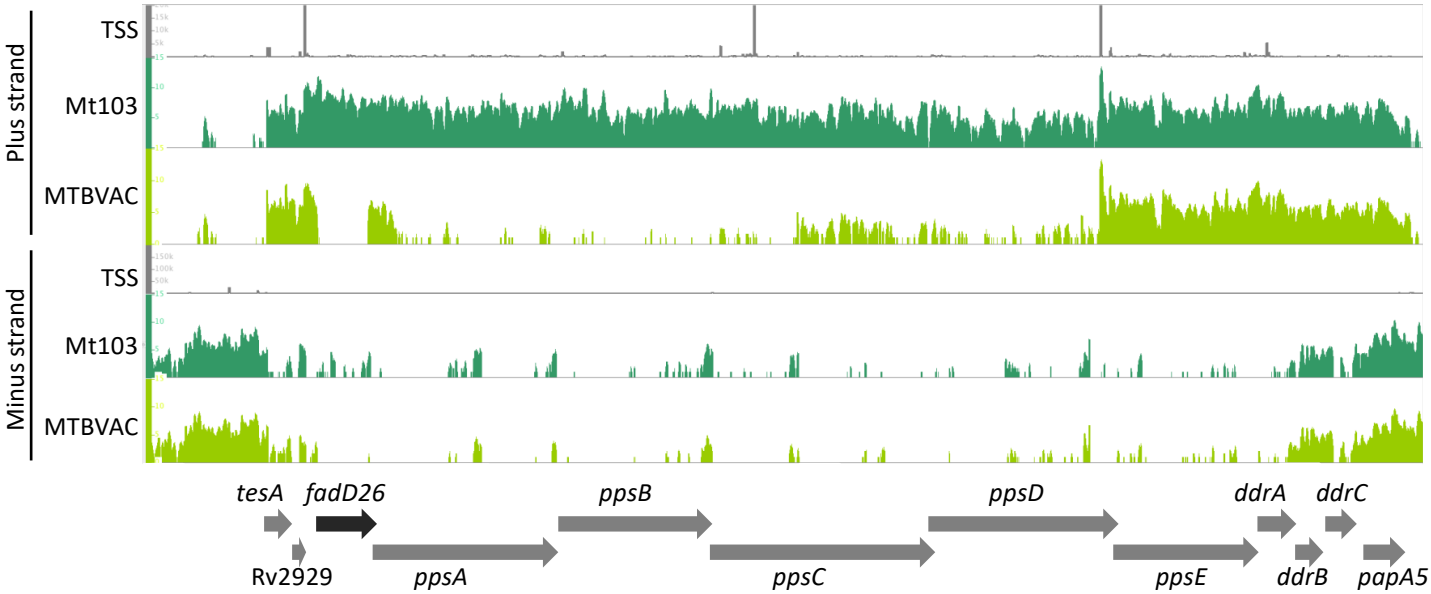

Figure S6. RNA-seq of the PDIM operon region. The figure shows the transcription start sites (TSS) and transcript counts in plus and minus strands of Mt103 (dark green) and MTBVAC (light green). Note the decrease in gene expression of *ppsA-D* (3´*fadD26*-downstream genes) in MTBVAC compared to Mt103.

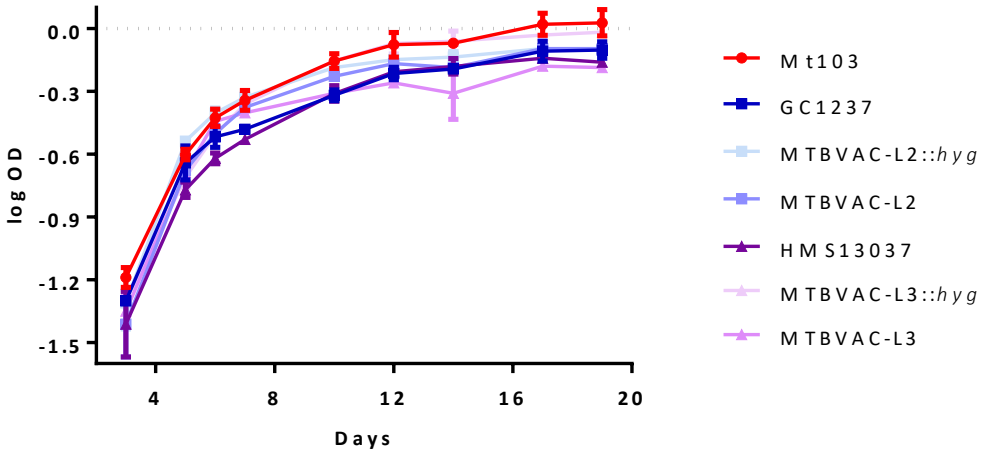

Figure S7. Growth of the new MTBVAC-L2 and MTBVAC-L3 strains constructed in this study. OD_{600} measurements from liquid cultures of parental strains (lineages 2, 3 and 4) and double *phoP*, *fadD26* marked and unmarked mutants in lineages 2 and 3 of *M. tuberculosis* (MTBVAC-L2::*hyg,* MTBVAC-L2, MTBVAC-L3::*hyg* and MTBVAC-L3).