

Supplementary Information

The *PPE25 (Rv1787)* - *PE18 (Rv1788)* - *PPE26 (Rv1789)* gene cluster encodes an interacting protein pair and is involved in immune evasion by *Mycobacterium tuberculosis*

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Running title: Immune evasion by the *M.tb PPE25-PE18-PPE26* operon

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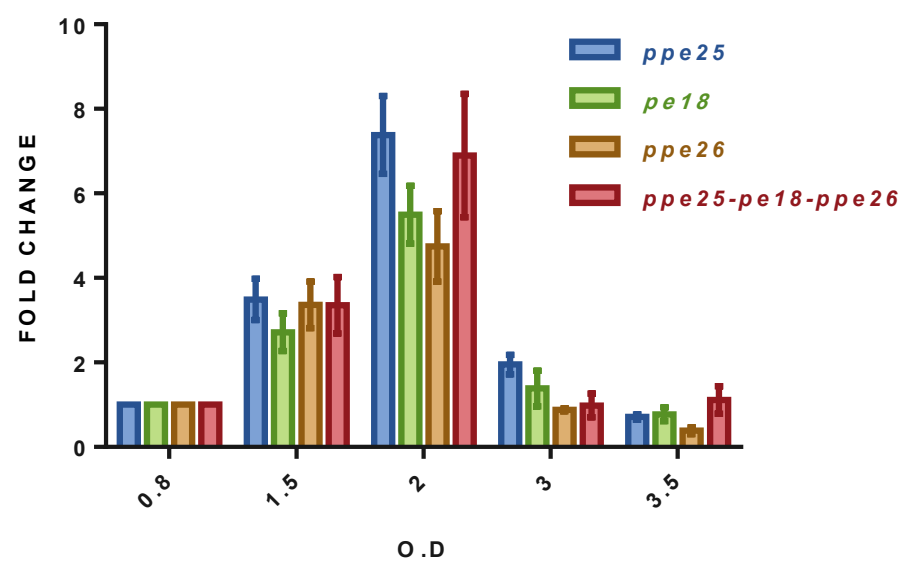


Fig S1. Real-time RT-PCR quantification of *PPE25*, *PE18*, *PPE26* and *PPE25-PE18-PPE26* transcripts as a function of *M. tb* H37Rv growth. Transcript levels are shown relative to the cognate gene's mRNA levels at 0.8 OD, which is given a value of 1. The data is representative of two biological replicates, with three technical replicates in each data point.

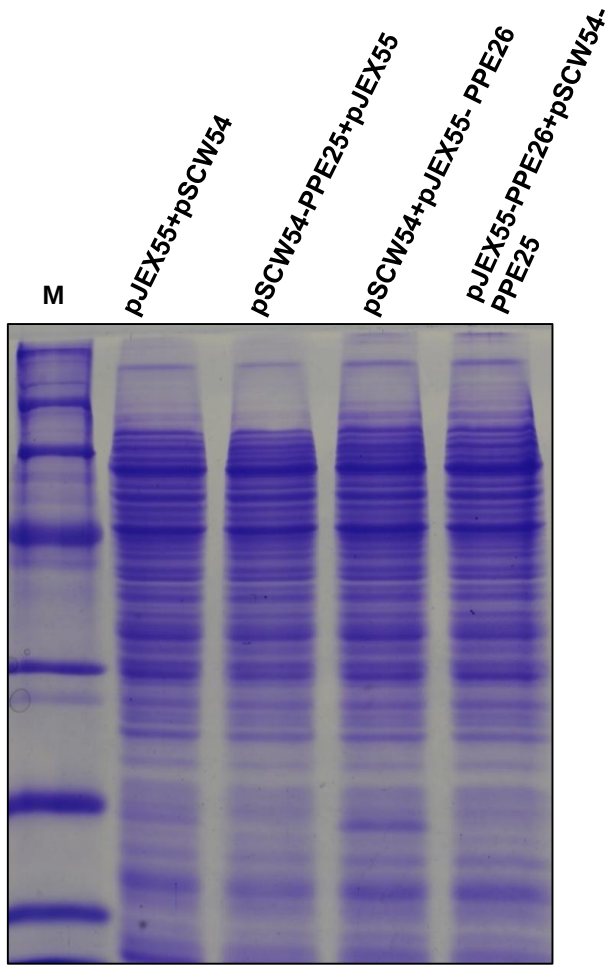


Fig. S2: *In vivo* co-IP of PPE25-PPE26. SDS-PAGE profiles showing equal loading for *in vivo* co-IP reactions of *M. smegmatis* expressing PPE25-6xHis and PPE26-c-myc, precipitated with an anti-c-myc antibody and probed with an anti-His antibody.

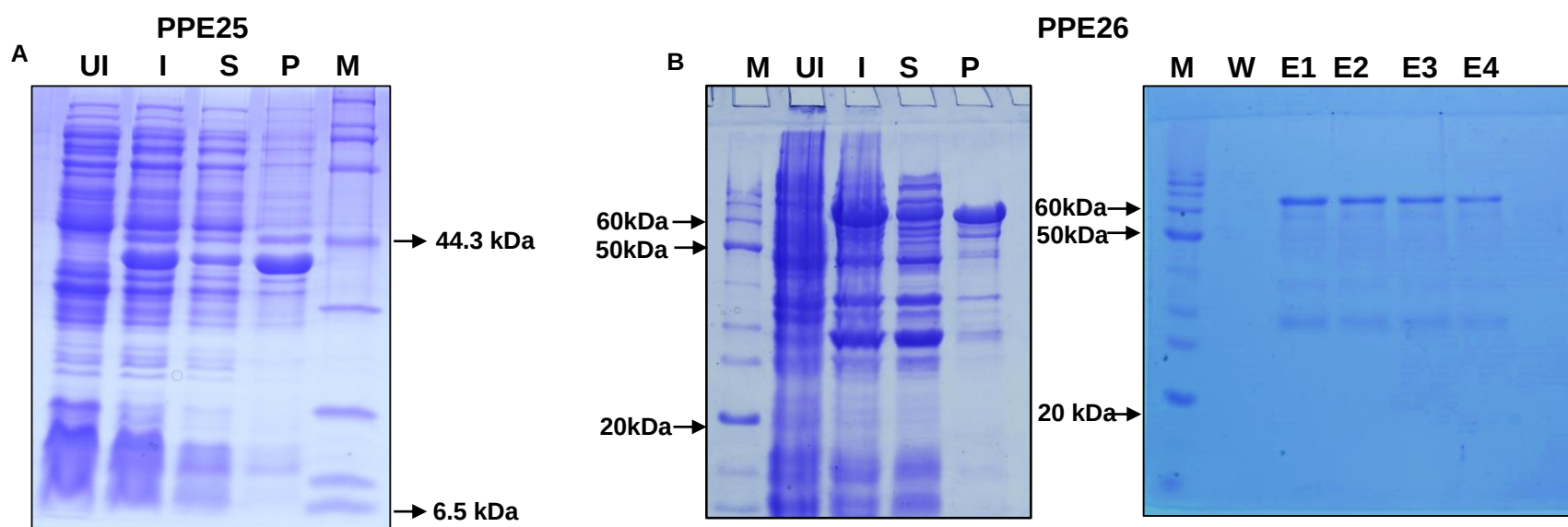


Fig. S3: Expression profile of PPE25 and PPE26. (A) Expression profile of PPE25 in *E. coli* BL21DE3. (B) Expression (left panel), and GSH based affinity chromatography purification profile (right panel) of PPE26 expressed in *E. coli* BL21DE3. UI - Uninduced sample, I - Induced sample, S - Supernatant, P - Pellet Fraction, M - Marker, W - Wash, E - Eluate.

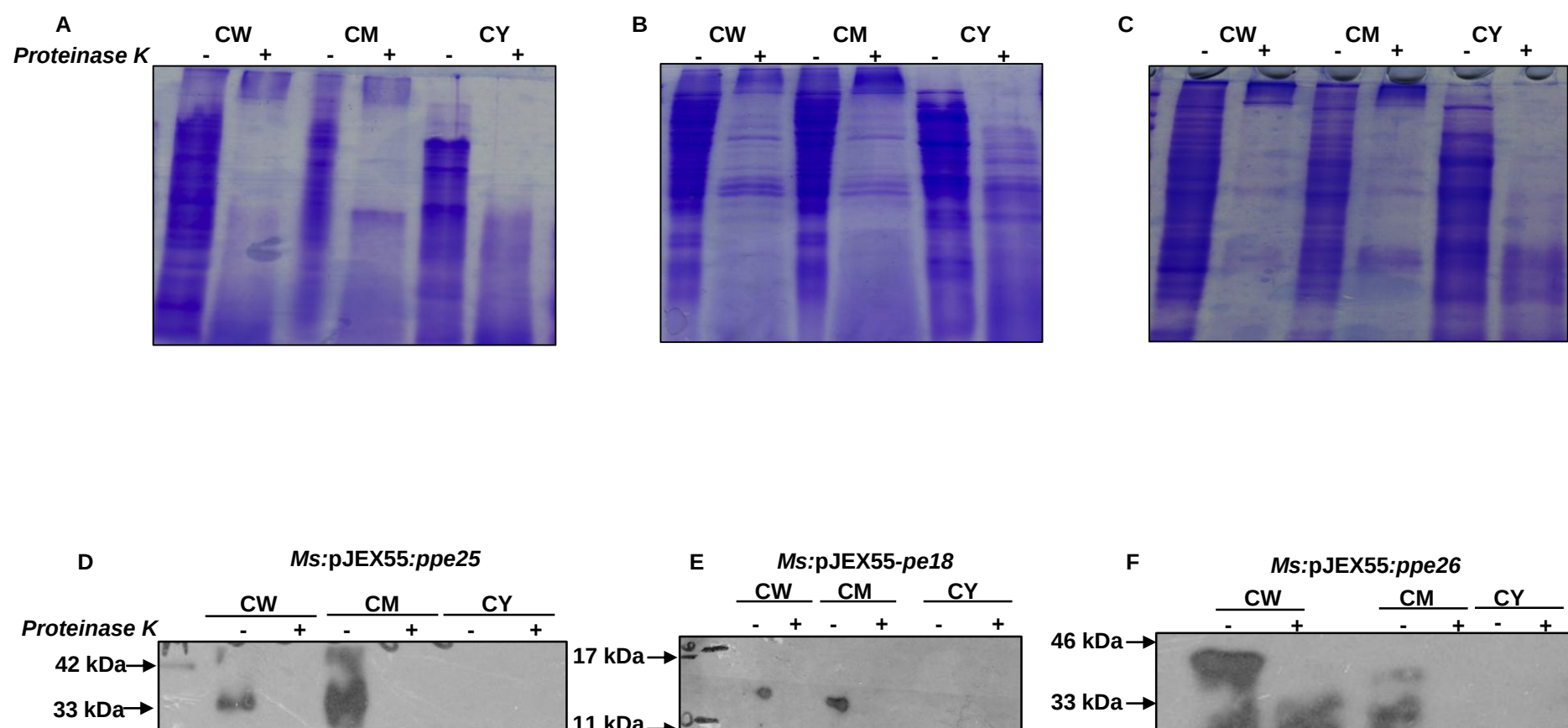


Fig. S4 Subcellular localisation and surface accessibility. Coomassie blue stained SDS-PAGE profiles of Proteinase K treated *M. smegmatis* expressing PPE25-c-myc (A), PE18-c-myc (B), and PPE26-c-myc (C), demonstrating equivalent loading of the sub-cellular fractions. Immunodetection of PPE25 (D), PE18 (E), and PPE26 (F), in subcellular fractions (CM - Cell Membrane, CW - Cell Wall, CY - Cytoplasm). An anti-c-myc monoclonal antibody was used to identify all proteins.

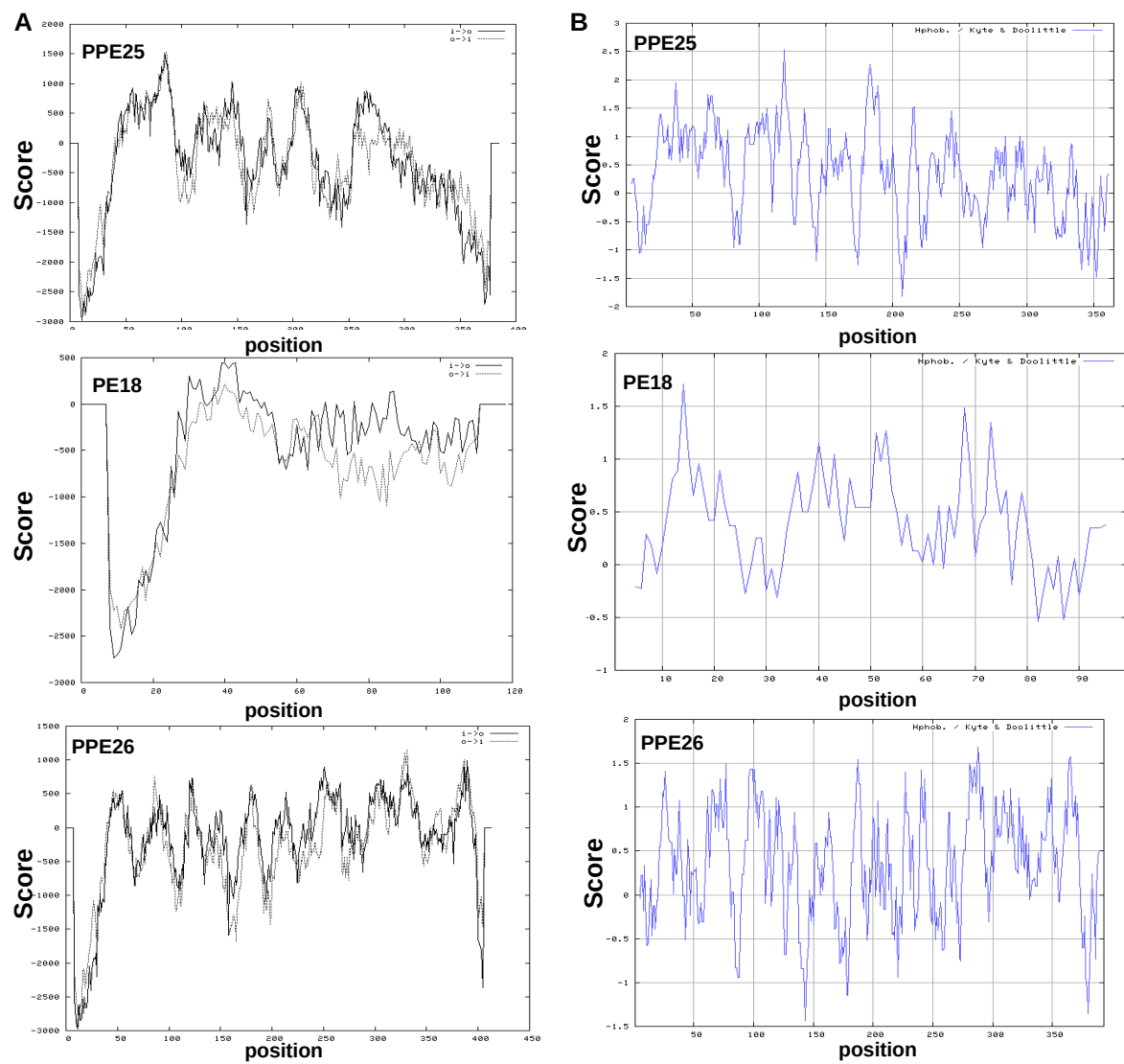


Fig. S5: *in silico* protein sequence analysis of *M. tb* PPE25, PPE18, and PPE26. Analyses of PPE25, PPE18, and PPE26 protein sequences for transmembrane prediction (A) and hydrophobicity (B).

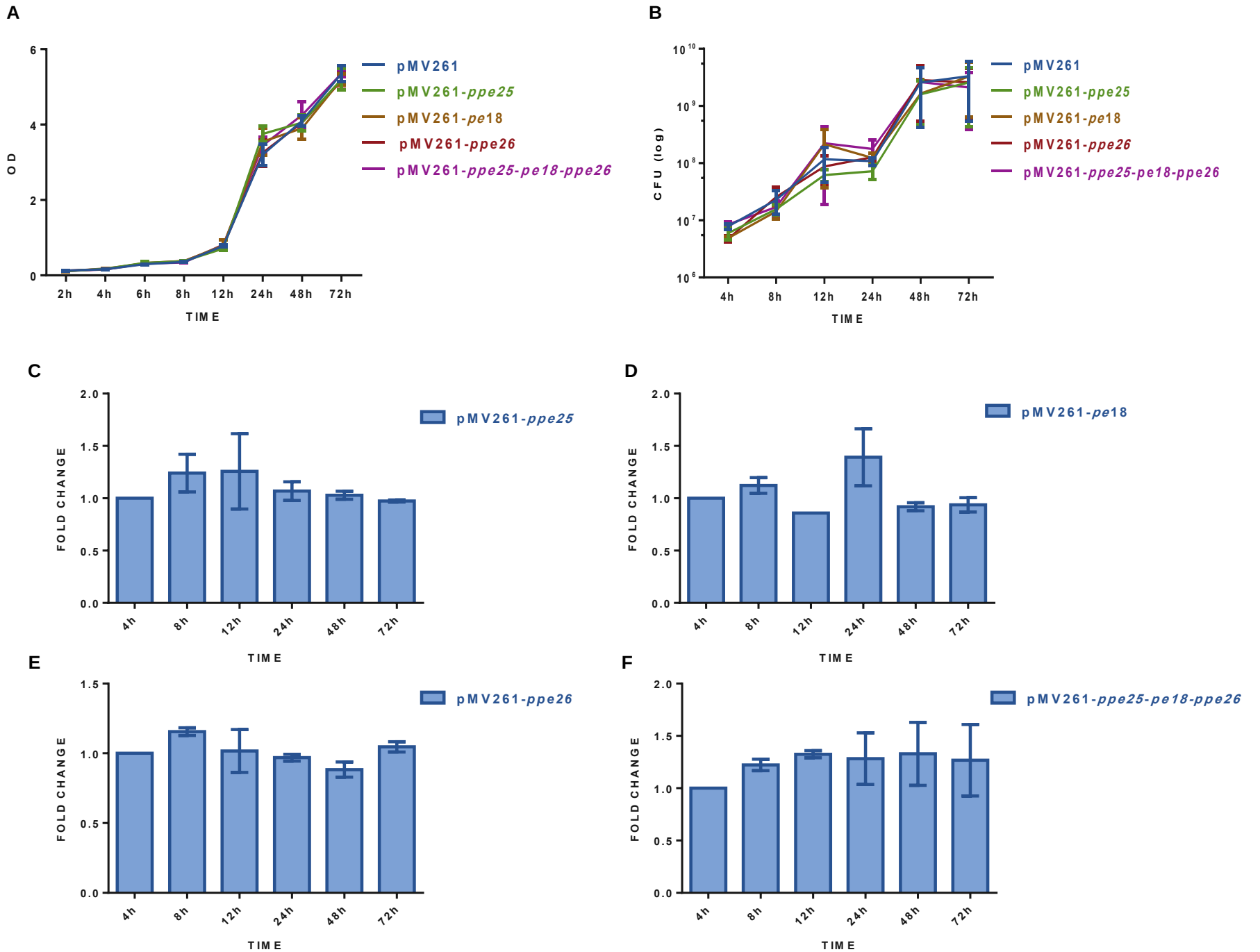


Fig. S6: Gene expression and growth analysis of recombinant *Mycobacterium smegmatis* strains expressing *PPE25*, *PPE18*, and *PPE26*. *In vitro* growth profiles of *M. smegmatis* expressing pMV261, *PPE25*, *PE18*, *PPE26*, and *PPE25-PE18-PPE26* (A) OD, (B) CFU counts. Transcript levels of *PPE25* (C), *PPE18* (D), *PPE26* (E), and *PPE25-PE18-PPE26* (F), were measured by real-time RT-PCR as a function of growth. Data are shown as the mean $\pm$ SEM of transcript levels normalised to the mRNA level of the relevant gene at time 4h (value = 1).