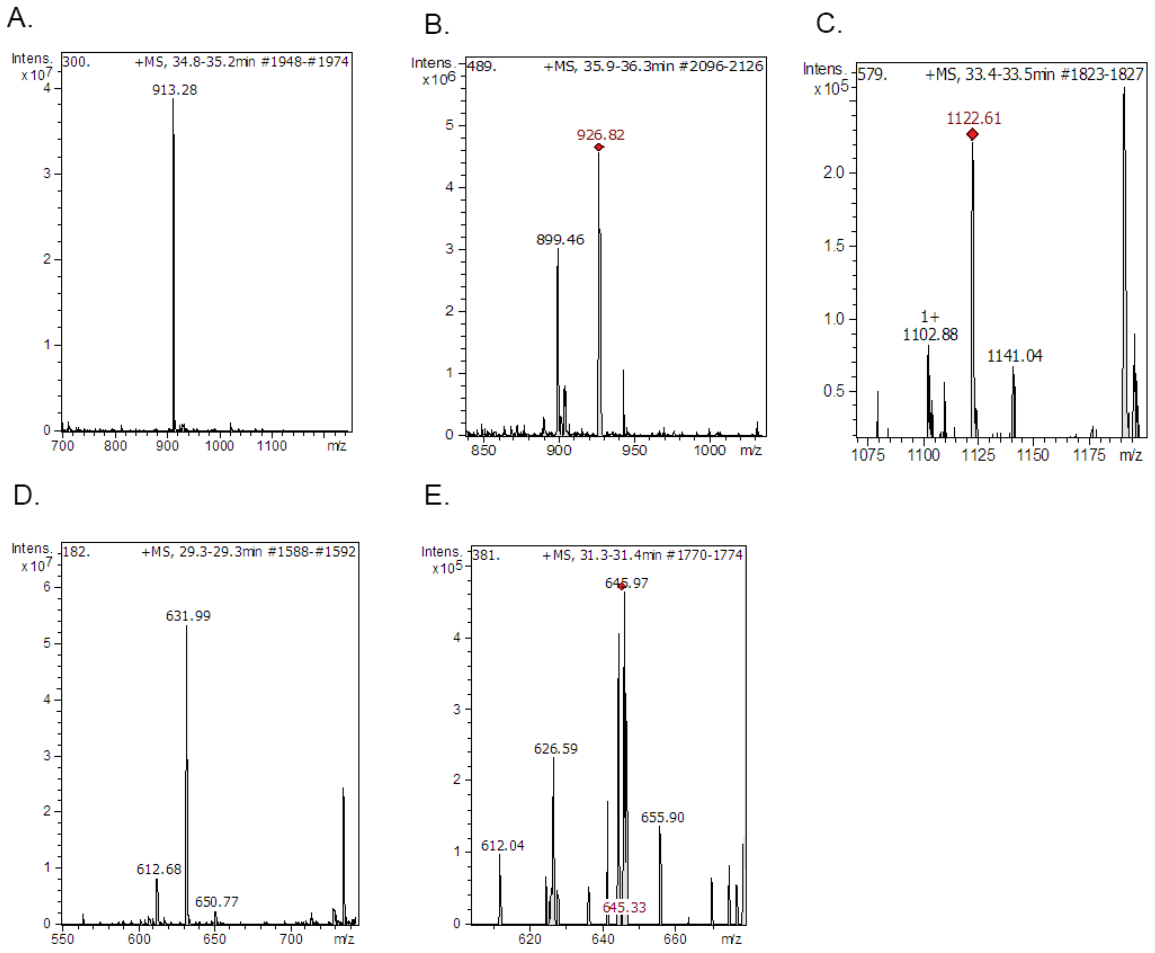
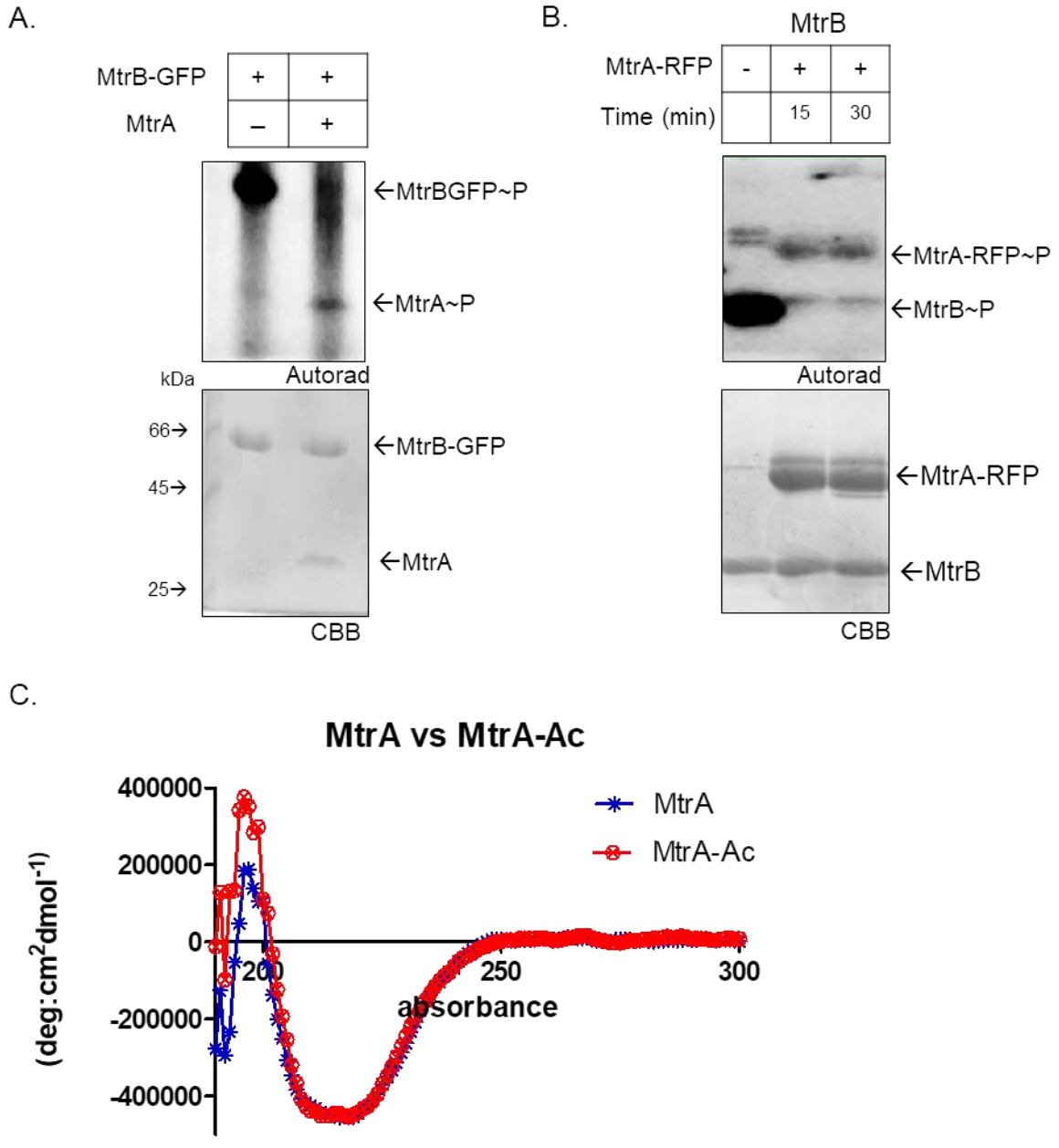
Supplementary Figure 1.



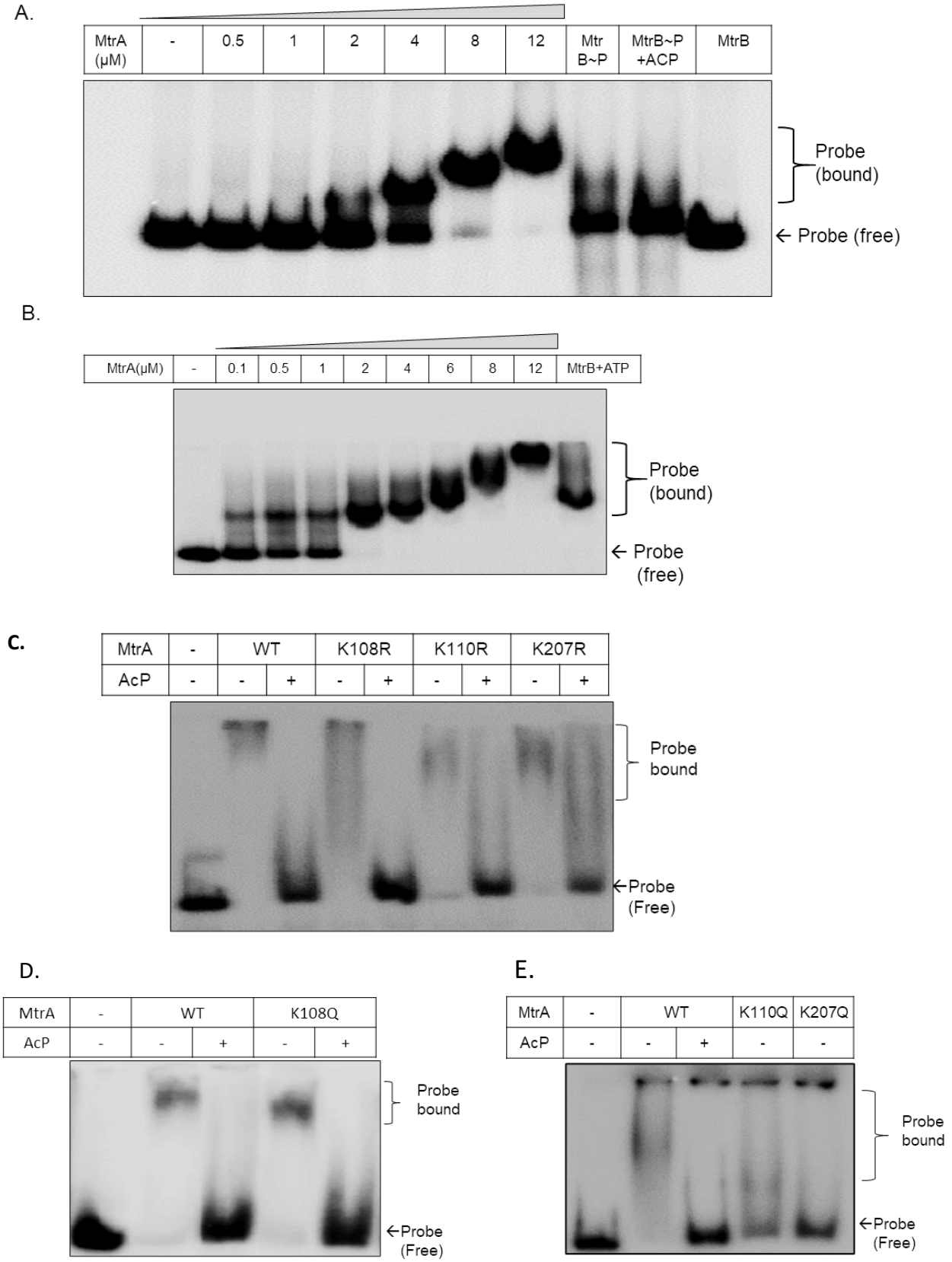
Supplementary Figure 1. A-E. **Identification of lysines acetylated in MtrA protein through AcP by MS/MS analysis**. Precursor ion spectra for **A.** MtrA-K108; **B.** MtrA-K110; **C.** MtrA-K108 acetylated; **D.** MtrA-K110 acetylated and **E.** MtrA-K207 acetylated. For all MS analysis, n=3 from independent samples.

Supplementary Figure 2.

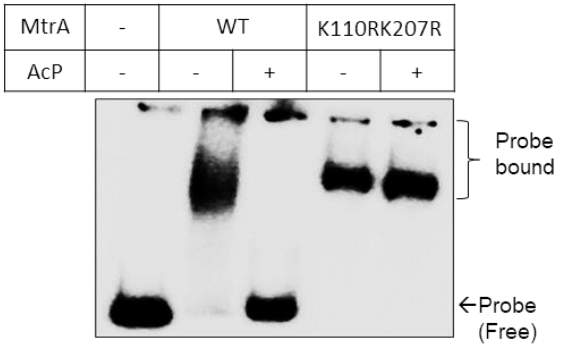


Supplementary Figure 2. A. Autophosphorylation and phosphotransfer analysis of GFP-tagged MtrB protein to MtrA. Top, autoradiogram; bottom, CBB stained gel. **B.** Autophosphorylation and phosphotransfer analysis of MtrB protein to RFP tagged MtrA protein. Top, autoradiogram; bottom, CBB stained gel. All the experiments were performed as described in the materials and methods section**. C.** Circular dichroism analysis of MtrA and MtrA with AcP proteins. MtrA proteins were evaluated using a Jasco Spectropolarimeter. Ellipticity was measured from 200 nm to 300 nm in 1 mm pathlength and 50nm/ sec scanning speed. The data were analyzed through K2D2 Dichroweb software.

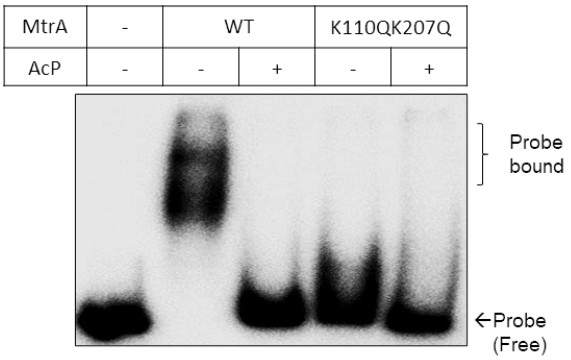
Supplementary Figure 3.



F.



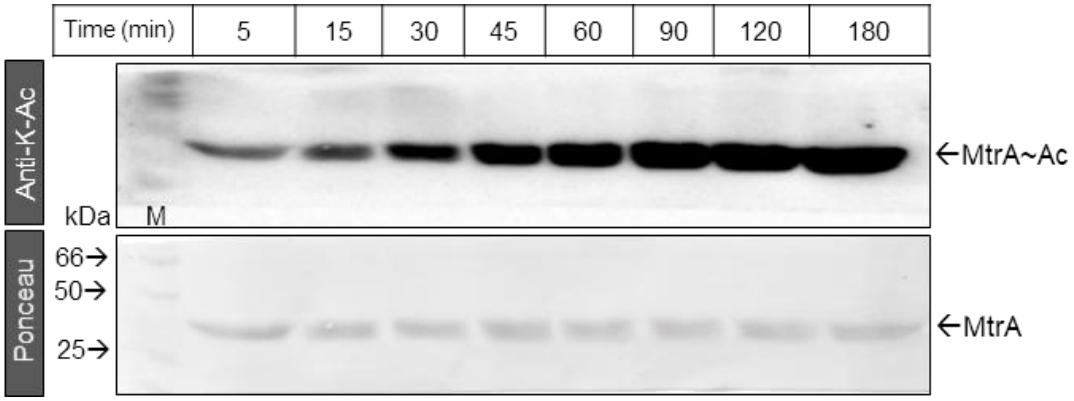
G.



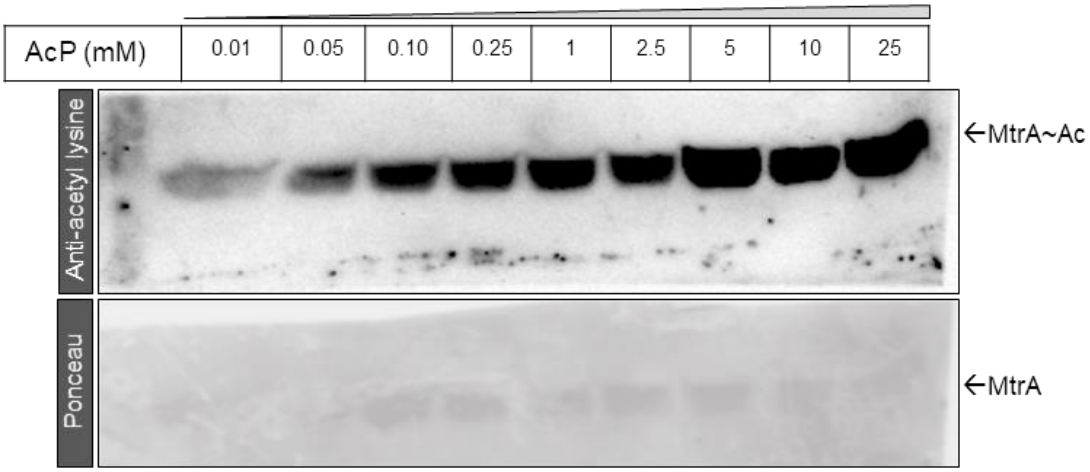
Supplementary Figure 3. Electrophoretic mobility shift assay (EMSA) with *oriC* promoter and *rpfB* promoter for dose titration on DNA binding activity of MtrA. **A.** A 526 bp DNA fragment corresponding to the *oriC* was PCR amplified from *M. tuberculosis* H37Rv genomic DNA template using specific primers. EMSA was performed using MtrA treated with various agents, as indicated. The concentration gradient indicates the amount of MtrA used in the assay. **B.** EMSA with *rpfB* gene promoter region using MtrA. The concentration gradient indicates the amount of MtrA used in the assay. **C-G. Effect of acetylation DNA binding ability of MtrA on *oriC* promoter**. Acetylation was performed using acetyl phosphate (AcP) as the donor. Acetylated and non-acetylated wild type MtrA proteins are used as controls. **C** Analysis of DNA binding activities of all three lysine defective mutants (K108R, K110R, and K207R) in the presence and absence of AcP; **D.** Analysis of K108Q lysine mimic mutant; **E.** K110Q or K207Q lysine mimic mutants in the absence of AcP. Analysis of **F.** MtrA K110RK207R (defective) and **G.** K110QK207Q (mimic) double mutants in the presence or absence of AcP. All the experiments were performed as described in the materials and methods section**.** N = 3.

Supplementary Figure 4.

A.

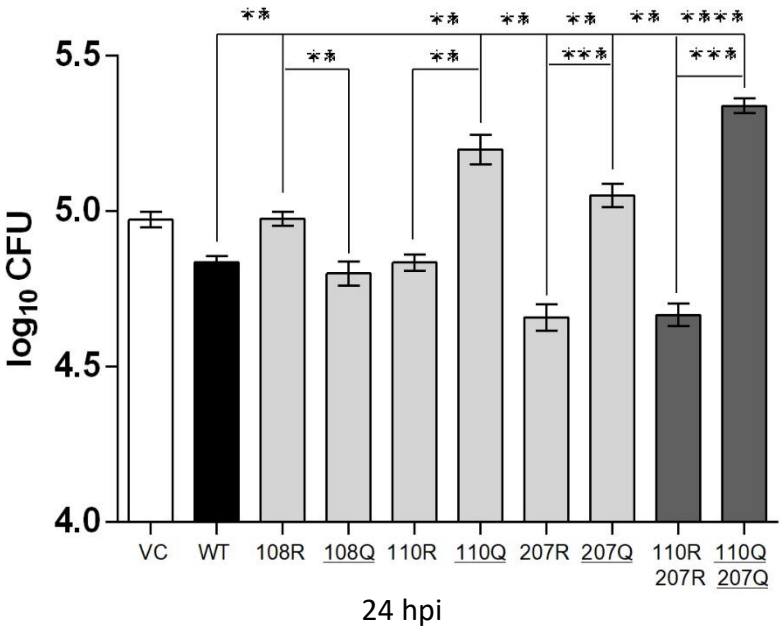


B.



Supplementary Figure 4. A. MtrA acetylation analysis using 5 mM AcP over a time course. This was performed in parallel with EMSA described in Figure 3A by anti-acetyl western blot analysis. B**.** MtrA acetylation analysis with various concentrations of AcP. The analysis was done in parallel with the EMSA experiment depicted in Figure 3E. All the experiments were performed as described in the materials and methods section**.** N = 3.

Supplementary Figure 5.



Supplementary Figure 5. Infection was performed in A549 alveolar epithelial cells with various H37Ra strains containing pMV261 plasmid with various variants of MtrA (as indicated) (VC; vector control), following which CFU was determined at 24 hours post-infection. Log_{10} CFU was further calculated for each strain and compared with each other. *P*- values were <0.05 for R and Q mutants by Student's *t*-test.