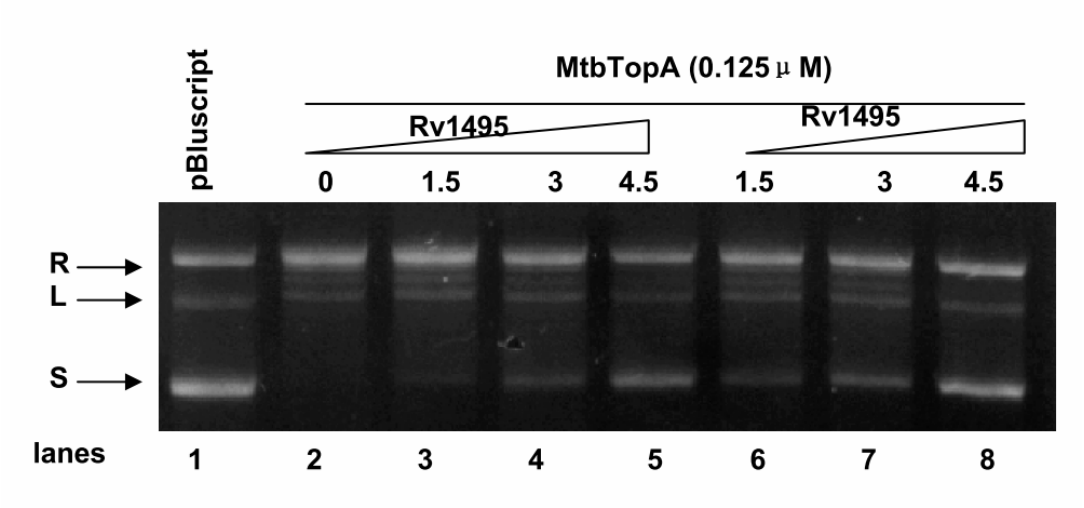
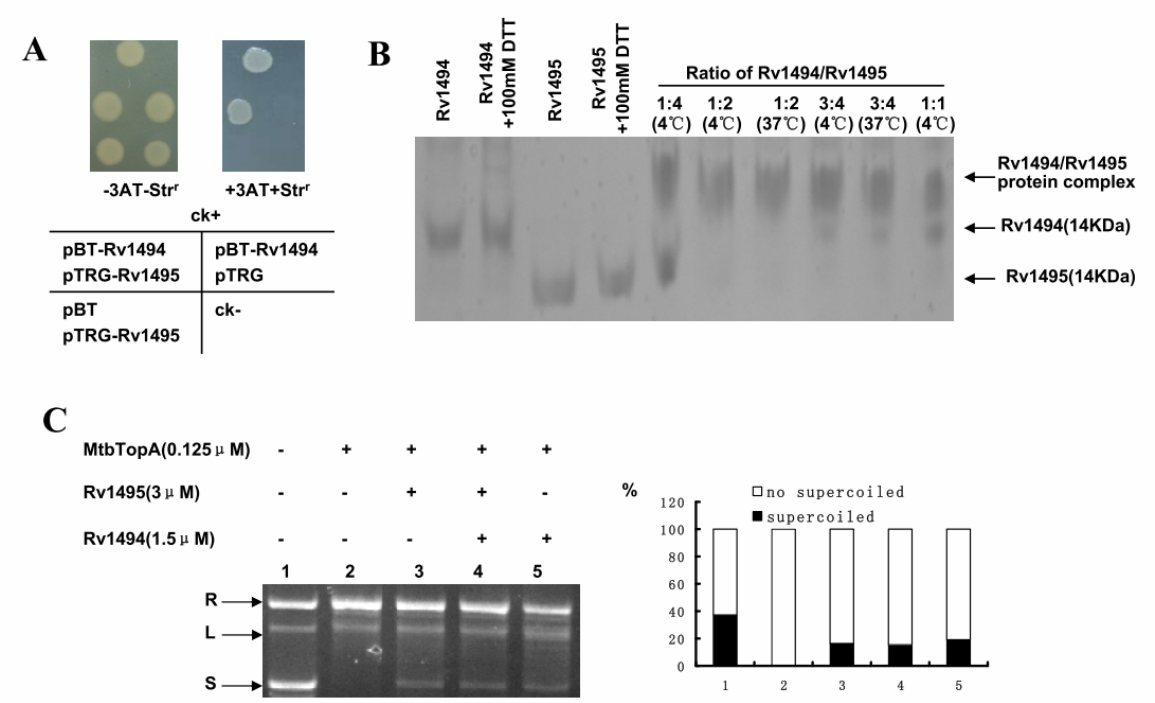
Supplemental figures

Figure S1



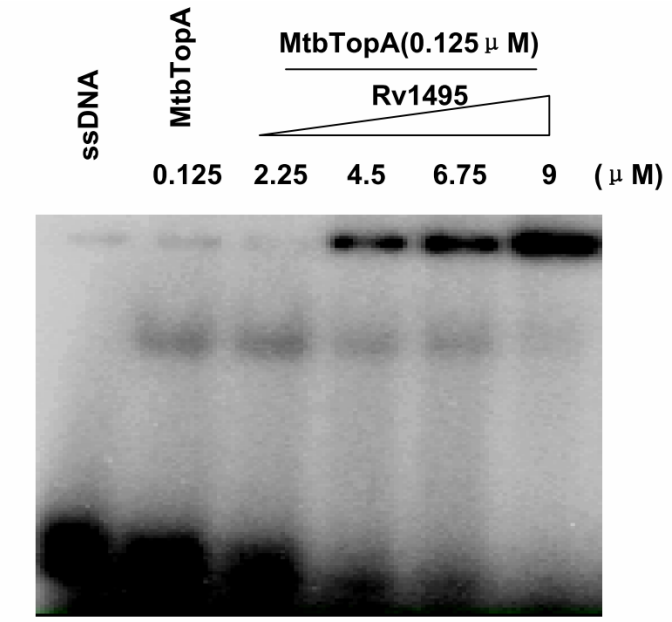
Effect of preincubation of MtbTopA with Rv1495 on the inhibition of MtbTopA activity. Topoisomerase activity assays were performed as described under "Materials and Methods". The plasmid pBluescript was used as DNA substrate in all reaction mixtures. Lanes 6-8 represent the activity assays of TopA under 10-min preincubation with Rv1495 on ice (Lanes 6-8) before the pBluescript DNA was added into the reactions. Lanes 2-5 represent no preincubation.

Figure S2



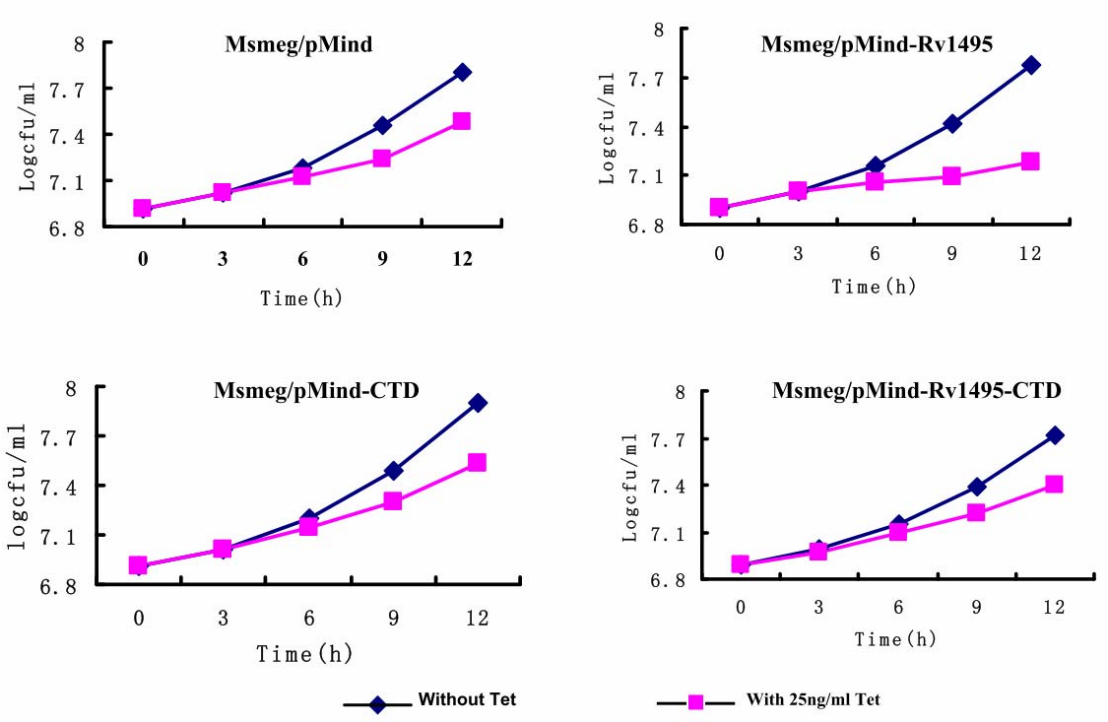
Assay for the toxin-antitoxin (Rv1495-Rv1494) interaction and their effect on the toxin-TopA interaction. **(A)** Bacterial two-hybrid assays (Stratagene) for the interactions of Rv1494 and Rv1495, which were performed as described under "Materials and Methods". **(B)** Native PAGE assay for the interaction between Rv1494 and 1495 and the protein complex. Different ratios of Rv1494/Rv1495 were co-incubated under 4℃ and 37℃. The mixtures were resolved by the native 12 % PAGE electrophoresis and stained by Coomassie Blue. The sites of single Rv1494, Rv1495 and Rv1494/Rv1495 protein complex were indicated by arrows on the right of the figure panel. **(C)** Effects of single Rv1494 or Rv1495 and Rv1494/Rv1495 complex on topoisomerase activities of Rv3646. Lanes 1, no topoisomerase as a negative control. L, linearized plasmid; R, relaxed plasmid; S, supercoiled plasmid. The supercoiled and no supercoiled plasmids were quantified using commercial plasmid as control. Their relative percentages were then calculated and depicted in the right panel.

Figure S3



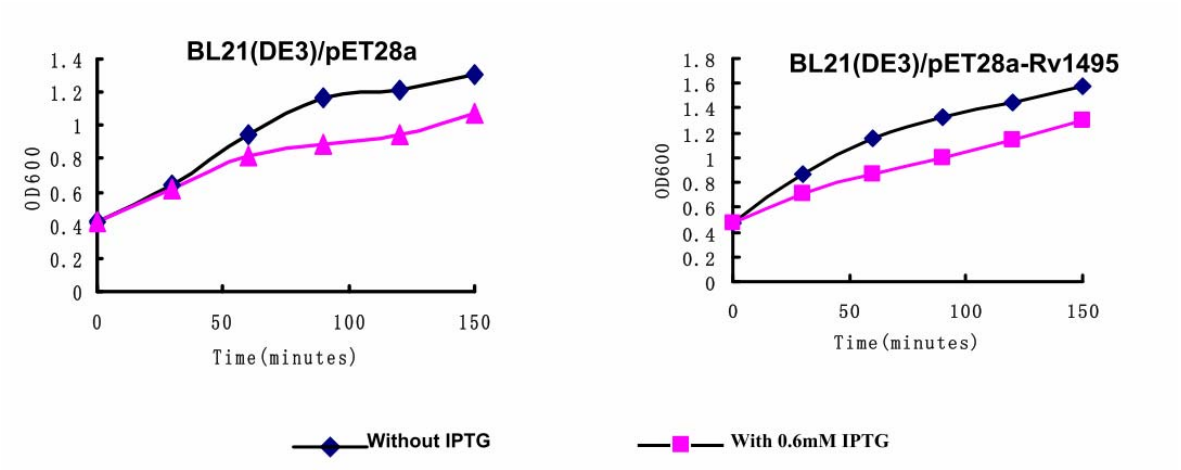
Effect of Rv1495 on the DNA-binding activity of MtbTopA. EMSA assays were performed as described under “Materials and Methods”. The concentrations of proteins are indicated on top of the panel.

Figure S4



Effects of the Rv1495 expression alone or coexpression with the C-terminus of MtbTopA on the growth of *M. smegmatis*. The Rv1495 gene and C-terminal domain of MtbTopA (CTD) alone, or their fusion form (Rv1495-CTD) were cloned into a TetR-controlled expression plasmid pMind. The effects of the Rv1495 expression alone or coexpression with the C-terminus of MtbTopA on the growth of *M. smegmatis* were analyzed as described in the “Materials and Methods”. The growth of these recombinant mycobacterial strains were examined in the presence (induction) or absence (no induction) of 25 ng/ml tetracycline (Tc). Aliquots were taken at the indicated times and the CFU was measured.

Figure S5



Effect of the Rv1495 expression on the growth of *E. coli*. Rv1495 gene from *M. tuberculosis* was amplified using its specific PCR primers and cloned into the prokaryotic expression vector pET28a. The recombinant plasmid pET28a-Rv1495 or control plasmid pET28a was transformed into *E. coli* BL21 (Novagen). The growth curves of recombinant strains were examined in the presence (induction) or absence (no induction) of 0.6 mM IPTG.