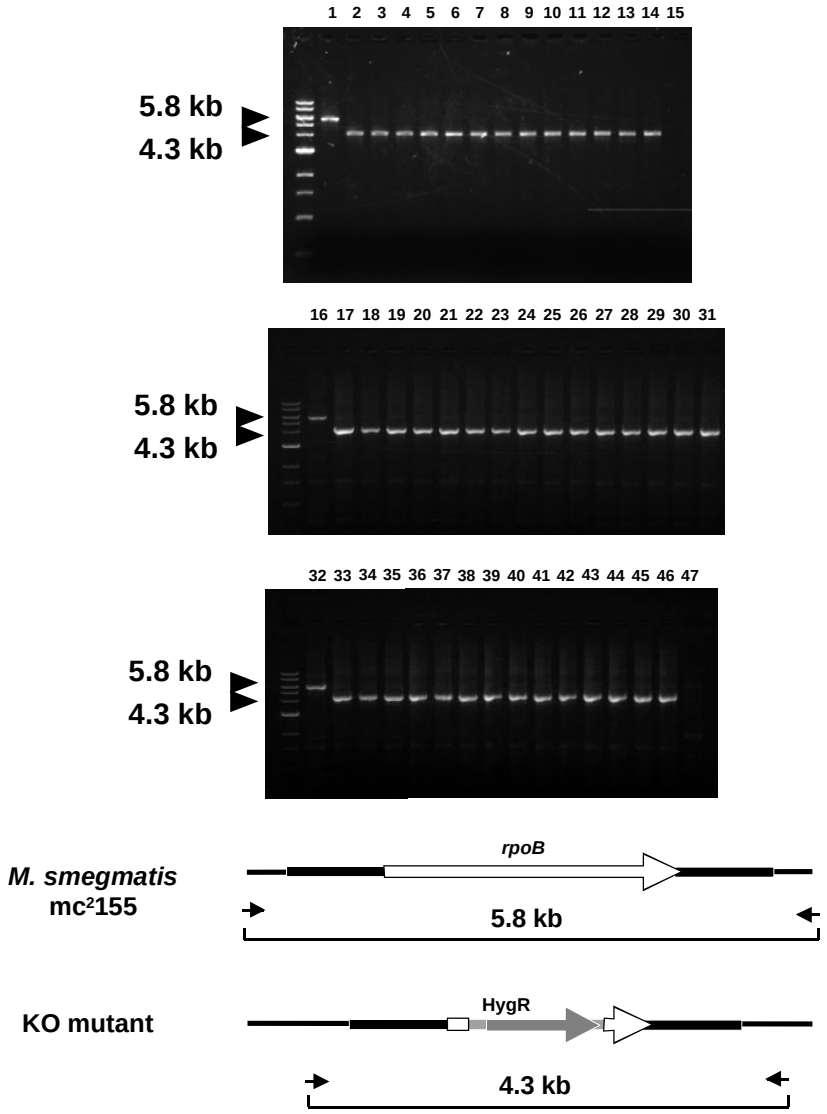

Figure S1. PCR analysis to confirm the disruption of *rpoB*. Black arrows represent primers MSRF and MSRBR for the PCR amplification. Lanes 1, 16, and 32, *M. smegmatis* mc^{2}155; lanes 2–14, *M. smegmatis* strains carrying the *M. leprae rpoB* without mutation and with mutations GGC507→GGG, GGC507→AGC, ACC508→ACA, CAG513→GTG, GAT516→AAT, CAG517→CAT, CAC526→TAC, TCG531→TTG, TCG531→TGG, GCG532→TCG, CTG533→CCG, and GTC547→ATC, respectively; lane 17–31 and 33–46, *M. smegmatis* strains carrying the *M. tuberculosis rpoB* without mutation and with mutations GGC507→AGC, GGC507→GAT, ACC508→CAC, ACC508→GCC, CAG510→CAT, CTG511→CCG, CAA513→AAT, CAA513→GAA, GAC516→GAG, GAC516→CAC, GAC516→GTC, CTG521→ATG, TCG522→TTG, GGG523→GCG, GGG523→GGC, CAC526→CTC, CAC526→TAC, CAC526→GAC, CAC526→TTC, CAC526→AAC, CAC526→CGC, CAC526→CAA, CGA529→AAA, TCG531→TTC, T531TTG, 506-508del, 514insTTC, and 518del, respectively; lanes 15 and 47, negative control. KO, knockout.

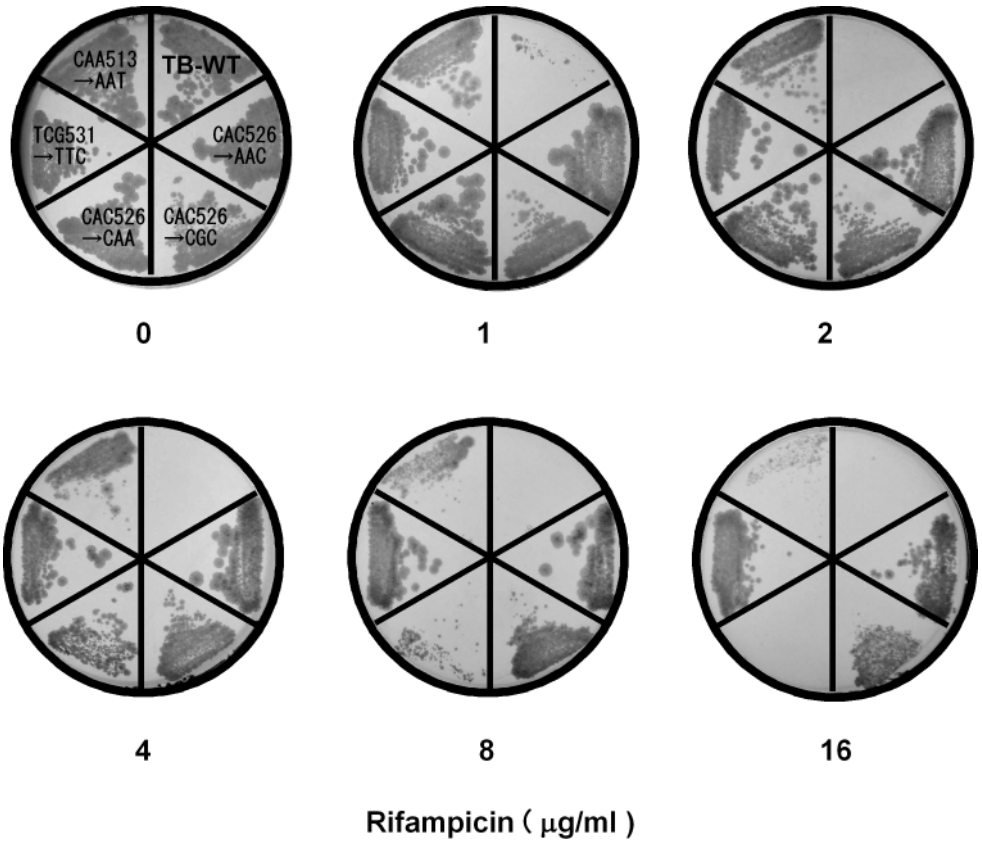

Figure S2. Rifampicin susceptibility of recombinant *M. smegmatis* strains. Approximately 1×10^3 bacterial cells were spread on 7H10 agar plates containing various concentrations of rifampicin. Growth was compared three days after inoculation. The rifampicin concentration is depicted below each plate. TB-WT, *M. tuberculosis* wild type sequence.