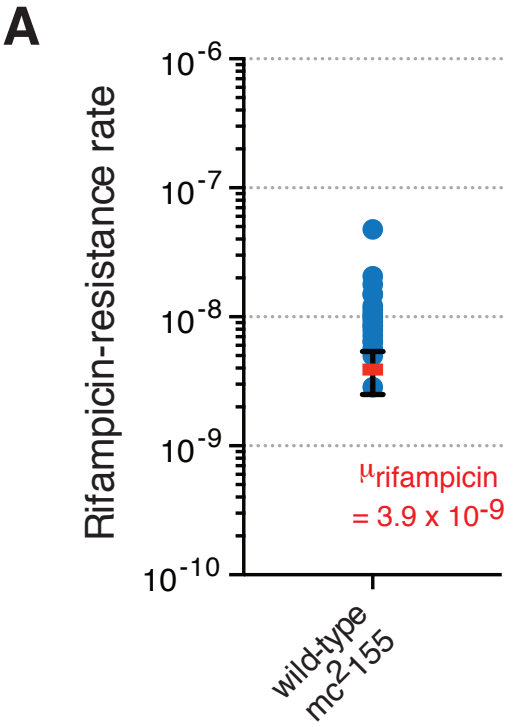

B

Mutation	Amino acid change
CAG>AAG	Q429K
CAG>CTG	Q429L
GAC>GTC	D432V
TCG>TTG	S438L
CAC>CGC	H442R
CAC>CCC	H442P
CAC>TAC	H442Y
CAC>GAC	H442D
TCG>TTG	S447L
TCG>TGG	S447F

$\mu_{rifampicin}$	3.9×10^{-9}
$\mu_{rifampicin}$ 95% CI	$(2.5 - 5.4) \times 10^{-9}$
Rifampicin taget size (bp)	10
$\mu_{in\ vitro}$	3.9×10^{-10}
$\mu_{in\ vitro}$ 95% CI	$(2.5 - 5.4) \times 10^{-10}$

Supplemental Figure 6. The per base pair mutation rate of *M. smegmatis* estimated from fluctuation analysis.

(A) Fluctuation analysis was used to determine the rates at which wild-type *M. smegmatis* acquired resistance to rifampicin. Circles represent mutant frequency (number of rifampicin-resistant mutants per cell plated in a single culture). The red bar represents the estimated mutation rate (mutations conferring rifampicin resistance per generation), with error bars representing the 95% confidence interval (CI). **(B)** The number of mutations in *rpoB (Ms1367)* that confer rifampicin resistance in our fluctuation analysis was determined by sequencing 150 independent rifampicin-resistant isolates. This analysis identified ten unique mutations. The per base pair mutation rate, $\mu_{in\ vitro}$, was determined by dividing $\mu_{rifampicin}$ by the target size.