## SUPPLEMENTARY MATERIAL

## Supplementary Table

Oligonucleotide	Sequence (5'-3')
pMS-Seq1	CGTTCTCGGCTCGATGATCC
MspA_SD	CG <u>GCATGC</u> AGAAA <i>GGAGG<u>TTAATTAA</u>TGAAGGCAATCAGTCGGGT</i>
rv0194_F4	TAATGCGCACGAATTGCTGGTGG
rv0194_Hind2	GC <u>AAGCTT</u> GTCAACTCGCCACCCATTCG
Salgd	TAGCTTATTCCTCAAGGCACGAGC
IS2	GAGGCGGCAGAAAGTCGTCAGGTCAG
Tn-mut_seq2	CAACGTGCGAGTCACGCTGTC
Tn_mut_seq4	CTTCTGCAGCAACGCCAGGTCCACACTG
Rv0194_F1	GGCAAATCCACGTTGGCGTC
Rv0194_rev_T7	CTAATACGACTCACTATAGGGAGACGGCAGAGGTCGGGTCGTCC
16SNbfw	TGCTACAATGGCCGGTACAAA
16SrevT7Prom	CTAATACGACTCACTATAGGGAGACGCTTCCGGTACGGCTACCT
tnpA_rev	CGAAGGTCAGCGGGTGCTCA
Aph2	CTCACCGAGGCAGTTCCATA

## Table S1. Oligonucleotides used in this work.

Restriction sites are underlined. The sequence shown in italics is the Shine-Dalgarno sequence of the *mspA* gene. Recognition site for T7 RNA polymerase is shown in bold.



**Fig. S1. Susceptibility of** *M. bovis* **BCG ML1034 to ampicillin.** The susceptibilities of wt *M. bovis* BCG (black bars) and of the ML1034 mutant (white bars) to ampicillin were determined by the microplate Alamar blue assay in triplicates. The percentage of survival is shown with standard deviations.



Fig. S2.  $\beta$ -lactamase activity of wild-type *M. bovis* BCG and the ML1034 mutant. Hydrolysis of nitrocefin by whole-cell lysates (black bars) and culture filtrates (grey bars) was measured as absorption at 490 nm. The  $\beta$ -lactamase activity is shown as  $A_{490}$  per min and mg of total protein. The background activity was determined using PBS as a negative control. All assays were performed in triplicate. Error bars represent standard deviations.