



Fig. S2 PCR-based genotyping of the *M. tuberculosis* *accD6* mutants **(A)** (top) Schematic showing the genomic orientation of the *accD6*_{Mtb} gene (thick gray arrow). The internal deletion of *accD6* introduced using a gene replacement vector is marked with a black rectangle. GR1-GR2/GR3-GR4 denote the gene-flanking fragments that were amplified and cloned together in the p2NIL suicide vector to obtain the mutated $\Delta accD6$ allele for gene replacement. The small gray arrows indicate the binding sites for the primers that were used for PCR-based genotyping. The thin black arrows indicate the length of the product amplified from wild-type *accD6* (1486 bp) and the length of the deleted fragment (853 bp, yielding a 633 bp PCR product). (bottom) The genotypes of selected *M. tuberculosis* mutants were confirmed by PCR. Lanes: 1, wild-type *M. tuberculosis*; 2, single crossover (SCO) strain carrying both wild-type and mutated genes; 3, one of the wild-type DCO strains exclusively found after selection. **(B)** (top) Schematic showing the region that was amplified in order to select proper $\Delta accD6_{Mtb}-P_{fasII} accD6_{Mtb}$ and $\Delta accD6_{Mtb}-P_{fasII} FASII_{Mtb}$ mutant strains. (bottom) The SCO strain was enriched with an intact *accD6* supplied on the pFD6Tb1 and pFASTb2 constructs. After further processing, DCO strains carrying wild-type *accD6* or $\Delta accD6$ were obtained. Lanes: 1, wild-type *M. tuberculosis*; 2, SCO strain; 3, $\Delta accD6_{Mtb}-P_{fasII} accD6_{Mtb}$; 4, $\Delta accD6_{Mtb}-P_{fasII} FASII_{Mtb}$.