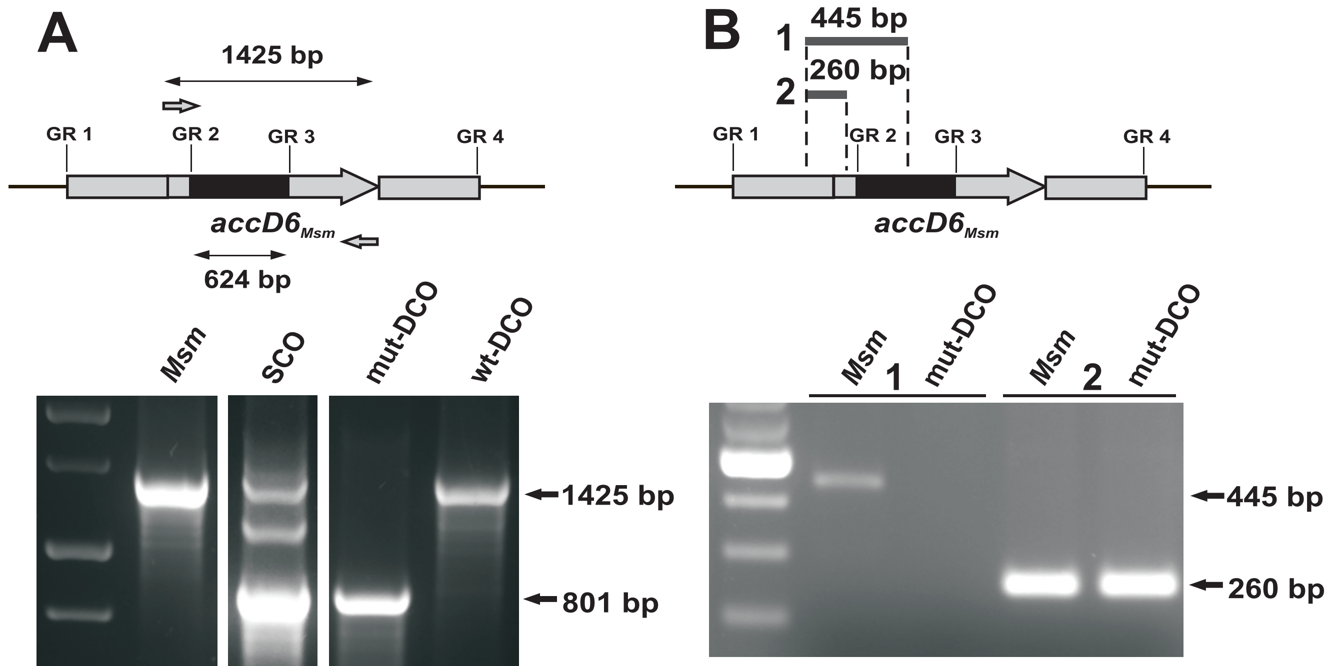

Fig. S3 PCR analysis of *M. smegmatis* Δ*accD6* DCO mutants **(A)** (top) Map showing the genomic orientation of the *accD6*$_{Msm}$ gene (thick gray arrow), and the internal deletion introduced into *accD6* (black rectangle). GR1-GR2/GR3-GR4 are the gene-flanking fragments that were amplified and cloned together into the suicide vector to obtain the mutated Δ*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* (1425 bp) and the length of the fragment deleted from the mutated gene (624 bp) to give a PCR product of 801 bp. (bottom). PCR-based genotyping. Lanes: *Msm*, wild-type *M. smegmatis*; SCO, single-crossover strain; mut-DCO, one of three obtained *M. smegmatis* Δ*accD6* mutants; wt-DCO, wild-type DCO mutant. **(B)** Confirmatory PCR showing the loss of the internal 624 bp region of the *accD6* gene in the Δ*accD6* mutant. Two sets of primers were used to amplify two products from the chromosomal DNA of wild-type *M. smegmatis* and an Δ*accD6* mutant. (top) Schematic showing the analytic strategy. In the first reaction, the reverse primer was complementary to a sequence within the deleted fragment (black rectangle), so the 445 bp product could not be amplified from the mutant. In the control reaction, the reverse primer was complementary to a sequence that was present in both wild-type and mutant strains, so a 260 bp product was amplified from both templates. (bottom) The results from this PCR-based analysis. All PCR reactions were performed using a single master mix. Product No. 1 was amplified using primers Ms-kasB-s-pol and MsD6pr2, while product No. 2 was amplified using primers Ms-kasB-s-pol and Ms-accD6-r-pol.