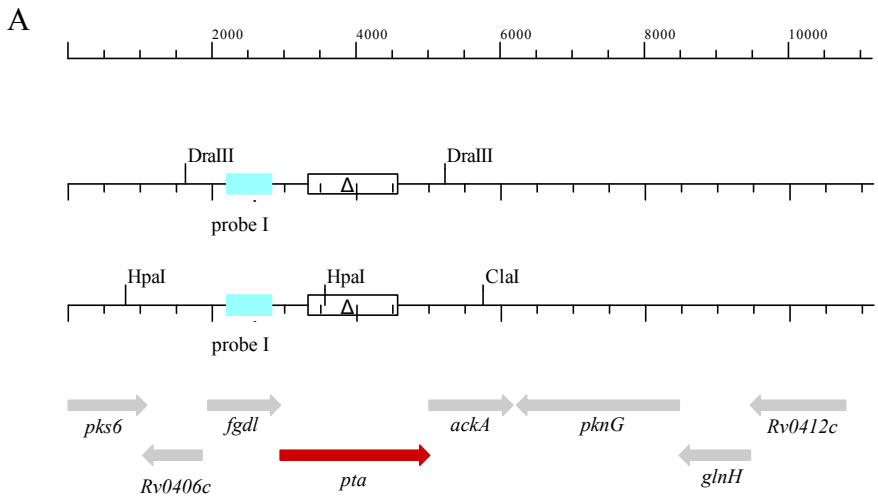
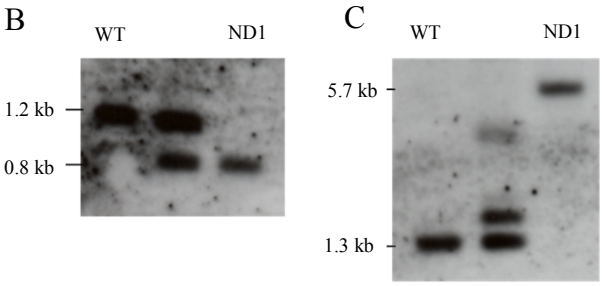
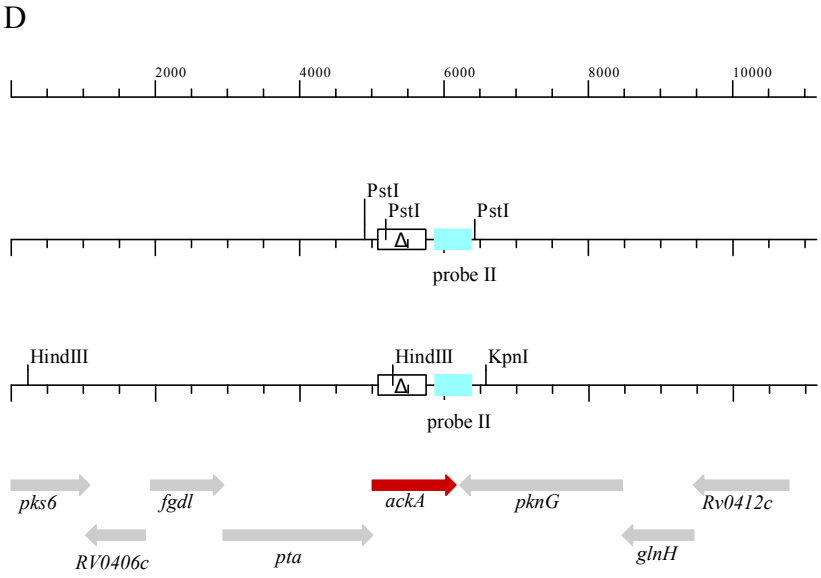
SUPPLEMENTARY MATERIAL

Supplementary Figure S1







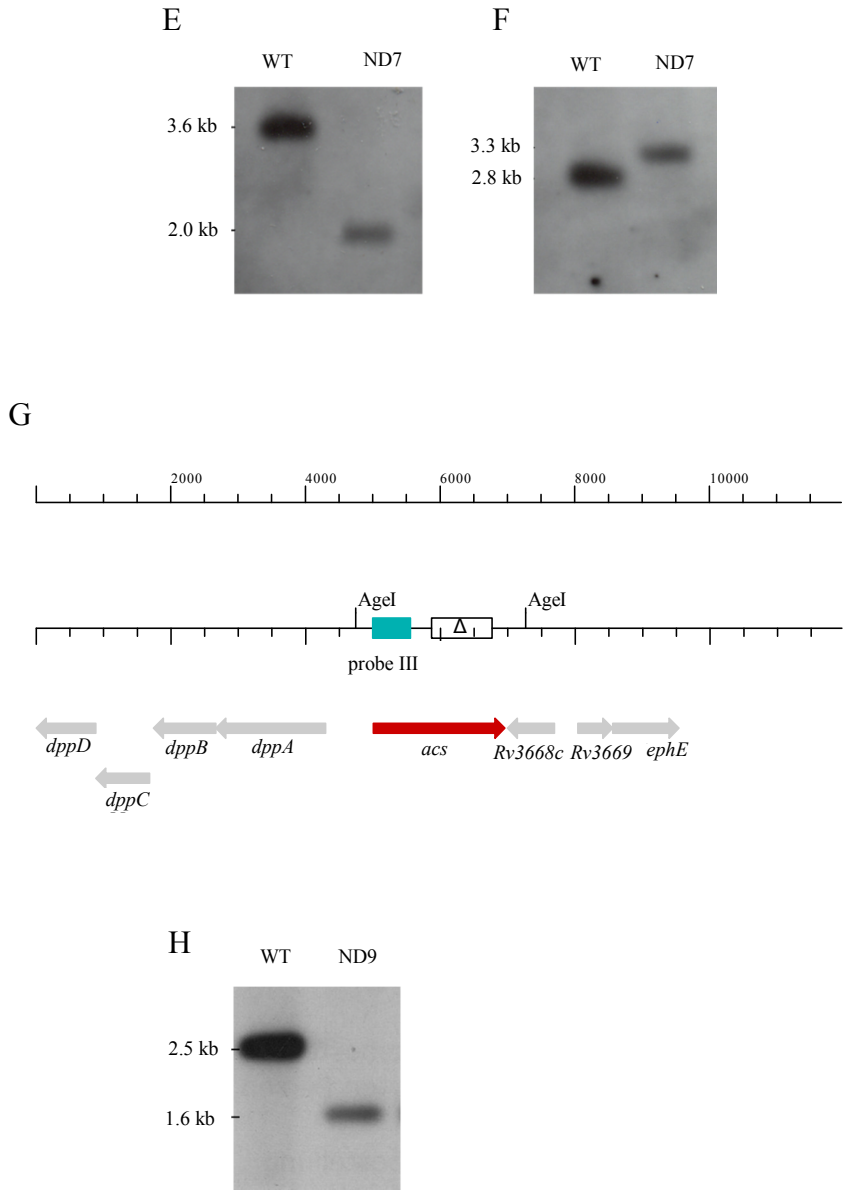


Figure S1: Knock-out strains were validated via Southern Blot analysis. Genomic DNA of *Mtb* H37Rv wild type (WT), Δ*pta,* Δ*ackA* and Δ*acs* mutant was isolated and digested as described below and further analyzed with Southern blot analysis. Genes are written in italics and the gene of interest is highlighted in red. The restriction sites important for Southern blot analysis are depicted in the map. The deleted sequences are indicated with a box labelled Δ, while the blue boxes represent probe binding sites. A) Explains the two different strategies used to validate the Δ*ackA* mutant (ND1): *Pst* I digestion and *Hind* III-*Kpn* I double digestion. B) Southern blot showing the results of the *Pst* I digestion. C) Southern blot showing the results of the *Hind* III and *Kpn* I double digestion. D) Explains the two different strategies

used to validate the Δ*pta* mutant (ND7): *Dra* III digestion and *Hind* III-*Kpn* I double digestion. E) Southern blot showing the result of the *Dra* III digestion. F) Southern blot showing the results of the *Hpa* I-*Cla* I double digestion. G) Explains the strategy used to validate the Δ*acs* mutant (ND9): the *Age* I digestion. H) Southern blot showing the result of the *Age* I digestion. For further details see method section.

METHOD

Verification of Δ*ackA*, Δ*pta* and Δ*acs* knock-out mutants in *Mtb* by Southern Blot analysis. To analyze the knock-out mutants for gene deletion by allelic exchange, different DNA probes were generated by PCR from chromosomal DNA of *Mtb* H37Rv. To amplify a 501 bp probe corresponding to the sequence downstream of the *ackA* gene the following primers were used: 5'-caccggttgcgcaagtac-3', and 5'-gcaccaaccacatcctcg-3'. The 621 bp probe corresponding to the sequence upstream of the *pta* gene was amplified by the following primers: 5'-ggctttcgccaccatggg-3', and 5'-ttcgacggcttcgtcggg-3'. The primer pair 5'-gcggtgatcagcagcttg-3', and 5'-cacatccgtgagtgagtc-3' was used to design a DNA probe to analyze the Δ*acs* mutant. The PCR products were labelled with digoxigenin dUTP (Roche Applied Science) and were used to detect the size change in fragments of digested genomic DNA in the wild type and the mutant strains. *Pst* I-digestion of chromosomal DNA produced a 1,235 bp fragment in the wild type, and an 861 bp fragment in the Δ*ackA* mutant. Double digestion of genomic DNA by *Hind* III and *Kpn* I produced a 1,294 bp fragment in the wild type and a 5,681 bp fragment in the Δ*ackA* mutant. *Dra* III digested chromosomal DNA produced a 3,593 bp fragment in the wild type and a 1,973 bp fragment in the Δ*pta* mutant. The *Hpa* I and *Cla* I digestion produced a 2,761 bp fragment in the wild type and a 3,331 bp fragment in the Δ*pta* mutant. *Age* I digested chromosomal DNA produced a 2,524 bp fragment in the wild type and a 1,627 bp fragment in the Δ*acs* mutant. For detection, the fragments were separated by agarose gel electrophoresis, transferred to a nylonmembrane, and

hybridized with the respective probe. Southern blotting and DNA hybridization were performed according to the manufacturer's instructions (Roche Applied Science).

Supplementary Figure S2

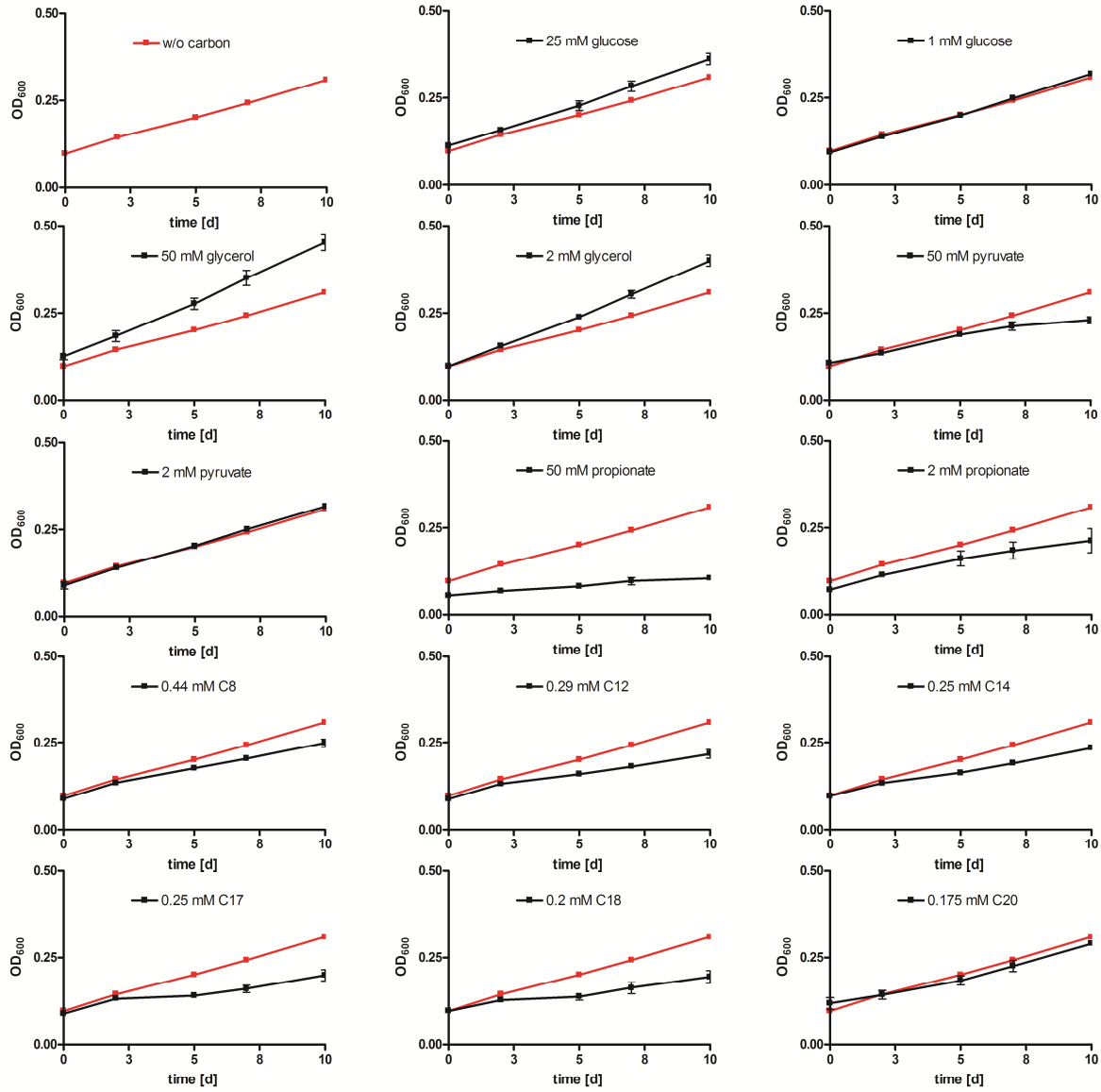


Figure S2: Growth of *Mtb* wild type strain on different carbon substrates. *Mtb* H37Rv was grown for 10 days in standing culture tubes in a standard growth medium containing 7H9 broth supplemented with 0.5% AS and 0.05% tween 80 (red squares). To analyze the impact of the carbon source on growth, different substrates were added to the standard medium (black squares) and growth was followed by measuring OD_{600}. Growth on standard medium with and without additional carbon source was compared for each substrate. Data represents the mean of three independent experiments, error bars indicate the SD.

Supplementary Figure S3

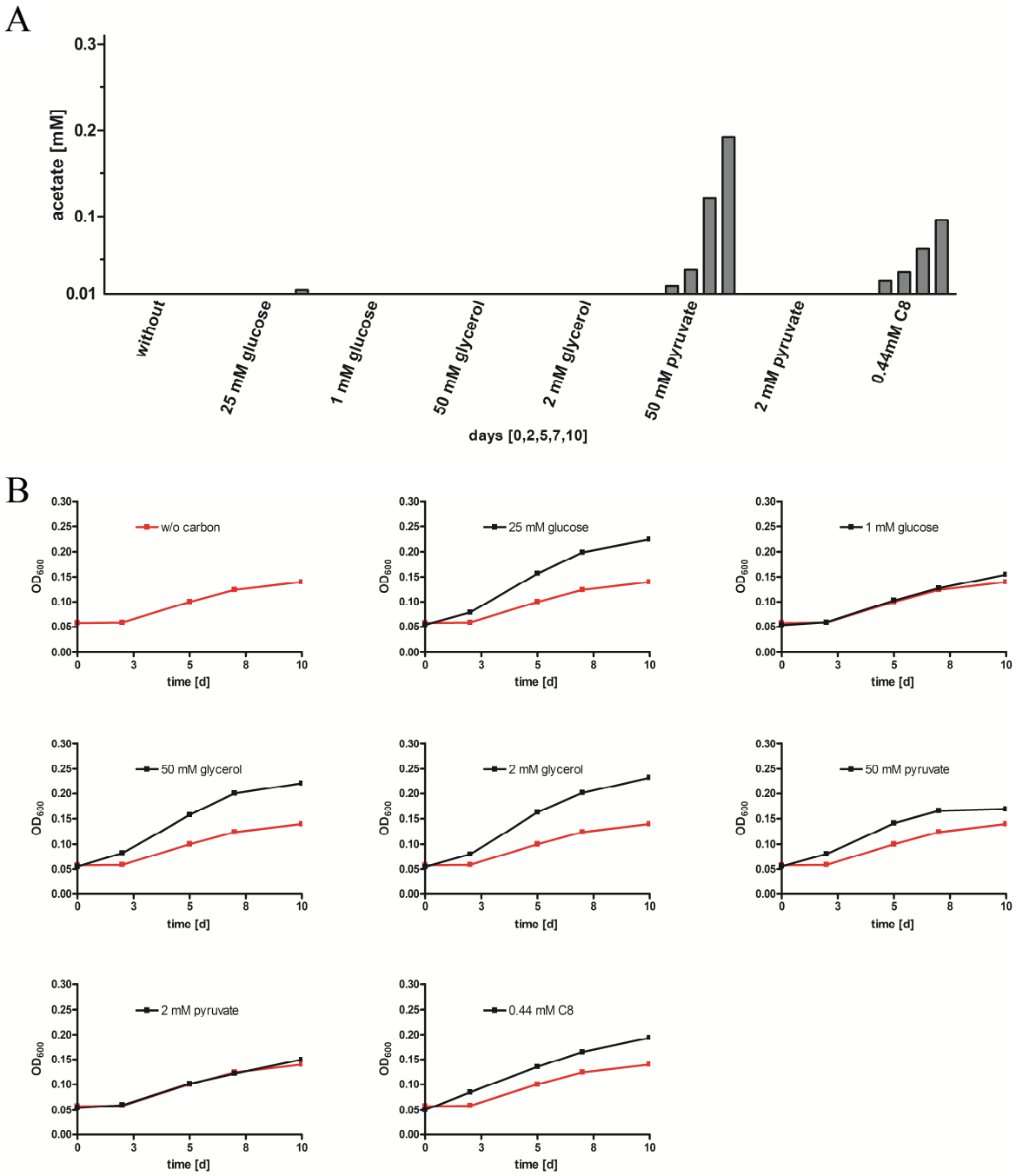


Figure S3: Impact of a defined basic medium on growth and acetate secretion in the *Mtb* wild type strain. *Mtb* H37Rv was grown for 10 days in standing cultures. The 7H9 based medium contained 0.5% AS of a fatty acid free albumin and 0.05% tyloxapol. The medium was supplemented with different carbon substrates and acetate secretion (A) and growth (B) were detected at indicated time points. To analyze the effect of carbon supplementation, growth of *Mtb* on the basic medium (red squares) was compared to growth in the presence of

the respective carbon source (black squares). Data shows one representative of two independent experiments.