**SUPPORTING INFORMATION (SI)**

Validation of CoaBC as a bactericidal target in the coenzyme A pathway of *Mycobacterium tuberculosis*

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METHODS

Bacterial strains and growth conditions

All mutant strains used in this study (Table S2) were derived from *Mtb* H37RvMA<sup>1</sup>, which is a virulent, PDIM-producing strain. All strains were grown in Difco Middlebrook 7H9 broth (BD) supplemented with ADC enrichment (BD), 0.2% glycerol (Sigma-Aldrich) and 0.05% Tween-80, or on Difco Middlebrook 7H10 agar (BD) supplemented with OADC enrichment and 0.5% glycerol. Hygromycin (Hyg), kanamycin (Kan) and gentamicin (Gm) were used at concentrations of 50, 25 and 2.5 µg/ml, and pantothenate (Sigma-Aldrich) and pantetheine (Sigma-Aldrich) were used at 25 µg/ml and 2.5 mg/ml, respectively. Conditional mutants carrying pGMCK3-OX38-T10, which results in strong induction of TetR (Tet-ON<sub>S</sub>) and pGMCK3-OX21-T10, which results in intermediate-level expression of TetR (Tet-ON<sub>M</sub>) were grown in the presence of the appropriate exogenous supplement in order to prevent the emergence of suppressors, where possible, or ATc (200ng/ml), as required.

In order to repress the expression of target genes in cells expressing wt-TetRs, cells were grown to OD<sub>600</sub> ~ 0.2 in the presence of the appropriate supplement or ATc (200 ng/ml), harvested by centrifugation and washed with an equal volume of supplement-free 7H9 broth (2×) prior to dilution into 7H9 broth containing the desired concentration of ATc. Mutants harbouring pGMCK3-OX38-T28 (revTetR) were routinely grown in the absence of supplementation to OD<sub>600</sub> ~ 0.2 prior to dilution in 7H9 broth containing the desired concentration of ATc. To avoid inactivation of the inducer, all cultures containing ATc were incubated in the dark and exposure of the cultures to light was minimised.

Construction and genotypic characterisation of promoter replacement mutants
The integrative plasmids used for generation of the promoter replacement mutants were constructed as previously described\textsuperscript{2-3}. Briefly, the ribosomal binding site and the first 570, 522, 627, 582, 390 and 609 bp of *panB*, *panE*, *panK* (*coaA*), *coaBC*, *coaD* and *coaE*, respectively, were amplified by PCR prior to restriction with *Sph*\textsubscript{I} and *Nol*\textsubscript{I}, and ligation into the similarly restricted pSE100\textsuperscript{4} (Table S2). The resulting suicide plasmids were electroporated into *Mtb* H37Rv and transformants were selected on 7H10 agar containing Hyg. Individual colonies of each mutant were grown to mid-logarithmic phase in 7H9 broth, following which genomic DNA was extracted from the harvested cells. The site-specificity of homologous recombination was confirmed by Southern hybridisation using the ECL Direct Nucleic Acid Labelling and Detection System (Amersham Biosciences) (Fig. S1).

Conditional mutants were generated by electroporation of pGMCK3-OX38-T10, pGMCK3-OX21-T10 and pGMCK3-OX38-T28\textsuperscript{5} into each of the promoter replacement mutants. Integration into the chromosome was facilitated by co-delivery of an additional suicide vector, pGA-OXP15-intL5, transiently expressing integrase\textsuperscript{5}. Transformants were selected on 7H10 agar supplemented with Hyg and Kan, in the presence and absence of ATc (200ng/ml). Dose-dependence of growth was determined by spotting on 7H10 agar containing doubling dilutions of ATc ranging from 200 to 1.6 ng/ml as well as in the absence of ATc. The ability of pantothenate to restore growth of the *panB*, *panC* and *panE* conditional mutants and pantethine to restore growth of the *coaBC* conditional mutants\textsuperscript{6} on 7H10 was also determined.

**RNA extraction, DNase-treatment, cDNA synthesis and ddPCR primer/probe design**

Total cellular RNA was extracted from two independent cultures of *M. tuberculosis* H37Rv and from two independent cultures of each of the conditional mutants, except the *coaBC* conditional mutants, from which RNA was extracted from three independent cultures, using
the FastRNA® Pro Blue Kit (MP Biomedicals) and FastPrep-24 system (MP Biomedicals) according to the manufacturer’s instructions. DNase treatment of 2 µg of total RNA was carried out at 37°C for 60 minutes using 4U TURBO™ DNase (Ambion), following which the DNase-treated RNA was purified by acid phenol:chloroform:isoamyl alcohol (125:24:1) (Ambion) extraction and ethanol precipitation.

A total of 60 ng DNase-treated RNA and 0.5 µg random hexamers were denatured at 65°C for 2 minutes using a T100™ Thermal Cycler (Bio-Rad). Following immediate incubation on ice for 1 minute, RNA was reverse transcribed into cDNA using 100 U MMLV High Performance Reverse Transcriptase (Epicentre, Illumina, WI) in the presence of 1 × MMLV Reaction Buffer, 10 mM DTT, 0.5 mM of each dNTP, 20 U RiboGuard™ RNase Inhibitor (Epicentre, Illumina, WI) and distilled H₂O to a final volume of 20 µl. The reaction was incubated at room temperature (22°C) for 10 minutes prior to incubation at 37°C for 60 minutes and termination at 85°C for 5 minutes.

TaqMan® minor groove binder (MGB) probes and primers (Applied Biosystems) (Table S3) were designed using Primer Express® Software v3.0 (Applied Biosystems). In order to exclusively quantify transcript expressed from the Tet-regulatable promoter, Pmcy1tetO, only the 3’ region of the gene of interest was amplified by primers located either downstream of or flanking the homologous region. Amplification of M. tuberculosis H37Rv sigA was used as the reference gene for quantification normalisation. In order to facilitate multiplexing of the assay, probes homologous to the genes of interest (panB, panC, panE, panK, coaBC, coaD and coaE) were 5’-FAM-labelled, while the probe directed against sigA was 5’-VIC-labelled. The efficiency of amplification of each primer/probe set was determined by generating standard curves using 10-fold serial dilutions of M. tuberculosis H37Rv genomic DNA. From the slope of the standard curve, PCR efficiency was calculated using the formula $E = 10^{[-1/\text{slope}]}$, where a slope of -3.3 corresponds to 100% amplification.
efficiency. The coefficient of determination, $R^2$, of the slopes produced by all primer/probe sets was $\geq 0.98$.

**Gene expression analysis of conditional knockdown mutants using ddPCR**

A volume of 2 µl of cDNA was used as the template in multiplex droplet digital PCR (ddPCR) assays consisting of 1 × ddPCR™ Supermix for Probes (Bio-Rad), 375 nM of each primer (Table S3), 250 nM of each TaqMan® MGB probe and distilled H$_2$O to a final volume of 20 µl. The reaction mix was emulsified with droplet generator oil (Bio-Rad, Hercules, CA) and partitioned into 20000 nanoliter-sized droplets using a QX-200™ Droplet Generator according to the manufacturer’s instructions. The droplets were transferred to a 96-well reaction plate (Eppendorf, Hauppauge, NY) prior to heat-sealing the plates with pierceable foil plate seals (Bio-Rad) using a PX1™ PCR plate sealer (Bio-Rad). PCR amplification was performed using a T100™ Thermal Cycler (Bio-Rad) and cycling conditions consisted of enzyme activation at 95°C for 10 minutes, 40 cycles of denaturation at 94°C for 30 seconds and annealing/extension at 60°C for 1 minute and enzyme deactivation at 98°C for 10 minutes. Following completion of PCR amplification, droplets were immediately analysed using a QX-200™ Droplet Reader (Bio-Rad, Hercules, CA), in which each individual droplet from each well is analysed using a two-colour detection system (in this case, FAM and VIC).

Fluorescence data for each well were analysed using QuantaSoft software (Bio-Rad, Hercules, CA). Where necessary, thresholds were determined manually according to the negative controls, which included RT negative controls for each sample tested, and no template controls for each primer/probe combination tested. For each sample, the absolute concentration of transcript (copies/ml) of the gene of interest was determined relative to *sigA.*
All data are representative of two biological replicates, except coaBC, which is representative of three biological replicates, for each of which two technical replicates were performed.

**Effect of depletion of CoA pathway enzymes on viability of Mtb**

Conditional mutants were grown to OD$_{600}$ ~ 0.2 in 7H9 medium as described above. Following a 500-fold dilution in 7H9 medium containing no supplementation, a volume of 50 µl of diluted inoculum was added to round-bottom 96-well microtitre plates containing 50 µl 7H9, with appropriate antibiotic supplementation, in both the presence and absence of ATc (200 ng/ml), and plates were incubated at 37°C for either 9 or 32 days. Following washing in ATc-free 7H9 medium to ensure no carry-over of inducer, serial dilutions of the starting inoculum (Day 0) were plated onto 7H10 agar with and without ATc (200 ng/ml) in order to quantitate the number of colony forming units (CFU) inoculated into the microtitre plates. Aliquots of 100 µl of serially diluted bacteria incubated in the presence and absence of ATc (200 ng/ml) were plated on 7H10 agar with and without ATc (200 ng/ml) every 24 hours over a period of 9 or 32 days. *Mtb* H37Rv and all promoter replacement mutants corresponding to the knockdown mutants tested were included as controls (Table S2), and CFUs were enumerated following 3 weeks’ incubation at 37°C.

**Genetic validation of CoaBC in vivo**

Female C57BL/6 mice (Jackson Laboratory) were infected with early-log-phase *Mtb* cultures by aerosol using an inhalation exposure system (Glas-Col). Single-cell suspensions were created in PBS in order to deliver 100 to 200 bacilli per mouse. Doxycycline containing food (2000 ppm, Research Diets) was fed to the mice starting at either the day before infection, day 8 post-infection or day 35 post-infection. Serial dilutions of lung and spleen homogenates were cultured on 7H10 plates, both in the presence and absence of appropriate antibiotic
selection, and CFUs were enumerated at the indicated time points. Lung sections from mice infected with \textit{coaBC} Tet-OFF and fed doxy-containing chow starting from the day before infection, day 8 post-infection or day 35 post-infection were stained with hematoxylin and eosin.
Table S1. Droplet digital PCR analysis of transcript levels in all conditional mutants

| Gene | Mtb Strain | Concentration of transcript normalised to $\text{sigA}$ (copies/ml) | Fold-change in transcript levels in SCO relative to H37Rv | Decrease in transcript levels relative to H37Rv (%) |
|------|------------|--------------------------------------------------|-----------------------------------|-----------------------------------|
| panB | H37Rv      | 171.5                                            | NA                               | NA                                |
|      | panB-SCO   | 513.0                                            | + 3.0                             | NA                                |
|      | panB Tet-ONM + | 465.0                               | NA                               | NA                                |
|      | panB Tet-ONM - | 11.3                                         | NA                               | 93.4                              |
|      | panB Tet-OFF + | 25.1                                          | NA                               | 85.4                              |
|      | panB Tet-OFF - | 556.3                                          | NA                               | NA                                |
| panC | H37Rv      | 221.8                                            | NA                               | NA                                |
|      | panC-SCO   | 128.8                                            | - 1.7                             | NA                                |
|      | panC Tet-ONM + | 109.5                                | NA                               | NA                                |
|      | panC Tet-ONM - | 11.9                                          | NA                               | 94.6                              |
|      | panC Tet-OFF + | 14.1                                           | NA                               | 93.6                              |
|      | panC Tet-OFF - | 133.5                                          | NA                               | NA                                |
| panE | H37Rv      | 31.8                                             | NA                               | NA                                |
|      | panE-SCO   | 180.8                                            | + 5.7                             | NA                                |
|      | panE Tet-ON S + | 143.8                               | NA                               | NA                                |
|      | panE Tet-ON S - | 54.1                                          | NA                               | ND                                |
|      | panE Tet-ONM + | 127.0                                          | NA                               | NA                                |
|      | panE Tet-ONM - | 34.5                                           | NA                               | ND                                |
|      | panE Tet-OFF + | 52.1                                          | NA                               | ND                                |
|      | panE Tet-OFF - | 122.0                                          | NA                               | NA                                |
| panK | H37Rv      | 103.3                                            | NA                               | NA                                |
|      | panK-SCO   | 87.9                                             | - 1.2                             | NA                                |
|      | panK Tet-ON S + | 85.4                                        | NA                               | NA                                |
|      | panK Tet-ON S - | 43                                            | NA                               | 58.4                              |
|      | panK Tet-ONM + | 90.7                                           | NA                               | NA                                |
|      | panK Tet-ONM - | 55.5                                           | NA                               | 46.3                              |
|      | panK Tet-OFF + | 51.2                                           | NA                               | 50.4                              |
|      | panK Tet-OFF - | 91.6                                           | NA                               | NA                                |
|    | Variable | H37Rv | Tet-ON_M | Tet-OFF_M | Tet-OFF_S | Tet-ON_S |
|----|----------|-------|----------|----------|----------|----------|
| **coaBC** |          |       |          |          |          |          |
|    |          | 341.8 | NA       | NA       | NA       |          |
|    | coaBC-SO | 357.0 | + 1.0    | NA       | NA       |          |
|    | coaBC Tet-ON_M + | 401.8 | NA       | 88.3     | 81.9     |          |
|    | coaBC Tet-ON_M - | 39.8  | NA       |          |          |          |
|    | coaBC Tet-OFF + | 61.7  | NA       |          |          |          |
|    | coaBC Tet-OFF - | 371.0 | NA       |          |          |          |
| **coaD** |          |       |          |          |          |          |
|    |          | 103.4 | NA       | NA       | NA       |          |
|    | coaD-SO  | 1655.0| + 16.0   | NA       | NA       |          |
|    | coaD Tet-ON_S + | 1735.0| NA       |          |          |          |
|    | coaD Tet-ON_S - | 36.5  | NA       | 64.7     |          |          |
|    | coaD Tet-ON_M + | 1997.5| NA       |          |          |          |
|    | coaD Tet-ON_M - | 374.0 | NA       | ND       |          |          |
|    | coaD Tet-OFF + | 105.5 | NA       | ND       |          |          |
|    | coaD Tet-OFF - | 1680.0| NA       |          |          |          |
| **coaE** |          |       |          |          |          |          |
|    |          | 546.0 | NA       | NA       | NA       |          |
|    | coaE-SO  | 278.3 | - 2.0    | NA       | NA       |          |
|    | coaE Tet-ON_M + | 155.5 | NA       |          |          |          |
|    | coaE Tet-ON_M - | 84.3  | NA       | 84.6     |          |          |
|    | coaE Tet-OFF + | 115.6 | NA       | 78.8     |          |          |
|    | coaE Tet-OFF - | 287.0 | NA       |          |          |          |

*Data are representative of two biological replicates performed in duplicate, except coaBC, which represents three biological replicates performed in duplicate. +, strains cultured in the presence of ATc (200ng/ml); -, strains cultured in the absence of ATc; NA, not applicable; ND, not determinable.
### Table S2. Bacterial strains and plasmids used in this study

| Strain or plasmid | Description | Reference |
|-------------------|-------------|-----------|
| **Mtb Strains**   |             |           |
| H37RvMA           | *Mtb* H37Rv isolate from the laboratory of Dr. C Sassetti; ATCC 27294 virulent laboratory strain | Ioerger et al., 2010 |
| panC SCO          | H37RvMA derivative in which expression of *panC* is controlled by P\textsubscript{myc1tetO} | Abrahams et al., 2012 |
| panB SCO          | H37RvMA derivative in which expression of *panB* is controlled by P\textsubscript{myc1tetO} | This study |
| panE SCO          | H37RvMA derivative in which expression of *panE* is controlled by P\textsubscript{myc1tetO} | This study |
| panK SCO          | H37RvMA derivative in which expression of *panK* is controlled by P\textsubscript{myc1tetO} | This study |
| coaBC SCO         | H37RvMA derivative in which expression of *coaBC* is controlled by P\textsubscript{myc1tetO} | This study |
| coaD SCO          | H37RvMA derivative in which expression of *coaD* is controlled by P\textsubscript{myc1tetO} | This study |
| coaE SCO          | H37RvMA derivative in which expression of *coaE* is controlled by P\textsubscript{myc1tetO} | This study |
| panC Tet-ON\textsubscript{M} | *panC* SCO containing pMC2m integrated at the L5 *attB* site and pPanC-Comp at the Tweety *attB* site | Abrahams et al., 2012 |
| panC Tet-OFF      | *panC* SCO containing pTEK-4SOX and pPanC-Comp integrated at the Tweety *attB* site | Abrahams et al., 2012 |
| panB Tet-ON\textsubscript{M} | *panB* SCO containing pGMCK3-OX21-T10 integrated at the L5 *attB* site | This study |
| panB Tet-OFF      | *panB* SCO containing pGMCK3-OX38-T28 integrated at the L5 *attB* site | This study |
| panE Tet-ON\textsubscript{S} | *panE* SCO containing pGMCK3-OX38-T10 integrated at the L5 *attB* site | This study |
| panE Tet-ON\textsubscript{M} | *panE* SCO containing pGMCK3-OX21-T10 integrated at the L5 *attB* site | This study |
| panE Tet-OFF      | *panE* SCO containing pGMCK3-OX38-T28 integrated at the L5 *attB* site | This study |
| panK Tet-ON\textsubscript{S} | *panK* SCO containing pGMCK3-OX38-T10 integrated at the L5 *attB* site | This study |
| panK Tet-ON\textsubscript{M} | *panK* SCO containing pGMCK3-OX21-T10 integrated at the L5 *attB* site | This study |
| Gene   | Description                                                                 | Integration Site       | Reference           |
|--------|------------------------------------------------------------------------------|------------------------|---------------------|
| panK   | SCO containing pGMCK3-OX38-T28 integrated at the L5 attB site                 | This study             |
| coaBC  | SCO containing pGMCK3-OX21-T10 integrated at the L5 attB site                 | This study             |
| coaD   | SCO containing pGMCK3-OX38-T10 integrated at the L5 attB site                 | This study             |
| coaE   | SCO containing pGMCK3-OX21-T10 integrated at the L5 attB site                 | This study             |

**E. coli Strains**

DH5α  
F- y80lacZAM15 Δ(lacZYA-argF)U169 recA1 endA1 hsdR17 (rk-,mk+) phoAsupE44 thi-1 gyrA96 relA1 λ-

**Plasmids**

| Plasmid               | Description                                                                                     | Reference          |
|-----------------------|--------------------------------------------------------------------------------------------------|--------------------|
| pSE100                | E. coli-Mycobacterium shuttle vector carrying P\textsubscript{myc}\textsubscript{1}\textsubscript{tet}\textsubscript{O}; Hyg\textsuperscript{r} | Guo et al., 2007   |
| pGMCK3-OX38-T10       | L5-based integration vector harboring P\textsubscript{myc1}\textsubscript{tetR}; Kan\textsuperscript{r} | Klotzsche et al, 2009 |
| pGMCK3-OX21-T10       | L5-based integration vector harboring P\textsubscript{myc1}\textsubscript{tetR}; Kan\textsuperscript{r} | Klotzsche et al, 2009 |
| pGMCK3-OX38-T28       | L5-based integration vector harboring P\textsubscript{myc1}\textsubscript{tetR} r1.7; Kan\textsuperscript{r} | Klotzsche et al, 2009 |
| pGA-OXP15-intL5       | Suicide plasmid harboring L5 integrase; Amp\textsuperscript{r}                                   | Klotzsche et al, 2009 |
| pPanC-SCO             | Suicide plasmid for generating panC SCO. pSE100 derivative in which the mycobacterial origin of replication was replaced by the first 596 bp of panC | Abrahams et al., 2012 |
| pPanB-SCO             | Suicide plasmid for generating panB SCO. pSE100 derivative in which the mycobacterial origin of replication was replaced by the first 570 bp of panB | This study          |
| pPanE-SCO             | Suicide plasmid for generating panE SCO. pSE100 derivative in which the mycobacterial origin of replication | This study          |
was replaced by the first 522 bp of *panE*

| Plasmid | Description | Reference |
|---------|-------------|-----------|
| **pPanK-SCO** | Suicide plasmid for generating *panK* SCO. pSE100 derivative in which the mycobacterial origin of replication was replaced by the first 627 bp of *panK* | This study |
| **pCoaBC-SCO** | Suicide plasmid for generating *coaBC* SCO. pSE100 derivative in which the mycobacterial origin of replication was replaced by the first 582 bp of *coaBC* | This study |
| **pCoaD-SCO** | Suicide plasmid for generating *coaD* SCO. pSE100 derivative in which the mycobacterial origin of replication was replaced by the first 390 bp of *coaD* | This study |
| **pCoaE-SCO** | Suicide plasmid for generating *coaE* SCO. pSE100 derivative in which the mycobacterial origin of replication was replaced by the first 609 bp of *coaE* | This study |
| Name           | Sequence (5’-3’)                                      | Application (Reference)                                      |
|----------------|------------------------------------------------------|-------------------------------------------------------------|
| **Construction of promoter replacement mutants** |                                                      |                                                             |
| panB-SphI-For  | CGGCATGCACGACCGAATTAGGACAGTG                       | Forward primer used to amplify a non-functional, 5’-fragment of the panB gene |
| panB-NotI-Rev  | TTGGCGGCGCTCATCATTGGCGACGCGA TCGCG                  | Reverse primer used to amplify a non-functional, 5’-fragment of the panB gene |
| panE-SphI-For  | ACGCATGCGAGACGCGACAGACCCCATC                       | Forward primer used to amplify a non-functional, 5’-fragment of the panE gene |
| panE-NotI-Rev  | GTCGGGCGCTCATCGGGCACCTCAGCGCG                     | Reverse primer used to amplify a non-functional, 5’-fragment of the panE gene |
| panK-SphI-For  | CAGCATGCGTCCCTGCAAGACTGACACTGACG                  | Forward primer used to amplify a non-functional, 5’-fragment of the panK gene |
| panK-NotI-Rev  | CTGGCGCCGCTCAGCCGGTGCTGCAAGA GTGTTG               | Reverse primer used to amplify a non-functional, 5’-fragment of the panK gene |
| coaBC-SphI-For | CTGGCATGCGAGTAGCACGGGCCAGGCCTG                     | Forward primer used to amplify a non-functional, 5’-fragment of the coaBC gene |
| coaBC-NotI-Rev | ATGGCGGCGCTCATGCGCCCGAGATCGTAG                    | Reverse primer used to amplify a non-functional, 5’-fragment of the coaBC gene |
| coaD-SphI-For  | AGGCATGCACGCGATGAGAGGAGGA GC                       | Forward primer used to amplify a non-functional, 5’-fragment of the coaD gene |
| coaD-NotI-Rev  | ATGGCGGCGCTCAGTACGCCGACAGGAGCACGTCGCG             | Reverse primer used to amplify a non-functional, 5’-fragment of the coaD gene |
| coaE-SphI-For  | GTGGCATGCTTGCAGGTCGTACGCCGTTGCA CG                 | Forward primer used to amplify a non-functional, 5’-fragment of the coaE gene |
| coaE-NotI-Rev  | GAGCGGCGCTCAATTGGGCCAGGTGCGCG                     | Reverse primer used to amplify a non-functional, 5’-fragment of the coaE gene |
| Gene (ORF) | Primer/Probe Sequence | Notes |
|-----------|-----------------------|-------|
| sigAF     | CGAGCCGATCTCGTTGGA    | Forward primer used in ddPCR analysis of *sigA* |
| sigAR     | TTCGATGAAATCGCAAGCT   | Reverse primer used in ddPCR analysis of *sigA* |
| *sigA*    | ACGAGGGGCAAGC       | *TaqMan MGB probe used in ddPCR analysis of *sigA*; 5’-VIC-labelled |
| panBF     | GAAGCCGAGCGTTTGC     | Forward primer used in ddPCR analysis of *panB* |
| panBR     | AATGGTAAGCTTGCAGCTTATGC | Reverse primer used in ddPCR analysis of *panB* |
| *panB*    | TCGTGATGGAGATGGT     | *TaqMan MGB probe used in ddPCR analysis of *panB*; 5’-FAM-labelled |
| panCF     | CCCGATGCCGCTCAAC     | Forward primer used in ddPCR analysis of *panC* |
| panCR     | GCGCAGAGTTCCGATTCTTCA | Reverse primer used in ddPCR analysis of *panC* |
| *panC*    | TGGTTGCTGCCCAGGC     | TaqMan MGB probe used in ddPCR analysis of *panC*; 5’-FAM-labelled |
| panEF     | GGTGCCGAAGTCTGAGT    | Forward primer used in ddPCR analysis of *panE* |
| panER     | CAGCATCGAGGTGACCA    | Reverse primer used in ddPCR analysis of *panE* |
| *panE*    | ACGTCGTCAGGAAGT      | TaqMan MGB probe used in ddPCR analysis of *panE*; 5’-FAM-labelled |
| panKF     | CCGGATCGAGGACATCGA   | Forward primer used in ddPCR analysis of *panK* |
| panKR     | GGTGCGCATGGCCAAA     | Reverse primer used in ddPCR analysis of *panK* |
| *panK*    | CAGTGGTACGTATCAGGG   | TaqMan MGB probe used in ddPCR analysis of *panK*; 5’-FAM-labelled |
| coaBCF    | TTTCAACCGAGCTAACTG   | Forward primer used in ddPCR analysis of *coaBC* |
| Gene | Forward Primer | Reverse Primer | TaqMan MGB Probe | Notes |
|------|----------------|----------------|-----------------|-------|
| **coaBCFR** | GACGGCATTGACGACTAACAGA | **Reverse primer used in ddPCR analysis of coaBC**<sup>1</sup> | | |
| **coaBC probe** | ACGCAAAGGCTGCG | **TaqMan MGB probe used in ddPCR analysis of coaBC; 5’-FAM-labelled**<sup>1</sup> | | |
| **coaDF** | TTCGTTCTGTCGTGTCCTCACT | **Forward primer used in ddPCR analysis of coaD**<sup>1</sup> | | |
| **coaDR** | CGGTTCCGGGCAGCAACT | **Reverse primer used in ddPCR analysis of coaD**<sup>1</sup> | | |
| **coaD probe** | CCAAAAGAAGTCGCGATGC | **TaqMan MGB probe used in ddPCR analysis of coaD; 5’-FAM-labelled**<sup>1</sup> | | |
| **coaEF** | TCGCGTGCGGGCAATA | **Forward primer used in ddPCR analysis of coaE**<sup>1</sup> | | |
| **coaER** | ACGGCCTTGACCCCAAT | **Reverse primer used in ddPCR analysis of coaE**<sup>1</sup> | | |
| **coaE probe** | CCTTGCAGTTGACCA | **TaqMan MGB probe used in ddPCR analysis of coaE; 5’-FAM-labelled**<sup>1</sup> | | |

<sup>1</sup>Restriction sites are shown in bold; in-frame stop codons are underlined; †All primer/probe combinations were designed downstream of, or flanking, the homologous region in order to ensure that only transcript driven from P<sub>myc1tetO</sub> was quantified; ‡VIC – 4',7,2'-trichloro-7'-phenyl-6-carboxyfluorescein; §FAM – 6-carboxyfluorescein.
Figure S1. Verification of the genotypes of all SCO recombinant strains by Southern hybridization. The probe binding position is indicated by a solid black line.
Figure S2. A schematic representation of the genetic context of all genes investigated in this study.

Figure S3. Exogenous supplementation with P-PantSH is unable to rescue the growth of CoaBC-deficient Mtb on 7H10 agar in 24-well microtitre plates. P-PantSH – 4’-phosphopantetheine; ATc – anhydrotetracycline; PantS – pantethine.
Figure S4. Absolute quantitation of all Pan and CoA biosynthetic genes in Mtb. Total mRNA was extracted from exponentially growing cultures of all strains following 24 hours exposure to ATc, as indicated. All values (copies/ml) are normalized to sigA and data are representative of two biological replicates performed in duplicate, except coaBC, which represents three biological replicates performed in duplicate. Error bars represent standard deviation; SCO, promoter replacement mutant; ATc, anhydrotetracycline (ng/ml).

Figure S5. Absolute quantitation of transcript levels of all Pan and CoA biosynthesis pathway genes in H37Rv. Total mRNA was extracted from exponentially growing cultures of H37Rv and wildtype basal expression levels of each gene were determined. All values (copies/ml) are normalized to sigA and data are representative of two biological replicates performed in duplicate. Error bars indicate the standard deviation (SD).
**Figure S6.** The time-dependent metabolomic profiles of *Mtb* CoA pathway mutant strains. Heat map profile showing intracellular pool size changes of 69 metabolites following ATc-triggered transcriptional silencing. Columns indicate the individual targeted gene in the CoA biosynthesis pathway. D1.5 and D3.0 labels indicate the duration of ATc treatment for each *Mtb* strain. The labels 1, 2, 3 designate biological replicates. Rows show the individual metabolites measured. Data were processed by Pearson’s correlation with centroid linkage clustering in program Cluster 3.0 and visualized using Java TreeView 1.0. Data are shown on log$_2$ scale relative to the without ATc treated control for each mutant strain.
Figure S7. Metabolomic profiles of *Mtb* CoA pathway mutant strains from two independent experiments. Profiles are as described in Figure S6, but taken from 2 independent experiments following 3 days of ATc treatment.
Figure S8. CoaBC is required in order for Mtb to establish an infection in mouse lungs. Mice were infected with coaBC Tet-ONM (a) and Tet-OFF (b) and received food with and without doxycycline starting from the day of infection. The limit of detection was 4 CFU in lungs and spleens. The data are representative of four mice per time point; error bars represent standard deviation (SD).

Figure S9. Depletion of CoaBC results in decreased lung pathology in mice. Lung sections stained with hematoxylin and eosin from mice infected with coaBC Tet-OFF and fed doxy-containing chow starting from the indicated time-points. The magnification of each section is depicted in boxed areas. Scale bar (1 mm).
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