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[1], 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$_S$) and pGMCK3-OX21-T10, which results in intermediate-level expression of TetR (Tet-ON$_M$) 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_{600} ~ 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_{600} ~ 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[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*I and *Not*I, and ligation into the similarly restricted pSE100[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[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[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[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 treatement 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 H_2O 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, P_{myc1}*tetO*, 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 $E = 10^{[-1/slope]}$, 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 ≥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_2O 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 *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.

TABLES

Table S1. Droplet digital PCR analysis of transcript levels in all conditional mutants

Gene	*Mtb* Strain	*Concentration of transcript normalised to *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-ON$_M$ +	465.0	NA	NA
	panB Tet-ON$_M$ -	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-ON$_M$ +	109.5	NA	NA
	panC Tet-ON$_M$ -	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-ON$_M$ +	127.0	NA	NA
	panE Tet-ON$_M$ -	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-ON$_M$ +	90.7	NA	NA
	panK Tet-ON$_M$ -	55.5	NA	46.3
	panK Tet-OFF +	51.2	NA	50.4
	panK Tet-OFF -	91.6	NA	NA

coaBC	H37Rv	341.8	NA	NA
	coaBC-SCO	357.0	+ 1.0	NA
	coaBC Tet-ON$_M$ +	401.8	NA	NA
	coaBC Tet-ON$_M$ -	39.8	NA	88.3
	coaBC Tet-OFF +	61.7	NA	81.9
	coaBC Tet-OFF -	371.0	NA	NA
coaD	H37Rv	103.4	NA	NA
	coaD-SCO	1655.0	+ 16.0	NA
	coaD Tet-ON$_S$ +	1735.0	NA	NA
	coaD Tet-ON$_S$ -	36.5	NA	64.7
	coaD Tet-ON$_M$ +	1997.5	NA	NA
	coaD Tet-ON$_M$ -	374.0	NA	ND
	coaD Tet-OFF +	105.5	NA	ND
	coaD Tet-OFF -	1680.0	NA	NA
coaE	H37Rv	546.0	NA	NA
	coaE-SCO	278.3	- 2.0	NA
	coaE Tet-ON$_M$ +	155.5	NA	NA
	coaE Tet-ON$_M$ -	84.3	NA	84.6
	coaE Tet-OFF +	115.6	NA	78.8
	coaE Tet-OFF -	287.0	NA	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_{myc1}*tetO*	Abrahams et al., 2012
panB SCO	H37RvMA derivative in which expression of *panB* is controlled by P_{myc1}*tetO*	This study
panE SCO	H37RvMA derivative in which expression of *panE* is controlled by P_{myc1}*tetO*	This study
panK SCO	H37RvMA derivative in which expression of *panK* is controlled by P_{myc1}*tetO*	This study
coaBC SCO	H37RvMA derivative in which expression of *coaBC* is controlled by P_{myc1}*tetO*	This study
coaD SCO	H37RvMA derivative in which expression of *coaD* is controlled by P_{myc1}*tetO*	This study
coaE SCO	H37RvMA derivative in which expression of *coaE* is controlled by P_{myc1}*tetO*	This study
panC Tet-ON_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_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_S	*panE* SCO containing pGMCK3-OX38-T10 integrated at the L5 *attB* site	This study
panE Tet-ON_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_S	*panK* SCO containing pGMCK3-OX38-T10 integrated at the L5 *attB* site	This study
panK Tet-ON_M	*panK* SCO containing pGMCK3-OX21-T10 integrated at the L5 *attB* site	This study

panK Tet-OFF	*panK* SCO containing pGMCK3-OX38-T28 integrated at the L5 *attB* site	This study
coaBC Tet-ON_M	*coaBC* SCO containing pGMCK3-OX21-T10 integrated at the L5 *attB* site	This study
coaBC Tet-OFF	*coaBC* SCO containing pGMCK3-OX38-T28 integrated at the L5 *attB* site	This study
coaD Tet-ON_S	*coaD* SCO containing pGMCK3-OX38-T10 integrated at the L5 *attB* site	This study
coaD Tet-ON_M	*coaD* SCO containing pGMCK3-OX21-T10 integrated at the L5 *attB* site	This study
coaD Tet-OFF	*coaD* SCO containing pGMCK3-OX38-T28 integrated at the L5 *attB* site	This study
coaE Tet-ON_M	*coaE* SCO containing pGMCK3-OX21-T10 integrated at the L5 *attB* site	This study
coaE Tet-OFF	*coaE* SCO containing pGMCK3-OX38-T28 integrated at the L5 *attB* site	This study
E. coli Strains		
DH5α	F- *ψ80lacZΔM15* Δ(*lacZYA-argF*)U169 *recA1 endA1 hsdR17*(rk-,mk+) *phoAsupE44 thi*-1 *gyrA96 relA1* λ-	Invitrogen
Plasmids		
pSE100	*E. coli-Mycobacterium* shuttle vector carrying P_{myc1}*tetO*; Hyg^r	Guo et al., 2007
pGMCK3-OX38-T10	L5-based integration vector harboring P_{smyc}-tetR; Kan^R	Klotzsche et al, 2009
pGMCK3-OX21-T10	L5-based integration vector harboring P_{imyc}-tetR; Kan^R	Klotzsche et al, 2009
pGMCK3-OX38-T28	L5-based integration vector harboring P_{smyc}-tetR r1.7; Kan^R	Klotzsche et al, 2009
pGA-OXP15-intL5	Suicide plasmid harboring L5 integrase; Amp^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*	
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

Table S3. Oligonucleotides used in this study

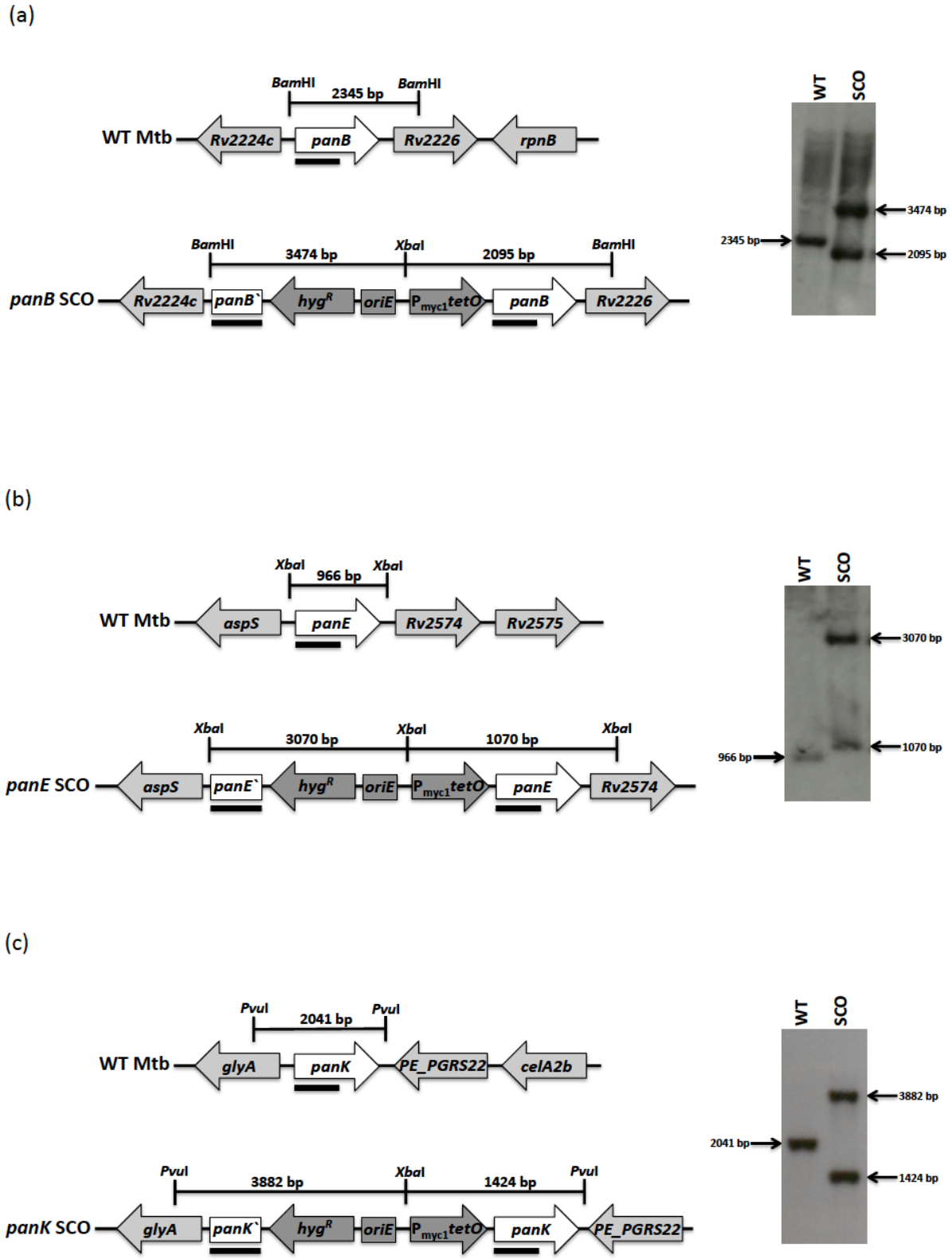
Name	*Sequence (5`-3`)	Application (Reference)
Construction of promoter replacement mutants		
panB-SphI-For	CG**GCATGC**CGACCACCATTAGGGACAG TG	Forward primer used to amplify a non-functional, 5'-fragment of the *panB* gene
panB-NotI-Rev	TT**GCGGCCGC**TCATTCGGCGACGGCGA TCGCG	Reverse primer used to amplify a non-functional, 5'-fragment of the *panB* gene
panE-SphI-For	AC**GCATGC**AGACGGCGCAGACCCCATC	Forward primer used to amplify a non-functional, 5'-fragment of the *panE* gene
panE-NotI-Rev	GT**GCGGCCGC**TCATCGGGCACCCTCAG CGCG	Reverse primer used to amplify a non-functional, 5'-fragment of the *panE* gene
panK-SphI-For	CA**GCATGC**GTCCCTGCAAGACTGACAC C	Forward primer used to amplify a non-functional, 5'-fragment of the *panK* gene
panK-NotI-Rev	CT**GCGGCCGC**TCAGCCGGTCTGCAAGA CGTTG	Reverse primer used to amplify a non-functional, 5'-fragment of the *panK* gene
coaBC-SphI-For	CT**GCATGC**GAGTAGCAGGGCAGGCCTG	Forward primer used to amplify a non-functional, 5'-fragment of the *coaBC* gene
coaBC-NotI-Rev	AT**GCGGCCGC**TCATCGCCCCGCGAGAT CGTAG	Reverse primer used to amplify a non-functional, 5'-fragment of the *coaBC* gene
coaD-SphI-For	AG**GCATGC**ACGCAGCGATGAGGAGGA GC	Forward primer used to amplify a non-functional, 5'-fragment of the *coaD* gene
coaD-NotI-Rev	AT**GCGGCCGC**TCAGTCACCGCCCAGCA TCGCG	Reverse primer used to amplify a non-functional, 5'-fragment of the *coaD* gene
coaE-SphI-For	GT**GCATGC**TTGCAGCTGATCGCGTTCA CG	Forward primer used to amplify a non-functional, 5'-fragment of the *coaE* gene
coaE-NotI-Rev	GA**GCGGCCGC**TCATTGGGCCAGGTTGT GCGCG	Reverse primer used to amplify a non-functional, 5'-fragment of the *coaE* gene

[†]ddPCR analysis of conditional knockdowns		
sigAF	CGAGCCGATCTCGTTGGA	Forward primer used in ddPCR analysis of *sigA*
sigAR	TTCGATGAAATCGCCAAGCT	Reverse primer used in ddPCR analysis of *sigA*
sigA probe	ACGAGGGCGACAGC	[‡]TaqMan MGB probe used in ddPCR analysis of *sigA*; 5'-VIC-labelled
panBF	GAAGCCGGAGCGTTTGC	Forward primer used in ddPCR analysis of *panB*
panBR	AATGGTAAGCTTGCCGGTGAT	Reverse primer used in ddPCR analysis of *panB*
panB probe	TCGTGATGGAGATGGT	[§]TaqMan MGB probe used in ddPCR analysis of *panB*; 5'-FAM-labelled
panCF	CCCGATGCCGCTCAAC	Forward primer used in ddPCR analysis of *panC*
panCR	GGCGAAAGTTCCGATTTCAA	Reverse primer used in ddPCR analysis of *panC*
panC probe	TGGTTGCTGCCCGGC	TaqMan MGB probe used in ddPCR analysis of *panC*; 5'-FAM-labelled
panEF	GGTGCCCGACTCGATGA	Forward primer used in ddPCR analysis of *panE*
panER	CAGCATCGAGGTGCCCA	Reverse primer used in ddPCR analysis of *panE*
panE probe	ACGTCGTCGACGAAGT	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 probe	CAGTGGTACGTATCACGG	TaqMan MGB probe used in ddPCR analysis of *panK*; 5'-FAM-labelled
coaBCF	TTTCATGCCCGAGCTAAACTG	Forward primer used in ddPCR analysis of *coaBC*

coaBCFR	GACGGCATTGACGACTAACAGA	Reverse primer used in ddPCR analysis of *coaBC*
coaBC probe	ACGCAAAGGCTGCG	TaqMan MGB probe used in ddPCR analysis of *coaBC*; 5'-FAM-labelled
coaDF	TTCGTTCGTGTCGTCCTCACT	Forward primer used in ddPCR analysis of *coaD*
coaDR	CGGTTCGGGCAGCAACT	Reverse primer used in ddPCR analysis of *coaD*
coaD probe	CCAAAGAAGTCGCGATGC	TaqMan MGB probe used in ddPCR analysis of *coaD*; 5'-FAM-labelled
coaEF	TCGCGTGCGGGCATA	Forward primer used in ddPCR analysis of *coaE*
coaER	ACGGCGGTTGACCCAAT	Reverse primer used in ddPCR analysis of *coaE*
coaE probe	CCTTGCGAGTTGACC	TaqMan MGB probe used in ddPCR analysis of *coaE*; 5'-FAM-labelled

[*]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_{myc1}*tetO* was quantified; [‡]VIC – 4,7,2'-trichloro-7'-phenyl-6-carboxyfluorescein; [§]FAM – 6-carboxyfluorescein.

FIGURES


(a)
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BamHI
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Rv2224c
panB
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rpnB
BamHI
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BamHI
panB SCO
Rv2224c
panB`
hygR
oriE
Pmyc1tetO
panB
Rv2226
WT
SCO
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966 bp
XbaI
WT Mtb
aspS
panE
Rv2574
Rv2575
XbaI
3070 bp
XbaI
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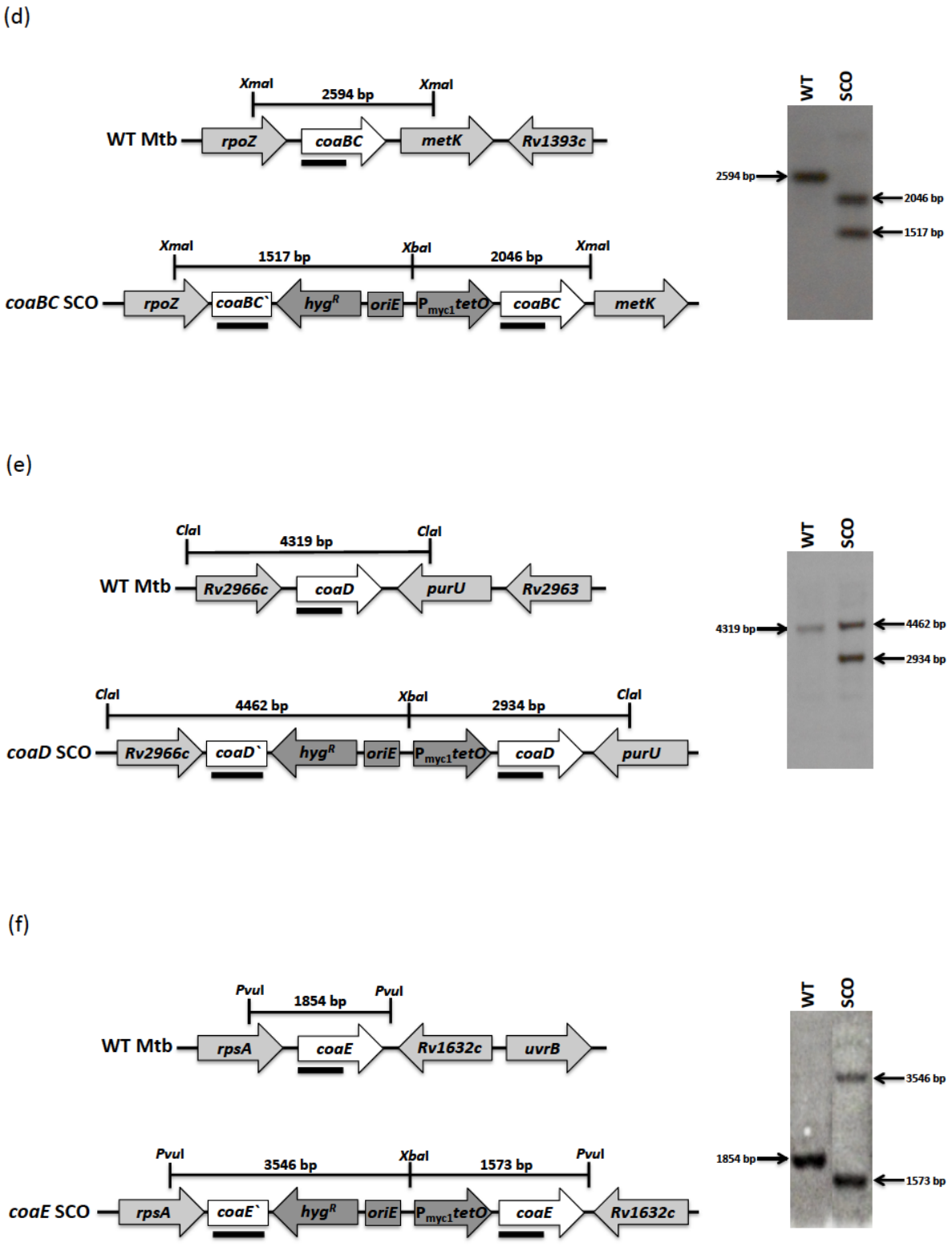

Figure S1. Verification of the genotypes of all SCO recombinant strains by Southern hybridization. The probe binding position is indicated by a solid black line.

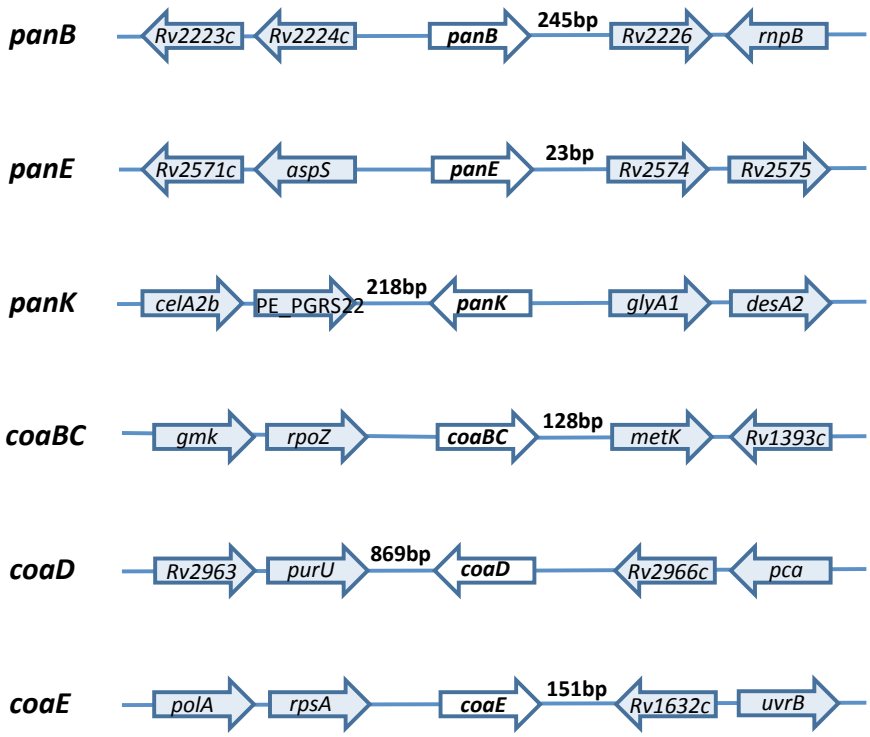

Figure S2. A schematic representation of the genetic context of all genes investigated in this study.

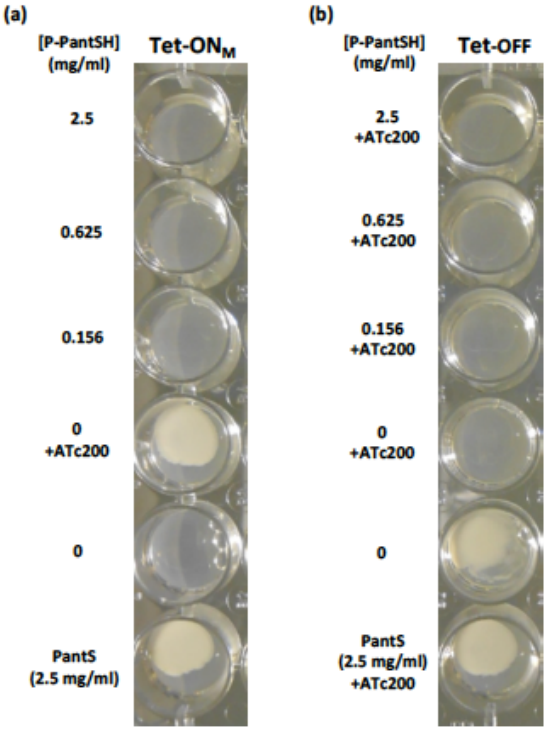

Figure S3. Exogenous supplementation with P-PantSH is unable to rescue the growth of CoaBC-deficient *Mtb* on 7H10 agar in 24-well microtitre plates. P-PantSH – 4`-phsphopantetheine; ATc – anhydrotetracycline; PantS – pantethine.

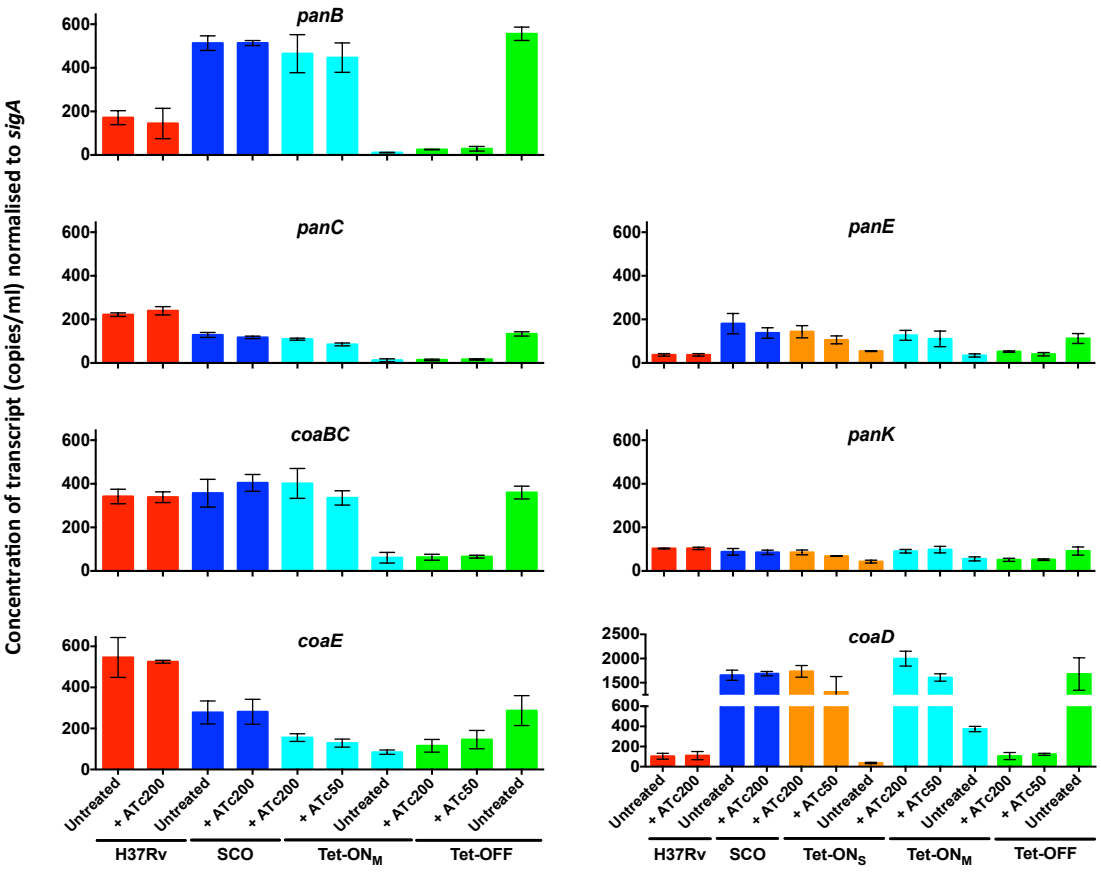

Figure S4. Absolute quantitation of all Pan and CoA biosynthetic genes in *Mtb*. Total mRNA was extracted from exponentially growing cultures of all strains following 24 hours exposure to ATc, as indicated. All values (copies/ml) are normalized to *sigA* and data are representative of two biological 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).

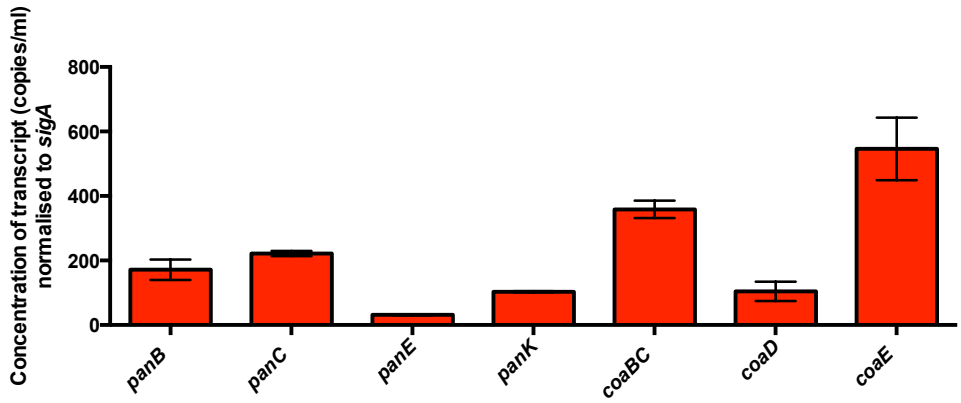

Figure S5. Absolute quantitation of transcript levels of all Pan and CoA biosynthesis pathway genes in H37Rv. Total mRNA was extracted from exponentially growing cultures of H37Rv and wildtype basal expression levels of each gene were determined. All values (copies/ml) are normalized to *sigA* and data are representative of two biological replicates performed in duplicate. Error bars indicate the standard deviation (SD).

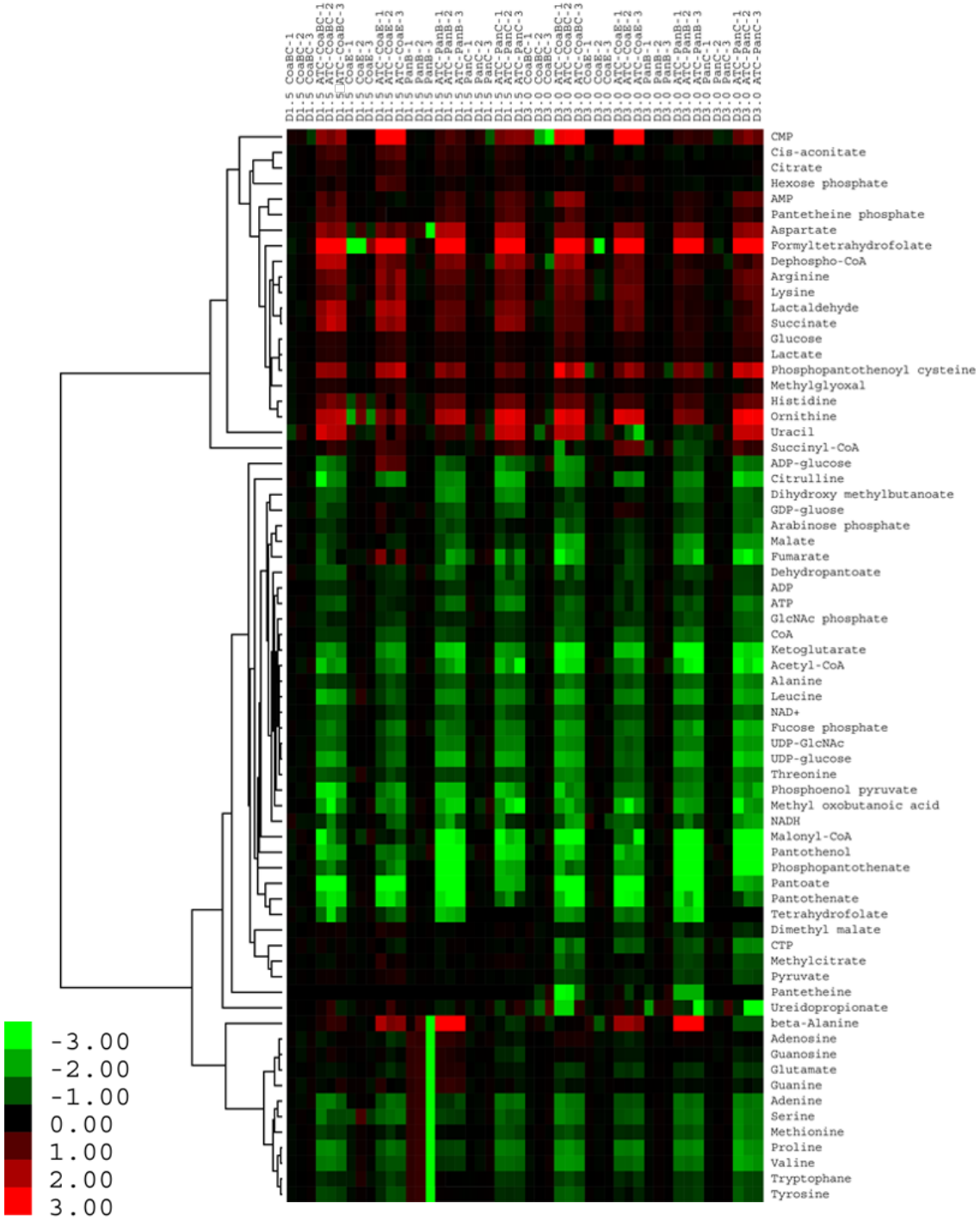

Figure S6. The time-dependent metabolomic profiles of *Mtb* CoA pathway mutant strains. Heat map profile showing intracellular pool size changes of 69 metabolites following ATc-triggered transcriptional silencing. Columns indicate the individual targeted gene in the CoA biosynthesis pathway. D1.5 and 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.

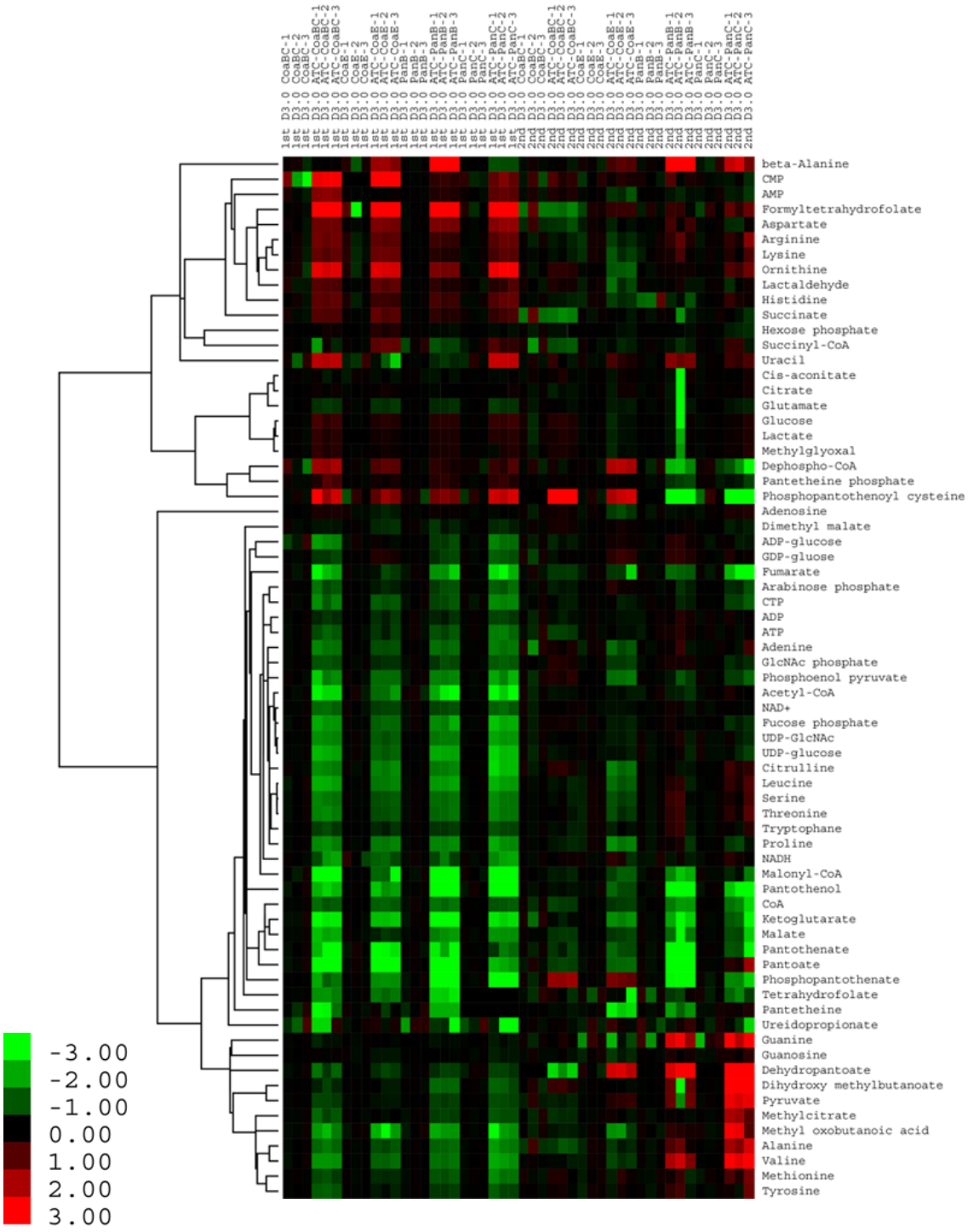

Figure S7. Metabolomic profiles of *Mtb* CoA pathway mutant strains from two independent experiments. Profiles are as described in Figure S6, but taken from 2 independent experiments following 3 days of ATc treatment.

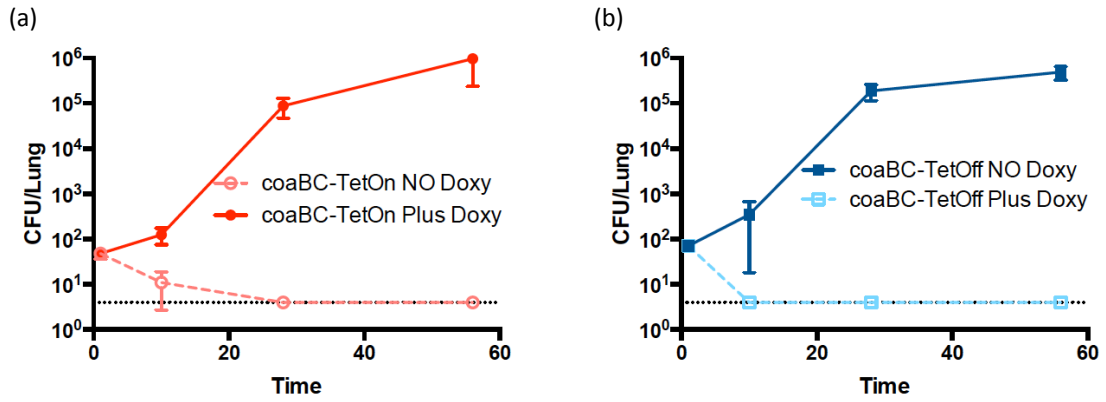

Figure S8. CoaBC is required in order for *Mtb* to establish an infection in mouse lungs. Mice were infected with *coaBC* Tet-ON$_M$ (a) and Tet-OFF (b) and received food with and without doxycycline starting from the day of infection. The limit of detection was 4 CFU in lungs and spleens. The data are representative of four mice per time point; error bars represent standard deviation (SD).

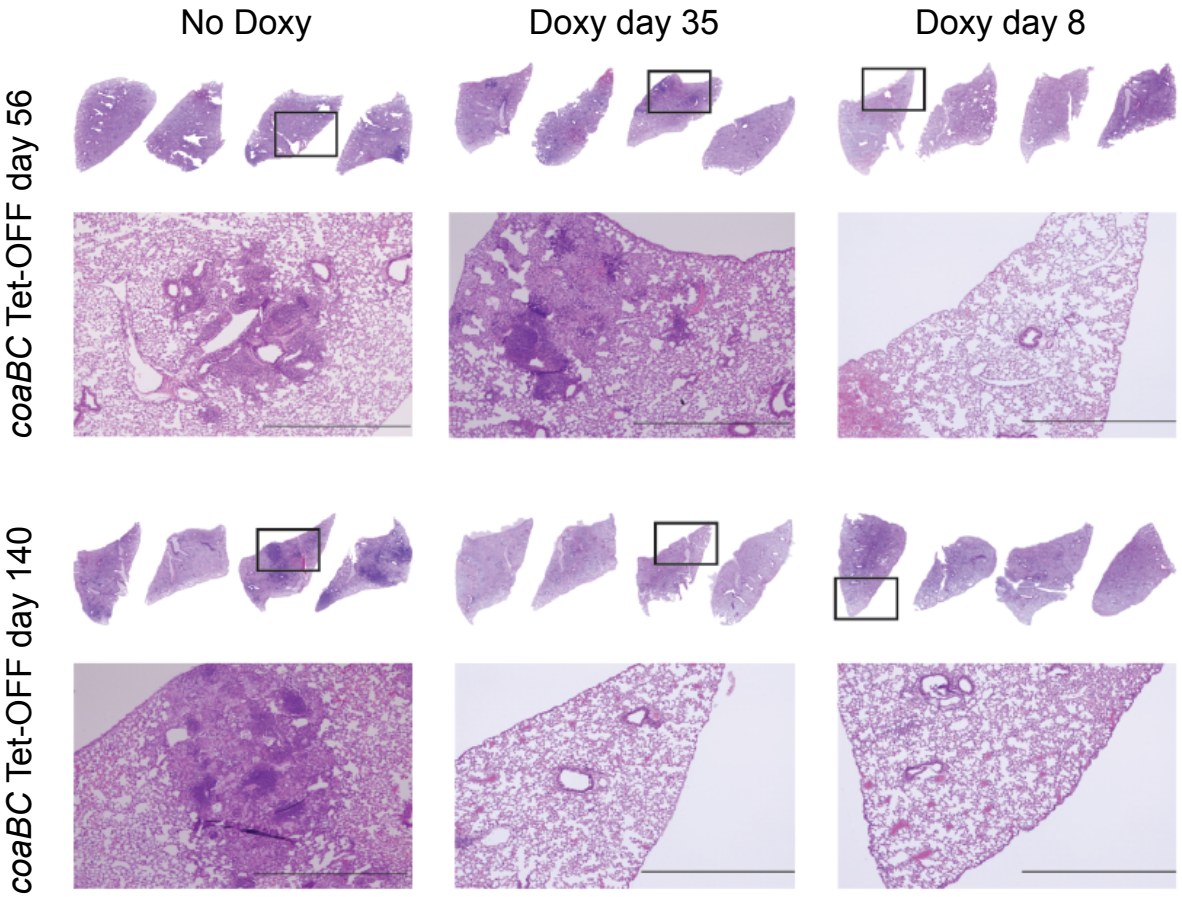

Figure S9. Depletion of CoaBC results in decreased lung pathology in mice. Lung sections stained with hematoxylin and eosin from mice infected with *coaBC* Tet-OFF and fed doxy-containing chow starting from the indicated time-points. The magnification of each section is depicted in boxed areas. Scale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

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