Supporting Information for

The Reversible Acetylation and Inactivation of *Mycobacterium tuberculosis* Acetyl-CoA Synthetase is Dependent on cAMP †

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Table S1: Oligonucleotides

Name	Sequence [a]
*Ms*Pat_F	5' ggaattc*catatg*gcggaactgaccgaggttc 3'
*Ms*Pat_R	5' atagttta*gcggccgcc*tggctcaccgcccggatc 3'
Rv0998_F	5’ ggaattc*catatg*ttggacgggatagccgaat 3’
Rv0998_R	5’ cgc*ggatcc*gccgacggcctcgatcactt 3’
Rv1151c_F	5' atcccgct*catatg*cgagtggcggtgctcagc 3'
Rv1151c_R	5' atcccgct*ctcgag*ctatttcagcagggcgggcag 3'
*Ms*ACS_F	5’ ggaattc*catatg*tgctgcgcgatctggag 3’
*Ms*ACS_R	5’ ccc*aagctt*ctacttgctcgcgcggatca 3’
*Mt*ACS_F	5' ggaattc*catatg*agtgagtccacccccgaag 3'
*Mt*ACS_R	5' ccc*aagctt*ctacttggcggcccggatcgc 3'
*Mt*ACS K617A_F	5' gactcgtagcggcgcaatcatgcgtcgac 3'
*Mt*ACS K617A_R	5' gtcgacgcatgattgcgccgctacgagtc 3'

[a] Restriction sites are italicized, while mutation sites are indicated in underline.

Table S2: Mass spectrometric analysis of the substrate of *Ms*Pat

GI: 118467355
acetyl-coenzyme A synthetase [*Mycobacterium smegmatis* str. mc^2 155]
16 unique peptides, 20 unique spectra, 41 total spectra, 200/940 amino acids (21% coverage)

Peptide Sequence	Probability	Modifications	Observed	Actual Mass	z	Delta AMU
AANALTDLGLVAGDR	95%		729.07	1,456.12	2	0.35
AASLKEAVDEAVADQPSVK	95%		964.70	1,927.39	2	0.40
ASAHGGPENMIEELR	95%		805.59	1,609.17	2	-0.58
EAVDEAVADQPSVK	95%		729.19	1,456.36	2	-0.34
ELGDTSTLVDPSVFEAIR	95%		975.24	1,948.47	2	0.49
FAEQGWYFAGDGAR	95%		787.76	1,573.51	2	-0.19
IDDVMNVSGHR	95%		414.48	1,240.43	3	-1.15
LLGSVGEPINPEAWR	95%		819.78	1,637.54	2	0.69
INVAYNcVDR	95%	Carbamidomethylation (Cys)	612.22	1,222.42	2	-0.16
LVITSDGQYR	95%		576.24	1,150.47	2	-0.13
PASHDLSSLR	95%		542.02	1,082.02	2	0.47
SITYAELKDEVcK	95%	Carbamidomethylation (Cys)	778.51	1,555.01	2	0.25
VAIHWEGEPVGDAR	95%		512.25	1,533.73	3	-1.02
WYREHIGGGK	95%		601.76	1,201.50	2	-0.10
YDSDGHIWVLGR	95%		709.63	1,417.24	2	0.57
YGVTIYYTAPTLIR	95%		816.15	1,630.28	2	0.40

Figure S1: Sequence alignment of protein acetyltransferases in mycobacteria.

Multiple sequence alignment of MSMEG_5458 (*Ms*Pat) from *M. smegmatis* and Rv0998 (*Mt*Pat) from *M. tuberculosis* was performed using ClustalW. The two proteins exhibit 56% sequence identity and 70% similarity.

Figure S2: SDS-PAGE analysis of recombinant proteins.

Lane 1: wild type *Mt*ACS; lane 2: *Ms*Pat purified under native conditions; lane 3: *Mt*ACS K617A; lane 4, renatured *Ms*Pat (apo *Ms*Pat); lane 5, Rv1151c; lane 6: protein ladder.

Figure S3: Characterization of the filtrate after denaturing MSMEG_5458.

(A) UV-Visible spectrum of the filtrate. (B) HPLC analysis of the filtrate. The chromatograms of the filtrate (solid line), cAMP standard (dotted line) and 5'-AMP (dashed line) are shown.

Figure S4: cAMP Standard Curve.

Various amounts of cAMP were analyzed by reverse phase HPLC using Phenomenex Gemini-C18 column (250 mm × 4.6 mm). The peak area was plotted against the amount of cAMP.

Figure S5: Sequence alignment of sirtuin-like deacetylases.

Multiple sequence alignment of CobB from *S. enterica*, MSMEG_4620, MSMEG_5175 and Rv1151c was performed using ClustalW. Rv1151c is only homologue of CobB in *M. tuberculosis*, which is 33% identical to CobB. The two CobB homologues in *M. smegmatis*, MSMEG_5175 and MSMEG_4620 share 36% and 24% sequence identity to CobB, respectively.

Figure S1: Sequence alignment of protein acetyltransferases in mycobacteria.

```
MsPat           ----MAELTEVRAADLAALEFFTGCRPSALEPLATQLRPLKAEPGQVLIRQGDPALTFML 56
Rv0998          -LDGIAELTGARVEDLAGMDVFQGCPAEGLVSLAASVQPLRAAAGQVLLRQGEPAVSFLL 59
                    :**** .*. ***.::.* ** ...* .**:.::**:* .****:***:**::*:*

MsPat           I-ESGRVQVSHAVADGPPIVLDIEPGLIIGEIALLRDAPRTATVVAAEPVIGWVGDRDAF 115
Rv0998          I-SSGSAEVSHVGDDGVAIIARALPGMIVGEIALLRDSPRSATVTTIEPLTGWTGGRGAF 118
                * .** .:***.  ** .*:    **:*:********:**:***.: **: **.*.*.**

MsPat           DT-ILHLPGMFDRLVRIARQRLAAFITPIPVQVRTGEWFYLRPVLPGDVERTLNGPVEFS 174
Rv0998          AT-MVHIPGVGERLLRTARQRLAAFVSPIPVRLADGTQLMLRPVLPGDRERTVHGHIQFS 177
                 * ::*:**: :**:* ********::****::  *  : ******** ***::* ::**

MsPat           SET-LYRRFQSVRKPTRALLEYLFEVDYADHFVWVMTEGALGPVIADARFVREGHNATMA 233
Rv0998          GET-LYRRFMSARVPSPALMHYLSEVDYVDHFVWVVTDGSD--PVADARFVRDETDPTVA 234
                .** ***** *.* *: **:.** ****.******:*:*:    :*******:  :.*:*

MsPat           EVAFTV-GDDYQGRGIGSFLMGALIVSANYVGVQRFNARVLTDNMAMRKIMDRLGAVWVR 292
Rv0998          EIAFTV-ADAYQGRGIGSFLIGALSVAARVDGVERFAARMLSDNVPMRTIMDRYGAVWQR 293
                *:**** .* **********:*** *:*.  **:** **:*:**:.**.**** **** *

MsPat           EDLGVVM-TEVDVPPVDTVPFEPELIDQIRDATRKVIRAVSQ 333
Rv0998          EDVGVIT-TMIDVPGPGELSLGREMVDQINRVARQVIEAVG- 333
                **:**:  * :***  . :.:  *::***. .:*:**.**.
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Figure S2: SDS-PAGE analysis of recombinant proteins.

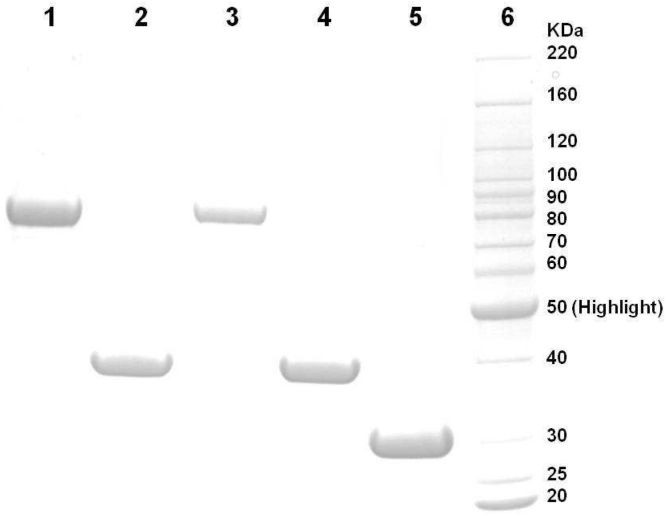

Figure S3: Characterization of the filtrate after denaturing MSMEG_5458.

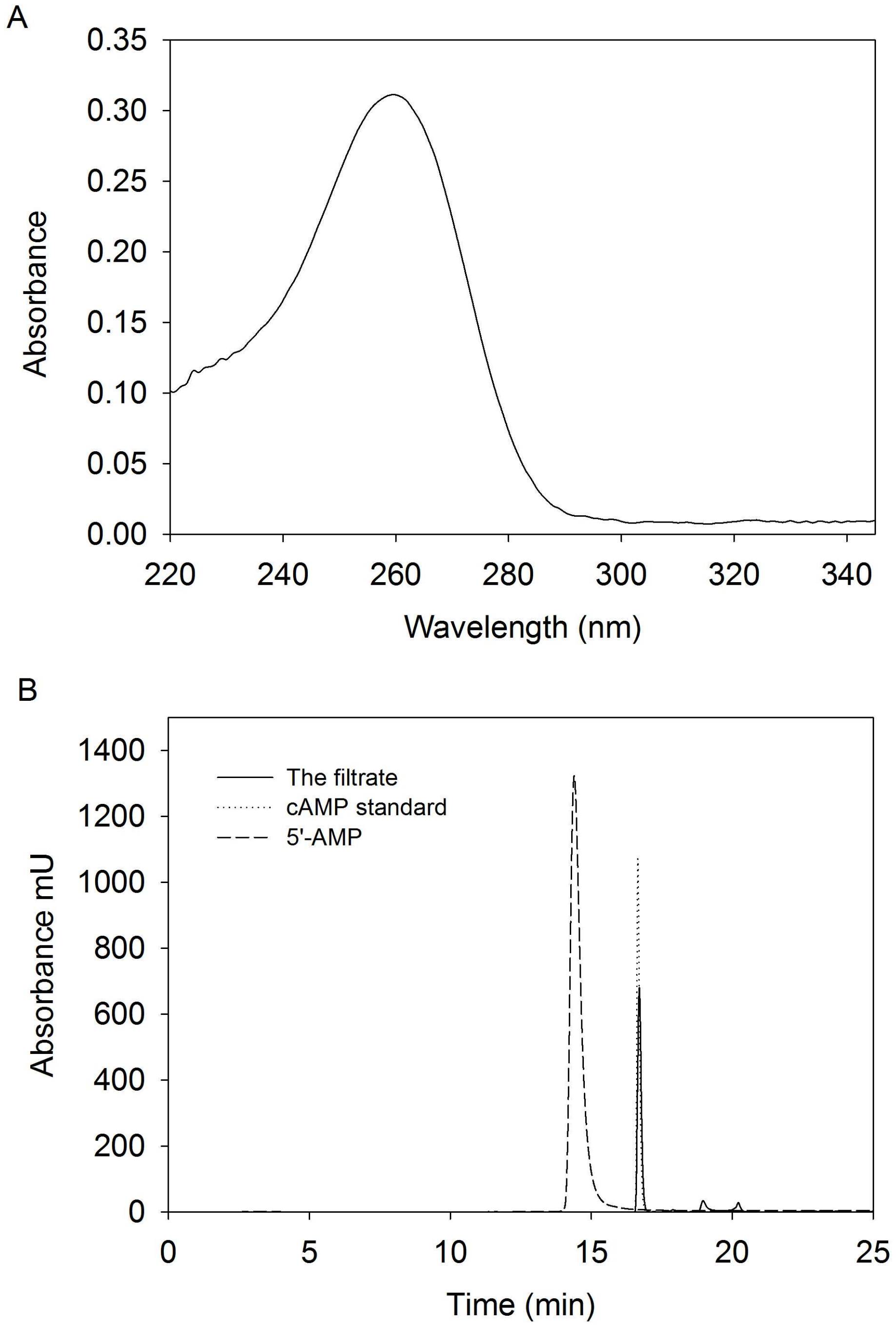

Figure S4: cAMP standard curve.

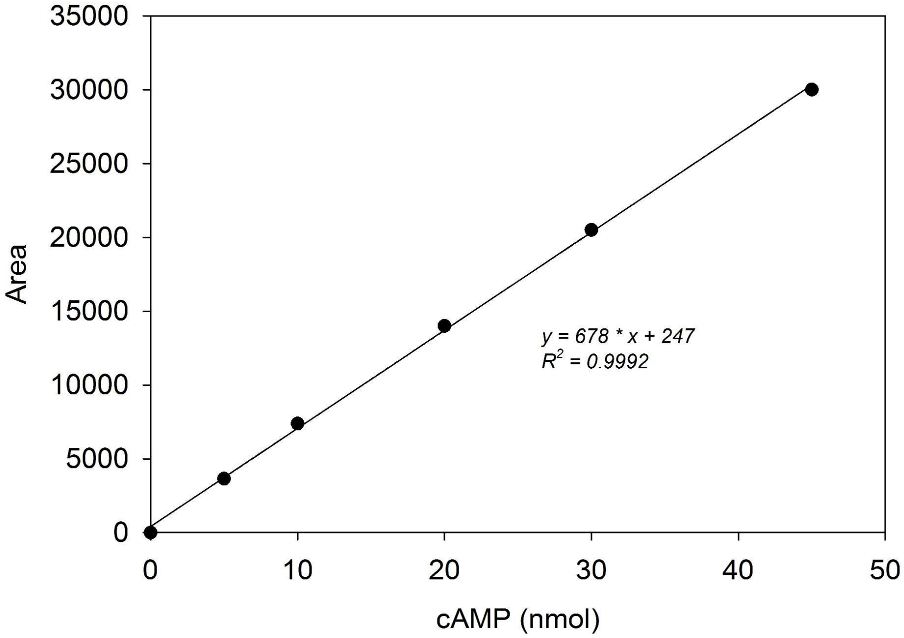

Figure S5: Sequence alignment of sirtuin-like deacetylases.

```
Rv1151c         ----------------------------------------MRVAVLSGAGISAESGVPTF 20
MSMEG5175       ----------------------------------------MQVTVLSGAGISAESGVPTF 20
cobB            MQSRRFHRLSRFRKNKRLLRERLRQRIFFRDRVVPEMMENPRVLVLTGAGISAESGIRTF 60
MSMEG4620       ----------------------------MDAPELVALLQGRRIVALTGAGMSTDSGIPDY 32
                                                         :: .*:***:*::**:  :

Rv1151c         RDDKNGLWARFDPYELSSTQGWLRNPERVWGWYLWRHYLVAN--VEPNDGHRAIAAWQDH 78
MSMEG5175       RDAETGLWAQVDPYEISSTDGWQRNPEKVWAWYLWRHYMMAR--VAPNEAHRTVAAWEDH 78
cobB            R-AADGLWEEHRVEDVATPEGFARNPGLVQTFYNARRQQLQQPEIQPNAAHLALAKLEEA 119
MSMEG4620       RGPDSPPSNPMTIQQFTSDP-VFRQRYWARNHVGWRHMDETQ----PNAGHRALAAMEAS 87
                *             :.::     *:   .      *:    .    ** .* ::*  :

Rv1151c         AEVS--VITQNVDDLHERAGSGAVHHLHGSLFEFRCA-----------RCGVPYTDALP- 124
MSMEG5175       LDVR--VVTQNIDDLHERAGSTNVYHLHGSLFEFRCD-----------ACGSAFEGNLP- 124
cobB            LGDRFLLVTQNIDNLHERAGNRNIIHMHGELLKVRCS-----------QSGQILEWNGD- 167
MSMEG4620       GVVAG-VITQNVDLLHTKAGSREVINLHGTYAQVVCLNPDCGHTMSRAALAVMLEEANPG 146
                      ::***:* ** :**.  : ::**   :. *              .

Rv1151c         -------------------EMPEPAIEVEPPVCDCGGLIRPDIVWFGEPLPEEPWRSAVE 165
MSMEG5175       -------------------EMPEPVETIDPPVCPCSGLIRPSVVWFGEPLPDAAWNRSVL 165
cobB            -------------------VMPE----DKCHCCQFPAPLRPHVVWFGE-MP-LGMDEIYM 202
MSMEG4620       FLARAESVGGIAVAPDADAMITDTASFVVVDCPMCGGMLKPDIVYFGDSVPKTRVEQAYS 206
                                    :.:             . ::* :*:**: :*

Rv1151c         ATGSADVMVVVGTSAIVYPAAGLPDLALARGTAVIEVNPEPTPLSGSATISIRESASQAL 225
MSMEG5175       AVSSADVVIVVGTSSIVYPAAGLPEAALAAGKPVIEVNPERTPLSDSATVSLRETASEAL 225
cobB            ALSMADIFIAIGTSGHVYPAAGFVHEAKLHGAHTVELNLEPSQVGSEFEEKHYGPASQVV 262
MSMEG4620       LVDSADALLVAGSSLTVFSGYRFVRHAAARGIPVGIVNRGPTRGDDLAAVKVHSGCSEML 266
                  . ** .:. *:*  *:..  :   *   *  .  :*   :  ..    .    .*: :

Rv1151c         PGLLERLPALLK---- 237
MSMEG5175       PTLLQRLPELLNRSA- 240
cobB            PEFVDKFLKGL----- 273
MSMEG4620       TLLAGELTRTYTASPG 282
                . :  .:
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