SUPPLEMENTAL FIGURES AND TABLES

Biosynthesis and regulation of sulfomenaquinone, a metabolite associated with virulence in *Mycobacterium tuberculosis**

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FIGURE S1: Mass spectra of TLE from *M. smegmatis* strains expressing SMK biosynthetic genes showing region m/z 880-886.

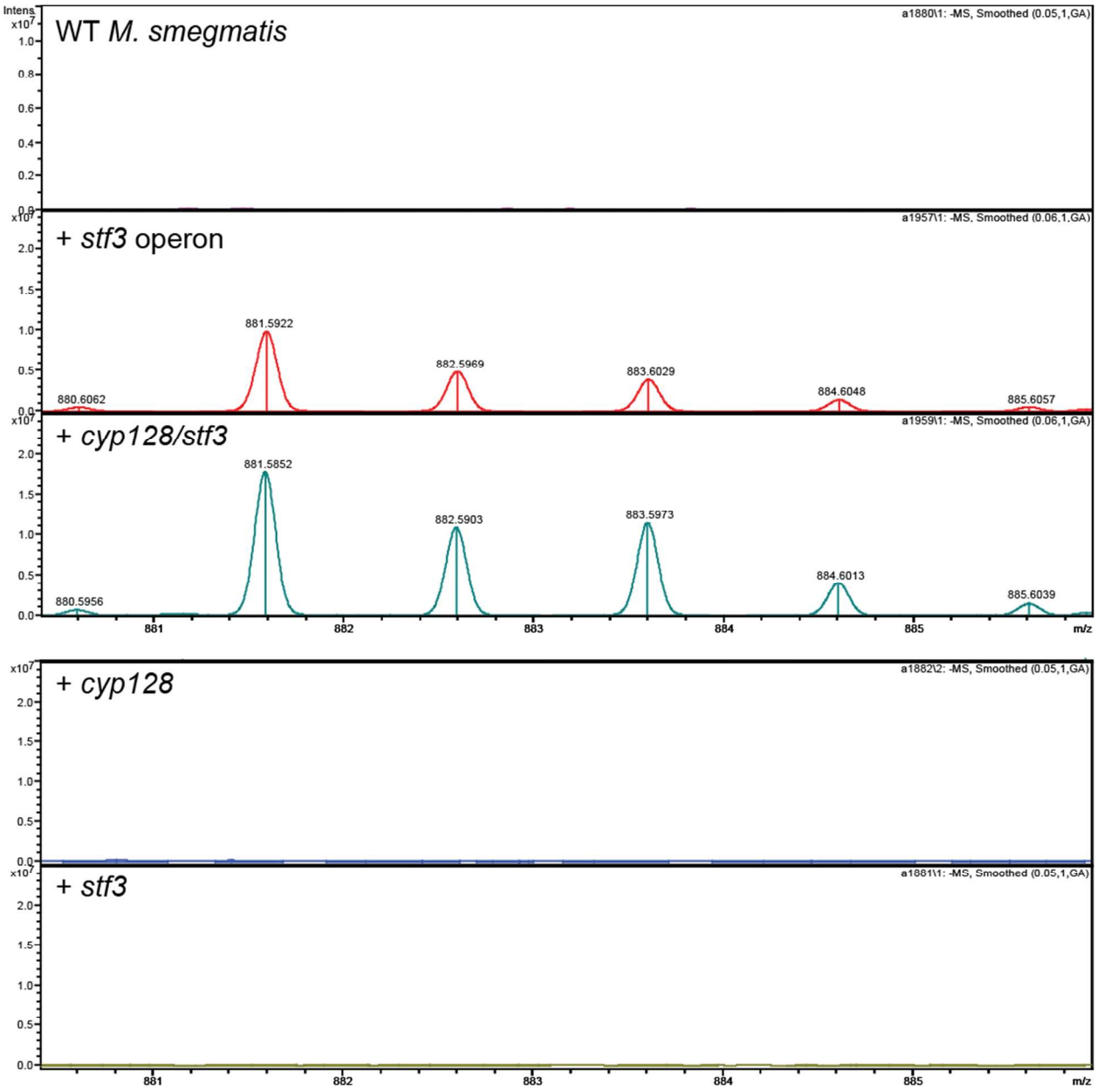

FIGURE S2: DNA gel from WT *M. tuberculosis* and Δ*cyp128* using primers for either *cyp128* or *hygromycin*.

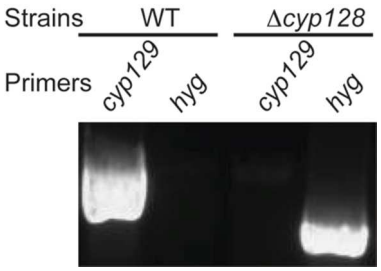

FIGURE S3: A) TLC analysis of TLE from *M. tuberculosis* strains grown on ^{35}S-sulfate: (I) WT, (II) Δ*cyp128*, (III) Δ*cyp128*::*cyp128*, (IV) Δ*stf3*, (V) Δ*stf3*::*stf3.* Arrow indicates spot corresponding to SMK. B) Growth of Δ*cyp128* and complement compared to WT in 7H9 liquid media.

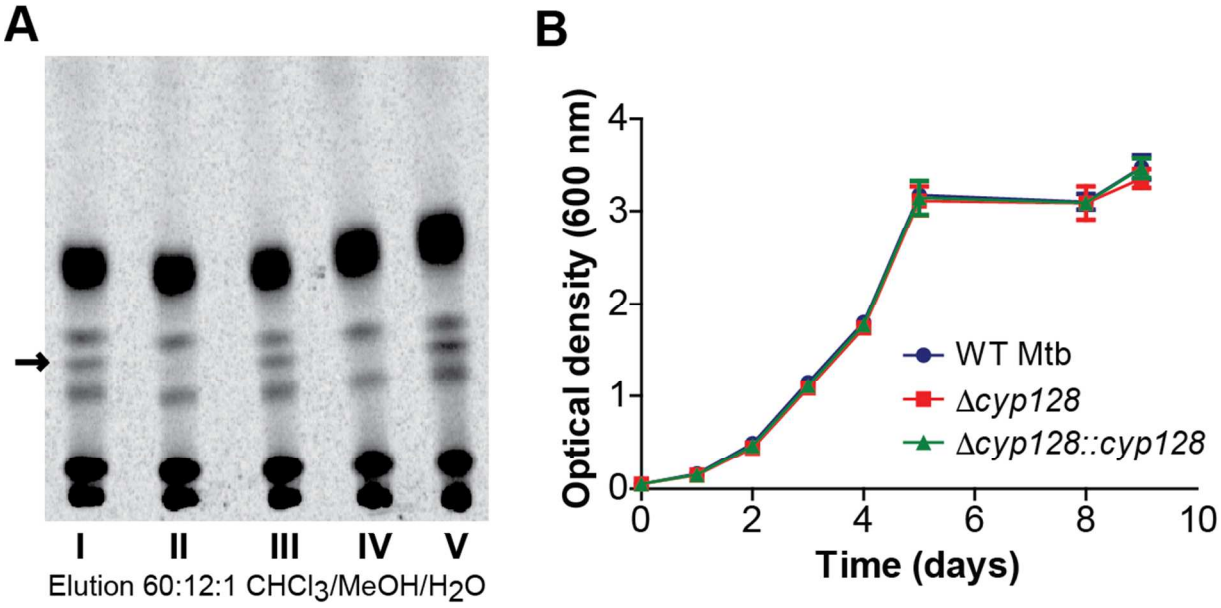

FIGURE S4: Mass spectra from TLE of *M. tuberculosis* WT and SMK deletion mutants with region m/z 880-886 shown.

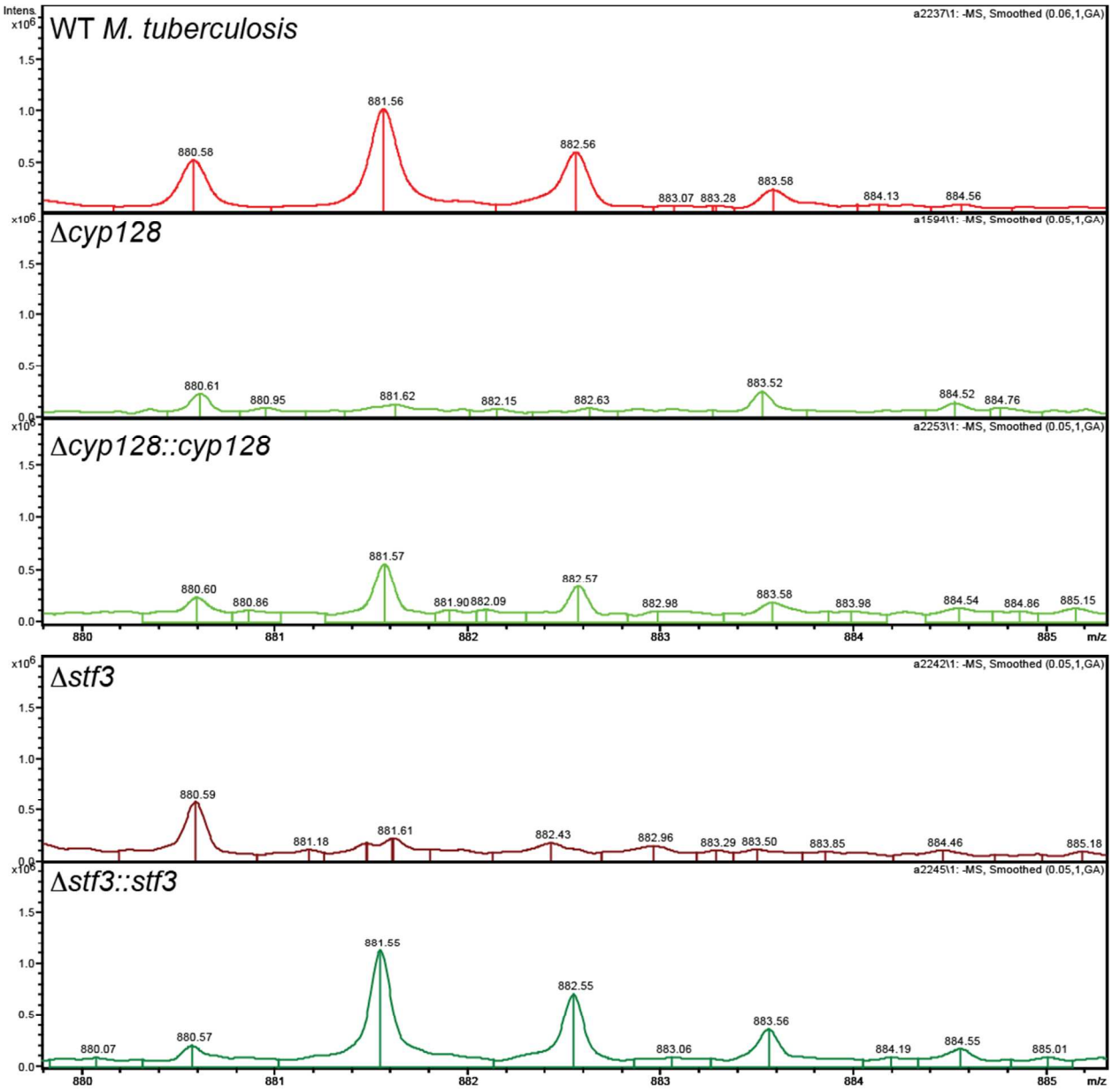

FIGURE S5: Scheme depicting promoters for *cyp128*.

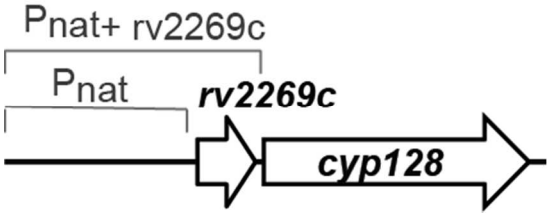

FIGURE S6: Mass spectra of TLE from Δ*cyp128* complementation strains with *cyp128* under control of three different promoters with region m/z 880-886 shown.

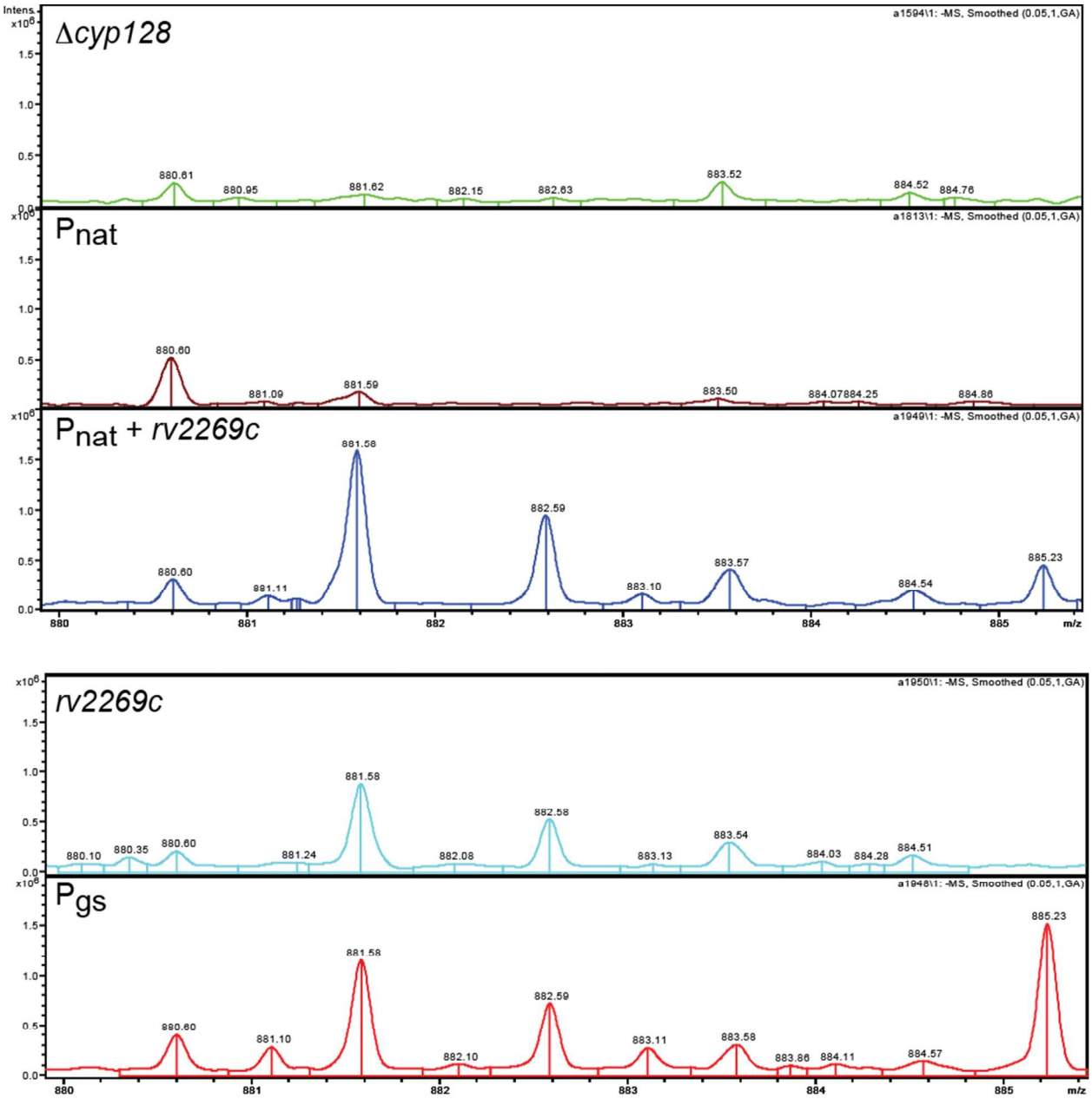

FIGURE S7: Structure of menaquinone-9 (MK-9).

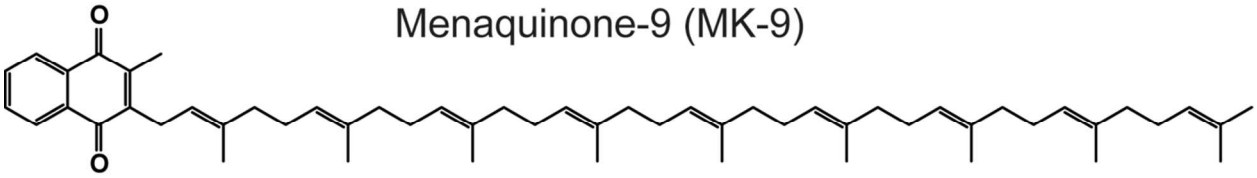

FIGURE S8: Schematic of *M. tuberculosis* electron transport chain and the inhibitors screened against WT *M. tuberculosis* and SMK mutants.

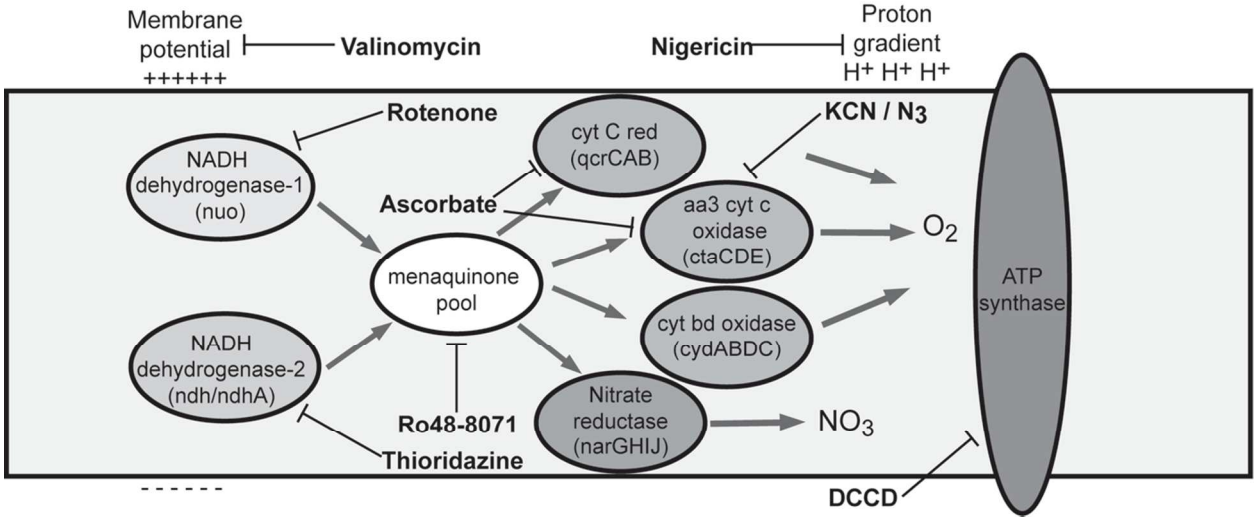

TABLE S1: Minimum inhibitory concentrations (MIC_{90}) values for WT, SMK mutants and complements.

Chemical stress	MIC	Cell wall inhibitors	MIC
H_2O_2	110 mM	INH	0.06 µg/ml
$NaNO_3$, pH 5.5	5 mM	ETA	5 µM
SDS	0.025%	ETH	6 µM

H_2O_2 hydrogen peroxide; $NaNO_3$ sodium nitrate, SDS sodium dodecyl sulfate, INH isoniazid, ETA ethionamide, ETH ethambutol.

TABLE S2: Strains used in this study

Strains		Genotype	Source
M. smegmatis			
mc^2155		Wild type	
mc^2155	*rv2269c*	pKMS101; Kn^r, contains *rv2269c*	This study
mc^2155	*cyp128*	pKMS102; Kn^r, contains *cyp128*	This study
mc^2155	*stf3*	pKMS103; Kn^r, contains *stf3*	This study
mc^2155	*stf3* operon	pKMS104; Kn^r, contains rv2269c, *cyp128, stf3*	This study
mc^2155	*cyp128, stf3*	pKMS105; Kn^r, contains *cyp128* and *stf3*	This study
M. tuberculosis			
H37Rv		Wild type	
H37Rv	Δ*cyp128*	Hyg^r, hyg cassette disrupting *cyp128*	This study
H37Rv	Δ*cyp128::cyp128*	Hyg^r, Kan^r, complemented strain of Δ*cyp128*	This study
H37Rv	Δ*stf3*	Hyg^r, stf3 interrupted by hyg resistance cassette	Ref [1]
H37Rv	Δ*stf3::stf3*	Hyg^r, Kan^r, complement with stf3 under the glutamine synthase promoter, modified pMV306 [2]	Ref [1]
H37Rv	Δ*rv2269c*	Hyg^r, *hsp60* promoter disrupting *rv2269c*	This study

TABLE S3: Plasmids used in this study

Reference name	Description	Source
pMV261	Kn^r, pAL5000 origin, ColE1 origin, multiple cloning site, Phsp60 promoter	Ref [2]
pMV306	Kn^r, A derivative of pMV261 lacking the Phsp60 promoter	Ref [2]
pKMS101	pMV261 derivative; contains *rv2269c*	This study
pKMS102	pMV261 derivative; contains *cyp128*	This study
pKMS103	pMV261 derivative; contains *stf3*	This study
pKMS104	pMV261 derivative; contains *rv2269c*, *cyp128*, and *stf3*	This study
pKMS105	pMV261 derivative; contains *cyp128* and *stf3*	This study
pKMS110	Plasmid used for *cyp128* disruption with hyg cassette	This study
pKMS109	Plasmid used for *rv2269c* disruption with hyg cassette	This study
pKMS133	Kn^r, a derivative of pMV306 encoding *cyp128* with *rv2269c* promoter.	This study
pKMS130	Kn^r, a derivative of pMV306 encoding *cyp128* with P_{nat} (upstream 1 kb of the first gene in the putative operon) + *rv2269c* as the promoter.	This study
pKMS118	Kn^r, a derivative of pMV306 encoding *cyp128* with P_{nat}.	This study

TABLE S4: Primers used in this study. Restriction enzymes sequences are in bold and enzyme in parentheses.

Primer name	Sequence	Description
okms102	cacttcgcaa**tggcca**acgatgcgcgacccttagcg	5' pKMS101, pKMS104 (MscI)
okms109	actgttctacgcctctctga**atcgat**agggtcatga	3' pKMS101 (ClaI)
okms100	ccagcgtcagaaacaatgtg	5’ pKMS102, pKMS105
okms101	cgtgacaacgggctgcttag	3' pKMS102
okms103	cacttcgcaa**tggcca**acgatgcgcgacccttagcg	5’ pKMS103 (MscI)
okms112	actgttctacgcctctctga**atcgat**gtcg	3’ pKMS103, pKMS104, pKMS105 (ClaI)
okms126	ccgtacgt**ctcgag**gtgagcaactgaccg	pKMS110 KO 5' cyp128 (XhoI)
okms127	caccatg**aagctt**ggtcagaccaacgtcgggc	pKMS110 KO 5' cyp128 (HindIII)
okms128	ccg**ggtacc**gaatagaggtggtcgagc	pKMS110 KO 3' cyp128 (KpnI)
okms129	cggta**cttaag**cgaacgtcggttgttgc	pKMS110 KO 3' cyp128 (AflII)
okms213	cgc**ggtacc**gtggccaacgatgcgcg	5’ pKMS133 (KpnI)
okms196	gtcgac**atcgat**gcacggcgaagcggttac	3’ pKMS133 (ClaI)
okms179	**ttcgaa**atgaccgcgacacagtccc	5' pKMS118 (BstBI)
okms180	gac**atcgat**tgcgcggtcagaccaac	3' pKMS118 cyp128 (ClaI)
okms181	gc**ggtacc**gtggcttgccatgtcgttatgag	5' pKMS130 (KpnI)
okms196	gtcgac**atcgat**gcacggcgaagcggttac	3’ pKMS130 (ClaI)
okms122	gtacgt**ctcgag**ttgtaggccctcggccagcg	pKMS109 KO 5' rv2269 (XhoI)
okms123	gatcca**gatatc**aactgggccgactgtgtagg	pKMS109 KO 5' rv2269 (EcoRV)
okms124	gacaggac**tctaga**cgcaattattgcgatgcccg	pKMS109 KO 3' rv2269 (XbaI)
okms125	gactagag**ggtacc**agcagtgctctcatag	pKMS109 KO 3' rv2269 (KpnI)

References:

(1) Mougous, J. D.; Senaratne, R. H.; Petzold, C. J.; Jain, M.; Lee, D. H.; Schelle, M. W.; Leavell, M. D.; Cox, J. S.; Leary, J. A.; Riley, L. W.; et al. A sulfated metabolite produced by stf3 negatively regulates the virulence of Mycobacterium tuberculosis. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103* (11), 4258–4263 DOI: 10.1073/pnas.0510861103.

(2) Stover, C. K.; la Cruz, de, V. F.; Fuerst, T. R.; Burlein, J. E.; Benson, L. A.; Bennett, L. T.; Bansal, G. P.; Young, J. F.; Lee, M. H.; Hatfull, G. F. New use of BCG for recombinant vaccines. *Nature* **1991**, *351* (6326), 456–460 DOI: 10.1038/351456a0.