

1 **Supplementary Table 1.** CLUSTAL O(1.2.4) multiple sequence alignment of 11 probable acetyltransferases from *Mycobacterium tuberculosis*
2 H37Rv

Rv2851c	-----	0
Rv2775	-----	0
Rv3027c	-----	0
Rv0730	-----MHGARTGVSFYAYAMT-----DHDQTAARR	25
Rv0919	-----	0
Rv0133	-----	0
Rv3216	-----	0
Rv0428c	MVSWPGLGTRVTVRYRRPAGSMPPPLTDAVGRLLAVDPTVRVQTKTGTIVEFSPVDVVALR	60
Rv2867c	-----MSAPPISRLVGERQV-----SVVRDAAAVWR	26
Rv2170	-----	0
Rv2669	-----	0
Rv2851c	-----	0
Rv2775	-----	0
Rv3027c	-----MSIA-SVLI-----PS	10
Rv0730	E-----IADALLAALERRHEVADAIVEA-----ANKA---	52
Rv0919	-----	0
Rv0133	-----	0
Rv3216	-----	0
Rv0428c	VLTDAPVRTAAIRALEHAAAAWPGVERTWLD--GWLLRAGHGA-----VLAANSAPVPL	112
Rv2867c	VLDDDPPIESCMVAAR-----VADHGIDPNAIGGELWTRRGAHESLCFAGANLIPLRGGPI	81
Rv2170	-----MA---IFLIDL--PPS	11
Rv2669	-----	0
Rv2851c	-----MTEALR----RV-----WAKDL-----	13
Rv2775	-----MHYPV-----WRQSW-----	10
Rv3027c	DKPHGVATGS-STGPRYSLLLSTDPSMVEAAQRLRYDV-----FSTTP-----	52
Rv0730	-----AAVEAIVNLLGTSHLAAEAVMSMSFDQ-LTQDARTKIIAELDDLNKQLS	100
Rv0919	-----VSGYSAPRRISDADD-----	15
Rv0133	-----MTPQARPARRADVRELSRTMA	21
Rv3216	-----VTDT-----	4
Rv0428c	DISAHTNTI-----TE----ISAWYAS-----RDLQPWLA	138
Rv2867c	DLNAFADVAM-STPRRCSSLVGRADLV----LPMWQRLEP--VWGPAR--DVRDNQPLMA	132
Rv2170	DMERRLGDALTVYVDAMRYPRGTETLR----APMWLEHIRRRGWQAVAAVEVTAAEQAEA	67
Rv2669	-----VTDADLAA	9

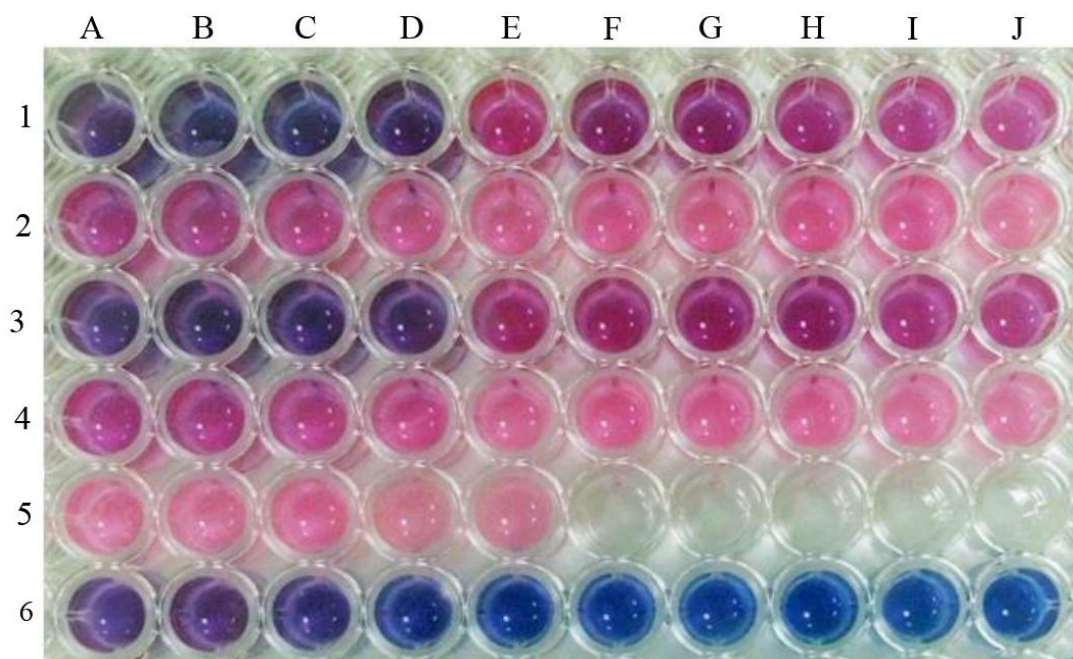
Rv2851c	-DARALYELLKL---RVEVFVVEQACPYPELDGRDLLAETRHFWLETPD--GEVTCTLRL	67
Rv2775	-TGILDPYLLDM---I-----GSP-----KLWVEESYPQSLKRGGWSM	44
Rv3027c	--GFALPAAADT---R-----RDGDRFDEYCDHLLVRDDDT-GELVGCYRM	92
Rv0730	FTVKERPASS---GEGE-----ELRPFSPDEDRDIFARTE-----	133
Rv0919	-----VTSFSSGE-----PSLDDYLKRA----L--ANHV-QGGSRCF	46
Rv0133	RAFYDDPVMSWLLSNDN-----ARTARLTRLFATIV----R--HQHL-AGGGVEV	64
Rv3216	-----IRRATPADTADIVAMIHA--LAEFEYAADQCTVTET	38
Rv0428c	VPDRLLPLPADLAGERREQVLVRDVSTGEPDRSVTLLDHPDDTWLRLYHQRLPLDMATPV	198
Rv2867c	LA--THPSCAIDTGVRQ-----VRPEELDSY-----LVAAVDM	163
Rv2170	ADTTALPSAA---ELS-----NAPMLGVAY-----	89
Rv2669	VAARTFPLAC---PPA-----VAPEHIASF-----VDAN---	35
Rv2851c	MEEH-----AGGEK-----	76
Rv2775	WIAE-----SGGQPIG-----MTMFG-----	60
Rv3027c	L-AP-----AGAIAGGLYT---ATEFDV-----CA	114
Rv0730	EMGAAGDGS---GGPAGS-----VDDEIRAAQKRVDDEEAWFVAVDSGVKVG	179
Rv0919	VTCDR-----GRVVGIFYALASGSVAHADAPGRV-----RR	76
Rv0133	ARGAAGIGGAALWDPPDRWRESRRQQLAMTPGFLRVFGFRTAKARAALDV-----MM	116
Rv3216	QIHTALFGD-FPTM-RGH-----VAEVN-----GGVAA---MALWFL-----NF	72
Rv0428c	IDGELAFGS-YLGV-AVA---R-AAVTDAPDGTRWVGLSA---MRAAD-----	237
Rv2867c	FIGEVGVDP-RLGD-GGRGYRRR-V-AGLIAAGRAWARFEH---GQVIFK-----AE	208
Rv2170	-----G-YPGA-PGQWWQQQ-VVLGLQR-----SGFPR-----LA	116
Rv2669	-LSSARFAE-YLTD-PRR-----AILTARHDGRI-VGYA-----M	66
Rv2851c	-----VFRI-----GRLCTKRDARGQGHSNRLLCALAEEVG DYPC---	111
Rv2775	PDIAHPDRIQIDALYVAENSQRHGIGGRLLNRLHSHPSADMILWCAEKNSKA-----	113
Rv3027c	FDPLRPSLVEM-----GRAVVREGHRNGGVLLMWAGILAYLDRYGYDYV	159
Rv0730	VFGELVHGEVDVRIWIHPDHRKKGYG-----TAALRKSRSEM-AWAFPAV	223
Rv0919	NMPDPVPVILLSRLAVDRKEQG RGLG-----SHLLRDAIGRCVQAADSIG	121
Rv0133	RVHPEEPHWYLAAGSDPTVRQG GFG-----QVLMRSRLDRCDAEHCPAY	161
Rv3216	STWDGVAGIYVEDLFVWPRFRRRGLA-----RGLLSTLARECVDNRYTR-	116
Rv0428c	--EQSATGSAGRQLWE--ALLGWGAG-----RGATRG-----	265
Rv2867c	VGSQSPAVGQIQGVVHPEWRGIGLG-----TAGTATLAAVIVGSGR-I-	251
Rv2170	IARLMTSYFELTELHILPRAQGRGLG-----EALARLLAGRDEDNVLL-	160
Rv2669	LIRGDDRDVELSKLYLLPGYHGTGAA-----AALMHKVLATAADWGALR-	110

Rv2851c	-----RIDAQAYLTAMYAQHGFVRDGDDEFLLDDG---IP	141
Rv2775	-----RGFYEEKDFHIDGRTFTWK-PLSGV	137
Rv3027c	TGCVSVPIGGDGETPGSRLRGVRDFILNRHAAPPQCQVYPYRPVRVDGRSLDDILPPRP	219
Rv0730	-----PMVARAPAAQPAQPGSAGR-----	242
Rv0919	L-----RAI-LVHALHDEARAFYVHDFEISPTDPL-----HLM	154
Rv0133	-----LESTKPENVPYYQRFGRVTRTRELALPDAGPPLW	194
Rv3216	-----LAW-SVLNWNSDAIALYDRIGGQPQHEWTIYRLSGPRL	153
Rv0428c	-----YVRVHDTATSVLAESLGFRLLHHHCERYLPAQSV--	297
Rv2867c	-----ASL-YVNSFNTVARAAYARVGFKKEIGTFATVLLD----	284
Rv2170	-----STP-ETNGEDNRAWRLYRRLGFTDII-RGYHFAGDPR-	195
Rv2669	-----VWL-GVNQKNQRAQRFYAKTGFKINGTRTFRLLGAHHEN	147
Rv2851c	HVPMLRPGS-----GQVERP-----	156
Rv2775	NVPH--VGYRLYRSAPPG-----	153
Rv3027c	AVPPLMRGYLRLGARACGEPAHDPDFGVGDFCLLLDKDHADTRYLRRLRSVAAASEMVND	279
Rv0730	-----	242
Rv0919	-----LLMKDARALIGD-----	166
Rv0133	-----AMWREPR-----	201
Rv3216	-----AALAAPR-----	160
Rv0428c	-----GWDTF-----	302
Rv2867c	-----	284
Rv2170	-----AFAILGRTLPL-----	206
Rv2669	-----DYVMV-RELV-----	156
Rv2851c	--	156
Rv2775	--	153
Rv3027c	AR	281
Rv0730	--	242
Rv0919	--	166
Rv0133	--	201
Rv3216	--	160
Rv0428c	--	302
Rv2867c	--	284
Rv2170	--	206
Rv2669	--	156

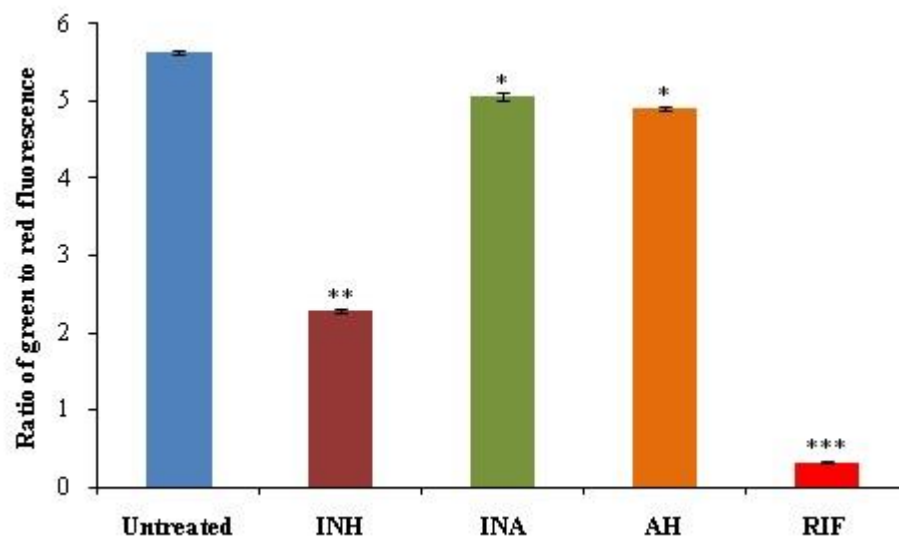
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Supplementary Table 2. List of primers used in this study

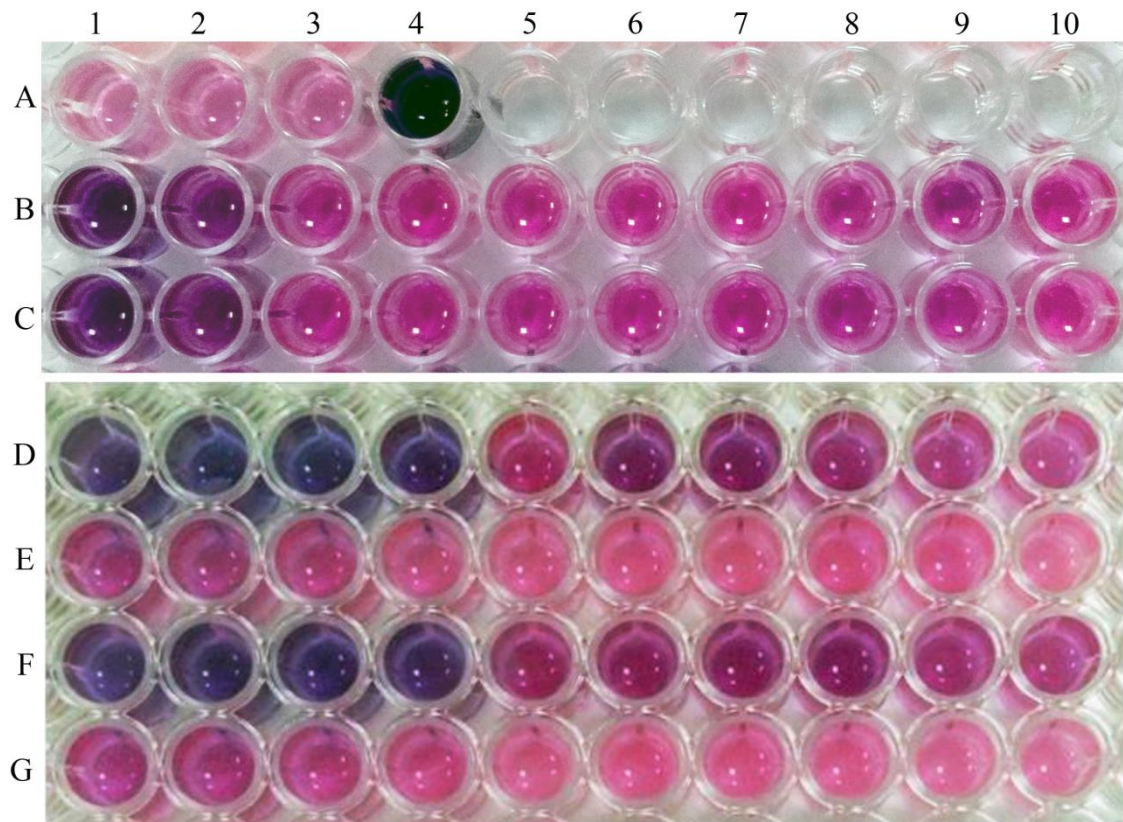
Gene	Primer sequence (5'-3')
<i>Rv2170F</i>	GGAATTCCATATGCATCACCATCACCATCA CTTGGCGATA TTCCTCATCGA
<i>Rv2170R</i> <i>Rv0428cF</i>	CGCGGATCC TTAGAGCGGTAGCGTGCGAC GATCCATATGCATCACCATCACCATCACAT GGATCTCGTG GCCGGGG
<i>Rv0428R</i> <i>MSMEG_4238F</i>	GATCAAGCTTCTAGAAAGGTATCCCAGCCG GATCCATATGCATCACCATCACCATCACAT GGCGGCGATG GCG
<i>MSMEG_4238R</i> <i>Rv2170PBENF</i>	GATCAAGCTTCTACAGGGGCAGGGGCC GATC GGATCCTTGGCGATATTCCTCATCGA
<i>Rv2170PBENR</i>	GATCAAGCTT TTAGAGCGGTAGCGTGCGAC
<i>Rv2170(R16A)F</i> <i>Rv2170(R16A)R</i> <i>Rv2170(Y24A)F</i> <i>Rv2170(Y24A)R</i> <i>Rv2170(R16A/Y24A/E129A)R</i> <i>Rv2170(E129A)F</i> <i>Rv2170(E129A)R</i>	GATATGGAGCGCGCCCTCGGTGATG GCTGGGCGGCAGATCGATGAG CCTGACGGTGGCCGTCGACGCG GCATCACCGAGGCGGCGCTCC GCATCACCGAGGCGGCGCTCC CGAGTTGACTGCCTTGACATCC AAGTAGCTGGTCATCAGTCGG



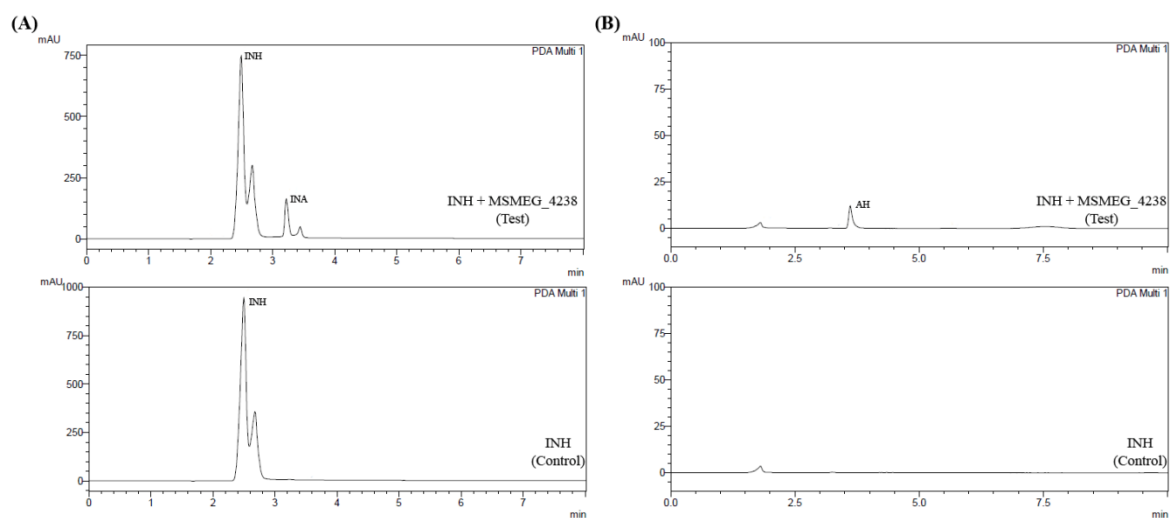
Supplementary Figure 1. Resazurin microtiter assay (REMA) to determine the minimum inhibitory concentration (MIC) of isoniazid against *M. smegmatis*. Wells of rows 1 and 2 (1A to 2J) and 3 and 4 (3A to 4J) contain *M. smegmatis* treated with decreasing concentrations of isoniazid (64 $\mu\text{g/mL}$ to 0.122 ng/mL). Wells 5A to 5E contain bacteria without any drug, and the remaining wells in row 5 contain sterile water. Wells of row 6 contain medium with isoniazid (64 $\mu\text{g/mL}$ to 0.125 $\mu\text{g/mL}$) alone. Well 1D represents the MIC (8 $\mu\text{g/mL}$) of isoniazid against *M. smegmatis*.



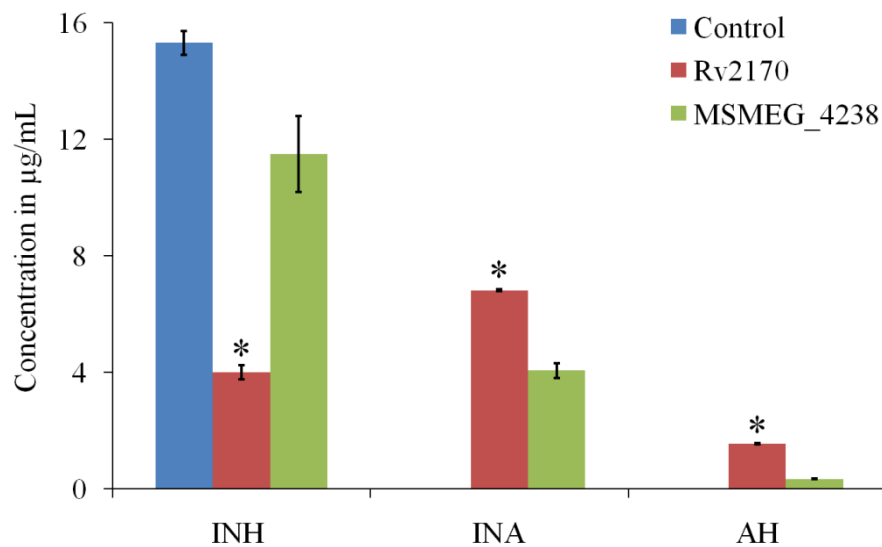
Supplementary Figure 2. Analysis of relative viability of *M. smegmatis* by measuring the ratio of green to red fluorescence. *M. smegmatis* incubated with 8 µg/mL each of isoniazid (INH), isonicotinic acid (INA), and acetylhydrazine (AH). Rifampicin (RIF) 1 µg/mL was used as positive control. The experiments were performed in triplicate and values were plotted as mean ± standard deviation. *significance of difference from control sample, $p \leq 0.05$



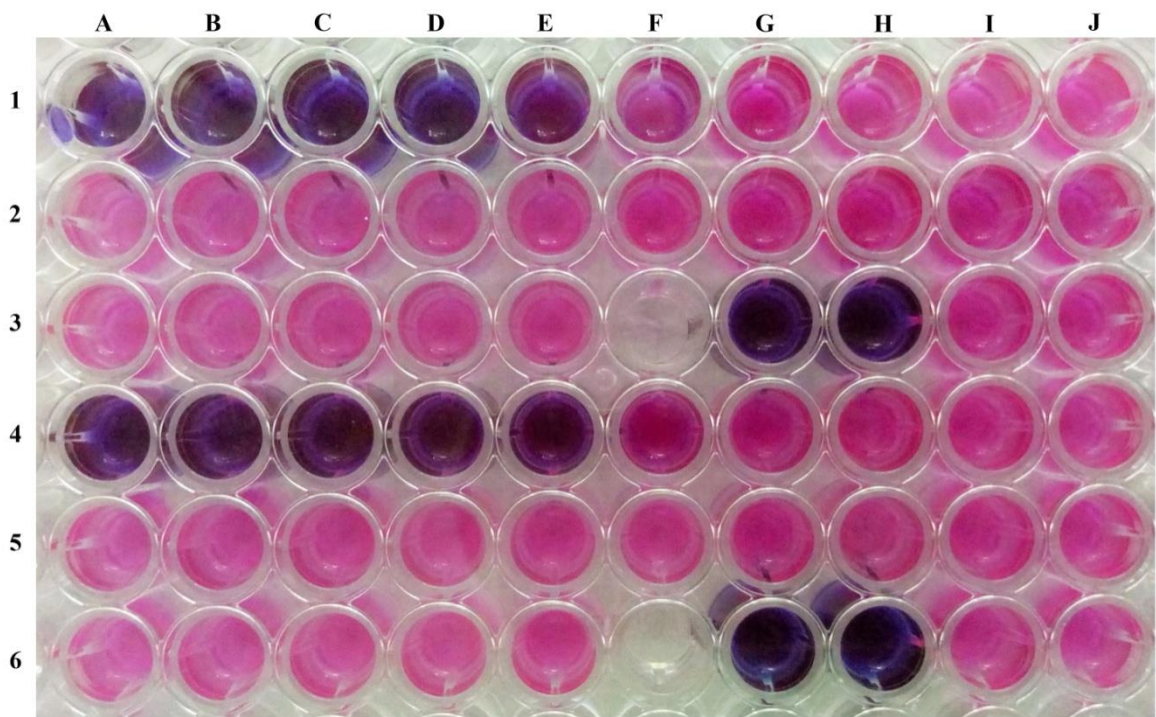
Supplementary Figure 3. Resazurin microtiter assay (REMA) to determine the toxicity of acetylisoniazid (AcINH) and isoniazid (INH) against *M. smegmatis*. Wells A1 to A3 contain bacteria without any drug, and A4 contains bacteria treated with rifampicin (1 $\mu\text{g/mL}$). The remaining wells in row A contain sterile water. Wells of row B (B1 to B10) and 3 (C1 to C10) contain *M. smegmatis* treated with decreasing concentrations of AcINH (16 $\mu\text{g/mL}$ to 0.03125 $\mu\text{g/mL}$). Wells of rows D1 to E10 and F1 to G10 contain *M. smegmatis* treated with decreasing concentrations of isoniazid (64 $\mu\text{g/mL}$ to 0.122 ng/mL).



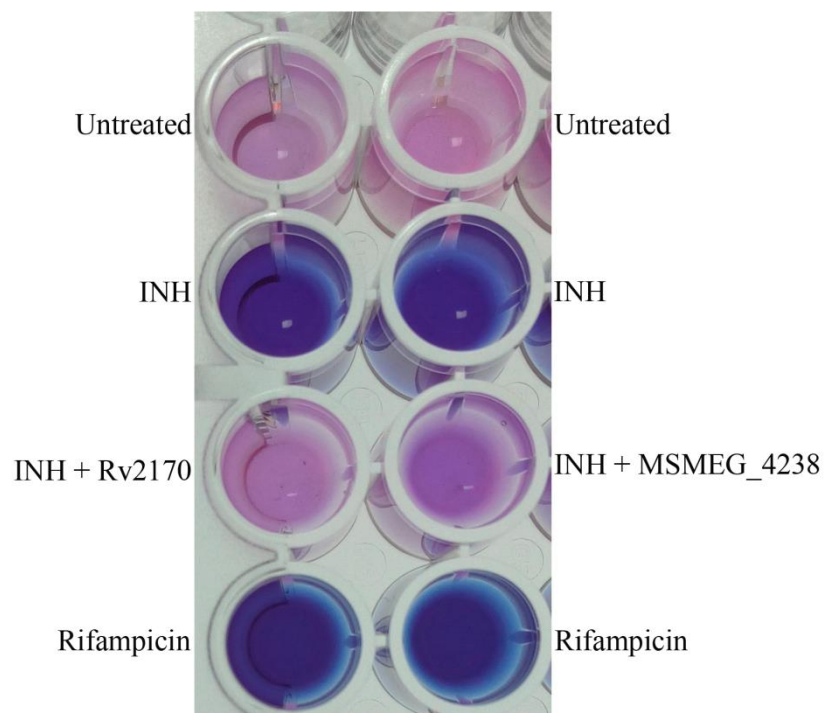
Supplementary Figure 4. HPLC analysis of *in vitro* acetylation of isoniazid [INH] by MSMEG_4238 shows formation of very little of (A) isonicotinic acid [INA], and (B) acetylhydrazine [AH] in test samples (INH + MSMEG_4238).



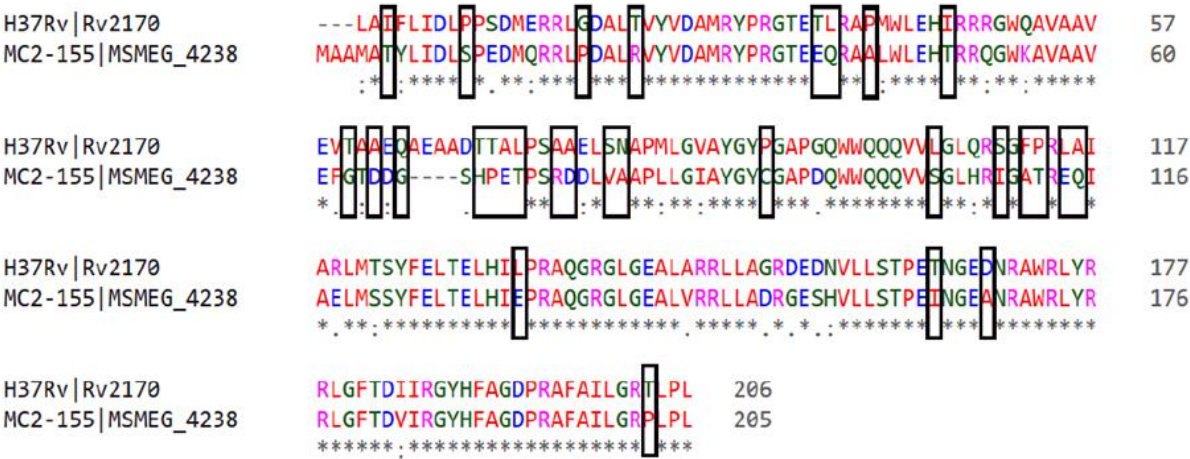
Supplementary Figure 5. Quantification of isoniazid (INH), isonicotinic acid (INA) and acetylhydrazine (AH) after *in vitro* acetylation of INH by Rv2170 based on HPLC analysis. The experiments were performed in triplicate and values were plotted as mean $\pm$ standard deviation. *Rv2170 significantly different from control and MSMEG_4238, $p \leq 0.05$



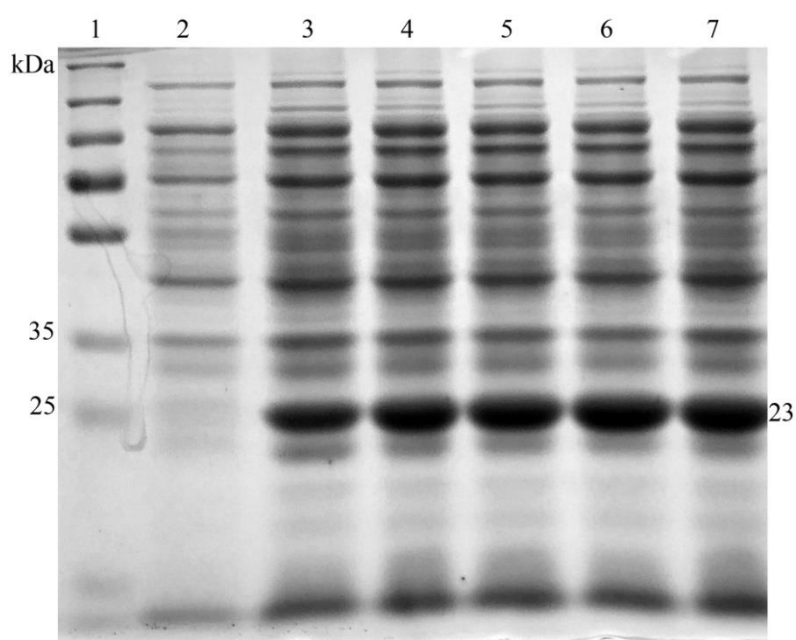
Supplementary Figure 6. Resazurin microtiter assay (REMA) to determine the minimum inhibitory concentration (MIC) of isoniazid against MTB H37Rv. Wells of rows 1 to 3 (1A to 3E) contain MTB H37Rv treated with decreasing concentrations of isoniazid (4 $\mu\text{g/mL}$ to 0.000237 ng/mL). Wells 4A to 6E represent duplicates of the same. Wells 3G, 3H, 6G, and 6H contain bacteria treated with rifampicin (1 $\mu\text{g/mL}$). Wells 3I, 3J, 6I and 6J contain bacteria without any drug. Wells 3F and 6F contain sterile water. Wells 1E and 4E represent the MIC (0.25 $\mu\text{g/mL}$) of isoniazid against MTBH37Rv.



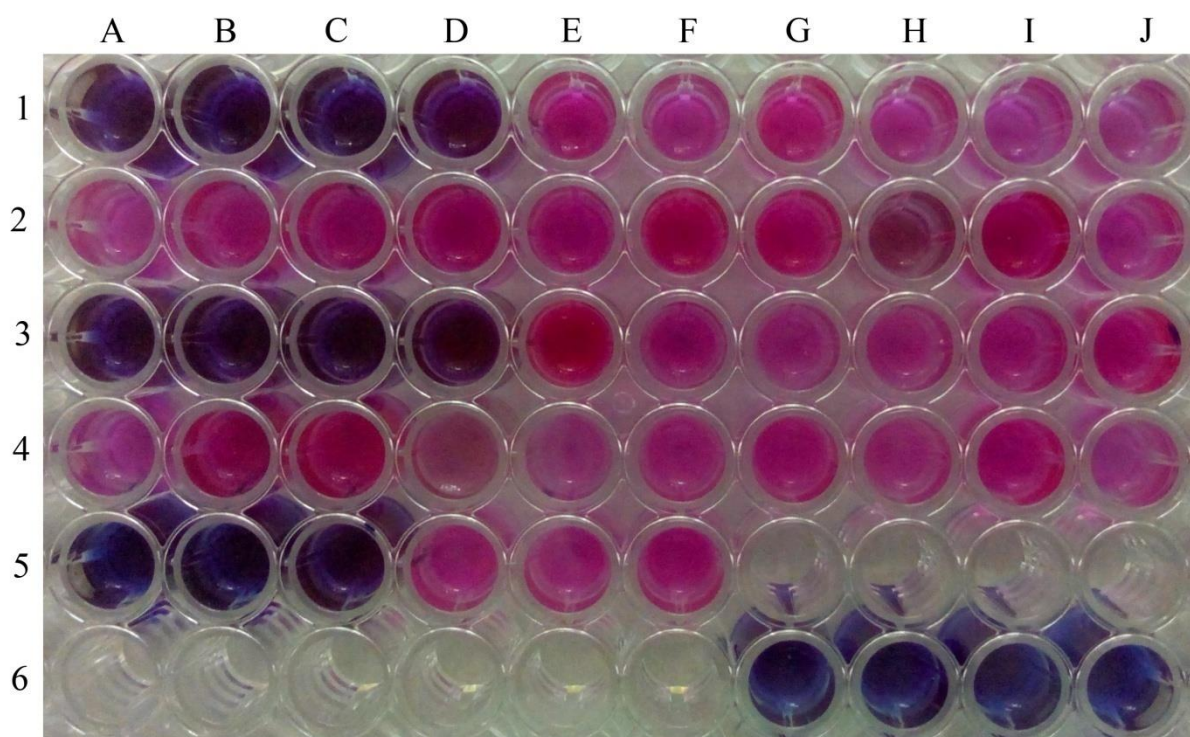
Supplementary Figure 7. REMA of MTB H37Rv in the presence of INH treated with Rv2170 (Lane 1-Test) and MSMEG_4238 (Lane 2-Test).



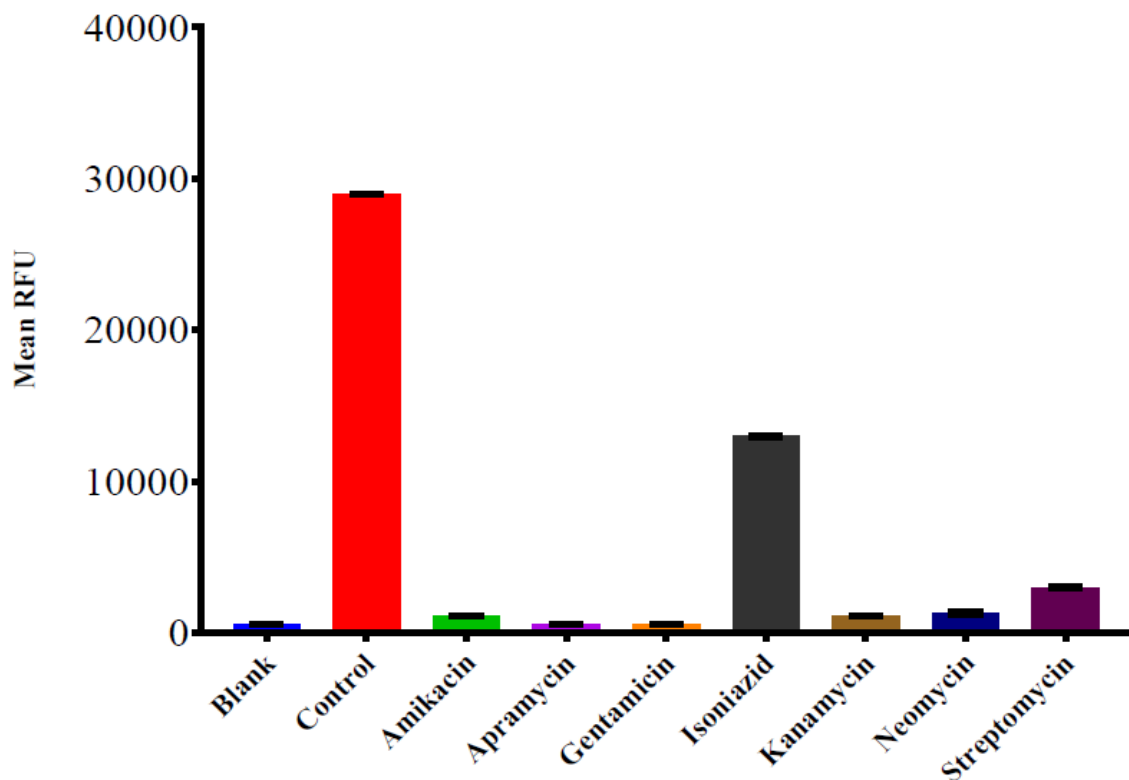
Supplementary Figure 8. Sequence alignment of Rv2170 and its orthologous protein MSMEG_4238. Amino acid substitutions are indicated in boxes.



Supplementary Figure 9. Lysates of *E. coli* Rosetta BL21(DE3) overexpressing Rv2170 (wild type and mutants) after induction with IPTG. Lane 1 - protein ladder, Lane 2 - uninduced (representative), Lane 3 - Rv2170 (R16A), Lane 4 - Rv2170 (Y24A), Lane 5 - Rv2170 (E129A), Lane 6 - Rv2170 (R16A/Y24A/Y129A) and Lane 7 - Rv2170 (wild type).



Supplementary Figure 10. Resazurin microtiter assay (REMA) to determine the minimum inhibitory concentration (MIC) of isoniazid against MTB H37Ra. Wells of rows 1 and 2 (1A to 2J) and 3 and 4 (3A to 4J) contain MTB H37Ra treated with decreasing concentrations of isoniazid (4 $\mu\text{g/mL}$ to 0.0076 ng/mL). Wells 3A to 4J represent duplicates of the same. Wells 5A to 5C contain bacteria treated with rifampicin (1 $\mu\text{g/mL}$). Wells 5D to 5F contain bacteria without any drug. Wells 5G to 6F contain sterile water. Wells 6G to 6J contain medium with isoniazid (4 to 0.5 $\mu\text{g/mL}$) alone. Well 1D represents the MIC (0.5 $\mu\text{g/mL}$) of isoniazid against MTB H37Ra.



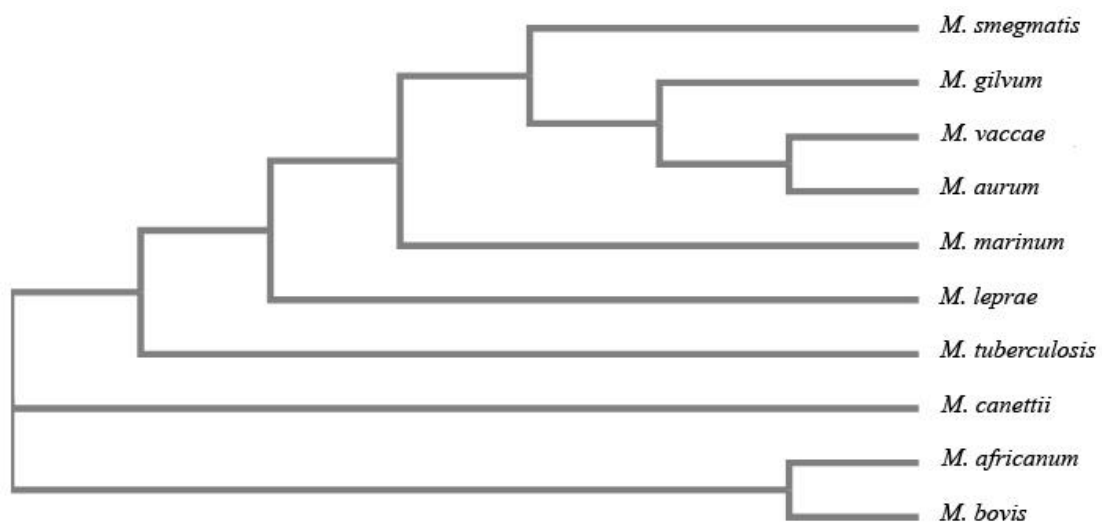
Supplementary Figure 11. Fluorometric acetyltransferase assay to determine the acetyltransferase activity of Rv2170 using different antibiotics as substrates. A ready-to-use positive control provided by the kit manufacturer to ensure the activity of kit components was used as control for this particular experiment. The experiment was performed in triplicate and values were plotted as mean $\pm$ standard deviation. *represents the significance of difference from the blank.

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<u>M. smegmatis</u>	MAAMATYILIDLSPEDMQRRLPDALRVYVDAMRYPRGTEEQRAALWLEHTRRQGWKAVAAV	60
<u>M. gilvum</u>	-----MQQRLGDALAVYVDAMRYPRGTEDQRAASMWLEHIRRHGWKAVAAV	45
<u>M. vaccae</u>	---MAARLIDLSPGEMQQRLGDALAVYVDAMRYPRGTEDQRAASMWLEHIRRHGWKAVAAI	57
<u>M. aurum</u>	---MATRLIDLSPSDMQQRLRDALAVYVDAMHYPRGTEEQRAASMWLEHIRRHGWKAVAAI	57
<u>M. leprae</u>	---LAIFLINLSPNEMERRLNEALEVYVDAMRYPRNTEENLRAGIWLEHIRRPGWQAVAAV	57
<u>M. marinum</u>	---LTIFVIDLPPDEMERRLREALTVYVDAMRYPRGTENQRAAMWLEHVRSGWKAVAAV	57
<u>M. canettii</u>	---MAIFLIDLPPSDMERRLGDALTVYVDAMRYPRGTETLRAPMWLEHIRRHGWQAVAAV	57
<u>M. tuberculosis</u>	---LAIFLIDLPPSDMERRLGDALTVYVDAMRYPRGTETLRAPMWLEHIRRHGWQAVAAV	57
<u>M. africanum</u>	---MAIFLIDLPPSDMERRLGDALTVYVDAMRYPRGTETLRAPMWLEHIRRHGWQAVAAV	57
<u>M. bovis</u>	---MAIFLIDLPPSDMERRLGDALTVYVDAMRYPRGTETLRAPMWLEHIRRHGWQAVAAV	57
	*::** :** *****:**. ** ** :**** ** **::****:	
<u>M. smegmatis</u>	EFGTDDGSH-----PETPSRDDLVAAPLLGIAYGYCGAPDQWWQQQVVSGLHRIG	110
<u>M. gilvum</u>	EVSGDPT-----DADHLPARAPLLGIAYGYCGAPDQWWQQQVI SGLRRNG	90
<u>M. vaccae</u>	EVPDSAEVA-----DAPEPSAAAPMLGIAYGYCGAPDQWWQQQVVSGLQSRG	104
<u>M. aurum</u>	EVTD-----PATPQADAPMLGIAYGYCGAPDQWWQQQVVAAGLRRSG	98
<u>M. leprae</u>	EVRIEV--ADVADMDGPAHPAPSADELNNAPLRGVAYGYPGAPGQWWQQQVVSGLQSRG	115
<u>M. marinum</u>	DVPQTQPSTQLPDII GADPNHELPAADLANAPMVGVAYGYPGAPGQWWQQQVVLGLQRRG	117
<u>M. canettii</u>	EVTAAEQAE-----AADTAALPSAAELSNAPMLGVAYGYPGAPGQWWQQQVVLGLQSRG	111
<u>M. tuberculosis</u>	EVTAAEQAE-----AADTTALPSAAELSNAPMLGVAYGYPGAPGQWWQQQVVLGLQSRG	111
<u>M. africanum</u>	EVTAAEQAE-----AADTTALPSAAELSNAPMLGVAYGYPGAPGQWWQQQVVLGLQSRG	111
<u>M. bovis</u>	EVTAAEQAE-----AADTTALPSAAELSNAPMLGVAYGYPGAPGQWWQQQVVLGLQSRG	111
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<u>M. smegmatis</u>	ATREQIAELMSYFELTELHIEPRAQGRGLGEALVRRLLADRGE SHVLLSTPE INGEANR	170
<u>M. gilvum</u>	ADADRISIELMSYFELTELHHPRAQGHGLGEALIRLLQDRTERQVLLSTPE INGEANR	150
<u>M. vaccae</u>	ADRSHIAELMQSYFELTELHHPRAQGRGLGEALIRLLDDRPEQHVVLLSTPE IVGEDNR	164
<u>M. aurum</u>	ADGARISDLMSYFELTELHHPRAQGRGLGEALIRLLDDRSEQQVLLSTPE INGEANR	158
<u>M. leprae</u>	LSTLEIARLMNSYFELTELHHPHTQGRGIGALTRLLAHRRENNVLLSTPE TNGETNR	175
<u>M. marinum</u>	FFPMHAIARVMSSYFELTELHHPNAQGRGIGALTRLLAARGEESVLLSTPE SNGEDNR	177
<u>M. canettii</u>	FPRLAIARLMTSYFELTELHILPRAQGRGLGEALARLLAGRDEDNVLLSTPE TNGEDNR	171
<u>M. tuberculosis</u>	FPRLAIARLMTSYFELTELHILPRAQGRGLGEALARLLAGRDEDNVLLSTPE TNGEDNR	171
<u>M. africanum</u>	FPRLAIARLMTSYFELTELHILPRAQGRGLGEALARLLAGRDEDNVLLSTPE TNGEDNR	171
<u>M. bovis</u>	FPRLAIARLMTSYFELTELHILPRAQGRGLGEALARLLAGRDEDNVLLSTPE TNGEDNR	171
	*: * ***** *.:**:***** **** * * ***** ** **	
<u>M. smegmatis</u>	AWRLYRRLGFTDVIRGYHFAGDPRAFAILGRPLPL	205
<u>M. gilvum</u>	AWRLYRRLGFTDVIREYHFAGDPRAFAILGRPLPL	185
<u>M. vaccae</u>	AWRLYRRLGFTDVIRGYHFSGDPRAFAILGRALPL	199
<u>M. aurum</u>	AWRLYRRLGFTDVIRGYHFAGDPRAFAILGRSLPL	193
<u>M. leprae</u>	AWRLYRRLGFTDI IRRHYFAGDPRAFAILGRTLPL	210
<u>M. marinum</u>	AWRLYRRLGFTDI LRGYFAGDPRAFAILGRALPL	212
<u>M. canettii</u>	AWRLYRRLGFTDI IRGYHFAGDPRAFAILGRTLPL	206
<u>M. tuberculosis</u>	AWRLYRRLGFTDI IRGYHFAGDPRAFAILGRTLPL	206
<u>M. africanum</u>	AWRLYRRLGFTDI IRGYHFAGDPRAFAILGRTLPL	206
<u>M. bovis</u>	AWRLYRRLGFTDI IRGYHFAGDPRAFAILGRTLPL	206
	***** *: * :*:***** **	

252 **Supplementary Figure 12.** Amino acid sequence alignment of Rv2170 and its orthologs
 253 present in pathogenic and non-pathogenic mycobacteria. Significant amino acid substitutions
 254 are highlighted.

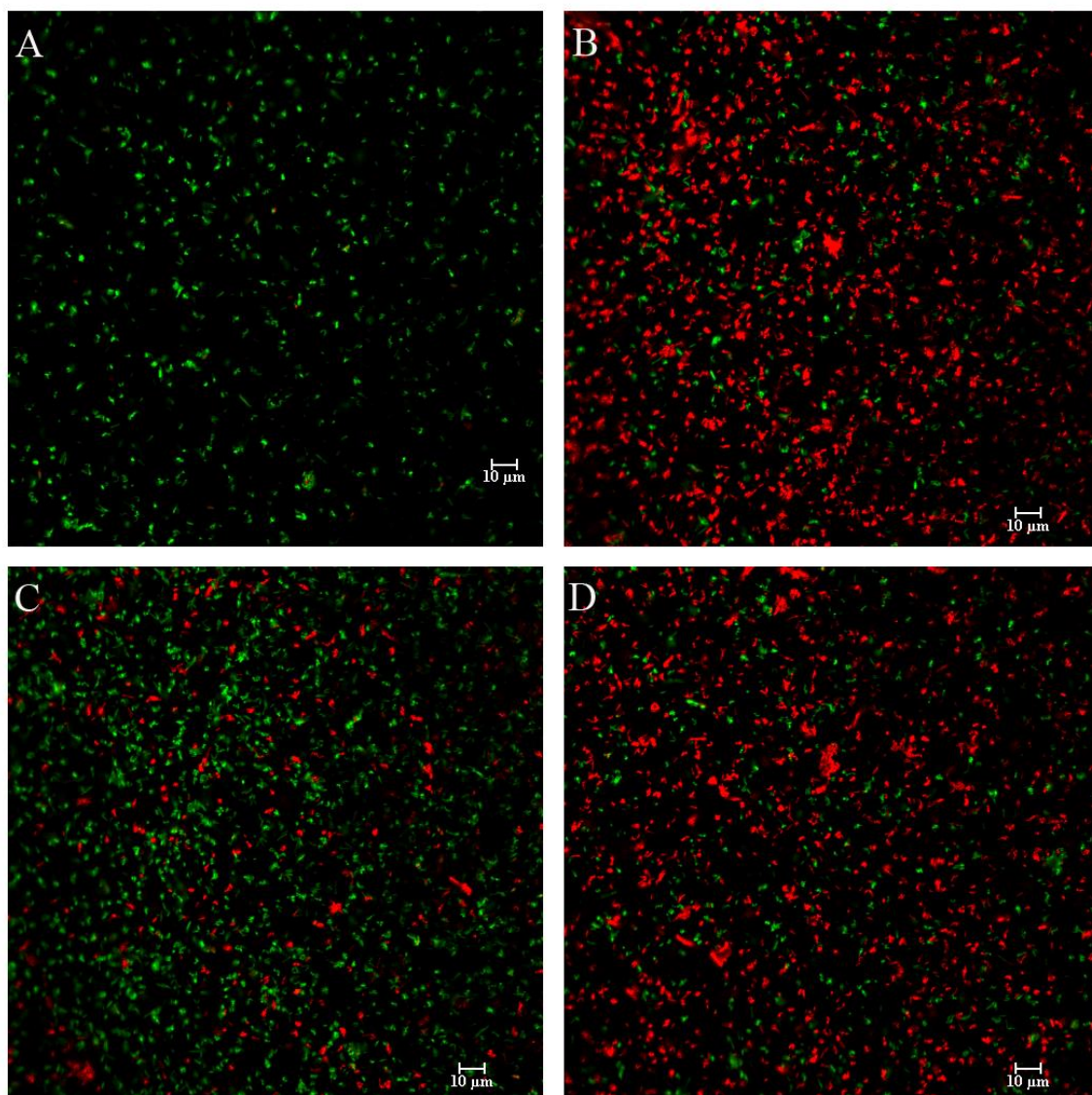
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Supplementary Figure 13. Cladogram of Rv2170 of MTB H37Rv and its orthologs in pathogenic and non-pathogenic mycobacteria.

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293 **Supplementary Figure 14.** Representative confocal microscopy images showing the
294 viability of *M. smegmatis* after it was incubated with (A) untreated, (B) INH-treated (C) INH
295 + Rv2170-treated and (D) INH + MSMEG_4238-treated. The live and dead bacteria are
296 stained green and red, respectively. Scale bar - 10 µm.

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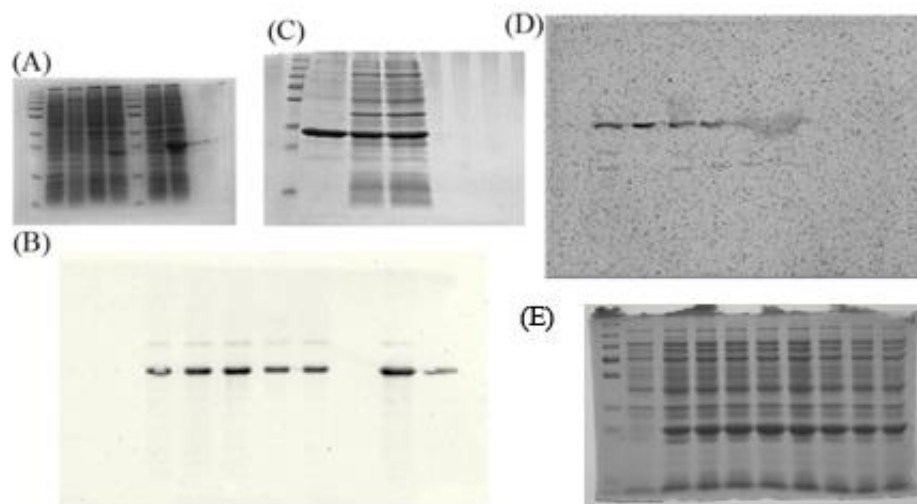
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Supplementary Figure 15. Original images of gel and blots of Figure 1 (A, B, C, D), Figure 7 (B) and Supplementary Figure 9 (E).