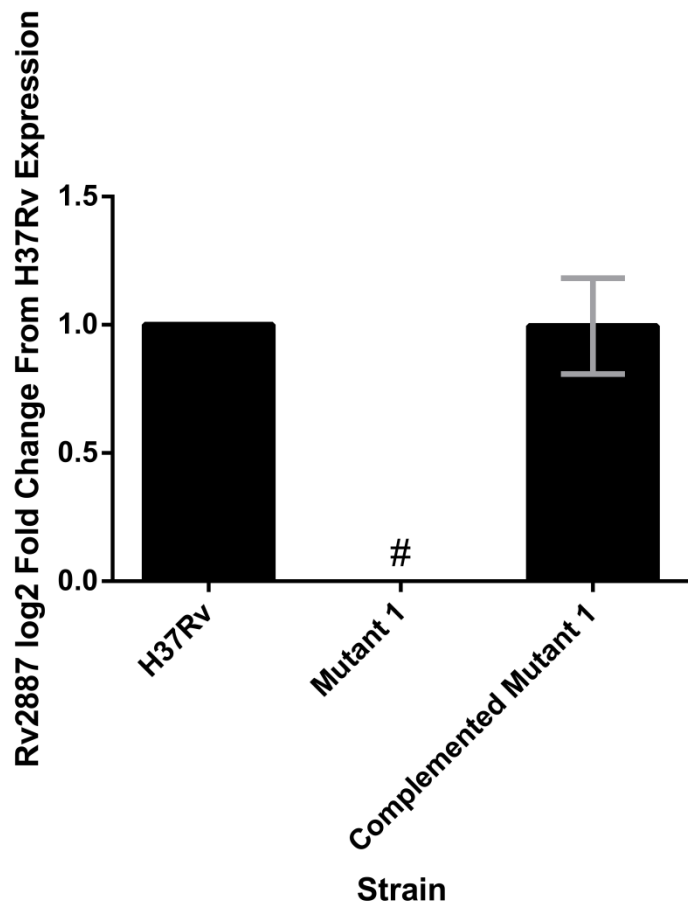


Supplemental Figure S1. Novel compounds used in this study. Chemical structure of (A) MP-III-71 and (B) N-Methyl derivative of MP-III-71.



Supplemental Figure S2. Expression of *Rv2887*. Quantitative reverse transcription PCR of *Rv2887*, normalized to *sigA*. This shows *Rv2887* expression is abolished in mutant 1 but restored to wild-type levels in the complement.

Whole Genome Sequencing							
	<u>Sample</u>	<u>Accession Number</u>	<u>Sequencing Platform</u>	<u>Number of reads</u>	<u>Average read length (bp)</u>	<u>Average coverage</u>	<u>Purpose</u>
	H37Rv	SRS889709	Ion Torrent PGM	1118469	118	23.90x	WGS
	mutant 1	SRS891424	Ion Torrent PGM	1128556	113	20.30x	WGS
	mutant 2	SRS891455	Ion Torrent PGM	2085715	101	33.90x	WGS
	H37Rv	SRS891666	SOLiD	25731984	50	266.14x	WGS
	mutant 3	SRS893802	SOLiD	24748378	50	264.32x	WGS
RNA-seq							
	<u>Sample</u>	<u>Accession Number</u>	<u>Sequencing Platform</u>	<u>Number of reads</u>	<u>Average read length (bp)</u>		<u>Purpose</u>
Replicate 1	mutant 1 + MP-III-71	SRS891491	Ion Torrent PGM	962352	119		RNA-seq
	mutant 1 + DMSO	SRS891670	Ion Torrent PGM	999721	120		RNA-seq
	mutant 1 complement + MP-III-71	SRS891672	Ion Torrent PGM	973824	124		RNA-seq
	mutant 1 complement + DMSO	SRS891793	Ion Torrent PGM	929050	130		RNA-seq
Replicate 2	mutant 1 + MP-III-71	SRS891794	Ion Torrent PGM	2304483	85.5		RNA-seq
	mutant 1 + DMSO	SRS891795	Ion Torrent PGM	1027257	99		RNA-seq
	mutant 1 complement + MP-III-71	SRS891796	Ion Torrent PGM	1657712	94		RNA-seq
	mutant 1 complement + DMSO	SRS891857	Ion Torrent PGM	1229293	199		RNA-seq
Replicate 3	mutant 1 + MP-III-71	SRS891859	Ion Torrent PGM	899239	89		RNA-seq
	mutant 1 + DMSO	SRS891860	Ion Torrent PGM	1057459	120		RNA-seq
	mutant 1 complement + MP-III-71	SRS891862	Ion Torrent PGM	681799	103		RNA-seq
	mutant 1 complement + DMSO	SRS891863	Ion Torrent PGM	1448013	101		RNA-seq

Supplemental Table S1. Next generation sequencing metrics. Statistics from all sequencing runs. WGS = whole genome sequencing.

SNPs					
<u>Mutation Position in H37Rv</u>	<u>Reference Base</u>	<u>Observed Base</u>	<u>Mutation Annotation</u>	<u>Mutation Effect</u>	<u>Strain Containing Mutation</u>
342542	A	G	Rv0282	D138G	mutant 1 and mutant 2
666418	G	A	Rv0573c	L275L	mutant 1 and mutant 2
2170598	C	T	Rv1918c	V5V	mutant 1 and mutant 2
2654064	A	G	Rv2374c	X344R	mutant 1 and mutant 2
3894771	A	G	Rv3478	T116A	mutant 1 and mutant 2
3894773	C	T	Rv3478	T116T	mutant 1 and mutant 2
4374865	C	A	Rv3892c	V273V	mutant 1 and mutant 2
2979619	A	G	Rv2658c	W24R	mutant 2
4110523	C	T	Rv3668c	intergenic	mutant 2
3196612	T	C	Rv2887	V61A	mutant 3
INDELS					
<u>Mutation Position in H37Rv</u>	<u>Reference Base</u>	<u>Observed Base</u>	<u>Mutation Annotation</u>	<u>Mutation Location</u>	<u>Strain Containing Mutation</u>
55533	TGCC	T	Rv0050	CDS	mutant 1 and mutant 2
131174	T	TG	Rv0108c	Intergenic	mutant 1 and mutant 2
234496	C	CGT	Rv0197	CDS	mutant 1 and mutant 2
424320	T	TC	Rv0354c	CDS	mutant 1 and mutant 2
1274805	CG	C	Rv1146	Intergenic	mutant 1
1780586	C	CG	Rv1575	CDS	mutant 1
3580636	CT	C	Rv3203	Intergenic	mutant 1
3590686	G	GC	Rv3213c	Intergenic	mutant 1
3635239	GC	G	Rv3255c	CDS	mutant 1
3862472	GA	G	Rv3443c	Intergenic	mutant 1
4232224	AGGTGGAGACAG TGGGCT	A	Rv3785	CDS	mutant 1
3196829	GGA	G	Rv2887	intergenic	mutant 2

Supplemental Table S2. SNPs and indels identified in whole genome sequencing not present in parent H37Rv strain.

<u>Strain</u>	<u>Gene Mutated</u>	<u>length of protein</u>	<u>transposon point of insertion</u>	<u>Reason tested</u>	<u>MIC MP-III-71</u>
CDC1551	NA				0.125-0.25 ug/mL
JHU2658c-26	Rv2658c	369	26	Mutated in mutant sequence	0.125 ug/mL
JHU2658c-30	Rv2658c	369	30	Mutated in mutant sequence	0.125 ug/mL
JHU3668c-441	Rv3668c	699	441	Mutated in mutant sequence	0.25-0.125 ug/mL
JHU0559c-124	Rv0559c	339	124	RNA-seq hit	0.25 ug/mL
JHU0559c-202	Rv0559c	339	202	RNA-seq hit	0.25 ug/mL
JHU2463-1183	Rv2463	1185	1183	RNA-seq hit	0.25 ug/mL

Supplemental Table S3. MIC of MP-III-71 against select transposon mutants. MICs are given in $\mu\text{g}/\text{mL}$.

A: MICs of <i>M. tuberculosis</i> strains (ug/mL)			
<u>Drug</u>	<u>H37Rv</u>	<u>mutant 1</u>	<u>complemented mutant 1</u>
MP-III-71	0.125-0.5	1-2	0.125-0.5
Me-MP-III-71	>32	>32	>32
Isoniazid	0.04	0.04	0.04
Rifampin	0.125	0.125	0.125
Ethambutol	1-2	1-2	1-2
Kanamycin	2	2	2
Verapamil	200	200	200
Chlorpromazine	10	10	10
Salicylate	250-500	250-500	250-500
CCCP	8	8	8

B: Fold change in MIC compared with MP-III-71 alone (MIC of MP-III-71 + drug/MIC of MP-III-71 alone)			
<u>Drug combination</u>	<u>H37Rv</u>	<u>mutant 1</u>	<u>complemented mutant 1</u>
MP-III-71 + 50 ug/mL Verapamil	0.125	0.5-1	0.125
MP-III-71 + 2.5 ug/mL Chlorpromazine	0.256	1	0.256
MP-III-71 + 62.5 ug/mL Salicylate	0.5-1	0.25-0.5	1
MP-III-71 + 1 ug/mL CCCP	1-4	0.5-1	2-4

Supplemental Table S4. MIC of select compounds against H37Rv, mutant 1 and mutant 1 complement.

MICs are given in $\mu\text{g/mL}$ (A) or as fold change over MP-III-71 alone (B). CCCP=carbonyl cyanide 3-chlorophylhydrazone.

	Hit (Subject)	% identity	Alignment length	Number of mismatches	Number of gap starts	Query start	Query end	Subject Start	Subject end	Expect value	Bit score
MarR (gi 13384163) as query	Rv1404	34.67	75	49	0	37	111	42	116	4.00E-07	47.4
	Rv0880	27.27	110	69	2	4	113	9	107	3.00E-05	41.2
	Rv2887	25.38	130	96	1	13	142	8	136	2.00E-04	38.1
	Rv0737	27.16	81	57	1	33	111	49	129	3.00E-04	37.7
	Rv0042c	32.39	71	48	0	37	107	82	152	0.027	31.2
	Rv2922c	33.33	45	30	0	97	141	723	767	2.4	24.6
	Rv1442	33.33	24	16	0	72	95	49	72	3.9	24.3
	Rv1663	28.57	35	25	0	103	137	322	356	4.6	23.9
MarA (gi 190905560) as query	Rv1931c	30.49	82	55	2	28	108	175	255	4.00E-09	53.5
	Rv3833	26.51	83	60	1	18	100	156	237	1.00E-05	42
	Rv3736	28.92	83	56	2	27	108	246	326	0.017	31.6
	Rv2463	44.83	29	12	1	42	70	168	192	0.23	27.7
	Rv0543c	30.23	43	30	0	7	49	55	97	1.7	25
	Rv0672	25.4	63	38	4	24	86	22	75	6.2	23.1

Supplemental Table S5. Results of using BLAST to compare *E. coli* MarR and MarA to all H37Rv proteins.