

Supporting information

Figure legends:

Figure S1. Generation and confirmation of *mcr11* knock-out and complementation strains. A. A diagram of the *mcr11* locus and the relative position of the hygromycin resistance cassette. Lines with arrows indicated the DNA sequence included in the various listed complementation strains. B. PCR confirmation of *mcr11* knockout and complementation strains of BCG. C. PCR confirmation of *mcr11* knockout and complementation strains of Mtb. D. Northern blot analysis of the indicated strains of BCG from 3 μg of total RNA harvested from late stationary phase cultures grown in hypoxic (1.3% O_2 , 5% CO_2), shaking conditions. E. Northern blot analysis of the indicated strains of Mtb from 5 μg of total RNA harvested from late-log phase cultures grown in hypoxic (1.3% O_2 , 5% CO_2), shaking conditions. The number above the lane indicates the identity of each sample. The column to the left of the rows indicates which gene was amplified or which RNA was probed by Northern blot.

Figure S2. FACS analysis of TSEs in Msm. FACS analysis of GFPv fluorescence of 20,000 events for each sample as indicated by the label to the left of the histogram. Representative of three independent repeats.

Figure S3. *abmR* contributes to the termination of Mcr11 transcripts. A. GFPv fluorescence assay used to measure *mcr11* promoter activity in mid-log phase Mtb in hypoxic (1.3% O_2 , 5% CO_2), shaking conditions. Fluorescence is normalized to the OD_{620} of each sample. B. The % termination in mid-log phase.

The various TSE constructs tested are indicated underneath the corresponding bar. C. Promoter reporter assay as in (A), but in late stationary phase. D. The % termination, as in (B), but in late stationary phase. Results representative of 3 biological replicates. Statistical analysis conducted with a one way ANOVA with Bonferroni correction for multiple comparisons. . Comparison made versus *MtbΔabmR* in A. and C., and versus Wt in B. and D. Asterisks indicate significance as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Figure S4. Termination at a sub-optimal TSE does not require a trans-gene for stabilization of Mcr11. A. A schematic of the transgene fusion constructs. *Mcr11* complementation was provided with (1) or without (2) the transgene GFPv fused to the end of *mcr11* downstream of sub-optimal terminator TSE1. An *Mcr11* complementation construct was also tested with TSE3 (3). B. Northern blot analysis of *Mcr11* expression in *Mtb* grown to late stationary phase in hypoxia (1.3% O₂, 5% CO₂). HisT was used as a loading control. Results of densitometry analysis are presented below the corresponding blot. The red line delineates the limit of detection. Results are the representative of 2-3 biological replicates.

Figure S5. Secondary structure of Mcr11 RNA generated by multiple modeling algorithms. A. The CentroidFold secondary structure using the CONTRAfold inference engine. B. The -54.40 kcal/mol MFE structure generated using Mfold. C. The -45.40 kcal/mol MFE structure generated using NUPACK. D.

The RNAfold centroid structure. E. The -54.00 kcal/mol MFE structure generated using RNAfold. F. The -53.80 kcal/mol MFE structure generated using RNAstructure. G. The ensemble centroid structure generated using Sfold. H. The -54.40 kcal/mol MFE structure generated using Sfold.

Figure S6. Regulation of Rv3280 and Rv3281 expression in Mtb. Mtb was grown for 12 days in under hypoxic, shaking conditions in -OA media. Gene expression was measured by qRT-PCR and normalized to the reference gene *sigA*. Comparison made of each strain versus *Mtb* Δ *mcr11*. Representative of three biological repeats.

Figure S7. Regulation of predicted targets of Mcr11 is condition specific in BCG and Mtb. A. BCG grown for 12 days in under hypoxic (1.3% O₂, 5% CO₂), shaking conditions in Middlebrook 7H9 + 0.2% glycerol, 10% OADC, and 0.05% Tyloxapol (+OA). Gene expression was measured by qRT-PCR and normalized to the reference gene *sigA*. G.-H. Mtb was grown for 7 days in under hypoxic (1.3% O₂, 5% CO₂), shaking conditions in Middlebrook 7H9 + 0.2% glycerol, 10% OADC, and 0.05% Tween (+OA) and treated with vehicle control (Control) or 10 mM dibutyryl cAMP on day 3 (+db cAMP). Gene expression was measured by qRT-PCR and normalized to the reference gene *sigA*. Representative of 2-3 biological repeats. Statistical analysis conducted with an unpaired, 2-tailed Student's t-test. Comparison made of each strain versus Δ *mcr11*. Asterisks indicate significance as follows: * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

Figure S1.

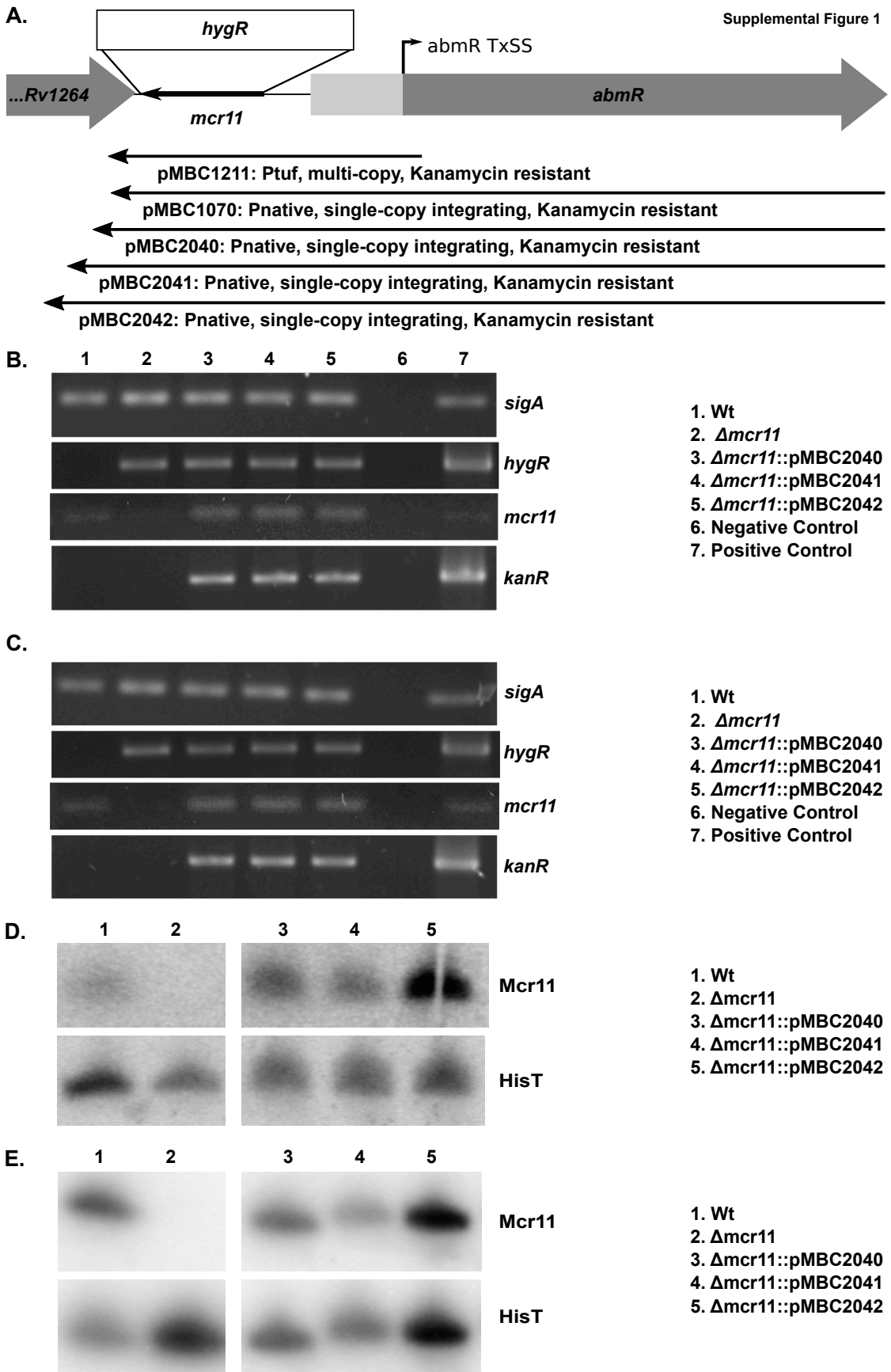


Figure S2.

Supplemental Figure 2

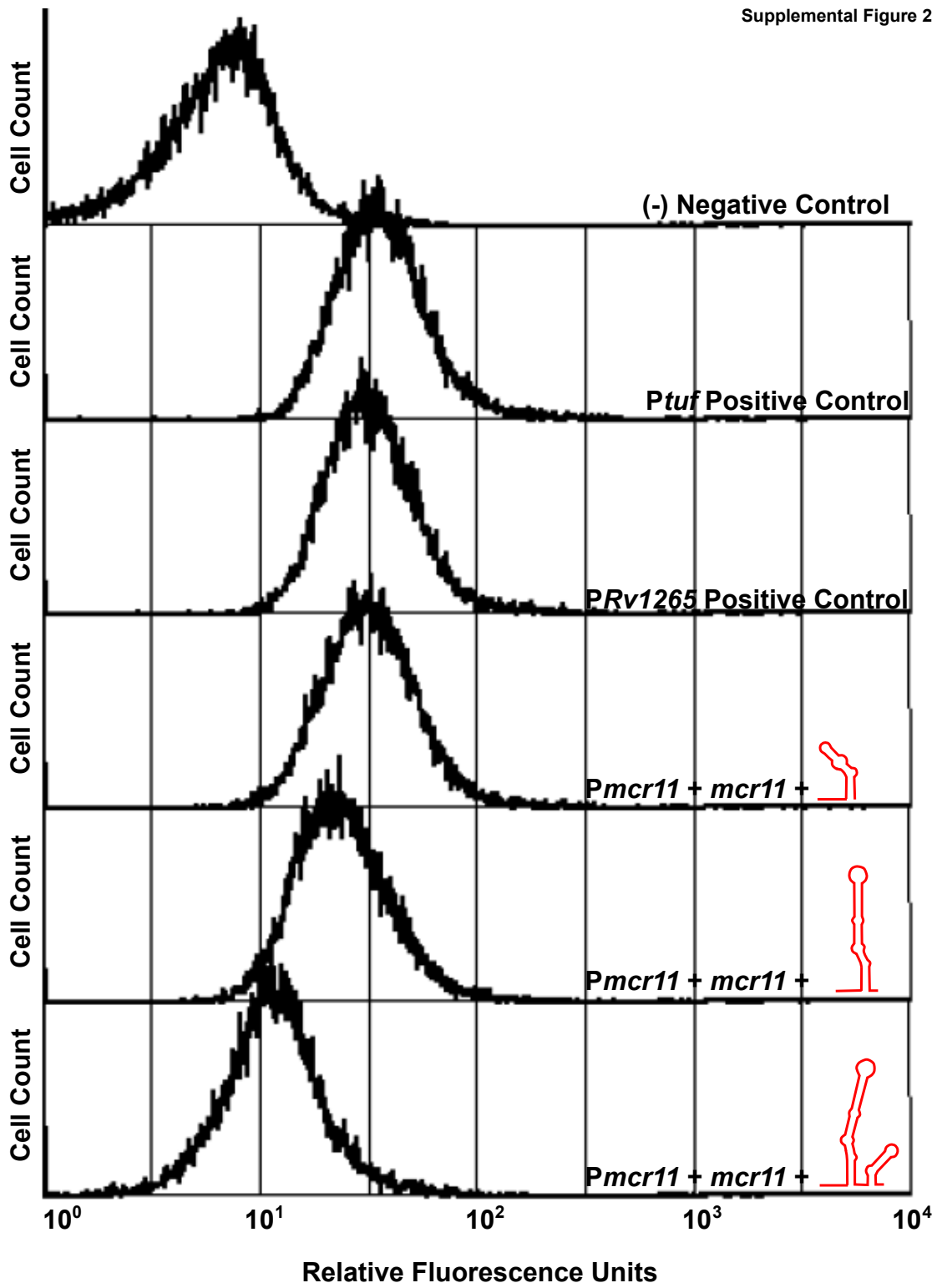


Figure S3.

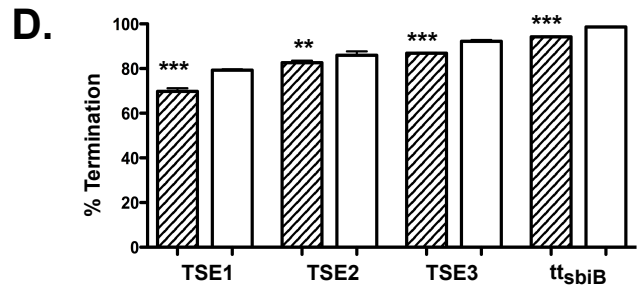
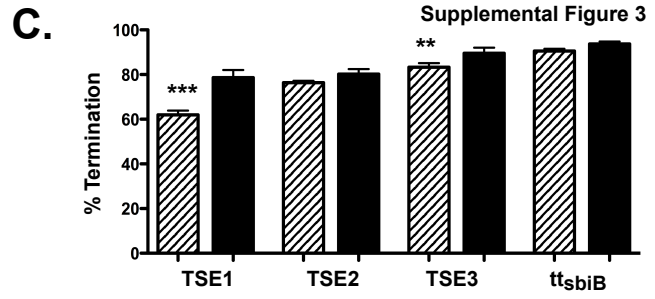
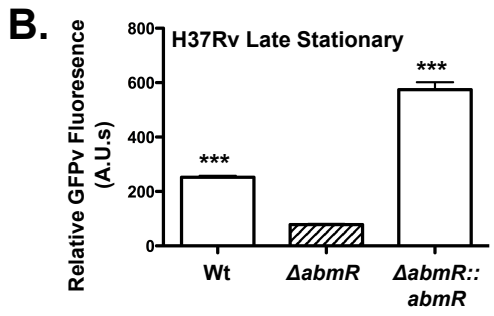
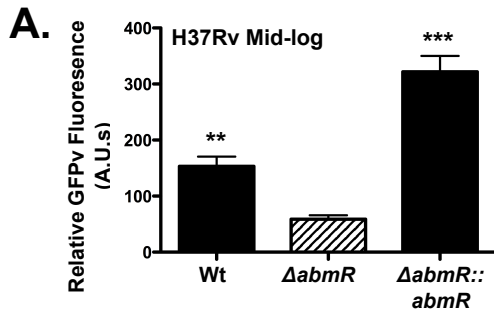
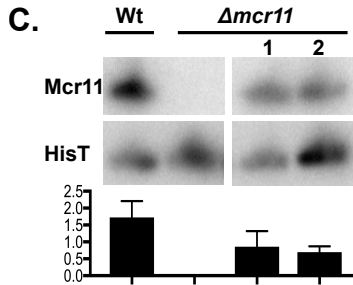
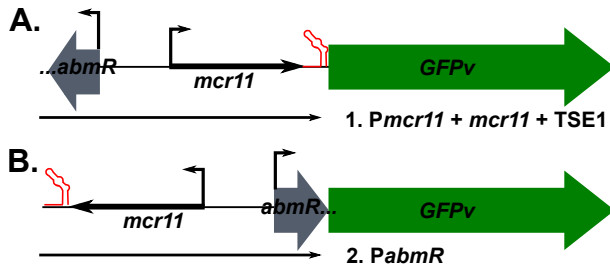
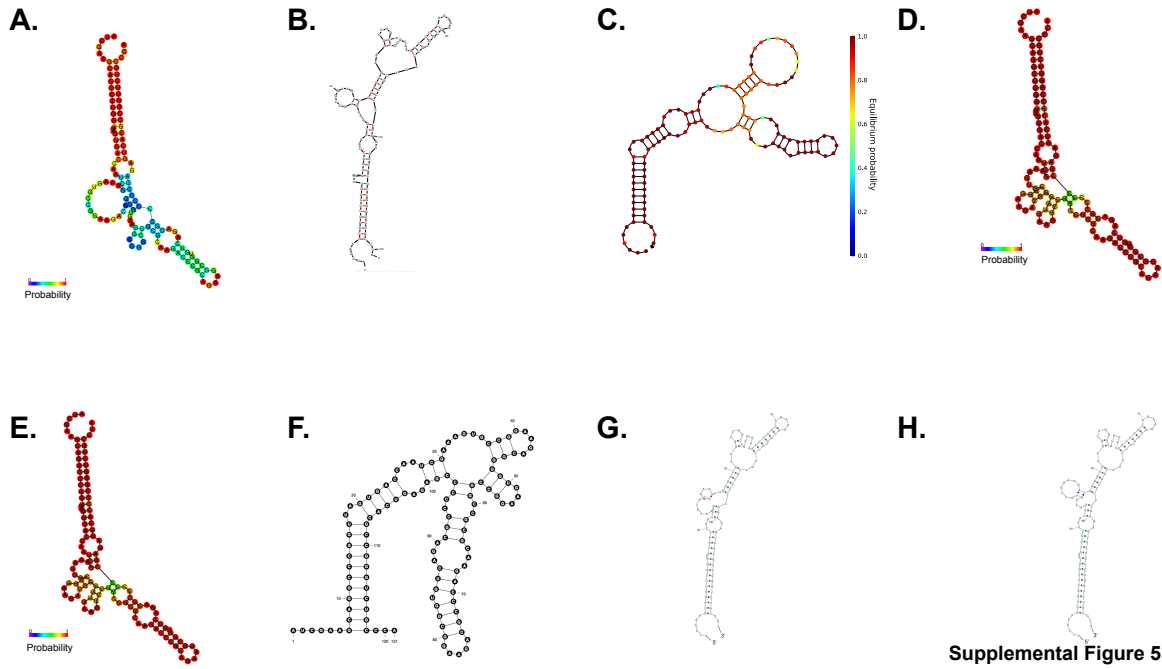


Figure S4.



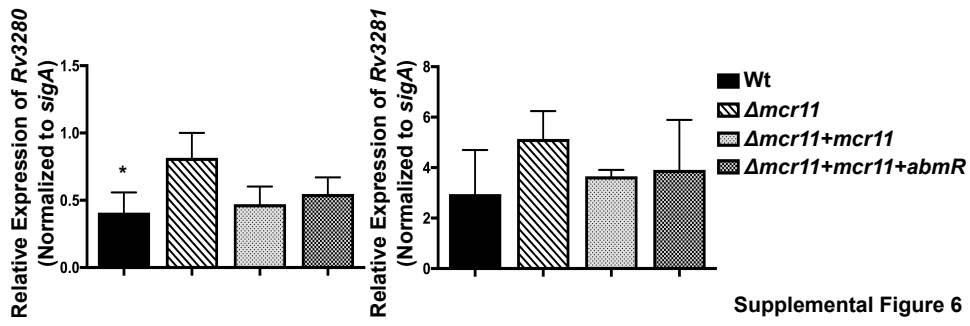
Supplemental Figure 4

Figure S5.



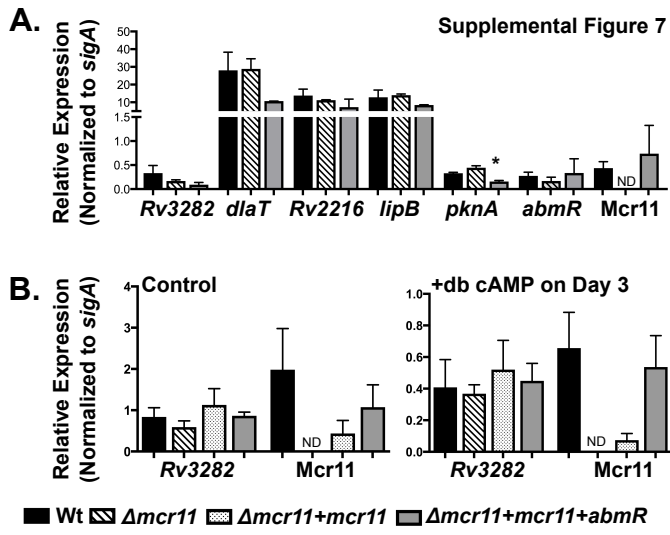
Supplemental Figure 5

Figure S6.



Supplemental Figure 6

Figure S7.



Supplemental Tables:

Table S1. Plasmids used in the study.

Supplemental Table 1 Plasmids used in this study

Plasmid	Description	Selective Markers	Insert Primers	Vector Backbone	E. Coli	Msm	BCG	Mtb
pMBC0562	lacZ gene with a S.D. downstream of a BamHI site	Hyg	None	pLACint	DH50	N/A	Wt	Wt
pMBC0775	PgyrB fused to lacZ reporter	Hyg	KM3829-3830	pMBC562	DH50	N/A	Wt	Wt
pMBC0819a	Rv1265 oriented promoter fusion from the end of Rv1265	Hyg	KM1065-1066	pMBC562	DH50	N/A	Wt	Wt
pMBC1070	Short hairpin mcr11 terminator, mcr11, and the Rv1265 ORF for mcr11 complementation	Kan	KM2658-2659	pMBC409	DH50	N/A	mcr11 KO	N/A
pMBC1211	Ptuf driving mcr11 gene with short hairpin terminator	Kan	KM2927-2928	pMBC280	DH50	N/A	N/A	mcr11 KO
pMBC1216	For disruption of mcr11 by homologous recombination	Hyg, Cab, SucSensitive	KM2919-2920 and KM2921-2922	phAB159	DH50	N/A	mcr11KO	mcr11KO
pMBC1510	mcr11 oriented promoter fusion to lacZ comprised of R	Hyg	KM1128-445	pMBC562	DH50	N/A	Wt	Wt
pMBC1566	Rv1265 oriented promoter fusion from mid-mcr11 to cor	Hyg	KM1128-445	pMBC562	DH50	N/A	Wt	Wt
pMBC1798	Promoterless control of GFPv expression reporter	Kan	none	pMBC304	DH50	Wt	Rv1265KO	Rv1265KO
pMBC1873	Ptuf fused to GFPv reporter	Kan	KM512-513	pMBC1798	DH50	Wt	Wt, Rv1265KO	Wt, Rv1265KO
pMBC1877	PgyrB fused to GFPv reporter	Kan	KM3829-3830	pMBC1798	DH50	N/A	Wt, mcr11 KO	Wt, mcr11KO, Rv1265KO
pMBC1902	Pmcr11 with the short hairpin terminator fused to GFPv reporter	Kan	KM1066-1128	pMBC1798	DH50	Wt	Wt, mcr11 KO	Wt, mcr11KO, Rv1265KO
pMBC1903	PRv1265 fused to GFPv, included mcr11 short hairpin terminator	Kan	KM1066-1128	pMBC1798	DH50	N/A	N/A	mcr11KO
pMBC1911	Pmcr11 with the uneven "U" terminator fused to GFPv reporter	Kan	KM4516-1128	pMBC1798	DH50	Wt	Wt, mcr11 KO	Wt, mcr11KO, Rv1265KO
pMBC1914	Pmcr11 with the "I" terminator fused to GFPv reporter	Kan	KM4515-1128	pMBC1798	DH50	Wt	mcr11 KO	Wt, Rv1265KO
pMBC1988	FlipB internal promoter fused to GFPv reporter	Kan	KM4032-4033	pMBC1798	DH50	N/A	N/A	Wt
pMBC2007	FlipB internal promoter with mutation made to Mcr11 interaction sequence fused to GFPv reporter	Kan	KM4032-4033 and 4109-4110	pMBC1798	DH50	N/A	N/A	Wt
pMBC2008	Pmcr11 with the uneven "V" poly A terminator fused to GFPv reporter	Kan	KM4517-1128	pMBC1798	DH50	Wt	Wt, mcr11 KO	mcr11KO
pMBC2040	Short "I" mcr11 terminator, mcr11, and the Rv1265 ORF for mcr11 complementation	Kan	KM4514-3498	pMBC409	DH50	N/A	mcr11 KO	mcr11 KO
pMBC2041	Uneven "U" mcr11 terminator, mcr11, and the Rv1265 ORF for mcr11 complementation	Kan	KM4515-3498	pMBC409	DH50	N/A	mcr11 KO	mcr11 KO
pMBC2042	Uneven "V" poly A mcr11 terminator, mcr11, and the Rv1265 ORF for mcr11 complementation	Kan	KM4517-3498	pMBC409	DH50	N/A	mcr11 KO	mcr11 KO
pMBC2066	Pmcr11 with the synthetic terminator fused to GFPv	Kan	KM5031-1128	pMBC1798	DH50	Wt	mcr11 KO	Wt, mcr11KO, Rv1265KO
pMBC2067	Uneven "V" poly A mcr11 terminator, mcr11 and Rv1265 ORF	Kan	KM4517-3498	pMBC1798	DH50	Wt	N/A	N/A
pMBC2069	Pmcr11 fused to GFPv reporter	Kan	KM4451-1128	pMBC1798	DH50	Wt	Wt	Wt, Rv1265KO
pMBC2071	Pmcr11 with the Rv1265 ORF behind it, fused to GFPv	Kan	KM4451-3498	pMBC1798	DH50	Wt	N/A	Rv1265KO

Table S2. Primers used in the study.

Supplemental Table 2 Primers used in this study		
Primer	Purpose	Sequence 5' to 3'
KM0512	Ptuf for promoter fusion studies	GGATCCACACCCGAGGACTACATGGG
KM0513	Ptuf for promoter fusion studies	GGATCCTGGTCCGATGTTGACGTGG
KM0816	Kanamycin resistance cassette	AGCAGGTAGCTTGCAAGTGGG
KM0817	Kanamycin resistance cassette	CGGCCACAGTCGATGAATCC
KM1066	For short hairpin terminator of mcr11	GGATCCCCGGCTCGTACCCTAGGC
KM1128	mcr11-Rv1265 intergenic region for promoter fusion assays	GCGGATCCCGTCATGTGATGGTGCC
KM1309	sigA ORF, used to QC RNA, as a PCR control	GATGACCCGAGCTTAGCGAGC
KM1310	sigA ORF, used to QC RNA, as a PCR control	CGTAGTGGAGAACTTGATACC
KM2327	Hygromycin resistance cassette	CTCGCGGTCCCGGGAAGGC
KM2328	Hygromycin resistance cassette	AGCCGGACCCGGTGATCAAGC
KM2658	For overexpression of Rv1265 from its native promoter	AAGCTTGGATTTCAGTGGTCTTCGC
KM2659	For overexpression of Rv1265	AAGCTTCCGTTTAGCCGGAAGATCC
KM2919	For mcr11 KO, upstream sequence	GCGGCCGCAATAAGGCCAAGACCTACC
KM2920	For mcr11 KO, upstream sequence	GCGGCCGCAAAAAATACAACGTCGAATGG
KM2921	For mcr11 KO, downstream sequence	CCATAGATTGGAGAGTCACGCCGGTCTGCC
KM2922	For mcr11 KO, downstream sequence	CCATCTTTTGGACACGGCACCTACGTATCC
KM2927	Rv1264-165 intergenic region	GGATCCGCCCGTCATGTGATGG
KM2928	Rv1264-165 intergenic region for run-off transcription	AAGCTTGGCGGCTCGTACCCTAG
KM3378	mcr11 sequence for checking KO and complementation	ATCGAAGCAGGCCGGTTAGTGAC
KM3379	mcr11 sequence for checking KO and complementation	ACACCGGTACACATGGGCAGAC
KM3498	For overexpression of Rv1265	GGATCCCCGTTTAGCCGGAAGATCC
KM3829	PgyrB for promoter fusion studies	ACGAAGCGGATCCGTTATG
KM3830	PgyrB for promoter fusion studies	AGGATCCGAATACTCTCC
KM4404	Probe for HisT on Northern Blots	AACCCGGGACAGCCAGGATCACAAAC
KM4451	mcr11-Rv1265 intergenic region for promoter fusion assays	GGATCCGATACAAAAATACAACGCT
KM4504	PpknA/B for promoter fusion studies	GGATCCGACAGCCCGCACTAGGG
KM4505	PpknA/B for promoter fusion studies	GGATCCGCTCATGGTTCCTCCCTGCA
KM4514	Reverse primer with short "I" terminator for mcr11	GGATCCCGGCCCAAGACGACGATTTG
KM4515	Reverse primer for long "I" terminator for mcr11	GGATCCCTCCGGCGCCGCCGCCAA
KM4516	Reverse primer for uneven "U" terminator for mcr11	GGATCCGTCGGCGGAGGGGC
KM4517	Reverse primer for uneven "V" with poly A terminator for mcr11	GGATCCTTTTTCGAGTCCGGCGAGGGGC
KM4521	Probe for Mcr11 on Northern Blots	GCGTGACTCTCGGGGGCGTCTGA
KM5031	Synthetic terminator for mcr11	GGATCCAAAAAAAAGCCCGCAATCGCGGGCACCCGGTACACATGG
KM5295	For mapping Mcr11's 3' end	GAAGCAGGCCGGTGTAGTACCAATCGAAAGTGCC
KM4653	sigA ORF qRT-PCR	TCAAACAGATCGGCAAGGTAG
KM4654	sigA ORF qRT-PCR	CGCTAAGCTCGGTATCAG
KM4690	mcr11 qRT-PCR	ATCGAAAGTCCGGAAGAC
KM4691	mcr11 qRT-PCR	CGGTACACATGGGCAGAC
KM4694	gyr ORF qRT-PCR	CTGCACCCGACAAAGTTAAG
KM4695	gyr ORF qRT-PCR	GTCCACGATGCTGCTATGAA
KM4698	pknA ORF qRT-PCR	CGTCTATTCACTGGGAGTTGTT
KM4699	pknA ORF qRT-PCR	GCTCCTTGATGTGCTTCATTG
KM4846	tuf ORF qRT-PCR	CGAGGACGCTCTTCAACATTAC
KM4847	tuf ORF qRT-PCR	ATGCCGACGATCTCAACTTC
KM5168	Rv2216 ORF qRT-PCR	GGGTTCTGTGGCTTGAT
KM5169	Rv2216 ORF qRT-PCR	GGGATCCAGTCAGTCTT
KM5170	lipB ORF qRT-PCR	CGACACTGCACGGGTTT
KM5171	lipB ORF qRT-PCR	CTCGCGGTCCTACTGAT
KM5192	Rv3282 ORF qRT-PCR	TACATCGAAGGCAGGCTACT
KM5193	Rv3282 ORF qRT-PCR	TTGTCTCGCAACCGGATAAC
KM5032	dlaT ORF qRT-PCR	GACCTTCTGCGGTTCTTC
KM5033	dlaT ORF qRT-PCR	CTGTTGTAGCTAGCGTTGAT
KM5287	Rv3280 ORF qRT-PCR	ACCTGCGACTGCTTCAATATC
KM5288	Rv3280 ORF qRT-PCR	GGATGATGCCGTTGTATCTCT
KM5291	Rv3281 ORF qRT-PCR	CGGGAACGAGACGAACAAT
KM5292	Rv3281 ORF qRT-PCR	CGTTCCGCTCACTCACTTC

Table S3a. Impact of stress on efficiency of *mcr11* termination in H37Rv.

Table S3b. Impact of stress on efficiency of *mcr11* termination in BCG.

Table S3c. Impact of stress on efficiency of *mcr11* termination in delta H37Rv_delta_ *abmR*

Supplemental Table 3A Impact of stress on *mcr11* termination in H37Rv

H37Rv	TSE1	TSE2	TSE3	ttsbiB
Mid-Log	71.1 ± 2.54	79.6 ± 7.62	88.8 ± 2.05	95.0 ± 2.80
Late Stationary	78.8 ± 1.62	84.8 ± 1.94	92.4 ± 0.78	98.5 ± 0.23
DMSO	77.1 ± 3.08	82.4 ± 3.03	91.7 ± 1.17	97.9 ± 0.47
DETA-NO	73.3 ± 2.18	75.2 ± 6.83	88.4 ± 4.23	97.3 ± 1.24
BDQ	73.8 ± 6.24	81.9 ± 3.91	91.6 ± 1.88	97.6 ± 1.03
RIF	76.2 ± 8.25	82.7 ± 3.95	90.9 ± 2.89	95.6 ± 3.35

Supplemental Table 3B Impact of stress on *mcr11* termination in BCG

BCG	TSE1	TSE3	TSE4
Mid-Log	39.6 ± 4.35	72.3 ± 2.87	66.6 ± 1.69
Late Stationary	69.6 ± 2.54	84.8 ± 3.16	78.4 ± 7.54
DMSO	69.8 ± 0.89	84.6 ± 2.95	78.4 ± 5.89
DETA-NO	67.7 ± 4.84	83.8 ± 2.70	74.8 ± 8.45
BDQ	71.8 ± 3.29	85.1 ± 3.53	82.0 ± 5.39
RIF	67.1 ± 4.37	84.0 ± 1.65	78.7 ± 2.65

Supplemental Table 3C Impact of stress on *mcr11* termination in Δ*abmR* H37Rv

Condition	H37Rv TSE1	Δ <i>abmR</i> TSE1
Mid-Log	71.1 ± 2.54	47.7 ± 2.57
Late Stationary	78.8 ± 1.62	68.27 ± 2.85
DMSO	77.1 ± 3.08	62.1 ± 7.36
DETA-NO	73.3 ± 2.18	64.9 ± 0.15
BDQ	73.8 ± 6.24	69.1 ± 1.67
RIF	76.2 ± 8.25	62.8 ± 3.56

(Comparison of the mean % termination of each TSE with standard deviation of 3 biological replicates after 24 hour exposure to a variety of stress conditions: (DMSO vehicle control, nitric oxide (DETA-NO), bedaquiline (BDQ), or rifampicin (RIF)).

Table S4. Results of TargetRNA and TargetRNA2 predictions of Mcr11 regulatory targets.

Supplemental Table 4 Bioinformatically predicted targets of Mcr11 regulation

Rv #	Gene	Gene Description	Mapped 3' Ends	Mapped 5' Ends*	Size Estimates
McRv11264c	Mcr11	Stable non-coding RNA	1413106	1413224	117nt
			1413107	1413227	121nt

*DiChiara, 2010

TargetRNA Hits

Rank	Rv #	Gene	Gene Description	Score	P-value	Base Pairing in Mcr11 Start	Base Pairing in Mcr11 Stop	Base pairing in mRNA relative to the Start Codon Start	Base pairing in mRNA relative to the Start Codon Stop	TSS Category (Position of Gene/ Number of Genes in Operon)	Relative Position of Putative Base-Pairing	5' mRNA Start	Gene Start	Gene Stop	Essentiality
1*	Rv1265	CHP	Regulatory Protein	-79 ^b	0	14	34	-67	-67	Leaderless (1/1)	otherwise in the 5' UTR	1413371 ^c	1413260	1413950	Non-essential
2	Rv1074c	fadA3	Beta-ketoadyl CoA thiolase	-59	0.00246	40	54	-33	-19	S'UTR (1/1)	In the S'UTR	1199411	1198156	1199373	Non-essential
3	Rv2317	lipB	Lipoate biosynthesis protein B	-54	0.00685	41	54	-51	-38	SD Operon (3/3)	Interruption between Rv2216 stop codon and lipB start codon	2481870	2484584	2485276	Essential

Target RNA2 Hits

Rank	Rv #	Gene	Gene Description	Energy	P-value	Base Pairing in Mcr11 Start	Base Pairing in Mcr11 Stop	Base pairing in mRNA relative to the Start Codon Start	Base pairing in mRNA relative to the Start Codon Stop	TSS Category (Position of Gene/ Number of Genes in Operon)	Relative Position of Putative Base-Pairing	5' mRNA Start	Gene Start	Gene Stop	Essentiality
1	Rv1265	CHP	Regulatory Protein	-20.11 ^d	0.000	14	34	-67	-67	Leaderless (1/1)	otherwise in the 5' UTR	1413149	1413260	1413940	Non-essential
2	Rv1138c	CHP	Oxidoreductase	-17.54	0.005	44	60	-75	-59	Unknown	Unknown	1265472	1266488		Non-essential
3	Rv2831	echA16	Enoyl-CoA hydratase	-11.42	0.011	43	57	-70	-55	Leaderless	No overlap if leaderless otherwise in the 5' UTR	3137271	3137271	3138020	Non-essential
4	Rv2103c	vapC37	Ribonuclease	-10.46	0.018	6	19	-79	-65	Leaderless Operon (2/2)	Within the open reading frame of the preceding gene (vapB37)	2364781	2364086	2364520	Non-essential
5	Rv0291	mycP3	Membrane-anchored mucosin, ES6-3	-10.03	0.023	36	56	-55	-36	SD Operon (8/8)	Within the open reading frame of the preceding gene (eccD3)	345432	354498	355883	Essential
6	Rv1079c	CHP	Conserved hypothetical protein	-9.86	0.025	53	65	-57	-65	Unknown	Unknown	Unknown	341770	349257	Non-essential
7	Rv0924c	mntH	Divalent metal cation transporter	-9.71	0.027	49	67	-68	-51	Unknown	Unknown	Unknown	1030578	1031864	Non-essential
8	Rv1953	vapC14	Ribonuclease	-9.41	0.031	38	57	-18	2	S' UTR Operon (2/2)	Within the open reading frame of the preceding gene (vapB14) and overlapping the start codon of vapC14	2200712	2200938	2201249	Non-essential
9	Rv2645	CHP	Conserved hypothetical protein	-9.05	0.036	47	60	-78	-64	S' UTR (1/5)	In the S'UTR	2969669	2970123	2970554	Non-essential
10	Rv1991A	maf66	Antitoxin to MazF6/MazE63	-8.91	0.039	36	51	-42	-26	Leaderless Operon (1/4)	No overlap if leaderless otherwise in the 5' UTR	2234891	2234643	2234891	Unknown
11	Rv2382	CHP	Similarity to septum-site inhibition protein Hsf	-8.72	0.042	39	55	-60	-44	Leaderless Operon (3/3)	Within the open reading frame of the preceding gene (eccE5)	3662062	3664219	3664887	Slow growth mutant in vitro
12	Rv0979c	CHP	Conserved hypothetical protein	-8.6	0.044	45	62	-22	-6	Unknown	Unknown	Unknown	1094670	1094804	Non-essential

a Targets are ranked in order of ascending P-value

b Score of the predicted sRNA:mRNA interaction, as computed by TargetRNA

c P-value for a target corresponds to the likelihood of observing as strong an interaction by chance

d Transcriptional boundaries tabulated from Cortes et al, 2013

e Thermodynamic energy (kcal/mol) of hybridization between the two RNA molecules

Red Indicates inhibits with leaderless transcripts, possibly lacking the putative Mcr11 interaction sequence due to leaderless feature

Blue Genes selected for follow-up in this study