Figure S1. Sequence of *dcas9* (*cas9* D10A and H840A) codon-optimized for *M. tuberculosis*.*

ATGGACAAGAAGTACAGCATCGGCCTG**GCG**ATCGGCACGAACTCGGTGGGCTGGGCGGTCATCACGGACGAGTACAA
GGTCCCCTCCAAGAAGTTCAAGGTCCTGGGCAACACCGACCGGCACAGCATCAAGAAGAACCTGATCGGCGCCCTGC
TGTTCGACTCCGGCGAGACCGCCGAGGCGACGCGCCTGAAGCGGACCGCGCGGCGCCGATACACGCGGCGCAAGAAC
CGCATCTGCTACCTGCAGGAAATCTTCTCGAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACCGGCTGGAGGA
GTCCTTCCTGGTGGAGGAGGACAAGAAGCACGAGCGCCACCCGATCTTCGGCAACATCGTGGACGAGGTGGCCTACC
ACGAGAAGTACCCCACGATCTACCACCTGCGCAAGAAGCTGGTGGACAGCACCGACAAGGCGGACCTGCGGCTGATC
TACCTGGCCCTGGCGCACATGATCAAGTTCCGCGGCCACTTCCTGATCGAGGGCGACCTGAACCCGGACAACTCGGA
CGTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAGAACCCCATCAACGCCTCCGGCG
TGGACGCCAAGGCGATCCTGTCGGCGCGCCTGTCCAAGTCGCGGCGCCTGGAGAACCTGATCGCCCAGCTGCCGGGC
GAGAAGAAGAACGGCCTGTTCGGCAACCTGATCGCGCTGAGCCTGGGCCTGACGCCCAACTTCAAGTCCAACTTCGA
CCTGGCCGAGGACGCGAAGCTGCAGCTGTCGAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCCCAGATCG
GCGACCAGTACGCGGACCTGTTCCTGGCCGCCAAGAACCTGAGCGACGCCATCCTGCTGTCCGACATCCTGCGGGTC
AACACCGAGATCACGAAGGCCCCGCTGAGCGCGTCCATGATCAAGCGGTACGACGAGCACCACCAGGACCTGACCCT
GCTGAAGGCGCTGGTGCGCCAGCAGCTGCCCGAGAAGTACAAGGAAATCTTCTTCGACCAGTCGAAGAACGGCTACG
CCGGCTACATCGACGGCGGCGCGAGCCAGGAGGAGTTCTACAAGTTCATCAAGCCGATCCTGGAGAAGATGGACGGC
ACGGAGGAGCTGCTGGTCAAGCTGAACCGCGAGGACCTGCTGCGCAAGCAGCGGACCTTCGACAACGGCTCGATCCC
GCACCAGATCCACCTGGGCGAGCTGCACGCCATCCTGCGGCGCCAGGAGGACTTCTACCCCTTCCTGAAGGACAACC
GCGAGAAGATCGAGAAGATCCTGACCTTCCGCATCCCGTACTACGTGGGCCCCCTGGCCCGCGGCAACTCGCGGTTC
GCGTGGATGACCCGGAAGAGCGAGGAGACCATCACGCCGTGGAACTTCGAGGAGGTCGTGGACAAGGGCGCCTCGGC
GCAGAGCTTCATCGAGCGCATGACCAACTTCGACAAGAACCTGCCGAACGAGAAGGTCCTGCCCAAGCACAGCCTGC
TGTACGAGTACTTCACCGTGTACAACGAGCTGACGAAGGTCAAGTACGTGACCGAGGGCATGCGGAAGCCGGCCTTC
CTGTCGGGCGAGCAGAAGAAGGCGATCGTGGACCTGCTGTTCAAGACCAACCGCAAGGTCACGGTGAAGCAGCTGAA
GGAGGACTACTTCAAGAAGATCGAGTGTTTCGACTCGGTCGAGATCAGCGGCGTGGAGGACCGCTTCAACGCCAGCC
TGGGCACCTACCACGACCTGCTGAAGATCATCAAGGACAAGGACTTCCTGGACAACGAGGAGAACGAGGACATCCTG
GAGGACATCGTCCTGACCCTGACGCTGTTCGAGGACCGCGAGATGATCGAGGAGCGGCTGAAGACGTACGCCCACCT
GTTCGACGACAAGGTGATGAAGCAGCTGAAGCGGCGCCGATACACCGGCTGGGGCCGCCTGTCGCGGAAGCTGATCA
ACGGCATCCGGGACAAGCAGTCCGGCAAGACCATCCTGGACTTCCTGAAGTCGGACGGCTTCGCGAACCGCAACTTC
ATGCAGCTGATCCACGACGACAGCCTGACCTTCAAGGAGGACATCCAGAAGGCCCAAGTGAGCGGCCAGGGCGACTC
CCTGCACGAGCACATCGCCAACCTGGCGGGCTCGCCGGCGATCAAGAAGGGCATCCTGCAGACCGTCAAGGTCGTGG
ACGAGCTGGTCAAGGTGATGGGCCGCCACAAGCCCGAGAACATCGTGATCGAGATGGCCCGGGAGAACCAGACCACG
CAGAAGGGCCAGAAGAACAGCCGCGAGCGGATGAAGCGCATCGAGGAGGGCATCAAGGAGCTGGGCTCGCAGATCCT
GAAGGAGCACCCGGTCGAGAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAACGGCCGCGACA
TGTACGTGGACCAGGAGCTGGACATCAACCGGCTGAGCGACTACGACGTGGAC**GCC**ATCGTGCCGCAGTCGTTCCTG
AAGGACGACAGCATCGACAACAAGGTCCTGACCCGCTCGGACAAGAACCGGGGCAAGTCGGACAACGTGCCCAGCGA
GGAGGTCGTGAAGAAGATGAAGAACTACTGGCGCCAGCTGCTGAACGCCAAGCTGATCACGCAGCGCAAGTTCGACA
ACCTGACCAAGGCCGAGCGGGGCGGCCTGAGCGAGCTGGACAAGGCGGGCTTCATCAAGCGCCAGCTGGTCGAGACC
CGGCAGATCACGAAGCACGTGGCGCAGATCCTGGACTCGCGGATGAACACGAAGTACGACGAGAACGACAAGCTGAT
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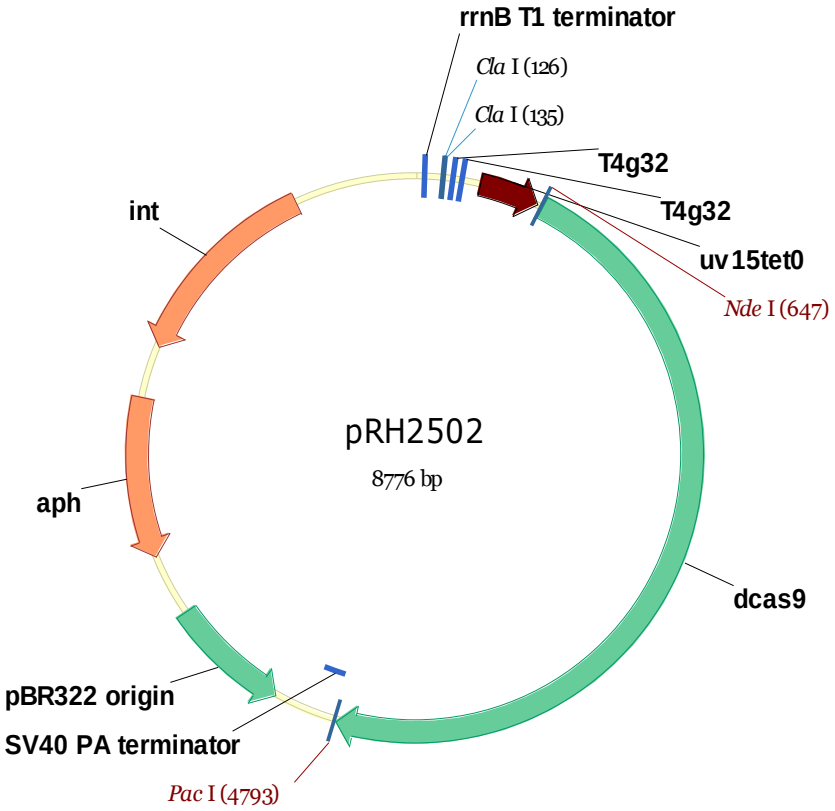
GGGAGATCAACAACTACCACCACGCCCACGACGCGTACCTGAACGCCGTCGTGGGCACCGCGCTGATCAAGAAGTAC
CCGAAGCTGGAGTCGGAGTTCGTCTACGGCGACTACAAGGTCTACGACGTGCGCAAGATGATCGCCAAGAGCGAGCA
GGAGATCGGCAAGGCCACCGCGAAGTACTTCTTCTACTCCAACATCATGAACTTCTTCAAGACCGAGATCACGCTGG
CCAACGGCGAGATCCGCAAGCGCCCGCTGATCGAGACCAACGGCGAGACGGGCGAGATCGTCTGGGACAAGGGCCGC
GACTTCGCGACCGTCCGGAAGGTGCTGAGCATGCCGCAGGTCAACATCGTGAAGAAGACCGAGGTGCAGACGGGCGG
CTTCTCCAAGGAGTCGATCCTGCCCAAGCGCAACTCGGACAAGCTGATCGCCCGGAAGAAGGACTGGGACCCGAAGA
AGTACGGCGGCTTCGACTCCCCCACCGTCGCCTACTCGGTGCTGGTCGTGGCGAAGGTCGAGAAGGGCAAGTCCAAG
AAGCTGAAGTCGGTGAAGGAGCTGCTGGGCATCACCATCATGGAGCGCTCGTCGTTCGAGAAGAACCCGATCGACTT
CCTGGAGGCGAAGGGCTACAAGGAGGTCAAGAAGGACCTGATCATCAAGCTGCCCAAGTACAGCCTGTTCGAGCTGG
AGAACGGCCGCAAGCGGATGCTGGCCTCCGCGGGCGAGCTGCAGAAGGGCAACGAGCTGGCCCTGCCGTCGAAGTAC
GTCAACTTCCTGTACCTGGCGTCGCACTACGAGAAGCTGAAGGGCAGCCCCGAGGACAACGAGCAGAAGCAGCTGTT
CGTGGAGCAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGCGGGTCATCCTGGCCG
ACGCGAACCTGGACAAGGTGCTGTCGGCCTACAACAAGCACCGCGACAAGCCGATCCGGGAGCAGGCGGAGAACATC
ATCCACCTGTTCACCCTGACGAACCTGGGCGCCCCCGCCGCGTTCAAGTACTTCGACACCACGATCGACCGCAAGCG
GTACACCTCCACGAAGGAGGTGCTGGACGCGACCCTGATCCACCAGTCGATCACCGGCCTGTACGAGACGCGCATCG
ACCTGAGCCAGCTGGGCGGCGACTCCCGGGCGGACCCGAAGAAGAAGCGGAAGGTGTGA

*Mutated codons 10 and 840 are shown in red and underlined.

Figure S2. a) Integrating vector for expression *dcas9*; b) Replicating vector for sgRNA and *tetR* expression. The integrating vector pRH2502 for induced expression of *dcas9* is derived from pTC-0X-1L (1), obtained from Addgene. The PacI site near the T4g32 sites was removed. PacI sites were introduced 15 bp after the NdeI site encompassing the start codon of *lacZI*, and 5' of the SV40 PA terminator. *lacZ* was removed and *dcas9* was inserted under the control of uv15tetO promoter at NdeI and PacI cloning sites. The replicative vector for sgRNA and TetR expression pRH2521 was derived from pTE-10M-0X (1) obtained from Addgene. The sequence P*myc1*tetO (2) followed by 2 BbsI sites and the sgRNA sequences were synthesized and cloned in pTE-10M-0X at a ClaI site ro produce pRH2521.

a



b

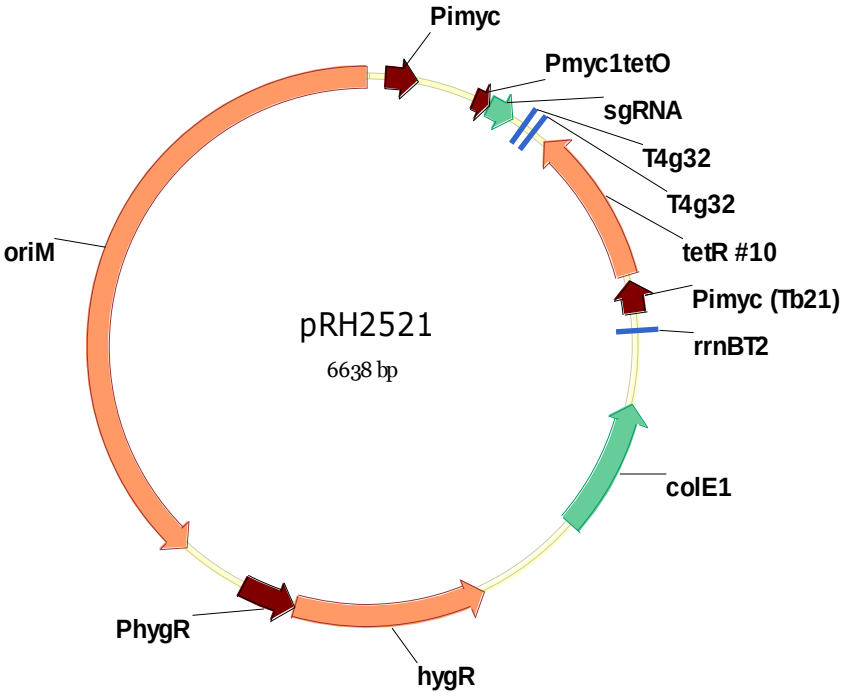


Figure S3. Cloning strategy for construction of vectors to express sgRNAS to target genes of interest. The example below incorporates sequences to target *M. smegmatis sigH*. The identical strategy is used to target genes in *M. tuberculosis* or *M. smegmatis.*

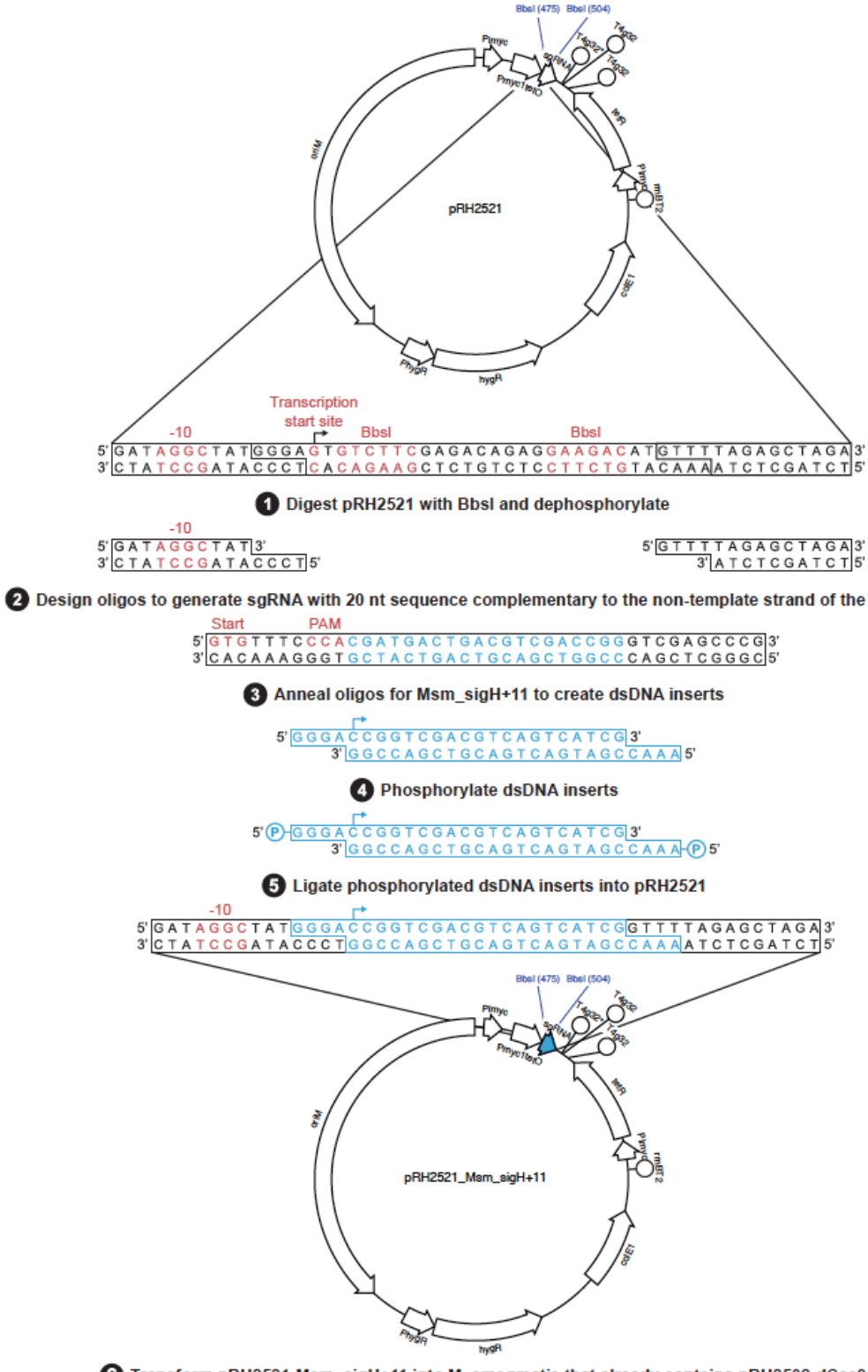


Figure S4. a. Transcription start sites identified by 5' RACE. b. Locations targeted by sgRNAs in 4 essential genes of *M. tuberculosis.* The locations in each gene targeted by CRISPRi are indicated by the vertical purple line. Numbering is the position of the first nt of the targeted sequence relative to the initiation codon.

a

	-35	-10	+1		
Rv1484-*inhA*	GATGAGC**GGATTC**AGCAGGGGGCGCTGCAA**TTTATC**CCAGCGAAG**C**GGGTCGG			-127/ATG	This study
Rv2763c-*dfrA*	CGCGGTATGAGGCGCGCCGACGACAATGAAGTCGGCTCGGACAG**C**CTTCAAGC			-36/ATG	This study
Rv2145c-*wag31*	TGGCGCGCACGATTTCAAATCTAAGGATTGTGATTTATTTGGTCTT**A**GAGTTTGA			-147/ATG	This study
Rv2150c-*ftsZ*	ACTTGACATAACTCTAAGCCTATGGTTGAGGTTGAGAGTTTGCCAGCA**G**ACACA			-43/ATG	(3)

b

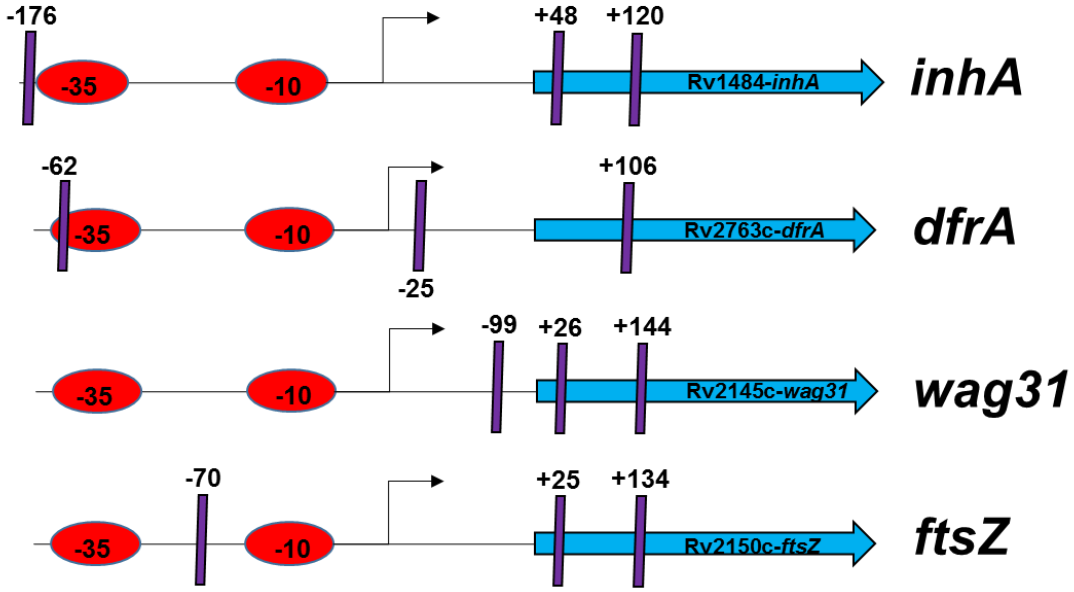


Figure S5. Determination of minimum inhibitory concentration of (a) isoniazid (INH) and (b) methotrexate (MTX) in *M. tuberculosis* strains expressing *dcas9* and either gene-specific sgRNAs or the sgRNA vector control. The Microplate Alamar Blue Assay (MABA) was performed as described in the materials and methods section.

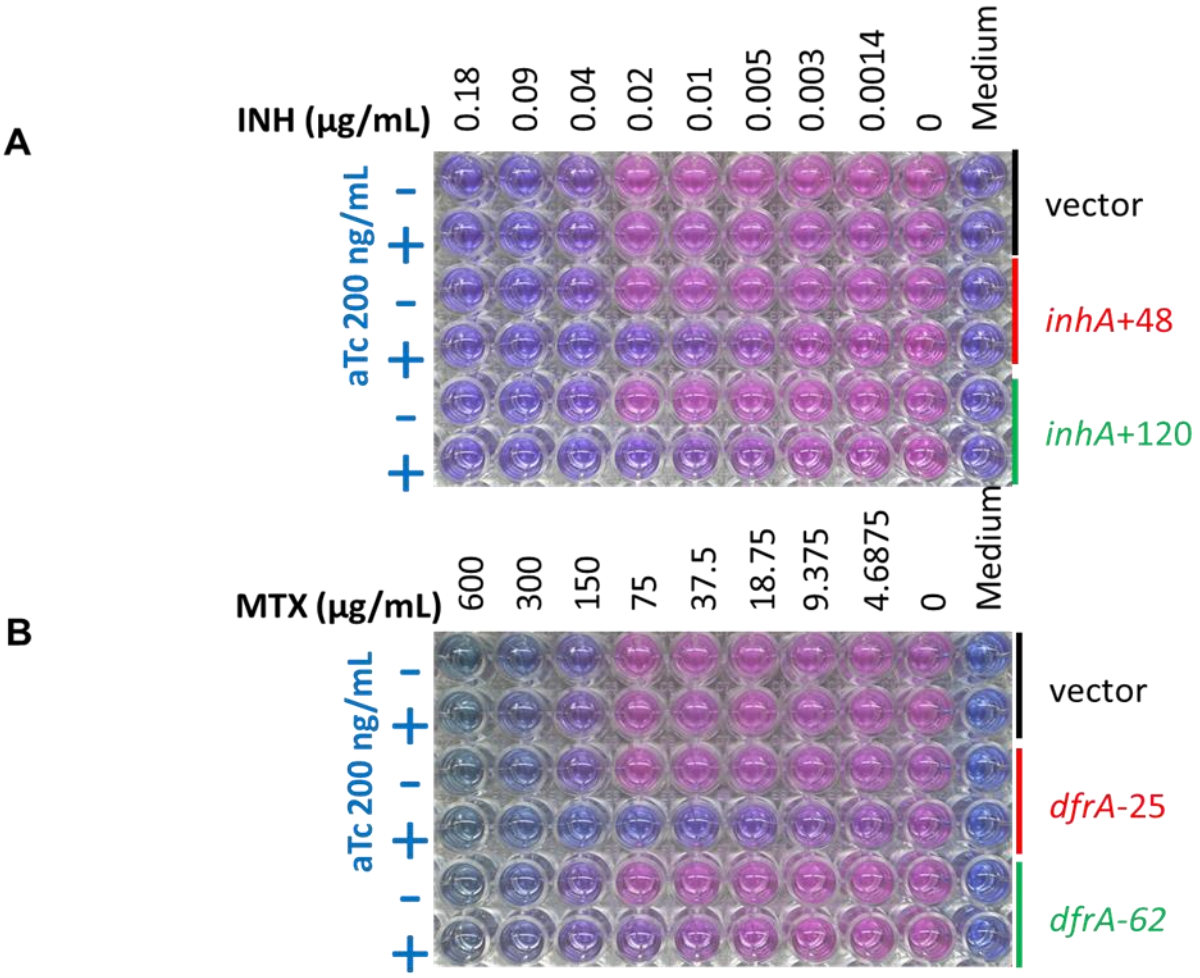


Figure S6. Effect of CRISPRi on off target genes that differ from the specific target sequence including the PAM by 2, 4 or 5nt. Expression of all genes containing a sequence that diverged by 2 or 4 nt, regardless of the presence of an intact PAM, was measured. Of the genes containing a sequence that differed by 5 nt, expression was measured only for those that had an intact PAM sequence. **Panel a**, alignment of target gene sequences and the sequences for the off-target genes for which expression following *dcas9* and sgRNA induction was measured. Nucleotides that differ are shown in red. The PAM sequence is underlined. **Panels b-j**, expression of the off-target genes following induction of dcas9 and the sgRNA indicated above each panel. No significant differences were observed at 24 or 48h for any of the 9 genes tested. **Table 1** below shows the number of mismatches for each off-target site compared the site targeted by the sgRNA indicated.

a

***sigH*-222** 5′-*CCC*GCTGGCGAACACGGTTGATT-3′
Rv3618-ATG-812nt- *CCC*GTTGGCGAACACCGTGGAAG-341nt-TGA

***inhA*+48** 5′-*CCG*ACTCGTCGATCGCGTTTCAC-3′
Rv0402c -GTG-1396nt-*ACC*ACTCGTCGATCCCGTTTCAG-1451nt-TAG

***inhA*+120** 5′-*CCG*GCTGCGGCTGATTCAGCGCA-3′
Rv0352 -*ACG*GCTGCGGCCGATGCGGCGCA-594nt-ATG

***dfrA*-25** 5′-*CCG*GAATCAAGGAGTGACGCGTG-3′
Rv1778c -ATG-340nt- *CAG*GAATCTACGAGTGGCGCGTG-81nt-TAG

***dfrA*-25** 5′-*CCG*GAATCAAGGAGTGACGCGTG-3′
Rv2885c-ATG-145nt -*CCG*ACATCAAGGCGTGGCGCGCG-1206nt-TGA

***Wag31*+144** 5′-*CCG*ATCTGCGTCAGAGGATCAAC-3′
Rv2880c-ATG-157nt -*CCG*ATCCGCGTCAGATGACCGAC-642nt-TGA

***ftsZ*+25** 5′-*CCG*TCATCAAGGTCGTGGGTATC-3′
Rv2277c-ATG-781nt -*CCG*GCATGAAGGTCATGGGTTTC-96nt-TAA

***ftsZ*+134** 5′-*CCA*GGCGTTGTTGATGAGCGATG-3′
Rv3307-GTG-389nt -*CCA*GCCGGTGCTGATCAGCGATC-389nt-TAA

***ftsZ*+134** 5′-*CCA*GGCGTTGTTGATGAGCGATG-3′
Rv0186-ATG-134nt -*CCC*GGCCTTGTTGATGAGCGATG-1913nt-TGA

Figure S6. (Continued)

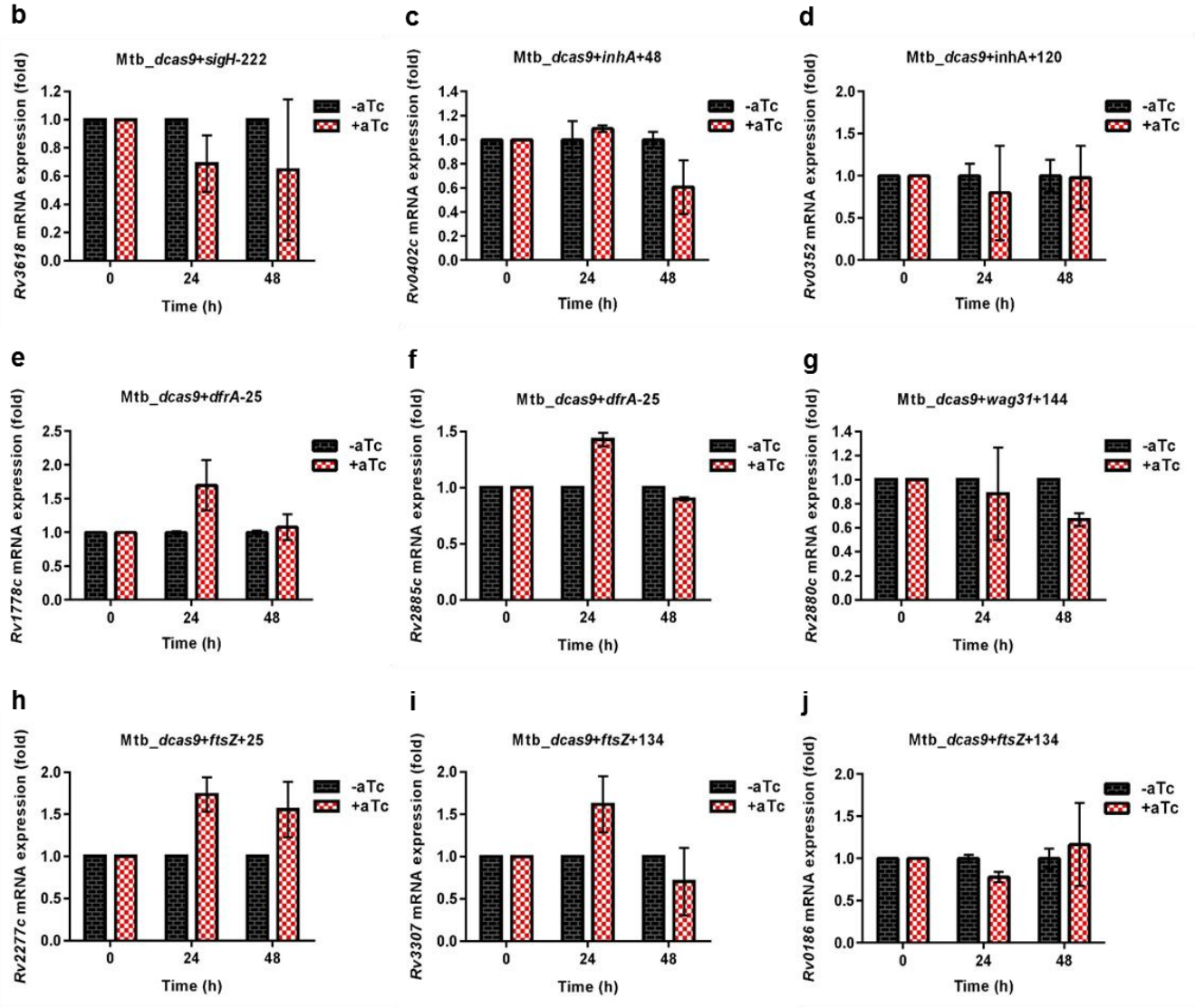
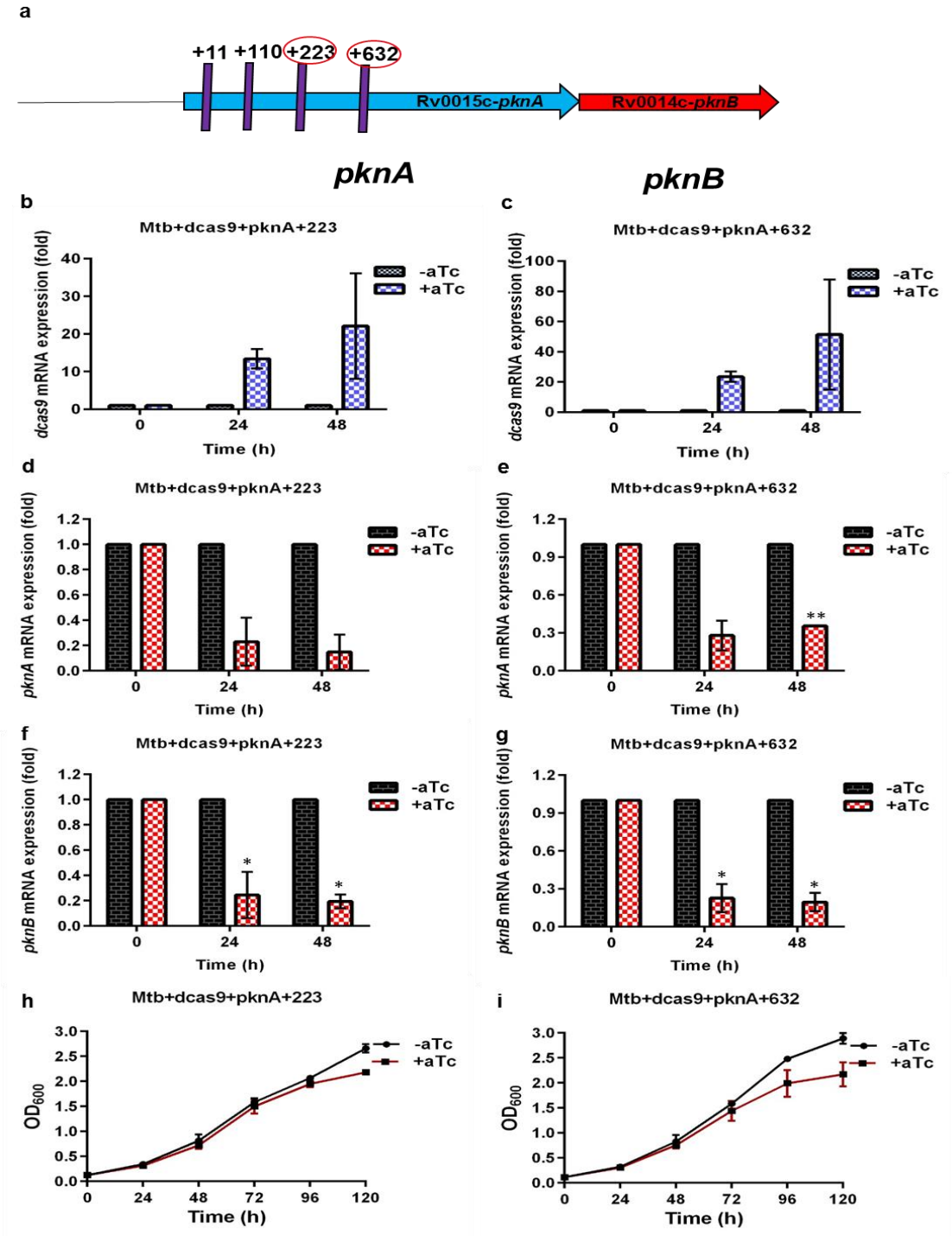


Figure S7. Polar effects on *pknB* (Rv0014c) of CRISPRi targeting *pknA* (Rv0015c) a. Position of target sequences, shown by purple vertical lines. Numbering refers to the first nucleotide in the target sequence relative to annotated start of coding sequence. Circled sgRNAs are those for which expression data are shown**. b,c.** Expression of *dcas9* with and without induction of *dcas9* and the sgRNA indicated. **d,e.** Expression of *pknA* without induction of *dcas9* and the *pknA*-targeting sgRNA indicated. **f, g.** Expression of *pknB* without induction of *dcas9* and the *pknA*-targeting sgRNA indicated. **h,i.** Growth curves of culture with and without induction of *dcas9* and the sgRNA indicated.



Supplementary Table 1: Potential off-target sequences for sgRNAs.

S.N.	Gene	Position of sgRNA relative to translation start codon of target gene	Number of mismatches/gene with mismatch				
			1	2	3	4	5
1	*sigH*	-222	0	0	0	0	Rv2226, Rv3618
2		-11	0	0	0	0	0
3		+9	0	0	0	0	0
4	*pknB*	+8	0	0	0	0	Rv0691c
5		+51	0	0	0	0	Rv1904
6		+143	0	0	0	0	Rv2115c
7	*pknA*	+223	0	0	0	0	0
8		+632	0	0	0	0	Rv0753c
9	*inhA*	+48	0	0	0	Rv0402c	Rv0680c
10		+120	0	0	0	Rv0352	Rv0050,0130c,0676c
11	*dfrA*	-62	0	0	0	0	Rv3031
12		-25	0	0	0	Rv1778c	Rv2885c
13	*wag31*	+26	0	0	0	0	0
14		+144	0	0	0	Rv2880c	0
15	*ftsZ*	+25	0	0	0	Rv2277c	0
16		+134	0	Rv0186	0	0	Rv1050,Rv0934,Rv3307

Expression of off-target genes highlighted in red was measured in strains with and without induction of *dcas9* and the sgRNA that contains a similar sequence.

Supplementary Table 2: List of oligonucleotides and primers used in this study.

oligonucleotides used for sgRNA		
sgRNA	**Forward Primer**	**Reverse Primer**
Msm_sigH+11	GGGACCGGTCGACGTCAGTCATCG	AAACCGATGACTGACGTCGACCGG
Msm_pknB+8	GGGAGTCGGAAAGGTGCTGAGGCG	AAACCGCCTCAGCACCTTTCCGAC
Mtb_pknB+8	GGGAGTCGGACAGGTGGGAAGGGG	AAACCCCCTTCCCACCTGTCCGAC
Mtb_pknB+51	GGGACGGACATGCCCCCAAATCCA	AAACTGGATTTGGGGGCATGTCCG
Mtb_pknB+143	GGGAAAGGTAAAAACTGGGATCGC	AAACGCGATCCCAGTTTTTACCTT
Mtb_inhA+48	GGGAGTGAAACGCGATCGACGAGT	AAACACTCGTCGATCGCGTTTCAC
Mtb_inhA+120	GGGATGCGCTGAATCAGCCGCAGC	AAACGCTGCGGCTGATTCAGCGCA
Mtb_dfrA-62	GGGAGGCGCGCCTCATACCGCGAC	AAACGTCGCGGTATGAGGCGCGCC
Mtb_dfrA-25	GGGACACGCGTCACTCCTTGATTC	AAACGAATCAAGGAGTGACGCGTG
Mtb_gyrA-110	GGGAGCGTCGACGTCCTCGCCCAT	AAACGATGGGCGAGGACGTCGACG
Mtb_gyrA-25	GGGACCTCGTTTGCAATCGAACGC	AAACGCGTTCGATTGCAAACGAGG
Mtb_wag31+26	GGGACTTACTGAACGCCACATTGG	AAACCAATGTGGCGTTCAGTAAGC
Mtb_wag31+144	GGGAGTTGATCCTCTGACGCAGAT	AAACATCTGCGTCAGAGGATCAAC
Mtb_ftsZ+25	GGGAGATACCCACGACCTTGATGA	AAACTCATCAAGGTCGTGGGTATC
Mtb_ftsZ+134	GGGACATCGCTCATCAACAACGCC	AAACGGCGTTGTTGATGAGCGATG
Mtb_pknA+223	GGGATTCGCCGTAGTCGTGCACGC	AAACGCGTGCACGACTACGGCGAA
Mtb_pknA+632	GGGATCCCAGTGAATAGACGTCGC	AAACGCGACGTCTATTCACTGGGA
Gene	**Primers used for qRT PCR**	
dcas9	AAGAAGTACAGCATCGGCCTGG	TTCTTGCGCCGCGTGTATCG
Msm_sigH	GAGACAGATGCCGAGCTCAC	AGGCCTTCACCATCGTTTCC
Msm_pknB	CATGGAGTACGTCGACGGTGTGAC	CTTGACATCGCGGTGGATGATG
Msm_sigA	TGCCGATCTGCTTGAGGTAGG	TTCGTGTGGGACGAGGAAGAG
Msm _5'sigA	GACCTTGAGGTGACCGACG	CTTCTTCCTCGTCCTCGACTG
Msm _3'sigA	TCGACGAGATCGGCCAGG	TAGTCCAGGTAGTCGCGCAG
Mtb_pknB	ACATTGTCCACACCGAAGGG	GCTGATCATGATGTTCGCCG
Mtb_inhA	ATCCACATCTCGGCGTATTC	ACGAACCTGTTGACCGACTC
Mtb_dfrA	AGATCACCATGGGGCACAC	GCCATAAAGTCAGCTTGGCG
Mtb_gyrA	TGACATCGAGCAGGAGATGC	AAGCCGGAATCGAACATTGC
Mtb_wag31	TAAGCCGCCTATCGGCAAAC	AGCTCGTTGATCCTCTGACG
Mtb_ftsZ	CAAGGACGAGATCGAAGAGC	GGAATCACGATGAGGGTGTC
Mtb_pknA	AAAGCCAGATGAACGGGGAG	TCGAGCATGTCCAGTGCG
Mtb_5'sigA	GAGGACCTCGACCTTGACG	GGCCGCCTCAGCTTCG
Mtb_3'sigA	ACCCACCGAAAAGGACAAGG	CTGTTTGAGGTAGGCGCGAA
Rv0402c	GTCCTCATCTGGCAGCACAT	GGCCCCGATTTCTTCTTGGA
Rv0352	AGACCGAGTTGGATTTCGTG	TGCAAGTGGGACACACCTTT
Rv1778c	ATCCTCTTTGAAGCCGCCG	GCCACTCGTAGATTCCTGGT
Rv0186	GGATCCTGCGGTCGATGTTT	TTTTGCAGCAGCACGATTCC
Rv2880c	AATCGGTGCTGATGCGCTA	CCGTCGATAGGTTGCGAGTC
Rv2277c	GTGAATGCCGAGTTGTGGGA	ACCGCATCGAGTCCGATTTT
Rv3307	GAGATCCGGATGTTGCAGA	GGATACGCCCAGTACCTCAG
Rv3618	AGTCGTCTCGGGAATTCGT	CAGTAGCAACGTTCCGAAGC
Rv2885c	CAAAGACCAGGTGTGTGTCA	CAGCTCTGCCAGTTCCAATA
Primers used for 5' RACE		
Adapter	GCUGAUGGCGAUGAAUGAACACUGCGUUUGCUGGCUUUGAUGAAA	
Outer forward	GCTGATGGCGATGAATGAACACTG	
Inner forward	CGCGGATCCGAACACTGCGTTTGCTGGCTTTGATG	
Rv No.	**GSP1 (Outer PCR Reverse)**	**GSP2 (Inner PCR Reverse)**
Rv1484	GATGATTCCGCTAACCAGAATCCG	GCCGCCGTCGACCGGGATGA
Rv2763c	ATGTGCGCCGGCCCATCACGA	TGTCGCTTGAGCCCAGATCAG
Rv2145c	CCGATAGGCGGCTTACTGAACGCC	ATAAGGACCAATTTCAAACTCTAAG

References

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2. Ehrt, S., Guo, X.V., Hickey, C.M., Ryou, M., Monteleone, M., Riley, L.W. and Schnappinger, D. (2005) Controlling gene expression in mycobacteria with anhydrotetracycline and Tet repressor. *Nucleic Acids Res*, **33**, e21.

3. Kiran, M., Chauhan, A., Dziedzic, R., Maloney, E., Mukherji, S.K., Madiraju, M. and Rajagopalan, M. (2009) *Mycobacterium tuberculosis ftsH* expression in response to stress and viability. *Tuberculosis*, **89**, S70-S73.