Supplemental Table S1 Primers used in this study

Name	Sequence 5'–3'	Enzyme	Usage
Ms6564f	GACGGAATTCCCATGACCACCGCTGAAGCG	*EcoRI*	Clone and expression
Ms6564r	ATATTCTAGATCAGGGGCGCAGGCCGGCCA	*XbaI*	Clone and expression
Ms3452f	ATATGAATTCCGATGGCCAACCCGACGCGC	*EcoRI*	Clone to pMV261
Ms3452r	GACGTCTAGATCATGTGTCTGGGCGCGTGT	*XbaI*	Clone to pMV261
Ms2389f	GCGCGAATTCATGAACAAAGCGGAGCTCAT	*EcoRI*	Clone to pMV261
Ms2389r	TAAGTCTAGATTACCTGCGGCCCTTCTTGG	*XbaI*	Clone to pMV261
Ms6564UPf	AGTATTAATTAAGGCTCGACACCGACTGCATC	*PacI*	Knock out
Ms6564UPr	AGTTACTAGTCGCAGGCCTTTACACGAAAA	*SpeI*	Knock out
Ms6564DNf	AGTAAAGCTTGTTCAGCGCTGGGGCACGGCA	*HindIII*	Knock out
Ms6564DNr	AGTTGCTAGCGCGCTTCCCGGTGGTTCTCTC	*NheI*	Knock out
Ms4232pLf	ATATTCTAGAAGTCAGGACATCGGCCGCCA	*XbaI*	Clone to pMV261
Ms4232pLr	AGATGAATTCGCAACAGTACCCATCGGCGA	*EcoRI*	Clone to pMV261
Ms4235pLf	ATAATCTAGACCACCTTTCCCCACGGGTCG	*XbaI*	Clone to pMV261
Ms4235pLr	AGACGAATTCTGAACACACCTCCGCAACGT	*EcoRI*	Clone to pMV261
Ms6302pLf	ATATTCTAGACCCTCCATGTGGTCGTAGCG	*XbaI*	Clone to pMV261
Ms6302pLr	GCGCTGAATTCCCTTCCCATTCTTACCTCCT	*EcoRI*	Clone to pMV261
Ms6806pLf	ATATTCTAGAACCGGCGACCCGATCTGCCA	*XbaI*	Clone to pMV261
Ms6806pLr	AGACGAATTCCGGGACCATTGTTCACGAGT	*EcoRI*	Clone to pMV261
Ms6564pLf	ATAGTCTAGAGGCGACATGACGTGCATGAG	*XbaI*	Clone to pMV261
Ms6564pLr	CGCTGAATTCCGCAGGCCTTTACACGAAAA	*EcoRI*	Clone to pMV261
Rv757pLf	AATTTCTAGATGGCCATGTCAACCGCCGCG	*XbaI*	Clone to pMV261
Rv757pLr	CCGGGAATTCTTGGTTGAACGTTACCTTCA	*EcoRI*	Clone to pMV261
LacZf	ATTCAAGCTTATGAGGATGAGGGAAGCAAG	*HindIII*	Clone to pMV261
LacZr	ATGCGCTAGCTTATTTTTGACACCAGACCA	*NheI*	Clone to pMV261
Ms1003pf	ATATGAATTCGGTCCCAGCGGTGCGACGAT	*EcoRI*	Clone and ChIP
Ms1003pr	GCGCTCTAGAGTCGAGGTCTTTCAGATGGA	*XbaI*	Clone and ChIP
Ms1015pf	ATATATGAATTCGGTCTCGTCGTGCGCCCACA	*EcoRI*	Clone and ChIP
Ms1015pr	GCGCGCTCTAGACTGTGAATTTCTGTGAATCC	*XbaI*	Clone and ChIP
Ms1432pf	AGATGAATTCCTTCGGGCGGAACCAGTCGG	*EcoRI*	Clone and ChIP
Ms1432pr	ATATTCTAGAGCGCTGAGCCTACTGGGCCG	*XbaI*	Clone and ChIP
Ms1930pf	ATATGAATTCGGCGGCCATGCTGGCCATGT	*EcoRI*	Clone and ChIP
Ms1930pr	GCGCTCTAGAATGCGGAGGAGATAGCCTTT	*XbaI*	Clone and ChIP
Ms2294pf	ATATATGAATTCGGTCTCGTCGTGCGCCCACA	*EcoRI*	Clone and ChIP
Ms2294pr	GCGCGCTCTAGACTGTGAATTTCTGTGAATCC	*XbaI*	Clone and ChIP
Ms2312pf	ATATATCTCGAGCGCACCGGCCGGCAGCAACG	*XhoI*	Clone and ChIP
Ms2312pr	ATCTATTCTAGACGTCCGCCGGCGACCTCGCG	*XbaI*	Clone and ChIP
Ms2389pf	ATATATCTCGAGATCTTCCGGGGGAACGCGGG	*XhoI*	Clone and ChIP
Ms2389pr	GCGCGCTCTAGACCAAAACCTCCGAAACCAGT	*XbaI*	Clone and ChIP
Ms2402pf	ATATGAATTCCCCTGTTACCCGGCCGGATC	*EcoRI*	Clone and ChIP
Ms2402pr	AGATTCTAGACGGACCTCCAAGTTCACGCC	*XbaI*	Clone and ChIP
Ms2417pf	ATATGAATTCCAGACCGCATGGTCGCCGAG	*EcoRI*	Clone and ChIP
Ms2417pr	AGATTCTAGAACCGCAATCCTACGGCGACG	*XbaI*	Clone and ChIP

continued

Ms2723pf	ATATGAATTCGTCGGCGCTGCCGCACGGGC	*EcoRI*	Clone and ChIP
Ms2723pr	GCGCTCTAGAGGTGGTGCCTCTCCGAGTAG	*XbaI*	Clone and ChIP
Ms2943pf	AGATGAATTCCGGCTCGATGACGAATGTGG	*EcoRI*	Clone and ChIP
Ms2943pr	AGATTCTAGAGCGAACCCCTTCGTCAGAAC	*XbaI*	Clone and ChIP
Ms3172pf	ATATACTCGAGGGAAGTCGACCGGCCTGGAG	*XhoI*	Clone and ChIP
Ms3172pr	GCGCGTCTAGAACCTGCAGATGCTAAAGGGC	*XbaI*	Clone and ChIP
Ms3673pf	ATATGAATTCACCGTGAGGGTGACCCCGAC	*EcoRI*	Clone and ChIP
Ms3673pr	AGATTCTAGAGGCGTCAATCTTGGGGTGCC	*XbaI*	Clone and ChIP
Ms3984pf	AGACGAATTCACGACATCGGCACCAAGGTC	*EcoRI*	Clone and ChIP
Ms3984pr	ATATTCTAGACGCATCGCCTCCTTGGCTCG	*XbaI*	Clone and ChIP
Ms4072pf	ATATGAATTCGCGGTCGGTTGAGCTCCGAC	*EcoRI*	Clone and ChIP
Ms4072pr	GCGCTCTAGATGTGGATGACGGAATACACC	*XbaI*	Clone and ChIP
Ms4084pf	AGACGAATTCCGACGAGATTCAAGGCCATC	*EcoRI*	Clone and ChIP
Ms4084pr	GACGTCTAGAGTCGTCAGTGATATCCCTGA	*XbaI*	Clone and ChIP
Ms4307pf	ATATGAATTCCGCCGAGCCCCAGCGAGGTC	*EcoRI*	Clone and ChIP
Ms4307pr	AGACTCTAGACTCAGCACCTCCGTCACGGC	*XbaI*	Clone and ChIP
Ms4674pf	ATATGAATTCTCAGCTCGACCTCGTCCCAG	*EcoRI*	Clone and ChIP
Ms4674pr	GCGCTCTAGACTTGTGCTCCTTGATGTCTT	*XbaI*	Clone and ChIP
Ms4925pf	ATATGAATTCCCGCCGTCGAGGTGGTCGAA	*EcoRI*	Clone and ChIP
Ms4925pr	ATATTCTAGACGAAGTGGTGCCGCTGTGGC	*XbaI*	Clone and ChIP
Ms5082pf	ATATGAATTCGCAGGCCGCTCAAGGTCAGC	*EcoRI*	Clone and ChIP
Ms5082pr	AGACTCTAGAGCTTCCGATACTGCCTCACG	*XbaI*	Clone and ChIP
Ms5253pf	ATATGAATTCTCGACACCGAGGAGCACCGA	*EcoRI*	Clone and ChIP
Ms5253pr	GCGCTCTAGATCGTTTCTCCTTATCTGGTG	*XbaI*	Clone and ChIP
Ms5399pf	AGATCTCGAGATCAACGTCATGGCCATCTC	*XhoI*	Clone and ChIP
Ms5399pr	ATATTCTAGAGGGTGGTGGCTACCCGGACC	*XbaI*	Clone and ChIP
Ms5402pf	AGATGAATTCGACAGCCTCGGGCACCATCG	*EcoRI*	Clone and ChIP
Ms5402pr	ATATTCTAGAGGGTGGTGGCTACCCGGACC	*XbaI*	Clone and ChIP
Ms5451pf	GCGCGAATTCAACCCCATTTTCACCTGAAG	*EcoRI*	Clone and ChIP
Ms5451pr	ATATTCTAGAGCGCGCGAGTGTATCGCACA	*XbaI*	Clone and ChIP
Ms6157pf	ATATGAATTCGGAGATCGCCCGCACCGAGG	*EcoRI*	Clone and ChIP
Ms6157pr	GACGTCTAGACTGTGCCTACGCTCCACTTC	*XbaI*	Clone and ChIP
Ms6302pf	ATATATGAATTCCCGGCATCCGCGAGGGCGGT	*EcoRI*	Clone and ChIP
Ms6302pr	GCGCGCTCTAGACCTTCCCATTCTTACCTCCT	*XbaI*	Clone and ChIP
Ms6304pf	ATATATGAATTCGCCAGCGCCTCGCGGCGCAC	*EcoRI*	Clone and ChIP
Ms6304pr	GCGCGCTCTAGACCGATGACATTCCCACGTTC	*XbaI*	Clone and ChIP
Ms6443pf	ATATCTCGAGCGTCGAAGGAAGCGGTGCTG	*XhoI*	Clone and ChIP
Ms6443pr	GACGTCTAGAATGTTCGCATCGAGGGGCGA	*XbaI*	Clone and ChIP
Ms6600pf	ATATCTCGAGCTGCACCGCGGTATCGCCGA	*XhoI*	Clone and ChIP
Ms6600pr	GCGCTCTAGAGAAGACCTCCGAGATCGGTG	*XbaI*	Clone and ChIP
Ms6806pf	AGACGAATTCACCAAGGGCGCCATCTACAA	*EcoRI*	Clone and ChIP
Ms6806pr	AGACTCTAGACGGGACCATTGTTCACGAGT	*XbaI*	Clone and ChIP

Notes: Restriction enzyme sites are underlined.

Supplemental Table S2 DNA substrate fragment used in this study

Name	Length and Source	Sequence or primers used to amplify long segments 5'–3'
Ms1003p	500bp, PCR	f: ATATGAATTCGGTCCCAGCGGTGCGACGAT
		r: GCGCTCTAGAGTCGAGGTCTTTCAGATGGA
Ms1015p	500bp, PCR	f: ATATATGAATTCGGTCTCGTCGTGCGCCCACA
		r: GCGCGCTCTAGACTGTGAATTTCTGTGAATCC
Ms1536p	500bp, PCR	f: ATATGAATTCCCGCGGCCATCCCCACCGCA
		r: GCGCTCTAGACAACGTTTGGACGCACGCGA
Ms1854p	500bp, PCR	f: ATATGAATTCAGATGGCACGGCGGCGTTGC
		r: AGATTCTAGACCCCTCGATCTGTGGCGATC
Ms2089p	500bp, PCR	f: ATATGAATTCTGCGCACGTCCACGGTGGTG
		r: ATATTCTAGACACGGCACGCCAGTGTAGCG
Ms2294p	500bp, PCR	f: ATATATGAATTCGGTCTCGTCGTGCGCCCACA
		r: GCGCGCTCTAGACTGTGAATTTCTGTGAATCC
Ms2389p	500bp, PCR	f: ATATATCTCGAGATCTTCCGGGGGAACGCGGG
		r: GCGCGCTCTAGACCAAAACCTCCGAAACCAGT
Ms2402p	500bp, PCR	f: ATATGAATTCCCCTGTTACCCGGCCGGATC
		r: AGATTCTAGACGGACCTCCAAGTTCACGCC
Ms2423p	500bp, PCR	f: AGATGAATTCGGTCTACCCGCACCTTGAGG
		r: ATATTCTAGAGACGCCTTACCCTACCGCCG
Ms2619p	500bp, PCR	f: ATATGAATTCAGGGCGGCGATCCGTTCGTG
		r: GACGTCTAGAAACCGGTCACCTTACCGGAA
Ms4232p	500bp, PCR	f: ATATGAATTCCAAGTCCACCATCGCGCCTG
		r: AGATTCTAGAGCAACAGTACCCATCGGCGA
Ms4925p	500bp, PCR	f: ATATGAATTCCCGCCGTCGAGGTGGTCGAA
		r: ATATTCTAGACGAAGTGGTGCCGCTGTGGC
Ms5083p	500bp, PCR	f: ATATGAATTCGCAGGCCGCTCAAGGTCAGC
		r: AGACTCTAGAGCTTCCGATACTGCCTCACG
Ms5402p	500bp, PCR	f: AGATGAATTCGACAGCCTCGGGCACCATCG
		r: ATATTCTAGAGGGTGGTGGCTACCCGGACC
Ms6157p	500bp, PCR	f: ATATGAATTCGGAGATCGCCCGCACCGAGG
		r: GACGTCTAGACTGTGCCTACGCTCCACTTC
Ms6201p	500bp, PCR	f: ATATGAATTCACGGCAGCGCGCCTGCGACA
		r: GACGTCTAGAACTTGCGAGAAGGCAGCACC
Ms6302p	500bp, PCR	f: ATATATGAATTCCCGGCATCCGCGAGGGCGGT
		r: GCGCGCTCTAGACCTTCCCATTCTTACCTCCT
Ms6304p	500bp, PCR	f: ATATATGAATTCGCCAGCGCCTCGCGGCGCAC
		r: GCGCGCTCTAGACCGATGACATTCCCACGTTC
Ms6443p	500bp, PCR	f: ATATCTCGAGCGTCGAAGGAAGCGGTGCTG
		r: GACGTCTAGAATGTTCGCATCGAGGGGCGA
Ms6806p	500bp, PCR	f: AGACGAATTCACCAAGGGCGCCATCTACAA
		r: AGACTCTAGACGGGACCATTGTTCACGAGT

continued

Ms6821p	500bp, PCR	f: ATATATGAATTCCATGCGCGCGTCGAGTCCGG r: GCGCGCTCTAGAACGGATTGTCACGATCCTTT
Ms6464p1	132bp, PCR	f: CTCTGTGGCCGGTCCACTTT r: CACGAACGTCGACGGCCTGA
Ms6464p2	136bp, PCR	f: TTCGTGCGCCACAGCGGCGT r: GTGTGGACGTCCGTCACGGC
Ms6464p3	135bp, PCR	f: GGACGTCCACACGGTCGATC r: TGATCGGGGCCAGCGGATTT
Ms6464p4	134bp, PCR	f: TTAAATCCGCTGGCCCCGAT r: CGCAGGCCTTTACACGAAAA
Ms6464p4-1	189bp, PCR	f: TTAAATCCGCTGGCCCCGAT labeled with FITC r: ATGGCCACCCGCGACTTCTC
Ms6464p4-2	187bp, PCR	f: TTAAATCCGCTGGCCCCGAT r: ATGGCCACCCGCGACTTCTC labeled with FITC
Ms6464p5	60bp, synthesized directly	**5'-AAATCCGCTGGCCCCGATCACCAGTTTCGTCGCTGTCA TAGGCCCTTGCGCTCGTGTCCT-3'** **3'**-AGGACACGAGCGCAAGGGCCTATGACAGCGACGAAACTG GTGATCGGGGCCAGCGGATTT-**5'**
Ms6464p6	39bp, synthesized directly	**5'-CGGGTCATAAACGAGACGGTACGTCTCGTCTTGTGGCA G-3'** **3'**-CTGCCACAAGACGAGACGTACCGTCTCGTTTATGACCCG-**5'**
Ms6464p7	45bp, synthesized directly	**5'-CTTGTGGCAGAGTACGGGGTGTCACTTTTCGTGTAAAG GCCTGCG-3'** **3'**-CGCAGGCCTTTACACGAAAAGTGACACCCCGTACTCTGCC ACAAG-**5'**
Ms6564p8	39bp,synthesized directly	**5'-CGGGTCATAACTTGACTAGTATTCAGTGCCTTGTGGCA G-3'** **3'**-CTGCCACAAGGCACTGAATACTAGTCAAGTTATGACCCG-**5'**

Supplemental Table S3 Primers for qRT-PCR

Name	Sequence 5'–3'	Enzyme	Usage
RT-MS1003f	AACACTGCCAACAACGCCGT		Reverse transcription -PCR
RT-MS1003r	AGAACCCGTCCATGGCAACA		Reverse transcription -PCR
RT-MS1014f	GAACAGGTCATGGGACTGCT		Reverse transcription -PCR
RT-MS1014r	CGCTGCTTGTTGTCACTGAT		Reverse transcription -PCR
RT-MS1536f	ACACAACTGCACGACGTGAT		Reverse transcription -PCR
RT-MS1536r	GAACACGTCACCGTAGGGAT		Reverse transcription -PCR
RT-MS2089f	CGACGATGTCTCGTTGAAGA		Reverse transcription -PCR
RT-MS2089r	GACATGGAACTTCGACACCC		Reverse transcription -PCR
RT-MS2294f	GAACAGGTCATGGGACTGCT		Reverse transcription -PCR
RT-MS2294r	CGCTGCTTGTTGTCACTGAT		Reverse transcription -PCR
RT-MS2389f	GTTCGGTGTCTTCGAGCAG		Reverse transcription -PCR
RT-MS2389r	CCAGAGATAACCGCCTTGAA		Reverse transcription -PCR
RT-MS2419f	AATTGACCGAGATGGTCACG		Reverse transcription -PCR
RT-MS2419r	GTCCAGCAGTTGACGTTTGA		Reverse transcription -PCR
RT-MS2423f	GCAAGAAGATCAAACGCCTC		Reverse transcription -PCR
RT-MS2423r	TCCATGACGTAGAACGGTGA		Reverse transcription -PCR
RT-MS3172f	GTGGTACCCGTCCTCGAAC		Reverse transcription -PCR
RT-MS3172r	TCTTGGCGATCTGTTTACCC		Reverse transcription -PCR
RT-MS4222f	ACTACCTCGCGGTCATCAAG		Reverse transcription -PCR
RT-MS4222r	AGACCACGGGTGGAGTCAC		Reverse transcription -PCR
RT-MS4225f	GATCCGACGAAATCCGTTC		Reverse transcription -PCR
RT-MS4225r	GGGTGAAGTAGAGCAGCAGG		Reverse transcription -PCR
RT-MS4226f	CGGGTGTTCGACGACTATG		Reverse transcription -PCR
RT-MS4226r	CGCTGTGCGTGAGTACAAGT		Reverse transcription -PCR
RT-MS4227f	GGACAACAACCACAGCGAC		Reverse transcription -PCR
RT-MS4227r	GGGTGATCAGTTCCAGGTCA		Reverse transcription -PCR
RT-MS4228f	CGCACAACGACTTCATCTTC		Reverse transcription -PCR
RT-MS4228r	CGACGTTGATGAACATCTGG		Reverse transcription -PCR
RT-MS4229f	TCGATGAGCTGGTACGTCAG		Reverse transcription -PCR
RT-MS4229r	ACTCATTTGTCCCAAGCACC		Reverse transcription -PCR
RT-MS4232f	AGATCGTCGAGATCGGTGAC		Reverse transcription -PCR
RT-MS4232r	CATGAACTTCGGTCCCTTGT		Reverse transcription -PCR
RT-MS4233f	CGAGACCTTCGTCTACCTCG		Reverse transcription -PCR
RT-MS4233r	GTACTGGCGCAGATCCTGTC		Reverse transcription -PCR
RT-MS4925f	GAGGATTTCGACCGCTGTTA		Reverse transcription -PCR
RT-MS4925r	CAACTGGGCCTGCAGTAGAT		Reverse transcription -PCR
RT-MS5082f	ATGGCCAAGGAACTCAAGC		Reverse transcription -PCR
RT-MS5082r	CAGATCTTGACCATGTCGGA		Reverse transcription -PCR
RT-MS6157f	GTCGAGCAGAAGCCCTACAC		Reverse transcription -PCR
RT-MS6157r	CGGTACGCATGTAGGTGATG		Reverse transcription -PCR
RT-MS6201f	TCGACCATCGAACAGCAGTA		Reverse transcription -PCR
RT-MS6201r	TTGGTGAACGTCTTGTCGAG		Reverse transcription -PCR

continued

RT-MS6301f	GGTGGGTTATCCCAAGACCT	Reverse transcription -PCR
RT-MS6301r	TGTAGTCGATGAACAACCGC	Reverse transcription -PCR
RT-MS6302f	AAGGATCGCATCAAACTGCT	Reverse transcription -PCR
RT-MS6302r	TTCCCTCCATCTGGTCGTAG	Reverse transcription -PCR
RT-MS6304f	GTATGAACCCAAGTGGGACG	Reverse transcription -PCR
RT-MS6304r	CTCGAAGTCGAGGTGGTTGT	Reverse transcription -PCR
RT-MS6443f	GACCTCGACTCGTTCTACGC	Reverse transcription -PCR
RT-MS6443r	GTAGGCCTTGGCCTCGTAAC	Reverse transcription -PCR
RT-MS6806f	CGACATCCTCATCACACTGG	Reverse transcription -PCR
RT-MS6806r	GGAGAACGTGCCGTTGTAGT	Reverse transcription -PCR
RT-MS6900f	GGTTCGTCGTTCAAGGTGTT	Reverse transcription -PCR
RT-MS6900r	GAGCATGAGCCGGTAGTAGC	Reverse transcription -PCR

Supplemental Table S4 Strains and plasmids used in this study

Strain or plasmid	Relevant genotype or features	Source or reference
Strains		
E.coli		
DH5a	Host for plasmid construction	TaKaRa
BL21	Host for overexpression	TaKaRa
XR	Host for bacteria one-hybrid()	Stratagene
M. smegmatis mc^2155		5
Msm/WT	M. smegmatis mc2155	5
Msm/pMV261	$mc^2155$with pMV261	This study
Msm/pMV261-Ms6564	$mc^2155$with pMV261::6564	This study
Msm/pMV261-Ms3452	$mc^2155$with pMV261::3452	This study
Msm/Ms6564::hyg	mc^2155 Ms6564 replaced by hyg	This study
Ms6564 complementation	$mc^2155$with pMind::6564	This study
△Y0	Msm/Ms6564::hyg with pMV261::LacZ	This study
△Y1	Msm/Ms6564::hyg with pMV::4232P::LacZ	This study
△Y2	Msm/Ms6564::hyg with pMV::4235P::LacZ	This study
△Y3	Msm/Ms6564::hyg with pMV::6806P::LacZ	This study
△Y4	Msm/Ms6564::hyg with pMV::6564P::LacZ	This study
△Y5	Msm/Ms6564::hyg with pMV::757P::LacZ	This study
△Y6	Msm/Ms6564::hyg with pMV::GroEL1P::LacZ	This study
Y0	$mc^2155$with pMV261::LacZ	This study
Y1	$mc^2155$with pMV::4232P::LacZ	This study
Y2	$mc^2155$with pMV::4235P::LacZ	This study
Y3	$mc^2155$with pMV::6806P::LacZ	This study
Y4	$mc^2155$with pMV::6564P::LacZ	This study
Y5	$mc^2155$with pMV::757P::LacZ	This study
Y6	$mc^2155$with pMV::GroEL1P::LacZ	This study
Plasmids		
pMV261	Kan^r, pAL5000 replicon	27
pMV261::6564	Ms6564 in EcoRI-XbaI site of pMV261	This study
pMV261::3452	Ms3452 in EcoRI-XbaI site of pMV261	This study
pMV261::2389	Ms2389 in EcoRI-XbaI site of pMV261	This study
pMV261:: LacZ	LacZ in HindIII-NheI site of pMV261	This study
pMV261::4232P::LacZ	Ms4232P in EcoRI-HindIIIsite of pMV261:: LacZ	This study
pMV261::4235P::LacZ	Ms4235P in EcoRI-HindIIIsite of pMV261:: LacZ	This study
pMV261::6806P::LacZ	Ms6806P in EcoRI-HindIIIsite of pMV261:: LacZ	This study
pMV261::6564P::LacZ	Ms6564P in EcoRI-HindIIIsite of pMV261:: LacZ	This study
pMV261::757P::LacZ	Rv757P in EcoRI-HindIIIsite of pMV261:: LacZ	This study
pMV261::GroEL1P::LacZ	GroEL1P in EcoRI-HindIIIsite of pMV261:: LacZ	This study
pMind	Kan^r, pAL5000 replicon	26
pMind::6564	Ms6564 in BamHI-PacI site of pMind	This study
pBXcmT	$chlo^r$, p15A replicon, lac-UV5 promoter	2
p6902	Ms6902p in XbaI sites of pBXcmT	This study

continued

P6564	Ms6564p in XbaI sites of pBXcmT	This study
P2312	Ms2312p in XbaI sites of pBXcmT	This study
P4925	Ms4925p in XbaI sites of pBXcmT	This study
pTRG	tet^r, ColE1 replicon, lpp/lac-UV5 promoter	Stratagene
pTRG-6564	Ms6564 in EcoRI-XbaI sites of pTRG	This study
pTRG-0349	Ms0349 in EcoRI-XbaI sites of pTRG	This study
pET28a(+)	Kan^r, T7 lac promoter, N-terminal His_6	Novagen
pET-6564	Ms6564 in EcoRI-XbaI sites of pET28a	This study

Supplemental Fig. S1

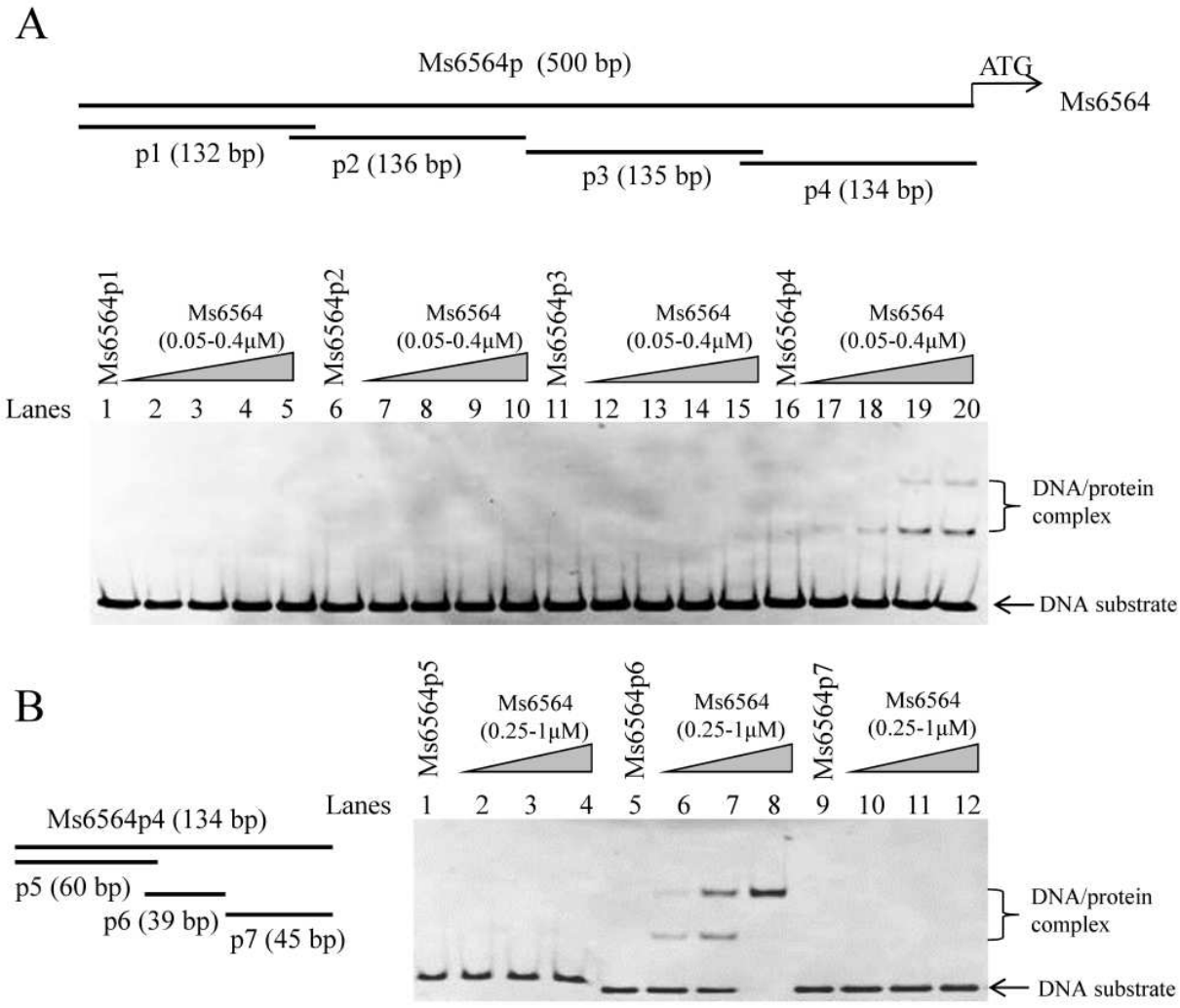

Fig. S1 EMSA assays for mapping the DNA-binding region within the Ms6564 promoter by Ms6564 protein. (A) Several short duplex DNA substrates derived from the Ms6564 promoter DNA, designated as p1, p2, p3, and p4, were synthesized and labeled. Each of these different DNA substrates was co-incubated with 0.05-0.4μM Ms6564 protein. **(B)** Several further truncated DNA substrates, p5, p6, and p7, covering different regions of Ms6564p4 were synthesized. Each of the DNA substrates was co-incubated with 0.25 - 1 μM Ms6564 protein. The free DNA substrate and DNA/protein complex are indicated.

Supplemental Fig. S2

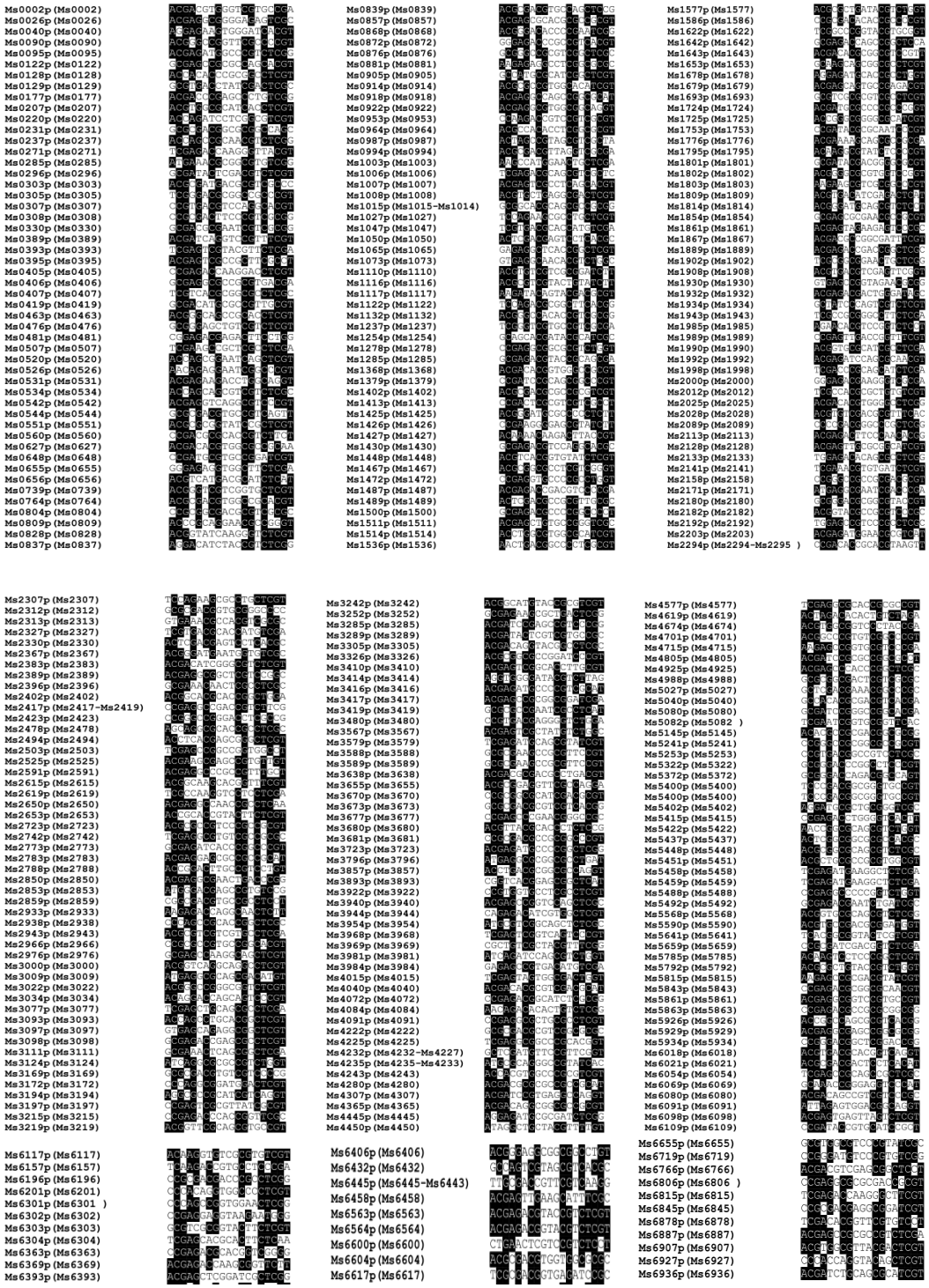

Fig. S2 The potential target genes of Ms6564 and motif sequence analysis. The ACGAGACGGTACGTCTCGT sequence was used to search for the *M. smegmatis* genomes. The identified motifs are listed.

Supplemental Fig. S3

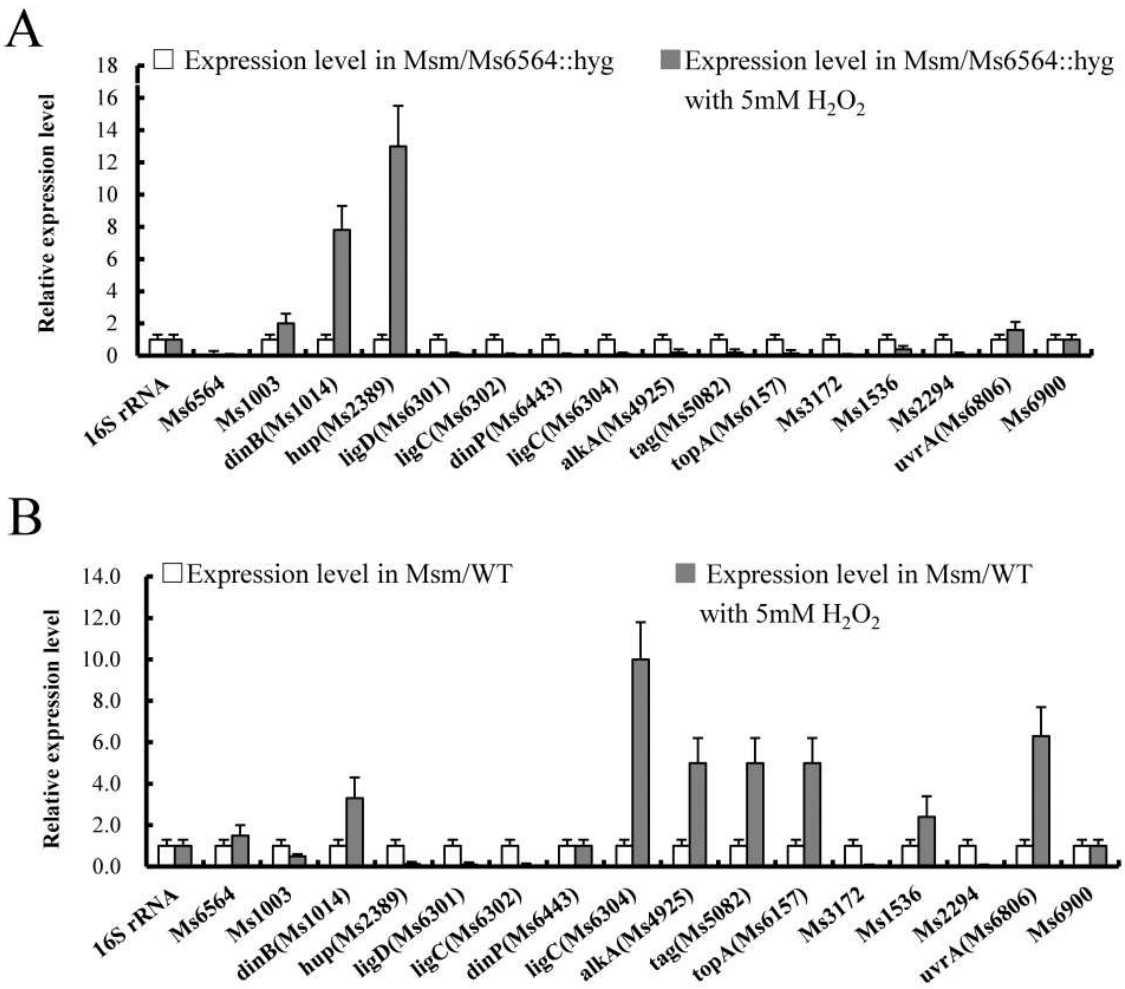

Fig. S3 Expression assays of DNA damage and repair genes in wild-type and Ms6564-deleted mutant strains after induction by 5 mM H_2O_2. qRT-PCR assay for the relative expression levels of DNA damage and repair genes in ΔMs6564 *M. smegmatis* strains **(A)** and in wildtype strains **(B)** before and after induction by 5 mM H_2O_2 for 3 h. The mycobacterial cDNA was amplified as described in "Materials and Methods". The relative expression levels of the genes were normalized using 16S rRNA gene as an invariant transcript, and an unrelated promoter gene Ms6900 was used as negative control. Data were analyzed using the $2^{\Delta\Delta Ct}$ method as described previously (30).

Supplemental Fig. S4

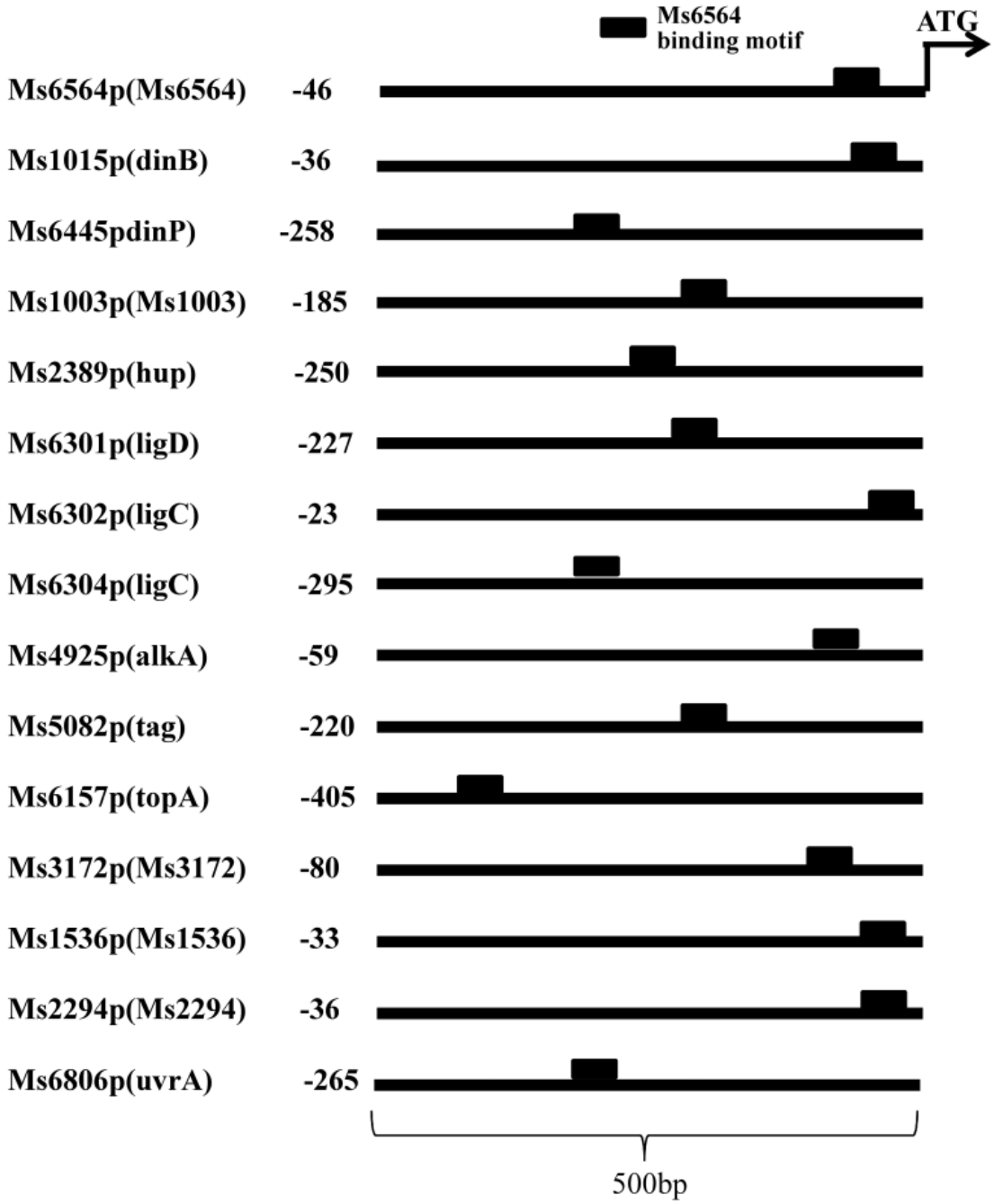

Fig. S4. Assays for the locations of binding site for Ms6564 with respect to the promoters of target genes. The distance (bp) to the start code ATG are indicated.

Supplemental Fig. S5

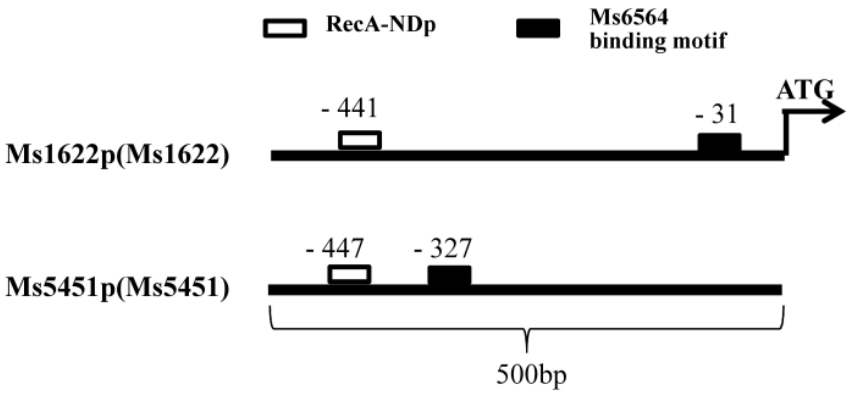

Fig. S5. Comparative assays for the locations of binding site for Ms6564 and ClpR regulators with respect to the promoters of target genes. The distance (bp) to the start code ATG are indicated on top of the sites.