A novel stress-inducible CmtR-ESX3-Zn²⁺ regulatory pathway essential for survival of *Mycobacterium bovis* under oxidative stress

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Running title: A novel CmtR-triggered antioxidant pathway in mycobacteria

Keywords: Mycobacterium bovis; oxidative stress; ESX-3; zinc ion; CmtR

Supplemental Information

Figure S1. Physical map of the *cmtR* gene region and amino acid sequence alignment of CmtR in *M. tuberculosis* H37Rv.

Figure S2. Assays for evaluating the DNA-binding ability of CmtR and the effect of H_2O_2 on the intracellular DNA-binding activity of EthR in *M. bovis* BCG.

Figure S3. Assays for studying the effects of H_2O_2 on the DNA-binding ability of CmtR and its mutant variants.

Figure S4. Assays for studying the effect of CmtR (C24S) on the growth of *M. bovis* BCG under H_2O_2 stress.

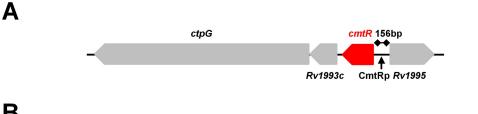
Figure S5. The functional categories of the potential target genes of CmtR in *M*. *tuberculosis* H37Ra.

Figure S6. Assays for studying the effects of H_2O_2 on the expression of *esx-3* operon genes in *M. bovis* BCG strains.

Figure S7. Assays for studying the growth of *M. bovis* BCG strains in 7H9 medium.

Figure S8. Assays for studying the survival of *M. bovis* BCG strains under H_2O_2 stress.

Table S1. Primers used in this study.Table S2. Strains and plasmids used in this study.



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Mtu:	Rv1994c	MLTCEMRESALARLGRALADPTRCRILVALLDGVCYPGQLAAHLGLTR
Mra:	MRA_2010	MLTCEMRESALARLGRALADPTRCRILVALLDGVCYPGQLAAHLGLTR
Mbb:	BCG_2011c	MLTCEMRESALARLGRALADPTRCRILVALLDGVCYPGQLAAHLGLTR
Msm:	MSMEG_5603	-MQTALHTDALARFGHALSDVTRTRILLSLNESPNYPADLAEQLGVSR
Mmi:	MMAR_2139	-MPMKIADDAPPTASPREDLAGAVALFHSLSDPTRLAIARRLADGERRVVDLTRELGLPQ
Mtu:	Rv1994c	SNVSNHLSCLRGCGLVVATYEGRQVRYALADSHLARALGELVQVVLAVDTDQPCVAE
Mra:	MRA_2010	SNVSNHLSCLRGCGLVVATYEGRQVRYALADSHLARALGELVQVVLAVDTDQPCVAE
Mbb:	BCG_2011c	SNVSNHLSCLRGCGLVVATYEGRQVRYALADSHLARALGELVQVVLAVDTDQPCVAE
Msm:	MSMEG_5603	QTLSNHLACLRGCGLVVAVPEGRRTRYELADARIGRALDDLMGLVLDVDPECRCVGP
Mmi:	MMAR_2139	STVSSHLACLRDCGLIAGRPEGRQVFYALAVPDLLDLFAAAETVLAATGNAVTLCPNYGT
Mtu:	Rv1994c	RAASGEAVEMTGS 118
Mra:	MRA_2010	RAASGEAVEMTGS 118
Mbb:	BCG_2011c	RAASGEAVEMTGS 118
Msm:	MSMEG_5603	DGAVCGCG 112
Mmi:	MMAR_2139	RPTRASASRRRGVKRAAPGVADWSPGR 146

Figure S1. Physical map of the *cmtR* gene region and amino acid sequence alignment of CmtR in *M. tuberculosis* H37Rv. (A) The *cmtR*(*Rv1994c*) operon genes in the *M. tuberculosis* H37Rv genome are shown. The CmtR-regulated region (*cmtRp*) are indicated by black arrows. (B) Amino acid sequence alignment (generated using ClustalW) of CmtR for the analysis of cysteine residue conservation among different mycobacterial species. The sequences shown are Rv1994c from *M. tuberculosis* H37Rv (Mtu), MRA_2010 from *M. tuberculosis* H37Ra (Mra), BCG_2011c from *M. bovis* BCG (Mbb), MSMEG_5603 from *Mycobacterium smegmatis* (Msm), and MMAR_2139 from *Mycobacterium marinum* (Mmi). The terms marked in red represent different cysteine residue sites in the sequence.

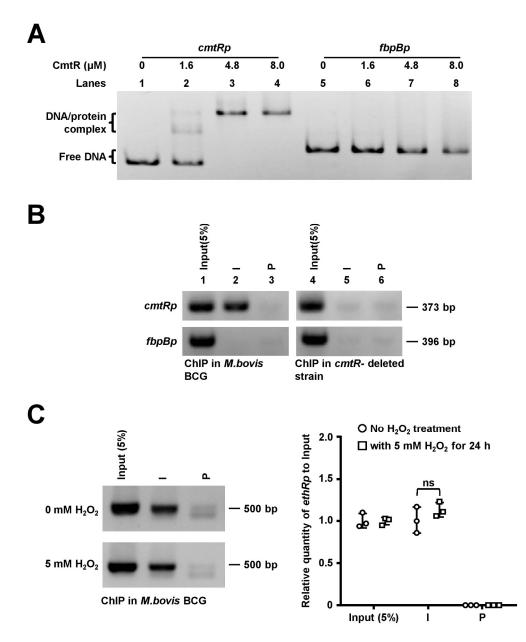
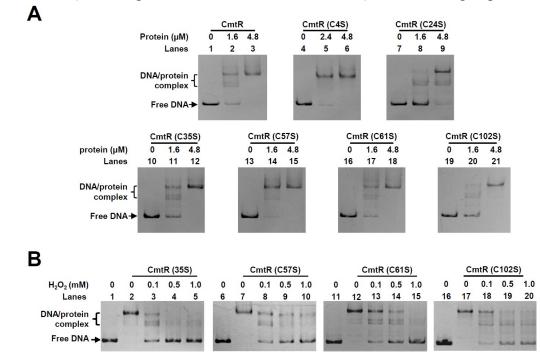
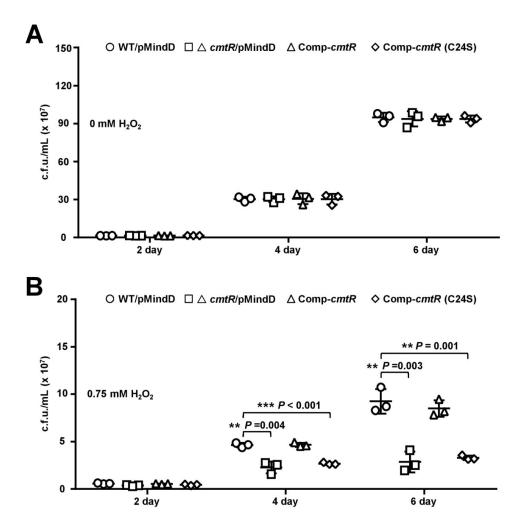


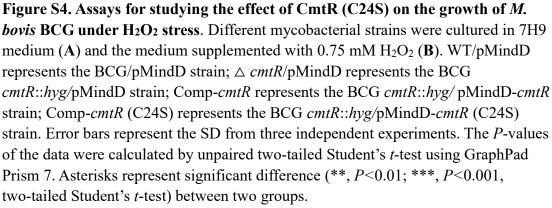
Figure S2. Assays for evaluating the DNA-binding ability of CmtR and the effect of H₂O₂ on the intracellular DNA-binding activity of EthR in *M. bovis* BCG. (A) EMSA. Either *cmtRp* promoter DNA (lanes 1-4) or *fbpBp* (lanes 5-8) were co-incubated with increasing quantities of CmtR, and the DNA-binding activities of the regulator were determined. (B) ChIP assays. ChIP using preimmune (P) or immune sera (I) against *cmtRp*. The mycobacterial promoter *fbpBp* was used as a negative control. The experiments were performed either in *M. bovis* BCG or in the *cmtR*-deleted strains. (C) Quantitative ChIP assays for evaluating the effect of H₂O₂ on the intracellular DNA-binding activity of EthR in *M. bovis* BCG. The Input (5%) indicates that the supernatant of the culture containing disrupted cells was diluted to 5% and used as the template for PCR. ChIP using P or I sera against EthR. Quantification was performed using qPCR (the right panel). Error bars represent the SD from three independent experiments. The *P*-values of the data were calculated by two-tailed Student's *t*-test using GraphPad Prism 7. Asterisks represent significant



difference (ns, not significant, two-tailed Student's *t*-test) between two groups.

Figure S3. Assays for studying the effects of H₂O₂ on the DNA-binding ability of CmtR and its mutant variants. (A) EMSA for studying the DNA-binding ability of CmtR and its mutant variants. CmtR and its mutant variants were co-incubated with the *cmtRp* DNA substrate; increasing concentrations (1.6-4.8 μ M) of the proteins are indicated at the top of the panels. (B) EMSA for studying the effects of H₂O₂ on the DNA-binding activity of CmtR mutant proteins. The protein concentration was 3.2 μ M. The concentration (0.1-1 mM) of H₂O₂ is indicated at the top of the panels. The protein/DNA complexes are indicated by arrows on the left side of the panels.





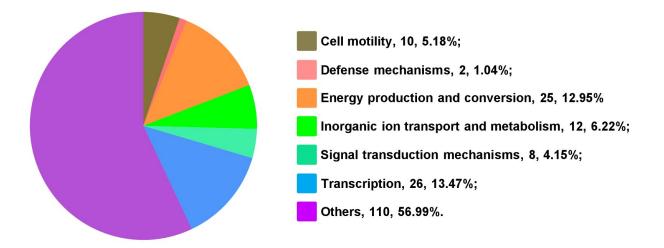


Figure S5. The functional categories of the potential target genes of CmtR in *M. tuberculosis* H37Ra. A functional classification of differentially expressed genes between wild-type and *cmtR*-deleted *M. tuberculosis* H37Ra strains in the context of Cluster of Orthologous Groups categories.

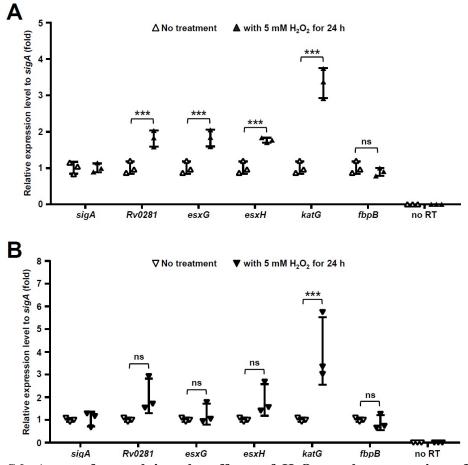


Figure S6. Assays for studying the effects of H₂O₂ on the expression of *esx-3* operon genes in *M. bovis* BCG strains. (A and B) qRT-PCR assays for studying the expression patterns of different genes (*Rv0281*, *esxG*, *esxH*, and *cmtR*) in *M. bovis* BCG WT (A) or *cmtR*-deleted strains (B) under treatment with indicated concentrations (0/5 mM) of H₂O₂ for 24 h. *KatG* and *fbpB* were used as control genes. Relative expression levels of the genes were normalized to those of *sigA*, which was used as an invariant transcript control using the $2^{-\Delta\Delta Ct}$ method. Error bars represent the SD from three independent experiments. The *P*-values of the data were calculated by unpaired two-tailed Student's *t*-test using GraphPad Prism 7. Asterisks represent significant difference (***, *P*<0.001; ns, not significant, two-tailed Student's *t*-test) between two groups.

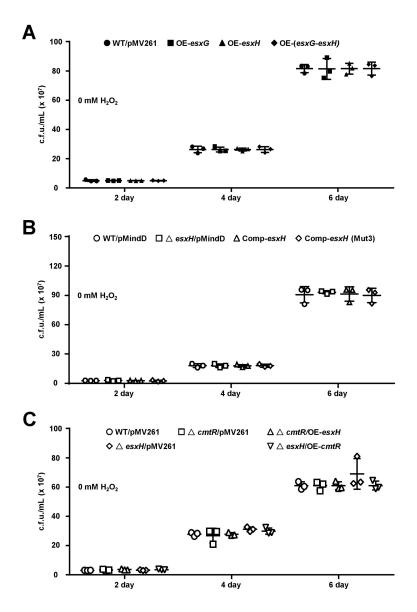
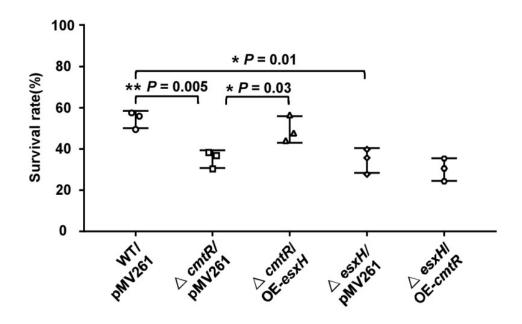
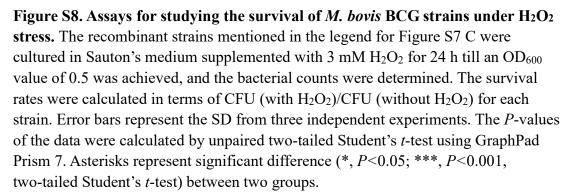


Figure S7. Assays for studying the growth of *M. bovis* BCG strains in 7H9 medium. (A-C) The recombinant mycobacterial strains were cultured in 7H9 medium for 6 days, and the bacterial counts were determined every 2 days. (A) WT/pMV261 represents the BCG/pMV261 strain; OE-*esxG*, OE-*esxH*, and OE-(*esxG-esxH*) represent the BCG/pMV261-*esxG*, BCG/pMV261-*esxH*, and BCG/pMV261-(*esxG-esxH*) strains, respectively; (B) WT/pMindD represents the BCG/pMindD strain; \triangle *esxH*/pMindD represents the BCG *esxH*::*hyg*/pMindD strain; Comp-*esxH* represents the BCG *esxH*::*hyg*/pMindD-*esxH* strain; Comp-*esxH*-Mut3 represents the BCG *esxH*::*hyg*/pMindD-*esxH* (Mut3) (Mut3: H14A, H70A, H76A) strain. (C) WT/pMV261 represents the BCG/pMV261 strain; \triangle *cmtR*/pMV261 represents the BCG *cmtR*::*hyg*/pMV261 strain; \triangle *cmtR*/OE-*esxH* represents the BCG *esxH*::*hyg*/pMV261-*esxH* strain; \triangle *esxH*/OE-*cmtR* represents the BCG *esxH*::*hyg*/pMV261 strain; \triangle *esxH*/OE-*cmtR* represents the BCG





	Usage	ConstructPrimer name		Sequence (from 5' to 3')
(pMV261 or pET-28a)Rv1994crCCGATCTAGATCTAGATCTAGCTACCTGGACTTGACloning and expression (pET-28a-Sumo)Rw2711frCCTGGAATTCCCAGACTGCGACCTGGACGAGTGGGA(pET-28a-Sumo)Rw1994cfTTTGATGGATCCATGCTGACCTGGAGATGCG(pET-28a-Sumo)Rw2359rCCAAAAGCTTTTAGCTGCCGGCAGTGGA(pET-28a-Sumo)Rw242fATTAGGATCCGTGAAGAGACAATCAGTTGGG(pET-28a-Sumo)Rw242fATTAGGATCCGGAAGAGCACATCAGTGGG(pET-28a-Sumo)Rw242fATTAGGATCCGGAAGAGCCGGTGGGATAGT(pET-28a-Sumo)Rw242fATTAGGATCCGGATGGGGGCGGGATAGGT(pGm) and expression (pET-28a-Sumo)Rw242fCCCCGGAATTCGGATGGCGCCCCATTGGATGGCGCCGATAGT(PGm) and expression (pET-28a-Sumo)Rw248fCCCTCCGAATTCGGATGGCGCCCCATTGGCTGGA(PGm) and expression (pET-28a-Sumo)Rw28fCCCTCCGAATTCGGATGGCGCCCCATTGGCTGGA(PGm) and expression (pET-28a-Sumo)Rw28fCCCTCCGAATTCGGATGGCGCCCCATTGGCTGGA(PGm) and expression (pET-28a-Sumo)Rw18CCCTCCGAATTCGGATGGCGCCCCATTGGC(PGm) and expression (pET-28a-Sumo)CufsCCCTCCGAATTCGGATGGCGCCCCCATTGGC(PGm) and expression (pET-28a-Sumo)CufsCCCTCCGAATTCGGATGCGCCCCCATTGGC(PGm) and expression (pET-28a-Sumo)CufsCCCCCCCCCGAATTCGGATGCCCCCATTGGC(PGm) and expression (pET-28a-Sumo)CufsCCCCCCCCGGATAGGCCCCCCATTGGC(PGm) and expression (CATSCufsCCCCCCCCGGATAGGACACCCCCGCAATC(PGm) and expression (PGT-2745CufsCGCACCCCCCCCCCGAAACC(PGT) and expression (PGT)CufsCATCGCCCCCCCCCCGGATAGGACACC <t< td=""><td>Cloning and expression</td><td rowspan="2">CmtR</td><td>Rv1994cf</td><td>CCAC<u>GAATTC</u>GTATGCTGACGTGTGAGATGCG</td></t<>	Cloning and expression	CmtR	Rv1994cf	CCAC <u>GAATTC</u> GTATGCTGACGTGTGAGATGCG
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(pET-28a-Sumo)Rv2242rATTAAAGCTTTCAGTGCGGCGTCGGATAGTCloning and expressionEscRv0287rCTCCGAATTCGGATGAGACCTTTTGGATGCTGARv010 and expressionEscHRv0288rCCCTCCGAATTCGGATGTCGAAATCATGTACRu01 and expressionEscHRv0288rTAACTCTAGAGGTAGCGCGCCCATTGGCsite-directed mutagenessiCdSCGSCCGACGCGGACGCGCGGATTCGGru11 contextCMRC4SrCCGACGCGGACCGGGACTCGGsite-directed mutagenessiCutRC24SrCCGACGCGGACCGGCGGGATCGGru12 contextCutRC24SrCAGACGCCGGACGGCGGGACCCGGCGGGru13 contextCutRC3SSrCTGGCCGGGATAGGAAACGCCATCru14 contextCutRC3SSrCTGGCCGGGATAGGAAACGCCATCru14 contextCutRCSTSrCAGCCCCGCAAAGAGGACAGAGGGru14 contextCutRC1SSCAGCCCCGCGAAAGCACAGAGGru14 contextCutRC102SrCTCCGGGACCCGGAGGATAGru14 contextCutRCU2SrCTCGGGGCCACAAGAGGGTGGTGGru14 contextCutRSssr/(H1A)ATATCCCGGGGACACAAGAGGATAGAACru14 contextSssr/(H1A)CGGGGCCATGGCGGAGACAAACru14 contextSssr/(H1A)CGGGGCCATGGCGGAAGAGGCCGCAAACru14 contextSssr/(H1A)CGGGGCCATGGCGGAAGAGGCCGCAAACru14 contextSssr/(H1A)CGGGGCCATGGCGGAAGAGGCCGAAACru14 contextSssr/(H1A)CGGGGCCATGGCGAAACACCAACACru14 contextSssr/(H1A)CGGGGCCATGGCGAAACACCAAACru14 contextSssr/(H1A)GGCCATAGCACAAAACCACAAACru14 contextSssr/(Cloning and expression	MID	Rv2242f	ATTA <u>GGATCC</u> GTGAACGACAATCAGTTGGC
Cloning and expressionExcRv0287rTACCTCTAGAGGTTAGAAACCCGGTATAGGTCGCloning and expressionExtRv0288fCCCTCCGAATTCGGATGTCGCAAATCATGTACRv0288rTAACTCTAGAGTTAGCCGCCCCATTTGGCsite-directed mutagenesisCmRC4SrCGCATCTCAGACGTCAGCATCTGAGATGCGsite-directed mutagenesisCmRC4SrCGCACCCGGGATCCGGGATCTGrettereted mutagenesisCmRC4SrCGAGCGGTTCCTATCCCGGCAGCrettereted mutagenesisCmRC3SSrCGGCCGGGATAGGAAACGCCATCrettereted mutagenesisCmRCSTSrCAGCCCGCGAAAGAGAAACGCCATCrettereted mutagenesisCmRCSTSrCAGCCCCGCAAAGACGACAGATGrettereted mutagenesisCmRC1SSrCAGCCCCGCAAAGAGGACGAGAGAGrettereted mutagenesisCmRC1SSrCAGCCCCGCAAAGAGGCCCGGAAAACrettereted mutagenesisCmRC102SrCTCGCGGACAGAGGGTTGGTCGrettereted mutagenesisCmRC102SrCTCGGCGACAGAGGGTTGGTCGrettereted mutagenesisCmRC102SrCTCGGCGACAGAGGGTTGGTCGrettereted mutagenesisCmRSxH (H1A)ATGTTGGGTGCCGCGGGGATATrettereted mutagenesisSmASxH (H1A)CTGCGGCCCCGGGGATATrettereted mutagenesisKmASxH (H1A)CTGCGGCCCGCGGGGATATrettereted mutagenesisSmASxH (H1A)TGCCGGCCCGCGGGAGCCCAAACACrettereted mutagenesisKmASxH (H1A)CGGCGCCCGCGGGAGCCCAAACACrettereted mutagenesisKmASxH (H1A)TGCGGGCCCGCGGGGAGATATrettereted mutagenesisKmASxH (H1A	(pET-28a-Sumo)	Марк	Rv2242r	ATTA <u>AAGCTT</u> TCAGTGCGGCGTCGGATAGT
Rv0287r TACCTCIAGAGGTTAGAACCCGGTATAGGTCG Cloning and expression ExH Rv0288f CCCTCCGAATTCGGATGTCGCAAATCATGTAC Rv0288r TAATCTAGAGCTAGCGCCCCATTTGGC Rv0288r TAATCGAGGTAGCGGCGCCCCATTGGC site-directed mutagenesis CmR C4Sr CGCACCGGGTCCGGAGTCTGAGCGTCGGAGTCGG site-directed mutagenesis CmR C24Sr CGAATCCGGGACCGCGGCGCGGCAGG site-directed mutagenesis CmR C35Sr CAGGCGGGTTGCTATGCCGGGCCGGGAGG site-directed mutagenesis C57Sr CAGCCCGGAAAGGACAACGCAACAGAGG site-directed mutagenesis C57Sr CAGCCCGGAAGGCGCGGGGGGGGGGGAGA site-directed mutagenesis C61Sr CTACCAGCCCGGAAGAGGCCGCGAGAAC site-directed mutagenesis C102Sr CTGGCGGCACCGAAAAC site-directed mutagenesis SaxH (H14A) ATGTCCCGGGGGCACCCAAACA site-directed mutagenesis ExxH SaxH (H14A) ATGCCCGGCAGCAGCAGAGAGA site-directed mutagenesis ExxH SaxH (H14A) ATGCCCGGCACCAAACAC site-directed mutagenesis ExxH GCGGGCCCGGGAGCCCAAACAC CAGCACCGCGCAGCAGAAACACCACAAAC site-directed mutagenesis		F C	Rv0287f	CTCC <u>GAATTC</u> GGATGAGCCTTTTGGATGCTCA
Cloning and expressionExHRv0288rTAACTCTAGAGTTAGCCGCCCCATTTGGCsite-directed mutagenessisCdSCGCATCTCAGAGGTCAGAGGTCGAGAGTGGGsite-directed mutagenessisCuRC24SrCCGACGCGGGCCCGGGATCCGGGCsite-directed mutagenessisCmRC35SrCTGGCCGGAAAGGAAACGCCATCsite-directed mutagenessisCmRC35SrCTGGCCGGAAAGGAAACGCCAGTGsite-directed mutagenessisCmRC57SrCAGCCCCGCAAAAGACGACAGATGsite-directed mutagenessisCmRC57SrCAGCCCCGCAAAAGACGACAGATGsite-directed mutagenessisCmRC102SrCTACCAGCCCGGAAGCGCCGGAAACGACGAGAGAGAGAGA	Cloning and expression	ESXG	Rv0287r	TACCTCTAGAGGTTAGAACCCGGTATAGGTCG
RA0288rTAACICTAGAGTTAGCCGCCCCATTGGCArring GMRC4SfTATGGATCCATGCTGACGTCGAGATGCGArring (C4S)C4SrCGCATCTCAGACGTCAGCATArring (C4S)C4SrCGCACCGGGACCCGGATCTGArring (C4S)C4SrCGACGCGGTCCCGGATTCTGArring (C4S)C4SrCGACACCGGGACCGCGCGGCGArring (C4S)C4SrCAGAATCCGGGACCGCGCGGCGGArring (C4S)C3SSCAGGCGTTTCCTATCCCGGCGCAGArring (C4S)C5SFCAGCCCGGAAAGACGCACAGATGArring (C4S)C57SCACCCGCGAAAGACGACAGATGArring (C4S)C61SrCACCACCCCGGAGCCCGCGAAACArring (C4S)C102SCGACCAACCCTCTGTCGCGGAGAArring (C4S)C102SrCTGGGGACAGAGGGTGGTGGArring (C402)C102SrCTCGGGGCACCCAAACACArring (C402)EsxH (H1AA)ATATCCCCGGCGGCACCCAAAATArring (C403)EsxH (H1AA)TGGACATCGCAGGAGAGCCGCAAArring (C404)EsxH (H70A)TGGACATCGCAGCATAGCCCGCAArring (C404)EsxH (H70A)TGGACATCGCAGCATAGCCCGCAArring (C405)EsxH (H70A)TGGCGATCACGCGGAACCACACArring (C404)Arring (C405)ArringArring (C405)EsxH (H70A)TGGCGACACCACACACACACArring (C405)EsxH (H70A)TGGCGACACCACAGAGGCTGCGAArring (C404)EsxH (H70A)TGGCCGACACACACACACACACArring (C405)EsxH (H70A)TGGCCGACACACACACACACACACArring (C405)EsxH (H70A)TGGCCGACACACACACACACACACACArrin		F 11	Rv0288f	CCCTCC <u>GAATTC</u> GGATGTCGCAAATCATGTAC
site-directed mutagenesisCMRC4SrCGCATCTAGACGTCAGCATsite-directed mutagenesisCmRC24SrCCGACGCGGGCCCGGATCTGcC4SC24SrCAGAATCCGGGACCGCGCGGCAGsite-directed mutagenesisCmRC35SfGATGGCGTTTCCTATCCCGGCCAGcC1C3SC35SrCTGGCCGGGATAGGAAACGCCATCcC1C3SC57SrCAGCCCGCAAAGACGACAGATGcC1C3CC57SrCAGCCCCGCAAAGCGACGACGAGAGsite-directed mutagenesisC61SrCTACCAGCCCGGAGCCCGGAGAGcC1C3CC102SrCTACCAGCCCGGAGCGCCGGAGAGsite-directed mutagenesisC102SrCTCGGCGACAGAGGGTTGGTCGc110C102SrCTCGGCGACAGAGGGTTGGTCGsite-directed mutagenesisEsxH (H14A)ATGTTGGGTGCCCCCGGAGATATsite-directed mutagenesisEsxH (H14A)rATATCCCCGGCGGCACCCAAACATsite-directed mutagenesisEsxH (H70A)rTGGACATCGCAGCAGCAGCAGCAGAGAGAGAGAGAGAGAG	Cloning and expression	EsxH	Rv0288r	TAAC <u>TCTAGA</u> GTTAGCCGCCCCATTTGGC
(c4s)C4srCGCATCTCAGACGTCAGCAT3ite-directed mutagenelCmRC24SrCCGACGCGGTCCCGGATTCTG3ite-directed mutagenelCmRC35SrGATGGCGTTTCCTATCCCGGCCAG3ite-directed mutagenelCmRC57SrCGGCCGGGATAGGAAAGCGCATC3ite-directed mutagenelCmRC57SrCAGCCCGCAAAGACGACGGTGGTGG3ite-directed mutagenelC61SrCTTGCGGGGCTCGGGGCGGGAG3ite-directed mutagenelC61SrCTACCAGCCCGGAGGCCGGAGAG3ite-directed mutagenelC102SrCGACCAACCCTCTGTCGCCGAG3ite-directed mutagenelC102SrCTCGGCGACAGAGGGTTGGTCG3ite-directed mutagenelEsxHC102SrCTCGGCGACAGAGGGTTGGTCG3ite-directed mutagenelEsxHSitt/H14A)ATGTCGCGGGGCACCAACAT3ite-directed mutagenelEsxHEsxH (H14A)CGGGCCTATGCTGCGGGGATAT3ite-directed mutagenelEsxHEsxH (H14A)GCGGGCCTATGCTGCGAGGCCGCA3ite-directed mutagenelEsxHGACCAACCCCGGGGACCCAACAT3ite-directed mutagenelEsxHGTGCAGCCGCGGCAGCACAACAC3ite-directed mutagenelEsxHGTGCAGCCCGCGGGGCTGGAGC3ite-directed mutagenelEsxHGSCGACCACCGCGAGACCAACAC3ite-directed mutagenelEsxHGTACAGTCCAGCCGCAAAACAC3ite-directed mutagenelEsxHGTACAGTCGGGCGCGGGGGGGGGGGGGGGGCGGAC3ite-directed mutagenelEsxHGSCGACCAGCACACACGCGGAAACACGTGGGCGCAGCAGCAGAGAGAGAGAGAGAGAGAGAGAGAG	·. ·· · · · ·	CmtR	C4Sf	TTAT <u>GGATCC</u> ATGCTGACGTCTGAGATGCG
site-directed mutageness(C24S)C24SrCAGAATCCGGGACCGCGTCG3te-directed mutagenessCmRG35SfGATGGCGGGATAGGAAACGCCATC3te-directed mutagenessCmRCATCGTCGTCTTGCGGGGCTG6CMRC57SfCAGCCCGGAAAGAGGACGACAGATG6CMRC1SSrCAGCCCGGCAAAGACGACAGATG6CMRC1SSrCAGCCCGGCAAAGACGACGAGAG6CMRC102SCAGCCCGCGAAGCCCCGCAAAC6CMRC102SCOCGGCGACAGAGGGTTGGTGG6CMRExxH (H14A)ATGTGGGGGCACCGAGAGAGGGTGGTGA61edreted mutagenessExXH (H14A)ATGTCCCGGGGGACACACACAC61edreted mutagenessExXH (H14A)GCGGGCCTATGCTGCGGAGAGAGAGGAGAGAGAGAGAGAG	site-directed mutagenesis	(C4S)	C4Sr	CGCATCTCAGACGTCAGCAT
(C24S)(C24S)C24SrCAGAATCCGGGACCGCGTCGGsite-directed mutagenessCmRC35SfGATGGCGTTCCTATCCCGGCCAGsite-directed mutagenessCmRC57SfCAGCCCGCAAAGACGACAGATGsite-directed mutagenessCmRC61SfGTTTGCGGGGCTCCGGGCTGGTAGsite-directed mutagenessCmRC102SfCGACCAACCCTCTGTGCGCGAAACsite-directed mutagenessCmRC102SfCGACCAACCCTCTGTGCGCGAGsite-directed mutagenessCmRC102SfCGACCAACCCTCTGTGGCGAGAGCCCGAAACsite-directed mutagenessEsxHC102SrCTCGGGGGCCACCCAACAGAGGGTTGGTCGsite-directed mutagenessEsxHKSxH (H14A)fATGTTGGGTGCCGCCGGGAATATsite-directed mutagenessEsxHEsxH (H14A)fATGTCGCGGGGCACCCAACACsite-directed mutagenessEsxHSxH (H70A)fTGGACATCGCAGCAGAGGGTTGGTCGsite-directed mutagenessEsxHKSxH (H70A)fTGGACATCGCAGCAACACACACACACACACACACACACAC	·. ·· · · · ·	CmtR	C24Sf	CCGACGCGGTCCCGGATTCTG
site-directed mutagenesis(C35S)C35SrCTGGCCGGGATAGGAAACGCCATCat-directed mutagenesisC37SCATCTGTCGTCTTGCGGGGCTGbit-directed mutagenesisC41CC45SrCAGCCCCGCAAAGACGACAGATGbit-directed mutagenesisC102SrCTACCAGCCCGGAGGCTCCGGGGATATc102SrC102SrCTCGGCGACAGAGGGTTGGTCGc102SrC1CGGCGACAGAGGGTTGGTCGc102SrCTCGGCGACAGAGGGTTGGTCGc102SrCTCGGCGACGCGGGGATATc102SrCTCGGCGACAGAGGGTTGGTCGc102SrCTCGGCGACGCGCGGGGATATc102SrCTCGGCGACGCGCGGGGATATc102SrCTCGGCGCCGCGGGGATATc102SrCTCGGCGCCGCGGGGATATc102SrCTCGGCGCCGCGGGCACCAACATc102SrCTGGCGCCCGCGGGCACCAACATc102SrCTGGCGCCCGCGGCACCAACACc102SrCTGGCGCCCGCGGGCACCCAACACc102SrCTCCGCGCACGCGGGCACCACACACc102SrCTCCGCGCACACACACCACACACc102SrCTCCGCTAAACCCGCGGGCCCGCAc102SrCTCGCGTAAACCCACAGAGGc102SrCATCGCCGCACAACACc102SrCATCGCCATGCACACACACACACc102SrCATCGCCATGCACACACACACACACACACACc102SrCATCGCCACACACACACACACACACACACACACACACACA	site-directed mutagenesis	(C24S)	C24Sr	CAGAATCCGGGACCGCGTCGG
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site-directed mutagenesis(C57S)CAGCCCGCAAAGACGACAGATGAtter directed mutagenesisCmtRC61SfCTACCAGCCCGGAGCCCCGCAAACAtter directed mutagenesisCmtRC102SfCGACCAACCCTCTGTCGCCGAGAtter directed mutagenesisEsxHC102SrCTCGGCGACAGAGGGTTGGTCGAtter directed mutagenesisEsxHATGTTGGGTGCCGCGGGAAATAtter directed mutagenesisEsxHATGTTGGGTGCCGCGGGAACACACACACACACACACACAC	site-directed mutagenesis	(C35S)	C35Sr	CTGGCCGGGATAGGAAACGCCATC
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site-directed mutagenesis(C61S)C61SrCTACCAGCCCGGAGCCCCGCAAAC $a_{\rm He}$ ChtRC102SrCGACCAACCCTCTGTCGCCGAG $(C102)$ C102SrCTCGGCGACAGAGGGTTGGTCG $a_{\rm He}$ ExxH(H14A)ATGTTGGGTGCCGCGGGGATAT $a_{\rm He}$ ExxH(H14A)ATATCCCCGGCGCACCCAACAT $a_{\rm He}$ ExxH(H70A)TGGGGCCTATGCTGCGATGTCCA $a_{\rm He}$ ExxH(H70A)TGGACATCGCAGCATAGGCCCGCA $a_{\rm He}$ ExxH(H70A)TGGACATCGCAGCATAGGCCCGCA $a_{\rm He}$ ExxH(H70A)GTCCAGCACCGCTGAAGCCAACAC $a_{\rm He}$ ExxH(H76A)GTCCAGCACCGCTGAAGCCAACAC $a_{\rm He}$ ExxH(H76A)GTGTTGGCTTCAGCGGTGCTGGAC $a_{\rm He}$ ExxH(H76A)GTGTGGCTAAACCACAGAGGA $a_{\rm He}$ ExxH(H76A)GTGCGATAACCACAGAGGA $a_{\rm Ho}$ ExxHCATGGCTAAACCACAGAGGA $a_{\rm Ho}$ ExxH(H76A)GTGCGATAACCACAGAGGA $a_{\rm Ho}$ ExxH(H76A)GTGCGATAACCACAGAGAGA $a_{\rm Ho}$ ExxH(H76A)GTGCGATAACACACAGAGAGA $a_{\rm Ho}$ ExxH(H76A)CATGCCAATGCAC	site-directed mutagenesis CmtR C35Sf GATGGCGTTTCC CTGGCCGGGATA C35Si C35Sr CTGGCCGGGATA CmtR C57Sf CATCTGTCGTCTT CAGCCCCGCAAA CmtR C61Sf GTTTGCGGGGCT	CAGCCCCGCAAAGACGACAGATG		
(C61S)C61SrCTACCAGCCCGGAGGCCCCGCAAACsite-directed mutagenesisCmtRC102SrCGACCAACCCTCTGTCGCCGAG $(102S)$ C102SrCTCGGCGACAGAGGGTTGGTCG $(102S)$ C102SrATGTTGGGTGCCGCGGGGATAT $(114A)$ EsxHH14A)rATATCCCCGGCGGCACCCAACAT $(114A)$ EsxH (H14A)rATATCCCCGGCGGCACCCAACAT $(114A)$ EsxH (H10A)rTGCGGGCCTATGCTGCGATGTCCA $(114A)$ EsxH (H70A)rTGGACATCGCAGCATAGGCCCGCA $(114A)$ EsxH (H70A)rTGGACATCGCAGCATAGGCCCGCA $(114A)$ EsxH (H70A)rTGGACATCGCAGCATAGGCCCGCA $(114A)$ EsxH (H70A)rGTGCTGACGCGCAGAGCCAACAC $(114A)$ EsxH (H76A)rGTGTTGGCTTCAGCGGTGCTGGAC $(114A)$ EsxH (H76A)rGTGTTGGCTAAACCACAGAGGA $(114A)$ EsxH (H76A)rGTGTGGCTAAACCACAGAGGA $(114A)$ EsxH (H76A)rGTGTGGCTAAACCACAGAGGA $(114A)$ EsxH (H76A)rGTGTGGCTAAACCACAACAGAGGA $(114A)$ EsxH (H76A)rGTGTGGCTAAACCACAGAGGA $(114A)$ EsxH (H76A)rGTGTGGCTAAACCACAGAGGAGA $(114A)$ EsxH (H76A)rGTGCGATCAACCACAAGAGGA $(114A)$ EsxH (H76A)rGTGCGGCTAAACCACAAACACACAAACACAAACACAAAA $(114A)$ EsxH (H76A)rGTGCGATAAACACCAAAACAAAAAAAAAAAAAAAAAAAA	(C35S) C35Sr CTGGCCGGGATAGGAAACGCCATC site-directed mutagenesis CmtR C57Sf CATCTGTCGTCTTTGCGGGGGCTG site-directed mutagenesis CmtR C61Sf CATCTGCGGGGCTCCGGGGCTGGTAG site-directed mutagenesis CmtR C61Sf GTTTGCGGGGCTCCGGGGCTGGTAG site-directed mutagenesis CmtR C102Sf CGACCAACCCTCTGTCGCCGAG	GTTTGCGGGGCTCCGGGCTGGTAG		
site-directed mutagenesis(C102S)C102SrCTCGGCGACAGAGGGTTGGTCG a_{B} EsxHEsxHATGTTGGGTGCCGCGGGATAT a_{B} EsxHEsxHATATCCCCGGCGCACCAACAT a_{H} EsxHH14A)rATATCCCGGCGCACCCAACAT a_{H} EsxHH14A)rTGCGGGCCTATGCTGCGATGTCCA a_{H} EsxHH170A)rTGGACATCGCAGCAGCATAGGCCCGCA a_{H} EsxHH170A)rTGGACATCGCAGCATAGGCCCGCA a_{H} EsxHH170A)rGTGTTGGCTTCAGCGGTGCTGGAC a_{H} EsxHH170A)rGTGTTGGCTTCAGCGGTGCTGGAC a_{H} EsxHH170A)rGTGTTGGCTTCAGCGGTGCTGGAC a_{H} a_{H} EsxHH170A)r a_{H} a_{H} EsxH		CTACCAGCCCGGAGCCCCGCAAAC		
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	site-directed mutagenesis(C57S)C57SrCAGCCCCGCAAAGACGACAGATGsite-directed mutagenesisCmtRC61SfGTTTGCGGGGCTCCGGGGCTGGTAG(C61S)C61SrCTACCAGCCCGGAGGCCCCGCAAACsite-directed mutagenesisCmtRC102SfCGACCAACCCTCTGTCGCCGAG(C102S)C102SrCTCGGCGACAGAGGGTTGGTCG	CGACCAACCCTCTGTCGCCGAG		
site-directed mutagenesis(H14A) $Exvt(H14A)$ ATATCCCCGGCGGCACCCAACAT $H14A$ $Exvt(H14A)$ ATATCCCCGGCGGCACCCAACAT $H14A$ $Exvt(H10A)$ TGGCGGCCTATGCTGCGATGTCCA $H14A$ $Exvt(H70A)$ TGGACATCGCAGCATAGGCCCGCA $H10A$ $Exvt(H70A)$ TGGACATCGCAGCATAGGCCCGCA $H10A$ $Exvt(H76A)$ GTCCAGCACCGCTGAAGCCAACAC $H10A$ $Exvt(H76A)$ GTGTTGGCTTCAGCGGTGCTGGAC $H10A$ $Exvt(H76A)$ GTGTTGGCTAAATCCGTTGCCG $H10A$ $Exvt(H76A)$ GTGTGGCTAAATCCGTGCG $H10A$ $Exvt(H76A)$ GTGTGGCTAAATCCGTGCG $H10A$ $Exvt(H76A)$ GTGTGGCTAAATCCGTGCG $H10A$ $Exvt(H76A)$ GTGCGATAAATCCGTGCG $H10A$ $Exvt(H76A)$ $H10A$ $H10A$ $H10A$ $Exvt(H76A)$ $H10A$ $H10A$ $Exvt(H10A)$ $H10A$ $H10A$ $H10A$ $H10A$ $H10A$ $H10A$ $H10A$		(C102S)	C102Sr	CTCGGCGACAGAGGGTTGGTCG
(H14A) $EsxH$ (H14A)ATATCCCCGGCGCACCCAACAT $H14A$ $EsxH$ (H14A)ATATCCCCGGCGCACCCAACAT $H14A$ $EsxH$ (H70A)TGCGGGCTATGCTGCGATGTCCA $H14A$ $EsxH$ (H70A)TGGACATCGCAGCATAGGCCCGCA $H14A$ $EsxH$ (H70A)TGGACATCGCAGCATAGGCCCGCA $H14A$ $EsxH$ (H70A)TGGACATCGCAGCATAGGCCCGCA $H14A$ $EsxH$ (H70A)GTCCAGCACCGCTGAAGCCAACAC $H14A$ $EsxH$ (H70A)GTGTTGGCTTCAGCGGTGCTGGAC $H14A$ $EsxH$ (H70A)GTGTTGGCTTCAGCGGTGCTGGAC $H14A$ $EsxH$ (H70A)GTGTTGGCTTCAGCGGTGCTGGAC $H14A$ $EsxH$ (H70A)GTGTTGGCTAAATCCGTTGCAG $H14A$ $EsxH$ (H70A) $H14A$ $H14A$ $H1$	· · · · · · ·		<i>EsxH</i> (H14A)f	ATGTTGGGTGCCGCCGGGGATAT
site-directed mutagenesis $(H70A)$ $EsxH$ $(H70A)r$ TGGACATCGCAGCATAGGCCCGCAsite-directed mutagenesis $EsxH$ $EsxH$ $(H76A)f$ GTCCAGCACCGCTGAAGCCAACAC $(H76A)$ $EsxH$ $(H76A)r$ GTGTTGGCTTCAGCGGTGCTGGAC $H76A)$ $Rv0280pf$ ACTGGCTAAATCCGTTGCCG $EMSA$ $Rv0280pf$ AGCCGTAAACACCACAGAGGG $EMSA$ $Rv0282pf$ CTACGCTGTTGAACGACTAC $EMSA$ $Rv0282pf$ CTACGCTGTTGAACGACTAC $EMSA$ $Rv0282pf$ CATGCCAATGTCATCAC $ChIP/EMSA$ $PbBp$ $Rv1886cpf$ CATCGCACGCCACAAAC $Rv1886cpr$ GTGCCGATCATCATCG $Rv1886cpr$	site-directed mutagenesis	(H14A)	EsxH (H14A)r	ATATCCCCGGCGGCACCCAACAT
$ \begin{array}{c} \mbox{(H70A)} & EsxH (H70A)r & TGGACATCGCAGCATAGGCCCGCA \\ \hline \mbox{Fishedimentagenesis} & EsxH (H70A)r & GTCCAGCACCGCTGAAGCCAACAC \\ \hline \mbox{Fishedimentagenesis} & EsxH (H76A)r & GTGTTGGCTTCAGCGGTGCTGGAC \\ \hline \mbox{(H70A)} & EsxH (H76A)r & GTGTTGGCTTCAGCGGTGCTGGAC \\ \hline \mbox{(H70A)} & EsxH (H76A)r & GTGTTGGCTTCAGCGGTGCTGGAC \\ \hline \mbox{(H70A)} & Rv0280pr & ACTGGCTAAATCCGTTGCCG \\ \hline \mbox{(H70A)} & Rv0280pr & AGCCGTAAACACCACAGAGGG \\ \hline \mbox{(H70A)} & Rv0280pr & CTACGCTGTGAACGACTAC \\ \hline \mbox{(H70A)} & Rv0282pr & CTACGCTGTGAACGACTAC \\ \hline \mbox{(H70A)} & Rv0282pr & CATGCCAATGTCATCAC \\ \hline \mbox{(H70A)} & Rv1886cpr & CATCGCACGACAAAC \\ \hline \mbox{(H70A)} & Rv1886cpr & GTGCCGATCATCAATCG \\ \hline \mbox{(H70A)} & Rv1886cpr & GTGCCGATCATCAATCG \\ \hline \mbox{(H70A)} & Rv1886cpr & GTGCCGATCATCAATCG \\ \hline \mbox{(H70A)} & Rv1886cpr & GTGCCGATCAATCG \\ \hline \mbox{(H70A)} & Rv1886cpr & Rv1886cpr & Rv1886cpr \\ \hline \mbox{(H70A)} & Rv1886cpr & Rv1886cpr & Rv1886cpr \\ \hline \mbox{(H70A)} & Rv1886cpr & Rv1886cpr \\ \hline \mbox{(H70A)} & Rv1886cpr & Rv1886cpr & Rv1886cpr \\ \hline \mbox{(H70A)} & Rv1886cpr & Rv1886cpr & Rv1886cpr \\ \hline \mbox{(H70A)} & Rv1886cpr & Rv1886cpr & Rv1886cpr \\ \hline \mbox{(H70A)} & Rv1886cpr & Rv1886cpr \\ \hline \mbox{(H70A)} & Rv1886cpr & Rv1886cpr & Rv1886cpr \\ \hline \mbox{(H70A)} & Rv1886cpr & Rv1886cpr & Rv1886cpr \\ \hline \mbox{(H70A)} & Rv1886cpr & Rv1886cpr & Rv1886cpr & Rv1886cpr & Rv1886cpr & Rv1886cpr \\ \hline \mbox{(H70A)} & Rv1886cpr & Rv$	· · · · · · ·	EsxH	EsxH (H70A)f	TGCGGGCCTATGCTGCGATGTCCA
site-directed mutagenesis $(H76A)$ $EsxH(H76A)r$ GTGTTGGCTTCAGCGGTGCTGGAC $EMSA$ $Rv0280pf$ $ACTGGCTAAATCCGTTGCCG$ $EMSA$ $Rv0280pr$ $AGCCGTAAACACCACAGAGGG$ $EMSA$ $Rv0282pf$ $CTACGCTGTTGAACGACTAC$ $EMSA$ $Rv0282pf$ $CATGCCAATGTCATCAC$ $ChIP/EMSA$ $PbPP$ $Rv1886cpr$ $CATCGCACGCCACAAAC$	site-directed mutagenesis	(H70A)	EsxH (H70A)r	TGGACATCGCAGCATAGGCCCGCA
$ \begin{array}{c} (H76A) & EsxH (H76A)r & GTGTTGGCTTCAGCGGTGCTGGAC \\ \\ \hline \\ EMSA & Rv0280p & ACTGGCTAAATCCGTTGCCG \\ \hline \\ Rv0280pr & AGCCGTAAACACCACAGAGGG \\ \\ \hline \\ Rv0282p & CTACGCTGTTGAACGACTAC \\ \hline \\ Rv0282pr & CATGCCAATGTCATCAC \\ \\ \hline \\ Rv0282pr & CATGCCACGCACAAAC \\ \\ \hline \\ Rv1886cpr & GTGCCGATCATCATCG \\ \end{array} $	EsxH EsxH (H76A)f GTCCAGCACCGCTGAAGCCAA		EsxH (H76A)f	GTCCAGCACCGCTGAAGCCAACAC
EMSA $Rv0280p$ $AGCCGTAAACACCACAGAGG$ EMSA $Rv0282p$ $AGCCGTAAACACCACAGAGG$ EMSA $Rv0282p$ $CTACGCTGTTGAACGACTAC$ $Rv0282p$ $CATGCCAATGTCATCAC$ $ChIP/EMSA$ $fbpBp$ $Rv1886cpf$ $Rv1886cpr$ $GTGCCGATCATCAATCG$			GTGTTGGCTTCAGCGGTGCTGGAC	
$\frac{1}{100} Rv0280 pr \qquad AGCCGTAAACACCACAGAGG \\ EMSA \qquad \qquad$	EMCA	D0280		ACTGGCTAAATCCGTTGCCG
EMSA $Rv0282p$ $CATGCCAATGTCATCAC$ ChIP/EMSA $fbpBp$ $Rv1886cpf$ CATCGCACGCCACAAACRv1886cprGTGCCGATCATCAATCG	EMSA	Kv0280p	Rv0280pr	AGCCGTAAACACCACAGAGG
Rv0282pr CATGCCAATGTCATCAC ChIP/EMSA fbpBp Rv1886cpr CATCGCACGCCACAAAC Rv1886cpr GTGCCGATCATCAATCG		Rv0282p	Rv0282pf	CTACGCTGTTGAACGACTAC
ChIP/EMSA fbpBp Rv1886cpr GTGCCGATCATCAATCG	EMSA		Rv0282pr	CATGCCAATGTCATCAC
Rv1886cpr GTGCCGATCATCG	CLID/EN/CA	fbpBp	Rv1886cpf	CATCGCACGCCACAAAC
ChIP/EMSA cmtRp Rv1994cpf GGGACTTCCTGTTCCGG	UNIP/EMISA		Rv1886cpr	GTGCCGATCATCAATCG
	ChIP/EMSA	cmtRp	Rv1994cpf	GGGACTTCCTGTTCCGG

Table S1. Primers used in this study

		Rv1994cpr	GATTCCCGCATCTCACAC
Clana ta mMV261 laa7	Dv0280m	Rv0280pLf	GACG <u>GAATTC</u> ACTGGCTAAATCCGTTGCCG
Clone to pMV261-lacZ	Rv0280p	Rv0280pLr	GACG <u>TCTAGA</u> AGCCGTAAACACCACAGAGG
$C_{1} = 1$	Rv0282p	Rv0282pLf	GAGG <u>GAATTC</u> CTACGCTGTTGAACGACTAC
Clone to pMV261-lacZ		Rv0282pLr	GGGTG <u>TCTAGA</u> CATGCCAATGTCATCAC
	fbpBp	Rv1886cpLf	GATG <u>GAATTC</u> CATCGCACGCCACAAAC
Clone to pMV261-lacZ		Rv1886cpLr	GCAC <u>TCTAGA</u> GTGCCGATCATCAATCG
$C_{1} = 1$	cmtRp	Rv1994cpLf	TTTC <u>GAATTC</u> GGGACTTCCTGTTCCGG
Clone to pMV261-lacZ		Rv1994cpLr	GGTC <u>TCTAGA</u> GATTCCCGCATCTCACAC
$C_{1} = 1$	LacZ	lacZf	TTC <u>AAGCTT</u> ATGAGGATGAGGGAAGCAAG
Clone to pMV261-lacZ		lacZr	ATGC <u>GCTAGC</u> TTATTTTTGACACCAGACCA
knock out of <i>cmtR</i>	CmtR	BCG_2011cUPf	TCACAC <u>TTAATTAA</u> GTGTACTACCGACGTTAGCCAG
KNOCK OUT OI CMIR		BCG_2011cUPr	GGCG <u>ACTAGT</u> AGATCAAATAGTACACC
knock out of <i>cmtR</i>	CmtR	BCG_2011cDNf	CCCG <u>AAGCTT</u> GCAGCAGACGACACGACTTGTG
KHOCK OUT OI CMIR		BCG_2011cDNr	AAAC <u>GCTAGC</u> GCGATGGTCATCAGGGTGC
knock out of <i>esxH</i>	EavII	BCG_0328UPf	CCCGAC <u>TTAATTAA</u> GTCGCGGTGGTATCAAGG
KHOCK OUT OI ESXH	EsxH	BCG_0328UPr	GGTG <u>ACTAGT</u> CATCACAAGTCCTCTCG
knock out of <i>esxH</i>	EsxH	BCG_0328DNf	TTAG <u>AAGCTT</u> CTAGCTCGCGCTACATGG
KHOCK OUT OI ESXII	ЕЗХП	BCG_0328DNr	TTATTTTC <u>GCTAGC</u> GCAAACGGTGTCCCAGG
qRT-PCR	SigA	RT-sigAf	TCGCGCCTACCTCAAACAG
qKI-I CK	SigA	RT-sigAr	CGTACAGGCCAGCCTCGAT
aDT DCD	CmtR	RT-CmtRf	TGCTGGATGGCGTTTGCT
qRT-PCR		RT-CmtRr	GGCCCTCATAGGTTGCGACTA
qRT-PCR	EhepD	RT-fbpBf	CGCGACATCAAGGTTCAGTT
qKI-FCK	FbpB	RT-fbpBr	CCGGCATGACTATCGACAGT
	Dv0281	RT-Rv0281f	GGGCTGCTGATCTATCTCCC
qRT-PCR	Rv0281	RT-Rv0281r	TCGCTCGTTGTAGACCAGTT
aDT DCD	Earc	RT-esxGf	CGGCTCAGGCGTTTCAC
qRT-PCR	EsxG	RT-esxGr	CCGCCGCCACAAACC
qRT-PCR	EavU	RT-esxHf	CAGGCCGCGTTGCA
ЧКІ-РСК	EsxH	RT-esxHr	CTGCCACGCCTGATACGT
aDT DCD	KatG	RT-katGf	CCGAGATTGCCAGCCTTAA
qRT-PCR		RT-katGr	GTTGACCTCCCACCCGACT

Notes: Restriction enzyme sites are underlined.

Plasmid or Strain	Relevant genotype or feature	Source or
		reference
Plasmid		
pET28a	Kanr, lacZ operon, T7 promotor, His-Tag	Novagen
pET28a- <i>cmtR</i>	cmtR inserted in EcoRI-XbaI of pET28a	This study
pET28a- <i>cmtR</i> (C4S)	cmtR (C4S) inserted in EcoRI-XbaI of pET28a	This study
pET28a- <i>cmtR</i> (C24S)	cmtR (C24S) inserted in EcoRI-XbaI of pET28a	This study
pET28a- <i>cmtR</i> (C35S)	cmtR (C35S) inserted in EcoRI-XbaI of pET28a	This study
pET28a- <i>cmtR</i> (C57S)	cmtR (C57S) inserted in EcoRI-XbaI of pET28a	This study
pET28a- <i>cmtR</i> (C61S)	cmtR (C61S) inserted in EcoRI-XbaI of pET28a	This study
pET28a-cmtR(C102S)	cmtR (C102S) inserted in EcoRI-XbaI of pET28a	This study
pET28a- <i>ideR</i>	ideR inserted in EcoRI-XbaI of pET28a	This study
pET28a-Sumo	Kan ^r , lacZ operon, T7 promotor, His-Tag	Novagen
pET28a-Sumo- <i>cmtR</i>	cmtR inserted in BamHI-HindIII of pET28a-Sumo	This study
pET28a-Sumo- <i>mabR</i>	mabR inserted in BamHI-HindIII of pET28a-Sumo	This study
pET28a-Sumo- <i>zur</i>	zur inserted in BamHI-HindIII of pET28a-Sumo	This study
pMV261	Kan ^r , pAL5000 replicon	This study
pMV261-cmtR	cmtR inserted in EcoRI-XbaI of pMV261	This study
pMV261-esxG	esxG inserted in EcoRI-XbaI of pMV261	This study
pMV261-esxH	esxH inserted in EcoRI-XbaI of pMV261	This study
pMV261-esxG-esxH	esxG-esxH inserted in EcoRI-XbaI of pMV261	This study
pMV261-lacZ	<i>lacZ</i> fused with null-promoter	
pMV261-hsp60-lacZ	hsp60 promoter fused with <i>lacZ</i>	This study
pMV261-Rv0280p-lacZ	Rv0280p promoter fused with <i>lacZ</i>	This study
pMV261-Rv0282p- <i>lacZ</i>	Rv0282p promoter fused with <i>lacZ</i>	This study
pMV261-fbpBp-lacZ	fbpBp promoter fused with <i>lacZ</i>	This study
pMV261-cmtRp- <i>lacZ</i>	cmtRp promoter fused with <i>lacZ</i>	This study
pMindD	Kan ^r , tet ^r , pAL5000 replicon	
pMindD-cmtR	cmtR inserted in EcoRI-XbaI of pMindD	This study
pMindD-cmtR(C24S)	cmtR (C24S) inserted in EcoRI-XbaI of pMindD	This study
pMindD-esxH	esxH inserted in EcoRI-XbaI of pMindD	This study
pMindD- esxH (Mut3)	esxH (H14A, H70A, H76A) inserted in EcoRI-XbaI of pMindD	This study
Strain		
E.coli		
DH5a	Host for plasmid construction	TaKaRa
BL21(λDE3)	Host for protein expression	TaKaRa
M.bovis BCG		
BCG/WT	M. bovis BCG wild-type	ATCC
BCG/cmtR::hyg	BCG with <i>cmtR</i> replaced by hyg	This study
BCG/esxH::hyg	BCG with <i>esxH</i> replaced by <i>hyg</i>	This study
		2

Table S2. Strains and plasmids used in this study

BCG cmtR::hyg/pMV261	cmtR replaced by hyg in BCG, cmtR-deleted strain with pMV261	This study
BCG cmtR::hyg/pMV261-esxH	<i>cmtR</i> replaced by <i>hyg</i> in BCG, <i>cmtR</i> -deleted strain with pMV261- <i>esxH</i>	This study
BCG esxH::hyg/pMV261	esxH replaced by hyg in BCG, esxH-deleted strain with pMV261	This study
BCG esxH::hyg/pMV261-cmtR	esxH replaced by hyg in BCG, esxH-deleted strain with pMV261-cmth	RThis study
BCG/pMV261-cmtR	cmtR-overexpression Strain	This study
BCG/pMV261-esxG	esxG-overexpression Strain	This study
BCG/pMV261-esxH	esxH-overexpression Strain	This study
BCG/pMV261-esxG-esxH	esxG-esxH-overexpression Strain	This study
BCG/pMinD	BCG with pMinD	This study
BCG cmtR::hyg/pMinD	cmtR replaced by hyg in BCG, cmtR-deleted strain with pMinD	This study
BCG cmtR::hyg/pMinD-cmtR	cmtR replaced by hyg in BCG, cmtR-deleted strain with pMinD-cmtR	This study
BCG cmtR::hyg/pMinD-cmtR(C24S)	<i>cmtR</i> replaced by <i>hyg</i> in BCG, <i>cmtR</i> -deleted strain with pMinD- <i>cmtR</i> (C24S)	This study
BCG esxH::hyg/pMinD	esxH replaced by hyg in BCG, esxH-deleted strain with pMinD	This study
BCG esxH::hyg/pMinD-esxH	esxH replaced by hyg in BCG, esxH-deleted strain with pMinD-esxH	This study
BCG	esxH replaced by hyg in BCG, esxH-deleted strain with pMinD-esxH	
esxH::hyg/pMinD-esxH(Mut3)	(H14A, H70A, H76A)	This study
BCG/pMVL1	BCG with pMV261- <i>lacZ</i> , negative control of β - galactosidase assays	This study
BCG/pMVL2	BCG with pMV261-hsp60- <i>lacZ</i> , positive control of β -galactosidase assays	This study
BCG/pMVL3	BCG with pMV261-Rv0280p- <i>lacZ</i>	This study
BCG/pMVL4	BCG with pMV261-Rv0282p- <i>lacZ</i>	This study
BCG/pMVL5	BCG with pMV261-fbpBp- <i>lacZ</i>	This study
BCG/pMVL6	BCG with pMV261-cmtRp- <i>lacZ</i>	This study
BCG cmtR::hyg/pMVL1	<i>cmtR</i> replaced by hyg in BCG, <i>cmtR</i> -deleted strain with pMV261- <i>lacZ</i> , negative control of β - galactosidase assays	This study
BCG cmtR::hyg/pMVL2	<i>cmtR</i> replaced by hyg in BCG, <i>cmtR</i> -deleted strain with pMV261-hsp60- <i>lacZ</i> , positive control of β -galactosidase assays	This study
BCG cmtR::hyg/pMVL3	<i>cmtR</i> replaced by hyg in BCG, <i>cmtR</i> -deleted strain with pMV261-Rv0280p- <i>lacZ</i>	This study
BCG cmtR::hyg/pMVL4	<i>cmtR</i> replaced by hyg in BCG, <i>cmtR</i> -deleted strain with pMV261-Rv0282p- <i>lacZ</i>	This study
BCG cmtR::hyg/pMVL5	<i>cmtR</i> replaced by hyg in BCG, <i>cmtR</i> -deleted strain with pMV261-fbpBp- <i>lacZ</i>	This study
BCG cmtR::hyg/pMVL6	<i>cmtR</i> replaced by hyg in BCG, <i>cmtR</i> -deleted strain with pMV261-cmtRp- <i>lacZ</i>	This study
M. tuberculosis H37Ra		
H37Ra/WT	Wild-type <i>M.bovis</i> H37Ra	ATCC
H37Ra/cmtR::hyg	H37Ra with <i>cmtR</i> replaced by <i>hyg</i>	This study