Appendix Data for

Cellular redox poise in *Mycobacterium tuberculosis* is modulated by a novel actinomycetes-specific transcription factor AosR

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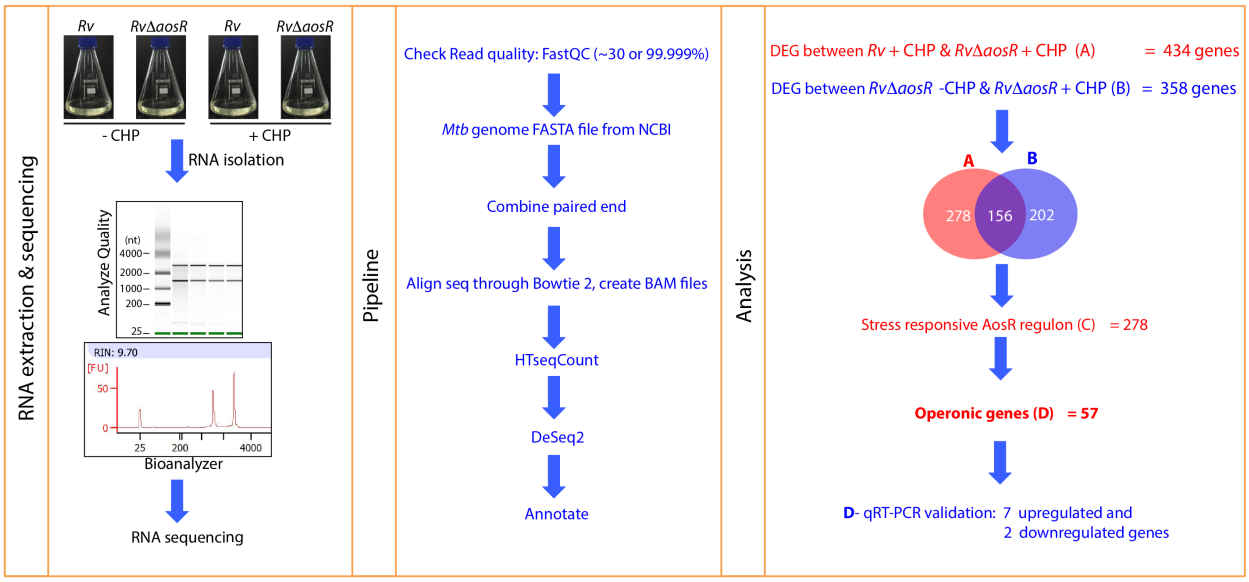
This PDF file includes:

Appendix Figure Legends

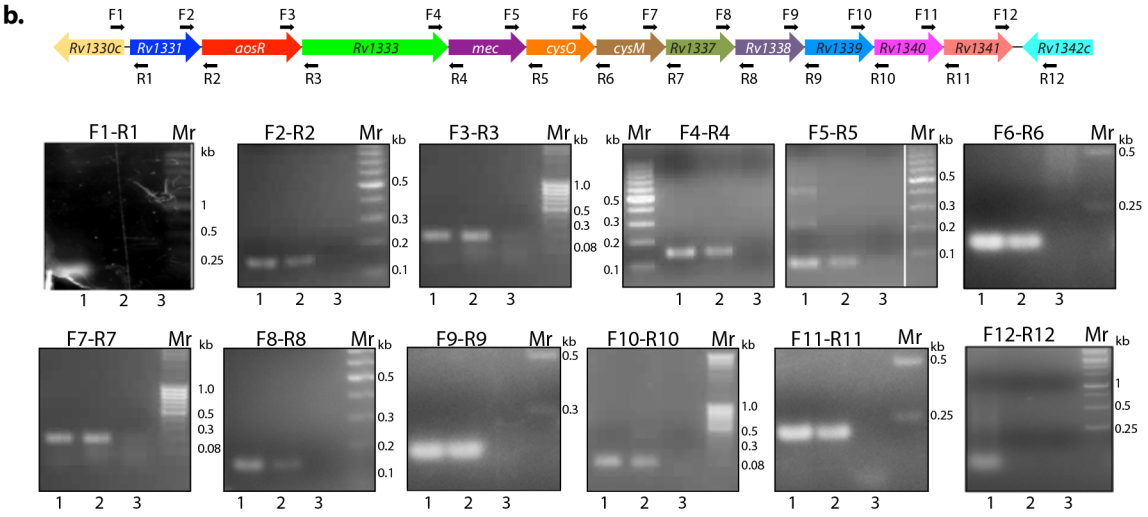


Appendix Figure S1 | Outline of processing of samples and data analysis for RNA seq.

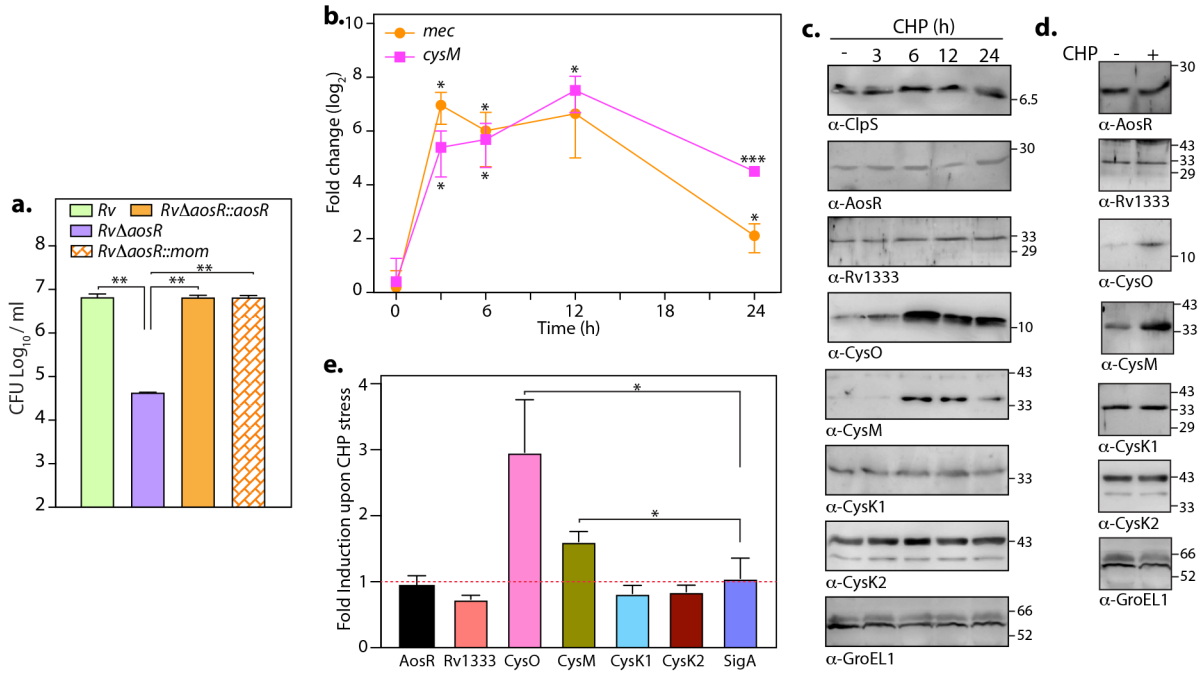
a.

Operon No.	Gene Name	Locus Name	Position	Gene Product
818	Rv1331	397673174	15006671 - 15009721	ATP dependent protease adapter protein (ClpS)
818	Rv1332	397673175	15009321 - 15015882	Transcriptional regulator
818	Rv1333	397673176	15016051 - 15026393	Amide hydrolase
818	Rv1334	397673177	15026471 - 15030874	Hydrolase (mec)
818	Rv1335	397673178	15031091 - 15033905	Sulfur carrier protein (CysO)
818	Rv1336	397673179	15034001 - 15043716	Cysteine synthase B (CysM)
818	Rv1337	397673180	15043621 - 15050847	Membrane protein
818	Rv1338	397673181	15050811 - 15058968	glutamate racemase (MurI)
818	Rv1339	397673182	15050231 - 15067449	Hypothetical protein
818	Rv1340	397673183	15067611 - 150754010	Ribonuclease (RhpA, RNase PH)
818	Rv1341	397673184	15075791 - 150819311	Nucleoside-triphosphatase

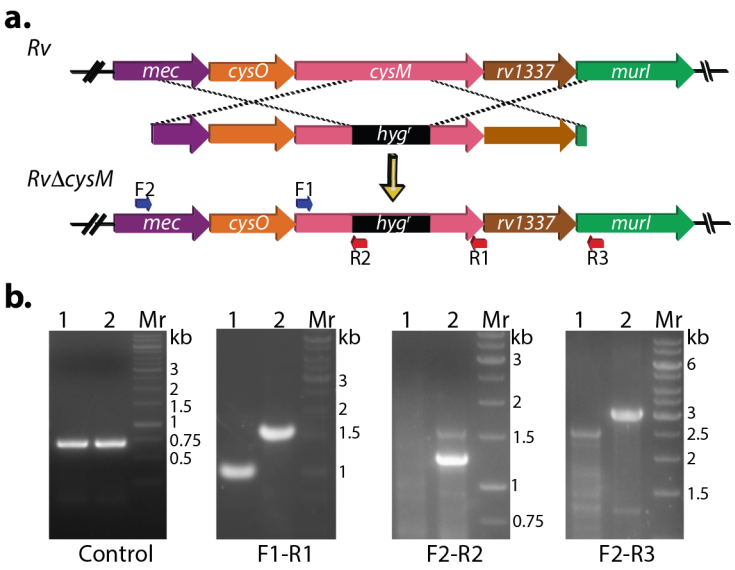
b.



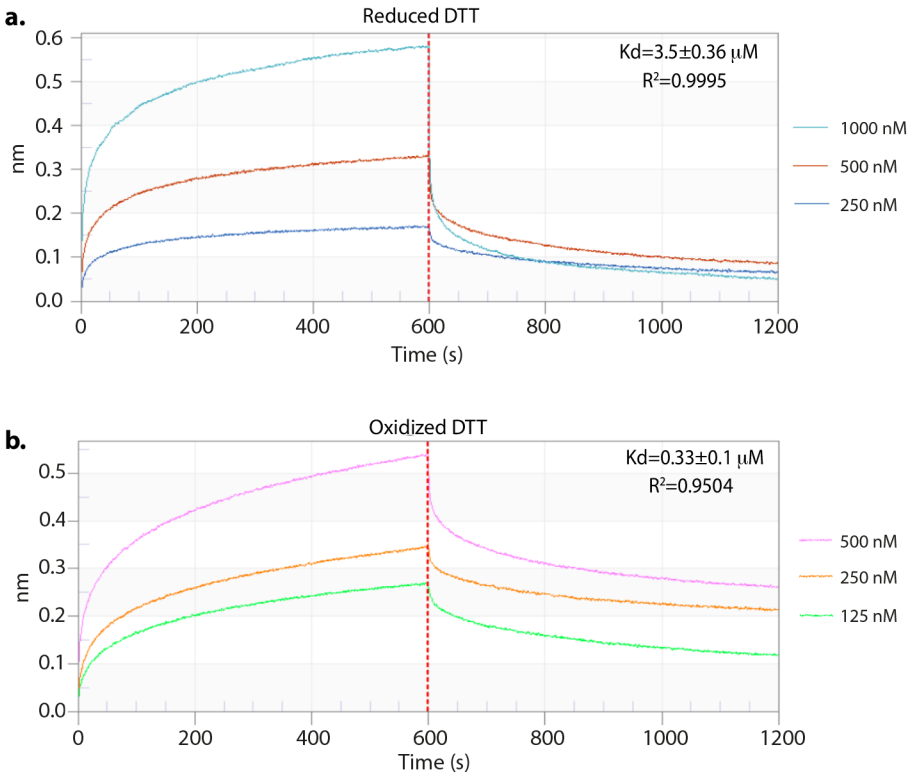
Appendix Figure S2 | Genetic organization of *aosR*. a. The transcriptional organization of the *Mtb* AosR was predicted by proOpDB (https://omictools.com/proopdb-tool), which suggested it to be a part of an eleven-gene operon. **b.** The operonic arrangement was confirmed by PCR with the help of intergenic primers. *Rv* genomic DNA (lane 1) was used as the positive control and mRNA that was not reverse transcribed was used as the negative control (lane 3). The PCR products from cDNA (lane 2) indicate that the adjacent genes are present on the same mRNA transcript and are, thus, operonic. Mr, molecular size marker.



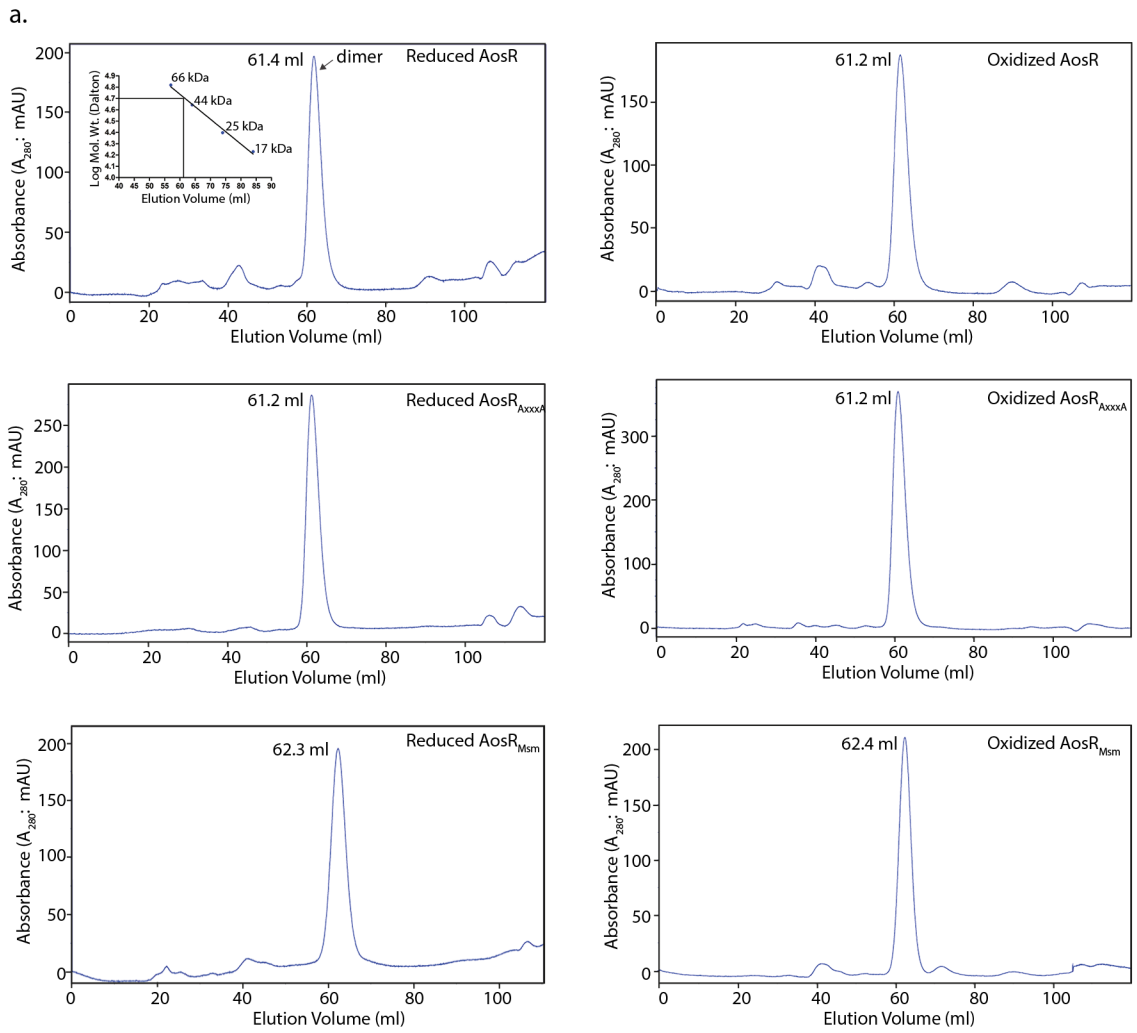
Appendix Figure S3|AosR regulates alternate cysteine biosynthesis pathway genes during oxidative stress. a. Bacterial strains were treated with 50 μM CHP for 24 h and the CFUs (mean ±SEM; n=3) were enumerated. Statistical significance was analyzed using one-way ANOVA followed by post hoc test (Tukey test; GraphPad prism). **b.** Early log phase of *Rv* was treated with 50 μM CHP and total RNA was extracted at indicated time points. The graph indicates the relative expression of *mec* and *cysM* quantitated by qRT-PCR compared with the untreated sample. Data was normalized with respect to 16s rRNA and results are expressed as mean log2 fold change ± SD of three independent replicates and is representative of one of two biological replicates. Statistical significance was analysed using the student's t-test (two tailed, unpaired). **c.** Early log phase of *Rv* was treated with 50 μM CHP and WCLs were prepared at indicated time points. The lysates were resolved on 12-15% SDS-PAGE, transferred to nitrocellulose membrane and probed with indicated antibodies. **d.** WCLs of *Rv* subjected to 0 or 50 μM of CHP for 6 h were resolved on 12-15% SDS-PAGE, transferred to nitrocellulose membrane and probed with indicated antibodies. **e.** Densitometric analysis of relative expression of indicated proteins upon treatment with 50 μM of CHP for 6 h as compared to untreated samples. The results were quantified from three biological replicates of panel **(d)**. The expression of each protein in a sample was normalized with respect to the expression of SigA for that sample. Statistical significance was analyzed using the student's t-test (two tailed, unpaired). *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$.



Appendix Figure S4|Generation of *RvΔcysM*. **a.** Pictorial representation of genomic DNA region in *Rv* and *RvΔcysM*, showing replacement *cysM* with *hygr*. Multiple primer sets used for the PCR based confirmation are depicted. **b.** PCR amplicons from 1. *Rv*; 2. *RvΔcysM*were resolved on 1% agarose gels. Primer pairs used for the amplification are indicated. First panel (control): ~0.7 kb amplicon of *aosR* in both the strains. Second panels show differential amplification of *cysM* in *Rv* (0.97 kb) and *RvΔcysM* (1.5 kb). Third panel shows amplicons (~1.2 kb) obtained only in *RvΔcysM* and the fourth panel shows differential amplicons sizes using beyond AES primers in *Rv* (~2.3 kb) and *RvΔcysM* (~3.1 kb). Mr represents 1 kb gene ruler ladder.



Appendix Figure S5| Bio-Layer Interferometry analysis of the interaction between SigH and AosR. BLI sensorgrams are shown for immobilised SigH interacting with increasing concentrations of AosR in the presence of either 4 mM of reduced DTT or oxidised DTT in running buffer (10 mM HEPES pH 7.4, 150 mM NaCl, and 3 mM EDTA pH 8.0). Association and dissociation time were 600 sec.



Appendix Figure S6| Dimeric state of AosR is independent of CxxxC residues and external redox environment. GFC of AosR, AosR$_{AxxxA}$, and AosR$_{Msm}$ performed in a buffer containing either 2mM oxidized or reduced DTT. Protein eluting at 61 ml and standard graph of HiLoad 16/60 Superdex 75 column shows the molecular weight of the protein to be around 50 kDa, which corresponds to its dimeric state.

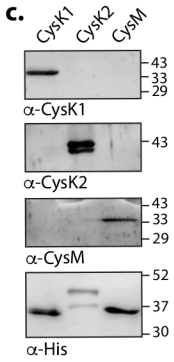
a.

```
Rv2334|cysK1  ---------------------MSIAEDITQLIGRTPLVRLRRVT-------DGAVADIVAK
Rv1336|cysM   ---------------------MTRYDSLLQALGNTPLVGLQRLSPRWDDGRDGPHVRLWAK
Rv0848|cysK2  VRSRQTRDRYRLLPEGYQVTPGRNRHPGTMVGNTPVLWIPELSG-----TSDPDRGFWAK
                                           :*.**:: : .::       ...   : **

Rv2334|cysK1  LEFFNPANSVKDRIGVAMLQAAEQAGLIKPDTIILEPTSGNTGIALAMVCAARGYRCVLT
Rv1336|cysM   LEDRNPTGSIKDRPAVRMIEQAEADGLLRPGATILEPTSGNTGISLAMAARLKGYRLICV
Rv0848|cysK2  LEGFNPGG-MKDRPALYMVECARARGDIAPGAAIVESTGGTLGLGLALAGKVYRHPVTLV
              **  ** . :*** .: *:: *.  * : *.: *:*.*.*. *:.**:.     :    .

Rv2334|cysK1  MPETMSLERRMLLRAYGAELILTPGADGMS----GAIAKAEELAKTDQRYFVPQQFENPA
Rv1336|cysM   MPENTSVERRQLLELYGAQIIFSAAEGGSN----TAVATAKELAATNPSWVMLYQYGNPA
Rv0848|cysK2  TDPGLEPIIARMLTAYGAGVDMVTQPHPVGGWQQARKDRVAQLMAEYPGAWNPNQYGNPD
                    .     :*  *** : : .     .         . :*           *: **

Rv2334|cysK1  NPAIHRVTTAEEVWRDTDGKVDIVVAGVGTGGTITGVAQVIKERKPSARFVAVEPAASPV
Rv1336|cysM   NTDSHYCGTGPELLADLP-EITHFVAGLGTTGTLMGTGRFLREHVANVKIVAAEPRYG--
Rv0848|cysK2  NVGAYRSLALELVAQLGR--IDVLVCSVGTGGHSAGVARVLREFNPDMRLIGVDTIGSTI
              *   :   :   :       :  .*..:** *   *..:.::*  .. :::..:.  .

Rv2334|cysK1  LSGGQKGPHPIQGIGAGFVPPVLDQDLVDEIITVGNEDALNVARRLAREEGLLVGISSGA
Rv1336|cysM   -----EGVYALRNMDEGFVPELYDPEILTARYSVGAVDAVRRTRELVHTEGIFAGISTGA
Rv0848|cysK2  FG-QPASNRLMRGLGSSIYPRNVDYRAFDEVHWVAPPEAVWACRSLAATHYASGGWSVGA
                    .   ::.:. .: *   *   .     *.  :*:   * *.  .    * * **

Rv2334|cysK1  ATVAALQVARRPENAGK--LIVVVLPDFGERYLSTPLFADVAD-----------------
Rv1336|cysM   VLHAALGVGAGALAAGERADIALVVADAGWKYLSTGAYAGSLDDAETALEGQLWA-----
Rv0848|cysK2  VALVAGWAARNLPADTT---IAAVFPDGPQRYFDTIYNDAYCNEHELLGGQPPTEPDEIA
              .  .*  ..           *. *..*   :*:.*       :

Rv2334|cysK1  -------------------------
Rv1336|cysM   -------------------------
Rv0848|cysK2  SPLDAVVTRWTRSTTVIDPTQVVS
```

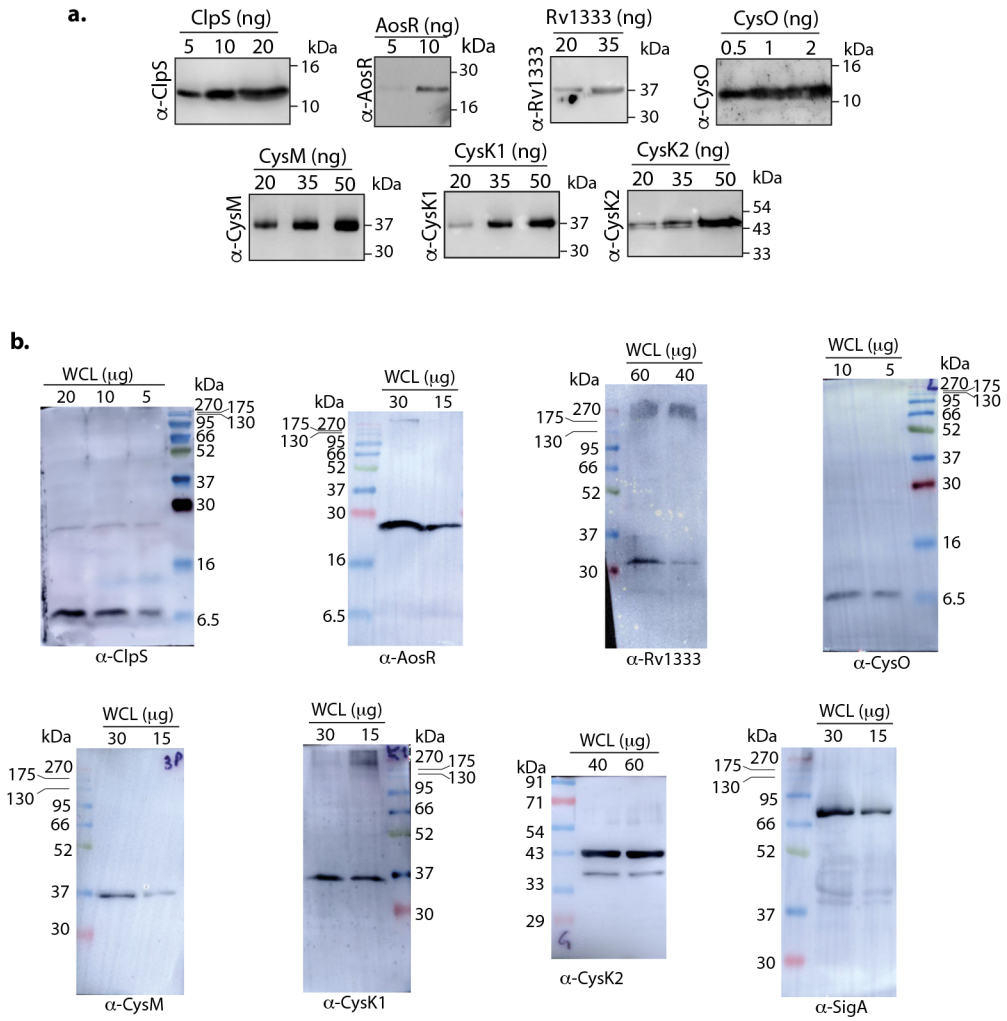
b.

	CysK1	CysK2	CysM
CysK1	100	21.93	35.8
CysK2		100	23.5
CysM			100

c.



Appendix Figure S7|Cysteine synthases in *Mtb*. a. Multiple sequence alignment of *Mtb* cysteine synthases, CysK1, CysK2 and CysM by Clustal W (https://www.genome.jp/tools-bin/clustalw) using sequences obtained from mycobrowser (https://mycobrowser.epfl.ch/).**b.** Percentage identity between *Mtb* cysteine synthases determined by Clustal W. **c.**35 ng of purified His tagged CysK1, CysK2 and CysM were resolved on 12-15% SDS-PAGE, transferred to nitrocellulose membrane and probed with indicated polyclonal sera to determine the specificity of antibodies.



Appendix Figure S8|Antibody characterization. a-b. Indicated amounts of purified His tagged purified protein (a) and WCL of *Rv* (b) were resolved on 12-15% SDS-PAGE, transferred to a nitrocellulose membrane, and probed with indicated polyclonal sera to determine the sensitivity and specificity.

Appendix Table 1| Essentiality of *Mtb* transcription factors

TFs that are essential *in vitro* but not *in vivo.*

TFs that are essential *in vivo* but not *in vitro* (are bold)

Transcription Factor	Name	Family	*In vitro(DeJesus et al, 2017)*			*In vivo* essential*(Sassetti & Rubin, 2003)*
			Essential	Growth Defect	Growth Advantage	
Rv0019c	FhaB		No			No
Rv0020c	FhaA		Yes			No
Rv0022c	WhiB5	WhiB	No			No
Rv0023	Rv0023	HTH		GD		No
Rv0038	Rv0038		No			No
Rv0042c	Rv0042c	MarR	No			No
Rv0043c	Rv0043c	GntR	No			No
Rv0047c	Rv0047c	PadR	No			No
Rv0054	Ssb	ssDNA binding	Yes			No
Rv0067c	Rv0067c	TetR	No			No
Rv0078	Rv0078	TetR	No			No
Rv0081	Rv0081	ArsR	No			No
Rv0117	OxyS	OxyR	No			No
Rv0135c	Rv0135c	TetR	No			No
Rv0144	Rv0144	TetR	No			No
Rv0158	Rv0158	TetR			GA	No
Rv0165c	Mce1R	GntR	No			No
Rv0182c	SigG	Sigma factor	No			No
Rv0195	Rv0195	2CRR			GA	No
Rv0196	Rv0196	TetR	No			No
Rv0212c	NadR	NadR	No			No
Rv0232	Rv0232	TetR			GA	No
Rv0238	Rv0238	TetR	No			No
Rv0260c	Rv0260c	2CRR	No			No
Rv0273c	Rv0273c	TetR	No			No
Rv0275c	Rv0275c	TetR	No			No
Rv0302	Rv0302	TetR	No			No
Rv0324	Rv0324	ArsR	No			No
Rv0328	Rv0328	TetR	No			No
Rv0330c	Rv0330c	TetR	No			No
Rv0339c	Rv0339c	HTH	No			No
Rv0348	Rv0348	HTH	No			No

Rv0353	HspR	HspR	No			No
Rv0377	Rv0377	LysR	No			No
Rv0386	Rv0386	Cyclase	No			No
Rv0440	GroEL2	Hsp	Yes			No
Rv0445c	SigK	sigma factor	No			No
Rv0452		TetR	No			No
Rv0465c		XRE	No			No
Rv0472c		TetR			GA	No
Rv0474		XRE			GA	No
Rv0485		NagC/XylR/ROK	No			No
Rv0491	RegX3	2CRR	No			No
Rv0494		GntR	No			No
Rv0576		ArsR	No			No
Rv0586	Mce2R	GntR	No			No
Rv0599c	VapB27	VapB	No			No
Rv0602c	TcrA	2CRR	No			No
Rv0608	VapB28	VapB	No			No
Rv0623	VapB30	VapB	No			No
Rv0653c		TetR	No			No
Rv0674		PaaX/ArsR (?)	No			No
Rv0678		Whth	No			No
Rv0681		TetR	No			No
Rv0691c		TetR	No			No
Rv0735	SigL	Sigma factor	No			No
Rv0737		MarR	No			No
Rv0744c		2CRR/HTH	No			No
Rv0757	PhoP	2CRR	No			No
Rv0767c		TetR	No			No
Rv0792c		GntR	No			No
Rv0818		OmpR			GA	No
Rv0821c	phoY2	PhoU			GA	No
Rv0823c					GA	No
Rv0827c	KmtR	ArsR	No			No
Rv0844c	NarL	LuxR	No			No
Rv0880		MarR	No			No
Rv0890c		LuxR	No			No
Rv0891c		Cyclase	No			No
Rv0894		LuxR	No			No
Rv0903c	PrrA	2CRR	Yes			No
Rv0967	CsoR	CsoR	No			No
Rv0981	MprA	2CRR	No			No

Rv1019		TetR	No			No
Rv1027c	KdpE	KdpE	No			No
Rv1033c	TrcR	2CRR			GA	No
Rv1049		HTH	No			No
Rv1129c		XRE	No			No
Rv1151c	Rv1151c	SIR2	No			No
Rv1152	Rv1152	GntR	No			No
Rv1167c	Rv1167c	TetR	No			No
Rv1176c	Rv1176c	PadR	No			No
Rv1186c	Rv1186c	HTH	No			No
Rv1189	SigI	Sigma factor	No			No
Rv1219c	Rv1219c	TetR	No			No
Rv1221	SigE	Sigma factor	No			No
Rv1255c	Rv1255c	TetR/AcrR	No			No
Rv1267c	EmbR	EmbR	No			No
Rv1287	Rv1287	Rrf2	No			No
Rv1332	**Rv1332**	**HTH**	**No**			**Yes**
Rv1353c	Rv1353c	TetR	No			No
Rv1358	Rv1358	LuxR	No			No
Rv1359	Rv1359	Cyclase	No			No
Rv1379	PyrR	PyrR	No			No
Rv1395	Rv1395	AraC	No			No
Rv1404	Rv1404	MarR	No			No
Rv1423	WhiA	WhiA	Yes			No
Rv1453	Rv1453	HTH			GA	No
Rv1460	**Rv1460**	**SufR**	**No**			**Yes**
Rv1473A	Rv1473A		No			No
Rv1474c	Rv1474c	TetR	No			No
Rv1534	Rv1534	TetR	No			No
Rv1556	Rv1556	TetR	No			No
Rv1560	**VapB11**	**VapB**	**No**			**Yes**
Rv1626	Rv1626	2CRR	No			No
Rv1657	ArgR	ArgR	No			No
Rv1674c	Rv1674c	ArsR	No			No
Rv1675c	Cmr	CRP/Fnr	No			No
Rv1719	Rv1719	IclR	No			No
Rv1725c	Rv1725c	HxlR	No			No
Rv1740	VapB34	VapB	No			No
Rv1773c	Rv1773c	IclR	No			No
Rv1776c	Rv1776c	TetR	No			No
Rv1816	Rv1816	TetR	No			No

Rv1828	Rv1828	MerR		GD		No
Rv1830	Rv1830	MerR	Yes			No
Rv1846c	BlaI	BlaI	No			No
Rv1909c	FurA	Fur	No			No
Rv1931c	**Rv1931c**	**AraC**	**No**			**Yes**
Rv1956	HigA	Hig/Cro-C1			GA	No
Rv1960c	ParD1	Antitoxin	No			No
Rv1963c	Mce3R	TetR	No			No
Rv1985c		LysR	No			No
Rv1990c		HTH	Yes			No
Rv1994c	CmtR	ArsR	No			No
Rv2009	VapB15	VapB	No			No
Rv2011c		MarR	No			No
Rv2017		HTH	Yes			No
Rv2021c		XRE	No			No
Rv2034		ArsR	No			No
Rv2069	SigC	Sigma factor	No			No
Rv2160A		TetR	No			No
Rv2160c		TetR	No			No
Rv2175c		wHTH	No			No
Rv2242		HTH	Yes			No
Rv2250c		TetR	No			No
Rv2258c		HTH_methyltransferase	No			No
Rv2282c		LysR	No			No
Rv2324		AsnC	No			No
Rv2358	SmtB	ArsR	No			No
Rv2359	**Zur/furB**	**FUR**	**No**			**Yes**
Rv2374c	**HrcA**	**HrcA**	**No**			**Yes**
Rv2478c		ssDNA binding	No			No
Rv2488c		LuxR	No			No
Rv2506		TetR	No			No
Rv2595	VapB40	VapB			GA	No
Rv2621c		Whth	No			No
Rv2640c		ArsR	No			No
Rv2642		ArsR	No			No
Rv2703	SigA	Sigma factor	Yes			No
Rv2710	SigB	Sigma factor	No			No
Rv2711	IdeR	IdeR	Yes			No
Rv2720	LexA	LexA		GD		No
Rv2745c	ClgR	XRE	No			No
Rv2760c	VapB42	VapB	No			No

Rv2779c		AsnC	No			No
Rv2788	SirR	MntR	No			No
Rv2827c		wHTH		GD		No
Rv2884		GlnR	No			No
Rv2887		MarR	No			No
Rv2912c		**TetR**	**No**			**Yes**
Rv2919c	GlnB		No			No
Rv2986c	HupB	HU		GD		No
Rv2989		IclR	No			No
Rv3050c		**TetR**	**No**			**Yes**
Rv3055		TetR			GA	No
Rv3058c		TetR			GA	No
Rv3060c		GntR	No			No
Rv3066		TetR	No			No
Rv3082c	VirS	AraC	No			No
Rv3095		HxlR	No			No
Rv3124	MoaR1	RedD	No			No
Rv3133c	DevR	2CRR	No			No
Rv3143		2CRR/HTH	No			No
Rv3160c		TetR	No			No
Rv3167c		TetR	No			No
Rv3173c		TetR	No			No
Rv3183		XRE	No			No
Rv3197A	WhiB7	WhiB	No			No
Rv3208		TetR	No			No
Rv3219	WhiB1	WhiB		GD		No
Rv3223c	SigH	Sigma factor	No			No
Rv3246c	MtrA	2CRR	Yes			Yes
Rv3249c		TetR	Yes			No
Rv3260c	WhiB2	WhiB	Yes			No
Rv3286c	SigF	Sigma factor			GA	No
Rv3291c	LrpA	AsnC		GD		No
Rv3295		TetR			GA	No
Rv3301c	PhoY1	PhoU	No			No
Rv3328c	SigJ	Sigma factor	No			No
Rv3334		MerR	No			No
Rv3405c		TetR	No			No
Rv3414c	SigD	Sigma factor	No			No
Rv3416	WhiB3	WhiB	No			No
Rv3417c	GroEL1	Not a traditional TF	No			No
Rv3488		PadR	No			No

Rv3557c		TetR	No			No
Rv3574	**KstR**	**TetR**	**No**			**Yes**
Rv3575c		LacI	No			No
Rv3583c		CRP/FNR		GD		No
Rv3597c	Lsr2	Lsr2		GD		Yes
Rv3676	Crp	cAMP binding	No			No
Rv3681c	WhiB4	WhiB	No			No
Rv3736	Rv3736	AraC	No			No
Rv3744	NmtR	ArsR	No			No
Rv3765c	TcrX	2CRR	No			No
Rv3830c	Rv3830c	TetR	No			No
Rv3833	Rv3833	AraC	No			No
Rv3840	Rv3840	LytR			GA	No
Rv3849	EspR		No			No
Rv3852	Hns	Hns	No			No
Rv3855	**EthR**	**TetR**	**No**			**Yes**
Rv3862c	WhiB6	WhiB	No			No
Rv3911	SigM	Sigma factor	No			No

Appendix Table S2| Strains used in the study

Strains	Description	Antibiotic Resistance	Source
DH5α	*E. coli* strain used for cloning plasmid constructs	-	Invitrogen
Rv	Laboratory strain of virulent *Mtb* (H37Rv)	-	Kind gift from Prof. Av-Gay (Cowley *et al*, 2004)
RvΔaosR	H37Rv *aosR*gene replacement mutant	Hyg	This study
RvΔaosR::aosR	Complementation strain, *RvΔaosR* electroporated with pST-*aosR*	Hyg, Kan	This study
RvΔaosR::aosRΔN	*RvΔaosR* electroporated with pNiT3xFlag-*aosR-ΔN*(missing 22 amino acids from N terminus)	Hyg, Kan	This study
RvΔaosR::aosR$_{Msm}$	*RvΔaosR* electroporated with pNiT3xFlag -*Msm*	Hyg, Kan	This study
RvΔaosR::aosR$_{AxxxA}$	*RvΔaosR* electroporated with pST-*aosR*$_{AxxxA}$	Hyg, Kan	This study
RvΔaosR::mom	*RvΔaosR* electroporated with pNiT3xFlag -*mec-cysO-cysM*	Hyg, Kan	This study
RvΔaosR::cysM	*RvΔaosR* electroporated with pNiT3xFlag -*cysM*	Hyg, Kan	This study
RvΔcysM	H37Rv *cysM* mutant	Hyg	This study
RvΔcysM::cysM	Complementation strain, *RvΔcysM* electroporated with pNiT-*cysM*	Hyg, Kan	This study
RvΔsigH	H37Rv *sigH*gene replacement mutant	Kan	(Manganelli *et al*, 2002)
RvΔsigH::sigH	Complementation strain, *RvΔsigH* electroporated with *sigH*	Hyg, Kan	(Manganelli *et al*., 2002)
Rv::pN-F-SigH	*Rv* electroporated with pNit3F-SigH	Kan	This study
Rv::mec-luciferase	*Rv*electroporated with 500 bp upstream of *mec* fused to luciferase cloned in a integrative vector	Apramycin	This study
Rv::100bpmec-luciferase	*Rv*electroporated with 100 bp upstream of *mec* fused to luciferase cloned in a integrative vector	Apramycin	This study
Rv::sigA-luciferase	*Rv*electroporated with 500 bp upstream of *sigA* fused to luciferase cloned in a integrative vector	Apramycin	This study
RvΔaosR::mec-luciferase	*RvΔaosR*electroporated with 500 bp upstream of *mec* fused to luciferase cloned in a integrative vector	Hyg, Apramycin	This study
RvΔaosR::pN	*RvΔaosR* electroporated with pNit3F	Hyg, Kan	This study
RvΔaosR::pN-aosR	*RvΔaosR*electroporated with pNit3F*aosR*	Hyg, Kan	This study
Msm-FLAG-sigH	*M. smegmatis mc2155* electroporated with pNit3F-SigH	Kan	This study
Msm-FLAG-sigH-AosR-HA	*Msm-FLAG-sigH* electroporated with AosR cloned in a giles integrative vector containing C terminus HA tag	Kan, Apramycin	This study
Msm-FLAG-sigH-AosR$_{AxxxA}$*-HA*	*Msm-FLAG-sigH* electroporated with AosR$_{AxxxA}$ cloned in a giles integrative vector containing C terminus HA tag	Kan, Apramycin	This study
Rv::mrx1-roGFP2	*Rv* electroporated with pST-K-mrx1-roGFP2	Kan	(Khan *et al*, 2017)
RvΔaosR::mrx1-roGFP2	*RvΔaosR* electroporated with pST-K-mrx1-roGFP2	Hyg, Kan	This study

Appendix Table S3| Primers used in the study

aosR-AES-LHS forward	DraIII, SnaBI	CACCTTTTTTCACAAAGTGTACGTAGCCAGCAACTCG CACGCGTC
aosR-AES-LHS reverse	DraIII	TTTTTTTTTCACTTCGTGCGGCGAAGAAGAGTCGCG ATC
aosR-AES-RHS forward	DraIII	CACCTTTTTCACAGAGTGCTTCGGCTGGCGCTCGGA GTG
aosR-AES-RHS reverse	DraIII, SnaBI	TTTTTTTTCACCTTGTGTACGTAACTGCGCCAGCGCA GCAATC
*aosR*forward	NdeI	CACCCATATGATGCCGCCGGTTTGTGGGC
aosR reverse	HindIII	AGCTAAGCTTTCATCGAGACCCCATCAGC
RvΔaosR screening forward		GATATCGCCGCGGAACCG
RvΔaosR screening reverse		CAATCACACCGATCGTCG
-765 bp of *aosR* for promoter cloning	ScaI	CACCAGTACTGACGCATTCGGCGAAGCTGC
-265 Reverse of *aosR* for promoter cloning	SapI	TTTTTTTTGCTCTTCAGCGGGCCCCAGGATACCCAGC GC
aosR forward	SapI	CACCTTTTGCTCTTCACGCATGCCGCCGGTTTGTGGG
aosR cys5/9→ala mutation forward		ATGCCGCCGGTTGCTGGGCGACGAGCCAGCAGGAC CGG
aosR cys5/→ala mutation reverse		CCGGTCCTGCTGGCTCGTCGCCCAGCAACCGGCGGC AT
aosR cys5/→ala mutation forward	NdeI	CACCCATATGCCGCCGGTTGCTGGGCGA
*aosRΔ*N (22 aa) forward	SapI	CACCGCTCTTCACGCATGGTGCGCAGGTGGAAGCGC
*aosRΔ*N reverse	SapI	CACCGCTCTTCACGCATGGCGCTTCCACCTGCGCAC
aosRMsm forward	NdeI	CACCCATATGGTGCGCAAGTGGAAGCGGGT
aosRMsm reverse	HindIII	TTTTAAGCTTCTACTTCCCCACCAGTTCGA
*cysK2*forward	NdeI	CACCCATATGAGGTCGCGGCAGACCCG
*cysK2*reverse	HindIII	ATCGAAGCTTACGACACCACCTGGGTTG
cysK1 forward	NdeI	CACCCATATGAGCATCGCCGAGGACAT
cysK1 reverse	HindIII	ATCGAAGCTTAGTCAGCCACGTCGGCGA
cysM-AES-LHS forward	SfiI, SnaBI	CACCTTTTGGCCTAAATGGCCTACGTACACCCCGACG AAGCCTGC
cysM-AES-LHS reverse	SfiI	TTTTTTTTGGCCTTTCTGGCCCTCGATCATCCGCACA GC
cysM-AES-RHS forward	SfiI	CACCTTTTGGCCTAGATGGCCAGACCGCTCTGGAAG GGCAA
cysM-AES-RHS reverse	SfiI, SnaBI	TTTTTTTTGGCCTCTTTGGCCTACGTACGGCGAATTC ATAACTTC
*cysM*forward	NdeI	CACCCATATGACACGATACGACTCGCT
*cysM*reverse	HindIII	ATCGAAGCTTATGCCCATAGTTGCCCTTCC
*Rv⊥cysM*screening forward		CACCCTACGTGCTGGTGATTCG
*Rv⊥cysM*screening reverse		TTTTTGATGGCCCGCGCGACCG
*gp91*phox-/-genotyping (oMIR0517)		AAGAGAAACTCCTCTGCGTGAA
*gp91*phox-/-genotyping (oMIR0518)		CGCACTGGAACCCCTGAGAAAGG
*gp91*phox-/-genotyping (oMIR0519)		GTTCTAATTCCATCAGAAGCTTATCG
sigH forward	NdeI	CACCCATATGGCCGACATCGATGGTGTAA
sigH reverse	HindIII	AGCTAAGCTTATGACGACACCCCCTCGT
500 bp upstream of *rv1334*	ScaI	CACCAGTACTAAATGCCGCTGGCAACGT
*rv1331*forward	NdeI	CACCCATATG GCTGTTGTGTCAGCGCCC
rv1331 reverse	HindIII	TTTTAAGCTTTCACCGGTCCTGCTGCAT
rv1333 forward	NdeI	CACCCATATG AACTCCATCACCGACGTC
rv1333 reverse	HindIII	TTTTAAGCTT TCAGGACCCGAATGCTCC
*Mec*forward	NdeI	CACCCATATGTTGCTTAGGAAAGGAACC
mec reverse	HindIII	TTTTAAGCTTTCAGTACTGCTCGACGAC
*cysO*forward	NdeI	CACCCATATGAACGTCACCGTATCCATT
cysO reverse	HindIII	TTTTAAGCTTTCACCCACCGGCCACGGC

Oligo 1 for EMSA		AGTCGTGCGGTGCTGGCCGGCGTGCTCAATGCTCAG CCGGTAGCCGGAATACCGACCTACCGTGACATGTTT TCCCGGAGCATTCGGGTCTTGAAACTCA
Oligo 2 for EMSA		TATGAGTTTCAGGACCCGAATGCTCCCCGGGAAACA TGTCACGGTAGGTCGGTATTCCGGCTACCGGCTGAG CATTGAGCACGCCGGCCAGCCACCGCACGACT
Luciferase Reverse	HindIII	TTTTAAGCTTTCTAGAGGTACCTTACAATT
Luciferase Forward	NdeI	CACCCATATGGAAGACGCCAAAAACATAAAG

Appendix Table S4|qRT-PCR primers used in the study

pncB1-rv1331 intergenic forward	GAATCCCCCGGCGGCCGCAAAGAG
pncB1-rv1331 intergenic reverse	CAACCCGGATGCCACCAGCTG
*rv1331-aosR*intergenic forward	CATGCCACCAAGCTGATGTT
rv1331-aosR intergenicreverse	CTCGAATCTCACCGGTCCTG
aosR-rv1333 intergenic forward	CGGAGTGATGCTTGAGATCG
aosR-rv1333 intergenic reverse	TCGGTGATGGAGTTCATCGG
rv1333-mec intergenic forward	GGAATACCGACCTACCGTGA
rv1333-mec intergenic reverse	CGAATCACCAGCACGTAGAC
mec-cysO intergenic forward	ACCGAGGAACCTGTCAATGT
mec-cysO intergenic reverse	GGATACGGTGACGTTCATGC
cysO-cysM intergenic forward	GTGACTCGGTCACCATCCTC
cysO-cysM intergenic reverse	CTGCAACAGCGAGTCGTATC
cysM-rv1337 intergenic forward	TCTGGAAGGGCAACTATGGG
cysM-rv1337 intergenic reverse	AAGGTGAGGATCGTCGT
rv1337-murI intergenic forward	TTCCGGGCATCCGAAGTTAT
rv1337-murI intergenic reverse	CCGACGTAGACGATGTCCT
rv1338-rv1339 intergenic forward	CACCCATCGCGCATTCATTA
rv1338-rv1339 intergenic reverse	CTACTGTGCCATGCCCGATA
rv1339-rphA intergenic forward	TGGTATGCGACGAGACGTT
rv1339- rphA intergenic reverse	GTCTTCTCGCTTGGACACG
rphA-rv1341 intergenic forward	GGATAAGCTGCTGGACATGG
rphA-Rv1341 intergenic reverse	GACCAGAAGCTTGGTCACAA
rv1341-rv1342c intergenic forward	GGATAAGCTGCTGGACATGG
rv1341-rv1342c intergenic reverse	GACCAGAAGCTTGGTCACAA
*rv1331*forward	GTCGACGTGACGGACAGGGC
rv1331 reverse	GCTCGCTGTAGCCGAACAAC
aosR forward	CTGTTGCCGGATTTCTACCG
aosR reverse	TTGTCCGGAACCGTGTCTAA
rv1333 forward	ATTTCGGCTATTCGGCCTGT
rv1333 reverse	GGCATTTACCACAGCAAGGA
mec forward	CAGACCTGGTGAATGCGATG
*mec*reverse	CCACACCTTCAGTTGCTCAC
cysO forward	CAGACCTGGTGAATGCGATG
cysO reverse	CCACACCTTCAGTTGCTCAC
cysM forward	GTGATGCCGGAGAACACATC
cysM reverse	CGTACTGGTACAGCATCACC
cysK1 forward	CCGAGACGATGAGTCTGGAG
cysK1 reverse	GTAGCGTTGATCGGTCTTGG
16s forward	GGATTGACGGTAGGTGGAGAAGAA
16s reverse	GTGAGATTTCACGAACAACGCGAC
*rv1503c*forward	CAACGCCCACATGTACTACG
*rv1503c*reverse	AGCGGCACGTAATGAAAGAC
rv1355 forward	AACTGGATTCCGGCGAATTG
rv1355 reverse	GTTGTCTTCGAGGGCTGTTC
rv3529 forward	CATCGGCTACCAAACTGGTG
rv3529 reverse	TTCATCTTGCTGCCCAACAC
rv1663 forward	TGTGTCAGCGTTTGTGTTGT
rv1663 reverse	CGTTCGCTGCAGCATAATTG
rv1936 forward	AAAGTACTGGAACGCCAACG
rv1936 reverse	GCTTCTGGTACGGCTTGATG
rv0152c forward	TTCATTTCCGACCTCAACGC
rv0152c reverse	GATTCTCGGTGCGGATGATG
rv3716c forward	GAGACCTTGCAGGACCTGAT
rv3716c reverse	CCATCTTCGTCACCTGCTG
rv1386 forward	GTTCAGCGTTCACGGTAGTC
rv1386 reverse	GCATAACTGGCACCCGATTC

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