The oxidation-sensing regulator (MosR) is a new redox dependent transcription factor in *Mycobacterium tuberculosis**

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* Running title: *MosR: oxidation sensor in Mtb*

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INVENTORY OF SUPPLEMENTAL DATA

SUPPLEMENTAL FIGURES

Fig. S1. Similarity among oxidation-sensing regulators. Numbers indicate the percentage identity over the number of amino acids.

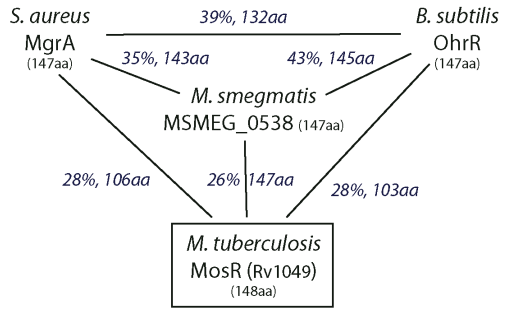
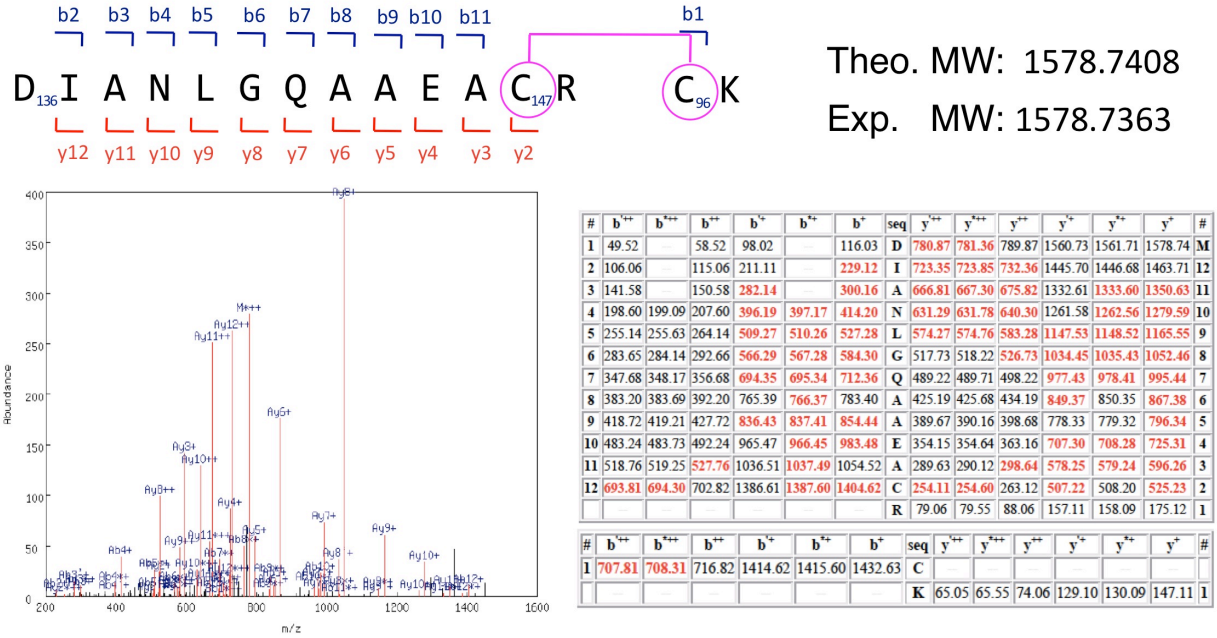


Fig. S2. LC/MS-MS characterization of C96-C147 peptide. MS/MS spectrum of peptide D_{136}-R_{148}–$C_{96}K_{97}$ (m/z_{obs} = 1578.7363). MS/MS theoretical fragmentation table with identified fragments in red.



#	b^{*++}	b^{*+++}	b^{++}	b^{*+}	b^{**+}	b^{+}	seq	y^{*++}	y^{*+++}	y^{++}	y^{*+}	y^{**+}	y^{+}	#
1	49.52		58.52	98.02		116.03	D	780.87	781.36	789.87	1560.73	1561.71	1578.74	M
2	106.06		115.06	211.11		229.12	I	723.35	723.85	732.36	1445.70	1446.68	1463.71	12
3	141.58		150.58	282.14		300.16	A	666.81	667.30	675.82	1332.61	1333.60	1350.63	11
4	198.60	199.09	207.60	396.19	397.17	414.20	N	631.29	631.78	640.30	1261.58	1262.56	1279.59	10
5	255.14	255.63	264.14	509.27	510.26	527.28	L	574.27	574.76	583.28	1147.53	1148.52	1165.55	9
6	283.65	284.14	292.66	566.29	567.28	584.30	G	517.73	518.22	526.73	1034.45	1035.43	1052.46	8
7	347.68	348.17	356.68	694.35	695.34	712.36	Q	489.22	489.71	498.22	977.43	978.41	995.44	7
8	383.20	383.69	392.20	765.39	766.37	783.40	A	425.19	425.68	434.19	849.37	850.35	867.38	6
9	418.72	419.21	427.72	836.43	837.41	854.44	A	389.67	390.16	398.68	778.33	779.32	796.34	5
10	483.24	483.73	492.24	965.47	966.45	983.48	E	354.15	354.64	363.16	707.30	708.28	725.31	4
11	518.76	519.25	527.76	1036.51	1037.49	1054.52	A	289.63	290.12	298.64	578.25	579.24	596.26	3
12	693.81	694.30	702.82	1386.61	1387.60	1404.62	C	254.11	254.60	263.12	507.22	508.20	525.23	2
							R	79.06	79.55	88.06	157.11	158.09	175.12	1

#	b^{*++}	b^{*+++}	b^{++}	b^{*+}	b^{**+}	b^{+}	seq	y^{*++}	y^{*+++}	y^{++}	y^{*+}	y^{**+}	y^{+}	#
1	707.81	708.31	716.82	1414.62	1415.60	1432.63	C							
							K	65.05	65.55	74.06	129.10	130.09	147.11	1

Fig. S3. Electrophoretic mobility shift assays. (A) Increasing concentrations of MosR incubated with 5 nM DNA. K_d ~ 30 nM. (B) Incubation of MosR (25 nM) with DNA (5 nM) in the presence of increasing concentrations of NO-precursor spermine NONOate.

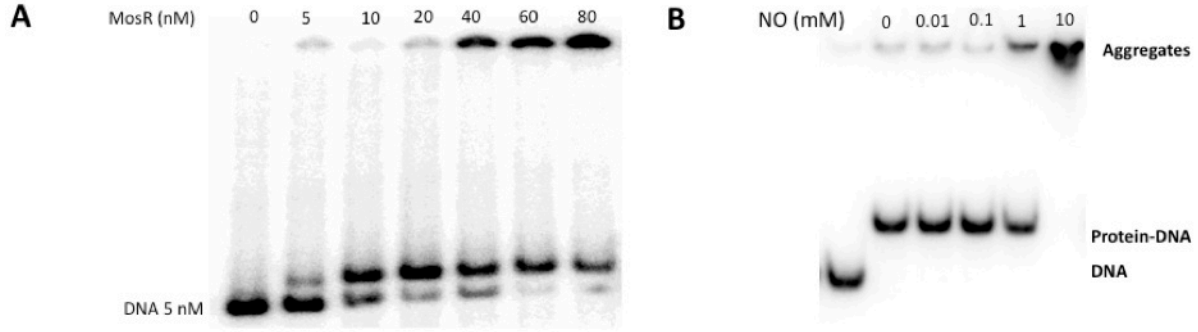
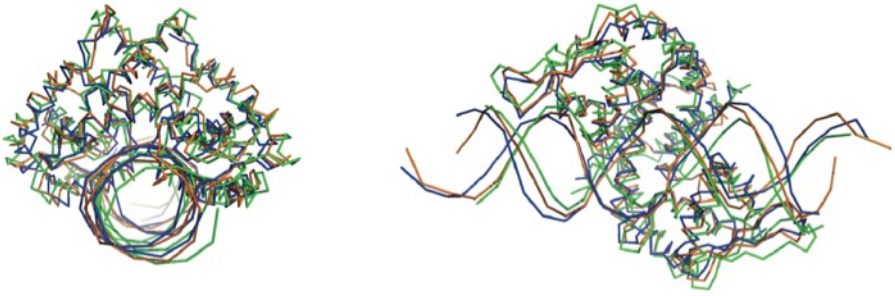


Fig. S4. MosR-DNA interactions. (A) Overlay of apo-MosR (blue) with apo-SlyA (green, 3QPT) (186 atoms, 3.17 Å^2) and apo-OhrR (orange, 1Z91) (73 atoms, RMSD = 1.84 Å^2). (B) Overlay of MosR-DNA (blue) with SlyA-DNA (green, 3Q5F) (197 atoms, RMSD = 3.75 Å^2) and OhrR-DNA (orange, 1Z9C) (188 atoms, RMS = 2.20 Å^2). (C) Scheme of MosR-DNA interactions. Residues from different monomers are shown by regular and oblique labels. Solid lines indicate side-chain interactions and dashed lines indicate mainchain interactions. (D) Stereoview of the recognition helix α4. Amino acid residues are labeled in black, DNA bases in blue.

A

B



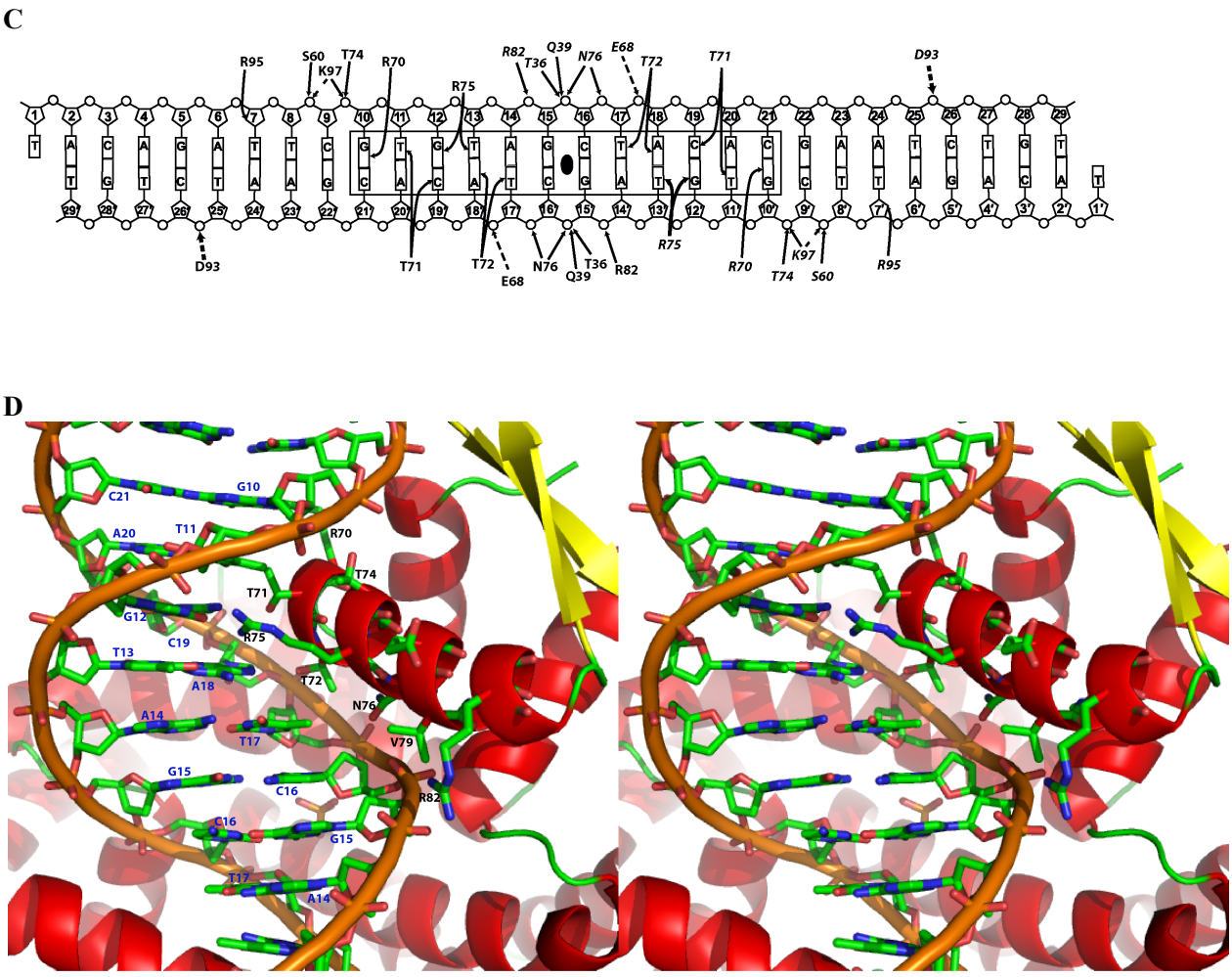


Fig. S5. Characterization of *mosR* deletion. (A) PCR test for deletion. Primers were chosen upstream and downstream of *mosR (rv1049)* so that the amplified product would be 1,116 bp for the wild-type strain and 675 bp for Δ*mosR* strain. (B) Southern blotting. Restriction enzymes (Sma I and EcoR V) were chosen so that the fragments would be 1,256 bp for wild-type, and 815 bp for Δ*mosR* strain.

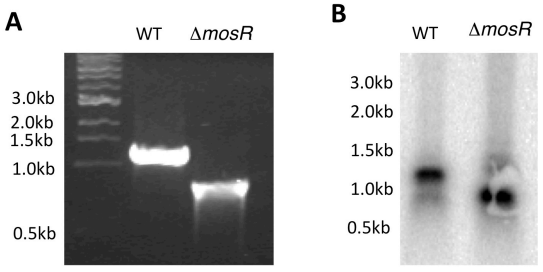


Fig. S6. Growth curves. Klett units over time for aereated *Mtb* cultures of WT, Δ*mosR*, MosR-complementation, MosR-C10S complementation and MosR-C12S complementation strains.

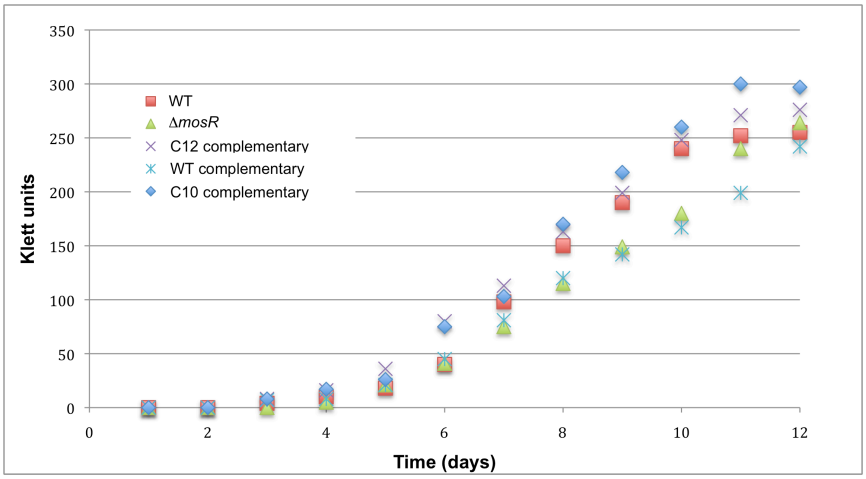
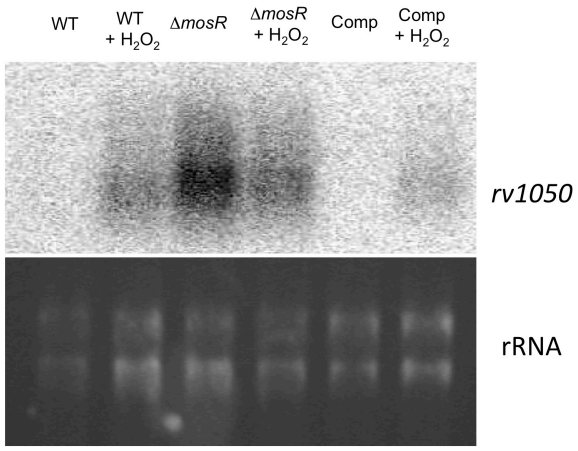


Fig. S7. Northern blot for *mosR* (*rv1050)*. In wild-type (WT), Δ*mosR*, and complementation (Comp) strains. Top: *rv1050* mRNA. Bottom: ribosomal RNA.



SUPPLEMENTAL TABLES

Table S1. Plasmids used in this study

Plasmid	Description	Source
pET28a	Vector for protein expression in *E. coli* Kan^R	Novagen
pET-mosR	*mosR* was cloned into pET28a Kan^R	This study
pET-mosR(5-148)	*mosR* (5-148) cloned into pET28a for crystallization Kan^R	This study
p2NIL	Gene manipulation vector for *Mtb* Kan^R	Parish and Stocker, 2000 (1)
pGOAL19	Delivery vector *Mtb* Hyg^R, Amp^R	Parish and Stocker, 2000 (1)
pGOAL-D*mosR*	Δ*mosR* suicide delivery vector pGOAL19 Hyg^R, Amp^R	This study
pUC-Int-GM	Complementation vector for *Mtb* Gm^R	Mahenthiralingam *et al*, 1998 (2)
pUC-mosR	*mosR* cloned into pUC-Int-GM vector Gm^R	This study
pUC-mosRC12S	C12S mosR cloned into pUC-Int-GM vector Gm^R	This study

Table S2. Primers used in this study

Description	Forward Primer	Reverse Primer
MosR expression	TTAGATCATATGGGTAAAGGCGCTGCATTC	ATAAAGCTTAGCGACACGCCTCAGC
MosR C10S	CTGCATTCGACGAAAGCGCATGTTATACTAC	GTAGTATAACATGCGCTTTCGTCGAATGCAG
MosR C12S	CATTCGACGAATGCGCATCTTATACTACTCG	CGAGTAGTATAAGATGCGCATTCGTCGAATG
MosR C10S-C12S	CATTCGACGAAAGCGCATCTTATACTACTCG	CGAGTAGTATAAGATGCGCTTTCGTCGAATG
mosR promoter	GGTCAAGCAACACCAGGTC	CCTTTGCCCATTACTCCAGA
rv1050 promoter	ATCGCGAATCTGGGTCAG	ACACCACCTGGTCACGAAAT
narX promoter	CCGGTCGACAACGACTACAC	GTGTCACCGTCACCTGGAC
narL promoter	TCGGAGTGCAAATCAATGTC	CGAAATAACGGGTGGTCGT
esxR promoter	ATATCGCGCAAGCCAATTT	CGACATCACAAACCCCTTTT
ndhA promoter	ACAAAAAGTTACGCCGTTGG	TCCACTACCGATGATGACCA
rv1050 RT-PCR	CGAGTTTGGTCGCATTGAC	CTTCATGATCGGCAAGACCT
sigA RT-PCR	CCTACGCTACGTGGTGGATT	TGGATTTCCAGCACCTTCTC
ΔmosR step 1	ATAAATCGTACCACGCCGGCAGTCGGAT	ATACTGCAGCGACGTGGAGCCTGGCT
ΔmosR step2	CTACACGAGTCTGGAGTAATG	TGATCTTTTTTGCGCATATATGTGTAG
mosR complementation	AATAGAATTCACTAAGGGACGATACTCTACTG	AATAGGATCCTCAGTTGGGTGTAACTACACA
mosR compl. C10S	CGTTCGACGAATCCGCTTGCTACACC	GGTGTAGCAAGCGGATTCGTCGAACG
mosR compl. C12S	GACGAATGCGCTTCCTACACCACCCG	CGGGTGGTGTAGGAAGCGCATTCGTC

Table S3. X-ray data collection and refinement statistics

	MosR-DNA complex (PDB: 4FX4)	**apo-MosR (PDB: 4FX0)**
Space group	R3	P212121
Unit-cell parameters	a = b = 146.6, c = 53.9 Å α = β = 90º, γ = 120 º	a = 52.0 b = 66.7 c = 79.1 Å α = β = γ = 90 º
Wavelength (Å)	0.979	1.033
Resolution range (Å)	30.0-3.20 (3.21-3.10)	20.0-2.70 (2.80-2.70)
Completeness (%)	98.5 (99.0)	99.6 (99.9)
Redundancy	3.2 (3.1)	3.5 (3.2)
R_{mrgd-F} (%)*	0.079 (0.376)	0.125 (0.735)
I/σ(I)	15.8 (3.6)	10.3 (1.8)
Number of reflections (total/test)	7799/360	7602/348
R_{work}/R_{free} (%)	24.0/28.0	24.9/27.7
Number of atoms		
Protein	2071	1952
DNA	1045	
Ions/Waters	5	0
Mean B value ($Å^2$)	87.5	53.2
r.m.s. deviations		
Bond length (Å)	0.003	0.002
Bond angle (°)	0.818	0.529
Ramachandran plot (%)	99.2/0.8/0.0	99.5/0.5/0.0

Table S4. Sensitivity range to antituberculosis drugs and oxidants of the *Mtb* strains used in this study

	WT	**ΔmosR**	**Comp**	**C10S-comp**	**C12S-comp**
Isoniazid (mM)	0.25-0.5	0.25-0.5	0.25-0.5	0.25-0.5	0.25-0.5
Ethambutol (mM)	4-8	4-8	4-8	4-8	4-8
Rifampin (mM)	0.05-0.2	0.05-0.2	0.05-0.2	0.05-.2	.05-.2
H_2O_2 (mM)	4-8	2-4	4-8	4-8	4-8
Diamide (mM)	3-6	3-6	3-6	3-6	3-6

Table S5. RNA microarray results

WT *vs*. Δ*Rv1049* (no H_2O_2)

Rv	Gene	Fold induction	Gene product
Rv1050	Rv1050	352	probable oxidoreductase
Rv3478	PPE	2.5	PPE-family protein
Rv0350	dnaK	-2.4	70 kD heat shock protein
Rv1361c	PPE	-9.1	PPE-family protein

WT *vs*. Δ*Rv1049* (10 mM H_2O_2)

Rv	Gene	Fold induction	Gene product
Rv0243	fadA2	3.1	acetyl-CoA C-acetyltransferase
Rv0839	Rv0839	2.9	conserved hypothetical protein
Rv1884c	Rv1884c	3.6	conserved hypothetical protein
Rv2558	Rv2558	2.4	conserved hypothetical protein
Rv3614c	Rv3614c	4.3	conserved hypothetical protein
Rv3615c	Rv3615c	5.0	conserved hypothetical protein
Rv0705	rpsS	-2.7	30S ribosomal protein S19
Rv0708	rplP	-2.2	50S ribosomal protein L16
Rv0710	rpsQ	-3.3	30S ribosomal protein S17

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Protein expression and purification. mosR gene was codon-optimized for expression in *E. coli* and cloned into pET28a (Novagen) between *Nco*I and *Xho*I sites. A thrombin cutting site was introduced in the C-terminus by site directed mutagenesis to facilitate removal of the his-tag after purification. *E. coli* BL21(DE3)star cells were transformed with the plasmid by heat-shock and positive colonies were selected on plates containing 30 mg/mL of Kanamycin. A single colony was used to inoculate a 10 mL culture that was grown overnight. Next day, the overnight culture was added to 1 L of LB media and grown at 37 ºC for approximately 3.5h until $OD_{(600nm)}$ ~ 0.6. At that time, the temperature was reduced to 16 ºC and 15 min later, 1 mM IPTG was added to induce protein expression. 12 h later cells were collected by centrifugation at 6,000 rpm for 7 min and the pellets were stored at -80 ºC. Subsequent protein purification was performed at 4 ºC. The frozen pellet was suspended in 30 mL of buffer A (300 mM NaCl, 10 mM tris-HCl pH 7.4, 10% glycerol, 10 mM β-mercaptoethanol). 2 units of DNAse I and 1 mM PMSF were added to facilitate DNA digestion and prevent protein degradation by proteases. Cells were lysed by sonication using 30 seconds on/off intervals for a total sonication time of 10 min. After sonication, the cells were centrifuged at 13,000 g for 30 min and the supernatant was passed through a 2 micron syringe filter (Amicon). Immediately afterwards, the supernatant was loaded into a 5 mL NiNTA column (GE Healthcare) and the column was washed with 5 volumes of 90% buffer A and 10% buffer B (same as buffer A plus 500 mM imidazole). Subsequently, the column was washed with 5 volumes of 90% 2 M $MgCl_2$ and 10% buffer B to facilitate DNA elution. The protein was then eluted from the column using a linear gradient from 10% to 100% buffer B over 40 mL. The purity of the protein was analyzed on a 12% non-reducing SDS-PAGE gel using loading buffer without DTT or β-mercaptoethanol. Fractions containing pure protein were combined and run on a desalting column with buffer A. The protein solution was concentrated down to ~50 μM, added 30% glycerol, and aliquots stored at – 80 ºC for future use.

Protein crystallization. For crystallization purposes a truncated version of the protein ($\Delta_{2\text{-}5}$MosR) was used. The protein was expressed an purified as detailed above except that immediately after the desalting step the protein was subjected to thrombin digestion and further purification. 1 unit of thrombin per mg of purified protein was added to the protein solution and incubated for 12h at 4 ºC to cut the his-tag. Afterwards, the protein solution was passed through a Ni-NTA column to remove the undigested protein. Then the protein was run on a Superdex-75 size exclusion column using buffer A to remove trace impurities. The protein fractions were combined and concentrated to 10 mg/mL for crystallization. Crystallization trials were performed using the hanging drop method screening conditions from Hampton research. Apo-MosR crystallized after 24h at 10 mg/mL at 16 ºC with a reservoir solution consisting of 0.2 M Sodium citrate pH 5.5, 20% PEG 3,350. For protein-DNA complex, the selenomethionine form of the protein was used. The selenomethionine form was expressed under the same conditions except that it was grown in minimum media (per liter: 12.8 g $Na_2HPO_4{\cdot}7H_2O$, 3 g KH_2PO_4, 0.5 g NaCl, 1 g NH_4Cl, 4 g glucose, 0.5 mg thiamine, 0.1 mM $CaCl_2$, 2 mM $MgSO_4$). When the culture reached $OD_{(600nm)}$ = 0.6 the following amino acids were added: 100 mg lysine, 100 mg threonine, 100 mg phenylalanine, 50 mg leucine, 50 mg valine, and 60 mg of selenomethionine. Protein expression was induced 15 min later with 1 mM IPTG and the temperature was reduced to 16 ºC. Every purification step thereafter was identical to the wild-type protein. Prior to crystallization 10 μL of pre-annealed double stranded DNA at 1.4 mM were added to 40 μL of protein dimer (MW 31,878 Da) at 10 mg/mL (313 μM) in a ~1.2 molar ratio DNA/protein. Crystallization trials were performed similarly to the protein alone. Crystals of MosR-DNA complex appeared after 72h at room temperature with a reservoir solution consisting of 1.4 M Sodium phosphate monobasic monohydrate/Potassium phosphate dibasic pH 5.6. Crystals were frozen in liquid nitrogen following cryoprotection with the reservoir solution containing 20% glycerol. Data were collected at the Advanced Photon Source at Argonne National Laboratory beamline 24-ID, and all images were indexed, integrated, and scaled using HKL2000 (3). The structure of the protein-DNA complex was solved at 3.1 Å resolution by molecular replacement using Phaser (4) with search model based on the

structure of OhrR with DNA (PDB ID 1Z9C). The structure of the protein alone was solved at 2.7 Å by molecular replacement using the newly obtained structure of the MosR-DNA complex as the search model. Both models were built using the program Coot (5) and refined with PHENIX (6) and CCP4 (7). Data collection and refinement statistics can be found in Table S3. The atomic coordinates and structure factors have been deposited in the Protein Data Bank with accession codes: 4FX0 and 4FX4.

Mass-spectrometry characterization. Purified recombinant MosR (20 μM) was treated with either 1 mM DTT, air oxidation for 1h on ice or 5 mM H_2O_2 on ice for 20 min. Afterwards, the free thiols were alkylated with iodoacetamide and sent for mass-spectrometry. The samples were digested with trypsin (2 μg) and run by LC-MS/MS on the LTQ-Orbitrap Velos (2 h gradient). The data was searched using MassMatrix which can search for disulfide bonds.

Site-directed mutagenesis. Site-directed mutagenesis was carried out using the site-directed mutagenesis kit from Agilent and the primers specified in Table S2.

Electrophoretic mobility shift assays. A 100-300 bp DNA upstream of the gene under study was amplified from *Mtb* genomic DNA using Phusion polymerase (New England Biolabs) and purified using PCR purification kit (Qiagen). 5 ng of DNA were radioactively labeled with γ-P32 ATP using T4 polynucleotide kinase (NEB) following the manufacturers instructions and the excess of ATP removed using a Bio-Spin column (Biorad). An aliquot of MosR protein was thawed and buffer exchanged using Bio-Spin columns to remove β-mercaptoethanol. The protein concentration was then estimated from the absorbance at 280 nm using the calculated extinction coefficient (13,940 M^{-1} cm^{-1}). Finally, the protein (50-100 nM final concentration) was combined with DNA (10 nM final concentration) in buffer 100 mM KCl, 3 mM $MgCl_2$, 10 mM tris HCl pH 7.4, 10% glycerol for 30 min at room temperature. To some of the samples H_2O_2, cumene hydropheroxide (CHP), *tert*-butyl hydropheroxide or spermine-NONOate were added to concentrations ranging from 10 μM to 50 mM. 1 h incubation was used to assess the difference between WT MosR, C10S_MosR and C12S_MosR (Fig. 3D). The samples were run on a 6 % polyacrylamide native gel for 90 min at 100 V. Afterwards, the gels were dried, exposed for 12 h to a Phosphor screen and the images were collected using a phosphorimager.

DNase I footprinting. DNase I footprinting assay was carried out using the protocol from Leblanc and Moss (8). Briefly, DNA corresponding to the 120 bp upstream region of *mosR* was amplified by PCR using one of the two primers labeled with P32 to yield DNA labeled on one end only. 50 nM of DNA was mixed with 500 nM of protein in buffer C (10 mM tris HCl pH 7.4, 100 mM NaCl, 3 mM $MgCl_2$ and 0.5 mM DTT) and with 25 mL of cofactor solution (10 mM $MgCl_2$, 5 mM $CaCl_2$). Next, 0.5 units of DNase I were added to the mix and allowed to react for 4 min when the reaction was stop by addition of 50 mL of STOP solution (1 % SDS, 200 mM NaCl, 20 mM EDTA, 40 μg/mL tRNA). The DNA was then purified by phenol-chloroform extraction and ethanol precipitation. Sequencing reactions were performed using the sequenase kit from USB. Both samples were run alongside on a 20 x 40 cm 6 % polyacrylamide gel at 40 W for 3 h. The gel was dried, exposed to a Phosphor screen and the images acquired using a Phosphorimager.

Construction of ΔmosR, Complementation, and C12S-Complementation strains. Δ*mosR* strain was constructed using the pGOAL/p2NIL system developed by Parish and Stoker (1). Briefly, 1 kb regions upstream and downstream of *mosR* were cloned into p2NIL plasmid (Kan^R) between BamHI and KpnI sites. p2NIL and pGOAL19 (LacZ and Hyg^R) were digested with PacI and ligated together to generate the delivery plasmid (LacZ, Hyg^R, Kan^R). This plasmid was transformed into freshly prepared *Mtb* competent cells. Cells carrying single-crossovers (SCOs) were isolated by selection for blue hygR kanR colonies on Middlebrook 7H11 agar containing OADC supplement (Difco) and 77 mM inositol. One SCO colony was plated onto agar containing sucrose (2%), inositol (77 mM) and tryptophan (50 μg/ml) to isolate bacteria with a second crossover, which will lead to mutant or wild-type cells depending on the location of the

recombination event. Mutant colonies were verified by PCR and Southern analysis (Figure S5 A, B). For complementation strains, *mosR* plus 318 bases upstream were cloned into pUC-GM-Int (2). Plasmids used in this study are in Table S1.

RNA extraction. RNA extraction was performed according to the protocol reported by Voskuil *et al*. (9). Briefly, 100 mL of culture at early log phase (~70 Klett units) was divided into two parts. One part was treated with 10 mM H_2O_2 for 30 min while the other part was left untreated as a control. After 30 min, the cultures were spun down, the media discarded, cells suspended in 1mL of trizol, transferred to a 2 mL tube with glass beads, frozen on dry ice and stored at -80 ºC. Samples were thawed and subjected to 3 x 45 s bead beating pulses keeping on ice in between, lysate was transferred to a tube containing phase lock gel and 350 mL of chloroform, mixed well and centrifuged 10 min at 14,000 g. The aqueous phase was then transferred to tubes containing 540 mL of isopropanol, taken out of BSL-3 and precipitated overnight at 4 C. Next day the tubes were centrifuged at 14,000 g for 10 min and the RNA pellets purified using the RNeasy kit from Qiagen with on column DNase I digestion.

RT-PCR. RT-PCR was performed using SuperScript One-Step RT-PCR System (Invitrogen). 10 ng of RNA was used for each reaction. Three samples were used for each strain (WT, Δ*mosR*, Comp and C12S) and condition (0, 5, 10 mM H_2O_2 treatment). Threshold cycle was determined for *rv1050* (probes) and *sigA* (control) using the primers in Table S2. Each reaction contained 10 ng of RNA.

Microarray. Microarray was performed as reported previously (9). cDNA Labeling and Microarray Hybridization: Both a PCR gene product microarray and a 70-mer oligonucleotide-based microarray (tuberculosis oligonucleotide set [Qiagen]) were used. Labeled cDNA was prepared as follows: 1.5 μg of total RNA and 4.4 μg of random oligonucleotide hexamers were incubated 2 min at 98°C, cooled on ice, combined with Stratascript RTase buffer, 0.5 mM dATP, dGTP, and dCTP, 0.02 mM dTTP, 1.5 nmol Cy3 or Cy5-dUTP (Amersham), and 1.8 μl Stratascript RTase (Stratagene) in a total volume of 25 μl and incubated 10 min at 25°C followed by 90 min at 42°C. Labeled cDNA was diluted in 400 μl TE and purified by microcon-10 (Amicon) filtration. Ten microliter hybridization solution (labeled cDNA, 5 μg tRNA, 3.8× SSC, 0.27% SDS) was sealed under a coverslip with rubber cement and hybridized overnight at 65°C for the DNA microarray. Oligonucleotide microarrays were first prehybridized for 1 h in 5X SSC, 1% BSA, and 0.1% SDS and washed with H2O and isopropanol. Following the prehybridization, 10 μl hybridization solution (labeled cDNA, 5 μg tRNA, 2× SSC, 25% formamide, 0.1% SDS) was hybridized overnight at 54°C. Slides were washed once for 2 min in 0.05% SDS, 1× SSC, washed twice for 2 min in 0.06× SSC, centrifuged at 600 rpm for 2 min, and were immediately scanned.

Microarray data analysis: Microarrays were scanned using a GenePix 4000A (Axon Instruments). The intensities of the two dyes at each spot were quantified using ScanAlyze (Michael Eisen1). All gene specific spots on the microarray other than those whose induction ratio was in the top or bottom 5% were used to normalize the intensities of Cy3 and Cy5 from each spot. After Cy3 and Cy5 channel normalization we eliminated large percentage fluctuations in low background spot values by adjusting low signal intensity spots to a minimum noise value. The noise value for each channel was determined by calculating the average intensity value for the 20% lowest intensity spots, and then every value below this average noise value was raised to the noise value. Microarray-determined ratios were calculated from three biological replicates and one to two microarrays for each biological replicate. Significance analysis of microarrays (SAM)2 was used to determine statistically significant regulated genes (10). Summary of results is presented in Table S5.

Southern and Northern Hybridization. Southern blot: Genomic DNA was extracted from *Mtb* strains and purified using Qiagen genomic purification kit. Purified DNA was subjected to simultaneous digestion with EcoR V and Sma I restriction enzymes. The DNA was run on a 1% agarose gel then transferred to a Hybond N+ membrane overnight by capillary blotting. The DNA on the membrane was hybridized with

^{32}P labeled DNA corresponding to the upstream region of *mosR* and exposed to the Phospor screen. *Northern blot*: 5 mg of purified RNA was run on a 1.5% agarose gel, transferred to the membrane, hybridized with P32-labeled DNA corresponding to *Rv1050* and exposed to the Phospor screen.

Culture conditions. 1 mL of 2-4 weeks old seed cultures were used to inoculate 100 mL of 7H9 medium supplemented with 10% homemade OAD (no catalase) and 0.2% glycerol and 0.1% tween-80. The cultures were grown for 2-3 weeks at 37 ºC, shaking at 120 rpm and monitored daily using a colorimeter. Flasks were opened daily to ensure oxygen availability.

Antibiotic susceptibility assays. Antibiotic susceptibility assays were performed using the MABA assay previously reported (11,12). Two hundred microliters of sterile deionized water was added to all outer-perimeter wells of sterile 96-well plates (Falcon 3072; Becton Dickinson, Lincoln Park, N.J.) to minimize evaporation of the medium in the test wells during incubation. The wells in rows B to G in columns 3 to 11 received 100 µl of 7H9GC broth. One hundred microliters of 2× drug solutions were added to the wells in rows B to G in columns 2 and 3. By using a multichannel pipette, 100 µl was transferred from column 3 to column 4, and the contents of the wells were mixed well. Identical serial 1:2 dilutions were continued through column 10, and 100 µl of excess medium was discarded from the wells in column 10. One hundred microliters of *Mtb* inoculum was added to the wells in rows B to G in columns 2 to 11 by using an Eppendorf repeating pipette (yielding a final volume of 200 µl per well). Thus, the wells in column 11 served as drug-free (inoculum-only) controls. Fifty microliters of a freshly prepared 1:1 mixture of 10× Alamar Blue (Accumed International, Westlake, Ohio) reagent and 10% Tween 80 was added to all wells. The plates were reincubated at 37°C for 24 h and the colors of all wells were recorded. A blue color in the well was interpreted as no growth, and a pink color was scored as growth. A few wells appeared violet after 24 h of incubation, but they invariably changed to pink after another day of incubation and thus were scored as growth (while the adjacent blue wells remained blue). The MIC was defined as the lowest drug concentration, which prevented a color change from blue to pink.

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