Figure S1

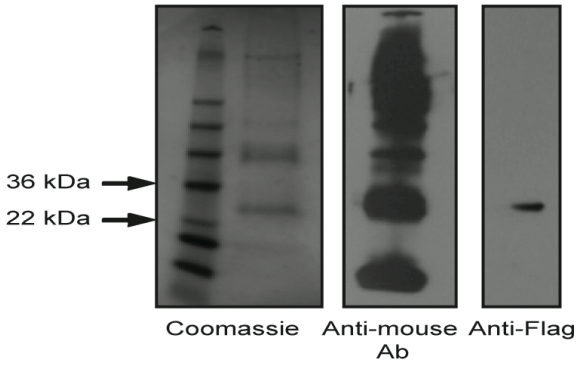

Figure S1, Related to Figure 2. FLAG-tagged DoxX pull down from *M. smegmatis* (*Msm*) was analyzed by SDS-PAGE and stained for total protein with coomassie blue. Western blot analysis was performed with anti-mouse antibody and anti-FLAG antibody to differentiate IgG from the affinity matrix and the purified antigen.

Figure S2

A

	Diameter of inhibition zone (plumbagin)	Diameter of inhibition zone (menadione)
WT	12+0.2 mm	15+0.2 mm
ΔMsSodA	19+0.2 mm	20+0.1 mm
ΔMsSseA	11+0.2 mm	14+0.2 mm
ΔMsDoxX	12+0.2 mm	16+0.1 mm

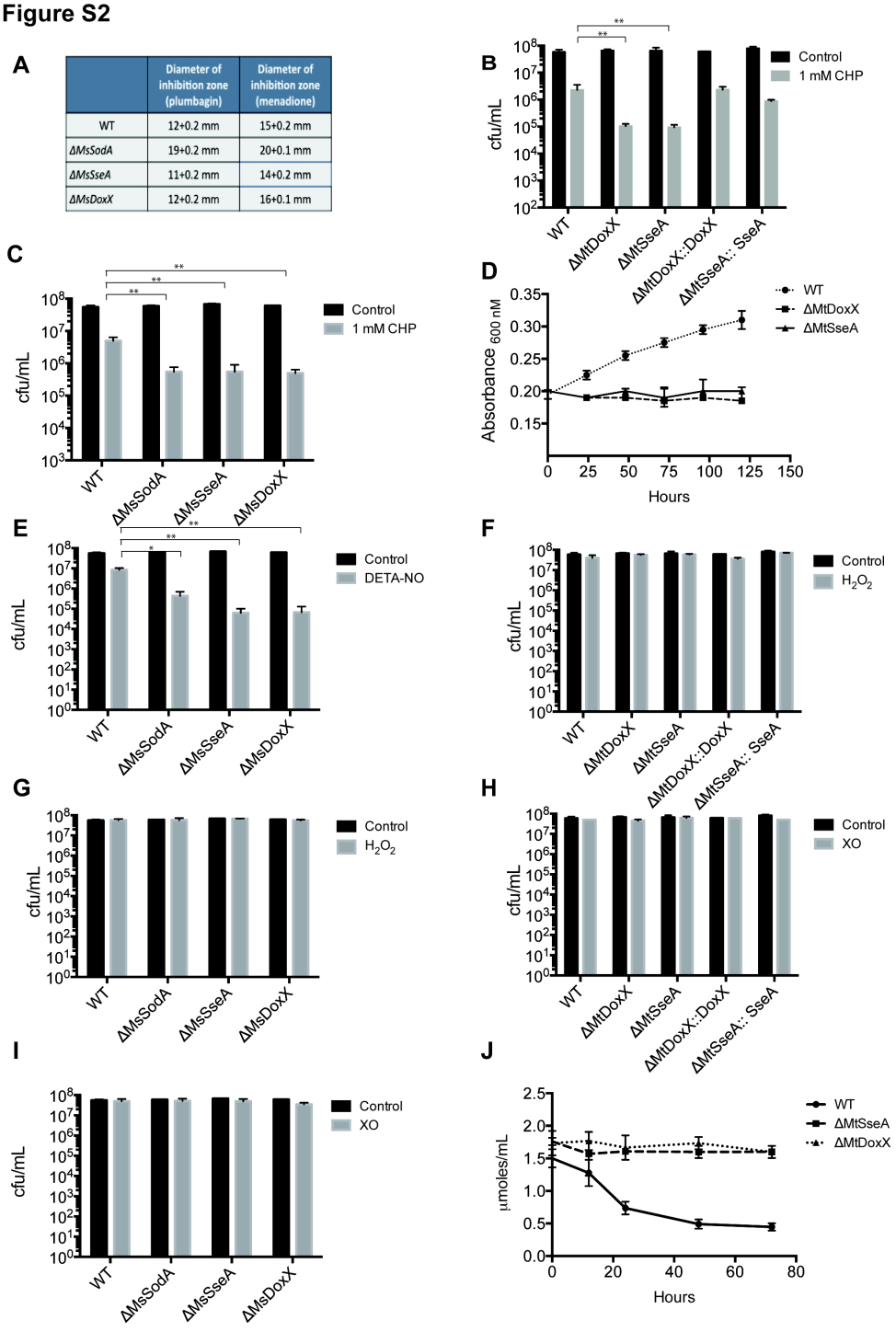

Figure S2, Related to Figure 3. A] Sensitivity to superoxide generators was determined using an agar diffusion assay. *Msm* and mutant strains were plated on 7H10 and incubated at 37°C in the presence of 5 mM plumbagin or 5 mM menadione-containing filter discs (8 mm in diameter). Data are the mean ± S.E. (error bars) of three independent experiments. Effect of CHP on viability of *Mtb* WT and mutant strains B] and *Msm* WT and mutant strains C]. Log phase cultures of the indicated strains were

exposed to 1 mM CHP for 1 h and CFU were enumerated. Values represent the mean ± S.E. of duplicate determinations of experiments repeated twice. Statistical significance was calculated using unpaired student's t test (** indicates $p < 0.01$). The effect of DETA-NO on *Mtb* WT and mutant strains D] and *Msm* WT and mutant strains E] was determined. Values shown represent the mean ± S.E. of duplicate determinations of experiments repeated twice. Statistical significance was calculated using unpaired student's t test (* indicates $p < 0.05$ and ** indicates $p < 0.01$). Effect of H_2O_2 on viability of *Mtb* WT and mutant strains F] and *Msm* WT and mutant strains G]. Log phase cultures of the indicated strains were exposed to 1 mM H_2O_2 for 1 h and CFU were enumerated. Values represent the mean ± S.E. of duplicate determinations of experiments repeated twice. Effect of extracellular redox stressors like xanthine oxidase (XO) on viability of *Mtb* WT and mutant strains H] and *Msm* WT and mutant strains I] are shown. J] Conversion of diamide to hydrazine was measured spectrophotometrically at 390 nM at the indicated time points. Diamide was added to cultures of equal optical density at the zero time point. Data are the mean ± S.E. (error bars) of two independent experiments.

Figure S3

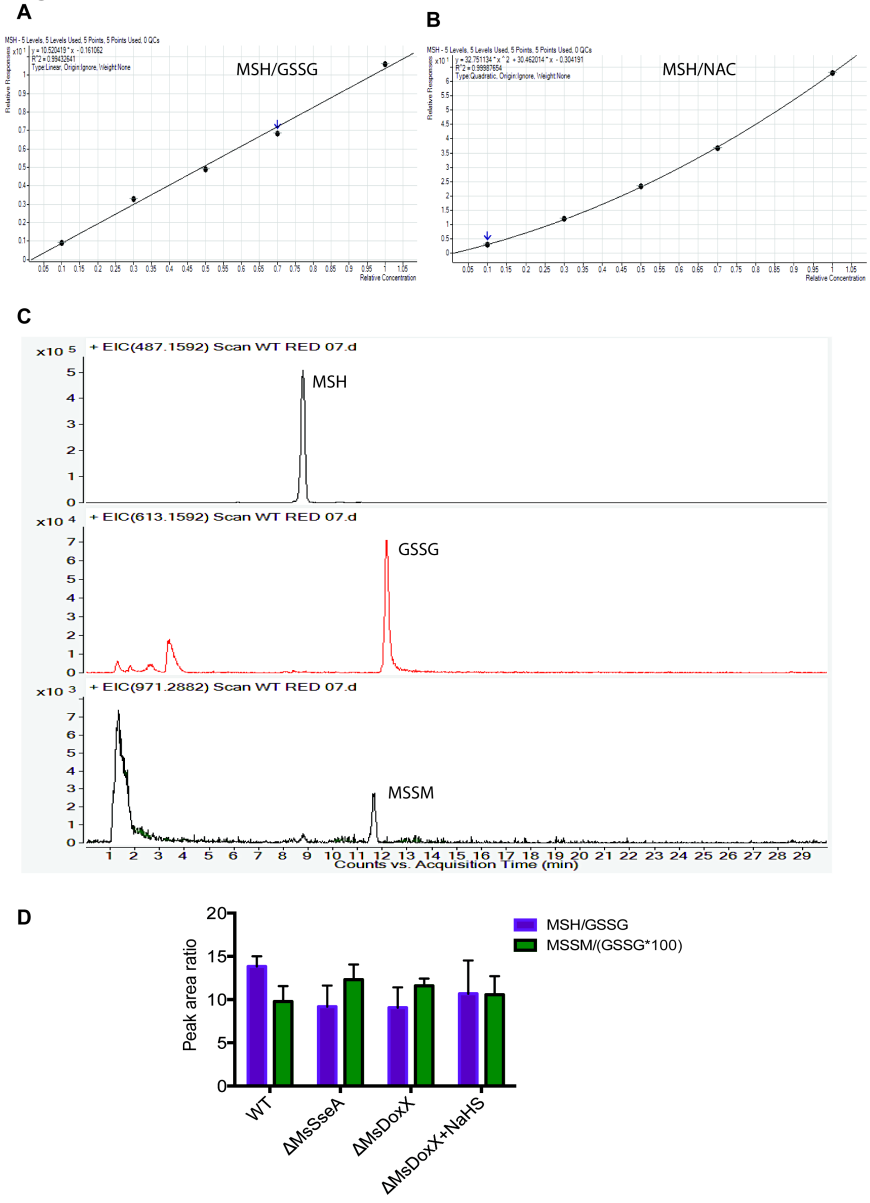

Figure S3, Related to Figure 3. LC/MS assay to verify mycothiol homeostasis defects in MRC mutants. This assay was validated using a pure analytical standard of MSH. A] Calibration curves to determine the most robust internal standard for MSH quantification. Use of oxidized glutathione (GSSG) as an internal standard produced an assay with a linear dose-response curve, and this standard was used for relative quantification of MSH (panel D). B] The use of N-acetylchitobiose (NAC) as an internal standard did not produce a linear dose-response curve. C] Extracted ion chromatogram of mycothiol (MSH) (m/z 487.1592), oxidized glutathione (GSSG) (m/z 613.1592) and oxidized mycothiol (MSSM) (m/z 971.2882). D] Average relative levels of MSH and MSSM in WT, *ΔMsDoxX*, *ΔMsSseA* and *ΔMsDoxX* pretreated with 100 µM NaHS for 1 h.

Values shown represent the mean ± S.E. of experiments repeated thrice. Statistical significance was calculated using unpaired student's t test (* indicates $p < 0.05$)

Figure S4

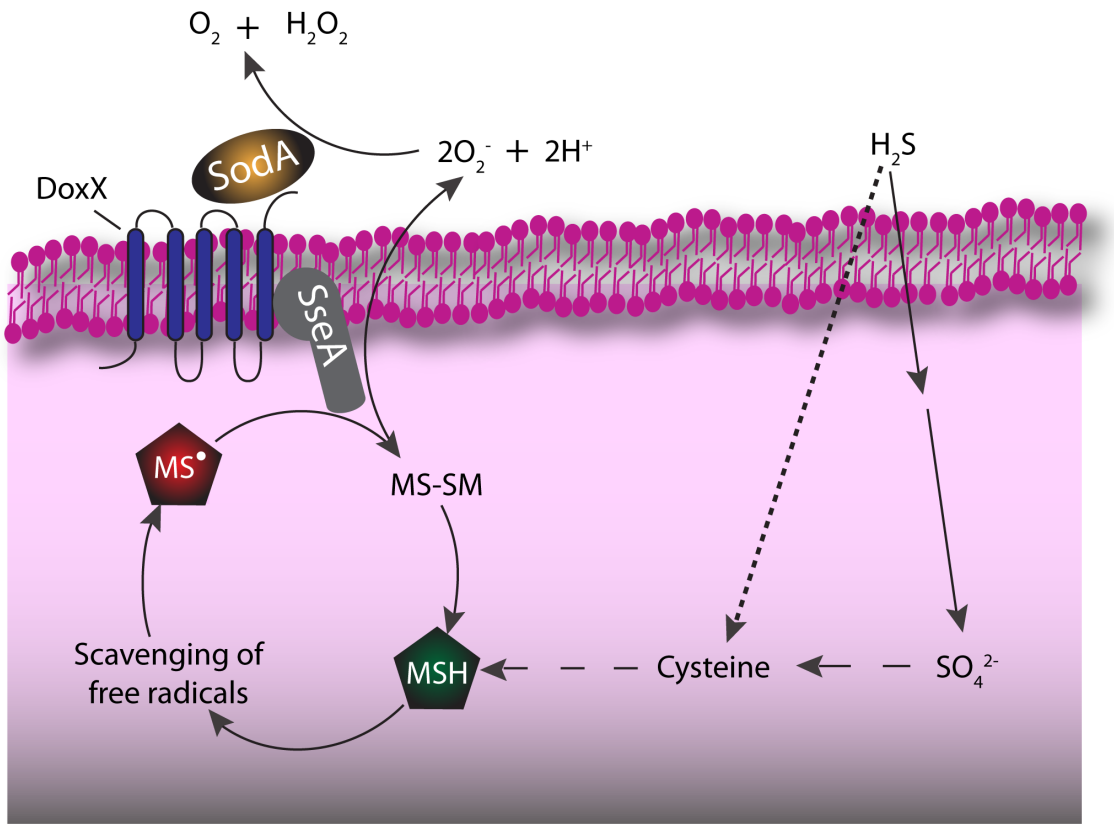

Figure S4, Related to Figure 4. Proposed mechanism of coordinated radical detoxification by the MRC. The known or predicted membrane topologies of MRC components suggest that this complex spans the membrane. SodA is secreted by the SecA2 system, DoxX is an integral membrane protein, and SseA lacks any secretion signals and is predicted to reside in the cytosol. This topology is reminiscent of the DoxABCD sulfur respiration system of *Acidianus ambivalens*, which shares limited homology with the MtDoxX protein. In this chemotrophic organism, the DoxA and DoxD form a thiosulfate oxido-reductase that oxidizes cytosolic thiosulfate and uses a quinone co-factor for electron transfer to a *doxBD*-encoded terminal oxidase. While it is unclear if MtDoxX interacts with quinones, the use of such a cofactor could explain the coupling of cytosolic oxidoreductase reactions with extracellular SOD activity that is implied by the transmembrane topology of the MRC. Mycothiol thiyl radicals are generated by the free radical scavenging activity of mycothiol. The DoxX and SseA facilitate the conversion of this radical to oxidized mycothiol. Oxidized mycothiol is then recycled by mycothione reductase activity. The superoxide anions that might be generated as a result of these activities can be detoxified by the associated superoxide dismutase, SodA. Cysteine, sulfate and H_2S supplementation can restore the reduced mycothiol pool.

Supplemental Table 1, Related to Figure 1. Summary of CtpC genetic interaction data. The TNseq data derived from the experiment depicted in Figure 1A are listed for the whole Mtb genome. "#TA in orf" indicated the total number of possible himar1 insertion sites in the indicated gene, " #TA with insertion" indicates the number of potential insertion sites in which an insertion is detected, "Template count/TA" indicates the number of uniquely barcoded amplicons (i.e. "templates") corresponding to an open reading frame divided by the number of potential insertion sites, " Relative representation (Log2 *ctpC*/WT)" indicates the ratio of template count/TA in the two libraries, and "Q-val" represents a corrected p value determined by resampling.

Supplemental Table 2. Related to Figure 1. Raw CtpC genetic interaction data. The number of uniquely barcoded amplicons (i.e. "templates") corresponding to each indicated insertion site are listed.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Plasmids

Plasmid	Description	Source
pDE43-MEK	Replicating destination vector adapted from Gateway cloning system, Kan^R	Dirk Schnappinger
pDE43-MCZ	Integrating destination vector adapted from Gateway cloning system, Zeo^R	Dirk Schnappinger
pMEK-FLAG-Rv3005c	pMEK containing FLAG-Rv3005c expressed from p46 promoter, Kan^R	This study
pMEK-FLAG-Rv3283	pMEK containing FLAG-Rv3283 expressed from p46 promoter, Kan^R	This study
pMEK-5'-hyg-3'-Rv3005c	pMEK containing Hyg^R flanked by 500bp upstream and downstream region of Rv3005c	This study
pMEK-5'-hyg-3'-Rv3283	pMEK containing Hyg^R flanked by 500bp upstream and downstream region of Rv3283	This study
pMV762-Mrx1-roGFP2	Plasmid containing redox sensitive mycoredoxin sensor, Hyg^R	Amit Singh

Primers used in this study

Primer name	Sequence
Adapter 1.2	TACCACGACCA-NH2
Adapter 2.2 BarB	ATGATGGCCGGTGGATTTGTGNNANNANNNTGGTCGTGGTAT
JEL_AP1	ATGATGGCCGGTGGATTTGTG
T7	TAATACGACTCACTATAGGGTCTAGAG
Sol_AP1_tagged_930A	CAAGCAGAAGACGGCATACGAGATTGTTCCGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCAATGATGGCCGGTGGATTTGTG
Sol_AP1_tagged_930B	CAAGCAGAAGACGGCATACGAGATTGTTCCGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGTCCATGATGGCCGGTGGATTTGTG
Sol_AP1_tagged_930C	CAAGCAGAAGACGGCATACGAGATTGTTCCGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAGTCCCATGATGGCCGGTGGATTTGTG
Sol_AP1_tagged_930D	CAAGCAGAAGACGGCATACGAGATTGTTCCGAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAGTGGATGATGGCCGGTGGATTTGTG
Sol_AP1_tagged_949A	CAAGCAGAAGACGGCATACGAGATTTCCGGAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCAATGATGGCCGGTGGATTTGTG
Sol_AP1_tagged_949B	CAAGCAGAAGACGGCATACGAGATTTCCGGAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGTCCATGATGGCCGGTGGATTTGTG
Sol_AP1_tagged_949C	CAAGCAGAAGACGGCATACGAGATTTCCGGAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAGTCCCATGATGGCCGGTGGATTTGTG
Sol_AP1_tagged_949D	CAAGCAGAAGACGGCATACGAGATTTCCGGAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAGTGGATGATGGCCGGTGGATTTGTG
Sol_Mar	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCGGGGACTTATCAGCCAACC
Sol_Mar1b	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTCGGGGACTTATCAGCCAACC
Sol_Mar4b	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGATACGGGGACTTATCAGCCAACC
Sol_Mar5b	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTATCTACGGGGACTTATCAGCCAACC
F-Rv3005c-23	GGGGACAGCTTTCTTGTACAAAGTGGCCGTGACCAGTTCGAATGACTCACATT
R-Rv3005c-23	GGGGACAACTTTGTATAATAAAGTTGAGTCATCATCAGGCCAGCGGATTTGCCCCGTTG
F-Rv3005c up-41	GGGGACAACTTTGTATAGAAAAGTTGCGGCTCGGCGCTGATAGAGATCTCC

R-Rv3005c up-41	GGGGACTGCTTTTTTGTACAAACTTGTCGTGTCAGGGTAAGGCCATTTAGT
F-Rv3005c down-23	GGGGACAGCTTTCTTGTACAAAGTGGACGCTGTCATCGGTACGGGTTGGGG
R-Rv3005c down-23	GGGGACAACTTTGTATAATAAAGTTGAGTCATCACCGCGGTGTCAGTCTTGATCCGCGA
F-Rv3283 up-41	GGGGACAACTTTGTATAGAAAAGTTGCATTGTGGCCGCGGATTGCGTTGTCG
R-Rv3283 up-41	GGGGACTGCTTTTTTGTACAAACTTGATCATCGAGCCTACCGCTGGCGACC
F-Rv3283 down-23	GGGGACAGCTTTCTTGTACAAAGTGGCCGCGCCCGCGAGCCTGCCCGCGCC
R-Rv3283 down-23	GGGGACAACTTTGTATAATAAAGTTGAGTCATCACCCGACAAGACTTGTAAGAAAATCT
F-Rv3283-23	GGGGACAGCTTTCTTGTACAAAGTGGCCGTGCCGCTTCCCGCAGACCCTAGCC
R-Rv3283-23	GGGGACAACTTTGTATAATAAAGTTGAGTCATCATCATACGACGGGTACCACTCCTGG
forA-SodA-Del	TCCACAATCACATATGAGGAAGGAATCTCGTGGCTGAATACACCCTGCCGCTCTAGAACTAGTGGATCC
ForB-SodA-Del	TTGCGGAGTGCGCTGCCATGGAGCAATGTTGACGATGCTTTCGGAGCGTCGATCTTTCTGACGTCCACAGCTCCACAATCACATATGAGGAAGG
RevA-SodA-Del	CGGGTGTCAGATGCCTCTTTGTGAGCGGATCAGCCGAAGATCAGACCGCGGTCGACGGTATCGATAAGC
RevB-SodA-Del	GGTCCCGAAAACGAGGGCTGCGAATCGCGCGAATCGCCCTTCGTTCACCAGATCAGGCGGTCGGTGCCGACCGGGTGTCAGATGCCTCTTTGTGAGC
ForA-TST-Del	CCCGTGCTCTCGGCGGTAGGCTCATGGTGTGTCGCTGCCTGCAGATCCCGCTCTAGAACTAGTGGATCC
ForB-TST-Del	GCCGCCGAACTGGGCGAACGTGCAGTGATTCGGGGCGGCGATCAGGCGTTACGCCCCAGTGCCCCGACAACACCCGTGCTCTCGGCGGTAGG
RevA-TST-Del	GACCACCTCTGCCAGGGCAGCCGGCATGGTCACGGTGCCGAACCCGGGTGGTCGACGGTATCGATAAGC
RevB-TST-Del	GGCGGCAGCTCGTTGGCGAACTCCAGCAACAGTTGCAGCTTGTCCTGACCGGCCACTTCCTGGAAGTCCGAGACCACCTCTGCCAGGGCAGC
ForA-DoxX-Del	CCGAGGAAGTTGCACTTACCCTGGCAGCGTGACCAGTTCCTCGCAGGACGCTCTAGAACTAGTGGATCC
ForB-DoxX-Del	CCGGGCAGGGGGCTCGTGACGCGGAATTTCGCTCCAATACCAGGCGGAAGCGCCGTGGTAAATACCAGGATCCCGAGGAAGTTGCACTTACC
RevA-DoxX-Del	GATCACGCGTAACGGTCCCGAAAGGCGAATCAGCTCAGCGGGTTGCCGCGGTCGACGGTATCGATAAGC
RevB-DoxX-Del	CGTGACGTTCGCCACGCTGCCGCTGCTCGCGGAGGTCAGCGGAGGACGGGTTCCGAACCCATACGCGTGACGATCACGCGTAACGGTCCCGAAAGG

Generation of mycobacterial mutants

Msm and Mtb mutants were constructed following the procedures described previously (Gee et al., 2012; Murphy et al., 2015; van Kessel and Hatfull, 2007) . Briefly, the indicated primers in the preceding table were used to assemble an allelic exchange construct for each gene. Transformation of linear DNA substrate into strains expressing the phage Che9c recombinase resulted in the replacement of the desired gene with a hygromycin-resistance cassette.

Genetic interactions with the *ctpC* mutant during infection

Transposon libraries were made using the pMycoMarT7 transposon in WT and *ctpC::hyg* strains (Sassetti et al., 2001). 10^6 colony-forming units (cfu) of WT or *ctpC* mutant libraries were introduced intravenously by tail vein injection into four groups of three C57BL/6 mice (8-10 weeks old). Housing and experimentation were in accordance with the guidelines set forth by the Department of Animal Medicine of University of Massachusetts Medical School and Institutional Animal Care and Use Committee. At days 0 and 32, the mice were sacrificed and the surviving bacteria isolated by plating spleen homogenates.

The WT and mutant libraries isolated from mice were collected separately by scraping the plates and then extracting the chromosomal DNA. The DNA was sheared by sonication with a Covaris S220 sonicator to approximately 500 bp in length. Damaged ends were repaired (Epicentre DNA End Repair Kit), and A-tailed with Choice-Taq DNA polymerase (Denville) to allow ligation of barcoded adapters with T-overhangs. The transposon chromosomal junctions were amplified for 20 cycles (95^{o}C, 30 sec; 58^{o}C, 30 sec; 72^{o}C, 45 sec) using a transposon specific primer (T7-1) (5' TAATACGACTCACTATAGGGTCTAGAG 3') and an adapter specific primer (JEL-AP1) (5' ATGATGGCCGGTGGATTTGTG 3'). The PCR product for each library was run on a 2% gel at 50V. Genomic DNA located between 300 and 500 bp was then extracted from the gel. Following gel-extraction, the PCR fragments were further amplified for 10 cycles (95^{o}C, 30 sec; 58^{o}C, 30 sec; 72^{o}C, 45 sec) with primers that were compatible with Illumina sequencing and paired-end, 100-base sequence reads were obtained on the Illumina HiSeq platform.

The raw sequence data in fastq format was processed using custom Perl scripts. Read 1 sequences were trimmed at the TGTTA (transposon insertion site), retaining sequence from TA onward. Mate reads were searched for GTGNNANNANNNTGG (adapter barcode and flanking sequence; barcode underlined) and trimmed 18 bases after the start of the barcode (the junction between ligated adapter and genomic DNA), retaining exactly 50 bases. The barcode sequence was captured and appended to the read ID in the fastq-formatted output file, effectively treating it as a read group. The trimmed sequences were aligned using BWA (version 0.6.1-r104) with options -q 5 -l 32 -k 2 -o 1. The resulting alignment file was parsed to obtain read counts mapping to each TA dinucleotide in the genome, ignoring reads containing a mismatched base at the insertion site TA. Each read was assigned an ID corresponding to the TA to which it aligned, strand, length of insert (corresponds to the sonication breakpoint of the original genomic DNA molecule), and barcode. Reads with identical ID's were assumed to be PCR duplicates and only counted once (Table S2).

The differential effect of selection between WT and *ctpC* mutant was assessed by determining the relative recovery of insertion events. For each annotated gene, the total number of insertions was summed over all TA's in the gene (excluding the translation terminator) and a $\log_2$-transformed ratio (R) calculated, where $R = (N_c + 1) / (N_w + 1)$. N_c and N_w are, respectively, the combined sum of insertions in a given gene in both replicates of the *ctpC* and WT libraries recovered after growth in mice. Negative values of $\log_2(R)$ indicate growth disadvantage of the *ctpC* mutant relative to WT and positive values, growth advantage. Statistical significance of the magnitude of $\log_2(R)$ was determined by permutation test. Insertion counts at all TA's (excluding terminator) in the test gene from all four libraries were pooled, randomly assigned to two bins, and a $\log_2(R)$ calculated. This was repeated for a total of 10,000 trials, and the number of times a $\log_2(R)$ value as extreme or more extreme than that observed in the original data was counted (x). The corresponding P value was x / 10,000. Cases where x equaled zero were arbitrarily assigned a P value of 10^{-5}. Benjamini-Hochberg correction was applied to account for multiple tests (Table S1).

Physical interactions

FLAG-tagged version of DoxX was expressed ectopically in both *M. smegmatis* and *ΔleuD ΔpanCD* double auxotroph *Mtb* and used for immunoprecipitation to characterize interacting proteins. Lysates from expression strains were extracted with 2% octyl glucoside, DoxX was immunoprecipiated using anti-FLAG M2 affinity gel, and bound proteins were then subjected to mass spectrometric analysis (proteomics facility, University of Massachusetts Medical school).

Thiosulfate oxidation assay

Thiosulfate oxidation activity was determined spectrophotometrically by measuring the reduction of potassium ferricyanide at 420 nm (Müller et al., 2004). The reaction mixture contained 50 mM Bis Tris, 1.25 mM potassium ferricyanide, 1.25 mM citric acid at pH 6.0, 10 mM thiosulfate and enzyme solution affinity purified using FLAG-tagged DoxX or FLAG-tagged SseA. The reaction was initiated by adding the thiosulfate solution.

Redox stressor sensitivity assay

Mtb WT, mutant and complemented strains were grown to an OD of ~1.0. For tBHP sensitivity, 7H10-OAD was used instead of 7H10-OADC and cells were treated with 1 mM tBHP or CHP or H_2O_2 for 1 h. Serial dilutions were plated. CFUs were assessed after 18 days of incubation at 37°C. *Msm*, mutant and complement strains were grown in 7H9-AD medium to an OD of ~1.0 and the same procedure was followed as with *Mtb*. For plumbagin and menadione sensitivity assay, a disk diffusion method was used.

Briefly, agar plates were prepared by pouring log phase bacterial cultures with 0.6% agarose on 7H10 plates. 5 µL of 5 mM plumbagin or 5 mM menadione were spotted on filter paper discs and placed on agar plates containing bacteria. For analyzing the sensitivity to xanthine oxidase, strains were grown in 7H9 medium to an OD of ~1.0. The cells were incubated for 120 min with 0.1 unit/ml xanthine oxidase and 250 µm hypoxanthine. Serial dilutions were plated and CFU were quantified. For nitrosative stress, *Mtb* and mutant strains were grown to an OD of 0.2 and then 1 mM DETA-NO was added to the culture. Absorbance at 600 nm was measured over time. For nitrosative stress in *Msm*, the different strains were grown to an OD of 0.5 and cells were treated with 1 mM DETA-NO for 1 h. Serial dilutions were plated and CFU were quantified. For diamide stress, aliquots of cultures 12 h after addition of 5 mM diamide were serially diluted and plated. For measurement of conversion of diamide to hydrazine, aliquots of cultures at different time points were taken after addition of 5 mM diamide, the cultures were centrifuged and supernatant was collected. Diamide conversion was measured at 390 nM.

Measurement of Lipid hydroperoxides

Lipid hydroperoxides (LHPOs) were measured on total lipid extract using FOX2 reagent (Griffiths et al., 2000). Wet cell pellets obtained from different strains of *M. tuberculosis* and *M. smegmatis* grown to an OD of ~1 were used to extract lipids. Cell pellets were suspended in 1.9 mL of (1:2 chloroform/methanol) and mixed by vortexing. Then 630 µL of chloroform and 630 µL of water were added, and the sample was vortexed. The samples were then subjected to centrifugation (5 min, 4000× *g*, 4^0C) for phase separation. The organic phase was collected and washed twice with 2 volumes of water. Organic phase (200 µL) was mixed with 1 mL of FOX2 reagent, and incubated in the dark for 1 h at 22^0C. Lipid hydroperoxides were quantified spectrophotometrically at 560 nm.

Macrophage infections

Immortalized C57BL/6 bone marrow derived macrophages seeded at 1×10^5 cells per well in 24-well plates were infected with *Mtb* at an MOI of 3 and incubated for 4 h, at 37°C in 5% CO_2. Extracellular bacteria were removed by washing twice with PBS. At day 7, infected macrophages were washed with PBS and lysed with 1% saponin for 5 min. Serial dilutions were plated and CFU was assessed. IFN-γ was added at a concentration of 25 ng/mL. NaHS treatment was carried out at a concentration of 100 µM.

Mouse infections

C57BL/6 and C3HeB/FeJ were infected using an aerosol generation chamber (Glas-Col) standardized to deliver ~200 cfu of *Mtb* per mouse. At four weeks postinfection, groups of mice had a sub-cutaneous osmotic pump implanted to administer the buffer (control group) or NaHS (treatment group). The pumps were purchased from Durect Corporation ALZET Osmotic Pumps (Cupertino, CA, USA). The treatment group mice were implanted with ALZET pump filled with 250 µL of NaHS (2 mM). These pumps are designed to deliver 6 µL/day. After six weeks of treatment, both the groups of mice were sacrificed to measure lung cfu.

Measurement of MSH:MSSH using the Mrs1-roGFP2 reporter

The plasmid pMV762-Mrx1-roGFP2 was transformed into the indicated strains. At an OD of ~1.0, 200 µl of each culture was transferred to a 96-well microplate and fluorescence emission was read at 510 nm after excitation at 390 nm and 490 nm. Cysteine, sulfate and NaHS treatments were carried out for 1 h at a concentration of 100 µM.

LC/MS/MS assay for mycothiol

Mycothiol analysis in cell extracts was carried out using a Agilent 6520 Accurate-Mass QTOF LC/MS system (Agilent Technologies Inc, Marshfield, MA, USA) on a ZIC-cHILIC (Merck Millipore, Billerica, MA, USA) column (100 mm x 2.1 mm, 3.0 µm d_p, 100 Å pore size). Mobile phases included an aqueous mobile phase A (10% acetonitrile in 5 mM ammonium acetate) and an organic mobile phase B (90% acetonitrile in 5 mM ammonium acetate). A linear gradient was performed from 94% B to 50% B over 10 min, held at 50% B for 5 min, and further held at 20% B for 5 min. The percentage of mobile phase B was then reduced to the initial condition and held for 5 min for column equilibration. The flow rate was 0.2 mL/min and the injection volume was 5 µL. The mass spectrometry (MS) was operated in the positive mode with full scan condition from *m/z* 50 to 3200. The instrument was calibrated for accurate mass before every analysis. All dependent MS parameters were optimized based on infusion experiments. Data were acquired and analyzed using Masshunter qualitative and quantitative analysis software version B.05.00.

Quantification of MSH:MSSM

A volume of 200 µL of acetonitrile with 2.5% formic acid/water (80/20) was added to each tube containing cell pellets. The mixtures were vortexed, incubated in -20°C for 15 min, then centrifuged at 16000x*g* for 10 min. The supernatant was transferred into a 1.5 mL centrifuge tube and further diluted with a 7:10 dilution. This dilution factor was selected from a serial dilution since the signal response of MSH and MSSM was in detectable and linear ranges. GSSG was selected as an internal standard for both analytes. The results were presented as peak area ratio between each analyte and the internal standard.

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