Figure EV1. Phylogenetic conservation of Rv1332.

A Pictorial representation of domain architecture of Rv1332.
B Phylogenetic tree depicting actinomycetes encoding for orthologs of Rv1332 (Huerta-Cepas et al, 2016).
Figure EV1.
Figure EV2. Generation and characterization of RvΔ1332.

A Schematic representation of loci in Rv and RvΔ1332 strains, depicting replacement aosR with hgyR. Multiple primer sets used for the PCR-based confirmation are depicted.

B PCR amplicons from Rv and RvΔaosR were resolved on 1% agarose gels. Primer pairs used for the amplification are indicated. First panel (control): ~0.97 kb amplicons of sigB in both the strains. The second panel shows amplicons (~1.2 kb) obtained only in RvΔ1332. The third panel shows differential amplicon sizes with primers beyond AES in Rv (~2.1 kb) and RvΔ1332 (~3.1 kb) to confirm site-specific recombination, and fourth panel shows differential amplification of Rv1332 gene in Rv (0.67 kb) and RvΔaosR (1.6 kb). Mr represents molecular marker.

C 35 µg WCLs prepared from Rv, RvΔaosR, and RvΔaosR::aosR were resolved on 15% SDS–PAGE, transferred to nitrocellulose membrane, and probed with α-AosR and α-SigA antibodies.

D, E Logarithmic phase cultures of Rv, RvΔaosR, and RvΔaosR::aosR were inoculated at (D) A600 ~0.05 in 7H9-ADC and CFU (mean ± SEM; n = 3) were enumerated at days 0, 2, and 4 or (E) at A600 ~0.01 in Sauton’s media and CFU (mean ± SEM; n = 3) were enumerated at days 0, 3, and 6.

Source data are available online for this figure.
**Figure EV3. AosR specifically mitigates oxidative stress.**

A. To assay the sensitivity of Mtb strains to acidic stress, single-cell suspensions of Rv, RvΔaosR, and RvΔaosR:aosR were inoculated in 7H9-ADC acidified to pH 4.5 and CFUs were enumerated after a week (n = 3).

B. Early-log-phase culture of Rv, RvΔaosR, and RvΔaosR:aosR was washed and resuspended in PBS containing 0.1% Tween 80 at A600 ~ 0.3. Bacterial survival was examined after a week (n = 3).

C. For surfactant stress, mid-logarithmic phase bacterial cultures were grown in 7H9-ADC broth containing 0.1% SDS for 3 h, and CFUs were enumerated (n = 3).

D, E. Logarithmic phase bacterial strains were inoculated at A600 ~ 0.3 in 7H9-ADS (D) containing 1 mM DETA-NO for 2 days or (E) 50 µM CHP for 24 h (n = 3).

F. Bacterial strains inoculated at A600 ~ 0.2 in the presence or absence of 50 µM CHP for 24 h were streaked on 7H11-OADS plates.

G. Logarithmic phase bacterial strains were treated with increasing indicated concentrations of CHP for 24 h. The data depicts mean ± SEM, representative of two independent experiments performed in triplicates.

Data information: (A–E) CFU obtained at day 0 was set as 100%, and percent survival was calculated. Data are plotted as mean percent survival (± SEM). Statistical significance was analyzed using one-way ANOVA followed by post hoc test (Tukey test, GraphPad prism). *P < 0.05, **P < 0.005, and ***P < 0.005.

Source data are available online for this figure.
Figure EV4. Pathways of ROS production and degradation.

A NADPH phagocyte oxidase (phox) activity is enhanced by IFN-γ. It reduces oxygen (O₂) to superoxide (O₂⁻), which can either react with nitric oxide to generate peroxynitrite (ONOO⁻) or be converted to less reactive species hydrogen peroxide (H₂O₂) by superoxide dismutase. H₂O₂ is either reduced to water by catalase or glutathione reaction or react with Fe²⁺ to form highly potent hydroxyl radicals (HO•) via Fenton reaction. Generation of O₂⁻ is additionally contributed by xanthine oxidase, mitochondria, and uncoupled nitric oxide synthase.

B CFU obtained for Rv in each set (Fig 2D) was normalized to 100%, and mean percent survival ± SEM (n = 3) of RvΔaosR and RvΔaosR::aosR was calculated.

C CFU obtained for Rv in each mice strain (Fig 2E) was normalized to 100%, and mean percent survival ± SEM (n = 3) of RvΔaosR and RvΔaosR::aosR was calculated.

Data information: Statistical significance was analyzed using one-way ANOVA followed by a post hoc test (Tukey test; GraphPad prism).

Source data are available online for this figure.
Figure EV5. Cysteine biosynthesis pathways in Mtb.

There are three de novo cysteine biosynthesis pathways in Mtb. The canonical cysteine synthase (CysK1) utilizes O-acetyl-L-serine derived from serine and sulfide derived from the reductive branch of APS pathway as substrates. CysM pathway is unique to actinomycetes and uses O-phospho-L-serine as acceptor substrate and thiocarboxylated CysO as a sulfur donor. CysK2 pathway generates either \( \text{L-}
\)cysteine when sulfide is used or \( \text{S-}
\)sulfocysteine when thiosulfide is used. The latter can be reduced to \( \text{L-}
\)cysteine or used as an oxidative stress response-signaling molecule.