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MAJOR ARTICLE

Histone Methyltransferase SET8 Epigenetically Reprograms Host Immune Responses to Assist Mycobacterial Survival

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NQO1 and TRXR1 are important host reductases implicated in the regulation of inflammation and apoptosis. Although the transcriptional machinery governing these processes have been extensively investigated, the associated epigenetic regulatory events remain unclear. Here, we report that SET8, a histone H4 lysine 20 monomethylase (H4K20me1), is highly induced during *Mycobacterium tuberculosis* infection that orchestrates immune evasion strategies through the induction of NQO1 and TRXR1 in vivo. SET8, along with FoxO3a, mediates an active NQO1-PGC1-α complex, which promotes the anti-inflammatory M2 macrophage phenotype, and assists TRXR1-regulated arrest of tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis. Strikingly, the loss-of-function analysis in an in vivo mouse tuberculosis model further corroborated the pivotal role of SET8-responsive NQO1 and TRXR1 in mycobacterial survival. Thus, augmenting host immune responses against *Mycobacterium tuberculosis* by harnessing the SET8-NQO1/TRXR1 axis with its specific and potent inhibitors could lead to promising host-directed therapeutic adjuvants for tuberculosis treatment.

Keywords. SET8; methyltransferase; Mycobacteria; NQO1; TRXR1; inflammation; apoptosis.

Even in the face of accessible chemotherapy, tuberculosis, caused by *Mycobacterium tuberculosis* (Mtb), continues to be an alarming threat to humans, claiming 1.4 million lives per year [1]. Engagement of Toll-like receptors (TLRs) upon infection with mycobacteria or its derivatives elicits diverse immune responses, including autophagy, apoptosis and inflammation, in an attempt to clear the infection [2–5]. Cellular sentinels such as macrophages exhibit considerable plasticity and are known to be polarized antithetically in contrasting infectious scenarios [6]. *Mycobacterium tuberculosis* intercepts the host cellular machinery, circumventing the protective immune responses, and subsequently establishes an efficacious infection, making it one of the most successful human pathogens. Emerging evidence suggests epigenetic remodeling as yet another crucial mechanism modulating host immune responses [7–9]. Genome-wide studies revealed the role of Lys methyltransferases (KMTs) in regulating a variety of cellular functions. For example, H3K9 methyltransferase, G9a (also known as EHMT2), is a well-established negative regulator of genes that encode for type I interferons (IFNs), whereas H3K27 demethylase Jumonji domain-containing 3 (JMJD3) directs the M2 polarization of macrophages upon TLR stimulation [10–12]. Further, SETDB2 methyltransferase was shown to repress the expression of CXC-chemokine ligand 1 (CXCL1) [13]. In addition, SET8 methyltransferase, which causes specific monomethylation on K20 of H4 (H4K20me1), is associated with oxidative stress and apoptosis [14, 15]. However, there is a dearth of information on the role of SET8 in modulating host immune responses during mycobacterial infection. A recent study identified genes belonging to the oxidoreductase family as major players promoting mycobacterial entry and/or survival in macrophages, and NAD(P)H dehydrogenase quinone 1 (NQO1) inhibitor was particularly highlighted as a promising host-directed therapeutic against tuberculosis [16]. NQO1 is known to detoxify cellular quinones, reduce oxidative stress, and in general promote an anti-inflammatory phenotype [17, 18]. Yet the molecular circuitry governing this important mycobacterial target remains largely unexplored. Additionally, sporadic reports indicate the importance of other host-derived reductases such as thioredoxin reductase (TRXR) in suppressing apoptosis [19, 20]. Although avirulent strain of mycobacteria like *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG) was found to induce apoptosis in different cell lines [21], it is suggested that pathogenic mycobacteria suppress apoptosis by inducing antiapoptotic genes *Mcl1* and *A1* [22, 23]. Thus, TRXR may prove to be another interesting target among the antioxidant family of enzymes and warrants further investigation.

In line with these observations, we show that the Mtb-induced expression of NQO1 and TRXR1 is regulated by miR-30e-3p–stabilized H4K20me1 methyltransferase, SET8. Notably,

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depletion of NQO1 and TRXR1 through use of specific short interfering RNA (siRNA) revealed the important roles of NQO1 and TRXR1 in regulating inflammatory responses and apoptosis during mycobacterial infection. Furthermore, treatment with dicoumarol (NQO1 inhibitor) and auranofin (TRXR1 inhibitor) during mycobacterial infection of macrophages suggested that anti-inflammatory responses and inhibition of apoptosis are dependent on the reductase activity of NQO1 and TRXR1, respectively. Interestingly, in an experimental mouse tuberculosis model, mice administered dicoumarol and auranofin showed a considerably reduced mycobacterial organ burden. This was also corroborated by lung histopathology, which indicated the reduced severity of tuberculosis with inhibitor treatment through the analysis of granuloma, thereby underscoring the importance of NQO1 and TRXR1 in tuberculosis pathogenesis.

Collectively, we suggest a crucial role for miR-30e-3p-FoxO3a-SET8 axis in regulating host-derived reductases NQO1 and TRXR1 and thus suppressing host immune responses such as cytokine signature and apoptosis. Hence, epigenetic reprogramming of the host cell by SET8 promotes Mtb survival in macrophages by regulating inflammation and apoptosis.

MATERIALS AND METHODS

Cells, Mice, and Bacteria

The RAW 264.7 mouse macrophage cell line was obtained from the National Centre for Cell Science, Pune, India, and was cultured in Dulbecco's modified Eagle medium (Gibco-Life Technologies) supplemented with 10% heat-inactivated fetal bovine serum (Gibco-Life Technologies) and maintained at 37°C in a 5% carbon dioxide incubator. Primary macrophages were obtained from peritoneal exudates of C57BL/6 wild-type (WT) and BALB/cJ mice. All strains of mice were purchased from the Jackson Laboratory and maintained in the Central Animal Facility, Indian Institute of Science. *Mycobacterium tuberculosis* H37Rv cultures were grown to mid-log phase, and single-cell suspensions of mycobacteria were obtained by passing mid-log phase culture through syringes of different gauge sizes. They were used at the indicated multiplicity of infection. All studies that involved virulent mycobacteria were carried out in the BSL-3 facility at the Centre for Infectious Disease Research, Indian Institute of Science.

Ethics Statement

All studies that involved mice, virulent mycobacteria, and human peripheral blood mononuclear cells (PBMCs) were carried out after the appropriate approvals from the Institutional Ethics and Biosafety committees. The animal care and use protocol adhered to were approved by national guidelines of the Committee for the Purpose of Control and Supervision of Experiments and Animals (CPCSEA), Government of India.

Please see the Supplmentary Materials for additional information.

RESULTS

SET8, a Histone Methyl Transferase, Facilitates M2 Macrophage Polarization and Regulates Apoptosis

Mycobacterium tuberculosis polarizes macrophage population majorly to the M2 phenotype and modulates apoptosis during infection [6, 22, 24]. However, mechanisms that regulate these 2 parallel events during mycobacterial infection require extensive investigation. Recent reports highlighted the role of pathogenic mycobacteria in modulating the transcriptional profile of host defense-associated genes by influencing diverse epigenetic factors [25, 26]. Although the H4K20me3 mark is implicated during inflammation, deposition of the H4K20me1 signature during Mtb infection on immune genes is undefined [27]. Hence, H4K20me1-associated methyltransferase SET8 was analyzed to explore the epigenetic regulation of inflammation during Mtb infection. Primary murine macrophages infected with Mtb were found to display elevated levels of SET8 and H4K20me1 (Figure 1A, left panel). Corroborating this, cells from lung tissue of Mtb-infected mice showed increased expression of SET8 (Figure 1A, right panel). Further, siRNA-mediated interference of *Setd8* transcripts resulted in reduced expression of Mtb-induced M2-phenotypic markers (*Arg1, Il-10, Il-13, Il-4,* and secreted interleukin 10 [IL-10]) (Figure 1B; Supplementary Figure 1*A* and 1*B*) and ARGINASE 1 (Supplementary Figure 1*C*) with concomitant upregulation of M1 phenotypic markers (interleukin 1β [IL-1β], interleukin 12 [IL-12], and tumor necrosis factor α [TNF-α]) (Figure 1B). Thus, the data indicate an important role of SET8 in promoting anti-inflammatory responses during mycobacterial infection.

Pathogenic Mtb evolved mechanisms to escape or suppress apoptosis [23, 28]. However, the molecular events involved in such processes are not clear. Of note, SET8 is suggested to suppress apoptosis during stress [14]. In this context, we sought to assess the impact of SET8 in regulating TNF-related apoptosis inducing ligand (TRAIL)–induced apoptosis during mycobacterial infection. The ability of TRAIL to induce apoptosis was assessed using annexin V staining by the confocal assay. We demonstrated that Mtb failed to inhibit TRAIL-induced apoptosis in SET8-depleted macrophages (Figure 1C). Importantly, *Setd8* siRNA-transfected macrophages showed reduced intracellular Mtb colony-forming units (CFUs) (Figure 1D), whereas SET8 over expression-transfected macrophages displayed increased intracellular Mtb CFUs (Supplementary Figure 1D). This evidence strongly conveys the significance of SET8 in regulating inflammation and apoptosis within the macrophages and its assistance in intracellular mycobacterial survival.

Epigenetic Regulation of Host Reductases NQO1 and TRXR1

A recent study based on short hairpin RNA screening revealed the importance of host oxidoreductase NQO1 in regulating in vitro intracellular survival of vaccine strain *M. bovis* BCG by modulating inflammatory responses [16]. However, the status

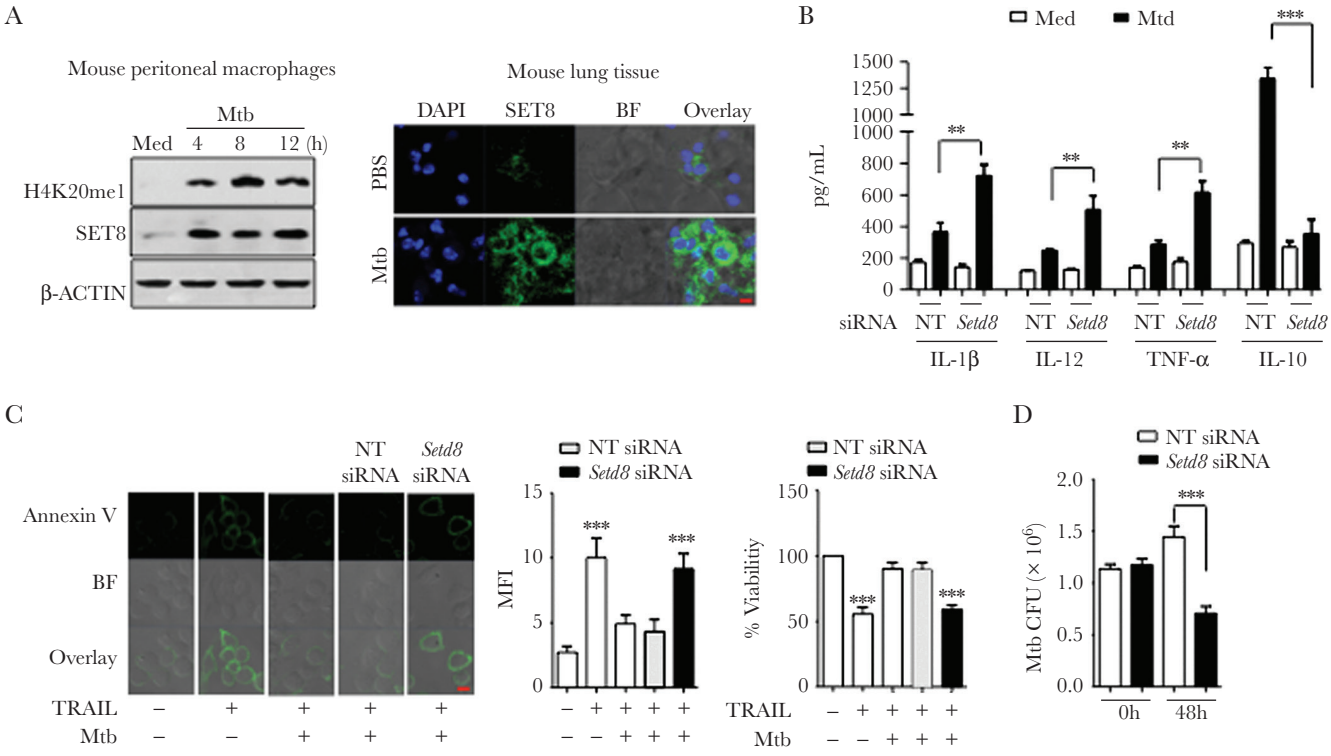


Figure 1. Regulatory role of SET8 in promoting alternative M2 macrophage phenotype and limiting apoptosis during mycobacterial infection. *A,* Mouse peritoneal macrophages were infected with *Mycobacterium tuberculosis* (Mtb) for the indicated time points Immunoblot analysis was performed for H4K20me1 and SET8 *(left panel).* Representative IF images of SET8 in the cryosections of lung tissue isolated from mice challenged with phosphate-buffered saline or Mtb (n = 6, each group) *(right panel). B,* Murine RAW264.7 macrophages were transiently transfected with nontargeting (NT) small interfering RNA (siRNA) or *Setd8* siRNA followed by Mtb infection for 12 hours. M1 (tumor necrosis factor α [TNF-α], interleukin 1β [IL-1β], and interleukin 12 [IL-12]) and M2 (interleukin 10 [IL-10]) phenotypic markers were analyzed by enzyme-linked immunosorbeant assay. *C,* TNF-related apoptosis-inducing ligand-induced apoptosis was assessed in NT siRNA or *Setd8* siRNA-transfected murine RAW264.7 macrophages by annexin V staining upon Mtb infection for 12 hours, and based on the immunofluorescence images, MFIs were calculated (n = 200 cells, each treatment). Percentage viability was determined by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. *D,* Nontargeting siRNA or *Setd8* siRNA-transfected murine peritoneal macrophages were infected with Mtb for the indicated time points, and colony-forming units (CFUs) were analyzed. All data represent the mean ± SEM for 6 values from 3 independent experiments, $^{**}P < .005$; $^{***}P < .0001$ (1-way analysis of variance). The cells were infected with bacteria at multiplicity of infection (MOI) 1:10 in all of the experiments, except at MOI 1:1 in CFU experiments. Scale bar: 5 μm. Abbreviations: BF, bright field; CFU, colony-forming unit; DAPI, 4′,6-diamidino-2-phenylindole; IF, immunofluorescence; IL-1β, interleukin 1β; IL-10, interleukin 10; IL-12, interleukin 12; Med, medium; MFI, mean fluorescence intensity; Mtb, *Mycobacterium tuberculosis*; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); NT, nontargeting; OE, overexpression. PBS, phosphate-buffered saline; siRNA, small interfering RNA; TNF-α, tumor necrosis factor α; TRAIL, TNF-related apoptosis-inducing ligand; WT, wild type.

and regulation of a select set of reductases during infection with virulent strains of mycobacteria remain obscure. In this perspective, we set out to delineate roles for reductases NQO1 and TRXR1 during virulent Mtb infection. Interestingly, TRXR1 is a well-established negative regulator of apoptosis [19]. In this context, both NQO1 and TRXR1 displayed a robust increase at the transcript and protein levels in conjunction with their increased enzyme activity in Mtb-infected macrophages (Supplementary Figure 2*A*–*C*). Further, analysis of the redox state of the enzymes in Mtb-infected macrophages by using a thiol reactive compound, 4-acetamido-4'-maleimidylstilbene-2,2'-disulfonic acid, disodium salt, showed increased levels of reduced form of TRXR1 and NQO1 (Supplementary Figure 2*D*). In addition, cells from lungs of Mtb-infected mice showed increased expression of NQO1 and TRXR1 (Figure 2A). Recent reports suggest the presence of epigenetic impressions on genes of several immune effectors [8, 10]. Having established that SET8 modulates inflammatory and apoptotic responses during mycobacterial infection, we sought to investigate the role of SET8 in regulating the expression of NQO1 and TRXR1. *Mycobacterium tuberculosis*–infected macrophages demonstrated the recruitment of SET8 at promoters of *Nqo1* and *Txnrd1* as well as H4K20me1 enrichment on the promoters of these genes (Figure 2B). Substantiating this observation, SET8-specific siRNA-transfected macrophages demonstrated reduced expression of Mtb-induced NQO1 and TRXR1 at the protein and transcript levels (Figure 2C; Supplementary Figure 2*E*), emphasizing the fact that SET8 catalytic activity is required to regulate target gene expression. To explore the role of its catalytic activity, macrophages that were transfected with SET8 catalytically inactive construct and then infected with Mtb displayed decreased expression of NQO1 and TRXR1 (Figure 2D). These results underscore the ability of Mtb-triggered SET8 to regulate the expression of NQO1 and TRXR1.

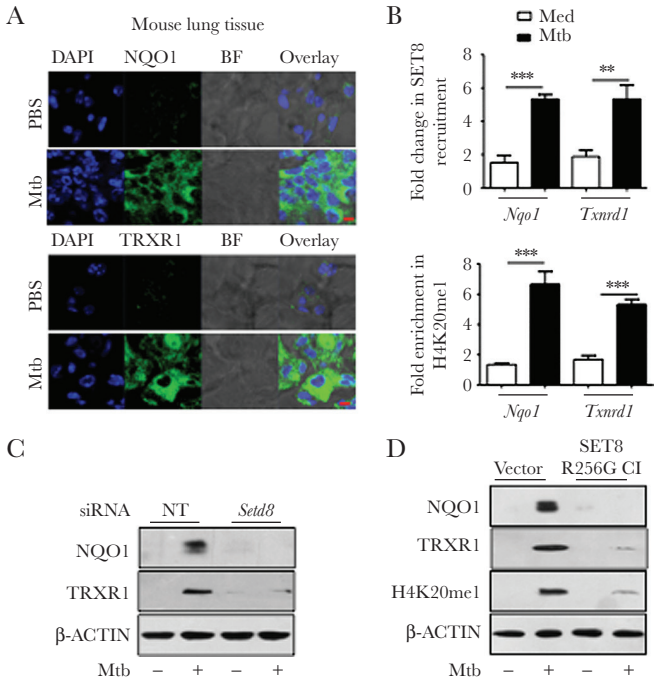


Figure 2. SET8 mediates H4K20me1 epigenetic modification on *Mycobacterium tuberculosis* (Mtb)–triggered NQO1 and TRXR1 promoters. *A,* Representative immunofluorescence images of NQO1 (upper panel) and TRXR1 (lower panel) in the cryosections of lung tissue isolated from mice challenged with phosphate-buffered saline or Mtb (n = 6, each group). *B,* The recruitment of SET8 (upper panel) and fold enrichment of H4K20me1 (lower panel) at mouse *Nqo1* and *Txnrd1* promoters were evaluated by chromatin immunoprecipitation assay upon Mtb infection for 12 hours*C* and *D*, Nontargeting (NT) small interfering RNA (siRNA) or *Setd8* siRNA transfected (*C*) and SET8 R256G CI construct transfected (*D*) murine RAW264.7 macrophages were infected with Mtb for 12 hours. After infection, protein levels of NQO1, TRXR1, and H4K20me1 were analyzed by immunoblotting. All data represent the mean ± SEM for 6 values from 3 independent experiments. $^{**}P < .005$; $^{***}P < .0001$ (*t* test). All blots are representative of 3 independent experiments. The cells were infected with bacteria at multiplicity of infection 1:10 in all experiments. Scale bar: 5 µm. Abbreviations: BF, bright field; CI, catalytically inactive; DAPI, 4′,6-diamidino-2-phenylindole; IF, immunofluorescence; Med, medium; Mtb, *Mycobacterium tuberculosis*; PBS, phosphate-buffered saline; NT, nontargeting; siRNA, small interfering RNA.

NQO1 Facilitates M2 Macrophage Polarization

The contribution of NQO1 in suppressing inflammation during cancer is well established [17, 18, 29]. However, NQO1 involvement in regulating inflammatory responses during mycobacterial infection needs further investigation. Interestingly, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α) stabilized by NQO1 is known to modulate inflammatory responses [30, 31]. Therefore, we examined the influence of PGC1-α in regulating Mtb-triggered inflammatory responses. *Mycobacterium tuberculosis*–infected macrophages displayed increased expression of PGC1-α (Supplementary Figure 3*A*), and interaction between NQO1 and PGC1-α was validated by immune pull-down assay (Supplementary Figure 3*B*). Further, Mtb-induced expression of M2 phenotypic markers was abrogated in *Ppargc1a*-specific siRNA-transfected macrophages (Figure 3A; Supplementary Figure 3*C*). In line with this observation, PGC1-α–depleted macrophages exhibit substantially elevated expression of M1 phenotypic markers compared with that of Mtb-infected macrophages (Figure 3A). These results suggest that Mtb-induced PGC1-α promotes M2 macrophage polarization.

Again, dicoumarol (NQO1 inhibitor)–treated macrophages abolished NQO1 and PGC1-α interaction, which indicates the essential role of NQO1 reductase activity for binding with PGC1-α (Figure 3B). To examine the role of NQO1 reductase activity in vivo, mice were challenged with Mtb, and the infection was allowed to establish for 4 weeks. Further, mice were administered dicoumarol (30 mg/kg) every other day up to 16 days. *Mycobacterium tuberculosis*–infected mice treated with dicoumarol demonstrated reduced secretion of anti-inflammatory IL-10 (Figure 3C) and increased secretion of proinflammatory TNF-α, IL-1β, and IL-12 (Figure 3D) in the lungs and spleen. In summary, these results suggest the direct involvement of PGC1-α, stabilized by NQO1 reductase activity, in directing the polarization of M2 macrophages in an in vivo mouse tuberculosis model.

Mycobacteria-Induced TRXR1 Regulates Apoptosis

TRXR1 is known to negatively regulate apoptosis by inhibiting apoptosis signal-regulating kinase 1 (ASK1) activity upon their interaction [20]. However, the contribution of TRXR1 in regulating apoptosis during mycobacterial infection is unexplored. At the outset, TRXR1 and ASK1 interaction was validated by co-immunoprecipitation (Supplementary Figure 4*A*). Next, the activity of ASK1 to induce apoptosis was analyzed through annexin V staining and cleavage of CASPASE-3. The ability of ASK1 to induce apoptosis was compromised in Mtb-infected

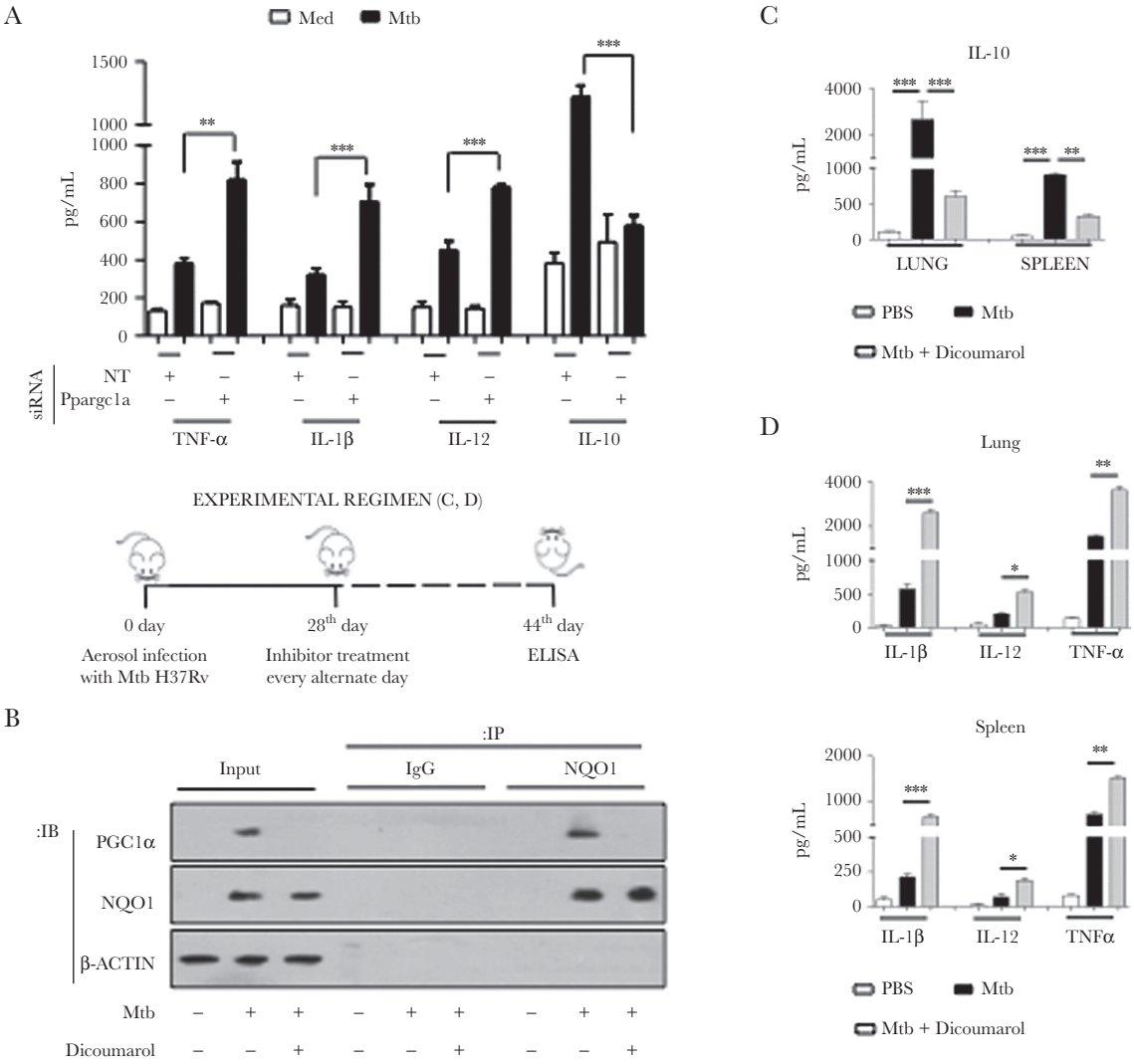


Figure 3. NQO1 synergizes with PGC1-α to facilitate the alternative M2 macrophage phenotype. *A*, Murine RAW264.7 macrophages transfected with nontargeting (NT) small interfering RNA (siRNA) or *Ppargc1a* siRNA were infected with *Mycobacterium tuberculosis* (Mtb) for 12 hours. Transcript analysis of M1 and M2 phenotypic markers was done by enzyme-linked immunosorbent assay (ELISA). *B*, Anti-NQO1 immunoprecipitation (IP) was performed in mouse peritoneal macrophages pretreated with dicoumarol (NQO1 inhibitor) followed by Mtb infection for 12 hours, and immunoprecipitates were subjected to immunoblotting using the indicated antibodies. *C* and *D*, Lungs and spleens of Mtb-infected or dicoumarol-treated mice were homogenized in sterile phosphate-buffered saline, and supernatant was analyzed for interleukin 10 (*C*), interleukin 1β, interleukin 12, and tumor necrosis factor α (*D*) by ELISA. All data represent the mean ± SEM for 6 values from 3 independent experiments. $^{**}P < .005$; $^{***}P < .0001$ (1-way analysis of variance). Blots are representative of 3 independent experiments. The cells were infected with bacteria at multiplicity of infection 1:10 in all of the experiments except in vivo experiments where approximately 200 bacilli were aerosolized. Abbreviations: ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; IL-10, interleukin 10; IL-12, interleukin 12; IP, immunoprecipitation; Med, medium; Mtb, *Mycobacterium tuberculosis*; NT, nontargeting; PBS, phosphate-buffered saline; siRNA, small interfering RNA; TNF-α, tumor necrosis factor α.

macrophages. However, macrophages depleted of TRXR1 during mycobacterial infection failed to inhibit ASK1-induced apoptosis (Figure 4A and 4B).

Auranofin has been identified as a highly effective inhibitor of selenoprotein thioredoxin reductase in vitro as well as in vivo [19, 32]. Therefore, we analyzed the effect of auranofin on ASK1-induced apoptosis during Mtb infection. Interestingly, Mtb-suppressed apoptosis in ASK1-transfected macrophages was rescued in auranofin-treated cells (Figure 4C and 4D). Taken together, these results demonstrate the ability of TRXR1 to modulate apoptosis during the course of Mtb infection.

SET8-Mediated NQO1 and TRXR1 Aid *Mycobacterium tuberculosis* Survival

To examine the potential of NQO1 and TRXR1 as targets for host-directed therapeutics, a mouse TB model was used. Importantly, mice aerosolized with Mtb and then treated with dicoumarol (30 mg/kg) (Figure 5A) and auranofin (2 mg/kg) (Figure 5B) displayed a 10-fold reduction of Mtb CFUs in their lungs and spleen [19, 33]. Further, in vitro CFU analysis suggested an additive effect of dicoumarol and auranofin because there was an appended reduction in Mtb CFUs (Supplementary Figure 5*A*). Furthermore, histopathological evaluation of

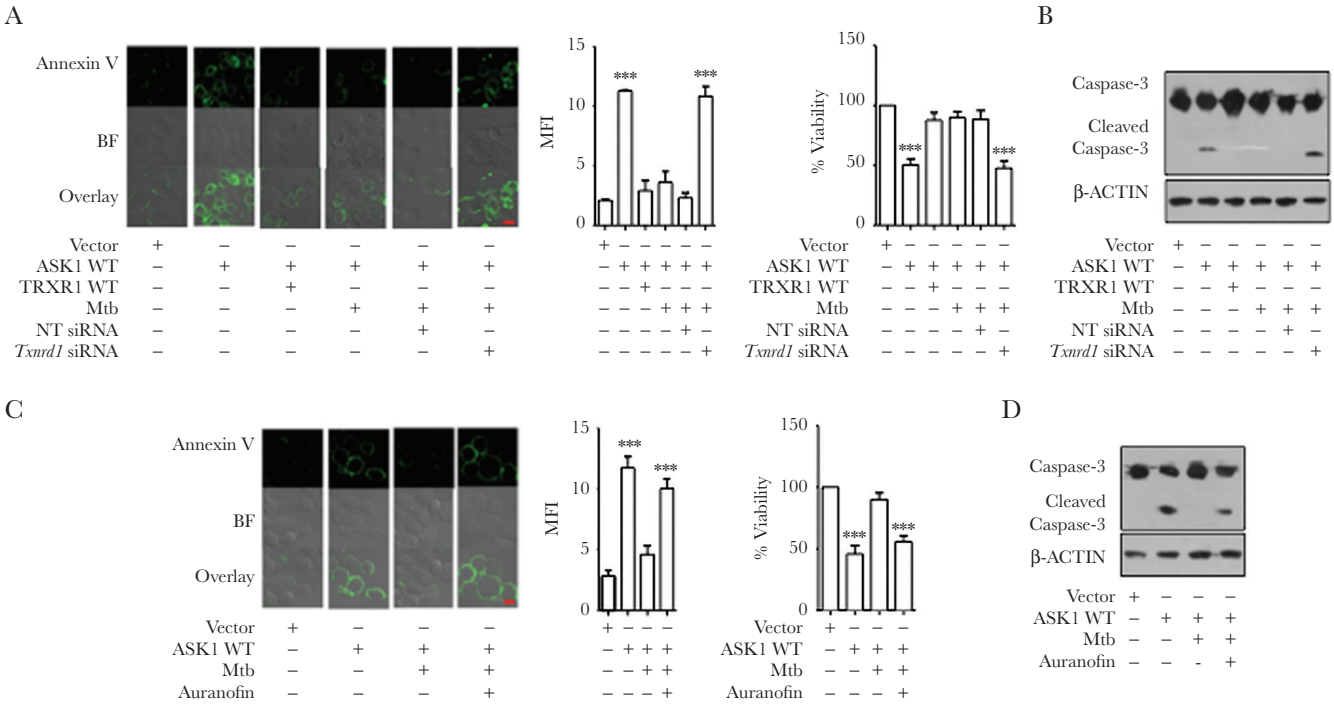
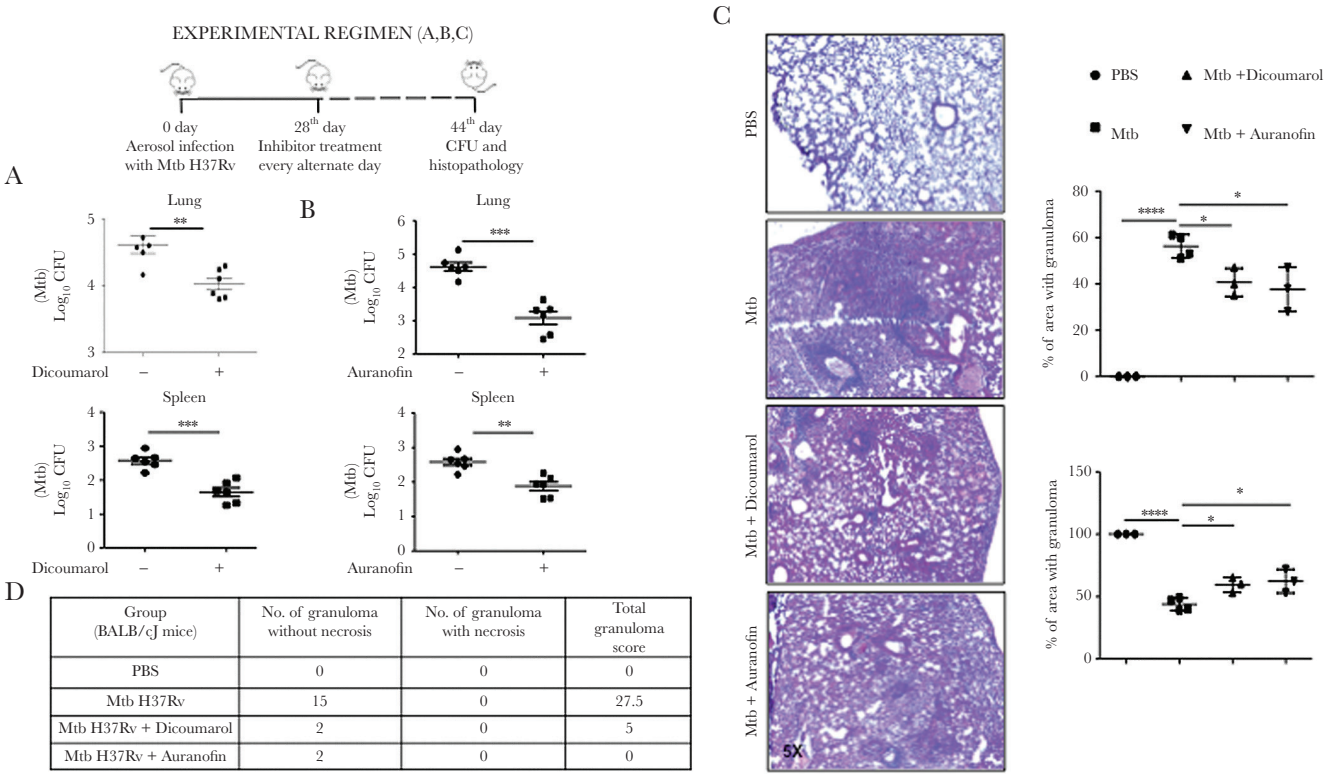


Figure 4. TRXR1 finetunes ASK1-induced apoptosis. *A* and *B*, Murine RAW264.7 macrophages transfected with indicated constructs or nontargeting (NT) small interfering RNA (siRNA) or *Txnrd1* siRNA were infected with Mycobacterium tuberculosis (*Mtb*). Annexin V staining was performed, and based on the immunofluorescence (IF) images, mean fluorescence intensities (MFIs) were calculated (n = 200 cells, each treatment). Percentage viability was determined by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay (*A*) and cleaved CASPASE-3 was analyzed by immunoblotting (*B*). *C* and *D*, Murine RAW264.7 macrophages transfected with indicated constructs were pretreated with auranofin (TRXR1 inhibitor) followed by Mtb infection (12 hours). Annexin V staining was performed, and based on the IF images, MFIs were calculated (n = 200 cells, each treatment). Percentage viability was determined by MTT assay (*C*). Cleaved CASPASE-3 was analyzed by immunoblotting (*D*). All data represent the mean ± SEM for 6 values from 3 independent experiments. ***$P < .0001$ (1-way analysis of variance). All blots are representative of 3 independent experiments. The cells were infected with bacteria at multiplicity of infectoin 1:10 in the experiments. Scale bar: 5 µm. Abbreviations: BF, bright field; IF, immunofluorescence; MFI, mean fluorescence intensity; Mtb, *Mycobacterium tuberculosis*; NT, nontargeting; siRNA, small interfering RNA; WT, wild type.

percentage of area with granuloma, percentage of area free of lesion (Figure 5C), and granuloma scoring (Figure 5D) strongly supported reduced mycobacterial pathologies. Dicoumarol did not affect the growth of Mtb in 7H9 broth, whereas auranofin showed a very modest inhibition in the growth pattern of Mtb in 7H9 broth. Neither of the compounds showed any toxicity on mouse macrophages (Supplementary Figure 5*B* and 5*C*). To further assess whether the killing of Mtb is due to the inhibition of TRXR1 in macrophages by auranofin and not by direct mycobactericidal activity, Mtb CFUs in mouse macrophages were assessed upon treatment with TRAIL in the presence and absence of auranofin. There was increased reduction in CFUs in the presence of TRAIL and auranofin compared with Mtb treatment alone, thereby accentuating the role of TRXR1 in mycobacterial survival (Supplementary Figure 5*D*). Altogether, these findings revealed the therapeutic value of host-derived reductases like NQO1 and TRXR1 in tuberculosis pathogenesis.

Mycobacteria-Triggered miR-30e-3p Regulates NQO1 and TRXR1

After establishing the contribution of SET8 in regulating Mtb-induced expression of NQO1 and TRXR1 (Figure 2), we sought to investigate the SET8 regulators during the course of Mtb infection. Interestingly, a previous study suggested a role for CDT2 (E3 ubiquitin ligase) in restricting SET8 during cell cycle progression [34]. In this perspective, primary macrophages infected with Mtb displayed reduced levels of CDT2 protein, but its transcript levels remained unchanged (Figure 6A). These data strongly advocated the possibility of posttranscriptional modification of CDT2 such as that regulated by microRNAs (miRNAs). Extensive bioinformatic analysis (Target Scan, miRanda, miRWalk 2.0) identified *Dtl*, encoding for CDT2, as a potential target of miR-30e-3p. To examine a possible role for miR-30e-3p, macrophages infected with Mtb were assessed for the expression of the selected miRNA. It was found that mouse macrophages elicit robust expression of miR-30e-3p upon mycobacterial infection (Figure 6B). Importantly, Mtb infection or transfection of mouse macrophages with miR-30e-3p mimics markedly reduced WT *Dtl* 3'UTR luciferase activity, whereas a mutant for miR-30e-3p binding at *Dtl* 3'UTR did not display any marked reduction in luciferase activity (Figure 6C). This strongly suggested *Dtl* as a direct target of miR-30e-3p. Notably, depletion of miR-30e-3p by using specific inhibitor in Mtb-infected macrophages showed diminished expression of SET8 and substantially decreased expression of NQO1 and TRXR1



D

Group (BALB/cJ mice)	No. of granuloma without necrosis	No. of granuloma with necrosis	Total granuloma score
PBS	0	0	0
Mtb H37Rv	15	0	27.5
Mtb H37Rv + Dicoumarol	2	0	5
Mtb H37Rv + Auranofin	2	0	0

Figure 5. NQO1 and TRXR1 promote mycobacterial survival and metastasis. *A* and *B*, Mice were aerosolized with 200 bacilli of Mycobacterium tuberculosis (Mtb) or Mtb in combination with dicoumarol (NQO1 inhibitor) (*A*) or auranofin (TRXR1 inhibitor) (*B*). Colony-forming units were evaluated on the $\log_{10}$ scale in lungs and spleens of mice (n = 6, each group). Paraformaldehyde-fixed mice lung tissue sections (5 µm) were subjected to hematoxylin and eosin staining, and microscopic analysis was performed to evaluate tuberculosis granuloma architecture. *C*, Percentage of area with granuloma and percentage of area free of lesion were calculated. *D*, The table represents histopathological evaluation and granuloma scoring. All data represent the mean ± SEM from 6 mice. $^{**}P < .005$; $^{***}P < .0001$ (*t* test). Abbreviations: CFU, colony-forming unit; Mtb, *Mycobacterium tuberculosis*; PBS, phosphate-buffered saline.

at the transcript and protein levels (Figure 6D; Supplementary Figure 6*A*). Further, mouse macrophages transfected with miR-30e-3p mimic showed an appreciable increase in Mtb CFUs compared with macrophages transfected with negative control mimic (Supplementary Figure 6*B*). Together these results unveil the role of miR-30e-3p in the regulation of *Dtl* and SET8-mediated expression of NQO1 and TRXR1 during Mtb infection and its survival.

Regulation of SET8-Responsive NQO1 and TRXR1

The FoxO family of transcription factors are known to coordinate with epigenetic modulators to regulate target gene expression [15, 35]. Thus, we investigated the role of FoxO3a in modulating SET8 responses. *Mycobacterium tuberculosis*–infected macrophages clearly indicated the interaction of SET8 with FoxO3a by immune pull-down experiments (Figure 7A). Interestingly, Mtb-infected macrophages clearly demonstrate the recruitment of FoxO3a at the promoters of *Nqo1* and *Txnrd1* (Supplementary Figure 7*A*). Although the MST1-FoxO pathway is associated with various cellular responses [36], the status of the MST1-FoxO3a signaling cascade during mycobacterial infection remains to be explored. Thus, we investigated the possible role of the MST1/2-FoxO3a axis in regulating Mtb-induced NQO1 and TRXR1 expression. In this context, siRNA medaited depletion of MST1/2 in murine macrophages elicited decreased expression of NQO1 and TRXR1 even upon mycobacterial infection (Figure 7B). Supporting this observation, Mtb-induced recruitment of FoxO3a on the promoters of NQO1 and TRXR1 was compromised in MST1/2-deficient macrophages (Figure 7C). In vitro CFU analysis of Mtb in cells transfected with *Foxo3a* siRNA showed marked reduction (Supplementary Figure 7*C*). These results indicate that MST1/2-FoxO3a axis plays an important role in regulating the expression of SET8-responsive NQO1 and TRXR1.

Evaluation of the Signaling Axis and Its Regulation in Human Peripheral Blood Mononuclear Cells

To further correlate our findings in the mice model with humans, we assessed the protein levels of TRXR1, NQO1, SET8, PGC1-α, H4K20me1, and CDT2 and the transcript levels of *TXNRD1*, *NQO1*, *SETD8*, *PPARGC1A*, and *DTL* in human PBMCs infected with Mtb. Corroborating our earlier results, we observed an increase in the protein and transcript levels of TRXR1, NQO1, SET8, and PGC1-α. There was also

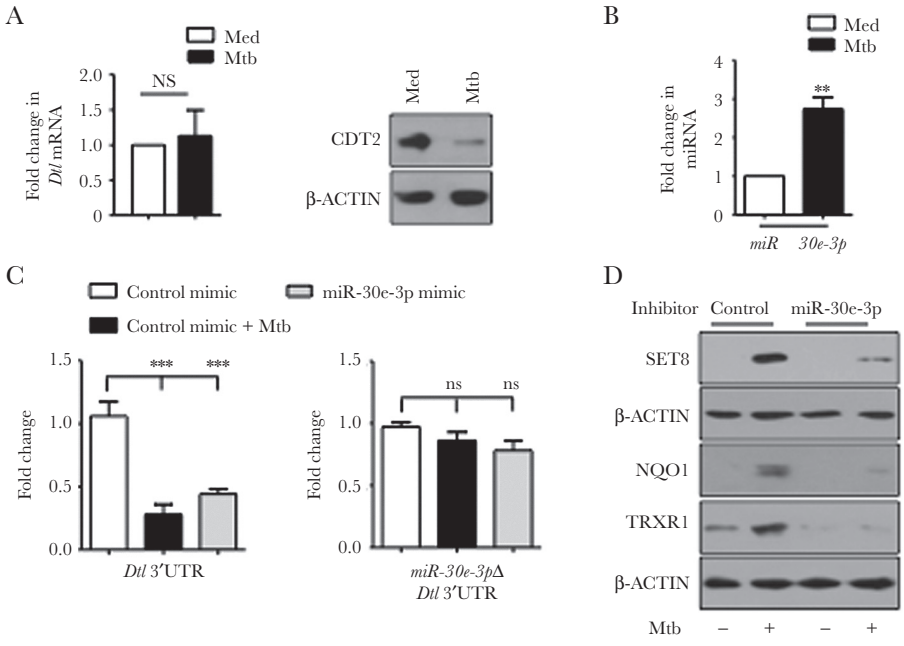


Figure 6. miR-30e-3p regulates NQO1 and TRXR1 by stabilizing SET8. *A*, Mouse peritoneal macrophages were infected with *Mycobacterium tuberculosis* (Mtb) for 12 hours. CDT2 was analyzed at the transcript level and protein level by quantitative real-time reverse-transcription polymerase chain reaction (RT-PCR) and immunoblotting, respectively. *B*, Peritoneal macrophages were infected with Mtb, and quantitative real-time RT-PCR analysis was performed on total RNA isolated using miR-30e-3p–specific primers. *C*, Murine RAW264.7 macrophages were transfected with wild-type or mutant (miR-30e-3pΔ) *Dtl* 3′UTR luciferase construct in combination with miR-30e-3p mimic or Mtb where indicated. Luciferase assay was performed. *D*, Murine RAW264.7 macrophages were transiently transfected with nontargeting inhibitor or miR-30e-3p inhibitor followed by Mtb infection for 12 hours. Protein levels of NQO1, TRXR1, and SET8 were analyzed by immunoblotting. All data represent the mean ± SEM for 6 values from 3 independent experiments. $^{**}P < .005$; $^{***}P < .0001$ (1-way analysis of variance for C and *t* test). All blots are representative of 3 independent experiments. The cells were infected with bacteria at multiplicity of infection 1:10 in all of the experiments. Abbreviatons: Med, medium; miRNA, microRNA; mRNA, messenger RNA; Mtb, *Mycobacterium tuberculosis*; NS, not significant.

an increase in the levels of H4K20me1 brought about by SET8. There was a decrease in the protein level of CDT2 but no appreciable change in its transcript level, thereby providing aevidence for posttranscriptional regulation brought about by miRNA (Supplementary Figure 8*A* and 8*B*). Analysis of the levels of miR-30e-3p in human PBMCs infected with Mtb indicated substantial upregulation (Supplementary Figure 8*C*). We evaluated the ability of TRXR1 to regulate apoptosis by assessing cleaved CASPASE-3 levels in human PBMCs treated with TRAIL in the presence or absence of auranofin. TRAIL-induced cleaved CASPASE-3 levels were reduced upon infection with Mtb, but the ability of Mtb to inhibit apoptosis was compromised upon treating the cells with auranofin (Supplementary Figure 8*D*). Further, we analyzed the role of NQO1 in regulating the M2 macrophage phenotype in human PBMCs. Upon treatment with dicoumarol, PBMCs showed decreased levels of IL-10 as opposed to elevated levels of TNF-α, IL-1β, and IL-12 when compared with their respective levels in case of Mtb infection alone. These experiments further validate our finding that elevated levels of miR-30e-3p and SET8 regulate the expression of TRXR1 and NQO1, which, in turn, play cardinal roles in regulating apoptosis and alternative M2 phenotype, respectively, upon Mtb infection in macrophages.

DISCUSSION

The notorious rise of mycobacteria as a major global threat has been aided by the pathogen's extended generation time, its capacity for dormancy, its ability to wield host responses, and growing drug resistance. In this respect, Mtb-driven granuloma formation and its dynamics has become a topic of focused study, which may support the development of more effective antituberculosis drugs [37, 38]. At present, available chemotherapy for tuberculosis has a limited impact on patients due to the complexity of this disease and the ability of Mtb to hijack host defense mechanisms, which include apoptosis and inflammation [22, 23, 28, 39]. At this point, the mechanism of host-directed therapeutics that target important host cellular signaling nodes involved in promoting mycobacterial survival seems to be an attractive target [1, 40, 41]. Thus, these fine immune regulatory host networks, which determine the fate of Mtb infection, require an extensive investigation. In this context, FoxO transcription factor–mediated defenses against oxidative stress, a major deterrent of mycobacterial persistence, have been well established [42–44]. In this study, we identified the role of the MST1/2-FoxO3a axis in regulating the expression of NQO1 and TRXR1 in Mtb-infected macrophages. Simultaneously, we have established that SET8, a histone methyltransferase

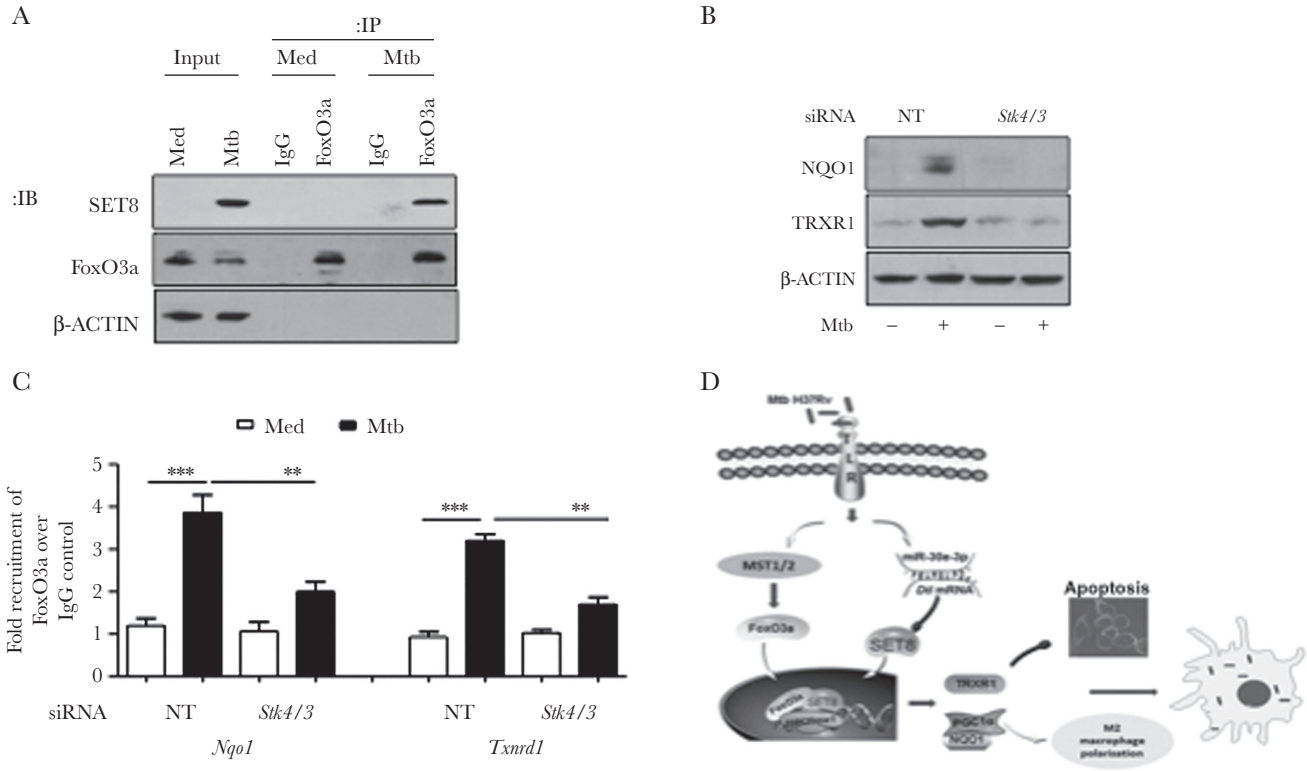


Figure 7. SET8-mediated expression of NQO1 and TRXR1 is regulated by the MST1/2-FoxO3a axis. A, Anti-FoxO3a immunoprecipitation (IP) was performed in Mycobacterium tuberculosis (Mtb)–infected (12 hour) mouse peritoneal macrophages, and immunoprecipitates were subjected to immunoblotting (IB) using the indicated antibodies. *B*, Murine RAW264.7 macrophages were transiently transfected with *Mst1/2* (*Stk4/3*) small interfering RNA (siRNA) followed by Mtb infection for 12 hours, and protein expression of NQO1 and TRXR1 was analyzed by immunoblotting. *C*, The recruitment of FoxO3a at mouse *Nqo1* and *Txnrd1* promoters in nontargeting (NT) siRNA and *Stk4/3* siRNA transiently transfected murine RAW264.7 macrophages upon infection with Mtb (12 hours) was evaluated by chromatin immunoprecipitation assay. *D*, Model, Pathogenic mycobacteria hijack SET8 to epigenetically reprogram host inflammatory and apoptotic responses in an MST1/2-FoxO3a–regulated NQO1- and TRXR1-dependent manner. All data represent the mean ± SEM for 6 values from 3 independent experiments. $^{**}P < .005$; $^{***}P < .0001$ (1-way analysis of variance). All blots are representative of 3 independent experiments. The cells were infected with bacteria at multiplicity of infection 1:10 in all of the experiments. Abbreviations: IB, immunoblotting; IgG, immunoglobulin G; IP, immunoprecipitation; Med, medium; Mtb, *Mycobacterium tuberculosis*; NT, nontargeting.

(H4K20me1), is an epigenetic regulator of NQO1 and TRXR1, supporting the notion of the FoxO family of transcription factors regulating the expression of its target genes through the recruitment of epigenetic modifiers [15, 35].

Whereas the evidence for the role of host-derived NQO1 in negatively regulating inflammation in mycobacteria-infected mice lungs was further strengthened, in the current study; TRXR1 was found to curb apoptosis in this scenario. Furthermore, reduced Mtb CFUs in the lungs and spleen of mice treated with dicoumarol (NQO1 inhibitor) and auranofin (TRXR1 inhibitor) provide strong evidence for the importance of these host reductases in assisting Mtb survival in the mouse tuberculosis model. Interestingly, auranofin was shown to inhibit bacterial TrxR of *Staphylococcus aureus* and *M. tuberculosis*. Due to its ability to inhibit both bacterial and host TrxR, auranofin may emerge as a strong antimicrobial candidate. Further, the role of auranofin during in vivo Mtb pathogenesis as a function of bacterial CFUs was unexplored [45]. Hence, our findings have further strengthened the evidence for auranofin's role in regulating host TRXR1 during Mtb pathogenesis. Additionally, lung histopathology of dicoumarol- and auranofin-treated mice showed reduced severity of tuberculosis through a granuloma analysis. Our results related to NQO1 are complemented by recent findings of Li and colleagues, who demonstrated that NQO1 facilitates intracellular mycobacterial survival [16].

Validation of the functional role of SET8 in vivo was hampered by the fact that mice deficient in SET8 are embryonically lethal [46, 47]. Therefore, extensive in vitro experiments were used to investigate the impact of SET8 on intracellular Mtb CFUs. Interestingly, we observed that SET8 facilitated intracellular mycobacterial survival, which indicated a close association of SET8 with tuberculosis pathogenesis. Keeping in mind the reversible nature of histone methylation, development and validation of a SET8-specific inhibitor would thus provide more insights into understanding the role of SET8 during Mtb infection. Several reports also suggest a crucial role of miRNA in mediating posttranscriptional regulation of genes during mycobacterial infection [48–50]. In the current context, Mtb-induced miR-30e-3p was found to target *Dtl* (a negative regulator of SET8), thus stabilizing SET8 and facilitating the

upregulation of NQO1 and TRXR1. Analogous observations in human PBMCs infected with Mtb further substantiated the premise of this study (Supplementary Figure 8).

In summary, our findings identified the host reductases NQO1 and TRXR1 as novel targets of Mtb pathogenesis that assist mycobacterial growth in a mouse tuberculosis model. Mycobacteria-induced NQO1 and TRXR1 are linked to the control of 2 fundamental host defense strategies—namely, inflammatory cytokine responses and apoptosis (Figure 7D). Importantly, NQO1 and TRXR1 were established as bonafide targets of Mtb-induced SET8. Thus, reinforcing host innate immune responses by the deregulation of Mtb-modulated SET8-NQO1/TRXR1 offers encouraging adjuvants to the existing frontline mycobacterial drugs.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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V. S. and K. N. B. conceived and designed all experiments. V. S., K. N. B., P. P., K. M., and S. M. B. wrote the manuscript. V. S., P. P., and S. M. B. performed the experiments and analyzed data. V. S. and R. S. R. performed in vivo experiments of mycobacterial colony-forming units.

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Kaufmann SH, Lange C, Rao M, et al. Progress in tuberculosis vaccine development and host-directed therapies—a state of the art review. Lancet Respir Med **2014**; 2:301–20.
2. Arcila ML, Sánchez MD, Ortiz B, Barrera LF, García LF, Rojas M. Activation of apoptosis, but not necrosis, during *Mycobacterium tuberculosis* infection correlated with decreased bacterial growth: role of TNF-alpha, IL-10, caspases and phospholipase A2. Cell Immunol **2007**; 249:80–93.
3. Deffert C, Cachat J, Krause KH. Phagocyte NADPH oxidase, chronic granulomatous disease and mycobacterial infections. Cell Microbiol **2014**; 16:1168–78.
4. Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy is a defense mechanism inhibiting BCG and *Mycobacterium tuberculosis* survival in infected macrophages. Cell **2004**; 119:753–66.
5. Nambi S, Long JE, Mishra BB, et al. The oxidative stress network of *Mycobacterium tuberculosis* reveals coordination between radical detoxification systems. Cell Host Microbe **2015**; 17:829–37.
6. Huang Z, Luo Q, Guo Y, et al. *Mycobacterium tuberculosis*–induced polarization of human macrophage orchestrates the formation and development of tuberculous granulomas in vitro. PLoS One **2015**; 10:e0129744.
7. Álvarez-Errico D, Vento-Tormo R, Sieweke M, Ballestar E. Epigenetic control of myeloid cell differentiation, identity and function. Nat Rev Immunol **2015**; 15:7–17.
8. Holla S, Balaji KN. Epigenetics and miRNA during bacteria-induced host immune responses. Epigenomics **2015**; 7:1197–212.
9. Yoshida K, Maekawa T, Zhu Y, et al. The transcription factor ATF7 mediates lipopolysaccharide-induced epigenetic changes in macrophages involved in innate immunological memory. Nat Immunol **2015**; 16:1034–43.
10. Fang TC, Schaefer U, Mecklenbrauker I, et al. Histone H3 lysine 9 di-methylation as an epigenetic signature of the interferon response. J Exp Med **2012**; 209:661–9.
11. Holla S, Prakhar P, Singh V, et al. MUSASHI-Mediated expression of JMJD3, a H3K27me3 demethylase, is involved in foamy macrophage generation during mycobacterial infection. PLoS Pathog **2016**; 12:e1005814.

12. Satoh T, Takeuchi O, Vandenbon A, et al. The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. Nat Immunol **2010**; 11:936–44.
13. Schliehe C, Flynn EK, Vilagos B, et al. The methyltransferase Setdb2 mediates virus-induced susceptibility to bacterial superinfection. Nat Immunol **2015**; 16:67–74.
14. Dhami GK, Liu H, Galka M, et al. Dynamic methylation of Numb by Set8 regulates its binding to p53 and apoptosis. Mol Cell **2013**; 50:565–76.
15. Serrano L, Martínez-Redondo P, Marazuela-Duque A, et al. The tumor suppressor SirT2 regulates cell cycle progression and genome stability by modulating the mitotic deposition of H4K20 methylation. Genes Dev **2013**; 27:639–53.
16. Li Q, Karim AF, Ding X, et al. Novel high throughput pooled shRNA screening identifies NQO1 as a potential drug target for host directed therapy for tuberculosis. Sci Rep **2016**; 6:27566.
17. Rushworth SA, MacEwan DJ, O'Connell MA. Lipopolysaccharide-induced expression of NAD(P)H:quinone oxidoreductase 1 and heme oxygenase-1 protects against excessive inflammatory responses in human monocytes. J Immunol **2008**; 181:6730–7.
18. Thapa D, Meng P, Bedolla RG, Reddick RL, Kumar AP, Ghosh R. NQO1 suppresses NF-κB-p300 interaction to regulate inflammatory mediators associated with prostate tumorigenesis. Cancer Res **2014**; 74:5644–55.
19. Fan C, Zheng W, Fu X, Li X, Wong YS, Chen T. Enhancement of auranofin-induced lung cancer cell apoptosis by selenocystine, a natural inhibitor of TrxR1 in vitro and in vivo. Cell Death Dis **2014**; 5:e1191.
20. Saitoh M, Nishitoh H, Fujii M, et al. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. EMBO J **1998**; 17:2596–606.
21. Ghorpade DS, Leyland R, Kurowska-Stolarska M, Patil SA, Balaji KN. MicroRNA-155 is required for *Mycobacterium bovis* BCG-mediated apoptosis of macrophages. Mol Cell Biol **2012**; 32:2239–53.
22. Olsen A, Chen Y, Ji Q, et al. Targeting *Mycobacterium tuberculosis* tumor necrosis factor alpha–downregulating genes for the development of antituberculous vaccines. mBio **2016**; 7:e01023–15.
23. Sly LM, Hingley-Wilson SM, Reiner NE, McMaster WR. Survival of *Mycobacterium tuberculosis* in host macrophages involves resistance to apoptosis dependent upon induction of antiapoptotic Bcl-2 family member Mcl-1. J Immunol **2003**; 170:430–7.
24. Marino S, Cilfone NA, Mattila JT, Linderman JJ, Flynn JL, Kirschner DE. Macrophage polarization drives granuloma outcome during *Mycobacterium tuberculosis* infection. Infect Immun **2015**; 83:324–38.
25. Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. Nat Rev Genet **2016**; 17:487–500.
26. Gómez-Díaz E, Jordà M, Peinado MA, Rivero A. Epigenetics of host-pathogen interactions: the road ahead and the road behind. PLoS Pathog **2012**; 8:e1003007.
27. Stender JD, Pascual G, Liu W, et al. Control of proinflammatory gene programs by regulated trimethylation and demethylation of histone H4K20. Mol Cell **2012**; 48:28–38.
28. Miller JL, Velmurugan K, Cowan MJ, Briken V. The type I NADH dehydrogenase of *Mycobacterium tuberculosis* counters phagosomal NOX2 activity to inhibit TNF-alpha-mediated host cell apoptosis. PLoS Pathog **2010**; 6:e1000864.
29. Oh GS, Kim HJ, Choi JH, et al. Pharmacological activation of NQO1 increases NAD^+ levels and attenuates cisplatin-mediated acute kidney injury in mice. Kidney Int **2014**; 85:547–60.
30. Adamovich Y, Shlomai A, Tsvetkov P, et al. The protein level of PGC-1α, a key metabolic regulator, is controlled by NADH-NQO1. Mol Cell Biol **2013**; 33:2603–13.
31. Eisele PS, Salatino S, Sobek J, Hottiger MO, Handschin C. The peroxisome proliferator-activated receptor γ coactivator 1α/β (PGC-1) coactivators repress the transcriptional activity of NF-κB in skeletal muscle cells. J Biol Chem **2013**; 288:2246–60.
32. Sachweh MC, Stafford WC, Drummond CJ, et al. Redox effects and cytotoxic profiles of MJ25 and auranofin towards malignant melanoma cells. Oncotarget **2015**; 6:16488–506.
33. Begleiter A, Leith MK, Thliveris JA, Digby T. Dietary induction of NQO1 increases the antitumour activity of mitomycin C in human colon tumours in vivo. Br J Cancer **2004**; 91:1624–31.
34. Abbas T, Shibata E, Park J, Jha S, Karnani N, Dutta A. CRL4(Cdt2) regulates cell proliferation and histone gene expression by targeting PR-Set7/Set8 for degradation. Mol Cell **2010**; 40:9–21.
35. Bakker WJ, Blázquez-Domingo M, Kolbus A, et al. FoxO3a regulates erythroid differentiation and induces BTG1, an activator of protein arginine methyl transferase 1. J Cell Biol **2004**; 164:175–84.
36. Choi J, Oh S, Lee D, et al. Mst1-FoxO signaling protects naive T lymphocytes from cellular oxidative stress in mice. PLoS One **2009**; 4:e8011.
37. Arbex MA, Varella Mde C, Siqueira HR, Mello FA. Antituberculosis drugs: drug interactions, adverse effects, and use in special situations. Part 1: first-line drugs. J Bras Pneumol **2010**; 36:626–40.
38. Arbex MA, Varella Mde C, Siqueira HR, Mello FA. Antituberculosis drugs: drug interactions, adverse effects, and use in special situations. Part 2: second line drugs. J Bras Pneumol **2010**; 36:641–56.

39. Hinchey J, Lee S, Jeon BY, et al. Enhanced priming of adaptive immunity by a proapoptotic mutant of *Mycobacterium tuberculosis*. J Clin Invest **2007**; 117:2279–88.
40. Kiran D, Podell BK, Chambers M, Basaraba RJ. Host-directed therapy targeting the *Mycobacterium tuberculosis* granuloma: a review. Semin Immunopathol **2016**; 38:167–83.
41. Mayer-Barber KD, Andrade BB, Oland SD, et al. Host-directed therapy of tuberculosis based on interleukin-1 and type I interferon crosstalk. Nature **2014**; 511:99–103.
42. Akasaki Y, Alvarez-Garcia O, Saito M, Caramés B, Iwamoto Y, Lotz MK. FoxO transcription factors support oxidative stress resistance in human chondrocytes. Arthritis Rheumatol **2014**; 66:3349–58.
43. Ambrogini E, Almeida M, Martin-Millan M, et al. FoxO-mediated defense against oxidative stress in osteoblasts is indispensable for skeletal homeostasis in mice. Cell Metab **2010**; 11:136–46.
44. Kops GJ, Dansen TB, Polderman PE, et al. Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. Nature **2002**; 419:316–21.
45. Harbut MB, Vilcheze C, Luo X, et al. Auranofin exerts broad-spectrum bactericidal activities by targeting thiol-redox homeostasis. Proc Natl Acad Sci U S A **2015**; 112:4453–8.
46. Oda H, Okamoto I, Murphy N, et al. Monomethylation of histone H4-lysine 20 is involved in chromosome structure and stability and is essential for mouse development. Mol Cell Biol **2009**; 29:2278–95.
47. Sakaguchi A, Steward R. Aberrant monomethylation of histone H4 lysine 20 activates the DNA damage checkpoint in *Drosophila melanogaster*. J Cell Biol **2007**; 176:155–62.
48. Dorhoi A, Iannaccone M, Farinacci M, et al. MicroRNA-223 controls susceptibility to tuberculosis by regulating lung neutrophil recruitment. J Clin Invest **2013**; 123:4836–48.
49. Ghorpade DS, Sinha AY, Holla S, Singh V, Balaji KN. NOD2-nitric oxide-responsive microRNA-146a activates Sonic hedgehog signaling to orchestrate inflammatory responses in murine model of inflammatory bowel disease. J Biol Chem **2013**; 288:33037–48.
50. Holla S, Kurowska-Stolarska M, Bayry J, Balaji KN. Selective inhibition of IFNG-induced autophagy by Mir155- and Mir31-responsive WNT5A and SHH signaling. Autophagy **2014**; 10:311–30.