Published in final edited form as:

Sci Immunol. 2017 March ; 2(9): . doi:10.1126/sciimmunol.aaj1789.

Host sirtuin 1 regulates mycobacterial immunopathogenesis and represents a therapeutic target against tuberculosis

Catherine Y. Cheng¹, Nuria M. Gutierrez², Mardiana B. Marzuki¹, Xiaohua Lu¹, Taylor W. Foreman³, Bhairav Paleja¹, Bennett Lee¹, Akhila Balachander¹, Jinmiao Chen¹, Liana Tsenova^{4,5}, Natalia Kurepina⁴, Karen W. W. Teng¹, Kim West², Smriti Mehra⁶, Francesca Zolezzi^{1,*}, Michael Poidinger¹, Barry Kreiswirth⁴, Deepak Kaushal³, Hardy Kornfeld², Evan W. Newell¹, and Amit Singhal^{1,7,†}

¹Singapore Immunology Network, Agency for Science, Technology and Research (A* STAR), Singapore 138648, Singapore

²Department of Medicine, University of Massachusetts Medical School, Worcester, MA 01655, USA

³Tulane National Primate Research Center, Covington, LA 70433, USA

⁴Public Health Research Institute of Rutgers Biomedical and Health Sciences, Rutgers University, Newark, NJ 07103, USA

⁵New York City College of Technology, Brooklyn, NY 11201, USA

⁶Louisiana State University School of Veterinary Medicine, Baton Rouge, LA 70803, USA

⁷Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore 308232, Singapore

Abstract

Mycobacterium tuberculosis (*Mtb*) executes a plethora of immune-evasive mechanisms, which contribute to its pathogenesis, limited efficacy of current therapy, and the emergence of drug-resistant strains. This has led to resurgence in attempts to develop new therapeutic strategies/targets against tuberculosis (TB). We show that *Mtb* down-regulates sirtuin 1 (SIRT1), a nicotinamide adenine dinucleotide (NAD⁺)-dependent deacetylase, in monocytes/macrophages, TB animal models, and TB patients with active disease. Activation of SIRT1 reduced intracellular

[†]Corresponding author. amit_singhal@immunol.a-star.edu.sg.

^{*}Present address: Galderma R&D, 06902 Sophia Antipolis Cedex, France.

Author contributions: C.Y.C. and A.S. conceived the idea, designed the study, performed the experiments, and analyzed the data. N.M.G., M.B.M., X.L., B.P., K.W.W.T., and K.W. performed the experiments. N.M.G., L.T., and H.K. performed the histopathological and morphometric analysis and experiments with KO mice. B.L. and M.P. performed the statistical and bioinformatics analysis. J.C., A.S., and E.W.N. analyzed the CyTOF data. F.Z. performed the microarray studies. T.W.F., S.M., and D.K. performed and analyzed the macaque experiments. A.B. performed and analyzed the confocal microscopy data. N.K. and B.K. performed the experiments with the MDR strains. A.S. oversaw the study. C.Y.C. and A.S. wrote the manuscript. All authors discussed the results and commented on the manuscript.

Competing interests: C.Y.C. and A.S. have filed a patent (PCT/SG2017/050021) with respect to the use of SIRT1 as a target for controlling mycobacterial infection. The other authors declare that they have no competing interests.

Data and materials availability: Microarray data have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus database under the accession number GSE78233.

growth of drug-susceptible and drug-resistant strains of *Mtb* and induced phagosome-lysosome fusion and autophagy in a SIRT1-dependent manner. SIRT1 activation dampened *Mtb*-mediated persistent inflammatory responses via deacetylation of RelA/p65, leading to impaired binding of RelA/p65 on the promoter of inflammatory genes. In *Mtb*-infected mice, the use of SIRT1 activators ameliorated lung pathology, reduced chronic inflammation, and enhanced efficacy of anti-TB drug. Mass cytometry-based high-dimensional analysis revealed that SIRT1 activation mediated modulation of lung myeloid cells in *Mtb*-infected mice. Myeloid cell-specific SIRT1 knockout mice display increased inflammatory responses and susceptibility to *Mtb* infection. Collectively, these results provide a link between SIRT1 activation and TB pathogenesis and indicate a potential of SIRT1 activators in designing an effective and clinically relevant host-directed therapies for TB.

Introduction

Tuberculosis (TB) pathogenesis is driven by a complex interplay between the host immune system and the survival strategies of the bacterium (1). The ability of *Mycobacterium tuberculosis* (*Mtb*) to persist in protected niches within the body delays the efficacy of current antibiotic therapy, contributing to the emergence of multidrug-resistant (MDR) strains. Therefore, the development of new therapeutic strategies, such as host-directed therapies (HDTs) that enhance *Mtb*-specific immunity, are urgently needed (2, 3).

An effective host immune response is important for the containment of persistent *Mtb* infection (4), and this is intimately linked to the metabolic programs of the host (5). The engagement of cellular immunometabolic circuits is predominantly regulated by sensors, including mTOR (mammalian target of rapamycin), AMPK (adenosine monophosphate-activated protein kinase), and the sirtuins (silent mating type information regulation 2 homologs) (6–8). Perturbations in mTOR and AMPK signaling have been associated with *Mtb* virulence (9, 10). Targeting the mTOR pathway with specific inhibitors has been shown to stimulate autophagy induction, leading to increased mycobacterial clearance (3, 11). Similarly, activation of AMPK using the antidiabetic drug metformin leads to improved *Mtb* control (12). This highlights the potential of targeting functional connections between host immune defense and metabolism to modulate *Mtb* pathogenicity.

The sirtuins are a family of nicotinamide adenine dinucleotide (NAD⁺)-dependent class III histone deacetylases, consisting of seven members present in nearly all subcellular compartments (8). Among these, sirtuin 1 (SIRT1) is involved in a range of cellular processes important for the maintenance of human health, including stress response, cellular metabolism, and aging (13, 14). SIRT1 is known to be important in the prevention of viral diseases (13, 15); however, its role in chronic bacterial infections is unknown.

In the present study, we show that SIRT1 expression is down-regulated during active *Mtb* infection and that enhancement of SIRT1 activity using specific activators inhibits the intracellular growth of *Mtb*, normalizes the inflammatory response, enhances the efficacy of isoniazid (INH; a first-line anti-TB drug), and limits disease immunopathology.

Results

Mtb infection down-regulates SIRT1 expression

Understanding *Mtb* evasion strategies is warranted for the advancement of TB HDTs (1, 3). This led us to investigate the effect of *Mtb* infection on sirtuins, an important family of host energy sensors. A comparison of *Mtb*-infected and uninfected THP-1 cells revealed a time-dependent *Mtb*-mediated down-regulation of *SIRT1* gene (fig. S1A and Fig. 1A) and protein (Fig. 1B). This is consistent with published microarray data sets of *Mtb*-infected cells (fig. S1, B and C) (16). A time-dependent reduction of *SIRT1* mRNA expression was also observed in the lung tissues of *Mtb*-infected mice (Fig. 1C). Higher SIRT1 expression was observed in the lesions of *Mtb*-infected macaques with latent TB compared with active disease (Fig. 1D). Immunostaining of the granulomas of *Mtb*-infected macaque lung sections further showed higher levels of SIRT1 in the bacillus Calmette-Guérin (BCG)-vaccinated macaques than in unvaccinated animals (Fig. 1, E and F). These SIRT1-expressing cells were mainly macrophages and not T cells (fig. S2).

To evaluate the clinical significance of *Mtb*-mediated SIRT1 down-regulation, we performed a pairwise comparison of *SIRT1* mRNA expression profiles derived from peripheral blood of active TB (ATB) patients, latent TB individuals, and healthy controls from seven different cohorts (table S1) (17–22). In the UK 2010, South Africa 2010 (17), and South Africa 2013 (22) cohorts, *SIRT1* mRNA levels were lowest in ATB patients compared with healthy and latent TB individuals (Fig. 1, G and H, and fig. S1D). *SIRT1* mRNA levels were also reduced in HIV patients with TB as co-morbidity compared with those with HIV alone (South Africa 2014 cohort; Fig. 1I) (21). In TB patients undergoing chemotherapy (18, 20), *SIRT1* mRNA levels increased after 2 to 3 months of therapy and reached levels comparable with those observed in healthy/latent TB individuals by the sixth month of treatment (Fig. 1J and fig. S1, E and F). Furthermore, human monocyte-derived macrophages (hMDMs) from ATB patients also displayed reduced *SIRT1* mRNA expression (fig. S1G) (19). In all, these results indicate (i) an important disease-related effect of *Mtb* infection on SIRT1 expression and (ii) an association of lower SIRT1 expression with TB pathogenesis.

SIRT1 activation restricts intracellular *Mtb* growth and induces autophagy and phagosome-lysosome fusion

To determine whether SIRT1 is essential for controlling mycobacterial growth, SIRT1-deficient cells were infected with *Mtb* and were found to display higher bacillary loads (Fig. 2A), suggesting that activating SIRT1 may control mycobacterial growth. Enhancing SIRT1 activity by resveratrol (RES; a natural activator) and SRT1720 (SRT; a synthetic activator) (23, 24) using concentrations that are not toxic for cells (fig. S3, sets of *Mtb*-infected cells (fig. S1, B and C) (16). A time-dependent reduction of *SIRT1* mRNA expression was also observed in the lung tissues of *Mtb*-infected mice (Fig. 1C). Higher SIRT1 expression was observed in the lesions of *Mtb*-infected macaques with latent TB compared with active disease (Fig. 1D). Immunostaining of the granulomas A and B) inhibited the growth of intracellular BCG (fig. S3C) and *Mtb* in THP-1 cells (Fig. 2, B to D) and hMDMs (fig. S3D) in a dose- and time-dependent manner. This effect was abolished in cells where SIRT1 was genetically (Fig. 2E) or chemically (fig. S3E) inactivated. RES and SRT also restricted

intracellular replication of *Mtb* MDR strains (Fig. 2F). Inhibition of mycobacterial growth by SIRT1 activation was further validated with SA3, an additional SIRT1 activator (fig. S3F). SIRT1 activity is primarily dependent on the availability of intracellular NAD⁺ (25, 26). In our experiments, *Mtb* infection reduced the intracellular NAD⁺/NADH ratio, which was reversed by the addition of SIRT1-activating compounds (fig. S3G). Notably, SRT activation increased the levels of *SIRT1* mRNA in *Mtb*-infected cells (fig. S3H), consistent with earlier studies (23, 27).

To identify the possible mechanism(s) underlying the restriction of intracellular *Mtb* growth by SIRT1 activators, we performed a genome-wide transcriptional analysis using total RNA isolated from *Mtb*-infected THP-1 cells in the presence or absence of RES. At 24 hours after infection, a total of 8766 genes were differentially expressed in response to infection (fig. S4A). RES treatment altered the expression of 6325 genes in *Mtb*-infected cells (fig. S4A), of which 71% (4486 of 6325) overlapped with those affected by infection. RES reversed the expression pattern of 3062 of these genes (quadrants X and Y in fig. S4B and table S2). Analysis of these 3062 genes identified the enrichment of genes that regulate autophagy and phagosome-lysosome fusion ($P = 4.76 \times 10^{-5}$), indicating the induction of innate antimicrobial cellular functions upon SIRT1 activation (Fig. 2G). Indeed, in mycobacteria-infected cells, SRT-mediated SIRT1 activation induced autophagy as assessed by up-regulation of LC3 (Fig. 2H and fig. S5A) and generation of its lipidated form (Fig. 2I). This SRT-mediated autophagy induction was not observed in *Mtb*-infected SIRT1^{-/-} cells (Fig. 2, H and I), and blocking autophagy abolished SRT-mediated restriction of mycobacterial growth (Fig. 2J). Furthermore *Mtb*-infected SIRT1^{-/-} cells were found to display less LC3 puncta (fig. S5B). SIRT1 activation by SRT or RES also led to an increase in fluorescent BCG accumulation in LysoTracker Red (LTR)-labeled compartments (Fig. 2, K and L, and fig. S5, C and D), whereas this was not observed in cells where SIRT1 was chemically (Fig. 2, K and L) or genetically (fig. S5E) inactivated. Together, these data indicate that SIRT1 activation contributes to the control of mycobacterial growth by inducing autophagy and phagosome-lysosome fusion.

SIRT1 activation normalizes *Mtb*-induced inflammatory responses by deacetylating RelA/p65

To further delineate the effect of SIRT1 activation, we performed Gene Ontology (GO) analysis on 3062 RES-modulated genes (fig. S4B). This indicated enrichment of GOs involved in the inflammatory response, DNA damage restoration, telomere maintenance, and viral defense responses (Fig. 3, A and B, and table S3). A similar trend was observed when 3062 genes were analyzed using Ingenuity Pathway Analysis (IPA). Pathways such as triggering receptor expressed by myeloid cell 1 (TREM-1) signaling, liver X receptor (LXR) activation, interferon signaling, and nuclear factor κB (NF-κB) signaling were associated with genes up-regulated by infection and down-regulated by RES (fig. S4C and table S4). These data suggest that RES results in the normalization of inflammation-associated genes and pathways, modulated by *Mtb* (12, 17, 28). Consistent with the effects of RES, treatment of *Mtb*-infected THP-1 cells with SRT reduced mRNA and protein expression of inflammatory cytokines (Fig. 3, C and D) and RelA/p65 (Fig. 3, E to G), which is a subunit of NF-κB, a known target of SIRT1 (29). Here, SRT treatment led to decreased RelA/p65

acetylation in *Mtb*-infected THP-1 cells (Fig. , F and G3). Chromatin immunoprecipitation (ChIP) assays revealed that *Mtb* infection enhanced the occupancy of RelA/p65 on *tumor necrosis factor- α* (*TNF α*), *interleukin1B* (*IL1B*), *IL6*, and *monocyte chemoattractant protein-1* (*MCP-1*) promoters, which was reversed by SRT (Fig. 3H). These results indicate that SIRT1 activation-mediated deacetylation of RelA/p65 is important for the unoccupancy of RelA/p65 on the promoter of proinflammatory genes, leading to the attenuation of *Mtb*-induced inflammatory responses.

SIRT1 activation restricts *Mtb* growth in mice

To determine whether SIRT1 is essential for controlling *Mtb* growth in vivo, myeloid cell-specific SIRT1 knockout (Mac-SIRT1 KO) mice (30) were infected with *Mtb* and were found to display higher bacillary loads compared with wild-type controls (Fig. 4A). We next evaluated the efficacy of RES and SRT in mouse models of acute and chronic TB (12). In an acute model of *Mtb* infection, RES and SRT inhibited the growth of *Mtb* in the lungs and spleens of infected wild-type mice (Fig. 4, B and C, and fig. S6A). When SRT was administered in combination with INH, a greater proportion of mice with no detectable colony-forming units (CFU) in the tissues was detected compared with mice receiving INH alone (Table 1). Consistent with our findings in vitro (fig. S3H), we also observed an increase in *SIRT1* mRNA levels in the lungs of *Mtb*-infected mice upon SRT treatment (fig. S6B). In a chronic model of TB, SRT alone inhibited the growth of *Mtb* in the lungs (Fig. 4D), and mice treated with INH in combination with SRT showed decreased bacillary loads in the lungs compared with mice receiving INH alone (Table 1). These results indicate that activation of SIRT1 restricts *Mtb* growth and can enhance *Mtb* clearance by anti-TB drugs.

SIRT1 activators reduce *Mtb*-mediated tissue pathology and inflammation in mice

We next assessed the effect of RES and SRT treatments on tissue pathology. Histologic examination of the lungs from untreated control mice at 35 days after infection revealed diffuse coalescent lesions, with numerous infiltrating macrophages and lymphocytes, and large number of intracellular acid-fast bacilli (AFB; Fig. 5A). At this time, RES-treated mice displayed reduced levels of AFB and slightly smaller granulomatous lesions (Fig. 5A), previously linked with improved *Mtb* control in mice (12, 31, 32). Morphometric analysis showed the reduced percentage of lung parenchyma area involvement in the pathology in RES-treated mice compared with untreated animals (43.8% versus 55.6%, respectively; Fig. 5B). Similarly, tissues of SRT-treated, *Mtb*-infected mice were smaller (fig. S6C) and had lower levels of cellular infiltrate (fig. S5D), with a reduced involvement of lung parenchyma compared with untreated mice (33.7% versus 44.3%, respectively; Fig. 5C). Lung tissue of SRT-treated mice also showed reduced mRNA and protein expression of inflammatory cytokines and chemokines (Fig. 5, D and E). In contrast, *Mtb*-infected Mac-SIRT1 KO mice showed increased expression of inflammatory cytokines and chemokines (Fig. 5, F and G, and fig. S6E). Together, these results emphasize the role of SIRT1 in regulating immunopathology during *Mtb* infection.

SIRT1 activation modulates the lung myeloid landscape in *Mtb*-infected mice

Infiltration of myeloid cells expressing inflammatory cytokines and chemokines into the tissues is a major characteristic of TB progression in mice (33). Because we observed

reduced pathological inflammation in the lungs of SRT-treated, *Mtb*-infected mice (Fig. 5), we investigated the profile of pulmonary myeloid cells. Lung cells from infected and uninfected mice, with or without SRT treatment, were stained with a panel of 37 markers (table S5) and analyzed using cytometry by time-of-flight (CyTOF) (34, 35). We first verified the panel antibodies for their binding to the lung cells (fig. S7) (35). Analysis of CD45⁺CD90⁺CD3⁺CD19⁺ lung cells using nonlinear dimensionality reduction technique tSNE (t-distributed stochastic neighbor embedding) in conjunction with a machine-learning updated clustering algorithm (DensVM) (35) identified 28 distinct cell clusters with shared surface marker expression characteristics (Fig. 6A and fig. S8). We assessed the accuracy of the machine-learning automated gating by validating each cluster using manual gating (fig. S9A), overlaying each cluster on its respective parent population. For example, dendritic cell (DC) clusters (clusters 7, 17, and 29; fig. S8), when overlaid on the gated CD11c⁺MHCII⁺ population, were distributed according to CD11b and CD103 expression (fig. S10A). Similar results were obtained when four Ly6C^{lo} and five Ly6C⁺ clusters (fig. S8) were assessed for Ly6C expression (fig. S10B). All four Ly6C^{lo} clusters (clusters 8, 9, 18, and 19) were further categorized into individual clusters on the basis of CD11b, CD44, and CD38 expression (fig. S10B), as identified by tSNE analysis (Fig. 6A and fig. S8).

Unbiased analysis of 28 clusters distinguished uninfected, infected, and SRT-treated, infected animals (Fig. 6B and fig. S11A). Nineteen of 28 clusters were found to be statistically significant enriched or depleted in *Mtb*-infected animals (Fig. 6B). These differentiated clusters included diverse phenotypes, that is, Ly6C⁺ (clusters 23 to 25) and Ly6C^{lo} (clusters 9, 18, and 19) monocytes, CD11b⁺ DCs (cluster 7), plasmacytoid DCs (cluster 11), CD64⁺MerTK⁺Siglec-F⁺ macrophages (clusters 13, 15, and 20), Ly6G⁺ neutrophils (clusters 4 and 10), eosinophils (cluster 1), and innate lymphoid cells (ILCs; clusters 21 and 34) (Fig. 6, A and B, and fig. S8). We confirmed the differential abundance of these cell subsets using manual gating (Fig. 6C and fig. S11, B and C). SRT treatment had minimal effect on uninfected animals as indicated by mixed clustering of cells from uninfected animals, with or without SRT treatment (Fig. 6B).

SIRT1 activation impairs Ly6C monocytes in the mouse lung

Our high-dimensional analysis revealed that SRT treatment of *Mtb*-infected animals normalized 6 of the 19 disease-associated clusters toward the frequency present in the lungs of uninfected mice (Fig. 6, B and D). Phenotyping of these six clusters identified them as Ly6C^{lo}CD11b^{lo}F4/80⁺ monocytes (clusters 18 and 19), Ly6C⁺CD11b^{lo}F4/80⁺ monocytes (cluster 23), Ly6G⁺CD11b^{lo} neutrophils (cluster 10), CD25⁺CD11b⁺Sca-1⁺ ILCs (cluster 21), and Siglec-F⁺ eosinophils (cluster 1) (Fig. 6D and fig. S11D). Of particular interest was the decreased abundance of CD11b^{lo}F4/80⁺ monocytes (Ly6C^{lo} or Ly6C⁺) in the lungs of SRT-treated, *Mtb*-infected mice compared with untreated, *Mtb*-infected mice, confirmed by manual gating (Fig. 6E) and verified using fluorescence flow cytometry (Fig. 6, F and G, and fig. S9B). In contrast, *Mtb*-infected Mac-SIRT1 KO mice displayed an increased percentage and number of CD11b^{lo}F4/80⁺ (Ly6C^{lo} or Ly6C⁺) monocytes in the lungs compared with wild-type mice as early as day 3 after infection (fig. S11E and Fig. 6H, respectively). Overall, our data indicate an association of SIRT1 activity/expression with

myeloid cell frequency in the lungs of *Mtb*-infected mice, relevant for the control of *Mtb* growth and inflammation.

Discussion

Mtb has coevolved with the human immune system, acquiring an unexpected ability to exploit cellular host factors and circumvent immunity for its own survival (1). In this study, we show that active *Mtb* infection suppresses SIRT1 (a conserved mammalian NAD⁺-dependent deacetylase) expression and reduces cellular NAD⁺ pool, which is required for SIRT1 activity (26, 36, 37). Depletion of cellular NAD⁺ could be due to a toxin secreted by *Mtb* that leads to host cell death and *Mtb* dissemination (38). We showed that SIRT1-activating compounds inhibited the intracellular growth of *Mtb*; activated host cell autophagy and phagosome-lysosome fusion; and reduced *Mtb* growth, tissue pathology, and chronic inflammation in mice. SIRT1 has been described to play a role in the regulation of autophagy (39) and can also directly affect AMPK activity (40), which we and others have recently shown to be clinically beneficial (11, 12, 41). Thus, the capacity of both SIRT1 and AMPK to modulate innate immune mechanism and inflammation (42, 43) emphasizes the importance of the SIRT1-AMPK axis in (i) rewiring the host antimicrobial arsenal and (ii) skewing the immunopathological balance toward protective immunity against *Mtb*, leading to enhanced host benefits (2, 3, 12).

TB pathogenesis is associated with early infiltration of myeloid cells into infected tissues (33). Inconsistencies in the naming and working definitions of myeloid cell subsets have complicated the objective description and quantification of cellular innate immune responses. Using a previously described unbiased high-dimensional analysis (35), we characterized the myeloid compartment in the lungs of *Mtb*-infected mice and found that SIRT1 activation modulated the infiltration of Ly6C^{lo}/Ly6C⁺ CD11b^{lo}F4/80⁺ monocytes. Cells fitting this description are important for early pathogen defense but, if sustained, can contribute to higher bacterial burdens and accelerate mortality in mice (44). Because *Mtb* persists and disseminates by infecting newly recruited monocytes/macrophages (45), reduced recruitment of Ly6C^{+/lo} monocytes upon SIRT1 activation could also favor SIRT1-mediated *Mtb* control. The recruitment of Ly6C monocytes into the tissues occurs via engagement of the chemokine receptor CCR2 with MCP-1 (CCL2) (46), a chemokine regulated by SIRT1. Together, our data provide new insights into the plasticity/heterogeneity of myeloid cells during *Mtb* infection (47) and suggest a role of SIRT1 activators in controlling *Mtb* growth/pathogenesis by regulating myeloid cell infiltration.

SIRT1 activators have a favorable safety profile and are currently being tested in phase 2 human trials (<https://ClinicalTrials.gov>) (48). Therefore, their usage as TB HDT could have important clinical implications through (i) avoiding the development of resistance by targeting the host and not the pathogen, (ii) shortening the course of multidrug therapy for TB, and (iii) improving anti-*Mtb* immune responses to facilitate pathogen clearance. Also, because SIRT1 activators modulate chronic inflammation, they could prevent long-term pulmonary morbidity due to lung destruction in TB patients (47). However, to fully decipher the applicability of SIRT1 activators as potential TB HDTs, several issues remain to be addressed. First, our study does not describe the effect of SIRT1 activators on the adaptive

immune response, which is important in controlling *Mtb* infection. Second, the exact dialog between SIRT1-deficient myeloid cells and TB pathogenicity remains unclear. Third, studies in other animal models such as macaques are warranted, possibly through the use of next-generation SIRT1 synthetic activators.

Overall, our study demonstrates an important role of SIRT1 in the *Mtb* infection-associated immunopathogenesis. Moreover, the study adds to our understanding of the lung myeloid cell heterogeneity and possible cross-talk of immunologic and metabolic pathways during *Mtb* infection, and suggests the potential of targeting SIRT1 for the development of innovative next-generation HDTs to improve TB treatment outcomes.

Materials and Methods

Study design

The objective of this study was to assess the effect of *Mtb* infection on host sirtuins, which regulate the range of immunometabolic processes. Our experiments indicated that *Mtb* modulates SIRT1 in monocytes/macrophages, mice, and macaques. Next, we evaluated the efficacy of SIRT1 activators against *Mtb* infection in mouse TB model and performed detailed mechanistic studies using in vitro and mouse models to precisely delineate the antimicrobial and anti-inflammatory function of SIRT1. Last, the clinical significance of *Mtb*-mediated SIRT1 down-regulation was evaluated by mining the *SIRT1* mRNA expression profiles from peripheral blood of ATB patients, latent TB individuals, and healthy controls from seven different cohorts. All experiments were performed at least twice. Each in vitro experiment was performed in duplicates/triplicates, and data of three to five independent experiments were presented. In mouse experiments, animals were randomly distributed among different groups. For histopathological analysis, pathologist was blinded for the groups.

Reagents

The following chemicals were used: SRT HCl (ApexBio, #A4180), RES (Sigma-Aldrich, #711004), SA3 (Santa Cruz Biotechnology, #SC-222315), EX527 (Selleckchem, #S1541), and INH (Sigma-Aldrich, #I3377). The following antibodies were used: anti-SIRT1 (Millipore, #07-131; Abcam, #E104), anti-NF- κ B p65 (Santa Cruz Biotechnology, #SC-372), anti-NF- κ B p65 (acetyl K310) (Abcam, #ab19870), anti-LC3B (Cell Signaling Technology, #3868), anti-CD3 (Dako, #A0452), anti-CD68 (Dako, #M0814), anti-CD163 (Serotec, #MCA1853), anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (14C10) (Cell Signaling Technology, #2118), anti-rabbit immunoglobulin G (IgG), horseradish peroxidase (HRP)-linked antibody (Cell Signaling Technology, #7074), and mouse anti-rabbit IgG (conformation-specific) monoclonal antibody (Cell Signaling Technology, #3678). Stealth SIRT1 small interfering RNAs (siRNAs) (set of three: #HSS118729, #HSS177403, and #HSS177404) and control siRNAs (#1299001) were from Life Technologies and Integrated DNA Technologies, respectively.

THP-1 cell culture

Human monocyte THP-1 cells from the American Type Culture Collection were maintained in RPMI 1640 (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% sodium pyruvate, 1% L-glutamine, 1% nonessential amino acids, and 1% kanamycin at 37°C in a 5% CO₂ humidified atmosphere. In infection experiments, no antibiotic was used.

Preparation of hMDMs

Peripheral blood mononuclear cells were isolated from total blood using a Ficoll gradient followed by enrichment of monocytes using CD14 magnetic beads (Miltenyi Biotec). The purified CD14⁺ monocytes were resuspended in RPMI 1640 with 10% FBS, 1% penicillin, 2 mM L-glutamine, and human macrophage colony-stimulating factor (M-CSF; 100 ng/ml; R&D Systems) and incubated at 37°C in 5% CO₂ for 6 days. The medium was changed on the fourth day. On day 6, differentiated macrophages were harvested and cultured overnight without M-CSF. On day 7, cells were washed and used for experiments. Blood collection from healthy volunteers was approved by the Institutional Review Board, National Healthcare Group Domain (Singapore).

Culture of mycobacterial strains

Mtb H37Rv, HN878, CDC1551, Erdman, W148, AH30, CC13, AI10, and KY strains; *Mycobacterium bovis* BCG; and *M. bovis* BCG–green fluorescent protein (GFP) were grown in Middlebrook 7H9 broth (BBL Microbiology Systems) supplemented with albumin-dextrose-catalase (ADC; Difco Laboratories) and 0.05% Tween 80 at 37°C for 5 to 7 days to an OD₆₀₀ (optical density at 600 nm) of 0.4 to 0.5. After this, mycobacterial cells were pelleted, resuspended in fresh 7H9 broth with 20% glycerol, and stored at -80°C. One vial of the stock was thawed to enumerate CFU per milliliter. On the day of infection, the cells were thawed, washed, and sonicated before use. Drug-resistant strains were maintained at the Public Health Research Institute (Newark, NJ).

Infection of cells with mycobacteria

Frozen *Mycobacterium* strains were thawed, washed, resuspended in antibiotic-free RPMI 1640 with 10% FBS, and used to infect cells with a multiplicity of infection of 5. The infected cells were incubated at 37°C in 5% CO₂ for 3 hours. After this, cells were washed twice with antibiotic-free medium, counted, seeded in triplicate, and either left untreated or treated with different compounds for the indicated time periods. For inhibition experiments, mycobacteria-infected cells were pretreated with the SIRT1 inhibitor EX527 or autophagy inhibitors for 0.5 hours before addition of SIRT1 activators. At predetermined time points after infection, the cells were lysed for enumerating mycobacteria. THP-1 cells were differentiated for 16 to 18 hours using 4 μM phorbol 12-myristate 13-acetate (Sigma) before infection with MDR strains of *Mtb*.

NAD⁺/NADH ratio measurement

Quantification of NAD⁺ and NADH was carried out using the NAD⁺/NADH Assay Kit (Abcam, #ab65348) according to the manufacturer's instructions. NAD⁺ and NADH values were normalized by protein concentration.

Transfection of siRNAs

THP-1 cells were transfected with siRNAs using the Lipofectamine 2000 Kit (Invitrogen) according to the manufacturer's protocol. Knockdown was confirmed by Western blotting. All transfection data are means $\pm$ SEM of three independent experiments, performed in duplicate.

Western blot

Protein lysates from cells were obtained by lysis in radioimmuno-precipitation assay buffer (Sigma) with protease (Roche) and phosphatase (Roche) inhibitors. A Micro BCA Protein kit (Thermo Scientific) was used to measure protein levels, and equal amounts of proteins were resolved by electrophoresis on 12% tris-HCl gels (Mini-PROTEAN TGX gels; Bio-Rad) and transferred onto polyvinylidene difluoride membranes (Trans-Blot Turbo Transfer Pack; Bio-Rad). Membranes were developed using the indicated primary antibody at a 1:1000 dilution and secondary antibodies at a 1:3000 dilution in blocking solution. This was followed by incubation with Chemiluminescent HRP detection reagent (Millipore) for 1 min before image acquisition.

ChIP assay

ChIP assays were performed using the anti-NF- κ B p65 antibody (C-20) or normal rabbit IgG, with the SimpleChIP Plus Enzymatic Chromatin IP Kit (Cell Signaling Technology, #9005), following the standard protocol. DNA fragments were subjected to quantitative reverse transcription polymerase chain reaction (qRT-PCR) using primers flanking NF- κ B response elements on various targets. ChIP qRT-PCR data analysis was performed as previously described (49).

Measurement of *M. bovis* BCG in lysosomes

THP-1 cells were infected with GFP-conjugated BCG. After infection, cells were incubated with 500 nM LTR (Invitrogen) in the presence or absence of drugs for 4 or 24 hours. Cells were then washed with phosphate-buffered saline (PBS) and fixed in 4% paraformaldehyde for 20 min at room temperature. Fixed cells were then washed with PBS. Fluorescence intensities of GFP-BCG and LTR were analyzed using an Olympus FV1000 confocal microscope. Z-stacks of cells were acquired and quantitated.

Autophagy analysis

Anti-LC3 antibody was used for flow cytometric analysis and Western blotting at a dilution of 1:250 and 1:1000, respectively. As a positive control, cells were treated with 2.5 μ M rapamycin or 1 μ M INH for 4 or 24 hours.

Mouse models of infection with *Mtb*

The antitubercular activity of SRT and RES was evaluated in an acute and chronic infection model of C57BL/6 mice (12). Female C57BL/6 mice (6 to 8 weeks old) were infected aerosolically with *Mtb* H37Rv or Erdman. Three to four animals were sacrificed on day 1 to determine the number of bacteria implanted in the lungs. Treatment was initiated 7 or 40 days after infection in the acute and chronic model, respectively. Drugs were administered

by gavage once a day, 6 days a week. Mice were sacrificed at predetermined time points followed by harvesting of tissues and enumeration of *Mtb* CFU. A total of seven experiments (five acute and two chronic) were performed. All mice were housed in a biosafety level 3 (BSL3) laboratory and treated humanely. The Mac-SIRT1 KO mice (30) were a gift from X. Li (U.S. National Institute of Environmental Health Sciences). A total of three experiments were performed with Mac-SIRT1 KO and their littermate controls.

Enumeration of *Mtb* CFU in infected mice

The mycobacterial load in the lung and spleen of infected mice was quantified by plating tissue homogenates on Middlebrook 7H11 agar supplemented with oleic acid-ADC (OADC). At predetermined time points, mice were euthanized, and organs were aseptically excised, washed in PBS, and homogenized in PBS containing 0.25% Tween 80 using a MACS tissue dissociator (Miltenyi Biotec). A series of dilutions of tissue homogenate were plated in triplicate. Agar plates were incubated at 37°C for 3 weeks, after which colonies were counted visually. CFU obtained from two or three dilutions were used to calculate the total number of CFU per tissue per mouse.

CyTOF marker labeling, flow cytometry, data acquisition, and analysis

Lung cells were stained with heavy metal isotope-labeled antibodies (table S5) (50), barcoded, and acquired on CyTOF 1 (Fluidigm). Samples were debarcoded using manual gating in FlowJo, and analysis of live CD45⁺CD3⁻CD90⁻CD19⁻ myeloid cells was carried out using the tSNE dimension reduction and DensVM density-based clustering algorithm (35). Flow cytometry was performed to validate CyTOF data (in independent experiment) and to assess the infiltration of myeloid cells in *Mtb*-infected Mac-SIRT1 KO mice (see Supplementary Methods for details on mass cytometry and flow cytometry).

Histology and morphometry

The upper right lobes of mouse lungs were fixed in 10% buffered formalin and paraffin-embedded. Sections were stained with hematoxylin and eosin (H&E) or Ziehl-Neelsen acid-fast stain and were photographed in a Nikon Microphot-FX photomicrographic system with NIS-Elements F3.0 software (Nikon Instruments Inc.). Morphometric analysis of lung involvement in pathology was performed using PathScan Enabler IV slide scanner (Meyer Instruments) and SigmaScan Pro 5 (SPSS Science Inc.) image analysis system. The area of all lesions in a section was measured first, and then the percentage from the area of the whole section was calculated (lung involvement).

Immunohistochemistry of macaque lung sections

Slides (5 µm thick) were cut from formalin-fixed, paraffin-embedded tissue blocks; subjected to double-antigen retrieval; and stained with SIRT1, CD3, CD68, and CD163 antibodies. Detection was performed with an anti-rabbit MACH 3 Rabbit AP-Polymer Kit (Biocare), and slides were counterstained with YO-PRO nuclei stain (Molecular Probes). The images were taken on a CRi microscope and counted using INFORM software. Only those cells that exhibited a SIRT1 signal at least 15 arbitrary units bright from each nucleus were considered positive and included for quantification.

Real-time quantitative PCR

Cells were lysed in TRIzol (Invitrogen), and total RNA was isolated using the RNeasy Mini Kit (Qiagen). RNA was reverse-transcribed using the iScript cDNA Synthesis Kit (Bio-Rad). Complementary DNA (cDNA) was used for real-time quantitative PCR (RT-qPCR) using 2× iQ SYBR Green Supermix and iCycler (Bio-Rad). The primers are described in table S6. The mRNA expression levels in infected cells per tissue were normalized to GAPDH expression, and fold induction was calculated by the C_T method relative to those in uninfected cells per tissue. RT-qPCR was performed in triplicate.

Microarray analysis

Total RNA from uninfected and *Mtb*-infected THP-1 cells, treated or untreated with 100 μM RES for 24 hours, was extracted using the mirVana Isolation Kit (Life Technologies). RNA quality was confirmed using the Agilent Bioanalyzer, and only samples with RNA integrity number >7 were processed. Biotinylated complementary RNA was prepared from 100 ng of total RNA using the Epicentre TargetAmp Nano-g Biotin-aRNA Labeling Kit for the Illumina system, followed by hybridization on Illumina human arrays. Raw expression data were extracted by GenomeStudio Gene Expression v1.9.0 and processed with quantile normalization (51). Hierarchical clustering analysis with complete linkage algorithms was performed using R (52). Heat maps were plotted using Spotfire (TIBCO Software Inc.; <http://spotfire.tibco.com/>). Differential expression analysis was performed using Linear Models for Microarray Data (LIMMA) (52). GO analysis was carried out by DAVID. Pathway analysis was carried out using IPA (Ingenuity Systems; www.ingenuity.com).

Gene expression analysis of macaque granulomas

Granuloma samples were collected from animals infected with a moderate dose of *Mtb* CDC1551 (50 to 100 CFU) via aerosol, which resulted in the development of either ATB or latent TB infection (LTBI). These samples were used for isolation of total RNA and subjected to host transcriptomics using rhesus macaque-specific microarrays (Agilent) relative to normal lung tissue derived from uninfected macaques as baseline, as described earlier (53). Control lung samples were labeled with Cy3, whereas lesion samples were labeled with Cy5 and cohybridized on arrays (54). Data were normalized using locally weighted scatterplot smoothing, and data specific for SIRT1 were analyzed across the matrix and hierarchically clustered using Spotfire DecisionSite for microarray analysis (<http://spotfire.tibco.com/>).

Cytokine analysis

Enzyme-linked immunosorbent assays (ELISAs) for IL-1β, IL-6, and MCP-1 were performed on THP-1 cell culture supernatants using human cytokine/chemokine-specific ELISA kits (eBioscience and BioLegend) as per manufacturers' instructions. Lung homogenates were assayed by Luminex 100 using Exponent 3.2 software and Milliplex MAP for Luminex xMAP Technology Assay (MCYTOMAG-70K Mouse Cytokine 32-Plex; Millipore).

Human cohort

SIRT1 gene expression profiles were retrieved from Gene Expression Omnibus. Eight publicly available clinical data sets of whole-blood gene expression profiles from ATB patients ($n = 201$), individuals with LTBI ($n = 191$), healthy controls ($n = 18$), patients with TB undergoing therapy at various time points ($n = 38$), and patients with other diseases ($n = 110$) were analyzed (table S1).

Statistical analysis

All values are means $\pm$ SEM of individual samples. Data analysis was performed with GraphPad Prism Software (GraphPad Software Inc.) using Student's *t* test and Mann-Whitney *U* test (when samples are not normally distributed) for two groups and analysis of variance (ANOVA) for multiple groups. Human expression data were analyzed by Mann-Whitney *U* test between patient groups or by paired Wilcoxon signed-rank test.

Study approval

The study was approved by the Institutional Biosafety Committee and the Institutional Animal Care and Use Committee of the Biological Resource Council (BRC), the Agency for Science, Technology and Research (A*STAR; Singapore), the Defence Science Organisation (DSO; Singapore), and the University of Massachusetts Medical School.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank laboratory personnel at BRC and DSO BSL3, and A. J. X. Lee for assistance. We also thank K. McLaughlin of Insight Editing London for revising the manuscript.

Funding: This research was supported by Singapore Immunology Network A*STAR; Biomedical Research Council A*STAR Young Investigator Grant 1518251030; A*STAR JCO-CDA 15302FG151; Singapore-India joint grant 1518224018; and NIH grants R01HL081149, OD011104, AI089323, HL106790, and AI111943.

References and Notes

1. Cambier CJ, Falkow S, Ramakrishnan L. Host evasion and exploitation schemes of *Mycobacterium tuberculosis*. *Cell*. 2014; 159:1497–1509. [PubMed: 25525872]
2. Uhlin M, Andersson J, Zumla A, Maeurer M. Adjunct immunotherapies for tuberculosis. *J Infect Dis*. 2012; 205:S325–S334. [PubMed: 22457298]
3. Wallis RS, Hafner R. Advancing host-directed therapy for tuberculosis. *Nat Rev Immunol*. 2015; 15:255–263. [PubMed: 25765201]
4. O'Garra A, Redford PS, McNab FW, Bloom CI, Wilkinson RJ, Berry MPR. The immune response in tuberculosis. *Annu Rev Immunol*. 2013; 31:475–527. [PubMed: 23516984]
5. Ganeshan K, Chawla A. Metabolic regulation of immune responses. *Annu Rev Immunol*. 2014; 32:609–634. [PubMed: 24655299]
6. Weichhart T, Hengstschläger M, Linke M. Regulation of innate immune cell function by mTOR. *Nat Rev Immunol*. 2015; 15:599–614. [PubMed: 26403194]
7. Viollet B, Andreelli F. AMP-activated protein kinase and metabolic control. *Handb Exp Pharmacol*. 2011; 203:303–330.

8. Chang HC, Guarente L. SIRT1 and other sirtuins in metabolism. *Trends Endocrinol Metab.* 2014; 25:138–145. [PubMed: 24388149]
9. Kumar D, Nath L, Kamal MA, Varshney A, Jain A, Singh S, Rao KVS. Genome-wide analysis of the host intracellular network that regulates survival of *Mycobacterium tuberculosis*. *Cell.* 2010; 140:731–743. [PubMed: 20211141]
10. Kumar D, Rao KVS. Regulation between survival, persistence, and elimination of intracellular mycobacteria: A nested equilibrium of delicate balances. *Microbes Infect.* 2011; 13:121–133. [PubMed: 20971210]
11. 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; 119:753–766. [PubMed: 15607973]
12. Singhal A, Jie L, Kumar P, Hong GS, Leow MKS, Paleja B, Tsenova L, Kurepina N, Chen J, Zolezzi F, Kreiswirth B, Poidinger M, Chee C, Kaplan G, Wang YT, De Libero G. Metformin as adjunct antituberculosis therapy. *Sci Transl Med.* 2014; 6:263ra159.
13. Herranz D, Muñoz-Martin M, Cañamero M, Mulero F, Martinez-Pastor B, Fernandez-Capetillo O, Serrano M. Sirt1 improves healthy ageing and protects from metabolic syndrome-associated cancer. *Nat Commun.* 2010; 1:3. [PubMed: 20975665]
14. Guarente L. Calorie restriction and sirtuins revisited. *Genes Dev.* 2013; 27:2072–2085. [PubMed: 24115767]
15. Koyuncu E, Budayeva HG, Miteva YV, Ricci DP, Silhavy TJ, Shenk T, Cristea IM. Sirtuins are evolutionarily conserved viral restriction factors. *mBio.* 2014; 5:e02249–14. [PubMed: 25516616]
16. Karim AF, Chandra P, Chopra A, Siddiqui Z, Bhaskar A, Singh A, Kumar D. Express path analysis identifies a tyrosine kinase Src-centric network regulating divergent host responses to *Mycobacterium tuberculosis* infection. *J Biol Chem.* 2011; 286:40307–40319. [PubMed: 21953458]
17. Berry MPR, Graham CM, McNab FW, Xu Z, Bloch SAA, Oni T, Wilkinson KA, Banchereau R, Skinner J, Wilkinson RJ, Quinn C, Blankenship D, Dhawan R, Cush JJ, Mejias A, Ramilo O, Kon OM, Pascual V, Banchereau J, Chaussabel D, O'Garra A. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature.* 2010; 466:973–977. [PubMed: 20725040]
18. Bloom CI, Graham CM, Berry MPR, Wilkinson KA, Oni T, Rozakeas F, Xu Z, Rossello-Urgell J, Chaussabel D, Banchereau J, Pascual V, Lipman M, Wilkinson RJ, O'Garra A. Detectable changes in the blood transcriptome are present after two weeks of antituberculosis therapy. *PLOS ONE.* 2012; 7:e46191. [PubMed: 23056259]
19. Thuong NT, Dunstan SJ, Chau TTH, Thorsson V, Simmons CP, Quyen NTH, Thwaites GE, Thi Ngoc Lan N, Hibberd M, Teo YY, Seielstad M, Aderem A, Farrar JJ, Hawn TR. Identification of tuberculosis susceptibility genes with human macrophage gene expression profiles. *PLOS Pathog.* 2008; 4:e1000229. [PubMed: 19057661]
20. Cai Y, Yang Q, Tang Y, Zhang M, Liu H, Zhang G, Deng Q, Huang J, Gao Z, Zhou B, Feng CG, Chen X. Increased complement C1q level marks active disease in human tuberculosis. *PLOS ONE.* 2014; 9:e92340. [PubMed: 24647646]
21. Dawany N, Showe LC, Kossenkova AV, Chang C, Ive P, Conradie F, Stevens W, Sanne I, Azzoni L, Montaner LJ. Identification of a 251 gene expression signature that can accurately detect *M. tuberculosis* in patients with and without HIV co-infection. *PLOS ONE.* 2014; 9:e89925. [PubMed: 24587128]
22. Kaforou M, Wright VJ, Oni T, French N, Anderson ST, Bangani N, Banwell CM, Brent AJ, Crampin AC, Dockrell HM, Eley B, Heyderman RS, Hibberd ML, Kern F, Langford PR, Ling L, Mendelson M, Ottenhoff TH, Zgambo F, Wilkinson RJ, Coin LJ, Levin M. Detection of tuberculosis in HIV-infected and -uninfected African adults using whole blood RNA expression signatures: A case-control study. *PLOS Med.* 2013; 10:e1001538. [PubMed: 24167453]
23. Milne JC, Lambert PD, Schenk S, Carney DP, Smith JJ, Gagne DJ, Jin L, Boss O, Perni RB, Vu CB, Bemis JE, Xie R, Disch JS, Ng PY, Nunes JJ, Lynch AV, Yang H, Galonek H, Israelian K, Choy W, Iffland A, Lavu S, Medvedik O, Sinclair DA, Olefsky JM, Jirousek MR, Elliott PJ, Westphal CH. Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. *Nature.* 2007; 450:712–716. [PubMed: 18046409]

24. Borra MT, Smith BC, Denu JM. Mechanism of human SIRT1 activation by resveratrol. *J Biol Chem*. 2005; 280:17187–17195. [PubMed: 15749705]
25. Revollo JR, Li X. The ways and means that fine tune Sirt1 activity. *Trends Biochem Sci*. 2013; 38:160–167. [PubMed: 23394938]
26. Yang T, Sauve AA. NAD metabolism and sirtuins: Metabolic regulation of protein deacetylation in stress and toxicity. *AAPS J*. 2006; 8:E632–E643. [PubMed: 17233528]
27. Bai L, Pang WJ, Yang YJ, Yang GS. Modulation of Sirt1 by resveratrol and nicotinamide alters proliferation and differentiation of pig preadipocytes. *Mol Cell Biochem*. 2008; 307:129–140. [PubMed: 17851734]
28. Zumla A, Rao M, Parida SK, Keshavjee S, Cassell G, Wallis R, Axelsson-Robertsson R, Doherty M, Andersson J, Maeurer M. Inflammation and tuberculosis: Host-directed therapies. *J Intern Med*. 2015; 277:373–387. [PubMed: 24717092]
29. Yang SR, Wright J, Bauter M, Seweryniak K, Kode A, Rahman I. Sirtuin regulates cigarette smoke-induced proinflammatory mediator release via RelA/p65 NF- κ B in macrophages in vitro and in rat lungs in vivo: Implications for chronic inflammation and aging. *Am J Physiol Lung Cell Mol Physiol*. 2007; 292:L567–L576. [PubMed: 17041012]
30. Schug TT, Xu Q, Gao H, Peres-da-Silva A, Draper DW, Fessler MB, Purushotham A, Li X. Myeloid deletion of SIRT1 induces inflammatory signaling in response to environmental stress. *Mol Cell Biol*. 2010; 30:4712–4721. [PubMed: 20647536]
31. Koo MS, Manca C, Yang G, O'Brien P, Sung N, Tsenova L, Subbian S, Fallows D, Muller G, Ehrt S, Kaplan G. Phosphodiesterase 4 inhibition reduces innate immunity and improves isoniazid clearance of *Mycobacterium tuberculosis* in the lungs of infected mice. *PLOS ONE*. 2011; 6:e17091. [PubMed: 21364878]
32. Lyadova IV, Eruslanov EB, Khaidukov SV, Yermeev VV, Majorov KB, Pichugin AV, Nikonenko BV, Kondratieva TK, Apt AS. Comparative analysis of T lymphocytes recovered from the lungs of mice genetically susceptible, resistant, and hyperresistant to *Mycobacterium tuberculosis*-triggered disease. *J Immunol*. 2000; 165:5921–5931. [PubMed: 11067954]
33. Lyadova IV, Tsiganov EN, Kapina MA, Shepelkova GS, Sosunov VV, Radaeva TV, Majorov KB, Shmitova NS, van den Ham HJ, Ganusov VV, De Boer RJ, Racine R, Winslow GM. In mice, tuberculosis progression is associated with intensive inflammatory response and the accumulation of Gr-1^{dim} cells in the lungs. *PLOS ONE*. 2010; 5:e10469. [PubMed: 20454613]
34. Bandura DR, Baranov VI, Ornatsky OI, Antonov A, Kinach R, Lou X, Pavlov S, Vorobiev S, Dick JE, Tanner SD. Mass cytometry: Technique for real time single cell multitarget immunoassay based on inductively coupled plasma time-of-flight mass spectrometry. *Anal Chem*. 2009; 81:6813–6822. [PubMed: 19601617]
35. Becher B, Schlitzer A, Chen J, Mair F, Sumatoh HR, Teng KWW, Low D, Ruedl C, Riccardi-Castagnoli P, Poidinger M, Greter M, Ginhoux F, Newell EW. High-dimensional analysis of the murine myeloid cell system. *Nat Immunol*. 2014; 15:1181–1189. [PubMed: 25306126]
36. Desquiere-Dumas V, Gueguen N, Leman G, Baron S, Nivet-Antoine V, Chupin S, Chevrollier A, Vessires E, Ayer A, Ferr M, Bonneau D, Henrion D, Reynier P, Procaccio V. Resveratrol induces a mitochondrial complex I-dependent increase in NADH oxidation responsible for sirtuin activation in liver cells. *J Biol Chem*. 2013; 288:36662–36675. [PubMed: 24178296]
37. Cote CD, Rasmussen BA, Duca FA, Zadeh-Tahmasebi M, Baur JA, Daljeet M, Breen DM, Filippi BM, Lam TK. Resveratrol activates duodenal Sirt1 to reverse insulin resistance in rats through a neuronal network. *Nat Med*. 2015; 21:498–505. [PubMed: 25849131]
38. Sun J, Siroy A, Lokareddy RK, Speer A, Doornbos KS, Cingolani G, Niederweis M. The tuberculosis necrotizing toxin kills macrophages by hydrolyzing NAD. *Nat Struct Mol Biol*. 2015; 22:672–678. [PubMed: 26237511]
39. Lee IH, Cao L, Mostoslavsky R, Lombard DB, Liu J, Bruns NE, Tsokos M, Alt FW, Finkel T. A role for the NAD-dependent deacetylase Sirt1 in the regulation of autophagy. *Proc Natl Acad Sci USA*. 2008; 105:3374–3379. [PubMed: 18296641]
40. Wang Y, Liang Y, Vanhoutte PM. SIRT1 and AMPK in regulating mammalian senescence: A critical review and a working model. *FEBS Lett*. 2011; 585:986–994. [PubMed: 21130086]

41. Mihaylova MM, Shaw RJ. The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nat Cell Biol.* 2011; 13:1016–1023. [PubMed: 21892142]
42. Salminen A, Hyttinen JMT, Kaarniranta K. AMP-activated protein kinase inhibits NF- κ B signaling and inflammation: Impact on healthspan and lifespan. *J Mol Med.* 2011; 89:667–676. [PubMed: 21431325]
43. Yang H, Zhang W, Pan H, Feldser HG, Lainez E, Miller C, Leung S, Zhong Z, Zhao H, Sweitzer S, Considine T, Riera T, Suri V, White B, Ellis JL, Vlasuk GP, Loh C. SIRT1 activators suppress inflammatory responses through promotion of p65 deacetylation and inhibition of NF- κ B activity. *PLOS ONE.* 2012; 7:e46364. [PubMed: 23029496]
44. Antonelli LRV, Gigliotti Rothfuchs A, Goncalves R, Roff E, Cheever AW, Bafica A, Salazar AM, Feng CG, Sher A. Intranasal Poly-IC treatment exacerbates tuberculosis in mice through the pulmonary recruitment of a pathogen-permissive monocyte/macrophage population. *J Clin Invest.* 2010; 120:1674–1682. [PubMed: 20389020]
45. McClean CM, Tobin DM. Macrophage form, function and phenotype in mycobacterial infection: Lessons from tuberculosis and other diseases. *Pathog Dis.* 2016; 74:ftw068. [PubMed: 27402783]
46. Serbina NV, Pamer EG. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat Immunol.* 2006; 7:311–317. [PubMed: 16462739]
47. Srivastava S, Ernst JD, Desvignes L. Beyond macrophages: The diversity of mononuclear cells in tuberculosis. *Immunol Rev.* 2014; 262:179–192. [PubMed: 25319335]
48. Samsami-Kor M, Daryani NE, Asl PR, Hekmatdoost A. Anti-inflammatory effects of resveratrol in patients with ulcerative colitis: A randomized, double-blind, placebo-controlled pilot study. *Arch Med Res.* 2015; 46:280–285. [PubMed: 26002728]
49. Chakrabarti SK, James JC, Mirmira RG. Quantitative assessment of gene targeting in vitro and in vivo by the pancreatic transcription factor, Pdx1. Importance of chromatin structure in directing promoter binding. *J Biol Chem.* 2002; 277:13286–13293. [PubMed: 11825903]
50. Newell EW, Sigal N, Nair N, Kidd BA, Greenberg HB, Davis MM. Combinatorial tetramer staining and mass cytometry analysis facilitate T-cell epitope mapping and characterization. *Nat Biotechnol.* 2013; 31:623–629. [PubMed: 23748502]
51. Bolstad BM, Irizarry RA, Astrand M, Speed TP. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics.* 2003; 19:185–193. [PubMed: 12538238]
52. Smyth GK. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol.* 2004; 3(Article3)
53. Mehra S, Alvarez X, Didier PJ, Doyle LA, Blanchard JL, Lackner AA, Kaushal D. Granuloma correlates of protection against tuberculosis and mechanisms of immune modulation by *Mycobacterium tuberculosis*. *J Infect Dis.* 2013; 207:1115–1127. [PubMed: 23255564]
54. Kaushal D, Foreman TW, Gautam US, Alvarez X, Adekambi T, Rangel-Moreno J, Golden NA, Johnson AMF, Phillips BL, Ahsan MH, Russell-Lodrigue KE, Doyle LA, Roy CJ, Didier PJ, Blanchard JL, Rengarajan J, Lackner AA, Khader SA, Mehra S. Mucosal vaccination with attenuated *Mycobacterium tuberculosis* induces strong central memory responses and protects against tuberculosis. *Nat Commun.* 2015; 6:8533. [PubMed: 26460802]

Fig. 1. *Mtb* Infection down-regulates SIRT1 expression

(A) *SIRT1* mRNA was assessed by qRT-PCR in *Mtb*-infected THP-1 cells. *SIRT1* expression normalized to GAPDH expression, relative to uninfected (UN) cells, is shown. (B) Western blot analysis of SIRT1 and GAPDH (control) of *Mtb*-infected THP-1 cells as in (A). Uncropped image is provided in fig. S12A. (C) *SIRT1* mRNA expression in the lungs of *Mtb*-infected mice. d, day; UN, uninfected mice ($n = 4$). (D) *SIRT1* mRNA expression in the lungs of macaques with active or latent TB. Fold change of *SIRT1* expression in lesions versus normal lung tissue is shown ($n = 10$ to 12). (E) Immunostaining of lung tissue of a representative uninfected and *Mtb*-infected macaques (either BCG-vaccinated or unvaccinated). Red, SIRT1 staining; green, nuclear staining. NC, necrotic core of granuloma. Magnification, ×20. Scale bars, 100 μm. (F) Percentage of pulmonary cells expressing SIRT1 from (E) ($n = 8$ to 20). (G to J) Raw intensity values for *SIRT1* mRNA expression in the data set of different cohorts, that is, UK 2010, South Africa 2010, South

Africa 2014, and China 2014. Red bars in (G) to (J) indicate the median. Data in (A) to (C) are representative of three to four independent experiments, expressed as means $\pm$ SEM, and analyzed by two-tailed Student's *t* test. Data in (D) and (F) to (I) are analyzed by Mann-Whitney *U* test. Data in (J) are analyzed by paired Wilcoxon signed-rank test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001. Exact *P* values are provided in table S7.

Fig. 2. SIRT1 activators enhance control of *Mtb* growth

(A) *Mtb* growth after 24 hours in scrambled control [wild-type (WT)] and SIRT1 knockdown (SIRT1^{-/-}) THP-1 cells presented as fold change compared with uninfected cells. An average of three independent experiments is shown. (B) *Mtb* growth after treatment of THP-1 cells with vehicle control or 100 μM RES. (C) *Mtb* growth after 24 hours in THP-1 cells treated with different doses of SRT. (D) *Mtb* growth after treatment of THP-1 cells with 5 μM SRT. (E) *Mtb* growth after 24 hours in WT and SIRT1^{-/-} THP-1 cells treated with 5 μM SRT or 100 μM RES. Data are presented as fold change relative to vehicle control. (F) Growth of *Mtb* MDR strains after 72 hours in THP-1 cells treated with 100 μM INH, 5 μM SRT, or 100 μM RES. The name of each strain is indicated. Growth restriction by SRT and RES is significant over control by ANOVA. (G) Scatterplot of differentially expressed autophagy genes in *Mtb*-infected THP-1 cells (I) versus uninfected cells (U), and *Mtb*-infected THP-1 cells treated with RES (R) versus untreated infected cells (I). Positive and

negative regulators of autophagy are enriched in upper left and lower right quadrants, respectively. Ifc, log fold change. **(H)** *Mtb*-infected (IN) WT or SIRT1^{-/-} THP-1 cells were treated with 5 μ M SRT, 100 μ M RES, or 2.5 μ M rapamycin (Rapa) (positive control) for 4 hours, stained for LC3, and analyzed by flow cytometry. Representative histogram plot of five independent experiments is shown. Gray histogram, untreated cells. Quantification data of LC3B MFI (mean fluorescence intensity) are presented. **(I)** WT (DT), WT, or SIRT1^{-/-} THP-1 cells were infected with *Mtb* and treated with 5 μ M SRT for 24 hours. Cell lysates were subjected to immunoblot analysis for LC3 and GAPDH. LC3 II/LC3 I ratio is indicated. Uncropped image is provided in fig. S12B. **(J)** BCG growth after 24 hours in THP-1 cells treated or not with 5 μ M SRT and 3-methyladenine (3-MA; 10 mM) or wortmannin (Wt; 5 μ M). **(K)** THP-1 cells were infected with BCG-GFP and incubated with or without 5 μ M SRT, 1 μ M EX527 (EX) and EX + SRT, and 500 nM LTR for 4 hours before fixation. Scale bars, 5 μ m. Zoomed view of boxed section is presented in the last column. **(L)** Quantification of LTR-positive BCG-GFP as in (K). Data in (B) to (E) and (I) to (L) are representative of three independent experiments. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001 by two-tailed Student's t test. Exact P values are provided in table S8.

Fig. 3. SIRT1 activators normalize *Mtb*-induced inflammatory responses

(A) Circos figure depicting GO pathways modulated in *Mtb*-infected THP-1 cells treated or not with RES (24 hours). Bars in the gray circle represent the number of genes in each GO category. Bars in the yellow circle represent the number of genes in particular GO category that were significantly changed upon infection [I (infected) versus U (uninfected)]. Bars in the blue circle show the number of genes significantly modulated by RES treatment in the infected cells [R (RES-treated, infected) versus I (infected)]. A cutoff of 1×10^{-4} was used for the GO pathways. Black lines indicate pathways sharing at least 50% of genes. The thickness of the line shows the extent of overlaps between the two ontologies. (B) Comparison of 149 genes from inflammatory response (GO:0006954) shown in (A), present within 3062 genes that are modulated by RES (see fig. S4, A and B). Heat map displays absolute expression values of the up-regulated (yellow) and down-regulated (blue) genes in the respective analysis. (C) mRNA expression of *IL6*, *TNFα*, *MCP-1*, and *IL1B* in *Mtb*-

infected THP-1 cells, treated or not with 5 μ M SRT over a period of 72 hours. **(D)** Estimation of IL-1 β , IL-6, and MCP-1 in the culture supernatant of *Mtb*-infected THP-1 cells at 24 hours after infection, treated with 5 μ M SRT or control [dimethyl sulfoxide (DMSO)], measured by ELISA. **(E)** mRNA expression of *RelA/p65* as in (C). **(F)** Immunoblots of cell lysates from uninfected (U N), lipopolysaccharide (LPS; 100 ng/ml)–stimulated, and *Mtb*-infected THP-1 cells, treated or not with 5 μ M SRT over a period of 72 hours. Uncropped image is provided in fig. S12C. **(G)** Relative protein band density of RelA/p65 and acetylated RelA/p65 normalized to GAPDH at 72 hours. **(H)** Effect of SRT treatment on RelA/p65 recruitment to *TNF α* , *IL1B*, *IL6*, and *MCP-1* promoters in *Mtb*-infected THP-1 cells. Aliquots of chromatin were obtained before (input) or after immunoprecipitation. Isolated chromatin was quantified by real-time PCR. Data are expressed as the fold change over the levels detected in the uninfected cells after correcting for differences in the amount of starting (input) chromatin material. Representative data from three (C to E) and two (F to H) independent experiments are shown. Data are means $\pm$ SEM performed in triplicate. * P < 0.05, ** P < 0.01 by two-tailed Student's t test. Exact P values are provided in table S9.

Fig. 4. SIRT1 activation reduces *Mtb* growth in mice

(A) Bacillary load in the lungs of control (WT) and Mac-SIRT1 KO mice infected with *Mtb* on day 21 after infection. Bar indicates the median. (B) *Mtb*-infected mice were treated with RES (50 or 100 mg/kg) starting 7 days after infection. Bacillary loads were enumerated in the lungs and spleen on days 1, 7, 21, and 35 after infection. Control, untreated infected mice. (C) *Mtb*-infected mice were treated with SRT (100 mg/kg) starting 7 days after infection. Bacillary loads were enumerated in the lungs and spleen on days 1, 7, 21, and 35 after infection. (D) *Mtb*-infected mice were treated with SRT (100 mg/kg) starting 40 days after infection. Bacillary loads in the lungs were enumerated on days 1, 7, 40, 55, and 68 after infection. $n = 8$ to 12 mice per group per time point. Data are means $\pm$ SEM. (A to D) Combined data of two independent experiments are shown. * $P < 0.05$, ** $P < 0.01$ by Mann-Whitney *U* test. Exact *P* values are provided in table S10.

Fig. 5. SIRT1 activation reduces TB-associated tissue pathology and inflammation in mice
(A) Light micrographs of H&E staining of representative lung sections on day 35 after infection from *Mtb*-infected mice, treated or not with RES (100 mg/kg). Scale bars, 500 μ m ($\times 4$ magnification) and 50 μ m ($\times 40$ magnification). IN, *Mtb*-infected mice; IN + RES, RES-treated, *Mtb*-infected mice. **(B)** Percentage of lung area involved in disease pathology in IN and IN + RES mice. **(C)** Percentage of lung area involved in disease pathology of IN mice treated or not with SRT (100 mg/kg) on day 35 after infection. IN + SRT, SRT-treated, *Mtb*-infected mice. **(D)** mRNA expression was assessed by qRT-PCR in the lung RNA from IN and IN + SRT mice on day 35 after infection. **(E)** Absolute values of the inflammatory chemokines and cytokines from the lungs of *Mtb*-infected mice as in (D), measured by Luminex. **(F)** WT (control) and Mac-SIRT1 KO mice were infected with *Mtb*. On day 14, lung mRNA expression was assessed by qRT-PCR. **(G)** Estimation of IL-1 β , TNF α , and MCP-1 in the lung homogenate of mice in (F) as measured by ELISA. Bar lines in (B) and (C) represent the median. Data in (D) to (G) are representative of two independent experiments. Data are means $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$ by Mann-Whitney U test. Exact P values are provided in table S11.

Fig. 6. SIRT1 activation modulates the lung myeloid landscape in *Mtb*-infected mice
 (A) tSNE analysis of single-cell data from lung tissues of analyzed mice. Cells were plotted and color-coded by the 28 “unsupervised” DensVM clusters. A grouped description of each cluster is indicated. Detailed characterization of each cluster is shown in fig. S8. IMs, interstitial macrophages; dim, dimension; pDC, plasmacytoid DC; AM, alveolar macrophage. (B) Heat plot summary of normalized cluster frequency (unsupervised clustering) across the different groups of mice, treated or not with SRT (100 mg/kg). UN, uninfected mice; UN + SRT, uninfected mice treated with SRT; IN, *Mtb*-infected mice; IN + SRT, *Mtb*-infected mice treated with SRT. *, clusters that were significantly altered in IN versus UN; +, clusters that were significantly altered in IN + SRT versus IN. (C) Mass cytometry data were analyzed by manual gating strategy. MQs, macrophages. (D) tSNE analysis of cellular composition of lung tissues derived from UN, IN, and IN + SRT mice, highlighting six SIRT1 modulated clusters. Each plot represents a pool of three to four lung

tissue cells. Ungrouped cells (remaining) are shown in gray. **(E)** Mean frequencies of Ly6C subsets among CD45⁺ myeloid cells. Mass cytometry data were analyzed by manual gating strategy. **(F and G)** Fluorescent flow cytometric analysis of lung cells from SRT-treated, *Mtb*-infected animals. PerCP, peridinin chlorophyll; PE/Cy7, phycoerythrin/cyanin 7. **(H)** Number of lung monocytes in WT and Mac-SIRT1 KO mice on day 3 after infection. Data are means $\pm$ SEM. $n = 4$ to 6 mice per group per time point. * $P < 0.05$, ** $P < 0.01$, # $P < 0.005$ by Mann-Whitney U test. Exact P values are provided in tables S12 and S13. FACS, fluorescence-activated cell sorting.

Table 1
SRT treatment enhances efficacy of INH in *Mtb*-infected mice

Mouse Model	Lungs		Spleen	
	INH [*]	SRT + INH [†]	INH	SRT + INH
Acute [‡]				
Experiment 1	2/4 (50%) [§]	4/5 (80%)	4/5 (80%)	5/5 (100%)
Experiment 2	1/4 (25%)	1/4 (25%)	0/5 (0%)	5/5 (100%)
Chronic	10.7 ± 2.5 × 10 ⁴ [¶]	4.9 ± 2.8 × 10 ⁴	Not performed	Not performed

^{*} *Mtb*-infected mice were administered with INH (10 mg/kg).

[†] *Mtb*-infected mice were coadministered with INH and SRT (100 mg/kg).

[‡] Acute TB model proportion of mice with no detectable CFU in the lungs and spleen during INH therapy with and without SRT, after 4 weeks of treatment in two different experiments.

[§] Number of mice with no detectable CFU out of total number of animals in that group (in bracket, percentage information is provided).

^{||} In a chronic TB model bacterial load (CFU) in the lung of INH or SRT + INH-treated mice after 2 weeks of treatment.

[¶] Mean CFU values ($n = 5$). Difference in CFU values between INH and SRT + INH group is significant ($P = 0.0317$ by Mann-Whitney U test).