CinA mediates multidrug tolerance in *Mycobacterium tuberculosis*
Killing 1.5 million people last year, *Mycobacterium tuberculosis* (*Mtb*) remains the leading cause of death by a single infectious bacterium. Partly responsible for the lack of success in fighting tuberculosis (TB) is the length and complexity of the required drug regimen, which still consists of at least 6 months of combination therapy with up to four drugs when TB of the required drug regimen, which still consists of at least two conditions in which tolerance to the drug is well documented: adaptation to IFNγ-activated primary mouse bone marrow-derived macrophages (BMDM) and starvation in phosphate-buffered saline (PBS). In BMDMs, the mutant library was subjected to two cycles of infection. Each time, isoniazid treatment was initiated 24 h post infection at which point the bacteria have been internalized by the macrophages. Comparing the relative abundance of each mutant in the input pool to the mutant pool recovered after the second BMDM infection identified 18 mutants that were significantly underrepresented after intracellular exposure to isoniazid (Supplementary Fig. 1a and Supplementary Data 1). Seventeen of these mutants were also underrepresented following infection of DMSO-treated control BMDMs, leaving a single isoniazid-specific hit with transposon insertions in *cinA* (rv1901). Similarly, *cinA* mutants were the only mutants underrepresented with statistical significance after treatment of PBS-starved *Mtb* with isoniazid (Supplementary Fig. 1b).

**CinA confers isoniazid tolerance in macrophages and in vitro.** To confirm that CinA imparts isoniazid tolerance to non-replicating *Mtb*, we constructed a *cinA* deletion mutant (Δ*cinA*) and confirmed loss of CinA by immunoblot (Supplementary Fig. 2a). Both, wild-type *Mtb* and the Δ*cinA* mutant survived equally well during nutrient starvation in PBS and as expected, isoniazid did not kill wild-type *Mtb* in PBS. In contrast, the Δ*cinA* mutant suffered from a marked loss in viability when exposed to isoniazid during PBS starvation (Fig. 1a). The Δ*cinA* mutant was also killed in resting and IFNγ-activated macrophages by a concentration of isoniazid that affected neither wild-type *Mtb* nor the complemented mutant (Fig. 1b, c). In contrast to its impact on isoniazid, deletion of *cinA* did not affect the activity of rifampicin against *Mtb* in PBS or macrophages (Fig. 1a–c).

In standard liquid culture, the minimum inhibitory concentrations (MICs) of isoniazid and rifampicin were similar for the Δ*cinA* mutant and wild-type *Mtb* (Fig. 1d), indicating that CinA does not mediate intrinsic resistance to isoniazid. However, the kinetics of isoniazid-mediated killing in liquid cultures were faster for the Δ*cinA* mutant than for wild-type *Mtb*. Although the kill kinetics maintained the characteristic biphasic pattern revealing phenotypically tolerant persistier cells, the persistor population was reduced up to 61-fold in the Δ*cinA* mutant compared to wild-type *Mtb* (Fig. 1e).

Deletion of CinA potentiates isoniazid treatment during chronic mouse infection. In mice, the Δ*cinA* mutant replicated normally during acute infection and survived equally well as wild-type when *Mtb* entered the chronic phase of infection (Fig. 2a and Supplementary Fig. 3a). In vivo isoniazid treatment leads to effective killing of *Mtb* during the acute phase of infection; however, after chronic infection is established isoniazid becomes substantially less potent. When infected mice were treated with isoniazid for 12 weeks, starting 6 weeks post aerosol infection, the Δ*cinA* mutant was killed more efficiently than wild-type *Mtb* (Fig. 2b–d and Supplementary Fig. 3b–e). Isoniazid at 10 mg/kg/day reduced bacterial burden of wild-type *Mtb* in lungs by 200-fold in 12 weeks, whereas Δ*cinA* mutant titers declined almost 6000-fold from two million CFU at the beginning of isoniazid treatment to 360 CFU (Fig. 2b and Supplementary Fig. 3e). At 5 mg/kg/day viability of the Δ*cinA* mutant decreased by three orders of magnitude (1200-fold) while wild-type *Mtb* decreased
less than 10-fold (Fig. 2c and Supplementary Fig. 3e). Even isoniazid concentrations as low as 2.5 mg/kg/day, which did not affect the viability of wild-type Mtb at all, markedly reduced viability of the ΔcinA mutant (Fig. 2d and Supplementary Fig. 3e). This is relevant because standard doses of isoniazid failed to sustain therapeutic levels in the majority of distinct pulmonary lung lesions including caseum where isoniazid-tolerant persisters reside25. The heightened susceptibility to isoniazid was also observed for ΔcinA mutant bacilli residing in mouse spleens (Supplementary Fig. 3b–e) and has been observed in a study that screened transposon mutants for altered susceptibility to the first-line antibiotics in mice26. Clearly, deletion of cinA potentiates the activity of isoniazid during chronic infection in mice.

CinA requires an intact pyrophosphatase domain to confer tolerance to isoniazid, ethionamide, delamanid, and pretomanid. As CinA consists of two independent functional domains, an N-terminal COG1058 domain and a C-terminal...
PncC domain, we sought to identify which domain confers isoniazid tolerance. We complemented the ΔcinA mutant with CinA proteins containing point mutations that abrogate the enzymatic activity of either the COG1058 pyrophosphatase domain (CinAD308A) or the PncC deamidase domain (CinAK323A) (Supplementary Fig. 2b)20,21,27. Both CinAD308A and CinAK323A were well expressed in the ΔcinA mutant (Supplementary Fig. 2b), but only CinAK323A complemented the tolerance defect of PBS-starved ΔcinA (Fig. 3a). This suggested that isoniazid tolerance mediated by CinA is due to its pyrophosphatase activity.

The NADH pyrophosphatase (NudC) of M. bovis can cleave the isoniazid-NAD adduct into AMP and isoniazid-nicotinamide mononucleotide, and its expression in M. smegmatis caused resistance to isoniazid and ethionamide28. In Mtb NudC is inactive due to a polymorphism at a highly conserved residue, P237, that is critical for activity28. We speculated that the pyrophosphatase domain of CinA might confer tolerance to isoniazid through direct cleavage of the isoniazid-NAD adduct. If so, CinA might also mediate tolerance to other antibiotics whose activity depends on the presence of an NAD adduct, such as ethionamide28. Indeed, ethionamide killed PBS-starved ΔcinA bacilli but did not affect viability of PBS-starved wild-type Mtb (Fig. 3a). Two other drugs approved for treatment of TB were recently shown (delamanid) or speculated (pretomanid) to act through an NAD adduct22. Delamanid and pretomanid both killed non-replicating ΔcinA bacilli better than wild-type Mtb; however, the differences observed for pretomanid were not as drastic as those observed for the other three drugs (Fig. 3a).

Mtb residing in caseum is extremely drug tolerant23. Therefore, we tested the impact of isoniazid and pretomanid on ΔcinA residing in a caseum mimetic30. Following a 4-week incubation in lipid-rich caseum mimic, non-replicating bacteria were treated with increasing concentrations of isoniazid and pretomanid for 7 days (Fig. 3b). The minimal bactericidal concentrations of isoniazid and pretomanid required to reduce viability of ΔcinA 10-fold (MBCcinA) were 10- and 15-fold lower, respectively, compared to the MBCcinA against wild-type Mtb and the complemented strain. Of note, the impact of deletion of cinA on the pretomanid bactericidal activity was concentration dependent. Above 8 μM, the difference in kill kinetics between wild-type and ΔcinA disappeared.

As observed for isoniazid, deletion of cinA also accelerated killing of replicating Mtb by ethionamide, delamanid and, to a lesser extent, pretomanid, but not rifampicin (Fig. 3c). Because the MIC of pretomanid was approximately fourfold lower against ΔcinA than wild-type Mtb (Fig. 3d), we used pretomanid at two concentrations, 15 × MIC against ΔcinA (1 μg/ml) and 15 × MIC against wild-type (4.5 μg/ml). The difference in pretomanid kill kinetics was concentration dependent similar to what we observed in the caseum mimetic; at 1 μg/ml pretomanid selectively killed ΔcinA but did not affect viability of wild-type Mtb, while increasing the pretomanid concentration to 4.5 μg/ml resulted in a similar killing of wild-type and ΔcinA (Supplementary Fig. 4a). Prolonged exposure to pretomanid and isoniazid demonstrated that drug-resistant mutants emerged around the same time (Supplementary Fig. 4a, b). However, since ΔcinA was killed more rapidly before resistant mutants took over, the absolute number of resistant ΔcinA bacteria was smaller than the number of resistant wild-type Mtb at every time point.

The MICs of rifampicin, isoniazid, ethionamide, and delamanid in liquid culture were not significantly affected by deletion of cinA (Fig. 3d and Supplementary Table 1). Increased killing of ΔcinA by these drugs was thus due to a reduction in drug tolerance. In the case of pretomanid a small MIC shift (~4.5-fold) was observed indicating increased susceptibility of the mutant which might be a result of CinA contributing to intrinsic resistance to that drug (Fig. 3d and Supplementary Table 1). All phenotypes of ΔcinA were complemented by CinA and CinAK323A but not CinAD308A (Fig. 3 and Supplementary Table 1). Thus, CinA, via its pyrophosphatase domain, confers tolerance to isoniazid, ethionamide, delamanid, and partial resistance to pretomanid.

CinA overexpression increases isoniazid tolerance. To assess the impact of CinA overexpression on Mtb drug tolerance and resistance, we expressed cinA under the control of an hydrotetracycline (atc) inducible promoter (cinA-TetON). This resulted in CinA overexpression in both the absence and presence of atc, likely because of leaky transcription by the TetR-controlled promoter (Fig. 4a). CinA-TetON was as tolerant to isoniazid as wild-type Mtb and in the presence of atc cinA-TetON survived isoniazid exposure without loss of viability while CFU of wild-type and the uninduced cinA-TetON declined 10-fold (Fig. 4b). Treatment of cinA-TetON with atc resulted in a significant growth defect (Supplementary Fig. 4c, d) and did not increase the MIC of isoniazid more than observed with the uninduced strain. We therefore determined MICs of other drugs using cinA-TetON without the addition of atc. This demonstrated that CinA overexpression resulted in a small and reproducible increase of the isoniazid and ethionamide MICs, but did not affect the MICs of delamanid, pretomanid and rifampicin compared to those against wild-type Mtb (Fig. 4c).

Deletion of CinA results in accumulation of NAD adducts in isoniazid and ethionamide treated Mtb. To substantiate the hypothesis that CinA is capable of cleaving NAD-drug adducts, we measured the impact of deleting cinA on the accumulation of isoniazid-NAD adducts in Mtb. We first exposed Mtb to isoniazid and prepared extracts for analysis by liquid chromatography-coupled tandem mass spectrometry (LC-MS/MS). This analysis revealed a molecule that: (i) matched both the predicted mass of the isoniazid-NAD adduct ([M + H] = 771.153) and observed chromatographic retention time and mass of a chemically synthesized isoniazid-NAD standard with a mass error <10 ppm, (ii) was only observed in isoniazid-treated samples; and (iii) exhibited a fragmentation pattern consistent with its predicted chemical structure and observed with a chemical standard synthesized by chemical oxidation of INH with stoichiometric amounts of manganese pyrophosphate and NAD(H) (Fig. 5a)31,32. The observed high-resolution fragmentation pattern specifically included mass ions corresponding to the adenosine diphosphate ([M + H] = 428.03540) and adenosine ([M + H] = 136.063) moieties of the isoniazid-NAD adduct. The intensity of these peaks increased in a dose-dependent fashion, with the mutant accumulating approximately threefold higher levels of adduct than wild-type (Fig. 5b). As anticipated, this accumulation was only restored to wild-type levels by complementation with a CinA allele harboring a functional pyrophosphatase domain (Fig. 5c) and overexpression of CinA resulted in a further reduction in isoniazid-NAD adduct levels (Supplementary Fig. 4e). Mtb exposed to ethionamide revealed a similar mass corresponding to an ethionamide-NAD adduct—fragmentation of which also confirmed the presence of an adenosine diphosphate and adenine moiety—suggesting the mutant also accumulated higher levels of the ethionamide-NAD adduct than wild-type Mtb (Supplementary Fig. 5).

Given the apparent role of CinA-mediated cleavage of NAD-drug adducts in tolerance to both isoniazid and ethionamide, we tested the role of this activity in mediating tolerance to the nitroimidazole class of anti-tubercular compounds. To do so, we exposed Mtb to pretomanid and sought evidence of molecules that met the following criteria: they were specific to pretomanid-
Fig. 3 CinA requires an intact pyrophosphatase domain to confer tolerance to isoniazid, ethionamide, delamanid, and pretomanid. a Bacteria were starved in PBS for 21 days and then exposed to 0.5 μg/ml isoniazid (INH), 50 μg/ml ethionamide (ETH), or were starved for 14 days and then exposed to 0.5 μg/ml and 1 μg/ml delamanid (DMD), 1 μg/ml pretomanid (PMD) or equal amount of DMSO for 7 days and cultured on agar plates for CFU enumeration. Data are means ± SEM from two independent experiments each with triplicate cultures, except for PMD data, which are from one experiment with triplicate cultures. Statistical significance was assessed by one-way ANOVA followed by Dunnett’s multiple comparison test. **P < 0.01, ***P < 0.001, ****P < 0.0001.

b CFU quantification of the indicated strains after incubation in caseum mimetic for 4 weeks followed by 1 week treatment with isoniazid or pretomanid at the indicated concentrations. The stippled lines indicate a 10-fold reduction of wild-type and ΔcinA relative to DMSO-treated cultures and were used to determine the MBC90 for these strains. Data are means ± SD of triplicate cultures and representative of two experiments.

c CFU quantification of the indicated strains after incubation with 0.5 μg/ml isoniazid, 4.5 μg/ml ethionamide, 50 μg/ml delamanid, 1 μg/ml pretomanid, and 5 μg/ml rifampicin in standard growth media. Data are means ± SEM from one or two independent experiments each with triplicate cultures. Statistical significance of the difference between wild-type and ΔcinA was assessed by two-tailed, unpaired t-test, *P < 0.05; ****P < 0.0001.

d Impact of antibiotics on growth of the indicated strains. Data are means ± SEM of triplicate cultures and representative of at least two experiments. Source data are provided as a Source Data file.
treated *Mtb*, as reported by their absence in both untreated cells and cells treated with INH or ETH; displayed a dose-dependent increase in cells exposed to pretomanid and were structurally consistent with addition to a NAD molecule. We identified one such ion ([M + H] = 979.1955) that matched these criteria and yielded fragmentation products corresponding to an in silico prediction of a putative pretomanid-NAD adduct33, as a chemical standard could not be synthesized (Fig. 5d, e). Analogous to the isoniazid- and ethionamide-NAD adducts, this included fragmentation products corresponding to the adenine, adenosine, and nicotinamide riboside-pretomanid moieties of NAD/H ([M + H] = 136.062, 250.093, 632.124, respectively)33. Relative quantitation of the pretomanid-NAD adduct in pretomanid-treated *Mtb* revealed accumulation in ΔcinA, which was reduced in the complemented strain. *Mtb* expressing CinAΔ329A (non-functional pyrophosphatase domain) accumulated pretomanid-NAD similar to ΔcinA, while expression of CinAΔK323A (with an inactive PncC deamidase domain) resulted in levels similar to those in the complemented strain (Fig. 5e). These results were consistent with those obtained in isoniazid-treated *Mtb* (Fig. 5c) and corresponded to the phenotypic susceptibility of these strains to pretomanid (Fig. 3c, d). Taken together, these results support a model in which CinA mediates tolerance to isoniazid, ethionamide, and the nitroimidazole pretomanid via cleavage of their corresponding NAD adducts through its pyrophosphatase domain.

**Absence of CinA potentiates the efficacy of BPaL.** The Nix-TB trial revealed that short course treatment with a combination of bedaquiline, pretomanid, and linezolid (BPaL) had a favorable outcome in 90% of patients with multidrug-resistant TB23. To test if CinA inactivation affects the efficacy of BPaL, we infected mice with wild-type *Mtb* or ΔcinA and 4 weeks later treated them with a BPaL regimen for 4 weeks (Fig. 6 and Supplementary Fig. 6a). This resulted in substantially more killing of ΔcinA relative to wild-type *Mtb* especially in mouse lungs where ΔcinA CFU declined over 100-fold, while wild-type *Mtb* CFU declined 10-fold. CinA deletion thus also potentiates the efficacy of BPaL, an FDA-approved regimen for multidrug-resistant forms of TB, in mice.

**Discussion**

Isoniazid, a corner stone of standard *Mtb* drug treatment, kills replicating bacilli in vitro and in vivo. However, when *Mtb* enters a slow- or non-replicating state, for example, due to nutrient starvation, hypoxia, or in response to adaptive immunity, the entire population becomes isoniazid tolerant and persists even in the presence of high isoniazid concentrations24,34. Little is known about the mechanisms leading to isoniazid tolerance when *Mtb* ceases to replicate. To identify genetic factors that aid the survival of *Mtb* in conditions that render the whole population refractory to isoniazid treatment we performed genome-wide screens and identified a single gene, cinA.

Deletion of cinA or inactivation of CinA’s pyrophosphatase domain not only increased killing in conditions where the entire *Mtb* population exhibits isoniazid tolerance, it also resulted in a substantially smaller persister subpopulation within actively replicating *Mtb* cultures. During axenic growth isoniazid rapidly kills more than 99% of the replicating *Mtb* population; however, a small subpopulation of isoniazid persister cells is only killed slowly resulting in a biphasic kill curve35. Stochastic repression of KatG activity, asymmetric cell division, and reversible increases in drug efflux or posttranslational modification can all lead to isoniazid-tolerant persisters36–40. The fraction of persisters present in replicating *Mtb* may thus consist of yet smaller, mechanistically distinct subpopulations. These subpopulations refractory to drug treatment are of particular interest as they are thought to contribute to the unusually long TB treatment and targeting these subpopulations has the potential to shorten TB chemotherapy40. The absence of CinA did not prevent the
emergence of drug-resistant bacteria, but the drug-resistant population of ΔcinA was smaller than the one that emerged in drug-treated wild-type Mtb. This suggests that CinA inactivation could delay the appearance of drug-resistant Mtb. This work also underscores that drug tolerance does not always rely on slowly or non-growing cells, because the pyrophosphatase activity of CinA impaired drug mediated killing of actively replicating Mtb.

CinA mediated tolerance not only to isoniazid but also to ethionamide, delamanid, and pretomanid. The MICs of isoniazid, ethionamide, and delamanid were not different between wild-type Mtb and the cinA mutant, indicating that CinA mediates tolerance and not resistance to these drugs. In the case of pretomanid, however, a small MIC shift was observed leaving the possibility that CinA also confers intrinsic resistance to this drug. Moreover,
Fig. 5 Inactivation of CinA results in accumulation of NAD adducts in isoniazid (INH) and pretomanid (PMD) treated Mtb. a MS/MS fragmentation spectra of a chemically synthesized isoniazid-NAD adduct and corresponding metabolite observed only in isoniazid-treated Mtb with the predicted mass of the INH-NAD adduct. Structures and annotated fragments provide confirmatory evidence of mass matching to the predicted parent adduct (Δppm = 10) and fragments corresponding to the ADP and adenine moieties of the adduct. Boxed masses additionally indicate matching collision energy-dependent fragmentation products observed in both the chemical standard and INH-treated Mtb. Additional non-matching masses correspond to likely fragmentation products of co-gating parent masses that entered the collision cell with the INH-NAD adduct as observed in the D V spectra. b Quantification of the isoniazid-NAD adduct as a function of the isoniazid concentration that the indicated strains were exposed to. c Accumulation of isoniazid-NAD is reversed by expression of CinA with a functional pyrophosphatase domain. Data are means ± SD of six (b) or three (c) cultures. d MS/MS fragmentation spectra of a mass ion corresponding to the predicted mass of a PMD-NAD adduct observed in pretomanid-treated Mtb. Structures and annotated masses provide confirmatory evidence of mass matching to the predicted parent adduct (Δppm = 10) and fragments corresponding to the adenine, adenosine, and nicotinamide riboside-pretomanid moieties of the PMD-NAD adduct. e Quantification of the relative abundance of pretomanid-NAD adduct in the indicated strains. Data are from six independent cultures that were pooled to ensure robust detection of potential differences in adduct levels. Source data are provided as a Source Data file.

![Fig. 5 Inactivation of CinA results in accumulation of NAD adducts in isoniazid (INH) and pretomanid (PMD) treated Mtb.](https://example.com/fig5)

**Fig. 6 Deletion of cinA potentiates the efficacy of BPaL.** Balb/C mice were infected with wild-type and ΔcinA and treated with BPaL (bedaquiline 20 mg/kg/day, pretomanid 50 mg/kg/day, and linezolid 100 mg/kg/day) starting after 4 weeks of infection. CFU recovered from lungs and spleens are normalized to the beginning of BPaL treatment. Data are means ± SD of six mice. Source data are provided as a Source Data file.

![Fig. 6 Deletion of cinA potentiates the efficacy of BPaL.](https://example.com/fig6)

overexpression of CinA increased the MICs of isoniazid and ethionamide but had no impact on the activities of pretomanid or delamanid. All four drugs are pro-drugs whose activation requires a bacterial enzyme. For isoniazid, ethionamide, and delamanid it has been demonstrated that activation leads to NAD-drug adducts; adduct formation of pretomanid has been predicted because of the similarity of this drug to delamanid. We confirmed this prediction by identifying the pretomanid-NAD adduct in live Mtb. This supports our hypothesis that CinA mediates tolerance through cleavage of formed NAD-drug adducts, which is further substantiated by our finding that in the absence of CinA NAD-drug adducts accumulated inside the bacilli during isoniazid, ethionamide, and pretomanid treatment. The different impact of CinA expression on drug tolerance and resistance suggest that the antibiotics we tested inhibit growth and kill Mtb by more than one mechanism and that the NAD adduct is more important for killing than growth inhibition. Similarly, the concentration-dependent hypersusceptibility of ΔcinA to pretomanid implies that the pretomanid-NAD adduct contributes to its bactericidal activity at low concentrations, while at higher concentrations other mechanisms dominate.

In this study we identified a single protein, CinA, as a mediator of tolerance to four TB drugs in clinical use. CinA-mediated tolerance is a direct result of its pyrophosphatase domain facilitating cleavage of NAD-drug adducts. Hence, targeting CinA with inhibitors of its pyrophosphatase domain has the potential to shorten treatment of drug-sensitive TB by potentiating the first-line drug isoniazid or enhancing treatment regimens for multi-and extensively drug-resistant TB by potentiating delamanid and pretomanid. Furthermore, targeting CinA could also complement and extend ongoing efforts to improve the clinical value of ethionamide containing regimens. Clinical precedents for this strategy exist, for example, in the form of Zavicefta in which the β-lactamase inhibitor avibactam strongly potentiates the activity of the cephalosporin ceftazidime to the extent that it is effective against Mtb including highly drug-resistant strains.

**Methods**

**Bacterial strains and media.** Mycobacterium tuberculosis H37Rv was used as the parental strain for all mutants throughout the study. Bacteria were cultured using Middlebrook 7H9 liquid medium supplemented with 0.2% glycerol, 0.05% Tween-80, and ADN (0.5% bovine serum albumin, 0.2% dextrose, 0.085% NaCl) or Middlebrook 7H10 solid agar supplemented with 0.2% glycerol, 10% Middlebrook oleic acid-albumin-dextrose-catalase (OADC) (Becton, Dickinson) and if necessary supplemented with 0.4% activated charcoal. Antibiotics for selection of genetically modified strains were added at the following concentrations: hygromycin (50 µg/ml) and kanamycin (25 µg/ml).

**Drugs.** Isoniazid (INH), Rifampicin (RIF), Ethionamide (ETH), and Pretomanid (PMD) were purchased from Sigma; Delamanid (DMD) was procured from MedKoo Biosciences. All antibiotics were dissolved in DMSO with exception of INH, which when used for treatment of mice, was dissolved in H2O.

**Preparation of murine BMDMs.** Bone marrow cells were harvested from female C57BL/6 mice and differentiated into BMDMs by culturing the cells in the presence of 20% L-cell conditioned medium. Macrophages were seeded at 1 × 10⁶ cells per T75 flasks overnight prior to infection and maintained in INH for the remainder of the infection. Bacteria were grown to early log phase, washed in PBS-Tween-80 0.05% prior to being diluted into DMEM, and added to macrophages at a multiplicity of infection (MOI) of 0.1. After 4 h macrophages were washed twice with warm PBS to remove extracellular bacteria. Intracellular bacteria were enumerated by lysing macrophages with 0.01% Triton X-100 and plating serial dilutions of lysates on 7H10 agar supplemented with 0.4% charcoal.

**TnSeq screen.** Transposon mutant libraries were constructed in wild-type Mtb H37Rv by himar1 mutagenesis. Transposon mutant library stocks were cultured using Middlebrook 7H9 liquid medium supplemented with 0.2% glycerol, 0.05% Tween-80, and ADN (0.5% bovine serum albumin, 0.2% dextrose, 0.085% NaCl) or Middlebrook 7H10 solid agar supplemented with 0.2% glycerol, 10% Middlebrook oleic acid-albumin-dextrose-catalase (OADC) (Becton, Dickinson) and if necessary supplemented with 0.4% activated charcoal. Antibiotics for selection of genetically modified strains were added at the following concentrations: hygromycin (50 µg/ml) and kanamycin (25 µg/ml).

**Transposon mutant libraries were constructed in wild-type Mtb H37Rv by himar1 mutagenesis. Transposon mutant library stocks were cultured using Middlebrook 7H9 liquid medium for 4 days with agititation (100 rpm) to allow recovery. For PBS starvation experiments cultures were washed twice in PBS containing 0.05% tyloxapol and adjusted to an optical density (OD₅₆₀) of 0.2. Libraries were starved for 14 days prior to exposure to 0.5 µg/ml INH or an equal amount of DMSO. After a total of 28 days of starvation libraries were cultured on 7H10 agar supplemented with 0.4% charcoal.

For infection of BMDMs 1 × 10⁷ INH-activated BMDMs were infected with transposon mutant libraries at a MOI of 1. At 24 h post infection (p.i.) 0.1 µg/ml INH or an equivalent amount of DMSO was added to infected BMDMs.

Macrophages were lysed 120 h p.i. and lysates cultured on 7H10 agar supplemented with charcoal. After 21 days of outgrowth colonies recovered from infected macrophages were collected, transferred into liquid medium and grown for 4 days prior to re-infection of BMDMs as before.

Genomic DNA was extracted from the recovered transposon mutant libraries and transposon–chromosome junctions were amplified and sequenced to determine the relative abundance of each transposon mutant in input and INH-treated libraries as described previously. Sequencing reads were processed and
analyzed using TPP and TRANSIT tools from the TRANSIT TnSeq analysis platform, respectively, and transposon–chromosome junctions were mapped to the \textit{Mtb} \textit{H37Rv} reference genome (GenBank accession number NC_018413.1)\textsuperscript{39}.



**Mutant generation and complementation.** The \(\Delta\text{cin}A\) mutant was constructed in \textit{Mtb} by replacing \textit{cinA} (\textit{rv1901}) with a hygromycin cassette through homologous recombination as described elsewhere\textsuperscript{60,61}. The first 100 bp of the open reading frame of \textit{cinA} were retained to avoid disruption of the \textit{rv1900c} promoter. Specifically, nucleotides 101–1195 of \textit{cinA} were replaced with a hygromycin-resistance cassette. To achieve this, \textit{Mtb} \textit{H37Rv} transformed with pNir-RecET-sacb-kan\textsuperscript{−} was grown to mid log phase and expression of the RecET proteins was induced by addition of 10 \(\mu\)M isoviolanolactone. After 8 h of RecET induction the cells were treated with glycine and incubated for 16 h as described\textsuperscript{61}. A DNA fragment containing the hygromycin-resistance cassette flanked by 500 bp of the upstream region including the first 100 bp of \textit{cinA} and 500 bp of the sequence downstream of \textit{cinA} was synthesized (GenScript Biotech) and transformed by electroporation. Knockout candidates were selected on hygromycin-containing agar. Deletion of \textit{cinA} was confirmed by PCR and western blot analysis. Plasmid pNirET-scac-kan was cured by counterselection on 10% sucrose containing agar. A complemented strain was constructed by replacing the attL5 attachment site under the control of a hsp60 promoter. For this, we used plasmids pDE43-MCK (kan\textsuperscript{R}, multisite Gateway destination vector), pEN41A-T02 (ampR, multisite Gateway entry plasmid; containing a hsp60 promoter) and the phage attP sites for site-specific integration into the mycobacterial chromosome. We used plasmids pDE43-MCK (kan\textsuperscript{R}, multisite Gateway destination plasmid), pEN41A-T02 (ampR, multisite Gateway entry plasmid), pDE43-MCK (ampR, multisite Gateway entry plasmid; containing a hsp60 promoter)\textsuperscript{39,62}, and pEN23A-cina (ampR, multisite Gateway entry plasmid; containing \textit{cinA}, nucleotides 28–1193)\textsuperscript{39}. Complementation strains expressing \textit{cinA} harboring point mutations (R80A = 1239G; K323A = arg → gug 967) rendering either the pyrophosphatase or the deamidase domain of the gene inactive were obtained by the same approach\textsuperscript{20,27}. For overexpression we cloned \textit{cinA} downstream of the anhydrotretracycline inducible promoter P606 (ref. \textsuperscript{66}). All DNA constructs were obtained from GenScript Biotech and plasmids were generated using Gateway Cloning Technology (Life Technologies).

**Mouse infection.** The mouse experiments to assess virulence of the \(\Delta\text{cin}A\) mutant and the impact of INH during infection were conducted following guidelines for care and use of laboratory animals provided by the National Institute of Health and with approval from the Institutional Animal Care and Use Committee of Weill Cornell Medicine (IACUC protocol 060–414A). Female 8-week-old C57BL/6 mice (Jackson Laboratory) were infected with 100–200 CFU/mouse using an Inhalation Exposure System (Glaxo). The mice were housed in HEPA-filtered cages within the animal facilities. C57BL/6 mice (Jackson Laboratory) were infected with 8×10\textsuperscript{6} cells in 200 \(\mu\)l of PBS containing 0.5% Tween-80, and dissolved in 20 mM TRIS-HCl pH 7.5 containing 8 M urea. To achieve this, mAbs directed against the caseum mimetic\textsuperscript{30,55} were prepared with the following modifications: to induce lipid droplet formation, PMA-differentiated THP-1 macrophages were infected with irradiated \textit{Mtb} (BEI Resources) at an approximate MOI of 1:50. The foamy macrophages were washed three times with PBS, followed by three freeze–thaw cycles to lyse the cells and incubation at 75 °C for 20–30 min to denature proteins in the matrix\textsuperscript{35}. The lysate was allowed to rest at 37 °C for 3 days, prior to freezing at −80 °C until inoculation. After 4 weeks of \textit{Mtb} incubation in the casemate mimetic, drugs were added at concentrations ranging from 0.125 to 5 μM, in fourfold increment, the samples as well as no-drug controls were incubated for 7 days, and serial dilutions were cultured on 7H11 agar medium for 6 weeks prior to CFU enumeration.

**CinA purification and antibody generation.** Full-length \textit{cinA} was cloned into pET28b vector and transformed into BL21(DE) \textit{E. coli}. \textit{C. bovis} were grown to an \(\text{OD}_{600}\) of 0.6 in LB media supplemented with 50 μg/mL kanamycin. Subsequently, cultures were cooled to 16 °C and \textit{cinA} expression was induced by addition of 0.5 mM IPTG for 16h. \textit{E. coli} cells were harvested by centrifugation and lysed by French Press in 20 mM TRIS-HCl pH 7.5, 0.2 M NaCl in the presence of protease inhibitor cocktail (Roche) and DNAase. Lysates containing \textit{CinA} inclusion bodies were pelleted at 55,000 x g for 30 min, washed twice in 20 mM TRIS-HCl pH 7.5, 0.2 M NaCl, 0.1% β-mercaptoethanol, and dialyzed against 20 mM TRIS-HCl pH 7.5 containing 8 M urea. The insoluble fraction of the sample was removed by centrifugation and unfolded protein was purified using a Ni affinity column under denaturing conditions, maintaining 8 M urea throughout all steps. \textit{C. bovis} was eluted with 500 mM imidazole. The concentration of the eluted \textit{CinA} was adjusted to 0.5 mg/mL in 20 mM TRIS-HCl pH 7.5 containing 8 M urea. The eluate was dialyzed against 50 mM NAcitrate-phosphate pH 7.6, 500 mM NaCl, 1 mM MgCl\textsubscript{2}, 10% glycerol, 4 M urea overnight at 4 °C. The urea concentration was reduced by step dialysis at room temperature, by twofold diluting the dialysis buffer every 2 h in urea-free buffer until a urea concentration of 0.25 M was achieved. Subsequently, the eluate was dialyzed against urea-free buffer overnight at 4 °C. Any precipitated protein was pelleted by centrifugation and the soluble fraction was concentrated to 0.5 mg/mL. Rabbit polyclonal antisera against recombinant \textit{CinA} was generated by Thermo Fisher Scientific.

**Affinity purification of polyclonal anti-\textit{CinA} serum.** To purify \textit{CinA}-specific antibody from anti-\textit{CinA} serum 50 μg of purified \textit{CinA} was resolved through SDS-page and transferred to nitrocellulose membrane. The membrane was incubated with anti-\textit{CinA} serum (1:6 dilution) at 4 °C overnight. \textit{CinA}-specific antibody was eluted with 10 mM glycine pH 2.7 for 2 min and the eluate was immediately neutralized with 300 μL of 1.5 M Tris-HCl pH 8.8. Subsequently, the antibody was dialyzed against PBS at 4 °C.

**Immunoblot analysis of \textit{CinA}.** Protein extracts were prepared from wild-type \(\Delta\text{cin}A, \text{cin}A, \Delta\text{cin}A; \text{cin}A_{\text{abs1}}, \text{cin}A_{\text{abs2}}; \text{cin}A_{\text{Tet}O}\) strains by mechanical lysis with 0.1 mm zirconia/silica beads in PBS in presence of protease inhibitor cocktail (Roche). Subsequently, lysates were filtered through 0.22 μm Spin-X columns (Corning). Protein concentrations were determined using a DC Protein Assay Kit (Bio-Rad). Approximately 20 μg of protein was resolved through SDS-page, transferred to nitrocellulose membrane and probed with anti-\textit{CinA} serum (1:50 dilution) and anti-PckA (1:10,000 dilution). Following incubation with anti-rabbit secondary antibody, protein was resolved through SDS-page, transferred to nitrocellulose membrane and probed with anti-\textit{CinA} serum (1:50 dilution) and anti-PckA (1:10,000 dilution). Following incubation with anti-rabbit secondary antibody (LI-COR Biosciences), protein bands were visualized using the Odyssey Infrared Imaging System (LI-COR Biosciences).

**Drug metabolite extract preparation.** Drug metabolite extracts of drug-treated \textit{Mtb} were prepared as described\textsuperscript{69} with some modifications. Briefly, bacteria were grown to an \(\text{OD}_{600}\) of 1.0 and 1 mL of culture was inoculated onto 0.22 μm polyvinylidene fluoride membrane filters (Millipore Sigma) using vacuum filtration.
Filters were transferred to 7H10 agar and incubated for 5 days to allow for sufficient biomass to accumulate for drug adduct detection. Subsequently, filters were placed on top of inverted 15 ml centrifuge caps containing 7H9 liquid medium for 24 h. After 24 h the medium inside the caps was replaced with 7H-containing antibiotics at the following concentrations: INH 0.03 µg/ml or 0.3 µg/ml, ETH 3 µg/ml, PDM 3 µg/ml or an equal volume of DMSO. Small molecules were extracted after 24 h of drug exposure by bead-beating in acetonitrile/methanol/water (40:40:26, v/v/v)5. Samples were stored at −80°C until drug adduct detection by LC-MS/MS.

Isoniazid-NAD adduct synthesis. INH-NAD adducts were synthesized by reacting INH and NAD+ in the presence of MnIII pyrophosphate, as described by Nguyen et al.26, MnIII pyrophosphate was prepared by stirring an aqeous solution of sodium pyrophosphate dehydrate (200 mM), MnIII acetate dihydtrate (50 mM), and pyrophosphoric acid (added to pH 4.5) for 24 h at room temperature58,59. Next, NAD+ (0.1 ml of a 20 mM stock; Roche) and INH (0.1 ml of a 20 mM stock) were combined in 0.9 ml of phosphate buffer (100 mM, pH 7.5), followed by the addition of MnIII pyrophosphate (10 consecutive additions of 8 µl of the above solution, 2 min apart, while mixing at 1000 rpm at room temperature). Twenty minutes after the last addition, the reaction mixture was clarified by centrifugation and stored at −80°C.

Drug adduct detection. Putative NAD adducts of isoniazid and ethionamide were separated based on a previously published chromatography method25,28, using an Agilent 1290 Infinity LC system coupled to a 6545 quadrupole-time-of-flight mass spectrometer for high-resolution MS/MS fragmentation analysis and a 6495 triple quadrupole mass spectrometer with iFunnel for quantification (Agilent Technologies). Samples were diluted 10-fold into mobile phase A (see below), injected (10 μl) onto an Accucore C18 (150 × 2.1 mm, 2.6 μm particle size; Thermo Scientific), and separated using a 0.2 ml/min gradient of acetonitrile with 0.1% acetic acid (mobile phase B) in water with 0.1% acetic acid and 20 mM ammonium acetate (mobile phase A) as follows: 0–6 min: 3–20% B, 6.01–8 min: 100% B, followed by 4 min re-equilibration at 3% B.

Adduct quantification was performed by multiple reaction monitoring in the positive ionization mode (INH-NAD: 771 to 428, 771 to 136; ETH-NAD: 799 to 428, 779 to 136; PDM: 979 to 250, 979 to 632 and 979 to 136) at a collision energy (CE) of 30 and 55 V, respectively, with a cell accelerator voltage of 5, a dwell time of 100 ms and the following source settings: gas temperature: 200°C, gas flow: 12 l/min, nebulizer: 15 psi, sheath gas temperature: 395°C, sheath gas flow: 12 l/min, capillary voltage: 3000 V, nozzle voltage: 1500 V.

For putative identification of the adducts, pooled extracts from Mtb exposed to a drug at 10× MIC were concentrated twofold (ETH) or 10-fold (INH, PDM) by centrifuging (5 min at 21,130 g). Fragmentation spectra were generated using a fragmentation modeling algorithm33. All chemicals used for LC-MS/MS fragmentation spectra (ETH and PDM) using an in silico competitive model49. Definitions and guidelines for research on antibiotic persistence. Nat. Rev. Microbiol. 50, 1–8 (2019).

McCune, R. M. & TOMPSETT, R. Fate of Mycobacterium tuberculosi in mouse tissues as determined by the microbial enumeration technique. I. The persistence of drug-susceptible tubercle bacilli in the tissues despite prolonged antimicrobial therapy. J. Exp. Med. 104, 737–762 (1956).

Manina, G., Dhar, N. & McKinney, I. B. Stress and host immunity amplify Mycobacterium tuberculosis phenotypic heterogeneity and induce nongrowing metabolically active forms. Cell Host Microbe 17, 32–46 (2015).

Liu, Y. et al. Immune activation of the host cell induces drug tolerance in Mycobacterium tuberculosis both in vitro and in vivo. J. Exp. Med. 213, 763–805 (2016).

Sarahy, J. P. et al. Extreme drug tolerance of Mycobacterium tuberculosis in casum. Antimicrob. Agents Chemother. 62, 2149–11 (2018).

Levin-Reisman, I. et al. Antibiotic tolerance facilitates the evolution of resistance. Science 355, 826–830 (2017).

Mishra, R. et al. Targeting redox heterogeneity to counteract drug tolerance in replicating Mycobacterium tuberculosis. Sci. Transl. Med. 11, eaaw6633 (2019).

Rozwarski, D. A., Grant, G. A., Barton, D. H., Jacobs, W. R. & Sacchettini, J. C. Modification of the NADH of the isoniazid target (InhA) from Mycobacterium tuberculosis. Science 279, 98–102 (1998).

Zhang, Y., Heym, B., Allen, B., Young, D. & Cole, S. The catalase-peroxidase gene and isoniazid resistance of Mycobacterium tuberculosis. Nature 358, 591–593 (1992).

Vilchèze, C. et al. Transfer of a point mutation in Mycobacterium tuberculosis inhA resolves the target of isoniazid. Nat. Med. 12, 1027–1029 (2006).

Muñoz-Elias, E. J. et al. Replication dynamics of Mycobacterium tuberculosis in chronically infected mice. Infect. Immun. 73, 546–551 (2005).

Gill, W. P. et al. A replication clock for Mycobacterium tuberculosis. Nat. Med. 15, 211–214 (2009).

McCune, R. M., McDermott, W. & Tompsett, R. The fate of Mycobacterium tuberculosis in mouse tissues as determined by the microbial enumeration technique. II. The conversion of tuberculosis infection to the latent state by the administration of pyrazinamide and a companion drug. J. Exp. Med. 104, 591–593 (1992).

Frida, Y., Mudgett, M. J., Fried, C., Dey, S. K. & Cole, S. T. The catalase-peroxidase gene of Mycobacterium tuberculosis. Proc. Natl Acad. Sci. U. S. A 114, 3137–3143 (2017).

Galeazzi, L. et al. Identification of nicotinamide mononucleotide deamidase of the bacterial pyridine nucleotide cycle reveals a novel broadly conserved amido hydrolyase family. J. Biol. Chem. 286, 40365–40375 (2011).

Calabrini, L. et al. Genomics-guided analysis of NAD recycling yields functional elucidation of COG1058 as a new family of pyrophosphatases. PLoS ONE 8, e53955–12 (2013).

Karupiah, V., Thistlreathaite, A., Dajani, R., Warwick, J. & Derrick, J. P. Structure and mechanism of the bifunctional CinA enzyme from Thermus thermophilus. J. Biol. Chem. 289, 33187–33197 (2014).

Hayashi, M. et al. Adduct formation of delamanid with NAD in mycobacteria. Antimicrob. Agents Chemother. 64, 113 (2020).

Conradie, F. et al. Treatment of highly drug-resistant pulmonary tuberculosis. N. Engl. J. Med. 382, 893–902 (2020).

Gengenbacher, M., Rao, S. P. S., Pethe, K. & Dick, T. Nutrient-starved, non-replicating Mycobacterium tuberculosis requires respiration, ATP synthase and isocitrate lyase for maintenance of ATP homeostasis and viability. Microbiology 156, 81–87 (2010).

Strydom, N. et al. Tuberculosis drugs’ distribution and emergence of resistance in patient’s lung lesions: a mechanistic model and tool for regimen and dose optimization. PLoS Med. 16, e1002773 (2019).

Bellerose, M. M. et al. Distinct bacterial pathways influence the efficacy of antibiotics against Mycobacterium tuberculosis. mSystems 5, 325 (2020).

Martinez-Moñino, A. B. et al. Characterization and mutational analysis of a nicotinamide mononucleotide deamidase from Agrobacterium tumefaciens showing high thermal stability and catalytic efficiency. PLoS ONE 12, e0174759–20 (2017).

Wang, X.-D. et al. Comparative analysis of mycobacterial NADH pyrophosphatase isoforms reveals a novel mechanism for isoniazid and ethionamide inactivation. Mol. Microbiol. 82, 1375–1391 (2011).
29. Wang, F. et al. Mechanism of thiocarboxamide drug action against tuberculosis and leprosy. J. Exp. Med. 204, 73–78 (2007).
30. Sarathy, J. P. et al. Prediction of drug penetration in tuberculosis lesions. ACS Infect. Dis. 2, 552–563 (2016).
31. Argyrou, A., Vetting, M. W., Aladegbami, B. & Blanchard, J. S. Mycobacterium tuberculosis dihydrofolate reductase is a target for isoniazid. Nat. Struct. Mol. Biol. 13, 408–413 (2006).
32. Nguyen, M., Claparols, C., Bernadou, J. & Meunier, B. A fast and efficient metal-mediated oxidation of isoniazid and identification of isoniazid-NAD(H) adducts. ChemBioChem 2, 877–883 (2001).
33. Djoumbou-Feunang, Y. et al. CEFM-ID 3.0: Significantly improved ESI-MS/MS prediction and compound identification. Metabolites 9, 72 (2019).
34. Baek, S.-H., Li, A. H. & Sassetti, C. M. Metabolic regulation of mycobacterial growth and antibiotic sensitivity. PLoS Biol. 9, e1001065 (2011).
35. Vilchèze, C. & Jacobs, W. R. The isoniazid paradigm of killing, resistance, and persistence in Mycobacterium tuberculosis. J. Mol. Biol. 341, 3450–3461 (2019).
36. Wakamoto, Y. et al. Dynamic persistence of antibiotic-stressed Mycobacteria. Science 339, 91–95 (2013).
37. Aldridge, B. B. et al. Asymmetry and aging of mycobacterial cells lead to variable growth and antibiotic susceptibility. Science 335, 100–104 (2012).
38. Rego, E. H., Audette, R. E. & Rubin, E. J. Deletion of a mycobacterial divisome factor collapses single-cell phenotypic heterogeneity. Nature 546, 153–157 (2017).
39. Adams, K. N. et al. Drug tolerance in replicating Mycobacteria mediated by a macrophage-induced efflux mechanism. Cell 145, 59–53 (2011).
40. Sakatos, A. et al. Posttranslational modification of a histone-like protein regulates phenotypic resistance to isoniazid in mycobacteria. Sci. Adv. 4, eaao1478 (2018).
41. Blondiaux, N. et al. Reversion of antibiotic resistance in Mycobacterium tuberculosis by spirosoxazole SMART-420. Science 355, 1206–1211 (2017).
42. Castricovino, J. et al. Combination therapy for tuberculosis treatment: pulmonary administration of ethionamide and booster co-loaded nanoparticles. Sci. Rep. 7, 5390 (2017).
43. Shirley, M. Ceftazidime-Avibactam: a review in the treatment of serious pneumonia. Drugs 78, 675–692 (2018).
44. Deshpande, D. et al. Ceftazidime-avibactam has potent sterilizing activity against highly drug-resistant tuberculosis. Sci. Adv. 3, e1701102 (2017).
45. Healy, C., Gouzy, A. & Ehrn, S. Peptidoglycan hydrolases RlpA and Am1 are critical for replication and persistence of Mycobacterium tuberculosis in the host. mBio 11, 11–14 (2020).
46. Long, J. et al. Identifying essential genes in Mycobacterium tuberculosis by global phenotypic profiling. Methods Mol. Biol. 1279, 79–95 (2015).
47. Xu, W. et al. Chemical genetic interaction profiling reveals determinants of intrinsic antibiotic resistance in Mycobacterium tuberculosis. Antimicrob. Agents Chemother. 61, 159–15 (2017).
48. Dejesus, M. et al. Comprehensive essentiality analysis of the Mycobacterium tuberculosis genome via saturating transposon mutagenesis. mBio 8, e02133–16 (2017).
49. Dejesus, M. A., Ambadpudi, C., Baker, R., Sassetti, C. & Ioerger, T. R. TRANSIT-A software tool for Himar1 TnSeq analysis. PLoS Comput. Biol. 11, e1004401 (2015).
50. Gee, C. L. et al. A phosphorylated pseudokinase complex controls cell wall synthesis in mycobacteria. Sci. Signal. 5, ra7–ra7 (2012).
51. Murphy, K. C., Murphy, K. C., Papavinassandumar, K. & Sassetti, C. M. Mycobacterial recombineering. Methods Mol. Biol. 1285, 177–199 (2015).
52. Schnappiger, D., O’Brien, K. M. & Ehrn, S. in Mycobacteria Protocols (eds. Parish, T. & Brown, A. C.) 1285, 151–175 (Springer, 2015).
53. Grover, S. et al. Two-way regulation of MmpL3 expression identifies and validates inhibitors of MmpL3 function in Mycobacterium tuberculosis. ACS Infect. Dis. 7, 141–152 (2021).
54. Xu, J. et al. Contribution of pretomanid to novel regimens containing bedaquiline with either linezolid or moxifloxacin and pyrazinamide in murine models of tuberculosis. Antimicrob. Agents Chemother. 63, e183–14 (2019).
55. Sarathy, J. P. et al. An in vitro caseum binding assay that predicts drug penetration in tuberculosis lesions. J. Vis. Exp. e55559, https://doi.org/10.3791/55559 (2017).
56. Nandakumar, M., Prosser, G. A., de Carvalho, L. P. S. & Rhee, K. Metabolomics of Mycobacterium tuberculosis. Methods Mol. Biol. 1285, 105–115 (2015).
57. Nandakumar, M., Nathan, C. & Rhee, K. Y. Isocturate lyase mediates broad antibiotic tolerance in Mycobacterium tuberculosis. Nat. Commun. 5, 4306 (2014).
58. Mahapatra, S. et al. A novel metabolite of antibacterial therapy demonstrates host activation of isoniazid and formation of the isoniazid-NAD + adduct. Antimicrob. Agents Chemother. 56, 28–35 (2011).
59. Trouwborst, R. E., Clement, B. G., Tebo, B. M., Glazer, B. T. & Luther, G. W. Soluble Min(III) in suboxic zones. Science 313, 1955–1957 (2006).

Acknowledgements
We thank Curtis Engelhart, Carolina Trujillo, Rodrigo Aguilera Olvera, Claire Healy, Heather Kim, Paula A. Pino Tamayo, Jennifer McConnell, Roseline Anthus-Sainte, Anderson Watson, Katherine LoMauro, and Yan Pan for technical help. This work was funded by the NIH (Tri-Institutional TB Research Units U19AI111143 to D.S. and S.E., R25AI140472 to K.Y.R., P01AI095208 to J.C.S.), the Bill & Melinda Gates Foundation (OPP1177930 to K.Y.R.), and the Welch Foundation (A-0015 to J.C.S.).

Author contributions
K.M.K., R.S.J., T.E.H., A.G., R.W., I.V.K., J.P.S., M.X., M.D.Z., and M.G. performed the experiments. M.G., V.D., J.C.S., K.Y.R., D.S., and S.E. designed the experiments. K.M.K., D.S., and S.E. wrote the manuscript with input from the other authors.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-022-29832-1.

Correspondence and requests for materials should be addressed to Dirk Schnappiger or Sabine Ehrt.

Peer review information Nature Communications thanks the anonymous reviewers for their contribution to the peer review of this work. Peer reviewer reports are available.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2022