Supplemental Methods

Bacterial growth conditions. Wild-type Mtb strain H37Rv (ATCC 25618) was grown in replicating conditions consisting of Middlebrook 7H9 broth supplemented with 10% oleic acid, dextrose and catalase (OADC) (BD Difco, USA), 0.5% glycerol, and 0.2% tyloxapol (Sigma) and incubated in 5% CO_2, 20% O_2 at 37 °C. *BCG* Pasteur IP1173P2 was cultivated in Middlebrook 7H9 medium (4.7 g/L Middlebrook 7H9 Broth, Difco) and supplemented with 0.4% (v/v) glycerol, 0.05% (v/v) Tween 80 and 10% (v/v) Middlebrook OADC enrichment, (0. 6% v/v oleic acid, 2 mg/mL dextrose, 5 mg/mL bovine serum albumin, 3 µg/mL catalase), or on solid medium 7H10 (19 g/L Middlebrook 7H10 Agar, Difco) adjusted to pH 6.6 and supplemented with 10% Middlebrook OADC enrichment and 0.5% glycerol. For non-replicating conditions, replicating Mtb cells were diluted in non-replicating medium composed of modified Sauton's base at a pH 5.0, butyrate as the carbon source, and 0.5 mM $NaNO_2$. The cells were then incubated in 5% CO_2 and 1.0% O_2 at 37 °C. In the nutrient starvation (Loebel) model of non-replication (Loebel, R. O., Shorr, E. & Richardson, H. B. *J Bacteriol* **26**, 167-200,1933), Mtb cells were incubated for 4 weeks in PBS-Tyl solution without $CaCl_2$ and $MgCl_2$ in 5% CO_2 and 20% O_2 at 37 °C ("starvation"). After a month of starvation, cells were exposed to compounds for 7 days, after which cell survival was assessed.

MIC and CARA assays. Minimum inhibitory concentration (MIC) values for Mtb cells were determined by serially diluting compounds in dimethylsulfoxide (DMSO) and dispensing into 96-well plate. Final DMSO concentrations did not exceed 1%. For the replicating MIC, bacterial suspensions were diluted to an OD_{580} of 0.01 and added to

24 the plate containing the compound. For non-replicating cells, NR MIC was determined
25 as described (Warrier, T., et. al., *ACS Infect Dis* **1**, 580-585, 2015) by resuspending
26 cells from the NR plates and transferred to a second plate containing supplemented
27 7H9 broth and incubated under replicating conditions for 10-12 days before recording
28 the OD_{580}. For *BCG* MIC, 96-well plates were incubated for 6 days at 37 °C and 5%
29 CO_2 in plastic bags. Alamar Blue (Promega) with 5% v/v Tween80 was added to the
30 BCG cells to determine cell viability; fluorescence was measured (λex = 530 nm, λem =
31 590 nm) after 16-24 hrs. IC_{50} or IC_{90} were calculated using the in-house analysis
32 program, Speedlogic (Sanofi). CARA plates were prepared and assayed as described
33 (Gold, B., et. al., *Antimicrob Agents Chemother* **59**, 6521-6538, 2015; Gold, B., *J Vis*
34 *Exp*.,118, 2016; doi: 10.3791/54690). Briefly, after 7 days of compound exposure, cells
35 were resuspended and CARA plates containing agar with 4g/L charcoal were inoculated
36 with 10 µL from the culture plate. After incubating the plates for 7 days (replicating
37 assay) or 12-14 days (NR assay), 50 µl of resazurin solution was added to each well.
38 **Whole cell screens.** We conducted two whole-cell screens independently. 673,640
39 chemical compounds from the Sanofi library were screened at 10 µM against *M.*
40 *smegmatis* under replicating conditions. In addition, 90,000 compounds from the Sanofi
41 collection were screened at 10 µM using *M. tuberculosis* strain $mc^2$6220
42 (Δ*panCD*Δ*lysA*) (Larsen, M. H., et. al., *Vaccine* **27**, 4709-4717, 2009) under both
43 replicating conditions and the non-replicating conditions of the 4-stress model (Gold, B.,
44 et. al., *Antimicrob Agents Chemother* **59**, 6521-6538, 2015).
45 **Generation of resistant mutants.** Resistant Mtb mutants for 2178, 2504, and 2150
46 were generated using 7H11 agar plates supplemented with 10% OADC and 0.5%

47 glycerol and made to contain 2X, 4X, 8X, or 10X the replicating MIC of the compound.
48 These plates were inoculated with 10^9, 10^8, 10^7, or 10^6 CFUs. After 2 months of
49 incubation, colonies were detected only on the 2X and 4X plates. SAR1 resistant *BCG*
50 was selected on 7H10 at 2.5X and 10X the MIC. Resistant colonies were confirmed on
51 10X the SAR1 MIC. The genomes of seven independently isolated Mtb mutants were
52 purified using the cetyltrimethylammonium bromide (CTAB, Sigma-Aldrich) extraction
53 method (Amaro, A., et. al, *Lett Appl Microbiol* **47**, 8-11, 2008) followed by three phenol
54 chloroform extractions to yield clean high quality DNA for whole genome sequencing.
55 The three BCG genomes were isolated using Invitrogen Genomic DNA mini kit
56 (Invitrogen, USA).
57 **MD simulations.** MD simulations of the wild type and the mutated A317S
58 homopentamer were prepared, executed and analysed with Amber18 and
59 AmberTools18 software packages (D.A. Case, I.Y. et. al., AMBER2018, University of
60 California, San Francisco, 2018). CHARMM-GUI membrane builder (Lee, el. al., *J*
61 *Chem Theory Comput* **12**, 405-413, 2016) was used for the embedding of the
62 transmembrane part of CorA in the 1,2-dimyristoyl-sn-glycero-3-phosphocholine
63 (DMPC) bilayer. Both systems were optimized with series of subsequent energy
64 minimizations followed by low temperature short simulations, which gradually increased
65 up to 300K simulations. The simulations were performed with the ff14SB and Lipid14
66 force fields (Dickson, C.J., et. al., *J Chem Theory Comput* **10**, 865-879, 2014; Maier, J.
67 A., et. al., *J Chem Theory Comput* **11**, 3696-3713, 2015) . Explicitly solvated TIP3P
68 waters (Jorgensen, W. L., Chandrasekhar, J., Madura, JD., *The Journal of Chemical*
69 *Physics* **79**, 926, 1983) in NPT ensemble, and 2fs integration steps were used for

70 generation of more than 400 ns production runs in total with snapshots saved every 20
71 ps. Simulations were carried out with all 10 magnesium ions bound to their known sites
72 in the intracellular part of the protein, and additional sodium ions were added for the
73 system total charge neutralization.

74 **ICP-MS.** Agilent MassHunter for ICP-MS software was used for data reduction and
75 processing. Instrument parameters are listed below. An independent reference
76 material was analyzed immediately after calibration to verify instrument accuracy. All
77 QA/QC samples met a priori acceptance criteria. All samples were diluted fifteen-fold in
78 2% (v/v) nitric acid to normalize acid content with the calibration standards. Final
79 graphical values were expressed as concentrations in the 30% nitric acid extract in
80 which ppm (μg/ mL) or ppb (ng/ mL) were converted to μM.

Agilent 7800 ICP-MS Instrument Settings

	Hydrogen Mode	Helium Mode
RF Power (Watts)	1600	1600
Sample Depth (mm)	8	8
Nebulizer Gas (L\min)	0.75	0.75
Dilution Gas (L\min)	0.4	0.4
OctP Bias (V)	-20	-20
KED (V)	4	4
Cell Gas Flow (ml\min)	6	5

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82 **Metal complexation using Infrared spectroscopy**. We attempted to isolate potential
83 metal complexes by adding a solution of 2178 or 2504 (50 mg, 1eq) in 20 mL
84 dichloromethane (DCM) and 3 mL of methanol to a solution of magnesium chloride
85 hexahydrate (27.5 mg, 1 eq) or cobalt (II) chloride hexahydrate (31.5 mg, 1 eq) in 1 mL
86 of methanol. The resulting solution was left open in a fume hood for 4-6 hours until a

87 suspension formed after about half the volume evaporated. The solid was filtered and
88 vacuum dried. Characterization was performed using Infrared (IR) spectrometry and
89 elemental analysis (EA). Compound and compound-metal complex spectra were
90 generated in a Nexus FT-IR spectrometer (Thermo Fisher) using solid state samples
91 prepared from cesium iodide chips for the far IR spectra or by Attenuated Total
92 Reflection (ATR) for the medium IR spectra. Elemental analysis was carried out by
93 introducing 1 mg of sample into a reaction tube maintained at 1000 °C using a Flash
94 2000 system (Thermo Fisher). Combustion gases (CO_2, H_2O, N_2O and SO_2) were then
95 separated and dosed by chromatography. Water content was determined by Karl-
96 Fischer coulometric titration.
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