Supplementary Materials and Methods

1. Proteomic procedure

1.1. Protein extraction

The protein preparation for the differential label-free proteomic analyses was performed as described previously (1). The biomass was treated with a lysis buffer (6 M guanidine HCl, 50 mM K2HPO4/KH2PO4, pH 8.5) followed by ultrasonication (3x10 s, 20% amplitude; U50 IKA Technik). 50 mg of extracted proteins were reduced and alkylated. The proteins were recovered by acetone precipitation and digested with 0.005% (w/v) trypsin (Promega V5111) in 25 mM (w/v) NH4HCO3 (pH 8.5). The trypsin treatment was stopped by adding 0.1% formic acid (v/v).

1.2. Separation of peptides

Prior to mass spectrometry (MS) analysis, reverse phase chromatography was used to separate the extracts. The reverse-phase column (length 15 cm, diameter 75 mm, flow 300 nl/min; PepMap C18, Dionex) was equilibrated with 4% (v/v) acetonitrile for 20 min and peptide elution was carried out over an acetonitrile gradient from 4% to 35% (v/v) for 120 min. The separated peptides were then analyzed online by TripleTOF 5600 mass spectrometer (AB Sciex, USA).

1.3. SWATH acquisition

Peptide spectra were acquired in a data-dependent (DDA) and data-independent (DIA) acquisitions modes. The MS/MS library was acquired in the DDA mode and analyzed by ProteinPilot soft-ware (version 4.5, AB Sciex, USA) using the algorithm Paragon (version 4.5.0.0, AB Sciex, USA). Briefly, the trypsin was chosen as the cleavage specificity and alkylation (C) set to iodoacetamide, carbamidomethylation as fixed modifications, oxidation (M) and deamination (N, Q) as variable modifications were set. All biological modifications and amino acid substitutions were considered and a thorough ID search was applied with a peptide confidence set at 0.99. The raw spectral data

obtained served as the input for ProteinPilot against the Mycobacterium bovis Uniprot database. For the database search, the cut-off peptide confidence limit was set at 95%. ProteinPilot provided a global false discovery rate of 1% and a local false discovery rate of 5%. Accumulation time was set to 0.1 s for MS1 scan and 65 ms for MS2 scan, with total cycle time being approximately 3.8 s.

For the SWATH analysis (DIA, AB Sciex), 32 incremental steps defined as windows of 25 m/z containing 1 m/z for the overlap of window was passed over the full mass range (400–1250 m/z). Peak intensity method was used for the quantitation of peptides. The ion chromatogram of top six fragmented peptides was extracted, and their area was integrated over the 15 min on six transitions. The tolerance was set at 100 ppm. The SWATH data were processed with PeakView software (version 2.1.0.11041, AB Sciex, USA). The retention time (RT) was calculated manually from a group of 15 selected peptides with RT in the range of 20-100 min. Software MarkerView (version 1.2.1, AB Sciex, USA) was used for analysis of the relative abundance of the peptides. For all experiments, proteins identified with one peptide were rejected for interpretation.

Reference

1. Deschoenmaeker F, Bayon-Vicente G, Sachdeva N, Depraetere O, Cabrera Pino JC, Leroy B, Muylaert K, Wattiez R. 2017. Impact of different nitrogen sources on the growth of Arthrospira sp. PCC 8005 under batch and continuous cultivation - A biochemical, transcriptomic and proteomic profile. Bioresour Technol 237:78-88.

Supplementary Figures and Legends

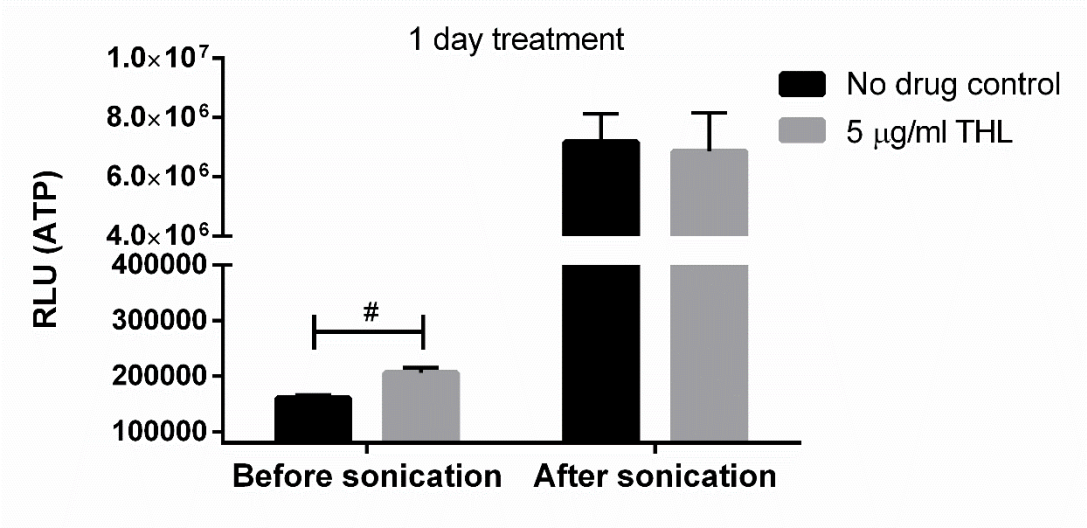

Figure S1. THL did not increase mycobacterial ATP. *M. bovis* BCG was treated with 5 μg/ml THL for 1 day and assessed for ATP both before and after sonication. #, $p<0.0001$ by unpaired t test. This experiment was performed twice, each in triplicate. Data from one representative experiment are shown. The error bar indicates standard deviation.

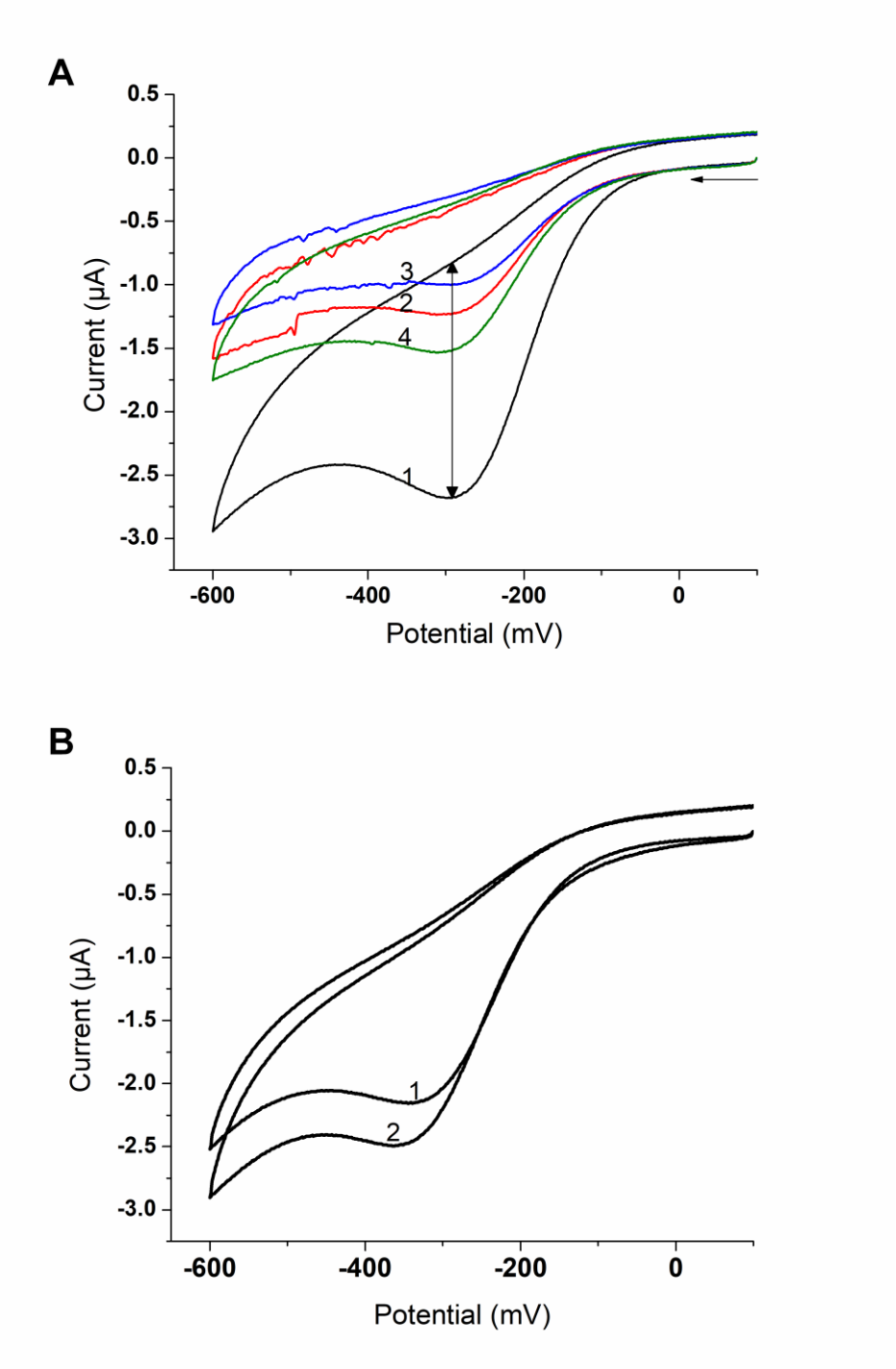

Figure S2. Determination of oxygen content by cyclic voltammetry. (A) Cyclic voltammetry analysis of oxygen content of fresh DTA medium (curve 1), *M. bovis* BCG culture (shaking for 5 hours, no drug control, curve 2), 0.4 μg/ml INH-treated *M. bovis* BCG (5 hours, curve 3) and

bacteria treated with 0.2 µg/ml rifampicin (5 hours, curve 4). The double-arrowed line reflects the intensity of the oxygen peak for curve 1, which indicates the initial oxygen content. (B) Cyclic voltammetry of oxygen content of 0.4 µg/ml INH-treated *M. bovis* BCG culture before (curve 1) and after (curve 2) sample reoxygenation by tube opening and shaking. The experiments were performed 3 times and data from one representative experiment are shown.

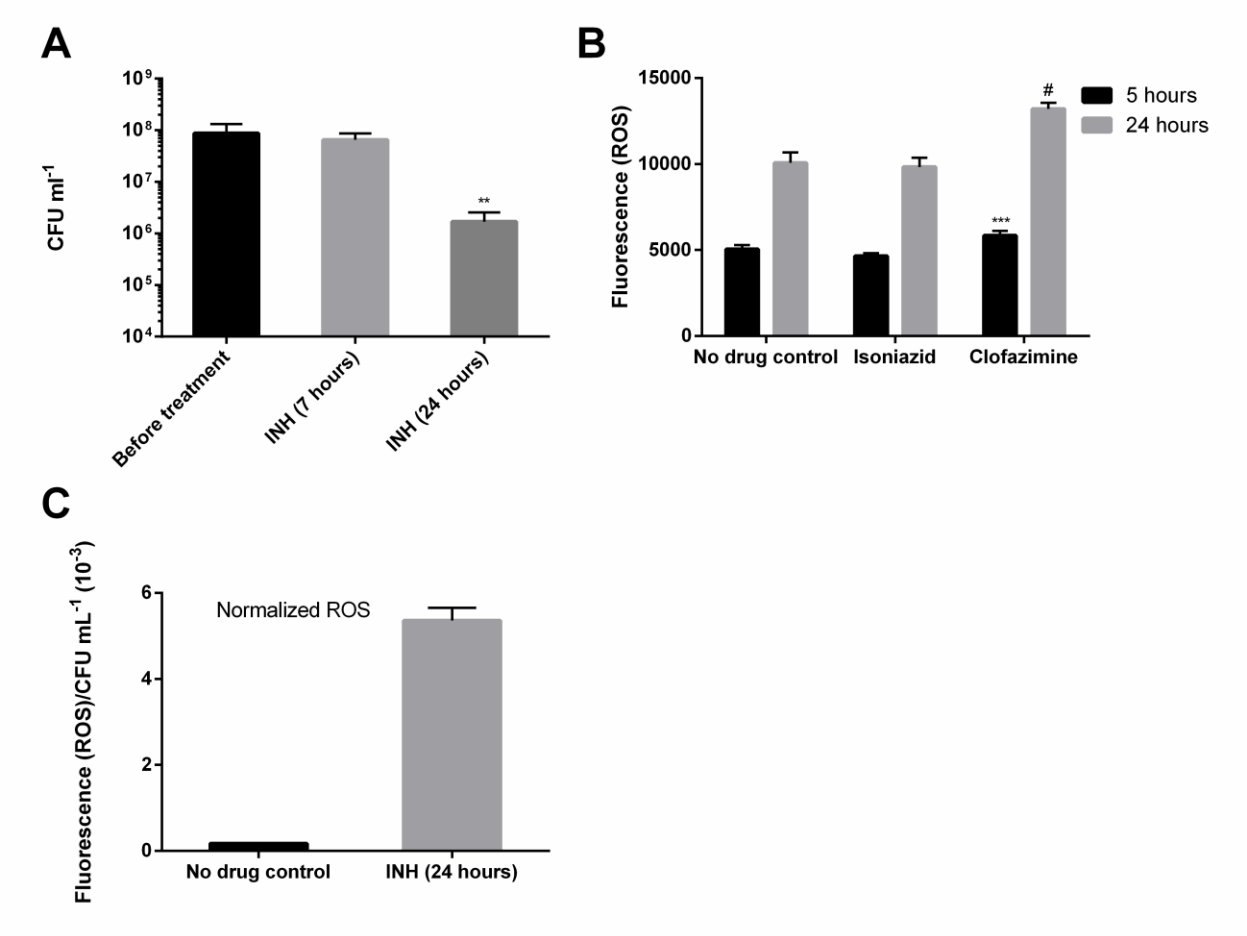

Figure S3. ROS determination. (A) WT *M. bovis* BCG was treated with 0.4 µg/ml INH for 7 and 24 hours before viability determination. **, $p<0.01$ relative to viability before treatment by unpaired t test. (B) ROS was measured after 5 and 24 hours of treatment with either 0.4 µg/ml INH or 16 µg/ml clofazimine. *** and #, $p<0.001$ and 0.0001 relative to no drug control by unpaired t test. (C) The ROS data was normalized by dividing by viability after 24 hours treatment with 0.4 µg/ml INH. This experiment was performed twice, each in triplicate. Data from one representative experiment are shown. The error bar indicates standard deviation.

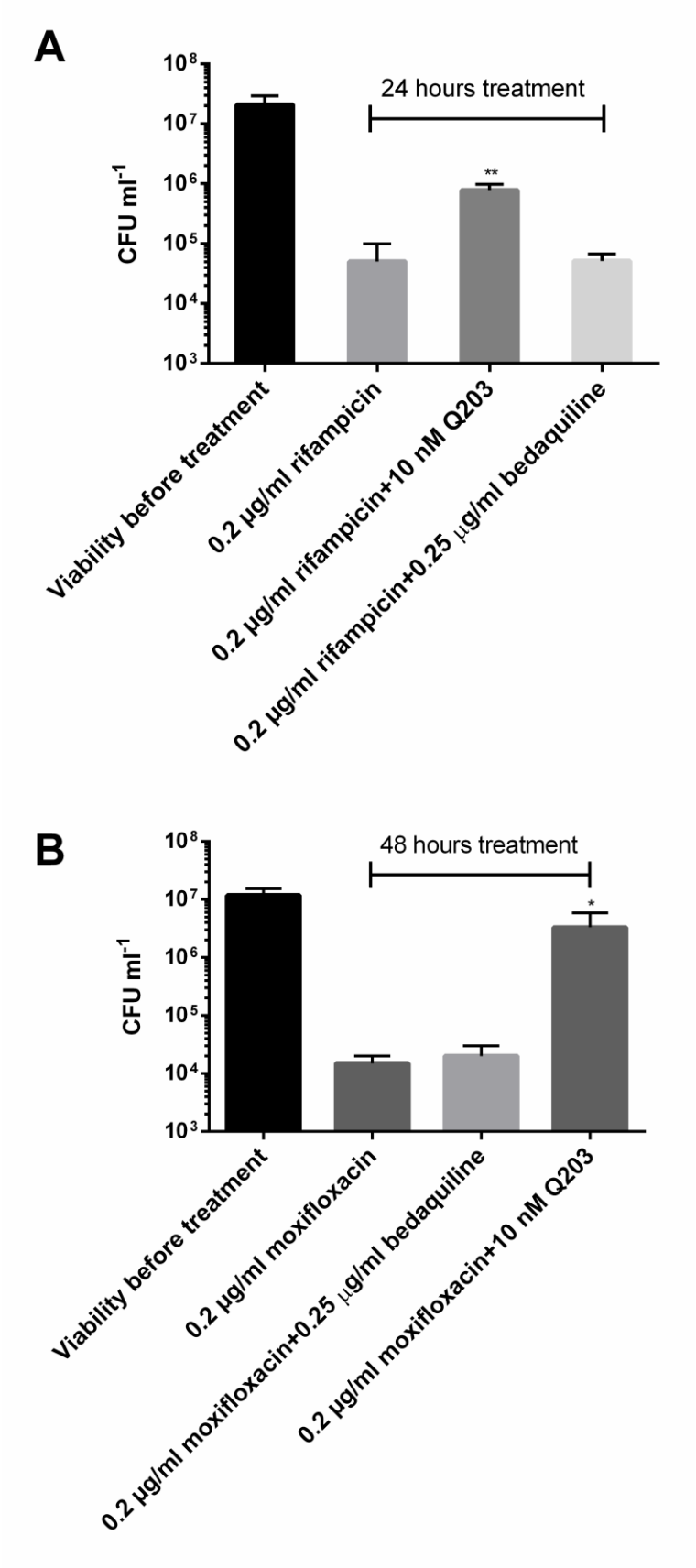
A
24 hours treatment
CFU ml-1
10^8
10^7
10^6
10^5
10^4
10^3
**
Viability before treatment
0.2 µg/ml rifampicin
0.2 µg/ml rifampicin+10 nM Q203
0.2 µg/ml rifampicin+0.25 µg/ml bedaquiline
B
48 hours treatment
CFU ml-1
10^8
10^7
10^6
10^5
10^4
10^3
*
Viability before treatment
0.2 µg/ml moxifloxacin
0.2 µg/ml moxifloxacin+0.25 µg/ml bedaquiline
0.2 µg/ml moxifloxacin+10 nM Q203

Figure. S4. Q203 but not bedaquiline compromised the bactericidal activity of rifampicin and moxifloxacin. (A) WT *M. bovis* BCG was treated with rifampicin (±Q203 or bedaquiline) for 1 day before viability determination. **, $p<0.01$ relative to the rifampicin group by unpaired t test. (B) WT *M. bovis* BCG was treated with moxifloxacin (±Q203 or bedaquiline) for 2 days before viability determination. *, $p<0.05$ relative to the moxifloxacin group by unpaired t test. This experiment was performed twice, each in triplicate. Data from one representative experiment are shown. The error bar indicates standard deviation.

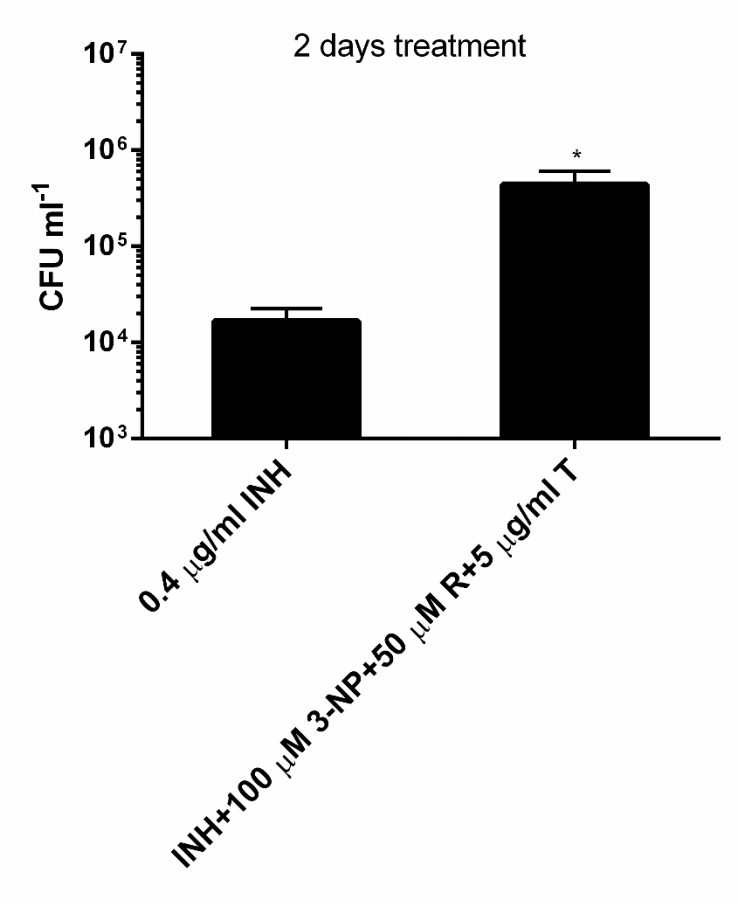

Figure. S5. Lower concentrations of NDHs and SDHs inhibitors slightly protected mycobacteria from INH's killing. WT *M. bovis* BCG cultures were treated with 0.4 μg/ml INH

(±100 μM 3-NP, 50 μM rotenone and 5 μg/ml thioridazine) for 2 days before the viability determination. R and T denote rotenone and thioridazine, respectively. *, $p<0.05$ by unpaired t test. This experiment was performed 3 times, each in triplicate. Data from one representative experiment are shown. The error bar indicates standard deviation.

Supplementary Tables

Table S1. Selected proteins with altered expression relative to no drug control after 7 hours treatment with 0.4 µg/ml INH

H37Rv locus*	**Name**	**Description**	**P-value[a]**	**Fold change[#] (INH/control)**
Lipid metabolism				
Rv0243	FadA2	3-ketoacyl-CoA thiolase involved in lipid degradation	0.00049	1.213665635
Rv3139	FadE24	acyl-CoA dehydrogenase	0.01887	1.378313522
Rv3140	FadE23	acyl-CoA dehydrogenase	0.0192	1.574635143
Rv0154c	FadE2	acyl-CoA dehydrogenase	0.02625	0.587104126
Rv1193	FadD36	fatty-acid--CoA ligase; lipid degradation	0.03369	0.785317436
Rv2928	TesA	thioesterase; PDIM/PGL synthesis	0.04854	0.727048845

Mycolic acid synthesis				
Rv2247	AccD6	acetyl-/propionyl-CoA carboxylase subunit beta; fatty acid (mycolic acid) biosynthesis	0.00543	1.91036958
Rv2243	FabD	malonyl CoA-acyl carrier protein transacylase	0.0123	3.214445307
Rv2246	KasB	3-oxoacyl-ACP synthase 2	0.02386	1.469420587
Rv2245	KasA	3-oxoacyl-ACP synthase 1	0.02559	1.620538068
(trans)membrane protein				
Rv0513	NA	transmembrane protein	0.01188	1.343272489
Rv2037c	NA	Conserved transmembrane protein	0.01803	0.204448975
Rv1779c	NA	integral membrane protein	0.0239	2.946885124

Rv2203	NA	membrane protein	0.03886	0.199758375
Rv2721c	NA	Possible conserved transmembrane alanine and glycine rich protein	0.0452	1.608722976
DNA/RNA metabolism				
Rv3221A	RshA	anti-sigma factor; Probably involved in survival following heat shock and oxidative stress	0.00148	0.259113456
Rv3731	LigC	DNA ligase C	0.00252	4.912052855
Regulator				
Rv1429	NA	PucR family transcriptional regulator	0.00542	2.17147255
Rv0757	PhoP	two component system response transcriptional positive regulator	0.02149	0.780424496
Others				

Rv1637c	NA	MBL fold metallo-hydrolase by conserved domain	0.00101	0.284988496
Rv2190c	NA	endopeptidase	0.00312	0.233800776
Rv0097	NA	oxidoreductase; taurine catabolism dioxygenase	0.01607	21.33419521
Rv3614c	EspD	ESX-1 secretion-associated protein	0.02145	1.895233455
Rv3670	EphE	epoxide hydrolase involved in detoxification reactions following oxidative damage to lipids	0.02301	0.498562464
Rv2633c	NA	hemerythrin domain-containing protein (oxygen transport?)	0.03147	1.742590203
Rv1589	BioB	biotin synthetase involved in biotin synthesis	0.03338	0.480737678
Rv2537c	AroD	3-dehydroquinate dehydratase involved	0.04819	0.348749772

		in chorismate biosynthesis		

*, The proteomic analysis was performed with *M. bovis* BCG. To locate corresponding homology proteins in *M. tuberculosis* H37Rv, NCBI protein blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) was used. a, only proteins with a p-value of <0.05 were analyzed. #, only proteins with a fold change of <0.8 or >1.2 were analyzed.

Table S2. Selected proteins with altered expression relative to INH after 7 hours treatment with Q203 plus INH

H37Rv locus*	Name	Description	P-value[a]	Fold change# (Q203 plus INH /INH)
Lipid metabolism				
Rv3130c	Tgs1	diacyglycerol O-acyltransferase	0.00306	1.400794483
Rv2931	PpsA	phthiocerol synthesis polyketide synthase type I	0.00843	0.681145818
Rv2247	AccD6	acetyl-/propionyl-CoA carboxylase subunit beta	0.01593	0.675351232
Rv2950c	FadD29	long-chain-fatty-acid--AMP ligase	0.01976	0.715231894
Rv2246	KasB	3-oxoacyl-ACP synthase 2	0.03162	0.72384135
Rv2930	FadD26	fatty-acid--CoA ligase	0.03427	0.61693415
Rv2243	FabD	malonyl CoA-acyl carrier protein transacylase	0.0411	0.608302157
Rv2933	PpsC	phthiocerol synthesis polyketide synthase type I	0.04313	0.762018148
Rv2524c	Fas	fatty acid synthase	0.0475	0.689531479

Rv2483c	PlsC	bifunctional L-3-phosphoserine phosphatase/1-acyl-sn-glycerol-3-phosphate acyltransferase; involved in phospholipid and serine biosynthesis	0.04794	1.517658617
Metabolic pathways				
Rv0097	NA	oxidoreductase; taurine catabolism dioxygenase	0.01514	0.047145124
Rv2503c	ScoB	succinyl-CoA:3-ketoacid-CoA transferase subunit B; succinyl-CoA + a 3-oxo acid = succinate + a 3-oxo-acyl-CoA	0.01548	2.29533991
Rv3319	SdhB	succinate dehydrogenase iron-sulfur protein subunit	0.01975	1.277809719
Rv0211	PckA	phosphoenolpyruvate carboxykinase	0.02199	1.308747283
Rv3432c	GadB	glutamate decarboxylase, catalyzing the production of GABA	0.04877	1.693843265

Rv2447c	FolC	folylpolyglutamate synthase	0.04896	1.823512674
Stress tolerance				
Rv1636	TB15.3	iron-regulated universal stress protein	0.01705	5.893120602
Rv0186A	MymT	metallothionein; coordinates Cu(I) ions into a Cu(I)-thiolate core; protects cell from copper toxicity	0.01798	4.0493237
Rv3841	BfrB	bacterioferritin; involved in iron storage; ferritin is an intracellular molecule that stores iron in a soluble, nontoxic, readily available form.	0.02415	1.839171396
(trans)membrane protein				
Rv1410c	NA	aminoglycosides/tetracycline-transport integral membrane protein; MFS-type drug efflux transporter P55	0.0047	3.785172145
Rv1363c	NA	membrane protein	0.01318	1.582604441

Rv3921c	YidC	membrane protein insertase; Probable conserved transmembrane protein	0.02647	1.215279175
Rv2203	NA	membrane protein	0.04113	5.121286615
DNA/RNA metabolism				
Rv2024c	NA	hypothetical protein; DNA endonuclease or helicase by conserved domain	0.0202	0.688502482
Rv1020	Mfd	transcription-repair coupling factor involved in nucleotide excision repair; necessary for strand-specific repair.	0.03014	0.550694931
Rv3211	RhlE	ATP-dependent RNA helicase	0.03049	0.577395926
Rv2703	SigA	RNA polymerase sigma factor	0.03401	0.558599797
Regulator/sensor kinase				
Rv3291c	LrpA	transcriptional regulator	0.00922	0.608085908
Rv1429	NA	PucR family transcriptional regulator	0.014	0.358270123

Rv0117	OxyS	oxidative stress response regulatory protein	0.01697	14.07978108
Others				
Rv0061c	NA	hypothetical protein	2.12E-05	5.962587805
Rv3290c	Lat	L-lysine-epsilon aminotransferase; catalytic activity: L-lysine + 2-oxoglutarate = 2-aminoadipate 6-semialdehyde + L-glutamate	0.00049	2.6780465
Rv2031c	HspX	alpha-crystallin	0.00145	1.282273248
Rv3241c	NA	ribosomal subunit interface protein; ribosome hibernation promotion factor	0.00305	2.620505432
Rv2964	PurU	formyltetrahydrofolate deformylase involved in purine biosynthesis	0.00971	0.256657936
Rv3768	NA	nuclear transport factor 2 family protein	0.01598	1.901407129
Rv0125	PepA	serine protease	0.01978	1.290189841
Rv3293	Pcd	piperideine-6-carboxylic acid dehydrogenase involved in L-	0.03284	1.383134105

		alpha-aminoadipic acid synthesis with Lat protein		
Rv2911	DacB2	penicillin-binding protein involved in peptidoglycan synthesis	0.03628	8.002129874
Rv2697c	Dut	deoxyuridine 5'-triphosphate nucleotidohydrolase involved in nucleotide metabolism	0.03753	2.475979793
Rv1299	PrfA	peptide chain release factor	0.04403	0.755629208
Rv0505c	SerB1	phosphoserine phosphatase	0.04958	1.450477053

*, The proteomic analysis was performed with *M. bovis* BCG. To locate corresponding homology proteins in *M. tuberculosis* H37Rv, NCBI protein blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) was used. a, only proteins with a p-value of <0.05 were analyzed. #, only proteins with a fold change of <0.8 or >1.2 were analyzed.