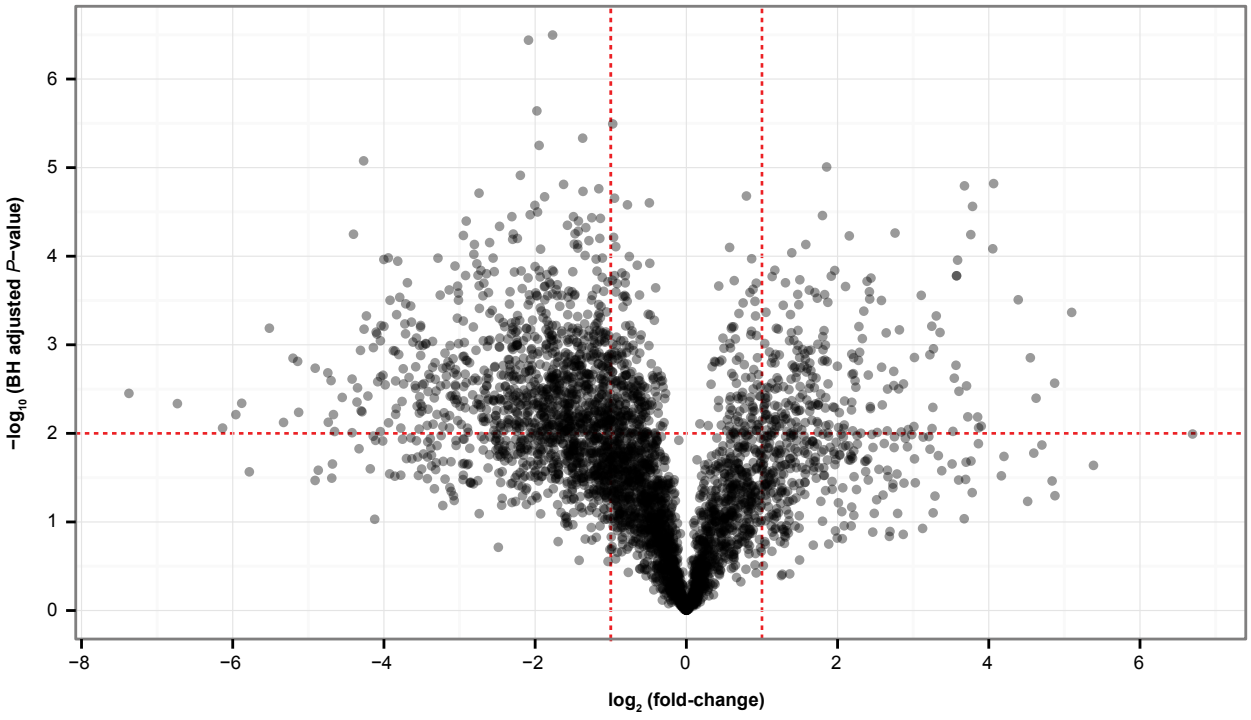
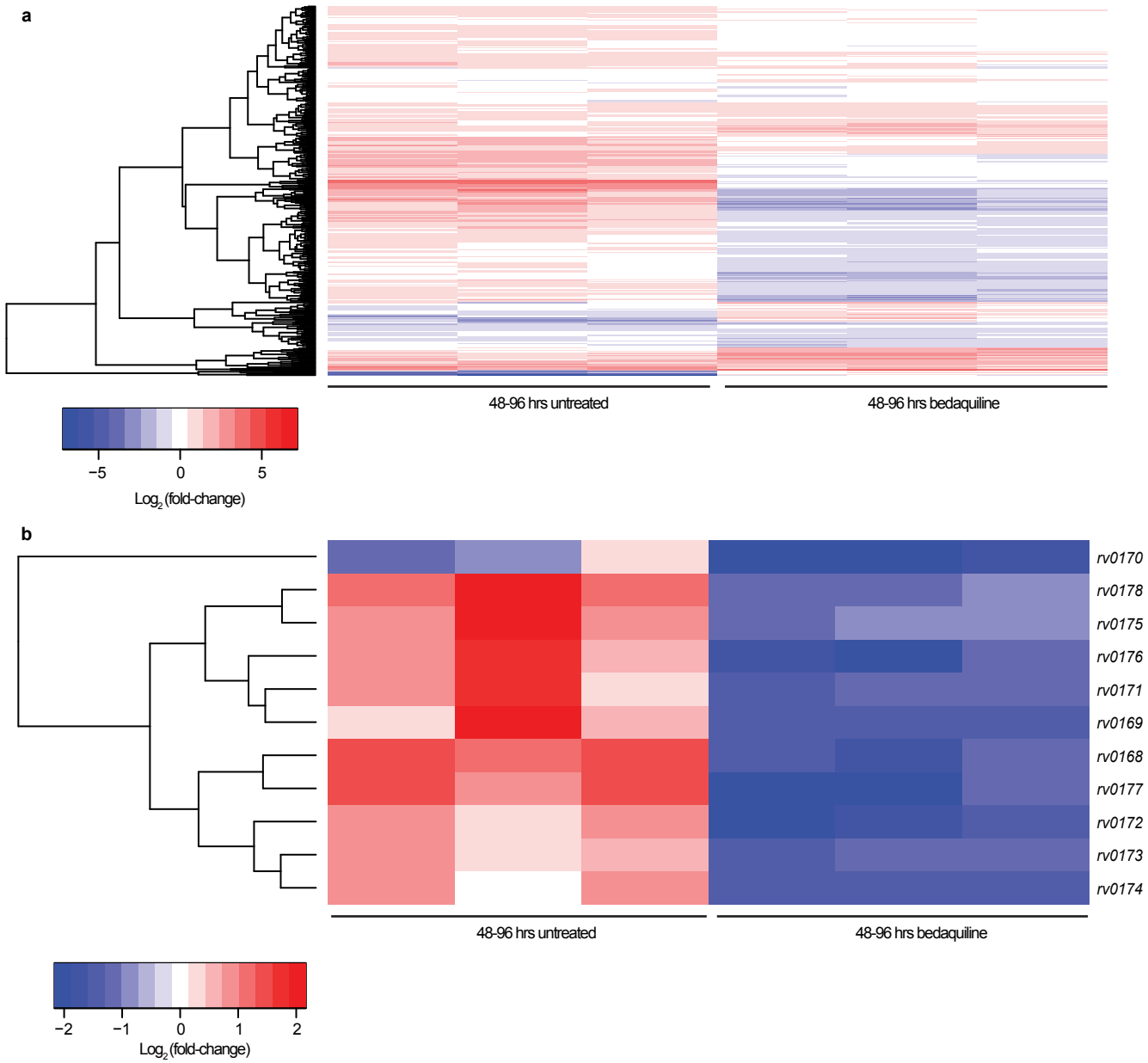
Network analysis identifies *Rv0324* and *Rv0880* as regulators of bedaquiline tolerance in *Mycobacterium tuberculosis*

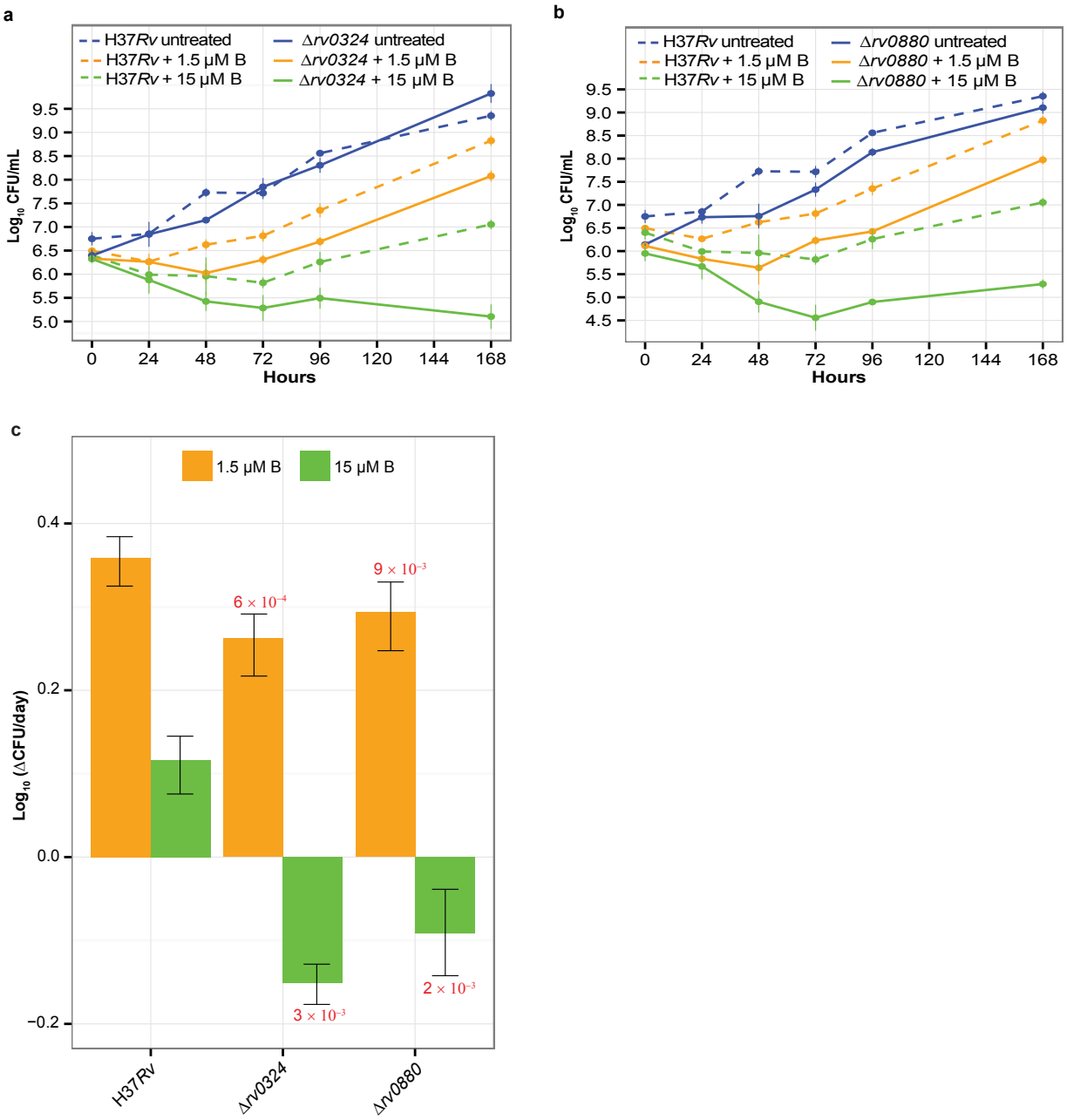
Supplementary Information



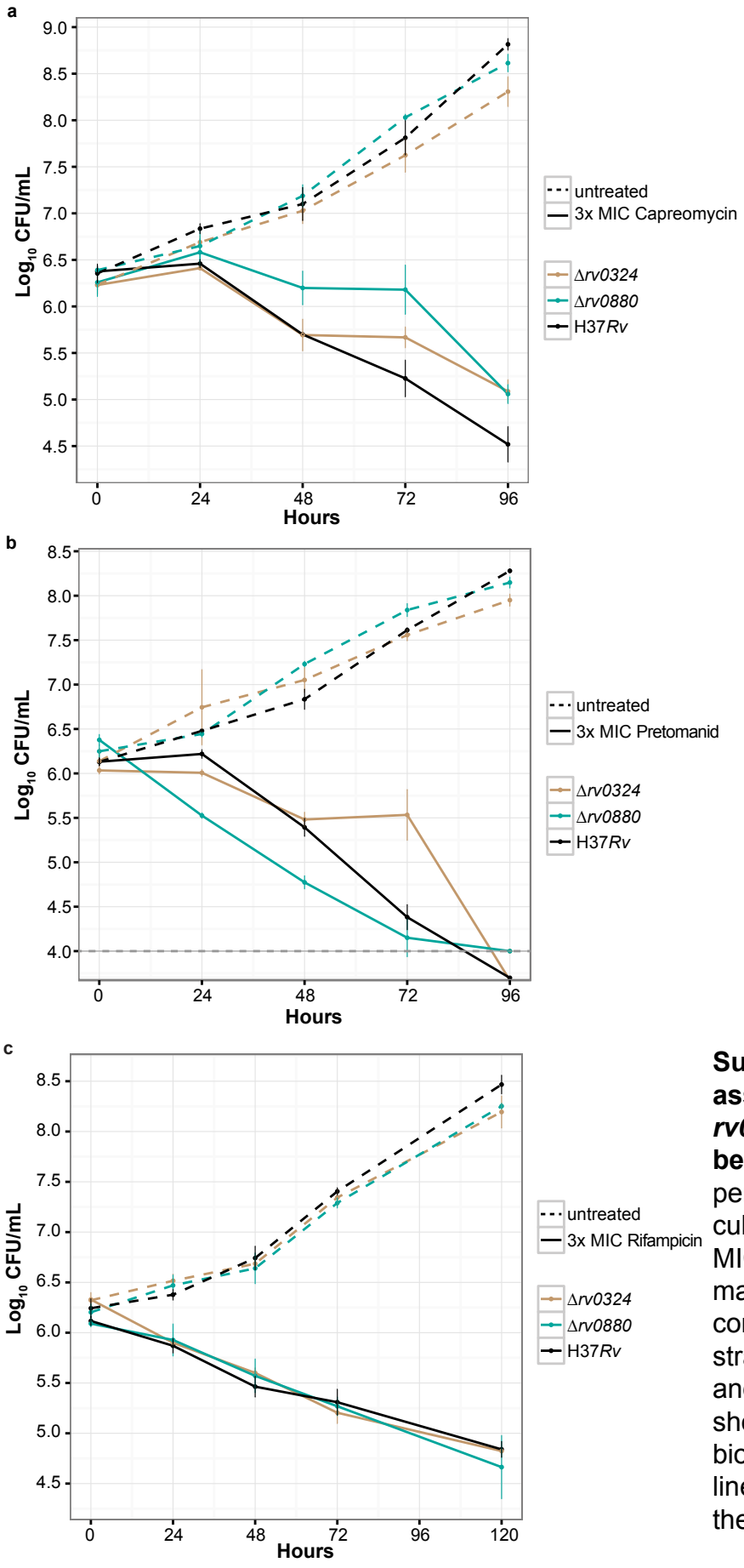
Supplementary Figure S1. Transcriptional response of MTB to bedaquiline treatment. Volcano plot for the MTB bedaquiline transcriptome data. The fold-change is between the average of triplicate samples treated with bedaquiline and the average of untreated triplicate samples. The Benjamini Hochberg (BH) adjusted *P*-value comes from a *t*-test comparison of the sample groups.



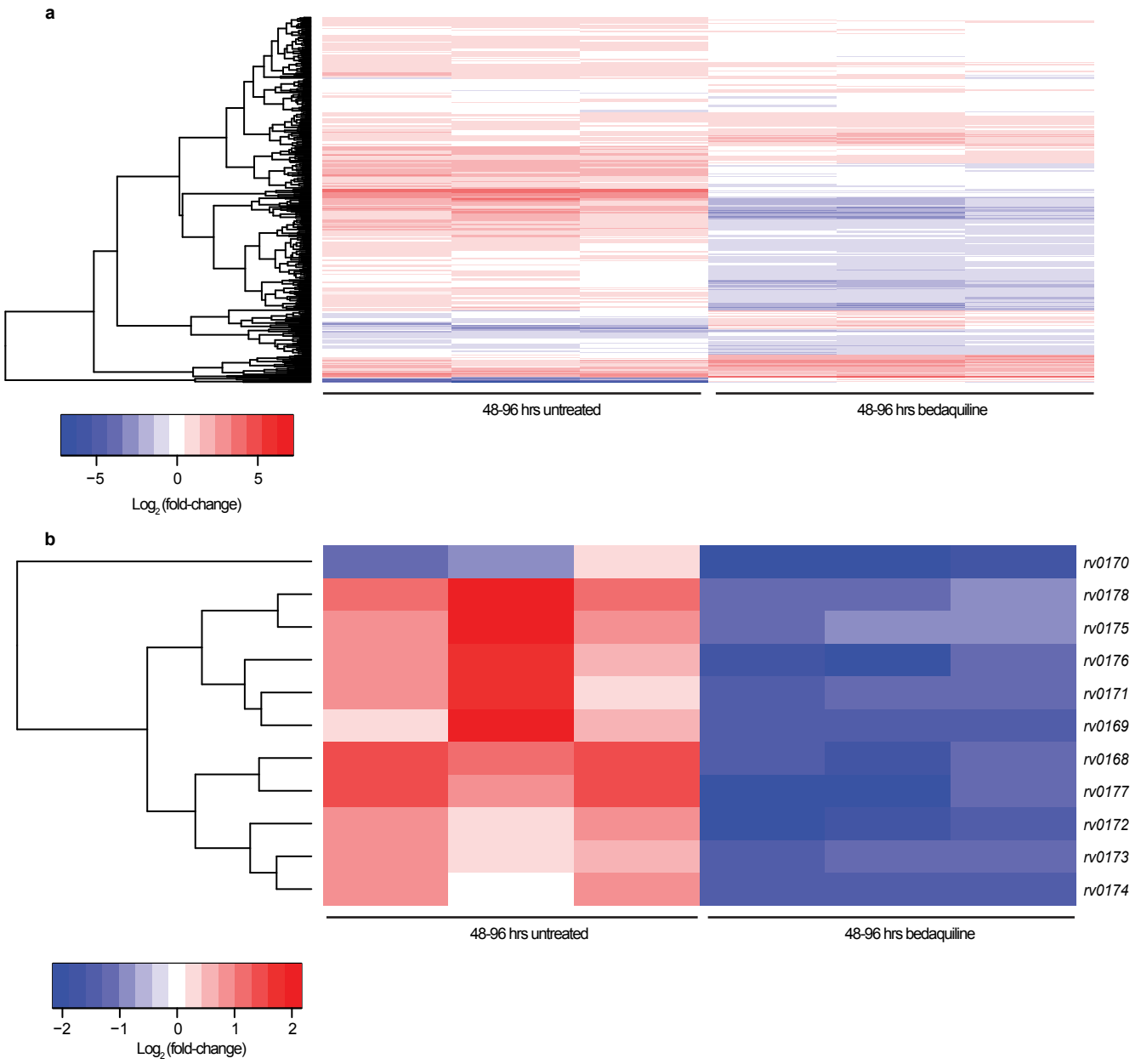
Supplementary Figure S2. Transcriptional response of *dosR* regulon and ATP synthase operon. Heat maps showing the log2 fold-change in gene expression of the *dosR* regulon (**a**) and ATP synthase operon (**b**) induced by bedaquiline treatment for 48-96 hrs as compared to untreated control from three biological samples. The color scale represents log2 fold-change values.



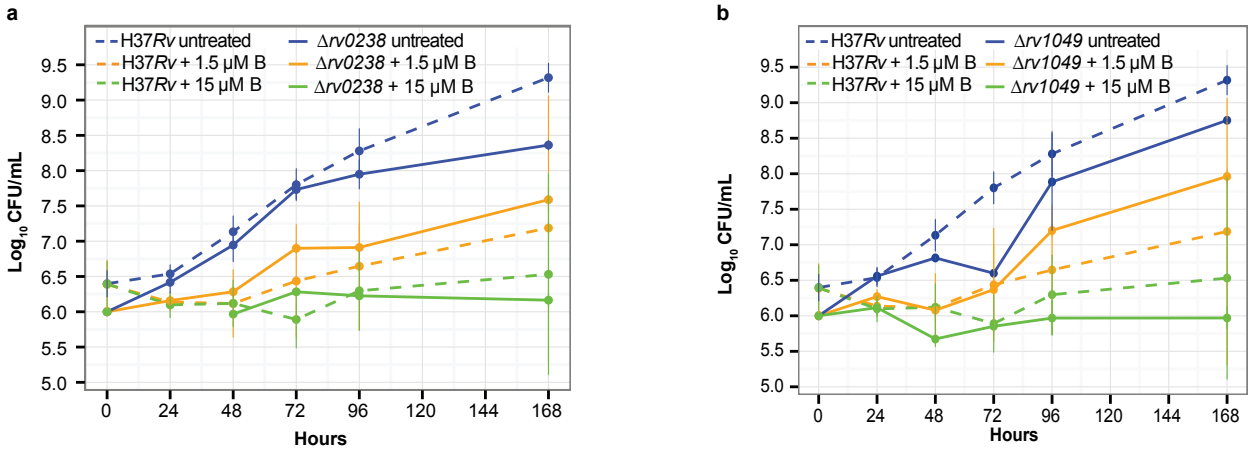
Supplementary Figure S3. Time-kill assays of wild type, Δ*rv0324* and Δ*rv0880* MTB strains with bedaquiline. Raw CFUs over a period of 168 hours (7 days) with no bedaquiline (untreated, blue), 1.5 µM bedaquiline (orange) or 15 µM bedaquiline (green) comparing the H37*Rv* wild type (dashed lines) with the mutant strains (solid lines) , Δ *rv0324* (**a**) and Δ*rv0880* (**b**). First-order rate kinetics were calculated by linear regression of CFUs from H37*Rv* wild type, Δ*rv0324*, and Δ*rv0880* strains obtained over 7 days of bedaquiline treatment (**c**). Statistical significance of rate kinetics between wild type and mutant strains were calculated using *t*-test, Benjamini Hochberg adjusted *P*-values are numbers in red. Error bars show the standard deviation from three biological samples (**a** and **b**) and the standard error of the linear regression (**c**). Representative results from two experiment repeats are presented. The same data (H37*Rv* wild type and 15 µM bedaquiline) is shown for 96 hours in **Figure 2** of the manuscript. Bedaquiline, B.



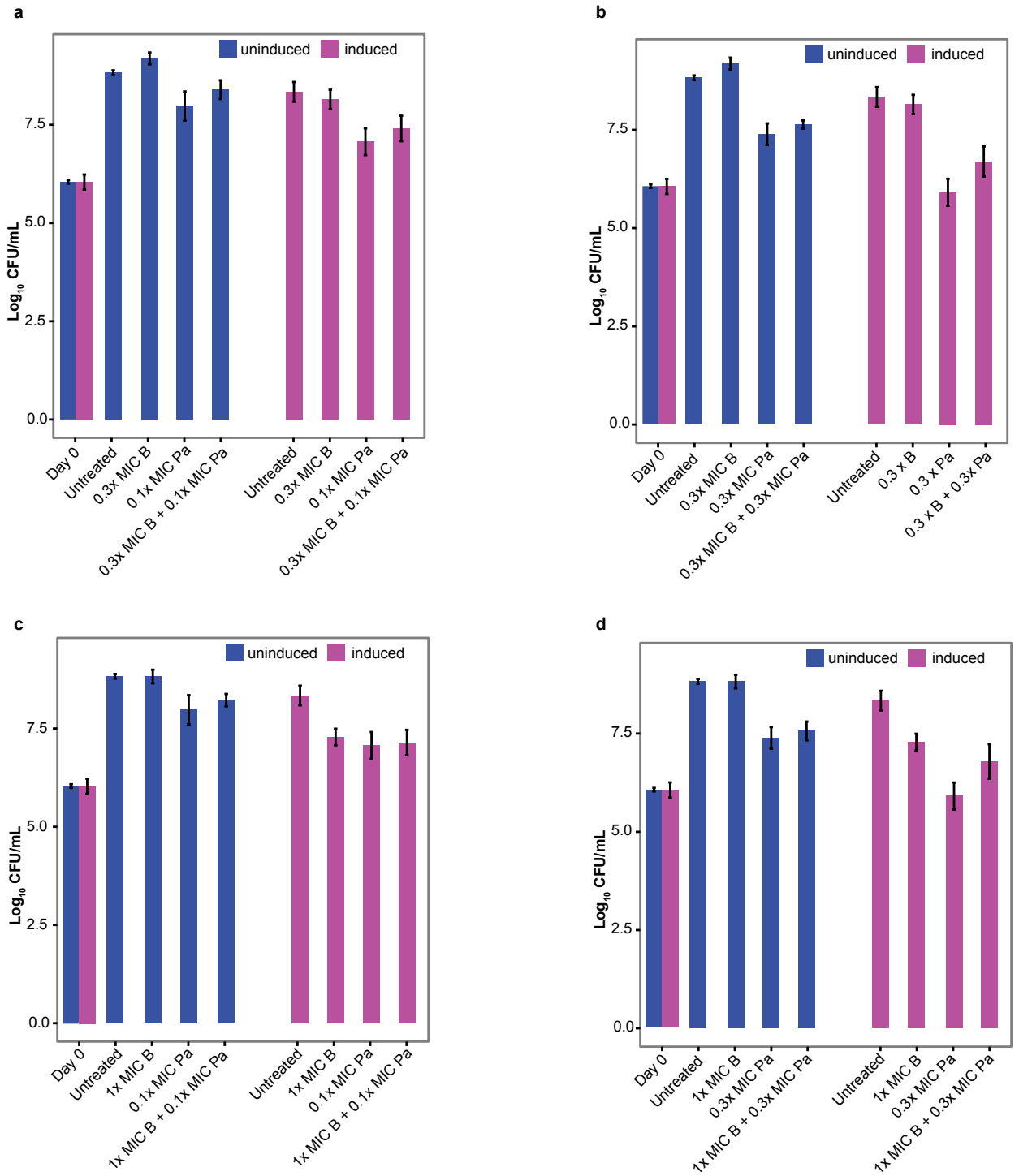
Supplementary Figure S4. Time-kill assays of wild type, Δ*rv0324* and Δ *rv0880* MTB strains with other antitubercular drugs. Raw CFUs over a period of 96-120 hours with no antitubercular drug (untreated, dashed lines), 3× MIC of capreomycin (**a**), 3× MIC of pretomanid (**b**) or 3× MIC of rifampicin (**c**) comparing the H37*Rv* wild type MTB strain (black) with the Δ*rv0324* (brown) and Δ*rv0880* (teal) strains. Error bars show the standard deviation from three biological samples and the grey dashed line represents the limit of detection for the assay.



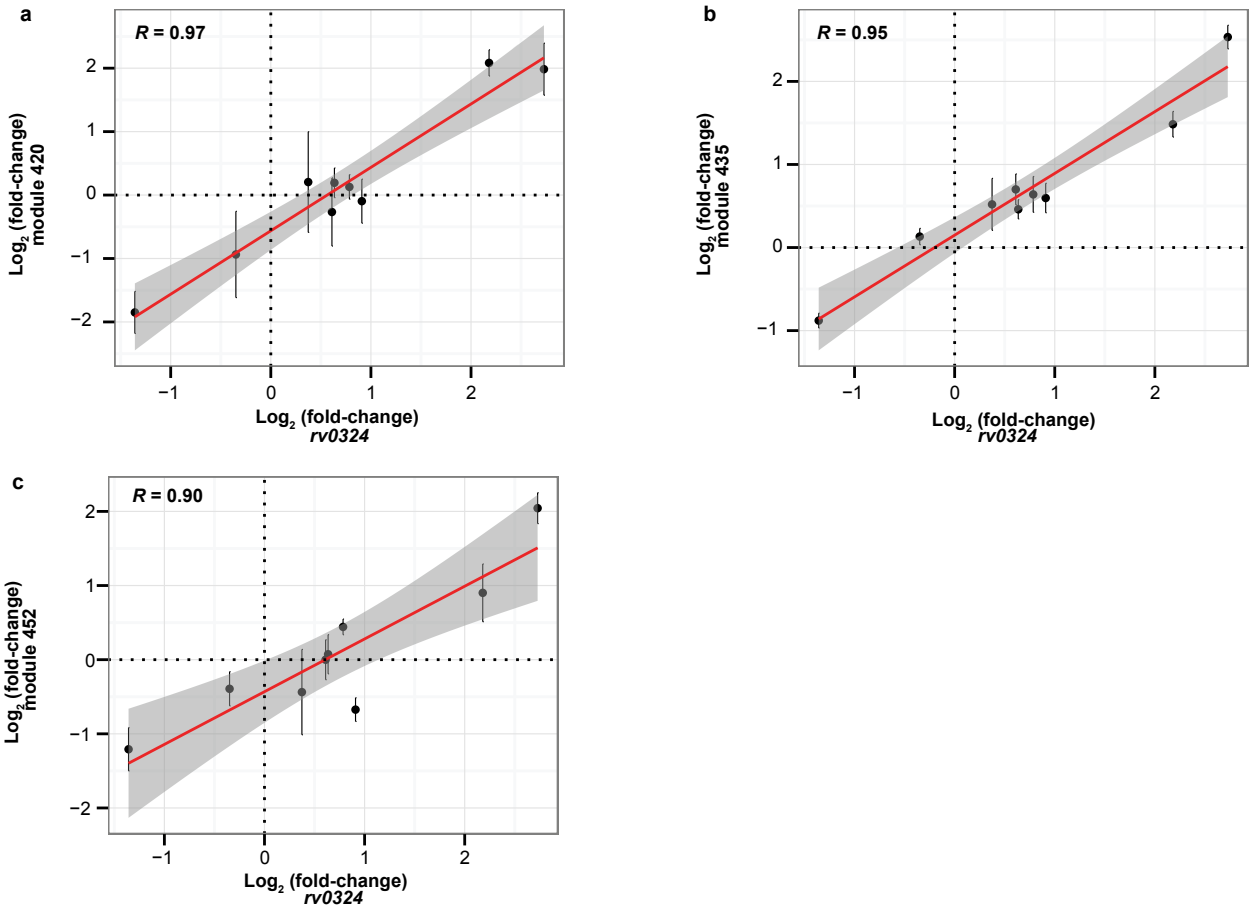
Supplementary Figure S5. Transcriptional response of *rv0328* and *rv1049* regulons. Heat maps showing the log2 fold-change in gene expression of the *rv0238* regulon (**a**) and *rv1049* regulon (**b**) induced by bedaquiline treatment for 48-96 hrs as compared to untreated control from three biological samples. The color scale represents log2 fold-change values.



Supplementary Figure S6. Killing of Δ*rv0238* and Δ*rv1049* MTB strains by bedaquiline. CFUs over a period of 168 hours (7 days) with no bedaquiline (untreated, blue), 1.5 µM bedaquiline (orange) or 15 µM bedaquiline (green) comparing the H37*Rv* wild type (dashed lines) with the mutant strains (solid lines), Δ*rv0238* (**a**) and Δ*rv1049* (**b**). Error bars show the standard deviation from three biological samples. Bedaquiline, B.



Supplementary Figure S7. Treatment of un-induced and induced rv0880 overexpression strain with bedaquiline-alone, pretomanid-alone, and bedaquiline and pretomanid in combination. Mean log10 CFU counts after 7 days of treatment with 0.3× MIC bedaquiline and 0.1× MIC pretomanid (**a**), 0.3× MIC bedaquiline and 0.3× MIC pretomanid (**b**), 1× MIC bedaquiline and 0.1× MIC pretomanid (**c**), and 1× MIC bedaquiline and 0.3× MIC pretomanid (**d**). Error bars show the standard deviation from three biological samples. Representative results from two experiment repeats are presented. Bedaquiline, B. Pretomanid, Pa.



Supplementary Figure S8. The nutrient-limited conditional regulation of modules by *rv0324*. The scatter plots show the correlation of log2 fold-change in gene expression for *rv0324* versus the median of gene members of (**a**) module 420 (R = 0.97, P-value = 1.4 × 10-5), (**b**) module 435 (R = 0.95, P-value = 9.8 × 10-5), and (**c**) module 452 (R = 0.90, P-value = 0.003) under limited nutrient growth conditions. Error bars show the standard deviation of module gene expression. The red line estimates the linear correlation between *rv0324* and module genes' expression; the grey area describes the standard error of the relationship.

	H37*Rv*	Δ*rv0324*	Δ*rv0880*	*rv0880 uninduced*	*rv0880 induced*
Bedaquiline MIC (μM)	2	2	2	0.5	0.5
Capreomycin MIC (μM)	2	1	2	0.5	0.5
Isoniazid MIC (μM)	1.8	1.8	1.8	1.8	1.8
Pretomanid MIC (μM)	0.42	0.42	0.42	0.42	0.21

Supplementary Table S4. Comparative in vitro activity of antitubercular agents with H37*Rv* wild type and TF perturbation strains. The MICs of four antitubercular agents for MTB wild type H37*Rv*, Δ*rv0324*, Δ*rv0880*, and un-induced and induced rv0880 overexpression strains. MICs were determined by the commercially available Bac Titer-Glo microbial cell viability assay kit. The MIC determinations were done with replicates in two (*rv0880* overexpression strain) or three (H37*Rv*, Δ*rv0324*, and Δ*rv0880* strains) independent experiments.

	rv0324	*rv0880*
Capreomycin 2× MIC	0.14	0.32
Capreomycin 4× MIC	2.83	0.82
Moxifloxacin 2× MIC	0.56	N.S.
Moxifloxacin 4× MIC	0.65	N.S.
Moxifloxacin 8× MIC	1.62	N.S.
Rifampin 1× MIC	N.S.	-0.23
Rifampin 2× MIC	N.S.	-0.11
Rifampin 4× MIC	N.S.	-0.6
Pretomanid 2× MIC	N.S.	-0.57
Pretomanid 4× MIC	N.S.	-0.73
Pretomanid 8× MIC	N.S.	-1.15
i030 2× MIC	0.15	N.S.
i030 4× MIC	0.17	N.S.
i030 8× MIC	0.64	N.S.
i031 2× MIC	0.71	N.S.
i031 4× MIC	1.6	N.S.
i047 2× MIC	0.63	N.S.
i047 4× MIC	0.65	N.S.

Supplementary Table S5. Antitubercular agents with significant differential expression of *rv0324* or *rv0880* in MTB. The table displays prioritized drugs from mining transcriptome data of 36 antitubercular agents for significant alteration of the expression of *rv0324* or *rv0880* (*P*-value < 0.01). The IsoGene R package was used to test the monotone relationship between gene expression and drug dosage. *P*-values were calculated based on a resampling procedure in which the distribution of the statistic under the null hypothesis is approximated using permutations. The values in the table are log2 fold-change (GSE71200). Not significant, N.S.