

1 **Transcriptomic signatures predict regulators of drug synergy and**
2 **clinical regimen efficacy against Tuberculosis**

3
4 Shuyi Ma^{1,2}, Suraj Jaipalli³, Jonah Larkins-Ford^{4,5,6}, Jenny Lohmiller¹, Bree B. Aldridge^{4,5,7}, David
5 R. Sherman^{1,2,9*}, Sriram Chandrasekaran^{3,8*}

6
7 ¹ Center for Global Infectious Disease Research, Seattle Children's Research Institute, Seattle
8 WA, USA

9 ² Pathobiology Program, Department of Global Health, University of Washington, Seattle, WA,
10 USA

11 ³ Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA

12 ⁴ Department of Molecular Biology and Microbiology, Tufts University School of Medicine,
13 Boston, MA, USA

14 ⁵ Laboratory of Systems Pharmacology, Harvard Medical School, Boston, MA, USA

15 ⁶ Sackler School of Graduate Biomedical Sciences, Tufts University School of Medicine, Boston,
16 MA, USA

17 ⁷ Department of Biomedical Engineering, Tufts University School of Engineering, Medford, MA,
18 USA.

19 ⁸ Center for Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI,
20 USA

21 ⁹ Current address: Microbiology Department, University of Washington, Seattle, WA, USA

22 *Correspondence: csriram@umich.edu, dsherman@uw.edu

23

24 **Classification:** BIOLOGICAL SCIENCES: Microbiology; Biophysics and Computational Biology

25

26 **Keywords:** Tuberculosis; drug combinations; transcription factors; drug synergy;
27 transcriptomics

28

29

30

31

32

33

34 **ABSTRACT**

35

36 The rapid spread of multi-drug resistant strains has created a pressing need for new drug
37 regimens to treat tuberculosis (TB), which kills 1.8 million people each year. Identifying new
38 regimens has been challenging due to the slow growth of the pathogen *M. tuberculosis* (MTB),
39 coupled with large number of possible drug combinations. Here we present a computational
40 model (INDIGO-MTB) that identified synergistic regimens featuring existing and emerging anti-
41 TB drugs after screening *in silico* over 1 million potential drug combinations using MTB drug
42 transcriptomic profiles. INDIGO-MTB further predicted the gene Rv1353c as a key
43 transcriptional regulator of multiple drug interactions, and we confirmed experimentally that
44 Rv1353c up-regulation reduces the antagonism of the bedaquiline-streptomycin combination.
45 Retrospective analysis of 57 clinical trials of TB regimens using INDIGO-MTB revealed that
46 synergistic combinations were significantly more efficacious than antagonistic combinations (p-
47 value = 1×10^{-4}) based on the percentage of patients with negative sputum cultures after 8
48 weeks of treatment. Our study establishes a framework for rapid assessment of TB drug
49 combinations and is also applicable to other bacterial pathogens.

50

51 **IMPORTANCE**

52

53 Multi-drug combination therapy is an important strategy for treating tuberculosis, the world's
54 deadliest bacterial infection. Long treatment durations and growing rates of drug resistance
55 have created an urgent need for new approaches to prioritize effective drug regimens. Hence,
56 we developed a computational model called INDIGO-MTB, which identifies synergistic drug
57 regimens from an immense set of possible drug combinations using pathogen response
58 transcriptome elicited by individual drugs. Although the underlying input data for INDIGO-MTB
59 was generated under *in vitro* broth culture conditions, the predictions from INDIGO-MTB
60 correlated significantly with *in vivo* drug regimen efficacy from clinical trials. INDIGO-MTB also
61 identified the transcription factor Rv1353c as a regulator of multiple drug interaction outcomes,
62 which could be targeted for rationally enhancing drug synergy.

63

64

65

66

67

68

69

70

71

72 INTRODUCTION

73 Tuberculosis (TB) is a global health threat of staggering proportions, taking a human life every
74 30 seconds (1). To ensure adequate treatment and combat onset of resistance, TB patients
75 receive multidrug therapy. However, the frontline regimen of four drugs and six months
76 treatment has not changed in 50 years, and resistance is spreading. In response, experts have
77 called for entirely new regimens to combat the TB pandemic (2). While some new anti-TB
78 agents are beginning to emerge (3), optimizing individual agents into effective regimens remains
79 a significant challenge.

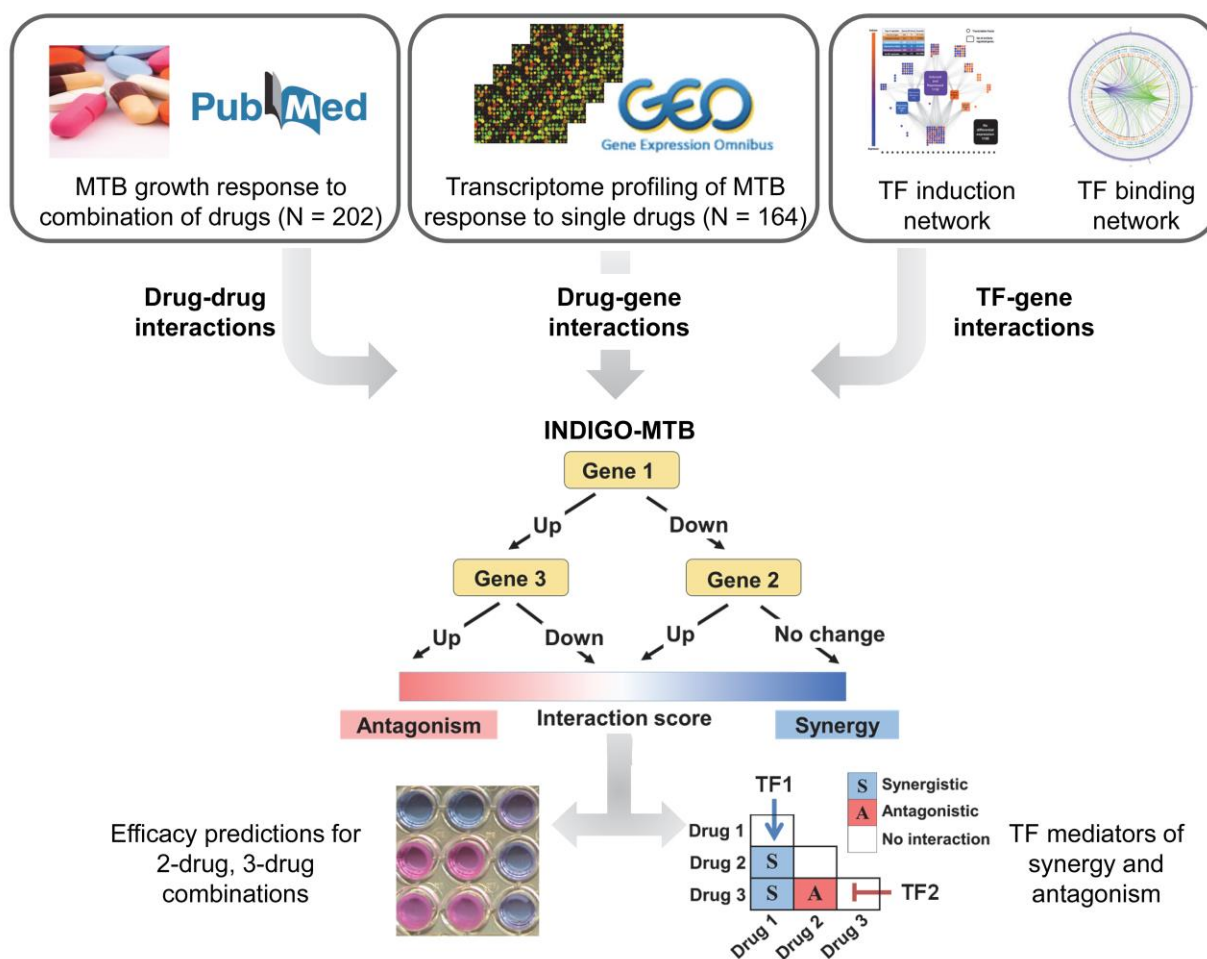
80 At present, combinations are designed and tested empirically, driven in part by clinical intuition.
81 A standard approach to evaluate drug interactions experimentally utilizes checkerboard assays,
82 which involves exposing the pathogen to different dose combinations of constituent drugs in a
83 regimen. New approaches have been developed to increase throughput of checkerboard
84 assays, either by reducing the number of doses required or by using computational optimization
85 to find optimal doses (4-6).

86 Even with these developments, the enormous and expanding number of potential drug
87 combinations renders regimen optimization by comprehensive experimental testing infeasible.
88 The 28 drugs used to treat TB (7-10) could be assembled into nearly 24,000 different 3- or 4-
89 drug combinations. Adding just two new agents to that list increases the number of different
90 combinations to almost 32,000. Thus, there is a need for high-throughput approaches that can
91 prioritize new drug combinations based on data generated from individual drugs. For example, a
92 feedback-based approach was recently used to determine the optimal dosing of multi-drug
93 regimens (4, 5). However, this approach still requires hundreds of dose-specific measurements
94 for training the algorithm, all of which must be re-done whenever a new agent is under
95 consideration. Computational tools such as metabolic modeling, kinetic modeling, and statistical
96 modeling (11-13) have limited power in this context because direct targets are not known for
97 many compounds. Existing approaches are also limited in the scale at which potential
98 combinations could be evaluated computationally — currently around hundreds. Furthermore,
99 empirical approaches based on drug similarity (or dissimilarity) are less effective in predicting
100 interaction outcomes for new drugs classes, and they also lack a model for antagonism (14).
101 Drugs with similar targets can have both synergistic and antagonistic outcomes (14).

102 To address this challenge, here we extend an *in silico* tool that we recently created —Inferring
103 Drug Interactions using chemo-Genomics and Orthology, (INDIGO) (14)— to predict
104 synergy/antagonism in combinations of two or more drugs. The original INDIGO model used
105 chemogenomic profiling data under exposure to individual drugs (15, 16) as input data to
106 identify drug-response genes (14). The scientific premise underlying INDIGO is that drug
107 synergy and antagonism arise because of coordinated, systems-level molecular changes
108 involving multiple cellular processes. Importantly, INDIGO can learn patterns from known drug
109 interactions, which can then be used to forecast outcomes for new drugs and conditions.
110 INDIGO can thus provide insights on underlying mechanism of drug interactions in an unbiased
111 fashion. INDIGO can assess millions of combination regimens without requiring information
112 about the drug target or mode of action. Once an optimal drug regimen can be determined using
113 INDIGO, the dose regimes could be further optimized using feedback-based dose optimization
114 techniques (4, 5).

115 The goal of this study is to identify antibiotic combinations that are most promising for TB drug
116 development. We have adapted INDIGO to make use of transcriptomics data to identify drug-
117 response genes, which are more widely available than chemogenomics data for most non-
118 model organisms, including *Mycobacterium tuberculosis* (MTB) (**Figure 1**). We then harness a
119 large compendium of publicly available and in-house generated transcriptomics data to show

120 that INDIGO can successfully estimate drug interactions in MTB. We further integrate INDIGO
 121 with known MTB gene regulatory networks to identify transcription factors (TFs) that influence
 122 the extent of synergy between drugs. False positives and outliers from our model represent
 123 existing knowledge gaps and can inform future drug interaction experiments. The significant
 124 correlation of INDIGO-MTB predictions with both *in vitro* validations of novel predictions and *in*
 125 *vivo* efficacy metrics from clinical trials indicate that the INDIGO-MTB model has great promise
 126 for selecting novel TB drug regimens. INDIGO-MTB further provides unbiased insights on
 127 underlying cellular processes that influence drug interactions.



128
 129 **Figure 1. Schematic of INDIGO-MTB.** INDIGO uses drug-gene associations inferred from transcriptomic
 130 data and experimentally measured drug-drug interactions as inputs to train a computational model that
 131 can infer interactions between new combinations of drugs. It does this by learning patterns in the drug-
 132 gene associations that are correlated with synergy and antagonism. In the example above, MTB
 133 upregulation of both gene 1 and gene 3 in response to the drugs measured in monotherapy is predictive
 134 of antagonism when the drugs are combined. By perturbing individual genes and known targets of
 135 Transcription Factors (TFs) in the model, we can infer the impact of individual gene and TF activity
 136 respectively on drug interactions and subsequently engineer interaction outcomes.

137
 138

139 RESULTS

140 Construction of INDIGO-MTB model from drug response transcriptomes

141 The INDIGO approach requires a list of drug-gene associations and known drug-drug
142 interaction data as input for building a chemical-genetic model of drug interactions. A gene is
143 assumed to have a chemical-genetic association with a drug if a change in its expression leads
144 to a statistically significant alteration in sensitivity to the drug of interest. A drug-gene
145 association network is created by integrating chemogenomic profiling data from hundreds of
146 drugs. This static network is then converted into a predictive model by leveraging the powerful
147 statistical learning tool, Random Forest (17). This algorithm builds decision-trees using genes in
148 the drug-gene association network and identifies those that are predictive of drug interaction
149 outcomes using a training data set. The training data comprises known drug interactions. This
150 trained network model can be used to forecast interactions for novel drug combinations (**Figure**
151 **1, Figure S1**).

152 While in the prior study, drug-gene associations were obtained from chemogenomic profiles of
153 *E. coli*, these comprehensive gene deletion/drug response data are difficult to generate
154 experimentally for most pathogens. We hence hypothesized that transcriptomics data, which
155 quantifies the responses of every gene to a given perturbation, could provide a readily available
156 alternate resource for analysis. This solution could circumvent the limitation that chemogenomic
157 data are not available for most pathogens, including MTB. Generating gene expression data for
158 response to monotherapy drug exposure is straightforward, and there are already publicly
159 available transcriptomic profiles for many anti-TB agents.

160 We compiled transcriptome data profiling MTB response to different compounds and metabolic
161 perturbations from the literature. We augmented this compendium by generating MTB
162 transcriptomic response profiles for emerging TB agents (Methods, **Table S1A**). In addition to
163 these transcriptomic data, we also used chemogenomics data from *Escherichia coli* (16), with *E.*
164 *coli* genes matched to corresponding orthologous genes in the MTB genome. Our prior study
165 showed that INDIGO can infer interactions in MTB with significant accuracy using orthologous
166 gene mapping (correlation $R = 0.54$; p -value = 0.006). This was based on the observation that
167 genes predictive of drug-drug interactions were surprisingly conserved between *E. coli* and
168 MTB. In cases where multiple datasets profiled the same compound, we prioritized data from
169 MTB profiled with the latest transcriptomics technology whenever possible. We normalized this
170 drug response compendium using the ComBat algorithm (18) to account for inter-study and
171 technology-specific (i.e. microarray, RNAseq) variation in transcriptomics data (Methods).
172 Overall, this compendium contains data for 164 compounds and 65 metabolic perturbations
173 (see **Table S1A** for full list)(12, 19, 20).

174 To train INDIGO-MTB, we compiled drug interaction values in MTB for 202 drug combination
175 regimens from the literature, featuring compounds with available chemogenomic or
176 transcriptomic profiles (**Table S1B**). The drugs in the training set consist of well-established
177 anti-TB drugs, including rifampicin (RIF), isoniazid (INH), streptomycin (STM), several
178 fluoroquinolones, as well as new drugs such as bedaquiline (BDQ). The extent of interaction
179 between drugs was quantified in these studies by the standard Fractional Inhibitory
180 Concentration (FIC) index (21), or the DiaMOND interaction score (6). In both of these metrics,
181 synergy implies that the same amount of growth inhibition is achieved with a lower dose when
182 both drugs are combined. We used statistical data normalization to combine these datasets,
183 similar to our approach for combining transcriptomics data from various studies and platforms
184 (Methods). This allowed us to account for the new technology-specific variation in drug
185 interaction score distribution. If separate studies in literature provided conflicting interaction
186 scores for a drug combination, we included both values to incorporate this experimental
187 uncertainty into the model.

188 **Experimental validation of INDIGO-MTB model**

189 The INDIGO-MTB model trained on these drug interaction data was used to infer interaction
190 outcomes for new drugs and regimens. Given that our compendium has 164 compounds and 65
191 perturbations, INDIGO-MTB estimated all 26,106 potential pairwise interactions and all
192 1,975,354 potential three-way interactions. **Table S1C** shows the entire list of pairwise
193 combinations and interaction scores.

194 We observed striking associations between specific compounds and interactions that were
195 highly synergistic or antagonistic. In particular, combinations containing the drugs
196 chlorpromazine and verapamil were highly enriched for synergistic interactions; 77% of
197 chlorpromazine-containing combinations and 80% of verapamil-containing combinations were
198 found to interact synergistically (FIC < 0.9) (**Figure S2**). Verapamil is an efflux pump inhibitor
199 that influences membrane potential (22) and has been previously been shown to potentiate the
200 activity of several anti-TB drugs (23-25). In contrast, all pairwise combinations featuring
201 sutezolid were found to be antagonistic.

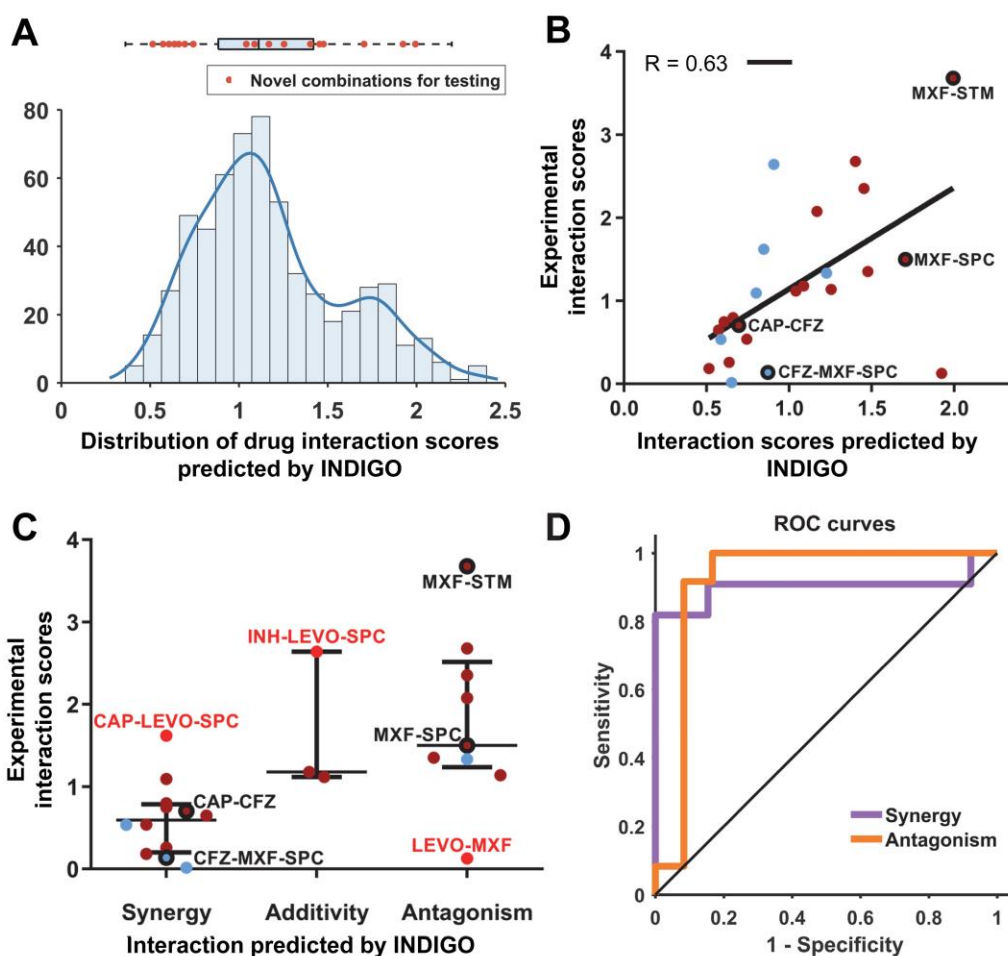
202 Previous work had found combinations of bacteriostatic drugs paired with bactericidal drugs
203 were likely to be antagonistic against *E. coli* (26). INDIGO-MTB uncovered a similar trend in the
204 MTB drug interactions; combinations featuring a bacteriostatic drug and a bactericidal drug had
205 significantly more antagonistic interaction scores than combinations featuring only bacteriostatic
206 drugs ($p < 10^{-12}$). Interestingly, combinations featuring only bactericidal drugs also had
207 significantly more antagonistic interaction scores than combinations featuring only bacteriostatic
208 drugs ($p < 10^{-12}$) (**Figure S2**).

209 To evaluate the accuracy of INDIGO-MTB, we experimentally measured interactions between a
210 set of two-drug and three-drug combinations, and we compared these measurements against
211 the interaction scores from INDIGO-MTB. The compounds featured in the tested combinations
212 are all FDA-approved agents that have diverse mechanisms of action, and are either part of
213 current first- and second-line TB therapy, or have been previously studied for their anti-
214 tubercular activity. The interaction outcomes for the test set combinations spanned the entire
215 range of INDIGO-MTB predicted interaction scores, enabling a rigorous assessment of INDIGO-
216 MTB (**Figure 2A, Figure S3**). We quantified the interaction outcome either by traditional
217 checkerboard assays or the high-throughput DiaMOND method for three-way combinations
218 (Methods). Given the diverse methodologies used in literature for measuring drug interactions,
219 we included combinations frequently measured in prior literature involving INH, RIF and STM as
220 reference combinations in our test set. In addition, among the test set combinations, 10
221 combinations involved pairwise subsets of three-way combinations that were measured using
222 DiaMOND methodology to infer 3-way interactions. Overall, among the 36 combinations in the
223 experimental validation set, 24 combinations were completely “novel”, i.e. never seen by
224 INDIGO. The sample size ($N = 24$ combinations) for the test set chosen for experimental
225 validation is sufficiently powered to significantly assess the accuracy of INDIGO’s correlation
226 with the experimental data (Methods).

227 We first classified experimentally measured combinations as synergistic, additive, or
228 antagonistic. INDIGO-MTB predicted interaction scores were significantly different between
229 these three classes (p -value = 0.0064, Kruskal-Wallis Rank Sum Test, **Figure 2C**). In addition,
230 there was a significant difference between INDIGO-MTB predictions for synergistic and
231 antagonistic combinations (p -value = 0.0009, non-parametric Komolgorov-Smirnov test).
232 Receiver Operating Curve (ROC) analysis of INDIGO-MTB predictive performance yielded an
233 area under the curve (AUC) of 0.89 ($p = 1.2 \times 10^{-3}$) and 0.91 ($p = 6.7 \times 10^{-4}$) for detecting
234 synergy and antagonism in the validation set, respectively (**Figure 2D**). These results are robust
235 to the choice of thresholds used for classifying interactions as synergistic or antagonistic
236 (**Figure S4**). We next performed a quantitative comparison between INDIGO-MTB interaction

237 scores and the corresponding *in vitro* experimentally measured FIC indices using the scale
238 invariant metric, spearman's rank correlation (R) (**Figure 2B**). We observed a high degree of
239 correlation between model prediction and experimental measurements for all combinations (R =
240 0.63, $p = 9.5 \times 10^{-4}$), and also after separating pairwise ($R=0.62 \pm 0.03$, $p = 9 \times 10^{-3}$) and three-
241 way interactions ($R=0.64 \pm 0.1$, $p = 8 \times 10^{-2}$). The correlation with INDIGO-MTB predictions is
242 identical for both the novel set (rank correlation $R = 0.63$) and for the total validation set, ($R =$
243 0.64 for all 36 combinations). Thus, not only can INDIGO *qualitatively* differentiate synergy and
244 antagonism, but it can also *quantitatively* separate regimens based on their extent of synergy.

245 Of note, we validated the INDIGO-MTB prediction that the combination of moxifloxacin (MXF)
246 and spectinomycin (SPC) are pairwise-antagonistic (DiaMOND FIC = 1.50) but could be made
247 more synergistic with the addition of clofazimine (CFZ) (DiaMOND FIC = 0.14). The synergy
248 identified between capreomycin (CAP) and CFZ (DiaMOND FIC = 0.70) and strong antagonism
249 between STM and moxifloxacin (MXF) (FIC = 3.68) were also experimentally confirmed. These
250 results, along with tenfold cross validation analysis of the training data (**Figure S4**), show that
251 INDIGO-MTB can successfully infer novel interactions among drugs with known transcriptome
252 profiles.



253
254 **Figure 2. INDIGO- MTB accurately predicts novel drug interactions.** (A) Drug combinations chosen
255 for experimental testing span the entire range of drug interaction predictions by INDIGO. The histogram
256 and box plot above it show the distribution of pairwise drug interaction scores for the 35 high interest TB
257 agents (the edges of the box plot demarcate the 25th and 75th percentile, and the dashed lines extend
258 between the 1st and 99th percentile). The interaction scores of the combinations chosen for testing are

259 shown as red dots. The 35 high interest agents contain drugs either currently used to treat TB or have
260 been used in the past to treat TB (27). **(B)** Comparison of INDIGO-MTB interaction scores with
261 experimental *in vitro* interaction scores. Each dot indicates a specific drug combination. Dark red dots
262 mark two-drug regimens ($R = 0.62$, $p = 9.3 \times 10^{-3}$), and blue dots mark three-drug regimens ($R = 0.64$, $p =$
263 8.81×10^{-2}). The specific combinations mentioned in the text are highlighted in the plot. For both
264 experimental and INDIGO-MTB scores, values less than 0.9 indicate synergy, values between 0.9 and
265 1.1 denote additivity, and values greater than 1.1 indicate antagonism. **(C)** Dot plot of experimentally
266 measured drug interaction scores versus the INDIGO-MTB predicted drug interaction type. The dots
267 labeled in red font denote outlier combinations that were misclassified by INDIGO-MTB. The interaction
268 scores were significantly different between predicted synergistic and antagonistic combinations ($p =$
269 0.0009 , KS test). The horizontal lines in the box plot represent the median and the first and third quartiles.
270 **(D)** ROC curves plotting sensitivity vs specificity for INDIGO-MTB predictions of synergy and antagonism
271 for both 2-drug and 3-drug combinations in the validation set. Sensitivity measures the true positive rate,
272 which is the fraction of true positive interactions correctly identified; specificity measures the true negative
273 rate. The area under the ROC (AUC) values provides an estimate of the sensitivity and specificity of
274 model predictions over a range of thresholds. The AUC values are 0.89 and 0.91 for synergy and
275 antagonism respectively. (Sensitivity = 90.9% and Specificity = 84.6% for synergy, Sensitivity = 66.6%,
276 Specificity = 91.7% for predicting antagonism).

277

278 While most predictions were confirmed experimentally, there were systematic inconsistencies
279 between the model and experiment for some individual drugs. For example, half of the
280 inconsistencies arose in combinations featuring spectinomycin (SPC). Although SPC has been
281 found to synergize with several anti-TB drugs with multiple modes of action (28, 29), the model
282 tends to overpredict synergy for combinations that include SPC. This may be in part because
283 SPC predictions were based on chemogenomic data from *E. coli* rather than MTB response
284 transcriptomes.

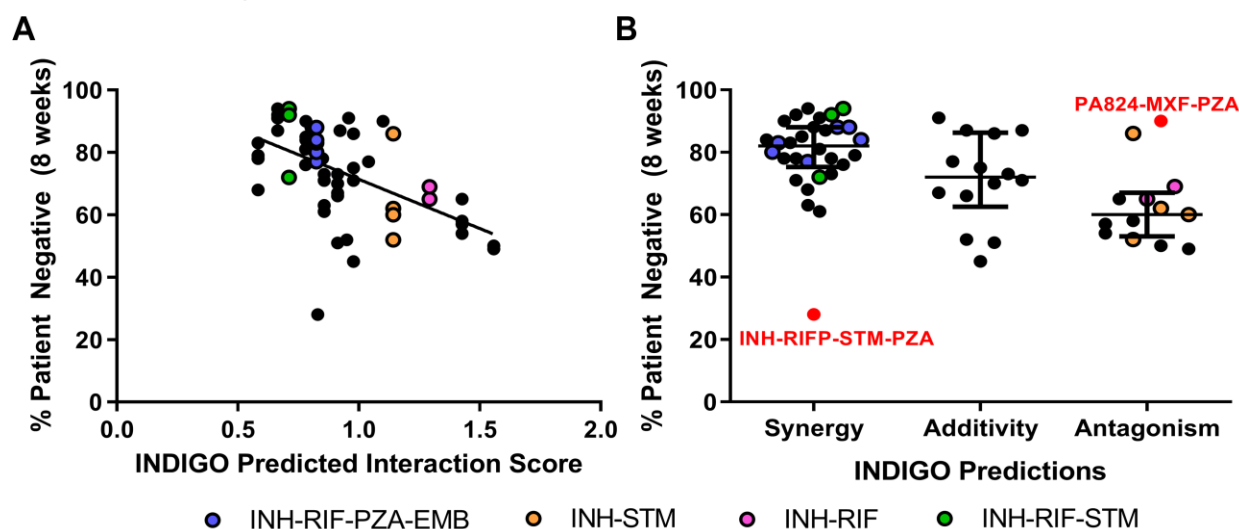
285 Given the high accuracy of our model for both pairwise and multi-drug combinations, we inferred
286 interactions for 35 promising TB drugs using INDIGO-MTB. The resulting compendium of 6545
287 three-way, 52,360 four-way, and the top 100 synergistic and antagonistic combinations from
288 324,632 five-way combinations is provided as a supplement to serve as a resource for guiding
289 future drug combination screens (**Table S2, Table S3**).

290 ***In vitro* drug synergy is correlated with a surrogate marker of clinical efficacy**

291 We next tested if *in vitro* drug interaction outcomes would be predictive of clinical efficacy. A
292 systematic evaluation of the clinical relevance of *in vitro* drug interactions on treatment efficacy
293 is lacking (30). We therefore compared INDIGO-MTB *in vitro* drug interaction predictions with a
294 meta-analysis of data assembled from 57 phase 2 clinical trials (31). These trials reported
295 regimen efficacy outcomes by sputum culture conversion rates of TB patients at two months. If
296 separate clinical studies reported conflicting efficacy scores for a drug regimen, we used both
297 values for comparison with INDIGO-MTB to incorporate this uncertainty.

298 We found a highly significant degree of correlation between the INDIGO-MTB interaction scores
299 and the sputum culture conversion rates for the corresponding combinations ($R = -0.55 \pm 0.04$,
300 $p \sim 10^{-5}$, see **Figure 3A, Table S1D**). The results show that regimens predicted to have greater
301 synergy performed better in the clinical trials. For example, the INH-RIF-STM regimen (green)
302 was predicted to be synergistic *in vitro*, and this combination conferred high patient culture
303 negativity (~94%) at two months (**Figure 3A**). In contrast, the pairwise combinations of INH-
304 STM (yellow) and INH-RIF (pink) were identified as antagonistic, and both drug pairs resulted in
305 low sputum conversion rates. There was a highly significant difference in sputum conversion
306 between synergistic and antagonistic combinations ($p \sim 10^{-4}$, **Figure 3B**), the difference in
307 clinical outcome for synergistic-additive ($p = 0.038$) and additive-antagonism ($p = 0.016$)
308 interactions were significant as well.

309 Among the combinations assessed clinically, only four two-way and two three-way drug
310 combinations had experimental *in vitro* drug interaction data. We next compared the correlation
311 of *in vitro* experimentally measured drug interaction with the corresponding sputum conversion
312 rates. We found that *in vitro* experimental drug interaction scores also correlated significantly (R
313 = -0.52 ± 0.1 , $p = 0.01$) with clinical sputum conversion by sampling analysis (**Figure S5**). This
314 correlation is comparable to the value observed with INDIGO-MTB across all 57 clinical trials.



315
316 **Figure 3. INDIGO-MTB drug interaction scores correlate with sputum culture negativity at 2**
317 **months. (A)** Comparison of model predictions with sputum conversion rates in human patients after 8
318 weeks of treatment in clinical trials ($R = -0.55$, $p \sim 10^{-5}$). Higher patient negative percentages indicate
319 more effective regimens. Each dot indicates a specific drug combination reported from a specific clinical
320 trial. Dots highlighted in the legend are drug combinations of interest mentioned in the text. **(B)** Dot plot of
321 sputum conversion rates against the INDIGO-MTB predicted drug interaction type. The dots labeled in
322 red font denote outlier combinations that were misclassified by INDIGO-MTB. The horizontal lines
323 represent the 1st quartile, 3rd quartile, and median (the widest horizontal line). The colored dots
324 correspond to combinations highlighted in the legend.

325
326 Despite the strong overall concordance between *in vitro* synergy and *in vivo* sputum culture
327 conversion rates, we found some outlier combinations that were inferred to be synergistic but
328 had poor clinical outcomes. All the outlier regimens contained pyrazinamide (PZA), whose
329 interaction scores were estimated based on transcriptomes that were generated under acidic
330 conditions, which were unlike the conditions of the other drug profiles. Furthermore, the RIF-
331 MXF combination was identified to be antagonistic by both our model and experiments but has
332 good *in vivo* efficacy. It is hypothesized to be effective because of its ability to suppress
333 resistance despite being antagonistic (32). Hence, synergy alone does not always imply clinical
334 efficacy. Numerous other factors can impact treatment outcome. Combinations can perform well
335 despite being antagonistic. Overall, our results suggest that drug synergy is significantly
336 correlated with treatment efficacy at 8 weeks, and identifying synergistic drug interactions is a
337 promising strategy to prioritize combination regimens.

338 **Inferring molecular mediators of drug synergy**

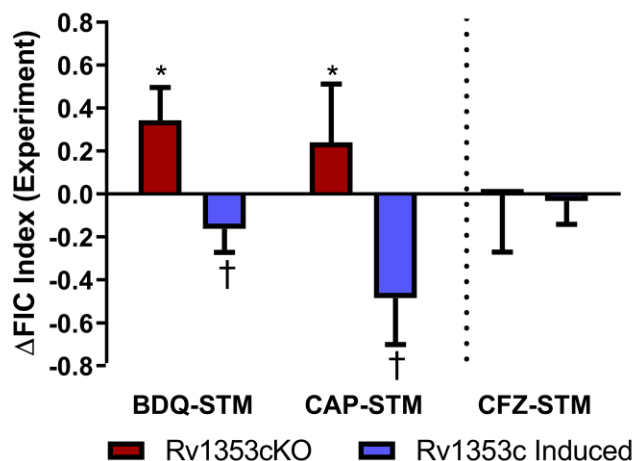
339 To interrogate what molecular processes underlie INDIGO-MTB's predictive ability, we identified
340 genes in the INDIGO-MTB model that most strongly influenced drug interaction scores. Genes
341 were *in silico* "deleted" from the INDIGO-MTB model (i.e., excluded from the model prediction)
342 and assigned an importance score by INDIGO-MTB proportional to their relative contribution in

343 calculating drug interaction scores. The top 500 genes sorted based on their importance score
344 accounted for 97% of INDIGO-MTB's predictive ability. We performed pathway enrichment
345 analysis using literature-curated pathways from the KEGG database (33, 34) to determine over-
346 represented pathways among the top 500 informative genes (**Table S1E**). Metabolic pathways
347 were highly enriched overall, and the most overrepresented pathway was oxidative
348 phosphorylation, which is targeted by BDQ. The model thus suggests that targeting this
349 pathway might have an impact on drug interaction outcomes.

350 We hypothesized that we could gain further insights into the genetic regulation of drug
351 interaction outcomes. To do this, we analyzed the INDIGO-MTB model in the context of the
352 MTB transcriptional regulatory network (TRN). The TRN was reconstructed by transcriptome
353 profiling of a comprehensive library of transcription factor induction strains (TFI)(35, 36). The
354 regulon (i.e., set of functional targets) for each transcription factor (TF) was defined as those
355 genes that significantly changed expression upon chemical induction of the TF expression.

356 To assess the systems-level impact of each TF on drug interactions, we performed *in silico*
357 deletions of entire regulon-defined gene sets and assessed the effect on the INDIGO-MTB
358 interaction scores. We identified regulon deletions that disrupt a specific drug interaction and
359 those that influence multiple drug interaction outcomes. For this analysis, we considered all 36
360 pairwise combinations comprising the drugs: INH, RIF, STM, MXF, CFZ, BDQ, capreomycin
361 (CAP), ethionamide, and pretomanid (PA824). The drugs tested are all current first- and
362 second-line TB agents that can be prescribed together as part of therapy and have differing
363 mechanisms of action. From this analysis, INDIGO-MTB identified the transcription factor
364 Rv1353c as having the highest impact on drug interactions among all the TFs (**Figure S6A**).
365 INDIGO-MTB estimated that Rv1353c would shift the interaction scores for almost every
366 pairwise interaction toward synergy upon induction ($\Delta\text{score} = -0.6 \pm 0.1$). The exception was the
367 combination CFZ-STM, for which INDIGO-MTB predicted minimal interaction shift associated
368 with TF induction ($\Delta\text{score} = -0.2$) (**Figure S6B**).

369 We tested these model predictions by comparing the interactions of three representative drug
370 combinations with the following three genetic perturbations: (1) TF induction, measured in the
371 TFI strain with the presence of chemical induction; (2) TF disruption, measured in a knockout
372 strain (see Methods); and (3) baseline TF levels, measured in the genetic wildtype strain,
373 H37Rv and the TFI strain in the absence of chemical induction. We selected two drug
374 combinations for which strong interaction shifts were inferred upon TF induction (BDQ-STM,
375 $\Delta\text{score} = -0.7$; CAP-STM; $\Delta\text{score} = -0.7$), as well as the CFZ-STM combination for which the
376 model estimated minimal interaction shift. The baseline interactions between the drug
377 combinations differ substantially (BDQ-STM is additive, whereas CAP-STM and CFZ-STM are
378 both antagonistic, **Figure S6C**). **Figure 4** shows the difference in experimentally measured
379 interaction scores of each drug combination for the genetic perturbation conditions, relative to
380 the wildtype (Methods). The results show that when Rv1353c is induced, interactions for both
381 BDQ-STM and CAP-STM shift toward synergy ($\Delta\text{FIC} = -0.2 \pm 0.1$, $p = 0.03$ for BDQ-STM; ΔFIC
382 $= -0.5 \pm 0.2$, $p = 0.01$ for CAP-STM), and when Rv1353c is disrupted, interactions for both BDQ-
383 STM and CAP-STM shift toward antagonism ($\Delta\text{FIC} = 0.3 \pm 0.2$, $p = 0.001$ for BDQ-STM; ΔFIC
384 $= 0.2 \pm 0.2$, $p = 0.04$ for CAP-STM). In contrast, there appears to be no significant shifts in
385 interaction for CFZ-STM with either induction or disruption of Rv1353c ($\Delta\text{FIC} = -0.0004 \pm 0.3$, p
386 $= 0.5$ for disruption; $\Delta\text{FIC} = -0.03 \pm 0.1$, $p = 0.03$ for induction). Collectively, these results confirm
387 the INDIGO-MTB predictions.



388

389 **Figure 4. Rv1353c influences interactions between drug combinations.** The *in vitro* experimentally
390 measured drug interaction scores are quantified for the three selected drug interactions, plotted as the
391 difference in FIC score of the gene perturbation relative to the wildtype (H37Rv). The red bars denote
392 values for the knockout strain, and the blue bars show values for the strain with Rv1353c induced.
393 Negative values indicate shifts toward synergy, and positive values indicate shifts toward antagonism.
394 The (*) and (†) indicate that differences are significantly greater or less than zero, respectively ($p < 0.05$,
395 one-tailed one-sample t-test). The error bars represent the standard deviation between replicates.

396 DISCUSSION

397 Here, we constructed an INDIGO-MTB model to predict *in vitro* synergy and antagonism of anti-
398 tuberculosis drug combinations using transcriptomics data. Our model complements existing
399 experimental strategies by increasing throughput and by identifying potential drug interaction
400 mechanisms. Our analysis using INDIGO-MTB revealed novel synergy between clinically
401 promising drug combinations, uncovered the role of the TF Rv1353c in influencing drug
402 interaction outcomes, and found a significant association between *in vitro* drug interaction
403 outcomes and clinical efficacy. These results suggest that using INDIGO-MTB to identify
404 synergistic regimens is a promising strategy for prioritizing combination therapies. While
405 significant challenges exist, constructing a high-quality model of drug interactions *in vitro* is the
406 first step towards inferring *in vivo* efficacy. No theoretical method currently exists that can
407 comprehensively screen thousands of combinations even *in vitro*. The significant correlation
408 between INDIGO interaction scores with both *in vitro* data and clinical efficacy data supports the
409 utility of our approach.

410 INDIGO-MTB outperforms existing strategies in terms of throughput. The largest studies in MTB
411 have so far analyzed up to two hundred unique drug combinations (37). Here, we have
412 estimated outcomes for 13,366 pairwise and 721,764 three-way combinations of 164 drugs with
413 significant accuracy based on our prospective validation. While many of the drugs might have
414 poor anti-TB activity on their own, they may greatly enhance synergy when added to existing
415 regimens. For example, we found chlorpromazine, originally used for treating psychiatric
416 disorders, synergizes with BDQ, resulting in four-fold reductions in inhibitory concentrations
417 (**Figure S7A**). Thus, INDIGO can facilitate repurposing of drugs to treat TB.

418 INDIGO complements other preclinical methods such as mouse models in prioritizing regimens
419 for clinical evaluation. A systematic comparison across multiple mouse studies is challenging
420 due to the lack of quantitative raw data and variation in metrics reported in the literature.
421 Nevertheless, combinations identified by INDIGO to be highly synergistic (top 0.01%, **Table S2**,
422 **Table S3**) were also found to be highly efficacious in recent mouse studies. Combinations

423 involving BDQ and CFZ alone or in a three-drug combination with PZA, Ethambutol (EMB), RIF,
424 or INH were all found to be synergistic by INDIGO and showed high bactericidal activity in
425 mouse models (5, 38-40). Four-way drug combinations involving BDQ, CFZ and PZA with EMB
426 or SQ109 were also synergistic in mouse studies (5, 38-40). In addition to these combinations
427 studied in mouse models, INDIGO-MTB also uncovered highly synergistic novel 4-drug and 5-
428 drug combinations that are promising candidates for pre-clinical evaluation, such as the
429 combination with BDQ, CFZ, RIF, CLA and the anti-malarial antifolate compound P218, and a 4-
430 drug combination involving BDQ, RIF, PA824, and the anti-psychotic drug thioridazine (**Table**
431 **S3**).

432 Since numerous factors could impact *in vivo* efficacy that are not considered during *in vitro*
433 studies, it is not *a priori* clear if there should be a significant correlation between *in vitro* synergy
434 and *in vivo* efficacy. Thus, we performed a systematic comparison of *in vitro* drug interaction
435 scores with clinical efficacy of drug combination regimens. Notably, here we observed a
436 statistically significant correlation between *in vitro* drug interaction scores and the percentage of
437 TB patients showing negative sputum culture after 2 months treatment in clinical trials, with
438 synergistic drug combinations showing greater clinical efficacy. Negative sputum culture at eight
439 weeks is a useful early measure of TB treatment efficacy that correlates well with relapse rates
440 (41, 42). The correlation that we observed between *in vitro* INDIGO-MTB predictions and
441 sputum conversion rates is notable, given the huge variability between clinical studies.

442 While existing high-throughput approaches are strictly non-mechanistic, INDIGO can reveal the
443 relative contribution of underlying cellular pathways on drug interaction outcomes. Our analysis
444 suggests that drug transporters and central metabolic pathways may play a role in influencing
445 drug interaction outcomes. This is consistent with recent studies on the role of bacterial
446 metabolic state in impacting drug interaction outcomes (43, 44). Contextualizing INDIGO-MTB
447 with the MTB transcriptional regulatory network revealed genetic regulators of drug interaction
448 response. This analysis uncovered the role of the transcription factor, Rv1353c as a broad
449 regulator of drug interaction outcomes. Rv1353c is an uncharacterized nonessential helix-turn-
450 helix type transcriptional regulator (45-49) that has previously been found to be deleted in
451 several clinical isolates (50). When induced under log-phase growth, Rv1353c activates 44
452 genes enriched for fatty acid biosynthesis and represses 50 genes, including two of the top five
453 most informative INDIGO-MTB predictor genes (Rv1857 and Rv1856c)(35). Interestingly,
454 INDIGO-MTB simulations suggest minimal shifts in drug interaction scores upon perturbing
455 either Rv1857 or Rv1856 individually, suggesting that the underlying molecular mechanisms
456 mediating drug interactions may be partially epistatic in nature. Collectively, this suggests that
457 knowledge of the underlying mechanism of drug interaction can be used to engineer synergy
458 between combination regimens. Our approach provides a rational strategy to identify genetic
459 targets that enhance synergy between existing regimens and introduces a potentially new way
460 to engineer effective regimens by modifying the interactions between the constituent drugs.

461 While the INDIGO approach has demonstrated significant utility in predicting synergy and
462 antagonism of drug combinations, it nevertheless has several key limitations. First, INDIGO-
463 MTB requires as input transcriptome data profiling of MTB response to each drug for which drug
464 interaction predictions are necessary. Transcriptomes are significantly faster and cheaper to
465 generate than the chemogenomic profiles used to power the original INDIGO models. This has
466 enabled us to use species-specific data to build INDIGO-MTB. Among the 35 TB drugs of
467 interest, the input data for only 10 drugs (28%) are derived from *E. coli* chemogenomics data.
468 The correlation observed in the current study, wherein the model was constructed using MTB
469 response transcriptomes elicited by drug exposure, is higher than the correlation observed in
470 our prior study, which used chemogenomic data to infer interactions ($R = 0.62$ for pairwise and
471 0.64 for three-way interactions for the current study, versus $R = 0.52$ for pairwise and 0.56 for

472 three-way interactions in the *E. coli* chemogenomic study (14, 51)). Notably, while predictions
473 using *E. coli* data were statistically significant, many of the incorrect predictions from our model,
474 such as drug combinations involving spectinomycin, might be attributed to challenges of
475 extrapolating predictions from *E. coli* using gene orthology information alone. Our results
476 suggest that gene expression changes encapsulate molecular response information that is as
477 informative of drug interaction phenotypes as gene deletion studies. The updated INDIGO
478 approach can hence be applied to other pathogens that lack chemogenomic data. With reduced
479 sequencing costs, transcriptomics data is unlikely to be a substantial limitation in the future.
480 Further, while the number of possible combinations increases exponentially with the number of
481 drugs, the number of transcriptomes required only increases linearly. Hence, INDIGO-MTB and
482 other methods that use responses elicited by individual drugs will be more cost and time
483 effective.

484 A second limitation stems from the fact that INDIGO-MTB predictions are currently based on
485 data gathered from log-phase *in vitro* broth culture conditions, which are markedly different from
486 the *in vivo* microenvironments. Outliers from our experimental validation involving PZA (which is
487 relatively more active under low pH conditions) substantiate the notion that the underlying
488 environmental context can influence the model accuracy. The INDIGO algorithm is currently
489 blind to MTB molecular responses to drugs in the host context. Training our model using MTB
490 transcriptome profiling data generated using an appropriate environmental condition (e.g., MTB
491 in a macrophage or mouse infection model) might address this limitation in the future. A recent
492 study has expanded the INDIGO model to enable *in silico* prediction of the impact of different
493 microenvironments in *E. coli* (51). Hence building an accurate INDIGO model for MTB can
494 provide a foundation for addressing this *in vivo* complexity.

495 Finally, while synergy is associated with a better treatment outcome on average, other factors
496 such as resistance evolution, toxicity, and drug pharmacokinetics will also influence treatment
497 success. In addition, there is considerable heterogeneity in clinical trial efficacy based on patient
498 population, dose and location. The curation of numerous clinical studies and ability to predict
499 interactions in high throughput provided us with sufficient statistical power to test the association
500 between synergy and *in vivo* efficacy despite this heterogeneity. In the future, incorporating
501 additional factors associated with drug behavior in the host may further improve the correlation
502 between model predictions and clinical outcomes.

503

504 **METHODS**

505 **Culture conditions**

506 MTB strains were cultured in Middlebrook 7H9 with the oleic acid, bovine albumin, dextrose and
507 catalase (OADC) supplement (Difco), and 0.05% Tween80 at 37 °C under aerobic conditions
508 with constant agitation to mid-log phase, as described previously (35, 52). Strains containing the
509 anhydrotetracycline (ATc)-inducible expression vector were grown with the addition of 50 µg/mL
510 hygromycin B to maintain the plasmid. To induce expression of the transcription factor Rv1353c,
511 20ng/µL of ATc was added to the culture media. Growth was monitored by the optical density at
512 600 nm (OD600).

513 The Rv1353c overexpression strain was generated previously (35, 36). Briefly, the Rv1353c
514 gene was cloned into a tagged, inducible vector that placed the gene under control of a
515 tetracycline-inducible promoter (53) and added a C-terminal FLAG epitope tag. This construct
516 was transformed into MTB H37Rv using standard methods. The strain is available from the BEI
517 strain repository at ATCC ((54), NR-46512).

518 Phage Knockout Strain Generation

519 The H37Rv $\Delta Rv1353c$ strain was constructed by a specialized transduction method(55) using a
520 gene-specific specialized transducing phage phasmid DNA provided by the Jacobs lab and the
521 previously described protocol (55). Briefly, high-titer phage stocks were generated by
522 transfecting the phasmid DNA into *Mycobacterium smegmatis* mc²155 at 30°C, and growing the
523 resulting phage plaques on an agar pad with a lawn of mc²155. Transduction-competent H37Rv
524 was incubated with high-titer phage stock for 24 hours at 37°C, and the transduced bacteria
525 were plated on 7H10 supplemented with 50 µg/mL hygromycin B to select for deletion-
526 substitution mutants.

527

528 Drug susceptibility and checkerboard drug-drug interaction experiments

529 Strains were grown to log phase (OD600 ≈ 0.3), diluted to a final OD600 ≈ 0.005 (equivalent to
530 10⁶ (CFU)/mL), and dispensed into 96-well flat-bottom plates (Corning, Acton, MA) at a final
531 volume of 200µL, containing 1% DMSO and varying concentrations of drugs in the different
532 wells. On each plate, control wells for each of the strains studied were included, containing: 1)
533 no drug and 1% DMSO vehicle; and 2) 1% culture and no drug with 1% DMSO vehicle, to
534 measure viability in the absence of drug exposure.

535 For drug susceptibility assays to measure the MIC, serial 2-fold dilutions of an individual drug
536 were arrayed in the different columns. For checkerboard drug interaction assays, 2-fold dilutions
537 of the first drug were arrayed in the columns and 2-fold dilutions of a second drug were arrayed
538 in the rows.

539 Plates were incubated at 37°C for 7 days. Cellular viability was assayed on day 7 by the
540 BacTiter Glo (Promega, Madison, WI) and Alamar Blue cell proliferation assays (Bio-Rad,
541 Hercules, CA) according to manufacturer recommendations. Briefly, we added 20µL of culture
542 from each well to 20µL of BacTiter-Glo Microbial Cell Viability Assay Reagent, incubated at
543 room temperature protected from direct light for 20 minutes, and read luminescence intensity
544 using a FluoStar Omega plate reader (BMG Lab Tech, Cary, NC). For Alamar Blue, we added
545 20µL of Alamar Blue reagent to 180µL of culture, incubated for 12 hours protected from direct
546 light, and read fluorescence intensity at emission wavelength 590nm after excitation at 544nm.
547 **Figure S7B** shows the strong concordance between the two methods - BacTiter-Glo and
548 Alamar Blue.

549 For drug susceptibility assays, the MIC was determined as the lowest drug concentration that
550 resulted in MTB viability comparable to the 1% culture control. For checkerboard assays, the
551 drug interaction was quantified by the Fractional Inhibitory Concentration (FIC) index, equal to:

552 $FIC = \frac{C_A}{MIC_A} + \frac{C_B}{MIC_B}$, where C_A is the concentration of drug A when combined with drug B yielding
553 an iso-effective inhibition comparable to the MIC, and C_B is the concentration of drug B when
554 combined with drug A yielding an iso-effective inhibition. The value for FIC can be extended to
555 any arbitrary number of drug combinations as follows

$$\sum FIC_N = FIC_1 + FIC_2 + \dots + FIC_n$$
$$\sum FIC_N = \frac{MIC_1(\text{in combination})}{MIC_1(\text{alone})} + \frac{MIC_2(\text{in combination})}{MIC_2(\text{alone})} + \dots + \frac{MIC_n(\text{in combination})}{MIC_n(\text{alone})}$$

556

557 Each MIC and checkerboard experiment was performed 2 times, with 2 biological replicates per
558 experiment. The mean FIC index across all iso-effective concentrations was calculated for each
559 biological replicate to determine reproducibility, and data across biological replicates were

560 summarized by averaging (**Figure S1**).

561 **DiaMOND drug-drug interaction experiments**

562 DiaMOND drug interaction experiments were performed in biological triplicate as previous
563 described (6). Rather than sampling the entire set of dose combinations used in a traditional
564 checkerboard assay, DiaMOND samples a subset of dose responses and approximates the
565 shape of the contour of the chosen phenotype (e.g. where 50% growth inhibition is observed,
566 IC₅₀). For example, a two-drug combination requires three dose responses (each individual
567 drug dose response and an equipotent drug combination dose response) rather than the entire
568 set of possible dose combinations.

569 Individual drug dose response ranges were chosen for each drug such that the IC₅₀ dose was
570 close to the center and doses were linearly spaced to provide high resolution IC₅₀
571 determination. Drug combination dose response ranges contained equipotent mixtures of two or
572 three drugs (e.g. a two-drug combination would contain $\frac{1}{2}$ of the IC₅₀ dose for each drug and a
573 three-drug combination would contain $\frac{1}{3}$ of the IC₅₀ of each drug).

574 Briefly, MTB strain H37Rv cultures were grown to mid-log phase (OD₆₀₀ \approx 0.6), diluted to
575 OD₆₀₀ \approx 0.05 and added to drug containing plates. Drugs were dispensed into 384-well plates
576 using a digital drug dispenser (D300e Digital Dispenser, HP) and 50 μ L diluted MTB cultures
577 were overlaid. Drug treatment plates were incubated in humidified containers for 5 days at 37 °C
578 without agitation. Growth was measured by OD₆₀₀ using a plate reader (Synergy Neo2,
579 Biotek). Two technical replicates were performed, and the average of each technical replicate
580 was used to calculate FIC scores.

581 The FIC for a drug combination was calculated as the ratio between the observed and expected
582 IC₅₀ dose of the drug combination as previously described (6). FICs from each of three
583 biological replicates were calculated to determine reproducibility, and data across biological
584 replicates were summarized by averaging. Briefly, the growth measurements were normalized
585 (background subtracted, normalized to untreated) and the observed IC₅₀ doses were calculated
586 for each individual and combination drug dose response. The expected IC₅₀ dose for the drug
587 combination was then calculated using the IC₅₀ of the individual drugs, based on the null
588 hypothesis that the interaction is additive. For two-drug combinations the expected IC₅₀ dose is
589 defined as the intersection of the line (additivity line) drawn between the IC₅₀ doses for each
590 individual drug. For three-drug combinations, the expected IC₅₀ dose is defined as the
591 intersection of the drug combination dose response and the plane (additivity plane) created by
592 connecting the IC₅₀ doses for each individual drug (**Figure S1**).

593 **RNA-seq transcriptome profile data generation**

594 To profile the MTB transcriptome response to exposure of individual drugs, cultures were diluted
595 to OD₆₀₀ \sim 0.2 (equivalent to 10⁸ colony-forming units (CFU)/mL) and exposed to a minimum
596 inhibitory concentration (MIC)-equivalent dose of drug for approximately 16 hours.

597 RNA was isolated from these cultures as described previously (35, 52). Briefly, cell pellets in
598 Trizol were transferred to a tube containing Lysing Matrix B (QBiogene) and vigorously shaken
599 at maximum speed for 30 s in a FastPrep 120 homogenizer (QBiogene) three times, with
600 cooling on ice between shakes. This mixture was centrifuged at maximum speed for 1 min and
601 the supernatant was transferred to a tube containing 300 μ L chloroform and Heavy Phase Lock
602 Gel (Eppendorf), inverted for 2 minutes and centrifuged at maximum speed for 5 minutes. RNA
603 in the aqueous phase was then precipitated with 300 μ L isopropanol and 300 μ L high salt
604 solution (0.8 M Na citrate, 1.2 M NaCl). RNA was purified using a RNeasy kit following the
605 manufacturer's recommendations (Qiagen) with one on-column DNase treatment (Qiagen).
606 Total RNA yield was quantified using a Nanodrop (Thermo Scientific).

607 To enrich the mRNA, ribosomal RNA was depleted from samples using the RiboZero rRNA
608 removal (bacteria) magnetic kit (Illumina Inc, San Diego, CA). The products of this reaction were
609 prepared for Illumina sequencing using the NEBNext Ultra RNA Library Prep Kit for Illumina
610 (New England Biolabs, Ipswich, MA) according to manufacturer's instructions, and using the
611 AMPure XP reagent (Agencourt Bioscience Corporation, Beverly, MA) for size selection and
612 cleanup of adaptor-ligated DNA. We used the NEBNext Multiplex Oligos for Illumina (Dual Index
613 Primers Set 1) to barcode the DNA libraries associated with each replicate and enable
614 multiplexing of 96 libraries per sequencing run. The prepared libraries were quantified using the
615 Kapa qPCR quantification kit, and were sequenced at the University of Washington Northwest
616 Genomics Center with the Illumina NextSeq 500 High Output v2 Kit (Illumina Inc, San Diego,
617 CA). The sequencing generated an average of 75 million base-pair paired-end raw read counts
618 per library.

619 Read alignment was carried out using a custom processing pipeline that harnesses the Bowtie 2
620 utilities(56, 57), which is available at <https://github.com/sturkarslan/DuffyNGS>, and
621 <https://github.com/sturkarslan/DuffyTools>. The RNA-seq data profiling response to drug
622 exposure generated for this study are publicly available at the Gene Expression Omnibus
623 (GEO) at **GSE119585**.

624 **Gene expression data analysis**

625 The RNA-seq transcriptome profiling data that we generated were supplemented with
626 microarray and RNA-seq transcriptome profiling datasets from literature that were downloaded
627 from GEO, along with associated gene accession identifiers. The \log_2 -transformed fold change
628 values of average gene expression in each treatment group were determined for all studies,
629 relative to the experiment's negative control. All genes that significantly change by more than 2-
630 fold (up or down) after each drug treatment were used as input features for INDIGO-MTB. The
631 results are robust to the thresholds chosen for finding differentially expressed genes (**Table**
632 **S1G**).

633 ComBat (18) normalization was used to minimize batch effects in the data, which uses empirical
634 Bayes approach to estimate each batch's corrected mean and variance. The effectiveness of
635 normalization was checked using principal component analysis. This version of the
636 transcriptomic/chemogenomic matrix represented the drug-gene network that was required to
637 build the INDIGO-MTB model.

638 The drug-gene interaction profiles for each drug are then used by INDIGO to create a "joint"
639 interaction profile for a drug combination (**Figure S1**). INDIGO assumes that cellular response
640 to drug combinations is a linear function of the cellular response to individual drugs. This
641 assumption is based on prior experimental studies that found that a linear model best explained
642 transcriptional response of cells treated with drug combinations (58, 59). Further, in our prior
643 study in *E. coli*, we found that other models of profile integration, such as correlation or profile
644 overlap performed poorly in predicting drug interactions compared to the linear integration
645 model (14).

646

647 **Quantifying drug-drug interaction scores for model training**

648 To train INDIGO-MTB, checkerboard FIC indices of drug combinations were collected after
649 conducting literature search (n=140). We also included FIC50 indices that were calculated using
650 the DiaMOND approach (n=62)(6). Since the DiaMOND study had a distinct distribution from
651 other checkerboard studies from literature (Mean = 1.05 and 0.99, Standard deviation = 0.32
652 and 0.81 for DiaMOND and checkerboard respectively), we statistically transformed the
653 DiaMOND scores so that the overall distribution of the DiaMOND-measured scores had the
654 same mean and standard deviation as the remaining checkerboard datasets. The normalized

655 scores were used for training INDIGO-MTB (**Table S1F**). Similarly, the DiaMOND data
656 generated in this study for validation was normalized using the same approach prior to
657 comparison with INDIGO-MTB predictions. The average interaction score in the final training set
658 was 1.01, suggesting that the training data set is not significantly biased towards synergy or
659 antagonism.

660 **Statistical Analyses**

661 Our experimental test set is sufficiently powered statistically to significantly assess the accuracy
662 of INDIGO's correlation with the experimental data. For example, the probability of getting a
663 correlation of 0.62 achieved by INDIGO by random chance is less than 1 in 10^3 . We statistically
664 estimated that we only need 14 samples to detect a correlation of 0.6 ($R \geq 0.6$) with a p-value of
665 0.01. Our test set sample size is significantly larger than this number.

666 Spearman rank correlations were computed using the statistical software R. Differences
667 between the means of each group in box plots were compared using two-sample one-tailed K-S
668 tests in R. To further assess the robustness of our results to variation in clinical trials, we
669 performed sampling analysis by choosing one representative clinical trial randomly for each
670 regimen. We observed a significant correlation between predicted interaction scores and the
671 sputum culture conversion rates (mean rank correlation $R = -0.38$ average of 100 random
672 sampling trials) (**Figure S7B**).

673 The significance of the AUC values from the ROC analysis was calculated by randomly
674 permuting the class labels (synergy or antagonism) of the test data 1000 times. The difference
675 in accuracy of the actual model with the random permuted models was compared using a t-test.

676 We used the RandomForest algorithm that is part of the Machine learning toolbox in MATLAB.
677 The regression random forest algorithm was used with default parameters for the number of
678 predictors sampled (default value – $N/3$, where N is the number of variables). Hyperparameter
679 tuning of parameters in the training set instead of using default parameters also resulted in a
680 similar accuracy in the test set (**Table S1H**). Random forests are perfectly suited for our
681 analysis as they can achieve high accuracy even with small sample sizes and can be easily
682 interpreted. The training set used here is relatively small for deep neural networks which
683 require thousands of samples. On the other hand, SVM and decision trees can be built with
684 small sample sizes but do not achieve high accuracy as Random Forests. The accuracy using
685 these approaches with default parameters is lower than Random Forests with default
686 parameters (**Table S1H**).

687 The INDIGO-MTB model and associated data sets are available from the Synapse
688 bioinformatics repository (Synapse ID: syn18824984) (https://www.synapse.org/INDIGO_MTB)
689 (DOI: 10.7303/syn18824984).

690 **ACKNOWLEDGMENTS**

691 The data reported in the paper are available in the Supplementary Materials. The RNA-seq data
692 generated for this study are available on the Gene Expression Omnibus [GSE119585].

693 We gratefully acknowledge the laboratory of William R. Jacobs Jr. for providing the phage that
694 enabled us to make the Rv1353c knockout strain. We gratefully acknowledge Andréanne
695 Lupien, Anthony Vocat, and Stewart T. Cole for providing us a sample of PBTZ169 for our
696 study. We also gratefully acknowledge the laboratory of Timothy Sterling for providing us with
697 ofloxacin resistant clinical strains. We also thank Jessica Winkler, Laura Green, and Reiling
698 Liao, for their technical assistance, and Awanti Sambarey for her feedback on the manuscript.

699 This work was supported by the National Institutes of Health [grant numbers U 19 AI106761
700 (DS, SC); U19 AI11276 (DS); U19 AI135976 (DS); 5T32AI007509 (SM); P50 GM107618-01A1

701 (BA); UL1TR002240 (SC); NIH Director's New Innovator Award 1DP2LM011952-01(BA); the
702 University of Michigan Precision Health and MCUBED (SC).

703 **AUTHOR CONTRIBUTIONS:**

704 S.M. conceived of the study, led the design, generated experimental checkerboard and RNA-
705 seq data, analyzed the experimental data, and drafted the manuscript. S.J. co-developed the
706 INDIGO-MTB model, generated the model predictions. J.L-F. generated DIAMOND
707 experimental drug interaction data and drafted the manuscript. J.L. generated experimental
708 checkerboard drug interaction data and RNA-seq data. B.A. organized the DIAMOND
709 experimental drug interaction data generation and drafted the manuscript. D.S. and SC
710 conceived of the study, led the design, organized the data analysis, and drafted the manuscript,
711 and SC also co-developed the INDIGO-MTB model.

712

713

714 **REFERENCES**

- 715 1. World Health Organization. 2016. Global Tuberculosis Report 2016.
- 716 2. Mdluli K, Kaneko T, Upton A. 2015. The tuberculosis drug discovery and development
717 pipeline and emerging drug targets. *Cold Spring Harb Perspect Med* 5.
- 718 3. Zumla A, Chakaya J, Centis R, D'Ambrosio L, Mwaba P, Bates M, Kapata N, Nyirenda
719 T, Chanda D, Mfinanga S, Hoelscher M, Maeurer M, Migliori GB. 2015. Tuberculosis
720 treatment and management--an update on treatment regimens, trials, new drugs, and
721 adjunct therapies. *Lancet Respir Med* 3:220-34.
- 722 4. Silva A, Lee BY, Clemens DL, Kee T, Ding X, Ho CM, Horwitz MA. 2016. Output-driven
723 feedback system control platform optimizes combinatorial therapy of tuberculosis using a
724 macrophage cell culture model. *Proc Natl Acad Sci U S A* 113:E2172-9.
- 725 5. Lee BY, Clemens DL, Silva A, Dillon BJ, Maslesa-Galic S, Nava S, Ding X, Ho CM,
726 Horwitz MA. 2017. Drug regimens identified and optimized by output-driven platform
727 markedly reduce tuberculosis treatment time. *Nat Commun* 8:14183.
- 728 6. Cokol M, Kuru N, Bicak E, Larkins-Ford J, Aldridge BB. 2017. Efficient measurement
729 and factorization of high-order drug interactions in *Mycobacterium tuberculosis*. *Sci Adv*
730 3:e1701881.
- 731 7. World Health Organization, Initiative ST. 2010. Treatment of tuberculosis: guidelines.
- 732 8. World Health Organization. 2016. WHO treatment guidelines for drug-resistant
733 tuberculosis 2016 update.
- 734 9. Dheda K, Gumbo T, Gandhi NR, Murray M, Theron G, Udwadia Z, Migliori GB, Warren
735 R. 2014. Global control of tuberculosis: from extensively drug-resistant to untreatable
736 tuberculosis. *Lancet Respir Med* 2:321-38.
- 737 10. Falzon D, Schunemann HJ, Harausz E, Gonzalez-Angulo L, Lienhardt C, Jaramillo E,
738 Weyer K. 2017. World Health Organization treatment guidelines for drug-resistant
739 tuberculosis, 2016 update. *Eur Respir J* 49.
- 740 11. Ekins S, Pottorf R, Reynolds RC, Williams AJ, Clark AM, Freundlich JS. 2014. Looking
741 back to the future: predicting in vivo efficacy of small molecules versus *Mycobacterium*
742 tuberculosis. *J Chem Inf Model* 54:1070-82.
- 743 12. Ma S, Minch KJ, Rustad TR, Hobbs S, Zhou SL, Sherman DR, Price ND. 2015.
744 Integrated Modeling of Gene Regulatory and Metabolic Networks in *Mycobacterium*
745 tuberculosis. *PLoS Comput Biol* 11:e1004543.
- 746 13. Peterson EJR, Ma S, Sherman DR, Baliga NS. 2016. Network analysis identifies Rv0324
747 and Rv0880 as regulators of bedaquiline tolerance in *Mycobacterium tuberculosis*.
748 *Nature Microbiology* doi:10.1038/nmicrobiol.2016.78.
- 749 14. Chandrasekaran S, Cokol-Cakmak M, Sahin N, Yilancioglu K, Kazan H, Collins JJ,
750 Cokol M. 2016. Chemogenomics and orthology-based design of antibiotic combination
751 therapies. *Mol Syst Biol* 12:872.

- 752 15. Bredel M, Jacoby E. 2004. Chemogenomics: an emerging strategy for rapid target and
753 drug discovery. *Nat Rev Genet* 5:262-75.
- 754 16. Nichols RJ, Sen S, Choo YJ, Beltrao P, Zietek M, Chaba R, Lee S, Kazmierczak KM,
755 Lee KJ, Wong A, Shales M, Lovett S, Winkler ME, Krogan NJ, Typas A, Gross CA.
756 2011. Phenotypic landscape of a bacterial cell. *Cell* 144:143-56.
- 757 17. Breiman L. 2001. Random Forests. *Machine Learning* 45:5-32.
- 758 18. Johnson WE, Li C, Rabinovic A. 2007. Adjusting batch effects in microarray expression
759 data using empirical Bayes methods. *Biostatistics* 8:118-27.
- 760 19. Boshoff HI, Myers TG, Copp BR, McNeil MR, Wilson MA, Barry CE, 3rd. 2004. The
761 transcriptional responses of *Mycobacterium tuberculosis* to inhibitors of metabolism:
762 novel insights into drug mechanisms of action. *J Biol Chem* 279:40174-84.
- 763 20. Iøerger TR, O'Malley T, Liao R, Guinn KM, Hickey MJ, Mohaideen N, Murphy KC,
764 Boshoff HI, Mizrahi V, Rubin EJ, Sasseti CM, Barry CE, 3rd, Sherman DR, Parish T,
765 Sacchettini JC. 2013. Identification of new drug targets and resistance mechanisms in
766 *Mycobacterium tuberculosis*. *PLoS One* 8:e75245.
- 767 21. Berenbaum MC. 1989. What is synergy? *Pharmacol Rev* 41:93-141.
- 768 22. Chen C, Gardete S, Jansen RS, Shetty A, Dick T, Rhee KY, Dartois V. 2018. Verapamil
769 Targets Membrane Energetics in *Mycobacterium tuberculosis*. *Antimicrob Agents*
770 *Chemother* 62.
- 771 23. Gupta S, Tyagi S, Almeida DV, Maiga MC, Ammerman NC, Bishai WR. 2013.
772 Acceleration of tuberculosis treatment by adjunctive therapy with verapamil as an efflux
773 inhibitor. *Am J Respir Crit Care Med* 188:600-7.
- 774 24. Gupta S, Cohen KA, Winglee K, Maiga M, Diarra B, Bishai WR. 2014. Efflux inhibition
775 with verapamil potentiates bedaquiline in *Mycobacterium tuberculosis*. *Antimicrob*
776 *Agents Chemother* 58:574-6.
- 777 25. Demitto Fde O, do Amaral RC, Maltempe FG, Siqueira VL, Scodro RB, Lopes MA,
778 Caleffi-Ferracioli KR, Canezin PH, Cardoso RF. 2015. In vitro activity of rifampicin and
779 verapamil combination in multidrug-resistant *mycobacterium tuberculosis*. *PLoS One*
780 10:e0116545.
- 781 26. Ocampo PS, Lazar V, Papp B, Arnoldini M, Abel zur Wiesch P, Busa-Fekete R, Fekete
782 G, Pal C, Ackermann M, Bonhoeffer S. 2014. Antagonism between bacteriostatic and
783 bactericidal antibiotics is prevalent. *Antimicrob Agents Chemother* 58:4573-82.
- 784 27. MD JL, Boshoff HI, Barry CE, 3rd. 2018. The present state of the tuberculosis drug
785 development pipeline. *Curr Opin Pharmacol* 42:81-94.
- 786 28. Robertson GT, Scherman MS, Bruhn DF, Liu J, Hastings C, McNeil MR, Butler MM,
787 Bowlin TL, Lee RB, Lee RE, Lenaerts AJ. 2017. Spectinamides are effective partner
788 agents for the treatment of tuberculosis in multiple mouse infection models. *J Antimicrob*
789 *Chemother* 72:770-777.
- 790 29. Lee RE, Hurdle JG, Liu J, Bruhn DF, Matt T, Scherman MS, Vaddady PK, Zheng Z, Qi J,
791 Akbergenov R, Das S, Madhura DB, Rathi C, Trivedi A, Villellas C, Lee RB, Rakesh,

- 792 Waidyarachchi SL, Sun D, McNeil MR, Ainsa JA, Boshoff HI, Gonzalez-Juarrero M,
793 Meibohm B, Bottger EC, Lenaerts AJ. 2014. Spectinamides: a new class of
794 semisynthetic antituberculosis agents that overcome native drug efflux. *Nat Med* 20:152-
795 158.
- 796 30. Franzblau SG, DeGroot MA, Cho SH, Andries K, Nuermberger E, Orme IM, Mdluli K,
797 Angulo-Barturen I, Dick T, Dartois V, Lenaerts AJ. 2012. Comprehensive analysis of
798 methods used for the evaluation of compounds against *Mycobacterium tuberculosis*.
799 *Tuberculosis (Edinb)* 92:453-88.
- 800 31. Bonnett LJ, Ken-Dror G, Koh G, Davies GR. 2017. Comparing the Efficacy of Drug
801 Regimens for Pulmonary Tuberculosis: Meta-analysis of Endpoints in Early-Phase
802 Clinical Trials. *Clin Infect Dis* 65:46-54.
- 803 32. Drusano GL, Sgambati N, Eichas A, Brown DL, Kulawy R, Louie A. 2010. The
804 combination of rifampin plus moxifloxacin is synergistic for suppression of resistance but
805 antagonistic for cell kill of *Mycobacterium tuberculosis* as determined in a hollow-fiber
806 infection model. *MBio* 1.
- 807 33. Kanehisa M, Goto S. 2000. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic
808 Acids Res* 28:27-30.
- 809 34. Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M. 2016. KEGG as a
810 reference resource for gene and protein annotation. *Nucleic Acids Res* 44:D457-62.
- 811 35. Rustad TR, Minch KJ, Ma S, Winkler JK, Hobbs S, Hickey M, Brabant W, Turkarlan S,
812 Price ND, Baliga NS, Sherman DR. 2014. Mapping and manipulating the *Mycobacterium
813 tuberculosis* transcriptome using a transcription factor overexpression-derived regulatory
814 network. *Genome Biol* 15:502.
- 815 36. Galagan JE, Minch K, Peterson M, Lyubetskaya A, Azizi E, Sweet L, Gomes A, Rustad
816 T, Dolganov G, Glotova I, Abeel T, Mahwinney C, Kennedy AD, Allard R, Brabant W,
817 Krueger A, Jaini S, Honda B, Yu WH, Hickey MJ, Zucker J, Garay C, Weiner B, Sisk P,
818 Stolte C, Winkler JK, Van de Peer Y, Iazzetti P, Camacho D, Dreyfuss J, Liu Y, Dorhoi
819 A, Mollenkopf HJ, Drogaris P, Lamontagne J, Zhou Y, Piquenot J, Park ST, Raman S,
820 Kaufmann SH, Mohny RP, Chelsky D, Moody DB, Sherman DR, Schoolnik GK. 2013.
821 The *Mycobacterium tuberculosis* regulatory network and hypoxia. *Nature* 499:178-83.
- 822 37. Singh R, Ramachandran V, Shandil R, Sharma S, Khandelwal S, Karmarkar M, Kumar
823 N, Solapure S, Saralaya R, Nanduri R, Panduga V, Reddy J, Prabhakar KR,
824 Rajagopalan S, Rao N, Narayanan S, Anandkumar A, Balasubramanian V, Datta S.
825 2015. In silico-based high-throughput screen for discovery of novel combinations for
826 tuberculosis treatment. *Antimicrob Agents Chemother* 59:5664-74.
- 827 38. Lee BY, Clemens DL, Silva A, Dillon BJ, Maslesa-Galic S, Nava S, Ho CM, Horwitz MA.
828 2018. Ultra-rapid near universal TB drug regimen identified via parabolic response
829 surface platform cures mice of both conventional and high susceptibility. *PLoS One*
830 13:e0207469.
- 831 39. Almeida D, Ioerger T, Tyagi S, Li SY, Mdluli K, Andries K, Grosset J, Sacchettini J,
832 Nuermberger E. 2016. Mutations in *pepQ* Confer Low-Level Resistance to Bedaquiline
833 and Clofazimine in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 60:4590-
834 9.

- 835 40. Tasneen R, Li SY, Peloquin CA, Taylor D, Williams KN, Andries K, Mdluli KE,
836 Nuermberger EL. 2011. Sterilizing activity of novel TMC207- and PA-824-containing
837 regimens in a murine model of tuberculosis. *Antimicrob Agents Chemother* 55:5485-92.
- 838 41. Mitchison DA. 1996. Modern methods for assessing the drugs used in the chemotherapy
839 of mycobacterial disease. *Soc Appl Bacteriol Symp Ser* 25:72S-80S.
- 840 42. Mitchison DA. 1993. Assessment of new sterilizing drugs for treating pulmonary
841 tuberculosis by culture at 2 months. *Am Rev Respir Dis* 147:1062-3.
- 842 43. Chevereau G, Bollenbach T. 2015. Systematic discovery of drug interaction
843 mechanisms. *Mol Syst Biol* 11:807.
- 844 44. Lobritz MA, Belenky P, Porter CB, Gutierrez A, Yang JH, Schwarz EG, Dwyer DJ, Khalil
845 AS, Collins JJ. 2015. Antibiotic efficacy is linked to bacterial cellular respiration. *Proc*
846 *Natl Acad Sci U S A* 112:8173-80.
- 847 45. Kapopoulou A, Lew JM, Cole ST. 2011. The MycoBrowser portal: a comprehensive and
848 manually annotated resource for mycobacterial genomes. *Tuberculosis (Edinb)* 91:8-13.
- 849 46. UniProt Consortium T. 2018. UniProt: the universal protein knowledgebase. *Nucleic*
850 *Acids Res* 46:2699.
- 851 47. DeJesus MA, Gerrick ER, Xu W, Park SW, Long JE, Boutte CC, Rubin EJ,
852 Schnappinger D, Ehrt S, Fortune SM, Sasseti CM, Ioerger TR. 2017. Comprehensive
853 Essentiality Analysis of the Mycobacterium tuberculosis Genome via Saturating
854 Transposon Mutagenesis. *MBio* 8.
- 855 48. Sasseti CM, Boyd DH, Rubin EJ. 2003. Genes required for mycobacterial growth
856 defined by high density mutagenesis. *Mol Microbiol* 48:77-84.
- 857 49. Griffin JE, Gawronski JD, DeJesus MA, Ioerger TR, Akerley BJ, Sasseti CM. 2011. High-
858 resolution phenotypic profiling defines genes essential for mycobacterial growth and
859 cholesterol catabolism. *PLoS Pathog* 7:e1002251.
- 860 50. Tsolaki AG, Hirsh AE, DeRiemer K, Enciso JA, Wong MZ, Hannan M, Goguet de la
861 Salmoniere YO, Aman K, Kato-Maeda M, Small PM. 2004. Functional and evolutionary
862 genomics of Mycobacterium tuberculosis: insights from genomic deletions in 100 strains.
863 *Proc Natl Acad Sci U S A* 101:4865-70.
- 864 51. Cokol M, Li C, Chandrasekaran S. 2018. Chemogenomic model identifies synergistic
865 drug combinations robust to the pathogen microenvironment. *PLoS Comput Biol*
866 14:e1006677.
- 867 52. Rustad TR, Harrell MI, Liao R, Sherman DR. 2008. The enduring hypoxic response of
868 Mycobacterium tuberculosis. *PLoS One* 3:e1502.
- 869 53. Ehrt S, Guo XV, Hickey CM, Ryou M, Monteleone M, Riley LW, Schnappinger D. 2005.
870 Controlling gene expression in mycobacteria with anhydrotetracycline and Tet repressor.
871 *Nucleic Acids Res* 33:e21.
- 872 54. BEI Resources Strain Depository. www.beiresources.org.

- 873 55. Jain P, Hsu T, Arai M, Biermann K, Thaler DS, Nguyen A, Gonzalez PA, Tufariello JM,
874 Kriakov J, Chen B, Larsen MH, Jacobs WR, Jr. 2014. Specialized transduction designed
875 for precise high-throughput unmarked deletions in Mycobacterium tuberculosis. MBio
876 5:e01245-14.
- 877 56. Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nat
878 Methods 9:357-9.
- 879 57. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G,
880 Durbin R, Genome Project Data Processing S. 2009. The Sequence Alignment/Map
881 format and SAMtools. Bioinformatics 25:2078-9.
- 882 58. Geva-Zatorsky N, Dekel E, Cohen AA, Danon T, Cohen L, Alon U. 2010. Protein
883 dynamics in drug combinations: a linear superposition of individual-drug responses. Cell
884 140:643-51.
- 885 59. Pritchard JR, Bruno PM, Gilbert LA, Capron KL, Lauffenburger DA, Hemann MT. 2013.
886 Defining principles of combination drug mechanisms of action. Proc Natl Acad Sci U S A
887 110:E170-9.

888

889 SUPPLEMENTAL MATERIALS

890

891 **Figure S1: Schematic of INDIGO-MTB modeling workflow (A-C) and the experimental**
892 **assays to measure drug interaction (D-F).** (A) The input datasets for training the INDIGO
893 algorithm are: [1] the transcriptomic profiles of drugs, and [2] the corresponding FIC scores of
894 known drug-drug interactions. From each transcriptome profile of MTB response to an individual
895 drug, we defined a corresponding drug-gene interaction matrix by assigning a value of 1 to
896 genes that changed in expression by more than 2-fold (up or down) after exposure to drug, and
897 setting all other genes to a value of 0. Only genes that are up-regulated are shown in the
898 remaining panels for simplicity. (B) For each drug combination, INDIGO calculates a “joint”
899 drug-gene interaction matrix using a linear combination of the drug-gene interaction matrices of
900 each constituent individual drug. The joint profile captures both the similarity and uniqueness in
901 the transcriptome response profiles of the individual drugs in each combination. The INDIGO
902 algorithm then uses a machine learning approach called Random Forest to create a
903 mathematical model that associates the FIC score of each drug combination to its
904 corresponding joint drug-gene interaction matrix. Random Forest builds a series of decision
905 trees to identify specific patterns in the drug-gene interaction matrices that significantly
906 associate with the value of the corresponding drug-drug interaction FIC scores. (C) Once built,
907 the INDIGO-MTB model requires only the transcriptomic response profile elicited by a new
908 compound of interest as input to predict FIC scores of combinations featuring the compound of
909 interest. (D) Representative checkerboard assay experiments of a synergistic and antagonistic
910 drug pair. Cultures were exposed to serial dilutions of drugs (designated in the rows and
911 columns) for 7 days, and bacterial viability was quantified by measuring ATP levels with the
912 BacTiter Glo reagent. The thick black boxes denote the individual drug MIC wells, and the
913 boxes with numbers denote concentrations that yielded iso-equivalent inhibition (each of the
914 numbers represent the FIC score calculated based on the drug concentrations associated with
915 corresponding well). (E-F) Representative DiaMOND assay experiments of a synergistic and
916 antagonistic drug pair (E) or triplet (F). Cultures were exposed to drugs in 384-well plates and
917 growth was measured by OD600. The FIC for a drug combination was calculated as the ratio

918 between the observed and expected IC50 dose of the drug combination. For two-drug
919 combinations the expected IC50 dose is defined as the intersection of the line (additivity line)
920 drawn between the IC50 doses for each individual drug. For three-drug combinations, the
921 expected IC50 dose is defined as the intersection of the drug combination dose response and
922 the plane (additivity plane) created by connecting the IC50 doses for each individual drug.

923

924 **Figure S2. Distributions of INDIGO-MTB interaction scores for combinations featuring**
925 **different drugs. (A)** Box plots of interaction scores of combinations featuring only bacteriostatic
926 drugs (blue) is shifted toward synergy (interaction score = 1.03 ± 0.2), relative to combinations
927 featuring bactericidal drugs (red) (interaction score = 1.25 ± 0.3). Combinations featuring
928 bactericidal drugs appear to have the most antagonistic INDIGO-MTB interaction scores. **(B)**
929 Distribution of INDIGO-MTB interaction scores for combinations involving Verapamil (VER) or
930 Chlorpromazine (CPZ). The distribution of interaction scores for these drugs is significantly
931 lower than the interaction score distribution for combinations featuring other drugs (p -value $< 1 \times$
932 10^{-16} , non-parametric Kolmogorov-Smirnov test), suggesting that combinations featuring these
933 drugs are enriched for synergy. The box plots display the first quartile (1Q), median, and the
934 third quartile (3Q) of the distribution of INDIGO-MTB scores for pairs of drugs, at least one of
935 which are VER or CPZ compared against all possible combinations excluding these two
936 antibiotics.

937

938 **Figure S3: INDIGO accurately forecasts interactions among the 36 test-set drug**
939 **combinations against MTB.** This figure shows the comparison between INDIGO-MTB
940 interaction scores and experiments for all 36 combinations in the validation set. This figure
941 complements Figure 2 which compares INDIGO predictions with the 24 novel combinations
942 (subset of 36 test combinations). **(A)** Drug combinations chosen for experimental testing span
943 the entire range of drug interaction predictions by INDIGO. The histogram and box plot above it
944 show the distribution of pairwise drug interaction scores for the 35 high interest TB agents (the
945 boundaries of the box plot denote the 25th and 75th percentiles of the distribution, and the
946 dashed lines extend between 1st and 99th percentiles). The red dots denote the combinations
947 selected for experimental validation. **(B)** Comparison of INDIGO-MTB interaction scores with *in*
948 *vitro* interaction scores. For both experimental and model-predicted scores, values less than 0.9
949 indicate synergy, values between 0.9 and 1.1 denote additivity, and values greater than 1.1
950 indicate antagonism. Each dot indicates a specific drug combination. Dark red dots mark two-
951 drug regimens ($R = 0.63$, $p \sim 10^{-4}$), and blue dots mark three-drug regimens ($R = 0.68$, $p \sim 10^{-2}$).
952 The specific combinations mentioned in the text are highlighted in the plot. **(C)** Dot plot of
953 experimentally measured drug interaction scores versus the INDIGO-MTB predicted drug
954 interaction type. The dots labeled in red font denote outlier combinations that were misclassified
955 by INDIGO-MTB. The interaction scores were significantly different between predicted
956 synergistic and antagonistic combinations ($p = 6 \times 10^{-5}$). The horizontal lines in the box plot
957 represent the median and the first and third quartiles. **(D)** Sensitivity vs specificity curves for
958 INDIGO-MTB predictions of synergy and antagonism for both 2-drug and 3-drug combinations
959 in the validation set. The AUC values are 0.89 ($p = 4 \times 10^{-5}$) and 0.91 ($p = 4.1 \times 10^{-5}$) for synergy
960 and antagonism respectively. (Sensitivity = 88.4% and Specificity = 86.5% for synergy,
961 Sensitivity = 79.4%, Specificity = 90% for predicting antagonism).

962

963 **Figure S4: Receiver operating curves (ROC) for INDIGO-MTB predictions of synergy and**
964 **antagonism for various thresholds of synergy and antagonism. (A-D)** ROC curves
965 generated from the independent test data. Sensitivity measures the true positive rate, which is
966 the fraction of true positive interactions correctly identified; specificity measures the true
967 negative rate. The area under the ROC curve (AUC) values provides an estimate of the
968 sensitivity and specificity of model predictions over a range of thresholds. At the highest
969 threshold for synergy (i.e. FIC < 0.5), the accuracy of the model is reduced likely due to majority
970 of the interactions in the test set being classified as neutral. **(E-H)** ROC curves INDIGO-MTB
971 tested using ten-fold cross validation analysis. In cross validation analysis, 10% of the training
972 dataset is blinded and predictions are made using an INDIGO-MTB model trained using the
973 remaining 90% of the training data. Performance metrics are then calculated based on
974 prediction on the withheld data. This analysis is repeated 10 times to cover the entire training
975 dataset. The plots show the sensitivity vs specificity curves for INDIGO-MTB predictions of
976 synergy and antagonism for various thresholds of synergy and antagonism. The cross-validation
977 accuracy is surprisingly lower than the test set accuracy as the training set comprises data from
978 15 different studies that were done in diverse labs and batches with different drugs and
979 methodologies. In our prior study (14), the training and test data were obtained from a single
980 source, and consequently the cross-validation accuracy matched the test set accuracy.

981

982 **Figure S5. Impact of variation between clinical trials and experimental drug interaction**
983 **studies on correlation between drug synergy and clinical efficacy.** Since multiple clinical
984 trials and experimental studies had measured the clinical efficacy outcome (sputum conversion
985 rates) and *in vitro* FIC scores for drug combinations, we assessed the robustness of correlations
986 between clinical efficacy data and both experimentally measured **(A-B)** and model-predicted **(C-**
987 **D)** interaction scores by performing sampling analysis. **(A).** Distribution of the correlation
988 between *in vitro* experimentally measured drug interaction scores with corresponding sputum
989 conversion rates. Since multiple experimental studies had measured the *in vitro* interaction
990 outcome for these combinations, we performed sampling analysis by randomly choosing one
991 representative study for each combination to determine the average correlation. We found that
992 the *in vitro* experimental drug interaction scores correlated significantly (mean R = -0.52, p ~
993 0.01, average of 100 trials) with clinical sputum conversion by this sampling analysis. Panel **(B)**
994 shows one representative trial with R = -0.52. For comparison, INDIGO-MTB achieved a similar
995 correlation across all 57 clinical trials (R = -0.55, p ~ 10⁻⁵). **(B)** Comparison of experimental FIC
996 scores with sputum conversion rates in human patients after 8 weeks of treatment in clinical
997 trials, with each regimen represented by FIC data from a single experimental study (R = -0.52, p
998 = 0.01). Data shown for four two-way and two three-way drug combinations that had both
999 experimental *in vitro* drug interaction data and sputum conversion rates. Each dot indicates a
1000 specific drug combination reported from a specific clinical trial. The combinations corresponding
1001 to each dot is provided in the legend. **(C)** Histogram visualizing the distribution of the
1002 correlations between INDIGO-MTB predictions and clinical efficacy, based on sampling
1003 analysis. We performed sampling analysis by randomly choosing one representative clinical trial
1004 for each combination to determine the average correlation with INDIGO-MTB predicted
1005 interaction scores. We observed a significant correlation between interaction scores and the
1006 sputum culture conversion rates (mean R = -0.38 average of 100 random sampling trials, p-
1007 value = 0.001). **(D)** shows data from one representative trial with R = -0.37.

1008

1009 **Figure S6: Impact of Transcription Factor (TF) deletion on drug interaction scores. (A)**
1010 The average predicted impact of deleting each of the 206 TFs on all 36 pairwise combinations

1011 comprising INH, RIF, STM, MXF, CFZ, BDQ, CAP, ETA, and PA824 is shown. Rv1353c had the
1012 biggest average impact on all the combinations and was chosen for experimental validation. **(B)**
1013 Predicted impact of Rv1353c deletion on drug interaction scores. The plot shows the relative
1014 absolute difference in FIC scores on all 36 pairwise combinations comprising INH, RIF, STM,
1015 MXF, CFZ, BDQ, CAP, ETA, and PA824. Interestingly, combinations with STM showed both the
1016 highest and lowest change in interaction score. The interactions in bold were chosen for
1017 experimental testing. All the chosen combinations were also antagonistic or additive, thus
1018 changing the activity of Rv1353c could be used to make these combinations more synergistic.
1019 **(C)** *in vitro* experimentally measured drug interaction scores for Rv1353c genetic perturbation
1020 strains exposed to drug combinations. The error bars represent the standard deviation between
1021 replicates.

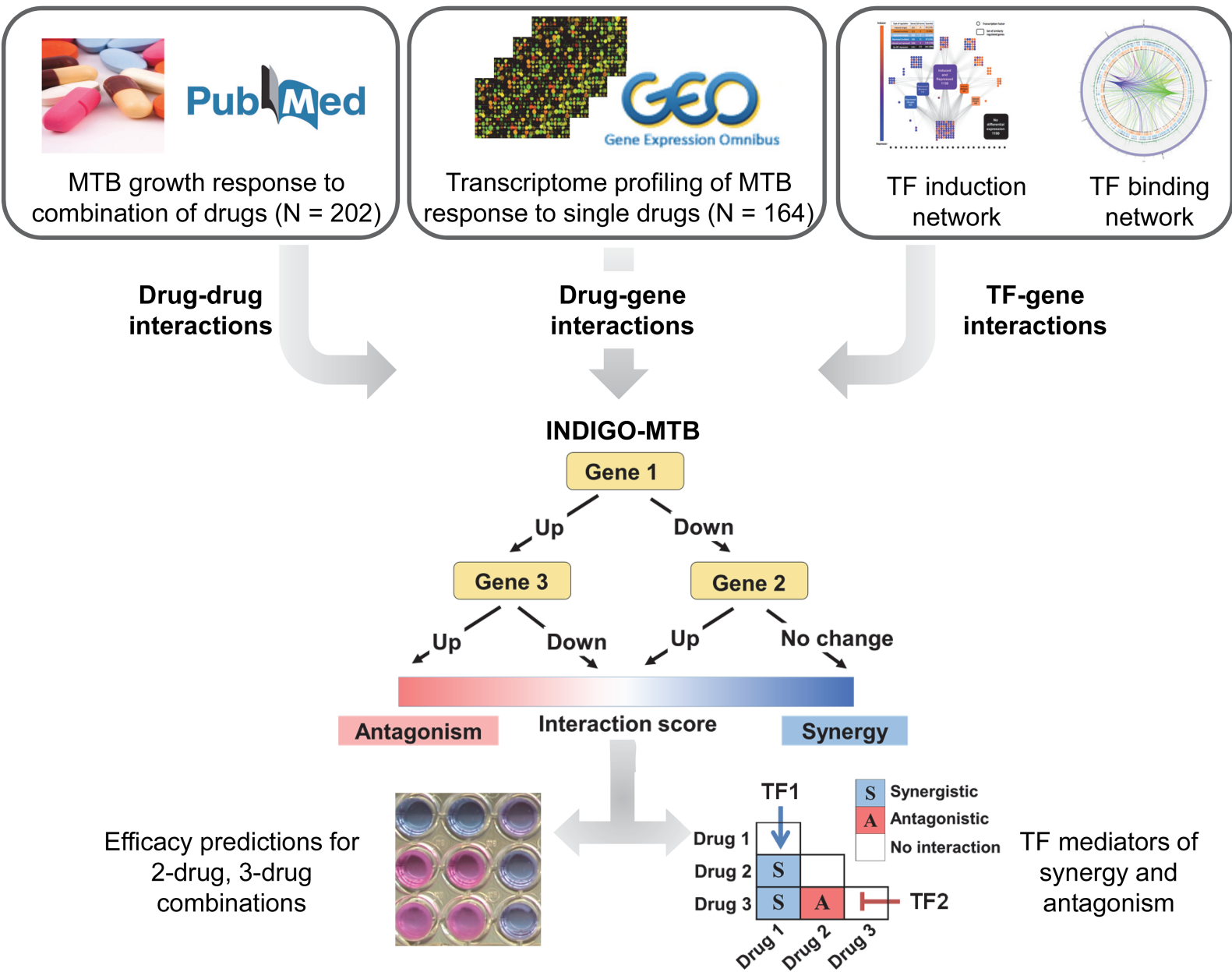
1022 **Figure S7. (A)** *in vitro* experimentally measured interaction score measured between
1023 bedaquiline (BDQ) and chlorpromazine (CPZ). The heatmap shows a representative
1024 checkerboard assay experiment, in which interaction MTB H37Rv cultures were exposed to
1025 different pairwise concentrations of BDQ and CPZ in 96-well plate format, designated in the
1026 columns and rows, respectively. Cultures were exposed to drugs for 7 days, and bacterial
1027 viability was quantified by measuring ATP levels with the BacTiter Glo reagent. The thick black
1028 boxes denote the individual drug MIC wells, and the boxes with numbers denote concentrations
1029 that yielded iso-equivalent inhibition (each of the numbers represent the FIC score calculated
1030 based on the drug concentrations associated with corresponding well). **(B)** Correlation analysis
1031 of FIC average values calculated from checkerboard assays measured by AlamarBlue or
1032 BacTiter Glo. Each point represents the average of the FIC indices of equivalently inhibited
1033 concentrations on an individual checkerboard plate. Pearson's R for this is .78 ($p < 0.0001$).

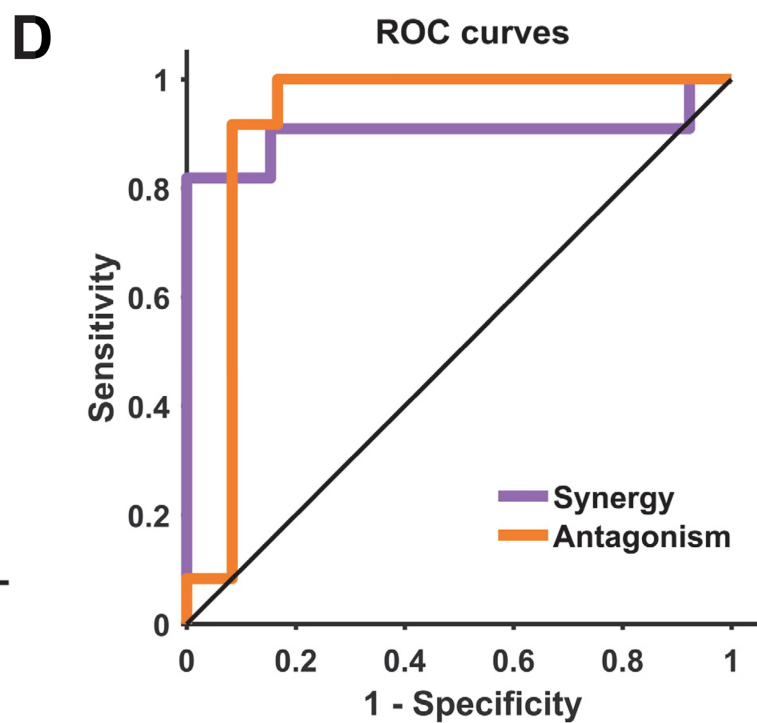
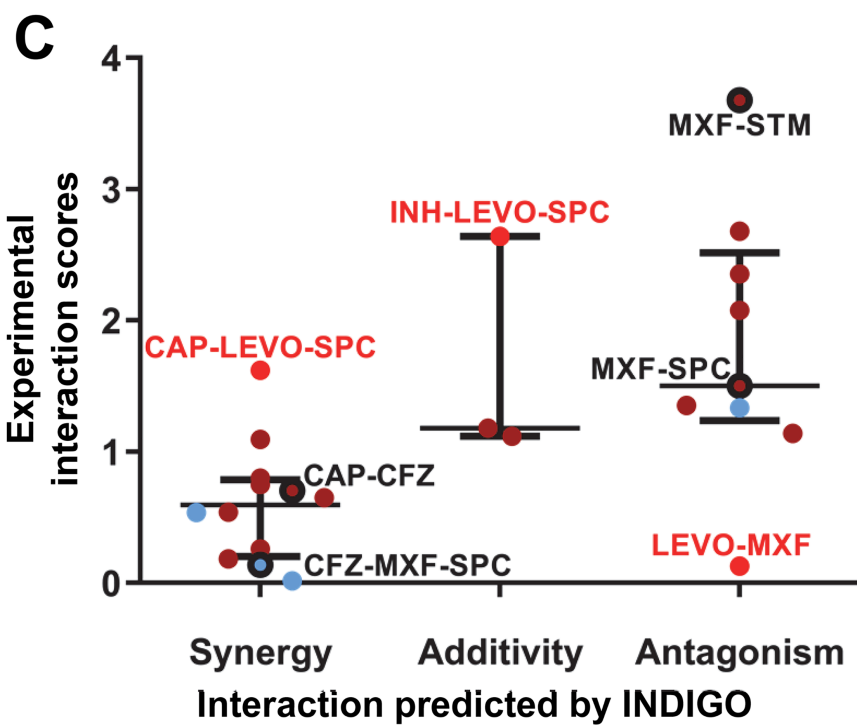
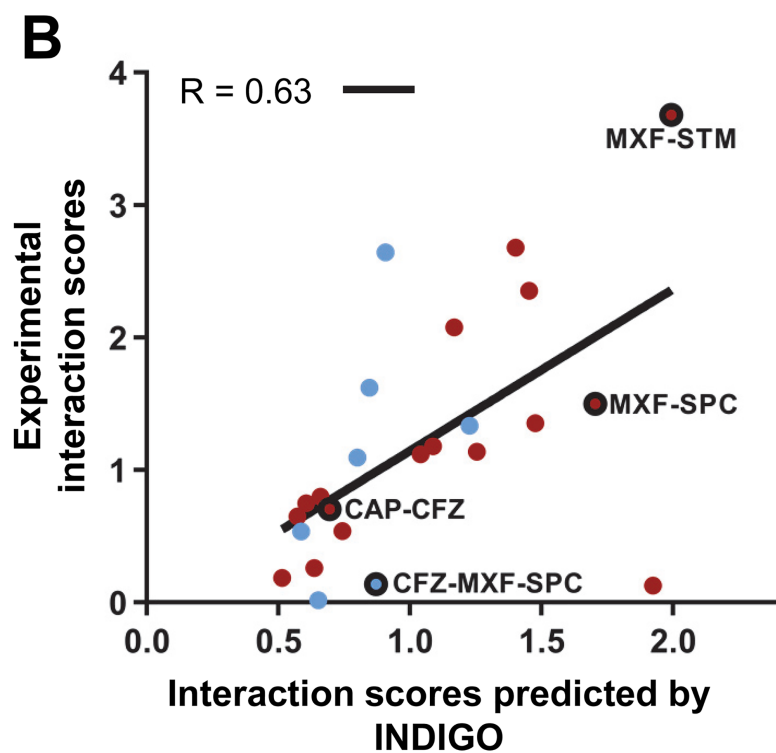
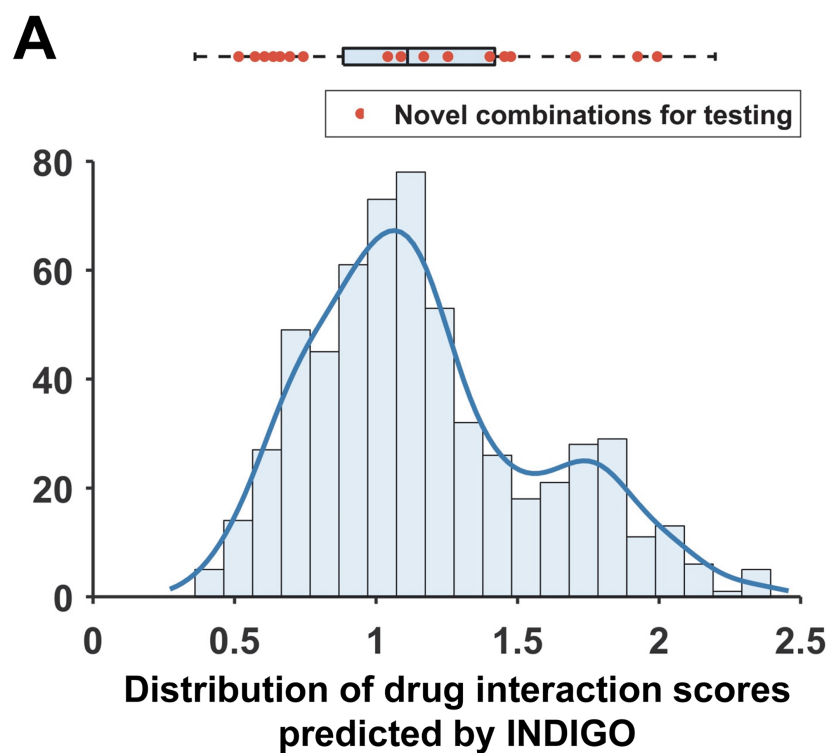
1034

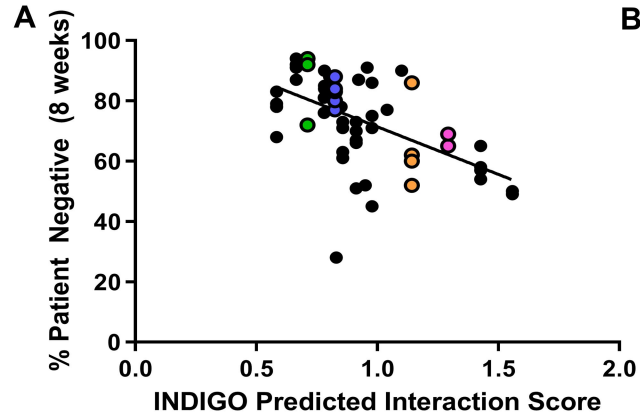
1035 **Table S1: (A)** Compendium of transcriptomics/chemogenomic data for INDIGO-MTB. **(B)**
1036 Training combinations with associated FIC interaction indices. **(C)** List of all possible pairwise
1037 interaction scores (164 compounds and 65 perturbations). **(D)** INDIGO-MTB predictions along
1038 with corresponding experimental (*in vitro*) and clinical validation data. Highlighted combinations
1039 are novel i.e. not used in training set. **(E)** Pathway enrichment analysis using the top 500
1040 predictive genes. **(F)** DiaMOND Distribution Transformation. **(G)** Impact of various thresholds for
1041 finding differentially expressed genes after drug treatment. Changing the log fold change
1042 threshold from 4- to 32-fold showed similar correlation with the experimental test set interactions
1043 as the default threshold (2-fold). The use of a fold change threshold to binarize the data was
1044 performed to reduce the noise in the datasets. Overall, this analysis shows that the INDIGO
1045 model is robust to the thresholds used. At the highest threshold (32-fold), a slightly higher
1046 correlation was observed, although it was not significantly better than the default settings based
1047 on partial correlation analysis (p -value > 0.05). **(H)** Impact of using various machine learning
1048 algorithms for predicting drug interactions.

1049 **Table S2: 2-way, 3-way, INDIGO-MTB scores for 35 high-interest TB drugs.**

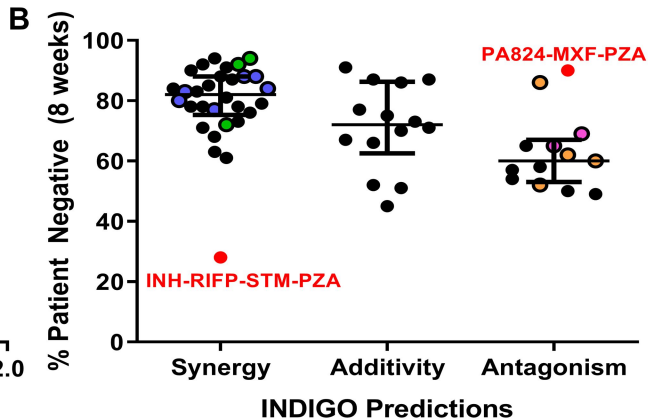
1050 **Table S3: 4-way, and 5-way INDIGO-MTB scores for 35 high-interest TB drugs.**







● INH-RIF-PZA-EMB ● INH-STM



● INH-RIF ● INH-RIF-STM

