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Supplementary Materials for

Efficient measurement and factorization of high-order drug interactions in *Mycobacterium tuberculosis*

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at advances.sciencemag.org/cgi/content/full/3/10/e1701881/DC1)

- Source data (Microsoft Excel format).

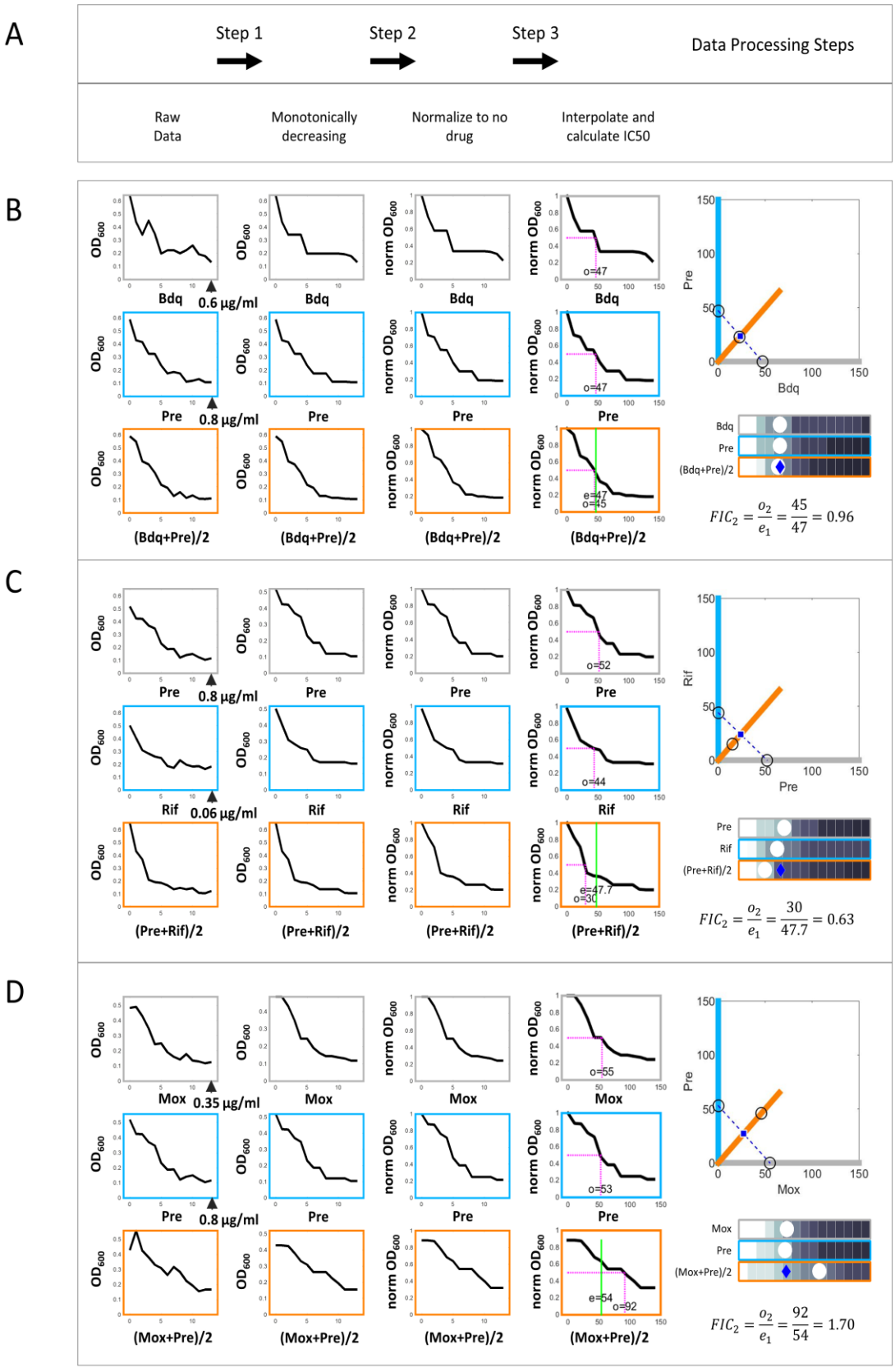

A
Step 1
Step 2
Step 3
Data Processing Steps
Raw Data
Monotonically decreasing
Normalize to no drug
Interpolate and calculate IC50
B
OD600
norm OD600
Bdq
0.6 μg/ml
Pre
0.8 μg/ml
(Bdq+Pre)/2
o=47
e=47
o=45
Bdq
Pre
(Bdq+Pre)/2
$FIC_2 = \frac{o_2}{e_1} = \frac{45}{47} = 0.96$
C
Pre
0.8 μg/ml
Rif
0.06 μg/ml
(Pre+Rif)/2
o=52
o=44
e=47.7
o=30
Rif
Pre
(Pre+Rif)/2
$FIC_2 = \frac{o_2}{e_1} = \frac{30}{47.7} = 0.63$
D
Mox
0.35 μg/ml
Pre
0.8 μg/ml
(Mox+Pre)/2
o=55
o=53
e=54
o=92
Mox
Pre
(Mox+Pre)/2
$FIC_2 = \frac{o_2}{e_1} = \frac{92}{54} = 1.70$

fig. S1. Data analysis pipeline to calculate FIC_2 scores from dose-response growth data. A) Outline of data analysis steps. Raw growth data are converted to be monotonically decreasing (Step 1), are normalized to the growth measured in the control without drug (Step 2), and finally the IC_{50} (50% growth inhibition) values are interpolated from each normalized dose-response curve (Step 3). The dose-responses are sampled linearly and therefore the x-axes are given as dose units (and an arrow also indicates the absolute concentration for each drug). In step 3, the x-axis units are in one-tenth increments of the dose units from steps 1-2, e.g. there are 140 steps from which the IC_{50} is interpolated. **B** to **D)** Examples of additive (B, bedaquiline + pretomanid), synergistic (C: pretomanid + rifampicin), and antagonistic (D: moxifloxacin + pretomanid) pairwise interactions illustrate the data analysis process from dose-response data to FIC_2 calculation. For each pairwise interaction, DiaMOND requires measurement of three dose-responses: each single (gray and blue colors) and the two-drug dose-response (orange). The expected dose of the two-drug combination (e_1 of the IC_{50}, for example) is calculated as the intersection of this two-drug dose-response in the dose-combination space with the straight line defined by the IC_{50} values of each single drug dose-response (blue dotted line). This expected dose (e_1; blue diamond) is calculated as shown in Supplementary Methods 1. The observed dose of the combination (o_2; white circle in the two-drug dose-response) is the interpolated IC_{50} of the two-drug dose-response (indicated by intersection of magenta dotted lines on the interpolated dose-response curve). The FIC_2 is then calculated as the ratio of o_2 to e_1.

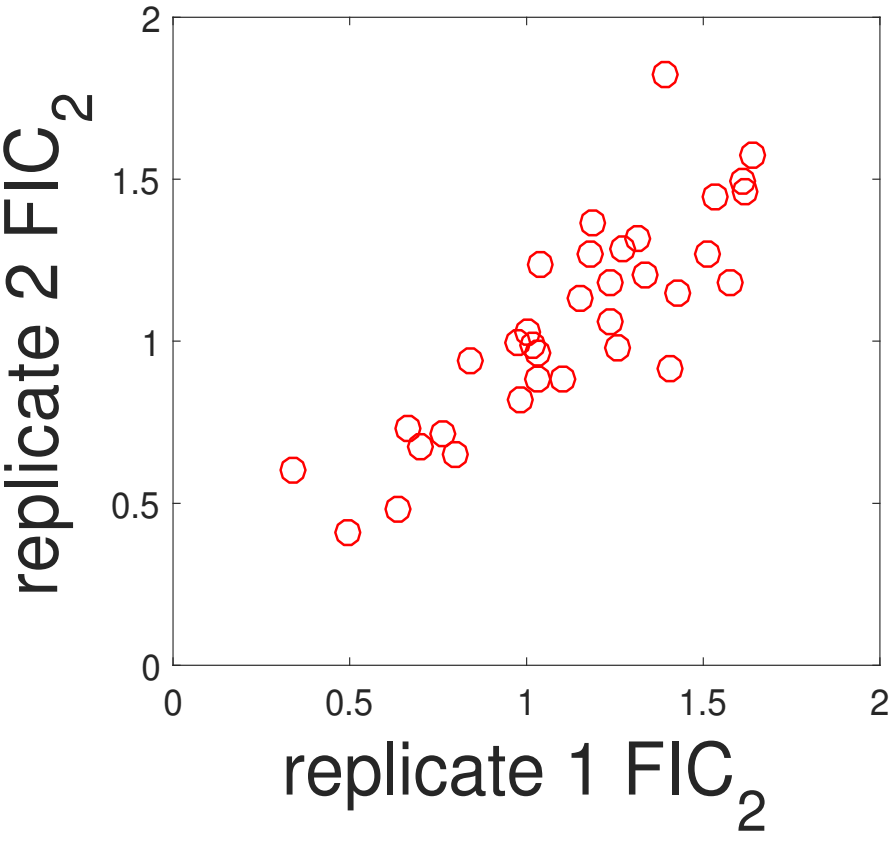


fig. S2. Scatterplot of replicate interaction scores (FIC_2) for all pairwise drug combinations shown in Fig. 1 (C and D). Pairwise interaction scores (FIC_2) for each biological replicate of each drug combination were calculated as described in equation 1 using the IC_{50} calculated from the individual and pairwise drug dose-responses. Reproducibility of interaction scores was assessed by calculating correlation between replicate FIC_2 scores (Spearman's correlation $r = 0.86$, $p < 3.7 \times 10^{-9}$). Each open circle represents a pairwise drug combination. These replicate scores are reported as the geometric average in Figures 1C-D.

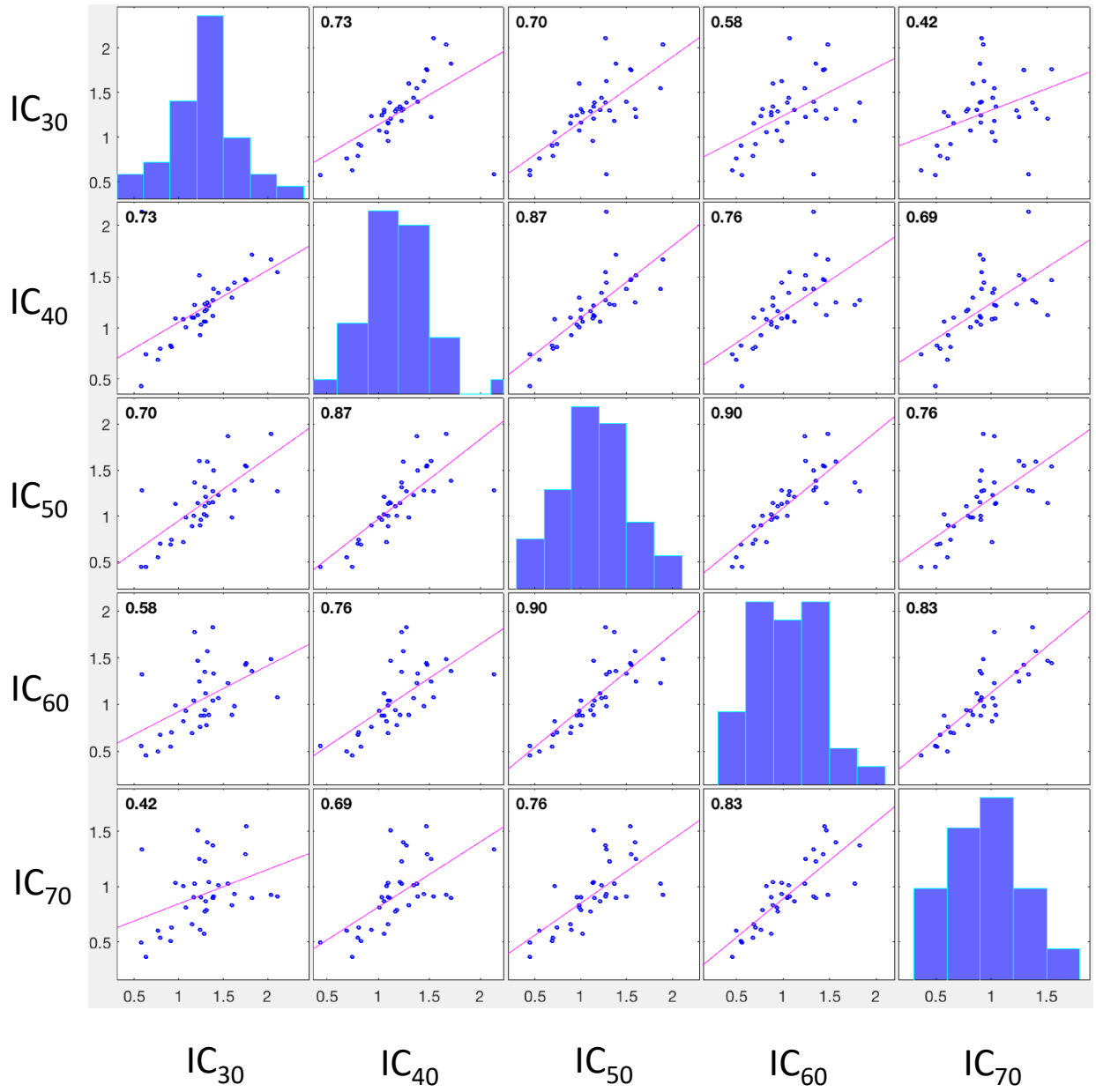


fig. S3. Correlation among FIC_2 scores calculated at different levels of growth inhibition (IC_{30}, IC_{40}, IC_{50}, IC_{60}, and IC_{70}) from the pairwise interaction data set described in Fig. 1 (C and D). Spearman's correlations are reported in the upper left of each scatter plot with regression line in magenta. On the diagonal are histograms of the FIC_2 scores at each level of growth inhibition.

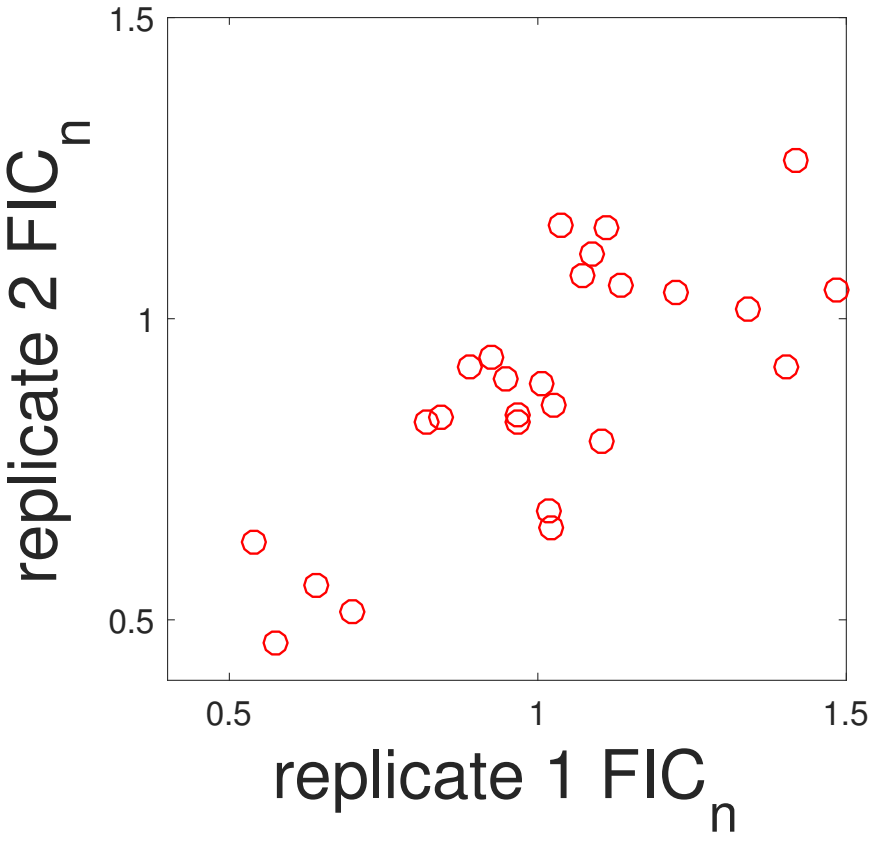


fig. S4. Scatterplot of replicate interaction scores (FIC_n) obtained for all two-way (FIC_2), three-way (FIC_3), four-way (FIC_4), and five-way (FIC_5) drug combinations in two replicates for the experiment shown in Fig. 2B. Interaction scores (FIC) for each biological replicate of each drug combination were calculated as described in equation 3 using the IC_{50} calculated from the individual and n-way drug dose-responses. Reproducibility of interaction scores was assessed by calculating correlation between replicate FIC scores (Spearman's correlation $r = 0.56$, $p < 3x10^{-3}$). Each open circle represents an n-way drug combination (FIC_2 n = 10, FIC_3 n = 10, FIC_4 n = 5, and FIC_5 n = 1).

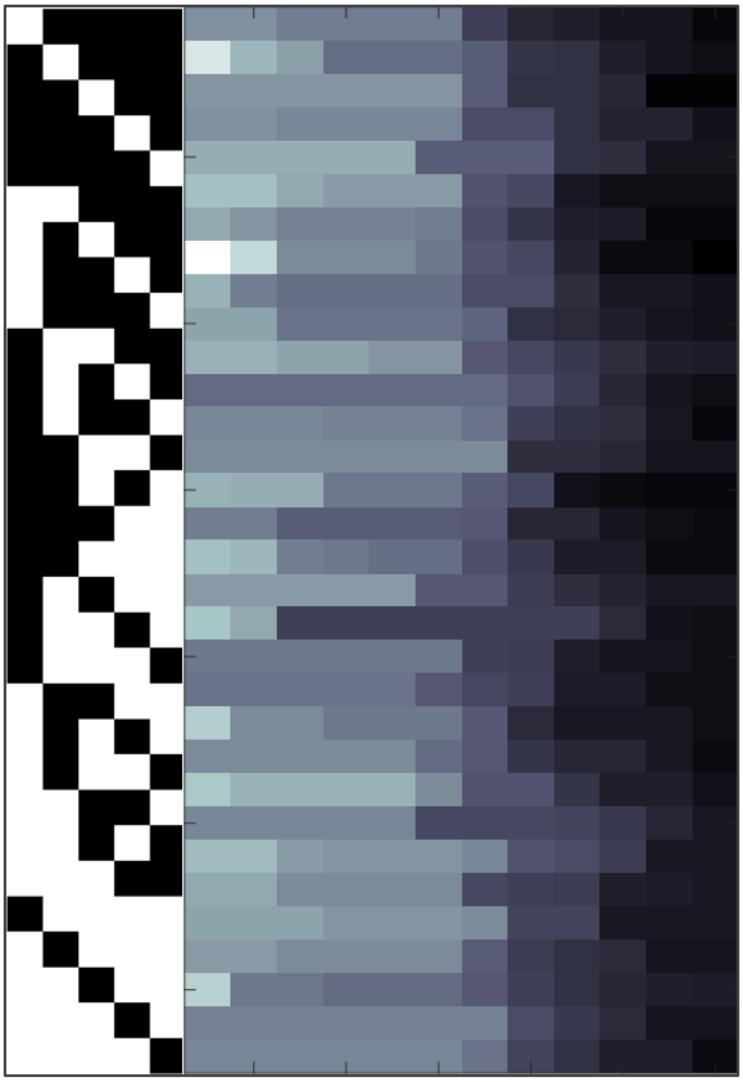


fig. S5. Dose responses of isoniazid in combination with itself in one-way, two-way, three-way, four-way, and five-way combinations. These dose-responses were used to generate the statistical error model of additivity. The drug dose is increasing from left to right and the black and white barcode on the right indicates the order of the self-self isoniazid combination, where the number of white squares represents the complexity of the combination (going from one-way at the top to five-way at the bottom).

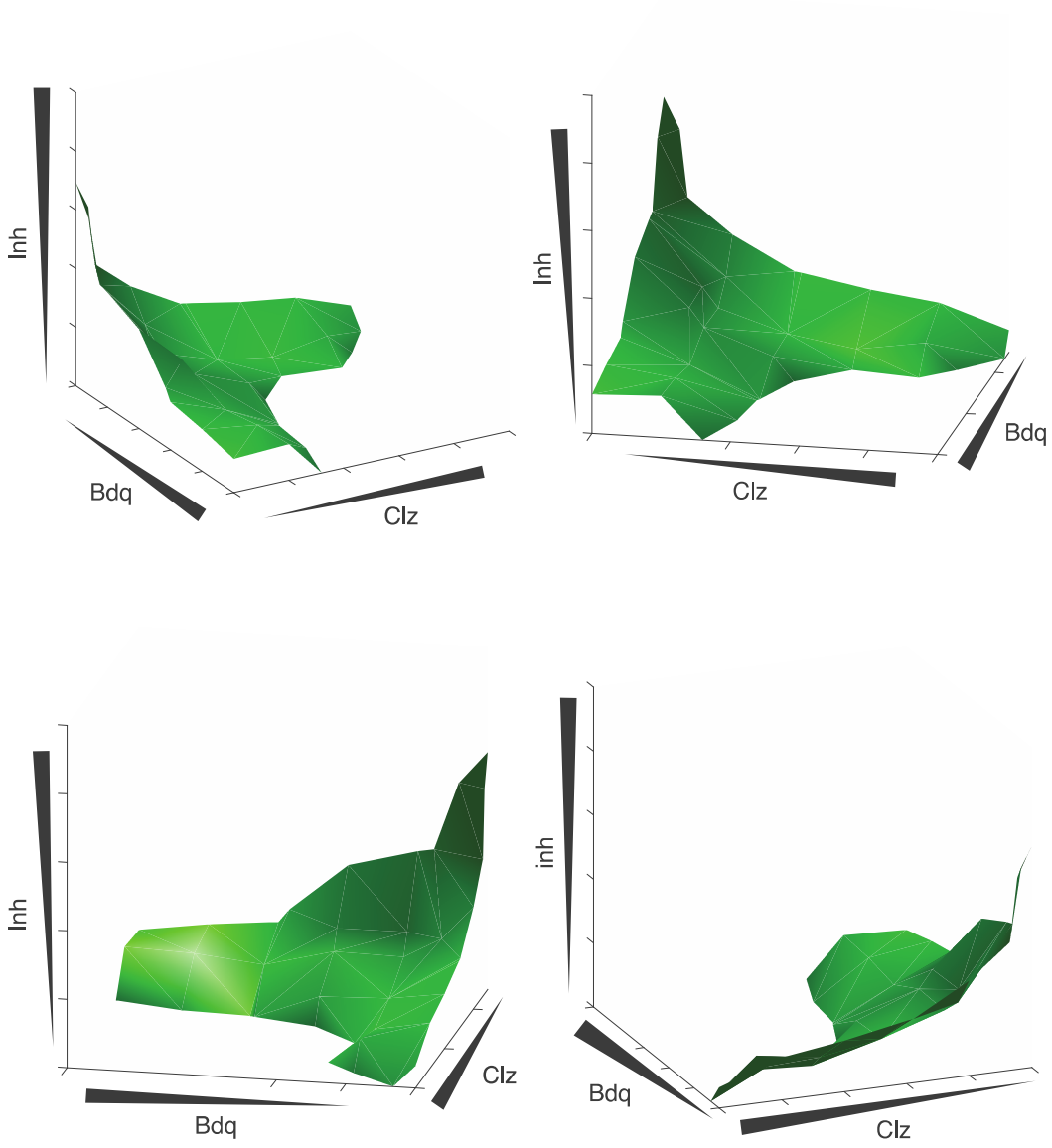


fig. S6. 3D isobole of the checkerboard assay for the isoniazid + clofazimine + bedaquiline interaction. Drug dose combinations were sampled linearly in a 6x6x6 dose matrix, with top concentrations as indicated in Table 1. The isobole (contour of the same phenotype) at IC_{80} is shown as a green surface. The color gradient is due to simulated lighting (MATLAB, 'camlight' on the 'isosurface' function) to visualize the shape of the surface. Four different angles of the same surface are shown.

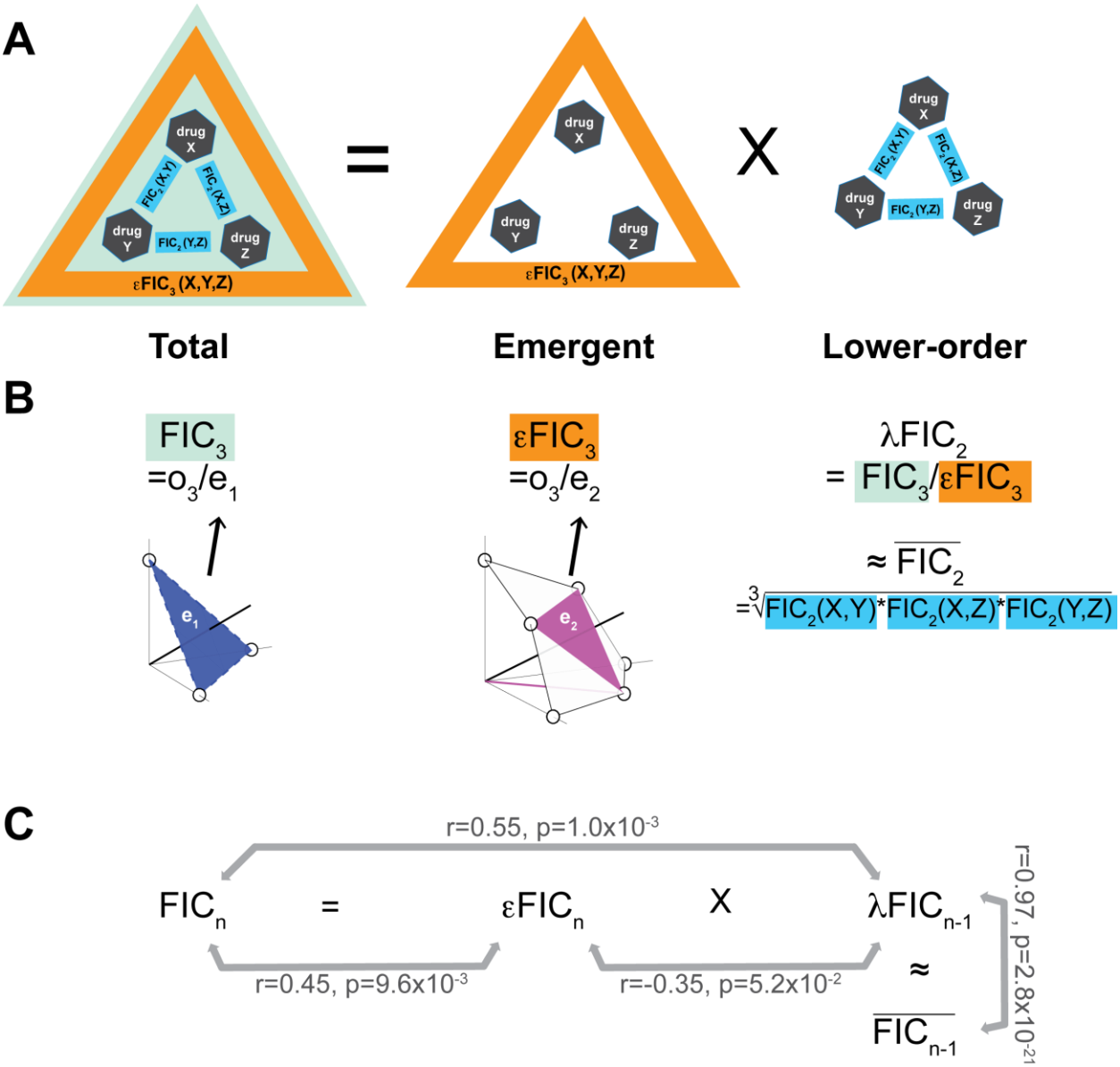


fig. S7. DiaMOND factorization model schematic. A to B) Visual representation (A) of the total, emergent, and lower-order interactions, measurements (A-B) and calculations (B) for a three-way interaction. Measured FICs are color-coded. The lower-order interaction score may be calculated from the total and emergent interaction scores, or it may be approximated by the geometric average of the component lower-order interactions, as shown. The measurement of FIC3, εFIC_3, all three FIC_2 scores requires the measurement of only seven dose-responses (X, Y, Z, X+Y, X+Z, Y+Z, and X+Y+Z). For example, if X, Y and Z have IC_{50} at 10 units, while X+Y, X+Z and Y+Z have IC_{50} at 5 units, and then DiaMOND reports that all pairwise combinations are synergistic, with an FIC_2 of 0.5 for

each pairwise interaction. Imagine that for the same example, the combination dose-response for X+Y+Z has an IC_{50} of 10 units. For the FIC_3 calculation, the IC_{50} of X+Y+Z (10 units) would be compared to the plane defined by the IC_{50} of X, Y and Z (10 units), and because X+Y+Z's IC_{50} is same with additive expectation, we conclude an FIC_3 score of 1. However, the calculation of εFIC_3 requires the comparison of the IC50 of X+Y+Z (10 units) with the plane defined by the IC_{50} of the X+Y, X+Z and Y+Z two-drug dose-responses (5 units). Because the three-way combination's IC_{50} is two times higher than the additive expectation from pairwise IC_{50}'s, DiaMOND concludes that $\boldsymbol{\varepsilon}FIC_3$ of this combination is 2. Therefore, we find $FIC_3 = 1$, and $\boldsymbol{\varepsilon}FIC_3 = 2$, thus the three-way combination is additive, and it is more antagonistic than a sum of pairwise synergies because the pairwise interactions were overall synergistic. The contribution of pairwise interactions to FIC_3 can be directly quantified by $FIC_3/\boldsymbol{\varepsilon}FIC_3$, by equation 4, we defined this quantity as $\boldsymbol{\lambda}FIC_2$; for this example, $FIC_3/\boldsymbol{\varepsilon}FIC_3 = 1 / 2 = 0.5$, indicating that the overall contribution of pairwise interactions is synergy. $\boldsymbol{\lambda}FIC_2$ can also be approximated by the geometric mean of all pairwise interactions, as used in equation 5 (shown in B above). In this example, FIC_2 score for all pairwise interactions were 0.5, so their geometric mean is 0.5, perfectly approximating the $\boldsymbol{\lambda}FIC_2$. **C)** Spearman correlation coefficients (r) and p values among the measured and calculated interaction scores in the factorization formula (equation 6).

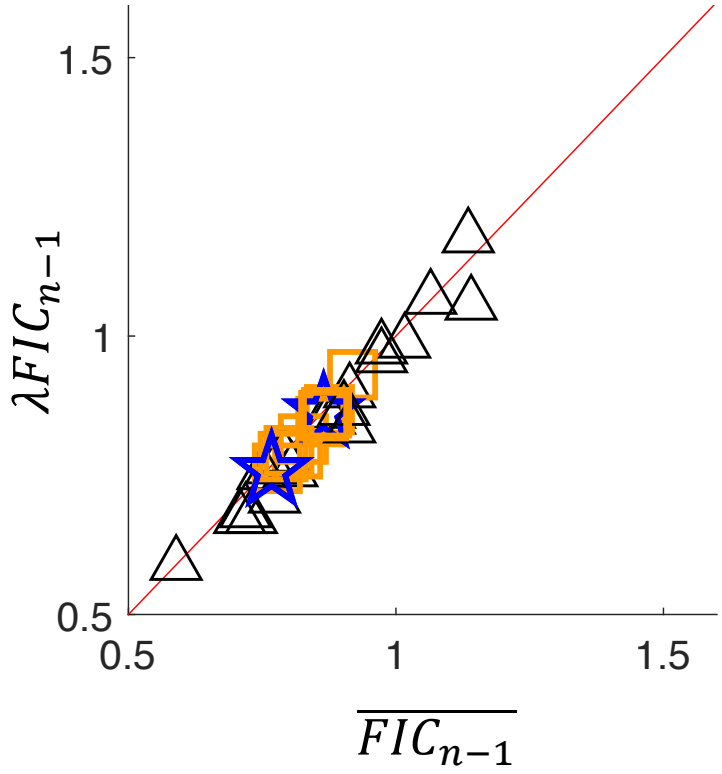
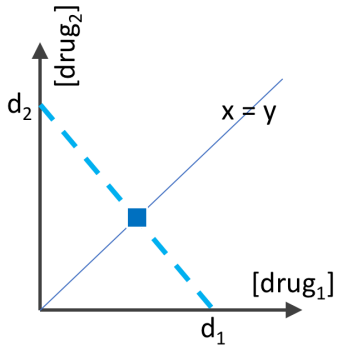


fig. S8. Scatterplot of the calculated lower-order (λFIC$_{n-1}$) interaction scores and the geometric mean of FIC$_{n-1}$ scores ($\overline{FIC_{n-1}}$) from the high-order measurements described in Figs. 2 and 3. λFIC$_{n-1}$ values were calculated by equation 6 ($FIC_n/\varepsilon FIC_n$). $\overline{FIC_{n-1}}$ was calculated as the geometric mean of the lower order interactions. Agreement between the calculated and measured scores was assessed by calculating Spearman's correlation (r = 0.97, p = 2.8x10^{-21}). Three-, four- or five-way interaction experiments are shown with triangles, squares or stars, respectively.

method S1. Derivation and formulas to calculate expectation doses.

The key to DiaMOND is the efficiency of using a combination dose-response instead of an exhaustive search of cell behaviors in all dose combinations of the drugs in consideration. Geometrically, a combination dose-response is a diagonal sampling of the traditional checkerboard (Figure 1A). To estimate the isobole (contour) and quantify the drug interaction (by the FIC), we calculate the expected value (of the IC_{50}, for example): e_n. The null hypothesis is that drugs are additive, therefore the checkerboard contour is expected to be a straight line in 2D (here), an uncurved plane in 3D (section 1.1), or an uncurved hyper-plane in n-D (section 1.2). Then the expected value of the diagonal sampling (the combination dose-response) is the intersection of the combination dose-response (outlined in orange in Figures 1A-B) and the additivity line or plane, which is uncurved. We may derive this intersection (e_n) using Euclidean geometry, and calculate the FIC_n by dividing the observed value by e_n. The derivations and equations are shown below for two drugs (section 1.1), three drugs (section 1.2), and n drugs (section 1.3).

method S1.1. Expectation for two drugs



Line equation is given as follows

$$\frac{1}{d_1}x + \frac{1}{d_2}y = 1$$

intersection of this line with x = y is found by

$$\frac{1}{d_1}x + \frac{1}{d_2}x = 1$$

$$\left(\frac{1}{d_1} + \frac{1}{d_2}\right)x = 1$$

$$x = \frac{d_1 \cdot d_2}{d_1 + d_2}$$

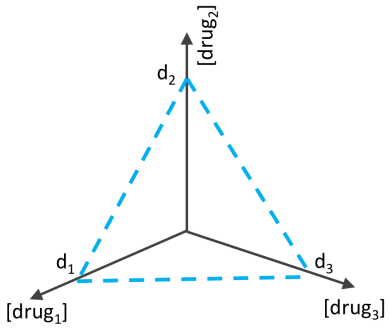
distance from origin is

$$\sqrt{2} \cdot \frac{d_1 \cdot d_2}{d_1 + d_2}$$

This distance is multiplied by $\sqrt{2}$ to find the expected position in two-drug dose-response

$$e_2 = 2 \cdot \frac{d_1 \cdot d_2}{d_1 + d_2}$$

method S1.2. Expectation for 3 drugs



Plane equation is given as follows

$$\frac{1}{d_1}x + \frac{1}{d_2}y + \frac{1}{d_3}z = 1$$

intersection of plane with x = y = z is found by

$$\frac{1}{d_1}x + \frac{1}{d_2}x + \frac{1}{d_3}x = 1$$

$$\left(\frac{1}{d_1} + \frac{1}{d_2} + \frac{1}{d_3}\right)x = 1$$

$$x = \frac{1}{\left(\frac{1}{d_1} + \frac{1}{d_2} + \frac{1}{d_3}\right)}$$

distance from origin is

$$\sqrt{3} \cdot \frac{1}{\left(\frac{1}{d_1} + \frac{1}{d_2} + \frac{1}{d_3}\right)}$$

This distance is multiplied by $\sqrt{3}$ to find the expected position in three-drug dose-response

$$e_3 = 3 \cdot \frac{1}{\left(\frac{1}{d_1} + \frac{1}{d_2} + \frac{1}{d_3}\right)}$$

method S1.3. Expectation for n drugs

Hyper-plane equation where each drug i intersects one axis at d_i is given as follows

$$\frac{1}{d_1}x_1 + \frac{1}{d_2}x_2 + \cdots + \frac{1}{d_n}x_n = 1$$

$$\sum_{i=1}^{n} \frac{1}{d_i} x_i = 1$$

intersection with line x1 = x2 = ⋯ = x_n is found by

$$\sum_{i=1}^{n} \frac{1}{d_i} x = 1$$

$$x = \frac{1}{\sum_{i=1}^{n} \frac{1}{d_i}}$$

distance from origin is

$$\sqrt{n} \cdot \frac{1}{\sum_{i=1}^{n} \frac{1}{d_i}}$$

This distance is multiplied by $\sqrt{n}$ to find the expected position in n-drug dose-response

$$e_n = n \cdot \frac{1}{\sum_{i=1}^{n} \frac{1}{d_i}}$$

method S2. DiaMOND equation for four-drug combination derived from Eq. 5, approximation, and recursion.

$$\mathrm{FIC}_4 = \frac{o_4}{e_1} = \frac{o_4}{e_3} \cdot \frac{e_3}{e_1} = \varepsilon\mathrm{FIC}_4 \cdot \lambda\mathrm{FIC}_3 \approx \varepsilon FIC_4 \cdot \overline{FIC_3}$$

each FIC_3 can be approximated as $\varepsilon FIC_3 \cdot \overline{FIC_2}$, therefore

$$\mathrm{FIC}_4 \approx \varepsilon FIC_4 \cdot \overline{FIC_3} = \varepsilon FIC_4 \cdot \overline{\varepsilon FIC_3 \cdot \overline{FIC_2}} = \varepsilon FIC_4 \cdot \overline{\varepsilon FIC_3 \cdot \overline{\varepsilon FIC_2}} .$$

To demonstrate the recursion, we will consider the interaction among 4 drugs a, b, c and d. ε(abc) or ε(ab) denotes the emergent interaction of 3-drug combo (abc) or 2-drug combo (ab), respectively

$$= \varepsilon FIC_4 \cdot \overline{\varepsilon FIC_3 \cdot \overline{\varepsilon FIC_2}}$$

$$= \varepsilon FIC_4 \cdot \sqrt[4]{\varepsilon FIC_3 \cdot \sqrt[3]{\varepsilon FIC_2}}$$

$$= \varepsilon FIC_4 \cdot \sqrt[4]{\varepsilon FIC_3 \cdot \sqrt[3]{\varepsilon FIC_2}}$$

$$= \varepsilon FIC_4 \cdot \sqrt[4]{\begin{matrix}\left[\varepsilon(abc) \cdot \sqrt[3]{\varepsilon(ab) \cdot \varepsilon(ac) \cdot \varepsilon(bc)}\right] \cdot \left[\varepsilon(abd) \cdot \sqrt[3]{\varepsilon(ab) \cdot \varepsilon(ad) \cdot \varepsilon(bd)}\right] \cdot \\ \left[\varepsilon(acd) \cdot \sqrt[3]{\varepsilon(ac) \cdot \varepsilon(ad) \cdot \varepsilon(cd)}\right] \cdot \left[\varepsilon(bcd) \cdot \sqrt[3]{\varepsilon(bc) \cdot \varepsilon(bd) \cdot \varepsilon(cd)}\right]\end{matrix}}$$

$$= \varepsilon FIC_4 \cdot \sqrt[4]{\varepsilon(abc) \cdot \varepsilon(abd) \cdot \varepsilon(acd) \cdot \varepsilon(bcd)}$$

$$\cdot \sqrt[4]{\begin{matrix}\left[\sqrt[3]{\varepsilon(ab) \cdot \varepsilon(ac) \cdot \varepsilon(bc)}\right] \cdot \left[\sqrt[3]{\varepsilon(ab) \cdot \varepsilon(ad) \cdot \varepsilon(bd)}\right] \cdot \\ \left[\sqrt[3]{\varepsilon(ac) \cdot \varepsilon(ad) \cdot \varepsilon(cd)}\right] \cdot \left[\sqrt[3]{\varepsilon(bc) \cdot \varepsilon(bd) \cdot \varepsilon(cd)}\right]\end{matrix}}$$

$$= \varepsilon FIC_4 \cdot \overline{\varepsilon FIC_3} \cdot \sqrt[4]{\left[\sqrt[3]{\varepsilon(ab) \cdot \varepsilon(ac) \cdot \varepsilon(ad) \cdot \varepsilon(bc) \cdot \varepsilon(bd) \cdot \varepsilon(cd)}\right]^2}$$

$$= \varepsilon FIC_4 \cdot \overline{\varepsilon FIC_3} \cdot \sqrt[6]{\varepsilon(ab) \cdot \varepsilon(ac) \cdot \varepsilon(ad) \cdot \varepsilon(bc) \cdot \varepsilon(bd) \cdot \varepsilon(cd)}$$

$$= \varepsilon FIC_4 \cdot \overline{\varepsilon FIC_3} \cdot \overline{\varepsilon FIC_2}$$

$$= \prod_{k=2}^{4} \overline{\varepsilon FIC_k}$$