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I. Methods

A. Cell lines, strains, and reagents.

<u>Bacteria</u>

A table of bacterial strains is given in Table S1.

Mammalian Cells

HCC827 parental (WT) and Gefitinib-resistant (GR6) cells, the latter of which were evolved by stepwise selection in increasing concentrations of Gefitnib, were obtained from J. Engelman (Massachusetts General Hospital) and grown in RPMI with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin¹. HMLE cells stably expressing lentiviral short hairpin RNAs (shRNA) against GFP (control) and E-Cadherin were obtained from P. Gupta (Whitehead Institute for Biomedical Research) and grown in media consisting of equal parts (1) complete MEGM media (Lonza) and (2) DMEM with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin². A375 parental (WT) cells were obtained from ATCC and grown in RPMI with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin². A375 parental (WT) cells were obtained from ATCC and grown in RPMI with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin². A375 parental (WT) cells were obtained from ATCC and grown in RPMI with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. PLX4720-resistant A375 cells were engineered by stably overexpressing the kinase C-RAF, which can confer resistance to PLX4720 by overriding B-RAF dependence³. C-RAF-expressing lentiviruses were produced as previously described^{4, 5}. A375 parental cells were infected at a 1:10 dilution of virus in 6-well plates in the presence of 7.5 µg/ml polybrene and centrifuged at 1200g for 1 hour at 37° C. Twenty-four hours after infection blasticidin (10 µg/ml) was added and cells were selected for 72 hours, after which blasticidin was removed and growth inhibition assays were performed.

Table S1: Bacterial Strains

Organism	Cell Line / Strain	Source	Parental Strain	Comments
E. coli	BW25113	6	***	Keio parent strain
E. coli	kwcmcip (1 - 15)	this work (Table S3)	BW25113	lab evolved strain
E. coli	<i>coli</i> kwcmcipseq (1-6) this work (Table S3)		BW25113	lab evolved strain
E. coli	kwdoxery (1-3)	this work (Table S3)	BW25113	lab evolved strain
E. coli	748k0.1	7	***	clinical isolate
E. coli	1 - 748MM	7	748k0.1	fluoroquinolone resistant
E. coli	1 - 748	7	748k0.1	fluoroquinolone resistant
S. aureus	Newman	8	***	
S. aureus	snnorr-1	this work	Newman	norfloxacin resistant
E. faecalis	V583	9	***	vancomycin resistant clinical

*** Strain is the drug sensitive "wild type"

Organism	Cell Line / Strain	Source	Parental Strain	Comments
				isolate
E. faecalis	Dap-A	9	V583	daptomycin resistant
E. faecalis	Dap-B	9	V583	daptomycin resistant
E. faecalis	Dap-C	9	V583	daptomycin resistant
S. cerevisiae	yjhk112	10	****	
S. cerevisiae	yMM8	10	yjhk112	cycloheximide resistant

B. Drugs

Drug solutions were made from solid stocks (Table S2). All antibiotic stock solutions were stored in the dark at -20° C in single-use daily aliquots. All drugs were thawed and diluted in sterilized media for experimental use.

Table 52. Drugs, Abbi	teviations, and moues (Action
Drug (source)	Abbreviation	Class
Chloramphenicol (MP Biomedicals)	Cm	Protein synthesis inhibitor (50 S target)
Doxycycline (Sigma Aldrich)	Dox	Protein synthesis inhibitor (30 S target)
Erythromycin (Sigma Aldrich)	Ery	Protein synthesis inhibitor (macrolide)
Lincomycin (MP Biomedicals)	Linc	Protein synthesis inhibitor (50 S target)
Ciprofloxacin (Sigma Alderich)	Cip	DNA synthesis inhibitor
Ofloxacin (Sigma Aldrich)	Ofl	DNA synthesis inhibitor
Trimethoprim (Sigma Aldrich)	Tmp	Folic acid synthesis inhibitor
Salicylate (Sigma Aldrich)	Sal	Pain reliever
Ampicillin (Sigma Aldrich)	Amp	Cell wall synthesis inhibitor
Daptomycin (Enzo Life Sciences)	Dap	Lipopeptide
Norfloxacin	Nor	DNA synthesis inhibitor

Table S2: Drugs, Abbreviations, and Modes of Action

Drug (source)	Abbreviation	Class
(Sigma Aldrich		
Linezolid (Selleck Chemicals)	Linez	Oxazolidinone
Cycloheximide (Ampresco)	Cyclo	Eukaryotic protein synthesis inhibitor
5-Fluorocytosine (Sigma Aldrich)	5-Fluor	Antimycotic (DNA synthesis / RNA biosynthesis inhibitor)
PLX4720 (Selleck Chemicals)	PLX	B-Raf inhibitor
Gefitinib (Selleck Chemicals)	Gef	EGFR inhibitor
17-AAG (Selleck Chemicals)	17-AAG	Hsp90 inhibitor
Dacarbazine (Sigma Aldrich)	Dacarb	Alkylating agent
Etoposide (Sigma Aldrich)	Etop	Topoisomerase inhibitor
5-Fluorouracil (Sigma Aldrich)	5-FU	Antimetabolite (apoptosis inhibitor)
Paclitaxel (Sigma Aldrich)	Pac	Mitotic inhibitor

C. Growth Inhibition Assays

Growth Assay for Bacteria

We inoculated media (LB for E. coli, TSB for S. aureus, BHI for E. faecalis) from a single colony and grew the cells overnight (12 h at 30°C with shaking at 200 rpm for E. coli, S. aureus; no shaking for E. faecalis). Following overnight growth, stationary phase cells were diluted (~5000 fold for E. coli, S. aureus; ~1000 fold for E. faecalis) in media. Following the initial dilution, S. aureus and E. faecalis were grown in drug free media for 1 hour prior to adding drugs and transferring to 96 well plates. We transferred E. coli to 96-well plates (round bottom, polystyrene, Corning) immediately following dilution. For each experiment, we set up a twodimensional matrix of 1 or 2 drug combinations in each of four 96-well plates (165-190 µl media per well). For the remainder of the experiment after the addition of drugs ($\sim 10-12$ h), cells were grown at 30°C (with shaking at 1000 rpm on four identical vibrating plate shakers for E. coli; no shaking for E. faecalis). A₆₀₀ (absorbance at 600 nm, proportional to optical density OD) was measured at 15-25 min intervals (with one exception; see below) using a Wallac Victor-2 1420 Multilabel Counter (PerkinElmer) combined with an automated robotic system (Twister II, Caliper Life Sciences) to transfer plates between shakers and the reader. Growth rates in bacteria were determined by fitting background-subtracted growth curves (A₆₀₀ vs. time) in early exponential phase (approximately $0.01 < A_{600} < 0.1$) to an exponential function (MATLAB 7.6.0 curve fitting toolbox, The Mathworks). For S. aureus with Nor-Cm (Figure 5), effective exponential growth rates were estimated using background subtracted A₆₀₀ measurements at times t = 2 hours and t = 6 hours; true exponential growth curves are therefore not required for this

particular assay, which is instead similar to traditional viability assays that compare cell number at the end of the experiment (see mammalian growth assays, below). Growth rates were normalized by the growth of cells in the absence of drugs. Error bars, unless otherwise noted, are taken to represent +/- one standard error of the fitted parameter.

Growth Assay for Yeast

We inoculated media (SAB) from a single colony and grew the cells overnight (12 h at 30°C with shaking at 200 rpm). Following overnight growth, stationary phase cells were diluted (~5000 fold) in media. Because *S. cervesaie* experiments required longer incubation times (~24 hours), evaporation was not negligible, and we therefore measured growth curves using Bioscreen C instrument (Growth Curves USA), which uses specialized plates that prevent evaporation. For these experiments, we used 100-well plates specific to the Bioscreen C instrument and set up a two-dimensional matrix of 1 or 2 drug combinations in each plate (165-190 μ l media per well). For the remainder of the experiment after the addition of drugs (18+ hours for *S. cervesaie*), cells were grown at 30°C. A₆₀₀ was measured at 15-25 min intervals using the Bioscreen C (Growth Curves USA). Growth rates were determined by fitting background-subtracted growth curves (A₆₀₀ vs. time) in early exponential phase (approximately 0.01 < A₆₀₀ <0.1) to an exponential function (MATLAB 7.6.0 curve fitting toolbox, The Mathworks).

Growth assay for Mammalian Cells

Cells were trypsinized, counted, and seeded into 96-well plates at 2,500 cells/well. Twenty-four later, DMSO or concentrated dilutions of indicated drugs (in DMSO) were added to cells (1:1,000 in standard media) to yield the indicated final drug concentrations. Cell viability was measured 4 days after drug addition using the Cell Titer Glo® luminescent viability assay (Promega). Viability was calculated as the percentage of control (untreated cells) after background subtraction. Three replicates were performed for each drug/concentration.

D. Estimation of Experimental Uncertainty and Example Growth Curves

Bacteria

In bacteria, growth inhibition assays are based on exponential fits to time series of absorbance (see Section IC). Because of the large number of drug dosages needed to estimate response surfaces, it is not practical to perform a large number of replicates at each dosage. However, to estimate the experimental uncertainty in these measurements, particularly for high drug dosages, we performed replicate experiments for each of the drugs most commonly used in the study. In each experiment, we exposed approximately 10 replicate cultures to the same concentration of a drug and then estimated the exponential growth rate for all replicates. In all cases in bacteria, the standard deviation, σ , of the growth rate measurements was small ($\sigma < 0.06$ in terms of relative growth rate, and typically much smaller). The uncertainty, which represents experiment-toexperiment variability-was small even in cases where the drugs have steep dose-response curves (e.g. ciprofloxacin, $\sigma \le 0.03$, and ampicillin, $\sigma \le 0.06$) and even for high concentrations of the drugs. In Figures 5 and S4, we have included error bars that indicate the largest standard deviation measured for the two drugs in each given experiment. While the current study is limited the study of monophasic responses, some drugs used here (e.g. ciprofloxacin) are known to produce biphasic responses under some conditions. Therefore, we note that extending this scaling approach to include biphasic responses remains an interesting avenue for future work.

Cancer Cells

Growth estimates in cancer cells rely on a standard luminescence based cell viability assay (Cell Titer Glo®). At each dosage in the study, we performed at least 3 replicates. Reported growth estimates are a mean of replicates, and uncertainties are estimated as the standard error of the

mean. In Figure S4, error bars are typically smaller than the size of the data points. Errors are slightly larger for NSCLC in Figure 5 and for cancer stem cells in Figure 6.

E. Evolved Drug Resistant Mutants in E. coli and S. aureus

Drug resistant E. *coli* mutants were isolated by first diluting liquid cultures of wild-type cells (BW25113 in stationary phase) 1000-fold into 96 individual 1 mL cultures on a single deep well plate, with each well supplemented with a combination of chloramphenicol and ciprofloxacin or doxycycline and erythromycin (Table S3). Cultures were grown at 30°C with shaking and diluted 1000x into fresh media (with drugs) every 24 hours for a total of 6 days (approximately 60 generations). Aliquots (1 µl) of each culture were streaked onto drug free agar plates and grown overnight at 30 degrees. A single colony from each plate was then selected, grown in LB for approximately 12 hours, and then frozen in 15% glycerol at -80°C. Frozen cultures were used to streak LB plates, and all experiments were performed on cultures grown from a single colony isolated from these plates. S. aureus norfloxacin resistant mutants were isolated on TSA (Trypric soy agar, BD) plates containing 4 ug/ml Nor, followed by spreading of overnight culture of Newman strain.

	Table S3: Mutant Selection Conditions	
Mutant	Selection Condition (gens = generations)	Drug Pair Used For Experimental Response Surface
kwcmcip1	60 gens in [Cm]=1.65 ug/mL	Sal-Cm
kwcmcip2	60 gens in [Cm]=1.55 ug/mL and [Cip]=3 ng/mL	Sal-Cm
kwcmcip3	60 gens in [Cm]=1.65 ug/mL	Sal-Cm
kwcmcipseq1	60 gens in [Cip]= 9 ng/mL, then 60 gens in [Cm]=0.375 ug/mL	Cm-Cip
kwcmcipseq2	60 gens in [Cip]= 10 ng/mL, then 60 gens in [Cm]=0.775 ug/mL	Cm-Cip
kwcmcipseq3	60 gens in [Cip]= 8 ng/mL, then 60 gens in [Cm]=1.3 ug/mL	Cm-Cip
kwcmcipseq4	60 gens in [Cm]=1.55 ug/mL, then 60 gens in [Cip]= 3 ng/mL	Cm-Cip
kwcmcip4	60 gens in [Cip]=7 ng/mL	Cm-Cip
kwcmcip5	60 gens in [Cm]=0.375 ug/mL and [Cip]=9 ng/mL	Cm-Cip
kwcmcip6	60 gens in [Cm]=0.775 ug/mL and [Cip]=10 ng/mL	Cm-Cip
kwcmcip7	60 gens in [Cm]=1.3 ug/mL and [Cip]=8 ng/mL	Cm-Cip
kwcmcip8	60 gens in [Cm]=1.55 ug/mL and [Cip]=3 ng/mL	Cm-Cip
kwcmcip9	60 gens in [Cm]=1.65 ug/mL	Cm-Cip
kwcmcip10	60 gens in [Cip]=7 ng/mL	Cm-Cip
kwcmcip11	60 gens in [Cm]=0.375 ug/mL and [Cip]=9 ng/mL	Cm-Cip

kwcmcip12	60 gens in [Cm]=0.375 ug/mL and [Cip]=9 ng/mL	Cm-Cip
kwcmcip13	60 gens in [Cm]=0.775 ug/mL and [Cip]=10 ng/mL	Cm-Cip
kwcmcip14	60 gens in [Cm]=1.3 ug/mL and [Cip]=8 ng/mL	Cm-Cip
kwcmcip15	60 gens in [Cm]=1.55 ug/mL and [Cip]=3 ng/mL	Cm-Cip and Sal-Cm
kwcmcipseq5	60 gens in [Cm]=0.375 ug/mL, then 60 gens in [Cip]= 9 ng/mL	Cm-Cip
kwcmcipseq6	60 gens in [Cm]=0.775 ug/mL, then 60 gens in [Cip]= 10 ng/mL	Cm-Cip
kwdoxery1	60 gens in [Dox]=0.6 ug/mL	Dox-Ery
kwdoxery2	60 gens in [Dox]=0.3 ug/mL and [Ery]=11.3 ug/mL	Dox-Ery
kwdoxery3	60 gens in [Dox]=0.15 ug/mL and [Ery]=22.5 ug/mL	Dox-Ery

F. Alternative Measures of Drug Interactions

In an effort solely to illustrate the diversity of possible dose-response surfaces (figure 1), we quantified drug interactions relative to Bliss independence^{11, 12}. The choice of Bliss independence as a null model is purely illustrative, and our general scaling model does not use Bliss independence to make predictions. However, we note that there are alternative ways to quantify and illustrate drug interactions once the response surface is known. In Figure S1 we re-plot the interactions from Figure 1 relative to Loewe additivity¹³, another common reference model (see below). The specific drug interactions may depend on the null model used (Bliss, as in Figure 1, or Loewe, as in Figure S1). However, in the context of this work, the choice of Bliss or Loewe as a reference model is used only to illustrate that response surfaces—and therefore traditional measures of drug interactions—do not appear to follow any simple pattern upon acquisition of resistance. Again, we stress that our general scaling model allows us to calculate the full dose response surface and do not depend on whether Bliss or Loewe is chosen to illustrate the interactions.

Quantifying Drug Interactions Based on Loewe Additivity

In addition to the Bliss-based interaction metric used in Figure 1, one can quantify drug interactions relative to Loewe additivity. Interactions based on Loewe additivity are closely related to the shape of the isoboles in 2-drug space¹⁴. Straight lines represent non-interacting drugs, and this model is widely known to be appropriate for the hypothetical experiment where one takes a single drug, divides it into two samples, and treats each sample as an individual drug. Mathematically, Loewe additivity corresponds to¹³

$$I = \frac{D_1}{D_{1,g}} + \frac{D_2}{D_{2,g}} = 1$$

where $D_{1,g}(D_{2,g})$ is the concentration of drug 1 (2) alone that leads to given level of growth, g, and $D_1(D_2)$ is the concentration of drug 1 (2) in a mixture of both drugs that leads to that same level of growth. I<1 corresponds to synergy, and I>1 corresponds to antagonism. Because the single-drug dose response curves in this study can be approximated as Hill-like functions, it is straightforward to calculate I for each point in the dose-response space—that is, at each measured value of growth—by inverting the single-drug response functions to get $D_{1,g}$ and $D_{2,g}$.

For comparison with Figure 1, we plot the interaction as I-1 (positive is antagonism, negative is

synergy) in Figure S1. The interaction is, by definition, zero when only one drug is present or when the drugs are Loewe additive.

II. Model for Two-Drug Interactions

A. Description of the Model

To evaluate the effect of drug-pair interaction, it is standard in pharmacology to use a null hypothesis such as Bliss independence^{11, 13, 15}. Bliss independence consists of treating the effect of the two drugs independently in a probabilistic sense. Under this condition, the growth rate of two non-interacting drugs is:

$$g_{12} = g_1(D_1)g_2(D_2)$$

where g_i represents the growth rate of cells in presence of the drug i, normalized by the growth rate in the absence of drug. Here the term 'growth' is loosely defined and may represent different measures of cell proliferation depending on the species under study^{13, 15, 16} (see Section I, above). When two drugs interact, the resulting growth rate is either larger or smaller than that obtained from the null model. Here we extend this null model and explicitly account for drug interactions by introducing a drug-drug coupling function that modifies the concentration of drug D₂ into an *effective* concentration, D_{2,eff}, that depends on drug 1.

$$g_{12} = g_1(D_1)g_2(D_{2,eff})$$

The model is fully specified by three one-dimensional functions. The two toxicity functions, g_1 and g_2 , depend on D_1 and D_2 , respectively, and describe the toxic effects of the drugs alone on growth. The drug-drug coupling function, D_{2eff}/D_2 , depends only on D_1 and accounts for drug interactions by allowing the presence of one drug (D_1) to modify the effective concentration, and hence the toxicity, of the other (D_2). We refer to the set of functions g_1 , g_2 , and D_{2eff}/D_2 as "basis" functions because they define the entire two-dimensional response surface g_{12} .

We have chosen to break the symmetry between the two drugs (D_1 affects D_{2eff} , but not vice versa) because in many observed drug interactions, such as the suppression between antibiotics^{14, 17}, one drug asymmetrically affects the other. However, this model is also appropriate for many symmetric drug interactions (see Section III for examples). We note that this model would need to be generalized if the drugs mutually suppressed one another, as describing such a growth surface would require two coupling functions. However, to our knowledge, no mutually suppressive drug interactions have been reported. Toxicity functions are described by Hill functions, which are common models of dose-response curves¹³ and may reflect the binding of drugs to some intracellular substrate. We stress that the form of Equation (1), where the effect of the two drugs can be decomposed into a product, arises in several contexts where mechanistic details are known, such as multi-antibiotic resistance¹⁸ and, more generally, in models of single enzyme inhibition¹⁹ (see Section III). While the model (Equation 1, main text) provides an excellent description of these well-characterized systems, molecular details and the associated form of D_{2eff}/D_2 (or $C(D_1)$) are not, in general, known. In the absence of mechanistic insight, we use the model but must *infer* the coupling function D_{2eff}/D_2 directly from experimental growth data (below). Importantly, this model does not introduce the usual artifact arising from the standard Bliss independence model. For example, it is well-known that standard Bliss independence introduces an artifactual result when one attempts to measure the interaction of drug with itself (more in Section IV). However, the model presented in this paper uses a coupling function to encapsulate drug interaction, which circumvents this artifactual behavior.

B. Estimation of Model Parameters and Coupling Functions for Pairwise Drug Interactions

Single drug toxicity functions (relative growth vs. drug concentration) were fit to Hill functions (see Fig 2) using nonlinear least squares fitting. Drug-drug coupling functions were then found by using a series of 5 distinct parameterizations (and an additional non-interacting model, Bliss independence) for the effective concentration of one drug. To infer the coupling functions from the data, both single drug toxicities were constrained to be of the Hill form previously measured. For each pair of drugs, parameters for the coupling function for each of the 5 parameterizations were determined by nonlinear least squared fitting, either assuming uni-directional drug 1 to drug 2 coupling or by assuming uni-directional drug 2 to drug 1 coupling. The Akaike Information Criteria (AIC)²⁰ was therefore determined for each of the 2*5+1 (Bliss) = 11 parameterizations, and the coupling function was taken to be the AIC best parameterization among the 11 options. We note that the drug-drug coupling can be extended to a bi-directional coupling, but the uni-directional coupling provides an excellent fit for all drug pairs tested (Table S4, S5). We also stress that each parameterization of the coupling function in Table S4 can be written in the form $D_{i,eff}/D_i = (1+C(D_j))^{-1}$. The forms given in Table S4 are rearranged for notational convenience. Additional technical details are covered below.

C. Model Selection and Multi-Model Inference

In order to select a parameterization of a two-drug interaction supported by the data, we use Akaike Information Criteria (AIC) methods to achieve an appropriate balance between parsimony and accuracy²⁰. For a given two-drug interaction, we begin by fitting the two single drug toxicity functions ($g_1(D_1)$ and $g_2(D_2)$) to a Hill form common in pharmacology literature¹³:

$$g_i(x) = \frac{1}{1 + (x/K_i)^{n_i}}$$
(S1)

where K_i is the concentration at which the drug has half-maximal effect (often known as the IC₅₀; we choose K for economy of notation). With the single drug toxicity functions determined, we then infer the drug-drug coupling function using a series of parameterizations, as described above.

Parameterization	Abbreviation	Equation	n = Number parameters (c _i)
Pointwise D ₁	PW1	$\frac{D_{2,eff}}{D_2} = \frac{1}{1+c_i}$	Number of D ₁ concentrations
Polynomial 2 D ₁	P21	$\frac{D_{2,eff}}{D_2} = 1 + c_1 D_1 + c_2 D_1^2$	2
Polynomial 3 D ₁	P31	$\frac{D_{2,eff}}{D_2} = 1 + c_1 D_1 + c_2 D_1^2 + c_3 D_1^3$	3

Table S4: Parameterizations for Drug-Drug Coupling

Parameterization	Abbreviation	Equation	n = Number parameters (c _i)
Saturating D ₁	Sat1	$\frac{D_{2,eff}}{D_2} = \frac{1}{1 + c_1 \left(\frac{D_1}{D_1 + c_2}\right)}$	2
Tanh D ₁	Tanh1	$\frac{D_{2.eff}}{D_2} = 1 + c_1 \tanh(c_2 D_1)$	2
Pointwise D ₂	PW2	$\frac{D_{1,eff}}{D_1} = \frac{1}{1+c_i}$	Number of D ₂ concentrations
Polynomial 2 D ₂	P22	$\frac{D_{1,eff}}{D_1} = 1 + c_1 D_2 + c_2 D_2^2$	2
Polynomial 3 D ₂	P32	$\frac{D_{1,eff}}{D_1} = 1 + c_1 D_2 + c_2 D_2^2 + c_3 D_2^3$	3
Saturating D ₂	Sat2	$\frac{D_{1,eff}}{D_1} = \frac{1}{1 + c_I \left(\frac{D_2}{D_2 + c_2}\right)}$	2
Tanh D ₂	Tanh2	$\frac{D_{1,eff}}{D_1} = 1 + c_1 \tanh(c_2 D_2)$	2
Bliss independent	Bliss	$\frac{D_{1,eff}}{D_1} = 1; \frac{\overline{D_{2,eff}}}{D_2} = 1;$	0

** For all models, we constrain $(D_{i,eff} / D_i)$ to be greater than or equal to 0. The expressions in the table represent one simple way of writing each parameterization. Note, however, that each model can be trivially re-written in the form $D_{i,eff}/D_i = (1+C(D_j))^{-1}$. For example, model P21 is equivalent to $D_{2,eff}/D_2 = (1+C(D_1))^{-1}$, with $C(D_1)=(1+c_1 D_1 + c_2 D_1^2)^{-1}-1$. Note also that c_i variables (lower case c) are fitting parameters and should not be confused with the function $C(D_1)$.

For each model listed above (Table S4), we calculate the AIC. AIC is given by

$$AIC = -2\log(L(\hat{c}_i \mid y)) + 2n'$$
(S2)

where $\log(L(\hat{c}_i | y))$ is the log likelihood function evaluated at its maximum value, y is the data, and \hat{c}_i denotes the maximum likelihood estimates for the entire set of parameters. Note that n' = n+1 is the true number of parameters of the model, because each model has an implicit parameter that corresponds to the variance of the error distribution. The AIC is an estimate of the expectation value of the relative Kullback-Leibler (KL) divergence between the fitted model and the "true mechanism" generating the observed data. The model with the lowest AIC value among a set of models is considered the best model in that it minimizes the KL divergence between the model and statistical mechanism underlying the data. For the least squares case, AIC reduces to

$$AIC = -N\log(\hat{\sigma}^2) + 2n'$$
(S3)

with N the number of observations and $\hat{\sigma}^2$ the maximum likelihood estimator of the error variance. In practice, we use a small sample estimator of AIC which includes an additional bias correction term

$$AIC = -2\log(L(\hat{c}_i \mid y)) + 2n' + \frac{2n'(n'+1)}{N-n'-1}$$
(S4)

While the absolute value of the AIC has little meaning, the difference in AIC values between two models does contain important information. These differences can be converted to Akaike weights, w_i , defined by

$$w_i = \frac{\exp(-\delta_i/2)}{\sum \exp(-\delta_j/2)}$$
(S5)

where $\delta_i = AIC_i - AIC_{min}$ and the index i runs over the set of models. Because $\exp(-\delta_i/2)$ is proportional to the likelihood of model i given the data, each Akaike weight can be interpreted as a measure of the evidence in favor of model i, given that one model in the set is KL best model of the set. Table S5 provides the AIC weights for each of the models listed above for 20 different two-drug combinations. Example coupling functions are shown in Figure 2.

In Figure 3, the pointwise models (PW1 or PW2) are used for directly comparing the coupling functions from wild type and mutant cells, regardless of Akaike weight, because these models are more directly connected with the raw data and do not involve fitting to some preassigned functional form. Instead, the pointwise models define the drug-drug coupling for each concentration of D_i . While these pointwise functions may be suffer from statistical overfitting, they nevertheless offer a direct comparison that requires no assumptions about the structure of the noise or any pre-defined functional forms (other than Equation 1, which defines the overall model). We also note that using the Akaike-best coupling functions for *E. coli* mutants in Figure 3A reduces the size of the error bars and does not modify the qualitative scaling result.

Finally, we note that when directly comparing the coupling functions of mutants and wild type cells, we do not consider the coupling function at large concentrations of the inducer ($D_i > K_i$). At those high concentrations, the growth is dominated by the toxicity of the inducing drug, and the value of the coupling function has relatively little effect on the overall growth. Therefore, the coupling function is under-constrained in that region and is difficult to estimate accurately.

Table S5: Two-drug Fitting Data (E. coli)

Drug 1	Drug 2	PW1	P21	P31	Tanh1	Sat1	PW2	P22	P32	Tanh2	Sat2	Bliss	R2	R2 Resp Surf	AIC Diff	AIC Weight
Cm	Tmp	0.00	0.74	0.26	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.94	0.91	41.17	1
Cm	Ofl	0.00	0.04	0.21	0.57	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.95	0.62	183.72	1
Cm	Cip	0.00	0.55	0.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.94	0.64	349.45	1
Tmp	Ofl	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.97	0.93	49.91	1
Tmp	Linc	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.86	0.54	112.49	1
Sal	Linc	0.01	0.01	0.98	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.93	0.36	213.15	1
Sal	Ery	0.00	0.00	0.00	0.00	0.00	0.19	0.62	0.18	0.00	0.00	0.00	0.94	0.84	83.42	1
Sal	Dox	0.00	0.03	0.23	0.00	0.73	0.00	0.00	0.00	0.00	0.00	0.00	0.85	***	182.81	1
Sal	Cm	0.01	0.00	0.00	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.96	0.59	205.36	1
Ofl	Linc	0.00	0.00	0.00	0.00	0.00	0.01	0.65	0.34	0.00	0.00	0.00	0.97	0.80	205.09	1
Ery	Tmp	0.00	0.00	0.00	0.00	0.00	0.01	0.73	0.26	0.00	0.00	0.00	0.98	0.92	118.46	1
Ery	Linc	0.00	0.00	0.00	0.00	0.00	0.02	0.38	0.60	0.00	0.00	0.00	0.94	0.41	217.96	1
Dox	Linc	0.00	0.00	0.00	0.00	0.00	0.04	0.69	0.28	0.00	0.00	0.00	0.98	0.86	180.05	1
Ery	Dox	0.00	0.00	0.00	0.00	0.00	0.04	0.29	0.11	0.21	0.36	0.00	0.98	0.98	8.76	0.98
Ery	Cm	0.01	0.02	0.50	0.00	0.48	0.00	0.00	0.00	0.00	0.00	0.00	0.96	0.94	37.32	1
Dox	Cm	0.01	0.30	0.10	0.24	0.35	0.00	0.00	0.00	0.00	0.00	0.00	0.99	0.96	120.68	1
Dox	Tmp	0.01	0.05	0.95	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.87	0.78	43.89	1
Dox	Ofl	0.00	0.72	0.24	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.96	0.81	167.44	1
Cm	Linc	0.00	0.00	0.99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.96	0.86	116.32	1
Sal	Cip	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.11	0.32	0.24	0.00	0.98	0.08	368.19	1

The Akaike weights (columns 3-13) for the model (Equation 1) with each of the drug-drug coupling models in Table S4 are given for 20 different two-drug combinations. The column R2 is the coefficient of determination (R^2) for the Akaike best model from Table S4. The column R2 Resp Surf is the corresponding R^2 value for the popular response surface model from pharmacology¹³. In the response surface model, which is based on Loewe additivity, the single drug responses are Hill-like functions and the coupling between drugs is captured by a single additive term with an interaction parameter typically denoted by α (see Equation 5 in reference ¹³). For both our model (Equation 1) and the response surface model, the parameters of the single drug dose-response surfaces are fit first, followed by an estimate of the interaction parameter(s). AIC Diff gives the AIC difference between the response surface model and the model (Equation 1) with Akaike-best coupling, and the last column (AIC weight) gives the Akaike weight in favor of Equation 1 (against the response surface model).

D. Scaling Relations and Estimation of Model Parameters for Drug-Resistant Mutants Because resistant mutants represent a perturbation of the wild-type phenotype, we hypothesized that mutant toxicity functions and coupling functions were re-scalable to wild type toxicity and coupling functions under the following one-parameter transformations

 $D_1 \rightarrow D_1' = a_1 D_1$ $D_2 \rightarrow D_2' = a_2 D_2$ $C \rightarrow C' = a_2 C$

where a_1 , a_2 , and a_3 are scaling parameters describing the increase/decrease in resistance to drug 1, the increase/decrease in resistance to drug 2, and the increase/decrease in drug-drug coupling at a given drug toxicity, respectively in the resistant mutant. Note that contributions to the growth response g_{12} from the coupling function $C(D_1)$ become negligible as D_1 increases because the toxicity of D_1 dominates the response.

III. Mechanistic Models and Biochemical Simulations

Throughout this study, we model the interactions between drugs by assuming that the presence of one drug increases or decreases the effective concentration of the other drug (Equation 1, main text). In the following sections, we describe two well-studied examples where this functional form naturally arises from mechanistic considerations (sections A and B). Finally, we demonstrate that the model also describes more complex models of biochemical reaction networks (sections C-D). We note that Figure panels S2B-E (and previous work²¹) suggest a

relationship between underlying network topology and the drug-drug coupling function and therefore provide a possible intuitive basis for our scaling results.

A. Multiple Antibiotic Resistance System

In the case of two drugs interacting through the multiple antibiotic resistance (MAR) operon²², an efflux-based system of intrinsic bacterial resistance, the effects of the drugs are well-described by a model of the form Equation 1 over the entire two-dimensional space of dosage combinations¹⁸. In this specific system, $C(D_1)$ is a binding function whose form is determined entirely by the (independently measured) *mar* promoter activity¹⁸. In fact, the Akaike best ad-hoc coupling function $C(D_1)$ fit directly from the growth data—without any prior knowledge of mechanism—is functionally identical to the mar promoter activity induction curve (Figure S2B). Notably, the functional form of $C(D_1)$ also describes the effects of mutations that increase or decrease efflux pumping¹⁸, and only the magnitude (maximum value) of $C(D_1)$ is changed. In this case, this magnitude reflects the microscopic factors such as efflux dynamics and substrate specificity that determine the rate at which the second drug is removed from the cell¹⁸.

B. Inhibitors of a Single Enzymatic Reaction

A model of the form Equation 1 also arises in perhaps the simplest possible biochemical scenario: that of two linear inhibitors of a single enzymatic reaction. Following ¹⁹, we consider the case of two inhibitors, D₁ and D₂, and assume that parameters α and β describe how the binding of inhibitors D₁ and D₂, respectively, affect the affinity of the enzyme for its substrate, S (α >1 or β >1 hinders binding, α <1 or β <1 facilitates binding). In addition, the parameter γ describes how the binding of one inhibitor affects the binding of another (γ >1 hinders binding, γ <1 facilitates binding). Following typical equilibrium assumptions¹⁹, it is straightforward to show that the normalized reaction velocity, g₁₂, in the presence of the two inhibitors together is given by

$$g_{12} = g_1(D_1)g_2(D_{2,eff}) = g_1(D_{1,eff})g_2(D_2),$$
(S6)

where $g_i = (1+D_i/K_i)^{-1}$ is the normalized reaction velocity in the presence of inhibitor i, K_i is the concentration of drug at which velocity is half maximal, $D_{i,eff}=D_i(1+C(D_j))^{-1}$ and

$$C(D_i) = \frac{(\kappa - 1)D_j}{D_j + \kappa}$$
(S7)

with $\kappa = \gamma (1+S/\alpha K_s) (1+S/\beta K_s) (1+S/\alpha \beta K_s)^{-1} (1+S/K_s)^{-1}$ and K_s is the binding constant of enzyme to substrate. Note that changing the microscopic parameters S, α , β , and γ can alter the scale (in D_j) and the magnitude (κ -1) of C(D_j); however, the functional form S7 remains the same (a hyperbolic binding function). For $\kappa > 1$ the interaction between the drugs is antagonistic, and the presence of one drug decreases the effective concentration of the other. For $\kappa < 1$, the interaction is synergistic and the presence of one drug increases the effective concentration of the other. Note that this model includes both Bliss independence¹¹ ($\gamma = 1$ and either $\alpha = 1$ or $\beta = 1$) as well as Loewe additivity²³ ($\gamma \rightarrow \infty$), the two most commonly used null models for non-interacting drugs, as special cases.

We stress that drug interactions in this simple model can be described by an effective concentration reduction of one drug, and it therefore takes the form of Equation 1 (or S6).

Despite the inherent symmetry in the microscopic model, an asymmetric functional form like S6 naturally arises, and the symmetry allows one to choose either $D_{1,eff}$ or $D_{2,eff}$ as the coupling function. We find that some drug pairs targeting the same cellular process (e.g. translation by the ribosome), such as doxycycline and erythromycin or doxycycline and chloramphenicol, are in fact well-described by a drug-drug coupling function similar to S7 ($\kappa \sim 0.35$ or 0.65, respectively) that is approximately symmetric in the choice of drug for $D_{i,eff}$ (Figure S2C). Note, however, that in these cases, because the single drug response functions $g_i(D_i)$ are given by Hill-like functions with Hill coefficients $n_i>1$, one should be cautious applying a direct microscopic interpretation of κ in terms of S, α , β , and γ . Interestingly, however, the binding of chloramphenicol has been shown, in vitro, to increase the binding of erythromycin to ribosomes²⁴, consistent with this interpretation. Under in vivo conditions, these drug pairs are approximately kinetically equivalent^{25, 26} and exhibit Loewe additive isoboles. Therefore, the mathematical effect of finite κ in S7 is to offset the inherent curvature of the g_i functions and produce linear isoboles. Therefore, while the functional form S7 remains an excellent model of the drug interaction (Figure S2C), the model does not necessarily indicate that synergy arises from facilitated binding at the ribosome.

C. Inhibitors of Larger Enzymatic Networks

In addition to the MAR system and the case of single enzyme inhibitors, the model, Equation 1 (main text), can be used to describe mutual inhibition of more complex biochemical networks. To explore this question, we numerically simulated the effects of simultaneous inhibitors on the toy network model in ref.²¹. The network consists of two pathways (A and B) and includes common network motifs, including negative feedback, bypass, and parallel reactions (²¹ and Figure S2D). Drugs are taken to be competitive inhibitors of the enzymatic reactions defining the network. Indeed, we find that a model of the form Equation 1 provides an excellent description of the effects of mutual inhibitors in to all reactions in this network. Interestingly, we find that the inferred drug-drug coupling functions $D_{i,eff}/D_i$ (which, as a reminder, are related to $C(D_j)$ through $D_{i,eff}=D_i(1+C(D_j))^{-1}$), are specific to the underlying network architectures of the targeted reactions (S15). For example, inhibitors that target two necessary and parallel pathways (such as A and B) lead to monotonically decreasing D_{2eff} (Figure S2D, e.g. drug pairs 15 and 10, or 14 and 4), inhibitors targeting parallel but not simultaneously required pathways lead to monotonically increasing D_{2eff} (Figure S2D, e.g. drug pairs 5 and 2, or 5 and 3).

D. Non-monotonic Drug-Drug Coupling: Negative Feedback and Steep Dose Response

Interestingly, we find non-monotonic drug-drug coupling functions can arise in at least two situations: 1) the targeted nodes involve negative feedback among reactions, and 2) the individual drugs have steep dose response curves. In case 1, the specific type of negative feedback appears to play a role in the form of the drug-drug coupling (Figure S2E). In case 2, steep single-drug dose response profiles can lead to non-monotonic coupling functions, even when the response surface, overall, is constrained to be very simple, such as Loewe additive. In that case, the curvature of the coupling functions compensates for the sigmoidal shape of the dose response curves, so the growth isoboles remain straight lines. Previous work has shown that the measured responses to drug pairs can provide clues to underlying network architecture²¹; our results are consistent with these findings, with the effects of different architectures reflected in the functional form of $D_{i,eff}/D_i$.

IV. Additional Experimental Examples of Growth Surface Rescaling in Drug-Resistant Mutants

A. Scaling Examples in E. faecalis, E. coli, and Human Cancer Cells

Figure S4 provides additional examples of growth surface rescaling in resistant mutants from *E*. *faecalis*, *E. coli*, *S. cerevisiae*, and human cancer cells.

Given the three functions describing the wild type response to a given drug pair, a mutant's entire response surface was predicted based on simultaneous nonlinear least squared fitting of the parameters a_1 , a_2 , and a_3 to growth rate data (Figures 5, S4). For each mutant, we also computed the best fit for a_1 and a_2 when a_3 is set to 1 (that is, when the drug interaction does not change, as in ¹⁴). We then compared the 3-parameter model with the 2-parameter model using AIC. If the data did not support the addition of the third parameter (a_3) with a weight of at least 0.75, we set $a_3=1$ and used the simpler two-parameter model (see, for example, Figure S4C-E for Dap-Linz). In total, 34 of the 42 mutants in this work were best described (AIC weight > 0.75) by the three-parameter model (Figure S4O). As part of a larger statistical analysis in section V (below) we extend this analysis to cases where either a_1 or a_2 is set to 1, but we find these simplifications are not statistically valid to describe any of our experimental mutants.

B. Scaling in Drug with Itself Experiment

To demonstrate the self-consistency of the model, we also applied our scaling analysis to a classic mock experiment where a drug is combined with itself. Since the interaction of a drug with itself should be the same when the drugs are applied to either drug sensitive or drug-resistant cells, the interactions derived from the scaling analysis should also remain the same.

Consider, without loss of generality, the case where daptomycin is combined with itself in a mock 2-drug experiment in both drug sensitive and dapatomycin-resistant cells (Figure S5A-D). First, one should note that solely the single-drug dose response in wild type and mutant cells differ by a rescaling of the daptomycin concentration (Figure S5A, B; see also ¹⁴ for examples with other drugs). This elementary scaling property has emerged as a general feature of both our scaling results and previous work¹⁴.

Using this daptomycin response date, we constructed 2-d response surfaces for mock two-drug experiments in wild type and Dap-C mutants. The contours of constant growth in the space of drug concentrations are straight lines, which corresponds to Loewe additivity (as expected in a drug-with-itself experiment). Because the single-drug dose response curves are identical up to a rescaling of drug concentration, the wild type and mutant growth surfaces are also identical up to a rescaling of drug concentration.

Using these response surfaces, we inferred the drug-drug coupling functions for both wild-type and mutant cells (Figure S5C-D). We found that the magnitude of the interaction does not change from wild-type to mutant; specifically, the scaling parameter $a_3=1$ in this mock experiment.

In any drug with itself experiment, the specific shape of the coupling function will be determined by the steepness of the individual dose response curves. However, the steepness of the dose response curves does not change following mutation (Figure S5A-B), so the shape of the coupling functions will also not change following mutation (Figure S5C-D).

In general, the drug with itself experiment will always correspond to a rescaling where a_1 and a_2 are equal—because the increase in resistance is equal for the two identical drugs—and the interaction does not change ($a_3=1$). Therefore, our model reduces to the elementary scaling model of Chait et al¹⁴, independent of which drug is chosen for the mock combination. Our

general scaling model is therefore entirely self-consistent because the interaction scaling parameter $(a_3=1)$ indicates no new interactions in the mutant.

VI. Statistical Analysis and Null Models

A. Null Model Based on Random Response Surface Ensemble

To precisely evaluate the statistical significance of our results, we have developed a null model that allows us to quantify the probability of observing our scaling results merely by chance. First, we created a random ensemble of smooth and plausible response surfaces from the basis functions (g_1 , g_2 , and D_{2eff}/D_2) that we obtained experimentally. We shuffled these functions to create an ensemble of approximately 350 arbitrary drug-response surfaces. Specifically, to create each surface in the ensemble, we randomly selected the single drug response parameters K_i and n_i for each drug from the collection of experimentally-measured parameters from all wild-type cells. Then, we randomly choose a single coupling function D_{2eff}/D_2 from the set of measured drug-drug coupling functions from all wild-type cells. We compute each arbitrary drug-response surface using equation 2 (main text) with these three functions (g_1 and g_2 , based on the parameters K_i and n_i , and the coupling function D_{2eff}/D_2). Each surface in this random ensemble is a smooth two-dimensional surface with properties of a typical drug-response surface (maximum growth at the origin, zero growth at high drug concentrations). However, because the three basis functions are randomly selected, the corresponding response surfaces do not correspond to any surface initially measured in the study.

We used this ensemble of random drug-response surfaces to quantify the statistical significance of our experimental results. The goal is to measure how well we can scale an experimentally measured surface to match any random drug-response surface. First, we determine the best scaling parameters to reproduce the random surfaces using the experimental basis functions from wild type (drug sensitive) cells associated with each mutant in the study. This procedure is equivalent to considering each random surface as a mutant of an experimentally measured wild type drug-response surface. For example, for comparison with the *E. coli* mutants in Figure 3A, we attempted to scale the same basis functions (those of Cm-Cip in wild type *E.coli*) to fit each surface in the random ensemble. We then compared the scaling results we observed experimentally with those in the random ensemble.

To quantify the goodness-of-fit, we used two statistical quantities. The first quantity is the coefficient of determination, R^2 , which provides an empirical measure of how well the scaling captures the variance in the data. However, it is possible that a model can provide a high R^2 value but still lead to predictions that deviate systematically from the data. These deviations will manifest themselves as spatial deviations in residuals. In traditional fits of 1-d functions, there are various correlation metrics available to assess the magnitude of theses correlations. In our case, because the response surfaces are two-dimensional, we must account for spatial correlations on a 2-d grid. To do so, we use the Geary C, which is a well-studied quantity and is commonly used to measure 2-d spatial correlation (see, for example, 27). The Geary C, GC, is given by

$$GC = \frac{(N-1)\sum_{i}\sum_{j}w_{ij}(X_{i} - X_{j})^{2}}{2W\sum_{i}(X_{i} - \overline{X})^{2}} ,$$

where N is the total number of observations, w_{ij} is a matrix of spatial weights that determines the

spatial connection between data points i and j, X_i is the variable of interest (in this case, residuals), overbar denotes an average, and W is the sum of all entries of w_{ij} . We take the weight matrix to be inversely related to the so-called "city block" distance measure. This matrix reports the distance between points on a grid in terms of total horizontal and vertical deviation. For example, position (1,1) on a 2-d grid is a distance of 1 from positions (1,2) and (2,1), and a distance of 2 from position (2,2).

GC ranges from 0 to 2, with GC=1 indicating no spatial correlation, and deviations from 1 indicating positive or negative correlation. To convert this number to a "goodness-of-fit" with a range similar to R^2 , we define

$$Z = 1 - |1 - GC|$$

Z ranges from 0 to 1, with 1 indicating no spatial correlation in residuals and 0 indicating strong spatial correlation / anti-correlation of residuals. We take Z as a qualitative measure of goodness of fit, similar to R^2 . Statistical quantities based on other spatial correlation, such as Morran's I, yield qualitatively similar results, but we choose this one because it is more sensitive to local spatial correlations, which seems most appropriate for a goodness-of-fit test of residuals.

We compare goodness-of-fit using R^2 or Z using either artificial mutants taken from a random ensemble of smooth surfaces or experimentally measured drug-resistant mutant from our study. From this procedure, we calculate for each mutant the probability p of observing, for a randomly selected surface, a fit equal to or better than what we found experimentally in our mutants. Hence, the quantity p is similar to a p value and describes the likelihood of observing our experimental scaling results by chance. Please note that because some basis functions from different drug pairs are similar, some surfaces in the ensemble can be similar to those of the resistant mutant. The surfaces in the ensemble also do not include any noise. Therefore, this p value is a conservative estimate because it may overestimate the probability p for fitting a truly random surface.

Figure S5E shows the value of p calculated using R^2 (closed symbols) or Z (open symbols). The majority of mutants in the study correspond to experimental results that are highly unlikely (p <<1) from chance alone. A small number of mutants correspond to scaling results that, while providing good quantitative descriptions of the observed experiments ($R^2>0.8$), are more likely to arise purely by chance (p~0.75 when using R^2 or Z alone). In these cases, we can only conclude that scaling the wild type basis functions provides an excellent description of the surface; we cannot say whether this agreement arises by chance or because our scaling hypothesis is correct. Nevertheless, when the data set is taken as a whole, with each mutant considered independent, the global p value is extraordinarily small (p<<10⁻¹⁰).

To further explore the previous points, we quantified each wild type basis set in our study according to how readily it can be scaled to fit an arbitrary surface (Figures S5F). To do so, we attempted to scale each basis set to fit all surfaces in the random ensemble. We then calculated the goodness-of-fit using R^2 for each surface in the ensemble. Figure S5F show the cumulative distribution functions for R^2 from these ensembles. Each curve corresponds to a single basis set. We find that some basis sets are highly "permissive"; that is, they can be scaled to provide a good fit to many different surfaces. For example, the basis set from Dox-Ery in *E.coli* (light blue circles, panel A) can provide fits with high R^2 values for many of the surfaces in the ensemble ($R^2 > 0.8$ for approximately 60% of the surfaces). This permissivity therefore underlies the high p value for the Dox-Ery scaling results in panel S5E. Interestingly, such highly permissive basis sets may prove useful as approximations to generic response surfaces when high degrees of accuracy are not required. By contrast, other basis sets are highly specific to the drug pairs and/or

cell lines used and are unlikely to provide a good fit to arbitrary surfaces. In these cases, such as the Cm-Cip basis, the scaling results we observe are highly unlikely to arise by chance. Qualitatively similar results are obtained when we use Z instead of R^2 .

B. AIC-Based Scaling of Mutants to determine Uniqueness of Basis Functions

Mutant	Cm- Tmp	Cm- Cip	Sal- Ery	Sal- Cm	Ery- Linc	Dox- Ofl
kwcmcipseq1			,			
(cm-cip 1)	0.00	1.00	0.00	0.00	0.00	0.00
kwcmcipseq2						
(cm-cip 2)	0.00	1.00	0.00	0.00	0.00	0.00
kwcmcipseq3						
(cm-cip 3)	0.00	1.00	0.00	0.00	0.00	0.00
kwcmcipseq4						
(cm-cip 4)	0.00	1.00	0.00	0.00	0.00	0.00
kwcmcip4						
(cm-cip 5)	0.00	1.00	0.00	0.00	0.00	0.00
kwcmcip5						
(cm-cip 6)	0.00	1.00	0.00	0.00	0.00	0.00
kwcmcip6						
(cm-cip 7)	0.00	1.00	0.00	0.00	0.00	0.00
kwcmcip7						
(cm-cip 8)	0.00	1.00	0.00	0.00	0.00	0.00
kwcmcip8						
(cm-cip 9)	0.00	1.00	0.00	0.00	0.00	0.00
kwcmcip9						
(cm-cip 10)	0.00	1.00	0.00	0.00	0.00	0.00
kwcmcip10						
(cm-cip 11)	0.31	0.01	0.59	0.00	0.10	0.00
kwcmcip11						
(cm-cip 12)	0.00	1.00	0.00	0.00	0.00	0.00
kwcmcip12						
(cm-cip 13)	0.00	1.00	0.00	0.00	0.00	0.00
kwcmcip13						
(cm-cip 14)	0.00	0.29	0.00	0.00	0.00	0.71
kwcmcip14						
(cm-cip 15)	0.00	0.11	0.00	0.00	0.00	0.89
kwemeip15	0.00		0.00	0.00	0.00	0.40
(cm-cip 16)	0.00	0.87	0.00	0.00	0.00	0.13
kwcmcipseq5	0.00	4.00	0.00	0.00	0.00	0.00
(cm-cip 17)	0.00	1.00	0.00	0.00	0.00	0.00
kwcmcipseq6	0.00	4.00	0.00	0.00	0.00	0.00
(cm-cip 18)	0.00	1.00	0.00	0.00	0.00	0.00

Table S6: Scaling Mutants to determine the Uniqueness of Basis Functions

Table S6: The table provides Akaike weights for models of the two-drug response of E. coli mutants (Table S3). For each mutant, the two-drug response was measured for the combination

of Cm-Cip. The WT basis functions from six representative drug pairs (including Cm-Cip) were then rescaled using scaling parameters a_i to provide the best fit for the mutant response. For all 18 mutants, the Akaike weight for the "true" basis functions (those associated with the drug pair Cm-Cip) was nonzero (> 0.01). For 15 of the 18 mutants, the WT basis corresponding to Cm-Cip was associated with a high Akaike weight (>0.87), indicating that the basis functions from other drug pairs do not provide an equally valid model for the data. In 2 of the remaining mutants, there is some evidence (nonzero Akaike weight) that WT basis functions for Dox-Ofl, a mechanistically similar drug pair, could provide the best model for the data; this is not surprising, since the toxicity functions and coupling functions for this drug pair are qualitatively similar to those for Cm-Cip. In the final case (kwcmcip10), the Cm-Cip basis has non-zero weight, but other drug pairs with similar, though not identical, basis functions provide the best model. This data demonstrates that all sets of basis functions are not equally valid models and the basis functions cannot be used interchangeably to describe an arbitrary response surface.

C. Comparison with Interpolation

Our scaling results allow us to estimate the full dose response surface of a drug-resistant mutant using only a small number of data points. To determine the improvement scaling provides over conventional fitting techniques, we compared the results of our scaling model to a standard numerical interpolation (Figure S5G-J). For four representative organisms, we estimated the full response surface using 4 data points and either 1) scaling or 2) numerical interpolation. For the latter, we assumed that growth = 1 at the origin and growth=0 at high drug concentrations corresponding to $(D_{1},D_{2})=1.5*(D_{1,max}, D_{2,max}), (D_{1},D_{2})=1.5*(D_{1,max}, 0), (D_{1},D_{2})=1.5*(0, D_{2,max}),$ where $D_{1,max}$ ($D_{2,max}$) is the highest concentration of drug 1 (2) used in the experiment. Note that these limits high and low growth limits are naturally satisfied in the scaling model.

To estimate the growth surface, we chose the following 4 data points on each surface: $(D_1,D_2)=(1/2)*(0, D_{2,max}), (D_1,D_2)=(1/2)*(D_{1,max},0), (D_1,D_2)=(1/2)*(D_{1,max}, D_{2,max}),$ and finally, the data point corresponding to the second highest concentration of each drug. We therefore have one data point from the single drug dose response curves and two data points with nonzero concentrations of both drugs that falls roughly on the diagonal through the space of drugs. Interpolation is performed using interpolation implemented with Matlab's griddata function (v4 algorithm), which estimates the 2-d surface from samples that need not be evenly spaced on the spatial grid. The interpolation is required to match the data at the sampled points.

Figure S5G-J shows that the scaling method always performs as well or better than the interpolation when there are a small number of data points. In most cases, interpolation requires approximately an order of magnitude more data points to achieve fits of similar quality (Figure S5H-J). However, in one case (Figure S5G), the interpolation provides a good approximation with roughly the same number of data points. We note that the scaling method typically outperforms interpolation when data points are randomly chosen, and as expected, the interpolation performs much worse when points are more clustered in the space. Histograms in figure 5 (main text) show the distribution of fit qualities with the scaling method for randomly sampled data points.

D. Sequential Fitting of Scaling Parameters

In addition to simultaneously fitting a_1 , a_2 , and a_3 for each mutant, we repeated the scaling analysis by first fitting the parameters a_1 , a_2 using single drug response data and then fitting a_3 using only the two drug response data. The fit quality is only very slightly reduced using this approach, providing evidence that the simultaneous fit of all 3 parameters does not suffer from overfitting (see Figure S5K). The right panels in figure S5K show the two examples from the main panel where R^2 is most dramatically reduced by using sequential fitting; even in these cases, the change in fit quality is modest (change in R^2 is less than 0.09).

In addition, we tested whether all 3 parameters are statistically justified for each mutant. Specifically, for each mutant we attempted to scale the wild type basis functions in four ways:

- 1. By using all 3 scaling parameters (a_1, a_2, a_3)
- 2. By using only (a₁, a₂) (drug interaction does not change)
- 3. By using only (a₁, a₃) (resistance to drug 2 does not change)
- 4. By using only (a₂, a₃) (resistance to drug 1 does not change)

We then compared the four cases (1-4) using standard AIC-based model selection techniques. We found that there are a small number of cases where scaling 2 provides the best model (see Table S7). By contrast, we found no examples where scaling 3 or 4 provide the best model. There are, indeed, cases where models 3 and 4 are good models by empirical measures ($R^2 > 0.9$ for the (a_1 , a_3) fit in E coli k01.48 Cm-Cip 1, for example). However, models 3 and 4 are never chosen to be the AIC superior model from the set.

		Parameters	to Fit	-
Mutant	(a1,a2,a3)	(a1,a2)	(a1,a3)	(a2,a3)
E.coli k01.48 Cm-Cip 1	1.00	0.00	0.00	0.00
E. coli k01.48 Cm-Cip 2	1.00	0.00	0.00	0.00
E. coli BW25113 Cm-Cip 1	1.00	0.00	0.00	0.00
E. coli BW25113 Cm-Cip 2	1.00	0.00	0.00	0.00
E. coli BW25113 Cm-Cip 3	1.00	0.00	0.00	0.00
E. coli BW25113 Cm-Cip 4	1.00	0.00	0.00	0.00
E. coli BW25113 Cm-Cip 5	0.96	0.04	0.00	0.00
E. coli BW25113 Cm-Cip 6	0.90	0.10	0.00	0.00
E. coli BW25113 Cm-Cip 7	0.93	0.07	0.00	0.00
E. coli BW25113 Cm-Cip 8	0.84	0.16	0.00	0.00
E. coli BW25113 Cm-Cip 9	1.00	0.00	0.00	0.00
E. coli BW25113 Cm-Cip				
10	1.00	0.00	0.00	0.00
E. coli BW25113 Cm-Cip				
11	0.54	0.46	0.00	0.00
E. coli BW25113 Cm-Cip				
12	1.00	0.00	0.00	0.00
E. coli BW25113 Cm-Cip				
13	0.82	0.18	0.00	0.00
E. coli BW25113 Cm-Cip				
14	1.00	0.00	0.00	0.00
E. coli BW25113 Cm-Cip				
15	1.00	0.00	0.00	0.00
E. coli BW25113 Cm-Cip				
16	1.00	0.00	0.00	0.00
E. coli BW25113 Cm-Cip				
17	0.89	0.11	0.00	0.00
E. coli BW25113 Cm-Cip	1.00	0.00	0.00	0.00

Table S7: AIC Weights for 3-parameter and 2-parameter scalings

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E. faecalis DapA Amp-Dap	0.93	0.07	0.00	0.00
E. faecalis DapB Amp-Dap	0.22	0.78	0.00	0.00
E. faecalis DapC Amp-Dap	1.00	0.00	0.00	0.00
E. faecalis DapA Linz-Dap	0.33	0.67	0.00	0.00
E. faecalis DapB Linz-Dap	0.75	0.25	0.00	0.00
E. faecalis DapC Linz-Dap	0.39	0.61	0.00	0.00
S. aureus Newman Cm-Nor				
1	0.44	0.56	0.00	0.00
S. aureus Newman Cm-Nor				
2	0.43	0.57	0.00	0.00
E. coli BW25113 Sal-Cm 1	1.00	0.00	0.00	0.00
E. coli BW25113 Sal-Cm 2	1.00	0.00	0.00	0.00
E. coli BW25113 Sal-Cm 3	1.00	0.00	0.00	0.00
E. coli BW25113 Sal-Cm 4	1.00	0.00	0.00	0.00
E. coli BW25113 Dox-Ery 1	0.97	0.03	0.00	0.00
E. coli BW25113 Dox-Ery 2	1.00	0.00	0.00	0.00
E. coli BW25113 Dox-Ery 3	0.33	0.67	0.00	0.00
Melanoma PLX – Dacarb	0.30	0.70	0.00	0.00
NSCLC 17AAG-Gef	1.00	0.00	0.00	0.00
NSCLC Gef-Pac	1.00	0.00	0.00	0.00

Table S7: AIC weights in favor of scaling models with all 3 parameters (1^{st} column) and with only two parameters (columns 2-4) for each mutant.

VII. Supplemental Figures and Figure Captions

Figure S1: Resistance events can alter Bliss- or Loewe-based interactions between drugs in prokaryotic and eukaryotic cells

Similar to Figure 1, heat maps quantify the drug interaction and classify it as synergistic or antagonistic across a range of active concentrations for both wild type and mutant cells. Full growth surfaces are shown below each plot. To quantify the drug interaction at each point on the response surface, we use the interaction parameter I based on Loewe additivity, which is positive (blue) for antagonistic, negative (red) for synergistic interactions, and 0 when there is no interaction (Loewe additivity). In addition to modifying the resistance of cells to one or more drugs, resistance events can also modify the interactions between drug pairs. A. Gefitinib resistance in non-small cell lung cancer (NSCLC). B. Chloramphenicol resistance in E. coli. C. Chloramphenicol and ciprofloxacin resistance in E. coli. D. Daptomycin resistance in E. faecalis. E. Erythromycin and doxycycline resistance in E. coli. F. Norfloxacin resistance in S. aureus.

Figure S2: Comparison with other response surface models and application to well-studied systems and network models.

A. The model from Equation 1 (blue) with AIC-best drug-drug coupling function is compared to the classic response surface model¹³ (red) for different drug pairs. In the response surface model, which is based on Loewe additivity, the single drug responses are Hill-like functions and the coupling between drugs is captured by a single additive term with an interaction parameter

typically denoted by a (see Equation 5 in reference ¹³). For both our model (Equation 1) and the response surface model, the parameters of the single drug dose-response surfaces are fit first, followed by an estimate of the interaction parameter(s). In some cases (e.g. dox-ery, row 4, column 2), both models provide excellent descriptions of the data, though in other cases (e.g. cm-ofl, row 1, column 2), Equation 1 outperforms the response surface model. In each case, Equation 1 provides a better statistical model of the data according to model selection (Table S5). Drug combinations are, from left to right in each row, Cm-Tmp, Cm-Ofl, Cm-Cip, Tmp-Ofl (row 1); Sal-Ofl, Sal-Linc, Sal-Ery, Sal-Dox (row 2); Sal-Cm, Linc-Ofl, Tmp-Ery, Linc-Ery (row 3); Dox-Linc, Dox-Ery, Ery-Cm, Dox-Cm (row 4); Dox-Tmp, Dox-Ofl, Cm-Linc, Sal-Cip (row 4).

B. The function $C(D_1)$ (blue line) is proportional to mar activity (red squares) when D_1 is salicylate and D_2 is tetracycline. Mar promoter activity (relative to untreated wild type cells) was measured in E. coli Frag-1B strain with PZS*2 MAR-YFP plasmid¹⁷. Promoter activity was determined by first correcting raw YFP fluorescence to account for changing background fluorescence as cells grow. Mar promoter activity was taken to be the background-corrected fluorescence concentration (fluorescence/absorbance), averaged over steady state, times the growth rate k.

C. Two examples of approximately symmetric drug interactions (Loewe additive).

D. Drug-drug coupling functions are shown for drug pairs targeting two reactions in the toy biochemical network (upper right, ²⁰). For parameter values and simulation details, see ²⁰. For each coupling function, the presence of drug i alters the effective concentration of drug j. Green curves: drug associated to a given row is drug i. Blue curves: drug associated with a given column is drug i; red curve: either drug (from row or column) can be chosen as drug i. For example, in row 4, column two, the curve is blue, which indicates that the presence of drug 2 increases the effective concentration of drug 4.

E. Non-monotonic coupling functions can arise when there is negative feedback, but the specific form depends on the specific architecture of the feedback loop. Top, model from ²⁰ (see Figure S15, S16). Bottom, negative feedback model from Figure 5 in ¹².

Figure S3: Example Growth Curves and Isobole Rescaling

A. Absorbance time series for a representative E. coli mutant, resistant to both ciprofloxacin and chloramphenicol, exposed to various concentrations of both drugs (see Figure 3). Green lines: exponential fits whose slopes are the estimated growth rates. Drug concentration increases linearly along each axis.

B. Full heat map of relative growth rates corresponding to the growth curves in A.

C. Contours of constant growth (isoboles) for S. aureus in drug sensitive (wild type) and drug resistant cells exposed to chloramphenicol and norfloxacin can be collapsed onto one another by a simple rescaling of the two drug concentrations, indicating that the morphology of the response surface, and the drug interaction, is the same in both strains (top panels). Similar results have been shown for other drug pairs in E. coli¹⁶. By contrast, growth isoboles for drug sensitive non-small cell lung cancer (NSCLC) cells (bottom panels) exposed to Gefitinib and 17-AAG have different curvatures (left) and cannot be collapsed onto one curve by a simple rescaling of drug concentrations (right). Instead, the drugs interact suppressively in the wild type and synergistically in the mutant.

Figure S4: Rescaled wild type basis functions describe resistant mutants from multiple organisms

Basis functions extracted from the responses of drug sensitive cells can be rescaled to describe the response of drug resistant mutants to the same drug pairs. Scaling parameters (a_1, a_2, a_3) and R^2 values are given below.

Panels A-E, daptomycin-resistant mutants (Dap A, Dap B, Dap C)⁹ exposed to daptomycin and either ampicillin or linezolid. For amp-dap combinations, a_1 is associated with daptomycin and a_2 with ampicillin. For dap-linez combinations, a_1 is associated with linezolid and a_2 with daptomycin.. A. $(a_1, a_2, a_3) = (0.0078 \pm 0.0004, 1.40 \pm 0.02, 0.70 \pm 0.06)$; $R^2 = 0.93$. B. $(a_1, a_2, a_3) = (0.015 \pm 0.0007, 2.26 \pm 0.04, 0.88 \pm 0.03)$; $R^2 = 0.96$. In both DapA and DapB mutants, the cell are strongly resistant to daptomycin $(a_1 <<1)$, slightly sensitized to ampicillin $(a_2>1)$, and the synergy between drugs has decreased $(a_3<1)$. C. $(a_1, a_2) = (1.27 \pm 0.03, 0.0032 \pm 0.0004)$; $R^2 = 0.95$; Additional parameter a_3 not supported by data (see description of model selection, above). D. $(a_1,a_2) = (0.82 \pm 0.03, 0.012 \pm 0.0007)$; $R^2 = 0.90$; Additional parameter a_3 not required (see description of model selection, above). E. $(a_1, a_2) = (0.73 \pm 0.01, 0.002 \pm 0.0001)$; $R^2 = 0.96$; Additional parameter a_3 not required. DapA mutants show slightly dereased resistance to linezolid $(a_1>1)$, while DapB and DapC mutants show slightly increased resistance to linezolid $(a_1<1)$.

Panels F-K, Fluoroquinolone-resistant E. coli exposed to chloramphenicol-ciprofloxacin (F-G), mutant BW25113 E. coli strains exposed to salicylate-chloramphenicol (H-I) and to doxycycline-erythromycin (J-K). Mutants in F-G are fluoroquinolone-resistant clinical isolates⁷, mutants in H-I are mutants kwcmcip1 and kwcmcip2 (Table S3), and mutants in J-K were selected by growing BW25113 cells for 60 generation in [Dox]=0.3 and [Ery]=15 ug/mL (J) or [Dox]=0.15 and [Ery]=30 ug/mL (K). Scaling parameters (a_1 , a_2 , a_3) and R^2 values are: F. (a_1 , a_2 , a_3)=(1.09±0.01, 0.05±0.0004, 0.61±0.03); R^2 =0.98. G. (a_1 , a_2 , a_3)=(1.14±0.02, 0.10±0.0009, 0.49±0.04); R^2 =0.968. H. (a_1 , a_2 , a_3)=(0.98±0.01, 0.23±0.005, 0.003±0.02); R^2 =0.99. I. (a_1 , a_2 , a_3)=(0.98±0.02, 0.25±0.005, -0.11±0.02); R^2 =0.98. J. (a_1 , a_2 , a_3)=(0.15±0.009, 0.17±0.007, 1.92±0.09); R^2 =0.87. K. (a_1 , a_2 , a_3)=(0.22±0.009, 0.25±0.009, 1.63±0.06); R^2 =0.93. In F-G, resistance to chloramphenicol is unchanged ($a_1 \sim 1$) but antagonism between the drugs has decreased ($a_3 < 1$). In H-I, resistance to chloramphenicol is increased ($a_2 < 1$) and the antagonism has dramatically decreased ($a_3 < 0$). In J-K, resistance to both drugs has increased and the synergy between drugs has increased ($a_3 > 1$).

Panel L, cycloheximide-resistant S. cerevisiae exposed to cycloheximide and 5-fluoroscytosine; $(a_1, a_2, a_3) = (0.9 \pm 0.05, 0.0043 \pm 0.0002, -0.22 \pm 0.02).$

Panels M-N, PLX4720-resistant A375 melanoma cells exposed to PLX-Dacarb and Gefitinibresistant GR6 cells exposed to gefitinib-paclitaxel. M. $(a_1, a_2) = (2.02\pm0.14, 0.06\pm0.004)$; $R^2 = 0.91$; parameter a_3 not needed (see section on model selection methods). We found that PLX4720, an inhibitor of the oncogene B-RAF which was recently shown to elicit dramatic clinical responses followed by the emergence of therapeutic resistance²⁶ and dacarbazine, a standard-of-care alkylating agent, act antagonistically on A375 melanoma cells. To explore effects of resistance on the two-drug response surface, we engineered PLX4720-resistant A375 cells by stably overexpressing the kinase C-RAF, which can confer resistance to PLX4720 by overriding B-RAF dependence.³ In these resistant cells, the resistance to dacarbazine has decreased $(a_1 > 1)$, the resistance to PLX4720 has increased dramatically $(a_2 << 1)$, and the coupling between drugs has slightly decreased, but not in a statistically significant way. Interestingly, in this case, one can describe the mutant isoboles by a simple rescaling of the wildtype contours, indicating that the drug interaction does not change in a significant way in the mutant. N. $(a_1, a_2, a_3) = (1.26 \pm 0.03, 0.01 \pm 0.0009, 4.68 \pm 0.6); R^2 = 0.8$. Gefitinib-resistant (GR6) cells show slightly increased sensitivity to paclitaxel $(a_1 > 1)$, increased resistance to gefitinib $(a_2 << 1)$, and a strong increase in the antagonism between the drugs $(a_3 > 1)$. See SI Section I for estimation of experimental error bars.

Panel O, the scaling parameter a_3 is greater than 1 when the magnitude of the drug interaction has increased in the mutant, less than 1 but greater than 0 when the magnitude of the drug interaction has decreased in the mutant, and less than 0 when the interaction has changed from synergistic to antagonistic or vice versa. Circles: E coli (blue, k01.48 mutants exposed to Cm-Cip; red, BW25113 mutants exposed to Cm-Cip; black, BW25113 mutants exposed to Sal-Cm; light blue, BW25113 mutants exposed to Dox-Ery). Squares: E. faecalis (blue, V583 mutants exposed to Linez-Dap; red, V583 mutants exposed to Dap-Amp). Upper triangles: S. aureus (blue, nor-R mutants exposed to Cm-Nor; red, nor-R mutants exposed to Cm-Cip Right triangles, cancer cells (blue: melanoma exposed to PLX-Dacarb; red: lung cancer exposed to Gef-17-AAG; black, lung cancer exposed to Gef-Pac).

Figure S5: Statistical controls: drug with itself, null model, comparison with interpolation, sequential fitting of parameters

A. Single drug dose-response functions for daptomycin in drug sensitive and drug resistant mutants.

B. Dose-response functions in panel A rescaled by the MIC, defined as the concentration at which the inhibition is 50%. Solid curve, Hill-like function with n=1.15, K=1. Response surfaces for drug-with-itself experiments are generated as Loewe additive combinations of two drugs (both daptomycin) characterized by single drug dose-response functions with parameters K, n estimated from data (panel A).

C. Drug-drug coupling function for wild-type (black) and Dap-C (cyan) response surfaces.

D. Drug-drug coupling functions following rescaling of daptomycin concentration by MIC. No scaling of the interaction magnitude is required $(a_3=1)$.

E. Probability p of observing a fit equal to or better than experiment in a random ensemble of surfaces. Fits are quantified using coefficient of determination, R²(closed symbols) or Z (open symbols). Each point represents a single mutant. Circles: E coli (blue, k01.48 mutants exposed to Cm-Cip; red, BW25113 mutants exposed to Cm-Cip; black, BW25113 mutants exposed to Sal-Cm; light blue, BW25113 mutants exposed to Dox-Ery). Squares: E. faecalis (blue, V583 mutants exposed to Linez-Dap; red, V583 mutants exposed to Dap-Amp). Upper triangles: S. aureus (blue, nor-R mutants exposed to Cm-Nor; red, nor-R mutants exposed to Cm-Cip). Right triangles, cancer cells (blue: melanoma exposed to PLX-Dacarb; red: non small cell lung cancer exposed to Gef-17-AAG; black, lung cancer exposed to Gef-Pac; light blue, cancer stem cells exposed to etoposide and PLX4720). Inset, histogram of all mutants for the R² measure.

F. Cumulative distribution functions of R^2 for each wild type basis set used in the study. Markers are the same as in panel E. For comparison, the open blue circles represent the cumulative distribution function (CDF) of R^2 for our entire experimental data set.

G-J. Comparison of scaling method (stars) and interpolation (circles or squares) using

coefficient of determination (R^2 , red) and root mean squared error (RMSE, blue) to quantify goodness of fit. Scaling is performed using exactly four data points, as described in Section V. Interpolation is performed using the same four data points in addition to N randomly chosen data points. N ranges from 0 to 76. R^2 and RMSE are means over 50 independent trials, with each trial corresponding to a particular choice of N additional data points used for interpolation. Error bars are +/- standard error of the mean.

K. Main panel: R^2 from scaling that fits (a_1, a_2, a_3) simultaneously (solid symbols) and from scaling that first fits (a_1, a_2) and then fits a_3 . Each point represents a single mutant. Circles: E coli (blue, k01.48 mutants exposed to Cm-Cip; red, BW25113 mutants exposed to Cm-Cip; black, BW25113 mutants exposed to Sal-Cm; light blue, BW25113 mutants exposed to Dox-Ery). Squares: E. faecalis (blue, V583 mutants exposed to Linez-Dap; red, V583 mutants exposed to Dap-Amp). Upper triangles: S. aureus (blue, nor-R mutants exposed to Cm-Nor; red, nor-R mutants exposed to Cm-Cip). Right triangles, cancer cells (blue: melanoma exposed to PLX-Dacarb; red: non small cell lung cancer exposed to Gef-17-AAG; black, lung cancer exposed to Gef-Pac; Right panels, examples where R^2 is most decreased by sequential fits (E. coli with Dox-Ery, top; lung cancer with Gef-Pac, bottom).



Е





Figure S3





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