Supplementary Information for

Metrics other than potency reveal systematic variation in responses to cancer drugs

Mohammad Fallahi-Sichani[1], Saman Honarnejad[1], Laura M. Heiser[2], Joe W. Gray[2] and Peter K. Sorger[1]

[1]HMS LINCS Center, Department of Systems Biology, Harvard Medical School, Boston, MA 02115

[2]Department of Biomedical Engineering, Oregon Health and Science University, Portland, OR 97239

Address correspondence to:

Peter K. Sorger , Email: peter_sorger@hms.harvard.edu

Supplementary Results

Nature Chemical Biology: doi:10.1038/nchembio.1337

Supplementary Table 1: List of anti-cancer drugs used in multi-parametric dose-response analysis and their nominal targets or mechanisms of action

Drug	Nominal target	Drug class in similarity score analysis
Sigma AKT1-2 inhibitor	AKT	AKT inhibitor
Triciribine	AKT	
GSK1070916	AURK	AURK inhibitor
VX-680	AURK/FLT3/ABL/JAK2	
Olomoucine II	CDK1	CDK1 inhibitor
NU6102	CDK1/2	
Carboplatin	DNA cross-linker	DNA cross-linking
Cisplatin	DNA cross-linker	
Oxaliplatin	DNA cross-linker	
AG1478	EGFR	EGFR inhibitor
Erlotinib	EGFR	
Gefitinib	EGFR	
LBH589	HDAC	HDAC inhibitor
Oxamflatin	HDAC	
Vorinostat	HDAC	
Trichostatin A	HDAC	
Valproic acid	HDAC	
Geldanamycin	HSP90	HSP90 inhibitor
17-AAG	HSP90	
Everolimus	MTOR	mTOR inhibitor
PP242 hydrate	MTOR	
Temsirolimus	MTOR	
GSK1059615	PI3K	PI3K inhibitor
GSK2119563A	PI3K	
GSK2126458A	PI3K	
CGC-11047	Polyamine analogue	Polyamine analogue
CGC-11144	Polyamine analogue	
Bortezomib	Proteasome	Proteasome inhibitor
MG-132	Proteasome	
Z-Leu-Leu-Leu-al	Proteasome	
Z-Leu-Leu-Norvalinal	Proteasome	
CPT-11	TOP1	TOP1 inhibitor

Nature Chemical Biology: doi:10.1038/nchembio.1337

Topotecan	TOP1	
Doxorubicin	TOP2	TOP2 inhibitor
Epirubicin	TOP2	
Etoposide	TOP2	
Docetaxel	TUBB	TUBB inhibitor
Paclitaxel	TUBB	
Vinorelbine	TUBB	
Ixabepilone	TUBB	
5-FUdR	TYMS	TYMS inhibitor
5-FU	TYMS	
Imatinib	ABL/KIT/PDGFR	N/A
NSC663284	CDC25	N/A
Fascaplysin	CDK4	N/A
GSK923295	CENPE	N/A
TCS2312 dihydrochloride	CHK1	N/A
BIBW2992	EGFR/HER2	N/A
XRP44X	ELK3	N/A
Tamoxifen	ESR1	N/A
Ibandronate sodium salt	FDPS	N/A
PD173074	FGFR1/3	N/A
Lestaurtinib	FLT3/JAK2/TrkA	N/A
Gemcitabine	DNA replication	N/A
GSK1838705A	IGF1R	N/A
IKK 16	IKK	N/A
SB-715992	KSP	N/A
Baicalein	Lipoxygenase	N/A
Nutlin 3a	MDM2	N/A
GSK1120212	MEK	N/A
Bosutinib	Src	N/A
MLN4924	NAE1	N/A
Sunitinib Malate	PDGFR/VEGFR/KIT/FLT3	N/A
GSK461364	PLK1	N/A

Nature Chemical Biology: doi:10.1038/nchembio.1337

Supplementary Table 2: Empirical mutual information scores (and their corresponding *P* values) between each of the estimated key dose-response parameters and the set of anti-cancer compounds and the breast cell line panel

Parameter	All drugs[1]		Drugs excluding cell cycle inhibitors	
	***I* (parameter; drugs)**	***I* (parameter; cell lines)**	***I* (parameter; drugs)**	***I* (parameter; cell lines)**
$\log_{10}(EC_{50})$	1.52 ($P < 10^{-4}$)	0.12 ($P = 0.99$)	1.46 ($P < 10^{-4}$)	0.17 ($P = 0.99$)
$\log_{10}(IC_{50})$	1.40 ($P < 10^{-4}$)	0.16 ($P = 0.05$)	1.42 ($P < 10^{-4}$)	0.17 ($P = 0.99$)
Hill slope	0.66 ($P < 10^{-4}$)	0.18 ($P = 0.11$)	0.70 ($P < 10^{-4}$)	0.25 ($P = 0.80$)
E_{max}	0.69 ($P < 10^{-4}$)	0.23 ($P < 10^{-4}$)	0.69 ($P < 10^{-4}$)	0.25 ($P = 0.03$)
E_{inf}	0.57 ($P < 10^{-4}$)	0.19 ($P < 10^{-4}$)	0.53 ($P < 10^{-4}$)	0.24 ($P = 0.02$)

Notes:

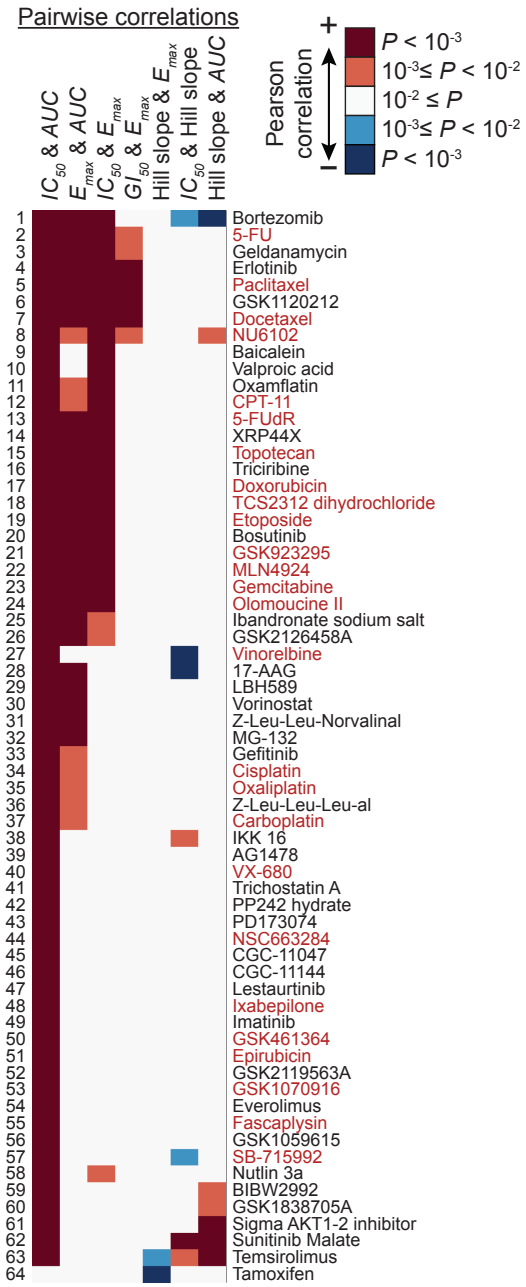
1. Mutual information for the parameters in the first column across all 64 drugs in the data set

Nature Chemical Biology: doi:10.1038/nchembio.1337

Supplementary Table 3: Pharmacokinetic data (*MTD* and C_{max}) for a selection of anti-cancer drugs and the metric suggested based on our analysis for comparison of drug sensitivity for breast cancer lines exposed to C_{max}

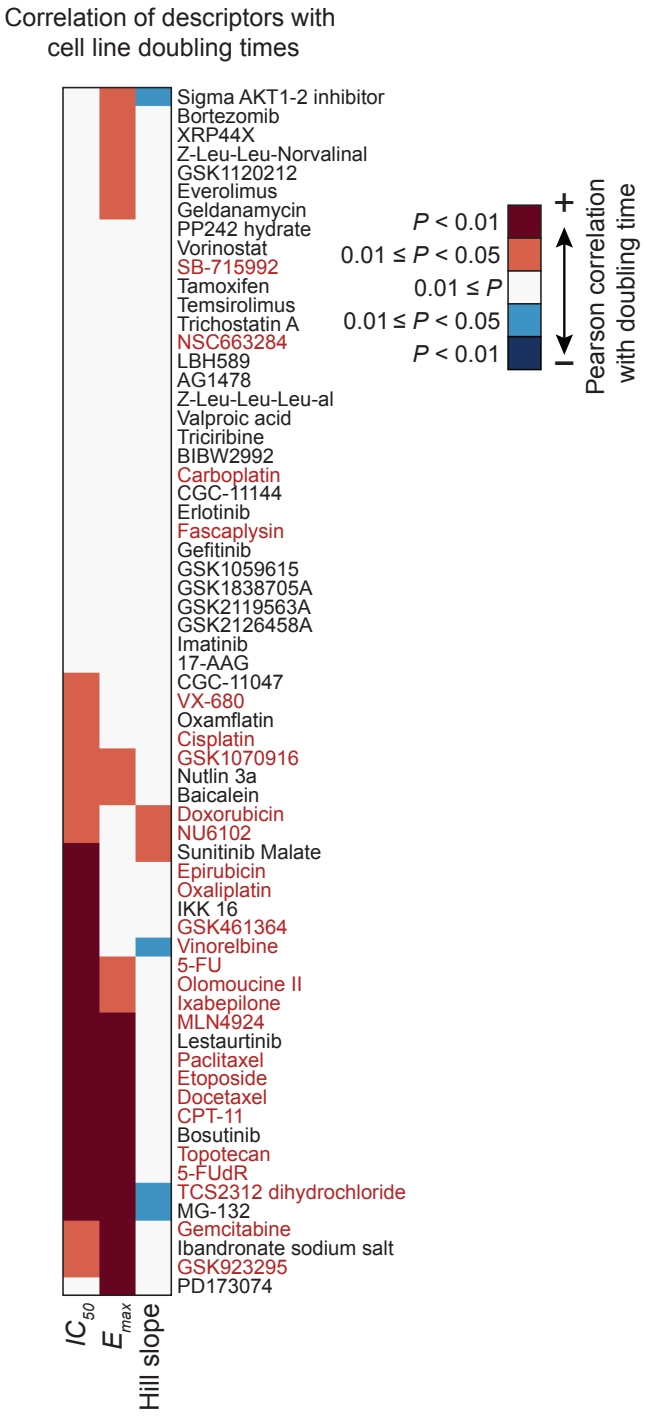
Drug	**Proposed *MTD***	**Route of administration**	**C_{max} at proposed MTD (μM)**	**IC_{50} of the most breast cancer cell line (μM)**	**Reference**	**Suggested metric for breast cancer analysis**
17-AAG	21 mg/m2	1-2 h infusion	8.5±4.7	0.026	1	AUC
BIBW2992	70 mg/day	Oral	0.37±0.13	0.017	2	IC50
Bortezomib	1.6 mg/m2	i.v.	0.11±0.20	0.021	3	EC50/AUC
Epirubicin	150 mg/m2	i.v.	5.68±2.76	0.083	4	IC50/Emax
Gefitinib	2000 mg/twice weekly	Oral	5.55±3.16	0.24	5	AUC
Everolimus	70mg/wk	Oral	0.18±0.05	0.04	6	AUC
GSK923295	190mg/m2	1 hr infusion	12±7.2	0.022	7	AUC/Emax
LBH589	14 mg/m2	30 min infusion	1.62±1.29	0.044	8	AUC/Emax
SB-715992	12 mg/m2	1 hr infusion	0.48±0.26	0.02	9	IC50
Topotecan	1.5 mg/m2	30 min infusion	0.073±0.023	0.005	10	AUC
Valproic acid	60 mg/kg	1 hr infusion	1248±208	1136	11	EC50
Vinorelbine	30 mg/m2	15 min infusion	1	0.004	12	IC50
5-FU	15 mg/kg	i.v.	461	15	12	IC50
Carboplatin	330 mg/m2	30 min infusion	123	12	12	IC50
Cisplatin	100 mg/m2	i.v.	8.3	2.1	12	EC50
Doxorubicin	60 mg/m2	i.v.	1.1	0.028	12	Emax
Etoposide	290 mg/m2	i.v.	58.1	0.45	12	IC50/Emax
Gemcitabine	790mg/m2	30 min infusion	57	0.003	12	Emax
Paclitaxel	175mg/m2	3 hr infusion	4.27	0.004	12	Emax

Nature Chemical Biology: doi:10.1038/nchembio.1337



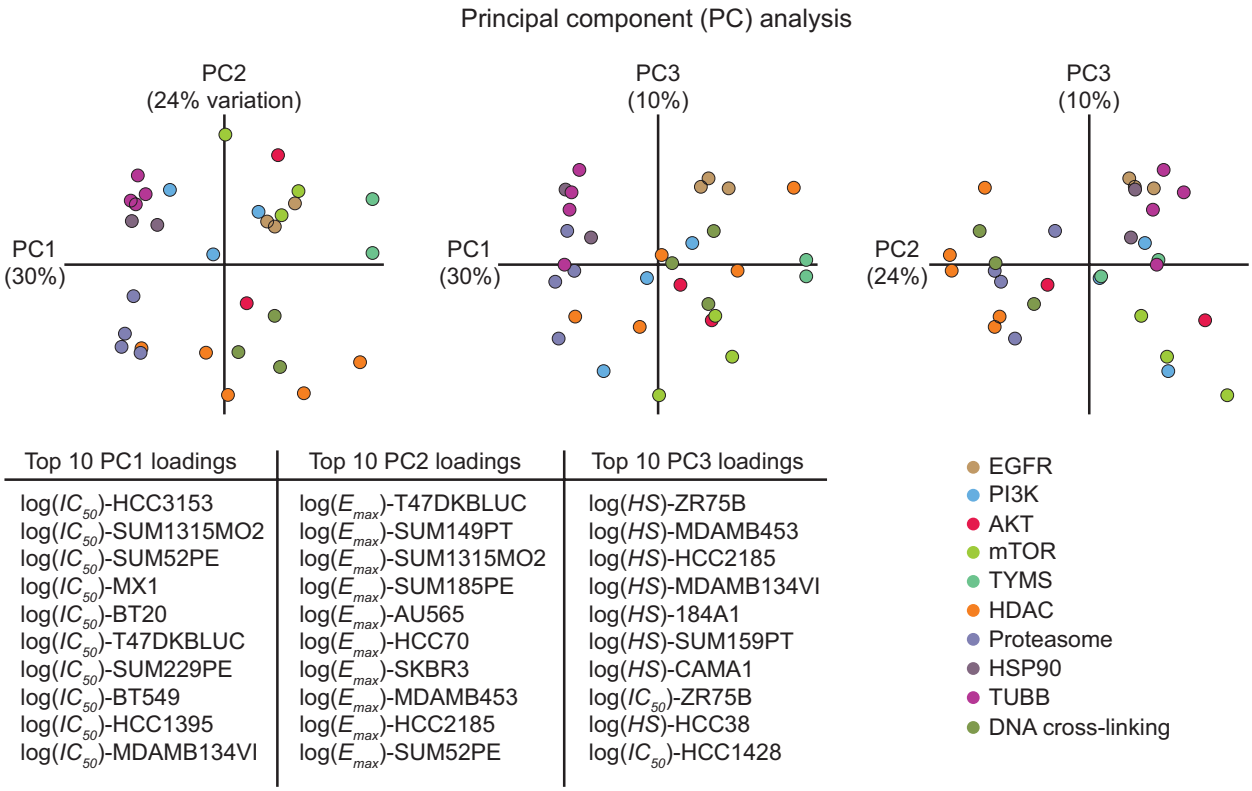
Supplementary Figure 1:
Different dose-response parameters representing drug sensitivity do not always correlate with each other. Pairwise correlation between different dose-response parameters estimated for each drug across the breast cell lines are shown and cell-cycle phase-specific cytotoxic compounds are denoted by red. *P* values were corrected using the Holm-Bonferroni method.

Nature Chemical Biology: doi:10.1038/nchembio.1337



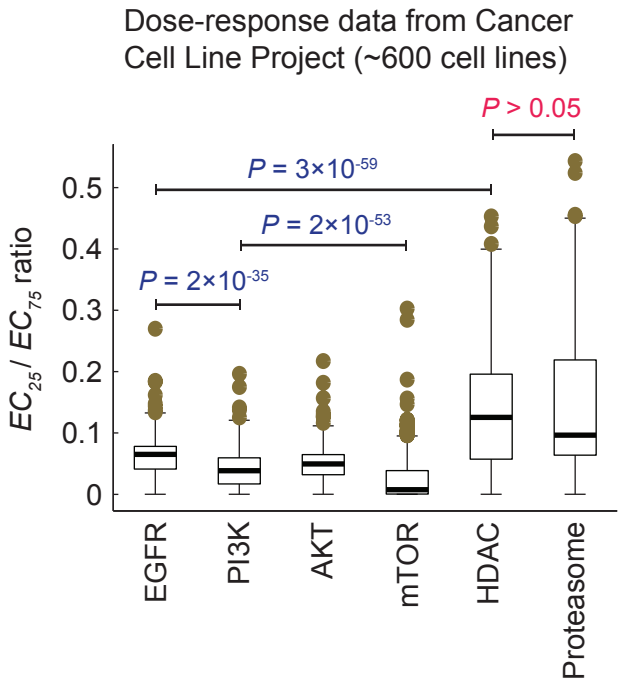
Supplementary Figure 2:
Correlation between different dose-response parameters estimated for the set of anti-cancer compounds and the doubling times across the breast cell lines. Cell-cycle phase-specific cytotoxic compounds are denoted by red. *P* values were corrected using the Benjamini-Hochberg method.

Nature Chemical Biology: doi:10.1038/nchembio.1337



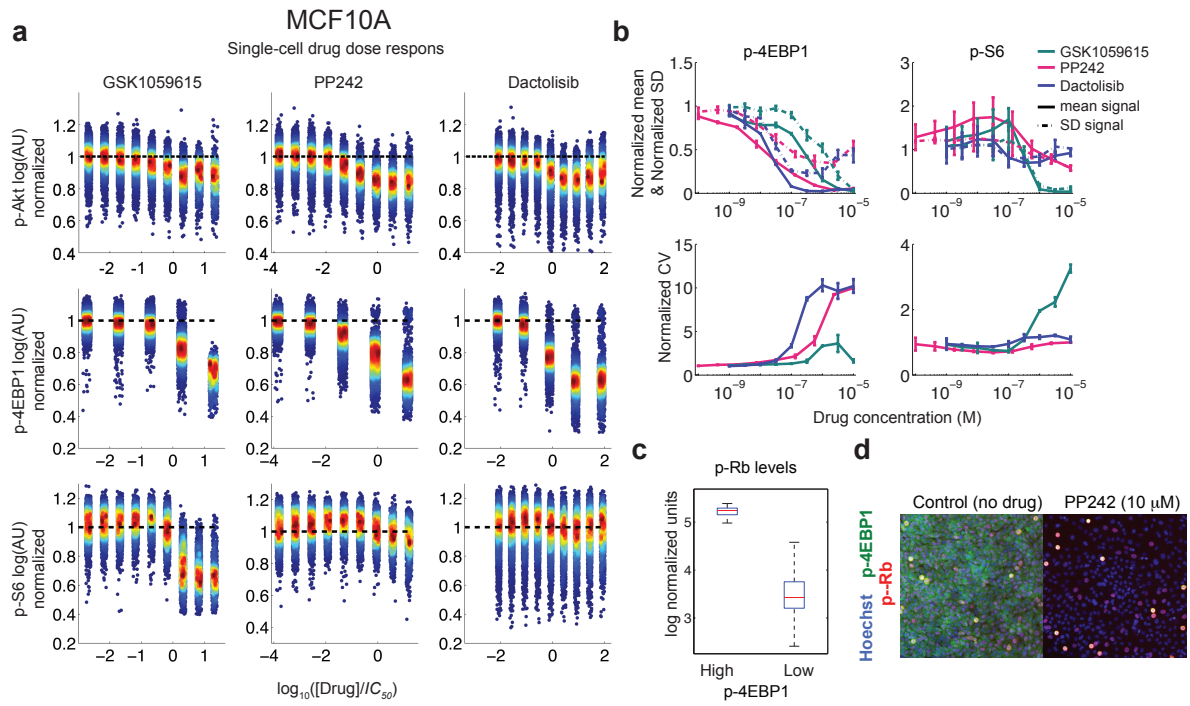
Supplementary Figure 3:
Principal component analysis of dose-response data (IC_{50}, E_{max} and Hill slope) for 31 drugs and 53 cell lines. PCA recognizes the multi-dimensional dose-response relationship between drugs and their targets into principal components that can be plotted to indicate the relatedness of each individual parameter (IC_{50}, E_{max} or HS across all the cell lines) to drug target. Drugs from most classes cluster based on values of IC_{50}, E_{max} and HS parameters. The top 10 loadings for each of principal components 1, 2, and 3 are shown.

Nature Chemical Biology: doi:10.1038/nchembio.1337



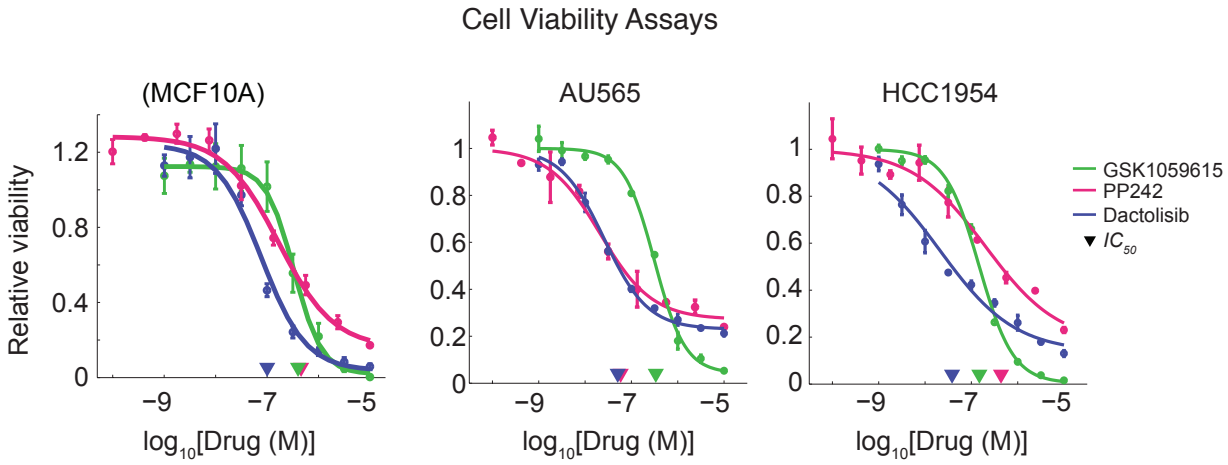
Supplementary Figure 4:
Association of Hill slope (approximated by the EC_{25}/EC_{75} ratio) with drug class is shown based on dose-response data on ~600 cell lines from the Cancer Cell line Project.

Nature Chemical Biology: doi:10.1038/nchembio.1337



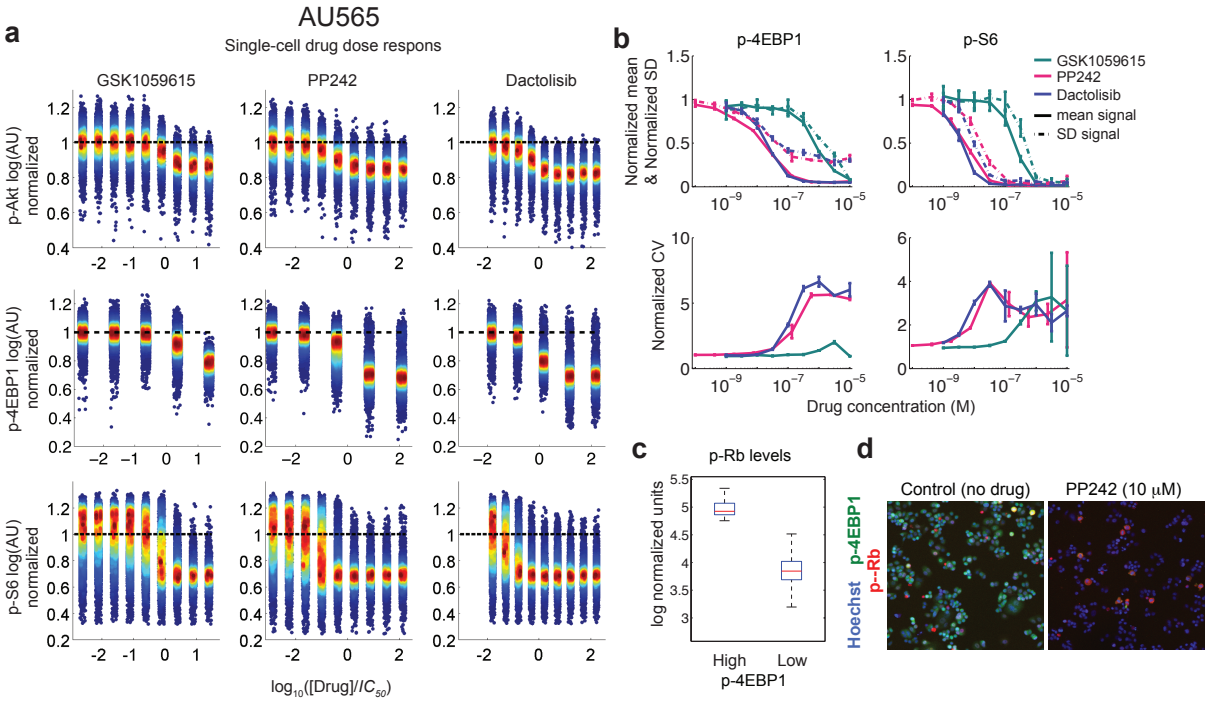
Supplementary Figure 5:
Single-cell analysis of drug dose response of the MCF10A cells to pharmacological inhibition of PI3K/Akt/mTOR pathway. (a) Single cell drug dose-response data measured for p-Akt (Ser473), p-4EBP1 (Thr37/46), and p-S6 (Ser235/236) after treatment with nine doses of GSK1059615, PP242 or dactolisib. (b) Dose-dependent variation of the mean signal, standard deviation (SD) and coefficient of variation (CV; the standard deviation for single cell measurements divided by the population-average) for single cell p-4EBP1 and p-S6 levels after treatment with GSK1059615, PP242 or dactolisib. (c) Cells with high p-4EBP1 levels 24 hr after exposure to 10 μM PP242 exhibit ~10 times higher levels of p-Rb than low-p4EBP1 cells (cells with p-4EBP1 levels below the population average p-4EBP1 level in the absence of drug). (d) Selected immunofluorescence images of p-4EBP1 (green), p-Rb (red) and Hoechst (blue) staining of cells in the absence of drug and 24 hr after exposure to 10 μM PP242.

Nature Chemical Biology: doi:10.1038/nchembio.1337



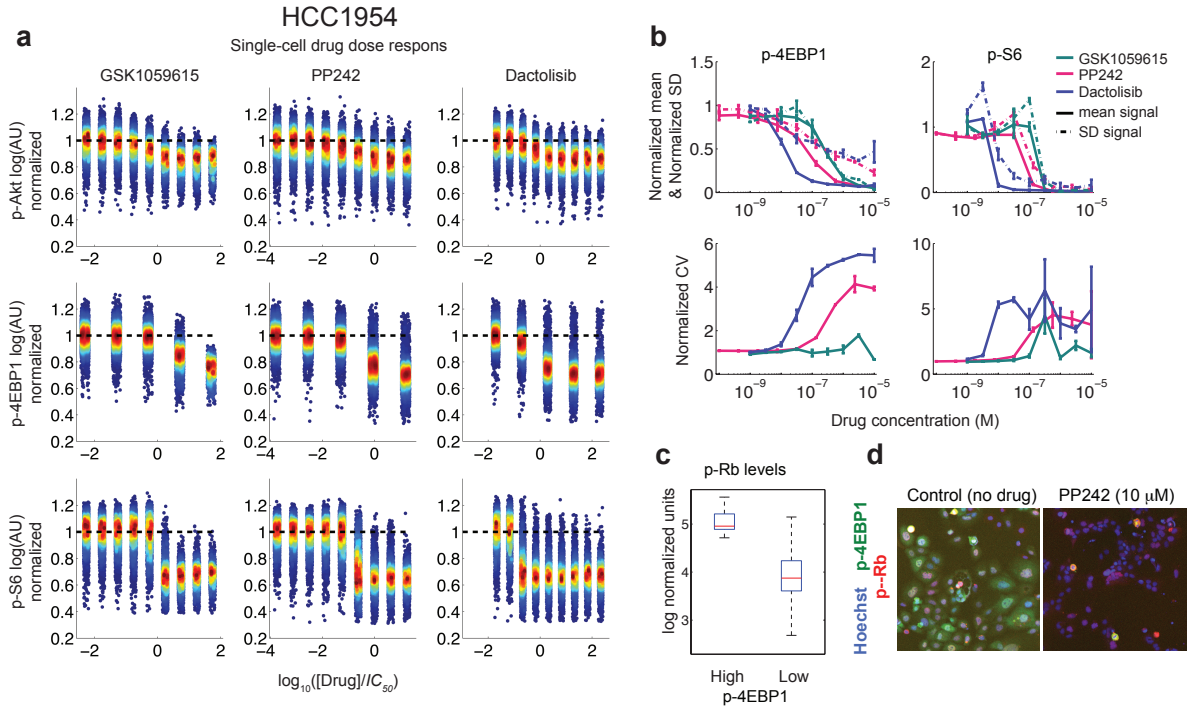
Supplementary Figure 6: Cell viability measurements using microscopy. Experimental data confirm $HS < 1$ and $E_{max} > 0$ for the drugs PP242 and dactolisib and $HS \sim 1$ for GSK1059615 across the tested cell lines.

Nature Chemical Biology: doi:10.1038/nchembio.1337



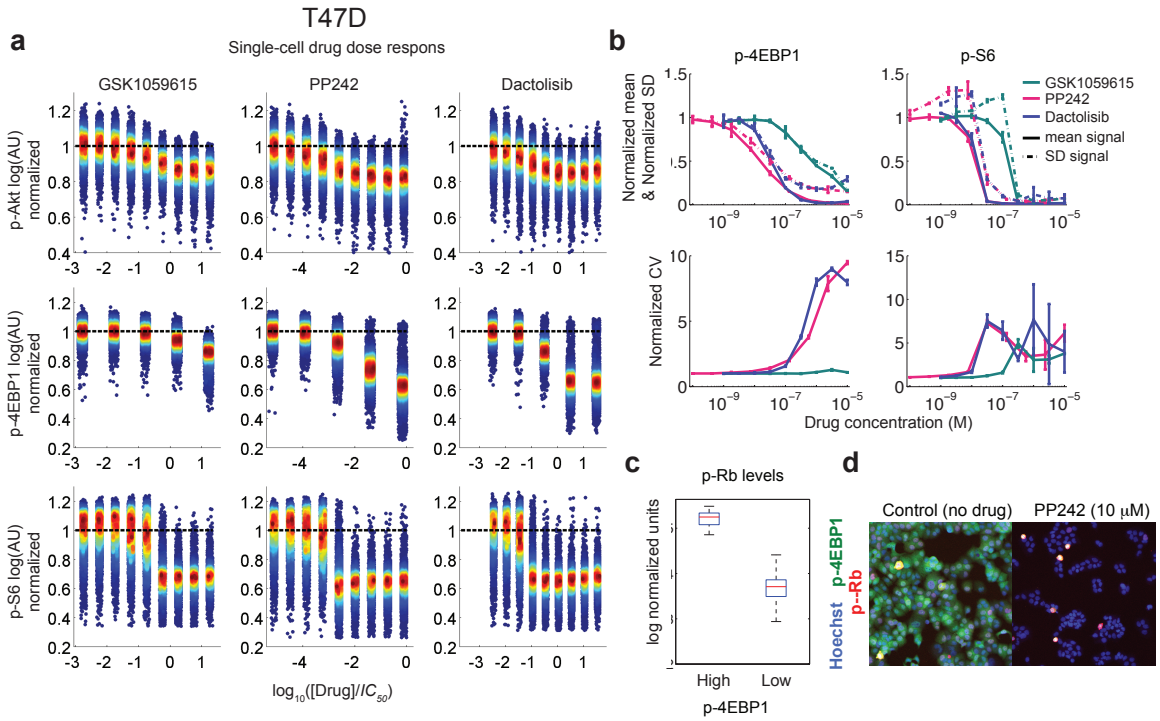
Supplementary Figure 7:
Single-cell analysis of drug dose response of the AU565 cells to pharmacological inhibition of PI3K/Akt/mTOR pathway. (a) Single cell drug dose-response data measured for p-Akt (Ser473), p-4EBP1 (Thr37/46), and p-S6 (Ser235/236) after treatment with nine doses of GSK1059615, PP242 or dactolisib. (b) Dose-dependent variation of the mean signal, standard deviation (SD) and coefficient of variation (CV; the standard deviation for single cell measurements divided by the population-average) for single cell p-4EBP1 and p-S6 levels after treatment with GSK1059615, PP242 or dactolisib. (c) Cells with high p-4EBP1 levels 24 hr after exposure to 10 μM PP242 exhibit ~10 times higher levels of p-Rb than low-p4EBP1 cells (cells with p-4EBP1 levels below the population average p-4EBP1 level in the absence of drug). (d) Selected immunofluorescence images of p-4EBP1 (green), p-Rb (red) and Hoechst (blue) staining of cells in the absence of drug and 24 hr after exposure to 10 μM PP242.

Nature Chemical Biology: doi:10.1038/nchembio.1337



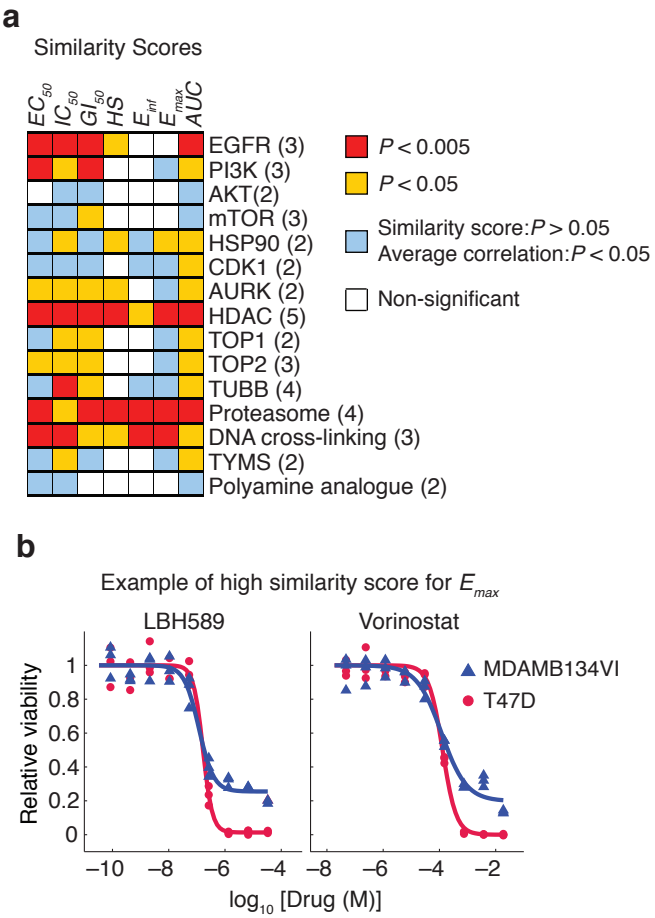
Supplementary Figure 8:
Single-cell analysis of drug dose response of the HCC1954 cells to pharmacological inhibition of PI3K/Akt/mTOR pathway. (a) Single cell drug dose-response data measured for p-Akt (Ser473), p-4EBP1 (Thr37/46), and p-S6 (Ser235/236) after treatment with nine doses of GSK1059615, PP242 or dactolisib. (b) Dose-dependent variation of the mean signal, standard deviation (SD) and coefficient of variation (CV; the standard deviation for single cell measurements divided by the population-average) for single cell p-4EBP1 and p-S6 levels after treatment with GSK1059615, PP242 or dactolisib. (c) Cells with high p-4EBP1 levels 24 hr after exposure to 10 μM PP242 exhibit ~10 times higher levels of p-Rb than low-p4EBP1 cells (cells with p-4EBP1 levels below the population average p-4EBP1 level in the absence of drug). (d) Selected immunofluorescence images of p-4EBP1 (green), p-Rb (red) and Hoechst (blue) staining of cells in the absence of drug and 24 hr after exposure to 10 μM PP242.

Nature Chemical Biology: doi:10.1038/nchembio.1337



Supplementary Figure 9:
Single-cell analysis of drug dose response of the T47D cells to pharmacological inhibition of PI3K/Akt/mTOR pathway. (a) Single cell drug dose-response data measured for p-Akt (Ser473), p-4EBP1 (Thr37/46), and p-S6 (Ser235/236) after treatment with nine doses of GSK1059615, PP242 or dactolisib. (b) Dose-dependent variation of the mean signal, standard deviation (SD) and coefficient of variation (CV; the standard deviation for single cell measurements divided by the population-average) for single cell p-4EBP1 and p-S6 levels after treatment with GSK1059615, PP242 or dactolisib. (c) Cells with high p-4EBP1 levels 24 hr after exposure to 10 μM PP242 exhibit ~10 times higher levels of p-Rb than low-p4EBP1 cells (cells with p-4EBP1 levels below the population average p-4EBP1 level in the absence of drug). (d) Selected immunofluorescence images of p-4EBP1 (green), p-Rb (red) and Hoechst (blue) staining of cells in the absence of drug and 24 hr after exposure to 10 μM PP242.

Nature Chemical Biology: doi:10.1038/nchembio.1337



Supplementary Figure 10: Different dose-response parameters capture cell line to cell line variation associated with different mechanisms of action. (a) Similarity score matrix showing significant similarities between patterns of response across the breast cell line panel for all drug classes that have at least two different compounds. The number of compounds within each class is shown in parentheses. (b) Dose-response relationships for two HDAC inhibitors (LBH589 and vorinostat) and two breast cancer cell lines (MDAMB134VI and T47D) that represent high similarity score of E_{max} for HDAC inhibitors. Each data-point represents one of the three replicates of the dose-response experiment.

Nature Chemical Biology: doi:10.1038/nchembio.1337

References cited in Supplementary Table 3:

1. Kummar, S. *et al*. Phase I trial of 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG), a heat shock protein inhibitor, administered twice weekly in patients with advanced malignancies. *Eur. J. Cancer* **46**, 340-347 (2010).

2. Eskens, F. A. *et al*. A phase I dose escalation study of BIBW 2992, an irreversible dual inhibitor of epidermal growth factor receptor 1 (EGFR) and 2 (HER2) tyrosine kinase in a 2-week on, 2-week off schedule in patients with advanced solid tumours. *Br. J. Cancer* **98**, 80-85 (2008).

3. Papandreou, C. N. *et al*. Phase I trial of the proteasome inhibitor bortezomib in patients with advanced solid tumors with observations in androgen-independent prostate cancer. *J. Clin. Oncol.* **22**, 2108-2121 (2004).

4. Tjuljandin, S. A. *et al*. Pharmacokinetics and toxicity of two schedules of high dose epirubicin. *Cancer Res.* **50**, 5095-5101 (1990).

5. Gross, M. E., Leichman, L., Lowe, E. S., Swaisland, A. & Agus, D. B. Safety and pharmacokinetics of high-dose gefitinib in patients with solid tumors: results of a phase I study. *Cancer Chemother. Pharmacol.* **69**, 273-280 (2012).

6. O'Donnell, A. *et al*. Phase I pharmacokinetic and pharmacodynamic study of the oral mammalian target of rapamycin inhibitor everolimus in patients with advanced solid tumors. *J. Clin. Oncol.* **26**, 1588-1595 (2008).

7. Chung, V. *et al*. First-time-in-human study of GSK923295, a novel antimitotic inhibitor of centromere-associated protein E (CENP-E), in patients with refractory cancer. *Cancer Chemother. Pharmacol.* **69**, 733-741 (2012).

8. Giles, F. *et al*. A phase I study of intravenous LBH589, a novel cinnamic hydroxamic acid analogue histone deacetylase inhibitor, in patients with refractory hematologic malignancies. *Clin. Cancer Res.* **12**, 4628-4635 (2006).

9. Gomez, H. L. *et al*. Phase I dose-escalation and pharmacokinetic study of ispinesib, a kinesin spindle protein inhibitor, administered on days 1 and 15 of a 28-day schedule in patients with no prior treatment for advanced breast cancer. *Anticancer Drugs* **23**, 335-341 (2012).

10. van Warmerdam, L. J. *et al*. Pharmacokinetics and pharmacodynamics of topotecan administered daily for 5 days every 3 weeks. *Cancer Chemother. Pharmacol.* **35**, 237-245 (1995).

11. Atmaca, A. *et al*. Valproic acid (VPA) in patients with refractory advanced cancer: a dose escalating phase I clinical trial. *Br. J. Cancer* **97**, 177-182 (2007).

12. Fridborg, H., Nygren, P. & Larsson, R. Relationship between pharmacokinetic parameters in patients and cytotoxicity in vitro of standard and investigational anticancer drugs. *Anticancer Drugs* **6**, 64-69 (1995).

Nature Chemical Biology: doi:10.1038/nchembio.1337