Supplementary Information

An integrated platform of genomic assays reveals small molecule bioactivities

Shawn Hoon, Andrew Smith, Iain M. Wallace, Sundari Suresh, Molly Miranda, Eula Fung, Michael Proctor, Kevan M. Shokat, Chao Zhang, Ronald W. Davis, Guri Giaever, Robert P. St.Onge, Corey Nislow
**Supplementary Figure 1.** Multicopy Suppression Profiling Validation.

(a) Schematic of MSP. A multicopy pool is generated by transforming a cloned genomic library into a yeast strain of choice. Following the growth of pooled transformants, plasmids are isolated and the genomic inserts are amplified by PCR using common primers and hybridized to a TAG4 array. (b) Distribution showing the number of ORFs present in the multicopy suppressor pool as a function of chip intensity cutoff. At 2-fold above a conservative estimate of background intensity (500), at least 4307 ORFs are estimated to be in the pool. (c) A genetic complementation screen using the cdc28-AS analog sensitive kinase. A diploid strain harboring the cdc28-AS allele as the sole source of CDC28 was transformed with a genomic library as described in text. Transformants were pooled in 700ul of selective media and grown in the presence or absence of 500nM 1-NM-PP1(1) which resulted in a ~50% inhibition of growth rate with inhibitor. The chemical structure of 1-NM-PP1 is shown on the left. (d) The fold change (log2(1-NM-PP1-treated/untreated)) in microarray signal intensity is plotted on the y-axis for ~6000 genes (arranged alphabetically on the x-axis). CDC28 and three genes flanking the CDC28 locus (CSH1, IFA38, TOS1) were overrepresented in the 1-NM-PP1 treated pool. (insert) Genomic location of the 4 genes highlighted. (e) Specificity of DSP. (left) The specificity of 1-NM-PP1 was assessed by screening a pool containing ~6000 heterozygous deletion strains and a single cdc28-AS strain with 1-NM-PP1. (right) The fold change in tag hybridization intensity (log2(control/1-NM-PP1)) is plotted on the y-axis for each strain (arranged alphabetically on the x-axis). Only the cdc28-AS strain exhibited sensitivity to the inhibitor.
Supplementary Figure 2. (a-c) Vector analysis results for (a) latrunculin A, (b) nocodazole and (c) MMS are presented. For latrunculin A, the only gene identified in both assays was MAK10, a N-terminal acetyltransferase. For nocodazole, only TUB3, one of 2 genes encoding alpha-tubulin was identified from both assays. TUB1, the other alpha-tubulin, was filtered from the analysis because of poor tag intensity. TUB2, the beta-tubulin was not sensitive nor identified as a suppressor. For MMS, HEM1, an essential gene encoding 5-aminolevulinate synthase was identified in both assays. (d) Isolating genomic fragment that suppresses rapamycin sensitivity. The genomic fragment coding the C-terminus of TOR2 and EAP1 isolated as a suppressor of rapamycin (black line). This fragment contains both the PI3/PI4 kinase domain and the FKBP12-rapamycin binding domain (FRB) of TOR2 and is sufficient to suppress rapamycin sensitivity in an isogenic culture (bottom middle), relative to the vector control (bottom left). A truncated fragment (red line) lacking the FRB domain fails to suppress rapamycin sensitivity (bottom right).
Supplementary Figure 3. Cantharidin and calyculin A have distinct effects in vivo (a) (top) BY4743 (wild-type) and a GLC7 heterozygous deletion were grown in the presence of DMSO, cantharidin and calyculin A in YPD. In the presence of either compound, the deletion strain grew slower than wild-type. (bottom) BY4743 (wild-type) containing either vector (pRS426) or a plasmid containing GLC7 (pRS426-GLC7) was grown in the presence of DMSO, cantharidin and calyculin A in URA- media. The strain harboring multiple copies of GLC7 conferred resistance to calyculin A but not cantharidin.(c) Clustergram of deletion sensitivity profiles of reference compounds used in this study. Log ratios from deletion sensitivity experiments were hierarchically clustered using Pearson’s correlation with average linkage. (d) Functional enrichment, determined by GO-slim analysis, of the top 50 strains sensitive to cantharidin and calyculin A were compared. Enrichment difference between both compounds was calculated by taking the difference between the fraction of genes mapped to each GO-slim annotation.
Supplementary Figure 4. CRG1 is a major suppressor of cantharidin sensitivity. Expression of CRG1 from a high-copy vector under its own promoter confers resistance to cantharidin but not calyculin A sensitivity.
Supplementary Figure 5. Cantharidin and calyculin A are synergistic in inhibiting growth. (a) Dose response matrix of cantharidin treatment with 8 other compounds in addition to mock treatment with itself. Interaction was determined using a Bliss model of independence. Treatment of cantharidin with calyculin A was highly synergistic. Representative growth curves are shown for different pairs of drug concentrations. Treatment of cantharidin against itself is a control for a purely additive interaction. The best-fit shape model assigned to this combination by surface response analysis is indicated (see Supplementary Methods online). (b-i) Growth curves, fitness heatmaps and interaction heatmaps for each compound.
b

Growth

Cantharidin (µM)

Calyculin A (µM)

Cantharidin (µM)

Antagonism

Synergy

Potentiation

Interaction

Fitness

Growth
Cantharidin ($\mu$M)

Fitness

Antagonism

Synergy

Loewe

Growth
Growth

![Graph showing the interaction between MMS (%) and Cantharidin (µM) on Growth.

Cantharidin (µM)

- MMS (%)
- Cantharidin (µM)
- Fitness
- Antagonism
- Synergy

Bliss Boost (saturating)
Growth

Cantharidin (µM)

Nocodazole (µg/ml)

Fitness

Cantharidin (µM)

Antagonism

Synergy

Not assigned

Interaction

-0.5

-0.4

-0.3

-0.2

-0.1

0

0.1

0.2

0.3

0.4

0.5
<table>
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<tr>
<th>Methotrexate (µM)</th>
<th>Cantharidin (µM)</th>
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<tr>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>0.2</td>
<td>0.3</td>
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<td>0.5</td>
<td>0.8</td>
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<td>1</td>
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**Growth**

- Methotrexate
- Cantharidin

**Fitness**

- Antagonism
- Synergy
- Interaction

**Loewe**

- Growth
- Methotrexate
- Cantharidin
Mechlorethamine (µM)

Cantharidin (µM)

Growth

Antagonism

Synergy

Loewe
**Supplementary Figure 6** Mechanistic insights into drug interactions. (a) (left) Strains identified as sensitive to either cantharidin (100uM) or calyculin A (2.5uM) at the original concentrations were mapped to an interaction network consisting of 15 interactions. (right) Strains identified as sensitive to the cocktail were overlaid onto the original network on the left. White nodes represent deletion strains not identified as sensitive in the cocktail screen. Red nodes represent strains that were identified as sensitive in both the original and cocktail screen. Blue nodes represent strains that were found sensitive only in the cocktail screen. The Venn diagram illustrates the number of strains in each category. (b) Distribution of epsilon e, a metric that quantifies the degree of interaction between cantharidin and calyculin A for each strain (see Methods). (c) Cantharidin boosts calyculin A sensitivity. Strains identified as synergistic or antagonistic in the cocktail experiment (Cantharind 33uM; calyculin A 1.6uM) were used in calculating the average sensitivity to cantharidin and calyculin A at the original screening concentration (Cantharidin 100uM; calyculin A 2.5uM). Boxplot of the distribution of scores are shown. Synergistic strains are significantly more sensitive to calyculin A than catharidin.
**Supplementary Figure 7** (a) Confirmation growth curves for 4130-1278. (Top row) Growth of HO strain compared to sec14+/- in the presence of DMSO or 4130-1278. (Bottom row) Growth of wild type strain with plasmids containing either empty vector or SEC14 under control of endogenous promoter. (b) Statistical analysis of chemogenomic profile and compound structure correlation. Spearman correlation coefficient for rank ordered chemogenomic profile similarities and compound similarities were determined for DSP (left) and MSP (right). 1000 random compound similarity matrices were generated by shuffling the rows and columns of the original structure similarity matrix. For each random matrix, the spearman correlation coefficient was computed. The scatterplots compare the original correlations and with the correlations involving the 1000 randomly generated compound similarity matrices. P-values for each (t-test)
**Supplementary Table 1.** Reference compounds used in this study

<table>
<thead>
<tr>
<th>Compound</th>
<th>DSP Concentration</th>
<th>MSP Concentration</th>
<th>Known mechanism of action</th>
<th>Protein Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 1-NM-PP1</td>
<td>500nM</td>
<td>500nM</td>
<td>Inhibits analog sensitive kinase $cdc28$-AS$^3$</td>
<td>$cdc28$-AS</td>
</tr>
<tr>
<td>2 Methotrexate</td>
<td>200µM</td>
<td>810µM</td>
<td>Inhibitor of folic acid biosynthesis$^2$</td>
<td>Dihydrofolate Reductase</td>
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<tr>
<td>3 Fluconazole</td>
<td>33.516 µM</td>
<td>37.5 µM</td>
<td>Inhibitor of Lanosterol 14-alpha-demethylase$^3, 4$</td>
<td>Erg11</td>
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<tr>
<td>4 Rapamycin</td>
<td>0.35nM</td>
<td>10nM</td>
<td>Inhibitor of TOR signaling$^5$</td>
<td>Tor1, Tor2</td>
</tr>
<tr>
<td>5 Cantharidin</td>
<td>100µM</td>
<td>100µM</td>
<td>Protein phosphatase inhibitor$^6, 7$</td>
<td>PP1, PP2a</td>
</tr>
<tr>
<td>6 Calyculin A</td>
<td>2.5µM</td>
<td>10µM</td>
<td>Protein phosphatase inhibitor$^8$</td>
<td>PP1, PP2a</td>
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<tr>
<td>7 Methyl methanesulfonate (MMS)</td>
<td>0.002%</td>
<td>0.015%</td>
<td>DNA-alkylating agent$^9$</td>
<td>Unknown</td>
</tr>
<tr>
<td>8 Latrunculin A</td>
<td>2.5µM</td>
<td>5µM</td>
<td>Actin-depolymerizing agent$^{10}$</td>
<td>Actin monomer</td>
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<tr>
<td>9 Nocodazole</td>
<td>15µM</td>
<td>33.2µM</td>
<td>Microtubule-depolymerizing agent$^{11}$</td>
<td>Alpha-beta tubulin dimer</td>
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</table>

**References**


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Supplementary Methods

Strains and Media. Yeast were maintained in YPD media\(^1,2\) at 30°C unless stated otherwise. Strains and plasmids used for individual analysis in this study are listed below were obtained from the yeast deletion collection or constructed \textit{de novo} using PCR-based gene replacement\(^3\). Multicopy suppressor ORFs are listed below and were cloned by gap-repair as described by Oldenburg K.R. et al. \(^4\). Primers used for cloning are listed in the following table:

Primers for cloning ORFs by gap-repair:

<table>
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<tr>
<th>ORF</th>
<th>Left Primer</th>
<th>Right Primer</th>
<th>Amplicon Size</th>
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<tr>
<td>GLC7</td>
<td>5' - TAG TGG ATC CCC CPG GCT GCA GGA ATT CGA TAT CAA GCT TCA GTG GCT GTT TGC TGA CAT - 3'</td>
<td>5' - GCG TAA TAC GAC TCA CTA TAG GGC GAA TTG GGT ACC GGG CCC AAA GGA AGA CGT GAC CAT - 3'</td>
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<tr>
<td>CRG1</td>
<td>5' - TAG TGG ATC CCC CPG GCT GCA GGA ATT CGA TAT CAA GCT TAA TGA ATG CGG CAA GAT ACC - 3'</td>
<td>5' - GCG TAA TAC GAC TCA CTA TAG GGC GAA TTG GGT ACC GGG CCC GGA AAC AGC TTT CTG AAG - 3'</td>
<td>2083</td>
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<tr>
<td>HEM1</td>
<td>5’ – TAG TGG ATC CCC CPG GCT GCA GGA ATT CGA TAT CAA GCT TGT TGG TGC TGC TGG TTT TGG TGA – 3’</td>
<td>5’ – GCG TAA TAC GAC TCA CTA TAG GGC GAA TTG GGT ACC GGG CAC TTC TAA GTG GGC CGC TGA – 3’</td>
<td>2806</td>
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<tr>
<td>orf19.633</td>
<td>5’ – TAG TGG ATC CCC CPG GCT GCA GGA ATT CGA TAT CAA GCT TCA AAA TGT CCA TGT GAT GCC – 3’</td>
<td>5’ - GCG TAA TAC GAC TCA CTA TAG GGC GAA TTG GGT ACC GGG CAT TCC AAT TTG CCA TAC CCA</td>
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Individual strains and plasmids used in this study:

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<th>Genotype</th>
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<td>BY4743</td>
<td>MATα/α his3ΔI/his3ΔI leu2Δ0/leu2Δ0 lys2Δ0/LYS2 MET15/met15Δ0 ura3Δ0/ura3Δ0</td>
<td>Giaever et al. 2002³</td>
</tr>
<tr>
<td>HHY100</td>
<td>BY4743; cdc28Δ::Kan/cdc28-as::Nat</td>
<td>This study⁴</td>
</tr>
<tr>
<td>HHY101</td>
<td>BY4743; glc7Δ::Kan/GLC7</td>
<td>Giaever et al. 2002⁵</td>
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<tr>
<td>HHY102</td>
<td>BY4743; crg1Δ::Kan/CRG1</td>
<td>Giaever et al. 2002⁵</td>
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<tr>
<td>KT1112</td>
<td>MATα leu2 ura3-52 his3</td>
<td>Baker et al. 1997⁶</td>
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<td>KT1638</td>
<td>MATα leu2 ura3-52 his3 glc7-109</td>
<td>Baker et al. 1997⁶</td>
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<td>KT1623</td>
<td>MATα leu2 ura3-52 his3 glc7-127s</td>
<td>Baker et al. 1997⁶</td>
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</table>

¹ Plasmid pJAU1 containing the cdc28-as allele harboring a F88G as described by Bishop A.C. et al. ⁷ was used to integrate the cdc28-as allele into the wild-type CDC28 locus using a pop-in-pop-out strategy².
### Plasmids

<table>
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<th>Plasmids</th>
<th>Characteristics</th>
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<td>pRS426</td>
<td>High copy number, URA3 marker</td>
<td>Sikorski R.S. et al.⁸</td>
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<tr>
<td>YEplac195</td>
<td>High copy number, URA3 marker</td>
<td>Gietz R.D. et al.⁹</td>
</tr>
<tr>
<td>pRS426-CRG1</td>
<td>High copy number, URA3 marker, CRG1-ORF plus regulatory regions</td>
<td>This study⁸</td>
</tr>
<tr>
<td>pRS426-HEM1</td>
<td>High copy number, URA3 marker, HEM1-ORF plus regulatory regions</td>
<td>This study⁸</td>
</tr>
<tr>
<td>pRS426-GLC7</td>
<td>High copy number, URA3 marker, GLC7-ORF plus regulatory regions</td>
<td>This study⁸</td>
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<tr>
<td>pRS426-orf19.633</td>
<td>High copy number, URA3 marker, orf19.633-ORF plus regulatory regions</td>
<td>This study⁸</td>
</tr>
<tr>
<td>YEplac195-EAP1-TOR2</td>
<td>High copy number, URA3 marker, 3.3kb genomic Sau3A fragment containing truncated C-terminal fragments of EAP1 and TOR2 (chrXI:54294-57644)</td>
<td>This study⁷</td>
</tr>
<tr>
<td>YEplac195-EAP1-TOR2-trunc</td>
<td>High copy number, URA3 marker, 3.3kb genomic Sau3A fragment containing truncated C-terminal fragments of EAP1 and TOR2 (chrXI:54294-56736)</td>
<td>This study⁷</td>
</tr>
</tbody>
</table>

⁸ ORFs along with their regulatory regions were cloned by gap-repair as described by Oldenburg K.R. et al.⁴
⁹ A truncated fragment was generated by digesting YEplac195-EAP1-TOR2 with *kpnl* to excise the fragment of TOR2 containing the rapamycin domain. Next the linearized plasmid was gel-purified and self-ligated to yield YEplac195-EAP1-TOR2-trunc

### Individual strain growth analysis.

For individual strain growth, yeast strains were grown to saturation (~20 h). Cells were then diluted to an OD₆₀₀ of 0.02 in a final volume of 100ul. Normalized cultures were grown in the presence of drug or diluent control in 96-well plates (Nunc, Rochester, New York, United States) using Tecan GENios microplate readers (Tecan, Austria) for up to 30 h. The growth rate of each culture was monitored by measuring the OD₆₀₀ every 15 minutes and the average doubling time (AvgG) was calculated as previously described¹⁰. Growth experiments for deletion strains were performed in YPD while those for multicopy suppressors were performed in synthetic complete media lacking uracil (SCM URA⁻).

### Genomic DNA preparation, TAG PCR, and microarray hybridization.

Genomic
DNA preparation, PCR amplification of molecular tags, and microarray hybridization were performed as previously described\textsuperscript{11} with the following modification. Cells collected from the essential heterozygous deletion pool and the homozygous deletion pool were combined in 1:2 ratio before genomic DNA preparation, TAG PCR and microarray hybridization.

**Plasmid isolation and insert PCR amplification and microarray hybridization.**

For MSP screens, plasmids were isolated using the Zymoprep II plasmid isolation kit (Zymoresearch; Catalog number D2004). The inserts were amplified by PCR with the FailSafe\textsuperscript{TM} PCR System (EPICENTRE Biotechnologies) using common M13 primers (M13 forward primer: 5' - GTT GTA AAA CGA CGG CCA GT - 3'; M13 reverse primer: 5' - CAG GAA ACA GCT ATG ACC - 3'). PCR cycling conditions were: 95°C 2min; 95°C 0.5min; 58°C 0.5min; 68°C 10min; 30 cycles; 68°C 15 min. The PCR products were purified using QIAquick PCR purification kit (Qiagen; Catalog number 28104) and labeled with biotin using the BioPrime (Invitrogen; Catalog number 18094-011) labeling kit. Labeled products were hybridized to Affymetrix TAG4 arrays using the same protocols as described for TAG hybridizations\textsuperscript{11}.

**Microarray Analysis.** Both DSP and MSP were analyzed using a high-density oligonucleotide tag array manufactured by Affymetrix\textsuperscript{13}. MSP for *C. albicans* was analyzed using a custom high-density oligonucleotide Genechip\textsuperscript{©} also manufactured by Affymetrix\textsuperscript{12} (PN=510556). For Deletion Sensitivity Profiling (DSP), barcode probe intensities were extracted and processed as previously described\textsuperscript{11}. Each array was mean normalized and fold change (log\textsubscript{2} control/treatment) was calculated by comparing to a set of control arrays. Tags from the homozygous pool were normalized separately from tags from the heterozygous essential pool, as were the upstream tags (uptag) and downstream tags (downtag). At least two biological replicates were carried out for each treatment condition. The log\textsubscript{2} ratios of both tags were averaged to generate a single score for each gene. For multicopy suppression profiling (MSP), ORF probe intensities were extracted and
processed in the same way as the barcode probes. Each ORF is represented by at least 2 probes and the log$_2$ ratios of each probe were averaged to generate a single score for each gene. To identify each suppressor locus, the log$_2$ ratio of intensities were ordered by each ORF’s genomic location and analyzed using a sliding window to identify locus that have at least 2 adjacent ORFs with log$_2$ ratios $\geq$ 1.6. The same analysis was applied to the C. albicans expression array. Vector analysis was used to identify strains that were significant in both assays$^{13}$. Z-scores of log$_2$ ratios from both assays are represented jointly as a vector in a Cartesian plane and various sectors of the plane correspond to different response patterns. An average vector $V_{\text{REP}}$ was calculated as described previously$^{13}$. Significance was determined using a procedure described by Breitling et al. $^{13}$. Array labels were shuffled and vector analysis was carried out. This was repeated 1000 times to generate a null distribution of the average vectors $V_{\text{REP}}$. We then counted the number of simulated $V_{\text{REP}}$ vectors, $x(V_{\text{REP}})$, that have lengths greater than or equal to a given experimental $V_{\text{REP}}$ length and calculated the average expected value $E(V_{\text{REP}}) = x(V_{\text{REP}})/1000$. Subsequently for each gene, we calculated an estimate of the percentage of false-positives if this gene was considered as significantly represented in both assays $q_i = E(V_{\text{REP}})/\text{rank}(g)$ where rank(g) represents the position of ORF $g$ in a list of all genes sorted by decreasing $|V_{\text{REP}}|$ values. Activity scores for DSP and MSP in Table 1 were calculated independently using log ratios of array intensities. For each gene $i$ with compound $j$, two activity scores were calculated: $A_{i,j,\text{DSP}} = D_{i,j} \cdot \sigma(D_j)$ and $A_{i,j,\text{MSP}} = M_{i,j} \cdot \sigma(M_j)$ where $D_{i,j}$ is the log$_2$ ratio of gene $i$, screened in compound $j$ in DSP, $M_{i,j}$ is the log$_2$ ratio of gene $i$, screened in compound $j$ in MSP and $\sigma(D_j)$ and $\sigma(M_j)$ are the standard deviation of log ratios for all genes screened in compound $j$ for DSP and MSP respectively. The two activity scores were then combined by multiplying them: $A_{i,j,\text{Combined}} = A_{i,j,\text{DSP}} \cdot A_{i,j,\text{MSP}}$. For, Table 1, activity scores were filtered such that only gene-compound with activity scores $> 5$ for both DSP and MSP were reported. All analyses were performed in MATLAB (MathWorks). GO-slim analysis of deletion sensitivity profiles in Supplementary Fig. 3c online was performed using the
GO-slim tool at the Saccharomyces Genome Database \(^1\) website. Enrichment difference between both compounds was calculated by taking the difference between the fraction of genes mapped to each GO-slim annotation.

**Affinity precipitation of Glc7.** Microcystin-LR agarose was obtained from Upstate Biotechnologies (Millipore). The Glc7 protein was tagged at the C-terminus with 13 copies of the myc-epitope by integrating a PCR product containing the epitope and a Kan\(^{\text{r}}\) marker at the GLC7 locus. Correct insertion of the epitope was confirmed by PCR, and immunoblotting with antibodies directed against the myc epitope (Santa Cruz Biotechnology). 10ml cultures of the GLC7-myc strain were grown to an OD\(_{600}\) of 1.0. Cells were pelleted and washed 2X in cold RIPA buffer containing a yeast protease inhibitor cocktail (RIPA-I, Sigma; Catalog number P8215). Cells were then resuspended in 2ml of RIPA containing \(\frac{1}{4}\) volume of glass beads (500microns, Sigma; Catalog number G9268) and disrupted by vortexing 5X (30 sec vortexing followed by 30 sec on ice). Lysates were clarified by centrifugation at 14,000XG for 15min. The supernatant was pre-cleared by incubation with unconjugated agarose, followed by centrifugation. 200\(\mu\)l aliquots of lysate were mixed with each compound (10\(\mu\)M final concentration), and then 50\(\mu\)l of microcystin-conjugated agarose beads were added. Lysates were incubated for 1h at 4\(^{\circ}\)C, then beads were washed 5X in RIPA-I and resuspended in 50\(\mu\)lSDS-PAGE sample buffer. Proteins were separated by SDS-PAGE on a 10% Tris-Glycine acrylamide gel (Invitrogen Catalog number EC60752BOX) and electroblotted to PVDF using tank transfer. Membranes were immunoblotted with an anti-myc mouse monoclonal antibody and HRP-labelled goat anti-mouse secondary antibodies, with the signal captured on hyperfilm (Amersham).

**Specificity of DSP and MSP.** To demonstrate the efficacy of our approach, we performed
a genetic complementation screen using a well-characterized and highly specific kinase inhibitor. Specifically, mutation of the highly conserved ‘gatekeeper’ residue in the ATP binding pocket of the CDC28 kinase renders this allele specifically sensitive to inhibition by the ATP analog 1-NM-PP1(1). A multicopy suppressor pool was generated in a heterozygous diploid strain that contained a single cdc28 analog-sensitive allele and no other endogenous source of CDC28. This pool was grown in a concentration of 1-NM-PP1 that slowed growth by roughly 50% (Supplementary Fig. 1c online) We plotted the fold change in response to 1-NM-PP1 for all genes and found 4 genes (CDC28, CSH1, IFA38 and TOS1) to be highly overrepresented in the 1-NM-PP1-treated sample (Supplementary Fig. 1d online). While the identification of CDC28 likely reflects its ability to confer resistance to 1-NM-PP1, CSH1, IFA38 and TOS1 were not predicted to confer resistance. These genes all flank the CDC28 locus (Supplementary Fig. 1d online) and thus were likely amplified from the same, or multiple CDC28-containing genomic clones. Even though genomic library inserts (~5kb in size) may contain several genes from the same linkage group, integrating deletion sensitivity data (see below) can often identify the true genetic modifier of drug resistance. In practice, the co-occurrence of neighboring genes is also used to support the detection of the relevant gene. Moreover, for genes of interest, the screen results are confirmed by repeating the experiments using isogenic cultures.

We also used the engineered target, cdc28-AS, to assess the noise in the DSP assay. The cdc28-AS strain described above, containing a unique barcode, was added to the heterozygous deletion pool and screened with 1-NM-PP1. Because only one strain in the pool contains the analog sensitive allele, we expected that only this strain would be sensitive to the inhibitor and by extension, any other strain identified would represent the noise inherent in our assay. As predicted, only the cdc28-AS strain was sensitive to the inhibitor (Supplementary Fig. 1e online). In contrast, the inhibitor had no effect on the heterozygous deletion strain containing the wildtype copy of CDC28 or the other 6000 heterozygous strains in the pool, demonstrating that both assays have very low technical
noise. It is important to note that this extremely "clean" profile represents a departure from the norm; most compounds exhibit multiple sensitive strains, strengthening the argument for integrating complementary assays.

**Dose response surface analysis.** We provide a description of the methods used to analyze the dose response surfaces generated in Fig. 4a and Supplementary Fig. 5 online. For detailed methods and theoretical explanations of the models, the reader is kindly referred to the paper by Lehár et al. 15 from which we based the analysis described herein.

Data:
For each drug, 1.25-fold serial drug dilutions were performed in DMSO. Wildtype yeast (BY4743) were grown overnight to saturation and diluted into YPD media to an OD$_{600}$ of 0.2 and aliquoted in an 8x8 matrix (96-well plate). Each drug combination pair was then added to yield the dose response matrix and grown using Tecan GENios microplate readers for up to 30h. At least two replicates were conducted for each growth condition. We used area under the growth curve (AUGC) as a metric to capture both defects in growth rate and carrying capacity. For each well, we measured AUGC and normalized it to the AUGC of the no-drug control to produce an 8x8 growth fitness matrix (available as supplementary data on the authors’ website). For the analysis described here, this matrix is converted into inhibition matrix using the equation $I = 1 - D$ where $I$ is the inhibition matrix and $D$ the growth fitness matrix.

**Response Surface Modeling:**

Sigmoidal dose response $I = E.C^n / (S^n + C^n)$ as a function of concentration $C$ were fitted to single drug data where $E$ is the limiting response at high concentration, $S$ is the effective concentration, and $a$ is the Hill coefficient determining the steepness of transition. We used these fitted curves for the surface model analysis.

The HSA model is based on the concept that an interaction exists when an effect of the combination exceeds the maximum effect exerted by any single components. i.e. $I_{HSA} = \max(I_x, I_y)$ where $I_x$ and $I_y$ are effects of the single agents at their respective concentrations.
of X and Y. Loewe additivity is the concept that zero interaction occurs when the response produced by the combination is the additive response of the single components.

An iterative approach was used to find the inhibition $I_{Loewe}$ that satisfied the Loewe additivity combination index (CI) equation: $(X/X_i) + (Y/Y_i) = 1$ where $X_i$ and $Y_i$ are the single-agent effective concentrations. For each concentration pair, the single agent curves were interpolated to find $X_i$ and $Y_i$ that produced I and the corresponding combination index CI was calculated. The equation was solved iteratively using nonlinear least squares (lsqnonlin) with MATLAB to converge on a value of I that produced $CI = 1$.

We used the Lehár et al.\textsuperscript{15} ‘Bliss boosting’ model for boost levels other than multiplicative. Dose response surfaces were fit to the equation: $I_{BLISS} = I_X + I_Y + (\beta - E_{\text{min}})(I_X/I_Y/E_X E_Y)$, where $E_{\text{min}} = \min(E_X, E_Y)$ and $E_X$ and $E_Y$ are the limiting single agent response. $\beta$ is the amount of boosting above $E_{\text{MAX}}$, the greater of the single agent response. The type of boosting can be further classified by using different reference levels: $\beta = -E_{\text{max}}$ is ‘canceling’, $\beta = E_{\text{min}} - E_{\text{max}}$ is ‘suppressing’, $\beta = 0$ is ‘masking’, $\beta = E_{\text{min}}(1 - E_{\text{max}})$ is multiplicative (Bliss independence) and $\beta = (1 - E_{\text{max}})$ is ‘saturating’. The reference level that most closely matches the $\beta$ value obtained from the fit is assigned. Since Bliss boosting can only be used provided that the single agent responses increases monotonically, nocodazole was excluded from this analysis as inhibition for this compound decreases after 8.2ug/ml.

Finally, a power-law potentiation model was used to describe a combination where one drug’s curve is shifted with a power-law slope $p$ above an enhancer concentration $Y_{POT}$.\textsuperscript{15} The inhibition $I_{POT} = \max(I_X(C), I_Y)$ where $I_X(C)$ is the single agent response curve of the potentiated compound at a shifted concentration $C = X[1 + (Y/Y_{POT})^p]\text{sign}(p)$ where $\text{sign}(p)$ is a unit sign with values (-1, 0, +1). The free parameters of this model $p$ and $Y_{POT}$ are estimated using the Nelder-Mead method (the fminsearch function in MATLAB was used). For Bliss boosting and potentiation models, parameters were estimated by minimizing sum
of the squared errors between the model and the observed dose response surface: $SS = \Sigma_{data} \left(I_{data} - I_{model}\right)^2$. For each compound pair, all four model surfaces were evaluated and the model with the overall best fit with the least sum of squared errors was assigned to that combination. A best fit was chosen from HSA, Loewe, Bliss boosting, and potentiation in that order depending on which was first consistent with $SS_{min}$, the minimum sum-of-squares error. Consistency was defined using a tolerance threshold defined as: $SS - SS_{min} < 0.1$. The results from these analysis is summarized in the following table:

<table>
<thead>
<tr>
<th>Combination</th>
<th>Model Assigned</th>
<th>$SS$</th>
<th>Parameter fits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canth-Canth</td>
<td>Loewe Additivity</td>
<td>0.079</td>
<td>-</td>
</tr>
<tr>
<td>Canth-Caly. A</td>
<td>Potentiation</td>
<td>0.66</td>
<td>$p = 0.84, Y_{POT} = 41\mu M$</td>
</tr>
<tr>
<td>Canth-Rapa</td>
<td>Bliss Boost (suppressing)</td>
<td>1.16</td>
<td>-0.66</td>
</tr>
<tr>
<td>Canth–Meth</td>
<td>Loewe</td>
<td>0.20</td>
<td>-</td>
</tr>
<tr>
<td>Canth-Noc</td>
<td>Not assigned</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Canth–Mech</td>
<td>Loewe</td>
<td>0.23</td>
<td>-</td>
</tr>
<tr>
<td>Canth–Lat. A</td>
<td>Loewe</td>
<td>0.13</td>
<td>-</td>
</tr>
<tr>
<td>Canth–MMS</td>
<td>Bliss Boost (saturating)</td>
<td>0.20</td>
<td>$\beta = 0.28$</td>
</tr>
</tbody>
</table>

Caly. A – Calyculin A  
Rapa – Rapamycin  
Meth – Methorexate  
Noc – Nocodazole  
Mech – Mechlorethamine  
Lat. A – Latrunculin A  
MMS - Methyl methanesulfonate

**Yeast Interaction Network.** Genetic, physical interaction data for *S. cerevisiae* were obtained from BioGRID. For this analysis, physical interactions included interactions
identified by affinity capture, biochemical activity, co-crystal, co-fractionation, co-purification, reconstituted complex. Genetic interactions included interactions identified by synthetic lethality, synthetic rescue, synthetic growth defect, dosage rescue, dosage lethality and dosage growth defect. Genetic congruency interactions were determined from synthetic lethal interactions using a congruency score >= 10 as described by Ye et al.\textsuperscript{17}. Co-fitness interactions were obtained by calculating the correlations of strain fitness in a compendium of chemical-genetic fitness profiles (Hillenmeyer et al.,\textit{submitted}). For the purpose of this analysis, we combined all interactions to create a merged network to contextualize the effect of the drug cocktail (\textbf{Fig. 4d, Supplementary Fig. 6a} online). Networks were visualized in Cytoscape\textsuperscript{18} and the MCODE plugin\textsuperscript{19} was used to identify highly connected modules using the default parameters. The search for modules was restricted to the set of physical interactions to identify complexes. For each module identified, we calculated an average drug interaction score $S_j = \frac{1}{n} \sum_{i=1}^{n} \epsilon_i$, where $\epsilon_i$ represents the drug interaction value for gene $i$ in module $j$ and $n$ represents the number of nodes in that module. Nodes without an assigned $\epsilon$ (gray nodes) were excluded from this analysis.

References


2. Sherman, F., Fink, G. R., Hicks, J. B. & Cold Spring Harbor Laboratory. in \textit{Laboratory course manual for methods in yeast genetics} 186 (Cold Spring Harbor Laboratory, New
York, N.Y., 1986).


