Figure S1

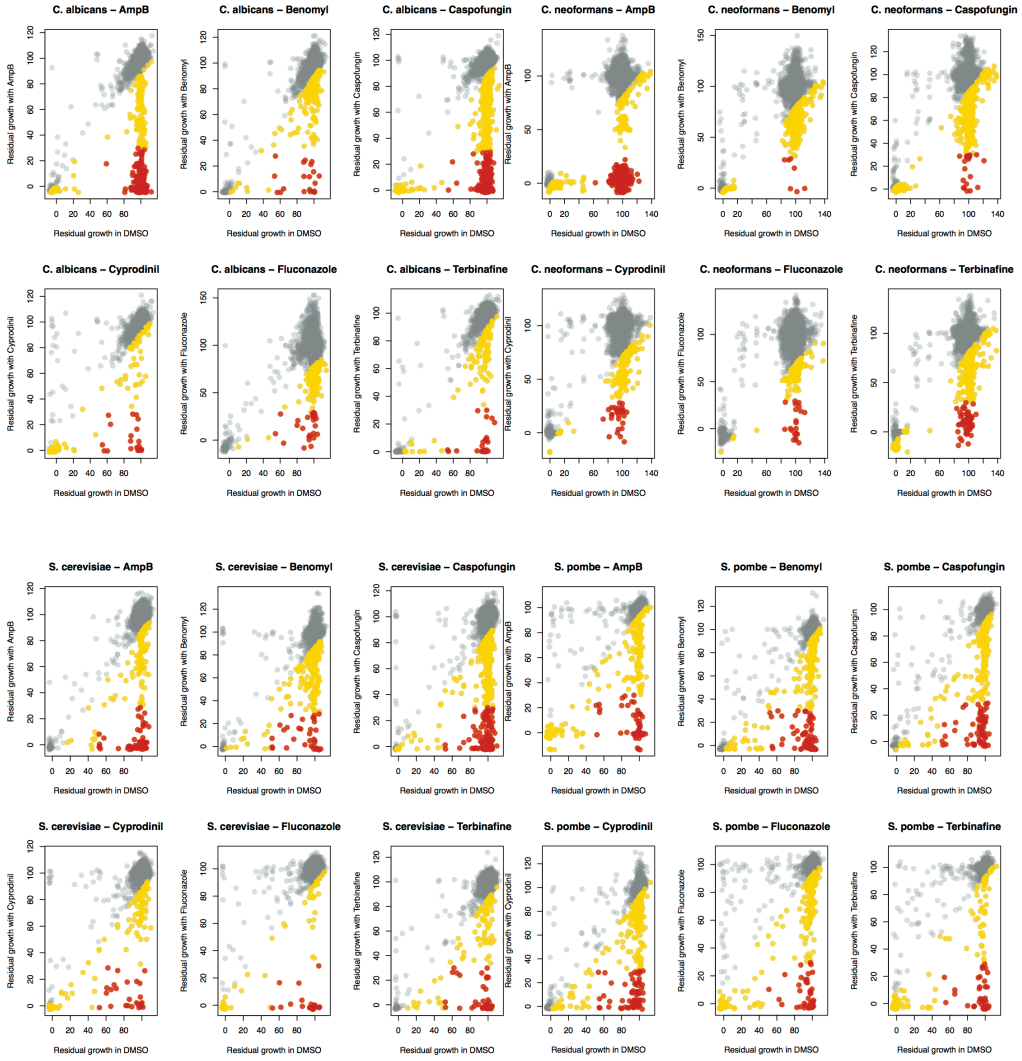

C. albicans – AmpB
C. albicans – Benomyl
C. albicans – Caspofungin
C. neoformans – AmpB
C. neoformans – Benomyl
C. neoformans – Caspofungin
C. albicans – Cyprodinil
C. albicans – Fluconazole
C. albicans – Terbinafine
C. neoformans – Cyprodinil
C. neoformans – Fluconazole
C. neoformans – Terbinafine
S. cerevisiae – AmpB
S. cerevisiae – Benomyl
S. cerevisiae – Caspofungin
S. pombe – AmpB
S. pombe – Benomyl
S. pombe – Caspofungin
S. cerevisiae – Cyprodinil
S. cerevisiae – Fluconazole
S. cerevisiae – Terbinafine
S. pombe – Cyprodinil
S. pombe – Fluconazole
S. pombe – Terbinafine
Residual growth in DMSO

Figure S2

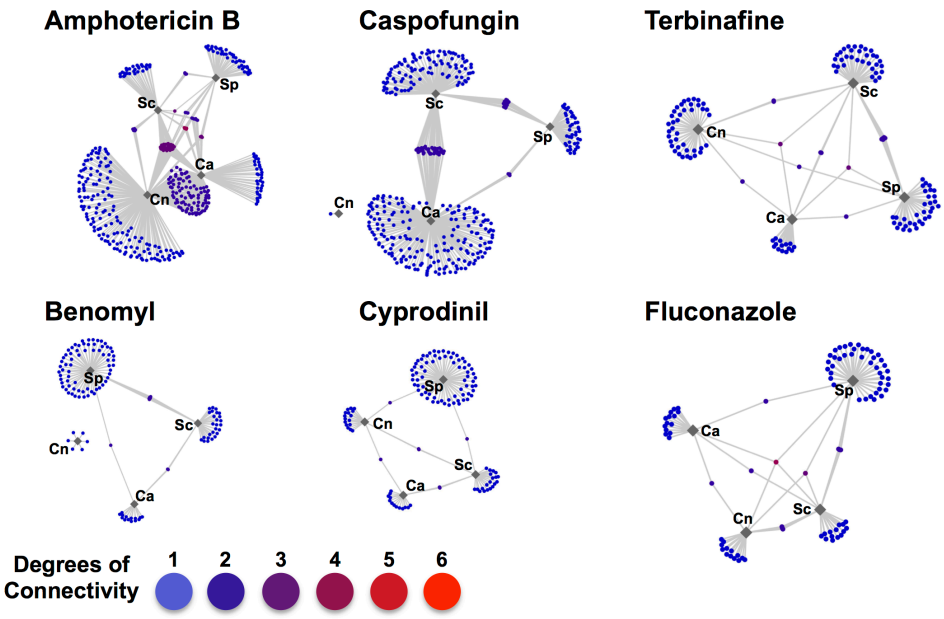

Amphotericin B
Sp
Sc
Ca
Cn
Caspofungin
Sc
Sp
Cn
Ca
Terbinafine
Sc
Cn
Sp
Ca
Benomyl
Sp
Sc
Cn
Ca
Cyprodinil
Sp
Cn
Sc
Ca
Fluconazole
Sp
Ca
Cn
Sc
Degrees of Connectivity
1
2
3
4
5
6

Figure S3

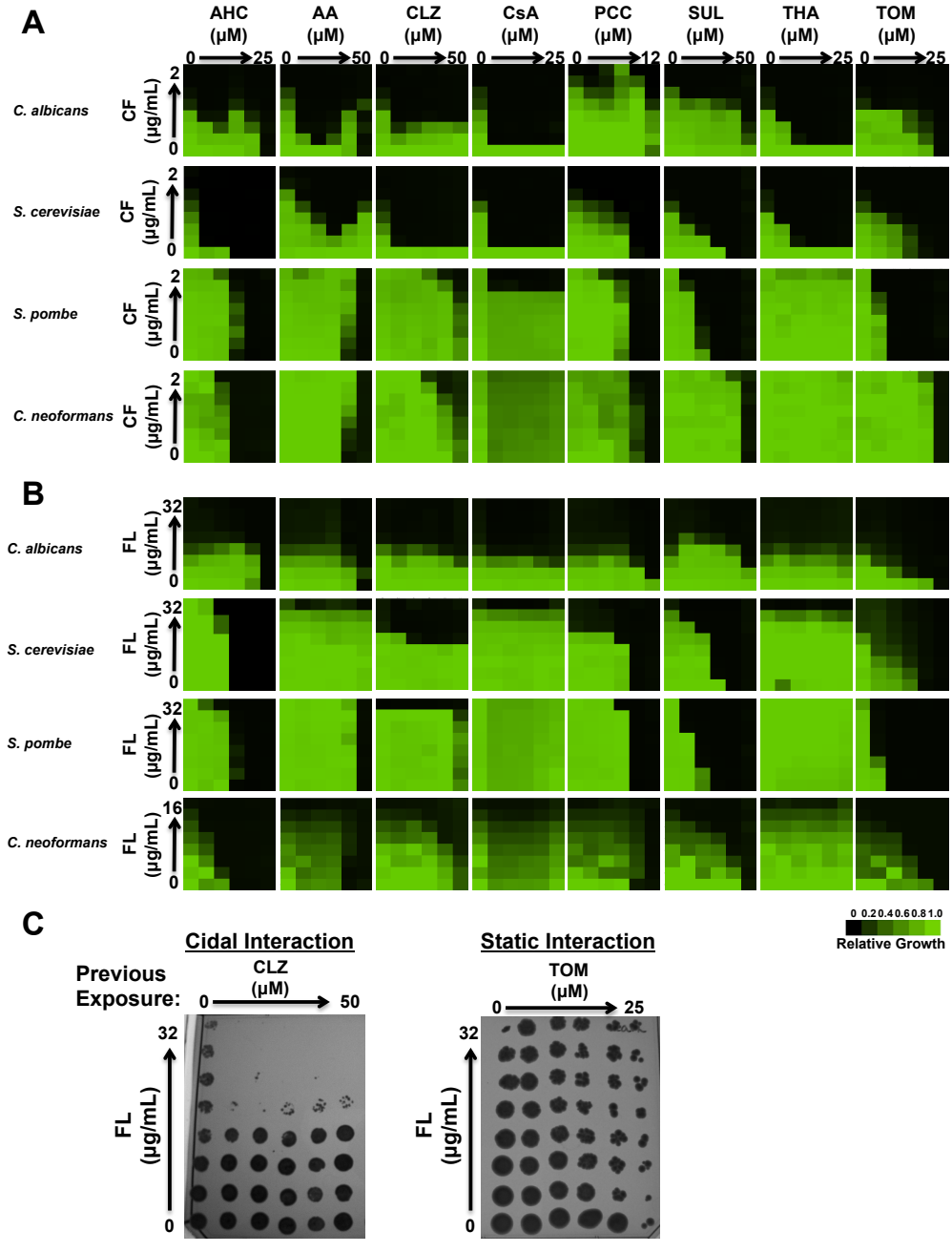

A
AHC (μM)
AA (μM)
CLZ (μM)
CsA (μM)
PCC (μM)
SUL (μM)
THA (μM)
TOM (μM)
0 → 25
0 → 50
0 → 50
0 → 25
0 → 12
0 → 50
0 → 25
0 → 25
C. albicans
S. cerevisiae
S. pombe
C. neoformans
CF (μg/mL)
2
0
B
FL (μg/mL)
32
16
0
C
Cidal Interaction
Static Interaction
Previous Exposure:
CLZ (μM)
0 → 50
TOM (μM)
0 → 25
0 0.2 0.4 0.6 0.8 1.0
Relative Growth

Figure S4

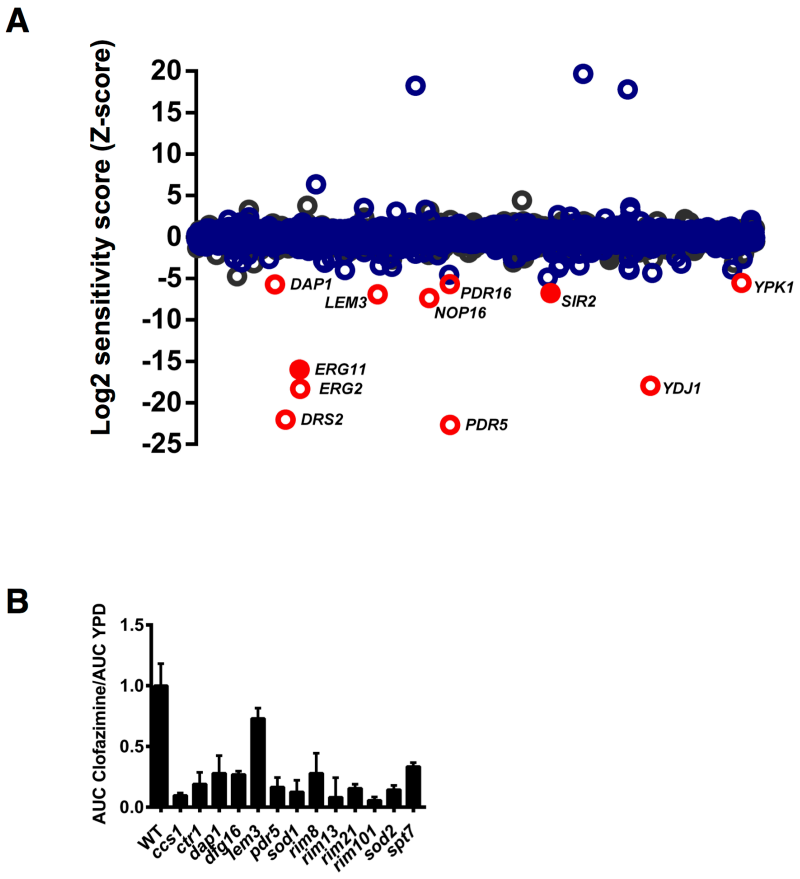

A
Log2 sensitivity score (Z-score)
20
15
10
5
0
-5
-10
-15
-20
-25
DAP1
LEM3
PDR16
NOP16
SIR2
YPK1
ERG11
ERG2
DRS2
PDR5
YDJ1
B
AUC Clofazimine/AUC YPD
1.5
1.0
0.5
0.0
WT
ccs1
ctr1
dap1
dig16
lem3
pdr5
sod1
rim8
rim13
rim21
rim101
sod2
spt7

Figure S5

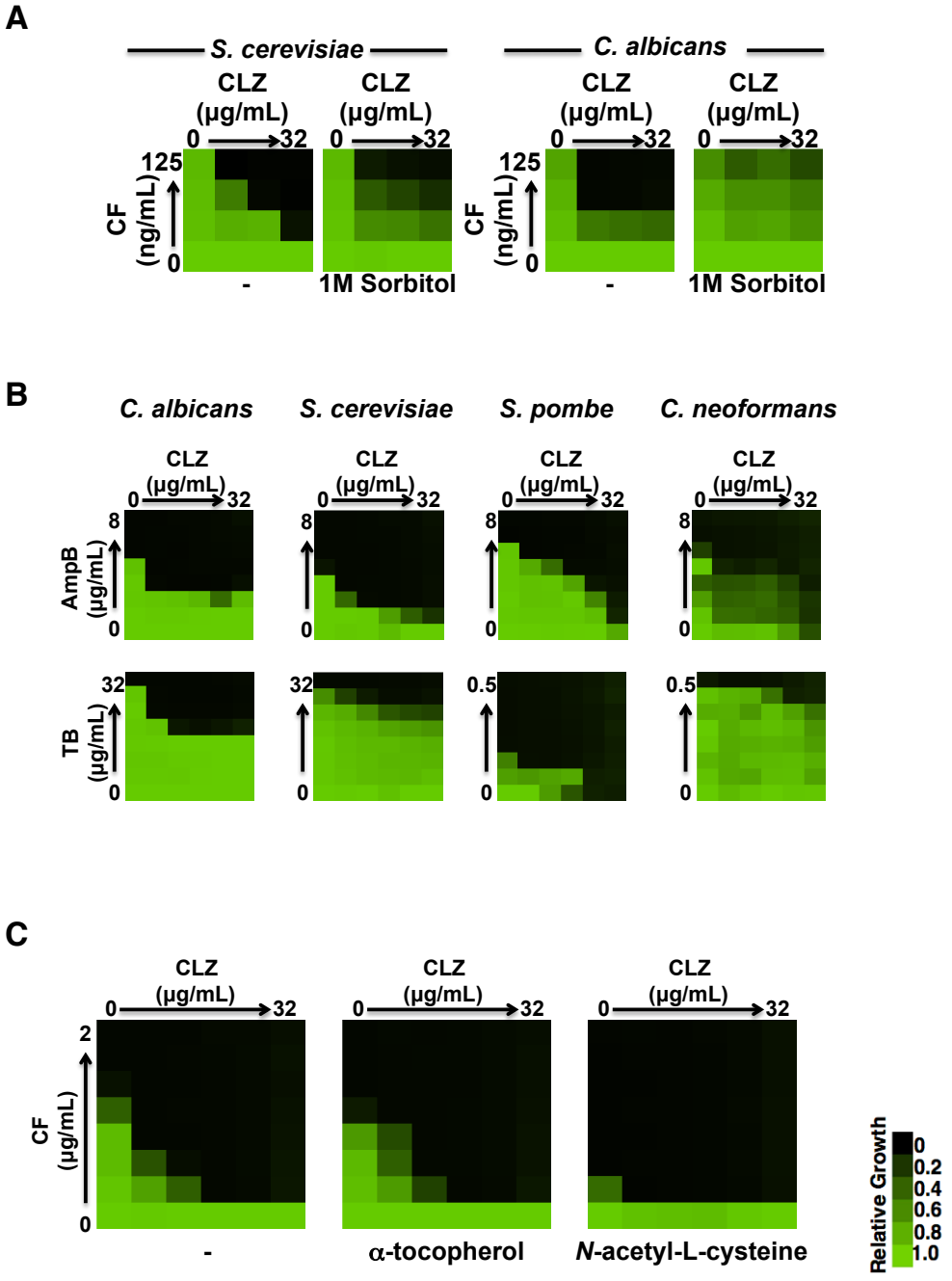
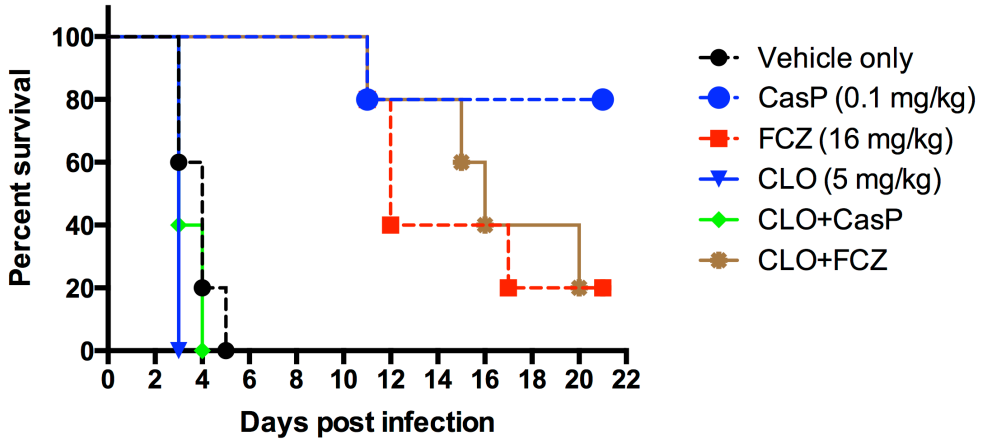

A
S. cerevisiae
C. albicans
CLZ (μg/mL)
0
32
125
0
CF (ng/mL)
-
1M Sorbitol
B
C. albicans
S. cerevisiae
S. pombe
C. neoformans
AmpB (μg/mL)
8
TB (μg/mL)
32
0.5
C
2
α-tocopherol
N-acetyl-L-cysteine
Relative Growth
0
0.2
0.4
0.6
0.8
1.0

Figure S6


Percent survival
100
80
60
40
20
0
0 2 4 6 8 10 12 14 16 18 20 22
Days post infection
Vehicle only
CasP (0.1 mg/kg)
FCZ (16 mg/kg)
CLO (5 mg/kg)
CLO+CasP
CLO+FCZ

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Primary screening data for bioactive compounds that potentiate antifungals in four fungal species. The scatterplots shows the normalized residual growth of *C. albicans* (top left), *C. neoformans* (top right), *S. cerevisiae* (bottom left), and *S. pombe* (bottom right) for each compound in the absence (x axis) and presence of antifungal (y axis). Those compounds that are 3 MADs below the diagonal were colored yellow. Compounds that were 3 MADs below the diagonal, inhibited growth less than 50% without antifungal, and inhibited growth greater than 80% with antifungal are colored red. Red compounds were classified as hits and were used for comparative analysis. Related to Figure 1 and Figure 2.

Figure S2. Chemical-species interaction networks. Chemical-species networks are generated for each individual antifungal. Compounds that potentiated an antifungal are depicted as circles and are connected by edges to the species in which synergy was observed. Each circle is color-coded to reflect its degree of connectivity (frequency of occurrence in the screens). Species in which the screen was conducted are shown as gray diamonds. (*C. albicans* (Ca), *C. neoformans* (Cn), *S. cerevisiae* (Sc) or *S. pombe* (Sp)). Related to Figure 1 and Figure 2.

Figure S3. ACM hit validation and characterization. A-B) Checkerboard assays confirm drug interactions between those compounds identified as hits during screening and caspofungin (CF) **(A)** or fluconazole (FL) **(B)**. Two-fold serial dilutions of CF or FL were combined with two-fold dilutions of the following compounds: Amiodarone Hydrochloride (AHC), Asiatic Acid (AA), Clofazimine (CLZ), Cyclosporin A (CsA), Palmitoyl-DL-Carnitine Chloride (PCC), Suloctidil (SUL), Thapsigargin (THA), and

Tomatidine (TOM). Growth was measured by OD_{600} and data was quantitatively displayed with color using Treeview (see color bar). **C)** Examples of cidal and static drug interactions. Checkerboard assays with two-fold dilutions of FL in combination with CLZ or TOM in *C. albicans* were performed in SC and incubated for 48 hours at 30°C. Cells from the checkerboard assays were spotted onto YPD medium and incubated at 30°C for 48 hours before plates were photographed. Related to Figure 3 and Table S5.

Figure S4. Genome-wide chemical-genetic interactions for fluconazole and validation of clofazimine sensitive mutants. A) Sensitivity of heterozygous essential deletion strains (gray circles) and homozygous deletion strains (blue circles) to clofazimine as assessed by haploinsuffucency (HIP) and homozygous deletion profiling (HOP). Those deletion strains that have a Z-score more significant than three standard-deviations below the mean are highlighted in red, filled circles represent heterozygous strains and open circles represent homozygous strains. *ERG11*, the known target of fluconazole, was the most sensitive heterozygous deletion strain. The fluconazole exporter *PDR5,* as well as other genes known to be sensitive to fluconazole were identified by HOP analysis. **B)** Deletion strains identified in the HIP-HOP analysis were confirmed to be sensitive to CLZ by growth curve analysis. Individual mutants were grown in the absence and presence of CLZ over 48 hours and the area under the curve (AUC) was calculated. Data are means $\pm$ standard deviation of triplicates. Related to Figure 5.

Figure S5. Clofazimine is potentiates antifungals in yeast due to perturbation of membranes, not through the generation of oxidative stress. A) Two-fold serial dilutions of caspofungin (CF) were combined with two-fold dilutions of clofazimine

(CLZ) in the absence or presence of 1M sorbitol in SC media. Growth was measured by OD_{600} and data was quantitatively displayed with color using Treeview (see color bar). **B)** Two-fold serial dilutions of amphotericin B (AmpB) or terbinafine (TB) were combined with two-fold dilutions of clofazimine (CLZ). Growth was analyzed as in part A. **C)** Two-fold serial dilutions of caspofungin (CF) were combined with two-fold dilutions of CLZ in the absence and presence of antioxidants α-tocopherol (12.5μg/mL) or *N*-acetyl-L-cysteine (5mM). Data was analyzed as described in part A. Related to Figure 6.

Figure S6. Clofazimine does not potentiate antifungals in a mammalian model of fungal infection. CD1 mice were infected with an inoculum of 200 μL of 1 x 10^6 CFU/mL of SC5314. Fluconazole (16 mg/kg) and caspofungin (0.1 mg/kg) were administered i.p. and clofazimine (5 mg/kg) was administered by oral gavage. Drugs were given 4 h, 24 h, 48 h, and 72 h post-infection. Viability was scored daily. Related to Figure 7.

Table S1. ¼ Minimum Inhibitory Concentration (MIC) of antifungals against different fungal species used for the generation of the ACM. Related to Figure 1.

Compound	*C. albicans* MIC (μg/mL)	*C. neoformans* MIC (μg/mL)	*S. cerevisiae* MIC (μg/mL)	*S. pombe* MIC (μg/mL)
Amphotericin B	0.25	1	0.25	0.25
Benomyl	8	2	2	1
Caspofungin	0.13	2*	0.13	0.5
Cyprodinil	16	16	8	2
Fluconazole	0.5	8	8	64
Terbinafine	4	1	16	0.02

* Since we were unable to calculate a MIC for caspofungin against *C. neoformans* we used a concentration of 2 μg/mL for the primary screen.

Table S2. Normalized Primary Screening Data. Related to Figure 1 and Figure 2.

Table S3. Compounds Identified as Antifungal Potentiators in the Antifungal Combination Matrix. Related to Figure 1 and Figure 2.

Table S4. Compounds Identified in the ACM that Potentiated Indicated Antifungal. Related to Figure 3.

Compound	Therapeutic Use	Screen Identified (Species: Antifungal)
Amiodarone Hydrochloride	• Na^+/K^+ channel blocker • Antiarrhythmic	*C. albicans*: AmpB, CF *C. neoformans*: AmpB, FL *S. cerevisiae*: AmpB, CF, FL
Asiatic Acid	• Anticancer	*C. albicans*: AmpB, CF, FL *C. neoformans*: AmpB *S. cerevisiae*: AmpB, CF *S. pombe*: AmpB
Clofazimine	• Antileprosy agent • Antimycobacterial agent	*C. albicans*: AmpB, CF, TB *C. neoformans*: AmpB
Cyclosporin A	• Immunosuppressant • Calcineurin inhibitor	*C. albicans*: AmpB, CF, TB *C. neoformans*: AmpB, Cyp, FL, TB *S. cerevisiae*: AmpB, CF, TB
Palmitoyl-DL-Carnitine Chloride	• Protein Kinase C inhibitor	*S. cerevisiae*: AmpB, Ben, CF, Cyp, FL, TB
Suloctidil	• Vasodilator • Hypocholesterolemic drug	*C. albicans*: CF *C. neoformans*: AmpB, Cyp, FL *S. cerevisiae*: CF, FL, TB *S. pombe*: FL, TB
Thapsigargin	• Sarco-endoplasmic reticulum Ca(2+)-ATPases inhibitor	*C. albicans*: AmpB, CF *C. neoformans*: AmpB *S. cerevisiae*: AmpB, Ben, CF, FL, TB
Tomatidine	• Antifungal	*C. albicans*: Ben, CF, Cyp, FL, TB *C. neoformans*: FL *S. cerevisiae*: Ben, CF, Cyp, FL, TB *S. pombe*: FL, TB

Table S5. ACM compound chemical interactions with antifungals. Calculated Fractional Inhibitory Concentration Index. Related to Figure 3.

Strain	ACM Compound	MIC ACM Compound (μM)	FIC[1] ACM Compound	MIC caspofungin (μg/mL)	FIC[1] caspofungin	FIC Index[2]
C. albicans	AHC	25	0.13	0.5	0.13	0.3
C. albicans	Asiatic Acid	50	0.13	0.5	0.06	0.2
C. albicans	Clofazimine	≥100	0.03	0.5	0.13	0.2
C. albicans	Cyclosporin A	≥50	0.06	0.5	0.06	0.1
C. albicans	PCC	25	0.5	0.5	0.5	1
C. albicans	Suloctidil	≥100	0.5	0.5	0.06	0.6
C. albicans	Thapsigargin	≥50	0.5	0.5	0.06	0.6
C. albicans	Tomatidine	25	0.5	0.5	0.06	0.6
S. cerevisiae	AHC	6.25	0.25	0.5	0.06	0.3
S. cerevisiae	Asiatic Acid	≥50	0.25	0.5	0.13	0.4
S. cerevisiae	Clofazimine	≥100	0.03	0.5	0.06	0.1
S. cerevisiae	Cyclosporin A	≥50	0.06	0.5	0.06	0.1
S. cerevisiae	PCC	6.25	0.5	0.5	0.25	0.8
S. cerevisiae	Suloctidil	25	0.5	0.5	0.06	0.6
S. cerevisiae	Thapsigargin	≥50	0.5	0.5	0.06	0.6
S. cerevisiae	Tomatidine	12.5	0.25	0.5	0.25	0.5
S. pombe	AHC	6.25	0.5	4	0.5	1
S. pombe	Asiatic Acid	50	1	4	0.5	1.5
S. pombe	Clofazimine	≥100	0.5	4	0.13	0.6
S. pombe	Cyclosporin A	≥50	0.06	4	0.25	0.3
S. pombe	PCC	6.25	0.5	4	0.25	0.8
S. pombe	Suloctidil	12.5	0.5	4	0.02	0.5
S. pombe	Thapsigargin	≥50	1	4	1	2
S. pombe	Tomatidine	3.125	0.5	4	0.13	0.6
C. neoformans	AHC	6.25	0.5	≥4	0.5	1
C. neoformans	Asiatic Acid	25	2	≥4	0.5	2.5
C. neoformans	Clofazimine	≥100	0.25	≥4	0.13	0.4
C. neoformans	Cyclosporin A	≥50	1	≥4	1	2
C. neoformans	PCC	12	1	≥4	1	2
C. neoformans	Suloctidil	50	0.5	≥4	0.5	1
C. neoformans	Thapsigargin	≥50	1	≥4	1	2
C. neoformans	Tomatidine	25	1	≥4	1	2

Strain	ACM Compound	MIC ACM Compound (μM)	FIC[1] ACM Compound	MIC fluconazole (μg/mL)	FIC[1] fluconazole	FIC Index[2]
C. albicans	AHC	25	0.5	2	1	1.5
C. albicans	Asiatic Acid	50	0.5	2	1	1.5
C. albicans	Clofazimine	≥100	1	2	1	2
C. albicans	Cyclosporin A	≥50	0.5	2	0.5	1
C. albicans	PCC	25	0.5	2	0.25	0.8
C. albicans	Suloctidil	≥100	0.5	2	0.5	1
C. albicans	Thapsigargin	≥50	1	2	1	2
C. albicans	Tomatidine	25	0.13	2	0.25	0.4
S. cerevisiae	AHC	6.25	0.5	32	0.5	1

S. cerevisiae	Asiatic Acid	≥50	1	32	1	2
S. cerevisiae	Clofazimine	≥100	0.06	32	0.25	0.3
S. cerevisiae	Cyclosporin A	≥50	1	32	1	2
S. cerevisiae	PCC	6.25	0.5	32	0.13	0.6
S. cerevisiae	Suloctidil	25	0.5	32	0.02	0.5
S. cerevisiae	Thapsigargin	≥50	0.5	32	0.5	1
S. cerevisiae	Tomatidine	12.5	0.25	32	0.03	0.3
S. pombe	AHC	6.25	0.5	64	0.5	1
S. pombe	Asiatic Acid	50	1	64	1	2
S. pombe	Clofazimine	≥100	0.5	64	0.25	0.8
S. pombe	Cyclosporin A	≥50	1	64	1	2
S. pombe	PCC	6.25	0.5	64	0.5	1
S. pombe	Suloctidil	12.5	0.5	64	0.02	0.5
S. pombe	Thapsigargin	≥50	1	64	1	2
S. pombe	Tomatidine	3.125	0.5	64	0.13	0.6
C. neoformans	AHC	6.25	0.5	16	0.02	0.5
C. neoformans	Asiatic Acid	25	1	16	1	2
C. neoformans	Clofazimine	≥100	0.25	16	0.13	0.4
C. neoformans	Cyclosporin A	≥50	1	16	1	2
C. neoformans	PCC	12	1	16	1	2
C. neoformans	Suloctidil	50	0.5	16	0.06	0.6
C. neoformans	Thapsigargin	≥50	1	16	1	2
C. neoformans	Tomatidine	25	0.25	16	0.06	0.3

[1] Fractional Inhibitory Concentration (FIC) = [X]/MICx, where [X] is the lowest inhibitory concentration of drug in the presence of the co-drug.
[2] FIC index = $FIC_{ACM\ compound} + FIC_{antifungal}$

Table S6. Dataset for Haploinsufficiency Profiling and Homozygous Deletion Profiling. Calculated Z-scores from all diploid and haploid deletion strain sensitivity profile. Related to Figure 5.

Table S7. Strains used in this study. Related to Figure 1.

Strain Name	Description	Genotype	Source
GDW39	*C. albicans* NCCLS 11	Wild type	ATCC # 90028
GDW361	*S. cerevisiae* BY4741	*MAT **a** his3Δ leu2Δ met15Δ ura3Δ*	(Giaever et al., 2002)
GDW508	*C.albicans* Y537	Amphotericin B Resistant Clinical Isolate	ATCC # 200955
GDW509	*C. tropicalis*	Wild type	ATCC # 200956
GDW655	*C. neoformans* H99	Wild type	Strain received from James W. Kronstad
GDW1004	*S. pombe*	Wild type	ATCC # 38366
GDW1584	*C. parapsilosis*	Wild type	ATCC # 22019
GDW1585	*C. albicans* Fluconazole Resistant Clinical Isolate	CAP-F-1-2008 LL#99383	Deborah Yamamura, St Joseph's Hospital, Hamilton ON
GDW1586	*C. albicans* Fluconazole Resistant Clinical Isolate	CAP-F-07-2007 LL#99359	Deborah Yamamura, St Joseph's Hospital, Hamilton ON
GDW1587	*C. glabrata*		Deborah Yamamura, St Joseph's Hospital, Hamilton ON
GDW2295	*A. fumigatus* Af293	Wild type	(Nierman et al. 2005)
GDW2590	*C. albicans,* SN95	*arg4Δ/arg4Δhis1Δ/his1Δ URA3/ura3Δ::imm434 IRO1/iro1Δ::imm434*	(Noble, SM and Johnson AD. 2005)
GDW2592	*C. albicans pkc1/pkc1*	As SN95, *CaTAR::HIS3 pkc1::FRT/ pkc1::FRT*	(Lafayette et. al.2010)
GDW2593	*C. albicans pkc1Δ/pkc1Δ + PKC1*	As SN95, *CaTAR::HIS3 pkc1::FRT/ pkc1::FRT::CaPKC1-FRT*	(Lafayette et. al.2010)
GDW2597	*S. cerevisiae*, SLT2-GFP	*MAT **a** his3Δ leu2Δ met15Δ ura3Δ*	(Huh, W-K et al. 2003)
Sc *bck1*	*S. cerevisiae* BY4741 *bck1Δ*	As BY4741, *bck1::KAN*	Homozygous Deletion Library
Sc *dfg16*	*S. cerevisiae* BY4741 *dfg16Δ*	As BY4741, *dfg16::KAN*	Homozygous Deletion Library

Sc *mkk2*	*S. cerevisiae* BY4741 *mkk2Δ*	As BY4741, *mkk2::KAN*	Homozygous Deletion Library
Sc *PKC1/pkc1*	*S. cerevisiae* BY4743 *PKC1/pkc1Δ*	As BY4743, *PKC1/pkc1::KAN*	Heterozygous Deletion Library
Sc *rim101*	*S. cerevisiae* BY4741 *rim101Δ*	As BY4741, *rim101::KAN*	Homozygous Deletion Library
Sc *rim13*	*S. cerevisiae* BY4741 *rim13Δ*	As BY4741, *rim13::KAN*	Homozygous Deletion Library
Sc *rim20*	*S. cerevisiae* BY4741 *rim20Δ*	As BY4741, *rim20::KAN*	Homozygous Deletion Library
Sc *rim21*	*S. cerevisiae* BY4741 *rim21Δ*	As BY4741, *rim21::KAN*	Homozygous Deletion Library
Sc *rim8*	*S. cerevisiae* BY4741 *rim8Δ*	As BY4741, *rim8::KAN*	Homozygous Deletion Library
Sc *rlm1*	*S. cerevisiae* BY4741 *rlm1Δ*	As BY4741, *rlm1::KAN*	Homozygous Deletion Library
Sc *slt2*	*S. cerevisiae* BY4741 *slt2Δ*	As BY4741, *slt2::KAN*	Homozygous Deletion Library
Sc *swi4*	*S. cerevisiae* BY4741 *swi4Δ*	As BY4741, *swi4::KAN*	Homozygous Deletion Library
Sc *swi6*	*S. cerevisiae* BY4741 *swi6Δ*	As BY4741, *swi6::KAN*	Homozygous Deletion Library

Table S8. Oligonucleotides used in this study. Related to Figure 4 and Figure 5.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Strains and Culture Conditions

Archives of *C. albicans* and *S. cerevisiae* were maintained at -80C in 25% glycerol. Strains were grown in either synthetic complete medium (SC: 0.67% Difco™ yeast nitrogen base w/o amino acids, 0.08% amino acid add back and 2% glucose) or in yeast peptone dextrose (YPD, 1% yeast extract, 2% bactopeptone, 2% glucose) as indicated. 2% agar was added for solid media. Strains used in this study are listed in Table S7.

High-Throughput Screening Conditions

The McMaster Bioactives collection, a compilation of chemicals purchased from Prestwick, Biomol, Sigma, and Microsource, was screened at a final concentration of 12.5 μM in the presence and absence of ¼ MIC antifungal (Amphotericin B, Benomyl, Caspofungin, Cyprodinil, Fluconazole and Terbinafine). Screens were performed using *C. albicans* (ATCC 90028)*, C. neoformans* (H99)*, S. cerevisiae* (BY4741)*,* and *S. pombe* (ATCC 38366). For each assay plate, the 32 high and 32 low growth controls were used to calculate residual growth within each well on the same assay plate as follows:

$$RG = ((A_{600} - (\mu - c)) \div ((\mu + c) - (\mu - c))$$

where μ+c, and μ-c are the A600 averages of the high-growth (+c) and low-growth (-c) controls. The robustness of the screen was measured by calculating the Z′ score and all screens achieved a Z' above 0.5 indicating an adequate screening window (Zhang, Chung et al. 1999). Screens were conducted in duplicate in 384-well flat bottom microtitre plates (ThermoScientific) with a final volume of 80 μL. A Beckman Biomek FX liquid handler (Beckman Coulter Inc., Fullerton, CA) was used to dispense 1 μL of McMaster Bioactive compound at a 1mM concentration and 1 μL of antifungal at 80X concentration used for the screen. This was followed by the addition of 78 μL of diluted cell culture prepared in SC. For preparation of diluted yeast culture, liquid overnight cultures in were diluted to an OD_{600} of 0.14, followed by a 1:1000 dilution in SC media. Positive growth controls (DMSO only) and negative growth controls (antifungal above the MIC) were included in rows 1, 2, 23 and 24 of each plate for normalization. Plates were incubated at 30°C. The OD_{600} was measured after 48 h of growth for *S. cerevisiae*

and *C. albicans* or 72 h of growth for *C. neoformans* and *S. pombe*. Growth controls on each plate were used to generate percentage growth data, and replicates were plotted against each other to identify outliers. All data was normalized for plate- and row/column-specific effects as described previously (Spitzer, Griffiths et al. 2011). To identify compounds that potentiated antifungals, growth in the presence of the antifungal was plotted against growth in the absence of the antifungal and data was fit to a linear model. Hits were considered those compounds that were 3 median absolute deviations (MADs) below the diagonal, inhibited growth on their own less than 50%, and resulted in at least 80% growth inhibition in the presence of the antifungal. Chemical-chemical interactions were generated using the program Cytoscape v3.2.1 (http://www.cytoscape.org).

Drug Susceptibility Assays

MIC and checkerboard assays were performed in U-bottom, 96-well microtiter plates (Fisher Scientific) using a modified broth microdilution protocol as described (LaFayette et al., 2010). In brief, assays were set up in SC media in a total volume of 0.2 mL/well with 2-fold dilutions of drug. Plates were incubated in the dark for 48 h-72 h, and absorbance was determined at 600 nm using a spectrophotometer (Molecular Devices). Each strain was tested in duplicate on at least two occasions. Data was quantitatively displayed with color using the program Java TreeView 1.1.6 (http://jtreeview.sourceforge.net). For growth curve analysis, overnight cultures were diluted to OD_{600} of 0.0625 with or without compound as indicated and grown at 30°C with continuous shaking, using the TECAN Sunrise. Optical density was measured at

595 nm every 15 min over 24 h – 48 h. Data was plotted and analyzed using GraphPad Prism.

Sequencing Analysis for Chemical Genomic Assays

The Illumina MiSeq platform and MiSeq Reagent kit v3 (2 x 75 bp) were used for sequencing. To avoid problems with cluster identification due to low diversity during the first 18 cycles, which correspond to the common deletion strain primer sequences, we started the run with 'dark cycle sequencing' through these first 18 nucleotides (sequencing chemistry occurs, but no imaging). Next Gen sequencing data were trimmed and counted with R scripts. PatMaN software was used to match sequences to barcodes (Prufer, Stenzel et al. 2008). All sequences with up to 3 mismatches to barcodes were kept. Log2 ratios of compound-treated versus DMSO-treated samples were generated and data was normalized by calculating Z-scores.

Sequencing of Fluconazole-Resistant Isolate of *C. albicans*

The fluconazole-resistant isolate of *C. albicans* was grown in YPD medium to late log-phase at which time gDNA was extracted using phenol-chloroform extraction methods. *ERG11, TAC1* and *UPC2* were amplified using primer combination indicated in Table S8. PCR products were sent for sequencing at the Farncombe Metagenomics Facility at McMaster University. Sequencing results were aligned relative to reference sequences obtained from *C. albicans* SC5314 Assembly 22.

Murine Model of *C. albicans* Infection.

For murine exposure, male CD1 mice (Charles River Laboratories) aged 5-6 weeks (20-25 g) were infected via the tail vein with 200 µl of a 1×10^6 CFU/ml PBS suspension of *C. albicans* SC5314. Each treatment group consisted of 5 mice. Fluconazole is purchased from the Duke Pharmacy Storeroom (Pfizer, 16 mg/kg) and caspofungin, also from Duke Pharmacy, (Merck, 0.1 mg/kg) were administered i.p. whereas clofazimine (Sigma, 5 mg/kg) was administered by oral gavage. Drugs were given injected at 4 h, 24 h, 48 h, and 72 h post infection. Mice were observed daily for signs of illness. All murine work was performed under a protocol, approved by the Institutional Animal Use and Care Committee at Duke University Medical Center.

Ethics Statement

Animals studies were conducted in the Division of Laboratory Animal Resources (DLAR) facilities at Duke University Medical Center (DUMC) in good practice as defined by the United States Animal Welfare Act and in full compliance with the guidelines of the DUMC Institutional Animal Care and Use Committee (IACUC). The vertebrate animal experiments were reviewed and approved by the DUMC IACUC under protocol number A114-14-05.

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