**Supplementary material** for: "Cross-species Discovery of Syncretic Drug Combinations that Potentiate the Antifungal Fluconazole" by M. Spitzer, E. Griffiths, K.M. Blakely, J. Wildenhain, L. Ejim, L. Rossi, G. De Pascale, J. Curak, E. Brown, M. Tyers, and G.D. Wright

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## Supplementary methods

### Fungal strains and culture conditions

All strains were stored at -80°C. The strains employed in this study were *Saccharomyces cerevisiae* BY4741, *Candida albicans* Caf2-1, *Candida albicans* F-1-2008 (clinical isolate), *Candida albicans* F-07-2007 (clinical isolate), *Candida parapsilosis* ATCC 22019, *Candida glabrata*, *Cryptococcus neoformans* H99 and *Cryptococcus gattii* R265. *S. cerevisiae* strains were maintained on synthetic complete (SC) agar (0.67% Difco™ yeast nitrogen base w/o amino acids, 0.08% amino acid add back, 2% glucose, 1.6% agar). *C. albicans* and *Cryptococcus* species were maintained on Sabauroud dextrose agar (Difco™). *Saccharomyces* and *Cryptococcus* strains were incubated at 30°C, while *Candida* strains were incubated at 37°C.

### Antifungal agents and chemicals

Fluconazole (Sandoz, Quebec, Canada) was purchased as a 2 mg/mL solution in water, sodium chloride and hydrochloric acid and/or sodium hydroxide. Albendazole (Sigma, Oakville, Canada), amphotericin B (Sigma), azaperone (Prestwick Chemical, Delaware, USA), clofazimine (Sigma), clomiphene citrate (Sigma), daunorubicin HCL (Sigma), ebselen (Sigma), ellipticine (Sigma), hyamine (Sigma), kawain (Sigma), ketoconazole (Sigma), L-cycloserine

(Sigma), lynestrenol (Prestwick Chemicals), mevinolin (Sigma), mitoxantrone dihydrochloride (Sigma), nitrofurantoin (Sigma), sertraline (Sigma), suloctidil (Sigma), tamoxifen citrate (Sigma), trifluoperazine dihydrochloride and zaprinast (Sigma) were purchased as standard powders. All stock solutions were prepared in 100% dimethyl sulfoxide (DMSO) to a final concentration of 12.8 mg/mL with the exception of daunorubicin HCl, terbinafine, trifluoroperazine dihydrochloride and ellipticine, which had a final concentration of 6.4 mg/mL. Fluconazole was maintained at room temperature, all other stock solutions were stored at -20°C.

**Data analysis of Prestwick library screen**

For each plate, 32 high growth controls and 12 low growth controls were used to establish a Z' score for each screen. Assay absorbance values were corrected using these controls and converted to percent residual activity. Replicate plots were performed on the duplicates to assess the reproducibility of the screens. Residual activity data was then averaged between the duplicate screens. Bias in the screening data was removed by normalizing the data plate-wise (Figure S1). The diagonal (y=x) was used to identify hits from the screen data with versus without fluconazole. Compounds that were more than 2 median absolute deviations (MADs) below the diagonal were defined as hits.

**Genome-wide deletion strain sensitivity profiles**

Deletion pools were generated by growing up the collections on XY glucose plates (YEPD +100 mg/L adenine and 200 mg/L tryptophan) containing 200 mg/L G418. Colonies were grown for 2 days and then condensed (384 format) onto XY agar plates and grown for another 2 days. Cells were then scraped into 15% glycerol, aliquots of the deletion pools were stored at -80°C.

For each of the drugs (trifluoperazine, clomiphene, sertraline, tamoxifen, suloctidil, L-cycloserine), concentrations that cause up to ~30% growth inhibition were determined. A 5x5

matrix of increasing concentrations of each drug (concentrations that cause less than 25% growth inhibition) and fluconazole (0-8 μg/mL, corresponding to 0-25% growth inhibition) was prepared in 10 mL cultures as well as single drug cultures with up to 30% growth inhibition, and solvent-only controls (DMSO and water).

**Data analysis of barcode arrays**

Identification of sensitive and resistant deletion strains was performed with custom R scripts and the limma, gplots, DBI and RMySQL packages for R. Low intensity and low quality spots were excluded from further analysis. Intensities of duplicate spots were averaged and log2 fold change ratios (drug treated/DMSO control) were used to calculate Z-scores for up and down barcode tags: $Z= (x-\mu)/\sigma$ with $\mu$ the mean log2 fold change for the whole array and $\sigma$ the standard deviation. These two scores were then averaged for each deletion strain to give the final Z-score.

***In vivo* imaging of effects of syncretic compounds**

Concentrations of drugs required to inhibit cell growth for microscopy studies were determined using a higher inoculum than described for determining standard MIC values in order to provide a sufficient number of cells for analysis. Growth in MIC assay plates was monitored spectrophotometrically for at least 24 h. Drug concentrations that produced a significant decrease in cell growth were selected for imaging. Overnight cultures of *S. cerevisiae* BY4741 were diluted to $OD_{600}$ of 2.4, then further diluted 1/20 in SC media. Drugs were added at mid-log phase and cells harvested for staining after 30-60 min of incubation. Hoechst dye (Bisbenzimide H 33258, Sigma#B2883) was prepared as a 20 mM stock solution in distilled water. Cells were harvested by centrifugation at 12,000 rpm for 2 min, and resuspended in 40 μL of YPD and 10 μL of 200 μM Hoechst. Cells were incubated with stain at 30°C for 15 min, washed once with $dH_2O$ and resuspended in 15 μL of $dH_2O$ for slide mounting. Calcofluor White M2R

(Fluorescent Brightener 28, Sigma#F3543) was dissolved in distilled water to a working concentration of 3.5 mg/mL with the addition of 1 drop of 5 M NaOH to aid in solubility. Cells were harvested by centrifugation and resuspended in 198 µL of 0.1 M Tris/HCl, pH 8 to which 2 µL of prepared Calcofluor White M2R was added. Cells were incubated with stain at 30°C for 15 min, washed with $dH_2O$, resuspended in 15 µL of $dH_2O$ and mounted. Mitotracker Green FM (Molecular Probes #M7514) was dissolved to a 1 mM stock solution in DMSO. Stain was added to YPD to a final concentration of 500 nM and warmed to 30°C. Cells were harvested and resuspended in the prewarmed media plus Mitrotracker Green FM, incubated at 30°C for 45 min with shaking, then washed with 200 µL PBS, resuspended in 15 µL of $dH_2O$ and mounted. FM4-64 (N-(3-triethylammoniumpropyl)-4-(6-(4-(diethylamino)phenyl)hexatrienyl)pyridinium dibromide) (Molecular Probes#T13320) was dissolved to a working stock solution of 1 mg/mL with DMSO. Cells were harvested by centrifugation and resuspended in 40L of YPD with 1 µL of 1 mg/mL stock solution of FM4-64. Cells were incubated at 30°C for 15 min, centrifuged, resuspended in 15 µl PBS and mounted.

**Insect larvae inoculation and injection**

*C. neoformans* H99 inocula used for injections were standardized as follows. Overnight cultures grown at 30°C were centrifuged, rinsed and diluted to an $OD_{530}$ of 1.3 in sterile PBS. A 10 µL aliquot of this inoculum contained 8 x $10^3$ cfu. Caterpillars were injected with 10 µL of the *Cryptococcus* preparation using a 250 µL barrel volume Hamilton luer lok syringe, a 30 gauge needle and a Hamilton repeating dispenser. *Cryptococcus* was injected in the lowest left abdominal proleg of each worm. Infected caterpillars were incubated in a dedicated incubator for 24 h at 37°C prior to injection of compound, fluconazole, combinations and/or DMSO and PBS controls. Sets of caterpillars were also injected with sterile PBS in place of *Cryptococcus* to

observe the effects of the saline buffer alone on survival rate. Uninjected controls were also observed to ensure that injection did not affect mortality.

A concentration of 1 μg fluconazole in saline/worm resulted in a sub-MIC pattern of mortality over 7 days, mirroring that of the *Cryptococcus*-alone controls. Suloctidil, sertraline, tamoxifen citrate, trifluoperazine dihydrochloride and fenpropidin were administered as injections of 25.6 μg/worm, alone and in combination with fluconazole. Caterpillars were then examined visually over a 1 week period for discoloration due to melanization and for failure to respond to touch as an inviability endpoint. PBS control caterpillars were unaffected by fluconazole, compound or combination injections. Uninfected caterpillars were not affected by DMSO injection. Drug combinations were also administered to PBS injected controls to observe the effects of the drugs alone on healthy non-infected caterpillars. P values were determined using the independent two-sample p-test, where equal sample sizes and equal variance were assumed:

$$p = \frac{\mu_1 - \mu_2}{s/\sqrt{(2/n)}}$$

where $\mu_1$ represents the average percent of caterpillars surviving on day 7 in the presence of *Cryptococcus*, sertraline and fluconazole, $\mu_2$ represents the average percent of caterpillars in the presence of *Cryptococcus* and fluconazole; $s = \sqrt{[(s_1^2 + s_2^2)/2]}$, where $s_1$ is the standard deviation between the sertraline/fluconazole triplicate dataset and $s_2$ is the standard deviation between the fluconazole triplicate dataset; n is the number of degrees of freedom (number of degrees of freedom determined as 2 x (number of participants per group)-2 ).

## Supplementary data files

***Dataset_HaploidTransformedData*** – Z-scores from chemical-genetic profiles of single drugs (syncretic synergizers and fluconazole) as well as drug combinations with fluconazole. Concentrations are provided in the column name for each screen. 4820 haploid deletions strains were screened.

***Dataset_HetEssTransformedData*** – Sensitivity Z-scores of 1101 heterozygous essential deletion strains to five of the syncretic drugs and fluconazole, as assessed by barcode microarray hybridization. Concentrations were as follows: trifluoperazine 2.25 μg/mL, tamoxifen 1 μg/mL, L-cycloserine 60 μg/mL, sertraline 1.2 μg/mL and clomiphene 0.8 μg/mL. Two screens were performed for fluconazole, one at 8 μg/mL and one at 10 μg/mL.

## Supplementary tables

***Table S1*** – Supplementary file (TableS1_ScreenData.xls) with normalized screen data. For each of the four species, all compounds are listed with plate position and name (columns 1-4). Columns 5 & 6 contain the residual activity (averaged over the two replicates) for the screens with Prestwick library alone and in the presence of fluconazole. The $7^{th}$ and $8^{th}$ column contain the level of additional inhibition in the presence of fluconazole and the residuals for each compound, respectively. The term 'yes' in column 9 indicates that a compound was classified as a hit. Compounds with a residual larger than 2*MAD and a residual activity in the Prestwick screen in the presence of fluconazole below the respective cut-off (median residual activity – 2*MAD of residual activity values) were defined as hits. The MAD and the residual activity cut-offs were as follows: *C. gattii* - 71.03 & 31.44, *C. neoformans* - 69.25 & 36.80, *C. albicans* - 89.47 & 11.88, *S. cerevisiae* - 87.86 & 12.71.

| ***Compound*** | *S. cerevisiae BY4741* | *C. albicans DSY654* | *C. neoformans H99* | *C. gattii R265* |
|---|---|---|---|---|
| Fluconazole | 32 | 8 | 64 | 32 |
| Albendazole | >128 | >128 | 0.5 | 0.5 |
| Amphotericin B | 2 | 2 | 1 | 1 |
| Azaperone | >128 | >128 | >128 | >128 |
| Caspofungin | 0.25 | 1 | 32 | 32 |
| Clofazimine | 32 | 32 | >128 | >128 |
| Clomiphene | 16 | 64 | 16 | 8 |
| L-cycloserine | 128 | >128 | >128 | >128 |
| Fenpropidin | 0.5 | 256 | 32 | >128 |
| Kawain | >128 | >128 | >128 | >128 |
| Ketoconazole | 8 | <0.12 | 4 | 4 |
| Lynestrenol | >128 | 2 | 8 | 4 |
| Mitoxantrone | 32 | >128 | 64 | 128 |
| Sertraline | >128 | >128 | >128 | >128 |
| Suloctidil | 8 | 64 | 32 | 32 |
| Tamoxifen | 32 | 32 | 16 | 8 |
| Terbinafine | 64 | 4 | 1 | 2 |
| Trifluoperazine | 256 | >128 | 64 | 128 |

***Table S2*** – Minimum inhibitory concentrations of hit compounds in each fungal species. Values are given in μg/mL.

| *Compound* | *S. cerevisiae* | *C. albicans* | *C. neoformans H99* | *C. gattii R265* |
|---|---|---|---|---|
| Albendazole | 0.504 | 2 | 1 | 0.531 |
| Amphotericin B | 0.75 | 0.563 | 1 | 0.625 |
| Azaperone | 0.75 | 2 | 0.75 | 2 |
| Caspofungin | 2 | 0.078 | 0.625 | 0.75 |
| Clofazimine | 0.375 | 2 | 0.75 | 0.562 |
| Clomiphene | 0.375 | 0.75 | 0.75 | 2 |
| L-cycloserine | 0.375 | 2 | 2 | 2 |
| Fenpropidin | 0.375 | 2 | 0.5 | 0.75 |
| Kawain | 2 | 2 | 2 | 1.5 |
| Ketoconazole | 1 | 0.5 | 0.75 | 0.75 |
| Lynestrenol | 0.563 | 0.508 | 2 | 2 |
| Mitoxantrone | 0.5 | 2 | 0.625 | 0.625 |
| Sertraline | 0.5 | 0.313 | 0.313 | 0.281 |
| Suloctidil | 0.375 | 0.625 | 0.375 | 0.375 |
| Tamoxifen | 0.375 | 1 | 0.375 | 0.5 |
| Terbinafine | 0.156 | 0.156 | 0.625 | 0.5 |
| Trifluoperazine | 0.313 | 0.375 | 0.375 | 0.313 |

***Table S3*** – Fractional inhibitory concentration index of hit compounds in each fungal species. Values are given in μg/mL.

| | *p-value* | *Difference* |
|---|---|---|
| Fluconazole 2µg/mL | 9.05E-08 | 1.98 |
| Fluconazole 4µg/mL | 3.25E-10 | 1.86 |
| Fluconazole 6µg/mL | - | 0.00 |
| Fluc. 8µg/mL | 1.59E-17 | -1.91 |
| Fluconazole 8µg/mL | 5.27E-14 | -1.79 |
| Fluc. 6 µg/mL & L-cycl. 40µg/mL | 5.85E-05 | -1.00 |
| Fluc. 7µg/mL & Tri. 1.2µg/mL | 3.28E-05 | -1.04 |
| Fluc. 6µg/mL & Tam. 1µg/mL | 2.39E-06 | -1.09 |
| Fluc. 6µg/mL & Sertr. 0.75µg/mL | 1.36E-05 | -1.22 |
| Fluc. 6µg/mL & Sertr. 0.75µg/mL | 1.71E-04 | -1.12 |
| Fluc. 6µg/mL & Clom. 0.2µg/mL | 3.07E-08 | -1.43 |
| Fluc. 6µg/mL & Clom. 0.2 µg/mL | 3.53E-08 | -1.58 |
| Fluc. 6µg/mL & Clom. 0.3 µg/mL | 4.91E-07 | -1.45 |
| DMSO 1 (E)/ DMSO 2 (E) | 4.46E-08 | 2.29 |
| L-cycloserine 40µg/mL | 9.21E-07 | 1.98 |
| L-cycloserine 50µg/mL | 7.07E-09 | 2.27 |
| Trifluoperazine 1.75µg/mL | 8.34E-05 | 1.78 |
| Tamoxifen 1µg/mL | 1.53E-05 | 1.75 |
| Tamoxifen 1.2µg/mL | 1.45E-04 | 1.51 |
| Sertraline 0.75µg/mL | 1.90E-05 | 1.59 |
| Sertraline 1µg/mL | 1.69E-05 | 1.63 |
| Sertr. 0.75µg/mL | 1.95E-06 | 1.77 |
| Clomiphene 0.5µg/mL | 2.27E-08 | 2.10 |
| Clomiphene 0.6µg/mL | 2.33E-06 | 1.84 |
| Clomiphene 0.3µg/mL | 9.38E-09 | 2.11 |
| Suloctidil 0.15µg/mL | 1.07E-07 | 2.31 |

***Table S4*** – P-values for mean Z-scores of fluconazole-specific deletion strains in indicated chemical-genetic profiles.

| | L-cycloserine | Trifluoperazine | Tamoxifen | Sertraline | Clomiphene | Suloctidil |
|---|---|---|---|---|---|---|
| *MDM10* | 1.19 | -1.15 | -1.63 | -0.49 | -3.05 | -2.96 |
| *DRS2* | 0.55 | -3.84 | -6.05 | -3.45 | -2.47 | -2.13 |
| *SWH1* | -2.13 | -1.81 | -3.63 | -2.16 | -1.14 | -0.96 |
| *YAR044W* | -1.56 | -3.04 | -3.87 | -1.97 | -1.83 | -1.86 |
| *SLM5* | -1.65 | -3.2 | -2.48 | -1.63 | -2.33 | -1.64 |
| *CDC50* | -0.16 | -4.17 | -3.59 | 0.24 | -3.53 | 0.34 |
| *IPT1* | -0.28 | -4.55 | -5.38 | 0.35 | -0.44 | -0.99 |
| *RRP8* | -0.13 | -1.46 | -1.88 | -3.52 | -2.1 | -0.57 |
| *SUM1* | 0.73 | -1.67 | -3.31 | -3.83 | -0.62 | 1.9 |
| *YPS7* | -1.27 | -0.99 | -1.4 | -3.53 | -0.6 | -1.96 |
| *VPS74* | -1.65 | -1.94 | -4.45 | -1.15 | -0.02 | 0.46 |
| *SNF1* | -0.46 | -0.45 | -0.2 | -0.85 | 0.01 | -3.46 |
| *PDR1* | 0.1 | -1.79 | -3.04 | -1.68 | -3.27 | -1.53 |
| *SKN1* | 0.02 | -1.85 | -3.97 | -0.35 | -0.84 | -0.18 |
| *KRE11* | 1.23 | 0.11 | -3.02 | -0.99 | -0.92 | -0.02 |
| *SMI1* | 0.52 | -1.58 | -3.18 | -1.28 | -0.63 | -0.15 |
| *SLT2* | 0.9 | -0.74 | -5.47 | -4.67 | -1.68 | -1.8 |
| *PEP8* | 0.15 | -1.82 | -3.14 | -1.48 | -2.05 | -0.76 |
| *BCK1* | 1.99 | -2.2 | -4.5 | -3.62 | -1.8 | -3.31 |
| *VPS35* | 1.27 | -3.34 | -2.17 | -0.89 | -0.52 | -0.16 |
| *RCY1* | 0.16 | -1.17 | -2.1 | 0.56 | -3.22 | -0.22 |
| *LAA1* | -0.4 | -3.38 | -4.22 | -0.94 | -1.42 | -0.78 |
| *SAC1* | 0.34 | -3.53 | -3.33 | -2.81 | -2.41 | -4.17 |
| *YLR065C* | -1.6 | 0.48 | -0.22 | 1.44 | -3.86 | -3.52 |
| *NYV1* | -0.54 | -1.48 | -3.48 | -1.21 | -0.88 | -0.96 |
| *YKE2* | -0.36 | 0.15 | -3.47 | 0.68 | 0.61 | 1.19 |
| *VPS38* | 0.27 | -5.63 | -2.23 | -1.13 | -0.32 | -0.11 |
| *ERG6* | 0.22 | -2.09 | -2.34 | -2.83 | -1.43 | -4.94 |
| *TSA1* | -3.77 | -0.23 | -0.1 | -1.02 | -0.68 | -0.28 |
| *RAD52* | -3.37 | -1.34 | 0.28 | -1.01 | -0.85 | -0.9 |
| *YMR073C* | -3.42 | -0.55 | -1.06 | -1.69 | -1.2 | 0.57 |
| *ERG2* | 2.06 | -1.31 | -3.95 | -0.72 | -0.09 | -2.23 |
| *PDR16* | -0.8 | -0.22 | 0.04 | -1.08 | -3.55 | -4.9 |
| *BRE5* | 0.31 | -0.28 | -0.94 | -3.37 | 0.08 | 0.38 |
| *MDM12* | -1.03 | -2.31 | -1.97 | -1.97 | -4.32 | -2.75 |
| *CKB2* | -0.51 | -2.74 | -4.17 | -0.22 | -0.03 | 0.4 |
| *YOR072W* | 0.22 | -4.59 | -6.1 | -7.08 | -1.95 | -1.54 |
| *PDR5* | -0.42 | -1.04 | -8.08 | -0.33 | -6.29 | -2.45 |
| *RFM1* | 0.28 | -0.78 | -1.36 | -3.21 | -0.09 | 0.4 |
| *SUR1* | 0.18 | -2.57 | -4.58 | 1.11 | -0.81 | 1.46 |
| *VPS30* | 1.01 | -4.14 | -1.29 | -0.69 | 0.22 | -0.74 |
| *ATS1* | 3.5 | -0.76 | -0.06 | 0.1 | 0.7 | 0.58 |
| *TAT1* | 4.44 | 2.16 | 1.77 | 2.18 | 2.43 | 1.15 |
| *RGP1* | 3.62 | 2.88 | 4.03 | 3.44 | 3.57 | 2.77 |
| *SWA2* | 1.89 | 4.35 | 3.66 | 5.33 | 5.95 | 1.73 |
| *VPS52* | 3.16 | 1.08 | 1.4 | 2.9 | 1.07 | 1.42 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *RHR2* | 0.58 | 0.7 | 0.04 | 0.58 | 0.54 | 3.4 |
| *RIC1* | 3.62 | 2.3 | 3.4 | 2.87 | 3.03 | 2.5 |
| *NHA1* | 3.17 | 0.59 | 0.06 | 1.1 | 0.96 | 2.41 |
| *YPT6* | 3.75 | 1.81 | 2.45 | 2.12 | 2.93 | 1.21 |
| *ROM2* | 3.75 | 0.44 | 1.29 | 0.28 | 2.45 | 0.78 |
| *OSW1* | 2.49 | 2.11 | 2.04 | 2.16 | 2.01 | 3.29 |
| *SNC2* | 2.81 | 0.92 | 1.9 | 1.66 | 2.07 | 3.3 |
| *BTS1* | 1.16 | 2.37 | -0.92 | 0.48 | 2.23 | 3.1 |

***Table S5*** – Z-scores for haploid deletion strains that significantly affect growth in at least one drug screen (Z-score of ±3).

| ***Drug*** | ***CGS p-value*** | ***PPP p-value*** |
|---|---|---|
| L-cycloserine | 0.0869 | 0.9283 |
| Clomiphene | 0.0468 | 0.9609 |
| Sertraline | 0.0001 | 0.4168 |
| Tamoxifen | $<10^{-7}$ | 0.327 |
| Trifluoperazine | $<10^{-7}$ | 0.001 |
| Suloctidil | 0.0006 | 0.2724 |
| Signature strains | $<10^{-7}$ | 0.0056 |

***Table S6*** – P-values for the CGS and PPP simulations using the 50 most sensitive strains from the six drug profiles (L-cycloserine, clomiphene, sertraline, tamoxifen, trifluoperazine & suloctidil) as well as the signature deletion strain set.

| ***Species*** | *$MIC_{Fluconazole}$* | *$MIC_{Sertraline}$* | *Lowest $MIC_{Fluconazole\ \&\ Sertraline}$* | *FICI* |
|---|---|---|---|---|
| *C. albicans* 2008 | 64 | >128 | 4 | <0.5 |
| *C. albicans* 2007 | 64 | >128 | 2 | <0.5 |
| *C. glabrata* | >128 | >128 | 8 | 0.625 |
| *C. parapsilosis* | 32 | >128 | 2 | 0.375 |

***Table S7*** – MIC and FICI data for the fluconazole - sertraline drug combination against drug resistant *Candida* strains. Values are in μg/mL.

# Supplementary Figures

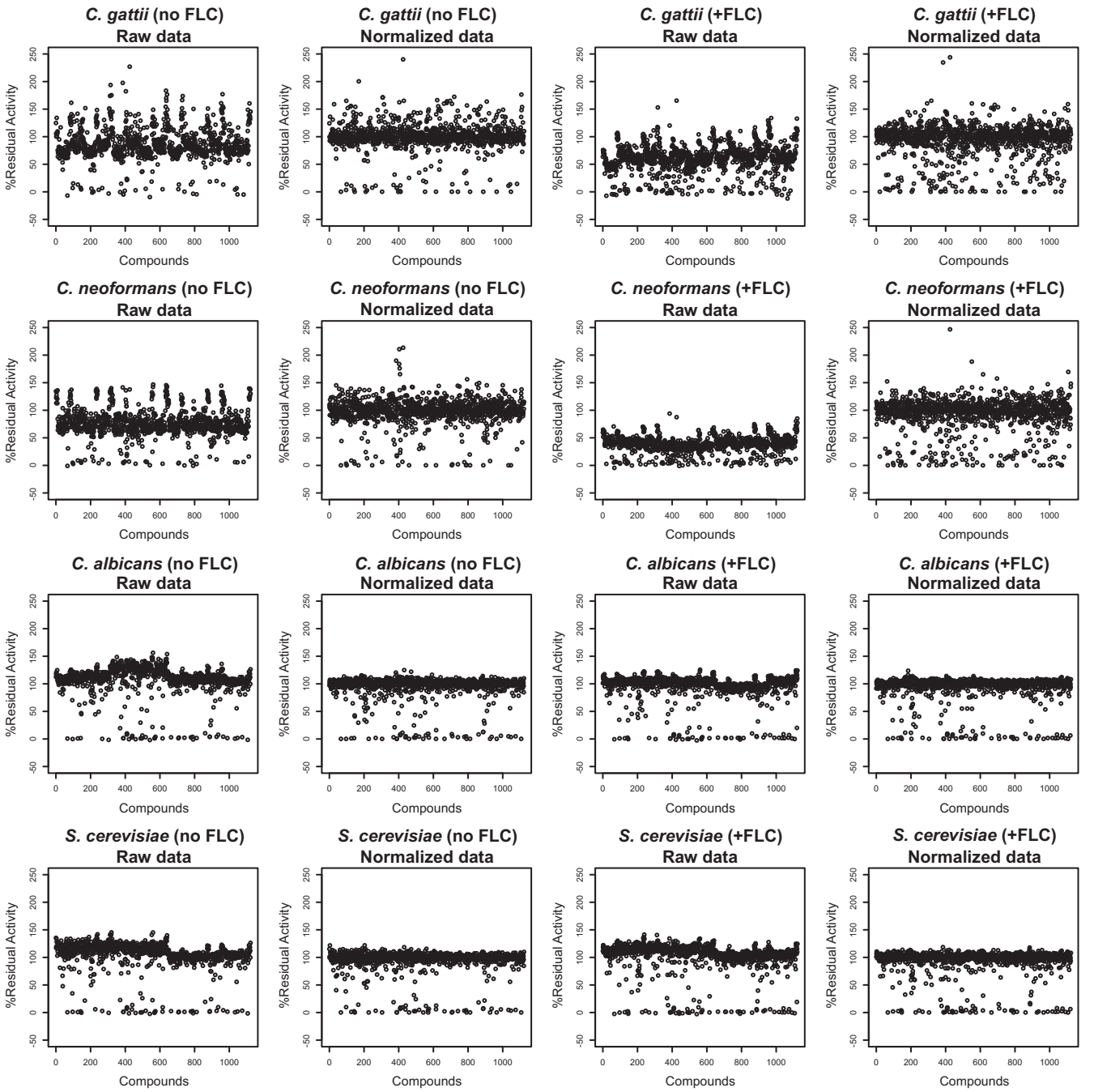



***Figure S1*** – Normalization of residual growth data. Inter-plate variation and spatial effects within each plate were removed by application of column-, row- and plate-wise normalization.

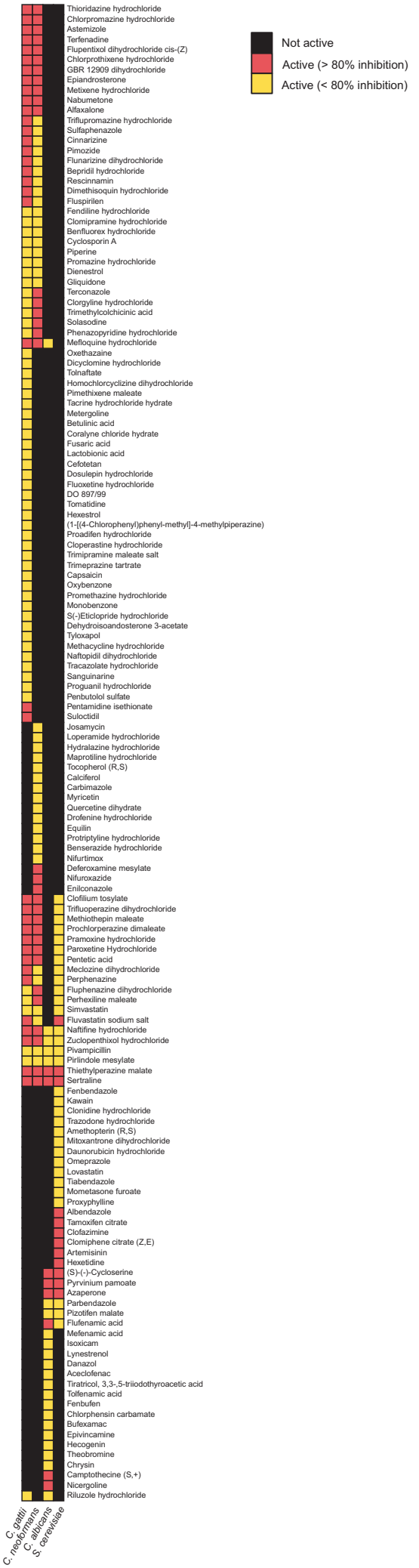



***Figure S2*** – Activity of the 148 hit compounds in Prestwick library screens against *Cryptococcus gattii* R265, *Cryptococcus neoformans* H99, *Candida albicans* Caf2-1 and *Saccharomyces cerevisiae* BY4741. Color indicates the level of growth inhibition in the presence of fluconazole compared to drug alone.

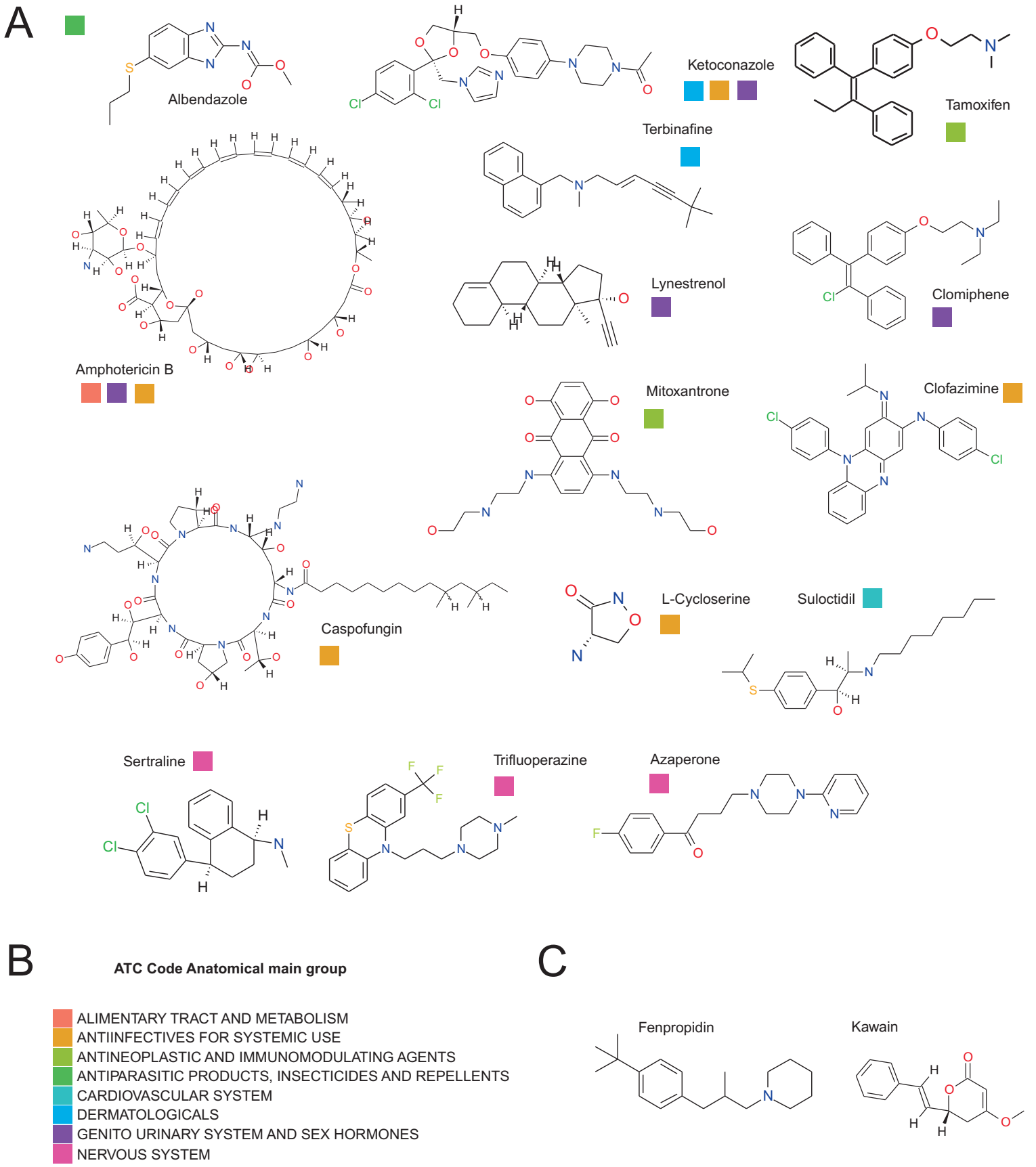



***Figure S3*** – Chemical structures and application classification of 17 compounds selected for synergy confirmation experiments. (A) Approved drugs and their application; (B) ATC anatomical main group; (C) the pesticide fenpropidin and the kavalactone kawain.

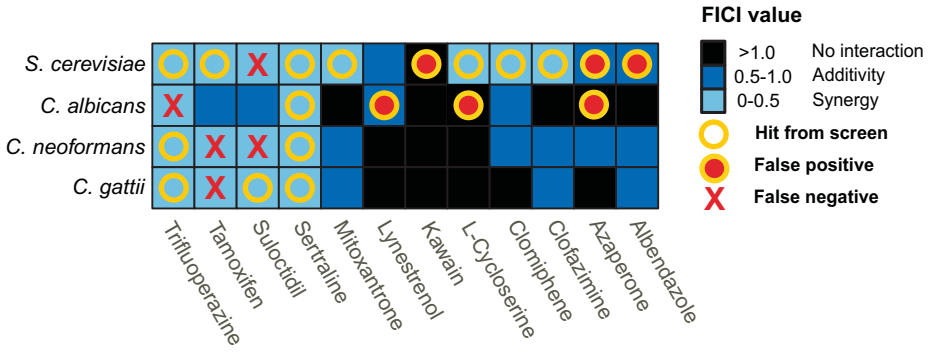
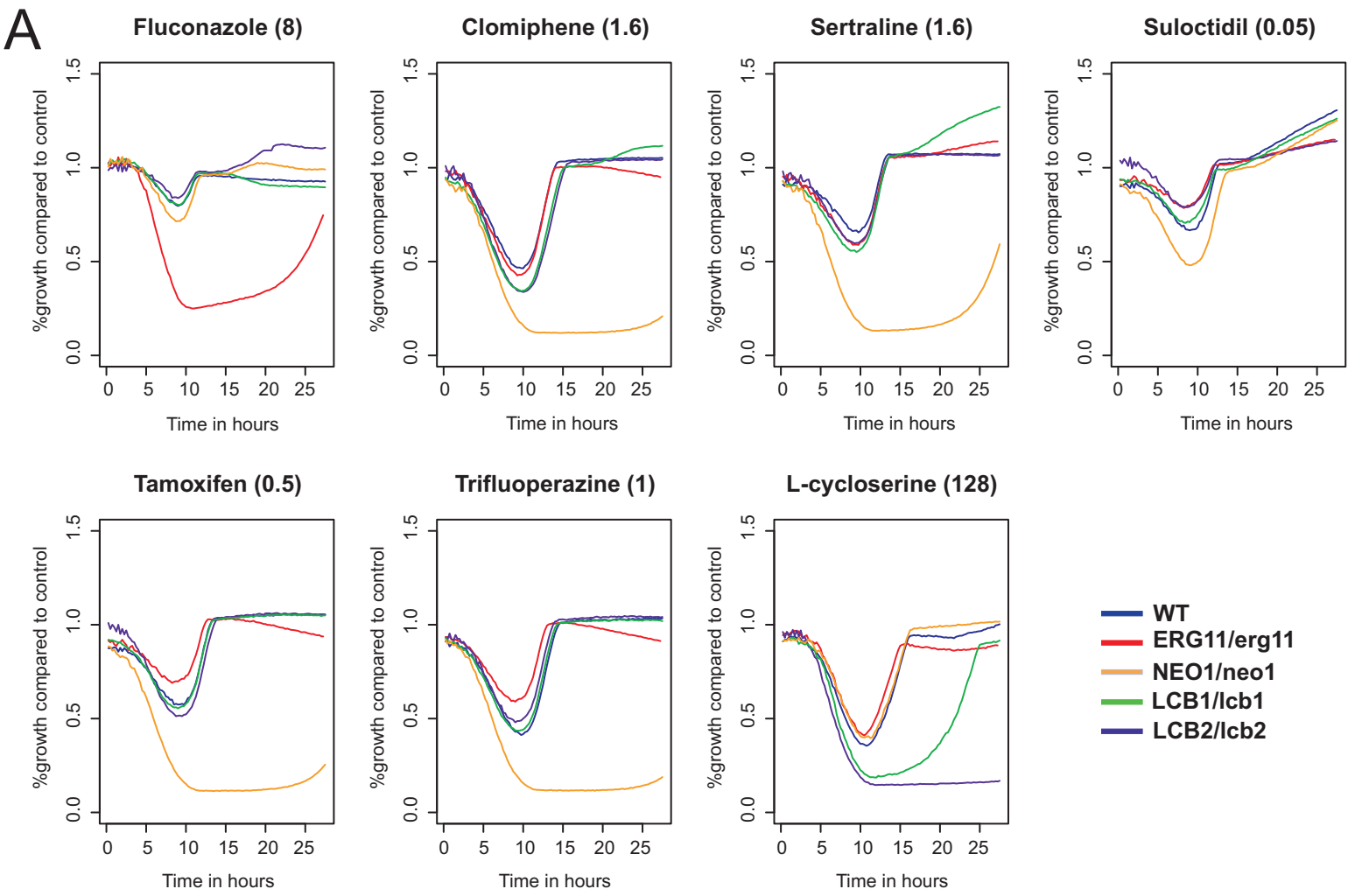



***Figure S4*** –Assessment of primary high throughput screen performance. Heatmap represents drug interactions with fluconazole as confirmed in the four fungal species. Dark blue indicates additive effects (FICI of 0.5 to 1) and light blue represents synergy (FICI < 0.5). Symbols indicate agreement with high throughput screen data.



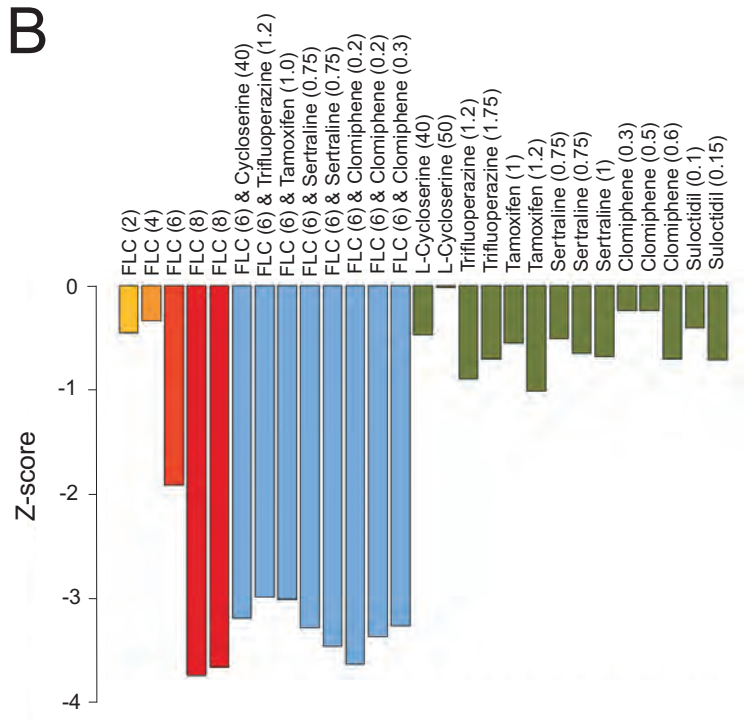




***Figure S5*** – (A) Confirmation of hits from haplo-insufficiency chemical genetic profiles. Based on barcode microarray array data for the heterozygous essential genes, *ERG11/erg11Δ*, *NEO1/neo1Δ* , *LCB1/lcb1Δ* and *LCB2/lcb2Δ* were selected for individual growth curve assays in the presence of the indicated drugs.

(B) Average Z-scores of fluconazole-sensitive deletion strains in the indicated haploid chemical-genetic screens. Red indicates fluconazole alone; blue indicates fluconazole with syncretic drug; green indicates syncretic drug alone. Values in parentheses indicate drug concentration in µg/mL.

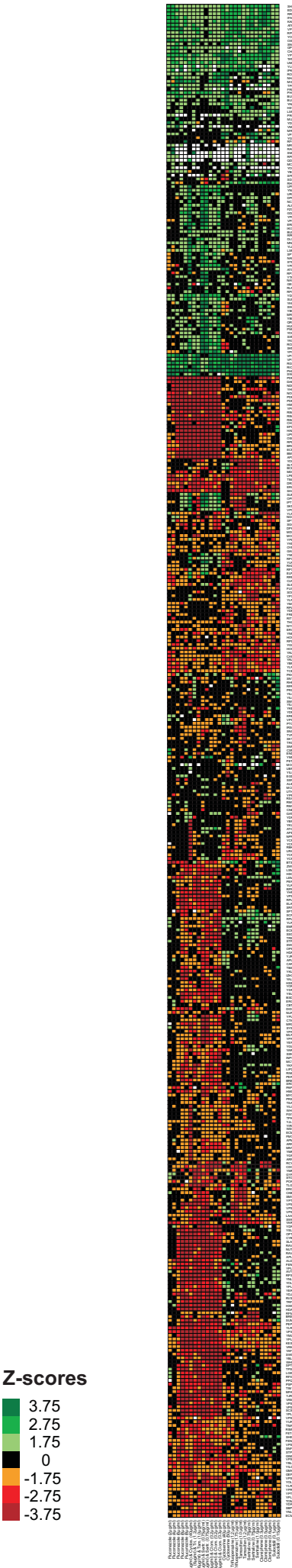



***Figure S6*** – Global heatmap representing all haploid deletion strain chemical-genetic profiles in this study. The haploid deletion strain pool for ~5000 non-essential genes was screened for sensitivity to sertraline, clomiphene, tamoxifen, L-cycloserine, trifluoperazine and suloctidil alone and in combination with fluconazole. Screens are represented along the x-axis and deletion strains along the y-axis. Screens and deletion strains were clustered hierarchically according to Pearson correlation between chemical-genetic profiles. Negative Z-scores (red) indicate sensitive deletion strains whereas positive Z-scores (green) represent resistant deletion strains. White squares indicate no data for a particular strain in that experiment.

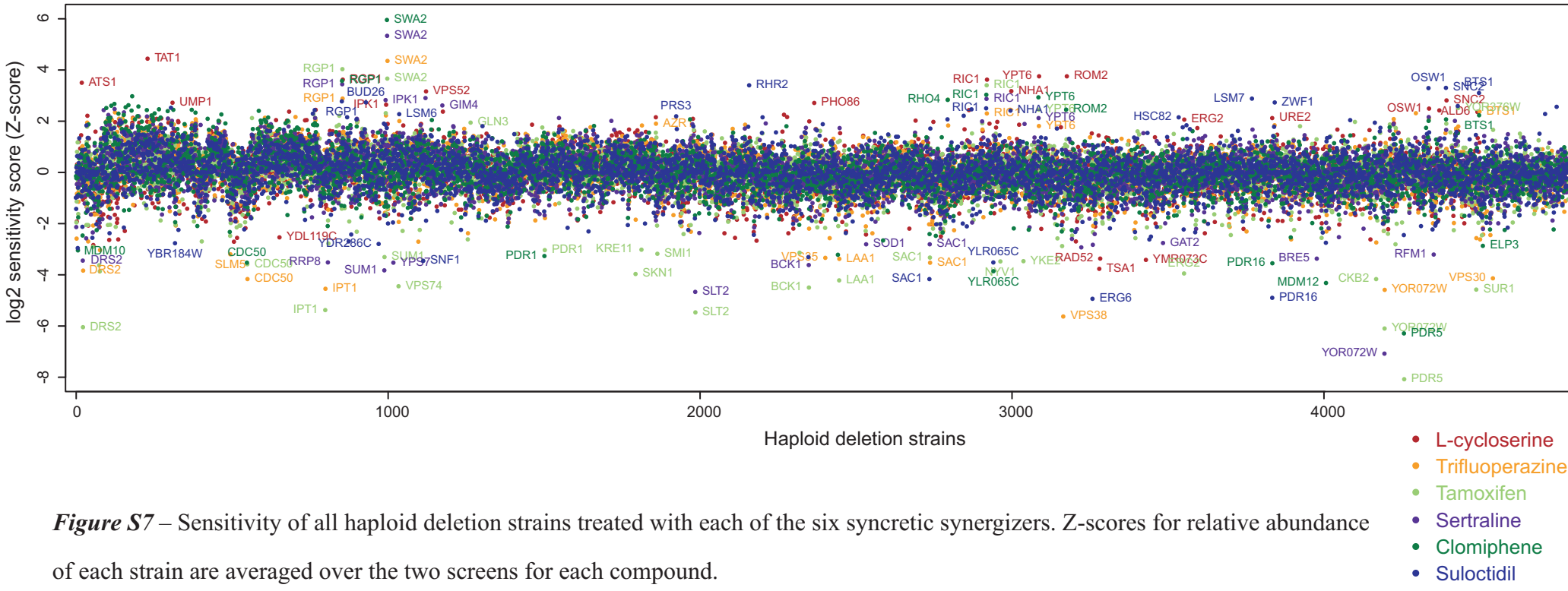



***Figure S7*** – Sensitivity of all haploid deletion strains treated with each of the six syncretic synergizers. Z-scores for relative abundance of each strain are averaged over the two screens for each compound.

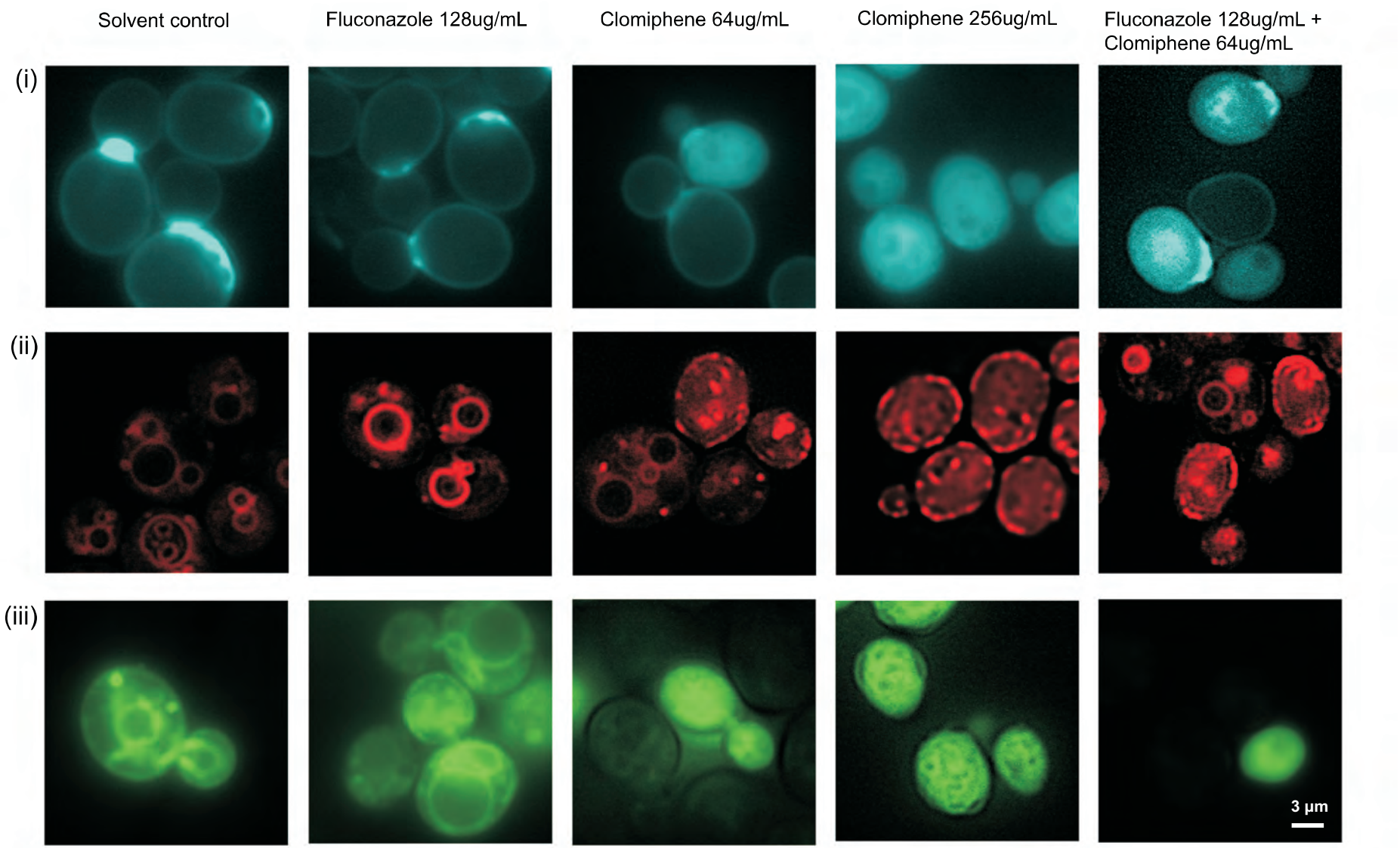



***Figure S8A*** - Images of wild type *S. cerevisiae* treated with either no drug, fluconazole (128 µg/mL), clomiphene (64 µg/mL and 256 µg/mL), or a combination of fluconazole (128 µg/mL) and clomiphene (64 µg/mL). The fluorescent dyes used were: (i) Calcofluor White M2R, (ii) FM4-64, and (iii) Mitrotracker Green FM. Exposure times for the images in row (iii) were as follows, from left to right: 2 seconds, 3 seconds, 35 milliseconds, 15 milliseconds and 15 milliseconds. Due to the high amount of dye in the cells treated with clomiphene, the exposure time was greatly reduced for clomiphene-treated samples in rows (i) and (iii) to obtain images that were not overexposed.

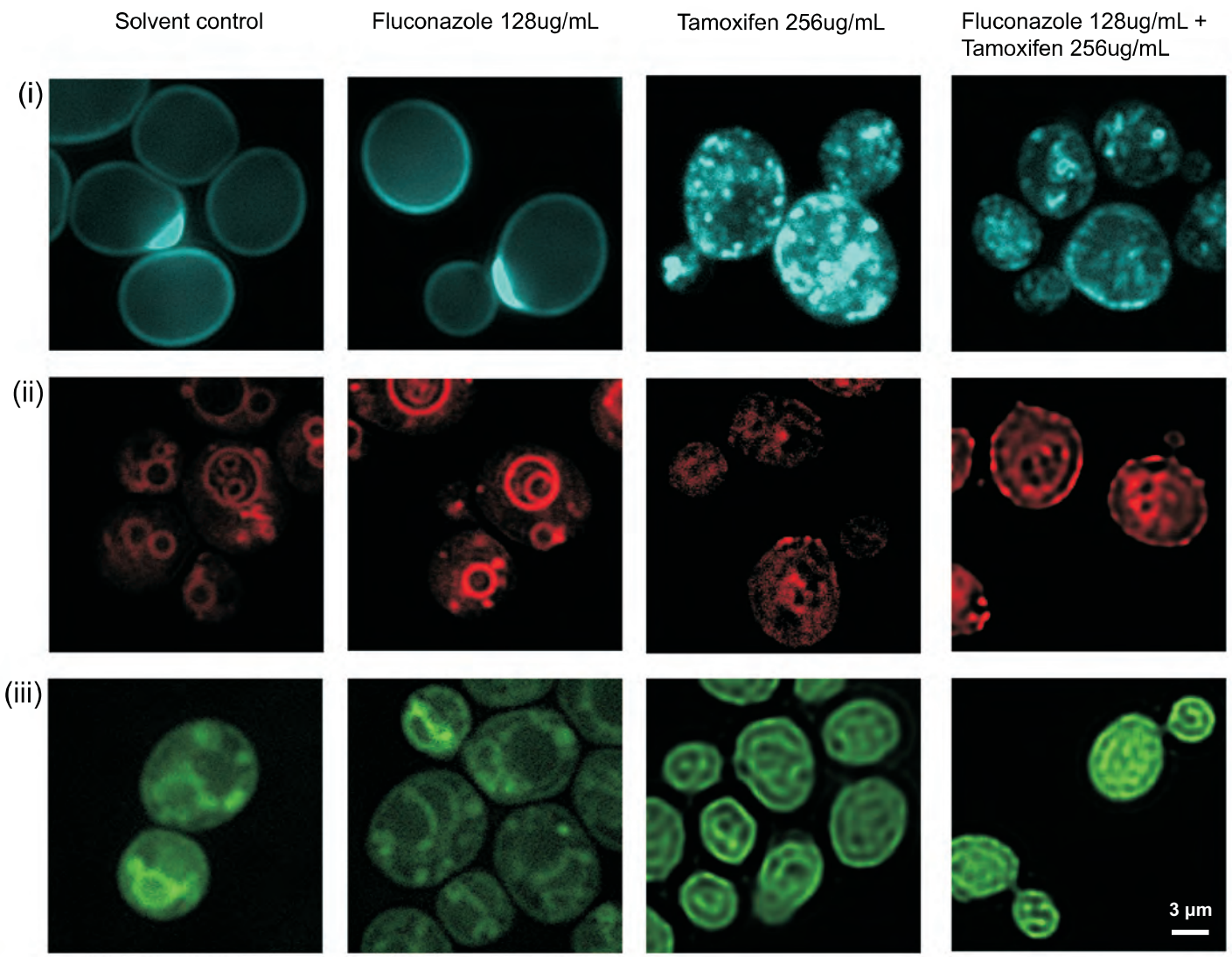



***Figure S8B*** - Images of wild type *S. cerevisiae* treated with either no drug, fluconazole (128 µg/mL), tamoxifen (256 µg/mL), or a combination of fluconazole (128 µg/mL) and tamoxifen (256 µg/mL). The fluorescent dyes used were: (i) Calcofluor White M2R, (ii) FM4-64, and (iii) Mitrotracker Green FM. Exposure times for row (i) were, from left to right, 2 seconds, 2 seconds, 100 milliseconds, 100 miliseconds. For row (iii) they were, from left to right, 8 seconds, 4 seconds, 180 milliseconds, and 180 milliseconds. The exposure times in rows (i) and (iii) were greatly reduced for tamoxifen-treated samples to obtain images that were not overexposed.

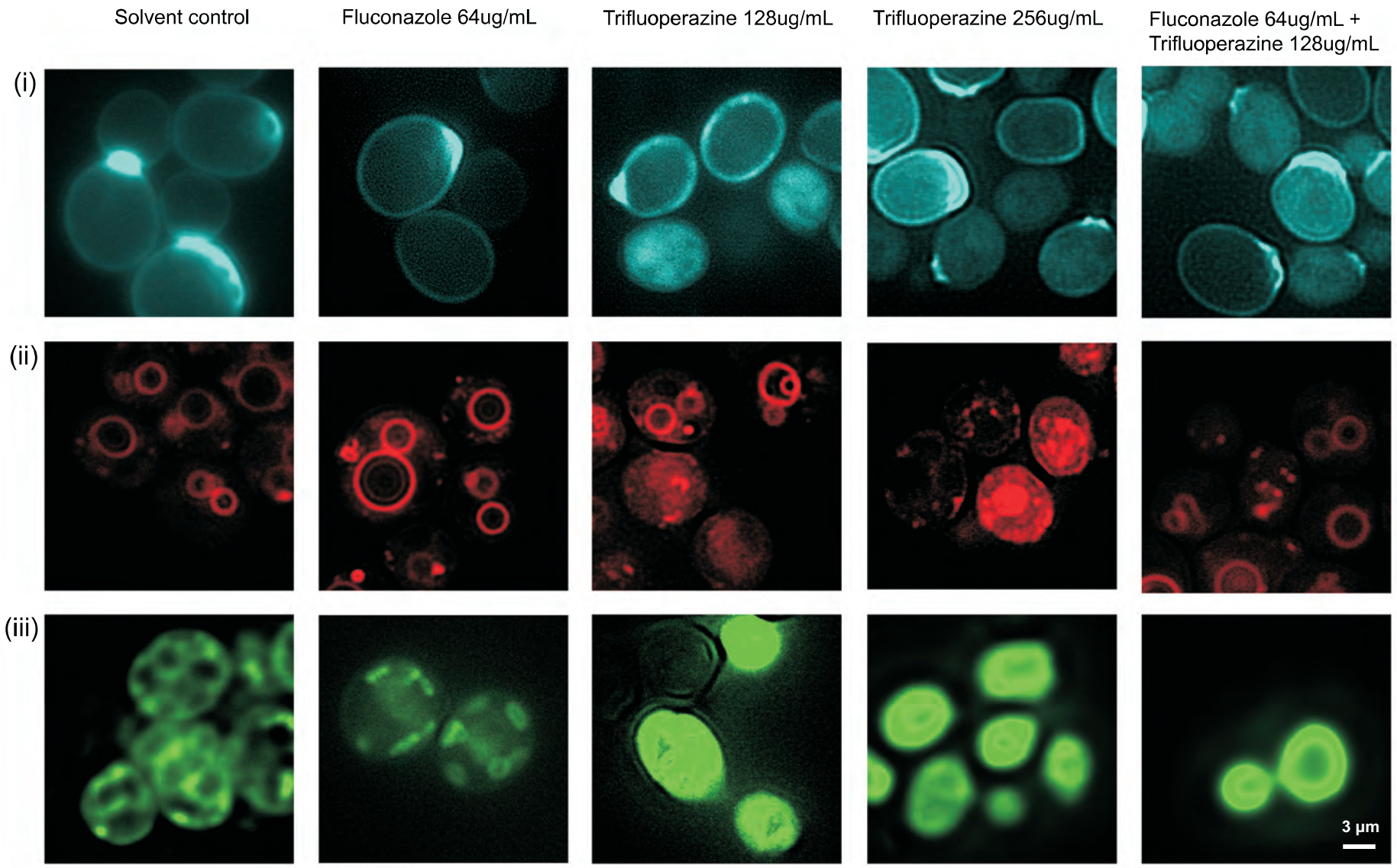



***Figure S8C*** - Images of wild type *S. cerevisiae* treated with either no drug, fluconazole (64 µg/mL), trifluoperazine (128 µg/mL and 256 µg/mL), or a combination of fluconazole (64 µg/mL) and trifluoperazine (128 µg/mL). The fluorescent dyes used were: (i) Calcofluor White M2R, (ii) FM4-64, and (iii) Mitrotracker Green FM. Exposure times for row (iii) were, from left to right, 2 seconds, 1 second, 100 milliseconds, 100 milliseconds, 100 milliseconds. Exposure times for rows (i) and (iii) were reduced for trifluoperazine-treated samples to obtain images that were not overexposed.

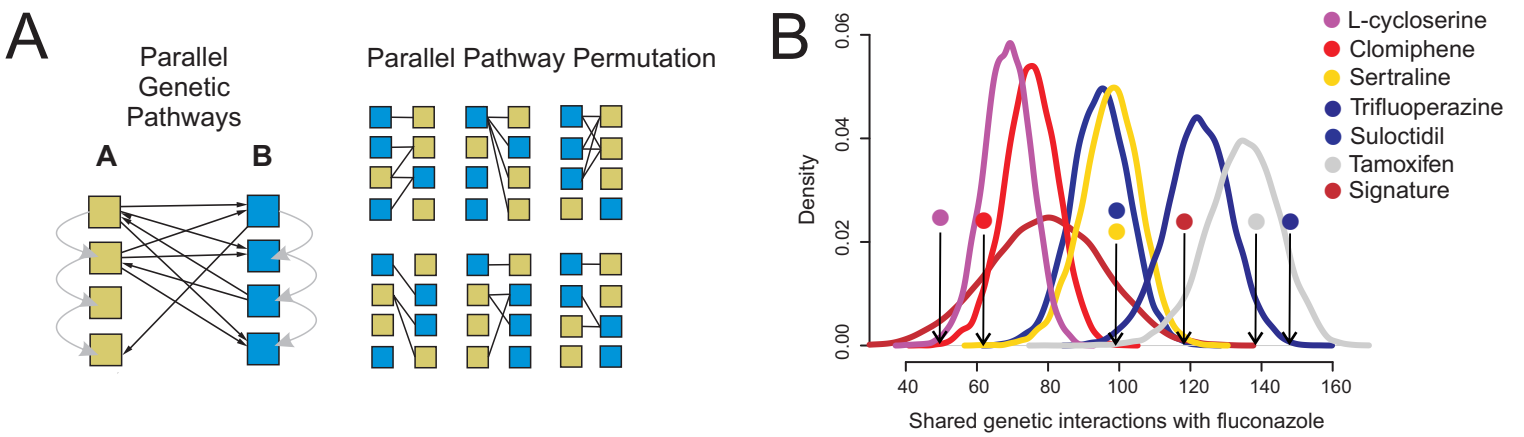



***Figure S9*** – Significance of genetic interaction enrichments between fluconazole and synergistic drugs by parallel pathway permutation test. (A) Scheme for parallel pathway permutation (PPP) simulations. The chemical-genetic interactors of fluconazole and each of the synergistic drugs can be considered to represent parallel pathways (left). For the PPP simulations, genetic interactions for fluconazole and each of the six drugs were pooled and randomly assigned to two groups. (B) PPP simulations with the 50 most sensitive haploid deletion mutants for each of the six synergizers as well as the signature deletion strain set. Coloured curves represent background distributions for each of the drugs; arrows indicate the number of actual genetic interactions for the different drugs. The signature set and trifluoperazine genetic interactions are significantly enriched (p-values < 0.006 and 0.001, respectively).