Supplemental Information for:

The Antifungal Occidiofungin Triggers an Apoptotic Mechanism of Cell Death in Yeast

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Table S1.

A.

	G1		S		G2/M		Other	
Occidiofungin	**-**	**+**	**-**	**+**	**-**	**+**	**-**	**+**
0.5 µg/mL								
0 hr	45	42	18	19	31	31	5	7
1.5 hr	44	46	17	15	26	26	14	14
1 µg/mL								
0 hr	34	42	22	24	27	19	17	16
2 hr	40	24	21	24	18	26	21	26
2 µg/mL								
0 hr	35	35	19	19	34	28	12	18
2 hr	40	33	21	24	23	25	16	18

B.

	log_{10} (CFUs/mL)	
Occidiofungin	**-**	**+**
0.5 µg/mL		
0 hr	6.56	6.49
1.5 hr	6.63	6.53
1 µg/mL	**-**	**+**
0 hr	6.62	6.53
2 hr	6.9	5.69
2 µg/mL	**-**	**+**
0 hr	6.58	6.48
2 hr	6.81	5.60

Impact of occidiofungin on cell cycle distribution. Occidiofungin was added to a logarithmically growing culture of *Candida albicans* to achieve concentrations of 0.5µg/mL, 1µg/mL, and 2µg/mL final. At the indicated times, cells were removed and processed for cell morphology (A) and cell viability by CFU determination (B). Data are from three independent experiments with the average percent of cells at each stage of the cell cycle given. Cell morphology was scored based on the following morphological criteria: unbudded cells are in G1; small budded cells are in S; medium and large budded cells are in G2/M. Cells placed in the 'other' category had multiple buds or a filamentous morphology.

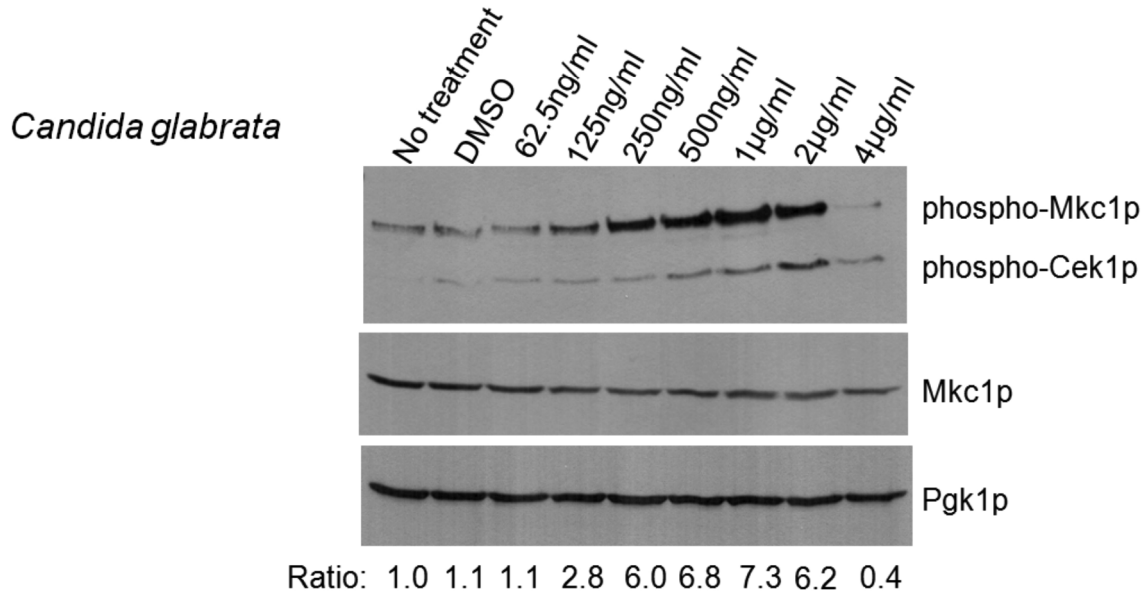

Figure S1. Western blot detection of MAPK activation in *Candida glabrata*. Cells were treated with increasing concentrations of occidiofungin (0-4 µg/mL) for 20 minutes. Cell extracts were analyzed by immunoblotting with antibodies against phospho-Mkc1p, total Mkc1p, and phospho-Hog1p. Detection of phosphoglycerate kinase (Pgk1p) was used to verify equal protein loading. The relative ratios of phosphorylated Mkc1p to total Mkc1p are indicated with the ratio obtained for untreated cells set to one.

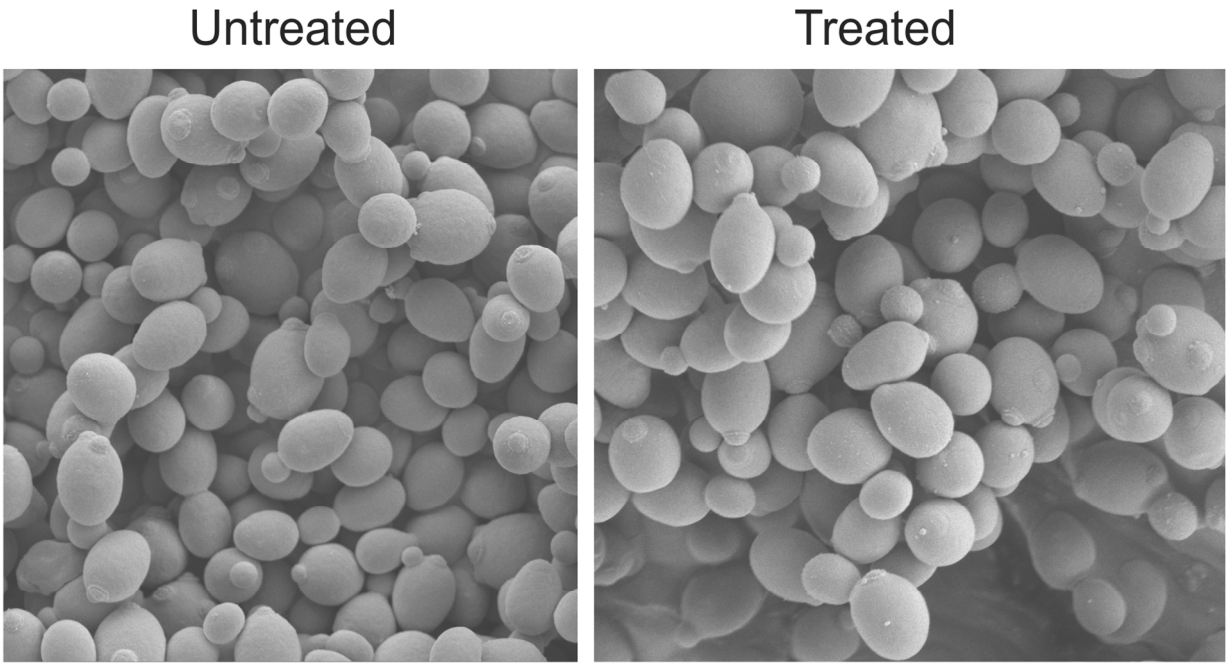

Figure S2. Scanning electron microscopy (SEM) images of *Candida albicans*: untreated (a) and occidiofungin treated (b) cells. *C. albicans* cells were propagated at 35˚C in RPMI until reaching an OD_{600} of 1.0. Occidiofungin (2 µg/mL), or an equivalent volume of DMSO, was added and cells returned to 35˚C for 30 minutes. Cells were isolated by centrifugation and processed for SEM as described in Materials and Methods. 4000X magnification is shown.

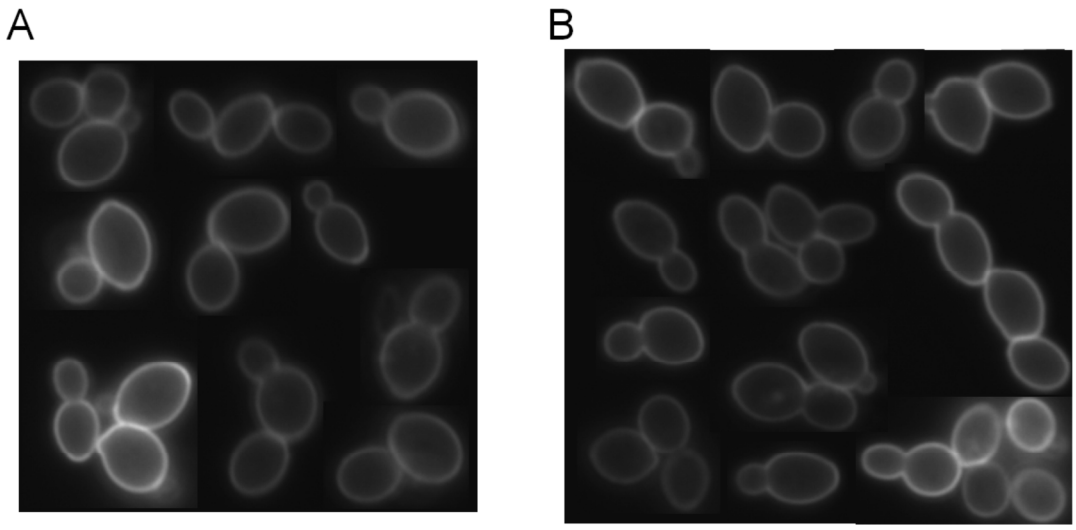

Figure S3. Cell wall mannoprotein distribution in *Candida glabrata* treated with occidiofungin remains unchanged. *C. glabrata* cells were grown for 24hr in the absence (A) or presence (B) of a sublethal concentration of occidiofungin. 1,3-b-glucan was visualized with concanavalinA-FITC staining by fluorescence microscopy. A montage of cells is shown for each treatment.

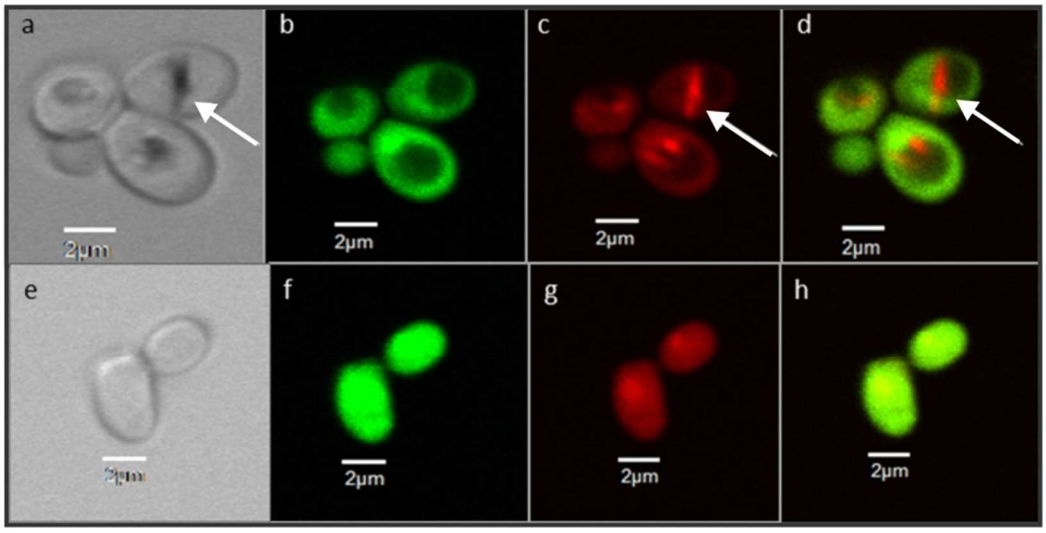

Figure S4. FUN-1 Assay. DIC and fluorescence images of yeast cells stained with FUN-1. Panels “a-d” are untreated yeast cells. CIVS stained red with FUN-1(shown by arrows). Panels “e-h” are occidiofungin treated cells (2µg/ml). CIVS not present in cells. Panels “a” and “e" are DIC images. Panels “b” and “f” are the green emission, while “c” and “g” are the red emission channels. Panels “d” and “h” are overlays of “a-c” and “e-g”, respectively.

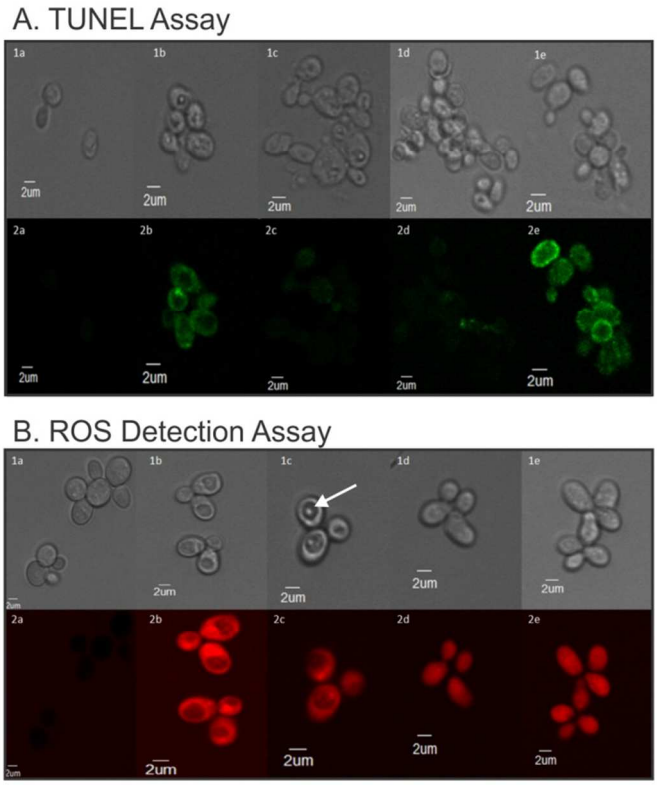

Figure S5. Fluorescent Microscopy Studies on *S. cerevisiae*. (A) TUNEL Assay. Rows "1" and "2" are DIC and fluorescence images, respectively. Column "a" shows cells treated with the solvent blank, "b" shows cells treated with 5mM H_2O_2 and "c-e" correspond to cells treated with 1 µg/ml, 4 µg/ml and 8 µg/ml of occidiofungin, respectively. (B) ROS Detection Assay. Rows "1" and "2" are DIC and fluorescence images, respectively. Column "a" corresponds to treatment with solvent blank, "b" corresponds to cells treated with 5mM H_2O_2, and "c-e" show cells treated with 1µg/ml, 2 µg/ml and 4 µg/ml of occidiofungin, respectively.

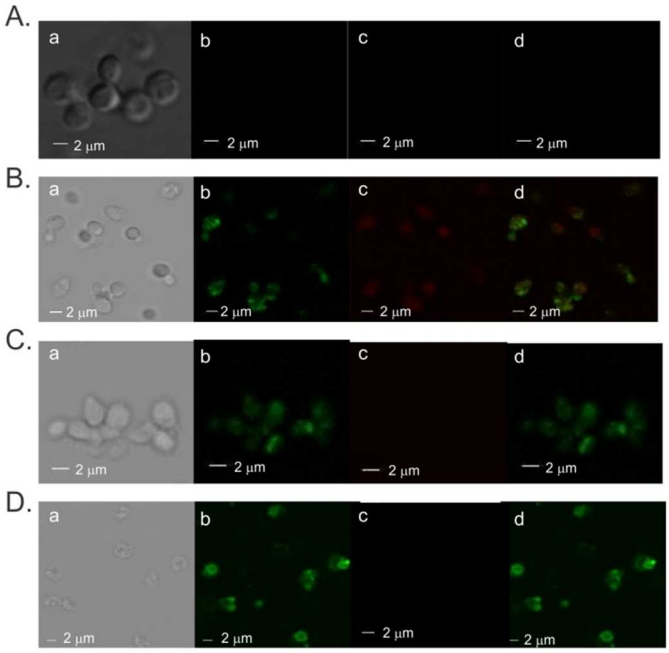

Figure S6. Phosphatidylserine Detection Assay on *S. cerevisiae*. Rows A-D are negative control (treatment with DMSO with no occidiofungin), positive control (5mM H_2O_2), cells treated with 1 μg/ml, 4 μg/ml of occidiofungin, respectively. Columns "a-d" correspond to DIC image, annexin fluorescence image, propidium iodide fluorescence image, and overlay of annexin and propidium iodide fluorescence images, respectively.

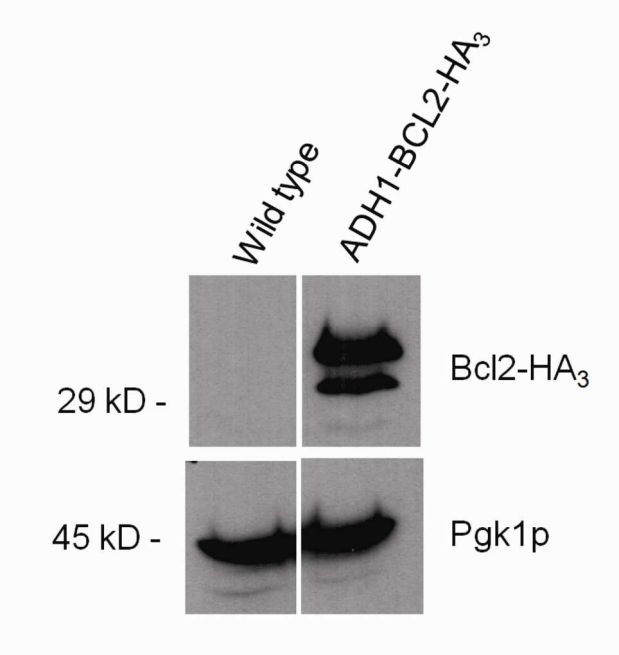

Figure S7. Western blot analysis. Constitutive expression of HA_3 tagged Bcl2 protein was observed. Anti-HA antibodies was used to detect HA_3 tagged Bcl2 protein and anti-3-phosphoglycerate kinase (Pgk1p) antibody was used as a loading control.

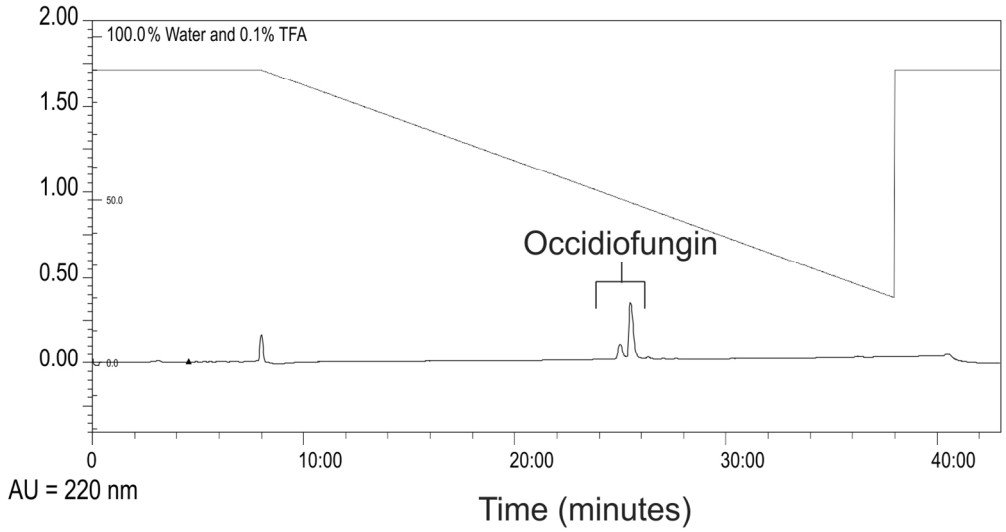

Figure S8. RP-HPLC chromatogram of 50 μg of purified occidiofungin loaded onto a 4.6 × 250 mm C18 column (Grace-Vydac, catalog 201TP54).