Electronic Supplementary Information (ESI)

Copper Potentiates Azole Antifungal Activity in a Way that Does Not Involve Complex Formation

Elizabeth W. Hunsaker and Katherine J. Franz*

Department of Chemistry, Duke University
French Family Science Center
124 Science Drive
27708, Durham, NC, USA.
E-mail: katherine.franz@duke.edu; Tel: +1 919-660-1541

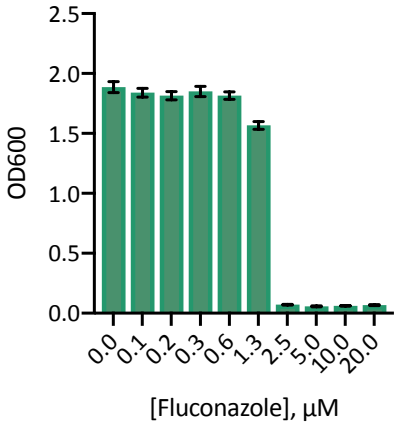

Figure S1 | 24-h growth of cells treated with fluconazole. The MIC of fluconazole at 24 h is 2.5 µM.

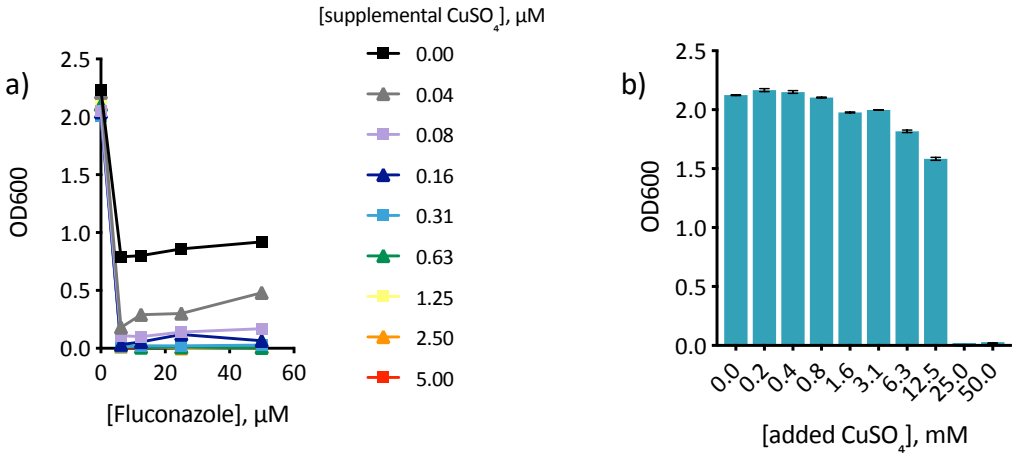

Figure S2 | Fluconazole activity is potentiated with as little as 0.04 µM supplemental Cu in YPD medium, several orders of magnitude below the MIC of Cu. (**a**) A checkerboard assay was used to determine the minimal amount of Cu needed to inhibit growth of fluconazole-treated cells more than fluconazole alone. Addition of 0.04 µM $CuSO_4$ supplemented into YPD was sufficient to observe improved 48 h growth inhibition by fluconazole. Total basal Cu of this batch of YPD was determined to be 0.168 µM ± 0.011. (**b**) The Cu tolerance of *C. albicans* in YPD was determined via a standard growth assay.

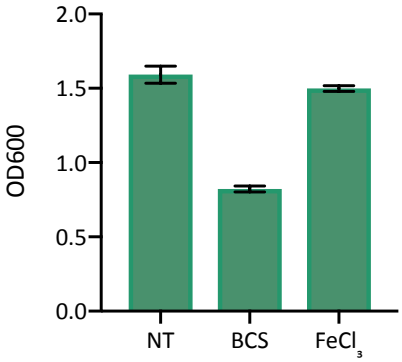

Figure S3 | Growth of *C. albicans* in the presence of BCS or $FeCl_3$. Treatment with 500 µM BCS slows cell growth, reducing it to ~50% of the untreated control at 48 h. Treatment with 250 µM $FeCl_3$ does not impact 48 h growth.

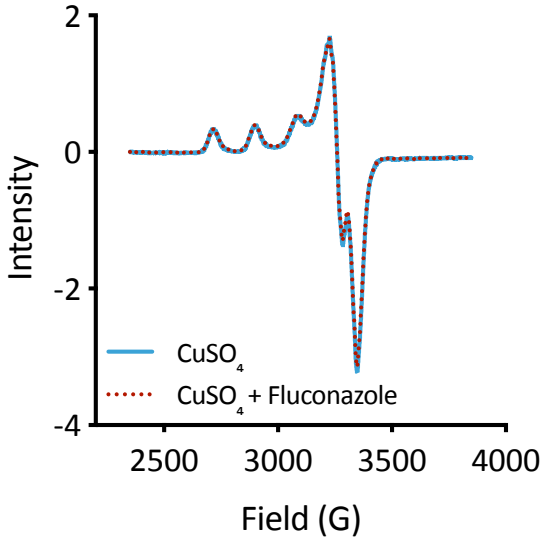

Figure S4 | Fluconazole does not bind Cu(II) in YPD. EPR spectra of fluconazole and Cu in YPD. Addition of Cu to YPD gives rise to an EPR spectrum (light blue trace) that is unchanged by addition of fluconazole (dotted red trace). Conditions: [FluC] = 300 μM, [$CuSO_4$] = 200 μM in YPD with 20% glycerol. Data collected at 77 K.

Table S1 | Metal Content (μM) of YPD Medium lot no. 2005064

Trial	Mg	Ca	Cu	Fe	Mn	Zn	Co	Ni
1	187	89.7	0.163	9.94	0.135	22.29	0.190	0.133
2	172	88.8	0.180	10.2	0.125	22.03	0.176	0.127
3	173	87.2	0.161	10.2	0.132	22.01	0.178	0.123
Average ± SD	177 ± 8	88.6 ± 1.3	0.168 ± 0.011	10.1 ± 0.2	0.13 ± 0.01	22.11 ± 0.15	0.181 ± 0.008	0.128 ± 0.005