Electronic Supplementary Material (ESI) for Metallomics.

Supplementary Information

Chemical and functional properties of metal chelators that mobilize copper to elicit fungal killing of *Cryptococcus neoformans*

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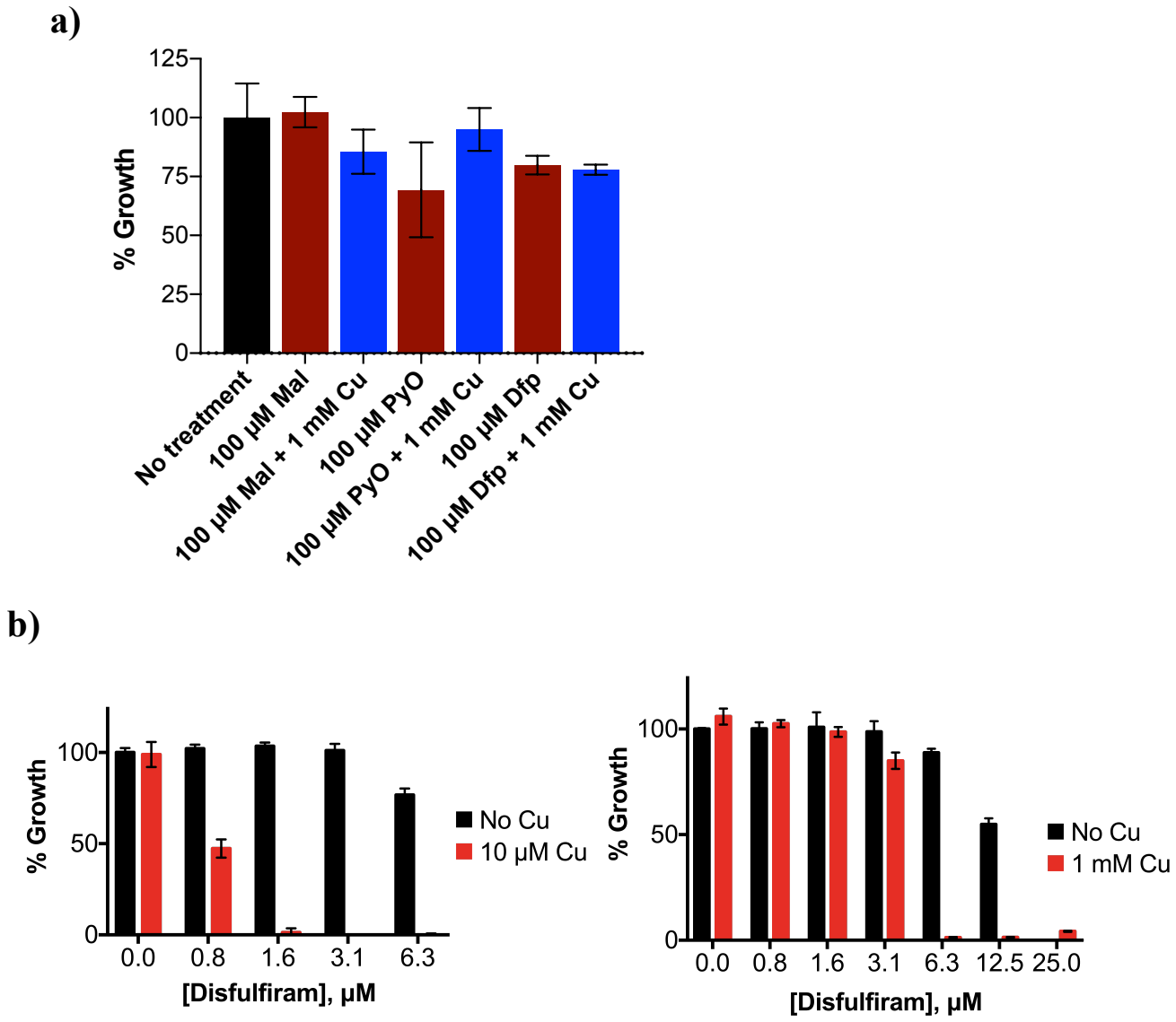

Figure S1. Percent growth of wild-type *C. neoformans* grown in SC medium at 30 °C for 48 h with (a) 100 µM maltol (Mal), 2-pyridinol-1-oxide (PyO), and deferiprone (Dfp) with and without 1 mM supplemental $CuSO_4$ or as a function of (b) disulfiram (DSF) with or without 0.01 or 1 mM supplemental $CuSO_4$.

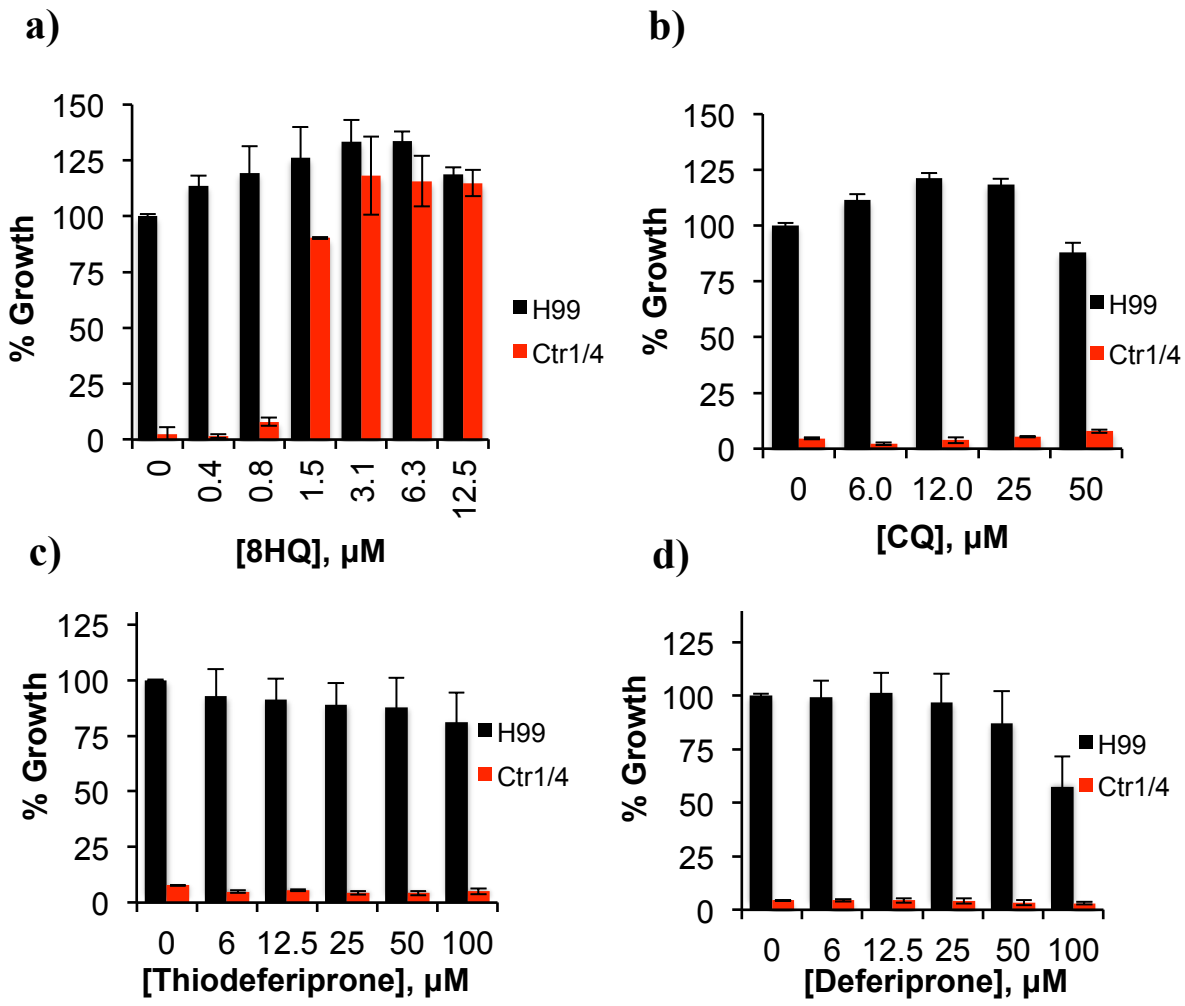

Figure S2. Comparison of the growth of WT *C. neoformans* to the *ctr1*Δ*ctr4Δ* strain as a function of (a) 8HQ, (b) CQ, (c) ThDfp, and (d) Dfp in YPEG medium without supplemental Cu. Due to the loss of cell-surface Cu transporter to import Cu, *ctr1*Δ*ctr4Δ* cells are unable to grow in these conditions. 8HQ is able to overcome this growth defect, suggesting it is able to transport trace Cu from the medium.

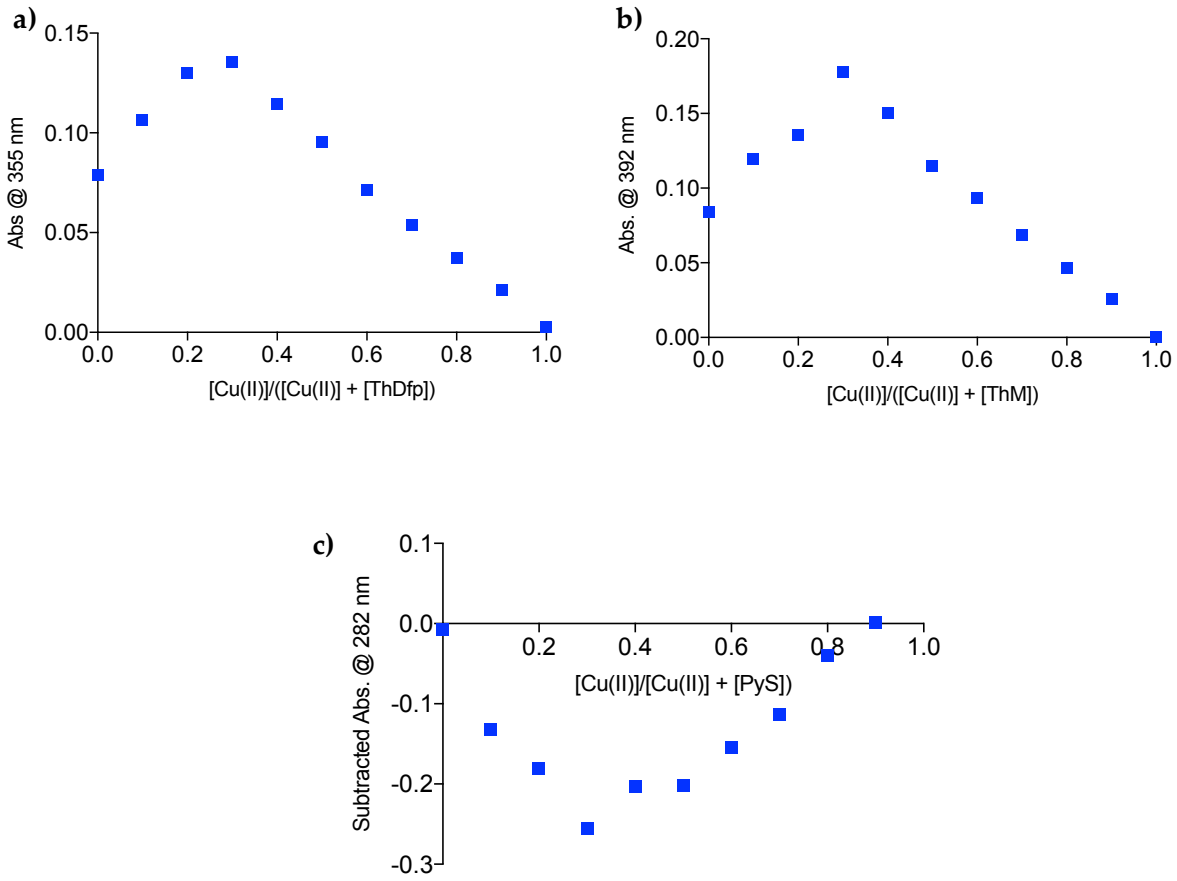

Figure S3. Stoichiometry of Cu(II) complexes of O,S donor ligands ThDfp, ThM, and PyS. Plots of the absorbance of (a) ThDfp, (b) ThM, (c) PyS at 355, 392 and 282 nm, respectively versus the mole fraction ([Cu(II)]/([Cu(II)]+[Ligand]) in universal buffer pH 7.4 based on the Job's plot method (see Figure 7 of main text for spectra of complexes). In the case that the complex absorbance overlapped with the absorbance of the ligand (i.e. PyS), absorbance measurements of the ligand at the appropriate concentration were subtracted, as indicated by a negative absorbance value. The inflection at 0.3 indicates 2L:1Cu stoichiometry for all three complexes.

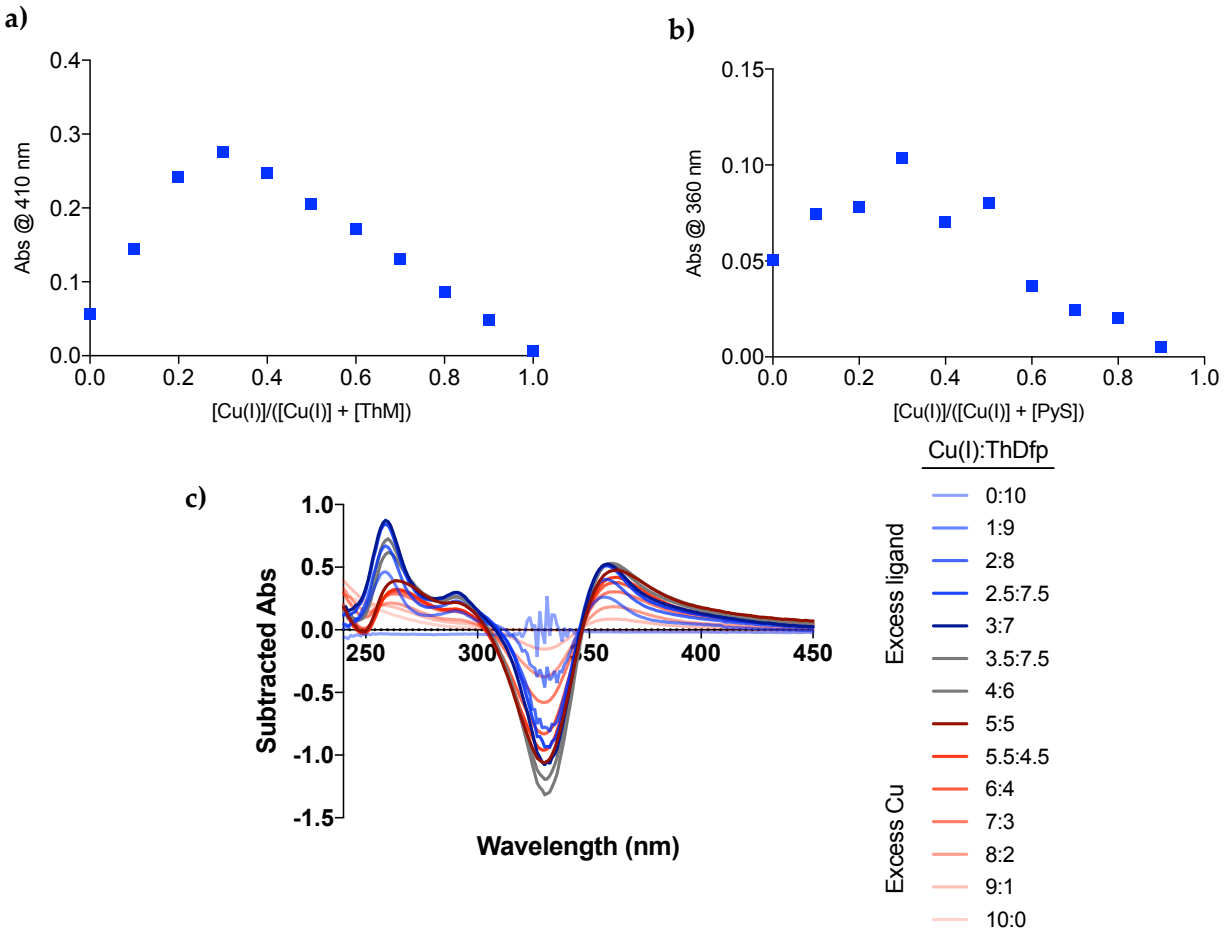

Figure S4. Stoichiometry of Cu(I) complexes of O, S donor ligands ThM, PyS and ThDfp. Plots of the absorbance of **(a)** ThM at 410 and **(b)** PyS at 360 nm versus the mole fraction ([Cu(I)]/([Cu(I)]+[Ligand]) in universal buffer pH 7.4 based on the Job's plot method (see Figure 7 of main text for spectra of complexes). The inflection at 0.3 indicates 2L:1Cu stoichiometry for ThM, while the inflections at both 0.3 and 0.5 for PyS indicate the presence of both 2:1 and 1:1 species under these conditions. Both 2:1 and 1:1 species are also evident in the case of ThDfp, as shown by the difference spectra in **(c)**, for which the absorbance spectrum of the ligand at the appropriate concentration was subtracted from each spectrum of the Cu(I):ThDfp sample. As shown by the color-coding, spectral features for samples with conditions of excess ligand that favor 2:1 stoichiometry (blue) are distinct from those with conditions of excess Cu(I) that favor 1:1 stoichiometry (red).

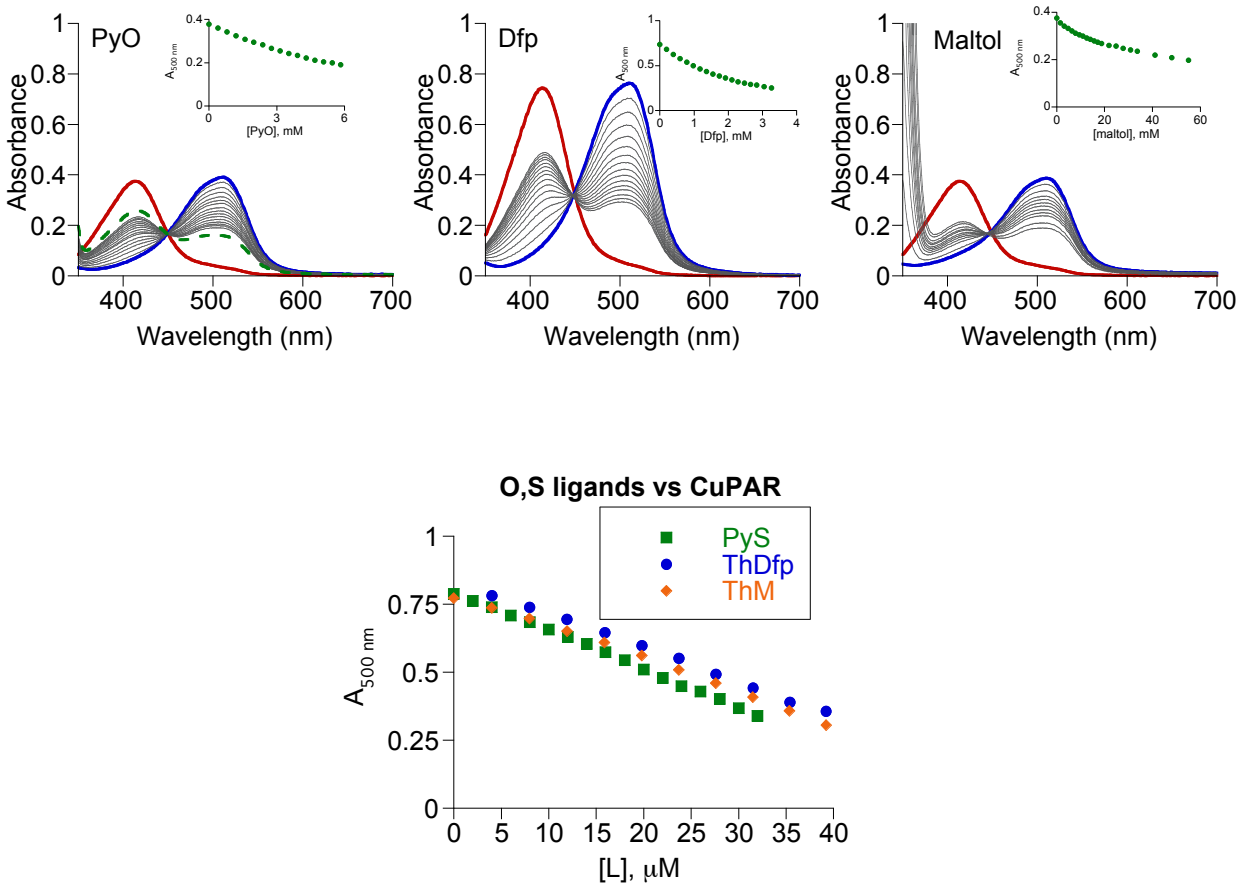

Figure S5. (Top): UV-vis spectra of competitive titrations of PAR-Cu(II) upon addition of PyO, Dfp, and Maltol. Equimolar solutions of PAR and $CuSO_4$ (10 or 20 μM) were allowed to equilibrate in 5 % DMSO universal buffer, pH 7.4, (initial, blue spectra; red spectra represent free PAR without metal). Ligands were added in aliquots of 2–4 μM with 5 min equilibration time between each addition, up to final concentrations indicated in the insets. The conditional binding constants in Table 2 of the main text were calculated as the average and standard deviation obtained by fitting three individual titrations to a 2L:1Cu model in Specfit. (Bottom): titrations of O,S ligands PyS, ThDfp and ThM against Cu(II)PAR resulted in complete exchange of Cu(II) from PAR to each ligand. These titrations provide a lower limit to the conditional binding affinity of the O,S ligands.

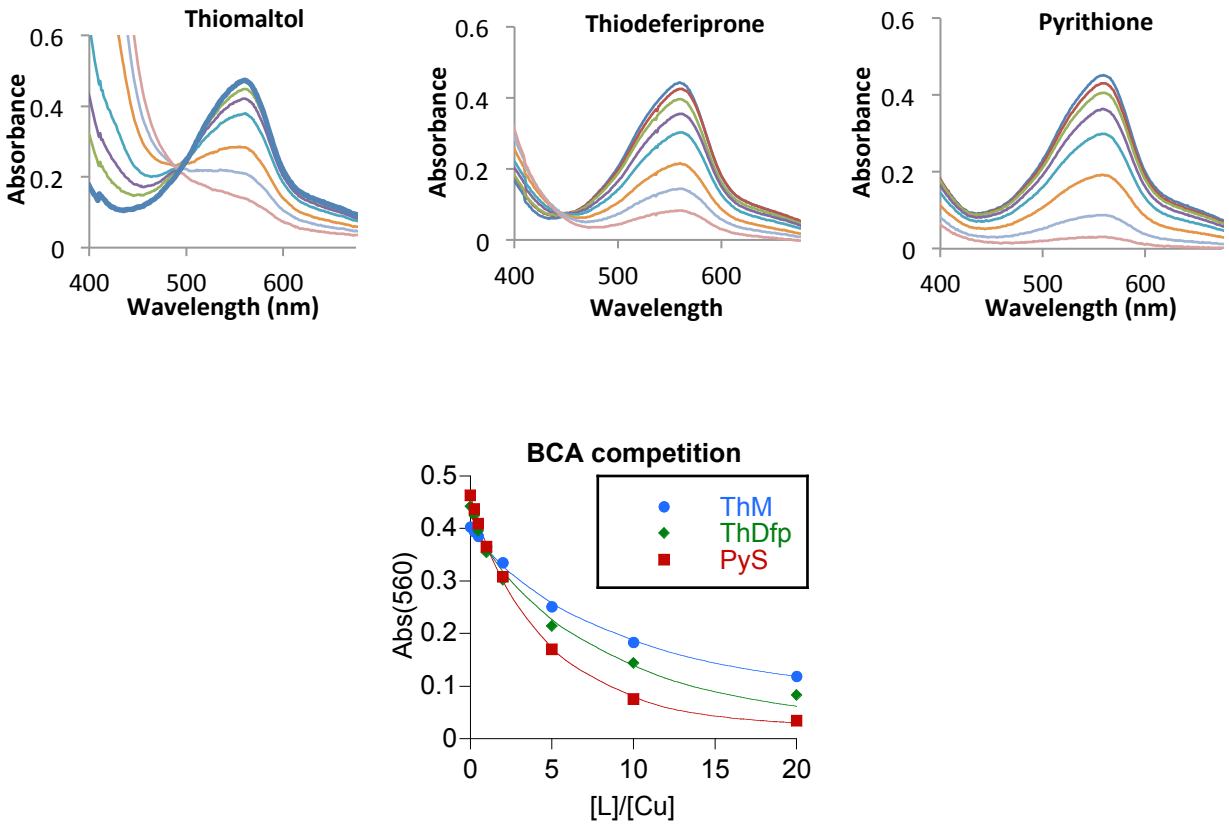

Figure S6. (top) Representative UV-vis spectra of $[Cu(BCA)_2]$ upon titration with increasing concentrations of competing ligand (L): thiomaltol (ThM), thiodeferiprone (ThDfp) and pyrithione (PyS). Conditions: 125 µM BCA and 50 µM ($[Cu(CH_3CN)_4]PF_6$) pre-equilibrated in deoxygenated universal buffer, pH 7.4 with 5% DMSO, in a nitrogen-filled glove box. Thm, ThDfp, and PyS were added in aliquots of 0.5, 1, 2 5, 10 and 20 equiv of Cu with 5 min equilibration time between each addition. (bottom): Plot of the maximum absorbance peak of $[Cu(BCA)_2]$ at 562 nm as a function of added ligand concentration. Lines represent best fits to the data obtained using a 2:1 L:Cu(I) model in Specfit, as described in the Experimental section. Log K_{ML2} values from these fits reported in Table 2 of the main text are the averages and standard deviations of 3 independent experiments for each L.

Table S1. List of Compounds in Biological Screen of *C. neoformans*

	Name of Compound	Commercial Vendor/Synthesized from Noted Reference
1	2-aminothiophenol	Sigma Aldrich
2	ammonium tetrathiomolybdate (TTM)	Sigma Aldrich
3	bathocuproinedisulfonic acid (BCS)	Sigma Aldrich
4	clioquinol (CQ)	Sigma Aldrich
5	deferasirox (Exjade)	Sigma Aldrich
6	deferiprone (Dfp)	Sigma Aldrich
7	deferoxamine (DFO)	Sigma Aldrich
8	dimethyl salicylaldehyde thiosemicarbazone	Bal-Demirci, T.; Akkurt, M.; Yalçin, S. P.; Büyükgüngör, *Trans. Met. Chem.* **2010,** *35*, 95-102.
9	ethylenediaminetetraacetic acid (EDTA)	Sigma Aldrich
10	((E)-N′-[1-(2-hydroxyphenyl)ethylene]isonicotinoylhydrazide (HAPI)	Hruskova, K.; Kovarikova, P.; Bendova, P.; Haskova, P.; Mackova, E. *et al.*, *Chem. Res. Toxicol.* **2011,** *24*, 290-302.
11	6-hydroxyquinoline (6HQ)	Sigma Aldrich
12	8-hydroxyquinoline (8HQ)	Sigma Aldrich
13	kojic acid	Sigma Aldrich
14	maltol	Sigma Aldrich
15	2 – methyl thioaniline	Sigma Aldrich
16	MEH 53	M. Helsel PhD dissertation, Duke University
17	$mepy^{Me}$	Graciously provided by Prof. Daniel Rabinovich, UNC Charlotte
18	mpy^{Me}	Graciously provided by Prof. Daniel Rabinovich, UNC Charlotte
19	N-methyl-salicylaldehyde thiosemicarbazone	Bal-Demirci, T.; Akkurt, M.; Yalçin, S. P.; Büyükgüngör, *Trans. Met. Chem.* **2010,** *35*, 95-102.
20	neocuproine	Sigma Aldrich
21	2-phenyl thioaniline	Sigma Aldrich
22	nitrilotriacetic acid (NTA)	Sigma Aldrich
23	$(2\text{-pic})_2m$	Graciously provided by Prof. Daniel Rabinovich, UNC Charlotte
24	2-pyridinol-1-oxide	Sigma Aldrich
25	$(2\text{-py})_2m$	Graciously provided by Prof. Daniel Rabinovich, UNC Charlotte
26	salicylaldehyde thiosemicarbazone	Sigma Aldrich
27	salicylaldehyde isonicotinoyl hydrazine (SIH)	Charkoudian, L.K.; Dentchev, T.; Lukinova, N.; Wolkow, N.; Franz, K.J., *J. Inorg. Biochem.* **2008,** *102*, 2130-2135.
28	sodium pyrithione (PyS)	Sigma Aldrich
29	thiodeferiprone (ThDfp)	Lewis, J.A, Cohen, S.M. *Inorg. Chem*. **2004**, *43*, 6534-6536.
30	thiomaltol (ThM)	Lewis, J.A., Puerta, D.T., Cohen, S.M. *Inorg. Chem.*, **2003**, *42,* 7455–7459.
31	trientine	Sigma Aldrich
32	disulfiram (DSF)	Sigma Aldrich

Table S2. Extinction coefficient of ligands and their bis-Cu(II) complex in 0.01 M Hepes, pH 7.4 and *n*-octanol.

	λ_{max} (ε) nm (M^{-1} cm^{-1})			
	0.01 M Hepes, pH 7.4		n-Octanol	
Ligand	L	$[Cu^{II}L_2]$	L	$[Cu^{II}L_2]$
Maltol	274 (9200)	306 (13900)	279 (7400)	331 (3600)
PyO	307 (4300)	300 (7400)	307 (4500)	308 (9000)
Dfp	279 (14300)	299 (20400)	314 (15100)	314 (8600)
CQ	#	#	329 (2320)	350 (4010)
8HQ	306 (3800)	372 (5900)	317 (2500)	394 (3500)
PyS	333 (4000)	320 (11900)	353 (4500)	327 (12200)
ThM	357 (15700)	384 (14400)	360 (16600)	393 (17500)
ThDfp	330 (22600)	320 (26600)	350 (26900)	367 (25800)

not measured due to insolubility