

Supporting Information

for

A Copper Hyperaccumulation Phenotype Correlates with Pathogenesis in *Cryptococcus neoformans*

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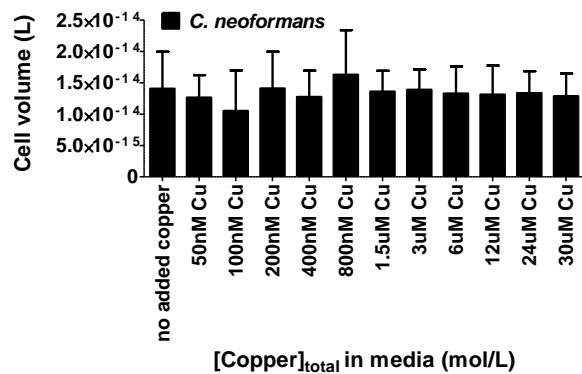
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Table S1 Cell Volumes of *C. neoformans* Grown with Increasing Copper

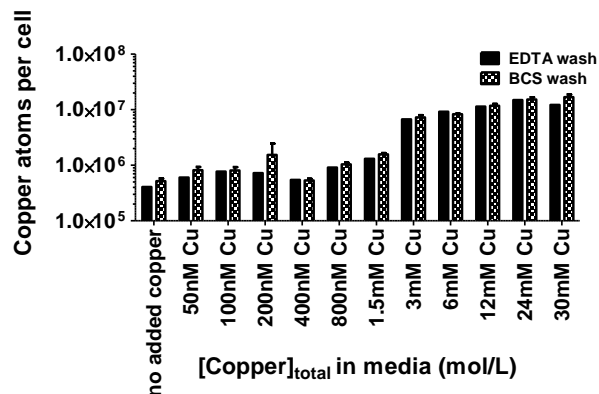
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Figure S1. Cell Volumes Remain Constant In the Face of Increasing Copper Availability



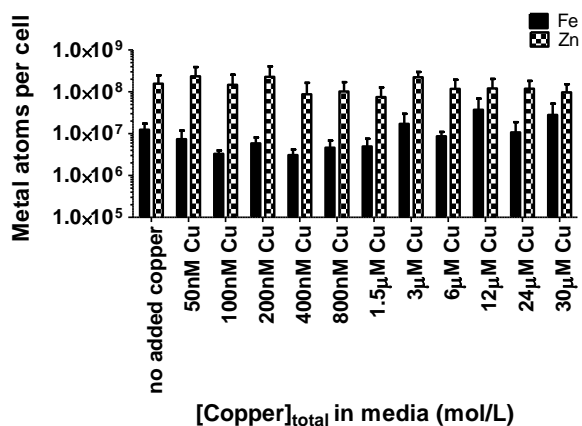
Cell volumes remain constant with increasing copper in the growth media. Despite varying copper availability during growth, there was no significant change in the cell volume of *C. neoformans*.

Figure S2 Verification of Intracellular Copper



Elevated intracellular copper is not due to adventitious binding of exogenous copper from the media. To verify that the observed increasing copper concentration was not due extracellular contamination from the elevated content in the media, a BCS wash was added to the ICP-MS preparation. *C. neoformans* was grown in YNB containing a broad range of copper concentrations and cells were washed to remove excess metal contamination from the media. In both cases, the cells were washed twice in YNB supplemented with 1mM EDTA. A third wash was done with ultra-pure laboratory grade water supplemented with either 1mM EDTA or 1mM BCS. There was no difference in measured copper between the two washes as determined by ICP-MS, confirming that the metal is intracellular. Mean \pm SEM is shown (n=2)

Figure S3 Iron and Zinc in *C. neoformans*



Iron and Zinc content do not significantly increase with increasing extracellular copper. *C. neoformans* was grown in YNB containing a broad range of copper concentrations and metal content was determined with ICP-MS. Mean ± SEM is shown (n=3). “No added copper” represents a copper-depleted medium in which metals were removed with Chelex-100 resin. Metals were then added back to pre-chelexed levels (~0.9µM Fe and ~1.5µM Zn) except for copper (n=2 for 25 nM and n=1 for 33 nM total media copper). No significant trend was observed in the iron or zinc content in the cells.

TABLE S1: Cell Volumes of *C. neoformans* Grown with Increasing Copper.

Cells were grown in YNB media with increasing copper and volumes were measured. Mean \pm SEM is shown (n=3).

Copper in YNB media	Cell Volume (L)
no added copper +1mM BCS	$1.2 \pm 0.1 \times 10^{-13}$
no added copper +1 μ M BCS	$1.4 \pm 0.1 \times 10^{-13}$
no added copper	$1.4 \pm 0.3 \times 10^{-14}$
50 nM	$1.3 \pm 0.2 \times 10^{-14}$
100 nM	$1.1 \pm 0.4 \times 10^{-14}$
200 nM	$1.4 \pm 0.3 \times 10^{-14}$
400 nM	$1.3 \pm 0.2 \times 10^{-14}$
800 nM	$1.6 \pm 0.4 \times 10^{-14}$
1.5 μ M	$1.4 \pm 0.2 \times 10^{-14}$
3 μ M	$1.4 \pm 0.2 \times 10^{-14}$
6 μ M	$1.3 \pm 0.3 \times 10^{-14}$
12 μ M	$1.3 \pm 0.3 \times 10^{-14}$
24 μ M	$1.3 \pm 0.2 \times 10^{-14}$
30 μ M	$1.3 \pm 0.2 \times 10^{-14}$

Note: Data shown is the same data plotted in Figure 5 and Figure S1

TABLE S2: Iron, Copper, and Zinc in *C. neoformans*. Cells were grown in YNB media with the appropriate copper content and analyzed with ICP-MS. Mean \pm SEM (n=3).

Copper in media	Fe in cell (atoms/cell)	Cu in cell (atoms/cell)	Zn in cell (atoms/cell)
no added copper	$1.3 \pm 0.5 \times 10^7$	$3.2 \pm 0.6 \times 10^5$	$1.6 \pm 0.9 \times 10^8$
50 nM	$7 \pm 5 \times 10^6$	$6 \pm 2 \times 10^5$	$2 \pm 2 \times 10^8$
100 nM	$3.3 \pm 0.7 \times 10^6$	$6.3 \pm 0.2 \times 10^5$	$1 \pm 1 \times 10^8$
200 nM	$6 \pm 2 \times 10^6$	$9 \pm 3 \times 10^5$	$2 \pm 1 \times 10^8$
400 nM	$3 \pm 1 \times 10^6$	$1.4 \pm 0.8 \times 10^6$	$8 \pm 8 \times 10^7$
800 nM	$5 \pm 2 \times 10^6$	$2 \pm 1 \times 10^6$	$1 \pm 0.7 \times 10^8$
1.5 μ M	$5 \pm 3 \times 10^6$	$1 \pm 1 \times 10^7$	$7 \pm 5 \times 10^7$
3 μ M	$2 \pm 1 \times 10^7$	$4 \pm 3 \times 10^7$	$2.2 \pm 0.8 \times 10^8$
6 μ M	$9 \pm 2 \times 10^6$	$9 \pm 8 \times 10^7$	$1.2 \pm 0.8 \times 10^8$
12 μ M	$3 \pm 3 \times 10^7$	$1 \pm 1 \times 10^8$	$1.2 \pm 0.8 \times 10^8$
24 μ M	$1.1 \pm 0.8 \times 10^7$	$2 \pm 2 \times 10^8$	$1.2 \pm 0.6 \times 10^8$
30 μ M	$3 \pm 2 \times 10^7$	$3 \pm 2 \times 10^8$	$1.0 \pm 0.5 \times 10^8$

Note: Data shown is the same data plotted in Figure S3