Supplementary Information

Prochelators modulate azole activity against *Candida albicans* in a metal-dependent manner

Madeline Ann Merriman,[a] Catherine A. Denning-Jannace,[a] Andrea Barboza Hurtado,[a] Francesca A. Vaccaro,[a] Yu-Shien Sung,[b] Elisa Tomat,[b,c] Katherine J. Franz*[a]

[a] Department of Chemistry, Duke University, French Family Science Center, 124 Science Drive, Durham, North Carolina 27708, United States

[b] The University of Arizona, Department of Chemistry and Biochemistry, Tucson, Arizona 85721-0041, United States

[c] University of Vienna, Faculty of Chemistry, Institute of Inorganic Chemistry, 1090 Wien, Austria

*corresponding author
Email: Katherine.franz@duke.edu

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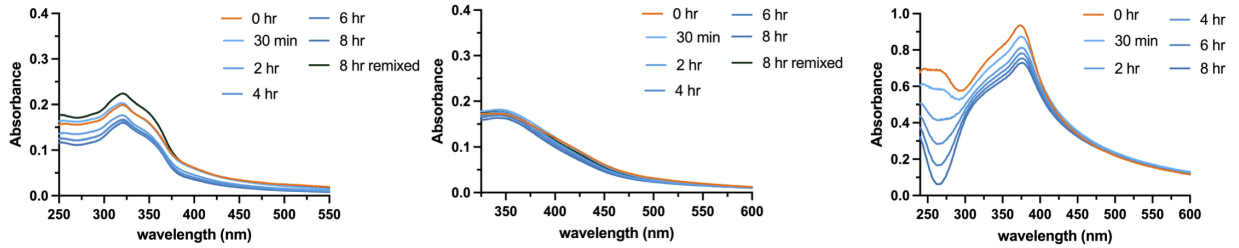

Figure S1. UV-visible spectra of 15 μM $(AH1\text{-}S)_2$ (left), 15 μM $(IT1\text{-}S)_2$ (middle), and $(TC1\text{-}S)_2$ (right) in MOPS buffer (1 % DMSO) over 8 h. Due to visible precipitation of $(244mTC\text{-}S)_2$ when diluted from DMSO stock into MOPS buffer with 1 % DMSO, spectra not shown.

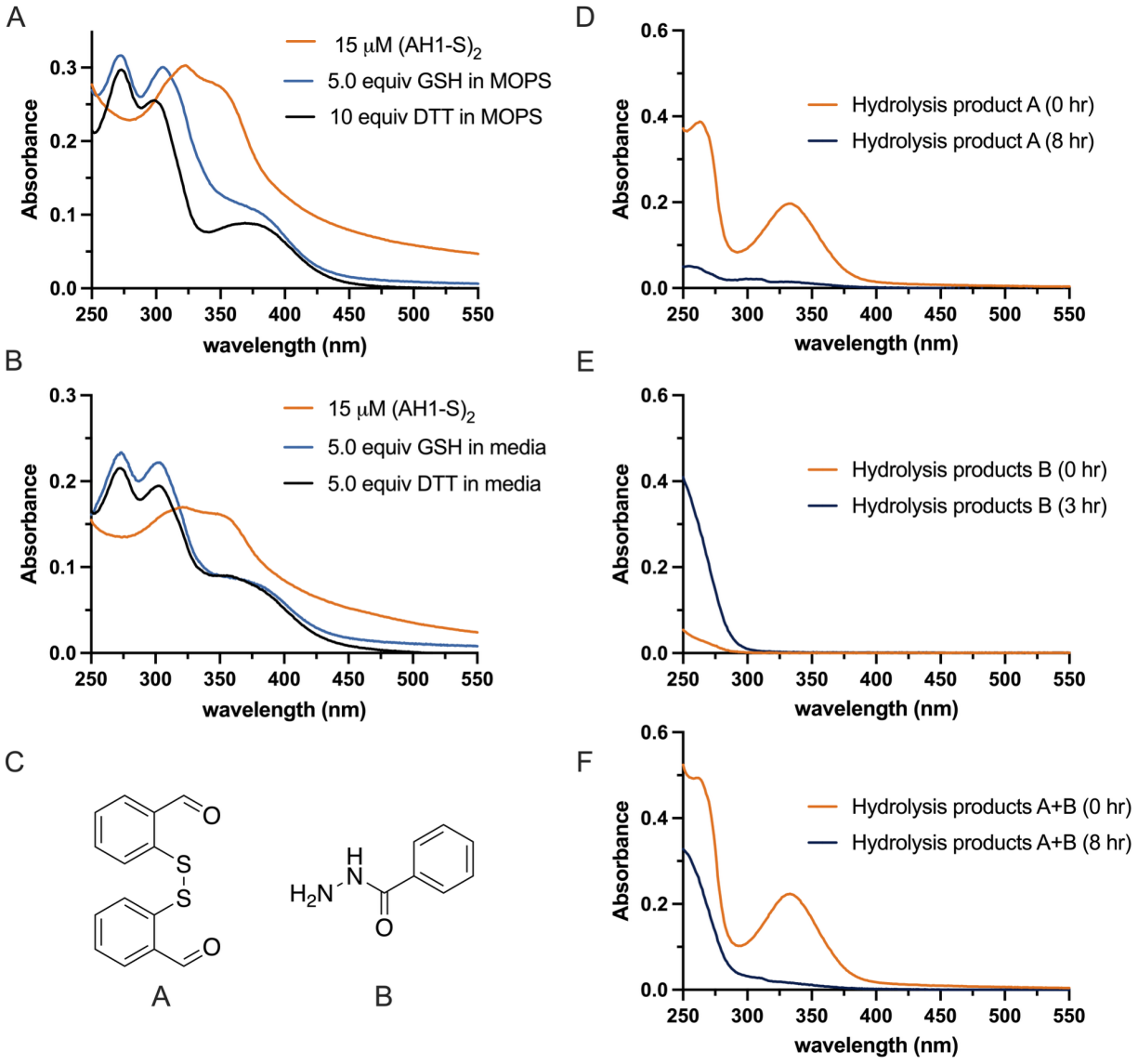

Figure S2. UV-visible spectra of 15 μM $(AH1\text{-}S)_2$ in **(A)** MOPS buffer or **(B)** SD medium before and after addition of 5 equivalents of glutathione (GSH, light blue) or dithiothreitol (DTT, dark blue). Solutions contain no more than 1 % DMSO. **(C)** Structures of hydrolysis products A (2'2'-dithioldibenzyl aldehyde) and B (benzoylhydrazine). **(D)** UV-visible spectra of 15 μM hydrolysis product A, **(E)** 30 μM hydrolysis product B, or **(F)** a combination of A + B in SD medium (1 % DMSO) over time.

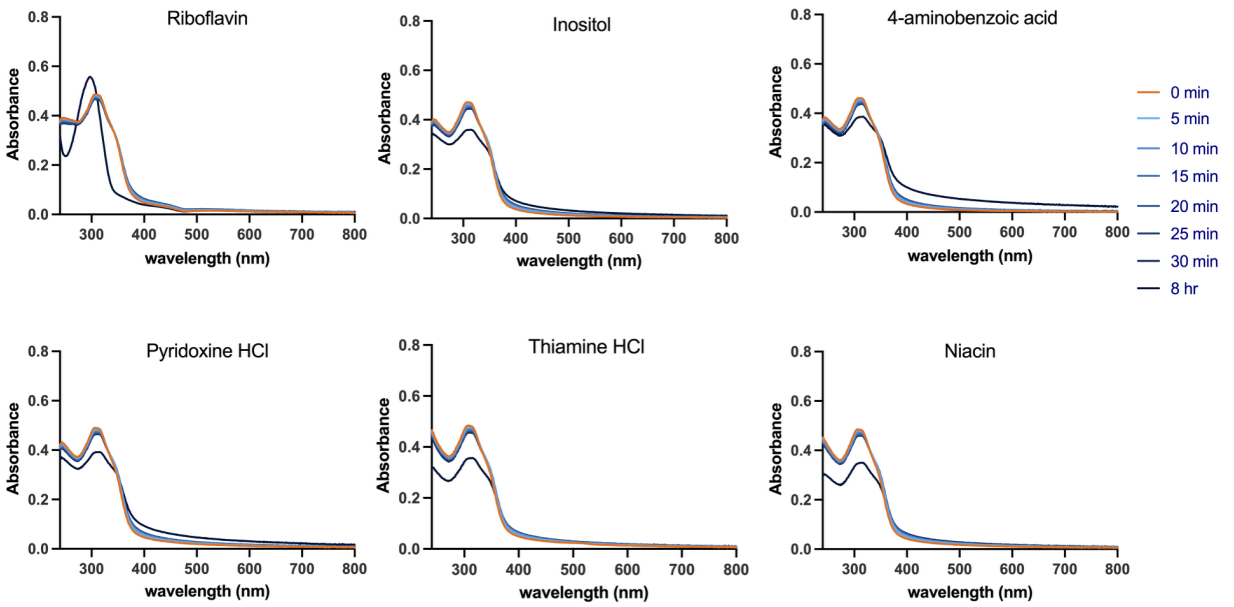

Figure S3. UV-visible spectra of 15 μM $(AH1\text{-}S)_2$ in MOPS buffer (1 % DMSO) with the addition of either 5.3 μM riboflavin, 14.6 μM 4-aminobenzoic acid, 111 μM inositol, 19.4 μM pyridoxine HCl, 10.7 μM thiamine HCl, or 32.5 μM niacin. Concentration of vitamins were chosen based upon the amount in SD medium. Scans were taken every 5 min up to 30 min, and a final scan was taken at 8 h. To account for changes in absorbance due to vitamin alone, each spectrum was adjusted by subtracting the absorbance of the vitamin's spectrum.

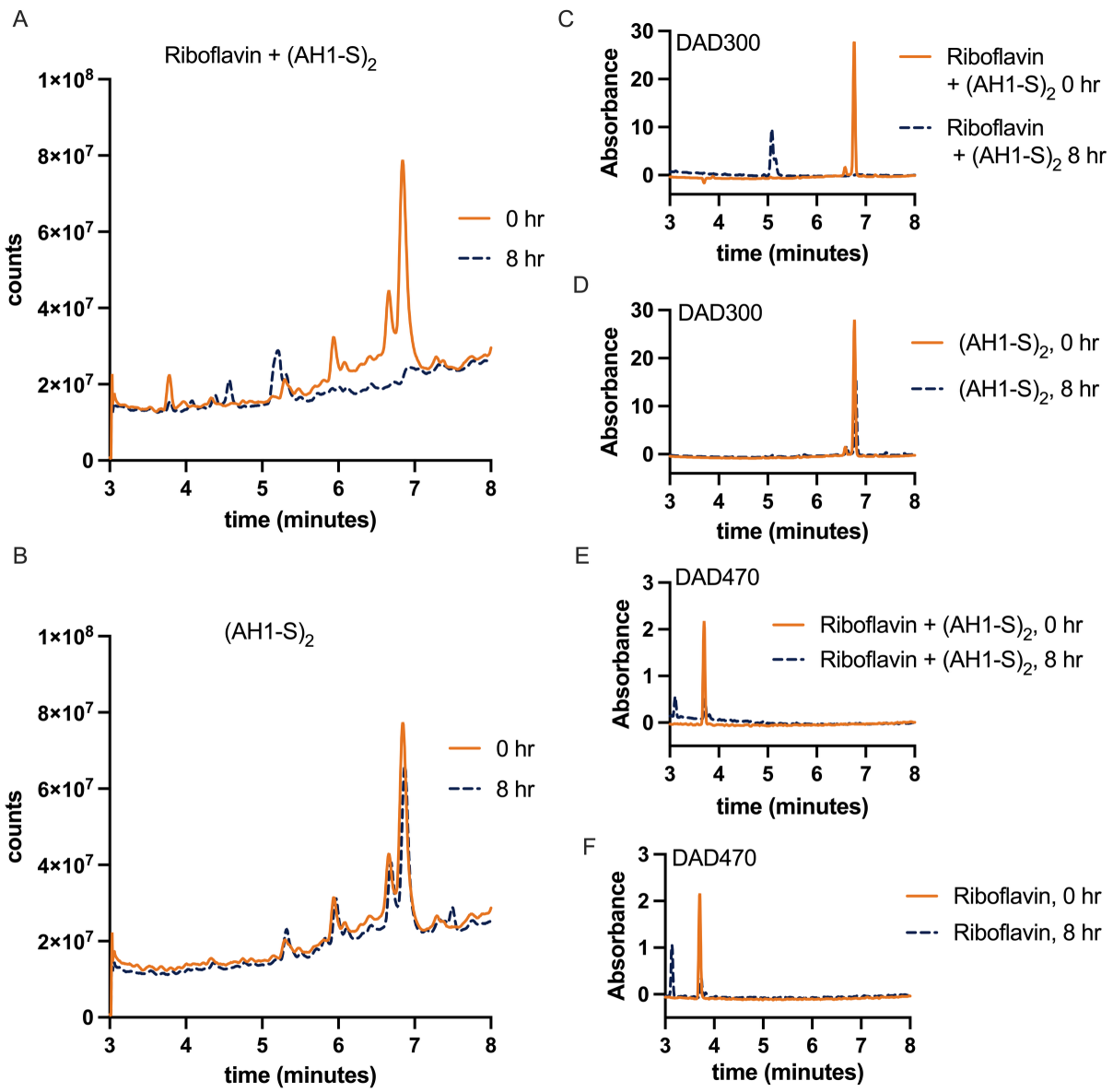

Figure S4. LC-MS analysis shows that riboflavin promotes the light-dependent degradation of $(AH1\text{-}S)_2$. Overlaid total ion chromatograms (TICs) of **(A)** 15 μM $(AH1\text{-}S)_2$ + 5.5 μM riboflavin or **(B)** $(AH1\text{-}S)_2$ alone in MOPS buffer at 0 h and after 8 h exposed to daylight in an Eppendorf tube. The peak corresponding to $(AH1\text{-}S)_2$ at 6.7 min disappears over time in the combined sample in (A). **(C-D)** Diode array detector (DAD) signals at 300 nm. $(AH1\text{-}S)_2$ elutes ~6.7 min and the unidentified photoproduct elutes at ~ 5.0 min. **(E-F)** DAD signal at 470 nm, corresponding to riboflavin, which elutes ~3.7 min.

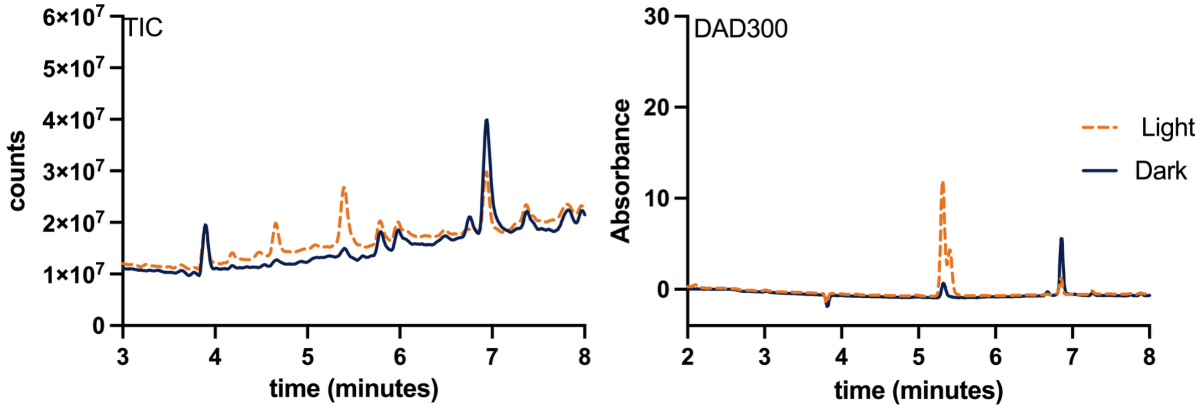

Figure S5. LC-MS traces of 15 μM $(AH1\text{-}S)_2$ + 5.5 μM riboflavin after 24 h in MOPS buffer under ambient light vs dark (wrapped in foil). Ambient light exposure promotes the degradation to a greater degree than when kept in the dark in MOPS buffer.

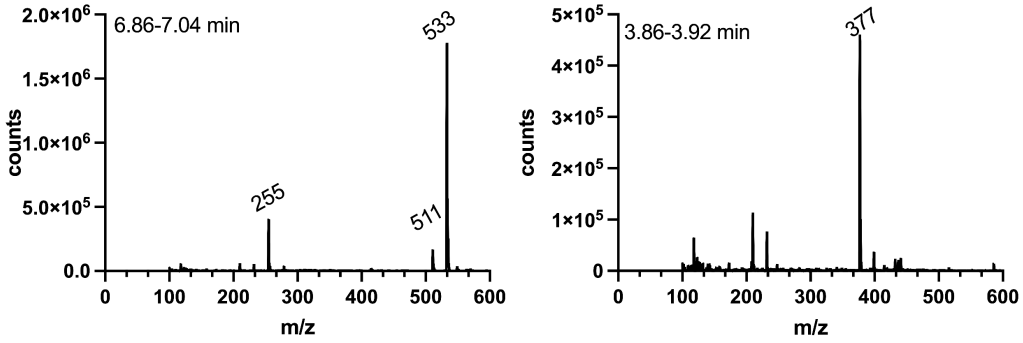

Figure S6. Representative mass spectra extracted from TIC traces in Figs. S4A of compounds that elute at t_R = 6.8 min (left) and t_R = 3.8 min (right). The molecular ion peak with a *m/z* of 533 corresponds to the sodium adduct of $(AH1\text{-}S)_2$; the molecular ion peak with an *m/z* of 377 corresponds to riboflavin.

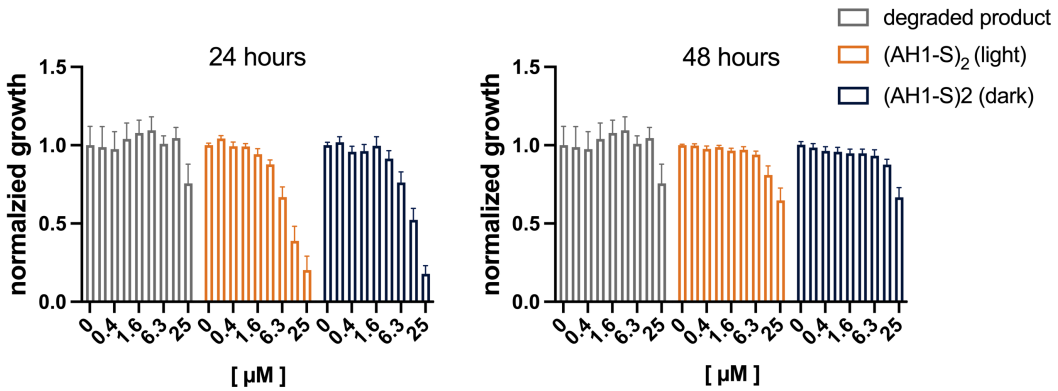

Figure S7. $(AH1\text{-}S)_2$ alone inhibits *C. albicans* growth in the dark and light at 24 and 48 h. Degraded product refers to a light-irradiated solution of $(AH1\text{-}S)_2$ and riboflavin in MOPS, with degradation verified by mass spectrometry. Cells were grown in SD medium (1 % DMSO) and growth was measured by OD_{600}. (n = 2–11 biological replicates composed of 1–3 technical replicates). Error bars represent SEM.

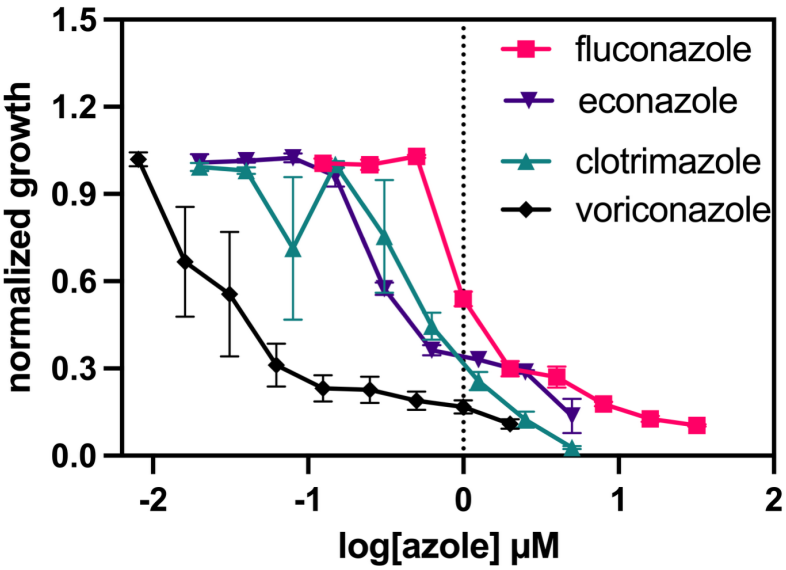

Figure S8. Microdilutions of fluconazole, econazole, clotrimazole, and voriconazole against *C. albicans* SC5314 in SD medium. 48 h timepoints are shown. Error bars represent SEM. (n=1 biological replicate of n=3 technical replicates).

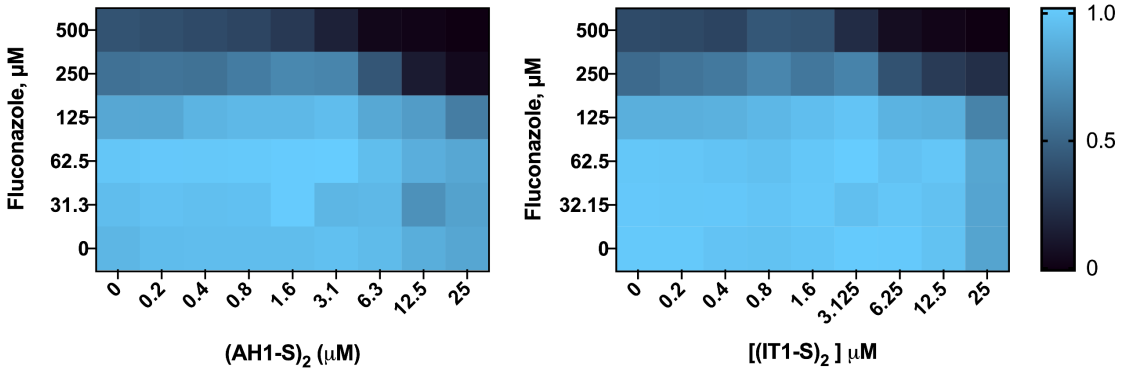

Figure S9. Fluconazole and prochelators $(AH1\text{-}S)_2$ (left) and $(IT1\text{-}S)_2$ (right) against *C. albicans* Y172. Cell growth in SD medium (1% DMSO) by OD_{600} after 48 h; n=2 biological replicates of 3 technical replicates each.

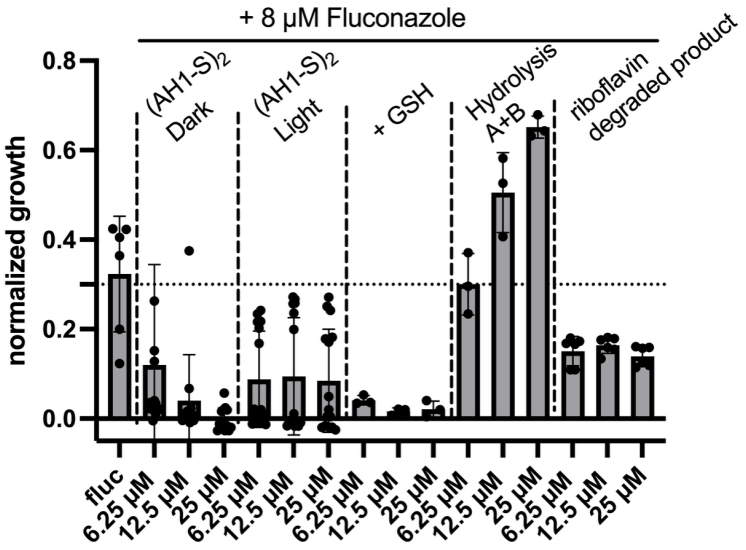

Figure S10. Growth assays of *C. albicans* SC5314 treated with 8 µM fluconazole and various controls of $(AH1\text{-}S)_2$: $(AH1\text{-}S)_2$ protected from light exposure (dark), $(AH1\text{-}S)_2$ exposed to ambient room light during the course of the experiment, $(AH1\text{-}S)_2$ treated with 5x GSH to generate AH1 in situ, 2'2'-dithioldibenzyl aldehyde and benzoylhydrazine (hydrolysis products A and B, Fig S2), and a solution of riboflavin and $(AH1\text{-}S)_2$ pre-exposed to sunlight to develop photodegradation products. Growth was measured at 48 h by OD_{600} and normalized to the non-treated cells; (n=1-6) biological replicates of 1-3 technical replicates.

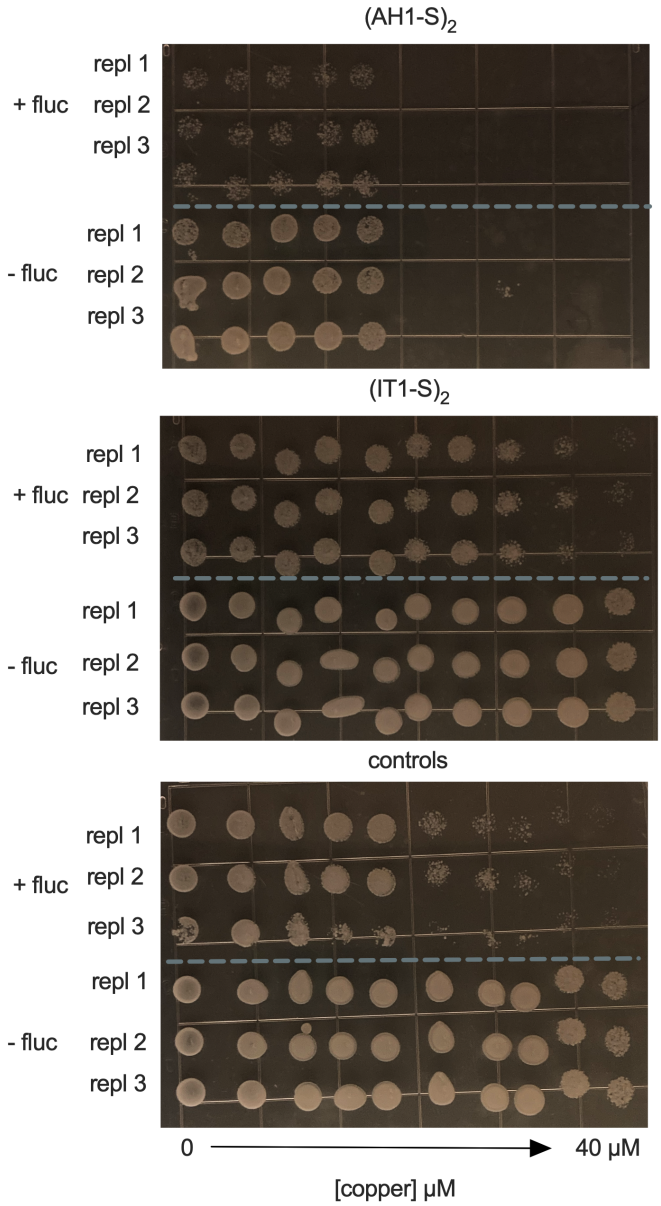

Figure S11. Cidality assays on YPD agar plates after 48 hours. (Top) 15 µM $(AH1\text{-}S)_2$ (middle) $(IT1\text{-}S)_2$ (bottom) fluconazole and no treatment.

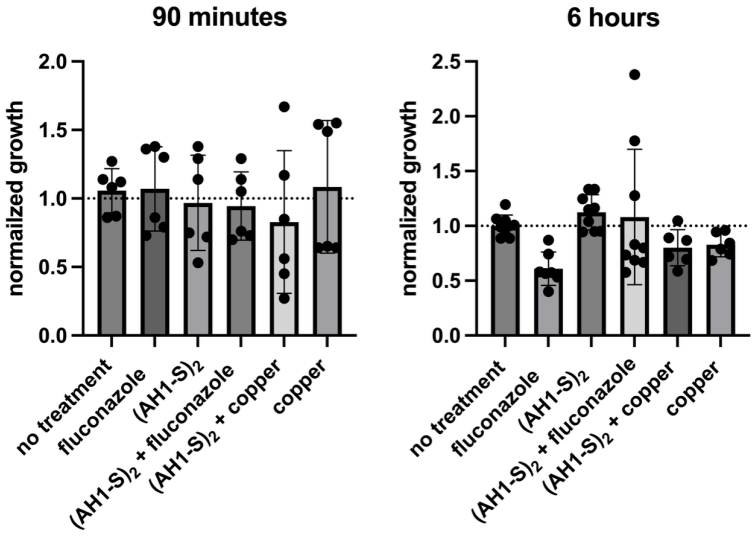

Figure S12. Comparison of relative growth levels of *C. albicans* SC5314 in YPD in the large culture volumes (25–100 mL) used for ICP-MS experiments. Treatment conditions: 25 μM $(AH1\text{-}S)_2$, 10 μM fluconazole, 10 μM copper, or a combination. Under these experimental conditions, fluconazole-treated cells lag in growth compared to non-treated cells at 6 h. $(AH1\text{-}S)_2$ + fluconazole did not on average impact growth compared to non-treated cells. However, the standard deviation for $(AH1\text{-}S)_2$ + fluconazole treated cells is much larger than the non-treated cells at 6 h post-treatment. Growth was measured by OD_{600} and normalized to no treatment. (n=2-3 biological replicates each of n=1-3 technical replicates).

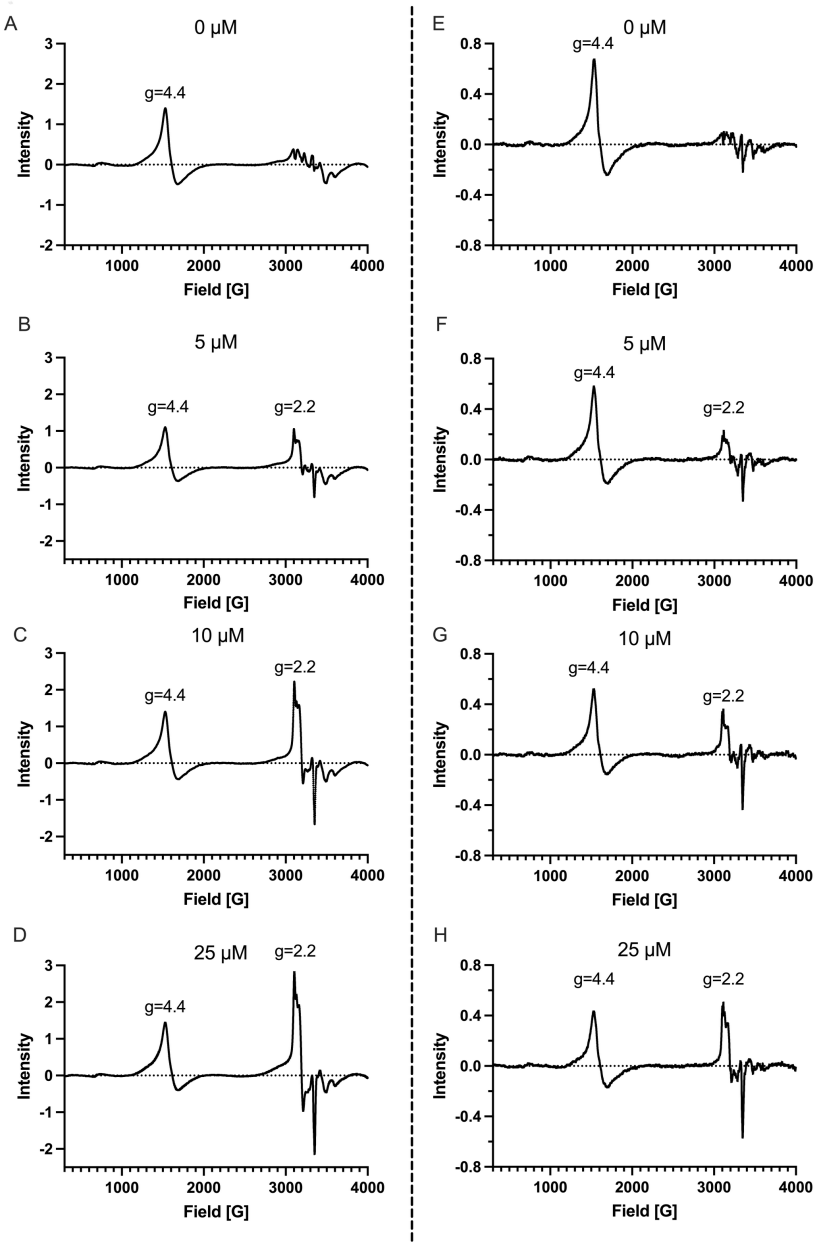

Figure S13. Concentration-dependence of whole cell EPR spectra of *C. albicans* treated with 0–50 µM $(AH1\text{-}S)_2$ after 90 min (left) and 6 h (right). An increase in the signal at g ~ 2.2–2.0 as a function of $(AH1\text{-}S)_2$ concentration is observable at both timepoints. For each spectrum, 2×10^9 cells were harvested; data collected at 77 K.

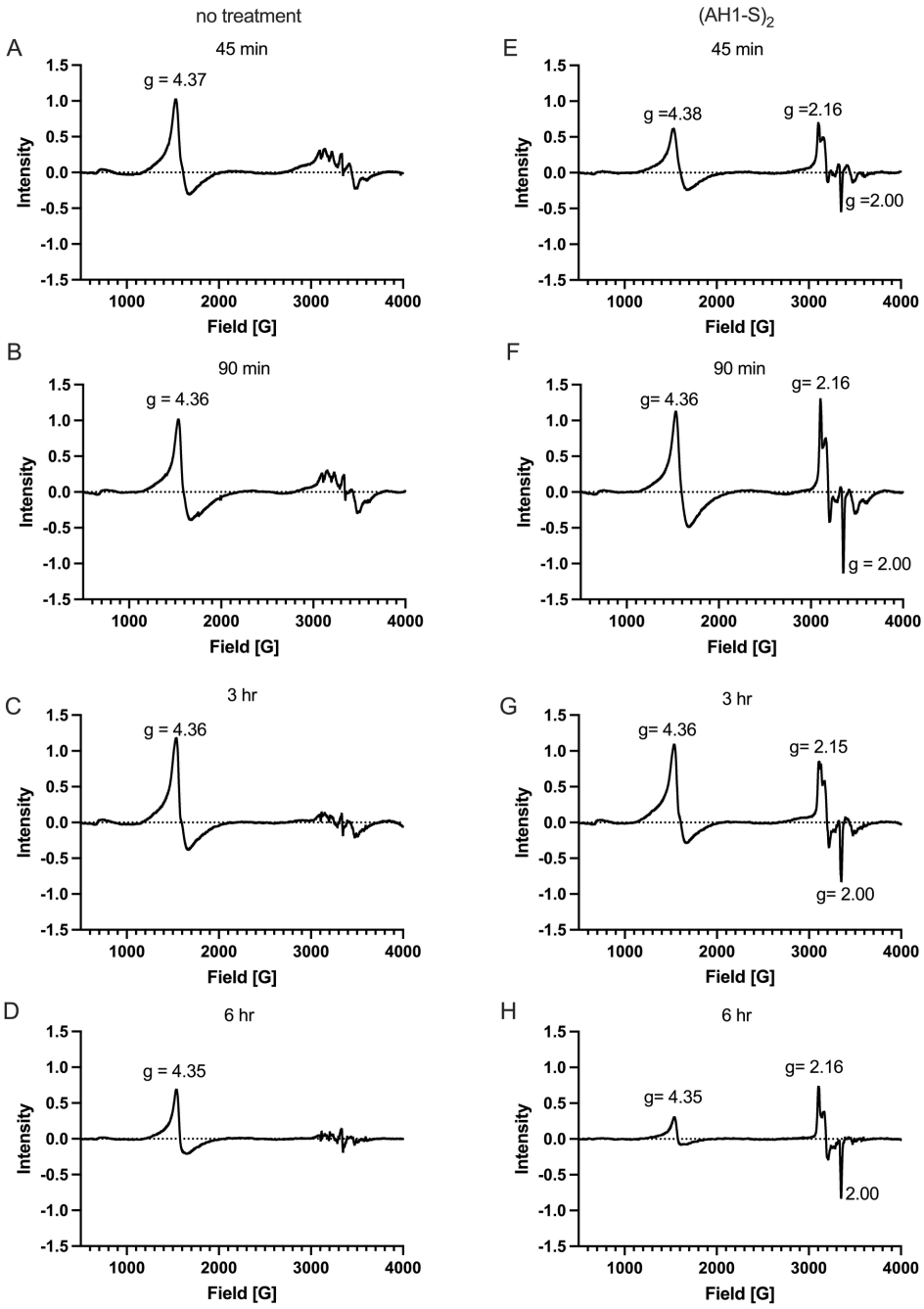

Figure S14. Time dependence of whole cell EPR spectra of non-treated *C. albicans* (**A–D**, left column) compared to treatment with 25 μM $(AH1\text{-}S)_2$ (**E–H)**, right column) for 45 min (A,E), 90 min (B, F), 3 h (C, G), and 6 h (D, H). In non-treated cells, the labile iron and manganese signals diminish over 6 h. In $(AH1\text{-}S)_2$ treated cells, the signal at g = 2.16–2.00 characteristic of low-spin Fe(III)AH1 complexes can be seen as early as 45 min, with the highest signal occurring at 90 min, then diminishing over time. The complex is still observed by 6 h. While both manganese and the Fe(III)AH1 complex give EPR signals ~g=2, the manganese signal is distinguished by its unique hyperfine structure, and the rhombic signal attributed to the Fe(III)AH1 complex has g values characteristic of previously characterized, isolated complexes.

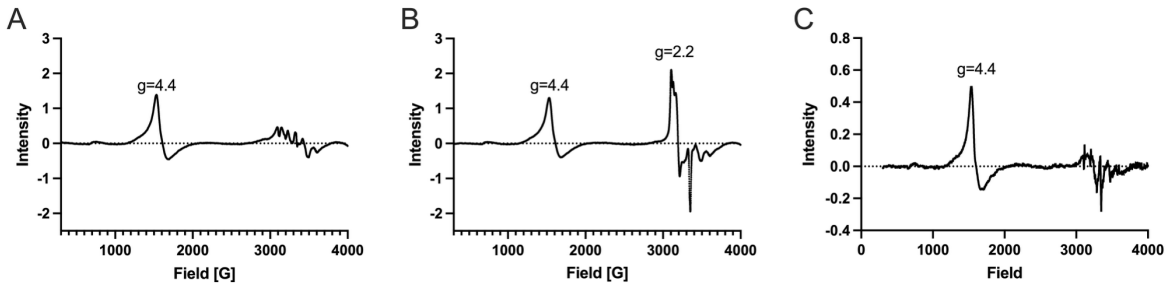

Figure S15. Whole cell EPR spectra of *C. albicans* treated with **(A)** 10 μM fluconazole and **(B)** 10 μM fluconazole +25 μM $(AH1\text{-}S)_2$ after 90 min. Neither fluconazole nor the combined treatment decreases the intensity of the labile iron signal (g=4.4) at this timepoint, in contrast to the 6 h data shown in Figures 7B and 7E in the main text. In the combined treatment, the signal corresponding to the low-spin Fe(III) complex with AH1 is observable by 90 min. **(C)** Control experiment showing whole cell EPR spectrum of *C. albicans* 6 h after treatment with 10 μM copper (comparison to Cu + $(AH1\text{-}S)_2$ spectrum shown in Fig. 7 F).

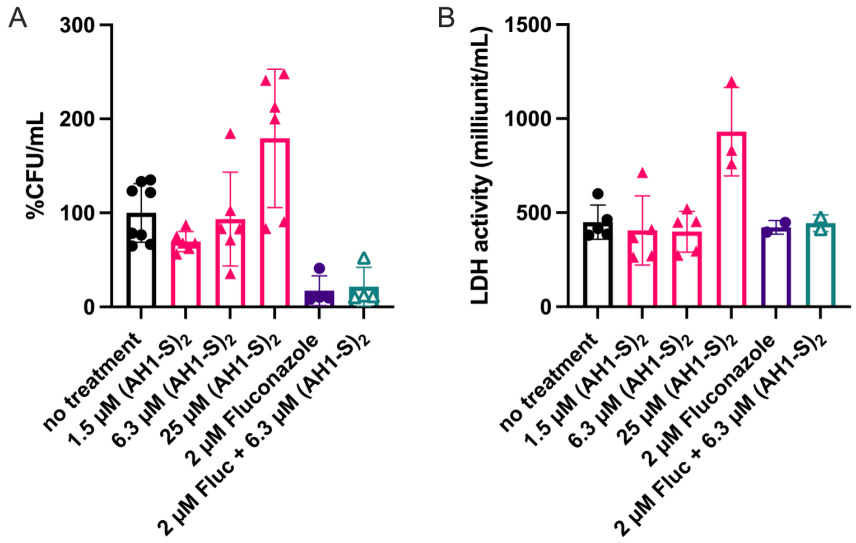

Figure S16. (A) Percent colony forming units (CFU) of *C. albicans* SC5314 under monoculture conditions (without macrophages) relative to the CFU of the non-treated monoculture. **(B)** Macrophage cytotoxicity as measured by the LDH assay under monoculture conditions.

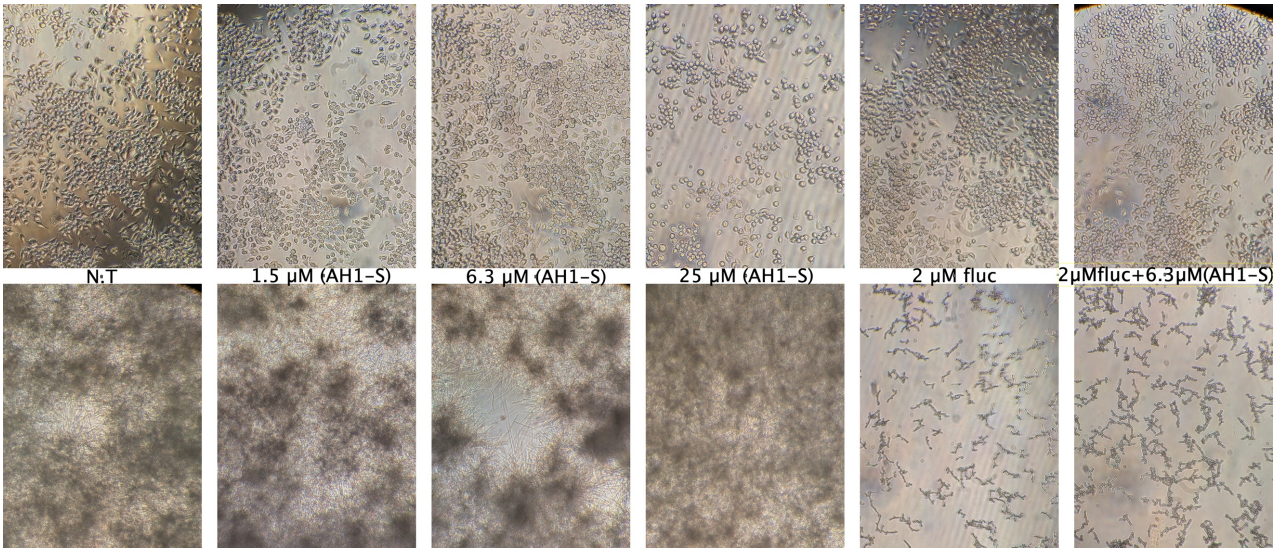

Figure S17. Representative images of macrophages grown in mono-culture (upper) and mono-culture *C. albicans* (lower). Cells were treated with 0-25 µM $(AH1\text{-}S)_2$, 2 µM fluconazole, or a combination in mammalian cell culture conditions.

Supplementary Table S1. Vitamins, essential biometals, and other ingredients in SD complete media

		10x concentration µM* or g/L[+]	SD concentration µM* or g/mL[+]
Vitamins	Biotin	0.0819*	0.00819*
	Calcium Pantothenate	8.39*	0.839*
	Folic acid	0.048*	0.0048*
	Inositol	111*	11.1*
	Niacin	32.5*	3.25*
	Para-aminobenzoic acid	14.6*	1.46*
	Pyridoxine HCl	19.4*	1.94*
	Riboflavin	5.3*	0.53*
	Thiamine HCl	10.7*	1.07*
Salts / other	Ammonium sulfate	50[+]	5 [+]
	Boric acid	80.8*	8.08*
	Calcium chloride	1.0[+]	0.1 [+]
	Manganese sulfate	5.0[+]	0.5 [+]
	Potassium iodide	6.02*	0.602*
	Potassium phosphate	10 [+]	0.1 [+]
	Sodium chloride	1.0[+]	0.1 [+]
Metals	Copper Sulfate	N/A	0.250
	Iron Chloride	N/A	0.740
	Manganese sulfate	N/A	2.4
	Zinc Sulfate	N/A	2.2
	Sodium Molybdate	8.27 *	0.827*
Sugar	D-(+)-glucose	200[+]	20 [+]

Supplementary Table S2. Average values with standard deviations of cellular metals detected by ICP-MS (µmol/ cell)

	No-treatment	$(AH1\text{-}S)_2$	$(AH1\text{-}S)_2$ + fluconazole	$(AH1\text{-}S)_2$ + copper	Fluconazole	Copper
Iron (90 min)	4.32E-07 ± 1.13E-07	4.50E-07 ±1.70E-07	4.20E-07 ±1.71E-07	3.65E-07 ±6.97E-07	4.67E-07 ±1.29E-07	5.11E-07 ±3.62E-07
Iron (6 h)	2.13E-08 ± 4.94E-09	1.43E-08 ±4.02E-09	2.19E-08 ±9.55E-09	3.61E-08 ±1.64E-08	4.15E-08 ±9.10E-09	2.99E-08 ±1.43E-08
Copper (90 min)	1.10E-08 ±4.99E-09	1.66E-08 ±1.22E-08	1.68E-08 ±1.27E-08	5.76E-08 ±1.10E-07	1.31E-08 7.70E-09	1.97E-08 ±8.06E-09
Copper (6 h)	1.42E-09 ±1.25E-09	1.19E-09 ±1.35E-09	2.70E-09 ±3.43E-09	1.29E-08 ±5.32E-09	2.85E-09 ±2.65E-09	9.53E-09 ±1.33E-09
Zinc (90 min)	2.49E-07 ±3.96E-08	2.90E-07 ±8.88E-08	2.48E-07 ±6.38E-08	4.18E-07 ±3.94E-07	2.25E-07 ±5.88E-08	2.45E-07 ±9.97E-08
Zinc (6 h)	4.53E-08 ±1.81E-08	3.63E-08 ±1.78E-08	3.43E-08 ±1.78E-08	6.05E-08 ±1.49E-08	5.41E-08 ±1.39E-08	6.19E-08 ±8.25E-09
Manganese (90 min)	2.10E-08 ±2.13E-08	2.03E-08 ±3.49E-09	2.13E-08 ±4.93E-09	2.64E-08 ±2.23E-08	2.02E-08 ±2.76E-09	2.03E-08 ±7.95E-09
Manganese (6 h)	1.02E-09 ±5.55E-10	7.81 E-10 ±3.77E-10	1.30E-09 ±5.69E-10	2.04E-09 ±1.36E-09	2.08E-09 ±6.06E-10	1.38E-09 ±4.25E-10

Data is composed of n=2-3 biological replicates, each of n=1-3 technical replicates.