

## Supplementary Material

### Antifungal Effect of Vitamin D<sub>3</sub> against *Cryptococcus neoformans* Coincides with Reduced Biofilm Formation, Compromised Cell Wall Integrity, and Increased Generation of Reactive Oxygen Species

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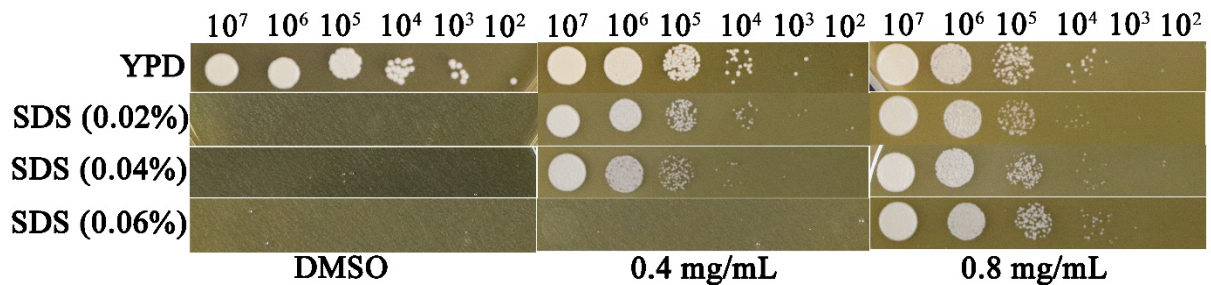
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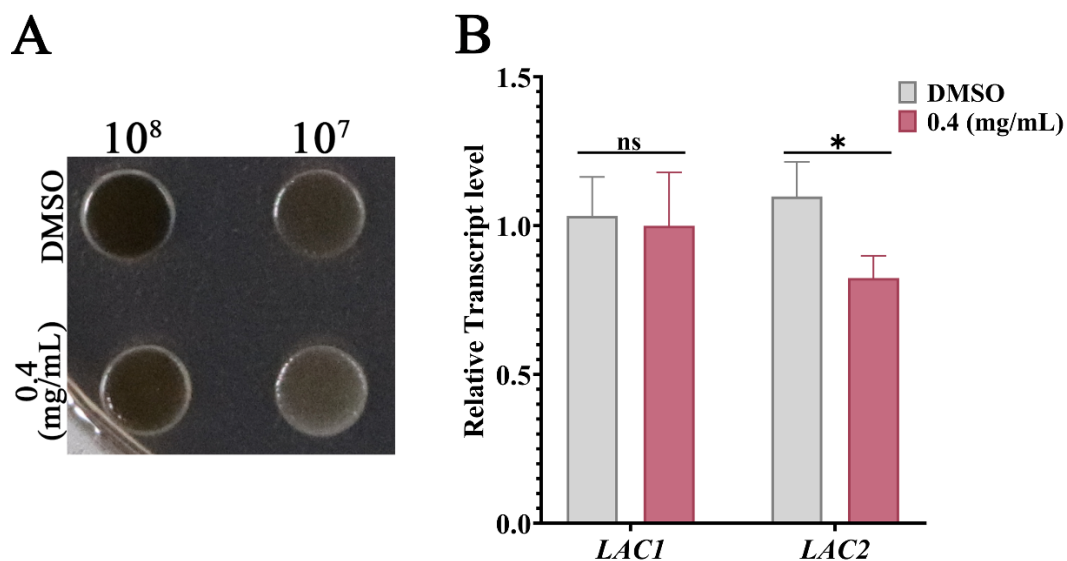
**Table S1.** Nucleotide sequences of primers used for RT-qPCR analysis.

Gene	Primer Sequence (5'-3')
<i>GPD1 F</i>	AGTATGACTCCACACATGGTCG
<i>GPD1 R</i>	AGACAAACATCGGAGCATCAGC
<i>CHS3 F</i>	ACCCAGGTCTGGCATTCC
<i>CHS3 R</i>	AGGATCAACATTGGAAGC
<i>CHS4 F</i>	CGGTCTTCAGGCATTGATTT
<i>CHS4 R</i>	TTCGGAGTGAAGTGATGCTG
<i>CHS5 F</i>	GCTTGATGATCTTCTATATCTG
<i>CHS5 R</i>	TACCTTCATCATGGATGACA
<i>CHS6 F</i>	TTGACCCTTGGCACATCT
<i>CHS6 R</i>	GTTGGCATAAGTATCCTT
<i>CDA1 F</i>	TCGAGCTATTGCTGCTCAGA
<i>CDA1 R</i>	GCTGGTAGATGTCGTGCTCA
<i>CDA2 F</i>	GTAACGAGGTCGTCTTTG
<i>CDA2 R</i>	TGTAGTTGGTGAGCTCGT
<i>CDA3 F</i>	ATGTGGCCGATGCTTTTAAC
<i>CDA3 R</i>	GAAGTGAGAAGGCCTGTTGG
<i>CDA4 F</i>	CGAACTCTCCAAGGTCGAAG
<i>CDA4 R</i>	GCGTCTCCAGTGTGCATCAGA
<i>CHI22 F</i>	AATGTGAGTGGGTCGGAAAG
<i>CHI22 R</i>	TAGCCGCCGAGTCTGTAGAT
<i>AGS1 F</i>	ATCCTTATCCGTTATTCC
<i>AGS1 R</i>	AGCTGTTCTCTAGCGAGC
<i>FKS1 F</i>	TGGACTGGTGTGTTGGTTCAA
<i>FKS1 R</i>	GTACAAAAGACCGTACTTG
<i>SKN1 F</i>	CTGGACAATGTATGCGGATG
<i>SKN1 R</i>	TCCGCAGTGGGATAATCTTC
<i>KRE6 F</i>	GTCTCGGAAGGCGACTCAT

<i>KRE6 R</i>	TCAACTCATTCTTTGGGAAGG
<i>LAC1 F</i>	TACAACTTTCCCCGACCTC
<i>LAC1 R</i>	GATGGAGAAGGTGAGCGTC
<i>LAC2 F</i>	TGTATGGCGCAAGGGGTTACT
<i>LAC2 R</i>	AGAACACGACTCTCCAAAGC



**Figure S1.** VD<sub>3</sub> reduced the stress of SDS with high concentrations. An overnight culture of *C. neoformans* strain H99 was washed and diluted to  $1 \times 10^7$  cells/mL in phosphate-buffered saline (PBS). Sensitivity to cell surface stress was evaluated with 0.02%, 0.04%, 0.06% SDS (Sigma-Aldrich Corporation). In brief, the cell suspensions were serially diluted in YPD medium containing different concentrations of VD<sub>3</sub> (0, 0.4 and 0.8 mg/mL) with or without the stressors, incubated at 37°C for 3 days, and photographed.



**Figure S2.** Effects of VD<sub>3</sub> on melanin formation. *C. neoformans* was cultured in YPD medium containing 0.4 mg/mL DMSO or VD<sub>3</sub> for 6 h. (A) VD<sub>3</sub> inhibited melanization on minimal medium containing 0.5 mM L-DOPA (3,4-dihydroxy-L-phenylalanine; Sigma-Aldrich Corporation). Images were captured after incubation for 3 days. (B) RT-qPCR quantification of the expression of genes related to melanin biosynthesis. Relative expression levels of genes were calculated using  $2^{-\Delta\Delta CT}$  method against *GPD1* as the housekeeping gene. Data were analyzed using t-test (ns,  $p > 0.05$ ; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; \*\*\*\*,  $p < 0.0001$ ).