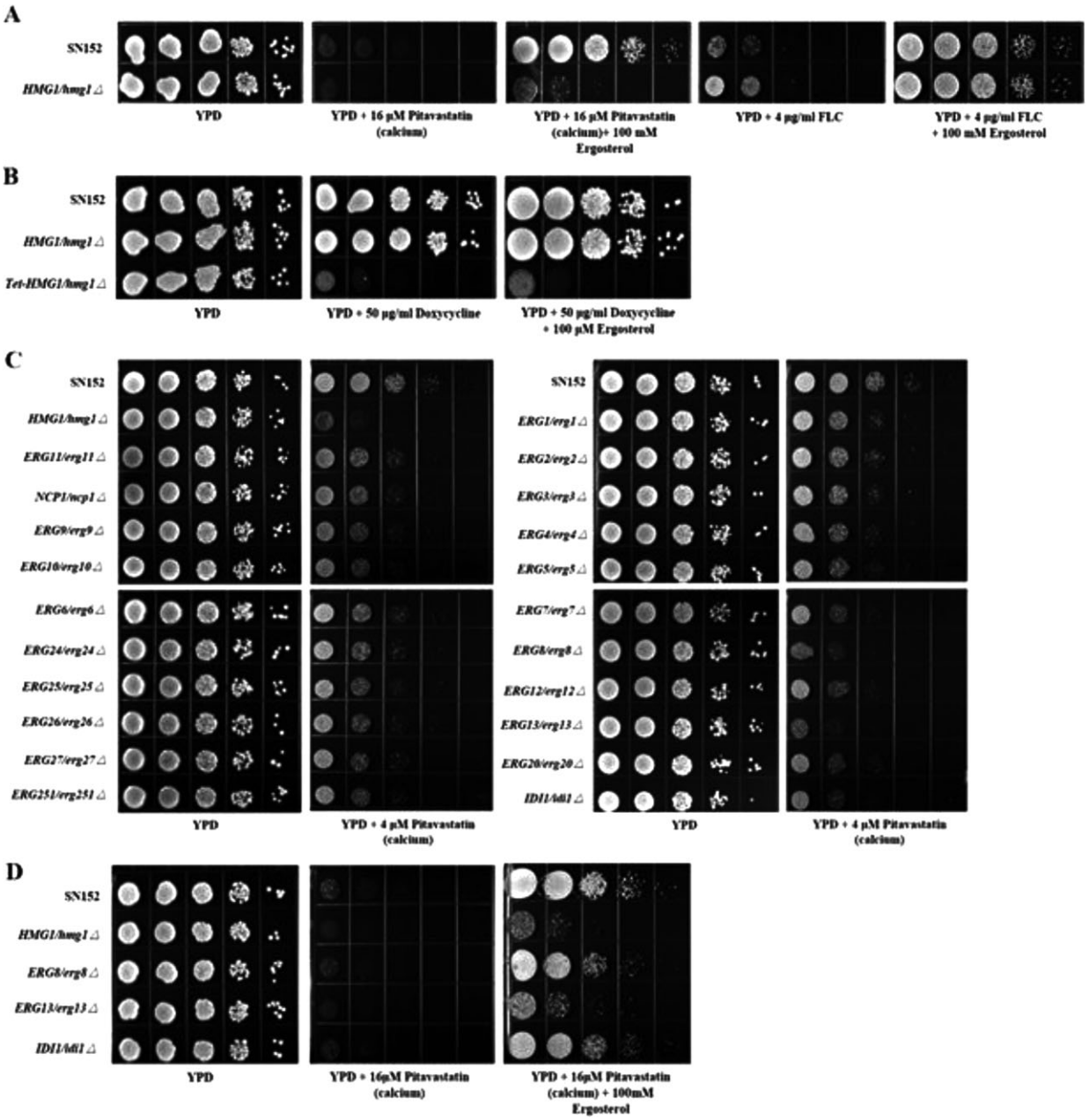

(Fig. 2b). This paradoxical phenotype suggests that Hmg1 is unlikely to be a target of PIT. We found four heterozygous deletion mutants increased susceptibility to PIT (Fig. 2c) and the growth defect of ERG8/erg8Δ and IDI1/idi1Δ mutants exposed to PIT can be restored by ergosterol (Fig. 2d), suggesting that Erg8 and Idi1 are potential targets of PIT.

Conclusions: Our study demonstrates that PIT has a better synergistic lethal effect with FLC by inhibiting ergosterol synthesis and higher safety and suggests that PIT may be repurposed as a promising adjuvant to make FLC fungicidal and enhance the efficacy of FLC in treating invasive fungal infections caused by pathogenic fungi with high FLC tolerance.

S3.4b

Lactoferrin, a natural source of peptides that potentiate the antifungal activity of amphotericin B

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S3.4 Free oral paper session, September 21, 2022, 4:45 PM - 6:15 PM

Objectives: It is notoriously difficult to prevent and treat fungal infections, however, the natural world has come up with remedies that are non-toxic, effective, and evade resistance. Here we investigate lactoferrin, an iron-binding glycoprotein found in milk, tears, and sweat, for its capacity to inhibit fungi and to synergize with commonly used antifungal drugs, with the aim of determining its mode of action.

Methods: Lactoferrin (LF) was obtained from a commercial supplier and two dairy companies. LF was tested on suite of pathogenic yeast and mold species for inhibition using CLSI microdilution methods. Synergy was determined with antifungal drugs amphotericin B (AMB), nystatin (NYS), fluconazole (FLC), itraconazole (ITC), voriconazole (VRC), and 5-fluorocytosine (5FC). The effect of LF on fungal cells was analyzed using scanning electron microscopy (SEM). The active peptide/s within LF were then predicted from pepsin and *in silico* digestion, synthesized, and tested for synergy with amphotericin B (AMB). Tethered synthetic membranes were produced and were loaded with ergosterol or cholesterol to test the nature and specificity of membrane binding by LF and the synthetic peptide.

Results: LF demonstrated antifungal activity against yeast species *Cryptococcus*, *Candida*, and *Saccharomyces* and was much less effective against molds. Good synergy was achieved with AMB but not azole or echinocandin drugs. While the iron-chelating capacity of LF was important for the antifungal activity it was not involved in synergy. SEM revealed cell damage suggesting an interaction between AMB, LF and the fungal membrane or cell wall. A 30-residue peptide from the C lobe of LF was synthesized and tested for activity and synergy. This peptide, dubbed lactofungin (LFG) was inactive alone but was potently synergistic with AMB, indicating a direct role in augmenting AMB activity. Synthetic membranes loaded with ergosterol but not cholesterol were disrupted by AMB + LFG, demonstrating that activity was fungal-specific and was mediated through ergosterol binding.

Conclusion: LF is a complex molecule that causes fungal inhibition via iron binding and when cleaved by pepsin can produce active peptides. As AMB is a highly toxic treatment, the use of LFG as a synergent could help increase activity while lowering the effective dose, thereby reducing undesirable side effects. The action of AMB + LFG appears dependent on ergosterol, suggesting inhibition will be highly fungal-specific.

S3.4c

A pipeline toward the identification of novel antifungal compounds derived from the microbial dark matter

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S3.4 Free oral paper session, September 21, 2022, 4:45 PM - 6:15 PM

Background: The current armamentarium of antifungal drugs and the restricted variety in antifungal drug classes combined with the ever-rising threat of resistant fungal pathogens highlighted the urgent need for novel antifungal compounds. Natural