

# Cas5 Influences the Activity of Fluconazole Against *Candida albicans*

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## Introduction

Azole antifungals, such as fluconazole (FLC), are the most commonly utilized antifungal agent in the treatment of *Candida* infections.<sup>1</sup> Unfortunately, efficacy is limited due to both the fungistatic nature of this class and rising rates of resistance.<sup>2</sup> Identifying strategies to circumvent these limitations could lead to enhancement of azole activity. The purpose of this study is to identify transcription factors (TFs) and corresponding transcriptional pathways essential for the fungistatic quality of the azoles in order to identify potential drug targets to enhance azole activity.

## Results

TABLE 1 Library hits with reduced MICs and MFCs in both RPMI (48 hr) and YPD (24 hr)

Clone ID	CGD	orf19 designation	MIC ( $\mu\text{g/ml}$ )		MFC ( $\mu\text{g/ml}$ )	
			RPMI	YPD	RPMI	YPD
WT	--	--	0.25	0.5	>4	>64
TF3	<i>RPN4</i>	orf19.1069	0.0625	0.25	0.125	32
TF33	<i>CAS5</i>	orf19.4670	0.125	0.25	4	2
TF77	<i>UPC2</i>	orf19.391	0.03125	0.125	1	1
TF104	<i>CZF1</i>	orf19.3127	0.0625	0.5	0.25	8

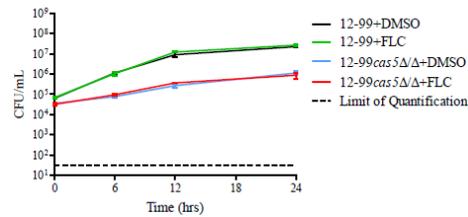


Figure 2.  
**CAS5** disruption in a highly azole resistant clinical isolate containing multiple resistance mechanisms did not alter susceptibility.

## Methods

- A transcription factor knock out library was screened for genes without which MFC was reduced.
  - Identified *cas5Δ/Δ* as having a marked reduction in MFC in both RPMI and YPD media.
- CAS5* was disrupted independently in SC5314 using the *SAT1* flipper method and assessed by broth microdilution, MIC/MFC testing, E-test, antifungal spot assays, and time-kill analysis.<sup>3-5</sup>
- Expression of *CDR1*, *CDR2*, *MDR1*, and *ERG11* was measured by qPCR.<sup>6</sup>

## Conclusions

These data suggest that *Cas5* regulates a transcriptional network that influences FLC activity against *C. albicans*. Further delineation of this transcriptional network may identify potential targets for co-therapeutic strategies to impart enhanced activity to the azole class of antifungals.

## References

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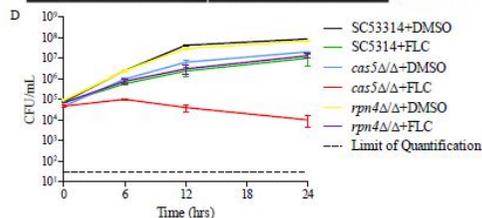
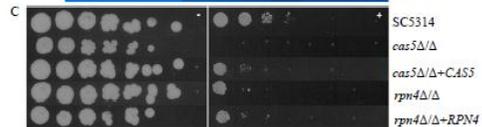
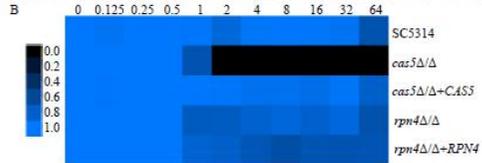
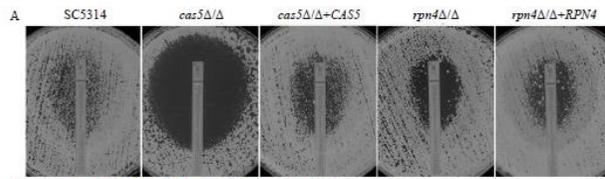


Figure 1.  
The *cas5Δ/Δ* mutant shows reduced susceptibility and a clear zone of inhibition by Etest, is unable to recover from fluconazole exposure at concentrations as low as 2  $\mu\text{g/ml}$  after 72 hours in YPD medium, is unable to grow on solid media containing 10  $\mu\text{g/ml}$  fluconazole, and exhibits increased susceptibility by time-kill analysis.

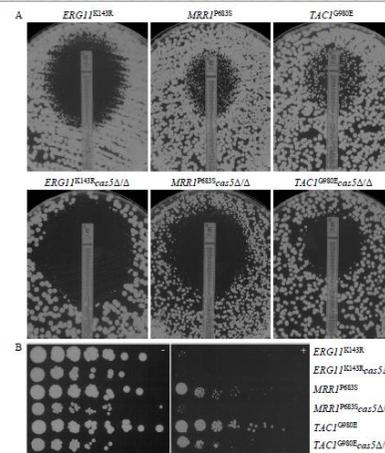


Figure 3.  
**CAS5** deletion in strains with specific mutations in *ERG11*, *MRR1*, or *TAC1* show increased susceptibility to fluconazole by Etest and a decreased ability to grow in the presence of fluconazole at 10  $\mu\text{g/ml}$ .

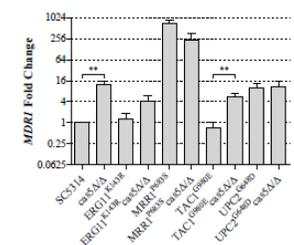
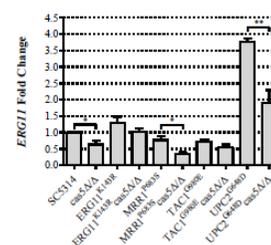
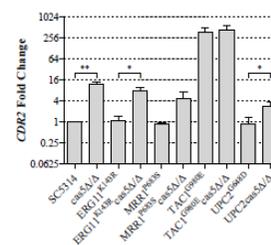
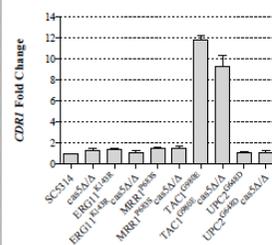


Figure 4.  
The moderate effect on susceptibility observed upon disruption of *CAS5* does not appear to be due to altered expression of the efflux pumps.