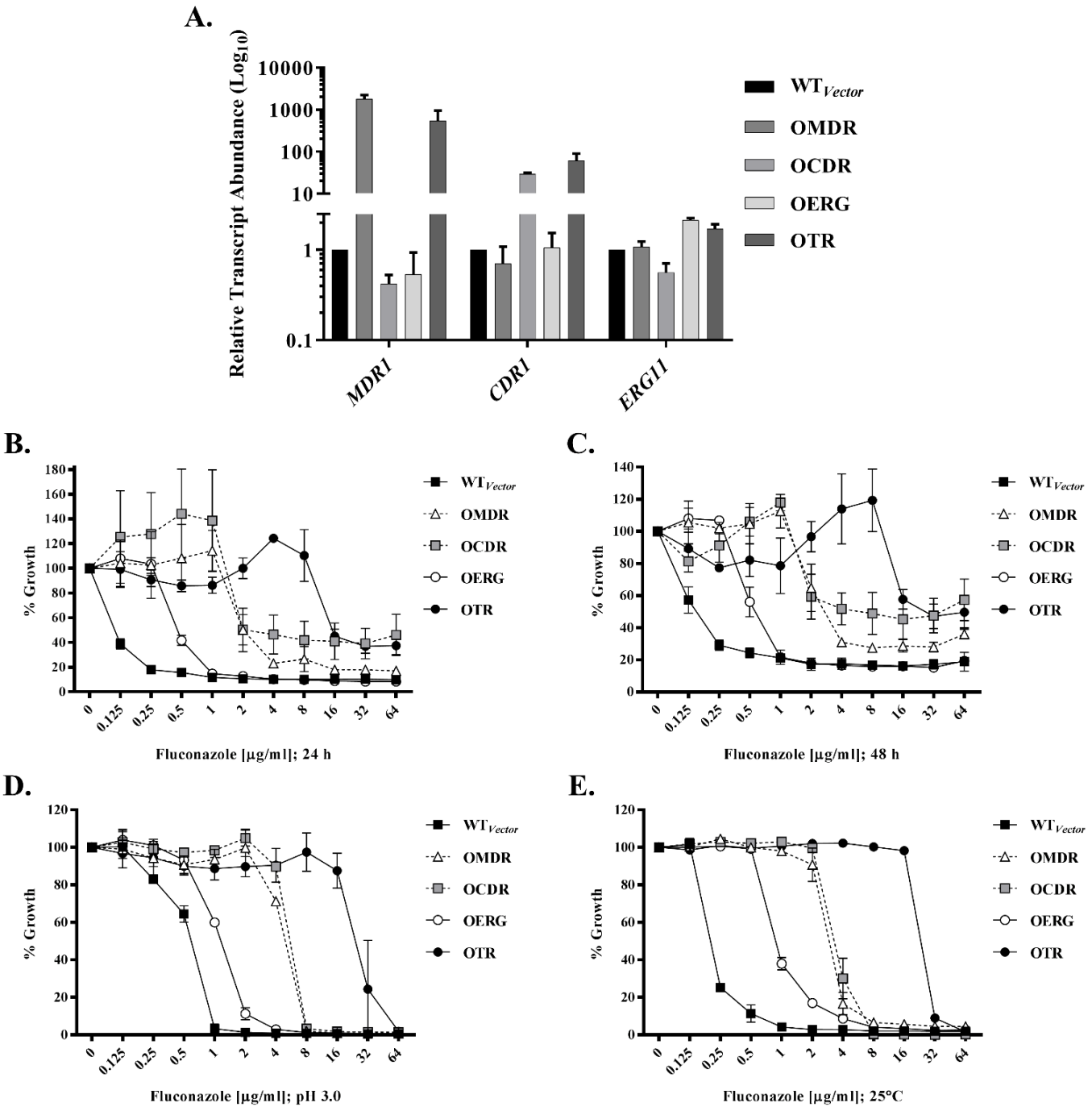
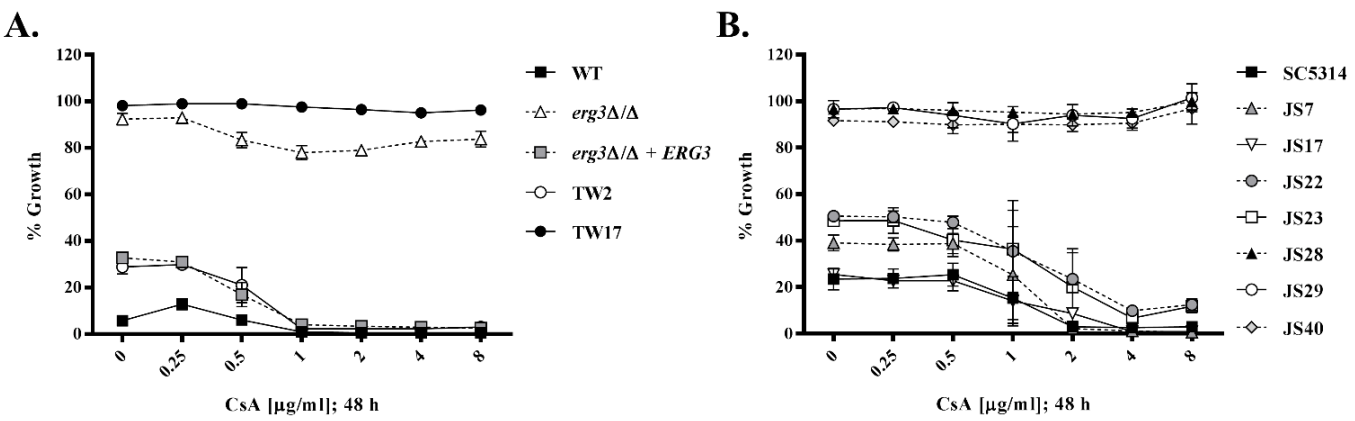
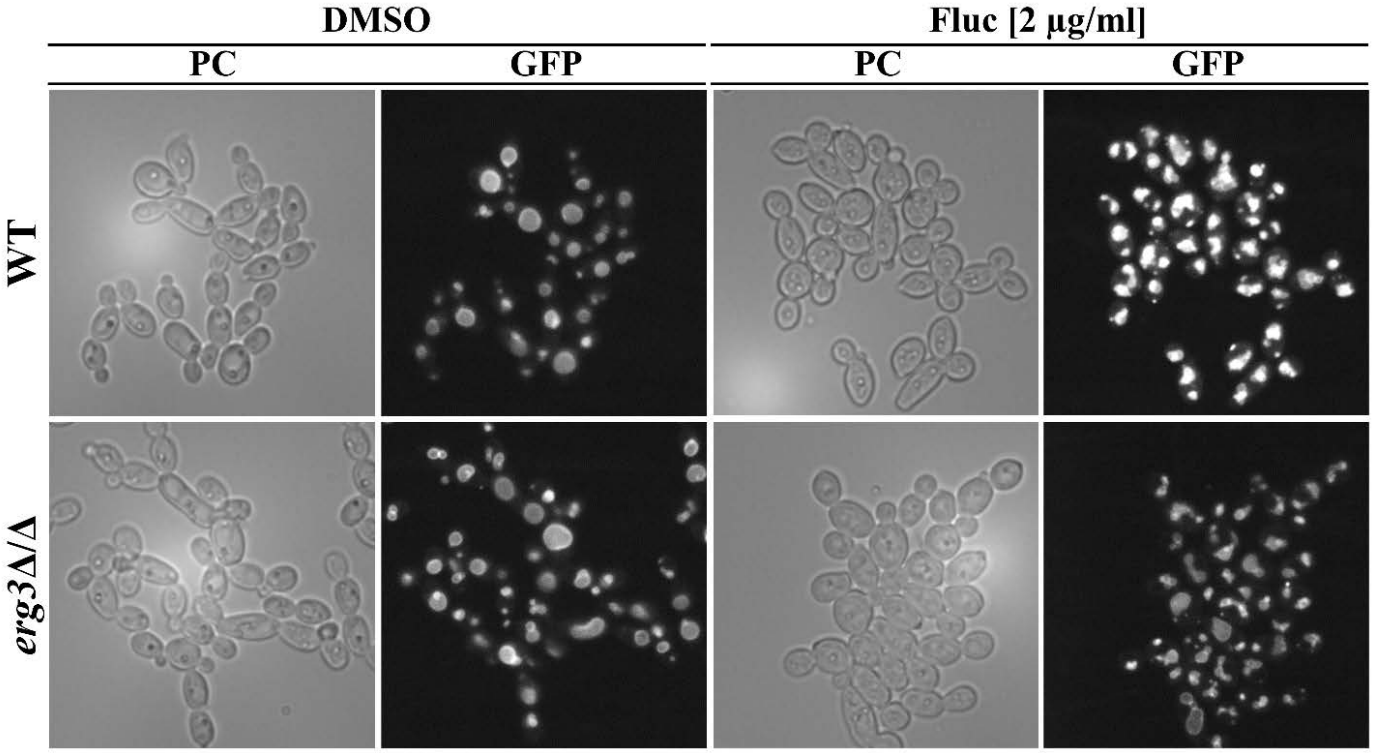


Supplemental Figure 1. Azole resistance due to overexpression of efflux pumps or Erg11p is not eliminated under conditions that abolish trailing growth. The wild-type (WT_{Vector}, XCTRL), OCDR (overexpressing Cdr1p), OMDR (overexpressing Mdr1p), OERG (overexpressing Erg11p), and OTR (overexpressing Cdr1p, Mdr1p and Erg11p) strains were grown overnight in YPD at 30°C then sub-cultured to an OD_{600nm} of 0.2 and then incubated at 30°C with shaking for 6 hours. RNA was then extracted and *MDR1*, *CDR1*, and *ERG11* transcript abundance quantified by qRT-PCR using the $2^{-\Delta\Delta}$*CT* method, and normalized to that of

ACT1 for each sample (A). The mean and standard error of the mean from two biological replicates, each with technical triplicates are shown for each strain. Relative transcript levels for each gene are expressed relative to the wild-type (WT_{Vector}) strain. The susceptibility of the wild-type (WT_{Vector}), OCDR (overexpressing Cdr1p), OMDR (overexpressing Mdr1p), OERG (overexpressing Erg11p), and OTR (overexpressing Cdr1p, Mdr1p and Erg11p) to fluconazole was evaluated using the CLSI broth microdilution protocol. Growth was measured as OD_{600nm} after 24 (B) or 48 (C) hours and expressed as a percentage of the growth in the minus drug (DMSO alone) control wells for each strain. Fluconazole susceptibility was also evaluated with RPMI medium with a pH of 3.0 (D), or with incubation at 25°C (E) and growth was compared after 48 hours of incubation. The mean ± standard deviation of three biological replicates are indicated.



Supplemental Figure 2. Growth of the *Candida albicans erg3*Δ/Δ mutant in the presence of fluconazole does not dependent upon calcineurin signaling. The growth of the indicated strains was compared in dose response experiments with the calcineurin inhibitor cyclosporine A (CsA) in the absence or presence of 1 µg/ml fluconazole, according to the conditions in the CLSI broth microdilution protocol. The growth of each strain was measured as OD_{600nm} after 48 hours incubation and expressed as a percentage of the growth in the minus drug (DMSO) control wells. The mean ± standard deviation of three biological replicates is shown in each panel.



Supplemental Figure 3. The *Candida albicans erg3*Δ/Δ mutant exhibits less vacuole fragmentation than wild-type following fluconazole treatment. The wild-type (WT) strain CAI4 and *erg3*Δ/Δ mutant were transformed with plasmid pKE1:GFP-YPT72. The resulting strains were grown overnight in YPD, and subcultured in YNB plus 2 µg/ml of fluconazole or 0.5% DMSO. After incubation at 35°C for 8 hours, cells were imaged using a 60X objective. PC, phase contrast.

Supplemental Table 1. List of oligonucleotides used in this study**.**

Primer	Sequence (5'-3')
ACT1-FWD-qRT-PCR	ACGGTGAAGAAGTTGCTGCTTTAGTT
ACT1prom-ClaI-F	TGGACA**ATCGAT**CCAGCCTCGTTTATAATAAACTTAGTC
ACT1-RVS-qRT-PCR	CGTCGTCACCGGCAAAA
ADH1-3'UTRR2-ApaI	TCATCA**GGGCCC**GAAAACTTGAAACTTGAAAACACC
ADH1term-SpeI-R	TGACA**ACTAGT**GAAAACTTGAAACTTGAAAACACCG
ARG4DETF7	TTTTCAACAAAAGCTGTTGCG
ARG4DETR7	ACCAGTAGAATAAGCATCAGC
BMR1-F	ACATAAATACTTTGCCCATCCAGAA
BMR1-R	AAGAGTTGGTTTGTAATCGGCTAAA
CaCDR1-F qPCR	ATTCTAAGATGTCGTCGCAAGATG
CaCDR1-R qPCR	AGTTCTGGCTAAATTCTGAATGTTTTC
CaERG11 FWD qRT-PCR	CCCCTATTAATTTTGTTTTCCCTAATTTAC
CaERG11 RVS qRT-PCR	CACGTTCTCTTCTCAGTTTAATTTCTTTC
HIS1DETF9	CATCAAGAGACGAAATAACCC
HIS1DETR5	AAGTAGAGACAGTAGCGGCCC
LUXINTDETF	CTGACCTTTAGTCTTTCCTGC
LUXINTDETR	CAGTAGTACTTGTTGTTGTATCG
MDR1ORFF-EagI	TCA**CGGCCG**ATGCATTACAGATTTTTGAGAG
MDR1ORFR-MluI	TCA**ACGCGT**ATAGGAAAACAATGACACCTC
TEF1prF-BamHI	TCA**GGATCC**TGCAAATCTGTTTGCTGATGG

* Engineered restriction enzyme sites are highlighted in bold text and underlined.

Supplemental Table 2. List of strains used in this study.

Strain	Relevant Genotype	Reference
BWP17	*ura3*Δ/Δ *his1*Δ/Δ *arg4*Δ/Δ	(1)
CAI4 ("Wild-type")	*ura3*Δ/Δ *HIS1/HIS1 ARG4/ARG4*	(2)
GP1 ("Wild-type")	*ura3*Δ/Δ*:URA3 his1*Δ/Δ*:HIS1 arg4*Δ/Δ*:ARG4*	(3)
*erg3*Δ/Δ	*ura3*Δ/Δ*:URA3 his1*Δ/Δ *arg4*Δ/Δ *erg3*Δ*:HIS1/erg3*Δ*:ARG4*	(3)
*erg3*Δ/Δ + *ERG3*	*ura3*Δ/Δ*:URA3: pr*$_{1000bp}$*ERG3 his1*Δ/Δ *arg4*Δ/Δ *erg3*Δ*:HIS1/erg3*Δ*:ARG4*	(3)
XCTRL (WT$_{Vector}$)	*ura3*Δ/Δ*:URA3 his1*Δ/Δ*:HIS1 arg4*Δ/Δ*:ARG4*	This study
OCDR	*ura3*Δ/Δ*:URA3: P*$_{TEF1}$*CDR1 his1*Δ/Δ*:HIS1 arg4*Δ/Δ*:ARG4*	This study
OMDR	*ura3*Δ/Δ*:URA3 his1*Δ/Δ*:HIS1: P*$_{TEF1}$*MDR1 arg4*Δ/Δ*:ARG4*	This study
OERG	*ura3*Δ/Δ*:URA3 his1*Δ/Δ*:HIS1 arg4*Δ/Δ*:ARG4: P*$_{TEF1}$*ERG11*	This study
OTR	*ura3*Δ/Δ*:URA3: P*$_{TEF1}$*CDR1 his1*Δ/Δ*:HIS1: P*$_{TEF1}$*MDR1 arg4*Δ/Δ*:ARG4: P*$_{TEF1}$*ERG11*	This study
SC5314 (Azole^S)	*ERG3/ERG3*	(4)
TW2 (Azole^S)	*ERG3/ERG3*	(5)
TW17 (Azole^R)	*ERG3/ERG3*	(5)
CA12 (Azole^R)	*ERG3*^{W332R}*/ERG3*^{W332R}	(6)
CA488 (Azole^R)	*ERG3*$^{H243N, T330A, A351V}$*/ERG3*$^{H243N, T330A, A351V}$	(6)
CA490 (Azole^R)	*ERG3*$^{D147G, T330A, A351V}$*/ERG3*$^{D147G, T330A, A351V}$	(6)
CA1008 (Azole^R)	*ERG3*$^{K97E, L193P, V237A, A351V, A353T}$*/ERG3*$^{K97E, L193P, V237A, A351V, A353T}$	(6)
JS7, JS17, JS22, JS23, JS28, JS29, and JS40	Clinical *C. albicans* isolates from patients with vaginal candidiasis	Jack Sobel

Azole^S: Azole susceptible clinical isolate.
Azole^R: Azole resistant clinical isolate.

1. Wilson RB, Davis D, Mitchell AP. Rapid hypothesis testing with *Candida albicans* through gene disruption with short homology regions. J Bacteriol. 1996;181(6):1868 - 74.
2. Fonzi WA, Irwin MY. Isogenic strain construction and gene mapping in *Candida albicans*. Genetics. 1993;134(3):717-28.
3. Luna-Tapia A, Willems HME, Parker JE, Tournu H, Barker KS, Nishimoto AT, et al. Loss of Upc2p-Inducible ERG3 Transcription Is Sufficient To Confer Niche-Specific Azole Resistance without Compromising Candida albicans Pathogenicity. MBio. 2018;9(3). Epub 2018/05/24.

4. Maestrone G, Semar R. Establishment and treatment of cutaneous *Candida albicans* infection in the rabbit. Naturwissenschaften. 1968;55(2):87-8.
5. White TC. Increased mRNA levels of *ERG16*, *CDR*, and *MDR1* correlate with increases in azole resistance in *Candida albicans* isolates from a patient infected with human immunodeficiency virus. Antimicrob Agent Chemother. 1997;41(7):1482-7.
6. Martel CM, Parker JE, Bader O, Weig M, Gross U, Warrilow AG, et al. Identification and characterization of four azole-resistant *erg3* mutants of *Candida albicans*. Antimicrob Agents Chemother. 2010;54(11):4527-33. Epub 2010/08/25.