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Secretory IgA reduced the ergosterol contents of *Candida albicans* to repress its hyphal growth and virulence

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Supplementary figures and tables

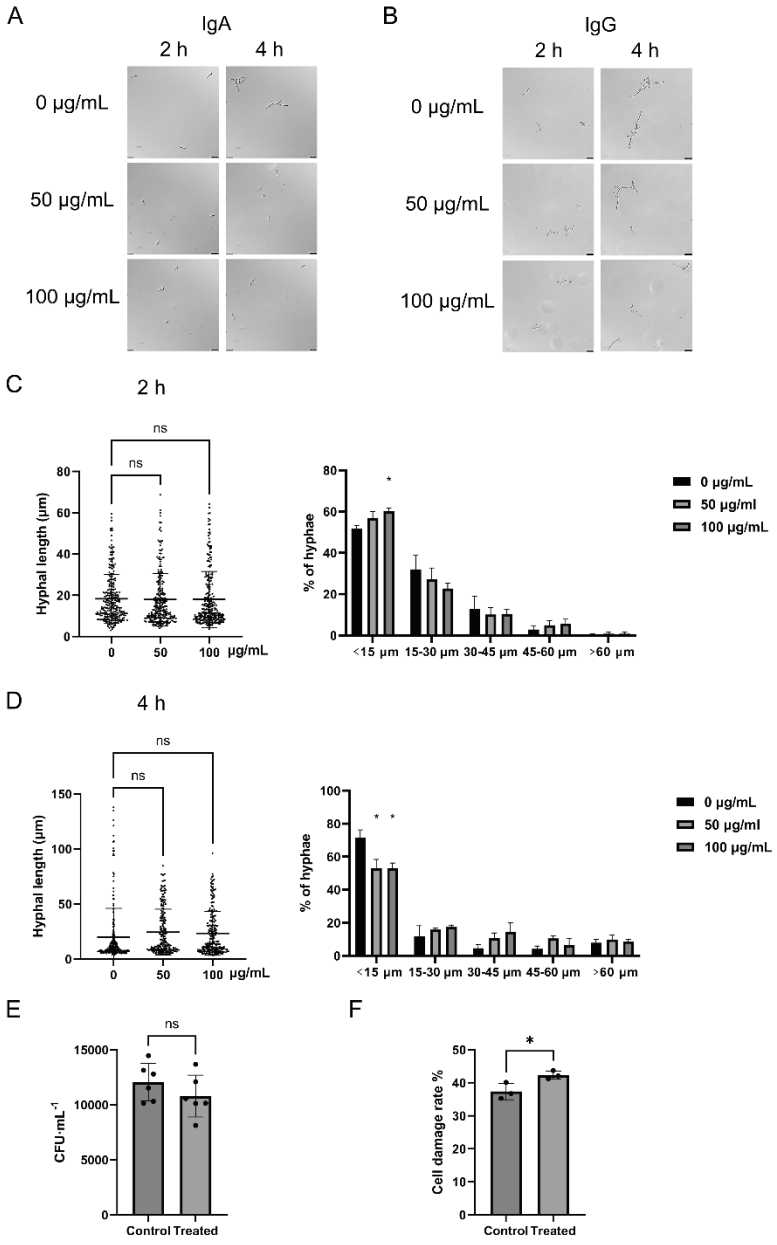

Fig. S1 The hyphal development and virulence inhibition by sIgA and IgG. A. The DIC (differential interference contrast) images of the hyphal formation of *C. albicans* SC5314 treated by 0, 50 and 100 μg/ml sIgA for 2 h and 4 h; **B.** The DIC images of the hyphal formation of *C. albicans* SC5314 treated by 0, 50 and 100 μg/ml IgG for 2 h and 4 h; **C.** The hyphal length and distribution of strain SC5314 treated by 0, 50 and 100 μg/ml IgG for 2 h; **D.** The hyphal length and distribution of strain SC5314 treated by 0, 50 and 100 μg/ml IgG for 4 h; **E.** The *C. albicans*

SC5314 adhesion to oral epithelial cell HOK with or without 100 μg/mL IgG; **F.** The cell damage of oral epithelial cell HOK caused by *C. albicans* SC5314 with or without 100 μg/mL IgG. All of the experiments were performed at least in three distinct replicates and the data are presented as the means ± SD, * $p < 0.05$, no significance (ns) $p > 0.05$.

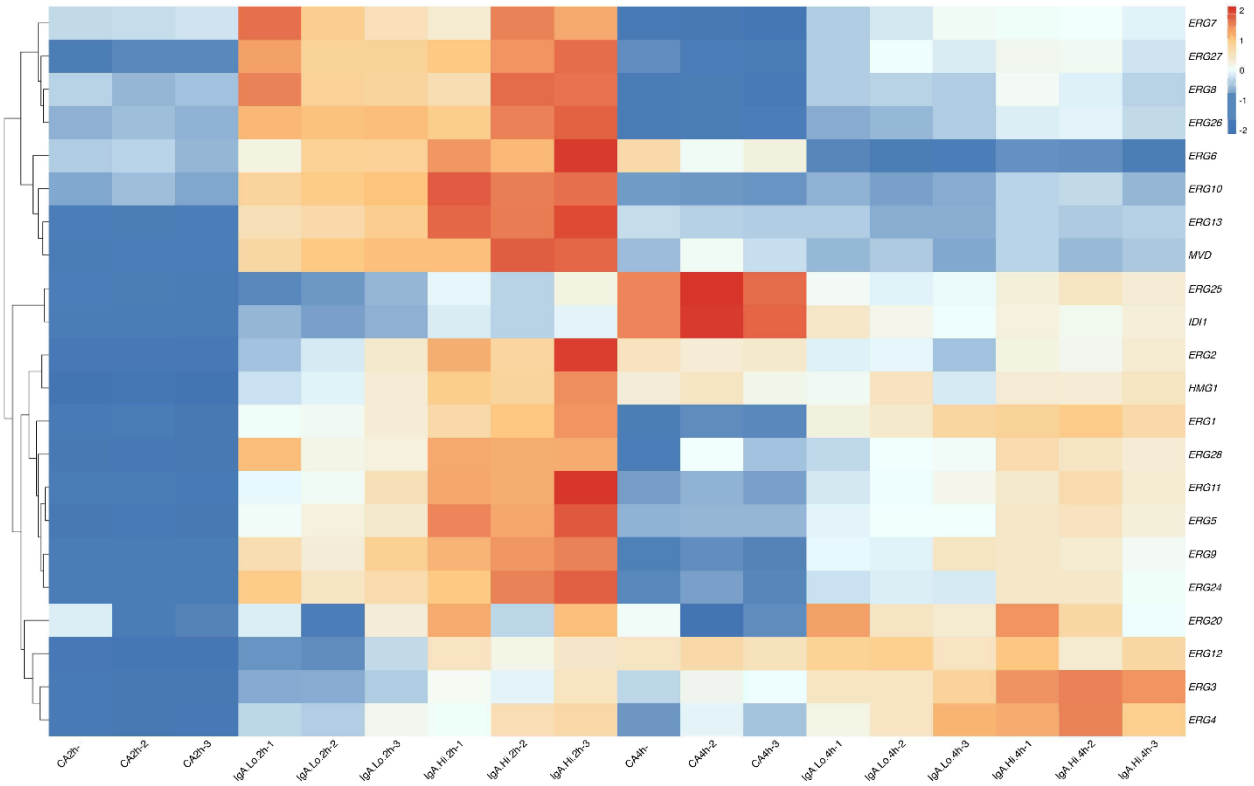

Fig. S2 The expression of genes from the ergosterol biosynthesis pathway. The heatmap indicated the different expressions of the genes from the ergosterol biosynthesis pathway of *C. albicans* treated by 0, 50 and 100 μg/ml sIgA for 2 h and 4 h.

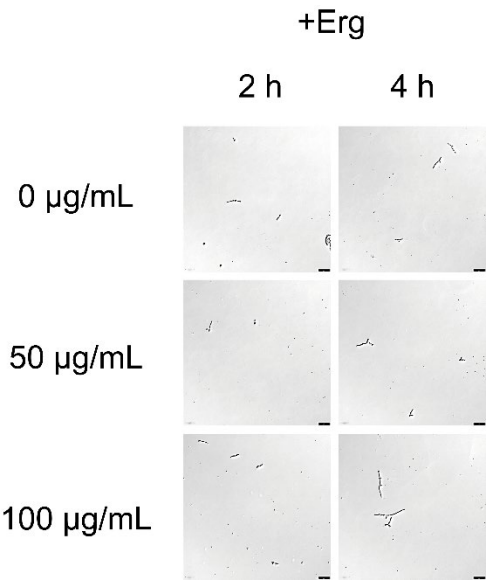

Fig. S3 The addition of ergosterol restored the hyphal development inhibited by sIgA. The DIC images of the hyphal formation of strain SC5314 treated by 0, 50 and 100 μg/ml sIgA for 2 h and 4 h with the addition of 10 μg/ml ergosterol.

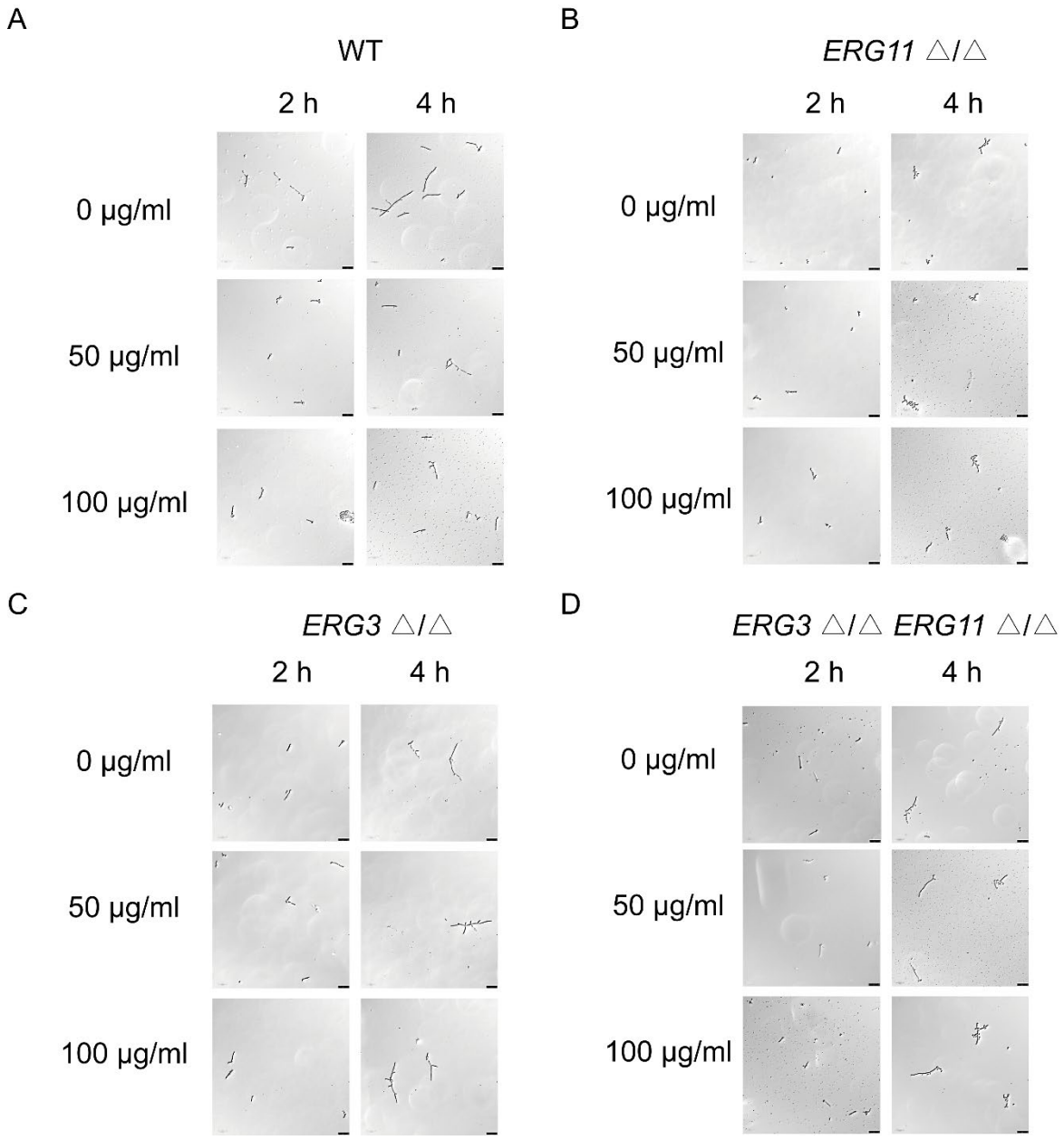

Fig. S4 sIgA lost the hyphal development inhibitory abilities on ergosterol biosynthesis pathway mutants. **A.** The DIC images of the hyphal formation of wild-type strain CAF2-1 treated by 0, 50 and 100 μg/ml sIgA for 2 h and 4 h; **B.** The DIC images of the hyphal formation of *erg11Δ/Δ* treated by 0, 50 and 100 μg/ml sIgA for 2 h and 4 h; **C.** The DIC images of the hyphal formation of *erg3Δ/Δ* treated by 0, 50 and 100 μg/ml sIgA for 2 h and 4 h; **D.** The DIC images of the hyphal formation of *erg3Δ/Δ erg11Δ/Δ* treated by 0, 50 and 100 μg/ml sIgA for 2 h and 4 h.

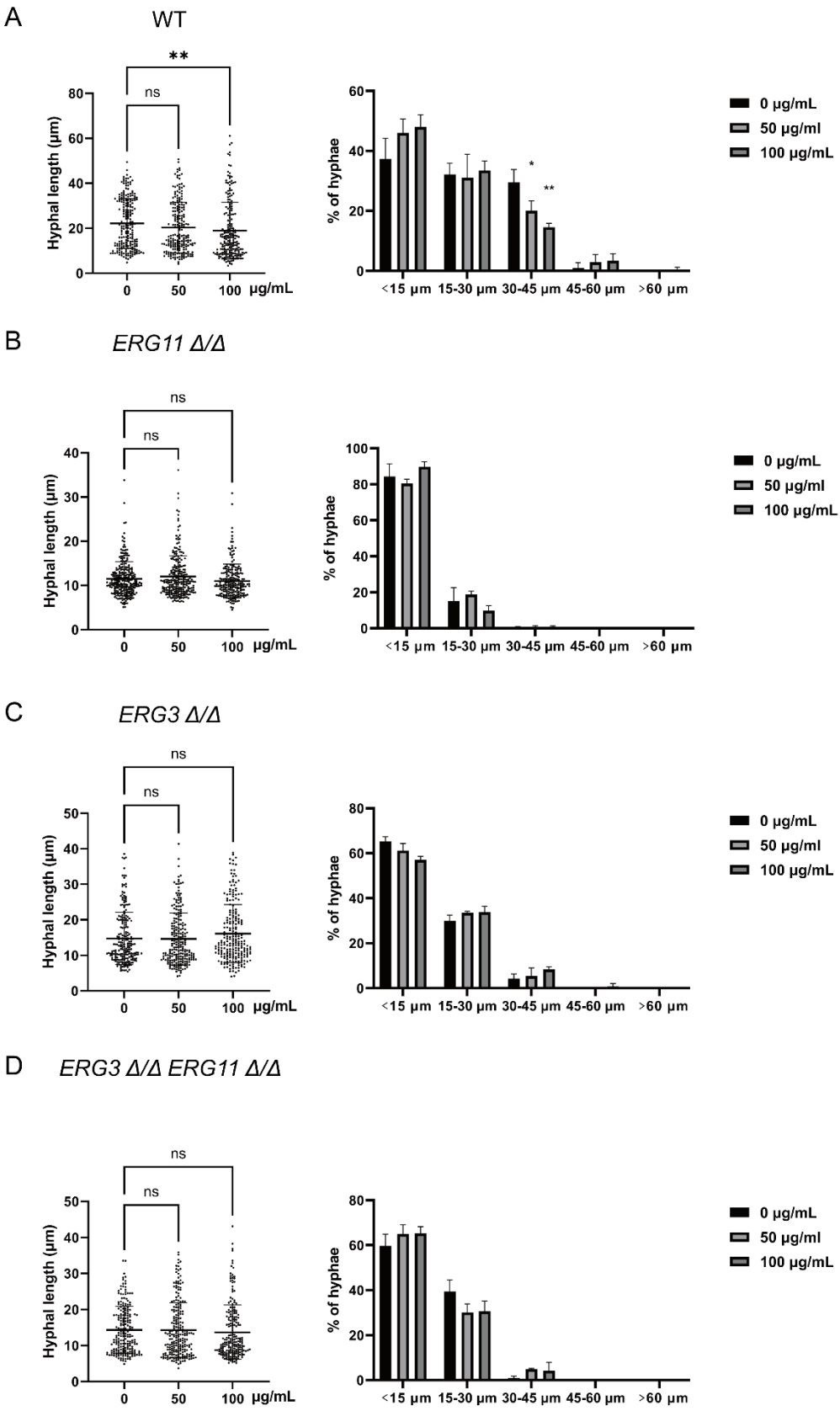
A
WT
**
ns
Hyphal length (µm)
0
20
40
60
80
0
50
100
µg/mL
% of hyphae
0
20
40
60
*
**
<15 µm
15-30 µm
30-45 µm
45-60 µm
>60 µm
0 µg/mL
50 µg/ml
100 µg/mL
B
ERG11 Δ/Δ
ns
ns
Hyphal length (µm)
0
10
20
30
40
0
50
100
µg/mL
% of hyphae
0
20
40
60
80
100
<15 µm
15-30 µm
30-45 µm
45-60 µm
>60 µm
0 µg/mL
50 µg/ml
100 µg/mL
C
ERG3 Δ/Δ
ns
ns
Hyphal length (µm)
0
10
20
30
40
50
0
50
100
µg/mL
% of hyphae
0
20
40
60
80
<15 µm
15-30 µm
30-45 µm
45-60 µm
>60 µm
0 µg/mL
50 µg/ml
100 µg/mL
D
ERG3 Δ/Δ ERG11 Δ/Δ
ns
ns
Hyphal length (µm)
0
10
20
30
40
50
0
50
100
µg/mL
% of hyphae
0
20
40
60
80
<15 µm
15-30 µm
30-45 µm
45-60 µm
>60 µm
0 µg/mL
50 µg/ml
100 µg/mL

Fig. 5 sIgA lost the hyphal development inhibitory abilities on *C. albicans* ergosterol pathway mutants. The hyphal length and distribution of wild-type strain CAF2-1 **(A)**, *erg11Δ/Δ* **(B)**, *erg3Δ/Δ* **(C)**, *erg3Δ/Δ erg11Δ/Δ* **(D)** treated by 0, 50 and 100 μg/mL sIgA for 2 h. The experiments were conducted with a minimum of three separate replicates, * $p < 0.01$, ** $p < 0.01$, no significance (ns) $p > 0.05$

Table S1. *Candida albicans* strains used in this study.

Stain	Genotype	Reference
SC5314	Parent strain, ATCC MYA-2876	(Gillum et al. 1984)
WT[a]	SC5314, *Δura3*::*imm434/URA3*	(Fonzi and Irwin 1993)
CAF2-1		
erg11Δ/Δ	WT[a], *erg3AΔ*::*hisG/ERG3*	(Sanglard et al. 2003)
	erg11Δ::*hisG/erg11Δ*::*hisG*	
erg3Δ/Δ	WT[a], *erg3AΔ*::*hisG/erg3BΔ*::*hisG-URA3-hisG;*	(Sanglard et al. 2003)
	erg11Δ::*hisG/ERG11*	
erg3Δ/Δ	WT[a], *erg3AΔ*::*hisG/erg3BΔ*::*hisG*	(Sanglard et al. 2003)
erg11Δ/Δ	*erg11Δ*::*hisG/erg11Δ*::*hisG-URA3-hisG*	

[a]Wild type

Table S2. Real-time PCR primers used in this study.

Primers	**Nucleotide Sequence (5'-3')**
18S rRNA	FW-CTAGGGATCGGTTGTTGTTCT
	RV-TTGTGTCTGGACCTGGTGAGT
ERG10	FW-ATCCATGACCAACACGCCAT
	RV-ATTTTTCAGCGGCAACACCC
ERG13	FW-TGCCTTCCATGTGCCAACTT
	RV-GGGACTTGCAAAGCTGGTTG
HMG1	FW-TCGACCATGCGTGGTTGTAA
	RV-TTCATCCCCATGGCATCACC
ERG12	FW-ATTGACAGGTGCTGGAGGTG
	RV-CCTCAATAGCGGCACGATCT
ERG8	FW-TGAGCCTTTGACTGTTGCGA
	RV-ACGGTCCAACAACTGGGTTT

MVD	FW-CCGTCAACATCGCCGTAAGT
	RV-CACGCTTGAGTACGTGGAGT
IDI1	FW-GGCCAGAAGTTACCCCCTTG
	RV-GCACGATGTAACAACCCAGC
ERG20	FW-ACCCGTGGCATTAGCAATGTA
	RV-GACGTTGTTCAGGGGTAGCA
ERG9	FW-ATGGTGTTGCCACCGTTGAA
	RV-GCAAATTCTTTGGCAGGGGT
ERG1	FW-ATTGGGAGGACATGCACCAG
	RV-ACTGCATCGTTAGCAGCAGA
ERG7	FW-CTTCCAGACGGTGGATGGTC
	RV-ACCCTATCAAAGCCCAAGCC
ERG11	FW-ACTCATGGGGTTGCCAATGT
	RV-AGCAGCATCACGTCTCCAAT
ERG24	FW-ACATGCTGGCTGGCCTATTT
	RV-GAGAGCTGTCGCTGTTCAGT
ERG25	FW-TGCTGCTCCATTTGGATTGG
	RV-AATGAGCATCAACGGCTTGG
ERG26	FW-TTTGTTTACGTCCTGCGGGT
	RV-GTGCTGCCAAAACATGAGCA
ERG27	FW-TTCGTTTGTTGGAGGGGCTT
	RV-CGCCAGTCCAATCTATGCCT
ERG6	FW-TTGTGGTGTAGGTGGTCCTG
	RV-TGAACGGTAGCTTCAATGGCA
ERG2	FW-GGTGGTGCCATGGGTACAAT
	RV-GCACCAGGATAAGCTGCTCT
ERG3	FW-AACGTGCCACTACTGCCATT
	RV-ACAGATGGCCAGTGTAACCA
ERG4	FW-CCAATGCTTGTGCCAAAGGT
	RV-ACTGAATGGAACCCCAGCAA
ERG5	FW-TGCTGGTCCCCGTTTCAAAT

RV-AAAGAACGGCGGTAATCGGT

References:

Fonzi WA, Irwin MY (1993) Isogenic strain construction and gene mapping in *Candida albicans*. Genetics 134(3):717-728 doi:10.1093/genetics/134.3.717

Gillum AM, Tsay EY, Kirsch DR (1984) Isolation of the *Candida albicans* gene for orotidine-5'-phosphate decarboxylase by complementation of *S. cerevisiae ura3* and *E. coli pyrF* mutations. Mol Gen Genet 198(2):179-182 doi:10.1007/bf00328721

Sanglard D, Ischer F, Parkinson T, Falconer D, Bille J (2003) *Candida albicans* mutations in the ergosterol biosynthetic pathway and resistance to several antifungal agents. Antimicrob Agents Chemother 47(8):2404-2412 doi:10.1128/aac.47.8.2404-2412.2003