

Novel pH-responsive monomer inhibit *Candida albicans* via dual antifungal modes of action

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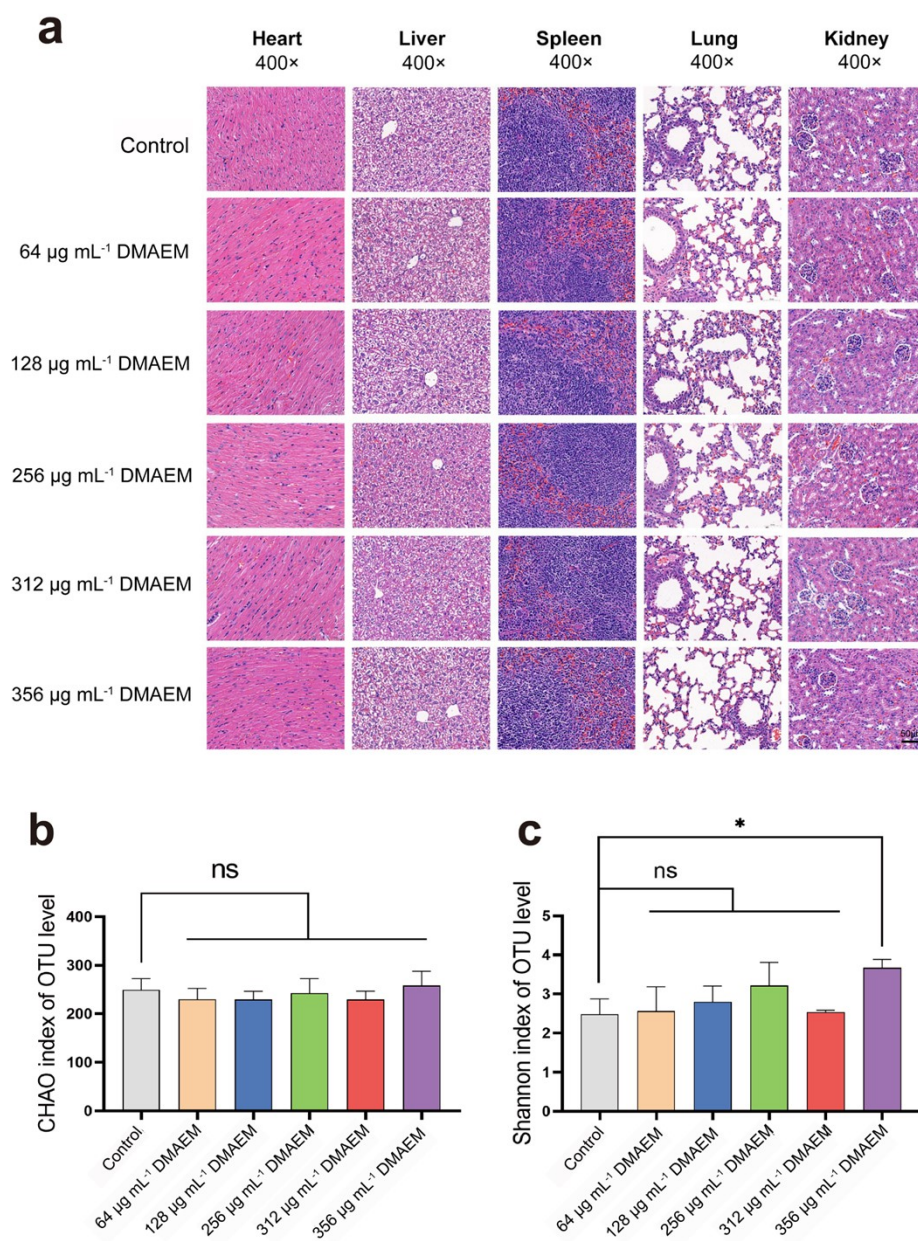


Fig. S1. DMAEM exhibited excellent biosafety *in vivo*. (a) Histological sections of mouse main organs tissue with H&E staining (nucleus in blue, and cytoplasm in purple) after 2-day topical administration with saline or DMAEM. (b) CHAO index of gut microbiome after 2-day topical administration of saline or DMAEM (n = 4, mean values ± s.d.). (c) Shannon index of gut microbiome after 2-day topical administration of saline or DMAEM (n = 4, mean values ± s.d.). Significance was assessed using Mann–Whitney U testing (b, c). **p* < 0.05, ns, not significant.

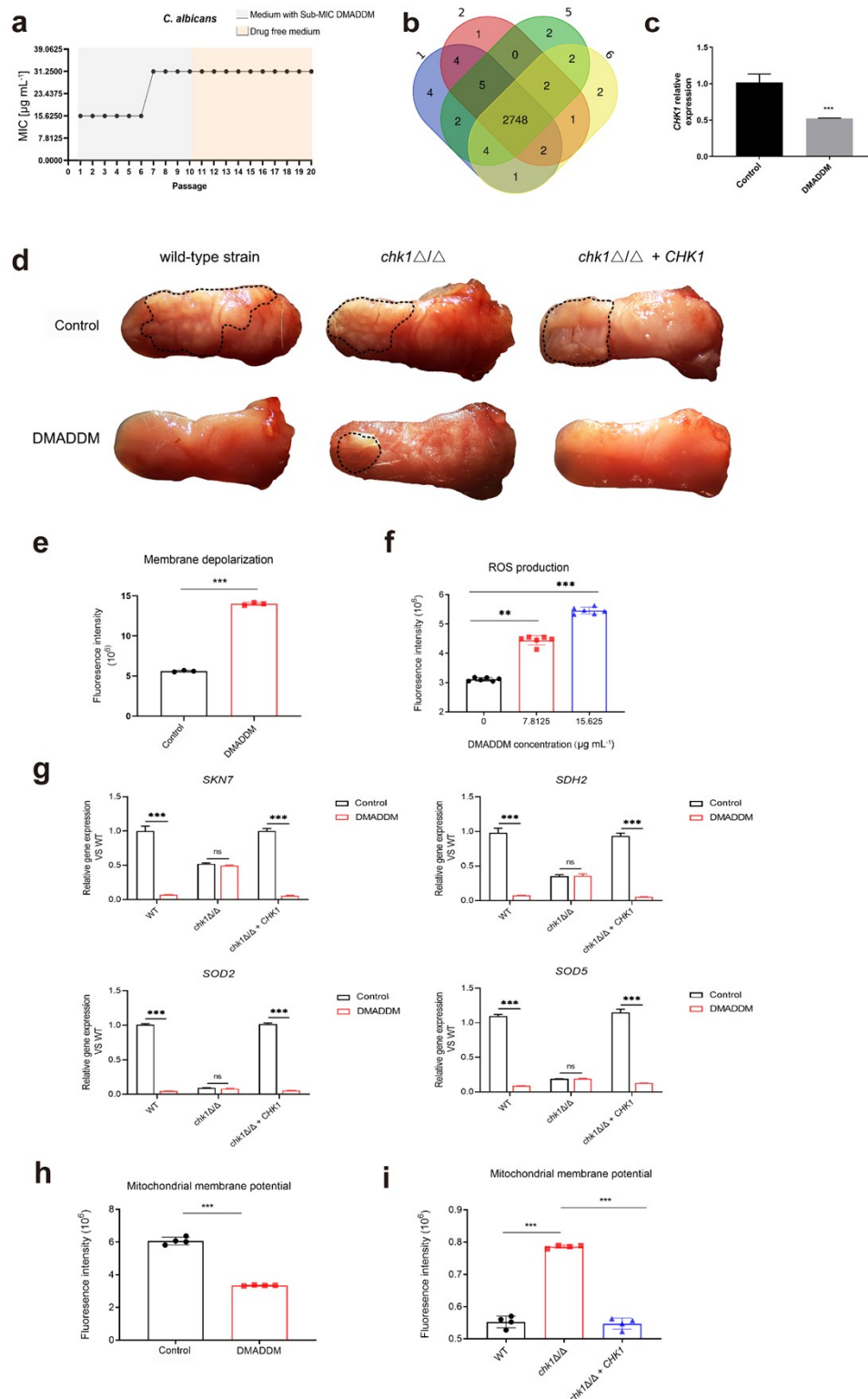


Fig. S2. DMAEM protonated into a cationic fungicide sharing similar structural and functional characteristics with DMADDM, which exerted fungicidal activities by targeting a two-component system *CHK1* which induces downstream accumulation of reactive oxygen species (ROS) and fungal apoptosis. (a) Sub-inhibitory concentration stimulation to induce DMADDM-resistant strains. (b) Single nucleotide polymorphisms (SNPs) in the four DMADDM-resistant strains. (c) *CHK1* relative expression in wild-type strain. (d) Photograph of mice tongues infected with *C. albicans*. The area enclosed by the black dotted indicates the pseudomembranous lesion caused by *C. albicans* infection. (e) Membrane depolarization of wild-type *C. albicans* strain after treatment with 250 $\mu\text{g mL}^{-1}$ DMADDM ($n = 6$, mean values \pm s.d.). (f) Intracellular ROS in wild-type strain, *chk1* Δ/Δ , *chk1* Δ/Δ +*CHK1* after DMADDM exposure. (g) Relative Gene expression in strains including *SKN7* and other ROS-scavenging genes (*SDH2*, *SOD2*, *SOD5*). (h) Mitochondrial membrane potential of wild-type *C. albicans* strain after treatment with 15.625 $\mu\text{g mL}^{-1}$ DMADDM ($n = 4$, mean values \pm s.d.). (i) Mitochondrial membrane potential of wild-type strain, *chk1* Δ/Δ , *chk1* Δ/Δ +*CHK1* after 15.625 $\mu\text{g mL}^{-1}$ DMADDM exposure ($n = 4$, mean values \pm s.d.). Student's t-tests (c, e, g, h) and one-way ANOVA with Tukey post-test (f, i) were performed. ** $p < 0.01$, *** $p < 0.001$, ns, not significant.

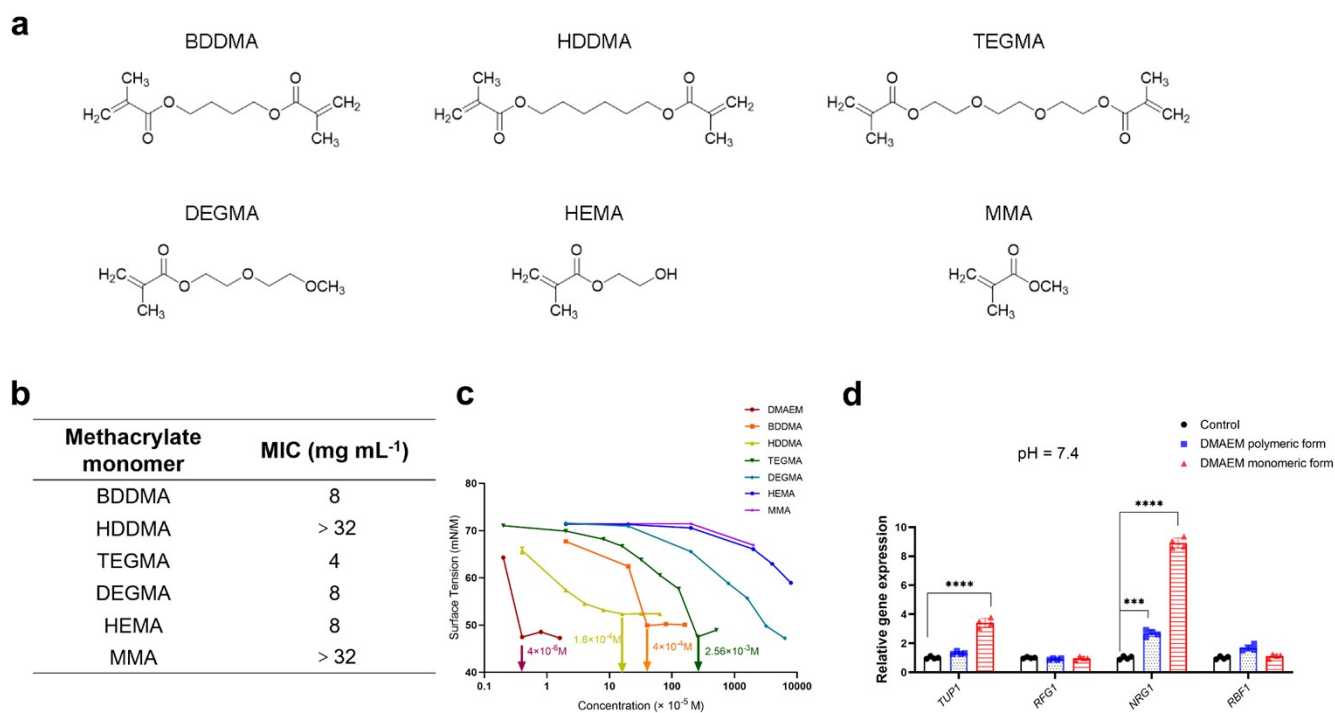


Fig. S3. The double-bond structure, together with hydrophobicity, are key factors in DMAEM inhibition of filamentation. (a) Structures of methacrylate compounds. (b) MIC of methacrylate compounds against *C. albicans* at neutral pH. (c) The critical micelle concentration values were obtained at the intersection of the surface tension curve versus the logarithmic value of the methacrylates concentration at pH 7.4. (d) Expression of filamentation-associated genes in *C. albicans* after 4 h exposure to PBS, DMAEM in either monomeric form or polymer form ($n = 4$, mean values \pm s.d.). Student's t-tests was performed. *** $p < 0.001$, **** $p < 0.0001$.

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

Strain name	genotype
SC5314	Parent strain, ATCC MYA-2876
Wild type (WT)	SC5314, Δ <i>ura3::imm434/URA3</i>
Δ <i>chk1</i>	<i>Dura3::imm434/Dura3::imm434</i> <i>Dchk1::hisG/Dchk1::hisG-URA3-hisG</i>
Δ <i>chk1/chk1+</i>	<i>Dura3::imm434/Dura3::imm434</i> <i>Dchk1::hisG/CHK1::URA3-hisG</i>

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

Primers	Nucleotide Sequence (5'to3')
<i>18S</i> rRNA-F	TCTTTCTTGATTTTGTGGGTGG
<i>18S</i> rRNA-R	TCGATAGTCCCTCTAAGAAGTG
<i>CHK1</i> -F	ATCCGGTGAAAGCGGAAGAG
<i>CHK1</i> -R	AACTGCTGCTGCTCGAAGAT
<i>SKN7</i> -F	GGTCCAATATCATCAGATACAGCAT
<i>SKN7</i> -R	AGATTCTGTCCAAGTGACTGTTGTT
<i>SDH2</i> -F	GGTTCGTGTGCCATGAACATT
<i>SDH2</i> -R	ATCAAGACTGCTGGACCCAAG
<i>SOD2</i> -F	ACAAGCCGTTGAAGCCAAATC
<i>SOD2</i> -R	AGGTAACCACCACCGTTGAA
<i>SOD5</i> -F	CTCCTCAAGGTAATGGCGCT
<i>SOD5</i> -R	AGGCAGCAATGACACCAACTA
<i>TUP1</i> -F	CTTGGAGTTGGCCCATAGAA
<i>TUP1</i> -R	TGGTGCCACAATCTGTTGTT
<i>RFG1</i> -F	AACCCTGAAGTTTCCCGAGAA
<i>RFG1</i> -R	CAGCAAGATTATTCCAATGTTTCCTT
<i>NRG1</i> -F	CCAAGTACCTCCACCAGCAT
<i>NRG1</i> -R	GGGAGTTGGCCAGTAAATCA
<i>RBF1</i> -F	CATGTCAGAGATGCGCTCACT
<i>RBF1</i> -R	ATGACCAGGTTTCATCGGGTT

F: forward; R: reverse.

Table S3. Function information of high-frequency mutated genes.

Gene name	Location	Description
<i>DAL1</i>	scaffold127/exonic/C1013T	Putative allantoate permease, involved in the regulation of nitrogen-degradation genes; required for yeast cell adherence to silicone substrate; Spider biofilm induced
<i>SIR2</i>	scaffold226/exonic/C11059C	Required for wild-type lifespan, asymmetric inheritance of oxidatively damaged proteins, rDNA silencing; regulates phenotypic switch in strain 3153A, not WO-1; partially complements <i>S. cerevisiae</i> sir2 mating defect; Spider biofilm induced
<i>RRN6</i>	scaffold47/exonic/C29547T	Predicted component of the core factor rDNA transcription factor complex; required for transcription of 35S rRNA genes by RNA polymerase I in <i>S. cerevisiae</i> ; possibly an essential gene, disruptants not obtained by UAU1 method
<i>CHS5</i>	scaffold107/exonic/T10626C	Putative chitin biosynthesis protein; fungal-specific; repressed upon yeast-to-hypha switch; rat catheter biofilm repressed
<i>YAT2</i>	scaffold144/exonic/G21815A	Peroxisomal carnitine acetyl transferase; no obvious metabolic, hyphal, virulence defects in Ura ⁺ strain; induced by macrophage engulfment, hyphal growth, starvation, nonfermentable carbon sources; rat catheter, Spider biofilm induced
<i>CCZI</i>	scaffold233/exonic/T1293C	Protein involved in oxidative stress response and autophagy; mutants have fragmented mitochondria and

		attenuated virulence
<i>NUP188</i>	scaffold140/exonic/T12028C	Putative nuclear pore complex subunit; transcript regulated by Nrg1 and Mig1
<i>DPB11</i>	scaffold627/exonic/T1337C	Ortholog(s) have DNA polymerase binding, protein kinase activator activity, protein-containing complex scaffold activity
<i>SEC16</i>	scaffold351/exonic/T440C	Ortholog(s) have protein membrane anchor activity and role in COPII vesicle coating, macroautophagy, protein localization to endoplasmic reticulum exit site
<i>NUP120</i>	scaffold187/exonic/C3860A	Ortholog(s) have structural constituent of nuclear pore activity
<i>ESPI</i>	scaffold447/exonic/A6390G	Putative caspase-like cysteine protease; mutation confers increased sensitivity to nocodazole; periodic mRNA expression, peak at cell-cycle S/G2 phase; mRNA binds She3
<i>NUP133</i>	scaffold405/exonic/A3228G	Ortholog(s) have structural constituent of nuclear pore activity
<i>RIF1</i>	scaffold31/exonic/A30649G	Protein involved in sub-telomeric silencing and regulation of biofilm formation; involved in regulation of telomere length; mutants display increased colonization of mouse kidneys relative to wild-type; required for silencing at MTL3
<i>SAP2</i>	scaffold239/exonic/A13890T	Secreted aspartyl proteinase; acts in utilization of protein as nitrogen source; assessment of virulence role

		complicated by URA3 effects; regulated by growth phase, alpha-pheromone; produced by opaque cells
<i>EIF4G</i>	scaffold750/exonic/A759G	Putative translation initiation factor eIF4G; overexpression causes hyperfilamentation; hyphal- and macrophage-induced; genes encoding some translation factors are downregulated upon phagocytosis by murine macrophage
<i>UTP20</i>	scaffold42/exonic/C5210A	Putative snoRNA-binding protein; <i>S. cerevisiae</i> Utp20 ortholog; likely essential for growth; repressed in core stress response; mutation confers resistance to 5-fluorocytosine (5-FC) and parnafungin
<i>SWR1</i>	scaffold252/exonic/G8503C	Component of the SWR1 complex, which has a role in exchanging histone variant H2AZ for histone H2A in chromatin; required for proper nucleosome positioning on WOR1 promoter
<i>NAF1</i>	scaffold82/exonic/G25130A	Putative RNA-binding protein; role in assembly of box H/ACA snoRNPs and thus pre-rRNA processing; Spider biofilm induced
<i>UME6</i>	scaffold184/exonic/A17463C	Zn(II)2Cys6 transcription factor; has a long 5'-UTR that regulates translational efficiency and controls transition to filamentous growth; stability controlled by Grr1p, Ubr1p, Ptc2p in response to CO ₂ and O ₂ levels
<i>ATG2</i>	scaffold6/exonic/G83123A	Protein described as similar to <i>S. cerevisiae</i> sporulation protein; ortholog of <i>S. cerevisiae</i> Atg2, an autophagic

		vesicle formation protein; up-regulation associated with azole resistance; Spider biofilm induced
<i>BMS1</i>	scaffold305/exonic/T4731A	Putative GTPase; Hap43-induced gene; mutation confers resistance to 5-fluorocytosine (5-FC); flucytosine induced; repressed by prostaglandins; Spider biofilm induced
<i>GPI7</i>	scaffold104/exonic/A1379G	Protein involved in attachment of GPI-linked proteins to cell wall; member of major facilitator superfamily; phosphodiesterase/nucleotide pyrophosphatase domain; similar to <i>S. cerevisiae</i> Gpi7p
<i>FOL1</i>	scaffold90/exonic/A14527G	Putative dihydroneopterin aldolase (dihydro-6-hydroxymethylpterin pyrophosphokinase); fungal-specific (no human or murine homolog)
<i>NUP82</i>	scaffold764/exonic/C256T	Linker nucleoporin of the nuclear pore complex; role in mRNA and export from nucleus, protein import into nucleus, ribosomal large subunit export from nucleus, ribosomal small subunit export from nucleus; rat catheter biofilm repressed
<i>MEC1</i>	scaffold117/exonic/A18901G	Cell cycle checkpoint protein with a role in genome integrity; RNA abundance regulated by tyrosol and cell density
<i>PIF1</i>	scaffold117/exonic/G19795T	DNA helicase and 3'-5' exonuclease; decreased transcription is observed upon fluphenazine treatment
<i>PEX14</i>	scaffold279/exonic/G10836A	Ortholog(s) have peroxisome matrix targeting signal-1

		binding, peroxisome matrix targeting signal-2 binding, protein binding, bridging activity
<i>APC5</i>	scaffold216/exonic/C2387T	Ortholog(s) have ubiquitin protein ligase activity, role in anaphase-promoting complex-dependent catabolic process, chromatin assembly, protein ubiquitination and anaphase-promoting complex localization
<i>RAD50</i>	scaffold192/exonic/G11349C	Putative DNA double-strand break repair factor; involved in response to oxidative stress and drug resistance; flow model biofilm repressed
<i>VPS41</i>	scaffold173/exonic/C1964T	Protein similar to <i>S. cerevisiae</i> Vps41p, which is involved in vacuole organization and biogenesis; transposon mutation affects filamentous growth; regulated by Gcn4p; induced in response to amino acid starvation (3-aminotriazole treatment)
<i>ALG6</i>	scaffold435/exonic/T967C	Putative glucosyltransferase involved in cell wall mannan biosynthesis; transcription is elevated in <i>chk1</i> , <i>nik1</i> , and <i>sln1</i> homozygous null mutants; repressed by nitric oxide; possibly essential gene, disruptants not obtained by UAU1 method
<i>PANI</i>	scaffold28/exonic/G29181A	Essential protein involved in endocytosis and polarized growth; ortholog of <i>S. cerevisiae</i> Pan1, which is a part of a complex that regulates actin cytoskeleton; Spider biofilm repressed
<i>LEU3</i>	scaffold178/exonic/A10984T	Zn(II)2Cys6 transcription factor; predicted regulator

		branched-chain amino acid biosynthesis genes; alkaline induced; induced by Msn11 under weak acid stress; required for yeast cell adherence to silicone substrate; Spider biofilm induced
<i>SSK2</i>	scaffold67/exonic/T18922A	MAP kinase kinase kinase (MAPKKK); regulates Hog1 activation and signaling; repressed by ciclopirox olamine MAP
<i>CHK1</i>	scaffold28/exonic/C45325T	Histidine kinase; 2-component signaling, cell wall synthesis; hyphal growth defect; avirulent in mouse, not rat vaginal infection; phagocytosis rate increased; Spider biofilm induced; required for RPMI biofilm; Bcr1-induced in a/a biofilm
<i>NAB3</i>	scaffold236/exonic/A818T	Putative nuclear polyadenylated RNA-binding protein; flucytosine repressed
<i>APC1</i>	scaffold11/exonic/G35646A	Putative Anaphase-Promoting Complex/Cyclosome subunit; essential for growth; periodic mRNA expression, peak at cell-cycle S/G2 phase
<i>ARO1</i>	scaffold73/exonic/C718T	Putative pentafunctional arom enzyme; required for aromatic amino acid biosynthesis; involved in cell wall integrity and formation of biofilm; fungal-specific (no human or murine homolog); Gcn2p-, Gcn4p-regulated
<i>TBF1</i>	scaffold107/exonic/C18397T	Essential transcription factor; induces ribosomal protein genes and the rDNA locus; acts with Cbf1 at subset of promoters; recruits Fhl1 and Ifh1 to promoters; role is

		analogous to that of <i>S. cerevisiae</i> Rap1; Spider biofilm induced
<i>PLB1</i>	scaffold23/exonic/A66147C	Phospholipase B; host cell penetration and virulence in mouse systemic infection; Hog1-induced; signal sequence, N-glycosylation, and Tyr phosphorylation site; induced in fluconazole-resistant strains; rat catheter biofilm repressed
<i>MBP1</i>	scaffold136/exonic/C19606T	Putative component of the MBF transcription complex involved in G1/S cell-cycle progression; non-periodic mRNA expression; predicted, conserved MBF binding sites upstream of G1/S-regulated genes
<i>ORC4</i>	scaffold178/exonic/T4338C	Phosphorylated protein similar to <i>S. cerevisiae</i> Orc4, subunit of the origin recognition complex (ORC); induced by alpha pheromone in SpiderM medium
<i>LAS1</i>	scaffold6/exonic/A80729G	Putative bud formation and morphogenesis protein; mutation confers hypersensitivity to 5-fluorocytosine (5-FC), 5-fluorouracil (5-FU); macrophage-induced; Spider biofilm induced
<i>PEX1</i>	scaffold302/exonic/A6702G	Peroxisomal ATPase involved in import of peroxisomal matrix proteins; involved in oxidative stress tolerance
<i>SWE1</i>	scaffold245/exonic/C10171T	Putative protein kinase with a role in control of growth and morphogenesis, required for full virulence; mutant cells are small, rounded, and sometimes binucleate; not required for filamentous growth; mutant is hypersensitive

		to caspofungin
<i>VPS51</i>	scaffold416/exonic/T6077C	Protein with a role in vacuolar function; null mutant has defect in damaging oral epithelial and vascular endothelial cells; required for normal hyphal growth and stress resistance; induced in presence of host oral or vascular cells
<i>DDC1</i>	scaffold256/exonic/T10407A	Putative DNA damage checkpoint protein; transcript induced during filamentous growth; regulated by Nrg1, Tup1
<i>TFC3</i>	scaffold33/exonic/G69417A	Putative DNA-binding transcription factor; merged with orf19.3833 in Assembly 20
<i>SKO1</i>	scaffold146/exonic/T13511G	bZIP transcription factor involved in cell wall damage response; represses the yeast-to-hypha transition; mutants are caspofungin sensitive; induced by osmotic stress via Hog1; activated by Rlm1p; induced by Mnl1 under weak acid stress
<i>TMT1</i>	scaffold442/exonic/G5730A	Ortholog(s) have trans-aconitate 3-methyltransferase activity and cytosol localization