Supplementary Information

The ume6 Δ/ume6Δ mutant is partially defective for biofilm formation

The ability of *C. albicans* to form biofilms on both inert and biological substrates is particularly problematic for patients undergoing treatment for candidiasis since *C. albicans* biofilms are highly resistant to antifungal drugs and can form on catheters during intravenous therapy (Ramage *et al.*, 2006). In order to determine the effect of the *ume6Δ/Δ* mutation on this important process we measured 24-hour biofilm formation of wild-type, *ume6Δ/+*, *ume6Δ/ume6Δ* and *ume6Δ/ume6Δ::UME6* strains on polystyrene wells using a colorimetric XTT-reduction assay (see Methods). As shown in Figure S1 , the *ume6Δ/ume6Δ* mutant showed a partial, but significant defect in *C. albicans* biofilm formation. This defect appeared to be the result of a loss of *UME6* function since the *ume6Δ/ume6Δ::UME6* add-back strain formed biofilms at a level equivalent to that of *ume6Δ/+* and wild-type strains.

These results are consistent with earlier studies which have indicated that filamentation is important for *C. albicans* biofilm formation (Ramage *et al.*, 2005). Microscopic examination revealed that biofilms formed by the *ume6 Δ/ume6Δ* mutant contained short, stubby filaments. In contrast, wild-type *C. albicans* biofilms contain extended hyphal filaments. Our results suggest that hyphal filament extension may be specifically important for *C. albicans* to achieve complete biofilm formation at least on an inert substrate.

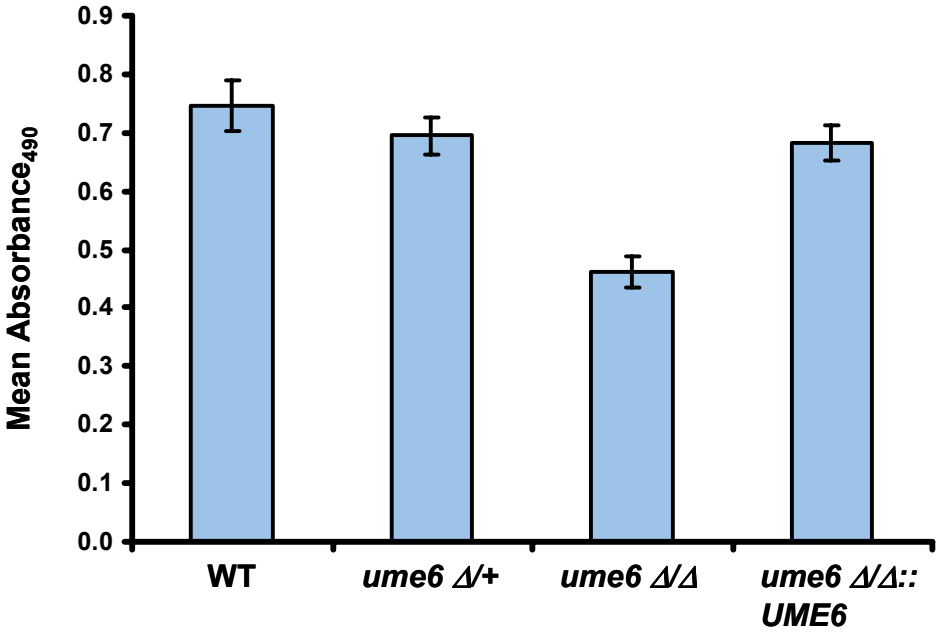

Figure S1. Partial defect in biofilm formation by the *ume6Δ/Δ* mutant. 24-hour biofilm formation of the indicated strains on polystyrene wells following growth at 37°C in RPMI medium was measured by an XTT reduction assay. Values represent the mean absorbance (at 420 nm) measured from 22 independent biofilms for each strain (error bars indicate standard deviation).

Methods

24-hour biofilm formation following growth at 37°C in RPMI medium was measured on polystyrene wells using an XTT-reduction assay as described previously (Ramage *et al.*, 2001).

References

Ramage, G., Martinez, J.P., and Lopez-Ribot, J.L. (2006). Candida biofilms on implanted biomaterials: a clinically significant problem. FEMS Yeast Res 6, 979-986.

Ramage, G., Saville, S.P., Thomas, D.P., and Lopez-Ribot, J.L. (2005). Candida biofilms: an update. Eukaryot Cell 4, 633-638.

Ramage, G., Vande Walle, K., Wickes, B.L., and Lopez-Ribot, J.L. (2001). Standardized method for in vitro antifungal susceptibility testing of Candida albicans biofilms. Antimicrob Agents Chemother 45, 2475-2479.

Table S1 **Primers used in this study**

#	Primer Name	Sequence	Description
1	DKO225	CTTTGCTTTACATAATTGGTGATAGG	*UME6* 5' flank upstream for fusion PCR
2	DKO226	cacggcgcgcctagcagcggCTAATTGGAAGTAAATTGAGGATATTG	*UME6* 5' flank downstream for fusion PCR
3	DKO229	gtcagcggccgcatccctgcAAGAATTAACAGGTTGACGGTTG	*UME6* 3' flank upstream for fusion PCR
4	DKO230	CGGGAAAAGTTGCAAGAGTTGGTG	*UME6* 3' flank downstream for fusion PCR
5	RZO37	ccgctgctaggcgcgccgtgACCAGTGTGATGGATATCTGC	5’ vector primer for *LEU2*, *ARG4*, *HIS1* markers (designed by R. Zordan)
6	RZO38	gcagggatgcggccgctgacAGCTCGGATCCACTAGTAACG	3’ vector primer for *LEU2*, *ARG4*, *HIS1* markers (designed by R. Zordan)
7	DKO414	gcatgaactcgagggatccGAGAGTTTTAATCAATTAGAAACC	5’ primer for *UME6* add-back
8	DKO419	tcgaacctggatccctcgagGGGAATGAGTTACAGTTTATCGGG	3’ primer for *UME6* add-back
9	MBO13	AGCTAGCAATGAACCAAACGG	5’ *NRG1* flank upstream primer for Δ*nrg1::ARG4* fusion
10	MBO14	cacggcgcgcctagcagcggGATTCTTAATGAAACTAGCAGGG	5’ *NRG1* flank downstream primer for Δ*nrg1::ARG4* fusion
11	MBO15	gtcagcggccgcatccctgcTGGATGGTTAATTGCTTGGG	3’ *NRG1* flank upstream primer for Δ*nrg1::ARG4* fusion
12	MBO16	AGGAGAGAAGATCTATGGCAATGC	3’ *NRG1* flank downstream primer for Δ*nrg1::ARG4* fusion
13	MBO5	tagctaaggtaccAGCTAGCAATGAACCAAACGG	5’ *NRG1* flank upstream primer for cloning into pSFS2

14	MBO6	agtcgatctcgagGATTCTTAATGAAACTAGCAGGG	5’ *NRG1* flank downstream primer for cloning into pSFS2
15	MBO7	tacgttagcggccgcTGGATGGTTAATTGCTTGGG	3’ *NRG1* flank upstream primer for cloning into pSFS2
16	MBO8	gtcgataccgcggAGGAGAGAAGATCTATGGCAATGC	3’ *NRG1* flank downstream primer for cloning into pSFS2
17	DKO491	TACAACCACCAACACATCCC	5’ *RFG1* flank upstream primer for Δ*rfg1::ARG4* fusion
18	DKO493	cacggcgcgcctagcagcggAATGGTGTGATGGTTTGC	5’ *RFG1* flank downstream primer for Δ*rfg1::ARG4* fusion
19	DKO495	gtcagcggccgcatccctgcTAGATACATATGAATTGAACC	3’ *RFG1* flank upstream primer for Δ*rfg1::ARG4* fusion
20	DKO497	TGCTCAAGCGTGCACACACC	3’ *RFG1* flank downstream primer for Δ*rfg1::ARG4* fusion
21	DKO492	actgacctgagaggtaccTACAACCACCAACACATCCC	5’ *RFG1* flank upstream primer for cloning into pSFS2
22	DKO494	tgcatgaacgatctcgagAATGGTGTGATGGTTTGC	5’ *RFG1* flank downstream primer for cloning into pSFS2
23	DKO496	agactgatcgtacgatgcggccgcTAGATACATATGAATTGAACC	3’ *RFG1* flank upstream primer for cloning into pSFS2
24	DKO498	caggtgacctctccgcggtGCTCAAGCGTGCACACACC	3’ *RFG1* flank downstream primer for cloning into pSFS2
25	DKO531	gatcactgacgtgactgcagTAGAATTTCCCGGGAGTTGC	5' *UME6* flank upstream primer for cloning into pBB510
26	DKO532	tgcatgaacgatggatccCTAATTGGAAGTAAATTGAGG	5’ *UME6* flank downstream primer for cloning into pBB510

27	DKO533	agactgatcgtacgatagatctaCAGGTTGACGGTTGACGGTGG	3' *UME6* flank upstream primer for cloning into pBB510
28	DKO534	caggtgacctctggtaccCGGGAAAAGTTGCAAGAGTTGGTG	3' *UME6* flank downstream primer for cloning into pBB510
29	DKO433	accaaacggtaCCTAGAATTTCCCGGGAGTTGC	5' *UME6* flank upstream primer for cloning into pSFS2
30	DKO527	tgcatgaacgatctcgagCTAATTGGAAGTAAATTGAGG	5' *UME6* flank downstream primer for cloning into pSFS2
31	DKO528	agactgatcgtacgatgcggccgcaCAGGTTGACGGTTGACGGTGG	3' *UME6* flank upstream primer for cloning into pSFS2
32	DKO529	caggtgacctctccgcggCGGGAAAAGTTGCAAGAGTTGGTG	3' *UME6* flank downstream primer for cloning into pSFS2
33	DKO400	GACATATTGACCGACATAAT	5' WT *ARG4* add-back primer
34	DKO401	CTAATGACTGAATTTGATGTA	3' WT *ARG4* add-back primer
35	DKO408	GAGGAGACAGAAGTTAGTAG	5' WT *HIS1* add-back primer
36	DKO409	TATGGTGCTCATGGCTACGC	3' WT *HIS1* add-back primer
37	DKO523	GTTGACCGAAGCTCCAATGAATCC	5' primer for *ACT1* probe
38	DKO526	CAGCAATACCTGGGAACATGG	3' primer for *ACT1* probe
39	DKO239	GTTGGGACTAGGATTGGTAAAGC	5' primer for *UME6* probe
40	DKO240	GATGTGGAGTAGACTTGGATAATGG	3' primer for *UME6* probe
41	DTO15	GACCAAGCACCTACTGTTCC	5' primer for *ECE1* probe
42	DTO16	GATCTAGTAATGAGTTGTGG	3' primer for *ECE1* probe
43	DTO17	CAACTGAACAAAGCATCACG	5' primer for *HYR1* probe
44	DTO18	CCTTCAGAACCTTCAGTTGAACCG	3' primer for *HYR1* probe
45	DTO19	CAGGAAGAACCTTGTGATTACC	5' primer for *HWP1* probe

46	DTO20	GTTGGAACAGAAGTGTTTGG	3’ primer for *HWP1* probe
47	DTO23	GTGAAGAACATACCTATGG	5’ primer for *RBT4* probe
48	DTO24	GTCCTTACCAGCTTCTTCG	3’ primer for *RBT4* probe
49	DTO21	GAGAGAAGCTGAAATTGCC	5’ primer for *RBT1* probe
50	DTO22	GGCTTTGACTTGAGCATTG	3’ primer for *RBT1* probe
51	DK258	CAACAATCACCTCACTTGCAACCC	5’ primer for *NRG1* probe
52	DK251	GCTTCTAAAGTCCTGTGTTGTTGTC	3’ primer for *NRG1* probe

Note: Lowercase bases indicate flanking sequences within primers that either contain restriction sites for cloning or were used as annealing sequences for fusion PCR fragments.