Supplemental Material

Supplemental Table S1   Strains used in this study.

Supplemental Table S2.   Oligonucleotides used in this study.

Supplemental Figure S1.   White cells of *ste3Δ* lose the cohesion response to **a**-pheromone exhibited by wild type P57072 cells, and white *ste3Δ/STE3* cells regain the response.   A, B, C. Aggregation of white P57072, *ste3Δ* and *ste3Δ/STE3* cells, respectively, in the presence (+) of a 1% mixture of opaque P37005 (**a/a**) cells and opaque WO-1 (α/α) cells. D, E, F. Aggregation of white P57072, *ste3Δ* and *ste3Δ/STE3* cells, respectively, in the absence (-) of the 1% mixture of opaque cells. G. Average number of cells (error bar represents standard deviation) computed from 20 aggregates. Scale bar represents 5 μm. Details of the method for inducing cohesion can be found in Materials and Methods, and in the legend to Figure 4.

Supplemental Figure S2.   White cells of *ste3Δ* lose the adhesive response to **a**-pheromone exhibited by wild type P57072, and white *ste3Δ/STE3* cells regain the response. Pseudocolor images in orange are provided. A, B, C. Representative images of the dish bottoms of *ste3Δ*, *ste3Δ/STE3* and P57072 cultures, respectively, in the presence (+) of the 1% mixture of opaque P37005 and WO-1 cells. D, E. F. A repeat of A, B, C, respectively. G, H, I. Representative images of the dish bottoms of *ste3Δ*, *ste3Δ/STE3* and P57072 cultures, respectively, in the absence (-) of the 1% mixture of opaque cells. J. Quantitation of cells adhering to the dish bottom. The average number was computed from three wells. Error bar represents standard deviation.   Note that the level attained by white P57072 cells is lower than that by white P37005 cells in response to pheromone. This may be due either to strain differences or **a/a**, α/α differences. The reason for the difference is now under investigation. Details of the method for inducing adhesion can be found in Materials and Methods, and in the legend to Figure 5.

Supplemental Figure S3.   White cells of *ste3Δ* lose enhancement of biofilm thickness in response to opaque cells of P37005, and white *ste3Δ/STE3* cells regain the enhancement response. A, B,

C. Representative LSCM Z-series projections of P57072, *ste3Δ* and *ste3Δ/STE3* biofilms, respectively. D. Average thickness (± standard deviation) of biofilms, computed from 9 measurements. See Materials and Methods and the legend to Figure 6 for details.

Supplemental Figure S4. Northern analysis reveals that pheromone up-regulation of *CPH1* is regained in the complemented strain *far1Δ/FAR1*. (-), absence of α-pheromone; (+) presence of α-pheromone. See legend to Figure 8 for details. 18S rRNA hybridization demonstrated equivalent loading (data not shown).

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

Strain	Parent	*MTL*	Relevant Genotype	Reference or source
P37005	−	**a/a**	Wild type	Lockhart et al. (2002)
WO-1	−	α/α	Wild type	Slutsky et al. (1987)
P57072	−	α/α	Wild type	Lockhart et al. (2002)
ste2Δ	P37005	**a/a**	*ste2Δ*::FRT/*ste2Δ*::FRT	This study
ste3Δ	P57072	α/α	*ste3Δ*::FRT/*ste3Δ*::FRT	This study
ste4Δ	P37005	**a/a**	*ste4Δ*::FRT/*ste4Δ*::FRT	This study
cek1Δ	P37005	**a/a**	*cek1Δ*::FRT/*cek1Δ*::FRT	This study
cek2Δ	P37005	**a/a**	*cek2Δ*::FRT/cek2Δ::FRT	This study
cek1Δ cek2Δ	*cek2Δ*	**a/a**	*cek1Δ*::FRT/*cek1Δ*::FRT cek2Δ::FRT/*cek2Δ*::FRT	This study
cph1Δ	P37005	**a/a**	*cph1Δ*::FRT/*cph1Δ*::FRT	This study
far1Δ	P37005	**a/a**	*far1Δ*::FRT/*far1Δ*::FRT	This study
far1Δ (WO-1)	WO-1	α/α	*far1Δ*::FRT/*far1Δ*::FRT	This study
ste2Δ/STE2	*ste2Δ*	**a/a**	*ste2Δ*::FRT/*STE2-GFP-SAT^R*	This study
ste3Δ/STE3	*ste3Δ*	α/α	*ste3Δ*::FRT/*STE3-GFP-SAT^R*	This study
ste4Δ/STE4	*ste4Δ*	**a/a**	*ste4Δ*::FRT/*STE4-GFP-SAT^R*	This study
cek1Δ/CEK1	*cek1Δ*	**a/a**	*cek1Δ*::FRT/*CEK1-GFP-SAT^R*	This study
cek2Δ/CEK2	*cek2Δ*	**a/a**	*cek2Δ*::FRT/*CEK2-GFP-SAT^R*	This study
cph1Δ/CPH1	*cph1Δ*	**a/a**	*cph1Δ*::FRT/*CPH1-GFP-SAT^R*	This study
far1Δ/FAR1	*far1Δ*	**a/a**	*far1Δ*::FRT/*FAR1-GFP-SAT^R*	This study

Supplemental Table S2.   Oligonucleotides used in this study

| Primer	Gene/Purpose	Sequence
STE2f1	*STE2* heterozygote	5'-TCTATTGTGTAAACTATTAC-3'
STE2r1	*STE2* heterozygote	5-GTGT<u>CCCGGG</u>AATCAATGCCTAGTCGATC-3'
STE2f2	*STE2* heterozygote	5'--TGTA<u>CCCGGG</u>CAAATCACCATCAAAAGA-3'
STE2r2	*STE2* heterozygote	5'-CTTGTACTGGTTCAGCAACC-3'
STE2f3	*STE2* homozygote	5'-GATCGACTAGGCATTGATTTTTG-3'
STE2r3	*STE2* homozygote	5'-TCAT<u>CCCGGG</u>TCTTCTTATGTTGAACAC-3'
STE2f4	*STE2* homozygote	5'-TCTT<u>CCCGGG</u>CTCAAACTGCTAATAAT-3'
STE2r4	*STE2* homozygote	5'-CACTCTTTTGATGGTGATTTG-3'
STE3f1	*STE3* heterozygote	5'-TGAATCTACTTTGGGCAGAG-3'
STE3r1	*STE3* heterozygote	5'-CCAA<u>CCCGGG</u>ATTTTCCTCTTGGTTTT-3'
STE3f2	*STE3* heterozygote	5'-ACAA<u>CCCGGG</u>GTCTTCGCCTGCAACATTA-3'
STE3r2	*STE3* heterozygote	5'-CACAAATGCAGATGTTGTCG-3'
STE3f3	*STE3* homozygote	5'-AAAACCAAGAGGAAAATCCC-3'
STE3r3	*STE3* homozygote	5'-ACTT<u>CCCGGG</u>TGCCATAAAAATGGCGG-3'
STE3f4	*STE3* homozygote	5'-ACAG<u>CCCGGG</u>CAACTGTATTCTTTCTGT-3'
STE3r4	*STE3* homozygote	5'-GCAGGCGAAGACTGGAGTTG-3'
STE4f1	*STE4* heterozygote	5'-ATGGTTAACTCGAACAT-3'
STE4r1	*STE4* heterozygote	5'-TCA<u>CCCGGG</u>TTGTAAAACAGATCCCA-3'
STE4f2	*STE4* heterozygote	5'-TCA<u>CCCGGG</u>AAGAGCGAGACTGAGGGTA-3'
STE4r2	*STE4* heterozygote	5'-AAGGTGCCATGAAAGGTA-3'
STE4f3	*STE4* homozygote	5'-ATGTCCGATTATCTTGC-3'
STE4r3	*STE4* homozygote	5'-TCA<u>CCCGGG</u>CATTTGATAGGTTCCATT-3'
STE4f4	*STE4* homozygote	5'-TCA<u>CCCGGG</u>CTGATCCGGTTATTCGAT-3'
STE4r4	*STE4* homozygote	5'-GACGGACCAAACTTTGAT-3'
CEK1f1	*CEK1* heterozygote	5'-ATTCCCGAGAATATATGA-3'
CEK1r1	*CEK1* heterozygote	5'-TCG<u>CCCGGG</u>TAAATAATATATAAGTTGA-3'
CEK1f2	*CEK1* heterozygote	5'-TCG<u>CCCGGG</u>TAAAGTTGAAGTTAAGTA-3'
CEK1r2	*CEK1* heterozygote	5'-GAGAGGTTTATTTGGTAGA-3'
CEK1f3	*CEK1* homozygote	5'-TTAAATTTACTATCCCAAA-3'
CEK1r3	*CEK1* homozygote	5'-TCG<u>CCCGGG</u>TGAGTTCTAATGACTCGAT-3'
CEK1f4	*CEK1* homozygote	5'-TCG<u>CCCGGG</u>TTGGTCAGTTGGTTGTAT-3'
CEK1r4	*CEK1* homozygote	5'-TCAAAACCTATACAACAA-3'
CEK2f1	*CEK2* heterozygote	5'-TAACGACAACTGCAGGAC-3'
CEK2r1	*CEK2* heterozygote	5'-TCA<u>CCCGGG</u>TTGGTCAGGTATTGTAA-3'
CEK2f2	*CEK2* heterozygote	5'-TCA<u>CCCGGG</u>CTTTACTTAATTAATTAC-3'
CEK2r2	*CEK2* heterozygote	5'-ACAATGGAGCACAATGCT-3'

CEK2f3	*CEK2* homozygote	5'-CTTCCTGTTACCATGTTA-3'
CEK2r3	*CEK2* homozygote	5'-TCA<u>CCCGGG</u>CATGTATTCCTGAATAA-3'
CEK2f4	*CEK2* homozygote	5'-TCA<u>CCCGGG</u>TTGAGTGCATCCAATTAT-3'
CEK2r4	*CEK2* homozygote	5'-CGACATGACTATTTCGA-3'
CPH1f1	*CPH1* heterozygote	5'-TTGAAATTAATCTAGAATC-3'
CPH1r1	*CPH1* heterozygote	5'-TCG<u>CCCGGG</u>CTAAAACTAAGACCAAAAC-3'
CPH1f2	*CPH1* heterozygote	5'-TAA<u>CCCGGG</u>TAGATGAATAGATACAGA-3'
CPH1r2	*CPH1* heterozygote	5'-AACGTGAGGTGATGTTTC-3'
CPH1f3	*CPH1* homozygote	5'-TCTTAGTTTTAGTTTGAC-3'
CPH1r3	*CPH1* homozygote	5'-TCG<u>CCCGGG</u>TAAACAATACAACGGACA-3'
CPH1f4	*CPH1* homozygote	5'-TCG<u>CCCGGG</u>AACAAGCCCAACCAATAA-3'
CPH1r4	*CPH1* homozygote	5'-TGGAATTCACAACATCAT-3'
FAR1f1	*FAR1* heterozygote	5'-TTGATAATGTCACCCAA-3'
FAR1r1	*FAR1* heterozygote	5'-TCA<u>CCCGGG</u>GTCTAACACTTTAAGTGGT-3'
FAR1f2	*FAR1* heterozygote	5'-TCA<u>CCCGGG</u>TTGTGCTGGTGCAACCAT-3'
FAR1r2	*FAR1* heterozygote	5'-TTGATTTGATCCGTAGA-3'
FAR1f3	*FAR1* homozygote	5'-ATGCGCAAACTGTTCCA-3'
FAR1r3	*FAR1* homozygote	5'-TCA<u>CCCGGG</u>ATAGACACACCAATGCCA-3'
FAR1f4	*FAR1* homozygote	5'-TCA<u>CCCGGG</u>TCAGTTGACACTTACTAT-3'
FAR1r4	*FAR1* homozygote	5'-TATTAAACTATTCATCA-3'
STE2Q1XhF	*STE2* complementation	5'-TCC<u>CTCGAG</u>TCTATTGTGTAAACTATTAC-3'
STE2Q1BhR	*STE2* complementation	5'-TCC<u>GGATCC</u>CACTCTTTTGATGGTGATTT-3'
STE2Q2BhF	*STE2* complementation	5'-TCC<u>GGATCC</u>AAATCGTATTCAAGTATCTT-3'
STE2Q2XhR	*STE2* complementation	5'-TCC<u>CTCGAG</u>CTTGCTCCATTGGGAAGTTT-3'
STE3Q1XhF	*STE3* complementation	5'-TCC<u>CTCGAG</u>TGAAGAGGCTAAAGACGTTG-3'
STE3Q1BhR	*STE3* complementation	5'-TCC<u>GGATCC</u>GTTATCATACGATTTCAGTT-3'
STE3Q2BhF	*STE3* complementation	5'-TCC<u>GGATCC</u>TCCATCGTATCCTGTTACTT-3'
STE3Q2XhR	*STE3* complementation	5'-TCC<u>CTCGAG</u>GAAACCAGAGGCTGGAATG-3'
STE4Q1XhF	*STE4* complementation	5'-TCA<u>CTCGAG</u>ATGGTTAACTCGAACAT-3'
STE4Q1BhR	*STE4* complementation	5'-TCA<u>GGATCC</u>GACGGACCAAACTTTGAT-3'
STE4Q2BhF	*STE4* complementation	5'-TCA<u>GGATCC</u>AAGAGCGAGACTGAGGGTA-3'
STE4Q2XhR	*STE4* complementation	5'-TCA<u>CTCGAG</u>AAGGTGCCATGAAAGGTA-3'
CEK1Q1XhF	*CEK1* complementation	5'-TCC<u>CTCGAG</u>ATTCCCGAGAATATATGA-3'
CEK1Q1BgR	*CEK1* complementation	5'-TCC<u>AGATCT</u>AATGGCTTCATAATCTCT-3'
CEK1Q2BgF	*CEK1* complementation	5'-TCC<u>AGATCT</u>TAAAGTTGAAGTTAAGTA-3'
CEK1Q2XhR	*CEK1* complementation	5'-TCC<u>CTCGAG</u>GAGAGGTTTATTTGGTAGA-3'
CEK2Q1XhF	*CEK2* complementation	5'-TCA<u>CTCGAG</u>TAACGACAACTGCAGGAC-3'
CEK2Q1BhR	*CEK2* complementation	5'-TCA<u>GGATCC</u>CGACATGACTATTTCGA-3'
CEK2Q2BhF	*CEK2* complementation	5'-TCA<u>GGATCC</u>CTTTACTTAATTAATTAC-3'

|		
CEK2Q2XhR	*CEK2* complementation	5'-TCACTCGAGACAATGGAGCACAATGCT-3'
CPH1Q1StF	*CPH1* complementation	5'-TCGAGGCCTTTGAAATTAATCTAGAATC-3'
CPH1Q1BhR	*CPH1* complementation	5'-TCGGGATCCTGTTTGTGACTGTTTTACTT-3'
CPH1Q2BhF	*CPH1* complementation	5'-TCGGGATCCTAGATGAATAGATACAGA-3'
CPH1Q2StR	*CPH1* complementation	5'-TCGAGGCCTAACGTGAGGTGATGTTTC-3'
FAR1Q1StF	*FAR1* complementation	5'-TCAAGGCCTTTGATAATGTCACCCAA-3'
FAR1Q1BgR	*FAR1* complementation	5'-TCAAGATCTTATTAAACTATTCATCA-3'
FAR1Q2BgF	*FAR1* complementation	5'-TCAAGATCTTTGTGCTGGTGCAACCAT-3'
FAR1Q2StR	*FAR1* complementation	5'-TCAAGGCCTTTGATTTGATCCGTAGA-3'
SATBgF1	*GFP-SAT1* PCR	5'-TCAAGATCTTCCATCATAAAATGTCGA-3'
GFBhF1	*GFP-SAT1* PCR	5'-TCAGGATCCATGTCTAAAGGTGAAGAA-3'
STE2f	Deletion probe for Southern	5'-GTGTTCAACATAAGAAGA-3'
STE2r	Deletion probe for Southern	5'-ATTATTAGCAGTTTGAGC-3'
STE3f	Deletion probe for Southern	5'-CCGCCATTTTTATGGCAC-3'
STE3r	Deletion probe for Southern	5'-TACAGTTGACCAATCTGT-3'
STE4f	Deletion probe for Southern	5'-AGGCAACTTTTATCAATC-3'
STE4r	Deletion probe for Southern	5'-AGCACAAAACATCTCCTGA-3'
CEK1f	Deletion probe for Southern	5'-ATCTGATGATCATATACA-3'
CEK1r	Deletion probe for Southern	5'-ACATCAATAGCAGTAGTA-3'
CEK2f	Deletion probe for Southern	5'-AAGGATTAAAGATGATTC-3'
CEK2r	Deletion probe for Southern	5'-TGATTTCTGGAGCTCGATA-3'
FAR1f	Deletion probe for Southern	5'-ACTAGTTAACCAAACATC-3'
FAR1r	Deletion probe for Southern	5'-TGATGTTGTACGTGGAAT-3'
CPH1f	Southern and Northern probe	5'-AGCATTATCATTCCATTA-3'
CPH1r	Southern and Northern probe	5'-TATTGACTTGGTGTGGCTT-3'
MFA1f	Northern probe	5'-ATGGCTGCTCAACAACAA-3'
MFA1r	Northern probe	5'-TTACATAACAGAACAAGT-3'
CSH1f	Northern probe	5'-TCGACTCTGAAAAAACTA-3'
CSH1r	Northern probe	5'-CATGCCAATGAAACTTGC-3'

ste3Δ (α/α) Cohesion Assay

A. P57072 (+)
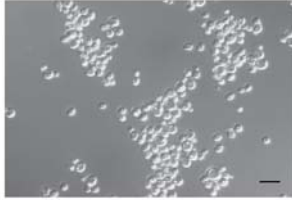
B. ste3Δ (+)

C. ste3Δ/STE3 (+)
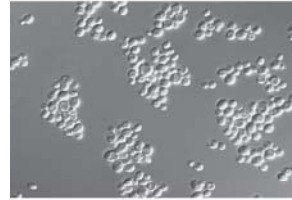
D. P57072 (-)
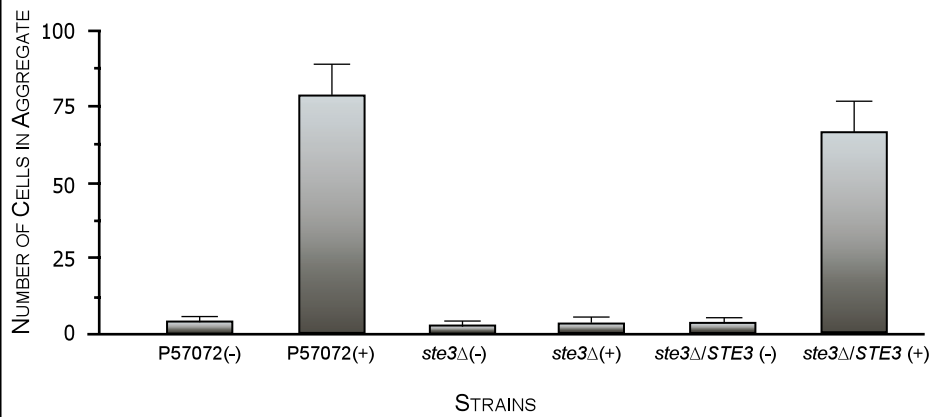
E. ste3Δ (-)

F. ste3Δ/STE3 (-)
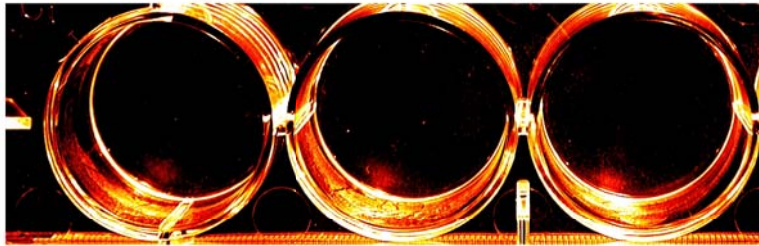
G.

ste3Δ (α/α) Adhesion Assay

A. *ste3Δ*(+)   B. *ste3Δ/STE3*(+)   C. P57072(+)

D. *ste3Δ*(+)   E. *ste3Δ/STE3*(+)   F. P57072(+)

G. *ste3Δ*(-)   H. *ste3Δ/STE3*(-)   I. P57072(-)

J.

Legend:
- ☐ Wh minus 1% Op Mixture
- ■ Wh plus 1% Op Mixture

Y-axis: Number of Adherent Cells
(5×10^8, 5×10^7, 5×10^6, 5×10^5, 5×10^4, 5×10^3)

X-axis: Strains (P57072, *ste3Δ*, *ste3Δ/STE3*)

A. P57072 wh & 10 % op

B. *ste3*Δ wh & 10% op

C. *ste3*Δ/*STE*3 wh & 10% op

50 μm

D. Ave. thickness: (μm)

36.4 ±3.1   21.3 ±2.0   36.7 ±1.4