Supporting Information

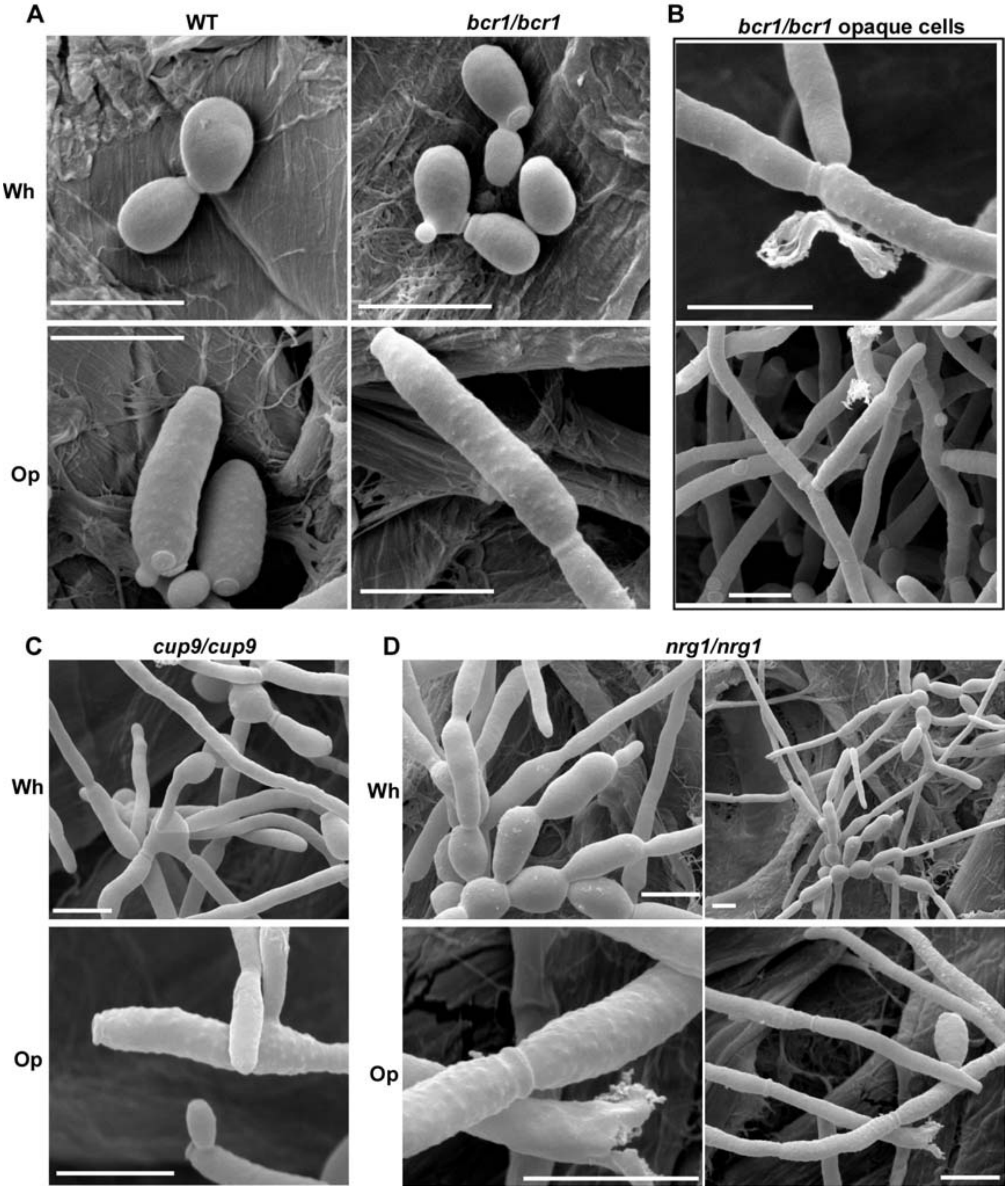

Fig. S1. SEM images of white and opaque cells.

A. Images of white and opaque cells of the WT and *bcr1/bcr1* mutant. B. Two more examples of opaque cells of the *bcr1/bcr1* mutant at different magnifications. C. Images of white and opaque cells of the *cup9/cup9* mutant. D. Images of white and opaque cells of the *nrg1/nrg1* mutant. Scale bar, 5 μm.

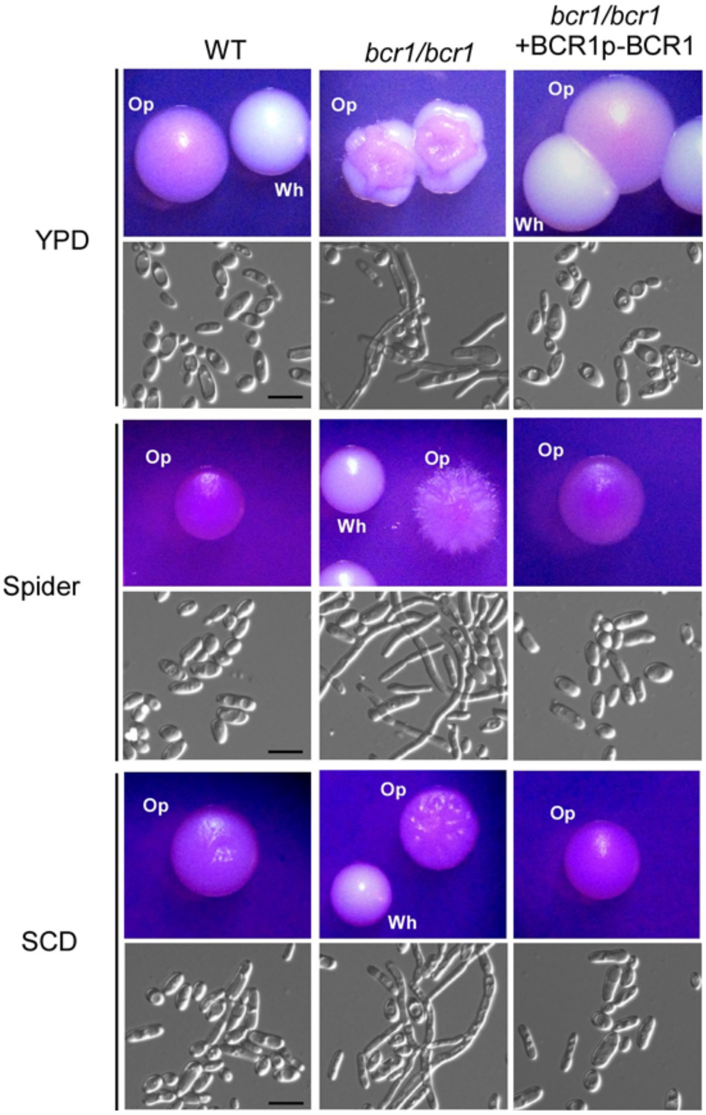

Fig. S2. Opaque cell filamentation of the *bcr1/bcr1* mutant in YPD, Spider and SCD medium.

Cells were plated onto agar plates containing different media as indicated and cultured at 25°C for 5 days. Colony and cellular morphology images of the WT, *bcr1/bcr1* mutant and *bcr1/bcr1*+BCR1p-BCR1 reconstituted strain are shown. Wh, white colony; op, opaque colony. Scale bar, 10 μm.

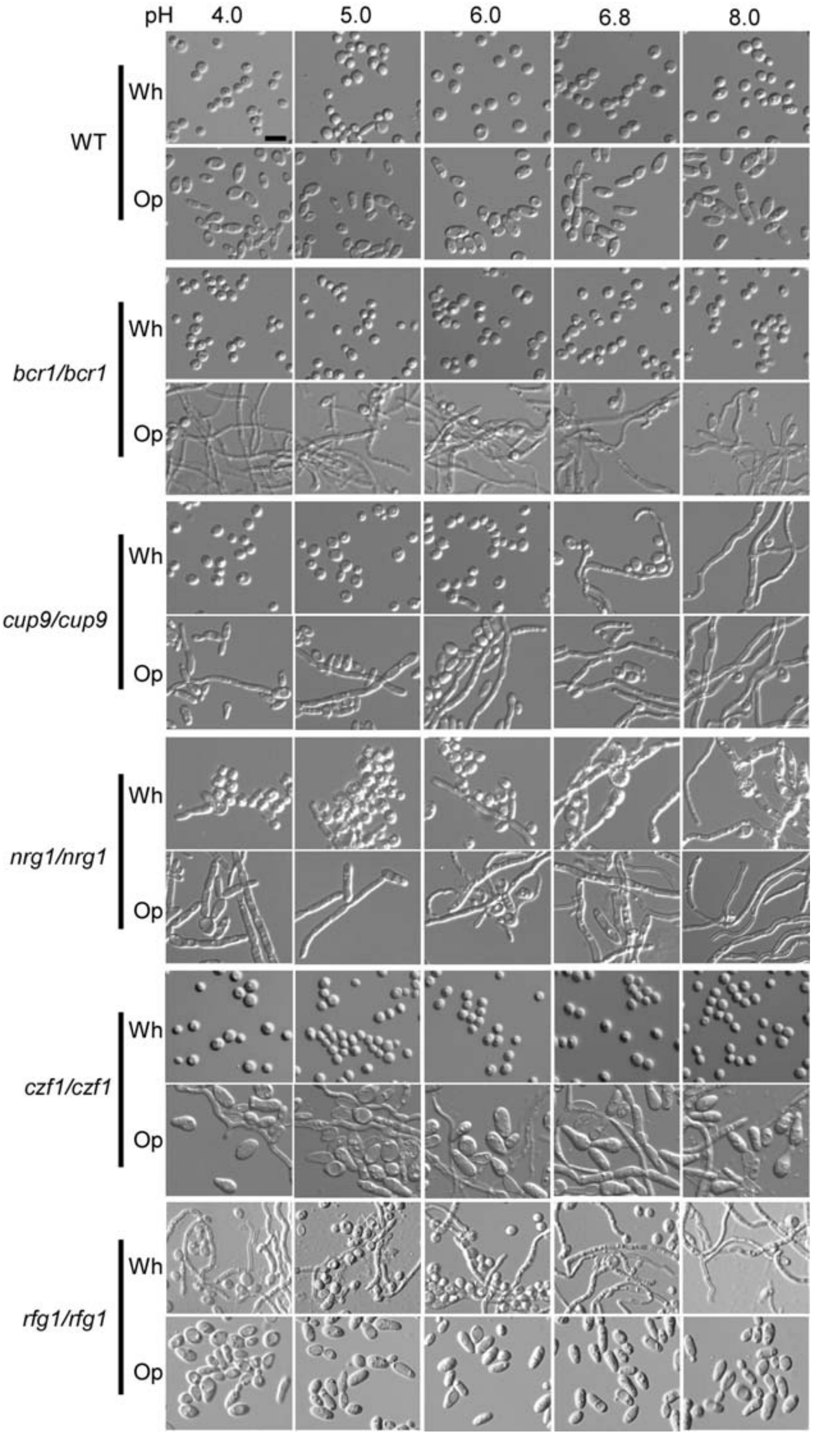

Fig. S3. Cellular morphology of the WT, *bcr1/bcr1*, *cup9/cup9*, *nrg1/nrg1*, *czf1/czf1* and *rfg1/rgf1* mutants.

White and opaque cells from a representative colony of each mutant under pH 4.0 to 8.0 are shown. This Fig. corresponds to the colony morphology presented in **Fig. 6**.

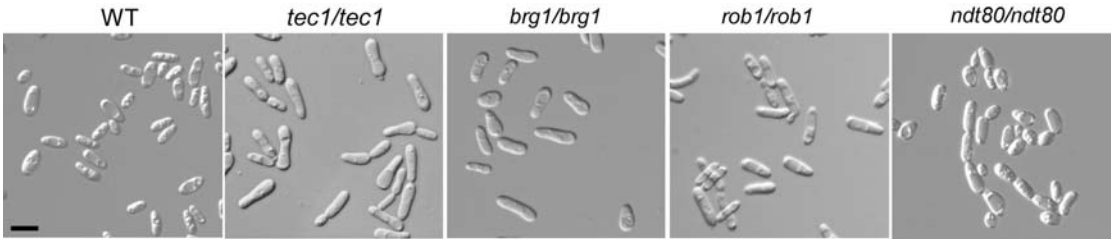

Fig. S4. Opaque cellular morphology of the *rob1/rob1* and *ndt80/ndt80* mutants.

WT, SN152a (*MTL***a**/**a**), a derivative of SN152. The *tec1/tec1*, *brg1/brg1*, *rob1/rob1* and *ndt80/ndt80* mutants are homozygous at the *MTL* locus (Du et al., *Mol Biol Cell*, 2012).

Table S1. White-to-opaque switching frequencies in the *bcr1/bcr1*, *cup9/cup9* and *czf1/czf1* mutants.

Strain	**White-to-opaque Swit. Freq.(%)**
WT	<0.8
bcr1/bcr1	1.9±0.5
cup9/cup9	1.6±0.8
czf1/czf1	<0.7

WT, JSM167, a derivative of SN250, *MTL*α/α. White-opaque switching assay was performed at 25°C in air. The genotypes of the *bcr1/bcr1*, *cup9/cup9* and *czf1/czf1* mutants are listed in **Table S3**. Lee's medium (pH6.8) was used for cell growth.

Table S2. Mating efficiency of the WT, *bcr1/bcr1*, *cup9/cup9* and *nrg1/nrg1* mutants.

Experimental strain	Mating efficiency of white cells	Mating efficiency of opaque cells
WT	<1 x 10^{-6}	1.5 x 10^{-3}
bcr1/bcr1	<1 x 10^{-6}	1.7 x 10^{-2}
cup9/cup9	<1 x 10^{-7}	3.5 x 10^{-2}
nrg1/nrg1	1.7 x 10^{-5}	1.9 x 10^{-2}

White and opaque cells of the experimental strains were crossed with opaque cells of a WT *MTL* opposite strain. *MTL***a**/**a** and *MTL*α/α cells in white or opaque phase were mixed and cultured on Lee's glucose plates for 4 days at 25°C. About 1 x 10^6 cells of each mating mixture were resuspended and plated onto selectable SCD plates. The mating efficiencies were calculated and shown. White and opaque cells of the WT (JSM167, *MTL*α/α, *arg4-*), *bcr1/bcr1*(*MTL*α/α, *arg4-*) and *cup9/cup9* (*MTL*α/α, *arg4-*) mutants were crossed with the WT opaque tester (GH1012, *MTL***a**/**a**, *ura3-*). White and opaque cells and white cells of the nrg1/nrg1 mutant were crossed with the WT opaque test (WUM5A, *MTL*α/α, *ura3-*). A representative selectable plate (depleted with uridine and L-arginine) of the WT and *bcr1/bcr1* mutant mating testing crosses was imaged and shown in **Fig. 4**.

Table S3. Strains used in this study

Strain	Parent strain	Genotype	Reference
GH1012	CAI4	*MTL**a**/**a** ura3::imm434/ura3::imm434*	[1]
GH1013	BWP17	*MTL**a/a** ura3::imm434/ura3::imm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG*	[1]
GH1109	GH1013	*MTL**a**/**a** ura3::imm434/ura3::imm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG cyr1::ARG4/cyr1::HIS1*	[1]
GH1296	CCF3	*MTL**a/ạ** ura3:: imm434/ura3:: imm434 flo8::hisG/flo8::hisG-URA3-hisG*	[2]
MMY620	CAI4	*MTL**a**/mtlα1::HisG mtlα2::HisG ura3::imm434/ura3::imm434 efg1::dpl200/efg1::URA3-dpl200*	[3]
SN250	CAI4	*MTL**a**/α ura3::imm434::URA3-IRO1/ura3::imm434 arg4::hisG/arg4::hisG his1::hisG/his1::hisG leu2::hisG::CdHIS1/leu2::hisG::CmLEU2*	[4]
SN250αΔ	SN250	*MTL**a**/ mtlα::FRT-SAT1-FRTura3::imm434::URA3-IRO1/ura3::imm434 arg4::hisG/arg4::hisG his1::hisG/his1::hisG leu2::hisG::CdHIS1/leu2::hisG::CmLEU2*	This study
SN250α	SN250	*MTLα/α ura3::imm434::URA3-IRO1/ura3::imm434 arg4::hisG/arg4::hisG his1::hisG/his1::hisG leu2::hisG::CdHIS1/leu2::hisG::CmLEU2*	This study
TF137	SN152	*MTL**a**/α ura3::imm434::URA3-IRO1/ura3::imm434 arg4::hisG/arg4::hisG his1::hisG/his1::hisG leu2::hisG /leu2::hisG bcr1::CdHIS1/bcr1::CmLEU2*	[5]
TF137α	TF137	*MTLα/α ura3::imm434::URA3-IRO1/ura3::imm434 arg4::hisG/arg4::hisG his1::hisG/his1::hisG leu2::hisG /leu2::hisG bcr1::CdHIS1/bcr1::CmLEU2*	This study
TF137αΔ	TF137	*MTL**a**/ mtlα::FRT-SAT1-FRT ura3::imm434::URA3-IRO1/ura3::imm434 arg4::hisG/arg4::hisG his1::hisG/his1::hisG leu2::hisG /leu2::hisG bcr1::CdHIS1/bcr1::CmLEU2*	This study
CJN702	BWP17	*MTL**a**/α ura3::imm434arg4::hisGhis1::hisG::pHIS1bcr1::ARG4/ura3::imm434 arg4::hisG his1::hisG bcr1::URA3*	[6]
CJN702a	CJN702	*MTL**a/a** ura3::imm434arg4::hisGhis1::hisG::pHIS1bcr1::ARG4/ura3::imm434 arg4::hisG his1::hisG bcr1::URA3*	This study
GH1501	GH1109	*MTL**a**/**a** ura3::imm434/ura3::imm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG cyr1::ARG4/cyr1::HIS1 bcr1::FRT /bcr1::URA3*	This study
GH1502	GH1296	*MTL**a/ạ** ura3:: imm434/ura3:: imm434 flo8::hisG/flo8::hisG bcr1::FRT/bcr1::URA3*	This study

GH1503	MMY620	*MTL**a**/mtlα1::HisG mtlα2::HisG ura3::imm434/ura3::imm434 efg1::dpl200/efg1::dpl200 bcr1::FRT /bcr1::URA3*	This study
GH1281C	WYZ12	*MTLα/α ura3:: imm434/ura3:: imm434 hgc1::AGR4/hgc1::HIS1*	[2]
hgc1/hgc1 bcr1/bcr1	GH1281 α	*MTLα/α ura3:: imm434/ura3:: imm434 hgc1::AGR4/hgc1::HIS1 bcr1::FRT-SAT1-FRT/bcr1::URA3*	This study
TF61	SN152	*MTL**a**/α ura3::imm434::URA3-IRO1/ura3::imm434 arg4::hisG/arg4::hisG his1::hisG/his1::hisG leu2::hisG /leu2::hisG cup9::CdHIS1/cup9::CmLEU2*	[5]
TF61C	TF61	*MTLα/α ura3::imm434::URA3-IRO1/ura3::imm434 arg4::hisG/arg4::hisG his1::hisG/his1::hisG leu2::hisG /leu2::hisG cup9::CdHIS1/cup9::CmLEU2*	This study
WCZF1M4B	WUM5A	*MTLα/α czf1::FRT/czf1::FRT*	[7]
E5.3I-3		*MTLα/α, czf1::HisG/czf1::HisG-ura3-HisG*	Kumamoto C.A., et al., (unpublished)
nrg1/nrg1	GH1013	*MTL**a**/**a** ura3::imm434/ura3::imm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG cyr1::ura3/nrg1::HIS1*	This study
rfg1/rfg1	CaWY5	*MTLα/α CaWY5 rfg1::HIS3 / rfg1::URA3*	[2]
SN152a	SN152	*MTL**a**/**a** ura3::imm434::URA3-IRO1/ura3::imm434 arg4::hisG/arg4::hisG his1::hisG/his1::hisG leu2::hisG /leu2::hisG*	[2]
CJN1787a	CJN1787	*As CJN1787, but MTL**a**/α::SAT1*	This study
tec1/tec1 (TF115α)		*MTLα/α ura3::imm434::URA3-IRO1/ura3::imm434 arg4::hisG/arg4::hisG his1::hisG/his1::hisG leu2::hisG /leu2::hisG tec1::CdHIS1/tec1::CmLEU2*	[2]
tec/tec1 bcr1/bcr1		*MTLα/α ura3::imm434::URA3-IRO1/ura3::imm434 arg4::hisG/arg4::hisG his1::hisG/his1::hisG leu2::hisG /leu2::hisG tec1::CdHIS1/tec1::CmLEU2 bcr1::FRT-SAT1-FRT/bcr1::ARG4*	This study
TF22		*MTL**a**/α ura3::imm434::URA3-IRO1/ura3::imm434 arg4::hisG/arg4::hisG his1::hisG/his1::hisG leu2::hisG /leu2::hisG brg1::CdHIS1/brg1::CmLEU2*	[5]
TF22α		*MTL**a**:: FRT-SAT1-FRT/α ura3::imm434::URA3-IRO1/ura3::imm434 arg4::hisG/arg4::hisG his1::hisG/his1::hisG leu2::hisG /leu2::hisG brg1::CdHIS1/brg1::CmLEU2*	This study
TF22α1		*MTL**a**:: FRT/α ura3::imm434::URA3-IRO1/ura3::imm434 arg4::hisG/arg4::hisG his1::hisG/his1::hisG leu2::hisG /leu2::hisG brg1::CdHIS1/brg1::CmLEU2*	This study
brg1/brg1 bcr1/bcr1		*MTL**a**:: FRT/α ura3::imm434::URA3-IRO1/ura3::imm434 arg4::hisG/arg4::hisG his1::hisG/his1::hisG leu2::hisG*	This study

		/leu2::hisG brg1::CdHIS1/brg1::CmLEU2 *bcr1::FRT-SAT1-FRT/bcr1::ARG4*	
brg1/brg1		*MTLα/α ura3::imm434::URA3-IRO1/ura3::imm434* *arg4::hisG/arg4::hisG his1::hisG/his1::hisG leu2::hisG* */leu2::hisG brg1::CdHIS1/brg1::CmLEU2*	[2]
rob1/rob1		*MTL***a***/***a** *ura3::imm434::URA3-IRO1/ura3::imm434* *arg4::hisG/arg4::hisG his1::hisG/his1::hisG leu2::hisG* */leu2::hisG rob1::CdHIS1/rob1::CmLEU2*	[2]
ndt80/ndt80		*MTLα/α ura3::imm434::URA3-IRO1/ura3::imm434* *arg4::hisG/arg4::hisG his1::hisG/his1::hisG leu2::hisG* */leu2::hisG ndt80::CdHIS1/ndt80::CmLEU2*	[2]
cph1/cph1	JKC18	*MTL***a***/α ura3::imm434 /ura3::imm434 cph1::hisG/cph1::hisG*	[8]
cph1/cph1	JKC18**a**	*MTL***a***/***a** *ura3::imm434/ura3::imm434 cph1::hisG/cph1::hisG*	This study
cph1/cph1 *bcr1/bcr1*		*MTL***a***/***a** *ura3::imm434 /ura3::imm434 cph1::hisG/cph1::hisG* *bcr1::URA3/bcr1::FRT-SAT1-FRT*	This study

References：

1. Huang, G., Srikantha, T., Sahni, N., Yi, S., and Soll, D.R. (2009). CO(2) regulates white-to-opaque switching in Candida albicans. Curr Biol *19*, 330-334.
2. Du, H., Guan, G., Xie, J., Cottier, F., Sun, Y., Jia, W., Muhlschlegel, F.A., and Huang, G. (2012). The transcription factor Flo8 mediates CO2 sensing in the human fungal pathogen Candida albicans. Molecular biology of the cell *23*, 2692-2701.
3. Zordan, R.E., Miller, M.G., Galgoczy, D.J., Tuch, B.B., and Johnson, A.D. (2007). Interlocking transcriptional feedback loops control white-opaque switching in Candida albicans. PLoS Biol *5*, e256.
4. Noble, S.M., French, S., Kohn, L.A., Chen, V., and Johnson, A.D. (2010). Systematic screens of a Candida albicans homozygous deletion library decouple morphogenetic switching and pathogenicity. Nature genetics *42*, 590-598.
5. Homann, O.R., Dea, J., Noble, S.M., and Johnson, A.D. (2009). A phenotypic profile of the Candida albicans regulatory network. PLoS Genet *5*, e1000783.
6. Nobile, C.J., Andes, D.R., Nett, J.E., Smith, F.J., Yue, F., Phan, Q.T., Edwards, J.E., Filler, S.G., and Mitchell, A.P. (2006). Critical role of Bcr1-dependent adhesins in C. albicans biofilm formation in vitro and in vivo. PLoS Pathog *2*, e63.
7. Ramirez-Zavala, B., Reuss, O., Park, Y.N., Ohlsen, K., and Morschhauser, J. (2008). Environmental induction of white-opaque switching in Candida albicans. PLoS Pathog *4*, e1000089.
8. Liu, H., J. Kohler & G. R. Fink, (1994) Suppression of hyphal formation in Candida albicans by mutation of a STE12 homolog. Science 266: 1723-1726.

Table S4. Primers used in this study

Name	**Sequence (5’ to 3’)**	**Purpose**
probeECE1F	CCATGCTCCAGAATTCAACA	Northern probes
probeECE1R	TACTGAGCCGGCATCTCTTT	
probeOP4F	CTCGGTGGCAGCTCTAGTTC	
probeOP4R	CAGCTTGACCAGCAGCATTA	
probeWOR1F	AGTGGTGGTAATGGCAATGG	
probeWOR1R	CGACCCAGAAGAATTTCCAA	
probeHWP1F	TCCAGAAGCTTCCATTCCAC	
probeHWP1R	GCAGCACCGAAAGTCAATCT	
BCR1-fwd	AATCTTGTCGACAGAGAGCAGGGTCATACTTGA	bcr1/bcr1+BCR1p-BCR1 complemented plasmid
BCR1-rev	AATCTTAGATCTCACTGTCGTCACCCCAATACTGT	
BCR1-3’fwd	AATCTTCTCGAG ACAGTATTGGGGTGACGACAGTG	
BCR1-3’rev	AATCTTGTCGAC CAACAATCTCCCTTCCCAAATAC	
BCR1-UP FWD	TTATATGGGCCCAACCACAACAAATCAACCAACC	pSFS2a-BCR1KO
BCR1-UP REV	AATCATCTCGAGGTTGTTGTAGTTGTTGTTTTTG	
BCR1-DOWN FWD	TAATATCCGCGGACAGTATTGGGGTGACGACAGTG	
BCR1-DOWN REV	ATATACGAGCTCCAACAATCTCCCTTCCCAAATAC	
BCR1-L FWD	TCACAGCACGCATCTATGGC	Fusion PCR for *BCR1* KO
BCR1-L REV	CACGGCGCGCCTAGCAGCGGAGATGAACTTGATGCCGACG	
BCR1-R FWD	GTCAGCGGCCGCATCCCTGCCCTCATCCTAATTGGGGCTC	
BCR1-R REV	TTGTTGTTGGTGTCAGTAGTGG	
LT31	CCGCTGCTAGGCGCGCCGTGGTTTTCCCAGTCACGACGTT	
LT32	GCAGGGATGCGGCCGCTGACTGTGGAATTGTGAGCGGATA	
NRG1-L FWD	GCTTTCATCTTGTTTGTCAC	Fusion PCR for NRG1 KO
NRG1-L REV	CACGGCGCGCCTAGCAGCGGTGAGATGAGAAACAGGATAACG	
NRG1-R FWD	GTCAGCGGCCGCATCCCTGCTGGTTACTGGTTCATGTATAGG	
NRG1-R REV	AGAGAAGATCTATGGCAATGC	

NRG1-5DR	CCCTGCTAGTTTCATTAAGAATCAAACAATCATTATGCTTTATCAACAATCATATCCAATGTTTTCCCAGTCACGACGTT	Deletion of the second copy of *NRG1* gene
NRG1-3DR	TTTGTTTTTCCCATTGGTATGAGTCTTGTAATGTTGATTACAATTATCTTGACGAGCAAAGTGGAATTGTGAGCGGATA	
NRG1-CHKF	ACACTACAACAACCTCAGCC	
NRG1-CHKR	ATGTCCTGAAGTAGTAAACG	
CZF1-F	ATTTGCGTTGTACTTCCCAG	ChIP-PCRs
CZF1-R	CTGTGTATATGTCTTGGGTGAC	
CUP9-F	AGATGAGATAGGTGTCATACAC	
CUP9-R	TTAATCTTTGTGTCAGGCCATC	
NRG1-F	TATACCTAGTAGCTGCATCC	
NRG1-R	TTCAACAATGGTGGAGAATG	
EFG1-1BF	AATCACTATTCTATCCCTTGC	
EFG1-1BR	TGTGTTTACGTGTCTCTGAC	
EFG1-2BF	ACTTGGTTCAAAGAAAGGAACG	
EFG1-2BR	TTAGGTTGTAGTGAAAGCTTGC	
HGC1-NF	TGTATGTGGATAGGGAAAAG	
HGC1-NR	ATTCTCTCTCTCTCTCGTTC	
OP4(5') FWD:	AACTGTGCCTGCACATAAAC	For construction of Op4p controlled cartTA expression strains
OP4(5') REV:	CTTTTATCTAATCTAGACATTATTTGTATGTGTGTAGGAG	
CARTTA FWD:	ATGTCTAGATTAGATAAAAG	
CARTTA REV:	CACGGCGCGCCTAGCAGCGGCTATTTACAATCAAAGGTGGTC	
ARG4 FWD:	CCGCTGCTAGGCGCGCCGTGGTTTTCCCAGTCACGACGTT	
ARG4 REV:	GCAGGGATGCGGCCGCTGACTGTGGAATTGTGAGCGGATA	
OP4(3') FWD :	GTCAGCGGCCGCATCCCTGCCTCCAACTGTTGATACTTCTG	
OP4(3') REV:	GCAAAGCATAGAGTGATCTC	