Supporting Information

Lu et al. 10.1073/pnas.1318690111

SI Materials and Methods

Plasmid and Strain Construction. The *Candida albicans* strains used in this study are listed in Table S1. Primer sequences are listed in Table S2. A 1.9-kb PCR product (primers 1 and 2) containing the C-terminal *SOK1* coding region and a 0.9-kb PCR product (primers 5 and 6) containing the C-terminal *CUP9* coding region were inserted into the *BamH*I-*Mlu*I sites of pPR673 (1). These two plasmids were digested with PstI or SpeI, respectively, to target integration into their own loci to express Sok1-13Myc and Cup9-13Myc. The pMAL2-SOK1-13MYC, pMAL2-CUP9-13MYC, and pMAL2-MGT1-13MYC plasmids were constructed by amplifying *SOK1* (primers 3 and 4), *CUP9* (primers 7 and 8), and *MGT1* (primers 13 and 14) to replace *NRG1* from the pMAL2-NRG1-MYC (2) plasmid. The resulting plasmids were digested with *Asc*I to target integration into *ADE2* locus to express Sok1-13Myc, Cup9-13Myc, and Mgt1-13Myc. The *SOK1* coding sequence was amplified using primers 15 and 16. The resulting PCR product was digested with *BamH*I and *Mlu*I and inserted into the *BamH*I-*Mlu*I site of pPR671-SAT1 (3) to create pACT1-SOK1-13MYC. *UBR1* was deleted based on the method described previously (4). The disruption was confirmed by PCR. *NRG1* was deleted based on *SAT1*-flipping strategy (5). Upstream (primers 9 and 10) and downstream (primers 11 and 12) sequences of *NRG1* were cloned as ApaI-XhoI and NotI-SacII fragments, respectively, on both sides of the *SAT1* flipper cassette to obtain the plasmid pSFS2-NRG1 for *NRG1* disruption. To disrupt the endogenous copies of *NRG1* in *sok1*/*sok1* and *tpk2*/*tpk2* mutant strains, pSFS2-NRG1 was linearized using ApaI and SacII, which was followed by two sequential rounds of transformation, selection, and recycling of the *SAT1* marker as described in the work by Reuss et al. (5).

Quantitative PCR Expression Analysis. Methods for RNA isolation were carried out as previously described (6); 10 μg total RNA were DNase-treated at 37 °C for 1 h using the RNase-Free DNase Kit (Qiagen). cDNA was synthesized using the SuperScript II Reverse Transcriptase Kit (Invitrogen), and quantitative PCR was done using iQ SYBR Green Supermix (Bio-Rad).

1. Lu Y, et al. (2008) Efg1-mediated recruitment of NuA4 to promoters is required for hypha-specific Swi/Snf binding and activation in *Candida albicans*. *Mol Biol Cell* 19(10): 4260–4272.
2. Lu Y, Su C, Wang A, Liu H (2011) Hyphal development in *Candida albicans* requires two temporally linked changes in promoter chromatin for initiation and maintenance. *PLoS Biol* 9(7):e1001105.
3. Lu Y, Su C, Solis NV, Filler SG, Liu H (2013) Synergistic regulation of hyphal elongation by hypoxia, $CO_{(2)}$, and nutrient conditions controls the virulence of *Candida albicans*. *Cell Host Microbe* 14(5):499–509.
4. Wilson RB, Davis D, Mitchell AP (1999) Rapid hypothesis testing with *Candida albicans* through gene disruption with short homology regions. *J Bacteriol* 181(6):1868–1874.
5. Reuss O, Vik A, Kolter R, Morschhäuser J (2004) The SAT1 flipper, an optimized tool for gene disruption in *Candida albicans*. *Gene* 341:119–127.
6. Lane S, Birse C, Zhou S, Matson R, Liu H (2001) DNA array studies demonstrate convergent regulation of virulence factors by Cph1, Cph2, and Efg1 in *Candida albicans*. *J Biol Chem* 276(52):48988–48996.

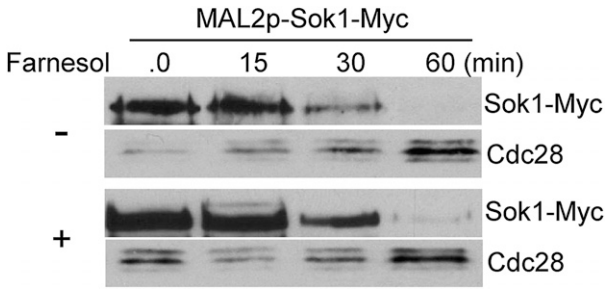

Fig. S1. The protein stability of Sok1 is not regulated by farnesol. Sok1 stability is monitored by *MAL2* promoter shutdown. Western blot of WT cells carrying Sok1-Myc under the *MAL2* promoter inoculated from overnight culture into fresh yeast extract peptone dextrose (YPD) medium at 30 °C with or without 100 μM farnesol.

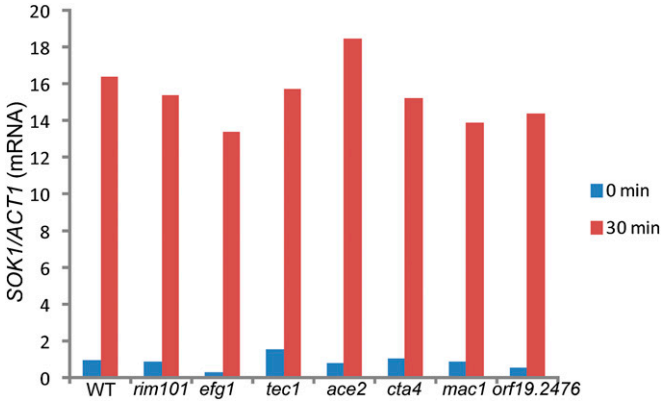

Fig. S2. The mutants defective in germ-tube formation show no defect in activation of *SOK1* expression. Quantitative RT-PCR analysis of *SOK1* expression in WT or indicated mutants. Cells were diluted into prewarmed YPD medium at 37 °C for 30 min. *SOK1* mRNA levels were determined as described in Fig. 4*B*. The 0 h normalized value of *SOK1/ACT1* for the WT was set to be 1.00.

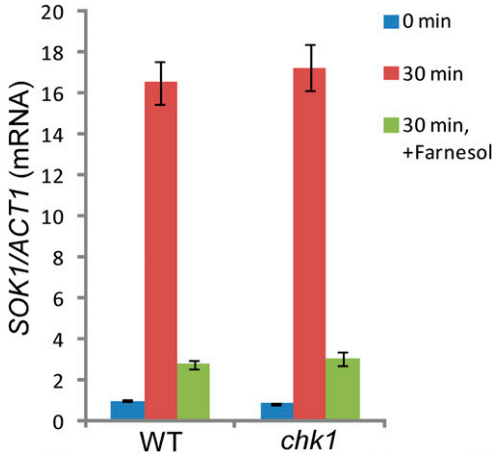

Fig. S3. Deletion of *CHK1* has no effect on *SOK1* expression. Quantitative RT-PCR analysis of *SOK1* expression in WT or *chk1* mutant. Cells were diluted into prewarmed YPD medium at 37 °C in the presence or absence of 100 μM farnesol. *SOK1* mRNA levels were determined as described in Fig. 4*B*. The 0 h normalized value of *SOK1/ACT1* for the WT was set to be 1.00.

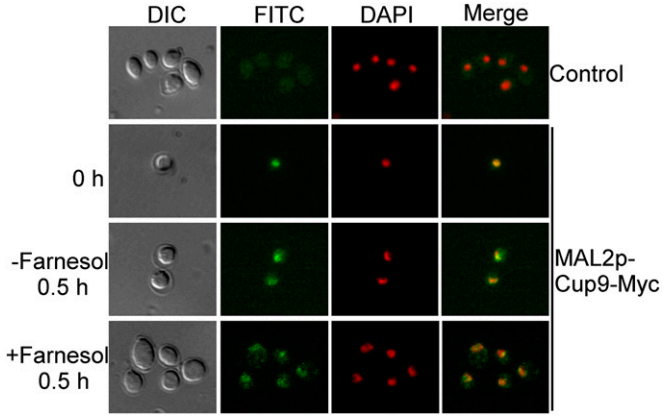

Fig. S4. Cup9 is constitutively localized in the nucleus. WT strain expressing Cup9-Myc under the *MAL2* promoter was grown in YPD medium at 37 °C in the presence or absence of 100 μM farnesol. Cells were fixed and processed for indirect immunofluorescence as described (1) with 9E10 mouse antibodies and FITC-conjugated secondary antibodies. DNA was stained with DAPI. An untagged control (SC5314) was included.

1. Wang A, et al. (2007) Temporal and spatial control of *HGC1* expression results in Hgc1 localization to the apical cells of hyphae in Candida albicans. *Eukaryot Cell* 6(2):253–261.

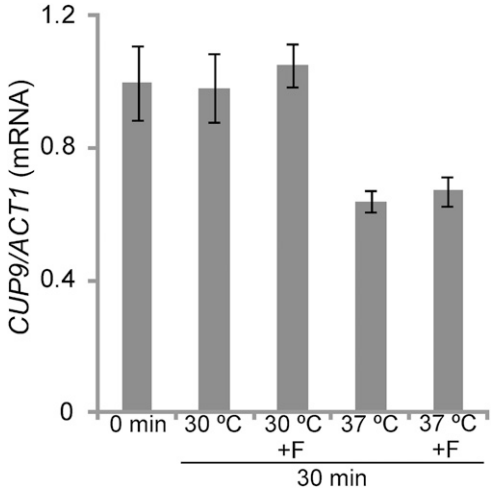

Fig. S5. Farnesol does not regulate the expression levels of *CUP9*. Quantitative RT-PCR analysis of *CUP9* expression in WT cells under the same growth condition as described in Fig. 4*C*. F, farnesol.

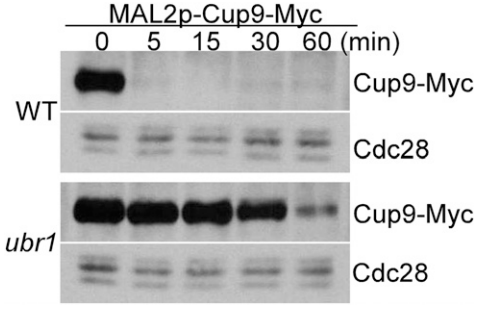

Fig. S6. Cup9 is degraded rapidly by Ubr1 in response to release from farnesol inhibition. WT and *ubr1* mutant cells carrying Cup9-Myc under the *MAL2* promoter were grown in yeast extract–peptone plus 2% maltose medium overnight; 2% glucose was added into the overnight cultures to shut off the promoter for 20 min. The overnight cultures were then inoculated into fresh YPD media at 30 °C. The protein degradation of Cup9 was assessed as described in Fig. 5*A*.

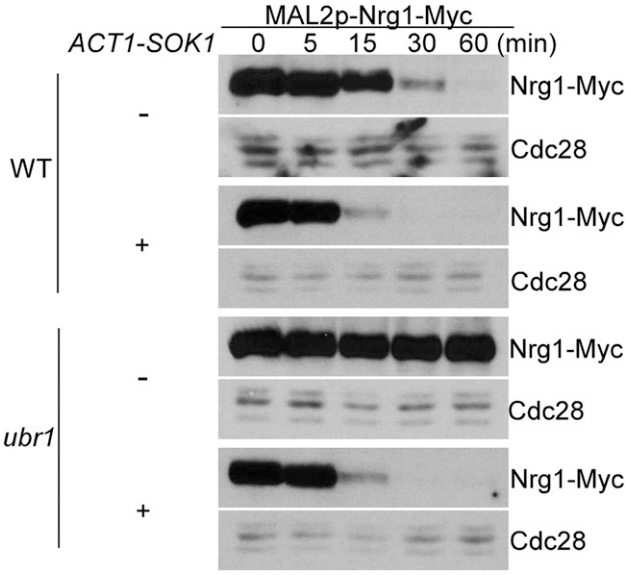

Fig. S7. Ubr1 is not directly responsible for the degradation of Nrg1. A Western blot of WT and *ubr1* mutant cells carrying Cup9-Myc under the *MAL2* promoter with or without *ACT1* promoter-driven *SOK1* inoculated from overnight cultures into fresh YPD media at 30 °C.

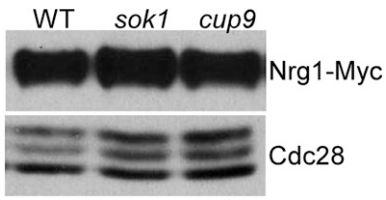

Fig. S8. There is no dramatic change in Nrg1 protein level in overnight culture of *sok1* or *cup9* mutant compared with that in WT cells.

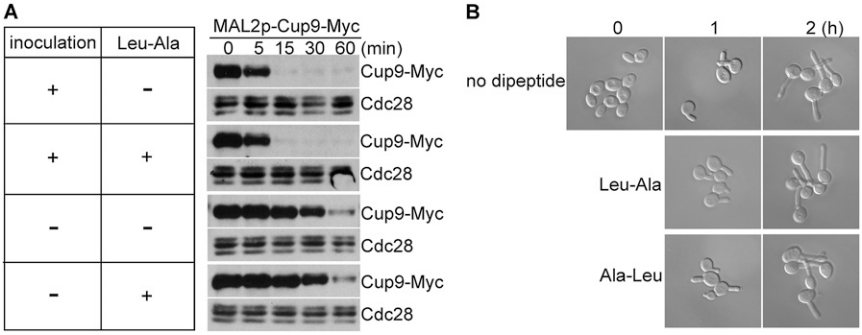

Fig. S9. Dipeptides have no effect on Cup9 degradation and germ-tube formation in *C. albicans*. (*A*) Cup9 stability is monitored by *MAL2* promoter shutdown. For the experiments with inoculation, WT cells carrying Cup9-Myc under the *MAL2* promoter were inoculated from overnight culture into fresh minimal medium of 2% glucose, 0.5% allantoin, and 0.17% yeast nitrogen base (YNB) at 30 °C with or without 10 mM Leu-Ala. For the experiments without inoculation, WT cells containing Cup9-Myc under the *MAL2* promoter were grown in minimal medium of 2% maltose, 0.5% allantoin, and 0.17% YNB with or without 10 mM Leu-Ala for 4 h to induce the expression of Cup9-Myc at 30 °C. Glucose (2%) was added to shut off the promoter. (*B*) Overnight culture of WT cells was diluted into fresh buffered minimal medium of 2% glucose, 0.5% allantoin, and 0.17% YNB (pH 7) with or without indicated dipeptides (10 mM) at 37 °C for cell morphology analysis.

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

Strain	Parent/ background	Genotype	Source
SC5314		WT	1
CAI4	SC5314	*ura3::1 imm434/ura3::1 imm434*	1
BWP17	SC5314	*ura3::1 imm434/ura3::1 imm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG*	2
HLY3922	CAI4	*ura3::1 imm434/ura3::1 imm434 NRG1/NRG1-13MYC-URA3*	3
CR276	CAI4	*ura3::1 imm434/ura3::1 imm434 cyr1::hisG/cyr1::hisG*	4
AS1	CAI4	*ura3::1 imm434/ura3::1 imm434 tpk2::hisG/tpk2::hisG*	5
HLY 4031	CAI4	*ura3::1 imm434/ura3::1 imm434 ADE2/ade2::MAL2p-NRG1-13MYC-URA3*	3
HLY4178	CAI4	*ura3::1 imm434/ura3::1 imm434 SOK1/SOK1-13MYC-URA3*	This study
HLY4179	CAI4	*ura3::1 imm434/ura3::1 imm434 CUP9/CUP9-13MYC-URA3*	This study
HLY4080	BWP17	*ubr1::ARG4/ubr1::HIS1 ura3::1 imm434/ura3::1 imm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG*	This study
HLY4278	CAI4	*ura3::1 imm434/ura3::1 imm434 ADE2/ade2::MAL2p-MGT1-13MYC-URA3*	This study
HLY4279	HLY4031	*ura3::1 imm434/ura3::1 imm434 ADE2/ade2::MAL2p-NRG1-13MYC-URA3 RP10::ACT1p-SOK1-13MYC-SAT1*	This study

1. Fonzi WA, Irwin MY (1993) Isogenic strain construction and gene mapping in *Candida albicans*. *Genetics* 134(3):717–728.
2. Wilson RB, Davis D, Mitchell AP (1999) Rapid hypothesis testing with *Candida albicans* through gene disruption with short homology regions. *J Bacteriol* 181(6):1868–1874.
3. Lu Y, Su C, Wang A, Liu H (2011) Hyphal development in *Candida albicans* requires two temporally linked changes in promoter chromatin for initiation and maintenance. *PLoS Biol* 9(7): e1001105.
4. Rocha CR, et al. (2001) Signaling through adenylyl cyclase is essential for hyphal growth and virulence in the pathogenic fungus *Candida albicans*. *Mol Biol Cell* 12(11):3631–3643.
5. Sonneborn A, et al. (2000) Protein kinase A encoded by *TPK2* regulates dimorphism of *Candida albicans*. *Mol Microbiol* 35(2):386–396.

Table S2. Primers used in this study

Primer	Sequence	Purpose and feature
1	5′-CGGGATCCCCAAGTGCATTTTATCCTCATC	pPR673-SOK1
2	5′-GGCGACGCGTCGTGATAACATACTTAATGGGGC	
3	5′-CTGTCTAGAATGACTTCAACAAATGGACA	pMAL2-SOK1-MYC
4	5′-CTGACGCGTTGATAACATACTTAATGGGGC	
5	5′-CGGGATCCCCTCTGGTAATGCTCCATTGC	pPR673-CUP9
6	5′-GGCGACGCGTCGAAAGTTCAAACGCTGTTGTTG	
7	5′-CTGTCTAGAATGAAGTTAGATCAATTAATTA	pMAL2-CUP9-MYC
8	5′-CTGACGCGTAAAGTTCAAACGCTGTTGTTG	
9	5′-CTAGGGCCCTAGTGCTCACTATACTTG	pSFS2-NRG1
10	5′-GTACTCGAGCTTAATGAAACTAGCAGG	
11	5′-ATAAGAATGCGGCCGCCCTAGTATAGATGTGGTC	
12	5′-TCCCCGCGGGATCTATGGCAATGCAAG	
13	5′-CTGTCTAGAATGTCTAACCTATATTATACTG	pMAL2-MGT1-MYC
14	5′-CTGACGCGTAATACCTATACCTTCCTGCTTC	
15	5′-CGGGATCCCATGACTTCAACAAATGGACA	pPR671-SOK1-SAT1
16	5′-GGCGACGCGTCGTGATAACATACTTAATGGGGC	

Restriction sites are underlined.