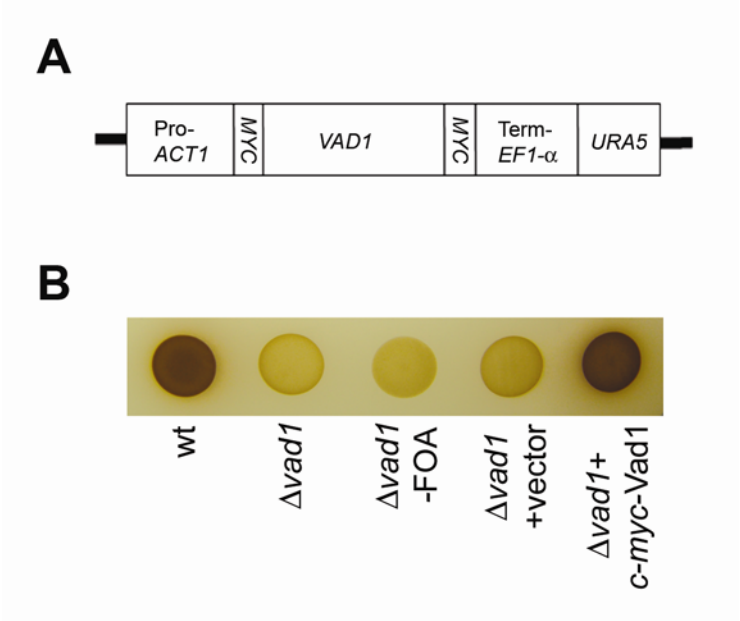

Figure S1. Expression of *c-myc*-tagged Vad1 in *C. neoformans*. (A) Scheme of expression construct of *c-myc*-Vad1 fusion protein. (B) Laccase expression was determined by melanin production. Cells were spotted onto minimal media containing norepinephrine and incubated for 2 d at 30°C.

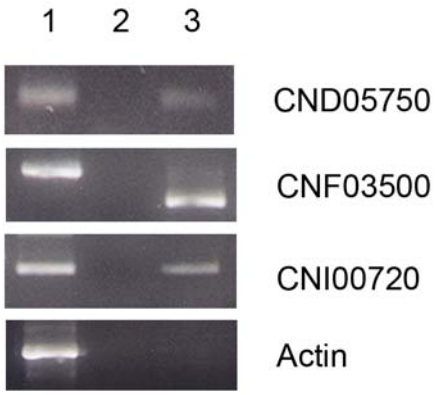

Figure S2. Reverse transcriptase-PCR analysis of mRNA after RNA immunoprecipitation. Photograph showing the amplified products in a 1% agarose gel stained with ethidium bromide. Input, 1; genomic DNA, 2; H99-RIP (control), 3; myc-Vad1-RIP. CND05750; MFα1, CNF03500; cell wall organization and biogenesis putative, CNI00720; transporter putative.

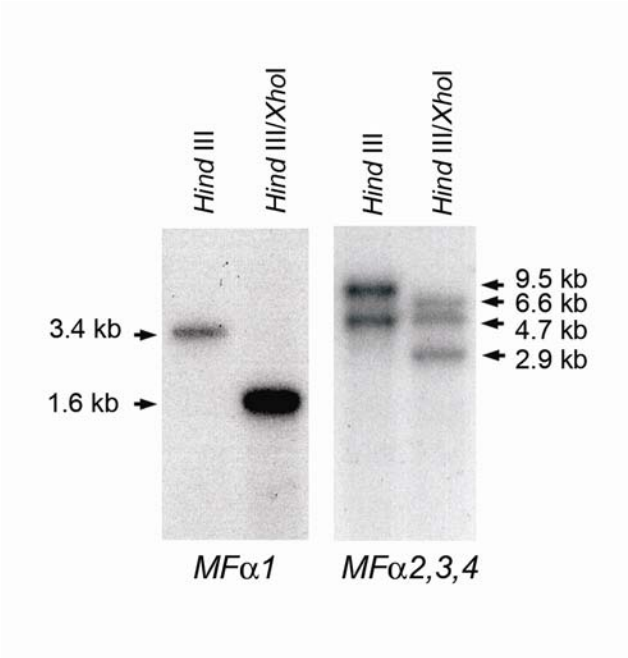

Figure S3. Southern blot analysis of *MFα* genes of *C. neoformans*. Southern blot analysis of wild type DNA using the indicated enzymes, hybridized with *MFα1*- (left panel) or *MFα2,3,4*-specific probes (right panel) consisting of a 200-bp region of the 3'UTR of *MFα1* or *MFα2*, respectively.

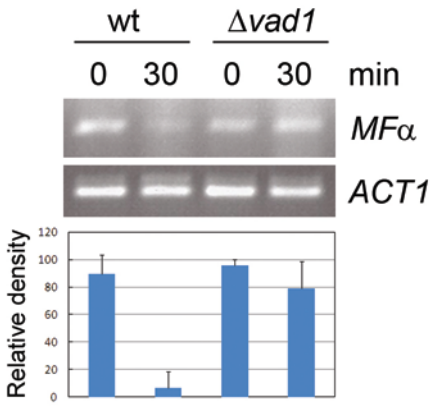

Figure S4. **Capped RNAs accumulate in a Δ*vad1* mutant.** RNA Ligase Mediated Rapid Amplication of cDNA ENDs (RLM-RACE) analysis was performed with total RNA isolated from the indicated strains at the indicated times after transcription repression by 1,10-phenanthroline by using the FirstChoice RLM-RACE kit according to manufacturer's protocol (Applied Biosystems). PCR amplification was performed by using the gene specific primer MF-mid-a (Supplementary Table 3) and a universal primer (supplied by the manufacturer) for *MFα*, The actin gene, *ACT1*, was used as loading control using primers ACT-70 and ACT-a (Supplementary Table 3). This analysis was performed in three independent experiments, and *MFα/ACT1* ratios were calculated for the indicated strains by densitometry (UN-SCAN-IT gel 6.1, Silk Scientific Corporation).

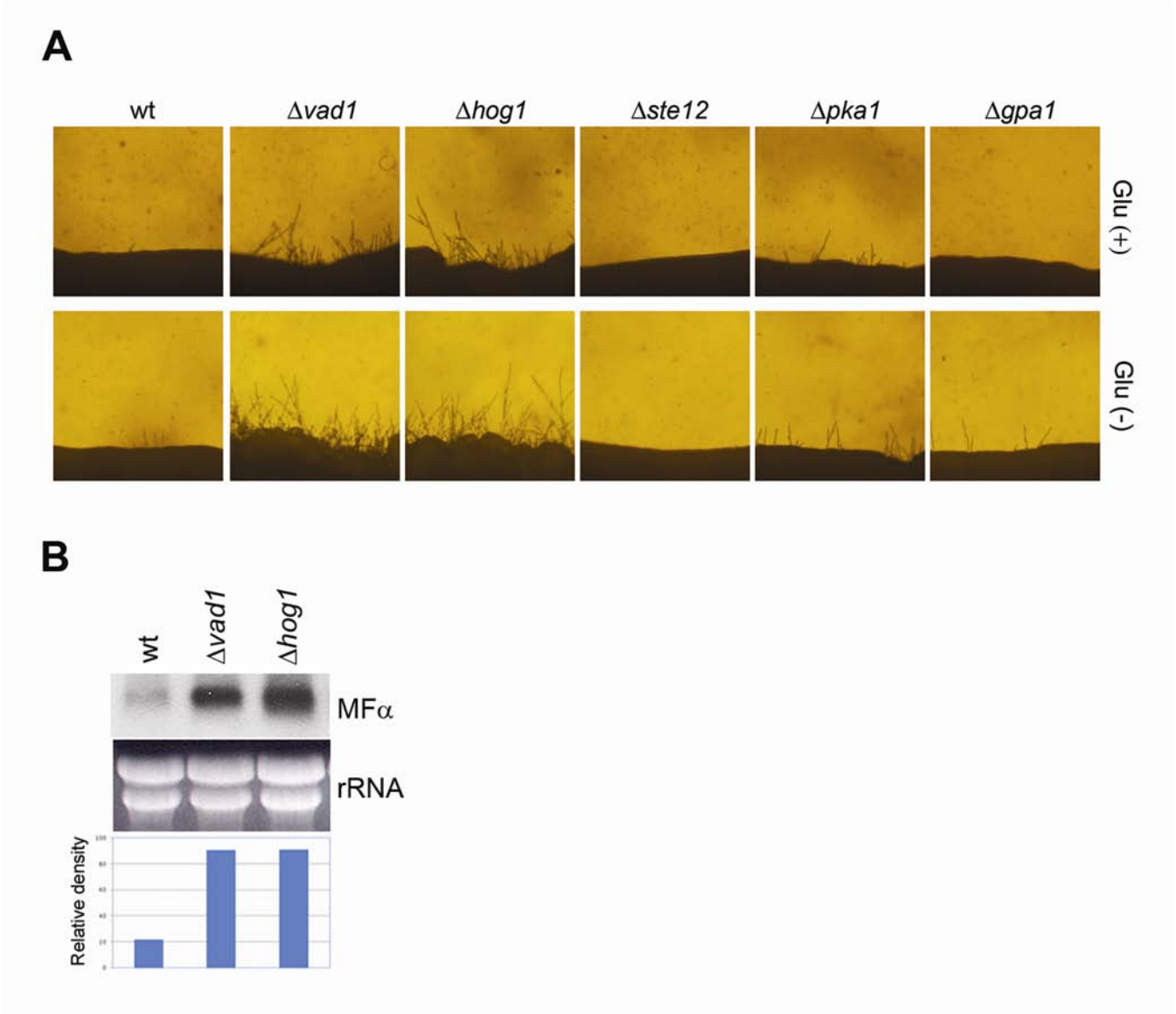

Figure S5. **Comparison of mating phenotypes and *MFα* transcript levels of Δ*vad1* and signal transduction mutant strains.** (A) Indicated strains were cocultured on V8 mating media with or without glucose and incubated for 4 d at room temperature in the dark. Representative edges of the mating patches were photographed at 40x magnification. (B) Northern blot analysis was performed with total RNA isolated from wild type or Δ*hog1* strains grown to mid-log phase. The blot was hybridized with the coding region from *MFα*. The graph demonstrates relative quantitative measurement of transcript levels of *MFα* normalized to the rRNA loading control by UN-SCAN-IT-GEL 6.1 (Silk Scientific).

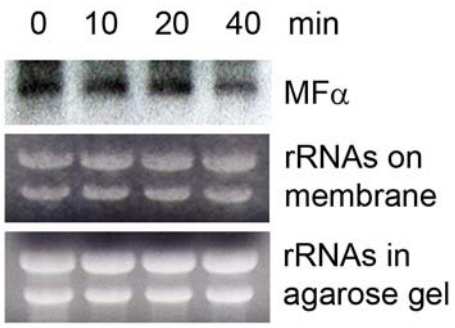

Figure S6. Hog1 does not alter stability of *MFα* mRNA in Δ*vad1* mutant cells. Northern blot analysis was performed with total RNA isolated from a Δ*vad1 + HOG1* strain. The blot was hybridized with an ORF region from *MFα* genes (at the indicated time after transcriptional repression by 1,10-phenanthroline). rRNAs levels were used as loading and transfer control within gels and membranes, respectively and visualized by ethidium bromide.

Supplementary Table S1. Strains used in this study. All of strains are serotype A of *C. neoformans*.

Name	Genotype	Reference
H99	*MATα*	Perfect et al, 1993
KN99	*MAT***a**	Nielsen et al, 2003
VAD1	*MATα vad1Δ::URA5 ura5*	Panepinto et al, 2005
YP513	*MATα ura5* (5-FOA^r)	This study
YP510	*MATα vad1Δ ura5* (5-FOA^r)	This study
YP501	*MATα vad1Δ ura5 ACT1*$_{pro}$*-MYC-VAD1-MYC-EF1α*$_{ter}$ *URA5*	This study
YP503	*MATα ura5* empty vector (*URA5*)	This study
YP519	*MATα vad1Δ ura5* empty vector (*URA5*)	This study
YP514	*MATα ura5 ACT1*$_{pro}$*-MFα1-EF1α*$_{ter}$ *URA5*	This study
YP515	*MATα ura5 ACT1*$_{pro}$*-MFα1-3'UTR*$_{ter}$ *URA5*	This study
YP532	*MATα ura5 GPD1*$_{pro}$*-GFP-MFα1-3'UTR*$_{ter}$ *URA5*	This study
YP533	*MATα vad1Δ ura5 GPD1*$_{pro}$*-GFP-MFα1-3'UTR*$_{ter}$ *URA5*	This study
YP527	*MATα ura5 GPD1*$_{pro}$*-MFα1 5'UTR#1-GFP-EF1α*$_{ter}$ *URA5*	This study
YP530	*MATα ura5 GPD1*$_{pro}$*-MFα1 5'UTR#4-GFP-EF1α*$_{ter}$ *URA5*	This study
YP538	*MATα ura5 GPD1*$_{pro}$*-MFα1 5'UTR#1-GFP-MFα1-3'UTR*$_{ter}$ *URA5*	This study
YP539	*MATα vad1Δ ura5 GPD1*$_{pro}$*-MFα1 5'UTR#1-GFP-MFα1-3'UTR*$_{ter}$ *URA5*	This study
STE12	*MATα ste12Δ::ADE2 ade2*	Yue et al, 1999
CDC40	*MATα pka1Δ::ADE2 ade2*	Hicks et al, 2004
YSB83	*MATα gpa1Δ::NAT-STM#177*	Bahn et al, 2004
YSB121	*MAT***a** *NEO*	Bahn et al, 2004
YP548	*MATα ura5 NAT* empty vector (*URA5*)	This study
YP549	*MATα ura5 NAT ACT1-VAD1 URA5*	This study
YSB64	*MATα hog1Δ::NAT-STM#177*	Bahn et al, 2005
YSB81	*MAT***a** *hog1Δ::NEO*	Bahn et al, 2005
YP508	*MATα hog1Δ::NAT-STM#177 ura5* (5-FOA^r)	This study
YP521	*MATα hog1Δ::NAT-STM#177 VAD1-URA5*	This study
YP522	*MATα hog1Δ::NAT-STM#177 ura5* empty vector (*URA5*)	This study
YP540	*MATα vad1Δ ura5 ACT1*$_{pro}$*-HOG1 URA5*	This study

Supplementary Table S2. Complete list of target genes showing Vad1 binding by RNA Immunoprecipitation.

Locus	Name	SDabove[a]	p-Value[b]
CNA05770	hypothetical protein	53.2	0
CND04720	hypothetical protein	34.5	0
CNB00010	hypothetical protein	23.6	0
CND05750	pheromone alpha	18.4	0
CNE00990	ubiquinone biosynthesis-related protein putative	16.7	0
CNM00900	conserved hypothetical protein	16.1	0
CNG01120	expressed protein	14.3	0
CNB03820	expressed protein	12.7	0
CND05690	MFalpha3	12.2	0
CNF03500	cell wall organization and biogenesis-related protein putative	12.0	0
CNJ00710	hypothetical protein	11.1	0
CND00920	mitochondrion protein putative	9.9	0
CNI02610	hypothetical protein	9.7	0
CNI03530	expressed protein	9.0	0
CNAG07413	171 3.seq.159 start:391516 end:392957 strand + 440	8.9	0
CND03890	asparagine-tRNA ligase putative	8.1	0
CNA05980	cytoplasm protein putative	7.8	1.87E-12
CNL06420	conserved hypothetical protein	6.7	4.08E-09
CNE04100	cyclin-dependent protein kinase regulator putative	6.3	5.06E-08
CNA05980	cytoplasm protein putative	6.3	5.06E-08
CNN02220	Epoxide hydrolase 1 (EC 3.3.2.3) putative	6.1	1.78E-07
CNG04690	hypothetical protein	6.0	3.75E-07
CNA04180	hypothetical protein	5.5	6.61E-06
CNC04320	conserved hypothetical protein	5.4	8.22E-06
CNM01490	hypothetical protein	5.4	8.42E-06
CNI01880	ATP-dependent RNA helicase putative	5.2	2.62E-05
CNA05270	conserved hypothetical protein	4.4	0.001509
CNF04720	pim1 protein (poly(a)+ RNA transport protein 2) putative	4.0	0.009513
CNAG00597	170 3.seq.175 start:417948 end:418924 strand - 36	4.0	0.009513
CNL05600	pyruvate dehydrogenase e1 component beta subunit mitochondrial precursor (ec 1.2.4.1) putative	3.7	0.024502
CNAG04006	171 3.seq.157 start:385200 end:388284 strand + 54	3.7	0.028622
CND01090	hypothetical protein	3.6	0.038358

[a]SDabove indicates the number of standard deviations (based on 4 microarray analyses) that the indicated gene signal was above background signal, the later defined as the mean level of signal of all genes in the immunoprecipitated RNA sample. [b]p-Values were corrected for False Discovery Rate (FDR) using the Benjamini-Hochberg method.

Supplementary Table S3. Primers used in this study.

Name	Sequence (5' to 3')[a]	Restriction enzyme	Relative strain or purpose
myc-VAD1-S[b]	GGAGGACAATTGATG*GAGCAGAAGCTCATAAGCGAGGA GGACCTC* **ATGGCTTCTTCCTCAACGTAAGCC**	*Mun* I	YP101
myc-VAD1-A[b]	GTGGTCAATTGTTA*GAGG TCCTCCTCG CTTATG AGC TTC TGC TCG GCTTGAG* **CCTGTTGGCTCTGGCTTGCACCG**	*Mun* I	YP101
MFα1-ORF-S1	ATATAAGAATTCCGACGCCTTCACTGCCATCTTC	*Eco* RI	YP104, YP108
MFα1-ORF-A	CCGTATGAATTCTTAGGCGATGACACAAAG	*Eco* RI	YP104
MFα1-3'UTR-A	AATATTACCGGTTGCTTGAGTGACGGATGC	*Age* I	YP108, YP119, YP126
MFα1-3'UTR-NS	GTTCCCAAGCAGTCAAGCCG		Southern & northern
MFα1-3'UTR-NA	GAACAAGGCGATGTTGGCTG		Southern & northern
MFα2-3'UTR-NS	ACATCCCCTCTGCGTGTTAG		Southern & northern
MFα2-3'UTR-NA	AGTGGGGTTCCGATTGAGTC		Southern & northern
ACT1-70	ACCGCGTCTCCTAATATGC		RT-PCR
ACT1-a	CATGAATTCAGACATGTTGGGCGAG		PCR
MF-mid-a	GTTGCGAGGGACTTCAGAAG		PCR
MFα1-A	TTAGGCGATGACACAAAG		RT-PCR
MFα1-5'UTR-S	GCGGAGAATTCTTCAACGTGTTCTATTG	*Eco* RI	YP111, YP114, YP126
MFα1-5'UTR-A4	GGCAGCGAATTCCTTGGAACTTTTTATATG	*Eco* RI	YP114
MFα1-5'UTR-A1	GATATAGAATTCTGTAGCGGATTATTGAGTG	*Eco* RI	YP111
MFα1-ORF-S2	TTATGTAGATCTATGGACGCCTTCACTTCCATC	*Bgl* II	YP119, YP126
GFP-S	GAAATTGAATTCCATGTCCAAGGGTGAGGAGCTC	*Eco* RI	YP111, YP114, YP119, YP126
GFP-A	ATATATAGATCTGAGGTCCTCCTCGGAGATGAG	*Bgl* II	YP111, YP114, YP119, YP126
HOG1-S	ATATAAGAATTCGGCCGATTTTGTCAAGCTCTCC	*Eco* RI	YP127
HOG1-A	ATATATGAATTCTTAGCTGGCAGGAGCAGCG	*Eco* RI	YP127

[a]Restriction site is underlined.
[b]c-myc and VAD1 are in italic and bold types, respectively.

SUPPLEMENTARY REFERENCE

Perfect, J.R., Toffaletti, D.L., and Rude, T.H. 1993. The gene encoding phosphoribosylaminoimidazole carboxylase (*ADE2*) is essential for growth of *Cryptococcus neoformans* in cerebrospinal fluid. Infect Immun **61**(10): 444

Nielsen, K., Cox, G.M., Wang, P., Toffaletti, D.L., Perfect, J.R., and Heitman, J. 2003. Sexual Cycle of *Cryptococcus neoformans* var. *grubii* and Virulence of Congenic a and {alpha} Isolates. Infect Immun **71**(9): 4831-4841.

Yue, C., Cavallo, L.M., Alspaugh, J.A., Wang, P., Cox, G.M., Perfect, J.R., and Heitman, J. 1999. The STE12{alpha} Homolog Is Required for Haploid Filamentation But Largely Dispensable for Mating and Virulence in *Cryptococcus neoformans*. Genetics **153**(4): 1601-1615.