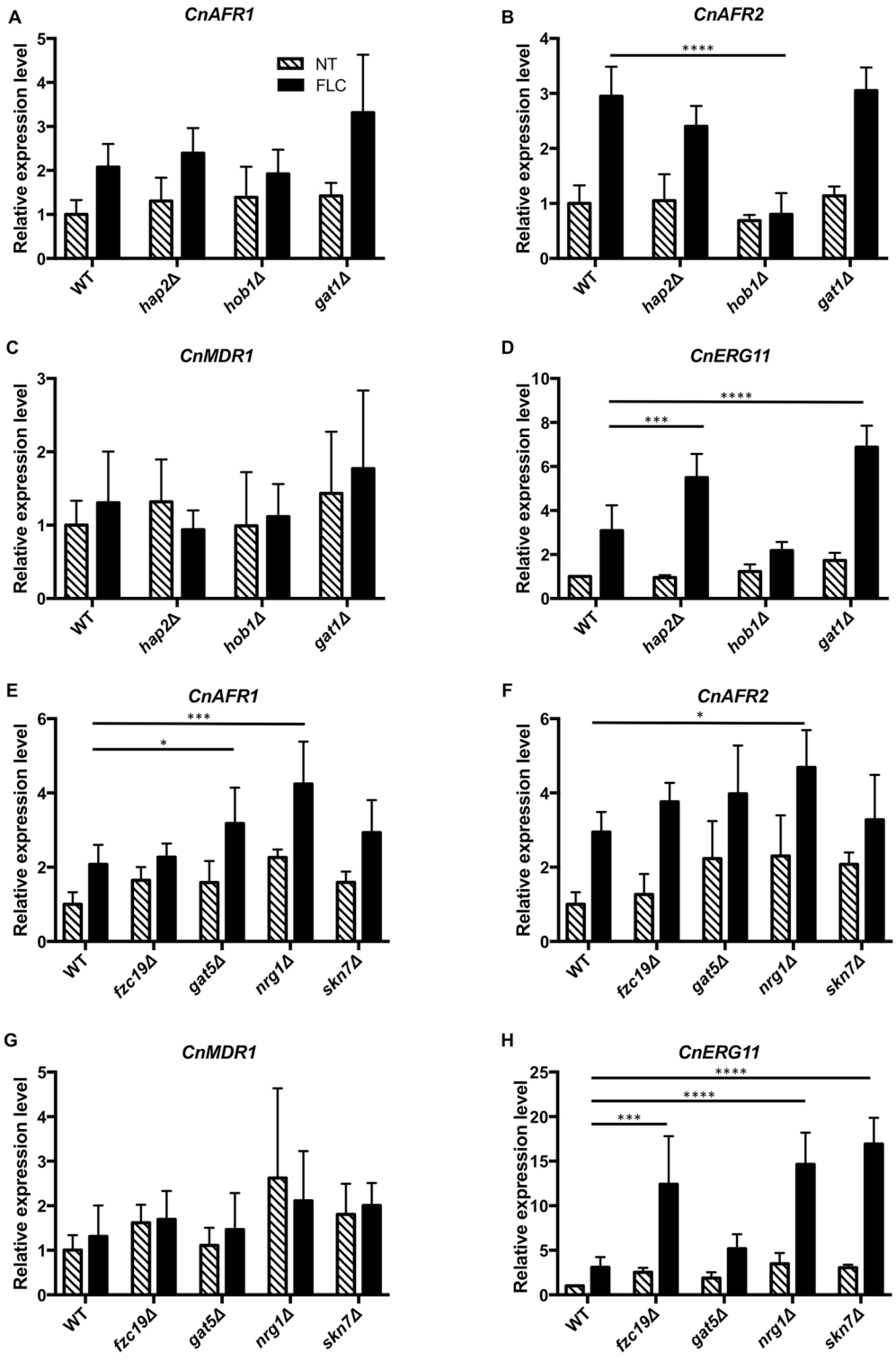

Fig. S1. The expression levels of *AFR1*, *AFR2*, *MDR1* and *ERG11* in the FLC resistance mutants (A-D) and mutants with increased frequency of FLC heteroresistance (E-H). The exponential growth phase cells of transcription factor mutant strains were either untreated (NT) or treated (FLC) with 32g/ml FLC for 2h. RNA was isolated and the expression levels of each gene were determined by RT-qPCR. The expression levels of each gene were normalized with that of actin gene and compared to the wild type without FLC treatment. Values represent the means ± standard deviation of three biological replicates. * represents $p< 0.0476$; *** represents $p< 0.0006$; **** represents $p< 0.0001$ (uncorrected Fisher's LSD)

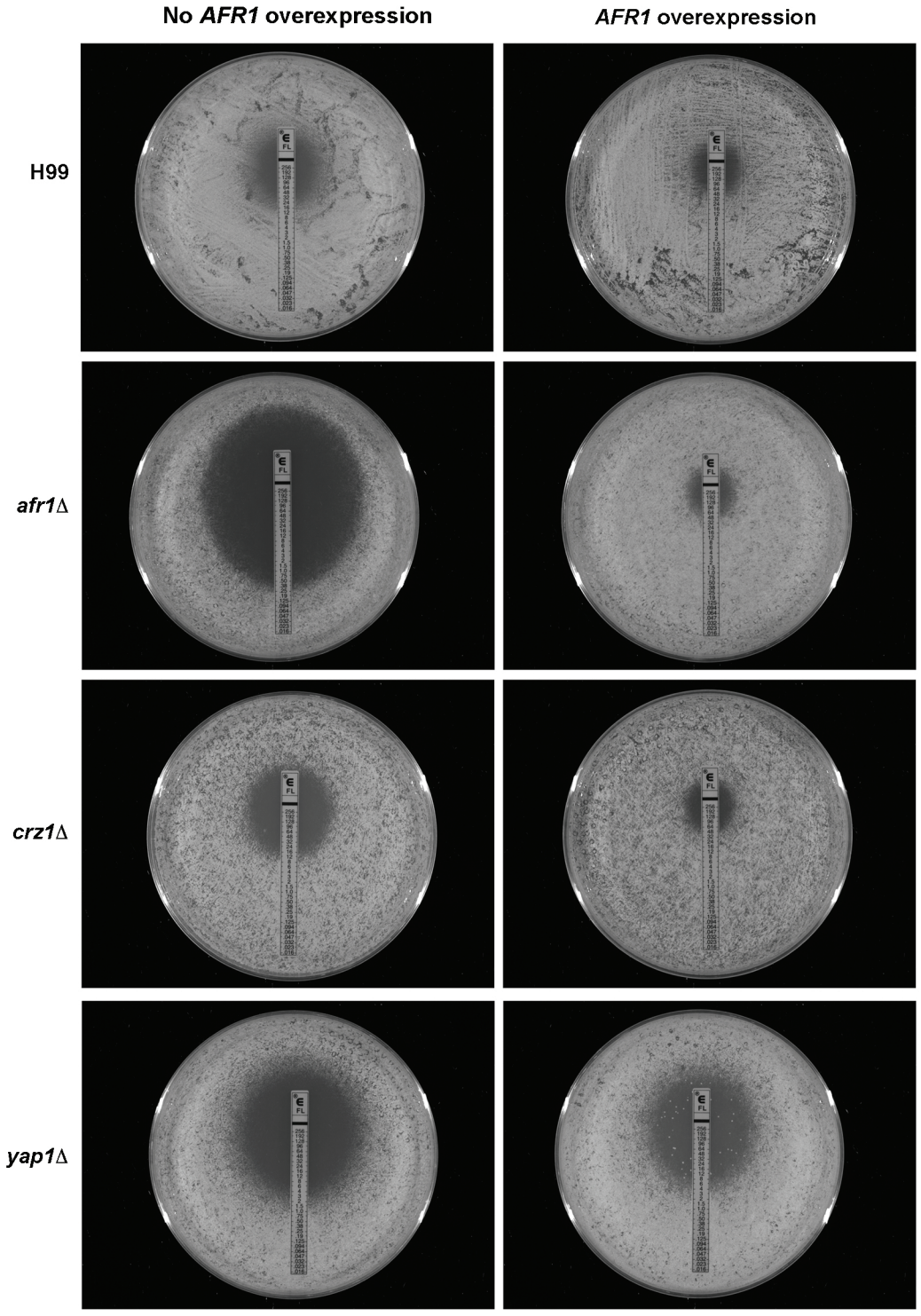

Fig. S2. Overexpression of *AFR1* results in a decrease of susceptibility to FLC. *AFR1* was placed under the control of *GPD1* promoter and transformed into indicated strains. E-test was used to measure the FLC susceptibility and photographs were taken after 3 days of incubation.

Table S1. List of strains used in this study.

Strain	Genotype	Parent	Reference
H99 (*C. neoformans*)	MATα serotype A		(1)
afr1Δ	*MATα afr1Δ::NEO*	H99	(2)
afr2Δ	*MATα afr2Δ::NEO*	H99	This study
mdr1Δ	*MATα mdr1Δ::NEO*	H99	This study
afr1Δ/afr2Δ	*MATα afr1Δ::NEO afr2Δ::HYG*	H99	This study
afr1Δ/mdr1Δ	*MATα afr1Δ::NEO mdr1Δ::NAT*	H99	This study
afr2Δ/mdr1Δ	*MATα afr2Δ::NEO mdr1Δ::NAT*	H99	This study
afr1Δ/afr2Δ/mdr1Δ	*MATα afr1Δ::NEO afr2Δ::HYG mdr1Δ::NAT*	H99	This study
YSB815	*MATα yap1Δ::NAT*	H99	Y. Bahn
YSB1416	*MATα yap2Δ::NAT*	H99	Y. Bahn
YSB1099	*MATα bzp3Δ::NAT*	H99	Y. Bahn
YSB1249	*MATα pip2Δ::NAT*	H99	Y. Bahn
YSB1263	*MATα crz1Δ::NAT*	H99	Y. Bahn
YSB2493	*MATα sre1Δ::NAT*	H99	Y. Bahn
YSB2622	*MATα zfc2Δ::NAT*	H99	Y. Bahn
YSB2702	*MATα bzp2Δ::NAT*	H99	Y. Bahn
YSB1104	*MATα hap2Δ::NAT*	H99	Y. Bahn
YSB2308	*MATα hob1Δ::NAT*	H99	Y. Bahn
YSB2972	*MATα gat1Δ::NAT*	H99	Y. Bahn
YSB3096	*MATα nrg1Δ::NAT*	H99	Y. Bahn
R265 (*C. gattii*)	MATα VGII serotype B		(3)
afr1Δ	*MATα afr1Δ::NEO*	R265	This study
afr2Δ	*MATα afr2Δ::NEO*	R265	This study
mdr1Δ	*MATα mdr1Δ::NEO*	R265	This study
afr1Δ/afr2Δ	*MATα afr1Δ::NEO afr2Δ::NAT*	R265	This study
afr1Δ/mdr1Δ	*MATα afr1Δ::NEO mdr1Δ::NAT*	R265	This study
afr2Δ/mdr1Δ	*MATα afr2Δ::NEO mdr1Δ::NAT*	R265	This study
afr1Δ/afr2Δ/mdr1Δ	*MATα afr1Δ::NEO afr2Δ::NAT mdr1Δ::HYG*	R265	This study

1. **Perfect JR, Ketabchi N, Cox GM, Ingram CW, Beiser CL.** 1993. Karyotyping of *Cryptococcus neoformans* as an epidemiological tool. J Clin Microbiol **31:**3305-3309.
2. **Sionov E, Lee H, Chang YC, Kwon-Chung KJ.** 2010. *Cryptococcus neoformans* overcomes stress of azole drugs by formation of disomy in specific multiple chromosomes. PLoS Pathog **6:**e1000848.
3. **Kidd SE, Hagen F, Tscharke RL, Huynh M, Bartlett KH, Fyfe M, Macdougall L, Boekhout T, Kwon-Chung KJ, Meyer W.** 2004. A rare genotype of *Cryptococcus gattii* caused the cryptococcosis outbreak on Vancouver Island (British Columbia, Canada). Proc Natl Acad Sci U S A **101:**17258-17263.

Table S2. Primers used in this study.

Primer name	Sequence (5' to 3')	Purpose
CNAG_AFR2_A	CAAATTCGAGGAGCTTGGAG	PCR for deletion
CNAG_AFR2_B	GCTAGTTTCTACATCTCTTCCGTGCGGTCGTAAGTACCCAAACC	PCR for deletion
CNAG_AFR2_C	CGCCGCTCTCCAGCTCACATCCTCGTCTCGTTCCAGCGAAAGAC	PCR for deletion
CNAG_AFR2_D	GAAAAGGGCCTATGCAATGA	PCR for deletion
CNAG_AFR2_E	TCTTGTCGTCATTCCCCTTC	PCR for deletion
CNAG_AFR2_F	TAGGTAAAGGGCCGAAAGGT	PCR for deletion
CNAG_MDR1_A	AAATGCGTAGGTGGCAAAAA	PCR for deletion
CNAG_MDR1_B	GCTAGTTTCTACATCTCTTCCGTGGCAGTGAGTCCTGGAGAAGC	PCR for deletion
CNAG_MDR1_C	CGCCGCTCTCCAGCTCACATCCTCCGCGAGGCAGGACTACTATC	PCR for deletion
CNAG_MDR1_D	TCGCAAAGTAGCAGTGAACAA	PCR for deletion
CNAG_MDR1_E	GCTCTGTTTGATGGATTTTGC	PCR for deletion
CNAG_MDR1_F	AACGAGTGCCACACTTAAAAAG	PCR for deletion
CNBG_AFR1_A	GCGACGACAGCAATAAATGA	PCR for deletion
CNBG_AFR1_B	GCTAGTTTCTACATCTCTTCCGTGCCTGACGGATTCTGAGTGGT	PCR for deletion
CNBG_AFR1_C	CGCCGCTCTCCAGCTCACATCCTCAACCAGGTGGAGAGATTTCG	PCR for deletion
CNBG_AFR1_D	CAACGTCATTGCTTTGATGG	PCR for deletion
CNBG_AFR1_E	GTCTTGGTCGGTTGGTGACT	PCR for deletion
CNBG_AFR1_F	GTTGCTTCGGGTGATCAAAT	PCR for deletion
CNBG_AFR2_A	GATTCCCCCTCAGAGCTTTC	PCR for deletion
CNBG_AFR2_B	GCTAGTTTCTACATCTCTTCCGTGACGGTTTTCTTCCGTTTGAG	PCR for deletion
CNBG_AFR2_C	CGCCGCTCTCCAGCTCACATCCTCCGGAGGACGTACAGGAGAAG	PCR for deletion
CNBG_AFR2_D	GGATGACGATAAACGGCAAT	PCR for deletion
CNBG_AFR2_E	GCCTATGCAGACGACTGTGA	PCR for deletion
CNBG_AFR2_F	TTCGATTGCCTCAAGATTCC	PCR for deletion
CNBG_MDR1_A	ATAATGCAGCGGTGGAAAAA	PCR for deletion
CNBG_MDR1_B	GCTAGTTTCTACATCTCTTCCGTGAGAATCATGGGCAAGAGCAT	PCR for deletion
CNBG_MDR1_C	CGCCGCTCTCCAGCTCACATCCTCGAGCATGGAACACACCAAGA	PCR for deletion
CNBG_MDR1_D	TGCAGATCAAAGCAACAGGT	PCR for deletion
CNBG_MDR1_E	CGAAGCAAGGAAAGATGGAG	PCR for deletion
CNBG_MDR1_F	TTCCGATCGATTACCGATGT	PCR for deletion
CNAG_AFR1_For	ACTGTCTGCTCGTGGGATAACTC	RT-PCR
CNAG_AFR1_Rev	GAAGTCGAAGAGATTTGGCGTAGT	RT-PCR
CNAG_AFR2_For	GGCCCATTTCACAGACACTCA	RT-PCR
CNAG_AFR2_Rev	CTTCGCGAGCACGGTGTT	RT-PCR
CNAG_MDR1_For	TCCCGGATGCGTCCAA	RT-PCR
CNAG_MDR1_Rev	CATTGTCGATGCTTCGGAAGA	RT-PCR
CNAG_ERG11_For	TGGCAAGACGCCAAAGTCT	RT-PCR
CNAG_ERG11_Rev	GGCGGCAAATCCCTTTTC	RT-PCR
CNBG_AFR1_For	GCCACTCAGCATGACGAACA	RT-PCR
CNBG_AFR1_Rev	GACCGACTTATCGCCCTCTTC	RT-PCR
CNBG_AFR2_For	CTGATTTTCTGCGGTGTATTGG	RT-PCR
CNBG_AFR2_Rev	AACGGCGAAACACGATACATAA	RT-PCR
CNBG_MDR1_For	CAAGCCGGAAACGTCTTCA	RT-PCR
CNBG_MDR1_Rev	TGGAAGCGGCCGATGA	RT-PCR
CNAG_Actin_For	CTTGCCTCTTTGTCTACCTTCCA	RT-PCR
CNAG_Actin_Rev	GGACGATTGAGGGACCAGACT	RT-PCR
CNBG_Actin_For	CGAGGGCGACCAACAATAGA	RT-PCR
CNBG_Actin_Rev	ATGTGCAAGGCTGGTTTCG	RT-PCR

Table S3. Primers used for qPCR assays.

Probe name	Chromosome location	Locus name	Primer forward	Probe
chr1A	Chr1	CNAG_00040	forward 5'-TGGCAAGACGCCAAAGTCT-3' reverse 5'-GGCGGCAAATCCCTTTTC-3'	AACCCTGCCCG ATGGCACGA
chr3A	Chr3	CNAG_02959	forward 5'-GAAGATGGCGAGCCTGACA-3' reverse 5'-CACTTCGAGCCTTCTTCTTCATG-3'	CACCCAGGAGG AGCTCGCCGA
chr5B	Chr5	CNAG_00976	forward 5'-CGGCTTTCTTTGTGTAAGTCTATCC-3' reverse 5'-CATACTGCATCGCCAAGATCA-3'	TGCTTTTCGCAC TCCAACTGTTTCCG