Two functionally redundant FK506-binding proteins regulate multidrug resistance gene expression and govern azole antifungal resistance

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Table S1: MIC80 of fluconazole (µg/ml) for wild-type and *Cgfpr*Δ strains.	
Strain	**MIC_{80} (µg/ml)**
wt	16
Cgfpr3 Δ	16
Cgfpr4 Δ	16
*Cgfpr3*Δ*4* Δ	64

Table S2: MIC80 of fluconazole (µg/ml) for *Cgrph1Δ* and *Cgset2Δ* mutants.	
Strain	**MIC_{80} (µg/ml)**
wt	16
Cgrph1Δ	8
Cgset2Δ	32

Table S3: List of *C. glabrata* strains used in the study.

Yeast strain	Genotype	Reference
YRK19	*ura3Δ::Tn903 G418R* (BG14)	Cormack and Falkow, 1999
YRK20	*URA3* (BG462)	De Las Peñas *et al*., 2003
YRK1948	*ura3Δ::Tn903 G418R Cgfpr3Δ::nat1*	This study
YRK1951	*URA3 Cgfpr3Δ::nat1*	This study
YRK1874	*ura3Δ::Tn903 G418R Cgfpr4Δ::nat1*	This study
YRK1877	*URA3 Cgfpr4Δ::nat1*	This study
YRK2015	*ura3Δ::Tn903 G418R Cgfpr3Δ4Δ::nat1*	This study
YRK2308	*URA3 Cgfpr3Δ4Δ::nat1*	This study
YRK4188	*URA3 Cgrph1::nat1*	This study
YRK4183	*URA3 Cgset2::nat1*	This study
YRK809	*ura3Δ::Tn903 G418R Cgpdr1Δ::nat1*	Kaur Laboratory
YRK1503	*URA3 Cgcdr1Δ::nat1*	Kaur Laboratory
YRK4162	*ura3Δ::Tn903 G418R Cgfpr3Δ4Δcdr1Δ::nat1*	This study
YRK4180	*ura3Δ::Tn903 G418R Cgfpr3Δ4Δpdr1Δ::nat1*	This study
YRK3896	*ura3Δ::Tn903 G418R* (YRK19)*/pRK2006*	This study
YRK3900	*ura3Δ::Tn903 G418R* (YRK19)*/pRK2010*	This study
YRK3934	*ura3Δ::Tn903 G418R Cgfpr3Δ4Δ::nat1 (YRK2015)/pRK2006*	This study
YRK3936	*ura3Δ::Tn903 G418R Cgfpr3Δ4Δ::nat1 (YRK2015)/pRK2008*	This study
YRK3938	*ura3Δ::Tn903 G418R Cgfpr3Δ4Δ::nat1 (YRK2015)/pRK2010*	This study
YRK3940	*ura3Δ::Tn903 G418R Cgfpr3Δ4Δ::nat1 (YRK2015)/pRK2012*	This study
YRK4191	*ura3Δ::Tn903 G418R Cgfpr3Δ4Δcdr1Δ::nat1 (YRK4162)/pRK1108*	This study
YRK4293	*ura3Δ::Tn903 G418R Cgfpr3Δ4Δpdr1Δ::nat1 (YRK4180)/pRK945*	This study

Table S4: List of plasmids used in the study.

Plasmid	Description	Reference
pRK1018	pGRB2.3 plasmid	Addgene (Plasmid #45343)
pRK2006	*CgFPR3* (1.314 kb) cloned in *XbaI-SpeI* sites of pRK1018 plasmid	This study
pRK2008	*CgFPR3* (1.041 kb) lacking catalytic domain cloned in XbaI-SpeI sites of pRK1018 plasmid	This study
pRK2010	*CgFPR4* (1.197 kb) cloned in *XbaI-SpeI* sites of pRK1018 plasmid	This study
pRK2012	*CgFPR4* (0.918 kb) lacking catalytic domain cloned in *XbaI-SpeI* sites of pRK1018 plasmid	This study
pRK1108	*CgCDR1* (4.500 kb) cloned in *SpeI-XmaI* sites of pRK1018 plasmid	Kaur Laboratory
pRK945	pSF4, pCgACU5-derived plasmid containing *CgPDR1* from wild-type strain	Sanglard laboratory
pRK70	pRD16, *C. glabrata* CEN/ARS plasmid for expressing *S. cerevisiae FLP1*	Cormack laboratory
pRK625	NAT cassette cloned in pCR2.1 plasmid	Cormack laboratory

Table S5 : List of primers used in the study.

Primer	Sequence (5'-3')	Description	Restriction enzyme	Reference
For generation of deletion strains				
OGRK2312	CGATAAACTTCCGCGTTAGC	*CgFPR4* _5' UTR Forward		
OGRK2313	gcgtcgacctgcagcgtacgTTCAGGAGTAACCTTTCCTTCC	*CgFPR4* _5' UTR Reverse		
OGRK2314	cgacggtgtcggtctcgtagCAAAGCTCATTTCTAGTTATTGTATGC	*CgFPR4* _3' UTR Forward		
OGRK2315	GCTCGATAGCCTTGGCAAAT	*CgFPR4* _3' UTR Reverse		
OGRK2316	TGGGAAGTGTGTCAAACCAA	*CgFPR4* _5' Integration check Forward		
OGRK2317	TCGAACCAACCTCAATTTCC	*CgFPR4* _3' Integration check Reverse		
OGRK2318	GCTATTTCCGAGGGTGATGA	*CgFPR4* _Internal check Forward		
OGRK2319	TCTACGCTCACCCCCAATAG	*CgFPR4* _Internal check Reverse		
OgRK2517	GGCTGCTTCCTTCTCTTTGA	*CgFPR3* _5' UTR Forward		
OgRK2518	gcgtcgacctgcagcgtacgCGCCACTAATAACCAACCTCA	*CgFPR3* _5' UTR Reverse		
OgRK2519	cgacggtgtcggtctcgtagGAACTGCTCTGGCGTTCATT	*CgFPR3* _3' UTR Forward		
OgRK2520	TTGGTGTAGCTTCGTTCTTTCA	*CgFPR3* _3' UTR Reverse		
OgRK2521	CTCCTCCAAGCAAGTCAAGG	*CgFPR3* _5' Integration check Forward		
OgRK2522	GGTAGATGCGGCTATTGCAT	*CgFPR3* _3' Integration check Reverse		
OgRK2523	TGACATGGACGATAGCGAAG	*CgFPR3* _Internal check Forward		
OgRK2524	GACGTCCCATCCCTTGATAA	*CgFPR3* _Internal check Reverse		
OgRK4102	CTTCACCGGACATGATGTTG	*CgRPH1* _5' UTR Forward		
OgRK4103	gcgtcgacctgcagcgtacgCAGCTCACCCCAGTTGGTAT	*CgRPH1* _5' UTR Reverse		
OgRK4104	cgacggtgtcggtctcgtagGACGAAGGGAGATTGCAAAG	*CgRPH1* _3' UTR Forward		
OgRK4105	GCCTGTCTCTCAGTTCTTGGA	*CgRPH1* _3' UTR Reverse		
OgRK4106	AATCCACCTCGACGTCAAAC	*CgRPH1* _5' Integration check Forward		
OgRK4107	CATCATCCTTACCGGCAACT	*CgRPH1* _3' Integration check Reverse		
OgRK4108	GTCCCTGGTGTGGCTAAAAA	*CgRPH1* _Internal check Forward		
OgRK4109	ATAGCCCCATGTCAAGCAAC	*CgRPH1* _Internal check Reverse		
OgRK4139	GGTGTTAACCCAGCTTCTGCTCATTC	*CgSET2* _5' UTR Forward		
OgRK4140	gcgtcgacctgcagcgtacgCAGACATGTTATCGAAGGTATG	*CgSET2* _5' UTR Reverse		
OgRK4141	cgacggtgtcggtctcgtagCTTGCTTGATATTCCTGCCATAC	*CgSET2* _3' UTR Forward		
OgRK4142	GAGCATGTTGACATGCCAGGACAC	*CgSET2* _3' UTR Reverse		
OgRK4143	GGCTCCAATAGGCCCATGGGATG	*CgSET2* _5' Integration check Forward		
OgRK4144	CATTGGTTCAGTGATCGACCCAG	*CgSET2* _3' Integration check Reverse		
OgRK1724	TGTCATCGAATGAGGAGCTG	*CgSET2* _Internal check Forward		
OgRK1725	AACAATTTCCCAACCAGGTG	*CgSET2* _Internal check Reverse		
OgRK4063	CCTTCGCGTAATAGGACTGC	*CgCDR1* _5' UTR Forward		
OgRK4064	GGTCAAAAGGTGTGGCAACT	*CgCDR1* _3' UTR Reverse		
For gene cloning				
OgRK2525	TCGA*TCTAGA* ATGTCTGATATGCTACCA	*CgFPR3* _Forward	XbaI	
OgRK2527	TCGA*ACTAGT* TTTGATGGACACCAAC	*CgFPR3* _Reverse_without stop codon	SpeI	
OGRK3835	TCGAACTAGTAGCTGGACCATCGCCAACGG	*CgFPR3* _Reverse_without stop codon_Catalytic domain deletion	SpeI	
OGRK2320	ATGC*TCTAGA* ATGTCTGATATGTTACCG	*CgFPR4* _Forward	XbaI	
OGRK2322	ATGC*ACTAGT* TTTTAGAGAAACTAAC	*CgFPR4* _Reverse_without stop codon	SpeI	
OGRK3837	ATGC*ACTAGT* ACCCTCACCTATCTTACGG	*CgFPR4* _Reverse_without stop codon_Catalytic domain deletion	SpeI	
For qRT-PCR				
OgRK127	TGCAGGACCAAGTCAGACAG	*CgCDR1* _Forward		Borah et al.,2011
OgRK128	CTCATCGGAAGTAGGGTCCA	*CgCDR1* _Reverse		Borah et al.,2011
OgRK129	CGAGGAGGAAGACGACTACG	*CgCDR2* _Forward		Borah et al.,2011
OgRK130	GCAGGTTCAGGAAAGTGCTC	*CgCDR2* _Reverse		Borah et al.,2011
OgRK131	ACGACCAATCAATGCAACAA	*CgSNQ2* _Forward		Borah et al.,2011
OgRK132	ACACCACCTCTGGAAAATGC	*CgSNQ2* _Reverse		Borah et al.,2011
OgRK133	ACGGTACCAAGCCATACGAG	*CgERG11* _Forward		Borah et al.,2011
OgRK134	GAACACTGGGGTGGTCAAGT	*CgERG11* _Reverse		Borah et al.,2011
OgRK135	AAAGGGAGTGACAGCGAGAA	*CgPDR1* _Forward		Borah et al.,2011
OgRK136	CTCAATGGCGTCAATGGATGA	*CgPDR1* _Reverse		Borah et al.,2011
OgRK191	TTTCAGAGTGCCAACTGTCG	*CgTDH3* _Forward		Borah et al.,2011
OgRK192	TGAAACAACAGCGTCCTCAG	*CgTDH3* _Reverse		Borah et al.,2011
For amplification of *nat1* cassette for *C. glabrata* gene disruption				
OGRK340	cgtacgctgcaggtcgacgcCTTCCGCTGCTAGGCGCGCCGTG	5' NAT half_Forward		Kaur Laboratory
OGRK343	TCTGTTCCAACCAGAATAAG	5' NAT half_Reverse		Kaur Laboratory
OGRK342	GTCTACTACTTTGGATGATAC	3' NAT half_Forward		Kaur Laboratory
OGRK341	ctacgagaccgacaccgtcgGGCCGCTGACGAAGT	3' NAT half_Reverse		Kaur Laboratory
OGRK344	TGCGCACGTCAAGACTGTCAAGG	5' integration check_Forward		Kaur Laboratory
OGRK345	TGTGAATGCTGGTCGCTATACTGC	3' integration check_Reverse		Kaur Laboratory

 # Nucleotide sequences written in small case letters represent overhangs.

*** The underlined sequences correspond to cleavage sites of restriction enzymes.**

Table S6 : List of antibodies used in the study.

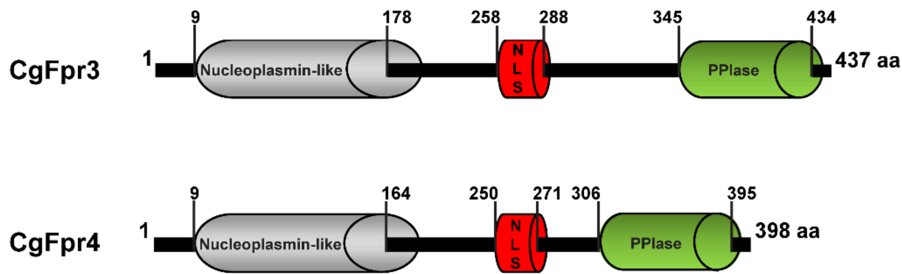
Name	Dilution used	Clonality	Company	Catalog number
Primary antibodies				
Anti-histone H3	1:5,000	Polyclonal	Abcam	ab1791
Anti-histone H4	1:5,000	Polyclonal	Abcam	ab10158
Anti-H3K36me3	1:5,000	Polyclonal	Abcam	ab9050
Anti-GAPDH	1:10,000	Polyclonal	Abcam	ab22555
Anti-GFP	1:10,000	Polyclonal	Abcam	ab290
Secondary antibodies				
Anti-rabbit	1:10,000		Cell Signaling Technology	7076S

References

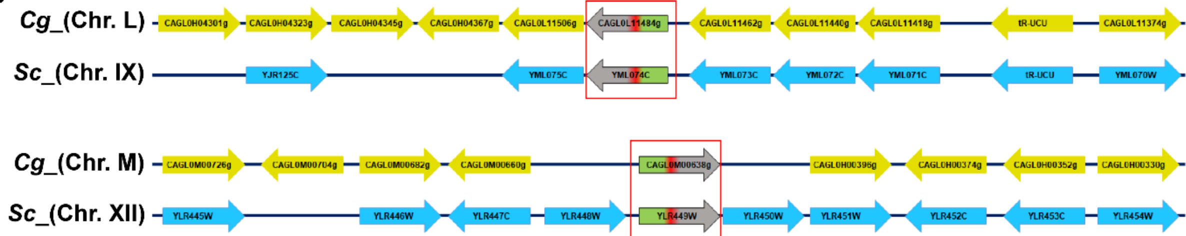
1. Cormack BP, Falkow S. 1999. Efficient homologous and illegitimate recombination in the opportunistic yeast pathogen *Candida glabrata*. Genetics 151:979–987.
2. De Las Peñas A, Pan SJ, Castaño I, Alder J, Cregg R, Cormack BP. 2003. Virulence-related surface glycoproteins in the yeast pathogen *Candida glabrata* are encoded in subtelomeric clusters and subject to RAP1- and SIR-dependent transcriptional silencing. Genes Dev 17:2245-58.
3. Borah S, Shivarathri R, Kaur R. 2011. The Rho1 GTPase-activating protein CgBem2 is required for survival of azole stress in *Candida glabrata*. J Biol Chem 286:34311–34324.

Figure S1

A



B



C

	Candida glabrata		*Saccharomyces cerevisiae*	
	Cagl0L11484p (CgFpr3)	Cagl0M00638p (CgFpr4)	YML074C (Fpr3)	YLR449W (Fpr4)
Cagl0L11484p	100	80	84.21	83.93
Cagl0M00638p	80	100	73.38	80.34

Figure S1: CgFpr3 and CgFpr4 possess peptidyl-prolyl cis-trans isomerase domain.

A. A schematic illustration of domains, as predicted by the Pfam tool (http://pfam.xfam.org), in CgFpr4 and CgFpr3 proteins. The nucleoplasmin-like domain and the PPIase (peptidyl-prolyl cis/trans isomerase) domains were identified at the C- and the N-termini, respectively, of CgFpr3 and CgFpr4 proteins. The NLS mapper tool (http://nls-mapper.iab.keio.ac.jp) predicted one bipartite nuclear localization signal (NLS) in the middle region of both proteins. The NLS region was marked based on the maximum score obtained by the NLS mapper tool. The FKBP_C peptidyl-prolyl cis-trans isomerase domain spanned 89 amino acids at the C-terminal region of proteins.

B. A schematic depicting syntenic nature of the *FPR3* and *FPR4* gene loci in *C. glabrata* and *S. cerevisiae* that share 77% identity with each other at the nucleotide level. The YGOB tool (http://ygob.ucd.ie) was used to determine the synteny between genomic regions of *C. glabrata* and *S. cerevisiae* that contain *FPR3* and *FPR4* ORFs. *C. glabrata* and *S. cerevisiae* CgFpr3- and CgFpr4 -encoding ORFs are marked in red-colored boxes, with color-coded individual domain-encoding sequences.

C. A summary table of amino acid sequence similarity between *C. glabrata* and *S. cerevisiae* Fpr3 and Fpr4 proteins. Sequence homology among proteins was determined using BLASTP analysis, after retrieving protein sequences of Fpr3 and Fpr4 of *C. glabrata* and *S. cerevisiae* from Candida Genome Database (CGD) and Saccharomyces Genome Database (SGD) respectively. *C. glabrata* Fpr3 and Fpr4 were used as a query sequence for the BLASTP analysis.

Figure S2

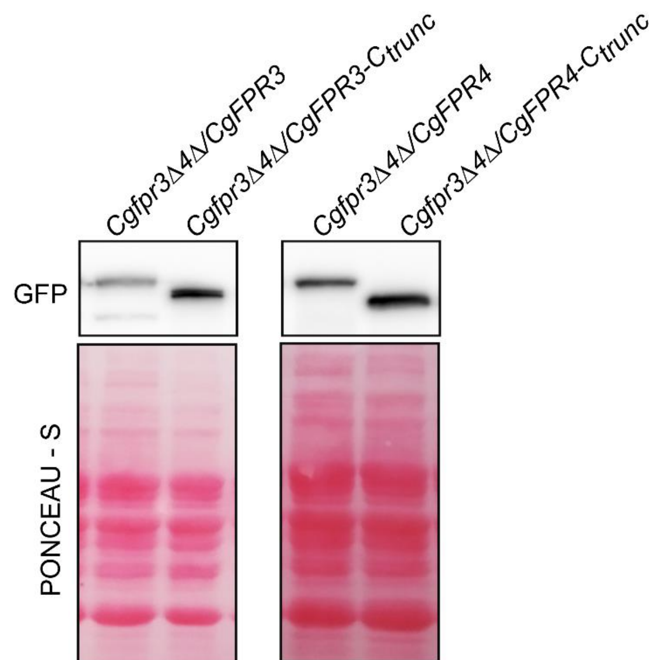


Figure S2: CgFpr3-C_{trunc}-GFP and CgFpr4-C_{trunc}-GFP proteins are expressed well in the *Cgfpr3Δ4Δ* mutant. An immunoblot showing expression of GFP-tagged full-length and C-terminally-truncated CgFpr3 and CgFpr4 proteins, that lack PPIase domain. The *Cgfpr3Δ4Δ* mutant expressing full length or truncated CgFpr3 and CgFpr4 proteins was grown in CAA medium for 4 h at 30°C. These log-phase cells were harvested and whole-cell extracts were prepared by glass bead lysis. 50 µg protein were resolved on 15% SDS-PAGE and probed with anti-GFP antibody. The bands of approximately 95 kDa for *Cgfpr3Δ4Δ//CgFPR3-GFP*, 85 kDa for *Cgfpr3Δ4Δ/CgFPR3-Ctrunc-GFP,* 90 kDa for *Cgfpr3Δ4Δ/CgFPR4-GFP* and 80 kDa for *Cgfpr3Δ4Δ/CgFPR4-Ctrunc-GFP* were observed. Ponceau S-stained membrane was used as loading control.

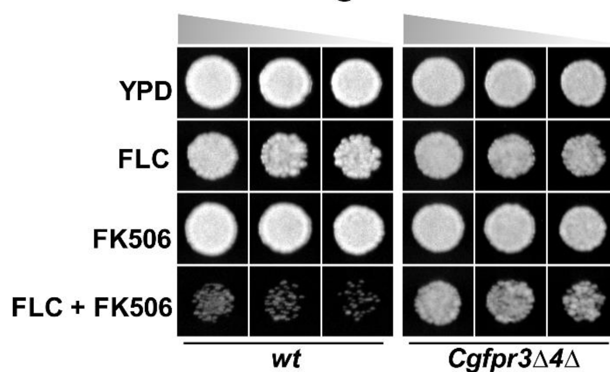


Figure S3: Fluconazole and FK506 combination is not cidal for *Cgfpr3Δ4Δ* mutant.
Liquid medium-based growth analysis of indicated *C. glabrata* strains to assess the sensitivity towards the combinatorial treatment with fluconazole and FK506. Wild-type (*wt*) and *Cgfpr3Δ4Δ* strains were grown in YPD medium lacking (YPD) or containing fluconazole (FLC; 128 μg/ml), FK506 (2 μg/ml) or fluconazole (128 μg/ml) plus FK506 (2 μg/ml) [FLC+FK506] at 30ºC. After 24 h incubation, cultures were diluted in PBS, and 3 μl of 100-, 250- and 500-fold diluted cultures were spotted on YPD medium. Images were captured after 1 day of growth at 30°C.

Figure S4

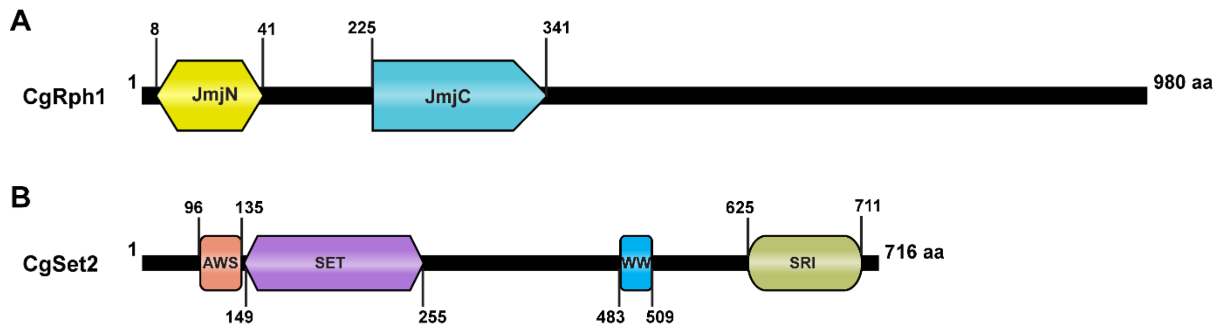


Figure S4: CgRph1 contains JmjN and JmjC domains at the N-terminus.

A. Schematic depiction of the domain composition of the CgRph1 protein, as predicted by the pfam tool. While the JmjC domain is known to function in the highly conserved histone demethylation mechanism, the JmjN and JmjC domains as two non-adjacent domains have often been found in the jumonji family of transcription factors.

B. Schematic depiction of the domain organization of the CgSet2 protein, as predicted by the pfam tool. The SET domain is a characteristic feature of protein lysine methyltransferase enzymes, while AWS and SRI domains refer to Associated with SET domain and Set2-Rpb1 interacting domain, respectively. The WW domain containing two signature tryptophan residues is implicated in binding to proline-rich motifs and mediate protein-protein interaction.

Figure S5

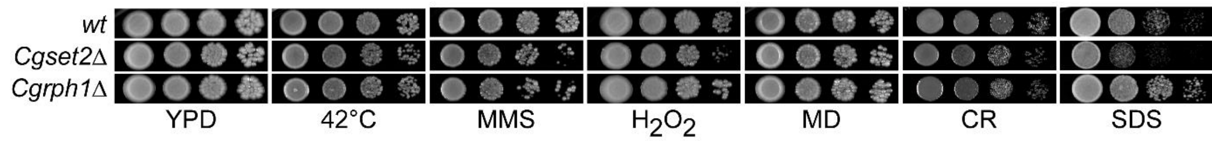


Figure S5: Serial dilution spotting analysis illustrating susceptibility of *Cgset2Δ* and *Cgrph1Δ* to varied stresses. Methyl methane sulfonate (MMS), hydrogen peroxide (H_2O_2), menadione (MD), congo red (CR) and sodium dodecyl sulfate (SDS) were used at a concentration of 0.03%, 25 mM, 100 µM, 2 mg/ml and 0.05%, respectively. All plates were incubated at 30ºC, unless indicated otherwise. Images were captured after 1-2 days of incubation.