Supplementary information for

Regional centromeres protect organisms from lethal mutations

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This file contains

- Supplementary figures S1-S3 with legends,
- Supplementary tables S1-S4

Supporting figures and tables

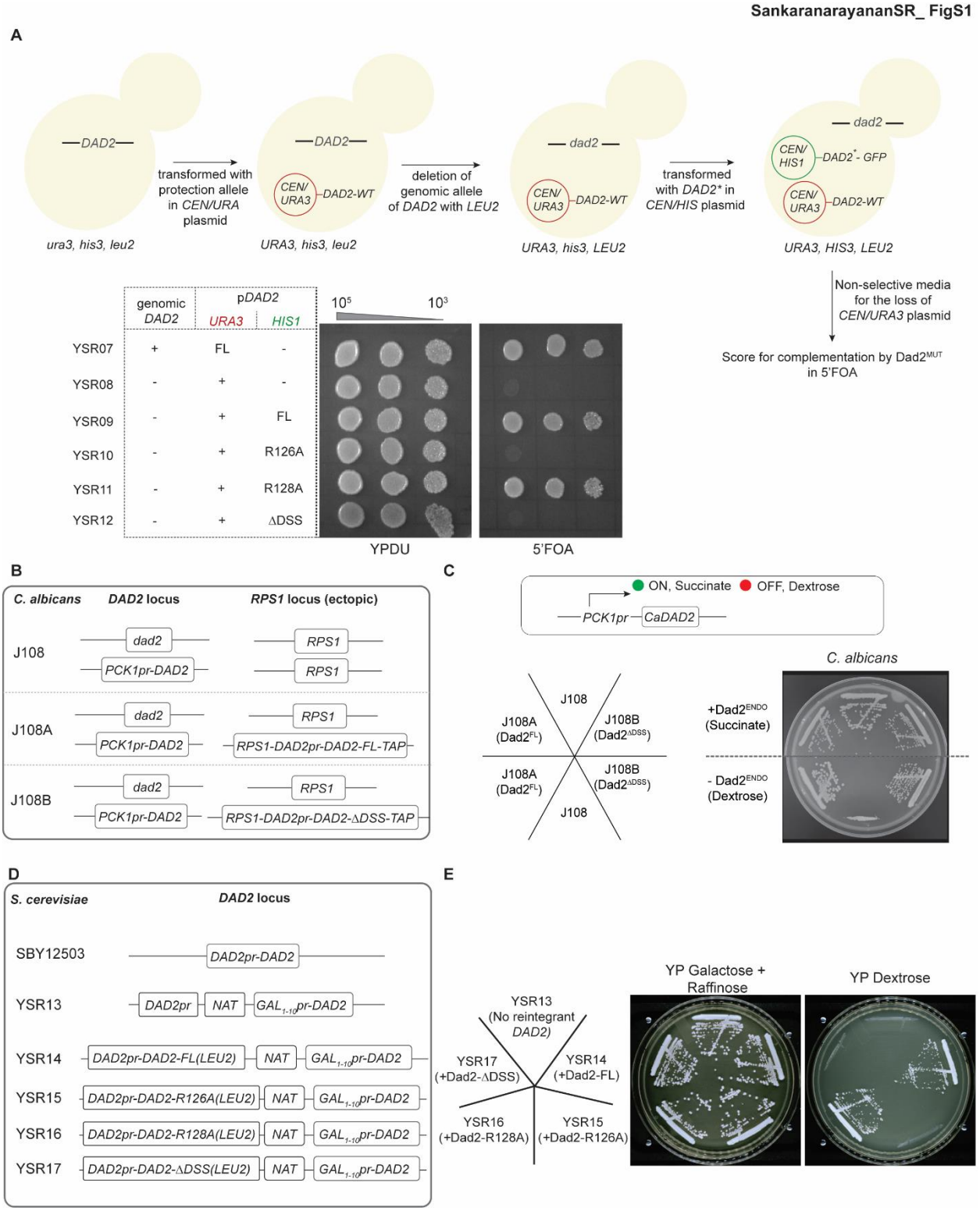

Figure S1. Essentiality of the DSS is not conserved across point and regional centromeres. (A) Schematic depicting the construction of strains YSR07 through YSR12 engineered to test for essentiality of the DSS for viability of *S. cerevisiae* at 30°C. The genomic allele of *DAD2* was deleted after protecting the essential Dad2 function with ScDad2-FL from the plasmid pRS316 (*CEN/URA3*). This strain was then transformed with pRS313 (*CEN/HIS3*) containing GFP-tagged full length or the mutant versions of *DAD2* to create strains YSR09 through YSR12. These strains were grown on nonselective media for 12 h, serially diluted ten-fold (10^5 to 10^3), and cells were spotted on YPDU and YPDU+5FOA plates. The plates were incubated at 30°C and imaged after 48 h. (B) The genomic locus of endogenous and ectopic *DAD2* in strains J108, J108A, and J108B engineered to test for the DSS function in *C. albicans* is depicted in a line diagram. (C) Cells from the parent strain J108 (no ectopic CaDad2) along with reintegrant strains J108A (ectopic CaDad2-FL) and J108B (ectopic CaDad2-ΔDSS) were streaked on plates with media permissive or non-permissive for the *PCK1* promoter-driven expression. Plates were imaged post-incubation at 30°C for 48 h. The box above the plate photograph schematically represents the regulation of *PCK1* promoter-driven expression. (D) Line diagrams depict the genomic locus of *DAD2* in the strains SBY12503, YSR13, and the reintegrant strains YSR14 through YSR17 engineered to test for the DSS function in *S. cerevisiae*. (E) Cells from the Dad2 conditional mutant (YSR13) and reintegrant strains expressing indicated versions of Dad2 (YSR14 through YSR17) were streaked on plates with media permissive and non-permissive for expression of the $GAL_{1\text{-}10}$ promoter that drives the expression of endogenous Dad2. The plates were photographed after incubation at 30°C for 48 h.

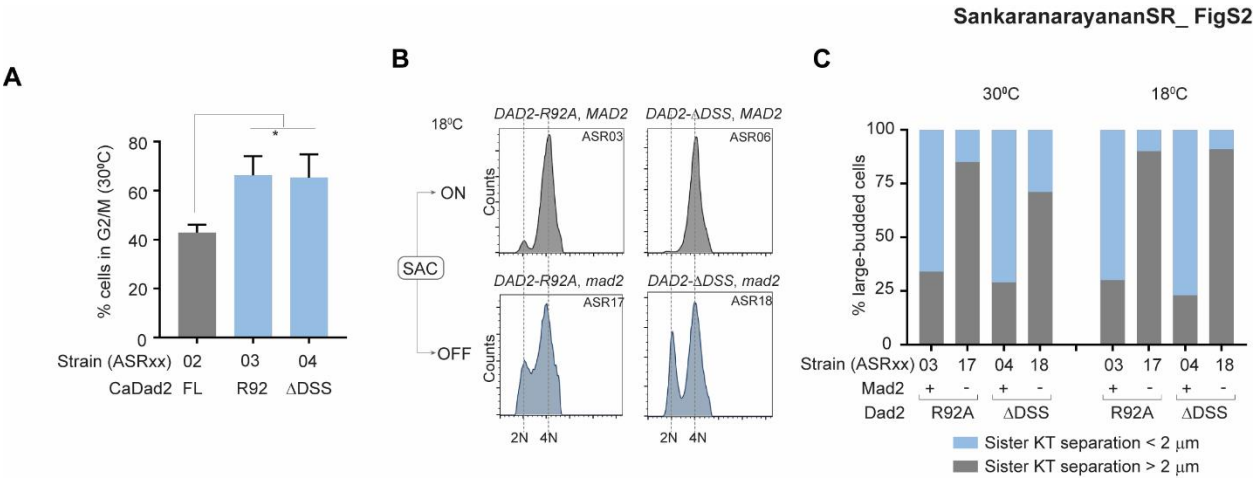

Figure S2. **The conserved DSS is essential for timely cell cycle progression in *C. albicans.*** (A) The bar plot depicts the percentage of cells in the G2/M stage in the indicated strains grown at 30°C (from Figure 3B). The percentage of G2/M cells in each strain was estimated using the cell cycle analysis tool in the software FCS Express 7. Statistical significance was tested by one-way ANOVA (*$p < 0.013$, N=3, 30000 cells per experiment). (B) Histograms depict the distribution of cells with 2N and 4N DNA content (*x*-axis) in the indicated strains after growth at 18°C as analyzed by flow cytometry. ON and OFF respectively indicate the ability or inability of strains to activate the spindle assembly checkpoint (SAC). (C) The percentage of large-budded cells with sister kinetochore separation less than 2 µm (blue) or greater than 2 µm (gray) in the indicated strains upon growth at 30°C and 18°C is plotted. The signs + and – in the *x*-axis indicate the presence or absence of *MAD2* in each strain respectively.

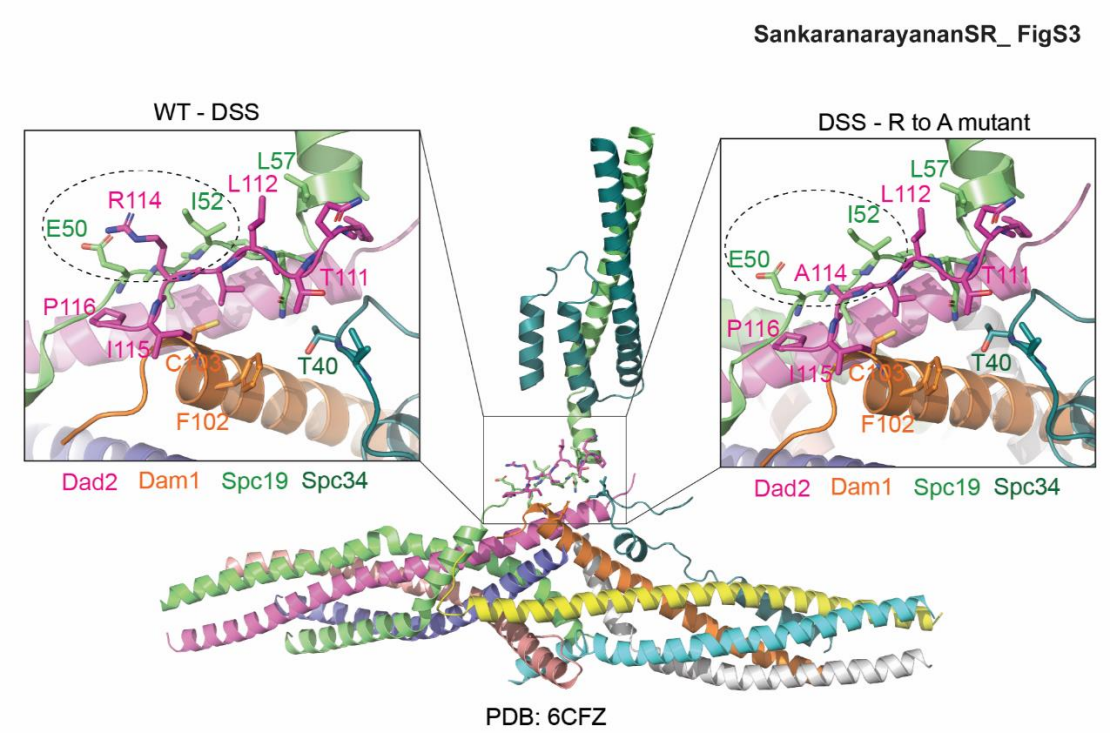

Figure S3. Neighborhood of the DSS and the conserved arginine residue based on the known structure of the Dam1 complex (PDB 6CFZ). The location of the DSS in the Dam1 complex monomer is highlighted in the box. The left inset represents the wild-type DSS motif. The residues making steric contacts with the conserved arginine in the DSS (R114 in *Chaetomium thermophilum* Dad2) is shown in dashed circle. The right inset represents the alanine susbsitution of the conserved arginine (A114) and the potential loss of interactions with the residues E50 and I52 of Spc19.

Table S1. Distribution of point and regional centromere structures across Eukaryota.

Taxonomic position		Species	*CEN* structure	*CEN* size	Ref
Opisthokonta	Fungi (Ascomycota)	*Saccharomyces cerevisiae*	point	125 bp	(Fitzgerald-Hayes et al. 1982)
		Naumovozyma castellii	point	125 bp	(Kobayashi et al. 2015)
		Naumovozyma dairenensis	point	125 bp	(Kobayashi et al. 2015)
		Saccharomyces bayanus	point	125 bp	(Gordon et al. 2011)
		Candida glabrata	point	125 bp	(Gordon et al. 2011)
		Vanderwalozyma polyspora	point	125 bp	(Gordon et al. 2011)
		Zygosaccharomyces rouxii	point	125 bp	(Gordon et al. 2011)
		Lachancea kluveri	point	125 bp	(Gordon et al. 2011)
		Ashbya gossypii	point	125 bp	(Gordon et al. 2011)
		Kluyveromyces lactis	point	125 bp	(Gordon et al. 2011)
		Candida albicans	regional	3-5 kb	(Sanyal et al. 2004)
		Candida dubliniensis	regional	3-5 kb	(Padmanabhan et al. 2008)
		Candida tropicalis	regional	10-18 kb	(Chatterjee et al. 2016)
		Candida parapsilosis	regional	1-2.6 kb	(Guin et al. 2020a; Ola et al. 2020)
		Candida viswanathii	regional	11-16 kb	(Guin et al. 2020a)
		Candida sojae	regional	7-26 kb	(Guin et al. 2020a)
		Candida auris	regional	2-3 kb	(Narayanan et al. 2021)
		Candida lusitaniae	regional	4-4.6 kb	(Kapoor et al. 2015)
		Candida haemulonii	regional	2-3 kb	(Narayanan et al. 2021)
		Candida duobushaamulonii	regional	2-3 kb	(Narayanan et al. 2021)
		Candida pseudohaemulonii	regional	2-3 kb	(Narayanan et al. 2021)
		Kuraishia capsulata	regional	3.5-6 kb	(Marie-Nelly et al. 2014)
		Ogataea polymorpha	regional	<10 kb	(Ravin et al. 2013)
		Blastobotrys adeninivorans	regional	~6 kb	(Kunze et al. 2014)
		Yarrowia lipolytica	regional	<200 bp	(Fournier et al. 1993)
		Komgaetaella phaffii	regional	3-5 kb	(Coughlan et al. 2016)
		Schefferosomyces. stipites	regional	~10 kb	(Coughlan and Wolfe 2019)
		Zymoseptoria tritici	regional	5-12.5 kb	(Schotanus et al. 2015)
		Neurospora crassa	regional	170-300 kb	(Cambareri et al. 1998)
		Magnaporthe oryzae	regional	57-109 kb	(Yadav et al. 2019)
		Schizosaccharomyces pombe	regional	35-110 kb	(Nakaseko et al. 1986; Fishel et al. 1988)
	Fungi (Basidiomycota)	*Cryptococcus neoformans*	regional	27-64 kb	(Janbon et al. 2014; Yadav et al. 2018)
		Cryptococcus deuterogattii	regional	8-22 kb	(Janbon et al. 2014; Yadav et al. 2018)
		Malassezia sympodialis	regional	3-5.5 kb	(Sankaranarayanan et al. 2020)
		Malassezia furfur	regional	3-5.5 kb	(Sankaranarayanan et al. 2020)

		Ustilago maydis	regional	7-38 kb	(Yadav et al. 2018)
	Fungi (Mucoromycota)	*Mucor circinelloides*	mosaic of point and regional	~1 kb	(Navarro-Mendoza et al. 2019)
	Animals	*Drosophila melanogaster*	regional	200-500 kb	(Sun et al. 1997)
		Equus asinus	regional	54-345 kb	(Nergadze et al. 2018)
		Gallus gallus	regional	>30 kb	(Shang et al. 2010)
		Mus musculus	regional	300-500 kb	(Kipling et al. 1991)
		Homo sapiens	regional	0.3-5 Mb	(Lo et al. 2001)
Amoebozoa	Amoeba (Slime mold)	*Dictyostelium discoideum*	regional	171-361 kb	(Glockner and Heidel 2009)
Stramenopila-Alveoloata-Rhizaria (SAR)	Stramenopiles	*Phaeodactylum tricornutum*	regional	2-5.6 kb	(Diner et al. 2017)
		Phytopthora sojae	regional	211-356 kb	(Fang et al. 2020)
	Alveolates	*Plasmodium falciparum*	regional	2-2.5 kb	(Kelly et al. 2006)
		Toxoplasma gondii	regional	13-20 kb	(Brooks et al. 2011)
Archeaplastida	Red algae	*Cyanidioschyzon merolae*	regional	1-4 kb	(Kanesaki et al. 2015)
	Land plants (Angiosperms)	*Oryza sativa*	regional	420-820 kb	(Yan et al. 2008)
		Arabidopsis thaliana	regional	40-148 kb	(Maluszynska and Heslop-Harrison 1991)
		Brassica campestris	regional	20.2-10 Mb	(Harrison and Heslop-Harrison 1995)
		Sorghum bicolor	regional	ND	(Jiang et al. 1996)
		Triticum aestivum	regional	ND	(Kishii et al. 2001)
		Zea mays	regional	2-10 Mb	(Ananiev et al. 1998)
		Solanum tuberosum	regional	1-3 Mb	(Gong et al. 2012)
Excavata	Kinetoplastid	*Trypanosoma bruceii*	regional	20-120 kb	(Obado et al. 2007; Echeverry et al. 2012)

ND, not determined

Table S2- List of strains used in this study

Strain	Parent	Genotype
S. cerevisiae strains		
CJY077		*MAT*a *Δdad2::KanMX6 ura3-52 lys2-801 ade2-101 trp1Δ63 leu2Δ1::pCJ055(DAD2^{TS}, LEU2) his3Δ200* [23]
YSR01	CJY077	*MAT*a *Δdad2::KanMX6 ura3-52 lys2-801 ade2-101 trp1Δ63 leu2Δ1::pCJ055 his3Δ200 pRS313G (GFP, HIS3)*
YSR02	CJY077	*MAT*a *Δdad2::KanMX6 ura3-52 lys2-801 ade2-101 trp1Δ63 leu2Δ1::pCJ055 his3Δ200 pSR01 (DAD2-FL-GFP, HIS3,CEN6)*
YSR03	CJY077	*MAT*a *Δdad2::KanMX6 ura3-52 lys2-801 ade2-101 trp1Δ63 leu2Δ1::pCJ055 his3Δ200 pSR02 (DAD2-R126A-GFP, HIS3,CEN6)*
YSR04	CJY077	*MAT*a *Δdad2::KanMX6 ura3-52 lys2-801 ade2-101 trp1Δ63 leu2Δ1::pCJ055 his3Δ200 pSR03 (DAD2-R128A-GFP, HIS3,CEN6)*
YSR05	CJY077	*MAT*a *Δdad2::KanMX6 ura3-52 lys2-801 ade2-101 trp1Δ63 leu2Δ1::pCJ055 his3Δ200 pSR04 (DAD2-ΔDSS-GFP, HIS3,CEN6)*
BY4741		*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*
YSR06	BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SPC42::SPC42-mCherry-KanMX4*
YSR07	YSR06	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SPC42::SPC42-mCherry-KanMX4 pSR05(Dad2-FL,URA3,CEN6)*
YSR08	YSR07	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SPC42::SPC42-mCherry-KanMX4 Δdad2::LEU2 pSR05(Dad2-FL,URA3,CEN6)*
YSR09	YSR08	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SPC42::SPC42-mCherry-KanMX4 Δdad2::LEU2 pSR05(Dad2-FL,URA3,CEN6) pSR01 (DAD2-FL-GFP, HIS3,CEN6)*
YSR10	YSR08	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SPC42::SPC42-mCherry-KanMX4 Δdad2::LEU2 pSR05(Dad2- FL,URA3,CEN6) pSR02 (DAD2-R126A-GFP, HIS3,CEN6)*
YSR11	YSR08	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SPC42::SPC42-mCherry-KanMX4 Δdad2::LEU2 pSR05(Dad2-FL,URA3,CEN6) pSR03(DAD2-R128A-GFP, HIS3,CEN6)*
YSR12	YSR08	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SPC42::SPC42-mCherry-KanMX4 Δdad2::LEU2 pSR05(Dad2-FL,URA3,CEN6) pSR04 (DAD2-ΔDSS-GFP, HIS3,CEN6)*

SBY12503		*MAT a pCUP1-GFP12-LacI12:HIS3 CEN3::33LacO:KanMX SPC110-mCherry:hphMX HSK3-3V5-IAA7:KanMX bar1-1 ade3*Δ *(UMBRIET N. et al., Nat Commun, 2014)*
YSR13	SBY12503	*MAT a pCUP1-GFP12-LacI12:HIS3 CEN3::33LacO:KanMX SPC110-mCherry:hphMX HSK3-3V5-IAA7:KanMX bar1-1 ade3*Δ *dad2::GAL$_{1-10}$prDAD2 (NAT)*
YSR14	YSR13	*MAT a pCUP1-GFP12-LacI12:HIS3 CEN3::33LacO:KanMX SPC110-mCherry:hphMX HSK3-3V5-IAA7:KanMX bar1-1 ade3*Δ *dad2::GAL$_{1-10}$prDAD2 (NAT) DAD2pr-DAD2-FL (LEU2)*
YSR15	YSR13	*MAT a pCUP1-GFP12-LacI12:HIS3 CEN3::33LacO:KanMX SPC110-mCherry:hphMX HSK3-3V5-IAA7:KanMX bar1-1 ade3*Δ *dad2::GAL$_{1-10}$prDAD2 (NAT) DAD2pr-DAD2-R126A (LEU2)*
YSR16	YSR13	*MAT a pCUP1-GFP12-LacI12:HIS3 CEN3::33LacO:KanMX SPC110-mCherry:hphMX HSK3-3V5-IAA7:KanMX bar1-1 ade3*Δ *dad2::GAL$_{1-10}$prDAD2 (NAT) DAD2pr-DAD2-R128A (LEU2)*
YSR17	YSR13	*MAT a pCUP1-GFP12-LacI12:HIS3 CEN3::33LacO:KanMX SPC110-mCherry:hphMX HSK3-3V5-IAA7:KanMX bar1-1 ade3*Δ *dad2::GAL$_{1-10}$prDAD2 (NAT) DAD2pr-DAD2-ΔDSS (LEU2)*
C.albicans strains		
SN148		*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG* (Noble and Johnson, 2005)
J108		*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG dad2::HIS1/PCK1pr-DAD2 (URA3)* (Thakur and Sanyal, 2011)
J108A	J108	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG dad2::HIS1/PCK1pr-DAD2 (URA3) RPS1/RPS1:DAD2pr-DAD2^{FL}-TAP(NAT)*
J108B	J108	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG dad2::HIS1/PCK1pr-DAD2 (URA3) RPS1/RPS1:DAD2pr-DAD2^{ΔDSS}-TAP(NAT)*
ASR01	SN148	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG dad2::HIS1/DAD2*
ASR02 (CaDad2-FL)	ASR01	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG dad2::HIS1/DAD2-FL-GFP(URA3)*
ASR03 (CaDad2-R92A)	ASR01	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG dad2::HIS1/DAD2-R92A-GFP(URA3)*

ASR04 (CaDad2-ΔDSS)	ASR01	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG dad2::HIS1/DAD2-ΔDSS-GFP(URA3)*
ASR07 (CaDad2-FL,CENP-A-TAP)	ASR02	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG dad2::HIS1/DAD2-FL-GFP(URA3) CSE4/CSE4-TAP (LEU2)*
ASR08 (CaDad2-R92A, CENP-A-TAP)	ASR03	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG dad2::HIS1/DAD2-R92A-GFP(URA3)*
ASR09 (CaDad2-ΔDSS, CENP-A-TAP)	ASR04	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG dad2::HIS1/DAD2-ΔDSS-GFP(URA3) CSE4/CSE4-TAP (LEU2)*
ASR12 (CaDad2-FL, Tub4-mCherry)	ASR02	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG dad2::HIS1/DAD2-FL-GFP(URA3) TUB4/TUB4-mCherry(NAT)*
ASR13 (CaDad2-R92A, Tub4-mCherry)	ASR03	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG dad2::HIS1/DAD2-R92A-GFP(URA3) TUB4/TUB4-mCherry(NAT)*
ASR14 (CaDad2-ΔDSS, Tub4-mCherry)	ASR04	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG dad2::HIS1/DAD2-ΔDSS-GFP(URA3) TUB4/TUB4-mCherry(NAT)*
ASR03M	ASR03	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG dad2::HIS1/DAD2-R92A-GFP(URA3) mad2::LEU2/MAD2*
ASR17 (CaDad2-R92A, *mad2*)	ASR03M	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG dad2::HIS1/DAD2-R92A-GFP(URA3) mad2::LEU2/mad2::ARG4*
ASR04M	ASR04	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG dad2::HIS1/DAD2-ΔDSS-GFP(URA3) mad2::LEU2/MAD2*
ASR18 (CaDad2-ΔDSS, *mad2*)	ASR04M	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG dad2::HIS1/DAD2-ΔDSS-GFP(URA3) mad2::LEU2/mad2::ARG4*
ASR19 (CaDad2-FL, Ndc80-mCherry)	ASR02	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG dad2::HIS1/DAD2-FL-GFP(URA3) NDC80/NDC80-mCherry-ARG4*

ASR20 (CaDad2-R92A, Ndc80-mCherry)	ASR03	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG dad2::HIS1/DAD2-R92A-GFP(URA3) NDC80/NDC80-mCherry-ARG4*
ASR21 (CaDad2-ΔDSS, Ndc80-mCherry)	ASR04	*Δura3::imm434/Δura3::imm434,Δhis1::hisG/Δhis1::hisG Δarg4::hisG/Δarg4::hisG, Δleu2::hisG/Δleu2::hisG dad2::HIS1/DAD2-ΔDSS-GFP(URA3) NDC80/NDC80-mCherry-ARG4*
C. neoformans strains		
CNV108		*α H99::GFP-H4-NAT (Kozubowski et al., mBio, 2013)*
CNSD169	CNVY108	*α H99::GFP-H4-NAT DAD2::DAD2p-DAD2-mCherry-NEO*
CNSD170	CNVY108	*α H99::GFP-H4-NAT DAD2::DAD2p-DAD2-R102A-mCherry-NEO*
CNSD171	CNVY108	*α H99::GFP-H4-NAT DAD2::DAD2p-DAD2-ΔDSS-mCherry-NEO*
SHR741		*α H99::GFP-H4-NAT, mad2::NEO (Sridhar et al., Nat. Commun., 2021)*

Table S3- List of plasmids used in this study

Plasmid	***Parent***	***Description***
pRS313		*CEN6/ARS/HIS3 plasmid*
pRS313G	*pRS313*	*GFP along with CYC1 terminator cloned into pRS313 in BamHI-ClaI sites*
pSR01	pRS313G	*ScDAD2-FL cloned in pRS313G in SacII-BamHI sites*
pSR02	pRS313G	*ScDAD2-R126A cloned in pRS313G in SacII-BamHI sites*
pSR03	pRS313G	*ScDAD2-R128A cloned in pRS313G in SacII-BamHI sites*
pSR04	pRS313G	*ScDAD2-ΔDSS cloned in pRS313G in SacII-BamHI sites*
pRS316		*CEN6/ARS/URA3 plasmid*
pSR05	pRS316	*ScDAD2-FL with native promoter and terminator cloned in pRS316 in SacII-SacI sites*
pYM-N25		*Plasmid used to amplify cassette to place DAD2 under GAL_{1-10} promoter with NAT marker*
pUG73		*Plasmid to amplify DAD2 deletion cassette with LEU2 marker, also used to generate reintegration plasmids*
pSR06	pUG73	*ScDAD2-FL cloned in SacII-SacI sites*
pSR07	pUG73	*ScDAD2-R126A cloned in SacII-SacI sites*
pSR08	pUG73	*ScDAD2-R128A cloned in SacII-SacI sites*
pSR09	pUG73	*ScDAD2-ΔDSS cloned in SacII-SacI sites*
pBS-RN	pBS-NAT	*CaRPS1 cloned in pBS NAT plasmid in NotI site*
pRN-$Dad2^{FL}$	pBS-RN	*CaDAD2pr-CaDAD2-FL-TAP cloned in SalI site*
pRN-$Dad2^{\Delta DSS}$	pBS-RN	*CaDAD2pr-CaDAD2-ΔDSS -TAP cloned in SalI site*
pRN-92	pBS-RN	*CaDAD2pr-CaDAD2-R92A-TAP cloned in SalI site*
pCse4TAPLeu		*CSE4-TAP with LEU2 marker (Varshney and Sanyal, 2019)*
pTub4-mCherryNAT	pDam1-mCherryNAT	*TUB4 ORF cloned in pDam1-mCherryNAT in SacII-SpeI sites*
pMad2-2		*MAD2 deletion cassette with LEU2 (Thakur and Sanyal, 2011)*
pMad2-3		*MAD2 deletion cassette with ARG4 (Thakur and Sanyal, 2011)*
pSR10	pBS-GFPUra	*3'UTR of CaDAD2 cloned in pBS-GFPUra in XhoI-KpnI sites*
pSR11 (CaDad2-FL)	pSR10	*CaDAD2-FL cloned in pSR10 in SacII-SpeI sites*
pSR12 (CaDad2-R'A)	pSR10	*CaDAD2-R92A cloned in pSR10 in SacII-SpeI sites*

pSR15 (CaDad2-ΔDSS)	pSR10	*CaDAD2-ΔDSS cloned in pSR10 in SacII-SpeI sites*
pLK25		*Amplification of mCherry-Neomycin for C. neoformans*

Table S4- List of primers used in this study

S. cerevisiae		
ScGFP-F	CATGGATCCATGGTGAGCAAGGGCG	Cloning GFP into pRS313
ScGFP-R	GCTGATCGATGCAAATTAAAGCCTTCGAGC	
SR282	TCCCGCGGCATTGCGGCAGGTAAAATATC	Amplification of Dad2$^{FL/MUT}$ as SacII/BamHI fragment to clone into pRS313G
ScDad2R	GAGGGATCCTTCGTTACCATCTACCCTAATTCTG	
Sc126A-R	GAGGGATCCTTCGTTACCATCTACCCTAATTGCGACCATTGTTTCC	
Sc128A-R	GAGGGATCCTTCGTTACCATCTACGGCAATTCTGACCATTGTTTCC	
ScΔDSS-R	GAGGGATCCTTCGTTACCATCCAAGGGTACCAGATCTTC	
ScDad2Pr F	TGACCGCGGGTACAATGGTCCTAACTTAATG	Cloning ScDad2-FL into pRS316
ScDad2R	ATCGAGCTCCCAACACTGTAGAATACTAATATC	
ScDad2delF	GAATATATCTAAAAAACTATTGAATAGGTTTGAAAAACTCATAATTCAGACAGTTATTGCTGTGAAGATCCCAGCAAAGG	Deletion of genomic allele of DAD2 with *LEU2* marker
ScDad2delR	ATGTATATGCTATTATCGCAACTGCCTTCTTCCGATTTATATAAGATCCTTTTCTTTCTGTGCAGGCTAACCGGAACCTGT	
ScDad2F	GGTTAGAGGGCGACAATAC	Confirmatory primers
Leu2R	CACCAGTGTTCAACTCAACAAG	
GalDad2FP	CTAAAAAACTATTGAATAGGTTTGAAAAACTCATAATTCAGACAGTTATTCGACATGGAGGCCCAGAATAC	Cassette to place *DAD2* under *GAL* promoter with *NAT* marker
GalDad2RP	GACTGAAGTTCTTTTCGCTTTATAGCAATTTGTTCATCTATTGAATCCATTTTGTACAATTCATCCATACCATGG	
Dad2pr-SacII-F	TGACCGCGGGTACAATGGTCCTAACTTAATG	Overlap PCR primers to amplify DAD2$^{FL/MUT}$ for cloning into pUG73 for reintegration
Dad2pr-SacI-R	ATCGAGCTCCCAACACTGTAGAATACTAATATC	
ScTer FP	GATGGTAACGAATGAACAGAAAG	
Sc126A-R	CTTTCTGTTCATTCGTTACCATCTACCCTAATTGCGACCATTGTTTCC	
Sc128A-R	CTTTCTGTTCATTCGTTACCATCTACGGCAATTCTGACCATTGTTTCC	
ScΔDSS-R	CTTTCTGTTCATTCGTTACCCAAGGGTACCAGATCTTC	
C. albicans		
RP10F	ATAAGAATGCGGCCGCTAGATCCAACTCAAGTACAACATGGC	amplification and cloning of RPS1 locus into pBS-NAT
RP10R	ATAAGAATGCGGCCGCGGATCCCCCAGATCATTA	

	TCC	
AD02	ACGCGTCGACATTTTGACTAGT TCTCAAATGGTTC	Overlap PCR primers to amplify CaDAD2$^{FL/MUT}$ and clone into pBS-RP10-NAT
AD03	CCATCGATCGATATCAAGCTTCAGGTTG	
Dad2delR	GTCTTGATTTTCTTCATTTGATTGTCCGAAAGCCTCTTTGT TATTATC	
Dad2delF	GATAATAACAAAGAGGCTTTCGGACAATCAAATGAAGA AAATCAAGAC	
R92A FP	GAACCATTAGTAGCAGTGCGTGTTGGACAATCA	
R92A RP	TGATTGTCCAACACGCACTGCTACTAATGGTTC	
Dad2DS-F	GGCCTCGAGGTGTACATATAATAACTCTAAATTCTGGC	Amplification of Dad2 3'UTR homology
Dad2DS-R	ACCGGTACCGAATTTTGTCAACCAAGAAATAGAC	
Dad2FP	TACCCGCGGATGCTGAAAACAAATACTGCTATATACC	Amplification of DAD2 ORF tagging with GFP
Dad2GFP-RP	CGACTAGTTTCCGTGGATTCTTCAACTTC	
Dad2cFP	TCAATACCCACCACAAAACC	Confirmatory primer
C. neoformans		
SD118	TGCATGCATTCTCGTCAAAATAGGCTGC	Overlap PCR primers to amplify CnDAD2$^{FL/R102A}$
SD119	TCGCCGCCGTATGCTAAGGCCACGAGACAAGGGAGAGG	
SD120	CCTCTCCCTTGTCTCGTGGCCTTAGCATACGGCGGCGAA	
VYP152	CTCGCCCTTGCTCACCATTTGTTGTTTTGTTTTATCAGATG CG	
VYP153	CTGATAAAACAAAACAACAAATGGTGAGCAAGGGCGAG	Amplification of mCherry-NEO from pLK25
VYP154	CTATTGGTCGTCATCAGCAGGCCAAGCTTGGTACCGAGC TC	
VYP155	GAGCTCGGTACCAAGCTTGGCCTGCTGATGACGACCAAT AG	amplification CnDAD2 3'UTR homology
VYP156	CCCAAGCTTCTCCATATCGTGTCTCAATTTCATCTC	
VYP157	CTTGTACAGCTCGTCCATGC	Confirmatory primer