1 Table S1. PCR primers

Primer	Use	Sequence (5' → 3')
JC17	*SAT1* flipper	GGCCCCCCCTCGAGGAAGTT
JC18	*SAT1* flipper	GCTCTAGAACTAGTGGATCT
JC57	5'NCR of *CNA1*	AATGATGGAGTTTATGGGAA
JC58	5'NCR of *CNA1*	AACTTCCTCGAGGGGGGGCCGAAAAAAAAAAAGGGGGGGG
JC59	3'NCR of *CNA1*	AGATCCACTAGTTCTAGAGCTTTCTCTTCCTCCTCCTCCTC
JC60	3'NCR of *CNA1*	TGCCCTTTTCTTTGTTTTTG
JC61	*CNA1* overlap	AACAACAATAAGGTGGATGGG
JC62	*CNA1* overlap	TATCTTACCCCTACCTGGTGG
JC63	*CNA1* ORF	GTCAGGAAATACTGTTCAACG
JC64	*CNA1* ORF	CTTCATATAATTGTTCCGA
JC48	Disruption confirmation	ACAATCAAAGGTGGTCCT
JC81	Disruption confirmation	AACTTCCTCGAGGGGGGGCC
JC82	5'NCR of *CNB1*	TGTTGTTGGGTAGGTTGCTGT
JC83	5'NCR of *CNB1*	AACTTCCTCGAGGGGGGGCCTTGTTGAAGTTGGATTGGTTG
JC86	3'NCR of *CNB1*	AGATCCACTAGTTCTAGAGCAGCACCTGTAAGATGGTTTAG
JC87	3'NCR of *CNB1*	TGTCCAAAAACGGTTTCCAG
JC88	*CNB1* overlap	TGCCTTTCTGTTCGATTGAT
JC89	*CNB1* overlap	CGACAATAATTGGAATGATGA
JC79	*CNB1* ORF	ATGGGGGCTAACGCAAGTATT
JC80	*CNB1* ORF	TTTAAGGTCAAAGTGTTGGCA
JC100	5'NCR of *CRZ1*	AATATAGAGACGGTGTGTGGG
JC101	5'NCR of *CRZ1*	AACTTCCTCGAGGGGGGGCCGGACAAAATGAAATATAGC
JC102	3'NCR of *CRZ1*	AGATCCACTAGTTCTAGAGCTCAACTTGTTGGTTTGTCTTT
JC103	3'NCR of *CRZ1*	CTAATGTCATGACTTCCCCA
JC104	*CRZ1* overlap	TGAGACATAAAAAGGCGAC
JC105	*CRZ1* overlap	TGATCTCGATATGATTGTCC
JC106	*CRZ1* ORF	TAATCCCTATCCACAGGACGA
JC107	*CRZ1* ORF	ATCACTGGGGGAACAAAACT
JC320	*CRZ1* complementation	AAAAAACCGCGGTCAACTTGTTGGTTTGTCTTT
JC321	*CRZ1* complementation	AAAAAAGAGCTCGTGATGAATCATGAGTCGGA
JC288	*CRZ1* complementation	AAGGTACCAATATAGAGACGGTGTGTGGG
JC289	*CRZ1* complementation	AAAAGCTTAGTAATTTCAACACCACTACT
JC292	qPCR *ACT1* ORF	AGCTCCAGAAGCTTTGTTCAGACC
JC293	qPCR *ACT1* ORF	TGCATACGTTCAGCAATACCTGGG
JC345	qPCR *CNA1* ORF	GCTTCACCTCATCCTTATTGGT
JC346	qPCR *CNA1* ORF	GGTGACACTGGTGTTGTTGTTT
JC329	qPCR *CNB1* ORF	TGAAGAGATCGATAGATTGCG
JC330	qPCR *CNB1* ORF	TTCCCGAGAATGCAGATAACC
JC347	qPCR *CRZ1* ORF	CACAACAATGGCGATGGTAATA
JC348	qPCR *CRZ1* ORF	ACCTTGATGCATGACTTTTCCT
JC337	qPCR *PMC1* ORF	TTGAAACAAGGTGTTGCTGAAG
JC338	qPCR *PMC1* ORF	ATTGTGGTCCTTCCATACATGA
JC341	qPCR *PMR1* ORF	TGAGTACCAGTATCGCTGCATT
JC342	qPCR *PMR1* ORF	GATCACCTGTCGGGTAAGAATC
JC362	qPCR *CCH1* ORF	TGTCACTGATGAAGCTGGCAAA
JC363	qPCR *CCH1* ORF	TGATTAAGCTTAGCCACTGGTGTG
JC364	qPCR *MID1* ORF	TGCCGTAACTATACCTCGATGTTC

JC365	qPCR *MID1* ORF	GGGCATAAAAATCCAAAATCAGC

1 Underline indicates sequences complementary to the *SAT1* flipper

2

Figure S1. *C. dubliniensis CNA1, CNB1,* and *CRZ1* genes were disrupted with the *SAT1* flipper.

(A) Structure of the *CNA1* disruption allele that was made by overlap PCR (see materials and methods for details). PpuMI was used to digest genomic DNAs extracted from specific strains. The PpuMI restriction sites are indicated 5' (-) and 3' (+) of the *CNA1* gene. The probe is marked as a bold line. The thick dark arrows represent the FRT sites for *FLP* recombinase. The thinner arrow on a raised line represents *C. albicans MAL2* promoter and the stick with a ball represents *C. albicans ACT1* terminator. The Southern blot is shown in the right panel. Sizes of each band were labeled. The strains are WT (CD36), *CNA1/cna1*::*SAT1-FLP* (YC31), *CNA1/cna1* (YC36), *cna1/cna1::SAT1-FLP* (YC40), *CNA1/cna1*::*SAT1-FLP* (YC29), *CNA1/cna1* (YC73), and *cna1/cna1*::*SAT1-FLP* (YC94). **(B)** Genomic DNA from *CNB1* disruptants was digested with HpaI. The strains are WT (CD36), *CNB1/cnb1*::*SAT1-FLP* (YC47), *CNB1/cnb1* (YC69), *cnb1/cnb1::SAT1-FLP* (YC87), *CNB1/cnb1*::*SAT1-FLP* (YC41), *CNB1/cnb1* (YC82), and *cnb1/cnb1*::*SAT1-FLP* (YC96). **(C)** Genomic DNA from *CRZ1* disruptants was digested with EcoRV. The strains are WT (CD36), *CRZ1/crz1*::*SAT1-FLP* (YC81), *CRZ1/crz1* (YC102), *crz1/crz1::SAT1-FLP* (YC107), *CRZ1/crz1*::*SAT1-FLP* (YC80), *CRZ1/crz1* (YC100), and *crz1/crz1*::*SAT1-FLP* (YC108).

Figure S2. Complementation by *CRZ1* rescues *crz1/crz1* mutants in various stresses. (A) Structure of the *CRZ1* complementation allele in plasmid pYC393 (see materials and methods for details). EcoRV was used to digest genomic DNA extracted from specific strains. The probe is marked as a red bold line. The Southern blot is shown in the right panel. Sizes of each band are labeled. The strains are WT (CD36), *crz1/crz1*::*SAT1-FLP* (YC108), *crz1/crz1* (YC280), and *crz1/crz1 + CRZ1* (YC512). **(B)** Cells were grown overnight in YPD at 30°C, five-fold serially

diluted, and spotted onto YPD medium containing SDS, fluconazole (FL), or $CaCl_2$ at the concentrations indicated. The plates were incubated at 30°C for 48 hr and photographed. **(C)** Cells were grown overnight and washed twice with ddH_2O. One hundred microliters containing ~100 cells were spread on Spider media and incubated at 37°C for 9 days. Scale bar=1 mm.

Figure S3. *C. dubliniensis* calcineurin is dispensable for growth at elevated temperature.

(A) A set of wild type and calcineurin mutants of *C. dubliniensis* and *C. neoformans* were grown overnight at 24°C, five-fold serially diluted, and spotted onto YPD to grow for 48 hr at various temperatures. **(B)** The growth curve of *C. dubliniensis* wild type and mutant strains at 30°C. Cells were grown overnight at 30°C, washed twice with ddH_2O, diluted to 0.2 OD/ml in fresh YPD media and incubated at 30°C with shaking at 250 rpm. The OD_{600} of cultures were measured at 0, 3, 6, 9, 24, and 30 hr. The experiments were performed in triplicate, and data was plotted using Prism 5.03.

Figure S4. Calcineurin controls increased tolerance to new generation azoles in *C. dubliniensis*.

Cells were grown overnight in YPD at 30°C, five-fold serially diluted, and spotted onto YPD medium ± posaconazole (POS) or voriconazole (VOR) at the indicated concentrations. The plates were incubated at 30°C for 48 hr and photographed.

Figure S5. *crz1/crz1* mutants are hypersensitive to $CaCl_2$ at elevated temperature in *C. dubliniensis* and *C. albicans*.

Cells were grown overnight in YPD at 30°C, five-fold serially diluted, and spotted onto YPD medium ± $CaCl_2$ at the indicated concentrations. The plates were incubated at 24°C, 30°C, and 37°C for 48 hr and photographed.

Figure S6. Calcineurin is required for germ tube formation in *C. dubliniensis* but not in *C. albicans*.

(A) Germ tube formation of wild type and mutant strains in liquid spider medium ± FK506 (1 μg/ml) at 37°C incubated for 2 hr on 96-well polystyrene plates. Arrow heads indicate cells with germ tube formation defects. Scale bar=20 μm. **(B)** Bar graph showing quantitative percentage of germ tube formation in wild type and mutant strains of *C. dubliniensis* and *C. albicans*. At least 200 cells were evaluated in each experiment. The experiments were repeated three times, and error bars represent the mean ± SD. The asterisks represent a statistically significant difference (P<0.05) between wild type and FK506 (1 μg/ml)-treated wild type, *cna1/cna1*, or *cnb1/cnb1* mutants.

Figure S7. *C. dubliniensis* is as virulent as *C. albicans* in a heterologous insect model.

Survival of *G. mellonella* after injection of 10^6 CFU/larva of *C. dubliniensis* or *C. albicans* was similar. All infected larva died within 3 days after infection.

Figure S8. *C. dubliniensis* exhibits less hyphal growth and damage to oral epithelial cell compared with *C. albicans*.

(A) FaDu oral epithelial cells were incubated with *C. dubliniensis* or *C. albicans* for 2, 4, or 6 hr, after which the morphology was examined by differential interference contrast microscopy. Scale bar=10 μm.

(B) FaDu oral epithelial cells were incubated with *C. dubliniensis* or *C. albicans* for 2, 4, and 6 hr, after which the extent of host cell damage was determined using a ^{51}Cr release assay. Results are the mean ± SD of three independent experiments. *P<0.05, **P<0.0005 compared with *C. albicans*.

Figure S1

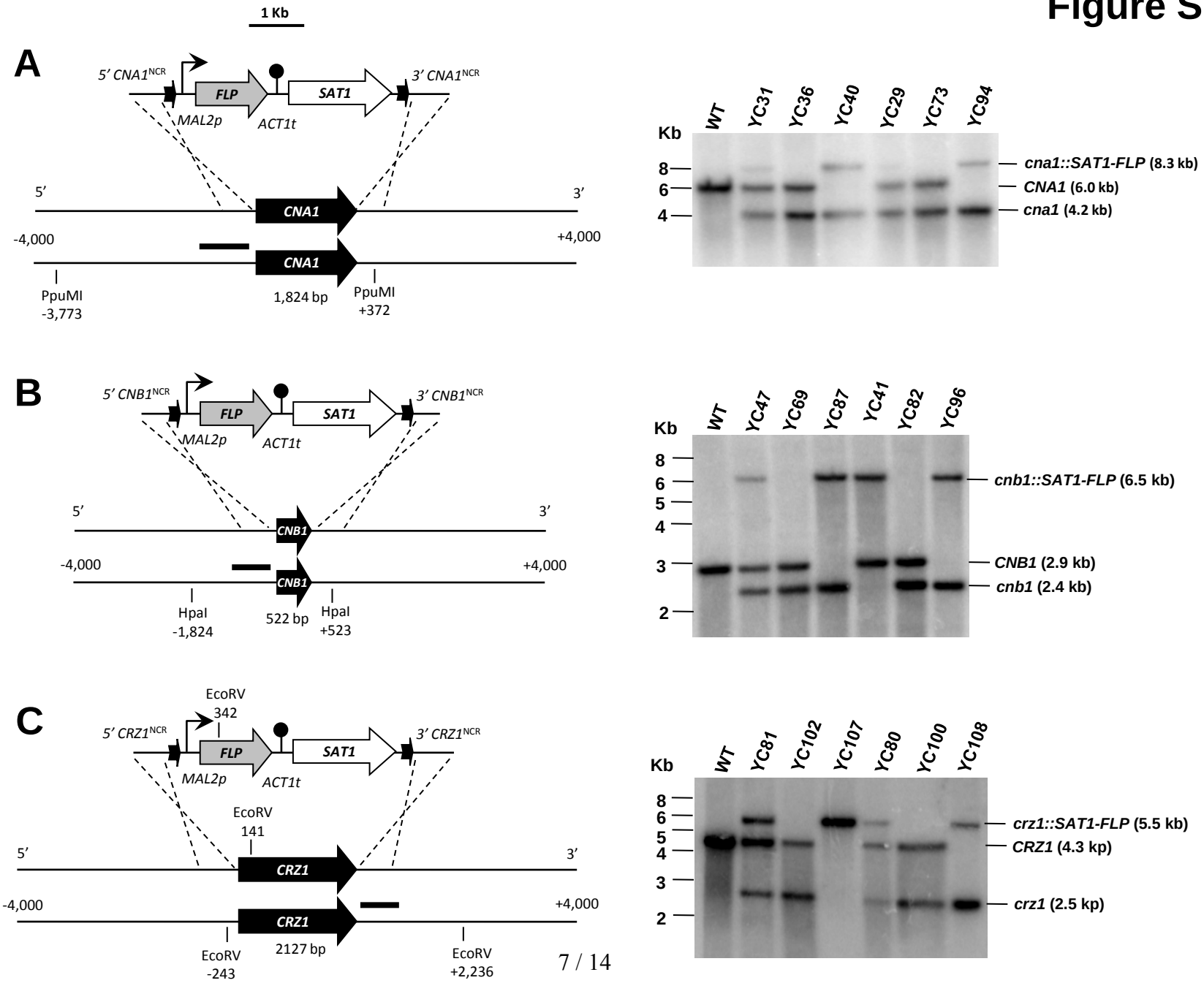
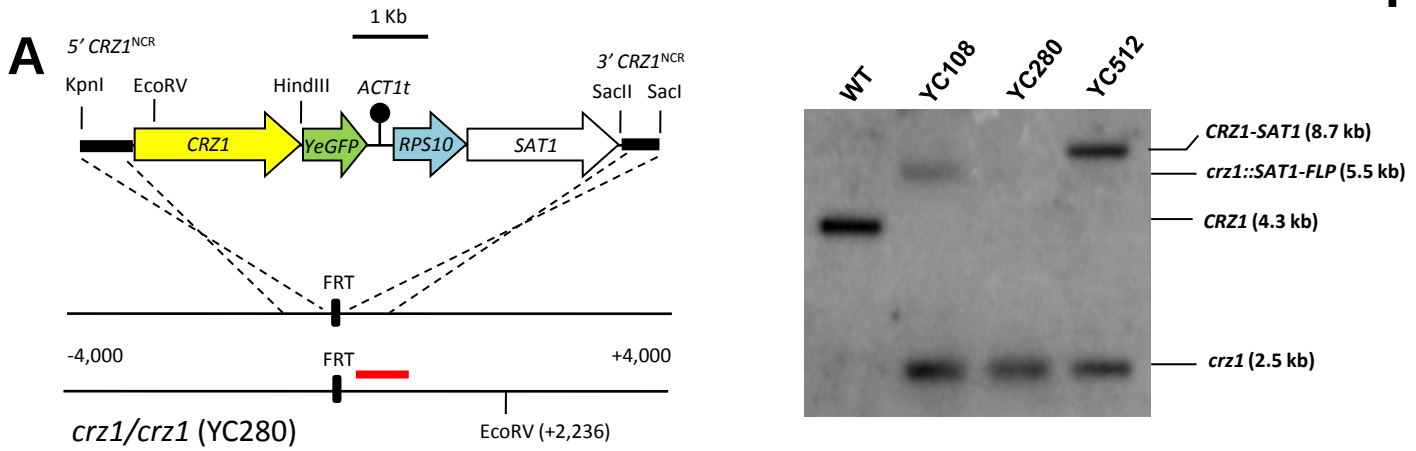
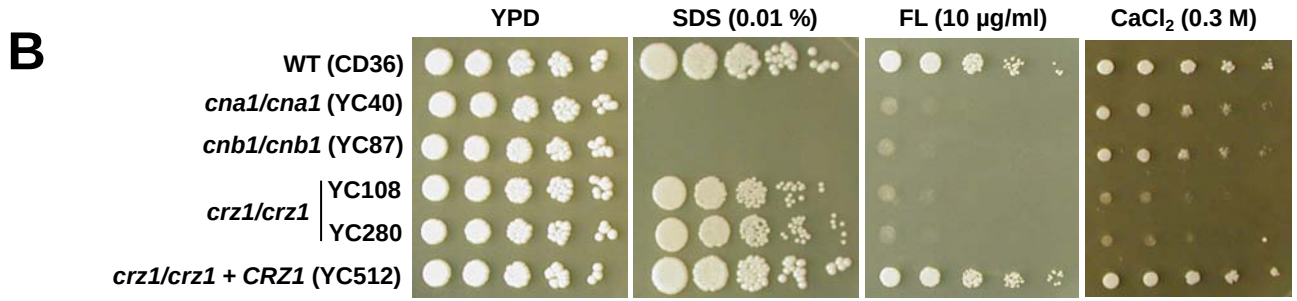

A
1 Kb
5′ CNA1NCR
FLP
SAT1
3′ CNA1NCR
MAL2p
ACT1t
5′
3′
CNA1
-4,000
+4,000
CNA1
PpuMI
-3,773
1,824 bp
PpuMI
+372
WT
YC31
YC36
YC40
YC29
YC73
YC94
Kb
8
6
4
cna1::SAT1-FLP (8.3 kb)
CNA1 (6.0 kb)
cna1 (4.2 kb)
B
5′ CNB1NCR
FLP
SAT1
3′ CNB1NCR
MAL2p
ACT1t
5′
3′
CNB1
-4,000
+4,000
CNB1
HpaI
-1,824
522 bp
HpaI
+523
WT
YC47
YC69
YC87
YC41
YC82
YC96
Kb
8
6
5
4
3
2
cnb1::SAT1-FLP (6.5 kb)
CNB1 (2.9 kb)
cnb1 (2.4 kb)
C
EcoRV
342
5′ CRZ1NCR
FLP
SAT1
3′ CRZ1NCR
MAL2p
ACT1t
EcoRV
141
5′
3′
CRZ1
-4,000
+4,000
CRZ1
EcoRV
-243
2127 bp
EcoRV
+2,236
WT
YC81
YC102
YC107
YC80
YC100
YC108
Kb
8
6
5
4
3
2
crz1::SAT1-FLP (5.5 kb)
CRZ1 (4.3 kp)
crz1 (2.5 kp)

Figure S2


A
1 Kb
5′ CRZ1NCR
3′ CRZ1NCR
KpnI
EcoRV
HindIII
ACT1t
SacII
SacI
CRZ1
YeGFP
RPS10
SAT1
FRT
-4,000
FRT
+4,000
crz1/crz1 (YC280)
EcoRV (+2,236)
WT
YC108
YC280
YC512
CRZ1-SAT1 (8.7 kb)
crz1::SAT1-FLP (5.5 kb)
CRZ1 (4.3 kb)
crz1 (2.5 kb)


B
YPD
SDS (0.01 %)
FL (10 µg/ml)
CaCl2 (0.3 M)
WT (CD36)
cna1/cna1 (YC40)
cnb1/cnb1 (YC87)
crz1/crz1
YC108
YC280
crz1/crz1 + CRZ1 (YC512)

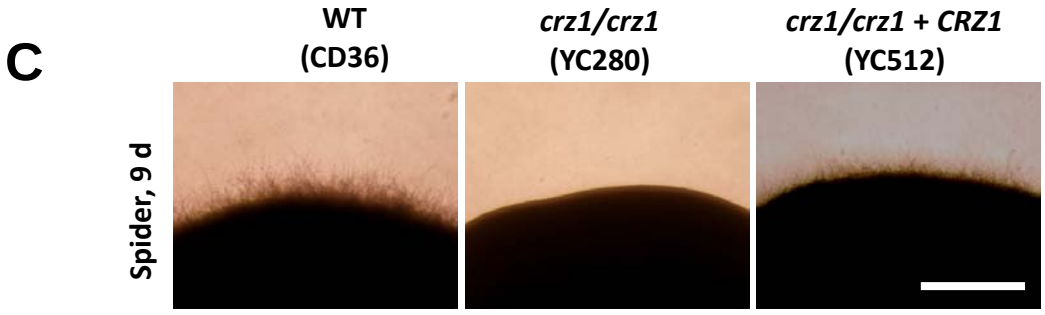

C
WT
(CD36)
crz1/crz1
(YC280)
crz1/crz1 + CRZ1
(YC512)
Spider, 9 d

Figure S3

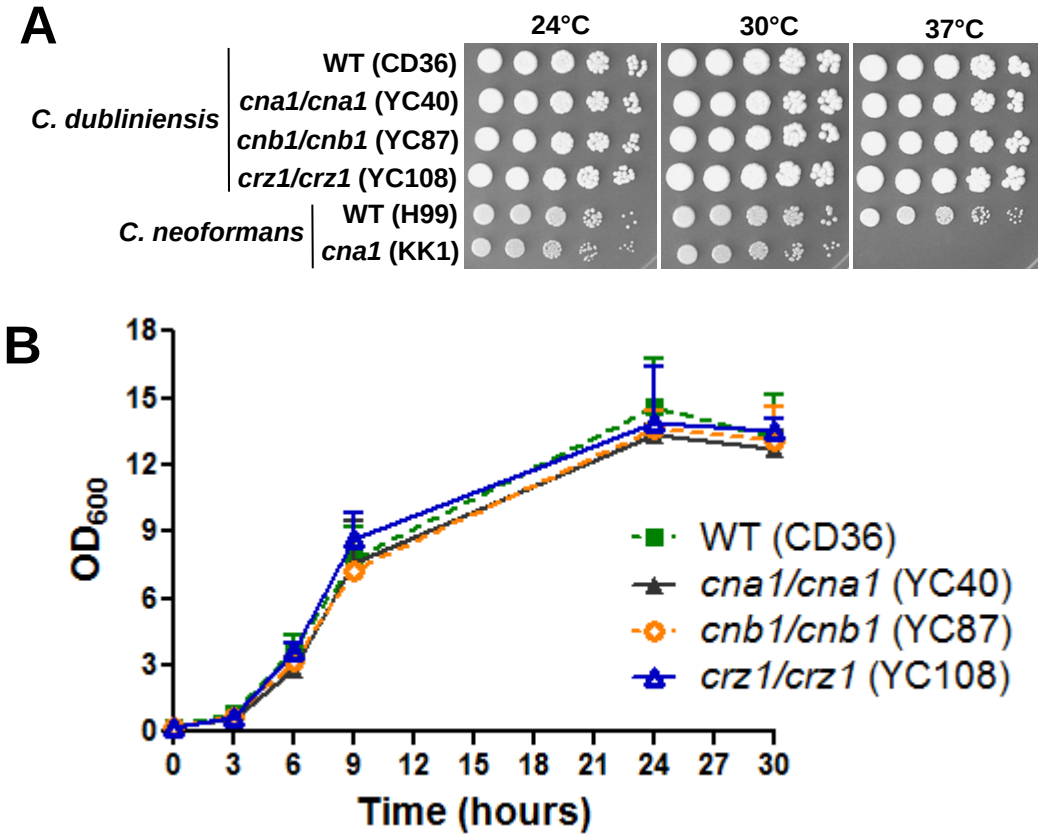

A
24°C
30°C
37°C
C. dubliniensis
WT (CD36)
cna1/cna1 (YC40)
cnb1/cnb1 (YC87)
crz1/crz1 (YC108)
C. neoformans
WT (H99)
cna1 (KK1)
B
OD600
Time (hours)
WT (CD36)
cna1/cna1 (YC40)
cnb1/cnb1 (YC87)
crz1/crz1 (YC108)

Figure S4

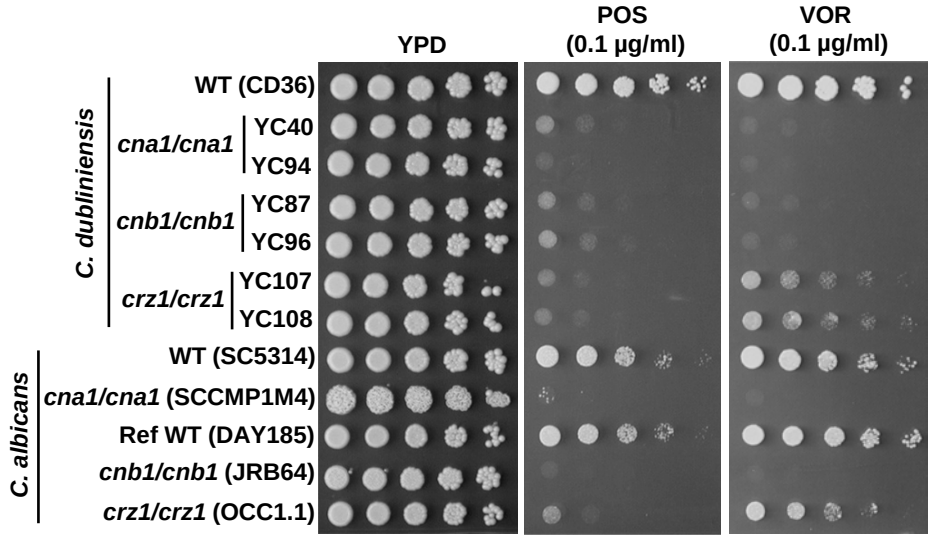

YPD
POS
(0.1 µg/ml)
VOR
(0.1 µg/ml)
C. dubliniensis
WT (CD36)
cna1/cna1
YC40
YC94
cnb1/cnb1
YC87
YC96
crz1/crz1
YC107
YC108
C. albicans
WT (SC5314)
cna1/cna1 (SCCMP1M4)
Ref WT (DAY185)
cnb1/cnb1 (JRB64)
crz1/crz1 (OCC1.1)

Figure S5

C. dubliniensis	*C. albicans*
WT (CD36)	WT (DAY185)
cna1/cna1 (YC40)	*cnb1/cnb1* (JRB64)
cna1/cna1 (YC94)	*cnb1/cnb1 + CNB1* (MCC85)
cnb1/cnb1 (YC87)	*crz1/crz1* (OCC1.1)
cnb1/cnb1 (YC96)	*crz1/crz1 + CRZ1* (OCC7)
crz1/crz1 (YC107)	WT (CAF2-1)
crz1/crz1 (YC108)	*cna1/cna1* (DSY2091)
crz1/crz1 + CRZ1 (YC512)	*cna1/cna1 + CNA1* (DSY2115)
	crz1/crz1 (DSY2195)
	crz1/crz1 + CRZ1 (MKY268)

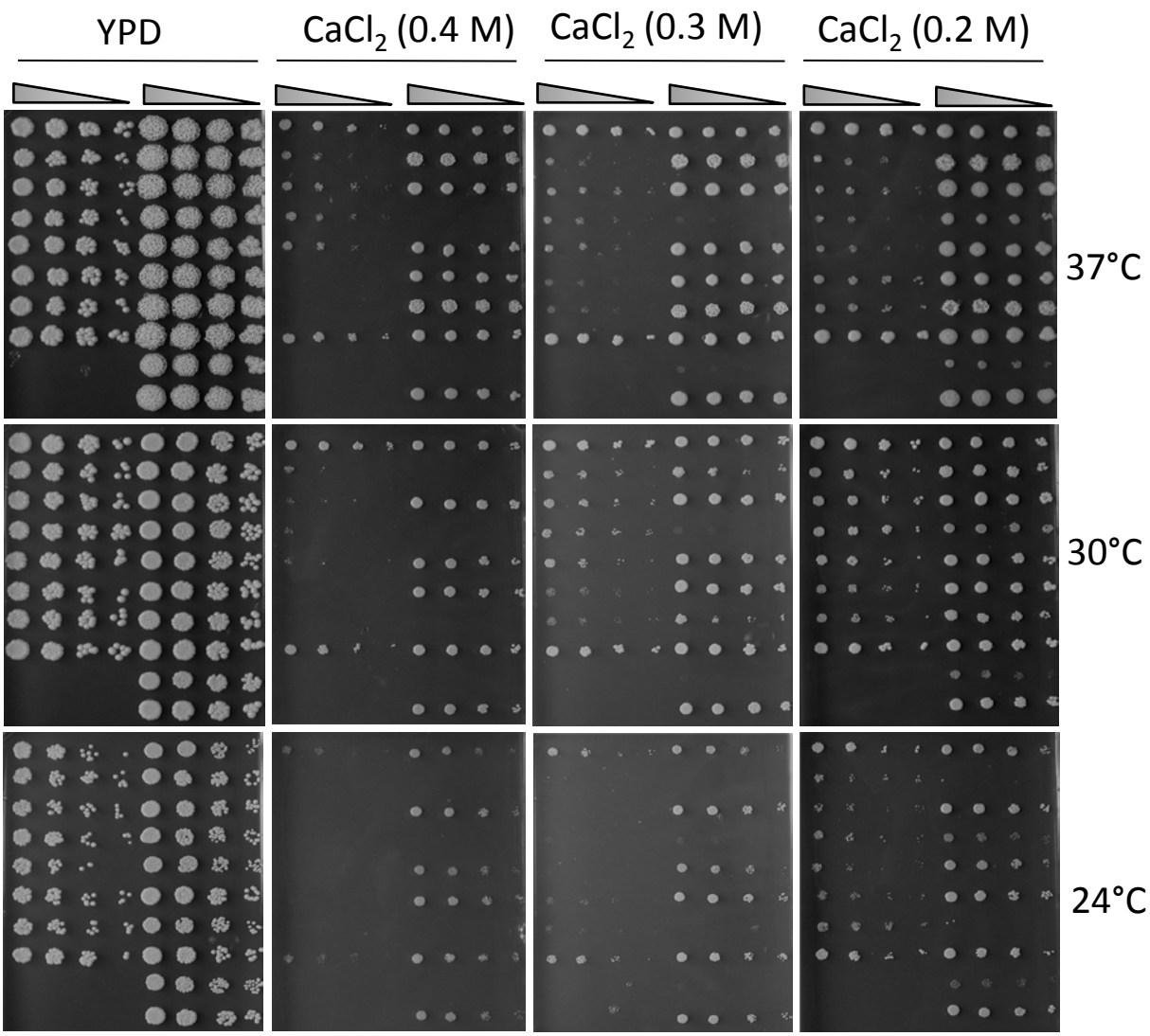


Figure S6

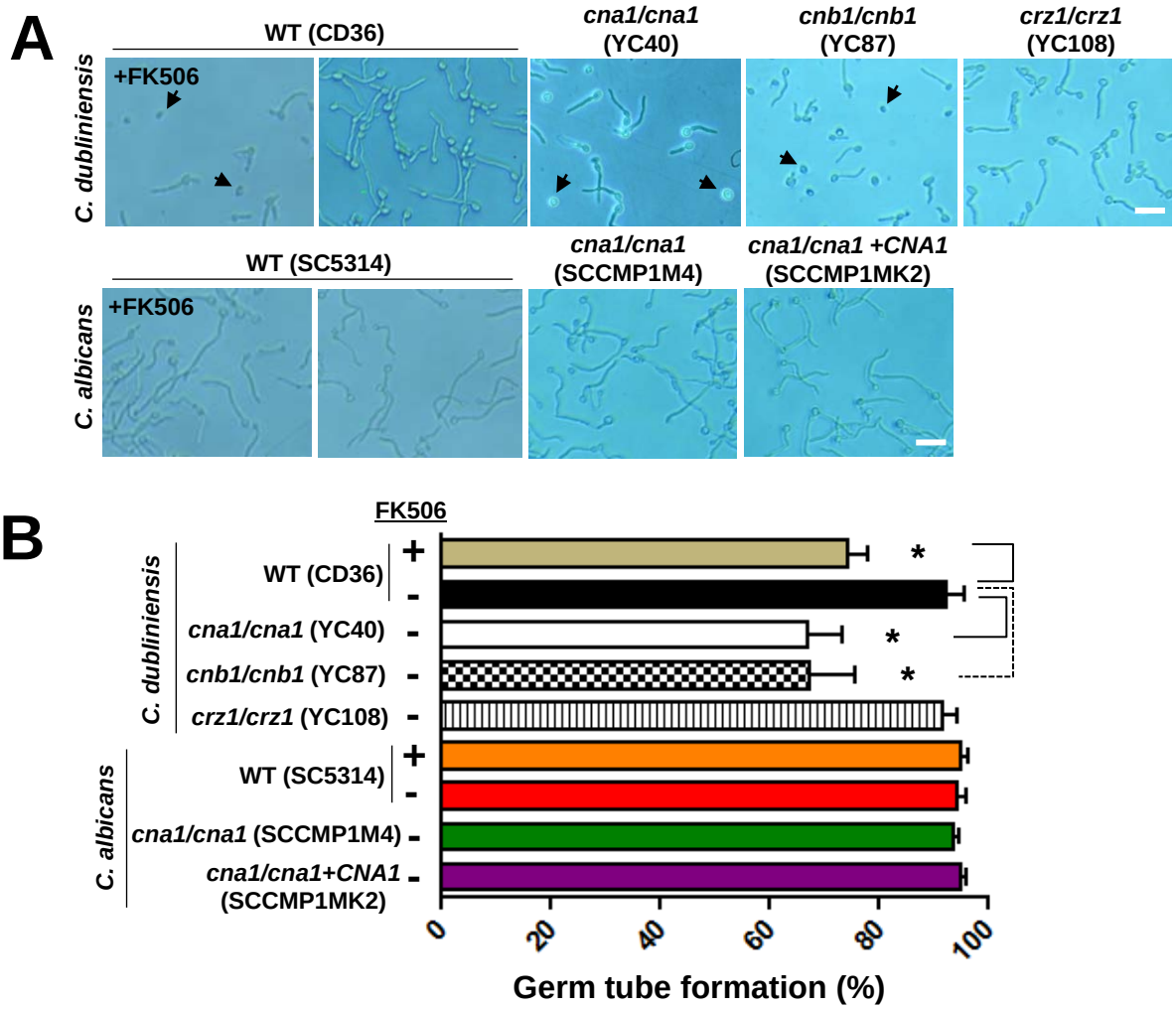

A
C. dubliniensis
WT (CD36)
+FK506
cna1/cna1 (YC40)
cnb1/cnb1 (YC87)
crz1/crz1 (YC108)
C. albicans
WT (SC5314)
+FK506
cna1/cna1 (SCCMP1M4)
cna1/cna1 +CNA1 (SCCMP1MK2)
B
FK506
C. dubliniensis
WT (CD36)
cna1/cna1 (YC40)
cnb1/cnb1 (YC87)
crz1/crz1 (YC108)
C. albicans
WT (SC5314)
cna1/cna1 (SCCMP1M4)
cna1/cna1+CNA1 (SCCMP1MK2)
0
20
40
60
80
100
Germ tube formation (%)

Figure S7

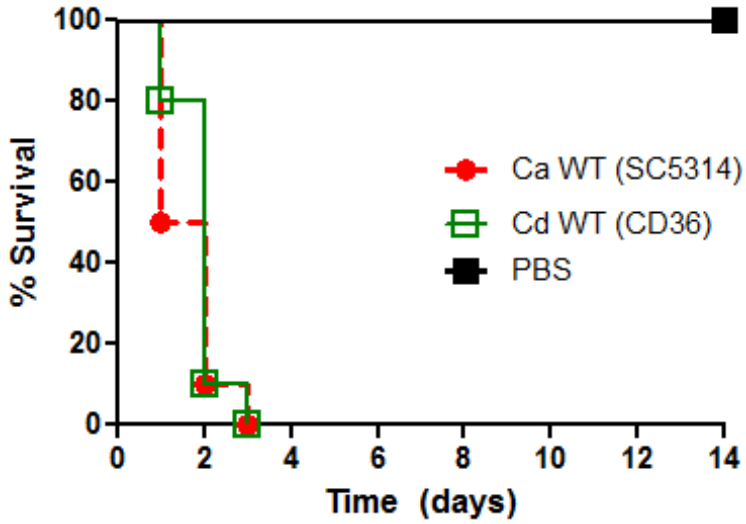
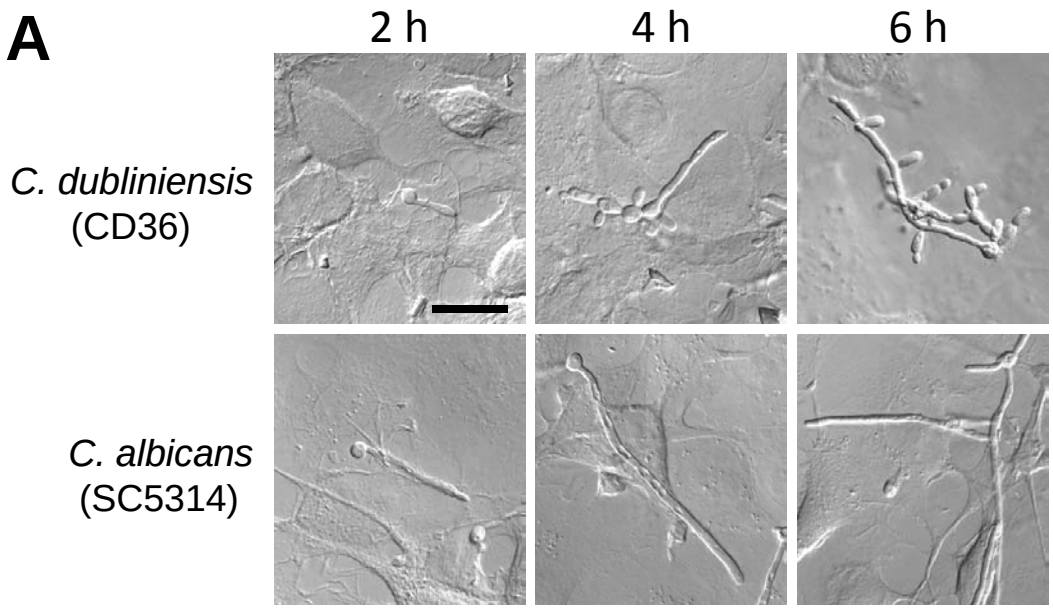
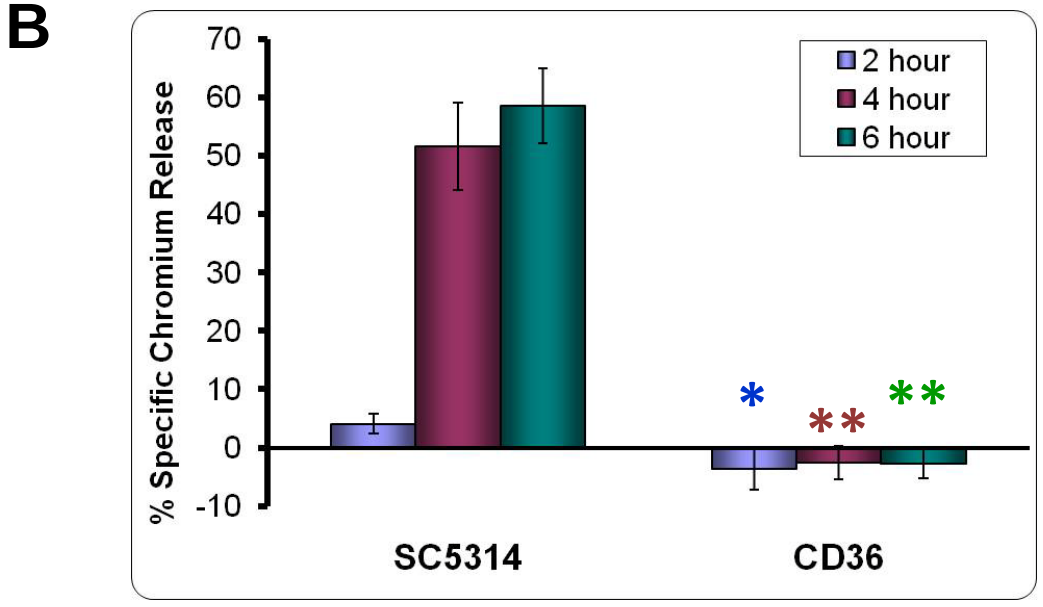

100
80
60
40
20
0
% Survival
0
2
4
6
8
10
12
14
Time (days)
Ca WT (SC5314)
Cd WT (CD36)
PBS

Figure S8


A
2 h
4 h
6 h
C. dubliniensis
(CD36)
C. albicans
(SC5314)


B
% Specific Chromium Release
70
60
50
40
30
20
10
0
-10
2 hour
4 hour
6 hour
*
**
**
SC5314
CD36