SUPPLEMENTAL TABLE

Table S1. PCR primers

Primer	Use	Sequence (5' → 3')
JC17	*SAT1* flipper	GGCCCCCCCTCGAGGAAGTT
JC18	*SAT1* flipper	GCTCTAGAACTAGTGGATCT
JC182	5'NCR of *CNB1*	TGCATCATTCGAAGACATGG
JC183	5'NCR of *CNB1*	AACTTCCTCGAGGGGGGGCCGTAGATATCGTTACGAGTG
JC184	3'NCR of *CNB1*	AGATCCACTAGTTCTAGAGCCGATTGTTTACTTGTTTCATG
JC185	3'NCR of *CNB1*	CGTGAGTTGATACATAACCA
JC186	*CNB1* overlap	TGGGAAACCAGCATTGTTGT
JC187	*CNB1* overlap	TCGGAAGAAGTGTTGTGACA
JC188	*CNB1* ORF	ATGGGGGCCAATTCAAGTAT
JC189	*CNB1* ORF	CGTCAATGTGTTTGCAATGG
JC48	Disruption confirmation	ACAATCAAAGGTGGTCCT
JC81	Disruption confirmation	AACTTCCTCGAGGGGGGGCC
JC400	3' NCR of 2nd allele of *CNB1*	AGATCCACTAGTTCTAGAGCCCATTGCAAACACATTGACG
JC402	5' NCR of 2nd allele of *CNB1*	AACTTCCTCGAGGGGGGGCCCGATGTATTTCTTCAATACT
JC215	5'NCR of *CRZ1*	AGTATAATTCAACTGACTTCA
JC216	5'NCR of *CRZ1*	AACTTCCTCGAGGGGGGGCCGAAAATTGACTAAACGGG
JC217	3'NCR of *CRZ1*	AGATCCACTAGTTCTAGAGCATTCATTTCTATGTGTTTGT
JC218	3'NCR of *CRZ1*	GGAAATATCATTAATTGATGC
JC219	*CRZ1* overlap	CACATGATCTGAAATATCTGA
JC220	*CRZ1* overlap	CCTTTTCAGCAGATGTTAGTG
JC221	*CRZ1* ORF	TAATATCCGTCAGGATGAGGA
JC222	*CRZ1* ORF	ACATCGGAATATGCAGTTGG
JC405	5' NCR of 2nd allele of *CRZ1*	AACTTCCTCGAGGGGGGGCCTCCTCATCCTGACGGATATT
JC406	3' NCR of 2nd allele of *CRZ1*	AGATCCACTAGTTCTAGAGCTCAATTGCTAACCTCATCC

Sequences complementary to the *SAT1* flipper are underlined.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Amino acid identity and pairwise alignment of calcineurin regulatory subunit (Cnb1) from *C. tropicalis*, *C. albicans*, and *S. cerevisiae*. Amino acid identity and multiple sequence alignments are depicted using ClustalW software (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_clustalw.html). **(A)** The % identity shared between the full-length proteins is shown in red. **(B)** The conserved amino acids and four Ca^{2+}-binding EF-hand motifs are indicated with green-shading and red-underlining, respectively. The ScCnb1 amino acids are numbered.

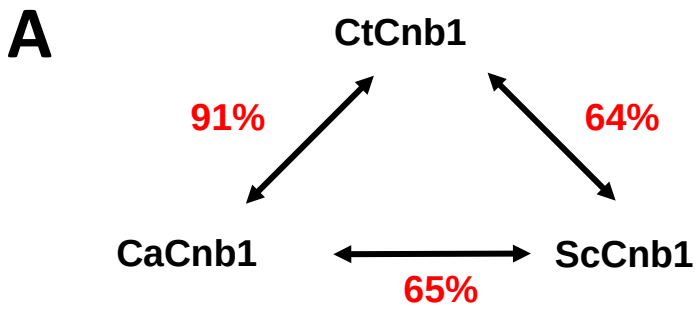
Figure S2. Amino acid identity and pairwise alignment of calcineurin downstream target Crz1 from *C. tropicalis*, *C. albicans*, and *S. cerevisiae*. (A) The % identity of the full-length proteins is shown in red. **(B)** The conserved amino acids and two zinc finger motifs (X_2-Cys-$X_{2,4}$-Cys-X_{12}-His-$X_{3,4,5}$-His; Cys_2His_2 type) are indicated with green-shading and red-underlining, respectively.

Figure S3. *C. tropicalis* is able to form hyphae on corn meal solid agar plate. *C. tropicalis* MYA3404 and *C. albicans* SC5314 strains were grown on cornmeal solid plate for 7 days. Filamentous cells on the edge of the colonies were excised with a sterile scalpel. Excised filamentous cells were mixed with 100 µl of calcofluor white solution (1 mg/ml; Fluorescent Brighter 28) and incubated for 5 minutes at room temperature. The cell mixtures were washed three times with 1 ml of dH_2O, and resuspended in 100 µl of dH_2O. Stained cell suspensions were spotted onto a slide and visualized at 400X magnification under bright field and UV, then photographed. The arrows represent calcofluor white staining site between two cells. Scale bar = 25 µm.

Figure S4. Mouse TLR4 is not required for protection from *C. tropicalis* in a murine systemic infection model. The survival of C3H/HeJ (TLR4 -/-; group of 5) or C3H/HeOuJ (TLR4 +/+, group of 5) mice following intravenous challenge with 5 x 10^6 *C. tropicalis* wild-type MYA3404 yeast cells was monitored for 5 days. The p value calculated by the Log-rank (Mantel-Cox) test method between the two groups was 0.602.

Figure S5. The loss of calcineurin or Crz1 does not affect cell growth at 37°C. (A) The calcineurin and *crz1/crz1* mutants exhibited wild-type growth curves in YPD medium at 37°C. Cells were grown overnight at 30°C, washed twice with dH_2O, diluted to 0.1 OD_{600}/ml in fresh YPD medium, and incubated at 37°C with shaking at 200 rpm. The OD_{600} of cultures was measured at 0, 3, 6, 9, 12, 24, 48, 72, and 96 hours. **(B)** The calcineurin and *crz1/crz1* mutants exhibited wild-type doubling times (~1.2 hr) in YPD medium at 37°C.

Figure S1



B

```
                1                                                          60
CtCnb1   MGAN-SSILNGFMEDTNFSIEEIHRMRKRFMKLDKDGSGEIDKQEFLSIPGISSNPLATR
CaCnb1   MGAN-ASILDGFIEDTNFSIEEIDRLRKRFMKLDKDGSGQIDKQEFLSIPGISSNPLATR
ScCnb1   MGAAPSKIVDGLLEDTNFDRDEIERLRKRFMKLDRDSSGSIDKNEFMSIPGVSSNPLAGR
               61                                                         120
CtCnb1   LMDVFDTDGDGRIDFEEFITGLSAFSGKSDNLTKLKFAFNIYDIDRDGYIGNGELFIVMK
CaCnb1   LMDVFDKDGDGSIDFEEFITGLSAFSGKSDNLNKLRFAFNIYDIDRDGYIGNGELFIVMK
ScCnb1   IMEVFDADNSGDVDFQEFITGLSIFSGRGSKDEKLRFAFKIYDIDKDGFISNGELFIVLK
             121                                                        175
CtCnb1   MMVGKNLQDEELQQIVDKTIMEADLDGDGKLNFEEFQKAVNTDSIANTLTLNLF-
CaCnb1   MMVGKNLKDEELQQIVDKTLMEADLDGDGKLNFEEFKNAVNTDTIANTLTLNMF-
ScCnb1   IMVGSNLDDEQLQQIVDRTIVENDSDGDGRLSFEEFKNAIETTEVAKSLTLQYDV
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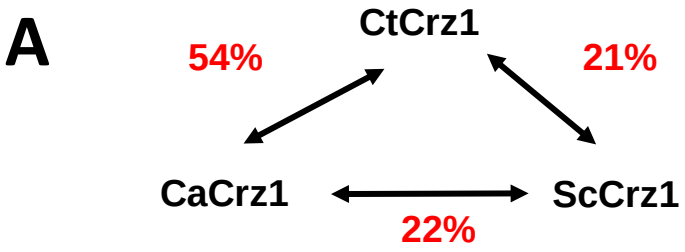
Figure S2

A



B

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          1                                                           60
CtCrz1    --------MSSNNIRQDEDKQIYDVFNLSPPSITIK---EETTNQSIDRTILSQPENSNQ
CaCrz1    --------MSNNPHPQDDGSQLYDNFEISPPSIVIR---KADTDQSLNKIMLNQESQDIN
ScCrz1    MSFSNGNMASYMTSSNGEEQSINNKNDIDDNSAYRRNNFRNSSNSGSHTFQLSDLDLDVD

CtCrz1    MHYYQ--------YNEAQALSFPSGSHQQPHLQQNQ----------EYSES-----FEP
CaCrz1    NYYTENVKNDNNPNNSYQDYTFSGNSSNQQHQQQQQQHLYEDLPTQFHYSNNS---FFEP
ScCrz1    MRMDS--------ANSSEKISKNLSSGIPDSFDSNVNSLLSPSSG--SYSADLNYQSLYK

CtCrz1    PPAPTVELTTDPLPNFSMPSGGIYMTDNASDISLNTKDLPQFTTNDYLSPSSQVSNQFSP
CaCrz1    PPAPTVELTDDPLPNFNYPPSNIYINDNASDISLNTKDLPQFTTNEFLSPTSQLSTPFSP
ScCrz1    PDLPQQQLQQQQLQQQQQQQQQQQQQQQKQTPTLKVEQSDTFQWDDILTPADNQHRPSLT

CtCrz1    GHYQQSSQDFLQVN----SNLNVNNNSNSNLLNPRSPSQYSTHSLYSENSSQPASPYLDA
CaCrz1    GHYSQSSQDFLQVNHTNGSGNNNNNNNNNNLLNPRSPSQYSSHSLYSDNSSQPASPFLDA
ScCrz1    NQFLSPRSNYDGTTRSS-GIDSNYSDTESNYHTPYLYPQDLVSSPAMSHLTANNDDFDDL

CtCrz1    VSHVSNNS-FIPPNIPTAYSDVGSNRGGVPGSNNNLVPN-HFDTVNDFL--TAEIALGGS
CaCrz1    ASHVSNNS-FIPPVIPTALSDVGS-QNLDPSHNLGLSANQHFDSVNEFLS-TGEIQLGQS
ScCrz1    LSVASMNSNYLLPVNSHGYKHISNLDELDDLLSLTYSDNNLLSASNNSDFNNSNNGIINT

CtCrz1    ISSTNLPAMEN--------AKQDGFGNSNFMENKPYNQES--------FTVPQFKTEPE-
CaCrz1    VSSTNLPSMEEDSIKWGGGNGQEAYTSLAMMEQRASADNSGMRLATHQFSETQIKQEDQQ
ScCrz1    ADTQNSTIAIN--------KSKVGTNQKMLLTIPTSSTPSP---STHAAPVTPIISIQEF

CtCrz1    ---GDQYIFSNPQMNFDFDITVTPPPQ-SESKQFPP---------------QGSQFDIMS
CaCrz1    TNMNHQYTFSNPQMNFDFDITVTPPPQQLEVKPFGNDKDMNNSSGTTNNNNNNSQFDIVS
ScCrz1    N-EGHFPVKNEDDGTLQLKVRDNESYSATNNNNLLR---------P-----DDNDYNNEA

CtCrz1    TAATNNSNQLLTENNLTNYNQLQ-SRKGTDEN-LEVERDATGIIISINQAPEVIAAKTPS
CaCrz1    TAATNNSNQLLTENNLSNYNQLQRTEQGNDNDSLQIHRDATGIIISINQAPEEIAAKTPS
ScCrz1    LSDIDRSFEDIINGRKLKLKKSRRRSSQTSNNSFTSRRSSRSRSISPDEKAKSISANREK

CtCrz1    LFSNSSANSSMHNS---PRSDVESNTNNNGNNNNSSNGLIPNSQLLSPSANS-------G
CaCrz1    LFSNSSANSSIHNS---PRSDIDNKSGQYYNNGGDGNSLVPNSQLLPSSPNSNNDNYGGG
ScCrz1    LLEMADLLPSSENDNNRERYDNDSKTSYNTINSSNFNEDNNNNNLLTSKPKIES-----G

CtCrz1    GDNKDS---LSPEEFQSMKRGRRKSHASKS--NP-SVSPRSKS---------------PN
CaCrz1    GSSNDENNLLNPEEFQSVKRGRRKSHASRTSTNPNSLSPRSRSRSRSSAKSSNDAVISDN
ScCrz1    IVNIKN-------ELDDTSKDLGILLDIDSLGQFEQKVGFKND---------------DN

CtCrz1    DESEEKLQSREKMLELALPTSSSKRTQKHPSLYGCHLCEKRFTRPYNLKSHIRTHTQEKP
CaCrz1    DESDDVLQSREKMLELALPSSSSKRTQKHPSLYACHLCDKRFTRPYNLKSHIRTHTQEKP
ScCrz1    HENNDNGTFSVKKNDNLEKLDSVTNNRKNPANFACDVCGKKFTRPYNLKSHLRTHTNERP

CtCrz1    FICSRCGKSFARSHDKKRHELLHQGVKNFKCEGFLQDGTKWGCGKSFARADALRRHFQTE
CaCrz1    FICSKCGKSFARSHDKKRHELLHQGIKNFKCEGYLQDGTRWGCGKSFARADALRRHFQTE
ScCrz1    FICSICGKAFARQHDRKRHEDLHTGKKRYVCGGKLKDGKPWGCGKKFARSDALGRHFKTE

CtCrz1    AGKQCVKRLLEEENSNNILKEATNGGDVDRGGGNDDGDDDDDNNDDQLLTSSSSKSTYS
CaCrz1    AGKQCVKRLLLEEQAN-----------------------SSGKPLATSSGVEIT--
ScCrz1    SGRRCITPLYEEARQE-------------------------------KS--GQES---
```

Calcofluor white Brightfield

Figure S3

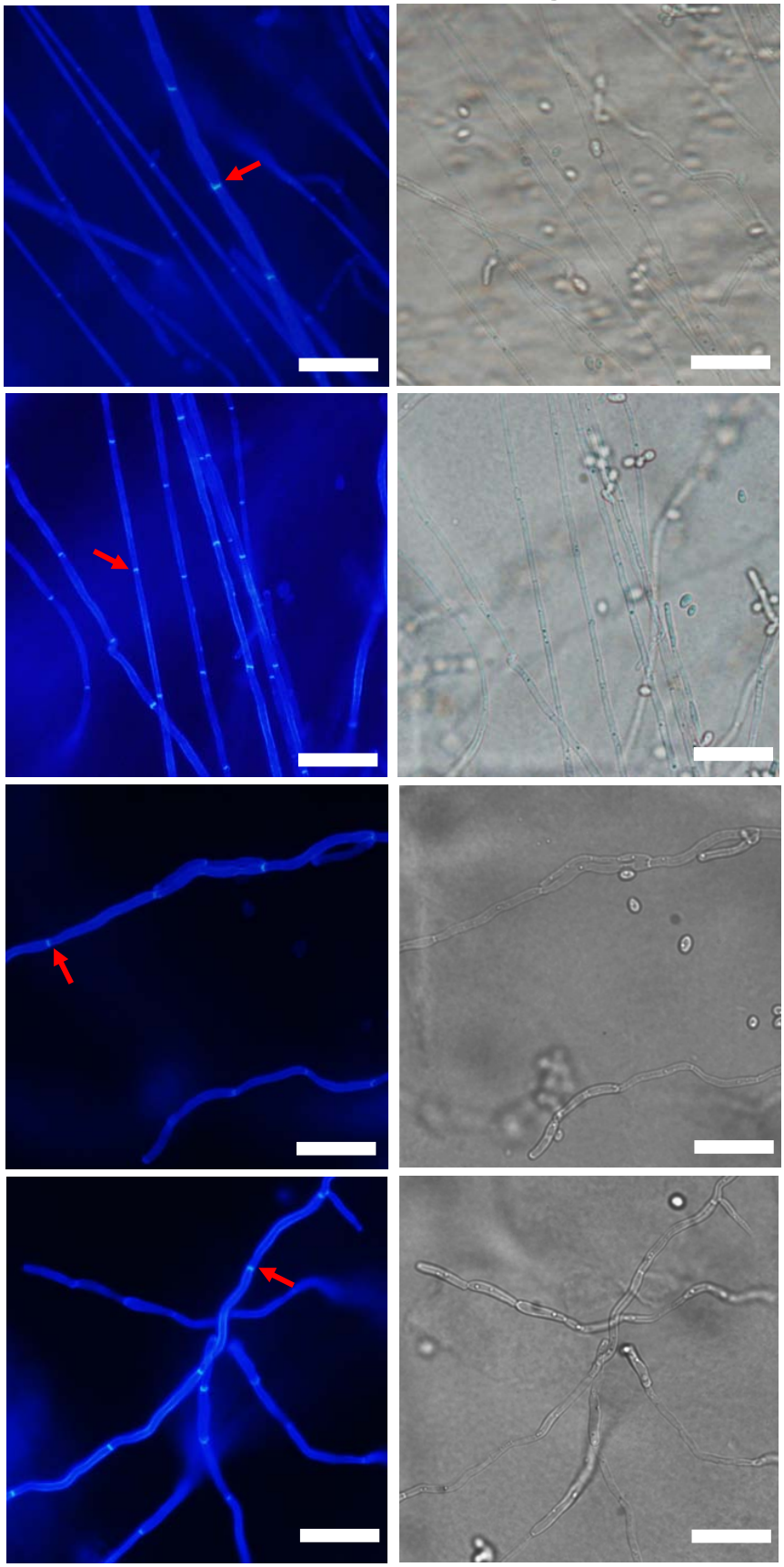


Figure S4

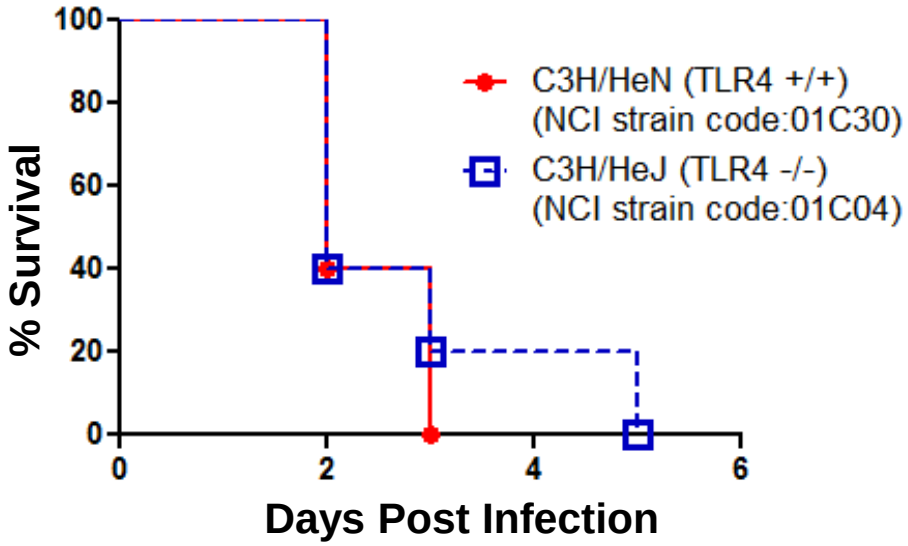
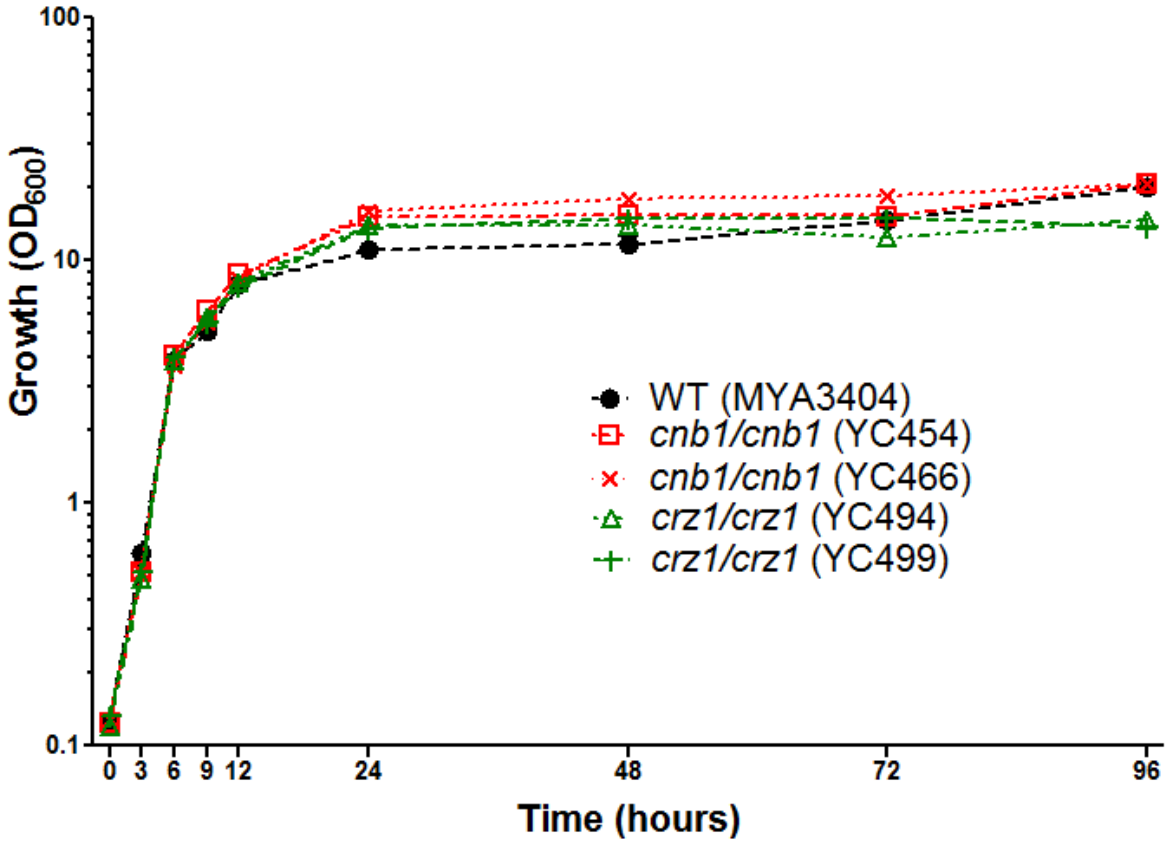

100
80
60
40
20
0
% Survival
0
2
4
6
Days Post Infection
C3H/HeN (TLR4 +/+)
(NCI strain code:01C30)
C3H/HeJ (TLR4 -/-)
(NCI strain code:01C04)

Figure S5

A



B

Strains	Doubling time (hr; in 6 hrs)
WT (MYA3404)	1.20 ± 0.02
cnb1/cnb1 (YC454)	1.18 ± 0.02
cnb1/cnb1 (YC466)	1.23 ± 0.02
crz1/crz1 (YC494)	1.19 ± 0.03
crz1/crz1 (YC499)	1.21 ± 0.03