

Table S1. Strains used in this study

Strains	Genotype
WT (BWP17)	ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG
ice2Δ/Δ	ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG ice2::ARG4/ ice2::dpl200
ice2Δ/Δ+ICE2	ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG ice2::ARG4/ ice2::dpl200, pDDB78-ICE2
WT+Ice2-GFP Erg6-RFP	ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG act1:: PACT1-ICE2-GFP-URA3 his:: pDDB78-ERG6-RFP
WT+Ice2-GFP PH3-RFP	ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG act1:: PACT1-ICE2-GFP-URA3 his:: pDDB78-PH3-RFP
WT+GFP-Dga2	ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG act1:: PACT1-GFP-DGA2-URA3
ice2Δ/Δ+GFP-Dga2	ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG ice2::ARG4/ ice2::dpl200 act1:: PACT1- DGA2-GFP-URA3
WT+GFP-Cho2	ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG act1:: PACT1-GFP-CHO2-URA3
ice2Δ/Δ+GFP-Cho2	ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG ice2::ARG4/ ice2::dpl200 act1:: PACT1- CHO2-GFP-URA3
WT+Erg6-GFP	ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG act1:: PACT1-ERG6-GFP-URA3
ice2Δ/Δ+Erg6-GFP	ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG ice2::ARG4/ ice2::dpl200 act1:: PACT1- ERG6-GFP-URA3
ice2Δ/Δ+ICE2+Erg6-GFP	ura3Δ::λimm434/ura3Δ::λimm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG ice2::ARG4/ ice2::dpl200, pDDB78-ICE2 act1:: PACT1-ERG6-GFP-URA3
WT ^a	URA3/ura3Δ::limm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG
ice2Δ/Δ ^a	URA3/ura3Δ::limm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG ice2::ARG4/ice2::dpl200

Table S2. Plasmids used in this study

Plasmids	Genotype
pRS-ArgΔ <i>Spe</i> I	Amp ^R ARG4
pDDB57	Amp ^R URA3
pGFP-URA3	Amp ^R GFP- <i>URA3</i>
pDDB78-Erg6-RFP	Amp ^R Erg6-RFP <i>HIS1</i>
pDDB78-PH3-RFP	Amp ^R PH3-RFP <i>HIS1</i>
pAU34M-GFP-Dga2	Amp ^R GFP-Dga2 <i>URA3</i>
pAU34M-GFP-Cho2	Amp ^R GFP-Cho2 <i>URA3</i>
pAU34M-Ice2-GFP	Amp ^R Ice2-GFP <i>URA3</i>
pAU34M-Erg6-GFP	Amp ^R Erg6-GFP <i>URA3</i>

Table S3. Primers used in this study

Name	Sequence (5'-3')
ICE2-5DR	TCTTTTGGATTCTTTCAGTTCATCACTCACATGTTTATATATTATTCCATTTATCA TCTTTCCAGTCACGACGTT
ICE2-3DR	ATCATTACTCAGTAATTCAGCAGCATAAAGTATTAATGTTGTTCCCATATTAATCCA ATTTGGAATTGTGAGCGGATA
ICE2-5DET	CACAACAATATTTTAGTATT
ICE2-3DET	CTACATTATCCTTTAGTAAT
ICE2-5com	TCCCCCGGGCAATAATGAAGATATTTACA
ICE2-3com	ATTTGCGGCCGAGCATTAAATTAATAACTAA
ICE2-5inner-1,2	TGTTTAGTGCTTCGTTGTTGGGA
ICE2-3inner-1	AACTTGACGTGAACCTGGTTGAG
ICE2-3inner-2	GGTTGAGGAGTTTTTGGTGGTGT
URA3-5inner	CGCGGGATTGGATGGTAT
URA3-3inner	TCTTGGCTCTTGGTTGGTG
URA3-5DET	GCCAGTGAATTGTAATACGAC
URA3-3DET	TCACACAGGAAACAGCTATGA
ICE2-5GFP	CCGCTCGAGATGCCATAATTAAACTAT
ICE2-3GFP	TCCCCCGGGTTCTACTTTCCAATGACTAG
DGA2-5GFP	TCCCCCGGGATGACAGACACATCTGACCT
DGA2-3GFP	CGCGGATCCTTATTCGACGATACTGAATT
Erg6-5RFP	CTAGCTAGCTAGATGTCTCCAGTTCAATTAGC
Erg6-3RFP	TCCCCCGGGGAATCTTTCTTTTCTAATGGTT
CHO2-5GFP	TCCCCCGGGATGACTTTCACAATCAATAA
CHO2-3GFP	CGCGGATCCTTATACATCTTTTTTATCAG

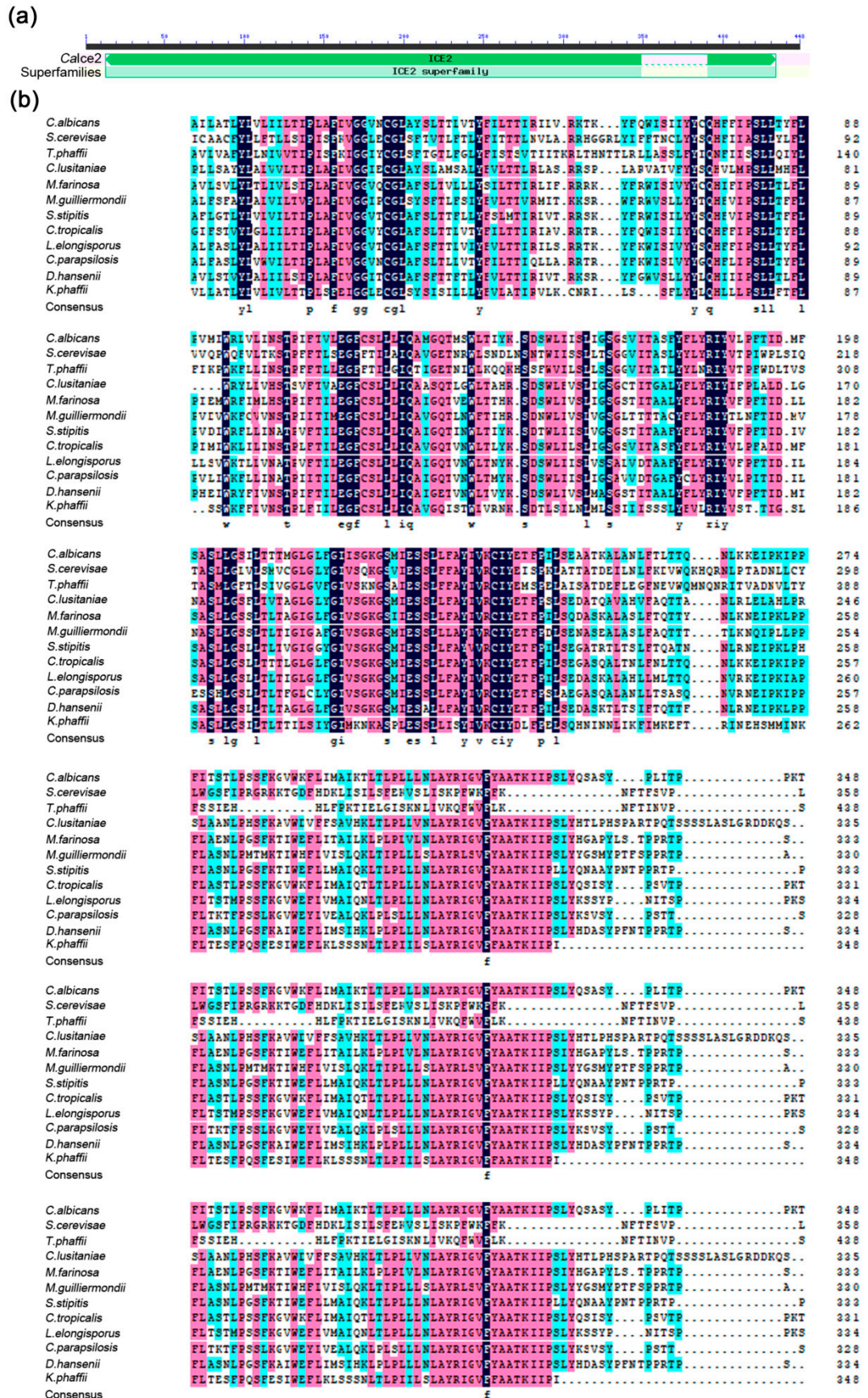


Figure S1. Conserved domain and sequence of Calce2. (a) Calce2 belongs to the ICE2 superfamily

and contains conserved Ice2 domain. Domain architecture ID: 10552640. (b) Protein sequence alignment result of *Calce2* with homologous proteins of Ice2 superfamily. The species to which the sequence belongs are shown on the left.

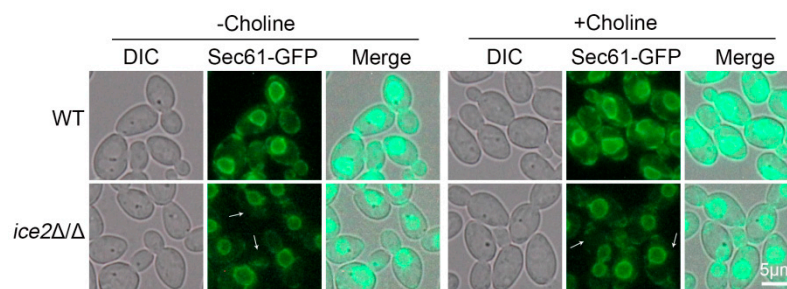


Figure S2. Addition of choline had no impact on ER morphology. Sec61-GFP: marker of ER. Cells were cultured in SC medium with 1 mM choline or without choline for 5 h. White arrow denote cortical ER deficiency.

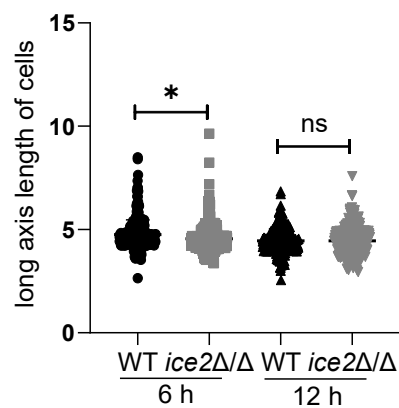


Figure S3. Long axis length of cells of WT and *ice2Δ/Δ* cultured in SC medium for 6 h and 12 h. One-way ANOVA method was used for statistical analysis, ns, no significance, *, $p < 0.05$.

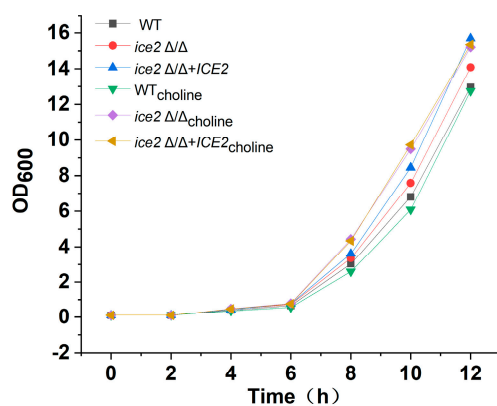


Figure S4. Growth curve of WT, *ice2Δ/Δ* and *ice2Δ/Δ+ICE2* with and without choline addition. All strains were cultured in SC medium at a constant temperature of 30°C and 160 rpm.

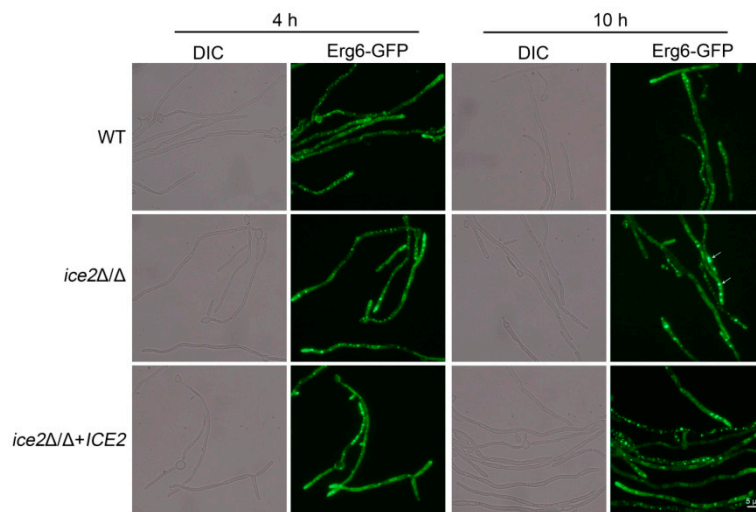


Figure S5. LDs in hyphae of WT, *ice2Δ/Δ* and *ice2Δ/Δ+ICE2*. The cells were cultured in the RPMI 1640 medium at 37 °C for 4 h and 10 h, respectively, followed by fluorescence microscopy. Erg6-GFP, the marker of LD. The white arrows indicate the LD aggregates.

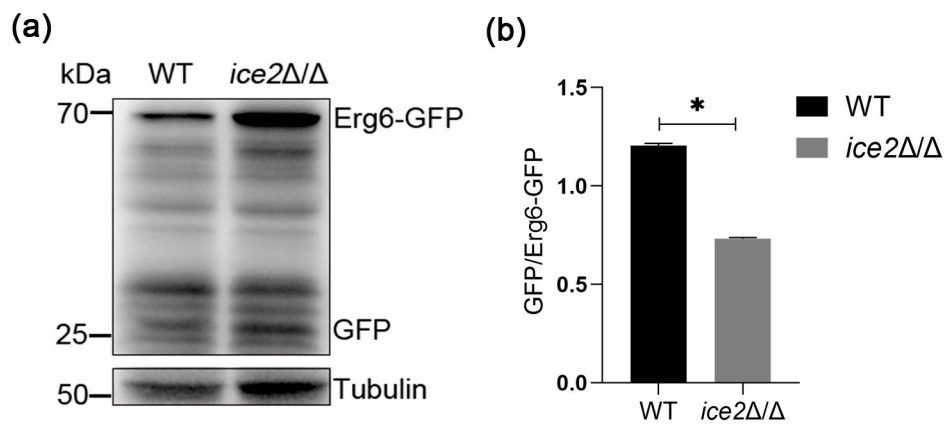


Figure S6. Deficiency of *ICE2* inhibits LD autophagy. (a) Western blots indicating the degradation of Erg6-GFP. Erg6-GFP, the marker of LD. The cells were cultured in SC medium for 5 d, followed by Western blotting. (b) The ratio of free GFP to Erg6-GFP. *, $p < 0.05$.

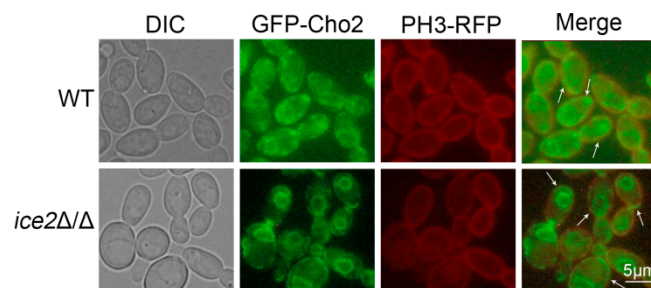


Figure S7. Co-localization of GFP-cho2 and PH3-RFP. PH3-RFP: marker of the PM. The white arrows denote localization of GFP-Cho2 on the PM.

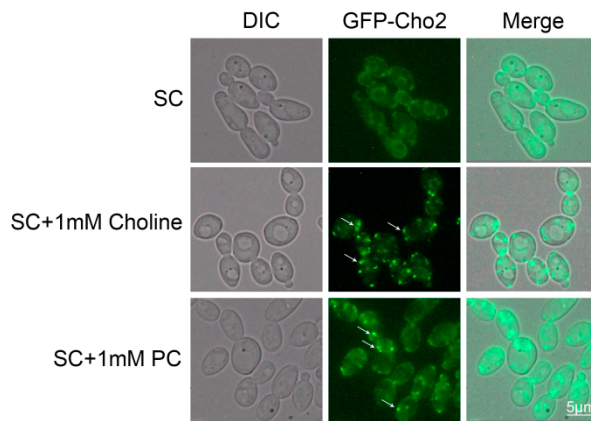


Figure S8. Addition of choline had no impact on ER morphology. Sec61-GFP: marker of ER. Cells were cultured in SC medium with 1 mM choline and without choline for 5 h. White arrow denote cortical ER deficiency.

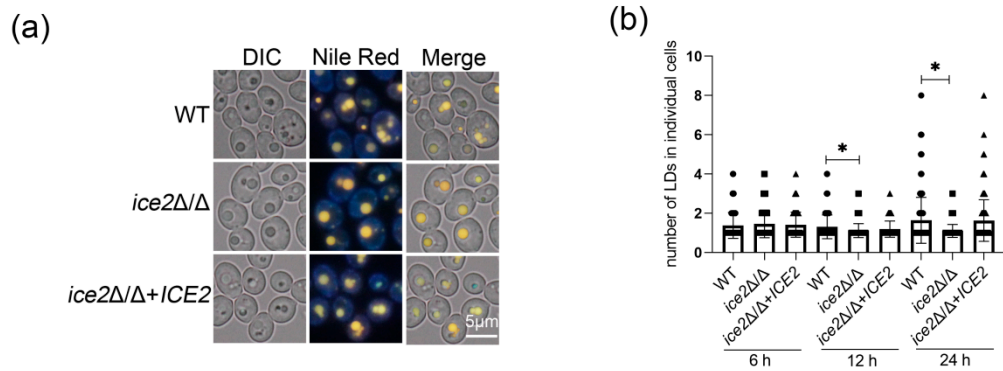


Figure S9. LD morphology at the later stage and statistical analysis of LD numbers in the tested strains. (a) LD morphology of the WT, *ice2Δ/Δ* and *ice2Δ/Δ+ICE2* cells cultured in SC medium for 24 h. (b) Number of LDs in more than 300 individual cells of WT, *ice2Δ/Δ* and *ice2Δ/Δ+ICE2* cultured in SC medium for 6 h, 12 h and 24 h. One-way ANOVA was used for statistical analysis, *, $p < 0.05$.

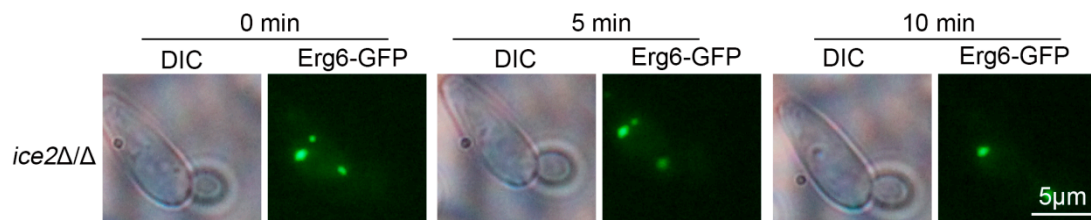


Figure S10. Dynamic LD fusion in the *ice2Δ/Δ* cells. The cells were cultured in the choline-rich SC medium for 5 h and then transferred to the choline-free SC medium for further observation. Erg6-GFP: the marker of LD.

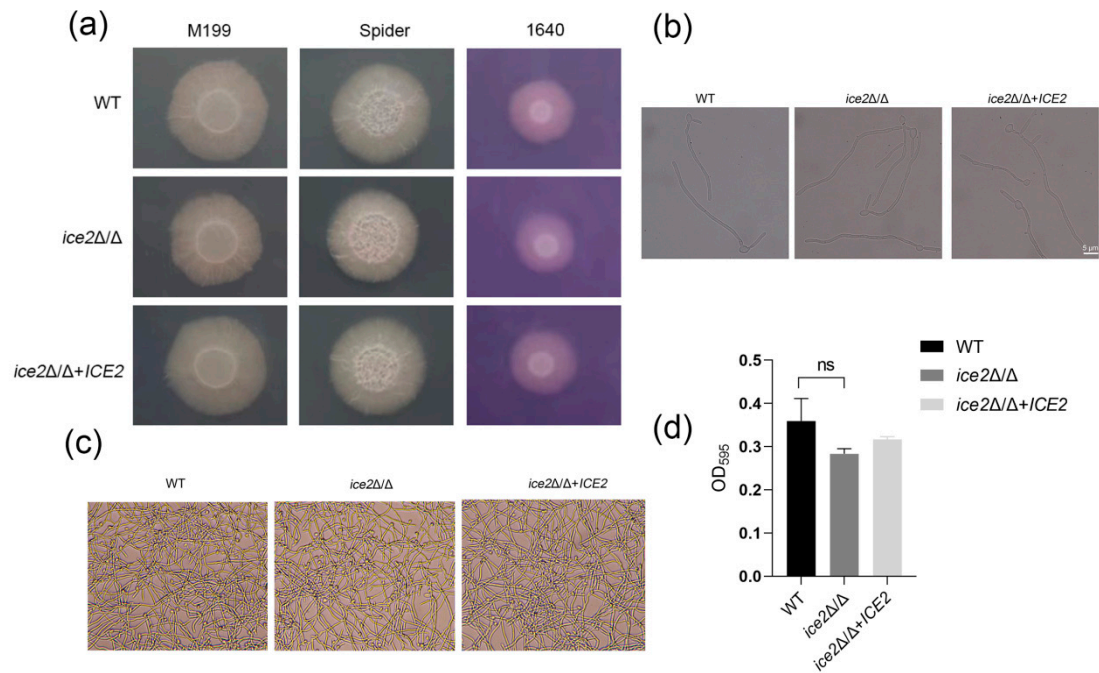


Figure S11. Deletion of *ICE2* has no impact on morphogenesis of *C. albicans*. (a) and (b) Deletion of *ICE2* did not affect mycelial development. For solid hyphal induction, 3 μ L cell solution of WT, *ice2Δ/Δ* and *ice2Δ/Δ+ICE2* with OD₆₀₀ of 0.3 were added to M199, Spider and 1640 solid medium and cultured at 37 °C for 5 d. For liquid hyphal induction, cells were culture in 1640 medium at 37 °C for 4 h. (c) *ICE2* deficiency did not affect the adhesion of *C. albicans* in vitro. The polyethylene porous plate was used for adhesion in vitro. (d) Biomass determination of adhesion cells of *C. albicans*. The adherent cells stained with crystal violet were washed from the polyethylene pore plate with glacial acetic acid, and the supernate was taken to measure OD₅₉₅, ns, no significance.

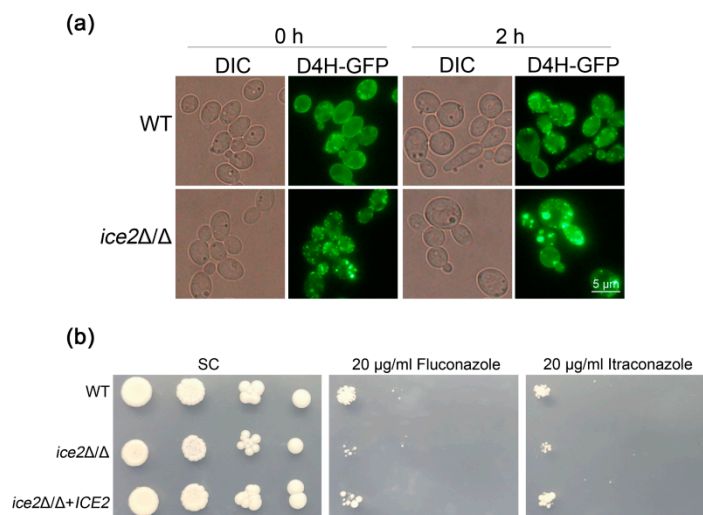


Figure S12. Effect of *ICE2* deletion on sterol distribution and sensitivity to azole. (a) Loss of *ICE2* leads to intracellular accumulation of sterols. D4H-GFP[63], the marker of sterols. Cells were cultured in SC medium. (b) Loss of *ICE2* leads to the increased sensitivity to fluconazole and itraconazole.