

Table S1. Comparison of amino acid sequences of P-body and SG components in *C. boidinii* with those in *S. cerevisiae*.

	protein	identity (%)	similarity (%)
P-body components	Edc3	25	57
SG components	Pab1	56	76
	Pbp1	23	55

Table S2. Intracellular localization of CbHog1-mCherry and CbEdc3-Venus derived from quantification of 50 cells after high temperature stress for 30 min.

	Dot number / 50 cells
Colocalized	43.3 ± 4.0
CbHog1-mCherry only	248.0 ± 10.5
Pab1-Venus only	113.7 ± 6.0

Errors represent the S.D. of triplicate measurements.

Table S3. Yeast strains used in this study.

Strain	Genotype	Reference
<i>S. cerevisiae</i>		
BY4741	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	(Brachmann et al., 1998)
HS01	BY4741, <i>Schog1::ScHOG1-GFP, LEU2</i>	This study
HS02	BY4741, (<i>P_{ScHOG1}CbHOG1-Venus, LEU2</i>	This study
HS03	BY4741, <i>Schog1::ScHOG1(1-350)-Venus, LEU2</i>	This study
<i>C. boidinii</i>		
AOU1	Wild type	(Tani et al., 1985)
TK62	<i>ura3</i>	(Sakai et al., 1991)
HC01	TK62, <i>Cbhog1Δ::URA3</i>	This study
HC02	HC01, <i>ura3</i>	This study
HC03	HC02, <i>ura3::(P_{CbHOG1}CbHOG1-Venus, URA3)</i>	This study
HC04	HC02, <i>ura3::(P_{CbACT1}ScHOG1-Venus, URA3)</i>	This study
HC05	HC02, <i>ura3::(P_{CbHOG1}CbHOG1Δ(1-10)-Venus, URA3)</i>	This study
HC06	HC02, <i>ura3::(P_{CbHOG1}CbHOG1Δ(1-17)-Venus, URA3)</i>	This study
HC07	HC02, <i>ura3::(P_{CbHOG1}CbHOG1Δ(1-20)-Venus, URA3)</i>	This study
HC08	HC02, <i>ura3::(P_{CbHOG1}CbHOG1Δ(1-30)-Venus, URA3)</i>	This study
HC09	HC02, <i>ura3::(P_{CbHOG1}CbHOG1Δ(1-41)-Venus, URA3)</i>	This study
HC10	HC02, <i>ura3::(P_{CbHOG1}CbHOG1Δ(348-398)-Venus, URA3)</i>	This study
HC11	HC02, <i>ura3::(P_{CbHOG1}CbHOG1Δ(318-398)-Venus, URA3)</i>	This study
HC12	HC02, <i>ura3::(P_{CbHOG1}CbHOG1Δ(300-398)-Venus, URA3)</i>	This study
HC13	HC02, <i>ura3::(P_{CbHOG1}CbHOG1(1-20)-Venus, URA3)</i>	This study
HC14	HC02, <i>ura3::(P_{CbHOG1}CbHOG1(1-30)-Venus, URA3)</i>	This study
HC15	HC02, <i>ura3::(P_{CbHOG1}CbHOG1(1-40)-Venus, URA3)</i>	This study
HC16	HC02, <i>ura3::(P_{CbHOG1}CbHOG1(1-50)-Venus, URA3)</i>	This study
HC17	HC02, <i>ura3::(P_{CbHOG1}CbHOG1(346-398)-Venus, URA3)</i>	This study
HC18	HC02, <i>ura3::(P_{CbHOG1}CbHOG1Δ(25-28)-Venus, URA3)</i>	This study
HC19	HC02, <i>ura3::(P_{CbHOG1}CbHOG1Δ(35-40)-Venus, URA3)</i>	This study
HC20	HC02, <i>ura3::(P_{CbHOG1}CbHOG1Δ(38-45)-Venus, URA3)</i>	This study
HC21	HC02, <i>ura3::(P_{CbHOG1}CbHOG1Δ(39-45)-Venus, URA3)</i>	This study
HC22	HC02, <i>ura3::(P_{CbHOG1}CbHOG1Δ(40-45)-Venus, URA3)</i>	This study
HC23	HC02, <i>ura3::(P_{CbHOG1}CbHOG1Δ(43-45)-Venus, URA3)</i>	This study
HC24	HC02, <i>ura3::(P_{CbACT1}ScHOG1-K52R-Venus, URA3)</i>	This study
HC25	HC02, <i>ura3::(P_{CbACT1}ScHOG1-T174A/Y176F-Venus, URA3)</i>	This study
HC26	HC02, <i>ura3::(P_{CbACT1}ScHOG1 Venus-CAAXRas2, URA3)</i>	This study
HC27	HC02, <i>ura3::(P_{CbACT1}ScHOG1(1-350)-Venus, URA3)</i>	This study
HC28	HC02, <i>ura3::(P_{CbHOG1}CbHOG1-Sc(351-435)-Venus, URA3)</i>	This study
BUL	<i>ura3, leu2</i>	(Sakai and Tani 1992)
SK01	BUL, <i>leu2::(P_{CbPAB1}CbPAB1-Venus, LEU2), ura3::(P_{CbPBP1}CbPBP1-mCherry, URA3)</i>	This study
HC30	BUL, <i>leu2::(P_{CbPAB1}CbPAB1-Venus, LEU2), ura3::(P_{CbHOG1}CbHOG1-mCherry, URA3)</i>	This study
HC31	HC02, <i>ura3::(P_{CbHOG1}CbHOG1(K50R)-Venus, URA3)</i>	This study
HC32	BUL, <i>leu2::(P_{CbPAB1}CbPAB1-Venus, LEU2), ura3::(P_{CbHOG1}CbHOG1(K50R)-mCherry, URA3)</i>	This study
HC33	HC02, <i>ura3::(P_{CbEDC3}CbEDC3-Venus, URA3)</i>	This study
HC34	BUL, <i>leu2::(P_{CbEDC3}CbEDC3-Venus, LEU2), ura3::(P_{CbHOG1}CbHOG1(K50R)-mCherry, URA3)</i>	This study
HC35	HC02, <i>ura3::(P_{CbPAB1}CbPAB1-Venus, URA3)</i>	This study
<i>P. pastoris</i>		
PPY12	<i>arg4 his4</i>	(Sakai et al., 1998)
HP01	PPY12, <i>Pphog1Δ::Zeo^r</i>	This study
HP02	HP01, <i>arg4::(P_{PpHOG1}PpHOG1-YFP, ARG4)</i>	This study
<i>S. pombe</i>		
FY14931	<i>ade6-216 leu1-32 lys1-131 ura4-D18 sty1::sty1-GFP-HA, Kan^r</i>	Purchased from NBRP

Table S4. Primers used in this study.

Designation	DNA sequence (5' → 3')
CbHOG1_UP_Fw	GGGCGAATTGGGTACGGCGCCGATGGAAATATGGAAACAGAGATG
CbHOG1_UP_Rv	GGGGGGGCCCGGTACCACCCATACCAACTGGATTTAAA
CbHOG1_DOWN_Fw	CACCGCGGTGGAGCTCAACAACAGCTGTAACAATAATTCAAG
CbHOG1_DOWN_Rv	ACAAAAGCTGGAGCTGAATTCACATGTCCAACAACAACAAC
CbHOG1_SPR_Fw	GATGGAAATATGGAAACAGAGATG
CbHOG1_SPR_Rv	CAACATGTCCAACAACAACAAC
CbHOG1_Vn_Fw	CTTTTGCTCACATGTGATGGAAATATGGAAACAGAGATGAAG
CbHOG1_Vn_Rv	AGAAACCATGTCGACCAGCTGTTGTTGTTGTTGTTGTT
P _{CbACT1} ScHOG1_Vn_Fw	ATATTACAAAAGTCGACATGACCACTAACGAGGAATTCATTAG
P _{CbACT1} ScHOG1_Vn_Rv	TTAGAAACCATGTCGACCTGTTGGAACCTATTAGCGTACTG
CbHOG1_Vn_Δ(1-10)_Fw	TTTGTTACTATATTTGAAACAACAATAAGATACTC
CbHOG1_Vn_Δ(1-17)_Fw	ACAAATAGATACTCAGATTTAAATCCAG
CbHOG1_Vn_Δ(1-20)_Fw	TACTCAGATTTAAATCCAGTTGGTATGG
CbHOG1_Vn_Δ(1-30)_Fw	GCATTTGGTTTAGTTTGTGCAGC
CbHOG1_Vn_Δ(1-41)_Fw	TTAACAAATCAAATGTTGCAATTAATAAAAAGTTATGAAACCTTTTT C
CbHOG1_Vn_Δ(348-398)_Rv	TTCAACTTGATGGAAATCTAAGATTTAC
CbHOG1_Vn_Δ(318-398)_Rv	ATCGAATTTTTCTTCAGCAACTGGTTC
CbHOG1_Vn_Δ(300-398)_Rv	GTATTCATGTTCTAAAGCTTGTCTGC
CbHOG1_Vn_ΔN_Rv	CATTTTATTTATATCTATATATATATATATGTCTATTGTATATGTCTATT GTC
HOG1_Vn_ΔC_Fw	GTCGACATGGTTTCTAAAGGTG
CbHOG1_Vn_(1-20)_Rv	TCTATTTGTTGTTTCAAATATAGTACCAAATATTTGAGTTC
CbHOG1_Vn_(1-30)_Rv	ACCCATACCAACTGGATTTAAATCTG
CbHOG1_Vn_(1-40)_Rv	ATCCTTTGCTGCACAACTAAACC
CbHOG1_Vn_(1-50)_Rv	TTTAATTGCAACATTTTGATTTGTTAATTTATCCTTTGC
CbHOG1_Vn_(346-398)_Rv	GGTGCTGAAGCTGATGCTTTA
CbHOG1_Vn_Δ(25-28)_Fw	AGATTTAATGGGTGCATTTGGTTTAGTTG
CbHOG1_Vn_Δ(25-28)_Rv	TGCACCCATTAATCTGAGTATCTATTTGTTGTTCAAATATAGTAC
CbHOG1_Vn_Δ(35-40)_Fw	TGGTTTAAAATTAACAAATCAAATGTTGCAATTAATAAAAAGTTATG AA
CbHOG1_Vn_Δ(35-40)_Rv	GTTAATTTTAAACCAAATGCACCCATACCAA
CbHOG1_Vn_Δ(38-45)_Fw	TTGTGCAAATGTTGCAATTAATAAAAAGTTATGAAACCTTTTTCA
CbHOG1_Vn_Δ(38-45)_Rv	GCAACATTTGCACAACTAAACCAAATGCAC
CbHOG1_Vn_Δ(39-45)_Fw	TGCAGCAAATGTTGCAATTAATAAAAAGTTATGAAACCTTTTTCA
CbHOG1_Vn_Δ(39-45)_Rv	GCAACATTTGCTGCACAACTAAACCAAATG
CbHOG1_Vn_Δ(40-45)_Fw	GCAGCAAAGAATGTTGCAATTAATAAAAAGTTATGAAACCTTTTTCAA
CbHOG1_Vn_Δ(40-45)_Rv	GCAACATTTCTTTGCTGCACAACTAAACCAAAT
CbHOG1_Vn_Δ(43-45)_Fw	TAAATTAATGTTGCAATTAATAAAAAGTTATGAAACCTTTTTCAA
CbHOG1_Vn_Δ(43-45)_Rv	GCAACATTTAATTTATCCTTTGCTGCACAACTAAAC
CbHOG1_Vn_(K50R)_Fw	GCAATTAATAAAAAGTTATGAAACCTTTTTCAACTGCTGTATTAG
CbHOG1_Vn_(K50R)_Rv	AACTTTTTTAATTGCAACTGGCTGCACAACTAAAC
CbHOG1_Vn_(1-398)_Fw	CTGCAGGGAATTTAATCATTTTCAA
CbHOG1_Vn_(1-398)_Rv	CAGCTGTTGTTGTTGTTGTT
CbPab1_Fw	CTTTTGCTCACATGTGGCGCTTAATTTTGGCTGAATC
CbPab1_Rv	AGAAACCATGTCGACGCGGATTCACCCTTCTTC
CbPbp1_Fw	CTTTTGCTCACATGTGACGCAATTGTTGGAATAATC
CbPbp1_Rv	ACTAACCATGTCGACAAATTTATAATGTCTCTTGAACCC
CbEde3_Fw	CTTTTGCTCACATGCTACTCTTCATTCTATAGCATG
CbEde3_Rv	AGAAACCATGTCGACAGATTGATAATTTCTAAAAGTGT
ScHOG1_KD_Fw	AAAATCATGAAACCTTTTTCCACTGCA
ScHOG1_KD_Rv	TCTAATGGCAACTGGCTGAGATG

ScHOG1_nP_Fw	GGCTTTGTTTCCACTAGATACTACAGG
ScHOG1_nP_Rv	AGCCATTTGAGGGTCTTGAATTCTTGCTAG
ScHOG1_CAAX_Fw	GGTTCTGGTGGTTGTTGTATTATTTCTTAACTGCAGGGAATTTAATC ATTTTCAAC
ScHOG1_CAAX_Rv	TTTATATAATTCATCCATACCTAAAGTAATACCAG
ScHOG1_350_Fw	ATCACTGCCACCAATCTTATGG
ScHOG1CTag_Fw	CGGTAACCAGGCCATACAGTACGCTAATGAGTTCCAACAGGTGAG CAAGGGCGAGGAGCT
ScHog1CTag_Rv	GAAGTAAGAATGAGTGGTTAGGGACATTAACAAAAACACGTGACC TGTATCGCTCAAAAG
ScHog1_(351-435)_Fw	CAACAACAACAGCTGGGACAGATTGATATATCTGCC
ScHog1_(351-435)_Rv	TTAAATTCCCTGCAGTTATTTATATAATTCATCCATACCTAAAGTAA TAC
Zhog1_UPfw	CGTTTAATAAAGCCAGCCATTTAGATCGTCTAGACTAACCTATACG GGTTTAATGCC
Zhog1_UPrv	TGAAGCTATGGTGTGGAAGACTGCCGGTTTATCCTC
Zhog1_DWfw	TTTGGTCATGAGATCCTGTTCTGCCAAGGACAAGC
Zhog1_DWrv	GGCATTAAACCCGTATAGGTTAGTCTAGACGATCTAAATGGCTGGC TTTATTAACG
pPICZ_Zeo_fw	CACACCATAGCTTCAAATGTTTCTAC
pPICZ_Zeo_rv	GATCTCATGACCAAAATCCCTTAAC
PpHog1IF_fw	GGTACCCGGGGATCCCTAACCTATACGGGTTTAATGCC
PpHog1IF_rv	CAGCTCGAGACTAGTTTGATCCTCAACTTGATGGAAGTC

Table S5. Plasmids used in this study.

Designation	Description	Reference
<i>S. cerevisiae</i>		
pMO152	EGFP <i>LEU2</i>	Oku <i>et al.</i> (In press)
<i>C. boidinii</i>		
SK+SPR	<i>URA3</i>	(Sakai & Tani 1992)
pHC100	Hog1-UPregion <i>URA3</i>	This study
pHC101	Hog1-UPregion Hog1-DOWNregion <i>URA3</i>	This study
pKK001	P _{CbACT1} Venus <i>URA3</i>	(Kawaguchi <i>et al</i> 2011)
pHC200	P _{CbHOG1} <i>CbHOG1</i> -Venus <i>URA3</i>	This study
pHC300	P _{CbACT1} <i>ScHOG1</i> -Venus <i>URA3</i>	This study
pHC201	P _{CbHOG1} <i>CbHOG1</i> Δ(1-10)-Venus <i>URA3</i>	This study
pHC202	P _{CbHOG1} <i>CbHOG1</i> Δ(1-17)-Venus <i>URA3</i>	This study
pHC203	P _{CbHOG1} <i>CbHOG1</i> Δ(1-20)-Venus <i>URA3</i>	This study
pHC204	P _{CbHOG1} <i>CbHOG1</i> Δ(1-30)-Venus <i>URA3</i>	This study
pHC205	P _{CbHOG1} <i>CbHOG1</i> Δ(1-41)-Venus <i>URA3</i>	This study
pHC206	P _{CbHOG1} <i>CbHOG1</i> Δ(348-398)-Venus <i>URA3</i>	This study
pHC207	P _{CbHOG1} <i>CbHOG1</i> Δ(318-398)-Venus <i>URA3</i>	This study
pHC208	P _{CbHOG1} <i>CbHOG1</i> Δ(300-398)-Venus <i>URA3</i>	This study
pHC209	P _{CbHOG1} <i>CbHOG1</i> (1-20)-Venus <i>URA3</i>	This study
pHC210	P _{CbHOG1} <i>CbHOG1</i> (1-30)-Venus <i>URA3</i>	This study
pHC211	P _{CbHOG1} <i>CbHOG1</i> (1-40)-Venus <i>URA3</i>	This study
pHC212	P _{CbHOG1} <i>CbHOG1</i> (1-50)-Venus <i>URA3</i>	This study
pHC213	P _{CbHOG1} <i>CbHOG1</i> (346-398)-Venus <i>URA3</i>	This study
pHC214	P _{CbHOG1} <i>CbHOG1</i> Δ(25-30)-Venus <i>URA3</i>	This study
pHC215	P _{CbHOG1} <i>CbHOG1</i> Δ(35-40)-Venus <i>URA3</i>	This study
pHC216	P _{CbHOG1} <i>CbHOG1</i> Δ(38-45)-Venus <i>URA3</i>	This study
pHC217	P _{CbHOG1} <i>CbHOG1</i> Δ(39-45)-Venus <i>URA3</i>	This study
pHC218	P _{CbHOG1} <i>CbHOG1</i> Δ(40-45)-Venus <i>URA3</i>	This study
pHC219	P _{CbHOG1} <i>CbHOG1</i> Δ(43-45)-Venus <i>URA3</i>	This study
pHC220	P _{CbHOG1} <i>CbHOG1</i> (K50R)-Venus <i>URA3</i>	This study
pHC221	P _{CbHOG1} <i>CbHOG1</i> (K50R)-mCherry <i>URA3</i>	This study
pSPM001	P _{CbACT1} mCherry <i>URA3</i>	(Shiraishi <i>et al</i> 2015)
pHC400	P _{CbHOG1} <i>CbHOG1</i> -mCherry <i>URA3</i>	This study
pHC401	P _{CbPAB1} <i>CbPAB1</i> -Venus <i>URA3</i>	This study
pHC402	P _{CbPAB1} <i>CbPAB1</i> -Venus <i>LEU2</i>	This study
pHC403	P _{CbPAB1} <i>CbPBPI</i> -mCherry <i>URA3</i>	This study
pHC404	P _{CbPAB1} <i>CbEDC3</i> -Venus <i>URA3</i>	This study
pHC405	P _{CbPAB1} <i>CbEDC3</i> -Venus <i>LEU2</i>	This study
pHC500	P _{CbACT1} <i>ScHOG1</i> -Venus <i>URA3</i>	This study
pHC501	P _{CbACT1} <i>ScHOG1</i> -K52R-Venus <i>URA3</i>	This study
pHC502	P _{CbACT1} <i>ScHOG1</i> -T174A/Y176F-Venus <i>URA3</i>	This study
pHC503	P _{CbACT1} <i>ScHOG1</i> -Venus-CAAX ^{Ras2} <i>URA3</i>	This study
pHC504	P _{CbACT1} <i>ScHOG1</i> (1-350)-Venus <i>URA3</i>	This study
pHC505	P _{CbHOG1} <i>CbHOG1</i> - <i>ScHOG1</i> (351-435)-Venus <i>URA3</i>	This study
<i>P. pastoris</i>		
pPICZ A	<i>P. pastoris</i> expression vector, Zeo ^r	Purchased from Invitrogen
pHP001	PpHog1-UPregion PpHog1-DOWNregion Zeocin	This study
nNT204	pIB1 <i>ARG4</i>	(Tamura <i>et al</i> 2010)
pHP100	P _{PpHOG1} <i>PpHOG1</i> -YFP <i>ARG4</i>	This study

References for Tables S3, S4, and S5

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SUPPLEMENTARY FIGURES

Fig. S1

A

CbHog1	-MAQE-FTRTQIFGTIFETTNRYSDLNVPVGMGAFGLVCAAKDKLTNQNVAIKVKMKPFST	58
ScHog1	MTTNEEFIRTQIFGTVFEITNRYNDLNPVGMGAFGLVCSATDTLT SQPVAIKKIMKPFST	60
PpHog1	-MSQEKFTRTQIFGTIFETTARYDELNVPVGMGAFGLVCSAKDKLTEQQVAIKKIMKPFST	59
SpSty1	--MAEFIRTQIFGTCFEITTRYSDLQPIGMGAFGLVCSAKDQLTGMNVAVKKIMKPFST	57
	* ***** ** * ** .: : : *****: . * ** **: : : *****	
CbHog1	AVLAKRTYRELKLLNHLRHENLISLDDIFLSPLEDIYFVTELOQGTDLHRLTSTRPLEKQF	118
ScHog1	AVLAKRTYRELKLLKHLRHENLISLQDIFLSPLEDIYFVTELOQGTDLHRLTSTRPLEKQF	120
PpHog1	PVLAKRTYRELKLLNHLRHENLITLTDIFLSPLEDIYIVTELOQGTDLHRLTSTRPLEKQF	119
SpSty1	PVLAKRTYRELKLLKHLRHENIISLSDIFISPFEDIYFVTELOQGTDLHRLTSTRPLETQF	117
	.*****:*****:* * ***:**:* ***:**:* ***:**:* ***:**:* ***:**:*	
CbHog1	IQYFLYQILRGLKVFVHSSGVIHRDLKPSNILINENCDLKICDFGLARVQDPQMTGYVSTR	178
ScHog1	VQYFLYQILRGLKYVHSAGVIHRDLKPSNILINENCDLKICDFGLARIQDPQMTGYVSTR	180
PpHog1	IQYFLYQILRALKYVHSAGVIHRDLKPSNILINENCDLKICDFGLARIQDHQMTGYVSTR	179
SpSty1	IQYFLYQILRGLKVFVHSAGVIHRDLKPSNILINENCDLKICDFGLARIQDPQMTGYVSTR	177
	:*****.**:***:*****:*****:*****:*****:*****:*****:*****	
CbHog1	YYRAPEIMLTWQKYDTEVDIWSAGCIFAEMIEGKPLFPKGDHVFQFSIITELLGSPPKDV	238
ScHog1	YYRAPEIMLTWQKYDVEVDIWSAGCIFAEMIEGKPLFPKGDHVFQFSIITDLLGSPPKDV	240
PpHog1	YYRAPEIMLTWQKYDTEVDIWSAGCIFAEMIEGKPLFPKGDHINQFSIITELLGSPPTDV	239
SpSty1	YYRAPEIMLTWQKYNVEVDIWSAGCIFAEMIEGKPLFPGRDHVNQFSIITELLGTPPMEV	237
	*****:*****:*****:*****:*****:*****:*****:*****:*****	
CbHog1	IDTICSENTLRFVQSLPHRDPPIPFNEKFKGVEPEAIDLKMLVDFPRKRVTAEQALEHE	298
ScHog1	INTICSENTLKFVTSPLPHRDPPIPFSEKFKTVEPDAVDLLEKMLVDFPKKRITAADALAHF	300
PpHog1	IDTICSENTLRFVQSLPHREPVPLIERFQGVPEAIDLLEKMLVDFDARKKRITAEE SLAHE	299
SpSty1	IETICSKNTLRFVQSLPQKEKVPFAEKFKNADPD AIDLLEKMLVDFPRKRISAADALAHN	297
	*:****:* ***:** * ***: : : * *: * *: . : * * : * * : * * : * * : * * *	
CbHog1	YLSPYHDPDTPVAEEKFDWSFNADLDPVDTWRVMMYSEILDFHQVEGAEADALQQVQVG	358
ScHog1	YSAPYHDPDTPVADAKFDWHFNADLDPVDTWRVMMYSEILDFHKIGGSDGQIDISATFD	360
PpHog1	YLEPYHDPDTPVAEEKFDWSFNADLDPVDTWRVMMYSEILDFHQVEDQ-----	348
SpSty1	YLAPYHDPDTPVADEVFDWSFQDNDLDPVETWKVMMYSEVLSFHNMDNELQS-----	349
	* ***** ** * * : * * : * * : * * : * * : * * : * * : * * : * * :	
CbHog1	QYEHSMTLQQQQQHQIQKQQELQENELLQQQQQQQQQL-----	398
ScHog1	DQVAAATAAAQAQAQAQVQLNMAAHSNGAGTTGNDHSDIAGGNKVS DHVAANDTIT	420
PpHog1	-----	
SpSty1	-----	
CbHog1	-----	
ScHog1	DYGNQAIQYANEFQQ	435
PpHog1	-----	
SpSty1	-----	

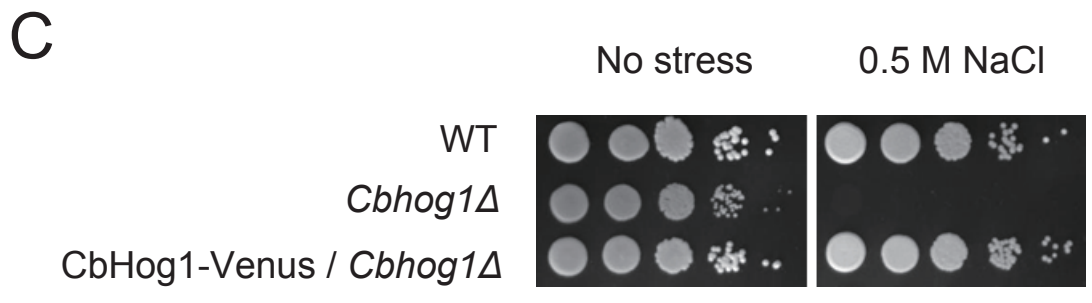
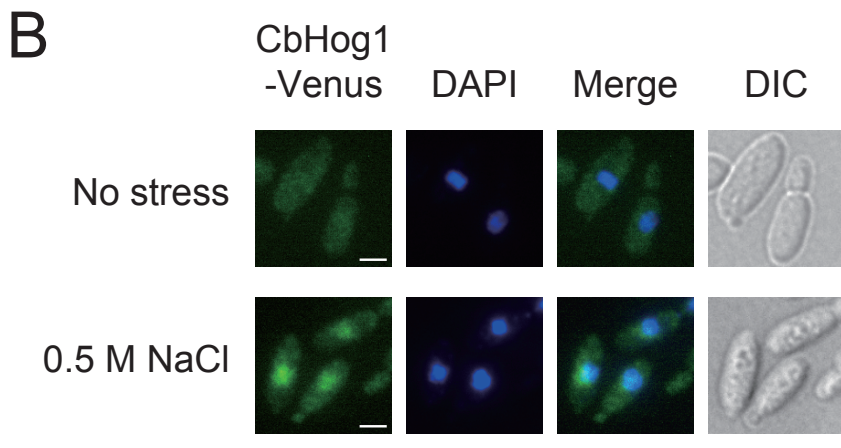


Fig. S1. CbHog1 is required for tolerance to high salt stress. (A) Alignment of the amino acid sequences of Hog1 homolog proteins from four yeast species, *C. boidinii*, *S. cerevisiae*, *P. pastoris*, and *S. pombe*. The CLUSTLW program was used to align the amino acid sequences of CbHog1, ScHog1, PpHog1 and SpSty1. The residues mutated in this study are marked in red. (B) Microscopic images of the *C. boidinii hog1Δ* strain expressing CbHog1-Venus. Cells were grown to early log phase in SD medium and shifted to SD medium supplemented with 0.5 M NaCl as high-osmolarity stress. DAPI was used for nuclear staining. Merged images were generated by combining the Venus (green) and DAPI (blue) fluorescence images. (C) Growth assay under high osmotic condition. The wild-type and the indicated mutant strains of *C. boidinii* were grown to early log phase, adjusted to $OD_{610} = 1$, and 3 μ L of each ten-fold serial dilutions were dropped onto YPD plates with or without 0.5 M NaCl. Cells were incubated at 28°C and cell growth was scored after 2 days.

Fig. S2

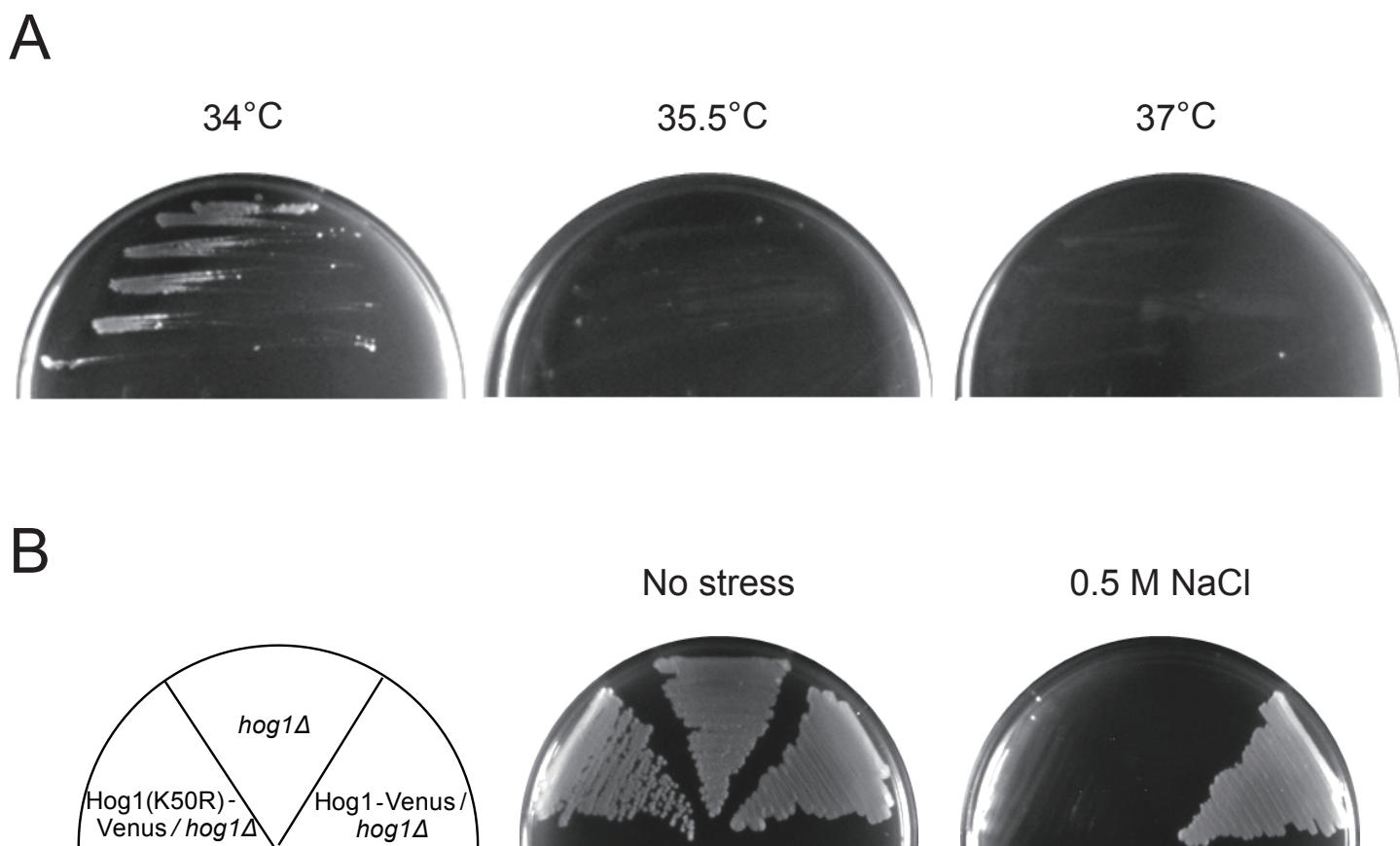
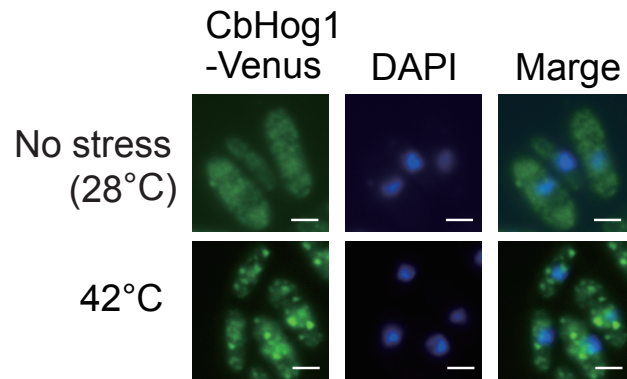


Fig. S2. Growth of *C. boidinii* strains under high-temperature or high-salt conditions.

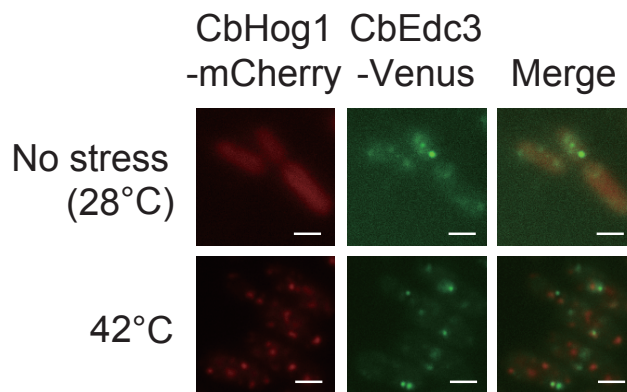
(A) Growth of *C. boidinii* under high-temperature conditions. Cells spread on the YPD plates were incubated at 34, 35.5 or 37°C and cell growth was scored after 2 days. (B) Growth assay of the *Cbhog1Δ* strain and the *Cbhog1Δ* strains expressing CbHog1-Venus or CbHog1(K50R)-Venus under high-salt condition. Cells were grown to early log phase, adjusted to $OD_{610} = 1$, and 3 μ L of each ten-fold serial dilutions were dropped onto YPD plates without or with 0.5 M NaCl. Cells were incubated at 28°C and cell growth was scored after 3 days.

Fig. S3

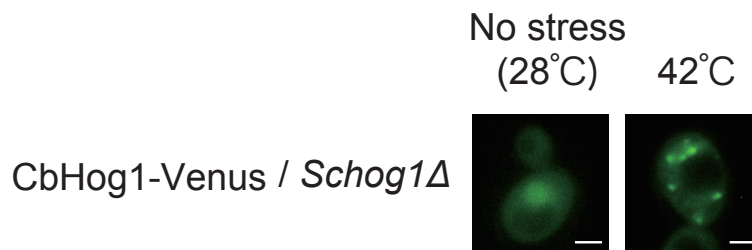
A



B



C



D

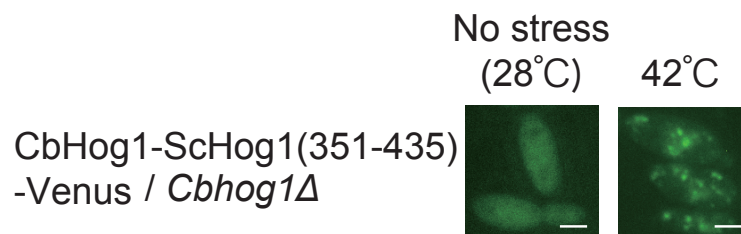


Fig. S3. Microscopic images of various strains. (A) Microscopic images of the *C. boidinii* strain expressing CbHog1-Venus. Cells were grown to early log phase, and treated with indicated high-temperature stress for 30 min. Merged images were generated by combining the Venus (green) and DAPI (blue) fluorescence images. (B) Microscopic images of the *C. boidinii* strain expressing CbHog1-mCherry and CbEdc3-Venus. Cells were grown to early log phase, and treated with indicated high-temperature stress for 30 min. Merged images were generated by combining the mCherry (red) and Venus (green) fluorescence images. (C) Microscopic images of the *S. cerevisiae hog1Δ* strain expressing CbHog1-Venus under the control of the *ScHog1* promoter. Cells were grown to early log phase in SD medium, and treated with high-temperature stress (42°C, 30 min). (D) Microscopic images of the *Cbhog1Δ* strain expressing CbHog1-ScHog1(351-435)-Venus. Cells were grown to early log phase in SD medium, and treated with high-temperature stress (42°C, 30 min).

Fig. S4

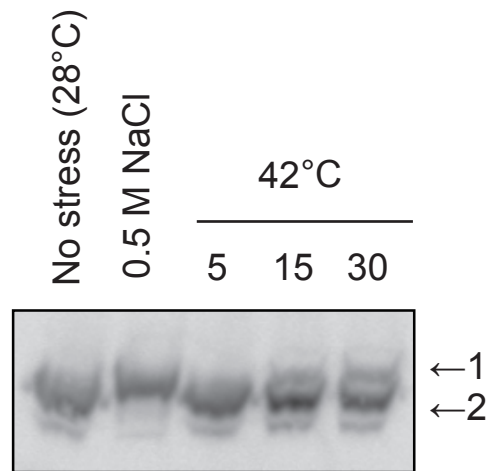


Fig. S4. CbHog1 is partially phosphorylated upon high-temperature stress. Immunoblot analysis of Venus-tagged Hog1 with Phos-tag SDS-PAGE under high-osmolarity (0.5 M NaCl, 5 min) or high-temperature stress (42°C, 5, 15 or 30 min). 1, phosphorylated CbHog1-Venus; 2, CbHog1-Venus.