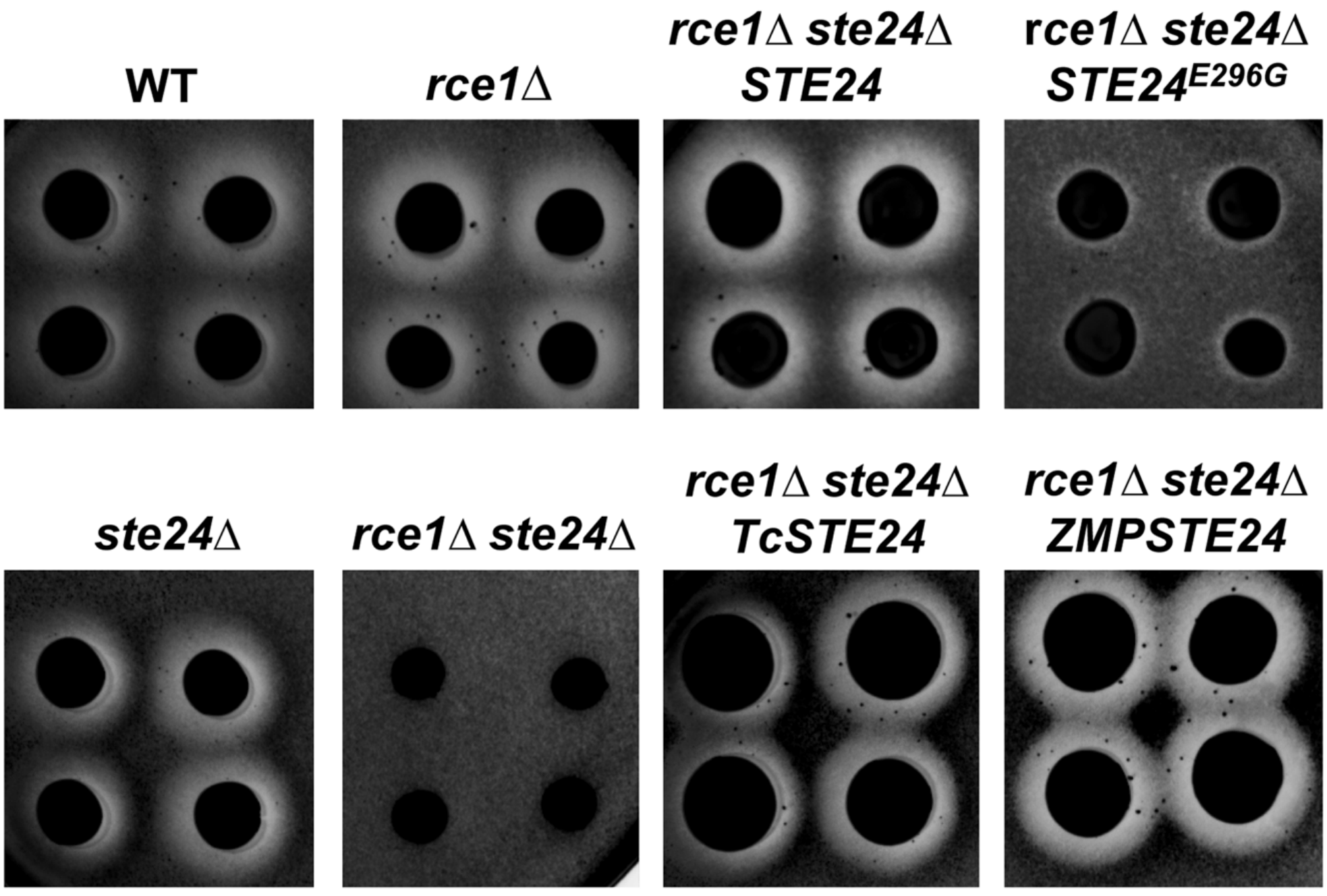
FIGURE LEGENDS SUPPLEMENTARY DATA

Figure S1. *Halo assay with catalytic active and inactive Ste24* - Different *MAT*a *cells* (*wild-type*, *ste24*Δ, *rce1*Δ, *ste24*Δ *rce1*Δ, *ste24*Δ *rce1*Δ *pRS415ste24*, *ste24*Δ*rce1*Δ, *pRS415ste24*^{E296G}, *ste24*Δ *rce1*Δ *pJJH71-ZMPSTE24* and *ste24*Δ *rce1*Δ *pJJH71-TcSTE24)* were grown overnight in liquid YPD medium and adjusted to a final concentration of 5×10^9 cells/ml. Then, 1,5 µl of each suspension was spotted onto YPD media at 42°C containing 0.04% Triton X-100 and 2×10^6 cells of the *MAT*α *sst2*Δ strain cells. Cells were incubated at 30°C for 3 days. Grayscales were inverted for a better visualization of the halos.

Figure S2. *Immunoblot detecting Chs3-13Myc in transformed wild-type and ste24*Δ *cell extracts*. Total cellular extracts obtained from wild-type (WT) and *ste24*Δ cells expressing Chs3-13Myc from centromeric plasmids were subjected to SDS-PAGE and subsequently blotted onto nitrocellulose membranes for immunostaining with anti-cMyc antibodies. Standard proteins are indicated with molecular masses given in kDa. MC, mock control using wild-type yeast cell transformed with the empty pRS415 vector.


WT
rce1Δ
rce1Δ ste24Δ STE24
rce1Δ ste24Δ STE24E296G
ste24Δ
rce1Δ ste24Δ
rce1Δ ste24Δ TcSTE24
rce1Δ ste24Δ ZMPSTE24

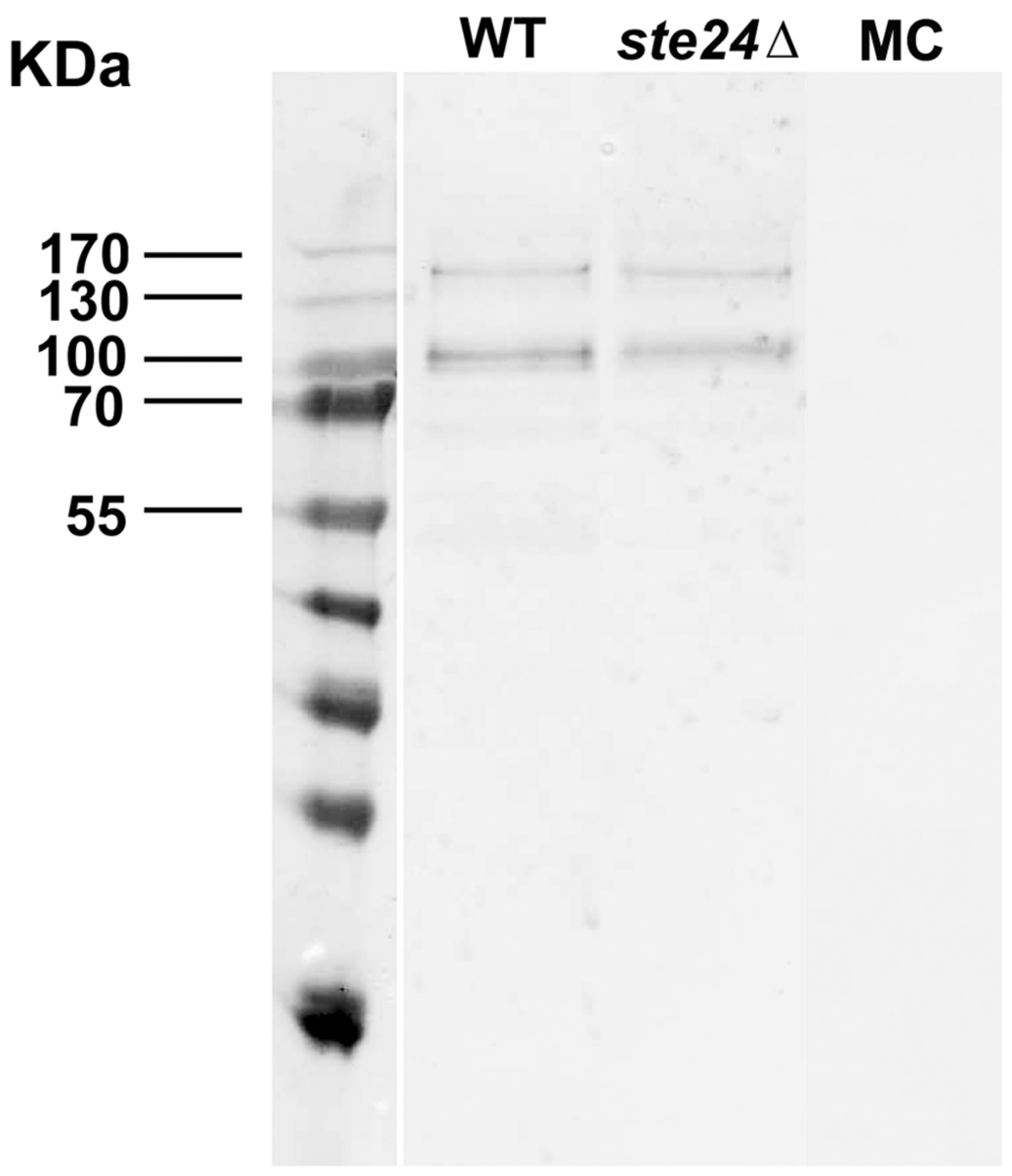

KDa
WT
ste24Δ
MC
170
130
100
70
55

Table S1. Oligonucleotide primers

Primer	Sequence 5' →3'	Amplified sequence
Primer for amplifying different cDNAs		
chs3pro(*Kpn*I)-F	TACTCAGGTACCAAAACGCGTTATTGGCGCTGG	*chs3* and *chs3* promoter
chs3(*Sac*II)-R	TACTCACCGCGGTGCAACGAAGGAGTCACTTTC	*chs3* without stop codon
chs3(*EcoR*I)-R	TACTCAGAATTCAAAACGCGTTATTGGCGCTGG	*chs3*
13Myc(*Sac*II)-F	TACTCACCGCGGCCCGGGTTAATTAACGGT	13Myctag
13Myc(*Sac*I)-R	TACTCAGAGCTCTTCACTAGTGATTGATAAATT	13Myctag
GALpro(*Kpn*I)-F	TACTCAGGTACCAGATCTGTAAAGAGCCCCATT	GAL1 promoter
GALpro_ATG_Flag (*Sal*I)-R	TACTCAGTCGACCTTGTCGTCATCGTCTTT GTAGTCAGACATTTTGAGATCCGGGTT	GAL1 promoter
GFP(*Sac*II)-F	TACTCACCGCGGCGGATCCCCGGGTTAATTAA	*gfp*
GFP(*Sac*I)-R	TACTCAGAGCTCTATTCTGGGCCTCCATGTCGC	*gfp*
ste24-F	TACTCAGTCGACTTTGATCTTAAGACGATTCTC	*ste24*
ste24-R	TACTCACCGCGGTTAGTTTTTCTTCTTTTCACT	*ste24*
ste24-F(BamHI)	TACTCAGGATCCATGTTTGATCTTAAGACGATT	*ste24*
ste24_9His-R(SpeI)	TACTCAGCATGCCTAGTGGTGGTGGTGGTGGTGG TGGTGGTGGTTTTTCTTCTTTTCACT	*ste24*
Zmpste24(BamHI)-F	TACTCAGGATCCATGGGGATGTGGGCATCGCTG	*Zmpste24*
Zmpste24(SphI)-R	TACTCAGCATGCTCAGTGTTGCTTCATAGTTTT	*Zmpste24*
Tcste24(BamHI)-F	TACTCAGGATCCATGATCACCTTAAACGAGGTT	*Tc_ste*24
TcSTE24(SphI)-R	TACTCAGCATGCTTAGTCCTTCTTCTGCAGTGC	*Tc_ste*24
Primer for homologous recombination		
chs4^{C693S}-F	AGAAGGATAAACAAGGTAAAAAAAAAAAAGA CTCTGTAATTATGTAGGGCGCGCCAC TTCTAAA	*chs4*^{C693S}
chs4^{C693S}-R	GTGTAAACTGTTGCACCTATAAAGAATGAAAAC AATCTAGCCGCATAGGCCACTAGTGGATCTG	*chs4*^{C693S}
Primer for site directed mutagenesis		
ste24^{E269G}-F	CGGCTGTTTTGGCCCATGGAATCGGTCACTGGC	*ste24*^{E269G}
ste24^{E269G}-R	GCCAGTGACCGATTCCATGGGCCAAAACAGCCG	*ste24*^{E269G}
Primer for yeast two-hybrid analyses		
chs3-C1 (*EcoR*I)-F	TAGTCAGAATTCATGACCGGCTTGAATGGAGAT	*chs3*-C1 (aa 1-165)
chs3-C1 (*BamH*I)-R	TACTCAGGATCCTGTATCATTCGTTTCCTTTCT	*chs3*-C1 (aa 1-165)
chs3-C3 (*EcoR*I)-F	TAGTCAGAATTCACCGTTTGTAGTAGTTCGAAA	*chs3*-C3 (aa 226-452)
chs3-C3 (*BamH*I)-R	TAGTCAGGATCCATCAGAGGCAATACAACCGAC	*chs3*-C3 (aa 226-452)
chs3-C4 (*EcoR*I)-F	TAGTCAGAATTCCGTTGGACTGTAGCTAGGAAA	*chs3*-C4 (aa 476-1000)
chs3-C4 (*BamH*I)-R	TAGTCAGGATCCCGTAGAATTAATCCATCTTCG	*chs3*-C4 (aa 476-1000)
chs3-C7 (*EcoR*I)-F	TAGTCAGAATTCAAATTTGATGACTTCTCATGG	*chs3*-C7 (aa 1109-1165)
chs3-C7 (*BamH*I)-R	TAGTCAGGATCCTGCAACGAAGGAGTCACTTTC	*chs3*-C7 (aa 1109-1165)
ste24-S2 (*EcoR*I)-F	TAGTCAGAATTCAGACAGTACCAGAAGCTATCT	*ste24*-S2 (aa 36–95)
ste24-S2 (*BamH*I)-R	TACTCAGGATCCTTTAGGGAAGAGGTCGTATTT	*ste24*-S2 (aa 36–95)
ste24-S6 (*EcoR*I)-F	TAGTCAGAATTCTTTAATAAGTTCACTCCATTG	*ste24*-S6 (aa 221-304)
ste24-S6 (*BamH*I)-R	TACTCAGGATCCTTTTTGCCAGTGACCGATTTC	*ste24*-S6 (aa 221-304)
ste24-S8 (*EcoR*I)-F	TAGTCAGAATTCAGTTTAATTTCCAGAACTCAT	*ste24*-S8 (aa 384-453)
ste24-S8 (*BamH*I)-R	TACTCAGGATCCGTTTTTCTTCTTTTCACTAAC	*ste24*-S8 (aa 384-453)

Table S2. Plasmids

Plasmid	Description	Source/reference
pAG503	centromeric, G418 resistance	Laboratory collection
pAG503 *CHS3*	Chs3 under endogenous promoter control	This study
pAG503 *GAL-STE24*↑	Ste24 under Gal1 promoter control	This study
pRS415	centromeric, *Leu2*	Stillman D. J.
pRS415ste24	Ste24, endogenous promoter	This study
pRS415ste24^{E269G}	Ste24^{E269G}, endogenous promoter	This study
pRS415 *CHS3*	Chs3, endogenous promoter control	This study
pRS415 *CHS3* Myc	Chs3, endogenous promoter, C-terminal 13Myc-tag	This study
pGREG576	centromeric, G418 resistance, *Ura3*, Gal promoter, GFP	Euroscarf (Jansen *et al.*, 2005)
pGREG576 CHS4	Chs4, Gal promoter, N-terminal GFP	This study
pGREG576 CHS4^{C693S}	Chs4^{C693S}, Gal promoter, N-terminal GFP	This study
pJJH71	2μ,*PFK2* promoter, *Ura3*	(Raben *et al.*, 1995)
pJJH71 ScSTE24	STE24, *PFK2* promoter	This study
pJJH71 ZMPSTE24	ZMPSTE24, *PFK2* promoter	This study
pJJH71 TcSTE24	TcSTE24, *PFK2* promoter	This study
pFA6a-GFP(S65T)-KanMX4	G418 for homologous recombination	Laboratory collection
pGADT7	Bait vector, Amp^r, *LEU2,* DNA-activation domain	Clontech
pGAD-C1	Bait vector, chs3 aa 1-165 (C1)	This study
pGAD-C3	Bait vector, chs3 aa 226-452 (C3)	This study
pGAD-C4	Bait vector, chs3 aa 476-1000 (C4)	This study
pGAD-C7	Bait vector, chs3 aa 1109-1165 (C7)	This study
pGAD-S2	Bait vector, ste24 aa 36–95 (S2)	This study
pGAD-S6	Bait vector, ste24 aa 221-304 (S6)	This study
pGAD-S8	Bait vector, ste24 aa 384-453 (S8)	This study
pGBRT7	Prey vector, Kan^r, *TRP1*, DNA-binding domain	Clontech
pGBR-C1	Prey vector, chs3 aa 1-165 (C1)	This study
pGBR-C3	Prey vector, chs3 aa 226-452 (C3)	This study
pGBR-C4	Prey vector, chs3 aa 476-1000 (C4)	This study
pGBR-C7	Prey vector, chs3 aa 1109-1165 (C7)	This study
pGBR-S2	Prey vector, ste24 aa 36–95 (S2)	This study
pGBR-S6	Prey vector, ste24 aa 221-304 (S6)	This study
pGBR-S8	Prey vector, ste24 aa 384-453 (S8)	This study