SI Appendix

Supplementary Materials and Methods

Chromatin immunoprecipitation

Cells were grown to an OD600 of 0.6 – 0.9 at 23ºC, diluted to an OD600 of 0.2 and shifted to 37ºC for 4 hours. 30 OD cells were cross-linked with 1% formaldehyde for 30 min at room temperature. The cross-linking reaction was stopped by adding glycine to a final concentration of 125 mM. Cells were washed twice with TBS and once with spheroplasting buffer (1 M sorbitol, 100 mM KPO_4 and 30 mM β-mercaptoethanol) and then resuspended in spheroplast buffer with 40 unit/ml zymolyase and incubated for 30 minutes at 30°C. Spheroplasts were washed twice with spheroplast buffer and then resuspended in MNase digestion buffer (10 mM Tris–HCl [pH 7.5], 50 mM NaCl, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.075% NP-40, 1 mM β-mercaptoethanol) supplemented with 40 units of micrococcal nuclease. Chromatin was digested for 30 minutes at 30°C. The digestion was stopped by placing the samples on ice and adding EDTA to a final concentration of 10 mM. 1 ml of ice-cold lysis buffer (50 mM HEPES, 5 mM EDTA, 140 mM NaCl, 1% Triton X-100 and 0.1% sodium deoxycholate) containing protease inhibitors was added to the samples. To remove cell debris, the lysate was centrifuged and the supernatant retained. From fragmented chromatin samples, aliquots were taken as an input control for the quantitative real-time PCR and as a control for the average chromatin fragment size. The remaining chromatin samples were pre-cleared with Dynabeads protein G (Thermo Fisher) for 2 h at 4°C and incubated overnight with 4 µl of α-Myc antibody. Dynabeads Protein G was subsequently added, and samples were incubated for 2 hours at 4ºC. The immunoprecipitates were recovered with a magnet and washed with 1 ml of the following buffers: 1) low salt solution (0.1% (v/v) SDS, 1% (v/v) Triton X-100, 2 mM EDTA, 20 mM Tris (pH 8.1) 150 mM NaCl); 2) high salt solution (0.1% (v/v) SDS, 1% Triton (v/v) X-100, 2 mM EDTA, 20 mM Tris (pH 8.1) 500 mM NaCl); 3) LiCl buffer (0.25 M LiCl, 1% (v/v) Nonidet P-40, 1% (w/v) sodium deoxycholate, 1 mM EDTA, 10mM Tris pH 8.1), twice 1x TE. The precipitated chromatin was resuspended in 100 μl 10% Chelex 100 resin (Bio-Rad) and boiled for 10 minutes to reverse cross-linking. Protein was digested with proteinase K, and samples were then RNase-treated, and IP DNA was obtained by collecting the supernatant after centrifugation. To extract the input DNA, the samples were incubated overnight at

65°C to reverse cross-linking. They were then treated 1 h with proteinase K and 1 h with RNase following by extraction with 10% Chelex 100 resin. Samples were subsequently analyzed by quantitative real-time PCR. Primer sequences are available upon request.

Yeast protein extracts, co-immunoprecipitation, and Western blotting

For Western blot analysis, 8 OD of cells were harvested, washed once with TBS and resuspended in 100µl lysis puffer (1x PBS containing 0.1% NP-40, 1mM EDTA and protease inhibitor). Cells were lysed by bead beating (using a FastPrep 5G Homogenizer MP-biomedical) for 45 s at the homogenizing intensity. Loading buffer was added to each sample, and samples were heated for 5 min to 95°C. Protein amounts equivalent to 1 OD of cells were analyzed by Western blot. Antibodies used for western blotting were α-HA (Covance MMS-101P), α-c-Myc antibody (9E10, Invitrogen) and α-H2B (Active Motif 39237).

For co-immunoprecipitation, yeast strains were grown at 23°C or 30ºC and shifted for 5 h to 37°C as indicated. 200 OD yeast cells were harvested and lysed by bead-beating in 1 ml of cold IP lysis buffer (50 mM HEPES, 200mM sodium acetate, 0.25% Nonidet P-40, 1mM EDTA, 5 mM magnesium acetate, 5% glycerol, 3 mM DTT, 1 mM PMSF and protease inhibitors). The whole-cell lysate was cleared by centrifugation, and samples were normalized for their protein concentration before being used for the IP. An aliquot of 100 µl was taken as input control. 600 µl of each sample was incubated with 5µl of α-Myc overnight followed by 2 h incubation with 50 μl of Protein G dynabeads at 4°C. For immunoprecipitation of HA-tagged Cse4 using α-HA agarose, the resin was pre-washed 5 times with lysis buffer prior to overnight incubation with lysate. 70 µl of α-HA agarose (Sigma, A2095) was added to 600 µl samples. Protein-antibody-bead/agarose conjugates were washed 3 times with lysis buffer and suspended in 50µl of sample loading buffer (final concentration 62.5 mM Tris pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, 0.001% bromophenol blue). α-Myc antibody was obtained from Thermo scientific (MA1-980) and used at a 1:500 dilution. HA-antibody (Covance) was used at 1:250. The immunoblots were imaged on a Bio-Rad imaging system.

Flow cytometry

FACS analysis was performed as previously described (1). Briefly, strains were grown at 23°C in YPD overnight, shifted for 5 h to the restrictive temperatures (34°C or 37°C) and grown to mid-log phase. 0.5 mL of exponentially growing cells were fixed with 70% ethanol and prepared for flow-cytometry and staining with Sytox Green dye. 100,000 cells were analyzed using a BD Accuri C6 Flow Cytometer.

Table S1: *S. cerevisiae* strains used in this study

Strain	Genotype	Source*
AEY1	*MATα ade2-101 his3-11,15 trp1-1 leu2-3,112 ura3-1* (W303)	
AEY2	AEY1, but *MAT***a**	
AEY4846	*MATα cse4Δ::kanMX cbf1Δ::NatMX ade2 lys2,* W303 + pRS313-*3xHA-CSE4*	
AEY6115	*MAT***a** *okp1-5::TRP1 cse4-R37A::HisMX ade2 lys2,* W303	
AEY6239	AEY1 *yta7Δ::KanMX*	
AEY6253	*MAT*α *okp1-5::TRP1 yta7Δ::NatMX ade2 LYS2*, W303	
AEY6256	*MAT*α *cse4-R37A::HisMX okp1-5::TRP1 yta7Δ::KanMX ade2 LYS2,* W303	
AEY6295	*MATα ame1-4::TRP1 yta7Δ::KanMX ade2 lys2,* W303	
AEY6327	AEY1 *cnn1Δ::KanMX*	
AEY6329	AEY1 *wip1Δ::KanMX*	
AEY6331	AEY1 *nkp2Δ::KanMX*	
AEY6332	AEY1 *mcm16Δ::KanMX*	
AEY6334	AEY1 *mcm22Δ::KanMX*	
AEY6335	AEY1 *nkp1Δ::KanMX*	
AEY6392	*MAT***a** *dsn1-7*, W303	J.V. Kilmartin (2)
AEY6393	*MAT***a** *dsn1-8*, W303	
AEY6394	*MAT***a** *nnf1-77*, W303	
AEY6395	*MAT***a** *nuf2-61*, W303	

AEY6396	*MAT***a** *nsl1-5* W303	
AEY6397	*MAT***a** *nsl1-6* W303	
AEY6403	*MATα mcm16Δ::KanMX yta7Δ::NatMX ade2 lys2*, W303	
AEY6406	*MAT***a** *mcm22Δ::KanMX yta7Δ::NatMX ade2 lys2*, W303	
AEY6408	*MAT***a** *wip1Δ::KanMX yta7Δ::NatMX ade2 lys2*, W303	
AEY6410	*MAT***a** *cnn1Δ::KanMX yta7Δ::NatMX ade2 lys2*, W303	
AEY6411	*MATα nkp2Δ::KanMX yta7Δ::NatMX ade2 lys2*, W303	
AEY6414	*MATα nkp1Δ::KanMX yta7Δ::NatMX ade2 lys2*, W303	
AEY6425	MAT*α asf1Δ::KanMX cse4-R37A::HisMX okp1-5::TRP1 ade2 lys2*, W303	
AEY6428	*MAT***a** *ctf3Δ::KanMX yta7Δ::NatMX ade2 LYS2*, W303	
AEY6430	*MAT***a** *iml3Δ::KanMX yta7Δ::NatMX ade2 LYS2*, W303	
AEY6433	*MAT***a** *chl4Δ::KanMX yta7Δ::NatMX ade2 LYS2*, W303	
AEY6437	*MAT***a** *ctf19Δ::KanMX yta7Δ::NatMX ade2 LYS2*, W303	
AEY6438	*MAT***a** *mcm21Δ::KanMX yta7Δ::NatMX ade2 LYS2*, W303	
AEY6451	*MAT***a** *cbf1Δ::NatMX yta7Δ::KanMX ade2 LYS2*, W303	
AEY6452	*MAT***a** *mif2-3 yta7Δ::NatMX ADE2 LYS2*, W303	
AEY6455	*MAT***a** *dsn1-8 yta7Δ::NatMX ade2 LYS2*, W303	
AEY6457	*MAT***a** *nuf2-61 yta7Δ::NatMX ade2 LYS2*, W303	
AEY6459	*MAT***a** *spc25-1 yta7Δ::NatMX ade2 LYS2*, W303	
AEY6461	*MAT***a** *mtw1-11 yta7Δ::NatMX ade2 LYS2*, W303	
AEY6463	*MAT***a** *nsl1-6 yta7Δ::NatMX ade2 LYS2*, W303	
AEY6471	*MAT***a** *dsn1-7 yta7Δ::NatMX ade2 LYS2*, W303	
AEY6474	*MAT***a** *cep3-2 yta7Δ::NatMX ade2 LYS2*, W303	
AEY6475	*MAT***a** *ndc10-2 yta7Δ::NatMX ade2 LYS2*, W303	
AEY6488	*MATα cse4Δ::kanMX cbf1Δ::NatMX yta7Δ::URAMX ade2 lys2*, W303+ pRS313-*3xHA-CSE4*	
AEY6494	*MAT***a** *ndc10-1 yta7Δ::NatMX ade2 lys2*, W303	
AEY6497	*MATα ndc80-1 yta7Δ::NatMX ade2 lys2*, W303	
AEY6499	*MATα spc105-4 yta7Δ::NatMX ade2 lys2*, W303	
AEY6501	*MATα spc24-1 yta7Δ::NatMX ade2 lys2*, W303	

AEY6502	*MATα nnf1-77 yta7Δ::NatMX ade2 lys2*, W303	
AEY6520	*MAT***a** *ctf13-3 yta7Δ::NatMX ade2 lys2*, W303	
AEY6521	*MAT***a** *nsl1-5 yta7Δ::NatMX ade2 lys2*, W303	
AEY6533	AEY1 *YTA7-9xmyc::KanMX*	
AEY6536	*MATα YTA7-9xmycKanMX cse4Δ::NatMX ade2 lys2,* W303 + pRS426-*3xHA-CSE4*	
AEY6542	*MATα cep3-1 yta7Δ::NatMX ade2 lys2, W303*	
AEY2546	*MATα cse4Δ::kanMX yta7Δ::NatMX ade2 lys2,* W303 + pPY20-*cse4-103*	
AEY6552#	*scm3-1(ts)-9Myc::klTRP1 ura3 leu2 his3* (LLY002-2)	E. Schiebel (3)
AEY6560#	*MAT***a** *yta7Δ::NatMX scm3-1(ts)-9Myc-klTRP1 ade2-101 his3-11,15 trp1-1 leu2-3,112 ura3-lys2*	
AEY6573	*MAT***a** *cse4Δ::kanMX chl4Δ::KanMX ADE2 LYS2,* W303 + pRS313-*3xHA-CSE4*	
AEY6577	*MAT***a** *cse4Δ::kanMX chl4Δ::KanMX yta7Δ::NatMX ADE2 LYS2,* W303 + pRS313-*3xHA-CSE4*	
AEY6580	*MATα cbf1Δ::NatMX OKP1-9xMyc::KanMX ade2 lys2,* W303	
AEY6621	*MATα cse4Δ::kanMX okp1-5::TRP1 ADE2 lys2,* W303 + pRS313-*3xHA-CSE4*	
AEY6624	*MAT***a** *cse4Δ::kanMX yta7Δ:: NatMX okp1-5::TRP1 ade2 lys2,* W303 + pRS313-*3xHA-CSE4*	
AEY6649	*MAT***a** *chl4Δ::KanMX yta7Δ::NatMX hht2-hhf2Δ::HIS3 ade2 LYS2,* W303	
AEY6669	*MATα cbf1Δ::NatMX OKP1-9xMyc::KanMX yta7Δ::URAMX ade2 lys2,* W303	
AEY6678	*MATα cbf1Δ::NatMX hht1-hhf1Δ::LEU2 hht2-hhf2Δ::HIS3 ade2 lys2,* W303 + pNOY439-*HHF2 myc-HHT2*	
AEY6679	*MATα cbf1Δ::NatMX yta7Δ::URAMX hht1-hhf1Δ::LEU2 hht2-hhf2Δ::HIS3 ade2 lys2,* W303 + pNOY439-*HHF2 myc-HHT2*	
AEY6734	AEY1 *SCM3-6xHA-KanMX*	
AEY6746	*MAT***a** *YTA7-9xmycKanMX SCM3-6xHA::KanMX ADE2 LYS2,* W303	
AEY6750	*MATα cse4Δ::kanMX ade2 lys2 GALSpr-SCM3::natNT2*,	

	W303 pRS313-*3xHA-CSE4*	
AEY6672	*MAT***a** *hht1-hhf1Δ::LEU2 hht2-hhf2Δ::HIS3 ade2 lys2Δ::His4 his3-11,15 trp1-1 leu2-3,112 ura3-1 can1-100 + pNOY439 (CEN6 ARS4-TRP1 HHF2 MYC-HHT2)*	
AEY6682	*MAT*α *yta7Δ::UraMX hht1-hhf1Δ::LEU2 hht2-hhf2Δ::HIS3 ade2 lys2,* W303 *+ pNOY439 (CEN6 ARS4-TRP1 HHF2 MYC-HHT2)*	
AEY6687	*MAT***a** *cse4Δ::kanMX ade2 lys2,* W303 + pRS313-*3xHA-CSE4*	
AEY6692	*MAT***a** *cse4Δ::kanMX yta7::URAMX ade2 lys2,* W303 + pRS313-*3xHA-CSE4*	
AEY6801	*MAT***a** *GALSpr::natNT2::SCM3-6xHA::KanMX ade2 LYS2 CSE4-13myc::URA3,* W303	

*Unless indicated otherwise, strains were from the laboratory collection or were generated in the course of this study. All strains except those marked with # are isogenic to W303.

Table S2: Plasmids used in this study

Plasmid	Description	Source*
pAE615	pRS313-3xHA-*CSE4*	(4)
pAE1771	*TRP1*, ARS1, CEN6-CDEIΔ (d66)	P. Hieter (5)
pAE2684	pRS316-*YTA7*	J. Rine (6)
pAE2685	pRS316-*yta7-K460A*	
pAE2686	pRS316-*yta7-E519Q*	
pAE2786	pRS326-*YTA7*	
pAE2904	pNOY439-*HHF2 myc-HHT2*	A. Nourani (7)

*Unless indicated otherwise, plasmids were from the laboratory collection or were generated in the course of this study.

Supplementary figures

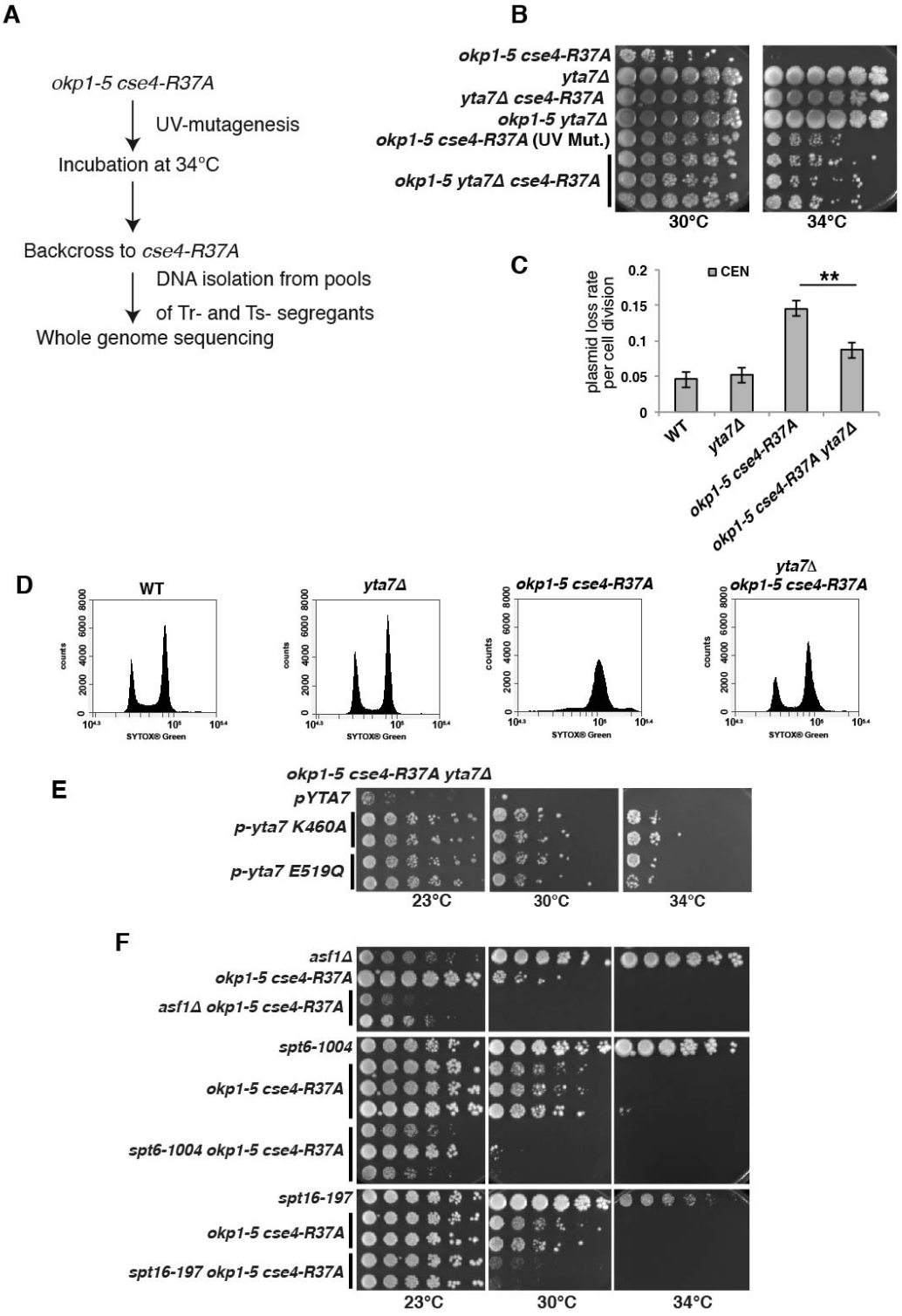

Fig. S1. *yta7Δ* suppressed the synthetic growth defects of mutation of Cse4-R37. (A) Schematic for the identification of extragenic suppressors of the growth defect of *okp1-5 cse4-R37A*. To eliminate any unlinked mutations, the temperature-resistant suppressor mutants were backcrossed to an unmutagenized parental strain, and the causative mutations were identified using whole-genome sequencing of pooled segregants. (B) *yta7Δ* suppressed the temperature-sensitive growth defect of *okp1-5*

cse4-R37A. Serial dilutions of the indicated strains were spotted on YPD medium and incubated for 3 days at the respective temperatures. The original *yta7* mutant from the suppressor screen is indicated as “UV Mut.”. (C) *yta7Δ* suppressed the plasmid maintenance defect of *okp1-5 cse4-R37A*. The loss rate of a CEN plasmid was measured in the indicated strains. Error bars indicate SD of at least three independent transformants. **, $P < 0.01$. (D) *yta7Δ* partially suppressed the G2/M arrest of *okp1-5 cse4-R37A* at the restrictive temperature. Cells were grown to early logarithmic phase at 23°C and shifted to 34°C for 5 h. Cellular DNA content was measured by FACS analysis using Sytox Green dye. (E) Mutations in the Walker A (K460A) or Walker B (E519Q) motifs of the AAA^+ ATPase domain of Yta7 caused a loss of Yta7 function in *okp1-5 cse4-R37A*. Representation as in B. (F) Mutations in the genes encoding the histone chaperones Spt16, Spt6, and Asf1 (*spt16-197*, *spt6-1004*, and *asf1Δ)* enhanced the growth defect of *okp1-5 cse4-R37A*. Representation as in B.

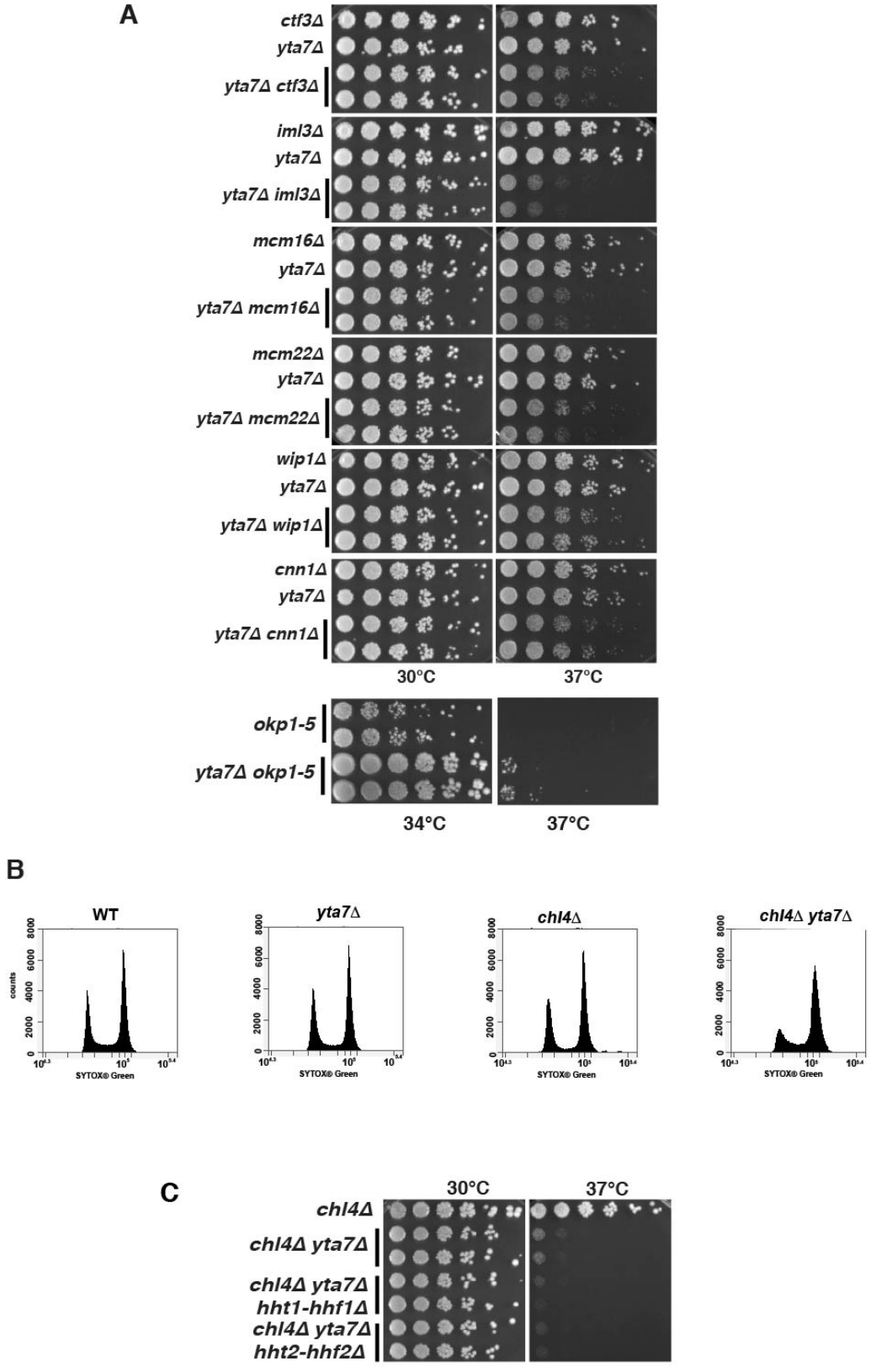

Fig. S2. *yta7Δ* causes defects in centromere function and cell-cycle progression. The synthetic growth defects of *yta7Δ* were caused by impairment of cell cycle progression. (A) Synthetic genetic interactions of *yta7Δ* with mutations in genes

encoding kinetochore components. Serial dilutions of the indicated strains were spotted on full medium and incubated for 3 days at indicated temperatures. (B) *yta7Δ* causes a G2/M cell cycle arrest in *chl4Δ* at the restrictive temperature. Cells were grown to early logarithmic phase at 23°C and shifted to 37°C for 5 h. Cellular DNA content was measured by FACS analysis using Sytox Green dye. (C) Decreased dosage of histone H3 and H4 did not suppress the defect caused by *yta7Δ* in *chl4Δ*, indicating that the effect of *yta7Δ* at centromeres is not due to increased levels of H3 and H4. Representation as in A.

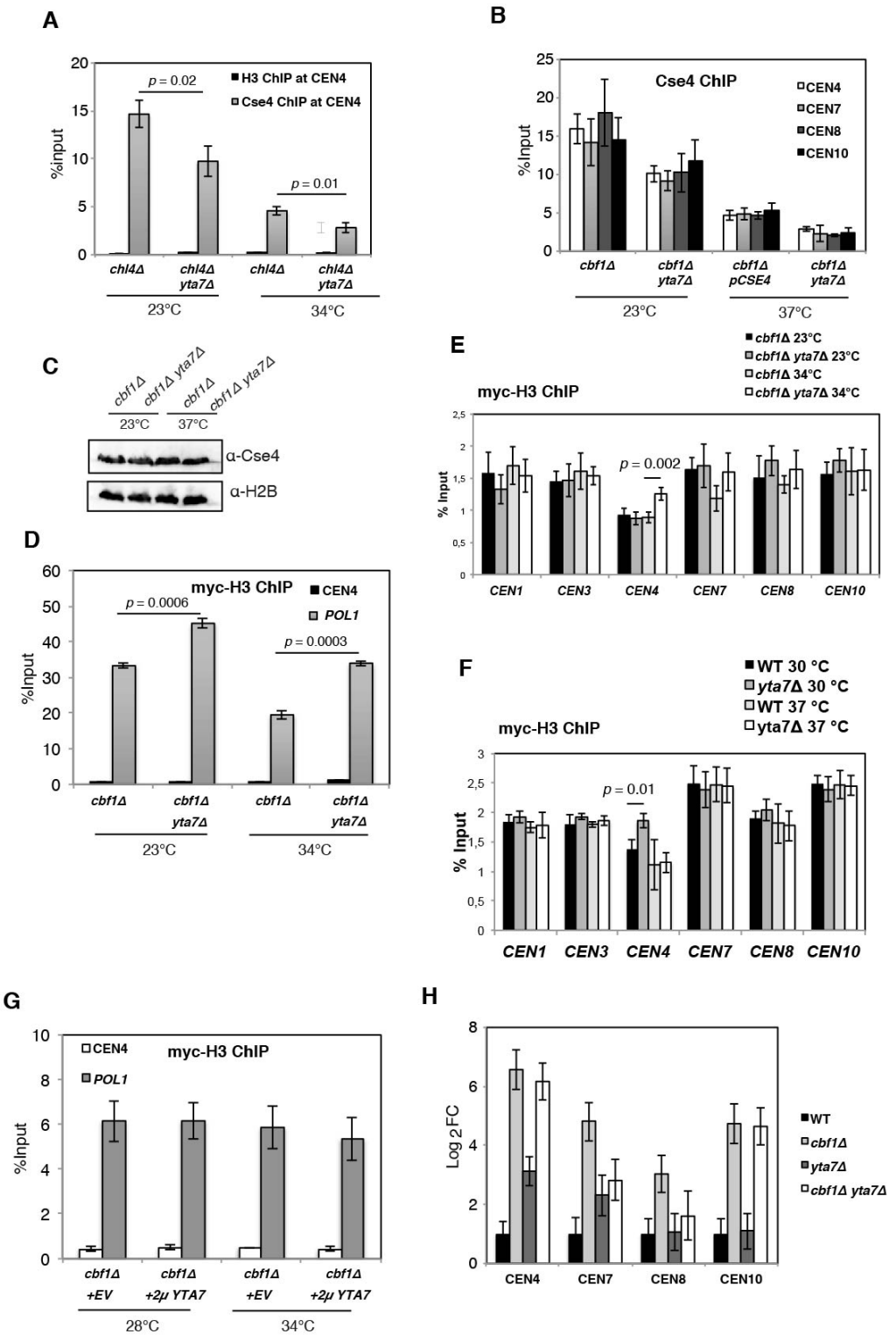

Fig. S3. Yta7 is required for proper localization of Cse4 to the centromere. (A) ChIP shows a significant decrease in Cse4 association with *CEN4* in the absence of Yta7 in *cbf1Δ* cells. The α-H3 antibody is used in ChIP analysis as a control. Analysis as in Fig. 2A. (B) A reduction in centromere-bound Cse4 at *CEN4*, *CEN7*, *CEN8*, and *CEN10* as detected by ChIP in *yta7Δ cbf1Δ* cells at both the permissive and restrictive temperatures. (C) *yta7Δ* did not reduce bulk Cse4 levels in *cbf1Δ* cells.

Western blot analysis of the amounts of HA-Cse4 and histone H2B (loading control) in whole-cell extracts. (D) *yta7Δ* caused increased levels of H3 at a non-centromeric region (*POL1*). ChIP was performed as in Fig. 2B. (E) *yta7Δ* caused a mild increase of H3 at *CEN4* in *cbf1Δ* cells at the restrictive temperature, but not at several other centromeres. (F) *yta7Δ* cells had mildly increased H3 levels at *CEN4* at 30ºC, but not at other centromeres, at 30ºC. (G) *YTA7* overexpression did not increase H3 levels at a non-centromeric site (*POL1*). The experiment was performed as in Fig. 2F. (H) *yta7Δ* did not cause increased transcription through the centromere in *cbf1Δ*. RT-qPCR analysis of cenRNA4, cenRNA7, cenRNA8, and cenRNA10 in the deletion mutants is shown relative to the WT. Means ± SD are given, n = 3 (*P*-values by Student's *t*-test).

Data information: (A,B, D - G) Yeast cells were grown at permissive temperature or were shifted to restrictive temperature for 4 hours before ChIP. Means ± SD, n = 3.

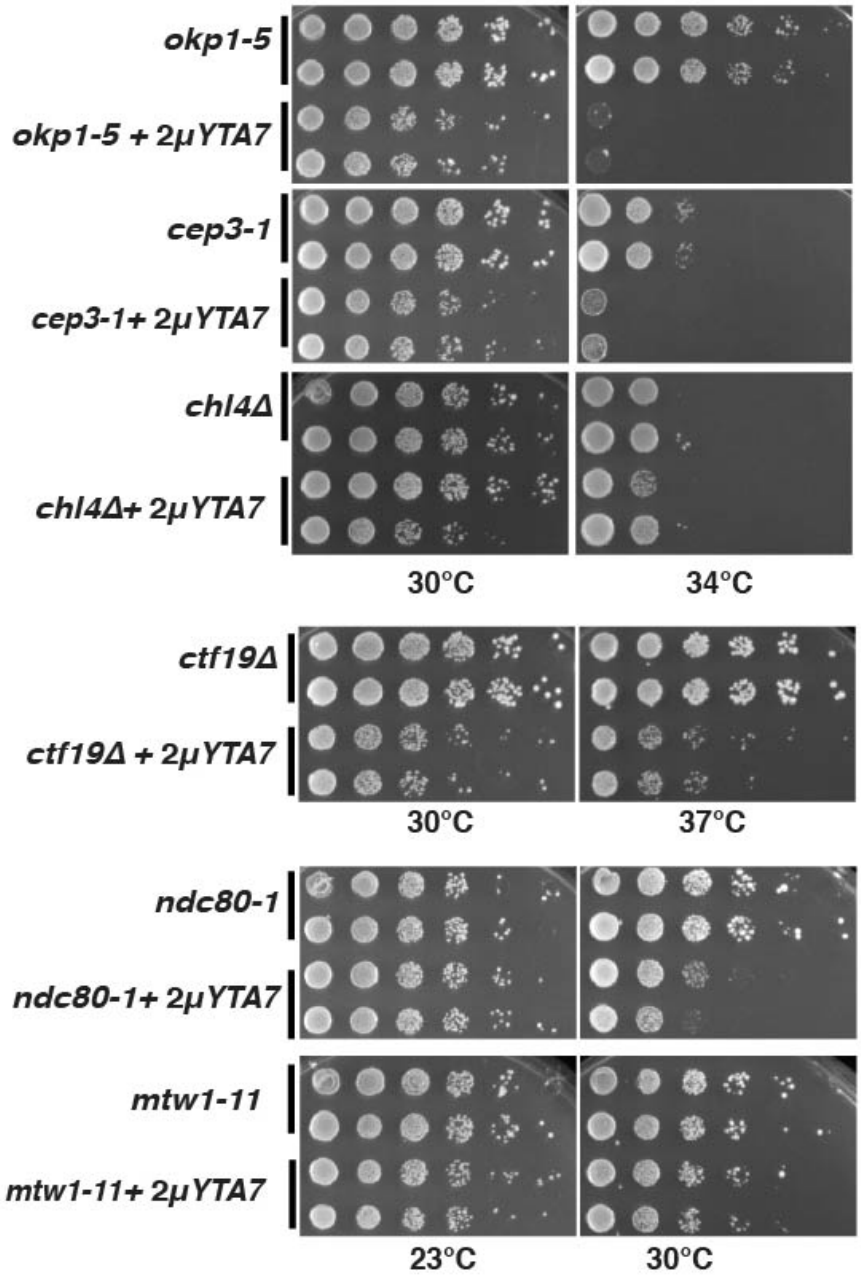

Fig. S4. Overexpression of *YTA7* causes a growth defect in kinetochore mutants. *YTA7* on a high-copy *2μ* plasmid and a control plasmid were introduced into the indicated strains, and serial dilutions of transformants were spotted on selective medium and grown at indicated temperatures for 3 days.

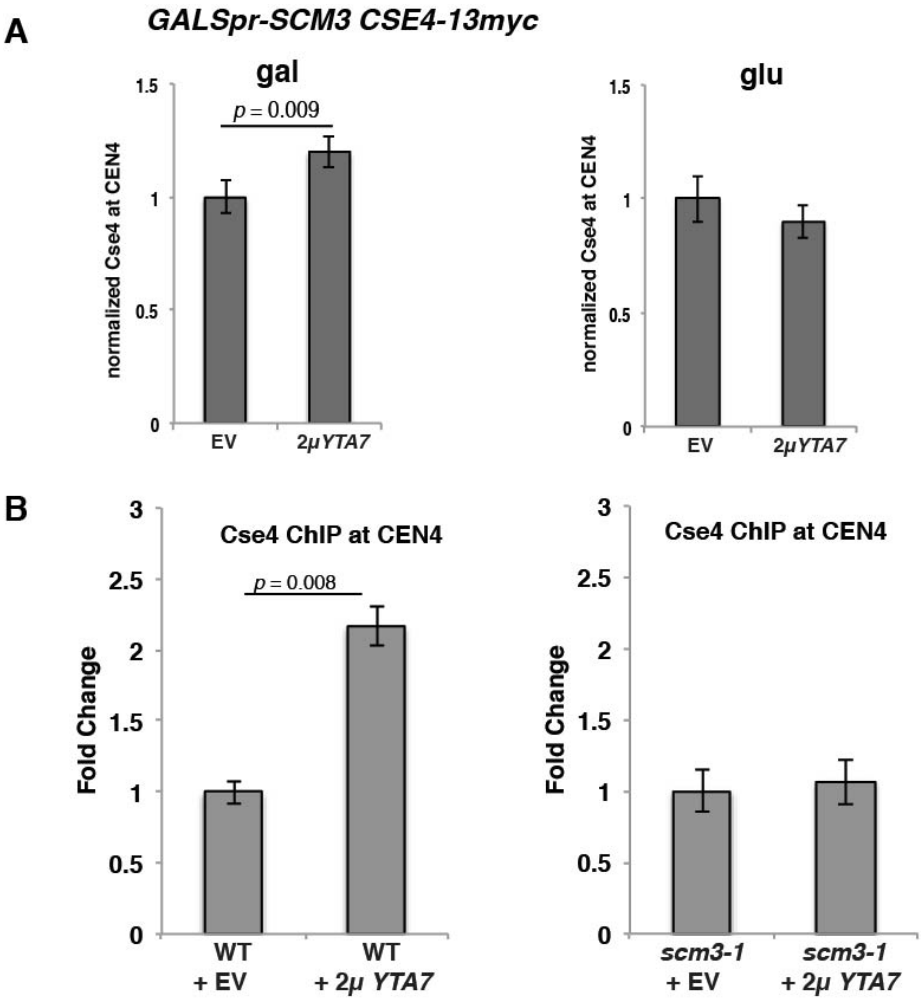

Fig. S5. *YTA7*-dependent Cse4 deposition at the centromere requires functional Scm3. (A) ChIP of 13myc-tagged Cse4 was conducted in cells with *GALSpr-SCM3* and *YTA7* overexpression (2*μ YTA7*) or vector control (EV). Cells were grown either in galactose (*SCM3* on, left) or glucose (*SCM3* off, right), and 13myc-Cse4 was precipitated with α-myc agarose. Values are normalized to the vector control. Means ± SD, n = 3 (*P*-values by Student's *t*-test) are shown. (B) ChIP of HA-Cse4 was conducted in wild-type (WT, left) or *scm3-1* cells and *YTA7* overexpression (2*μ* YTA7) or vector control (EV). Cells were grown 34ºC, and HA-Cse4 was precipitated with α-HA agarose. Values are normalized to the vector control. Means ± SD, n = 3 (P-values by Student's t-test) are shown.

Supplementary references

1. Anedchenko EA*, et al.* (2019) The kinetochore module Okp1(CENP-Q)/Ame1(CENP-U) is a reader for N-terminal modifications on the centromeric histone Cse4(CENP-A). *EMBO J* 38(1).

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