

1    **Legends to Supplementary Figures**

2    **Supplementary Figure S1**

3    Ctf18-RFC co-purifies with DNA polymerase epsilon. **(A)** Ctf18-TAP was  
4    purified from 4 litres of cell culture, and the final material was resolved in one  
5    lane of a 4-12% gradient gel, which was then cut into 60 bands, before  
6    analysis of the protein content of each band by mass spectrometry. The  
7    histograms show the number of detected peptides per band of the gel, for  
8    each of the indicated proteins, with Mascot scores indicated in brackets and  
9    bold. **(B)** Summary of mass spectrometry data after two-step purifications  
10   (IgG-Sepharose and release of bound material with TEV protease, then  
11   Calmodulin-affinity resin) of cell extracts from *DPB2-TAP* and control strains.  
12   For each protein detected in the mass spectrometry analysis, the number of  
13   spectral counts is shown for each strain, indicating that Ctf18-Dcc1-Ctf8 were  
14   specifically enriched upon purification of DNA polymerase epsilon.

15

16   **Supplementary Figure S2**

17   Ctf18 does not interact with the Dpb3 or Dpb4 subunits of Pol ε in the yeast  
18   two-hybrid assay. Two-hybrid analysis was performed as in Figure 2,  
19   indicating that Ctf18-RFC did not interact in this assay with Dpb3 or Dpb4 **(A)**.  
20   As a control, we confirmed that Dpb3 and Dpb4 were able to interact with  
21   each other in the same assay **(B)**.

22

1   **Supplementary Figure S3**

2   Pol2CT interacts with itself in the yeast two-hybrid assay. The indicated  
3   fragments were tested in the yeast two-hybrid assay, as above in Figure 2A.

4

5   **Supplementary Figure S4**

6   The Pol ε binding module of Ctf18-RFC comprises a conserved motif at the  
7   end of Ctf18 that interacts with Dcc1-Ctf8 and Pol2NT. (**A**) Alignment of the  
8   carboxy terminal half of Ctf18 from the indicated budding yeast species,  
9   generated as in Figure 3B. Asterisks denote 10 conserved hydrophobic  
10   residues at the end of Ctf18. (**B**) An equivalent alignment of the carboxy  
11   terminal half of Ctf18 from the indicated species (X.l. = *Xenopus laevis*; H.s =  
12   *Homo sapiens*; D.m. = *Drosophila melanogaster*; S.p. =  
13   *Schizosaccharomyces pombe*; O.s. = *Oryza sativa*; S.c. = *Saccharomyces*  
14   *cerevisiae*).

15

16   **Supplementary Figure S5**

17   Residues at the extreme carboxyl terminus of Ctf18 are important for  
18   interaction with Pol2NT and Dcc1. The indicated Ctf18 fragments were tested  
19   for their ability to interact with Pol2NT (**A**) and Dcc1 (**B**) in the two-hybrid  
20   assay, as in Figures 2 and 3.

21

22   **Supplementary Figure S6**

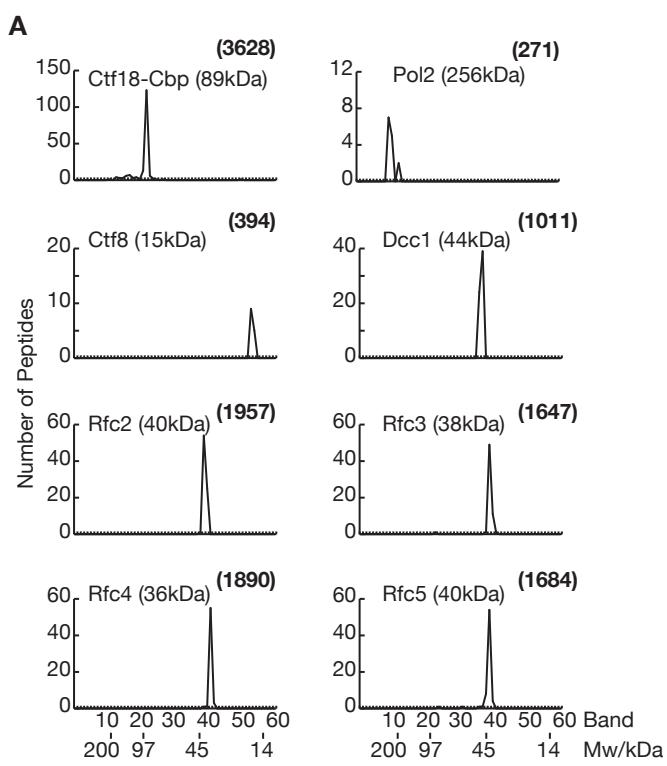
23   Comparison of the hydroxyurea sensitivity caused by various mutations in  
24   Ctf18-RFC. Cells were processed as for Figure 4A.

1

2 **Supplementary Figure S7**

3 Increased phosphorylation of Serine 129 of histone H2A indicates DNA  
4 damage in ctf18-2A upon release into S-phase in the presence of  
5 hydroxyurea. One of the replicates from Figure 5A was used to monitor DNA  
6 content by flow cytometry (upper panels) and  $\gamma$ -H2A by immunoblotting (lower  
7 panels). Ponceau-stained total protein provided a loading control.

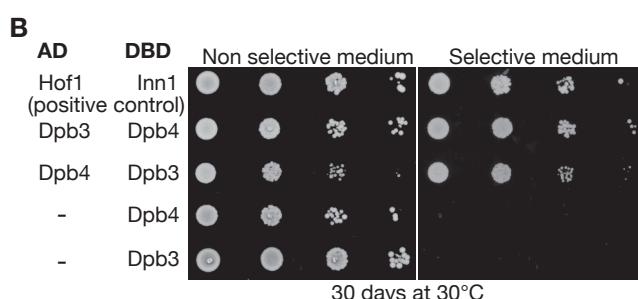
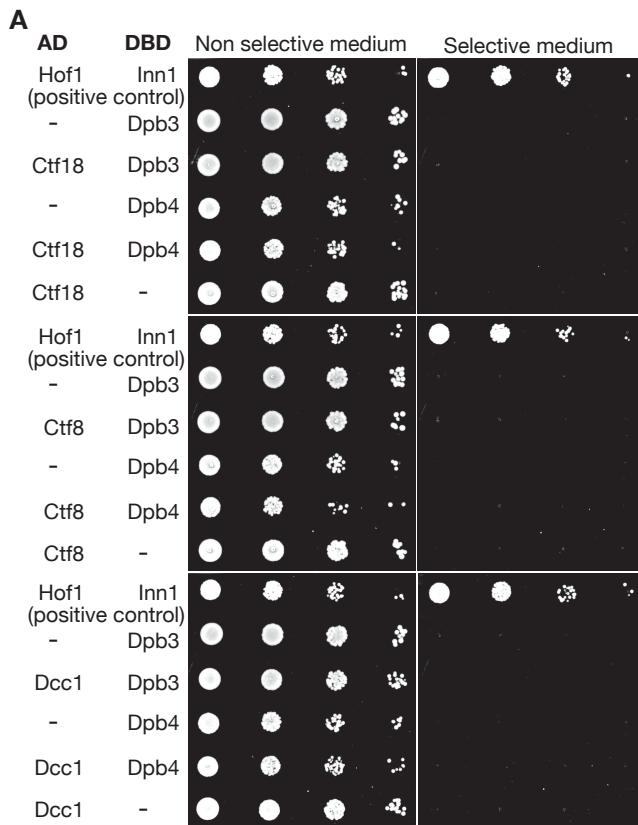
Garcia-Rodriguez Fig S1



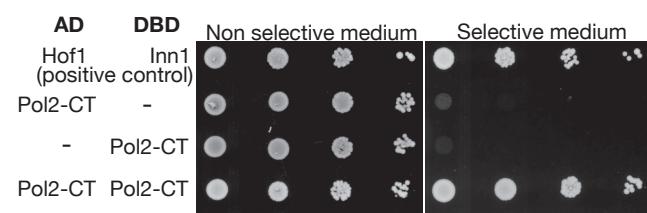
**B**

Protein	Mw (kDa)	Spectral counts (DPB2-TAP)	Spectral counts (Control)
Dpb2-TAP	99	1193	11
Dpb3	23	157	2
Dpb4	22	218	0
Pol2	256	4286	146
Ctf18	84	91	6
Dcc1	44	19	2
Ctf8	15	25	0

Garcia-Rodriguez Fig S2



**Garcia-Rodriguez Fig S3**



Garcia-Rodriguez Fig S4

**A**

S. mikatae Ctf18	506	<b>SALVPLVFFQTFGDIANKDDIRMKNSEYERRELRRANLDVVVDLIMRHISVQSPLMASFTN</b>
S. cerevisiae Ctf18	484	<b>SALVPLVFFQTFGDIANKDDIRMKNSEYEORELKTRANSDIVSLIMRHISVQSPLMASFTD</b>
S. bayanus Ctf18	484	<b>SSLVPLVFFQTFGDIANKDDIRMKNSEYEORELKTRANLDIVDSIIRHISVEQSPLMASFTD</b>
S. castellii Ctf18	481	<b>CSIVPLAFFQNFGDVANKEDDMRIKNAEFENREIQKSNAIDLSSLIHRISMENPKMMSFID</b>
S. kluyveri Ctf18	466	<b>SSLVPMAFFQLFSDIANKDDSLIKSVDYEVRRESIKHHNFSITGSLLDRT--SPRSRVLYN</b>
S. mikatae Ctf18	566	<b>RKSLIFEILPYLESMISSDFNKNIKNLKLKOVIMETLVOLLKSFQNLNLRSEIYDARSG</b>
S. cerevisiae Ctf18	544	<b>RKSLIFEILPYLDMSISSDFNKNIKRNKLKQAIMEELVQLLKSQNLNLRSEGFDVRGG</b>
S. bayanus Ctf18	544	<b>RNSLVFQILPYLDMSVSSDLNKVKNLKFQOTIIETLVQVMKNFQLSLMENRSDGFDVRGG</b>
S. castellii Ctf18	541	<b>RNTLIFDILPYVDRMLSSDISKVKDLTVKSAMLEDLLNINLYFVLKITQVPAFGMEIKNS</b>
S. kluyveri Ctf18	523	<b>KKNLILELEVPMYDIIITSDSLKIRSMPLRQSMVDNIPVPLKDFGLTLEQRHDPTTSRSM</b>
S. mikatae Ctf18	626	<b>LVIDPPIEEVVLNPKHISEVQQKRPNNLSSLLAKIEENKAKKRHIDQVTRDRLQSQEIH</b>
S. cerevisiae Ctf18	604	<b>LTIIDPPIDEVVLLNPKHINEVQHKRANNLSSLLAKIEENRAKKRHIDQVTEDERLQSQEMH</b>
S. bayanus Ctf18	604	<b>LIIDPPIDKVVLDPKHINKVQHKRPNNLNSLLAKIEENKVKKRHIDQVTKERFQSQEIH</b>
S. castellii Ctf18	601	<b>LTLDPPIDKVVLLEPSSVKATFKRPTMLHLLLAKIEERKVKKRIRDKEKOLOTEEEDR</b>
S. kluyveri Ctf18	581	<b>LCIQPPPLDEVTLLEKRVKEVATKRPGLLNFLLLAKVEECEVKRTIDKVNRERLENEE--</b>
S. mikatae Ctf18	686	<b>TKKAKTALTSSSSSTIDFFFKNQYGLLKQNQESFGTQKST-----VSDEPDQV-DDST</b>
S. cerevisiae Ctf18	664	<b>SKKVKTGLNSSSSSTIDFFFKNQYGLLKQTOELEETOKTI-----GSDETNQA-DDCN</b>
S. bayanus Ctf18	664	<b>TKKAKMASNSSSSSTIDFFFKNQYRIVNQTOEAASGGRKNV-----GSSEP---DENT</b>
S. castellii Ctf18	661	<b>ATKKSKISSSSGRGSTIDFFFKSOYSAINKETASSQDIPKQ-----NADAAGRAGSKNE</b>
S. kluyveri Ctf18	639	<b>-NRKRSKTSAVSTPVDFFKSQYSSIKTKSSDVSKRASTPELRRSRNFSEGSNTTTATNE</b>
S. mikatae Ctf18	736	<b>QKIKIWVKYNEGFSNAVRKNVTWNNLWE</b>
S. cerevisiae Ctf18	714	<b>QTVKIWVKYNEGFSNAVRKNVTWNNLWE</b>
S. bayanus Ctf18	711	<b>EEIKIWVKYKEGFSNAVRKNVTWNNLWE</b>
S. castellii Ctf18	712	<b>ETTRIWVKYKEGFSNAVRKNVTWESLWQ</b>
S. kluyveri Ctf18	698	<b>EKIRIWVKYKEGFSNAVRKNVNWETLWQ</b>

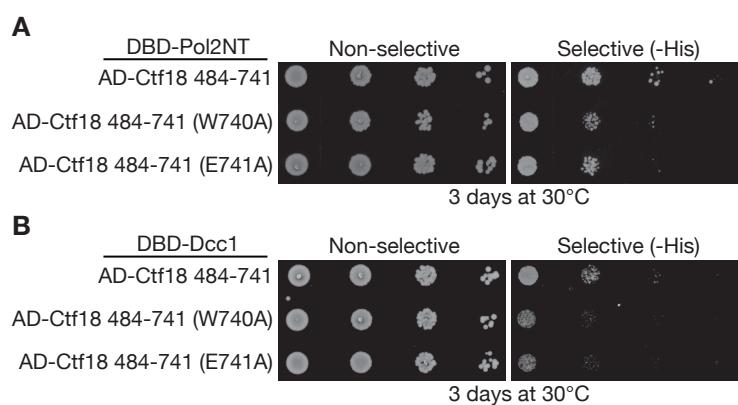
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**B**

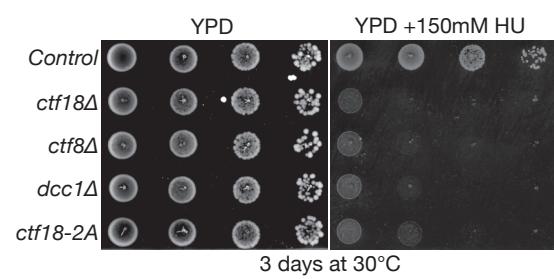
X.I. Ctf18	724	<b>LPFLPVAFHLLFAASNVP---RIAYPS-SHYEAQSKLNOMONLLNAMVSEISPAIRTRVG</b>
H.s. Ctf18	698	<b>PPFLPVAFHVLFASSHTP---RITFPS-SQOEAQNRMSQMRNLIQTLVSGIAPATRSRAT</b>
D.m. Ctf18	735	<b>LQYGFVAWHLLFATLAWP---KIAFP-T-RGFEFOQKSTNQRNIFQALCKGVTTSLALVGQ</b>
S.p. Ctf18	718	<b>IPYSIIHFHYLYATPEKC---RLPHPPRSDSLAEALKYRTRKEILDASFISTLNAYENQMH</b>
O.s. Ctf18	727	<b>OPPVAITISQLVAQVEKP---NIEWPK-SLQRCRTMLLEKKDKLKWTQNQMSPLISRHL</b>
S.c. Ctf18	484	<b>SALVPLVFFQTFGDIANKDDIRMKNSEYEORELKTRANSDIVSLIMRHISVQSPLMASFTD</b>
X.I. Ctf18	780	<b>PQSLVLDALCLLLVDLSP-KLRPVNTQOLFSTKEKQQLAELINTMLAYNLTYHQERTTMEQ</b>
H.s. Ctf18	754	<b>PQALLLDALCLLLVDLSP-KLRPVNTQOLFSTKEKQQLAELINTMLAYNLTYHQERTTMEQ</b>
D.m. Ctf18	791	<b>GGTLLLDLTVPLLKRLSP-QLRSVAVQOLLSPKEQODLRTHTIEVMVDSLGLTFQVKSOEGH</b>
S.p. Ctf18	775	<b>ERSILLELIRTILITINP-TLKQKEDSMSRPSALPSKIEHAINILNHYNLRFQOLPVGDGN</b>
O.s. Ctf18	783	<b>VESFVEDIASPFHIIISPSSLRVPVALNLLSEREKDELVQLVDTMVSYSITYRNTKLEPQE</b>
S.c. Ctf18	544	<b>RKSLIFEILPYLDMSISS-DFNKNIRN---LKLQAIAMEVQOLLKSFQNLNLRSEGFD</b>
X.I. Ctf18	839	<b>YVYK--LDPNVEDVCRFP--DLPVRKPLTYQTQKLIAREIELERMRRTEAFQOARNAGRD</b>
H.s. Ctf18	813	<b>YIYR--LEPVNVEELCRFP--ELPARKPLTYQTQKLIAREIEVEKMRRAEASARVENSPQV</b>
D.m. Ctf18	850	<b>YVFQ--TEPDLDLDSAAPP--GYTG-LSLPYFSRQKLIAREVDLERIRRAAPKGAPSAPAA</b>
S.p. Ctf18	834	<b>YVYR--LEPPLDELVWDA--PTSS----YSVRQMLSQELLKKRLLADIKKQTLTDNPSS</b>
O.s. Ctf18	843	<b>RISGSMVSPDVPSLSDLP--AINDIINFKVE-KOKIMKDSAGKLLNQANEQDKRNEVSVS</b>
S.c. Ctf18	600	<b>VRGGLTIDPPIDEVVLNPKHINEVQHKRANNLSSLLAKIEENRAKKRHIDQVTEDERLQ</b>
X.I. Ctf18	895	<b>NTTAAAVKTADPKGAKSAAKPAALNHEQRLENIMKKATFEEKPEKDFFG-RQIVKKVAA</b>
H.s. Ctf18	869	<b>DGSPPGLELLGGIGEKGVHRPAPRNHEQRLEHIMRRAAREEQPEKDFFG-RVVVRSTAV</b>
D.m. Ctf18	905	<b>KKKTSQAAAQLPNHLQTLKPKPISASNMH-----SAPKQOQLTKDFFG-RITHKSTST</b>
S.p. Ctf18	885	<b>NSSRKRKDFNSG-----KAIKRDFGG-RIISEPKSE</b>
O.s. Ctf18	900	<b>EKKSalvstksnpttLKMQLSSASSMSGKDPApAKKHSNHGGINFFD-RFRKERPVD</b>
S.c. Ctf18	660	<b>QEMHSKVKTG-----LNSSSSTIDFFKQYGLLQKQ</b>
X.I. Ctf18	954	<b>PVT-ASANQEESEVERRIGKAVGNSDVWFRFNEGVSNAVRRNIIYIKDLL--</b>
H.s. Ctf18	928	<b>LSAGDTAPEQDSVERRMGTAVGRSEEVWFRFNEGVSNAVRRSLYIRDLL--</b>
D.m. Ctf18	956	<b>-----NSAEEESKTDIAIVKSPIIWRYKEGFNNAVRKDVHIHELL--</b>
S.p. Ctf18	915	<b>AVT--SNNAALNTGDHPVSIKHAINIKFHDGFSNAVRKPISLNEILNF</b>
O.s. Ctf18	959	<b>AKA---RNDAGQQVATTLRDSRPLIFKYNEGFTNAVKRPVKVRDLLL-</b>
S.c. Ctf18	693	<b>ELEETQKTIIGSDETNQADDNCNQTVKIWVKYNEGFSNAVRKNVTWNNLWE-</b>

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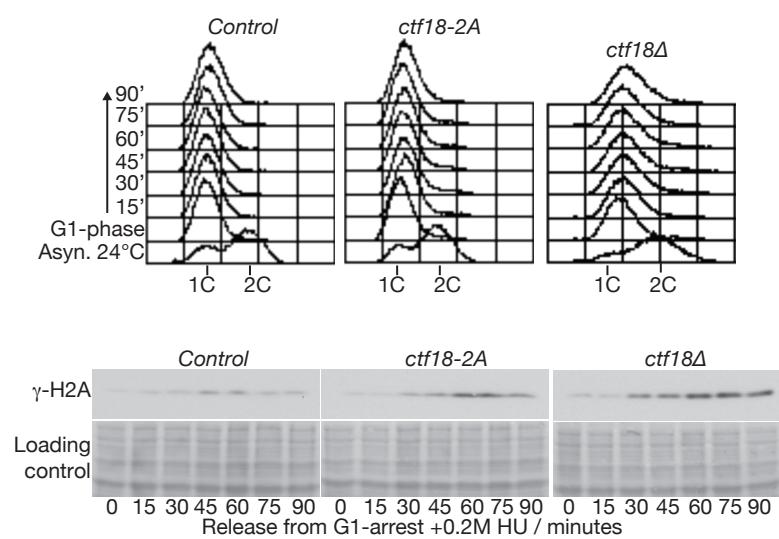
**Garcia-Rodriguez Fig S5**



Garcia-Rodriguez Figure S6



Garcia-Rodriguez Figure S7



Strain	Genotype	Source
W303-1a	<i>MAT<math>\alpha</math> ade2-1 ura3-1 his3-1 trp1-1 leu2-3, 112 can1-100</i>	R. Rothstein
YAC53	<i>MAT<math>\alpha</math> sml1Δ::HIS3 rad53Δ::ADE2</i>	This study
YASD375	<i>MAT<math>\alpha</math> TAP-SLD5 (KanMX) pep4Δ::URA3 ADE2</i>	This study
YCS394	<i>MAT<math>\alpha</math> MCM4-5-FLAG (hphNT) leu2-3,112:: pRS305-GAL-DPB2-9myc (LEU2) GAL TIR1-9myc (kITRP1) dpb2-IAA17 (kanMX) pep4Δ::ADE2</i>	This study
YCS396	<i>MAT<math>\alpha</math> MCM4-5-FLAG (hphNT) leu2-3,112:: pRS305-GAL-dpb2-NT (1-168)-9myc GAL TIR1-9myc (kITRP1) dpb2-IAA17 (kanMX) pep4Δ::ADE2</i>	This study
YCS1180	<i>MAT<math>\alpha</math> MCM4-5FLAG (hphNT) pep4Δ::ADE2</i>	This study
YGDP1970	<i>MAT<math>\alpha</math> CTF18-TAP (HIS3MX) pep4Δ::URA3 ADE2</i>	This study
YGDP1971	<i>MAT<math>\alpha</math> CTF18-TAP (HIS3MX) dcc1Δ::hphNT pep4Δ::URA3 ADE2</i>	This study
YGDP1972	<i>MAT<math>\alpha</math> DCC1-TAP (HIS3MX) pep4Δ::URA3 ADE2</i>	This study
YGDP1973	<i>MAT<math>\alpha</math> DCC1-TAP (HIS3MX) ctf18Δ::K.I.TRP1 pep4Δ::URA3 ADE2</i>	This study
YGDP993	<i>MAT<math>\alpha</math> MCM4-5FLAG (hphNT) mrc1Δ::K.I.TRP1 pep4Δ::ADE2</i>	This study
YLG33	<i>MAT<math>\alpha</math> TAP-SLD5 (KanMX) ctf18Δ::K.I.TRP1 pep4Δ::URA3 ADE2</i>	This study
YLG60	<i>MAT<math>\alpha</math> trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ ctf8Δ::URA3</i>	This study
YLG63	<i>MAT<math>\alpha</math> trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ dcc1Δ::URA3</i>	This study
YLG98	<i>MAT<math>\alpha</math> MRC1-18MYC (K.I.TRP1) pep4Δ::URA3 ADE2</i>	This study
YLG100	<i>MAT<math>\alpha</math> MRC1-18MYC (K.I.TRP1) CTF18-TAP (HIS3MX) pep4Δ::URA3 ADE2</i>	This study
YLG263	<i>MAT<math>\Delta</math> / MAT<math>\alpha</math> ade2-1 / ade2-1 ura3- 1 /ura3-1 his3-1 / his3-1 trp1-1 / trp1-1 leu2-3, 112 / leu2-3,112 can1-100 / can1-100 ctf18-2A (K.I.TRP1) / CTF18 ctf4Δ::KanMX / CTF4 pep4Δ::ADE2 / PEP4</i>	This study
YLG249	<i>MAT<math>\alpha</math> ctf18-2A (K.I.TRP1) pep4Δ::ADE2</i>	This study
YLG284	<i>MAT<math>\alpha</math> / MAT<math>\alpha</math> ade2-1 / ade2-1 ura3- 1 /ura3-1 his3-1 / his3-1 trp1-1 / trp1-1 leu2-3, 112 / leu2-3,112 can1-100 / can1-100 CTF18 (K.I.TRP1) / CTF18 ctf4Δ::KanMX / CTF4 pep4Δ::ADE2 / PEP4</i>	This study
YLG292	<i>MAT<math>\alpha</math> / MAT<math>\alpha</math> ade2-1 / ade2-1 ura3- 1 /ura3-1 his3-1 / his3-1 trp1-1 / trp1-1 leu2-3, 112 / leu2-3,112 can1-100 / can1-100 CTF18 (K.I.TRP1) / CTF18 mrc1Δ::hphNT / MRC1 pep4Δ::ADE2 / PEP4</i>	This study

YLG296	<i>MAT<sub>a</sub></i> / <i>MAT<sub>α</sub></i> <i>ade2-1</i> / <i>ade2-1 ura3-1</i> / <i>ura3-1 his3-1</i> / <i>his3-1 trp1-1</i> / <i>trp1-1 leu2-3, 112</i> / <i>leu2-3, 112 can1-100</i> / <i>can1-100 ctf18-2A (K.I.TRP1)</i> / <i>CTF18 mrc1Δ::hphNT</i> / <i>MRC1 pep4Δ::ADE2 PEP4</i>	This study
YLG301	<i>MAT<sub>a</sub></i> <i>DCC1-TAP (HIS3MX)</i> <i>CTF18 (K.I.TRP1)</i> <i>pep4Δ::URA3 ADE2</i>	This study
YLG303	<i>MAT<sub>a</sub></i> <i>DCC1-TAP (HIS3MX)</i> <i>ctf18-2A (K.I.TRP1)</i> <i>pep4Δ::URA3 ADE2</i>	This study
YLG316	<i>MAT<sub>a</sub></i> <i>CTF18 (K.I.TRP1)</i>	This study
YLG320	<i>MAT<sub>a</sub></i> <i>ctf18-2A (K.I.TRP1)</i>	This study
YLG421	<i>MAT<sub>a</sub></i> <i>MCM4-5-FLAG (hphNT)</i> <i>ctf18Δ::K.I.TRP1 pep4Δ::URA3 ADE2</i>	This study
YLG423	<i>MAT<sub>a</sub></i> <i>MCM4-5-FLAG (hphNT)</i> <i>ctf18-2A (K.I.TRP1) pep4Δ::URA3 ADE2</i>	This study
YLG426	<i>MAT<sub>a</sub></i> <i>MCM4-5FLAG (hphNT)</i> <i>CTF18 (K.I.TRP1) pep4Δ::URA3 ADE2</i>	This study
YLG445	<i>MAT<sub>a</sub></i> <i>CTF18 (K.I.TRP1) ura3::3xURA3-tetO112 leu2::tetR-GFP-LEU2 PDS1-mCherry (KanMX)</i>	This study
YLG447	<i>MAT<sub>a</sub></i> <i>ctf18-2A (K.I.TRP1) ura3::3xURA3-tetO112 leu2::tetR-GFP-LEU2 PDS1-mCherry (KanMX)</i>	This study
YLG449	<i>MAT<sub>a</sub></i> <i>ctf18Δ::K.I.TRP1 ura3::3xURA3-tetO112 leu2::tetR-GFP-LEU2 PDS1-mCherry (KanMX)</i>	This study
YVM164	<i>MAT<sub>a</sub></i> <i>ctf18Δ::K.I.TRP1</i>	This study
YVM657	<i>MAT<sub>a</sub></i> <i>CTF18-TAP (HIS3MX)</i> <i>POL2-9MYC (K.I.TRP1) RFC4-6HA (K.I.TRP1) pep4Δ::URA3 ADE2</i>	This study
YVM738	<i>MAT<sub>a</sub></i> <i>CTF18-TAP (HIS3MX)</i> <i>POL2-9MYC (K.I.TRP1) RFC4-6HA (K.I.TRP1) dcc1Δ::hphNT pep4Δ::URA3 ADE2</i>	This study
YVM740	<i>MAT<sub>a</sub></i> <i>CTF18-TAP (HIS3MX)</i> <i>POL2-9MYC (K.I.TRP1) RFC4-6HA (K.I.TRP1) ctf8Δ::hphNT pep4Δ::URA3 ADE2</i>	This study
YVM850	<i>MAT<sub>a</sub></i> <i>DCC1-TAP (HIS3MX)</i> <i>ctf18Δ::K.I.TRP1 pep4Δ::URA3 ADE2</i>	This study
PJ69-4A	<i>MAT<sub>a</sub></i> <i>trp1-901 leu2-3, 112 ura3-52 his3-200 gal4Δ gal80Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ</i>	H. Ulrich

### Supplementary Table S1

Strains used in this study – all the above strains are based on the W303 background, except for the yeast two-hybrid strain PJ69-4A and its derivatives YLG60 and YLG63.

Plasmid	Description	Source
pAD27	Gal4 DNA binding domain-MYC-Inn1	This study
pAD30	Gal4 Activation domain-HA-Hof1	This study
pGADT7	Gal4 Activation domain-HA	Clontech
pGBT7	Gal4 DNA binding domain-MYC	Clontech
pHM27	pGADT7-Mrc1	This study
pLG1	pGADT7-Ctf18	This study
pLG2	pGBT7-Ctf18	This study
pLG4	pGBT7-Ctf8	This study
pLG6	pGBT7-Dcc1	This study
pLG16	pGBT7-Pol2 1-1256	This study
pLG24	pGADT7-Ctf18 1-116	This study
pLG25	pGADT7-Ctf18 1-374	This study
pLG26	pGADT7-Ctf18 1-494	This study
pLG27	pGADT7-Ctf18 1-595	This study
pLG28	pGADT7-Ctf18 1-657	This study
pLG31	pGADT7-Ctf18 369-741	This study
pLG32	pGADT7-Ctf18 494-741	This study
pLG35	pGADT7-Ctf18 654-741	This study
pLG36	pGADT7-Ctf18 675-741	This study
pLG37	pGADT7-Ctf18 709-741	This study
pLG54	pGBT7-Pol2 1-227	This study
pLG55	pGBT7-Pol2 1-396	This study
pLG57	pGBT7-Pol2 1-933	This study
pLG59	pGBT7-Pol2 214-1265	This study
pLG60	pGBT7-Pol2 392-1265	This study
pLG61	pGBT7-Pol2 627-1265	This study
pLG62	pGBT7-Pol2 930-1265	This study

### Supplementary Table S2

Plasmids used in this study.