

Supplementary Table 1 Yeast strains used in this study.

All strains were derived from a w303 background: *MATa* or α , *ade2-1*, *trp1-1*, *can1-100*, *leu2-3,112*, *his3-11,15*, *ura3-52*, *GAL*, *psi*⁺

Figure 1

- (a,b) Y3564 *MATa*, *SMC3-Pk₃::HIS3*
Y3912 *MATa*, *SMC3-Pk₃::HIS3*, *ctf4Δ::TRP1*
Y3913 *MATa*, *SMC3-Pk₃::HIS3*, *ctf18Δ::TRP1*
Y3914 *MATa*, *SMC3-Pk₃::HIS3*, *tof1Δ::TRP1*
Y3915 *MATa*, *SMC3-Pk₃::HIS3*, *csm3Δ::TRP1*
Y3916 *MATa*, *SMC3-Pk₃::HIS3*, *chl1Δ::TRP1*
Y3917 *MATa*, *SMC3-Pk₃::HIS3*, *mrc1Δ::TRP1*
- (c) Y4019 *MATa*, *SMC3-Pk₃::HIS3*, *ECO1-HA₆::LEU2*
Y4352 *MATa*, *SMC3-Pk₃::HIS3*, *ECO1-HA₆::LEU2*, *ctf4Δ::TRP1*
Y4353 *MATa*, *SMC3-Pk₃::HIS3*, *ECO1-HA₆::LEU2*, *ctf18Δ::TRP1*
Y4354 *MATa*, *SMC3-Pk₃::HIS3*, *ECO1-HA₆::LEU2*, *tof1Δ::TRP1*
Y4355 *MATa*, *SMC3-Pk₃::HIS3*, *ECO1-HA₆::LEU2*, *csm3Δ::TRP1*
Y4356 *MATa*, *SMC3-Pk₃::HIS3*, *ECO1-HA₆::LEU2*, *chl1Δ::TRP1*
Y4357 *MATa*, *SMC3-Pk₃::HIS3*, *ECO1-HA₆::LEU2*, *mrc1Δ::TRP1*

Figure 2

- (a) as Fig. 1 and
Y4192 *MATa*, *eco1-1::TRP1*, *SMC3-Pk₃::HIS3*, *URA3::tetOs*, *HIS3::tetR-GFP*
- (b) K7100 *MATa*, *URA3::tetOs*, *HIS3::tetR-GFP*
Y3569 *MATa*, *URA3::tetOs*, *HIS3::tetR-GFP*, *eco1-1::TRP1*
Y3856 *MATa*, *URA3::tetOs*, *HIS3::tetR-GFP*, *chl1Δ::TRP1*
Y4161 *MATa*, *URA3::tetOs*, *HIS3::tetR-GFP*, *ctf4Δ::TRP1*

Figure 3

- (a) K7100 as Fig. 2b
Y3320 *MATa*, *URA3::tetOs*, *HIS3::tetR-GFP*, *eco1Δ::LEU2*, *wpl1Δ::kan^R*
Y3853 *MATa*, *URA3::tetOs*, *HIS3::tetR-GFP*, *ctf18Δ::TRP1*
Y3854 *MATa*, *URA3::tetOs*, *HIS3::tetR-GFP*, *tof1Δ::TRP1*

Y3855 *MATa*, *URA3::tetOs*, *HIS3::tetR-GFP*, *csm3Δ::TRP1*

Y3856 Fig. 2b

Y3857 *MATa*, *URA3::tetOs*, *HIS3::tetR-GFP*, *mrc1Δ::TRP1*

Y3830 *MATa*, *URA3::tetOs*, *HIS3::tetR-GFP*, *eco1Δ::LEU2*, *wpl1Δ::kan^R*,
ctf18Δ::TRP1

Y3832 *MATa*, *URA3::tetOs*, *HIS3::tetR-GFP*, *eco1Δ::LEU2*, *wpl1Δ::kan^R*,
tof1Δ::TRP1

Y3833 *MATa*, *URA3::tetOs*, *HIS3::tetR-GFP*, *eco1Δ::LEU2*, *wpl1Δ::kan^R*,
csm3Δ::TRP1

Y3805 *MATa*, *URA3::tetOs*, *HIS3::tetR-GFP*, *eco1Δ::LEU2*, *wpl1Δ::kan^R*,
chl1Δ::TRP1

Y3806 *MATa*, *URA3::tetOs*, *HIS3::tetR-GFP*, *eco1Δ::LEU2*, *wpl1Δ::kan^R*,
mrc1Δ::TRP1

(b) Y3320 as Fig. 3a

x

Y3794 *MATα*, *URA3::tetOs*, *HIS3::tetR-GFP*, *ctf4Δ::TRP1*

(c) as Fig. 3a and

Y4157 *MATa*, *URA3::tetOs*, *HIS3::tetR-GFP*, *eco1Δ::LEU2*, *WPL1::GAL1-WPL1-
PK9::kan^R;TRP1*

Y4161 as Fig. 2b

(d) K7100 and 4161 as Fig. 2b

as Fig. 3a and

Y933 *MATa*, *URA3::tetOs*, *HIS3::tetR-GFP*, *wpl1Δ::kan^R*

Y4151 *MATα*, *URA3::tetOs*, *HIS3::tetR-GFP*, *wpl1Δ::kan^R*, *ctf4Δ::TRP1*

Y4152 *MATα*, *URA3::tetOs*, *HIS3::tetR-GFP*, *wpl1Δ::kan^R*, *ctf18Δ::TRP1*

Y4153 *MATα*, *URA3::tetOs*, *HIS3::tetR-GFP*, *wpl1Δ::kan^R*, *tof1Δ::TRP1*

Y4154 *MATα*, *URA3::tetOs*, *HIS3::tetR-GFP*, *wpl1Δ::kan^R*, *csm3Δ::TRP1*

Y4155 *MATα*, *URA3::tetOs*, *HIS3::tetR-GFP*, *wpl1Δ::kan^R*, *chl1Δ::TRP1*

Y4156 *MATα*, *URA3::tetOs*, *HIS3::tetR-GFP*, *wpl1Δ::kan^R*, *mrc1Δ::TRP1*

Figure 4

(b) Y4298 *MATα*, *tor1-1*, *fpr1::nat^R*, *RPL13A-2xFKBP12::TRP1*, *Sccl-FRB-GFP::HIS3*,
ctf4Δ::LEU2

Y4299 *MATα*, *tor1-1*, *fpr1::nat^R*, *RPL13A-2xFKBP12::TRP1*, *Sccl-FRB-GFP::HIS3*,
chl1Δ::LEU2

Y4300 *MATα, tor1-1, fpr1::nat^R, RPL13A-2xFKBP12::TRP1, Scc1-FRB-GFP::HIS3, ctf18Δ::LEU2*

Y4340 *MATα, tor1-1, fpr1::nat^R, RPL13A-2xFKBP12::TRP1, Scc1-FRB-GFP::HIS3, ADH1-OsTIR1-myc9::URA3, eco1-aid::kan^R*

Y4342 *MATα, tor1-1, fpr1::nat^R, RPL13A-2xFKBP12::TRP1, Scc1-FRB-GFP::HIS3*

Figure 5

- (a) as Fig. 2b and
Y4227 *MATa, URA3::tetOs, HIS3::tetR-GFP, ctf4Δ::TRP1, chl1Δ::LEU2*
- (b) Y1451 *MATa, CTF4-HA₆::HIS3*
Y3615 *MATa, CTF4-HA₆::HIS3, CHL1-PK₃::TRP1*
Y3616 *MATa, CTF4-HA₆::HIS3, POL1-PK₃::TRP1*
- (c) Y2329 *MATa, SCC1-Pk₃::HIS3*
Y3619 *MATa, SCC1-Pk₃::HIS3, chl1Δ::LEU2*

Figure 6

- (a) Y4184 *MATa, URA3::tetOs, HIS3::tetR-GFP, fen1Δ::TRP1*
x
Y4165 *MATα, URA3::tetOs, HIS3::tetR-GFP, ctf4Δ::LEU2*
Y4184 as above
x
Y4169 *MATα, URA3::tetOs, HIS3::tetR-GFP, chl1Δ::LEU2*
Y4183 *MATa, dna2-2::LEU2*
x
Y3798 *MATα, URA3::tetOs, HIS3::tetR-GFP, chl1Δ::TRP1*
- (b) K7100 as Fig. 2b
Y3853 as Fig. 3a
Y4184 as Fig. 6a
Y4193 *MATa, URA3::tetOs, HIS3::tetR-GFP, dna2-2::LEU2*
- (c) yIW310 *MATa, cdc9::tetO₇-CDC9, cmv_LacI-nat^R*
yIW312 *MATa, cdc9::tetO₇-CDC9, cmv_LacI-nat^R, ctf4Δ::TRP1*
yIW313 *MATa, cdc9::tetO₇-CDC9, cmv_LacI-nat^R, chl1Δ::TRP1*
yIW314 *MATa, cdc9::tetO₇-CDC9, cmv_LacI-nat^R, ctf18Δ::TRP1*

(d) K7100 as Fig. 2b

Y4222 *MATa*, *URA3::tetOs*, *HIS3::tetR-GFP*, *asf1Δ::TRP1*

Y4348 *MATa*, *URA3::tetOs*, *HIS3::tetR-GFP*, *cac1Δ::TRP1*

Y4349 *MATa*, *URA3::tetOs*, *HIS3::tetR-GFP*, *cac2Δ::TRP1*

Supplementary Figure 1

Y4133 *MATa*, *ctf4Δ::TRP1*, YCplac33-*URA3-CTF4*

Y4134 *MATa*, *ctf4Δ::TRP1*, *eco1Δ::LEU2*, *wpl1Δ::kan^R*, YCplac33-*URA3-CTF4*

Y4135 *MATa*, *ctf4Δ::TRP1*, *ctf18Δ::HIS3*, YCplac33-*URA3-CTF4*

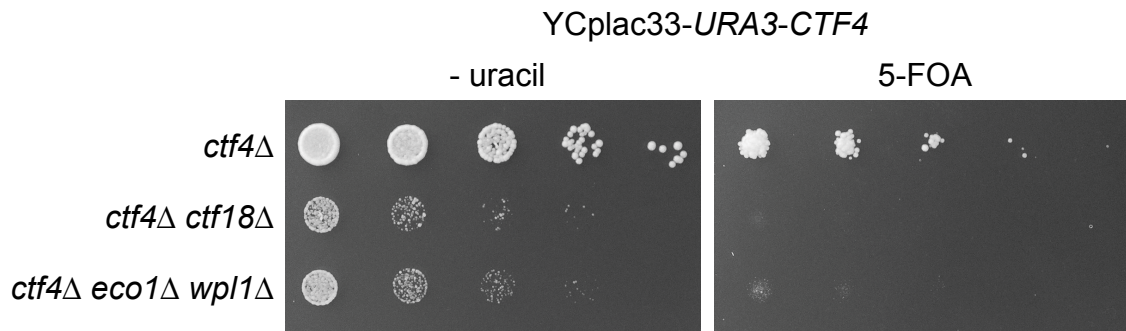
Supplementary Figure 2

K699 *MATa*, *ade2-1*, *trp1-1*, *can1-100*, *leu2-3,112*, *his3-11,15*, *ura3-52*, *GAL*, *psi⁺*

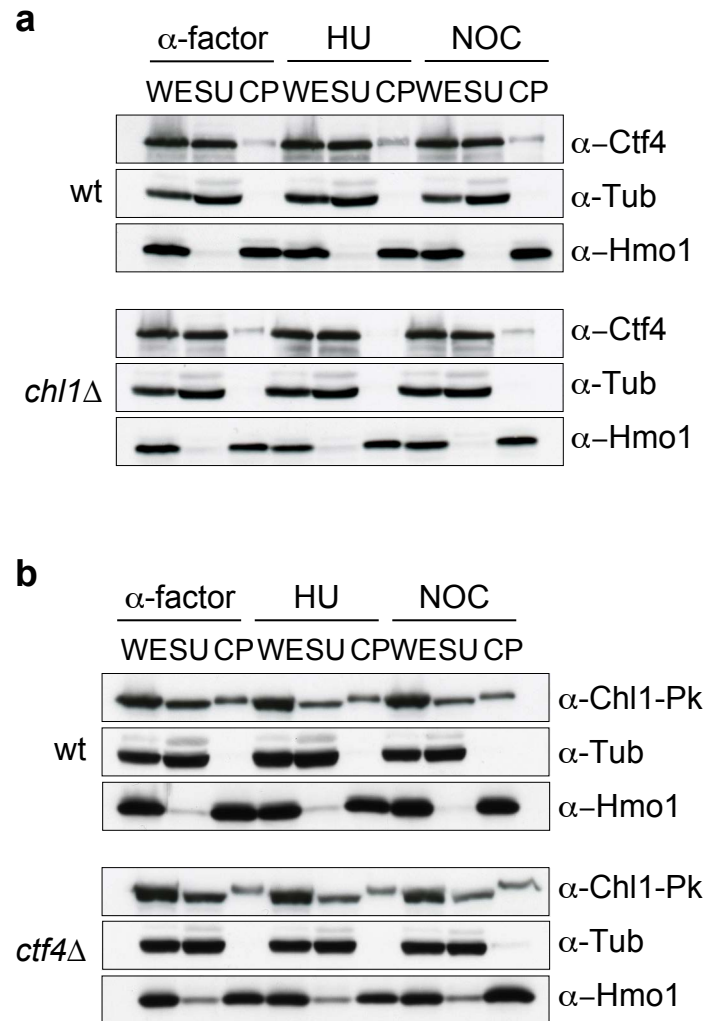
Y3856 as Fig. 2b

Y3612 *MATa*, *CHL1-PK₃::HIS3*

Y3617 *MATa*, *CHL1-PK₃::HIS3*, *ctf4Δ::TRP1*



Supplementary Fig. 1 *ctf4*Δ is synthetically lethal in combination with *eco1*Δ *wpl1*Δ. A plasmid loss assay was employed to assess synthetic interactions between *CTF4* and *ECO1*. 5-fold serial dilutions of cultures of the indicated genotypes were spotted onto medium lacking uracil or containing 5-FOA and incubated at 25°C. *eco1*Δ *wpl1*Δ *ctf4*Δ cells could not grow without the plasmid containing *CTF4*, while the plasmid was efficiently lost from cells containing the *ctf4*Δ deletion only. *ctf4*Δ *ctf18*Δ cells were included as a control that is known to exhibit synthetic lethality. Both *ctf4*Δ *ctf18*Δ and *ctf4*Δ *eco1*Δ *wpl1*Δ cells containing the *CTF4* plasmid grew poorly on synthetic complete medium lacking uracil, when compared to *ctf4*Δ cells.



Supplementary Fig. 2 Chl1 promotes Ctf4 recruitment to chromosomes in S-phase. **a** Wild type and *chl1* Δ cells were synchronized in G1 by α -factor treatment and released into either HU or nocodazole-containing medium. Whole cell extracts (WE) were separated into supernatant (SU) and chromatin (CP) fractions, and Ctf4 was detected by immunoblotting using an α -Ctf4 antibody. Tubulin and Hmo1 served as loading controls for the supernatant and chromatin fractions, respectively. The low but detectable Ctf4 enrichment on chromatin during S-phase, consistent with its role as part of the replisome progression complex, was not observed in the absence of Chl1. **b** Ctf4 is not required for chromatin association of Chl1. As **a**, but chromatin binding of Pk epitope-tagged Chl1 was compared between wild type and *ctf4* Δ cells. A constant low-level chromatin binding of Chl1 was observed that was independent of Ctf4.