SUPPLEMENTAL MATERIAL

The Prp19C/NTC subunit Syf2 and the Prp19C/NTC-associated protein Cwc15 function in TREX occupancy and transcription elongation

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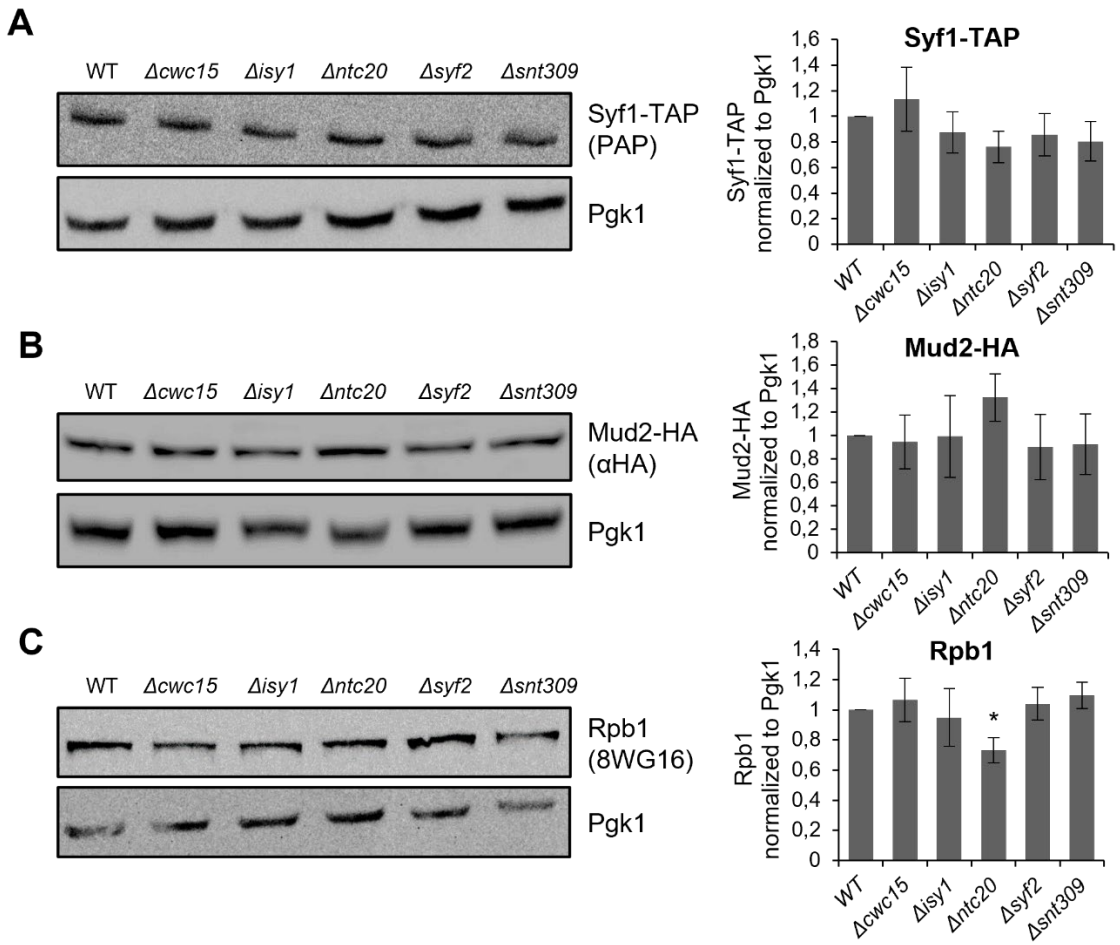
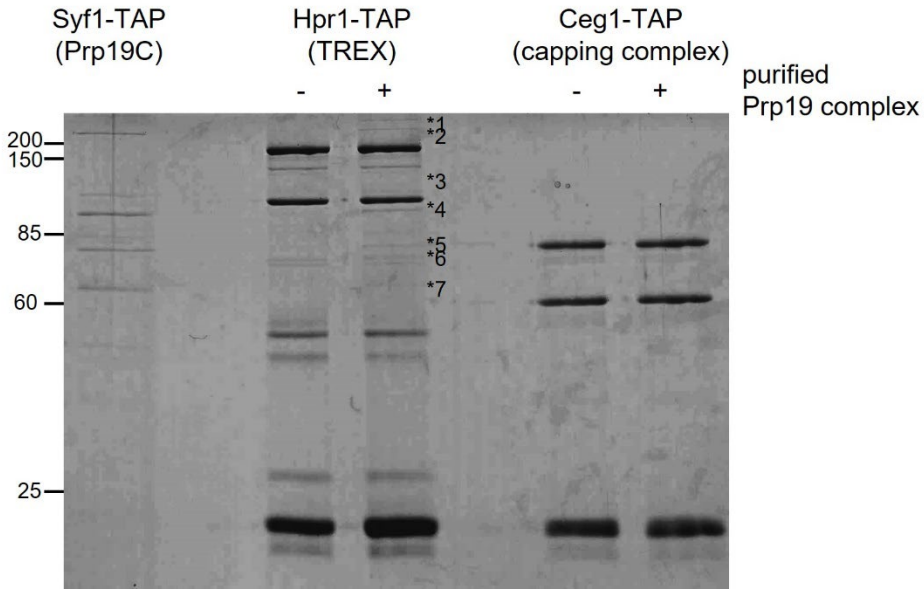
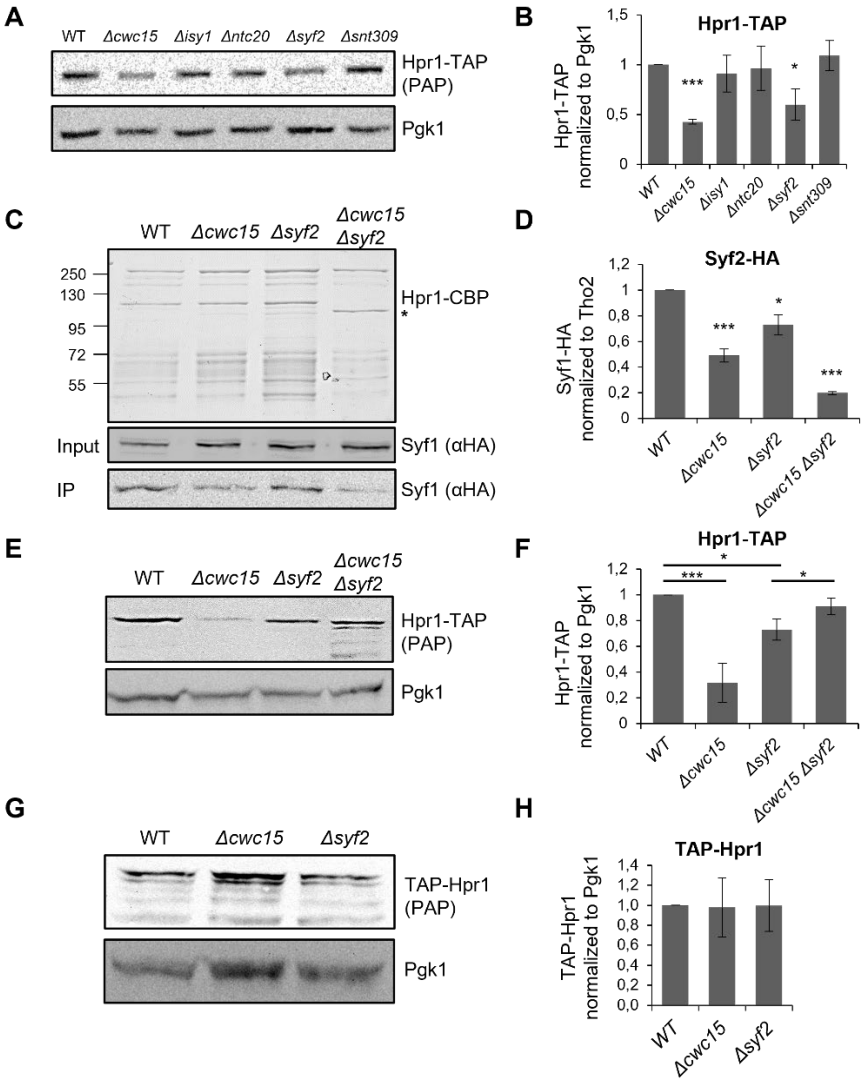


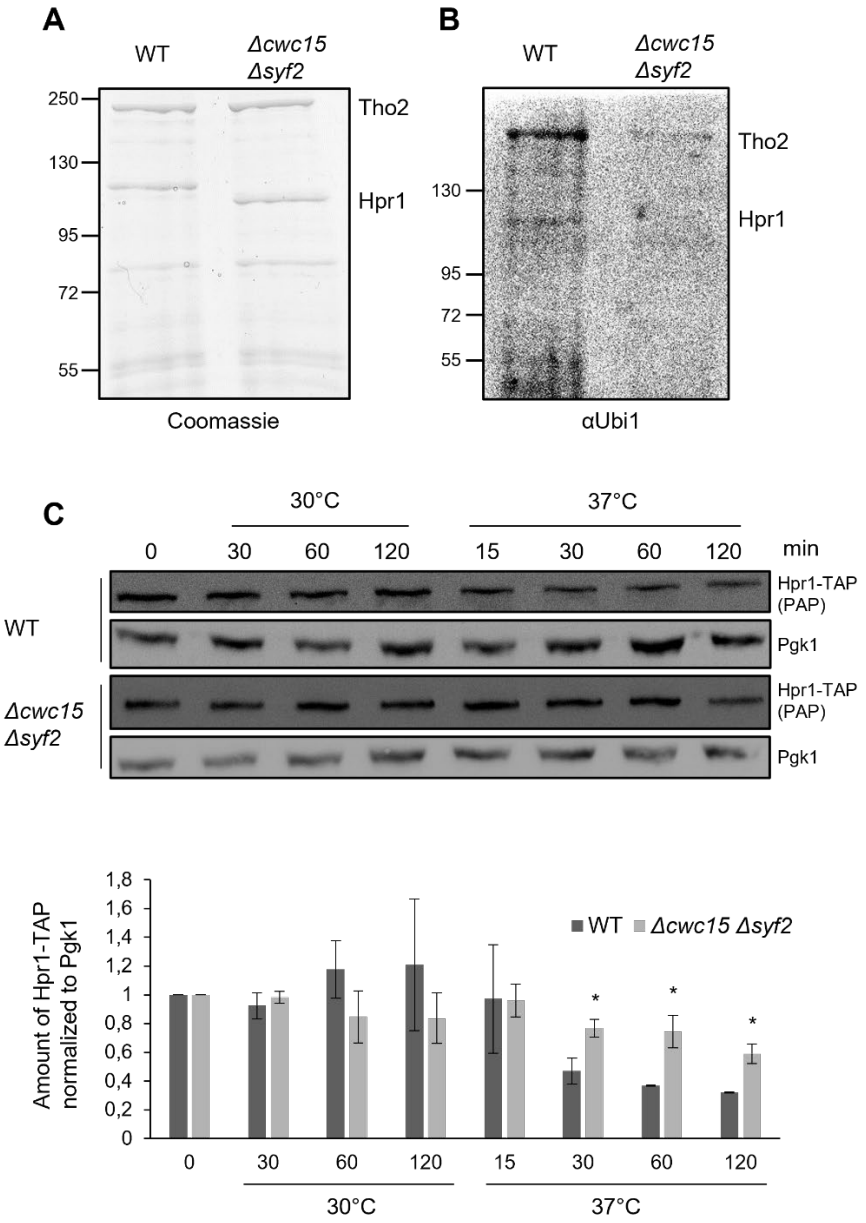
SUPPLEMENTAL FIGURE S1. Total levels of Syf1, Mud2 and Rpb1 do not change in deletion mutants of genes encoding the nonessential Prp19C components Cwc15, Isy1, Ntc20, Syf2 and Snt309. (*A*) Western blots (left panel) and quantification of three independent experiments (right panel) of total Syf1 levels in whole cell lysates of deletion mutants of the five nonessential Prp19C components. The amount of Syf1-TAP was quantified with an antibody directed against the protein A moiety of the TAP tag (PAP) and normalized to the amount of Pgk1. Values for wild-type (WT) cells were set to 1. (*B*) Western blots (left panel) and quantification (right panel) as in (A) except that the total levels of Mud2-HA were determined with an αHA antibody. (*C*) Western blots (left panel) and quantification (right panel) as in (A) except that the total levels of Rpb1 were determined with antibody 8WG16. Total Rpb1 levels slightly decrease in the *Δntc20* mutant.



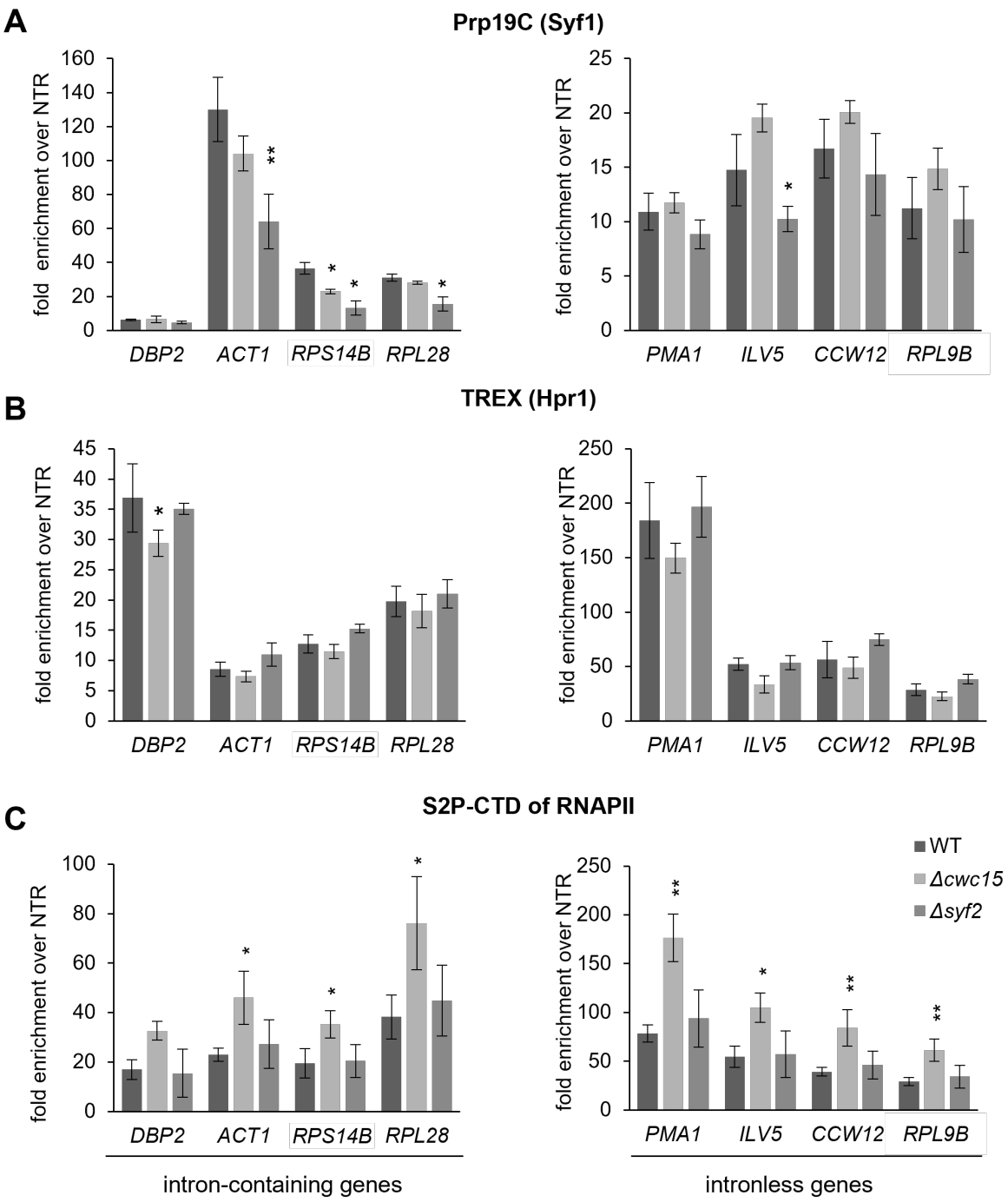
SUPPLEMENTAL FIGURE S2. Prp19C binds directly to the THO complex in an RNA-independent manner. Equal amounts of Prp19C were purified until the EGTA eluate from a strain expressing Syf1-TAP and incubated with purified THO and mRNA capping enzyme, a heterotetramer composed of two molecules of Ceg1 and Cet1 each that served as negative control, bound to IgG-coupled Sepharose beads. Beads were washed and the bound proteins were eluted by cleavage with TEV protease. Prp19C subunits that interact with the THO complex were identified by mass spectrometry: 1: Prp8, 2: Brr2, 3: Snu114, 4: Syf1, 5: Cef1, 6: Syf3, 7: Prp19.



SUPPLEMENTAL FIGURE S3. Deletion of *CWC15* or *SYF2* causes a decrease in total Hpr1-TAP but not TAP-Hpr1 levels. (*A*) Western blots to determine the amount of Hpr1-TAP in whole cell lysates using an antibody against the protein A moiety of the TAP tag (PAP) in the five deletion mutants of nonessential Prp19C components compared to wild-type (WT) cells. Pgk1 levels served as a loading control. (*B*) Quantification of three independent experiments as depicted in (A). (*C, D*) Deletion of *CWC15* and/or *SYF2* decreases the interaction between TREX and Prp19C. (*C*) EGTA eluates of TREX purifications using Hpr1-TAP from strains expressing HA-tagged Syf1 in wild-type (WT), *Δcwc15*, *Δsyf2* and *Δcwc15 Δsyf2* cells. The band corresponding to Hpr1-CBP in wild-type cells is marked Hpr1-CBP, the band corresponding to faster migrating Hpr1-CBP is indicated by an asterisk (*). Syf1-HA levels of extracts (Input) and eluates (IP) were assessed by Western blotting using antibody against the HA tag. (*D*) Quantification of three independent purifications as shown in (C). (*E,F*) Double deletion of *CWC15* and *SYF2* restores total Hpr1-TAP levels. (*E*) Western blots of whole cell lysates to determine the total levels of Hpr1-TAP in wild-type (WT), *Δcwc15*, *Δsyf2* and *Δcwc15 Δsyf2* cells. Pgk1 levels served as a loading control. (*F*) Quantification of three independent experiments as shown in (E). (*G,H*) Total TAP-Hpr1 levels are unaffected in *Δcwc15* and *Δsyf2* cells. (*G*) Western blots of whole cell lysate to determine the total levels of N-terminally TAP-tagged Hpr1 in wild-type (WT), *Δcwc15* and *Δsyf2* cells. Pgk1 levels served as a loading control. (*H*) Quantification of total TAP-Hpr1 levels normalized to Pgk1 of three independent experiments.



SUPPLEMENTAL FIGURE S4. Double deletion of *CWC15* and *SYF2* causes lower ubiquitylation levels and higher stability of Hpr1-TAP. (*A* and *B*) Hpr1-TAP is less ubiquitylated in *Δcwc15 Δsyf2* cells. (*A*) Coomassie gel of EGTA eluates of TAP purifications of C-terminally TAP-tagged Hpr1 from wild-type and *Δcwc15 Δsyf2* cells. Bands corresponding to TREX components Tho2 and Hpr1 are indicated. The band between 72 and 95 kD most likely corresponds to Hsp70, and the bands at 55 kD most likely contain the TREX components Hrb1, Gbp2, Mft1 and Sub2. (*B*) Western blots of the eluates shown in (A) using an antibody against ubiquitin (αUBI1). Interestingly, other TREX components such as Tho2 are also ubiquitylated in wild-type cells and to a lower extent in *Δcwc15 Δsyf2* cells. (*C*) Hpr1-TAP is more stable in the *Δcwc15 Δsyf2* double deletion mutant than in wild-type cells at 37°C. Cells were grown in YPD medium at 30°C and shifted to 37°C at an OD_{600} of 0.6, and samples were taken at the time points indicated. Upper panel: Western blots with antibodies directed against protein A moiety of the TAP tag to detect Hpr1-TAP (PAP) or against Pgk1, which served as loading control. Lower panel: Relative amount of Hpr1-TAP normalized to the amount of Pgk1 at t = 0 min, based on quantification of three independent experiments as depicted in the upper panel.



SUPPLEMENTAL FIGURE S5. (*A* and *B*) Prp19C occupancy is reduced in *Δsyf2* cells and TREX occupancy is reduced in *Δcwc15* cells. The occupancy of Syf1 (*A*) and Hpr1 (*B*) was assessed in wild-type cells (WT), *Δcwc15* and *Δsyf2* deletion mutants using chromatin immunoprecipitation (ChIP) at both intron-containing (left panels) and intronless genes (right panels). (*C*) The occupancy of S2-phosphorylated RNAPII increases in *Δcwc15* cells and is unchanged in *Δsyf2* cells. The occupancy of S2P was assessed with the antibody 3E10. ChIPs were quantified by Real Time PCR using primer pairs annealing to the 3' end of the respective genes.

SUPPLEMENTAL TABLE S1. Yeast strains

Strain	Strain Background	Genotype	Reference
wild-type	BY4741	*MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0*	(Strasser and Hurt 2000)
wild-type	W303	*MATa; ade2-1; his3-11, 15; ura3-1; leu2-3, 112; trp1-1; can1-100; rad5-535*	(Thomas and Rothstein 1989)
MUD2-HA	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; MUD2-HA::HIS3*	this study
SYF1-TAP MUD2-HA	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; SYF1-TAP::URA, MUD2-HA::HIS*	this study
SYF1-TAP MUD2-HA Δcwc15	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; SYF1-TAP::URA3; MUD2-HA::HIS3; Δcwc15::kanMX4*	this study
SYF1-TAP MUD2-HA Δisy1	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; SYF1-TAP::URA3; MUD2-HA::HIS3; Δisy1::kanMX4*	this study
SYF1-TAP MUD2-HA Δntc20	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; MUD2-HA::HIS3; SYF1-TAP::URA3; Δntc20::kanMX4*	this study
SYF1-TAP MUD2-HA Δsyf2	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; MUD2-HA::HIS3; SYF1-TAP::URA3; Δsyf2::kanMX4*	this study
SYF1-TAP MUD2-HA Δsnt309	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; MUD2-HA::HIS3; SYF1-TAP::URA3; Δsnt309::kanMX4;*	this study
SYF1-HA	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; SYF1-HA::HIS3*	this study
HPR1-TAP SYF1-HA	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; SYF1-HA::HIS3; HPR1-TAP::URA3*	this study
HPR1-TAP SYF1-HA Δcwc15	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; HPR1-TAP::URA3; SYF1-HA::HIS3; Δcwc15::kanMX4;*	this study
HPR1-TAP SYF1-HA Δisy1	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; HPR1-TAP::URA3; SYF1-HA::HIS3; Δisy1::kanMX4*	this study
HPR1-TAP SYF1-HA Δntc20	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; HPR1-TAP::HIS3; SYF1-HA::URA3; Δntc20::kanMX4;*	this study
HPR1-TAP SYF1-HA Δsyf2	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; HPR1-TAP::URA3; SYF1-HA::HIS3; Δsyf2::kanMX4*	this study
HPR1-TAP SYF1-HA Δsnt309	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; HPR1-TAP::URA3; SYF1-HA::HIS3; Δsnt309::kanMX4*	this study
HPR1-TAP SYF1-HA Δcwc15 Δsyf2	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; HPR1-TAP::URA3; SYF1-HA::HIS3; Δsyf2::kanMX4; Δcwc15::LEU2;*	this study
SYF1-TAP	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; SYF1-TAP::URA3;*	this study
SYF1-TAP Δcwc15	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; SYF1-TAP::URA3; Δcwc15::kanMX4*	this study
SYF1-TAP Δisy1	BY4741	*MATa; leu2Δ0; met15Δ0; his3Δ1; ura3Δ0, SYF1-TAP::URA3; Δisy1::kanMX4*	this study
SYF1-TAP Δntc20	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; SYF1-TAP::URA3; Δntc20::kanMX4*	this study
SYF1-TAP Δsyf2	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; SYF1-TAP::URA3; Δsyf2::kanMX4*	this study

SYF1-TAP Δsnt309	BY4741	*MATa; leu2Δ0; met15Δ0; ura3Δ0 SYF1-TAP::URA3; Δsnt309*::kanMX4	this study
HPR1-TAP	BY4741	*MATa, his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0; HPR1-TAP::URA3*	this study
HPR1-TAP Δcwc15	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; HPR1-TAP::URA3; Δcwc15::kanMX4*	this study
HPR1-TAP Δisy1	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; HPR1-TAP::URA3; Δisy1::kanMX4*	this study
HPR1-TAP Δntc20	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; HPR1-TAP::URA3; Δntc20::kanMX4*	this study
HPR1-TAP Δsyf2	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; Δsyf2::kanMX4; HPR1-TAP::URA3*	this study
HPR1-TAP Δsnt309	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; HPR1-TAP::URA3; Δsnt309::kanMX4*	this study
Δdst1	W303	*MATa; ura3-1; trp1-1; his3-11,15; leu2-3,112; ade2-1; can1-100; GAL+; Δdst1::HIS3*	this study
Δcwc15	W303	*MATa; ura3-1; trp1-1; his3-11,15; leu2-3,112; ade2-1; can1-100; GAL+; Δcwc15::kanMX4*	this study
Δisy1	W303	*MATa; ura3-1; trp1-1; his3-11,15; leu2-3,112; ade2-1; can1-100; GAL+; Δisy1::kanMX4*	this study
Δntc20	W303	*MATa; ura3-1; trp1-1; his3-11,15; leu2-3,112; ade2-1; can1-100; GAL+; Δntc20::kanMX4*	this study
Δsyf2	W303	*MATa; ura3-1; trp1-1; his3-11,15; leu2-3,112; ade2-1; can1-100; GAL+; Δsyf2::kanMX4*	this study
Δsnt309	W303	*MATa; ura3-1; trp1-1; his3-11,15; leu2-3,112; ade2-1; can1-100; GAL+; Δsnt309*::kanMX4	this study
Δcwc15 Δdst1	W303	*MATa; ura3-1; trp1-1; his3-11,15; leu2-3,112; ade2-1; can1-100; GAL+; Δdst1::HIS3; Δcwc15::kanMX4*	this study
Δisy1 Δdst1	W303	*MATa; ura3-1; trp1-1; his3-11,15; leu2-3,112; ade2-1; can1-100; GAL+; Δdst1::HIS3; Δisy1::kanMX4*	this study
Δntc20 Δdst1	W303	*MATa; ura3-1; trp1-1; his3-11,15; leu2-3,112; ade2-1; can1-100; GAL+; Δdst1::HIS, Δntc20::kanMX4*	this study
Δsyf2 Δdst1	W303	*MATa; ura3-1; trp1-1; his3-11,15; leu2-3,112; ade2-1; can1-100; GAL+; Δdst1::HIS3; Δsyf2::kanMX4*	this study
Δsnt309 Δdst1	W303	*MATa; ura3-1; trp1-1; his3-11,15; leu2-3,112; ade2-1; can1-100; GAL+; Δdst1::HIS3; Δsnt309::kanMX4*	this study
Δcwc15	BY4741	*MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0 Δcwc15::kanMX4*	Euroscarf
Δisy1	BY4741	*MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0 Δisy1::kanMX4*	Euroscarf
Δntc20	BY4741	*MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0 Δntc20::kanMX4*	Euroscarf
Δsyf2	BY4741	*MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0 Δsyf2::kanMX4*	Euroscarf
Δsnt309	BY4741	*MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0 Δsnt309::kanMX4*	Euroscarf
TAP-HPR1 SYF1-HA	BY4741	*MATa; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; TAP-HPR1; SYF1-HA::HIS3*	this study
TAP-HPR1 SYF1-HA Δcwc15	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; NTAP-HPR1; SYF1-HA::HIS3; Δcwc15::kanMX4*	this study
TAP-HPR1 SYF1-HA Δsyf2	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; NTAP-HPR1; SYF1-HA::HIS3; Δsyf2::kanMX4*	this study

TAP-HPR1 SYF1-HA Δcwc15 Δsyf2	BY4741	*MATa; ura3Δ0; leu2Δ0; his3Δ1; met15Δ0; NTAP-HPR1; SYF1-HA::HIS3; Δsyf2::kanMX4; Δcwc15::LEU24*	this study
HPR1-TAP	W303	*MATa; ura3-1; trp1-1; his3-11,15; leu2-3,112; ade2-1; can1-100; GAL+; HPR1-TAP::TRP1*	this study
HPR1-TAP Δcwc15 Δsyf2	W303	*MATa; ura3-1; trp1-1; his3-11,15; leu2-3,112; ade2-1; can1-100; GAL+; HPR1-TAP::TRP1; Δsyf2::kanMX4; Δcwc15::LEU2*	this study

SUPPLEMENTAL TABLE S2. Plasmids

Plasmid	Description	Reference
pBS1539	for C-terminal TAP-tagging of a protein by genomic integration with the *URA3* marker	(Puig et al. 2001)
pBS1776	for N-terminal TAP-tagging of a protein by genomic integration with the *LEU2* marker	(Puig et al. 2001)
pYM15	for C-terminal HA-tagging with the *HIS3MX6* marker	Euroscarf
pRS316-*GAL10::ACT1*	*GAL10* promoter sequence in front of *ACT1* ORF in pRS316	(Chanarat et al. 2012)

SUPPLEMENTAL TABLE S3. Oligonucleotides

Primer	Sequence (5′–3′)
YER-for	TGCGTACAAAAAGTGTCAAGAGATT
YER_rev	ATGCGCAAGAAGGTGCCTAT
PMA1-3'-for	CAGAGCTGCTGGTCCATTCTG
PMA1-3'-rev	GAAGACGGCACCAGCCAAT
DBP2-3'-for	CTTCACCGAACAAAACAAAGGTT
DBP2-3'-rev	TCGGGAGGAATATTTTGATTAGCT
ACT1-3'-for	TCAGAGCCCCAGAAGCTTTG
ACT1-3'-rev	TTGGTCAATACCGGCAGATTC
ILV5-3'-for	TGGTACCCAATCTTCAAGAATGC
ILV5-3'-rev	ACCGTTCTTGGTAGATTCGTACA
CCW12-3'-for	TGAAGCTCCAAAGAACACCACC
CCW12-3'-rev	AGCAGCAGCACCAGTGTAAG
RPL9B-3'-for	AGGACGAAATCGTCTTATCTGGT
RPL9B-3'-rev	CAGATTTGTTGCAAGTCAGCGG
RPL28-3'-for	TGGAAGCCAGTCTTGAACTTGG
RPL28-3'-rev	TTGGTCTCTCTTGTCTTCTGGGA
RPS14B-3'-for	AAGACCCCAGGACCAGGTG
RPS14B-3'-rev	GATACGGCCAATCCTCAAACCAG
ACT1-cy5	Cy5- TGGATTGAGCTTCATCACCAACG
GAL10-cy5_96	Cy5- ATTAGCTCTACCACAGTGTGTG
SCR1-cy5	Cy5-TTTACGACGGAGGAAAGACG

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