Supplementary Data

Degradation of DNA damage-independently stalled RNA polymerase II is independent of the E3 ligase Elc1

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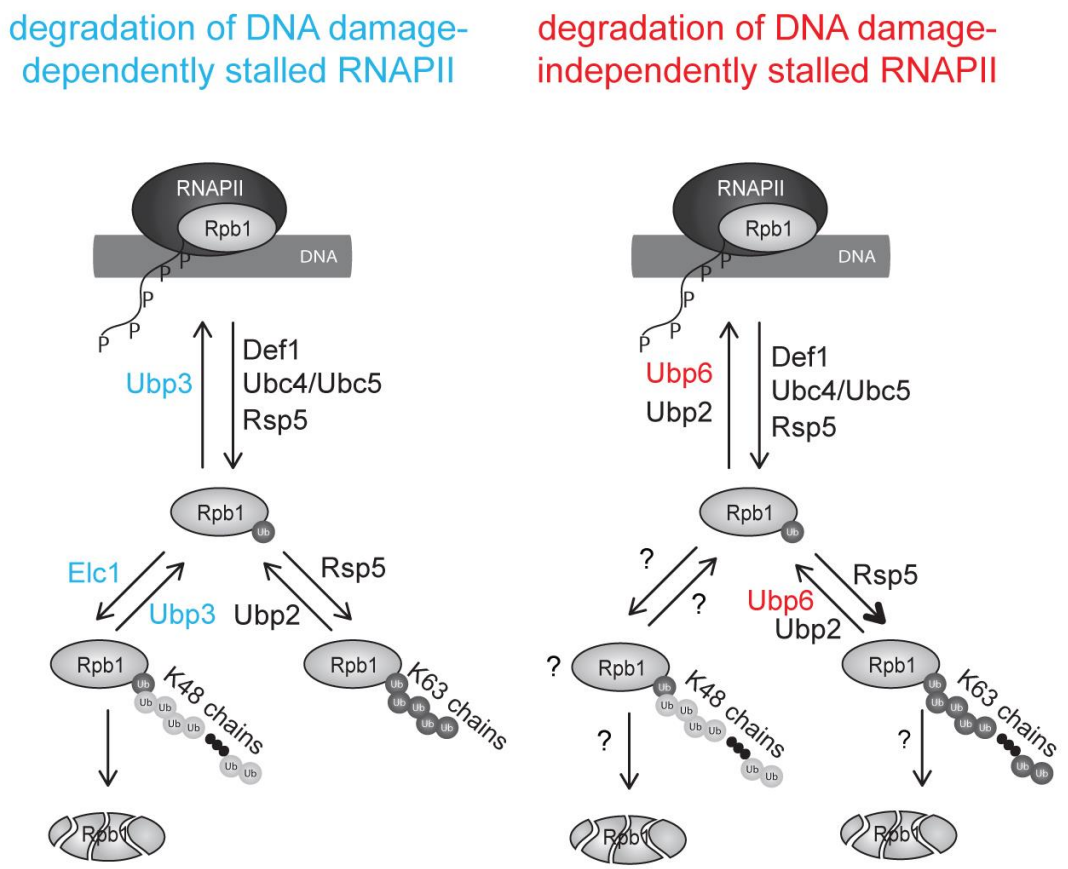

Figure S1 Models comparing the degradation of Rpb1 of persistently stalled RNAP II complexes due to different causes. The pathway for degradation upon DNA damage is shown on the left side and the pathway for degradation of DNA damage-independently stalled RNAPII is shown on the right side. See text for details.

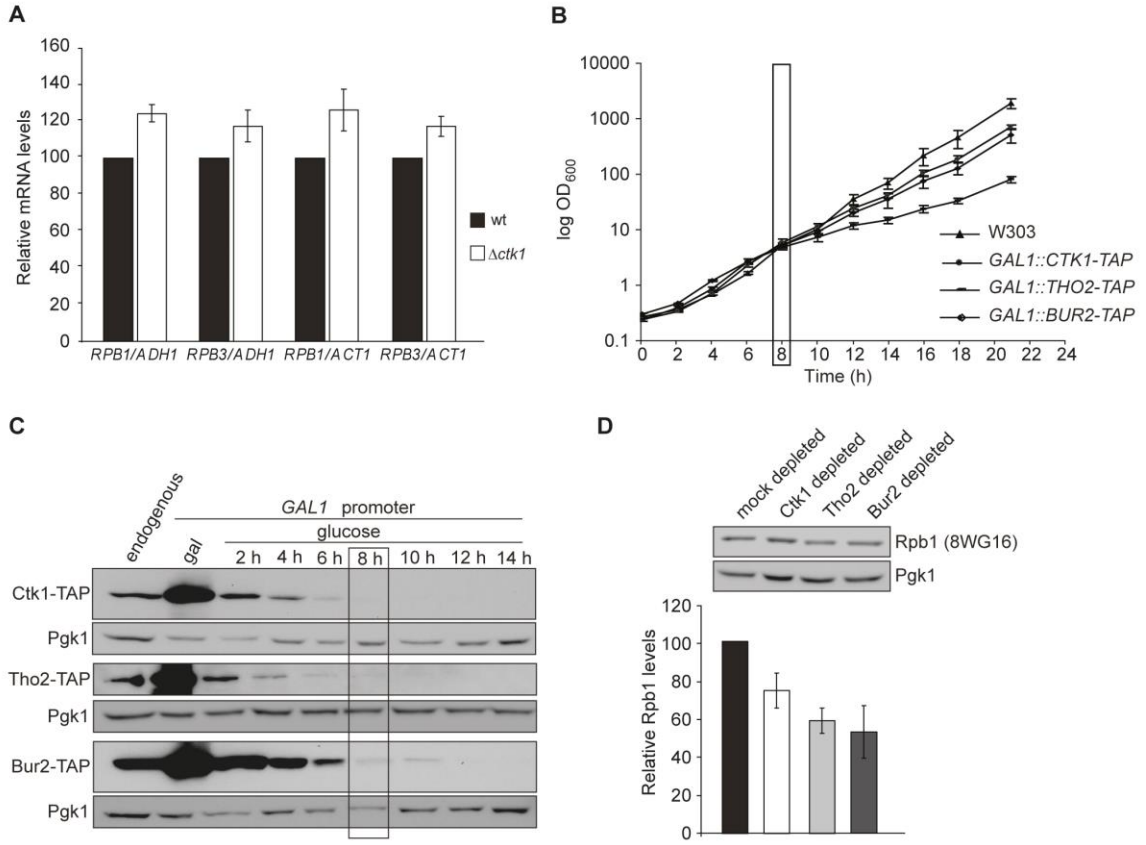

Figure S2 Depletion of transcription elongation factors causes lower total levels of Rpb1. (A) Levels of mRNAs encoding Rpb1 and Rpb3 are not reduced compared to mRNAs encoding the house-keeping proteins Adh1 and Act1 in *Δctk1* cells. To quantify total mRNA levels encoding Rpb1, Rpb3, Adh1 and Act1, RNA was extracted from yeast as previously described by (1). First strand cDNA synthesis was performed using oligo-dT and M-MuLV Reverse Transcriptase (Fermentas). The cDNA was analyzed by Real-Time PCR on an ABI Prism 7000 machine (Applied Biosystems). Reactions were performed as for the ChIP analysis. Relative *RPB1* and *RPB3* mRNA levels were calculated using a standard curve method as the ratio of the *RPB1* and *RPB3* over the *ADH1* or *ACT1* mRNA amount, respectively. Primer sequences are available on request. Columns and error bars represent the mean ± standard deviation from 3 independent experiments (bottom panels). (B) Growth curve of yeast cells depleted of Ctk1, Tho2, or Bur2. Growth is not impaired during the first 8 hours of depletion. (C) Protein levels of Ctk1, Tho2, and Bur2 after different depletion times. Depletion of TAP-tagged versions of these proteins was assessed by Western blotting using PAP antibody (Peroxidase Anti-Peroxidase; Sigma). (D) Total levels of Rpb1 were reduced after 8 hours of depletion of Ctk1, Tho2, or Bur2. Columns and error bars represent the mean ± standard deviation from 3 independent experiments (bottom panel).

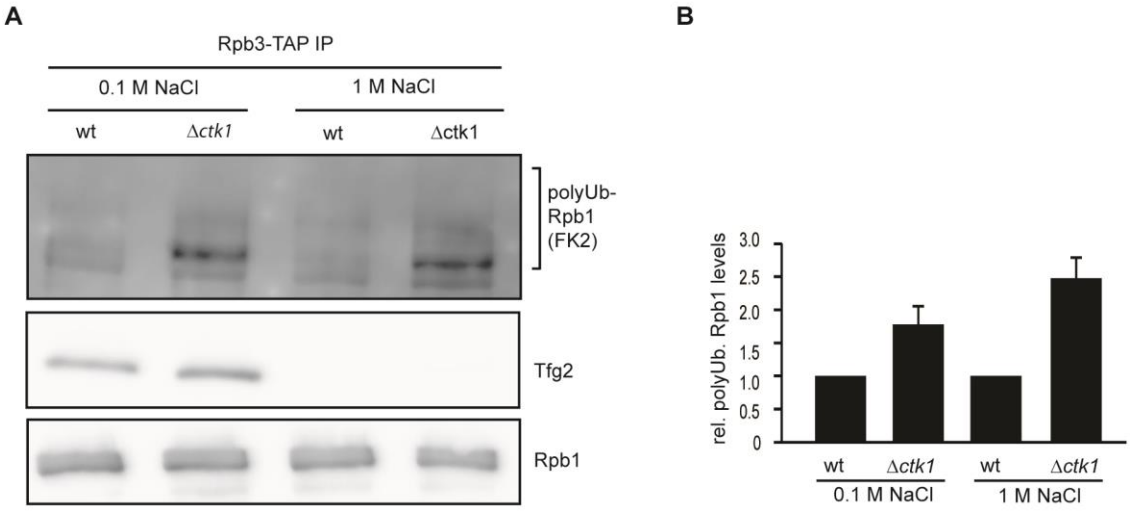

Figure S3 Ubiquitylation of Rpb1 is increased when transcription elongation is impaired by deletion of *CTK1*. (**A**) Equal amounts of RNAPII were purified from Rpb3 TAP-tagged wt and *Δctk1* strains with buffer containing 100 mM or 1 M NaCl (high salt) in the presence of deubiquitylase and proteasome inhibitors by using limiting amounts of beads. Polyubiquitylation of Rpb1 was assessed by Western blotting using a ubiquitin-specific antibody (upper panel) and normalized to the amount of purified Rpb1 (lower panel). Tfg2, a subunit of TFIIF, a transcription factor with the highest affinity for RNAPII, does not copurify with RNAPII under high salt conditions (middle panel). (**B**) Ubiquitylation of Rpb1 increased in the *Δctk1* strain compared to wt when RNAPII was purified under high salt conditions. The respective ubiquitin signal was normalized to the amount of Rpb1. Columns and error bars represent the mean ± standard deviation of three independent experiments.

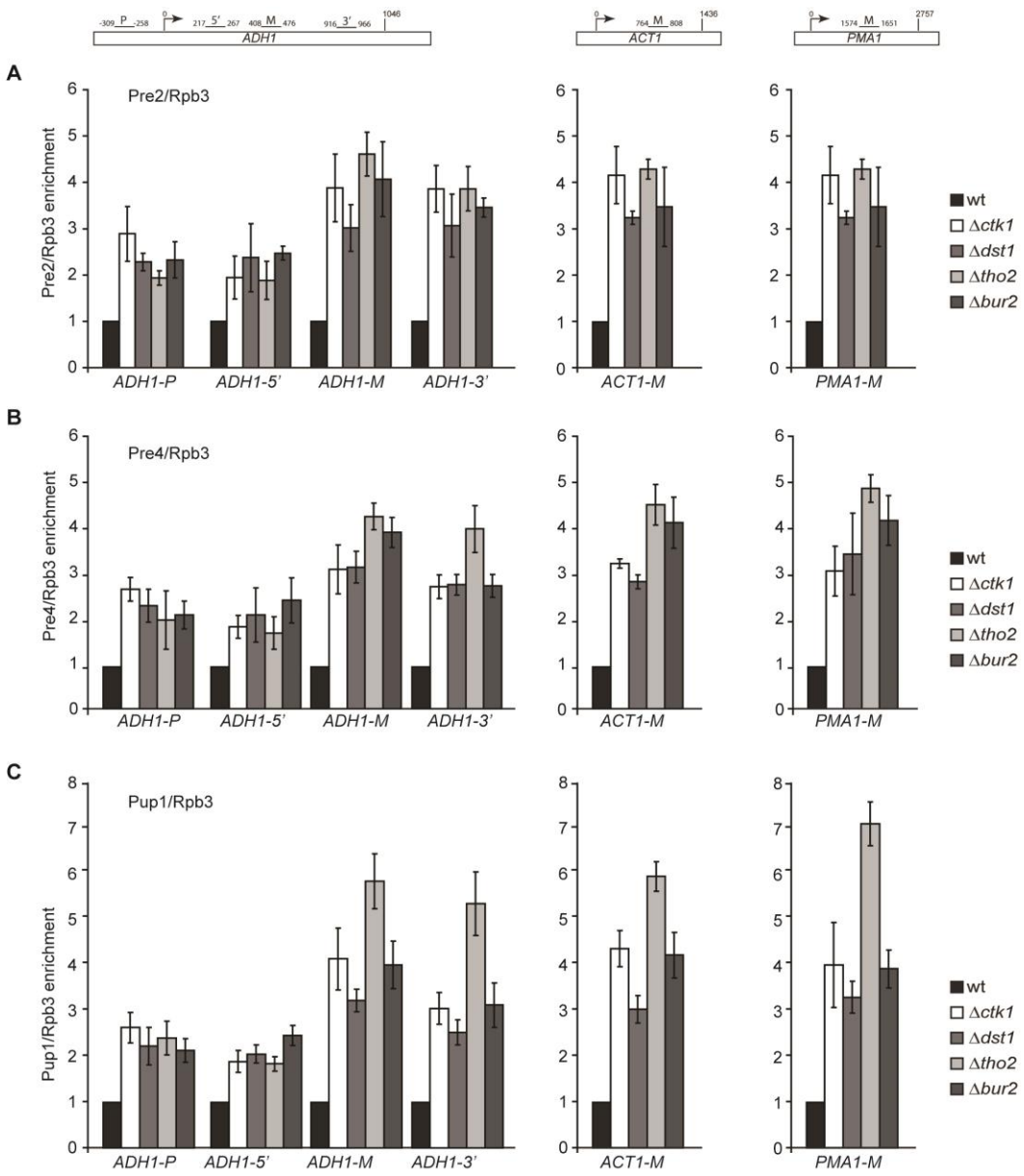

Figure S4 The proteasome is recruited to actively transcribed genes. ChIP experiments were performed with *PRE2*-, *PRE4*-, and *PUP1-TAP* strains and recruitment to *ADH1*, *ACT1*, and *PMA1* was quantified by RealTime-PCR with the indicated primers (upper panels) and calculated relative to the recruitment of Rpb3. Columns and error bars represent the mean ± standard deviation from 3 independent experiments (bottom panels).

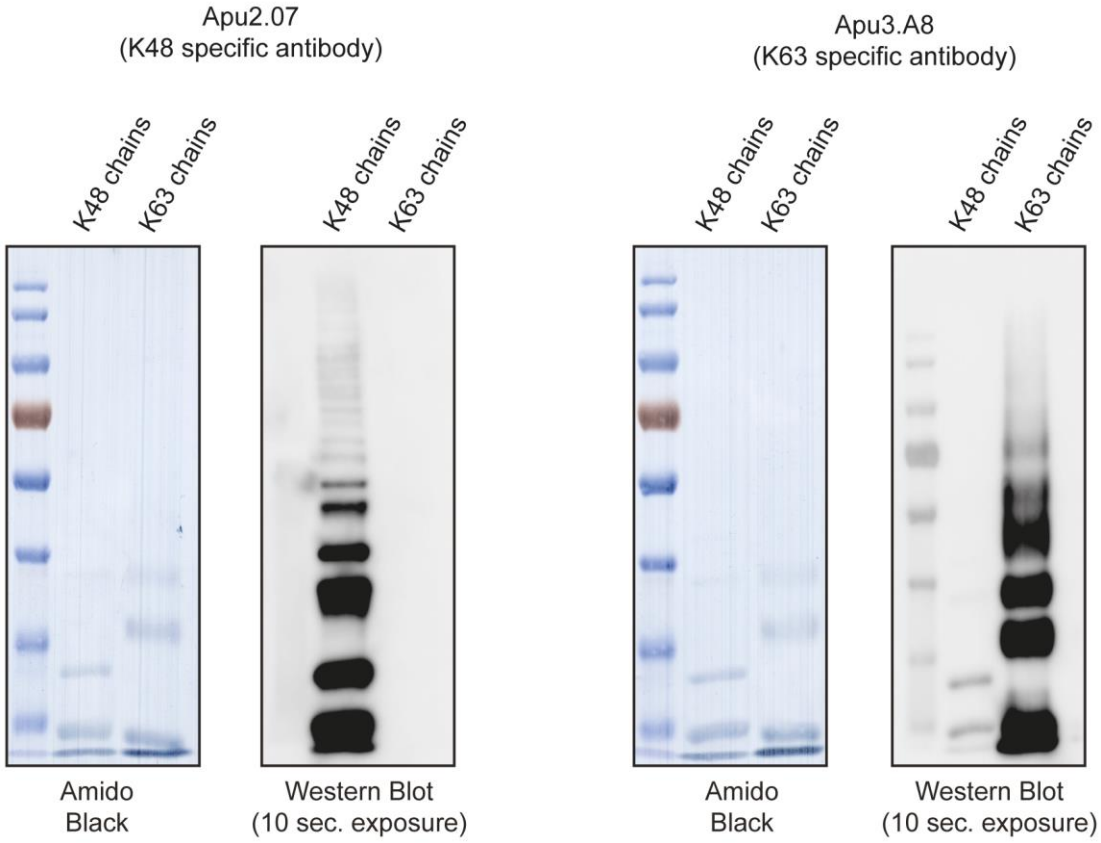

Figure S5 Specificity and affinity of the linkage-specific antibodies Apu2.07 (K48) and Apu3.A8 (K63). 5 µg of K48- or K63-chains (Sigma) were loaded, blotted and detected with the respective antibody. Each antibody is specific for the K48- or K63-linked chain, respectively, and with approximately equal affinity.

Table S1 Yeast strains

Name	Nr.	Back-ground	Genotype	Reference
W303	YKS8		*ura3-1, trp1-1, his3-11,15, leu2-3,112, ade2-1, can1-100, GAL+*	
CTK1 shuffle	YKS814	W303	*ctk1::HIS3* , pRS316-*CTK1*	(2)
DST1 shuffle	YKS1027	W303	*dst1::HIS3*, pRS316-*DST1*	This study
THO2 shuffle	YKS1108	W303	*tho2::kanMX6*, pRS316-*THO2*	This study
BUR2 shuffle	YKS1101	W303	*bur2::kanMX6*, pRS316-*BUR2*	This study
BY4741	YKS1470		*his3Δ, leu2Δ, lys2Δ, met15Δ, trp1Δ::hisG, URA::CMV-tTA*	(3)
rpb1-N488D	YKS1614	BY4741	*rpb1-N488D*	(3)
rpb1-E1103G	YKS1615	BY4741	*rpb1-E1103G*	(3)
RPB3-TAP	YKS1138	W303	*RPB3*-TAP*::TRP1*	This study
RPB3-TAP *CTK1* shuffle	YKS981	W303	*CTK1::HIS3, RPB3*-TAP*::TRP1*, pRS316-*CTK1*	This study
RPB3-TAP *DST1* shuffle	YKS1030	W303	*dst1::HIS3*, *RPB3*-TAP*::TRP1*, pRS316-*DST1*	This study
RPB3-TAP *THO2* shuffle	YKS1109	W303	*tho2::kanMX6, RPB3*-TAP*::TRP1*, pRS316-*THO2*	This study
RPB3-TAP *BUR2* shuffle	YKS1103	W303	*bur2::kanMX6, RPB3*-TAP*::TRP1*, pRS316-*BUR2*	This study
RPB3-TAP	YKS1613	BY4741	*RPB3*-TAP*::TRP1*	This study
RPB3-TAP *rpb1-N488D*	YKS1614	BY4741	*rpb1-N488D*, *RPB3*-TAP*::TRP1*	This study
RPB3-TAP *rpb1-E1103G*	YKS1615	BY4741	*rpb1-E1103G*, *RPB3*-TAP*::TRP1*	This study
PRE1-TAP *CTK1* shuffle	YKS982	W303	*ctk1::HIS3*, *PRE1*-TAP*::TRP1*, pRS316-*CTK1*	This study
PRE1-TAP *DST1* shuffle	YKS1033	W303	*dst1::HIS3*, *PRE1*-TAP*::TRP1*, pRS316-*DST1*	This study
PRE1-TAP *THO2* shuffle	YKS1110	W303	*tho2::kanMX6, PRE1*-TAP*::TRP1*, pRS316-*THO2*	This study
PRE1-TAP *BUR2* shuffle	YKS1104	W303	*bur2::kanMX6, PRE1*-TAP*::TRP1*, pRS316-*BUR2*	This study
RPB3-TAP *Δubc4*	YKS1649	W303	*ubc4::HIS3*, *RPB3*-TAP*::TRP1*	This study
RPB3-TAP *Δubc4 DST1* shuffle	YKS1650	W303	*ubc4::HIS3*, *dst1::HIS3, RPB3*-TAP*::TRP1*, pRS316-*DST1*	This study
RPB3-TAP *Δubc5*	YKS1652	W303	*ubc5::HIS3*, *RPB3*-TAP*::TRP1*	This study
RPB3-TAP *Δubc5 DST1* shuffle	YKS1653	W303	*ubc5::HIS3*, *dst1::HIS3, RPB3*-TAP*::TRP1*, pRS316-*DST1*	This study
RPB3-TAP *RSP5* shuffle	YKS1654	W303	*rsp5::HIS3*, *RPB3*-TAP*::TRP1*	This study
RPB3-TAP *Δdst1 RSP5* shuffle	YKS1655	W303	*rsp5::HIS3*, *dst1::HIS3, RPB3*-TAP*::TRP1*, pRS316-*DST1*	This study
RPB3-TAP *Δdef1*	YKS1656	W303	*def1::kanMX6*, *RPB3*-TAP*::TRP1*	This study
RPB3-TAP *Δdef1 DST1* shuffle	YKS1657	W303	*def1::kanMX6*, *dst1::HIS3, RPB3*-TAP*::TRP1*, pRS316-*DST1*	This study
RPB3-TAP Δrad26	YKS1840	W303	*rad26::kanMX6, RPB3-TAP::TRP1*	This study
RPB3-TAP Δrad26 DST1 shuffle	YKS1841	W303	*rad26::kanMX6, dst1::HIS3, RPB3-TAP::TRP1*, pRS316-*DST1*	This study
RPB3-TAP *Δubp3*	YKS1665	W303	*ubp3::HIS3*, *RPB3*-TAP*::TRP1*	This study
RPB3-TAP *Δubp3 DST1* shuffle	YKS1666	W303	*ubp3::HIS3*, *dst1::HIS3, RPB3*-TAP*::TRP1*, pRS316-*DST1*	This study
RPB3-TAP *Δubp2*	YKS1746	W303	*upb2::kanMX6, RPB3*-TAP*::TRP1*	This study
RPB3-TAP *Δubp2 DST1* shuffle	YKS1751	W303	*ubp2::kanMX6, dst1::HIS3, RPB3*-TAP*::TRP1*, pRS316-*DST1*	This study
RPB3-TAP *Δubp6*	YKS1696	W303	*ubp6::kanMX6*, *RPB3*-TAP*::TRP1*	This study
RPB3-TAP *Δubp6 DST1* shuffle	YKS1714	W303	*ubp6::kanMX6*, *dst1::HIS3, RPB3*-TAP*::TRP1*, pRS316-*DST1*	This study
RPB3-TAP *Δelc1*	YKS1745	W303	*elc1::kanMX6*, *RPB3*-TAP*::TRP1*	This study
RPB3-TAP *Δelc1 DST1* shuffle	YKS1747	W303	*elc1::kanMX6*, *dst1::HIS3, RPB3*-TAP*::TRP1*, pRS316-*DST1*	This study
GAL1::*CTK1*-TAP	YKS552	RS453	*HIS3::PGAL1::CTK1*-TAP*::URA3*	(2)

GAL1::*THO2*-TAP	YKS1617	RS453	*HIS3::PGAL1::THO2*-TAP*::TRP1*	This study
GAL1::*BUR2*-TAP	YKS1618	RS453	*HIS3::PGAL1::BUR2*-TAP*::TRP1*	This study
PRE2-TAP *CTK1* shuffle	YKS1042	W303	*ctk1::HIS3, PRE2*-TAP*::TRP1*, pRS316-*CTK1*	This study
PRE4-TAP *CTK1* shuffle	YKS983	W303	*ctk1::HIS3, PRE4*-TAP*::TRP1*, pRS316-*CTK1*	This study
PUP1-TAP *CTK1* shuffle	YKS1041	W303	*ctk1::HIS3, PUP1*-TAP*::TRP1*, pRS316-*CTK1*	This study
PRE2-TAP *DST1* shuffle	YKS1034	W303	*dst1::HIS3, PRE2*-TAP*::TRP1*, pRS316-*DST1*	This study
PRE4-TAP *DST1* shuffle	YKS1035	W303	*dst1::HIS3, PRE4*-TAP*::TRP1*, pRS316-*DST1*	This study
PUP1-TAP *DST1* shuffle	YKS1031	W303	*dst1::HIS3, PUP1*-TAP*::TRP1*, pRS316-*DST1*	This study
PRE2-TAP *THO2* shuffle	YKS1111	W303	*THO2::kanMX6, PRE2*-TAP*::TRP1*, pRS316-*THO2*	This study
PRE4-TAP *THO2* shuffle	YKS1112	W303	*THO2::kanMX6, PRE4*-TAP*::TRP1*, pRS316-*THO2*	This study
PUP1-TAP *THO2* shuffle	YKS1113	W303	*THO2::kanMX6, PUP1*-TAP*::TRP1*, pRS316-*THO2*	This study
PRE2-TAP *BUR2* shuffle	YKS1105	W303	*BUR2::kanMX6, PRE2*-TAP*::TRP1*, pRS316-*BUR2*	This study
PRE4-TAP *BUR2* shuffle	YKS1106	W303	*BUR2::kanMX6, PRE4*-TAP*::TRP1*, pRS316-*BUR2*	This study
PUP1-TAP *BUR2* shuffle	YKS1107	W303	*BUR2::kanMX6, PUP1*-TAP*::TRP1*, pRS316-*BUR2*	This study

Table S2 Plasmids

Name	*Plasmid Nr.*	*Reference*
pRS314	pKS6	(4)
pRS315	pKS7	(4)
pRS316	pKS8	(4)
pRS315-*CTK1*	pKS391	(5)
pRS316-*CTK1*	pKS491	(2)
pRS315-*DST1*	pKS621	This study
pRS316-*DST1*	pKS622	This study
pRS315-*THO2*	pKS181	A. Aguilera
pRS316-*THO2*	pKS520	This study
pRS315-*BUR2*	pKS395	This study
pRS316-*BUR2*	pKS431	This study
pRS316-*UBC4*	pKS1311	This study
pRS316-*UBC5*	pKS1314	This study
pRS316-*DEF1*	pKS1305	This study
pRS315-*RSP5*	pKS1316	This study
pRS316-*RSP5*	pKS1317	This study
pHJ80	pKS1569	(6)
pHJ81	pKS1570	(6)
pBSIIKS+-5'-*tho2-kanMX6*-3'	pKS1352	This study
pBSIIKS+-5'-*bur2-kanMX4*-3'	pKS 348	This study
pBSIIKS+-5'-*ubc4-HIS3*-3'	pKS1324	This study
pBSIIKS+-5'-*ubc5-HIS3*-3'	pKS1326	This study
pBSIIKS+-5'-*def1-kanMX4*-3'	pKS1302	This study
pBSIIKS+-5'-*rad26-kanMX4*-3'	pKS1569	This study
pBSIIKS+-5'-*rsp5-HIS3*-3'	pKS1328	This study
pBSIIKS+-5'-*elc1-kanMX6*-3'	pKS1552	This study
pBSIIKS+-5'-*ubp2-kanMX6*-3'	pKS1554	This study
pBSIIKS+-5'-*ubp3-kanMX6*-3'	pKS1376	This study
pBSIIKS+-5'-*ubp6-kanMX6*-3'	pKS1559	This study

Table S3 p-values

			p-value
Figure 1A			
wt	to	*Δctk1*	< 0.0001
wt	to	*Δdst1*	0.0011
wt	to	*Δtho2*	0.0067
wt	to	*Δbur2*	< 0.0001
wt + solvent	to	wt + 6AU	0.0006
Figure 2A			
wt	to	*Δctk1*	< 0.0001
wt	to	*Δdst1*	< 0.0001
wt	to	*Δtho2*	< 0.0001
wt	to	*Δbur2*	0.0028
Figure 3A			
wt	to	mock	0.0003
wt	to	*Δctk1*	0.0047
wt	to	*Δdst1*	0.0162
wt	to	*Δtho2*	0.0023
wt	to	*Δbur2*	0.0026
Figure 3D			
ADH1 P			
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δctk1*	0.0023
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δdst1*	0.0356
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δtho2*	< 0.0001
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δbur2*	0.0007
ADH1 5’			
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δctk1*	0.0689
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δdst1*	0.0463
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δtho2*	0.0005
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δbur2*	< 0.0001
ADH1 M			
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δctk1*	0.0004
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δdst1*	0.0063
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δtho2*	0.0020
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δbur2*	0.0005
ADH1 3’			
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δctk1*	0.0016
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δdst1*	0.0020
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δtho2*	0.0007
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δbur2*	0.0009
ACT1 M			
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δctk1*	0.0037
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δdst1*	0.0021
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δtho2*	0.0111
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δbur2*	0.0027
PMA1 M			
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δctk1*	0.0001
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δdst1*	0.0011
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δtho2*	0.0030
Pre1 vs Rpb3 wt	to	Pre1 vs Rpb3 *Δbur2*	< 0.0001

Figure 3G			
Pre1/Rpb3			
ADH1-P solvent	to	6-AU	0.1128
ADH1-5' solvent	to	6-AU	0.0537
ADH1-M solvent	to	6-AU	0.0005
ADH1-3' solvent	to	6-AU	0.0370
Figure 4A			
wt	to	*Δdst1*	0.0092
wt	to	*Δdef1*	< 0.0001
wt	to	*Δdst1 Δdef1*	0.9041
Δdst1	to	*Δdst1 Δdef1*	0.0002
Figure 4B			
wt	to	*Δdst1*	< 0.0001
wt	to	*Δubc4*	0.0729
wt	to	*Δubc5*	0.2298
wt	to	*Δdst1 Δubc4*	0.0030
wt	to	*Δdst1 Δubc5*	0.0064
Δdst1	to	*Δdst1 Δubc4*	0.0027
Δdst1	to	*Δdst1 Δubc5*	0.0017
Figure 4C			
wt	to	*Δdst1*	0.0023
wt	to	*Δrsp5*	0.1668
wt	to	*Δdst1 Δrsp5*	0.0106
Δdst1	to	*Δdst1 Δrsp5*	0.0030
Figure 4D			
wt - UV	to	wt + UV	0.0009
wt - UV	to	*Δdst1* - UV	0.0064
wt - UV	to	*Δdst1* + UV	0.0327
wt - UV	to	*Δelc1* - UV	0.9033
wt - UV	to	*Δelc1* + UV	0.2059
wt - UV	to	*Δelc1 Δdst1* - UV	0.0373
wt - UV	to	*Δelc1 Δdst1* + UV	0.6204
Δdst1 - UV	to	*Δdst1* + UV	0.2207
Δdst1 - UV	to	*Δelc1 Δdst1* - UV	0.5487
Δdst1 + UV	to	*Δelc1 Δdst1* + UV	0.1216
Δelc1 - UV	to	*Δelc1* + UV	0.6594
Δelc1 Δdst1 - UV	to	*Δelc1 Δdst1* + UV	0.5461
wt - UV	to	wt + UV	0.0009
wt - UV	to	*Δdst1* - UV	0.0064
wt - UV	to	*Δdst1* + UV	0.0327
wt - UV	to	*Δelc1* - UV	0.9033
Figure 4E			
wt	to	*Δdst1*	< 0.0001
wt	to	*Δcul3*	< 0.0001
wt	to	*Δdst1 Δcul3*	0.0026
Δdst1	to	*Δdst1 Δcul3*	0.0041
Figure 5			
K63-linked chains			
wt	to	*Δctk1*	0.0002
wt	to	*Δdst1*	0.0502
wt	to	*Δtho2*	0.0076
wt	to	*Δbur2*	0.0231
K48-linked chains			
wt	to	*Δctk1*	0.0011

wt	to	*Δdst1*	0.9443
wt	to	*Δtho2*	0.0445
wt	to	*Δbur2*	0.0238
Figure 6A			
wt	to	*Δdst1*	< 0.0001
wt	to	*Δubp6*	0.0008
wt	to	*Δdst1 Δubp6*	< 0.0001
wt	to	*Δubp3*	0.0028
wt	to	*Δdst1 Δubp3*	0.0593
wt	to	*Δubp2*	0.1105
wt	to	*Δdst1 Δubp2*	0.0024
Δdst1	to	*Δdst1 Δubp6*	< 0.0001
Δdst1	to	*Δdst1 Δubp3*	0.0010
Δdst1	to	*Δdst1 Δubp2*	0.0032
Figure 6B			
wt	to	*Δdst1*	< 0.0001
wt	to	*Δubp6*	0.0740
wt	to	*ubp6-C118A*	0.6516
wt	to	*Δdst1 Δubp6*	< 0.0001
wt	to	*Δdst1 ubp6-C118A*	< 0.0001
Δdst1	to	*Δdst1 Δubp6*	< 0.0001
Δdst1	to	*Δdst1 ubp6-C118A*	0.0022

SUPPLEMENTARY REFERENCES

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