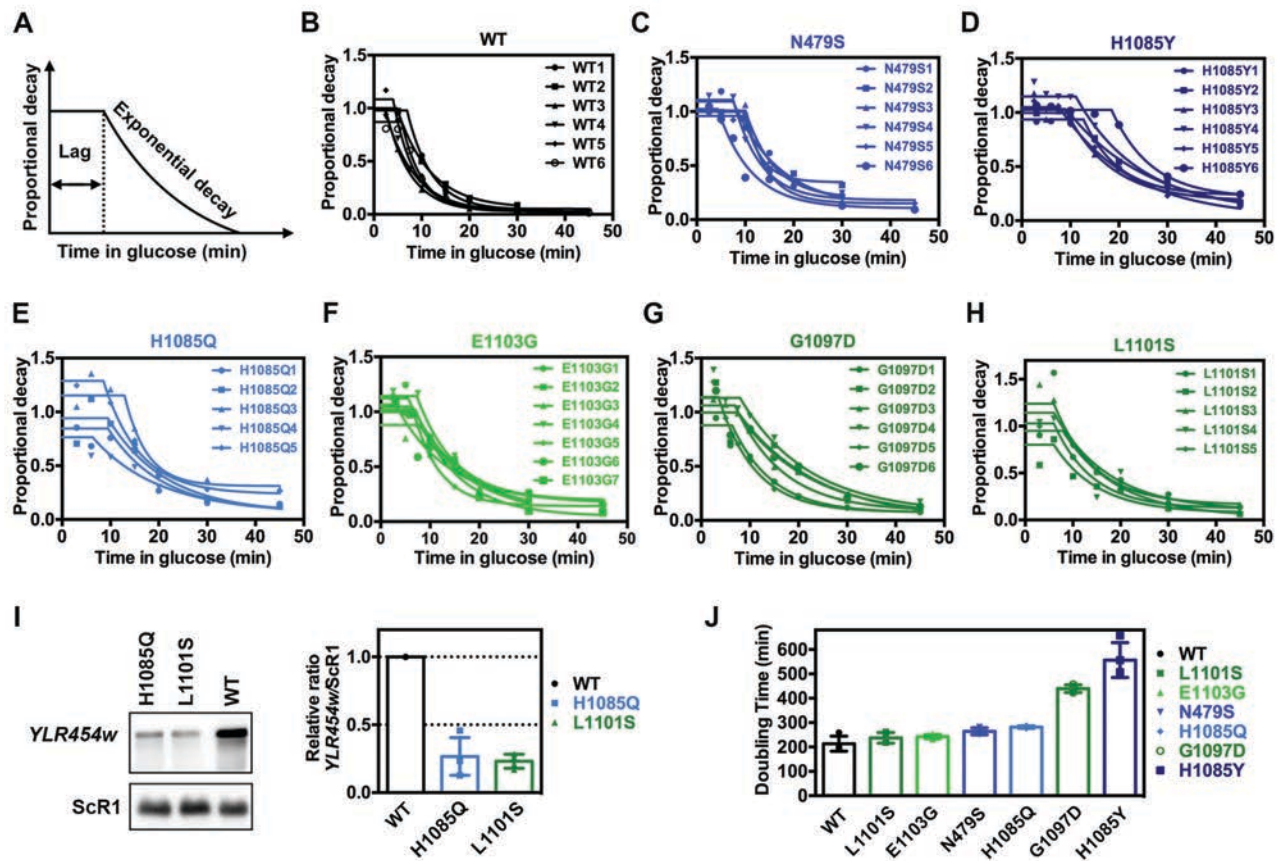
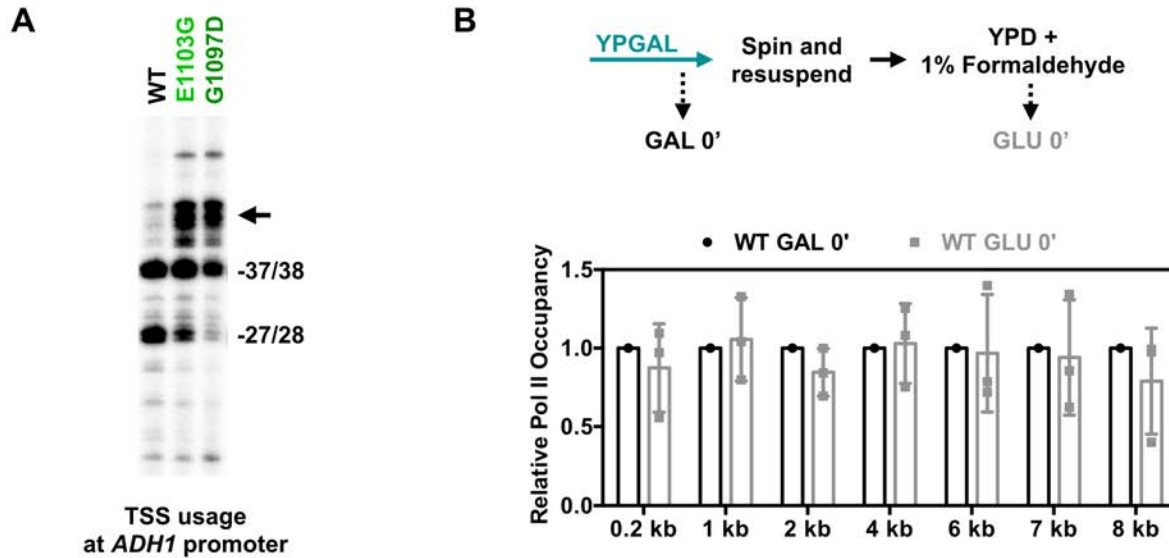


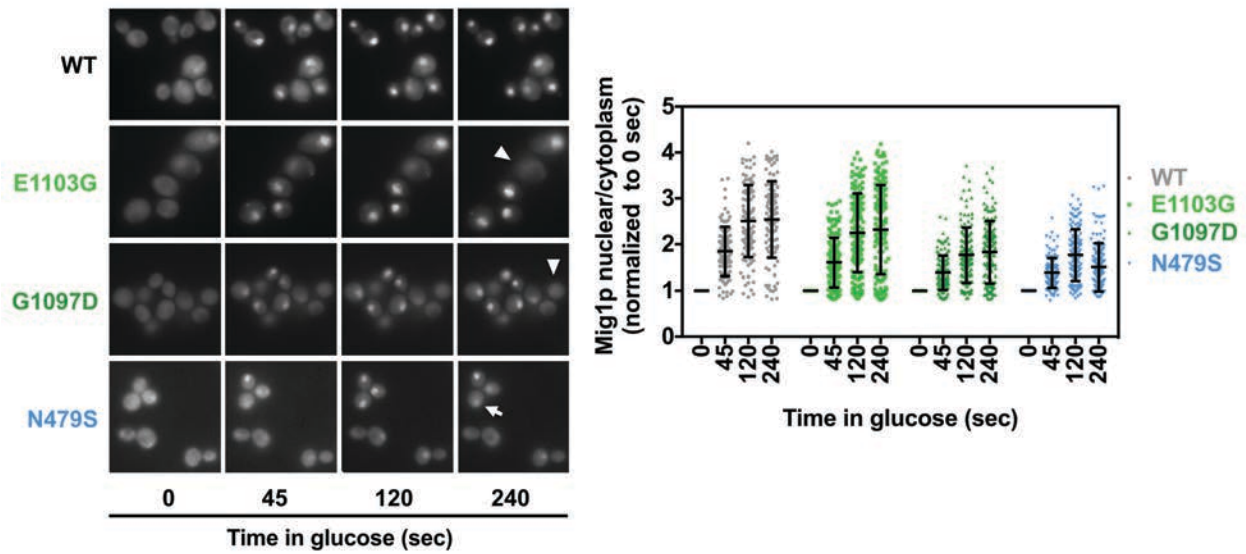
**Supplementary Figure 1. Modulation of reporter gene expression defect in pre-mRNA processing factor mutants.** (A) *GAL1p::YLR454w* reporter expression level in Pol II catalytic mutants in WT, *xrn1Δ*, or *rrp6Δ* backgrounds determined by Northern blotting. WT Pol II sample (*XRN1/RP6*) was run in parallel with *xrn1Δ* or *rrp6Δ* mutant samples on each blot for normalization purposes (**Figure 2A**) (B) *GAL1p::YLR454w* reporter expression level in Pol II catalytic mutants in WT and in *rat1-1* background at permissive (27°C) or restrictive (37°C) temperatures. Overnight grown cells were inoculated in fresh YPGal media to amplify to mid-log growth at 27°C, then shifted to 37°C to inactivate Rat1p. RNAs were isolated from half of each culture prior to temperature shift (27°C samples). Remaining cultures were washed and resuspended in pre-warmed YPGal media to grow for another 2 hrs at 37°C prior to RNA isolation. Relative *GAL1p::YLR454w* expression levels were normalized to WT (*RAT1*) 27°C value. Data shown are average of three biological repeats with error bars representing the standard deviation (SD) of the mean. Note: values were normalized to WT (*RAT1*) at 27°C, and are presented on logarithmic scale. (C) 10-fold serial dilutions of saturated cultures of Pol II catalytic mutants alone or in combination with *xrn1Δ/rat1-1* mutant were plated for growth at 27°C or 37°C on different media as indicated. Blue and green bars indicate LOF and GOF mutants, respectively.



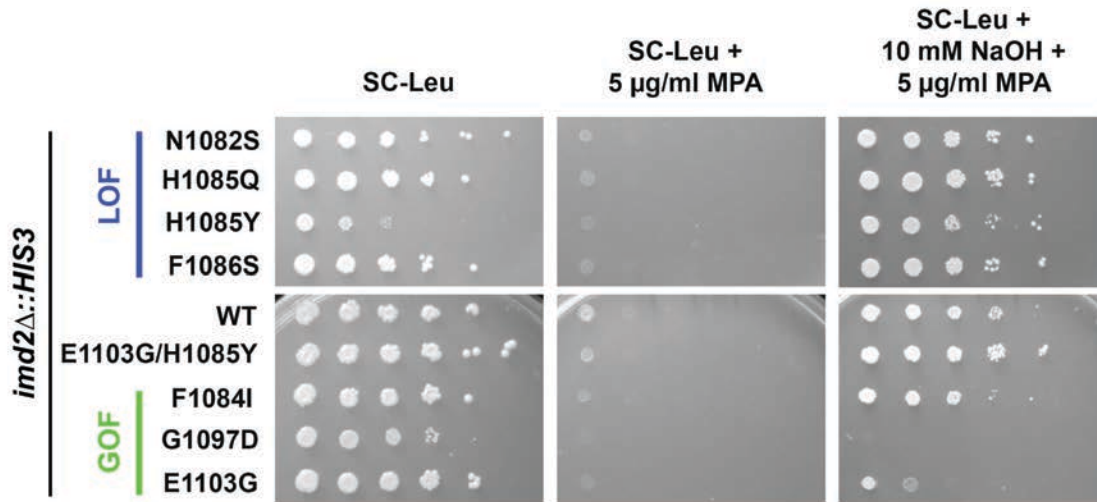
**Supplementary Figure 2. Pol II catalytic mutants alter *GAL1p::YLR454w* mRNA decay and growth in YPGal media** (A) Schematic of the mRNA decay curve fitting showing 'lag' and 'exponential decay' periods after transcriptional shut-off. (B-H) Individual decay curve for *GAL1p::YLR454w* decay in Pol II catalytic mutants plotted using non-linear regression using GraphPad prism. (I) *GAL1p::YLR454w* expression level in H1085Q and L1101S determined by Northern blotting. Values normalized to WT *GAL1p::YLR454w* expression level. Data shown are average of three biological repeats with error bars representing the standard deviation (SD) of the mean. (J) Doubling time of Pol II mutants in YPGal media determined using Tecan plate reader. Error bars represent average  $\pm$  SD of four replicate cultures.



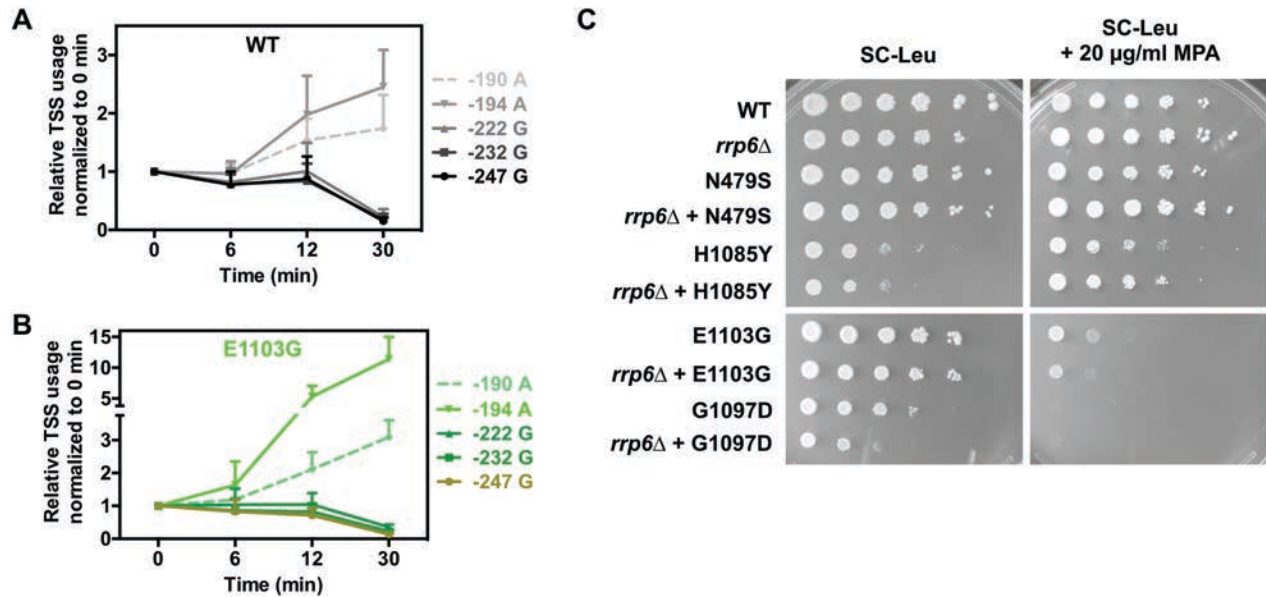
**Supplementary Figure 3. Validation of GOF mutant phenotypes and formaldehyde crosslinking kinetics.** (A) Primer extension analysis of start site usage at *ADH1* in WT and GOF catalytic mutants shows expected upstream shift (arrow) for GOF mutants. (B) Evaluating formaldehyde cross-linking efficiency during shut-off of *GAL1p::YLR454w* transcription by addition of 4% glucose. WT culture was grown to mid-log phase at 30°C and half of the culture was isolated and fixed with 1% formaldehyde as time 0 in YPGal (*GAL 0'*). Then, the rest half was quickly centrifuged and resuspended in YPD (4% dextrose) containing 1% formaldehyde to obtain time 0 in YPD + formaldehyde (*Glu 0'*). ChIP was performed to determine Pol II occupancy over *GAL1p::YLR454w* (see Fig. 5A) in *GAL 0'* versus *Glu 0'*. This comparative analysis allows us to determine if there is any apparent repression during cross-linking in the presence of glucose to inhibit *GAL1p::YLR454w* transcription. Bar graphs show average of three biological repeats +/- SD.



**Supplementary Figure 4. Impaired Mig1p nuclear import in G1097D.** Representative images of nuclear localization of Mig1p-GFP in WT and catalytic mutants upon glucose (4% final) addition (left) and average Mig1p nuclear localization +/- SD (right). Data from multiple cells (WT, n=104 cells from 6 individual experiments; E1103G, n= 147 cells from 8 individual experiments; G1097D, n= 152 from 5 individual experiments; N479S, n=129 from 9 individual experiments) used to make the histogram in **Figure 7**. Arrowhead showing example of non-responding cell that does not show Mig1p-GFP foci accumulation upon glucose addition and arrow showing cell that decays Mig1p-GFP signal over time.



**Supplementary Figure 5. Addition of NaOH in the media abrogates MPA effects.** 10-fold serial dilutions of saturated cultures of Pol II catalytic mutants were plated for growth at 30°C on synthetic media containing NaOH. All strains contain endogenous *IMD2* deletion (*imd2Δ::HIS3*), rendering them highly sensitive to MPA. Only G1097D (strongest GOF) shows sensitivity to MPA in presence of NaOH in the medium.



**Supplementary Figure 6. Stabilization of *IMD2* CUTs allows *IMD2* TSSs at intermediate downstream positions to be observed.** Kinetics of upstream 'G' start site loss and subsequently gain of presumptive novel 'A' sites upon MPA (20  $\mu$ g/ml) treatment in WT (A) and E1103G (B) cells containing *rrp6* $\Delta$ . Error bars represent average of three biological repeats  $\pm$  SD. Note difference in scale between (A) and (B). (C) Deletion of *RRP6* does not confer MPA resistance to MPA-sensitive mutants. 10-fold serial dilution of saturated cultures of Pol II mutants strains alone and in combination with *rrp6* $\Delta$  mutant plated on synthetic complete medium lacking leucine (SC-Leu) and SC-Leu medium containing 20  $\mu$ g/ml MPA (final) to determine MPA sensitivity.

**Supplementary Table1.** List of mutants with corresponding references that used glucose shut-off experiment to determine apparent in vivo elongation rate on *GAL1p::YLR454w* reporter.

| <b>Mutant/Condition tested</b>  | <b>Apparent In vivo elongation</b>  | <b>Reference</b> |
|---|---|------------------|
| <i>rpb2-10</i><br><i>hpr1, thp2, mft1, cdc73, rtf1, spt4, ctk1, ctk2</i><br>and <i>ppr2</i> . | <i>rpb2-10</i> apparent slower than WT<br>no detectable effect  | (1)              |
| <i>asf1</i>   | no detectable effect  | (2)              |
| <i>set2, pob3</i> and <i>set2/pob3</i>  | no detectable effect  | (3)              |
| <i>swi2</i>   | no detectable effect  | (4)              |
| <i>gcn5</i>   | <i>gcn5</i> apparent slower than WT   | (5)              |
| <i>esa1</i><br><i>gcn5</i><br><i>esa1/gcn5</i>  | <i>esa1</i> apparent slower than WT<br><i>gcn5</i> no detectable effect<br><i>esa1/gcn5</i> apparent slower than WT | (6)              |
| <i>chd1</i><br><i>spt5-242</i>  | <i>chd1</i> apparent slower than WT<br><i>spt5-242</i> apparent slower than WT                                      | (7)              |
| <i>rpb1</i> N488D   | <i>rpb1</i> N488D apparent slower than WT   | (8)              |
| <i>dhh1</i><br><i>ccr4</i><br><i>not4</i>   | <i>dhh1</i> apparent slower than WT<br><i>ccr4</i> apparent slower than WT<br><i>not4</i> apparent slower than WT   | (9)              |
| Temperature   | apparent elongation rate increases with temperature   | (10)             |
| <i>pdf1</i><br><i>dst1</i>  | <i>pdf1</i> apparent slower than WT<br><i>dst1</i> no detectable effect   | (11)             |
| <i>rpb1</i> E1103G  | <i>rpb1</i> E1103G apparent faster than WT  | (12)             |
| <i>rat1-1</i>   | <i>rat1-1</i> apparent faster than WT   | (13)             |
| <i>rpb2</i> K864G/K865G/ $\Delta$ 866-871   | no detectable effect  | (14)             |

**Supplementary Table 2.**

| <b>Experiment/Phenotype</b>   | <b>LOF/Slow mutant phenotype</b>  | <b>GOF/Fast mutant phenotype</b>   |
|---|---|--|
| Steady state Pol II occupancy (over <i>GAL1p::YLR454w</i> )   | Decreased overall Pol II occupancy  | Decreased overall Pol II occupancy   |
| Apparent Pol II processivity defect at the 3' end of <i>GAL1p::YLR454w</i>  | Apparent 3' end processivity defect in <i>rpb1</i> H1085Y   | Apparent 3' end processivity defect in <i>rpb1</i> G1097D  |
| Steady state Pol II occupancy in glucose vs galactose media   | Subtle increase in overall Pol II occupancy in galactose for <i>rpb1</i> H1085Y   | Decrease in overall Pol II occupancy in galactose for <i>rpb1</i> G1097D   |
| In vivo gene expression (Reporters- <i>GAL1p::YLR454w</i> , <i>TEF1p::YLR454w</i> , <i>TEF1</i> and <i>GAL1</i> ) | Gene expression decreases; most robust effect for <i>rpb1</i> H1085Y  | Gene expression decreases; most robust effect for <i>rpb1</i> G1097D   |
| <i>GAL1p</i> induction kinetics   | Induction is delayed  | Induction is delayed   |
| Genetic interaction with pre-mRNA processing factors  | Suppression of Ts- phenotype of <i>rat1-1</i> and <i>xrn1Δ/rat1-1</i> mutants   | Synthetic sick interactions with <i>xrn1Δ</i> and <i>rat1-1</i>  |
| mRNA decay rate of <i>GAL1p::YLR454w</i>  | Decay rate decreases  | Decay rate decreases   |
| In vivo elongation rate over <i>GAL1p::YLR454w</i> template   | Apparent elongation rate slower than WT on <i>GAL1p::YLR454w</i> (with caveats noted in main text)  | Apparent elongation rate slower than WT on <i>GAL1p::YLR454w</i> (with caveats noted in main text, especially for G1097D)                      |
| snR33 termination window  | Shorter termination window than WT  | Longer termination window than WT  |
| Ability of GTP-sensing in absence of endogenous <i>IMD2</i>   | Retain ability to sense GTP; MPA sensitivity is suppressed by addition of guanine   | Retain ability to sense GTP; MPA sensitivity poorly suppressed by addition of guanine  |
| Response to GTP depletion in absence of endogenous <i>IMD2</i>  | Constitutively use downstream functional 'A' start site at <i>IMD2</i> promoter; sense GTP depletion and can further shift TSS downstream to functional 'A' site upon MPA treatment | Sense GTP depletion but cannot shift TSS downstream to functional 'A' site; instead use novel TSSs that produce non-functional <i>IMD2</i> CUT |



**Supplementary Table 3.** List of yeast strains used in this study.

| Yeast strain | Relevant mutation  | Genotype   | Reference  | Relevant Figure |
|--------------|--------------------|--|------------|-----------------|
| CKY1124      | <i>RPB1</i> WT     | <i>MAT<math>\alpha</math> lys2-128<math>\delta</math> ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>0 or <math>\Delta</math>1 trp1<math>\Delta</math>63 met15<math>\Delta</math>0 rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w [pRS315 <i>RPB1</i> CEN LEU2]</i>        | This study | Figure 1-5      |
| CKY1110      | <i>rpb1</i> N479S  | <i>MAT<math>\alpha</math> lys2-128<math>\delta</math> ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>0 or <math>\Delta</math>1 trp1<math>\Delta</math>63 met15<math>\Delta</math>0 rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w [pRS315 <i>rpb1</i> N479S CEN LEU2]</i>  | This study | Figure 1-5      |
| CKY1111      | <i>rpb1</i> H1085Q | <i>MAT<math>\alpha</math> lys2-128<math>\delta</math> ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>0 or <math>\Delta</math>1 trp1<math>\Delta</math>63 met15<math>\Delta</math>0 rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w [pRS315 <i>rpb1</i> H1085Q CEN LEU2]</i> | This study | Figure 3, and 4 |
| CKY1112      | <i>rpb1</i> E1103G | <i>MAT<math>\alpha</math> lys2-128<math>\delta</math> ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>0 or <math>\Delta</math>1 trp1<math>\Delta</math>63 met15<math>\Delta</math>0 rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w [pRS315 <i>rpb1</i> E1103G CEN LEU2]</i> | This study | Figure 1-5      |
| CKY1113      | <i>rpb1</i> M1079R | <i>MAT<math>\alpha</math> lys2-128<math>\delta</math> ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>0 or <math>\Delta</math>1 trp1<math>\Delta</math>63 met15<math>\Delta</math>0 rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w [pRS315 <i>rpb1</i> M1079R CEN LEU2]</i> | This study | Figure 3        |
| CKY1114      | <i>rpb1</i> G1097D | <i>MAT<math>\alpha</math> lys2-128<math>\delta</math> ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>0 or <math>\Delta</math>1 trp1<math>\Delta</math>63 met15<math>\Delta</math>0 rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w [pRS315 <i>rpb1</i> G1097D CEN LEU2]</i> | This study | Figure 1-5      |
| CKY1116      | <i>rpb1</i> H1085Y | <i>MAT<math>\alpha</math> lys2-128<math>\delta</math> ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>0 or <math>\Delta</math>1 trp1<math>\Delta</math>63 met15<math>\Delta</math>0 rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w [pRS315 <i>rpb1</i> H1085Y CEN LEU2]</i> | This study | Figure 1-5      |
| CKY2064      | <i>rpb1</i> L1101S | <i>MAT<math>\alpha</math> lys2-128<math>\delta</math> ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>0 or <math>\Delta</math>1 trp1<math>\Delta</math>63 met15<math>\Delta</math>0 rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w [pRS315 <i>rpb1</i> L1101S CEN LEU2]</i> | This study | Figure 3, and 4 |
| CKY2065      | <i>rpb1</i> F1086S | <i>MAT<math>\alpha</math> lys2-128<math>\delta</math> ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>0 or <math>\Delta</math>1 trp1<math>\Delta</math>63 met15<math>\Delta</math>0 rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w [pRS315 <i>rpb1</i> F1086S CEN LEU2]</i> | This study | Figure 3        |
| CKY1132      | <i>RPB1</i> WT     | <i>MAT<math>\alpha</math> ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>1 or <math>\Delta</math>0 trp1<math>\Delta</math>63 met15<math>\Delta</math>0 lys2-128<math>\delta</math> rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::TEFp::YLR454w [pRS315 <i>RPB1</i> CEN LEU2]</i>         | This study | Figure 1 and 2  |

|         |                    |  |            |                    |
|---------|--------------------|--|------------|--------------------|
| CKY1133 | <i>rpb1</i> N479S  | <i>MATα ura3-52 his3Δ200 leu2Δ1 or Δ0 trp1Δ63 met15Δ0 lys2-128θ rpb1Δ::CLONATMX RPB3::3XFLAG::kanmX kanmx::TEFp::YLR454w [pRS315 rpb1 N479S CEN LEU2]</i>  | This study | Figure 2           |
| CKY1135 | <i>rpb1</i> H1085Y | <i>MATα ura3-52 his3Δ200 leu2Δ1 or Δ0 trp1Δ63 met15Δ0 lys2-128θ rpb1Δ::CLONATMX RPB3::3XFLAG::kanmX kanmx::TEFp::YLR454w [pRS315 rpb1 H1085Y CEN LEU2]</i> | This study | Figure 1 and 2     |
| CKY1136 | <i>rpb1</i> E1103G | <i>MATα ura3-52 his3Δ200 leu2Δ1 or Δ0 trp1Δ63 met15Δ0 lys2-128θ rpb1Δ::CLONATMX RPB3::3XFLAG::kanmX kanmx::TEFp::YLR454w [pRS315 rpb1 E1103G CEN LEU2]</i> | This study | Figure 2           |
| CKY1137 | <i>rpb1</i> G1097D | <i>MATα ura3-52 his3Δ200 leu2Δ1 or Δ0 trp1Δ63 met15Δ0 lys2-128θ rpb1Δ::CLONATMX RPB3::3XFLAG::kanmX kanmx::TEFp::YLR454w [pRS315 rpb1 G1097D CEN LEU2]</i> | This study | Figure 1 and 2     |
| CKY1492 | <i>RPB1</i> WT     | <i>MATα lys2-128θ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 rpb1Δ::CLONATMX kanmx::GAL1p::YLR454w rrp6Δ::kanmx [pRS315 RPB1 CEN LEU2]</i>                      | This study | Figure 3, 6 and 10 |
| CKY1493 | <i>rpb1</i> N479S  | <i>MATα lys2-128θ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 rpb1Δ::CLONATMX kanmx::GAL1p::YLR454w rrp6Δ::kanmx [pRS315 rpb1 N479S CEN LEU2]</i>                | This study | Figure 3 and 6     |
| CKY1494 | <i>rpb1</i> H1085Q | <i>MATα lys2-128θ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 rpb1Δ::CLONATMX kanmx::GAL1p::YLR454w rrp6Δ::kanmx [pRS315 rpb1 H1085Q CEN LEU2]</i>               | This study | Figure 3           |
| CKY1495 | <i>rpb1</i> F1086S | <i>MATα lys2-128θ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 rpb1Δ::CLONATMX kanmx::GAL1p::YLR454w rrp6Δ::kanmx [pRS315 rpb1 F1086S CEN LEU2]</i>               | This study | Figure 3           |
| CKY1496 | <i>rpb1</i> H1085Y | <i>MATα lys2-128θ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 rpb1Δ::CLONATMX kanmx::GAL1p::YLR454w rrp6Δ::kanmx [pRS315 rpb1 H1085Y CEN LEU2]</i>               | This study | Figure 3 and 6     |
| CKY1498 | <i>rpb1</i> M1079R | <i>MATα lys2-128θ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 rpb1Δ::CLONATMX kanmx::GAL1p::YLR454w rrp6Δ::kanmx [pRS315 rpb1 M1079R CEN LEU2]</i>               | This study | Figure 3           |
| CKY1499 | <i>rpb1</i> L1101S | <i>MATα lys2-128θ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 rpb1Δ::CLONATMX kanmx::GAL1p::YLR454w rrp6Δ::kanmx [pRS315 rpb1 L1101S CEN LEU2]</i>               | This study | Figure 3           |
| CKY1500 | <i>rpb1</i> E1103G | <i>MATα lys2-128θ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 rpb1Δ::CLONATMX kanmx::GAL1p::YLR454w rrp6Δ::kanmx [pRS315 rpb1 E1103G CEN LEU2]</i>               | This study | Figure 3, 6 and 10 |
| CKY1501 | <i>rpb1</i> G1097D | <i>MATα lys2-128θ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 rpb1Δ::CLONATMX</i>  | This study | Figure 3 and 6     |

|         |                    |   |            |          |
|---------|--------------------|---|------------|----------|
|         |                    | <i>kanmx::GAL1p::YLR454w rrp6Δ::kanmx [pRS315 rpb1 G1097D CEN LEU2]</i>   |            |          |
| CKY1503 | <i>RPB1 WT</i>     | <i>MATα lys2-128θ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 met15Δ0 rpb1Δ::CLONATMX kanmx::GAL1p::YLR454w rat1-1 xrn1Δ::kanmx [pRS315 RPB1 CEN LEU2]</i>        | This study | Figure 3 |
| CKY1504 | <i>rpb1 N479S</i>  | <i>MATα lys2-128θ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 met15Δ0 rpb1Δ::CLONATMX kanmx::GAL1p::YLR454w rat1-1 xrn1Δ::kanmx [pRS315 rpb1 N479S CEN LEU2]</i>  | This study | Figure 3 |
| CKY1505 | <i>rpb1 H1085Q</i> | <i>MATα lys2-128θ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 met15Δ0 rpb1Δ::CLONATMX kanmx::GAL1p::YLR454w rat1-1 xrn1Δ::kanmx [pRS315 rpb1 H1085Q CEN LEU2]</i> | This study | Figure 3 |
| CKY1506 | <i>rpb1 F1086S</i> | <i>MATα lys2-128θ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 met15Δ0 rpb1Δ::CLONATMX kanmx::GAL1p::YLR454w rat1-1 xrn1Δ::kanmx [pRS315 rpb1 N479S CEN LEU2]</i>  | This study | Figure 3 |
| CKY1507 | <i>rpb1 H1085Y</i> | <i>MATα lys2-128θ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 met15Δ0 rpb1Δ::CLONATMX kanmx::GAL1p::YLR454w rat1-1 xrn1Δ::kanmx [pRS315 rpb1 H1085Y CEN LEU2]</i> | This study | Figure 3 |
| CKY1509 | <i>rpb1 M1079R</i> | <i>MATα lys2-128θ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 met15Δ0 rpb1Δ::CLONATMX kanmx::GAL1p::YLR454w rat1-1 xrn1Δ::kanmx [pRS315 rpb1 M1079R CEN LEU2]</i> | This study | Figure 3 |
| CKY1510 | <i>rpb1 L1101S</i> | <i>MATα lys2-128θ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 met15Δ0 rpb1Δ::CLONATMX kanmx::GAL1p::YLR454w rat1-1 xrn1Δ::kanmx [pRS315 rpb1 L1101S CEN LEU2]</i> | This study | Figure 3 |
| CKY1511 | <i>rpb1 E1103G</i> | <i>MATα lys2-128θ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 met15Δ0 rpb1Δ::CLONATMX kanmx::GAL1p::YLR454w rat1-1 xrn1Δ::kanmx [pRS315 rpb1 E1103G CEN LEU2]</i> | This study | Figure 3 |
| CKY1512 | <i>rpb1 G1097D</i> | <i>MATα lys2-128θ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 met15Δ0 rpb1Δ::CLONATMX kanmx::GAL1p::YLR454w rat1-1 xrn1Δ::kanmx [pRS315 rpb1 G1097D CEN LEU2]</i> | This study | Figure 3 |
| CKY1519 | <i>RPB1 WT</i>     | <i>MATα lys2-128θ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 rpb1Δ::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w xrn1Δ::kanmx [pRS315 RPB1 CEN LEU2]</i>   | This study | Figure 3 |
| CKY1520 | <i>rpb1 N479S</i>  | <i>MATα lys2-128θ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 rpb1Δ::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w xrn1Δ::kanmx</i>                          | This study | Figure 3 |

|         |                    |  |            |          |
|---------|--------------------|--|------------|----------|
|         |                    | [pRS315 <i>rpb1</i> N479S CEN LEU2]  |            |          |
| CKY1521 | <i>rpb1</i> H1085Q | MATa <i>lys2-128<math>\theta</math> ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>0 or <math>\Delta</math>1 <i>trp1<math>\Delta</math>63 rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w xrn1<math>\Delta</math>::kanmx</i><br/>[pRS315 <i>rpb1</i> H1085Q CEN LEU2]</i> | This study | Figure 3 |
| CKY1522 | <i>rpb1</i> F1086S | MATa <i>lys2-128<math>\theta</math> ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>0 or <math>\Delta</math>1 <i>trp1<math>\Delta</math>63 rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w xrn1<math>\Delta</math>::kanmx</i><br/>[pRS315 <i>rpb1</i> F1086S CEN LEU2]</i> | This study | Figure 3 |
| CKY1523 | <i>rpb1</i> H1085Y | MATa <i>lys2-128<math>\theta</math> ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>0 or <math>\Delta</math>1 <i>trp1<math>\Delta</math>63 rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w xrn1<math>\Delta</math>::kanmx</i><br/>[pRS315 <i>rpb1</i> H1085Y CEN LEU2]</i> | This study | Figure 3 |
| CKY1525 | <i>rpb1</i> M1079R | MATa <i>lys2-128<math>\theta</math> ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>0 or <math>\Delta</math>1 <i>trp1<math>\Delta</math>63 rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w xrn1<math>\Delta</math>::kanmx</i><br/>[pRS315 <i>rpb1</i> M1079R CEN LEU2]</i> | This study | Figure 3 |
| CKY1526 | <i>rpb1</i> L1101S | MATa <i>lys2-128<math>\theta</math> ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>0 or <math>\Delta</math>1 <i>trp1<math>\Delta</math>63 rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w xrn1<math>\Delta</math>::kanmx</i><br/>[pRS315 <i>rpb1</i> L1101S CEN LEU2]</i> | This study | Figure 3 |
| CKY1527 | <i>rpb1</i> E1103G | MATa <i>lys2-128<math>\theta</math> ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>0 or <math>\Delta</math>1 <i>trp1<math>\Delta</math>63 rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w xrn1<math>\Delta</math>::kanmx</i><br/>[pRS315 <i>rpb1</i> E1103G CEN LEU2]</i> | This study | Figure 3 |
| CKY1528 | <i>rpb1</i> G1097D | MATa <i>lys2-128<math>\theta</math> ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>0 or <math>\Delta</math>1 <i>trp1<math>\Delta</math>63 rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w xrn1<math>\Delta</math>::kanmx</i><br/>[pRS315 <i>rpb1</i> G1097D CEN LEU2]</i> | This study | Figure 3 |
| CKY1533 | RPB1 WT            | MATa <i>ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>0 or <math>\Delta</math>1 <i>trp1<math>\Delta</math>63 rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w rat1-1</i> [pRS315 RPB1 CEN LEU2]</i>   | This study | Figure 3 |
| CKY1534 | <i>rpb1</i> N479S  | MATa <i>ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>0 or <math>\Delta</math>1 <i>trp1<math>\Delta</math>63 rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w rat1-1</i> [pRS315 <i>rpb1</i> N479S CEN LEU2]</i>  | This study | Figure 3 |
| CKY1535 | <i>rpb1</i> H1085Q | MATa <i>ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>0 or <math>\Delta</math>1 <i>trp1<math>\Delta</math>63 rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w rat1-1</i> [pRS315 <i>rpb1</i> H1085Q CEN LEU2]</i>   | This study | Figure 3 |
| CKY1536 | <i>rpb1</i> F1086S | MATa <i>ura3-52 his3<math>\Delta</math>200 leu2<math>\Delta</math>0 or <math>\Delta</math>1 <i>trp1<math>\Delta</math>63 rpb1<math>\Delta</math>::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w rat1-1</i> [pRS315</i>  | This study | Figure 3 |

|         |                    |   |            |          |
|---------|--------------------|---|------------|----------|
|         |                    | <i>rpb1 F1086S CEN LEU2</i>   |            |          |
| CKY1537 | <i>rpb1 H1085Y</i> | <i>MATa ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 rpb1Δ::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w rat1-1 [pRS315 rpb1 H1085Y CEN LEU2]</i>                            | This study | Figure 3 |
| CKY1539 | <i>rpb1 M1079R</i> | <i>MATa ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 rpb1Δ::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w rat1-1 [pRS315 rpb1 M1079R CEN LEU2]</i>                            | This study | Figure 3 |
| CKY1540 | <i>rpb1 L1101S</i> | <i>MATa ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 rpb1Δ::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w rat1-1 [pRS315 rpb1 L1101S CEN LEU2]</i>                            | This study | Figure 3 |
| CKY1541 | <i>rpb1 E1103G</i> | <i>MATa ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 rpb1Δ::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w rat1-1 [pRS315 rpb1 E1103G CEN LEU2]</i>                            | This study | Figure 3 |
| CKY1542 | <i>rpb1 G1097D</i> | <i>MATa ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 rpb1Δ::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w rat1-1 [pRS315 rpb1 G1097D CEN LEU2]</i>                            | This study | Figure 3 |
| CKY2066 | <i>RPB1 WT</i>     | <i>MATa lys2-128Δ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 met15Δ0 rpb1Δ::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w hph(hyg)::dxo1Δ [pRS315 RPB1 CEN LEU2]</i>        | This study | Figure 3 |
| CKY2067 | <i>rpb1 N479S</i>  | <i>MATa lys2-128Δ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 met15Δ0 rpb1Δ::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w hph(hyg)::dxo1Δ [pRS315 rpb1 N479S CEN LEU2]</i>  | This study | Figure 3 |
| CKY2068 | <i>rpb1 H1085Q</i> | <i>MATa lys2-128Δ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 met15Δ0 rpb1Δ::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w hph(hyg)::dxo1Δ [pRS315 rpb1 H1085Q CEN LEU2]</i> | This study | Figure 3 |
| CKY2069 | <i>rpb1 F1086S</i> | <i>MATa lys2-128Δ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 met15Δ0 rpb1Δ::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w hph(hyg)::dxo1Δ [pRS315 rpb1 F1086S CEN LEU2]</i> | This study | Figure 3 |
| CKY2071 | <i>rpb1 M1079R</i> | <i>MATa lys2-128Δ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 met15Δ0 rpb1Δ::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w hph(hyg)::dxo1Δ [pRS315 rpb1 M1079R CEN LEU2]</i> | This study | Figure 3 |
| CKY2072 | <i>rpb1 L1101S</i> | <i>MATa lys2-128Δ ura3-52 his3Δ200 leu2Δ0 or Δ1 trp1Δ63 met15Δ0 rpb1Δ::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w hph(hyg)::dxo1Δ</i>                               | This study | Figure 3 |

|         |                    |   |            |          |
|---------|--------------------|---|------------|----------|
|         |                    | [pRS315 <i>rpb1</i> L1101S CEN LEU2]  |            |          |
| CKY2073 | <i>rpb1</i> E1103G | MATa <i>lys2-128Δ ura3-52 his3Δ200 leu2Δ0</i> or $\Delta 1$ <i>trp1Δ63 met15Δ0 rpb1Δ::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w hph(hyg)::dxo1Δ</i> [pRS315 <i>rpb1</i> E1103G CEN LEU2] | This study | Figure 3 |
| CKY2074 | <i>rpb1</i> G1097D | MATa <i>lys2-128Δ ura3-52 his3Δ200 leu2Δ0</i> or $\Delta 1$ <i>trp1Δ63 met15Δ0 rpb1Δ::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w hph(hyg)::dxo1Δ</i> [pRS315 <i>rpb1</i> G1097D CEN LEU2] | This study | Figure 3 |
| CKY2075 | <i>rpb1</i> H1085Y | MATa <i>lys2-128Δ ura3-52 his3Δ200 leu2Δ0</i> or $\Delta 1$ <i>trp1Δ63 met15Δ0 rpb1Δ::CLONATMX RPB3::3XFLAG::kanmx kanmx::GAL1p::YLR454w hph(hyg)::dxo1Δ</i> [pRS315 <i>rpb1</i> H1085Y CEN LEU2] | This study | Figure 3 |
| CKY2076 | RPB1 WT            | MATa <i>ura3-52 his3Δ200 leu2Δ1</i> or $\Delta 0$ <i>trp1Δ63 met15Δ0 lys2-128Δ gal10Δ56 rpb1Δ::CLONATMX RPB3::TAP::KlacTRP1 rai1Δ::kanmx</i> [pRS315 RPB1 CEN LEU2]                               | This study | Figure 3 |
| CKY2077 | <i>rpb1</i> N479S  | MATa <i>ura3-52 his3Δ200 leu2Δ1</i> or $\Delta 0$ <i>trp1Δ63 met15Δ0 lys2-128Δ gal10Δ56 rpb1Δ::CLONATMX RPB3::TAP::KlacTRP1 rai1Δ::kanmx</i> [pRS315 <i>rpb1</i> N479S CEN LEU2]                  | This study | Figure 3 |
| CKY2078 | <i>rpb1</i> H1085Q | MATa <i>ura3-52 his3Δ200 leu2Δ1</i> or $\Delta 0$ <i>trp1Δ63 met15Δ0 lys2-128Δ gal10Δ56 rpb1Δ::CLONATMX RPB3::TAP::KlacTRP1 rai1Δ::kanmx</i> [pRS315 <i>rpb1</i> H1085Q CEN LEU2]                 | This study | Figure 3 |
| CKY2079 | <i>rpb1</i> F1086S | MATa <i>ura3-52 his3Δ200 leu2Δ1</i> or $\Delta 0$ <i>trp1Δ63 met15Δ0 lys2-128Δ gal10Δ56 rpb1Δ::CLONATMX RPB3::TAP::KlacTRP1 rai1Δ::kanmx</i> [pRS315 <i>rpb1</i> F1086S CEN LEU2]                 | This study | Figure 3 |
| CKY2081 | <i>rpb1</i> M1079R | MATa <i>ura3-52 his3Δ200 leu2Δ1</i> or $\Delta 0$ <i>trp1Δ63 met15Δ0 lys2-128Δ gal10Δ56 rpb1Δ::CLONATMX RPB3::TAP::KlacTRP1 rai1Δ::kanmx</i> [pRS315 <i>rpb1</i> M1079R CEN LEU2]                 | This study | Figure 3 |
| CKY2082 | <i>rpb1</i> L1101S | MATa <i>ura3-52 his3Δ200 leu2Δ1</i> or $\Delta 0$ <i>trp1Δ63 met15Δ0 lys2-128Δ gal10Δ56 rpb1Δ::CLONATMX RPB3::TAP::KlacTRP1 rai1Δ::kanmx</i> [pRS315 <i>rpb1</i> L1101S CEN LEU2]                 | This study | Figure 3 |
| CKY2083 | <i>rpb1</i> E1103G | MATa <i>ura3-52 his3Δ200 leu2Δ1</i> or $\Delta 0$ <i>trp1Δ63 met15Δ0 lys2-128Δ gal10Δ56 rpb1Δ::CLONATMX RPB3::TAP::KlacTRP1 rai1Δ::kanmx</i> [pRS315 <i>rpb1</i> E1103G CEN LEU2]                 | This study | Figure 3 |
| CKY2084 | <i>rpb1</i> G1097D | MATa <i>ura3-52 his3Δ200 leu2Δ1</i> or $\Delta 0$ <i>trp1Δ63 met15Δ0 lys2-128Δ gal10Δ56 rpb1Δ::CLONATMX RPB3::TAP::KlacTRP1 rai1Δ::kanmx</i> [pRS315 <i>rpb1</i> G1097D CEN LEU2]                 | This study | Figure 3 |

|            |                            |   |            |                 |
|------------|----------------------------|---|------------|-----------------|
| CKY2085    | <i>rpb1</i> H1085Y         | <i>MATa ura3-52 his3Δ200 leu2Δ1 or Δ0 trp1Δ63 met15Δ0 lys2-128Δ gal10Δ56 rpb1Δ::CLONATMX RPB3::TAP::KlacTRP1 rai1Δ::kanmx [pRS315 rpb1 H1085Y CEN LEU2]</i> | This study | Figure 3        |
| CKY283     | <i>RPB1</i> WT             | <i>MATa ura3-52 his3Δ200 leu2Δ1 or Δ0 trp1Δ63 met15Δ0 lys2-128Δ gal10Δ56 rpb1Δ::CLONATMX RPB3::TAP::KlacTRP1 [pRP112 RPB1 CEN URA3]</i>                     | (15)       | Figure 8        |
| CKY874     | <i>RPB1</i> WT             | <i>MATa leu2Δ0 or Δ1 ura3-52 his3Δ200 met15Δ0 trp1Δ63 lys2-128Δ RPB3::TAP::KlacTRP1 rpb1Δ::CLONAT imd2Δ::HIS3 [pRS315 RPB1 CEN LEU2]</i>                    | This study | Figure 8 and 10 |
| CKY876     | <i>rpb1</i> N1082S         | <i>MATa leu2Δ0 or Δ1 ura3-52 his3Δ200 met15Δ0 trp1Δ63 lys2-128Δ RPB3::TAP::KlacTRP1 rpb1Δ::CLONAT imd2Δ::HIS3 [pRS315 rpb1 N1082S CEN LEU2]</i>             | This study | Figure 8        |
| CKY877     | <i>rpb1</i> H1085Q         | <i>MATa leu2Δ0 or Δ1 ura3-52 his3Δ200 met15Δ0 trp1Δ63 lys2-128Δ RPB3::TAP::KlacTRP1 rpb1Δ::CLONAT imd2Δ::HIS3 [pRS315 rpb1 H1085Q CEN LEU2]</i>             | This study | Figure 8        |
| CKY878     | <i>rpb1</i> H1085Y         | <i>MATa leu2Δ0 or Δ1 ura3-52 his3Δ200 met15Δ0 trp1Δ63 lys2-128Δ RPB3::TAP::KlacTRP1 rpb1Δ::CLONAT imd2Δ::HIS3 [pRS315 rpb1 H1085Y CEN LEU2]</i>             | This study | Figure 8 and 10 |
| CKY879     | <i>rpb1</i> F1086S         | <i>MATa leu2Δ0 or Δ1 ura3-52 his3Δ200 met15Δ0 trp1Δ63 lys2-128Δ RPB3::TAP::KlacTRP1 rpb1Δ::CLONAT imd2Δ::HIS3 [pRS315 rpb1 F1086S CEN LEU2]</i>             | This study | Figure 8        |
| CKY882     | <i>rpb1</i> H1085Y /E1103G | <i>MATa leu2Δ0 or Δ1 ura3-52 his3Δ200 met15Δ0 trp1Δ63 lys2-128Δ RPB3::TAP::KlacTRP1 rpb1Δ::CLONAT imd2Δ::HIS3 [pRS315 rpb1 H1085Y/E1103G CEN LEU2]</i>      | This study | Figure 8        |
| CKY883     | <i>rpb1</i> F1084I         | <i>MATa leu2Δ0 or Δ1 ura3-52 his3Δ200 met15Δ0 trp1Δ63 lys2-128Δ RPB3::TAP::KlacTRP1 rpb1Δ::CLONAT imd2Δ::HIS3 [pRS315 rpb1 F1084I CEN LEU2]</i>             | This study | Figure 8        |
| CKY884     | <i>rpb1</i> G1097D         | <i>MATa leu2Δ0 or Δ1 ura3-52 his3Δ200 met15Δ0 trp1Δ63 lys2-128Δ RPB3::TAP::KlacTRP1 rpb1Δ::CLONAT imd2Δ::HIS3 [pRS315 rpb1 G1097D CEN LEU2]</i>             | This study | Figure 8        |
| CKY885     | <i>rpb1</i> E1103G         | <i>MATa leu2Δ0 or Δ1 ura3-52 his3Δ200 met15Δ0 trp1Δ63 lys2-128Δ RPB3::TAP::KlacTRP1 rpb1Δ::CLONAT imd2Δ::HIS3 [pRS315 rpb1 E1103G CEN LEU2]</i>             | This study | Figure 8 and 10 |
| CKY717/718 | <i>dst1Δ</i>               | <i>MATa ura3-52 his3Δ200 leu2Δ1 or Δ0</i>   | This       | Figure 9        |

|              |               |  |            |          |
|--------------|---------------|--|------------|----------|
|              |               | <i>trp1Δ63 met15Δ0 lys2-128θ gal10Δ56 rpb1Δ::CLONATMX RPB3::TAP::KlacTRP1 dst1Δ::KANMX [pRP112 RPB1 CEN URA3]</i>                                      | study      |          |
| CKY982/983   | <i>spt3Δ</i>  | <i>MATa ura3-52 his3Δ200 leu2Δ1 or Δ0 trp1Δ63 met15Δ0 lys2-128θ gal10Δ56 rpb1Δ::CLONATMX RPB3::TAP::KlacTRP1 spt3Δ::kanmx [pRP112 RPB1 CEN URA3]</i>   | This study | Figure 9 |
| CKY1164/1165 | <i>spt4Δ</i>  | <i>MATa ura3-52 his3Δ200 leu2Δ1 or Δ0 trp1Δ63 met15Δ0 lys2-128θ gal10Δ56 rpb1Δ::CLONATMX RPB3::TAP::KlacTRP1 spt4Δ::kanmx [pRP112 RPB1 CEN URA3]</i>   | This study | Figure 9 |
| CKY986/987   | <i>gal11Δ</i> | <i>MATa ura3-52 his3Δ200 leu2Δ1 or Δ0 trp1Δ63 met15Δ0 lys2-128θ gal10Δ56 rpb1Δ::CLONATMX RPB3::TAP::KlacTRP1 gal11Δ::kanmx [pRP112 RPB1 CEN URA3]</i>  | This study | Figure 9 |
| CKY736/737   | <i>sgf73Δ</i> | <i>MATa ura3-52 his3Δ200 leu2Δ1 or Δ0 trp1Δ63 met15Δ0 lys2-128θ gal10Δ56 rpb1Δ::CLONATMX RPB3::TAP::KlacTRP1 sgf73Δ::KANMX [pRP112 RPB1 CEN URA3]</i>  | This study | Figure 9 |
| CKY970/971   | <i>dst1Δ</i>  | <i>MATa leu2Δ0 or Δ1 ura3-52 his3Δ200 met15Δ0 trp1Δ63 lys2-128θ RPB3::TAP::KlacTRP1 rpb1Δ::CLONAT imd2Δ::HIS3 dst1Δ::kanmx [pRP112 RPB1 CEN URA3]</i>  | This study | Figure 9 |
| CKY966/967   | <i>spt3Δ</i>  | <i>MATa leu2Δ0 or Δ1 ura3-52 his3Δ200 met15Δ0 trp1Δ63 lys2-128θ RPB3::TAP::KlacTRP1 rpb1Δ::CLONAT imd2Δ::HIS3 spt3Δ::kanmx [pRP112 RPB1 CEN URA3]</i>  | This study | Figure 9 |
| CKY968/969   | <i>spt4Δ</i>  | <i>MATa leu2Δ0 or Δ1 ura3-52 his3Δ200 met15Δ0 trp1Δ63 lys2-128θ RPB3::TAP::KlacTRP1 rpb1Δ::CLONAT imd2Δ::HIS3 spt4Δ::kanmx [pRP112 RPB1 CEN URA3]</i>  | This study | Figure 9 |
| CKY974/975   | <i>gal11Δ</i> | <i>MATa leu2Δ0 or Δ1 ura3-52 his3Δ200 met15Δ0 trp1Δ63 lys2-128θ RPB3::TAP::KlacTRP1 rpb1Δ::CLONAT imd2Δ::HIS3 gal11Δ::kanmx [pRP112 RPB1 CEN URA3]</i> | This study | Figure 9 |
| CKY972/973   | <i>sgf73Δ</i> | <i>MATa leu2Δ0 or Δ1 ura3-52 his3Δ200 met15Δ0 trp1Δ63 lys2-128θ RPB3::TAP::KlacTRP1 rpb1Δ::CLONAT imd2Δ::HIS3 sgf73Δ::kanmx [pRP112 RPB1 CEN URA3]</i> | This study | Figure 9 |
| CKY728/729   | <i>paf1Δ</i>  | <i>MATa ura3-52 his3Δ200 leu2Δ1 or Δ0 trp1Δ63 met15Δ0 lys2-128θ gal10Δ56 rpb1Δ::CLONATMX RPB3::TAP::KlacTRP1 paf1Δ::KANMX [pRP112 RPB1 CEN URA3]</i>   | This study | Figure 9 |
| CKY990/991   | <i>bur2Δ</i>  | <i>MATa ura3-52 his3Δ200 leu2Δ1 or Δ0 trp1Δ63 met15Δ0 lys2-128θ gal10Δ56 rpb1Δ::CLONATMX RPB3::TAP::KlacTRP1 bur2Δ::kanmx [pRP112 RPB1 CEN URA3]</i>   | This study | Figure 9 |
| CKY988/989   | <i>pop2Δ</i>  | <i>MATa ura3-52 his3Δ200 leu2Δ1 or Δ0</i>  | This       | Figure 9 |



|              |                    |  |            |          |
|--------------|--------------------|--|------------|----------|
|              |                    | <i>trp1Δ63 met15Δ0 lys2-128Δ gal10Δ56 rpb1Δ::CLONATMX RPB3::TAP::KlacTRP1 pop2Δ::kanmx [pRP112 RPB1 CEN URA3]</i>                                      | study      |          |
| CKY976/977   | <i>paf1Δ</i>       | <i>MATa leu2Δ0 or Δ1 ura3-52 his3Δ200 met15Δ0 trp1Δ63 lys2-128Δ RPB3::TAP::KlacTRP1 rpb1Δ::CLONAT imd2Δ::HIS3 paf1Δ::kanmx [pRP112 RPB1 CEN URA3]</i>  | This study | Figure 9 |
| CKY980/981   | <i>bur2Δ</i>       | <i>MATa leu2Δ0 or Δ1 ura3-52 his3Δ200 met15Δ0 trp1Δ63 lys2-128Δ RPB3::TAP::KlacTRP1 rpb1Δ::CLONAT imd2Δ::HIS3 bur2Δ::kanmx [pRP112 RPB1 CEN URA3]</i>  | This study | Figure 9 |
| CKY978/979   | <i>pop2Δ</i>       | <i>MATa leu2Δ0 or Δ1 ura3-52 his3Δ200 met15Δ0 trp1Δ63 lys2-128Δ RPB3::TAP::KlacTRP1 rpb1Δ::CLONAT imd2Δ::HIS3 pop2Δ::kanmx [pRP112 RPB1 CEN URA3]</i>  | This study | Figure 9 |
| CKY2430/2431 | <i>RPB1 WT</i>     | <i>MATa his3Δ200 leu2Δ0 or 1 ura3-52 lys2-128Δ met15Δ0 trp1Δ63 RPB3::TAP::KlacTRP1 rpb1Δ::CLONATMX MIG1::EGFP::kanmx [pRS315 RPB1 CEN LEU2]</i>        | This study | Figure 7 |
| CKY2432/2433 | <i>rpb1 E1103G</i> | <i>MATa his3Δ200 leu2Δ0 or 1 ura3-52 lys2-128Δ met15Δ0 trp1Δ63 RPB3::TAP::KlacTRP1 rpb1Δ::CLONATMX MIG1::EGFP::kanmx [pRS315 rpb1 E1103G CEN LEU2]</i> | This study | Figure 7 |
| CKY2434/2435 | <i>rpb1 G1097D</i> | <i>MATa his3Δ200 leu2Δ0 or 1 ura3-52 lys2-128Δ met15Δ0 trp1Δ63 RPB3::TAP::KlacTRP1 rpb1Δ::CLONATMX MIG1::EGFP::kanmx [pRS315 rpb1 G1097D CEN LEU2]</i> | This study | Figure 7 |
| CKY2467/2468 | <i>rpb1 N479S</i>  | <i>MATa his3Δ200 leu2Δ0 or 1 ura3-52 lys2-128Δ met15Δ0 trp1Δ63 RPB3::TAP::KlacTRP1 rpb1Δ::CLONATMX MIG1::EGFP::kanmx [pRS315 rpb1 N479S CEN LEU2]</i>  | This study | Figure 7 |

**Supplementary Table 4.** List of plasmids used in this study.

| <b>Plasmid</b> | <b>Description</b>                  | <b>Genotype</b>                              | <b>Reference</b> |
|----------------|-------------------------------------|--|------------------|
| pCK518         | pRS316 <i>RPB1</i>                  | ampr ColE1 ori <i>URA3</i><br><i>CEN ARS</i> | (15)             |
| pCK859         | pRS315 <i>RPB1</i>                  | ampr ColE1 ori<br><i>LEU2 CEN ARS</i>        | (15)             |
| pCK856         | pRS315 <i>rpb1</i><br>N479S         | ampr ColE1 ori<br><i>LEU2 CEN ARS</i>        | (15)             |
| pCK864         | pRS315 <i>rpb1</i><br>L1101S        | ampr ColE1 ori<br><i>LEU2 CEN ARS</i>        | (15)             |
| pCK867         | pRS315 <i>rpb1</i><br>G1097D        | ampr ColE1 ori<br><i>LEU2 CEN ARS</i>        | (15)             |
| pCK870         | pRS315 <i>rpb1</i><br>H1085Y        | ampr ColE1 ori<br><i>LEU2 CEN ARS</i>        | (15)             |
| pCK871         | pRS315 <i>rpb1</i><br>F1086S        | ampr ColE1 ori<br><i>LEU2 CEN ARS</i>        | (15)             |
| pCK886         | pRS315 <i>rpb1</i><br>N1082S        | ampr ColE1 ori<br><i>LEU2 CEN ARS</i>        | (15)             |
| pCK887         | pRS315 <i>rpb1</i><br>H1085Q        | ampr ColE1 ori<br><i>LEU2 CEN ARS</i>        | (15)             |
| pCK890         | pRS315 <i>rpb1</i><br>H1085Y/E1103G | ampr ColE1 ori<br><i>LEU2 CEN ARS</i>        | (15)             |
| pCK955         | pRS315 <i>rpb1</i><br>F1084I        | ampr ColE1 ori<br><i>LEU2 CEN ARS</i>        | (15)             |
| pCK960         | pRS315 <i>rpb1</i><br>E1103G        | ampr ColE1 ori<br><i>LEU2 CEN ARS</i>        | (15)             |

**Supplementary Table 5.** List of primers used in this study.

| Primer  | Description / Relevant gene | Sequence                        | Function                                       | Reference  |
|---------|-----------------------------|---------------------------------|--|------------|
| CKO667  | KANMX - GAL1p/TEF1p F       | TAACGCCGCCATCCAGTGT<br>C        | ChIP/RT-PCR                                    | This study |
| CKO668  | KANMX -TEF1p/R              | GCGCGGAGTCCGAGAAAA<br>TC        | ChIP/RT-PCR                                    | This study |
| CKO669  | KANMX -GAL1p/R              | GCGAGGCACATCTGCGTT<br>TC        | ChIP/RT-PCR                                    | This study |
| CKO672  | YLR454w 0.2 kb F            | GTACCGTCAGGCTAAAATC<br>CGTTCCG  | ChIP/RT-PCR                                    | This study |
| CKO673  | YLR454w 0.2 kb R            | GACCCATTGAGCCAGTAT<br>TGTGA     | ChIP/RT-PCR                                    | This study |
| CKO653  | YLR454w 1 kb F              | ACAGGTTTCAGAAATGAGAT<br>GCCAG   | ChIP/RT-PCR<br>Northern probe<br>amplification | This study |
| CKO654  | YLR454w 1 kb R              | TTGGCGTGGCTTTGATGTT<br>TTCG     | ChIP/RT-PCR                                    | This study |
| CKO655  | YLR454w 2 kb F              | AGCGACTTCATGTTCCAGC<br>AACT     | ChIP/RT-PCR                                    | This study |
| CKO656  | YLR454w 2 kb R              | CTTGGCATAAAAACCGACC<br>TAGCAC   | ChIP/RT-PCR<br>Northern probe<br>amplification | This study |
| CKO657  | YLR454w 3 kb F              | TGGGGCCAACTAAAGGAG<br>TTAC      | ChIP/RT-PCR                                    | This study |
| CKO658  | YLR454w 3 kb R              | CCAAAAGTTTGGCTGCGTT<br>G        | ChIP/RT-PCR                                    | This study |
| CKO659  | YLR454w 4 kb F              | ACCTCCACTAAGCTCTACA<br>CAAAGT   | ChIP/RT-PCR                                    | This study |
| CKO660  | YLR454w 4 kb R              | TTCTGGGCACGAACAACG<br>AG        | ChIP/RT-PCR                                    | This study |
| CKO661  | YLR454w 5 kb F              | GTCCCAACGGGTTCAAGG<br>CATCC     | ChIP/RT-PCR                                    | This study |
| CKO662  | YLR454w 5 kb R              | ATCGGCATCAGCGTTGTG<br>GT        | ChIP/RT-PCR                                    | This study |
| CKO663  | YLR454w 6 kb F              | ACTGTTGAAATGGAACGAG<br>GACGC    | ChIP/RT-PCR                                    | This study |
| CKO664  | YLR454w 6 kb R              | CCTTCTGGTATCGCTTCCA<br>TACTCG   | ChIP/RT-PCR                                    | This study |
| CKO665  | YLR454w 7 kb F              | ACACAGTCGGTTTGGCGA<br>G         | ChIP/RT-PCR                                    | This study |
| CKO666  | YLR454w 7 kb R              | ACTCGACAAAGTGGTCTCA<br>ACG      | ChIP/RT-PCR                                    | This study |
| CKO1780 | YLR454w 8 kb F              | GAGGGTCACAGATCTATTA<br>CTTGCCC  | ChIP/RT-PCR                                    | (12)       |
| CKO1781 | YLR454w 8 kb R              | GTTGTGAGTTGCTTCAGTG<br>GTGAAGTG | ChIP/RT-PCR                                    | (12)       |
| CKO946  | TEL-VI 1                    | GCGTAACAAAGCCATAATG<br>CCTCC    | ChIP/RT-PCR                                    | (12)       |
| CKO947  | TEF-VI 2                    | CTCGTTAGGATCACGTTCCG<br>AATCC   | ChIP/RT-PCR                                    | (12)       |
| CKO1268 | TEF1 F                      | ATGGGTAAAGAGAAGTCTC             | Northern probe                                 | This study |

|         |                         |                                    |  |            |
|---------|-------------------------|------------------------------------|--|------------|
|         |                         | AC                                 | amplification                                |            |
| CKO1269 | TEF1 R                  | CAGCCTTTTGAGCAGCCTT<br>GGTA        | Northern probe<br>amplification              | This study |
| CKO1172 | SCR1 F                  | AGGCTGTAATGGCTTTCTG<br>GTGGGATGGGA | Northern probe<br>amplification              | (16)       |
| CKO1173 | SCR1 R                  | GATATGTGCTATCCCGGCC<br>GCCTCCATCAC | Northern probe<br>amplification              | (16)       |
| CKO1692 | GAL1 F                  | GTGCCCGAGCATAATTAAG<br>AAAT        | Northern probe<br>amplification              | This study |
| CKO1693 | GAL1 R                  | TGTAGTGACTIONCTACT<br>CTTA         | Northern probe<br>amplification              | This study |
| CKO1782 | snR33 +12 Top<br>Strand | CTCTTTGTACGATGGTGTC<br>ACTC        | Northern probe<br>amplification              | (12)       |
| CKO1783 | snR33 3'UTR low<br>+347 | AATTGTTAAATGCATTGGC<br>TCG         | Northern probe<br>amplification/la<br>beling | (12)       |

## Supplementary References

1. Mason, P.B. and Struhl, K. (2005) Distinction and relationship between elongation rate and processivity of RNA polymerase II in vivo. *Mol Cell*, **17**, 831-840.
2. Schwabish, M.A. and Struhl, K. (2006) Asf1 mediates histone eviction and deposition during elongation by RNA polymerase II. *Mol Cell*, **22**, 415-422.
3. Biswas, D., Dutta-Biswas, R., Mitra, D., Shibata, Y., Strahl, B.D., Formosa, T. and Stillman, D.J. (2006) Opposing roles for Set2 and yFACT in regulating TBP binding at promoters. *EMBO J*, **25**, 4479-4489.
4. Schwabish, M.A. and Struhl, K. (2007) The Swi/Snf complex is important for histone eviction during transcriptional activation and RNA polymerase II elongation in vivo. *Mol Cell Biol*, **27**, 6987-6995.
5. Govind, C.K., Zhang, F., Qiu, H., Hofmeyer, K. and Hinnebusch, A.G. (2007) Gcn5 promotes acetylation, eviction, and methylation of nucleosomes in transcribed coding regions. *Mol Cell*, **25**, 31-42.
6. Ginsburg, D.S., Govind, C.K. and Hinnebusch, A.G. (2009) NuA4 lysine acetyltransferase Esa1 is targeted to coding regions and stimulates transcription elongation with Gcn5. *Mol Cell Biol*, **29**, 6473-6487.
7. Quan, T.K. and Hartzog, G.A. (2010) Histone H3K4 and K36 methylation, Chd1 and Rpd3S oppose the functions of *Saccharomyces cerevisiae* Spt4-Spt5 in transcription. *Genetics*, **184**, 321-334.
8. Jimeno-Gonzalez, S., Haaning, L.L., Malagon, F. and Jensen, T.H. (2010) The yeast 5'-3' exonuclease Rat1p functions during transcription elongation by RNA polymerase II. *Mol Cell*, **37**, 580-587.
9. Kruk, J.A., Dutta, A., Fu, J., Gilmour, D.S. and Reese, J.C. (2011) The multifunctional Ccr4-Not complex directly promotes transcription elongation. *Genes Dev*, **25**, 581-593.
10. Miguel, A., Monton, F., Li, T., Gomez-Herreros, F., Chavez, S., Alepuz, P. and Perez-Ortin, J.E. (2013) External conditions inversely change the RNA polymerase II elongation rate and density in yeast. *Biochim Biophys Acta*, **1829**, 1248-1255.
11. Millan-Zambrano, G., Rodriguez-Gil, A., Penate, X., de Miguel-Jimenez, L., Morillo-Huesca, M., Krogan, N. and Chavez, S. (2013) The prefoldin complex regulates chromatin dynamics during transcription elongation. *PLoS Genet*, **9**, e1003776.
12. Hazelbaker, D.Z., Marquardt, S., Wlotzka, W. and Buratowski, S. (2013) Kinetic competition between RNA Polymerase II and Sen1-dependent transcription termination. *Mol Cell*, **49**, 55-66.
13. Jimeno-Gonzalez, S., Schmid, M., Malagon, F., Haaning, L.L. and Jensen, T.H. (2014) Rat1p maintains RNA polymerase II CTD phosphorylation balance. *RNA*, **20**, 551-558.
14. Barnes, C.O., Calero, M., Malik, I., Graham, B.W., Spahr, H., Lin, G., Cohen, A.E., Brown, I.S., Zhang, Q., Pullara, F. *et al.* (2015) Crystal Structure of a Transcribing RNA Polymerase II Complex Reveals a Complete Transcription Bubble. *Mol Cell*, **59**, 258-269.
15. Kaplan, C.D., Jin, H., Zhang, I.L. and Belyanin, A. (2012) Dissection of Pol II trigger loop function and Pol II activity-dependent control of start site selection in vivo. *PLoS Genet*, **8**, e1002627.
16. Maderazo, A.B., He, F., Mangus, D.A. and Jacobson, A. (2000) Upf1p control of nonsense mRNA translation is regulated by Nmd2p and Upf3p. *Mol Cell Biol*, **20**, 4591-4603.