Supplemental Figures and Methods

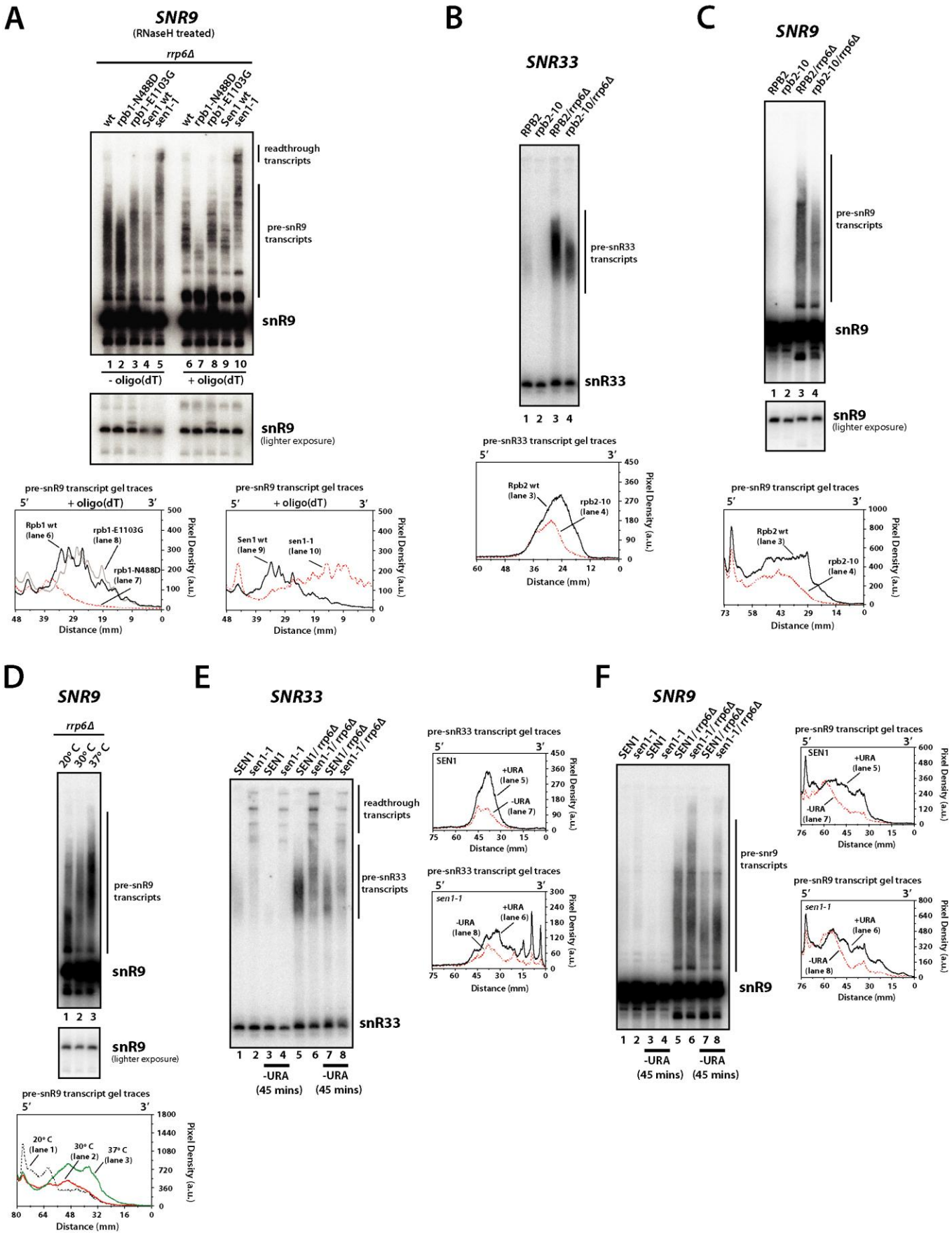

Figure S1. Effects of Pol II mutations, temperature and uracil depletion on non-coding RNA termination windows, related to Figure 2.

(A) Northern blot analysis of snoRNA gene *SNR9* with RNA from wild-type Rpb1 (wt) (YSB2697), *rpb1-N488D* (YSB2698), *rpb1-E1103G* (YSB2699), and wild-type Sen1 (YSB2859) and *sen1-1* (YSB2860) isogenic strains containing *rrp6*Δ grown at 30°C. RNA was treated with RNaseH in the absence or presence of oligo(dT).

(B, C) Northern blot analysis of *SNR33* and *SNR9* with RNA from *RPB2* (Z24), *rpb2-10* (Z428), *RPB2/rrp6*Δ (YSB2901) and *rpb2-10/rrp6*Δ (YSB2902) strains grown at 30°C.

(D) Northern blot analysis of snoRNA gene *SNR9* from wild-type Rpb1 (YSB2697) strains containing *rrp6*Δ grown at 20°C, 30°C, and 37°C.

(E, F) Northern blot analysis of *SNR33* and *SNR9* with RNA from *SEN1* WT (1971), *sen1-1* (FWY1), *SEN1/rrp6*Δ (YSB2859) and *sen1-1/rrp6*Δ (YSB2860) strains grown at 30°C in SC complete media (lanes 1, 2, 5, 6) or grown in SC complete and shifted to media lacking uracil (-URA) for 45 minutes prior to harvesting (lanes 3, 4, 7, 8).

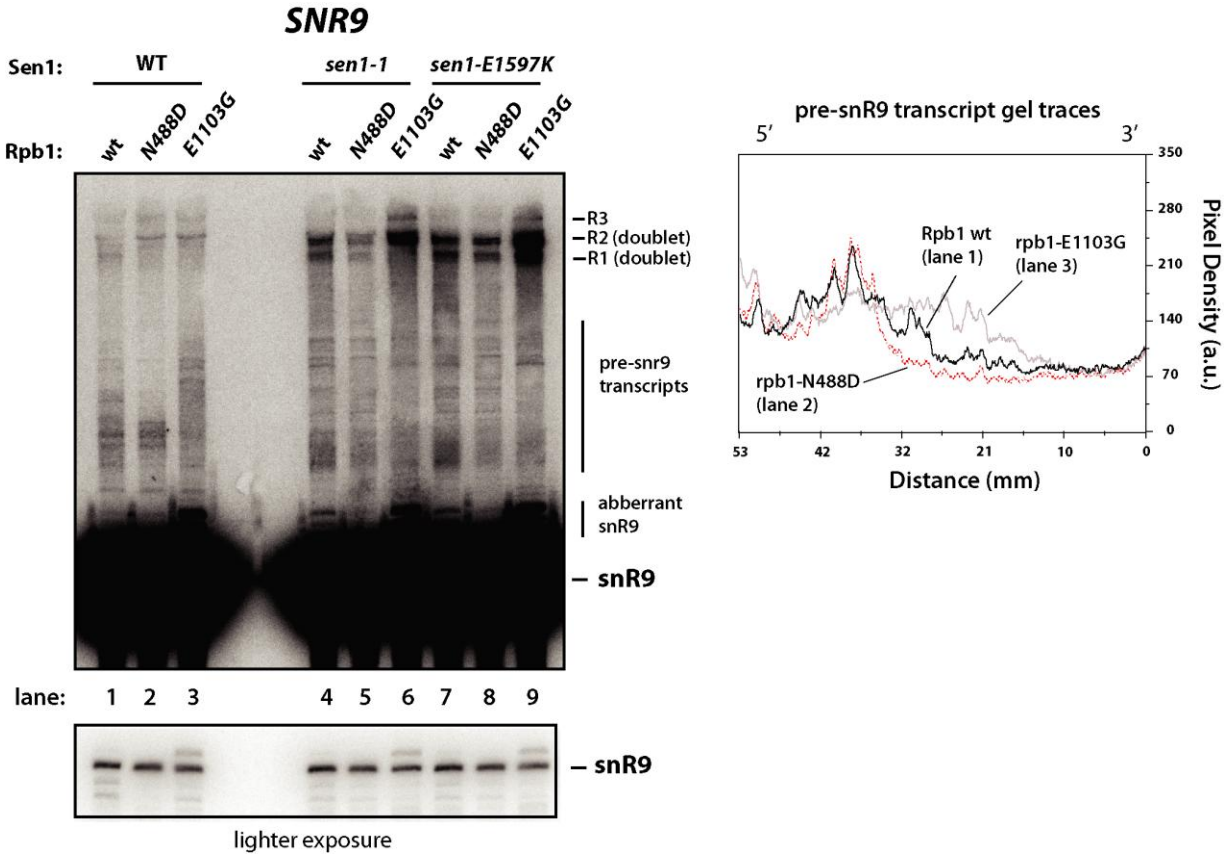

Figure S2. Effects of Pol II and Sen1 mutations on SNR9 termination, related to figure 4.
Northern blot analysis of snoRNA gene *SNR9* with RNA from WT (YSB2700), *rpb1-N488D* (YSB2701), *rpb1-E1103G* (YSB2702), *sen1-1* (YSB2704), *sen1-1/rpb1-N488D* (YSB2705), *sen1-1/rpb1-E1103G* (YSB2706), *sen1-E1597K* (YSB2707), *sen1-E1597K/rpb1-N488D* (YSB2708), and *sen1-E1597K/rpb1-E1103G* (YSB2709) strains grown at 30°C, wt; wild-type, R1-R3; readthrough/extended transcripts that increase in the presence of *sen1-1* and *sen1-E1597K* backgrounds.

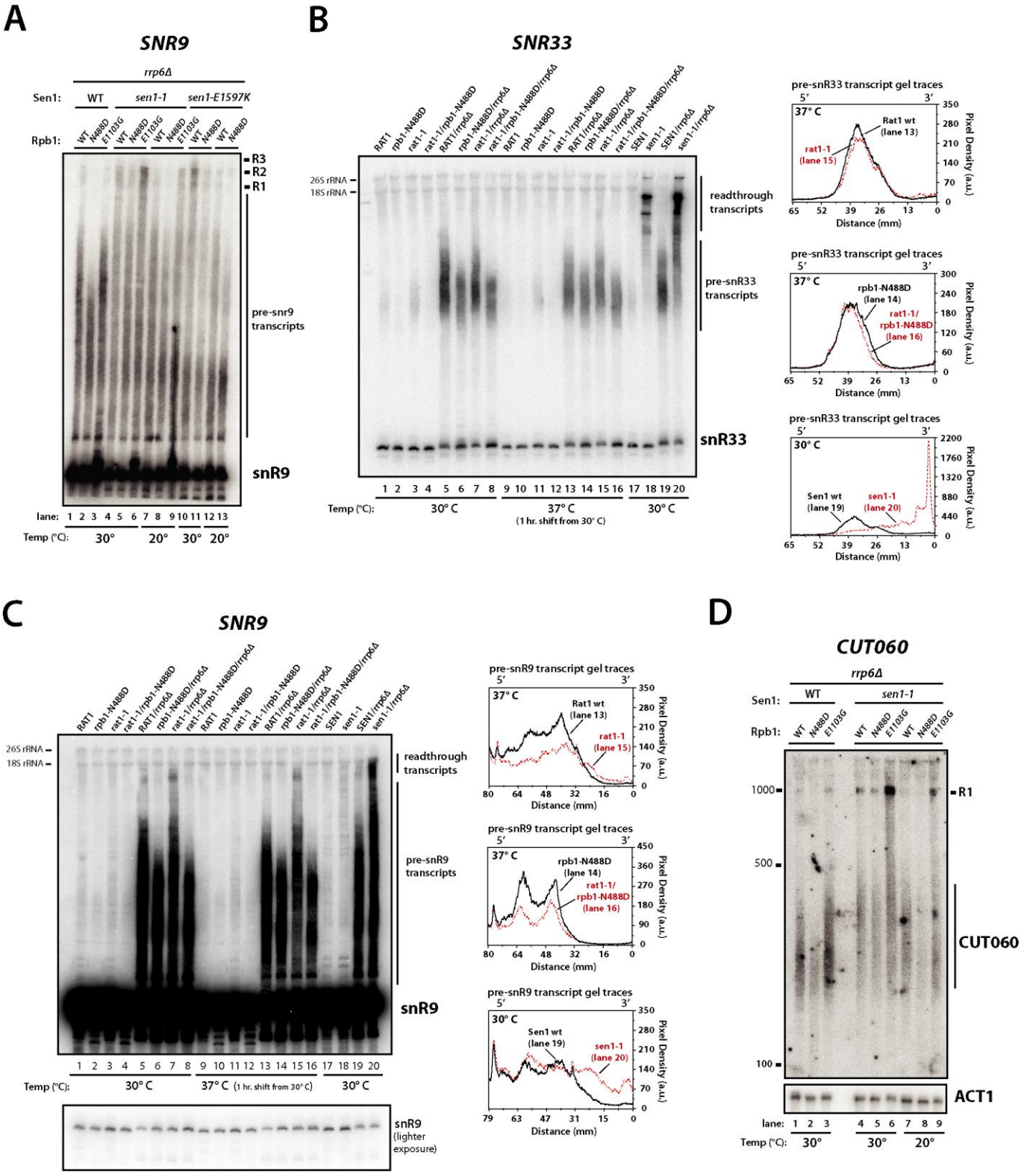
A
SNR9
rrp6Δ
Sen1:
WT
sen1-1
sen1-E1597K
Rpb1:
WT
N488D
E1103G
R3
R2
R1
pre-snr9 transcripts
snR9
lane: 1 2 3 4 5 6 7 8 9 10 11 12 13
Temp (°C): 30° 20° 30° 20°
B
SNR33
RAT1
rpb1-N488D
rat1-1
rat1-1/rpb1-N488D
RAT1/rrp6Δ
rpb1-N488D/rrp6Δ
rat1-1/rrp6Δ
rat1-1/rpb1-N488D/rrp6Δ
SEN1
sen1-1
SEN1/rrp6Δ
sen1-1/rrp6Δ
26S rRNA
18S rRNA
readthrough transcripts
pre-snR33 transcripts
snR33
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Temp (°C): 30° C 37° C (1 hr. shift from 30° C) 30° C
pre-snR33 transcript gel traces
5′ 3′
37° C
Rat1 wt (lane 13)
rat1-1 (lane 15)
Pixel Density (a.u.)
Distance (mm)
rpb1-N488D (lane 14)
rat1-1/ rpb1-N488D (lane 16)
30° C
Sen1 wt (lane 19)
sen1-1 (lane 20)
C
SNR9
pre-snR9 transcripts
snR9
snR9 (lighter exposure)
pre-snR9 transcript gel traces
D
CUT060
rrp6Δ
Sen1:
WT
sen1-1
Rpb1:
WT
N488D
E1103G
1000
500
100
R1
CUT060
ACT1
lane: 1 2 3 4 5 6 7 8 9
Temp (°C): 30° 30° 20°

Figure S3. Effects of Pol II, Sen1, and Rat1 mutations on non-coding RNA termination windows, related to Figure 5.

(A) Northern blot analysis of *SNR9* and with RNA from WT (YSB2697), *rpb1-N488D* (YSB2698), *rpb1-E1103G* (YSB2699), *sen1-1* (YSB2711), *sen1-1/rpb1-N488D* (YSB2712), *sen1-1/rpb1-E1103G* (YSB2713), *sen1-E1597K* (YSB2714), and *sen1-E1597K/rpb1-N488D* (YSB2715), strains containing *rrp6*Δ grown at 30°C and 20°C. R1-R3; readthrough/extended transcripts whose levels increase in *sen1-1* and *sen1-E1597K* backgrounds.
(B, C) Northern blot analysis of *SNR33* and *SNR9* with RNA from *RAT1* (THJ2400), *rpb1-N488D* (THJ2401), *rat1-1* (THJ2402), *rat1-1/rpb1-N488D* (THJ2403), *RAT1/rrp6*Δ (YSB2909), *rpb1-N488D/rrp6*Δ (YSB2906), *rat1-1/rrp6*Δ (YSB2907), *rat1-1/rpb1-N488D/rrp6*Δ (YSB2908), *SEN1* (1971), *sen1-1* (FWY1), *SEN1/rrp6*Δ (YSB2859), and *sen1-1/rrp6*Δ (YSB2860) grown at 30°C or then shifted to 37°C for 1 hour prior to harvesting.
(D) Northern blot analysis of *CUT060* with RNA from indicated in (A) grown at 30°C and 20°C. ACT1 mRNA and serves as a loading control. R1; readthrough/extended transcripts whose levels increase in *sen1-1* and *sen1-E1597K* backgrounds

Supplemental Experimental Procedures

Yeast Strains

RPB1 (GRY3020), *rpb1-N488D* (GRY3027), and *rpb1-E1103G* (GRY3028) strains were generously provided by Jeffrey Strathern. To introduce the *sen1-1* and *sen1-E1597K* alleles into this background, a C-terminal MYC13 tag marked with KanMX6 was integrated into strains FWY1 (*sen1-1*) (Rasmussen and Culbertson, 1998) and *nrd2-1* (sen1-E1597K) (Steinmetz and Brow, 1996), creating *sen1-1*-Myc and *sen1-E1597K*-Myc strains YSB2703 and YSB2573. DNA containing part of the Sen1 coding region harboring the *sen1* point mutations and the C-terminal MYC13::KanMX6 tag were amplified using PCR and used to transform *RPB1* (GRY3020), *rpb1-N488D* (GRY3027), and *rpb1-E1103G* (GRY3028) strains. Transformants were selected on G418 plates and the presence of the *sen1-1* or *sen1-E1597K* mutation was confirmed by conferral of the temperature sensitive phenotype and sequencing of the genomic *SEN1* locus. Comparison of Sen1 strains with and without the C-terminal Myc-tag show no discernable differences in snoRNA termination efficiency. Where indicated, cells were also transformed with either *rrp6*Δ::KANMX or *rrp6*Δ::NATMX cassettes.

Yeast Growth Assays and Spot Growth Tests.

For yeast growth rate assays, overnight cultures were grown at room temperature (20°C) in YPD media and then diluted to OD_{600} ~0.05 in YPD media and grown at 30°C. Samples were taken at indicated timepoints and optical densities were measured with a spectrophotometer. For spot growth tests, overnight cultures were grown at 20°C in YPD media and were diluted to OD_{600} ~0.5 and spotted in three-fold dilutions on YPD-complete plates and grown at 30°C for indicated times. For spot tests on galactose plates, overnight cultures were grown at 30°C, pelleted, washed with water, and diluted to OD_{600} ~0.1 and spotted in ten-fold dilutions on 2% galactose/SC-uracil/x-gal plates and grown at 30°C.

Chromatin Immunoprecipitation (ChIP) Analysis. For *in vivo* elongation rate measurement, strains GRY3020 (*RPB1*) and GRY3028 (*rpb1-E1103G*) were transformed with KpnI-digested YIplac204-Gal1-454 plasmid, containing a *TRP1* marker and the *GAL1* promoter fused to the *YLR454* open-reading frame (Mason and Struhl, 2005), generating strains YSB2716 and YSB2717. Overnight cultures in synthetic complete media with 2% raffinose were used to inoculate larger cultures of YP-Galactose (2% yeast extract, 4% bacto-peptone, 2% galactose) to an OD_{600} of 0.1. After growth at 30°C for 4-5 hours, pre-glucose induction samples were taken for the 0 min timepoint and crosslinked with formaldehyde. Remaining cells were centrifuged and washed with synthetic complete media lacking a carbon source and used to inoculate YP-Glucose media (2% yeast extract, 4% bacto-peptone, 4% glucose). Aliquots were removed after 2, 4, and 8 minutes as post-glucose samples and crosslinked. Chromatin preparations and immunoprecipations were performed as described previously (Keogh and Buratowski, 2004). Precipitated or input DNA from two-independent replicates was analyzed in triplicate by quantitative PCR reactions using a Roche Lightcycler 480. Fold-enrichment values were calculated by normalizing to a non-transcribed telomeric region as previously described (Marquardt et al., 2011).

RNaseH oligo(dT) Treatment

30 µg total RNA in the absence or presence of 750 ng of 28mer oligo(dT) were ethanol precipitated in 3 volumes 100% ethanol and 1/10 volume 3 M sodium acetate, pH 5.2 for overnight at -20°C. Pellets were resuspended in 9 µl water and 1 µl 10X hybridization buffer (0.25M Tris pH7.5, 10Mm EDTA, 0.5M NaCl) and placed in a 68°C water bath and cooled to 30°C. 10µl 2X RNase H buffer (40mM Tris pH7.5, 20mM $MgCl_2$, 100mM NaCl, 2mM DTT, 60 µg/ml BSA) and 1µl (1U) RNase H (Invitrogen) was added to the reactions and incubated for 60 min at 30°C. Reactions were stopped by the addition of 130 µl stop mix (40 µg/ml tRNA, 20mM EDTA, 30mM sodium acetate, pH 5.2 and extracted with phenol-choloform-isoamyl alcohol and resuspended in DEPC-treated water and subjected to Northern Analysis.

Supplemental References

Keogh, M.C., and Buratowski, S. (2004). Using chromatin immunoprecipitation to map cotranscriptional mRNA processing in Saccharomyces cerevisiae. Methods Mol Biol *257*, 1-16.

Marquardt, S., Hazelbaker, D.Z., and Buratowski, S. (2011). Distinct RNA degradation pathways and 3' extensions of yeast non-coding RNA species. Transcription *2*, 145-154.

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.

Rasmussen, T.P., and Culbertson, M.R. (1998). The putative nucleic acid helicase Sen1p is required for formation and stability of termini and for maximal rates of synthesis and levels of accumulation of small nucleolar RNAs in Saccharomyces cerevisiae. Mol Cell Biol *18*, 6885-6896.

Steinmetz, E.J., and Brow, D.A. (1996). Repression of gene expression by an exogenous sequence element acting in concert with a heterogeneous nuclear ribonucleoprotein-like protein, Nrd1, and the putative helicase Sen1. Mol Cell Biol *16*, 6993-7003.

Table S1: Yeast Strains

Identifier	Name
GRY3020	wild-type
GRY3027	rpb1-N488D
GRY3028	rpb1-E1103G
YSB2716	GAL1-YLR454
YSB2717	GAL1-YLR454/rpb1-E1103G
YSB2697	rrp6Δ
YSB2698	rrp6Δ/rpb1-N488D
YSB2699	rrp6Δ/rpb1-E1103G
YSB2700	Sen1-myc
YSB2701	Sen1-myc/rpb1-N488D
YSB2702	Sen1-myc/rpb1-E1103G
YSB2704	sen1-1-myc
YSB2705	sen1-1-myc/rpb1-N488D
YSB2706	sen1-1-myc/rpb1-E1103G
YSB2707	sen1(E1597K)-myc
YSB2708	sen1(E1597K)-myc/rpb1-N488D
YSB2709	sen1(E1597K)-myc/rpb1-E1103G
YSB2711	rrp6Δ/sen1-1-myc
YSB2712	rrp6Δ/sen1-1-myc/rpb1-N488D
YSB2713	rrp6Δ/sen1-1-myc/rpb1-E1103G
YSB2714	rrp6Δ/sen1(E1597K)-myc
YSB2715	rrp6Δ/sen1(E1597K)-myc/rpb1-N488D
FWY1	sen1-1
YSB2703	sen1-1-myc
nrd2-1	sen1-E1597K
YSB2573	sen1(E1597K)-myc
YSB2859	rrp6Δ
YSB2860	rrp6Δ/sen1-1
1971	SEN1
Z24	RPB2
Z428	rpb2-10
YSB2901	rrp6Δ/RPB2
YSB2902	rrp6Δ/rpb2-10
THJ2400	wild-type
THJ2401	rpb1-N488D
THJ2402	rat1-1
THJ2403	rpb1-N488D/rat1-1
YSB2909	rrp6Δ
YSB2906	rrp6Δ/rpb1-N488D
YSB2907	rrp6Δ/rat1-1
YSB2908	rrp6Δ/rpb1-N488D/rat1-1

Table S2: Oligonucleotides

Identifier	Name	Sequence 5' to 3'
1617	TEL-VI (1)	GCGTAACAAAGCCATAATGCCTCC
1618	TEL-VI (2)	CTCGTTAGGATCACGTTCGAATCC
1077	GAL1(-194)	CTGGGGTAATTAATCAGCGAAGCGATG
1078	YLR454(+35)	CACTTGTACAGTAGAACATTAATCGGAAAC
2824	YLR454w_0.5_Top	CGCAATTAGTCAACAACGATATCACGATTG
2825	YLR454w_0.5_Bot	CCTACTTGAAGTCCATCCTTCAGAGG
2826	YLR454w_1.0_Top	CAATACCAACAGGTTCAGAAATGAGATGC
2827	YLR454w_1.0_Bot	GAGAGAACAAATTGGTTTCGCCAAATATCG
1079	YLR454(+1986)	CATATCATCCACCCTAGGTGCTAGGTCGG
1080	YLR454(+2199)	GAGCTGACCAGACCTAACCATAGTAGCGTG
1081	YLR454(+4069)	AGATATTACTCGTTGTTCGTGCCCAG
1082	YLR454(+4268)	TCCCAAAACCCTAGTTTAACAGAAGG
1085	YLR454(+7701)	GAGGGTCACAGATCTATTACTTGCCC
1086	YLR454(+7850)	GTTGTGAGTTGCTTCAGTGGTGAAGTG
1309	snR13 Pro up -60	TTATAAATGGCATCTCAAATCGTC
1310	snR13 Pro low +124	GGTCAGATAAAAGTAAAAAAAGGTAGC
1316	TRS31 ORF low +922	ATCACGGCGCCTCATCTTTG
1324	YCR015C Pro low +556	CGGCAGTGATCTCGTTCCATTG
519	Adh1-CDS-Low#1	ATTTGACCCTTTTCCATCTTTTCGTAA
518	Adh1-CDS-Up#1	TTCAACCAAGTCGTCAAGTCCATCTCTA
1311	snR13 3UTR up +119	CTGACCTTTTAACTTCCCCGTAG
1312	snR13 3UTR low +359	CTGTCGCTTCCGTGTCTCTTGTCCTG
1619	snR33 3'UTR up +208	GTATTACTACTGAGCTTGTTTATCTGT
1322	snR33 3UTR low +288	TAAAGAAAACGATAAGAACTAACC
2436	snR33 +12 Top Strand	CTCTTTGTACGATGGTGTCACTC
1620	snR33 3'UTR low +347	AATTGTTAAATGCATTGGCTCG
2353	snR3 1F	GTACTAATCCACCGCATTAGACAGT
2354	snR3 1R	CTTCTGACACTCGAGTCTCATTCA
2658	SNR31_code_top	GCAAAATTACACCATGAGTTCC
2659	SNR31_code_bot	GTAGAACGAATCATGACCAAC
2357	snR9 1F	GAACTTTCTACGCCTTTTCCTCTATG
2358	snR9 1R	GTCTGAAGGACTAATGATAGGTGGG
2451	CUT_60_F	AGCTCTGGCCTTGCAATAAA
2452	CUT_60_R	CCCCTTCCTAGACGACTCCA
2509	SUT477_F	AAAACAGCTGGGCCTTCTCT
2510	SUT477_R	TAAGGCGCAATCTACCGAAG
1810	Act1 CDS 2F	TCCTTCTGTTTTGGGTTTGGAATC
1811	Act1 CDS 2R	CCAATCCAGACGGAGTACTTTCTTTC
Struhl Lab	Oligo(dT) 28 mer	TTTTTTTTTTTTTTTTTTTTTTTTTTTT
2985	SCR1_F	AGGCTGTAATGGCTTTCTGGTGGGA
2986	SCR1_R	ACGGGCTGCCCGCAAAGATCGA