# Expanded View Figures

**A**

| | | no tag | | | Sen1 | | | Dbl8 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Score | Coverage | # Unique Peptides | Score | Coverage | # Unique Peptides | Score | Coverage | # Unique Peptides | MW | RNA Pol |
| | Sen1 | 0,00 | 0,00 | 0,00 | 2066,75 | 39,18 | 63,00 | 0,00 | 0,00 | 0,00 | 192,55 kDa | |
| | Dbl8 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 551,74 | 16,00 | 25,00 | 222,08 kDa | |
| RNAP3-specific | Rpc1 | 0,00 | 0,00 | 0,00 | 1159,97 | 39,43 | 43,00 | 0,00 | 0,00 | 0,00 | 157,56 kDa | III |
| | Rpc11 | 0,00 | 0,00 | 0,00 | 181,26 | 66,06 | 4,00 | 0,00 | 0,00 | 0,00 | 12,82 kDa | III |
| | Rpc17 | 0,00 | 0,00 | 0,00 | 159,16 | 51,16 | 6,00 | 0,00 | 0,00 | 0,00 | 14,93 kDa | III |
| | Rpc2 | 0,00 | 0,00 | 0,00 | 873,66 | 42,66 | 39,00 | 0,00 | 0,00 | 0,00 | 130,24 kDa | III |
| | Rpc25 | 0,00 | 0,00 | 0,00 | 90,55 | 23,15 | 4,00 | 0,00 | 0,00 | 0,00 | 23,24 kDa | III |
| | Rpc31 | 0,00 | 0,00 | 0,00 | 228,89 | 42,38 | 9,00 | 0,00 | 0,00 | 0,00 | 24,34 kDa | III |
| | Rpc34 | 0,00 | 0,00 | 0,00 | 156,24 | 39,53 | 7,00 | 0,00 | 0,00 | 0,00 | 33,95 kDa | III |
| | Rpc37 | 0,00 | 0,00 | 0,00 | 627,02 | 57,85 | 14,00 | 0,00 | 0,00 | 0,00 | 27,38 kDa | III |
| | Rpc53 | 0,00 | 0,00 | 0,00 | 218,55 | 44,85 | 10,00 | 0,00 | 0,00 | 0,00 | 36,87 kDa | III |
| | Rpc82 | 0,00 | 0,00 | 0,00 | 552,12 | 36,55 | 22,00 | 0,00 | 0,00 | 0,00 | 68,37 kDa | III |
| RNAP1&RNAP3 | Rpc19 | 0,00 | 0,00 | 0,00 | 102,28 | 29,60 | 3,00 | 0,00 | 0,00 | 0,00 | 13,72 kDa | I, III |
| | Rpc40 | 0,00 | 0,00 | 0,00 | 294,39 | 40,23 | 11,00 | 0,00 | 0,00 | 0,00 | 39,15 kDa | I, III |
| RNAP1, RNAP2 & RNAP3 | Rpb5 | 0,00 | 0,00 | 0,00 | 87,31 | 11,43 | 2,00 | 0,00 | 0,00 | 0,00 | 23,92 kDa | I, II, III |
| | Rpb6 | 0,00 | 0,00 | 0,00 | 114,25 | 26,76 | 3,00 | 0,00 | 0,00 | 0,00 | 15,73 kDa | I, II, III |
| | Rpb8 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 14,30 kDa | I, II, III |
| | Rpb10 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 8,28 kDa | I, II, III |
| | Rpc10 | 0,00 | 0,00 | 0,00 | 181,26 | 66,06 | 4,00 | 0,00 | 0,00 | 0,00 | 7,20 kDa | I, II, III |
| RNAP1-specific | Rpa34 | 0,00 | 0,00 | 0,00 | 58,50 | 15,14 | 2,00 | 121,87 | 35,86 | 6,00 | 28,01 kDa | I |
| | Rpa49 | 0,00 | 0,00 | 0,00 | 48,75 | 5,88 | 2,00 | 33,05 | 5,18 | 2,00 | 48,07 kDa | I |
| | Rpa1/Nuc1 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 119,91 | 5,51 | 8,00 | 189,13 kDa | I |
| | Rpa12 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 77,00 | 27,73 | 2,00 | 13,1 kDa | I |
| | Ker1 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 16,98 kDa | I |
| | Rpa2 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 131,69 kDa | I |
| | Rpa43 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 19,39 kDa | I |
| DNA Repair | Pso2 | 0,00 | 0,00 | 0,00 | 198,92 | 19,82 | 10,00 | 0,00 | 0,00 | 0,00 | 63,08 kDa | |

**B**

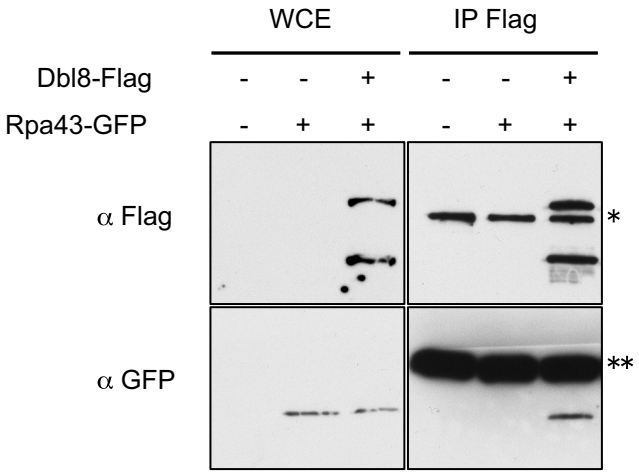


**Figure EV1. Sen1 and Dbl8 associate with different RNA polymerases.**

A Flag-tagged Sen1 and Dbl8 were affinity-purified and their associated proteins identified using mass spectrometry analysis. The table lists the RNA polymerase components that were recovered with either Sen1 or Dbl8. The full list of proteins identified is shown in Appendix Table S1.

B Co-immunoprecipitation between Flag-tagged Dbl8 and the GFP-tagged RNAP1 subunit Rpa43. * Aspecific protein recognized by the anti-Flag antibody; ** IgG.

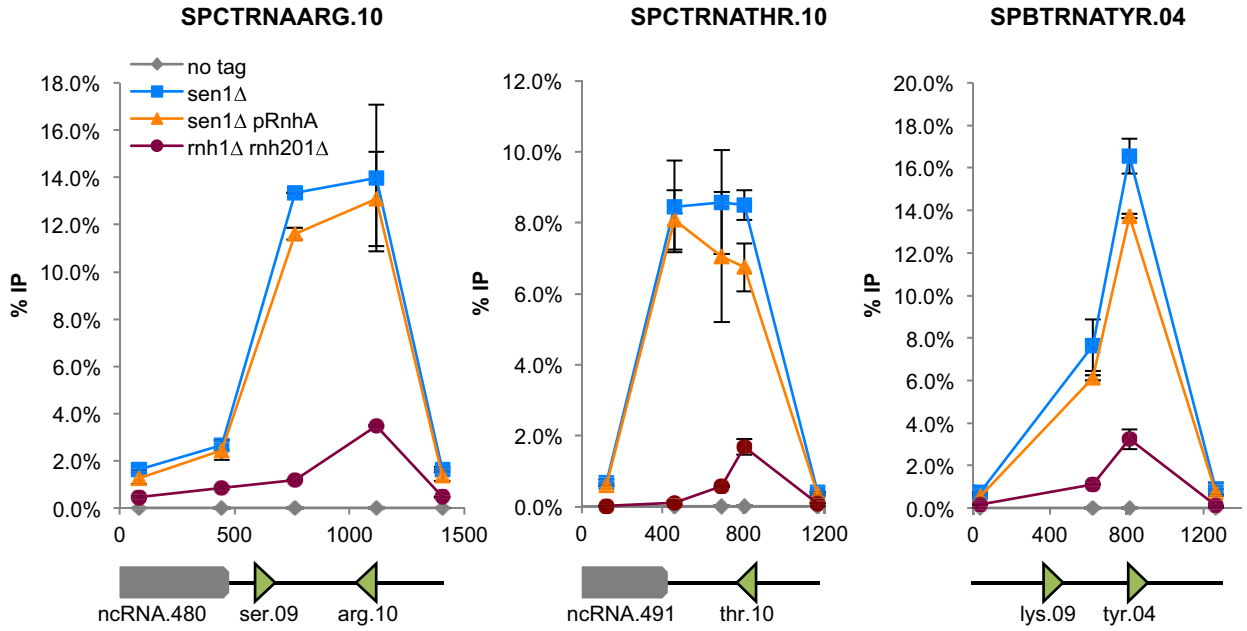


**Figure EV2. Loss of R-loops upon expression of RnhA does not impact the distribution of RNAP3 in the absence of Sen1.**
ChIP-qPCR analysis of the RNAP3 subunit Rpc25 in the indicated genotypes and at the indicated loci in a population of cycling cells (mean ± SD from two biological replicates).

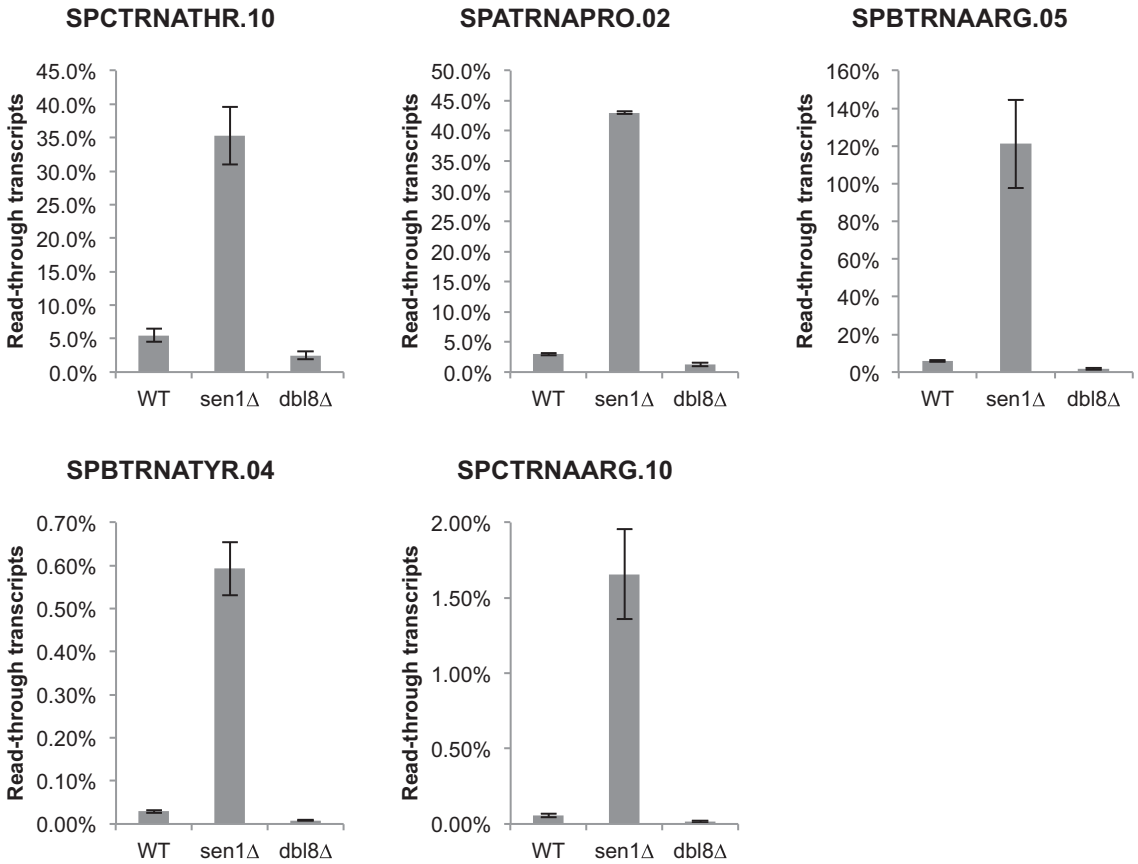


**Figure EV3.**

**Figure EV3. Lack of Sen1 but not lack of its close homologue Dbl8 results in read-through transcripts at tRNA genes.**
Strand-specific RT–qPCR was used to quantify the levels of read-through transcripts (see Materials and Methods). The mean ± SD from two biological replicates is represented here.

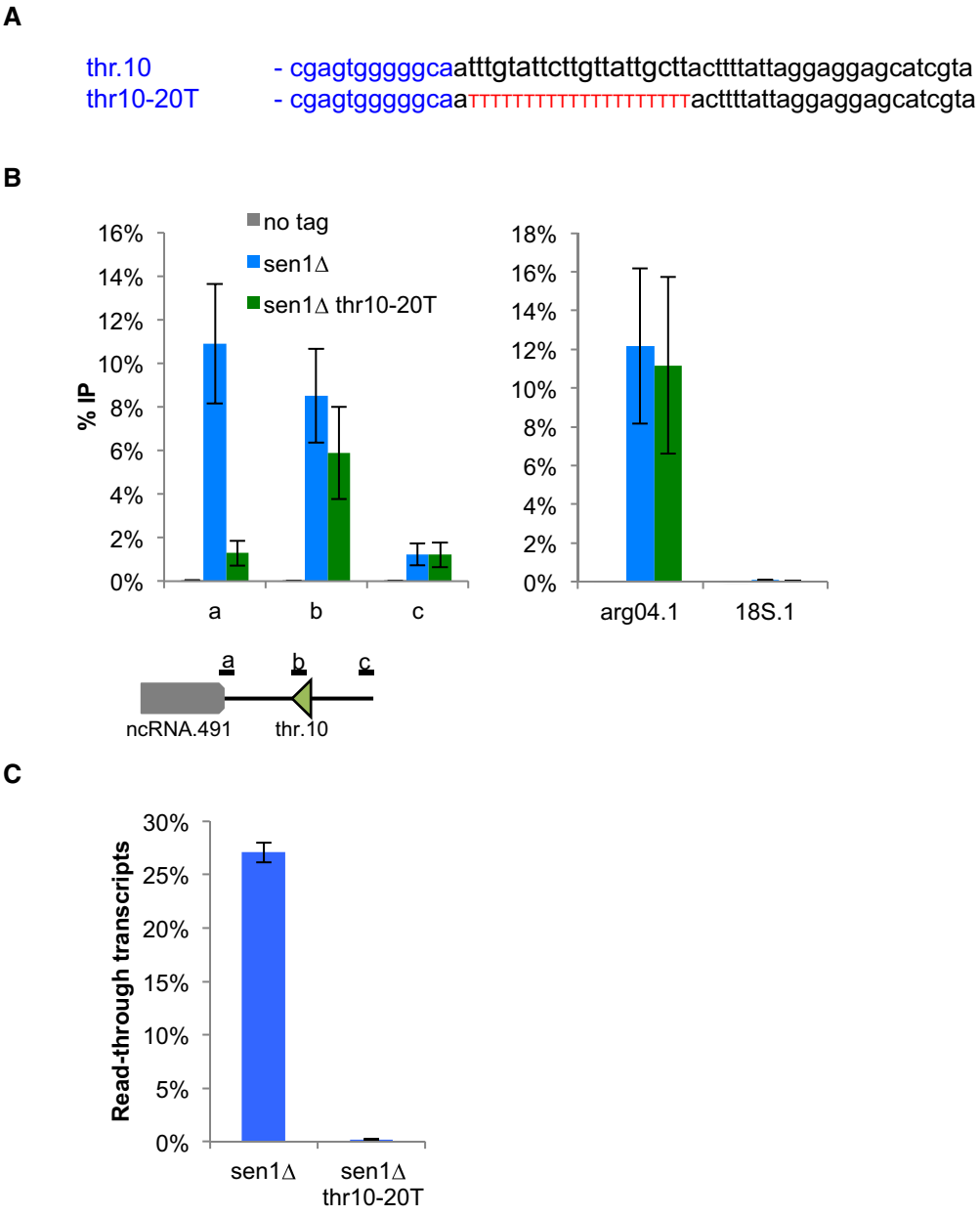


**Figure EV4. A super-terminator at *SPCTRNATHR.10* suppresses the accumulation of RNAP3 in 3′ when Sen1 is missing.**

A Sequences of the engineered strong super-terminator (*thr10-20T*) at the *SPCTRNATHR.10* gene.

B ChIP-qPCR analysis of Rpc37 around *SPCTRNATHR.10* gene in the strong terminator mutant (mean ± SD from four biological replicates). The RNAP3-transcribed *SPBTRNAARG.04* (arg.04) and the RNAP1-transcribed rDNA (18S) were used as specificity controls.

C RT–qPCR demonstrates the loss of read-through transcripts in the strong terminator mutant (mean ± SD from 3 biological replicates).

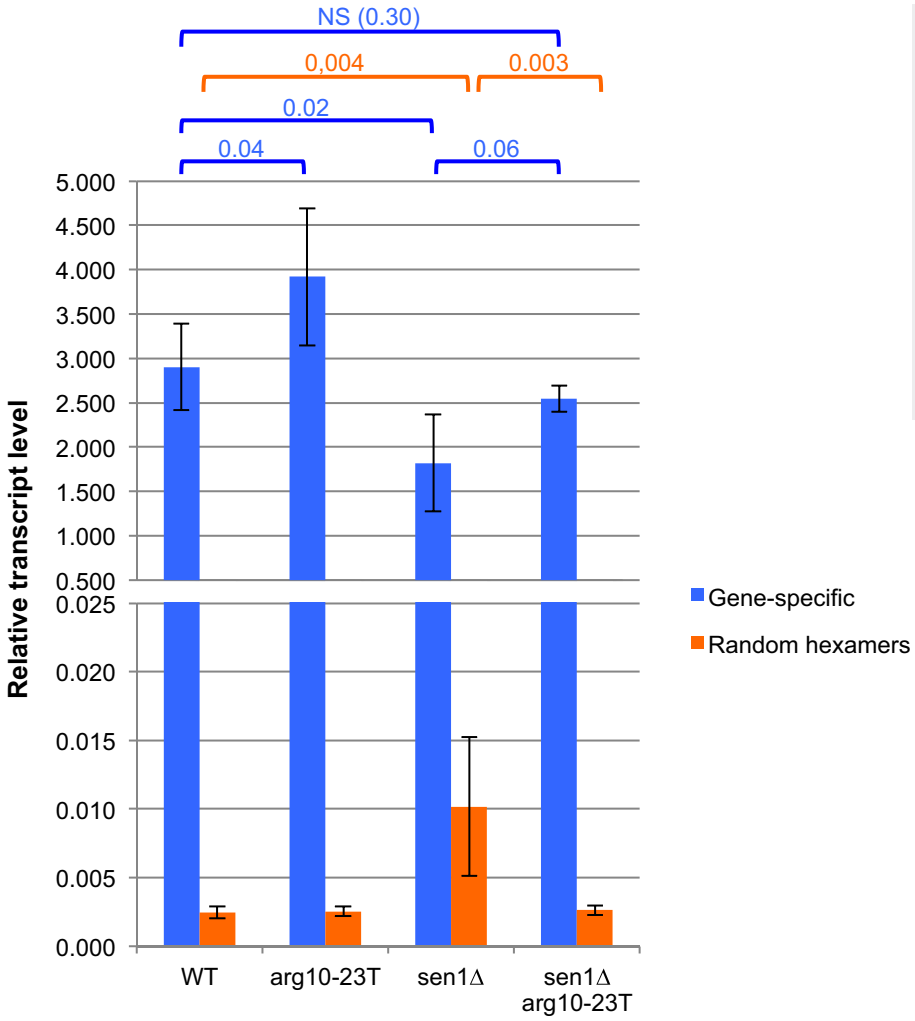


**Figure EV5. RT with random hexamers predominantly retro-transcribed the long read-through tRNA transcripts produced in the absence of Sen1.**
Total RNAs were extracted from the indicated strains, and RT–qPCR was performed using either random hexamers or a primer specific for *SPCTRNAARG.10*. The gene-specific primer gave much stronger signals than random hexamers. Random hexamers retro-transcribed predominantly the long read-through transcripts produced in the absence of Sen1, as shown by the fact that relative RNA levels dropped significantly when read-through transcription was prevented by the presence of a strong terminator (*arg10-23T*) (mean ± SD from 6 biological replicates; *P*-values from a Wilcoxon–Mann–Whitney test).