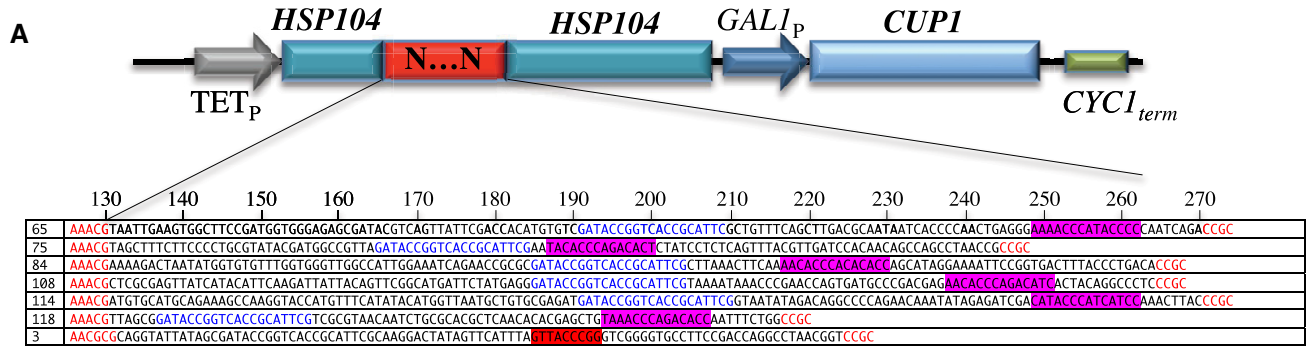
Expanded View Figures

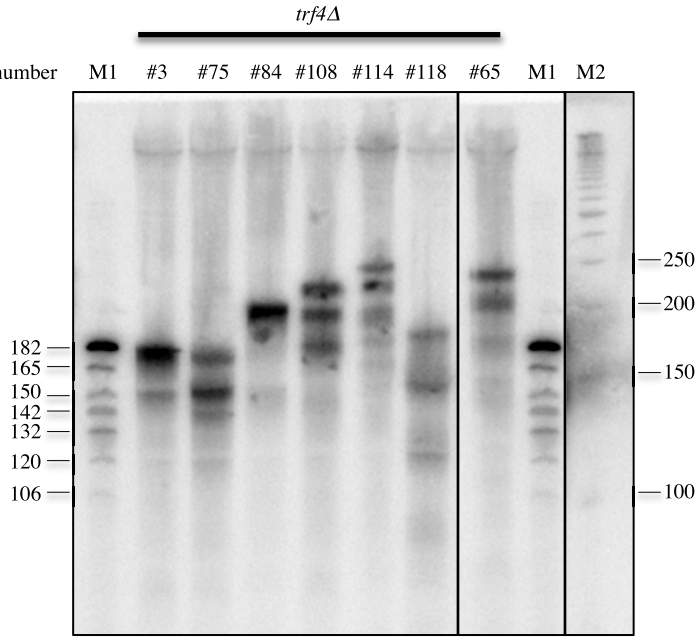
Figure EV1. 3′ end mapping of Rap1-terminated transcripts.

A Top: Schematic drawing of the reporter system used for selecting the RB terminators with the position of the insertion and the sequence of the selected clones containing a Rap1 site (purple). In blue the sequence of a constant linker used for constructing the pool. Flanking HSP104 sequences are indicated in red. Bottom: PAGE-northern blot analysis of RNAs produced by the different constructs after oligonucleotide-directed RNaseH cleavage at −130 nt from the start of the insertion. The position from the RNase H cleavage point is indicated above the sequences. The presence of several shorter RNAs might reveal the occurrence of termination at sites of RNAPII piling up. All analyses were done in an *trf4*Δ strain to detect unstable transcripts. All lanes are derived from the same gel; marker M1 is shown twice for clarity.

B Snapshots showing the RNAPII CRAC and RNAseq signal at intron-containing genes, illustrating the co-transcriptional nature of the CRAC signal.

Source data are available online for this figure.





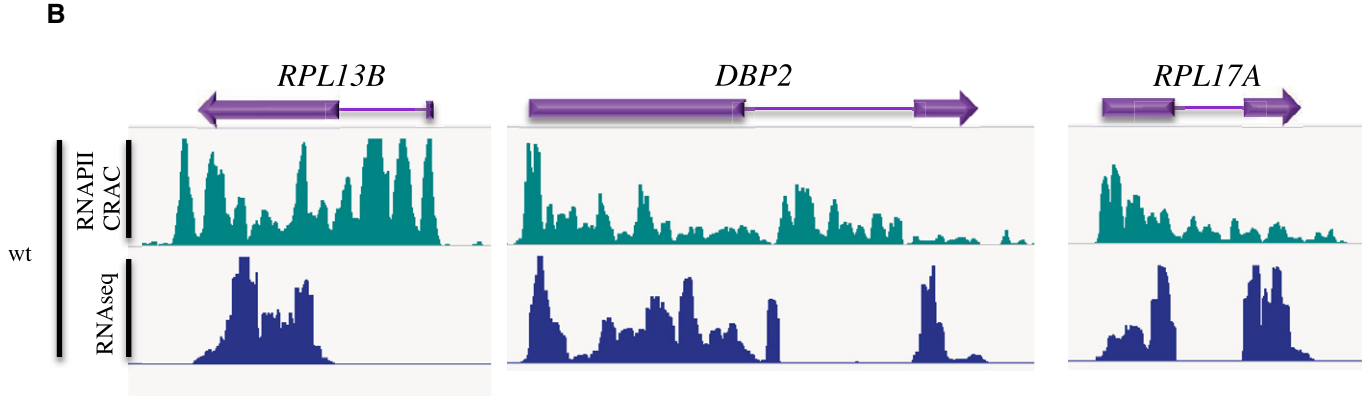


Figure EV1.

Figure EV2. Roadblock termination functions as a fail safe mechanism to limit constitutive readthrough.

A Aggregate plot showing the average RNAPII CRAC profile at sites of Reb1 occupancy located within 300 nt downstream of genes terminated by the CPF pathway as in Fig 3B. The plot demonstrates that in striking contrast to alteration of the CPF pathway, affecting NNS termination by nuclear depletion of Nrd1 has no significant effects on the accumulation of RNAPII at the site of roadblock. Sites used in these analyses are listed in Dataset EV1.

B Snapshots illustrating the presence of significant levels of intergenic RNAPII CRAC signals at three tandem gene loci. The transcription initiation sites (TSS, Malabat *et al*, 2015) detected in the regions shown are indicated on top of the RNAPII CRAC tracks. In all these cases, intergenic initiation cannot be detected, indicating that the intergenic RNAPII signal derives from the constitutive readthrough at the CPF-dependent terminator of the upstream gene. Note that the levels of the readthrough signal in these cases are comparable to the levels of transcription of the downstream gene.

C Aggregate plot showing the average RNAPII CRAC profile at sites of Abf1 occupancy located within 300 nt downstream of genes terminated by the CPF pathway as in Fig 3B. The plots show the average RNAPII occupancy in the wild-type and in *rna15-1* cells grown for 1.5 hours at the non-permissive temperature. Sites used in these analyses are listed in Dataset EV1.

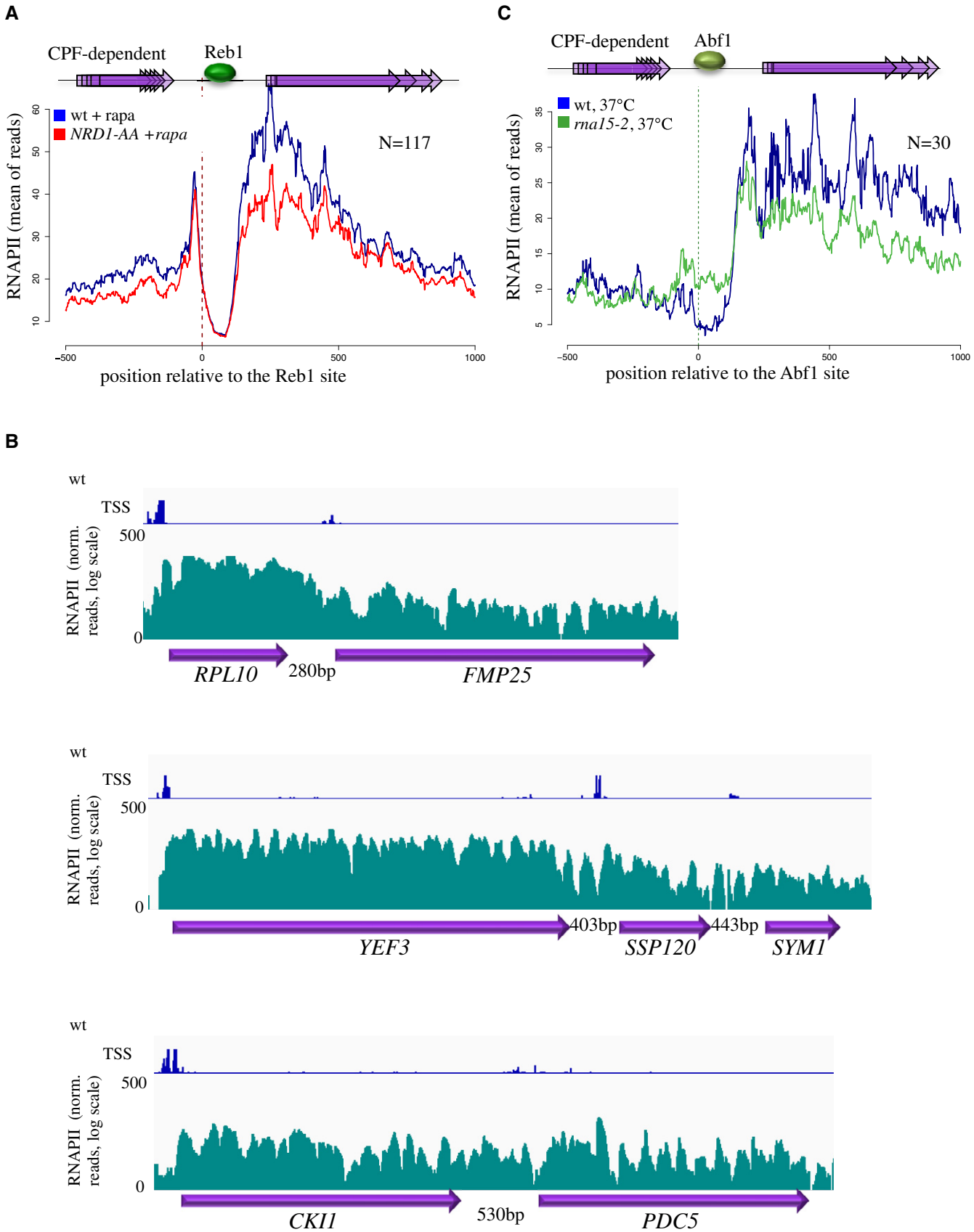


Figure EV2.

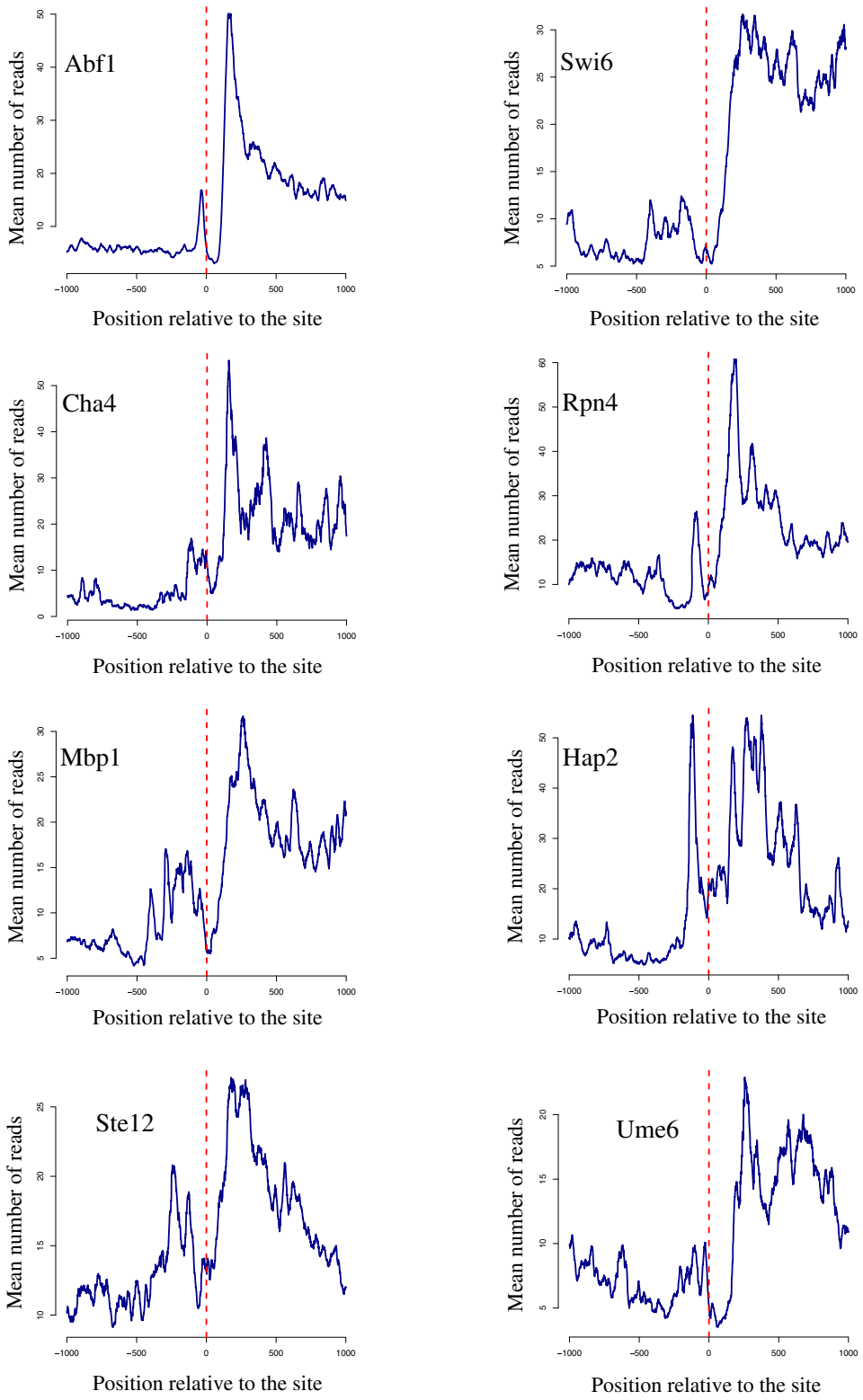


Figure EV3. Metasite analyses illustrating the profile of RNAPII CRAC signal at various transcription factor binding sites.

Aggregate plots showing the profile of RNAPII CRAC signal around sites of binding for the transcription factors indicated. The large peak after each binding site corresponds to downstream events of transcription initiation as for the plots in Fig 3A. The roadblock peak precedes the position of aligned binding sites. Sites used in these analyses are listed in Dataset EV1.

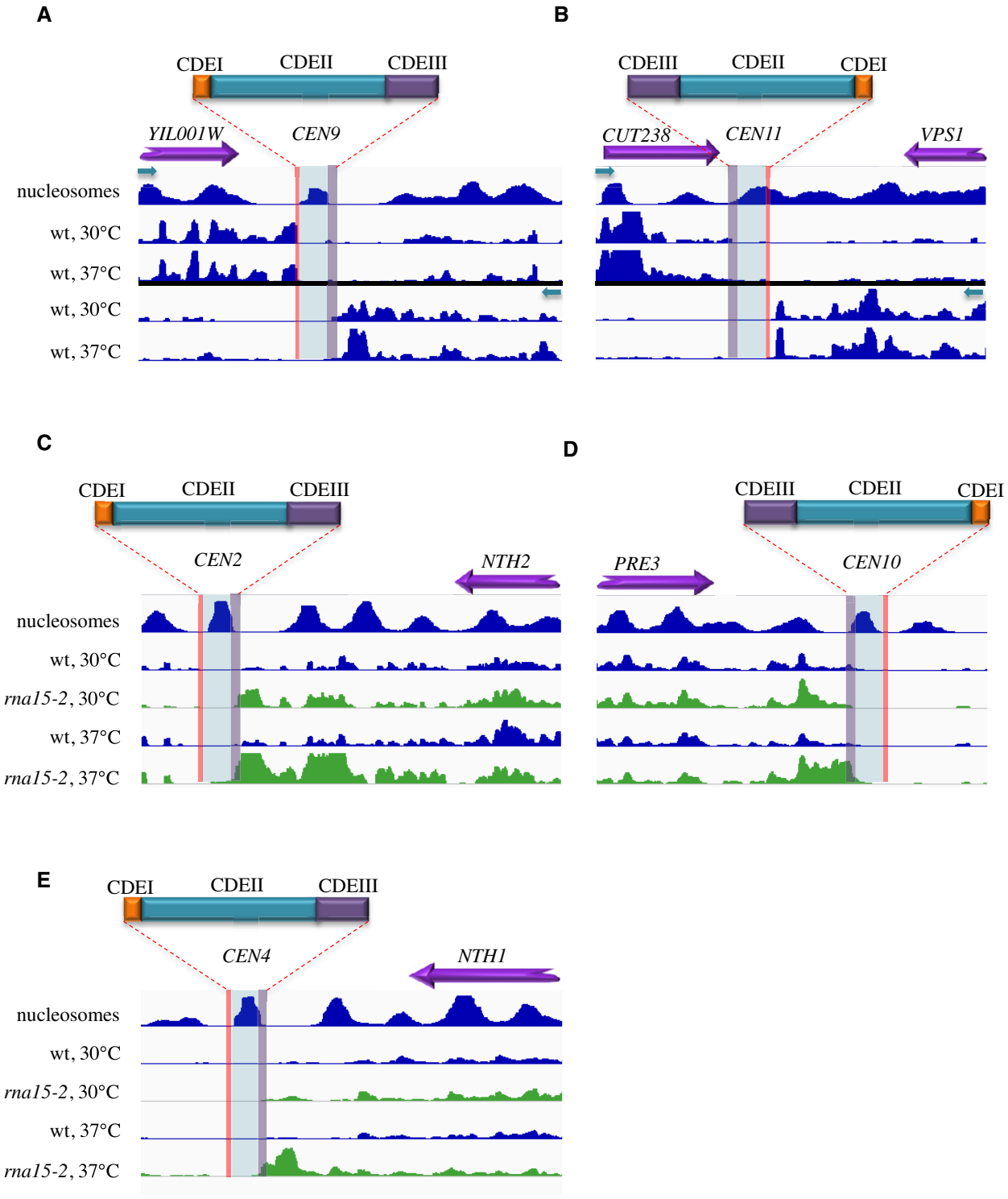


Figure EV4. Snapshots showing the RNAPII CRAC signal around representative centromeres.

A–E The structure of each centromere is schematically shown on top and reported, scaled, on the tracks. In (A, B), both strands are shown and the direction of transcription is indicated by a green arrow. In (C–E), only one strand is shown as no significant levels of transcription could be detected on the other strand. RNAPII CRAC tracks derived from *rna15-1* cells are shown at the permissive and non-permissive temperature to illustrate the effect of increased readthrough transcription at convergent genes, which leads to the accumulation of RNAPII signal at the roadblock.

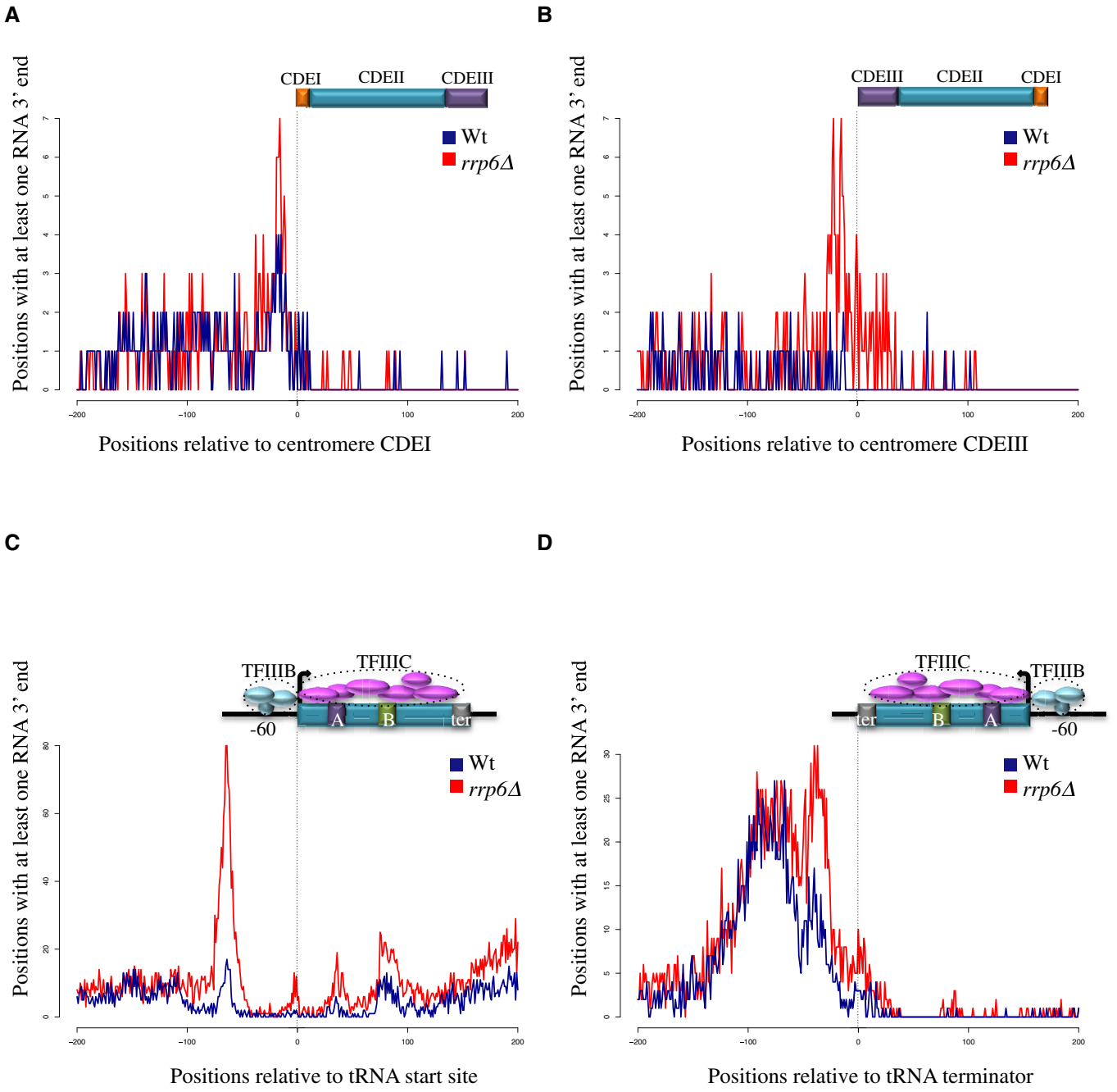


Figure EV5. Metasite analysis of RNA 3′ ends around centromeres and tRNAs.

A–D Aggregate plots showing the distribution of RNA 3′ ends around centromeres and tRNAs in wt and *rrp6*Δ cells to detect unstable RNAs. The orientation of centromeres and tRNA relative to transcription is shown by schematic drawings. Note the presence of 3′ ends peaks indicating the occurrence of transcription termination at sites of RB.