Expanded View Figures

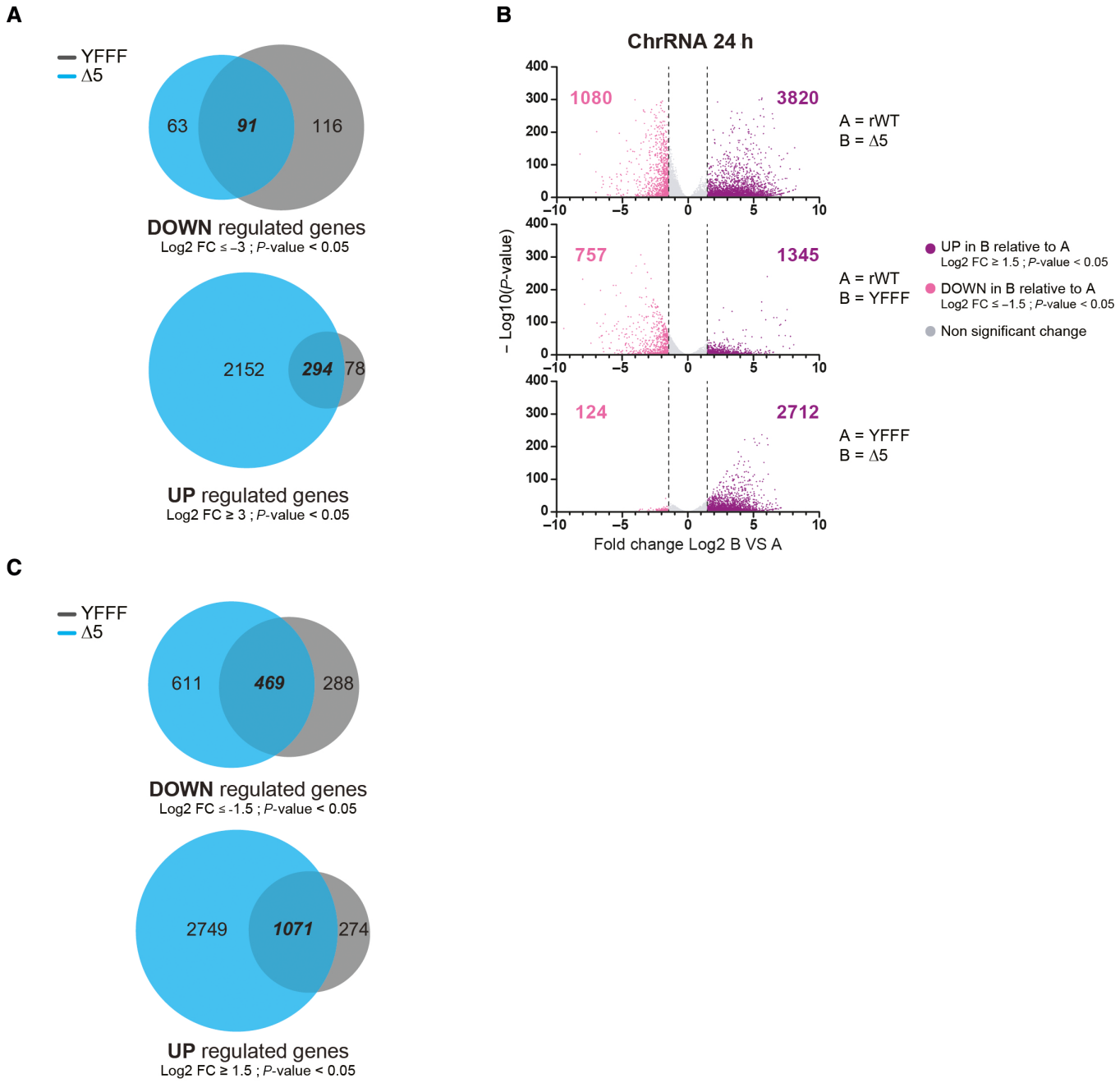

Figure EV1. CTD-Δ5 mutation impacts transcription to a higher extent than YFFF mutation.

A Venn diagram showing common genes UP and DOWN regulated between YFFF and CTD-Δ5 mutants compared with rWT at a threshold of log2 fold change ≥ 3 and *P*-value < 0.05. Experiments were done in biological duplicates.

B Volcano plot of differentially expressed genes in chromatin-associated (ChrRNA) RNA-seq datasets: protein-coding genes UP (purple) and DOWN (pink) regulated in B relative to A after 24-h α-amanitin treatment. Experiments were done in biological duplicates. (Threshold: log2 fold change ≥ 1.5, *P*-value < 0.05).

C Venn diagram showing common genes UP and DOWN regulated between YFFF and CTD-Δ5 mutants compared with rWT at a threshold of log2 fold change ≥ 1.5 and *P*-value < 0.05. Experiments were done in biological duplicates.

Figure EV2. ChrRNA read-through phenotype is more pronounced in CTD-Δ5 compare with YFFF mutant.

A Example of read-through phenotype in ChrRNA-seq datasets YFFF and CTD-Δ5 at the 5′ (antisense) and 3′ (sense) ends of the KBTBD8 gene compared with rWT.

B Average metagene profiles of ChrRNA-seq signals in sense and antisense directions (top and bottom, respectively) over the gene bodies of expressed protein-coding genes and the 20 kb upstream and downstream surrounding regions in rWT (dotted gray line), YFFF (dark gray line) and CTD-Δ5 (blue). All profiles are asinh transformed and normalized over the gene bodies of the rWT for the mutants. Experiments were done in biological duplicates. *P*-values were calculated using two-sided Wilcoxon tests. *P*-values associated with read-through in sense direction: rWT vs. YFFF = 2e-70, rWT vs. CTD-Δ5 = 8e-146, and CTD-Δ5 vs. YFFF = 1e-87. For read-though in antisense direction: rWT vs. YFFF = 9e-121, rWT vs. CTD-Δ5 = 5e-165, and CTD-Δ5 vs. YFFF = 2e-146.

C Average metagene profiles of ChrRNA-seq signals in sense and antisense directions (top and bottom, respectively) over the gene bodies of expressed protein-coding genes and the 20 kb upstream and downstream surrounding regions in rWT (dotted gray line), YFFF (dark gray line) and CTD-Δ5 (blue). Profiles are asinh transformed and non-normalized on gene bodies. Experiments were done in biological duplicates. *P*-values were calculated using two-sided Wilcoxon tests. *P*-values associated with read-through in sense direction: rWT vs. YFFF = 5e-109, rWT vs. CTD-Δ5 = 2e-112, and CTD-Δ5 vs. YFFF = 0.41. For read-though in antisense direction: rWT vs. YFFF = 8e-152, rWT vs. CTD-Δ5 = 4e-154, and CTD-Δ5 vs. YFFF = 4e-17.

D Density plots of ChrRNA-seq signals in rWT, YFFF, and CTD-Δ5 mutant on protein-coding genes (*a*) The antisense 20 kb regions upstream of genes (*b*) The sense gene body and (*c*) The sense 20 kb regions downstream of genes in rWT (light gray), YFFF (dark gray), and CTD-Δ5 (blue). Experiments were done in biological duplicates. *P*-values were calculated using two-sided Wilcoxon tests. RPKM, Reads per kilobase per million mapped reads.

E Boxplots of ChrRNA read-through (RT) indexes based on signal over 20 kb upstream (antisense) and downstream (sense) regions of coding genes in rWT (dotted gray line), YFFF (dark gray), and CTD-Δ5 mutant (blue). Units are asinh transformed. *P*-values were calculated using two-sided Wilcoxon tests. Experiments were done in biological duplicates. Boxplots represent minimal and maximal values, first and third quartiles with median value as central band. *P*-value associated with antisense RT index in rWT vs. YFFF = 2e-90, rWT vs. CTD-Δ5 = 4e-254, and CTD-Δ5 vs. YFFF = 8e-71. Concerning downstream RT indexes: rWT vs. YFFF = 5e-181, rWT vs. CTD-Δ5 < 2e-254, and CTD-Δ5 vs. YFFF = 2e-66.

F Examples of ChrRNA-seq signals showing potential transcription interference of SULF2 and RUNX3 in YFFF and CTD-Δ5 mutants due to RT in sense direction from NCOA3 and CLIC4, respectively.

Source data are available online for this figure.

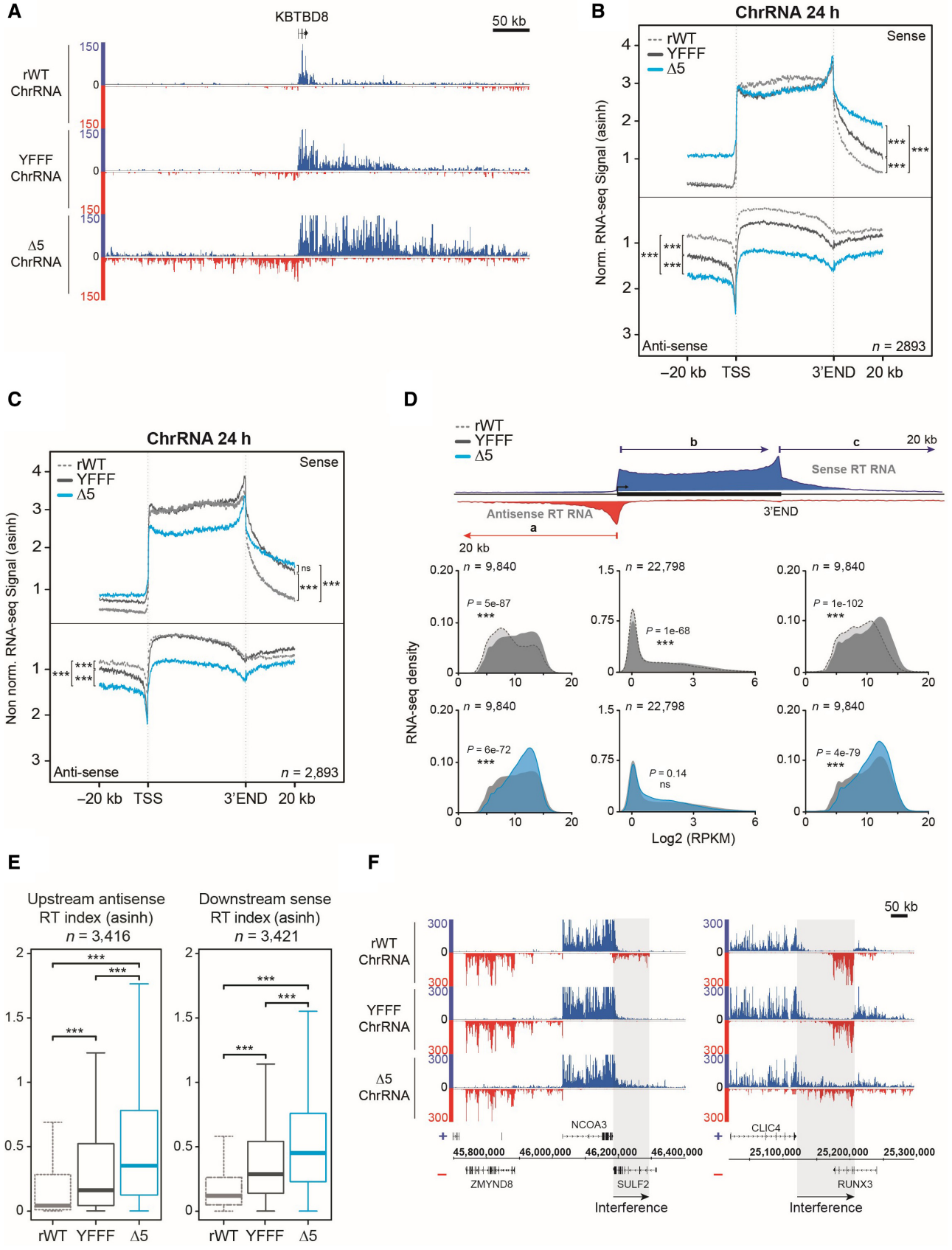

Figure EV2.

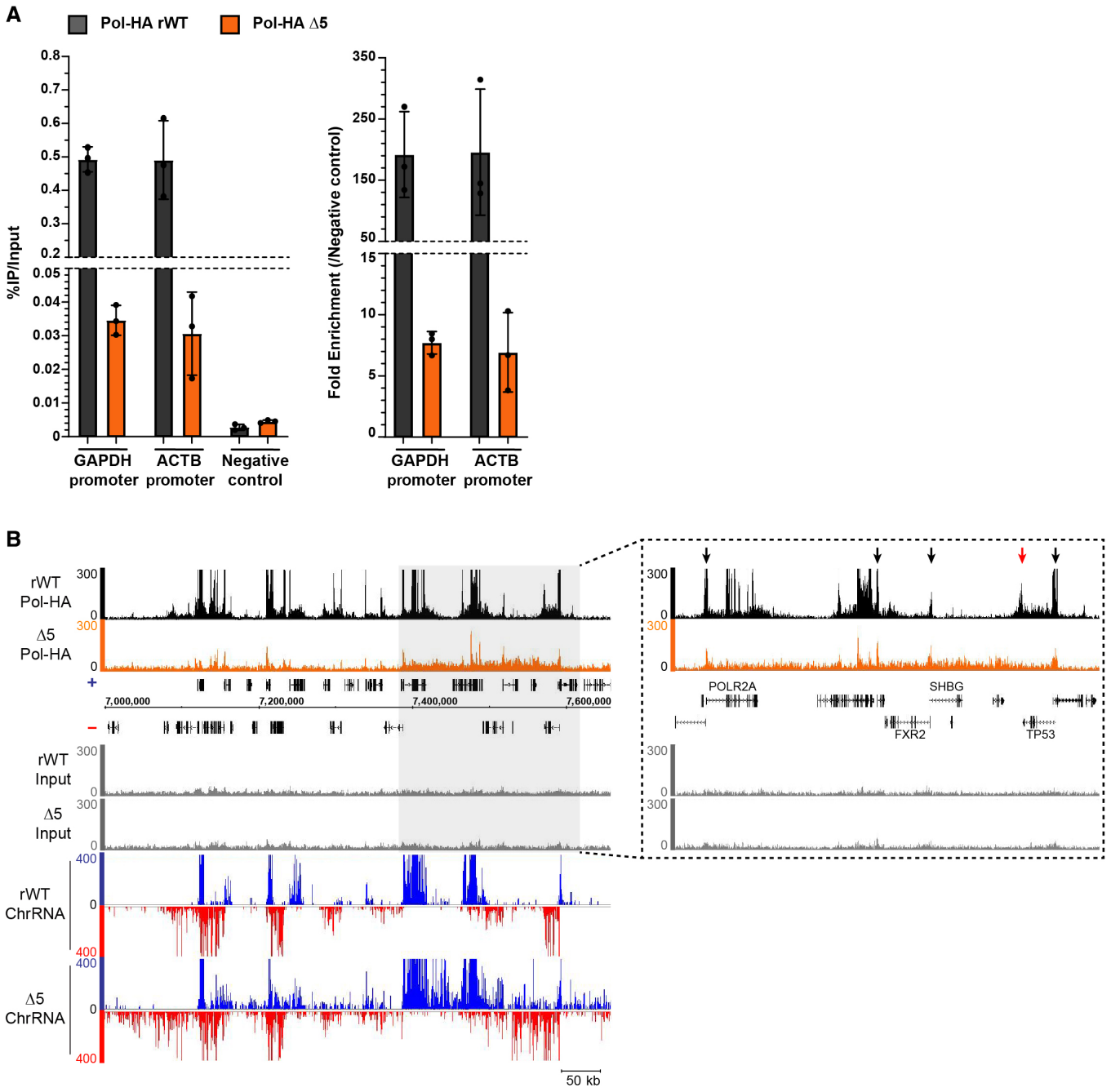

Figure EV3. Pol II accumulation is reduced at promoters in CTD-Δ5 context and lost at 3′ end of coding genes.

A qPCR showing the enrichment of Pol-HA ChIP on positive regions compared with negative control in rWT (black) and CTD-Δ5 (orange). Left: Percentage of IP over Input for each target. Right: Fold enrichment normalized on negative control. Error bars represent the mean with SD of 3 technical replicates.

B Example of Pol-HA ChIP-seq signal in rWT (black) and CTD-Δ5 (orange) as well as their associated Input profiles and ChrRNA signals. Light gray rectangle highlights a region in which there is a global accumulation of Pol-HA in CTD-Δ5 associated with read-through at ChrRNA level. Right: Extended view of the region highlighted in light gray. Black arrows show promoter-associated Pol-HA accumulation in both rWT and CTD-Δ5. Red arrow shows 3′-end Pol-HA accumulation in rWT, which is lost in CTD-Δ5 mutant. Experiments were done in biological duplicate, however, only the sample with the best signal-to-noise ratio was used for subsequent analysis.

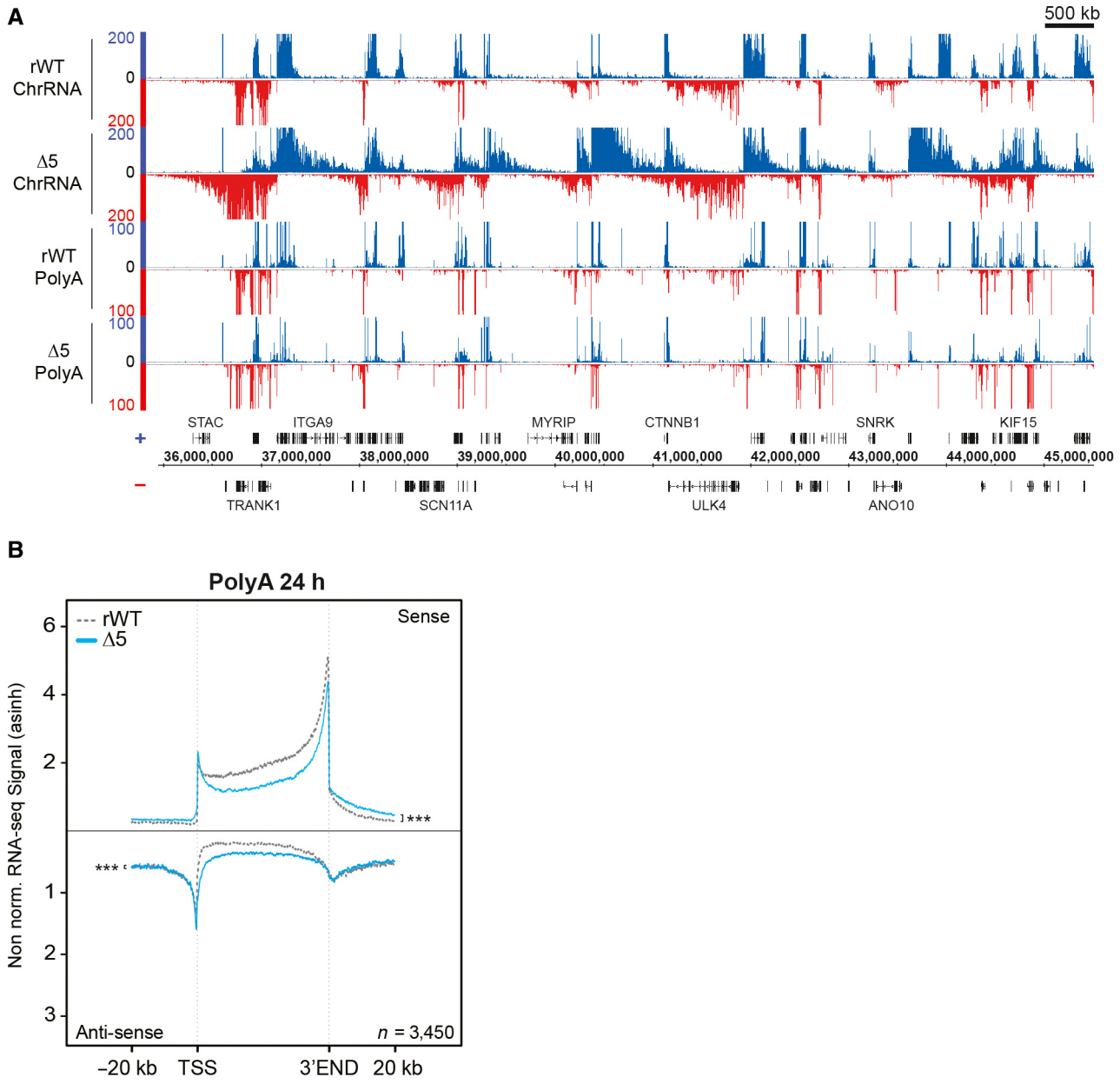

Figure EV4. Polyadenylated transcripts are less affected by CTD-Δ5 mutation.

A Wide view example of ChrRNA-seq and its associated polyA signal in rWT and CTD-Δ5.

B Average metagene profiles of polyA RNA-seq signals in sense and antisense directions (top and bottom, respectively) over the gene bodies of expressed protein-coding genes and the 20 kb upstream and downstream surrounding regions in rWT (dotted gray line) and CTD-Δ5 (blue). All profiles are asinh transformed and non-normalized over gene bodies. Experiments were done in biological duplicates. *P*-values associated with read-through were calculated using two-sided Wilcoxon tests: 4e-53 in sense and 9e-4 in antisense.

Figure EV5. CTD-Δ5 mutation leads to transcriptional misregulation of snRNA and histone genes associated with defects in polymerase II recruitment.

A Average metagene of sense ChrRNA signals at the top 50% of expressed U1, U2, U4, and U5 snRNA genes in rWT (dotted gray line) and CTD-Δ5 mutant (blue line) Gray rectangles indicate the corresponding gene size. Experiments were done in biological duplicates. *P*-values were calculated using two-sided Wilcoxon tests.

B Example of ChrRNA signal at U1 and U5 loci in showing the global downregulation of snrRNA in CTD-Δ5 mutant.

C Average metagene profiles of sense ChrRNA signal at the top 50% of expressed nonpolyadenylated histone genes in rWT (dotted gray line) and CTD-Δ5 (blue line). Experiments were done in biological duplicates. *P*-values were calculated using a two-sided Wilcoxon test.

D Example of ChrRNA signal at histone gene cluster on chromosome 6 illustrating the global upregulation in CTD-Δ5 comparison with rWT.

E Average metagene profiles of Pol II on top 50% of enriched snRNA in rWT (black) and CTD-Δ5 (orange). Experiments were done in biological duplicates. Boxplots represent minimal and maximal values, first and third quartiles with median value as central band. *P*-values were calculated using two-sided Wilcoxon tests.

F Average metagene profiles of Pol II on top 50% of most enriched histone genes in rWT (black) and CTD-Δ5 (orange). Experiments were done in biological duplicates. Boxplots represent minimal and maximal values, first and third quartiles with median value as central band. *P*-values were calculated using a two-sided Wilcoxon test.

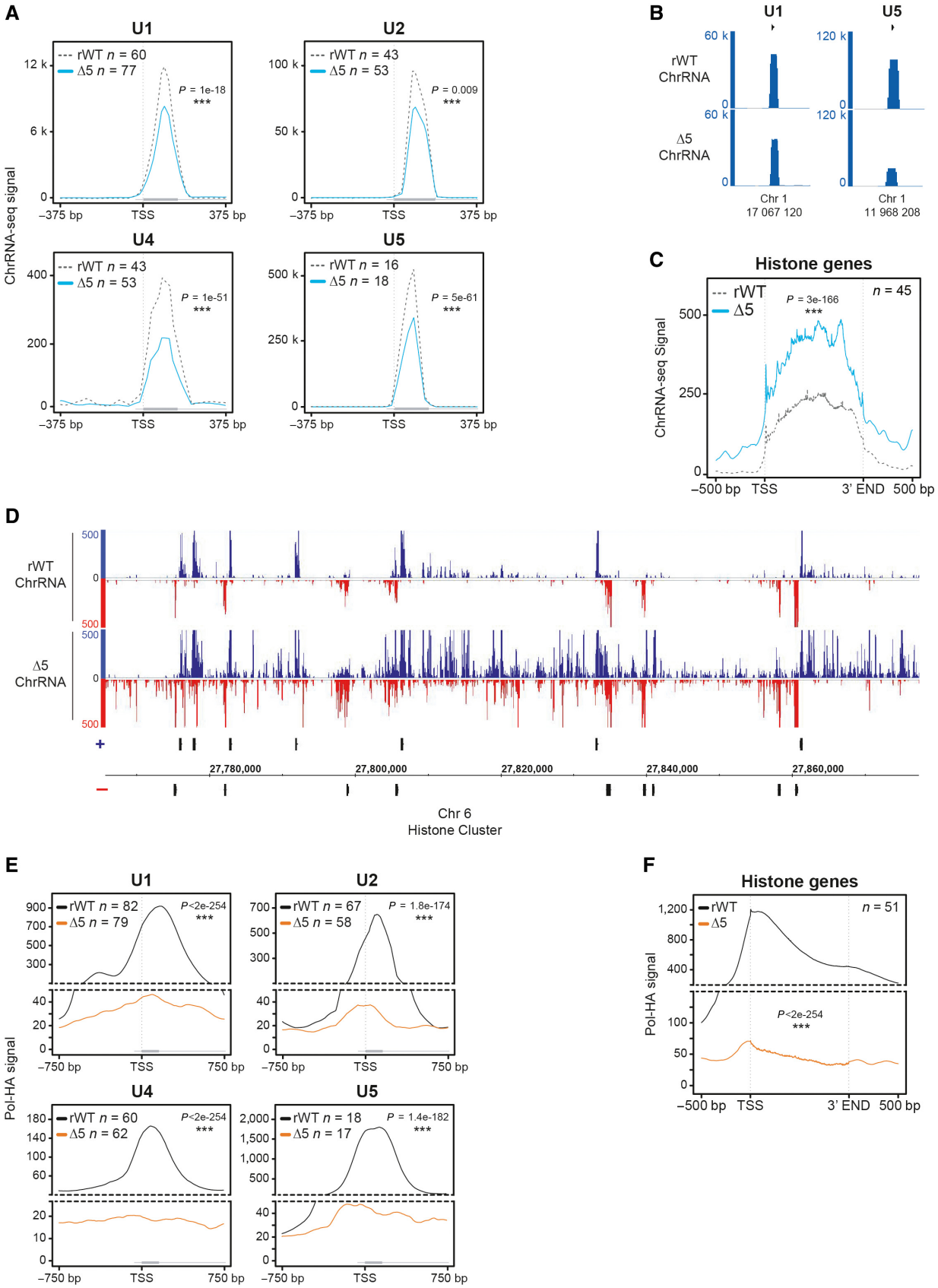

Figure EV5.