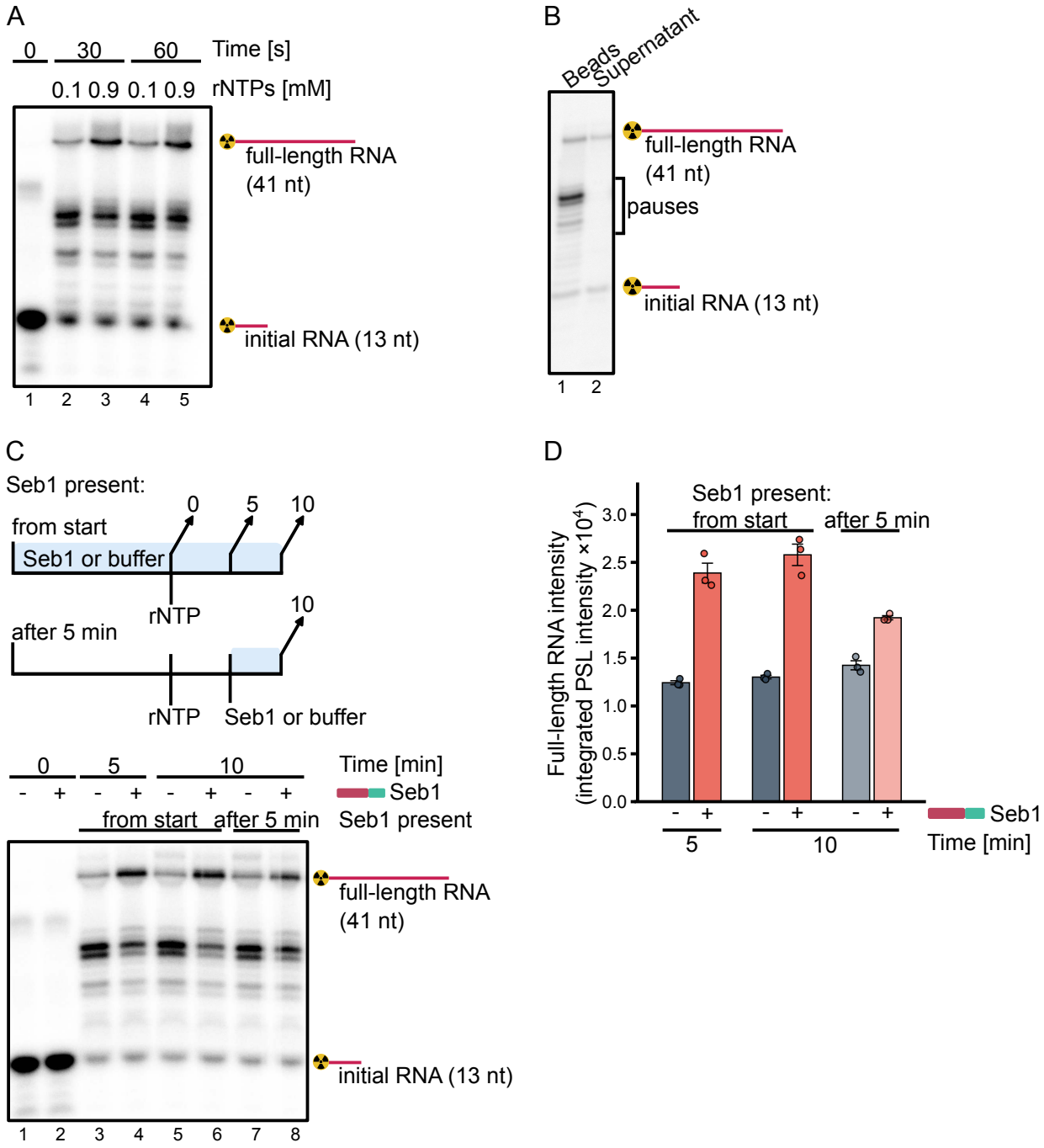
Supplemental Table 1. Nucleic acid templates used in this study

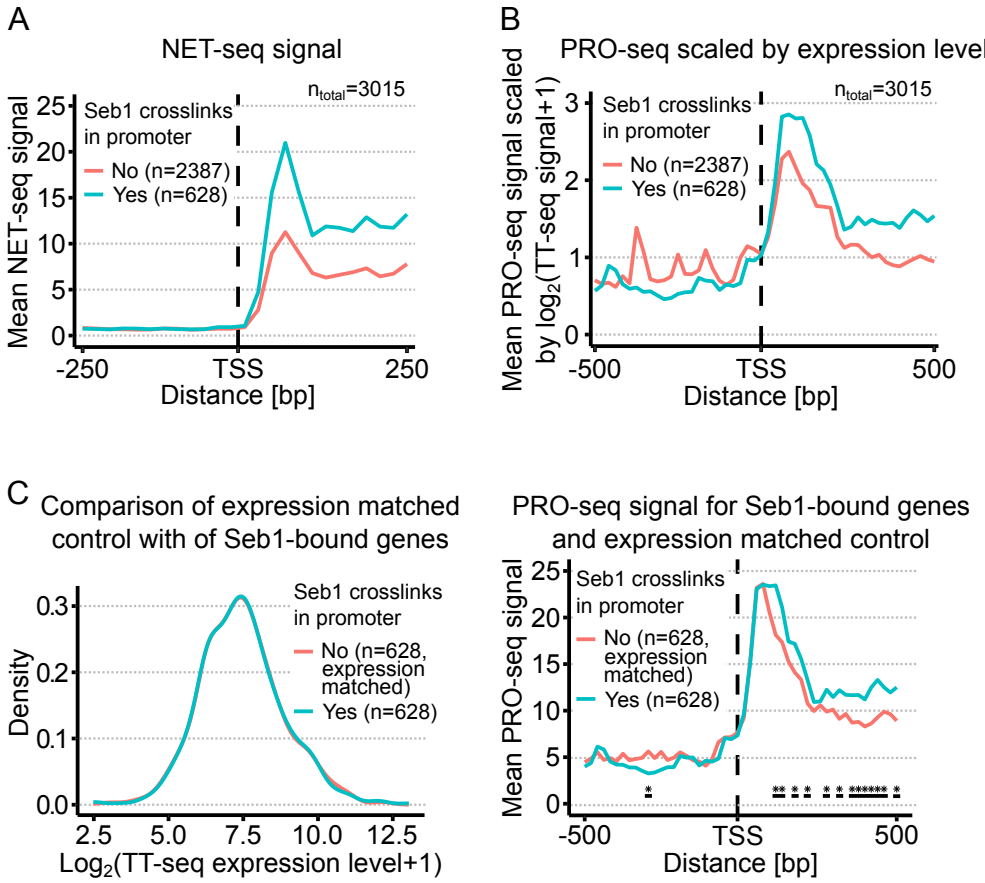
Name	Figure/Internal name	Sequence
In vitro **transcription allowing re-initiation (red highlights oligo ligated.)**		
T1_NT	Figure 1D	5'-GATGTTGTAATAAAGAAGTGGGATTTGGAGGATccctactagcagcgtccctcgcgttgttctggcgttcatatttcacggatagttacgattgtttccaaagtcggtctgcccgagaagctgcaggtcgccg-3'
T1_T	Figure 1D	3'-ATTAAAAAAAAACTACAACATTATTTCTTCACCCTAAACCTCCTAgggatgatcgtcgcagggagcgcaacaagaccgcaagtataaagtgcctatcaatgctaacaaaggtttcagccagacgggctcttcgacgtccagcggc-5'
T2_NT	Figure 1E	5'-GATGTTGTAAGATccctactagcagcgtccctcgcgttgttctggcgttcatatttcacggatagttacgattgtttccaaagtcggtctgcccgagaagctgcaggtcgccgaagggcgaattccagcacactggcggccgttactagtggatccgagctcggtaccaagcttgatgcatagcttgagtattctatagtgtcacctaaatagcttggcgtaatcatggtcatagctgtttcctgtgtgaaattgttatccgctc-3'
T2_T	Figure 1E	3'-ATTAAAAAAAAACTACAACATTCTAgggatgatcgtcgcagggagcgcaacaagaccgcaagtataaagtgcctatcaatgctaacaaaggtttcagccagacgggctcttcgacgtccagcggcttccgcttaaggtcgtgtgaccgccggcaatgatcacctaggctcgagccatggttcgaactacgtatcgaactcataagatatcacagtggatttatcgaaccgcattagtaccagtatcgacaaaggacacactttaacaataggcgag-5'
Single round ***in vitro*** **transcription**		
T-DNA	Figure 2B, C, D, F, Supplemental Figure 1A, B, C/HIV_T	5'-GCTTTATTGAGGCTTAAGCAGTGGGTTCCCTCTCGATGGCTGTAAGT-3'
NT-DNA	Figure 2B, C, D, F, Supplemental Figure 1A, B, C/HIV_NT	5'-ACTTACAGCCATCGAGAGGGAACCCACTGCTTAAGCCTCAATAAAGC-3'
RNA13	Figure 2B, C, D, F, Supplemental Figure 1A, B, C/HIV_R	5'-AAUAAUCGAGAGG-3'

Supplemental Figure 1



Supplemental Figure 1. Seb1 counteracts and resolves pausing *in vitro*. (A) Comparison of rNTP concentration effects on transcription output *in vitro*. Transcription was initiated with 100 or 900 µM rNTPs and 10 mM Mg^{2+}. (B) RNA of Pol II elongation complexes revealed by separation of the reaction into bead and supernatant fractions. (C) Seb1 promotes more efficient transcription, possibly by inhibiting and resolving Pol II pausing. The top panel shows schematics of the experimental setup. Transcription complexes were incubated with or without 1.3 µM Seb1 from the start or after 5 min of transcription initiation. Transcription was initiated with 100 µM rNTPs and 10 mM Mg^{2+}. (D) Quantification of full-length RNA for the results presented in panel (C). PSL intensity - photostimulated luminescence intensity.

Supplemental Figure 2



Supplemental Figure 2. Seb1 promoter-bound genes correlate with altered pausing. (A) NET-seq signal for genes stratified according to the presence of Seb1 crosslinks in the promoter. (B) Expression levels cannot fully explain the observed differences in pausing between genes with Seb1 crosslinking. Data shown in Figure 3C (right panel) were scaled for each gene by expression levels obtained from nascent transcription profiling (TT-seq) (Kuś et al. 2025). (C) Sampling of genes without Seb1 crosslinking in promoter regions to match expression levels of the Seb1-bound set - one sample is shown (left panel). Right panel depicts the mean PRO-seq signal from 100 samples of genes without Seb1 crosslinks in promoter regions, where these genes were selected to match the expression levels of Seb1-bound genes. Statistical analysis was performed using z-score statistics, and p-values were corrected for multiple comparisons using the Benjamini-Hochberg method. Regions showing significant differences are marked with stars. Related to Figure 3C and Supplemental Figure 2B.