Molecular Cell, Volume *81*

Supplemental information

Two distinct mechanisms of RNA polymerase II elongation stimulation *in vivo*

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Figure S1

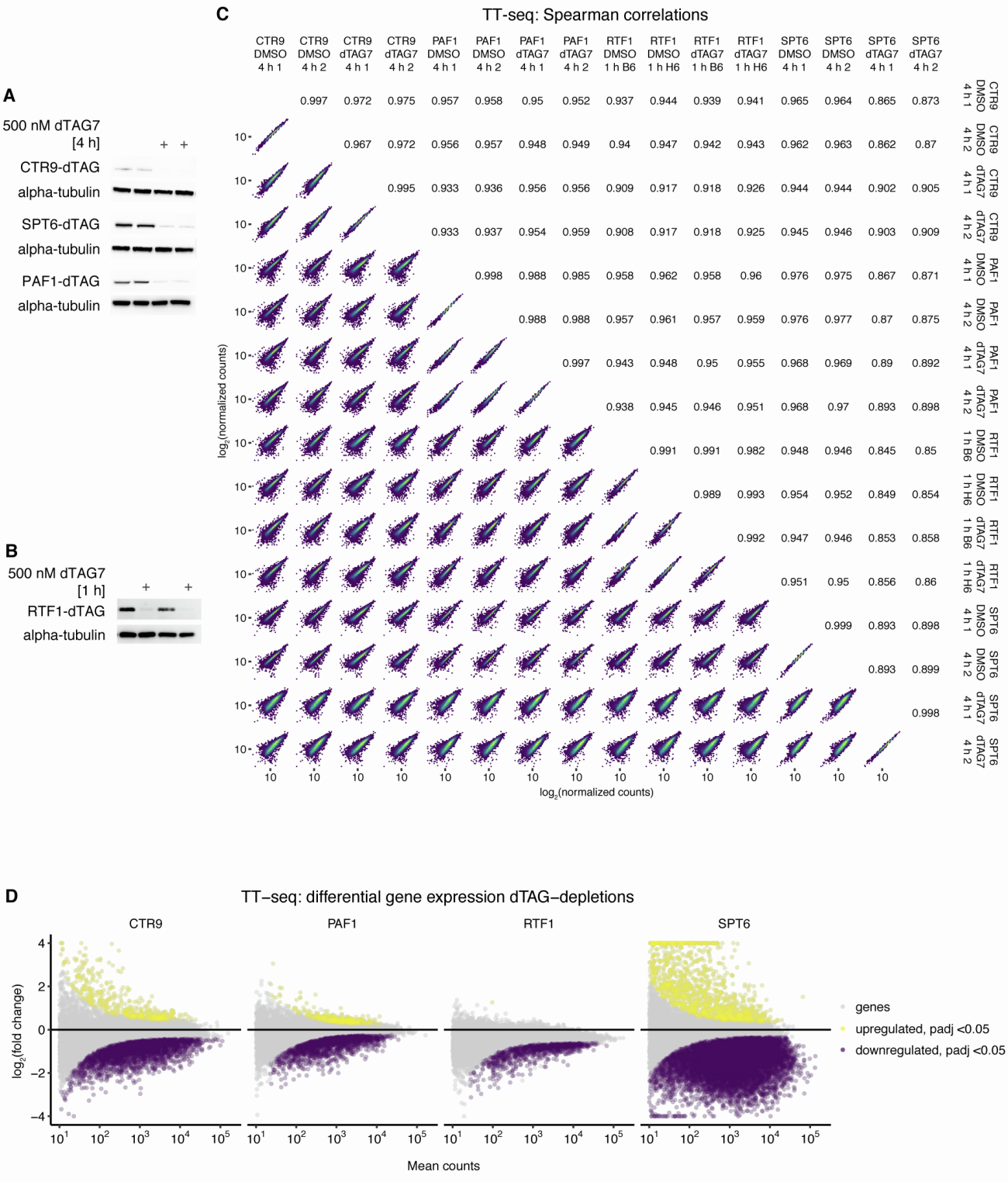

Figure S1. CTR9, PAF1, RTF1 and SPT6 are required for normal RNA synthesis in human cells - relates to Figure 1

A) Western blots of whole cell lysates from dTAG cell lines (CTR9, PAF1 and SPT6) showing the depletion of dTAG-ed factors (anti-HA) and loading control (anti-alpha-tubulin) after 4-h dTAG7 treatment times.

B) As in A) showing RTF1 depletion after 1-h dTAG7 treatment time.

C) Scatter plots of normalized TT-seq gene counts and Spearman correlation coefficients (ρ) of all sample pairs (n = 10378).

D) MAplots showing significantly differentially expressed genes (n = 10378) detected by TT-seq upon near complete depletions of CTR9, PAF1, RTF1 or SPT6. p-adj <0.05.

Figure S2

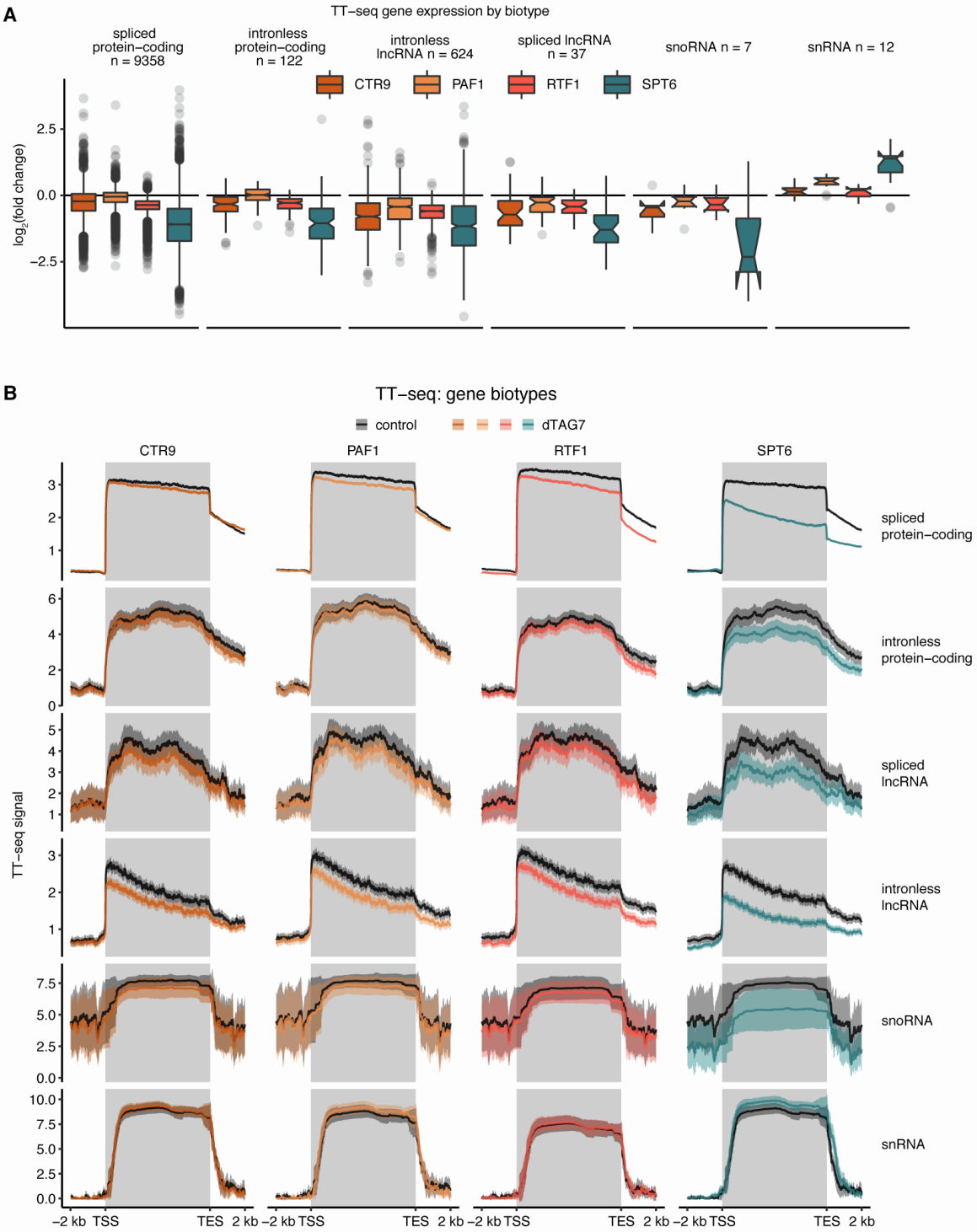

Figure S2. Gene biotypes show similar responses to PAF or SPT6 depletion - relates to Figure 1
A) Box plots showing changes in expression of main gene biotypes upon depletions of the individual factors. The thickened line represents the median fold change, the lower and upper hinges correspond to the first and third quartiles, respectively, and the notches extend to 1.58 times the inter quartile range divided by the square root of N. The whiskers represent the largest/smallest value within the 1.5 times inter-quartile range from the hinge, outliers are show in light gray and the number of genes in each group is indicated.
B) Scaled metagene profiles of TT-seq signal showing mean signal of main gene biotypes after individual depletions of CTR9, PAF1, RTF1 and SPT6. The corresponding shaded area represents the 95 % confidence interval of the mean. The gene region is shaded gray. The metagene window was expanded 2kb upstream and downstream. The TT-seq signal within the gene was scaled by binning. The y-axis represents the log2-transformed normalized TT-seq signal. The control samples are shown in black and the dTAG7-treated samples are shown in the respective color and only sense profiles are shown. Numbers of genes in each subset are as in A).

Figure S3

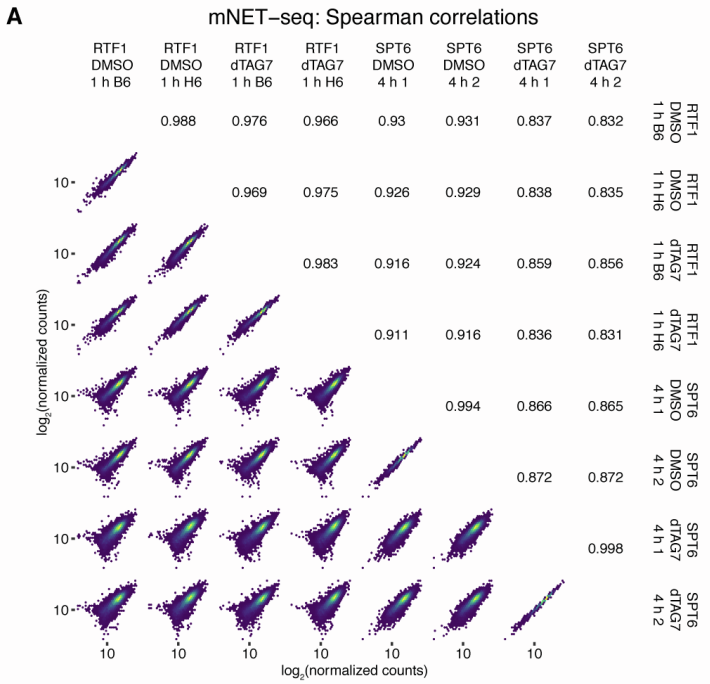

Figure S3. Analysis of Pol II occupancy with mNET-seq - relates to Figure 3
A) Scatter plots of normalized mNET-seq gene counts and Spearman correlation coefficient (ρ) of each sample pair after depletions of RTF1 or SPT6 (n = 10378).

Figure S4

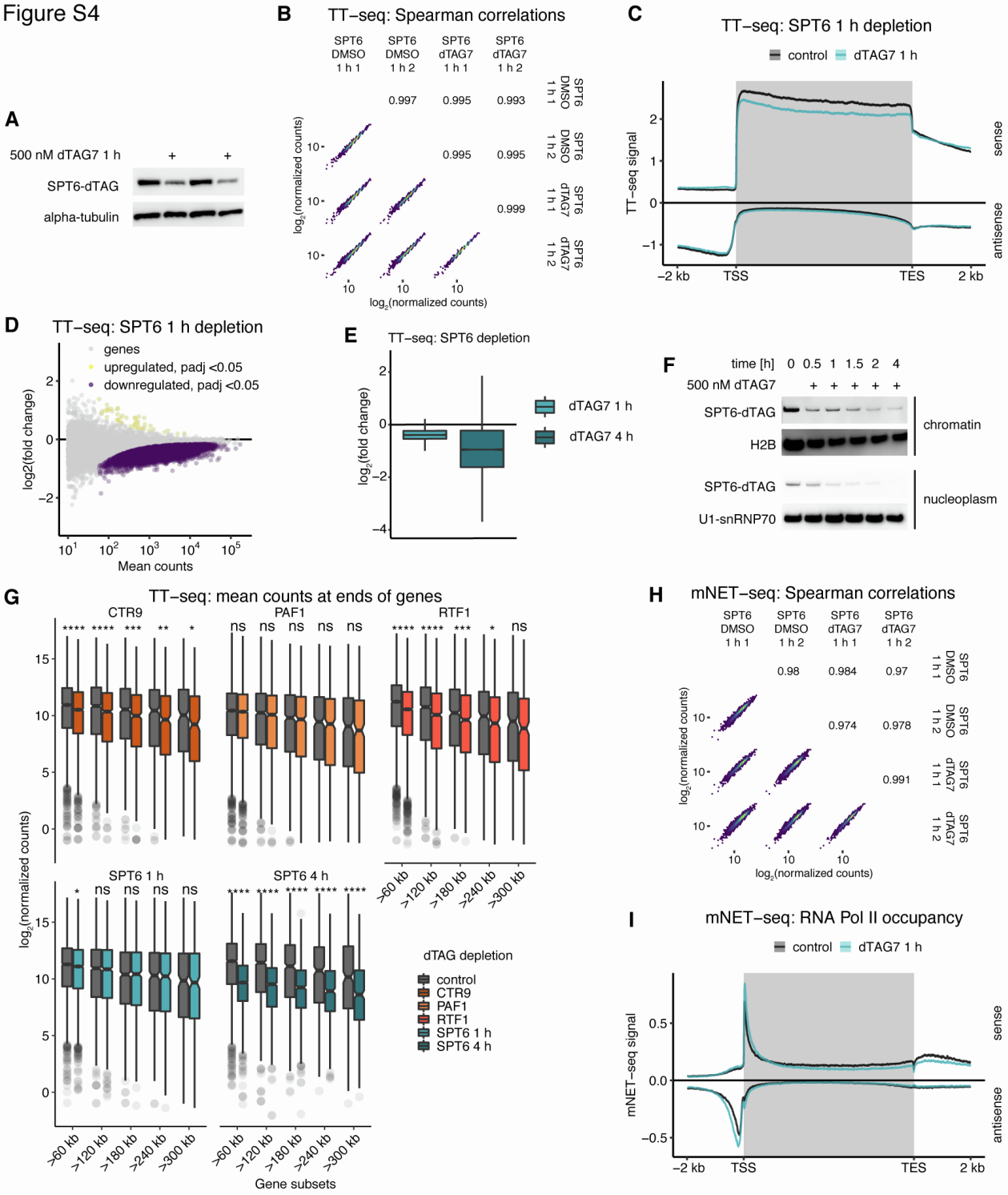

Figure S4. Evidence that SPT6 remains bound to elongating RNA Pol II - relates to Figure 4

A) Western blots of whole cell lysates from dTAG-SPT6 cell line showing partial depletion of SPT6 (1 h dTAG7 treatment, anti-HA) and loading control (anti-alpha-tubulin).

B) Scatter plots for normalized mNET-seq gene counts and Spearman correlation coefficient (ρ) of each sample pair after partial depletion of SPT6 (1 h dTAG7 treatment).

C) Scaled metagene profiles of TT-seq signal showing mean signal of all expressed genes (n = 10378) after partial depletion of SPT6 (1 h dTAG7 treatment). Representations as in Figure S2B, except that antisense signal is shown as negative values.

D) MAplot showing significantly differentially expressed genes detected by TT-seq upon partial depletion of SPT6 (1 h dTAG7 treatment). p-adj <0.05, n = 10378.

E) Box plots showing expression fold change upon partial and near complete SPT6 depletion (1 h and 4 h, respectively) of all expressed genes (n = 10378). Representations as in Figure S2A.

F) SPT6 depletion time course Western blots of nucleoplasmic and chromatin fractions. SPT6 levels were detected with anti-HA antibody and compared to loading control (anti-H2B and anti-U1-snRNP70, for chromatin and nucleoplasm fractions, respectively).

G) Box plots showing normalized counts on the ends of genes of gene subsets from Figure 4C upon dTAG-depletion. Representations as in Supplementary Figure S2A, except that outliers are shown in light gray. ns: $p > 0.05$, *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$, ****: $p \leq 0.0001$.

H) Scatter plots of normalized mNET-seq gene counts and Spearman correlation coefficient (ρ) of each pair upon partial depletion of SPT6.

I) Scaled metagene profiles of mNET-seq showing stranded occupancy for RNA Pol II of all expressed genes (n = 10378) after partial depletion of SPT6 (1 h dTAG7 treatment). Representations as in Figure S2A, except that antisense signal is shown as negative values.

Figure S5

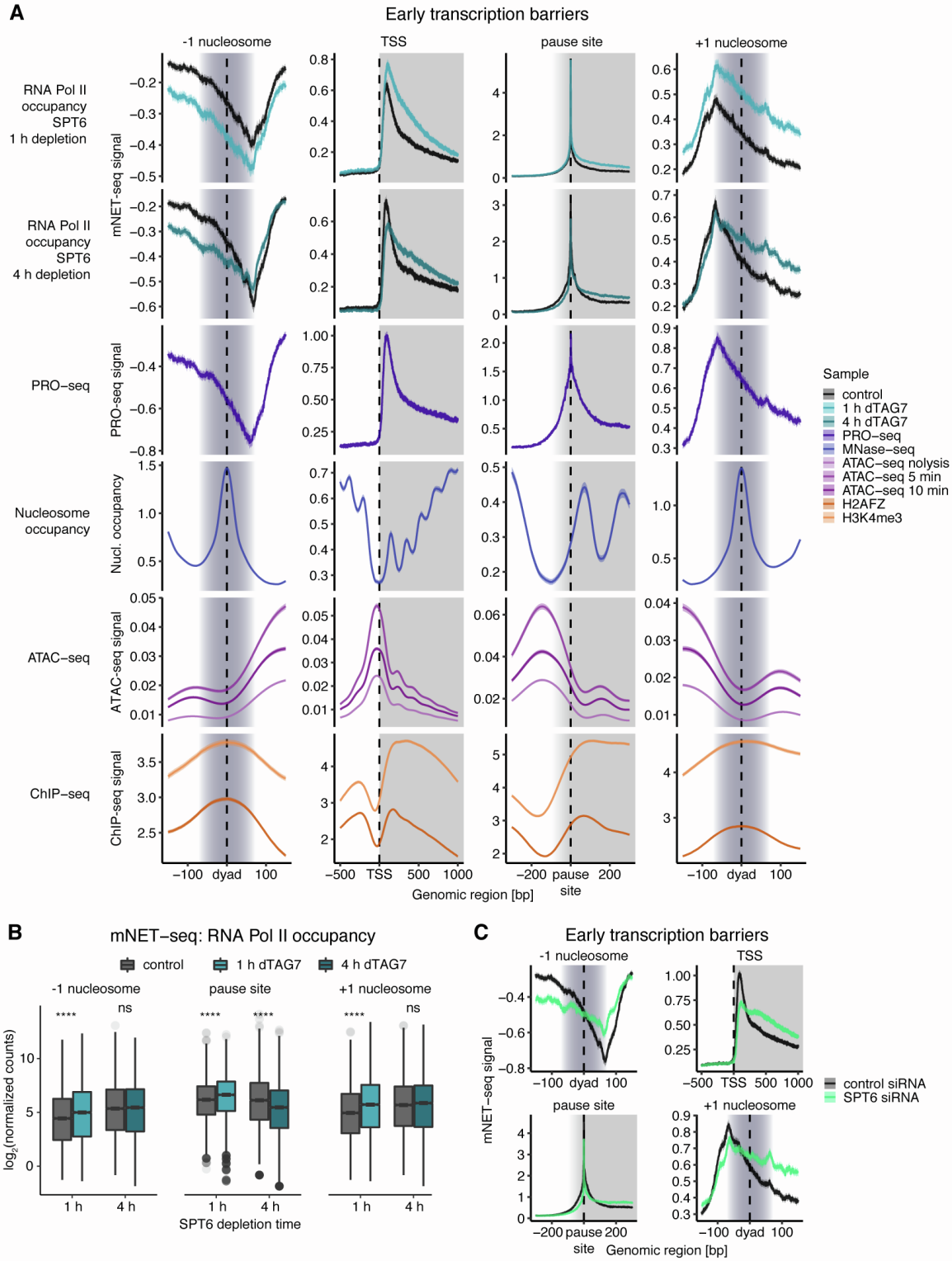

Figure S5. SPT6 depletion leads to Pol II accumulation in the +1 and -1 nucleosome regions - relates to Figure 5

A) Metagene profiles aligned at the -1 nucleosome dyad, TSS, pause site and +1 nucleosome dyad showing mean occupancy of RNA Pol II (mNET-seq) upon partial or near complete SPT6 depletion (1 h and 4 h dTAG7 treatment, respectively) and mean nucleosome occupancy (MNase-seq, ENCODE, (Kundaje et al., 2012)), PRO-seq signal (Core et al., 2014), ATAC-seq signal (Karabacak Calviello et al., 2019) and H2AFZ and H3K4me3 ChIP-seq signals (ENCODE, https://www.encodeproject.org/, ENCFF494WCA and ENCFF847JMY) in untreated K562 cells (n = 10273, 10378, 6383 and 10202, respectively). The corresponding shaded area represents the 95 % confidence interval of the mean. The gene region is shaded light gray and the nucleosome position is shaded mid gray. The y-axis represents the log2-transformed signal. The sense profiles are shown as positive values and the antisense profiles are shown as negative values. Only sense mNET-seq and PRO-seq profiles are shown at the TSS, pause site, +1 nucleosome dyad, and the antisense mNET-seq and PRO-seq profiles are shown at the -1 nucleosome dyad.

B) Box plots showing RNA Pol II occupancy at -1 nucleosomes (n = 10273), pause sites (n = 6383, ±5 nt) and +1 nucleosomes (n = 10202, ±72 nt) upon partial or near complete SPT6 depletion (1 h and 4 h, respectively). Representations as in Supplementary Figure S2A. Adjusted p-values were determined with Mann-Whitney U test and are indicated for each treatment set., ns: $p > 0.05$, *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$, ****: $p \leq 0.0001$.

C) Metagene profiles aligned at the -1 nucleosome dyad, TSS, pause site and +1 nucleosome dyad showing mean occupancy of RNA Pol II (mNET-seq) upon siRNA knockdown of SPT6 in the HeLa cell line (Nojima et al., 2018). n = 10273, 10378, 6383 and 10202, respectively. Representations as in A).

Figure S6

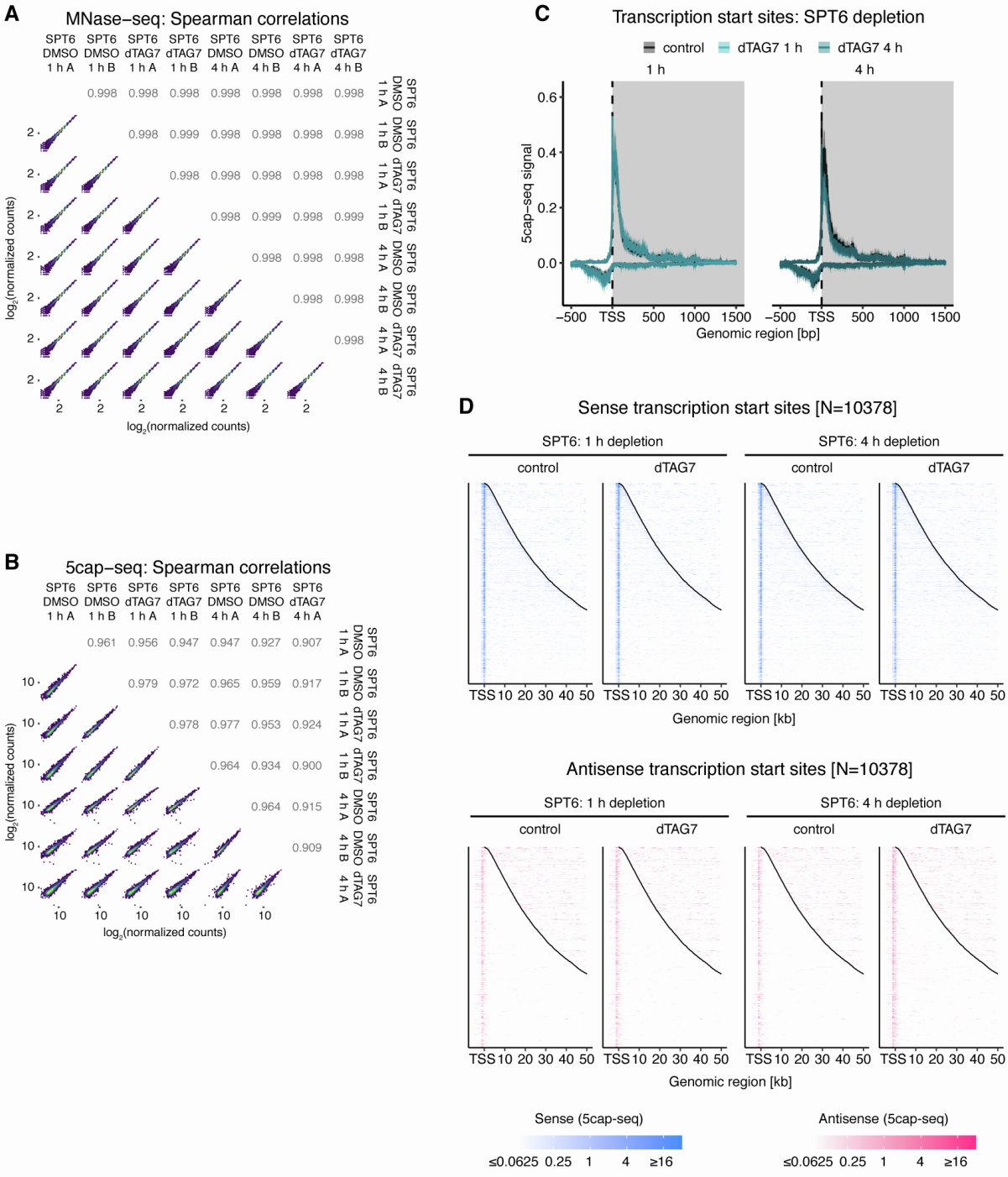

Figure S6. Nucleosome occupancy and transcription start sites are stable - relates to Figure 5
A) Scatter plots of normalized MNase-seq gene counts and Spearman correlation coefficient (ρ) of each pair upon partial (1 h) and complete (4 h) depletion of SPT6.
B) Scatter plots of normalized 5cap-seq gene counts and Spearman correlation coefficient (ρ) of each pair upon partial (1 h) and complete (4 h) depletion of SPT6.
C) Metagene profiles aligned at the TSS showing mean transcription start site signal (5cap-seq) upon partial or near complete SPT6 depletion (1 h and 4 h dTAG7 treatment, respectively) (n = 10378). The corresponding shaded area represents the 95 % confidence interval of the mean. The gene region is shaded light gray. The y-axis represents the $\log_2$-transformed signal. The sense profiles are shown as positive values and the antisense profiles are shown as negative values.
D) Heatmap analyses of transcription start site signal (5cap-seq) upon depletions of SPT6 (1 h and 4 h dTAG7 treatment). Expressed gene (n = 10378) regions (-5 to 50 kb) are aligned at the TSS and sorted by gene length. The black line represents the gene end, the sense (top) and antisense (bottom) signals are presented separately.

Figure S7

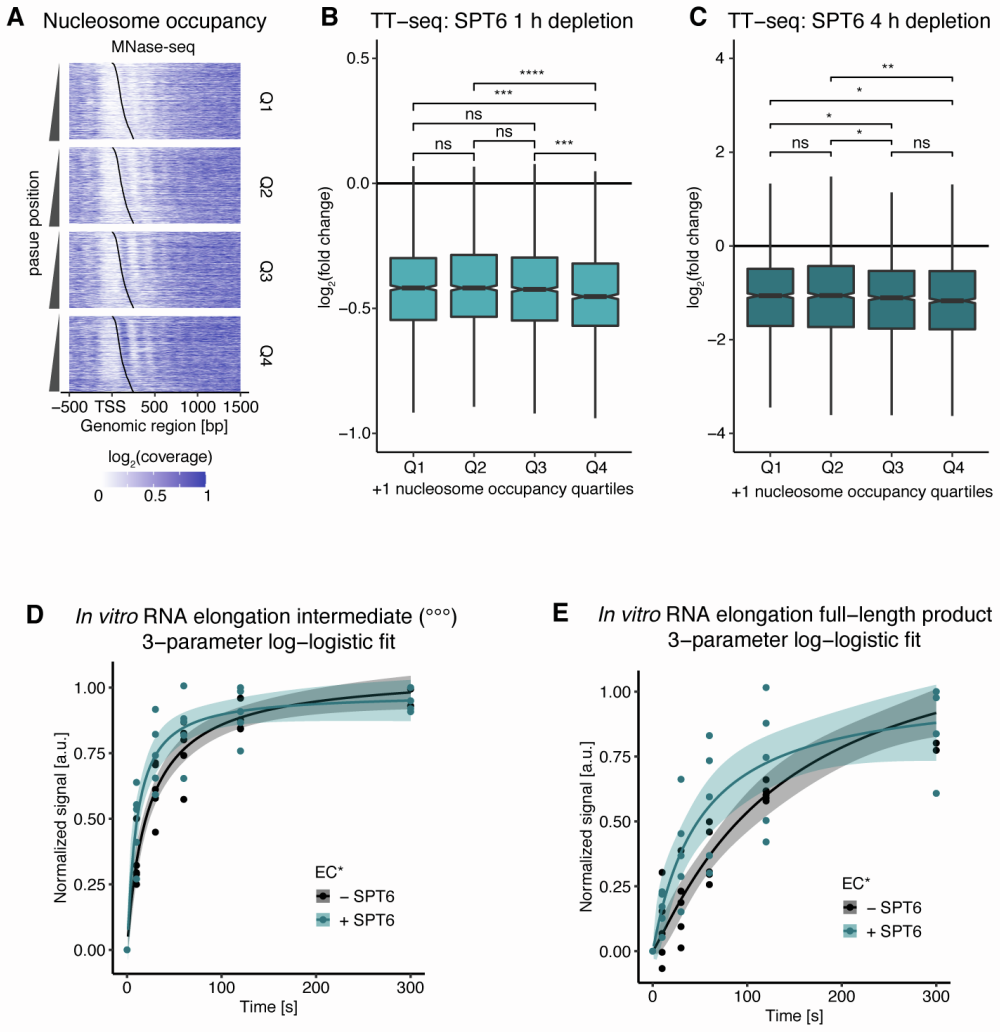

Figure S7. SPT6 facilitates nucleosome transcription - relates to Figure 6

A) Heatmap analyses of nucleosome occupancy signals (MNase-seq) of genes for the untreated K562 cell line. Genes with a pause site (n = 6383) were separated into +1 nucleosome occupancy quartiles and then sorted by distance of the pause site from the TSS. The heatmap representations are as in Figure 5C.

B) Box plots showing RNA synthesis fold change (TT-seq) of genes separated into +1 nucleosome occupancy quartiles upon partial SPT6 depletion (1 h dTAG7 treatment). Representations as in Figure 1C. Adjusted p-values were determined with Mann-Whitney U test and are indicated for each comparison. ns: $p > 0.05$, *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$, ****: $p \leq 0.0001$.

C) As in B) for near complete SPT6 depletion (4 h dTAG7 treatment).

D) Log-logistic model fit of in vitro RNA elongation full-length product quantification. The predicted values of the model with the 0.95 confidence interval are shown in the respective color for the experimental time range. The normalized measured values are shown in the respective color (closed dots).

E) Log-logistic model fit of in vitro RNA elongation intermediate (°°°) quantification, representations are as in A).

Table S1: Oligonucleotide sequences - relates to STAR Methods section

Name	Sequence	Note
sgSPT6-N-1	TTTTGTGGAAAGCGAGGCTG	sgRNA
RepSPT6-N-1_F	GCGTTACATAGCATCGTACGCGTACGTGTTTGG TCTGATTTTGTGGAAAGCGAGGCTATGACCGAG TACAAGCCCACG	HR template cloning primer; underlined = microhomology + frame correction
RepSPT6-N-1_R	AGCATTCTAGAGCATCGTACGCGTACGTGTTTG GGGTCTGGATGGTGGGCGCCATAGATCCGCC GCCACC	HR template cloning primer; underlined = microhomology + frame correction
SPT6_N_Fwd	AGCAGTGGCAGGTTGCTAAA	Genotyping primer
SPT6_N_Rev	TGCTGAGCCTGAGCTTTTCA	Genotyping primer
sgPAF1-N-1	CTGCCGTCGCCTCGTCGCTA	sgRNA
RepPAF1-N-1_F	GCGTTACATAGCATCGTACGCGTACGTGTTTGG CGGGCTGCCGTCGCCTCGTCGATGACCGAGTA CAAGCCCACG	HR template cloning primer; underlined = microhomology + frame correction
RepPAF1-N-1_R	AGCATTCTAGAGCATCGTACGCGTACGTGTTTG GGGTCTGGATGGTGGGCGCCATAGATCCGCC GCCACC	HR template cloning primer; underlined = microhomology + frame correction
PAF1_N_Fwd	CCCACCTCATCTCAACCCAC	Genotyping primer
PAF1_N_Rev	TGCTGCAACAGAAAAGCGTG	Genotyping primer
sgCTR9-N-1	ATGGAGCCCCGCGACATGAT	sgRNA
RepCTR9-N-1_F	GCGTTACATAGCATCGTACGCGTACGTGTTTGG TGCTCGCCTTTTGACCCCATCATGACCGAGTAC AAGCCCACG	HR template cloning primer; underlined = microhomology + frame correction
RepCTR9-N-1_R	AGCATTCTAGAGCATCGTACGCGTACGTGTTTG GCTCGATGGAGCCCCGCGACATAGATCCGCC GCCACC	HR template cloning primer; underlined = microhomology + frame correction
CTR9_N_Fwd	CTGGATTAGCCTGAAGCGGA	Genotyping primer
CTR9_N_Rev	GGGGCGATCGTACAAGTTCA	Genotyping primer
RTF1-N-sgRNA-1	CGGAGCGGAGCGCGCATGCG	sgRNA
microHD5'-RTF1-N-F1	GCGTTACATAGCATCGTACGCGTACGTGTTTGG GGGCGGAGCGGAGCGCGCATGACCGAGTACA AGCCCACG	HR template cloning primer; underlined = microhomology + frame correction
microHD3'-RTF1-C-R1	AGCATTCTAGAGCATCGTACGCGTACGTGTTTG GACCCACACAAAGGCGACCGCGAGATCCGCC GCCACC	HR template cloning primer; underlined = microhomology + frame correction
RTF1-N-gDNA-F1	CAGTCGCGGGTAAAGCCACC	Genotyping primer
RTF1-N-gDNA-R1	GTGTCCGAGTCGATCACGACG	Genotyping primer
RNA primer for transcription assay	/56-FAM/rUrUrArUrCrArCrUrGrUrC	
Template strand NCP substrate	TTGTGTTTGGTGTGTCTGGGTGGTGGCCGTTTT CGTTGTTTTTTTCTGTCTCGAACCTGGAGACTAG GGAGTAATCCCCTTGGCGGTTAAAACGCGGGG GACAGCGCGTACGTGCGTTTAAGCGGTGCTAG AGCTGTCTACGACCAATTGAGCGGCCTCGGCA CCGGGATTCTGAT	
rP5_RND	CTTTCCCTACACGACGCTCTTCCGATrCrUrNrNr NrNrNrNrNrN	UMI containing adapter
BioNotI-P5-PET	[Btn]TATAGCGGCCGCAATGATACGGCGACCAC CGAGATCTACACTCTTTCCCTACACGACGCTCT TCCGATCT	Biotinylated P5 adapter
P7MPX_linker_for	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC *T	P7-containing adapter
P7MPX_linker_rev	[Phos]GATCGGAAGAGCACACGTCTGAACTCCA GTCAC[AmC7]	P7-containing adapter
PE1.0#	ATGATACGGCGACCACCGAGATCTACACTCTTT CCCTACACGACGCTCTTCCGATC*T	Library amplification primer
PE2_MPX_01	CAAGCAGAAGACGGCATACGAGATCGTGATGT GACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	Library amplification primer with index

PE2_MPX_02	CAAGCAGAAGACGGCATACGAGATACATCGGT GACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	Library amplification primer with index
PE2_MPX_03	CAAGCAGAAGACGGCATACGAGATGCCTAAGT GACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	Library amplification primer with index
PE2_MPX_05	CAAGCAGAAGACGGCATACGAGATCACTGTGT GACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	Library amplification primer with index
PE2_MPX_07	CAAGCAGAAGACGGCATACGAGATGATCTGGT GACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	Library amplification primer with index
PE2_MPX_08	CAAGCAGAAGACGGCATACGAGATTCAAGTGT GACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	Library amplification primer with index
PE2_MPX_10	CAAGCAGAAGACGGCATACGAGATAAGCTAGT GACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	Library amplification primer with index
PE2_MPX_12	CAAGCAGAAGACGGCATACGAGATTACAAGGT GACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	Library amplification primer with index
rP5_RND	CTTTCCCTACACGACGCTCTTCCGATrCrUrNrNr NrNrNrNrNrN	UMI containing adapter
BioNotI-P5-PET	[Btn]TATAGCGGCCGCAATGATACGGCGACCAC CGAGATCTACACTCTTTCCCTACACGACGCTCT TCCGATCT	Biotinylated P5 adapter
P7MPX_linker_for	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC *T	P7-containing adapter
P7MPX_linker_rev	[Phos]GATCGGAAGAGCACACGTCTGAACTCCA GTCAC[AmC7]	P7-containing adapter
PE1.0	ATGATACGGCGACCACCGAGATCTACACTCTTT CCCTACACGACGCTCTTCCGATC*T	Library amplification primer
PE2_MPX_01	CAAGCAGAAGACGGCATACGAGATCGTGATGT GACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	Library amplification primer with index
PE2_MPX_02	CAAGCAGAAGACGGCATACGAGATACATCGGT GACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	Library amplification primer with index
PE2_MPX_03	CAAGCAGAAGACGGCATACGAGATGCCTAAGT GACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	Library amplification primer with index
PE2_MPX_05	CAAGCAGAAGACGGCATACGAGATCACTGTGT GACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	Library amplification primer with index
PE2_MPX_07	CAAGCAGAAGACGGCATACGAGATGATCTGGT GACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	Library amplification primer with index
PE2_MPX_08	CAAGCAGAAGACGGCATACGAGATTCAAGTGT GACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	Library amplification primer with index
PE2_MPX_10	CAAGCAGAAGACGGCATACGAGATAAGCTAGT GACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	Library amplification primer with index
PE2_MPX_12	CAAGCAGAAGACGGCATACGAGATTACAAGGT GACTGGAGTTCAGACGTGTGCTCTTCCGATC*T	Library amplification primer with index