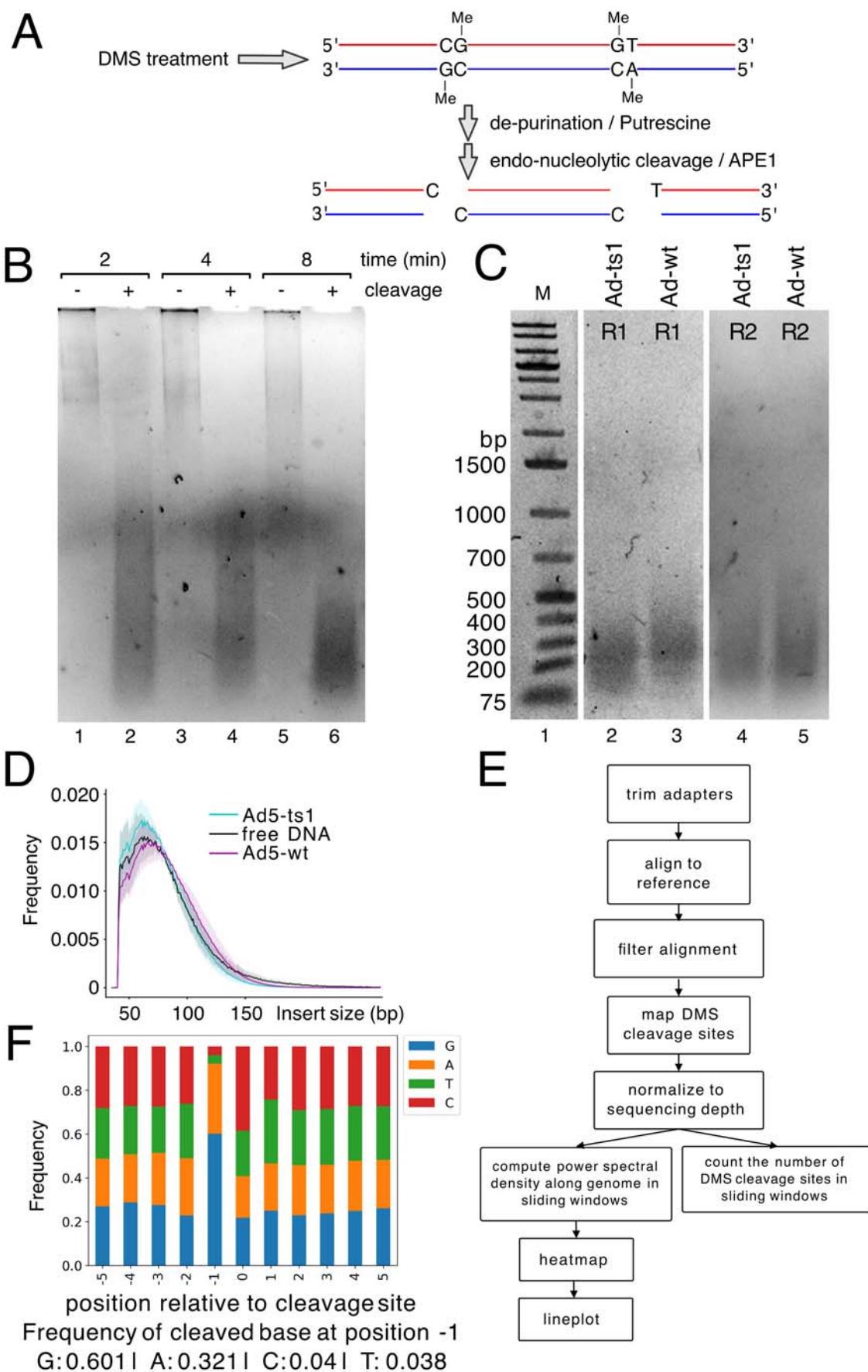


Expanded View Figures

Figure EV1. Depiction of DMS-seq workflow and controls.

(A) Scheme showing the DMS caused fragmentation of DNA by Putrescine and APE1. (B) Agarose gel (1.3%) of viral DNA was treated in the absence or presence of 5% DMS for the indicated time points. (C) Viral DNA was fragmented after treatment with 2% DMS for 4 min, and a subsequent cleavage reaction was used for library preparation. (D) Aligned fragment size distribution histogram of free DNA, Ad-wt, and Ad-ts1. (E) Overview of the pipeline for the processing of DMS-seq data. (F) Plot of nucleotide frequencies relative to fragment cleavage positions, as indicated.



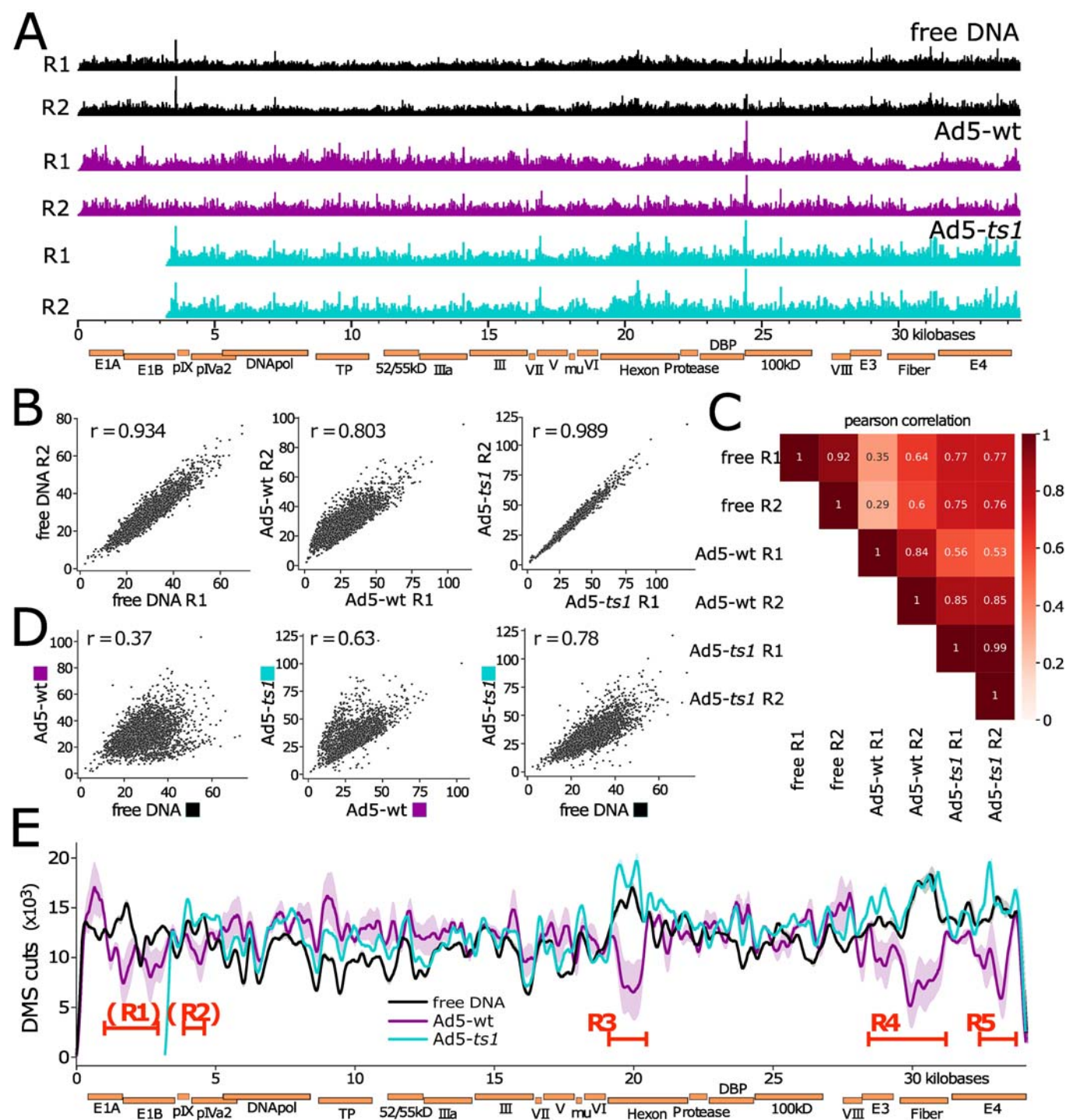


Figure EV2. DMS cleavage site distribution along the genome.

(A) Bar chart of cleavage sites along the reference genomes, individually displayed for each replicate. (B) Correlation between replicates in 10 bp bins along the genome. (C) Correlation matrix for the individual samples generated by deeptools ('multiBamSummary bins -bs 5'). (D) Correlation between samples (replicates combined) in 10 bp bins along the genome. (E) Count of DMS cut sites in a 400 bp sliding window with 100 bp step size across the reference genomes.

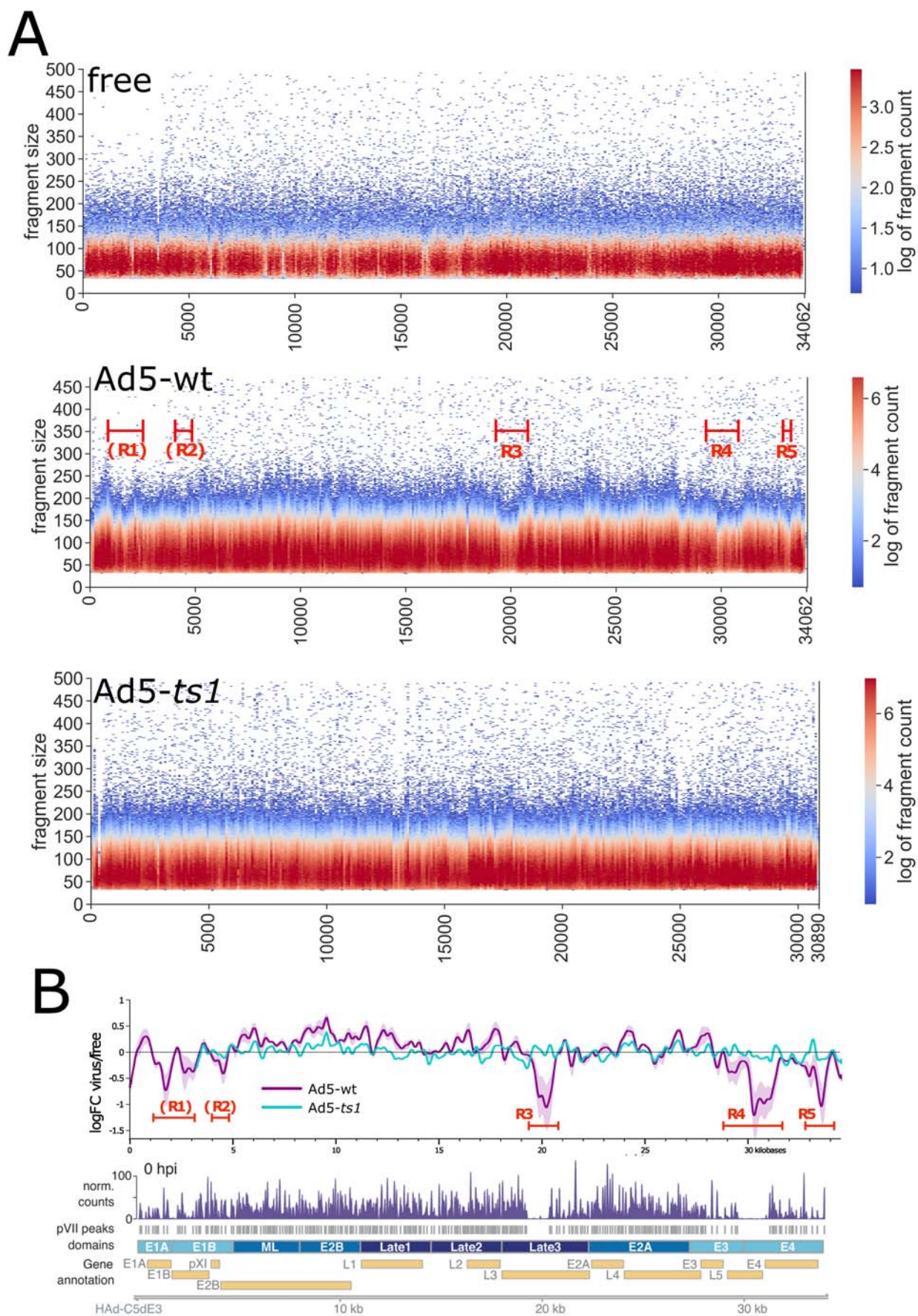


**Figure EV3. DNA size evaluation along the adenoviral genome.**

(A) V-plots (heatmaps) plotting the fragment size against the fragment midpoint for all samples. (B) Comparison of DMS-seq data with MNase-seq coverage along the adenovirus genome. Top panel: log fold change of DMS methylation sites of viral samples against free DNA (Fig. 1C). Bottom panel: MNase-seq coverage (adapted from Schwartz et al, 2023).

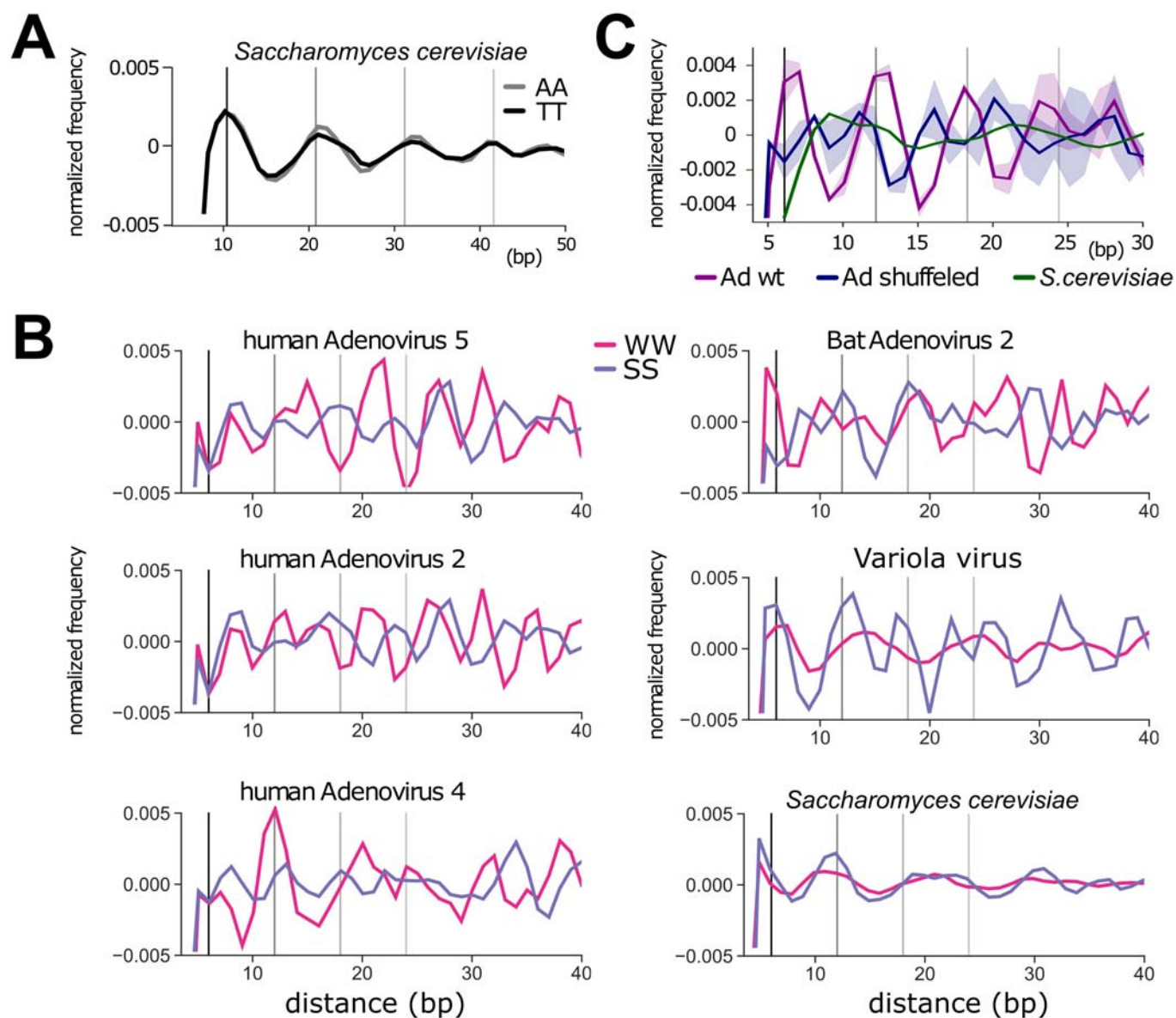


Figure EV4. Dinucleotide distance histograms.

(A) Nucleotide repeat patterns of ~11-bp AA and TT on the *S. cerevisiae* genome. Vertical lines are placed every 10.3 bp. (B) SS (G/C) and WW (A/T) dinucleotide sequence distance histograms on genomes from different adenovirus species, including Variola virus and the yeast genome. Vertical lines are placed every 6.1 bp. (C) Dinucleotide (~6-bp) periodicity of the T/C (YY) distance histogram on the human adenovirus 5 genome. The same analysis was performed with the shuffled Adenovirus genome and the *S. cerevisiae* genome. Vertical lines are drawn every 6.1 bp.

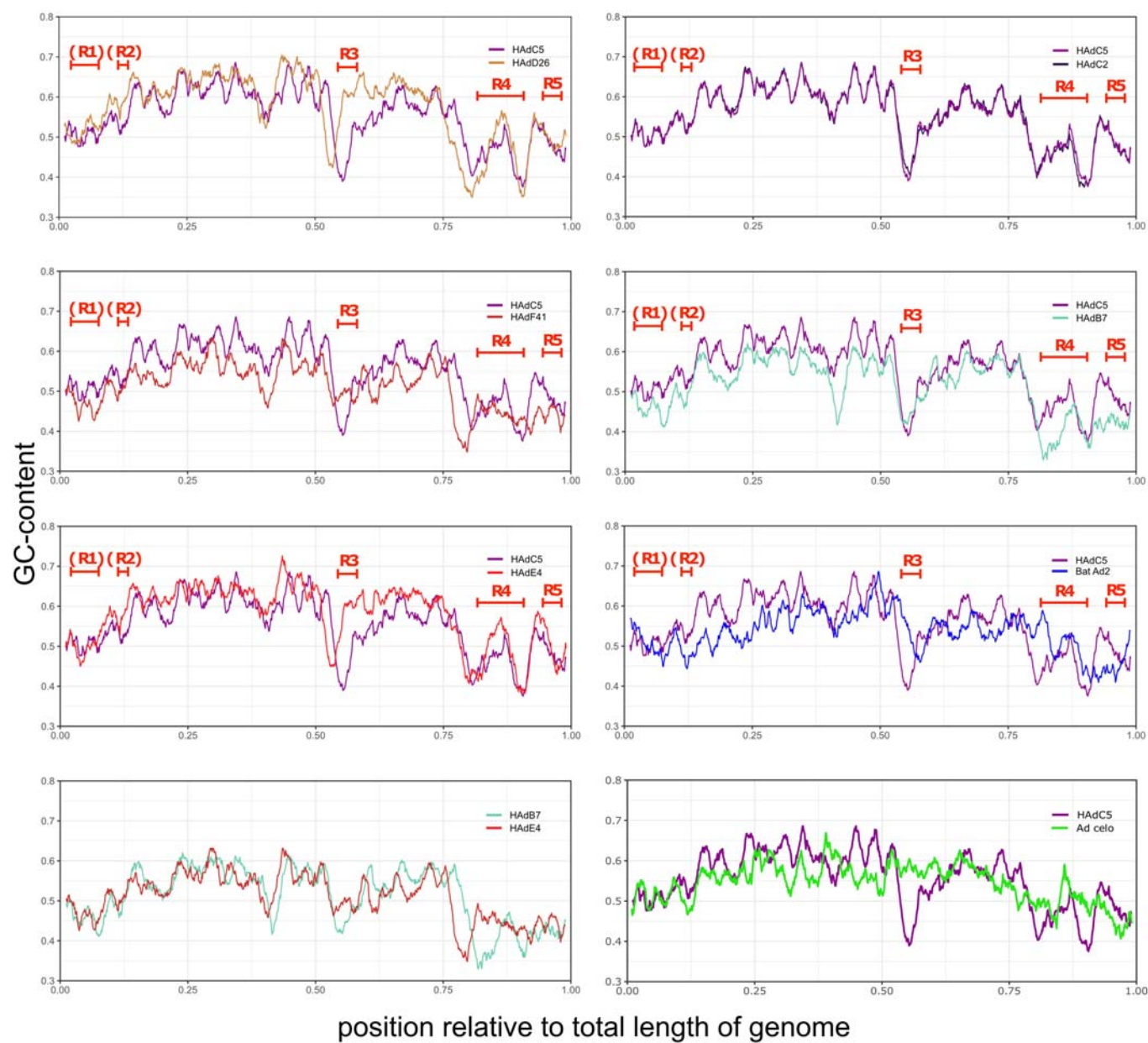


Figure EV5. Comparisons of GC-content distribution along various adenoviral genomes.

The Y-axis shows the GC-content. Because of the variable sizes of the tested genomes, the X-axis represents the relative positions along the entire length of the genome.