# Expanded View Figures

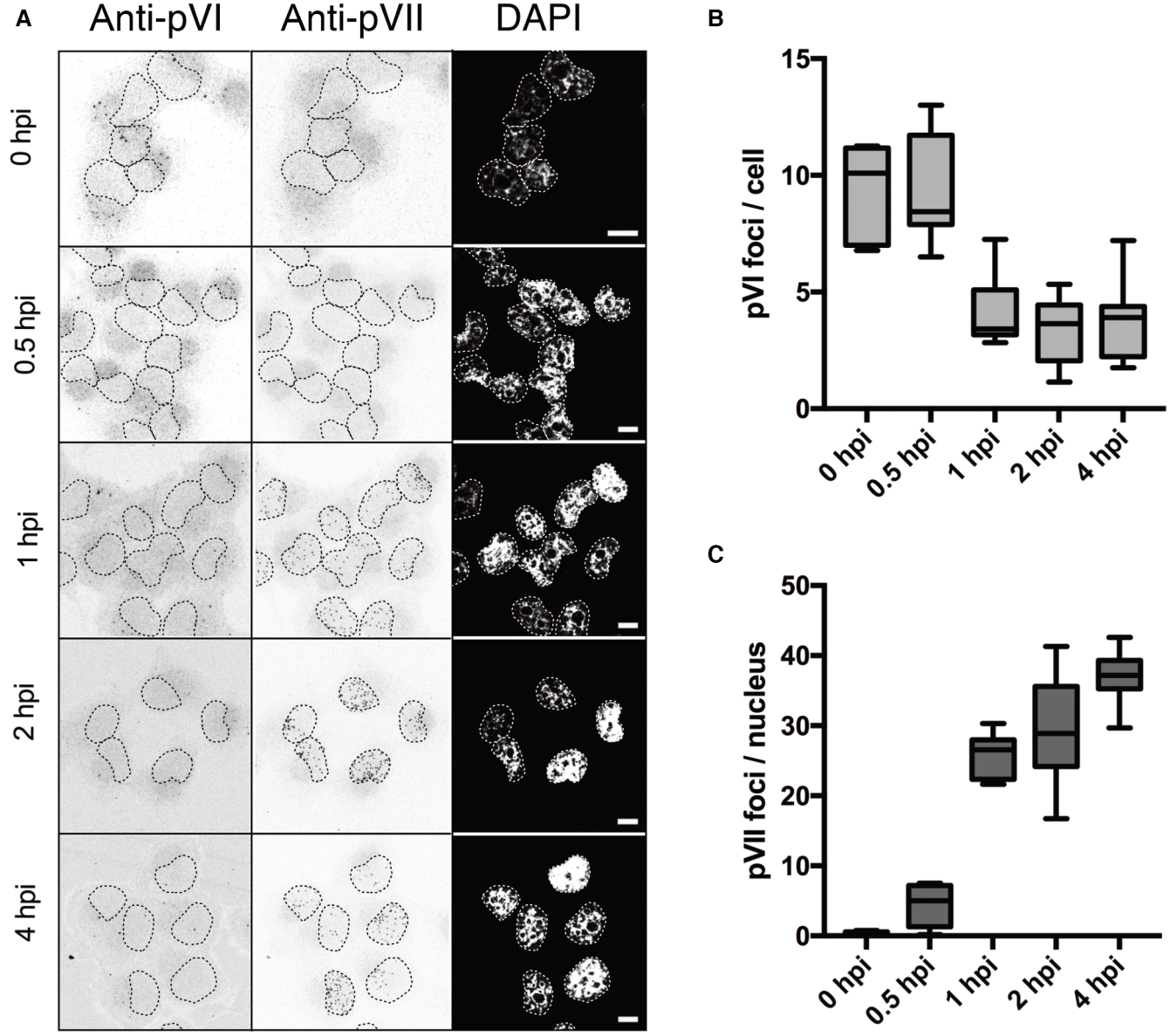


**Figure EV1. Spatio-temporal analysis of adenovirus infection using microscopy.**

A Images show representative confocal images at indicated time points stained with anti-pVI (left column) and anti-pVII (middle column) antibodies and DAPI detection of the nucleus (right column). Nuclei are indicated by dashed lines. Scale bar is 10 μm.

B Quantification of the pVI signal over time as indicated on the x-axis. The distribution of pVI foci per cell are shown as Box and Whiskers plot ($n > 44$ cells per time point). The central band shows the median, boxes the upper and lower quartile and whiskers the min and max.

C As in (B) showing the quantification of pVII foci per nucleus from the same experiment ($n > 44$ nuclei per time point). The central band shows the median, boxes the upper and lower quartile and whiskers the min and max.

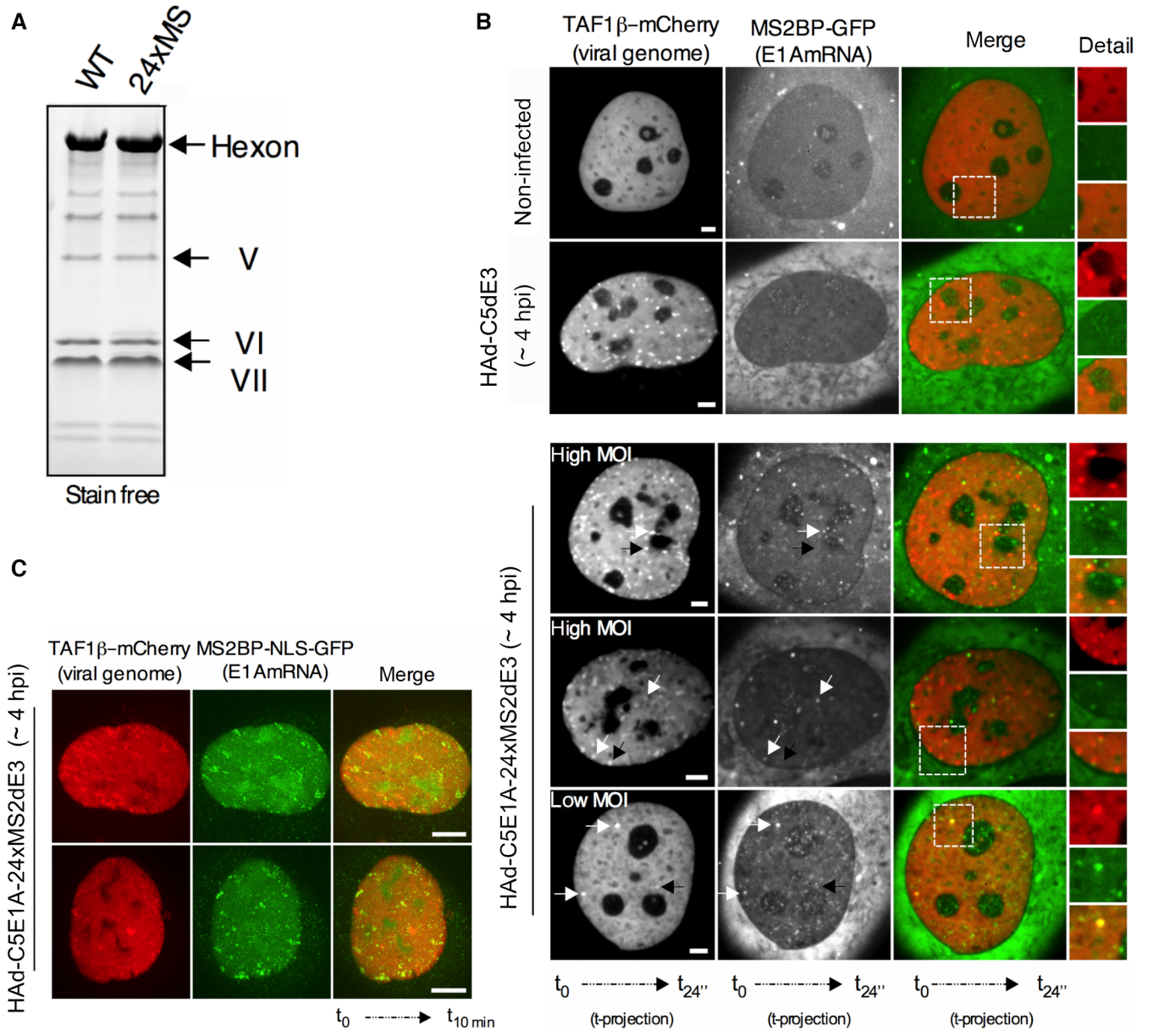


**Figure EV2. MS2-repeat tagged E1AmRNA transcription.**

A Stain free gel analysis of purified HAd-C5dE3 and HAd-C5E1A-24xMS2dE3. Major capsid proteins are indicated. Note that both viruses have the same profile. Infectious titer for each virus was determined in plaque assay to be 9 × 105 and 9.2 × 105 PFU/µl respectively.

B Representative images composed of t-projections of 24 frames imaged at ~4 hpi at a frame rate of 1 frame per second. Cells express TAF1β-mCherry showing viral genomes (first column, red signal in merge) and MS2BP-NES-GFP showing MS2-repeat tagged E1AmRNAs (second column, green signal in merge) and an overlay (third column, merge) of both channels. The first row shows non-infected control cells, the second row cells infected with untagged control virus and the third and fourth row examples of cells infected with virus expressing E1AmRNA tagged with MS2-repeats using a high MOI and the fifth row using a low MOI. Black arrows point at non-transcribing and white arrows at transcribing genomes. Details are shown as magnifications of the boxed area. Scale bar is 10 µm.

C Images as in (B) and Fig 3 showing two examples of a longer acquisition. Representative images composed of 10 min t-projections imaged at ~4 hpi. Cells express TAF1b-mCherry showing viral genomes (first column, red signal) and MS2BP-NLS-GFP showing MS2- repeat tagged E1AmRNAs (second column, green signal) and an overlay (third column, merge) of both channels. Transcribing genomes appear in yellow in the t-projection. Note that some displacement occurred during the longer acquisition time resulting in some blur. Scale bar is 20 µm.

**Figure EV3. MNase footprinting in virus particles.**

A Agarose gel after incremental chromatin digestions (from 10s to 180s) of genomic HAd-C5dE3 DNA (gDNA) or HAd-C5dE3 chromatin enclosed in virus particles using 5 U of MNase. gDNA was inserted into a bacterial artificial chromosome and mixed with generic DNA in ratio 1:14 (I). Virus particles were incubated for 6 min at 48°C to enable MNase infiltration. DNA from lane 5 and 9 (90s of MNase digestion for gDNA samples and 180s for virus particles, respectively) was isolated and subjected to next generation sequencing. MW, molecular weight marker; R, replicate; I, input.

B Kernel density plot of the fragment size distribution of paired-end reads mapped to the HAd-C5dE3 genome.

C MNase footprinting analysis of gDNA (black) or HAd-C5dE3 chromatin in virus particles (purple). The sites of preferential MNase cleavage were assessed as the number of 5′ ends of all DNA fragments along the HAd-C5dE3 genome. The MNase cutting frequency was normalized to counts per million sequenced fragments (CPM) and quantified in 500 bp sliding windows using a step size of 100 bp. The average of two biological replicates is shown.

D Boxplots showing preferential regions of MNase catalyzed DNA hydrolysis in viral chromatin compared to gDNA. The data is represented as log fold change (logFC) taken form the profile in (C). The box represents the interquartile range (IQR). The line inside the box represents the median of the dataset. The whiskers extend from the edges of the box to the first and third quartiles, but no further than 1.5 times of the IQR. The average score in n 500 bp windows was assessed in the corresponding domain: 17 E1A, 30 E1B, 33 ML, 30 E2B, 35 Late1, 35, Late2, 43 Late3, 50 E2A, 27 E3, 39 E4.

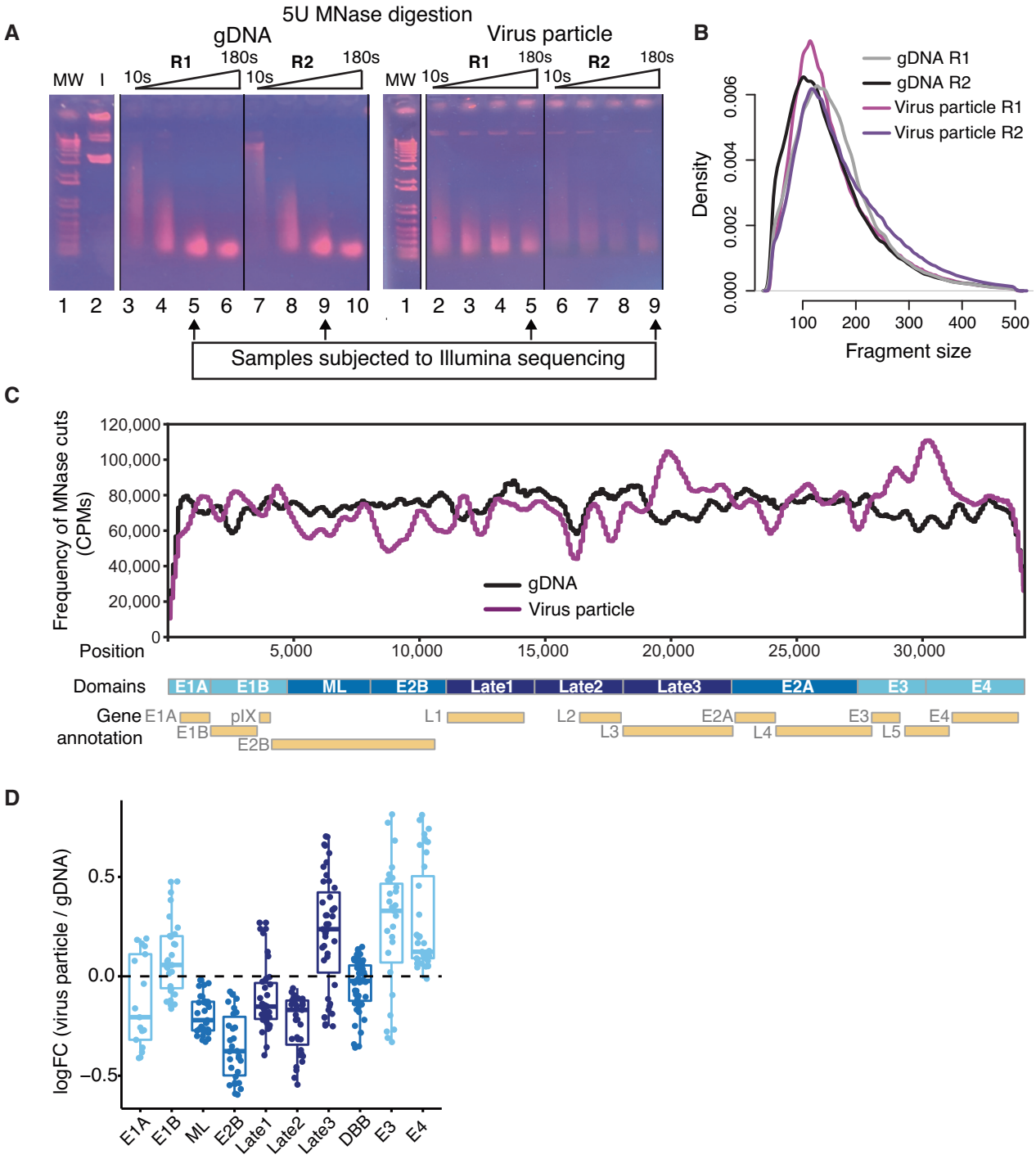
A
5U MNase digestion
gDNA
Virus particle
MW I
10s R1 180s
10s R2 180s
MW
1 2 3 4 5 6 7 8 9 10
1 2 3 4 5 6 7 8 9
Samples subjected to Illumina sequencing
B
gDNA R1
gDNA R2
Virus particle R1
Virus particle R2
Density
0.000 0.002 0.004 0.006
100 200 300 400 500
Fragment size
C
Frequency of MNase cuts (CPMs)
120,000 100,000 80,000 60,000 40,000 20,000 0
gDNA
Virus particle
Position
5,000 10,000 15,000 20,000 25,000 30,000
Domains
E1A E1B ML E2B Late1 Late2 Late3 E2A E3 E4
Gene annotation
E1A E1B pIX E2B L1 L2 L3 E2A L4 E3 L5 E4
D
logFC (virus particle / gDNA)
0.5 0.0 −0.5
E1A E1B ML E2B Late1 Late2 Late3 DBB E3 E4

**Figure EV3.**

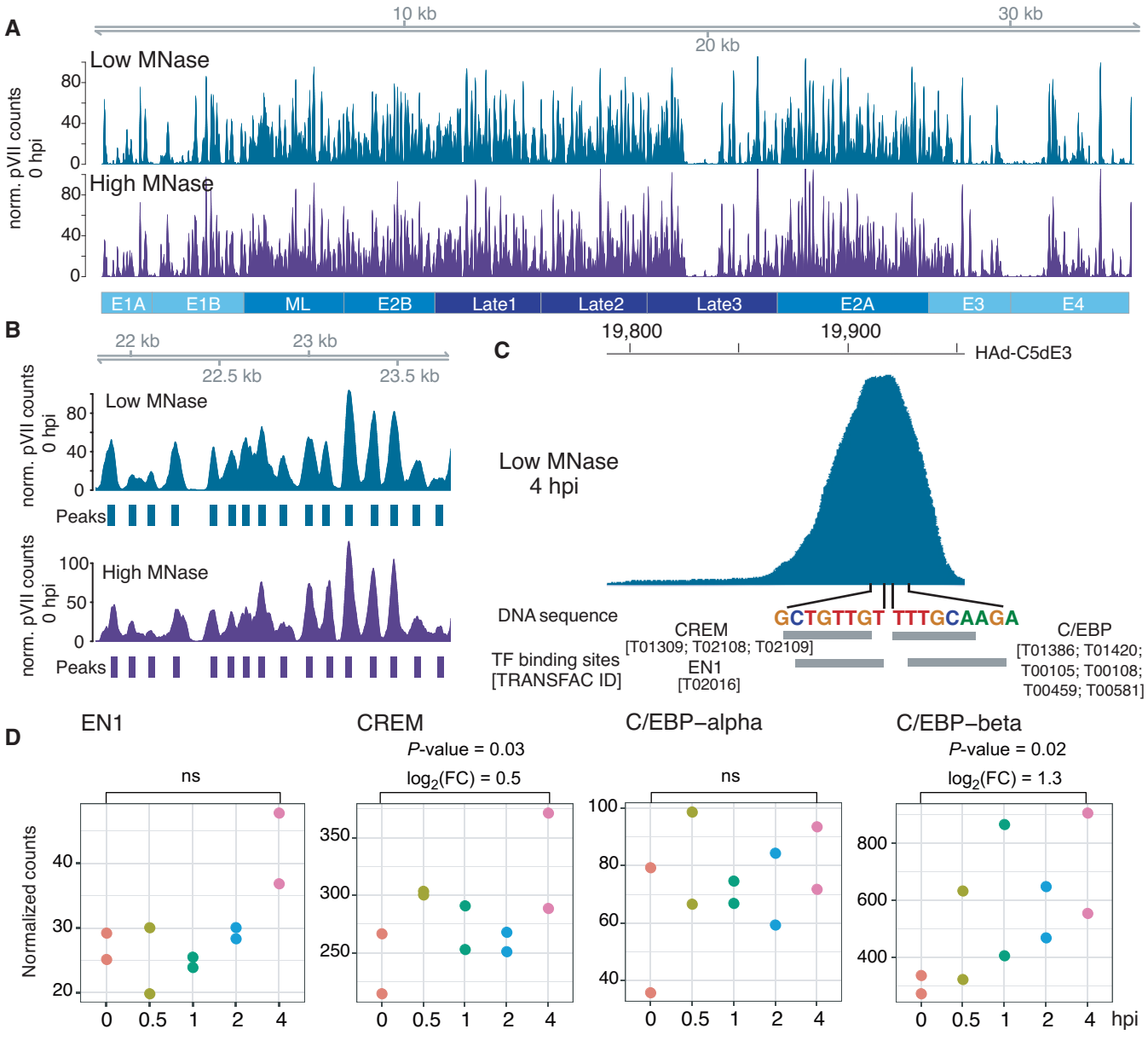


**Figure EV4. Assessment of transcription factor binding to Late3 region.**

A, B Comparison of pVII occupancy profile (A) and position calling (B) between high and low MNase digested chromatin at time point 0 hpi.

C Transcription factor motifs in the center of a low MNase extracted peak at 4 hpi. The genomic location is indicated at the top. The DNA sequences containing known binding motifs are highlighted. The sites of the obtained motifs are illustrated as gray box and their respective TRANSFAC ids are displayed in square brackets.

D Gene expression changes of transcription factors over the infection time course. *P*-values and $\log_2$(fold-changes) from differential gene expression analysis between 4 and 0 hpi using DESeq2 are indicated. Wald test; ns, not significant.

**Figure EV5. Histone H3.1 is not deposited onto Ad genomes in the first 4 h of infection.**

A Estimating the number of assembled nucleosomes onto the HAd-C5dE3 genome. A mixed distribution of pVII and nucleosome fragments was calculated to reassemble the fragment distribution at 2 and 4 hpi. The best match was obtained using 8%/14% of nucleosomal fragments at 2 hpi/4 hpi.

B Images show an overview of cells from individual clones stably expressing Flag-tagged histones H3.1 (top row), H3.3 (center row) and control cells (bottom row). Cells were stained with anti-flag antibodies and detected with Alexa488 secondary antibodies and counterstained with DAPI as indicated above each column. Scale bar is 10 μm.

C Cells as in (B) analyzed by western blot using Flag specific antibodies. The histone H3 specific signal is indicated (arrow). A loading control is shown at the bottom.

D Hierarchical clustering of the Pearson correlation coefficients of ChIP-seq samples. The average fragment coverage in 50 bp bins was calculated for each sample.

E H3.1 and H3K27ac profiles along the HAd-C5dE3 genome. Normalized coverage of Flag (red shades)/H3K27ac (orange shades) ChIP-seq experiments in U2OS31. The average signal of 2–3 replicates is shown and the 95% confidence interval is indicated.

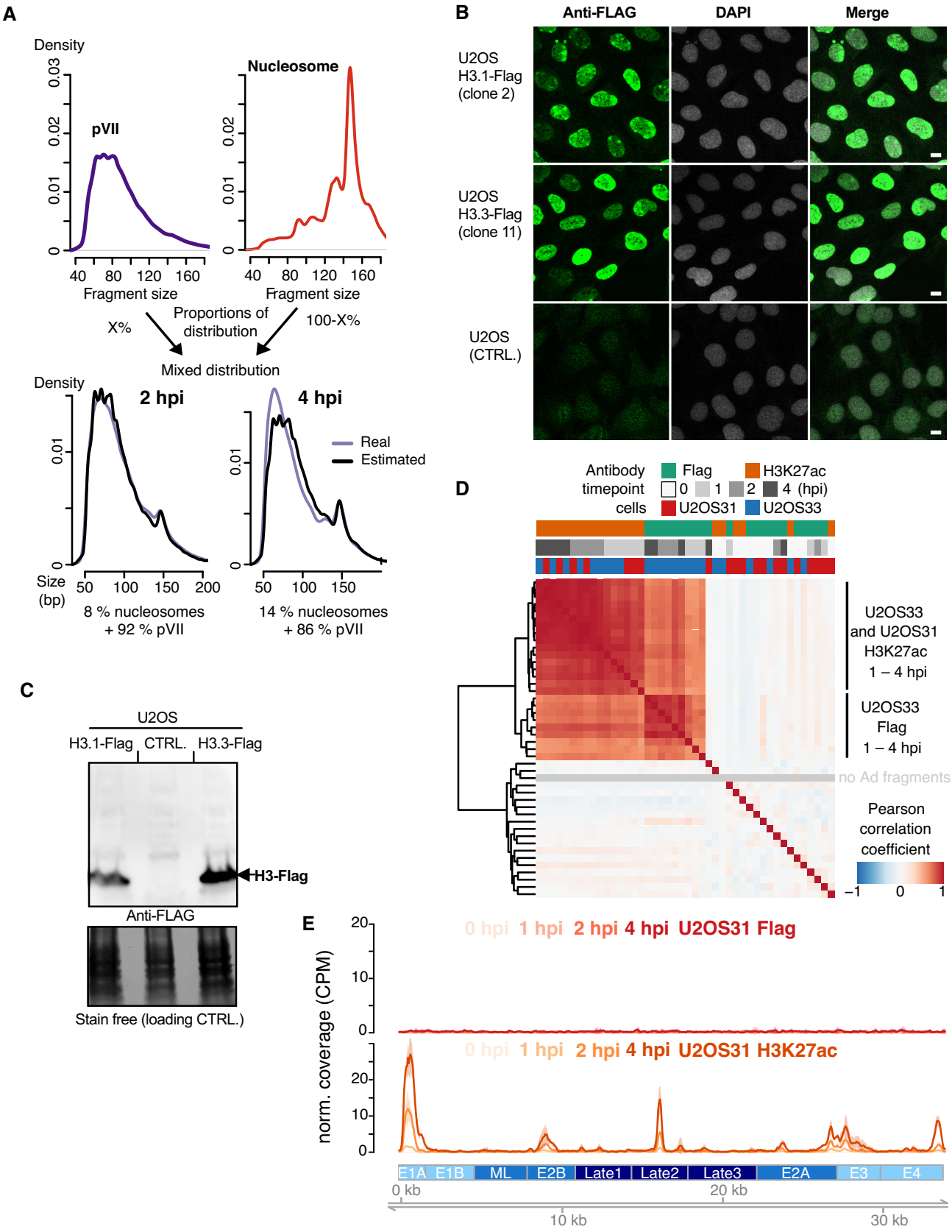


**Figure EV5.**