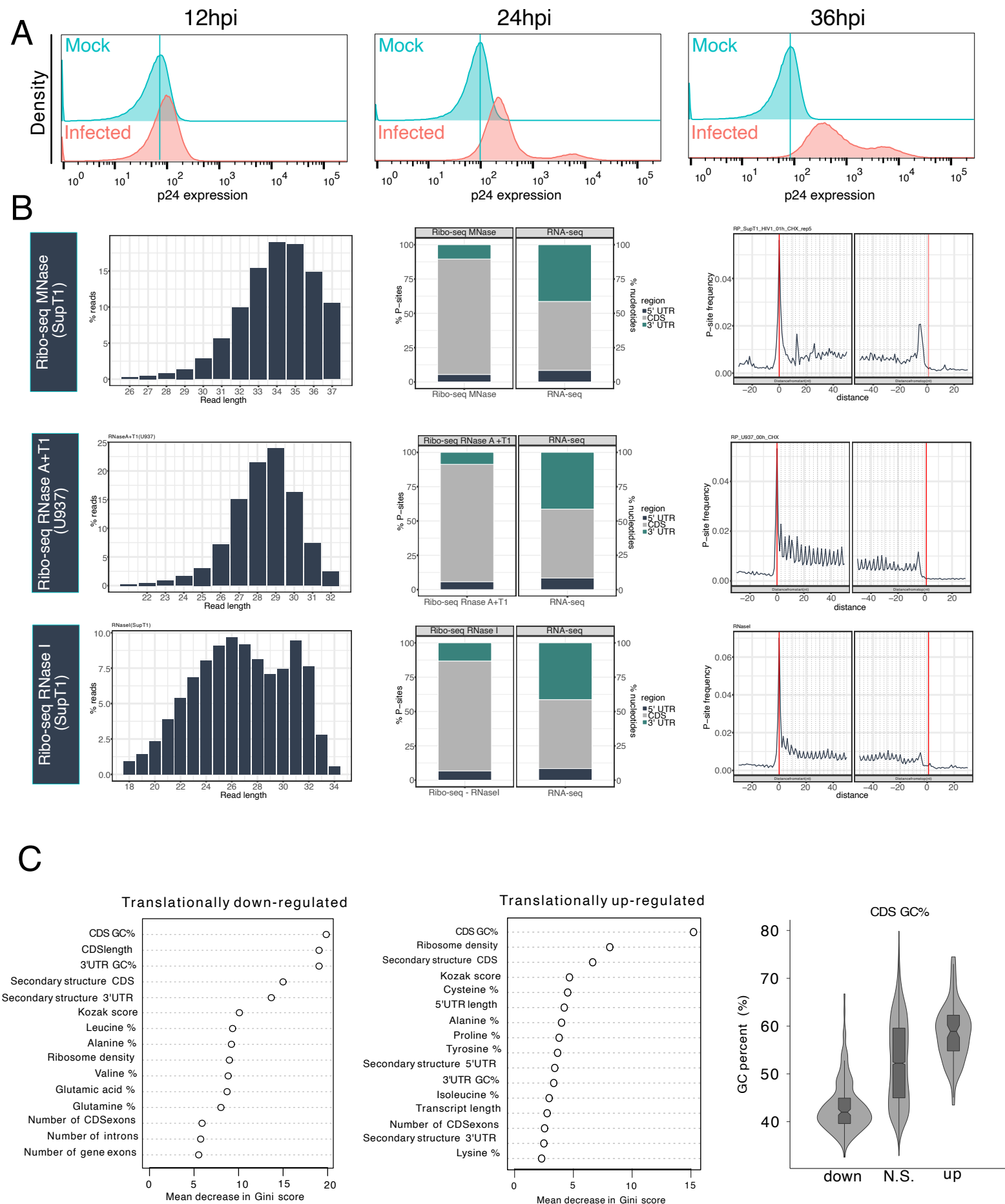
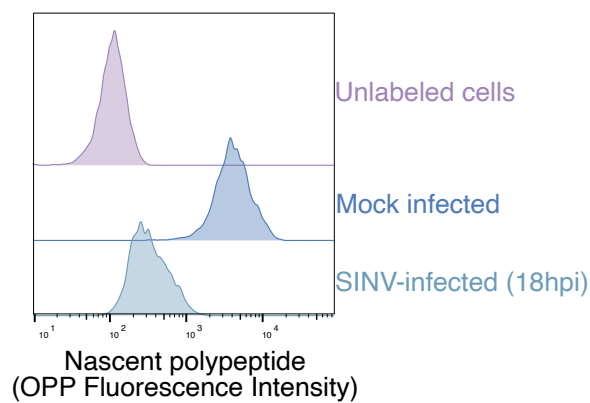


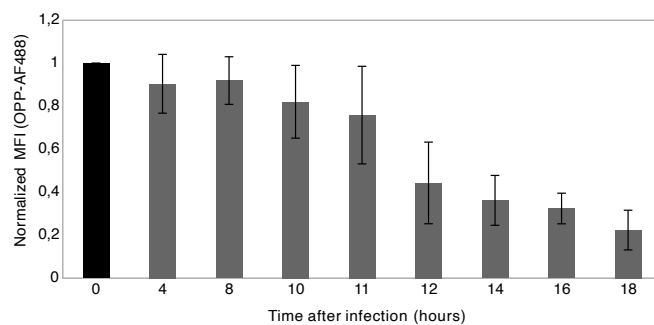


Supplementary figure S1. (A), Flow cytometry analysis of p24 expression in mock (blue) or HIV-1 infected (pink) SupT1 cells at 12, 24 or 36 hpi. n=1 biological replicate. (B), Distribution of ribosome P-sites in the -40 to +100 nucleotide region surrounding the annotated AUG start codons of all cellular and viral transcripts obtained from ribosome profiling libraries prepared from MNase, RNase A+T1 or RNase I-treated SupT1 cytoplasmic cell lysates. (C), Random forest analysis of features associated with down-translated (top panel), up-translated (middle panel) transcripts. (Bottom panel) Violin plots of CDS GC content of genes that undergo translational down-regulation (down), are not regulated (N.S.) or those that undergo translation up-regulation (up) 36 hours post infection.

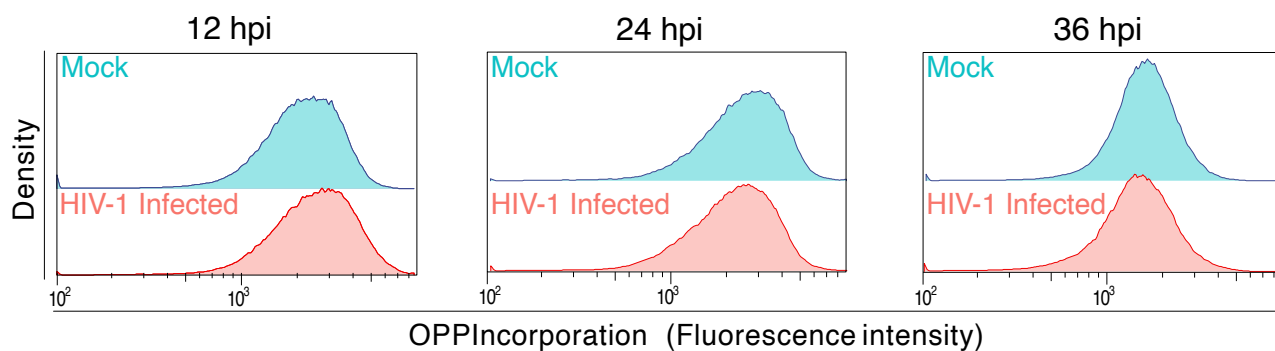
A



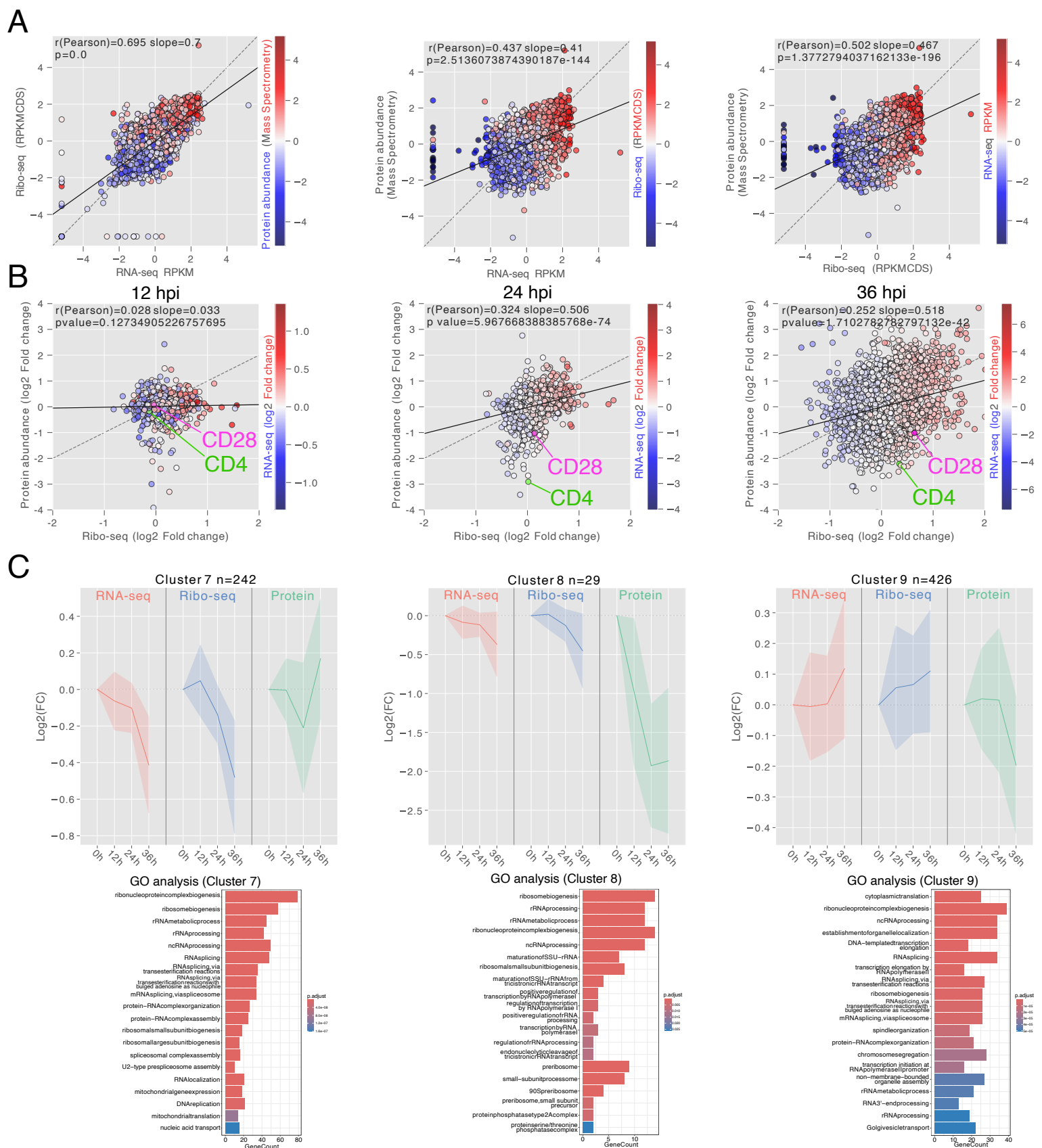
Translation efficiency upon Sindbis virus infection - HEK293T cells



B



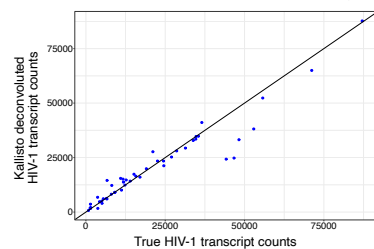
Supplementary Figure S2. (A), Density plot of flow cytometry signal for OPP incorporation in HEK293T cells infected with Sindbis virus (at MOI 5) from 0 to 18 hpi. (B), Flow cytometry analysis of OPP signal in SupT1 cells mock infected or infected with HIV-1 (NL4.3 strain at MOI 5) at 12, 24 and 36 hpi (results presented correspond to gating on live cells).



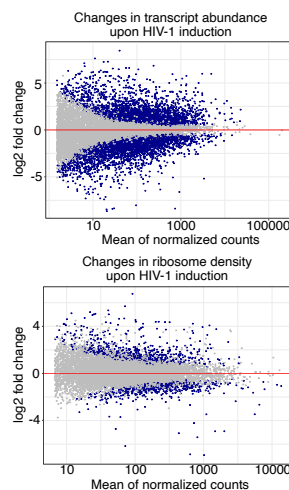
Supplementary figure S3. (A), Scatterplot of protein abundance (IBAQ score), RNA-seq (reads per kilobase per million of sequenced reads) and Ribo-seq (reads per kilobase of coding sequence per million of sequenced reads) expression levels per gene (n=4 independent replicates). Raw values were subjected to quantile and centred log-ratio transformation. (B), Scatter-plots of log2 fold changes in ribosome profiling (x axis) and protein abundance (y axis) between mock infected and HIV-1 infected cells at 12hpi (left panel), 24hpi (center panel) and 36hpi (right panel). Log2 fold-changes in RNA-seq expression is color coded for each gene (n=4 independent replicates). (C), (Top panels) Cluster Gene expression trajectories (RNA level in red, ribosome profiling in blue and protein abundance in green) for genes within specific identified clusters. (Bottom panels) Gene ontology analysis for each cluster of genes.

A

Deconvolution from simulated HIV-1 RNA-Seq dataset

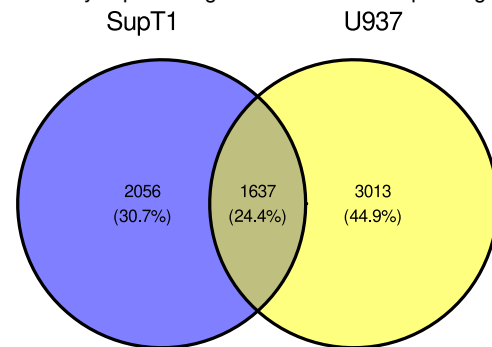


C

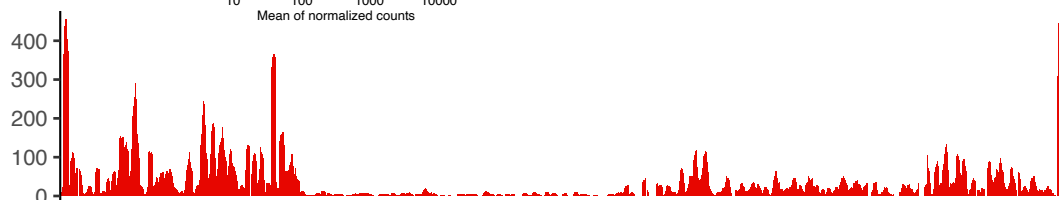
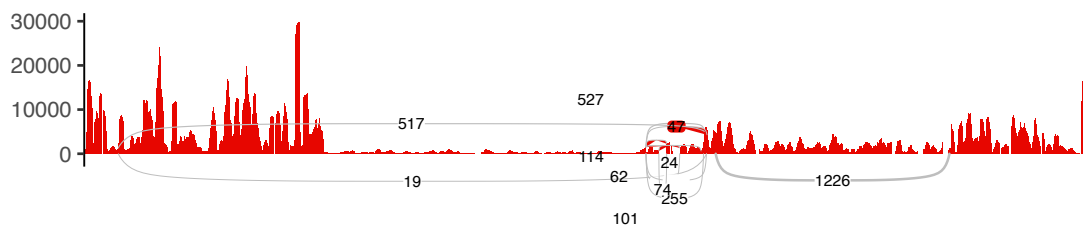
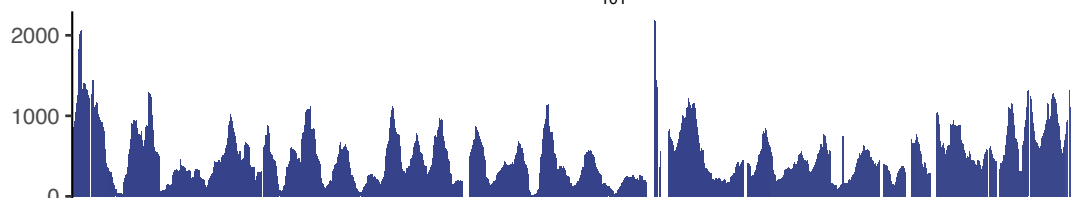
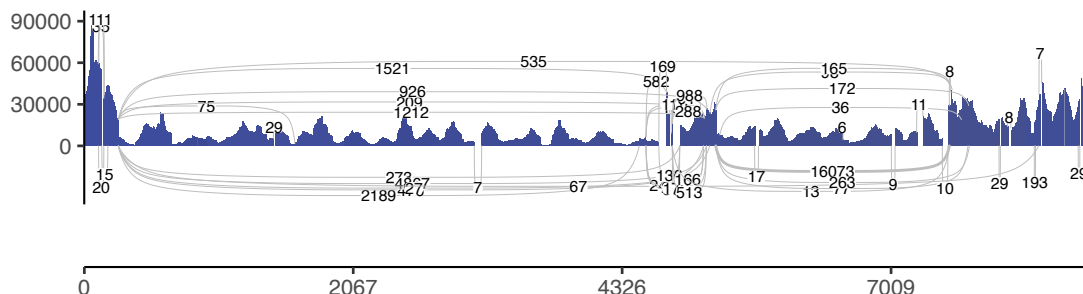
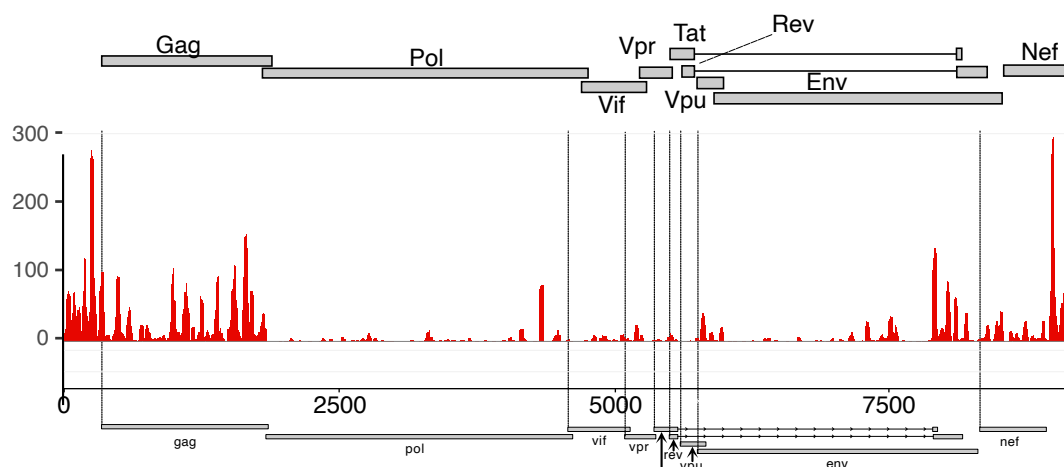


D

Differentially expressed genes from ribosome profiling data

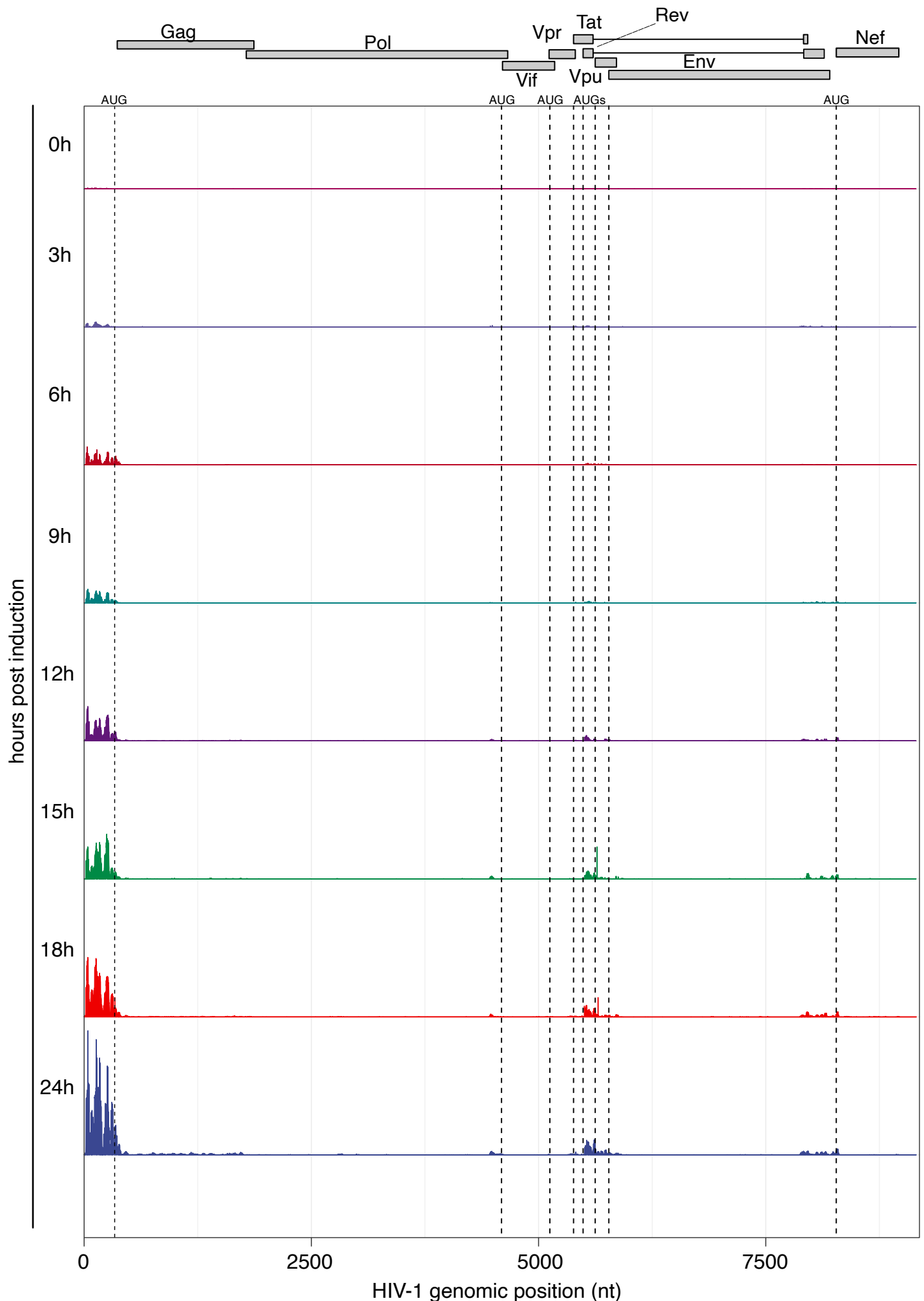


B

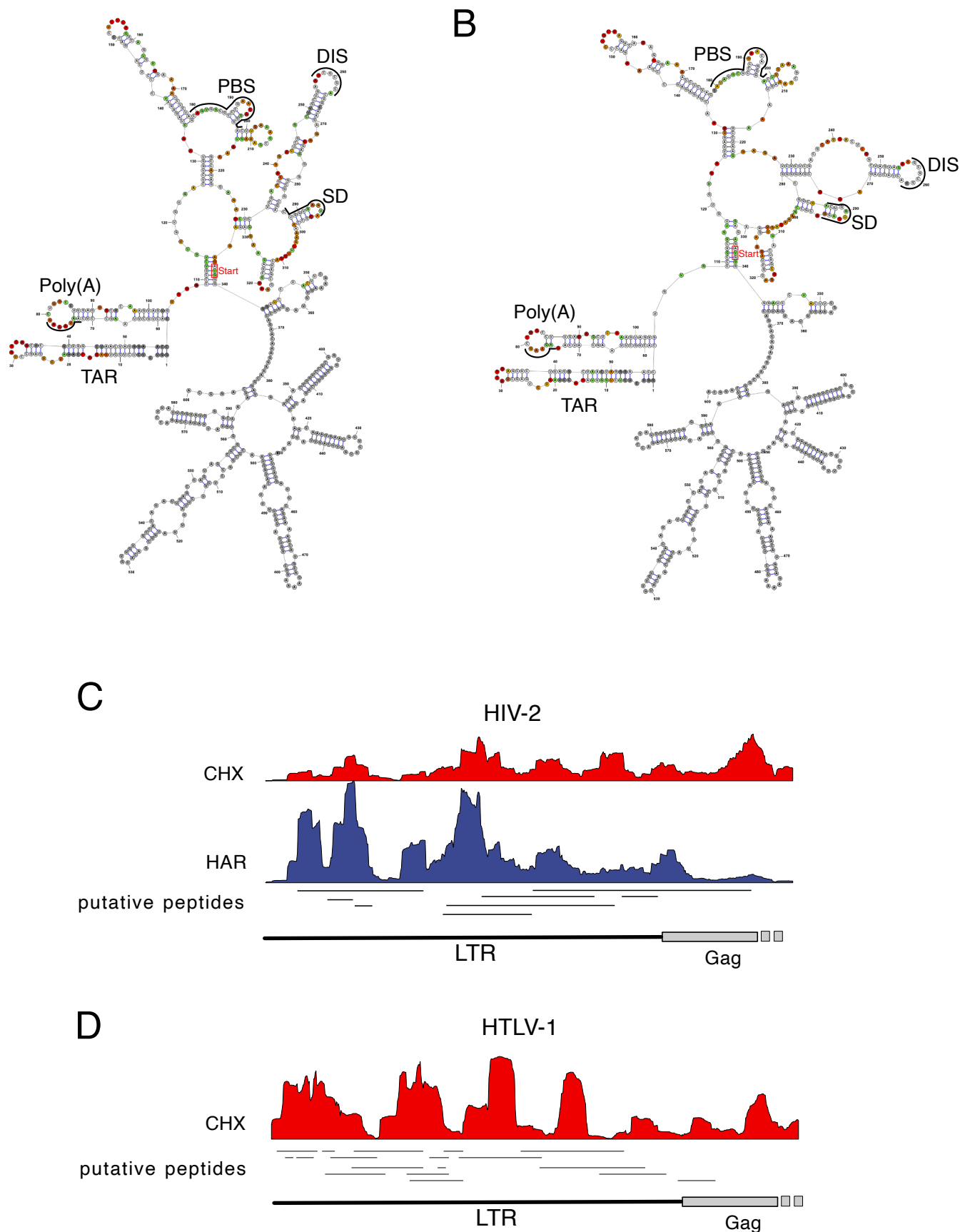
Ribosome Profiling
01 hour post infectionRibosome Profiling
24 hours post infectionRNA-seq
01 hour post infectionRNA-seq
24 hours post infectionRibosome Profiling
after ribosome-IP
01 hour post infection

Supplementary Figure S4(A), Scatterplot of a simulated HIV-1 RNA-seq dataset to test the Kallisto transcript-specific quantification pipeline based on annotated full-length transcripts obtained by Nanopore cDNA sequencing described in (2). True HIV-1 transcript counts are presented in the x-axis, while Kallisto deconvoluted transcript counts are presented in the y-axis. (B), Sashimi plot indicating all exon-exon junction mapping reads from our RNA-seq and Ribo-seq datasets at 1 and 24 hpi. $n = 4$ independent experiments. (C), MA-plot ($\log_2$ fold change Vs mean average) of cytoplasmic RNA-seq reads (Top panel) and ribosome profiling (bottom panel) from U937 cells with a latent HIV-1 integrated proviral DNA, 24 hours after induction of proviral DNA expression. Blue dots correspond to differentially expressed genes with an adjusted p -value < 0.05 . $n = 3$ independent experiments. (D). Venn-diagram of overlapping differentially expressed genes between HIV-1 infection and PMA-ionomycin induction.

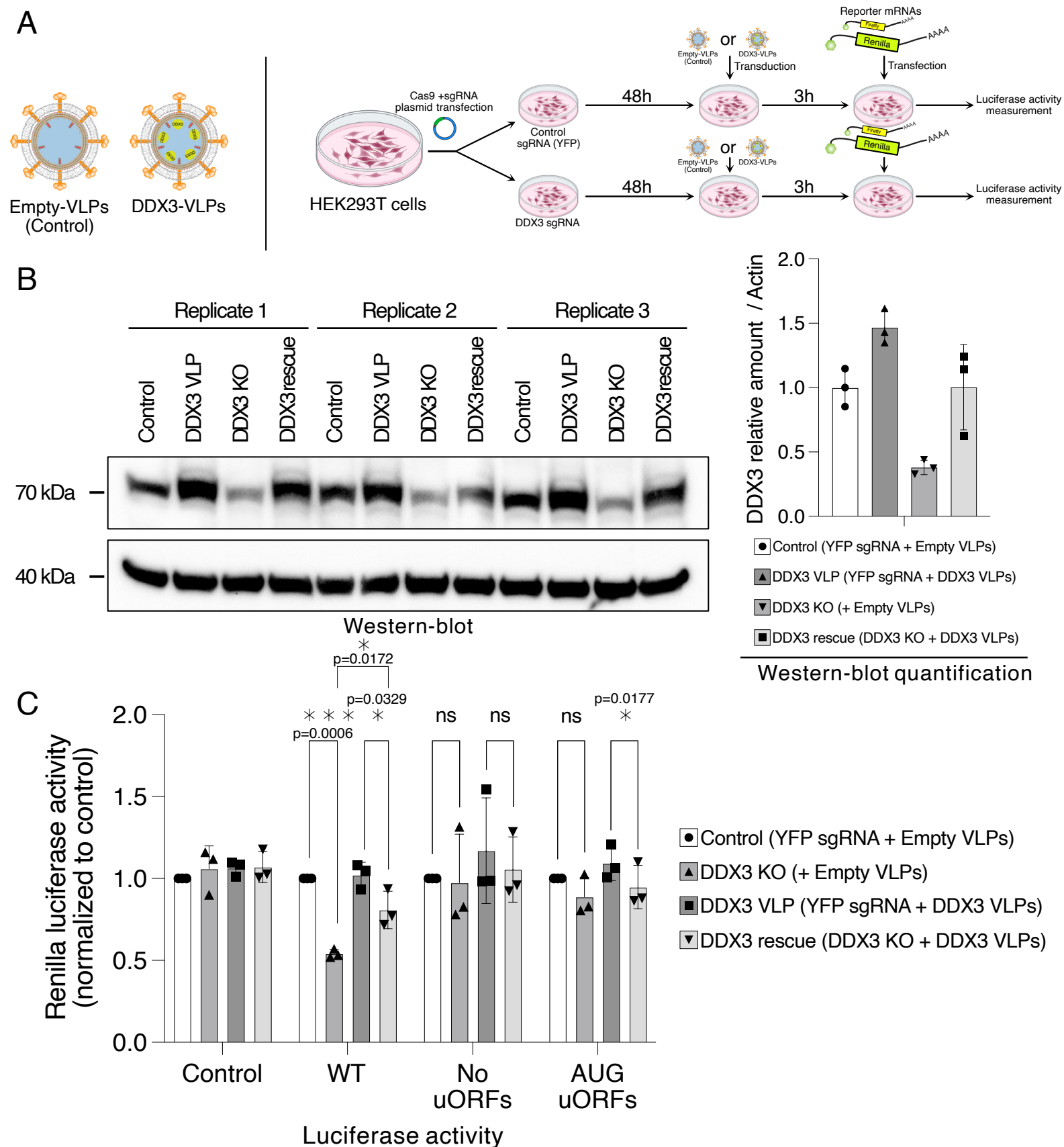
A Ribosome profiling in presence of Harringtonine from U937 cells induced with PMA and iomycin



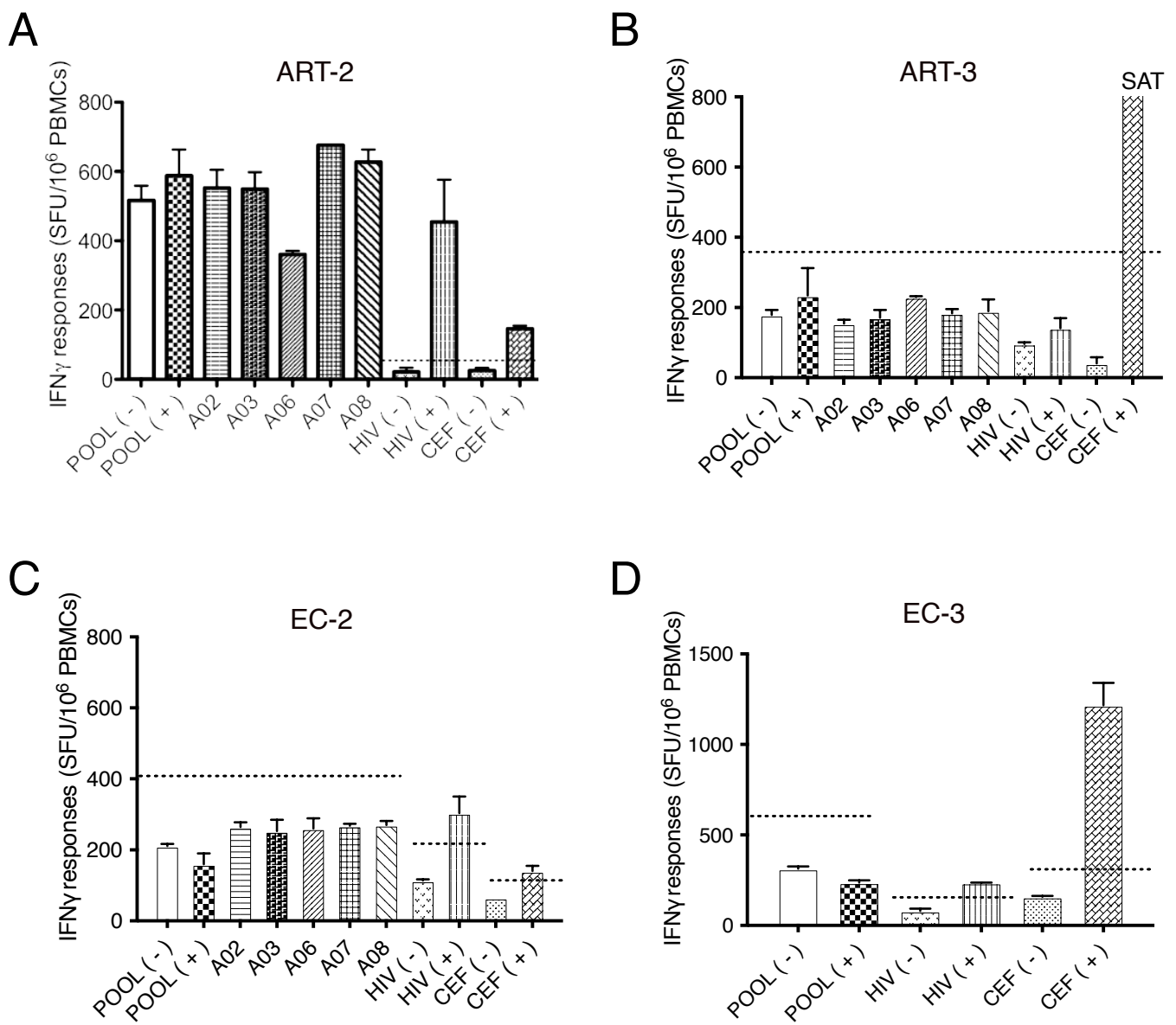
Supplementary Figure S5 (A), Distribution of ribosome profiling reads across the HIV-1 genome obtained from Harringtonine-treated U937 cells in which HIV-1 transcription was induced for 3, 6, 9, 12, 15, 18 and 24 h. The position of each viral gene and its corresponding canonical AUG start codon are depicted.



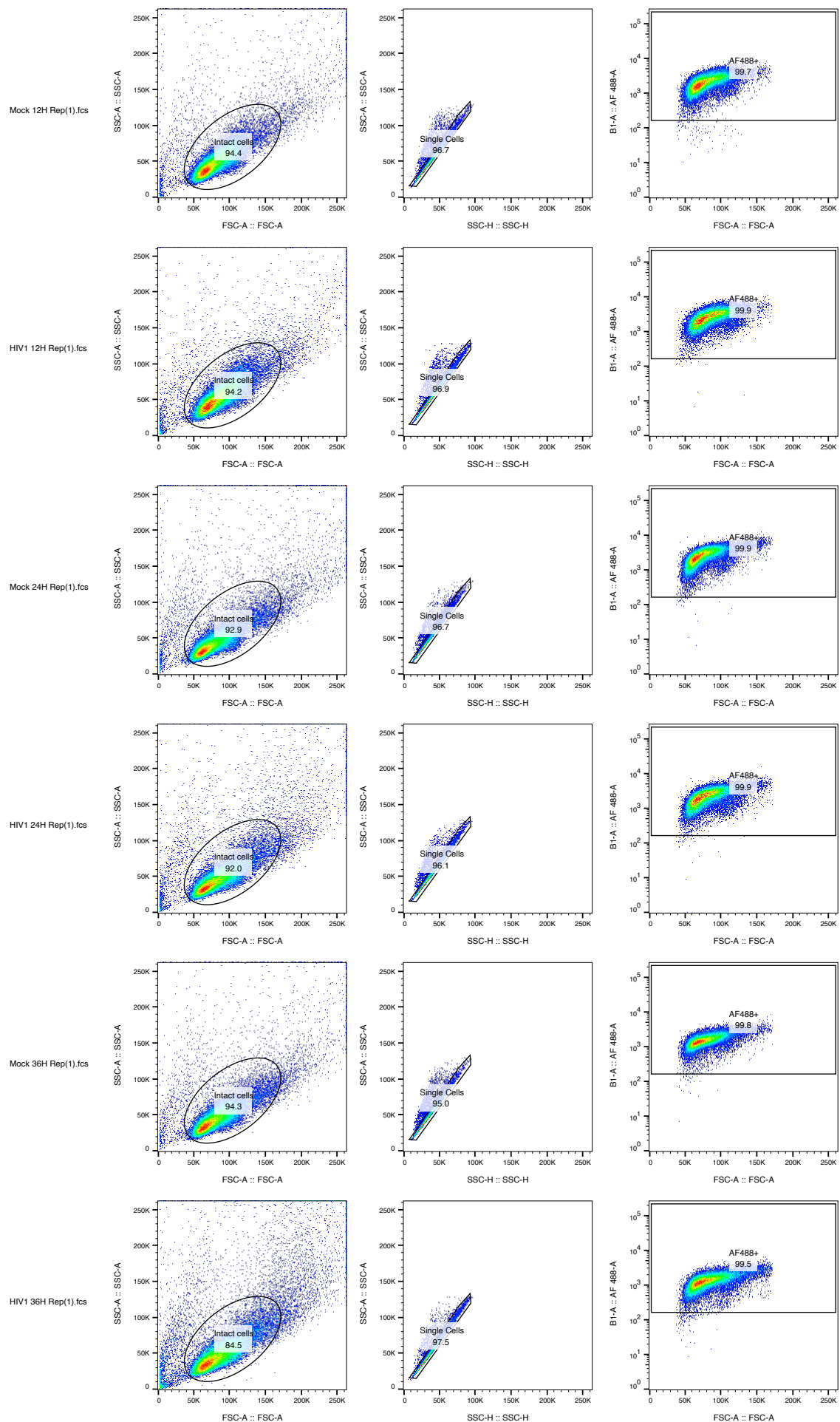
Supplementary figure S6 (A and B), Secondary structure of the HIV-1 wild-type 5'UTR (left) and No-uORFs 5'UTR (right) mutant followed by the renilla luciferase coding region as obtained by SHAPE-analysis. (C), Distribution of ribosome profiling reads across the 5'UTR of unspliced viral mRNAs in HIV-2 infected U937 cells incubated with cycloheximide or harringtonine before lysis ($n=3$ independent replicates). (D), Distribution of ribosome profiling reads across the 5'UTR of unspliced viral mRNA of HTLV-1 in immortalized T cells obtained from HTLV-1 infected humanized mice ($n=2$ independent replicates).



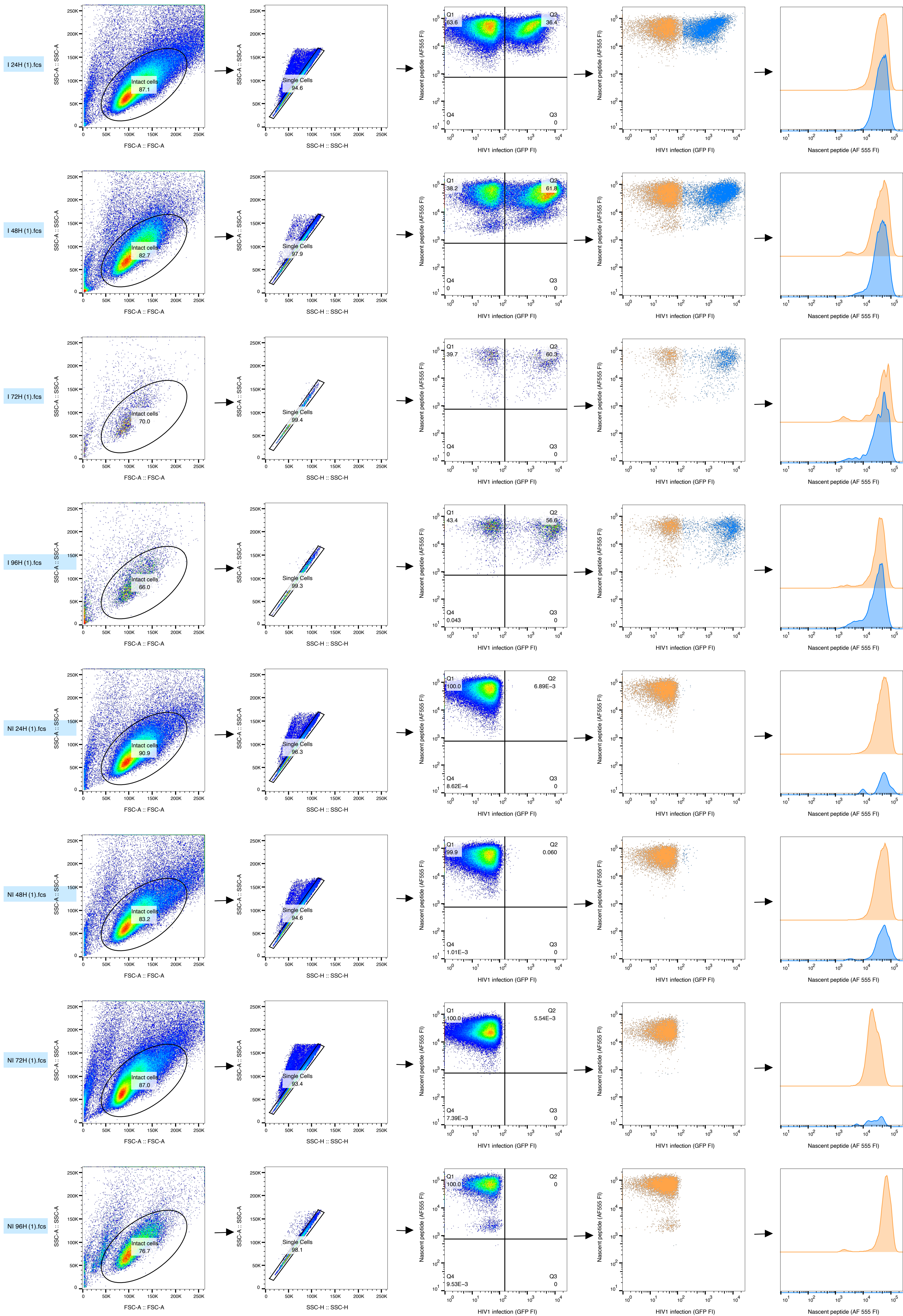
Supplementary Figure S7. Effect of DDX3 knockout and rescue upon delivery of ready-made DDX3 protein through Virus-like particles on HIV-1 5'UTR-dependent mRNA translation (A) Schemedepictingtheexperimental strategy (Schemewas partly created in BioRender. Ricci, E. (2022) BioRender.com/a59a503). Briefly, HEK293T were transfected with plasmids coding for Cas9 and sgRNAs targeting YFP or DDX3 coding sequences. After 48h cells were transduced either with empty VLPs or with VLPs loaded with human DDX3 ready-made proteins. 3h after transduction, cells are transfected with in vitro transcribed luciferase coding mRNAs driven by the globin 5'UTR or HIV-1 5'UTRs. A firefly coding mRNA is also transfected to normalize renilla luciferase counts. Cells are lysed 3h after transfection and luciferase activity measured. (B) Western-blot against DDX3 or beta-actin from total cell lysates (left panel) and its quantification (right panel). n=3 independent replicates. (C), Relative renilla luciferase activity for each 5'UTR normalized against the control condition (YFP sgRNA + Empty VLPs) n=3 independent replicates. A one-tailed Student t-test was performed to assess the differences between the mean values of compared conditions. Error bars correspond to the standard deviation of the mean values.



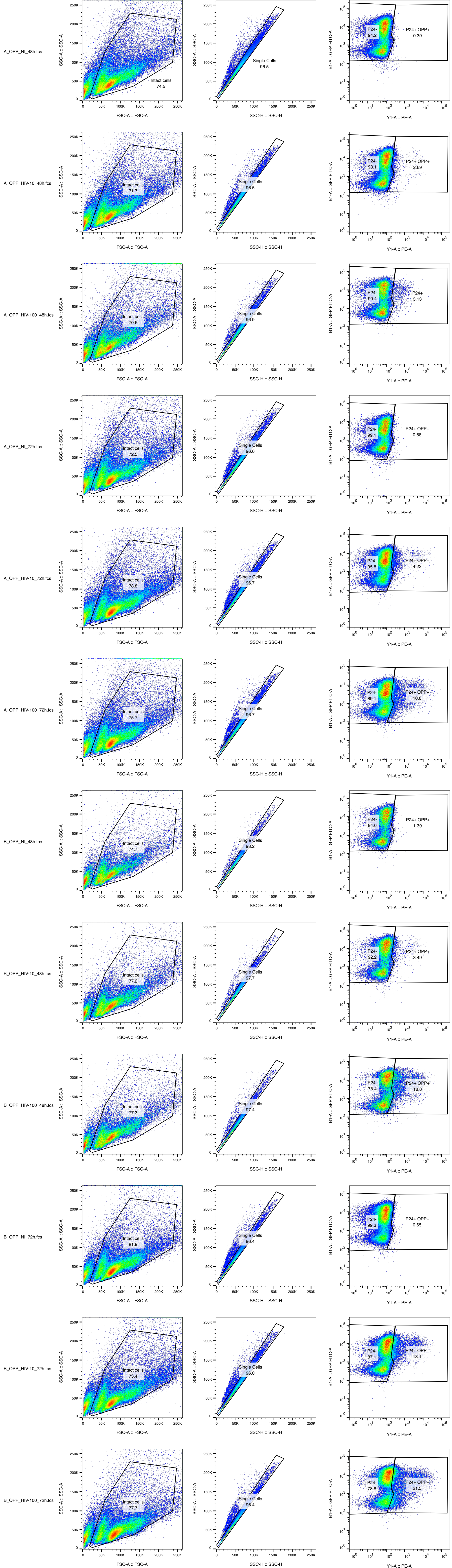
Supplementary figure S8 (A to D), Four HIV infected individuals with no 5'LTR uORF-specific T cell responses. IFN γ -Elispot data from the 4 individuals, from Figure 9C, presenting no T cell responses against 5'LTR uORF-derived peptides, expressed as spot forming units (SFU) per million PBMCs. As in Figure 9A to 9D, memory T cells from ART and EC were stimulated/expanded with the pool of uORF derived peptides (POOL), the HIV pool (HIV) or an additional control pool (CEF) composed of peptides derived from CMV, EBV and Flu viruses. (A, B, C), on day 12 T cell responses against the POOL, HIV and CEF pools as well as individual 5'LTR uORF-derived peptides (A02; A03; A06; A07; A08) were assessed using IFN γ -Elispot. (D), T cell responses of donor EC-3 were assessed on day 7 with the indicated pools. Since no reactivity to the pool of 5'LTR uORF-derived peptides (POOL) was detected, T cell responses to the individual peptides were not assessed on day 12 for donor EC-3. POOL (-) and POOL (+): PBMCs expanded with the pool of uORF peptides but restimulated on the day of the Elispot with medium or the POOL, respectively. A02; A03; A06; A07 and A08 name of individual uORF-derived peptides used for restimulation. HIV (-) and HIV (+): PBMCs expanded with the pool of Env- and Gag-derived peptides but restimulated for the Elispot assay with medium or the same pool of HIV peptides, respectively. CEF (-) and CEF (+) PBMCs expanded with the pool of virus-derived peptides but restimulated for the Elispot assay with medium or the same CEF pool, respectively. Responses were considered positive when IFN γ production was superior to 20 spots/10⁶ PBMCs and at least twofold higher than background production from cells restimulated with medium (dotted lines). SAT: saturated signal, where counts cannot be estimated due to overwhelm IFN γ secretion by activated T cells.



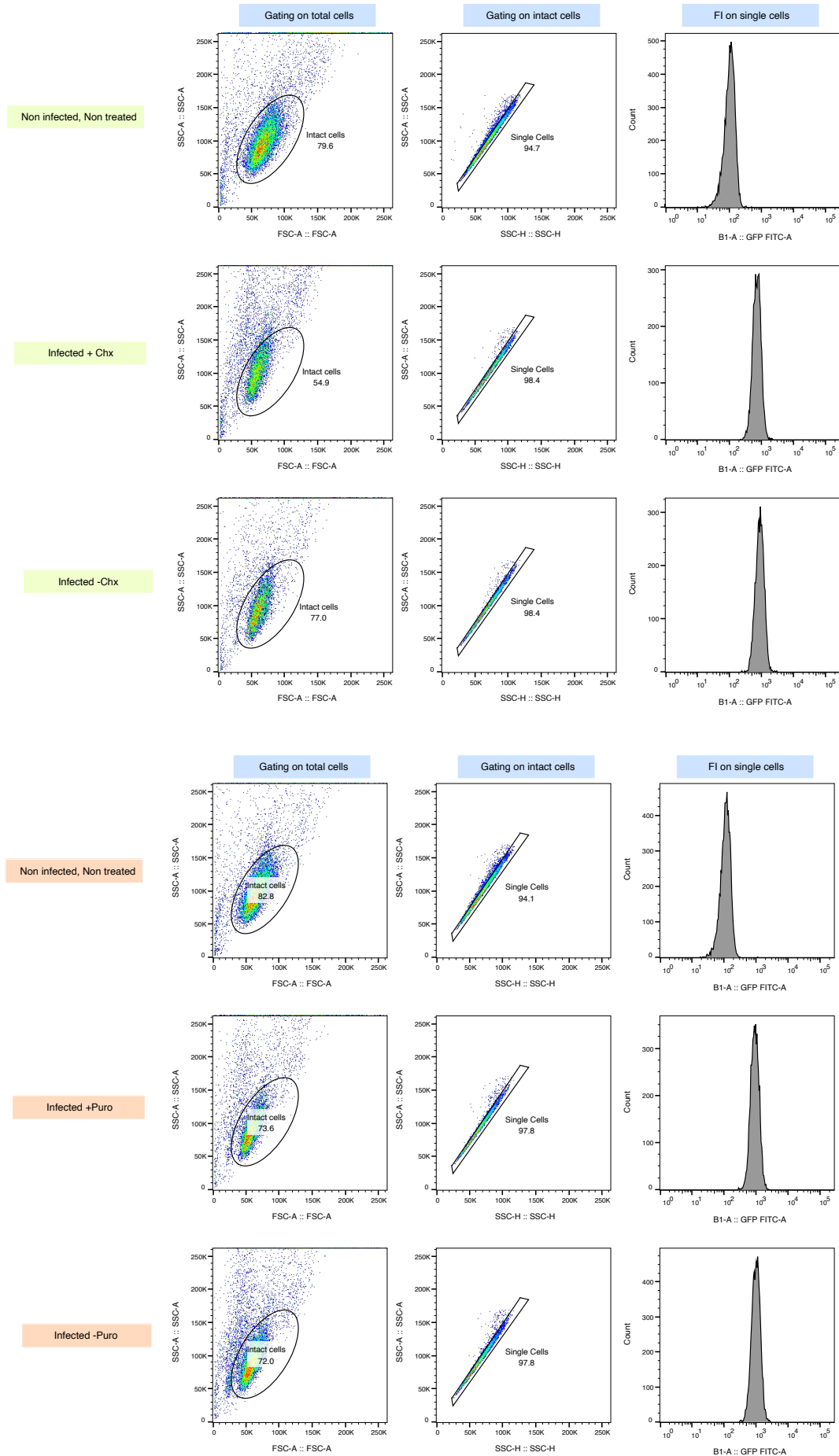
Supplementary Figure S9. Gating strategy of the flow cytometry results presented in Figure 2B.



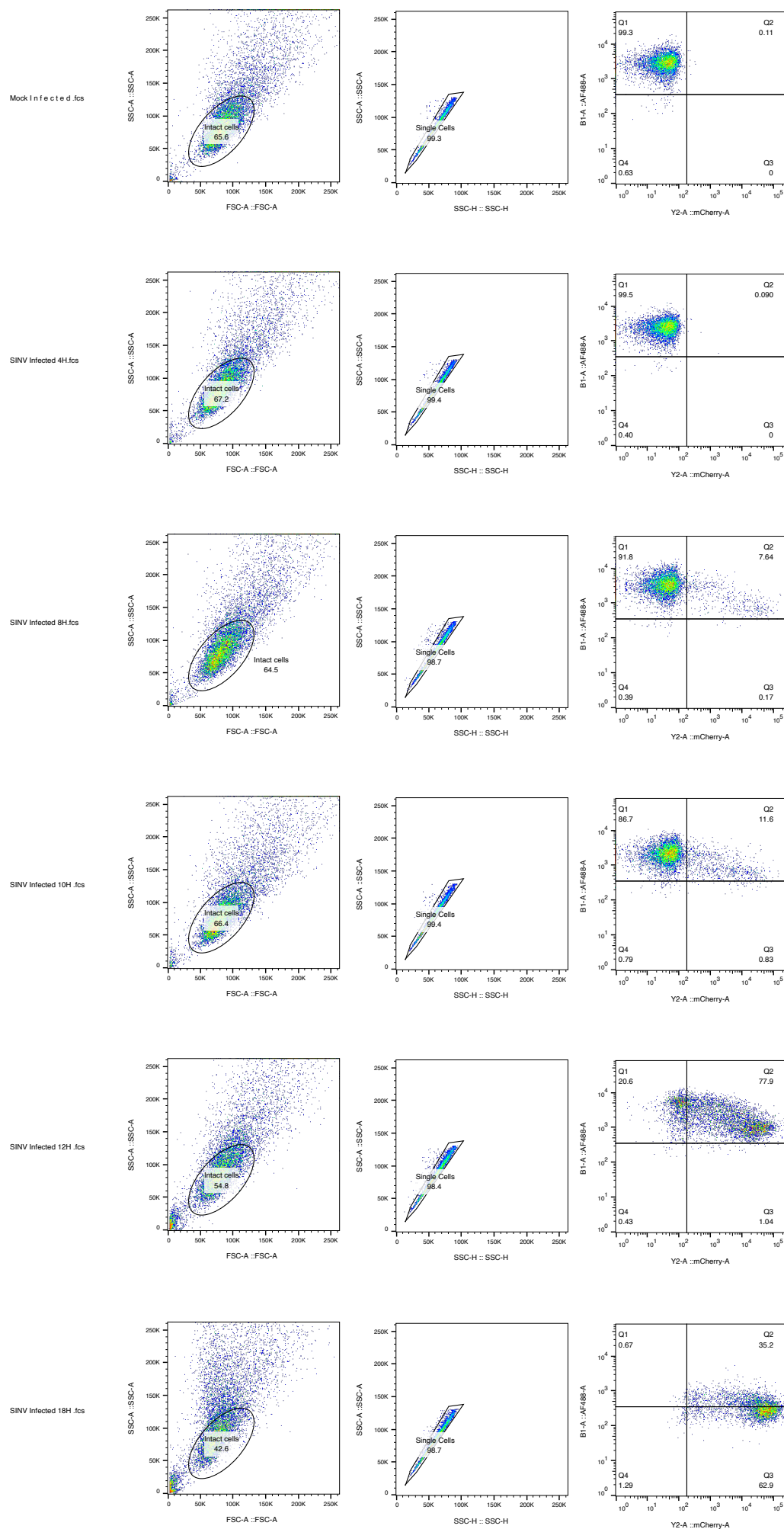
Supplementary Figure S10. Gating strategy of the flow cytometry results presented in Figure 2C.



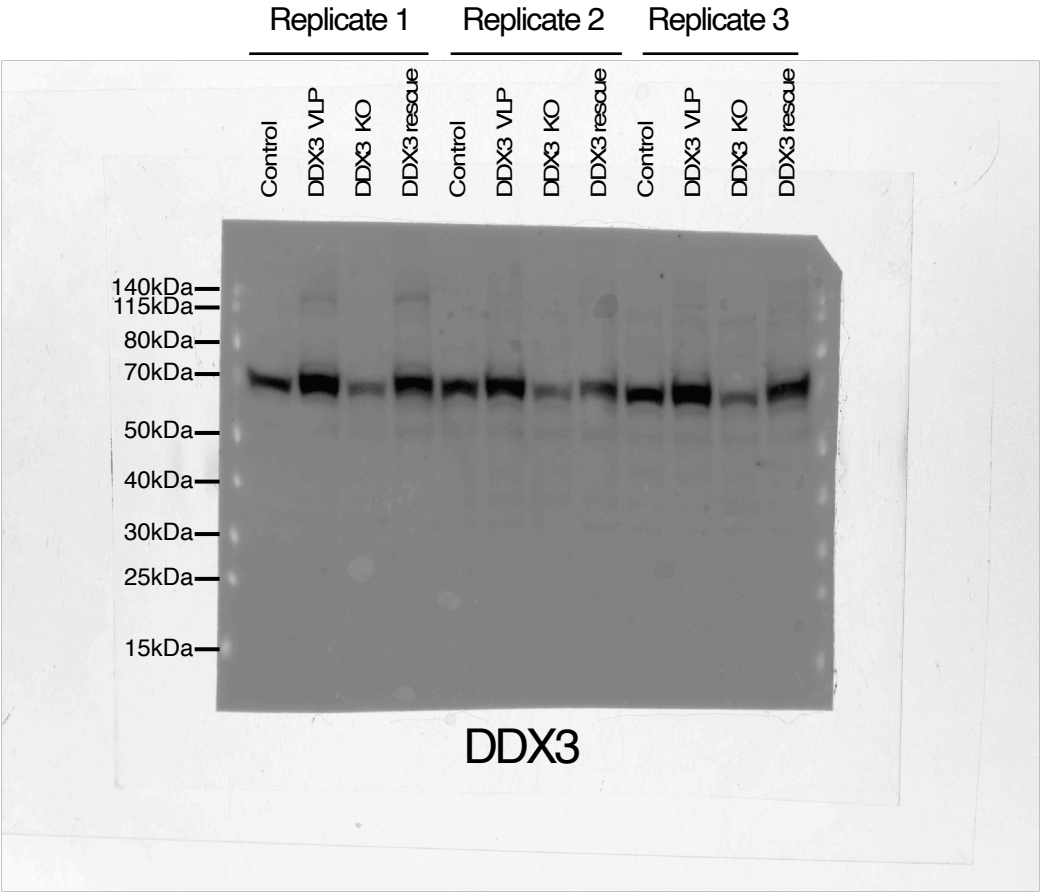
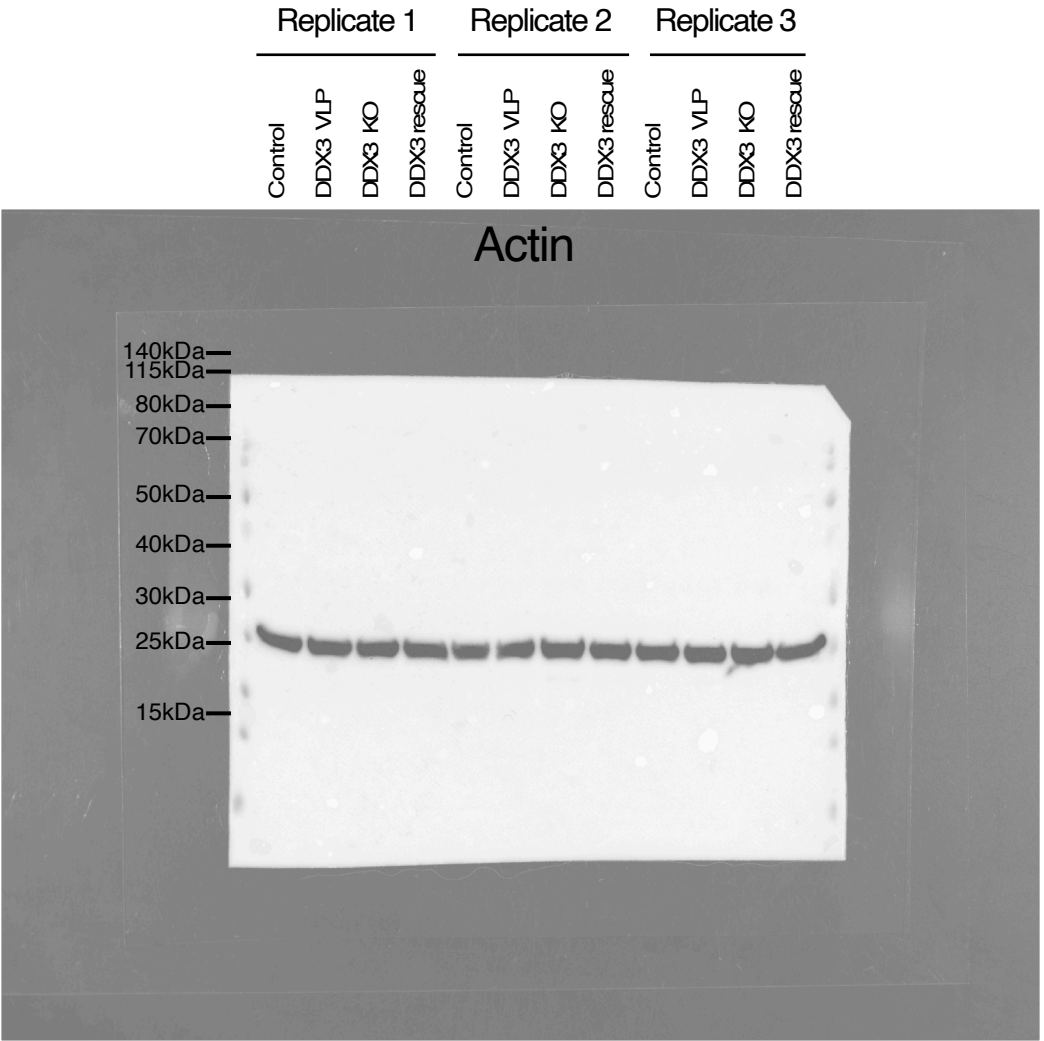
Supplementary Figure S11. Gating strategy of the flow cytometry results presented in Figure 2D.



Supplementary Figure S12. Gating strategy of the flow cytometry results presented in Figure 5D.



Supplementary Figure S13. Gating strategy of the flow cytometry results presented in Supplementary figure 2A.



Uncropped images of anti beta-actin and DDX3 western-blot presented in Supplementary Figure 7B