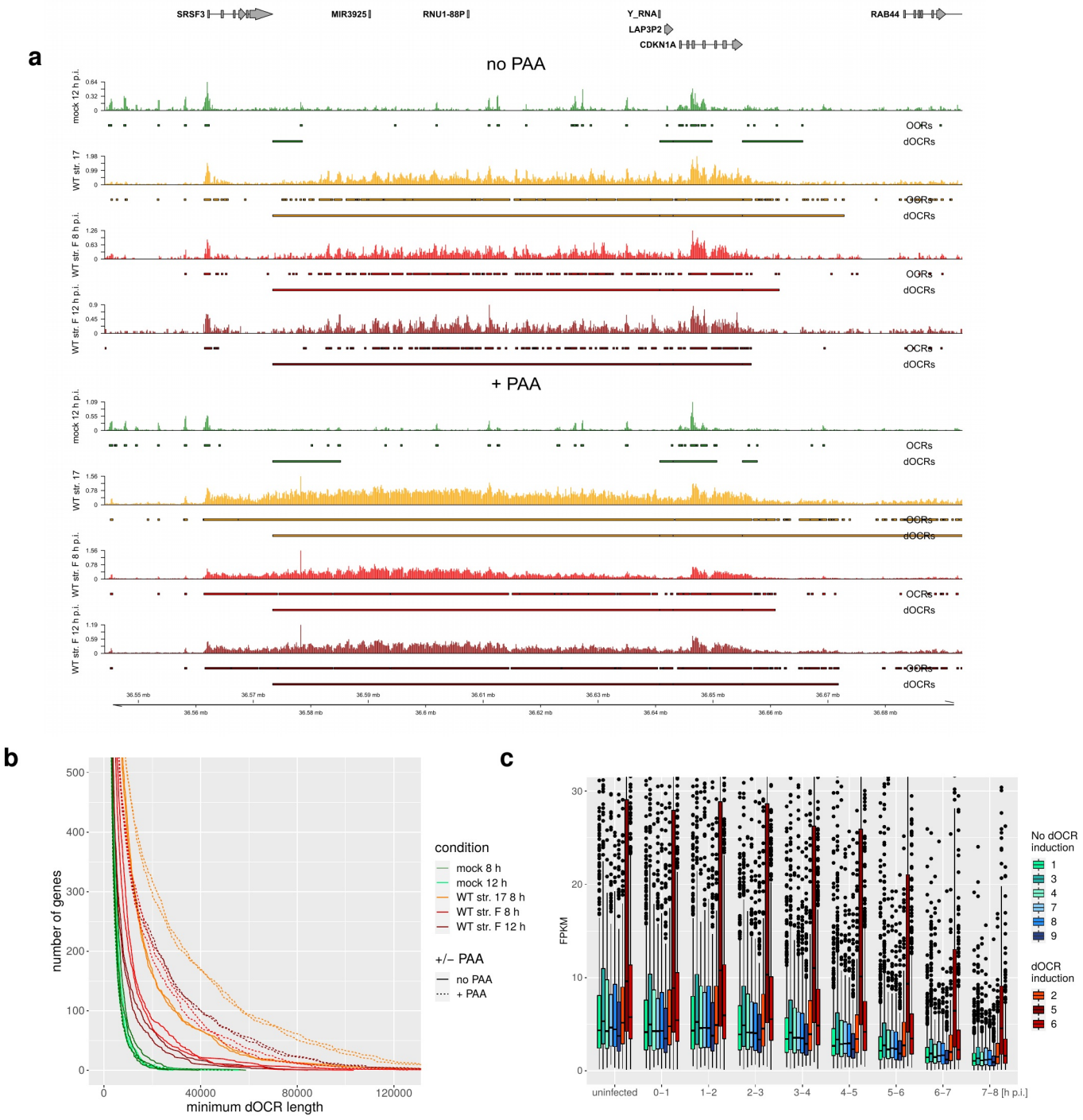
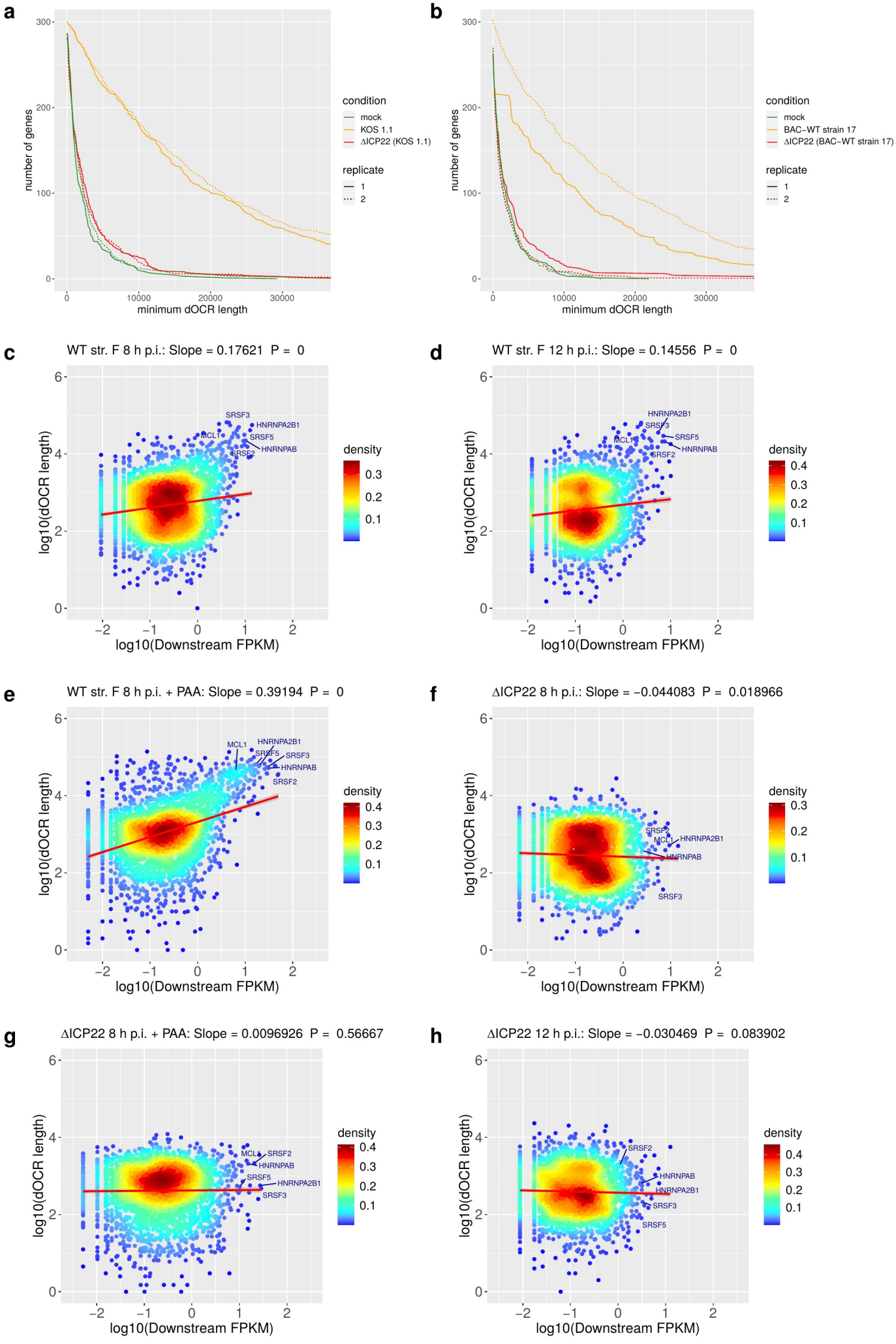
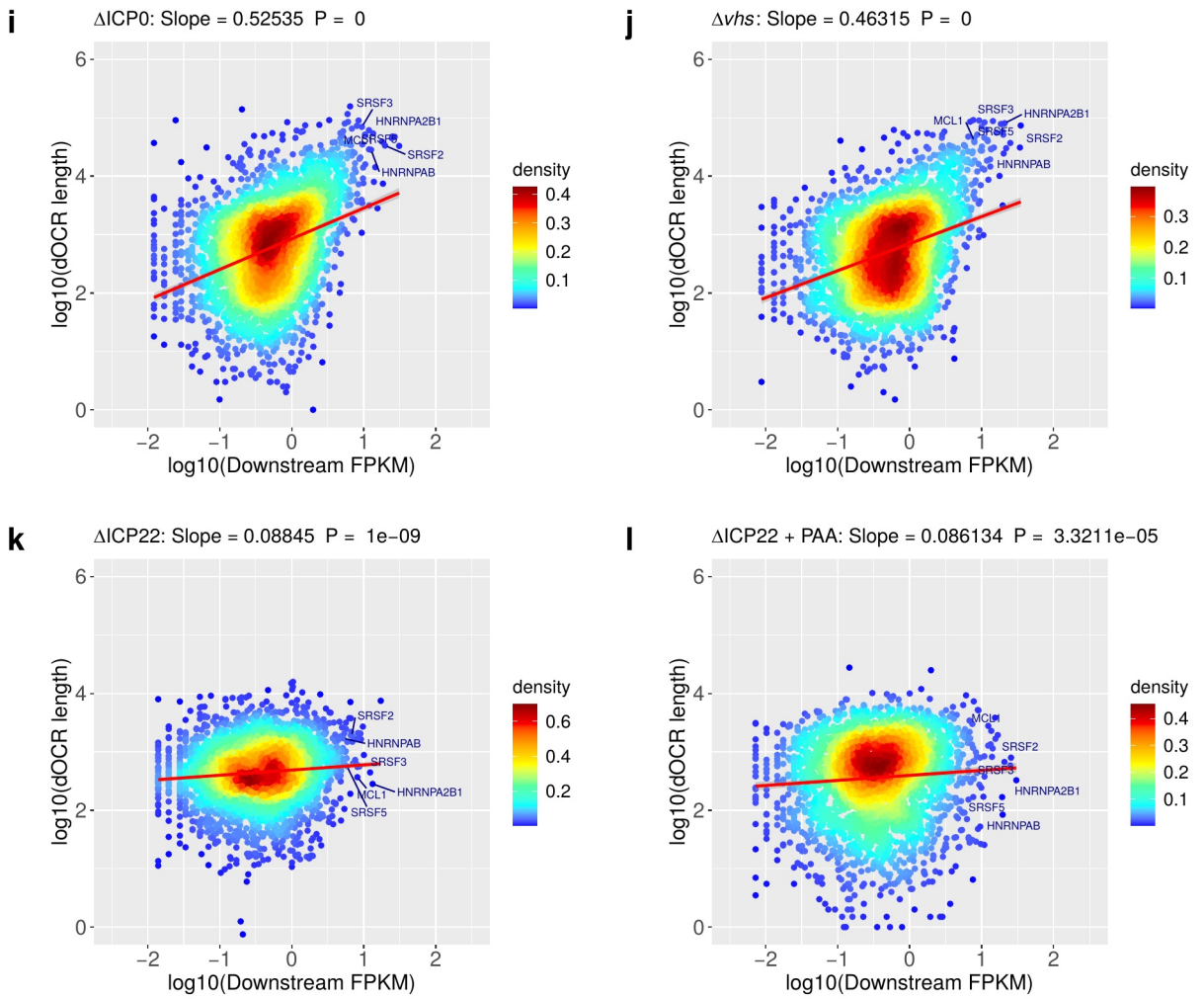
S1 File: Supplementary Figures



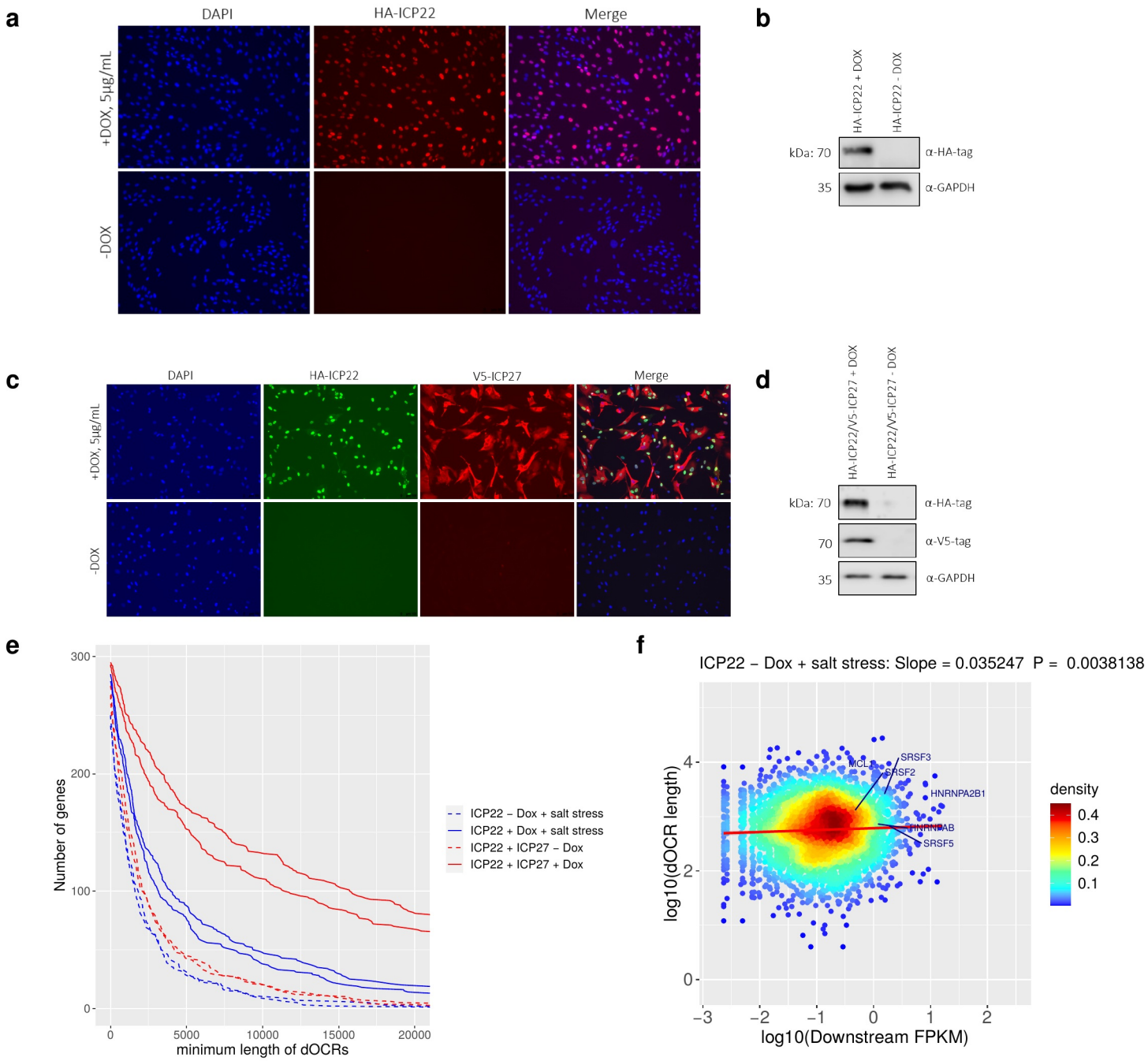
Sup. Fig. 1: (a) ATAC-seq data for mock, WT stain 17 and WT strain F infection without and with PAA treatment for an example gene (SRSF3). Tracks show ATAC-seq read coverage (normalized to total number of mapped human reads and averaged between replicates) followed by open chromatin regions (OCRs) identified with F-Seq as well as the dOCR regions calculated from the OCRs as described in Materials and Methods. OCRs and dOCRs are always shown only for the first replicate for simplification.Gene annotation is indicated at the top. Boxes represent exons and lines introns and strand is indicated by arrowheads. Genomic coordinates are shown on the bottom. **(b)** Number of genes (y-axis) for which dOCRs reach at least the length indicated on the x-axis in mock and WT strain 17 and F HSV-1 infection after down-sampling to approximately the same number of reads mapped to the human genome. **(c)** Boxplots show the distribution of gene expression (gene FPKM)

for the different clusters from Fig. 1b. Gene FPKM values were calculated from our published 4sU-seq time-course for the first 8 h of WT strain 17 infection [1]. Cluster 5 (strong induction of dOCRs) is characterized by substantially higher FPKM values than all other clusters already in uninfected cells and throughout infection. Clusters 2 shows no significant difference compared to clusters without induction of dOCRs, while cluster 6 exhibits slightly increased gene FPKMs throughout the whole time-course.


a
number of genes
minimum dOCR length
condition
mock
KOS 1.1
ΔICP22 (KOS 1.1)
replicate
1
2
b
number of genes
minimum dOCR length
condition
mock
BAC−WT strain 17
ΔICP22 (BAC−WT strain 17)
replicate
1
2
c
WT str. F 8 h p.i.: Slope = 0.17621 P = 0
log10(dOCR length)
log10(Downstream FPKM)
density
SRSF3
HNRNPA2B1
MCL1
SRSF5
HNRNPAB
SRSF2
d
WT str. F 12 h p.i.: Slope = 0.14556 P = 0
log10(dOCR length)
log10(Downstream FPKM)
density
HNRNPA2B1
SRSF3
MCL1
SRSF5
HNRNPAB
SRSF2
e
WT str. F 8 h p.i. + PAA: Slope = 0.39194 P = 0
log10(dOCR length)
log10(Downstream FPKM)
density
MCL1
HNRNPA2B1
SRSF5
SRSF3
HNRNPAB
SRSF2
f
ΔICP22 8 h p.i.: Slope = −0.044083 P = 0.018966
log10(dOCR length)
log10(Downstream FPKM)
density
SRSF2
MCL1
HNRNPA2B1
HNRNPAB
SRSF3
g
ΔICP22 8 h p.i. + PAA: Slope = 0.0096926 P = 0.56667
log10(dOCR length)
log10(Downstream FPKM)
density
MCL1
SRSF2
HNRNPAB
SRSF5
HNRNPA2B1
SRSF3
h
ΔICP22 12 h p.i.: Slope = −0.030469 P = 0.083902
log10(dOCR length)
log10(Downstream FPKM)
density
SRSF2
HNRNPAB
HNRNPA2B1
SRSF3
SRSF5

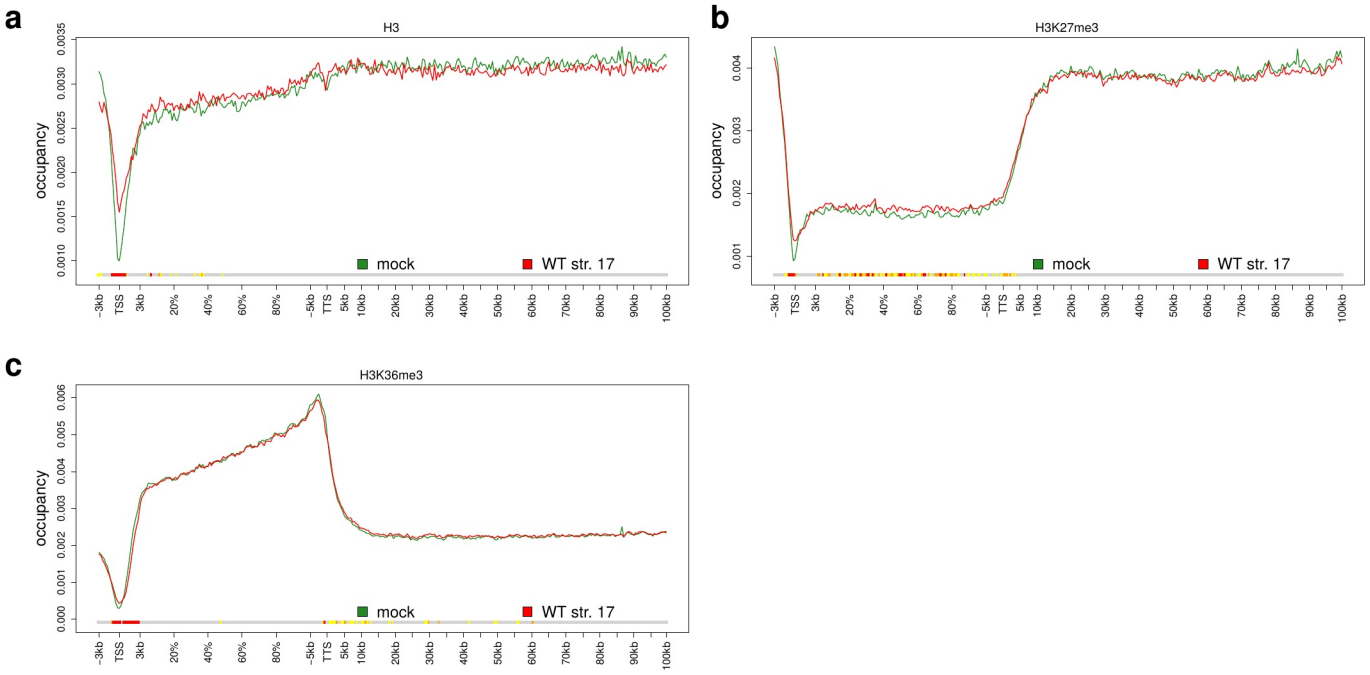


Sup. Fig. 2: (a-b) Number of genes in cluster 5 (y-axis) for which dOCRs reach at least the length indicated on the x-axis in **(a)** mock, WT strain KOS1.1 infection and infection with a ΔICP22 mutant derived from KOS1.1 (all with PAA treatment) and **(b)** mock, WT strain 17 (BAC-derived) infection and infection with a ΔICP22 mutant derived from BAC-WT strain 17 (no PAA treatment). **(c-h)** Scatter plots as in Fig. 2c,d correlating downstream FPKM in total RNA against dOCR length (average of two replicates). The red line indicates a linear fit of log10(dOCR length) against log10(downstream FPKM). Colors indicate density of points from high (red) to low (blue). Slope of the fit and p-value for the significance of the linear correlation are indicated on top of each figure. Example genes with strong induction of dOCRs in HSV-1 infection are highlighted. Results are shown for **(c-e)** WT strain F ±PAA and **(f-h)** ΔICP22 infection ±PAA. Scatter plots for 12 h p.i. WT strain F/ΔICP22 + PAA infection are shown in Fig. 2c,d. **(i-l)** Scatter plots as in Fig. 2e,f correlating downstream FPKM in 4sU-RNA against dOCR length (average of two replicates). Results are shown for (i) ΔICP0, (j) Δ*vhs* and (k-l) ΔICP22 infection without (k) and with (l) PAA treatment. Scatter plots for WT strain 17 infection and ΔICP27 infection are shown in Fig. 2e,f.

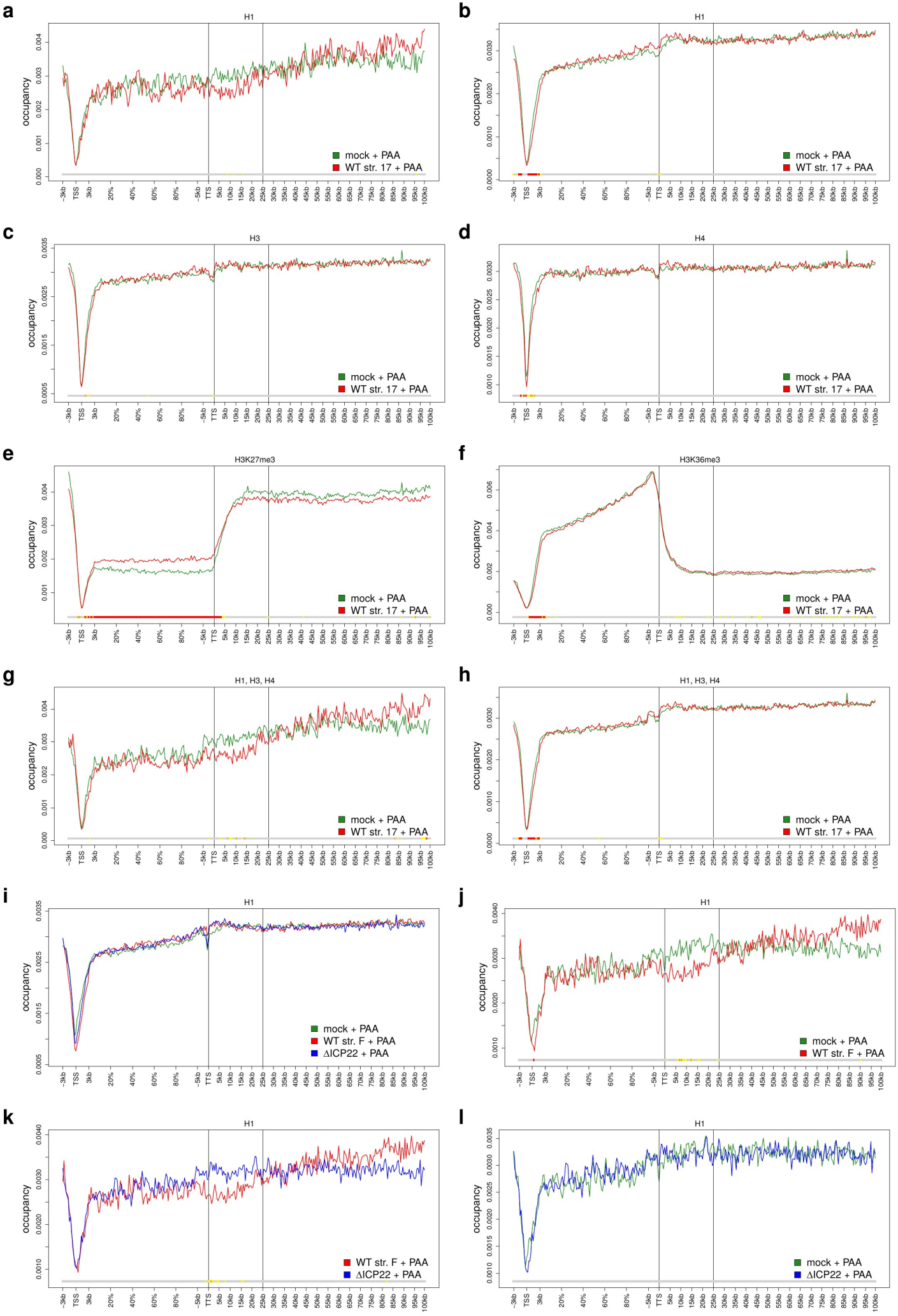


Sup. Fig. 3: (a-b) Generation of polyclonal HA-ICP22 doxycycline-inducible cells. T-HFs transduced with inducible HA-ICP22-expressing lentivirus were seeded with/without presence of 5μg/mL doxycycline and collected 48h post induction. Upon addition of doxycycline cells express HA-ICP22. Immunofluorescence and western blot images were obtained from the day of ATAC/Omni-ATAC-seq experiment. **(a)** HA-ICP22 protein is shown in red and DAPI depicts cell nuclei. Mean value of cells expressing HA-ICP22 was calculated from there different fields of the same experiment and is $\sim$88%. **(b)** Total cell lysates were collected 48h post Dox-induction and were probed for HA-tag. GAPDH was used as a loading control. **(c-d)** Generation of polyclonal HA-ICP22+V5-ICP27 doxycycline-inducible cells. V5-ICP27 doxycycline-inducible polyclonal cells transduced with inducible HA-ICP22-expressing lentivirus were seeded with/without presence of 5μg/mL doxycycline and collected 48h post induction. Upon addition of doxycycline cells express both HA-ICP22 and V5-ICP27. Immunofluorescence and western blot images were obtained from the day of ATAC/Omni-ATAC-seq experiment. (**c**) HA-ICP22 protein is shown in green, V5-ICP27 is shown in red and DAPI

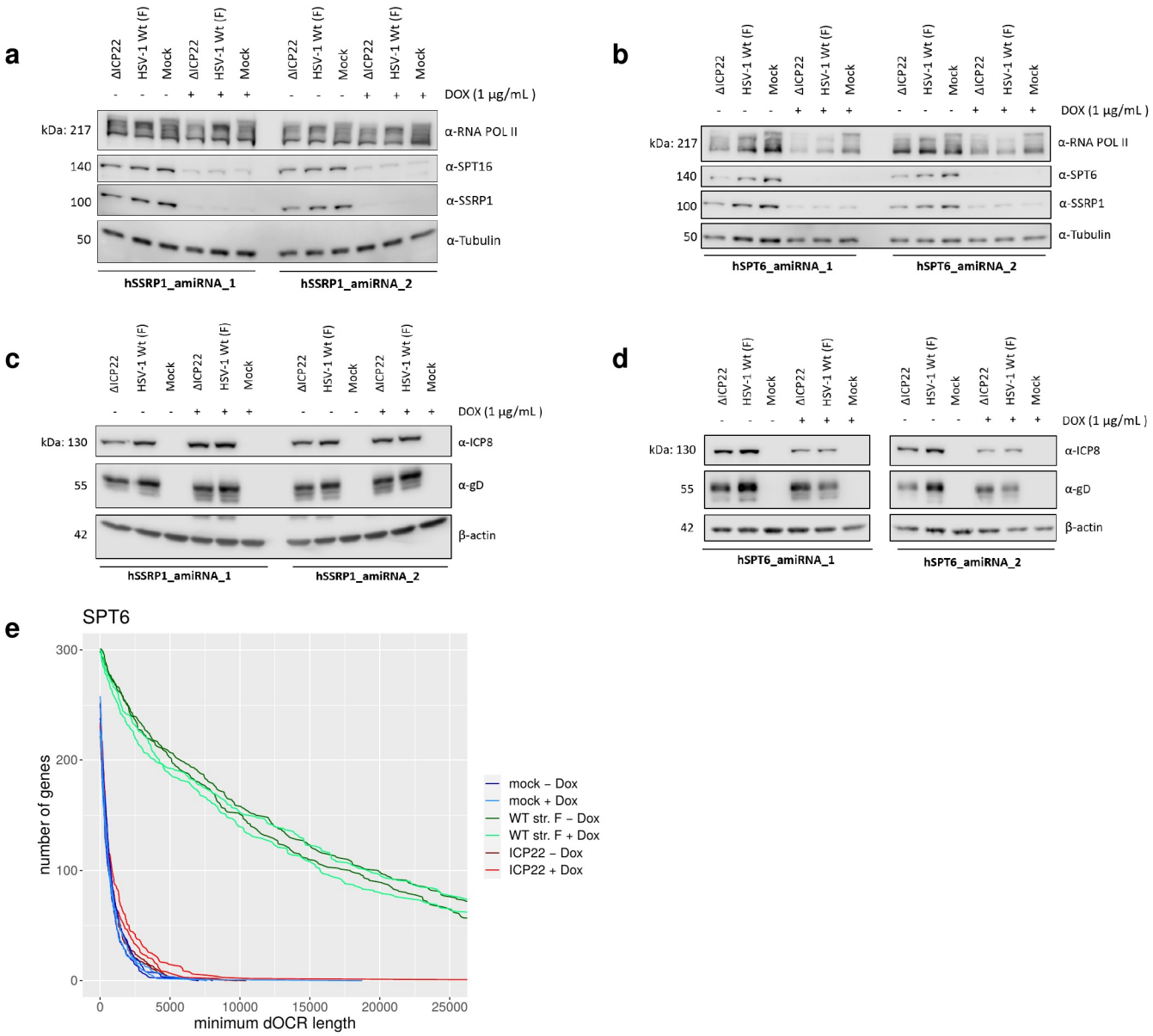
depicts cell nuclei. Mean value of cells expressing HA-ICP22, V5-ICP27, or HA-ICP22 and V5-ICP27 was calculated from three different fields of the same experiment and is $\sim$80%, 90%, 90% respectively. (**d**) Total cell lysates were collected 48h post Dox-induction and were probed with for HA-tag and V5-tag. GAPDH was used as a loading control. **(e)** Number of genes in cluster 5 (y-axis) for which dOCRs reach at least the length indicated on the x-axis in T-HFs-ICP22/ICP27 cells $\pm$ Dox (red) and T-HFs-ICP22 cells ($\pm$ Dox) exposed to 2 h salt stress (blue). Results are shown after down-sampling to approximately the same number of reads on the cellular genome. **(f)** Scatter plot as in Fig. 3c,d correlating downstream FPKM in total RNA (x-axis) against dOCR length (average of two replicates) for 2 h salt stress without Dox-induced ICP22 expression (T-HFs-ICP22 cells - Dox).



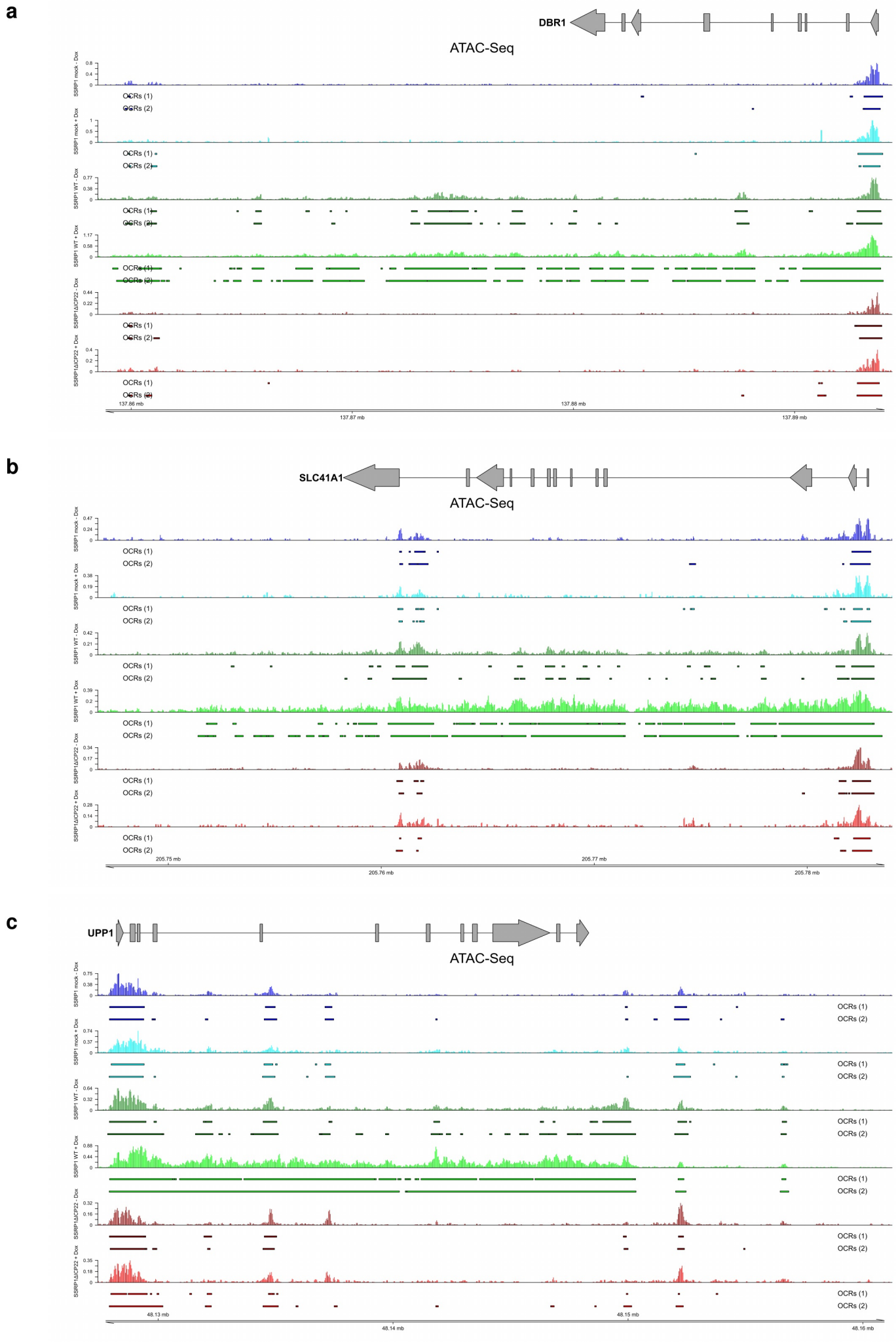
Sup. Fig. 4: Metagene plots as in **Fig. 4a-c** showing the distribution of **(a)** H3, **(b)** H3K27me3 and **(b)** H3K36me3 for genes without induction of dOCRs in mock and WT strain 17 infection. The color track at the bottom of each subfigure indicates the significance of paired Wilcoxon tests comparing the normalized transcript coverages of genes for each position between mock and WT infection. P-values are adjusted for multiple testing with the Bonferroni method within each subfigure; color code: red = adj. p-value $\leq 10^{-5}$, orange = adj. p-value $\leq 10^{-3}$, yellow = adj. p-value ≤ 0.05.


a
H1
occupancy
mock + PAA
WT str. 17 + PAA
b
H1
occupancy
mock + PAA
WT str. 17 + PAA
c
H3
occupancy
mock + PAA
WT str. 17 + PAA
d
H4
occupancy
mock + PAA
WT str. 17 + PAA
e
H3K27me3
occupancy
mock + PAA
WT str. 17 + PAA
f
H3K36me3
occupancy
mock + PAA
WT str. 17 + PAA
g
H1, H3, H4
occupancy
mock + PAA
WT str. 17 + PAA
h
H1, H3, H4
occupancy
mock + PAA
WT str. 17 + PAA
i
H1
occupancy
mock + PAA
WT str. F + PAA
ΔICP22 + PAA
j
H1
occupancy
mock + PAA
WT str. F + PAA
k
H1
occupancy
WT str. F + PAA
ΔICP22 + PAA
l
H1
occupancy
mock + PAA
ΔICP22 + PAA

Sup. Fig. 5: (a) Metagene plot showing the distribution of H1 for genes with strong induction of dOCRs (cluster 5) in mock and WT strain 17 infection with PAA treatment. **(b-f)** Metagene plots showing the distribution of **(b)** H1, **(c)** H3, **(d)** H4, **(e)** H3K27me3 and **(f)** H3K36me3 for genes without induction of dOCRs, i.e. all analyzed genes without clusters 2,5 and 6, in mock and WT strain 17 infection with PAA treatment. **(g,h)** Metagene plots combining histones H1, H3 and H4 for genes with strong induction of dOCRs (cluster 5) **(g)** and genes without induction of dOCRs **(h)**. **(i)** Metagene plot for histone H1 for genes without induction of dOCRs for mock, WT strain F and $\triangle$ICP22 infection. **(j-l)** Metagene plots for histone H1 for cluster 5 for the pairwise comparison of mock vs. WT strain F infection **(j)** , WT strain F vs. $\triangle$ICP22 infection **(k)** and mock vs. $\triangle$ICP22 infection **(l)** . (**(a-h,j-l)**) The color track at the bottom of each subfigure indicates the significance of paired Wilcoxon tests comparing the normalized transcript coverages for each position between mock and WT infection. P-values are adjusted for multiple testing with the Bonferroni method within each subfigure; color code: red = adj. p-value $\leq 10^{-5}$, orange = adj. p-value $\leq 10^{-3}$, yellow = adj. p-value ≤ 0.05.



Sup. Fig. 6: (a-b) Knock-down of SSRP1 **(a)** and SPT6 **(b)** in T-HFs by an inducible, lentiviral construct encoding an artificial miRNAs (amiRNAs). **(a)** Knock-down of SSRP1 was induced with 1 μg/mL doxycycline (hSSRP1_amiRNA_1 and hSSRP1_amiRNA_2) and compared to control cells. **(b)** Knock-down of SPT6 was induced with 1 μg/mL doxycycline (hSPT6_amiRNA_1 and hSPT6_amiRNA_2) and compared to control cells. In both cases, cells were infected with WT HSV-1 (F) or ΔICP22 at an MOI of 10. PAA was used at 350 μg/mL during the course of infection. Total cell lysates were collected at 72 hours post-induction with doxycycline and western blot was performed using antibodies, as indicated. α-Tubulin was used as a loading control. **(c-d)** Expression of HSV-1 proteins in SSRP1 **(c)** and SPT6 **(d)** knock-down cells. Cells were infected with WT HSV-1 (F) or ΔICP22 at an MOI of 10 or Mock infected for 8 hours. PAA was used at 350 μg/mL during the course of infection. Total cell lysates were collected at 72 hours post-induction with doxycycline. Western blots were probed for two HSV-1 viral proteins, ICP8 and glycoprotein D. β-actin was used as a loading control. **(e)** Number of genes in cluster 5 that exhibit dOCRs with at least a certain length (x-axis) in mock, WT strain F and ΔICP22 with or without Dox-induced knockdown of SPT6 after down-sampling of reads to approximately the same number of reads mapping to the human genome.


a
DBR1
ATAC-Seq
SSRP1 mock - Dox
SSRP1 mock + Dox
SSRP1 WT - Dox
SSRP1 WT + Dox
SSRP1ΔUCP22 - Dox
SSRP1ΔUCP22 + Dox
OCRs (1)
OCRs (2)
137.86 mb
137.87 mb
137.88 mb
137.89 mb
b
SLC41A1
ATAC-Seq
205.75 mb
205.76 mb
205.77 mb
205.78 mb
c
UPP1
ATAC-Seq
48.13 mb
48.14 mb
48.15 mb
48.16 mb

Sup. Fig. 7: Example genes (**(a)** DBR1, **(b)** SLC41A1, **(c)** UPP1) showing increased chromatin accessibility within the gene body in SSRP1-depleted cells in HSV-1 infection. Tracks show ATAC-seq read coverage (normalized to total number of mapped human reads; averaged between replicates) in mock, WT and $\triangle$ICP22 infection without and with Dox-induced SSRP1 depletion. Identified OCRs for both replicates are shown separately below the read coverage tracks. Gene annotation is indicated at the top. Boxes represent exons and lines introns and strand is indicated by arrowheads. Genomic coordinates are shown on the bottom.

References

[1] Rutkowski AJ, Erhard F, L'Hernault A, Bonfert T, Schilhabel M, Crump C, et al. Widespread disruption of host transcription termination in HSV-1 infection. Nature communications. 2015;6:7126. doi:10.1038/ncomms8126.