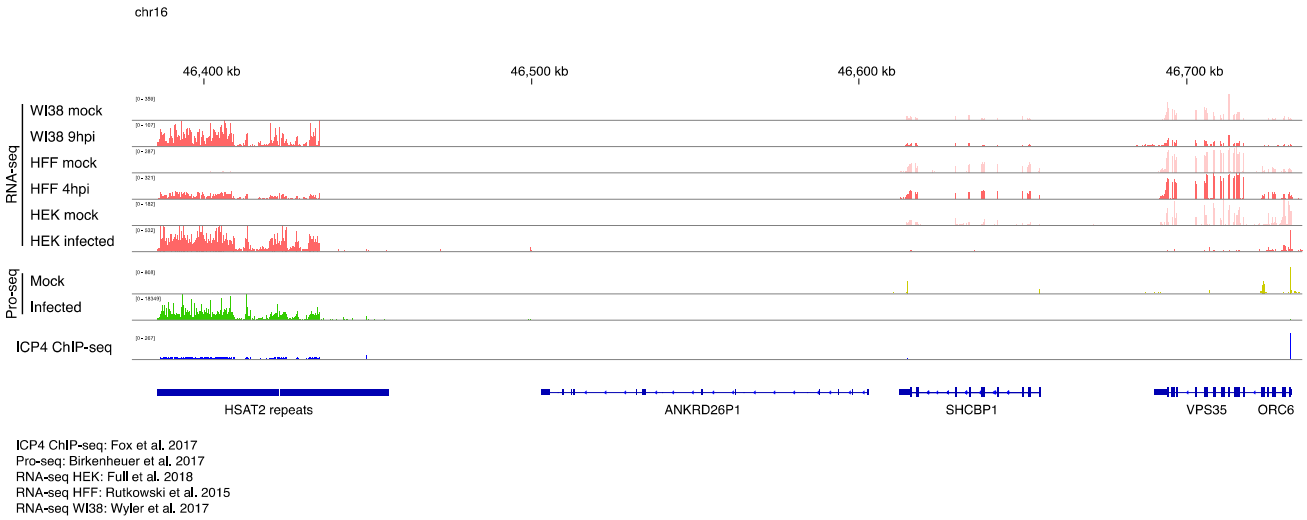
Supplemental Figure 1

A



B

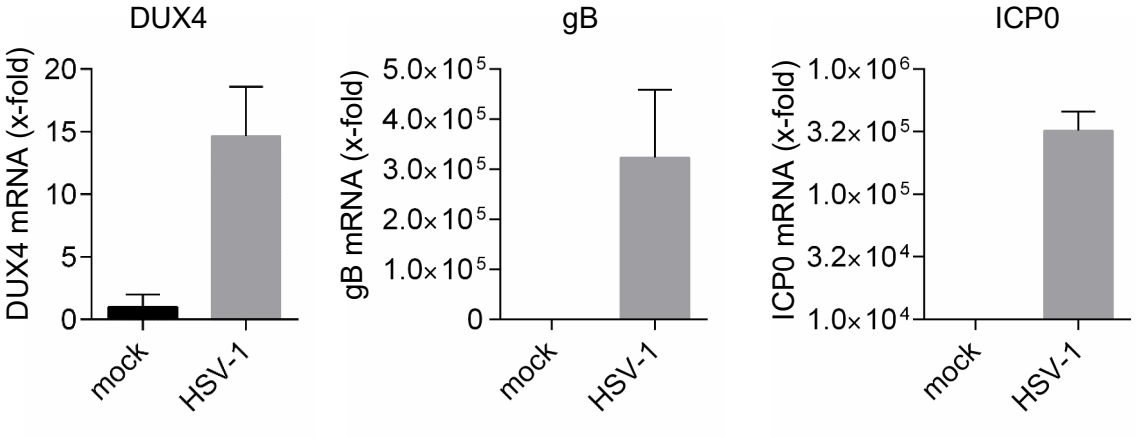


Fig. S1. A RNA-Seq. data of the HSATII repeats and neighbouring loci in WI38 9 hpi, HFF 4 hpi and HEK 293T cells 18 hpi. Pro-Seq. (Precision Run-On Sequencing) shows RNA-pol II occupancy at HSATII repeats. **B** qRT-PCR of cells infected with HSV-1 for 18 h (MOI 0.1). HSV-1 ICP0 and gB served as infection controls. Data represent mean and s.d. of *n* = 3 (biological replicates).

Supplemental Figure 2

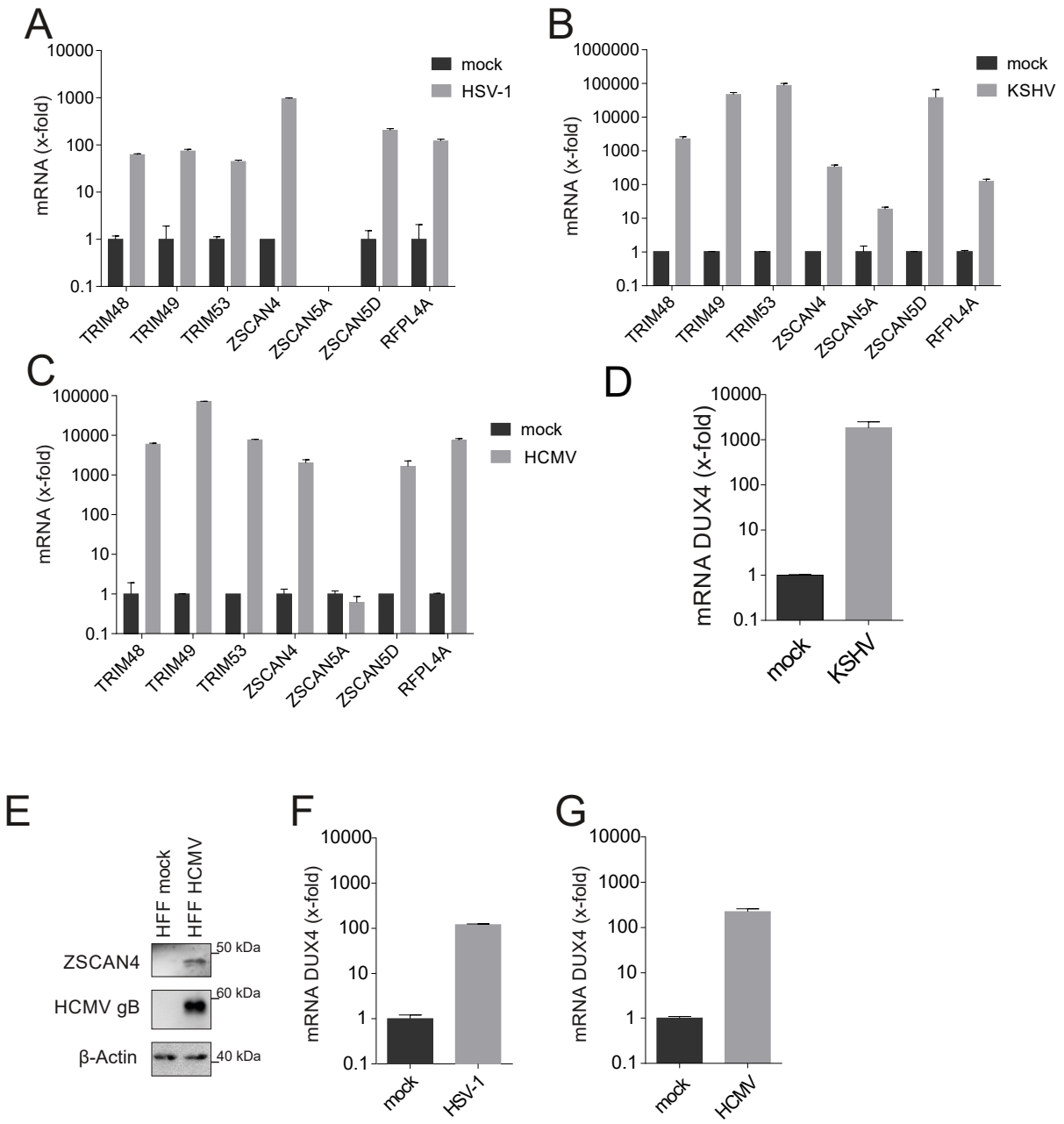


Fig. S2. A-C. qPCR analysis of DUX4 target gene expression upon infection of 293T cells with HSV-1 MOI 5 for 18 h **(A)**, upon expression of HFF cells with HCMV MOI 1 for 6d **(B)** and upon lytic reaction of KSHV in iSLK cells at 5d p.i. **(C).** x-fold activation of indicated genes relativ to HPRT**. D** mRNA expression of DUX4 in iSLK cells with reactivated KSHV infection, harvested after 5 d. x-fold activation of DUX4 relativ to HPRT. **E** HFF cells infected with HCMV for 6d with MOI 1. Western blot analysis of ZSCAN4. HCMV glycoprotein B (gB) was used as marker for infection. **F** mRNA expression of DUX4 in 293T upon infection with HSV-1 for 18 h with MOI 10. x-fold activation of DUX4 relativ to HPRT **G.** mRNA expression of DUX4 target genes in HFF infected with HCMV for 6 d with MOI 1. x-fold activation of DUX4 relativ to HPRT. In all experiments, one representative out of at least n=3 is shown.

Supplemental Figure 3

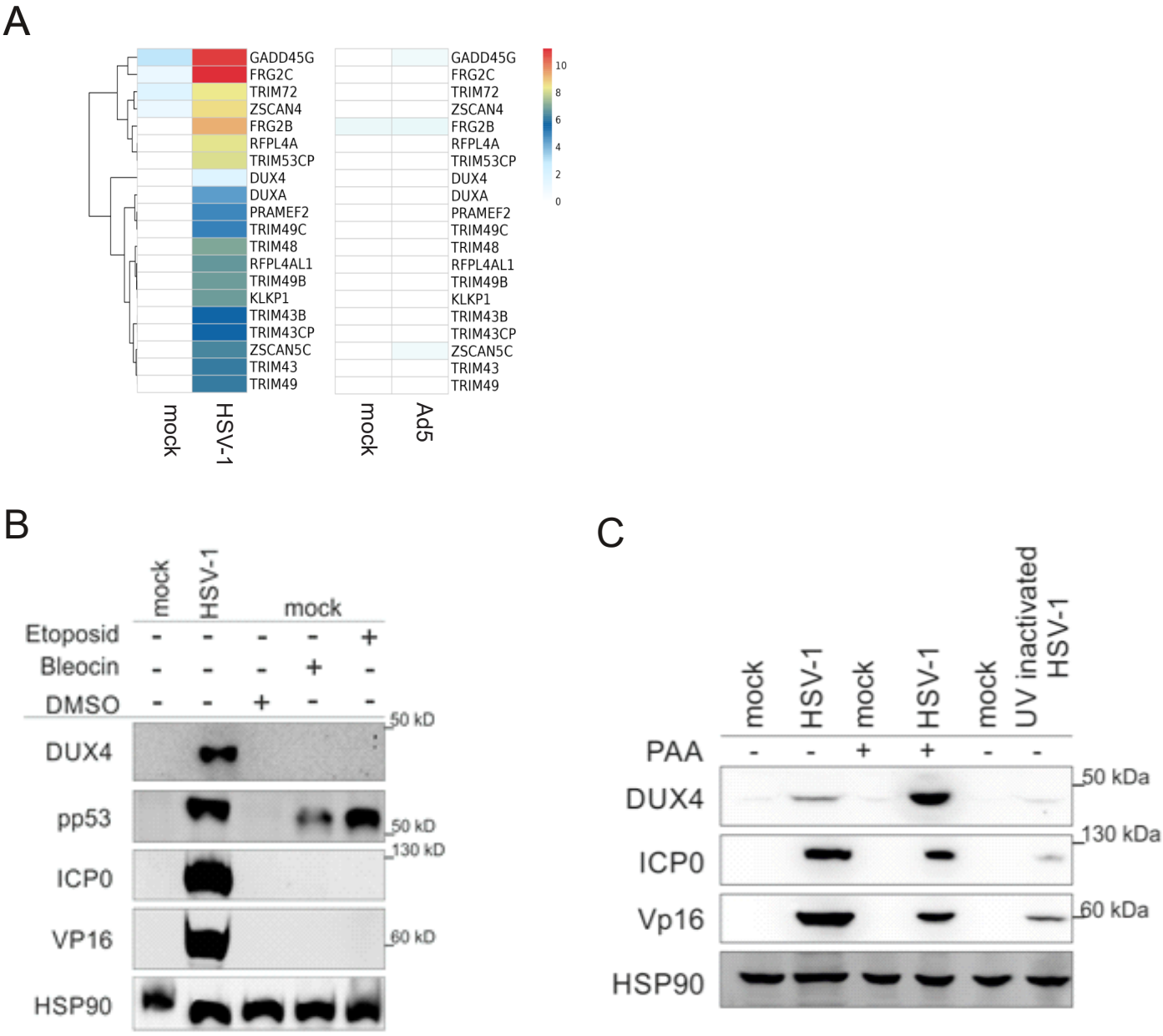


Fig. S3. DUX4 expression is independent of the DNA damage response (DDR) and viral DNA replication.A Heatmap of total RNA-Seq. experiments showing the number of normalized reads per gene (log2) focusing on DUX4 and DUX4-target genes. Left panel 293T cells infected with HSV-1 for 16h. Right panel A549 cells infected with Adenovirus 5 for 12h. **B** Western blot of DUX4 expression in 239T cells infected with HSV-1 for 18 h or treated with Bleocin or Etoposid. Bleocin and Etoposid were used to induce DNA damage in the cells and start the DDR. HSV-1 ICP0 and Vp16 were used as markers for infection. HSP90 was used as loading control. One representative out of n=3 **C** Western blot of DUX4 expression in 239T cells infected with wt HSV-1, UV-inactivated HSV-1 or treated with Polymerase inhibitor Phosphonoacetic acid (PAA). HSV-1 ICP0 and Vp16 were used as markers for infection. HSP90 was used as loading control. One representative out of n=3

Supplemental Figure 4

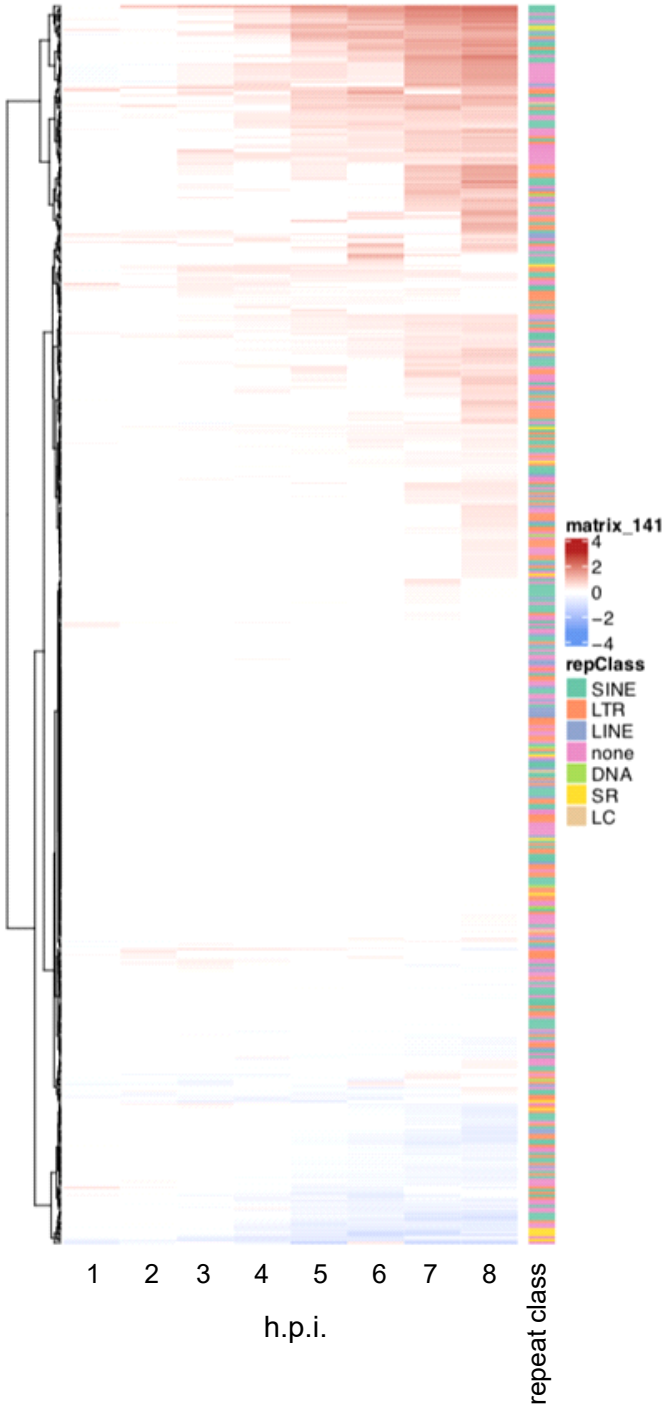


Fig. S4. DUX4 target genes have variable dynamics during infection. Genes are upregulated / downregulated with different kinetics (based on 4sU data by Rutkowski et al.). Whereas some genes are upregulated quite early, the bulk of upregulation happens at 3-4 hpi. Upregulated genes are enriched for methylation mediated chromatin silencing proteins, while the downregulated genes are enriched for antigen presentation. The most enriched tissue in the human ARCH4 expression database is the human zygote. The second most expressed gene is OOEP which is an early embryonic protein necessary for progression through the zygotic activation. h.p.i. (hours post infection)

Supplemental Figure 5

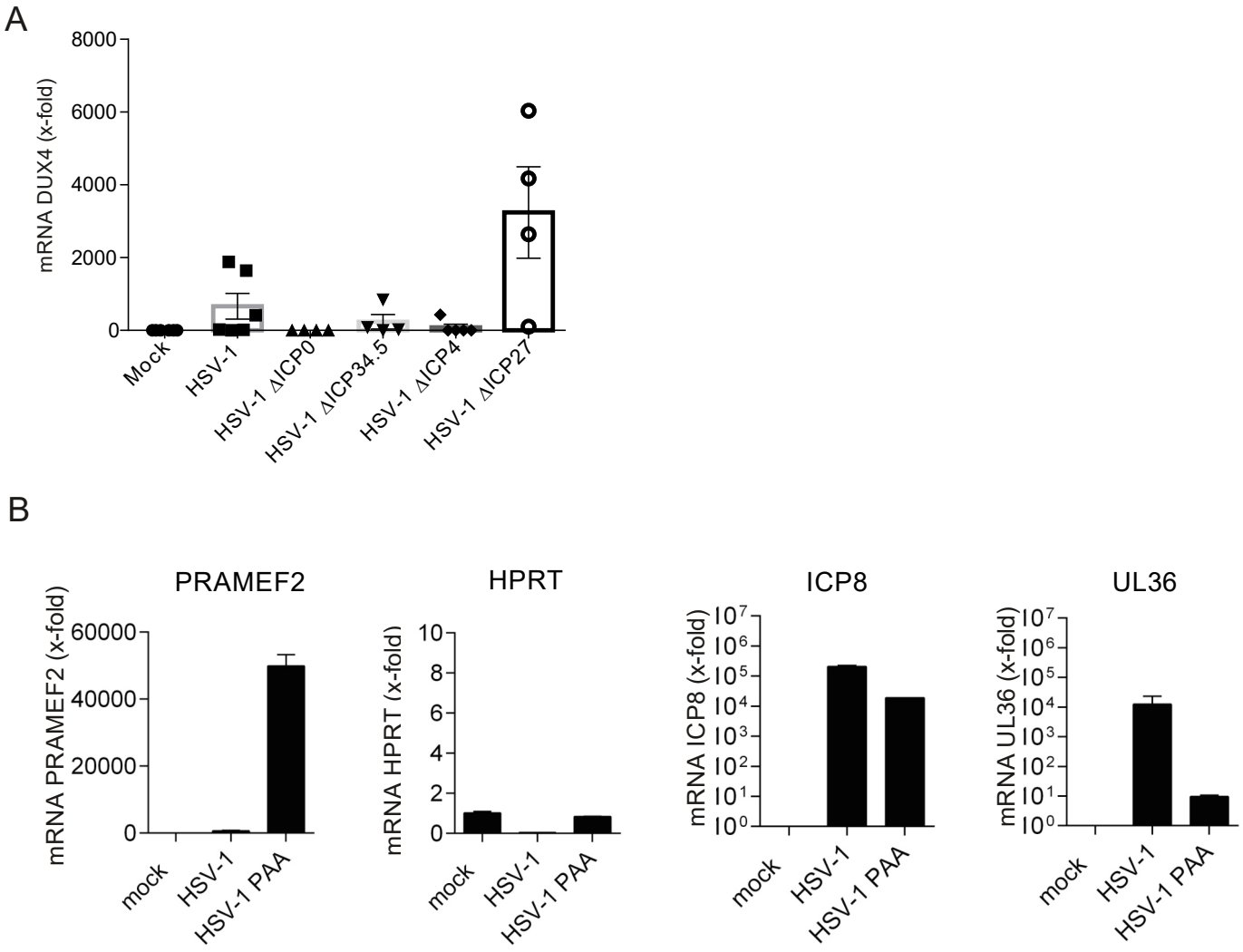
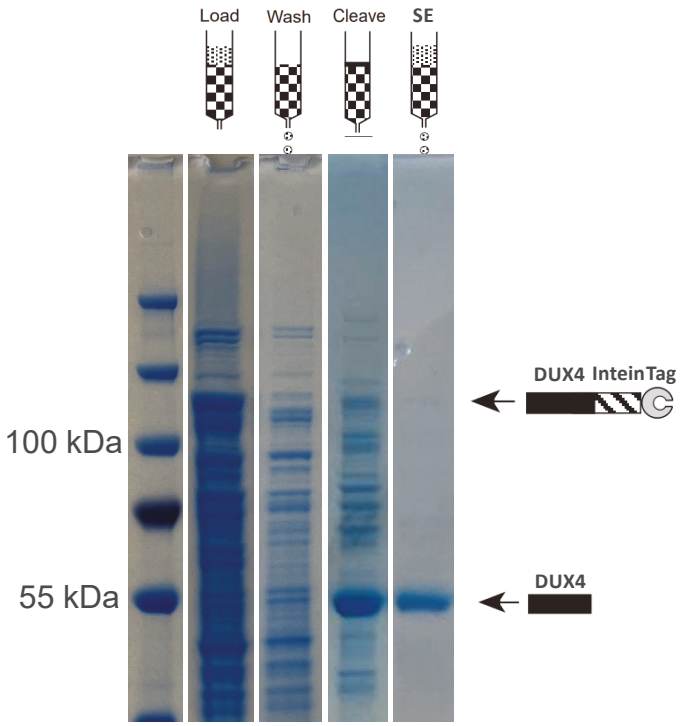


Figure S5. A qRT-PCR of DUX4 expression relative to HPRT from primary HFF cells infected with HSV-1, HSV-1 ΔICP0 (ICP4-YFP), HSV-1 ΔICP34.5, HSV-1 ΔICP4 for 24 h (MOI 10). **B** qRT-PCR analysis of cellular genes PRAMEF2 and HPRT as well as viral genes ICP8 and UL36 in HDF-TERT cells untreated or treated with PAA and infected with HSV-1 (MOI 0.1). Values are presented as fold induction (normalized to HPRT RNA) relative to uninfected control cells.

Supplemental Figure 6

A



B

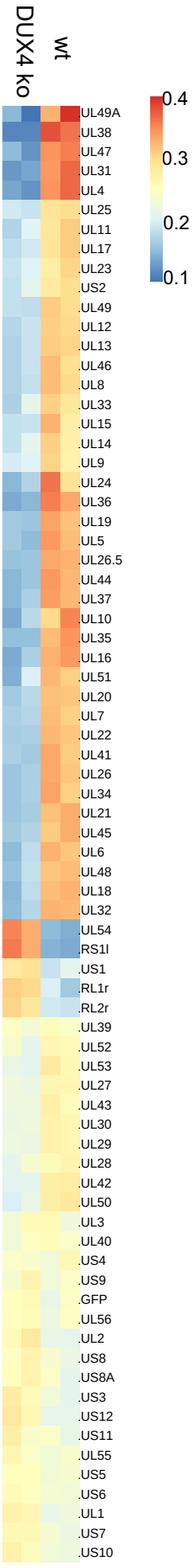


Fig. S6. A Expression and purification of full-length DUX4 protein in E.coli. Coloidal coomassie gels of purification steps. Intein-tagged DUX4 protein was expressed in E.coli and purified with chitin columns on a Äkta pure system. Cleavage of the Intein-tag was induced by DTT and the cleaved protein further purified by size-exclusion (SE) chromatography. **B** Expression of individual HSV-1 genes in DUX4 ko HAP1 cells vs. wt HAP1 cells. DUX4 ko HAP1 cells and wt HAP1 cells were infected with HSV-1 at MOI of 1 for 8h in duplicates. Heatmap shows the normalized expression of all HSV-1 genes.