

## SUPPLEMENTARY DATA

**Figure S1. Micrograph of a representative micrococcal nuclease digestion assay used for calculation of average nucleosome length.** DNA digestion pattern in BJAB and BJAB-EBNA1 nuclei digested with micrococcal nuclease for 4 min. The micrograph of the ethidium bromide stained gel illustrates the faster migration of nucleosome fragments in BJAB-EBNA1 cells as compared to BJAB cells, which is indicative of shorter nucleosome particles due to de-protection of the linker region. Only the size of N2 to N5 fragments was used for calculation of average nucleosome length.

**Figure S2. EBNA1 induces chromatin decondensation in the NIH2/4 reporter cell line.** a) NIH2/4 cells that carry multiple copies of the LacO sequence integrated in a heterochromatic region were transfected with mCherry-LacR, or mCherry-LacR-EBNA1. The size of the condensed array visualized in mCherry-LacR expressing cells is smaller than that detected in A03-1 cells (compare with Figure 3a,3b). Tethering of EBNA1 to the array induces decondensation, as judged by the formation of extended structures of irregular shape. Scale bar 5  $\mu$ m. b) Quantification of the array size; a 25-fold increase in size was induced by EBNA1.  $p < 0.0001$ .

**Figure S3. EBNA1 induces chromatin decondensation independently of the recruitment of USP7.** a) NIH2/4 cells transfected with mCherry-LacR-EBNA1 or mCherry-LacR-EBNA1-DBD were co-transfected with GFP-USP7. Recruitment of USP7 to the array was observed in cells expressing the EBNA1-DBD alone in spite of failure to induce chromatin decondensation. b) Localization profile confirming the recruitment of USP7 to both EBNA1 and EBNA1-DBD

**Figure S4. Sequence logo of the chromatin binding domains of the EBNA1 and HMGA protein families.** The sequence logo representation generated by ClustalW Multiple Sequence Alignment of the GR domains of EBNA1 and homologues encoded by the Baboon (CEHV-12), Rhesus (CEHV-15), Macaque (Si-IIA) and Marmoset (CalHV-3) lymphocryptoviruses, with the position of the repeats in the

EBNA1 sequence, are shown alongside the sequence logo and position of the AT-hooks of 17 HMGA family proteins from different species.

**Figure S5. HMGA1a promotes chromatin decondensation.** a) Targeting of HMGA1a fused to mCherry-LacR induces decondensation of the LacO array in the A03-1 reporter cell line. Scale bar 2  $\mu\text{m}$ . b) Quantification of the array size expressed as percentage of the nuclear area occupied by the array in 15 cells. LacR=  $2.39\pm 0.22$ ; LacR-HMGA1a=  $8.17\pm 0.75$ ,  $p < 0.0001$ .

**Figure S1**

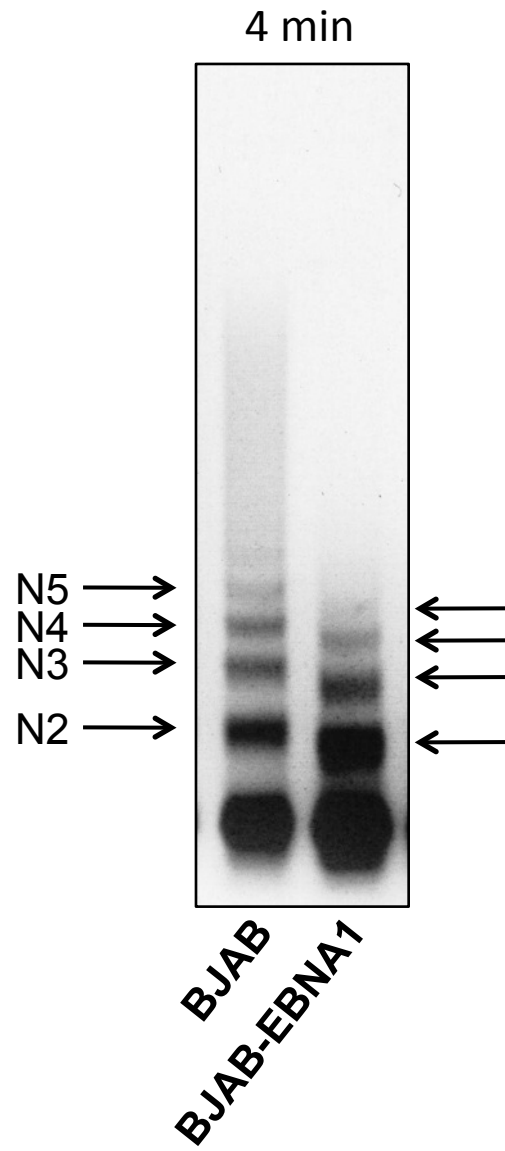
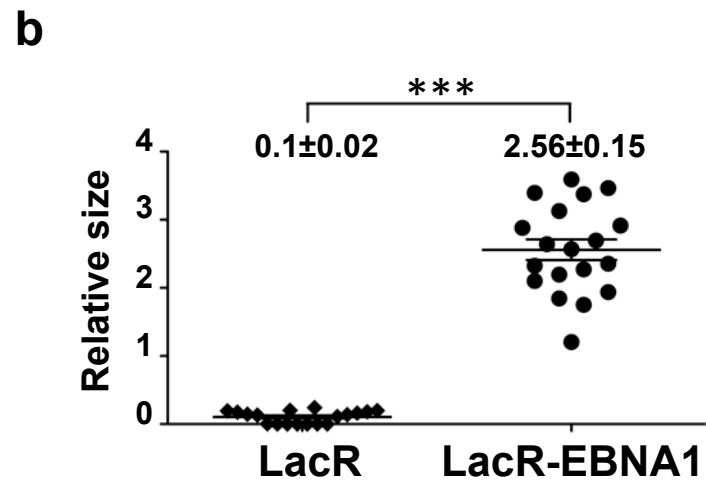
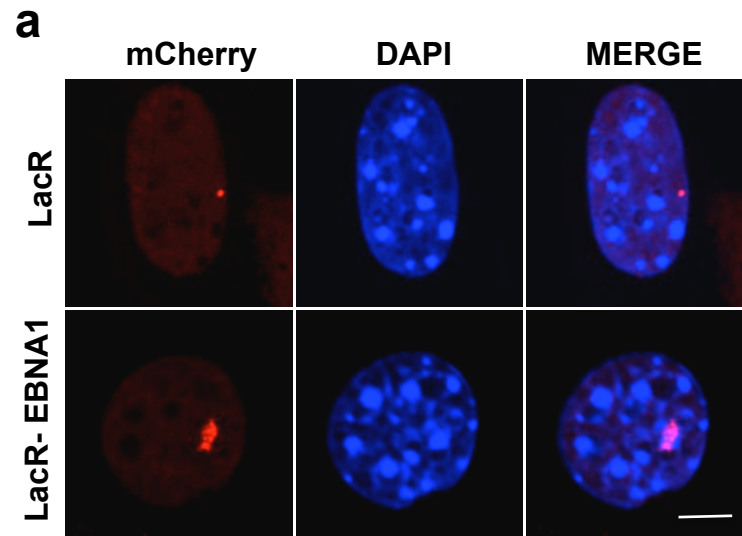


Figure S2



**Figure S3**

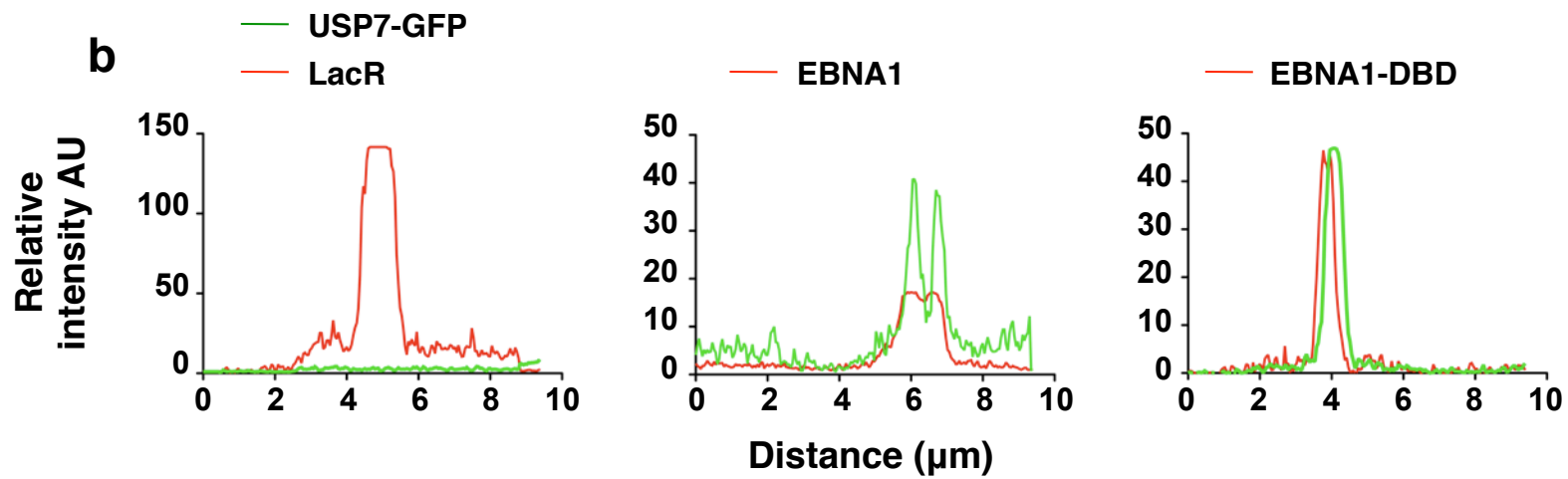
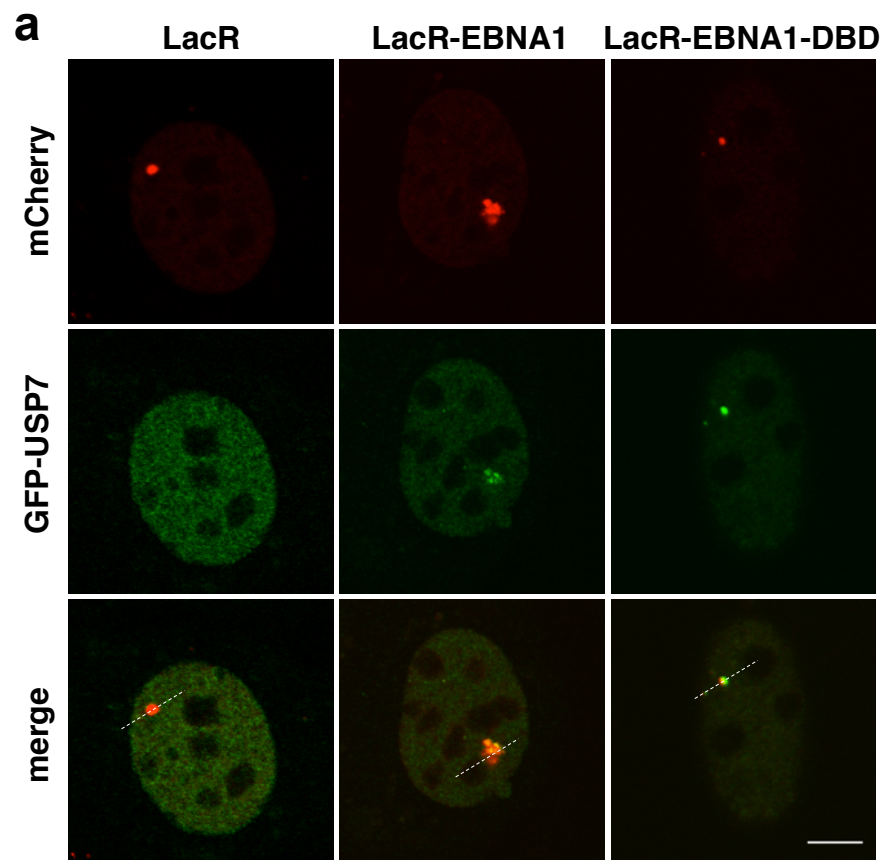
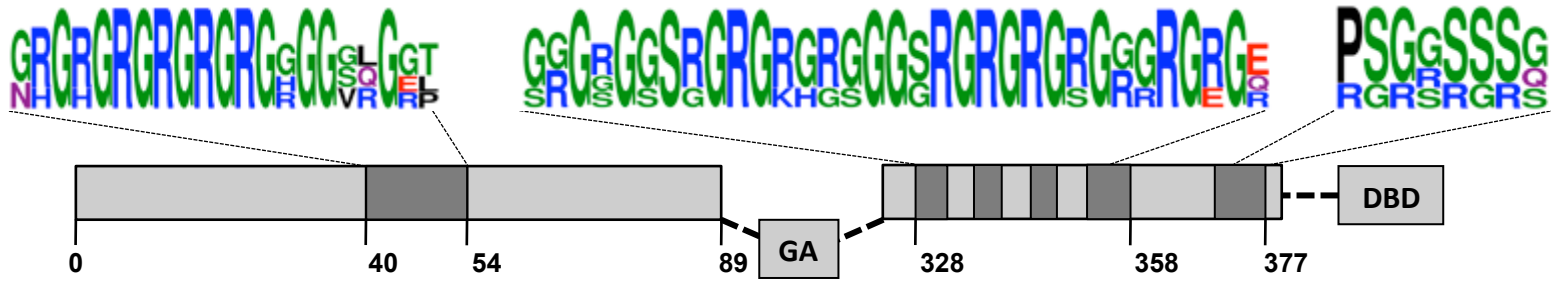
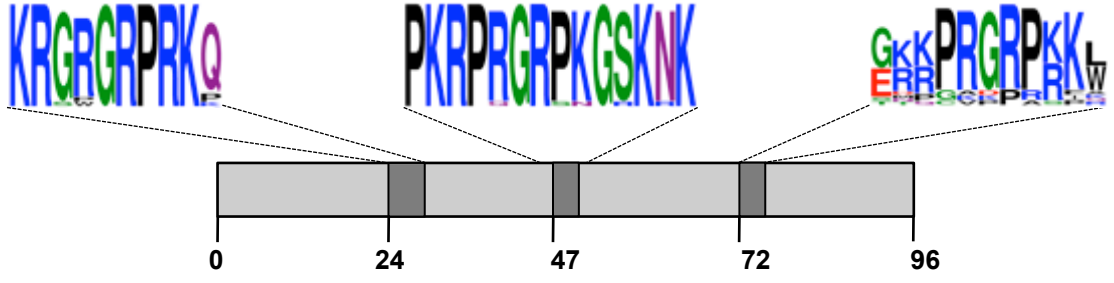


Figure S4

# EBNA1

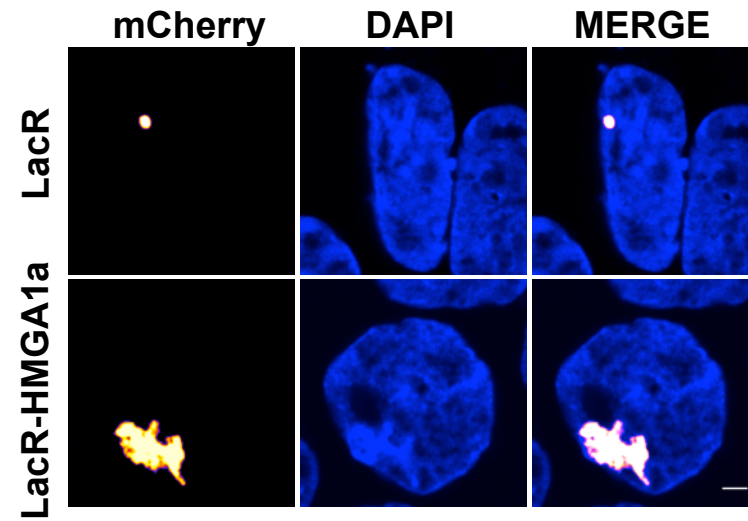


# HMGA



**Figure S5**

**a**



**b**

