Cell Reports, Volume *40*

Supplemental information

Herpesvirus-induced spermidine synthesis and eIF5A hypusination for viral episomal maintenance

Un Yung Choi, Jae Jin Lee, Angela Park, Kyle L. Jung, Shin-Ae Lee, Youn Jung Choi, Hye-Ra Lee, Chih-Jen Lai, Hyungjin Eoh, and Jae U. Jung

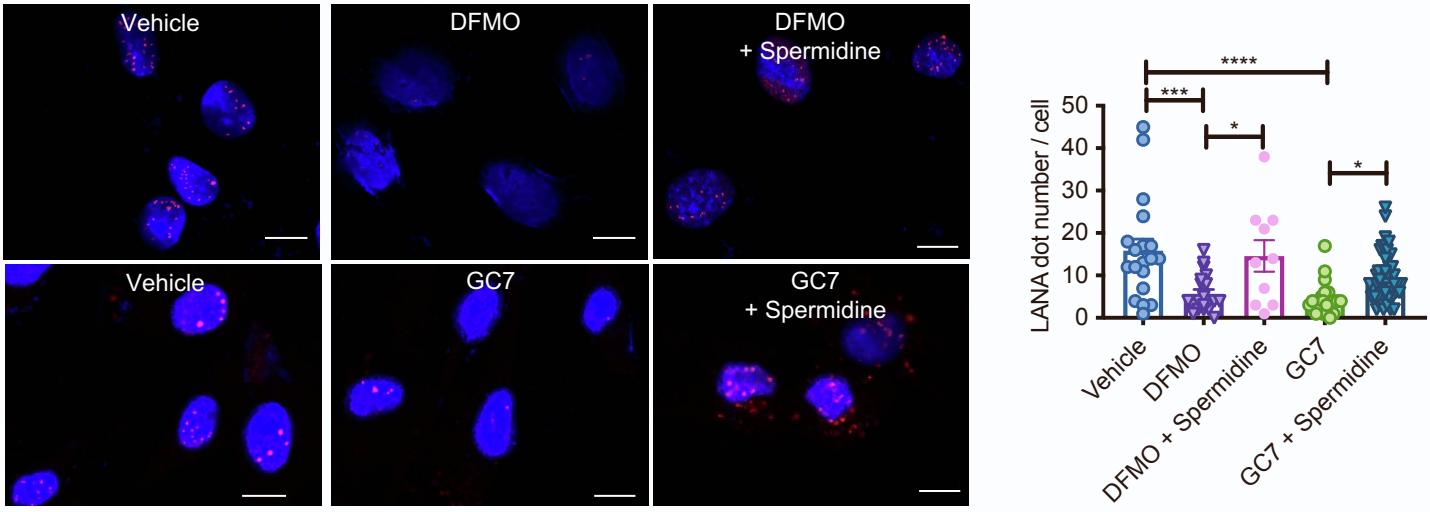

Figure S1. LANA Immunofluorescence of TIME cells cultured in 2D monolayer. Related to Figure 3.

KSHV-infected TIME cells in 2D culture were treated with 1 mM DFMO or 5 μM GC7, with or without the supplementation of 20 μM spermidine for 3 days, followed by immunofluorescence analysis with anti-LANA antibody (red) and Hoechst 33342 (blue) (biological replicates: 4). Scale bar = 10 μm. Right; Graph of the average number of LANA dots per cell. * $p<0.05$, *** $p=0.003$, **** $p<0.0001$ using one-way ANOVA with Tukey's multiple comparison test. Error bars represent SEM.

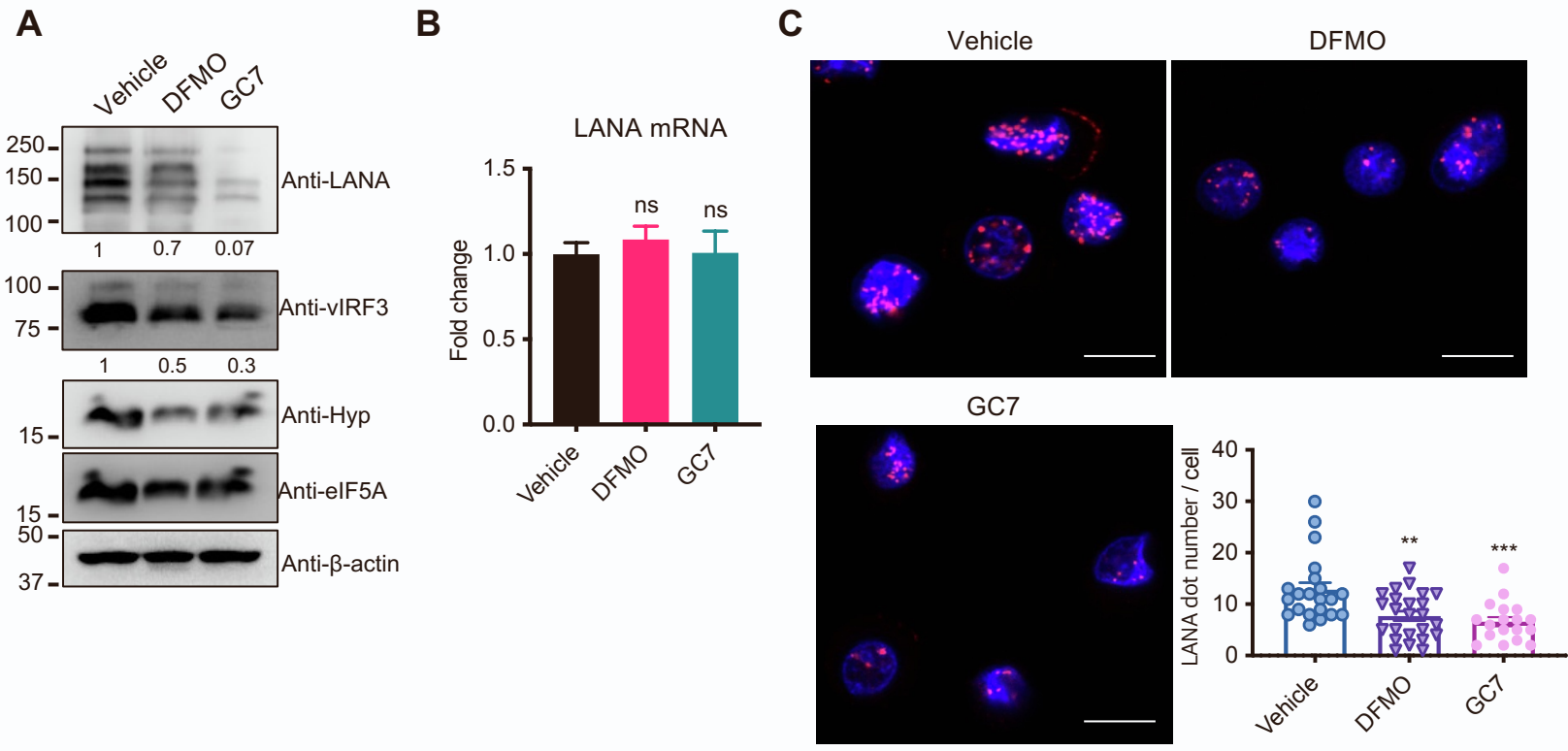

Figure S2. eIF5A hypusination is required for KSHV LANA expression and persistence in PEL cell. Related to Figure 3.

(A-B) BCBL-1 cells were treated with 1 mM DFMO or 10 μM GC7 (biological replicates: 3). (A) Whole-cell lysates were prepared at day 4 post-infection and probed for anti-LANA, anti-vIRF3, anti-hypusine, anti-eIF5A and anti-β-actin antibodies by immunoblotting. LANA and vIRF3 levels were quantified using Image Lab and normalized to β-actin. (B) RNAs extracted from cells above in (A) were subjected to qPCR analysis to measure LANA mRNA (biological replicates: 2; technical replicates: 2-3). (C) BCBL-1 cells were treated with 2 mM DFMO or 10 μM for 5 days and imaged by immunofluorescence with anti-LANA antibody (red) and Hoechst 33342 (blue). Scale bar = 10 μm. Average numbers of LANA dots per cell are presented in the graph (biological replicates: 3). ** $p<0.005$, *** $p=0.006$ using one-way ANOVA with Dunnett's multiple comparison test. Error bars represent SEM.

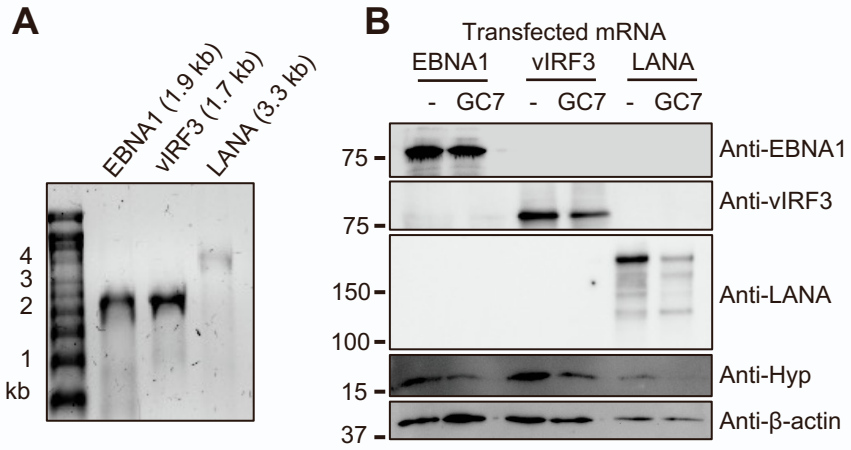

Figure S3. EBNA, vIRF3, and LANA expression from in vitro-transcribed 5'-capped and polyadenylated mRNA transfection. Related to Figure 3.

(A) Denaturing gel electrophoresis of in vitro-transcribed 5-capped and polyadenylated mRNAs. Each mRNA was electrophoresed on a denaturing formaldehyde agarose gel and visualized by SYBR Green II. (B) 5'-capped and polyadenylated mRNAs were transfected into HEK-293T cells and protein levels were determined at 24 hours post-transfection by immunoblotting with anti-EBNA1, anti-vIRF3, anti-LANA, anti-hypusine, and anti-β-actin antibodies (biological replicates: 2; technical replicates: 2-3).

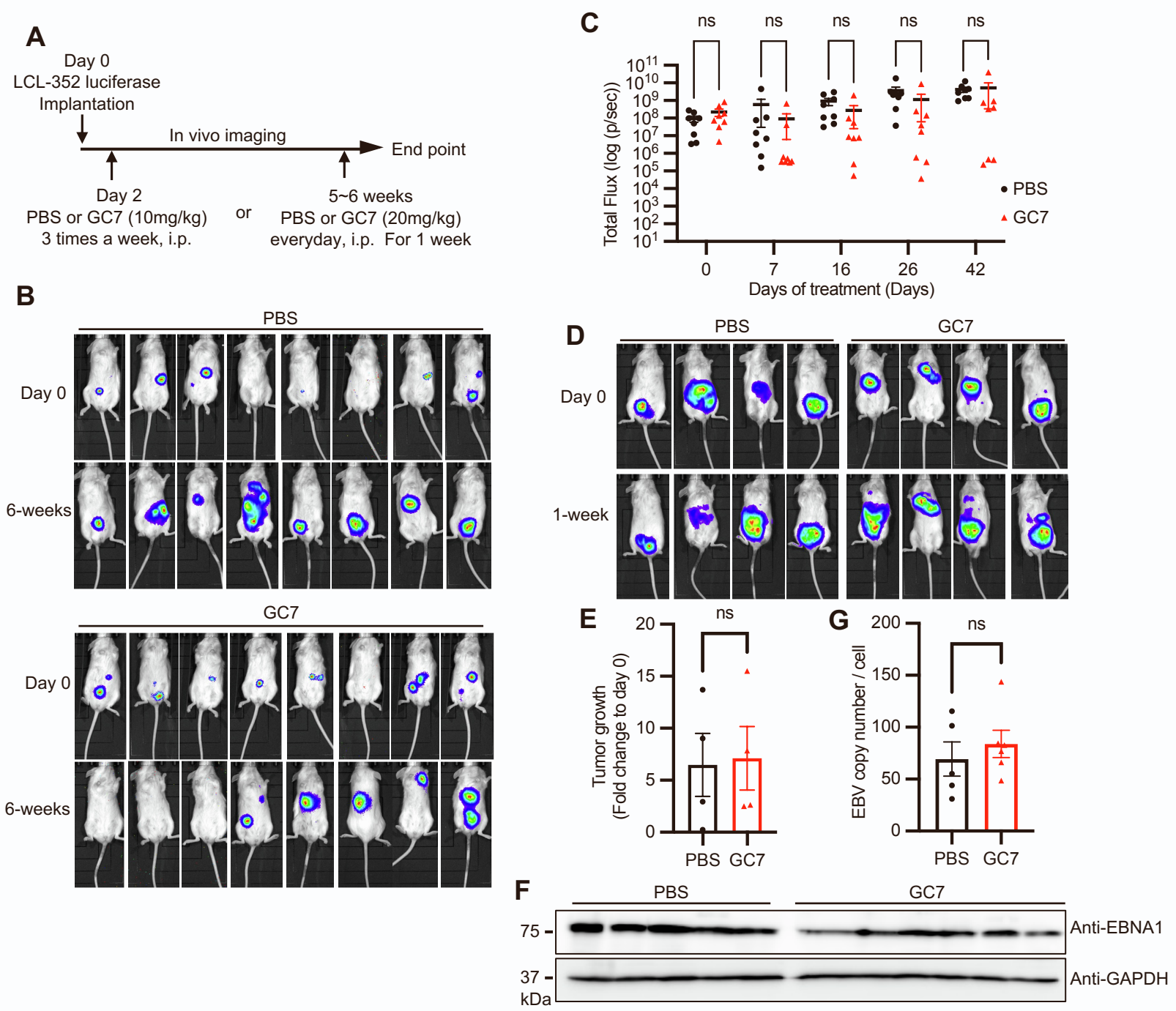

Figure S4. Effect of GC7 treatment in mice engrafted with EBV LCL352-luciferase cells. Related to Figure 5.

(A) NOD/SCID mice intraperitoneally (i.p) engrafted with 5x106 LCL352-luciferase cells and imaged using the IVIS Imaging system for bioluminescence signal intensity in the whole body. (B) At 2 days post-engraftment, mice were injected (i.p) with PBS or GC7 three times a week for 6 weeks. Mice are shown before treatment or after treatment. (C) Quantification of in vivo bioluminescence signal of the animals treated with PBS or GC7 for 6 weeks. (n = 8 in each group). Two-way ANOVA with Sidak's multiple comparisons test was performed to assess the significance. ns, non-significant. (D) After tumor development, mice were injected (i.p) with PBS or GC7 daily for a week. Mice are shown before or after treatment. (E) Tumor growth for each mouse from (D) was determined by in vivo bioluminescence signal measurement. Fold change of total flux for each mouse was graphed as a bar. (n = 4 in each group). Student's t-test was performed to assess the significance. ns, non-significant. Error bars represent SEM. (F-G) Tumor cells from mice (D) were isolated and analyzed for (F) immunoblotting to show EBNA1 and GAPDH (loading control) expression or (G) qPCR to measure intracellular EBV genome copy numbers. Student's t-test was performed to assess the significance. ns, non-significant. Error bars represent SEM.

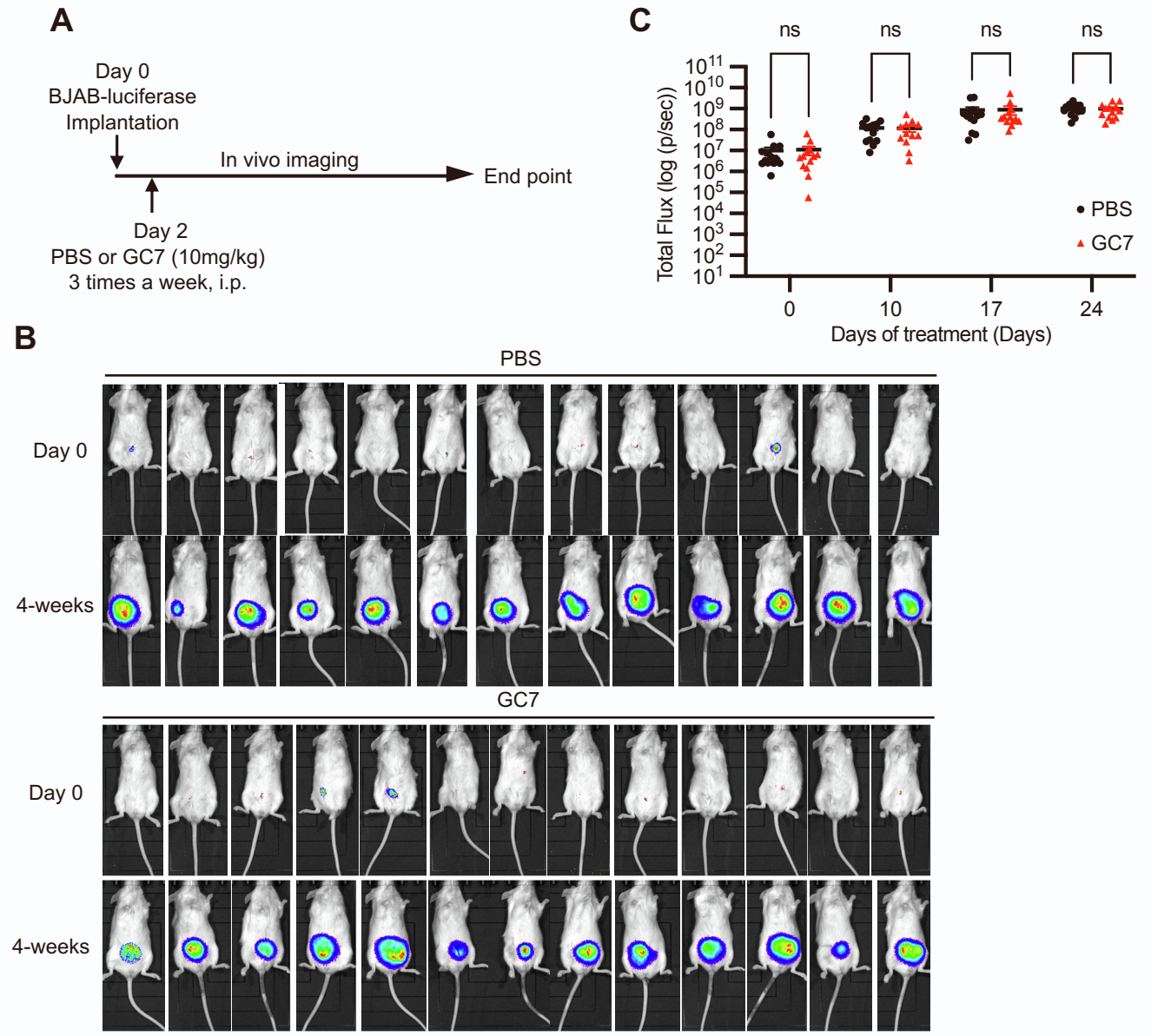

Figure 5S. *In vivo* bioluminescence imaging of mice engrafted with BJAB-luciferase cells. Related to Figure 5.

(**A**) NOD/SCID mice intraperitoneally (i.p) engrafted with 5x10^6 BJAB-luciferase cells and imaged using the IVIS Imaging system for bioluminescence signal intensity in the whole body. (**B**) At 2 days post-engraftment, mice were injected (i.p) with PBS or GC7 3 times a week for 4 weeks. Mice are shown before or after treatment. (**C**) Quantification of *in vivo* bioluminescence signal of the animals treated with PBS or GC7 during 6-week treatment period. (n = 13 in each group). Two-way ANOVA with Sidak's multiple comparisons test was performed to assess the significance. ns, not significant.

Primers list for qPCR	
hODC_q_F	ATATTGGCGGTGGCTTTCCT
hODC_q_R	TGTCCAACGCTGGGTTGATT
hSRM_q_F	GGTCCAGTGTGAGATCGACG
hSRM_q_R	CCCACATGTAGGGTCAGCTT
hSMS_q_F	GTGATGCGCAAGGCAAAGAA
hSMS_q_R	TCCTCGCACTATGGGTGGTA
hPAOX_q_F	CCAAGGACGTGGTTGAGGAA
hPAOX_q_R	GACTGGTTTATCGTGCCGGA
hSAT1_q_F	GGTTGCAGAAGTGCCGAAAG
hSAT1_q_R	TGCCAATCCACGGGTCATAG
hOAZ_q_F	CGGCAGCAGCAGTGAGAG
hOAZ_q_R	CCTGGGATCTTCAGGGGTGG
hRPS11_q_F	CATTCAGACTGAGCGTGCCT
hRPS11_q_R	GGAGCTTCTCCTTGCCAGTT
LANA_q_F	GAGTCTGGTGACGACTTGGAG
LANA_q_R	G AGGAAGGCCAGACTCTTCAAC
ORF11_F	GGCACCCATACAGCTTCTACGA
ORF11_R	CGTTTACTACTGCACACTGCA
EBNA1_F	TACAGGACCTGGAAATGGCC
EBNA1_R	TCTTTGAGGTCCACTGCCG
β-Globin_F	TGAGCCTTCACCTTAGGGTTGCCCA
β-Globin_R	GCCCTGGGCAGGTTGGTATCAAGGT

Supplementary Table 2. List of primers used for qPCR. Related to STAR methods