Supporting Information

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SI Materials and Methods

Lentiviruses. Lentiviral ICP4 expression plasmid pCDH-ICP4-puro was constructed by replacing the complete HCMV IE promoter of plasmid pCDH-CMV-MCS-EF1-Puro (Systembio) with the ICP4 promoter and coding region from plasmid S3 containing the SphI fragment from pICP4-lox-pac (1) (GenBank JQ673480.1 HSV-1 KOS, map positions 131,587–124,379) in the SphI site of pUC19. Lentiviral ICP27 expression plasmid pCDH-ICP27-blast was constructed, first, by replacement of the puromycin-resistance cassette of pCDH-CMV-MCS-EF1-Puro with the blasticidin-resistance cassette of pcDNA6/BioEase-DEST (Invitrogen) to create pCDH-CMV-MCS-SV40-blast. The ICP27 promoter and coding region were then isolated by digestion of plasmid PD7 containing the ICP27 gene and flanking sequences between EcoRV and SacI sites (GenBank JQ673480.1, map positions 110,580–115,666) with BamHI (map position 113,244) and SacI, and the isolated fragment was used to replace the CMV promoter in pCDH-CMV-MCS-SV40-blast.

Lentiviruses were produced using the ViraPower Lentiviral Packaging Mix (Invitrogen) according to the manufacturer's instructions. Briefly, 293T cells were transfected with pCDH-ICP4-puro or pCDH-ICP27-blast in ViraPower mix. Supernatants were harvested 2 d later, clarified, filtered through a 0.45-μm filter, and concentrated by centrifugation.

HSV–BAC Recombineering. All HSV–BAC constructs generated in this study and converted to virus particles are listed in Table 1 and were derived from KOS-37 BAC (2), a kind gift from D. Leib (Dartmouth Medical School, Hanover, NH). All BAC engineering was performed by scarless Red recombination with pRed/ET (Gene Bridges) and either pBAD-*I-sceI* plasmid (kindly provided by N. Osterrieder, Free University of Berlin, Berlin, Germany) or in *Escherichia coli* strain GS1783 (from G. Smith, Northwestern University, Chicago, IL), as described (3), or by in vitro Gateway (GW) recombination according to the Gateway Technology Manual (Invitrogen). All constructs were confirmed by PCR analysis, field inversion gel electrophoresis analysis of restriction enzyme digests, and targeted DNA sequencing. Targeting plasmids for Red recombination were constructed as described (4). The kanamycin-resistance gene flanked by an I-*Sce*I restriction site (I-*Sce*I-*aphAI* fragment) was amplified from pEPkan-S2 (also from N. Osterrieder, Free University of Berlin, Berlin, Germany) (4) by PCR with the different targeting primers specified below with reference to Table S2. All targeting fragments for Red recombination were purified by a Qiagen gel extraction kit (Qiagen) or SpinSmart Nucleic Acid Prep and Purification Columns (Denville Scientific).

To construct the JΔNI vectors, we first introduced the previously described hyperactive N/T double mutation (5) into the gB gene of KOS-37 BAC and deleted the internal repeat (joint) region. The I-*Sce*I-*aphAI* fragment was cloned into the SnaBI site of plasmid pgB1:D285N/A549T (5). The resulting plasmid, pgB:N/T-kan, was used as a template for amplification with primers R21 and R22, and the product was recombined with the native gB gene of KOS-37 BAC, followed by I-*Sce*I-enhanced deletion of the *aphAI* gene in pBAD-*I-sceI* plasmid-transformed bacteria. Next, we amplified I-*Sce*I-*aphAI* with nested forward primers R6 and R8 and reverse primer R7 for Red-mediated deletion of the joint region (GenBank JQ673480, positions 117,080–132,461), followed by removal of the *aphAI* gene.

Replacement of the ICP4 locus in the joint-deleted gB:N/T BAC with an mCherry expression cassette was achieved as follows. Plasmid pUbC-mCherry-SV40pA was constructed by cloning of the human ubiquitin C promoter (UbCp) from pBluescriptUB-Flag-mArt (a gift from H. Nakai, Oregon Health and Science University, Portland, OR), the mCherry gene from pEP-miR (Cell Biolabs), and the SV40 polyadenylation (polyA or pA) region from pEP4-EO2SCK2M-EN2L (plasmid 20924; Addgene) (6) into pBluescript KS+ (Stratagene). The I-*Sce*I-*aphAI* fragment was then cloned into the BamHI site of pUbC-mCherry-SV40pA at the boundary between UbCp and mCherry to generate pUbC-mCherry-SV40pA-KAN. The insert was PCR-amplified with primers R11 and R12 (Table S2) for Red-mediated recombination with the ICP4 target locus. The resulting construct was deleted for HSV-1 KOS positions 146,113–151,581 of the GenBank JQ673480 sequence, including the TAATGARAT motifs of the ICP22 promoter.

Replacement of the ICP0 and ICP27 IE promoters with the early (β) HSV-1 thymidine kinase (TK) promoter to generate JΔNI2 was initiated by PCR through the TK promoter in front of both the ICP0 and the ICP27 coding region in the JΔββ virus genome (7) with primer pairs R41/42 and R43/44, respectively. The products were cloned into pCRblunt (Invitrogen) to produce pCRBlunt-β0 and pCRBlunt-β27. Insertion of I-*Sce*I-*aphAI* amplified with primer pair R45/46 into the BglII site of pCRBlunt-β0 and with primer pair R47/48 into the HpaI site of pCRBlunt-β27 yielded pCRBlunt-β0-KAN and pCRBlunt-β27-KAN, respectively. The inserts were amplified with primer pairs R41/42 and R43/44, respectively, for Red recombination with the joint-deleted UbCp-mCherry gB:N/T BAC. JΔNI3 was then derived from JΔNI2 by Red-mediated deletion of the complete ICP0 coding sequence using nested targeting forward primers R1 and R2 and reverse primer R3 for I-*Sce*I-*aphAI* amplification. JΔNI5 was likewise derived from JΔNI3 by deletion of the ICP27 coding sequence using primer pair R4/R5 to produce the targeting I-*Sce*I-*aphAI* fragment.

JΔNI6GFP was generated by insertion of an EGFP expression cassette between the UL3 and UL4 genes of JΔNI5. First, plasmid pCAG-GFP was constructed by replacing the gH gene between the CAG promoter (CMV enhancer/chicken-β actin promoter/chimeric intron) and the rabbit β-globin polyA region in plasmid pPEP100 (a gift from P. Spear, Northwestern University, Evanston, IL) (8) with the EGFP gene from pEGFP-C1 (Clontech). I-*Sce*I-*aphAI* was then inserted into the SnaBI site of pCAG-GFP to create plasmid pCAG-GFPKAN. Separately, multistep PCR using KOS-37 BAC DNA as initial template was performed to generate a fragment that contained multiple new cloning sites (MCS) between the UL3 and UL4 polyA regions, as follows. First, extension PCR was performed to amplify the 3′ UTRs of UL3 and UL4 with primer pairs R17/18 and R19/20, respectively, that added an overlapping MCS region to each 3′ UTR fragment. The two PCR products were gel-purified, and 100 ng of each was used for overlapping PCR with primers R17 and R19 to create a continuous fragment. This product was cloned into pCRBlunt (Invitrogen), yielding plasmid pCRBluntUL3-4linker. The insert of pCAG-GFPKAN was then cloned between the AccI and PsiI sites in the MCS of pCRBluntUL3-4linker. The resulting plasmid was digested with MfeI and PpuMI, and the UL3-CAG-GFPKAN-UL4 fragment was isolated for recombination with the UL3-UL4 intergenic region of JΔNI5 followed by *aphAI* gene removal.

To create JΔNI7GFP, an XhoI fragment (~6.2 kb) containing the two CTRLs, LAP1 and LATP2, of the HSV-1 LAT locus was isolated from KOS-37 BAC DNA and cloned into pBluescript

KS+. An internal KpnI-SalI fragment extending from near the end of LATP2 to ~250 bp downstream of CTRL2 was isolated from this recombinant and cloned between the KpnI and SalI sites of pSP72 (Promega) to produce pSP72KOS-LAT. An MCS was then introduced between two BstXI sites located ~240 and ~430 bp downstream of the KpnI site, yielding pSP72KOS-LATlinker. Separately, plasmid pCAG-GW was constructed by replacing the gH gene of pPEP100 with a PCR-amplified modified GW recombination cassette (GW-Zeo; zeocin resistance instead of chloramphenicol resistance) (9). The insert of pCAG-GW was then cloned into the MCS of pSP72KOS-LATlinker to produce pSP72KOS-LATlinker-CAG-GW. The plasmid was digested with KpnI and HpaI to isolate the CAG-GW region with flanking LAT sequences for Red-mediated recombination with the LAT locus of JΔNI5 in *ccdB*-resistant HerpesHogs bacteria (9). Finally, the GW cassette in the resulting JΔNI5 recombinant BAC was replaced with the EGFP gene by Red-mediated recombination with an AatII-PsiI fragment, including CAG and polyA sequences, of plasmid pCAG-GFPKAN. JΔNI7GFP derivatives deleted for specific LAT region elements outside the EGFP cassette (CTRL1, GenBank JQ673480, positions 8,978–9,161; CTRL2, positions 5,694–5,857; LATP2, positions 6,123–7,530) were generated by Red-mediated recombination of JΔNI7GFP DNA with targeted I-*Sce*I-*aphAI* cassettes produced by PCR with the respective F1, F2, and R primers listed in Table S2.

For the construction of the different JΔNI9 and JΔNI10 vectors, the remainder of the LAT region, including CTRL1, LATP2, and CTRL2, was deleted from the JΔNI5 genome by recombination with LAT-targeted I-*Sce*I-*aphAI* generated by PCR with primers R38, R39, and R40, producing JΔNI5ΔL. GW-Zeo was then amplified with targeting primers for the intergenic region between UL45 and UL46 (R34/35) or UL50 and UL51 (R36/37), and the product of each reaction was recombined with JΔNI5ΔL BAC DNA to create JΔNI9GW and JΔNI10GW. JΔNI7GFP BAC DNA was digested with XhoI, and the ~7.2-kb CAG-GFP-containing fragment from the LAT locus was isolated and cloned into pENTR1A (pENTR-LAT-XhoI). The corresponding ~5.3-kb XhoI fragment of JΔNI7GFPΔC12LP2 was likewise isolated and cloned into pENTR1A (pENTR-LATΔ-XhoI), and, lastly, the insert of pCAG-GFP (see above) was transferred into pENTR1A (pENTR-CAG-GFP). In vitro LR Clonase (Invitrogen) reactions were then performed to recombine the different pENTR constructs with JΔNI10GW BAC DNA, producing JΔNI10LAT-GFP, JΔNI10ΔC12LP2-GFP, and JΔNI10GFP, respectively, and pENTR-LAT-XhoI and pENTR-CAG-GFP were recombined with JΔNI9GW BAC DNA to generate JΔNI9LAT-GFP and JΔNI9GFP.

For the construction of JΔNI7-GWL1, the GW-Zeo cassette was amplified with primers R49 and R50 and cloned into the MCS of pSP72KOS-LATlinker to create pSP72KOS-LATlinker-GW. The GW region with flanking homology arms was isolated by digestion with KpnI and HpaI and recombined with the LAT locus of JΔNI5 by Red-mediated recombination.

JΔNI7mDMD was derived from JΔNI7-GWL1. We first constructed plasmid pENTR-CAG-mcs-β-globin polyA by replacement of the EGFP gene-containing EcoRI/BglII fragment of pENTR-CAGGFP with a synthetic MCS. A full-length mouse dystrophin cDNA was then obtained from plasmid pCCL-DMD (10) (a kind gift from C. T. Caskey, Baylor College of Medicine, Houston, TX) by NotI digestion and cloned into the NotI site in the MCS of pENTR-CAG-mcs-β-globin polyA to generate pENTR-CAGmDMD (Fig. S6). Gateway LR recombination of pENTR-CAGmDMD with JΔNI7-GWL1 was then used to produce JΔNI7mDMD.

KNTc was constructed by introduction of the gB:N/T mutations into KOS-37 BAC as described above, and the UbCp-mCherry cassette into the intergenic region between UL3 and UL4 by Red recombination with the insert of pUbC-mCherry-SV40pA-KAN targeted by amplification with primers R13 and R14.

Viruses. JΔNI BAC DNAs were converted to infectious viruses by transfection of U2OS-based complementing cells. DNA in 500 μL of OptiMEM (Invitrogen) was incubated with 1 μL of Lipofectamine Plus Reagent (Invitrogen) for 5 min at room temperature, 6.25 μL of Lipofectamine LTX (Invitrogen) was added, and the mixture was incubated for 30 min at room temperature and added to cells. After incubation at 37 °C for 6 h, the transfection mix was removed, and the cells were cultured overnight at 37 °C with serum-free DMEM, transferred to a 33 °C incubator, and monitored for 100% cytopathic effect (CPE). Supernatants were titered and then amplified by infection of sequentially larger cultures at an MOI of 0.001 pfu per cell. KNTc infectious virus was produced by transfection of Vero cells using 3 μL of Lipofectamine Plus Reagent and 9 μL of Lipofectamine LTX for 4 h at 37 °C; an MOI of 0.01 pfu per cell was used for KNTc virus amplification on Vero cells. Complementing cells for transfections and/or virus growth were as follows: U2OS-ICP4 (JΔNI2, JΔNI3), U2OS-ICP4/27 (JΔNI5 and derivatives), and Vero-7b (QOZHG virus). All virus stocks were titered on U2OS-ICP4/27 cells (Table 2). Physical titers [genome copies (gc)/mL] were determined by quantitative real-time PCR as described below. Fluorescent images of infected cells were obtained with a Nikon Diaphot fluorescence microscope (Nikon) at 40× magnification or at 100× magnification (Fig. S3*A* only).

Virus Growth Curves. Replicate wells of Vero-7b, U2OS, U2OS-ICP4, and U2OS-ICP4/27 cells in a 24-well plate were infected at an MOI of 0.001 for 2 h, treated with 0.1 M glycine (pH 3.0) for 1 min to inactivate extracellular virus, and incubated at 37 °C and 5% CO_2. Media were harvested daily and titered by standard plaque assay on U2OS-ICP4/27 cells.

Cytotoxicity Assay. HDF and Vero cells (5×10^3) were seeded in a 96-well plate and infected at 25,000 gc per cell. Cell viabilities were determined at 1–3 or 5 dpi by MTT assay, essentially as described (11).

Western Blotting and Immunofluorescence. Cell lysate preparation and Western blotting were typically performed as described (12). For Western blotting of dystrophin, cell lysates were separated on NuPAGE Novex 3–8% Tris-Acetate Protein Gels (Invitrogen). Polyclonal rabbit anti-ICP0 antibodies were produced in our laboratory, anti-ICP27 (10-H44) was from Fitzgerald Industries International (www.fitzgerald-fii.com), anti-ICP22 was a gift from John Blaho (Mount Sinai School of Medicine, New York), anti-ICP4 (10F1) was from Santa Cruz Biotechnology, anti–α-tubulin (T6793) was from Sigma, and anti-dystrophin was from Millipore (clone 1808). Immunofluorescence using the same ICP4, ICP27, and dystrophin antibodies or anti-Cre antibody 2D8 (Millipore) was performed essentially as described (13) and was recorded under a Zeiss axiovert 200 fluorescence microscope (Zeiss) (anti-dystrophin) or a Nikon Diaphot fluorescence microscope (all others) at 100× magnification.

Quantitative Reverse Transcription-PCR and Genome PCR. For qRT-PCR, total RNA was typically extracted using an RNeasy kit (Qiagen). cDNA was synthesized with a RETROscript Kit (Ambion). Real-time PCR was carried out in triplicate by the StepOnePlus Real-Time PCR System (Applied Biosystems). For small numbers of cells, a Cells-to-cDNA II Kit (Ambion) was used for cell lysis and reverse transcription. Results for 18S ribosomal (r) RNA or viral particle number were used to normalize the data. All qRT-PCR primers used in this study are listed in Table S3 (Q1–Q36).

For the determination of physical (genome copy) titers of virus stocks, 5 μL of virus was incubated with 300 U/mL Benzonase nuclease (Sigma) for 1 h at 25 °C in the presence of 2 mM $MgCl_2$, and viral DNA was extracted by a DNeasy Blood and Tissue Kit (Qiagen). Then, gc titers were determined by qPCR for the glycoprotein D (gD) gene with the gD primers (Q37, Q38) and probe (Q39) listed in Table S3. Amounts of nuclear viral DNA were determined by rinsing the cells at 2 hpi, isolation of nuclei as described (14, 15), DNA extraction with the DNeasy Blood and Tissue Kit, and qPCR for the gD gene as above. Cellular 18S ribosomal DNA levels were measured with the use of TaqMan Ribosomal RNA Control Reagents (Invitrogen) and were used to normalize viral DNA amounts.

Generation of U2OS-ICP4/27-Cre Cells. Retroviral expression plasmid pCX4Hyg-Cre for stable Cre expression in U2OS-ICP4/27 cells was constructed as follows. A PCR-amplified Gateway recombination cassette (12) was inserted into the multicloning site of pCX4Hyg (a kind gift from T. Akagi, KAN Research Institute, Kobe, Japan; GenBank accession no. AB086387) to create plasmid pCX4Hyg-GW. The complete NLS-Cre coding sequence from plasmid pTurbo-Cre (ES Cell Core; Washington University School of Medicine; GenBank accession no. AF334827.1) was inserted between the *att*L1 and *att*L2 sites of pENTR1A (Invitrogen) and then transferred into pCX4Hyg-GW by LR Clonase-mediated Gateway recombination, creating pCX4Hyg-Cre. Retroviral particles were produced as described (16). Briefly, 293T cells were cotransfected with pCX4Hyg-Cre, pCL-GagPol, and pHCMV-VSV-G vectors (the last two kindly provided by N. Kiyokawa, H. Okita, and A. Umezawa, NRICHD, Tokyo), supernatant was collected 48 h later, filtered through a 0.45-μm filter, and concentrated by centrifugation. U2OS-ICP4/27 cells were infected with the purified Cre retrovirus and selected for resistance to puromycin (2 μg/mL), blasticidin (10 μg/mL), and hygromycin (200 μg/mL). Resistant clones were isolated and screened for Cre expression by immunostaining with anti-Cre antibody 2D8 (Millipore).

BAC Excision. BAC-deleted viruses (JΔNI5ΔB, JΔNI7GFPΔB, JΔNI7mDMDΔB) were generated by infection of U2OS-ICP4/27-Cre cells with JΔNI5, JΔNI7GFP, or JΔNI7mDMD virus at 0.001 gc per cell. At 100% CPE, supernatants were harvested and cleared, and individual recombinants were isolated by standard limiting dilution (17) on U2OS-ICP4/27 cells. Viruses were screened for loss of β-galactosidase activity by infection of U2OS-ICP4/27 cells at 10 gc per cell and X-gal staining (17) 48 h later. Accurate BAC deletion was confirmed by PCR across the remaining loxP site and sequencing of the product.

Statistics. All values are presented as the mean ± SD. Differences between pairs were analyzed by Student's *t* test using Microsoft Excel 14.4.1. *P* values below 0.05 ($P < 0.05$) were considered statistically significant.

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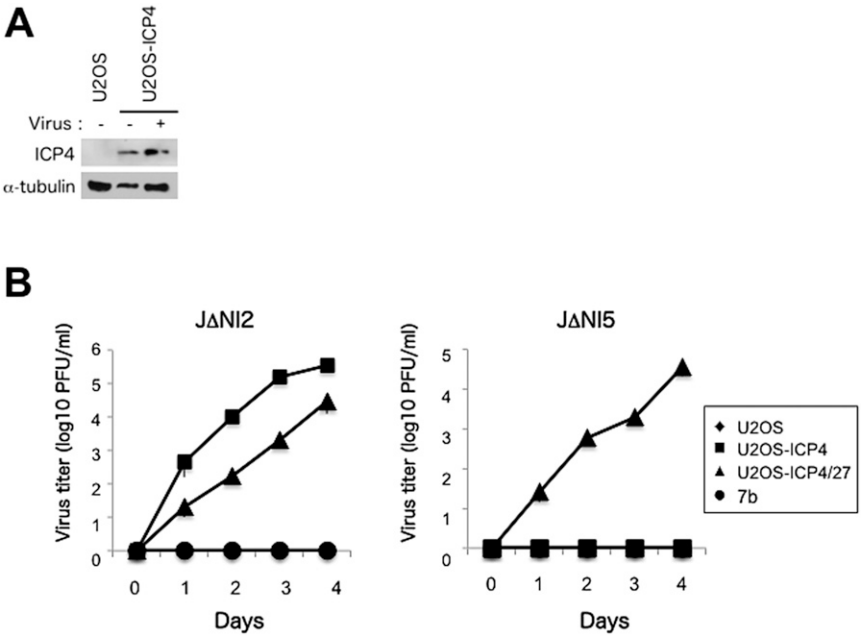

Fig. S1. U2OS-ICP4 cells and complementation requirements for JΔNI2 and JΔNI5 growth. (*A*) Western blot analysis of U2OS-ICP4 cells. Uninfected cells and cells infected with ICP4-deficient QOZHG virus (1) at an MOI of 1 were harvested at 24 hpi, and extracts were prepared for gel electrophoresis; an extract from uninfected U2OS cells was included as a control. The blot was probed with antibodies for ICP4 or α-tubulin as a loading control. The results show that ICP4 expression is independent of induction by virus infection. (*B*) JΔNI2 (*Left*) and JΔNI5 (*Right*) virus growth in U2OS, U2OS-ICP4, U2OS-ICP4/27, and Vero-7b (2) cells. Cells were infected at an MOI of 0.001, and extracellular virus from duplicate wells ($n = 2$) was harvested daily and titered on U2OS-ICP4/27 cells.

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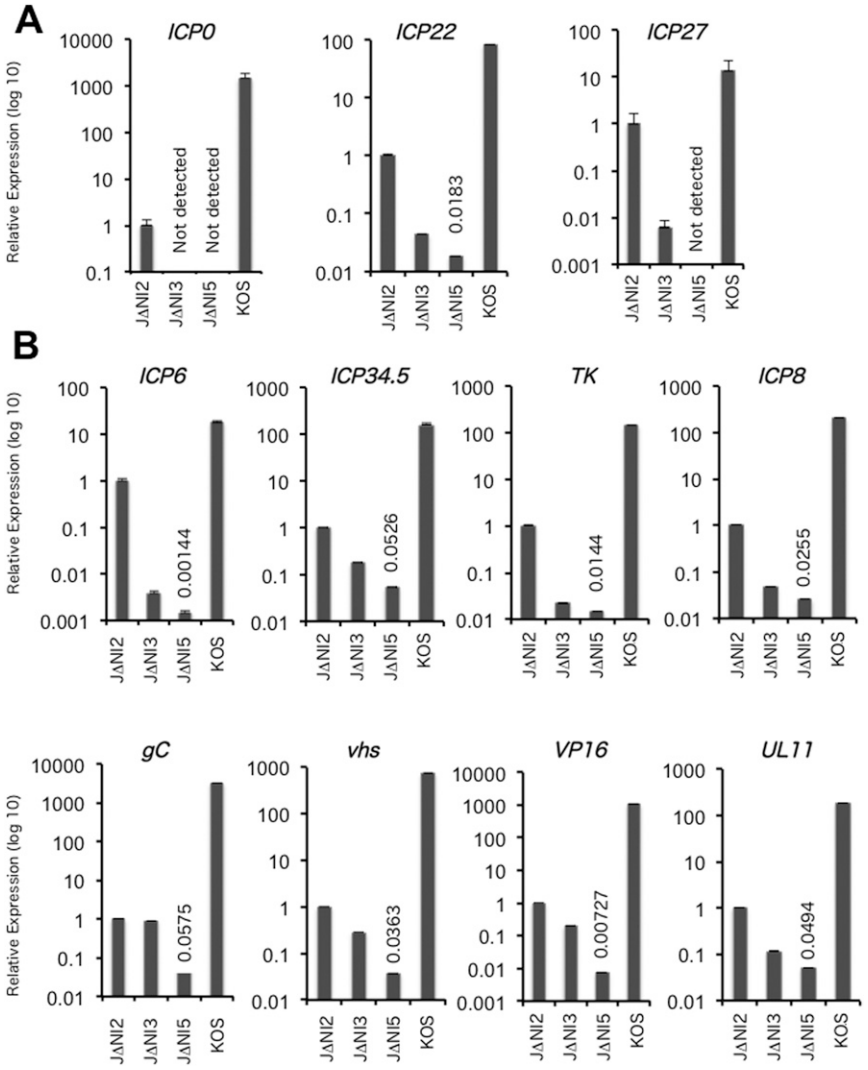

Fig. S2. JΔNI viral gene expression in noncomplementing cells. (*A*) JΔNI IE gene expression measured by qRT-PCR. HDFs were infected with the indicated viruses at 1,000 gc per cell. mRNA was isolated at 12 hpi and reverse-transcribed for qPCR determination of the cDNA levels for the genes listed at the top. Expression was normalized to 18S rRNA levels and is shown relative to JΔNI2-infected cells. (*B*) qRT-PCR analysis for expression of early (*Upper*) and late genes (*Lower*). HDFs were infected and processed as in *A*. ICP6 may be considered a delayed IE gene and is grouped here with the early genes because its expression is reportedly more dependent on ICP0 than on VP16 (1, 2). Data in *A* and *B* represent averages ± SD of two independent experiments.

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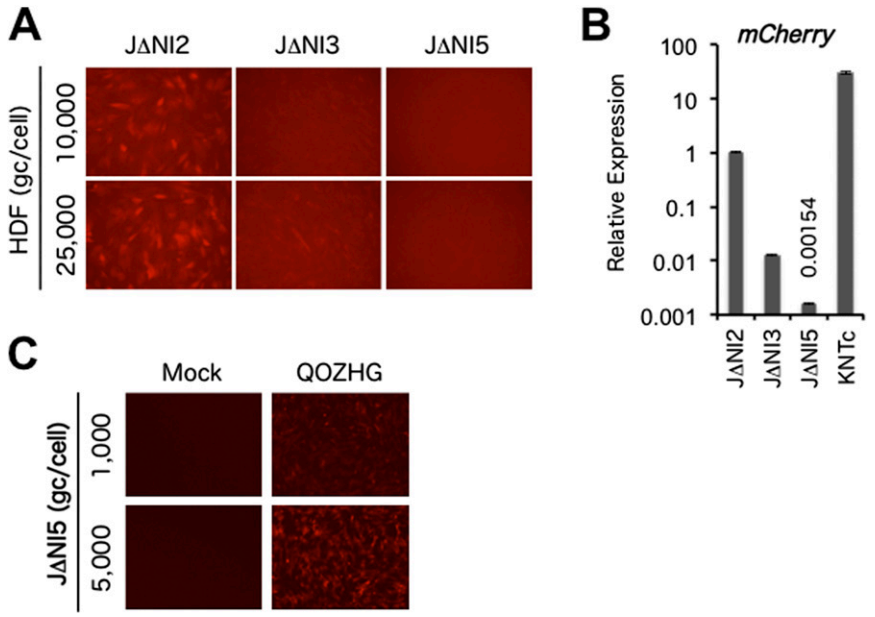

Fig. S3. Reporter-gene expression in JΔNI-infected HDFs. (*A*) mCherry fluorescence in infected HDFs. Cells were infected at the indicated gc per cell and photographed at 24 hpi and 100× magnification. (*B*) Relative mCherry mRNA levels in HDFs. Cells were infected at 5,000 gc per cell and harvested at 6 hpi for mRNA isolation and qRT-PCR with mCherry-specific primers listed in Table S3. Expression was normalized to 18S rRNA levels and is shown relative to JΔNI2-infected cells (averages ± SD of two independent experiments). KNTc is a replication-competent control vector that contains a UbC promoter-mCherry expression cassette in the intergenic region between the U_L3 and U_L4 genes and the entry-enhancing N/T double mutation in gB (5). (*C*) Induction of mCherry expression in JΔNI5-infected HDFs. Cells were infected at the indicted gc per cell, superinfected at 24 h with QOZHG at 5,000 gc per cell, and photographed 24 h later at 40× magnification.

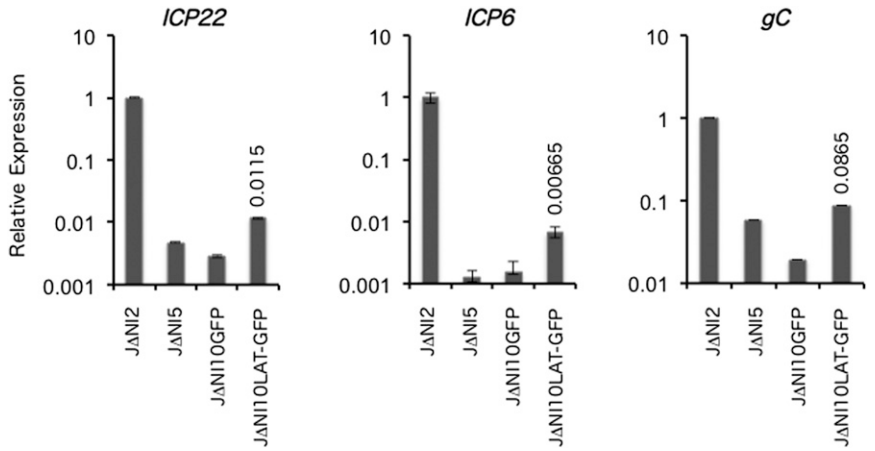

Fig. S4. Effect of repositioned LAT elements on viral gene expression. HDFs were infected with the indicated viruses at 1,000 gc per cell. mRNA was isolated at 12 hpi and reverse-transcribed for qPCR determination of the cDNA levels for the genes listed at the top. Expression was normalized to 18S rRNA levels and is shown relative to JΔNI2-infected cells (averages of two independent experiments ± SD).

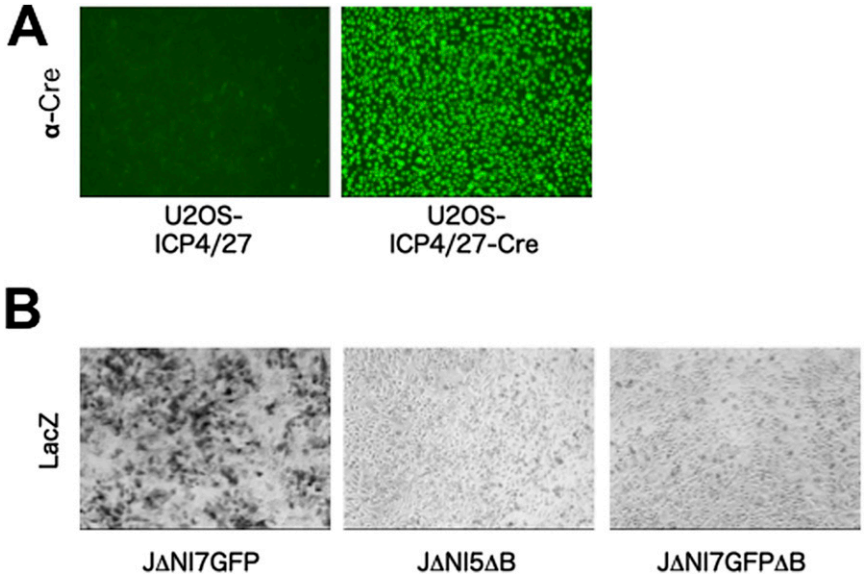

Fig. S5. Cre-expressing complementing cell line and its utilization for BAC excision. (*A*) Confirmation of Cre expression in U2OS-ICP4/27-Cre cells by anti-Cre immunofluorescent staining in comparison with the parental U2OS-ICP4/27 cell line. (*B*) Loss of *lacZ* gene expression after virus passage through U2OS-ICP4/27-Cre cells. Representative images of β-galactosidase activity in U2OS-ICP4/27 cells infected with JΔNI7GFP virus (BAC+) or U2OS-ICP4/27-Cre–passaged (BAC-excised) virus isolates (ΔB). Cells were infected at 10 gc per cell, fixed 48 h later, and stained with X-gal (dark cells).

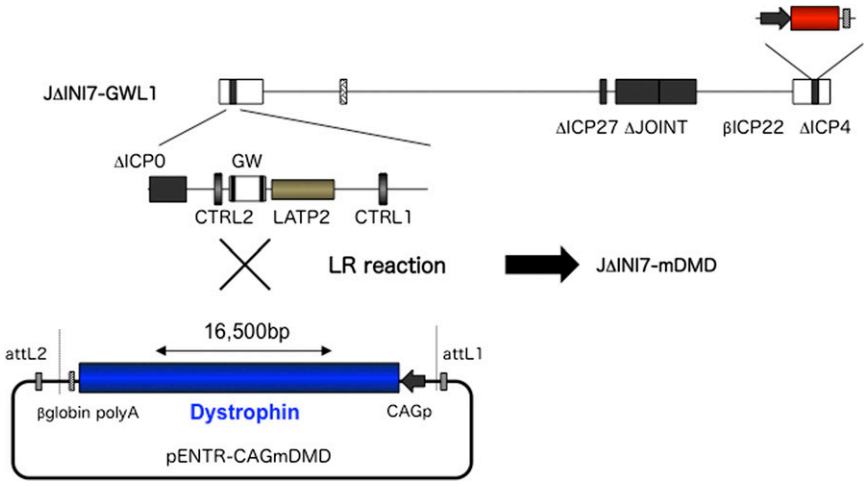

Fig. S6. Construction of vector JΔNI7mDMD. Red-mediated recombination was used to introduce a Gateway cassette between LATP2 and CTRL2 of JΔNI5, creating JΔNI7-GWL1 (*Upper*). A 16.5-kb expression cassette for full-length mouse dystrophin consisting of the CAG promoter, the complete mouse dystrophin cDNA, and the rabbit β-globin polyA region was assembled in a pENTR plasmid (*Lower*) and transferred into the LAT region of JΔNI7-GWL1 by GW recombination ("LR reaction") to generate JΔNI7mDMD.

Table S1. JΔNI5 plaquing efficiency on U2OS-ICP4/27 cells at different passage numbers

Passage no.	Plaque no. (mean ± SD)
p5	17.33 ± 6.35
p10	15 ± 2.64
p20	16 ± 4

U2OS-ICP4/27 cells were infected with JΔNI5 virus at 0.1 gc per cell, and the cells were overlayed with methylcellulose at 2 hpi. Plaques were counted at 4 dpi. Means ± SD of three determinations.

Table S2. Primers for Red-mediated recombination

Name	Target gene/locus	Forward (F)/reverse (R)	Sequence
R1	ΔICP0	F1	CCCGATATCCAATTGCGGGCGCTGGGTGGTCTCTGGCCGCGCCCACTACACCA-GCCAATCCGTGTAGGATGACGACGATAAGTAGGGATA
R2		F2	GATCTCGAGCTCAAGCTTCGAATTCTGCAGTCGACGGTACCGCGGGCCCCGAT-ATCCAATTGCGGGC
R3		R	ACACGGATTGGCTGGTGTAGTGGGCGCGGCCAGAGACCACCCAGCGCCCGCAA-CCAATTAACCAATTCTGATTAG
R4	ΔICP27	F	TATGGATCCCGGACCTGGTTAACCACCCGCCGGTCCTACGCGAACTGGAGGAT-AAGCGCAGGATGACGACGATAAGTAGGGATA
R5		R	CAGGAATTCGCGCTTATCCTCCAGTTCGCGTAGGACCGGCGGGTGGTTAACCA-GGTCCGCAACCAATTAACCAATTCTGATTAG
R6	ΔICP47 promoter and initiation codon	F1	GGGCCCTGGAAATGGCGGACACCTTCCTGGACACCATGCGGGTTGGGCCCAGG-ATGACGACGATAAGTAGGGATAACAGGG
R7		R	GGGCCCAACCCGCATGGTGTCCAGGAAGGTGTCCGCCATTTCCAGGGCCCCAA-CCAATTAACCAATTCTGATTAG
R8		F2	TTTATAACCCCGGGGGTCATTCCCAACGATCACATGCAATCTAACTGGCTGGG-CCCTGGAAATGGCGGACACC
R9	UbC-mCherry insertion	F1	TATGGATCCCCGCGGATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCAT-AGGATGACGACGATAAGTAGGG
R10		R1	AATCTGCAGGGATCCCTACAACCAATTAACCAATTCTGATTAG
R11		F2 (for ΔICP4)	CTTGGGGCGGTCCCGCCCGCCGGCCAATGGGGGGGCGGCAAGGCGGGCGGTGG-CGGCCGCTCTAGAAGATCTGGC
R12		R2 (for ΔICP4)	CCGCGGGGGGCCCGGGCTGCCACAGGTGAAACCAACAGAGCACGGCGCACGCT-GGGTACCGGGCCCCCCCTCGAG
R13		F3 (for UL3 and 4)	CCTCACTGCCCGTCGCGCGTGTTTGATGTTAATAAATAACACATAAATTTTGG-CGGCCGCTCTAGAAGATCTGGC
R14		R3 (for UL3 and 4)	CCGACACTGAAATGCCCCCCCCCCCTTGCGGGCGGTCCATTAAAGACAACGCT-GGGTACCGGGCCCCCCCTCGAG
R15	CAG-EGFP insertion	F1	TATTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTG-CTTAGGATGACGACGATAAGTAGGGATA
R16		R1	AATCTGCAGTACGTACTACAACCAATTAACCAATTCTGATTAG
R17		F2	GCAATTGGCTCTGCCCCGCCGTCCCCGTGTTCGTCC
R18		R2	TTTTTGCAAAAGCCTAGGCCTCCAATAACTAGTCAATAATCAATGTCGACTTA-TTTATTAACATCAAACACGCGC
R19		F3	GCACGCGTAGAGGTGCTGCGGGAGATTCAACTGAGC
R20		R3	TATTGACTAGTTATTGGAGGCCTAGGCTTTTGCAAAAAAGCTTATAATGGGTC-TTTAATGGACCGCCCGCAAGGG
R21	gB:N/T insertion	F	AACTGCATCGTCGAGGAGGTGGACGCGCGC
R22		R	GGAGACGGCCATCACGTCGCCGAGCATCCG
R23	ΔCTRL1	F1	TATACCCGTGACACCCGACGCTGGGGGGCGTGGCTGCCGGGAGGGGCCGCGTA-TGAGGATGACGACGATAAGTAGGGATA
R24		F2	CCACACAAGCCCCGTATCCCCGTTCCCGCGCTTTTCGTTGGTTTATATACCCG-TGACACCCGACGCTGGG
R25		R	CATACGCGGCCCCTCCCGGCAGCCACGCCCCCCAGCGTCGGGTGTCACGGCAA-CCAATTAACCAATTCTGATTAG
R26	ΔCTRL2	F1	AATCCAACACAGACAGGGAAAAGATACAAAAGTAAACCTTTATTTCCCAACAA-GGATGACGACGATAAGTAGGGATAACA
R27		F2	GTCAGGCAGCCCGGGCCGCGGCTCTGTGGTTAACACCAGAGCCTGCCCAATCC-AACACAGACAGGGAAAA
R28		R	TGTTGGGAAATAAAGGTTTACTTTTGTATCTTTTCCCTGTCTGTGTTGGACAA-CCAATTAACCAATTCTGATTAG
R29	ΔLATP2	F1	CAGATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCC-AGGATGACGACGATAAGTAGGGATAAC
R30		F2	AGACTTTCCGGGCGCGTCCGGGTGCCGCGGCTCTCCGGGCCCCCCTGCAGATA-GTAATCAATTACGGGGT
R31		R	GGAACTCCATATATGGGCTATGAACTAATGACCCCGTAATTGATTACTATCAA-CCAATTAACCAATTCTGATTAG
R32	GW	F1 (for LAT)	TGACAATTGACTAGTTATACAAGTTTGTACAAAAAAGCTGAAC
R33		R1 (for LAT)	CCATTATAACAATTGAGATCTCCACTTTGTACAAGAAAGCTGAACG
R34		F2 (for UL45 and 46)	CGGACCCAAAATAATAAACACACAATCACGTGCGATAAAAAGAACACGCGACA-AGTTTGTACAAAAAAGCTGAAC
R35		R2 (for UL45 and 46)	CCTGTTTGTCGACGAGATTTAATAAAAATAACCAAAAACACCACAGGGGACCA-CTTTGTACAAGAAAGCTGAACG

Table S2. Cont.

Name	Target gene/locus	Forward (F)/reverse (R)	Sequence
R36		F3 (for UL50 and 51)	AAACGTTTGTATCTCATCTTTCCTGTGTGTAGTTGTTTCTGTTGGATGCCATA-TCACAAGTTTGTACAAAAAAGC
R37		R3 (for UL50 and 51)	TGCGTGTTTTCATCCAACCCGTGTGTTCTGTGTTTGTGGGATGGAGGGGCCGA-GAAACGTAAAATGATATAAATA
R38	ΔLAT	F1	CCGGGGACACCGCCAGCAAACGCGAGCAACGGGCCACGGGGATGAAGCAGAGG-ATGACGACGATAAGTAGGGATA
R39		F2	CCACACAAGCCCCGTATCCCCGTTCCCGCGCTTTTCGTTGGTTTATATACCCG-GGGACACCGCCAGCAAACGCGA
R40		R	CTGCTTCATCCCCGTGGCCCGTTGCTCGCGTTTGCTGGCGGTGTCCCCGGCAA-CCAATTAACCAATTCTGATTAG
R41	Insertion of TK promoter	F1 (for ICP0 pro)	CCCGGCCAACCAGCGTCCGCCGAGTCTTCGG
R42		R1 (for ICP0 pro)	GCCGGTTCCAGTGTAAGGGTCGGGGGTCCC
R43		F2 (for ICP27 pro)	CCTGACAGAGCTGTATTGTA
R44		R2 (for ICP27 pro)	AGATCGCTGTCGGAGAGGTCC
R45		F3 (for ICP0 pro)	TATAGATCTCGAGCTCAAGCTTCGAATTCTGCAGTCGACGGTACCGCGGGCCC-AGGATGACGACGATAAGTAGGG
R46		R3 (for ICP0 pro)	AATCTGCAGAGATCTCTACAACCAATTAACCAATTCTGATTAG
R47		F4 (for ICP27 pro)	TATGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGT-AGGATGACGACGATAAGTAGGG
R48		R4 (for ICP27 pro)	AATCTGCAGGTTAACCTACAACCAATTAACCAATTCTGATTAG
R49		F	TGACAATTGACTAGTTATACAAGTTTGTACAAAAAAGCTGAAC
R50		R	CCATTATAACAATTGAGATCTCCACTTTGTACAAGAAAGCTGAACG

Table S3. qPCR primers used in this study

Name	Target gene	Forward (F)/reverse (R)	Sequence
Q1	Human S18	F	GCGGCGGAAAATAGCCTTTG
Q2		R	GATCACACGTTCCACCTCATC
Q3	EGFP	F	ATCATGGCCGACAAGCAGAAGAAC
Q4		R	GTACAGCTCGTCCATGCCGAGAGT
Q5	mCherry	F	CCTGTCCCCTCAGTTCATGT
Q6		R	GCTTCAAGTAGTCGGGGATG
Q7	ICP0	F	ACCACCATGACGACGACTC
Q8		R	AGCCCCGTCTCGAACAGT
Q9	ICP22	F	CGTGTGCAAGCTTCCTTGT
Q10		R	TCTCGAGATTACTAAGATCACACTCC
Q11	ICP27	F	AGTCGTGTCTGCGAGTTGAC
Q12		R	AAAACAGGGAGTTGCAATAAAAA
Q13	ICP4	F	ATGGGGTGGCTCCAGAAC
Q14		R	CTGCCGGTGATGAAGGAG
Q15	ICP34.5	F	GTAACCTCCACGCCCAACT
Q16		R	AGCAGCAGCGAACAAGAAG
Q17	UL11	F	ATGGGCCTCTCGTTCTCC
Q18		R	CGGTGATGAGGACGTTGTT
Q19	UL23 (TK)	F	TCATCTTCGACCGCCATC
Q20		R	TGCTGCCCATAAGGTATCG
Q21	UL27 (gB)	F	GTCAGCACCTTCATCGACCT
Q22		R	CAGGGGGACAAACTCGTG
Q23	UL29 (ICP8)	F	AGCTGCAGATCGAGGACTG
Q24		R	CCATCATCTCCTCGCTTAGG
Q25	UL30	F	GTCTGCTCTACGACCTGTCCA
Q26		R	AACCGAGCGAAAACAGGAG
Q27	UL41 (vhs)	F	TCCATCCCAATAACACCTACG
Q28		R	GGGGTCCAGTGACATTCG
Q29	UL44 (gC)	F	GAGGGTCAGCCGTTCAAG
Q30		R	AACTCCACGGGGTTACGC
Q31	UL48 (VP16)	F	GCGCTCTCTCGTTTCTTCC
Q32		R	GGCCAACACGGTTCGATA
Q33	UL39 (ICP6)	F	CAATCTGGGAAGCGTGAATC
Q34		R	CGCCCAAAGTCAAACGTC
Q35	Rat S18	F	CCACAGGAGGCCTACACG
Q36		R	CTGAAACTTCTCGGGGATCA
Q37	gD-TaqMAN	F	CCCCGCTGGAACTACTATGACA
Q38		R	GCATCAGGAACCCCAGGTT
Q39		Probe	FAM-TTCAGCGCCGTCAGCGAGGA-TAMRA