HSV-1 Remodels Host Telomeres to Facilitate Viral Replication

Supplemental Materials

Supplemental Experimental Procedures

Cells. BJ, BJ-hTERT, HeLa, U2OS, VERO, and human neuroblastoma SY5Y cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % fetal bovine serum and antibiotics in a 5 % CO_2 incubator at 37 °C. HepaRG-based cells, including HA-TetR, HA-cICP0, HA-FXE, and HA-IE1 cells, were grown in William medium E supplemented with 10% FBS, 2 mM L-glutamine, 0.5 μM hydrocortisone, and 5 μg/ml insulin in a 5 % CO_2 incubator at 37 °C (Everett et al., 2009). Human foreskin fibroblasts (HFFs) were grown in Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum, 250 mg/ml Amphotericin B, and antibiotics in a 5% CO_2 incubator at 37°C. Human lung epithelial cells (Calu-3) were cultured in MEM supplemented with 15% fetal bovine serum and antibiotics in a 5% CO_2 incubator at 37°C.

Plasmids and Lentiviral Transduction. Plasmid DNA transfections were performed by the use of Lipofectamine 2000 reagent (Invitrogen) using 2-5 μg of plasmid DNA for 1 X 10^6 cells, which were seeded in 6-cm plates 12 to 16 h prior to transfection. pLKO.1 vector-based shRNA constructs for PML, ATRX, and Daxx were gifts of Roger Everett and described previously (Lukashchuk and Everett, 2010). shControl and shTPP1 were generated in pLKO.1 vector with target sequence as followings: shCon, TTATCGCGCATATCACGCG; shTPP1-1, CCCAGAAACCTAGCCTGGAGTTCAA; shTPP1-2, GCAGCTGCTTGAGGTACTACA;

shTPP1-4, GACTTAGATGTTCAGAAAA; shTPP1-5, TGGAGTTCAAGGAGTTTGT. Lentiviruses were produced by the use of packaging vectors pMDLg/pRRE, RSV-Rev, and CMV-VSVG, and was used for infection experiments as described (Deng et al., 2012a). For shRNA depletion experiments, cells were infected twice on consecutive days, treated with 1 μg/ml Puromycin at 48 hrs after the first infection, and harvested or re-plated at 6 days post-infection for further analysis. shRNA depletion was assayed by western blotting and the knocking-down efficiency was examined by using Multi Gauge V3.0 software (Fuji Film). TPP1 or TRF2 levels were calculated relative to signals from β-Actin loading control and shRNA control, which was normalized to 1.

Viral Strains and Infection. HSV-1 wt strain 17+, and 17+ strain-derived ICP0-null mutant *dl*1403, ICP0 ring finger deletion mutant FXE, and its revertant FXER have been described elsewhere (Everett and Orr, 2009; Lukashchuk and Everett, 2010). Viruses were propagated in VERO cells and titrated in U2OS cells where ICP0 is not required for efficient HSV-1 replication (Harland and Brown, 1998; Yao and Schaffer, 1995). HSV-1 wt strain KOS1.1, and KOS1.1-derived ICP8-null mutant HD-2 and ICP8-GFP expressing virus HSV8GFP were described previously(Da Costa et al., 1997; Taylor et al., 2003). Varicella-zoster virus (VZV) was generated from recombinant VZV-BAC-Luc strain (Zhang et al., 2007). VZV (CPE > 80%) was used to infect HFFs at 1:10 ratio of infected cells to uninfected cells, or 1:2 ratio for high dose infection. hCMV strain Towne and adenovirus serotype 5 (Ad5) were used to infect BJ and U2OS cells. Viruses were adsorbed for 2 hrs in a 5 % CO_2 incubator at 37 °C, which was regarded as time 0. Influenza virus strain PR8 was used to infect 85% confluent Calu3 cells by incubating the cells with influenza virus (MOI=3) in 3 ml of serum-free medium for 1 hr at 37°C

incubator with gentle agitation every 15 min. Cells were collected at 4, 8, 24, 48, and 72 hrs after infection. For all HSV-1 infection experiments, cells were plated at about 60% confluence in growth medium the day before infection, and the amount of cells varied by cell types and plates size. After removing the growth medium, infection was carried by incubating HSV-1 virus or mock in a low volume of pre-warmed blank medium with cells for 1 hr at 37°C incubator. After 1 hr incubation, complete growth medium was added into the cells (time 0), and infected cells will be collected for further applications at indicated time points. For ICP0 restricted cell types, such as BJ and BJ-hTERT cells, ten times MOI difference of HSV-1 wild-type and ICP0 mutants were used for infection to achieve comparable levels of viral infection.

Western Blotting. Equal amounts of whole-cell extracts in 1 x Laemmli buffer were resolved by electrophoresis in 8–16% Tris·Glycine gels (Life Technologies), transferred onto a nitrocellulose membrane, blotted with the indicated antibodies, and visualized by use of the ECL plus kit (GE Healthcare) using either X-ray film or the LAS 3000 imager (Fuji Film). Primary antibodies to TPP1 (Bethyl Laboratories), PML, POT1 and GAPDH (Santa Cruz), phospho-Histone H2AX (γH2AX) (Millipore), ICP8 (Abcam), and α-Tublin, Daxx, and β-actin peroxidase conjugate (Sigma-Aldrich) were used according to manufacturer's specifications. Monoclonal antibodies to ICP0 and ICP4 were gifts from Roger Everett (Institute of Virology, Scotland). Rabbit polyclonal antibodies to TRF1, TRF2, and hRap1 were generated against recombinant proteins and affinity-purified.

RNA Preparation and Analysis. Total RNA was purified with Trizol reagent (Life Technologies) as described previously (Deng et al., 2012a). All RNA samples were treated with

DNase I for 45 min at 37 °C before further application. When indicated, RNA samples (~ 5-10 μg) were further treated with RNase A (Roche) at a final concentration of 100 μg/ml for 45 min at 37 °C before denaturation in sample loading buffer (Ambion). Northern blotting was performed using Church buffer (0.5N Na-phosphate, pH 7.2, 7% SDS, 1mM EDTA, 1% BSA) for 16-18 hrs at 50 °C, essentially as described previously (Deng et al., 2012a). The blots were first hybridized with a ^{32}P-labeled $(TAACCC)_4$ probe, then stripped, and probed with a ^{32}P-labeled 18S probe. Images were analyzed by phosphor-imager (Amersham Biosciences), processed with a Typhoon 9410 Imager (GE Healthcare), and quantified with ImageQuant 5.2 software (Molecular Dynamics). TERRA RNA levels were calculated relative to signals from 18S internal control and mock control. Quantitative RT-PCR experiments were performed using Super Script III Reverse Transcriptase (Invitrogen) and an ABI Prism 7900 Sequence Detection System (Applied Biosystems), essentially as described (Deng et al., 2009b; Deng et al., 2012b). Relative RT-PCR was determined using ΔΔCT methods relative to control samples and internal control *U1* snRNA. Primer sequences used for real time PCR have been validated and listed previously (Deng et al., 2012a). Nascent RNA was prepared by the use of Click-iT Nascent RNA capture Kit (Life Technologies) with minor modifications. Briefly, BJ cells were infected with mock or HSV-1 (MOI=1) for 2 hrs, and then EU was added in the final concentration of 0.2 mM for additional 4 hrs. Total RNA was prepared and subject to Click reaction. Nascent RNA was purified by using Streptavidin T1 magnetic beads, and was used as a template for cDNA synthesis using SuperScript VILO cDNA synthesis kit (Life Technologies) as specified by manufacturers. Relative RT-PCR was determined using ΔΔCT methods relative to mock-infected samples and internal control *U1*.

Immuno-FISH Assay. Indirect immunofluorescence (IF) combined with fluorescence *in situ* hybridization (FISH) analysis was performed as described (Rai and Chang, 2011) with some modifications. Briefly, cells grown on coverslips were fixed for 15 min in 2% paraformaldehyde/2% sucrose at RT, followed by permeabilization for 10 min in 1x PBS/0.5% Nonidet-P40 at RT. After washing with 1x PBS, cells were incubated for 60 min in blocking solution (0.5% BSA, 0.2% fish gelatin, 0.1% Triton X-100, 1 mM EDTA in 1x PBS) before immuno-staining. Primary antibodies were prepared in blocking solution as following dilutions: monoclonal anti-γH2AX (1:200), anti-ICP0 (1:500), anti-ICP8 (1:1000), and rabbit polyclonal TRF2 (1:1600). After IF, cells were fixed in 4% paraformaldehyde in 1x PBS for 10 min, washed in 1x PBS, dehydrated in ethanol series (70%, 95%, 100%), and air- dried. Coverslips were denatured for 5 min at 80-85 °C in hybridization mix (70% formamide, 10mM Tris-HCl, pH 7.2, and 0.5% blocking solution (Roche)) containing telomeric PNA-Tamra-$(CCCTAA)_3$ probe, and hybridization was continued for 2 hrs at room temperature in the dark moisturized chambers. Coverslips were washed twice for 15 min each with 70% formamide, 10 mM Tris-HCl (pH 7.2), and 0.1% BSA, and followed by three washes for 5 min each with 0.15 M NaCl, 0.1 M Tris-HCl (pH 7.2), and 0.08% Tween-20. Nuclei were counterstained with 0.1 μg/ml DAPI in blocking solution and slides were mounted with VectorShield (Vector Laboratories, Inc). Immuno-RNA FISH was performed essentially as described previously (Deng et al., 2009b). Images were captured either with a 100 X lens on a Nikon E600 Upright microscope (Nikon Instruments, Inc., Melville, NY) using ImagePro Plus software (Media Cybernetics, Silver Spring, MD) for image processing, or with a 63 X lens on a Leica SP5 II Confocal microscope (Leica Microsystems, Wetzler, Germany) using LAS AF software for image processing and quantification. Cells with five or more γH2AX foci colocalizing with telomere

DNA foci were scored as TIF positive. Colocalization for telomere DNA foci with ICP0 or ICP8 foci was quantified by counting the percent of telomere DNA foci colocalized with either ICP0 or ICP8 foci in HSV-1 infected cells from at least three independent Immuno-FISH experiments.

Metaphase Telomeric DNA FISH. FISH analysis on metaphase spreads was performed as described (Deng et al., 2009b) with some modifications. Briefly, BJ cells were infected with HSV-1 (MOI=0.1) or mock for 6 hrs, and then treated further with colcemid (0.1μg/ml; Gibco) for 90 min to accumulate mitotic cells. Cells were collected by centrifugation at 120 g for 10 min, resuspended in 75 mM KCl hypotonic solution at 37 °C for 25 min, and fixed in fresh 3:1 methanol/acetic acid for 4-5 times. Metaphase chromosome spreads were stained with 0.1 μg/ml 4', 6'-diamino-2-phenylindole (DAPI) and analyzed by telomeric DNA FISH using PNA-Tamra-$(CCCTAA)_3$ probe as described above. Images were taken with a 100 X lens on a Nikon E600 Upright microscope (Nikon Instruments, Inc., Melville, NY) using ImagePro Plus software (Media Cybernetics, Silver Spring, MD) for image processing. Statistical analysis was performed using paired two-tailed Student t Test from three independent metaphase spread FISH experiments.

Live Cell Imaging. Live cell imaging of HSV-1 infection was performed as described (Taylor and Knipe, 2009), with some modifications. U2OS cells (~0.5 x 10^6) were transfected with a plasmid (~0.5 μg) expressing either DsRed-TRF2 or Cherry-TRF1, and re-plated on a glass coverslip bottom culture chamber at 6 hrs post-transfection. At 20 hrs post-transfection, cells grown in culture chamber were infected with ICP8-GFP viruses at a MOI of 20, and placed onto a Leica SP5 II Confocal microscope stage prewarmed to 37 °C and filled with CO_2 for time-

lapse imaging. Multiple cells with either DsRed-TRF2 or Cherry-TRF1 foci were positioned for live cell imaging to visualize the colocalization of ICP8-GFP with TRFs starting at around 1 h post-infection. The phase and fluorescent images were captured at 5 min or 10 min intervals for cells transfected with DsRed-TRF2 or Cherry-TRF1, respectively, over a period of about 16 hrs.

Chromatin Immunoprecipitation (ChIP) Assays. ChIP assays were performed as described previously (Deng et al., 2012a). BJ cells infected with HSV-1 wt (MOI=0.1), ΔICP0 (MOI=1), or mock at 6 hrs post infection were used for ChIP. ChIP DNA at telomeres was quantified by dot blotting with probes specific for telomere repeat DNA or Alu repeat as described (Deng et al., 2012a). Briefly, ChIP DNA was denatured, dot blotted onto GeneScreen Plus blotting membranes (PerkinElmer) and crosslinked at 125 mJ. Oligonucleotide probes for telomere repeats (4 x TTAGGG or 4 x TAACCC) or Alu repeats (cggagtctcgctctgtcgcccaggctggagtgcagtggcgcga) were labeled with γ-[^{32}P]ATP (3,000 Ci/mmol) and T4 nucleotide kinase (New England Biolabs). The membrane was hybridized in Church hybridization buffer containing a ^{32}P-labeled probe at 42 °C overnight, washed three times in 0.04 N Na-phosphate, 1% SDS, 1 mM EDTA at 42 °C, developed with a Typhoon 9410 Imager (GE Healthcare) and quantified with ImageQuant 5.2 software (Molecular Dynamics). Quantification of ChIP DNA at subtelomeric regions was determined using real-time PCR and the Absolute Quantification program with an ABI 7900 Sequence Detection System (Applied Biosystems). Primers specific for 10q and XYq subtelomeres have been described previously (Deng et al., 2012a)), and primers specific for HSV-1 terminal repeats (TR-4) are as followings: forward, 5'- TGTTTGTTTATTCCGACATTGGTT -3'; reverse, 5'- CAGCCACACGCAAGAACAGA -3'. ChIP western analysis was performed essentially as

ChIP assay, except that ChIP eluted materials were incubated at 65 °C for 3 hrs to reverse cross-linking and assayed by western blot. Antibodies used in ChIP assay include: rabbit polyclonal antibodies to CTCF, histone H3 K4 di- or tri-methylation, histone H3 Ac, or total histone H3 (Millipore or Active Motif), ICP8 (Abcam), and rabbit IgG (Santa Cruz). Rabbit antibodies to TRF1 and TRF2 were generated against recombinant protein and affinity purified.

Telomere Length Assay. BJ or BJ-hTERT cells infected with HSV-1 wt (MOI=0.1), ΔICP0 (MOI=1), or mock at various time points were used for telomere length assay. For telomere length assay in U2OS or SY5Y cells, HSV-1 wt (MOI=1) or ΔICP0 (MOI=1) was used for infection. Genomic DNA was prepared using Wizard genomic DNA purification kit (Promega) as manufacturer's instruction. For in-gel hybridization, genomic DNA (~ 10 μg) was digested with *Alu*I+*Mbo*I restriction endonucleases, fractionated in a 0.7% agarose gel, and dried at 28 °C by a vacuum gel dryer (BioRad). The dried gel was hybridized in-gel at 42 °C with 5′-end-labeled ^{32}P-$(TAACCC)_4$ probe in Church buffer, and washed twice for 5 min with 0.2 M wash buffer (0.2 M Na_2HPO4 pH 7.2, 1 mM EDTA, and 2% SDS) at room temperature and once with 0.1 M wash buffer at 42 °C. The gel was further denatured in alkaline solution (0.5 M NaOH, 0.15 M NaCl) twice for 30 min each, neutralized in neutralization solution (0.5 M Tris-HCl, pH 7.5, 3 M NaCl) twice for 20 min each, and hybridized with ^{32}P-labeled $(TAACCC)_4$ or Alu probe as described above. An oligonucleotide probe specific for the HSV-1 terminal repeats (TR) region (HSV-1 genome coordinates: 151751-151790) contains the following sequence: gagttgttgc gcggtgagtt gtcgctgtga gttgtgttgt. For regular Southern blots, genomic DNA (~ 7.5 μg) digested with *Alu*I+*Mbo*I restriction endonucleases was fractionated in a 0.7% agarose gel, transferred onto a GeneScreen Plus hybridization membrane (PerkinElmer), and hybridized with

^{32}P-labeled $(TTAGGG)_4$ probe in Church buffer as described. The images were analyzed by Phosphor-imager, visualized by Typhoon 9410 Imager (GE Healthcare), and quantified with ImageQuant 5.2 software (Molecular Dynamics).

MNase Pattern Assay. Telomeric nucleosome patterns were analyzed by micrococcal nuclease (MNase) digestion. Briefly, nuclei isolated from BJ cells infected with HSV-1 wt (MOI=0.1), ΔICP0 (MOI=1), or mock at 6 hrs post infection were treated with indicated units of MNase in reaction buffer (25 mM KCl, 1 mM $CaCl_2$, 4 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.4, and 12.5% glycerol) for 5 min at 37 °C. The reaction was stopped immediately by adding an equal volume of stop buffer (2% SDS, 0.2 M NaCl, 10 mM EDTA, 10 mM EGTA, 50 mM Tris-HCl, pH 8.0, and 100 μg/ml proteinase K). DNA was isolated by phenol/chloroform extraction, fractionated in a 1.5% agarose gel, transferred onto a GeneScreen Plus membrane, and hybridized with ^{32}P-labeled $(TTAGGG)_4$ probe or ^{32}P-labeled Alu probe. The images were visualized by Typhoon 9410 Imager (GE Healthcare), and quantified with ImageQuant 5.2 software (Molecular Dynamics). The relative signal intensities for mono-, di-, or tri-nucleosomes were calculated by the ratio of signals from telomeric probe to corresponding signals from Alu probe at four MNase units (9, 15, 30, and 60 U), and the average value from the four reactions was used for graph.

Viral Copy Number and Plaque Assay. BJ cells depleted for TRF2, TPP1, or Control were infected with HSV-1 at an MOI of 0.1. For quantification of viral genome copy number, genomic DNA isolated from infected cells at time 0 and 16 or 24 hrs post-infection was assayed by qPCR using primers specific for either HSV-1 ICP0 promoter region (Forward: 5'-TAACTTATACCCCACGCCTTTC-3'; Reverse: 5'- TCCGGGTATGGTAATGAGTTTC-3') or

VP16 promoter region (Forward: 5'- GCCGCCCCGTACCTCGTGAC -3'; Reverse: 5'-CAGCCCGCTCCGCTTCTCG -3'). The relative numbers of viral genomes at 16 hrs post-infection was determined using ΔΔCT relative to time 0 samples and an actin control. For plaque assay, cell culture supernatants from infected BJ cells at time 0, 8, or 24 hrs post-infection were used to infect Vero cells in 24 –well plates for progeny virus titers. After virus adsorption for 1 h, the cells were overlaid with medium containing 0.5% carboxymethylcellulose. Plaques were stained with crystal violet at 3 days post-infection. For viral copy number analysis in TPP1-expressing cells, U2OS cells were first transfected with increasing amount of vectors expressing Flag-TPP1 or control vector. After 20 hrs post-transfection, cells were infected with HSV-1 at an MOI of 0.1 and assayed by qPCR using primers specific for either VP16 promoter region or control actin region at time 0 and 16hrs post-infection.

Supplemental Figure Legends

Figure S1. Telomeric DNA FISH analysis on metaphase spreads and TIF assay in HSV-1 infected cells (related to Figure 1). A) Additional representative images of telomeric DNA FISH on metaphase spreads in mock infected cells (i); HSV-1 infected cell with separated sister chromatids (ii); metaphase spreads with telomeric aberrations, including telomere free ends (arrowhead) and sister chromatid fusion (star) in HSV-1 infected cells (iii). Scale bar = 10 μm. **B)** Additional representative images of TIF assay in BJ cells infected with HSV-1 (MOI=1) or mock at 6 hrs post-infection. Scale bar at left equals to 5 μm, and scale bar in the enlarged image equals to 1 μm.

Figure S2. HSV-1 is unique in inducing high levels of TERRA RNA when compared to other viruses (related to Figure 2). A) Northern blot of TERRA RNA from BJ fibroblasts infected with HSV-1 (MOI=1), hCMV (MOI=3), or Adenovirus (MOI=25) at indicated time points. The blot was first hybridized with ^{32}P-labelled $(TAACCC)_4$ probe for TERRA RNA, then stripped and re-hybridized with ^{32}P-labelled 18S probe. The size of TERRA RNA was shown in Kb at left. The relative value represents TERRA signals relative to 18S rRNA signals and mock infection. **B)** The same as in (A), except that U2OS cells were used for infections. **C)** Northern blot of TERRA in HFF cells infected with VZV at indicated time points. **D)** Northern blot of TERRA in Calu-3 cells infected with PR8 strain of influenza virus (MOI=3) at indicated time points. **E)** Western blot analysis of viral protein expression. BJ fibroblasts and U2OS cells were infected with HSV-1 (MOI=1), hCMV (MOI=3), or Adenovirus (MOI=25) for 6 or 12 hrs, as shown in Fig. 1A and B. The infected cells were harvested and assayed by Western blot using antibodies specific for viral proteins, including HSV-1 ICP0, hCMV IE1 or IE2 (Virusys), or AdV DBP (a gift kindly provided by Arnold Levine). **F)** Cellular inflammatory response does not induce TERRA. BJ-hTERT and U2OS cells were treated with recombinant human interferon β 1A (IFNβ 1a) (Millipore) at a final concentration of 1000 U/ml to induce cellular inflammatory response. Western blot was performed to assay the cells harvested at indicated time points, using antibodies specific for ISG15 (Santa Cruz) or control β-actin (Upper panels). Northern blot analysis of TERRA RNA levels in cells treated with IFNβ 1a. The relative value of TERRA RNA was shown at the bottom (Lower panels). **G)** BJ cells were infected with increased titers of HSV-1 viruses (MOIs=0.01, 0.05, 0.1, 1, and 5) or mock for 6 hrs. Upper panels, western blot analysis of infected cells with antibodies specific for viral proteins ICP0, ICP8, or cellular proteins PML, γH2AX, and β-actin. Lower panels, infected cells were assayed by Northern blot

using ^{32}P-labeled $(TAACCC)_4$ probe for TERRA RNA or 18S probe for 18S rRNA. The numbers below shows the relative value of TERRA RNA to 18S RNA and mock infection.

Figure S3. ICP0-null mutant virus (ΔICP0) is compromised for TERRA induction in multiple cell types (related to Figure 3). A) Northern blotting analysis of TERRA RNA in U2OS cells infected with either HSV-1 wt (MOI=1) or ΔICP0 (MOI=1) at 9 or 24 hrs post-infection. TERRA signals were quantified as relative value to 18S rRNA signals and mock infection. RNase A treatment diminishes TERRA signals, indicating the signals are specific for RNA. Note: TERRA RNA was slightly induced in late stage (24 h) of infected U2OS cells by ΔICP0 mutant viruses. **B)** Western blotting analysis of infected U2OS cells as shown in (A) using antibodies specific for viral proteins ICP0, ICP4, or ICP8, and cellular factors TRF2, γH2AX or β-actin. **C)** Northern blotting analysis of TERRA RNA in SY5Y cells infected with either HSV-1 wt (MOI=1) or ΔICP0 (MOI=1) at 6 or 16 hrs post-infection. TERRA signals were quantified as relative value to 18S rRNA signals and mock infection, and shown at the bottom. **D)** Western blotting analysis of infected SY5Y cells as shown in (C) using antibodies specific for viral proteins ICP0, or ICP4, and cellular factors PML, γH2AX or β-actin.

Figure S4. ICP0 alone only moderately induces TERRA levels, and a fraction of ICP0 localizes to telomere DNA foci (related to Figure 3). A) HA-TetR, HA-cICP0, HA-FXE, and HA-IE1 cells were treated with tetracycline at 0.1 μg/ml for 24 or 48 hrs. Total RNA isolated from these cells were assayed by Northern blotting with ^{32}P-labeled $(CCCTAA)_4$ or 18S probe. TERRA signals were quantified as relative value to 18S rRNA signals and mock, as shown in numbers at the bottom. **B)** Western blotting analysis of HepaRG-based cells treated with

tetracycline, shown in (A), using antibodies to ICP0, PML, IE1, or β-actin. **C)** U2OS cells were infected with lentiviruses encoding YFP-ICP0, YFP-ICP0fxe, or vector control, and assayed by Northern blotting for TERRA levels at 24 hrs post-infection. **D)** Quantification of Northern blots from three independent experiments, a representative shown in (C). The bar graph represents mean TERRA levels and standard errors relative to 18S and vector control. P values were calculated by a two-tailed Student's t –Test. **E)** Western blot of YFP-ICP0 or -ICP0fxe expression in infected cells, as shown in (C). **F)** A portion of exogenous YFP-ICP0 or YFP-ICP0fxe localizes to telomere. Representative telomere DNA FISH analysis of U2OS cells infected with lentiviruses encoding YFP-ICP0, YFP-ICP0fxe, or vector. The infected cells were assayed by FISH with CCCTAA PNA probe (red) at 24 hrs post-infection. YFP-ICP0s are shown in green (middle panel), and Dapi (blue) and merge images are shown to the right. Images were taken with a 100 X lens on a Nikon E600 Upright microscope using ImagePro Plus software for image processing. Scale bar = 5 μm. **G)** Indirect immunofluorescence analysis of infected U2OS cells as shown in (F). TRF2 foci were detected using rabbit polyclonal antibody specific for TRF2 (1:1600 dilution), and shown in red (left panel). YFP-ICP0s are shown in green (middle panel), and Dapi (blue) and merge images are shown to the right. Scale bar = 5 μm.

Figure S5. HSV-1 infection alters TPP1 but not other shelterin proteins (related to Figure 4). A) BJ cells were infected with HSV-1 (MOI=3) and assayed by Western blotting at indicated time course, using antibodies against shelterin proteins TPP1, POT1, hRap1, TRF1, TRF2, or control Tubulin. **B)** HeLa cells were infected with HSV-1 (MOI=1) in the absence or presence of MG132 (5 μM) for indicated time and assayed by Western blotting using antibodies as

indicated. **C)** Western blot of infected HeLa cells the same as in (B), instead that HeLa cells were infected with either wild-type HSV-1 (MOI=1) or ΔICP0 (MOI=1) viruses. **D)** BJ cells were infected with lentiviruses expressing shRNA against TPP1, or negative control. The infected cells were selected with puromycin (1 μg/ml) and assayed by Western blot using indicated antibodies to examine the knock-down efficiency at day 6 post infection. Multiple shRNA against TPP1 were used in the assay to eliminate possible off-target effects. **E)** Effects of TPP1 depletion on viral DNA synthesis were assayed by qPCR to measure viral copy number. BJ cells depleted for TPP1 or control were infected with HSV-1 (MOI=0.1). Genomic DNA was isolated from infected cells at time 0 and 24 hrs post-infection, and used as a template for qPCR analysis with primers specific for HSV-1 ICP0 promoter or Actin. The bar graph represents the mean value (± SD) of ICP0 promoter region relative to Actin region and time 0 from three independent experiments by the use of ΔΔCT methods. **F)** BJ cells depleted for TPP1 or control were infected with HSV-1 (MOI=0.1), and culture mediums were collected at time 0 and 24 hrs post-infection and subject to plaque assay. The bar graph shows the fold-increase in viral titer in shTPP1 cells relative to that in shCon cells (designated as 1) at 24 hrs post-infection (Mean ± SD) from triplicate plaque assays.

Figure S6. HSV-1 infection leads to nucleosome eviction at telomeres (related to Figure 5). **A)** MNase pattern assay was performed in BJ cells infected with HSV-1 wt (MOI=0.1), ΔICP0 (MOI=1), or mock 6 hrs post-infection. Nuclei isolated from infected cells were treated with indicated units of MNase, and about 30 μg of purified DNA was fractionated on 1.5% agarose gel. After transfer, the blot was assayed by hybridization with ^{32}P-labeled $(TTAGGG)_4$ probe (middle panel), followed by striping, and re-hybridized with ^{32}P-labeled Alu probe (right panel).

B) Quantification of signal intensity for mono-, di-, tri-nucleosome at telomeres relative to that at Alu repeats in MNase pattern assay, shown in (A). The bar graph shows the mean of relative signals intensity (± SD) from four reactions at various MNase units, including 9, 15, 30, and 60 U. P values were calculated by a paired two-tailed Student's t –Test from the four reactions. **C)** Western blot after ChIP assay was performed to assess that the same amount of protein was pulled down in ChIP samples. ChIP assay was performed in BJ cells infected with HSV-1 wt (MOI=0.1), ΔICP0 (MOI=1), or mock at 6 hrs post-infection with antibodies specific to TRF2, histone H3, or control IgG. ChIP samples were further assayed by western blot with antibodies specific to TRF2, H3, or control β-actin. **D)** Western blot of BJ and U2OS cells infected with lentiviruses expressing shRNA against PML, Daxx, ATRX, or negative control on day 6, using antibodies specific for PML, Daxx, ATRX, or β-actin. **E)** Northern blot analysis of infected BJ and U2OS cells, as shown in (D). EtBr staining of the gel (lower panel) indicates RNA integrity (28S and 18S bands). About 7.5 μg of total RNA isolated from infected BJ or U2OS cells at 6 days post-infection was used for the assay. Numbers at the bottom show the value of TERRA signals relative to 18S RNA signals and shRNA control.

Figure S7. ICP8 foci colocalizes with telomeric DNA or TRF2 foci during HSV-1 infection (related to Figure 6). A) Representative confocal microscopy analysis of U2OS cells infected with wild-type HSV-1 (MOI=1), ΔICP0 (MOI=1) or mock. The infected cells were assayed by immuno-FISH with CCCTAA PNA probe (red) and antibody to ICP8 (green) at 6 hrs post infection. A subset of infected cells shows distinct but small ICP8 foci, indicating these cells were under earlier stage of infection. Telomere DNA foci was enlarged in U2OS cells infected with HSV-1 wild-type. Enlarged images from Dapi (blue) and merge images are shown to the

right. Images were taken with a Leica SP5 confocal microscope using a 63x lens with 4x zoom. Scale bar at left equals to 5 μm, and scale bar in the enlarged image equals to 1 μm. **B)** Quantification of infected U2OS cells with large telomere DNA foci. Infected cells positive for ICP8 staining were used for the score. The bar graph represents the mean (± SD) generated from more than four independent experiments with total cell numbers >100 for each infection. P values were calculated by a two-tailed Student's t –Test for HSV-1 wt or ΔICP0 infected cells. No cells with enlarged telomere foci were observed in mock infected U2OS cells. **C)** Indirect immunofluorescence analysis of infected U2OS cells infected with wild-type HSV-1 (MOI=1) or mock at 6 hrs post infection. The infected cells were stained with mouse monoclonal antibody to ICP8 (green) followed by rabbit polyclonal antibody specific for TRF2 (1:1600 dilution), as shown in red. Dapi (blue) and merge images are shown to the right. Scale bar = 5 μm. **D)** TRF2 foci localizes to telomere DNA foci in U2OS cells infected with HSV-1 in the presence or absence of PAA. IF-FISH was performed by IF with antibody specific for TRF2 (green) followed by FISH with CCCTAA PNA probe (red) at 6 hrs post-infection. Dapi (blue) and merge images are shown to the right. Images were taken with a 100 X lens on a Nikon E600 Upright microscope using ImagePro Plus software for image processing. Scale bar = 5 μm. Note that PAA treatment did not alter the colocalization of TRF2 foci with telomere DNA, and did not prevent the formation of enlarged telomere DNA foci.

Movie S1. Live cell imaging of ICP8 foci (ICP8-GFP, green) colocalization with cellular telomeres (DsRed-TRF2, red) in U2OS cells. (related to Figure 6). U2OS cells expressing DSRed-TRF2 were infected with HSV-1 ICP8-GFP virus (MOI=20), and subject to time-lapse imaging analysis using a Leica SP5 II Confocal microscope stage prewarmed to 37 °C and filled

with CO_2. The fluorescent images were captured at about 1 hr post-infection with 5 min intervals over a period of about 16 hrs.

Movie S2. Live cell imaging of ICP8 foci (ICP8-GFP, green) colocalization with cellular telomeres (Cherry-TRF1, red) in U2OS cells. (related to Figure 6). U2OS cells expressing Cherry-TRF1 were infected with HSV-1 ICP8-GFP virus (MOI=20), and subject to time-lapse imaging analysis using a Leica SP5 II Confocal microscope stage prewarmed to 37 °C and filled with CO_2. The fluorescent images were captured at about 1 hr post-infection with 10 min intervals over a period of about 16 hrs.

Figure S1

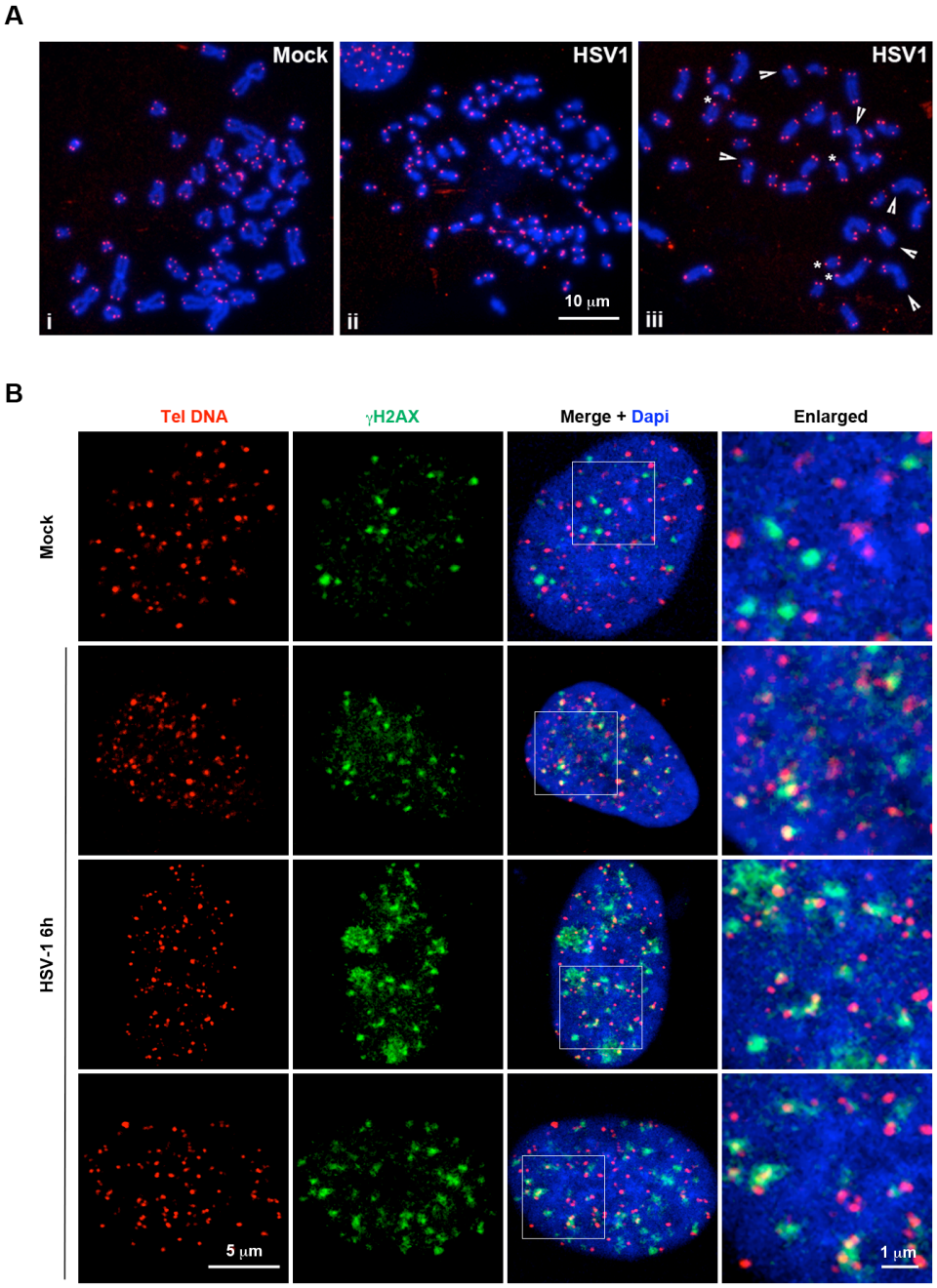

A
Mock
HSV1
HSV1
i
ii
iii
10 μm
B
Tel DNA
γH2AX
Merge + Dapi
Enlarged
Mock
HSV-1 6h
5 μm
1 μm

Figure S2

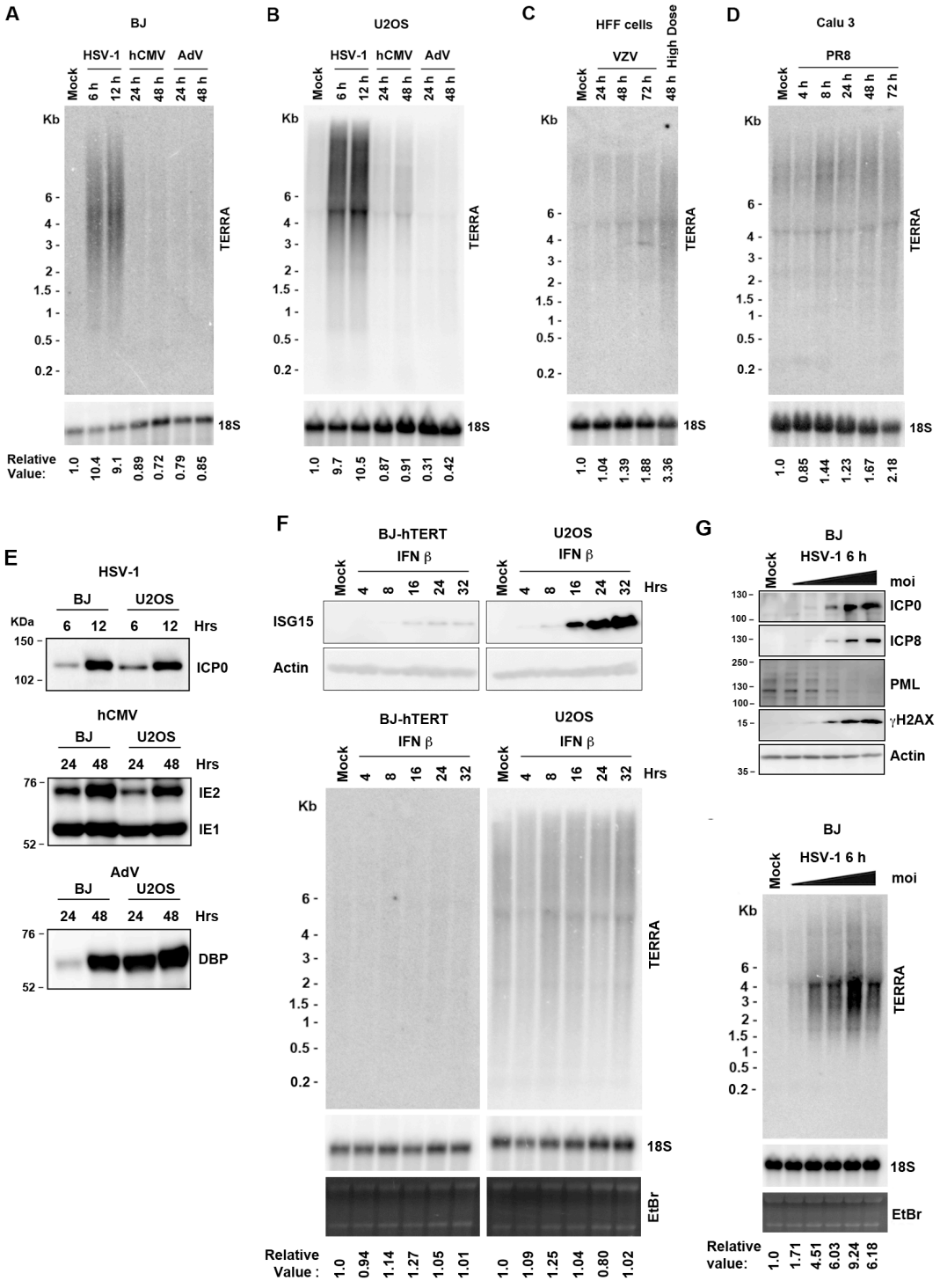

A
BJ
HSV-1 hCMV AdV
Mock 6 h 12 h 24 h 48 h 24 h 48 h
Kb
6 - 4 - 3 - 2 - 1.5 - 1 - 0.5 - 0.2 -
TERRA
18S
Relative Value: 1.0 10.4 9.1 0.89 0.72 0.79 0.85
B
U2OS
HSV-1 hCMV AdV
Mock 6 h 12 h 24 h 48 h 24 h 48 h
Kb
6 - 4 - 3 - 2 - 1.5 - 1 - 0.5 - 0.2 -
TERRA
18S
1.0 9.7 10.5 0.87 0.91 0.31 0.42
C
HFF cells
VZV
Mock 24 h 48 h 72 h 48 h High Dose
Kb
6 - 4 - 3 - 2 - 1.5 - 1 - 0.5 - 0.2 -
TERRA
18S
1.0 1.04 1.39 1.88 3.36
D
Calu 3
PR8
Mock 4 h 8 h 24 h 48 h 72 h
Kb
6 - 4 - 3 - 2 - 1.5 - 1 - 0.5 - 0.2 -
TERRA
18S
1.0 0.85 1.44 1.23 1.67 2.18
E
HSV-1
BJ U2OS
KDa 6 12 6 12 Hrs
150 - 102 - ICP0
hCMV
BJ U2OS
24 48 24 48 Hrs
76 - 52 - IE2 IE1
AdV
BJ U2OS
24 48 24 48 Hrs
76 - 52 - DBP
F
BJ-hTERT IFN β
U2OS IFN β
Mock 4 8 16 24 32 Hrs
ISG15
Actin
Kb
6 - 4 - 3 - 2 - 1.5 - 1 - 0.5 - 0.2 -
TERRA
18S
EtBr
Relative Value : 1.0 0.94 1.14 1.27 1.05 1.01 1.0 1.09 1.25 1.04 0.80 1.02
G
BJ
HSV-1 6 h
Mock moi
130 - ICP0
130 - ICP8
250 - PML
130 - 100 - γH2AX
15 - Actin
35 -
BJ
HSV-1 6 h
Mock moi
Kb
6 - 4 - 3 - 2 - 1.5 - 1 - 0.5 - 0.2 -
TERRA
18S
EtBr
Relative value: 1.0 1.71 4.51 6.03 9.24 6.18

Figure S3

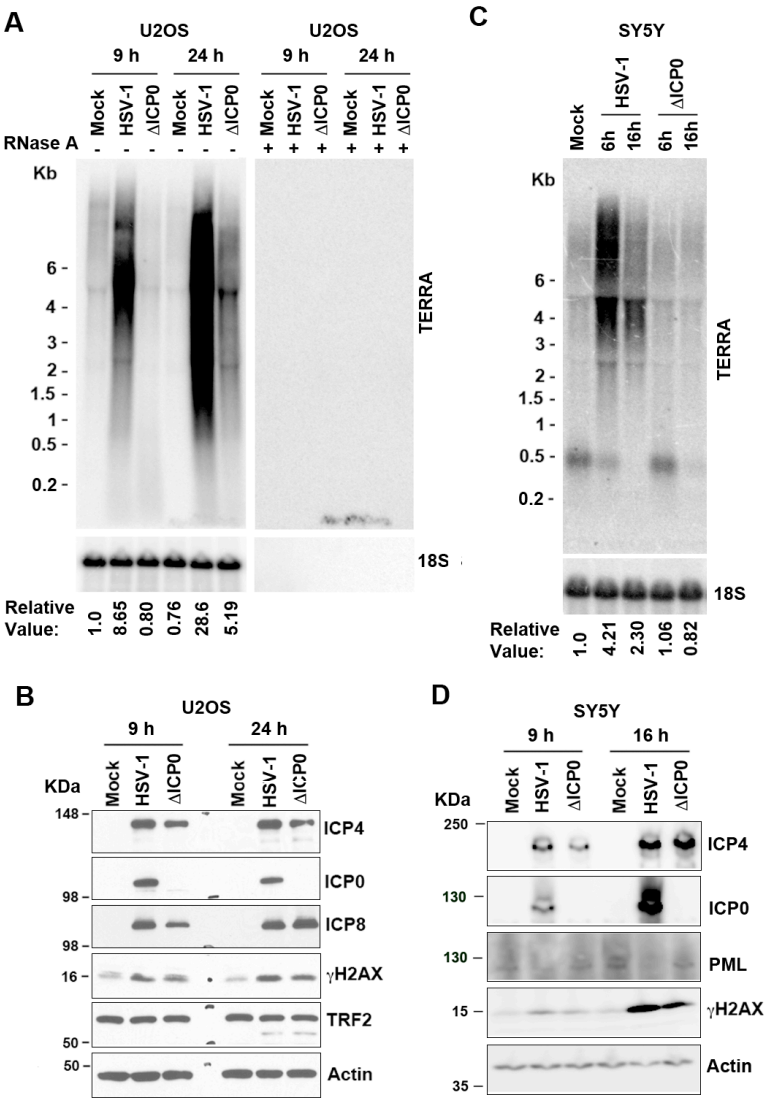

A
U2OS
9 h
24 h
U2OS
9 h
24 h
Mock
HSV-1
ΔICP0
RNase A
Kb
6
4
3
2
1.5
1
0.5
0.2
TERRA
18S
Relative Value:
1.0
8.65
0.80
0.76
28.6
5.19
C
SY5Y
Mock
6h
16h
HSV-1
ΔICP0
Kb
TERRA
18S
Relative Value:
1.0
4.21
2.30
1.06
0.82
B
U2OS
9 h
24 h
KDa
148
ICP4
ICP0
98
ICP8
98
16
γH2AX
TRF2
50
50
Actin
D
SY5Y
9 h
16 h
KDa
250
ICP4
130
ICP0
130
PML
15
γH2AX
35
Actin

Figure S4

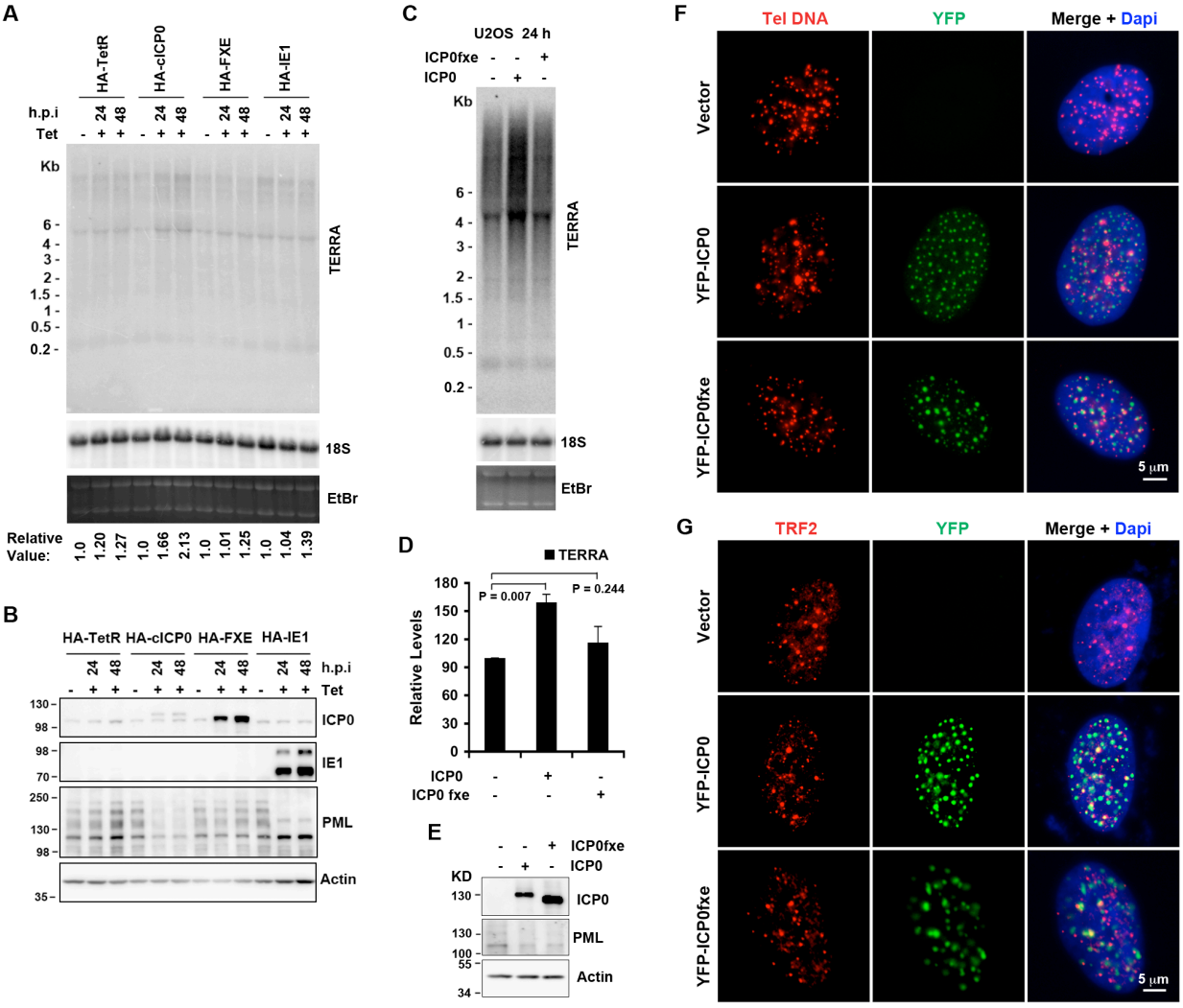

A
HA-TetR
HA-cICP0
HA-FXE
HA-IE1
h.p.i
Tet
Kb
TERRA
18S
EtBr
Relative Value:
1.0 1.20 1.27 1.0 1.66 2.13 1.0 1.01 1.25 1.0 1.04 1.39
B
HA-TetR HA-cICP0 HA-FXE HA-IE1
h.p.i
Tet
ICP0
IE1
PML
Actin
C
U2OS 24 h
ICP0fxe
ICP0
Kb
TERRA
18S
EtBr
D
TERRA
Relative Levels
P = 0.007
P = 0.244
ICP0
ICP0 fxe
E
ICP0fxe
ICP0
KD
ICP0
PML
Actin
F
Tel DNA
YFP
Merge + Dapi
Vector
YFP-ICP0
YFP-ICP0fxe
5 μm
G
TRF2
YFP
Merge + Dapi
Vector
YFP-ICP0
YFP-ICP0fxe
5 μm

Figure S5

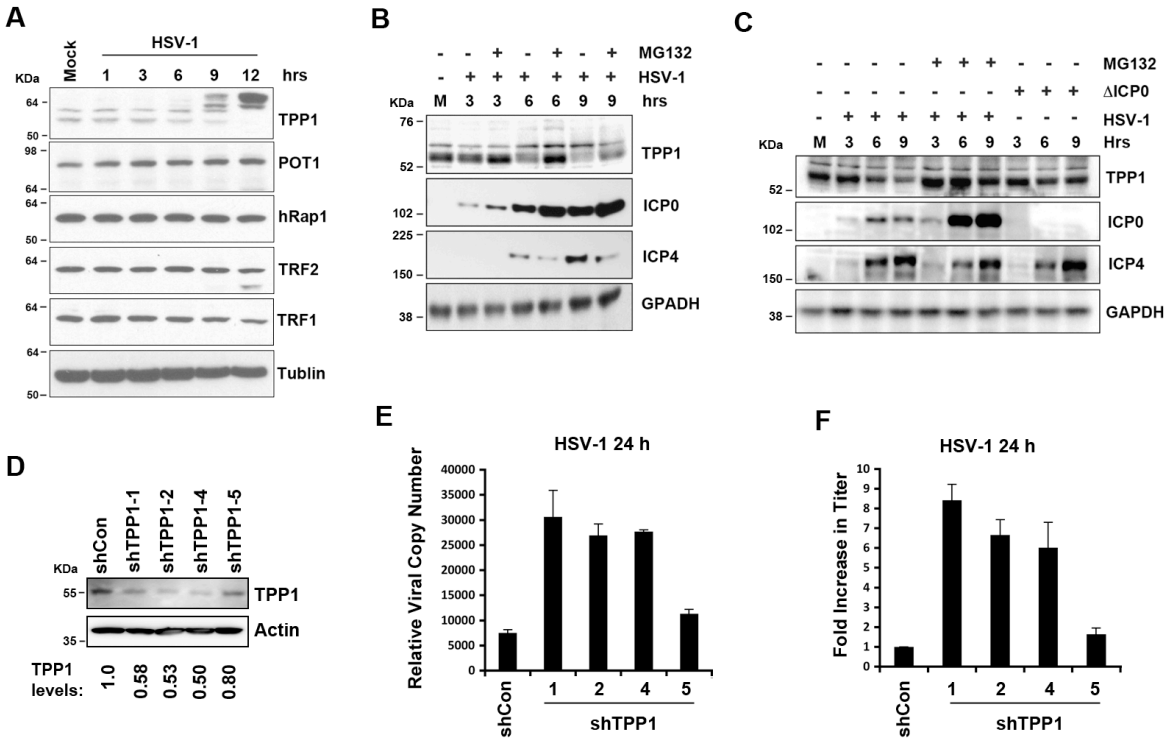

A
Mock
HSV-1
KDa
1 3 6 9 12 hrs
TPP1
POT1
hRap1
TRF2
TRF1
Tublin
B
MG132
HSV-1
KDa M 3 3 6 6 9 9 hrs
TPP1
ICP0
ICP4
GPADH
C
MG132
ΔICP0
HSV-1
KDa M 3 6 9 3 6 9 3 6 9 Hrs
TPP1
ICP0
ICP4
GAPDH
D
shCon
shTPP1-1
shTPP1-2
shTPP1-4
shTPP1-5
KDa
TPP1
Actin
TPP1 levels: 1.0 0.58 0.53 0.50 0.80
E
HSV-1 24 h
Relative Viral Copy Number
shCon
shTPP1
F
HSV-1 24 h
Fold Increase in Titer
shCon
shTPP1

Figure S6

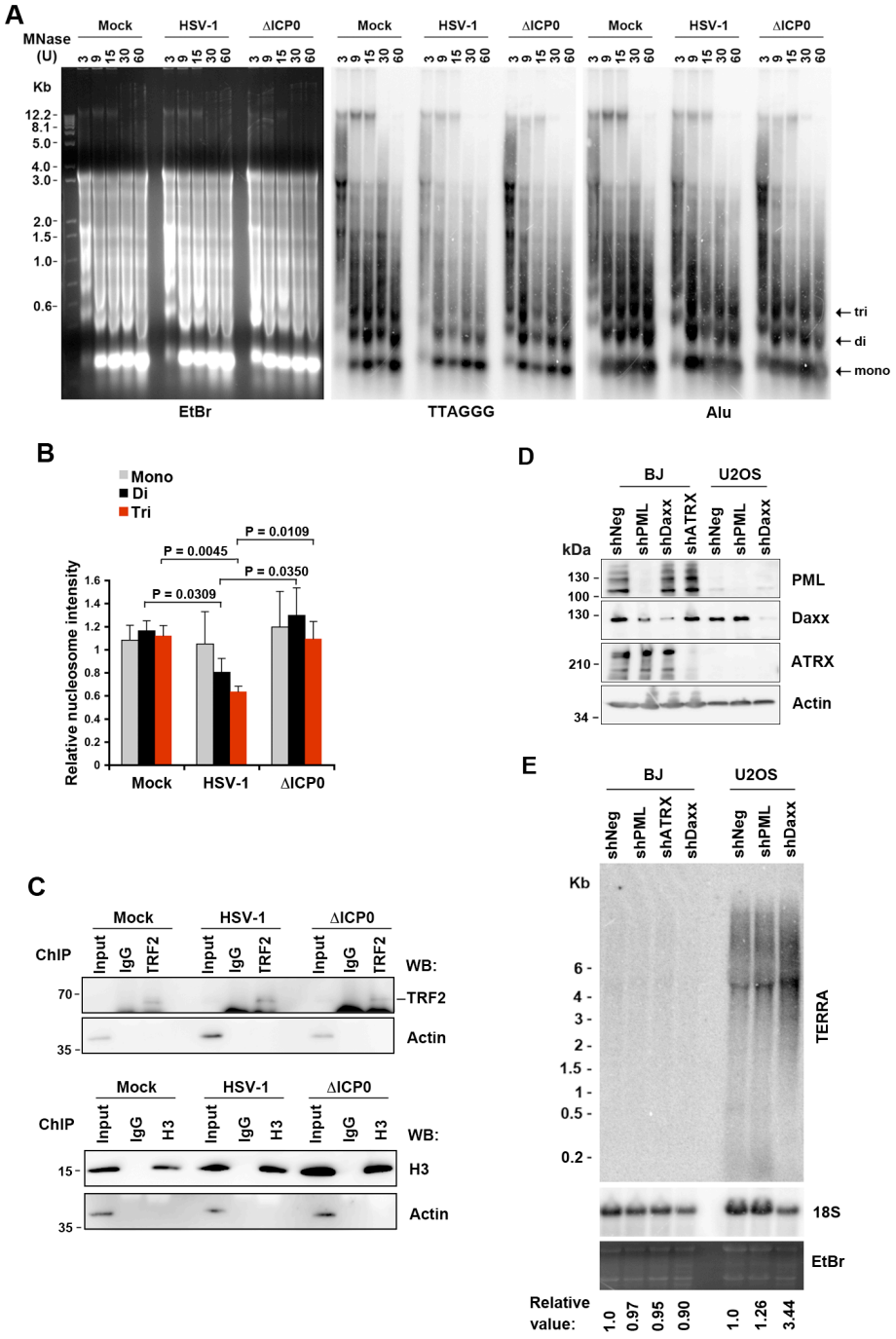
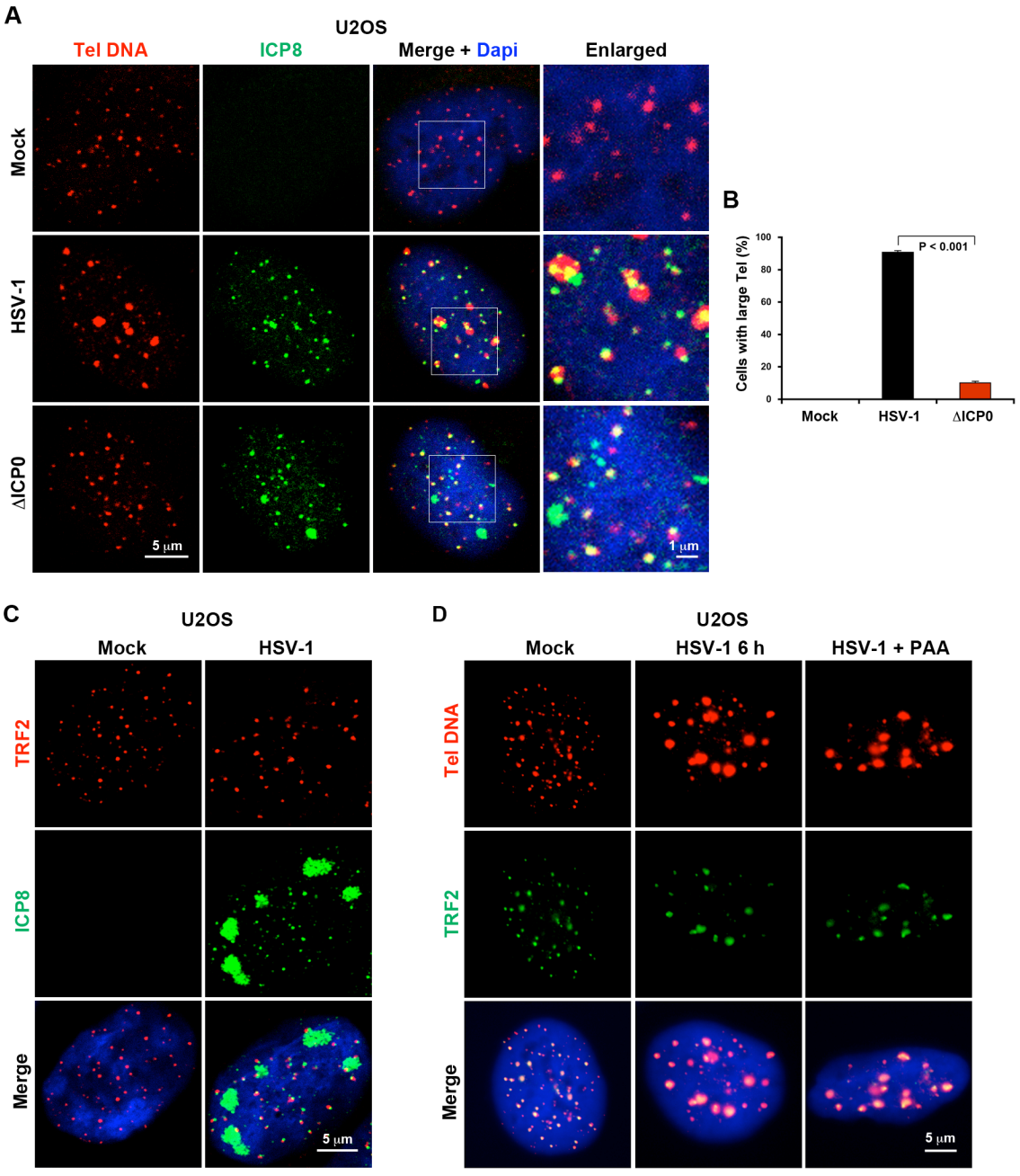

A
Mock
HSV-1
ΔICP0
MNase (U)
3 9 15 30 60
Kb
12.2
8.1
5.0
4.0
3.0
2.0
1.5
1.0
0.6
tri
di
mono
EtBr
TTAGGG
Alu
B
Mono
Di
Tri
P = 0.0045
P = 0.0109
P = 0.0350
P = 0.0309
Relative nucleosome intensity
Mock
HSV-1
ΔICP0
C
ChIP
Input
IgG
TRF2
H3
WB:
70
35
15
TRF2
Actin
H3
D
BJ
U2OS
shNeg
shPML
shDaxx
shATRX
kDa
130
100
210
34
PML
Daxx
ATRX
Actin
E
Kb
6
4
3
2
1.5
1
0.5
0.2
TERRA
18S
EtBr
Relative value:
1.0
0.97
0.95
0.90
1.0
1.26
3.44

Figure S7


A
U2OS
Tel DNA
ICP8
Merge + Dapi
Enlarged
Mock
HSV-1
ΔICP0
5 μm
1 μm
B
Cells with large Tel (%)
P < 0.001
Mock
HSV-1
ΔICP0
C
U2OS
Mock
HSV-1
TRF2
ICP8
Merge
5 μm
D
U2OS
Mock
HSV-1 6 h
HSV-1 + PAA
Tel DNA
TRF2
Merge
5 μm