SUPPLEMENTAL MATERIALS

Table S1

Percent Cells with Large Replication Compartments

	Serum-Starved MRC-5	Dividing MRC-5	Dividing HFF
WT	91	100	98
Δ97	38	72	86
Δ97-E7	91	100	94

Infected cells, analyzed as in Fig. 3, were counted and the percentages of cells containing large replication compartments (filling the nucleus) were calculated.

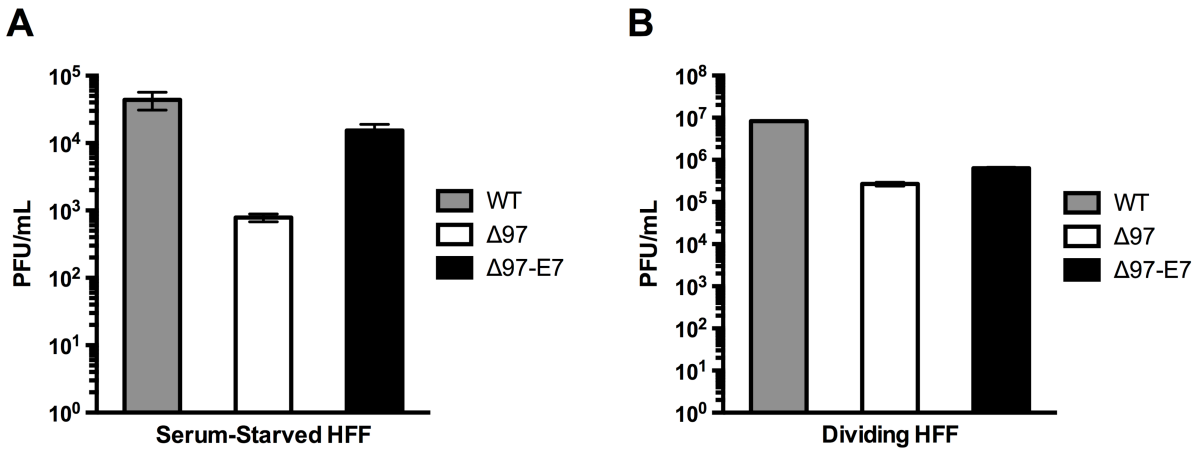

FIGURE S1. Replication of HCMV WT, Δ97, and Δ97-E7 viruses in HFF cells under different growth conditions. Serum-starved (maintained in medium containing 0.1% FBS 72 h prior to infection) or dividing (maintained in medium containing 10% FBS) HFF cells were infected with wild type HCMV (WT), *UL97*-null HCMV (Δ97), or *UL97*-null HCMV expressing HPV 16 E7 (Δ97-E7) at an MOI of 1. Titers (PFU/mL) were determined in triplicate at 120 h post-infection. Error bars indicate standard deviations.

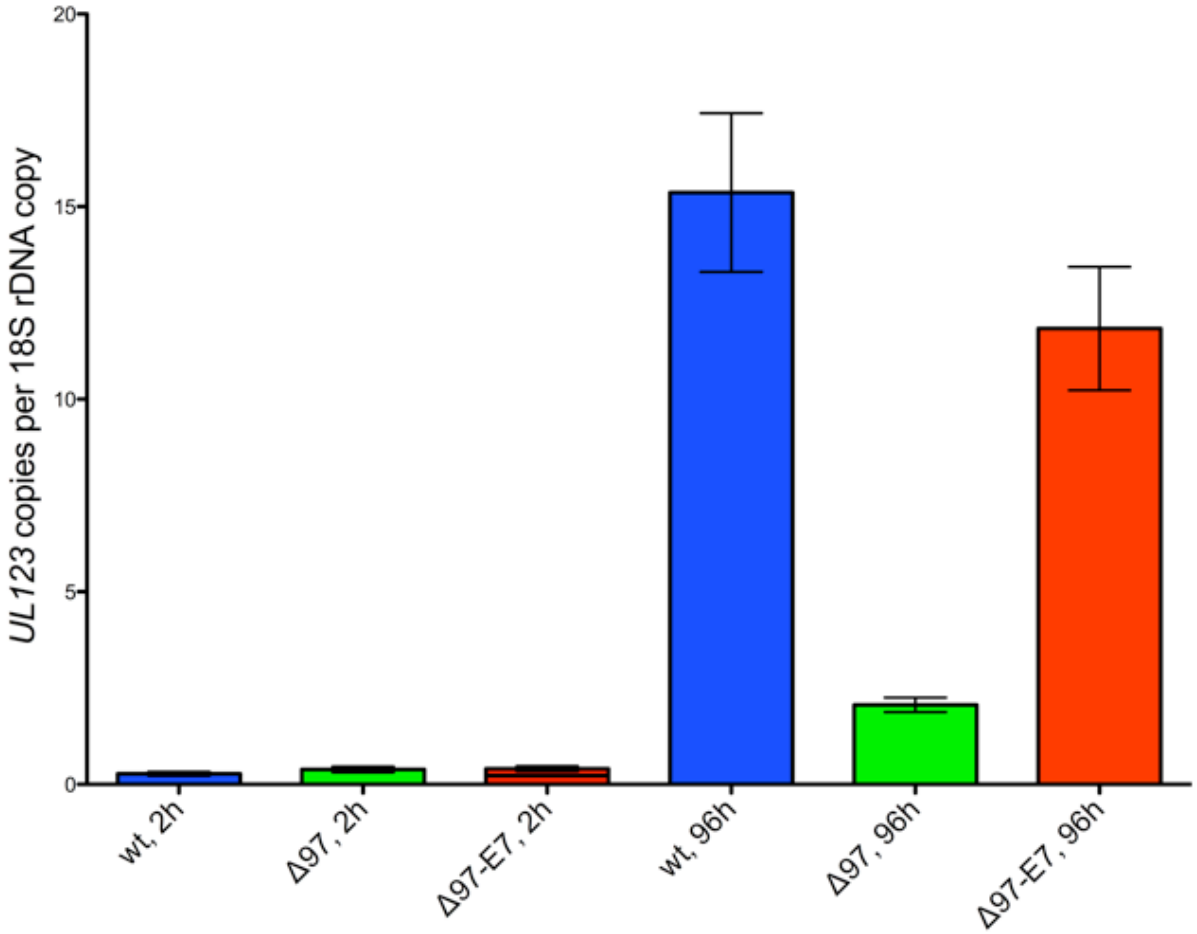

Figure S2. Viral DNA synthesis. Serum-starved MRC-5 fibroblasts were infected at MOI 1 with the indicated viruses, and were harvested for DNA isolation at 2 h post infection (2 hpi), and 96 h post infection (96 hpi). DNA samples were measured by real-time qPCR to determine absolute number of copies of the viral *UL123* gene and of the cellular *18S rDNA* gene (a multicopy allele) in triplicate 0.1 ng samples for each condition. The levels of viral DNA, indicated by *UL123* copies normalized to copies of the *18S rDNA* gene, are shown for each condition. Measurements of viral DNA present at 2 hpi were included to confirm that delivery of viral genomes during infection were similar for all three viruses. For *UL123*, real-time qPCR efficiency was 92%, and the R^2 value for the standard curve was 0.997. For *18S rDNA*, the corresponding values were 101% and 0.991, respectively.

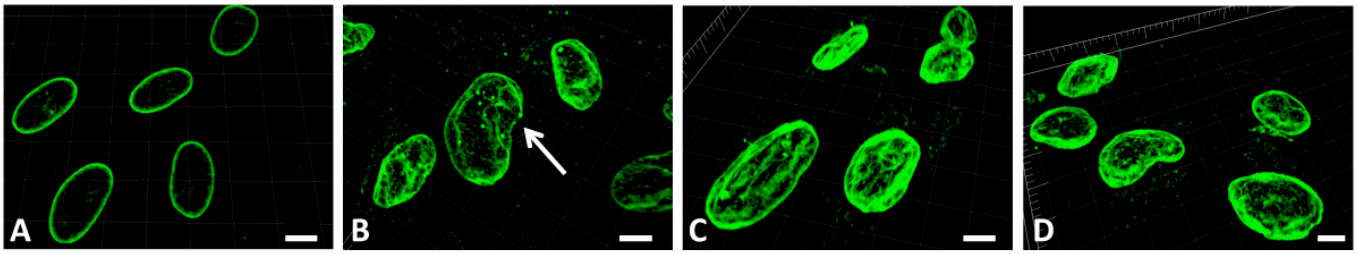

Figure S3. Gaps in nuclear lamina in HFF cells. Dividing HFF cells were mock-infected (panel A) or infected with WT HCMV (B), mutant Δ97 (C) or mutant Δ97-E7 (D) at an MOI of 3 under dividing conditions. At 96 hpi, cells were stained for lamin A/C (green). Serial optical sections were acquired by confocal microscopy. 3-D reconstruction images are shown. A gap in lamin A/C staining in WT-infected cells in panel B is shown by the white arrow.

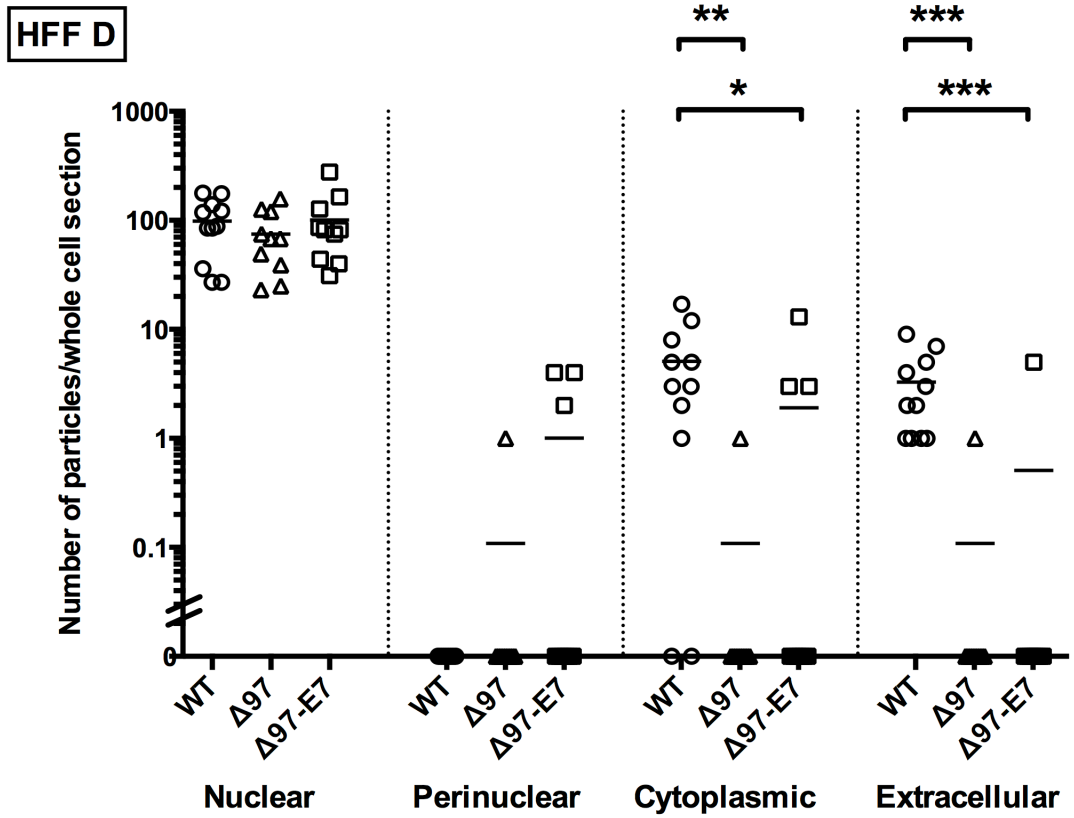

Figure S4. Viral particle distributions in HFF cells. Ten or eleven thin whole cell sections from dividing HFF infected with the indicated viruses, which were used for the experiment show in Fig. 5, were analyzed for the number of viral particles that were nuclear, perinuclear, cytoplasmic, and extracellular. The mean number of particles in each condition is indicated with a horizontal line. The data were analyzed by a Kruskal-Wallis test followed by Dunn's tests to compare each mutant to WT virus while correcting for multiple comparisons. Brackets indicate significant differences: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

SUPPLEMENTAL MATERIALS AND METHODS:

Quantitative PCR. Real-time quantitative PCR (qPCR) to determine levels of viral DNA, expressed as *IE1-72* (*UL123*) copies normalized to cellular *18S* rDNA copies, was performed as previously described (1), with the following modifications. Total DNA from 0.5 x 10^6 infected cells/sample was isolated at 96 hpi using a DNeasy Blood and Tissue mini kit (Qiagen). Absolute quantification on three replicates of 0.1 ng total DNA per sample was performed by running qPCR reactions alongside duplicate standard curves of serial ten-fold dilutions of the TB40/E BAC (39) (a generous gift of Christian Sinzger, Institute for Virology, Ulm), from 10^7 to 10^2 copies/well, for determination of viral copy number, and of pJK-*18S*, a plasmid containing a fragment of the human 18S rDNA gene, from 10^7 to 10^2 copies per well, for determination of cellular copy number. pJK-*18S* was constructed by performing 25 cycles of PCR using KOD hot start DNA polymerase (EMD Millipore) and the following DNA oligonucleotide primers, at 3 μM each, *18S* Fw: 5'-CTT TCG AGG CCC TGT AAT TG-3' and *18S* Rv 5'-ACC TCC GAC TTT CGT TCT TG-3.' 1 μg of genomic DNA isolated from human fibroblast cells was used as template, and the 507 bp PCR product was ligated into the pJET1.2 vector using the CloneJet PCR cloning kit (Fermentas), as per the manufacturer's instructions. The identity and fidelity of the cloned 18S rDNA sequence was confirmed by DNA sequencing (Genewiz).

REFERENCES:

1. Nogalski, M. T., G. Chan, E. V. Stevenson, S. Gray, and A. D. Yurochko. 2011. Human cytomegalovirus-regulated paxillin in monocytes links cellular pathogenic motility to the process of viral entry. J. Virol. **85:**1360-1369.