Supporting Information

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SI Materials and Methods

Construction of Recombinant Viruses. Wild-type and Δ*DYLC* HPV16 *E7* coding sequences (CDS) were PCR amplified from plasmids pOZ-C E7 and pOZ E7 DLYC (1), respectively, using primers 97subE7 Fw and 97subE7 Rv (Table S1), which added terminal *Bgl*II and *Bam*HI sites. PCR products were double-digested with *Bgl*II and *Bam*HI and inserted into the cloning plasmid pSP72 (Promega), yielding pE7 and pDLYC. The *I-SceI-AphAI* cassette from pEP-KanaS (2) was then PCR adapted with primers *Ban*II E7 *Aph*AI Fw and *Ban*II E7 *Aph*AI Rv, and digested with *Ban*II, which is predicted to cut at both ends but also once within the *AphAI* marker sequence. The resulting fragments of 784 and 248 bp were added to ligation reactions with pE7 and pDLYC, which had been opened with *Ban*II, yielding plasmids pE7-TSR and pDLYC-TSR. Plasmids were verified to be free of spurious mutations by DNA sequencing. All DNA oligonucleotides for construction of the two-step Red shuttle vector were custom synthesized and PAGE purified by the manufacturer (Integrated DNA Technologies). *E7-I-Sce AphAI* and *E7* Δ*DLYC–ISce-AphAI* cassettes were excised from pE7-TSR and pDLYC-TSR after *Bgl*II-*Bam*HI double digestion and electroporated into GS1783 *E. coli* (a generous gift of Greg Smith, Northwestern University) harboring AD169rv (3), a BAC clone of HCMV strain AD169. Kanamycin-resistant integrate colonies were obtained and resolved using two-step Red mutagenesis (2) as described previously (4), except *E. coli* strain GS1783, which expresses the I-*Sce*I homing endonuclease under arabinose control, was used instead of DY380.

For construction of viruses expressing wild-type or ΔDLYC mutant HPV16 E7 proteins from the intergenic region between *US9* and *US10*, primers Us10_UL97 promoter Fw and Us9_SV40polyA_E7 Rv (Table S1) were used to PCR amplify sequences spanning from 503 bp upstream of the *UL97* start codon to the *E7* stop codon from I-*Sce*I-*Aph*AI integrate BACs that were generated as intermediates during construction of Δ97-E7 and Δ97-DLYC. PCR products were electroporated into GS1783 cells harboring pp28-LUC (a generous gift of Gloria Komazin-Meredith, Harvard Medical School, Boston, MA), an AD169 BAC that encodes in the *US9-US10* intergenic region a copy of the pp28 (*UL99*) promoter driving expression of a firefly luciferase cassette bearing an SV40 polyadenylation signal from pGL4.13 (Promega, Inc.). pp28-LUC was derived from pBAC/AD169, a close predecessor of pAD/Cre (5) that was generously provided by Dong Yu and Thomas Shenk (Princeton University, Princeton, NJ). Kanamycin-resistant integrate colonies were resolved to yield WT-E7 and WT-DLYC BACs, in which a cassette comprised of a copy of approximately 0.5 kb of sequence immediately upstream of the native *UL97* ORF, a wild-type or a ΔDLYC mutant HPV16 *E7* CDS, respectively, had replaced the pp28 promoter and luciferase sequences between *US9* and *US10* in pp28-LUC, while leaving the SV40 polyadenylation signal in place (Fig. S4). These BACs were verified by DNA sequencing and agarose gel electrophoresis to be free of unexpected mutations or rearrangements, and were electroporated into HFF to allow for recovery of infectious virus.

Southern Blotting. Virus infected cells were collected at the indicated time points and DNA was extracted by standard procedures. One microgram of each DNA sample was digested with *Bam*HI, resolved on a 0.8% agarose gel and transferred to a nylon membrane. Hybridization with a digoxigenin-labeled probe directed against the UL83 gene and chemiluminescent detection were carried out using the DIG High Prime labeling and detection kit (Roche), as per the manufacturer's instructions.

RNA Quantification. To quantify mRNA levels of *TS*, *UMPS*, and *TBP*, RT-qPCR was performed. RNA was extracted from cells using the RNeasy Kit (Qiagen) according to the manufacturer's recommendations and 3 μg total RNA was treated with RQ1 RNase-free DNase (Promega) to remove contaminating DNA. cDNA was made using the SuperScript III First-Strand Kit (Invitrogen). qPCR was performed on an ABI 7900HT qPCR machine (Applied Biosystems) using TaqMan Universal PCR Master Mix and TaqMan Gene Expression Assay (Applied Biosystems) primer and probe sets for TS (Hs00426591_m1), UMPS (Hs00923717_m1), and TBP (Hs00427620_m1), as per the manufacturer's recommendations.

1. Huh KW, et al. (2005) Association of the human papillomavirus type 16 E7 oncoprotein with the 600-kDa retinoblastoma protein-associated factor, p600. *Proc Natl Acad Sci USA* 102:11492–11497.
2. Tischer BK, von Einem J, Kaufer B, Osterrieder N (2006) Two-step red-mediated recombination for versatile high-efficiency markerless DNA manipulation in *Escherichia coli*. *Biotechniques* 40:191–197.
3. Hobom U, Brune W, Messerle M, Hahn G, Koszinowski UH (2000) Fast screening procedures for random transposon libraries of cloned herpesvirus genomes: Mutational analysis of human cytomegalovirus envelope glycoprotein genes. *J Virol* 74:7720–7729.
4. Kamil JP, Coen DM (2007) Human cytomegalovirus protein kinase UL97 forms a complex with the tegument phosphoprotein pp65. *J Virol* 81:10659–10668.
5. Yu D, Smith GA, Enquist LW, Shenk T (2002) Construction of a self-excisable bacterial artificial chromosome containing the human cytomegalovirus genome and mutagenesis of the diploid TRL/IRL13 gene. *J Virol* 76:2316–2328.

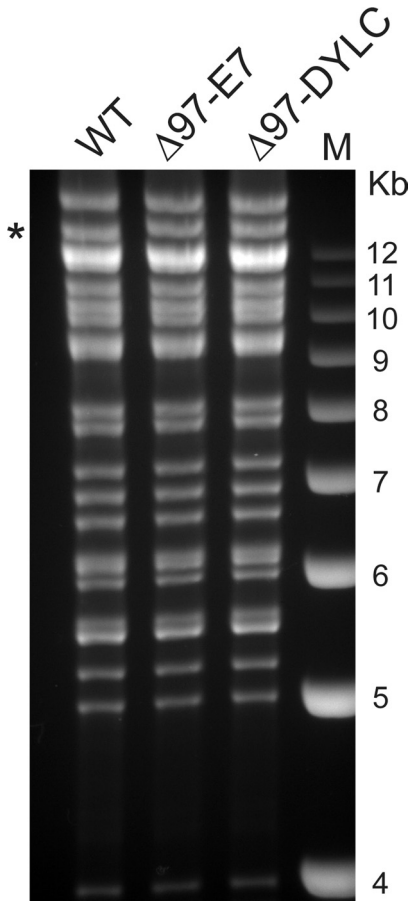

Fig. S1. Comparison of recombinant HCMV BAC DNA by agarose gel electrophoresis. BAC DNA (1.5 μg) from WT (AD169rv), Δ97-E7 and Δ97-DLYC were each digested with *Bam*HI and loaded onto a 0.7% agarose-0.5× Tris Borate EDTA (TBE) gel and electrophoresed overnight at 60 V. The gel was stained with 0.5× TBE containing 1 μg/mL ethidium bromide and documented using UV illumination using a CCD camera (Bio-Rad). An asterisk marks a restriction fragment of approximately 13.5 kb in the WT lane that is predicted to be approximately 250 bp larger in Δ97-E7 and Δ97-DLYC digests, owing to the loss of a *Bam*HI site in *UL97*. M: Invitrogen 1 kb DNA ladder.

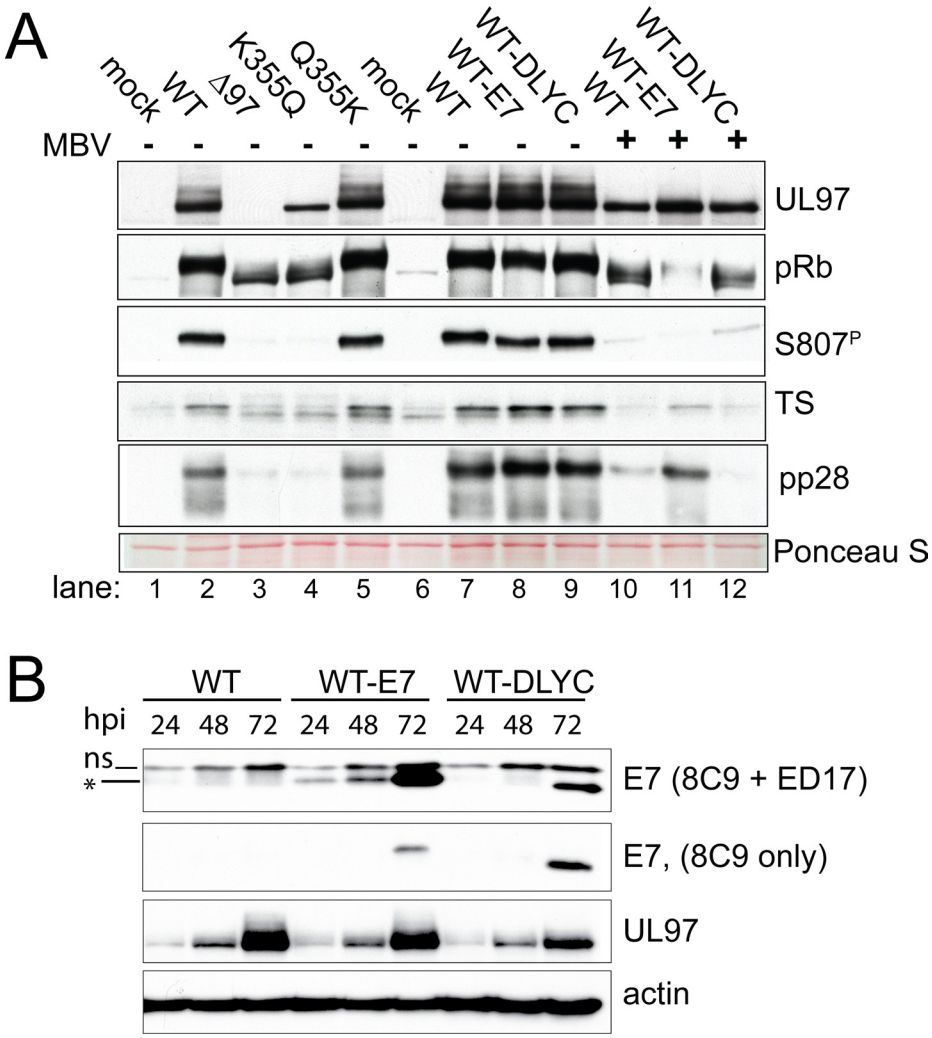

Fig. S2. Analysis of protein expression in cells infected with additional mutant viruses, and in the absence or presence of the UL97 inhibitor maribavir. (*A*) Quiescent, serum-deprived human fibroblasts were infected at an MOI of 1.0 PFU/cell with wild-type HCMV (WT), *UL97*-null HCMV (Δ97), an HCMV expressing a catalytically deficient point mutant of UL97 (K355Q), wild-type rescuant derived from K355Q (Q355K), WT expressing either wild-type (WT-E7) or ΔDLYC mutant (WT-DLYC) forms of HPV16 E7, or mock-infected (''mock''). Where indicated (+), 1 μM maribavir (MBV) was present during infection. Lysates collected at 72 hpi were compared for the expression of the following gene products: UL97, pRb, TS, and pp28. Phosphorylation of pRb was monitored using an antibody (S807^{P}) that detects pRb only when dually phosphorylated at Ser-807 and Ser-811. Ponceau S stain detection of a prominent band matching the expected relative mobility of beta-actin is shown as a loading control. Note: WT in lanes 7 and 10 is parental WT of WT-E7 and WT-DLYC, while WT in the lane 2 is parental WT of Δ97, K355Q, and Q355K. For infections analyzed in lanes 6–9, DMSO carrier (0.1% vol/vol final) was present instead of MBV. (*B*) After infection of serum-fed, subconfluent cells at an MOI of 1 PFU/cell, HPV16 E7 expression from WT-E7 and WT-DLYC was monitored using monoclonal antibody (mAb) 8C9, which detects both wild-type and ΔDLYC forms of HPV16, alone or in combination with mAb ED17, which detects only wild-type HPV16 E7 and cannot detect ΔDLYC mutant forms. UL97 and beta-actin levels were also monitored. Because wild-type HPV16 E7 polypeptides can bind both 8C9 and ED17 mAbs (presumably, at one distinct epitope per mAb), and ΔDLYC E7 is only detected by the 8C9 mAb, detection of HPV16 E7 from WT-E7 is likely more sensitive than that from WT-DLYC in the result shown in the top panel. In the top panel, a nonspecific band (indicated as ''ns'') was detected when 8C9 and ED17 were used in combination to detect E7; the species interpreted as E7 is indicated with an asterisk (*).

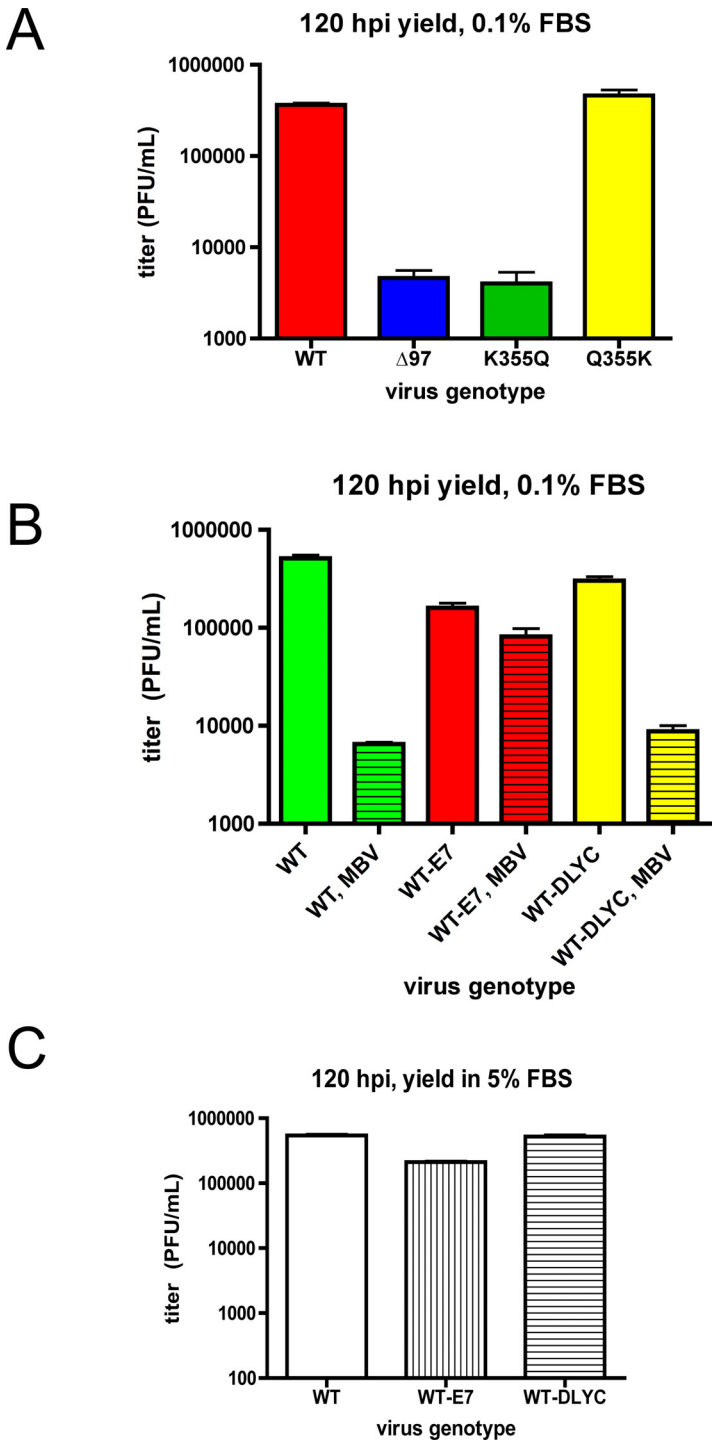

Fig. S3. Analysis of viral replication. (*A*) Quiescent, serum-deprived human fibroblasts were infected at an MOI of 1 PFU/cell with wild-type HCMV (WT), *UL97*-null HCMV (Δ97), an HCMV expressing a catalytically deficient point mutant of UL97 (K355Q), or a rescued virus derived from K355Q (Q355K), in which a wild-type *UL97* allele was restored, and compared for yield of infectious virus at 120 hpi. (*B*) Recombinant HCMVs expressing either wild-type (WT-E7) or ΔDLYC mutant (WT-DLYC) forms of HPV16 E7 were compared to parental WT virus (WT) from which they were derived, as above, except in the presence or absence or presence (MBV) of 1 μM maribavir. (*C*) Serum-fed, subconfluent fibroblasts were infected at an MOI of 1 PFU/cell with the same three viruses compared in panel *B*, above, and monitored for production of infectious virus at 120 hpi. For all three panels, the data represent the average measurement from three replicates per condition, with error bars representing standard deviations.

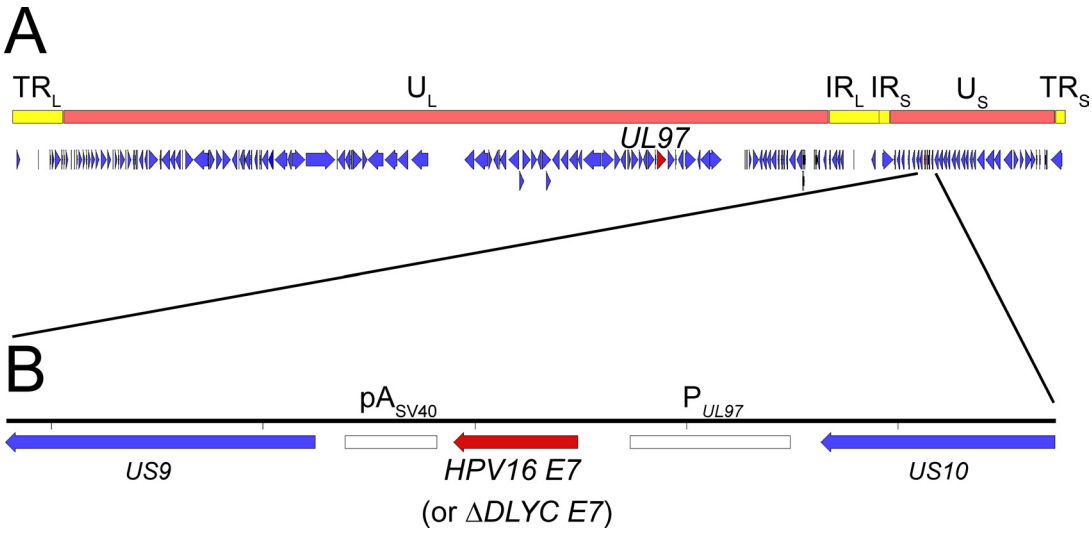

Fig. S4. Genome organization of WT-E7 and WT-DLYC viruses that express wild-type or ΔDLYC mutant HPV16 E7 in addition to UL97. (*A*) A schematic of the HCMV strain AD169 genome depicting genome segments (TR_L, U_L, IR_L, IR_S, U_S, and TR_S) and, below, viral genes (arrows), as designated by GenBank sequence file NC 001347. Repeat regions are colored in yellow, unique regions in light red, *UL97* in dark red. The intergenic region between *US9* and *US10* where an HPV16 E7 expression cassette was inserted is indicated by lines that transition to the bottom panel. (*B*) The *US9-US10* region is shown in greater detail, *US9* and *US10* are depicted as blue arrows, and a duplicated sequence identical to a 0.5 kb region immediately upstream of *UL97* (P_{UL97}) is shown as a white box, as is an SV40 polyA signal (pA_{SV40}). Sequences encoding HPV16 E7 are depicted as a red arrow.

Table S1. Oligonucleotides used to construct recombinant viruses

Primer name	Sequence (5′-to-3′)
BanII E7 AphAI Fw	TTTATTTTTAGAGCCCATTACAATATTGTAACCTTTAGGGATAACAGGGTAATCGATTT
BanII E7 AphAI Rv	CCTCCTTCTGGGCTCTGTCCGGTTCTGCTTGTCCGCCAGTGTTACAACCAATTAACC
97 subE7 Fw	AAAAAAAGATCTGCTCTTCGTGGTAGCTAGTGCAGCCTTAGGAACAGGGAAGACTGTCGCCACTATGCATGGAGATACACCTACA
97 subE7 Rv	TCCTCGGATCCGCTCTTCTGTTGCCTTTCCCCTCAGCAACCGTCACGTTCCGCGTCCCGGTTATGGTTTCTGAGAACAGATG
Us10_UL97 promoter Fw	CATTGTTGTTTACTGAAAAGGAATGTGCTTTCCCGGCATGGGCCCGATTCTGACGTCGGTCAACAAACAG
Us9_SV40polyA_E7 Rv	TGTCTGCTCGAAGCGGCCGGCCGCCCCGACTCTAGAATTATTATGGTTTCTGAGAACAGATGG