Supplementary Materials

Highlighting of a LAGLIDADG and a zing finger motifs located in the pUL56 sequence crucial for HCMV replication

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a

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AD169     CDRHHTVELQFTDNVRFSVSLATLNDIERFLCKMNYVYGILAPE
TB40/E    CDRHHTVELQFTDNVRFSVSLATLNDIERFLCKMNYVYGILAPE
Toledo    CDRHHTVELQFTDNVRFSVSLATLNDIERFLCKMNYVYGILAPE
Towne     CDRHHTVELQFTDNVRFSVSLATLNDIERFLCKMNYVYGILAPE
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b

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AD169     ARQEVYVEGTTCAQCYEELTIIPNQGRSLNKRLQGLLCNHIAVHRPSSQSDV
TB40/E    ARQEVYVEGTTCAQCYEELTIIPNQGRSLNKRLQGLLCNHIAVHRPSSQSDV
Toledo    ARQEVYVEGTTCAQCYEELTIIPNQGRSLNKRLQGLLCNHIAVHRPSSQSDV
Towne     ARQEVYVEGTTCAQCYEELTIIPNQGRSLNKRLQGLLCNHIAVHRPSSQSDV
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Figure S1: Identification of patterns in N-terminal part of pUL56 from 4 reference strains (AD169, TB40/E, Toledo, Towne). a. Sequences alignment of the LATLNDIERFL pattern. b. Sequences alignment of conserved regions IV and residues involved in metal-binding site. Key residues involved in the both patterns are shown as white letters on a black background.

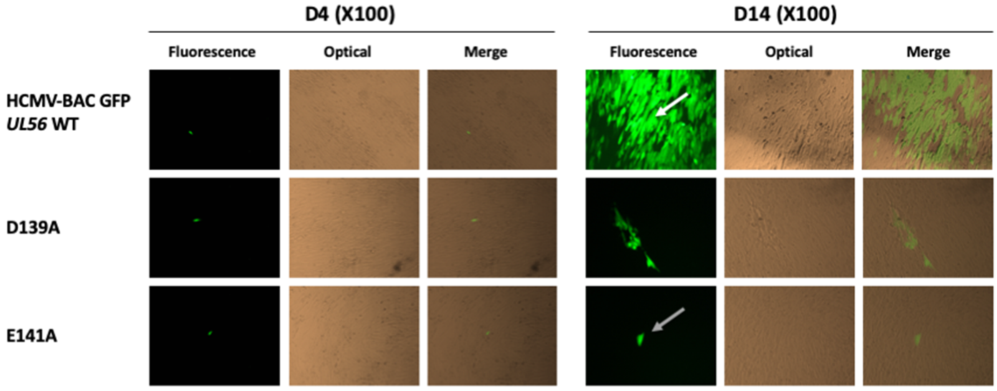

Figure S2: Effect of the terminase pUL56 D139A and E141A mutations on viral growth. A. Plaque formation assay using tranfection of HCMV-BAC AD169 and recombinant virus strains (amino acid probably involved in nuclease activity) in MRC-5 cells at day 4 and day 14. Green fluorescent foci (white arrow) were observed with the wild-type HCMV-BAC GFP and the D139A mutation. Single infected cells (grey arrow) were observed with the E141A mutation.

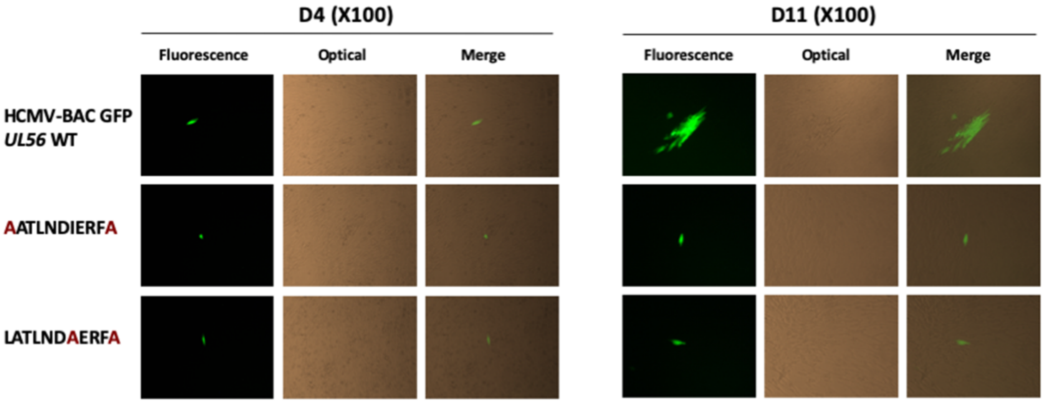

Figure S3: Effect of the terminase pUL56 mutations whithin the putative leucine-zipper on viral growth. Plaque formation assay using tranfection of HCMV-BAC AD169 and recombinants virus strain (amino acid probably involved in leucine zipper pattern) in MRC-5 cells.

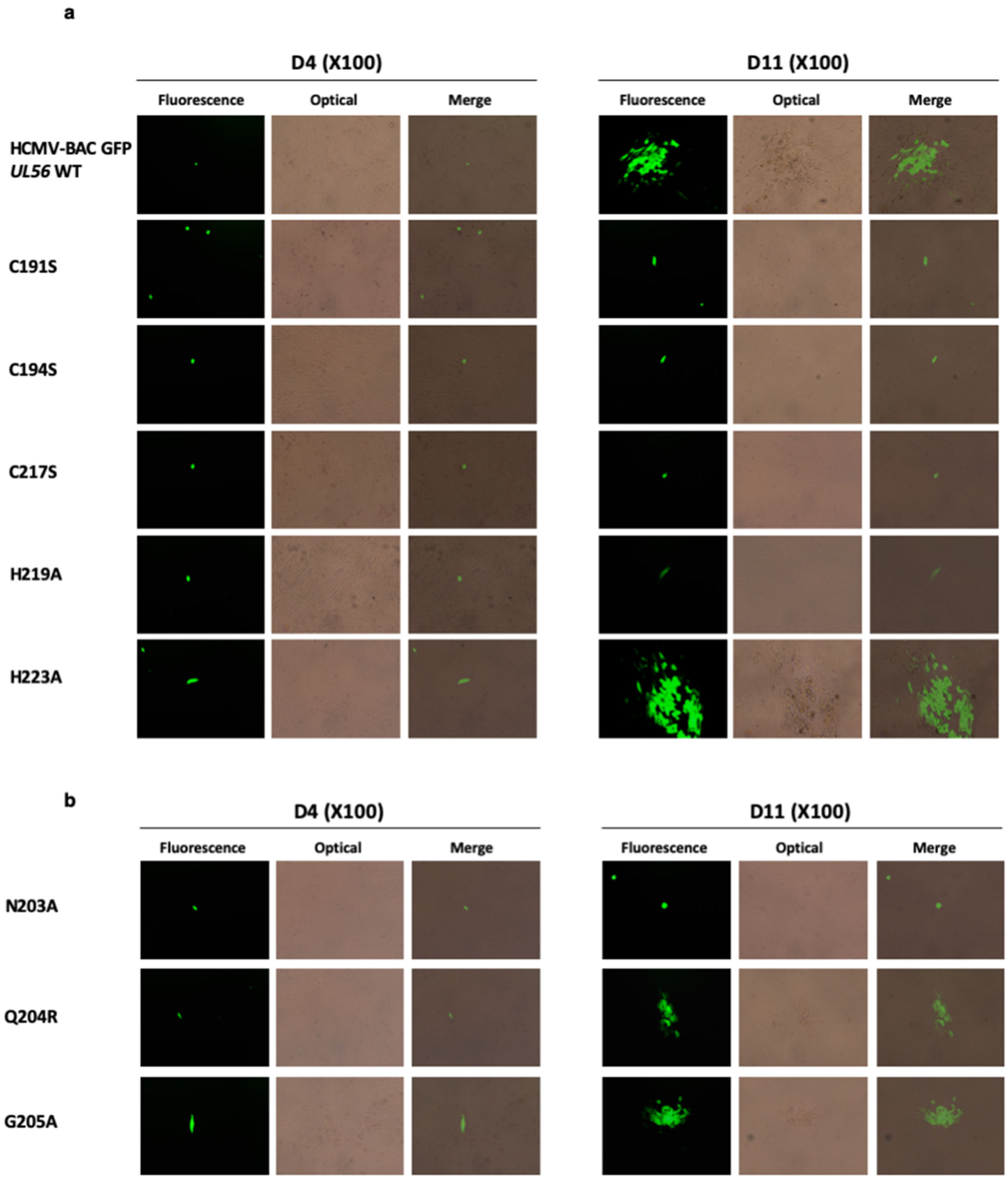

Figure S4: Effect of the terminase pUL56 mutations within the putative zinc finger on viral growth. A. Plaque formation assay using transfection of HCMV-BAC AD169 and recombinants virus strain (amino acid probably involved in zinc chelation of putative zinc finger pattern) in MRC-5 cells. B. Plaque formation assay using transfection of HCMV BAC AD169 and recombinant virus strains (amino acids in the loop of putative zinc finger pattern) in MRC-5 cells.

Table S1: Sequences used for alignment of pUL56 and homologues.

Identification	Sequence identification	Accession number	Reference
HSV_1	Human herpesvirus type 1 pUL28 packaging protein	CAA32321	Dolan et al., 1992
HSV_2	Human herpesvirus type 2 pUL28 packaging protein	CAB0675	Barnett et al., 1992
CeHV_1	Cercopithecine herpesvirus 1 pUL28	BAC58068	Pelyrigina et al., 2003
CeHV_2	Cercopithecine herpesvirus 2 pUL28	AAU84532	Tyler, Peters, and Severini, 2005
EHV_1	Equine herpesvirus 1 ORF32	YP_053077	Telford et al., 1992
GaHV_3	Gallid herpesvirus 3 pUL28	BAB16538	Izumiya et al., 2001
MeHV_1	Meleagrid herpesvirus 1 pUL28 processing and transport protein	AAG30068	Kingham et al., 2001
HHV_3	Human herpesvirus 3 (Varicella-Zooster virus)	CAA27913	Davison and Scott, 1986
SUID	Suid herpesvirus 1 (Pseudorabies virus) UL28	YP_068331	Klupp et al., 2004
GaHV_1	Gallid herpesvirus 1 UL28-like protein	YP_182357	Thureen et al., 2006
GaHV_2	Gallid herpesvirus 2 Homologue of HSV 1 ICP 18.5 packaging protein	AAF66763	Kato et al., 1999
AD169	Human cytomegalovirus AD169 strain pUL56	X17403	Chee et al., 1990
HHV_6_B	Human herpesvirus 6 type B transport protein	Q9WT24	Isegawa et al., 1999
HHV_6_A_GS	Human herpesvirus 3 type A transport protein	AHK06969	None
CCMV	Chimpanzee herpesvirus pUL56	AAM00906	Davison et al., 2003
RhCMV	Cercopithecine herpesvirus 8 (Rhesus cytomegalovirus) putative pUL56 DNA packaging protein	AAZ80592	None
MCMV	Murid herpesvirus 1 (Murine cytomegalovirus) major DNA-binding protein	CAA47415	Messerle et al., 1992
RCMV	Murid herpesvirus 2 (Rat cytomegalovirus Maastricht) pR56 protein	AAC56431	Beuken et al., 1996
HHV_8	Human herpesvirus 8 BALF3 EBV homolog, transport protein homologue	AAC57084	Moore et al., 1996
HHV_4_1	Human herpesvirus 4 type 1 BALF3	YP_401715	de Jesus et al., 2003
HHV_4_2	Human herpesvirus 4 type 2 BALF3	YP001129509	Dolan A et al., 2006

Table S2: Sequences of *UL56*-primers used for mutagenesis-PCR into putative LATLNDIERFL pattern.

Mutant BAC	Primer direction	Primer sequence
UL56 D139A	Forward	GGACAACGTCCGTTTCAGCGTCAGTCTGGCCACACTCAACGCCATCGAGCGCTTCCTGTGCAATAGGGATAACAGGGTAATCGATTT
	Reverse	TCCCGTACACGTAGTTCATTTTGCACAGGAAGCGCTCGATGGCGTTGAGTGTGGCCAGACTGAGCCAGTGTTACAACCAATTAACC
UL56 E141A	Forward	CGTCCGTTTCAGCGTCAGTCTGGCCACACTCAACGACATCGCCCGCTTCCTGTGCAAAATGAATAGGGATAACAGGGTAATCGATTT
	Reverse	CCAGGATCCCGTACACGTAGTTCATTTTGCACAGGAAGCGGGCGATGTCGTTGAGTGTGGCCAGCCAGTGTTACAACCAATTAACC
UL56 L134A	Forward	GGAGCTCCAGTTTACGGACAACGTCCGTTTCAGCGTCAGTGCGGCCACACTCAACGACATCGATAGGGATAACAGGGTAATCGATTT
	Reverse	TCATTTTGCACAGGAAGCGCTCGATGTCGTTGAGTGTGGCCGCACTGACGCTGAAACGGACGTGCCAGTGTTACAACCAATTAACC
UL56 L137A	Forward	GTTTACGGACAACGTCCGTTTCAGCGTCAGTCTGGCCACAGCCAACGACATCGAGCGCTTCCTTAGGGATAACAGGGTAATCGATTT
	Reverse	ACACGTAGTTCATTTTGCACAGGAAGCGCTCGATGTCGTTGGCTGTGGCCAGACTGACGCTGAGCCAGTGTTACAACCAATTAACC
UL56 I140A	Forward	CAACGTCCGTTTCAGCGTCAGTCTGGCCACACTCAACGACGCCGAGCGCTTCCTGTGCAAAATTAGGGATAACAGGGTAATCGATTT
	Reverse	GGATCCCGTACACGTAGTTCATTTTGCACAGGAAGCGCTCGGCGTCGTTGAGTGTGGCCAGACGCCAGTGTTACAACCAATTAACC
UL56 L144A	Forward	CAGCGTCAGTCTGGCCACACTCAACGACATCGAGCGCTTCGCGTGCAAAATGAACTACGTGTATAGGGATAACAGGGTAATCGATTT
	Reverse	CCTCCGGCGCCAGGATCCCGTACACGTAGTTCATTTTGCACGCGAAGCGCTCGATGTCGTTGAGCCAGTGTTACAACCAATTAACC
UL56 L134A L137A I140A L144A	Forward	GGAGCTCCAGTTTACGGACAACGTCCGTTTCAGCGTCAGTGCGGCCACAGCCAACGACGCCGAGCGCTTCGCGTGCAAAATGAACTACGTGTATAGGGATAACAGGGTAATCGATTT
	Reverse	CCTCCGGCGCCAGGATCCCGTACACGTAGTTCATTTTGCACGCGAAGCGCTCGGCGTCGTTGGCTGTGGCCGCACTGACGCTGAAACGGACGTGCCAGTGTTACAACCAATTAACC
UL56 I140A L144A	Forward	CAACGTCCGTTTCAGCGTCAGTCTGGCCACACTCAACGACGCCGAGCGCTTCGCGTGCAAAATGAACTACGTGTATAGGGATAACAGGGTAATCGATTT
	Reverse	CCTCCGGCGCCAGGATCCCGTACACGTAGTTCATTTTGCACGCGAAGCGCTCGGCGTCGTTGAGTGTGGCCAGACGCCAGTGTTACAACCAATTAACC
UL56 L134A L144A	Forward	GGAGCTCCAGTTTACGGACAACGTCCGTTTCAGCGTCAGTGCGGCCACACTCAACGACATCGAGCGCTTCGCGTGCAAAATGAACTACGTGTATAGGGATAACAGGGTAATCGATTT
	Reverse	CCTCCGGCGCCAGGATCCCGTACACGTAGTTCATTTTGCACGCGAAGCGCTCGATGTCGTTGAGTGTGGCCGCACTGACGCTGAAACGGACGTGCCAGTGTTACAACCAATTAACC

Table S3: Sequences of *UL56*-primers used for mutagenesis-PCR into putative zinc-finger pattern.

Mutant BAC	Primer direction	Primer sequence
UL56 C191S	Forward	GCCGGTGGCGCGTCAGGAAGTGTACGTCGAAGGGACGACATCTGCCCAATGCTACGAGGAGCTTAGGGATAACAGGGTAATCGATTT
	Reverse	CCTGATTCGGGATGATGGTCAGCTCCTCGTAGCATTGGGCAGATGTCGTCCCTTCGACGTACAGCCAGTGTTACAACCAATTAACC
UL56 C194S	Forward	GCGTCAGGAAGTGTACGTCGAAGGGACGACATGCGCCCAATCTTACGAGGAGCTGACCATCATTAGGGATAACAGGGTAATCGATTT
	Reverse	GCGAGCGGCCCTGATTCGGGATGATGGTCAGCTCCTCGTAAGATTGGGCGCATGTCGTCCCTTGCCAGTGTTACAACCAATTAACC
UL56 C217S	Forward	TCAGGGCCGCTCGCTGAACAAGCGGCTGCAGGGCTTGCTGTCTAACCATATAGCGGTCCACCGTAGGGATAACAGGGTAATCGATTT
	Reverse	CATCGGACTGGCTTGACGGACGGTGGACCGCTATATGGTTAGACAGCAAGCCCTGCAGCCGCTGCCAGTGTTACAACCAATTAACC
UL56 N203A	Forward	GACATGCGCCCAATGCTACGAGGAGCTGACCATCATCCCGGCTCAGGGCCGCTCGCTGAACAATAGGGATAACAGGGTAATCGATTT
	Reverse	ACAGCAAGCCCTGCAGCCGCTTGTTCAGCGAGCGGCCCTGAGCCGGGATGATGGTCAGCTCCTGCCAGTGTTACAACCAATTAACC
UL56 Q204R	Forward	ATGCGCCCAATGCTACGAGGAGCTGACCATCATCCCGAATAGAGGCCGCTCGCTGAACAAGCGTAGGGATAACAGGGTAATCGATTT
	Reverse	TGCACAGCAAGCCCTGCAGCCGCTTGTTCAGCGAGCGGCCTCTATTCGGGATGATGGTCAGCTGCCAGTGTTACAACCAATTAACC
UL56 G205A	Forward	CGCCCAATGCTACGAGGAGCTGACCATCATCCCGAATCAGGCCCGCTCGCTGAACAAGCGGCTTAGGGATAACAGGGTAATCGATTT
	Reverse	GGTTGCACAGCAAGCCCTGCAGCCGCTTGTTCAGCGAGCGGGCCTGATTCGGGATGATGGTCAGCCAGTGTTACAACCAATTAACC
UL56 H219A	Forward	CCGCTCGCTGAACAAGCGGCTGCAGGGCTTGCTGTGCAACGCTATAGCGGTCCACCGTCCGTCTAGGGATAACAGGGTAATCGATTT
	Reverse	TATTCACATCGGACTGGCTTGACGGACGGTGGACCGCTATAGCGTTGCACAGCAAGCCCTGCAGCCAGTGTTACAACCAATTAACC
UL56 H223A	Forward	CAAGCGGCTGCAGGGCTTGCTGTGCAACCATATAGCGGTCGCCCGTCCGTCAAGCCAGTCCGATAGGGATAACAGGGTAATCGATTT
	Reverse	CCACCGTCTGGATATTCACATCGGACTGGCTTGACGGACGGGCGACCGCTATATGGTTGCACAGCCAGTGTTACAACCAATTAACC