SUPPLEMENTAL TABLES

TABLE SI

Oligonucleotide primers used for generation of PCR products for BACmid recombination and siRNA sequences used for knockdown of pUL50 and pUL97 expression

[a]Nucleotide specification: additional bases (lower case letters), CODING SEQUENCE (capital letters), **RESTRICTION SITE** (capital letters, bold), ***NUCLEOTIDES DIFFERING FROM WILD-TYPE SEQUENCE*** (capital letters, bold, italic), I-*Sce*I site (white)

Designation	Size [bp]	Sequence
Primers[a]		
UL50L11Afor	86	tac**GGATCC**ACGCGCAGCTCGCTGGGACCCAACTTGAGGATACGCCGC GTGGCCTGCAC***CGC***TAGGGATAACAGGGTAATCGATTT
UL50L11Arev	135	tac**GAATTC**TCGGCGGTGGCGTCGGTGCGATGGAGATGAACAAGGTTC TCCATCAGGAT***GCG***GTGCAGGCCACGCGGCGTATCCTCAAGTTGGGT CCCAGCGAGCTGCGCGTGCCAGTGTTACAACCAATTAACC
UL50Q13Afor	86	tac**GGATCC**TCGGTGACGCGCAGCTCGCTGGGACCCAACTTGAGGATA CGCCGCGTGGC***CGC***TAGGGATAACAGGGTAATCGATTT
UL50Q13Arev	135	tac**GAATTC**GTGGCGTCGGTGCGATGGAGATGAACAAGGTTCTCCATC AGGATCTGGTG***GCG***GCCACGCGGCGTATCCTCAAGTTGGGTCCCAGC GAGCTGCGCGTCACCGAGCCAGTGTTACAACCAATTAACC
UL50E56Afor	86	tac**GGATCC**ATAAAACAAGGCACGTGGTCTGTGCGGCTCTCCCAGTAG CTGAGTAGATA***CGC***TAGGGATAACAGGGTAATCGATTT
UL50E56Arev	135	tac**GAATTC**ATTACTCGGTGTGCGACGCCATGCTCAAGACAGACACGGT CTATTGTGTC***GCG***TATCTACTCAGCTACTGGGAGAGCCGCACAGACCA CGTGCCTTGTTTTATGCCAGTGTTACAACCAATTAACC
E56AY57Afor UL50	89	tac**GGATCC**AAGATAAAACAAGGCACGTGGTCTGTGCGGCTCTCCCAG TAGCTGAGTAG***CGCCGC***TAGGGATAACAGGGTAATCGATTT
E56AY57Arev UL50	138	tac**GAATTC**ATTACTCGGTGTGCGACGCCATGCTCAAGACAGACACGGT CTATTGTGTC***GCGGCG***CTACTCAGCTACTGGGAGAGCCGCACAGACC ACGTGCCTTGTTTTATCTTGCCAGTGTTACAACCAATTAACC
UL50L116Afor	86	tac**GGATCC**CACTTCGTCAGCACTCCGTAGGCCGAGGGCTTGATCTCC TCGATGTCCTT***CGC***TAGGGATAACAGGGTAATCGATTT
UL50L116Arev	135	tac**GAATTC**ACGTAGGTGAGTTCAATGTGCTTAAGGTGAACGAGTCGCT CATCGTCACG***GCG***AAGGACATCGAGGAGATCAAGCCCTCGGCCTACG GAGTGCTGACGAAGTGGCCAGTGTTACAACCAATTAACC
siRNAs		
UL50a	21	GACAGACACGGUCUAUUGUUU (sense sequence) ACAAUAGACCGUGUCUGUCUU (antisense sequence)
UL50b	21	UUCGGCGUCGGUGUUCAACUU (sense sequence) GUUGAACACCGACGCCGAAUU (antisense sequence)
UL50A	21	GACAGACACGGUCUAUUGUUU (sense sequence) ACAAUAGACCGUGUCUGUCUU (antisense sequence)
UL50B	21	UUCGGCGUCGGUGUUCAACUU (sense sequence) GUUGAACACCGACGCCGAAUU (antisense sequence)
UL50C	21	UAUCUGCUCAGCUACUGGGUU (sense sequence) CCCAGUAGCUGAGCAGAUAUU (antisense sequence)
UL50D	21	UGUGCUUAAGGUGAACGAGUU (sense sequence) CUCGUUCACCUUAAGCACAUU (antisense sequence)
UL97a	21	UUUCUCAAUCACCAGUGUCUU (sense sequence) GACACUGGUGAUUGAGAAAUU (antisense sequence)
UL97b	21	GAUCUGUUAUGCCGUGGACUU (sense sequence) GUCCACGGCAUAACAGAUCUU (antisense sequence)

Table SII

List of NEC samples and corresponding negative control samples analyzed by MS/MS

No. of Exp.	Sample	Virus	IP antibody	dpi	Specificity/ Negative Control
I. MS/MS	NEC 1	UL53-FLAG	mAb-FLAG	7	pUL53
	NEC 2	UL53-FLAG + UL50-HA	mAb-FLAG + mAb-HA	7	pUL53 + pUL50
	Ctrl 1	UL53-FLAG	mAb-HA	7	Control for NEC 1
	Ctrl 2	UL53-FLAG + UL50-HA	mAb-GFP	7	Control for NEC 2
II. MS/MS	NEC 3	UL53-FLAG	mAb-FLAG	>7	pUL53
	NEC 4	UL50-HA	mAb-FLAG + mAb-HA	>7	pUL50
	Ctrl 3a	HCMV-GFP	mAb-FLAG	>7	Control for NEC 3
	Ctrl 3b	UL53-FLAG	mAb-HA	>7	Control for NEC 3
	Ctrl 4	UL50-HA	mAb-GFP	>7	Control for NEC 4
III. MS/MS	NEC 5	UL53-FLAG	mAb-FLAG	1	pUL53
	NEC 6	UL53-FLAG	mAb-FLAG	2	pUL53
	NEC 7	UL53-FLAG	mAb-FLAG	3	pUL53
	NEC 8	UL53-FLAG	mAb-FLAG	4	pUL53
	Ctrl 5	Mock	mAb-FLAG	1	Control for NEC 5
	Ctrl 6	Mock	mAb-FLAG	2	Control for NEC 6
	Ctrl 7	Mock	mAb-FLAG	3	Control for NEC 7
	Ctrl 8	Mock	mAb-FLAG	4	Control for NEC 8
IV. MS/MS	NEC 9	UL53-FLAG	mAb-FLAG	2	pUL53
	NEC 10	UL53-FLAG	mAb-FLAG	3	pUL53
	NEC 11	UL53-FLAG	mAb-FLAG	4	pUL53
	Ctrl 9a	Mock	mAb-FLAG	2	Control for NEC 9
	Ctrl 9b	UL53-FLAG	Fab fragment	2	Control for NEC 9
	Ctrl 10	UL53-FLAG	Fab fragment	3	Control for NEC 10
	Ctrl 11	UL53-FLAG	Fab fragment	4	Control for NEC 11

SUPPLEMENTAL FIGURES

Figure SI

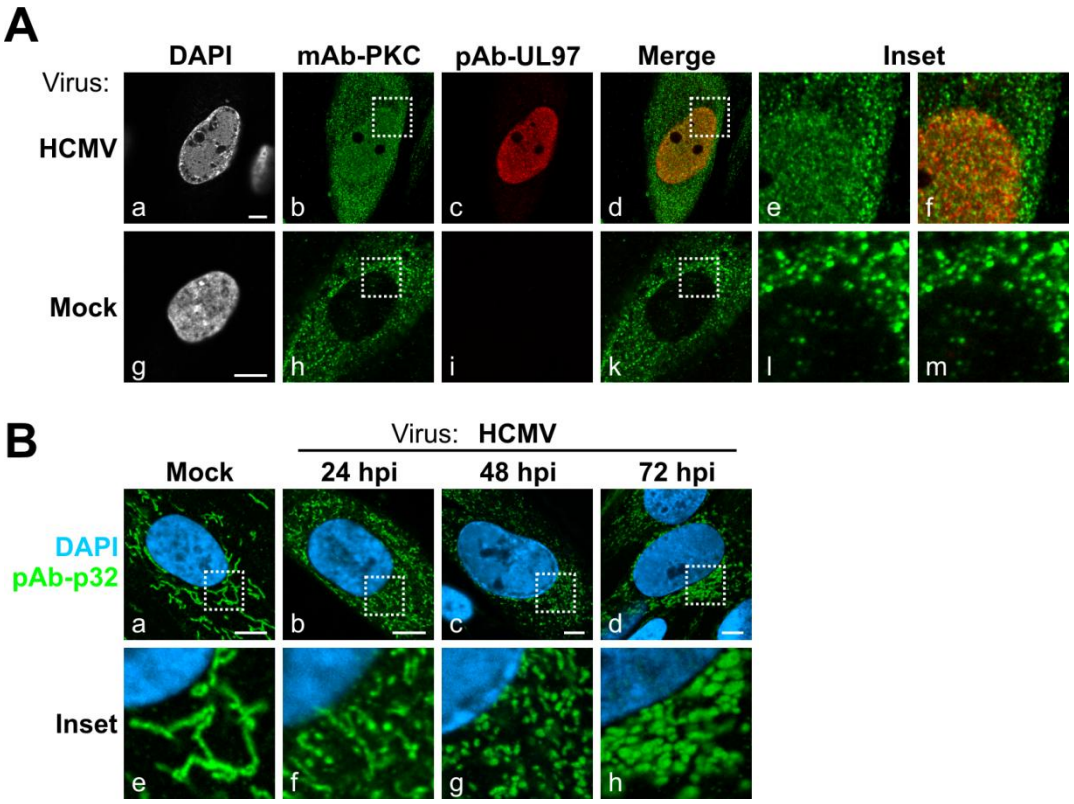

FIG. S1. **Intracellular localization of NEC-associated viral protein kinase pUL97, cellular PKC, and cellular protein p32/gC1qR in HCMV-infected primary fibroblasts.** HFFs were infected with HCMV strain AD169 (*A*, panels a-f and *B*) or remained uninfected (mock) (*A*, panels g-m). At 3 dpi (*A*) or at consecutive time-points post-infection (B), cells were fixed and coimmunostained with the indicated antibodies. *B, Dashed boxes* (panels a-d) are depicted in the insets (panels e-h). *DAPI,* 4',6-diamidino-2-phenylindole; s*cale bars*, 7.5 µm.

Figure SII

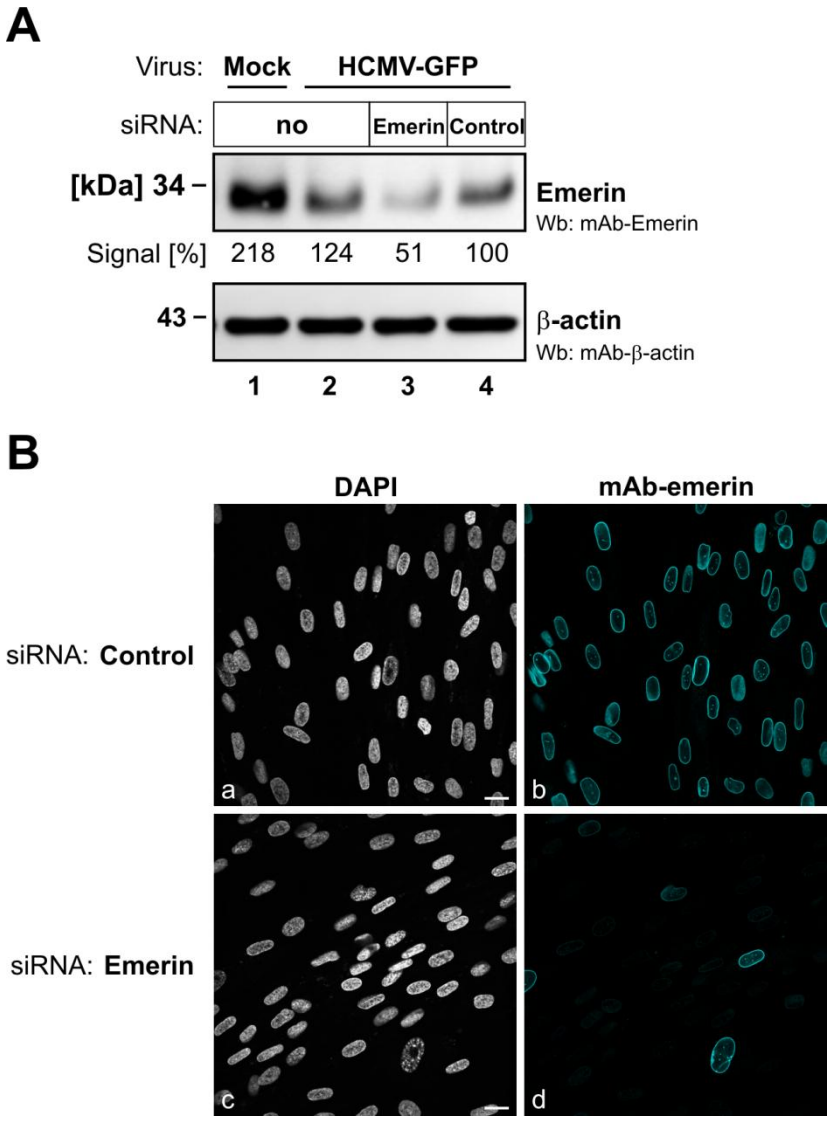

FIG. S2. **Knock-down efficiency of emerin-specific siRNAs in primary fibroblasts.** HFFs were transiently transfected with emerin-specific siRNAs or with a scrambled siRNA as a control. In addition, transfected cells were infected with the reporter virus HCMV-GFP (*A,* lanes 2-3) or remained uninfected (mock) (*A,* lane 1 and *B*). *A,* At 7 dpi, cells were harvested and HCMV replication efficiency was determined from total lysates by automated GFP fluorometry (see Fig. 6A). Thereafter, these samples were further subjected to Wb analysis to monitor the knock-down of emerin. Signal intensities of emerin expression were evaluated by densitometry and compared to the corresponding staining of the loading control β-actin. *B,* At 6 d post-transfection of siRNAs, cells were fixed and immunostained with mAb-emerin. Samples were subsequently analyzed by confocal laser scanning microscopy. Knock-down efficiency of emerin-specific siRNAs was determined by scoring six microscopic fields (415 cells). In ~80% of the scored cells, endogenous emerin levels were markedly reduced. *DAPI,* 4',6-di-amidino-2-phenylindole; s*cale bars*, 20 µm.