viruses

MDPI

Supplementary Materials

Review

Nuclear egress complexes of HCMV and other herpesviruses: solving the puzzle of sequence coevolution, conserved structures and subfamily-spanning binding properties

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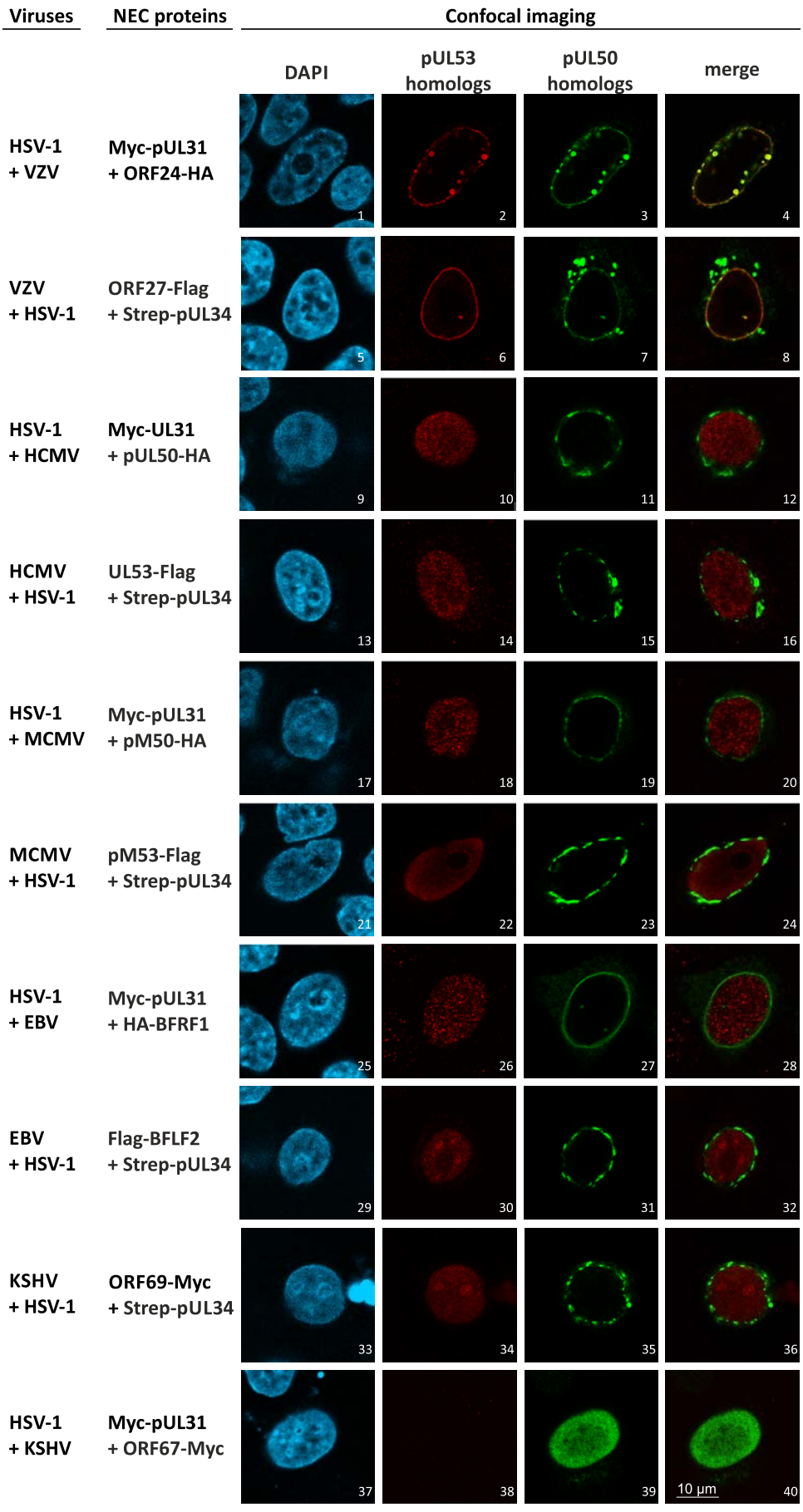
Table S1. Sequences of HCMV pUL53 and EBV BFLF2 hook peptides.

Peptide	**Sequence** (position numbers are based on pUL53 and BFLF2, respectively)
pUL53 hook peptide	Ac[a]-[59]LTLHDLHDIFREHPELELKYLNMMKMAIT[87]-Aoa[b]-Lys(Fluo[c])-NH_2
BFLF2 hook peptide	Ac-[78]DRSHFSLRDFFRGISANFELGKDFLREMNTPIH[110]-Aoa-Lys(Fluo)-NH_2

[a] Ac, acetyl; [b] Aoa, 8-amino-3,6-dioxaoctanoic acid; [c] Fluo, fluorescein

Figure S1. Video depiction of herpesviral 3D core NEC crystal structures:
(a) HSV-1, (b) PRV, (c) HCMV and (d) EBV.

(a)


Viruses
NEC proteins
Confocal imaging
DAPI
pUL53 homologs
pUL50 homologs
merge
HSV-1 + VZV
Myc-pUL31 + ORF24-HA
1
2
3
4
VZV + HSV-1
ORF27-Flag + Strep-pUL34
5
6
7
8
HSV-1 + HCMV
Myc-UL31 + pUL50-HA
9
10
11
12
HCMV + HSV-1
UL53-Flag + Strep-pUL34
13
14
15
16
HSV-1 + MCMV
Myc-pUL31 + pM50-HA
17
18
19
20
MCMV + HSV-1
pM53-Flag + Strep-pUL34
21
22
23
24
HSV-1 + EBV
Myc-pUL31 + HA-BFRF1
25
26
27
28
EBV + HSV-1
Flag-BFLF2 + Strep-pUL34
29
30
31
32
KSHV + HSV-1
ORF69-Myc + Strep-pUL34
33
34
35
36
HSV-1 + KSHV
Myc-pUL31 + ORF67-Myc
37
38
39
10 µm
40

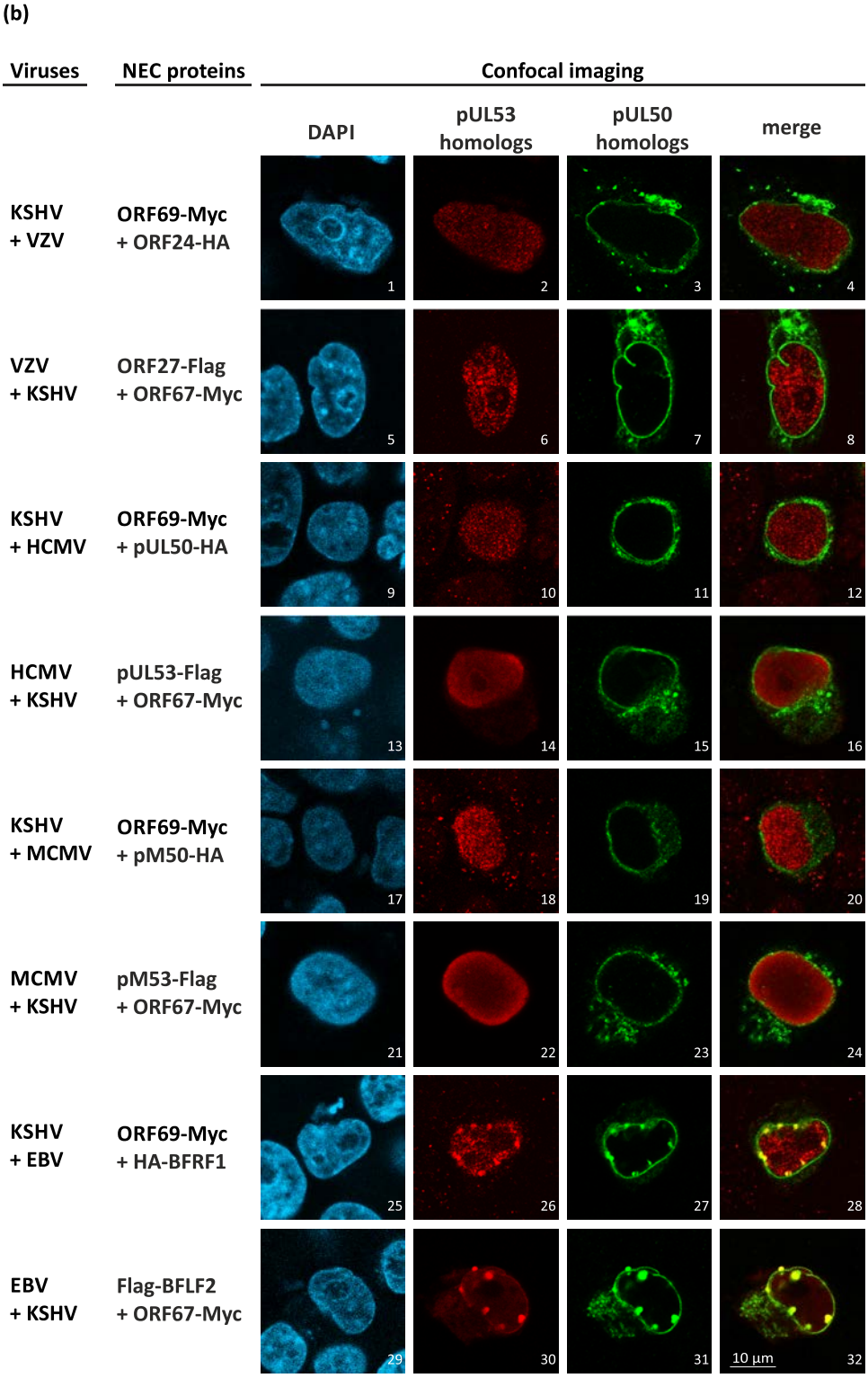


Figure S2. Primary data of confocal imaging analysis comparing autologous versus nonautologous α-/β-/γ-herpesviral core NEC interactions. HeLa cells were transiently transfected with constructs coding for tagged versions of the NEC proteins indicated. Cells were fixed and used for indirect immunostaining with tag-specific primary and fluorescence-labeled secondary antibodies. **(a)** Combinations between HSV-1-specific and various herpesviral NEC proteins. **(b)** Combinations between KSHV-specific and various herpesviral NEC proteins. Scale bar 10 µm.

Materials and Methods

The detailed mechanistic and structural analyses of herpesviral nuclear egress proteins, which have been performed by a great number of independent research groups, are reviewed in Marschall et al. [8]. Materials and Methods used for α-, β- and γ-herpesviruses are specifically referenced therein. For the sequence alignments, the crystal structures of the three subfamily prototypical NEC complexes from HSV-1 (PDB entry code: 4ZXS), HCMV (6T3X), and EBV (6T3Z) were structurally superimposed first and a structure-based sequence alignment deduced. Subsequently, subfamily-specific multiple sequence alignments were calculated and merged with the structure-based sequence alignment. Concerning the generation of primary data sets included in this review article, the Materials and Methods used were published previously [9-12,21-23,25-28]. Peptide synthesis methods [29], as well as the competitive pUL50-pUL53 binding assay to assess the inhibitory activity of the alanine and D-amino acid scan pUL53 hook peptides were previously described [22]. For the fluorescence polarization assay, C-terminally fluoresceinylated pUL53 and BFLF2 hook peptides (25 nM) were incubated with bacterially produced, recombinant pUL50 and BFRF1, respectively, at two-fold serial dilutions, starting at 10 μM (BFRF1) and 20 μM (pUL50), respectively. Fluorescence polarization was measured at 485 nm (excitation) and 535 nm (emission).