

Structural identification of conserved RNA binding sites in herpesvirus ORF57 homologs: Implications for PAN RNA recognition

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Table S1. PAN RNA interaction sites for KSHV ORF57 identified by Sztuba-Solinska et al. 2017.(1) Binding sites sequences are underlined with 2 flanking bases either site, those involved in base-pairing are italicized.

PAN sequence	Sequence	Is site base paired?
43-49 (NB in MRE and PAN17)	A <u>UUUU</u> GU	No
118-124	CU <u>UUU</u> GG	No
333-339	<i>CACU</i> ACC	Partially
336-343	G <u>CCCC</u> UU	No
479-485	UU <u>CUU</u> AG	No
587-593	<i>CAUUU</i> UA	Partially
600-606	<i>GUUUU</i> AG	Partially
774-783	UU <u>AAUGUUUU</u>	Partially
947-953	UU <u>UUU</u> AU	No

(A)

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KSHV -SPRFDDSIIPRHHGACFNVFIPAPPSHVPEVFTDRDITALIRAGGKDELINKKISAKK 245
HVS -LKLFDISILPKSGEPKLFPLVPSLPCQEAETNDKYVLAMAQRAMHDVPISSKQLTANL 206
      ** **:*: : : **: *.: * .*: : *: : . : * : .*:**:*:

KSHV IDHLHRQMLSFVTSRHNQAYWVSCRRETAAGGLQTLGAFVVEEQMTWAQTVVRHGGWFDE 305
HVS LPVKFKPLLSIVRYTPNYYYWVSMRKETIASANLCTVAAFLEDESLCWGQQYLKNDIFIFSE 266
      : .: :**:* * **** *:* * :..* *:*:*:*:*: *. * :... *. *

KSHV KDIDIILDTAIFVCNAFVTRFRLHLSC--VFDKQSELALIKQVAYLVAMGNRLVEACNL 363
HVS NGKDIILDTSSALLSQLVHKIKMLPF-CHCLMQTTPQDHIVKQVCYLIASNNRILDVRY 325
      :. *****: : . : * :****. * * :... : : ***.***:* .**:*:* .

KSHV LGEVKLNFRGGLLLAFVLTIPGMQSRRSISARGQELFRTLLEYRPGDVMGLLNIVIMEH 423
HVS LQTSVIKSPIVLLLAYAVCLPAAIICTKNETQLYSHCMRILKEYRPGDVMNILHESLTQH 385
      * : : ****:.: :*. . : : . :*: *****.:*: : : *

KSHV HSLCRNSECAAATRAAMGSAKFNKGLFFYPPLS 455
HVS LNKCPSTCAYTTRAIVGTKANTTGLFFLPTQ 417
      . * . * ** :*** :*: . ***** * .
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(B)

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KSHV SPRFDDSIIPRHHGACFNVFIPAPPSHVPEVFTDRDITALIRAGGKDELINKKISAKKI 246
HSV1 -----ADTI 245
      *..*

KSHV DHLHRQMLSFVTSRH-----NQAYWVSCRRETAAGGLQTLGAFVVEEQMTWAQTVVRHGG 301
HSV1 DATTRLVLRISERAAVDRISESFGRSAQVMHDPFG----GQFFPAANSPWAPVLAGQGG 301
      * * : * :..* .: : * : * : ** .. :**

KSHV WFDEKDIDII----- 311
HSV1 PFDAETRRVSWETLVAHGPSLYRTFAGNPRAASTAKAMRDCVLRQENFIEALASADETLA 361
      ** : :

KSHV -----LDTAIFVCNAFVTRFRLHLSCVFDKQSELALIKQVAYLVAM 353
HSV1 WCKMCIHHNLPLRPQDPIIGTTAAVLDNLATRLRPF-LQCYLKARGLC----- 408
      :.*: * : :.***: : *. * :. :.

KSHV GNRLVEACNLLGEVKLNFRGGLLLAFVLTIPGMQS---RRSI-----SARGQELFRTLLE 405
HSV1 --GLDELCSRRRLADIK----DIASFVFI LARLANRVERGVAEIDYATLG-VGVGEMKH 461
      * * *. .: : : **:.* . : .*: : : * . :.

KSHV YYRPGDVMGLLNIVIMEHSLCRNSECAAATRAAMGSAKFNKGLFFYPPLS-- 455
HSV1 FYLPGACMAGLIEILDTHRQECSSRVCEL TASHI-VAPPYVHGKYFYCNSLF 512
      :* ** *. * * : *:. * . * : : : : * :** *
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Figure S1. Alignment of KSHV ORF57 primary sequence with other IHDs of known structure. (A) With HVS ORF57, indicative of 30% identity with minimal gaps. (B) With HSV-1 ICP27, indicative of 16% sequence identity and large gaps. The CHCC zinc coordinating residues are highlighted yellow.

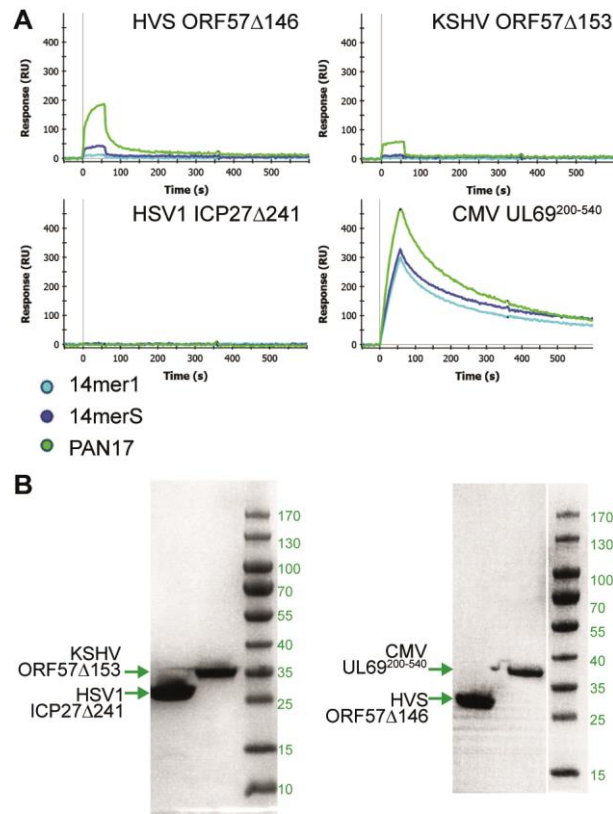


Figure S2. Surface plasmon resonance sensorgrams from RNA binding screen. (A) Each graph represents the response measured from a different protein construct vs each of the three immobilized RNA oligonucleotides 14mer1 (CCGUCCCCGCCGU), 14merS (CAGUCGCGAAGAGG) and PAN17 (CACCUAUGGAUUUUGUG), colored cyan, blue and green respectively. (B) Coomassie stained SDS-PAGE of purified proteins used in SPR.

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ORF57 -----GPLLKLF D 152
UL69 GPQQQMLALIDDELDAMDEDELQQLSRLIEKKKRARLQRGAASSGTSPTSSTSPVYDLQR 60
      *: .*

ORF57 -----ISILPKSGEPK L-----FLPVPSLPCQEAEKTNDKYVLAMAQRA-MHDVPISS 199
UL69 YTAESLRLAPYPADLKVPTAFPPQDHQPRGRILLSHDELMHTDYLLHIRQQFDWLEEP L L R 120
      : : * .: *: . * : .. * : .*:* : *: : *:

ORF57 KQLTANLLPV----KFKPLLSIVRYTPNYYYVW-----SMRKETIAS 237
UL69 KLVVEKIFAVYNAPNLHTLLAIIDETLSYMKYHLLHGLPVNPHDPYLETVGGMRQLLFNK 180
      * .. ::: * ::: **:*: * .* : .**: : .

ORF57 ANLCTVA AFLDES LCGQQYLKND FIFSENGKDIILD TSSALLS QLVHKIKMLPFCHCLM 297
UL69 LNNLDLGCILDH QDGDGHDHCSTL KRLVKKPGQMS-----A-----WLRDDVCDL 224
      * :.:**.. **: : . . :.: * : * * * :

ORF57 Q TTPQDHIVKQVCYLIASNNRILD AVRYLQTSVIKSPIVLLLAYAVCLPAAI-----I 350
UL69 QKRPPETF S-----QPMHRAMAYVCSFSRVAVSLRRRAL 258
      *. * : : .*: :**.. : : :

ORF57 CTKNETQLYSH--CMRILKEYRPGDVMNILHESLTQHLNKCPSSTCAYTTRAI VGTKANT 408
UL69 QVTGTPQFFDQFD TNNAMGTYRCGAVS D LILGALQC--HECQ NEMCELR IQRALAPYRF- 315
      ... *::: . : ** * * ::: :* ::* .. * : : .

ORF57 TGLFFLPTQ----- 417
UL69 -MIAYCPFDEQSLLDLTVFAGTTTTTA 341
      : : * :

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Figure S3. Alignment of the primary sequences of IHD regions from HVS ORF57Δ146 and CMV UL69 residues 200-540 reveals 17% identity with several large gaps. The U-bend binding residues conserved in γ -herpesviruses are colored green.

References

1. Sztuba-Solinska, J., Rausch, J.W., Smith, R., Miller, J.T., Whitby, D. and Le Grice, S.F.J. (2017) Kaposi's sarcoma-associated herpesvirus polyadenylated nuclear RNA: a structural scaffold for nuclear, cytoplasmic and viral proteins. *Nucleic Acids Res*, **45**, 6805-6821.