Supporting Information

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Fig. S1. Comparison of KSHV and HSV-1 MCPuds. The pseudoatomic model of KSHV MCPud was initially calculated by homology modeling with the crystal structure of HSV-1 MCPud (PDB ID code 1NO7) as template, and then adjusted according to the 6-Å resolution cryoEM structure of KSHV. The side-by-side presentations of KSHV and HSV-1 MCPud models demonstrate the similar overall fold of the two structures. The major differences between the two structures that might affect the SCP binding are marked with black arrows. A surface loop in HSV-1 has become a major helix in KSHV, creating a deep groove (dashed line) with other two helices (red arrows) for the binding of SCP stem helix.



Fig. S2. A stereo pair presentation of Fig. 5D.

KSHV:	MSNFKVRDPVIQERLDHDYAHHPLVARMNTLDQGNMSQAEYLVQKRHYLVFLIAHHYYEAYLRRMGGIQRRDHLQTLRDQKPRERA
EBV :	-MARRLPKPTLQGRLEADFPDSPLLPKFQELNQNNLPNDVFREAQRSYLVFLTSQFCYEEYVQRTFGVPRRQRAIDKRQRASVAGAGA
RRV :	MSSLRVKEPIVQGRLEHDYPNHPLVAEMNNLPQGDMSPAQYAIAKRNYLVFLTAKHHYDMYMQKKNGILRKDHLRGLRGKK
HVS :	MHRLRVTDPVVQGKLEESDSTHELVKRLEILPQNNMTPEEYTLMKRNYLVFLIAQYNFDQYIETTHGIKRKKHIEGLKANLKP
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KSHV:	DRVSAASAYDAGTFTVPSRPGPASGTTPGGQDSLGVSGSSITTLSSGPHSLSPASDILTTLSSTTETAAPA-VADARKPPSGKKK-
EBV :	AHLGGSSATPVQQAQAAASAGTGALASSAPSTAVAQSATPSVSSSISSLRAATSGATAAASAAAAVDTGSGGGGQPQDTAPRGARKKQ
RRV :	DASSSISGVLSGSGSAAPSVAPVASTLGSNSFTTISSGPHSLIGSMGPAPGGGGPGSVASSGIGSTSLSPSDATTLDTRRSSQNKKSK
HVS :	SALSADSASLSGLLSTSLTTPSLSSTPTSLTSMPGLSISGPSTTDTIDSKKKPKAK
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Fig. S3. SCPs of different gammaherpesviruses share conserved sequences and secondary structures in their N-terminal halves. Secondary structures of KSHV, EBV, RRV, and HVS SCP were predicted from the Iterative Threading Assembly Refinement (I-TASSER) server (1). The predicated helices are labeled in red, β -strands in blue, and coils in black. The protein sequences were also subjected to multiple sequence alignment with Clustal Omega (2), and level of conservation is indicated by symbols. Asterisk indicates identical amino acid in all sequences; colon indicates different but highly conserved amino acids; period indicates different amino acids that are somewhat similar.

1. Roy A, Kucukural A, Zhang Y (2010) I-TASSER: A unified platform for automated protein structure and function prediction. *Nat Protoc* 5(4):725–738. 2. Sievers F, et al. (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* 7:539.

Table S1. Statistic of number of capsids observed in thin sections of cells infected with WT or mutant KSHV

Cell type	No. of representative sections counted	No. of capsids in cell nucleus (mean \pm SD)
iSLK-WT	6	76 ± 34
iSLK-SCPnull	9	16 ± 9
iSLK-SCPN86	7	98 ± 42



Movie S1. KSHV virion capsid structure and SCP cross-linking in its hexons.

Movie S1



Movie S2. KSHV SCP binds in a surface groove of MCP via hydrophobic interactions.

Movie S2