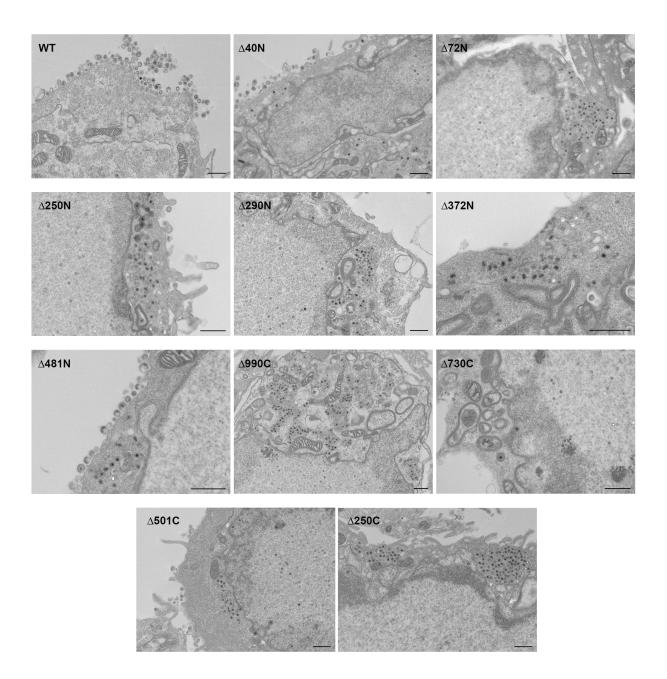


**Supplementary Figure S1.** Plating efficiency of mutant viruses following marker-rescue/marker-transfer transfections. BH31 (UL36/UL37) cells were co-transfected with K $\Delta$ 36/37CR and plasmids carrying UL37 wild-type (WT) and UL37-EGFP truncation mutations,  $\Delta$ 40N, and  $\Delta$ 501C. The transfection progeny was plated on BH31, BD45 (UL37) and Vero cells to demonstrate marker-rescue/marker-transfer. The transfection progeny contained mostly K $\Delta$ 36/37CR virus compared to the recombinant virus, at least 100 fold more. WT and  $\Delta$ 40N were able to plaque on all cells whereas  $\Delta$ 501C only replicated on the complementing cell lines. The arrow indicates increasing 10-fold serial dilutions down the plate starting at a 1 x 10<sup>-2</sup> dilution of the transfection virus stock.



**Supplementary Figure S2.** Truncations of pUL37 lead to a defect in cytoplasmic envelopment of virus particles. Representative TEM images of cells infected with each virus encoding a polypeptide truncation mutant of pUL37 are shown with scale bars of 1  $\mu$ m indicated.

Identity 1. HHV-1 strain KOS (JQ673480) 2. HHV-1 strain 17 (NC_001806) 3. HHV-2 (NC_001798) 4. HHV-3 (NC_001348) 5. McHV-1 (NC_004812) 6. AnHV-1 (JF999965)	1         10         20         30         40         50         60           Imvsptpppkegraatppk	70 1 A D R G L P S A M A D R G L P S A M S D S A L Q V P A P A A P P G D G R A P D S G G Q L S D N E Y
7. BoHV-1 (NC_001847) 8. BoHV-5 (NC_005261) 9. CeHV-9 (NC_002686) 10. EHV-1 (NC_001491) 11. EHV-4 (NC_001844) 12. FeHV-1 (FJ478159) 13. GaHV-2 (NC_002229) 14. GaHV-3 (NC_002577) 15. MeHV-1 (NC_002641) 16. SuHV-1 (NC_006151) Identity		140 150 74 A P A D A R A C W
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2. HHV-1 strain 17 (NC_001806) 3. HHV-2 (NC_001798) 4. HHV-3 (NC_001348) 5. McHV-1 (NC_001847) 6. AnHV-1 (JF999965) 7. BoHV-1 (NC_001847) 8. BoHV-5 (NC_005261) 9. CeHV-9 (NC_002686) 10. EHV-1 (NC_001491) 11. EHV-4 (NC_001844) 12. FeHV-1 (FJ478159) 13. GaHV-2 (NC_002229) 14. GaHV-3 (NC_002577) 15. MeHV-1 (NC_002641) 16. SuHV-1 (NC_006151) Identity 1. HHV-1 strain KOS (JQ673480)	A G A Q Q R L L A L L Q Q T W T L L Q N T N S P S V V N N T L J D A G F T P S H C T H Y L S A L E G F L Y         A G A Q Q R L L A L L Q Q T W A L V Q N S N S P S V V N N T L T D A G F T P A H C T Y Y I S A L E G F L Y         A G	A       G       V       P       A       R       T       P       T       G       H         A       G       V       P       A       R       T       P       P       G       H         A       G       V       P       A       R       T       P       P       G       H         G       F       I       H       G       S       V       V       A       D       E         T       G       G       A       S       R       G       L       A       G       P         M       Y       S       R       A       T       T       D       N       G       Y         A       Q       L       S       R       P       E       P       R       P       R       P         A       Q       L       S       R       P       E       P       R       P       R       P       R       P       R       P       R       P       R       P       R       P       R       D       N       S       N       N       N       S       N       R       N
<ol> <li>HHV-1 strain 17 (NC_001806)</li> <li>HHV-2 (NC_001798)</li> <li>HHV-3 (NC_001348)</li> <li>McHV-1 (NC_004812)</li> <li>AnHV-1 (JF999965)</li> <li>BoHV-1 (NC_001847)</li> <li>BoHV-5 (NC_005261)</li> <li>CeHV-9 (NC_002686)</li> <li>EHV-1 (NC_001844)</li> <li>FeHV-1 (FJ478159)</li> <li>GaHV-2 (NC_002229)</li> <li>GaHV-3 (NC_002577)</li> <li>MeHV-1 (NC_006151)</li> <li>Identity</li> <li>HHV-1 strain KOS (JQ673480)</li> </ol>	G L G E V Q Q L F G C I A L A G S N V F G L A R E Y G Y Y A N Y V K T F R R V Q G A S E H T H G R L C E A V G L S G G V L S Q T G L S E I Q Q L F G C I A L A G A N V F G L A R E Y G Y Y A N Y V K T F R R I Q G A S E H T H G R L C E A V G L S G G V L S Q T H I T D F Q Q L F G C I A L Y G G L A R Y Q L V E T Y A P T T E Y V L T Y T R T V N E T E K R Y E T L L P A L G L P P G G L G Q I G L S E I Q Q L F G C L A L Y G A N V F G L A R E Y G Y H S G Y V R A F R R I Q D A C E K A H A R L C E A G L T G G V L S Q T A L N D T Q Q L F G C L A L T G A N V F G L I D S Y G R D A D Y L D A Y A E A M S S L E S D S G D F L S A I G L P K G G I E Q T A L N D T Q Q V I G C V A W A G G V L F K L F D A Y G P S A D Y L A H Y T A T I A N L H P Y Y A D V L P L L G L P D G G L E Q T A L A D A L Q A V G C V A W A G G V L F K L F D A Y G P S A D Y L A H Y T A T I A N L H P Y Y A D V L P L L G L P D G G L E Q T A L A D A L Q A V G C V S L V G G L A Y Q L L E T Y A P T A H Y V S T Y T H I L S E T E K R Y E T L I P A L G L P P G G L E Q T K L T D F Q Q V G C V S L V G G V V F R L L L S Y G F G L D Y L R D Y T T T I S T L E P V Y N E L L L S A L G L A D K G V E Q T S L D Y A Q Q V V G C V S I V G G V F R L L M S Y G F G L D Y I R D Y T T T I S T L E P V Y N E L L L S A L G L A D K G V E Q T S L D Y A Q Q V V G C V A M V G S L I F K L Y T H Y G N G L D Y I R D Y T T T T I S T L E P V Y N E L L L S A L G L A D K G V E Q T S L D Y A Q Q V V G C V A M V G S L I F K L Y T H Y G N G L D Y I R D Y T T T T I S T L E P V Y N E L L L S A L G L A D K G V E Q T L L D E A Q Q A I G C V A M V G S L I F K L Y T H Y G N G L D Y I R D Y T T T T I S T L E P V Y N E L L L S A L G L A D K G V E Q T L L D E A Q Q V V G C V A M V G S L I F K L V T H Y G N G L D Y I R D Y T T T T I S T L E P V Y N E L L L S A L G L A D K G V E Q T I F D D V Q Q V V G C V A M V G S L I F K L V T H Y G N G L D Y I R D Y T T T T I S T L E P V Y N E L L L S A L G L A D K G V E Q T I F D D V Q Q V V G C V A M Y G S L I F K L Y T H Y G N G L D Y I R D N G L D Y I R D Y G S L P P E S D -	L A R I M G P A V P T A R I M G P A V P T M R R C F A P R P L I L A R V M G P V T P T I R H C M A P R P I T R H C M A P R P R T R H C M A P R P R T R H C M A P R P R T I R H C M A P R P V M I R R S M A P R P Y M L R R S M A P R P Y M I G R C I P P I P H E L G R C I P P N P H E L G R C I P P N P H E L G R C I P P N P H T 670
2. HHV-1 strain 17 (NC_001806) 3. HHV-2 (NC_001798) 4. HHV-3 (NC_001348) 5. McHV-1 (NC_004812) 6. AnHV-1 (JF999965) 7. BoHV-1 (NC_001847) 8. BoHV-5 (NC_005261) 9. CeHV-9 (NC_002686) 10. EHV-1 (NC_001844) 12. FeHV-1 (FJ478159) 13. GaHV-2 (NC_002229) 14. GaHV-3 (NC_002577) 15. MeHV-1 (NC_002641) 16. SuHV-1 (NC_006151) Identity 1. HHV-1 strain KOS (JQ673480)	50-52 50	9 D T T P T T P A T P L D T T P T T P A T P L G T T P T T P A T P L G T T P V A P A T P S G T E P P G N D E Q C G V A P P A A E A Q P G V A P P A A E A Q P G V A P P V D T Q L S G V R I P D R D T T S G V R I P D R D T T S G V R I P D R S N V L G T V P P E E L I G G G V P P E E N S N S T
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2. HHV-1 strain 17 (NC_001806) 3. HHV-2 (NC_001798) 4. HHV-3 (NC_001348) 5. McHV-1 (NC_004812) 6. AnHV-1 (JF999965) 7. BoHV-1 (NC_001847) 8. BoHV-5 (NC_005261) 9. CeHV-9 (NC_002686) 10. EHV-1 (NC_001844) 12. FeHV-1 (FJ478159) 13. GaHV-2 (NC_002229) 14. GaHV-3 (NC_002577) 15. MeHV-1 (NC_002641) 16. SuHV-1 (NC_006151) Identity 1. HHV-1 strain KOS (JQ673480)	S H E D N V L A V V R R E I H G F L S V V S G H A R A S K L S G D Q V P G F C F M G Q F A R W R R S - A C Y Q A S H E G N V L A V V R R E I H G F L S V V S G H A R A S K L S G D Q V P G F C F M G Q F A R W R R S - A C Y Q A S F D G N V L A V V R R E I H G F L S V V S G H A R A S K L S G D Q V P G F C F M G Q F A R W R R S - A C Y Q A S F D G N V L A V Y R R E I H G F L S V V S G H A R A S K L S G D Q V P G F C F M G Q F A R W R R S - A C Y Q A S F D G N V L A V Y R R E I H G F L S V V S G H A R A S K L S G D Q V P G F C F M G Q F A R W R R S - A C Y Q A S F D G N V L A V Y R R E I H G F L S V V S G H A R A S K L S G D Q V P G F C F M G Q F A R W R R S A T A H A G N S Y K R I F S T A S R V A F T V A G G H A R A S R L A G E H V P G F C F M G Q F A R W R R A T A H A A P G G G L D D A H A A L A G H S A V R A Q T A L A L A A G K V A G A E A P G L H E V G R S U Q R W D A L G A A L G R A P G G G G L D D A H H A L A G H S A V R A Q T A L A L A A G K V A G A E A P G L H E V G R S U Q R W D A L G A A L G R A S T C D S - I N L L F S T A S R Y A H F T Q E Q L D V L L R V N K V C G N C V P G L G N V D K U G R W S A A L G R A E G T R F G K L F N S L L T R H T Q L A R A Q R G L A L R A G K L U G G S E A P G L K H V N T F U Q R W G A S V M Y Q K A E G T R F G K L F N S L L T R H T Q L A R A Q R G L A L K A G K L U G G S E A P G L K H V N T F U Q R W G A S S S R Y Y K Y N A L G Q D A V K L F S V L G A D F A G L T R L Q N S L D L H V R A T S C S T P P G M Q D L C W L U G R W S T S S S R Y K Y N A L G Q D A V K L F S V L G A D F A G L T R L Q N S L D L H V R A T S C S T P P G M R S L S H L U D K W S T S E I N S S M F S D A A A R F F S D L G A D Y A G L Y R L Q T S M D L H V R M L T C S H P P G M R S L S H L U D K W S T S E I N S S M F S D A A A R F F S D L G A D Y A G L Y R L Q T S M D L H V R M L T C S H P P G M R S L S H L U D K W S T S E I N S S M F S D A A A R F F S D L G A D Y A G L Y R L Q T S M D L H V R M L T C S H P P G M R S L S H L U D K W S T S -	T A V P S A E S R T A T G P R R A L V K K T D E T L D D S A G E R D L D D R A G E R D A T T S P C V D F T S G S T P E V N T S G S T P E A N T S T A D N V P E A A T Y R D R S S H E T F R D K S S Q D S F R D K S S Q D
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2. HHV-1 strain 17 (NC_001806) 3. HHV-2 (NC_001798) 4. HHV-3 (NC_001348) 5. McHV-1 (NC_004812) 6. AnHV-1 (JF999965) 7. BoHV-1 (NC_001847) 8. BoHV-5 (NC_005261) 9. CeHV-9 (NC_002686) 10. EHV-1 (NC_001844) 12. FeHV-1 (FJ478159) 13. GaHV-2 (NC_002229) 14. GaHV-3 (NC_002577) 15. MeHV-1 (NC_006151) Identity	S L G P L G Q T T A V P D S V D L S R Q G L A V T L S M D W L L M N E L R V T D G V F R A S A F R P L A G P E S P R S L G P L G Q P T V V P D S V D L S P Q G L A A T L S M D W L L I N E L R V T D G V F R A S A F R P S A G P G A P G N A T F L H D N V K I T T T V R D V I S E A P T L I I G Q R W L R P D E L S N V D L R L G V P G N T S G S D P S M G P L G T T T V P P D S V D L S P Q G L A A T L S M D W L L I N E L R V T D G V F R A S A F R P S A G P G A P G N A T F L H D N V K I T T T V R D V I S E A P T L I I G Q R W L R P D E L S N V D L R L G V P G N T S G S D P S M G P L G T T T V P P D S V D L S P Q G L A V M L G M D W L L N N E M L Q V T D G V F R A S A F R P F P G P D P P R N Y A P L E M H T H K P P E V D P D N H - L D Y F L N S K R L T Q D D L S I V D K V F N A E H V D R G P L Q R R A A S A G A T T D A A A S A A A A A N T A A G P P W L T T E D L A A E V D G V C A S R P A A A R N V K I T T S G I R T P Q T T T A I H N A S S V L I E Q R W L M I D E L S E V D A Y L G L L K T K M D A L R K R V T M P A N I D S I R - G N D S L A T R E Y L K K E D L A A E I D A A F N N T K M D A L R K R V T M P A N I D S I R - G N D G L L T R E Y L K K E D L A A E I D A F N N T K Q E L D I L R K G V I A P N D V D L T S F A R D F L T K Q R ' T A D A V N I D T V F N N E R Q A R D G N A N F D T U D L D T V A H D D V D L A A F A K D F L K R R ' T A D A V N I I D T V F N N E R Q A R D G N A N F D T U D L D T V A H D	D L E V R D A G N S L D L E V R D A G N S L D L E A Q D A G G S T D L E V R E E G S L L
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**Supplementary Figure S3.** pUL37 alanine-scanning mutants (AAA) target conserved residues in the C-terminal half of the polypeptide. pUL37 amino acid sequences or homologs from different alphaherpesviruses were aligned using Geneious and amino acid conservation is indicated. pUL37 AAA mutated sequences are annotated on KOS pUL37 (Accession: JQ673480).

Supplementary Table S1. Primer sequences.

Primer name	Nucleotide sequence 5'-3'
BglII F	GAGCCGGGCGTCATGAGATCTGCAGACCGCGGTCTC
BglII R	GAGACCGCGGTCTGCAGATCTCATGACGCCCGGCTC
BsrGI <sup>s</sup> F	GGAATTCTACGTAAAAACTTTCAGGCG <i>TGTACA</i> GGGCGCCAGCGAGCACAC
BsrGI <sup>s</sup> R	GTGTGCTCGCTGGCGCCCTGTACACGCCTGAAAGTTTTTACGTAGAATTCC
WT BglII F	GGAAGATCTATGGCAGACCGCGGTCTCCCGTCC
∆40 BglII F	GGG <i>AGATCT</i> CCAACCCCCACGGCCGAGACGGC
∆72 BglII F	GGG <i>AGATCT</i> GGAACGGCCATCGCCCGGCAGAC
Δ250N BglII F	GGG <i>AGATCT</i> GCGCACCTCCAGCGCATAGACGAC
$\Delta 290$ BglII F	GGG <i>AGATCT</i> CTGTTGGCGCAGTTTCAGCACACC
∆372 BglII F	GGG <i>AGATCT</i> GCCGTCTCCAGCCTGCTGCAGCTC
$\Delta 481$ BglII F	GGG <i>AGATCT</i> GTAAAAACTTTCAGGCGGGTCCAG
WT BsrGI R	GTGCTCGCTGGCGCCCTGTACACG
∆990 SpeI R	CCACTAGTGTCGCGACGGCTCGTAAGCACGAC
∆730 SpeI R	GGACTAGTCTCGGGGGGGGGGGGGGGGGGGGGGGGGGGG
∆501 SpeI R	GGACTAGTCGCCTCGCAGAGCCGCCCGTGCGTG
$\Delta 250$ C SpeI R	GGACTAGTCGCCGAGAACAGCGTCACCCGATTAC
WT SpeI R	GGACTAGTTAACGGCAAGTCCGTGGGTGG
CMV-DsRed F	GGACTAGTTAGTTATTAATAGTAATCAATTAC
CMV-DsRed R	GGCGGCCGCAACCACAACTAGAATGCAGTG
550–52 F	AGTTCCGGTCAACCCGCGGCCGCCGCGAGACGGCGCTC
654–56 F	GCCCTGCGTGTGTGGCGGCCGCCCGGGACTTTGGGCTG
657–59 F	GTGTTGGCCTGGGCCGCGGCCGCTGGGCTGGGCTATCTC
667–69 F	GGCTATCTCCCCACGGCGGCCGCCCATCGCACCAAACTG
672–74 F	GTTGAGGGCCATCGCGCGGCGCGGGGCGCGCGCGCGCGCG
676–78 F	CGCACCAAACTGGGCGCGGCCGCCACCCTCCTCGAACCG
680–82 F	GGCGCGCTGATCACCGCGCCGCACCGGCCGCCCGGGGC
702–04 F	GACAACATAGAGCAGGCGGCGCGGGAGCTGTACGTGATC
704–06 F	ATAGAGCAGCTGCTCGCGGCCGCGTACGTGATCTCCAGG
713–15 F	GTGATCTCCAGGGGTGCGGCCGCGCAGCTGCGGCCCCTG
739-41 F	AGCCTCCTGTTGATTGCGGCCGCCGCCCTGGCCGCCCGG
906–08 F	GGTCAGTTTCTAGCGGCGGCGCGCGCGTCTGTCGGCCTGC
909 <b>—</b> 11 F	CTAGCGCGCTGGCGGGCGGCCGCGGCCTGCTATCAAGCC