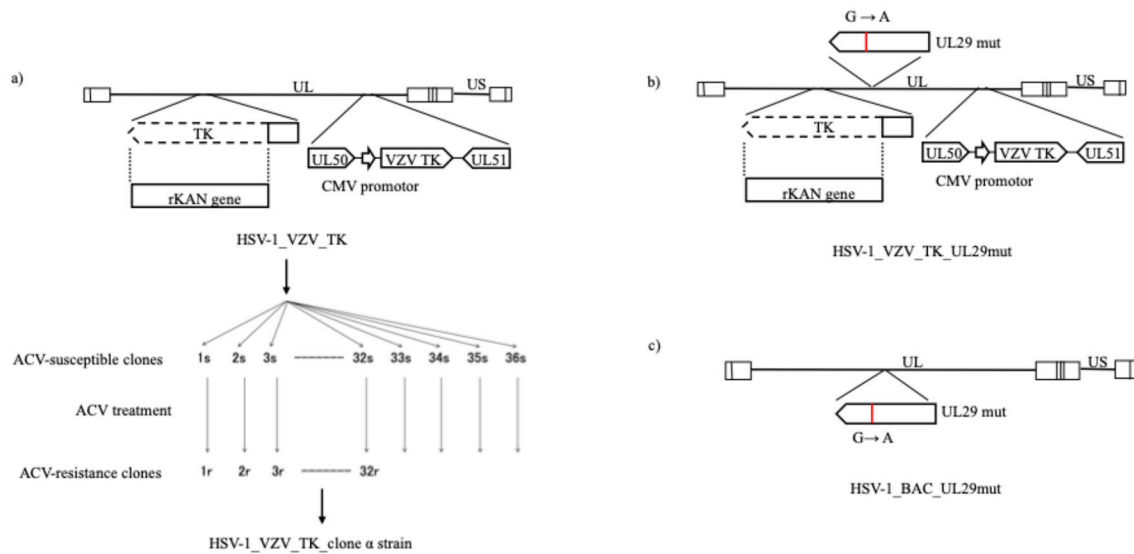


Supplemental Materials



Supplemental Figure S1. Structure of HSV-1_VZV_TK, HSV-1_VZV_TK_UL29mut, and HSV-1_BAC_UL29mut strains.

(a) The recombinant HSV-1_VZV_TK strain bears a VZV TK gene in the intergenic region between the UL50 and UL51 genes, and a Kan resistance gene was used to replace the partial HSV-1 TK gene. The HSV-1_VZV_TK_clone α strain was isolated by plaque purification under ACV supplementation conditions. (b) The HSV-1_VZV_TK_UL29mut strain was constructed based on HSV-1_VZV_TK using a two-step Red-mediated mutagenesis procedure with pUC-Zeo. (c) HSV-1_BAC_UL29mut was constructed from the parental HSV-1_BAC using a two-step Red-mediated mutagenesis procedure and pcDNA3-KanS.

Table. S1 Primers used for mutagenesis

| primer code | sequence (5' to 3') direction* | introduced alteration |
|-------------------------|---|-----------------------|
| HSV-1_VZV_TK_UL29mut F | GCGGTTGGTGATGATGGTCTCCAGCCGCCCATGGCCGTGgGGACCGCCTGGTCCACGTACTGTAAAACGACGGCCAGTGA | P597L |
| HSV-1_VZV_TK_UL29mut R | GCCGAACTCGAGACCCTGCAGTACGTGGACCAGGCGGTCCcCACGGCCATGGGGCGGCTGATTACGCCAAGCTTGCATGC | |
| HSV-1_BAC_UL29mut F | GCGGTTGGTGATGATGGTCTCCAGCCGCCCATGGCCGTGgGGACCGCCTGGTCCACGTACAGGATGACGACGATAAGTAGGG | P597L |
| HSV-1_BAC_UL29mut R | GCCGAACTCGAGACCCTGCAGTACGTGGACCAGGCGGTCCcCACGGCCATGGGGCGGCTGCAACCAATTAACCAATTCTGATTAG | |
| HSV-1_BAC_UL29mut_rev F | GCGGTTGGTGATGATGGTCTCCAGCCGCCCATGGCCGTGgGGACCGCCTGGTCCACGTACAGGATGACGACGATAAGTAGGG | L597P |
| HSV-1_BAC_UL29mut_rev R | GCCGAACTCGAGACCCTGCAGTACGTGGACCAGGCGGTCCcCACGGCCATGGGGCGGCTGCAACCAATTAACCAATTCTGATTAG | |

*Mutated and reversed sequences are shown in lower case and with underline, respectively.

Table. S2 Comparison of ACV susceptibility of all viruses in this study

| cells | IC ₅₀ (mean ± SD µg/ml) | | | | | | |
|--------------|------------------------------------|-------------|------------------|--------------------------|--------------------------|-------------------|-----------------------|
| | HSV-1 F strain | HSV-1_BAC | HSV-1_BAC_VZV_TK | HSV-1_BAC_VZV_TK_clone α | HSV-1_BAC_VZV_TK_UL29mut | HSV-1_BAC_UL29mut | HSV-1_BAC_UL29mut_rev |
| Vero cells | 0.42 ± 0.07 | 0.41 ± 0.06 | 2.6 ± 0.10 | 7.2 ± 1.50 | 7.2 ± 1.70 | 0.65 ± 0.09 | 0.47 ± 0.03 |
| HEL cells | 0.27 ± 0.11 | 0.60 ± 0.14 | NT | NT | NT | 1.39 ± 0.02 | 0.45 ± 0.21 |
| ARPE19 cells | 23.19 ± 2.11 | 24.7 ± 11.0 | NT | NT | NT | 113 ± 1.26 | 27.9 ± 9.20 |

NT, not tested