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(54) Title: SYNCYTIAL ONCOLYTIC HERPES SIMPLEX MUTANTS AS POTENT CANCER THERAPEUTICS

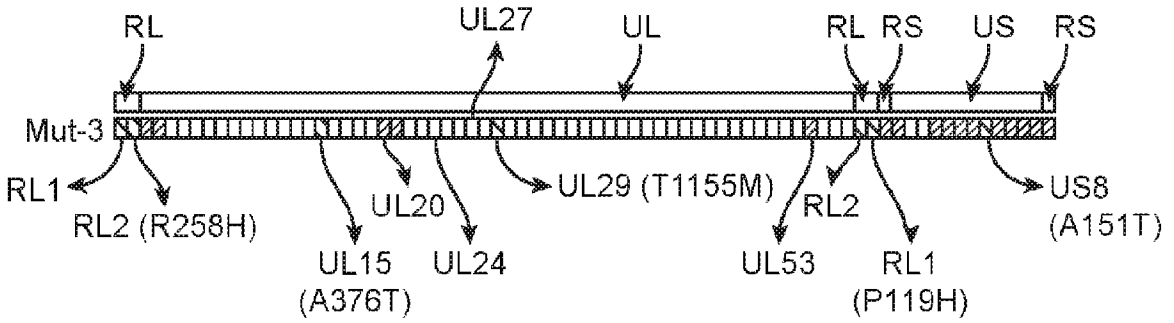

FIG. 1B

(57) Abrégé/Abstract:
The disclosure provides a non-natural herpes simplex virus ("HSV"), compositions comprising, or alternatively consisting essentially of, or yet further consisting of the HSV, and methods of producing the HSV, or infecting a cell with the HSV. Also provided herein are methods of treating cancer or inhibiting the growth or metastasis of cancer cell in a subject in need thereof.

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(54) Title: SYNCYTIAL ONCOLYTIC HERPES SIMPLEX MUTANTS AS POTENT CANCER THERAPEUTICS

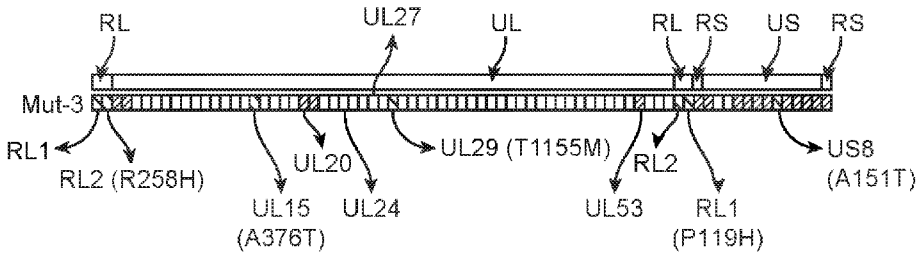

FIG. 1B

(57) Abstract: The disclosure provides a non-natural herpes simplex virus ("HSV"), compositions comprising, or alternatively consisting essentially of, or yet further consisting of the HSV, and methods of producing the HSV, or infecting a cell with the HSV. Also provided herein are methods of treating cancer or inhibiting the growth or metastasis of cancer cell in a subject in need thereof.

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SYNCYTIAL ONCOLYTIC HERPES SIMPLEX MUTANTS AS POTENT CANCER THERAPEUTICS

CROSS-REFERENCE TO RELATED APPLICATIONS

[1] This application claims priority under 35 U.S.C. §119(e) to U.S. Provisional Application Nos. 62/818,577 and 62/932,725, filed March 14, 2019 and November 8, 2019, respectively, the content of each of which is hereby incorporated by reference into this disclosure.

STATEMENT OF GOVERNMENT SUPPORT

[2] This invention was made with government support under the Grant No. CA223104 awarded by National Institute of Health and National Cancer Institute (NIH/NCI). The government has certain rights to the invention.

SEQUENCE LISTING

[3] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 13, 2020, is named 106887-7660_ST25.txt and is 245,014 bytes in size.

BACKGROUND

[4] The antitumor efficacy of oncolytic herpes simplex viruses (oHSVs) such as Imlygic™, recently FDA approved to treat melanoma, is very promising. These vectors have two major mechanisms of action: (1) a lytic phase, determined by direct infection and lysis of cells, and (2) an immunologic phase, driven by the stimulation of antitumor immunity. However, not all cancers respond similarly as virus spread is intrinsically slow in some cancers. In culture, cells vary in their levels of permissivity to viruses. In animals, variations in the tumor's stromal and immune cell composition lead to variations in the capacity for virus spread and immune reactions. Therefore, strategies to improve the potency of the lytic phase to reach optimal therapeutic benefit are still needed. This disclosure satisfies these needs and provide related advantages as well.

SUMMARY

[5] The present disclosure provides a non-natural herpes simplex virus ("HSV"), wherein the virus comprises, alternatively consists essentially of, or yet further consists of a mutation in one or more of: (a) a glycoprotein E ("gE")-encoding gene, (b) an Infected Cell Protein 0 ("ICP0")-encoding gene, (c) a DNA packaging terminase subunit 1-encoding gene, (d) an ICP8-encoding gene, or (e) an ICP34.5-encoding gene.

[6] In another aspect, the present disclosure provides a composition or a pharmaceutical composition that comprises, alternatively consists essentially of, or yet further consists of the non-natural HSV of this disclosure. In another aspect, provided in this disclosure is a method to infect a cell, comprising, or alternatively consisting essentially of, or yet further consisting of contacting the cell with the non-natural HSV or a composition or pharmaceutical composition containing the non-natural HSV.

[7] In one aspect, the present disclosure provides a method of preparing the non-natural HSV of this disclosure comprising, or alternatively consisting essentially of, or yet further consisting of mutating a gene in a non-natural HSV viral particle or introducing into the non-natural HSV a transgene. In another aspect, the method of producing the non-natural HSV vector, comprises, or alternatively consists essentially of, or yet further consists of: (a) introducing to a host cell a 17TermA HSV vector and an rRp450 HSV vector; (b) growing the host cell for at least 3 passages; and (c) isolating a HSV particle produced by the host cell.

[8] Also provided is a method for inhibiting the growth or metastasis of a cancer cell or a metastatic cancer cell, the method comprising, or consisting essentially of, or yet further consisting of, contacting the cell with an effective amount of the non-natural HSV vector or a composition or a pharmaceutical composition containing the non-natural HSV vector as described herein. The contacting is *in vitro* or *in vivo*. In one aspect, the contacting is *in vivo* by administration of the non-natural HSV or a composition or a pharmaceutical composition to a subject. *In vitro*, the method is practiced by placing the non-natural HSV in contact with the cell. The *in vitro* method can be used to test for new therapies or as a personalized assay to determine if the therapy is suitable for the cancer to be treated. Additional cancer therapies

can be combined with the therapy which can be concurrent or sequential to the disclosed methods.

[9] The cancer cell to be treated can be a solid tumor or blood cancer, e.g., carcinoma or sarcoma and non-limiting examples of such include pancreatic cancer, renal cancer, small cell lung cancer, brain cancer, neuroblastoma, neural cancer, bone cancer, lymphoma, myeloma, colon cancer, uterine cancer, breast cancer, leukemia, liver cancer, prostate cancer, skin cancer, or melanoma. The cell is of any species, *e.g.*, mammalian and human and when performed *in vitro*, it can be from a cultured cell line or a primary cell, *e.g.*, from a tissue biopsy. The cell can be an adult or juvenile cell or a cancer stem cell (*i.e.*, cancer cells possessing characteristics associated with normal stem cells, specially the ability to give rise to all cell types found in a particular cancer sample) or a cancer cell without such characteristics associated with normal stem cells. In one embodiment, the cell expresses N-myc proto-oncogene protein (MYCN), and/or expresses MYCN at a level higher than non-cancer cells.

[10] In another aspect, also provided in this disclosure is a method for treating cancer or a metastatic cancer, or inhibiting the growth or metastasis of a cancer cell in a subject in need thereof, comprising, or alternatively consisting essentially of, or yet further consisting of, administering to the subject an effective amount of the non-natural HSV, the composition or the pharmaceutical composition of this disclosure. The subject to be treated can be of any species, *e.g.*, mammalian and human, e.g., canine, equine, bovine, feline, simian, rat or murine. The administration can be as a first line therapy, a second line therapy, a third line therapy, a fourth line therapy, or a fifth line therapy. Additional cancer therapies can be combined with the therapy which can be concurrent or sequential to the disclosed methods. The cancer to be treated can be a solid tumor or blood cancer, e.g., carcinoma or sarcoma and non-limiting examples of such include pancreatic cancer, renal cancer, small cell lung cancer, brain cancer, neuroblastoma, neural cancer, bone cancer, lymphoma, myeloma, colon cancer,

uterine cancer, breast cancer, leukemia, liver cancer, prostate cancer, skin cancer, or melanoma.

[11] The method of this disclosure can be combined with appropriate diagnostics to monitor disease remission or progression. Several methods for such monitoring are known in the art.

[12] In one aspect, the disclosure provides a method of inducing cell lysis, comprising, or alternatively consisting essentially of, or yet further consisting of, contacting the cell with an effective amount of the non-natural HSV, the composition, and/or the pharmaceutical composition of this disclosure. The contacting is *in vitro* or *in vivo*. In one aspect, the contacting is *in vivo* by administration of the non-natural HSV or a composition or a pharmaceutical composition to a subject. *In vitro*, the method is practiced by placing the non-natural HSV in contact with the cell. The *in vitro* method can be used to test for new therapies or as a personalized assay to determine if the therapy is suitable for the subject to be treated. Additional cell lytic therapies can be combined with the therapy which can be concurrent or sequential to the disclosed methods.

[13] The cell to be treated can be a solid tumor or blood cancer, e.g., carcinoma or sarcoma and non-limiting examples of such include pancreatic cancer, renal cancer, small cell lung cancer, brain cancer, neuroblastoma, neural cancer, bone cancer, lymphoma, myeloma, colon cancer, uterine cancer, breast cancer, leukemia, liver cancer, prostate cancer, skin cancer, or melanoma. The cell is of any species, *e.g.*, mammalian and human and when performed *in vitro*, it can be from a cultured cell line or a primary cell, *e.g.*, from a tissue biopsy. The cell can be an adult or juvenile cell or a cancer stem cell or a cancer cell without characteristics associated with normal stem cells. The therapy can be combined with an appropriate assay to test for the effectiveness of the therapy, e.g., cancer remission or progression.

[14] In another aspect, the disclosure also provides a kit comprising, or alternatively consisting essentially of, or yet further consisting of the non-natural HSV, the composition, and/or the pharmaceutical composition of this disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[15] **FIGS. 1A-1B** show schematic diagram of generating Mut-3 and Mut-3Δ34.5 (**FIG. 1A**) and Mut-3 SNP (**FIG. 1B**). An HSV Mut-3 was isolated from the serial passage of mixing 17TermA and rRp450 in a non-permissive line ("Directed Evolution"); and an attenuated mutant Mut-3Δ34.5 was constructed via gene editing (labelled as the "CRISPR/Cas9" step) (**FIG. 1A**). **FIG 1B** shows the sequence comparison of Mut-3 with its parent viruses. Nonsynonamous mutations that differ from either parent in Mut-3 are shaded with backslashes, including UL15, UL29, US8, RL1 and RL2. Genome sequences that are identical to 17TermA are showed as blank boxes in the bottom panel; those identical to rRp450 are marked with forward slashes.

[16] **FIGS. 2A – 2C** show plaque sizes and receptor usage of Mut-3, Mut-3Δ34.5, rRp450 & 17TermA. Applicant performed plaque assay of the four viruses at the same time and scanned and analyzed the plaque images 3 days after via Keyence HS All-in-one Fluorescence Microscope BZ-II Analyzer. **(FIG. 2A)** Raw (top) and masked (bottom, numbered plaques) images of the four viruses. **(FIG. 2B)** The average plaque size of the four viruses (left) and the numbers of plaques were calculated (right). The plaque sizes of Mut-3 and Mut-3Δ34.5 are significantly larger than both parent viruses rRp450 and 17TermA. **(FIG. 2C)** *In vitro* cytotoxicity/MTS assay of CHO cell sets. Applicant infected CHO-K1 (as marked), CHO-Nectin-1 (as marked), CHO-Nectin-2 (as marked) and CHO-HVEM (as marked) with the four viruses with different multiplicity of infections (MOIs). Applicant measured cell survival colorimetric Cell Proliferation/MTS assay three days post-virus infections (pvi) relative to untreated control. Only CHO-Nectin-1 and CHO-HVEM but not CHO-K1 or CHO-Nectin-2 (mainly for HSV-2 entry) were sensitive to treatments of the four viruses, suggesting that Mut-3 and Mut-3Δ34.5 did not bypass the receptor barrier and could still relay on canonical HSV entry receptors to infect the cells.

[17] **FIGS. 3A – 3C: Mut-3Δ34.5 is more potent than 17TermA ($\gamma_1$34.5-null) at killing human and murine neuroblastoma cells, which is not due to increased yield of infectious virus particles *in vitro*.** (**FIG. 3A**) *In vitro* cytotoxicity/MTS assay of neuroblastoma cell lines. Applicant infected both human (SK-N-AS) and murine (975A2) neuroblastoma cells with Mut-3 (as marked), Mut-3Δ34.5 (as marked) or 17TermA (as marked) with different

(MOIs). Applicant measured cell survival via MTS assay 4 days pvi relative to untreated control. N=6, error bars represent *SEM*. **** $p<0.0001$, 2-way ANOVA. (**FIG. 3B**) *In vitro* virus replication assay. Applicant infected neuroblastoma cell lines with 17TermA (as marked) or Mut-3Δ34.5 (as marked) at MOI =0.1 (upper panel, human line SK-N-AS) or MOI=0.5 (lower panel, murine line 975A2) and washed the cells with PBS 2 hours pvi. Applicant harvested cell lysate 2, 24, 48 and 72 h pvi and determined the virus yields by plaque assays. N=3. Error bars represent *SD*. * $p<0.05$,** $p<0.001$,**** $p<0.0001$, 2-way ANOVA. (**FIG. 3C**) Comparing relative free released virions in Mut-3Δ34.5 vs. 17TermA infected neuroblastoma cultures. Applicant performed the assays similar as described in (**FIG. 3B**), by harvesting supernatant and pellet to perform the plaque assay in the two separated portions over the time instead of measuring as a whole culture in **FIG. 3B**. Applicant calculated the relative free virion as proportion of infectious particle in the supernatant over the whole culture (supernatant + pellet). Mut-3Δ34.5 shows significant more relative released virions than 17TermA at 48hrs. N=3. Error bars represent *SD*. * $p<0.05$.

[18] FIGS. 4A – 4H: TEM analysis of Mut3Δ34.5 and 17TermA uptake in neuroblastoma cells. Neuroblastoma cells SK-N-AS and 975A2 were infected with Mut-3Δ34.5 or 17TermA at MOI of 50 for 20mins at 37°C. **(Figs. 4A, 4B, 4E & 4F)** The insets show Mut-3Δ34.5 virions were mainly found in endocytic vesicles (arrowheads except the right one in FIG. 4B) with very few found fused with plasma membrane (the right arrowhead in FIG. 4B). (**Fig. 4C, 4D, 4G & 4H**) The insets show 17TermA virions were mainly found in endocytic vesicles (arrowheads except the bottom one in FIG. 4G), very few found fused with plasma membrane (the bottom arrowhead in FIG. 4G). TEM analysis was done via Hitachi H-7650 TEM. N: nucleus. Scale bar: 500 nm.

[19] FIGS. 5A – 5C: Attenuated 17Δ34.5 virions showed comparable potency to 17TermA in neuroblastoma cells. Applicant generated 17Δ34.5 via CRISPR-Cas9 gene editing technique to replace g134.5 gene in wild-type strain 17^+ with EGFP expressing cassette. **(FIG. 5A)** Plaque images of 17Δ34.5 clones on Vero cells. Both B4 and G1 clones produce 100% GFP positive (left panels) non-syncytia (right panels, phase contrast) plaques after 3 rounds of plaque purification. Two days pvi. Imagines were taken via EVOS® FL Imaging System. Scale bar: 400 μm **(FIG. 5B)** *In vitro* cytotoxicity/MTS assay of

neuroblastoma cell lines. Applicant infected neuroblastoma cell lines, SK-N-AS and 975A2, with wild-type strain 17^+ (as marked), 17TermA (as marked), 17Δ34.5 clone B4 (as marked) or 17Δ34.5 clone G1 (as marked) at different MOIs. Applicant measured cell survival via MTS assay 3 days pvi relative to untreated control. The potencies of both 17Δ34.5 clones were significantly lower than their wild-type counterpart, strain17+, but comparable to 17TermA. The EGFP cassette and EGFP DNA sequence are shown in **FIG. 5C** (SEQ ID NO: 11).

[20] FIGS. 6A – 6C: Mut-3Δ34.5 displays much faster viral gene transfer and cell killing compared to 17Δ34.5. Applicant treated (**FIG. 6A**) Vero (**FIG. 6B**) SK-N-AS (**FIG. 6C**) 975A2 cells with either mock CTL, Mut-3Δ34.5, or 17Δ34.5 at different MOIs and used IncuCyte ZOOM live cell to monitor GFP positive area (top panels) and cell confluence (bottom panels) over time. N= 6 wells per condition, and n=2 measurements per well for each time point. Post virus infection (pvi). Mann-Whitney U test is used to compare time until maximum GFP area between viruses for (**FIG. 6A**). Error bars represent *SD.*

[21] FIG. 7: Mut-3Δ34.5 is more effective than 17TermA to control human neuroblastoma growth *in vivo*. Athymic nude mice with sub-q SK-N-AS tumors were intratumorally injected with either phosphate-buffered saline (PBS) control (as marked, n=8), three doses of 1e7 pfu of 17TermA (as marked, n=8) or Mut-3Δ34.5 (as marked, n=9). Kaplan–Meier survival curves were plotted. Log-rank test was used to score the statistical significance between 17TermA and Mut-3Δ34.5. $*p<0.05$.

[22] FIGS. 8A – 8C: Mut-3ΔICP6, an attenuated version of Mut-3, induces superior cytotoxicity in the human neuroblastoma cell line CHP-134 compared to oncolytic herpes virus rRp450. (FIG. 8A) maps of Mut-3ΔICP6 constructed via a CRISPR-Cas9 gene editing strategy wherein the UL39 gene that encodes ICP-6 was replaced with a CMV-driven GFP reporter cassette. MTS cell viability assays comparing the cytotoxicity profiles of Mut-3, rRp450 and Mut3-ΔICP6/D7-1 in the human neuroblastoma cell lines. Cells were plated in 96-well dishes at 4000 cells/well, incubated at 37°C overnight and then infected with each listed virus at a multiplicity of infection (MOI) of 0.001, 0.01, 0.1, and 1 infectious viral particles per cell. The assays were performed using Cell Titer96 AQuesous Non-Radioactive Cell Proliferation Assay/MTS (Promega, Madison, WI) on days 3 or 4 post

infection per the manufacturer's instructions. Each sample group was run in quadruplicate with the results presented as percent cell survival relative to uninfected controls. Error bars represent standard deviation. Statistical significance was assessed using t test. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. **FIG. 8B** SK-N-AS and **FIG. 8C** CHP-134, graphically show the results.

[23] FIGS. 9A - 9B: Mut-3ΔICP6 induces superior cytotoxicity in the murine neuroblastoma cell lines Neuro-2a & 975A2 compared to rRp450. FIGS. 9A - 9B show the results of MTS cell viability assays comparing the cytotoxicity profiles of Mut-3, rRp450 and Mut3-ΔICP6/D7-1 in the murine neuroblastoma cell lines. Cells were plated in 96-well dishes at 4000 cells/well, incubated at 37°C overnight and then infected with each listed virus at a multiplicity of infection (MOI) of 0.001, 0.01, 0.1, and 1 infectious viral particles per cell. The assays were performed using Cell Titer96 AQuesous Non-Radioactive Cell Proliferation Assay/MTS (Promega, Madison, WI) on days 3 or 4 post infection per the manufacturer's instructions. Each sample group was run in quadruplicate with the results presented as percent cell survival relative to uninfected controls. Error bars represent standard deviation. Statistical significance was assessed using t test. **$p \leq 0.01$, ***$p \leq 0.001$. **FIG. 9A:** Neuro-2a and **FIG. 9B:** 975A2.

[24] FIGS. 10A – 10B: Mut-3ΔICP6 produces a significantly higher virus yield than rRp450 in the murine neuroblastoma cell line 975A2 over 48 and 72hrs infection time periods. Murine 975A2 **(FIG. 10A)** and human SK-N-AS **(FIG. 10B)** neuroblastoma cells were plated in 12-well dishes at 2 x 10^5 cells per well, incubated at 37°C overnight, and infected with each virus in 200 µL serum-free media at MOI 0.01 for SK-N-AS cells **(FIG. 10B)** or MOI 0.5 for 975A2 cells (FIG. 10A) for 2 hours with gentle shaking every 20 minutes. The cells were washed once with PBS and covered with 1 mL of complete media. The cells and supernatants were then collected at 2, 24, 48 and 72 hours post infection, freeze-thawed three times, and serially diluted and titrated on Vero cells to determine infectious virus yield by standard plaque assay. Each sample was assayed in triplicate. Error bars represent standard deviation. Statistical significance was assessed using t test. * $p \leq 0.05$, **$p \leq 0.01$.

[25] FIGS. 11A – 11B: Mut-3Δ34.5 & Mut-3ΔICP6 are significantly less potent than the Mut-3 virus against differentiated human keratinocyte cells. Human keratinocyte (HFK) cells were grown in EpiLife Media (Cascade Biologics) supplemented with human keratinocyte growth supplement according to the manufacturer's instructions. Undifferentiated HFKs **(FIG. 11A)** were seeded into a 96-well plate at a density of 2000 cells per well and cultured overnight. The cultures were then infected with Mut-3, Mut-3Δ34.5/C8G5 or Mut-3ΔICP6 D7-1 at MOIs of 0.0004, 0.004, 0.04, 0.4, and 4. Cell survival was determined by MTS assay after 3 days of virus infection. Differentiated HFKs were produced by adding 10% FBS and 1 mmol/l $CaCl_2$ to their culture medium 24 hours after seeding and allowing them to incubate an additional 48 hours prior to infection. Differentiated cell survival **(FIG. 11B)** was determined by MTS assay after 4 days of virus infection. Each sample was assayed in quadruplicate. Error bars represent standard deviation. Statistical significance was assessed using t test. $*p \leq 0.05$, $**p \leq 0.01$.

[26] FIG. 12: Intravenous injection of 1e6 pfu of wild-type strain KOS virus is lethal to naïve Blab/c mice. Description of Safety Studies for Mut-3Δ34.5 & Mut-3ΔICP6. Applicant initiated a viral biodistribution study by injecting naïve non-tumor bearing Balb/c mice (20 male, 20 female) with 1e8 plaque-forming units (pfu) of each virus via tail vein. Applicant then sacrificed these animals at days 1, 14, 28, 56, and 85 (n = 4 per time point) and collected their peripheral blood, testes (male), ovaries (female), spleens, lungs, kidneys, hearts, lungs and brains for analysis. Applicant observed each mouse daily for the first two weeks following virus administration, and then twice weekly until their scheduled day of sacrifice. The body weights of each mouse pre-virus were recorded and weekly thereafter. In parallel, the wild-type KOS virus was administered [dosage range: 1e5 to 1e7 pfu per mouse (n=3)] to groups of mice as the positive (non-safe) control. Applicant also performed pathological analysis of the harvested organs, comparing samples obtained from mice given the mutant viruses to those given the wild-type KOS virus. Applicant also utilized plaque assays to detect the replicative potential of these viruses. Result shows the survival curves of mice receiving a single dose of 1e5, 1e6 or 1e7 pfu of wild-type KOS virus.

[27] FIGS. 13A – 13B: Naïve Balb/c mice can tolerate intravenous injection of up to 1e8 pfu of Mut-3Δ34.5(C8G5) or Mut-3ΔICP6(D7-1). Applicant completed the safety and biodistribution study and found that mice receiving up to 1e8 pfu of Mut-3Δ34.5(C8G5) or

Mut-3ΔICP6(D7-1) remained healthy via physical examination until their designated day of sacrifice (up to day 85). In female mice, plaque assays revealed the presence of infectious virus in the heart, kidney, liver, ovary and spleen only at the 24 hour time point following infection. **FIG. 13A** and **FIG. 13B**, graphically show the results from female and male mice, respectively.

[28] FIG. 14 replicating HSV can be detected in the brains, kidneys and ovaries of mice receiving 1e6 pfu or more wild-type KOS virus. FIG. 14 is a Table summarizing the results of plaque assays from the tissues of mice injected with 1e6 or 1e7 pfu of wild-type KOS virus. The mice were sacrificed 5-6 days post virus injection (pvi) after they began displaying outward signs of morbidity (kyphosis, lethargy hind limb paralysis, etc.). A "+" indicates detectable plaque(s) and a "-" indicates their absence. A gray shaded box indicates that these data are not available.

[29] FIG. 15 shows that replicating HSV can be detected in almost all the harvested tissues except lung of mice receiving 1e8 pfu of C8G5 or D7-1 24hr pvi. The table summarizing the results of plaque assays from the tissues of mice injected with MutΔ34.5 or Mut3ΔICP6 24 hours post virus infection. A "+" indicates detectable plaque(s) and a "-" indicates their absence. A gray shaded box indicates that these data are not available.

[30] FIG. 16 shows that no replicating HSV can be detected in any of the harvested tissues of mice receiving 1e8 pfu of C8G5 or D7-1 14day pvi. Table summarizing the results of plaque assays from the tissues of mice injected with MutΔ34.5 or Mut3ΔICP6 14 post virus infection. A "-" indicates no detectable plaque(s).

[31] FIG. 17 shows that no replicating HSV can be detected in any of the harvested tissues of mice receiving 1e8 pfu of C8G5 or D7-1 28day pvi. Table summarizing the results of plaque assays from the tissues of mice injected with MutΔ34.5 or Mut3ΔICP6 28 post virus infection. A "-" indicates no detectable plaque(s). A gray shaded box indicates that these data are not available.

[32] FIG. 18 is a table summarizing that no replicating HSV can be detected in any of the harvested tissues of mice receiving 1e8 pfu of C8G5 or D7-1 56day pvi. The table summarizes the results of plaque assays from the tissues of mice injected with MutΔ34.5 or Mut3ΔICP6 56 post virus infection. A "-" indicates no detectable plaque(s).

[33] FIG. 19 shows that no replicating HSV can be detected in any of the harvested tissues of mice receiving 1e8 pfu of C8G5 or D7-1 85day pvi. Table summarizing the results of qPCR and plaque assays from the tissues of mice injected with MutΔ34.5 or Mut3ΔICP6 85 post virus infection. A "-" indicates no detectable plaque(s).

[34] FIGS. 20A – 20D show the result of a study showing plaque size of Mut-3ΔgE is much smaller compare to Mut-3 or Mut-3D34.5(C8G5). FIG. 20A: Mut-3ΔgE was constructed via a CRISPR-Cas9 gene editing strategy, wherein the Us8 gene that encodes glycoprotein gE is replaced with a CMV-driven GFP reporter cassette. **FIG. 20B:** Non-syncytial GFP positive plaque phenotype of Mut-3ΔgE clone 28D5-B4 & 28D5-H1 – Vero cells were infected with serially diluted 28D5-B4 or 28D5-H1 for 2 hours and covered in overlay medium. Photos of plaques were taken 3 days pvi. **FIG. 20C:** qPCR analysis of Mut-3ΔgE clone 28D5-B4 & 28D5-H1 reveals the loss of Us8/gE. Baby hamster kidney (BHK) cells were infected with Mut-3ΔgE clone 28D5-B4 or 28D5-H1 and their lysates were harvested when ≥50% cytopathic effect was observed. 50-100ng of genomic DNA isolated from virus infected BHK cell lysate was used in qPCR reaction. Results are presented as gE/Us8 or GFP fold relative to Us8a control HSV gene. **FIG. 20D:** A comparison of plaque sizes between Mut-3ΔgE clone 28D5-B4 & 28D5-H1 to Mut-3 or Mut-3Δ34.5/C8G5, taken from a standard plaque assay performed in Vero cells. Plaque photos of Mut-3, Mut-3Δ34.5, Mut-3ΔgE clone 28D5-B4 and clone 28D5-H1 were taken 3 days pvi.

[35] FIGS. 21A – 21D provide the result of a study showing plaque size of 17ΔgE is much smaller compared to 17syn+ or 17Δ34.5. FIG. 21A: 17ΔgE was constructed via a CRISPR-Cas9 gene editing strategy, wherein the Us8 gene that encodes glycoprotein gE is replaced with a CMV-driven GFP reporter cassette. **FIG. 21B:** Non-syncytial and small GFP positive plaque phenotype of 17ΔgE clone 12G5– Vero cells were infected with serially diluted 17ΔgE 12G5 for 2 hours and covered in overlay medium. Photos of plaques were taken 3 days pvi. **FIG. 21C:** qPCR analysis of 17ΔgE clone 12G5 reveals the loss of Us8/gE. Baby hamster kidney (BHK) cells were infected with 17ΔgE clone 12G5 and the lysate was harvested when ⩾50% cytopathic effect was observed. 50-100ng of genomic DNA isolated from virus infected BHK cell lysate was used in qPCR reaction. Results are presented as gE/Us8 or GFP fold relative to ICP6 control HSV gene. **FIG. 21D:** A comparison of plaque

sizes between 17ΔgE clone 12G5 17syn+ or 17Δ34.5 clone B4, taken from a standard plaque assay performed in Vero cells. Plaque photos were taken 3 days pvi.

DETAILED DESCRIPTION

[36] Embodiments according to the present disclosure will be described more fully hereinafter. Aspects of the disclosure may, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art. The terminology used in the description herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

[37] Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. It will be further understood that terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the present application and relevant art and should not be interpreted in an idealized or overly formal sense unless expressly so defined herein. While not explicitly defined below, such terms should be interpreted according to their common meaning.

[38] The terminology used in the description herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety.

[39] The practice of the present technology will employ, unless otherwise indicated, conventional techniques of tissue culture, immunology, molecular biology, microbiology, cell biology, and recombinant DNA, which are within the skill of the art.

[40] Unless the context indicates otherwise, it is specifically intended that the various features of the invention described herein can be used in any combination. Moreover, the disclosure also contemplates that in some embodiments, any feature or combination of features set forth herein can be excluded or omitted. To illustrate, if the specification states

that a complex comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, can be omitted and disclaimed singularly or in any combination.

[41] Unless explicitly indicated otherwise, all specified embodiments, features, and terms intend to include both the recited embodiment, feature, or term and biological equivalents thereof.

[42] All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (-) by increments of 1.0 or 0.1, as appropriate, or alternatively by a variation of +/- 15 %, or alternatively 10%, or alternatively 5%, or alternatively 2%. It is to be understood, although not always explicitly stated, that all numerical designations are preceded by the term "about". It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

[43] As used herein, the term "comparable" refers to having a level same with that of the reference or within a variation of +/- 50%, or alternatively 45%, or alternatively 40%, or alternatively 35%, or alternatively 30%, or alternatively 25%, or alternatively 20%, or alternatively 15 %, or alternatively 10%, or alternatively 5%, or alternatively 2% compared to the reference level

[44] Throughout this disclosure, various publications, patents and published patent specifications are referenced by an identifying citation or by an Arabic numeral. The full citation for the publications identified by an Arabic numeral are found immediately preceding the claims. The disclosures of these publications, patents and published patent specifications are hereby incorporated by reference into the present disclosure in their entirety to more fully describe the state of the art to which this invention pertains.

Definitions

[45] The practice of the present technology will employ, unless otherwise indicated, conventional techniques of organic chemistry, pharmacology, immunology, molecular biology, microbiology, cell biology and recombinant DNA, which are within the skill of the art. See, *e.g.*, Sambrook, Fritsch and Maniatis, Molecular Cloning: A Laboratory Manual,

2nd edition (1989); Current Protocols In Molecular Biology (F. M. Ausubel, *et al.* eds., (1987)); the series Methods in Enzymology (Academic Press, Inc.): PCR 2: A Practical Approach (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) Antibodies, a Laboratory Manual, and Animal Cell Culture (R.I. Freshney, ed. (1987)).

[46] As used in the description of the invention and the appended claims, the singular forms "a," "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise.

[47] As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but do not exclude others. As used herein, the transitional phrase consisting essentially of (and grammatical variants) is to be interpreted as encompassing the recited materials or steps and those that do not materially affect the basic and novel characteristic(s) of the recited embodiment. These features are recited in the method embodiments. Thus, the term "consisting essentially of" as used herein should not be interpreted as equivalent to "comprising." "Consisting of" shall mean excluding more than trace elements of other ingredients and substantial method steps for administering the compositions disclosed herein. Aspects defined by each of these transition terms are within the scope of the present disclosure.

[48] The term "about," as used herein when referring to a measurable value such as an amount or concentration and the like, is meant to encompass variations of 20%, 10%, 5%, 1 %, 0.5%, or even 0.1 % of the specified amount.

[49] The terms or "acceptable," "effective," or "sufficient" when used to describe the selection of any components, ranges, dose forms, etc. disclosed herein intend that said component, range, dose form, etc. is suitable for the disclosed purpose.

[50] As used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

[51] The term "cell" as used herein may refer to either a prokaryotic or eukaryotic cell, optionally obtained from a subject or a commercially available source.

[52] "Eukaryotic cells" all of the life kingdoms except monera. They can be easily distinguished through a membrane-bound nucleus. Animals, plants, fungi, and protists are eukaryotes or organisms whose cells are organized into complex structures by internal membranes and a cytoskeleton. The most characteristic membrane-bound structure is the nucleus. Unless specifically recited, the term "host" includes a eukaryotic host, including, for example, yeast, higher plant, insect and mammalian cells. Non-limiting examples of eukaryotic cells or hosts include simian, bovine, porcine, murine, rat, avian, reptilian and human, e.g., HEK293 cells and 293T cells.

[53] "Prokaryotic cells" that usually lack a nucleus or any other membrane-bound organelles and are divided into two domains, bacteria and archaea. In addition to chromosomal DNA, these cells can also contain genetic information in a circular loop called on episome. Bacterial cells are very small, roughly the size of an animal mitochondrion (about 1-2 μm in diameter and 10 μm long). Prokaryotic cells feature three major shapes: rod shaped, spherical, and spiral. Instead of going through elaborate replication processes like eukaryotes, bacterial cells divide by binary fission. Examples include but are not limited to *Bacillus* bacteria, *E. coli* bacterium, and *Salmonella* bacterium.

[54] The term "encode" as it is applied to nucleic acid sequences refers to a polynucleotide which is said to "encode" a polypeptide if, in its native state or when manipulated by methods well known to those skilled in the art, can be transcribed and/or translated to produce the mRNA for the polypeptide and/or a fragment thereof. The antisense strand is the complement of such a nucleic acid, and the encoding sequence can be deduced therefrom.

[55] The terms "equivalent" or "biological equivalent" are used interchangeably when referring to a particular molecule, biological, or cellular material and intend those having minimal homology while still maintaining desired structure or functionality. Non-limiting examples of equivalent polypeptides, include a polypeptide having at least 60%, or alternatively at least 65%, or alternatively at least 70%, or alternatively at least 75%, or alternatively 80%, or alternatively at least 85%, or alternatively at least 90%, or alternatively at least 95% identity thereto or for polypeptide sequences, or a polypeptide which is encoded by a polynucleotide or its complement that hybridizes under conditions of high stringency to a polynucleotide encoding such polypeptide sequences. Conditions of high stringency are

described herein and incorporated herein by reference. Alternatively, an equivalent thereof is a polypeptide encoded by a polynucleotide or a complement thereto, having at least 70%, or alternatively at least 75%, or alternatively 80%, or alternatively at least 85%, or alternatively at least 90%, or alternatively at least 95% identity, or at least 97% sequence identity to the reference polynucleotide, e.g., the wild-type polynucleotide. In one aspect, the equivalent polypeptide or polynucleotide has the same or substantially similar biological function as the reference polypeptide or polynucleotide, respectively, e.g., cytolytic function, anti-tumor, anti-metastatic, or anti-cancer function, as determined by the appropriate cell assay or animal model as described herein.

[56] Non-limiting examples of equivalent polypeptides, include a polynucleotide having at least 60%, or alternatively at least 65%, or alternatively at least 70%, or alternatively at least 75%, or alternatively 80%, or alternatively at least 85%, or alternatively at least 90%, or alternatively at least 95%, or alternatively at least 97%, identity to a reference polynucleotide. An equivalent also intends a polynucleotide or its complement that hybridizes under conditions of high stringency to a reference polynucleotide.

[57] A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) having a certain percentage (for example, 80%, 85%, 90%, or 95%) of "sequence identity" to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. The alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in Current Protocols in Molecular Biology (Ausubel et al., eds. 1987) Supplement 30, section 7.7.18, Table 7.7.1. In certain embodiments, default parameters are used for alignment. A non-limiting exemplary alignment program is BLAST, using default parameters. In particular, exemplary programs include BLASTN and BLASTP, using the following default parameters: Genetic code=standard; filter=none; strand=both; cutoff=60; expect=10; Matrix=BLOSUM62; Descriptions=50 sequences; sort by=HIGH SCORE; Databases=non-redundant, GenBank+EMBL+DDBJ+PDB+GenBank CDS translations+SwissProtein+SPupdate+PIR. Details of these programs can be found at the following Internet address: ncbi.nlm.nih.gov/cgi-bin/BLAST. Sequence identity and percent identity can be determined by incorporating them into clustalW (available at the web address: genome.jp/tools/clustalw/, last accessed on Jan. 13, 2017).

[58] "Homology" or "identity" or "similarity" refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence that may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. An "unrelated" or "non-homologous" sequence shares less than 40% identity, or alternatively less than 25% identity, with one of the sequences of the present disclosure.

[59] "Homology" or "identity" or "similarity" can also refer to two nucleic acid molecules that hybridize under stringent conditions.

[60] "Hybridization" refers to a reaction in which one or more polynucleotides react to form a complex that is stabilized via hydrogen bonding between the bases of the nucleotide residues. The hydrogen bonding may occur by Watson-Crick base pairing, Hoogstein binding, or in any other sequence-specific manner. The complex may comprise, or alternatively consist essentially of, or yet further consist of comprise, or alternatively consist essentially of, or yet further consist of two strands forming a duplex structure, three or more strands forming a multi-stranded complex, a single self-hybridizing strand, or any combination of these. A hybridization reaction may constitute a step in a more extensive process, such as the initiation of a PCR reaction, or the enzymatic cleavage of a polynucleotide by a ribozyme.

[61] Examples of stringent hybridization conditions include: incubation temperatures of about 25° C. to about 37° C.; hybridization buffer concentrations of about 6×SSC to about 10×SSC; formamide concentrations of about 0% to about 25%; and wash solutions from about 4×SSC to about 8×SSC. Examples of moderate hybridization conditions include: incubation temperatures of about 40° C. to about 50° C.; buffer concentrations of about 9×SSC to about 2×SSC; formamide concentrations of about 30% to about 50%; and wash solutions of about 5×SSC to about 2×SSC. Examples of high stringency conditions include: incubation temperatures of about 55° C. to about 68° C.; buffer concentrations of about 1×SSC to about 0.1×SSC; formamide concentrations of about 55% to about 75%; and wash solutions of about 1×SSC, 0.1×SSC, or deionized water. In general, hybridization incubation

times are from 5 minutes to 24 hours, with 1, 2, or more washing steps, and wash incubation times are about 1, 2, or 15 minutes. SSC is 0.15 M NaCl and 15 mM citrate buffer. It is understood that equivalents of SSC using other buffer systems can be employed.

[62] As used herein, "expression" refers to the process by which polynucleotides are transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently being translated into peptides, polypeptides, or proteins. If the polynucleotide is derived from genomic DNA, expression may include splicing of the mRNA in a eukaryotic cell.

[63] A "gene" refers to a polynucleotide containing at least one open reading frame (ORF) that is capable of encoding a particular polypeptide or protein after being transcribed and translated. A "gene product" or alternatively a "gene expression product" refers to the amino acid (e.g., peptide or polypeptide) generated when a gene is transcribed and translated.

[64] "Under transcriptional control" is a term well understood in the art and indicates that transcription of a polynucleotide sequence, usually a DNA sequence, depends on its being operatively linked to an element which contributes to the initiation of, or promotes, transcription. "Operatively linked" intends the polynucleotides are arranged in a manner that allows them to function in a cell. In one aspect, this invention provides promoters operatively linked to the downstream sequences, e.g., HSV virulence genes or their mutants.

[65] The term "encode" as it is applied to polynucleotides refers to a polynucleotide which is said to "encode" a polypeptide if, in its native state or when manipulated by methods well known to those skilled in the art, it can be transcribed and/or translated to produce the mRNA for the polypeptide and/or a fragment thereof. The antisense strand is the complement of such a nucleic acid, and the encoding sequence can be deduced therefrom.

[66] The term "isolated" as used herein refers to molecules or biologicals or cellular materials being substantially free from other materials.

[67] As used herein, the term "functional" may be used to modify any molecule, biological, or cellular material to intend that it accomplishes a particular, specified effect.

[68] As used herein, the terms "nucleic acid sequence" and "polynucleotide" are used interchangeably to refer to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to,

single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising, or alternatively consisting essentially of, or yet further consisting of purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases.

[69] The term "wild-type" refers to a gene or gene product having characteristics of that gene or gene product when isolated from a naturally occurring source. In some embodiments, the wild type genes or gene products, even for one viral strain, contain slight different sequences.

[70] The term "mutant" refers to a gene or gene product which displays modifications in sequence and or functional properties (i.e., altered characteristics) when compared to the wild-type gene or gene product or the gene or gene product from other mutant strain(s). In one embodiment, the other mutant strain comprise a 17TermA or an rR450 strain.

[71] The term “mutation” refers to a DNA sequence variation from a wild type or other mutant strain (s). A mutation produces or does not produce a function property in an organism. There are multiple types of mutations, including but not limited to an insertion, a deletion, a truncation, a frameshift, a substitution, or a point mutation.

[72] The term “point mutation” refers to a mutation with a single nucleotide base change, insertion, or deletion of the genetic material, DNA or RNA.

[73] “Deletion” refers to a mutation in which a part of chromosome or a sequence of DNA is missing.

[74] “Frameshift” refers to a mutation caused by indels (insertions or deletions) of a number of nucleotides in a DNA sequence that is not divisible by three.

[75] “Substitution” refers to a mutation with a substitution of one or a few nucleotides of a gene.

[76] “Truncation” refers to a mutation with elimination of the N- or C-terminal portion of a protein by proteolysis or manipulation of the structural gene, or premature termination of protein elongation due to the presence of a termination codon in its structural gene as a result of a nonsense mutation.

[77] In some embodiment, the mutation is a nonsynonymous mutation. The term "nonsynonymous mutation" refers to a mutation that alters the amino acid sequence of a protein, which is contrasted with a synonymous mutation that do not alter amino acid sequences.

[78] The term "promoter" as used herein refers to any sequence that regulates the expression of a coding sequence, such as a gene. Promoters may be constitutive, inducible, repressible, or tissue-specific, for example. A "promoter" is a control sequence that is a region of a polynucleotide sequence at which initiation and rate of transcription are controlled. It may contain genetic elements at which regulatory proteins and molecules may bind such as RNA polymerase and other transcription factors. Non-limiting exemplary promoters include Rous sarcoma virus (RSV) LTR promoter (optionally with the RSV enhancer), a cytomegalovirus (CMV) promoter, an SV40 promoter, a dihydrofolate reductase promoter, a β-actin promoter, a phosphoglycerol kinase (PGK) promoter, a U6 promoter, or an EF1 promoter. In some embodiments, the promoter is a chicken β-actin ("CBA") promoter.

[79] Additional non-limiting exemplary promoters with certain target specificity are provided herein below including but not limited to CMV, EF1a, SV40, PGK1 (human or mouse), P5, Ubc, human beta actin, CAG, TRE, UAS, Ac5, Polyhedrin, CaMKIIa, Gal1, TEF1, GDS, ADH1, CaMV35S, Ubi, H1, U6, and Alpha-1-antitrypsin. Synthetically-derived promoters may be used for ubiquitous or tissue specific expression. Further, virus-derived promoters, some of which are noted above, may be useful in the methods disclosed herein, e.g., CMV, HIV, adenovirus, and AAV promoters. In some embodiments, the promoter is coupled to an enhancer to increase the transcription efficiency.

[80] An enhancer is a regulatory element that increases the expression of a target sequence. A "promoter/enhancer" is a polynucleotide that contains sequences capable of providing both promoter and enhancer functions. For example, the long terminal repeats of retroviruses contain both promoter and enhancer functions. The enhancer/promoter may be "endogenous" or "exogenous" or "heterologous." An "endogenous" enhancer/promoter is one which is naturally linked with a given gene in the genome. An "exogenous" or "heterologous" enhancer/promoter is one which is placed in juxtaposition to a gene by means of genetic

manipulation (i.e., molecular biological techniques) such that transcription of that gene is directed by the linked enhancer/promoter.

[81] The term "tumor-specific promoter or tissue-specific promoter" as used herein means a promoter permitting expression of a gene, which is under control of the promoter, specifically in a desired tumor cell or tissue. Non-limiting examples of tissue-specific promoters that can be used in the invention include a prostate-specific antigen (PSA) promoter, a prostate-specific membrane antigen (PSMA) promoter, a casein promoter, an IgG promoter, a chorionic embryonic antigen promoter, an elastase promoter, a porphobilinogen deaminase promoter, an insulin promoter, a growth hormone factor promoter, an acetylcholine receptor promoter, an alcohol dehydrogenase promoter, and an α or β globin promoter.

[82] Non-limiting examples of tumor-specific promoters to be used in the present invention include the telomerase reverse transcriptase promoter, the glial fibrillary acidic protein promoter, an E2F promoter; a survivin promoter, a COX-2 promoter, an EGD-2 promoter; an ELF-1 promoter; a hypoxia-specific promoter; a carcinoembryonic antigen promoter, and the stromelysin 3 promoter.

[83] The term "cryopreservative" refers to a compound or material that is capable of, protecting the one or more tissues, virus, or other biological agents from being damaged or compromised. Examples of cryopreservatives include, but are not limited to, chondroitin sulfate, glycosaminoglycan dimethylsulfoxide, cell penetrating organic solutes, polysaccharides, glycerol, Dulbecco's minimum essential medium (DMEM), glutamine, D-glucose, sodium pyruvate, fetal calf serum, papaverine, DMSO, glycerol, trehalose, KH2PO4, K2HPO4, KCl, mannitol, NaHCO3, sodium ascorbate, 1,2-propanediol, formamide, 2,3-butanediol, probuchol, curcumin and mixtures thereof.

[84] The term "protein", "peptide" and "polypeptide" are used interchangeably and in their broadest sense to refer to a compound of two or more subunits of amino acids, amino acid analogs or peptidomimetics. The subunits may be linked by peptide bonds. In another aspect, the subunit may be linked by other bonds, e.g., ester, ether, etc. A protein or peptide must contain at least two amino acids and no limitation is placed on the maximum number of amino acids which may comprise, or alternatively consist essentially of, or yet further consist

of comprise, or alternatively consist essentially of, or yet further consist of a protein's or peptide's sequence. As used herein the term "amino acid" refers to either natural and/or unnatural or synthetic amino acids, including glycine and both the D and L optical isomers, amino acid analogs and peptidomimetics.

[85] As used herein, the term "vector" refers to a non-chromosomal nucleic acid comprising, or alternatively consisting essentially of, or yet further consisting of an intact replicon such that the vector may be replicated when placed within a cell, for example by a process of transformation. Vectors may be viral or non-viral. Viral vectors include retroviruses, adenoviruses, herpes simplex virus ("HSV"), baculoviruses, modified baculoviruses, papovirus, or otherwise modified naturally occurring viruses. Exemplary non-viral vectors for delivering nucleic acid include naked DNA; DNA complexed with cationic lipids, alone or in combination with cationic polymers; anionic and cationic liposomes; DNA-protein complexes and particles comprising, or alternatively consisting essentially of, or yet further consisting of DNA condensed with cationic polymers such as heterogeneous polylysine, defined-length oligopeptides, and polyethylene imine, in some cases contained in liposomes; and the use of ternary complexes comprising, or alternatively consisting essentially of, or yet further consisting of a virus and polylysine-DNA.

[86] A "viral vector" is defined as a recombinantly produced virus or viral particle that comprises, alternatively consists essentially of, or yet further consists of a polynucleotide to be delivered into a host cell, either in vivo, ex vivo or in vitro. Examples of viral vectors include retroviral vectors, HSV vectors, AAV vectors, lentiviral vectors, adenovirus vectors, alphavirus vectors and the like. Alphavirus vectors, such as Semliki Forest virus-based vectors and Sindbis virus-based vectors, have also been developed for use in gene therapy and immunotherapy. See, Schlesinger and Dubensky (1999) Curr. Opin. Biotechnol. 5:434-439 and Ying, et al. (1999) Nat. Med. 5(7):823-827.

[87] In another embodiment, the expression of an HSV virulence protein (*e.g.*, wild type or mutatnts) is regulated by a promoter that is an inducible promoter. In a specific related embodiment, the promoter is an inducible tetracycline promoter. The Tet-Off and Tet-On Gene Expression Systems give researchers ready access to the regulated, high-level gene expression systems described as Tet-Off and Tet-On. In the Tet-Off system, gene expression

is turned on when tetracycline (Tc) or doxycycline (Dox; a Tc derivative) is removed from the culture medium. In contrast, expression is turned on in the Tet-On system by the addition of Dox. Both systems permit gene expression to be tightly regulated in response to varying concentrations of Tc or Dox. Maximal expression levels in Tet systems are very high and compare favorably with the maximal levels obtainable from strong, constitutive mammalian promoters such as CMV. Unlike other inducible mammalian expression systems, gene regulation in the Tet Systems is highly specific, so interpretation of results is not complicated by pleiotropic effects or nonspecific induction. In *E. coli*, the Tet repressor protein (TetR) negatively regulates the genes of the tetracycline-resistance operon on the Tn10 transposon. TetR blocks transcription of these genes by binding to the tet operator sequences (tetO) in the absence of Tc. TetR and tetO provide the basis of regulation and induction for use in mammalian experimental systems. In the Tet-On system, the regulatory protein is based on a "reverse" Tet repressor (rTetR) which was created by four amino acid changes in TetR (Hillen & Berens, Mechanisms underlying expression of Tn10 encoded tetracycline resistance. Annu Rev Microbiol. 1994;48:345-69; Gossen et al., Transcriptional activation by tetracyclines in mammalian cells. Science. 1995 Jun 23;268(5218):1766-9). The resulting protein, rtTA (reverse tTA also referred to tetracycline activator protein), is encoded by the pTet-On regulator plasmid.

[88] In a related embodiment, the vector further comprises, or alternatively consists essentially of, or yet further consists of a nucleic acid encoding a tetracycline activator protein; and a promoter that regulates expression of the tetracycline activator protein.

[89] Other inducible systems useful in vectors, isolated cells, viral packaging systems, and methods described herein include regulation by ecdysone, by estrogen, progesterone, chemical inducers of dimerization, and isopropyl-beta-D1-thiogalactopyranoside (IPTG).

[90] As used herein, the term “recombinant expression system” or “recombinant vector” refers to a genetic construct or constructs for the expression of certain genetic material formed by recombination.

[91] A “gene delivery vehicle” is defined as any molecule that can carry inserted polynucleotides into a host cell. Examples of gene delivery vehicles are liposomes, micelles biocompatible polymers, including natural polymers and synthetic polymers; lipoproteins;

polypeptides; polysaccharides; lipopolysaccharides; artificial viral envelopes; metal particles; and bacteria, or viruses, such as baculovirus, adenovirus and retrovirus, bacteriophage, cosmid, plasmid, fungal vectors and other recombination vehicles typically used in the art which have been described for expression in a variety of eukaryotic and prokaryotic hosts, and may be used for gene therapy as well as for simple protein expression.

[92] A polynucleotide disclosed herein can be delivered to a cell or tissue using a gene delivery vehicle. "Gene delivery," "gene transfer," "transducing," and the like as used herein, are terms referring to the introduction of an exogenous polynucleotide (sometimes referred to as a "transgene") into a host cell, irrespective of the method used for the introduction. Such methods include a variety of well-known techniques such as vector-mediated gene transfer (by, e.g., viral infection/transfection, or various other protein-based or lipid-based gene delivery complexes) as well as techniques facilitating the delivery of "naked" polynucleotides (such as electroporation, "gene gun" delivery and various other techniques used for the introduction of polynucleotides). The introduced polynucleotide may be stably or transiently maintained in the host cell. Stable maintenance typically requires that the introduced polynucleotide either contains an origin of replication compatible with the host cell or integrates into a replicon of the host cell such as an extrachromosomal replicon (e.g., a plasmid) or a nuclear or mitochondrial chromosome. A number of vectors are known to be capable of mediating transfer of genes to mammalian cells, as is known in the art and described herein.

[93] A "plasmid" is an extra-chromosomal DNA molecule separate from the chromosomal DNA which is capable of replicating independently of the chromosomal DNA. In many cases, it is circular and double-stranded. Plasmids provide a mechanism for horizontal gene transfer within a population of microbes and typically provide a selective advantage under a given environmental state. Plasmids may carry genes that provide resistance to naturally occurring antibiotics in a competitive environmental niche, or alternatively the proteins produced may act as toxins under similar circumstances.

[94] "Plasmids" used in genetic engineering are called "plasmid vectors". Many plasmids are commercially available for such uses. The gene to be replicated is inserted into copies of a plasmid containing genes that make cells resistant to particular antibiotics and a multiple

cloning site (MCS, or polylinker), which is a short region containing several commonly used restriction sites allowing the easy insertion of DNA fragments at this location. Another major use of plasmids is to make large amounts of proteins. In this case, researchers grow bacteria containing a plasmid harboring the gene of interest. Just as the bacterium produces proteins to confer its antibiotic resistance, it can also be induced to produce large amounts of proteins from the inserted gene.

[95] The term "herpes simplex virus" or "HSV" as used herein means a herpes simplex virus that produces the effect of the present invention, which includes a wild type or mutant herpes simplex virus. In one embodiment, the mutant non-natural HSV is obtained by mutating or modifying any of the genes of wild-type HSV or by inserting any of exogenous genes. The serum type of HSV comprises, alternatively consists essentially of, or yet further consists of a type 1 HSV (or HSV-1) or a type 2 HSV (or HSV-2). The HSV-1 is an enveloped, double-stranded DNA virus. In one embodiment, the HSV-1 can infect a human cell. In another embodiment, a sequence, a gene or multiple genes can be incorporated to the HSV-1. The size of incorporated sequence can be approximate 1 base, 5 bases, 10 bases, 100 bases, 1kb, 10 kb, 100 kb, or 150 kb. HSV-1 can induce cell lysis at a relatively low multiplicity of infection (MOI), and its proliferation can be inhibited by anti-viral drugs. In one embodiment, the HSV viral DNA stays outside the chromosomes without being incorporated into the genome of host cells. The HSV-1 can encompass a variety of strains (e.g., KOS and McKrae). See Wang et al., (2013) Virus Res. 173(2):436–440. In one embodiment, the HSV-1 is an HSV-1 KOS strain. In another embodiment, the HSV-1 is an HSV-1 McKrae strain.

[96] There are several HSV mutants, for example, 17TermA HSV and rRp450 HSV. The term "17TermA HSV" refers to mutant HSV-1 virus that comprises the entire ICP34.5 gene, but with a termination codon inserted before 100 bp of coding region, resulting in early termination of protein expression and expression of a 30 amino acid truncated protein. The 17TermA HSV mutant displays a growth defect because of the truncated ICP34.5 protein. See Orvedahl et al., (2007) Cell Host & Microbe, 1:1, 23-25. The term "rRp450" refers to an attenuated herpes simplex 1 vector deficient in the viral-encoded ribonucleotide reductase or ICP6. See Aghi M et al., (1999) *Cancer Res.*, 59(16):3861-5.

[97] The HSV genome encodes multiple virulence proteins, which include but are not limited to glycoprotein E ("gE"), Infected Cell Protein 0 ("ICP0"), Infected Cell Protein 6 ("ICP6"), DNA packaging terminase subunit 1, Infected Cell Protein 8 ("ICP8"), and Infected Cell Protein 34.5 ("ICP34.5"). An exemplary HSV1 genome can be found at NCBI Reference Sequence: NC_001806.2, last accessed on March 13, 2020.

[98] The term "gE-encoding gene" refers to a gene or its DNA fragment encoding a gE protein. An exemplary gE-encoding gene can be identified at positions 33-2555 of the HSV-1 genome sequence at NCBI Reference Sequence: NC_001806.2. The term "ICP6 protein" refers to an infected cell protein 6 encoded by the HSV genome. ICP6 is a subunit of ribonucleotide reductase ("RR") and a key enzyme for nucleotide metabolism and viral DNA synthesis in non-dividing cells.

[99] A "dysfunctional" protein refers to a protein that has an impaired or no function of the original protein. In one embodiment, a dysfunctional protein is caused by deletion or substitution in the coding sequences. For example, with a dysfunctional ICP6 gene, be deletion or inactivation, HSV cannot replicate in normal non-dividing cells. In actively dividing cells with increased RR activity, however, the deficient enzyme activity of the virus is compensated, enabling the virus to replicate. The DNA and amino acid sequences of ICP 34.5 are provided in SEQ ID Nos: 1, 2, and 5-10. The DNA and amino acid sequences of gE are provided in SEQ ID Nos: 12-19. The DNA and amino acid sequences of ICP 0 are provided in SEQ ID Nos: 20-26. The DNA and amino acid sequences of DNA packaging terminase subunit 1 are provided in SEQ ID Nos: 35-42. The DNA and amino acid sequences of ICP 8 are provided in SEQ ID Nos: 27-34. The DNA and amino acid sequences of ICP 6 are provided in SEQ ID Nos: 43-50.

[100] The term "ICP0-encoding gene" refers to a gene or its DNA fragment encoding an ICP0 protein. Exemplary DNA and amino acid sequences of ICP 0 are provided in SEQ ID Nos: 20-26. The term "DNA packaging terminase subunit 1-encoding gene" refers to a gene or its DNA fragment encoding a DNA packaging terminase subunit 1 protein or peptide. Exemplary DNA and amino acid sequences of DNA packaging terminase subunit 1 are provided in SEQ ID Nos: 35-42. The term "ICP 8-encoding gene" refers to a gene or its DNA fragment encoding an ICP 8 protein. Exemplary DNA and amino acid sequences of

ICP 8 are provided in SEQ ID Nos: 27-34. The term "ICP 34.5-encoding gene" refers to a gene or its DNA fragment encoding an ICP 34.5 protein. Exemplary DNA and amino acid sequences of ICP 34.5 are provided in SEQ ID Nos: 1, 2, and 5-10. The term "glycoprotein E ("gE")-encoding gene" refers to a gene or its DNA fragment encoding an gE protein. Exemplary DNA and amino acid sequences of gE are provided in SEQ ID Nos: 12-19.

[101] The term "deletion or inactivation of a gene" means deletion of the whole or portion of the gene or suppression of expression of the gene through substitution of some bases, modification, insertion of an unnecessary sequence or the like. The deletion or inactivation of the HSV gene (*e.g.*, gE, ICP0, and ICP8) can be conducted by those skilled in the art in a known method or a method based thereon. For example, a method using homologous recombination can be employed. For example, it is possible to divide and inactivate the HSV gene by cloning a DNA fragment containing a portion of the HSV gene and a sequence unrelated to the HSV gene in a suitable plasmid vector and then introducing it into HSV to cause homologous recombination in some region of the HSV gene. Alternatively, the mutation or deletion of an HSV gene can be caused by spontaneous mutation in the viral passage.

[102] In aspects where gene transfer is mediated by a DNA viral vector, such as an herpes simplex virus, a vector construct refers to the polynucleotide comprising, or alternatively consisting essentially of, or yet further consisting of the viral genome or part thereof, and a transgene. Thus, in one aspect, the non-natural HSV further comprises a transgene coding for a therapeutic polynucleotide or protein.

[103] Vectors that contain both a promoter and a cloning site into which a polynucleotide can be operatively linked are well known in the art. Such vectors are capable of transcribing RNA *in vitro* or *in vivo*, and are commercially available from sources such as Agilent Technologies (Santa Clara, Calif.) and Promega Biotech (Madison, Wis.). In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5′ and/or 3′ untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus

ribosome binding sites can be inserted immediately 5′ of the start codon to enhance expression.

[104] Gene delivery vehicles also include DNA/liposome complexes, micelles and targeted viral protein-DNA complexes. Liposomes that also comprise, or alternatively consist essentially of, or yet further consist of comprise, or alternatively consist essentially of, or yet further consist of a targeting antibody or fragment thereof can be used in the methods disclosed herein. In addition to the delivery of polynucleotides to a cell or cell population, direct introduction of the proteins described herein to the cell or cell population can be done by the non-limiting technique of protein transfection, alternatively culturing conditions that can enhance the expression and/or promote the activity of the proteins disclosed herein are other non-limiting techniques.

[105] As used herein, the term “signal peptide” or “signal polypeptide” intends an amino acid sequence usually present at the N-terminal end of newly synthesized secretory or membrane polypeptides or proteins. It acts to direct the polypeptide to a specific cellular location, *e.g.* across a cell membrane, into a cell membrane, or into the nucleus. In some embodiments, the signal peptide is removed following localization. Examples of signal peptides are well known in the art. Non-limiting examples are those described in U.S. Patent Nos. 8,853,381, 5,958,736, and 8,795,965.

[106] In one aspect, the HSV are detectably labeled. As used herein, the term "label" intends a directly or indirectly detectable compound or composition that is conjugated directly or indirectly to the composition to be detected, *e.g.*, polynucleotide or protein such as an antibody so as to generate a "labeled" composition. The term also includes sequences conjugated to the polynucleotide that will provide a signal upon expression of the inserted sequences, such as green fluorescent protein (GFP) and the like. The label may be detectable by itself (*e.g.*, radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable. The labels can be suitable for small scale detection or more suitable for high-throughput screening. As such, suitable labels include, but are not limited to radioisotopes, fluorochromes, chemiluminescent compounds, dyes, and proteins, including enzymes. The label may be simply detected, or it may be quantified. A response that is simply detected

generally comprises, alternatively consists essentially of, or yet further consists of a response whose existence merely is confirmed, whereas a response that is quantified generally comprises, alternatively consists essentially of, or yet further consists of a response having a quantifiable *(e.g.*, numerically reportable) value such as an intensity, polarization, and/or other property. In luminescence or fluorescence assays, the detectable response may be generated directly using a luminophore or fluorophore associated with an assay component actually involved in binding, or indirectly using a luminophore or fluorophore associated with another (*e.g.*, reporter or indicator) component.

[107] Examples of luminescent labels that produce signals include but are not limited to bioluminescence and chemiluminescence. Detectable luminescence response generally comprises, alternatively consists essentially of, or yet further consists of a change in, or an occurrence of, a luminescence signal. Suitable methods and luminophores for luminescent labeling assay components are known in the art and described for example in Haugland, Richard P. (1996) Handbook of Fluorescent Probes and Research Chemicals (6th ed.). Examples of luminescent probes include, but are not limited to, aequorin and luciferases.

[108] Examples of suitable fluorescent labels include, but are not limited to, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malacite green, stilbene, Lucifer Yellow, Cascade Blue.TM., and Texas Red. Other suitable optical dyes are described in the Haugland, Richard P. (1996) Handbook of Fluorescent Probes and Research Chemicals (6th ed.).

[109] In another aspect, the fluorescent label is functionalized to facilitate covalent attachment to a cellular component present in or on the surface of the cell or tissue such as a cell surface marker. Suitable functional groups, including, but not are limited to, isothiocyanate groups, amino groups, haloacetyl groups, maleimides, succinimidyl esters, and sulfonyl halides, all of which may be used to attach the fluorescent label to a second molecule. The choice of the functional group of the fluorescent label will depend on the site of attachment to either a linker, the agent, the marker, or the second labeling agent.

[110] Attachment of the fluorescent label may be either directly to the cellular component or compound or alternatively, can by via a linker. Suitable binding pairs for use in indirectly

linking the fluorescent label to the intermediate include, but are not limited to, antigens/antibodies, *e.g.*, rhodamine/anti-rhodamine, biotin/avidin and biotin/strepavidin.

[111] The phrase "solid support" refers to non-aqueous surfaces such as "culture plates" "gene chips" or "microarrays." Such gene chips or microarrays can be used for diagnostic and therapeutic purposes by a number of techniques known to one of skill in the art. In one technique, oligonucleotides are attached and arrayed on a gene chip for determining the DNA sequence by the hybridization approach, such as that outlined in U.S. Patent Nos. 6,025,136 and 6,018,041. The polynucleotides of this invention can be modified to probes, which in turn can be used for detection of a genetic sequence. Such techniques have been described, for example, in U.S. Patent Nos. 5,968,740 and 5,858,659. A probe also can be attached or affixed to an electrode surface for the electrochemical detection of nucleic acid sequences such as described by Kayem et al. U.S. Patent No. 5,952,172 and by Kelley et al. (1999) Nucleic Acids Res. 27:4830-4837.

[112] A "composition" is intended to mean a combination of active polypeptide, polynucleotide or antibody and another compound or composition, inert (*e.g.*, a detectable label) or active (*e.g.*, a gene delivery vehicle).

[113] A "pharmaceutical composition" is intended to include the combination of an active polypeptide, polynucleotide or antibody with a carrier, inert or active such as a solid support, making the composition suitable for diagnostic or therapeutic use *in vitro*, *in vivo* or *ex vivo*.

[114] As used herein, the term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see Martin (1975) Remington's Pharm. Sci., 15th Ed. (Mack Publ. Co., Easton).

[115] A "subject" of diagnosis or treatment is a cell or an animal such as a mammal, or a human. A subject is not limited to a specific species and includes non-human animals subject to diagnosis or treatment and are those subject to infections or animal models, for example, simians, murines, such as, rats, mice, chinchilla, canine, such as dogs, cats, leporids, such as

rabbits, livestock, sport animals, and pets. Human patients are included within the term as well.

[116] The term "tissue" is used herein to refer to tissue of a living or deceased organism or any tissue derived from or designed to mimic a living or deceased organism. The tissue may be healthy, diseased, and/or have genetic mutations. The biological tissue may include any single tissue (e.g., a collection of cells that may be interconnected) or a group of tissues making up an organ or part or region of the body of an organism. The tissue may comprise, or alternatively consist essentially of, or yet further consist of comprise, or alternatively consist essentially of, or yet further consist of a homogeneous cellular material or it may be a composite structure such as that found in regions of the body including the thorax which for instance can include lung tissue, skeletal tissue, and/or muscle tissue. Exemplary tissues include, but are not limited to those derived from liver, lung, thyroid, skin, pancreas, blood vessels, bladder, kidneys, brain, biliary tree, duodenum, abdominal aorta, iliac vein, heart and intestines, including any combination thereof.

[117] As used herein, "treating" or "treatment" of a disease in a subject refers to (1) preventing the symptoms or disease from occurring in a subject that is predisposed or does not yet display symptoms of the disease; (2) inhibiting the disease or arresting its development; or (3) ameliorating or causing regression of the disease or the symptoms of the disease. As understood in the art, "treatment" is an approach for obtaining beneficial or desired results, including clinical results. For the purposes of the present technology, beneficial or desired results can include one or more, but are not limited to, alleviation or amelioration of one or more symptoms, diminishment of extent of a condition (including a disease), stabilized (i.e., not worsening) state of a condition (including disease), delay or slowing of condition (including disease), progression, amelioration or palliation of the condition (including disease), states and remission (whether partial or total), whether detectable or undetectable.

[118] As used herein the term "effective amount" intends to mean a quantity sufficient to achieve a desired effect. In the context of therapeutic or prophylactic applications, the effective amount will depend on the type and severity of the condition at issue and the characteristics of the individual subject, such as general health, age, sex, body weight, and

tolerance to pharmaceutical compositions. In the context of gene therapy, in some embodiments the effective amount is the amount sufficient to result in regaining part or full function of a gene that is deficient in a subject. In one aspect, an effective amount is an amount to provide a multiplicity of infection (MOI) of from 0.001 to 1 infectious viral particles per cell in ranges in between. Non-limiting examples include a multiplicity of infection (MOI) of at least 0.001, or at least 0.01, or at least 0.1 or at least 1, or from 0.01 to 1, or from 0.1 to 1, or from about 0.01 to 0.1, or less than 1, or less than 0.1, or less than 0.01 infectious viral particles per cell. In other embodiments, the effective amount of an HSV viral particle is the amount sufficient to result in cell lysis in a subject. In some embodiments, the effective amount is the amount required to increase galactose metabolism in a subject in need thereof. The skilled artisan will be able to determine appropriate amounts depending on these and other factors.

[119] In some embodiments the effective amount will depend on the size and nature of the application in question. It will also depend on the nature and sensitivity of the target subject and the methods in use. The skilled artisan will be able to determine the effective amount based on these and other considerations. The effective amount may comprise, or alternatively consist essentially of, or yet further consist of comprise, or alternatively consist essentially of, or yet further consist of one or more administrations of a composition depending on the embodiment.

[120] As used herein, the term "administer" or “administration” intends to mean delivery of a substance to a subject such as an animal or human. Administration can be affected in one dose, continuously or intermittently throughout the course of treatment, e.g. intratumorally or intravenously. Methods of determining the most effective means and dosage of administration are known to those of skill in the art and will vary with the composition used for therapy, the purpose of the therapy, as well as the age, health or gender of the subject being treated. Single or multiple administrations can be carried out with the dose level and pattern being selected by the treating physician or in the case of pets and animals, treating veterinarian. Suitable dosage formulations and methods of administering the agents are known in the art. Route of administration can also be determined and method of determining the most effective route of administration are known to those of skill in the art and will vary with the composition used for treatment, the purpose of the treatment, the health condition or

disease stage of the subject being treated and the target cell or tissue. Non-limiting examples of route of administration include direct and systemic, e.g., intravenous, intra-arterial, intramuscular, intracardiac, intrathecal, subventricular, epidural, intracerebral, intratumorally, intracranially, intracerebroventricular, sub-retinal, intravitreal, intraarticular, intraocular, intraperitoneal, intrauterine, intradermal, subcutaneous, transdermal, transmuccosal, and inhalation.

Oncolytic herpes simplex virus (oHSV)

[121] The efficacy of oHSVs against tumors is derived from direct cell killing (lytic phase) *and* enhancing anticancer immunity (immune phase). These viruses have been constructed in various ways to selectively target cancer cells. To achieve cancer selectivity, the most common mutation is deletion of the neurovirulence gene, $\gamma_1$34.5/RL1. Expression of ICP34.5, encoded by $\gamma_1$34.5/RL1, is essential for HSV-1 to counteract the host cell anti-viral protein kinase RNA-activated (PKR)-pathway that normally phosphorylates e-IF2α in response to virus infection and stops protein translation. ICP34.5 redirects cellular protein phosphatase-1 (PP1) to dephosphorylate e-IF2α, allowing productive virus replication. Many cancer cells are defective in the PKR response, and therefore support the replication of HSV vectors including $\gamma_1$34.5-null mutants. Some vectors are constructed by mutating critical metabolic viral genes such as ribonucleotide reductase (RR, the large subunit of which is encoded by the ICP6/UL39 gene). Since many malignant cells have increased expression and activity of RR, ICP6-null mutants selectively replicate in highly proliferating cancer cells because of the large ribonucleotide pools present.

HSV Entry, Cell-cell Spread and Syncytia Formation

[122] The entry of HSV-1 involves serial steps of viral glycoproteins interacting with cellular surface molecules. First, glycoproteins B and C (gB and gC) attach to cell heparan sulfate proteoglycans followed by binding of gD to virus entry receptors (*i.e.*, nectin-1, herpesvirus entry mediator (HVEM), or 3-O-sulfated heparan sulfate (3-OS-HS)). The process further allows gH/gL to interact with gB and trigger fusion of the viral envelope to the target cell membrane, resulting in release of the virion capsid and tegument proteins into the cell. Several reports suggest that membrane fusion is also crucial for subsequent lateral spread of virus between neighboring cells. Viral cell-cell spread takes place when virus

spread from infected cells to adjacent uninfected cells through areas of cell contact, which can even occur in the presence of virus neutralizing antibody. The presence of gE/gI enhances cell-cell spread, whereas gE hypomorph mutants reduce cell-cell fusion and plaque size. Syncytia are the result of multiple adjacent cells fusing together into multinucleated giant cells. Mutations that trigger syncytia have been discovered in at least four HSV viral genes (*i.e.*, gB, gK, UL20 and UL24).

[123] This disclosure provides a strategy to combine "directed evolution" and CRISPR/Cas9 technology for development of clinically relevant viral vectors. This effective combination represents a substantive departure from prior art approaches. Without being bound by theory, it is anticipated that unexpected mechanisms can lead to increased viral potency for killing tumors. This is anticipated to reveal previously unknown mutation(s) that cause a hyperfusogenic phenotype and increased potency, as well as the stepping stone for developing next generation oHSVs. The oHSVs so produced have an enhanced lytic phase, longer sustainability, and maximized therapeutic outcome, which is in line with the purpose of National Cancer Institute's Clinical and Translational Exploratory/Developmental Studies.

[124] Although the focus is on the "lytic phase" of virotherapy, a longer sustained virus response would boost the inflammatory response *in vivo*, benefitting the subsequent "immune phase" of virotherapy to reach optimal anti-tumor efficacy. Efficacy studies in pediatric cancer models can address the use of the non-natural HSV in addressing childhood cancers. The resulting improved viruses are anticipated to be active across a variety of adult cancers as well.

Modes for Carrying Out the Disclosure

[125] Despite HSVs can target or infect a broad range of cells to induce lysis, the viral infection itself has caused cytotoxicity and other disorders, *e.g.*, encephalitis, esophagitis, and pneumonitis. A large number of HSV genes affects pathogenicity. For example, γ34.5 (RL1) can cause neuropathogenicity. ICP6 (UL39), ribonucleotide reductase, thymidine kinase (UL23), uracil DNA glycosylase (UL2), dUTPase (UL50), and DNA polymerase (UL30) are involved in nucleotide metabolism and virulence of HSVs. Therefore, there is a need to produce an attenuated, yet replication-competent HSV particle to carry out its function of tumor inhibition, while minimizing its side-effects.

[126] Provided herein is a new virus, named Mut-3, that exhibits a giant syncytial plaque phenotype. Applicant isolated Mut-3 from the serial passage of mixing 17TermA and rRp450 in a non-permissive line ("Directed Evolution") and constructed the attenuated mutant Mut-3Δ34.5 via gene editing (the "CRISPR/Cas9" step) (**FIG. 1A**). Whole genome sequence analysis revealed that Mut-3 acquired intact copies of both $\gamma_1$34.5/RL1 and UL39 (which encodes ICP6), making its genotype resemble that of a wild type (WT) virus. Without being bound by theory, Applicants found that the lytic activity of Mut-3 is even greater than many WT viruses, suggesting other genomic alterations (in addition to its complete virus genome) may be involved in its increased potency. Five nonsynonymous mutations in Mut-3 that are different from either parent virus including an Alanine to Threonine mutation at position 151 (A151T) in the gene that encodes gE were found. An attenuated version of Mut-3 virus is provided that replaces $\gamma_1$34.5/RL1 with green fluorescent protein (GFP) via CRISPR/Cas9 gene editing, designated as Mut-3Δ34.5 (**FIG. 1A**, bottom, labeled as the "CRISPR/Cas9" step). See, FIG. 1B showing a result summary of sequence comparison of Mut-3 with its parent viruses. Non-synonamous mutations that differ from either parent in Mut-3 are shaded with back slashes, including UL15, UL29, US8, RL1 and RL2. Genome sequences that are identical to 17TermA are indicated as blank boxes; those identical to rRp450 are shaded with forward slashes.

[127] Therefore, this disclosure provides a non-natural herpes simplex virus ("HSV"), wherein the virus comprises, alternatively consists essentially of, or yet further consists of a mutation in a virulence gene that is one or more from the group of: (a) a glycoprotein E ("gE")-encoding gene, (b) an Infected Cell Protein 0 ("ICP0")-encoding gene, (c) a DNA packaging terminase subunit 1-encoding gene, (d) an ICP8-encoding gene, or (e) an ICP34.5-encoding gene. In one embodiment, the HSV further comprises, alternatively consists essentially of, or yet further consists of a gene encoding a dysfunctional ICP34.5 protein and/or a gene encoding a dysfunctional ICP6 protein. In another embodiment, the gene encoding the dysfunctional ICP34.5 protein comprises, alternatively consists essentially of, or yet further consists of a polynucleotide having a sequence at least 95% identical to SEQ ID No. 1, 5, 7, 9, or 51, and equivalents thereof with the proviso that the equivalent maintains the mutated or altered amino acid or nucleotides. In another embodiment, the gene encoding the dysfunctional ICP6 protein comprises, alternatively consists essentially of, or yet further

consists of, or consists essentially of, or yet further consists of, a polynucleotide having a sequence at least 95% identical to SEQ ID No. 43, 45, 47, or 49, and equivalents thereof with the proviso that the equivalent maintains the mutated or altered amino acid or nucleotides as compared to wt sequence.

[128] In certain embodiment, the gE encoding gene of the non-natural herpes simplex virus comprises, alternatively consists essentially of, or yet further consists of a polynucleotide having a sequence selected from SEQ ID NOs: 12, 14, 16, 18, a sequence at least 80%, 85%, 90%, 95%, 97% or 99% identical to any one of SEQ ID NOs: 12, 14, 16, and 18, and an equivalent thereof with the proviso that the equivalent maintains the mutated or altered amino acid or nucleotides as compared to wt sequence. In a further embodiment, the HSV comprising such equivalent enters cells and/or spread among the cells and/or replicates DNAs at a level comparable to a non-natural HSV comprising the mutated gE having an amino acid sequence of SEQ ID NO: 13. A non-limiting example of evaluating a HSV entering cells, spreading among the cells and replicating DNAs can be found in the Examples. In yet a further embodiment, the polynucleotide of the gE encoding gene encodes a polypeptide having an amino acid sequence selected from SEQ ID NOs: 13, 15, 17, and 19.

[129] In certain embodiment, the ICP0 encoding gene of the non-natural herpes simplex virus comprises, alternatively consists essentially of, or yet further consists of a polynucleotide having a sequence selected from SEQ ID NOs: 20, 22, 24, 25, and 53, a sequence of any one of SEQ ID NOs: 20, 22, 24, 25, and 53 free of one or both of introns, a sequence at least 80%, 85%, 90%, 95%, 97% or 99% identical to SEQ ID NOs: 20, 22, 24, 25, and 53, and an equivalent thereof with the proviso that the equivalent maintains the mutated or altered amino acid or nucleotides as compared to wt sequence. In one embodiment, the introns are noted in the Sequence Listing as well as in the following: nucleotide (nt) 58 to nt 861 of SEQ ID NO: 20, nt 1529 to nt 1663 of SEQ ID NO: 20, nt 58 to nt 822 of SEQ ID NO: 22, nt 1490 to nt 1625 of SEQ ID NO: 22, 58 to nt 862 of SEQ ID NO: 24, nt 1530 to nt 1668 of SEQ ID NO: 24, nt 58 to nt 861 of SEQ ID NO: 25, nt 1529 to nt 1663 of SEQ ID NO: 25, nt 58 to nt 822 of SEQ ID NO: 53, and nt 1490 to nt 1625 of SEQ ID NO: 53. In a further embodiment, the equivalent encodes a polypeptide of ICP0 having a function (for example, of promoting transcription from viral genes, disrupting structures in the nucleus known as nuclear dots or promyelocytic leukemia (PML) nuclear

bodies, and altering the expression of host and viral genes in combination with a neuron specific protein) at a level comparable to wild-type ICP0 or the mutated ICP0 having an amino acid sequence of SEQ ID NO: 21. Examples of evaluating such functions can be found, Lee HR, Kim DJ, Lee JM, et al. (June 2004). "Ability of the human cytomegalovirus IE1 protein to modulate sumoylation of PML correlates with its functional activities in transcriptional regulation and infectivity in cultured fibroblast cells". J. Virol. 78 (12): 6527–42; Gu H, Liang Y, Mandel G, Roizman B (May 2005). "Components of the REST/CoREST/histone deacetylase repressor complex are disrupted, modified, and translocated in HSV-1-infected cells". Proc. Natl. Acad. Sci. U.S.A. 102 (21): 7571–6; and Pinnoji RC, Bedadala GR, George B, Holland TC, Hill JM, Hsia SC (2007). "Repressor element-1 silencing transcription factor/neuronal restrictive silencer factor (REST/NRSF) can regulate HSV-1 immediate-early transcription via histone modification". Virol. J. 4: 56. Additionally or alternatively, the HSV comprising such equivalent enters cells and/or spread among the cells and/or replicates DNAs at a level comparable to a non-natural HSV comprising the mutated ICP0 having an amino acid sequence of SEQ ID NO: 21. A non-limiting example of evaluating a HSV entering cells, spreading among the cells and replicating DNAs can be found in the Examples. In yet a further embodiment, the polynucleotide of the ICP0 encoding gene encodes a polypeptide having an amino acid sequence selected from SEQ ID NOs: 21, 23, and 26.

[130] In certain embodiment, the ICP8 encoding gene of the non-natural herpes simplex virus comprises, alternatively consists essentially of, or yet further consists of a polynucleotide having a sequence selected from SEQ ID NOs: 27, 29, 31, 33, a sequence at least 80%, 85%, 90%, 95%, 97% or 99% identical to any one of SEQ ID NO: 27, 29, 31, and 33, and an equivalent thereof with the proviso that the equivalent maintains the mutated or altered amino acid or nucleotides as compared to wt sequence. In a further embodiment, the equivalent encodes a polypeptide of ICP8 having a function (for example, of annealing to single-stranded DNA (ssDNA), melting small fragments of double-stranded DNA, or destabilizing duplex DNA during initiation of replication) at a level comparable to wild-type ICP8 or the mutated ICP8 having an amino acid sequence of SEQ ID NO: 28. Such functions can be evaluated via methods available in the art, for example, Boehmer, PE; Lehman, IR (1993). "Herpes simplex virus type 1 ICP8: Helix-destabilizing properties". Journal of

Virology. 67 (2): 711–5. Additionally or alternatively, the HSV comprising such equivalent enters cells and/or spread among the cells and/or replicates DNAs at a level comparable to a non-natural HSV comprising the mutated ICP8 having an amino acid sequence of SEQ ID NO: 28. A non-limiting example of evaluating a HSV entering cells, spreading among the cells and replicating DNAs can be found in the Examples. In yet a further embodiment, the polynucleotide of the ICP8 encoding gene encodes a polypeptide having an amino acid sequence selected from SEQ ID NOs: 28, 30, 32, and 34.

[131] In certain embodiment, the DNA packaging terminase subunit 1 encoding gene of the non-natural herpes simplex virus comprises, alternatively consists essentially of, or yet further consists of a polynucleotide having a sequence selected from SEQ ID NOs: 35, 37, 39, 41, a sequence at least 80%, 85%, 90%, 95%, 97% or 99% identical to any one of SEQ ID NOs: 35, 37, 39, and 41, and an equivalent thereof with the proviso that the equivalent maintains the mutated or altered amino acid or nucleotides as compared to wt sequence. In a further embodiment, the HSV comprising such equivalent enters cells and/or spread among the cells and/or replicates DNAs at a level comparable to a non-natural HSV comprising the mutated DNA packaging terminase subunit 1 having an amino acid sequence of SEQ ID NO: 36. A non-limiting example of evaluating a HSV entering cells, spreading among the cells and replicating DNAs can be found in the Examples. In a further embodiment, the polynucleotide of the DNA packaging terminase subunit 1 encoding gene encodes a polypeptide having an amino acid sequence selected from SEQ ID NOs: 36, 38, 40, and 42.

[132] In certain embodiment, the HSV comprises, alternatively consists essentially of, or yet further consists of one or more of polynucleotide(s) having a sequence selected from SEQ ID NOs: 12, 20, 27, and 35. In a further embodiment, the HSV does not have a functional ICP34.5 (*i.e.*, encoding a dysfunctional ICP34.5 or not encoding an ICP34.5). Additionally or alternatively, the HSV does not have a functional ICP6 (i.e., encoding a dysfunctional ICP6 or not encoding an ICP6). In another embodiment, the mutation in the virulence gene comprises, or consists essentially of, or yet further consists of, an insertion, a deletion, a truncation, a frameshift, a substitution, or a point mutation, for example, of the ICP34.5 gene and/or of the ICP6 gene. In another embodiment, the HSV lacks a gene encoding a functional ICP34.5 protein and/or a functional ICP6 protein. In another embodiment, the mutation is a nonsynonymous mutation in the virulence gene.

[133] In one embodiment, the mutation on the non-natural HSV of the disclosure encodes one or more of: (a) an alanine-to-threonine mutation at position 151 of the gE protein, (b) an arginine-to-histidine mutation at position 258 of the ICP0 protein, (c) an alanine-to-threonine mutation at position 376 of the DNA packaging terminase subunit 1 protein, (d) a threonine-to-methionine mutation at position 1155 of the ICP8 protein, or (e) a proline-to-histidine mutation at position 119 of the ICP34.5 protein. In another embodiment, the non-natural HSV comprises, or alternatively consists essentially of, or yet further consists of one or more of SEQ ID No. 2, SEQ ID No. 13, SEQ ID No. 21, SEQ ID No. 28, or SEQ ID No. 36, and equivalents thereof with the proviso that the equivalent maintains the mutated or altered amino acid or nucleotides.

[134] In certain embodiment, provided is a non-natural herpes simplex virus ("HSV"), wherein the virus comprises, alternatively consists essentially of, or yet further consists of a mutation in one or more of: (a) a gE, (b) an ICP0, (c) a DNA packaging terminase subunit 1, (d) an ICP8, or (e) an ICP34.5. In certain embodiment, the HSV does not comprise a functional ICP34.5 protein (for example, an ICP34.5 of the 17TermA strain or of the rRp450 strain). In a further embodiment, the HSV does not comprise any ICP34.5 protein. Additionally or alternatively, the HSV does not comprise a functional ICP6 protein (for example, an ICP6 of the 17TermA strain or of the rRp450 strain). In yet a further embodiment, the HSV does not comprise any ICP6 protein.

[135] In one embodiment, the mutation(s) on the non-natural HSV of the disclosure is/are one or more of: (a) an alanine-to-threonine mutation at position 151 of the gE protein, (b) an arginine-to-histidine mutation at position 258 of the ICP0 protein, (c) an alanine-to-threonine mutation at position 376 of the DNA packaging terminase subunit 1 protein, (d) a threonine-to-methionine mutation at position 1155 of the ICP8 protein, or (e) a proline-to-histidine mutation at position 119 of the ICP34.5 protein.

[136] In certain embodiment, the gE of the non-natural HSV comprises, alternatively consists essentially of, or yet further consists of an amino acid sequence selected from SEQ ID NOs: 13, 15, 17 and 19. In a further embodiment, the non-natural HSV further comprises a polynucleotide encoding the amino acid sequence of the gE, for example, a polynucleotide having a sequence selected from SEQ ID NOs: 12, 14, 16, 18, and an equivalent thereof. In

certain embodiment, the ICP0 of the non-natural HSV comprises, alternatively consists essentially of, or yet further consists of an amino acid sequence selected from SEQ ID NOs: 21, 23, and 26. In a further embodiment, the non-natural HSV further comprises a polynucleotide encoding the amino acid sequence of the ICP0, for example, a polynucleotide having a sequence selected from SEQ ID NOs: 20, 22, 24, 25, 53 and an equivalent thereof. In certain embodiment, the ICP8 of the non-natural HSV comprises, alternatively consists essentially of, or yet further consists of an amino acid sequence selected from SEQ ID NOs: 28, 30, 32 and 34. In a further embodiment, the non-natural HSV further comprises a polynucleotide encoding the amino acid sequence of the ICP8, for example, a polynucleotide having a sequence selected from SEQ ID NOs: 27, 29, 31, 33, and an equivalent thereof. In certain embodiment, the DNA packaging terminase subunit 1 of the non-natural HSV comprises, alternatively consists essentially of, or yet further consists of an amino acid sequence selected from SEQ ID NOs: 36, 38, 40 and 42. In a further embodiment, the non-natural HSV further comprises a polynucleotide encoding the amino acid sequence of the DNA packaging terminase subunit 1, for example, a polynucleotide having a sequence selected from SEQ ID NOs: 35, 37, 39, 41, or an equivalent thereof.

[137] In certain embodiment, the non-natural HSV comprises, or alternatively consists essentially of, or yet further consists of one or more of polypeptide(s) having an amino acid sequence selected from SEQ ID NOs: 13, 21, 28, and 36. In a further embodiment, the non-natural HSV comprises, or alternatively consists essentially of, or yet further consists of one or more of polynucleotide(s) encoding one or more of an amino acid sequence selected from SEQ ID NOs: 13, 21, 28, and 36.

[138] In certain embodiment, the non-natural HSV comprises, or alternatively consists essentially of, or yet further consists of one or more of the following: (a) a polynucleotide encoding an amino acid sequence selected from SEQ ID NOs. 2, 6, 8, 10, and 52, and/or a polynucleotide having a sequence selected from SEQ ID NOs. 1, 5, 7, 9, and 51; (b) a polypeptide having an amino acid sequence selected from SEQ ID NOs. 2, 6, 8, 10, and 52; (c) a polynucleotide encoding an amino acid sequence selected from SEQ ID NOs. 13, 15, 17 and 19, and/or a polynucleotide having a sequence selected from SEQ ID NOs. 12, 14, 16 and 18; (d) a polypeptide having an amino acid sequence selected from SEQ ID NOs. 13, 15, 17 and 19; (e) a polynucleotide encoding an amino acid sequence selected from SEQ ID NOs.

21, 23 and 26, and/or a polynucleotide having a sequence selected from SEQ ID NOs. 20, 22, 24, 25, and 53, or a sequence thereof free of one or two or more introns; (f) a polypeptide having an amino acid sequence selected from SEQ ID NOs. 21, 23 and 26; (g) a polynucleotide encoding an amino acid sequence selected from SEQ ID NOs. 28, 30, 32 and 34, and/or a polynucleotide having a sequence selected from SEQ ID NOs. 27, 29, 31, and 33; (h) a polypeptide having an amino acid sequence selected from SEQ ID NOs. 28, 30, 32 and 34; (i) a polynucleotide encoding an amino acid sequence selected from SEQ ID NOs. 36, 38, 40, and 42, and/or a polynucleotide having a sequence selected from SEQ ID NOs. 35, 37, 39 and 41; (j) a polypeptide having an amino acid sequence selected from SEQ ID NOs. 36, 38, 40, and 42; (k) a polynucleotide encoding an amino acid sequence selected from SEQ ID NOs. 44, 46, 48 and 50, and/or a polynucleotide having a sequence selected from SEQ ID NOs. 43, 45, 47 and 49; (l) a poly peptide having an amino acid sequence selected from SEQ ID NOs. 44, 46, 48 and 50.

[139] In another embodiment, the non-natural HSV further comprises, or alternatively consists essentially of, or yet further consists of a polynucleotide having sequence that is identical to at least a fragment of a virulence gene from a 17TermA HSV and equivalents thereof. In another embodiment, the non-natural HSV further comprises, or alternatively consists essentially of, or yet further consists of a polynucleotide having sequence that is identical to at least a fragment of a virulent gene from an rRp450 HSV. The non-natural HSV, in some embodiments, is derived from an HSV type 1 ("HSV-1") or an HSV type 2 ("HSV-2") strain. In one embodiment, the non-natural HSV is derived from an HSV-1 KOS strain. In another embodiment, the non-natural HSV further comprises, or alternatively consists essentially of, or yet further consists of a transgene.

[140] Because the HSVs as disclosed here retains its lytic function, in another aspect, this disclosure provides a method for treating cancer or inhibiting the growth or metastasis of cancer cell in a subject in need thereof, comprising, or consisting essentially of, or yet further consisting of, administering to the subject an effective amount of the non-natural HSV or a composition comprising, or alternatively consisting essentially of, or yet further consisting of the non-natural HSV. In one aspect, the cancer comprises pancreatic cancer, renal cancer, small cell lung cancer, brain cancer, neural cancer, neuroblastoma, bone cancer, lymphoma, myeloma, colon cancer, uterine cancer, breast cancer, leukemia, liver cancer, prostate cancer,

skin cancer, or melanoma. The subject being treated can be an adult or a pediatric patient, *e.g.*, a mammal or a human patient. In another embodiment, non-natural HSV vector or the composition or pharmaceutical composition is administered by locally or systemically by injection, infusion, instillation, and/or inhalation. In another embodiment, the subject is a mammal. In some embodiment, the mammal is a mouse, a rat, a guinea pig, a non-human primate, a dog, a cat, a horse, a cow, a pig, a goat, or a sheep. In another embodiment, the subject is human.

[141] In another aspect, the disclosure provides a method for inducing cell lysis, which comprises, or consists essentially of, or yet further consists of, contacting the cell with a non-natural HSV of this disclosure or a composition comprising, or alternatively consisting essentially of, or yet further consisting of the non-natural HSV. In one embodiment, the cell is a cancer cell. In a further aspect, the cell is a cultured cell (for use as a pre-clinical model or pre-clinical assay) or a cell isolated from a subject. The cell can be cultured or within an isolated tissue. Non-limiting examples of such cells include cells from: pancreatic cancer, renal cancer, small cell lung cancer, brain cancer, neuroblastoma, neural cancer, bone cancer, lymphoma, myeloma, colon cancer, uterine cancer, breast cancer, leukemia, liver cancer, prostate cancer, skin cancer, or melanoma. The cells can be isolated from mammal, *e.g.*, humans can be adult or juvenile (pediatric).

[142] In another aspect, the disclosure provides a method to infect a cell, the method comprising, or alternatively consisting essentially of, or yet further consisting of, contacting the cell with the non-natural HSV. In one embodiment, the cell is a eukaryotic cell. In another embodiment, the cell is a lymphocyte. In one embodiment, the cell is a cancer cell such as a blood cancer or a solid tumor cells, e.g., carcinoma or sarcoma In a further aspect, the cell is a cultured cell (for use as a pre-clinical model or pre-clinical assay) or a cell isolated from a subject. The cell can be cultured or within an isolated tissue. Non-limiting examples of such cells include cells from: pancreatic cancer, renal cancer, small cell lung cancer, brain cancer, neuroblastoma, neural cancer, bone cancer, lymphoma, myeloma, colon cancer, uterine cancer, breast cancer, leukemia, liver cancer, prostate cancer, skin cancer, or melanoma. The cells can be isolated from mammal, e.g., humans can be adult or juvenile (pediatric).

[143] Applicants discovered that the conventional a group of Epstein-Barr virus ("EBV")-infected lymphocytes were resistant to conventional oncolytic HSVs ("oHSVs"). Without being bound by a theory, the resistance is at least partly due to a low level of expression of HSV entry receptors on the EBV-infected lymphocytes. Surprisingly, the HSV of the claimed invention were able to induce lysis in the EBV-infected lymphocytes, which are resistant to wild type strain 17, KOS and McKrae HSV viruses. Therefore, in one embodiment, the cell comprises, or alternatively consists essentially of, or yet further consists of a cell infected by EBV or alternatively a cell resistant to EBV. In another embodiment, the cell comprises, or alternatively consists essentially of, or yet further consists of a virulence element of an Epstein-Barr virus ("EBV"). In another embodiment, the cell comprises, or alternatively consists essentially of, or yet further consists of a lymphocyte infected by the EBV. In another embodiment, the HSV of the claimed invention has a higher infection rate to the EBV-infected cell compared to a control. In one embodiment, the control comprises, or alternatively consists essentially of, or yet further consists of a conventional oHSV. In another embodiment, the conventional oHSV comprises wild type strain 17 HSV, KOS HSV, or McKrae HSV.

Production of HSV mutants or derivatives

[144] The production of HSV mutants or derivatives involves a change or mutation of a gene or a combination of genes encoded by a parental HSV strain. For example, a derivative may have the sequence of a HSV-l or HSV-2 genome modified by nucleotide substitutions, for example from 1, 2 or 3 to 10, 25, 50 or 100 substitutions. The HSV-l or HSV-2 genome may alternatively or additionally be modified by one or more insertions and/or deletions and/or by an extension at either or both ends. The gene modification methods are known in the art, *e.g.*, CRISPR, recombinant construction, or point mutation. A person with ordinary skill in the art would know how to produce a HSV mutant based on need.

[145] In addition to the target genetic modification methods, a HSV mutant can be produced spontaneously. For example, the culture of viruses such as HSV involves a technique known as serial passage. To grow and maintain viruses, suitable cells are infected with the virus, the virus replicates within the cell and the virus is then harvested; fresh cells are then re-infected, this process constitutes one cycle of serial passage. Each such cycle may take, for example, a

few days in the case of HSV. As discussed above, such serial passaging may lead to changes in the properties or gene sequences of the virus strain, in that selection takes places for properties that would favor the clinical applications of the HSVs. For example, the enhanced properties can include rapid replication, or the capacity to travel along axons to infect human cells. In addition, the spontaneous mutation can be produced by infecting a cell with one HSV or more than one HSVs.

[146] Thus, the disclosure provides a method to prepare an HSV or its mutants or derivatives by mutating a gene in the HSV. In another embodiment, the method comprises, or alternatively consists essentially of, or yet further consists of inducing to the non-natural HSV a transgene.

[147] In another aspect, provided herein is a method of producing an HSV viral particle, the method comprising, or alternatively consisting essentially of, or yet further consisting of: (a) introducing to a host cell a 17TermA HSV vector and an rRp450 HSV vector; (b) growing the host cell for at least 3 passages; and (c) isolating a HSV particle produced by the host cell. In one embodiment, the HSV is introduced to the host cell by transfection, infection, transformation, electroporation, injection, microinjection, or the combination thereof. In another embodiment, the host cell is grown for at least 3 passages, 10 passages, 20 passages, 30 passages, 40 passages, or 50 passages. In some embodiment, the host cell comprises, or alternatively consists essentially of, or yet further consists of a complementing gene product to support replication of the introduced HSV vectors. In another embodiment, the complementing gene encodes an ICP6 protein and/or an ICP34.5 protein. In another embodiment, the HSV particle so produced comprises, or alternatively consists essentially of, or yet further consists of the HSV vector in this disclosure.

[148] In certain embodiment, provided herein is a method of producing a non-natural HSV viral particle of the disclosure. The method comprises, or alternatively consists essentially of, or yet further consists of: (a) introducing to a host cell a non-natural HSV vector; (b) growing the host cells; and (c) isolating a HSV particle produced by the host cell.

[149] In certain embodiment, provided herein is a method of producing a non-natural HSV viral particle of the disclosure comprising, or alternatively consisting essentially of, or yet further consisting of: (a) introducing to a host cell a polynucleotide encoding a viral genome

of the non-natural HSV vector; (b) growing the host cells; and (c) collecting and isolating the HSV particle produced by the host cell. In one embodiment, the nucleic acid sequence encoding the viral genome is introduced to the host cell by transfection, infection, transformation, electroporation, injection, microinjection, or the combination thereof. In one embodiment, the nucleic acid sequence encoding the viral genome is introduced to the host cell in a vector. In a further embodiment, the vector is a viral vector (such as an HSV) or a non-viral vector (such as a plasmid or a nanoparticle). In yet a further embodiment, the vector is an HSV. In some embodiment, the host cell comprises, or alternatively consists essentially of, or yet further consists of a complementing gene product to support replication of the introduced HSV vectors. In one embodiment, such complementing gene product is provided in the host cell via a helper virus. In another embodiment, the complementing gene encodes an ICP6 protein and/or an ICP34.5 protein. In another embodiment, the HSV particle so produced comprises, or alternatively consists essentially of, or yet further consists of the HSV vector in this disclosure.

[150] In one embodiment, the isolating step refers to a process of substantially separating the HSV from other materials, such as host cells, cell debris, culture medium or any other agent used in culturing the host cells, for example by centrifuge, filtration, chromatography, or any combination thereof. A non-limiting example can be found at Sia et al, Optimal purification method for Herpes-based viral vectors that confers minimal cytotoxicity for systemic route of vector administration. J Virol Methods. 2007 Feb;139(2):166-74.

Compositions

[151] In another aspect, the disclosure provides a composition comprising, or consisting essentially of, or yet further consisting of, the non-natural HSV described here. Compositions, including pharmaceutical compositions comprising, or alternatively consisting essentially of, or yet further consisting of the agents or viral particles described herein can be manufactured by means of conventional mixing, dissolving, granulating, levigating, emulsifying, encapsulating, entrapping, or lyophilization processes. The compositions can be formulated in conventional manner using one or more physiologically acceptable carriers, diluents, excipients, or auxiliaries which facilitate processing of the viral particles provided herein into preparations which can be used pharmaceutically.

[152] The agents and viral particles of the technology can be administered by parenteral (e.g., intramuscular, intraperitoneal, intravenous, intracerebroventricular ("ICV"), intracisternal injection or infusion, subcutaneous injection, or implant), oral, by inhalation spray nasal, vaginal, rectal, sublingual, urethral (e.g., urethral suppository) or topical routes of administration (e.g., gel, ointment, cream, aerosol, etc.) and can be formulated, alone or together, in suitable dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants, excipients, and vehicles appropriate for each route of administration.

[153] In one embodiment, this disclosure relates to a composition comprising, or consisting essentially of, or yet further consisting of: a non-natural HSV viral particle as described herein and a carrier.

[154] In another embodiment, this disclosure relates to a pharmaceutical composition comprising, or consisting essentially of, or yet further consisting of: a non-natural viral particle as described herein and a pharmaceutically acceptable carrier. In another embodiment, the composition comprises, or alternatively consists essentially of, or yet further consists of a cryopreservative that facilitates the freezing and thawing of the non-natural HSV without loss of significant virulence.

[155] In another embodiment, this disclosure relates to a pharmaceutical composition comprising, or alternatively consisting essentially of, or yet further consisting of a therapeutically effective amount of a non-natural HSV viral particle as described herein and a pharmaceutically acceptable carrier.

[156] The pharmaceutical compositions for the administration of the HSV viral particles can be conveniently presented in dosage unit form and can be prepared by any of the methods well known in the art of pharmacy. The pharmaceutical compositions can be, for example, prepared by uniformly and intimately bringing the HSV viral particles provided herein into association with a liquid carrier, a finely divided solid carrier or both, and then, if necessary, shaping the product into the desired formulation. In the pharmaceutical composition the compound provided herein is included in an amount sufficient to produce the desired therapeutic effect. For example, pharmaceutical compositions of this disclosure may take a form suitable for virtually any mode of administration, including, for example, topical,

ocular, oral, buccal, systemic, nasal, injection, infusion, transdermal, rectal, and vaginal, or a form suitable for administration by inhalation or insufflation.

[157] For topical administration, the non-natural HSV viral particles can be formulated as solutions, gels, ointments, creams, suspensions, etc., as is well-known in the art.

[158] Systemic formulations include those designed for administration by injection (e.g., subcutaneous, intravenous, infusion, intramuscular, intrathecal, or intraperitoneal injection) as well as those designed for transdermal, transmucosal, oral, or pulmonary administration.

[159] Useful injectable preparations include sterile suspensions, solutions, or emulsions of the HSV viral particles provided herein in aqueous or oily vehicles. The compositions may also contain formulating agents, such as suspending, stabilizing, and/or dispersing agents. The formulations for injection can be presented in unit dosage form, e.g., in ampules or in multidose containers, and may contain added preservatives.

[160] Alternatively, the injectable formulation can be provided in powder form for reconstitution with a suitable vehicle, including but not limited to sterile pyrogen free water, buffer, and dextrose solution, before use. To this end, the HSV viral particles provided herein can be dried by any art-known technique, such as lyophilization, and reconstituted prior to use.

[161] For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are known in the art.

[162] For oral administration, the pharmaceutical compositions may take the form of, for example, lozenges, tablets, or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone, or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose, or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc, or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulfate). The tablets can be coated by methods well known in the art with, for example, sugars, films, or enteric coatings.

[163] Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions, and such compositions may

contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents, and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the HSV viral particles provided herein in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients can be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents (e.g., corn starch or alginic acid); binding agents (e.g., starch, gelatin, or acacia); and lubricating agents (e.g., magnesium stearate, stearic acid, or talc). The tablets can be left uncoated or they can be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate can be employed. They may also be coated by the techniques well known to the skilled artisan. The pharmaceutical compositions of the technology may also be in the form of oil-in-water emulsions.

[164] Liquid preparations for oral administration may take the form of, for example, elixirs, solutions, syrups, or suspensions, or they can be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives, or hydrogenated edible fats); emulsifying agents (e.g., lecithin, or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol, cremophore TM, or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, preservatives, flavoring, coloring, and sweetening agents as appropriate.

[165] In one embodiment, provided is a method of preparing the non-natural HSV of this disclosure comprising, or alternatively consisting essentially of, or yet further consisting of mutating a gene in a non-natural HSV viral particle or introducing into the non-natural HSV a transgene. In another aspect, the method of producing the non-natural HSV vector, comprises, or alternatively consists essentially of, or yet further consists of: (a) introducing to

a host cell a 17TermA HSV vector and an rRp450 HSV vector; (b) growing the host cell for at least 3 passages; and (c) isolating a HSV particle produced by the host cell.

[166] Also provided is a method for inhibiting the growth or metastasis of a cancer cell or a metastatic cancer cell, the method comprising, or consisting essentially of, or yet further consisting of, contacting the cell with an effective amount of the non-natural HSV vector or a composition or a pharmaceutical composition containing the non-natural HSV vector as described herein. The contacting is *in vitro* or *in vivo*. In one aspect, the contacting is *in vivo* by administration of the non-natural HSV or a composition or a pharmaceutical composition to a subject. *In vitro*, the method is practiced by placing the non-natural HSV in contact with the cell. The *in vitro* method can be used to test for new therapies or as a personalized assay to determine if the therapy is suitable for the cancer to be treated. Additional cancer therapies can be combined with the therapy which can be concurrent or sequential to the disclosed methods.

[167] The cancer cell to be treated can be a solid tumor or blood cancer, e.g., carcinoma or sarcoma and non-limiting examples of such include pancreatic cancer, renal cancer, small cell lung cancer, brain cancer, neuroblastoma, neural cancer, bone cancer, lymphoma, myeloma, colon cancer, uterine cancer, breast cancer, leukemia, liver cancer, prostate cancer, skin cancer, or melanoma. The cell is of any species, *e.g.*, mammalian and human and when performed *in vitro*, it can be from a cultured cell line or a primary cell, *e.g.*, from a tissue biopsy. The cell can be an adult or juvenile cell or a cancer stem cell, from a tissue biopsy. The cell can be an adult or juvenile cell or a cancer stem cell (*i.e.*, cancer cells possessing characteristics associated with normal stem cells, specially the ability to give rise to all cell types found in a particular cancer sample) or a cancer cell without such characteristics associated with normal stem cells. In one embodiment, the cell expresses N-myc proto-oncogene protein (MYCN), and/or expresses MYCN at a level higher than non-cancer cells.

[168] Additionally provided in this disclosure is a method for treating cancer, or inhibiting the growth or metastasis of a cancer cell in a subject in need thereof, comprising, or alternatively consisting essentially of, or yet further consisting of, administering to the subject an effective amount of the non-natural HSV, the composition or the pharmaceutical composition of this disclosure. The subject to be treated can be of any species, *e.g.*,

mammalian and human, e.g., canine, equine, bovine, feline, simian, rat or murine. The administration can be as a first line therapy, a second line therapy, a third line therapy, a fourth line therapy, or a fifth line therapy. Additional cancer therapies can be combined with the therapy which can be concurrent or sequential to the disclosed methods. The cancer to be treated can be a solid tumor or blood cancer, e.g., carcinoma or sarcoma and non-limiting examples of such include pancreatic cancer, renal cancer, small cell lung cancer, brain cancer, neuroblastoma, neural cancer, bone cancer, lymphoma, myeloma, colon cancer, uterine cancer, breast cancer, leukemia, liver cancer, prostate cancer, skin cancer, or melanoma.

[169] The method of this disclosure can be combined with appropriate diagnostics to monitor disease remission or progression. Several methods for such monitoring are known in the art.

[170] Further provided is a method of inducing cell lysis, comprising, or alternatively consisting essentially of, or yet further consisting of, contacting the cell with an effective amount of the non-natural HSV, the composition, and/or the pharmaceutical composition of this disclosure. The contacting is *in vitro* or *in vivo*. In one aspect, the contacting is *in vivo* by administration of the non-natural HSV or a composition or a pharmaceutical composition to a subject. *In vitro*, the method is practiced by placing the non-natural HSV in contact with the cell. The *in vitro* method can be used to test for new therapies or as a personalized assay to determine if the therapy is suitable for the subject to be treated. Additional cell lytic therapies can be combined with the therapy which can be concurrent or sequential to the disclosed methods.

[171] The cell to be treated can be a solid tumor or blood cancer, e.g., carcinoma or sarcoma and non-limiting examples of such include pancreatic cancer, renal cancer, small cell lung cancer, brain cancer, neuroblastoma, neural cancer, bone cancer, lymphoma, myeloma, colon cancer, uterine cancer, breast cancer, leukemia, liver cancer, prostate cancer, skin cancer, or melanoma. The cell is of any species, *e.g.*, mammalian and human and when performed *in vitro*, it can be from a cultured cell line or a primary cell, *e.g.*, from a tissue biopsy. The cell can be an adult or juvenile cell or a cancer stem cell or a cancer cell without the characteristics associated with normal stem cells. The therapy can be combined with an

appropriate assay to test for the effectiveness of the therapy, e.g., cancer remission or progression.

Use of HSV viral particles for preparing medicaments

[172] The HSVs and compositions of the present invention are also useful in the preparation of medicaments to treat a variety of pathologies as described herein. The methods and techniques for preparing medicaments of a composition are known in the art. For the purpose of illustration only, pharmaceutical formulations and routes of delivery are detailed herein.

[173] Thus, one of skill in the art would readily appreciate that any one or more of the compositions described above, including the many specific embodiments, can be used by applying standard pharmaceutical manufacturing procedures to prepare medicaments to treat the many disorders described herein. Such medicaments can be delivered to the subject by using delivery methods known in the pharmaceutical arts.

Administration of additional therapeutic agents

[174] The methods disclosed herein can further comprise, or alternatively consist essentially of, or yet further consist of administration of an effective amount of additional therapeutic agents to augment or enhance the therapeutic efficacy of the disclosed methods. In one embodiment, the additional therapeutic agents comprise, or alternatively consist essentially of, or yet further consist of surgical resection of a tumor, an anti-tumor agent such as a small molecule or immunotherapy or cell lytic therapy.

[175] Administration of the therapeutic agent or substance of the present disclosure to a patient will follow general protocols for the administration of that particular primary or secondary therapy, taking into account the toxicity, if any, of the treatment. It is expected that the treatment cycles would be repeated as necessary. It also is contemplated that various standard therapies, as well as surgical intervention, may be applied in combination with the described therapy.

[176] As is apparent to those skilled in the art, the combination therapy can take the form of a combined therapy for concurrent or sequential administration.

Kits

[177] The agents or non-natural HSVs described herein may, in some embodiments, be assembled into pharmaceutical or diagnostic or research kits to facilitate their use in therapeutic, diagnostic, or research applications. A kit may include one or more containers housing the components of the invention and instructions for use. Specifically, such kits may include one or more agents described herein, along with instructions describing the intended application and the proper use of these agents. In certain embodiments, agents in a kit may be in a pharmaceutical formulation and dosage suitable for a particular application and for a method of administration of the agents. Kits for research purposes may contain the components in appropriate concentrations or quantities for running various experiments.

[178] The kit may be designed to facilitate use of the methods described herein and can take many forms. Each of the compositions of the kit, where applicable, may be provided in liquid form (*e.g.*, in solution), or in solid form, (*e.g.*, a dry powder). In certain cases, some of the compositions may be constitutable or otherwise processable (*e.g.*, to an active form), for example, by the addition of a suitable solvent or other species (for example, water or a cell culture medium), which may or may not be provided with the kit. In some embodiments, the compositions may be provided in a preservation solution (*e.g.*, cryopreservation solution). Non-limiting examples of preservation solutions include DMSO, paraformaldehyde, and CryoStor® (Stem Cell Technologies, Vancouver, Canada). In some embodiments, the preservation solution contains an amount of metalloprotease inhibitors.

[179] As used herein, "instructions" can define a component of instruction and/or promotion, and typically involve written instructions on or associated with packaging of the invention. Instructions also can include any oral or electronic instructions provided in any manner such that a user will clearly recognize that the instructions are to be associated with the kit, for example, audiovisual (*e.g.*, videotape, DVD, etc.), internet, and/or web-based communications, etc. The written instructions may be in a form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals or biological products, which instructions can also reflect approval by the agency of manufacture, use, or sale for animal administration.

[180] The kit may contain any one or more of the components described herein in one or more containers. As an example, in one embodiment, the kit may include instructions for mixing one or more components of the kit and/or isolating and mixing a sample and applying to a subject. The kit may include a container housing agents described herein. The HSVs may be in the form of a liquid, gel, or solid (powder). The HSVs may be prepared sterilely, packaged in syringe and shipped refrigerated. Alternatively, it may be housed in a vial or other container for storage. A second container may have other agents prepared sterilely. Alternatively, the kit may include the active agents premixed and shipped in a syringe, vial, tube, or other container. The kit may have one or more or all of the components required to administer the agents to a subject, such as a syringe, topical application devices, or IV needle tubing and bag.

Screening Assays

[181] This disclosure also provides screening assays to identify potential therapeutic agents of known and new compounds and combinations. For example, one of skill in the art can also determine if the HSV provides a therapeutic benefit in vitro by contacting the HSV with a sample cell or tissue to be treated. The cell or tissue can be from any species, e.g., simian, canine, bovine, ovine, rat, mouse or human.

[182] The contacting can also be performed in vivo in an appropriate animal model or human patient. When performed in vitro, the HSV can be directly added to the cell culture medium. When practiced in vitro, the method can be used to screen for novel combination therapies, formulations or treatment regimens, prior to administration to an animal or a human patient.

[183] In another aspect, the assay requires contacting a first sample comprising suitable cells or tissue ("control sample") with an effective amount of an HSV as disclosed herein and contacting a second sample of the suitable cells or tissue ("test sample") with the HSV, agent or combination to be assayed. In one aspect in the case of cancer, the inhibition of growth of the first and second cell samples are determined. If the inhibition of growth of the second sample is substantially the same or greater than the first sample, then the agent is a potential drug for therapy. In one aspect, substantially the same or greater inhibition of growth of the cells is a difference of less than about 1%, or alternatively less than about 5% or alternatively

less than about 10%, or alternatively greater than about 10%, or alternatively greater than about 20%, or alternatively greater than about 50%, or alternatively greater than about 90%. The contacting can be in vitro or in vivo. Means for determining the inhibition of growth of the cells are well known in the art.

[184] In a further aspect, the test agent is contacted with a third sample of cells or tissue comprising normal counterpart cells or tissue to the control and test samples and selecting agents that treat the second sample of cells or tissue but does not adversely affect the third sample. For the purpose of the assays described herein, a suitable cell or tissue is described herein such as cancer or other diseases as described herein. Examples of such include but are not limited to cancer cell or tissue obtained by biopsy or from blood.

[185] Efficacy of the test composition is determined using methods known in the art which include but are not limited to cell viability assays.

[186] In yet a further aspect, the assay requires at least two cell types, the first being a suitable control cell.

[187] The assays also are useful to predict whether a subject will be suitably treated by this disclosure by delivering an HSV to a sample containing the cell to be treated and assaying for treatment, which will vary with the pathology, or for screening for new drugs and combinations. In one aspect, the cell or tissue is obtained from the subject or patient by biopsy. This disclosure also provides kits for determining whether a pathological cell or a patient will be suitably treated by this therapy by providing at least one composition of this disclosure and instructions for use.

[188] The test cells can be grown in small multi-well plates and is used to detect the biological activity of test compounds. For the purposes of this disclosure, the successful HSV or other agent will block the growth or kill the cancer cell but leave the control cell type unharmed.

[189] The following examples are provided to illustrate and not limit the disclosure.

EXAMPLES

Production of HSV mutant

[190] A non-permissive cell line was infected with 17TermA and rRp450 ("directed evolution") and cultured after serial passage to isolate a HSV Mut-3 mutant that contains a WT-like genotype. The HSV Mut-3 mutant was then used to construct the attenuated mutant Mut-3Δ34.5 via gene editing (labelled as "CRISPR/Cas9") (FIG. 1A). The sequence comparison of Mut-3 with its parent viruses is shown in FIG. 1B. Nonsynonamous mutations that differ from either parent in Mut-3 are shaded with back slashes, including UL15, UL29, US8, RL1 and RL2. Genome sequences that are identical to 17TermA are indicated as blank boxes; those identical to rRp450 are shaded with slashes.

[191] The potent oHSV mutant, Mut-3 was isolated that contains a WT-like genotype. Both Mut-3 and the attenuated version Mut-3Δ34.5 rely on the canonical HSV entry proteins, nectin-1 or herpes virus entry mediator (HVEM), to achieve successful infection (not shown). The kinetics of Mut-3Δ34.5 viral gene transfer as measured by the onset of detectable GFP positive area is much earlier when compared to 17Δ34.5, a $\gamma_1$34.5-null virus derived from wildtype strain 17 via same CRISPR/Cas9 gene editing strategy. In addition, Mut-3Δ34.5 infection leads to more cell killing as measured by its less cell confluence area. However, Mut-3Δ34.5 appears to be less replicative whether compared to 17Δ34.5 or to the Mut-3 Δ34.5-null parent virus, 17TermA. These results indicate that even in the attenuated Mut-3Δ34.5 version, a unique genomic alteration(s) results in a syncytial phenotype (not shown) and enhanced potency remains. In a study of an epidermal growth factor receptor (EGFR)-retargeted HSV, it was reported that introducing a syncytial mutation does not impair the specificity of entry and spread. Without being bound by theory, Applicants believe that the underlying cause of a syncytial phenotypes in Mut-3Δ34.5 may result in altered kinetics upon virus fusion to the cell membrane that might affect entry and/or viral mediated cell-to-cell fusion, which leads to faster cell killing and diminished virus particle production.

[192] Mutations were not found in gB, gD, gH and gL, which are the canonical glycoproteins involved in HSV-1 entry. However, five genes contain nonsynonymous (NS) mutation in Mut-3 that are different from either parent: RL1, RL2, UL15, UL29 and Us8/gE.

Mut-3 and its attenuated version Mut-3Δ34.5 both display a fusogenic phenotype, suggesting this phenomenon is not RL1-associated. Whole-genome analysis also suggests that this phenotype is not linked to previously reported mutations since the amino acid sequences of Mut-3 are either identical to rRp450 and the reference strain 17 genome (not a syncytial virus) in gK/UL53 and UL20, or identical to 17TermA (not a syncytial virus) in gB/UL27 and UL24. The A151T mutation of gE/Us8 is the only glycoprotein (virion surface protein) in Mut-3 that has a NS alteration compared to its parent viruses. Although gE has not been linked to either virus attachment or entry, gE/gI dimerization mediates virus cell-to-cell spread as mutations in gE have been associated with smaller plaque sizes. **Applicant hypothesize that A151T of gE in Mut-3Δ34.5 may be the underlying cause of its syncytial and increased potency phenotypes. Based on applicant study priority (from top to bottom), in **Table 1** applicant list all five NS mutations found in five genes in Mut-3 as well as each gene's corresponding function, possible role associated with Mut-3 increased potency phenotype, and applicant proposed studies. The other three mutations (except for RL1) may also be determined, as was done for gE A151T.

Table 1 Mutations of HSV proteins and functions thereof nt: nucleotide; a.a: amino acid.

Gene	Protein Function	NS Mutation	Experiments
Us8	**glycoprotein E**	nt 451 g to a, a.a **A**151**T**	As detailed later, for example, under the sections titled "The Us8/gE revertant in Mut-3Δ34.5 for loss-of-function study" and "The Us8/gE A151T mutation in Mut-3 parent 17TermA for a gain-of-function"

Gene	Protein Function	NS Mutation	Experiments
RL2	**ICP0**, immediate early gene (IE) gene, ubiquitin E3 ligase. Regulate early genes expression	nt 1712 g to a, a.a **R**258**H**	Loss- and gain of function test: i) To validate faster entry or spread observed as detailed later, for example, under the section titled "Comparison of HSV mutants" ii) Evaluate the changes in HSV early gene (eg. ICP8 & TK) expression
UL15	**DNA packaging terminase subunit 1**. Co-localized UL28, UL33 with UL29/ICP8	nt 1126 g to a, a.a **A**376**T**	Loss- and gain of function test: iii) To validate faster entry or spread observed as detailed later, for example, under the section titled "Comparison of HSV mutants" iv) Evaluate the changes in HSV DNA replication
UL29	**ICP8**, early (E) gene, single-stranded DNA-binding protein	nt 3464 c to t, a.a **T**1155**M**	Loss- and gain of function test: i) To validate faster entry or spread observed as detailed later, for example, under the section titled "Comparison of HSV mutants" ii) Evaluate the changes in HSV DNA replication.

Gene	Protein Function	NS Mutation	Experiments
RL1	ICP34.5, neurovirulence protein	nt 356 c to a, **P**119**H,** H119 is same as 17+	Rule out, as Mut-3Δ34.5 remains syncytial

Comparison of HSV mutants

[193] Plaque assays were performed on the four viruses shown in **FIG 1A** at the same time and scanned and analyzed the plaque image 3 days after via Keyence HS All-in-one Fluorescence Microscope BZ-II Analyzer. As shown in **FIGS. 1A** and **1B**, the plaque sizes of Mut-3 and Mut-3Δ34.5 were significantly larger than both parent viruses rRp450 and 17TermA. In an in vitro cytotoxicity/MTS assay of CHO cell sets, the CHO-K1, CHO-Nectin-1, CHO-Nectin-2 and CHO-HVEM were infected with the four viruses with different multiplicity of infections (MOIs). The cell survival colorimetric cell proliferation and MTS assay were measured 3 days post-virus infections (pvi) relative to untreated control. Only CHO-Nectin-1 and CHO-HVEM but not CHO-K1 or CHO-Nectin-2 (mainly for HSV-2 entry) were sensitive to treatments of the four viruses (**FIG. 2C**). Without being bound by a theory, the results suggested that Mut-3 and Mut-3Δ34.5 do not by pass the receptor barrier still relay on canonical HSV entry receptors to infect the cells.

[194] The increased potency for Mut-3Δ34.5 in killing human and murine neuroblastoma cells as compared to 17TermA was not due to an increased yield of infectious virus (**FIG. 3**). An analysis by the transmission electron microscopy ("TEM") also revealed that after infecting neuroblastoma cells, Mut-3Δ34.5 virions were mainly found in endocytic vesicles, while 17TermA virions were mainly found in endocytic vesicles (**FIG. 4**).

[195] An attenuated 17Δ34.5 mutant was produced by CRISPR-Cas9 gene editing technique to replace g134.5 gene in wild-type strain 17+ with EGFP expressing cassette. 17Δ34.5 mutant had a lower potency than its wild-type strain 17+, but comparable to 17TermA (**FIG. 5**). The attenuation of 17Δ34.5 was further confirmed when Mut-3Δ34.5 displays much faster viral gene transfer and cell killing compared to 17 Δ34.5 (**FIG. 6**). In addition, Mut-

3Δ34.5 was more effective than 17TermA to control human neuroblastoma growth in vivo (**FIG. 7**)

The Us8/gE revertant in Mut-3Δ34.5 for loss-of-function study

[196] Introducing a single nucleotide change in the HSV genome solely via CRISPR/Cas9 technology is difficult due to multiple copies of the genome during virus replication and the inability to accompany this gene editing with a selection marker. One construct can be the result of a two-step process in conjunction with CRISPR/Cas9 technology: replacing the whole gE coding region with a reporter gene (such as mCherry or red fluorescent protein (RFP)) to construct a gE-null *Mut-3Δ34.5*; then 2) replacing the reporter gene with the WT gE coding region, resulting in the gE-WT Mut-3Δ34.5 revertant. Examination of the extent to which both the gE-null intermediate and this gE revertant Mut-3Δ34.5 loses the phenotype can be observed as above. This disclosure provides this construct as well.

The Us8/gE A151T mutation in Mut-3 parent 17TermA for a gain-of-function

[197] As above, the gE mutant 17TermA can be constructed in two steps: 1) completely knocking out the gE coding region and replace it with a reporter gene (e.g., GFP); then 2) replacing the reporter gene by knocking in the gE coding region containing the A151T mutation. The phenotype of the Mut-3Δ34.5 will be determined. This disclosure provides this construct as well.

[198] If gE A151T is only responsible for partial or none of the observed phenotypes, or even if so, other mutations in Mut-3 are provided by this disclosure. The mutants are constructed by systematically undertaking similar gain- and loss-of-function approaches as described herein, and as noted in **Table 1**, alone or in combination. Safety profile and efficacy of attenuated Mut-3Δ34.5 can be tested in mouse models. Thus, this disclosure provides the animal models used to test the mutants as well as the methods to do so.

[199] The Mut-3 strain, a potent and WT-like HSV mutant was constructed from the recombination of 17TermA and rRp450. An attenuated version, Mut-3Δ34.5, was created through the deletion of $\gamma_1$34.5/RL1, the viral virulence protein, to ensure its safety for clinical use. No adverse clinical signs or significant changes in body weight in Balb/c mice more than 85 days after intravenously administering up to 1e8 plaque-forming units (pfu) in

applicant pilot toxicology study were observed (not shown). In addition, Mut-3Δ34.5 also shows anti-tumor efficacy in a highly aggressive neuroblastoma model both in vitro (**FIG. 3A**) and in vivo (**FIG. 7**) as compared to 17TermA.

The biodistribution profile of Mut-3Δ34.5 in naïve non-tumor bearing mice

[200] Applicant's own study shows that naïve Balb/c mice can tolerate up to 1e8 pfu of intravenously (iv) delivered Mut-3Δ34.5 virus without any physical sign of illness for more than 85 days. A biodistruibution study was performed starting with iv administration of the previously tested highest dose (1e8 pfu of Mut-3Δ34.5 virus per mouse) to naïve non-tumor bearing Balb/c mice of both genders (30 mice per gender). Peripheral blood is collected and then the mice are sacrificed. Testes, ovaries, spleen, lung, kidneys, heart, lungs and brain are harvested at 24 h, 14 d, 28 d, 56 d and 85 d pvi (n=6 each point). Half of the organs are embedded in formalin to preserve for pathology analysis, and the other half is homogenized for qPCR analysis of HSV genome and plaque assay to access the viral load in each organ. The mice are observed daily for the first two weeks following virus administration, and then observes twice weekly until the scheduled sacrificed day. The body weight of each mouse is measured pre-virus and weekly thereafter. The mice are sacrificed to show the existence or non-existence of adverse clinical signs or with >20% weight loss and are analyzed for viral activity in the organs as described above. In parallel, the wild type KOS virus [dosage range: 1×10^5 to 1×10^7 pfu per mouse (n=3)] is administered to groups of mice as the positive (non-safe) control. Applicant previously found a dose of 1×10^6 pfu KOS virus was uniformly lethal within 2 to 3 days in FVBN mice. qPCR is performed to analyze HSV genome copies, plaque assay to evaluate virus activities and pathology analysis in the organs of positive control mice that show signs of illness. These results can serve as positive indicators/threshold to evaluate the data collected from Mut-3Δ34.5-treated mice. The pathological changes in tissues/organs in Mut-3Δ34.5-treated groups are further evaluated to show comparable viral loads to the positive control. 6 mice per group for each gender are used to assess the biodistribution and safety and tolerance over different period of time. The biodistribution of Mut-3Δ34.5 is measured by the numbers of HSV genome copies per nanogram of genomic DNA in different organs over different times with descriptive statistics and compared with univariate analyses (if applicable).

Cytotoxicity of Mut-3Δ34.5 with other γ134.5-null viruses (17TermA & T-VEC) in various pediatric cancer cell lines in vitro

[201] Superior killing by Mut-3Δ34.5 was observed compared to 17TermA in human and murine neuroblastoma cells (**FIG. 3A**). Using the same MTS in vitro assay shown in **FIG. 3A**, the same analysis is applied to other pediatric cancer cells, such as sarcomas, malignant peripheral nerve sheath tumor (MPNST) and brain tumors (applicant have access to numerous models) to determine if the increased potency phenotype is applicable across different tumor types. The most effective line from each cell type is used to perform in vivo efficacy studies.

Examine the efficacy of Mut-3Δ34.5 in human pediatric tumor models compared to other oHSV therapeutics, 17TermA & T-VEC.

[202] One of the highly responsive models of each tumor type (three total) is chosen to conduct efficacy studies in xenografts using 5 to 6-week-old female athymic nude mice. When tumors reach 150-300 mm^3, mice are pooled and randomized into 3 groups (n=11 each): i) phosphate-buffered saline (PBS) control, ii) Mut-3Δ34.5 virus, or iii)17TermA or T-VEC virus. For efficacy between viruses, a minimum of 11 mice per group are used to detect large differences (20% vs. 80% at d20) in survival and tumor growth with a minimum 80% power. Each mouse is treated intratumorally with either 1 X 10^7 pfu of virus in 100 µl of PBS or PBS only (control) every other day with three injections total treatment regimen based on applicant previous study. The mice are monitored for tumor volumes (twice weekly) and body weight (weekly) for 80 days following virus injections. The endpoint criteria include tumor volume exceeding 2500 mm^3, tumor diameter reaching 2 cm, or weight loss >20%. Animal survival can be displayed using Kaplan-Meier curves and survival can be compared between groups by log-rank tests.

Efficacy of Mut-3Δ34.5 in murine pediatric tumor models compared to other oHSV therapeutics (17TermA & T-VEC)

[203] One of the highly responsive murine tumor models of each tumor type (two total) is chosen to conduct applicant efficacy studies in (5-6)-week gender matched C57BL6 mice. Similarly, mice are pooled and randomized into 3 groups, n=11 each: i) PBS, ii) Mut-3Δ34, iii) 17TermA or T-VEC. Each mouse is treated intratumorally with either 1e8 pfu of virus in

100 µl of PBS or PBS only every other day with three injections total as applicant previous study. The endpoint criteria include tumor volume exceeding 2500 mm^3, tumor diameter reaching 2 cm, or weight loss >20%. Animal survival will be displayed using Kaplan-Meier curves and survival will be compared between groups by log-rank tests.

Summary of Pathology Analysis

[204] Tissues from 4 wild-type KOS injected mice (1e6 & 1e7 pfu, 2 mice per dose) were submitted for pathology analysis via the OSU Comparative Pathology & Mouse Phenotyping Shared Resource. Tissues from Mut-3D34.5/C8G5 and Mut-3DICP6 /D7-1 injected mice (24hr & 14d pvi time points, 2 mice per time point) were subsequently submitted for pathology analysis.

Pathology Report (female): KOS injected mice

[205] Lymphoplasmacytic encephalitis of the brainstem is consistent with published reports of CNS pathology in Balb/c mice injected with HSV-1. All mice examined in this submission had this lesion, though one submitted brain included only a small segment of the brainstem and thus the lesion appeared milder than in the other three mice. Sections of adrenal gland were present with the kidney section from two of the mice. Both of these mice had marked necrosis of both the adrenal cortex and medulla.

Pathology Report (female): 24hrs & 14days for both Mut-3Δ34.5/C8G5 and Mut-3ΔICP6/D7-1 injected mice

[206] Samples from mice sacrificed 24 hr following HSV infection all had the following lesions with moderate to marked severity: periportal to midzonal hepatic necrosis, including individual hepatocytes and necrosis of focally extensive hepatic cords; marked splenic red pulp necrosis and moderate white pulp necrosis with smaller than expected follicles. Several 24 hr mice had increased apoptosis/necrosis of cells in follicles and the corpus luteum in ovarian tissue. This was not noted beyond expected amounts in the control mice evaluated months ago, and in the mice noted here it was in excess of expected amounts.

[207] In day 14 mice, oval cell hyperplasia is a common chronic reaction of livers undergoing damage and is likely a response to your infection. A few samples of lung had

necrotic and inflammatory lesions which were not noted in 24 hr animals. Overall, day 14 mice had far fewer and much milder lesions than 24 hr animals.

[208] The mutant viruses developed in the lab may be contributing to necrosis of a variety of cell types, particularly at 24 hrs, including hepatocytes, splenocytes in red and white pulp, and cells in the ovary.

Equivalents

[209] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[210] The inventions illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising," "including," "containing," etc., shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed.

[211] Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification, improvement and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications, improvements and variations are considered to be within the scope of this invention. The materials, methods, and examples provided here are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention.

[212] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[213] All publications, patent applications, patents, and other references mentioned herein are expressly incorporated by reference in their entirety, including all formulas and figures, to the same extent as if each were incorporated by reference individually. In case of conflict, the present specification, including definitions, will control.

[214] Other embodiments are set forth within the following claims.

SEQ ID NO. 1: The DNA sequence of ICP34.5 in Mut 3 and Mut-3ΔICP6 viral strains. The c356a mutation is shown in bold and italic in the sequence below.

ATGGCCCGCCGCCGCCGCCATCGCGGCCCCCGCCGCCCCCGGCCGCCCGG
GCCCACGGGCGCGGTCCCAACCGCACAGTCCCAGGTAACCTCCACGCCCA
ACTCGGAACCCGTGGTCAGGAGCGCGCCCGCGGCCGCCCCGCCGCCGCCC
CCCGCCAGTGGGCCCCCGCCTTCTTGTTCGCTGCTGCTGCGCCAGTGGCT
CCACGTTCCCGAGTCCGCGTCCGACGACGACGACGACGACTGGCCGGACA
GCCCCCCGCCCGAGCCGGCGCCAGAGGCCCGGCCCACCGCCGCCGCCCCC
CGCCCCCGGTCCCCACCGCCCGGCGCGGGCCCGGGGGGCGGGGCTAACCC
CTCCC***A***CCCCCCCTCACGCCCCTTCCGCCTTCCGCCGCGCCTCGCCCTCC
GCCTGCGCGTCACCGCAGAGCACCTGGCGCGCCTGCGCCTGCGACGCGCG
GGCGGGGAGGGGGCGCCGAAGCCCCCCGCGACCCCCGCGACCCCCGCGAC
CCCCACGCGGGTGCGCTTCTCGCCCCACGTCCGGGTGCGCCACCTGGTGG
TCTGGGCCTCGGCCGCCCGCCTGGCGCGCCGCGGCTCGTGGGCCCGCGAG
CGGGCCGACCGGGCTCGGTTCCGGCGCCGGGTGGCGGAGGCCGAGGCGGT
CATCGGGCCGTGCCTGGGGCCCGAGGCCCGTGCCCGGGCCCTGGCCCGCG
GAGCCGGCCCGGCGAACTCGGTCTAA

SEQ ID NO. 2 The amino acid sequence of ICP34.5 in Mut3 & Mut-3ΔICP6. The P119H mutation is shown in bold and italic in the sequence below.

MARRRRHRGPRRPRPPGPTGAVPTAQSQVTSTPNSEPVVRSAPAAAPPPP
PASGPPPSCSLLLRQWLHVPESASDDDDDDWPDSPPPEPAPEARPTAAAP
RPRSPPPGAGPGGGANPS***H***PPSRPFRLPPRLALRLRVTAEHLARLRLRRA
GGEGAPKPPATPATPATPTRVRFSPHVRVRHLVVWASAARLARRGSWARE
RADRARFRRRVAEAEAVIGPCLGPEARARALARGAGPANSV

SEQ ID NO. 3: the DNA coding sequence of EGFP. The ICP34.5 coding region was replaced with a EGFP expression cassette (Fig. 5C, SEQ ID NO: 11) in Mut3ΔIC34.5

ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGT
CGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGG
GCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACC
ACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTA
CGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACT
TCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTC
TTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGG

CGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGG
ACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAAC
GTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAA
GATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACC
AGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCAC
TACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGA
TCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCA
TGGACGAGCTGTACAAGAAGCTTAGCCATGGCTTCCCGCCGGAGGTGGAG
GAGCAGGATGATGGCACGCTGCCCATGTCTTGTGCCCAGGAGAGCGGGAT
GGACCGTCACCCTGCAGCCTGTGCTTCTGCTAGGATCAATGTGTAG

SEQ ID NO. 4: the amino acid coding sequence of EGFP.

MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICT
TGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIF
FKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHN
VYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNH
YLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYKKLSHGFPPEVE
EQDDGTLPMSCAQESGMDRHPAACASARINV

SEQ ID NO. 5: the DNA sequence of ICP34.5 in the 17TermA strain.

ATGGCCCGCCGCCGCCGCCATCGCGGCCCCCGCCGCCCCCGGCCGCCCGG
GCCCACGGGCGCCGTCCCAACCGCACAGTCCCAGGTAACCTAGACTAGTC
TAGCGTAACCTCCACGCCCAACTCGGAACCCGCGGTCAGGAGCGCGCCCG
CGGCCGCCCCGCCGCCGCCCCCCGCCGGTGGGCCCCCGCCTTCTTGTTCG
CTGCTGCTGCGCCAGTGGCTCCACGTTCCCGAGTCCGCGTCCGACAACGA
CGATGACGACGACTGGCCGGACAGCCCCCCGCCCGAGCCGGCGCCAGAGG
CCCGGCCCACCGCCGCCGCCCCCCGGCCCCGGCCCCCACCGCCCGGCGTG
GGCCCGGGGGGCGGGGCTGACCCCTCCCACCCCCCCTCGCGCCCCTTCCG
CCTTCCGCCGCGCCTCGCCCTCCGCCTGCGCGTCACCGCGGAGCACCTGG
CGCGCCTGCGCCTGCGACGCGCGGGCGGGGAGGGGGCGCCGGAGCCCCCC
GCGACCCCCGCGACCCCCGCGACCCCCGCGACCCCCGCGACCCCCGCGCG
GGTGCGCTTCTCGCCCCACGTCCGGGTGCGCCACCTGGTGGTCTGGGCCT
CGGCCGCCCGCCTGGCGCGCCGCGGCTCGTGGGCCCGCGAGCGGGCCGAC
CGGGCTCGGTTCCGGCGCCGGGTGGCGGAGGCCGAGGCGGTCATCGGGCC
GTGCCTGGGGCCCGAGGCCCGTGCCCGGGCCCTGGCCCGCGGAGCCGGCC
CGGCGAACTCGGTCTAA

SEQ ID NO. 6: the amino acid sequence of ICP34.5 in the 17TermA strain.

MARRRRHRGPRRPRPPGPTGAVPTAQSQVT*

(*Stop codon – sequences after * are predicted no expressed)

SEQ ID NO. 7: the DNA sequence of ICP34.5 in the rRp450 strain.

ATGGCCCGCCGCCGCCATCGCGGCCCCCGCCGCCCCCGGCCGCCCGGGCC
CACGGGCGCGGTCCCAACCGCACAGTCCCAGGTAACCTCCACGCCCAACT
CGGAACCCGTGGTCAGGAGCGCGCCCGCGGCCGCCCCGCCGCCGCCCCCC
GCCAGTGGGCCCCCGCCTTCTTGTTCGCTGCTGCTGCGCCAGTGGCTCCA
CGTTCCCGAGTCCGCGTCCGACGACGACGACGACGACTGGCCGGACAGCC
CCCCGCCCGAGCCGGCGCCAGAGGCCCGGCCCACCGCCGCCGCCCCCCGC
CCCCGGTCCCCACCGCCCGGCGCGGGCCCGGGGGGGCGGGGCTAACCCCTC
CCCCCCCCCCTCACGCCCCTTCCGCCTTCCGCCGCGCCTCGCCCTCCGCC
TGCGCGTCACCGCAGAGCACCTGGCGCGCCTGCGCCTGCGACGCGCGGGC
GGGGAGGGGGCGCCGAAGCCCCCCGCGACCCCCGCGACCCCCGCGACCCC
CACGCGGGTGCGCTTCTCGCCCCACGTCCGGGTGCGCCACCTGGTGGTCT
GGGCCTCGGCCGCCCGCCTGGCGCGCCGCGGCTCGTGGGCCCGCGAGCGG
GCCGACCGGGCTCGGTTCCGGCGCCGGGTGGCGGAGGCCGAGGCGGTCAT
CGGGCCGTGCCTGGGGCCCGAGGCCCGTGCCCGGGCCCTGGCCCGCGGAG
CCGGCCCGGCGAACTCGGTCTAA

SEQ ID NO. 8: the amino acid sequence of ICP34.5 in the rRp450 strain.

MARRRHRGPRRPRPPGPTGAVPTAQSQVTSTPNSEPVVRSAPAAAPPPPP
ASGPPPSCSLLLRQWLHVPESASDDDDDDWPDSPPPEPAPEARPTAAAPR
PRSPPPGAGPGGGANPSPPPSRPFRLPPRLALRLRVTAEHLARLRLRRAG
GEGAPKPPATPATPATPTRVRFSPHVRVRHLVVWASAARLARRGSWARER
ADRARFRRRVAEAEAVIGPCLGPEARARALARGAGPANSV

SEQ ID NO. 9: the DNA sequence of ICP34.5 in the wildtype 17 strain.

ATGGCCCGCCGCCGCCGCCATCGCGGCCCCCGCCGCCCCCGGCCGCCCGG
GCCCACGGGCGCGGTCCCAACCGCACAGTCCCAGGTAACCTCCACGCCCA
ACTCGGAACCCGTGGTCAGGAGCGCGCCCGCGGCCGCCCCGCCGCCGCCC
CCCGCCAGTGGGCCCCCGCCTTCTTGTTCGCTGCTGCTGCGCCAGTGGCT
CCACGTTCCCGAGTCCGCGTCCGACGACGACGACGACGACTGGCCGGACA
GCCCCCCGCCCGAGCCGGCGCCAGAGGCCCGGCCCACCGCCGCCGCCCCC
CGCCCCCGGTCCCCACCGCCCGGCGCGGGCCCGGGGGGCGGGGCTAACCC
CTCCCACCCCCCCCTCACGCCCCTTCCGCCTTCCGCCGCGCCTCGCCCTCC
GCCTGCGCGTCACCGCAGAGCACCTGGCGCGCCTGCGCCTGCGACGCGCG
GGCGGGGAGGGGGCGCCGAAGCCCCCCGCGACCCCCGCGACCCCCGCGAC
CCCCACGCGGGTGCGCTTCTCGCCCCACGTCCGGGTGCGCCACCTGGTGG
TCTGGGCCTCGGCCGCCCGCCTGGCGCGCCGCGGCTCGTGGGCCCGCGAG
CGGGCCGACCGGGCTCGGTTCCGGCGCCGGGTGGCGGAGGCCGAGGCGGT
CATCGGGCCGTGCCTGGGGCCCGAGGCCCGTGCCCGGGCCCTGGCCCGCG
GAGCCGGCCCGGCGAACTCGGTCTAA

SEQ ID NO. 51: the DNA sequence of ICP34.5 in the wild type 17 strain.

ATGGCCCGCCGCCGCCGCCATCGCGGCCCCCGCCGCCCCCGGCCGCCCGGGCCCACGGGCGC
CGTCCCAACCGCACAGTCCCAGGTAACCTCCACGCCCAACTCGGAACCCGCGGTCAGGAGCG

CGCCCGCGGCCGCCCCGCCGCCGCCCCCCGCCGGTGGGCCCCCGCCTTCTTGTTCGCTGCTG
CTGCGCCAGTGGCTCCACGTTCCCGAGTCCGCGTCCGACGACGACGATGACGACGACTGGCC
GGACAGCCCCCCGCCCGAGCCGGCGCCAGAGGCCCGGCCCACCGCCGCCGCCCCCCGGCCCC
GGCCCCCACCGCCCGGCGTGGGCCCGGGGGGCGGGGCTGACCCCTCCCACCCCCCCTCGCGC
CCCTTCCGCCTTCCGCCGCGCCTCGCCCTCCGCCTGCGCGTCACCGCGGAGCACCTGGCGCG
CCTGCGCCTGCGACGCGCGGGCGGGGAGGGGGCGCCGGAGCCCCCCGCGACCCCCGCGACCC
CCGCGACCCCCCGCGACCCCCCGCGACCCCCGCGCGGGTGCGCTTCTCGCCCCACGTCCGGGTG
CGCCACCTGGTGGTCTGGGCCTCGGCCGCCCGCCTGGCGCGCCGCGGCTCGTGGGCCCGCGA
GCGGGCCGACCGGGCTCGGTTCCGGCGCCGGGTGGCGGAGGCCGAGGCGGTCATCGGGCCGT
GCCTGGGGCCCGAGGCCCGTGCCCGGGCCCTGGCCCGCGGAGCCGGCCCGGCGAACTCGGTC
TAA

SEQ ID NO. 10: the amino acid sequence of ICP34.5 in the wildtype 17 strain.

MARRRRHRGPRRPRPPGPTGAVPTAQSQVTSTPNSEPVVRSAPAAAPPPP
PASGPPPSCSLLLRQWLHVPESASDDDDDDWPDSPPPEPAPEARPTAAAP
RPRSPPPGAGPGGGANPSHPPSRPFRLPPRLALRLRVTAEHLARLRLRRA
GGEGAPKPPATPATPATPTRVRFSPHVRVRHLVVWASAARLARRGSWARE
RADRARFRRRVAEAEAVIGPCLGPEARARALARGAGPANSV

SEQ ID NO. 52: the amino acid sequence of ICP34.5 in the wildtype 17 strain.
MARRRRHRGPRRPRPPGPTGAVPTAQSQVTSTPNSEPAVRSAPA
AAPPPPPAGGPPPSCSLLLRQWLHVPESASDDDDDDDWPDSPPPEPAPEARPTAAAPR
PRPPPPGVGPGGGADPSHPPSRPFRLPPRLALRLRVTAEHLARLRLRRAGGEGAPEPP
ATPATPATPATPATPARVRFSPHVRVRHLVVWASAARLARRGSWARERADRARFRRRV
AEAEAVIGPCLGPEARARALARGAGPANSV

SEQ ID NO: 11: the DNA sequence of EGFP cassette.

AACACATTAATTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATA
AAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGT
TACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTC
ACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATG
TCTGCTCGAAGCGGCCGGCCGCCCCGACTCTAGACTACACATTGATCCTA
GCAGAAGCACAGGCTGCAGGGTGACGGTCCATCCCGCTCTCCTGGGCACA
AGACATGGGCAGCGTGCCATCATCCTGCTCCTCCACCTCCGGCGGGAAGC
CATGGCTAAGCTTCTTGTACAGCTCGTCCATGCCGAGAGTGATCCCGGCG
GCGGTCACGAACTCCAGCAGGACCATGTGATCGCGCTTCTCGTTGGGGTC
TTTGCTCAGGGCGGACTGGGTGCTCAGGTAGTGGTTGTCGGGCAGCAGCA
CGGGGCCGTCGCCGATGGGGGTGTTCTGCTGGTAGTGGTCGGCGAGCTGC
ACGCTGCCGTCCTCGATGTTGTGGCGGATCTTGAAGTTCACCTTGATGCC
GTTCTTCTGCTTGTCGGCCATGATATAGACGTTGTGGCTGTTGTAGTTGT
ACTCCAGCTTGTGCCCCAGGATGTTGCCGTCCTCCTTGAAGTCGATGCCC
TTCAGCTCGATGCGGTTCACCAGGGTGTCGCCCTCGAACTTCACCTCGGC
GCGGGTCTTGTAGTTGCCGTCGTCCTTGAAGAAGATGGTGCGCTCCTGGA
CGTAGCCTTCGGGCATGGCGGACTTGAAGAAGTCGTGCTGCTTCATGTGG

TCGGGGTAGCGGCTGAAGCACTGCACGCCGTAGGTCAGGGTGGTCACGAG
GGTGGGCCAGGGCACGGGCAGCTTGCCGGTGGTGCAGATGAACTTCAGGG
TCAGCTTGCCGTAGGTGGCATCGCCCTCGCCCTCGCCGGACACGCTGAAC
TTGTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCACCACCCC
GGTGAACAGCTCCTCGCCCTTGCTCACCATCCGGGAATTGCGGCCGCGGG
TACAATTCCGCAGCTTTTAGAGCAGAAGTAACACTTCCGTACAGGCCTAG
AAGTAAAGGCAACATCCACTGAGGAGCAGTTCTTTGATTTGCACCACCAC
CGGATCCGGGACCTGAAATAAAAGACAAAAAGACTAAACTTACCAGTTAA
CTTTCTGGTTTTTCAGTTCCTCGAGTACCGGATCCTCTAGAGTCCGGAGG
CTGGATCGGTCCCGGTGTCTTCTATGGAGGTCAAAACAGCGTGGATGGCG
TCTCCAGGCGATCTGACGGTTCACTAAACGAGCTCTGCTTATATAGACCT
CCCACCGTACACGCCTACCGCCCATTTGCGTCAATGGGGCGGAGTTGTTA
CGACATTTTGGAAAGTCCCGTTGATTTTGGTGCCAAAACAAACTCCCATT
GACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATC
CACGCCCATTGATGTACTGCCAAAACCGCATCACCATGGTAATAGCGATG
ACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTA
CTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGG
GCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTAC
CGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTAC
TATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCGTTGGGCG
GTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGACCTGCAGGCAT
GCAAGCTCGAATTCGAACACGCAGATGCAGTCGGGGCGGCAGATCTTAAT
TAATGGCTGGTTGTTTGTTGT

SEQ ID NO: 12: The DNA sequence of gE (glycoprotein E) in Mut-3, Mut-3Δ34.5 or Mut-3ΔICP6 viral strain. The g451a mutation is shown in bold and italic in the sequence below.

ATGGATCGCGGGGCGGTGGTGGGGTTTCTTCTCGGTGTTTGTGTTGTATC
GTGCTTGGCGGGAACGCCCAAAACGTCCTGGAGACGGGTGAGTGTCGGCG
AGGACGTTTCGTTGCTTCCAGCTCCGGGGCCTACGGGGCGCGGCCCGACC
CAGAAACTACTATGGGCCGTGGAACCCCTGGATGGGTGCGGCCCCTTACA
CCCGTCGTGGGTCTCGCTGATGCCCCCCAAGCAGGTGCCCGAGACGGTCG
TGGATGCGGCGTGCATGCGCGCTCCGGTCCCGCTGGCGATGGCGTACGCC
CCCCCGGCCCCATCTGCGACCGGGGGTCTACGAACGGACTTCGTGTGGCA
GGAGCGCGCGGCCGTGGTTAACCGGAGTCTGGTTATTCACGGGGTCCGAG
AGACGGACAGCGGCCTGTATACCCTGTCCGTGGGCGACATAAAGGACCCG
ACTCGCCAAGTGGCCTCGGTGGTCCTGGTGGTGCAACCGGCCCCAGTTCC
GACCCCACCCCCGACCCCAGCCGATTACGACGAGGATGACAATGACGAGG
GCGAGGACGAAAGTCTCGCCGGCACTCCCGCCAGCGGGACCCCCCGGCTC
CCGCCTCCCCCCGCCCCCCCGAGGTCTTGGCCCAGCGCCCCCGAAGTCTC
ACATGTGCGTGGGGTGACCGTGCGTATGGAGACTCCGGAAGCTATCCTGT
TTTCCCCCGGGGAGACGTTCAGCACGAACGTCTCCATCCATGCCATCGCC
CACGACGACCAGACCTACTCCATGGACGTCGTCTGGTTGAGGTTCGACGT
GCCGACCTCGTGTGCCGAGATGCGAATATACGAATCGTGTCTGTATCACC
CGCAGCTCCCAGAATGTCTGTCCCCGGCCGACGCGCCGTGCGCCGCGAGT

ACGTGGACGTCTCGCCTGGCCGTCCGCAGCTACGCGGGGTGTTCCAGAAC
AAACCCCCCACCGCGCTGTTCGGCCGAGGCTCACATGGAGCCCGTCCCGG
GGCTGGCGTGGCAGGCGGCCTCCGTCAATCTGGAGTTCCGGGACGCGTCC
CCACAACACTCCGGCCTGTATCTGTGTGTGGTGTACGTCAACGACCATAT
TCACGCCTGGGGCCACATTACCATCAGCACCGCGGCGCAGTACCGGAACG
CGGTGGTGGAACAGCCCCTCCCACAGCGCGGCGCGGATTTGGCCGAGCCC
ACCCACCCGCACGTCGGGGCCCCTCCCCACGCGCCCCCAACCCACGGCGC
CCTGCGGTTAGGGGCGGTGATGGGGGCCGCCCTGCTGCTGTCTGCGCTGG
GGTTGTCGGTGTGGGCGTGTATGACCTGTTGGCGCAGGCGTGCCTGGCGG
GCGGTTAAAAGCAGGGCCTCGGGTAAGGGGCCCACGTACATTCGCGTGGC
CGACAGCGAGCTGTACGCGGACTGGAGCTCGGACAGCGAGGGAGAACGCG
ACCAGGTCCCGTGGCTGGCCCCCCCGGAGAGACCCGACTCTCCCTCCACC
AATGGATCCGGCTTTGAGATCTTATCACCAACGGCTCCGTCTGTATACCC
CCGTAGCGACGGGCATCAATCTCGCCGCCAGCTCACAACCTTTGGATCCG
GAAGGCCCGATCGCCGTTACTCCCAGGCCTCCGATTCGTCCGTCTTCTGG
TAA

SEQ ID NO: 13: The amino acid sequence of gE (glycoprotein E) in Mut-3, Mut-3Δ34.5 or Mut-3ΔICP6 viral strain. The A151T mutation is shown in bold and italic in the sequence below.

MDRGAVVGFLLGVCVVSCLAGTPKTSWRRVSVGEDVSLLPAPGPTGRGPT
QKLLWAVEPLDGCGPLHPSWVSLMPPKQVPETVVDAACMRAPVPLAMAYA
PPAPSATGGLRTDFVWQERAAVVNRSLVIHGVRETDSGLYTLSVGDIKDP
TRQVASVVLVVQPAPVPTPPPTPADYDEDDNDEGEDESLAGTPASGTPRL
PPPPAPPRSWPSAPEVSHVRGVTVRMETPEAILFSPGETFSTNVSIHAIA
HDDQTYSMDVVWLRFDVPTSCAEMRIYESCLYHPQLPECLSPADAPCAAS
TWTSRLAVRSYAGCSRTNPPPRCSAEAHMEPVPGLAWQAASVNLEFRDAS
PQHSGLYLCVVYVNDHIHAWGHITISTAAQYRNAVVEQPLPQRGADLAEP
THPHVGAPPHAPPTHGALRLGAVMGAALLLSALGLSVWACMTCWRRRAWR
AVKSRASGKGPTYIRVADSELYADWSSDSEGERDQVPWLAPPERPDSPST
NGSGFEILSPTAPSVYPRSDGHQSRRQLTTFGSGRPDRRYSQASDSSVFW

SEQ ID NO: 14: the DNA sequence of gE in the 17TermA strain.

ATGGATCGCGGGGCGGTGGTGGGGTTTCTTCTCGGTGTTTGTGTTGTATC
GTGCTTGGCGGGAACGCCCAAAACGTCCTGGAGACGGGTGAGTGTCGGCG
AGGACGTTTCGTTGCTTCCAGCTCCGGGGCCTACGGGGCGCGGCCCGACC
CAGAAACTACTATGGGCCGTGGAACCCCTGGATGGGTGCGGCCCCTTACA
CCCGTCGTGGGTCTCGCTGATGCCCCCCAAGCAGGTGCCCGAGACGGTCG
TGGATGCGGCGTGCATGCGCGCTCCGGTCCCGCTGGCGATGGCGTACGCC
CCCCCGGCCCCATCTGCGACCGGGGGTCTACGAACGGACTTCGTGTGGCA
GGAGCGCGCGGCCGTGGTTAACCGGAGTCTGGTTATTCACGGGGTCCGAG
AGACGGACAGCGGCCTGTATACCCTGTCCGTGGGCGACATAAAGGACCCG

GCTCGCCAAGTGGCCTCGGTGGTCCTGGTGGTGCAACCGGCCCCAGTTCC
GACCCCACCCCCGACCCCAGCCGATTACGACGAGGATGACAATGACGAGG
GCGAGGACGAAAGTCTCGCCGGCACTCCCGCCAGCGGGACCCCCCGGCTC
CCGCCTCCCCCCGCCCCCCCGAGGTCTTGGCCCAGCGCCCCCGAAGTCTC
ACATGTGCGTGGGGTGACCGTGCGTATGGAGACTCCGGAAGCTATCCTGT
TTTCCCCCGGGGAGACGTTCAGCACGAACGTCTCCATCCATGCCATCGCC
CACGACGACCAGACCTACTCCATGGACGTCGTCTGGTTGAGGTTCGACGT
GCCGACCTCGTGTGCCGAGATGCGAATATACGAATCGTGTCTGTATCACC
CGCAGCTCCCAGAATGTCTGTCCCCGGCCGACGCGCCGTGCGCCGCGAGT
ACGTGGACGTCTCGCCTGGCCGTCCGCAGCTACGCGGGGTGTTCCAGAAC
AAACCCCCCACCGCGCTGTTCGGCCGAGGCTCACATGGAGCCCGTCCCGG
GGCTGGCGTGGCAGGCGGCCTCCGTCAATCTGGAGTTCCGGGACGCGTCC
CCACAACACTCCGGCCTGTATCTGTGTGTGGTGTACGTCAACGACCATAT
TCACGCCTGGGGCCACATTACCATCAGCACCGCGGCGCAGTACCGGAACG
CGGTGGTGGAACAGCCCCTCCCACAGCGCGGCGCGGATTTGGCCGAGCCC
ACCCACCCGCACGTCGGGGCCCCTCCCCACGCGCCCCCAACCCACGGCGC
CCTGCGGTTAGGGGCGGTGATGGGGGCCGCCCTGCTGCTGTCTGCACTGG
GGTTGTCGGTGTGGGCGTGTATGACCTGTTGGCGCAGGCGTGCCTGGCGG
GCGGTTAAAAGCAGGGCCTCGGGTAAGGGGCCCACGTACATTCGCGTGGC
CGACAGCGAGCTGTACGCGGACTGGAGCTCGGACAGCGAGGGAGAACGCG
ACCAGGTCCCGTGGCTGGCCCCCCCGGAGAGACCCGACTCTCCCTCCACC
AATGGATCCGGCTTTGAGATCTTATCACCAACGGCTCCGTCTGTATACCC
CCGTAGCGATGGGCATCAATCTCGCCGCCAGCTCACAACCTTTGGATCCG
GAAGGCCCGATCGCCGTTACTCCCAGGCCTCCGATTCGTCCGTCTTCTGG
TAA

SEQ ID NO: 15: the amino acid sequence of gE in the 17TermA strain.

MDRGAVVGFLLGVCVVSCLAGTPKTSWRRVSVGEDVSLLPAPGPTGRGPT
QKLLWAVEPLDGCGPLHPSWVSLMPPKQVPETVVDAACMRAPVPLAMAYA
PPAPSATGGLRTDFVWQERAAVVNRSLVIHGVRETDSGLYTLSVGDIKDP
ARQVASVVLVVQPAPVPTPPPTPADYDEDDNDEGEDESLAGTPASGTPRL
PPPPAPPRSWPSAPEVSHVRGVTVRMETPEAILFSPGETFSTNVSIHAIA
HDDQTYSMDVVWLRFDVPTSCAEMRIYESCLYHPQLPECLSPADAPCAAS
TWTSRLAVRSYAGCSRTNPPPRCSAEAHMEPVPGLAWQAASVNLEFRDAS
PQHSGLYLCVVYVNDHIHAWGHITISTAAQYRNAVVEQPLPQRGADLAEP
THPHVGAPPHAPPTHGALRLGAVMGAALLLSALGLSVWACMTCWRRRAWR
AVKSRASGKGPTYIRVADSELYADWSSDSEGERDQVPWLAPPERPDSPST
NGSGFEILSPTAPSVYPRSDGHQSRRQLTTFGSGRPDRRYSQASDSSVFW

SEQ ID NO. 16: the DNA sequence of gE in the rRp450 strain.

ATGGATCGCGGGGCGGTGGTGGGGTTTCTTCTCGGTGTTTGTGTTGTATC
GTGCTTGGCGGGAACGCCCAAAACGTCCTGGAGACGGGTGAGTGTCGGCG
AGGACGTTTCGTTGCTTCCAGCTCCGGGGCCTACGGGGCGCGGCCCGACC
CAGAAACTACTATGGGCCGTGGAACCCCTGGATGGGTGCGGCCCCTTACA

CCCGTCGTGGGTCTCGCTGATGCCCCCCAAGCAGGTGCCCGAGACGGTCG
TGGATGCGGCGTGCATGCGCGCTCCGGTCCCGCTGGCGATGGCGTACGCC
CCCCCGGCCCCATCTGCGACCGGGGGTCTACGAACGGACTTCGTGTGGCA
GGAGCGCGCGGCCGTGGTTAACCGGAGTCTGGTTATTCACGGGGTCCGAG
AGACGGACAGCGGCCTGTATACCCTGTCCGTGGGCGACATAAAGGACCCG
GCTCGCCAAGTGGCCTCGGTGGTCCTGGTGGTGCAACCGGCCCCAGTTCC
GACCCCACCCCCGACCCCAGCCGATTACGACGAGGATGACAATGACGAGG
GCGAGGACGAAAGTCTCGCCGGCACTCCCGCCAGCGGGACCCCCCGGCTC
CCGCCTCCCCCCGCCCCCCCGAGGTCTTGGCCCAGCGCCCCCGAAGTCTC
ACATGTGCGTGGGGTGACCGTGCGTATGGAGACTCCGGAAGCTATCCTGT
TTTCCCCCGGGGAGACGTTCAGCACGAACGTCTCCATCCATGCCATCGCC
CACGACGACCAGACCTACTCCATGGACGTCGTCTGGTTGAGGTTCGACGT
GCCGACCTCGTGTGCCGAGATGCGAATATACGAATCGTGTCTGTATCACC
CGCAGCTCCCAGAATGTCTGTCCCCGGCCGACGCGCCGTGCGCCGCGAGT
ACGTGGACGTCTCGCCTGGCCGTCCGCAGCTACGCGGGGTGTTCCAGAAC
AAACCCCCCACCGCGCTGTTCGGCCGAGGCTCACATGGAGCCCGTCCCGG
GGCTGGCGTGGCAGGCGGCCTCCGTCAATCTGGAGTTCCGGGACGCGTCC
CCACAACACTCCGGCCTGTATCTGTGTGTGGTGTACGTCAACGACCATAT
TCACGCCTGGGGCCACATTACCATCAGCACCGCGGCGCAGTACCGGAACG
CGGTGGTGGAACAGCCCCTCCCACAGCGCGGCGCGGATTTGGCCGAGCCC
ACCCACCCGCACGTCGGGGCCCCTCCCCACGCGCCCCCAACCCACGGCGC
CCTGCGGTTAGGGGCGGTGATGGGGGCCGCCCTGCTGCTGTCTGCGCTGG
GGTTGTCGGTGTGGGCGTGTATGACCTGTTGGCGCAGGCGTGCCTGGCGG
GCGGTTAAAAGCAGGGCCTCGGGTAAGGGGCCCACGTACATTCGCGTGGC
CGACAGCGAGCTGTACGCGGACTGGAGCTCGGACAGCGAGGGAGAACGCG
ACCAGGTCCCGTGGCTGGCCCCCCCGGAGAGACCCGACTCTCCCTCCACC
AATGGATCCGGCTTTGAGATCTTATCACCAACGGCTCCGTCTGTATACCC
CCGTAGCGACGGGCATCAATCTCGCCGCCAGCTCACAACCTTTGGATCCG
GAAGGCCCGATCGCCGTTACTCCCAGGCCTCCGATTCGTCCGTCTTCTGG
TAA

SEQ ID NO. 17: the amino acid sequence of gE in the rRp450 strain.

MDRGAVVGFLLGVCVVSCLAGTPKTSWRRVSVGEDVSLLPAPGPTGRGPT
QKLLWAVEPLDGCGPLHPSWVSLMPPKQVPETVVDAACMRAPVPLAMAYA
PPAPSATGGLRTDFVWQERAAVVNRSLVIHGVRETDSGLYTLSVGDIKDP
ARQVASVVLVVQPAPVPTPPPTPADYDEDDNDEGEDESLAGTPASGTPRL
PPPPAPPRSWPSAPEVSHVRGVTVRMETPEAILFSPGETFSTNVSIHAIA
HDDQTYSMDVVWLRFDVPTSCAEMRIYESCLYHPQLPECLSPADAPCAAS
TWTSRLAVRSYAGCSRTNPPPRCSAEAHMEPVPGLAWQAASVNLEFRDAS
PQHSGLYLCVVYVNDHIHAWGHITISTAAQYRNAVVEQPLPQRGADLAEP
THPHVGAPPHAPPTHGALRLGAVMGAALLLSALGLSVWACMTCWRRRAWR
AVKSRASGKGPTYIRVADSELYADWSSDSEGERDQVPWLAPPERPDSPST
NGSGFEILSPTAPSVYPRSDGHQSRRQLTTFGSGRPDRRYSQASDSSVFW

SEQ ID NO. 18: the DNA sequence of gE in the wildtype 17 strain.

ATGGATCGCGGGGCGGTGGTGGGGTTTCTTCTCGGTGTTTGTGTTGTATC
GTGCTTGGCGGGAACGCCCAAAACGTCCTGGAGACGGGTGAGTGTCGGCG
AGGACGTTTCGTTGCTTCCAGCTCCGGGGCCTACGGGGCGCGGCCCGACC
CAGAAACTACTATGGGCCGTGGAACCCCTGGATGGGTGCGGCCCCTTACA
CCCGTCGTGGGTCTCGCTGATGCCCCCCAAGCAGGTGCCCGAGACGGTCG
TGGATGCGGCGTGCATGCGCGCTCCGGTCCCGCTGGCGATGGCGTACGCC
CCCCCGGCCCCATCTGCGACCGGGGGTCTACGAACGGACTTCGTGTGGCA
GGAGCGCGCGGCCGTGGTTAACCGGAGTCTGGTTATTCACGGGGTCCGAG
AGACGGACAGCGGCCTGTATACCCTGTCCGTGGGCGACATAAAGGACCCG
GCTCGCCAAGTGGCCTCGGTGGTCCTGGTGGTGCAACCGGCCCCAGTTCC
GACCCCACCCCCGACCCCAGCCGATTACGACGAGGATGACAATGACGAGG
GCGAGGACGAAAGTCTCGCCGGCACTCCCGCCAGCGGGACCCCCCGGCTC
CCGCCTCCCCCCGCCCCCCCGAGGTCTTGGCCCAGCGCCCCCGAAGTCTC
ACATGTGCGTGGGGTGACCGTGCGTATGGAGACTCCGGAAGCTATCCTGT
TTTCCCCCGGGGAGACGTTCAGCACGAACGTCTCCATCCATGCCATCGCC
CACGACGACCAGACCTACTCCATGGACGTCGTCTGGTTGAGGTTCGACGT
GCCGACCTCGTGTGCCGAGATGCGAATATACGAATCGTGTCTGTATCACC
CGCAGCTCCCAGAATGTCTGTCCCCGGCCGACGCGCCGTGCGCCGCGAGT
ACGTGGACGTCTCGCCTGGCCGTCCGCAGCTACGCGGGGTGTTCCAGAAC
AAACCCCCCACCGCGCTGTTCGGCCGAGGCTCACATGGAGCCCGTCCCGG
GGCTGGCGTGGCAGGCGGCCTCCGTCAATCTGGAGTTCCGGGACGCGTCC
CCACAACACTCCGGCCTGTATCTGTGTGTGGTGTACGTCAACGACCATAT
TCACGCCTGGGGCCACATTACCATCAGCACCGCGGCGCAGTACCGGAACG
CGGTGGTGGAACAGCCCCTCCCACAGCGCGGCGCGGATTTGGCCGAGCCC
ACCCACCCGCACGTCGGGGCCCCTCCCCACGCGCCCCCAACCCACGGCGC
CCTGCGGTTAGGGGCGGTGATGGGGGCCGCCCTGCTGCTGTCTGCGCTGG
GGTTGTCGGTGTGGGCGTGTATGACCTGTTGGCGCAGGCGTGCCTGGCGG
GCGGTTAAAAGCAGGGCCTCGGGTAAGGGGCCCACGTACATTCGCGTGGC
CGACAGCGAGCTGTACGCGGACTGGAGCTCGGACAGCGAGGGAGAACGCG
ACCAGGTCCCGTGGCTGGCCCCCCCGGAGAGACCCGACTCTCCCTCCACC
AATGGATCCGGCTTTGAGATCTTATCACCAACGGCTCCGTCTGTATACCC
CCGTAGCGACGGGCATCAATCTCGCCGCCAGCTCACAACCTTTGGATCCG
GAAGGCCCGATCGCCGTTACTCCCAGGCCTCCGATTCGTCCGTCTTCTGG
TAA

SEQ ID NO. 19: the amino acid sequence of gE in the wildtype 17 strain.

MDRGAVVGFLLGVCVVSCLAGTPKTSWRRVSVGEDVSLLPAPGPTGRGPT
QKLLWAVEPLDGCGPLHPSWVSLMPPKQVPETVVDAACMRAPVPLAMAYA
PPAPSATGGLRTDFVWQERAAVVNRSLVIHGVRETDSGLYTLSVGDIKDP
ARQVASVVLVVQPAPVPTPPPTPADYDEDDNDEGEDESLAGTPASGTPRL
PPPPAPPRSWPSAPEVSHVRGVTVRMETPEAILFSPGETFSTNVSIHAIA
HDDQTYSMDVVWLRFDVPTSCAEMRIYESCLYHPQLPECLSPADAPCAAS
TWTSRLAVRSYAGCSRTNPPPRCSAEAHMEPVPGLAWQAASVNLEFRDAS
PQHSGLYLCVVYVNDHIHAWGHITISTAAQYRNAVVEQPLPQRGADLAEP
THPHVGAPPHAPPTHGALRLGAVMGAALLLSALGLSVWACMTCWRRRAWR
AVKSRASGKGPTYIRVADSELYADWSSDSEGERDQVPWLAPPERPDSPST
NGSGFEILSPTAPSVYPRSDGHQSRRQLTTFGSGRPDRRYSQASDSSVFW

SEQ ID NO. 20: the DNA sequence of ICP0 in Mut 3, Mut-3Δ34.5 and Mut-3ΔICP6 viral strains. The a848c (within intron) and g1712a (for R258H) mutations are shown in bold and italic in the following sequence. Two introns are included (*i.e.*, nucleotide (nt) 58 to nt 861, and nt 1529 to nt 1663 of the following sequence, also see the sequences within the brackets).

ATGGAGCCCCGCCCCGGAGCGAGTACCCGCCGGCCTGAGGGCCGCCCCCA
GCGCGAG(GTGAGGGGCCGGGCGCCATGTCTGGGGCGCCATGTCTGGGGCG
CCATGTCTGGGGCGCCATGTCTGGGGCGCCATGTTGGGGGGCGCCATGTT
GGGGGGCGCCATGTTGGGGGACCCCCGACCCTTACACTGGAACCGGCCGC
CATGTTGGGGGACCCCCACTCATACACGGGAGCCGGGCGCCATGTTGGGG
CGCCATGTTAGGGGGCGTGGAACCCCGTGACACTATATATACAGGGACCG
GGGGCGCCATGTTAGGGGGCGCGGAACCCCCTGACCCTATATATACAGGG
ACCGGGGTCGCCCTGTTAGGGGTCGCCATGTGACCCCCTGACTTTATATA
TACAGACCCCCAACACCTACACATGGCCCCTTTGACTCAGACGCAGGGCC
CGGGGTCGCCGTGGGACCCCCCTGACTCATACACAGAGACACGCCCCCAC
AACAAACACACAGGGACCGGGGTCGCCGTGTTAGGGGGCGTGGTCCCCAC
TGACTCATACGCAGGGCCCCCTTACTCACACGCATCTAGGGGGGTGGGGA
GGAGCCGCCCGCCATATTTGGGGGACGCCGTGGGACCCCCGACTCCGGTG
CGTCTGGAGGGCGGGAGAAGAGGGAAGAAGAGGGGTCGGGATCCAAAGGA
CGGACCCAGACCACCTTTGGTTGCAGACCCCTTTCTCCCCCCTCTTCCGA
GGCCAGCAGGGGGGCAGGACTTTGTGAGGCGGGGGGGGAGGGGGAACTCG
TGGGCGCTGATTGACGCGGGAAATCCCCCCATTCTTACCCGCCCCCC***C***TT
TTTCCCCTCAG)CCCGCCCCGGATGTCTGGGTGTTTCCCTGCGACCGAGAC
CTGCCGGACAGCAGCGACTCGGAGGCGGAGACCGAAGTGGGGGGGCGGGG
GGACGCCGACCACCATGACGACGACTCCGCCTCCGAGGCGGACAGCACGG
ACACGGAACTGTTCGAGACGGGGCTGCTGGGGCCGCAGGGCGTGGATGGG
GGGGCGGTCTCGGGGGGGAGCCCCCCCCGCGAGGAAGACCCCGGCAGTTG
CGGGGGCGCCCCCCCCTCGAGAGGACGGGGGGAGCGACGAGGGCGACGTGT
GCGCCGTGTGCACGGATGAGATCGCGCCCCACCTGCGCTGCGACACCTTC
CCGTGCATGCACCGCTTCTGCATCCCGTGCATGAAAACCTGGATGCAATT
GCGCAACACCTGCCCGCTGTGCAACGCCAAGCTGGTGTACCTGATAGTGG
GCGTGACGCCCAGCGGGTCGTTCAGCACCATCCCGATCGTGAACGACCCC
CAGACCCGCATGGAGGCCGAGGAGGCCGTCAGGGCGGGCACGGCCGTGGA
CTTTATCTGGACGGGCAATCAGCGGTTCGCCCCGCGGTACCTGACCCTGG
GGGGGCACACGGTGAGGGCCCTGTCGCCCACCCACCCGGAACCCACCACG
GACGAGGATGACGACGACCTGGACGACG(GTGAGGCGGGGGGCGGCAAGGA
CCCTGGGGGAGGAGGAGGAGGAGGGGGGGGAGGGAGGAATAGGCGGGCGG
GCGAGGAAAGGGCGGGCCGGGGAGGGGGCGTAACCTGATCGCGCCCCCCG
TTGTCTCTTGCAG)CAGACTACGTACCGCCCGCCCCCCGCCGGACGCCCCG
CGCCCCCCCAC**A**CAGAGGCGCCGCCGCGCCCCCCGTGACGGGCGGGGCGT
CTCACGCAGCCCCCCAGCCGGCCGCGGCTCGGACAGCGCCCCCCTCGGCG
CCCATCGGGCCACACGGCAGCAGTAACACCAACACCACCACCAACAGCAG
CGGCGGCGGCGGCTCCCGCCAGTCGCGAGCCGCGGCGCCGCGGGGGGCGT
CTGGCCCCTCCGGGGGGGTTGGGGTTGGGGTTGGGGTTGTTGAAGCGGAG
GCGGGGCGGCCGAGGGGCCGGACGGGCCCCCTTGTCAACAGACCCGCCCC
CCTTGCAAACAACAGAGACCCCATAGTGATCAGCGACTCCCCCCCGGCCT
CTCCCCACAGGCCCCCCGCGGCGCCCATGCCAGGCTCCGCCCCCCGCCCC
GGGCCCCCCGCGTCCGCGGCCGCGTCGGGACCCGCGCGCCCCCGCGCGGC
CGTGGCCCCGTGCGTGCGAGCGCCGCCTCCGGGGCCCGGCCCCCGCGCCC
CGGCCCCCGGGGCGGAGCCGGCCGCCCGCCCCGCGGACGCGCGCCGTGTG
CCCCAGTCGCACTCGTCCCTGGCTCAGGCCGCGAACCAAGAACAGAGTCT
GTGCCGGGCGCGTGCGACGGTGGCGCGCGGCTCGGGGGGGCCGGGCGTGG
AGGGTGGGCACGGGCCCTCCCGCGGCGCCGCCCCCTCCGGCGCCGCCCCG
CTCCCCTCCGCCGCCTCTGTCGAGCAGGAGGCGGCGGTGCGTCCGAGGAA

GAGGCGCGGGTCGGGCCAGGAAAACCCCTCCCCCCAGTCCACGCGTCCCC
CCCTCGCGCCGGCAGGGGCCAAGAGGGCGGCGACGCACCCCCCCTCCGAC
TCAGGGCCGGGGGGGCGCGGCCAGGGTGGGCCCGGGACCCCCCTGACGTC
CTCGGCGGCCTCCGCCTCTTCCTCCTCTGCCTCTTCCTCCTCGGCCCCGA
CCCCCGCGGGGGCCGCCTCTTCCGCCGCCGGGGCCGCGTCCTCCTCCGCT
TCCGCCTCCTCGGGCGGGGCCGTCGGTGCCCTGGGAGGGAGACAAGAGGA
AACCTCCCTCGGCCCCCGCGCTGCTTCTGGGCCGCGGGGGCCGAGGAAGT
GTGCCCGGAAGACGCGCCACGCGGAGACTTCCGGGGCCGTCCCCGCGGGC
GGCCTCACGCGCTACCTGCCCATCTCGGGGGTCTCTAGCGTGGTCGCCCT
GTCGCCTTACGTGAACAAGACGATCACGGGGGACTGCCTGCCCATCCTGG
ACATGGAGACGGGGAACATCGGGGCGTACGTGGTCCTGGTGGACCAGACG
GGAAACATGGCGACCCGGCTGCGGGCCGCGGTCCCCGGCTGGAGCCGCCG
CACCCTGCTCCCCGAGACCGCGGGTAACCACGTGATGCCCCCCGAGTACC
CGACGGCCCCCGCGTCGGAGTGGAACAGCCTCTGGATGACCCCCGTGGGG
AACATGCTGTTCGACCAGGGCACCCTAGTGGGCGCCCTGGACTTCCGCAG
CCTGCGGTCTCGGCACCCGTGGTCCGGGGAGCAGGGGGCGTCGACCCGGG
ACGAGGGAAAACAATAA

SEQ ID NO. 21: The amino acid sequence of ICP0 in Mut 3, Mut-3Δ34.5 and Mut-3ΔICP6. The R258H mutation is shown in italic and bold in the sequence below.

MEPRPGASTR RPEGRPQREP APDVWVFPCD RDLPDSSDSE AETEVGGRGD ADHHDDDSAS
EADSTDTELF ETGLLGPQGV DGGAVSGGSP PREEDPGSCG GAPPREDGGS DEGDVCAVCT
DEIAPHLRCD TFPCMHRFCI PCMKTWMQLR NTCPLCNAKL VYLIVGVTPS GSFSTIPIVN
DPQTRMEAEE AVRAGTAVDF IWTGNQRFAP RYLTLGGHTV RALSPTHPEP TTDEDDDDLD
DADYVPPAPR RTPRAPP***H***RG AAAPPVTGGA SHAAPQPAAA RTAPPSAPIG PHGSSNTNTT
TNSSGGGGSR QSRAAAPRGA SGPSGGVGVG VGVVEAEAGR PRGRTGPLVN RPAPLANNRD
PIVISDSPPA SPHRPPAAPM PGSAPRPGPP ASAAASGPAR PRAAVAPCVR APPPGPGPRA
PAPGAEPAAR PADARRVPQS HSSLAQAANQ EQSLCRARAT VARGSGGPGV EGGHGPSRGA
APSGAAPLPS AASVEQEAAV RPRKRRGSGQ ENPSPQSTRP PLAPAGAKRA ATHPPSDSGP
GGRGQGGPGT PLTSSAASAS SSSASSSSAP TPAGAASSAA GAASSSASAS SGGAVGALGG
RQEETSLGPR AASGPRGPRK CARKTRHAET SGAVPAGGLT RYLPISGVSS VVALSPYVNK
TITGDCLPIL DMETGNIGAY VVLVDQTGNM ATRLRAAVPG WSRRTLLPET AGNHVMPPEY
PTAPASEWNS LWMTPVGNML FDQGTLVGAL DFRSLRSRHP WSGEQGASTR DEGKQ

SEQ ID NO. 22: the DNA sequence of ICP0 in the 17TermA strain. Two introns are included (*i.e.*, nucleotide (nt) 58 to nt 822, and nt 1490 to nt 1625 of the following sequence, also see the sequences within the brackets)

ATGGAGCCCCGCCCCGGAGCGAGTACCCGCCGGCCTGAGGGCCGCCCCCA
GCGCGAG(GTGAGGGGCCGGGCGCCATGTCTGGGGCGCCATATTGGGGGGC
GCCATATTGGGGGGCGCCATGTTGGGGGACCCCCGACCCTTACACTGGAA
CCGGCCGCCATGTTGGGGGACCCCCACTCATACACGGGAGCCGGGCGCCA
TGTTGGGGCGCCATGTTAGGGGGCGTGGAACCCCGTGACACTATATATAC
AGGGACCGGGGGCGCCATGTTAGGGGGTGCGGAACCCCCTGACCCTATAT
ATACAGGGACCGGGGTCGCCCTGTTGGGGGTCGCCATGTGACCCCCTGAC
TTTATATATACAGACCCCCAACACATACACATGGCCCCTTTGACTCAGAC
GCAGGGCCCGGGGTCGCCGTGGGACCCCCTGACTCATACACAGAGACACG
CCCCCACAACAAACACACAGGGACCGGGGTCGCCGTGTTGGGGGCGTGGT
CCCCACTGACTCATACGCAGGCCCCCCTTACTCACACGCATCTAGGGGGG
TGGGGAGGAGCCGCCCGCCATATTTGGGGGACGCCGTGGGACCCCCGACT
CCGGTGCGTCTGGAGGGCGGGAGAAGAGGGAAGAAGAGGGGTCGGGATCC
AAAGGACGGACCCAGACCACCTTTGGTTGCAGACCCCTTTCTCCCCCCTC

TTCCGAGGCCAGCAGGGGGGCAGGACTTTGTGAGGCGGGGGGGGGAGAGG
GGGAACTCGTGGGTGCTGATTGACGCGGGAAATCCCCCCCCCATTCTTACC
CGCCCCCCTTTTTTCCCCTTAG)CCCGCCCCGGATGTCTGGGTGTTTCCCT
GCGACCGAGACCTGCCGGACAGCAGCGACTCTGAGGCGGAGACCGAAGTG
GGGGGGCGGGGGGACGCCGACCACCATGACGACGACTCCGCCTCCGAGGC
GGACAGCACGGACACGGAACTGTTCGAGACGGGGCTGCTGGGGCCGCAGG
GCGTGGATGGGGGGGCGGTCTCGGGGGGGAGCCCCCCCCGCGAGGAAGAC
CCCGGCAGTTGCGGGGGCGCCCCCCCTCGAGAGGACGGGGGGAGCGACGA
GGGTGACGTGTGCGCCGTGTGCACGGATGAGATCGCGCCCCACCTGCGCT
GCGACACCTTCCCGTGCATGCACCGCTTCTGCATCCCGTGCATGAAAACC
TGGATGCAATTGCGCAACACCTGCCCGCTGTGCAACGCCAAGCTGGTGTA
CCTGATAGTGGGCGTGACGCCCAGCGGGTCGTTCAGCACCATCCCGATCG
TGAACGACCCCCAGACCCGCATGGAGGCCGAGGAGGCCGTCAGGGCGGGC
ACGGCCGTGGACTTTATCTGGACGGGCAATCAGCGGTTCGCCCCGCGGTA
CCTGACCCTGGGGGGGCACACGGTGAGGGCCCTGTCGCCCACCCACCCGG
AACCCACCACGGACGAGGATGACGACGACCTGGACGACG(GTGAGGCGGGG
GGCGGCAAGGACCCTGGGGGAGGAGGAGGAGGAGGGGGGGGGAGGGAGGA
ATAGGCGGGCGGGCGAGGAAAGGGCGGGCCGGGGAGGGGGCGTAACCTGA
TCGCGCCCCCCGTTGTCTCTTGCAG)CAGACTACGTACCGCCCGCCCCCCG
CCGGACGCCCCGCGCCCCCCCACGCAGAGGCGCCGCCGCGCCCCCCGTGA
CGGGCGGGGCGTCTCACGCAGCCCCCCAGCCGGCCGCGGCTCGGACAGCG
CCCCCCTCGGCGCCCATCGGGCCACACGGCAGCAGTAACACCAACACCAC
CACCAACAGCAGCGGCGGCGGCGGCTCCCGCCAGTCGCGAGCCGCGGCGC
CGCGGGGGGCGTCTGGCCCCTCCGGGGGGGGTTGGGGTTGGGGTTGGGGTT
GTTGAAGCGGAGGCGGGGCGGCCGAGGGGCCGGACGGGCCCCCTTGTCAA
CAGACCCGCCCCCCCTTGCAAACAACAGAGACCCCATAGTGATCAGCGACT
CCCCCCCGGCCTCTCCCCACAGGCCCCCCGCGGCGCCCATGCCAGGCTCC
GCCCCCCGCCCCGGGCCCCCCCGCGTCCGCGGCCGCGTCGGGACCCGCGCG
CCCCCGCGCGGCCGTGGCCCCGTGCGTGCGAGCGCCGCCTCCGGGGCCCG
GCCCCCGCGCCCCGGCCCCCGGGGCGGAGCCGGCCGCCCGCCCCGCGGAC
GCGCGCCGTGTGCCCCAGTCGCACTCGTCCCTGGCTCAGGCCGCGAACCA
AGAACAGAGTCTGTGCCGGGCGCGTGCGACGGTGGCGCGCGGCTCGGGGG
GGCCGGGCGTGGAGGGTGGGCACGGGCCCTCCCGCGGCGCCGCCCCCTCC
GGCGCCGCCCCGCTCCCCTCCGCCGCCTCTGTCGAGCAGGAGGCGGCGGT
GCGTCCGAGGAAGAGGCGCGGGTCGGGCCAGGAAAACCCCTCCCCCCAGT
CCACGCGTCCCCCCCCTCGCGCCGGCAGGGGCCAAGAGGGCGGCGACGCAC
CCCCCCTCCGACTCAGGGCCGGGGGGGCGCGGCCAGGGTGGGCCCGGGAC
CCCCCTGACGTCCTCGGCGGCCTCCGCCTCTTCCTCCTCTGCCTCTTCCT
CCTCGGCCCCGACCCCCGCGGGGGCCGCCTCTTCCGCCGCCGGGGCCGCG
TCCTCCTCCGCTTCCGCCTCCTCGGGCGGGGCCGTCGGTGCCCTGGGAGG
GAGACAAGAGGAAACCTCCCTCGGCCCCCGCGCTGCTTCTGGGCCGCGGG
GGCCGAGGAAGTGTGCCCGGAAGACGCGCCACGCGGAGACTTCCGGGGCC
GTCCCCGCGGGCGGCCTCACGCGCTACCTGCCCATCTCGGGGGTCTCTAG
CGTGGTCGCCCTGTCGCCTTACGTGAACAAGACTATCACGGGGGACTGCC
TGCCCATCCTGGACATGGAGACGGGGAACATCGGGGCGTACGTGGTCCTG
GTGGACCAGACGGGAAACATGGCGACCCGGCTGCGGGCCGCGGTCCCCGG
CTGGAGCCGCCGCACCCTGCTCCCCGAGACCGCGGGTAACCACGTGATGC
CCCCCGAGTACCCGACGGCCCCCGCGTCGGAGTGGAACAGCCTCTGGATG
ACCCCCGTGGGGAACATGCTGTTCGACCAGGGCACCCTAGTGGGCGCCCT
GGACTTCCGCAGCCTGCGGTCTCGGCACCCGTGGTCCGGGGAGCAGGGGG
CGTCGACCCGGGACGAGGGAAAACAATAA

SEQ ID NO. 23: the amino acid sequence of ICP0 in the 17TermA strain.

MEPRPGASTR RPEGRPQREP APDVWVFPCD RDLPDSSDSE AETEVGGRGD ADHHDDDSAS
EADSTDTELF ETGLLGPQGV DGGAVSGGSP PREEDPGSCG GAPPREDGGS DEGDVCAVCT
DEIAPHLRCD TFPCMHRFCI PCMKTWMQLR NTCPLCNAKL VYLIVGVTPS GSFSTIPIVN
DPQTRMEAEE AVRAGTAVDF IWTGNQRFAP RYLTLGGHTV RALSPTHPEP TTDEDDDDLD
DADYVPPAPR RTPRAPPRRG AAAPPVTGGA SHAAPQPAAA RTAPPSAPIG PHGSSNTNTT
TNSSGGGGSR QSRAAAPRGA SGPSGGVGVG VGVVEAEAGR PRGRTGPLVN RPAPLANNRD
PIVISDSPPA SPHRPPAAPM PGSAPRPGPP ASAAASGPAR PRAAVAPCVR APPPGPGPRA
PAPGAEPAAR PADARRVPQS HSSLAQAANQ EQSLCRARAT VARGSGGPGV EGGHGPSRGA
APSGAAPLPS AASVEQEAAV RPRKRRGSGQ ENPSPQSTRP PLAPAGAKRA ATHPPSDSGP
GGRGQGGPGT PLTSSAASAS SSSASSSSAP TPAGAASSAA GAASSSASAS SGGAVGALGG
RQEETSLGPR AASGPRGPRK CARKTRHAET SGAVPAGGLT RYLPISGVSS VVALSPYVNK
TITGDCLPIL DMETGNIGAY VVLVDQTGNM ATRLRAAVPG WSRRTLLPET AGNHVMPPEY
PTAPASEWNS LWMTPVGNML FDQGTLVGAL DFRSLRSRHP WSGEQGASTR DEGKQ

SEQ ID NO. 24: the DNA sequence of ICP0 in the rRp450 strain. Two introns are included (*i.e.*, nucleotide (nt) 58 to nt 862, and nt 1530 to nt 1668 of the following sequence, also see the sequences within the brackets)

ATGGAGCCCCGCCCCGGAGCGAGTACCCGCCGGCCTGAGGGCCGCCCCCA
GCGCGAG(GTGAGGGGCCGGGCGCCATGTCTGGGGCGCCATGTCTGGGGCG
CCATGTCTGGGGCGCCATGTCTGGGGCGCCATGTTGGGGGGCGCCATGTT
GGGGGGCGCCATGTTGGGGGACCCCCGACCCTTACACTGGAACCGGCCGC
CATGTTGGGGGACCCCCACTCATACACGGGAGCCGGGCGCCATGTTGGGG
CGCCATGTTAGGGGGCGTGGAACCCCGTGACACTATATATACAGGGACCG
GGGGCGCCATGTTAGGGGGCGCGGAACCCCCTGACCCTATATATACAGGG
ACCGGGGTCGCCCTGTTAGGGGTCGCCATGTGACCCCCTGACTTTATATA
TACAGACCCCCAACACCTACACATGGCCCCTTTGACTCAGACGCAGGGCC
CGGGGTCGCCGTGGGACCCCCCTGACTCATACACAGAGACACGCCCCCAC
AACAAACACACAGGGACCGGGGTCGCCGTGTTAGGGGGCGTGGTCCCCAC
TGACTCATACGCAGGGCCCCCTTACTCACACGCATCTAGGGGGGTGGGGA
GGAGCCGCCCGCCATATTTGGGGGACGCCGTGGGACCCCCGACTCCGGTG
CGTCTGGAGGGCGGGAGAAGAGGGAAGAAGAGGGGTCGGGATCCAAAGGA
CGGACCCAGACCACCTTTGGTTGCAGACCCCTTTCTCCCCCCTCTTCCGA
GGCCAGCAGGGGGGCAGGACTTTGTGAGGCGGGGGGGGAGGGGGAACTCG
TGGGCGCTGATTGACGCGGGAAATCCCCCCATTCTTACCCGCCCCCCCTT
TTTTCCCCTCAG)CCCGCCCCGGATGTCTGGGTGTTTCCCTGCGACCGAGA
CCTGCCGGACAGCAGCGACTCGGAGGCGGAGACCGAAGTGGGGGGGCGGG
GGGACGCCGACCACCATGACGACGACTCCGCCTCCGAGGCGGACAGCACG
GACACGGAACTGTTCGAGACGGGGCTGCTGGGGCCGCAGGGCGTGGATGG
GGGGGCGGTCTCGGGGGGGAGCCCCCCCCGCGAGGAAGACCCCGGCAGTT
GCGGGGGCGCCCCCCCTCGAGAGGACGGGGGGAGCGACGAGGGCGACGTG
TGCGCCGTGTGCACGGATGAGATCGCGCCCCACCTGCGCTGCGACACCTT
CCCGTGCATGCACCGCTTCTGCATCCCGTGCATGAAAACCTGGATGCAAT
TGCGCAACACCTGCCCGCTGTGCAACGCCAAGCTGGTGTACCTGATAGTG
GGCGTGACGCCCAGCGGGTCGTTCAGCACCATCCCGATCGTGAACGACCC
CCAGACCCGCATGGAGGCCGAGGAGGCCGTCAGGGCGGGCACGGCCGTGG
ACTTTATCTGGACGGGCAATCAGCGGTTCGCCCCGCGGTACCTGACCCTG
GGGGGGCACACGGTGAGGGCCCTGTCGCCCACCCACCCTGAGCCCACCAC
GGACGAGGATGACGACGACCTGGACGACG(GTGAGGCGGGGGGGCGGCGAG
GACCCTGGGGGAGGAGGAGGAGGGGGGGGGGGGGGAGGAATAGGCGGGCG
GGCGGGCGAGGAAAGGGCGGGCCGGGGAGGGGGCGTAACCTGATCGCGCC
CCCCGTTGTCTCTTGCAG)CAGACTACGTACCGCCCGCCCCCCGCCGGACG
CCCCGCGCCCCCCCACGCAGAGGCGCCGCCGCGCCCCCCGTGACGGGCGG

GGCGTCTCACGCAGCCCCCCAGCCGGCCGCGGCTCGGACAGCGCCCCCCT
CGGCGCCCATCGGGCCACACGGCAGCAGTAACACTAACACCACCACCAAC
AGCAGCGGCGGCGGCGGCTCCCGCCAGTCGCGAGCCGCGGTGCCGCGGGG
GGCGTCTGGCCCCTCCGGGGGGGTTGGGGTTGTTGAAGCGGAGGCGGGGC
GGCCGAGGGGCCGGACGGGCCCCCTTGTCAACAGACCCGCCCCCCTTGCA
AACAACAGAGACCCCATAGTGATCAGCGACTCCCCCCCGGCCTCTCCCCA
CAGGCCCCCCGCGGCGCCCATGCCAGGCTCCGCCCCCCGCCCCGGTCCCC
CCGCGTCCGCGGCCGCGTCGGGCCCCGCGCGCCCCCGCGCGGCCGTGGCC
CCGTGTGTGCGGGCGCCGCCTCCGGGGCCCGGCCCCCGCGCCCCGGCCCC
CGGGGCGGAGCCGGCCGCCCGCCCCGCGGACGCGCGCCGTGTGCCCCAGT
CGCACTCGTCCCTGGCTCAGGCCGCGAACCAAGAACAGAGTCTGTGCCGG
GCGCGTGCGACGGTGGCGCGCGGCTCGGGGGGGCCGGGCGTGGAGGGTGG
ACACGGGCCCTCCCGCGGCGCCGCCCCCTCCGGCGCCGCCCCCTCCGGCG
CCCCCCCGCTCCCCTCCGCCGCCTCTGTCGAGCAGGAGGCGGCGGTGCGT
CCGAGGAAGAGGCGCGGGTCGGGCCAGGAAAACCCCTCCCCCCAGTCCAC
GCGTCCCCCCCTCGCGCCGGCAGGGGCCAAGAGGGCGGCGACGCACCCCC
CCTCCGACTCAGGGCCGGGGGGGCGCGGCCAGGGAGGGCCCGGGACCCCC
CTGACGTCCTCGGCGGCCTCCGCCTCTTCCTCCTCCGCCTCTTCCTCCTC
GGCCCCGACTCCCGCGGGGGCCACCTCTTCCGCCACCGGGGCCGCGTCCT
CCTCCGCTTCCGCCTCCTCGGGCGGGGCCGTCGGTGCCCTGGGAGGGAGA
CAAGAGGAAACCTCCCTCGGCCCCCGCGCTGCTTCTGGGCCGCGGGGGCC
GAGGAAGTGTGCCCGGAAGACGCGCCACGCGGAGACTTCCGGGGCCGTCC
CCGCGGGCGGCCTCACGCGCTACCTGCCCATCTCGGGGGTCTCTAGCGTG
GTCGCCCTGTCGCCTTACGTGAACAAGACGATCACGGGGGACTGCCTGCC
CATCCTGGACATGGAGACGGGGAACATCGGGGCGTACGTGGTCCTGGTGG
ACCAGACGGGAAACATGGCGACCCGGCTGCGGGCCGCGGTCCCCGGCTGG
AGCCGCCGCACCCTGCTCCCCGAGACCGCGGGTAACCACGTGACGCCCCC
CGAGTACCCGACGGCCCCCGCGTCGGAGTGGAACAGCCTCTGGATGACCC
CCGTGGGGAACATGCTGTTCGACCAGGGCACCCTAGTGGGCGCCCTGGAC
TTCCGCAGCCTGCGGTCTCGGCACCCGTGGTCCGGGGAGCAGGGGGCGTC
GACCCGGGACGAGGGAAAACAATAA

SEQ ID NO. 25: the DNA sequence of ICP0 in the wildtype 17 strain. Two introns are included (*i.e.*, nucleotide (nt) 58 to nt 861, and nt 1529 to nt 1663 of the following sequence, also see the sequences within the brackets)

ATGGAGCCCCGCCCCGGAGCGAGTACCCGCCGGCCTGAGGGCCGCCCCCA
GCGCGAG(GTGAGGGGCCGGGCGCCATGTCTGGGGCGCCATGTCTGGGGCG
CCATGTCTGGGGCGCCATGTCTGGGGCGCCATGTTGGGGGGCGCCATGTT
GGGGGGCGCCATGTTGGGGGACCCCCGACCCTTACACTGGAACCGGCCGC
CATGTTGGGGGACCCCCACTCATACACGGGAGCCGGGCGCCATGTTGGGG
CGCCATGTTAGGGGGCGTGGAACCCCGTGACACTATATATACAGGGACCG
GGGGCGCCATGTTAGGGGGCGCGGAACCCCCTGACCCTATATATACAGGG
ACCGGGGTCGCCCTGTTAGGGGTCGCCATGTGACCCCCTGACTTTATATA
TACAGACCCCCAACACCTACACATGGCCCCTTTGACTCAGACGCAGGGCC
CGGGGTCGCCGTGGGACCCCCCTGACTCATACACAGAGACACGCCCCCAC
AACAAACACACAGGGACCGGGGTCGCCGTGTTAGGGGGCGTGGTCCCCAC
TGACTCATACGCAGGGCCCCCTTACTCACACGCATCTAGGGGGGTGGGGA
GGAGCCGCCCGCCATATTTGGGGGACGCCGTGGGACCCCCGACTCCGGTG
CGTCTGGAGGGCGGGAGAAGAGGGAAGAAGAGGGGTCGGGATCCAAAGGA
CGGACCCAGACCACCTTTGGTTGCAGACCCCTTTCTCCCCCCTCTTCCGA
GGCCAGCAGGGGGGCAGGACTTTGTGAGGCGGGGGGGGAGGGGGAACTCG
TGGGCGCTGATTGACGCGGGAAATCCCCCCATTCTTACCCGCCCCCCTTT
TTTCCCCTCAG)CCCGCCCCGGATGTCTGGGTGTTTCCCTGCGACCGAGAC
CTGCCGGACAGCAGCGACTCGGAGGCGGAGACCGAAGTGGGGGGGCGGGG

GGACGCCGACCACCATGACGACGACTCCGCCTCCGAGGCGGACAGCACGG
ACACGGAACTGTTCGAGACGGGGCTGCTGGGGCCGCAGGGCGTGGATGGG
GGGGCGGTCTCGGGGGGGAGCCCCCCCCGCGAGGAAGACCCCGGCAGTTG
CGGGGGCGCCCCCCCTCGAGAGGACGGGGGGAGCGACGAGGGCGACGTGT
GCGCCGTGTGCACGGATGAGATCGCGCCCCACCTGCGCTGCGACACCTTC
CCGTGCATGCACCGCTTCTGCATCCCGTGCATGAAAACCTGGATGCAATT
GCGCAACACCTGCCCGCTGTGCAACGCCAAGCTGGTGTACCTGATAGTGG
GCGTGACGCCCAGCGGGTCGTTCAGCACCATCCCGATCGTGAACGACCCC
CAGACCCGCATGGAGGCCGAGGAGGCCGTCAGGGCGGGCACGGCCGTGGA
CTTTATCTGGACGGGCAATCAGCGGTTCGCCCCGCGGTACCTGACCCTGG
GGGGGCACACGGTGAGGGCCCTGTCGCCCACCCACCCGGAACCCACCACG
GACGAGGATGACGACGACCTGGACGACG(GTGAGGCGGGGGGCGGCAAGGA
CCCTGGGGGAGGAGGAGGAGGAGGGGGGGGAGGGAGGAATAGGCGGGCGG
GCGAGGAAAGGGCGGGCCGGGGAGGGGGCGTAACCTGATCGCGCCCCCCG
TTGTCTCTTGCAG)CAGACTACGTACCGCCCGCCCCCCGCCGGACGCCCCG
CGCCCCCCCCACGCAGAGGCGCCGCCGCGCCCCCCGTGACGGGCGGGGCGT
CTCACGCAGCCCCCCAGCCGGCCGCGGCTCGGACAGCGCCCCCCTCGGCG
CCCATCGGGCCACACGGCAGCAGTAACACCAACACCACCACCAACAGCAG
CGGCGGCGGCGGCTCCCGCCAGTCGCGAGCCGCGGCGCCGCGGGGGGCGT
CTGGCCCCTCCGGGGGGGTTGGGGTTGGGGTTGGGGTTGTTGAAGCGGAG
GCGGGGCGGCCGAGGGGCCGGACGGGCCCCCTTGTCAACAGACCCGCCCC
CCTTGCAAACAACAGAGACCCCATAGTGATCAGCGACTCCCCCCCGGCCT
CTCCCCACAGGCCCCCCGCGGCGCCCATGCCAGGCTCCGCCCCCCGCCCC
GGGCCCCCCGCGTCCGCGGCCGCGTCGGGACCCGCGCGCCCCCGCGCGGC
CGTGGCCCCGTGCGTGCGAGCGCCGCCTCCGGGGCCCGGCCCCCGCGCCC
CGGCCCCCGGGGCGGAGCCGGCCGCCCGCCCCGCGGACGCGCGCCGTGTG
CCCCAGTCGCACTCGTCCCTGGCTCAGGCCGCGAACCAAGAACAGAGTCT
GTGCCGGGCGCGTGCGACGGTGGCGCGCGGCTCGGGGGGGCCGGGCGTGG
AGGGTGGGCACGGGCCCTCCCGCGGCGCCGCCCCCTCCGGCGCCGCCCCG
CTCCCCTCCGCCGCCTCTGTCGAGCAGGAGGCGGCGGTGCGTCCGAGGAA
GAGGCGCGGGTCGGGCCAGGAAAACCCCTCCCCCCAGTCCACGCGTCCCC
CCCTCGCGCCGGCAGGGGCCAAGAGGGCGGCGACGCACCCCCCCTCCGAC
TCAGGGCCGGGGGGGCGCGGCCAGGGTGGGCCCGGGACCCCCCTGACGTC
CTCGGCGGCCTCCGCCTCTTCCTCCTCTGCCTCTTCCTCCTCGGCCCCGA
CCCCCGCGGGGGCCGCCTCTTCCGCCGCCGGGGCCGCGTCCTCCTCCGCT
TCCGCCTCCTCGGGCGGGGCCGTCGGTGCCCTGGGAGGGAGACAAGAGGA
AACCTCCCTCGGCCCCCGCGCTGCTTCTGGGCCGCGGGGGCCGAGGAAGT
GTGCCCGGAAGACGCGCCACGCGGAGACTTCCGGGGCCGTCCCCGCGGGC
GGCCTCACGCGCTACCTGCCCATCTCGGGGGTCTCTAGCGTGGTCGCCCT
GTCGCCTTACGTGAACAAGACGATCACGGGGGACTGCCTGCCCATCCTGG
ACATGGAGACGGGGAACATCGGGGCGTACGTGGTCCTGGTGGACCAGACG
GGAAACATGGCGACCCGGCTGCGGGCCGCGGTCCCCGGCTGGAGCCGCCG
CACCCTGCTCCCCGAGACCGCGGGTAACCACGTGATGCCCCCCGAGTACC
CGACGGCCCCCCGCGTCGGAGTGGAACAGCCTCTGGATGACCCCCGTGGGG
AACATGCTGTTCGACCAGGGCACCCTAGTGGGCGCCCTGGACTTCCGCAG
CCTGCGGTCTCGGCACCCGTGGTCCGGGGAGCAGGGGGCGTCGACCCGGG
ACGAGGGAAAACAATAA

SEQ ID NO. 53: the DNA sequence of ICP0 in the wildtype 17 strain. Two introns are included (*i.e.*, nucleotide (nt) 58 to nt 822, and nt 1490 to nt 1625 of the following sequence, also see the sequences within the brackets)

ATGGAGCCCCGCCCCGGAGCGAGTACCCGCCGGCCTGAGGGCCGCCCCCA
GCGCGAG(GTGAGGGGCCGGGCGCCATGTCTGGGGCGCCATATTGGGGGGC
GCCATATTGGGGGGCGCCATGTTGGGGGACCCCCGACCCTTACACTGGAA

CCGGCCGCCATGTTGGGGGACCCCCACTCATACACGGGAGCCGGGCGCCA
TGTTGGGGCGCCATGTTAGGGGGCGTGGAACCCCGTGACACTATATATAC
AGGGACCGGGGGCGCCATGTTAGGGGGTGCGGAACCCCCTGACCCTATAT
ATACAGGGACCGGGGTCGCCCTGTTGGGGGTCGCCATGTGACCCCCTGAC
TTTATATATACAGACCCCCAACACATACACATGGCCCCTTTGACTCAGAC
GCAGGGCCCGGGGGTCGCCGTGGGACCCCCTGACTCATACACAGAGACACG
CCCCCACAACAAACACACAGGGACCGGGGTCGCCGTGTTGGGGGCGTGGT
CCCCACTGACTCATACGCAGGCCCCCCTTACTCACACGCATCTAGGGGGG
TGGGGAGGAGCCGCCCGCCATATTTGGGGGACGCCGTGGGACCCCCGACT
CCGGTGCGTCTGGAGGGCGGGAGAAGAGGGAAGAAGAGGGGTCGGGATCC
AAAGGACGGACCCAGACCACCTTTGGTTGCAGACCCCTTTCTCCCCCCTC
TTCCGAGGCCAGCAGGGGGGCAGGACTTTGTGAGGCGGGGGGGGGAGAGG
GGGAACTCGTGGGTGCTGATTGACGCGGGAAATCCCCCCCCCATTCTTACC
CGCCCCCCTTTTTTCCCCTTAG)CCCGCCCCGGATGTCTGGGTGTTTCCCT
GCGACCGAGACCTGCCGGACAGCAGCGACTCTGAGGCGGAGACCGAAGTG
GGGGGGCGGGGGGGACGCCGACCACCATGACGACGACTCCGCCTCCGAGGC
GGACAGCACGGACACGGAACTGTTCGAGACGGGGCTGCTGGGGCCGCAGG
GCGTGGATGGGGGGGCGGTCTCGGGGGGGAGCCCCCCCCGCGAGGAAGAC
CCCGGCAGTTGCGGGGGCGCCCCCCCTCGAGAGGACGGGGGGAGCGACGA
GGGCGACGTGTGCGCCGTGTGCACGGATGAGATCGCGCCCCACCTGCGCT
GCGACACCTTCCCGTGCATGCACCGCTTCTGCATCCCGTGCATGAAAACC
TGGATGCAATTGCGCAACACCTGCCCGCTGTGCAACGCCAAGCTGGTGTA
CCTGATAGTGGGCGTGACGCCCAGCGGGTCGTTCAGCACCATCCCGATCG
TGAACGACCCCCAGACCCGCATGGAGGCCGAGGAGGCCGTCAGGGCGGGC
ACGGCCGTGGACTTTATCTGGACGGGCAATCAGCGGTTCGCCCCGCGGTA
CCTGACCCTGGGGGGGGCACACGGTGAGGGCCCTGTCGCCCACCCACCCGG
AGCCCACCACGGACGAGGATGACGACGACCTGGACGACG(GTGAGGCGGGG
GGCGGCAAGGACCCTGGGGGAGGAGGAGGAGGAGGGGGGGGGGAGGGAGGA
ATAGGCGGGCGGGCGAGGAAAGGGCGGGCCGGGGAGGGGGCGTAACCTGA
TCGCGCCCCCCGTTGTCTCTTGCAG)CAGACTACGTACCGCCCGCCCCCCG
CCGGACGCCCCGCGCCCCCCCACGCAGAGGCGCCGCCGCGCCCCCCGTGA
CGGGCGGGGCGTCTCACGCAGCCCCCCAGCCGGCCGCGGCTCGGACAGCG
CCCCCCTCGGCGCCCATCGGGCCACACGGCAGCAGTAACACCAACACCAC
CACCAACAGCAGCGGCGGCGGCGGCTCCCGCCAGTCGCGAGCCGCGGCGC
CGCGGGGGGCGTCTGGCCCCTCCGGGGGGGTTGGGGTTGGGGTTGGGGTT
GTTGAAGCGGAGGCGGGGCGGCCGAGGGGCCGGACGGGCCCCCTTGTCAA
CAGACCCGCCCCCCTTGCAAACAACAGAGACCCCATAGTGATCAGCGACT
CCCCCCCGGCCTCTCCCCACAGGCCCCCCGCGGCGCCCATGCCAGGCTCC
GCCCCCCGCCCCGGGCCCCCCGCGTCCGCGGCCGCGTCGGGACCCGCGCG
CCCCCGCGCGGCCGTGGCCCCGTGCGTGCGAGCGCCGCCTCCGGGGCCCG
GCCCCCGCGCCCCGGCCCCCGGGGCGGAGCCGGCCGCCCGCCCCGCGGAC
GCGCGCCGTGTGCCCCAGTCGCACTCGTCCCTGGCTCAGGCCGCGAACCA
AGAACAGAGTCTGTGCCGGGCGCGTGCGACGGTGGCGCGCGGCTCGGGGG
GGCCGGGCGTGGAGGGTGGGCACGGGCCCTCCCGCGGCGCCGCCCCCTCC
GGCGCCGCCCCGCTCCCCTCCGCCGCCTCTGTCGAGCAGGAGGCGGCGGT
GCGTCCGAGGAAGAGGCGCGGGTCGGGCCAGGAAAACCCCTCCCCCCAGT
CCACGCGTCCCCCCCTCGCGCCGGCAGGGGCCAAGAGGGCGGCGACGCAC
CCCCCCTCCGACTCAGGGCCGGGGGGGCGCGGCCAGGGTGGGCCCGGGAC
CCCCCTGACGTCCTCGGCGGCCTCCGCCTCTTCCTCCTCTGCCTCTTCCT
CCTCGGCCCCGACCCCCGCGGGGGCCGCCTCTTCCGCCGCCGGGGCCGCG
TCCTCCTCCGCTTCCGCCTCCTCGGGCGGGGCCGTCGGTGCCCTGGGAGG
GAGACAAGAGGAAACCTCCCTCGGCCCCCGCGCTGCTTCTGGGCCGCGGG
GGCCGAGGAAGTGTGCCCGGAAGACGCGCCACGCGGAGACTTCCGGGGCC
GTCCCCGCGGGCGGCCTCACGCGCTACCTGCCCATCTCGGGGGTCTCTAG
CGTGGTCGCCCTGTCGCCTTACGTGAACAAGACTATCACGGGGGACTGCC
TGCCCATCCTGGACATGGAGACGGGGAACATCGGGGCGTACGTGGTCCTG
GTGGACCAGACGGGAAACATGGCGACCCGGCTGCGGGCCGCGGTCCCCGG

CTGGAGCCGCCGCACCCTGCTCCCCGAGACCGCGGGTAACCACGTGATGC
CCCCCGAGTACCCGACGGCCCCCGCGTCGGAGTGGAACAGCCTCTGGATG
ACCCCCGTGGGGAACATGCTGTTCGACCAGGGCACCCTAGTGGGCGCCCT
GGACTTCCGCAGCCTGCGGTCTCGGCACCCGTGGTCCGGGGAGCAGGGGG
CGTCGACCCGGGACGAGGGAAAACAATAA

SEQ ID NO. 26: the amino acid sequence of ICP0 in the wildtype 17 strain.

MEPRPGASTR RPEGRPQREP APDVWVFPCD RDLPDSSDSE AETEVGGRGD ADHHDDDSAS
EADSTDTELF ETGLLGPQGV DGGAVSGGSP PREEDPGSCG GAPPREDGGS DEGDVCAVCT
DEIAPHLRCD TFPCMHRFCI PCMKTWMQLR NTCPLCNAKL VYLIVGVTPS GSFSTIPIVN
DPQTRMEAEE AVRAGTAVDF IWTGNQRFAP RYLTLGGHTV RALSPTHPEP TTDEDDDDLD
DADYVPPAPR RTPRAPPRRG AAAPPVTGGA SHAAPQPAAA RTAPPSAPIG PHGSSNTNTT
TNSSGGGGSR QSRAAAPRGA SGPSGGVGVG VGVVEAEAGR PRGRTGPLVN RPAPLANNRD
PIVISDSPPA SPHRPPAAPM PGSAPRPGPP ASAAASGPAR PRAAVAPCVR APPPGPGPRA
PAPGAEPAAR PADARRVPQS HSSLAQAANQ EQSLCRARAT VARGSGGPGV EGGHGPSRGA
APSGAAPLPS AASVEQEAAV RPRKRRGSGQ ENPSPQSTRP PLAPAGAKRA ATHPPSDSGP
GGRGQGGPGT PLTSSAASAS SSSASSSSAP TPAGAASSAA GAASSSASAS SGGAVGALGG
RQEETSLGPR AASGPRGPRK CARKTRHAET SGAVPAGGLT RYLPISGVSS VVALSPYVNK
TITGDCLPIL DMETGNIGAY VVLVDQTGNM ATRLRAAVPG WSRRTLLPET AGNHVMPPEY
PTAPASEWNS LWMTPVGNML FDQGTLVGAL DFRSLRSRHP WSGEQGASTR DEGKQ

SEQ ID NO. 27: The DNA sequence of ICP8 from Mut-3, Mut-3Δ34.5 and Mut-3ΔICP6 viral strains. The c3464t mutation is shown in bold and italic in the sequence below.

ATGGAGACAAAGCCCAAGACGGCAACCACCATCAAGGTCCCCCCCGGGCC
CCTGGGATACGTGTACGCTCGCGCGTGTCCGTCCGAAGGCATCGAGCTTC
TGGCGTTACTGTCGGCACGCAGCGGCGATTCCGACGTCGCCGTGGCGCCC
CTGGTCGTGGGCCTGACCGTGGAGAGCGGCTTTGAGGCCAACGTGGCCGT
GGTCGTGGGTTCTCGCACGACGGGGCTCGGGGGTACCGCGGTGTCCCTGA
AACTGACGCCCTCGCACTACAGCTCGTCCGTGTACGTCTTTCACGGCGGC
CGGCACCTGGACCCCAGCACCCAGGCCCCGAACCTGACGCGACTTTGCGA
GCGGGCACGCCGCCATTTTGGCTTTTCGGACTACACCCCCCGGCCCGGCG
ACCTCAAACACGAGACGACGGGGGAGGCGCTGTGTGAGCGCCTCGGCCTG
GACCCGGACCGCGCCCTCCTGTATCTGGTCGTTACCGAGGGCTTCAAGGA
GGCCGTGTGCATCAACAACACCTTTCTGCACCTGGGAGGCTCGGACAAGG
TAACCATAGGCGGGGCGGAGGTGCACCGCATACCCGTGTACCCGTTGCAG
CTGTTCATGCCGGATTTTAGCCGTGTCATCGCAGAGCCGTTCAACGCCAA
CCACCGATCGATCGGGGAGAATTTTACCTACCCGCTTCCGTTTTTTAACC
GCCCCCTCAACCGCCTCCTGTTCGAGGCGGTCGTGGGACCCGCCGCCGTG
GCACTGCGATGCCGAAACGTGGACGCCGTGGCCCGCGCGGCCGCCCACCT
GGCGTTTGACGAAAACCACGAGGGCGCCGCCCTCCCCGCCGACATTACGT
TCACGGCCTTCGAAGCCAGCCAGGGTAAGACCCCGCGGGGCGGGCGCGAC
GGCGGCGGCAAGGGCCCGGCGGGCGGGTTCGAACAGCGCCTGGCCTCCGT
CATGGCCGGAGACGCCGCCCTGGCCCTCGATTCTATCGTGTCGATGGCCG
TCTTTGACGAGCCGCCCACCGACATCTCCGCGTGGCCGCTGTTCGAGGGC
CAGGACACGGCCGCGGCCCGCGCCAACGCCGTCGGGGCGTACCTGGCGCG
CGCCGCGGGACTCGTGGGGGCCATGGTATTTAGCACCAACTCGGCCCTCC
ATCTCACCGAGGTGGACGACGCCGGCCCGGCGGACCCAAAGGACCACAGC
AAACCCTCCTTTTACCGCTTCTTCCTCGTGCCCGGGACCCACGTGGCGGC
CAACCCACAGGTGGACCGCGAGGGACACGTGGTGCCCGGGTTCGAGGGTC
GGCCCACCGCGCCCCTCGTCGGCGGAACCCAGGAATTTGCCGGCGAGCAC
CTGGCCATGCTGTGTGGGTTTTCCCCGGCGCTGCTGGCCAAGATGCTGTT
TTACCTGGAGCGCTGCGACGGCGGCGTGATCGTCGGGCGCCAGGAGATGG

ACGTGTTTCGATACGTCGCGGACTCCAACCAGACCGACGTGCCCTGTAAC
CTATGCACCTTCGACACGCGCCACGCCTGCGTACACACGACGCTCATGCG
CCTCCGGGCGCGCCATCCAAAGTTCGCCAGCGCCGCCCGCGGAGCCATCG
GCGTCTTCGGGACCATGAACAGCATGTACAGCGACTGCGACGTGCTGGGA
AACTACGCCGCCTTCTCGGCCCTGAAGCGCGCGGACGGATCCGAGACCGC
CCGGACCATCATGCAGGAGACGTACCGCGCGGCGACCGAGCGCGTCATGG
CCGAACTCGAGACCCTGCAGTACGTGGACCAGGCGGTCCCCACGGCCATG
GGGCGGCTGGAGACCATCATCACCAACCGCGAGGCCCTGCATACGGTGGT
GAACAACGTCAGGCAGGTCGTGGACCGCGAGGTGGAGCAGCTGATGCGCA
ACCTGGTGGAGGGGAGGAACTTCAAGTTTCGCGACGGTCTGGGCGAGGCC
AACCACGCCATGTCCCTGACGCTGGACCCGTACGCGTGCGGGCCGTGCCC
CCTGCTTCAGCTTCTCGGGCGGCGATCCAACCTCGCCGTGTACCAGGACC
TGGCCCTGAGTCAGTGCCACGGGGTGTTCGCCGGGCAGTCGGTCGAGGGG
CGCAACTTTCGCAATCAATTCCAACCGGTGCTGCGGCGGCGCGTGATGGA
CATGTTTAACAACGGGTTTCTGTCGGCCAAAACGCTGACGGTCGCGCTCT
CGGAGGGGGCGGCTATCTGCGCCCCCAGCCTAACGGCCGGCCAGACGGCC
CCCGCCGAGAGCAGCTTCGAGGGCGACGTTGCCCGCGTGACCCTGGGGTT
TCCCAAGGAGCTGCGCGTCAAGAGCCGCGTGTTGTTCGCGGGCGCGAGCG
CCAACGCGTCCGAGGCCGCCAAGGCGCGGGTCGCCAGCCTCCAGAGCGCC
TACCAGAAGCCCGACAAGCGCGTGGACATCCTCCTCGGACCGCTGGGCTT
TCTGCTGAAGCAGTTCCACGCGGCCATCTTCCCCAACGGCAAGCCCCCGG
GGTCCAACCAGCCGAACCCGCAGTGGTTCTGGACGGCCCTCCAACGCAAC
CAGCTTCCCGCCCGGCTCCTGTCGCGCGAGGACATCGAGACCATCGCGTT
CATTAAAAAGTTTTCCCTGGACTACGGCGCGATAAACTTTATTAACCTGG
CCCCCAACAACGTGAGCGAGCTGGCGATGTACTACATGGCAAACCAGATT
CTGCGGTACTGCGATCACTCGACATACTTCATCAACACCCTTACGGCCAT
CATCGCGGGGTCCCGCCGTCCCCCCAGCGTGCAGGCTGCGGCCGCGTGGT
CCGCGCAGGGCGGGGCGGGCCTGGAGGCCGGGGCCCGCGCGCTGATGGAC
GCCGTGGACGCGCATCCGGGCGCGTGGACGTCCATGTTCGCCAGCTGCAA
CCTGCTGCGGCCCGTCATGGCGGCGCGCCCCATGGTCGTGTTGGGGTTGA
GCATCAGCAAGTACTACGGCATGGCCGGCAACGACCGTGTGTTTCAGGCC
GGGAACTGGGCCAGCCTGATGGGCGGCAAAAACGCGTGCCCGCTCCTTAT
TTTTGACCGCACCCGCAAGTTCGTCCTGGCCTGTCCCCGGGCCGGGTTTG
TGTGCGCGGCCTCAAGCCTCGGCGGCGGAGCGCACGAAAGCTCGCTGTGC
GAGCAGCTCCGGGGCATTATCTCCGAGGGCGGGGCGGCCGTCGCCAGTAG
CGTGTTCGTGGCGACCGTGAAAAGCCTGGGGCCCCGCACCCAGCAGCTGC
AGATCGAGGACTGGCTGGCGCTCCTGGAGGACGAGTACCTAAGCGAGGAG
ATGATGGAGCTGACCGCGCGTGCCCTGGAGCGCGGCAACGGCGAGTGGTC
GACGGACGCGGCCCTGGAGGTGGCGCACGAGGCCGAGGCCCTAGTCAGCC
AACTCGGCAACGCCGGGGAGGTGTTTAACTTTGGGGATTTTGGCTGCGAG
GACGACAACGCGA***T***GCCGTTCGGCGGCCCGGGGGCCCCGGGACCGGCATT
TGCCGGCCGCAAACGGGCGTTCCACGGGGATGACCCGTTTGGGGAGGGGC
CCCCCGACAAAAAGGGAGACCTGACGTTGGATATGCTGTGA

SEQ ID NO. 28: The amino acid sequence of ICP8 in Mut-3, Mut-3Δ34.5 and Mut-3ΔICP6 viral strains. The T1155M mutation is shown in bold and italic in the sequence below.

METKPKTATTIKVPPGPLGYVYARACPSEGIELLALLSARSGDSDVAVAP
LVVGLTVESGFEANVAVVVGSRTTGLGGTAVSLKLTPSHYSSSVYVFHGG
RHLDPSTQAPNLTRLCERARRHFGFSDYTPRPGDLKHETTGEALCERLGL
DPDRALLYLVVTEGFKEAVCINNTFLHLGGSDKVTIGGAEVHRIPVYPLQ
LFMPDFSRVIAEPFNANHRSIGENFTYPLPFFNRPLNRLLFEAVVGPAAV
ALRCRNVDAVARAAAHLAFDENHEGAALPADITFTAFEASQGKTPRGGRD
GGGKGPAGGFEQRLASVMAGDAALALDSIVSMAVFDEPPTDISAWPLFEG
QDTAAARANAVGAYLARAAGLVGAMVFSTNSALHLTEVDDAGPADPKDHS
KPSFYRFFLVPGTHVAANPQVDREGHVVPGFEGRPTAPLVGGTQEFAGEH

LAMLCGFSPALLAKMLFYLERCDGGVIVGRQEMDVFRYVADSNQTDVPCN
LCTFDTRHACVHTTLMRLRARHPKFASAARGAIGVFGTMNSMYSDCDVLG
NYAAFSALKRADGSETARTIMQETYRAATERVMAELETLQYVDQAVPTAM
GRLETIITNREALHTVVNNVRQVVDREVEQLMRNLVEGRNFKFRDGLGEA
NHAMSLTLDPYACGPCPLLQLLGRRSNLAVYQDLALSQCHGVFAGQSVEG
RNFRNQFQPVLRRRVMDMFNNGFLSAKTLTVALSEGAAICAPSLTAGQTA
PAESSFEGDVARVTLGFPKELRVKSRVLFAGASANASEAAKARVASLQSA
YQKPDKRVDILLGPLGFLLKQFHAAIFPNGKPPGSNQPNPQWFWTALQRN
QLPARLLSREDIETIAFIKKFSLDYGAINFINLAPNNVSELAMYYMANQI
LRYCDHSTYFINTLTAIIAGSRRPPSVQAAAAWSAQGGAGLEAGARALMD
AVDAHPGAWTSMFASCNLLRPVMAARPMVVLGLSISKYYGMAGNDRVFQA
GNWASLMGGKNACPLLIFDRTRKFVLACPRAGFVCAASSLGGGAHESSLC
EQLRGIISEGGAAVASSVFVATVKSLGPRTQQLQIEDWLALLEDEYLSEE
MMELTARALERGNGEWSTDAALEVAHEAEALVSQLGNAGEVFNFGDFGCE
DDNA*M*PFGGPGAPGPAFAGRKRAFHGDDPFGEGPPDKKGDLTLDML

SEQ ID NO. 29: the DNA sequence of ICP8 in the 17TermA strain.

ATGGAGACAAAGCCCAAGACGGCAACCACCATCAAGGTCCCCCCCGGGCC
CCTGGGATACGTGTACGCTCGCGCGTGTCCGTCCGAAGGCATCGAGCTTC
TGGCGTTACTGTCGGCACGCAGCGGCGATTCCGACGTCGCCGTGGCGCCC
CTGGTCGTGGGCCTGACCGTGGAGAGCGGCTTTGAGGCCAACGTGGCCGT
GGTCGTGGGTTCTCGCACGACGGGGCTCGGGGGTACCGCGGTGTCCCTGA
AACTGACGCCCTCGCACTACAGCTCGTCCGTGTACGTCTTTCACGGCGGC
CGGCACCTGGACCCCAGCACCCAGGCCCCGAACCTGACGCGACTTTGCGA
GCGGGCACGCCGCCATTTTGGCTTTTCGGACTACACCCCCCGGCCCGGCG
ACCTCAAACACGAGACGACGGGGGAGGCGCTGTGTGAGCGCCTCGGCCTG
GACCCGGACCGCGCCCTCCTGTATCTGGTCGTTACCGAGGGCTTCAAGGA
GGCCGTGTGCATCAACAACACCTTTCTGCACCTGGGAGGCTCGGACAAGG
TAACCATAGGCGGGGCGGAGGTGCACCGCATACCCGTGTACCCGTTGCAG
CTGTTCATGCCGGATTTTAGCCGTGTCATCGCAGAGCCGTTCAACGCCAA
CCACCGATCGATCGGGGAGAATTTTACCTACCCGCTTCCGTTTTTTAACC
GCCCCCTCAACCGCCTCCTGTTCGAGGCGGTCGTGGGACCCGCCGCCGTG
GCACTGCGATGCCGAAACGTGGACGCCGTGGCCCGCGCGGCCGCCCACCT
GGCGTTTGACGAAAACCACGAGGGCGCCGCCCTCCCCGCCGACATTACGT
TCACGGCCTTCGAAGCCAGCCAGGGTAAGACCCCGCGGGGCGGGCGCGAC
GGCGGCGGCAAGGGCCCGGCGGGCGGGTTCGAACAGCGCCTGGCCTCCGT
CATGGCCGGAGACGCCGCCCTGGCCCTCGATTCTATCGTGTCGATGGCCG
TCTTTGACGAGCCGCCCACCGACATCTCCGCGTGGCCGCTGTTCGAGGGC
CAGGACACGGCCGCGGCCCGCGCCAACGCCGTCGGGGCGTACCTGGCGCG
CGCCGCGGGACTCGTGGGGGCCATGGTATTTAGCACCAACTCGGCCCTCC
ATCTCACCGAGGTGGACGACGCCGGCCCGGCGGACCCAAAGGACCACAGC
AAACCCTCCTTTTACCGCTTCTTCCTCGTGCCCGGGACCCACGTGGCGGC
CAACCCACAGGTGGACCGCGAGGGACACGTGGTGCCCGGGTTCGAGGGTC
GGCCCACCGCGCCCCTCGTCGGCGGAACCCAGGAATTTGCCGGCGAGCAC
CTGGCCATGCTGTGTGGGTTTTCCCCGGCGCTGCTGGCCAAGATGCTGTT
TTACCTGGAGCGCTGCGACGGCGGCGTGATCGTCGGGCGCCAGGAGATGG
ACGTGTTTCGATACGTCGCGGACTCCAACCAGACCGACGTGCCCTGTAAC
CTATGCACCTTCGACACGCGCCACGCCTGCGTACACACGACGCTCATGCG
CCTCCGGGCGCGCCATCCAAAGTTCGCCAGCGCCGCCCGCGGAGCCATCG

GCGTCTTCGGGACCATGAACAGCATGTACAGCGACTGCGACGTGCTGGGA
AACTACGCCGCCTTCTCGGCCCTGAAGCGCGCGGACGGATCCGAGACCGC
CCGGACCATCATGCAGGAGACGTACCGCGCGGCGACCGAGCGCGTCATGG
CCGAACTCGAGACCCTGCAGTACGTGGACCAGGCGGTCCCCACGGCCATG
GGGCGGCTGGAGACCATCATCACCAACCGCGAGGCCCTGCATACGGTGGT
GAACAACGTCAGGCAGGTCGTGGACCGCGAGGTGGAGCAGCTGATGCGCA
ACCTGGTGGAGGGGAGGAACTTCAAGTTTCGCGACGGTCTGGGCGAGGCC
AACCACGCCATGTCCCTGACGCTGGACCCGTACGCGTGCGGGCCGTGCCC
CCTGCTTCAGCTTCTCGGGCGGCGATCCAACCTCGCCGTGTACCAGGACC
TGGCCCTGAGTCAGTGCCACGGGGTGTTCGCCGGGCAGTCGGTCGAGGGG
CGCAACTTTCGCAATCAATTCCAACCGGTGCTGCGGCGGCGCGTGATGGA
CATGTTTAACAACGGGTTTCTGTCGGCCAAAACGCTGACGGTCGCGCTCT
CGGAGGGGGCGGCTATCTGCGCCCCCAGCCTAACGGCCGGCCAGACGGCC
CCCGCCGAGAGCAGCTTCGAGGGCGACGTTGCCCGCGTGACCCTGGGGTT
TCCCAAGGAGCTGCGCGTCAAGAGCCGCGTGTTGTTCGCGGGCGCGAGCG
CCAACGCGTCCGAGGCCGCCAAGGCGCGGGTCGCCAGCCTCCAGAGCGCC
TACCAGAAGCCCGACAAGCGCGTGGACATCCTCCTCGGACCGCTGGGCTT
TCTGCTGAAGCAGTTCCACGCGGCCATCTTCCCCAACGGCAAGCCCCCGG
GGTCCAACCAGCCGAACCCGCAGTGGTTCTGGACGGCCCTCCAACGCAAC
CAGCTTCCCGCCCGGCTCCTGTCGCGCGAGGACATCGAGACCATCGCGTT
CATTAAAAAGTTTTCCCTGGACTACGGCGCGATAAACTTTATTAACCTGG
CCCCCAACAACGTGAGCGAGCTGGCGATGTACTACATGGCAAACCAGATT
CTGCGGTACTGCGATCACTCGACATACTTCATCAACACCCTTACGGCCAT
CATCGCGGGGTCCCGCCGTCCCCCCAGCGTGCAGGCTGCGGCCGCGTGGT
CCGCGCAGGGCGGGGCGGGCCTGGAGGCCGGGGCCCGCGCGCTGATGGAC
GCCGTGGACGCGCATCCGGGCGCGTGGACGTCCATGTTCGCCAGCTGCAA
CCTGCTGCGGCCCGTCATGGCGGCGCGCCCCATGGTCGTGTTGGGGTTGA
GCATCAGCAAGTACTACGGCATGGCCGGCAACGACCGTGTGTTTCAGGCC
GGGAACTGGGCCAGCCTGATGGGCGGCAAAAACGCGTGCCCGCTCCTTAT
TTTTGACCGCACCCGCAAGTTCGTCCTGGCCTGTCCCCGGGCCGGGTTTG
TGTGCGCGGCCTCAAGCCTCGGCGGCGGAGCGCACGAAAGCTCGCTGTGC
GAGCAGCTCCGGGGCATTATCTCCGAGGGCGGGGCGGCCGTCGCCAGTAG
CGTGTTCGTGGCGACCGTGAAAAGCCTGGGGCCCCGCACCCAGCAGCTGC
AGATCGAGGACTGGCTGGCGCTCCTGGAGGACGAGTACCTAAGCGAGGAG
ATGATGGAGCTGACCGCGCGTGCCCTGGAGCGCGGCAACGGCGAGTGGTC
GACGGACGCGGCCCTGGAGGTGGCGCACGAGGCCGAGGCCCTAGTCAGCC
AACTCGGCAACGCCGGGGAGGTGTTTAACTTTGGGGATTTTGGCTGCGAG
GACGACAACGCGACGCCGTTCGGCGGCCCGGGGGCCCCGGGACCGGCATT
TGCCGGCCGCAAACGGGCGTTCCACGGGGATGACCCGTTTGGGGAGGGGC
CCCCCGACAAAAAGGGAGACCTGACGTTGGATATGCTGTGA

SEQ ID NO. 30: the amino acid sequence of ICP8 in the 17TermA strain.

METKPKTATTIKVPPGPLGYVYARACPSEGIELLALLSARSGDSDVAVAP
LVVGLTVESGFEANVAVVVGSRTTGLGGTAVSLKLTPSHYSSSVYVFHGG
RHLDPSTQAPNLTRLCERARRHFGFSDYTPRPGDLKHETTGEALCERLGL
DPDRALLYLVVTEGFKEAVCINNTFLHLGGSDKVTIGGAEVHRIPVYPLQ

LFMPDFSRVIAEPFNANHRSIGENFTYPLPFFNRPLNRLLFEAVVGPAAV
ALRCRNVDAVARAAAHLAFDENHEGAALPADITFTAFEASQGKTPRGGRD
GGGKGPAGGFEQRLASVMAGDAALALDSIVSMAVFDEPPTDISAWPLFEG
QDTAAARANAVGAYLARAAGLVGAMVFSTNSALHLTEVDDAGPADPKDHS
KPSFYRFFLVPGTHVAANPQVDREGHVVPGFEGRPTAPLVGGTQEFAGEH
LAMLCGFSPALLAKMLFYLERCDGGVIVGRQEMDVFRYVADSNQTDVPCN
LCTFDTRHACVHTTLMRLRARHPKFASAARGAIGVFGTMNSMYSDCDVLG
NYAAFSALKRADGSETARTIMQETYRAATERVMAELETLQYVDQAVPTAM
GRLETIITNREALHTVVNNVRQVVDREVEQLMRNLVEGRNFKFRDGLGEA
NHAMSLTLDPYACGPCPLLQLLGRRSNLAVYQDLALSQCHGVFAGQSVEG
RNFRNQFQPVLRRRVMDMFNNGFLSAKTLTVALSEGAAICAPSLTAGQTA
PAESSFEGDVARVTLGFPKELRVKSRVLFAGASANASEAAKARVASLQSA
YQKPDKRVDILLGPLGFLLKQFHAAIFPNGKPPGSNQPNPQWFWTALQRN
QLPARLLSREDIETIAFIKKFSLDYGAINFINLAPNNVSELAMYYMANQI
LRYCDHSTYFINTLTAIIAGSRRPPSVQAAAAWSAQGGAGLEAGARALMD
AVDAHPGAWTSMFASCNLLRPVMAARPMVVLGLSISKYYGMAGNDRVFQA
GNWASLMGGKNACPLLIFDRTRKFVLACPRAGFVCAASSLGGGAHESSLC
EQLRGIISEGGAAVASSVFVATVKSLGPRTQQLQIEDWLALLEDEYLSEE
MMELTARALERGNGEWSTDAALEVAHEAEALVSQLGNAGEVFNFGDFGCE
DDNATPFGGPGAPGPAFAGRKRAFHGDDPFGEGPPDKKGDLTLDML

SEQ ID NO. 31: the DNA sequence of ICP8 in the rRp450 strain.

ATGGAGACAAAGCCCAAGACGGCAACCACCATCAAGGTCCCCCCCGGGCC
CCTGGGATACGTGTACGCTCGCGCGTGTCCGTCCGAAGGCATCGAGCTTC
TGGCGTTACTGTCGGCGCGCAGCGGCGATGCCGACGTCGCCGTGGCGCCC
CTGGTCGTGGGCCTGACCGTGGAGAGCGGCTTTGAGGCCAACGTAGCCGT
GGTCGTGGGTTCTCGCACGACGGGGCTCGGGGGTACCGCGGTGTCCCTGA
AACTGACGCCATCGCACTACAGCTCGTCCGTGTACGTCTTTCACGGCGGC
CGGCACCTGGACCCCAGCACCCAGGCCCCAAACCTGACGCGACTCTGCGA
GCGGGCACGCCGCCATTTTGGCTTTTCGGACTACACCCCCCGGCCCGGCG
ACCTCAAACACGAGACGACGGGGGAGGCGCTGTGTGAGCGCCTCGGCCTG
GACCCGGACCGCGCCCTCCTGTATCTGGTCGTTACCGAGGGCTTCAAGGA
GGCCGTGTGCATCAACAACACCTTTCTGCACCTGGGAGGCTCGGACAAGG
TAACCATAGGCGGGGCGGAGGTGCACCGCATACCCGTGTATCCGTTGCAG
CTGTTCATGCCGGATTTTAGCCGGGTCATCGCCGAGCCGTTCAACGCCAA
CCACCGATCGATCGGGGAGAATTTTACCTACCCGCTTCCGTTTTTTAACC
GCCCCCTCAACCGCCTCCTGTTCGAGGCGGTCGTGGGACCCGCCGCCGTG
GCACTGCGATGCCGAAACGTGGACGCCGTGGCCCGCGCGGCCGCCCACCT
GGCGTTTGACGAAAACCACGAGGGCGCCGCCCTCCCCGCCGACATTACGT
TCACGGCCTTCGAAGCCAGCCAGGGTAAGACCCCGCGGGGTGGGCGCGAC
GGCGGCGGCAAGGGCCCGGCGGGCGGGTTCGAACAGCGCCTGGCCTCCGT
CATGGCCGGAGACGCCGCCCTGGCCCTCGAGTCTATCGTGTCGATGGCCG
TCTTCGACGAGCCGCCCACCGACATCTCCGCGTGGCCGCTGTGCGAGGGC
CAGGACACGGCCGCGGCCCGCGACAACGCCGTCGGGGCGTACCTGGCGCG
CGCCGCGGGACTCGTGGGGGCCATGGTATTTAGCACCAACTCGGCCCTCC
ATCTCACCGAGGTGGACGACGCCGGTCCGGCGGACCCAAAGGACCACAGC

AAACCCTCCTTTTACCGCTTCTTCCTCGTGCCCGGGACCCACGTGGCGGC
CAACCCACAGGTGGACCGCGAGGGACACGTGGTGCCCGGGTTCGAGGGTC
GGCCCACCGCGCCCCTCGTCGGCGGAACCCAGGAATTTGCCGGCGAGCAC
CTGGCCATGCTGTGTGGGTTTTCCCCGGCGCTGCTGGCCAAGATGCTGTT
TTACCTGGAGCGCTGCGACGGCGGCGTGATCGTCGGGCGCCAGGAGATGG
ACGTGTTTCGATACGTCGCGGACTCCAACCAGACCGACGTGCCCTGCAAC
CTGTGCACCTTCGACACGCGCCACGCCTGCGTACACACGACGCTCATGCG
CCTCCGGGCGCGCCATCCCAAGTTCGCCAGCGCCGCCCGCGGAGCCATCG
GCGTCTTCGGGACCATGAACAGCATGTACAGCGACTGCGACGTGCTGGGA
AACTACGCCGCCTTCTCGGCCCTGAAGCGCGCGGACGGATCCGAGACCGC
CCGGACCATCATGCAGGAGACGTACCGCGCGGCGACCGAGCGCGTCATGG
CCGAACTCGAGACCCTGCAGTACGTGGACCAGGCGGTCCCCACGGCCATG
GGGCGGCTGGAGACCATCATCACCAACCGCGAGGCCCTGCATACGGTGGT
GAACAACGTCAGGCAGGTCGTGGACCGCGAGGTGGAGCAGCTGATGCGCA
ACCTGGTGGAGGGGAGGAACTTCAAGTTTCGCGACGGTCTGGGCGAGGCC
AACCACGCCATGTCCCTGACGCTGGACCCGTACGCGTGCGGGCCATGCCC
CCTGCTTCAGCTTCTCGGGCGGCGATCCAACCTCGCCGTGTATCAGGACC
TGGCCCTGAGCCAGTGCCACGGGGTGTTCGCCGGGCAGTCGGTCGAGGGG
CGCAACTTTCGCAATCAATTCCAACCGGTGCTGCGGCGGCGCGTGATGGA
CATGTTTAACAACGGGTTTCTGTCGGCCAAAACGCTGACGGTCGCGCTCT
CGGAGGGGGCGGCTATCTGCGCCCCCAGCCTAACGGCCGGCCAGACGGCC
CCCGCCGAGAGCAGCTTCGAGGGCGACGTTGCCCGCGTGACCCTGGGGTT
TCCCAAGGAGCTGCGCGTCAAGAGCCGCGTGTTGTTCGCGGGCGCGAGCG
CCAACGCGTCCGAGGCCGCCAAGGCGCGGGTCGCCAGCCTCCAGAGCGCC
TACCAGAAGCCCGACAAGCGCGTGGACATCCTCCTCGGACCGCTGGGCTT
TCTGCTGAAGCAGTTCCACGCGGCCATCTTCCCCAACGGCAAGCCCCCGG
GGTCCAACCAGCCGAACCCGCAGTGGTTCTGGACGGCCCTCCAACGCAAC
CAGCTTCCCGCCCGGCTCCTGTCGCGCGAGGACATCGAGACCATCGCGTT
CATTAAAAAGTTTTCCCTGGACTACGGCGCGATAAACTTTATTAACCTGG
CCCCCAACAACGTGAGCGAGCTGGCGATGTACTACATGGCAAACCAGATT
CTGCGGTACTGCGATCACTCGACATACTTCATCAACACCCTCACGGCCAT
CATCGCGGGGTCCCGCCGTCCCCCCAGCGTGCAGGCGGCGGCCGCGTGGT
CCGCGCAGGGCGGGGCGGGCCTGGAGGCCGGGGCCCGCGCGCTGATGGAC
GCCGTGGACGCGCATCCGGGCGCGTGGACGTCCATGTTCGCCAGCTGCAA
CCTGCTGCGGCCCGTCATGGCGGCGCGCCCCATGGTCGTGTTGGGGTTGA
GCATCAGCAAATACTACGGCATGGCCGGCAACGACCGTGTGTTTCAGGCC
GGGAACTGGGCCAGCCTGATGGGCGGCAAAAACGCGTGCCCGCTCCTTAT
TTTTGACCGCACCCGCAAGTTCGTCCTGGCCTGTCCCCGGGCCGGGTTTG
TGTGCGCGGCCTCGAACCTCGGCGGCGGAGCGCACGAAAGCTCGCTGTGC
GAGCAGCTCCGGGGCATTATCTCCGAGGGCGGGGCGGCCGTCGCCAGTAG
CGTGTTCGTGGCGACCGTGAAAAGCCTGGGGCCCCGCACCCAGCAGCTGC
AGATCGAGGACTGGCTGGCGCTCCTGGAGGACGAGTACCTAAGCGAGGAG
ATGATGGAGCTGACCGCGCGTGCCCTGGAGCGCGGCAACGGCGAGTGGTC
GACGGACGCGGCCCTGGAGGTGGCGCACGAGGCCGAGGCCCTAGTCAGCC
AACTCGGCAACGCCGGGGAGGTGTTTAACTTTGGGGATTTTGGCTGCGAG
GACGACAACGCGACGCCGTTCGGCGGCCCGGGGGCCCCGGGACCGGCATT
TGCCGGCCGCAAACGGGCGTTCCACGGGGATGACCCGTTTGGGGAGGGGC

CCCCCGACAAAAAGGGAGACCTGACGTTGGATATGCTGTGA

SEQ ID NO. 32: the amino acid sequence of ICP8 in the rRp450 strain.

METKPKTATTIKVPPGPLGYVYARACPSEGIELLALLSARSGDADVAVAP
LVVGLTVESGFEANVAVVVGSRTTGLGGTAVSLKLTPSHYSSSVYVFHGG
RHLDPSTQAPNLTRLCERARRHFGFSDYTPRPGDLKHETTGEALCERLGL
DPDRALLYLVVTEGFKEAVCINNTFLHLGGSDKVTIGGAEVHRIPVYPLQ
LFMPDFSRVIAEPFNANHRSIGENFTYPLPFFNRPLNRLLFEAVVGPAAV
ALRCRNVDAVARAAAHLAFDENHEGAALPADITFTAFEASQGKTPRGGRD
GGGKGPAGGFEQRLASVMAGDAALALESIVSMAVFDEPPTDISAWPLCEG
QDTAAARDNAVGAYLARAAGLVGAMVFSTNSALHLTEVDDAGPADPKDHS
KPSFYRFFLVPGTHVAANPQVDREGHVVPGFEGRPTAPLVGGTQEFAGEH
LAMLCGFSPALLAKMLFYLERCDGGVIVGRQEMDVFRYVADSNQTDVPCN
LCTFDTRHACVHTTLMRLRARHPKFASAARGAIGVFGTMNSMYSDCDVLG
NYAAFSALKRADGSETARTIMQETYRAATERVMAELETLQYVDQAVPTAM
GRLETIITNREALHTVVNNVRQVVDREVEQLMRNLVEGRNFKFRDGLGEA
NHAMSLTLDPYACGPCPLLQLLGRRSNLAVYQDLALSQCHGVFAGQSVEG
RNFRNQFQPVLRRRVMDMFNNGFLSAKTLTVALSEGAAICAPSLTAGQTA
PAESSFEGDVARVTLGFPKELRVKSRVLFAGASANASEAAKARVASLQSA
YQKPDKRVDILLGPLGFLLKQFHAAIFPNGKPPGSNQPNPQWFWTALQRN
QLPARLLSREDIETIAFIKKFSLDYGAINFINLAPNNVSELAMYYMANQI
LRYCDHSTYFINTLTAIIAGSRRPPSVQAAAAWSAQGGAGLEAGARALMD
AVDAHPGAWTSMFASCNLLRPVMAARPMVVLGLSISKYYGMAGNDRVFQA
GNWASLMGGKNACPLLIFDRTRKFVLACPRAGFVCAASNLGGGAHESSLC
EQLRGIISEGGAAVASSVFVATVKSLGPRTQQLQIEDWLALLEDEYLSEE
MMELTARALERGNGEWSTDAALEVAHEAEALVSQLGNAGEVFNFGDFGCE
DDNATPFGGPGAPGPAFAGRKRAFHGDDPFGEGPPDKKGDLTLDML

SEQ ID NO. 33: the DNA sequence of ICP8 in the wildtype 17 strain.

ATGGAGACAAAGCCCAAGACGGCAACCACCATCAAGGTCCCCCCCGGGCC
CCTGGGATACGTGTACGCTCGCGCGTGTCCGTCCGAAGGCATCGAGCTTC
TGGCGTTACTGTCGGCACGCAGCGGCGATTCCGACGTCGCCGTGGCGCCC
CTGGTCGTGGGCCTGACCGTGGAGAGCGGCTTTGAGGCCAACGTGGCCGT
GGTCGTGGGTTCTCGCACGACGGGGCTCGGGGGTACCGCGGTGTCCCTGA
AACTGACGCCCTCGCACTACAGCTCGTCCGTGTACGTCTTTCACGGCGGC
CGGCACCTGGACCCCAGCACCCAGGCCCCGAACCTGACGCGACTTTGCGA
GCGGGCACGCCGCCATTTTGGCTTTTCGGACTACACCCCCCGGCCCGGCG
ACCTCAAACACGAGACGACGGGGGAGGCGCTGTGTGAGCGCCTCGGCCTG
GACCCGGACCGCGCCCTCCTGTATCTGGTCGTTACCGAGGGCTTCAAGGA
GGCCGTGTGCATCAACAACACCTTTCTGCACCTGGGAGGCTCGGACAAGG
TAACCATAGGCGGGGCGGAGGTGCACCGCATACCCGTGTACCCGTTGCAG
CTGTTCATGCCGGATTTTAGCCGTGTCATCGCAGAGCCGTTCAACGCCAA
CCACCGATCGATCGGGGAGAATTTTACCTACCCGCTTCCGTTTTTTAACC
GCCCCCTCAACCGCCTCCTGTTCGAGGCGGTCGTGGGACCCGCCGCCGTG
GCACTGCGATGCCGAAACGTGGACGCCGTGGCCCGCGCGGCCGCCCACCT
GGCGTTTGACGAAAACCACGAGGGCGCCGCCCTCCCCGCCGACATTACGT

TCACGGCCTTCGAAGCCAGCCAGGGTAAGACCCCGCGGGGCGGGCGCGAC
GGCGGCGGCAAGGGCCCGGCGGGCGGGTTCGAACAGCGCCTGGCCTCCGT
CATGGCCGGAGACGCCGCCCTGGCCCTCGATTCTATCGTGTCGATGGCCG
TCTTTGACGAGCCGCCCACCGACATCTCCGCGTGGCCGCTGTTCGAGGGC
CAGGACACGGCCGCGGCCCGCGCCAACGCCGTCGGGGCGTACCTGGCGCG
CGCCGCGGGACTCGTGGGGGCCATGGTATTTAGCACCAACTCGGCCCTCC
ATCTCACCGAGGTGGACGACGCCGGCCCGGCGGACCCAAAGGACCACAGC
AAACCCTCCTTTTACCGCTTCTTCCTCGTGCCCGGGACCCACGTGGCGGC
CAACCCACAGGTGGACCGCGAGGGACACGTGGTGCCCGGGTTCGAGGGTC
GGCCCACCGCGCCCCTCGTCGGCGGAACCCAGGAATTTGCCGGCGAGCAC
CTGGCCATGCTGTGTGGGTTTTCCCCGGCGCTGCTGGCCAAGATGCTGTT
TTACCTGGAGCGCTGCGACGGCGGCGTGATCGTCGGGCGCCAGGAGATGG
ACGTGTTTCGATACGTCGCGGACTCCAACCAGACCGACGTGCCCTGTAAC
CTATGCACCTTCGACACGCGCCACGCCTGCGTACACACGACGCTCATGCG
CCTCCGGGCGCGCCATCCAAAGTTCGCCAGCGCCGCCCGCGGAGCCATCG
GCGTCTTCGGGACCATGAACAGCATGTACAGCGACTGCGACGTGCTGGGA
AACTACGCCGCCTTCTCGGCCCTGAAGCGCGCGGACGGATCCGAGACCGC
CCGGACCATCATGCAGGAGACGTACCGCGCGGCGACCGAGCGCGTCATGG
CCGAACTCGAGACCCTGCAGTACGTGGACCAGGCGGTCCCCACGGCCATG
GGGCGGCTGGAGACCATCATCACCAACCGCGAGGCCCTGCATACGGTGGT
GAACAACGTCAGGCAGGTCGTGGACCGCGAGGTGGAGCAGCTGATGCGCA
ACCTGGTGGAGGGGAGGAACTTCAAGTTTCGCGACGGTCTGGGCGAGGCC
AACCACGCCATGTCCCTGACGCTGGACCCGTACGCGTGCGGGCCGTGCCC
CCTGCTTCAGCTTCTCGGGCGGCGATCCAACCTCGCCGTGTACCAGGACC
TGGCCCTGAGTCAGTGCCACGGGGTGTTCGCCGGGCAGTCGGTCGAGGGG
CGCAACTTTCGCAATCAATTCCAACCGGTGCTGCGGCGGCGCGTGATGGA
CATGTTTAACAACGGGTTTCTGTCGGCCAAAACGCTGACGGTCGCGCTCT
CGGAGGGGGCGGCTATCTGCGCCCCCAGCCTAACGGCCGGCCAGACGGCC
CCCGCCGAGAGCAGCTTCGAGGGCGACGTTGCCCGCGTGACCCTGGGGTT
TCCCAAGGAGCTGCGCGTCAAGAGCCGCGTGTTGTTCGCGGGCGCGAGCG
CCAACGCGTCCGAGGCCGCCAAGGCGCGGGTCGCCAGCCTCCAGAGCGCC
TACCAGAAGCCCGACAAGCGCGTGGACATCCTCCTCGGACCGCTGGGCTT
TCTGCTGAAGCAGTTCCACGCGGCCATCTTCCCCAACGGCAAGCCCCCGG
GGTCCAACCAGCCGAACCCGCAGTGGTTCTGGACGGCCCTCCAACGCAAC
CAGCTTCCCGCCCGGCTCCTGTCGCGCGAGGACATCGAGACCATCGCGTT
CATTAAAAAGTTTTCCCTGGACTACGGCGCGATAAACTTTATTAACCTGG
CCCCCAACAACGTGAGCGAGCTGGCGATGTACTACATGGCAAACCAGATT
CTGCGGTACTGCGATCACTCGACATACTTCATCAACACCCTTACGGCCAT
CATCGCGGGGTCCCGCCGTCCCCCCAGCGTGCAGGCTGCGGCCGCGTGGT
CCGCGCAGGGCGGGGCGGGCCTGGAGGCCGGGGCCCGCGCGCTGATGGAC
GCCGTGGACGCGCATCCGGGCGCGTGGACGTCCATGTTCGCCAGCTGCAA
CCTGCTGCGGCCCGTCATGGCGGCGCGCCCCATGGTCGTGTTGGGGTTGA
GCATCAGCAAGTACTACGGCATGGCCGGCAACGACCGTGTGTTTCAGGCC
GGGAACTGGGCCAGCCTGATGGGCGGCAAAAACGCGTGCCCGCTCCTTAT
TTTTGACCGCACCCGCAAGTTCGTCCTGGCCTGTCCCCGGGCCGGGTTTG
TGTGCGCGGCCTCAAGCCTCGGCGGCGGAGCGCACGAAAGCTCGCTGTGC
GAGCAGCTCCGGGGCATTATCTCCGAGGGCGGGGCGGCCGTCGCCAGTAG

CGTGTTCGTGGCGACCGTGAAAAGCCTGGGGCCCCGCACCCAGCAGCTGC
AGATCGAGGACTGGCTGGCGCTCCTGGAGGACGAGTACCTAAGCGAGGAG
ATGATGGAGCTGACCGCGCGTGCCCTGGAGCGCGGCAACGGCGAGTGGTC
GACGGACGCGGCCCTGGAGGTGGCGCACGAGGCCGAGGCCCTAGTCAGCC
AACTCGGCAACGCCGGGGAGGTGTTTAACTTTGGGGATTTTGGCTGCGAG
GACGACAACGCGACGCCGTTCGGCGGCCCGGGGGCCCCGGGACCGGCATT
TGCCGGCCGCAAACGGGCGTTCCACGGGGATGACCCGTTTGGGGAGGGGC
CCCCCGACAAAAAGGGAGACCTGACGTTGGATATGCTGTGA

SEQ ID NO. 34: the amino acid sequence of ICP8 in the wildtype 17 strain.

METKPKTATTIKVPPGPLGYVYARACPSEGIELLALLSARSGDSDVAVAP
LVVGLTVESGFEANVAVVVGSRTTGLGGTAVSLKLTPSHYSSSVYVFHGG
RHLDPSTQAPNLTRLCERARRHFGFSDYTPRPGDLKHETTGEALCERLGL
DPDRALLYLVVTEGFKEAVCINNTFLHLGGSDKVTIGGAEVHRIPVYPLQ
LFMPDFSRVIAEPFNANHRSIGENFTYPLPFFNRPLNRLLFEAVVGPAAV
ALRCRNVDAVARAAAHLAFDENHEGAALPADITFTAFEASQGKTPRGGRD
GGGKGPAGGFEQRLASVMAGDAALALDSIVSMAVFDEPPTDISAWPLFEG
QDTAAARANAVGAYLARAAGLVGAMVFSTNSALHLTEVDDAGPADPKDHS
KPSFYRFFLVPGTHVAANPQVDREGHVVPGFEGRPTAPLVGGTQEFAGEH
LAMLCGFSPALLAKMLFYLERCDGGVIVGRQEMDVFRYVADSNQTDVPCN
LCTFDTRHACVHTTLMRLRARHPKFASAARGAIGVFGTMNSMYSDCDVLG
NYAAFSALKRADGSETARTIMQETYRAATERVMAELETLQYVDQAVPTAM
GRLETIITNREALHTVVNNVRQVVDREVEQLMRNLVEGRNFKFRDGLGEA
NHAMSLTLDPYACGPCPLLQLLGRRSNLAVYQDLALSQCHGVFAGQSVEG
RNFRNQFQPVLRRRVMDMFNNGFLSAKTLTVALSEGAAICAPSLTAGQTA
PAESSFEGDVARVTLGFPKELRVKSRVLFAGASANASEAAKARVASLQSA
YQKPDKRVDILLGPLGFLLKQFHAAIFPNGKPPGSNQPNPQWFWTALQRN
QLPARLLSREDIETIAFIKKFSLDYGAINFINLAPNNVSELAMYYMANQI
LRYCDHSTYFINTLTAIIAGSRRPPSVQAAAAWSAQGGAGLEAGARALMD
AVDAHPGAWTSMFASCNLLRPVMAARPMVVLGLSISKYYGMAGNDRVFQA
GNWASLMGGKNACPLLIFDRTRKFVLACPRAGFVCAASSLGGGAHESSLC
EQLRGIISEGGAAVASSVFVATVKSLGPRTQQLQIEDWLALLEDEYLSEE
MMELTARALERGNGEWSTDAALEVAHEAEALVSQLGNAGEVFNFGDFGCE
DDNATPFGGPGAPGPAFAGRKRAFHGDDPFGEGPPDKKGDLTLDML

SEQ ID NO. 35: The DNA sequence of DNA packaging terminase sub1 from Mut-3, Mut-3Δ34.5 and Mut-3ΔICP6 viral strains. The g1126a mutation is shown in bold and italic in the sequence below.

ATGTTTGGTCAGCAGCTGGCGTCCGACGTCCAGCAGTACCTGGAGCGCCT
CGAGAAACAGAGGCAACTTAAGGTGGGCGCGGACGAGGCGTCGGCGGGCC
TCACCATGGGCGGCGATGCCCTACGAGTGCCCTTTTTAGATTTCGCGACC
GCGACCCCCAAGCGCCACCAGACCGTGGTCCCTGGCGTCGGGACGCTCCA
CGACTGCTGCGAGCACTCGCCGCTCTTCTCGGCCGTGGCGCGGCGGCTGC
TGTTTAATAGCCTGGTGCCGGCGCAACTAAAGGGGCGTGATTTCGGGGGC
GACCACACGGCCAAGCTGGAATTCCTGGCCCCCGAGTTGGTACGGGCGGT
GGCGCGACTGCGGTTTAAGGAGTGCGCGCCGGCGGACGTGGTGCCTCAGC

GTAACGCCTACTATAGCGTTCTGAATACGTTTCAGGCCCTCCACCGCTCC
GAAGCCTTTCGCCAGCTGGTGCACTTTGTGCGGGACTTTGCCCAGCTGCT
CAAAACCTCCTTCCGGGCCTCCAGCCTCACGGAGACCACGGGCCCCCCCA
AAAAACGGGCCAAGGTGGACGTGGCCACCCACGGCCGGACGTACGGCACG
CTGGAGCTGTTCCAAAAAATGATCCTTATGCACGCCACCTACTTTCTGGC
CGCCGTGCTCCTCGGGGACCACGCGGAGCAGGTCAACACGTTCCTGCGTC
TCGTGTTTGAGATCCCCCTGTTTAGCGACGCGGCCGTGCGCCACTTCCGC
CAGCGCGCCACCGTGTTTCTCGTCCCCCGGCGCCACGGCAAGACCTGGTT
TCTGGTGCCCCTCATCGCGCTGTCGCTGGCCTCCTTTCGGGGGATCAAGA
TCGGCTACACGGCGCACATCCGCAAGGCGACCGAGCCGGTGTTTGAGGAG
ATCGACGCCTGCCTGCGGGGCTGGTTCGGTTCGGCCCGAGTGGACCACGT
TAAAGGGGAAACCATCTCCTTCTCGTTTCCGGACGGGTCGCGCAGTACCA
TCGTGTTTGCCTCCAGCCACAACACAAACGGAATCCGAGGCCAGGACTTT
AACCTGCTCTTTGTCGACGAGGCCAACTTTATTCGCCCGGATGCGGTCCA
GACGATTATGGGCTTTCTCAACCAG**A**CCAACTGCAAGATTATCTTCGTGT
CGTCCACCAACACCGGGAAGGCCAGTACGAGCTTTTTGTACAACCTCCGC
GGGGCCGCAGACGAGCTTCTCAACGTGGTGACCTATATATGCGATGATCA
CATGCCGAGGGTGGTGACGCACACAAACGCCACGGCCTGTTCTTGTTATA
TCCTCAACAAGCCCGTTTTCATCACGATGGACGGGGCGGTTCGCCGGACC
GCCGATTTGTTTCTGGCCGATTCCTTCATGCAGGAGATCATCGGGGGCCA
GGCCAGGGAGACCGGCGACGACCGGCCCGTTCTGACCAAGTCTGCGGGGG
AGCGGTTTCTGTTGTACCGCCCCTCGACCACCACCAACAGCGGCCTCATG
GCCCCCGATTTGTACGTGTACGTGGATCCCGCGTTCACGGCCAACACCCG
AGCCTCCGGGACCGGCGTCGCTGTCGTCGGGCGGTACCGCGACGATTATA
TCATCTTCGCCCTGGAGCACTTTTTTCTCCGCGCGCTCACGGGCTCGGCC
CCCGCCGACATCGCCCGCTGCGTCGTCCACAGTCTGACGCAGGTCCTGGC
CCTGCATCCCGGGGCGTTTCGCGGCGTCCGGGTGGCGGTCGAGGGAAATA
GCAGCCAGGACTCGGCCGTCGCCATCGCCACGCACGTGCACACAGAGATG
CACCGCCTACTGGCCTCGGAGGGGGCCGACGCGGGCTCGGGCCCCGAGCT
TCTCTTCTACCACTGCGAGCCTCCCGGGAGCGCGGTGCTGTACCCCTTTT
TCCTGCTCAACAAACAGAAGACGCCCGCCTTTGAACACTTTATTAAAAAG
TTTAACTCCGGGGGCGTCATGGCCTCCCAGGAGATCGTTTCCGCGACGGT
GCGCCTGCAGACCGACCCGGTCGAGTATCTGCTCGAGCAGCTAAATAACC
TCACCGAAACCGTCTCCCCCAACACTGACGTCCGTACGTATTCCGGAAAA
CGGAACGGCGCCTCGGATGACCTTATGGTCGCCGTCATTATGGCCATCTA
CCTCGCGGCCCAGGCCGGACCTCCGCACACATTCGCTCCTATCATACGCG
TCTCGTGA

SEQ ID NO. 36: The amino acid sequence of DNA packaging terminase sub1 from Mut-3, Mut-3Δ34.5 and Mut-3ΔICP6 viral strains. The A376T mutation is shown in bold and italic in the sequence below.

MFGQQLASDVQQYLERLEKQRQLKVGADEASAGLTMGGDALRVPFLDFAT
ATPKRHQTVVPGVGTLHDCCEHSPLFSAVARRLLFNSLVPAQLKGRDFGG
DHTAKLEFLAPELVRAVARLRFKECAPADVVPQRNAYYSVLNTFQALHRS
EAFRQLVHFVRDFAQLLKTSFRASSLTETTGPPKKRAKVDVATHGRTYGT
LELFQKMILMHATYFLAAVLLGDHAEQVNTFLRLVFEIPLFSDAAVRHFR
QRATVFLVPRRHGKTWFLVPLIALSLASFRGIKIGYTAHIRKATEPVFEE
IDACLRGWFGSARVDHVKGETISFSFPDGSRSTIVFASSHNTNGIRGQDF
NLLFVDEANFIRPDAVQTIMGFLNQ***T***NCKIIFVSSTNTGKASTSFLYNLR
GAADELLNVVTYICDDHMPRVVTHTNATACSCYILNKPVFITMDGAVRRT
ADLFLADSFMQEIIGGQARETGDDRPVLTKSAGERFLLYRPSTTTNSGLM
APDLYVYVDPAFTANTRASGTGVAVVGRYRDDYIIFALEHFFLRALTGSA
PADIARCVVHSLTQVLALHPGAFRGVRVAVEGNSSQDSAVAIATHVHTEM
HRLLASEGADAGSGPELLFYHCEPPGSAVLYPFFLLNKQKTPAFEHFIKK
FNSGGVMASQEIVSATVRLQTDPVEYLLEQLNNLTETVSPNTDVRTYSGK

RNGASDDLMVAVIMAIYLAAQAGPPHTFAPIIRVS

SEQ ID NO. 37: The DNA sequence of DNA packaging terminase sub1 the 17TermA viral strain.

ATGTTTGGTCAGCAGCTGGCGTCCGACGTCCAGCAGTACCTGGAGCGCCT
CGAGAAACAGAGGCAACTTAAGGTGGGCGCGGACGAGGCGTCGGCGGGCC
TCACCATGGGCGGCGATGCCCTACGAGTGCCCTTTTTAGATTTCGCGACC
GCGACCCCCAAGCGCCACCAGACCGTGGTCCCTGGCGTCGGGACGCTCCA
CGACTGCTGCGAGCACTCGCCGCTCTTCTCGGCCGTGGCGCGGCGGCTGC
TGTTTAATAGCCTGGTGCCGGCGCAACTAAAGGGGCGTGATTTCGGGGGC
GACCACACGGCCAAGCTGGAATTCCTGGCCCCCGAGTTGGTACGGGCGGT
GGCGCGACTGCGGTTTAAGGAGTGCGCGCCGGCGGACGTGGTGCCTCAGC
GTAACGCCTACTATAGCGTTCTGAATACGTTTCAGGCCCTCCACCGCTCC
GAAGCCTTTCGCCAGCTGGTGCACTTTGTGCGGGACTTTGCCCAGCTGCT
CAAAACCTCCTTCCGGGCCTCCAGCCTCACGGAGACCACGGGCCCCCCCA
AAAAACGGGCCAAGGTGGACGTGGCCACCCACGGCCGGACGTACGGCACG
CTGGAGCTGTTCCAAAAAATGATCCTTATGCACGCCACCTACTTTCTGGC
CGCCGTGCTCCTCGGGGACCACGCGGAGCAGGTCAACACGTTCCTGCGTC
TCGTGTTTGAGATCCCCCTGTTTAGCGACGCGGCCGTGCGCCACTTCCGC
CAGCGCGCCACCGTGTTTCTCGTCCCCCGGCGCCACGGCAAGACCTGGTT
TCTGGTGCCCCTCATCGCGCTGTCGCTGGCCTCCTTTCGGGGGATCAAGA
TCGGCTACACGGCGCACATCCGCAAGGCGACCGAGCCGGTGTTTGAGGAG
ATCGACGCCTGCCTGCGGGGCTGGTTCGGTTCGGCCCGAGTGGACCACGT
TAAAGGGGAAACCATCTCCTTCTCGTTTCCGGACGGGTCGCGCAGTACCA
TCGTGTTTGCCTCCAGCCACAACACAAACGGAATCCGAGGCCAGGACTTT
AACCTGCTCTTTGTCGACGAGGCCAACTTTATTCGCCCGGATGCGGTCCA
GACGATTATGGGCTTTCTCAACCAGGCCAACTGCAAGATTATCTTCGTGT
CGTCCACCAACACCGGGAAGGCCAGTACGAGCTTTTTGTACAACCTCCGC
GGGGCCGCAGACGAGCTTCTCAACGTGGTGACCTATATATGCGATGATCA
CATGCCGAGGGTGGTGACGCACACAAACGCCACGGCCTGTTCTTGTTATA
TCCTCAACAAGCCCGTTTTCATCACGATGGACGGGGCGGTTCGCCGGACC
GCCGATTTGTTTCTGGCCGATTCCTTCATGCAGGAGATCATCGGGGGCCA
GGCCAGGGAGACCGGCGACGACCGGCCCGTTCTGACCAAGTCTGCGGGGG
AGCGGTTTCTGTTGTACCGCCCCTCGACCACCACCAACAGCGGCCTCATG
GCCCCCGATTTGTACGTGTACGTGGATCCCGCGTTCACGGCCAACACCCG
AGCCTCCGGGACCGGCGTCGCTGTCGTCGGGCGGTACCGCGACGATTATA
TCATCTTCGCCCTGGAGCACTTTTTTCTCCGCGCGCTCACGGGCTCGGCC
CCCGCCGACATCGCCCGCTGCGTCGTCCACAGTCTGACGCAGGTCCTGGC
CCTGCATCCCGGGGCGTTTCGCGGCGTCCGGGTGGCGGTCGAGGGAAATA
GCAGCCAGGACTCGGCCGTCGCCATCGCCACGCACGTGCACACAGAGATG
CACCGCCTACTGGCCTCGGAGGGGGCCGACGCGGGCTCGGGCCCCGAGCT
TCTCTTCTACCACTGCGAGCCTCCCGGGAGCGCGGTGCTGTACCCCTTTT
TCCTGCTCAACAAACAGAAGACGCCCGCCTTTGAACACTTTATTAAAAAG
TTTAACTCCGGGGGCGTCATGGCCTCCCAGGAGATCGTTTCCGCGACGGT
GCGCCTGCAGACCGACCCGGTCGAGTATCTGCTCGAGCAGCTAAATAACC
TCACCGAAACCGTCTCCCCCAACACTGACGTCCGTACGTATTCCGGAAAA

CGGAACGGCGCCTCGGATGACCTTATGGTCGCCGTCATTATGGCCATCTA
CCTCGCGGCCCAGGCCGGACCTCCGCACACATTCGCTCCTATCATACGCG
TCTCGTGA

SEQ ID NO. 38: The amino acid sequence of DNA packaging terminase sub1 the 17TermA viral strain.

MFGQQLASDVQQYLERLEKQRQLKVGADEASAGLTMGGDALRVPFLDFAT
ATPKRHQTVVPGVGTLHDCCEHSPLFSAVARRLLFNSLVPAQLKGRDFGG
DHTAKLEFLAPELVRAVARLRFKECAPADVVPQRNAYYSVLNTFQALHRS
EAFRQLVHFVRDFAQLLKTSFRASSLTETTGPPKKRAKVDVATHGRTYGT
LELFQKMILMHATYFLAAVLLGDHAEQVNTFLRLVFEIPLFSDAAVRHFR
QRATVFLVPRRHGKTWFLVPLIALSLASFRGIKIGYTAHIRKATEPVFEE
IDACLRGWFGSARVDHVKGETISFSFPDGSRSTIVFASSHNTNGIRGQDF
NLLFVDEANFIRPDAVQTIMGFLNQANCKIIFVSSTNTGKASTSFLYNLR
GAADELLNVVTYICDDHMPRVVTHTNATACSCYILNKPVFITMDGAVRRT
ADLFLADSFMQEIIGGQARETGDDRPVLTKSAGERFLLYRPSTTTNSGLM
APDLYVYVDPAFTANTRASGTGVAVVGRYRDDYIIFALEHFFLRALTGSA
PADIARCVVHSLTQVLALHPGAFRGVRVAVEGNSSQDSAVAIATHVHTEM
HRLLASEGADAGSGPELLFYHCEPPGSAVLYPFFLLNKQKTPAFEHFIKK
FNSGGVMASQEIVSATVRLQTDPVEYLLEQLNNLTETVSPNTDVRTYSGK
RNGASDDLMVAVIMAIYLAAQAGPPHTFAPIIRVS

SEQ ID NO. 39: The DNA sequence of DNA packaging terminase sub1 the rRp450 viral strain.

ATGTTTGGTCAGCAGCTGGCGTCCGACGTCCAGCAGTACCTGGAGCGCCT
CGAGAAACAGAGGCAACTTAAGGTGGGCGCGGACGAGGCGTCGGCGGGCC
TCACAATGGGCGGCGATGCCCTACGAGTGCCCTTTTTAGATTTCGCGACC
GCGACCCCCAAGCGCCACCAGACCGTGGTCCCGGGCGTCGGGACGCTCCA
CGACTGCTGCGAGCACTCGCCGCTCTTCTCGGCCGTGGCGCGGCGGCTGC
TGTTTAATAGCCTGGTGCCGGCGCAACTAAAGGGGCGTGATTTCGGGGGC
GACCACACGGCCAAGCTGGAATTCCTGGCCCCCGAGTTGGTACGGGCGGT
GGCGCGACTGCGGTTTAAGGAGTGCGCGCCGGCGGACGTGGTGCCTCAGC
GTAACGCCTACTATAGCGTTCTGAACACGTTTCAGGCCCTCCACCGCTCC
GAAGCCTTTCGCCAGCTGGTGCACTTTGTGCGGGACTTTGCCCAGCTGCT
TAAAACCTCCTTCCGGGCCTCCAGCCTCACGGAGACCACGGGCCCCCCAA
AAAAACGGGCCAAGGTGGACGTGGCCACCCACGGCCGGACGTACGGCACG
CTGGAGCTGTTCCAAAAAATGATCCTTATGCACGCCACCTACTTTCTGGC
CGCCGTGCTCCTCGGGGACCACGCGGAGCAGGTCAACACGTTCCTGCGTC
TCGTGTTTGAGATCCCCCTGTTTAGCGACGCGGCCGTGCGCCACTTCCGC
CAGCGCGCCACCGTGTTTCTCGTCCCCCGGCGCCACGGCAAGACCTGGTT
TCTAGTGCCCCTCATCGCGCTGTCGCTGGCCTCCTTTCGGGGGATCAAGA
TCGGCTACACGGCGCACATCCGCAAGGCGACCGAGCCGGTGTTTGAGGAG
ATCGACGCCTGCCTGCGGGGCTGGTTCGGTTCGGCCCGAGTGGACCACGT
TAAAGGGGAAACCATCTCCTTCTCGTTTCCGGACGGGTCGCGCAGTACCA
TCGTGTTTGCCTCCAGCCACAACACAAACGGAATCCGAGGCCAGGACTTT

AACCTGCTCTTTGTCGACGAGGCCAACTTTATTCGCCCGGATGCGGTCCA
GACGATTATGGGCTTTCTCAACCAGGCCAACTGCAAGATTATCTTCGTGT
CGTCCACCAACACCGGGAAGGCCAGTACGAGCTTTTTGTACAACCTCCGC
GGGGCCGCCGACGAGCTTCTCAACGTGGTGACCTATATATGCGATGATCA
CATGCCGCGGGTGGTGACGCACACAAACGCCACGGCCTGTTCTTGTTATA
TCCTCAACAAGCCCGTTTTCATCACGATGGACGGGGCGGTTCGCCGGACC
GCCGATTTGTTTCTGGCCGATTCCTTCATGCAGGAGATCATCGGGGGCCA
GGCCAGGGAGACCGGCGACGACCGGCCCGTTCTGACCAAGTCTGCGGGGG
AGCGGTTTCTGTTGTACCGCCCCTCGACCACCACCAACAGCGGCCTCATG
GCCCCCGATTTGTACGTGTACGTGGATCCCGCGTTCACGGCCAACACCCG
AGCCTCCGGGACCGGCGTCGCTGTCGTCGGGCGGTACCGCGACGATTATA
TCATCTTCGCCCTGGAGCACTTTTTTCTCCGCGCGCTCACGGGCTCGGCC
CCCGCCGACATCGCCCGCTGCGTCGTCCACAGTCTGACGCAGGTCCTGGC
CCTGCATCCCGGGGCGTTTCGCGGCGTCCGGGTGGCGGTCGAGGGAAATA
GCAGCCAGGACTCGGCCGTCGCCATCGCCACGCACGTGCACACAGAGATG
CACCGCCTACTGGCCTCGGAGGGGGCCGACGCGGGCTCGGGCCCCGAGCT
TCTCTTCTACCACTGCGAGCCTCCCGGGAGCGCGGTGCTGTACCCCTTTT
TCCTGCTCAACAAACAGAAGACGCCCGCCTTTGAACACTTTATTAAAAAG
TTTAACTCCGGGGGCGTCATGGCCTCCCAGGAGATCGTTTCCGCGACGGT
GCGCCTGCAGACCGACCCGGTCGAGTATCTGCTCGAGCAGCTGAATAACC
TCACCGAAACCGTCTCCCCCAACACGGACGTCCGTACGTATTCCGGAAAA
CGGAACGGCGCCTCGGATGACCTTATGGTCGCCGTCATTATGGCCATCTA
CCTTGCGGCCCAGGCCGGACCTCCGCACACATTCGCTCCCATCACACGCG
TTTCGTGA

SEQ ID NO. 40: The amino acid sequence of DNA packaging terminase sub1 the rRp450 viral strain.

MFGQQLASDVQQYLERLEKQRQLKVGADEASAGLTMGGDALRVPFLDFAT
ATPKRHQTVVPGVGTLHDCCEHSPLFSAVARRLLFNSLVPAQLKGRDFGG
DHTAKLEFLAPELVRAVARLRFKECAPADVVPQRNAYYSVLNTFQALHRS
EAFRQLVHFVRDFAQLLKTSFRASSLTETTGPPKKRAKVDVATHGRTYGT
LELFQKMILMHATYFLAAVLLGDHAEQVNTFLRLVFEIPLFSDAAVRHFR
QRATVFLVPRRHGKTWFLVPLIALSLASFRGIKIGYTAHIRKATEPVFEE
IDACLRGWFGSARVDHVKGETISFSFPDGSRSTIVFASSHNTNGIRGQDF
NLLFVDEANFIRPDAVQTIMGFLNQANCKIIFVSSTNTGKASTSFLYNLR
GAADELLNVVTYICDDHMPRVVTHTNATACSCYILNKPVFITMDGAVRRT
ADLFLADSFMQEIIGGQARETGDDRPVLTKSAGERFLLYRPSTTTNSGLM
APDLYVYVDPAFTANTRASGTGVAVVGRYRDDYIIFALEHFFLRALTGSA
PADIARCVVHSLTQVLALHPGAFRGVRVAVEGNSSQDSAVAIATHVHTEM
HRLLASEGADAGSGPELLFYHCEPPGSAVLYPFFLLNKQKTPAFEHFIKK
FNSGGVMASQEIVSATVRLQTDPVEYLLEQLNNLTETVSPNTDVRTYSGK
RNGASDDLMVAVIMAIYLAAQAGPPHTFAPITRVS

SEQ ID NO. 41: The DNA sequence of DNA packaging terminase sub1 the wild-type 17 strain.

ATGTTTGGTCAGCAGCTGGCGTCCGACGTCCAGCAGTACCTGGAGCGCCT
CGAGAAACAGAGGCAACTTAAGGTGGGCGCGGACGAGGCGTCGGCGGGCC
TCACCATGGGCGGCGATGCCCTACGAGTGCCCTTTTTAGATTTCGCGACC
GCGACCCCCAAGCGCCACCAGACCGTGGTCCCTGGCGTCGGGACGCTCCA
CGACTGCTGCGAGCACTCGCCGCTCTTCTCGGCCGTGGCGCGGCGGCTGC
TGTTTAATAGCCTGGTGCCGGCGCAACTAAAGGGGCGTGATTTCGGGGGC
GACCACACGGCCAAGCTGGAATTCCTGGCCCCCGAGTTGGTACGGGCGGT
GGCGCGACTGCGGTTTAAGGAGTGCGCGCCGGCGGACGTGGTGCCTCAGC
GTAACGCCTACTATAGCGTTCTGAATACGTTTCAGGCCCTCCACCGCTCC
GAAGCCTTTCGCCAGCTGGTGCACTTTGTGCGGGACTTTGCCCAGCTGCT
CAAAACCTCCTTCCGGGCCTCCAGCCTCACGGAGACCACGGGCCCCCCCA
AAAAACGGGCCAAGGTGGACGTGGCCACCCACGGCCGGACGTACGGCACG
CTGGAGCTGTTCCAAAAAATGATCCTTATGCACGCCACCTACTTTCTGGC
CGCCGTGCTCCTCGGGGACCACGCGGAGCAGGTCAACACGTTCCTGCGTC
TCGTGTTTGAGATCCCCCTGTTTAGCGACGCGGCCGTGCGCCACTTCCGC
CAGCGCGCCACCGTGTTTCTCGTCCCCCGGCGCCACGGCAAGACCTGGTT
TCTGGTGCCCCTCATCGCGCTGTCGCTGGCCTCCTTTCGGGGGATCAAGA
TCGGCTACACGGCGCACATCCGCAAGGCGACCGAGCCGGTGTTTGAGGAG
ATCGACGCCTGCCTGCGGGGCTGGTTCGGTTCGGCCCGAGTGGACCACGT
TAAAGGGGAAACCATCTCCTTCTCGTTTCCGGACGGGTCGCGCAGTACCA
TCGTGTTTGCCTCCAGCCACAACACAAACGGAATCCGAGGCCAGGACTTT
AACCTGCTCTTTGTCGACGAGGCCAACTTTATTCGCCCGGATGCGGTCCA
GACGATTATGGGCTTTCTCAACCAGGCCAACTGCAAGATTATCTTCGTGT
CGTCCACCAACACCGGGAAGGCCAGTACGAGCTTTTTGTACAACCTCCGC
GGGGCCGCAGACGAGCTTCTCAACGTGGTGACCTATATATGCGATGATCA
CATGCCGAGGGTGGTGACGCACACAAACGCCACGGCCTGTTCTTGTTATA
TCCTCAACAAGCCCGTTTTCATCACGATGGACGGGGCGGTTCGCCGGACC
GCCGATTTGTTTCTGGCCGATTCCTTCATGCAGGAGATCATCGGGGGCCA
GGCCAGGGAGACCGGCGACGACCGGCCCGTTCTGACCAAGTCTGCGGGGG
AGCGGTTTCTGTTGTACCGCCCCTCGACCACCACCAACAGCGGCCTCATG
GCCCCCGATTTGTACGTGTACGTGGATCCCGCGTTCACGGCCAACACCCG
AGCCTCCGGGACCGGCGTCGCTGTCGTCGGGCGGTACCGCGACGATTATA
TCATCTTCGCCCTGGAGCACTTTTTTCTCCGCGCGCTCACGGGCTCGGCC
CCCGCCGACATCGCCCGCTGCGTCGTCCACAGTCTGACGCAGGTCCTGGC
CCTGCATCCCGGGGCGTTTCGCGGCGTCCGGGTGGCGGTCGAGGGAAATA
GCAGCCAGGACTCGGCCGTCGCCATCGCCACGCACGTGCACACAGAGATG
CACCGCCTACTGGCCTCGGAGGGGGCCGACGCGGGCTCGGGCCCCGAGCT
TCTCTTCTACCACTGCGAGCCTCCCGGGAGCGCGGTGCTGTACCCCTTTT
TCCTGCTCAACAAACAGAAGACGCCCGCCTTTGAACACTTTATTAAAAAG
TTTAACTCCGGGGGCGTCATGGCCTCCCAGGAGATCGTTTCCGCGACGGT
GCGCCTGCAGACCGACCCGGTCGAGTATCTGCTCGAGCAGCTAAATAACC
TCACCGAAACCGTCTCCCCCAACACTGACGTCCGTACGTATTCCGGAAAA
CGGAACGGCGCCTCGGATGACCTTATGGTCGCCGTCATTATGGCCATCTA

CCTCGCGGCCCAGGCCGGACCTCCGCACACATTCGCTCCTATCATACGCG
TCTCGTGA

SEQ ID NO. 42: The amino acid sequence of DNA packaging terminase sub1 the wild-type 17 strain.

MFGQQLASDVQQYLERLEKQRQLKVGADEASAGLTMGGDALRVPFLDFAT
ATPKRHQTVVPGVGTLHDCCEHSPLFSAVARRLLFNSLVPAQLKGRDFGG
DHTAKLEFLAPELVRAVARLRFKECAPADVVPQRNAYYSVLNTFQALHRS
EAFRQLVHFVRDFAQLLKTSFRASSLTETTGPPKKRAKVDVATHGRTYGT
LELFQKMILMHATYFLAAVLLGDHAEQVNTFLRLVFEIPLFSDAAVRHFR
QRATVFLVPRRHGKTWFLVPLIALSLASFRGIKIGYTAHIRKATEPVFEE
IDACLRGWFGSARVDHVKGETISFSFPDGSRSTIVFASSHNTNGIRGQDF
NLLFVDEANFIRPDAVQTIMGFLNQANCKIIFVSSTNTGKASTSFLYNLR
GAADELLNVVTYICDDHMPRVVTHTNATACSCYILNKPVFITMDGAVRRT
ADLFLADSFMQEIIGGQARETGDDRPVLTKSAGERFLLYRPSTTTNSGLM
APDLYVYVDPAFTANTRASGTGVAVVGRYRDDYIIFALEHFFLRALTGSA
PADIARCVVHSLTQVLALHPGAFRGVRVAVEGNSSQDSAVAIATHVHTEM
HRLLASEGADAGSGPELLFYHCEPPGSAVLYPFFLLNKQKTPAFEHFIKK
FNSGGVMASQEIVSATVRLQTDPVEYLLEQLNNLTETVSPNTDVRTYSGK
RNGASDDLMVAVIMAIYLAAQAGPPHTFAPIIRVS

SEQ ID NO. 43: the DNA sequence of ICP6 in the Mut 3 and Mut-3Δ34.5 viral strains. The sequence is identical to that in the wild-type 17 strain.

ATGGCCAGCCGCCCAGCCGCATCCTCTCCCGTCGAAGCGCGGGCCCCGGT
TGGGGGACAGGAGGCCGGCGGCCCCAGCGCAGCCACCCAGGGGGAGGCCG
CCGGGGCCCCTCTCGCCCACGGCCACCACGTGTACTGCCAGCGAGTCAAT
GGCGTGATGGTGCTTTCCGACAAGACGCCCGGGTCCGCGTCCTACCGCAT
CAGCGATAGCAACTTTGTCCAATGTGGTTCCAACTGCACCATGATCATCG
ACGGAGACGTGGTGCGCGGGCGCCCCCAGGACCCGGGGGCCGCGGCATCC
CCCGCTCCCTTCGTTGCGGTGACAAACATCGGAGCCGGCAGCGACGGCGG
GACCGCCGTCGTGGCATTCGGGGGAACCCCACGTCGCTCGGCGGGGACGT
CTACCGGTACCCAGACGGCCGACGTCCCCACCGAGGCCCTTGGGGGCCCC
CCTCCTCCTCCCCGCTTCACCCTGGGTGGCGGCTGTTGTTCCTGTCGCGA
CACACGGCGCCGCTCTGCGGTATTCGGGGGGGAGGGGGATCCAGTCGGCC
CCGCGGAGTTCGTCTCGGACGACCGGTCGTCCGATTCCGACTCGGATGAC
TCGGAGGACACGGACTCGGAGACGCTGTCACACGCCTCCTCGGACGTGTC
CGGCGGGGCCACGTACGACGACGCCCTTGACTCCGATTCGTCATCGGATG
ACTCCCTGCAGATAGATGGCCCCGTGTGTCGCCCGTGGAGCAATGACACC
GCGCCCCTGGATGTTTGCCCCGGGACCCCCGGCCCGGGCGCCGACGCCGG
TGGTCCCTCAGCGGTAGACCCACACGCGCCGACGCCAGAGGCCGGCGCTG
GTCTTGCGGCCGATCCCGCCGTGGCCCGGGACGACGCGGAGGGGCTTTCG
GACCCCCGGCCACGTCTGGGAACGGGCACGGCCTACCCCGTCCCCCTGGA

ACTCACGCCCGAGAACGCGGAGGCCGTGGCGCGCTTTCTGGGAGATGCCG
TGAACCGCGAACCCGCGCTCATGCTGGAGTACTTTTGCCGGTGCGCCCGC
GAGGAAACCAAGCGTGTCCCCCCCAGGACATTCGGCAGCCCCCCTCGCCT
CACGGAGGACGACTTTGGGCTTCTCAACTACGCGCTCGTGGAGATGCAGC
GCCTGTGTCTGGACGTTCCTCCGGTCCCGCCGAACGCATACATGCCCTAT
TATCTCAGGGAGTATGTGACGCGGCTGGTCAACGGGTTCAAGCCGCTGGT
GAGCCGGTCCGCTCGCCTTTACCGCATCCTGGGGGGTTCTGGTGCACCTGC
GGATCCGGACCCGGGAGGCCTCCTTTGAGGAGTGGCTGCGATCCAAGGAA
GTGGCCCTGGATTTTGGCCTGACGGAAAGGCTTCGCGAGCACGAAGCCCA
GCTGGTGATCCTGGCCCAGGCTCTGGACCATTACGACTGTCTGATCCACA
GCACACCGCACACGCTGGTCGAGCGGGGGCTGCAATCGGCCCTGAAGTAT
GAGGAGTTTTACCTAAAGCGTTTTGGCGGGCACTACATGGAGTCCGTCTT
CCAGATGTACACCCGCATCGCCGGCTTTTTGGCCTGCCGGGCCACGCGCG
GCATGCGCCACATCGCCCTGGGGCGAGAGGGGTCGTGGTGGGAAATGTTC
AAGTTCTTTTTCCACCGCCTCTACGACCACCAGATCGTACCGTCGACCCC
CGCCATGCTGAACCTGGGGACCCGCAACTACTACACCTCCAGCTGCTACC
TGGTAAACCCCCAGGCCACCACAAACAAGGCGACCCTGCGGGCCATCACC
AGCAACGTCAGTGCCATCCTCGCCCGCAACGGGGGCATCGGGCTATGCGT
GCAGGCGTTTAACGACTCCGGCCCCGGGACCGCCAGCGTCATGCCCGCCC
TCAAGGTCCTTGACTCGCTGGTGGCGGCGCACAACAAAGAGAGCGCGCGT
CCGACCGGCGCGTGCGTGTACCTGGAGCCGTGGCACACCGACGTGCGGGC
CGTGCTCCGGATGAAGGGGGTCCTCGCCGGCGAAGAGGCCCAGCGCTGCG
ACAATATCTTCAGCGCCCTCTGGATGCCAGACCTGTTTTTCAAGCGCCTG
ATTCGCCACCTGGACGGCGAGAAGAACGTCACATGGACCCTGTTCGACCG
GGACACCAGCATGTCGCTCGCCGACTTTCACGGGGAGGAGTTCGAGAAGC
TCTACCAGCACCTCGAGGTCATGGGGTTCGGCGAGCAGATACCCATCCAG
GAGCTGGCCTATGGCATTGTGCGCAGTGCGGCCACGACCGGGAGCCCCTT
CGTCATGTTCAAAGACGCGGTGAACCGCCACTACATCTACGACACCCAGG
GGGCGGCCATCGCCGGCTCCAACCTCTGCACCGAGATCGTCCATCCGGCC
TCCAAGCGATCCAGTGGGGTCTGCAACCTGGGAAGCGTGAATCTGGCCCG
ATGCGTCTCCAGGCAGACGTTTGACTTTGGGCGGCTCCGCGACGCCGTGC
AGGCGTGCGTGCTGATGGTGAACATCATGATCGACAGCACGCTACAACCC
ACGCCCCAGTGCACCCGCGGCAACGACAACCTGCGGTCCATGGGAATCGG
CATGCAGGGCCTGCACACGGCCTGCCTGAAGCTGGGGCTGGATCTGGAGT
CTGTCGAATTTCAGGACCTGAACAAACACATCGCCGAGGTGATGCTGCTG
TCGGCGATGAAGACCAGCAACGCGCTGTGCGTTCGCGGGGCCCGTCCCTT
CAACCACTTTAAGCGCAGCATGTATCGCGCCGGCCGCTTTCACTGGGAGC
GCTTTCCGGACGCCCGGCCGCGGTACGAGGGCGAGTGGGAGATGCTACGC
CAGAGCATGATGAAACACGGCCTGCGCAACAGCCAGTTTGTCGCGCTGAT
GCCCACCGCCGCCTCGGCGCAGATCTCGGACGTCAGCGAGGGCTTTGCCC
CCCTGTTCACCAACCTGTTCAGCAAGGTGACCCGGGACGGCGAGACGCTG
CGCCCCAACACGCTCCTGCTAAAGGAACTGGAACGCACGTTTAGCGGGAA
GCGCCTCCTGGAGGTGATGGACAGTCTCGACGCCAAGCAGTGGTCCGTGG
CGCAGGCGCTCCCGTGCCTGGAGCCCACCCACCCCCTCCGGCGATTCAAG
ACCGCGTTTGACTACGACCAGAAGTTGCTGATCGACCTGTGTGCGGACCG
CGCCCCCTACGTCGACCATAGCCAATCCATGACCCTGTATGTCACGGAGA
AGGCGGACGGGACCCTCCCAGCCTCCACCCTGGTCCGCCTTCTGGTCCAC

GCATATAAGCGCGGACTAAAAACAGGGATGTACTACTGCAAGGTTCGCAA
GGCGACCAACAGCGGGGTCTTTGGCGGCGACGACAACATTGTCTGCACGA
GCTGCGCGCTGTGA

SEQ ID NO. 44: the amino acid sequence of ICP6 in the Mut 3 and Mut-3Δ34.5 viral strains.

MASRPAASSPVEARAPVGGQEAGGPSAATQGEAAGAPLAHGHHVYCQRVN
GVMVLSDKTPGSASYRISDSNFVQCGSNCTMIIDGDVVRGRPQDPGAAAS
PAPFVAVTNIGAGSDGGTAVVAFGGTPRRSAGTSTGTQTADVPTEALGGP
PPPPRFTLGGGCCSCRDTRRRSAVFGGEGDPVGPAEFVSDDRSSDSDSDD
SEDTDSETLSHASSDVSGGATYDDALDSDSSSDDSLQIDGPVCRPWSNDT
APLDVCPGTPGPGADAGGPSAVDPHAPTPEAGAGLAADPAVARDDAEGLS
DPRPRLGTGTAYPVPLELTPENAEAVARFLGDAVNREPALMLEYFCRCAR
EETKRVPPRTFGSPPRLTEDDFGLLNYALVEMQRLCLDVPPVPPNAYMPY
YLREYVTRLVNGFKPLVSRSARLYRILGVLVHLRIRTREASFEEWLRSKE
VALDFGLTERLREHEAQLVILAQALDHYDCLIHSTPHTLVERGLQSALKY
EEFYLKRFGGHYMESVFQMYTRIAGFLACRATRGMRHIALGREGSWWEMF
KFFFHRLYDHQIVPSTPAMLNLGTRNYYTSSCYLVNPQATTNKATLRAIT
SNVSAILARNGGIGLCVQAFNDSGPGTASVMPALKVLDSLVAAHNKESAR
PTGACVYLEPWHTDVRAVLRMKGVLAGEEAQRCDNIFSALWMPDLFFKRL
IRHLDGEKNVTWTLFDRDTSMSLADFHGEEFEKLYQHLEVMGFGEQIPIQ
ELAYGIVRSAATTGSPFVMFKDAVNRHYIYDTQGAAIAGSNLCTEIVHPA
SKRSSGVCNLGSVNLARCVSRQTFDFGRLRDAVQACVLMVNIMIDSTLQP
TPQCTRGNDNLRSMGIGMQGLHTACLKLGLDLESVEFQDLNKHIAEVMLL
SAMKTSNALCVRGARPFNHFKRSMYRAGRFHWERFPDARPRYEGEWEMLR
QSMMKHGLRNSQFVALMPTAASAQISDVSEGFAPLFTNLFSKVTRDGETL
RPNTLLLKELERTFSGKRLLEVMDSLDAKQWSVAQALPCLEPTHPLRRFK
TAFDYDQKLLIDLCADRAPYVDHSQSMTLYVTEKADGTLPASTLVRLLVH
AYKRGLKTGMYYCKVRKATNSGVFGGDDNIVCTSCAL

SEQ ID NO. 45: the DNA sequence of ICP6 in the 17TermA strain.

ATGGCCAGCCGCCCAGCCGCATCCTCTCCCGTCGAAGCGCGGGCCCCGGT
TGGGGGACAGGAGGCCGGCGGCCCCAGCGCAGCCACCCAGGGGGAGGCCG
CCGGGGCCCCTCTCGCCCACGGCCACCACGTGTACTGCCAGCGAGTCAAT
GGCGTGATGGTGCTTTCCGACAAGACGCCCGGGTCCGCGTCCTACCGCAT
CAGCGATAGCAACTTTGTCCAATGTGGTTCCAACTGCACCATGATCATCG
ACGGAGACGTGGTGCGCGGGCGCCCCCAGGACCCGGGGGCCGCGGCATCC
CCCGCTCCCTTCGTTGCGGTGACAAACATCGGAGCCGGCAGCGACGGCGG
GACCGCCGTCGTGGCATTCGGGGGAACCCCACGTCGCTCGGCGGGGACGT
CTACCGGTACCCAGACGGCCGACGTCCCCACCGAGGCCCTTGGGGGCCCC
CCTCCTCCTCCCCGCTTCACCCTGGGTGGCGGCTGTTGTTCCTGTCGCGA
CACACGGCGCCGCTCTGCGGTATTCGGGGGGGAGGGGGATCCAGTCGGCC
CCGCGGAGTTCGTCTCGGACGACCGGTCGTCCGATTCCGACTCGGATGAC
TCGGAGGACACGGACTCGGAGACGCTGTCACACGCCTCCTCGGACGTGTC
CGGCGGGGCCACGTACGACGACGCCCTTGACTCCGATTCGTCATCGGATG
ACTCCCTGCAGATAGATGGCCCCGTGTGTCGCCCGTGGAGCAATGACACC
GCGCCCCTGGATGTTTGCCCCGGGACCCCCGGCCCGGGCGCCGACGCCGG

TGGTCCCTCAGCGGTAGACCCACACGCGCCGACGCCAGAGGCCGGCGCTG
GTCTTGCGGCCGATCCCGCCGTGGCCCGGGACGACGCGGAGGGGCTTTCG
GACCCCCGGCCACGTCTGGGAACGGGCACGGCCTACCCCGTCCCCCTGGA
ACTCACGCCCGAGAACGCGGAGGCCGTGGCGCGCTTTCTGGGAGATGCCG
TGAACCGCGAACCCGCGCTCATGCTGGAGTACTTTTGCCGGTGCGCCCGC
GAGGAAACCAAGCGTGTCCCCCCCCAGGACATTCGGCAGCCCCCCTCGCCT
CACGGAGGACGACTTTGGGCTTCTCAACTACGCGCTCGTGGAGATGCAGC
GCCTGTGTCTGGACGTTCCTCCGGTCCCGCCGAACGCATACATGCCCTAT
TATCTCAGGGAGTATGTGACGCGGCTGGTCAACGGGTTCAAGCCGCTGGT
GAGCCGGTCCGCTCGCCTTTACCGCATCCTGGGGGGTTCTGGTGCACCTGC
GGATCCGGACCCGGGAGGCCTCCTTTGAGGAGTGGCTGCGATCCAAGGAA
GTGGCCCTGGATTTTGGCCTGACGGAAAGGCTTCGCGAGCACGAAGCCCA
GCTGGTGATCCTGGCCCAGGCTCTGGACCATTACGACTGTCTGATCCACA
GCACACCGCACACGCTGGTCGAGCGGGGGCTGCAATCGGCCCTGAAGTAT
GAGGAGTTTTACCTAAAGCGTTTTGGCGGGCACTACATGGAGTCCGTCTT
CCAGATGTACACCCGCATCGCCGGCTTTTTGGCCTGCCGGGCCACGCGCG
GCATGCGCCACATCGCCCTGGGGCGAGAGGGGTCGTGGTGGGAAATGTTC
AAGTTCTTTTTCCACCGCCTCTACGACCACCAGATCGTACCGTCGACCCC
CGCCATGCTGAACCTGGGGACCCGCAACTACTACACCTCCAGCTGCTACC
TGGTAAACCCCCAGGCCACCACAAACAAGGCGACCCTGCGGGCCATCACC
AGCAACGTCAGTGCCATCCTCGCCCGCAACGGGGGCATCGGGCTATGCGT
GCAGGCGTTTAACGACTCCGGCCCCGGGACCGCCAGCGTCATGCCCGCCC
TCAAGGTCCTTGACTCGCTGGTGGCGGCGCACAACAAAGAGAGCGCGCGT
CCGACCGGCGCGTGCGTGTACCTGGAGCCGTGGCACACCGACGTGCGGGC
CGTGCTCCGGATGAAGGGGGTCCTCGCCGGCGAAGAGGCCCAGCGCTGCG
ACAATATCTTCAGCGCCCTCTGGATGCCAGACCTGTTTTTCAAGCGCCTG
ATTCGCCACCTGGACGGCGAGAAGAACGTCACATGGACCCTGTTCGACCG
GGACACCAGCATGTCGCTCGCCGACTTTCACGGGGAGGAGTTCGAGAAGC
TCTACCAGCACCTCGAGGTCATGGGGTTCGGCGAGCAGATACCCATCCAG
GAGCTGGCCTATGGCATTGTGCGCAGTGCGGCCACGACCGGGAGCCCCTT
CGTCATGTTCAAAGACGCGGTGAACCGCCACTACATCTACGACACCCAGG
GGGCGGCCATCGCCGGCTCCAACCTCTGCACCGAGATCGTCCATCCGGCC
TCCAAGCGATCCAGTGGGGTCTGCAACCTGGGAAGCGTGAATCTGGCCCG
ATGCGTCTCCAGGCAGACGTTTGACTTTGGGCGGCTCCGCGACGCCGTGC
AGGCGTGCGTGCTGATGGTGAACATCATGATCGACAGCACGCTACAACCC
ACGCCCCAGTGCACCCGCGGCAACGACAACCTGCGGTCCATGGGAATCGG
CATGCAGGGCCTGCACACGGCCTGCCTGAAGCTGGGGCTGGATCTGGAGT
CTGCCGAATTTCAGGACCTGAACAAACACATCGCCGAGGTGATGCTGCTG
TCGGCGATGAAGACCAGCAACGCGCTGTGCGTTCGCGGGGCCCGTCCCTT
CAACCACTTTAAGCGCAGCATGTATCGCGCCGGCCGCTTTCACTGGGAGC
GCTTTCCGGACGCCCGGCCGCGGTACGAGGGCGAGTGGGAGATGCTACGC
CAGAGCATGATGAAACACGGCCTGCGCAACAGCCAGTTTGTCGCGCTGAT
GCCCACCGCCGCCTCGGCGCAGATCTCGGACGTCAGCGAGGGCTTTGCCC
CCCTGTTCACCAACCTGTTCAGCAAGGTGACCCGGGACGGCGAGACGCTG
CGCCCCAACACGCTCCTGCTAAAGGAACTGGAACGCACGTTTAGCGGGAA
GCGCCTCCTGGAGGTGATGGACAGTCTCGACGCCAAGCAGTGGTCCGTGG
CGCAGGCGCTCCCGTGCCTGGAGCCCACCCACCCCCTCCGGCGATTCAAG

ACCGCGTTTGACTACGACCAGAAGTTGCTGATCGACCTGTGTGCGGACCG
CGCCCCCTACGTCGACCATAGCCAATCCATGACCCTGTATGTCACGGAGA
AGGCGGACGGGACCCTCCCAGCCTCCACCCTGGTCCGCCTTCTGGTCCAC
GCATATAAGCGCGGACTAAAAACAGGGATGTACTACTGCAAGGTTCGCAA
GGCGACCAACAGCGGGGTCTTTGGCGGCGACGACAACATTGTCTGCATGA
GCTGCGCGCTGTGA

SEQ ID NO. 46: the amino acid sequence of ICP6 in the 17TermA strain.

MASRPAASSPVEARAPVGGQEAGGPSAATQGEAAGAPLAHGHHVYCQRVN
GVMVLSDKTPGSASYRISDSNFVQCGSNCTMIIDGDVVRGRPQDPGAAAS
PAPFVAVTNIGAGSDGGTAVVAFGGTPRRSAGTSTGTQTADVPTEALGGP
PPPPRFTLGGGCCSCRDTRRRSAVFGGEGDPVGPAEFVSDDRSSDSDSDD
SEDTDSETLSHASSDVSGGATYDDALDSDSSSDDSLQIDGPVCRPWSNDT
APLDVCPGTPGPGADAGGPSAVDPHAPTPEAGAGLAADPAVARDDAEGLS
DPRPRLGTGTAYPVPLELTPENAEAVARFLGDAVNREPALMLEYFCRCAR
EETKRVPPRTFGSPPRLTEDDFGLLNYALVEMQRLCLDVPPVPPNAYMPY
YLREYVTRLVNGFKPLVSRSARLYRILGVLVHLRIRTREASFEEWLRSKE
VALDFGLTERLREHEAQLVILAQALDHYDCLIHSTPHTLVERGLQSALKY
EEFYLKRFGGHYMESVFQMYTRIAGFLACRATRGMRHIALGREGSWWEMF
KFFFHRLYDHQIVPSTPAMLNLGTRNYYTSSCYLVNPQATTNKATLRAIT
SNVSAILARNGGIGLCVQAFNDSGPGTASVMPALKVLDSLVAAHNKESAR
PTGACVYLEPWHTDVRAVLRMKGVLAGEEAQRCDNIFSALWMPDLFFKRL
IRHLDGEKNVTWTLFDRDTSMSLADFHGEEFEKLYQHLEVMGFGEQIPIQ
ELAYGIVRSAATTGSPFVMFKDAVNRHYIYDTQGAAIAGSNLCTEIVHPA
SKRSSGVCNLGSVNLARCVSRQTFDFGRLRDAVQACVLMVNIMIDSTLQP
TPQCTRGNDNLRSMGIGMQGLHTACLKLGLDLESAEFQDLNKHIAEVMLL
SAMKTSNALCVRGARPFNHFKRSMYRAGRFHWERFPDARPRYEGEWEMLR
QSMMKHGLRNSQFVALMPTAASAQISDVSEGFAPLFTNLFSKVTRDGETL
RPNTLLLKELERTFSGKRLLEVMDSLDAKQWSVAQALPCLEPTHPLRRFK
TAFDYDQKLLIDLCADRAPYVDHSQSMTLYVTEKADGTLPASTLVRLLVH
AYKRGLKTGMYYCKVRKATNSGVFGGDDNIVCMSCAL

SEQ ID NO. 47: the DNA sequence of rat cyctochrome P450 2B1 in the rRp450 strain.

GAACCCCTTCGCCATGGAGCCCAGTATCTTGCTCCTCCTTGCTCTCCTTG
TGGGCTTCTTGTTACTCTTAGTCAGGGGACACCCAAAGTCCCGTGGCAAC
TTCCCACCAGGACCTCGTCCCCTTCCCCTCTTGGGGAACCTCCTGCAGTT
GGACAGAGGGGGCCTCCTCAATTCCTTCATGCAGCTTCGAGAAAAATATG
GAGATGTGTTCACAGTACACCTGGGACCAAGGCCTGTGGTCATGCTATGT
GGGACAGACACCATAAAGGAGGCTCTGGTGGGCCAAGCTGAGGATTTCTC
TGGTCGGGGAACAATCGCTGTGATTGAGCCAATCTTCAAGGAATATGGTG
TGATCTTTGCCAATGGGGAACGCTGGAAGGCCCTTCGGCGATTCTCTCTG
GCTACCATGAGAGACTTTGGGATGGGAAAGAGGAGTGTGGAAGAACGGAT
TCAGGAGGAAGCCCAATGTTTGGTGGAGGAACTGCGGAAATCCCAGGGAG
CCCCACTGGATCCCACCTTCCTCTTCCAGTGCATCACAGCCAACATCATC
TGCTCCATTGTGTTTGGAGAGCGCTTTGACTACACAGACCGCCAGTTCCT

GCGCCTGTTGGAGCTGTTCTACCGGACCTTTTCCCTCCTAAGTTCATTCT
CCAGCCAGGTGTTTGAGTTCTTCTCTGGGTTCCTGAAATACTTTCCTGGT
GCCCACAGACAAATCTCCAAAAACCTCCAGGAAATCCTCGATTACATTGG
CCATATTGTGGAGAAGCACAGGGCCACCTTAGACCCAAGCGCTCCACGAG
ACTTCATCGACACTTACCTTCTGCGCATGGAGAAGGAGAAGTCGAACCAC
CACACAGAGTTCCATCATGAGAACCTCATGATCTCCCTGCTCTCTCTCTT
CTTTGCTGGCACTGAGACCAGCAGCACCACACTCCGCTATGGTTTCCTGC
TGATGCTCAAGTACCCCCATGTCGCAGAGAAAGTCCAAAAGGAGATTGAT
CAGGTGATCGGCTCACACCGGCTACCAACCCTTGATGACCGCAGTAAAAT
GCCATACACTGATGCAGTTATCCATGAGATTCAGAGGTTTTCAGATCTTG
TCCCTATTGGAGTACCACACAGAGTCACCAAAGACACCATGTTCCGAGGG
TACCTGCTTCCCAAGAACACTGAAGTGTACCCCATCCTGAGTTCAGCTCT
CCATGACCCACAGTACTTTGACCACCCAGACAGCTTCAATCCTGAACACT
TCCTGGATGCCAATGGGGCACTGAAAAAGAGTGAAGCTTTCATGCCCTTC
TCCACAGGAAAGCGCATTTGTCTTGGCGAAGGCATTGCCCGAAATGAATT
GTTCCTCTTCTTCACCACCATCCTCCAGAACTTCTCTGTGTCAAGCCATT
TGGCTCCCAAGGACATTGACCTCACGCCCAAGGAGAGTGGCATTGGAAAA
ATACCTCCAACGTACCAGATCTGCTTCTCAGCTCGGTGATCCGGCTGAGG
CAGCCATGTGCCCCAGTTCTGTTGGGAATGGAACTTGTTTATTGCAGCTT
ATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCA
TTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATC
TTATCATGTCTGGATCCCCGGGCGAGCTCGAATTCCTCCTTTGAGGAGTG
GCTGCGATCCAAGGAAGTGGCCCTGGACTTTGGCCTGACGGAAAGGCTTC
GCGAGCACGAAGCCCAGCTGGTGATCCTGGCCCAGGCTCTGGACCATTAC
GACTGTCTGATCCACAGCACACCGCACACGCTGGTCGAGCGGGGGCTGCA
ATCGGCCCTGAAGTATGAGGAGTTTTACCTAAAGCGCTTTGGCGGGCACT
ACATGGAGTCCGTCTTCCAGATGTACACCCGCATCGCCGGCTTTTTGGCC
TGCCGGGCCACGCGCGGCATGCGCCACATCGCCCTGGGGCGAGAGGGGTC
GTGGTGGGAAATGTTCAAGTTCTTTTTCCACCGCCTCTACGACCACCAGA
TCGTACCGTCGACCCCCGCCATGCTGAACCTGGGGACCCGCAACTACTAC
ACCTCCAGCTGCTACCTGGTAAACCCCCAGGCCACCACAAACAAGGCGAC
CCTGCGGGCCATCACCAGCAACGTCAGCGCCATCCTCGCCCGCAACGGGG
GCATCGGGCTATGCGTGCAGGCGTTTAACGACTCCGGCCCCGGGACCGCT
AGCGTCATACCCGCCCTCAAGGTCCTCGACTCGCTGGTGGCGGCGCACAA
CAAAGAGAGCGCGCGTCCAACCGGCGCGTGCGTGTACCTGGAGCCGTGGC
ACACCGACGTGCGGGCCGTGCTCCGGATGAAGGGGGTCCTCGCCGGCGAA
GAGGCCCAGCGCTGCGACAATATCTTCAGCGCCCTCTGGATGCCAGACCT
GTTTTTCAAGCGCCTGATTCGCCACCTGGACGGCGAGAAGAACGTCACAT
GGACCCTGTTCGACCGGGACACCAGCATGTCGCTCGCCGACTTTCACGGG
GAGGAGTTCGAGAAGCTCTACCAGCACCTCGAGGTCATGGGGTTCGGCGA
GCAGATACCCATCCAGGAGCTGGCCTATGGCATTGTGCGCAGTGCGGCCA
CGACCGGGAGCCCCTTCGTCATGTTCAAAGACGCGGTGAACCGCCACTAC
ATCTACGACACCCAGGGGGCGGCCATCGCCGGCTCCAACCTCTGCACCGA
GATCGTCCATCCGGCCTCCAAGCGATCCAGTGGGGTCTGCAATCTGGGAA
GCGTGAATCTGGCCCGATGCGTCTCCAGGCAGACGTTTGACTTTGGGCGG
CTCCGCGACGCCGTGCAGGCGTGCGTGCTGATGGTGAACATCATGATCGA
CAGCACGCTACAACCCACGCCCCAGTGCACCCGCGGCAACGACAACCTGC

GGTCCATGGGAATCGGCATGCAGGGCCTGCACACGGCCTGCCTGAAGCTG
GGGCTGGATCTGGAGTCTGTCGAATTTCAGGACCTGAACAAACACATCGC
CGAGGTGATGCTGCTGTCGGCGATGAAGACCAGCAACGCGCTGTGCGTTC
GCGGGGCCCGTCCCTTCAACCACTTTAAGCGCAGCATGTATCGCGCCGGC
CGCTTTCACTGGGAGCGCTTTCCGGACGCCCGGCCGCGGTACGAGGGCGA
GTGGGAGATGCTACGCCAGAGCATGATGAAACACGGCCTGCGCAACAGCC
AGTTTGTCGCGCTGATGCCCACCGCCGCCTCGGCGCAGATCTCGGACGTC
AGCGAGGGCTTTGCCCCCCTGTTCACCAACCTGTTCAGCAAGGTGACCCG
GGACGGCGAGACGCTGCGCCCCAACACGCTCCTGCTAAAGGAACTGGAAC
GCACGTTTAGCGGGAAGCGCCTCCTGGAGGTGATGGACAGTCTCGACGCC
AAGCAGTGGTCCGTGGCGCAGGCGCTCCCGTGCCTGGAGCCCACCCACCC
CCTCCGGCGATTCAAGACCGCGTTTGACTACGACCAGAAGTTGCTGATCG
ACCTGTGTGCGGACCGCGCCCCCTACGTCGACCATAGCCAATCCATGACC
CTGTATGTCACGGAGAAGGCGGACGGGACCCTCCCAGCCTCCACCCTGGT
CCGCCTTCTGGTCCACGCATATAAGCGCGGACTAAAAACAGGGATGTACT
ACTGCAAGGTTCGCAAGGCGACCAACAGCGGGGTCTTTGGCGGCGACGAC
AACATTGTCTGCACGAGCTGCGCGCTGTGA

SEQ ID NO. 48: the amino acid sequence of rat cyctochrome P450 2B1 in the rRp450 strain.

MEPSILLLLALLVGFLLLLVRGHPKSRGNFPPGPRPLPLLGNLLQL
DRGGLLNSFMQLREKYGDVFTVHLGPRPVVMLCGTDTIKEALVGQAEDFS
GRGTIAVIEPIFKEYGVIFANGERWKALRRFSLATMRDFGMGKRSVEERI
QEEAQCLVEELRKSQGAPLDPTFLFQCITANIICSIVFGERFDYTDRQFL
RLLELFYRTFSLLSSFSSQVFEFFSGFLKYFPGAHRQISKNLQEILDYIG
HIVEKHRATLDPSAPRDFIDTYLLRMEKEKSNHHTEFHHENLMISLLSLF
FAGTETSSTTLRYGFLLMLKYPHVAEKVQKEIDQVIGSHRLPTLDDRSKM
PYTDAVIHEIQRFSDLVPIGVPHRVTKDTMFRGYLLPKNTEVYPILSSAL
HDPQYFDHPDSFNPEHFLDANGALKKSEAFMPFSTGKRICLGEGIARNEL
FLFFTTILQNFSVSSHLAPKDIDLTPKESGIGKIPPTYQICFSAR*
*Stop codon - sequences after * are predicted no expressed

SEQ ID NO. 49: the DNA sequence of ICP6 in the wild-type 17 strain.

ATGGCCAGCCGCCCAGCCGCATCCTCTCCCGTCGAAGCGCGGGCCCCGGT
TGGGGGACAGGAGGCCGGCGGCCCCAGCGCAGCCACCCAGGGGGAGGCCG
CCGGGGCCCCTCTCGCCCACGGCCACCACGTGTACTGCCAGCGAGTCAAT
GGCGTGATGGTGCTTTCCGACAAGACGCCCGGGTCCGCGTCCTACCGCAT
CAGCGATAGCAACTTTGTCCAATGTGGTTCCAACTGCACCATGATCATCG
ACGGAGACGTGGTGCGCGGGCGCCCCCAGGACCCGGGGGCCGCGGCATCC
CCCGCTCCCTTCGTTGCGGTGACAAACATCGGAGCCGGCAGCGACGGCGG
GACCGCCGTCGTGGCATTCGGGGGAACCCCACGTCGCTCGGCGGGGACGT
CTACCGGTACCCAGACGGCCGACGTCCCCACCGAGGCCCTTGGGGGCCCC
CCTCCTCCTCCCCGCTTCACCCTGGGTGGCGGCTGTTGTTCCTGTCGCGA
CACACGGCGCCGCTCTGCGGTATTCGGGGGGGAGGGGGATCCAGTCGGCC
CCGCGGAGTTCGTCTCGGACGACCGGTCGTCCGATTCCGACTCGGATGAC
TCGGAGGACACGGACTCGGAGACGCTGTCACACGCCTCCTCGGACGTGTC
CGGCGGGGCCACGTACGACGACGCCCTTGACTCCGATTCGTCATCGGATG
ACTCCCTGCAGATAGATGGCCCCGTGTGTCGCCCGTGGAGCAATGACACC

GCGCCCCTGGATGTTTGCCCCGGGACCCCCGGCCCGGGCGCCGACGCCGG
TGGTCCCTCAGCGGTAGACCCACACGCGCCGACGCCAGAGGCCGGCGCTG
GTCTTGCGGCCGATCCCGCCGTGGCCCGGGACGACGCGGAGGGGCTTTCG
GACCCCCGGCCACGTCTGGGAACGGGCACGGCCTACCCCGTCCCCCTGGA
ACTCACGCCCGAGAACGCGGAGGCCGTGGCGCGCTTTCTGGGAGATGCCG
TGAACCGCGAACCCGCGCTCATGCTGGAGTACTTTTGCCGGTGCGCCCGC
GAGGAAACCAAGCGTGTCCCCCCCAGGACATTCGGCAGCCCCCCTCGCCT
CACGGAGGACGACTTTGGGCTTCTCAACTACGCGCTCGTGGAGATGCAGC
GCCTGTGTCTGGACGTTCCTCCGGTCCCGCCGAACGCATACATGCCCTAT
TATCTCAGGGAGTATGTGACGCGGCTGGTCAACGGGTTCAAGCCGCTGGT
GAGCCGGTCCGCTCGCCTTTACCGCATCCTGGGGGTTCTGGTGCACCTGC
GGATCCGGACCCGGGAGGCCTCCTTTGAGGAGTGGCTGCGATCCAAGGAA
GTGGCCCTGGATTTTGGCCTGACGGAAAGGCTTCGCGAGCACGAAGCCCA
GCTGGTGATCCTGGCCCAGGCTCTGGACCATTACGACTGTCTGATCCACA
GCACACCGCACACGCTGGTCGAGCGGGGGCTGCAATCGGCCCTGAAGTAT
GAGGAGTTTTACCTAAAGCGTTTTGGCGGGCACTACATGGAGTCCGTCTT
CCAGATGTACACCCGCATCGCCGGCTTTTTGGCCTGCCGGGCCACGCGCG
GCATGCGCCACATCGCCCTGGGGCGAGAGGGGTCGTGGTGGGAAATGTTC
AAGTTCTTTTTCCACCGCCTCTACGACCACCAGATCGTACCGTCGACCCC
CGCCATGCTGAACCTGGGGACCCGCAACTACTACACCTCCAGCTGCTACC
TGGTAAACCCCCAGGCCACCACAAACAAGGCGACCCTGCGGGCCATCACC
AGCAACGTCAGTGCCATCCTCGCCCGCAACGGGGGCATCGGGCTATGCGT
GCAGGCGTTTAACGACTCCGGCCCCGGGACCGCCAGCGTCATGCCCGCCC
TCAAGGTCCTTGACTCGCTGGTGGCGGCGCACAACAAAGAGAGCGCGCGT
CCGACCGGCGCGTGCGTGTACCTGGAGCCGTGGCACACCGACGTGCGGGC
CGTGCTCCGGATGAAGGGGGTCCTCGCCGGCGAAGAGGCCCAGCGCTGCG
ACAATATCTTCAGCGCCCTCTGGATGCCAGACCTGTTTTTCAAGCGCCTG
ATTCGCCACCTGGACGGCGAGAAGAACGTCACATGGACCCTGTTCGACCG
GGACACCAGCATGTCGCTCGCCGACTTTCACGGGGAGGAGTTCGAGAAGC
TCTACCAGCACCTCGAGGTCATGGGGTTCGGCGAGCAGATACCCATCCAG
GAGCTGGCCTATGGCATTGTGCGCAGTGCGGCCACGACCGGGAGCCCCTT
CGTCATGTTCAAAGACGCGGTGAACCGCCACTACATCTACGACACCCAGG
GGGCGGCCATCGCCGGCTCCAACCTCTGCACCGAGATCGTCCATCCGGCC
TCCAAGCGATCCAGTGGGGTCTGCAACCTGGGAAGCGTGAATCTGGCCCG
ATGCGTCTCCAGGCAGACGTTTGACTTTGGGCGGCTCCGCGACGCCGTGC
AGGCGTGCGTGCTGATGGTGAACATCATGATCGACAGCACGCTACAACCC
ACGCCCCAGTGCACCCGCGGCAACGACAACCTGCGGTCCATGGGAATCGG
CATGCAGGGCCTGCACACGGCCTGCCTGAAGCTGGGGCTGGATCTGGAGT
CTGTCGAATTTCAGGACCTGAACAAACACATCGCCGAGGTGATGCTGCTG
TCGGCGATGAAGACCAGCAACGCGCTGTGCGTTCGCGGGGCCCGTCCCTT
CAACCACTTTAAGCGCAGCATGTATCGCGCCGGCCGCTTTCACTGGGAGC
GCTTTCCGGACGCCCGGCCGCGGTACGAGGGCGAGTGGGAGATGCTACGC
CAGAGCATGATGAAACACGGCCTGCGCAACAGCCAGTTTGTCGCGCTGAT
GCCCACCGCCGCCTCGGCGCAGATCTCGGACGTCAGCGAGGGCTTTGCCC
CCCTGTTCACCAACCTGTTCAGCAAGGTGACCCGGGACGGCGAGACGCTG
CGCCCCAACACGCTCCTGCTAAAGGAACTGGAACGCACGTTTAGCGGGAA
GCGCCTCCTGGAGGTGATGGACAGTCTCGACGCCAAGCAGTGGTCCGTGG

CGCAGGCGCTCCCGTGCCTGGAGCCCACCCACCCCCTCCGGCGATTCAAG
ACCGCGTTTGACTACGACCAGAAGTTGCTGATCGACCTGTGTGCGGACCG
CGCCCCCTACGTCGACCATAGCCAATCCATGACCCTGTATGTCACGGAGA
AGGCGGACGGGACCCTCCCAGCCTCCACCCTGGTCCGCCTTCTGGTCCAC
GCATATAAGCGCGGACTAAAAACAGGGATGTACTACTGCAAGGTTCGCAA
GGCGACCAACAGCGGGGTCTTTGGCGGCGACGACAACATTGTCTGCACGA
GCTGCGCGCTGTGA

SEQ ID NO. 50: the amino acid sequence of ICP6 in the wild-type 17 strain.

MASRPAASSPVEARAPVGGQEAGGPSAATQGEAAGAPLAHGHHVYCQRVN
GVMVLSDKTPGSASYRISDSNFVQCGSNCTMIIDGDVVRGRPQDPGAAAS
PAPFVAVTNIGAGSDGGTAVVAFGGTPRRSAGTSTGTQTADVPTEALGGP
PPPPRFTLGGGCCSCRDTRRRSAVFGGEGDPVGPAEFVSDDRSSDSDSDD
SEDTDSETLSHASSDVSGGATYDDALDSDSSSDDSLQIDGPVCRPWSNDT
APLDVCPGTPGPGADAGGPSAVDPHAPTPEAGAGLAADPAVARDDAEGLS
DPRPRLGTGTAYPVPLELTPENAEAVARFLGDAVNREPALMLEYFCRCAR
EETKRVPPRTFGSPPRLTEDDFGLLNYALVEMQRLCLDVPPVPPNAYMPY
YLREYVTRLVNGFKPLVSRSARLYRILGVLVHLRIRTREASFEEWLRSKE
VALDFGLTERLREHEAQLVILAQALDHYDCLIHSTPHTLVERGLQSALKY
EEFYLKRFGGHYMESVFQMYTRIAGFLACRATRGMRHIALGREGSWWEMF
KFFFHRLYDHQIVPSTPAMLNLGTRNYYTSSCYLVNPQATTNKATLRAIT
SNVSAILARNGGIGLCVQAFNDSGPGTASVMPALKVLDSLVAAHNKESAR
PTGACVYLEPWHTDVRAVLRMKGVLAGEEAQRCDNIFSALWMPDLFFKRL
IRHLDGEKNVTWTLFDRDTSMSLADFHGEEFEKLYQHLEVMGFGEQIPIQ
ELAYGIVRSAATTGSPFVMFKDAVNRHYIYDTQGAAIAGSNLCTEIVHPA
SKRSSGVCNLGSVNLARCVSRQTFDFGRLRDAVQACVLMVNIMIDSTLQP
TPQCTRGNDNLRSMGIGMQGLHTACLKLGLDLESVEFQDLNKHIAEVMLL
SAMKTSNALCVRGARPFNHFKRSMYRAGRFHWERFPDARPRYEGEWEMLR
QSMMKHGLRNSQFVALMPTAASAQISDVSEGFAPLFTNLFSKVTRDGETL
RPNTLLLKELERTFSGKRLLEVMDSLDAKQWSVAQALPCLEPTHPLRRFK
TAFDYDQKLLIDLCADRAPYVDHSQSMTLYVTEKADGTLPASTLVRLLVH
AYKRGLKTGMYYCKVRKATNSGVFGGDDNIVCTSCAL

WHAT IS CLAIMED IS:

1. A non-natural herpes simplex virus ("HSV"), wherein the virus comprises one or more mutation(s) in a virulence gene that is from the group of:

(a) a glycoprotein E ("gE")-encoding gene,

(b) an Infected Cell Protein 0 ("ICP0")-encoding gene,

(c) a DNA packaging terminase subunit 1-encoding gene,

(d) an ICP8-encoding gene, or

(e) an ICP34.5-encoding gene.

2. The non-natural HSV of claim 1, wherein the HSV further comprises a gene encoding a dysfunctional ICP34.5 protein and/or a gene encoding a dysfunctional ICP6 protein.

3. The non-natural HSV of claim 2, wherein the gene encoding the dysfunctional ICP34.5 protein comprises a polynucleotide having a sequence selected from SEQ ID NO. 5, SEQ ID NO. 7 and a sequence at least 95% identical to SEQ ID No. 5, or 7 and maintaining the mutation at the nucleotide in any one of SEQ ID No. 5, or 7.

4. The non-natural HSV of claim 2, wherein the gene encoding the dysfunctional ICP6 protein comprises a polynucleotide having a sequence selected from SEQ ID NO. 45, SEQ ID NO. 47, and a sequence at least 95% identical to SEQ ID No. 45, or 47 and maintaining the mutation at the nucleotide in any one of SEQ ID No. 45, or 47.

5. The non-natural HSV of any one of claims 1-4, wherein the mutation in the virulence gene comprises an insertion, a deletion, a truncation, a frameshift, a substitution, or a point mutation.

6. The non-natural HSV of any one of claims 1-5, wherein the HSV lacks a gene encoding a functional ICP34.5 protein and/or a functional ICP6 protein.

7. The non-natural HSV of any one of claims 1-6, wherein the mutation is a nonsynonymous mutation in the virulence gene.

8. The non-natural HSV of any one of claims 1-7 or an equivalent thereof, wherein the mutation or equivalent encodes:

(a) an alanine-to-threonine mutation at position 151 of the gE protein,

(b) an arginine-to-histidine mutation at position 258 of the ICP0 protein,

(c) an alanine-to-threonine mutation at position 376 of the DNA packaging terminase subunit 1 protein,

(d) a threonine-to-methionine mutation at position 1155 of the ICP8 protein, or

(e) a proline-to-histidine mutation at position 119 of the ICP34.5 protein.

9. The non-natural HSV of any one of claims 1-8, comprising one or more of the following:

(a) a polynucleotide encoding an amino acid sequence selected from SEQ ID NOs. 2, 6, 8, 10, and 52, and/or a polynucleotide having a sequence selected from SEQ ID NOs. 1, 5, 7, 9, and 51;

(b) a polypeptide having an amino acid sequence selected from SEQ ID NOs. 2, 6, 8, 10, and 52;

(c) a polynucleotide encoding an amino acid sequence selected from SEQ ID NOs. 13, 15, 17 and 19, and/or a polynucleotide having a sequence selected from SEQ ID NOs. 12, 14, 16 and 18;

(d) a polypeptide having an amino acid sequence selected from SEQ ID NOs. 13, 15, 17 and 19;

(e) a polynucleotide encoding an amino acid sequence selected from SEQ ID NOs. 21, 23 and 26, and/or a polynucleotide having a sequence selected from SEQ ID NOs. 20, 22, 24, 25, and 53, or a sequence thereof free of one or two or more introns;

(f) a polypeptide having an amino acid sequence selected from SEQ ID NOs. 21, 23 and 26;

(g) a polynucleotide encoding an amino acid sequence selected from SEQ ID NOs. 28, 30, 32 and 34, and/or a polynucleotide having a sequence selected from SEQ ID NOs. 27, 29, 31, and 33;

(h) a polypeptide having an amino acid sequence selected from SEQ ID NOs. 28, 30, 32 and 34;

(i) a polynucleotide encoding an amino acid sequence selected from SEQ ID NOs. 36, 38, 40, and 42, and/or a polynucleotide having a sequence selected from SEQ ID NOs. 35, 37, 39 and 41;

(j) a polypeptide having an amino acid sequence selected from SEQ ID NOs. 36, 38, 40, and 42;

(k) a polynucleotide encoding an amino acid sequence selected from SEQ ID NOs. 44, 46, 48 and 50, and/or a polynucleotide having a sequence selected from SEQ ID NOs. 43, 45, 47 and 49;

(l) a poly peptide having an amino acid sequence selected from SEQ ID NOs. 44, 46, 48 and 50.

10. The non-natural HSV of any one of claims 1-9, further comprising a polynucleotide having sequence that is identical to at least a fragment of a virulence gene from a 17TermA HSV.

11. The non-natural HSV of any one of claims 1-10, further comprising a polynucleotide having sequence that is identical to at least a fragment of a virulent gene from an rRp450 HSV.

12. The non-natural HSV of any one of claims 1-11, wherein the HSV is derived from a HSV type 1 (“HSV-1”) or a HSV type 2 (“HSV-2”) strain.

13. The non-natural HSV of any one of claims 1-11, wherein the HSV is derived from a HSV-1 KOS strain.

14. The non-natural HSV of any one of claims 1-13, further comprising a transgene.

15. A composition comprising the non-natural HSV of any one of claims 1-14 and a carrier.

16. The composition of claim 14, wherein the carrier is a pharmaceutically available carrier.

17. The composition of claim 15 or 16, further comprising a cryopreservative that facilitates the freezing and thawing of the non-natural HSV without loss of significant virulence.

18. A non-human mammal infected with the non-natural HSV of any one of claims 1-14.

19. A cell infected with the non-natural HSV of any one of claims 1-14, optionally a lymphocyte.

20. A method to infect a cell, comprising contacting the cell with the non-natural HSV of any one of claims 1-14 or the composition of any one of claims 15-17.

21. The method of claim 20, wherein the cell is a lymphocyte.

22. The method of claim 20 or 21, wherein the cell has been infected with an Epstein-Barr virus ("EBV").

23. A method to prepare a viral vector comprising introducing a transgene into the non-natural HSV of any one of claims 1-13.

24. A method for inhibiting the growth or metastasis of cancer cell, comprising contacting the cell with an effective amount of the non-natural HSV vector of any one of claims 1-14 or the composition of any one of claims 15-17.

25. The method of claim 24, wherein the contacting is *in vitro* or *in vivo*.

26. The method of claim 24, wherein the contacting is *in vivo* by administration of the non-natural to a subject.

27. A method for treating cancer in a subject, comprising administering to the subject an effective amount of the non-natural HSV vector of any one of claims 1-14 or the composition of any one of claims 15-17.

28. The method of any one of claims 24-27, wherein the cancer is of the type, or the cancer cell is selected from a cell of the type: pancreatic cancer, renal cancer, small cell lung cancer, brain cancer, neural cancer, bone cancer, lymphoma, myeloma, colon cancer, uterine cancer, breast cancer, leukemia, liver cancer, prostate cancer, skin cancer, or melanoma.

29. The method of claim 27 or 28, wherein the HSV vector or the pharmaceutical composition is administered by injection, infusion, instillation, and/or inhalation.

30. The method of any one of claims 27-28, wherein the administration comprises a therapy of the group of: a first line therapy, a second line therapy, a third line therapy, a fourth line therapy, or a fifth line therapy.

31. The method of claim 27, further comprising administering to the subject an effective amount of an anticancer therapy.

32. The method of any one of claims 27-31, wherein the subject is a mammal.

33. The method of claim 32, wherein the mammal is from the group of: a human, a mouse, a rat, a guinea pig, a non-human primate, a dog, a cat, a horse, a cow, a pig, a goat, or a sheep.

34. The method of claim 32, wherein the subject is human.

35. A method of producing the HSV vector of any one of claims 1-13, comprising
(a) introducing to a host cell a 17TermA HSV vector and an rRp450 HSV vector;
(b) growing the host cell for at least 3 passages; and
(c) isolating an HSV particle produced by the host cell.

36. The method of claim 35, wherein the HSV is introduced to the host cell by transfection, infection, transformation, electroporation, injection, microinjection, or the combination thereof.

37. The method of claim 35 or 36, wherein the host cell is grown for at least 3 passages, 10 passages, 20 passages, 30 passages, 40 passages, or 50 passages.

38. The method of any one of claims 35-37, wherein the host cell comprises a complementing gene product to support replication of the introduced HSV vectors.

39. The method of claim 38, wherein the complementing gene encodes an ICP6 protein and/or an ICP34.5 protein.

40. The method of any one of claims 35-39, further comprising introducing a transgene into the HSV vector.

41. The HSV particle produced by the method of any one of claims 35-40.

42. A method for inducing cell lysis, comprising contacting the cell with the non-natural HSV of any one of claims 1-14 or the composition of any one of claims 15-17.

43. The method of claim 42, wherein the cell is a cancer cell.

44. A kit comprising the HSV vector of any one of claims 1-14 or the composition of any one of claims 15-17, and instructions for use.

45. A method of producing the HSV vector of any one of claims 1-13, comprising

(a) introducing to a host cell a polynucleotide encoding a viral genome of the HSV vector;

(b) growing the host cell; and

(c) isolating an HSV particle produced by the host cell.

46. The method according to claim 45, wherein the polynucleotide is introduced to the host cell by transfection, infection, transformation, electroporation, injection, microinjection, or the combination thereof.

47. The method according to claim 45 or 46, wherein the nucleic acid sequence encoding the viral genome is introduced to the host cell in a vector.

48. The method according to claim 47, wherein the vector is an HSV or a plasmid.

49. The method according to any one of claims 45 to 48, wherein the host cell comprises a complementing gene product to support replication of the introduced HSV vectors.

50. The method according to any one of claims 45 to 49, wherein the isolated HSV is substantially free of host cells, cell debris, culture medium or any other agent used in culturing the host cells.

51. The method according to any one of claims 45 to 50, wherein the isolation is via centrifuge, filtration, chromatography, or any combination thereof.

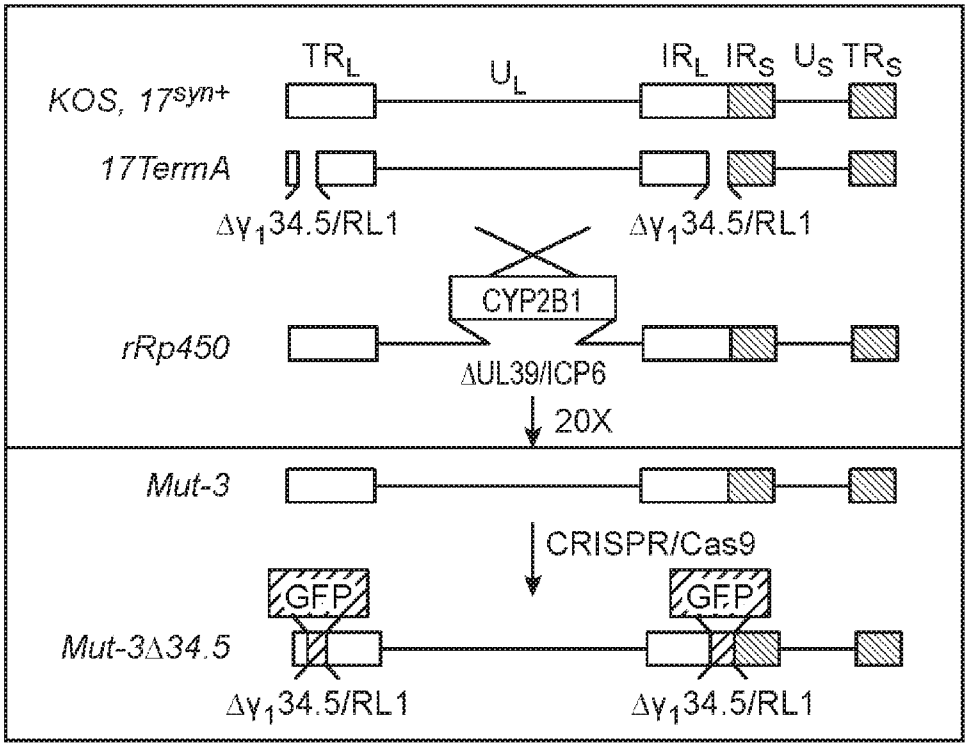

TRL
UL
IRL IRS US TRS
KOS, 17syn+
17TermA
Δγ134.5/RL1
Δγ134.5/RL1
CYP2B1
rRp450
ΔUL39/ICP6
20X
Mut-3
CRISPR/Cas9
GFP
GFP
Mut-3Δ34.5
Δγ134.5/RL1
Δγ134.5/RL1

FIG. 1A

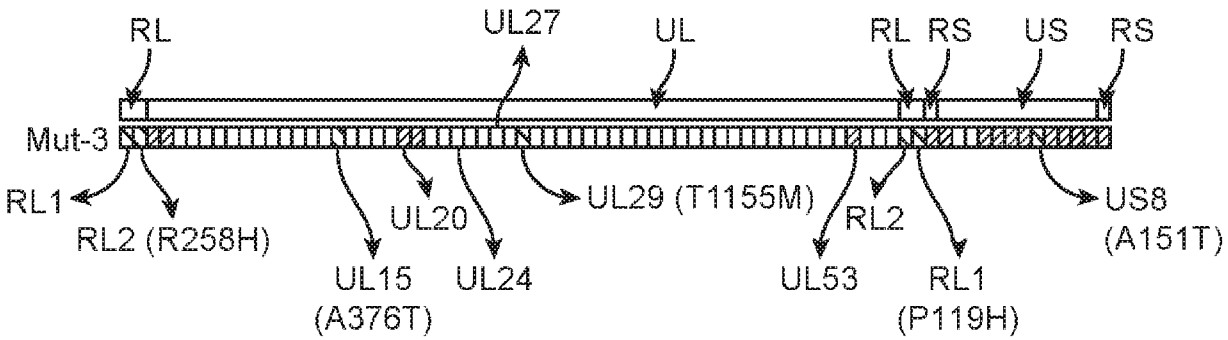

RL
UL27
UL
RL RS
US
RS
Mut-3
RL1
RL2 (R258H)
UL15
(A376T)
UL20
UL24
UL29 (T1155M)
UL53
RL2
RL1
(P119H)
US8
(A151T)

FIG. 1B

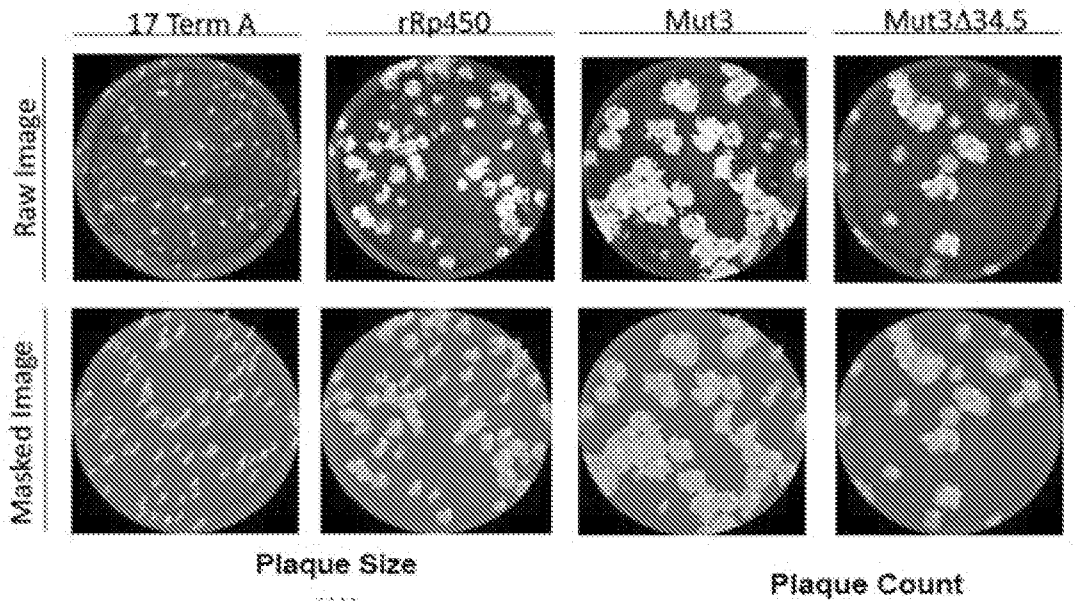

17 Term A
rRp450
Mut3
Mut3Δ34.5
Raw Image
Masked Image
Plaque Size
Plaque Count

FIG. 2A

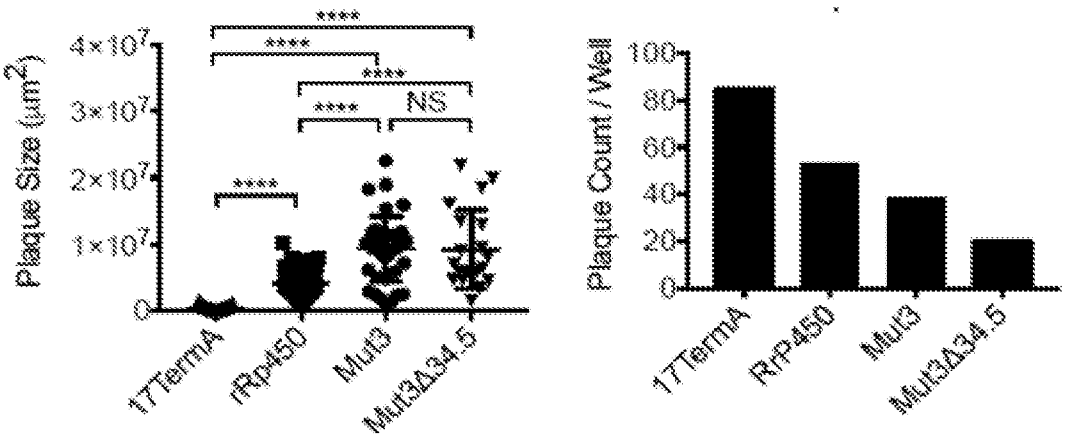

Plaque Size (μm²)
4×10⁷
3×10⁷
2×10⁷
1×10⁷
0
****
NS
17TermA
rRp450
Mut3
Mut3Δ34.5
Plaque Count / Well
100
80
60
40
20
0
17TermA
RrP450
Mut3
Mut3Δ34.5

FIG. 2B

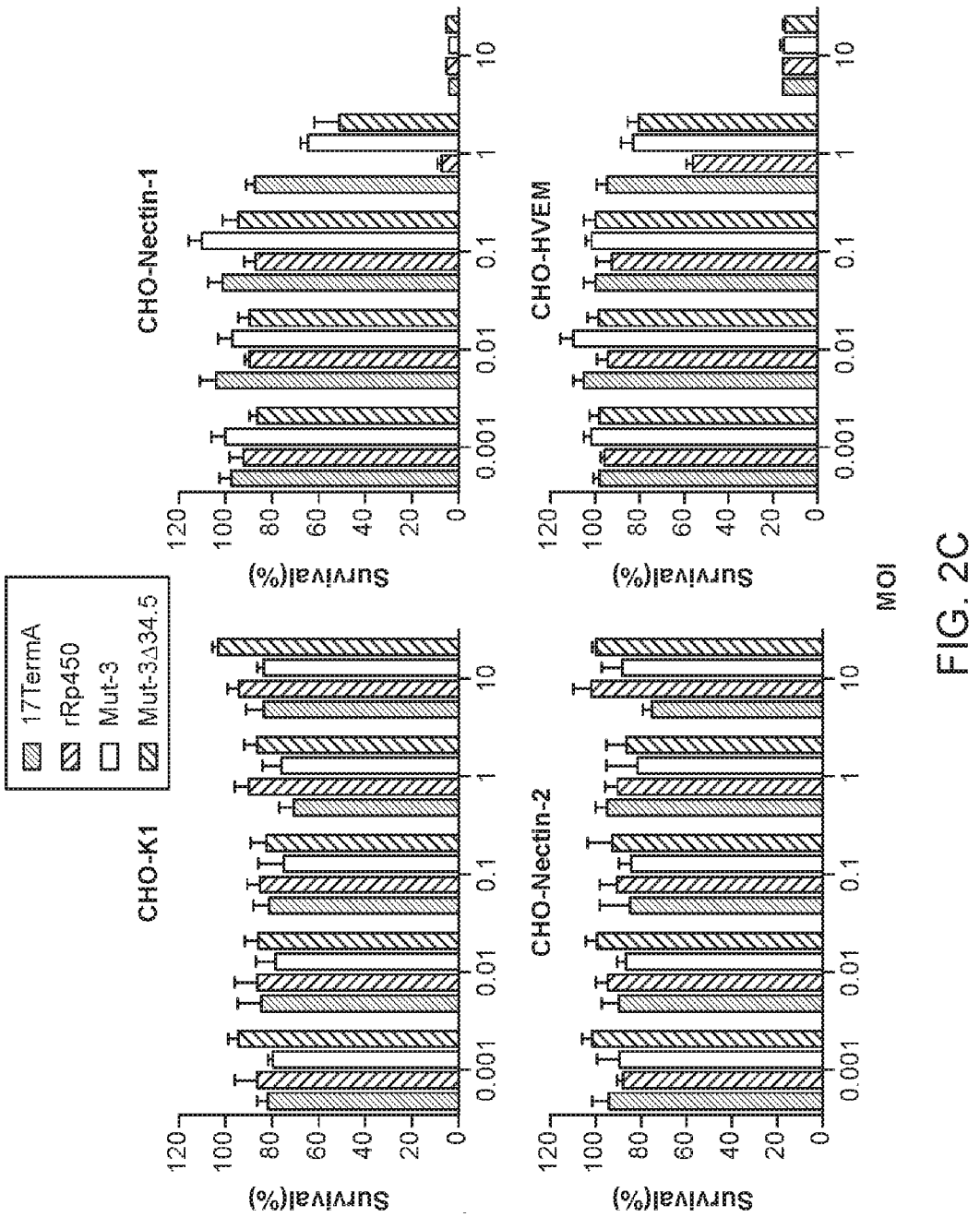

17TermA
rRp450
Mut-3
Mut-3Δ34.5
CHO-K1
CHO-Nectin-1
CHO-Nectin-2
CHO-HVEM
Survival(%)
MOI
0
20
40
60
80
100
120
0.001
0.01
0.1
1
10

FIG. 2C

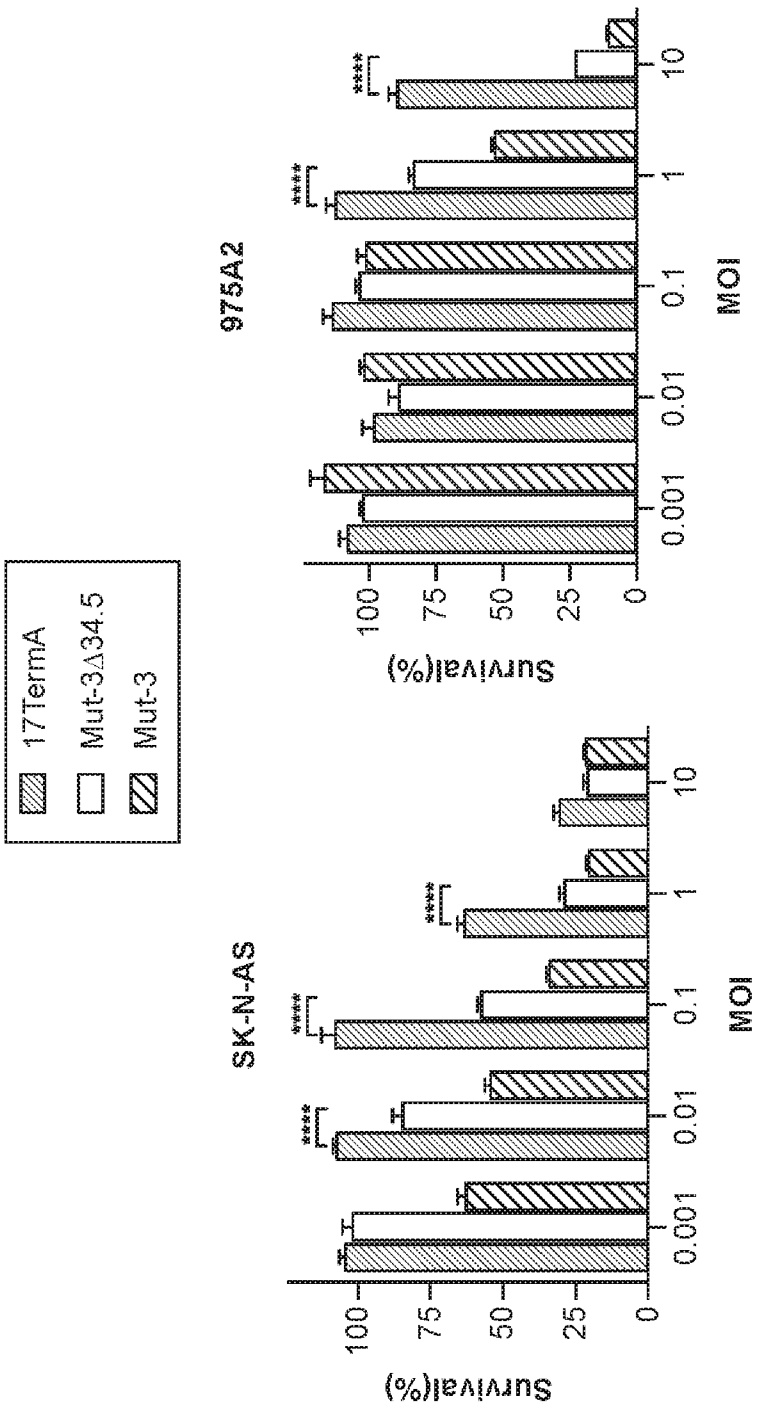

17TermA
Mut-3Δ34.5
Mut-3
975A2
SK-N-AS
Survival(%)
MOI
0
25
50
75
100
0.001
0.01
0.1
1
10
****

FIG. 3A

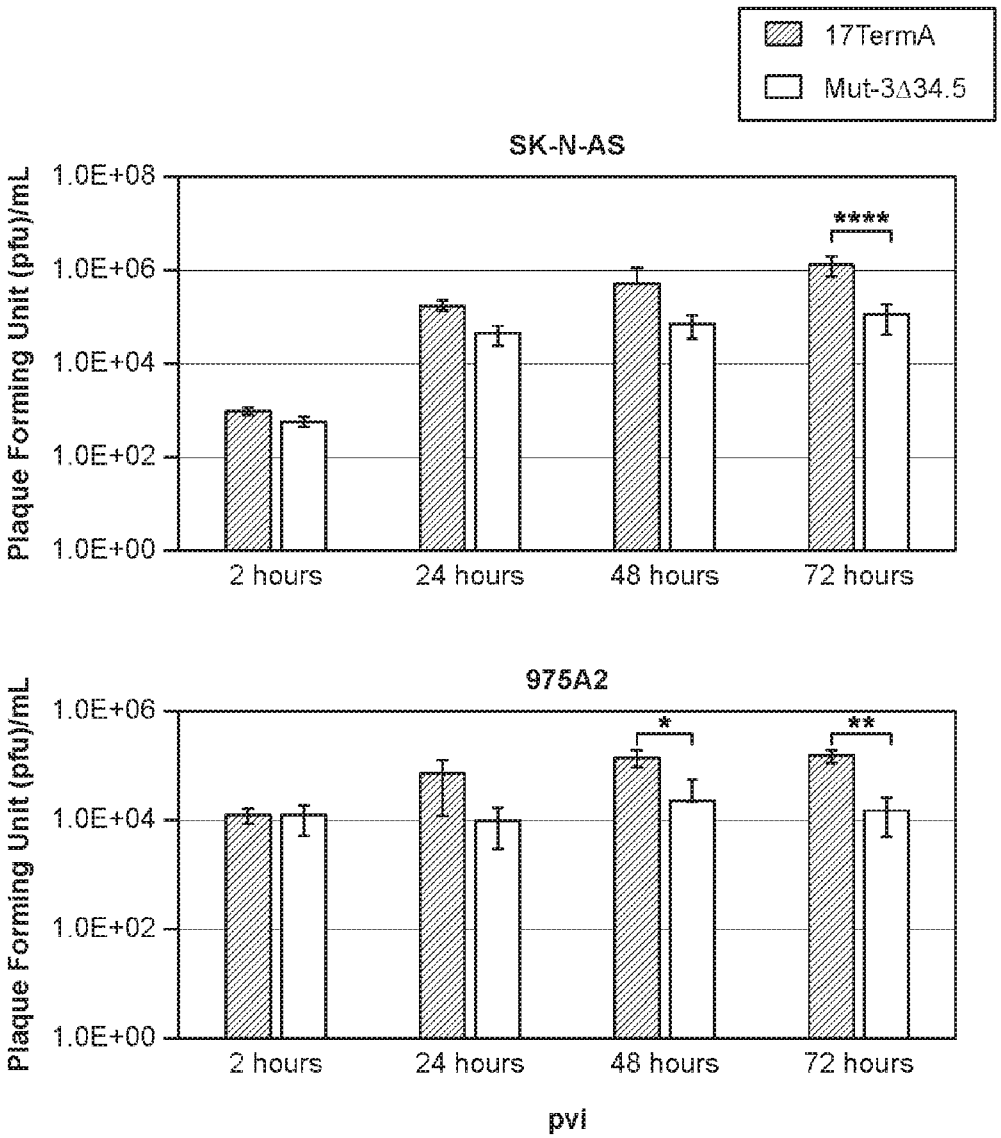

17TermA
Mut-3Δ34.5
SK-N-AS
Plaque Forming Unit (pfu)/mL
1.0E+08
1.0E+06
1.0E+04
1.0E+02
1.0E+00
****
2 hours
24 hours
48 hours
72 hours
975A2
Plaque Forming Unit (pfu)/mL
1.0E+06
1.0E+04
1.0E+02
1.0E+00
*
**
2 hours
24 hours
48 hours
72 hours
pvi

FIG. 3B

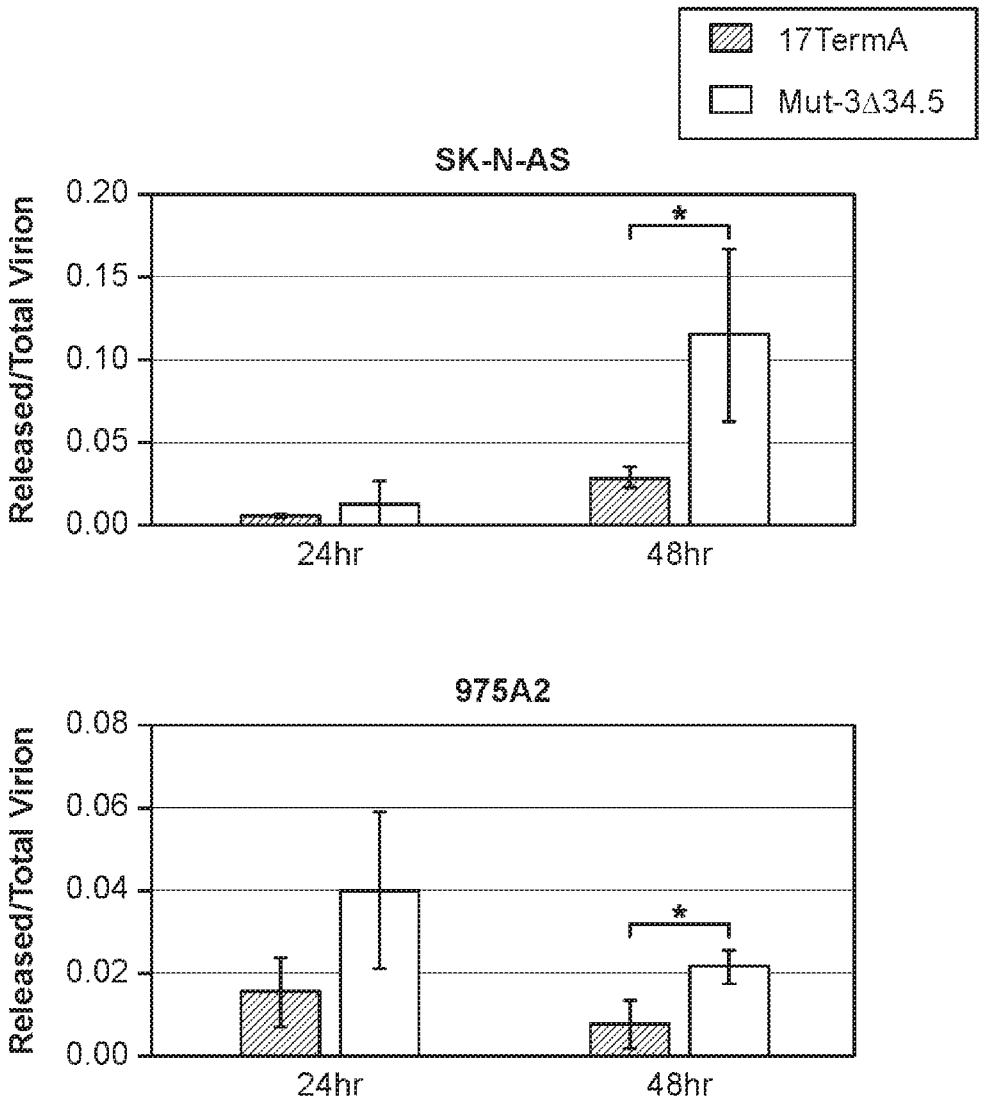
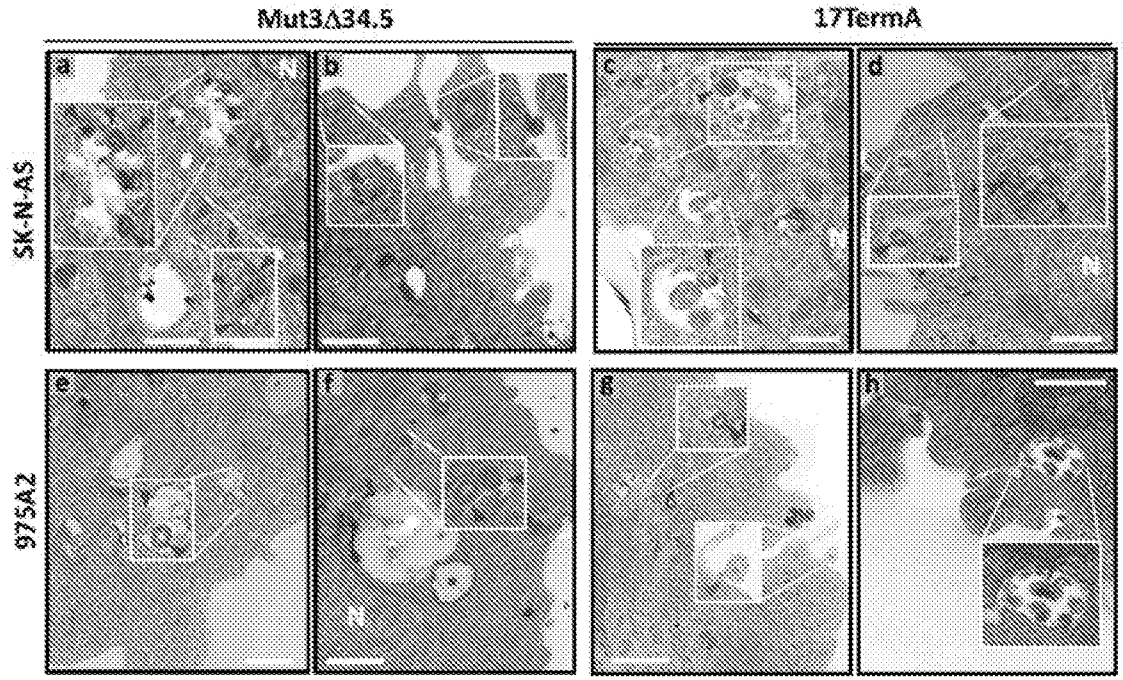

17TermA
Mut-3Δ34.5
SK-N-AS
Released/Total Virion
0.20
0.15
0.10
0.05
0.00
*
24hr
48hr
975A2
Released/Total Virion
0.08
0.06
0.04
0.02
0.00
*
24hr
48hr

FIG. 3C



FIGS. 4A – 4H

a

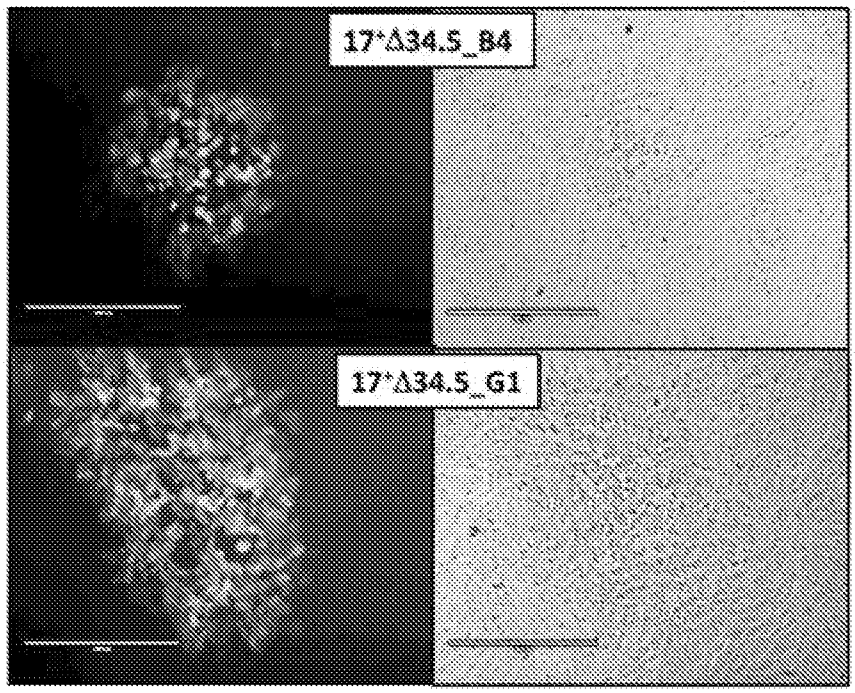


FIG. 5A

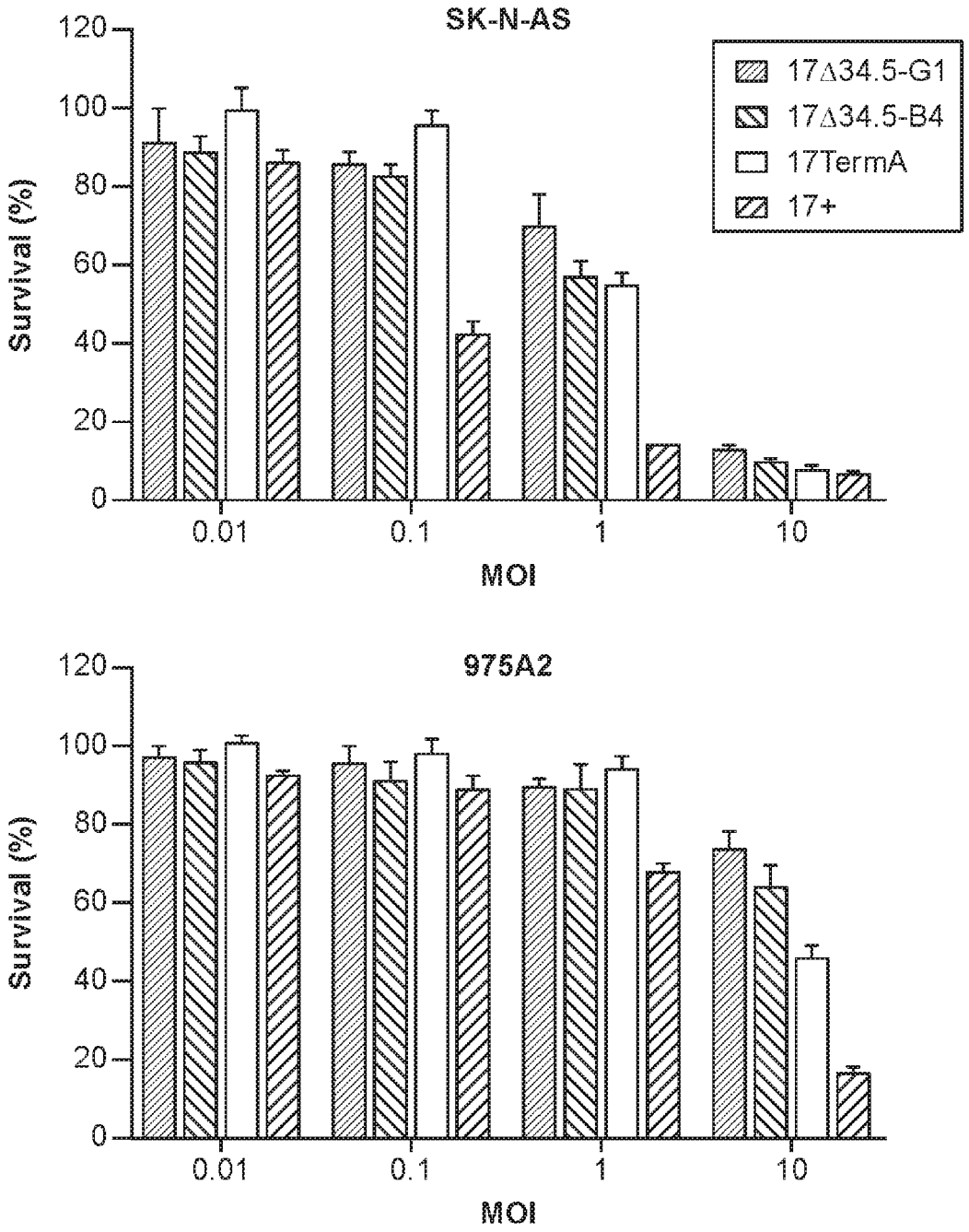

SK-N-AS
17Δ34.5-G1
17Δ34.5-B4
17TermA
17+
Survival (%)
120
100
80
60
40
20
0
0.01
0.1
1
10
MOI
975A2
Survival (%)
120
100
80
60
40
20
0
0.01
0.1
1
10
MOI

FIG. 5B

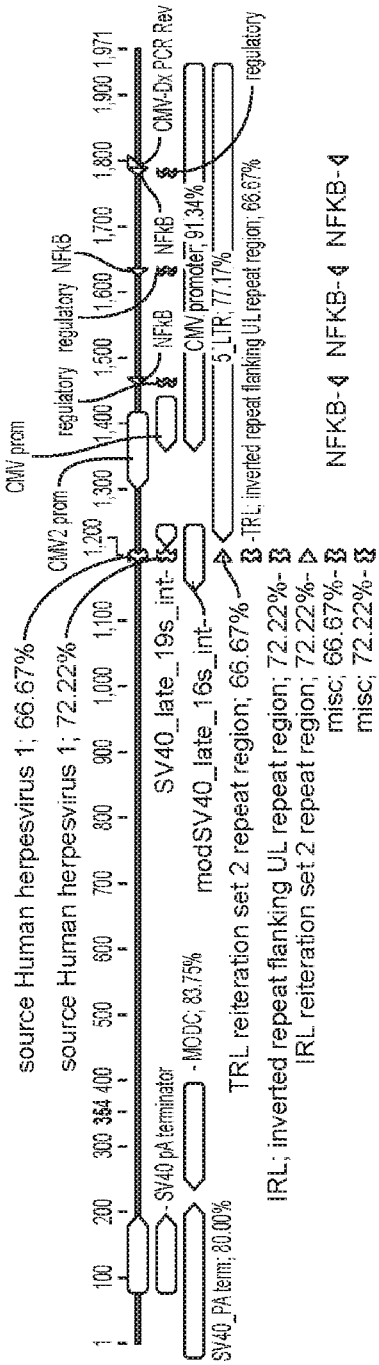

source Human herpesvirus 1; 66.67%
source Human herpesvirus 1; 72.22%
CMV prom
CMV2 prom
regulatory
regulatory NFkB
NFkB
NFkB
CMV promoter; 91.34%
5'LTR; 77.17%
CMV-Dx PCR Rev
regulatory
-TRL; inverted repeat flanking UL repeat region; 66.67%
SV40_late_19s_int-
modSV40_late_16s_int-
TRL reiteration set 2 repeat region; 66.67%
IRL; inverted repeat flanking UL repeat region; 72.22%-
IRL reiteration set 2 repeat region; 72.22%-
misc; 66.67%-
misc; 72.22%-
NFKB-4 NFKB-4 NFKB-4
SV40 pA terminator
- MODC; 83.75%
SV40_PA term; 80.00%
d2EGFP reporter
EGFP-F reporter; 93.23%
EYFP reporter
GFP_cyc3 reporter; GFP reporter; 72.52% -> 72.11%
d2EYFP reporter
EGFP reporter
1
100
200
300
364
400
500
600
700
800
900
1,000
1,100
1,200
1,300
1,400
1,500
1,600
1,700
1,800
1,900
1,971

FIG. 5C

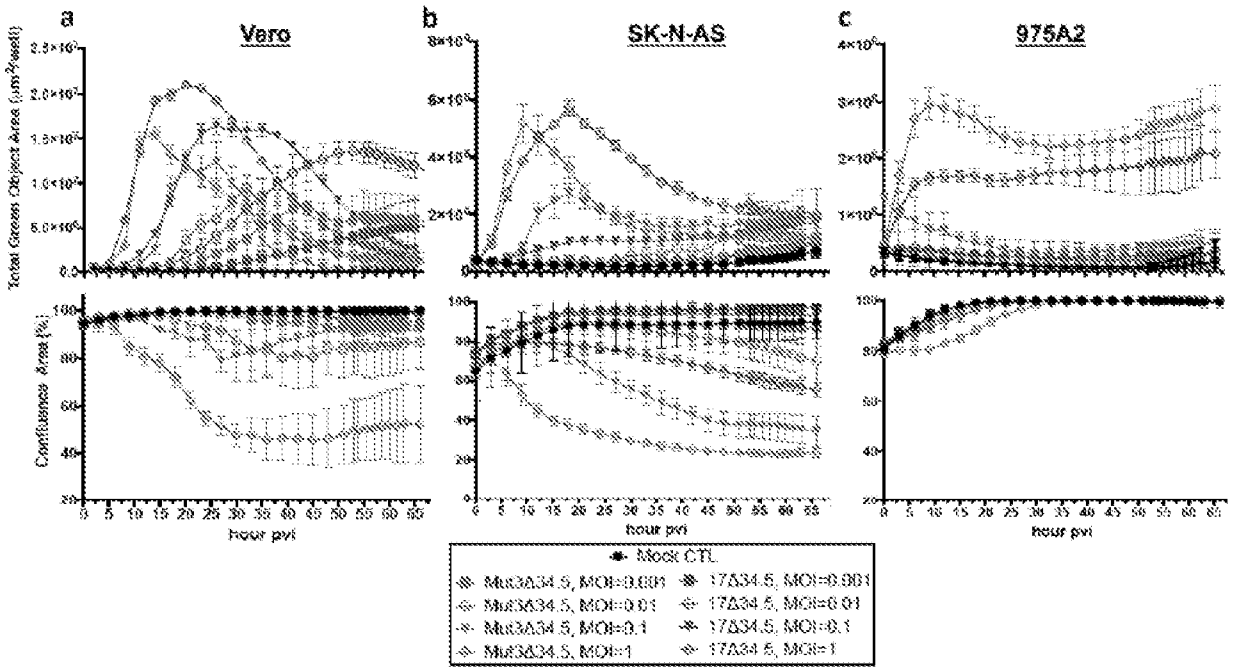
Sequence of EGFP cassette

AACACATTAATTAAAAAACCTCCCACACCTCCCCCTGAACCTGAAACATA
AAATGAATGCAATTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGT
TACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTC
ACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATG
TCTGCTCGAAGCGGCCGGCCGCCCCGACTCTAGACTACACATTGATCCTA
GCAGAAGCACAGGCTGCAGGGTGACGGTCCATCCCGCTCTCCTGGGCACA
AGACATGGGCAGCGTGCCATCATCCTGCTCCTCCACCTCCGGCGGGAAGC
CATGGCTAAGCTTCTTGTACAGCTCGTCCATGCCGAGAGTGATCCCGGCG
GCGGTCACGAACTCCAGCAGGACCATGTGATCGCGCTTCTCGTTGGGGTC
TTTGCTCAGGGCGGACTGGGTGCTCAGGTAGTGGTTGTCGGGCAGCAGCA
CGGGGCCGTCGCCGATGGGGGTGTTCTGCTGGTAGTGGTCGGCGAGCTGC
ACGCTGCCGTCCTCGATGTTGTGGCGGATCTTGAAGTTCACCTTGATGCC
GTTCTTCTGCTTGTCGGCCATGATATAGACGTTGTGGCTGTTGTAGTTGT
ACTCCAGCTTGTGCCCCAGGATGTTGCCGTCCTCCTTGAAGTCGATGCCC
TTCAGCTCGATGCGGTTCACCAGGGTGTCGCCCTCGAACTTCACCTCGGC
GCGGGTCTTGTAGTTGCCGTCGTCCTTGAAGAAGATGGTGCGCTCCTGGA
CGTAGCCTTCGGGCATGGCGGACTTGAAGAAGTCGTGCTGCTTCATGTGG
TCGGGGTAGCGGCTGAAGCACTGCACGCCGTAGGTCAGGGTGGTCACGAG
GGTGGGCCAGGGCACGGGCAGCTTGCCGGTGGTGCAGATGAACTTCAGGG

FIG. 5C (Cont. 1)

TCAGCTTGCCGTAGGTGGCATCGCCCTCGCCCTCGCCGGACACGCTGAAC
TTGTGGCCGTTTACGTCGCCGTCCAGCTCGACCAGGATGGGCACCACCCC
GGTGAACAGCTCCTCGCCCTTGCTCACCATCCGGGAATTGCGGCCGCGGG
TACAATTCCGCAGCTTTTAGAGCAGAAGTAACACTTCCGTACAGGCCTAG
AAGTAAAGGCAACATCCACTGAGGAGCAGTTCTTTGATTTGCACCACCAC
CGGATCCGGGACCTGAAATAAAAGACAAAAAGACTAAACTTACCAGTTAA
CTTTCTGGTTTTTCAGTTCCTCGAGTACCGGATCCTCTAGAGTCCGGAGG
CTGGATCGGTCCCGGTGTCTTCTATGGAGGTCAAAACAGCGTGGATGGCG
TCTCCAGGCGATCTGACGGTTCACTAAACGAGCTCTGCTTATATAGACCT
CCCACCGTACACGCCTACCGCCCATTTGCGTCAATGGGGCGGAGTTGTTA
CGACATTTTGGAAAGTCCCGTTGATTTTGGTGCCAAAACAAACTCCCATT
GACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATC
CACGCCCATTGATGTACTGCCAAAACCGCATCACCATGGTAATAGCGATG
ACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTA
CTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGG
GCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTAC
CGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTAC
TATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCGTTGGGCG
GTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGACCTGCAGGCAT
GCAAGCTCGAATTCGAACACGCAGATGCAGTCGGGGCGGCAGATCTTAAT
TAATGGCTGGTTGTTTGTTGT

FIG. 5C (Cont. 2)


a
Vero
b
SK-N-AS
c
975A2
Total Green Object Area (μm²/well)
Confluence Area (%)
hour pvi
Mock CTL
Mut3Δ34.5, MOI=0.001
Mut3Δ34.5, MOI=0.01
Mut3Δ34.5, MOI=0.1
Mut3Δ34.5, MOI=1
17Δ34.5, MOI=0.001
17Δ34.5, MOI=0.01
17Δ34.5, MOI=0.1
17Δ34.5, MOI=1

FIGS. 6A – 6C

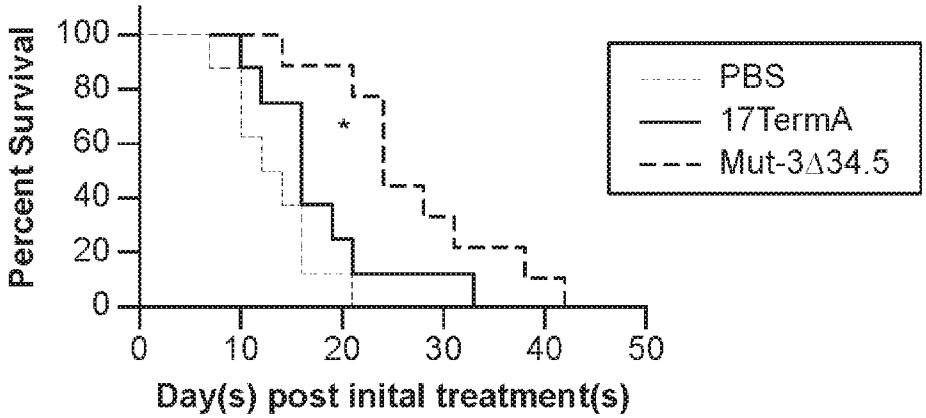
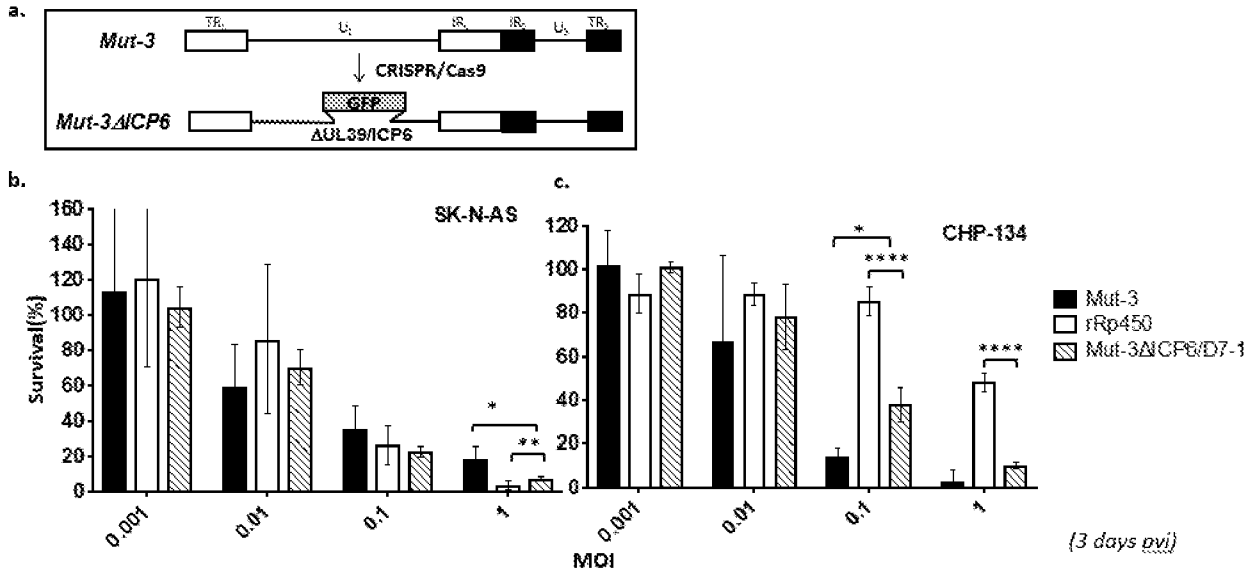

Percent Survival
100
80
60
40
20
0
0
10
20
30
40
50
Day(s) post inital treatment(s)
*
PBS
17TermA
Mut-3Δ34.5

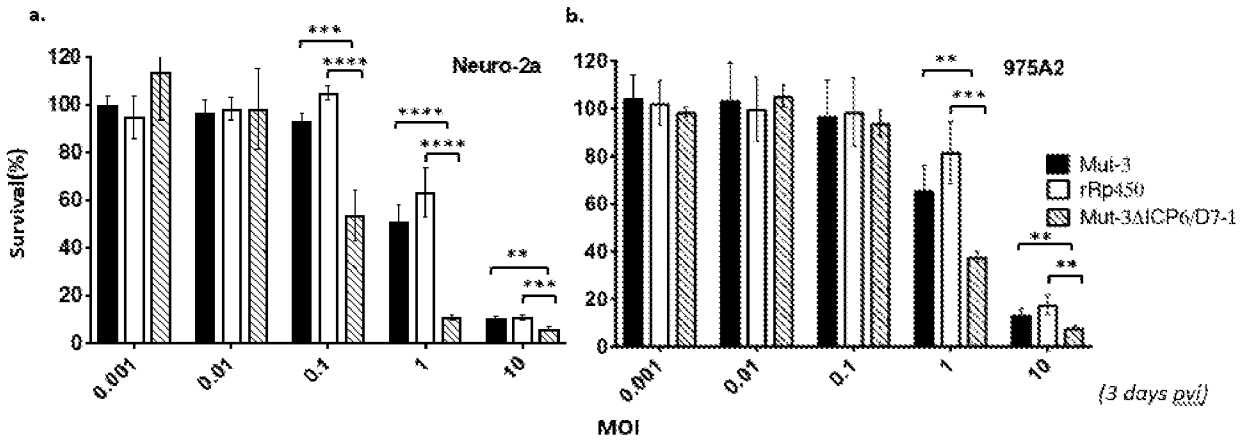
FIG. 7

Mut-3ΔICP6, an attenuated version of Mut-3, induces superior cytotoxicity in the human neuroblastoma cell line CHP-134 compared to oncolytic herpes virus rRp450



FIGS. 8A – 8C

Mut-3ΔICP6 induces superior cytotoxicity in the murine neuroblastoma cell lines Neuro-2a & 975A2 compared to rRp450



FIGS. 9A – 9B

Mut-3ΔICP6 produces a significantly higher virus yield than rRp450 in the murine neuroblastoma cell line 975A2 over 48 and 72hrs infection time periods

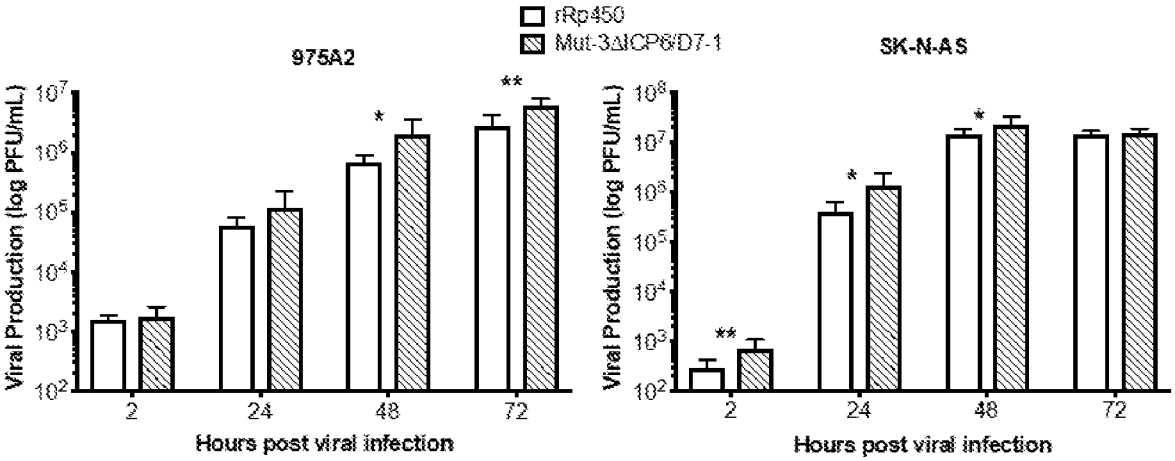


FIG. 10A FIG. 10B

Mut-3Δ34.5 & Mut-3ΔICP6 are significantly less potent than the Mut-3 virus against differentiated human keratinocyte cells

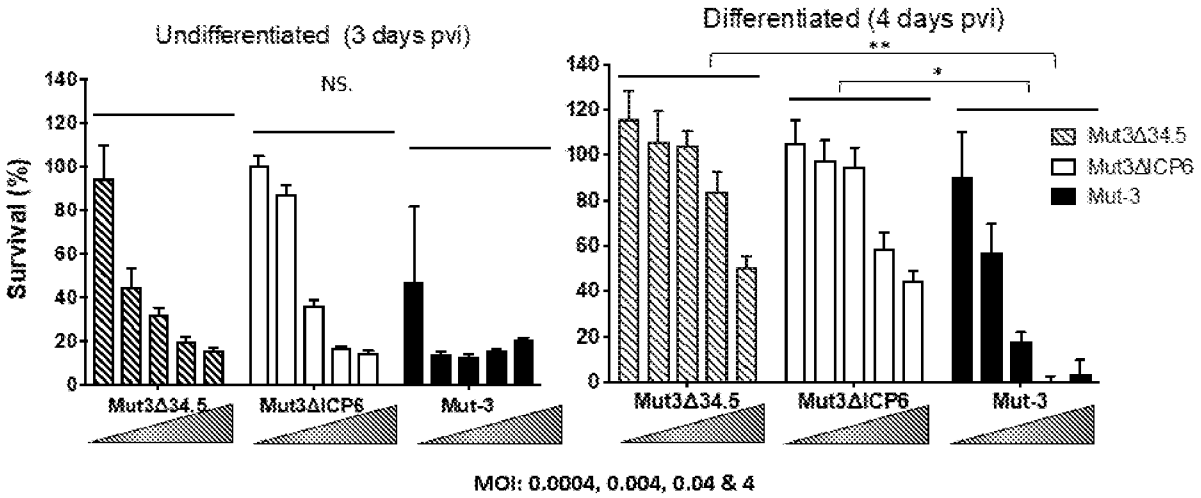


FIG. 11A FIG. 11B

Intravenous injection of 1e6 pfu of wild-type strain KOS virus is lethal to naïve Blab/c mice

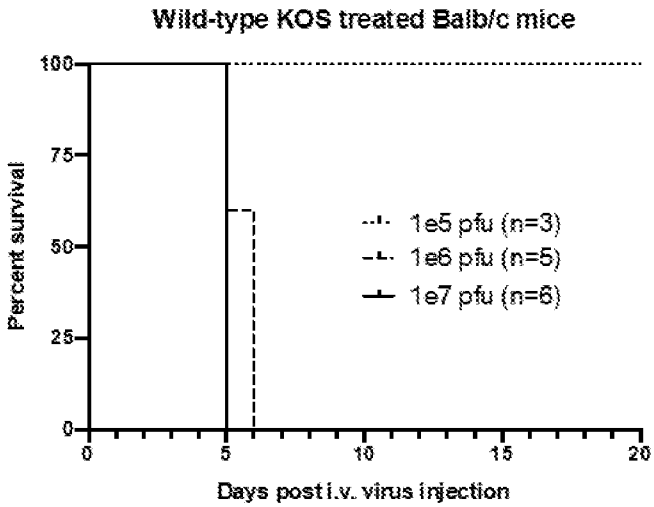
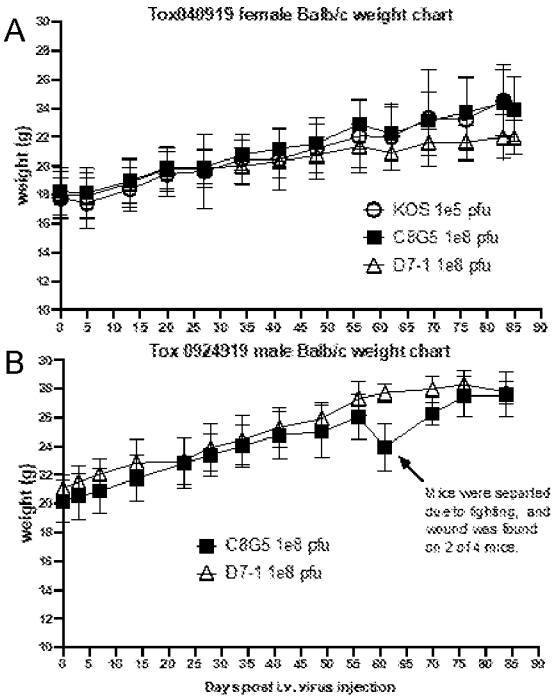


FIG. 12



Naïve Balb/c mice can tolerate intravenous injection of up to 1e8 pfu of Mut-3Δ34.5(C8G5) or Mut-3ΔICP6(D7-1)

FIGS. 13A – 13B

Wild-type KOS		Brain	Heart	Kidney	Liver	Lung	Ovary	Spleen
1e7_#1	plaque			-				-
1e7_#2	plaque	+	-	-	-	-	+	-
1e7_#3	plaque	+	-	+	-	-	+	-
1e7_#4	plaque	+	-	+	-	-	+	-
1e6_#1	plaque	+	-	+		-		-
1e6_#2	plaque	+	-	+	-	-	+	-
1e6_#3	plaque	-	-	+	-	-	+	-

FIG. 14

24hrs pvi		Brain	Heart	Kidney	Liver	Lung	Ovary	Spleen
C8G5 (Mut3-Δ34.5)								
#1	plaque	-	-	+	+	-	+	-
#2	plaque	-	+	-	-	-	-	-
#3	plaque	-	+	+	-	-	-	-
#4	plaque	-	-	+	-	-	-	-
D7-1 (Mut3-ΔICP6)								
#1	plaque	+	+	+	-		+	-
#2	plaque	-	+	+	+	-	+	-
#3	plaque	+	+	+	-	-	-	-
#4	plaque	-	-	+	+	-	+	-

FIG. 15

14 days pvi		Brain	Heart	Kidney	Liver	Lung	Ovary	Spleen
C8G5 (Mut3-Δ34.5)								
#1	plaque	-	-	-	-	-	-	-
#2	plaque	-	-	-	-	-	-	-
#3	plaque	-	-	-	-	-	-	-
#4	plaque	-	-	-	-	-	-	-
D7-1 (Mut3-ΔICP6)								
#1	plaque	-	-	-	-	-	-	-
#2	plaque	-	-	-	-	-	-	-
#3	plaque	-	-	-	-	-	-	-
#4	plaque	-	-	-	-	-	-	-

FIG. 16

28 days pvi		Brain	Heart	Kidney	Liver	Lung	Ovary	Spleen
C8G5 (Mut3-Δ34.5)								
#1	plaque	-	-					
#2	plaque	-	-	-	-	-	-	-
#3	plaque	-	-	-	-	-	-	-
#4	plaque	-	-	-	-	-	-	-
D7-1 (Mut3-ΔICP6)								
#1	plaque	-	-	-	-	-	-	-
#2	plaque	-	-	-	-	-	-	-
#3	plaque	-	-	-	-	-	-	-
#4	plaque	-	-	-	-	-	-	-

FIG. 17

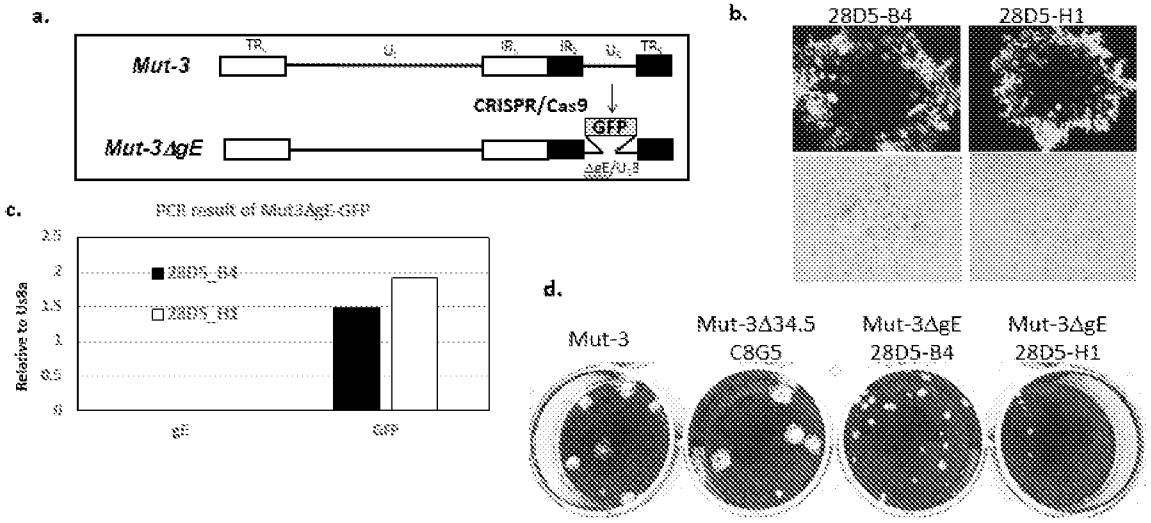
56 days pvi		Brain	Heart	Kidney	Liver	Lung	Ovary	Spleen
C8G5 (Mut3-Δ34.5)								
#1	plaque	-	-	-	-	-	-	-
#2	plaque	-	-	-	-	-	-	-
#3	plaque	-	-	-	-	-	-	-
#4	plaque	-	-	-	-	-	-	-
D7-1 (Mut3-ΔICP6)								
#1	plaque	-	-	-	-	-	-	-
#2	plaque	-	-	-	-	-	-	-
#3	plaque	-	-	-	-	-	-	-
#4	plaque	-	-	-	-	-	-	-

FIG. 18

85 days pvi		Brain	Heart	Kidney	Liver	Lung	Ovary	Spleen
C8G5 (Mut3-Δ34.5)								
#1	plaque	-	-	-	-	-	-	-
#2	plaque	-	-	-	-	-	-	-
#3	plaque	-	-	-	-	-	-	-
#4	plaque	-	-	-	-	-	-	-
D7-1 (Mut3-ΔICP6)								
#1	plaque	-	-	-	-	-	-	-
#2	plaque	-	-	-	-	-	-	-
#3	plaque	-	-	-	-	-	-	-
#4	plaque	-	-	-	-	-	-	-

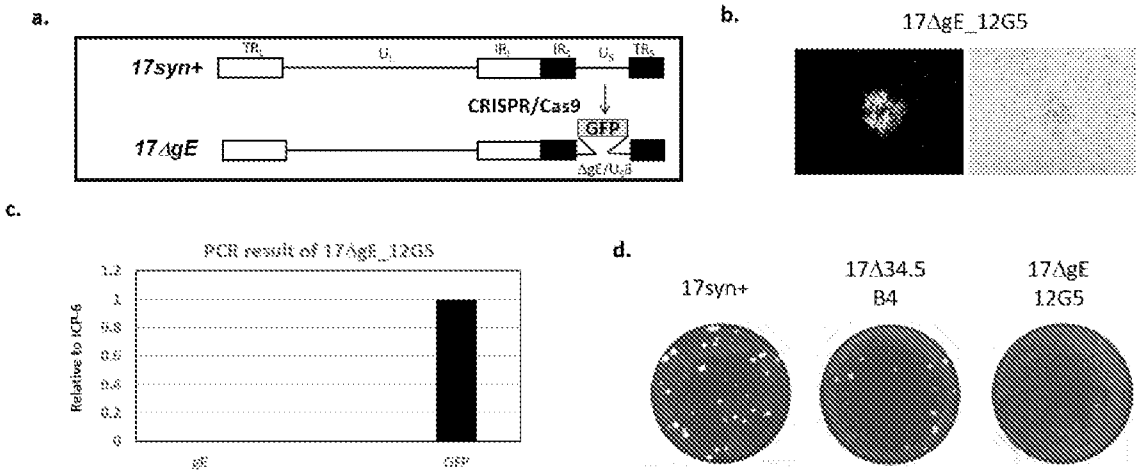
FIG. 19

Plaque size of Mut-3ΔgE is much smaller compare to Mut-3 or Mut-3Δ34.5(C8G5)



FIGS. 20A – 20D

Plaque size of 17ΔgE is much smaller compare to 17syn+ or 17Δ34.5



FIGS. 21A – 21D

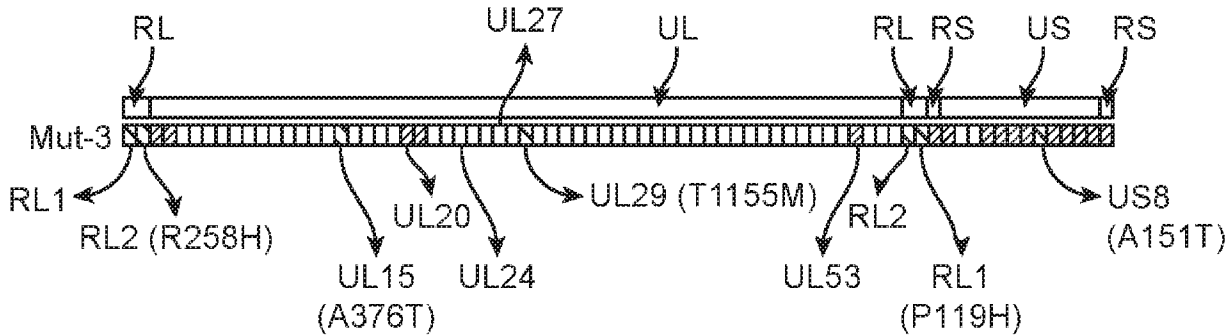

RL
UL27
UL
RL
RS
US
RS
Mut-3
RL1
RL2 (R258H)
UL20
UL29 (T1155M)
RL2
US8
(A151T)
UL15
(A376T)
UL24
UL53
RL1
(P119H)

FIG. 1B