US 20230082744A1

(19) **United States**

(12) **Patent Application Publication**

Smith et al.

(10) **Pub. No.: US 2023/0082744 A1**

(43) **Pub. Date: Mar. 16, 2023**

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(54) **NON-NEUROINVASIVE VIRUSES AND USES THEREOF**

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(21) Appl. No.: **17/932,320**

(22) Filed: **Sep. 15, 2022**

Related U.S. Application Data

(62) Division of application No. 16/868,243, filed on May 6, 2020, now Pat. No. 11,447,753, which is a division of application No. 15/555,873, filed on Sep. 5, 2017, now Pat. No. 10,647,964, filed as application No. PCT/US2016/020960 on Mar. 4, 2016.

(60) Provisional application No. 62/128,613, filed on Mar. 5, 2015.

Publication Classification

(51) **Int. Cl.**

C12N 7/00	(2006.01)
C07K 14/005	(2006.01)
A61K 39/12	(2006.01)
A61K 35/763	(2006.01)
A61K 39/245	(2006.01)
A61K 39/25	(2006.01)

(52) **U.S. Cl.**

CPC ***C12N 7/00*** (2013.01); ***C07K 14/005*** (2013.01); ***A61K 39/12*** (2013.01); ***A61K 35/763*** (2013.01); ***A61K 39/245*** (2013.01); ***A61K 39/25*** (2013.01); *A61K 2039/5254* (2013.01)

(57) **ABSTRACT**

Provided herein are compositions and methods for vaccination and research applications. In particular, provided herein are non-neuroinvasive herpesviruses and alpha herpesviruses and uses thereof.

Specification includes a Sequence Listing.

Figure 1

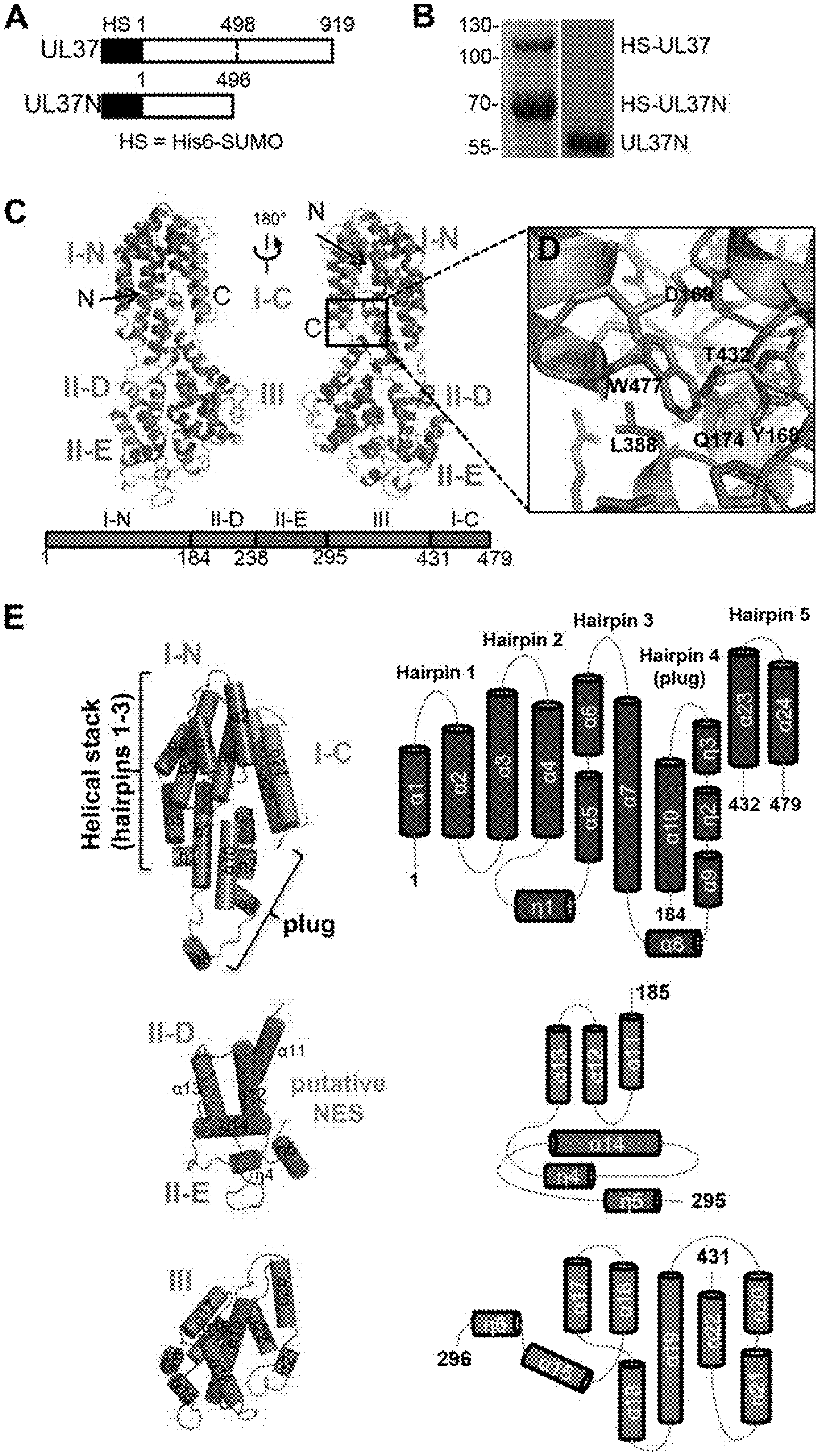

A
HS 1
498
919
UL37
1
496
UL37N
HS = His6-SUMO
B
130-
100-
70-
55-
HS-UL37
HS-UL37N
UL37N
C
I-N
N
C
180°
I-C
II-D
III
II-E
D
D168
T432
W477
L388
Q174
Y169
I-N
II-D
II-E
III
I-C
1
184
238
295
431
479
E
I-N
Helical stack (hairpins 1-3)
I-C
plug
Hairpin 1
Hairpin 2
Hairpin 3
Hairpin 4 (plug)
Hairpin 5
α1
α2
α3
α4
α5
α6
α7
η1
α8
α9
η2
η3
α10
α23
α24
1
184
432
479
II-D
α11
α13
putative NES
η4
II-E
185
α11
α12
α13
α14
η4
η5
295
III
296
431
α15
α16
α17
α18
α19
α20
α21
α22

Figure 2

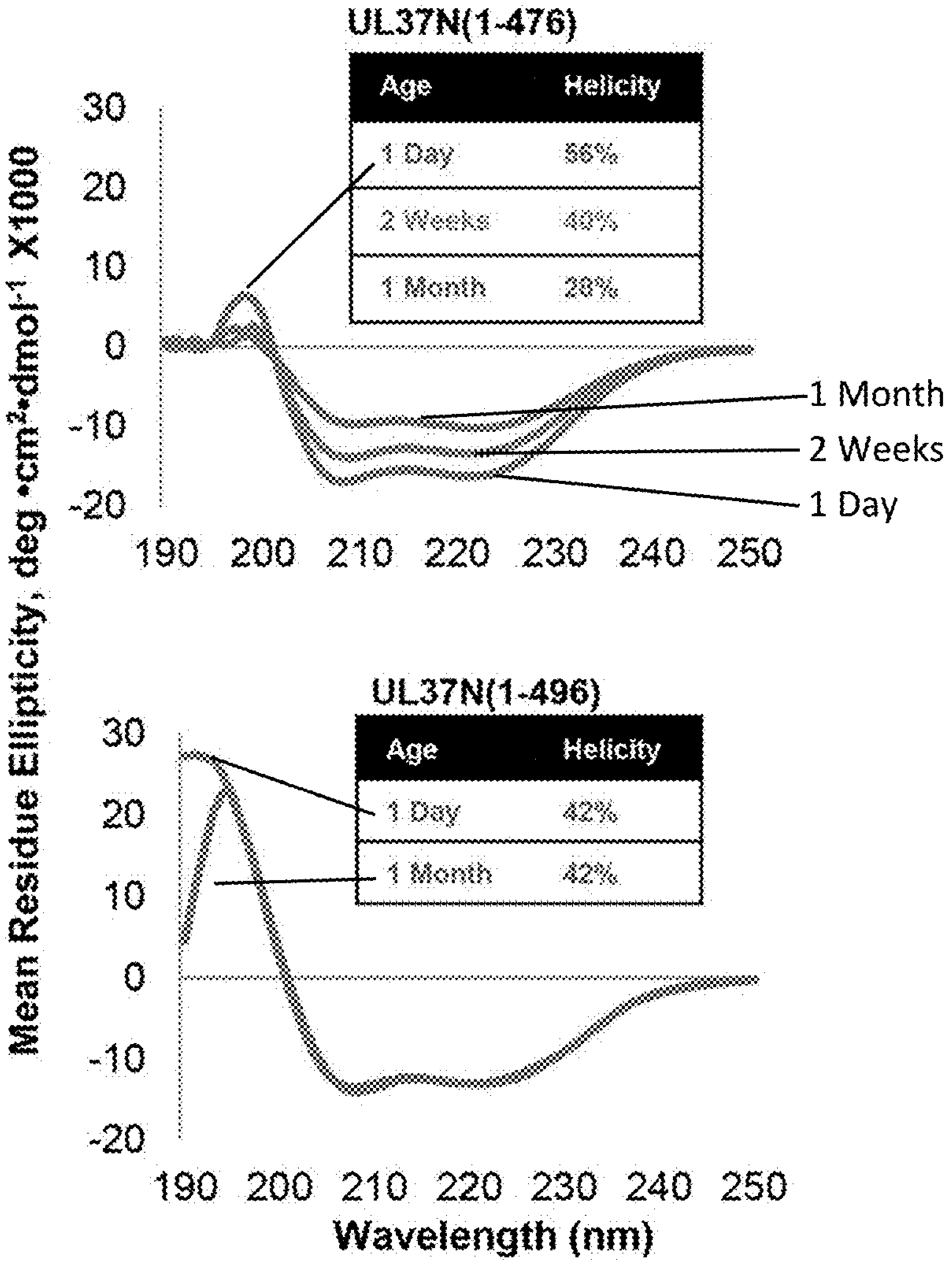

UL37N(1-476)
Age
Helicity
1 Day
56%
2 Weeks
40%
1 Month
28%
30
20
10
0
-10
-20
190
200
210
220
230
240
250
1 Month
2 Weeks
1 Day
Mean Residue Ellipticity, deg •cm²•dmol⁻¹ X1000
UL37N(1-496)
Age
Helicity
1 Day
42%
1 Month
42%
30
20
10
0
-10
-20
190
200
210
220
230
240
250
Wavelength (nm)

Figure 3

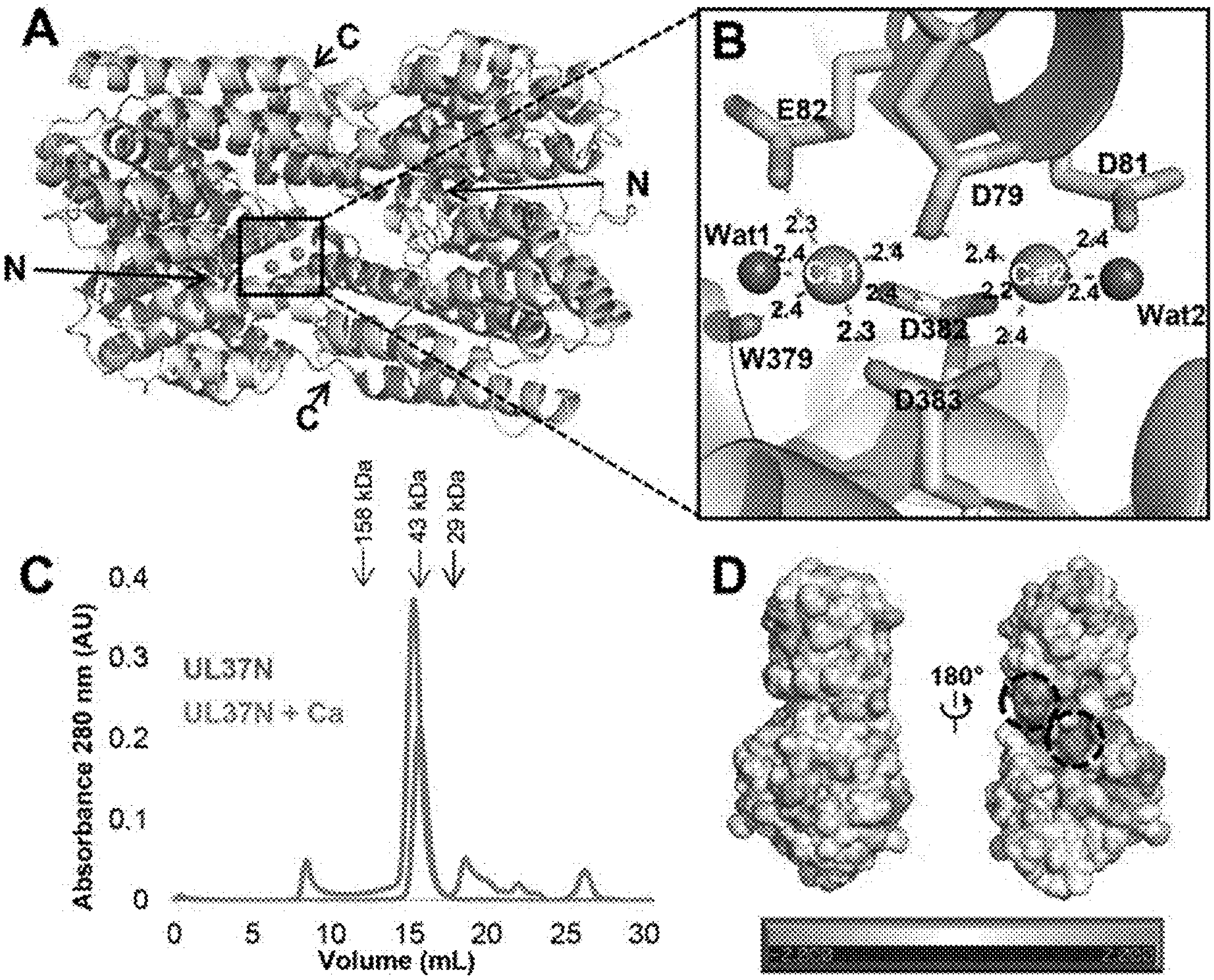

A
C
N
N
C
B
E82
D81
D79
Wat1
2.3
2.4
2.4
2.4
2.4
2.4
2.4
2.4
2.4
2.2
2.4
2.3
D382
2.4
Wat2
W379
D383
C
158 kDa
43 kDa
29 kDa
0.4
0.3
0.2
0.1
0
Absorbance 280 nm (AU)
UL37N
UL37N + Ca
0
5
10
15
20
25
30
Volume (mL)
D
180°

Figure 4

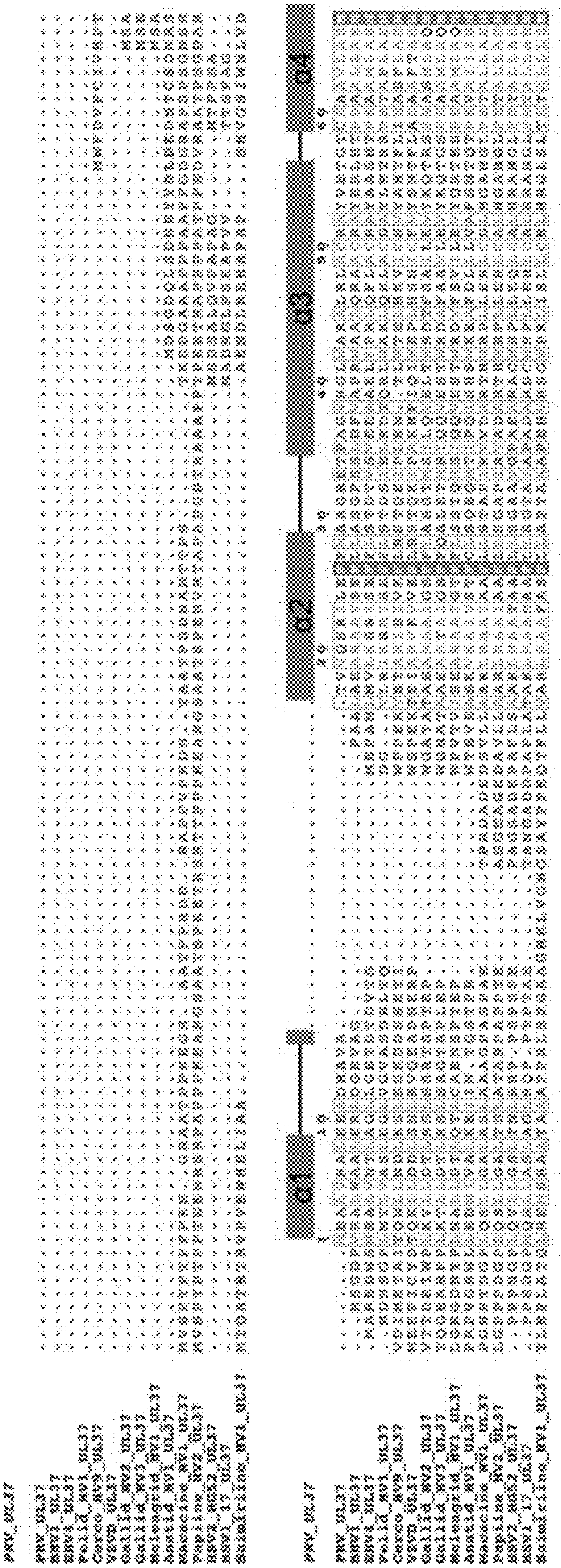


Figure 4 (Cont.)

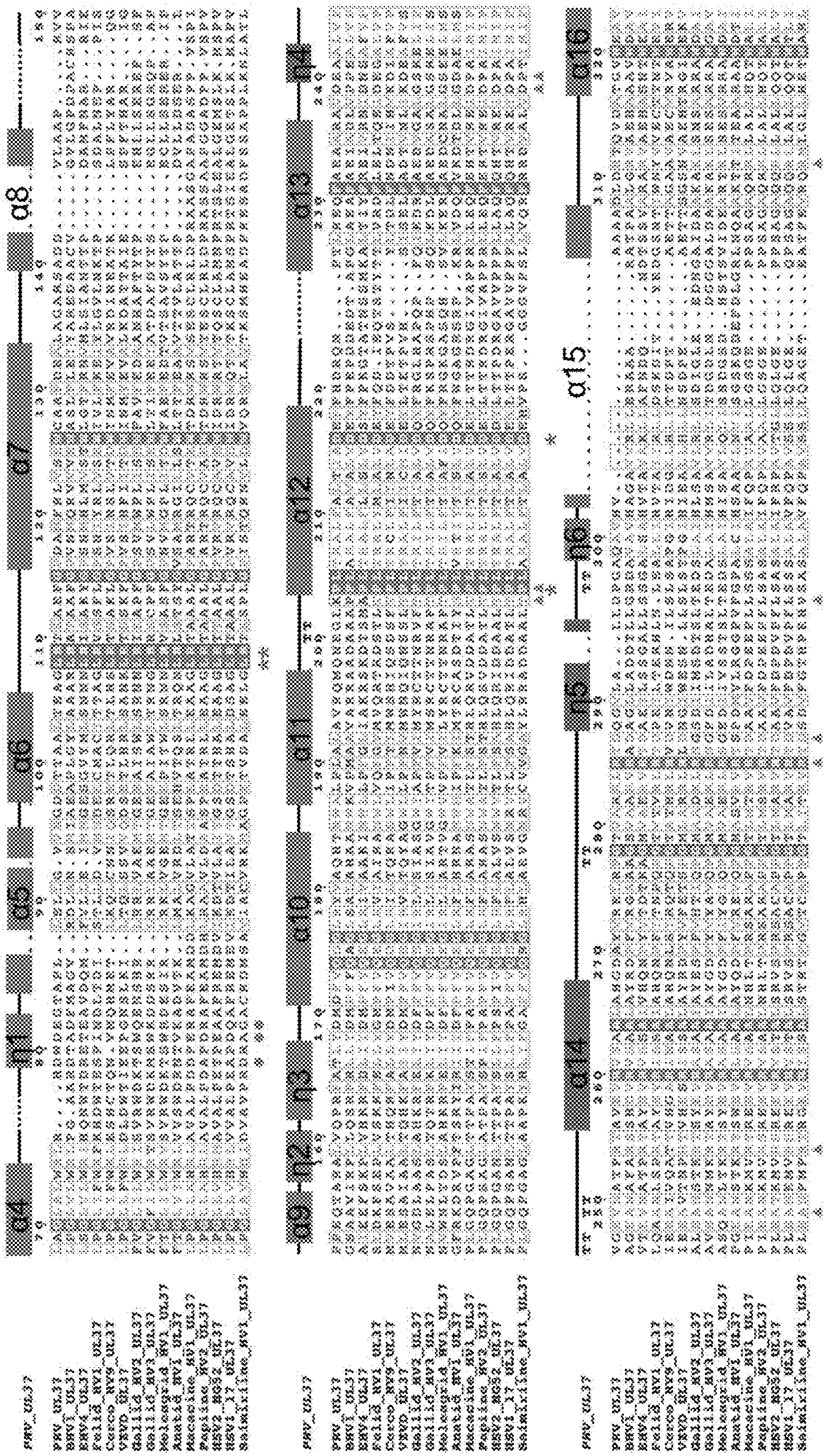


Figure 4 (Cont.)

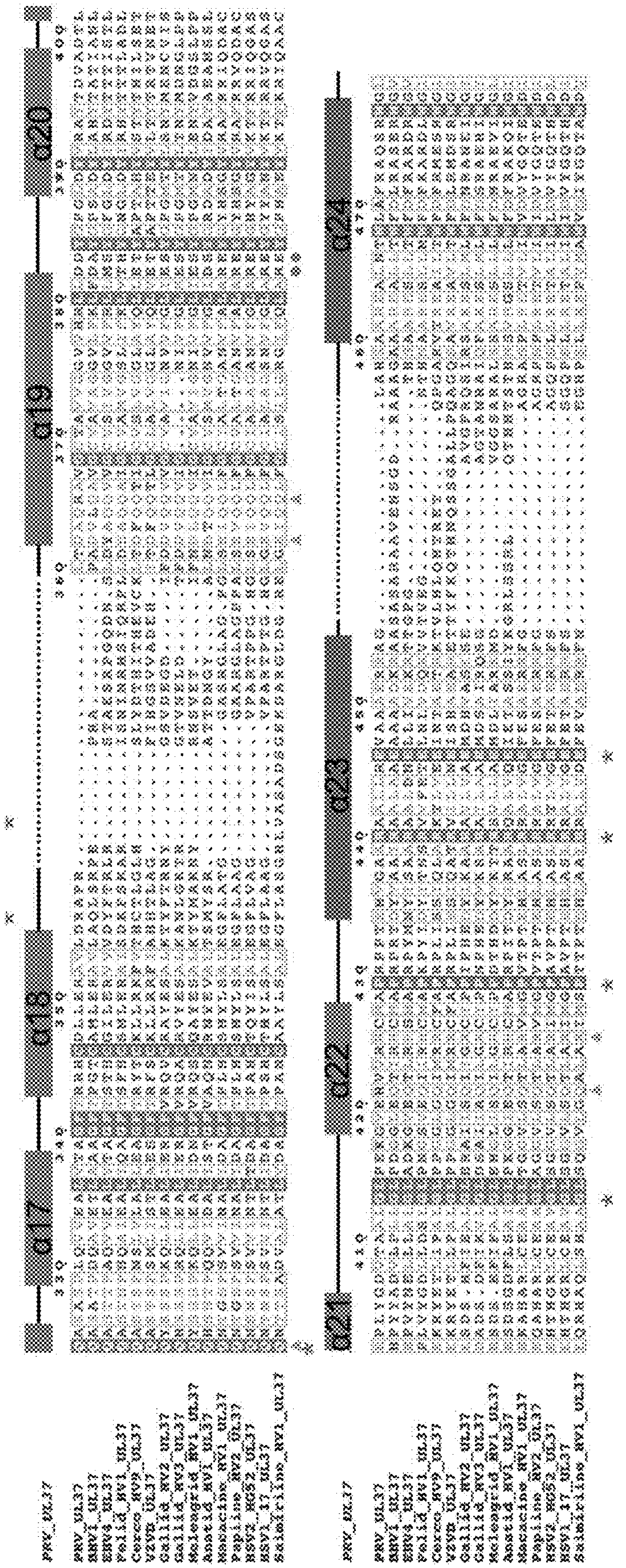


Figure 5

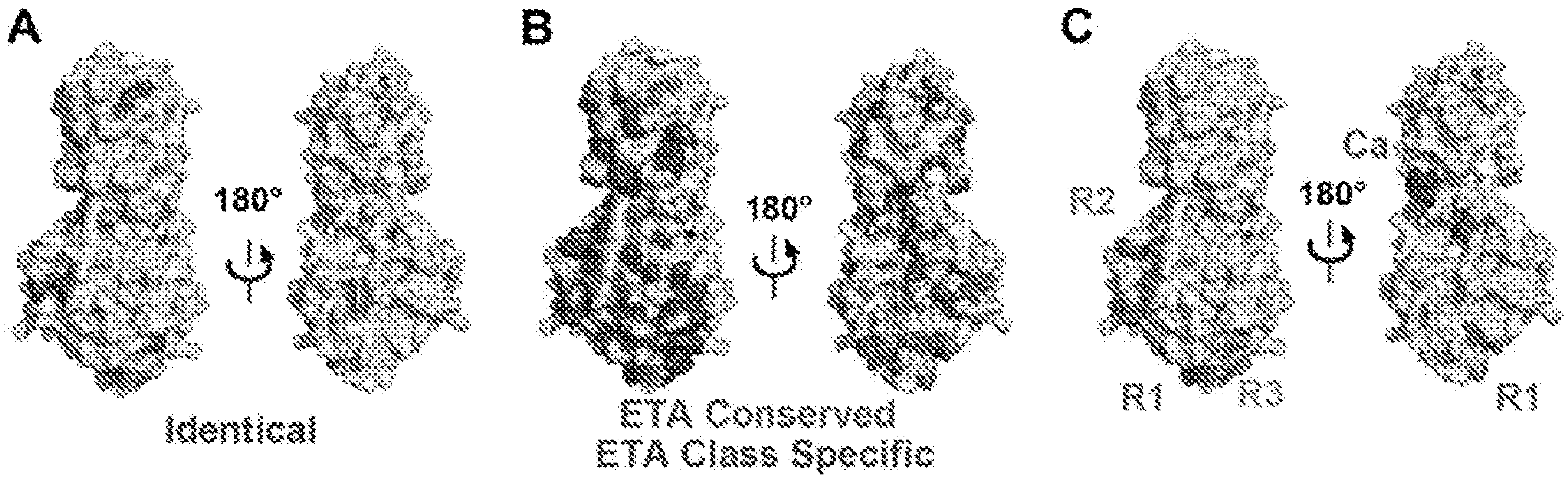


Figure 6

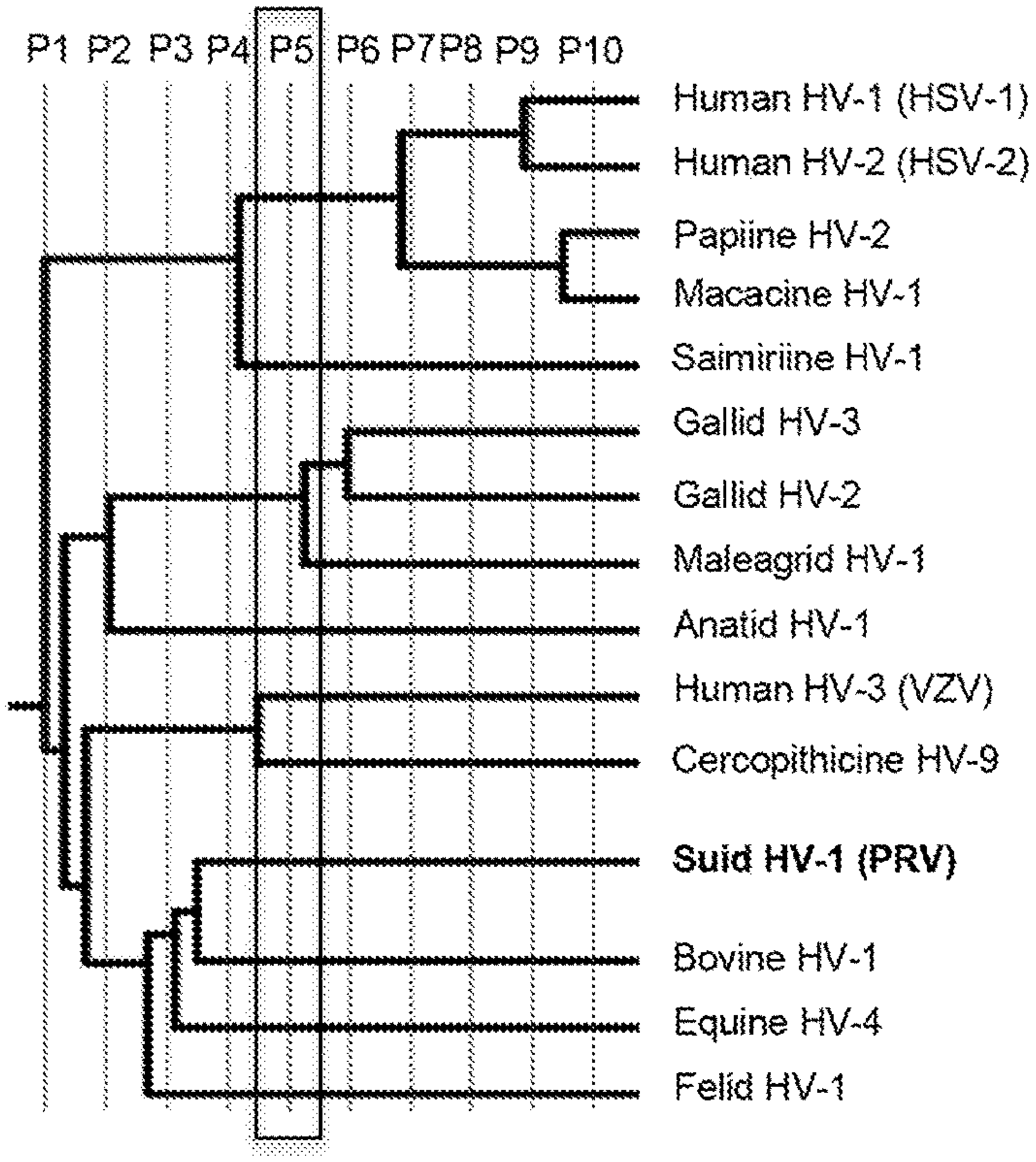


Figure 7

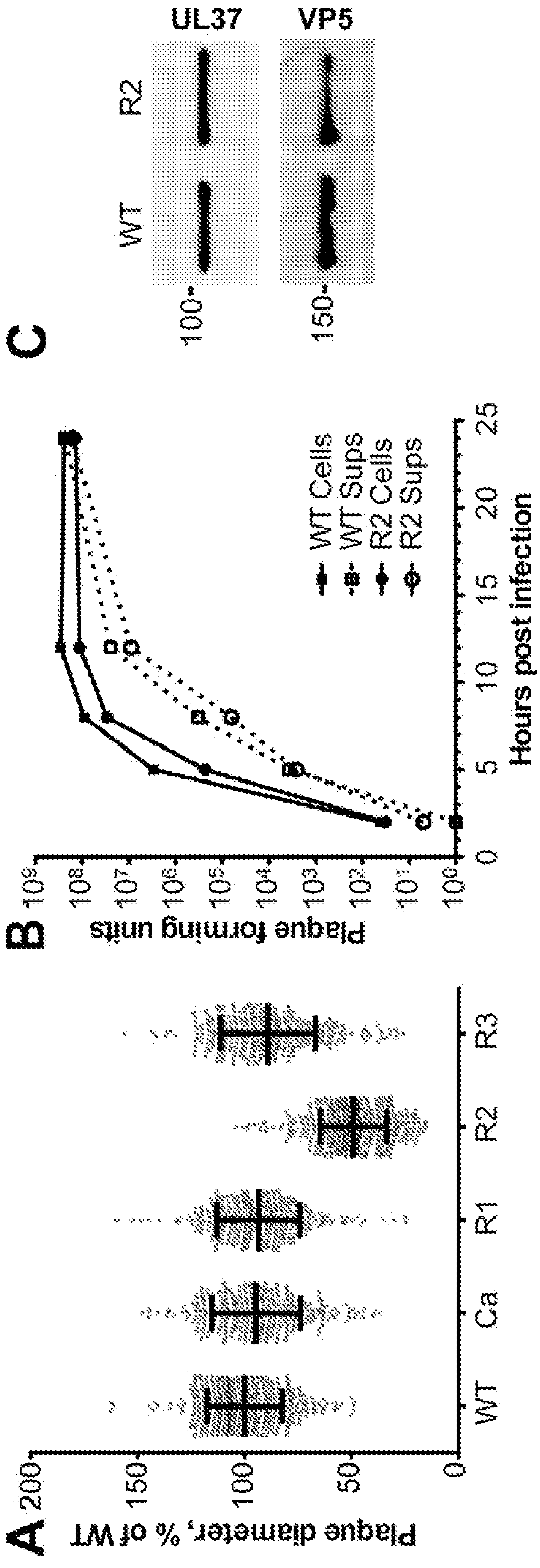

A
Plaque diameter, % of WT
200
150
100
50
0
WT
Ca
R1
R2
R3
B
Plaque forming units
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
0
5
10
15
20
25
Hours post infection
WT Cells
WT Sups
R2 Cells
R2 Sups
C
WT
R2
UL37
VP5
100-
150-

Figure 8

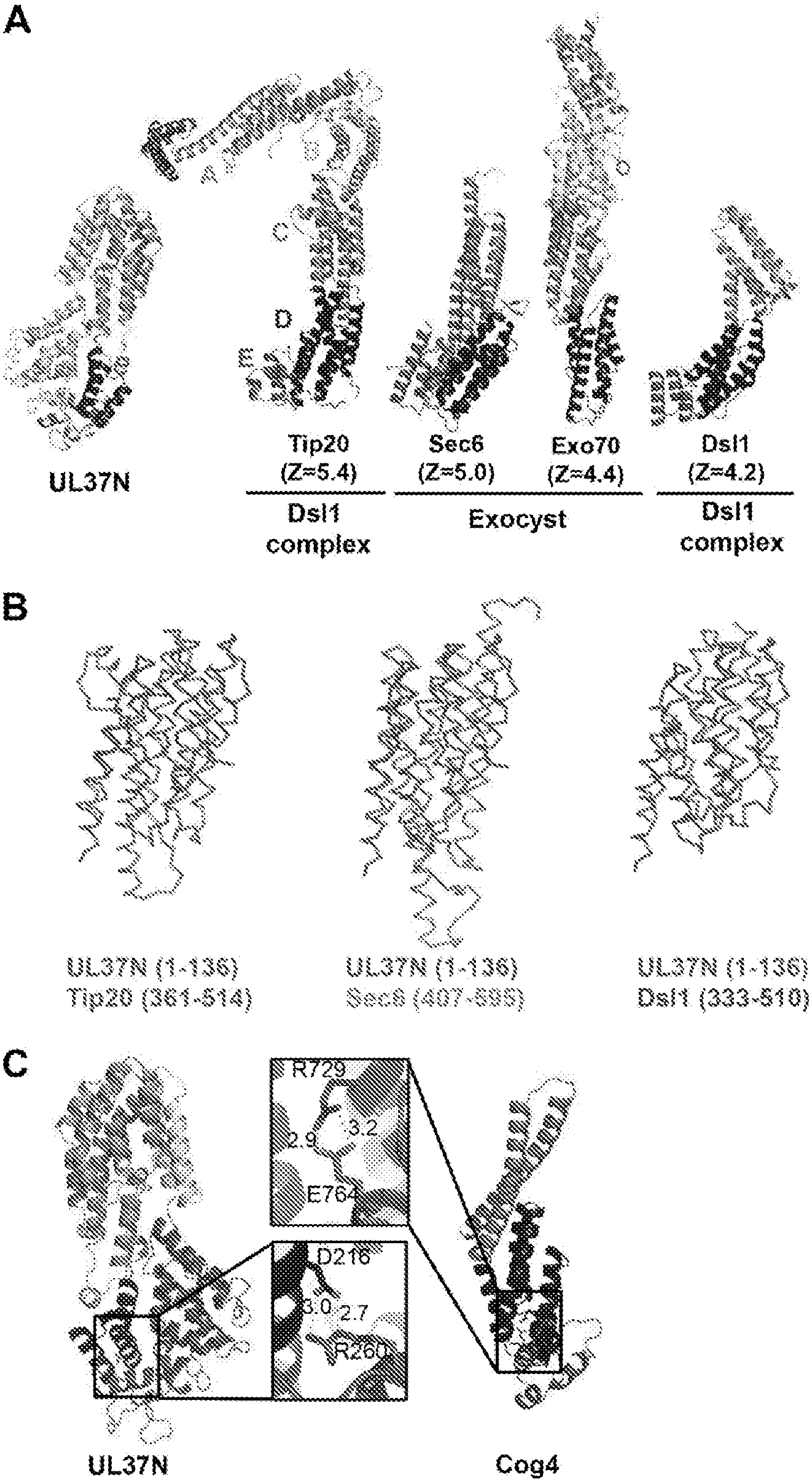

A
A
B
C
D
E
UL37N
Tip20
(Z=5.4)
Dsl1
complex
Sec6
(Z=5.0)
Exo70
(Z=4.4)
Exocyst
Dsl1
(Z=4.2)
Dsl1
complex
B
UL37N (1-136)
Tip20 (361-514)
UL37N (1-136)
Sec6 (407-595)
UL37N (1-136)
Dsl1 (333-510)
C
R729
2.9
3.2
E764
D216
3.0
2.7
R260
UL37N
Cog4

Figure 9

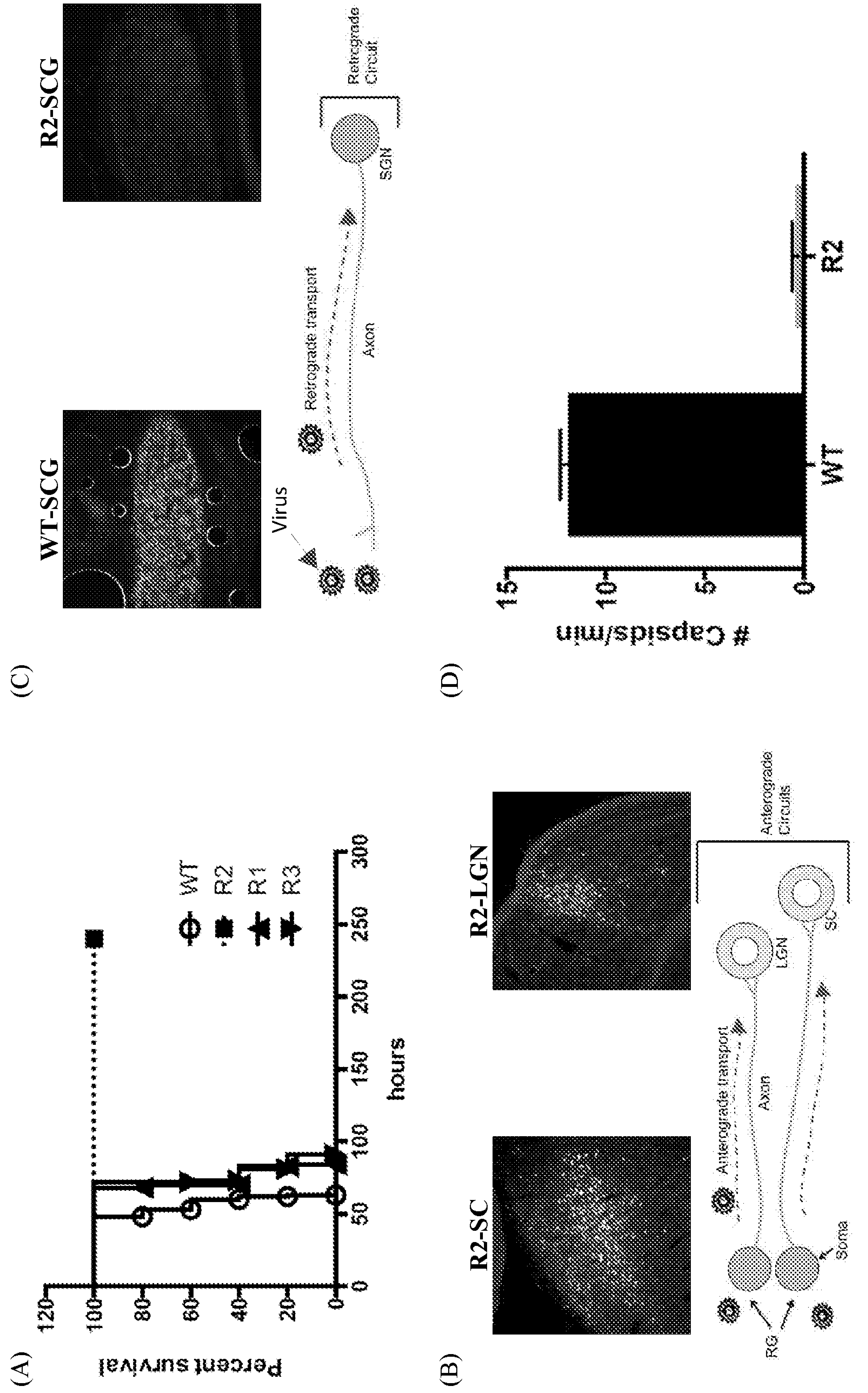

(A)
Percent survival
120
100
80
60
40
20
0
0
50
100
150
200
250
300
hours
WT
R2
R1
R3
(B)
R2-SC
R2-LGN
RG
Soma
Anterograde transport
Axon
LGN
SC
Anterograde Circuits
(C)
WT-SCG
R2-SCG
Virus
Retrograde transport
Axon
SGN
Retrograde Circuit
(D)
Capsids/min
15
10
5
0
WT
R2

Figure 10

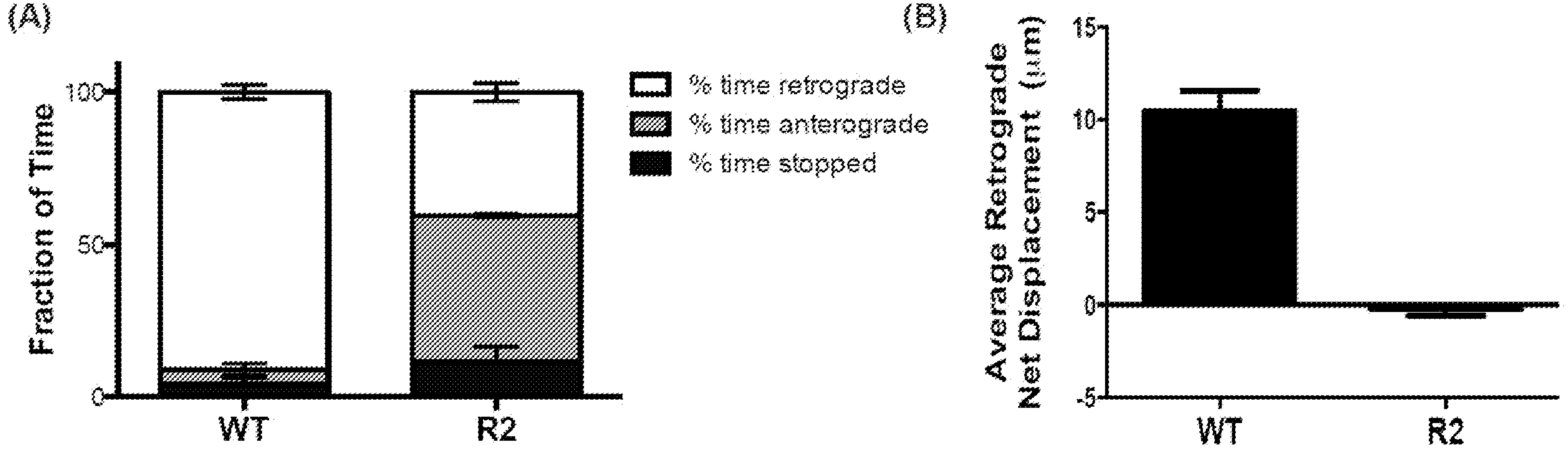

(A)
Fraction of Time
100
50
0
WT
R2
☐ % time retrograde
▨ % time anterograde
■ % time stopped
(B)
Average Retrograde Net Displacement (μm)
15
10
5
0
-5
WT
R2

Figure 11

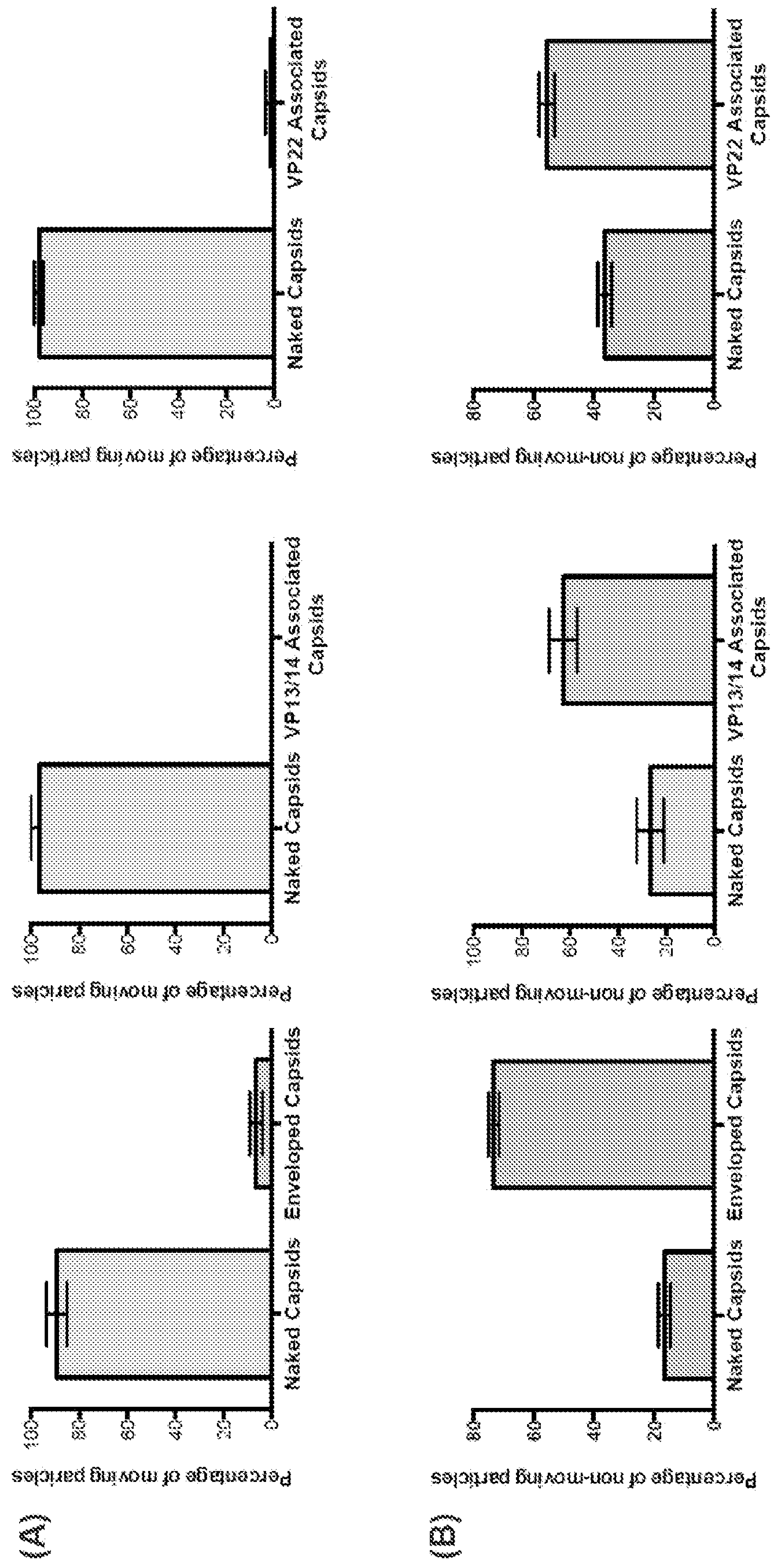

(A)
Percentage of moving particles
100
80
60
40
20
0
Naked Capsids
Enveloped Capsids
Percentage of moving particles
100
80
60
40
20
0
Naked Capsids
VP13/14 Associated Capsids
Percentage of moving particles
100
80
60
40
20
0
Naked Capsids
VP22 Associated Capsids
(B)
Percentage of non-moving particles
80
60
40
20
0
Naked Capsids
Enveloped Capsids
Percentage of non-moving particles
100
80
60
40
20
0
Naked Capsids
VP13/14 Associated Capsids
Percentage of non-moving particles
80
60
40
20
0
Naked Capsids
VP22 Associated Capsids

Figure 12

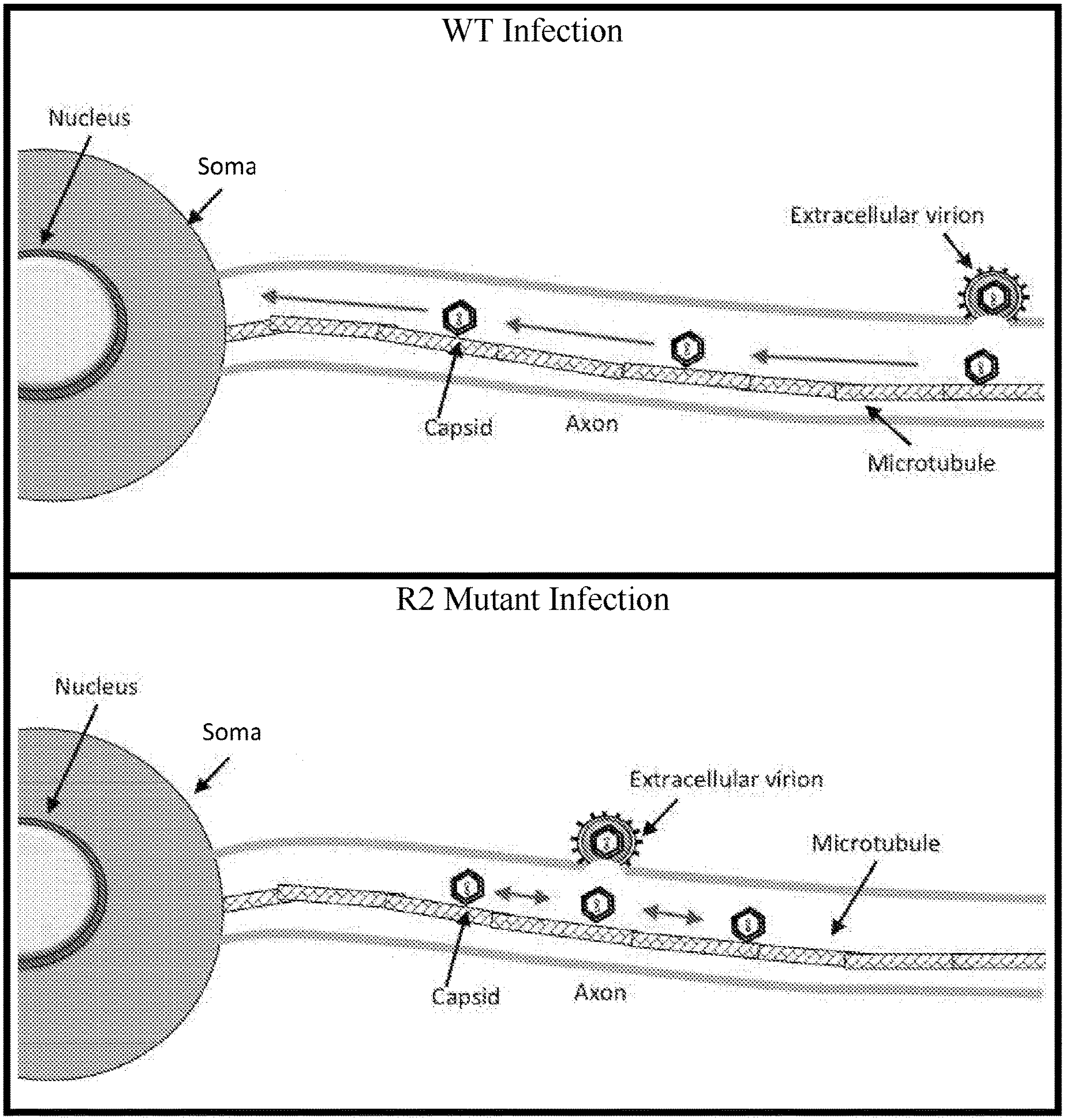

WT Infection
Nucleus
Soma
Extracellular virion
Capsid
Axon
Microtubule
R2 Mutant Infection
Nucleus
Soma
Extracellular virion
Microtubule
Capsid
Axon

Figure 13

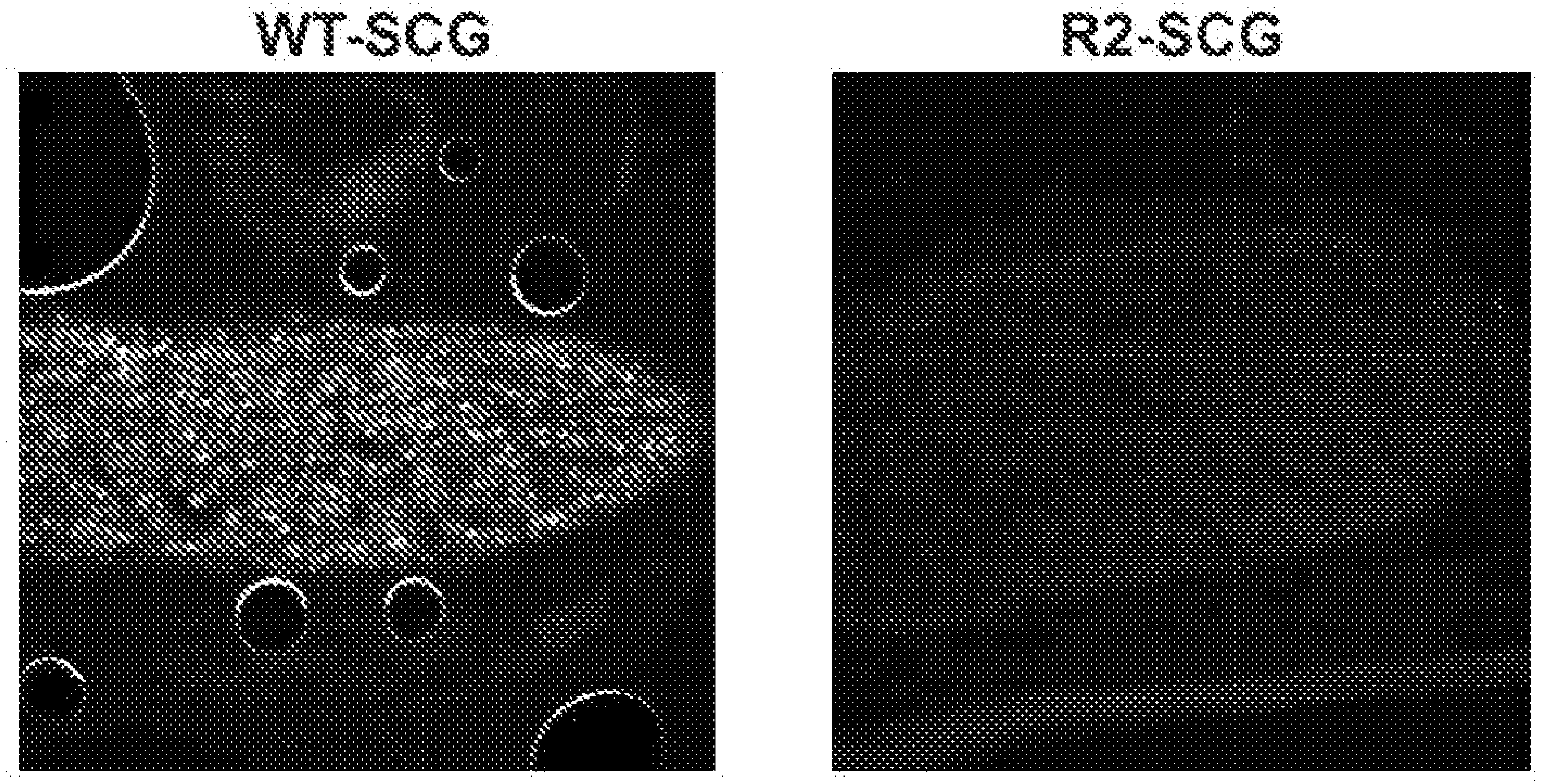


Figure 14

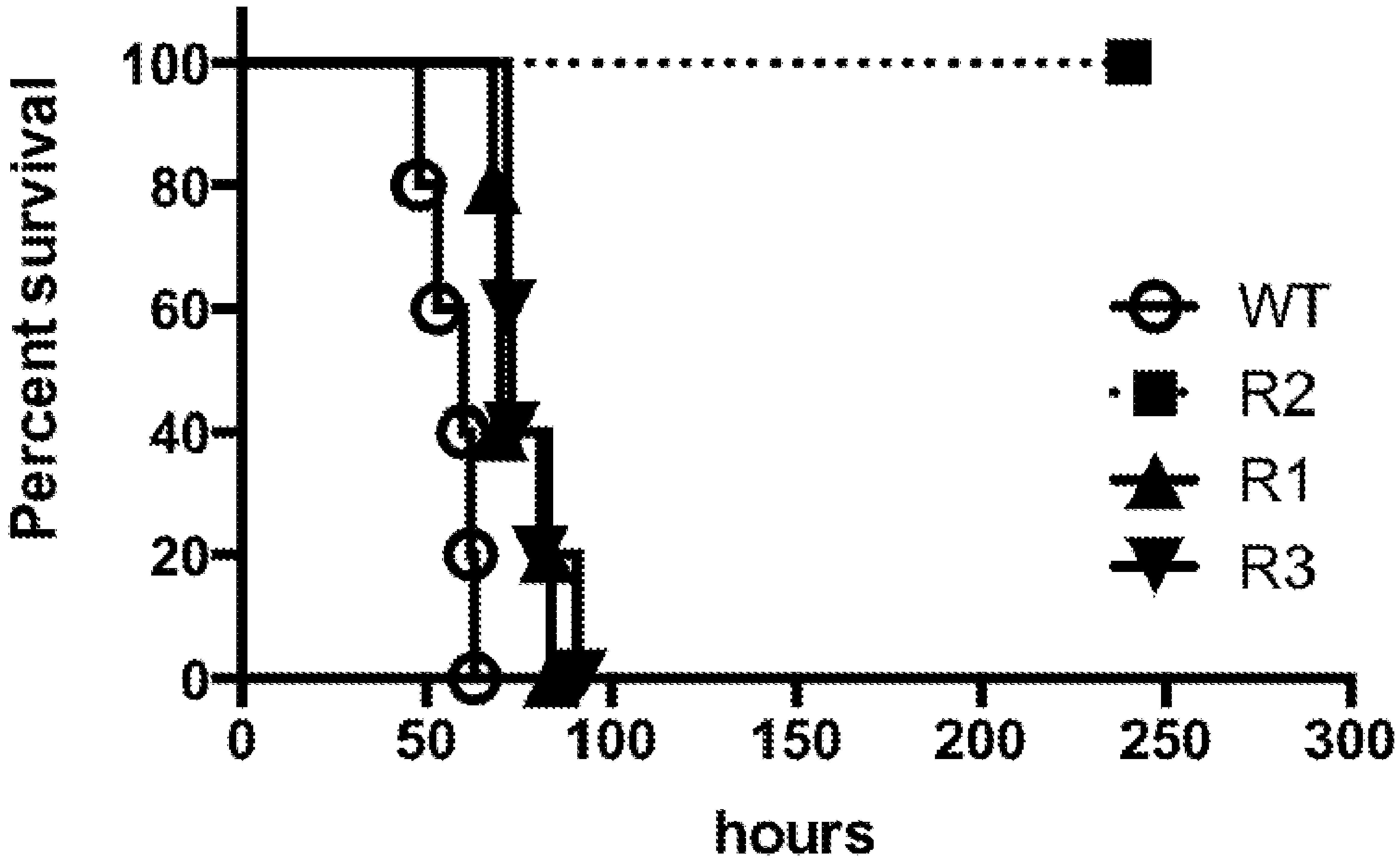


Figure 15

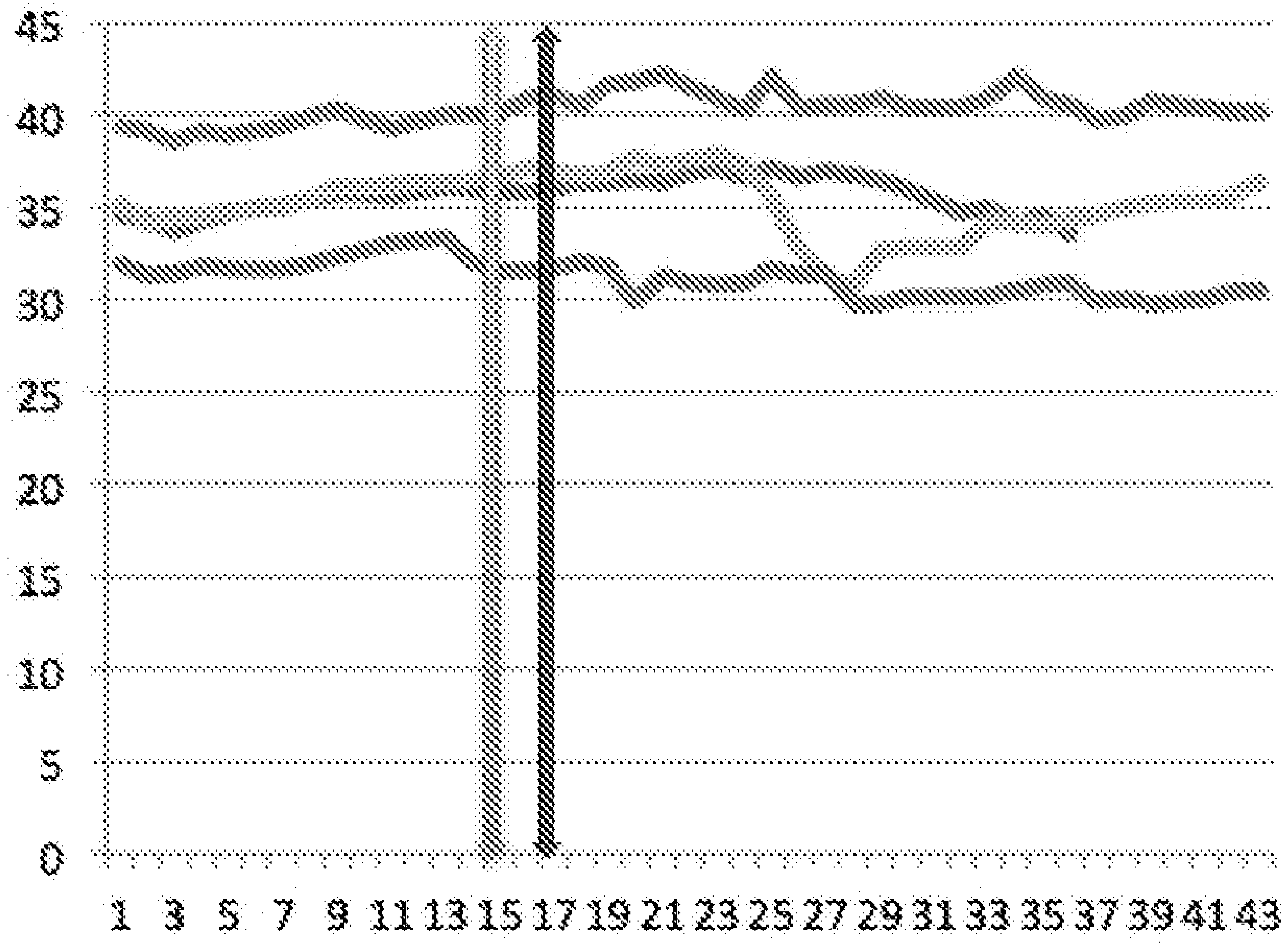


Figure 16

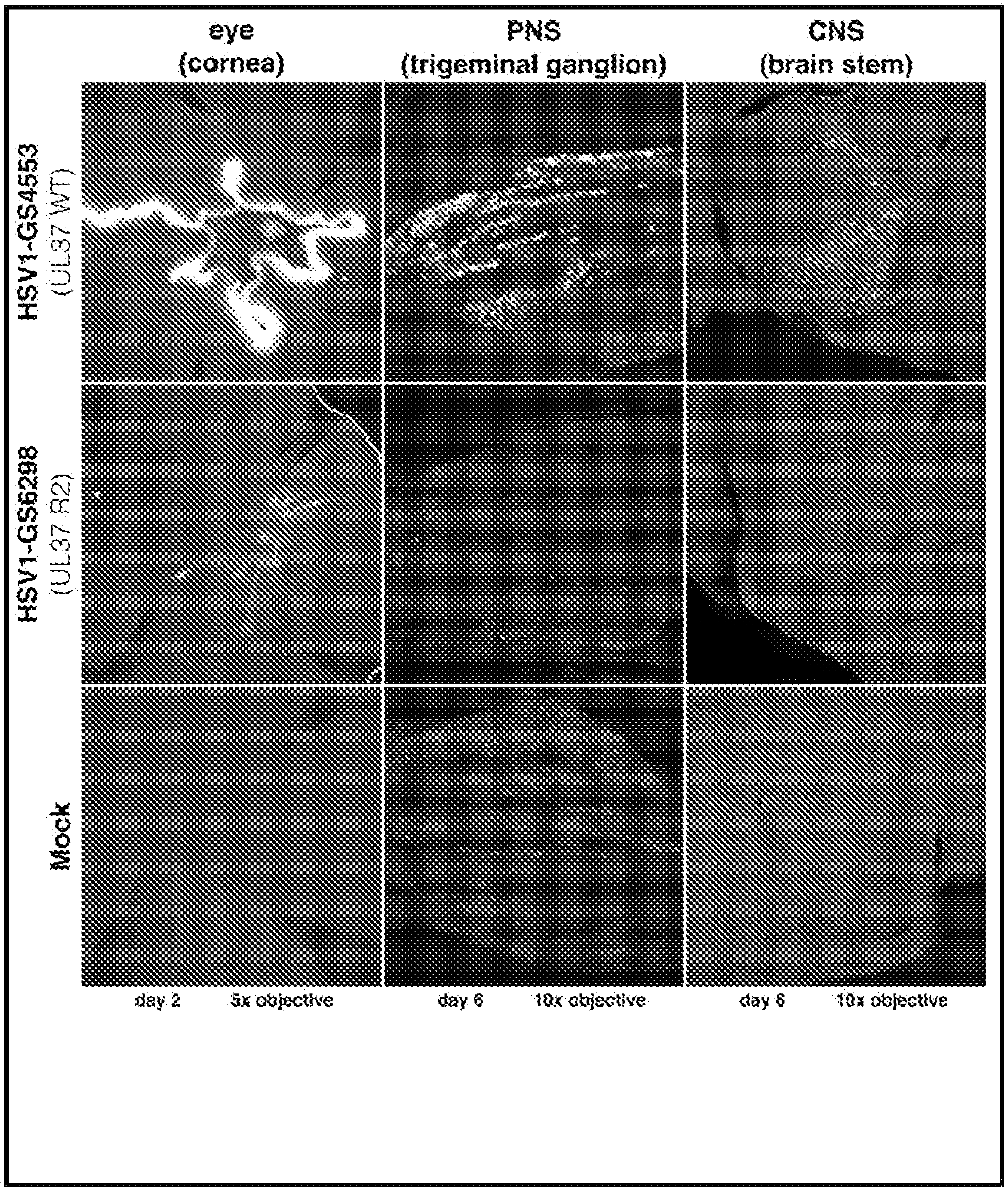
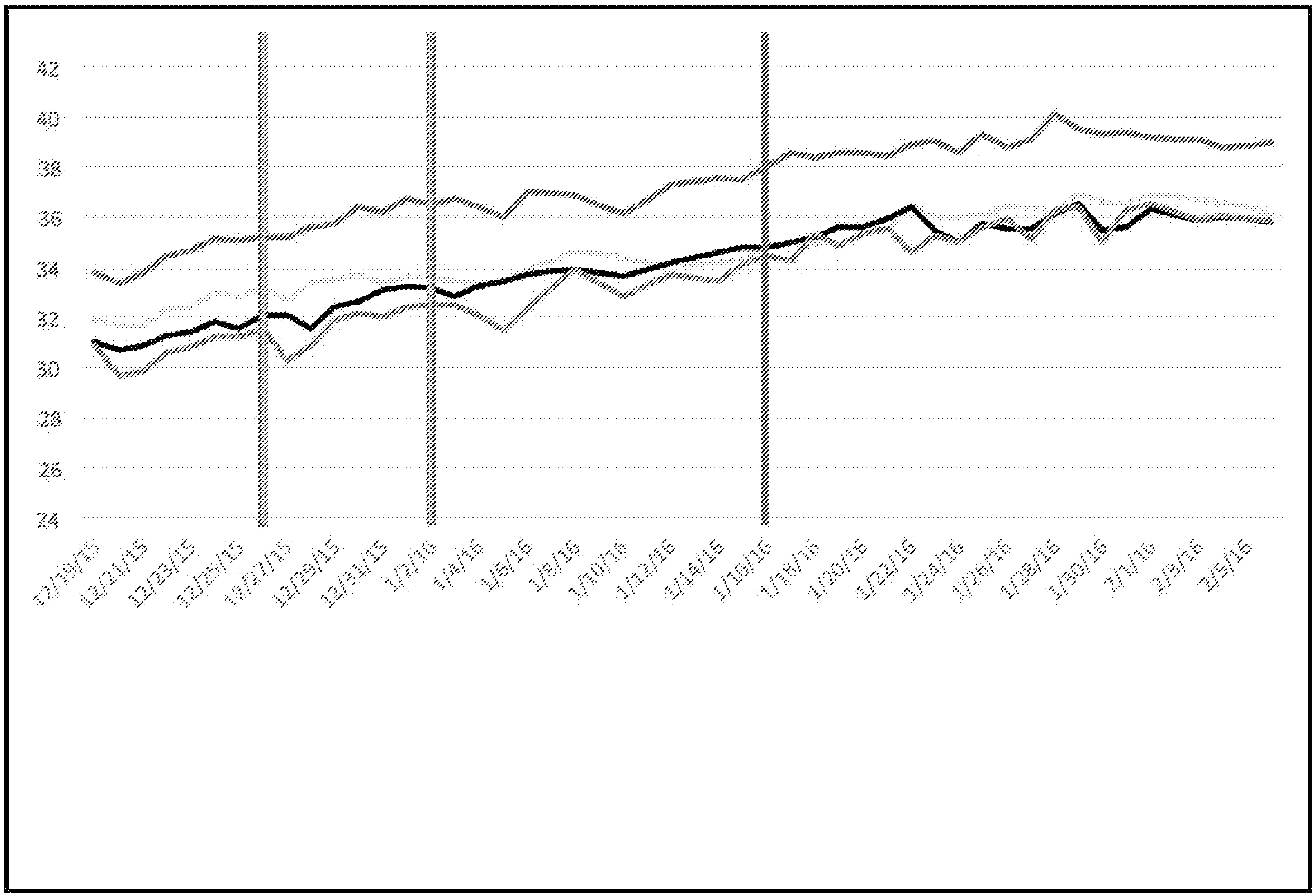


Figure 17


42
40
38
36
34
32
30
28
26
24
12/19/15
12/21/15
12/23/15
12/25/15
12/27/15
12/29/15
12/31/15
1/2/16
1/4/16
1/6/16
1/8/16
1/10/16
1/12/16
1/14/16
1/16/16
1/18/16
1/20/16
1/22/16
1/24/16
1/26/16
1/28/16
1/30/16
2/1/16
2/3/16
2/5/16

NON-NEUROINVASIVE VIRUSES AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present Application is a divisional of U.S. application Ser. No. 16/868,243, filed May 6, 2020, and issuing as U.S. Pat. No. 11,447,753 on Sep. 20, 2022, which is a divisional application of U.S. application Ser. No. 15/555,873, which issued on May 12, 2020 as U.S. Pat. No. 10,647,964, which is a national stage filing under 35 U.S.C. 371 of International Application No. PCT/US2016/020960, filed Mar. 4, 2016, which claims priority to U.S. Provisional Patent Application Ser. No. 62/128,613 filed Mar. 5, 2015, all of which are herein incorporated by reference in their entirety.

GOVERNMENT SUPPORT

[0002] This invention was made with government support under R01 AI056346 and OD001996 both of which were awarded by the National Institutes of Health. The government has certain rights in the invention.

REFERENCE TO SEQUENCE LISTING SUBMITTED VIA EFS-WEB

[0003] This application is being filed electronically via Patent Center and includes an electronically submitted Sequence Listing in .xml format. the .xml file contains a sequence listing entitled “702581.02009_sequence_listing.xml” created on Sep. 2, 2022 and is 29,607 bytes in size. The Sequence Listing contained in this .xml file is part of the specification and is hereby incorporated by reference herein in its entirety.

FIELD OF THE DISCLOSURE

[0004] Provided herein are compositions and methods for vaccination and research applications. In particular, provided herein are non-neuroinvasive herpesviruses and alpha herpesviruses and uses thereof.

BACKGROUND OF THE DISCLOSURE

[0005] Alphaherpesviruses are neuroinvasive pathogens of humans and livestock. All cause a range of disease manifestations from mild to severe, establish life-long infections by invading and residing in the nervous system, and once infected there is no cure.

[0006] Of the three human pathogens, varicella zoster virus (VZV) and herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), there is a vaccine only for VZV. The VZV vaccine is a live-attenuated virus that retains full neuroinvasive properties.

[0007] Additional vaccines for alphaherpesviruses are needed, preferably utilizing viruses without neuroinvasive properties.

SUMMARY OF THE DISCLOSURE

[0008] Provided herein are compositions and methods for vaccination and research applications. In particular, provided herein are non-neuroinvasive herpesviruses and alpha herpesviruses and uses thereof.

[0009] For example, in some embodiments, the present disclosure provides a non-neuroinvasive herepsvirus or alphaherpesvirus (e.g., HSV-1, HSV-2, PRV, VZV, BHV, or EHV). In some embodiments, the UL37 protein of the virus has at least 70% (e.g., at least 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, or 97%) sequence identity to a sequence selected, for example, SEQ ID NOS: 22-36.

[0010] In some embodiments, the virus comprises one or more mutations in the neuroinvasive (R2) domain. Examples include, for example, a variant herpes simplex virus 1 or 2 particle comprising a mutant UL37 protein, wherein said mutant UL37 protein comprises one or more mutations selected from Q403A, E452A, Q455A, Q511A, or R515A; a variant varicella zoster virus particle comprising a mutant UL37 protein, wherein said mutant UL37 protein comprises one or more mutations selected from Q363A, D413A, Q416A, Q472A, or R476A; and a variant pseudorabies virus particle comprising a mutant UL37 protein, wherein said mutant UL37 protein comprises one or more mutations selected from Q324A, D362A, R365A, H421A, or H425A. In some embodiments, the one or more mutations is two or more (e.g., three, four, or all) of the mutations. In some embodiments, the virus particle exhibits reduced neuro-invasiveness relative to a virus particle lacking the mutations. In some embodiments, the virus is avirulent.

[0011] Further embodiments provide a nucleic acid or vector encoding the virus particles described herein.

[0012] Additional embodiments provide a pharmaceutical composition or vaccine composition comprising the virus particle, nucleic acid, or vector described herein and a pharmaceutically acceptable carrier. In some embodiments, the composition further comprises an adjuvant.

[0013] Still other embodiments provide a method of inducing an immune response against a virus, comprising: administering the composition described herein to a subject under conditions such that said subject generates an immune response to the virus. In some embodiments, the subject exhibits immunity to the virus.

[0014] Yet other embodiments provide the use of the compositions described herein to generate an immune response against a virus in a subject.

[0015] Certain embodiments provide method of preventing infection by a virus, comprising: administering the compositions described herein to a subject under conditions such that the subject generates an immune response to the virus and is immune to infection by the virus. In some embodiments, the subject is human or a non-human animal (e.g., bovine, equine, companion animal, livestock, etc.).

[0016] In some embodiments, the present disclosure provides the use of the compositions described herein to prevent infection by a virus in a subject.

[0017] Still further embodiments provide a method or use of treating or preventing cancer, comprising: administering the composition described herein to a subject diagnosed with cancer under conditions such that the cancer is reduced or eliminated.

[0018] Additional embodiments are described herein.

DESCRIPTION OF THE DRAWINGS

[0019] FIG. **1** shows UL37N structure. (A) Linear diagram of UL37 constructs expressed. HS, the His6-SUMO tag. (B) Coomassie-stained SDS-polyacrylamide gel showing purified His6-SUMO-tagged UL37 contaminated with the His6-SUMO-tagged UL37N proteolytic cleavage product and purified monodisperse UL37N. (C) Crystal structure of a UL37N monomer shown in two orientations related by a 180-degree rotation around the vertical axis. (D) A close-up view of residue W477 and its surroundings. (E) UL37N domains are shown individually.

[0020] FIG. **2** shows that UL37N (1 to 496) is more stable over time than UL37N (1 to 476), as measured using circular dichroism on protein aged 1 day, 2 weeks, or 1 month.

[0021] FIG. **3** shows that UL37N is a calcium-dependent dimer in crystals but not in solution.

[0022] (A) Two UL37N monomers in the asymmetric unit. (B) A close-up view of the calcium-binding site at the dimer interface. (C) Overlay of size exclusion chromatograms of UL37N with or without 0.2 M CaCl2). (D) Electrostatic surface potential map of UL37N generated using the Charmm program.

[0023] FIG. **4** shows a sequence alignment of 15 UL37 homologs from alphaherpesviruses. The 15 UL37 homologs shown in the alignment are PRV_UL37 (SEQ ID NO: 22), BHV1_UL37 (SEQ ID NO: 23), EHV4_UL37 (SEQ ID NO: 24), Felid_HV1_UL37 (SEQ ID NO: 25), Cerco_HV9_UL37 (SEQ ID NO: 26), VZVD_UL37 (SEQ ID NO: 27), Gallid_HV2_UL37 (SEQ ID NO: 28), Gallid_HV3_UL37 (SEQ ID NO: 29), Meleagrid_HV1_UL37 (SEQ ID NO: 30), Anatid_HV1_UL37 (SEQ ID NO: 31), Macacine_HV1_UL37 (SEQ ID NO: 32), Papiine_HV2_UL37 (SEQ ID NO: 33), HSV2_HG52_UL37 (SEQ ID NO: 34), HSV1_17_UL37 (SEQ ID NO: 35), Saimiriine_HV1_UL37 (SEQ ID NO: 36).

[0024] FIG. **5** shows residue conservation on the surface of UL37N. (A) The UL37N structure is shown in surface representation. (B) ETA class conserved and class specific. (C) Mutated residues in R1, R2, R3, and the calcium-binding site (Ca).

[0025] FIG. **6** shows a phylogenetic tree from ETA using UL37 homologs from 15 alphaherpesviruses.

[0026] FIG. **7** shows propagation and spread of PRV encoding mutant forms of UL37. (A) Relative plaque diameters of mutant viruses Ca (PRV-GS5476), R1 (PRV-GS5321), R2 (PRV-GS5604), and R3 (PRV-GS5350). (B) Single-step growth curves comparing propagation of PRV-GS4284 (UL37 WT) and PRV-GS5604 (UL37 R2 mutant). (C) Western blot analysis of UL37 protein incorporation into WT and R2 mutant extracellular virions.

[0027] FIG. **8** shows that UL37N shares structural similarities with several subunits of cellular MTCs. (A) UL37N is shown side by side with the Tip20 (PDB accession no. 3FHN) and Dsl1 (PDB accession no. 3K8P) subunits of the Dsl1 complex and the Sec6 (PDB accession no. 2FJI) and Exo70 (PDB accession no. 2B7M) subunits of the exocyst complex, with the Z-score for each alignment displayed. (B) Overlays of regions of Tip20, Sec6, and Dsl1 that align onto residues 1 to 136 of UL37N. (C) The salt bridge in domain II between putative D and E subdomains of UL37N is strictly conserved among

[0028] FIG. **9** shows characterization of the neuroinvasive properties of the R2 mutant. (A) The R2 region is essential for virulence in a mouse model of infection. (B) The R2 region is essential for retrograde mediated neuroinvasion. (C) The R2 region is dispensable for anterograde spread through neurons. (D) The R2 mutant does not travel retrograde down axons upon infection.

[0029] FIG. **10** shows that R2 mutant particles display aberrant non-processive motion. (A) Mutation of the R2 region significantly increases the severity of stop and reversal events. (B) Aberrant motion of R2 mutant particles does not result in overall movement towards the soma

[0030] FIG. **11** shows that motion of R2 particles does not result from endocytosis of virions or retention of outer tegument proteins following entry. (A) Moving particles are not associated with the gD envelope protein or the outer tegument proteins VP22 and VP13/14. (B) Non-moving particles are predominantly associated with envelope and tegument proteins.

[0031] FIG. **12** shows modeling of wild-type and the R2 mutant trafficking in neuronal cells (Top) Herpesvirus replication occurs in the nucleus, this requires incoming particles to traverse the cytoplasm following entry into the cell. (Bottom) R2 mutant virions fuse with the plasma membrane of the axon however the released capsids alternate between motion towards (retrograde) and away (anterograde) from the soma.

[0032] FIG. **13** shows that PRV R2 lacks neuroinvasive properties.

[0033] FIG. **14** shows that PRV R2 is avirulent.

[0034] FIG. **15** shows that PRV R2 is a potent live-attenuated vaccine.

[0035] FIG. **16** shows that the HSV-1 R2 mutant (HSV1-GS6298) is incapable of transmitting into the nervous system following replication in the mouse cornea.

[0036] FIG. **17** shows protection of mice from lethal PRV challenge following vaccination with PRV encoding the R2 deletion.

DEFINITIONS

[0037] As used herein, the term "subject" refers to any animal (e.g., a mammal), including, but not limited to, humans, non-human primates, rodents, and the like, which is to be the recipient of a particular treatment. Typically, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

[0038] As used herein, the term "non-human animals" refers to all non-human animals including, but not limited to, vertebrates such as rodents, non-human primates, ovines, bovines, ruminants, lagomorphs, porcines, caprines, equines, canines, felines, aves, etc.

[0039] As used herein, the term "cell culture" refers to any in vitro culture of cells. Included within this term are continuous cell lines (e.g., with an immortal phenotype), primary cell cultures, transformed cell lines, finite cell lines (e.g., non-transformed cells), and any other cell population maintained in vitro.

[0040] As used herein, the term "eukaryote" refers to organisms distinguishable from "prokaryotes." It is intended that the term encompass all organisms with cells that exhibit the usual characteristics of eukaryotes, such as the presence of a true nucleus bounded by a nuclear membrane, within which lie the chromosomes, the presence of membrane-bound organelles, and other characteristics commonly observed in eukaryotic organisms. Thus, the term includes, but is not limited to such organisms as fungi, protozoa, and animals (e.g., humans).

[0041] As used herein, the term "in vitro" refers to an artificial environment and to processes or reactions that occur within an artificial environment. In vitro environments can consist of, but are not limited to, test tubes and cell culture. The term "in vivo" refers to the natural environment (e.g., an animal or a cell) and to processes or reaction that occur within a natural environment.

[0042] As used herein, the term "sample" is used in its broadest sense. In one sense, it is meant to include a specimen or culture obtained from any source, as well as biological and environmental samples. Biological samples may be obtained from animals (including humans) and encompass fluids, solids, tissues, and gases. Biological samples include blood products, such as plasma, serum and the like. Environmental samples include environmental material such as surface matter, soil, water, and industrial samples. Such examples are not however to be construed as limiting the sample types applicable to the present disclosure.

[0043] As used herein, the term "co-administration" refers to the administration of at least two agent(s) (e.g., a composition described herein and a anti-viral agent) or therapies to a subject. In some embodiments, the co-administration of two or more agents/therapies is concurrent. In other embodiments, a first agent/therapy is administered prior to a second agent/therapy. The appropriate dosage for co-administration can be readily determined by one skilled in the art. In some embodiments, when agents/therapies are co-administered, the respective agents/therapies are administered at lower dosages than appropriate for their administration alone. Thus, co-administration is especially desirable in embodiments where the co-administration of the agents/therapies lowers the requisite dosage of a known potentially harmful (e.g., toxic) agent(s).

[0044] As used herein, the term "pharmaceutical composition" refers to the combination of an active agent with a carrier, inert or active, making the composition especially suitable for diagnostic or therapeutic use in vivo, in vivo or ex vivo.

[0045] As used herein, the term "pharmaceutically acceptable carrier" refers to any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions (e.g., such as an oil/water or water/oil emulsions), and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants. (See e.g., Martin, Remington's Pharmaceutical Sciences, 15th Ed., Mack Publ. Co., Easton, Pa. [1975]).

[0046] As used herein, the term "immune response" refers to a response by the immune system of a subject. For example, immune responses include, but are not limited to, a detectable alteration (e.g., increase) in Toll receptor activation, lymphokine (e.g., cytokine (e.g., Th1 or Th2 type cytokines) or chemokine) expression and/or secretion, macrophage activation, dendritic cell activation, T cell activation (e.g., CD4+ or CD8+ T cells), NK cell activation, and/or B cell activation (e.g., antibody generation and/or secretion). Additional examples of immune responses include binding of an immunogen (e.g., antigen (e.g., immunogenic polypeptide)) to an MHC molecule and inducing a cytotoxic T lymphocyte ("CTL") response, inducing a B cell response (e.g., antibody production), and/or T-helper lymphocyte response, and/or a delayed type hypersensitivity (DTH) response against the antigen from which the immunogenic polypeptide is derived, expansion (e.g., growth of a population of cells) of cells of the immune system (e.g., T cells, B cells (e.g., of any stage of development (e.g., plasma cells), and increased processing and presentation of antigen by antigen presenting cells. An immune response may be to immunogens that the subject's immune system recognizes as foreign (e.g., non-self antigens from microorganisms (e.g., pathogens), or self-antigens recognized as foreign). Thus, it is to be understood that, as used herein, "immune response" refers to any type of immune response, including, but not limited to, innate immune responses (e.g., activation of Toll receptor signaling cascade) cell-mediated immune responses (e.g., responses mediated by T cells (e.g., antigen-specific T cells) and non-specific cells of the immune system) and humoral immune responses (e.g., responses mediated by B cells (e.g., via generation and secretion of antibodies into the plasma, lymph, and/or tissue fluids). The term "immune response" is meant to encompass all aspects of the capability of a subject's immune system to respond to antigens and/or immunogens (e.g., both the initial response to an immunogen (e.g., a pathogen) as well as acquired (e.g., memory) responses that are a result of an adaptive immune response).

[0047] As used herein, the term "immunity" refers to protection from disease (e.g., preventing or attenuating (e.g., suppression) of a sign, symptom or condition of the disease) upon exposure to a microorganism (e.g., pathogen) capable of causing the disease. Immunity can be innate (e.g., non-adaptive (e.g., non-acquired) immune responses that exist in the absence of a previous exposure to an antigen) and/or acquired (e.g., immune responses that are mediated by B and T cells following a previous exposure to antigen (e.g., that exhibit increased specificity and reactivity to the antigen)).

[0048] As used herein, the term "immunogen" refers to a molecule which stimulates a response from the adaptive immune system, which may include responses drawn from the group comprising an antibody response, a cytotoxic T cell response, a T helper response, and a T cell memory. An immunogen may stimulate an upregulation of the immune response with a resultant inflammatory response, or may result in down regulation or immunosuppression. Thus the T-cell response may be a T regulatory response. An immunogen also may stimulate a B-cell response and lead to an increase in antibody titer.

[0049] Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms, such as "polypeptide" or "protein" are not meant to limit the amino acid sequence to the complete, native amino acid sequence associated with the recited protein molecule.

[0050] As used herein, the term "peptide" refers to a polymer of two or more amino acids joined via peptide bonds or modified peptide bonds. As used herein, the term "dipeptides" refers to a polymer of two amino acids joined via a peptide or modified peptide bond.

[0051] The term "wild-type" refers to a gene or gene product that has the characteristics of that gene or gene product when isolated from a naturally occurring source. A wild-type gene is that which is most frequently observed in a population and is thus arbitrarily designed the "normal" or "wild-type" form of the gene. In contrast, the terms "modified", "mutant", and "variant" refer to a gene or gene product that displays modifications in sequence and or functional properties (i.e., altered characteristics) when compared to the wild-type gene or gene product. It is noted that naturally-occurring mutants can be isolated; these are identified by the fact that they have altered characteristics when compared to the wild-type gene or gene product.

[0052] As used herein, the term "neuroinvasive" refers to a property of a microorganism (e.g., virus) to enter the nervous system of a subject. In some embodiments, neuroinvasive viruses persist in the nervous system for an extended period of time (e.g., many years to an entire lifetime). In some embodiments, neuroinvasive viruses exhibit periods of dormancy followed by repeat disease.

[0053] As used herein, the term "non-neuroinvasive" refers to a virus or other microorganism that lacks neuroinvasive properties.

[0054] As used herein, the term "virulent" refers to a microorganism (e.g., virus) that is able to cause disease or symptoms of disease in a subject.

[0055] As used herein, the term "avirulent" refers to virus (e.g., variant herpesvirus or alphaherpesvirus of embodiments of the present disclosure) that has reduced or no virulence (e.g., does not cause disease or symptoms of disease).

DETAILED DESCRIPTION OF THE DISCLOSURE

[0056] Provided herein are compositions and methods for vaccination and research applications. In particular, provided herein are non-neuroinvasive herpesviruses and alpha herpesviruses and uses thereof.

[0057] Alpha-herpesviruses enter the nervous system following initial replication at exposed body surfaces. *Ibis* neuroinvasive property is a critical step to the establishment of life-long infection. However, this property is un-desirable for clinical applications such as vaccines and cancer therapy.

[0058] Accordingly, in some embodiments, the present disclosure provides a non-neuroinvasive herepsvirus or alphaherpesvirus. In some embodiments, the virus comprises one or more mutation in the R2 domain. Examples include, for example, a variant herpes simplex virus 1 or 2 particle comprising a mutant UL37 protein, wherein said mutant UL37 protein comprises one or more mutations selected from Q403A, E452A, Q455A, Q511A or R515A; a variant varicella zoster virus particle comprising a mutant UL37 protein, wherein said mutant UL37 protein comprises one or more mutations selected from Q363A, D413A, Q416A, Q472A, or R476A; and a variant pseudorabies virus particle comprising a mutant UL37 protein, wherein said mutant UL37 protein comprises one or more mutations selected from Q324A, D362A, R365A, H421A, or H425A. In some embodiments, the one or more mutations is two or more (e.g., three, four, or all) of the mutations. In some embodiments, the virus particle exhibits reduced neuroinvasiveness relative to a virus particle lacking the mutations. In some embodiments, the virus is avirulent.

[0059] The present disclosure is not limited to particular herpes or alpha herpes viruses. Examples include, but are not limited to, herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), pseudorabies virus (PRV), varicella-zoster virus (VRV), bovine herpesvirus-1 (BHV-1; causative agent of bovine infectious rhinotracheitis and pustular vulvovaginitis), bovine herpes virus 5 (BHV-5; causative agent of meningoencephalitis and respiratory disease in cattle and sheep); equine herpes virus 1 (EHV-1; causative agent of equine abortions and respiratory disease); equine herpes virus 3 (EHV-3; causative agent of equine coital exanthema); and equine herpes virus 4 (EHV-4; causative agent of equine rhinopneumonitis). Mutations in the R2 domain of the described viruses are identified using the methods described herein (e.g., by homology to PRV and as described in Examples 2-4).

[0060] The non-neuroinvasive viruses described herein find use in a variety of research, screening, and therapeutic applications (e.g., for use in preparing vaccine and oncolytic virus compositions).

I. Compositions

[0061] In some embodiments, the present disclosure provides vaccine and/or pharmaceutical compositions comprising a variant herpesvirus described herein. The present disclosure is not limited by the particular formulation of a composition. Indeed, a vaccine or pharmaceutical composition of the present disclosure may comprise one or more different agents in addition to the variant herpesvirus. These agents or cofactors include, but are not limited to, adjuvants, surfactants, additives, buffers, solubilizers, chelators, oils, salts, therapeutic agents, drugs, bioactive agents, antibacterials, and antimicrobial agents (e.g., antibiotics, antivirals, etc.). In some embodiments, a vaccine composition comprising a variant herpesvirus comprises an agent and/or co-factor that enhance the ability of the antigen to induce an immune response (e.g., an adjuvant). In some embodiments, the presence of one or more co-factors or agents reduces the amount of antigen required for induction of an immune response (e.g., a protective immune response (e.g., protective immunization)). In some embodiments, the presence of one or more co-factors or agents can be used to skew the immune response towards a cellular (e.g., T cell mediated) or humoral (e.g., antibody mediated) immune response. The present disclosure is not limited by the type of co-factor or agent used in a therapeutic agent of the present disclosure.

[0062] Adjuvants are described in general in Vaccine Design—the Subunit and Adjuvant Approach, edited by Powell and Newman, Plenum Press, New York, 1995. The present disclosure is not limited by the type of adjuvant utilized (e.g., for use in a composition (e.g., pharmaceutical composition). For example, in some embodiments, suitable adjuvants include an aluminium salt such as aluminium hydroxide gel (alum) or aluminium phosphate. In some embodiments, an adjuvant may be a salt of calcium, iron or zinc, or may be an insoluble suspension of acylated tyrosine, or acylated sugars, cationically or anionically derivatised polysaccharides, or polyphosphazenes.

[0063] In general, an immune response is generated to an antigen through the interaction of the antigen with the cells of the immune system. Immune responses may be broadly categorized into two categories: humoral and cell mediated immune responses (e.g., traditionally characterized by antibody and cellular effector mechanisms of protection, respectively). These categories of response have been termed Th-type responses (cell-mediated response), and B cell responses (humoral response).

[0064] Stimulation of an immune response can result from a direct or indirect response of a cell or component of the immune system to an intervention (e.g., exposure to an antigen or immunogen). Immune responses can be measured in many ways including activation, proliferation or differentiation of cells of the immune system (e.g., B cells; T cells; APCs such as for example dendritic cells and macrophages, NK cells, NKT cells etc.); up-regulated or down-regulated expression of markers and cytokines; stimulation of IgA, IgM, or IgG titer; splenomegaly (including increased spleen cellularity); hyperplasia and mixed cellular infiltrates in various organs. Other responses, cells, and components of the immune system that can be assessed with respect to immune stimulation are known in the art.

[0065] In some embodiments, the present disclosure provides a method of stimulating a Th1-type immune response in a subject comprising administering to a subject a composition comprising a non-neuroinvasive herpes or alphaherpes virus. However, in other embodiments, the present disclosure provides a method of stimulating a Th2-type immune response in a subject (e.g., if balancing of a T cell mediated response is desired) comprising administering to a subject a composition comprising a non-neuroinvasive herpes or alphaherpes virus. In further preferred embodiments, adjuvants can be used (e.g., can be co-administered with a composition of the present disclosure) to skew an immune response toward either a Th1 or Th2 type immune response. For example, adjuvants that induce Th2 or weak Th1 responses include, but are not limited to, alum, saponins, and SB-As4. Adjuvants that induce Th1 responses include but are not limited to MPL, MDP, ISCOMS, IL-12, IFN-γ, and SB-AS2.

[0066] Several other types of Th1-type immunogens can be used (e.g., as an adjuvant) in compositions and methods of the present disclosure. These include, but are not limited to, the following. In some embodiments, monophosphoryl lipid A (e.g., in particular 3-de-O-acylated monophosphoryl lipid A (3D-MPL)), is used. 3D-MPL is a well known adjuvant manufactured by Ribi Immunochem, Montana. Chemically it is often supplied as a mixture of 3-de-O-acylated monophosphoryl lipid A with either 4, 5, or 6 acylated chains. In some embodiments, diphosphoryl lipid A, and 3-O-deacylated variants thereof are used. Each of these immunogens can be purified and prepared by methods described in GB 2122204B, hereby incorporated by reference in its entirety. Other purified and synthetic lipopolysaccharides have been described (See, e.g., U.S. Pat. No. 6,005,099 and EP 0 729 473; Hilgers et al., 1986, Int. Arch. Allergy. Immunol., 79(4):392-6; Hilgers et al., 1987, Immunology, 60(1):141-6; and EP 0 549 074, each of which is hereby incorporated by reference in its entirety). In some embodiments, 3D-MPL is used in the form of a particulate formulation (e.g., having a small particle size less than 0.2 μm in diameter, described in EP 0 689 454, hereby incorporated by reference in its entirety).

[0067] In some embodiments, saponins are used as an adjuvant (e.g., Th1-type adjuvant) in a composition of the present disclosure. Saponins are well known adjuvants (See, e.g., Lacaille-Dubois and Wagner (1996) Phytomedicine vol 2 pp 363-386). Examples of saponins include Quil A (derived from the bark of the South American tree Quillaja Saponaria Molina), and fractions thereof (See, e.g., U.S. Pat. No. 5,057,540; Kensil, Crit Rev Ther Drug Carrier Syst, 1996, 12 (1-2):1-55; and EP 0 362 279, each of which is hereby incorporated by reference in its entirety). Also contemplated to be useful in the present disclosure are the haemolytic saponins QS7, QS17, and QS21 (HPLC purified fractions of Quil A; See, e.g., Kensil et al. (1991). J. Immunology 146, 431-437, U.S. Pat. No. 5,057,540; WO 96/33739; WO 96/11711 and EP 0 362 279, each of which is hereby incorporated by reference in its entirety). Also contemplated to be useful are combinations of QS21 and polysorbate or cyclodextrin (See, e.g., WO 99/10008, hereby incorporated by reference in its entirety.

[0068] In some embodiments, an immunogenic oligonucleotide containing unmethylated CpG dinucleotides ("CpG") is used as an adjuvant. CpG is an abbreviation for cytosine-guanosine dinucleotide motifs present in DNA. CpG is known in the art as being an adjuvant when administered by both systemic and mucosal routes (See, e.g., WO 96/02555, EP 468520, Davis et al., J. Immunol, 1998, 160(2):870-876; McCluskie and Davis, J. Immunol., 1998, 161(9):4463-6; and U.S. Pat. App. No. 20050238660, each of which is hereby incorporated by reference in its entirety). For example, in some embodiments, the immunostimulatory sequence is Purine-Purine-C-G-pyrimidine-pyrimidine; wherein the CG motif is not methylated.

[0069] Although an understanding of the mechanism is not necessary to practice the present disclosure and the present disclosure is not limited to any particular mechanism of action, in some embodiments, the presence of one or more CpG oligonucleotides activate various immune subsets including natural killer cells (which produce IFN-γ) and macrophages. In some embodiments, CpG oligonucleotides are formulated into a composition of the present disclosure for inducing an immune response. In some embodiments, a free solution of CpG is co-administered together with an antigen (e.g., present within a solution (See, e.g., WO 96/02555; hereby incorporated by reference). In some embodiments, a CpG oligonucleotide is covalently conjugated to an antigen (See, e.g., WO 98/16247, hereby incorporated by reference), or formulated with a carrier such as aluminium hydroxide (See, e.g., Brazolot-Millan et al., Proc. Natl. Acad Sci., USA, 1998, 95(26), 15553-8).

[0070] In some embodiments, adjuvants such as Complete Freunds Adjuvant and Incomplete Freunds Adjuvant, cytokines (e.g., interleukins (e.g., IL-2, IFN-γ, IL-4, etc.), macrophage colony stimulating factor, tumor necrosis factor, etc.), detoxified mutants of a bacterial ADP-ribosylating toxin such as a cholera toxin (CT), a pertussis toxin (PT), or an *E. Coli* heat-labile toxin (LT), particularly LT-K63 (where lysine is substituted for the wild-type amino acid at position 63) LT-R72 (where arginine is substituted for the wild-type amino acid at position 72), CT-S109 (where serine is substituted for the wild-type amino acid at position 109), and PT-K9/G129 (where lysine is substituted for the wild-type amino acid at position 9 and glycine substituted at position 129) (See, e.g., WO93/13202 and WO92/19265, each of which is hereby incorporated by reference), and other immunogenic substances (e.g., that enhance the effectiveness of a composition of the present disclosure) are used with a composition comprising a non-neuroinvasive herpes or alphaherpes virus of the present disclosure.

[0071] Additional examples of adjuvants that find use in the present disclosure include poly(di(carboxylatophenoxy) phosphazene (PCPP polymer; Virus Research Institute, USA); derivatives of lipopolysaccharides such as monophosphoryl lipid A (MPL; Ribi ImmunoChem Research, Inc., Hamilton, Mont.), muramyl dipeptide (MDP; Ribi) and threonyl-muramyl dipeptide (t-MDP; Ribi); OM-174 (a glucosamine disaccharide related to lipid A; OM Pharma SA, Meyrin, Switzerland); and Leishmania elongation factor (a purified Leishmania protein; Corixa Corporation, Seattle, Wash.).

[0072] Adjuvants may be added to a composition comprising a non-neuroinvasive herpes or alphaherpes virus, or, the adjuvant may be formulated with carriers, for example liposomes, or metallic salts (e.g., aluminium salts (e.g., aluminium hydroxide)) prior to combining with or co-administration with a composition.

[0073] In some embodiments, a composition comprising a non-neuroinvasive herpes or alphaherpes virus comprises a single adjuvant. In other embodiments, a composition comprises two or more adjuvants (See, e.g., WO 94/00153; WO 95/17210; WO 96/33739; WO 98/56414; WO 99/12565; WO 99/11241; and WO 94/00153, each of which is hereby incorporated by reference in its entirety).

[0074] In some embodiments, a composition comprising an antigen or immunogen comprises one or more mucoadhesives (See, e.g., U.S. Pat. App. No. 20050281843, hereby incorporated by reference in its entirety). The present disclosure is not limited by the type of mucoadhesive utilized. Indeed, a variety of mucoadhesives are contemplated to be useful in the present disclosure including, but not limited to, cross-linked derivatives of poly(acrylic acid) (e.g., carbopol and polycarbophil), polyvinyl alcohol, polyvinyl pyrollidone, polysaccharides (e.g., alginate and chitosan), hydroxypropyl methylcellulose, lectins, fimbrial proteins, and carboxymethylcellulose. Although an understanding of the mechanism is not necessary to practice the present disclosure and the present disclosure is not limited to any particular mechanism of action, in some embodiments, use of a mucoadhesive (e.g., in a composition comprising a non-neuroinvasive herpes or alphaherpes virus) enhances induction of an immune response in a subject (e.g., administered a composition of the present disclosure) due to an increase in duration and/or amount of exposure to a non-neuroinvasive herpes or alphaherpes virus that a subject experiences when a mucoadhesive is used compared to the duration and/or amount of exposure to a non-neuroinvasive herpes or alphaherpes virus in the absence of using the mucoadhesive.

[0075] In some embodiments, a composition of the present disclosure may comprise sterile aqueous preparations. Acceptable vehicles and solvents include, but are not limited to, water, Ringer's solution, phosphate buffered saline and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed mineral or non-mineral oil may be employed including synthetic mono-ordi-glycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables. Carrier formulations suitable for mucosal, subcutaneous, intramuscular, intraperitoneal, intravenous, or administration via other routes may be found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa.

[0076] A composition comprising a variant viral particle of the present disclosure can be used therapeutically (e.g., to enhance an immune response) or as a prophylactic (e.g., for immunization (e.g., to prevent signs or symptoms of disease)) or as an oncolytic virus. A composition can be administered to a subject via a number of different delivery routes and methods.

[0077] In some embodiments, compositions of the present disclosure are administered mucosally (e.g., using standard techniques; See, e.g., Remington: The Science and Practice of Pharmacy, Mack Publishing Company, Easton, Pa., 19th edition, 1995 (e.g., for mucosal delivery techniques, including intranasal, pulmonary, vaginal and rectal techniques), as well as European Publication No. 517,565 and Illum et al., J. Controlled Rel., 1994, 29:133-141 (e.g., for techniques of intranasal administration), including via cell, vesicles, and liposomes, each of which is hereby incorporated by reference in its entirety). Alternatively, the compositions of the present disclosure may be administered dermally or transdermally, using standard techniques (See, e.g., Remington: The Science arid Practice of Pharmacy, Mack Publishing Company, Easton, Pa., 19th edition, 1995). The present disclosure is not limited by the route of administration.

[0078] Although an understanding of the mechanism is not necessary to practice the present disclosure and the present disclosure is not limited to any particular mechanism of action, in some embodiments, mucosal vaccination is the preferred route of administration as it has been shown that mucosal administration of antigens has a greater efficacy of inducing protective immune responses at mucosal surfaces (e.g., mucosal immunity), the route of entry of many pathogens. In addition, mucosal vaccination, such as intranasal vaccination, may induce mucosal immunity not only in the nasal mucosa, but also in distant mucosal sites such as the genital mucosa (See, e.g., Mestecky, Journal of Clinical Immunology, 7:265-276, 1987). More advantageously, in further preferred embodiments, in addition to inducing mucosal immune responses, mucosal vaccination also induces systemic immunity. In some embodiments, non-parenteral administration (e.g., muscosal administration of vaccines) provides an efficient and convenient way to boost systemic immunity (e.g., induced by parenteral or mucosal vaccination (e.g., in cases where multiple boosts are used to sustain a vigorous systemic immunity)).

[0079] In some embodiments, a composition comprising a non-neuroinvasive herpes or alphaherpes virus of the present disclosure may be used to protect or treat a subject susceptible to, or suffering from, disease by means of administering a composition of the present disclosure via a mucosal route (e.g., an oral/alimentary or nasal route). Alternative mucosal routes include intravaginal and intra-rectal routes. In preferred embodiments of the present disclosure, a nasal route of administration is used, termed "intranasal administration" or "intranasal vaccination" herein. Methods of intranasal vaccination are well known in the art, including the administration of a droplet or spray form of the vaccine into the nasopharynx of a subject to be immunized. In some embodiments, a nebulized or aerosolized composition is provided. Enteric formulations such as gastro resistant capsules for oral administration, suppositories for rectal or vaginal administration also form part of this disclosure. Compositions of the present disclosure may also be administered via the oral route. Under these circumstances, a composition comprising a non-neuroinvasive herpes or alphaherpes virus may comprise a pharmaceutically acceptable excipient and/or include alkaline buffers, or enteric capsules. Formulations for nasal delivery may include those with dextran or cyclodextran and saponin as an adjuvant.

[0080] Compositions of the present disclosure may also be administered via a vaginal route. In such cases, a composition comprising a non-neuroinvasive herpes or alphaherpes virus may comprise pharmaceutically acceptable excipients and/or emulsifiers, polymers (e.g., CARBOPOL), and other known stabilizers of vaginal creams and suppositories. In some embodiments, compositions of the present disclosure are administered via a rectal route. In such cases, compositions may comprise excipients and/or waxes and polymers known in the art for forming rectal suppositories.

[0081] In some embodiments, the same route of administration (e.g., mucosal administration) is chosen for both a priming and boosting vaccination. In some embodiments, multiple routes of administration are utilized (e.g., at the same time, or, alternatively, sequentially) in order to stimulate an immune response.

[0082] For example, in some embodiments, a composition comprising a non-neuroinvasive herpes or alphaherpes virus is administered to a mucosal surface of a subject in either a priming or boosting vaccination regime. Alternatively, in some embodiments, the composition is administered systemically in either a priming or boosting vaccination regime. In some embodiments, a composition is administered to a subject in a priming vaccination regimen via mucosal administration and a boosting regimen via systemic administration. In some embodiments, a composition is administered to a subject in a priming vaccination regimen via systemic administration and a boosting regimen via mucosal administration. Examples of systemic routes of administration include, but are not limited to, a parenteral, intramuscular, intradermal, transdermal, subcutaneous, intraperitoneal or intravenous administration. A composition comprising a non-neuroinvasive herpes or alphaherpes virus may be used for both prophylactic and therapeutic purposes.

[0083] In some embodiments, compositions of the present disclosure are administered by pulmonary delivery. For example, a composition of the present disclosure can be delivered to the lungs of a subject (e.g., a human) via inhalation (e.g., thereby traversing across the lung epithelial lining to the blood stream (See, e.g., Adjei, et al. Pharmaceutical Research 1990; 7:565-569; Adjei, et al. Int. J. Pharmaceutics 1990; 63:135-144; Braquet, et al. J. Cardiovascular Pharmacology 1989 143-146; Hubbard, et al. (1989) Annals of Internal Medicine, Vol. III, pp. 206-212; Smith, et al. J. Clin. Invest. 1989; 84:1145-1146; Oswein, et al. "Aerosolization of Proteins", 1990; Proceedings of Symposium on Respiratory Drug Delivery II Keystone, Colorado; Debs, et al. J. Immunol. 1988; 140:3482-3488; and U.S. Pat. No. 5,284,656 to Platz, et al, each of which are hereby incorporated by reference in its entirety). A method and composition for pulmonary delivery of drugs for systemic effect is described in U.S. Pat. No. 5,451,569 to Wong, et al., hereby incorporated by reference; See also U.S. Pat. No. 6,651,655 to Licalsi et al., hereby incorporated by reference in its entirety)).

[0084] Further contemplated for use in the practice of this disclosure are a wide range of mechanical devices designed for pulmonary and/or nasal mucosal delivery of pharmaceutical agents including, but not limited to, nebulizers, metered dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art. Some specific examples of commercially available devices suitable for the practice of this disclosure are the Ultravent nebulizer (Mallinckrodt Inc., St. Louis, Mo.); the Acorn II nebulizer (Marquest Medical Products, Englewood, Colo.); the Ventolin metered dose inhaler (Glaxo Inc., Research Triangle Park, N.C.); and the Spinhaler powder inhaler (Fisons Corp., Bedford, Mass.). All such devices require the use of formulations suitable for dispensing of the therapeutic agent. Typically, each formulation is specific to the type of device employed and may involve the use of an appropriate propellant material, in addition to the usual diluents, adjuvants, surfactants, carriers and/or other agents useful in therapy. Also, the use of liposomes, microcapsules or microspheres, inclusion complexes, or other types of carriers is contemplated.

[0085] Thus, in some embodiments, a composition comprising an variant virus of the present disclosure may be used to protect and/or treat a subject susceptible to, or suffering from, a disease by means of administering the composition by mucosal, intramuscular, intraperitoneal, intradermal, transdermal, pulmonary, intravenous, subcutaneous or other route of administration described herein. Methods of systemic administration of the vaccine preparations may include conventional syringes and needles, or devices designed for ballistic delivery of solid vaccines (See, e.g., WO 99/27961, hereby incorporated by reference), or needleless pressure liquid jet device (See, e.g., U.S. Pat. Nos. 4,596,556; 5,993,412, each of which are hereby incorporated by reference), or transdermal patches (See, e.g., WO 97/48440; WO 98/28037, each of which are hereby incorporated by reference). The present disclosure may also be used to enhance the immunogenicity of antigens applied to the skin (transdermal or transcutaneous delivery, See, e.g., WO 98/20734; WO 98/28037, each of which are hereby incorporated by reference).

[0086] The present disclosure is not limited by the type of subject administered (e.g., in order to stimulate an immune response (e.g., in order to generate protective immunity (e.g., mucosal and/or systemic immunity) or to target cancer cells) a composition of the present disclosure. Indeed, a wide variety of subjects are contemplated to be benefited from administration of a composition of the present disclosure. In some embodiments, the subject is a human or non-human animal. In some embodiments, human subjects are of any age (e.g., adults, children, infants, etc.) that have been or are likely to become exposed to a microorganism (e.g., herpesvirus). In some embodiments, the human subjects are subjects that are more likely to receive a direct exposure to pathogenic microorganisms or that are more likely to display signs and symptoms of disease after exposure to a pathogen (e.g., immune suppressed subjects). In some embodiments, the general public is administered (e.g., vaccinated with) a composition of the present disclosure (e.g., to prevent the occurrence or spread of disease). For example, in some embodiments, compositions and methods of the present disclosure are utilized to vaccinate a group of people (e.g., a population of a region, city, state and/or country) for their own health (e.g., to prevent or treat disease). In some embodiments, the subjects are non-human mammals (e.g., pigs, cattle, goats, horses, sheep, or other livestock; or mice, rats, rabbits or other animal). In some embodiments, compositions and methods of the present disclosure are utilized in research settings (e.g., with research animals).

[0087] A composition of the present disclosure may be formulated for administration by any route, such as mucosal, oral, transdermal, intranasal, intramuscular, parenteral or other route described herein. The compositions may be in any one or more different forms including, but not limited to, tablets, capsules, powders, granules, lozenges, foams, creams or liquid preparations.

[0088] Topical formulations of the present disclosure may be presented as, for instance, ointments, creams or lotions, foams, and aerosols, and may contain appropriate conventional additives such as preservatives, solvents (e.g., to assist penetration), and emollients in ointments and creams.

[0089] Topical formulations may also include agents that enhance penetration of the active ingredients through the skin. Exemplary agents include a binary combination of N-(hydroxyethyl) pyrrolidone and a cell-envelope disordering compound, a sugar ester in combination with a sulfoxide or phosphine oxide, and sucrose monooleate, decyl methyl sulfoxide, and alcohol.

[0090] Other exemplary materials that increase skin penetration include surfactants or wetting agents including, but not limited to, polyoxyethylene sorbitan mono-oleoate (Polysorbate 80); sorbitan mono-oleate (Span 80); p-isooctyl polyoxyethylene-phenol polymer (Triton WR-1330); polyoxyethylene sorbitan tri-oleate (Tween 85); dioctyl sodium sulfosuccinate; and sodium sarcosinate (Sarcosyl NL-97); and other pharmaceutically acceptable surfactants.

[0091] In certain embodiments of the disclosure, compositions may further comprise one or more alcohols, zinc-containing compounds, emollients, humectants, thickening and/or gelling agents, neutralizing agents, and surfactants. Water used in the formulations is preferably deionized water having a neutral pH. Additional additives in the topical formulations include, but are not limited to, silicone fluids, dyes, fragrances, pH adjusters, and vitamins. Topical formulations may also contain compatible conventional carriers, such as cream or ointment bases and ethanol or oleyl alcohol for lotions. Such carriers may be present as from about 1% up to about 98% of the formulation. The ointment base can comprise one or more of petrolatum, mineral oil, ceresin, lanolin alcohol, panthenol, glycerin, bisabolol, cocoa butter and the like.

[0092] In some embodiments, pharmaceutical compositions of the present disclosure may be formulated and used as foams. Pharmaceutical foams include formulations such as, but not limited to, emulsions, microemulsions, creams, jellies and liposomes. While basically similar in nature these formulations vary in the components and the consistency of the final product. The compositions of the present disclosure may additionally contain other adjunct components conventionally found in pharmaceutical compositions. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the compositions of the present disclosure, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, preferably do not unduly interfere with the biological activities of the components of the compositions of the present disclosure. The formulations can be sterilized and, if desired, mixed with auxiliary agents (e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like) that do not deleteriously interact with the non-neuroinvasive herpes or alphaherpes virus or other components of the formulation. In some embodiments, immunostimulatory compositions of the present disclosure are administered in the form of a pharmaceutically acceptable salt. When used the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically acceptable salts thereof. Such salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulphuric, nitric, phosphoric, maleic, acetic, salicylic, p-toluene sulphonic, tartaric, citric, methane sulphonic, formic, malonic, succinic, naphthalene-2-sulphonic, and benzene sulphonic. Also, such salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts of the carboxylic acid group.

[0093] Suitable buffering agents include, but are not limited to, acetic acid and a salt (1-2% w/v); citric acid and a salt (1-3% w/v); boric acid and a salt (0.5-2.5% w/v); and phosphoric acid and a salt (0.8-2% w/v). Suitable preservatives may include benzalkonium chloride (0.003-0.03% w/v); chlorobutanol (0.3-0.9% w/v); parabens (0.01-0.25% w/v) and thimerosal (0.004-0.02% w/v).

[0094] In some embodiments, vaccine compositions are co-administered with one or more antibiotics or antiviral agents. There are an enormous amount of antimicrobial agents currently available for use in treating bacterial, fungal and viral infections. For a comprehensive treatise on the general classes of such drugs and their mechanisms of action, the skilled artisan is referred to Goodman & Gilman's "The Pharmacological Basis of Therapeutics" Eds. Hardman et al., 9th Edition, Pub. McGraw Hill, chapters 43 through 50, 1996, (herein incorporated by reference in its entirety). Generally, these agents include agents that inhibit cell wall synthesis (e.g., penicillins, cephalosporins, cycloserine, vancomycin, bacitracin); and the imidazole antifungal agents (e.g., miconazole, ketoconazole and clotrimazole); agents that act directly to disrupt the cell membrane of the microorganism (e.g., detergents such as polmyxin and colistimethate and the antifungals nystatin and amphotericin B); agents that affect the ribosomal subunits to inhibit protein synthesis (e.g., chloramphenicol, the tetracyclines, erthromycin and clindamycin); agents that alter protein synthesis and lead to cell death (e.g., aminoglycosides); agents that affect nucleic acid metabolism (e.g., the rifamycins and the quinolones); the antimetabolites (e.g., trimethoprim and sulfonamides); and the nucleic acid analogues such as zidovudine, gangcyclovir, vidarabine, and acyclovir which act to inhibit viral enzymes essential for DNA synthesis. Various combinations of antimicrobials may be employed.

[0095] In some embodiments, compositions comprising variant viruses are administered in combination with anticancer (e.g., chemotherapy agents). Various classes of antineoplastic (e.g., anticancer) agents are contemplated for use in certain embodiments of the present disclosure. Anticancer agents suitable for use with the present disclosure include, but are not limited to, agents that induce apoptosis, agents that inhibit adenosine deaminase function, inhibit pyrimidine biosynthesis, inhibit purine ring biosynthesis, inhibit nucleotide interconversions, inhibit ribonucleotide reductase, inhibit thymidine monophosphate (TMP) synthesis, inhibit dihydrofolate reduction, inhibit DNA synthesis, form adducts with DNA, damage DNA, inhibit DNA repair, intercalate with DNA, deaminate asparagines, inhibit RNA synthesis, inhibit protein synthesis or stability, inhibit microtubule synthesis or function, and the like.

[0096] In some embodiments, exemplary anticancer agents suitable for use in compositions and methods of the present disclosure include, but are not limited to: 1) alkaloids, including microtubule inhibitors (e.g., vincristine, vinblastine, and vindesine, etc.), microtubule stabilizers (e.g., paclitaxel (TAXOL), and docetaxel, etc.), and chromatin function inhibitors, including topoisomerase inhibitors, such as epipodophyllotoxins (e.g., etoposide (VP-16), and teniposide (VM-26), etc.), and agents that target topoisomerase I (e.g., camptothecin and isirinotecan (CPT-11), etc.); 2) covalent DNA-binding agents (alkylating agents), including nitrogen mustards (e.g., mechlorethamine, chlorambucil, cyclophosphamide, ifosphamide, and busulfan (MYLERAN), etc.), nitrosoureas (e.g., carmustine, lomustine, and semustine, etc.), and other alkylating agents (e.g., dacarbazine, hydroxymethylmelamine, thiotepa, and mitomycin, etc.); 3) noncovalent DNA-binding agents (antitumor antibiotics), including nucleic acid inhibitors (e.g., dactinomycin (actinomycin D), etc.), anthracyclines (e.g., daunorubicin (daunomycin, and cerubidine), doxorubicin (adriamycin), and idarubicin (idamycin), etc.), anthracenediones (e.g., anthracycline analogues, such as mitoxantrone, etc.), bleomycins (BLENOXANE), etc., and plicamycin (mithramycin), etc.; 4) antimetabolites, including antifolates (e.g., methotrexate, FOLEX, and MEXATE, etc.), purine antimetabolites (e.g., 6-mercaptopurine (6-MP, PURINETHOL), 6-thioguanine (6-TG), azathioprine, acyclovir, ganciclovir, chlorodeoxyadenosine, 2-chlorodeoxyadenosine (CdA), and 2'-deoxycoformycin (pentostatin), etc.), pyrimidine antagonists (e.g., fluoropyrimidines (e.g., 5-fluorouracil (ADRUCIL), 5-fluorodeoxyuridine (FdUrd) (floxuridine)) etc.), and cytosine arabinosides (e.g., CYTOSAR (ara-C) and fludarabine, etc.); 5) enzymes, including L-asparaginase, and hydroxyurea, etc.; 6) hormones, including glucocorticoids, antiestrogens (e.g., tamoxifen, etc.), nonsteroidal antiandrogens (e.g., flutamide, etc.), and aromatase inhibitors (e.g., anastrozole (ARIMIDEX), etc.); 7) platinum compounds (e.g., cisplatin and carboplatin, etc.); 8) monoclonal antibodies conjugated with anticancer drugs, toxins, and/or radionuclides, etc.; 9) biological response modifiers (e.g., interferons (e.g., IFN-α, etc.) and interleukins (e.g., IL-2, etc.), etc.); 10) adoptive immunotherapy; 11) hematopoietic growth factors; 12) agents that induce tumor cell differentiation (e.g., all-trans-retinoic acid, etc.); 13) gene therapy techniques; 14) antisense therapy techniques; 15) tumor vaccines; 16) therapies directed against tumor metastases (e.g., batimastat, etc.); 17) angiogenesis inhibitors; 18) proteosome inhibitors (e.g., VELCADE); 19) inhibitors of acetylation and/or methylation (e.g., HDAC inhibitors); 20) modulators of NF kappa B; 21) inhibitors of cell cycle regulation (e.g., CDK inhibitors); 22) modulators of p53 protein function; and 23) radiation. Any oncolytic agent used in a cancer therapy context finds use in the compositions and methods of the present invention. For example, the U.S. Food and Drug Administration maintains a formulary of oncolytic agents approved for use in the United States. International counterpart agencies to the U.S.F.D.A. maintain similar formularies.

[0097] In co-administration procedures, the agents may be administered concurrently or sequentially. In one embodiment, the compositions described herein are administered prior to the other active agent(s). The pharmaceutical formulations and modes of administration may be any of those described herein. In addition, the two or more co-administered agents may each be administered using different modes (e.g., routes) or different formulations. The additional agents to be co-administered (e.g., antibiotics, chemotherapy agents, adjuvants, etc.) can be any of the well-known agents in the art, including, but not limited to, those that are currently in clinical use.

[0098] In some embodiments, a composition comprising a non-neuroinvasive herpes or alphaherpes virus is administered to a subject via more than one route. For example, a subject that would benefit from having a protective immune response (e.g., immunity) towards a pathogenic microorganism may benefit from receiving mucosal administration (e.g., nasal administration or other mucosal routes described herein) and, additionally, receiving one or more other routes of administration (e.g., parenteral or pulmonary administration (e.g., via a nebulizer, inhaler, or other methods described herein). In some embodiments, administration via mucosal route is sufficient to induce both mucosal as well as systemic immunity towards the herpes or alphaherpes virus. In other embodiments, administration via multiple routes serves to provide both mucosal and systemic immunity. Thus, although an understanding of the mechanism is not necessary to practice the present disclosure and the present disclosure is not limited to any particular mechanism of action, in some embodiments, it is contemplated that a subject administered a composition of the present disclosure via multiple routes of administration (e.g., immunization (e.g., mucosal as well as airway or parenteral administration of the composition) may have a stronger immune response to a non-neuroinvasive herpes or alphaherpes virus than a subject administered a composition via just one route.

[0099] Other delivery systems can include time-release, delayed release or sustained release delivery systems. Such systems can avoid repeated administrations of the compositions, increasing convenience to the subject and a physician. Many types of release delivery systems are available and known to those of ordinary skill in the art. They include polymer based systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Pat. No. 5,075,109, hereby incorporated by reference. Delivery systems also include non-polymer systems that are: lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono-di- and tri-glycerides; hydrogel release systems; sylastic systems; peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which an agent of the disclosure is contained in a form within a matrix such as those described in U.S. Pat. Nos. 4,452,775, 4,675,189, and 5,736,152, each of which is hereby incorporated by reference and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Pat. Nos. 3,854,480, 5,133,974 and 5,407,686, each of which is hereby incorporated by reference. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

[0100] In some embodiments, a vaccine or pharmaceutical composition of the present disclosure is formulated in a concentrated dose that can be diluted prior to administration to a subject. For example, dilutions of a concentrated composition may be administered to a subject such that the subject receives any one or more of the specific dosages provided herein. In some embodiments, dilution of a concentrated composition may be made such that a subject is administered (e.g., in a single dose). Concentrated compositions are contemplated to be useful in a setting in which large numbers of subjects may be administered a composition of the present disclosure (e.g., an immunization clinic, hospital, school, etc.). In some embodiments, a composition comprising a non-neuroinvasive herpes or alphaherpes virus of the present disclosure (e.g., a concentrated composition) is stable at room temperature for more than 1 week, in some embodiments for more than 2 weeks, in some embodiments for more than 3 weeks, in some embodiments for more than 4 weeks, in some embodiments for more than 5 weeks, and in some embodiments for more than 6 weeks.

[0101] The present disclosure further provides kits comprising the vaccine or pharmaceutical compositions comprised herein. In some embodiments, the kit includes all of the components necessary, sufficient or useful for administering the vaccine. For example, in some embodiments, the kits comprise devices for administering the vaccine (e.g., needles or other injection devices), temperature control components (e.g., refrigeration or other cooling components), sanitation components (e.g., alcohol swabs for sanitizing the site of injection) and instructions for administering the vaccine.

II. Uses

[0102] The non-neuroinvasive viruses described herein find use in a variety of research, screening, and therapeutic applications.

[0103] Embodiments of the present disclosure provide vaccine compositions for use in the prevention of disease in human and non-human animals (e.g., livestock and companion animals).

[0104] In some embodiments, the non-neuroinvasive viruses described herein find use in cancer therapy (e.g., as oncolytic viruses). An oncolytic virus is a virus that preferentially infects and kills cancer cells. As the infected cancer cells are destroyed by lysis, they release new infectious virus particles to help destroy the remaining tumor. Oncolytic viruses are thought not only to cause direct destruction of the tumor cells, but also to stimulate host anti-tumor immune responses. Oncolytic herpesviruses are described, for example, in Varghese, et al. (Cancer Gene Therapy 9 (12): 967-78). In some embodiments, the modified non-neuroinvasive viruses described herein find use as oncolytic viruses (e.g., as described herein or with further modification).

[0105] In some embodiments, the viral compositions described herein find use in vaccination (e.g., against herpesvirus and alphaherpes virus infection).

[0106] In some embodiments, following an initial administration of a composition of the present disclosure (e.g., an initial vaccination), a subject may receive one or more boost administrations (e.g., around 2 weeks, around 3 weeks, around 4 weeks, around 5 weeks, around 6 weeks, around 7 weeks, around 8 weeks, around 10 weeks, around 3 months, around 4 months, around 6 months, around 9 months, around 1 year, around 2 years, around 3 years, around 5 years, around 10 years) subsequent to a first, second, third, fourth, fifth, sixth, seventh, eights, ninth, tenth, and/or more than tenth administration. Although an understanding of the mechanism is not necessary to practice the present disclosure and the present disclosure is not limited to any particular mechanism of action, in some embodiments, reintroduction of a non-neuroinvasive herpes or alphaherpes virus in a boost dose enables vigorous systemic immunity in a subject. The boost can be with the same formulation given for the primary immune response, or can be with a different formulation that contains the virus. The dosage regimen will also, at least in part, be determined by the need of the subject and be dependent on the judgment of a practitioner.

[0107] Dosage units may be proportionately increased or decreased based on several factors including, but not limited to, the weight, age, and health status of the subject. In addition, dosage units may be increased or decreased for subsequent administrations (e.g., boost administrations).

[0108] In some embodiments, the compositions described herein find use in research uses (e.g., to identify neurons in cells and non-human animals). For example, in some embodiments, the modified viruses described herein find use as anterograde-specific trans-synaptic tracers of the mammalian nervous system.

EXPERIMENTAL

[0109] The following examples are provided in order to demonstrate and further illustrate certain preferred embodiments and aspects of the present disclosure and are not to be construed as limiting the scope thereof.

Example 1

Crystal Structure of the Herpesvirus Inner Tegument Protein UL37

Materials and Methods

[0110] Cloning. Plasmid pGS3610 encodes the PRV Becker UL37 gene fused to an N-terminal His6-SUMO tandem tag. This was made by cutting the pETDuet-SUMO vector (a derivative of pETDuet-1 and a gift from Thomas Schwartz) and the pGS1740 subclone of UL37 with BamHI and HindIII. The pJP4 plasmid, which contains a His6-SUMO-PreScission tag in frame with the BamHI restriction site of the multiple-cloning site in a pET24b vector, was made through PCR of the His6-SUMO-PreScission tag from pETDuet-SUMO using the primers 5=-GGGAATTC-CATATGGGCAGCAGCCATCACCATCA (SEQ ID NO: 1) and 3=-CTAGGGATCCGGGCCCCTGGAACAGAACTT (SEQ ID NO: 2). The PCR product was subcloned into pET24b using NdeI and BamHI restriction sites. The PRV UL37 gene for *Escherichia coli* expression was synthesized by GeneArt. The N-terminal half (residues 1 to 496) of codon-optimized PRV UL37 (referred to as UL37N) was amplified by PCR from the full-length PRV codon-optimized UL37 gene using the primers 5=-CTAGGGATC-CATGGAAGCACTGGTTCGTGC (SEQ ID NO: 3) and 3=-CTAGAAGCTTCTAGGCTGCGCTGGTCGGTG (SEQ ID NO: 4). The PCR product was subcloned into pJP4 using the BamHI and HindIII restriction sites to yield plasmid pJP23.

[0111] Virus construction. All recombinant PRV (strain Becker) isolates were derived from a variant of the pBecker3 infectious clone, pGS4284, that encodes the mCherry red fluorescent protein fused in frame to the UL25 capsid protein (Bohannon K P, Sollars P J, Pickard G E, Smith G A. 2012. Fusion of a fluorescent protein to the pUL25 minor capsid protein of pseudorabies virus allows live-cell capsid imaging with negligible impact on infection. J. Gen. Virol. 93:124-129). Viruses were produced by electroporation of infectious clones into the pig kidney epithelial cell line PK15, as previously described (Luxton G W, Haverlock S, Coller K E, Antinone S E, Pincetic A, Smith G A. 2005. Targeting of herpesvirus capsid transport in axons is coupled to association with specific sets of tegument proteins. Proc. Natl. Acad. Sci. U.S.A 102:5832-5837). PK15 cells were maintained in Dulbecco modified Eagle medium (DMEM;

Invitrogen) supplemented with 10% bovine growth supplement (BGS; HyClone), which was reduced to 2% during transfection and infection. The harvested virus was passaged once to produce a high-titer stock by infecting a 10-cm dish of PK15 cells with 1 μl virus. Transfection of pGS4284 resulted in PRV-GS4284, which upon passage propagated to titers of >5×10^8 PFU/ml. To make PRV encoding the pentuple mutations D79A/D81A/E82A/D382A/D383A in the calcium-binding region (Ca) of UL37, codon changes were introduced through two rounds of en passant mutagenesis of pGS4284. The first set of primers, 5=CTCGCCGA GAACCTGGC CGGCCTGGCGCTGTGGCGCCTGCGCCACGCCTGGGCCGCGGGCACGGCCCCGCT GAGGA TGACGACGATAAGTAGGG(SEQ ID NO: 5) and 5=GTCGCCGTGACGACCCCCAGGAGCTCCAGCAG CGGGGCCGTGCCCGCGGCCCAGGCGTGGCGCAG GCGCCACAACCAATAACCAATCTGATAG (SEQ ID NO: 6), was used to generate the D382A/D383A mutations (mutated bases are in bold) and produced pGS5456. The second set of primers, 5=GTCGGCTGCACGGCGGTC GTC GGC GGCGTCGTGCACCGCCTCCTCGCCGCCT A CGGGCCCGGGCTGAGGATGACGACGATAAGTA G GG (SEQ ID NO: 7) and 5=CGCGACGTCCGTGTAGGCG CG CACGTAGTCCAGCCCGGGCCCGTAGGCGGCG A GGAGGCGGTGCACCAACCAATAACCAATCTGA1T AG (SEQ ID NO: 8), was used to generate the D79A/D81A/E82A mutations, which were introduced into pGS5456 to produce the final mutant, pGS5476. PRV-GS5476 typically propagated to a titer of >5×108 PFU/ml. The region 2 (R2) and region 3 (R3) mutant viruses were produced in the same manner as the Ca mutant. For R2, mutations were introduced into pGS4284 in three sequential rounds using primers 5=-CTCGACCACACGCAGGTGGACGCCACGGGCGT GTGGGAGGCGGTGGCGGCCAGCGCCTCGCCGAGGATGACGACGATAAGTAGGG (SEQ ID NO: 9) and 5=-CGCGGTCACGAGCGCCTCCACGACCTGCAGCG GCGAGGCGCTGGCCGCCACCGC CTCCCACACCAA CCAA1TAACCAATCTGATAG (SEQ ID NO: 10) (encoding Q324A), 5=-GACCTCCTCGAGCGCGCCGTGCTG GA CCGCGCGCCCCGCCTGACGGCCGCGCAG GCT GCCGTCGGCTGCACGAGGATGACGACGATAAGTA GGG (SEQ ID NO: 11) and 5=-GAGGCGGTGCACGAC GCCGCCGACGACCGCCGTGCAGCCGACGGCAGC CTGCGCGGCCGTCAGGCGGGGCGCCAACCAA1TA ACCAATCTGATAG (SEQ ID NO: 12) (encoding D362A/R365A), and 5=-GGGGACGTGAC GGCGGCG CTGG GGCTCCCCGAGAAGGGCGTGGAGGCCGTGGTGC GCGCTTGCATGGCGCCGCGCAGGATGACGACGATAAGTAGGG (SEQ ID NO: 13) and 5=-GCGCGCCGCG CCCACGTGCTCCGTGGGCGGGCGCGGCGCCATGC AAGCGCGCAC CACGGCCTCCACGCCCTCTCCAACCAATTAACCAATTCTGATTAG (SEQ ID NO: 14) (encoding H421A/H425A). The first PCR product was recombined into pGS4284, resulting in pGS5483. The second PCR product was then recombined into pGS5483, resulting in pGS5558. The final recombination was made into pGS5558, resulting in pGS5604. PRV-GS5604 typically propagated to a titer of >5×10^8 PFU/ml. The R3 mutations were introduced in two rounds using primers 5=-CTGCCGCTGGCGTG GCG GTGCGCCAGATGCAGAACGAGGGCCTGGCGC AGCTG ACGCGCGCGCTCAGGATGACGACGATAAG TAGGG (SEQ ID NO: 15) and 5=-GAAGAACTCGTCG GCGATCGTGAGGGCAAAGAGCGCGCGCGTCAGC TGCGCCAGGCCCTCGTTCTGCAACCAATAACCAA TTCTGATAG (SEQ ID NO: 16) (encoding D239A/E240A) and primers 5=AACCCGACGCTGCGCGAGCAGTCGCC GAGGCGGCGCGGGCCGTGGCCGCGG CGGCGCTGG TGCCCAGGATGACGACGATAAGTAGGG (SEQ ID NO: 17) and 5=-CGTGCGCGGCGTGGCGTGACCTCGCCCACGGGCACCAGCGCCGCCGCGGCCAC GGCC CGC GCCGCCAACCAATAACCAATTCTGATTAG (SEQ ID NO: 18) (encoding K203A/P204Q). The first PCR product was recombined into pGS4284, resulting in pGS5242. The second PCR product was then recombined into pGS5242, resulting in pGS5350. PRV-GS5350 typically propagated to a titer of >5×10 PFU/ml. The region 1 (R1) mutant virus (V249R/R254A/R285A/D287A/H311A) was generated using a modified two-step recombination. The region of the UL37 gene encoding amino acids 249 to 311 was first replaced with the kanamycin resistance cassette of pEP-kan-S using primers 5=-CCGAGGCGGCGCGG GCCGT GGACGAGGCGGCGCTGGTGCCCGTGGGCGAGACG CAGGTGGACGCCACGGGAGGATGACGACGATAA GTAGGG (SEQ ID NO: 19) and 5=-GAGGCGCTG GCCTGCACCGCCTCCCACACGCCCGTGGCGTCCA CCTGCGTCTCGC CCACGGGCACCAGCGCAACCAA TTAACCAATTCTGATTAG (SEQ ID NO: 20). The PCR product was recombined into pGS4284, resulting in the intermediate construct pGS5313. The deletion in pGS5313 was then repaired using a 489-bp synthetic DNA encoding the missing UL37 sequence with the five codon changes and 150 bp of flanking homologous sequence to each side (pGS5267; Integrated DNA Technologies). The synthetic DNA was released from a pIDTSmart vector using flanking HindIII sites and recombined into pGS5313. Recombination was carried out by growing *E. coli* strain GS1783 harboring pGS5313 in 30 ml of Luria Broth (LB) supplemented with 20 μg/ml chloramphenicol to an optical density at 600 nm(OD600) of 0.6 at 32° C. in a baffled flask.

[0112] At this point, 20 ml of LB supplemented with 20 μg/ml chloramphenicol and 2% L-arabinose was added, and the culture was incubated with shaking at 32° C. for 70 min. The culture was then transferred to a 42° C. shaking water bath for 15 min, and the contents were then transferred to a 50-ml conical tube and chilled on ice. The chilled bacteria were washed three times, and the final pellet was suspended in 300 ml double-distilled H_2O, of which 48 μl was used in an electroporation with 2 μl of the pGS5267 synthetic fragment. After recovery, the reaction mixture was plated on LB agar plates supplemented with 20 μg/ml chloramphenicol and 2% L-arabinose. The resulting isolate was saved as pGS5321. PRV-GS5321 typically propagated to a titer of >5×10^8 PFU/ml. The sequences of all genetic modifications in the infectious clones were confirmed.

[0113] Viral propagation kinetics, viral titers, and plaque size analysis. Quantitation of viral propagation kinetics was assessed by single-step growth in PK15 cells infected at a multiplicity of infection (MOI) of 10 for each viral stain. Viral titers from cells or medium supernatants harvested at 2, 5, 8, 12, or 24 h postinfection (hpi) were determined in duplicate by plaque assay, as previously described (Smith G A, Enquist L W. 1999. Construction and transposon mutagenesis in *Escherichia coli* of a full-length infectious clone of pseudorabies virus, an alphaherpesvirus. J. Virol. 73:6405-6414). Measurements of plaque diameters were obtained by infection of PK15 cells in 6-well trays with serial 10-fold dilutions for each virus. At 4 days postinfection, images were captured with a ×4 objective on a Nikon

TE2000 inverted fluorescence microscopy (Nikon Instruments) fitted with a CoolSnap HQ2 camera (Photometrics). Two orthogonal diameter measurements of each fluorescent plaque were obtained using the Metamorph software package (Molecular Devices) and averaged. The reported plaque diameters represented an average of more than 50 plaques per virus. Measurements of the plaque diameters of mutant viruses were always conducted side by side with measurement of the plaque diameter of PRV-GS4284 (the virus encoding wild-type [WT] UL37), and the diameters of the mutant viruses were normalized to that diameter. Single-step growth and plaque diameters were plotted using the Prism software package (GraphPad Software).

[0114] Virion protein incorporation. PK15 cells were infected with either PRV-GS4284 (WT) or PRV-GS604 (R2) at an MOI of 3. Infections were carried out in 15-cm dishes of confluent cells. Infected cells and extracellular media were harvested once all cells displayed a cytopathic effect, which was typically at 18 hpi. Cellular debris was removed by centrifugation at 5,000×g, and virions were concentrated from the supernatant by pelleting through a 10% Nycodenz cushion at 13,000 rpm in an SW28 rotor (Beckman). The resulting pellet was resuspended in 100 μl of TNE buffer (150 mM NaCl, 50 mM Tris [pH 7.4], 10 mM EDTA). Viral particles were dispersed by 10 1-s pulses of sonication in a cup horn ultrasonic processor (VCX-500; Sonics and Materials, Newtown, Conn.). The sample was loaded onto a 12 to 32% dextran gradient and centrifuged at 20,000 rpm for 1 h at 4° C. The heavy viral band was collected and spun at 25,000 rpm in a Beckman SW50.1 rotor at 4° C. for 30 min. The final pellet was resuspended in final sample buffer (10 mMTris [pH 7.4], 150 mMNaCl, 1% Triton X-100) containing 10% 0-mercaptoethanol, and the samples were boiled for 5 min prior to electrophoresis of 5 μl of each sample through an 8% sodium dodecyl sulfate (SDS)-polyacrylamide gel. Proteins were subsequently transferred onto an Immobilon polyvinylidene difluoride membrane (Millipore), and VP5 was detected using the 3C10 mouse monoclonal antibody (a gift of Lynn Enquist) at a 1:1,000 dilution. UL37 was detected using D1789, a rabbit antiserum raised against a peptide derived from the PRV UL37 sequence (REAADRVLGDYHE), at a 1:2,500 dilution. The secondary goat antimouse and antirabbit dye-labeled antibodies (LiCor) were used at 1:5,000 dilutions. Proteins were visualized and quantitated using an Odyssey Fc imager and ImageStudio software (LiCor). The ratio of UL37 to VP5 was quantified for four independent experiments and normalized to the average value obtained for the UL37-to-VP5 ratio for WT virus. Data were plotted using the Prism software package (GraphPad Software), and significance was determined using an unpaired Student's t test.

[0115] Protein expression and purification. Both UL37 and UL37N constructs were expressed as N-terminal His6-SUMO fusions in T7 Express *E. coli* (New England BioLabs). Freshly transformed cells were incubated at 37° C. overnight in 5 ml LB starter culture supplemented with 50 μg/ml kanamycin. The starter culture was diluted into 1 liter LB supplemented with 50 μg/ml kanamycin and grown at 37° C. until the OD600 reached 0.8 to 1.0. At this point, the temperature was shifted to 16° C. and the cells were induced with 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG). For production of UL37N, expression was induced for 16 to 20 h. Cells were harvested by centrifugation at 12,000×g for 40 min, resuspended in 25 ml 20 mM piperazine-N,N=-bis (2-ethanesulfonic acid) (PIPES), pH 7.0, 50 mM NaCl, 0.1% Igepal CA-630 (Sigma), 5% glycerol, 10 mM imidazole, 0.1 mM tris(2-carboxyethyl)phosphine (TCEP), and 1 EDTA free complete protease inhibitor cocktail tablet (Roche), and lysed by use of a French press. The insoluble fraction was removed by centrifugation of the whole-cell lysate at 14,000×g for 30 min at 4° C. Soluble lysate was loaded onto a 5-ml Ni-Sepharose 6B FF column (GE Healthcare). The column was subsequently washed with 10 column volumes (CVs) of 20 mM PIPES, pH 7.0, 50 mM NaCl, 0.1 mM TCEP (buffer A) containing increasing amounts of imidazole at 10 mM or 25 mM. Protein was eluted in buffer A containing 100 mM imidazole. The eluate was immediately concentrated, and the imidazole was removed by buffer exchange into buffer A using an Ultra-15 50-kDa-cutoff concentrator (Millipore). The protein concentration was determined from the absorbance at 280 nm using a calculated extinction coefficient. Glutathione S-transferase (GST)-tagged PreScission protease was added to the protein solution at a 1:50 protease-to-protein ratio, and the protein was cleaved overnight at 4° C. to remove the His6-SUMO tag. The protease-protein solution was sequentially applied to glutathione-Sepharose 4B (GE Healthcare) and Ni-Sepharose 6B to remove the GST-tagged PreScission protease and the His6-SUMO tag, respectively. Cleaved protein was present in the unbound and wash fractions. UL37N was further purified by size exclusion chromatography using a Superdex 200 column (GE Healthcare) and concentrated to 3.5 to 4.0 mg/ml using an Ultra-15 30-kDa-cutoff concentrator (Millipore). Protein purity was assessed by SDS-polyacrylamide gel electrophoresis (PAGE) and Coomassie G-250 staining. The final yield was 18 mg of pure protein per 1 liter of *E. coli* culture. All UL37N protein samples used for crystallization and biochemical studies were stored in 20 mM PIPES, pH 7.0, 50 mM NaCl, and 0.5 mM TCEP.

[0116] A BL21 *E. coli* strain expressing GST-tagged PreScission protease was a gift from Peter Cherepanov (London Research Institute, London, United Kingdom). Protein expression was induced with 0.5 mM IPTG at 30° C. for 4 h before the cells were harvested and lysed. The PreScission protease was purified over glutathione-Sepharose in a buffer containing 20 mM Tris, pH 8.0, 200 mM NaCl, and 1 mM TCEP. The column was washed 3 times with 10 CVs of the binding buffer, and protein was eluted from the column in binding buffer containing 5 mM reduced glutathione. The eluted protein was concentrated in a 30-kDa-cutoff concentrator (Millipore) and further purified over a Superdex 200 size exclusion column equilibrated with the binding buffer. The protein was concentrated to 1 mg/ml, flash frozen, and stored at −80° C.

[0117] Thermofluor assay. The optimal buffer composition and the optimal NaCl concentration for the stability of the UL37N protein (PIPES, pH 7.0, and 50 mM NaCl) were determined using the Thermofluor method (34). Protein was diluted to 0.15 mg/ml in the storage buffer, and a fluorescent dye, SYPRO orange (Invitrogen), was added at a 1:1,000 dilution. Ten microliters of the protein-dye solution was pipetted into each well of a 96-well PCR microplate. Next, 10 μl of buffer (from a custom-made screen containing buffers at pH 4.5 to 10.5 and NaCl concentrations ranging from 0 to 500 mM) was added to wells containing the protein dye solution. The plate was sealed and centrifuged for 1 min at 500×g and 25° C. Samples were analyzed on a Roche LightCycler 480 quantitative PCR machine using an excitation wavelength of 465 nm and detection of emission at 610 nm. The emission signal was analyzed from 25° C. to 95° C. at a continuous acquisition rate of 3 measurements per ° C. Data were analyzed using the ThermoQ software program. Conditions that stabilized UL37N further increased its solubility.

[0118] Mass spectrometry. For mass spectrometry analysis, the UL37 protein was analyzed using sinapinic acid (Agilent Technologies) as the matrix. Mass spectrometry measurements were performed on a Voyager DE-Pro matrix-assisted laser desorption ionization-time of flight mass spectrometer (Applied Biosystems).

[0119] Crystallization and structure determination. Crystals of UL37N were grown by vapor diffusion at room temperature in hanging drops using 1 μl protein and 1 μl well solution containing 24 to 26% polyethylene glycol 1000, 0.3 $MCa(CH_3COO)_2$, and 0.1M imidazole, pH8.0. Large plates formed in 3 to 8 days and were harvested 2 to 4 weeks later. For data collection, crystals were incubated in a solution identical to the well solution plus 10% glycerol for 30 s to 2 min prior to flash freezing in liquid N2. Heavy atom derivative crystals were obtained by soaking native crystals in well solution containing 5 mM thimerosal (Na salt of ethylmercurithiosalicylic acid or $C_9H_9H_9NaO_2S$) for 12 to 16 h. Derivative crystals were harvested and frozen using the protocol developed for the native crystals. X-ray diffraction data were collected at 100 K at the X25 beam line at the National Synchrotron Light Source. The data were processed using HKL2000 (Otwinowski Z, Minor W. 1997. Processing of X-ray diffraction data collected in oscillation mode. Methods Enzymol. 276:307-326) and indexed in space group P21 (Table 1). The native data set was processed up to a 2.0-Å resolution, and the single-wavelength anomalous dispersion (SAD) Hg data set was processed to a 2.3-Å resolution (Table 1). All 12 heavy atom sites were found using the phenix.autosol program, and the experimental density allowed the tracing of ~70% of the residues in the phenix.autobuild program. Additional residues were manually built using the Coot program (Emsley P, Cowtan K. 2004. Coot: model-building tools for molecular graphics. Acta Crystallogr. D Biol. Crystallogr. 60:2126-2132). There are two UL37N molecules in the asymmetric unit.

[0120] Before refinement of the heavy atom model, 10% of the data was set aside for cross-validation. The model was refined against the SAD Hg data set to 2.3-Å resolution using the phenix.refine program. Next, test set flags were transferred to the native data set; additionally, 10% of the native data between 2.3 and 2.0 Å was set aside for cross-validation. After several cycles of refinement in the phenix.refine program (Adams P D, Grosse-Kunstleve R W, Hung L W, Ioerger T R, McCoy A J, Moriarty N W, Read R J, Sacchettini J C, Sauter N K, Terwilliger T C. 2002. PHENIX: building new software for automated crystallographic structure determination. Acta Crystallogr. D Biol. Crystallogr. 58:1948-1954) and rebuilding in Coot (Emsley et al, supra), R work was 17.3% and R free was 22.0%. The final model contained all amino acids from residues 1 to 479, including 3 of the 4 linker residues left after protease cleavage of the N-terminal tag. The final model is missing residues 480 to 496 in both chains. The MolProbity server (Davis I W, Leaver-Fay A, Chen V B, Block J N, Kapral G J, Wang X, Murray L W, Arendall W B, III, Snoeyink J, Richardson J S, Richardson D C. 2007.

[0121] MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. Nucleic Acids Res. 35:W375-W383) was used to assess the stereochemical quality of all models. According to MolProbity, 99.0% of the residues lie in the most favored regions of the Ramachandran plot and 1% lie in the additionally allowed regions of the Ramachandran plot. Final statistics are listed in Table 1.

[0122] Structure analysis. The sequence alignment was generated and analyzed using the ClustalW (Larkin M A, Blackshields G, Brown N P, Chenna R, McGettigan P A, McWilliam H, Valentin F, Wallace I M, Wilm A, Lopez R, Thompson J D, Gibson T J, Higgins D G. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23:2947-2948) and ESPRIPT (Gouet P, Courcelle E, Stuart D I, Metoz F. 1999. ESPript: analysis of multiple sequence alignments in PostScript. Bioinformatics 15:305-308) programs. Interfaces were analyzed using the PISA program (Krissinel E, Henrick K. 2007. Inference of macromolecular assemblies from crystalline state. J. Mol. Biol. 372:774-797). Structural homology searches were performed using the Dali server (Holm L, Rosenstrom P. 2010. Dali server: conservation mapping in 3D. Nucleic Acids Res. 38:W545-W549), and the top hits were superposed onto the UL37N protein using the Dalilite pairwise comparison tool. The Evolutionary Trace server was used for evolutionary trace analysis. All structure figures were made in the PyMOL program.

[0123] Protein structure accession number. Atomic coordinates and structure factors for the UL37N structure have been deposited in the RCSB Protein Data Bank under accession number 4K70.

Results

[0124] Characterization of UL37N. Initially, full-length PRV UL37 was expressed with an N-terminal His6-SUMO tag in *E. coli* (FIG. **1**A). During expression, this protein underwent spontaneous proteolysis, which generated a fragment containing the His6-SUMO tag and the N terminus of UL37 (FIG. **1**B). Using mass spectrometry, the proteolytic site was localized around residue 498, which is approximately in the middle of the UL37 sequence. The difficulty in separating full-length UL37 from the truncated UL37 resulted in a very low yield of the purified full-length UL37, ~200 μg/liter cell culture. Unlike full-length UL37, which was prone to aggregation, the N-terminal product of proteolytic cleavage was readily soluble and was pursued further. A fragment containing residues 1 to 496 of UL37 (UL37N) plus an N-terminal His6-SUMO tag was expressed in *E. coli* (FIG. **1**A), purified to homogeneity, and the His6-SUMO tag was cleaved (FIG. **1**B), obtaining a yield of ~18 to 20 mg per liter of cell culture. The optimal buffer composition and NaCl concentration for protein stability, PIPES, pH7.0, and 50 mM NaCl, were determined using the Thermofluor method. These conditions further increased the solubility of UL37N. All UL37N protein samples used for crystallization and biochemical studies were stored in 20 mM PIPES, pH 7.0, 50 mM NaCl, and 0.5 mM TCEP.

[0125] Architecture of UL37N. The crystal structure of UL37N was determined using single anomalous dispersion and refined against a 2.0-Å native data set (Table 1). There are two monomers in the asymmetric unit, and the final model included residues 1 to 479 plus N-terminal linker residues PGS in both monomers (Table 1). The two monomers adopted very similar conformations, with the root mean square deviation (RMSD) being 0.4 for 482 common

C-α residues (Holm et al., supra). UL37N is an elongated molecule with dimensions of 99 by 42 by 26 Å composed of 24α helices and 6 310 helices arranged into a series of helical bundles (FIG. **1**C). The structure can be divided into three domains: domain I, residues 1 to 184 and 432 to 479; domain II, residues 185 to 295; and domain III, residues 296 to 431 (FIGS. **1**C and E).

[0126] Domain I is formed by two noncontiguous segments of the polypeptide chain, residues 1 to 184 and residues 432 to 479 (FIG. **1**E). Residue 479 is the last resolved residue; no electron density was observed for residues 480 to 496, and they are likely disordered. Domain I consists of five helical hairpins with the up-down topology which are formed by 12 α helices (α 1 to α 10, α 23, and α 24) and 3 310 helices (η1 to η3). Linker residues GS, which precede the start methionine, form the N terminus of helix α 1. Hairpins 1 through 3 form a helical stack (FIG. **1**E). Hairpin 1 consists of two short antiparallel helices, while hairpin 2 consists of two longer kinked helices, and hairpin 3 has two up helices followed by a loop and a single down helix. Helix η 1 connects hairpins 2 and 3. The last two helices, a 23 and a 24, form hairpin 5 (FIG. **1**E). Only the top part of hairpin 5 interacts with hairpins 1 to 3, an arrangement that results in a large U-shaped groove within domain I. Hairpin 4, formed by helix α 10 running antiparallel to helices α 9 and η2, forms a plug in the U-shaped groove in domain I. Helix η3 forms the tip of the plug. At the opposite end of the plug, a solitary helix, α 8, at the tip of a long extension interacts with domain II.

[0127] The Dali structural homology search (Holm et al., supra) revealed that domain I bears a structural resemblance to the helical bundle domains of several subunits of multisubunit tethering complexes. Conserved residue W477 plays a key role in the stability of not only domain I but also the entire UL37N because a shorter construct, UL37N from residues 1 to 476 [UL37N(1 to 476)], which lacks residue W477, has a lower thermal stability and progressively loses secondary structure during storage (FIG. **2**). W477 helps anchor the hairpin 4 plug in domain I through van der Waals interactions with several hydrophobic residues and a hydrogen bond with the carboxyl of D169 (FIG. **1**D), as well as van der Waals interactions with several hydrophobic residues in domain III. Domain II, residues 185 to 295, consists of helices α 11 to α 14 and two 310 helices (η4 and η5) (FIG. **1**E). Helices α 11 to α 13 form a helical bundle, in which the last turn of helix α 12 adopts a π-helix conformation. The putative nuclear export signal (NES), residues 263 to 273 in HSV-2 (Watanabe D, Ushijima Y, Goshima F, Takakuwa H, Tomita Y, Nishiyama Y. 2000. Identification of nuclear export signal in UL37 protein of herpes simplex virus type 2. Biochem. Biophys. Res. Commun. 276:1248-1254), maps to buried helix α 12 (FIG. **1**E) and is unlikely to be functional. Two long loops at the bottom of domain II are well structured (FIG. **1**E) and adopt similar conformations in the two UL37N molecules present within the crystal asymmetric unit. Helix α 14 appears to buttress both loops. Domain II does not have any structural homologs according to the Dali server (Holm et al., supra). Domain III, residues 296 to 431, is composed of helices α 15 to α 22 and one 310 helix (η6) (FIG. **1**E). This domain is also a helical bundle, with the a 19 central helix surrounded by the other six helices. This central helix maintains the structural integrity of domain III and is highly conserved.

[0128] UL37N is a dimer in crystals but a monomer in solution. Two UL37N monomers in the asymmetric unit form an X-shaped dimer (FIG. **3**A) that buries 1,734.8 Å2 of surface area. Four calcium ions are coordinated at the dimer interface as two symmetry related sets of two calcium ions. Each set is coordinated by carboxyl oxygens from the side chains of Asp79, Asp81, and Glu82 of one monomer, carboxyl oxygens from the side chains of Asp382 and Asp383 plus the carbonyl oxygen of Trp379 of the other monomer, and two water molecules (FIG. **3**B). As a result, one calcium ion is hexahedrally coordinated, while the second is pentahedrally coordinated.

[0129] Despite forming a dimer in crystals, UL37N is a monomer in solution. Crystal formation required the presence of at least 0.1M $Ca(CH_3COO)_2$, and the best crystals were obtained in the presence of 0.3 $MCa(CH_3COO)_2$. In solution, UL37N remained monomeric even in the presence of 0.2M $CaCl_2$, judging by its elution volume on size exclusion chromatography (FIG. **3**C). It was concluded that the dimerization of UL37N observed in crystals is likely induced by crystallization conditions (a high protein concentration and the presence of calcium). The coordination of four calcium ions at the dimer interface helps explain the importance of calcium ions in mediating crystal contacts. In the absence of calcium, the buried interface would have been smaller, 1,504.0 Å2 instead of 1,734.8 A2. Although UL37N does not dimerize in solution, the dimerization interface features multiple grooves and several negatively charged patches (FIG. **3**D). In full-length UL37, this area may participate in intramolecular contacts with the C-terminal half of UL37, which is also conserved among alphaherpesviruses and is predicted to be largely α helical.

[0130] ETA reveals several conserved surface dusters within UL37N. To analyze sequence conservation within UL37N, a sequence alignment of 15 UL37 homologs from alphaherpesviruses, a subfamily of herpesviruses that includes HSV and PRV, was generated. Thirty five strictly conserved residues (FIG. **4**) were identified. Most are located within the hydrophobic core and are used for maintaining the structural integrity of the protein, but 11 of these conserved residues are surface exposed (FIGS. **3** and **5**A) and are a logical choice for mutational analysis because surface-exposed conserved residues often participate in protein-protein interactions. None of these, however, clustered in a way that would help pinpoint regions of potential functional importance (FIG. **5**A).

[0131] To locate potentially important functional sites on the surface of UL37N, ETA (43) was performed on the same sequence alignment (FIG. **6**). ETA uses a sequence alignment of homologous proteins to generate a phylogenetic tree, which is then broken up into partitions, with more closely related sequences being grouped into classes. Within each partition, consensus sequences are generated for each set of sequences within a class. Each position within the sequence alignment is designated conserved, class specific, or neutral. Conserved residues have the same residue in all consensus sequences, whereas class-specific residues have a common residue for each closely related subgroup, but that residue is different among more divergent subgroups. Positions lacking consensus among the members of at least one subgroup are considered neutral. Clustering of conserved and class-specific residues on the protein surface may indicate regions of potential functional importance (Lichtarge O, Bourne H R, Cohen F E. 1996. An evolutionary trace method defines binding surfaces common to protein families. J. Mol. Biol. 257: 342-358). This method has been used to detect functional sites in a number of proteins (Sowa M E, He W, Slep K C, Kercher M A, Lichtarge O, Wensel T G. 2001. Prediction and confirmation of a site critical for effector regulation of RGS domain activity. Nat. Struct. Biol. 8:234-237; Chakravarty S, Hutson A M, Estes M K, Prasad B V. 2005. Evolutionary trace residues in noroviruses: importance in receptor binding, antigenicity, virion assembly, and strain diversity. J. Virol. 79:554-568).

[0132] ETA on UL37N revealed several surface clusters which contained both conserved and class-specific residues (FIGS. **5**A and B). Several of these were not considered further because they either contained salt bridges, which are likely essential for protein stability alone, or contained several residues with only partially exposed side chains. Three clusters were chosen for further analysis. To probe their functional roles, 4 to 5 residues within each cluster were mutated to either eliminate a bulky side chain or, in two cases, to replace a small side chain with a bulky one. Three mutants with the following mutations were generated: V249R/R254A/R285A/D287A/H311A (R1 mutant), Q324A/D362A/R365A/H421A/H425A (R2 mutant), and K203A/P204Q/D239A/E240A/D295A (R3 mutant) (FIG. **5**C). Mutated residues define three regions of potential functional importance, referred to as regions 1 through 3 (R1 to R3, respectively). R1 and R3 are located in domain II of UL37N, and R2 is located in domain III of UL37N. Additionally, residues involved in calcium binding were mutated to confirm that potential calcium-induced dimerization of UL37 is not essential for function, generating the mutant D79A/D81A/E82A/D382A/D383A (Ca).

[0133] UL37 region 2 is required for efficient viral spread. All mutations were introduced into the PRV strain Becker background, and each virus was propagated to wild-type titers. To further investigate these mutants, viral replication and spread were measured in single-step growth and plaque formation assays, respectively. The Ca, R1, and R3 mutants did not display any reduction in plaque size or viral replication. In contrast, the R2 mutant plaques were restricted to about half the diameter of virus encoding wild-type UL37 (FIG. **7**A). A defect in plaque formation can be the result of a defect in cell-cell spread or propagation kinetics. To address this question, the rates of cell-associated virus production and virus release into the supernatant were measured. The amount of virus released into the supernatant by the UL37 R2 mutant virus was similar to the amount released by the WT virus, indicating that the R2 mutations cause a defect in cell-cell spread (FIG. **7**B). There was no reduction in the structural incorporation of the UL37 R2 mutant protein relative to that of wild-type UL37 on the basis of immune detection in purified extracellular virions (FIG. **7**C). In fact, the R2 mutant protein was slightly increased in virions relative to the amount of wild-type UL37 (161%; n 4), but this was not statistically significant. It is contemplated that the R2 cluster serves as a binding site for as of yet unidentified cellular or viral proteins important for UL37 function in virus trafficking, which is essential in cell-cell spread.

[0134] UL37 shares structural similarity with subunits of the CATCHR family of tethering complexes. The UL37 proteins have no notable sequence homology to any viral or host proteins.

[0135] A Dali search (Holm et al., supra) revealed that domain I resembles several subunits of eukaryotic multisubunit tethering complexes (MTCs) (Jackson L P, Kummel D, Reinisch K M, Owen D J. 2012. Structures and mechanisms of vesicle coat components and multisubunit tethering complexes. Curr. Opin. Cell Biol. 24:475-483; Brocker C, Engelbrecht-Vandre S, Ungermann C. 2010. Multisubunit tethering complexes and their role in membrane fusion. Curr. Biol. 20: R943-R952). In intracellular trafficking pathways, MTCs tether vesicles to the target organelles to which they localize both to bring the vesicles closer to their target membranes and to help ensure the delivery of the vesicle to the correct target organelle (Brocker et al., supra). Several subunits of four MTCs, the Dsl1 complex, the exocyst complex, the Golgi-associated retrograde protein (GARP) complex, and the conserved oligomeric Golgi protein (COG) complex, share strong structural similarities, despite low sequence identity (Jackson et al., supra; Richardson B C, Smith R D, Ungar D, Nakamura A, Jeffrey P D, Lupashin V V, Hughson F M. 2009. Structural basis for a human glycosylation disorder caused by mutation of the COG4 gene. Proc. Natl. Acad. Sci. U.S.A 106:13329-13334; Tripathi A, Ren Y, Jeffrey P D, Hughson F M. 2009. Structural characterization of Tip20p and Dsl1p, subunits of the Dsl1p vesicle tethering complex. Nat. Struct. Mol. Biol. 16:114-123 Dong G, Hutagalung A H, Fu C, Novick P, Reinisch K M. 2005. The structures of exocyst subunit Exo70p and the Exo84p C-terminal domains reveal a common motif. Nat. Struct. Mol. Biol. 12:1094-1100), a finding which points to their common evolutionary origin and mechanistic similarities. Their structures consist of one to five helical bundle domains of similar folds. UL37N shares the highest structural similarity with several subunits of the Dsl1 complex and the exocyst, with Dali Z-scores of 4.2 to 5.4 (FIG. **8** and Table 2), while similarity to other MTC subunits is less pronounced. Although these similarity scores for the top hits are modest, they are comparable to the scores for some of the more distantly related MTC subunits. The structural similarity to MTC subunits is particularly remarkable because the sequence identity is under 10% (Holm et al., supra).

[0136] Residues 1 to 136 of domain I resemble the helical bundles of MTCs the most and typically align with domain C of MTCs (Jackson et al., supra), but the structural similarity between UL37N and MTCs extends beyond domain I and includes domain II (FIG. **8**A). The domain I and domain II module resembles domains C, D, and E of MTC subunits and has an overall J shape (FIG. **8**A), which is found in some tethering subunits as the result of an additional domain E that follows domains C and D. Although the tip of domain II of UL37N only remotely resembles domain E of MTC subunits, the folds of domain E diverge even among MTC subunits themselves (FIG. **8**A). The Cog4 subunit of the COG tethering complex has a salt bridge between domains D and E that involves a conserved arginine (48). UL37N also has a salt bridge between D216 and R260 in subdomains II-D and II-E, respectively (FIG. **8**C), that is strictly conserved among alphaherpesviruses (FIG. **4**). Despite noticeable similarity, the structure of UL37N differs from the structures of MTC subunits in several aspects. First, instead of multiple helical bundles of similar topology (51), it has only one helical bundle with a topology similar to the topologies found in MTC subunits. Second, unlike in MTC subunits, where domain D or E is C terminal, the polypeptide chain in UL37N continues into domain III and a hairpin in domain I. Thus, the structural resemblance of domains I and II of UL37N to the MTCs may be the result of convergent evolution.

TABLE 1

Data collection and refinement statistics

Parameter	Value for[a]: Native crystal	Native crystal soaked in thimerosal
Data collection		
Space group	$P2_1$	$P2_1$
Unit cell dimensions		
a, b, c (Å)	51.67, 159.59, 67.38	51.53, 156.30, 66.34
α, β, γ (°)	90, 91.33, 90	90, 91.78, 90
Resolution (Å)	43.12-2.00 (2.07-2.00)	48.91-2.05 (2.12-2.05)
R_{sym} or R_{merge}	0.086 (0.516)	0.097 (0.280)
I/σI	20.32 (2.74)	13.87 (2.18)
Completeness (%)	89.4 (49.5)	85.1 (35.31)
Redundancy	6.3 (4.4)	3.9 (2.1)
Refinement statistics		
Resolution range (Å)	43.12-2.00	
No. of reflections (free)	64,342 (2,347)	
R_{work}/R_{free}	17.30/22.01	
No. of atoms	7,983	
Protein	7,342	
Ligand/ion	45	
Water	596	
B-factors[b]	35.05	
Protein	35.03	
Ligand/ion	42.8	
Water	37.5	
RMSD		
Bond length (Å)	0.007	
Bond angle (°)	0.96	

[a] Values in parentheses are for the highest-resolution shell.
[b] B-factor, isotropic displacement parameter.

TABLE 2

Alignments of UL37N with components of MTCs[a]

Protein	Z-score	RMSD	No. of aligned residues	% identity
Tip20	5.4 (3.8)	10.1 (3.8)	186 (92)	4 (14)
Sec6	5.0 (5.1)	3.9 (3.7)	145 (111)	7 (8)
Exo70	4.4 (5.8)	15.7 (3.3)	182 (123)	6 (9)
Dsl1	4.2 (5.7)	11.1 (3.8)	159 (98)	6 (7)
Cog4	3.6 (3.8)	4.7 (4.6)	135 (107)	10 (10)
Sec15	3.2 (4.4)	3.4 (3.5)	72 (97)	4 (4)
Exo84	2.5 (5.4)	4.1 (4.0)	75 (102)	4 (9)

[a] All alignments were carried out using the Dali server (42). Either the entire UL37N or just the helical bundle from residues 1 to 136 (for which the data are given in parentheses) was used in the Dali search.

Example 2

[0137] A Surface-Exposed Region of the UL37 Protein that is Essential for Alphaherpesvirus Neuroinvasion

[0138] Alphaherpesviruses are pathogens that proficiently invade the peripheral nervous system of their host. Although infections are not typically associated with significant symptoms, debilitating diseases including shingles, encephalitis and blindness can arise from the active replication of these viruses coupled with their ability to spread within neural circuits (Levitz R E. Herpes simplex encephalitis: A review. Heart Lung. 1998 May-June; 27(3):209-12; Lichtarge O, Bourne H R, Cohen F E. An evolutionary trace method defines binding surfaces common to protein families. J Mol Biol. 1996 Mar. 29; 257(2):342-58). Unfortunately, the viral factors that contribute to neuroinvasion remain largely unknown. Transport of herpesvirus capsids from the cell periphery to the nucleus is useful for viral replication. In neuronal cells this distance often spans the length of the axon, eliminating passive diffusion as a viable means of delivery. The UL37 protein is a member of a small subset of viral proteins that remain associated with capsids as they travel down the axon towards the nucleus to begin replication (Schmitz J B, Albright A G, Kinchington P R, Jenkins F J. The UL37 protein of herpes simplex virus type 1 is associated with the tegument of purified virions. Virology. 1995 Feb. 1; 206(2):1055-65). Viruses deficient in this protein either fail to propagate or demonstrate delays in transport of capsids to the nucleus prior to replication and in morphogenesis post-replication (Desai P, Sexton G L, McCaffery J M, Person S. A null mutation in the gene encoding the herpes simplex virus type 1 UL37 polypeptide abrogates virus maturation. J Virol. 2001 November; 75(21): 10259-71; Klupp B G, Granzow H, Mundt E, Mettenleiter T C. Pseudorabies virus UL37 gene product is involved in secondary envelopment. J Virol. 2001 October; 75(19): 8927-36; Krautwald M, Fuchs W, Klupp B G, Mettenleiter T C. Translocation of incoming pseudorabies virus capsids to the cell nucleus is delayed in the absence of tegument protein pUL37. J Virol. 2009 April; 83(7):3389-96). This supports an essential role for UL37 in the intracellular transport of capsids during infection. A UL37N crystal structure (UL37N is the amino terminal half of the protein) identified three conserved surface-exposed regions (Pitts J D, Klabis J, Richards A L, Smith G A, Heldwein E E. Crystal structure of the herpesvirus inner segment protein UL37 supports its essential role in control of viral trafficking. J Virol. 2014 May; 88(10):5462-73). The data demonstrated that pseudorabies virus (PRV) mutated in one of these regions, designated R2, is ablated in a critical neuroinvasion property: retrograde axon transport. Although R2 mutant particles fuse with the axon plasma membrane to release the viral capsid, these capsids are defective at initiating long distance retrograde axon transport towards the nucleus to begin genome replication. Despite this dramatic neural defect, the R2 mutant propagates with wild-type kinetics in epithelial cells (Pitts et al., supra). UL37 performs effector functions that are required specifically during neural delivery. The UL37 R2 region is essential for long distance retrograde motion in both in vivo and in vitro systems R2 mutant particles exhibit short non-processive motion in axons The non-processive movement of R2 mutant particles is not the result of retention of envelope or outer tegument proteins following fusion with the cell membrane

[0139] Results are shown in FIGS. **9-12**. FIG. **9** shows characterization of the neuroinvasive properties of the R2 mutant. As shown in FIG. **9**A, the R2 region is essential for virulence in a mouse model of infection. CD-1 mice were infected by intranasal instillation of either wild-type (WT) PRV or PRV carrying mutations in the R1, R2, or R3 regions of the amino terminal portion of the UL37 protein. Mice infected with the R2 mutant did not present any symptoms of infection and were sacrificed at 240 hpi. A total of five mice were infected for each virus tested.

[0140] FIG. **9**B shows that the R2 region is essential for retrograde mediated neuroinvasion. The eye anterior chamber of Long-Evans rats was injected with wild-type (WT) or R2 mutant PRV encoding a fluorescent reporter. In this model, wild-type PRV initially replicates in the iris and ciliary body then invades autonomic nerve endings to spread by retrograde axon transport to the superior cervical ganglion (SCG). By 48 hpi the wild-type PRV was detected in neurons in the SCG, Following infection with the R2 mutant virus was not observed in the SCG, images were taked at 120 hpi. FIG. **9**C shows that the R2 region is dispensable for anterograde spread through neurons. For imaging of anterograde circuits virus was injected into the vitreous humor of the eye of the rat, which exposes the soma of retinal ganglion (RG) neurons to the inoculum. RG neurons project axons to the lateral geniculate nucleus (LGN) and superior colliculus (SC) therefore invasion of the LGN and SC by anterograde transport can be observed during infection. The R2 mutant was detected in both the SC and the LGN at 102 hpi. FIG. **9**D shows

[0141] That the R2 mutant does not travel retrograde down axons upon infection. Dorsal root ganglion (DRG) sensory neurons were isolated from embryonic chickens (embryonic day 8 [E8] to E10). Explants were cultured for three days prior to infection with 3.5×107 PFU/coverslip of both a RFP-tagged R2 mutant (R2) and a GFP-tagged wild-type (WT) virus. Mid segments of axons were imaged during the first hour post infection (hpi). The frequency of axon transport is reported as the average number of capsids entering the field of view per minute (error bars=SEM).

[0142] FIG. **10** shows that R2 mutant particles display aberrant non-processive motion. DRG explants were cultures as described in FIG. **9**. FIG. **10**A shows that mutation of the R2 region significantly increases the severity of stop and reversal events. Explants were infected were infected with 7.0×10^7 PFU/coverslip of either WT or R2 mutant virus and imaged at 3.5 hpi. Kymographs were generated using the Metamorph software package. Entire particle paths, whether moving, stalled, or reversing, were traced within the kymograph using the “Multi-line” tool. Fraction of time stopped and faction of time anterograde were calculated for each particle by dividing the total time the particle was either stopped or moving in the anterograde direction by the total time the particle was imaged. Greater than 30 particles were analyzed per virus for three replicate experiments and an average value calculated for each virus. Values reported represent the mean of the average values obtained. (error bars=SEM). FIG. **9**B shows that aberrant motion of R2 mutant particles does not result in overall movement towards the soma. The average net displacement of all moving virus particles over a 10 second period was calculated. Inset image is a montage of six frames from a subregion of a time-lapse recording of the R2 mutant. Each frame is a 100 ms exposure representing every fifteenth frame of the original recording (the montage represents a 7.5 s time window). Retrograde motion of the particle is indicated with a red arrow while anterograde motion is shown with a white arrow (error bars=SEM).

[0143] FIG. **11** shows that motion of R2 particles does not result from endocytosis of virions or retention of outer tegument proteins following entry. Fusion of extracellular enveloped virions with the plasma membrane results in separation of the viral capsid from the envelope and the majority of tegument proteins. To examine the role of the R2 region in these events a R2 mutant virus was generated with RFP fused to the capsid and GFP fused to either the gD envelope protein or the VP13/14 or VP22 tegument proteins. These “dual-fluorescent” viruses allow for monitoring of capsid entry and tegument disassociation as determined by loss of the GFP signal. DRG explants were cultured as described in FIG. **9**. Explants were infected were infected with 7.0×10^7 PFU/coverslip of the appropriate R2 mutant virus and imaged at 3.5 hpi. FIG. **11**A shows that moving particles are not associated with the gD envelope protein or the outer tegument proteins VP22 and VP13/14. Moving particles were scored as either “Naked capsids”: capsids lacking coincident GFP signal or as being associated with the respective GFP tagged protein gD (envelope), VP13/14, or VP22. Moving particles were defined as those that traveled >2.5 μm. Particles were tallied across two independent experiments with greater then three fields imaged per experiment. The fraction of the total number of moving particles that were positive for either only the RFP signal or both the RFP and GFP signals was calculated (error bars=SEM). FIG. **11**B shows that non-moving particles are predominantly associated with envelope and tegument proteins. As described in panel (A) non-moving particles were scored as either “Naked capsids”: capsids lacking coincident GFP signal or as being associated with the respective GFP tagged protein. Particles were tallied across two independent experiments with greater then three fields imaged per experiment. The fraction of the total number of non-moving particles that were positive for either only the RFP signal or both the RFP and GFP signals was calculated (error bars=SEM).

[0144] FIG. **12** shows modeling of wild-type and the R2 mutant trafficking in neuronal cells. The top view shows that herpesvirus replication occurs in the nucleus, this requires incoming particles to traverse the cytoplasm following entry into the cell. Wild-type virions fuse with the axon plasma membrane, which results in release of the capsid into the cell. Capsids travel along axonal microtubules towards the nucleus within the soma of the neuron (Sodeik B, Ebersold M W, Helenius A. Microtubule-mediated transport of incoming herpes simplex virus 1 capsids to the nucleus. J Cell Biol. 1997 Mar. 10; 136(5):1007-21). The bottom view shows that R2 mutant virions fuse with the plasma membrane of the axon however the released capsids alternate between motion towards (retrograde) and away (anterograde) from the soma. This “non-processive” motion prevents particles from reaching the soma to begin replication.

Example 3

Non-Neuroinvasive Herpesviruses for Vaccine and Oncolytic Vector Applications

[0145] This example describes a conserved feature in herpesviruses that, when mutated, eliminates the neuroinvasive property of the virus. This allows for the production of live-attenuated vaccine strains that lack the neuroinvasive property, thereby preventing the establishment of life-long infections while retaining the ability of the virus to replicate and spread in peripheral tissues to generate a robust sterilizing immune response.

[0146] Three conserved surface regions in the UL37 tegument protein were observed when the three dimensional structure of this protein was determined (Pitts et al., supra).

Mutation of one of these regions (region 2) reduced the capacity of PRV to spread in epithelial cells, but did not impact its replication.

[0147] The important feature of the UL37 R2 mutants described herein is robust infection at the peripheral site of inoculation to produce a robust immune response and immune memory, with no involvement of the nervous system (which prevents establishment of life-long latent infections and subsequent complications).

[0148] Identifying the sites to mutate in related viruses (such as HSV and VZV) was a two step process. First, the crystal structure of PRV UL37 (Example 1) was used as a base model to map out the corresponding amino acids from the other viruses (homology modeling and structure model analysis). Second, the relevance of the positions was confirmed based on primary sequence alignments.

[0149] The mutations that destroy neuroinvasive properties are generally conserved in all alpha herpesviruses, but are unique to each virus (Table 3).

TABLE 3

Mutations to eliminate the virus neuroinvasive properties	
Human pathogens	
Herpes simplex virus types 1 & 2	Q403A/E452A/Q455A/Q511A/R515A
Varicella zoster virus	Q363A/D413A/Q416A/Q472A/R476A
Veterinary pathogens	
Pseudorabies virus	Q324A/D362A/R365A/H421A/H425A

[0150] PRV mutants with the above mutations were engineered (PRV-R2). FIG. **13** shows that PRV R2 lacks neuroinvasive properties. Mice were exposed to either wild type PRV (WT) or the PRV UL37 R2 mutant (R2) by eye injection, and neuroinvasion was assessed by isolating the superior cervical ganglion (SCG) that innervates the iris. Virus activity marked by glowing neurons in the WT infection are absent in the R2 infection.

[0151] FIG. **14** shows that PRV R2 is avirulent. Groups of five mice were infected with either wild-type PRV (WT) or PRV mutated in one of the UL37 surface regions. The region 2 (R2) mutant strain of PRV was avirulent. The mice infected with R2 displayed no symptoms or weight loss during the course of the experiment.

[0152] FIG. **15** shows that PRV R2 is a potent live-attenuated vaccine. Four mice were administered the PRV R2 neuroinvasive mutant on day 1 (x-axis). The weight of the animals in grams (y-axis) was monitored daily. On day 14, the animals received a lethal challenge of wild-type PRV (blue vertical line). The red vertical line indicates the maximum life-expectancy for unvaccinated animals. All four test animals survived to day 35 with only minimal fluctuations in weight.

Example 4

Non-Invasive Herpes Simplex Virus

[0153] A herpes simplex virus type 1 (HSV-1) non-invasive mutant encoding five codon changes in the R2 effector region of the pUL37 tegument protein: Q403A, E452A, Q455A, Q511A, R515A was engineered.

[0154] The HSV-1 pUL37 R2 mutant (HSV1-GS6298) was confirmed unable to enter the peripheral (trigeminal ganglion) and central (brain stem) nervous system of mice following inoculation into the periphery (eye; corneal scarification model) (FIG. **16**). In addition, the R2 mutant also displayed attenuated spread in the cornea.

[0155] The R2 mutagenesis method results in HSV-1 lacking neuroinvasive properties, consistent with our original findings with PRV. This documents that a live-attenuated non-invasive vaccine strain of HSV-1 is useful. Furthermore, the neuroinvasive effector function of R2 is conserved in PRV and HSV-1, which is consistent with R2 functional conservation across the neuroinvasive herpesviruses given that the R2 sequence is conserved. Therefore, live-attenuated non-invasive vaccines of clinically- and agriculturally-relevant herpesvirus are produced using this technology (e.g., varicella-zoster virus, bovine herpesvirus, equine herpesvirus).

Example 5

R2 Mutation Design for Increased Safety

[0156] To simplify the production and stabilize R2 mutants, a new mutation design was developed based on an in-frame deletion and insertion of a 10 aa linker coding sequence (linker sequence: GSGSGSGSGS (SEQ ID NO: 21)). The linker was designed to span the cleft resulting from the deleted R2 region and thereby maintain proper folding of the pUL37 protein, based on predictions made from pUL37 structural data. Mutants of HSV-1 and PRV were produced, and the latter was tested in a vaccine model (FIG. **17**).

[0157] An improved non-invasive design intended to prevent spontaneous reversion of the vaccine strain is able to protect mice from lethal PRV challenge.

[0158] All publications, patents, patent applications and accession numbers mentioned in the above specification are herein incorporated by reference in their entirety. Although the disclosure has been described in connection with specific embodiments, it should be understood that the disclosure as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications and variations of the described compositions and methods of the disclosure will be apparent to those of ordinary skill in the art and are intended to be within the scope of the following claims.

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source                   1..94
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AQHTNWALPL ALAVRQMQNE GLKPLTRALF AHGDAYVREL RPGTVARRLR TDQGVLALLD  240
PGAQAVHVAA AADLDHTQVD ATGVWEAVQA SASPLQVVEA LVTAGFTRRH CDLLERAVLD  300
RAPRLTDAQR AVGCTAVVGG VVHRLLDDYG PGLDYVRAYT DVADTLEPLY GDVTAALGLP  360
EKGVEHVVRH CMAPRPPTEH VGAARAALLR EVAAAERRAG LAHSAAREAL NTWLAFRAQS  420
RWGL                                                               424

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LRATAAFGPV NMQRVVVEWA SLFLEIYARE DAACVGVLGP DPACRSPAGS AAVIRPLLQS  180
RFRLLYDMPF FQAGLSALAH AANWKVPMAA VARRAADAAA PPLARALFAV ALVDEYFPEP  240
DDEDTAPGLA EAFAEIADLV PPEALVPAGE ANAFARSSHD VRVSAALAYR DPFVRGAAAG  300
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VQASATPDQA VETLAAAGFT PGTCAMLERA VLAQLSRPEP RAPADVLQAV GCVAVAGGVL  420
FKLFDAYGPS ADYLAHYTAT IANLHPYYAD VLPLLGLPDG GLEQTIRHCM APRPRTDYVA  480
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GVYMLSNNNL RISAKYGPSN MHLMVSTWLG TFRNVMLSIA NTTPDAMFNA RRIEAIEEFS  180
KPLVHKRFDL IYDMPFVQEG LRIVAAKINW LLPFGLIAKR SKDTSMAPLT RALFLLSLVD  240
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DYAQQVVGCV SIVGGVVFRL LMSYGFGLDY IRDYTTTIST LEPVYNELLL ALGLADKGVE  480
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PGTVTVRLRT DPEILTERHL NLEALLIHVT AIIRLLDSKD ITYEDGSNTI WNYVVECTTN  360
TWEVIQASTN PHQAIEALIQ AGFTSFHCSM LERAISDKFS KARISNINRH SIQRPLLDEA  420
QQAIGCVAMV GSLIFKLVTH YGNGLDYIRH YTTTLADLPL VYGDLLDSLG LPNGSVEQII  480
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KCLFIINLAD EYFDDTPVSY LTDLFNDNII HVKDIAFVPI EEAIVQATTV HGARINAALA  300
HQNLSIRQTQ PGTATHRLRV DVNIWDNNIL SLSAPGIHID GLLHLITTDP TAETTAGAAV  360
AECVRVAWER VQASTSPNSL VLALLEAGFT RYTCKLLRKF VTHCTLGLHS LYDTHITHEV  420
CKLTDFQQTI GCVSLVGGLA YQLLETYAPT AHYVSTYTHI LSETEKRYET LIPALGLPPG  480
GLGQIMRRCF APRPLISSIQ LARKTLVEEI NTAETRKTVL HLQHTRETQP GARVTREAIL  540
TWFDFRMESR WGI                                                     553

SEQ ID NO: 27          moltype = AA  length = 547
FEATURE                Location/Qualifiers
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-continued

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source                 1..547
                       mol_type = protein
                       organism = Herpes virus
SEQUENCE: 27
MEEPICYDTQ KLLDDLSNLK VQEADNERPW SPEKTEIARV KVVKFLRSTQ KIPAKHFIQI  60
WEPLHSNICF VYSNTFLAEA AFTAENLPGL LFWRLDLDWT IEEPGNSLKI LTQLSSVVQD  120
SETLHRLSAN KLRTSSKFGP VSIHFIITDW INMYEVALKD ATTAIESPFT HARIGMLESA  180
IAALTQHKFA IIYDMPFVQE GIRVLTQYAG WLLPFNVMWN QIQNSSLTPL TRALFIICMI  240
DEYLTETPVH SISELFADTV NLIKDEAFVS IEEAVTNPRT VHESRISSAL AYRDPYVFET  300
SPGMLARRLR LDNGIWESNL LSLSTPGIHI EALLHLLNSD PEAETTSGSN VAEHTRGIWE  360
KVQASTSPSM LISTLAESGF TRFSCKLLRR FIAHHTLAGF IHGSVVADEH ITDFQQTLGC  420
LALVGGLAYQ LVETYAPTTE YVLTYTRTVN ETEKRYETLL PALGLPPGGL GQIMRRCFAP  480
RPLIESIQAT RVILLNEISH AEARETTYFK QTHNQSSGAL LPQAGQSAVR EAVLTWFDLR  540
MDSRWGI                                                            547

SEQ ID NO: 28          moltype = AA  length = 545
FEATURE                Location/Qualifiers
source                 1..545
                       mol_type = protein
                       organism = Herpes virus
SEQUENCE: 28
MSAVTTDEIW PLKVLLDTLR SLSSRTSPTE PWGATATAEA RAAIGSFFLA SGTMSILQVE  60
LTWRDTFSAI LEVYKQTRSP EASMLAQNFV GLILWRISVR WDKTSWQENS HRLRRLVAEM  120
TGEEAISWLS RNNLRISAPF GPSVMWPLIS EWFAVFEDAA NHAFTYTPEH LLSEREFSFN  180
VGDLAASLAH KRFELIYDFP FVQEGIRLVS IASGWIAPFV IMYRCTTNRV FTPLTRILFT  240
IALVDQYFRG LHAPQPFQIK DRFAEDVGAL GSKELIPALE ANSTKRTSYE VRASAAIAYE  300
SPFVHTIQPG MAADKLRNGS DIIMSDTSLT EDSLAIHLSA VLRLISDIGL EEDNGAIDAA  360
KAKLSNSARR AWDAIQYSSS PKQLLEALIE RGFVRQVCRA YESALKTYFT RNYGSVDEGD  420
IFDDVQQVVG CVAVIGNVVF GLIESYGPGM TYLSNYMENC VISESDSHFI EALGLERAII  480
SQIIGRCIPP IPHEDYIKAA RAVLVAEMDH VASKSEAVGF RQSIRSAKES LMLWFDNRAN  540
EIWGI                                                              545

SEQ ID NO: 29          moltype = AA  length = 539
FEATURE                Location/Qualifiers
source                 1..539
                       mol_type = protein
                       organism = Herpes virus
SEQUENCE: 29
MSETQGEARF PLKTLLDTLR SLSAGTAPLE PWGNATAAEA RTAIGSFFQA LETMSIQQVE  60
STWRDAFAAV LEVYKQTGSP EAAMLAQNFV GFILWRTSVR WDKMSWKDDS RRLRRLAAEM  120
TGEEAIAWLT RNGLRRSCPF GPSVLWPLIS EWLTIFEEIA TDAFDYTSEG LLSGRQPAPN  180
ALELPASLTQ TRFKLIYDFP FVQEGIRLIS IAVGWITPFV IMSRCTTNRA FTPLTRILFT  240
LALVDQYFKS PRSPHPSQLK DLFAEDASAL GSRELISAVE ANNMKRTAYD VRASAAIAYG  300
DPYVYAVQPG MVAEKLRNGP DIILADHALT EDALAIHMSA VVRLITDGDL NDGGGALDAA  360
KAKLSESARR AWGAVQHSSS PRQLLEALIE RGFVRQACRV YESALKANLG KTRGTVNELD  420
TFDDVQQVIG NIVFGLMESY GPGMTYLTNY MDNGLPPDAD SDFIKVLGLD SAIIAQILGR  480
CIPPNPHEDY VKSARAILAA EMDSAIRQSG AGTANRAIQF AKESLMLWFD SRAEHIWGI   539

SEQ ID NO: 30          moltype = AA  length = 544
FEATURE                Location/Qualifiers
source                 1..544
                       mol_type = protein
                       organism = Herpes virus
SEQUENCE: 30
MSALGKGDNY PLNALLDTLQ TLCAENSPTE PWPVTVISEA RAAIGTFFLS STQMSIQQVE  60
STWRDVFSVI LEVYQRTKSP EAAMLAQNFT GLILWRVSVR WDKTSWRDES IRLRKLVGEM  120
TGEEPITWLS RNNLRVSASF GPNVMGPLIT DWFAEFEDTV TSAVSYTPEC LLSERERIPN  180
VWNLTDSLAH KRFELIYDFP FVQEGIRLIA RTVGWVVPFV ILYRCTTNRA FTPLTRILFT  240
IAFIDQYFRG KGASQHSVLK ERFAEDCNAL GSEELMSASQ ANLTKRTSYE VRASAAIAYG  300
DPFIYGIQPG MVAERLRSGE DIIVSSTSLT EDSLAIHISA VLQLISSDGS DHSTSVIDEA  360
RTKLSESVRR AWDAIQYSSS PKQLLEALID NGFVRQSCQA YESALKTYMA KNYRNSVETI  420
FNDLQQVIGC VAVIGNIVFG LIESYGPGMN YLENYVDGSL PPESDSEFIF ALGLEHGLIS  480
QILGRCIPPD THDDYVKTTR SVLLAEMDLI ARKMDVGGSA RALSSARESL LLWFDHRAEV  540
IWGL                                                               544

SEQ ID NO: 31          moltype = AA  length = 573
FEATURE                Location/Qualifiers
source                 1..573
                       mol_type = protein
                       organism = Herpes virus
SEQUENCE: 31
MDSGDQLSDN EYYDLDEDNT CSDNRSPRPV GRWLLKDMIV ALKEIINTQS TPRWTEVEAS  60
KVKAIVSTFC LSQEQMTIPQ ISHSWKEAFD LLLVAFSNTQ TPEVAIIIEN FTGLVIWRLV  120
VSWDRNTVKA DVTKLMALVR DLTSEHVTQS LTRQNLRLST SYGVSAMRGI LLSWLTTFEA  180
AVTTVLATTP DVLLDSERLG FRKDRVPFTS RYIRIIYDFP FVQEGLRFLH RNANWMIPFK  240
IMTRCASDTI YSPLVRTIYT ISLVDQYFWG AGRSRPKRLV DQFVKDTDLL GDAELMSPGE  300
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ANSTKRTSWE VRLSAALAYQ DPFVREVQPG MASVRVRTSP DMVLRGGPVF GPALCIHSAA  360
VLNVISGSKQ DEFDLGRLNQ AAKTTITEAA RAAWDTIQHS NTPQQVIDAL ISTGFVAQNC  420
RNYEVALTSM YSRATTDNGY ALNDTQQVIG CVSMVGNVVF GLIDSYGRDA DYIDAYAEAM  480
SSLESDSGDF LSAIGLPKGG IEQTIRHCMA PRPITDYIRA ARQALVQEIE TASSIYKGRL  540
SSRLQTHHTS THNSVRGSLL LWFDFRAKQI WGI                                573

SEQ ID NO: 32          moltype = AA  length = 636
FEATURE                Location/Qualifiers
source                 1..636
                       mol_type = protein
                       organism = Herpes virus
SEQUENCE: 32
MVSPTPTPPP KEGRAATPPK EGRAATPPRD DRAPPVPKDN TAATPSDNAR TTPSTKEDGA  60
AAPPPAAPPG DGRAPSPSGN SRPGHPTDGP LQSLLGALAS LAAAGPASPA ETPRDADEDS  120
VLLAAKLRAA IAAFLLSTAP IRVVDARTHW RPLLERLCDL HGAHGLPETA LLAENLPGLL  180
AHRLAVALPD DPERAFEAMD DLKAGVLATT SPEATRLLEA AGLRTAAALG PARTRQCVTE  240
WTDRWRSVSE SCLRLDPRAA SGAPADASPP VSPIPLGQPG AGLTTPAYST IFPAPFVQEG  300
LRFLARASNW ATLFSTHLQR VDDATLTPLT RALFTLALVD EYLTTRDRGI VAPPRLLEQF  360
EHTVREIDPA IMIPPIEANK MVRTREEVRV SAALNHLTPR SARAPPGTLM TRVRTDAAVF  420
DPEEPLLSSS ALAIFQPAVA ALLGSGEPPS AGAQRRLLAL LHQTWALIQN TGSPSVVINA  480
LIDAGFTPLH CSHYLSALEG FLATGGASRG LAGPGLSEIQ QLFGCIALTG ANVFALAREY  540
GYHSGYVRAF RRIQDACEKA HARLCEAAGL TGGVLSQTLA RVMGPVTPTE HLASLRRALV  600
GEFESAERRF GAGRASPLRE TVLIWVDVYG QTEWDI                             636

SEQ ID NO: 33          moltype = AA  length = 655
FEATURE                Location/Qualifiers
source                 1..655
                       mol_type = protein
                       organism = Herpes virus
SEQUENCE: 33
MVSPTPTPPT EENRSRPAPP PKEARGSAAT SPKETRSRTT PPPKEARGSA ATSPENVRTA  60
PAPGDTRAAA PPTPEETRAP PPPATPPEDV RAATPSGDAR LGPPPDGPLQ SLLGALTSLA  120
TARPAPPTEA SGEAGEDAVL LAARLRAAIA AFLLSGAPIR VADARTHWRP LLERLCALHG  180
AHGLPETALL AENLPGLLAH RLAVALPDAP DRAFEAMDHL RAAVLDAASP EATRLLEAAG  240
LRTAAALGPA RTRQCVAEWT DRWRSVTESC LRLDPRASSA APGGADPPVS PVPLGQPSAG  300
LATPAYSPIF PAPFVQEGLR FLARASNWAT LFSTHLQSVD DATLTPLTRA LFTLSLVDEY  360
LTTRDRGIVA PPRLLEQFER TVREIDPAIM IPPIEANKMV RSREEVRVSA ALNHLTPRSA  420
RAPPGTLMSR VRTDAAVFDP EEPFLSASAL AIFQPAVAAL LGSGEPPSAG AQRRLLALLH  480
QTWALIQNTG SPSVVINALI DAGFTPLHCS HYLSALEGFL AAGGAARGLA GPPALSEVQQ  540
LFGCVALTGA NVFALAREYG YHSGYVRAFR RVQDACEQAH ARLCEAAGLA GGVLSQTLAR  600
VMGPVTPTEH LASLRRALVG EFESAERRFG AGRPSPLRET VLIWIDVYGQ TEWDI        655

SEQ ID NO: 34          moltype = AA  length = 570
FEATURE                Location/Qualifiers
source                 1..570
                       mol_type = protein
                       organism = Herpes virus
SEQUENCE: 34
MSDSALQVPA PAGMTPPSAP PPNGPLQVLL GSLTNLRRPP SPSSEPAGSA DEPAFLSAAK  60
LHAATAAFLL SGAAVGPAEA RACWHPLLEQ LCALHRAHGL PETALLAENL PGLLVHRMAV  120
ALPETPEAAF REMDVIKDTV LAITGSDTTH ALEAAGLRTT AALGPVRVRQ CAVEWIDRWR  180
TVTQSCLAMN PRTSLEALGE MSLKMSPVPL GQPGANLTTP AYSLLFPSPI VQEGLRFLAL  240
VSNWVTLFSA HLQRIDDAAL TPLTRALFTL ALVDDYLTTP DRGAVVPPPL LAQFQHTVRE  300
IDPAIMIPPL EATKMVRSRE EVRVSTALSR VSPRSACAPP GTLMARVRTD AAVFDPDVPF  360
LSASALAIFR PAVTGLLQLG EPPSAGAQQR LLALLQQTWA LVQNSNSPSV VINTLTDAGF  420
TPAHCTQYIS ALEGFLVAGV PARTPPGHGL SEIQQLFGCI ALAGANVFGL AREYGHYAGY  480
VKTFRRIQGA SEHTHGRLCE AVGLSGGVLS QTLARIMGPA VPTEHLASLR RTLVGEFETA  540
ERRFSAGQPS LLRETALIWL DVYGQTHWDL                                    570

SEQ ID NO: 35          moltype = AA  length = 570
FEATURE                Location/Qualifiers
source                 1..570
                       mol_type = protein
                       organism = Herpes virus
SEQUENCE: 35
MADRGLPSEA PVVTTSPAGP PSDGPMQRLL ASLAGLRQPP TPTAETANGA DDPAFLATAK  60
LRAAMAAFLL SGTAIAPADA RDCWRPLLEH LCALHRAHGL PETALLAENL PGLLVHRLVV  120
ALPEAPDQAF REMEVIKDTI LAVTGSDTSH ALDSAGLRTA AALGPVRVRQ CAVEWIDRWQ  180
TVTKSCLAMS PRTSIEALGE TSLKMAPVPL GQPSANLTTP AYSLLFPAPF VQEGLRFLAL  240
VSNRVTLFSA HLQRIDDATL TPLTRALFTL ALVDEYLTTP ERGAVVPPPL LAQFQHTVRE  300
IDPAIMIPPL EANKMVRSRE EVRVSTALSR VSPRSACAPP GTLMARVRTD VAVFDPDVPF  360
LSSSALAVFQ PAVSSLLQLG EQPSAGAQQR LLALLQQTWT LIQNTNSPSV VINTLIDAGF  420
TPSHCTHYLS ALEGFLAAGV PARTPTGHGL GEVQQLFGCI ALAGSNVFGL AREYGYYANY  480
VKTFRRVQGA SEHTHGRLCE AVGLSGGVLS QTLARIMGPA VPTEHLASLR RALVGEFETA  540
ERRFSSGQPS LLRETALIWI DVYGQTHWDI                                    570
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SEQ ID NO: 36          moltype = AA  length = 612
FEATURE                Location/Qualifiers
source                 1..612
                       mol_type = protein
                       organism = Herpesvirus saimiri
SEQUENCE: 36
MTQATRTRVP VEWHELIAAA ENDLREHAPA PSHVGSIWNL VDTLEPLATQ LREMSRAATA  60
AAPPRLSPGA AGRKLVGHGS AYPPEQTFLL VARLRAAFAS FLLAPTAAAP EHVRSGWPRL  120
ISLLCELHRG LSLTETALLL ENLPGLAVHH IDVAVPRDRA GACRDMSAVI ACVRKMAGPE  180
TVDALEELGL RTSSPLGPIS TQRNVLDWVQ RWLAVTKSMH EADPRESADF SSAPPLKNLA  240
TLPLGQPGAG LAAPKYHLIF GAPFVQRGLR HLAEVGNRVC VVGAYLRRAD DAALTPLARA  300
LFTLALVDEH VPSGGGVPSL LVQRFRRDVA LVDPTIMIPP LEANPMPRTR GEVRISSALS  360
TRTPGVTCAP PGTLITRVRT DSDVFGTHPE HVSASALAVF QPAVSSLLQA GETEATPEVR  420
QRMLGLLHET WARLQNTTSA DVALATLVDA GFTPANCAAY LSALEGFLAS GHLVASADSG  480
EKDARGLDGR ELGEIQQLFG CISILGRGIF QLAREYGPHA EYVKTFKRIQ AACEQRHAQL  540
SHAAGLSQGV LGQALARIMS PTTPTEHLAA LRRALVDEFE VAERRFNEGH PSLLREPVMA  600
WVDIYGQTAW DV                                                     612
```

1. A variant herpesvirus or alphaherpes virus particle comprising at least two mutations in a UL37 protein comprising a polypeptide having one of SEQ ID NOs: 22-27, 29-33, and 36 or a polypeptide having at least 90% identify to one of SEQ ID NOs: 22-27, 29-33, and 36, wherein the mutations are at positions selected from the group consisting of Q269, D307, R310, H366, and H370 in SEQ ID NO: 22 or at the corresponding positions in one of SEQ ID NOs: 23-27, 29-33, and 36, wherein said virus particle exhibits reduced neuro-invasiveness relative to a virus particle lacking said mutation, and wherein the ability of the virus particle to replicate in peripheral tissues is retained.

2. The virus particle of claim **1**, wherein said virus particle is selected from the group consisting of pseudorabies virus (PRV), varicella-zoster virus (VZV), bovine herpesvirus 1 (BHV-1), bovine herpes virus 5 (BHV-5); equine herpes virus 1 (EHV-1); equine herpes virus 3 (EHV-3); and equine herpes virus 4 (EHV-4).

3. (canceled)

4. (canceled)

5. The virus particle of claim **1**, wherein the UL37 protein of said virus has at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 22-27, 29-33 and 36.

6. (canceled)

7. (canceled)

8. (canceled)

9. The virus particle of claim **1**, wherein said two or more mutations is two mutations.

10. The virus particle of claim **1**, wherein said two or more mutations is three or more mutations.

11. The virus particle of claim **1**, wherein said two or more mutations is four or more mutations.

12. The virus particle of claim **1**, wherein said two or more mutations is all five of said mutations.

13. (canceled)

14. The virus particle of claim **1**, wherein said virus is avirulent.

15. A nucleic acid encoding the virus particle of claim **1**.

16. A vector comprising the nucleic acid of claim **15**.

17. A pharmaceutical composition comprising the virus particle of claim **1**, a nucleic acid encoding the virus particle, or a vector comprising the nucleic acid encoding the virus particle and a pharmaceutically acceptable carrier.

18. The pharmaceutical composition of claim **17**, wherein said composition further comprises an adjuvant.

19. A vaccine composition comprising the virus particle of claim **1**, h nucleic acid encoding the virus particle, or a vector comprising the nucleic acid encoding the virus particle and a pharmaceutically acceptable carrier.

20. The vaccine of claim **19**, wherein said composition further comprises an adjuvant.

21. A method of inducing an immune response against a virus, comprising: administering the composition of claim **17** to a subject under conditions such that said subject generates an immune response to said virus.

22. (canceled)

23. The method of claim **21**, wherein said subject is human.

24. The method of claim **21**, wherein said subject is a non-human animal

25. (canceled)

26. (canceled)

27. (canceled)

28. (canceled)

29. A method of preventing infection by a virus, comprising: administering the composition claim **17** to a subject under conditions such that said subject generates an immune response to said virus and is immune to infection by said virus.

30. (canceled)

31. A method of treating or preventing cancer, comprising: administering the composition of claim **17** to a subject diagnosed with cancer under conditions such that said cancer is reduced or eliminated.

32. (canceled)

* * * * *