US 20230285591A1

(19) **United States**
(12) **Patent Application Publication**
LEE et al.

(10) **Pub. No.: US 2023/0285591 A1**
(43) **Pub. Date: Sep. 14, 2023**

(54) **NIPAH VIRUS ENVELOPE PSEUDOTYPED LENTIVIRUSES AND METHODS OF THEIR USE**

(71) Applicant: **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA**, OAKLAND, CA (US)

(72) Inventors: **Benhur LEE**, New York, NY (US); **Karina PALOMARES**, Los Angeles, CA (US); **Olivier PERNET**, Los Angeles, CA (US)

(73) Assignee: **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA**, OAKLAND, CA (US)

(21) Appl. No.: **18/151,174**

(22) Filed: **Jan. 6, 2023**

Related U.S. Application Data

(63) Continuation of application No. 16/120,055, filed on Aug. 31, 2018, now Pat. No. 11,576,982, which is a continuation of application No. 15/330,826, filed on Nov. 7, 2016, now Pat. No. 10,064,958, which is a continuation of application No. 14/387,371, filed as application No. PCT/US2013/032197 on Mar. 15, 2013, now Pat. No. 9,486,539.

(60) Provisional application No. 61/615,534, filed on Mar. 26, 2012.

Publication Classification

(51) **Int. Cl.**

A61K 48/00	(2006.01)
A61K 39/12	(2006.01)
C12N 7/00	(2006.01)
C12N 15/86	(2006.01)

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

CPC ***A61K 48/0008*** (2013.01); ***A61K 39/12*** (2013.01); ***A61K 48/00*** (2013.01); ***A61K 48/0075*** (2013.01); ***C12N 7/00*** (2013.01); ***C12N 15/86*** (2013.01); *C12N 2740/15021* (2013.01); *C12N 2740/15032* (2013.01); *C12N 2740/15041* (2013.01); *C12N 2740/15043* (2013.01); *C12N 2740/15071* (2013.01); *C12N 2760/18222* (2013.01); *C12N 2760/18232* (2013.01); *C12N 2760/18234* (2013.01); *C12N 2760/18241* (2013.01); *C12N 2760/18245* (2013.01); *C12N 2760/18271* (2013.01); *C12N 2810/6027* (2013.01); *C12N 2810/6072* (2013.01)

(57) **ABSTRACT**

The present invention realtes to lentiviral particles which have been pseudotyped with Nipah virus (NiV) fusion (F) and attachment (G) glycoproteins (NiVpp-F/G). Additionally, the present invention relates to truncated NiV-F glyocproteins useful in producing such NiVpp lentiviral particles, as well as to additional variant peptides which enhance activity. Further, the present invention relates to methods of using such lentiviral particles or sequences, for example in the treatetment of cancer or CNS disorders.

Specification includes a Sequence Listing.

NiV-F (519-546 of SEQ ID NO: 1, plus AU tag (DTYRYI); 519-552 of SEQ ID NO: 2):	EKKRNTYSRLEDRRVRPTSSGDLYYIGTDTYRYI
NiV-F T234 (deletion of 525-546 of SEQ ID NO:1; plus AU tag(DTYRYI)):	EKKRNT.....DTYRYI
NiV-G (1-46 of SEQ ID NO: 10):	MGPAENKKVRFENTTSDKGKIPSKVIKSYYGTMDIKKINEGLLDSK
NiV-G Stop:	S.....MDIKKINEGLLDSK
NiV-G Δ5 (1-41 of SEQ ID NO: 13):	MG.....KVRFENTTSDKGKIPSKVIKSYYGTMDIKKINEGLLDSK
NiV-G Δ10 (1-36 of SEQ ID NO: 15):	MG.....NTTSDKGKIPSKVIKSYYGTMDIKKINEGLLDSK
NiV-G Δ15 (1-31 of SEQ ID NO: 17):	MG.....KGKIPSKVIKSYYGTMDIKKINEGLLDSK
NiV-G Δ20 (1-26 of SEQ ID NO: 19):	MG.....SKVIKSYYGTMDIKKINEGLLDSK
NiV-G Δ25 (1-21 of SEQ ID NO: 21):	MG.....SYYGTMDIKKINEGLLDSK
NiV-G Δ30 (1-16 of SEQ ID NO: 23):	.....GTMDIKKINEGLLDSK

FIG. 1

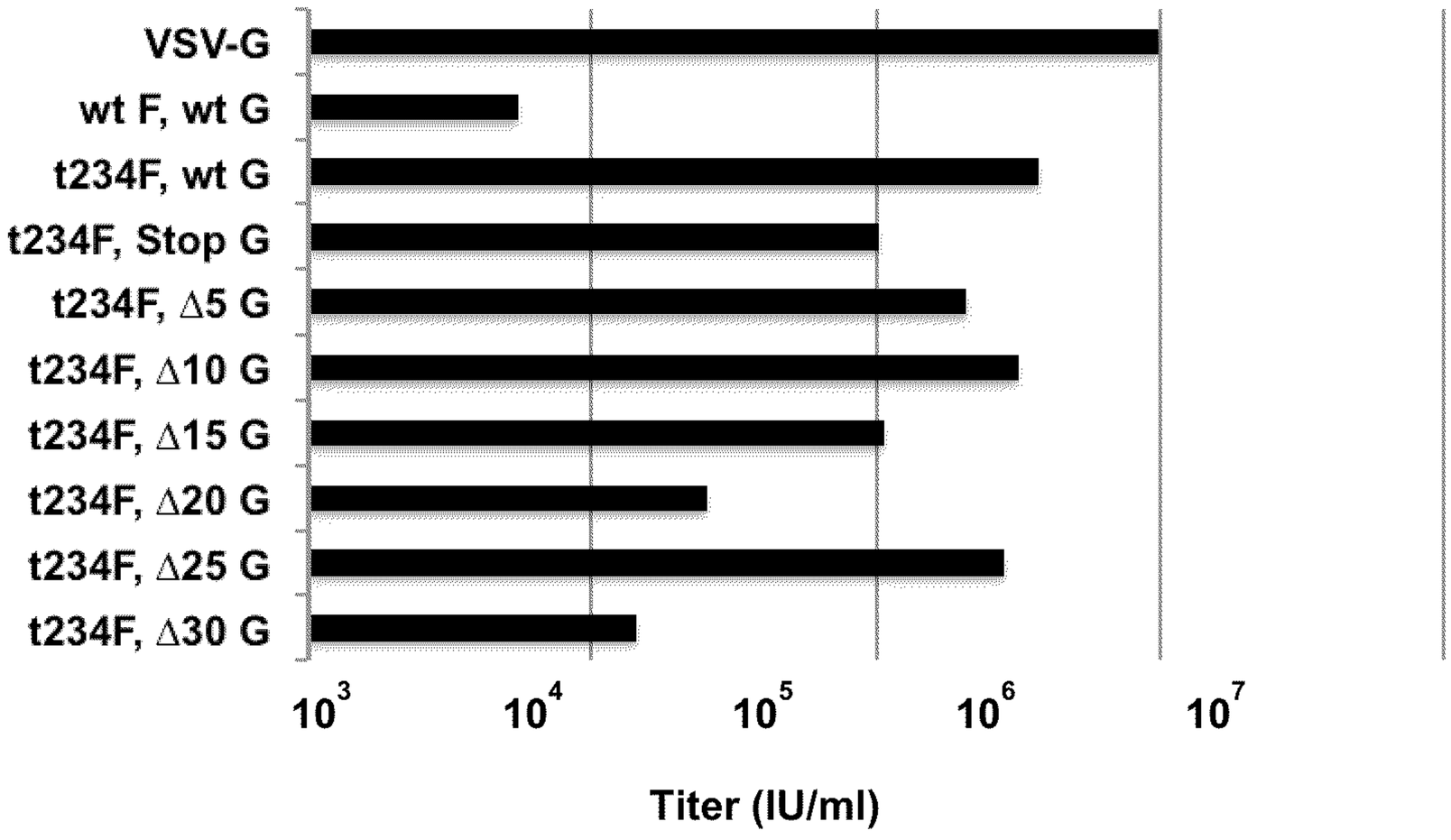
VSV-G
wt F, wt G
t234F, wt G
t234F, Stop G
t234F, Δ5 G
t234F, Δ10 G
t234F, Δ15 G
t234F, Δ20 G
t234F, Δ25 G
t234F, Δ30 G
10^3
10^4
10^5
10^6
10^7
Titer (IU/ml)

FIG. 2

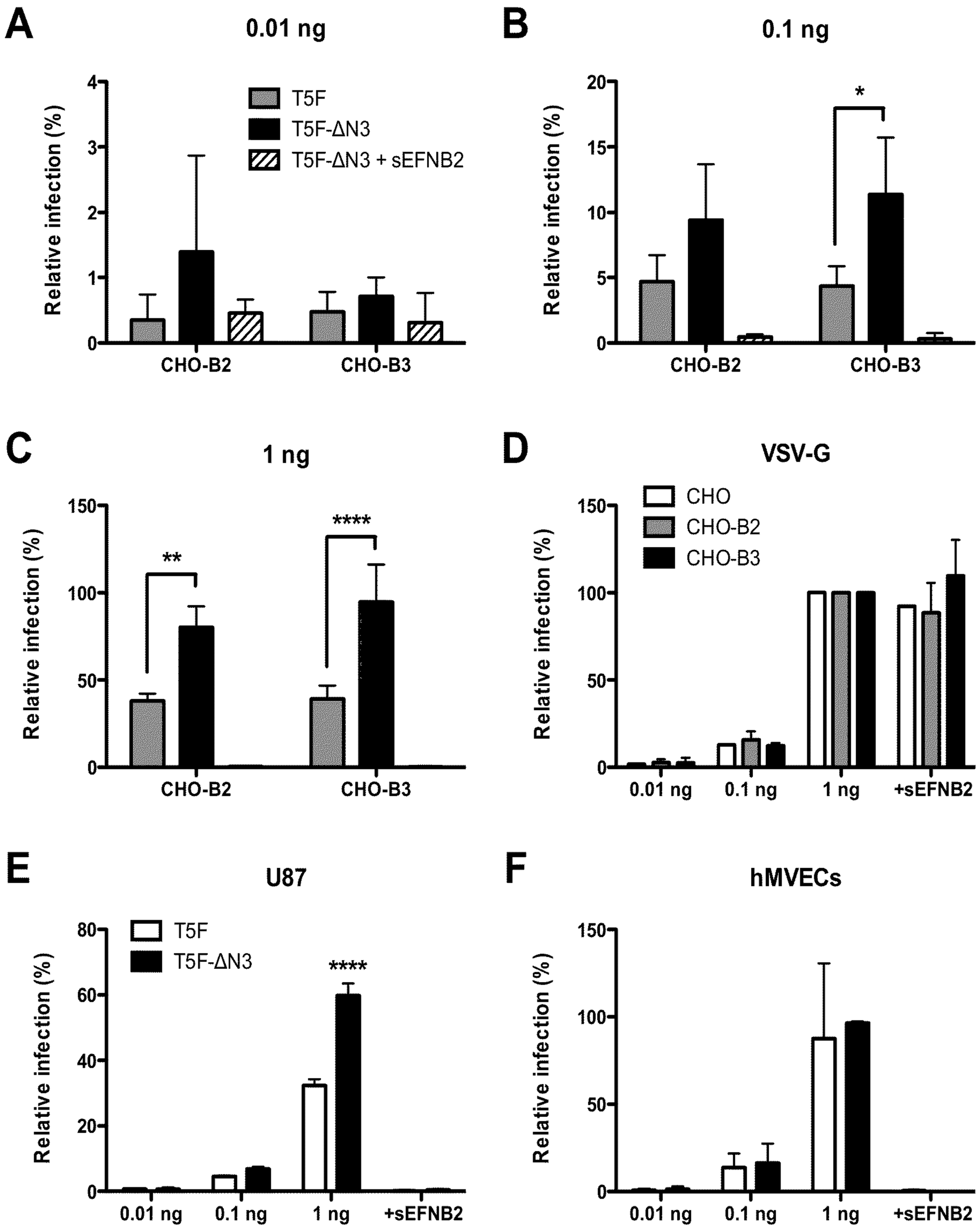
A
0.01 ng
Relative infection (%)
T5F
T5F-ΔN3
T5F-ΔN3 + sEFNB2
CHO-B2
CHO-B3
B
0.1 ng
*
C
1 ng
**
****
D
VSV-G
CHO
CHO-B2
CHO-B3
0.01 ng
0.1 ng
1 ng
+sEFNB2
E
U87
F
hMVECs

FIG. 3

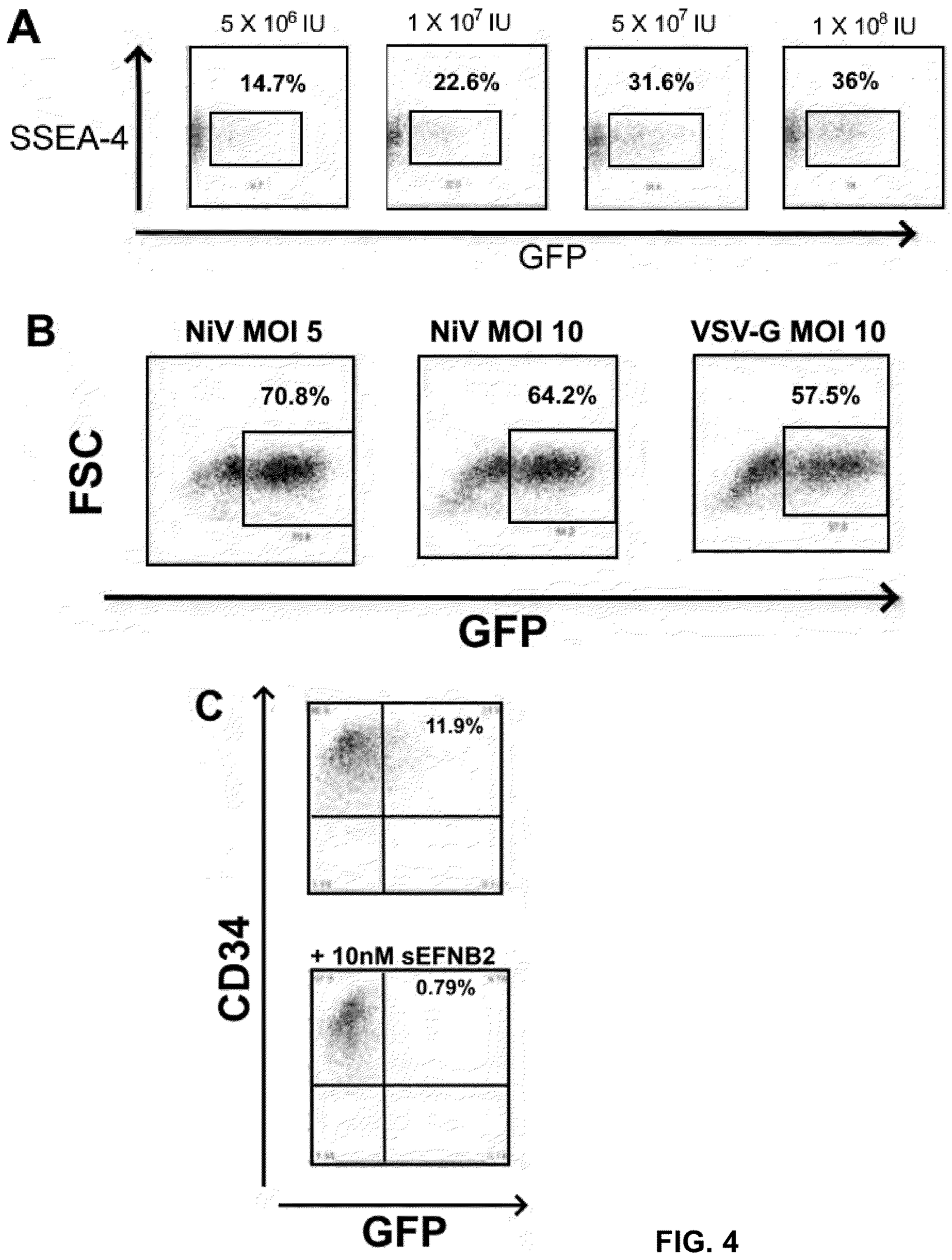
A
5 X 10^6 IU
1 X 10^7 IU
5 X 10^7 IU
1 X 10^8 IU
14.7%
22.6%
31.6%
36%
SSEA-4
GFP
B
NiV MOI 5
NiV MOI 10
VSV-G MOI 10
70.8%
64.2%
57.5%
FSC
GFP
C
11.9%
+ 10nM sEFNB2
0.79%
CD34
GFP

FIG. 4

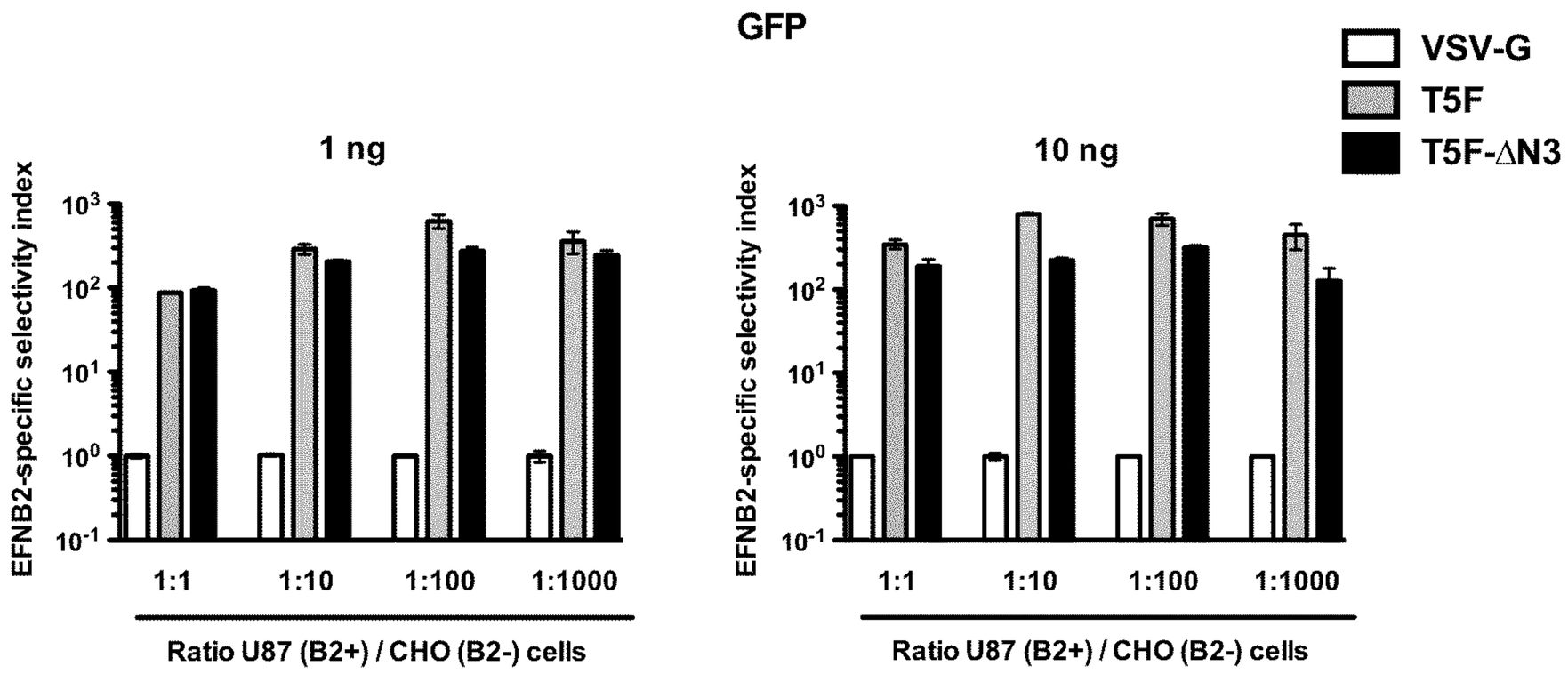
GFP
VSV-G
T5F
T5F-ΔN3
1 ng
10 ng
EFNB2-specific selectivity index
10^3
10^2
10^1
10^0
10^{-1}
1:1
1:10
1:100
1:1000
Ratio U87 (B2+) / CHO (B2-) cells

FIG. 5

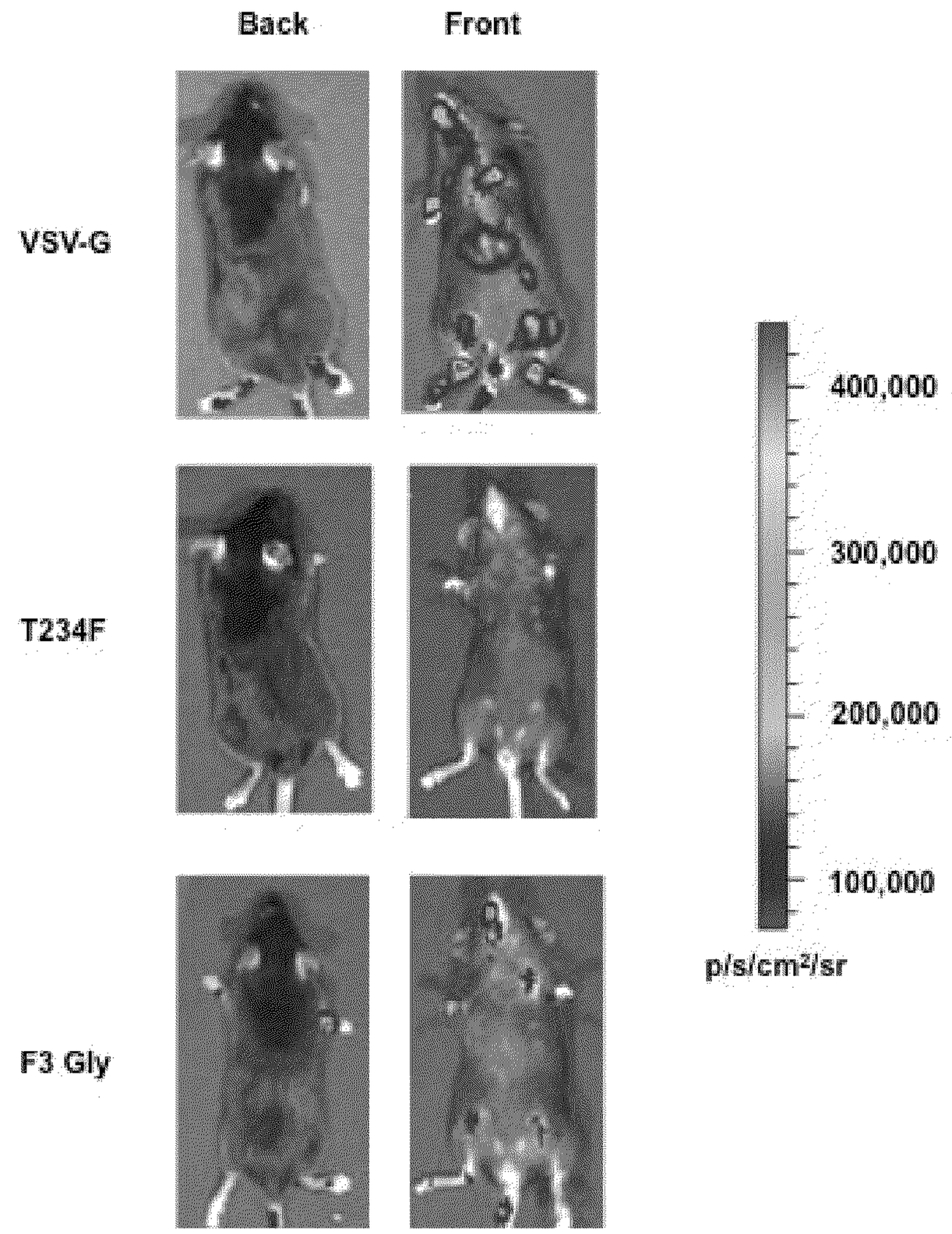
Back
Front
VSV-G
T234F
F3 Gly
400,000
300,000
200,000
100,000
p/s/cm²/sr

FIG. 6

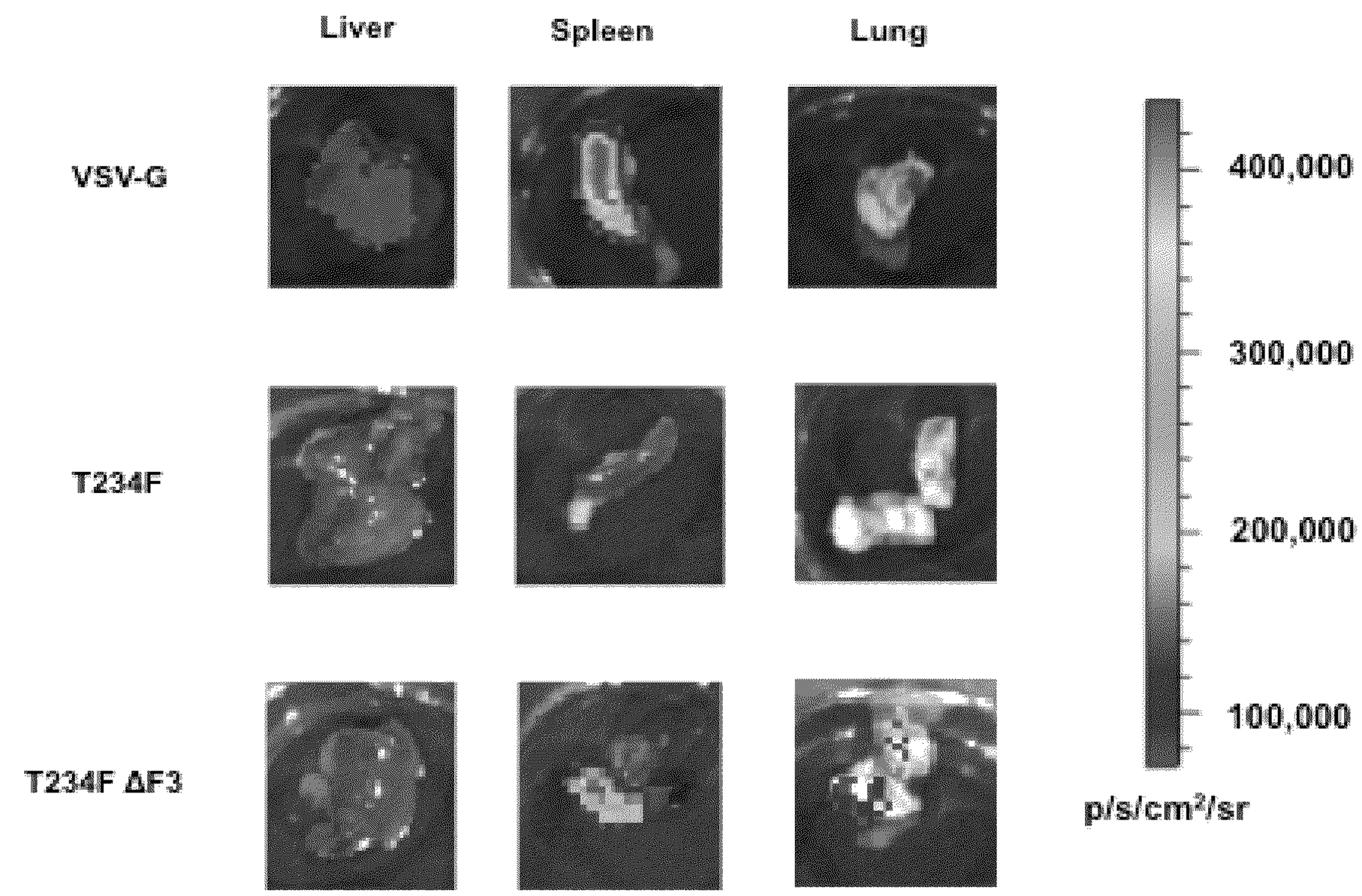
Liver
Spleen
Lung
VSV-G
T234F
T234F ΔF3
400,000
300,000
200,000
100,000
p/s/cm²/sr

FIG. 7

NIPAH VIRUS ENVELOPE PSEUDOTYPED LENTIVIRUSES AND METHODS OF THEIR USE

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0001] This invention was made with Government support under AI069317 awarded by the National Institutes of Health. The Government has certain rights in the invention.

CROSS REFERENCE TO RELATED APPLICATIONS

[0002] This application is a continuation of U.S. Application Serial No. 16/120,055, filed on Aug. 31, 2018, which is a continuation of U.S. Application Serial No. 15/330,826, filed on Nov. 7, 2016, now U.S. Pat. No. 10,064,958, which is a continuation of U.S. Application Serial No. 14/387,371, filed on Sept. 23, 2014, now U.S. Pat. No. 9,486,539, which is a 35 U.S.C. 371 national stage filing of PCT/US13/32197, filed on Mar. 15, 2013, which claims the benefit of U.S. Provisional Pat. Application No. 61/615,534, filed on Mar. 26, 2012, the entire contents of which are incorporated herein by reference.

REFERENCE TO A SEQUENCE LISTING

[0003] The content of the XML file of the sequence listing named "UCLA234_Seq", which is 56 kb in size, created on Jan. 6, 2023, and electronically submitted herewith the application, is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0004] Lentiviruses are common vectors used in gene therapy because they can transduce non-dividing cells and offer stable integration into a target cell's genome. The host range of lentivirus vectors can be altered by pseudotyping with glycoproteins derived from enveloped viruses. Current gene therapy typically employs lentiviral vectors pseudotyped with the VSV-G envelope proetin (VSV-Gpp), which has a ubiquitous host cell receptor, thereby allowing transduction of most cell types. However, VSV-G itself is known to be cytotoxic and the envelope cytotoxicity limits the amount of VSV-Gpp that can be concentrated and used for cell transduction. That is, while VSV-G envelope has great stability in the vector particle, and can be concentrated to high titers via ultracentrifugation, the toxicity of VSV-G itself limits the viral titer that can be used as too high a concentration of VSV-Gpp applied to the target cell population results in apoptotic cell death. In addition, because it has a ubiquitous host cell receptor, VSV-Gpp canot be targeted to specific populations of cells. Additionally, when VSV-Gpp is administered intravenously to mice, the majority is trapped in the liver, sometimes termed the "liver sink" effect, which is detrimental to the gene therapy unless the desired target cells reside in the liver.

[0005] To overcome these shortcomings of VSV-Gpp, other strategies have been devised for targeted lentiviral gene therapy. One common strategy involves pseudotyping lentiviral vectors with a modified Sindbis virus envelope that has been mutated to remove its own receptor binding site and engineered to dsiplay a "ZZ" motif from proteinA - a motif that binds to the Fc region of most antibodies. Incubation of the Sindbis-ZZ pseudotyped vectors with a specific monoclonal antibody theoretically should target the lentiviral particles to the cell-type in question. (See Morizono K et al., 2005, Nat Med Vol 11 (3):346-52). However, while the technique works well in vitro, in vivo the majority of the intravenously administered Sindbis-ZZ pseudoptyped vector is still trapped in the liver, regardless of the antibody used. As such, improved methods of overcoming the shortcomings of VSV-Gpp are still needed.

[0006] Nipah virus (NiV) is an emerging paramyxovirus that causes acute fatal encephalitis. Two envelope glycoproteins (the fusion and attachment glycoproteins) mediate cellular entry of Nipah virus. The attachment protein, NiV-G, functions in recognition of the receptor (EphrinB2 and EphrinB3). Binding of the receptor to NiV-G triggers a series of conformational changes that eventually lead to the triggering of NiV-F, which exposes the fusion peptide of NiV-F, allowing another series of conformational changes that lead to virus-cell membrane fusion. EphrinB2 was previously identified as the primary NiV receptor (Negrete et al., 2005), as well as ephrinB3 as an alternate receptor (Negrete et al., 2006). In fact, NiV-G has an extremely high affinity for ephrinB2 and B3, with affinity binding constants (Kd) in the picomolar range (Negrete et al., 2006) (Kd=0.06 nM and 0.58 nM for cell surface expressed ephrinB2 and B3, respectively). Significantly, residues important for ephrinB2/B3 interactions with their endogenous ephB receptors are also critical for their activity as NiV receptors, indicating that the NiV attachment glycoprotein (NiV-G) can block endogenous ephrinB2-ephB4 receptor interactions.

[0007] Ephrin receptor-ligand pairs (Eph-ephrin) are membrane associated receptor tyrosine kinases (RTKs) with well-established roles in development; they regulate cell boundaries during tissue formation, and provide guidance cues during neurogenesis and angiogenesis. (See Pasquale EB. Eph-ephrin bidirectional signaling in physiology and disease. *Cell*. 2008;133:38-52.) Cognate interactions activate both the Eph receptor (forward signaling) and ephrin ligand (reverse signaling) on opposing cells. These bi-directional signaling cascades result in cell-cell repulsion or attraction, depending on cell type or other microenvironmental cues.

[0008] EphrinB-ephB receptor-ligand interactions are a common regulator of multiple somatic stem cells, e.g., intestinal crypt stem cells and hematopoietic stem cells (Pasquale (2008) Cell 133:38-52; Poliakov et al. (2004) Dev. Cell. 7:465-480), where differentiation is a carefully choreographed molecular and cellular response to local environmental determinants. EphrinB2, in particular, has been identified as a molecular stem cell signature common to human embryonic, neural, and hematopoietic stem cells (hESC, hNSC and hHSC) (Ivanova et al. (2002) Science 298:601-604). Its cognate receptor, EphB4, has also been shown to affect mouse ESC fate. Despite much evidence from model systems that ephrinB2/ephB4 axis may be intimately involved in ESC fate (survival, self-renewal, and pluripotency), this particular axis has not been carefully studied in human ESC.

[0009] In mouse ESC, ephB4 inactivation results in bias against differentiation: ephB4-deficient mouse ESCs appear to remain in a more primitive state and are impaired in embryoid body (EB) formation in general and mesodermal

differentiation in particular. (Wang et al. (2004) Blood 103:100-109)). Conversely, over expression of ephB4 in umbilical cord blood CD34+ cells results in a loss of the most primitive progenitors (LTC-ICs and CD34+/CD38- cells) likely due to differentiation into more committed precursors. (Wang et al. (2002) Blood 99:2740-2747)). EphrinB-ephB ligand-receptor interactions are promiscuous, and the lack of highly specific yet versatile reagents to interrogate this axis has hampered the understanding of ephrinB2/ephB4′s role in hESC fate (pluirpotency, survival and self-renewal) and HSC lineage commitment. Understanding the regulation of this signaling axis could improve the culture of hESCs and the efficiency of HSC lineage differentiation, both previously key barriers in the field.

[0010] EphB4 and ephrinB2 are both expressed in ESC and likely contribute to some aspect of stem cell fate. However, while ephrinB2 is clearly also involved in ectoderm and endoderm differentiation, ephB4 is unique amongst ephB receptors for not being expressed in the central nervous system. Thus, ephrinB2 "reverse" signaling and ephB4 "forward" signaling likely play overlapping but distinct roles in germ layer commitment and differentiation. Understanding the relative contribution of each signaling pathway may result in more optimal conditions for directing the differentiation of specific cell types.

[0011] Finally, ephrinB-ephB usually follows a gradient of ligand-receptor interactions, and expression of ephrinB2 is indeed heterogeneous within an ESC colony. Understanding the basis for the heterogeneity seen in human ES cell cultures will lead to more robust culture conditions that give rise to more homogenous population of cells suitable for regenerative medicine.

[0012] Eph-ephrin RTK expression is dysregulated in multiple cancers, and various members of this RTK family have been implicated in cancer development, progression, and subsequent metastases (See Pasquale EB. Eph receptors and ephrins in cancer: bidirectional signaling and beyond. *Nat Rev Cancer*. 2010;10:165-180).

[0013] Deciphering the role of Eph signaling activities in cancer is confounded by the promiscuity of interactions between Eph-ephrin receptor-ligand pairs, and the complexity of the resultant signaling cascades. Nevertheless, the centrality of ephrinB2 in facilitating tumor angiogenesis and promoting invasion and metastasis is supported by a slew of studies that provide a sound mechanistic basis for its action (See Pasquale EB. Eph receptors and ephrins in cancer: bidirectional signaling and beyond. *Nat Rev Cancer*. 2010;10:165-180). As such, soluble EphB4 inhibits tumor growth in multiple xenograft models (see Kertesz N, Krasnoperov V, Reddy R, et al. The soluble extracellular domain of EphB4(sEphB4) antagonizes EphB4-EphrinB2 interaction, modulates angiogenesis, and inhibits tumor growth. *Blood*. 2006;107:2330-2338; Kumar SR, Scehnet JS, Ley EJ, et al. Preferential induction of EphB4 over EphB2 and its implication in colorectal cancer progression. *Cancer Res*. 2009;69:3736-3745; Spannuth WA, Mangala LS, Stone RL, et al. Converging evidence for efficacy from parallel EphB4-targeted approaches in ovarian carcinoma. *Mol Cancer Ther*. 2010;9:2377-2388), while molecular genetic evidence implicates ephrinB2 reverse signaling in the activation of VEGFR2 that leads to vessel sprouting (See Branco-Price C, Johnson RS. Tumor vessels are Eph-ing complicated. *Cancer Cell*. 2010;17:533-534; Sawamiphak S, Seidel S, Essmann CL, et al. Ephrin-B2 regulates VEGFR2 function in developmental and tumor angiogenesis. *Nature*. 2010;465:487-491). The latter point suggests the exciting possibility that blocking ephrinB2 signaling may synergize with anti-VEGF therapies. Furthermore, amongst all the ephrins examined, only ephrinB2 on stromal cells (fibroblast, endothelial cells, or pericytes) activates ephB3/ephB4 on invasive prostate cancer cells leading to loss of contact inhibition of locomotion (CIL), the tumor invasive phenotype responsible for cancer metastases (See Astin JW, Batson J, Kadir S, et al. Competition amongst Eph receptors regulates contact inhibition of locomotion and invasiveness in prostate cancer cells. *Nat Cell Biol*. 2010;12:1194-1204; Wang B. Cancer cells exploit the eph-ephrin system to promote invasion and metastasis: tales of unwitting partners. *Sci Signal*. 2011;4:pe28).

[0014] Use of Nipah virus in conjunction with a lentivirus vector has heretofor been hampered by the fact that paramyxoviral envelopes are known not to pseudotype functionally onto lentiviral particles, presumbly due to some incompatibility of the cytoplasmic tail of the fusion and attachment glycoproteins with the matrix (gag) protein of HIV.

[0015] There remains a need for improved gene therapy compositions and methods that allow for enhanced delivery of the gene product to the target cells or tissues.

BRIEF SUMMARY OF THE INVENTION

[0016] The present inventors have successfully pseudotyped NiV glycoproteins onto lentiviral particles (NiVpp) by using appropriate cytoplasmic tail truncations. The inventors found that efficient functional pseudotyping requires only truncation of the F protein cytoplasmic tail, while full-length NiV-G can be used. Additional variations can also be introduced into the NiV-F or NiV-G peptide sequence to impact the properties of the resulting NiVpp lentivirus, e.g., increasing or decreasing infectivity of the NiVpp lentivirus. Codon-optimization of the NiV-F and G genes also allows for high-level expression of F and G, which enables efficient pseudotyping of NiV-F/G onto lentiviral particles (NiVpp).

[0017] NiVpp can be specifically targeted to various ephrinB2 expressing primary cells. The normal biology of ephrinB2, which undergoes rapid endocytosis upon interactions with its cognate receptor (e.g., EphB4, another membrane assocaited receptor-tyrosine kinase), can also be exploited. Thus, NiVpp targeted to endothelial cells may also be transcytosed across the blood-brain barrier to deliver gene-therapeutic payloads globally across the CNS. This could be useful, for example, in the treatment of Hungtington's disease, which requires global correction of the gene at issue.

[0018] Additionally, the ephrinB2-ephB4 axis is dysregulated in many cancers. In some breast cancers, tumor angiogenic vessels that supply the breast cancer stroma over express ephrinB2, while in other cancers (e.g., prostate), over expression of ephrinB2 has been implicated in the loss of contact inhibition of locomotion and thus may be responsible for metastasis. As such, NiVpp could be used to target cancer cells or angiogenic vessels, for example, to treat or otherwise impact various tunors or cancers.

[0019] Finally, EphrinB2 has been implicated as a molecular signature of stemness (Ivanova, NB et al., 2002, Science, 298, 601), and the inventors have confirmed that

NiVpp can specifically target subpopulations of human embryonic stem cells (SSEA4+), human neuroprogenitor stem cells (nestin+), and human hematopoietic stem cells (CD34+).

[0020] Thus, NiVpp pseudotyped lentivirus has many potential uses, including but not limited to: (1) To deliver any gene to neurons or endothelial cells, which over-express ephrinB2; (2) To deliver any gene to ephrinB2+ embryonic, neural, and hematopoietic stem cell populations; (3) To target tumors over-expressing ephrinB2; (4) To target ephrinB2+ cell populations in vivo or in vitro, e.g., for better transduction of neural stem cells for eventual transplantation; (5) To deliver therapeutic genes across the blood-brain barrier to the CNS.

[0021] Given all of this, in one embodiment, the invention is directed to a Nipah virus envelope pseudotyped lentivirus. In another embodiment, the invention is directed to Nipah virus (NiV) glycoproteins pseudotyped onto lentiviral particles (NiVpp).

[0022] In another embodiment, the invention is directed to a method for specifically targeting or delivering a gene or peptide product to ephrinB2+ cells or cell populations using the foregoing pseudotyped lentivirus. Such methods may be used, for example, to treat cancer or to combat angiogenic vessels. In certain examples, the ephrinB2+ cells comprise embryonic, neural, or hematopoietic stem cells.

[0023] In another embodiment, the invention is directed to a method for delivering or transporting a gene or peptide product across the blood-brain barrier using the foregoing pseudotyped lentivirus.

[0024] In another embodiment, the invention is directed to a method for altering brain function in a subject comprising injection of NiVpp into specific areas of said subject's brain.

[0025] In another embodiment, the invention is directed to a method for delivering any gene or peptide product to neurons or endothelial cells which overexpress ephrinB2 using the foregoing pseudotyped lentivirus.

[0026] In another embodiment, the invention is directed to a method for targeting tumors using the foregoing pseudotyped lentivirus.

BRIEF DESCRIPTION OF THE FIGURES

[0027] This application file contains at least one drawing executed in color. Copies of this application with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0028] FIG. **1** shows relevant portions of the NiV-F and NiV-G glycoproteins and the mutations made thereto.

[0029] FIG. **2** shows the titer obtained from NiVpp pseudotyped lentivirus produced using various NiV-F and NiV-G truncated glycoproteins.

[0030] FIG. **3** shows the relative infection of various cell types by various forms of NiVpp pseudotyped lentivirus, in some cases in the presence of soluble ephrinB2. In some panels, infectivity of VSV-G is also shown.

[0031] FIG. **4** shows the ability of NiVp pseudotyped lentivirus to infect various cell types at various MOIs., in some cases in the presence of soluble ephrinB2.

[0032] FIG. **5** shows the selectivity index of various pseudotypes of lentivirus for ephrinB2+ cells, when those cells are co-cultured with ephrinB2- cells at different ratios (1:1, 1:10, 1:100, and 1:1000).

[0033] FIGS. **6** and **7** show the localization of various lentivirus pseudotypes when they are injected into the animal for an in vivo examination of infectivity.

DETAILED DESCRIPTION

[0034] Before describing the present invention in detail, it is to be understood that this invention is not limited to particular embodiments, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting. Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, organic chemistry, and nucleic acid chemistry and hybridization are those well-known and commonly employed in the art. Standard techniques are used for nucleic acid and polypeptide synthesis. Procedures used for genetic engineering are well known and can be found, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, N.Y.).

[0035] As used in this specification and the appended claims, terms in the singular and the singular forms "a," "an," and "the," for example, include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "polypeptide," "the polypeptide" or "a polypeptide" also includes a plurality of polypeptides. Additionally, as used herein, the term "comprises" is intended to indicate a non-exhaustive list of components or steps, thus indicating that the given composition or method includes the listed components or steps and may also include additional components or steps not specifically listed. As an example, a composition "comprising a polypeptide" may also include additional components or polypeptides. The term "comprising" is also intended to encompass embodiments "consisting essentially of" and "consisting of" the listed components or steps. Similarly, the term "consisting essentially of" is also intended to encompass embodiments "consisting of" the listed components or steps.

[0036] Numeric ranges recited within the specification are inclusive of the numbers defining the range (the end point numbers) and also are intended to include each integer or any non-integer fraction within the defined range.

[0037] In describing and claiming the present invention, the following terminology will be used in accordance with the definitions set out below.

[0038] The terms "polypeptide," "peptide," and "protein" are generally used interchangeably herein and they refer to a polymer in which the monomers are amino acids that are joined together through amide bonds. Additionally, unnatural amino acids, for example, β-alanine, phenylglycine, and homoarginine are also included. Amino acids that are not gene-encoded can also be used with the technology disclosed herein. Furthermore, amino acids that have been modified to include reactive groups, glycosylation sites, polymers, therapeutic moieties, biomolecules, and the like can also be used. All of the amino acids used herein can be either the D- or L- isomer. The L-isomer is generally preferred. As used herein, "polypeptide," "peptide," and "protein" refer to both glycosylated and unglycosylated forms.

[0039] The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. "Amino acid analogs" refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g. homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. "Amino acid mimetics" refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that function in a manner similar to a naturally occurring amino acid.

[0040] As used herein, "NiVpp," "NiVpp lentivirus," "NiVpp pseudotyped lentivirus," NiV pseudotyped lentivirus," or the like refers to a lentivirus particle which has been pseudotyped using Nipah virus envelope glycoproteins NiV-F and NiV-G. The NiV-F glycoprotein on such NiVpp lentivirus particles is a variant form which has been modified such that it possesses a cytoplasmic tail truncation. In certain examples, the truncation will be a deletion of amino acid residues 525-546 of the NiV-F peptide, which will be referred to herein as the "T5F" or "T234F" form of the NiV-F glycoprotein (see FIG. **1**; deletion of 525-546 of SEQ ID NO:1 plus AU tag [DTYRYI]). In other examples, the NiV-F glycoprotein will further include a mutation to an N-linked glycosylation site, more specifically a substitution of glutamine (Q) for asparagine (N) at amino acid position 99 of the NiV-F peptide, which will be referred to herein as the "DeltaN3" or "ΔN3" form of the NiV-F glycoprotein. The NiV-G glycoprotein can be either a wild-type form or a modified or variant form of the protein, such as a truncated NiV-G. Deletions of 5, 10, 15, 20, 25, and 30 amino acids at or near the N-terminus of the NiV-G peptide were constructed, which are referred to herein as "Δ5G," "Δ10G," "Δ15G," "Δ20G," "Δ25G," and "Δ30G," respectively. A partial amino acid sequence of the NiV-F and NiV-G peptides showing each one of these variations is shown in FIG. **1**.

[0041] The present inventors engineered the Nipah virus envelope glycoproteins to be efficiently pseudotyped onto lentiviruses, and such NiV pseudotyped lentiviruses can efficiently target ephrinB2 expressing cells in vitro and in vivo. In certain examples, the NiVpp can be used to target a subpopulation of ephrinB2+/SSEA-4+ human embryonic stem cells (hESC). In other examples, NiVpp can be used to deliver agents that antagonize EphB-ephrinB2 mediated signaling specifically to ephrinB2-expressing target cells.

[0042] Further, NiVpp is the first demonstration of any lentiviral vector administered intravenously that can bypass the liver sink, which allows for targeting of specific ephrinB2+ populations in vivo. In addition, the natural tropism of NiVpp can be altered by mutating the natural receptor binding site to make it more ephrinB2 or B3 specific, depending on the clinical context of its use. NiVpp opens up the possibility for therapeutic targeting of ephrinB2-overexpressing cells common in various solid cancers or their tumor angiogenic vessels (see E. Pasquale, 2011).

[0043] EphrinB2 and its endogenous receptor, EphB4, are both receptor tyrosine kinases that undergo bi-directional signaling as well as bidirectional endocytosis upon interaction with each other. NiVpp can take advantage of this biological property for transcytosis across the blood brain barrier. This is a critical barrier in CNS targeted gene therapy (by systemic administration). NiVpp can transcytose across functional microvascular endothelial cell layers to infect target cells at the bottom of the transwell chamber. Further, considering that NiVpp can transduce Nestin+ neural stem cells even more efficiently than VSV-Gpp, direct stereotatic injection of NiVpp into specific CNS areas where neurogenesis (proliferation of neurons from stem cell progenitors) is known to occur in the adult brain, such as the hippocampus and the subventricular zone, is possible.

[0044] The efficiency of NiVpp transduction can be improved by engineering hyperfusogenic mutations in one or both of NiV-F and NiV-G. Several such mutations have been previously described (see, e.g., Lee at al, 2011, Trends in Microbiology). This could be useul, for example, for maintaining the specificity and picomolar affinity of NiV-G for ephrinB2 and/or B3 while independently enhancing the entry efficiency of NiVpp. Additionally, mutations in NiV-G that completely abrogate ephrinB2 and B3 binding, but that do not impact the association of this NiV-G with NiV-F, have been identified. This could allow for specific targeting of other desired cell types that are not ephrinB2+ through the addition of a single chain variable fragment (scFV) directed against a different cell surface molecule

[0045] The inventors have generated several mutants of the NiV fusion protein (NiV-F), and have also generated stepwise truncations in the cytoplasmic tail of the attachment protein (NiV-G), and screened each in combination with the NiV-F variant(s) for the ability to pseudotype lentivirus. Infectivity has been examined using a variety of cell types, including 293T and CHO-B2 cells, both of which express the NiV primary receptor, ephrinB2. While many of the G-truncations were expressed and could be pseudotyped onto lentiviruses, the highest increase in viral transduction titers (~100-fold) was obtained with the NiV-F variant and wild-type NiV-G, indicating that only truncations in the cytoplasmic tail of NiV-F are critical for efficient pseudotyping. Infection was blocked using soluble ephrinB2, confirming specificity of NiV pseudotyped lentivirus for ephrinB2+ cells. Moreover, NiV pseudotyped lentiviruses can suitably transduce primary human neurons and microvascular endothelial cells. Thus, lentivirus pseudotyped with NiV envelope may be used for targeted gene therapy in situations where ephrinB2/B3 is upregulated in the diseased tissue, thereby overcoming limitations of current gene therapy.

[0046] The NiVpp psuedotyped lentivirus vectors disclosed herein could be used to deliver any desired nucleic acid encoding for any desired peptide to any cell that expresses an appropriate receptor for NiV. In certain examples, these nucleic acid "payloads" will be delivered to cells expressing ephrin, for example ephrinB2 or ephrin B3. In other examples, the payload may be a nucleic acid encoding for a peptide product that is absent from the gene, such as is commonly done in gene therapy. This could be useful, for example, for targeting a genetic payload to neural stem cells. In other examples, the payload may be a nucleic acid or peptide that is toxic to the cell, for example to combat cancer cells. In other examples, the payload may be an ephrin

antagonist, such as a soluble ephrinB2 or a nucleic acid capable of silencing or downregulating ephrinB2, such as an siRNA. Delivery of such ephrinB2 antagonists may be useful, for example, for impacting cell pluripotency or development, or for decreasing metastasis of certain cancer cells.

[0047] The following examples are offered to illustrate, but not to limit, the claimed embodiments. It is to be understood that the examples and embodiments described herein are for illustrative purposes only, and persons skilled in the art will recognize various parameters that can be altered without departing from the spirit of the disclosure or the scope of the appended claims.

EXAMPLES

EXAMPLE 1 - Generation of Truncated Glycoproteins and NiVpp Pseudotyped Lentivirus

[0048] Previous studies have shown that pseudotyping of lentiviral vectors with unmodified paramyxoviral glycoproteins is highly inefficient. In the present study, we obtained chemically-synthesized, codon-optimized wild-type NiV-F and NiV-G nucleotides. These codon-optimized NiV-F and NiV-G sequences included a tag at the 3′ end encoding an AU1 peptide tag (DTYRYI) or a hemaglutinin peptide tag (YPYDVPDYA), respectively. These were subcloned into pcDNA3.1 vectors for mutagenesis. Variants of NiV-F and NiV-G were produced using a QuickChange site directed mutagenesis kit (Stratagene, Cedar Creek, TX) with primers designed to correspond to the desired deletions. A NiV-F variant, termed T5F or T234F, with a truncation of the cytoplasmic tail, as discussed above, was produced (see, e.g., Aguilar et al. (2007) J. Virol. 81:4520-4532). NiV-G variants were produced by making stepwise truncations of the cytoplasmic tail of NiV-G. FIG. **1** shows the variant forms of NiV-F and NiV-G that were produced.

[0049] NiVpp lentiviral vectors were created using various combinations of these variant NiV-F and NiV-G glycoproteins. All lentiviral vectors were produced by calcium phosphate-mediated transient transfection of 293T cells. One day prior to transfection, 1.6×10^7 293T cells were seeded in a T175 flask. 7 μg of NiV-F (wild-type of variant), 7 μg of NiV-G (wild-type or variant), 12.5 μg of the packaging plasmid pCMVΔR8.9, and 12.5 μg of the lentiviral transfer vector plasmid FG12-GFP or FUhLucW were transfected into cells. After 8 h, the transfection medium was removed and fresh medium was added. 48 h post-transfection, the viral supernatant was harvested and concentrated by centrifugation at 28,000 rpm at 4° C. for 2 h over a 20% sucrose cushion. To determine viral titer, serial dilutions of concentrated viral stocks were added to 293T cells and incubated at 37° C. for 2 h. 3 days post-infection, the cells were analyzed by flow cytometry for eGFP expression. Titers are expressed as infectious units per mL (IU/mL).

[0050] Truncation of the NiV-F cytoplasmic tail alone resulted in a titer of ~10^6 IU/mL on 293T cells, a 100-fold increase in titer compared to wtF/wtG pseudotypes (FIG. **2**). With regard to the NiV-G variants, although the T234F/Δ10G and T234F/ Δ25G variants demonstrated similar titers to T234F/wtG, none of the NiV-G variants produced greater titers than T234F/wtG (FIG. **2**). Moreover, combinations of wt F with the NiV-G truncation variants produced extremely low titers (data not shown), indicating that truncations in NiV-F are critical for efficient pseudotyping. Following concentration, titers of ~10^8-10^9 were obtained, compared to 10^{10} for VSV-G (data not shown). These high titer NiV pseudotyped lentiviruses can be used for efficient infection of ephrinB2+ cells, including for infection of hESCs, to deliver marker genes to tag ephrinB2+ hESCs, or to deliver siRNAs or other genes to antagonize the ephrinB2-ephB4 axis on hESCs.

EXAMPLE 2 - In Vitro and in Vivo Infection Using NiVpp Pseudotyped Lentivirus

[0051] Increasing amounts of virus (based on MOI or p24 equivalent) were added to 1×10^5 cells of each cell type and centrifuged at 2,000 rpm at 37° C. for 2 hours. As a specificity control, 10 nM of soluble ephrinB2 (R&D Systems) was added to the infection medium in some studies. To exclude pseudotransduction, 5 μM of nevirapine (NVP; a reverse transcriptase inhibitor) was added in some studies. For stem cell transductions, 4 ng/ml of polybrene (Sigma) was added. Following an overnight incubation with virus, the infection medium was removed and replaced with fresh medium. 72 hours post-infection, the cells were harvested and analyzed by flow cytometry for eGFP expression. For transduction of a mixed population of cells, ephrinB2+ human U87 cells were mixed with ephrinB2-non-human Chinese hamster ovary (CHO) cells at different ratios (U87:CHO ratios = 1:1, 1:10, 1:100, and 1:1000), and seeded at a density of 50,000 cells per well in 24-well plates. The next day, cells were infected with 1 or 10 ng of NiV T5F/wt G, T5FΔN3/wt G, and VSV-G pseudotypes. 72 h post-infection, the cells were harvested, stained with the mouse W6/32 anti-human HLA-ABC monoclonal antibody (eBioscience), followed by Alexa 647-conjugated goat anti-mouse secondary antibodies. Samples were fixed and then analyzed by dual-color flow cytometry for human HLA and eGFP expression.

[0052] CHO, CHO-B2, and CHO-B3 cells were infected with 0.01 ng, 0.1 ng, and 1 ng (p24 equivalents) of NiVpp or VSV-Gpp lentiviral pseudotypes carrying the GFP reporter gene (FIG. **3**, panels A-D). Infectivity was determined by the percent of GFP+ cells at 48 h post-infection via FACS analysis. The % GFP+ cells in each of the CHO cell lines infected by VSV-Gpp at maximal viral input (1 ng) was set at 100%, and all other infections in that cell line were normalized to this value. For reference, at 1 ng, VSV-G infected 20.2% of CHO, 22.7% of CHO-B2, and 21.6% of CHO-B3 cells. U87 cells and HMVECs were infected with T5F/wt G and T5FΔN3/wt G pseudotypes as described for panels A-C but normalized to VSV-Gpp infection of the same cell line (U87 or HMVECs) at maximal viral input (1 ng) (FIG. **3**, panels E & F). For reference, at 1 ng, VSV-G infected 36.5% of U87 cells and 14.4% of HMVECs. Inhibition by 10 nM of soluble ephrinB2 (sEFNB2) was used to demonstrate specificity of NiV receptor-mediated entry. All pseudotyped particle infections, regardless of envelope used, were also abrogated by 5 μM niverapine (NVP), a reverse transcriptase inhibitor (data not shown). Data shown in FIG. **3** are averages ± standard deviations for three independent experiments. Statistical analyses were performed using a two-way ANOVA with Bonferroni post-test comparison using GraphPad PRISM™. *: $p < 0.05$, **: $p < 0.01$, ****: $p < 0.0001$. As this figure demonstrates, NiVpp pseudotyped lentiviurus is able to effectively infect all cell types tested in an

ephrinB2 dependent manner. Moreover, T5FΔN3 showed an improved infectivity versus T5F.

[0053] EphrinB2 is a functional marker of human embryonic, neural, and hematopoietic stem cells (hESC, hNSC and hHSC). To confirm that ephrinB2 is functionally expressed on hESC, hNSC and hHSC, and to confirm that T234F/wtG pseudotype can mediate transfer into these ephrinB2+ cells, we transduced human ESCs, HSCs, and NSCs with NiVpp pseudotyped lentiviruses carrying a marker gene for EGFP. FIG. **4** shows that NiV pseudotypes infected SSEA-4+ hESC (H1 line) (panel A), hNSC (Nestin+) (panel B), and a subpopulation of purified CD34+ cells from human fetal liver (panel C). More specifically, in panel A of FIG. **4**, increasing amounts of NiVpp were added to H9 hESCs. Cells were stained for the cell-surface pluripotency marker, SSEA-4, and examined for GFP expression 72 h post-transduction by FACS analysis. 1×10^8 IU of NiVpp produced an infection rate of approximately 36% of SSEA-4+ hESC (FIG. **4**, panel A). For panel B of FIG. **4**, progenitor cells derived from the medial temporal lobe of a 17-week human fetus were infected with NiVpp. 72 h post-transduction, cells were stained for nestin and GFP expression was quantified by FACS analysis. The results of this analysis suggest that the NiV pseudotypes may infect NSC more efficiently than VSV-G pseudotypes. In panel C of FIG. **4**, purified $CD34^+$ cells from human fetal liver were infected with NiVpp. 72 h post-transduction, cells were stained for the cell-surface marker CD34 and analyzed for GFP expression by FACS analysis. At a multiplicity of infection (MOI) of 10, T234F/wtG pseudotypes specifically transduced ~12% of purified CD34+ cells from human fetal liver. Moreover, this infection was inhibited with soluble ephrinB2 (10 nM) in all cases (data only shown for fetal liver CD34+ cells, which shows a reduction from ~12% to <1% infection in the presence of soluble ephrinB2).

[0054] In the ehprinB2+/B2- ratio study, U87 (ephrinB2+) cells were mixed with CHO (ephrinB2-) cells at different ratios (U87:CHO ratios = 1:1, 1:10, 1:100, and 1:1000) and seeded at a density of 50,000 cells per well in 24-well plates. The next day, cells were infected with 1 or 10 ng of NiV T5F/wt G, T5FΔN3/wt G, and VSV-G pseudotypes. 72 h post-infection, the cells were harvested and stained with the W6/32 anti-human HLA-ABC monoclonal antibody and the infection rate (GFP-positive cells) was determined by FACS analysis. Although the cells were seeded and infected at the indicated ratio, the CHO cells divided faster and outgrew the U87 cells by about ten-fold in each sample. Data from 300,000 cells were acquired for every condition used for analysis. To take into account the differential permissivity of U87 and CHO cells to lentiviral transduction, we first calculated the "cell-specific selectivity index" for U87 cells, the U87 Sl as {B/(A+B)}/{D/(C+D)} where B and D represents the % of infected (GFP+) U87 and CHO cells, respectively, and A and C represents their uninfected counterparts, such that the total fraction of U87 (A+B) and CHO (C+D) cells in any given admixture upon analysis must equal 100%. A U87 SI of >1 indicates a selective preference for infecting U87 over CHO cells. For VSV-Gpp, the U87 SI at 1 and 10 ng is 5.14 and 1.93, respectively. This likely reflects the receptor-independent preference for U87 over CHO cells due to the HIV-1 based vector backbone alone. The reduction in U87 SI at a higher inoculum of VSV-Gpp is also consistent with the known ability of VSV-G-delivered gag to saturate non-human post-entry restriction factors. Since VSV-G is not known to have a cell-type specific receptor, we calculated the "NiV receptor-specific selectivity index", or the "EphrinB2 SI" as the VSV-G or NiV Env specific U87 SI divided by the U87 SI for VSV-G. This normalizes for differences in the intrinsic permissiveness of U87 over CHO cells for lentiviral transduction. This formulation now allows one to evaluate the selectivity of NiVpp for infecting ephrinB2-expressing cells relative to VSV-Gpp under all conditions analysed. The values of the U87 SI and EphrinB2 SI for VSV-G, T5F, and T5FΔN3 pseudotypes are provided in Table 1 :

TABLE 1

Specificity Index	Infection rate	VSV-G	T5F	T5FΔN3
U87 SI	1 ng	5.14	258.7	292.5
U87 SI	10 ng	1.93	362.8	342.9
EphrinB2 SI	1 ng	1.00	50.3	56.9
Ephrin B2 SI	10 ng	1.00	188.0	177.7

[0055] The EphrinB2 Selectivity Index calculated for VSV-Gpp, and NiVpp bearing T5F or T5F-ΔN3 for all the indicated conditions is shown in FIG. **5**. Data shown are averages ± standard deviations for triplicates done at 1 ng, and average ± range for duplicates done at 10 ng. As these results demonstrate, the NiVpp pseudotyped lentivirus vectors have a greatly increased specificity for EphrinB2 bearing cells as compared to VSV-Gpp pseudotyped lentivirus.

[0056] For in vivo analysis, the FvcFlw (firefly luciferase) vector was pseudotyped with VSV-G and two variant NiV pseudotypes, T234F and T234FΔN3-as discussed above. 10 ng of p24 equivalents of each pseudotyped lentivirus was injected into C57/BL6 mice through the tail vein. At 5 days post-injection, luciferase expression was imaged. Following whole-body imaging (see FIG. **6**), each organ was isolated to image luciferase expression (see FIG. **7**). As the images demonstrate, the NiVpp pseudotyped lentivirus is able to avoid the liver sink and to effectively infect cells and deliver genetic payloads in other tissues.

[0057] In addition to the other publications cited throughout this application, the following references are incorporated herein in their entireties for all purposes:

[0058] 1. An DS, Donahue RE, Kamata M, et al. Stable reduction of CCR5 by RNAi through hematopoietic stem cell transplant in non-human primates. Proc Natl Acad Sci USA. 2007;104:13110-13115.

[0059] 2. Shimizu S, Kamata M, Kittipongdaja P, et al. Characterization of a potent non-cytotoxic shRNA directed to the HIV-1 co-receptor CCR5. Genet Vaccines Ther. 2009;7:8.

[0060] 3. Palmer A, Klein R. Multiple roles of ephrins in morphogenesis, neuronal networking, and brain function. Genes Dev. 2003;17:1429-1450.

[0061] 4. Bowden TA, Aricescu AR, Gilbert RJ, Grimes JM, Jones EY, Stuart DI. Structural basis of Nipah and Hendra virus attachment to their cell-surface receptor ephrin-B2. Nat Struct Mol Biol. 2008;15:567-572.

[0062] 5. Graf T, Stadtfeld M. Heterogeneity of embryonic and adult stem cells. Cell Stem Cell. 2008;3:480-483.

[0063] 6. Hough SR, Laslett AL, Grimmond SB, Kolle G, Pera MF. A continuum of cell states spans pluripo-

tency and lineage commitment in human embryonic stem cells. PLoS One. 2009;4:e7708.

[0064] 7. Kullander K, Klein R. Mechanisms and functions of Eph and ephrin signalling. Nat Rev Mol Cell Biol. 2002;3:475-486.

[0065] 8. Berges BK, Akkina SR, Folkvord JM, Connick E, Akkina R. Mucosal transmission of R5 and X4 tropic HIV-1 via vaginal and rectal routes in humanized Rag2-/- gammac -/- (RAG-hu) mice. Virology. 2008;373:342-351.

[0066] 9. Damoiseaux R, Sherman SP, Alva JA, Peterson C, Pyle AD. Integrated chemical genomics reveals modifiers of survival in human embryonic stem cells. Stem Cells. 2009;27:533-542.

[0067] 10. Pyle AD, Lock LF, Donovan PJ. Neurotrophins mediate human embryonic stem cell survival. Nat Biotechnol. 2006;24:344-350.

[0068] 11. Scehnet JS, Ley EJ, Krasnoperov V, et al. The role of Ephs, Ephrins, and growth factors in Kaposi sarcoma and implications of EphrinB2 blockade. Blood. 2009;113:254-263.

[0069] 12. Fortunel NO, Otu HH, Ng HH, et al. Comment on "'Stemness': transcriptional profiling of embryonic and adult stem cells" and "a stem cell molecular signature". Science. 2003;302:393; author reply 393.

[0070] 13. Levroney EL, Aguilar HC, Fulcher JA, et al. Novel innate immune functions for galectin- 1: galectin-1 inhibits cell fusion by Nipah virus envelope glycoproteins and augments dendritic cell secretion of proinflammatory cytokines. J Immunol. 2005;175:413-420.

[0071] 14. Arvanitis D, Davy A. Eph/ephrin signaling: networks. Genes Dev. 2008;22:416-429.

[0072] 15. Ng ES, Davis RP, Azzola L, Stanley EG, Elefanty AG. Forced aggregation of defined numbers of human embryonic stem cells into embryoid bodies fosters robust, reproducible hematopoietic differentiation. Blood. 2005;106:1601-1603.

SEQUENCE LISTING

Sequence total quantity: 24

SEQ ID NO: 1 moltype = AA length = 546
FEATURE Location/Qualifiers
source 1..546
mol_type = protein
organism = Nipah henipavirus

SEQ ID NO: 1

```
MVVILDKRCY CNLLILILMI SECSVGILHY EKLSKIGLVK GVTRKYKIKS NPLTKDIVIK  60
MIPNVSNMSQ CTGSVMENYK TRLNGILTPI KGALEIYKNN THDLVGDVRL AGVIMAGVAI  120
GIATAAQITA GVALYEAMKN ADNINKLKSS IESTNEAVVK LQETAEKTVY VLTALQDYIN  180
TNLVPTIDKI SCKQTELSLD LALSKYLSDL LFVFGPNLQD PVSNSMTIQA ISQAFGGNYE  240
TLLRTLGYAT EDFDDLLESD SITGQIIYVD LSSYYIIVRV YFPILTEIQQ AYIQELLPVS  300
FNNDNSEWIS IVPNFILVRN TLISNIEIGF CLITKRSVIC NQDYATPMTN NMRECLTGST  360
EKCPRELVVS SHVPRFALSN GVLFANCISV TCQCQTTGRA ISQSGEQTLL MIDNTTCPTA  420
VLGNVIISLG KYLGSVNYNS EGIAIGPPVF TDKVDISSQI SSMNQSLQQS KDYIKEAQRL  480
LDTVNPSLIS MLSMIILYVL SIASLCIGLI TFISFIIVEK KRNTYSRLED RRVRPTSSGD  540
LYYIGT                                                             546
```

SEQ ID NO: 2 moltype = AA length = 552
FEATURE Location/Qualifiers
REGION 1..552
note = Variant peptide
source 1..552
mol_type = protein
organism = synthetic construct

SEQ ID NO: 2

```
MVVILDKRCY CNLLILILMI SECSVGILHY EKLSKIGLVK GVTRKYKIKS NPLTKDIVIK  60
MIPNVSNMSQ CTGSVMENYK TRLNGILTPI KGALEIYKNN THDLVGDVRL AGVIMAGVAI  120
GIATAAQITA GVALYEAMKN ADNINKLKSS IESTNEAVVK LQETAEKTVY VLTALQDYIN  180
TNLVPTIDKI SCKQTELSLD LALSKYLSDL LFVFGPNLQD PVSNSMTIQA ISQAFGGNYE  240
TLLRTLGYAT EDFDDLLESD SITGQIIYVD LSSYYIIVRV YFPILTEIQQ AYIQELLPVS  300
FNNDNSEWIS IVPNFILVRN TLISNIEIGF CLITKRSVIC NQDYATPMTN NMRECLTGST  360
EKCPRELVVS SHVPRFALSN GVLFANCISV TCQCQTTGRA ISQSGEQTLL MIDNTTCPTA  420
VLGNVIISLG KYLGSVNYNS EGIAIGPPVF TDKVDISSQI SSMNQSLQQS KDYIKEAQRL  480
LDTVNPSLIS MLSMIILYVL SIASLCIGLI TFISFIIVEK KRNTYSRLED RRVRPTSSGD  540
LYYIGTDTYR YI                                                      552
```

SEQ ID NO: 3 moltype = DNA length = 1659
FEATURE Location/Qualifiers
misc_feature 1..1659
note = Variant nucleotide
source 1..1659
mol_type = other DNA

-continued

organism = synthetic construct

SEQ ID NO: 3

```
atggtggtga tcctggacaa gcggtgctac tgcaacctgc tgatcctgat cctgatgatc  60
agcgagtgca gcgtgggcat cctgcactac gagaagctga gcaagatcgg cctggtgaag  120
ggcgtgaccc ggaagtacaa gatcaagagc aacccccctga ccaaggacat cgtgatcaag  180
atgatcccca acgtgagcaa catgagccag tgcaccggca gcgtgatgga gaactacaag  240
acccggctga acggcatcct gacccccatc aagggcgccc tggagatcta caagaacaac  300
acccacgacc tggtgggcga cgtgcggctg gccggcgtga tcatggccgg cgtggccatc  360
ggcatcgcca cagccgccca gatcaccgcc ggagtggccc tgtacgaggc catgaagaac  420
gccgacaaca tcaacaagct gaagagcagc atcgagagca ccaacgaggc cgtggtgaag  480
ctgcaggaga ccgccgagaa aaccgtgtac gtgctgaccg ccctgcagga ctacatcaac  540
accaacctgg tgcccaccat cgacaagatc agctgcaagc agaccgagct gagcctggac  600
ctggccctga gcaagtacct gagcgacctg ctgttcgtgt tcggccccaa cctgcaggac  660
cccgtgagca acagcatgac catccaggcc atcagccagg ccttcggcgg caactacgag  720
accctgctgc ggaccctggg ctacgccacc gaggacttcg acgacctgct ggagagcgac  780
agcatcaccg gccagatcat ctacgtggac ctgagcagct actacatcat cgtgcgggtg  840
tacttcccca tcctgaccga gatccagcag gcctacatcc aggagctgct gcccgtgagc  900
ttcaacaacg acaacagcga gtggatcagc atcgtgccca acttcatcct ggtgcggaac  960
accctgatca gcaacatcga gatcggcttc tgcctgatca ccaagcggag cgtgatctgc  1020
aaccaggact acgccacccc catgaccaac aacatgcggg agtgcctgac cggcagcacc  1080
gagaagtgcc ccagggagct ggtggtgagc agccacgtgc cccggttcgc cctgagcaac  1140
ggcgtgctgt tcgccaactg catcagcgtg acctgccagt gccagaccac cggcagagcc  1200
atcagccaga gcggcgagca gaccctgctg atgatcgaca acaccacctg ccccaccgcc  1260
gtgctgggca acgtgatcat cagcctgggc aagtatctgg gcagcgtgaa ctacaacagc  1320
gagggcatcg ccatcggccc tcccgtgttc accgacaagg tggacatcag cagccagatc  1380
agcagcatga accagagcct gcagcagagc aaggattaca tcaaggaggc ccagcggctg  1440
ctggacaccg tgaaccccag cctgatcagc atgctgtcca tgatcatcct gtacgtgctg  1500
agcatcgcca gcctgtgcat cggcctgatc accttcatca gcttcatcat cgtggagaag  1560
aagcggaaca cctacagccg gctggaggac cggcgggtgc ggcccaccag cagcggcgac  1620
ctgtactaca tcggcaccga cacctaccgg tacatctaa                          1659
```

SEQ ID NO: 4 moltype = AA length = 532

FEATURE Location/Qualifiers

REGION 1..532

note = Variant peptide

source 1..532

mol_type = protein

organism = synthetic construct

SEQ ID NO: 4

```
MVVILDKRCY CNLLILILMI SECSVGILHY EKLSKIGLVK GVTRKYKIKS NPLTKDIVIK  60
MIPNVSNMSQ CTGSVMENYK TRLNGILTPI KGALEIYKNN THDLVGDVRL AGVIMAGVAI  120
GIATAAQITA GVALYEAMKN ADNINKLKSS IESTNEAVVK LQETAEKTVY VLTALQDYIN  180
TNLVPTIDKI SCKQTELSLD LALSKYLSDL LFVFGPNLQD PVSNSMTIQA ISQAFGGNYE  240
TLLRTLGYAT EDFDDLLESD SITGQIIYVD LSSYYIIVRV YFPILTEIQQ AYIQELLPVS  300
FNNDNSEWIS IVPNFILVRN TLISNIEIGF CLITKRSVIC NQDYATPMTN NMRECLTGST  360
EKCPRELVVS SHVPRFALSN GVLFANCISV TCQCQTTGRA ISQSGEQTLL MIDNTTCPTA  420
VLGNVIISLG KYLGSVNYNS EGIAIGPPVF TDKVDISSQI SSMNQSLQQS KDYIKEAQRL  480
LDTVNPSLIS MLSMIILYVL SIASLCIGLI TFISFIIVEK KRNTGTDTYR YI           532
```

SEQ ID NO: 5 moltype = DNA length = 1599

FEATURE Location/Qualifiers

misc_feature 1..1599

note = Variant nucleotide

source 1..1599

mol_type = other DNA

organism = synthetic construct

SEQ ID NO: 5

```
atggtggtga tcctggacaa gcggtgctac tgcaacctgc tgatcctgat cctgatgatc  60
agcgagtgca gcgtgggcat cctgcactac gagaagctga gcaagatcgg cctggtgaag  120
ggcgtgaccc ggaagtacaa gatcaagagc aacccccctga ccaaggacat cgtgatcaag  180
atgatcccca acgtgagcaa catgagccag tgcaccggca gcgtgatgga gaactacaag  240
acccggctga acggcatcct gacccccatc aagggcgccc tggagatcta caagaacaac  300
acccacgacc tggtgggcga cgtgcggctg gccggcgtga tcatggccgg cgtggccatc  360
ggcatcgcca cagccgccca gatcaccgcc ggagtggccc tgtacgaggc catgaagaac  420
gccgacaaca tcaacaagct gaagagcagc atcgagagca ccaacgaggc cgtggtgaag  480
ctgcaggaga ccgccgagaa aaccgtgtac gtgctgaccg ccctgcagga ctacatcaac  540
accaacctgg tgcccaccat cgacaagatc agctgcaagc agaccgagct gagcctggac  600
ctggccctga gcaagtacct gagcgacctg ctgttcgtgt tcggccccaa cctgcaggac  660
cccgtgagca acagcatgac catccaggcc atcagccagg ccttcggcgg caactacgag  720
accctgctgc ggaccctggg ctacgccacc gaggacttcg acgacctgct ggagagcgac  780
```

-continued

```
agcatcaccg gccagatcat ctacgtggac ctgagcagct actacatcat cgtgcgggtg  840
tacttcccca tcctgaccga gatccagcag gcctacatcc aggagctgct gcccgtgagc  900
ttcaacaacg acaacagcga gtggatcagc atcgtgccca acttcatcct ggtgcggaac  960
accctgatca gcaacatcga gatcggcttc tgcctgatca ccaagcggag cgtgatctgc  1020
aaccaggact acgccacccc catgaccaac aacatgcggg agtgcctgac cggcagcacc  1080
gagaagtgcc ccagggagct ggtggtgagc agccacgtgc cccggttcgc cctgagcaac  1140
ggcgtgctgt tcgccaactg catcagcgtg acctgccagt gccagaccac cggcagagcc  1200
atcagccaga gcggcgagca gaccctgctg atgatcgaca acaccacctg ccccaccgcc  1260
gtgctgggca acgtgatcat cagcctgggc aagtatctgg gcagcgtgaa ctacaacagc  1320
gagggcatcg ccatcggccc tcccgtgttc accgacaagg tggacatcag cagccagatc  1380
agcagcatga accagagcct gcagcagagc aaggattaca tcaaggaggc ccagcggctg  1440
ctggacaccg tgaaccccag cctgatcagc atgctgtcca tgatcatcct gtacgtgctg  1500
agcatcgcca gcctgtgcat cggcctgatc accttcatca gcttcatcat cgtggagaag  1560
aagcggaaca ccggcaccga cacctaccgg tacatctaa                         1599

SEQ ID NO: 6            moltype = AA  length = 552
FEATURE                 Location/Qualifiers
REGION                  1..552
                        note = Variant peptide
source                  1..552
                        mol_type = protein
                        organism = synthetic construct
SEQ ID NO: 6
MVVILDKRCY CNLLILILMI SECSVGILHY EKLSKIGLVK GVTRKYKIKS NPLTKDIVIK  60
MIPNVSNMSQ CTGSVMENYK TRLNGILTPI KGALEIYKQN THDLVGDVRL AGVIMAGVAI  120
GIATAAQITA GVALYEAMKN ADNINKLKSS IESTNEAVVK LQETAEKTVY VLTALQDYIN  180
TNLVPTIDKI SCKQTELSLD LALSKYLSDL LFVFGPNLQD PVSNSMTIQA ISQAFGGNYE  240
TLLRTLGYAT EDFDDLLESD SITGQIIYVD LSSYYIIVRV YFPILTEIQQ AYIQELLPVS  300
FNNDNSEWIS IVPNFILVRN TLISNIEIGF CLITKRSVIC NQDYATPMTN NMRECLTGST  360
EKCPRELVVS SHVPRFALSN GVLFANCISV TCQCQTTGRA ISQSGEQTLL MIDNTTCPTA  420
VLGNVIISLG KYLGSVNYNS EGIAIGPPVF TDKVDISSQI SSMNQSLQQS KDYIKEAQRL  480
LDTVNPSLIS MLSMIILYVL SIASLCIGLI TFISFIIVEK KRNTYSRLED RRVRPTSSGD  540
LYYIGTDTYR YI                                                     552

SEQ ID NO: 7            moltype = DNA  length = 1659
FEATURE                 Location/Qualifiers
misc_feature            1..1659
                        note = Variant nucleotide
source                  1..1659
                        mol_type = other DNA
                        organism = synthetic construct
SEQ ID NO: 7
atggtggtga tcctggacaa gcggtgctac tgcaacctgc tgatcctgat cctgatgatc  60
agcgagtgca gcgtgggcat cctgcactac gagaagctga gcaagatcgg cctggtgaag  120
ggcgtgaccc ggaagtacaa gatcaagagc aacccctga ccaaggacat cgtgatcaag  180
atgatcccca acgtgagcaa catgagccag tgcaccggca gcgtgatgga gaactacaag  240
acccggctga acggcatcct gaccccatc aagggcgccc tggagatcta caagcaraac  300
acccacgacc tggtgggcga cgtgcggctg gccggcgtga tcatggccgg cgtggccatc  360
ggcatcgcca cagccgccca gatcaccgcc ggagtggccc tgtacgaggc catgaagaac  420
gccgacaaca tcaacaagct gaagagcagc atcgagagca ccaacgaggc cgtggtgaag  480
ctgcaggaga ccgccgagaa aaccgtgtac gtgctgaccg ccctgcagga ctacatcaac  540
accaacctgg tgcccaccat cgacaagatc agctgcaagc agaccgagct gagcctggac  600
ctggccctga gcaagtacct gagcgacctg ctgttcgtgt tcggccccaa cctgcaggac  660
cccgtgagca acagcatgac catccaggcc atcagccagg ccttcggcgg caactacgag  720
accctgctgc ggaccctggg ctacgccacc gaggacttcg acgacctgct ggagagcgac  780
agcatcaccg gccagatcat ctacgtggac ctgagcagct actacatcat cgtgcgggtg  840
tacttcccca tcctgaccga gatccagcag gcctacatcc aggagctgct gcccgtgagc  900
ttcaacaacg acaacagcga gtggatcagc atcgtgccca acttcatcct ggtgcggaac  960
accctgatca gcaacatcga gatcggcttc tgcctgatca ccaagcggag cgtgatctgc  1020
aaccaggact acgccacccc catgaccaac aacatgcggg agtgcctgac cggcagcacc  1080
gagaagtgcc ccagggagct ggtggtgagc agccacgtgc cccggttcgc cctgagcaac  1140
ggcgtgctgt tcgccaactg catcagcgtg acctgccagt gccagaccac cggcagagcc  1200
atcagccaga gcggcgagca gaccctgctg atgatcgaca acaccacctg ccccaccgcc  1260
gtgctgggca acgtgatcat cagcctgggc aagtatctgg gcagcgtgaa ctacaacagc  1320
gagggcatcg ccatcggccc tcccgtgttc accgacaagg tggacatcag cagccagatc  1380
agcagcatga accagagcct gcagcagagc aaggattaca tcaaggaggc ccagcggctg  1440
ctggacaccg tgaaccccag cctgatcagc atgctgtcca tgatcatcct gtacgtgctg  1500
agcatcgcca gcctgtgcat cggcctgatc accttcatca gcttcatcat cgtggagaag  1560
```

-continued

```
aagcggaaca cctacagccg gctggaggac cggcgggtgc ggcccaccag cagcggcgac  1620
ctgtactaca tcggcaccga cacctaccgg tacatctaa                         1659

SEQ ID NO: 8           moltype = AA  length = 532
FEATURE                Location/Qualifiers
REGION                 1..532
                       note = Variant peptide
source                 1..532
                       mol_type = protein
                       organism = synthetic construct
SEQ ID NO: 8
MVVILDKRCY CNLLILILMI SECSVGILHY EKLSKIGLVK GVTRKYKIKS NPLTKDIVIK  60
MIPNVSNMSQ CTGSVMENYK TRLNGILTPI KGALEIYKQN THDLVGDVRL AGVIMAGVAI  120
GIATAAQITA GVALYEAMKN ADNINKLKSS IESTNEAVVK LQETAEKTVY VLTALQDYIN  180
TNLVPTIDKI SCKQTELSLD LALSKYLSDL LFVFGPNLQD PVSNSMTIQA ISQAFGGNYE  240
TLLRTLGYAT EDFDDLLESD SITGQIIYVD LSSYYIIVRV YFPILTEIQQ AYIQELLPVS  300
FNNDNSEWIS IVPNFILVRN TLISNIEIGF CLITKRSVIC NQDYATPMTN NMRECLTGST  360
EKCPRELVVS SHVPRFALSN GVLFANCISV TCQCQTTGRA ISQSGEQTLL MIDNTTCPTA  420
VLGNVIISLG KYLGSVNYNS EGIAIGPPVF TDKVDISSQI SSMNQSLQQS KDYIKEAQRL  480
LDTVNPSLIS MLSMIILYVL SIASLCIGLI TFISFIIVEK KRNTGTDTYR YI           532

SEQ ID NO: 9           moltype = DNA  length = 1599
FEATURE                Location/Qualifiers
misc_feature           1..1599
                       note = Variant nucleotide
source                 1..1599
                       mol_type = other DNA
                       organism = synthetic construct
SEQ ID NO: 9
atggtggtga tcctggacaa gcggtgctac tgcaacctgc tgatcctgat cctgatgatc  60
agcgagtgca gcgtgggcat cctgcactac gagaagctga gcaagatcgg cctggtgaag  120
ggcgtgaccc ggaagtacaa gatcaagagc aacccccctga ccaaggacat cgtgatcaag  180
atgatcccca acgtgagcaa catgagccag tgcaccggca gcgtgatgga gaactacaag  240
acccggctga acggcatcct gacccccatc aagggcgccc tggagatcta caagcaraac  300
acccacgacc tggtgggcga cgtgcggctg gccggcgtga tcatggccgg cgtggccatc  360
ggcatcgcca cagccgccca gatcaccgcc ggagtggccc tgtacgaggc catgaagaac  420
gccgacaaca tcaacaagct gaagagcagc atcgagagca ccaacgaggc cgtggtgaag  480
ctgcaggaga ccgccgagaa aaccgtgtac gtgctgaccg ccctgcagga ctacatcaac  540
accaacctgg tgcccaccat cgacaagatc agctgcaagc agaccgagct gagcctggac  600
ctggccctga gcaagtacct gagcgacctg ctgttcgtgt tcggccccaa cctgcaggac  660
cccgtgagca acagcatgac catccaggcc atcagccagg ccttcggcgg caactacgag  720
accctgctgc ggaccctggg ctacgccacc gaggacttcg acgacctgct ggagagcgac  780
agcatcaccg gccagatcat ctacgtggac ctgagcagct actacatcat cgtgcgggtg  840
tacttcccca tcctgaccga gatccagcag gcctacatcc aggagctgct gccccgtgagc  900
ttcaacaacg acaacagcga gtggatcagc atcgtgccca acttcatcct ggtgcggaac  960
accctgatca gcaacatcga gatcggcttc tgcctgatca ccaagcggag cgtgatctgc  1020
aaccaggact acgccacccc catgaccaac aacatgcggg agtgcctgac cggcagcacc  1080
gagaagtgcc ccagggagct ggtggtgagc agccacgtgc cccggttcgc cctgagcaac  1140
ggcgtgctgt tcgccaactg catcagcgtg acctgccagt gccagaccac cggcagagcc  1200
atcagccaga gcggcgagca gaccctgctg atgatcgaca acaccacctg ccccaccgcc  1260
gtgctgggca acgtgatcat cagcctgggc aagtatctgg gcagcgtgaa ctacaacagc  1320
gagggcatcg ccatcggccc tcccgtgttc accgacaagg tggacatcag cagccagatc  1380
agcagcatga accagagcct gcagcagagc aaggattaca tcaaggaggc ccagcggctg  1440
ctggacaccg tgaaccccag cctgatcagc atgctgtcca tgatcatcct gtacgtgctg  1500
agcatcgcca gcctgtgcat cggcctgatc accttcatca gcttcatcat cgtggagaag  1560
aagcggaaca ccggcaccga cacctaccgg tacatctaa                         1599

SEQ ID NO: 10          moltype = AA  length = 602
FEATURE                Location/Qualifiers
source                 1..602
                       mol_type = protein
                       organism = Nipah henipavirus
SEQ ID NO: 10
MGPAENKKVR FENTTSDKGK IPSKVIKSYY GTMDIKKINE GLLDSKILSA FNTVIALLGS  60
IVIIVMNIMI IQNYTRSTDN QAVIKDALQG IQQQIKGLAD KIGTEIGPKV SLIDTSSTIT  120
IPANIGLLGS KISQSTASIN ENVNEKCKFT LPPLKIHECN ISCPNPLPFR EYRPQTEGVS  180
NLVGLPNNIC LQKTSNQILK PKLISYTLPV VGQSGTCITD PLLAMDEGYF AYSHLERIGS  240
CSRGVSKQRI IGVGEVLDRG DEVPSLFMTN VWTPPNPNTV YHCSAVYNNE FYYVLCAVST  300
VGDPILNSTY WSGSLMMTRL AVKPKSNGGG YNQHQLALRS IEKGRYDKVM PYGPSGIKQG  360
DTLYFPAVGF LVRTEFKYND SNCPITKCQY SKPENCRLSM GIRPNSHYIL RSGLLKYNLS  420
```

-continued

```
DGENPKVVFI EISDQRLSIG SPSKIYDSLG QPVFYQASFS WDTMIKFGDV LTVNPLVVNW  480
RNNTVISRPG QSQCPRFNTC PEICWEGVYN DAFLIDRINW ISAGVFLDSN QTAENPVFTV  540
FKDNEILYRA QLASEDTNAQ KTITNCFLLK NKIWCISLVE IYDTGDNVIR PKLFAVKIPE  600
QC                                                                 602

SEQ ID NO: 11          moltype = AA  length = 611
FEATURE                Location/Qualifiers
REGION                 1..611
                       note = Variant peptide
source                 1..611
                       mol_type = protein
                       organism = synthetic construct
SEQ ID NO: 11
MGPAENKKVR FENTTSDKGK IPSKVIKSYY GTMDIKKINE GLLDSKILSA FNTVIALLGS  60
IVIIVMNIMI IQNYTRSTDN QAVIKDALQG IQQQIKGLAD KIGTEIGPKV SLIDTSSTIT  120
IPANIGLLGS KISQSTASIN ENVNEKCKFT LPPLKIHECN ISCPNPLPFR EYRPQTEGVS  180
NLVGLPNNIC LQKTSNQILK PKLISYTLPV VGQSGTCITD PLLAMDEGYF AYSHLERIGS  240
CSRGVSKQRI IGVGEVLDRG DEVPSLFMTN VWTPPNPNTV YHCSAVYNNE FYYVLCAVST  300
VGDPILNSTY WSGSLMMTRL AVKPKSNGGG YNQHQLALRS IEKGRYDKVM PYGPSGIKQG  360
DTLYFPAVGF LVRTEFKYND SNCPITKCQY SKPENCRLSM GIRPNSHYIL RSGLLKYNLS  420
DGENPKVVFI EISDQRLSIG SPSKIYDSLG QPVFYQASFS WDTMIKFGDV LTVNPLVVNW  480
RNNTVISRPG QSQCPRFNTC PEICWEGVYN DAFLIDRINW ISAGVFLDSN QTAENPVFTV  540
FKDNEILYRA QLASEDTNAQ KTITNCFLLK NKIWCISLVE IYDTGDNVIR PKLFAVKIPE  600
QCYPYDVPDY A                                                       611

SEQ ID NO: 12          moltype = DNA  length = 1836
FEATURE                Location/Qualifiers
misc_feature           1..1836
                       note = Variant nucleotide
source                 1..1836
                       mol_type = other DNA
                       organism = synthetic construct
SEQ ID NO: 12
atgggacctg ccgagaacaa gaaagtgcgg ttcgagaaca ccacaagcga caagggcaag  60
atccccagca aagtgatcaa gagctactac ggcaccatgg acatcaagaa gatcaacgag  120
ggcctgctgg acagcaagat cctgagcgcc ttcaacaccg tgatcgccct gctgggcagc  180
atcgtgatca ttgtgatgaa catcatgatc atccagaact acacccggag caccgacaac  240
caggccgtga tcaaggacgc cctgcaggga atccagcagc agatcaaggg cctggccgac  300
aagatcggca ccgagatcgg ccccaaagtg agcctgatcg acaccagcag caccatcacc  360
atccccgcca acatcggcct gctgggatcc aagatcagcc agagcaccgc cagcatcaac  420
gagaacgtga acgagaagtg caagttcacc ctgcccccc tgaagatcca cgagtgcaac  480
atcagctgcc ccaaccccct gccttccgg gagtaccggc cccagaccga gggcgtgagc  540
aacctggtgg gcctgcccaa caacatctgc ctgcagaaaa ccagcaacca gatcctgaag  600
cccaagctga tctcctacac cctgcccgtg gtgggccaga gcggcacctg catcaccgac  660
cccctgctgg ccatggacga gggctacttc gcctacagcc acctggagcg gatcggcagc  720
tgcagccggg gagtgagcaa gcagcggatc atcggcgtgg gcgaagtgct ggaccggggc  780
gacgaagtgc ccagcctgtt catgaccaac gtgtggaccc cccccaaccc caacaccgtg  840
taccactgca gcgccgtgta caacaacgag ttctactacg tgctgtgcgc cgtgagcacc  900
gtgggcgacc ccatcctgaa cagcacctac tggagcggca gcctgatgat gacccggctg  960
gccgtgaagc ctaagagcaa cggcggaggc tacaaccagc accagctggc cctgcggagc  1020
atcgagaagg gccggtacga caaagtgatg ccctacggcc ccagcggcat caagcagggc  1080
gacaccctgt acttccccgc cgtgggcttc ctggtgcgga ccgagttcaa gtacaacgac  1140
agcaactgcc ccatcaccaa gtgccagtac agcaagcccg agaactgccg gctgagcatg  1200
ggcatccggc ccaacagcca ctacatcctg cggagcggcc tgctgaagta caacctgagc  1260
gacggcgaga accccaaagt ggtgttcatc gagatcagcg accagagact gagcatcggc  1320
agccccagca agatctacga cagcctgggc cagcccgtgt tctaccaggc cagcttcagc  1380
tgggacacca tgatcaagtt cggcgacgtg ctgaccgtga acccctggt ggtgaactgg  1440
cggaacaata ccgtgatcag cagacccggc cagagccagt gccccggtt caacacctgc  1500
cccgagatct gctgggaggg cgtgtacaac gacgccttcc tgatcgaccg gatcaactgg  1560
atcagcgccg gagtgttcct ggatagcaac cagaccgccg agaatcccgt gttcaccgtg  1620
tttaaggaca acgagatcct gtacagagcc cagctggcca gcgaggacac caacgcccag  1680
aaaaccatca ccaactgctt cctgctgaag aataagatct ggtgcatcag cctggtggag  1740
atctacgata ccggcgacaa cgtgatcagg cccaagctgt tcgccgtgaa gatccccgag  1800
cagtgctacc cctacgacgt gcccgactac gcctga                             1836

SEQ ID NO: 13          moltype = AA  length = 606
FEATURE                Location/Qualifiers
REGION                 1..606
                       note = Variant peptide
source                 1..606
```

-continued

```
                     mol_type = protein
                     organism = synthetic construct
SEQ ID NO: 13
MGKVRFENTT SDKGKIPSKV IKSYYGTMDI KKINEGLLDS KILSAFNTVI ALLGSIVIIV  60
MNIMIIQNYT RSTDNQAVIK DALQGIQQQI KGLADKIGTE IGPKVSLIDT SSTITIPANI  120
GLLGSKISQS TASINENVNE KCKFTLPPLK IHECNISCPN PLPFREYRPQ TEGVSNLVGL  180
PNNICLQKTS NQILKPKLIS YTLPVVGQSG TCITDPLLAM DEGYFAYSHL ERIGSCSRGV  240
SKQRIIGVGE VLDRGDEVPS LFMTNVWTPP NPNTVYHCSA VYNNEFYYVL CAVSTVGDPI  300
LNSTYWSGSL MMTRLAVKPK SNGGGYNQHQ LALRSIEKGR YDKVMPYGPS GIKQGDTLYF  360
PAVGFLVRTE FKYNDSNCPI TKCQYSKPEN CRLSMGIRPN SHYILRSGLL KYNLSDGENP  420
KVVFIEISDQ RLSIGSPSKI YDSLGQPVFY QASFSWDTMI KFGDVLTVNP LVVNWRNNTV  480
ISRPGQSQCP RFNTCPEICW EGVYNDAFLI DRINWISAGV FLDSNQTAEN PVFTVFKDNE  540
ILYRAQLASE DTNAQKTITN CFLLKNKIWC ISLVEIYDTG DNVIRPKLFA VKIPEQCYPY  600
DVPDYA                                                             606

SEQ ID NO: 14          moltype = DNA  length = 1821
FEATURE                Location/Qualifiers
misc_feature           1..1821
                       note = Variant nucleotide
source                 1..1821
                       mol_type = other DNA
                       organism = synthetic construct
SEQ ID NO: 14
atgggaaaag tgcggttcga gaacaccaca agcgacaagg gcaagatccc cagcaaagtg  60
atcaagagct actacggcac catggacatc aagaagatca acgagggcct gctggacagc  120
aagatcctga gcgccttcaa caccgtgatc gccctgctgg gcagcatcgt gatcattgtg  180
atgaacatca tgatcatcca gaactacacc cggagcaccg acaaccaggc cgtgatcaag  240
gacgccctgc agggaatcca gcagcagatc aagggcctgg ccgacaagat cggcaccgag  300
atcggcccca aagtgagcct gatcgacacc agcagcacca tcaccatccc cgccaacatc  360
ggcctgctgg gatccaagat cagccagagc accgccagca tcaacgagaa cgtgaacgag  420
aagtgcaagt tcaccctgcc ccccctgaag atccacgagt gcaacatcag ctgccccaac  480
cccctgccct tccgggagta ccggccccag accgagggcg tgagcaacct ggtgggcctg  540
cccaacaaca tctgcctgca gaaaaccagc aaccagatcc tgaagcccaa gctgatctcc  600
tacaccctgc ccgtggtggg ccagagcggc acctgcatca ccgacccccc gctggccatg  660
gacgagggct acttcgccta cagccacctg gagcggatcg gcagctgcag ccggggagtg  720
agcaagcagc ggatcatcgg cgtgggcgaa gtgctggacc ggggcgacga agtgcccagc  780
ctgttcatga ccaacgtgtg gacccccccc aaccccaaca ccgtgtacca ctgcagcgcc  840
gtgtacaaca acgagttcta ctacgtgctg tgcgccgtga gcaccgtggg cgaccccatc  900
ctgaacagca cctactggag cggcagcctg atgatgaccc ggctggccgt gaagcctaag  960
agcaacggcg gaggctacaa ccagcaccag ctggccctgc ggagcatcga gaagggccgg  1020
tacgacaaag tgatgcccta cggccccagc ggcatcaagc agggcgacac cctgtacttc  1080
cccgccgtgg gcttcctggt gcggaccgag ttcaagtaca acgacagcaa ctgccccatc  1140
accaagtgcc agtacagcaa gcccgagaac tgccggctga gcatgggcat ccggcccaac  1200
agccactaca tcctgcggag cggcctgctg aagtacaacc tgagcgacgg cgagaacccc  1260
aaagtggtgt tcatcgagat cagcgaccag agactgagca tcggcagccc cagcaagatc  1320
tacgacagcc tgggccagcc cgtgttctac caggccagct tcagctggga caccatgatc  1380
aagttcggcg acgtgctgac cgtgaacccc ctggtggtga actggcggaa caataccgtg  1440
atcagcagac ccggccagag ccagtgcccc cggttcaaca cctgccccga gatctgctgg  1500
gagggcgtgt acaacgacgc cttcctgatc gaccggatca actggatcag cgccggagtg  1560
ttcctggata gcaaccagac cgccgagaat cccgtgttca ccgtgtttaa ggacaacgag  1620
atcctgtaca gagcccagct ggccagcgag gacaccaacg cccagaaaac catcaccaac  1680
tgcttcctgc tgaagaataa gatctggtgc atcagcctgg tggagatcta cgataccggc  1740
gacaacgtga tcaggcccaa gctgttcgcc gtgaagatcc ccgagcagtg ctacccctac  1800
gacgtgcccg actacgcctg a                                             1821

SEQ ID NO: 15          moltype = AA  length = 601
FEATURE                Location/Qualifiers
REGION                 1..601
                       note = Variant peptide
source                 1..601
                       mol_type = protein
                       organism = synthetic construct
SEQ ID NO: 15
MGNTTSDKGK IPSKVIKSYY GTMDIKKINE GLLDSKILSA FNTVIALLGS IVIIVMNIMI  60
IQNYTRSTDN QAVIKDALQG IQQQIKGLAD KIGTEIGPKV SLIDTSSTIT IPANIGLLGS  120
KISQSTASIN ENVNEKCKFT LPPLKIHECN ISCPNPLPFR EYRPQTEGVS NLVGLPNNIC  180
LQKTSNQILK PKLISYTLPV VGQSGTCITD PLLAMDEGYF AYSHLERIGS CSRGVSKQRI  240
IGVGEVLDRG DEVPSLFMTN VWTPPNPNTV YHCSAVYNNE FYYVLCAVST VGDPILNSTY  300
WSGSLMMTRL AVKPKSNGGG YNQHQLALRS IEKGRYDKVM PYGPSGIKQG DTLYFPAVGF  360
LVRTEFKYND SNCPITKCQY SKPENCRLSM GIRPNSHYIL RSGLLKYNLS DGENPKVVFI  420
```

-continued

```
EISDQRLSIG SPSKIYDSLG QPVFYQASFS WDTMIKFGDV LTVNPLVVNW RNNTVISRPG  480
QSQCPRFNTC PEICWEGVYN DAFLIDRINW ISAGVFLDSN QTAENPVFTV FKDNEILYRA  540
QLASEDTNAQ KTITNCFLLK NKIWCISLVE IYDTGDNVIR PKLFAVKIPE QCYPYDVPDY  600
A                                                                  601

SEQ ID NO: 16          moltype = DNA  length = 1806
FEATURE                Location/Qualifiers
misc_feature           1..1806
                       note = Variant nucleotide
source                 1..1806
                       mol_type = other DNA
                       organism = synthetic construct
SEQ ID NO: 16
atgggaaaca ccacaagcga caagggcaag atccccagca aagtgatcaa gagctactac  60
ggcaccatgg acatcaagaa gatcaacgag ggcctgctgg acagcaagat cctgagcgcc  120
ttcaacaccg tgatcgccct gctgggcagc atcgtgatca ttgtgatgaa catcatgatc  180
atccagaact acacccggag caccgacaac caggccgtga tcaaggacgc cctgcaggga  240
atccagcagc agatcaaggg cctggccgac aagatcggca ccgagatcgg ccccaaagtg  300
agcctgatcg acaccagcag caccatcacc atccccgcca acatcggcct gctgggatcc  360
aagatcagcc agagcaccgc cagcatcaac gagaacgtga acgagaagtg caagttcacc  420
ctgccccccc tgaagatcca cgagtgcaac atcagctgcc ccaacccccc gcccttccgg  480
gagtaccggc cccagaccga gggcgtgagc aacctggtgg gcctgcccaa caacatctgc  540
ctgcagaaaa ccagcaacca gatcctgaag cccaagctga tctcctacac cctgcccgtg  600
gtgggccaga gcggcacctg catcaccgac cccctgctgg ccatggacga gggctacttc  660
gcctacagcc acctggagcg gatcggcagc tgcagccggg gagtgagcaa gcagcggatc  720
atcggcgtgg gcgaagtgct ggaccggggc gacgaagtgc ccagcctgtt catgaccaac  780
gtgtggaccc cccccaaccc caacaccgtg taccactgca gcgccgtgta caacaacgag  840
ttctactacg tgctgtgcgc cgtgagcacc gtgggcgacc ccatcctgaa cagcacctac  900
tggagcggca gcctgatgat gacccggctg gccgtgaagc ctaagagcaa cggcggaggc  960
tacaaccagc accagctggc cctgcggagc atcgagaagg gccggtacga caaagtgatg  1020
ccctacggcc ccagcggcat caagcagggc gacaccctgt acttccccgc cgtgggcttc  1080
ctggtgcgga ccgagttcaa gtacaacgac agcaactgcc ccatcaccaa gtgccagtac  1140
agcaagcccg agaactgccg gctgagcatg ggcatccggc ccaacagcca ctacatcctg  1200
cggagcggcc tgctgaagta caacctgagc gacggcgaga accccaaagt ggtgttcatc  1260
gagatcagcg accagagact gagcatcggc agccccagca agatctacga cagcctgggc  1320
cagcccgtgt tctaccaggc cagcttcagc tgggacacca tgatcaagtt cggcgacgtg  1380
ctgaccgtga acccccctggt ggtgaactgg cggaacaata ccgtgatcag cagacccggc  1440
cagagccagt gccccccggtt caacacctgc cccgagatct gctgggaggg cgtgtacaac  1500
gacgccttcc tgatcgaccg gatcaactgg atcagcgccg gagtgttcct ggatagcaac  1560
cagaccgccg agaatcccgt gttcaccgtg tttaaggaca acgagatcct gtacagagcc  1620
cagctggcca gcgaggacac caacgcccag aaaaccatca ccaactgctt cctgctgaag  1680
aataagatct ggtgcatcag cctggtggag atctacgata ccggcgacaa cgtgatcagg  1740
cccaagctgt tcgccgtgaa gatccccgag cagtgctacc cctacgacgt gcccgactac  1800
gcctga                                                             1806

SEQ ID NO: 17          moltype = AA  length = 596
FEATURE                Location/Qualifiers
REGION                 1..596
                       note = Variant peptide
source                 1..596
                       mol_type = protein
                       organism = synthetic construct
SEQ ID NO: 17
MGKGKIPSKV IKSYYGTMDI KKINEGLLDS KILSAFNTVI ALLGSIVIIV MNIMIIQNYT  60
RSTDNQAVIK DALQGIQQQI KGLADKIGTE IGPKVSLIDT SSTITIPANI GLLGSKISQS  120
TASINENVNE KCKFTLPPLK IHECNISCPN PLPFREYRPQ TEGVSNLVGL PNNICLQKTS  180
NQILKPKLIS YTLPVVGQSG TCITDPLLAM DEGYFAYSHL ERIGSCSRGV SKQRIIGVGE  240
VLDRGDEVPS LFMTNVWTPP NPNTVYHCSA VYNNEFYYVL CAVSTVGDPI LNSTYWSGSL  300
MMTRLAVKPK SNGGGYNQHQ LALRSIEKGR YDKVMPYGPS GIKQGDTLYF PAVGFLVRTE  360
FKYNDSNCPI TKCQYSKPEN CRLSMGIRPN SHYILRSGLL KYNLSDGENP KVVFIEISDQ  420
RLSIGSPSKI YDSLGQPVFY QASFSWDTMI KFGDVLTVNP LVVNWRNNTV ISRPGQSQCP  480
RFNTCPEICW EGVYNDAFLI DRINWISAGV FLDSNQTAEN PVFTVFKDNE ILYRAQLASE  540
DTNAQKTITN CFLLKNKIWC ISLVEIYDTG DNVIRPKLFA VKIPEQCYPY DVPDYA      596

SEQ ID NO: 18          moltype = DNA  length = 1791
FEATURE                Location/Qualifiers
misc_feature           1..1791
                       note = Variant nucleotide
source                 1..1791
                       mol_type = other DNA
```

-continued

organism = synthetic construct

SEQ ID NO: 18

```
atgggaaagg gcaagatccc cagcaaagtg atcaagagct actacggcac catggacatc  60
aagaagatca acgagggcct gctggacagc aagatcctga gcgccttcaa caccgtgatc  120
gccctgctgg gcagcatcgt gatcattgtg atgaacatca tgatcatcca gaactacacc  180
cggagcaccg acaaccaggc cgtgatcaag gacgccctgc agggaatcca gcagcagatc  240
aagggcctgg ccgacaagat cggcaccgag atcggcccca aagtgagcct gatcgacacc  300
agcagcacca tcaccatccc cgccaacatc ggcctgctgg gatccaagat cagccagagc  360
accgccagca tcaacgagaa cgtgaacgag aagtgcaagt tcaccctgcc ccccctgaag  420
atccacgagt gcaacatcag ctgccccaac cccctgccct tccgggagta ccggccccag  480
accgagggcg tgagcaacct ggtgggcctg cccaacaaca tctgcctgca gaaaaccagc  540
aaccagatcc tgaagcccaa gctgatctcc tacaccctgc ccgtggtggg ccagagcggc  600
acctgcatca ccgacccccct gctggccatg gacgagggct acttcgccta cagccacctg  660
gagcggatcg gcagctgcag ccggggagtg agcaagcagc ggatcatcgg cgtgggcgaa  720
gtgctggacc ggggcgacga agtgcccagc ctgttcatga ccaacgtgtg gacccccccc  780
aaccccaaca ccgtgtacca ctgcagcgcc gtgtacaaca acgagttcta ctacgtgctg  840
tgcgccgtga gcaccgtggg cgaccccatc ctgaacagca cctactggag cggcagcctg  900
atgatgaccc ggctggccgt gaagcctaag agcaacggcg gaggctacaa ccagcaccag  960
ctggccctgc ggagcatcga gaagggccgg tacgacaaag tgatgcccta cggccccagc  1020
ggcatcaagc agggcgacac cctgtacttc cccgccgtgg gcttcctggt gcggaccgag  1080
ttcaagtaca acgacagcaa ctgccccatc accaagtgcc agtacagcaa gcccgagaac  1140
tgccggctga gcatgggcat ccggcccaac agccactaca tcctgcggag cggcctgctg  1200
aagtacaacc tgagcgacgg cgagaacccc aaagtggtgt tcatcgagat cagcgaccag  1260
agactgagca tcggcagccc cagcaagatc tacgacagcc tgggccagcc cgtgttctac  1320
caggccagct tcagctggga caccatgatc aagttcggcg acgtgctgac cgtgaacccc  1380
ctggtggtga actggcggaa caataccgtg atcagcagac ccggccagag ccagtgcccc  1440
cggttcaaca cctgccccga gatctgctgg gagggcgtgt acaacgacgc cttcctgatc  1500
gaccggatca actggatcag cgccggagtg ttcctggata gcaaccagac cgccgagaat  1560
cccgtgttca ccgtgtttaa ggacaacgag atcctgtaca gagcccagct ggccagcgag  1620
gacaccaacg cccagaaaac catcaccaac tgcttcctgc tgaagaataa gatctggtgc  1680
atcagcctgg tggagatcta cgataccggc gacaacgtga tcaggcccaa gctgttcgcc  1740
gtgaagatcc ccgagcagtg ctacccctac gacgtgcccg actacgcctg a            1791
```

SEQ ID NO: 19 moltype = AA length = 591

FEATURE Location/Qualifiers

REGION 1..591
note = Variant peptide

source 1..591
mol_type = protein
organism = synthetic construct

SEQ ID NO: 19

```
MGSKVIKSYY GTMDIKKINE GLLDSKILSA FNTVIALLGS IVIIVMNIMI IQNYTRSTDN  60
QAVIKDALQG IQQQIKGLAD KIGTEIGPKV SLIDTSSTIT IPANIGLLGS KISQSTASIN  120
ENVNEKCKFT LPPLKIHECN ISCPNPLPFR EYRPQTEGVS NLVGLPNNIC LQKTSNQILK  180
PKLISYTLPV VGQSGTCITD PLLAMDEGYF AYSHLERIGS CSRGVSKQRI IGVGEVLDRG  240
DEVPSLFMTN VWTPPNPNTV YHCSAVYNNE FYYVLCAVST VGDPILNSTY WSGSLMMTRL  300
AVKPKSNGGG YNQHQLALRS IEKGRYDKVM PYGPSGIKQG DTLYFPAVGF LVRTEFKYND  360
SNCPITKCQY SKPENCRLSM GIRPNSHYIL RSGLLKYNLS DGENPKVVFI EISDQRLSIG  420
SPSKIYDSLG QPVFYQASFS WDTMIKFGDV LTVNPLVVNW RNNTVISRPG QSQCPRFNTC  480
PEICWEGVYN DAFLIDRINW ISAGVFLDSN QTAENPVFTV FKDNEILYRA QLASEDTNAQ  540
KTITNCFLLK NKIWCISLVE IYDTGDNVIR PKLFAVKIPE QCYPYDVPDY A           591
```

SEQ ID NO: 20 moltype = DNA length = 1776

FEATURE Location/Qualifiers

misc_feature 1..1776
note = Variant nucleotide

source 1..1776
mol_type = other DNA
organism = synthetic construct

SEQ ID NO: 20

```
atgggaagca aagtgatcaa gagctactac ggcaccatgg acatcaagaa gatcaacgag  60
ggcctgctgg acagcaagat cctgagcgcc ttcaacaccg tgatcgccct gctgggcagc  120
atcgtgatca ttgtgatgaa catcatgatc atccagaact acacccggag caccgacaac  180
caggccgtga tcaaggacgc cctgcaggga atccagcagc agatcaaggg cctggccgac  240
aagatcggca ccgagatcgg ccccaaagtg agcctgatcg acaccagcag caccatcacc  300
atccccgcca acatcggcct gctgggatcc aagatcagcc agagcaccgc cagcatcaac  360
gagaacgtga acgagaagtg caagttcacc ctgcccccccc tgaagatcca cgagtgcaac  420
atcagctgcc ccaacccccct gcccttccgg gagtaccggc cccagaccga gggcgtgagc  480
aacctggtgg gcctgcccaa caacatctgc ctgcagaaaa ccagcaacca gatcctgaag  540
cccaagctga tctcctacac cctgcccgtg gtgggccaga gcggcacctg catcaccgac  600
```

-continued

```
cccctgctgg ccatggacga gggctacttc gcctacagcc acctggagcg gatcggcagc  660
tgcagccggg gagtgagcaa gcagcggatc atcggcgtgg gcgaagtgct ggaccggggc  720
gacgaagtgc ccagcctgtt catgaccaac gtgtggaccc cccccaaccc caacaccgtg  780
taccactgca gcgccgtgta caacaacgag ttctactacg tgctgtgcgc cgtgagcacc  840
gtgggcgacc ccatcctgaa cagcacctac tggagcggca gcctgatgat gacccggctg  900
gccgtgaagc ctaagagcaa cggcggaggc tacaaccagc accagctggc cctgcggagc  960
atcgagaagg gccggtacga caaagtgatg ccctacggcc ccagcggcat caagcagggc  1020
gacaccctgt acttccccgc cgtgggcttc ctggtgcgga ccgagttcaa gtacaacgac  1080
agcaactgcc ccatcaccaa gtgccagtac agcaagcccg agaactgccg gctgagcatg  1140
ggcatccggc ccaacagcca ctacatcctg cggagcggcc tgctgaagta caacctgagc  1200
gacggcgaga accccaaagt ggtgttcatc gagatcagcg accagagact gagcatcggc  1260
agccccagca agatctacga cagcctgggc cagcccgtgt tctaccaggc cagcttcagc  1320
tgggacacca tgatcaagtt cggcgacgtg ctgaccgtga acccccctggt ggtgaactgg  1380
cggaacaata ccgtgatcag cagacccggc cagagccagt gcccccggtt caacacctgc  1440
cccgagatct gctgggaggg cgtgtacaac gacgccttcc tgatcgaccg gatcaactgg  1500
atcagcgccg gagtgttcct ggatagcaac cagaccgccg agaatcccgt gttcaccgtg  1560
tttaaggaca acgagatcct gtacagagcc cagctggcca gcgaggacac caacgcccag  1620
aaaaccatca ccaactgctt cctgctgaag aataagatct ggtgcatcag cctggtggag  1680
atctacgata ccggcgacaa cgtgatcagg cccaagctgt tcgccgtgaa gatccccgag  1740
cagtgctacc cctacgacgt gcccgactac gcctga                             1776

SEQ ID NO: 21          moltype = AA  length = 586
FEATURE                Location/Qualifiers
REGION                 1..586
                       note = Variant peptide
source                 1..586
                       mol_type = protein
                       organism = synthetic construct
SEQ ID NO: 21
MGSYYGTMDI KKINEGLLDS KILSAFNTVI ALLGSIVIIV MNIMIIQNYT RSTDNQAVIK  60
DALQGIQQQI KGLADKIGTE IGPKVSLIDT SSTITIPANI GLLGSKISQS TASINENVNE  120
KCKFTLPPLK IHECNISCPN PLPFREYRPQ TEGVSNLVGL PNNICLQKTS NQILKPKLIS  180
YTLPVVGQSG TCITDPLLAM DEGYFAYSHL ERIGSCSRGV SKQRIIGVGE VLDRGDEVPS  240
LFMTNVWTPP NPNTVYHCSA VYNNEFYYVL CAVSTVGDPI LNSTYWSGSL MMTRLAVKPK  300
SNGGGYNQHQ LALRSIEKGR YDKVMPYGPS GIKQGDTLYF PAVGFLVRTE FKYNDSNCPI  360
TKCQYSKPEN CRLSMGIRPN SHYILRSGLL KYNLSDGENP KVVFIEISDQ RLSIGSPSKI  420
YDSLGQPVFY QASFSWDTMI KFGDVLTVNP LVVNWRNNTV ISRPGQSQCP RFNTCPEICW  480
EGVYNDAFLI DRINWISAGV FLDSNQTAEN PVFTVFKDNE ILYRAQLASE DTNAQKTITN  540
CFLLKNKIWC ISLVEIYDTG DNVIRPKLFA VKIPEQCYPY DVPDYA                  586

SEQ ID NO: 22          moltype = DNA  length = 1761
FEATURE                Location/Qualifiers
misc_feature           1..1761
                       note = Variant nucleotide
source                 1..1761
                       mol_type = other DNA
                       organism = synthetic construct
SEQ ID NO: 22
atgggaagct actacggcac catggacatc aagaagatca acgagggcct gctggacagc  60
aagatcctga gcgccttcaa caccgtgatc gccctgctgg gcagcatcgt gatcattgtg  120
atgaacatca tgatcatcca gaactacacc cggagcaccg acaaccaggc cgtgatcaag  180
gacgccctgc agggaatcca gcagcagatc aagggcctgg ccgacaagat cggcaccgag  240
atcggcccca aagtgagcct gatcgacacc agcagcacca tcaccatccc cgccaacatc  300
ggcctgctgg gatccaagat cagccagagc accgccagca tcaacgagaa cgtgaacgag  360
aagtgcaagt tcaccctgcc ccccctgaag atccacgagt gcaacatcag ctgccccaac  420
cccctgccct tccgggagta ccggccccag accgagggcg tgagcaacct ggtgggcctg  480
cccaacaaca tctgcctgca gaaaaccagc aaccagatcc tgaagcccaa gctgatctcc  540
tacaccctgc ccgtggtggg ccagagcggc acctgcatca ccgacccccct gctggccatg  600
gacgagggct acttcgccta cagccacctg gagcggatcg gcagctgcag ccggggagtg  660
agcaagcagc ggatcatcgg cgtgggcgaa gtgctggacc ggggcgacga agtgcccagc  720
ctgttcatga ccaacgtgtg gacccccccc aaccccaaca ccgtgtacca ctgcagcgcc  780
gtgtacaaca acgagttcta ctacgtgctg tgcgccgtga gcaccgtggg cgacccccatc  840
ctgaacagca cctactggag cggcagcctg atgatgaccc ggctggccgt gaagcctaag  900
agcaacggcg gaggctacaa ccagcaccag ctggccctgc ggagcatcga gaagggccgg  960
tacgacaaag tgatgcccta cggccccagc ggcatcaagc agggcgacac cctgtacttc  1020
cccgccgtgg gcttcctggt gcggaccgag ttcaagtaca acgacagcaa ctgccccatc  1080
accaagtgcc agtacagcaa gcccgagaac tgccggctga gcatgggcat ccggcccaac  1140
agccactaca tcctgcggag cggcctgctg aagtacaacc tgagcgacgg cgagaacccc  1200
aaagtggtgt tcatcgagat cagcgaccag agactgagca tcggcagccc cagcaagatc  1260
tacgacagcc tgggccagcc cgtgttctac caggccagct tcagctggga caccatgatc  1320
```

-continued

```
aagttcggcg acgtgctgac cgtgaacccc ctggtggtga actggcggaa caataccgtg  1380
atcagcagac ccggccagag ccagtgcccc cggttcaaca cctgccccga gatctgctgg  1440
gagggcgtgt acaacgacgc cttcctgatc gaccggatca actggatcag cgccggagtg  1500
ttcctggata gcaaccagac cgccgagaat cccgtgttca ccgtgtttaa ggacaacgag  1560
atcctgtaca gagcccagct ggccagcgag gacaccaacg cccagaaaac catcaccaac  1620
tgcttcctgc tgaagaataa gatctggtgc atcagcctgg tggagatcta cgataccggc  1680
gacaacgtga tcaggcccaa gctgttcgcc gtgaagatcc ccgagcagtg ctacccctac  1740
gacgtgcccg actacgcctg a                                            1761

SEQ ID NO: 23          moltype = AA  length = 581
FEATURE                Location/Qualifiers
REGION                 1..581
                       note = Variant peptide
source                 1..581
                       mol_type = protein
                       organism = synthetic construct
SEQ ID NO: 23
GTMDIKKINE GLLDSKILSA FNTVIALLGS IVIIVMNIMI IQNYTRSTDN QAVIKDALQG  60
IQQQIKGLAD KIGTEIGPKV SLIDTSSTIT IPANIGLLGS KISQSTASIN ENVNEKCKFT  120
LPPLKIHECN ISCPNPLPFR EYRPQTEGVS NLVGLPNNIC LQKTSNQILK PKLISYTLPV  180
VGQSGTCITD PLLAMDEGYF AYSHLERIGS CSRGVSKQRI IGVGEVLDRG DEVPSLFMTN  240
VWTPPNPNTV YHCSAVYNNE FYYVLCAVST VGDPILNSTY WSGSLMMTRL AVKPKSNGGG  300
YNQHQLALRS IEKGRYDKVM PYGPSGIKQG DTLYFPAVGF LVRTEFKYND SNCPITKCQY  360
SKPENCRLSM GIRPNSHYIL RSGLLKYNLS DGENPKVVFI EISDQRLSIG SPSKIYDSLG  420
QPVFYQASFS WDTMIKFGDV LTVNPLVVNW RNNTVISRPG QSQCPRFNTC PEICWEGVYN  480
DAFLIDRINW ISAGVFLDSN QTAENPVFTV FKDNEILYRA QLASEDTNAQ KTITNCFLLK  540
NKIWCISLVE IYDTGDNVIR PKLFAVKIPE QCYPYDVPDY A                      581

SEQ ID NO: 24          moltype = DNA  length = 1746
FEATURE                Location/Qualifiers
misc_feature           1..1746
                       note = Variant nucleotide
source                 1..1746
                       mol_type = other DNA
                       organism = synthetic construct
SEQ ID NO: 24
ggcaccatgg acatcaagaa gatcaacgag ggcctgctgg acagcaagat cctgagcgcc  60
ttcaacaccg tgatcgccct gctgggcagc atcgtgatca ttgtgatgaa catcatgatc  120
atccagaact acacccggag caccgacaac caggccgtga tcaaggacgc cctgcaggga  180
atccagcagc agatcaaggg cctggccgac aagatcggca ccgagatcgg ccccaaagtg  240
agcctgatcg acaccagcag caccatcacc atccccgcca acatcggcct gctgggatcc  300
aagatcagcc agagcaccgc cagcatcaac gagaacgtga acgagaagtg caagttcacc  360
ctgccccccc tgaagatcca cgagtgcaac atcagctgcc ccaaccccct gcccttccgg  420
gagtaccggc cccagaccga gggcgtgagc aacctggtgg gcctgcccaa caacatctgc  480
ctgcagaaaa ccagcaacca gatcctgaag cccaagctga tctcctacac cctgcccgtg  540
gtgggccaga gcggcacctg catcaccgac cccctgctgg ccatggacga gggctacttc  600
gcctacagcc acctggagcg gatcggcagc tgcagccggg gagtgagcaa gcagcggatc  660
atcggcgtgg gcgaagtgct ggaccggggc gacgaagtgc ccagcctgtt catgaccaac  720
gtgtggaccc cccccaaccc caacaccgtg taccactgca gcgccgtgta caacaacgag  780
ttctactacg tgctgtgcgc cgtgagcacc gtgggcgacc ccatcctgaa cagcacctac  840
tggagcggca gcctgatgat gacccggctg gccgtgaagc ctaagagcaa cggcggaggc  900
tacaaccagc accagctggc cctgcggagc atcgagaagg gccggtacga caaagtgatg  960
ccctacggcc ccagcggcat caagcagggc gacaccctgt acttccccgc cgtgggcttc  1020
ctggtgcgga ccgagttcaa gtacaacgac agcaactgcc ccatcaccaa gtgccagtac  1080
agcaagcccg agaactgccg gctgagcatg ggcatccggc ccaacagcca ctacatcctg  1140
cggagcggcc tgctgaagta caacctgagc gacggcgaga accccaaagt ggtgttcatc  1200
gagatcagcg accagagact gagcatcggc agccccagca agatctacga cagcctgggc  1260
cagcccgtgt tctaccaggc cagcttcagc tgggacacca tgatcaagtt cggcgacgtg  1320
ctgaccgtga acccccctggt ggtgaactgg cggaacaata ccgtgatcag cagacccggc  1380
cagagccagt gcccccggtt caacacctgc cccgagatct gctgggaggg cgtgtacaac  1440
gacgccttcc tgatcgaccg gatcaactgg atcagcgccg gagtgttcct ggatagcaac  1500
cagaccgccg agaatcccgt gttcaccgtg tttaaggaca acgagatcct gtacagagcc  1560
cagctggcca gcgaggacac caacgcccag aaaaccatca ccaactgctt cctgctgaag  1620
aataagatct ggtgcatcag cctggtggag atctacgata ccggcgacaa cgtgatcagg  1680
cccaagctgt tcgccgtgaa gatccccgag cagtgctacc cctacgacgt gcccgactac  1740
gcctga                                                            1746
```

What is claimed is:

1-11. (canceled)

12. A method of delivering a nucleic acid to a cell, the method comprising contacting a Nipah virus (NiV) envelope pseudotyped lentivirus particle comprising a nucleic acid for delivery with cells,

wherein the NiV pseudotyped lentivirus particle comprises Nipah Virus fusion (NiV-F) and Nipah Virus attachment (NiV-G) proteins,

wherein the NiV-F protein comprises a deletion in its cytoplasmic tail relative to a wild type NiV-F sequence, wherein the NiV-F protein comprises a cytoplasmic tail lacking amino residues 525-546 of SEQ ID NO: 1,

wherein the NiV-G protein is wild-type NiV-G or is a NiV-G protein that has a cytoplasmic tail truncation comprising a deletion in its cytoplasmic tail relative to wild type NiV-G sequence,

and wherein the NiV envelope pseudotyped lentivirus particle has increased viral transduction titer compared to a lentivirus particle pseudotyped with wild-type NiV-F and wild-type NiV-G.

13. The method of claim **12**, wherein the contacting is in vitro.

14. The method of claim **12**, wherein the contacting is in vivo.

15. A method of delivering a nucleic acid to a cell, the method comprising administering a therapeutically effective amount of a NiV pseudotyped lentivirus particle comprising a nucleic acid for delivery to a subject,

wherein the NiV pseudotyped lentivirus particle comprises Nipah Virus fusion (NiV-F) and Nipah Virus attachment (NiV-G) proteins,

wherein the NiV-F protein comprises a deletion in its cytoplasmic tail relative to a wild type NiV-F sequence, wherein the NiV-F protein comprises a cytoplasmic tail lacking amino residues 525-546 of SEQ ID NO: 1,

wherein the NiV-G protein is wild-type NiV-G or is a NiV-G protein that has a cytoplasmic tail truncation comprising a deletion in its cytoplasmic tail relative to wild type NiV-G sequence,

and wherein the NiV envelope pseudotyped lentivirus particle has increased viral transduction titer compared to a lentivirus particle pseudotyped with wild-type NiV-F and wild-type NiV-G.

16. The method of claim **12**, wherein the NiV-G protein comprises a deletion of at least 10 contiguous amino acid residues from the cytoplasmic tail.

17. The method of claim **12**, wherein the NiV-G protein comprises a deletion of at least 15 contiguous amino acid residues from the cytoplasmic tail.

18. The method of claim **12**, wherein the NiV-G protein comprises a deletion of at least 20 contiguous amino acid residues from the cytoplasmic tail.

19. The method of claim **12**, wherein the NiV-G protein comprises a deletion of at least 30 contiguous amino acid residues from the cytoplasmic tail.

20. The method of claim **12**, wherein the NiV-F protein or the NiV-G protein further comprises a hyperfusogenic mutation.

21. The method of claim **12**, wherein the NiV-G protein further comprises a mutation that abrogates ephrinB2 and B3 binding.

22. The method of claim **12**, wherein the nucleic acid for delivery encodes a payload chosen from: a gene therapy payload, a payload that is toxic to the cell, or an ephrin antagonist.

23. The method of claim **12**, wherein the NiV-G comprises a single chain variable fragment (scFV) directed against a cell surface molecule other than ephrinB2 and/or B3.

24. The method of claim **12**, wherein the viral transduction titer is increased greater than 2-fold.

25. The method of claim **15**, wherein the administration of the NiV envelope pseudotyped lentivirus particle comprising the nucleic acid effective for treating cancer.

26. The method of claim **15**, wherein the NiV envelope pseudotyped lentivirus particle is administered intravenously.

27. A pharmaceutical composition comprising a Nipah virus (NiV) envelope pseudotyped lentivirus particle, wherein the NiV pseudotyped lentivirus particle comprises Nipah Virus fusion (NiV-F) and Nipah Virus attachment (NiV-G) proteins,

wherein the NiV-F protein comprises a deletion in its cytoplasmic tail relative to a wild type NiV-F sequence, wherein the NiV-F protein comprises a cytoplasmic tail lacking amino residues 525-546 of SEQ ID NO: 1,

wherein the NiV-G protein is wild-type NiV-G or is a NiV-G protein that has a cytoplasmic tail truncation comprising a deletion in its cytoplasmic tail relative to wild type NiV-G sequence,

and wherein the NiV envelope pseudotyped lentivirus particle has increased viral transduction titer compared to a lentivirus particle pseudotyped with wild-type NiV-F and wild-type NiV-G.

28. The composition of claim **27**, wherein the NiV-G protein comprises a deletion of at least 10 contiguous amino acid residues from the cytoplasmic tail.

29. The composition of claim **27**, wherein the NiV-G protein comprises a deletion of at least 15 contiguous amino acid residues from the cytoplasmic tail.

30. The composition of claim **27**, wherein the NiV-G protein comprises a deletion of at least 20 contiguous amino acid residues from the cytoplasmic tail.

31. The composition of claim **27**, wherein the NiV-G protein comprises a deletion of at least 30 contiguous amino acid residues from the cytoplasmic tail.

32. The composition of claim **27**, wherein the NiV-F protein or the NiV-G protein further comprises a hyperfusogenic mutation.

33. The composition of claim **27**, wherein the NiV-G protein further comprises a mutation that abrogates ephrinB2 and B3 binding.

34. The composition of claim **27**, wherein the NiV-G comprises a single chain variable fragment (scFV) directed against a cell surface molecule other than ephrinB2 and/or B3.

35. The composition of claim **27**, wherein the viral transduction titer is increased greater than 2-fold.

36. A method of administering the pharmaceutical composition of claim **27**, the method comprising systemically administering a therapeutically effective amount of the NiV pseudotyped lentivirus particle to a subject in need thereof.

37. A method of producing a Nipah virus (NiV) envelope pseudotyped lentivirus particle, the method comprising culturing producer cells comprising one or more nucleic acids for the production of the lentiviral particle under conditions for producing the lentiviral particle by the host cells,

wherein the NiV pseudotyped lentivirus particle comprises Nipah Virus fusion (NiV-F) and Nipah Virus attachment (NiV-G) proteins,

wherein the NiV-F protein comprises a deletion in its cytoplasmic tail relative to a wild type NiV-F sequence,

wherein the NiV-F protein comprises a cytoplasmic tail lacking amino residues 525-546 of SEQ ID NO: 1,

wherein the NiV-G protein is wild-type NiV-G or is a NiV-G protein that has a cytoplasmic tail truncation comprising a deletion in its cytoplasmic tail relative to wild type NiV-G sequence,

and wherein the NiV envelope pseudotyped lentivirus particle has increased viral transduction titer compared to a lentivirus particle pseudotyped with wild-type NiV-F and wild-type NiV-G.

38. The method of claim **37**, wherein the pseudotyped lentivirus particle further comprises a nucleic acid for delivery that encodes a payload chosen from: a gene therapy payload, a payload that is toxic to the cell, or an ephrin antagonist.

39. The method of claim **37**, wherein the NiV-G protein comprises a deletion of at least 10 contiguous amino acid residues from the cytoplasmic tail.

40. The method of claim **37**, wherein the NiV-G protein comprises a deletion of at least 15 contiguous amino acid residues from the cytoplasmic tail.

41. The method of claim **37**, wherein the NiV-G protein comprises a deletion of at least 20 contiguous amino acid residues from the cytoplasmic tail.

42. The method of claim **37**, wherein the NiV-G protein comprises a deletion of at least 30 contiguous amino acid residues from the cytoplasmic tail.

43. The method of claim **37**, wherein the NiV-F protein or the NiV-G protein further comprises a hyperfusogenic mutation.

44. The method of claim **37**, wherein the NiV-G protein further comprises a mutation that abrogates ephrinB2 and B3 binding.

45. The method of claim **37**, wherein the NiV-G comprises a single chain variable fragment (scFV) directed against a cell surface molecule other than ephrinB2 and/or B3.

46. The method of claim **37**, wherein the viral transduction titer is increased greater than 2-fold.

* * * * *