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(54) **METHODS AND COMPOSITIONS FOR THE ACTIVATION OF GAMMA-DELTA T-CELLS**

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(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

The present invention relates generally to methods and compositions for gene therapy and immunotherapy that activate gamma delta T-cells, and in particular, can be used in the treatment of various cancers and infectious diseases.

**16 Claims, 15 Drawing Sheets**

**Specification includes a Sequence Listing.**

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USPTO; Non-Final Office Action dated Feb. 22, 2018 in U.S. Appl. No. 13/333,882.
USPTO; Notice of Allowance dated Mar. 26, 2018 in U.S. Appl. No. 15/668,223.
USPTO; Notice of Allowance dated Apr. 23, 2018 in U.S. Appl. No. 15/850,937.
USPTO; Notice Allowance dated Apr. 26, 2018 in U.S. Appl. No. 15/849,062.
USPTO; Non-Final Office Action dated Jun. 15, 2018 in U.S. Appl. No. 15/904,131.
USPTO; Requirement for Restriction dated Jul. 12, 2018 in U.S. Appl. No. 15/736,284.
USPTO; Invitation to Pay Additional Fees and, Where Applicable, Protest Fee dated Jul. 17, 2018 in Application No. PCT/US2018/25733.
USPTO; Requirement for Restriction dated Aug. 3, 2018 in U.S. Appl. No. 16/011,550.
USPTO; Notice of Allowance dated Aug. 10, 2018 in U.S. Appl. No. 15/904,131.
USPTO; Final Office Action dated Aug. 27, 2018 in U.S. Appl. No. 13/333,882.
USPTO; Non-Final Office Action dated Sep. 19, 2018 in U.S. Appl. No. 16/011,550.
USPTO; Invitation to Pay Additional Fees and, Where Applicable, Protest Fee dated Sep. 11, 2018 in Application No. PCT/US2018/37924.
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USPTO; Notice of Allowance dated Oct. 31, 2018 in U.S. Appl. No. 16/011,550.
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EPO; Extended Search Report dated Dec. 11, 2018 in EP Application No. 16822021.8.
PCT; Invitation to Pay Additional Fees in Application No. PCT/US2018/053919 dated Feb. 22, 2019.
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GenBank Sequence M65141.1 Retrieved from the Internet <URL: https://www.ncbi.ntm.nih.gov/nuccore/M65141.1. Especially Sequence, nt 301-420, (Retrieved Mar. 31, 2019).
PCT; International Search Report dated Apr. 12, 2019 in Application No. PCT/ US2018/053919.
PCT; Written Opinion dated Apr. 12, 2019 in Application No. PCT/US2018/053919.
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USPTO; Non-Final Office Action dated May 24, 2019 in U.S. Appl. No. 16/218,010.
EPO; Extended Search Report dated Jun. 6, 2019 in EP Application No. 17739028.3.

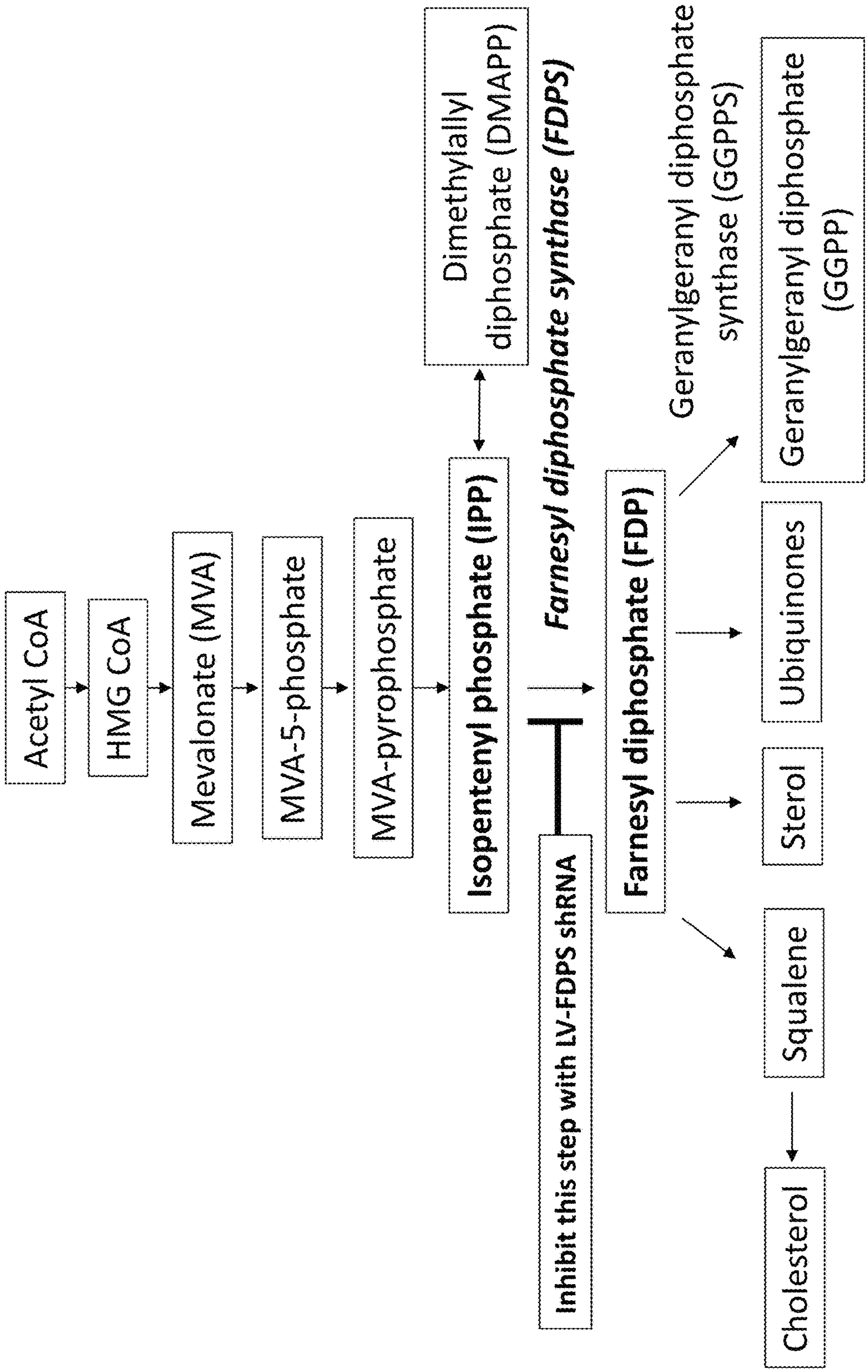
Acetyl CoA
HMG CoA
Mevalonate (MVA)
MVA-5-phosphate
MVA-pyrophosphate
Isopentenyl phosphate (IPP)
Dimethylallyl diphosphate (DMAPP)
Farnesyl diphosphate synthase (FDPS)
Inhibit this step with LV-FDPS shRNA
Farnesyl diphosphate (FDP)
Geranylgeranyl diphosphate synthase (GGPPS)
Geranylgeranyl diphosphate (GGPP)
Ubiquinones
Sterol
Squalene
Cholesterol

Figure 1

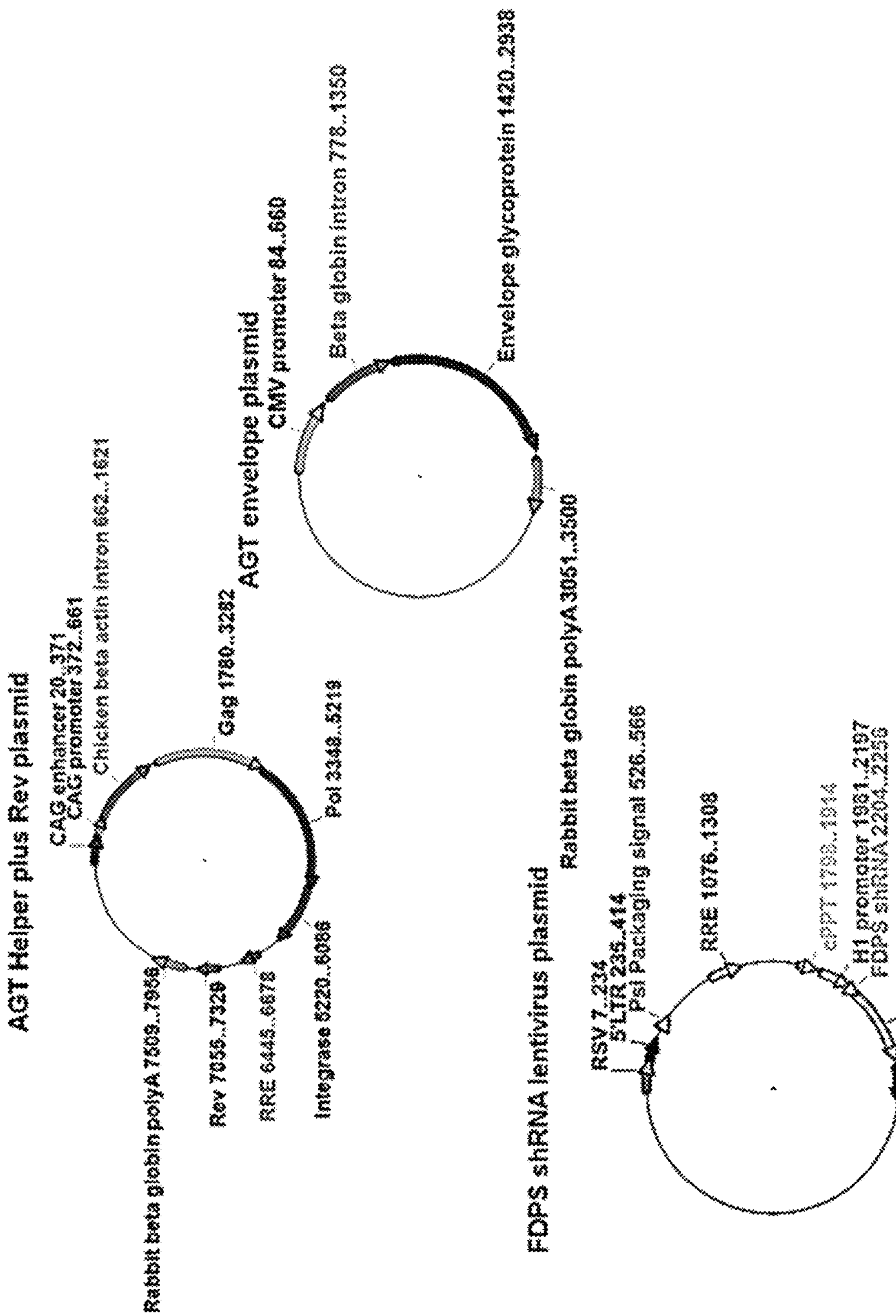
AGT Helper plus Rev plasmid
CAG enhancer 20..371
CAG promoter 372..661
Chicken beta actin intron 662..1621
Gag 1780..3282
Pol 3348..5219
Integrase 5220..6086
RRE 6445..6678
Rev 7055..7326
Rabbit beta globin polyA 7509..7956
AGT envelope plasmid
CMV promoter 84..660
Beta globin intron 778..1350
Envelope glycoprotein 1420..2938
Rabbit beta globin polyA 3051..3500
FDPS shRNA lentivirus plasmid
RSV 7..234
5'LTR 235..414
Psi Packaging signal 526..566
RRE 1076..1308
cPPT 1798..1914
H1 promoter 1951..2197
FDPS shRNA 2204..2256
WPRE 2289..2879
3'delta LTR 2951..3200

Figure 2

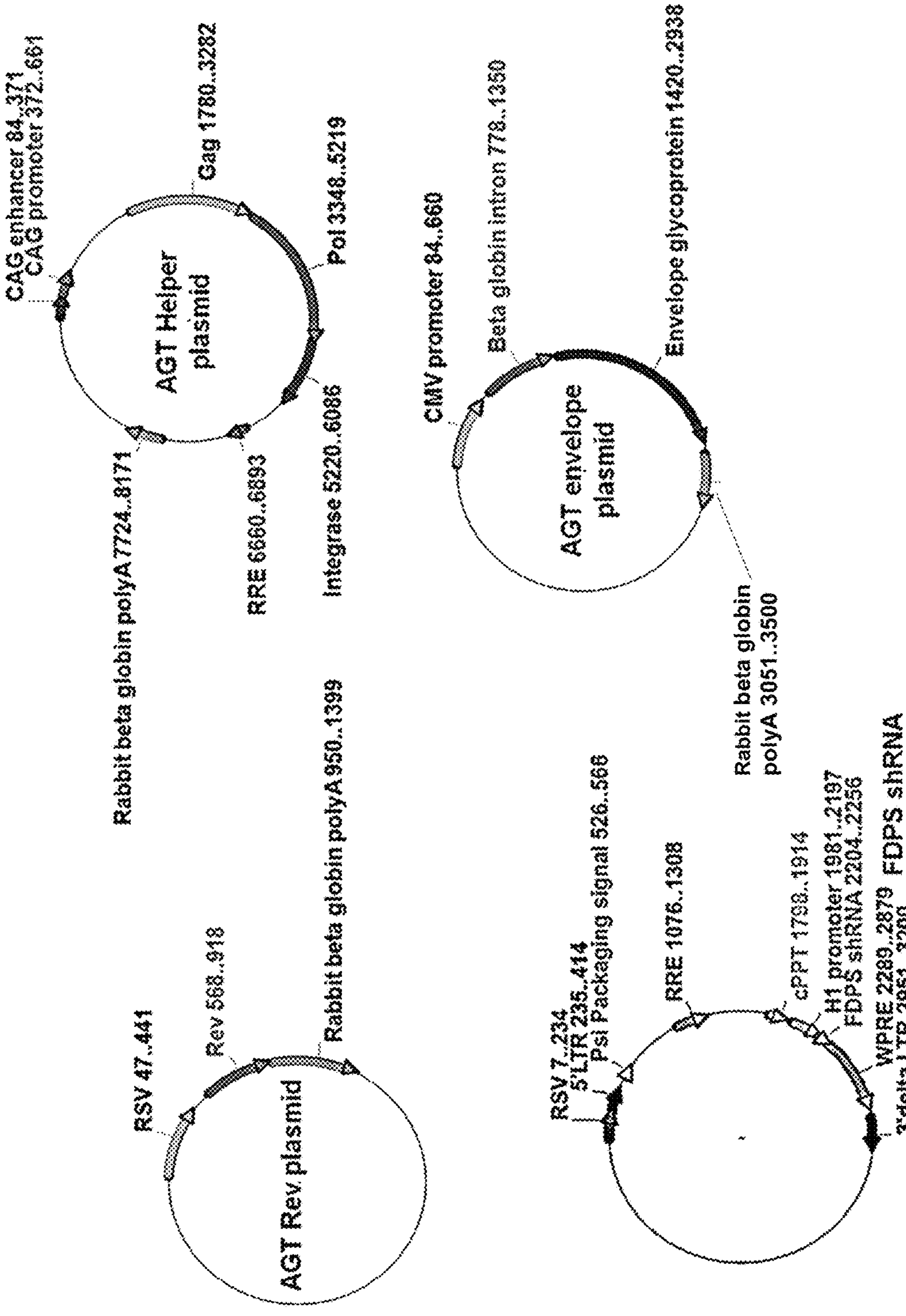
AGT Helper plasmid
CAG enhancer 84..371
CAG promoter 372..661
Gag 1780..3282
Pol 3348..5219
Integrase 5220..6086
RRE 6660..6893
Rabbit beta globin polyA 7724..8171
AGT envelope plasmid
CMV promoter 84..660
Beta globin intron 778..1350
Envelope glycoprotein 1420..2938
Rabbit beta globin polyA 3051..3500
AGT Rev plasmid
RSV 47..441
Rev 568..918
Rabbit beta globin polyA 950..1399
FDPS shRNA lentivirus plasmid
RSV 7..234
5'LTR 235..414
Psi Packaging signal 526..568
RRE 1076..1308
cPPT 1798..1914
H1 promoter 1981..2197
FDPS shRNA 2204..2256
WPRE 2289..2879
3'delta LTR 2951..3200

Figure 3

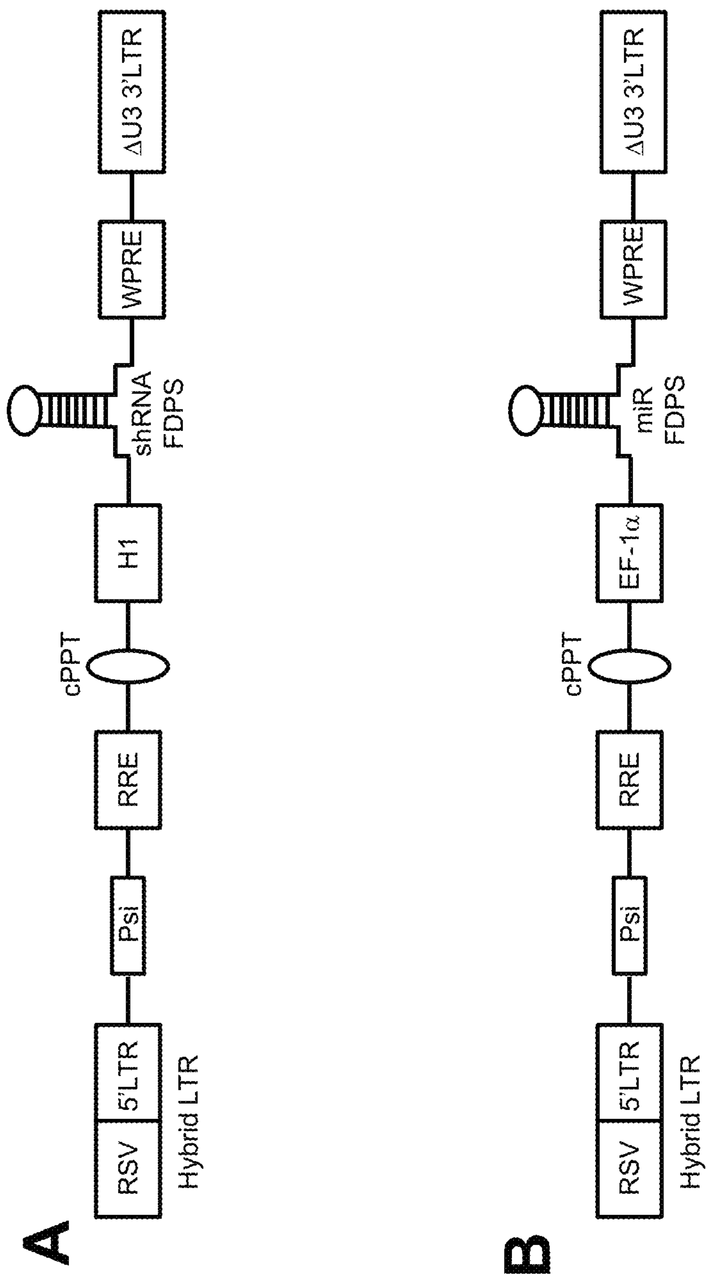
A
RSV
5'LTR
Hybrid LTR
Psi
RRE
cPPT
H1
shRNA
FDPS
WPRE
ΔU3 3'LTR
B
RSV
5'LTR
Hybrid LTR
Psi
RRE
cPPT
EF-1α
miR
FDPS
WPRE
ΔU3 3'LTR

Figure 4

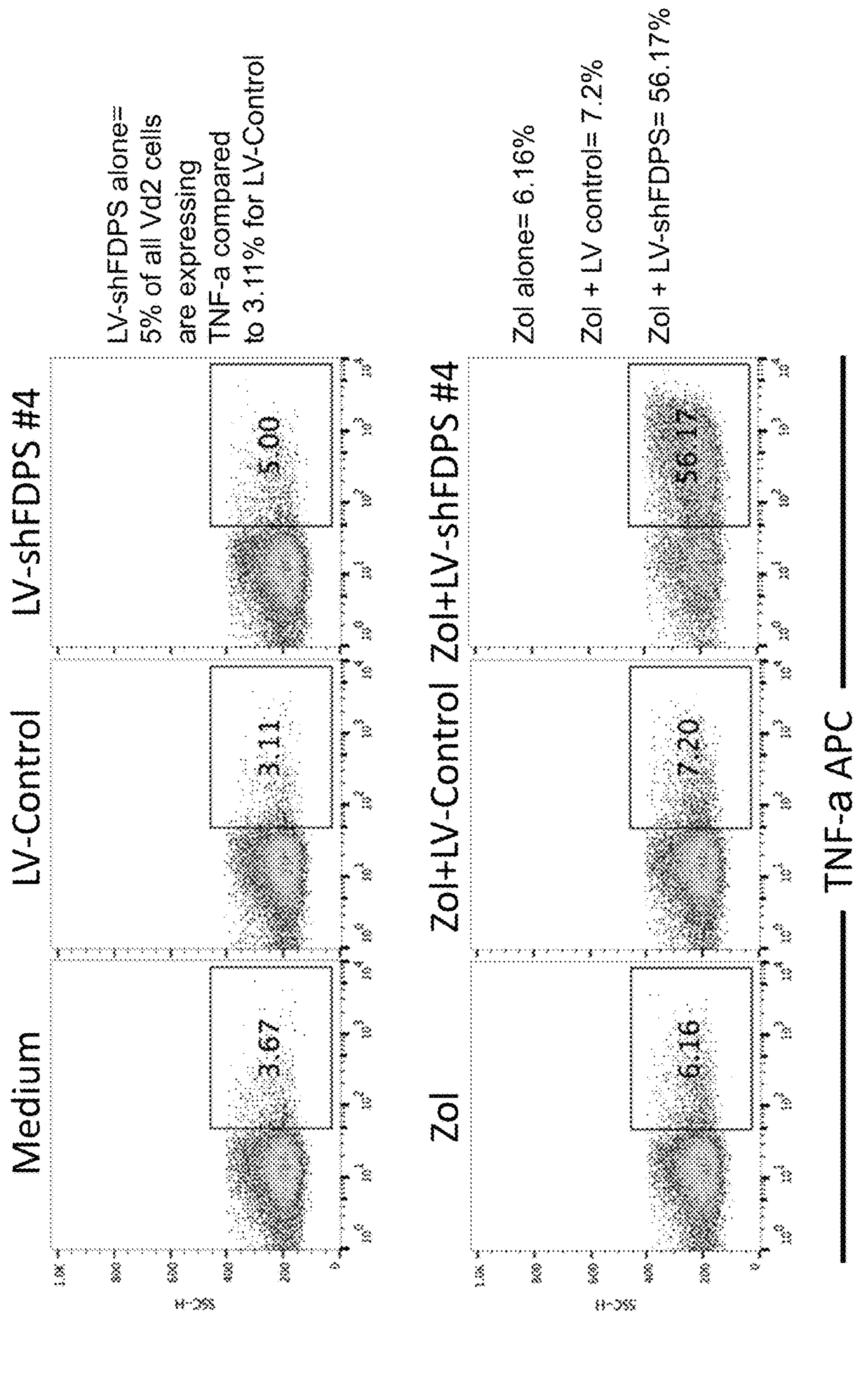
Medium
3.67
LV-Control
3.11
LV-shFDPS #4
5.00
LV-shFDPS alone= 5% of all Vd2 cells are expressing TNF-a compared to 3.11% for LV-Control
Zol
6.16
Zol+LV-Control
7.20
Zol+LV-shFDPS #4
56.17
Zol alone= 6.16%
Zol + LV control= 7.2%
Zol + LV-shFDPS= 56.17%
TNF-a APC
SSC-H

Figure 5

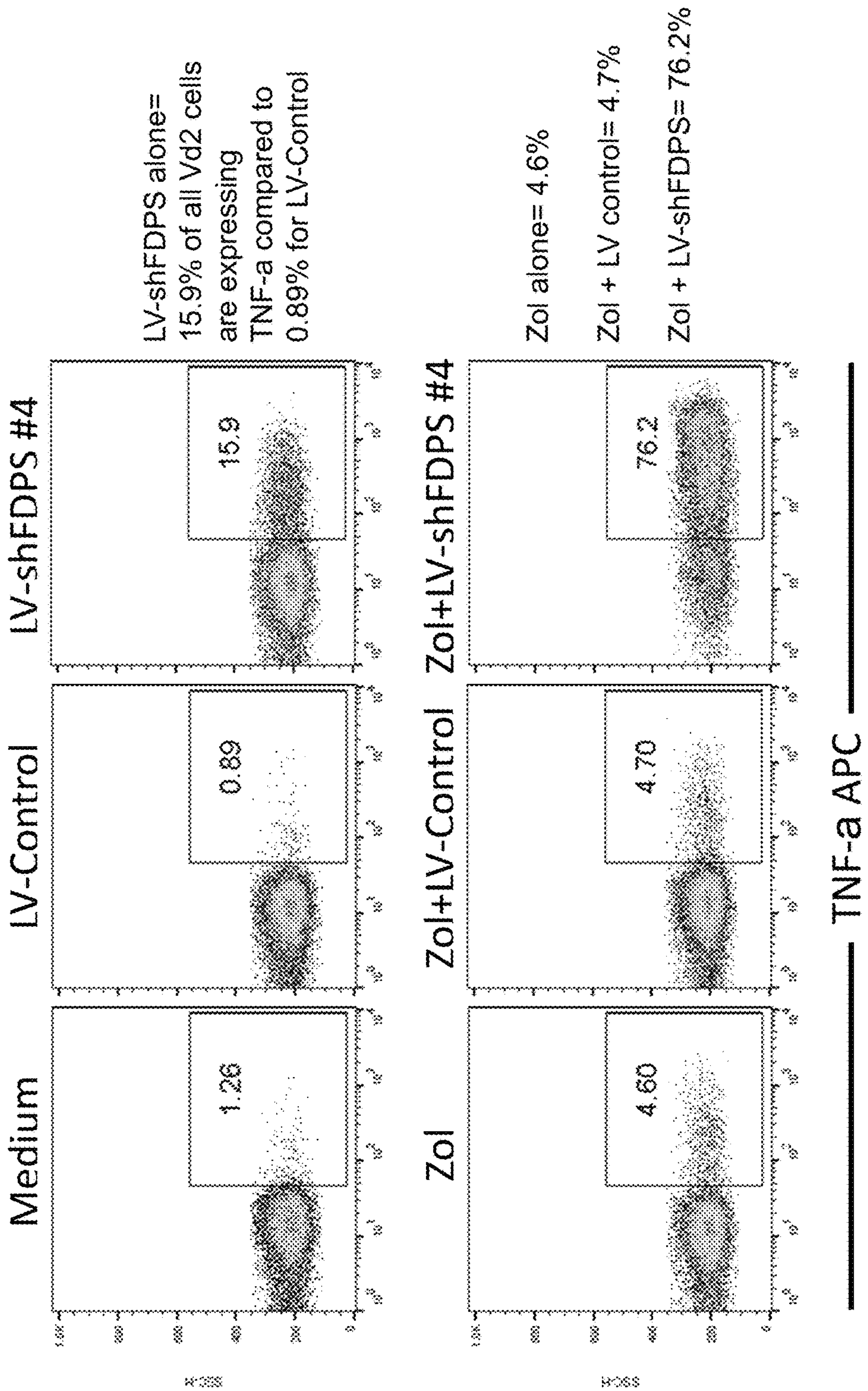
Medium
LV-Control
LV-shFDPS #4
1.26
0.89
15.9
LV-shFDPS alone= 15.9% of all Vd2 cells are expressing TNF-a compared to 0.89% for LV-Control
Zol
Zol+LV-Control
Zol+LV-shFDPS #4
4.60
4.70
76.2
Zol alone= 4.6%
Zol + LV control= 4.7%
Zol + LV-shFDPS= 76.2%
TNF-a APC

**Figure 6**

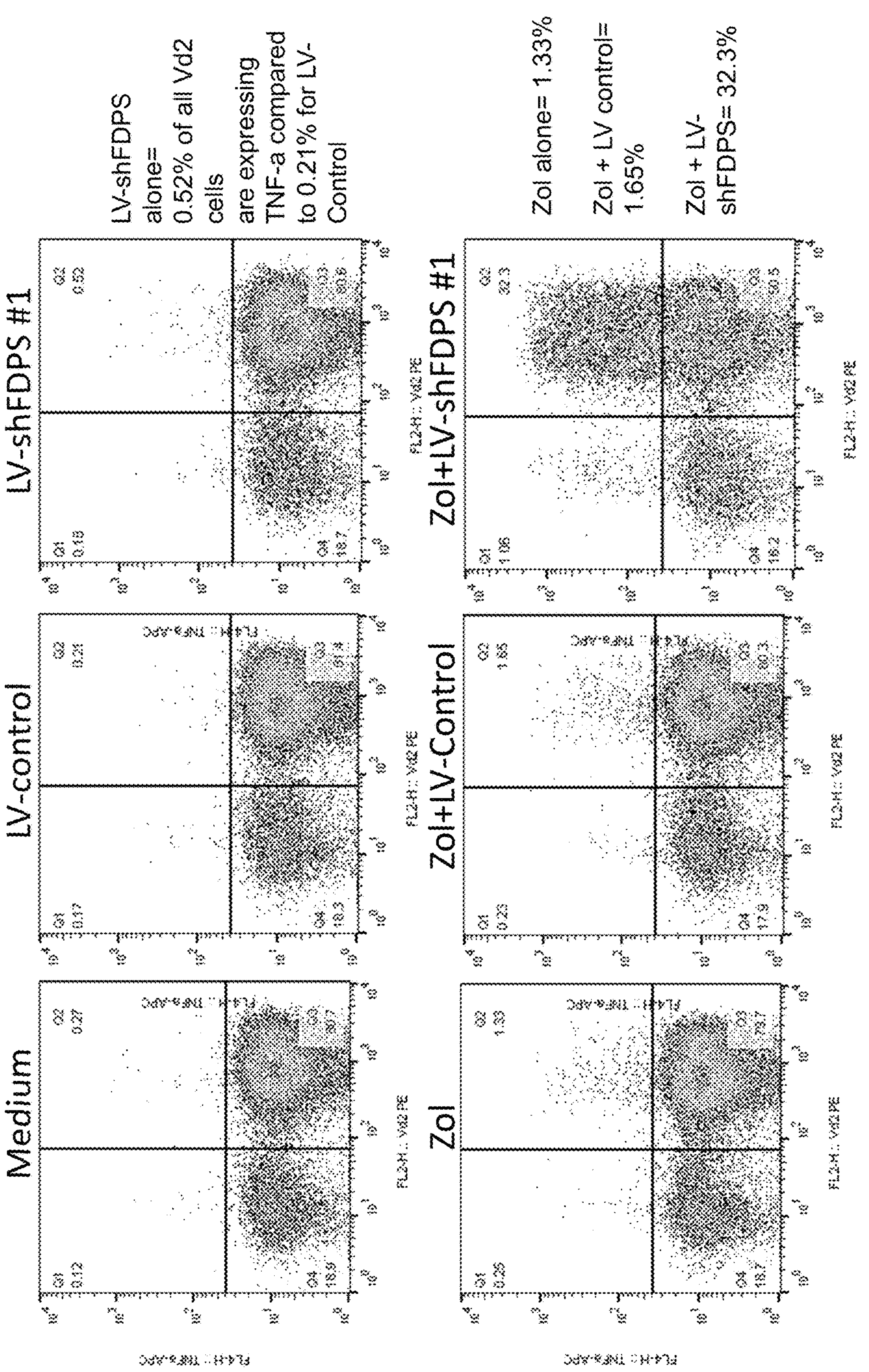
Medium
LV-control
LV-shFDPS #1
Zol
Zol+LV-Control
Zol+LV-shFDPS #1
LV-shFDPS alone= 0.52% of all Vd2 cells are expressing TNF-a compared to 0.21% for LV-Control
Zol alone= 1.33%
Zol + LV control= 1.65%
Zol + LV-shFDPS= 32.3%

Figure 7

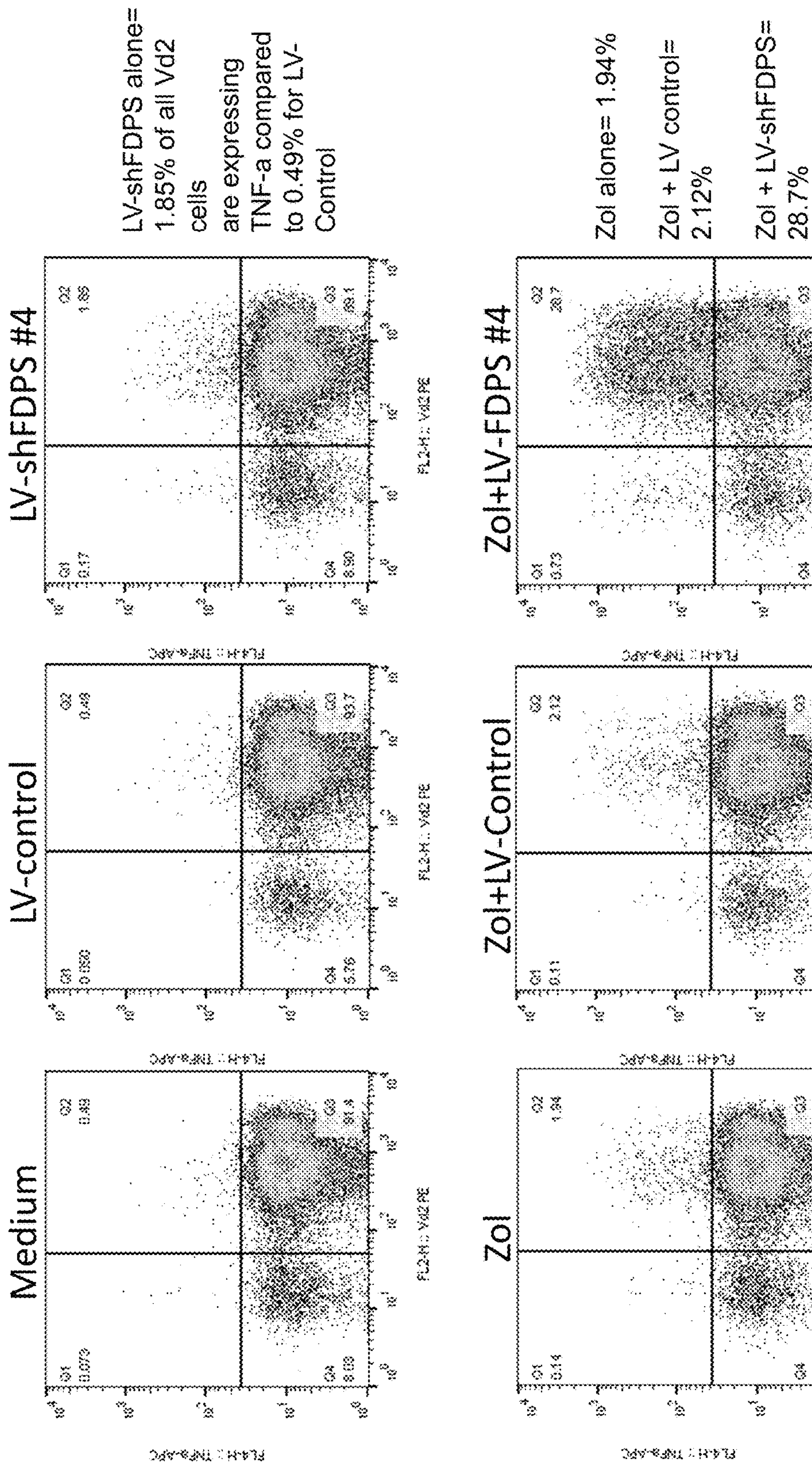
Medium
LV-control
LV-shFDPS #4
LV-shFDPS alone= 1.85% of all Vd2 cells are expressing TNF-a compared to 0.49% for LV-Control
Zol
Zol+LV-Control
Zol+LV-FDPS #4
Zol alone= 1.94%
Zol + LV control= 2.12%
Zol + LV-shFDPS= 28.7%

Figure 8

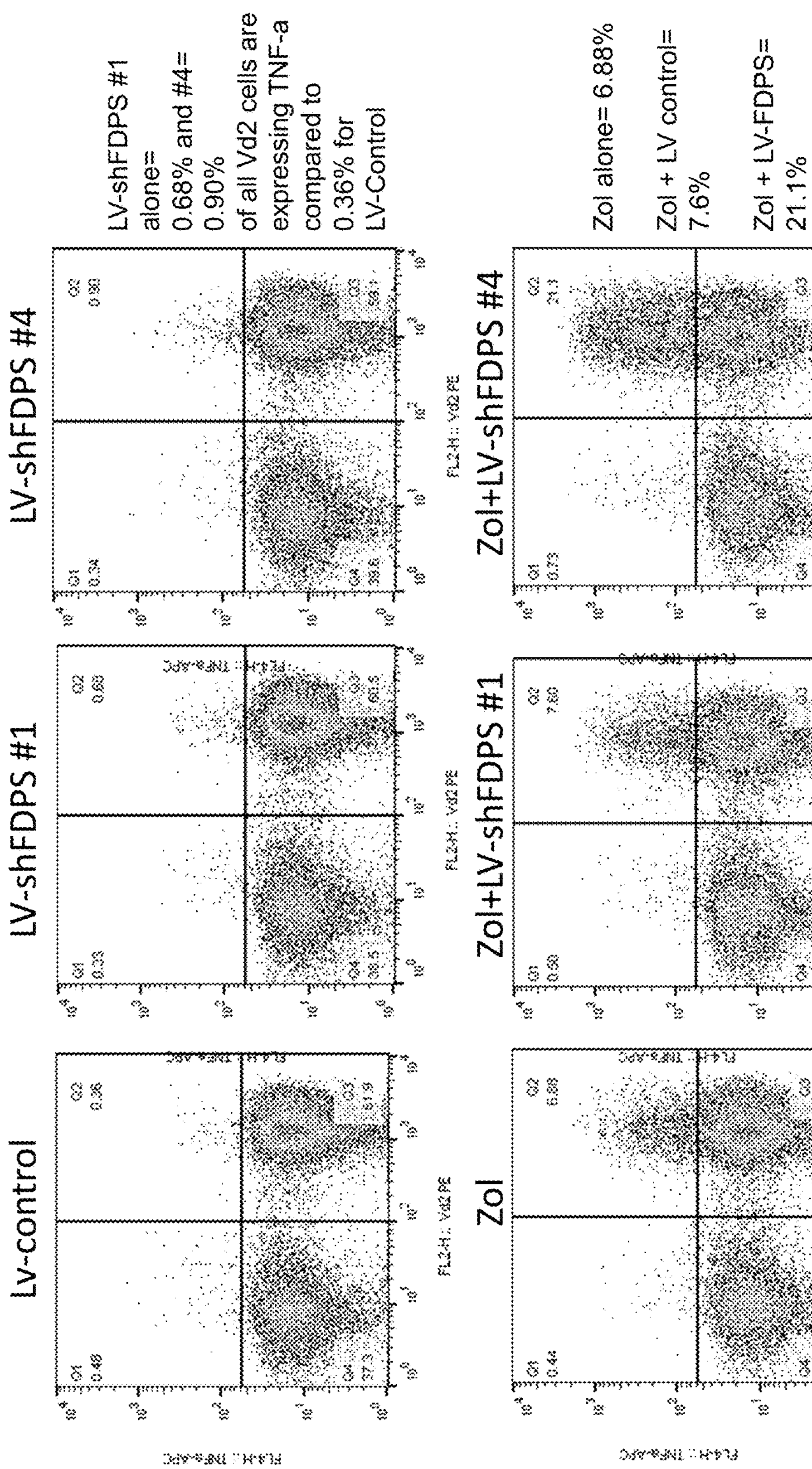
Lv-control
LV-shFDPS #1
LV-shFDPS #4
Zol
Zol+LV-shFDPS #1
Zol+LV-shFDPS #4
LV-shFDPS #1 alone= 0.68% and #4= 0.90% of all Vd2 cells are expressing TNF-a compared to 0.36% for LV-Control
Zol alone= 6.88%
Zol + LV control= 7.6%
Zol + LV-FDPS= 21.1%

Figure 9

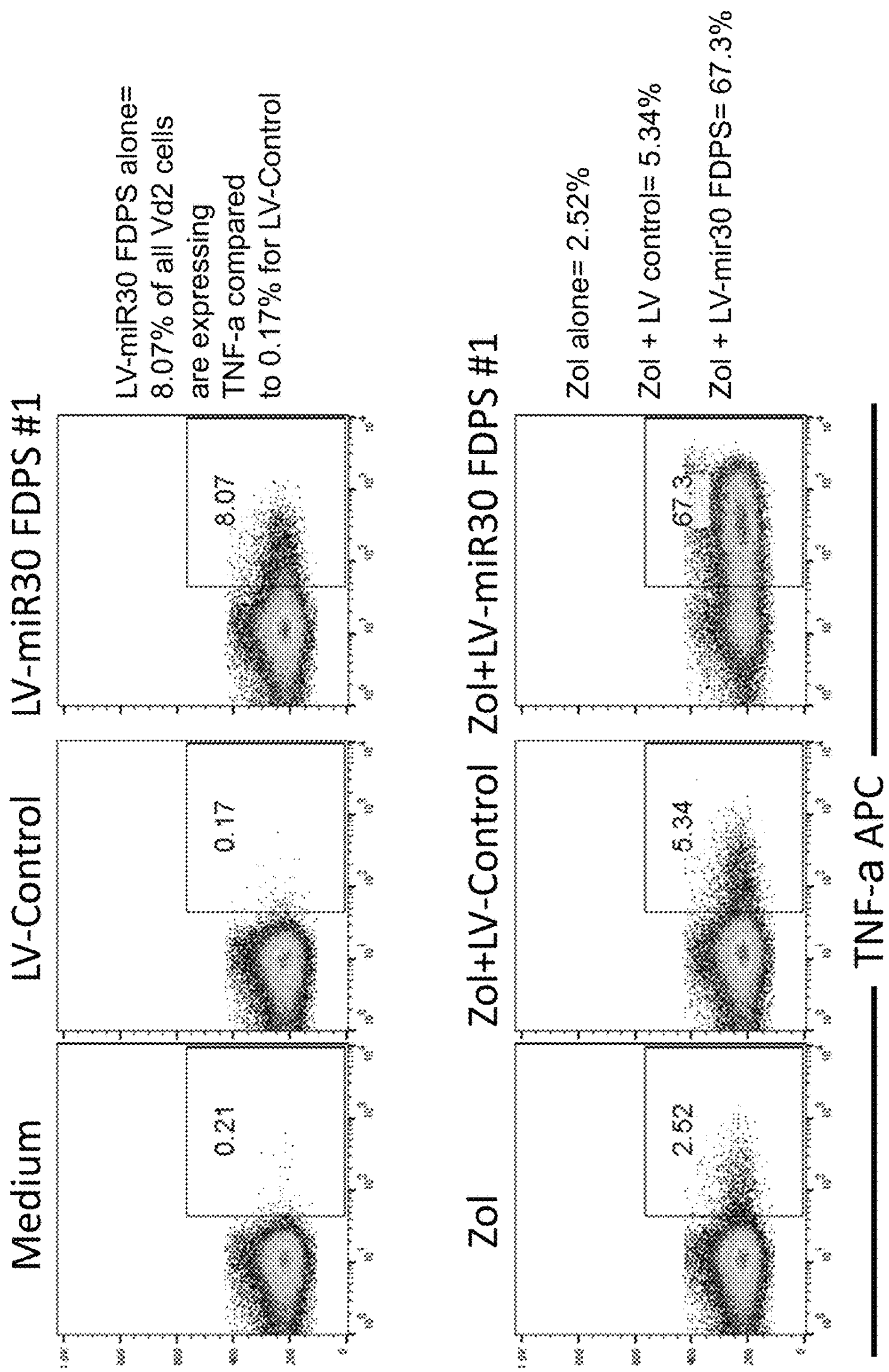
Medium
0.21
LV-Control
0.17
LV-miR30 FDPS #1
8.07
LV-miR30 FDPS alone= 8.07% of all Vd2 cells are expressing TNF-a compared to 0.17% for LV-Control
Zol
2.52
Zol+LV-Control
5.34
Zol+LV-miR30 FDPS #1
67.3
Zol alone= 2.52%
Zol + LV control= 5.34%
Zol + LV-mir30 FDPS= 67.3%
TNF-a APC

**Figure 10**

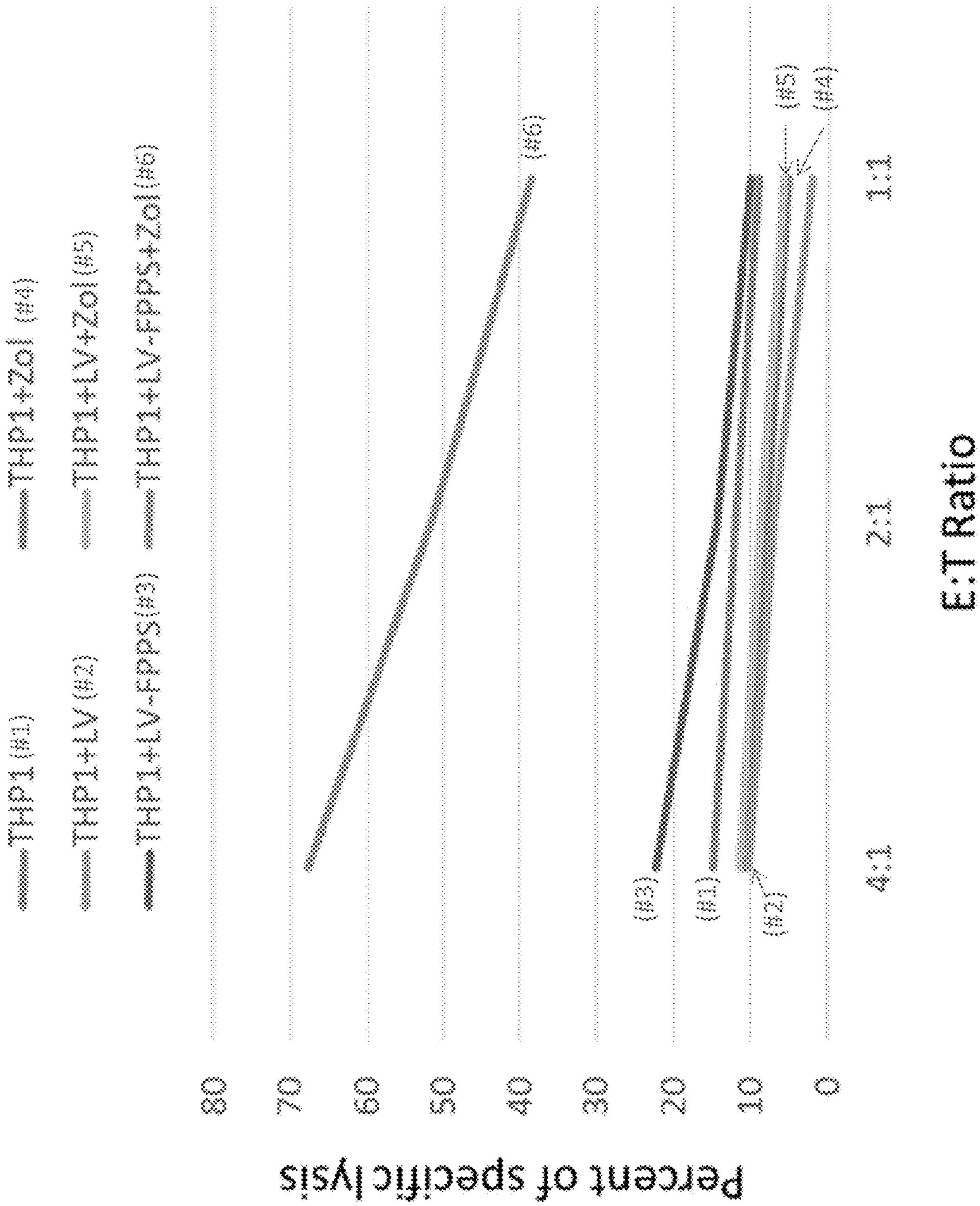
THP1 (#1)
THP1+LV (#2)
THP1+LV-FPPS(#3)
THP1+Zol (#4)
THP1+LV+Zol (#5)
THP1+LV-FPPS+Zol (#6)
Percent of specific lysis
80
70
60
50
40
30
20
10
0
4:1
2:1
1:1
E:T Ratio
(#1)
(#2)
(#3)
(#4)
(#5)
(#6)

Figure 11

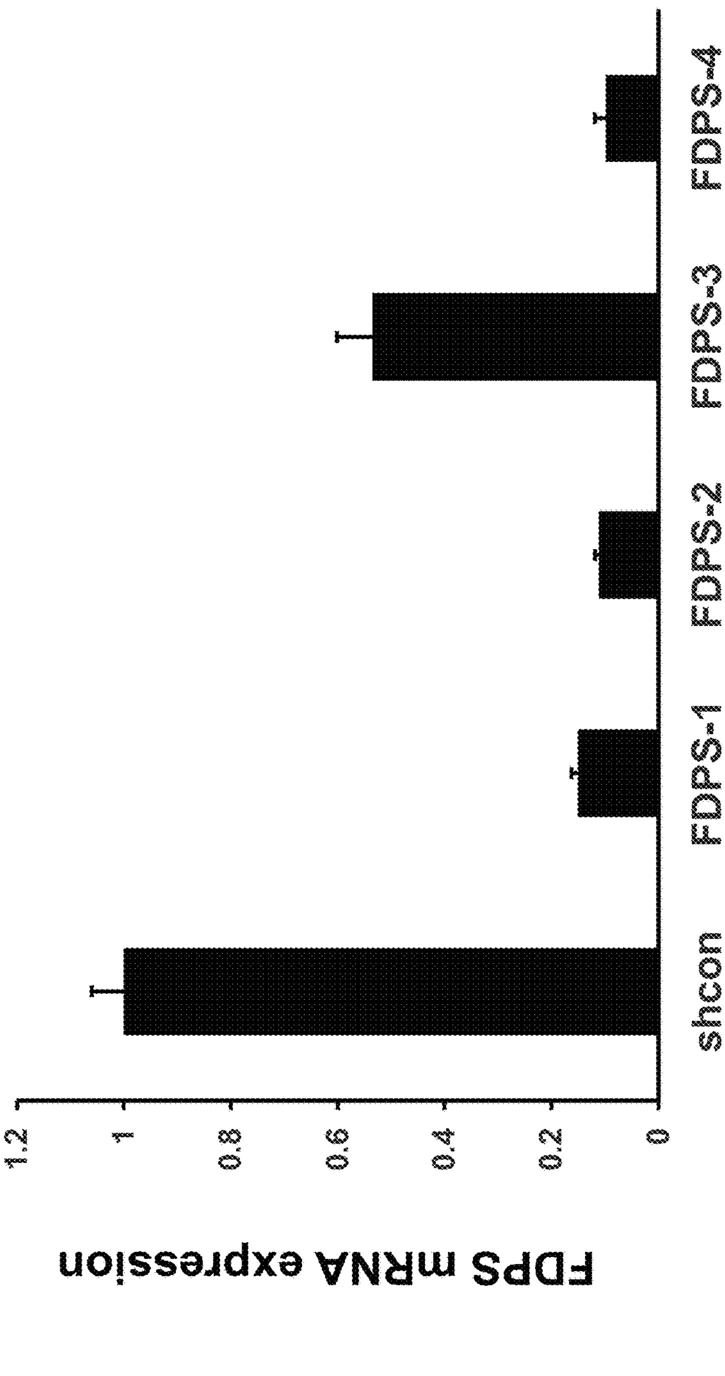
FDPS mRNA expression
1.2
1
0.8
0.6
0.4
0.2
0
shcon
FDPS-1
FDPS-2
FDPS-3
FDPS-4

Figure 12

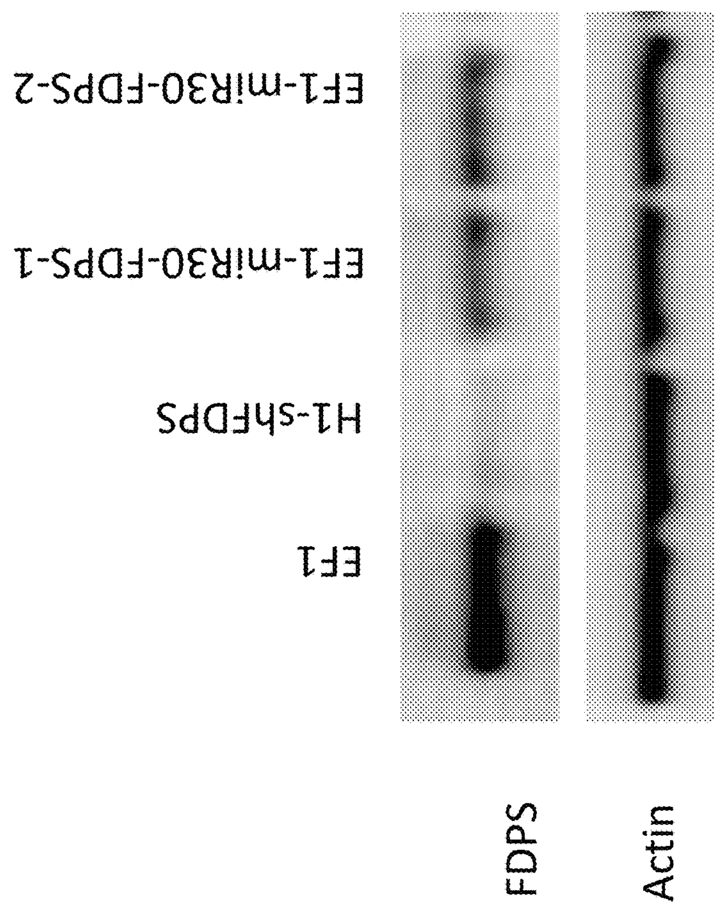


Figure 13

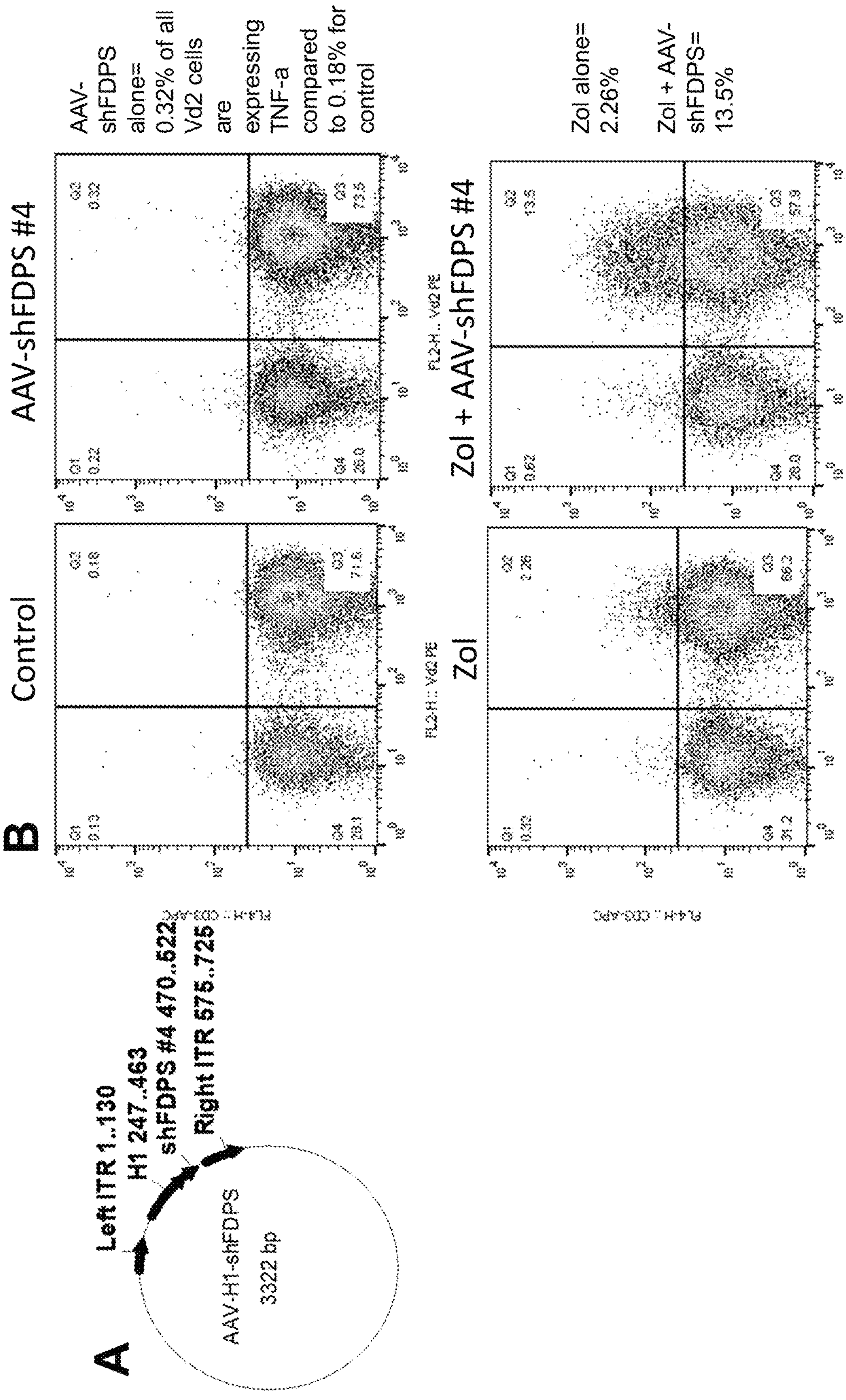
A
AAV-H1-shFDPS
3322 bp
Left ITR 1..130
H1 247..463
shFDPS #4 470..522
Right ITR 575..725
B
Control
AAV-shFDPS #4
Zol
Zol + AAV-shFDPS #4
AAV-shFDPS alone= 0.32% of all Vd2 cells are expressing TNF-a compared to 0.18% for control
Zol alone= 2.26%
Zol + AAV-shFDPS= 13.5%

Figure 14

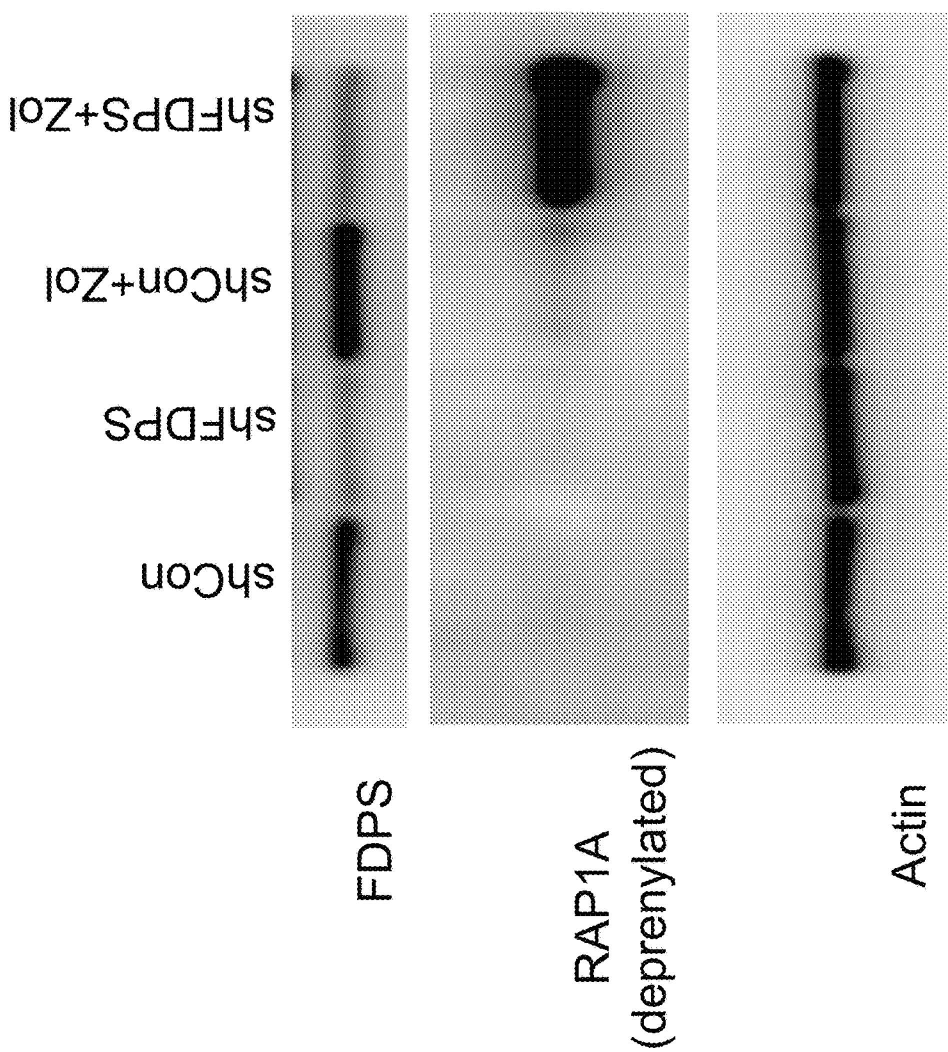
shCon
shFDPS
shCon+Zol
shFDPS+Zol
FDPS
RAP1A
(deprenylated)
Actin

Figure 15

# METHODS AND COMPOSITIONS FOR THE ACTIVATION OF GAMMA-DELTA T-CELLS

## CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation of U.S. patent application Ser. No. 16/008,991, filed on Jun. 14, 2018 entitled "Methods and Compositions for the Activation of Gamma-Delta T-cells," which is a continuation of U.S. patent application Ser. No. 15/850,937, filed on Dec. 21, 2017 entitled "Methods and Compositions for the Activation of Gamma-Delta T-cells," which is a continuation of U.S. patent application Ser. No. 15/652,080, filed on Jul. 17, 2017 entitled "Methods and Compositions for the Activation of Gamma-Delta T-cells" which is a continuation of International Application No. PCT/US17/13399 filed on Jan. 13, 2017, entitled "Methods and Compositions for the Activation of Gamma-Delta T-cells" which claims priority to U.S. Provisional Patent Application No. 62/279,474, filed on Jan. 15, 2016, and entitled "Methods and Compositions for the Activation of Gamma-Delta T-cells," the disclosures of which are incorporated herein by reference.

## FIELD OF THE INVENTION

The present disclosure relates generally to the fields of gene therapy and immunotherapy, specifically in relation to increased activation of gamma delta ("GD") T cells.

## BACKGROUND

Human T cells are distinguished on the basis of T cell receptor structure. The major populations, including CD4+ and CD8+ subsets, express a receptor composed of alpha and beta chains. A smaller subset expresses T cell receptor made from gamma and delta chains. Gamma delta ("GD") T cells make up 3-10% circulating lymphocytes, and Vδ2+ subset makes up 75% of GD T cells in blood. Vδ2+ cells recognize non-peptide epitopes and do not require antigen presentation by major histocompatibility complexes ("MHC") or human leukocyte antigen ("HLA"). The majority of Vδ2+ T cells also express a Vγ9 chain and are stimulated by exposure to 5-carbon pyrophosphate compounds that are intermediates in mevalonate and non-mevalonate sterol/isoprenoid synthesis pathways. The response to isopentenyl pyrophosphate (5-carbon) is universal among healthy human beings.

Another subset of GD T cells, Vδ+, make up a much smaller percentage of the T cells circulating in the blood, but Vδ+1 cells are commonly found in the epithelial mucosa and the skin.

In general, GD T cells have several functions, including killing tumor cells and pathogen-infected cells. Stimulation through their unique T cell receptor ("TCRs") composed of two glycoprotein chains, γ and δ, improves the capacity for cellular cytotoxicity, cytokine secretion and other effector functions. The TCRs of GD T cells have unique specificities and the cells themselves occur in high clonal frequencies, thus allowing rapid innate-like responses to tumors and pathogens.

Aminobisphosphonate drugs ("ABPs") and other inhibitors of farnesyl diphosphate synthase ("FDPS"), which are downstream from isopentenyl pyrophosphate ("IPP") in the mevalonate pathway (see, for e.g., FIG. **1**), have been used to treat various diseases, including cancers, specifically



those involving bone metastasis. ABPs include trade names such as Zometa® (Novartis) and Fosamax® (Merck).

ABPs have also been used to stimulate GD T cells. This may be because when FDPS is inhibited in myeloid cells, IPP begins to accumulate and geranylgeranyl pyrophosphate ("GGPP"), a downstream product of FDPS that suppresses activation of the inflammasome pathway, is reduced. The reduction in GGPP removes an inhibitor of the caspase-dependent inflammasome pathway and allows secretion of mature cytokines including interleukin-beta and interleukin-18, the latter being especially important for gamma delta T cell activation.

Thus, when FDPS is blocked, the increased IPP and decreased GGPP combine to activate Vδ2+ T cells. Vδ2+ cells activated by IPP or ABPs will proliferate rapidly, express a number of cytokines and chemokines, and can function to cytotoxically destroy tumor cells or cells infected with pathogenic microorganisms.

However, ABPs are associated with inflammation and osteonecrosis, as well as having poor bioavailability due to their chemistry. Likewise, IPP has a very short half-life and is difficult to synthesize. Both types of compounds require systemic administration in an individual. Accordingly, both ABPs in general, and IPP specifically, leave a great deal to be desired for therapeutic purposes.

## SUMMARY OF THE INVENTION

In one aspect, a method of activating a GD T cell is provided. The method includes infecting, in the presence of the GD T cell, a target cell with a viral delivery system that encodes at least one genetic element. In embodiments, the at least one genetic element includes a small RNA capable of inhibiting production of an enzyme involved in the mevalonate pathway. In embodiments, the enzyme is FDPS. In embodiments, when the enzyme is inhibited in the target cell, the target cell subsequently activates the GD T cell. In embodiments, the target cell is a cancer cell or a cell that has been infected with an infectious agent. In a preferred embodiment, the activation of the GD T cell results in the GD T cell killing the cancer cell or the cell infected with an infectious agent. In embodiments, the at least one encoded genetic element includes a microRNA or a shRNA. In further embodiments, the target cell is also contacted with an aminobisphosphonate drug. In embodiments, the aminobisphosphonate drug is zoledronic acid.

In another aspect, a method of treating cancer in a subject is provided. The method includes administering to the subject a therapeutically-effective amount of a viral delivery system that encodes at least one genetic element. In embodiments, the at least one genetic element includes a small RNA capable of inhibiting production of an enzyme involved in the mevalonate pathway. In further embodiments, when the enzyme is inhibited in a cancer cell in the presence of a GD T cell, the cancer cell activates the GD T cell, to thereby treat the cancer. In embodiments, the enzyme is FDPS. In embodiments, the at least one encoded genetic element includes a microRNA or a shRNA. In further embodiments, the target cell is also contacted with an aminobisphosphonate drug. In embodiments, the aminobisphosphonate drug is zoledronic acid.

In another aspect, a method of treating an infectious disease in a subject is provided. The method includes administering to the subject a therapeutically-effective amount of a viral delivery system that encodes at least one genetic element. In embodiments, the at least one genetic element includes a small RNA capable of inhibiting production of an enzyme involved in the mevalonate pathway. In further embodiments, when the enzyme is inhibited in a cell that is infected with an infectious agent in the presence of a

GD T cell, the infected cell activates the GD T cell, to thereby treat the infected cell, and the infectious disease. In embodiments, the enzyme is FDPS. In embodiments, the at least one encoded genetic element includes a microRNA or a shRNA. In further embodiments, the target cell is also contacted with an aminobisphosphonate drug. In embodiments, the aminobisphosphonate drug is zoledronic acid.

In another aspect, the at least one encoded genetic element includes a shRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with

(SEQ ID NO: 1)
GTCCTGGAGTACAATGCCATTCTCGAGAATGGCAT

TGTACTCCAGGACTTTTT

(SEQ ID NO: 2)
GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCT

GAACGAAATCCTGCTTTTT;

(SEQ ID NO: 3)
GCCATGTACATGGCAGGAATTCTCGAGAA

TTCCTGCCATGTACATGGCTTTTT;
or

(SEQ ID NO: 4)
GCAGAAGGAGGCTGAGAAAGTCTCGAGACTT

TCTCAGCCTCCTTCTGCTTTTT.

In a preferred embodiment, the shRNA includes

(SEQ ID NO: 1)
GTCCTGGAGTACAATGCCATTCTCGAGAATGGCAT

TGTACTCCAGGACTTTTT

(SEQ ID NO: 2)
GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCT

GAACGAAATCCTGCTTTTT;

(SEQ ID NO: 3)
GCCATGTACATGGCAGGAATTCTCGAGAA

TTCCTGCCATGTACATGGCTTTTT;
or

(SEQ ID NO: 4)
GCAGAAGGAGGCTGAGAAAGTCTCGAGACTT

TCTCAGCCTCCTTCTGCTTTTT.

In another aspect, the at least one encoded genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with

(SEQ ID NO: 5)
AGGTATATTGCTGTTGACAGTGAGCGACACTTTCTC

AGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGG

AGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCA

AGGGGCT;

(SEQ ID NO: 6)
AAGGTATATTGCTGTTGACAGTGAGCGACACT

TTCTCAGCCTCCTTCTGCGTGAAGCCACAGATG

GCAGAAGGGCTGAGAAAGTGCT

GCCTACTGCCTCGGACTTCAAGGGGCT;



-continued

(SEQ ID NO: 7)
TGCTGTTGACAGTGAGCGACTTTCTCAGCC

TCCTTCTGCGTGAAGCCACAGATGGCAGAAGG

AGGCTGAGAAAGTTGCCTACTGCCTCGGA;

(SEQ ID NO: 8)
CCTGGAGGCTTGCTGAAGGCTGTATGCTG

ACTTTCTCAGCCTCCTTCTGCTTTTGGCC

ACTGACTGAGCAGAAGGGCTGAGAAAGTC

AGGACACAAGGCCTGTTACTAGCACTCA;

(SEQ ID NO: 9)
CATCTCCATGGCTGTACCACCTTGTCGGG

ACTTTCTCAGCCTCCTTCTGCCTGTTG

AATCTCATGGCAGAAGGAGGCGAGAAAG

TCTGACATTTTGGTATCTTTCATCTGACCA;
or

(SEQ ID NO: 10)
GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCT

CAGCCTCCTTCTGCTGGTCCCCTCCCCGCAGAAGGA

GGCTGAGAAAGTCCTTCCC

TCCCAATGACCGCGTCTTCGTCG.

In a preferred embodiment, the microRNA includes

(SEQ ID NO: 5)
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTC

AGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAAGG

AGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCA

AGGGGCT;

(SEQ ID NO: 6)
AAGGTATATTGCTGTTGACAGTGAGCGACACT

TTCTCAGCCTCCTTCTGCGTGAAGCCACAGATG

GCAGAAGGGCTGAGAAAGTGCT

GCCTACTGCCTCGGACTTCAAGGGGCT;

(SEQ ID NO: 7)
TGCTGTTGACAGTGAGCGACTTTCTCAGCC

TCCTTCTGCGTGAAGCCACAGATGGCAGAAGG

AGGCTGAGAAAGTTGCCTACTGCCTCGGA;

(SEQ ID NO: 8)
CCTGGAGGCTTGCTGAAGGCTGTATGCTG

ACTTTCTCAGCCTCCTTCTGCTTTTGGCC

ACTGACTGAGCAGAAGGGCTGAGAAAGTC

AGGACACAAGGCCTGTTACTAGCACTCA;

(SEQ ID NO: 9)
CATCTCCATGGCTGTACCACCTTGTCGGG

ACTTTCTCAGCCTCCTTCTGCCTGTTG

AATCTCATGGCAGAAGGAGGCGAGAAAG

TCTGACATTTTGGTATCTTTCATCTGACCA;

-continued

or

(SEQ ID NO: 10)

GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCT

CAGCCTCCTTCTGCTGGTCCCCTCCCCGCAGAAGGA

GGCTGAGAAAGTCCTTCCC

TCCCAATGACCGCGTCTTCGTCG.

In another aspect, a viral vector comprising at least one encoded genetic element is provided. The at least one encoded genetic element includes a small RNA capable of inhibiting production of an enzyme involved in the mevalonate pathway. In embodiments, the enzyme involved in the mevalonate pathway is farnesyl diphosphate synthase (FDPS). In embodiments, the at least one encoded genetic element includes a microRNA or a shRNA.

In another aspect, the at least one encoded genetic element includes a shRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with v In a preferred embodiment, the shRNA includes SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4.

In another aspect, the at least one encoded genetic element includes a microRNA having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; or SEQ ID NO: 10. In a preferred embodiment, the microRNA includes SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; or SEQ ID NO: 10.

In embodiments, the viral vector is comprised of any vector that can effectively transduce the small RNA into a target cell. In embodiments, the viral vector is a lentiviral vector. In other embodiments, the viral vector is an adeno-associated virus vector.

In another aspect, the viral vector includes a second encoded genetic element. In embodiments, the second genetic element includes at least one cytokine or chemokine. In embodiments, the at least one cytokine is selected from the group consisting of: IL-18, TNF-α, interferon-γ, IL-1, IL-2, IL-15, IL-17, and IL-12. In embodiments, the at least one chemokine is a CC chemokine or a CXC chemokine. In further embodiments, the at least one chemokine is RANTES.

In another aspect, a lentiviral vector system for expressing a lentiviral particle is provided. The system includes a lentiviral vector, at least one envelope plasmid for expressing an envelope protein optimized for infecting a cell; and at least one helper plasmid for expressing gag, pol, and rev genes. When the lentiviral vector, the at least one envelope plasmid, and the at least one helper plasmid are transfected into a packaging cell, a lentiviral particle is produced by the packaging cell. In embodiments, the lentiviral particle is capable of infecting a targeting cell, and inhibiting an enzyme involved in the mevalonate pathway within the target cell. In embodiments, the enzyme involved in the mevalonate pathway is FDPS. In embodiments, the lentiviral vector system includes a first helper plasmid for expressing the gag and pol genes, and a second helper plasmid for expressing the rev gene. In embodiments, the envelope protein is preferably optimized for infecting a target cell. In embodiments, the target cell is a cancer cell. In other embodiments, the target cell is a cell that is infected with an infectious agent.

## BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** depicts an overview of the major steps in the mevalonate pathway for biosynthesis of steroids and isoprenoids.

FIG. **2** depicts an exemplary 3-vector lentiviral vector system in a circularized form.

FIG. **3** depicts an exemplary 4-vector lentiviral vector system in a circularized form.

FIG. **4** depicts: (A) a linear map of a lentiviral vector expressing a FDPS shRNA targeting sequence; and (B) a linear map of a lentiviral vector expressing a synthetic microRNA with a FDPS targeting sequence.

FIG. **5** depicts data demonstrating activation of Vδ2+ T cells THP-1 leukemia cells with a lentivirus expressing FDPS shRNA #4 (SEQ ID NO: 4), as described herein.

FIG. **6** depicts data demonstrating activation of Vδ2+ T cells by THP-1 leukemia cells with a lentivirus expressing FDPS shRNA #4 (SEQ ID NO: 4), as described herein.

FIG. **7** depicts data demonstrating activation of Vδ2+ T cells by PC3 prostate carcinoma cells with a lentivirus expressing FDPS shRNA #1 (SEQ ID NO: 1), as described herein.

FIG. **8** depicts data demonstrating activation of Vδ2+ T cells by PC3 prostate carcinoma cells with a lentivirus expressing FDPS shRNA #4 (SEQ ID NO: 4), as described herein.

FIG. **9** depicts data demonstrating activation of Vδ2+ T cells by HepG2 carcinoma cells with a lentivirus expressing FDPS shRNA #1 (SEQ ID NO: 1) or FDPS shRNA #4 (SEQ ID NO: 4), as described herein.

FIG. **10** depicts data demonstrating activation of Vδ2+ T cells by THP-1 leukemia cells with a lentivirus expressing miR30 FDPS #1 (SEQ ID NO: 5), as described herein.

FIG. **11** depicts data demonstrating the percent of specific lysis versus an E:T ratio for a variety of experimental conditions, as described herein.

FIG. **12** depicts data demonstrating lentiviral-delivered shRNA-based RNA interference targeting the human FDPS gene.

FIG. **13** depicts data demonstrating lentiviral-delivered miR-based RNA interference targeting the human FDPS gene.

FIG. **14** depicts data demonstrating activation of Vδ2+ T cells by HepG2 carcinoma cells with an adeno-associated virus expressing FDPS shRNA #4 (SEQ ID NO: 4), as described herein.

FIG. **15** depicts immunoblot data demonstrating lack of RAP1 prenylation in the cells transduced with LV-shFDPS and treated with zoledronic acid.

## DETAILED DESCRIPTION

### Overview of Disclosure

The present disclosure relates to gene therapy constructs and delivery of the same to cells, resulting in suppression of Farnesyl diphosphate synthase ("FDPS"), which is necessary to convert isopentenyl phosphate (IPP) to farnesyl diphosphate (FDP), as shown, for example, in FIG. **1**. In embodiments, one or more viral vectors are provided with microRNAs or short homology RNAs (shRNA) that target FDPS, thereby reducing expression levels of this enzyme. The viral vectors include lentiviral vectors and AAV vectors. A consequence of modulating expression of FDPS is to increase the accumulation of IPP, which is a stimulator of GD T cell proliferation and differentiation. Accordingly, the constructs provided herein are used to activate GD T cells, and are used to treat cancers and infectious diseases.

Definitions and Interpretation

Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The methods and techniques of the present disclosure are generally performed according to conventional methods well-known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g.: Sambrook J. & Russell D. Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2000); Ausubel et al., Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology, Wiley, John & Sons, Inc. (2002); Harlow and Lane Using Antibodies: A Laboratory Manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1998); and Coligan et al., Short Protocols in Protein Science, Wiley, John & Sons, Inc. (2003). Any enzymatic reactions or purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclature used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art.

As used in the description and the appended claims, the singular forms "a", "an" and "the" are used interchangeably and intended to include the plural forms as well and fall within each meaning, unless the context clearly indicates otherwise. Also, as used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the listed items, as well as the lack of combinations when interpreted in the alternative ("or").

All numerical designations, e.g., pH, temperature, time, concentration, and molecular weight, including ranges, are approximations which are varied (+) or (−) by increments of 0.1. It is to be understood, although not always explicitly stated that all numerical designations are preceded by the term "about". The term "about" also includes the exact value "X" in addition to minor increments of "X" such as "X+0.1" or "X−0.1." It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary and that equivalents of such are known in the art.

As used herein, the term "about" will be understood by persons of ordinary skill in the art and will vary to some extent depending upon the context in which it is used. If there are uses of the term which are not clear to persons of ordinary skill in the art given the context in which it is used, "about" will mean up to plus or minus 10% of the particular term.

The terms "administration of" or "administering" an active agent should be understood to mean providing an active agent to the subject in need of treatment in a form that can be introduced into that individual's body in a therapeutically useful form and therapeutically effective amount.

As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but not excluding others. "Consisting essentially of" when used to define compositions and methods, shall mean excluding other elements of any essential significance to the composition or method. "Consisting of" shall mean excluding more than trace elements of other ingredients for claimed compositions and substantial method steps. Embodiments defined by each of these transition terms are within the scope of this disclosure. Accordingly, it is intended that the methods and compositions can include additional steps and components (comprising) or alternatively including steps and compositions of no significance (consisting essentially of) or alternatively, intending only the stated method steps or compositions (consisting of).

As used herein, "expression," "expressed," or "encodes" refers to the process by which polynucleotides are transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently being translated into peptides, polypeptides, or proteins. Expression may include splicing of the mRNA in a eukaryotic cell or other forms of post-transcriptional modification or post-translational modification.

The term "farnesyl diphosphate synthase" may also be referred to herein as FDPS, and may also be referred to herein as farnesyl pyrophosphate synthase or FPPS.

The term "gamma delta T cell" may also be referred to herein as a γδ T cell, or further as a GD T cell. The term "gamma delta T cell activation" refers to any measurable biological phenomenon associated with a gamma delta T cell that is representative of such T cell being activated. Non-limiting examples of such a biological phenomenon include an increase of cytokine production, changes in the qualitative or quantitative composition of cell surface proteins, an increase in T cell proliferation, and/or an increase in T cell effector function, such killing or a target cell or assisting another effector cell to kill a target cell.

The terms "individual," "subject," and "patient" are used interchangeably herein, and refer to any individual mammal subject, e.g., bovine, canine, feline, equine, or human.

The term "miRNA" refers to a microRNA, and also may be referred to herein as "miR".

The term "packaging cell line" refers to any cell line that can be used to express a lentiviral particle.

The term "percent identity," in the context of two or more nucleic acid or polypeptide sequences, refer to two or more sequences or subsequences that have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described below (e.g., BLASTP and BLASTN or other algorithms available to persons of skill) or by visual inspection. Depending on the application, the "percent identity" can exist over a region of the sequence being compared, e.g., over a functional domain, or, alternatively, exist over the full length of the two sequences to be compared. For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally Ausubel et al., infra).

One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul et al., J. Mol. Biol. 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information website.

The percent identity between two nucleotide sequences can be determined using the GAP program in the GCG software package (available at http://www.gcg.com), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. The percent identity between two nucleotide or amino acid sequences can also be determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences can be determined using the Needleman and Wunsch (*J.* Mol. Biol. (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at http://www.gcg.com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

The nucleic acid and protein sequences of the present disclosure can further be used as a "query sequence" to perform a search against public databases to, for example, identify related sequences. Such searches can be performed using the NBLAST and) (BLAST programs (version 2.0) of Altschul, et al. (1990) *J Mol. Biol.* 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score=100, word length=12 to obtain nucleotide sequences homologous to the nucleic acid molecules provided in the disclosure. BLAST protein searches can be performed with the XBLAST program, score=50, wordlength=3 to obtain amino acid sequences homologous to the protein molecules of the disclosure. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov.

As used herein, "pharmaceutically acceptable" refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues, organs, and/or bodily fluids of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio.

As used herein, a "pharmaceutically acceptable carrier" refers to, and includes, any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. The compositions can include a pharmaceutically acceptable salt, e.g., an acid addition salt or a base addition salt (see, e.g., Berge et al. (1977) *J Pharm Sci* 66:1-19).

As used herein, the term "SEQ ID NO" is synonymous with the term "Sequence ID No."

As used herein, "small RNA" refers to non-coding RNA that are generally less than about 200 nucleotides or less in length and possess a silencing or interference function. In other embodiments, the small RNA is about 175 nucleotides or less, about 150 nucleotides or less, about 125 nucleotides or less, about 100 nucleotides or less, or about 75 nucleotides or less in length. Such RNAs include microRNA (miRNA), small interfering RNA (siRNA), double stranded RNA (dsRNA), and short hairpin RNA (shRNA). "Small RNA" of the disclosure should be capable of inhibiting or knocking-down gene expression of a target gene, generally through pathways that result in the destruction of the target gene mRNA.

The term "therapeutically effective amount" refers to a sufficient quantity of the active agents of the present disclosure, in a suitable composition, and in a suitable dosage form to treat or prevent the symptoms, progression, or onset of the complications seen in patients suffering from a given ailment, injury, disease, or condition. The therapeutically effective amount will vary depending on the state of the patient's condition or its severity, and the age, weight, etc., of the subject to be treated. A therapeutically effective amount can vary, depending on any of a number of factors, including, e.g., the route of administration, the condition of the subject, as well as other factors understood by those in the art.

As used herein, the term "therapeutic vector" includes, without limitation, reference to a lentiviral vector or an AAV vector.

"A treatment" is intended to target the disease state and combat it, i.e., ameliorate or prevent the disease state. The particular treatment thus will depend on the disease state to be targeted and the current or future state of medicinal therapies and therapeutic approaches. A treatment may have associated toxicities.

The term "treatment" or "treating" generally refers to an intervention in an attempt to alter the natural course of the subject being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects include, but are not limited to, preventing occurrence or recurrence of disease, alleviating symptoms, suppressing, diminishing or inhibiting any direct or indirect pathological consequences of the disease, ameliorating or palliating the disease state, and causing remission or improved prognosis.

## DESCRIPTION OF ASPECTS OF THE DISCLOSURE

In one aspect, a method of activating a GDT cell is provided. The method includes infecting, in the presence of the GD T cell, a target cell with a viral delivery system encoding at least one genetic element. In embodiments, the at least one encoded genetic element includes a small RNA capable of inhibiting production of an enzyme involved in the mevalonate pathway. In embodiments, the enzyme is FDPS. In embodiments, when the enzyme is inhibited in the target cell, the target cell activates the GD T cell. In embodiments, the target cell is a cancer cell or a cell that has been infected with an infectious agent. In embodiments, the at least one encoded genetic element includes a microRNA or a shRNA.

In embodiments, the at least one encoded genetic element includes a shRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with

(SEQ ID NO: 1)
GTCCTGGAGTACAATGCCATTCTCGAGAATGGCAT
TGTACTCCAGGACTTTTT

(SEQ ID NO: 2)
GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCT
GAACGAAATCCTGCTTTTT;

(SEQ ID NO: 3)
GCCATGTACATGGCAGGAATTCTCGAGAA
TTCCTGCCATGTACATGGCTTTTT;
or

(SEQ ID NO: 4)
GCAGAAGGAGGCTGAGAAAGTCTCGAGACTT
TCTCAGCCTCCTTCTGCTTTTT.

In a preferred embodiment, the shRNA includes

(SEQ ID NO: 1)
GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTACTCCAGGACTTT
TT;

(SEQ ID NO: 2)
GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCTGAACGAAATCCTGCTTT
TT;

(SEQ ID NO: 3)
GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGTACATGGCTTT
TT;
or

(SEQ ID NO: 4)
GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCTTT
TT.

In another aspect, the at least one encoded genetic element includes a microRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with

(SEQ ID NO: 5)
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCG
TGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCTCG
GACTTCAAGGGGCT;

(SEQ ID NO: 6)
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCG
TGAAGCCACAGATGGCAGAAGGGCTGAGAAAGTGCTGCCTACTGCCTCGGA
CTTCAAGGGGCT;

(SEQ ID NO: 7)
TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAG
ATGGCAGAAGGAGGCTGAGAAAGTTGCCTACTGCCTCGGA;

-continued

(SEQ ID NO: 8)
CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCTCAGCCTCCTTCTGCT
TTTGGCCACTGACTGAGCAGAAGGGCTGAGAAAGTCAGGACACAAGGCCTG
TTACTAGCACTCA;

(SEQ ID NO: 9)
CATCTCCATGGCTGTACCACCTTGTCGGGACTTTCTCAGCCTCCTTCTGCC
TGTTGAATCTCATGGCAGAAGGAGGCGAGAAAGTCTGACATTTTGGTATCT
TTCATCTGACCA;
or

(SEQ ID NO: 10)
GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCTCAGCCTCCTTCTGCT
GGTCCCCTCCCCGCAGAAGGAGGCTGAGAAAGTCCTTCCCTCCCAATGACC
GCGTCTTCGTCG.

In a preferred embodiment, the microRNA includes

(SEQ ID NO: 5)
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCG
TGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCTCG
GACTTCAAGGGGCT;

(SEQ ID NO: 6)
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCG
TGAAGCCACAGATGGCAGAAGGGCTGAGAAAGTGCTGCCTACTGCCTCGGA
CTTCAAGGGGCT;

(SEQ ID NO: 7)
TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACAG
ATGGCAGAAGGAGGCTGAGAAAGTTGCCTACTGCCTCGGA;

(SEQ ID NO: 8)
CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCTCAGCCTCCTTCTGCT
TTTGGCCACTGACTGAGCAGAAGGGCTGAGAAAGTCAGGACACAAGGCCTG
TTACTAGCACTCA;

(SEQ ID NO: 9)
CATCTCCATGGCTGTACCACCTTGTCGGGACTTTCTCAGCCTCCTTCTGCC
TGTTGAATCTCATGGCAGAAGGAGGCGAGAAAGTCTGACATTTTGGTATCT
TTCATCTGACCA;
or

(SEQ ID NO: 10)
GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCTCAGCCTCCTTCTGCT
GGTCCCCTCCCCGCAGAAGGAGGCTGAGAAAGTCCTTCCCTCCCAATGACC
GCGTCTTCGTCG.

In another aspect, the target cell is also contacted with an aminobisphosphonate drug. In a preferred embodiment, the aminobisphosphonate drug is zoledronic acid.

In another aspect, a method of treating cancer in a subject is provided. The method includes administering to the subject a therapeutically-effective amount of a viral delivery system encoding at least one genetic element. In embodiments, the at least one encoded genetic element includes a small RNA capable of inhibiting production of an enzyme involved in the mevalonate pathway. In further embodiments, when the enzyme is inhibited in a cancer cell in the presence of a GD T cell, the cancer cell activates the GD T cell, to thereby treat the cancer. In embodiments, the enzyme is FDPS. In embodiments, the at least one encoded genetic element includes a microRNA or a shRNA.

In another aspect, a method of treating an infectious disease in a subject is provided. The method includes administering to the subject a therapeutically-effective amount of a viral delivery system encoding at least one genetic element. In embodiments, the at least one encoded genetic element includes a small RNA capable of inhibiting production of an enzyme involved in the mevalonate pathway. In further embodiments, when the enzyme is inhibited in a cell that is infected with an infectious agent and is in the presence of a GD T cell, the infected cell activates the GD T cell, to thereby treat the infected cell, and the infectious disease. In embodiments, the enzyme is FDPS. In embodiments, the at least one encoded genetic element includes a microRNA or a shRNA.

In embodiments, the at least one encoded genetic element includes a shRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4. In a preferred embodiment, the shRNA includes SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4.

In other embodiments, the at least one encoded genetic element includes a microRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; or SEQ ID NO: 10. In a preferred embodiment, the microRNA includes SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; or SEQ ID NO: 10.

In another aspect, a viral vector comprising at least one encoded genetic element is provided. The at least one encoded genetic element includes a small RNA capable of inhibiting production of an enzyme involved in the mevalonate pathway. In embodiments, the enzyme involved in the mevalonate pathway is farnesyl diphosphate synthase (FDPS). In embodiments, the at least one encoded genetic element includes a microRNA or a shRNA.

In another aspect, the at least one encoded genetic element includes a shRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4. In a preferred embodiment, the shRNA includes SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 3; or SEQ ID NO: 4.

In another aspect, the at least one encoded genetic element includes a microRNA having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95% or more percent identity with SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; or SEQ ID NO: 10. In a preferred embodiment, the microRNA includes SEQ ID NO: 5; SEQ ID NO: 6; SEQ ID NO: 7; SEQ ID NO: 8; SEQ ID NO: 9; or SEQ ID NO: 10.

In embodiments, the viral vector includes any vector that can effectively transduce the small RNA. In embodiments, the viral vector is a lentiviral vector. In other embodiments, the viral vector is an adeno-associated virus (AAV) vector.

In another aspect, the viral vector includes a second encoded genetic element. In embodiments, the second genetic element includes at least one cytokine or chemokine. In embodiments, the at least one cytokine is selected from the group consisting of: IL-18, TNF-α, interferon-γ, IL-1, IL-2, IL-15, IL-17, and IL-12. In embodiments, the at least one chemokine is a CC chemokine, CXC chemokine, c CX3 chemokine or a XC chemokine. In a further embodiment, the at least one chemokine is the CC chemokine, RANTES.

In another aspect, a lentiviral vector system for expressing a lentiviral particle is provided. The system includes a lentiviral vector, at least one envelope plasmid for expressing an envelope protein optimized for infecting a cell; and at least one helper plasmid for expressing gag, pol, and rev genes. When the lentiviral vector, the at least one envelope plasmid, and the at least one helper plasmid are transfected into a packaging cell, a lentiviral particle is produced by the packaging cell. In embodiments, the lentiviral particle is capable of infecting a targeting cell, and inhibiting an enzyme involved in the mevalonate pathway within the target cell. In embodiments, the enzyme involved in the mevalonate pathway is FDPS. In embodiments, the lentiviral vector system includes a first helper plasmid for expressing the gag and pol genes, and a second helper plasmid for expressing the rev gene. In embodiments, the envelope protein is preferably optimized for infecting a target cell. In embodiments, the target cell is a cancer cell. In other embodiments, the target cell is a cell that is infected with an infectious disease.

Cancer

The compositions and methods provided herein are used to treat cancer. A cell, tissue, or target may be a cancer cell, a cancerous tissue, harbor cancerous tissue, or be a subject or patient diagnosed or at risk of developing a disease or condition. In certain aspects, a cell may be an epithelial, an endothelial, a mesothelial, a glial, a stromal, or a mucosal cell. The cancer cell population can include, but is not limited to a brain, a neuronal, a blood, an endometrial, a meninges, an esophageal, a lung, a cardiovascular, a liver, a lymphoid, a breast, a bone, a connective tissue, a fat, a retinal, a thyroid, a glandular, an adrenal, a pancreatic, a stomach, an intestinal, a kidney, a bladder, a colon, a prostate, a uterine, an ovarian, a cervical, a testicular, a splenic, a skin, a smooth muscle, a cardiac muscle, or a striated muscle cell, can also include a cancer cell population from any of the foregoing, and can be associated with one or more of carcinomas, sarcomas, myelomas, leukemias, lymphomas, mixed types or mixtures of the foregoing. In still a further aspect cancer includes, but is not limited to astrocytoma, acute myeloid leukemia, anaplastic large cell lymphoma, acute lymphoblastic leukemia, angiosarcoma, B-cell lymphoma, Burkitt's lymphoma, breast carcinoma, bladder carcinoma, carcinoma of the head and neck, cervical carcinoma, chronic lymphoblastic leukemia, chronic myeloid leukemia, colorectal carcinoma, endometrial carcinoma, esophageal squamous cell carcinoma, Ewing's sarcoma, fibrosarcoma, glioma, glioblastoma, gastrinoma, gastric carcinoma, hepatoblastoma, hepatocellular carcinoma, Kaposi's sarcoma, Hodgkin lymphoma, laryngeal squamous cell carcinoma, larynx carcinoma, leukemia, leiomyosarcoma, lipoma, liposarcoma, melanoma, mantle cell lymphoma, medulloblastoma, mesothelioma, myxofibrosarcoma, myeloid leukemia, mucosa-associated lymphoid tissue B cell lymphoma, multiple myeloma, high-risk myelodysplastic syndrome, nasopharyngeal carcinoma, neuroblastoma, neurofibroma, high-grade non-Hodgkin lymphoma, non-Hodgkin lymphoma, lung carcinoma, non-small cell lung carcinoma, ovarian carcinoma, oesophageal carcinoma, osteosarcoma, pancreatic carcinoma, pheochromocytoma, prostate carcinoma, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, salivary gland tumor, Schwanomma, small cell lung cancer, squamous cell carcinoma of the head and neck, testicular tumor, thyroid carcinoma, urothelial carcinoma, and Wilm's tumor.

The compositions and methods provided herein are also used to treat NSCLC (non-small cell lung cancer), pediatric malignancies, cervical and other tumors caused or promoted by human papilloma virus (HPV), melanoma, Barrett's esophagus (pre-malignant syndrome), adrenal and skin cancers and auto immune, neoplastic cutaneous diseases.

Infectious Diseases

The compositions and methods disclosed herein can be used to treat infectious diseases. The term "infectious disease" includes any disease that is caused by an infectious agent. An "infectious agent" includes any exogenous pathogen including, without limitation, bacteria, fungi, viruses, *mycoplasma*, and parasites. Infectious agents that may be treated with compositions provided for in this disclosure include any art-recognized infectious organisms that cause pathogenesis in an animal, including such organisms as bacteria that are gram-negative or gram-positive cocci or bacilli, DNA and RNA viruses, including, but not limited to, DNA viruses such as papilloma viruses, parvoviruses, adenoviruses, herpesviruses and vaccinia viruses, and RNA viruses, such as arenaviruses, coronaviruses, rhinoviruses, respiratory syncytial viruses, influenza viruses, picornaviruses, paramyxoviruses, reoviruses, retroviruses, and rhabdoviruses. Examples of fungi that may be treated with the compositions and methods of the disclosure include fungi that grow as molds or are yeastlike, including, for example, fungi that cause diseases such as ringworm, histoplasmosis, blastomycosis, aspergillosis, cryptococcosis, sporotrichosis, coccidioidomycosis, paracoccidio-idomycosis, and candidiasis. Compositions and methods provided for herein may be utilized to treat parasitic infections including, but not limited to, infections caused by somatic tapeworms, blood flukes, tissue roundworms, ameba, and *Plasmodium, Trypanosoma, Leishmania*, and *Toxoplasma* species.

Methods of GD T Cell Activation

Provided herein are compositions and methods for activating GD T cells in an individual, as well as methods for treating tumors and infectious diseases. For instance, in embodiments, the compositions and methods provided herein can be used in methods to treat all known cancers because activated GD T cells comprise a natural mechanism for immune surveillance of tumors (See for e.g.: Pauza et al. 2014 *Frontiers in Immunol.* 5:687). Likewise, in embodiments, the compositions and methods provided herein can be used to treat infectious diseases, including but not limited to flavivirus, influenza virus, human retrovirus, mycobacteria, plasmodia and a variety of other viral, fungal and bacterial infections. (See for e.g.: Pauza and Cairo, 2015 *Cell Immunol.* 296(1).

In general, a vector system is administered to an individual to transfect or transduce a target cell population with the disclosed constructs for decreasing expression of FDPS and, in other embodiments, increasing expression of chemokines or cytokines. Administration and transfection/transduction can occur in vivo or ex vivo, with the transfected cells later administered back into the subject in the latter scenario.

Administration of the disclosed vectors and transfection or transduction of the disclosed constructs into a subject's cells result in decreased expression of FDPS, increased expression of cytokines or chemokines, accumulation of IPP and in many cases, reduced growth rates for genetically modified tumor cells. All of these features work together to activate and co-localize GD T cells to the site of a tumor or infection.

The disclosed methods can also increase the capacity of NK cells to recognize and destroy tumor cells and/or infected cells. Crosstalk between GD T cells and NK cells is an important aspect of regulating the immune and inflammatory responses. Further, GD T cells are known to trigger dendritic cell maturation, recruit B cells and macrophages, and participate in a variety of cytolytic activities, such as secretion of interferon-γ and TNF-α.

In embodiments, the disclosed compositions and methods provided herein comprise a form of gene therapy for activating GD T cells at the site of tumor or infectious disease pathology. In an aspect, the compositions and methods provided herein activate GD T cells and support their proliferation, differentiation, and functional capacities by promoting the production of specific cytokines needed for cytolytic activity capable of killing cancer cells or treating infectious diseases.

In embodiments the gene therapy sequences (e.g., FDPS shRNAs) are carried by therapeutic vectors, including but not limited to viral vectors such as lentiviruses or adeno-associated viruses, although other viral vectors can also be suitable. Gene therapy constructs may also be delivered in the form of DNA or RNA, including but not limited to plasmid forms. In embodiments, the disclosed gene therapy constructs may also be delivered in the form of protein-nucleic acid complexes or lipid nucleic acid complexes and mixtures of these formulations. For instance, a protein-nucleic acid complex can comprise nucleic acids of interest in a complex with cationic peptides such as lysine and arginine. Lipid-nucleic acids complexes can comprise lipid emulsions, micelles, liposomes, and/or mixtures of neutral and cationic lipids such as DOTMA, DOSPA, DOTAP, and DMRIE.

In embodiments, therapeutic vectors may comprise a single construct or at least two, at least three, at least four, or at least five different constructs. When more than one construct is present in a vector the constructs may be identical, or they may be different. For instance, the constructs may vary in terms of their promoters, the presence or absence of an integrating elements, and/or their sequences. In some embodiments, a therapeutic vector will comprise at least one construct that encodes a small RNA capable of knocking down the expression of FDPS. In embodiments, the therapeutic vector will also encode a specific cytokine(s) and/or chemokine(s), including but not limited to TNF-α, interferon-γ, IL-1, IL-2, IL-15, IL-17, IL-18 or IL-12. In some embodiments, a single construct may encode both small RNAs capable of knocking down the expression of FDPS and specific cytokines or chemokines, including but not limited to TNF-α, interferon-γ, IL-1, IL-2, IL-15, IL-17, IL-18 or IL-12.

In embodiments, viral vectors may introduce nucleic acid constructs that become integrated into the host chromosome. Alternately, transient delivery vectors may be used to prevent chromosomal integration and limit the lifespan of gene therapy constructs.

In embodiments, the disclosed constructs and vectors comprise short homology region RNA ("shRNA"), micro RNA ("miRNA"), or siRNA capable of reducing or knocking down expression of FDPS and/or geranyl pyrophosphate synthase ("GPPS") and/or farnesyl transferase ("FT") genes. By down regulating these genes, which control steroid and isoprenoid synthesis, isopentenyl pyrophosphate ("IPP") levels are elevated. Elevation and accumulation of IPP is a known mechanism for increasing GD T cells activation. Further, down regulation of these pyrophosphate synthase genes removes an important negative regulator of inflammasome function that in turn results in increased expression of cytokines that are important for GD T cell activation and effector cell function.

In embodiments, the disclosed constructs are regulated by specific promoters that are capable of producing interleukin-2 and/or interleukin-15 to sustain GD T cell proliferation. In addition, the disclosed constructs may be regulated by specific promoters that are capable of producing interleukin-1 beta and/or interleukin-18 and/or interferon-gamma required for GD T cell differentiation and acquisition of all effector cell function. Desirable effector cell functions include the capacity for direct cytotoxic cell killing of tumors and/or infected cells, secretion of beneficial cytokines and/or chemokines, increased expression of NK receptors required to recognize cancerous or infected cells, and increased expression of Fc receptors needed to bind targeting antibodies in order to co-localize GD T cells with cancerous or infected cell targets.

In embodiments, the disclosed methods activate GD T cells, resulting in the indirect effect of increasing the capacity for NK cells to attack and destroy cancerous cells, tumors, or infected cells. The activation of NK cells requires GD T cells that are stimulated to proliferate and differentiate, and to express 4-1BBL costimulatory ligand needed to engage the 4-1BB costimulatory receptor on NK cells. This form of crosstalk is known as an important mechanism for activating NK cells and is achieved here through the action of the disclosed methods and compositions.

In another aspect, crosstalk between GD T cells and NK cells is an important mechanism for eliminating inflammatory dendritic cells that accumulate in diseased tissues. Alone, neither GD T cells nor NK cells are capable of destroying dendritic cells, but once the aforementioned crosstalk interactions have occurred, NK cells are altered to become cytotoxic against inflammatory dendritic cells. This immuno-regulatory mechanism depends on strong activation and proliferation of GD T cells.

In embodiments, the disclosed methods for activation of GD T cells further comprise a step of suppressing pathologic inflammatory responses that may include cellular proliferation leading to atherosclerosis, chronic immune activation that stimulates tumor growth, autoimmune diseases including psoriasis and other presentations in the epidermis, inflammatory diseases of the central nervous system, and arthritis and other diseases of unregulated immune responses.

In embodiments, therapeutic vectors are administered concurrently with aminobisphosphonate (ABP) drugs to achieve synergistic activation of gamma delta T cells. The synergism can allow alternate, modified or reduced doses of ABP and may decrease adverse reactions to ABP including acute inflammatory responses and chronic diseases.

Constructs for GD T Cell Activation

Inhibition of FDPS results in IPP accumulation, resulting in activation of Vδ2+GD T cells and expression of IL-18, which is also important in activating GD T cells. Inhibition of farnesyl transferase results in decreased prenylation of proteins. The disclosed constructs can be transfected or transduced into specific target cells, like tumor cells or infected cells, where they can express RNA sequences (i.e., siRNA, shRNA or microRNA) that will inhibit translation of FDPS as well as encode and express cytotoxic cytokines or chemokines.

Disclosed herein are constructs for decreasing expression of FDPS and/or FT, increasing expression of cytokines, and increasing expression of chemokines including RANTES. For instance, in some embodiments the constructs may encode for interferon-gamma, IL-1, IL-2, IL-15, IL-17, IL-18 or IL-12.

Expression of cytokines and chemokines, like those listed above, will result in localized cytotoxic destruction of tumor cells or cells infected with pathogenic organisms. Accordingly, expression of such constructs by a tumor cell or an infected cell will result in the unwanted cells assisting in its own destruction.

Likewise, if the disclosed constructs are expressed in a tumor cell or infected cell, decreasing the expression of FDPS and FT will result in activation and recruitment of GD T cells to the tumor site of site of cell infection. Increasing expression of RANTES will further attract GD T cells to intended tissue location. Because GD T cells can kill a broad range of tumors of epithelial origin as well as many leukemias and lymphomas, and are further able to produce high levels of the anti-tumor cytokine, IFNγ, recruitment of GD T cells to the site of a tumor can be a particularly effective means of inducing anti-tumor immunity.

Decreased Expression of FDPS can be Achieved Via shRNA, microRNA, siRNA, or other means known in the art. For instance, shRNAs according to SEQ ID NOS: 1, 2, 3, or 4, or variants thereof can be used in the disclosed constructs and methods, although this example is not limiting. The coding regions for RNAs to decrease expression of FDPS and FT and the coding regions of cytokine and chemokines may be in the same construct or on different constructs.

The classical approach for the production of recombinant polypeptides or gene regulatory molecules including small RNA is the use of stable expression constructs. These constructs are based upon chromosomal integration of a transduced expression plasmid (or at least a portion thereof) into the genome of the host cell, short-duration plasmid transfection, or non-integrating viral vectors also with limited half-life. The sites of gene integration are generally random, and the number and ratio of genes integrating at any particular site are often unpredictable; likewise, non-integrating plasmids or viral vectors also generate nuclear DNA but these species usually lack sequences required for DNA replication and continuous maintenance. Thus, constructs that rely on chromosomal integration result in permanent maintenance of the recombinant gene that may exceed the therapeutic interval.

An alternative to stable expression constructs for gene expression are transient expression constructs. The expression of the latter gene expression construct is based on non-integrated plasmids, and hence the expression is typically lost as the cell undergoes division or the plasmid vectors are destroyed by endogenous nucleases.

The disclosed constructs are preferably episomal constructs that are transiently expressed. Episomal constructs are degraded or diluted over time such that they do not make permanent changes to a subject's genome, nor are they incorporated into the chromosome of a target cell. The process of episomal replication typically incorporates both host cell replication machinery and viral trans-acting factors.

Avoiding chromosomal integration reduces certain barriers to in vivo gene delivery. However, even integration-defective constructs can have a background frequency of integration, and any DNA molecule can find rare homologies to recombine with host sequences; but these rates of integration are exceptionally rare and generally not clinically significant.

Thus, in some embodiments, the disclosed vectors support active gene and/or small RNA delivery over a period of about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, or about 12 weeks. In some embodiments, the disclosed vectors support active gene and/or small RNA delivery over a period of about 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, or longer. Any combination of these time periods can also be used in the methods of the invention, e.g., 1 month and 1 week, or 3 months and 2 weeks.

However, in some embodiments, the constructs comprise integrating elements that depend on a retroviral integrase gene, such that the construct becomes integrated into the subject's chromosome. Retrotransposition and transposition are additional examples of mechanisms whereby mobile genetic elements become integrated or inserted into the chromosome. Plasmids may become integrated into the chromosome by recombination, and gene editing technologies including CRISPR and TALEN utilize guide RNA sequences and alter chromosomal loci by gene conversion mechanisms.

Constructs may comprise specific promoters for expressing cytokines involved in the maintenance of GD T cells (i.e. IL-2, IL-7, IL-17, and IL-15). For example, promoters that may be incorporated into the disclosed constructs include but are not limited to TATA-box promoters, CpG-box promoters, CCAAT-box promoters, TTGACA-box promoters, BRE-box promoters, INR-box promoters, AT-based promoters, CG-based promoters, ATCG-compact promoters, ATCG-balanced promoters, ATCG-middle promoters, ATCG-less promoters, AT-less promoters, CG-less promoters, AT-spike promoters, and CG-spike promoters. See Gagniuc and Ionescu-Tirgoviste, Eukaryotic genomes may exhibit up to 10 generic classes of gene promoters, BMC GENOMICS 13:512 (2012).

Therapeutic Vectors

The construct can be delivered via known transfection and/or transduction vectors, including but not limited to lentiviral vectors, adeno-associated virus, poxvirus, herpesvirus vectors, protein and/or lipid complexes, liposomes, micelles, and the like.

Viral vectors can be preferentially targeted to cell types that are useful for the disclosed methods (i.e., tumor cells or myeloid cells). Viral vectors can be used to transduce genes into target cells owing to specific virus envelope-host cell receptor interactions and viral mechanisms for gene expression. As a result, viral vectors have been used as vehicles for the transfer of genes into many different cell types including whole embryos, fertilized eggs, isolated tissue samples, tissue targets in situ, and cultured cell lines. The ability to introduce and express foreign genes in a cell is useful for the study of gene expression, and the elucidation of cell lineages as well as providing the potential for therapeutic interventions such as gene therapy, somatic cell reprogramming of induced pluripotent stem cells, and various types of immunotherapy. Viral components from viruses like Papovaviridae (e.g. bovine papillomavirus or BPV) or Herpesviridae (e.g. Epstein Barr Virus or EBV) or Hepadnaviridae (e.g. Hepatitis B Virus or HBV) or pox vectors including vaccinia may be used in the disclosed vectors.

Lentiviral vectors are a preferred type of vector for the disclosed compositions and methods, although the disclosure is not specifically limited to lentiviral vectors. Lentivirus is a genus of viruses that can deliver a significant amount of viral nucleic acid into a host cell. Lentiviruses are characterized as having a unique ability to infect/transduce non-dividing cells, and following transduction, lentiviruses integrate their nucleic acid into the host cell's chromosomes.

Infectious lentiviruses have three main genes coding for the virulence proteins gag, pol, and env, and two regulatory genes including tat and rev. Depending on the specific serotype and virus, there may be additional accessory genes that code for proteins involved in regulation, synthesis, and/or processing viral nucleic acids and other replicative functions.

Moreover, lentiviruses contain long terminal repeat (LTR) regions, which may be approximately 600 nt long. LTRs may be segmented into U3, R, and U5 regions. LTRs can mediate integration of retroviral DNA into the host chromosome via the action of integrase. Alternatively, without functioning integrase, the LTRs may be used to circularize the viral nucleic acid.

Viral proteins involved in early stages of lentivirus replication include reverse transcriptase and integrase. Reverse transcriptase is the virally encoded, RNA-dependent DNA polymerase. The enzyme uses a viral RNA genome as a template for the synthesis of a complementary DNA copy. Reverse transcriptase also has RNaseH activity for destruction of the RNA-template. Integrase binds both the viral cDNA generated by reverse transcriptase and the host DNA. Integrase processes the LTR before inserting the viral genome into the host DNA. Tat acts as a trans-activator during transcription to enhance initiation and elongation. The rev responsive element acts post-transcriptionally, regulating mRNA splicing and transport to the cytoplasm.

Viral vectors, in general, comprise glycoproteins and the various glycoproteins may provide specific affinities. For instance, VSVG peptides can increase transfection into myeloid cells. Alternatively, viral vectors can also have targeting moieties, such as antibodies, attached to their shell peptides. Targeting antibodies can be specific for antigens that are overexpressed on a tumor, for instance, like HER-2, PSA, CEA, M2-PK, and CA19-9.

Other viral vector specificities are also known in the art and can be used to target particular populations of cells. For example, poxvirus vectors target to macrophages and dendritic cells.

Lentiviral Vector System

A lentiviral virion (particle) is expressed by a vector system encoding the necessary viral proteins to produce a virion (viral particle). There is at least one vector containing a nucleic acid sequence encoding the lentiviral pol proteins necessary for reverse transcription and integration, operably linked to a promoter. In another embodiment, the pot proteins are expressed by multiple vectors. There is also a vector containing a nucleic acid sequence encoding the lentiviral gag proteins necessary for forming a viral capsid operably linked to a promoter. In an embodiment, this gag nucleic acid sequence is on a separate vector than at least some of the pol nucleic acid sequence. In another embodiment, the gag nucleic acid is on a separate vector from all the pot nucleic acid sequences that encode pol proteins.

Numerous modifications can be made to the vectors, which are used to create the particles to further minimize the chance of obtaining wild type revertants. These include, but are not limited to deletions of the U3 region of the LTR, tat deletions and matrix (MA) deletions.

The gag, pol and env vector(s) do not contain nucleotides from the lentiviral genome that package lentiviral RNA, referred to as the lentiviral packaging sequence.

The vector(s) forming the particle preferably do not contain a nucleic acid sequence from the lentiviral genome that expresses an envelope protein. Preferably, a separate vector that contains a nucleic acid sequence encoding an envelope protein operably linked to a promoter is used. This env vector also does not contain a lentiviral packaging sequence. In one embodiment the env nucleic acid sequence encodes a lentiviral envelope protein.

In another embodiment the envelope protein is not from the lentivirus, but from a different virus. The resultant particle is referred to as a pseudotyped particle. By appropriate selection of envelopes one can "infect" virtually any cell. For example, one can use an env gene that encodes an envelope protein that targets an endocytic compartment such as that of the influenza virus, VSV-G, alpha viruses (Semliki forest virus, Sindbis virus), arenaviruses (lymphocytic choriomeningitis virus), flaviviruses (tick-borne encephalitis virus, Dengue virus, hepatitis C virus, GB virus), rhabdoviruses (vesicular stomatitis virus, rabies virus), paramyxoviruses (mumps or measles) and orthomyxoviruses (influenza virus). Other envelopes that can preferably be used include those from Moloney Leukemia Virus such as MLV-E, MLV-A and GAIN. These latter envelopes are particularly preferred where the host cell is a primary cell. Other envelope proteins can be selected depending upon the desired host cell. For example, targeting specific receptors such as a dopamine receptor can be used for brain delivery. Another target can be vascular endothelium. These cells can be targeted using a filovirus envelope. For example, the GP of Ebola, which by post-transcriptional modification become the GP, and $GP_2$ glycoproteins. In another embodiment, one can use different lentiviral capsids with a pseudotyped envelope (for example, FIV or SHIV [U.S. Pat. No. 5,654,195]). A SHIV pseudotyped vector can readily be used in animal models such as monkeys.

As detailed herein, a lentiviral vector system typically includes at least one helper plasmid comprising at least one of a gag, pol, or rev gene. Each of the gag, pol and rev genes may be provided on individual plasmids, or one or more genes may be provided together on the same plasmid. In one embodiment, the gag, pol, and rev genes are provided on the same plasmid (e.g., FIG. **2**). In another embodiment, the gag and pol genes are provided on a first plasmid and the rev gene is provided on a second plasmid (e.g., FIG. **3**). Accordingly, both 3-vector and 4-vector systems can be used to produce a lentivirus as described in the Examples section and elsewhere herein. The therapeutic vector, the envelope plasmid and at least one helper plasmid are transfected into a packaging cell line. A non-limiting example of a packaging cell line is the 293T/17 HEK cell line. When the therapeutic vector, the envelope plasmid, and at least one helper plasmid are transfected into the packaging cell line, a lentiviral particle is ultimately produced.

In another aspect, a lentiviral vector system for expressing a lentiviral particle is disclosed. The system includes a lentiviral vector as described herein; an envelope plasmid for expressing an envelope protein optimized for infecting a cell; and at least one helper plasmid for expressing gag, pol, and rev genes, wherein when the lentiviral vector, the envelope plasmid, and the at least one helper plasmid are transfected into a packaging cell line, a lentiviral particle is produced by the packaging cell line, wherein the lentiviral particle is capable of inhibiting production of chemokine receptor CCR5 or targeting an HIV RNA sequence.

In another aspect, and as detailed in FIG. **2**, the lentiviral vector, which is also referred to herein as a therapeutic vector, can include the following elements: hybrid 5' long terminal repeat (RSV/5' LTR) (SEQ ID NOS: 11-12), Psi sequence (RNA packaging site) (SEQ ID NO: 13), RRE (Rev-response element) (SEQ ID NO: 14), cPPT (polypurine tract) (SEQ ID NO: 15), H1 promoter (SEQ ID NO: 16), FDPS shRNA (SEQ ID NOS: 1, 2, 3, 4), Woodchuck Post-Transcriptional Regulatory Element (WPRE) (SEQ ID NO: 17), and 3' Delta LTR (SEQ ID NO: 18). In another aspect, sequence variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

In another aspect, and as detailed herein, a helper plasmid has been designed to include the following elements: CAG promoter (SEQ ID NO: 19); HIV component gag (SEQ ID NO: 20); HIV component pol (SEQ ID NO: 21); HIV Int (SEQ ID NO: 22); HIV RRE (SEQ ID NO: 23); and HIV Rev (SEQ ID NO: 24). In another aspect, the helper plasmid may be modified to include a first helper plasmid for expressing the gag and pol genes, and a second and separate plasmid for expressing the rev gene. In another aspect, sequence variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

In another aspect, and as detailed herein, an envelope plasmid has been designed to include the following elements being from left to right: RNA polymerase II promoter (CMV) (SEQ ID NO: 25) and vesicular stomatitis virus G glycoprotein (VSV-G) (SEQ ID NO: 26). In another aspect, sequence variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

In another aspect, the plasmids used for lentiviral packaging can be modified with similar elements and the intron sequences could potentially be removed without loss of vector function. For example, the following elements can replace similar elements in the plasmids that comprise the packaging system: Elongation Factor-1 (EF-1), phosphoglycerate kinase (PGK), and ubiquitin C (UbC) promoters can replace the CMV or CAG promoter. SV40 poly A and bGH poly A can replace the rabbit beta globin poly A. The HIV sequences in the helper plasmid can be constructed from different HIV strains or clades. The VSV-G glycoprotein can be substituted with membrane glycoproteins from feline endogenous virus (RD114), gibbon ape leukemia virus (GALV), Rabies (FUG), lymphocytic choriomeningitis virus (LCMV), influenza A fowl plague virus (FPV), Ross River alphavirus (RRV), murine leukemia virus 10A1 (MLV), or Ebola virus (EboV).

Of note, lentiviral packaging systems can be acquired commercially (e.g., Lenti-vpak packaging kit from OriGene Technologies, Inc., Rockville, Md.), and can also be designed as described herein. Moreover, it is within the skill of a person skilled in the art to substitute or modify aspects of a lentiviral packaging system to improve any number of relevant factors, including the production efficiency of a lentiviral particle.

Doses and Dosage Forms

The disclosed vectors allow for short, medium, or long-term expression of genes or sequences of interest and episomal maintenance of the disclosed vectors. Accordingly, dosing regimens may vary based upon the condition being treated and the method of administration.

In one embodiment, transduction vectors may be administered to a subject in need in varying doses. Specifically, a subject may be administered about $\geq 10^6$ infectious doses (where 1 dose is needed on average to transduce 1 target cell). More specifically, a subject may be administered about $\geq 10^7$, about $\geq 10^8$, about $\geq 10^9$, or about $\geq 10^{10}$ infectious doses, or any number of doses in-between these values. Upper limits of transduction vector dosing will be determined for each disease indication and will depend on toxicity/safety profiles for each individual product or product lot.

Additionally, a vector of the present disclosure may be administered periodically, such as once or twice a day, or any other suitable time period. For example, vectors may be administered to a subject in need once a week, once every other week, once every three weeks, once a month, every other month, every three months, every six months, every nine months, once a year, every eighteen months, every two years, every thirty months, or every three years.

In one embodiment, the disclosed vectors are administered as a pharmaceutical composition. In some embodiments, the pharmaceutical composition comprising the disclosed vectors can be formulated in a wide variety of dosage forms, including but not limited to nasal, pulmonary, oral, topical, or parenteral dosage forms for clinical application. Each of the dosage forms can comprise various solubilizing agents, disintegrating agents, surfactants, fillers, thickeners, binders, diluents such as wetting agents or other pharmaceutically acceptable excipients. The pharmaceutical composition comprising a vector can also be formulated for injection, insufflation, infusion, or intradermal exposure. For instance, an injectable formulation may comprise the disclosed vectors in an aqueous or non-aqueous solution at a suitable pH and tonicity.

The disclosed vectors may be administered to a subject via direct injection into a tumor site or at a site of infection. In some embodiments, the vectors can be administered systemically. In some embodiments, the vectors can be administered via guided cannulation to tissues immediately surrounding the sites of tumor or infection.

The disclosed vector compositions can be administered using any pharmaceutically acceptable method, such as intranasal, buccal, sublingual, oral, rectal, ocular, parenteral (intravenously, intradermally, intramuscularly, subcutaneously, intraperitoneally), pulmonary, intravaginal, locally administered, topically administered, topically administered after scarification, mucosally administered, via an aerosol, in semi-solid media such as agarose or gelatin, or via a buccal or nasal spray formulation.

Further, the disclosed vector compositions can be formulated into any pharmaceutically acceptable dosage form, such as a solid dosage form, tablet, pill, lozenge, capsule, liquid dispersion, gel, aerosol, pulmonary aerosol, nasal aerosol, ointment, cream, semi-solid dosage form, a solution, an emulsion, and a suspension. Further, the composition may be a controlled release formulation, sustained release formulation, immediate release formulation, or any combination thereof. Further, the composition may be a transdermal delivery system.

In some embodiments, the pharmaceutical composition comprising a vector can be formulated in a solid dosage form for oral administration, and the solid dosage form can be powders, granules, capsules, tablets or pills. In some embodiments, the solid dosage form can include one or more excipients such as calcium carbonate, starch, sucrose, lactose, microcrystalline cellulose or gelatin. In addition, the solid dosage form can include, in addition to the excipients, a lubricant such as talc or magnesium stearate. In some embodiments, the oral dosage form can be immediate release, or a modified release form. Modified release dosage forms include controlled or extended release, enteric release, and the like. The excipients used in the modified release dosage forms are commonly known to a person of ordinary skill in the art.

In a further embodiment, the pharmaceutical composition comprising a vector can be formulated as a sublingual or buccal dosage form. Such dosage forms comprise sublingual tablets or solution compositions that are administered under the tongue and buccal tablets that are placed between the cheek and gum.

In some embodiments, the pharmaceutical composition comprising a vector can be formulated as a nasal dosage form. Such dosage forms of the present invention comprise solution, suspension, and gel compositions for nasal delivery.

In some embodiments, the pharmaceutical composition comprising a vector can be formulated in a liquid dosage form for oral administration, such as suspensions, emulsions or syrups. In some embodiments, the liquid dosage form can include, in addition to commonly used simple diluents such as water and liquid paraffin, various excipients such as humectants, sweeteners, aromatics or preservatives. In particular embodiments, the composition comprising vectors can be formulated to be suitable for administration to a pediatric patient.

In some embodiment, the pharmaceutical composition can be formulated in a dosage form for parenteral administration, such as sterile aqueous solutions, suspensions, emulsions, non-aqueous solutions or suppositories. In some embodiments, the solutions or suspensions can include propyleneglycol, polyethyleneglycol, vegetable oils such as olive oil or injectable esters such as ethyl oleate.

The dosage of the pharmaceutical composition can vary depending on the patient's weight, age, gender, administration time and mode, excretion rate, and the severity of disease.

In some embodiments, the treatment of cancer is accomplished by guided direct injection of the disclosed vector constructs into tumors, using needle, or intravascular cannulation. In some embodiments, the disclosed vectors are administered into the cerebrospinal fluid, blood or lymphatic circulation by venous or arterial cannulation or injection, intradermal delivery, intramuscular delivery or injection into a draining organ near the site of disease.

The following examples are given to illustrate the present invention. It should be understood, however, that the invention is not to be limited to the specific conditions or details described in these examples. All printed publications referenced herein are specifically incorporated by reference.

## EXAMPLES

### Example 1: Development of a Lentiviral Vector System

A lentiviral vector system was developed as summarized in FIG. 4 (circularized form). Lentiviral particles were produced in 293T/17 HEK cells (purchased from American Type Culture Collection, Manassas, Va.) following transfection with the therapeutic vector, the envelope plasmid, and the helper plasmid. The transfection of 293T/17 HEK cells, which produced functional viral particles, employed the reagent Poly(ethylenimine) (PEI) to increase the efficiency of plasmid DNA uptake. The plasmids and DNA were initially added separately in culture medium without serum in a ratio of 3:1 (mass ratio of PEI to DNA). After 2-3 days, cell medium was collected and lentiviral particles were purified by high-speed centrifugation and/or filtration followed by anion-exchange chromatography. The concentration of lentiviral particles can be expressed in terms of transducing units/ml (TU/ml). The determination of TU was accomplished by measuring HIV p24 levels in culture fluids (p24 protein is incorporated into lentiviral particles), measuring the number of viral DNA copies per cell by quantitative PCR, or by infecting cells and using light (if the vectors encode luciferase or fluorescent protein markers).

As mentioned above, a 3-vector system (i.e., a 2-vector lentiviral packaging system) was designed for the production of lentiviral particles. A schematic of the 3-vector system is shown in FIG. **2**. Briefly, and with reference to FIG. **2**, the top-most vector is a helper plasmid, which, in this case, includes Rev. The vector appearing in the middle of FIG. **2** is the envelope plasmid. The bottom-most vector is the therapeutic vector, as described herein.

Referring more specifically to FIG. **2**, the Helper plus Rev plasmid includes a CAG enhancer (SEQ ID NO: 27); a CAG promoter (SEQ ID NO: 19); a chicken beta actin intron (SEQ ID NO: 28); a HIV gag (SEQ ID NO: 20); a HIV Pol (SEQ ID NO: 21); a HIV Int (SEQ ID NO: 22); a HIV RRE (SEQ ID NO: 23); a HIV Rev (SEQ ID NO: 24); and a rabbit beta globin poly A (SEQ ID NO: 29).

The Envelope plasmid includes a CMV promoter (SEQ ID NO: 25); a beta globin intron (SEQ ID NO: 30); a VSV-G (SEQ ID NO: 28); and a rabbit beta globin poly A (SEQ ID NO: 31).

Synthesis of a 2-Vector Lentiviral Packaging System Including Helper (Plus Rev) and Envelope Plasmids.

Materials and Methods:

Construction of the helper plasmid: The helper plasmid was constructed by initial PCR amplification of a DNA fragment from the pNL4-3 HIV plasmid (NIH Aids Reagent Program) containing Gag, Pol, and Integrase genes. Primers were designed to amplify the fragment with EcoRI and NotI restriction sites which could be used to insert at the same sites in the pCDNA3 plasmid (Invitrogen). The forward primer was (5'-TAAGCAGAATTC ATGAATTTGCCAGGAAGAT-3') (SEQ ID NO: 32) and reverse primer was (5'-CCATACAATGAATGGACACTAGGCGGCCGCACGAAT-3') (SEQ ID NO: 33).

The sequence for the Gag, Pol, Integrase fragment was as follows:

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                                          (SEQ ID NO: 34)
GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGGAAT

TGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCT

GCGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAAC

ATAATTGGAAGAAATCTGTTGACTCAGATTGGCTGCACTTTAAATTTTCC

CATTAGTCCTATTGAGACTGTACCAGTAAAATTAAAGCCAGGAATGGATG

GCCCAAAAGTTAAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTA

GTAGAAATTTGTACAGAAATGGAAAAGGAAGGAAAAATTTCAAAAATTGG

GCCTGAAAATCCATACAATACTCCAGTATTTGCCATAAAGAAAAAAGACA

GTACTAAATGGAGAAAATTAGTAGATTTCAGAGAACTTAATAAGAGAACT

CAAGATTTCTGGGAAGTTCAATTAGGAATACCACATCCTGCAGGGTTAAA

ACAGAAAAAATCAGTAACAGTACTGGATGTGGGCGATGCATATTTTTCAG

TTCCCTTAGATAAAGACTTCAGGAAGTATACTGCATTTACCATACCTAGT
```

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                    -continued
ATAAACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACA

GGGATGGAAAGGATCACCAGCAATATTCCAGTGTAGCATGACAAAAATCT

TAGAGCCTTTTAGAAAACAAAATCCAGACATAGTCATCTATCAATACATG

GATGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAA

AATAGAGGAACTGAGACAACATCTGTTGAGGTGGGGATTTACCACACCAG

ACAAAAAACATCAGAAAGAACCTCCATTCCTTTGGATGGGTTATGAACTC

CATCCTGATAAATGGACAGTACAGCCTATAGTGCTGCCAGAAAAGGACAG

CTGGACTGTCAATGACATACAGAAATTAGTGGGAAAATTGAATTGGGCAA

GTCAGATTTATGCAGGGATTAAAGTAAGGCAATTATGTAAACTTCTTAGG

GGAACCAAAGCACTAACAGAAGTAGTACCACTAACAGAAGAAGCAGAGCT

AGAACTGGCAGAAAACAGGGAGATTCTAAAAGAACCGGTACATGGAGTGT

ATTATGACCCATCAAAAGACTTAATAGCAGAAATACAGAAGCAGGGGCAA

GGCCAATGGACATATCAAATTTATCAAGAGCCATTTAAAAATCTGAAAAC

AGGAAAGTATGCAAGAATGAAGGGTGCCCACACTAATGATGTGAAACAAT

TAACAGAGGCAGTACAAAAAATAGCCACAGAAAGCATAGTAATATGGGGA

AAGACTCCTAAATTTAAATTACCCATACAAAAGGAAACATGGGAAGCATG

GTGGACAGAGTATTGGCAAGCCACCTGGATTCCTGAGTGGGAGTTTGTCA

ATACCCCTCCCTTAGTGAAGTTATGGTACCAGTTAGAGAAAGAACCCATA

ATAGGAGCAGAAACTTTCTATGTAGATGGGGCAGCCAATAGGGAAACTAA

ATTAGGAAAAGCAGGATATGTAACTGACAGAGGAAGACAAAAAGTTGTCC

CCCTAACGGACACAACAAATCAGAAGACTGAGTTACAAGCAATTCATCTA

GCTTTGCAGGATTCGGGATTAGAAGTAAACATAGTGACAGACTCACAATA

TGCATTGGGAATCATTCAAGCACAACCAGATAAGAGTGAATCAGAGTTAG

TCAGTCAAATAATAGAGCAGTTAATAAAAAAGGAAAAAGTCTACCTGGCA

TGGGTACCAGCACACAAAGGAATTGGAGGAAATGAACAAGTAGATAAATT

GGTCAGTGCTGGAATCAGGAAAGTACTATTTTTAGATGGAATAGATAAGG

CCCAAGAAGAACATGAGAAATATCACAGTAATTGGAGAGCAATGGCTAGT

GATTTTAACCTACCACCTGTAGTAGCAAAAGAAATAGTAGCCAGCTGTGA

TAAATGTCAGCTAAAAGGGGAAGCCATGCATGGACAAGTAGACTGTAGCC

CAGGAATATGGCAGCTAGATTGTACACATTTAGAAGGAAAAGTTATCTTG

GTAGCAGTTCATGTAGCCAGTGGATATATAGAAGCAGAAGTAATTCCAGC

AGAGACAGGGCAAGAAACAGCATACTTCCTCTTAAAATTAGCAGGAAGAT

GGCCAGTAAAAACAGTACATACAGACAATGGCAGCAATTTCACCAGTACT

ACAGTTAAGGCCGCCTGTTGGTGGGCGGGGATCAAGCAGGAATTTGGCAT

TCCCTACAATCCCCAAAGTCAAGGAGTAATAGAATCTATGAATAAAGAAT

TAAAGAAAATTATAGGACAGGTAAGAGATCAGGCTGAACATCTTAAGACA

GCAGTACAAATGGCAGTATTCATCCACAATTTTAAAAGAAAAGGGGGGAT

TGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACA

TACAAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTTTCGG

GTTTATTACAGGGACAGCAGAGATCCAGTTTGGAAAGGACCAGCAAAGCT

CCTCTGGAAAGGTGAAGGGGCAGTAGTAATACAAGATAATAGTGACATAA
```

-continued

AAGTAGTGCCAAGAAGAAAAGCAAAGATCATCAGGGATTATGGAAAACAG

ATGGCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAA

Next, a DNA fragment containing the Rev, RRE, and rabbit beta globin poly A sequence with XbaI and XmaI flanking restriction sites was synthesized by MWG Operon. The DNA fragment was then inserted into the plasmid at the XbaI and XmaI restriction sites The DNA sequence was as follows:

(SEQ ID NO: 35)

TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCATCAGAAC

AGTCAGACTCATCAAGCTTCTCTATCAAAGCAACCCACCTCCCAATCCCG

AGGGGACCCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGA

CAGAGACAGATCCATTCGATTAGTGAACGGATCCTTGGCACTTATCTGGG

ACGATCTGCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACTTA

CTCTTGATTGTAACGAGGATTGTGGAACTTCTGGGACGCAGGGGGTGGGA

AGCCCTCAAATATTGGTGGAATCTCCTACAATATTGGAGTCAGGAGCTAA

AGAATAGAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACT

ATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTC

TGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAAC

AGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGA

ATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTAGATCTTTT

TCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGA

CTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAAT

TTTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAA

ACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCT

GGCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAGTATATGAAACA

GCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGTT

AGATTTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTA

AAATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACT

ACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATCCCTCGACCTGC

AGCCCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTG

TTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTA

AAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGC

TCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCGGATCCGCAT

CTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCC

CCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTT

TTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAG

AAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAAC

TTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAA

TTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCA

AACTCATCAATGTATCTTATCAGCGGCCGCCCCGGG

Finally, the CMV promoter of pCDNA3.1 was replaced with the CAG enhancer/promoter plus a chicken beta actin intron sequence. A DNA fragment containing the CAG enhancer/promoter/intron sequence with MluI and EcoRI flanking restriction sites was synthesized by MWG Operon. The DNA fragment was then inserted into the plasmid at the MluI and EcoRI restriction sites. The DNA sequence was as follows:

(SEQ ID NO: 36)

ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCC

CATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGC

TGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCC

CATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATT

TACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT

ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGC

CCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTAT

TAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCTTCAC

TCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTT

TTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGCGCGCGCC

AGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTG

CGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCG

AGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGG

AGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCC

GCCCGCCCCGGCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGG

GACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCT

CGTTTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAGGGCC

CTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGT

GGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCTGCGG

GCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGC

CGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGGCTG

CGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCGG

TCGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGCACGG

CCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCG

TGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCG

CCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCGGAGCGCC

GGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATC

GTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGCGGAGCCGAA

ATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGTG

CGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGC

GCCGCCGTCCCCTTCTCCATCTCCAGCCTCGGGGCTGCCGCAGGGGGACG

GCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTG

ACCGGCGGGAATTC

Construction of the VSV-G Envelope Plasmid:

The vesicular stomatitis Indiana virus glycoprotein (VSV-G) sequence was synthesized by MWG Operon with flanking EcoRI restriction sites. The DNA fragment was then inserted into the pCDNA3.1 plasmid (Invitrogen) at the EcoRI restriction site and the correct orientation was determined by sequencing using a CMV specific primer. The DNA sequence was as follows:

```
                                        (SEQ ID NO: 37)
GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTTATTCATTGGGGTGAA
TTGCAAGTTCACCATAGTTTTTCCACACAACCAAAAAGGAAACTGGAAAA
ATGTTCCTTCTAATTACCATTATTGCCCGTCAAGCTCAGATTTAAATTGG
CATAATGACTTAATAGGCACAGCCTTACAAGTCAAAATGCCCAAGAGTCA
CAAGGCTATTCAAGCAGACGGTTGGATGTGTCATGCTTCCAAATGGGTCA
CTACTTGTGATTTCCGCTGGTATGGACCGAAGTATATAACACATTCCATC
CGATCCTTCACTCCATCTGTAGAACAATGCAAGGAAAGCATTGAACAAAC
GAAACAAGGAACTTGGCTGAATCCAGGCTTCCCTCCTCAAAGTTGTGGAT
ATGCAACTGTGACGGATGCCGAAGCAGTGATTGTCCAGGTGACTCCTCAC
CATGTGCTGGTTGATGAATACACAGGAGAATGGGTTGATTCACAGTTCAT
CAACGGAAAATGCAGCAATTACATATGCCCCACTGTCCATAACTCTACAA
CCTGGCATTCTGACTATAAGGTCAAAGGGCTATGTGATTCTAACCTCATT
TCCATGGACATCACCTTCTTCTCAGAGGACGGAGAGCTATCATCCCTGGG
AAAGGAGGGCACAGGGTTCAGAAGTAACTACTTTGCTTATGAAACTGGAG
GCAAGGCCTGCAAAATGCAATACTGCAAGCATTGGGGAGTCAGACTCCCA
TCAGGTGTCTGGTTCGAGATGGCTGATAAGGATCTCTTTGCTGCAGCCAG
ATTCCCTGAATGCCCAGAAGGGTCAAGTATCTCTGCTCCATCTCAGACCT
CAGTGGATGTAAGTCTAATTCAGGACGTTGAGAGGATCTTGGATTATTCC
CTCTGCCAAGAAACCTGGAGCAAAATCAGAGCGGGTCTTCCAATCTCTCC
AGTGGATCTCAGCTATCTTGCTCCTAAAAACCCAGGAACCGGTCCTGCTT
TCACCATAATCAATGGTACCCTAAAATACTTTGAGACCAGATACATCAGA
GTCGATATTGCTGCTCCAATCCTCTCAAGAATGGTCGGAATGATCAGTGG
AACTACCACAGAAAGGGAACTGTGGGATGACTGGGCACCATATGAAGACG
TGGAAATTGGACCCAATGGAGTTCTGAGGACCAGTTCAGGATATAAGTTT
CCTTTATACATGATTGGACATGGTATGTTGGACTCCGATCTTCATCTTAG
CTCAAAGGCTCAGGTGTTCGAACATCCTCACATTCAAGACGCTGCTTCGC
AACTTCCTGATGATGAGAGTTTATTTTTTGGTGATACTGGGCTATCCAAA
AATCCAATCGAGCTTGTAGAAGGTTGGTTCAGTAGTTGGAAAAGCTCTAT
TGCCTCTTTTTTCTTTATCATAGGGTTAATCATTGGACTATTCTTGGTTC
TCCGAGTTGGTATCCATCTTTGCATTAAATTAAAGCACACCAAGAAAAGA
CAGATTTATACAGACATAGAGATGAGAATTC
```

A 4-vector system (i.e., a 3-vector lentiviral packaging system) has also been designed and produced using the methods and materials described herein. A schematic of the 4-vector system is shown in FIG. **3**. Briefly, and with reference to FIG. **3**, the top-most vector is a helper plasmid, which, in this case, does not include Rev. The vector second from the top is a separate Rev plasmid. The vector second from the bottom is the envelope plasmid. The bottom-most vector is the previously described therapeutic vector.

Referring, in part, to FIG. **2**, the Helper plasmid includes a CAG enhancer (SEQ ID NO: 27); a CAG promoter (SEQ ID NO: 19); a chicken beta actin intron (SEQ ID NO: 28); a HIV gag (SEQ ID NO: 20); a HIV Pol (SEQ ID NO: 21); a HIV Int (SEQ ID NO: 22); a HIV RRE (SEQ ID NO: 23); and a rabbit beta globin poly A (SEQ ID NO: 29).

The Rev plasmid includes a RSV promoter (SEQ ID NO: 38); a HIV Rev (SEQ ID NO: 39); and a rabbit beta globin poly A (SEQ ID NO: 29).

The Envelope plasmid includes a CMV promoter (SEQ ID NO: 25); a beta globin intron (SEQ ID NO: 30); a VSV-G (SEQ ID NO: 28); and a rabbit beta globin poly A (SEQ ID NO: 29).

Synthesis of a 3-Vector Lentiviral Packaging System Including Helper, Rev, and Envelope Plasmids.

Materials and Methods:

Construction of the Helper Plasmid without Rev:

The Helper plasmid without Rev was constructed by inserting a DNA fragment containing the RRE and rabbit beta globin poly A sequence. This sequence was synthesized by MWG Operon with flanking XbaI and XmaI restriction sites. The RRE/rabbit poly A beta globin sequence was then inserted into the Helper plasmid at the XbaI and XmaI restriction sites. The DNA sequence is as follows:

```
                                        (SEQ ID NO: 67)
TCTAGAAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTA
TGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCT
GGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACA
GCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAA
TCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTAGATCTTTTT
CCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGAC
TTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATT
TTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAA
CATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTG
GCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAGTATATGAAACAG
CCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGTTA
GATTTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAA
AATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTA
CTCCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATCCCTCGACCTGCA
GCCCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGT
TATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAA
AGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCT
CACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCGGATCCGCATC
TCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCC
CTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTT
TTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGA
AGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAACT
TGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAAT
TTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAA
ACTCATCAATGTATCTTATCACCCGGG
```

Construction of the Rev Plasmid:

The RSV promoter and HIV Rev sequence was synthesized as a single DNA fragment by MWG Operon with flanking MfeI and XbaI restriction sites. The DNA fragment was then inserted into the pCDNA3.1 plasmid (Invitrogen) at the MfeI and XbaI restriction sites in which the CMV promoter is replaced with the RSV promoter. The DNA sequence was as follows:

```
                                       (SEQ ID NO: 40)
CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGGGGACTAGGGTG

TGTTTAGGCGAAAAGCGGGGCTTCGGTTGTACGCGGTTAGGAGTCCCCTC

AGGATATAGTAGTTTCGCTTTTGCATAGGGAGGGGGAAATGTAGTCTTAT

GCAATACACTTGTAGTCTTGCAACATGGTAACGATGAGTTAGCAACATGC

CTTACAAGGAGAGAAAAAGCACCGTGCATGCCGATTGGTGGAAGTAAGGT

GGTACGATCGTGCCTTATTAGGAAGGCAACAGACAGGTCTGACATGGATT

GGACGAACCACTGAATTCCGCATTGCAGAGATAATTGTATTTAAGTGCCT

AGCTCGATACAATAAACGCCATTTGACCATTCACCACATTGGTGTGCACC

TCCAAGCTCGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCAT

CCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCC

CTCGAAGCTAGCGATTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAG

CGACGAAGAACTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAA

GCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAAGGAATAGA

AGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTCGATTAGTGAACG

GATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCAGC

TACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGGAACT

TCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATCTCCTAC

AATATTGGAGTCAGGAGCTAAAGAATAGTCTAGA
```

The plasmids for the 2-vector and 3-vector packaging systems could be modified with similar elements and the intron sequences could potentially be removed without loss of vector function. For example, the following elements could replace similar elements in the 2-vector and 3-vector packaging system:

Promoters: Elongation Factor-1 (EF-1) (SEQ ID NO: 41), phosphoglycerate kinase (PGK) (SEQ ID NO: 42), and ubiquitin C (UbC) (SEQ ID NO: 43) can replace the CMV (SEQ ID NO: 25) or CAG promoter (SEQ ID NO: 19). These sequences can also be further varied by addition, substitution, deletion or mutation.

Poly A sequences: SV40 poly A (SEQ ID NO: 44) and bGH poly A (SEQ ID NO: 45) can replace the rabbit beta globin poly A (SEQ ID NO: 29). These sequences can also be further varied by addition, substitution, deletion or mutation.

HIV Gag, Pol, and Integrase sequences: The HIV sequences in the Helper plasmid can be constructed from different HIV strains or clades. For example, HIV Gag (SEQ ID NO: 20); HIV Pol (SEQ ID NO: 21); and HIV Int (SEQ ID NO: 22) from the Bal strain can be interchanged with the gag, pol, and int sequences contained in the helper/helper plus Rev plasmids as outlined herein. These sequences can also be further varied by addition, substitution, deletion or mutation.

Envelope: The VSV-G glycoprotein can be substituted with membrane glycoproteins from feline endogenous virus (RD114) (SEQ ID NO: 46), gibbon ape leukemia virus (GALV) (SEQ ID NO: 47), Rabies (FUG) (SEQ ID NO: 48), lymphocytic choriomeningitis virus (LCMV) (SEQ ID NO: 49), influenza A fowl plague virus (FPV) (SEQ ID NO: 50), Ross River alphavirus (RRV) (SEQ ID NO: 51), murine leukemia virus 10A1 (MLV) (SEQ ID NO: 52), or Ebola virus (EboV) (SEQ ID NO: 53). Sequences for these envelopes are identified in the sequence portion herein. Further, these sequences can also be further varied by addition, substitution, deletion or mutation.

In summary, the 3-vector versus 4-vector systems can be compared and contrasted, in part, as follows. The 3-vector lentiviral vector system contains: 1. Helper plasmid: HIV Gag, Pol, Integrase, and Rev/Tat; 2. Envelope plasmid: VSV-G/FUG envelope; and 3. Therapeutic vector: RSV 5'LTR, Psi Packaging Signal, Gag fragment, RRE, Env fragment, cPPT, WPRE, and 3'6 LTR. The 4-vector lentiviral vector system contains: 1. Helper plasmid: HIV Gag, Pol, and Integrase; 2. Rev plasmid: Rev; 3. Envelope plasmid: VSV-G/FUG envelope; and 4. Therapeutic vector: RSV 5'LTR, Psi Packaging Signal, Gag fragment, RRE, Env fragment, cPPT, WPRE, and 3'delta LTR. Sequences corresponding with the above elements are identified in the sequence listings portion herein.

## Example 2: Development of a Lentiviral Vector that Expresses FDPS

The purpose of this Example was to develop an FDPS lentivirus vector.

Inhibitory RNA Design:

The sequence of *Homo sapiens* Farnesyl diphosphate synthase (FDPS) (NM_002004.3) mRNA was used to search for potential siRNA or shRNA candidates to knockdown FDPS levels in human cells. Potential RNA interference sequences were chosen from candidates selected by siRNA or shRNA design programs such as from GPP Web Portal hosted by the Broad Institute (http://portals.broadinstitute.org/gpp/public/) or the BLOCK-iT RNAi Designer from Thermo Scientific (https://rnaidesigner.thermofisher.com/rnaiexpress/). Individual selected shRNA sequences were inserted into a lentiviral vector immediately 3 prime to a RNA polymerase III promoter such as H1 (SEQ ID NO: 16), U6 (SEQ ID NO: 54), or 7SK (SEQ ID NO: 55) to regulate shRNA expression. These lentivirus shRNA constructs were used to transduce cells and measure the change in specific mRNA levels. The shRNA most potent for reducing mRNA levels were embedded individually within a microRNA backbone to allow for expression by either the EF-1alpha or CMV RNA polymerase II promoters. The microRNA backbone was selected from mirbase.org. RNA sequences were also synthesized as synthetic siRNA oligonucleotides and introduced directly into cells without using a lentiviral vector.

Vector Construction: For FDPS shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by Eurofins MWG Operon. Overlapping sense and antisense oligonucleotide sequences were mixed and annealed during cooling from 70 degrees Celsius to room temperature. The lentiviral vector was digested with the restriction enzymes BamHI and EcoRI for one hour at 37 degrees Celsius. The digested lentiviral vector was purified by agarose gel electrophoresis and extracted from the gel using a DNA gel extraction kit from Thermo Scientific. The DNA concentrations were determined and vector to oligo (3:1 ratio) were mixed, allowed to anneal, and ligated. The ligation reaction was performed with T4 DNA ligase for 30 minutes at room temperature. 2.5 microliters of the ligation mix were added to 25 microliters of STBL3 competent bacterial cells. Transformation was achieved after heat-shock at 42 degrees Celsius. Bacterial cells were spread on agar plates containing ampicillin and drug-resistant colonies (indicating the presence of ampicillin-resistance plasmids) were recovered and expanded in LB broth. To check for insertion of the oligo sequences, plasmid DNA was extracted from harvested bacteria cultures with the Thermo Scientific DNA mini prep kit. Insertion of shRNA sequences in the lentiviral vector was verified by DNA sequencing using a specific primer for the promoter used to regulate shRNA expression. Using the following target sequences, exemplary shRNA sequences were determined to knock-down FDPS:

```
(FDPS target sequence #1; SEQ ID NO: 56)
GTCCTGGAGTACAATGCCATT;

(FDPS shRNA sequence #1; SEQ ID NO: 1)
GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTACTCCAGGAC
TTTTT;

(FDPS target sequence #2; SEQ ID NO: 57)
GCAGGATTTCGTTCAGCACTT;

(FDPS shRNA sequence #2; SEQ ID NO: 2)
GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCTGAACGAAATCCTGC
TTTTT;

(FDPS target sequence #3; SEQ ID NO: 58)
GCCATGTACATGGCAGGAATT;

(FDPS shRNA sequence #3; SEQ ID NO: 3)
GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGTACATGGC
TTTTT;

(FDPS target sequence #4; SEQ ID NO: 59)
GCAGAAGGAGGCTGAGAAAGT;
and

(FDPS shRNA sequence #4; SEQ ID NO: 4)
GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGC
TTTTT.
```

shRNA sequences were then assembled into a synthetic microRNA (miR) under control of the EF-1 alpha promoter. Briefly, a miR hairpin sequences, such as miR30, miR21, or miR185 as detailed below, was obtained from mirbase.org. The 19-22mer shRNA target sequence was used to construct the synthetic miR sequence. The miR sequence was arranged as an anti-sense-target-sequence-hairpin loop sequence (specific for each microRNA)-sense target sequence.

The following miR sequences were developed:

```
(miR30 FDPS sequence #1; SEQ ID NO: 5)
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCT

GCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACT

GCCTCGGACTTCAAGGGGCT

(miR30 FDPS sequence #2; SEQ ID NO: 6)
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCT

GCGTGAAGCCACAGATGGCAGAAGGGCTGAGAAAGTGCTGCCTACTGC

CTCGGACTTCAAGGGGCT

(miR30 FDPS sequence #3; SEQ ID NO: 7)
TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCGTGAAGCCA

CAGATGGCAGAAGGAGGCTGAGAAAGTTGCCTACTGCCTCGGA

(miR155 FDPS sequence #1; SEQ ID NO: 8)
CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCTCAGCCTCCTTCT

GCTTTTGGCCACTGACTGAGCAGAAGGGCTGAGAAAGTCAGGACACAA

GGCCTGTTACTAGCACTCA
```

-continued

```
(miR21 FDPS sequence #1; SEQ ID NO: 9)
CATCTCCATGGCTGTACCACCTTGTCGGGACTTTCTCAGCCTCCTTCT

GCCTGTTGAATCTCATGGCAGAAGGAGGCGAGAAAGTCTGACATTTTG

GTATCTTTCATCTGACCA

(miR185 FDPS sequence #1; SEQ ID NO: 10)
GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCTCAGCCTCCTTCT

GCTGGTCCCCTCCCCGCAGAAGGAGGCTGAGAAAGTCCTTCCCTCCCA

ATGACCGCGTCTTCGTCG
```

## Example 3—Knock-Down of FDPS for 3 Days in THP1 Monocytic Leukemia by shRNA #4

This Example illustrates that knock-down of FDPS in THP1 monocytic leukemia cells by lentiviral (LV)-expressing FDPS shRNA #4 stimulates TNF-α expression in gamma delta T cells, as shown in FIG. **5**.

THP1 cells ($1\times10^5$ cells) were transduced with LV-control or LV-FDPS shRNA #4 for 3 days. Two days after transduction, cells were treated with or without 1 μM zoledronic acid. After 24 hours, the transduced THP-1 cells were co-cultured with $5\times10^5$ PBMC cells and IL-2 in a round bottom 96 well plate for 4 hours. The PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated, and Vδ2+ and TNF-α+ cells were selected on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, LV-control stimulated 3.1% of TNF-α expressing Vγ9Vδ2 T cells and LV-FDPS shRNA #4 stimulated 5%. With zoledronic acid treatment, LV-control stimulated 7.2% of TNF-α expressing Vγ9Vδ2 T cells and LV-FDPS shRNA #4 stimulated 56.2%.

## Example 4—Knock-Down of FDPS for 14 Days in THP1 Leukemia Cells by shRNA #4

This Example illustrates that Knock-down of FDPS for 14 days in THP1 leukemia cells by lentiviral (LV)-expressing FDPS shRNA #4 stimulates TNF-α expression in GD T cells, as shown in FIG. **6**.

THP1 cells ($1\times10^5$ cells) were transduced with LV-control or LV-FDPS shRNA #4 for 14 days. Two days after transduction, cells were treated with or without 1 uM zoledronic acid. After 24 hours, the transduced THP-1 cells were co-cultured with $5\times10^5$ PBMC cells and IL-2 in a round bottom 96 well plate for 4 hours. The PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated, and Vδ2+ and TNF-α+ cells were selected on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, LV-control stimulated 0.9% of TNF-α expressing Vγ9Vδ2 T cells and LV-FDPS shRNA #4 (SEQ ID NO: 4) stimulated 15.9%. With zoledronic acid treatment, LV-control stimulated 4.7% of TNF-α expressing Vγ9Vδ2 T cells and LV-FDPS shRNA #4 (SEQ ID NO: 4) stimulated 76.2%.

Example 5—Knock-Down of FDPS for 3 Days in PC3 Prostate Carcinoma Cells by shRNA #1

This Example illustrates that knock-down of FDPS for 3 days in PC3 prostate carcinoma cells by lentiviral (LV)-expressing FDPS shRNA #1 stimulates TNF-α expression in GD T cells, as shown in FIG. **7**.

PC3 cells were transduced with LV-control or LV-FDPS shRNA #1 (SEQ ID NO: 1) for 3 days. Two days after transduction, cells were treated with or without 1 uM zoledronic acid. After 24 hours, the transduced PC3 cells were co-cultured with $5\times10^5$ PBMC cells and IL-2 in a round bottom 96 well plate for 4 hours. The PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated, and Vδ2+ and TNF-α+ cells were selected on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, LV-control stimulated 0.2% of TNF-α expressing Vγ9Vδ2 T cells and LV-FDPS shRNA #1 stimulated 0.5%. With zoledronic acid treatment, LV-control stimulated 1.7% of TNF-α expressing Vγ9Vδ2 T cells and LV-FDPS shRNA #1 (SEQ ID NO: 1) stimulated 32.2%.

Example 6—Knock-Down of FDPS for 3 Days in PC3 Prostate Carcinoma Cells by shRNA #4

This Example illustrates that Knock-down of FDPS for 3 days in PC3 prostate carcinoma cells by lentiviral (LV)-expressing FDPS shRNA #4 stimulates TNF-α expression in GD T cells, as shown in FIG. **8**.

PC3 cells were transduced with LV-control or LV-FDPS shRNA #4 (SEQ ID NO: 4) for 3 days. Two days after transduction, cells were treated with or without 1 uM zoledronic acid. After 24 hours, the transduced PC3 cells were co-cultured with $5\times10^5$ PBMC cells and IL-2 in a round bottom 96 well plate for 4 hours. The PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated, and Vδ2+ and TNF-α+ cells were selected on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, LV-control stimulated 0.5% of TNF-α expressing Vγ9Vδ2 T cells and LV-FDPS shRNA #4 (SEQ ID NO: 4) stimulated 1.9%. With zoledronic acid treatment, LV-control stimulated 2.1% of TNF-α expressing Vγ9Vδ2 T cells and LV-FDPS shRNA #4 stimulated 28.7%.

Example 7—Knock-Down of FDPS for 3 Days in HepG2 Liver Carcinoma Cells by shRNA #1 and #4

This Example illustrates that Knock-down of FDPS for 3 days in HepG2 liver carcinoma cells by lentiviral (LV)-expressing FDPS shRNA #1 (SEQ ID NO: 1) and shRNA#4 (SEQ ID NO: 4) stimulates TNF-α expression in GD T cells, as shown in FIG. **9**.

HepG2 cells were transduced with LV-control, LV-FDPS shRNA #1 (SEQ ID NO: 1), or LV-FDPS shRNA #4 (SEQ ID NO: 4) for 3 days. Two days after transduction, cells were treated with or without 1 uM zoledronic acid. After 24 hours, the transduced HepG2 cells were co-cultured with $5\times10^5$ PBMC cells and IL-2 in a round bottom 96 well plate for 4 hours. The PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated, and Vδ2+ and TNF-α+ cells were selected on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, LV-control stimulated 0.4% of TNF-α expressing Vγ9Vδ2 T cells and LV-FDPS shRNA #1 (SEQ ID NO: 1) and #4 (SEQ ID NO: 4) stimulated 0.7% and 0.9%, respectively. With zoledronic acid treatment, LV-control stimulated 6.9% of TNF-α expressing Vγ9Vδ2 T cells and LV-FDPS shRNA #1 and #4 stimulated 7.6% and 21.1%, respectively.

Example 8—Knock-Down of FDPS for 3 Days in THP1 Leukemia by microRNA-30

This Example illustrates that Knock-down of FDPS for 3 days in THP1 leukemia cells by lentiviral (LV)-expressing FDPS-targeted synthetic microRNA-30 stimulates TNF-α expression in gamma delta T cells, as shown in FIG. **10**.

THP1 cells ($1\times10^5$ cells) were transduced with LV-control or LV-miR30 FDPS #1 (SEQ ID NO: 5) for 3 days. Two days after transduction, cells were treated with or without 1 uM zoledronic acid. After 24 hours, the transduced THP-1 cells were co-cultured with $5\times10^5$ PBMC cells and IL-2 in a round bottom 96 well plate for 4 hours. The PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated, and Vδ2+ and TNF-α+ cells were selected on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, LV-control stimulated 0.2% of TNF-α expressing Vγ9Vδ2 T cells and LV-miR30 FDPS stimulated 8.1%. With zoledronic acid treatment, LV-control stimulated 5.3% of TNF-α expressing Vγ9Vδ2 T cells and LV-miR30 FDPS #1 (SEQ ID NO: 5) stimulated 67.3%.

Example 9: E:T Ratios Resulting from Mixture of THP-1 Cells, Cultured Human GD T Cells, and/or Zometa (Zol)

This Example demonstrates results from mixing treated THP-1 monocytoid tumor cells with cultured human GD T cells, as shown in FIG. **11**.

The monocytoid cell line THP-1 was treated with control lentivirus vector (LV), LV suppressing farnesyl diphosphate synthase gene expression (LV-FDPS), zoledronic acid (Zol) or combinations. The legend, as shown in FIG. **11**, was: lentiviral control vectors (LV-Control), lentiviral vectors expressing microRNA to down regulate FDPS (LV-FPPS), Zometa (Zol), Zometa plus lentiviral control (Zol+LV-Control), or Zometa plus lentiviral vectors expressing microRNA to down regulate FPPS (Zol+LV-FPPS).

Human GD T cells were cultured from an anonymous donor and added to treated THP-1 cells in 4:1, 2:1 or 1:1 ratios (GD T:THP-1) for 4 hours. Cell killing was measured by a fluorescence assay. When THP-1 cells were treated with a combination of LV-FDPS and Zol, cytotoxic T cell killing by GD T cells was increased greatly compared to either treatment alone. When LV-FDPS treatment alone was compared to Zol treatment alone, the LV-FDPS lead to greater killing but was >3-fold below tumor cell killing after combination treatment. The combined LV-FDPS plus Zol treatment caused nearly 70% tumor cell killing with 4:1 ratio; this was more than 3-fold higher than the second best treatment (LV-FDPS alone).

### Example 10—Lentiviral-Delivered shRNA-Based RNA Interference Targeting the Human Farnesyl Diphosphate Synthase (FDPS) Gene

HepG2 human hepatocellular carcinoma cells were infected with lentiviral vectors containing the H1 promoter and either a non-targeting or four different FDPS shRNA sequences, as shown in FIG. **12**. After 48 hours, RNA was extracted from the cells and converted to cDNA. Expression of FDPS cDNA was determined by quantitative PCR using SYBR Green and FDPS primers. FDPS expression was normalized to actin levels for each sample.

FDPS-targeting lentiviral vectors containing the H1 promoter and either a non-targeting sequence

```
                                      (SEQ ID NO: 60)
(5'-GCCGCTTTGTAGGATAGAGCTCGAGCTCTATCCTACAAAGCGGCTTT

TT-3')
```

or one of four different FDPS shRNA sequences

```
GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTACTCCAGGACTTT
TT
(FDPS shRNA sequence #1; SEQ ID NO: 1);

GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCTGAACGAAATCCTGCTTT
TT
(FDPS shRNA sequence #2; SEQ ID NO: 2);

GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGTACATGGCTTT
TT
(FDPS shRNA sequence #3; SEQ ID NO: 3);
and

GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCTTT
TT
(FDPS shRNA sequence #4; SEQ ID NO: 4)
```

were produced in 293 T cells.

HepG2 human hepatocellular carcinoma cells were then infected with lentiviral vectors to determine the efficacy of FDPS knock-down. After 48 hours, RNA was extracted from the cells using the RNeasy RNA isolation kit (Qiagen) and converted to cDNA with the SuperScript VILO cDNA synthesis kit (Thermo Scientific). Expression of FDPS cDNA was determined by quantitative PCR on an Applied Biosystems StepOne qPCR machine using a SYBR Green PCR mix (Thermo Scientific) and FDPS primers (Forward primer: 5'-AGGAATTGATGGCGAGAAGG-3' (SEQ ID NO: 61) and Reverse primer: 5'-CCCAAAGAGGTCAAGGTAATCA-3' (SEQ ID NO: 62)). FDPS expression was normalized to actin levels for each sample using the actin primers (Forward primer: 5'-AGCGCGGCTACAGCTTCA-3' (SEQ ID NO: 63) and Reverse primer: 5'-GGCGACGTAGCACAGCTTCT-3') (SEQ ID NO: 64). The relative FDPS RNA expression of the shCon sample is set at 100%. There was an 85% (FDPS sequence #1), 89% (FDPS sequence #2), 46% (FDPS sequence #3), and 98% (FDPS sequence #4) decrease in FDPS expression.

### Example 11—Lentiviral-Delivered miR-Based RNA Interference Targeting the Human Farnesyl Diphosphate Synthase (FDPS) Gene

As shown in FIG. **13**, HepG2 human hepatocellular carcinoma cells were infected with lentiviral vectors containing either the H1 promoter (SEQ ID NO: 16) the FDPS shRNA #4 (SEQ ID NO: 4) sequence or the EF-1α promoter (SEQ ID NO: 41) and miR30-based FDPS sequences. After 48 hours, cells were lysed and an immunoblot was performed using an anti-FDPS (Thermo Scientific) and an anti-actin (Sigma) antibody as a protein loading control.

More specifically, HepG2 human hepatocellular carcinoma cells were infected with lentiviral vectors containing either the H1 promoter (SEQ ID NO: 16) and the FDPS shRNA sequence

```
GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCTTT
TT
(FDPS shRNA sequence #4; SEQ ID NO: 4)
```

or the EF-1alpha promoter (SEQ ID NO: 41) and miR30-based FDPS sequences

```
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCG

TGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCTCG

GACTTCAAGGGGCT (miR30 FDPS sequence #1; SEQ ID NO:

5)
and

AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCG

TGAAGCCACAGATGGCAGAAGGGCTGAGAAAGTGCTGCCTACTGCCTCGGA

CTTCAAGGGGCT (miR30 FDPS sequence #2; SEQ ID NO:
6).
```

After 48 hours, cells were lysed with NP-40 lysis buffer and protein was quantified with the Bio-Rad protein assay reagent. Protein samples at 50 micrograms were electrophoresed on 4-12% Bis-Tris gels (Thermo Scientific and transferred to PVDF membranes (EMD Millipore). An immunoblot was performed using an anti-FDPS (Thermo Scientific) and an anti-actin (Sigma) antibody as a protein loading control. Antibodies were bound with HRP-conjugated secondary antibodies and detected with a Licor c-DiGit Blot scanner using the Immobilon Western ECL reagent (EMD Millipore). The densitometry of the immunoblot bands were quantified with the NIH image software. The LV control with the EF-1 promoter was set at 100%. There was a 68% (LV-shFDPS #4), 43% (LV-miR FDPS #1), and 38% (LV-miR FDPS #3) reduction of FDPS protein expression.

### Example 12—Knock-Down of FDPS for 3 Days in HepG2 Liver Carcinoma Cells by Adeno-Associated Virus (AAV)-Expressing FDPS shRNA #4

This Example illustrates that knock-down of FDPS for 3 days in HepG2 liver carcinoma cells by adeno-associated virus (AAV)-expressing FDPS shRNA #4 (SEQ ID NO: 4) stimulates TNF-α expression in GD T cells (FIG. **14**, Panel B).

HepG2 cells were transduced with control or AAV-FDPS shRNA #4 (SEQ ID NO: 8) for 3 days. Two days after transduction, cells were treated with or without 1 uM zoledronic acid. After 24 hours, the transduced HepG2 cells were co-cultured with $5\times10^5$ PBMC cells and IL-2 in a round bottom 96 well plate for 4 hours. The PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated, and Vδ2+ and TNF-α+ cells were selected on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of flow cytograms (FIG. **14**, Panel B).

AAV Vector Construction.

FDPS shRNA sequence #4 (SEQ ID NO: 4) was inserted into the pAAV plasmid (Cell Biolabs). FDPS oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by Eurofins MWG Operon. Overlapping sense and antisense oligonucleotide sequences were mixed and annealed during cooling from 70 degrees Celsius to room temperature. The pAAV was digested with the restriction enzymes BamHI and EcoRI for one hour at 37 degrees Celsius. The digested pAAV plasmid was purified by agarose gel electrophoresis and extracted from the gel using a DNA gel extraction kit from Thermo Scientific. The DNA concentrations were determined and vector to oligo (3:1 ratio) were mixed, allowed to anneal, and ligated. The ligation reaction was performed with T4 DNA ligase for 30 minutes at room temperature. 2.5 microliters of the ligation mix were added to 25 microliters of STBL3 competent bacterial cells. Transformation was achieved after heat-shock at 42 degrees Celsius. Bacterial cells were spread on agar plates containing ampicillin and drug-resistant colonies (indicating the presence of ampicillin-resistance plasmids) were recovered and expanded in LB broth. To check for insertion of the oligo sequences, plasmid DNA was extracted from harvested bacteria cultures with the Thermo Scientific DNA mini prep kit. Insertion of shRNA sequences in the pAAV plasmid was verified by DNA sequencing using a specific primer for the promoter used to regulate shRNA expression. An exemplary AAV vector with a H1 promoter (SEQ ID NO: 16), shFDPS sequence (e.g., SEQ ID NO: 4), Left Inverted Terminal Repeat (Left ITR; SEQ ID NO: 65), and Right Inverted Terminal Repeat (Right ITR; SEQ ID NO: 66) can be found in FIG. **14**, Panel A).

Production of AAV Particles.

The AAV-FDPS shRNA plasmid was combined with the plasmids pAAV-RC2 (Cell Biolabs) and pHelper (Cell Biolabs). The pAAV-RC2 plasmid contains the Rep and AAV2 capsid genes and pHelper contains the adenovirus E2A, E4, and VA genes. To produce AAV particles, these plasmids were transfected in the ratio 1:1:1 (pAAV-shFDPS: pAAV-RC2: pHelper) into 293T cells. For transfection of cells in 150 mm dishes (BD Falcon), 10 micrograms of each plasmid were added together in 1 ml of DMEM. In another tube, 60 microliters of the transfection reagent PEI (1 microgram/ml) (Polysciences) was added to 1 ml of DMEM. The two tubes were mixed together and allowed to incubate for 15 minutes. Then the transfection mixture was added to cells and the cells were collected after 3 days. The cells were lysed by freeze/thaw lysis in dry ice/isopropanol. Benzonase nuclease (Sigma) was added to the cell lysate for 30 minutes at 37 degrees Celsius. Cell debris were then pelleted by centrifugation at 4 degrees Celsius for 15 minutes at 12,000 rpm. The supernatant was collected and then added to target cells.

### Example 13—Decreased RAP1 Prenylation in the Cells Transduced with LV-shFDPS and Treated with Zoledronic Acid

This Example illustrates that lentiviral-delivered shRNA targeting the human farnesyl diphosphate synthase (FDPS) gene and zoledronic acid synergize to inhibit farnesyl diphosphate production.

FDPS is an enzyme in the isoprenoid synthesis pathway that catalyzes the production of farnesyl diphosphate. Inhibiting the enzyme activity of FDPS by zoledronic acid or reduced protein expression by shRNA-mediated knockdown will result in reduced farnesyl diphosphate levels. Farnesylation of cellular proteins requires farnesyl diphosphate. RAP1A is a protein that is modified by farnesylation, which can be used as a biomarker for levels of cellular farnesyl diphosphate. An antibody that specifically recognizes reduced RAP1A farnesylation was used to measure FDPS activity after transduction with LV-shFDPS alone or in combination with zoledronic acid. HepG2 human hepatocellular carcinoma cells were infected with lentiviral vectors containing FDPS shRNA sequence #4. For the zoledronic acid treated cells, zoledronic acid (Sigma) was added for the last 24 hours. After 48 hours, cells were lysed with NP-40 lysis buffer and protein was quantified with the Bio-Rad protein assay reagent. Protein samples at 50 micrograms were electrophoresed on 4-12% Bis-Tris gels (Thermo Scientific and transferred to PVDF membranes (EMD Millipore). An immunoblot was performed using an anti-FDPS (Thermo Scientific), anti-RAP1A (Santa Cruz), and an anti-actin (Sigma) antibody as a protein loading control. Antibodies were bound with HRP-conjugated secondary antibodies and detected with a Licor c-DiGit Blot scanner using the Immobilon Western ECL reagent (EMD Millipore). An increase in the RAP1A band intensity correlates with reduced farnesylation. RAP1A defarnesylation occurred only in the cells transduced with LV-shFDPS and treated with zoledronic acid.

### Example 14—Treatment of a Subject with Cancer

LV-FDPS is a Genetic Medicine Delivered by a Lentivirus Vector Via Local Administration to the Site of Late Stage, Non-Resectable Hepatocellular Carcinoma

A Phase I clinical trial will test safety and feasibility of delivering LV-FDPS to the site of hepatocellular carcinoma (HCC) using ultrasound guided cannulation of the liver in patients without concomitant radiotherapy or chemotherapy. It is rationally predicted that this study will result in the successful treatment of HCC. The study is an open label, 4×3 dose escalation (4 dose ranges, up to 3 subjects per dose) to identify the maximum tolerable dose of LV-FDPS in patients 18 years or older with Stage III/IV non-resectable HCC.

LV-FDPS is a genetic therapy designed to reduce expression in tumor cells of the enzyme farnesyl diphosphate synthase. Experimental studies show that tumor cells modified by LV-FDPS induce the anti-tumor activity of human gamma delta T cells, including the capacity for tumor killing by cellular cytotoxicity.

Subjects with target lesions ≥1 cm in longest diameter (measured by helical CT) and ≤4.9 cm maximum diameter and meeting inclusion and exclusion criteria detailed below, are enrolled into the next available dosing category. A maximum of 3 subjects are recruited for each dosage group. The dose is number of transducing units of LV-FDPS as described in the product release criteria, delivered via intrahepatic cannulation in a single bolus with volume not to exceed 25 mL. The minimum dose is $1\times10^9$ transducing units and escalation is 10-fold to a next dose of $1\times10^{10}$ transducing units, the next dose is $1\times10^{11}$ transducing units, and a maximum dose of $1\times10^{12}$ transducing units based on reported experience with recombinant adenovirus therapy for HCC (Sangro et al., A phase I clinic trial of thymidine kinase-based gene therapy in advanced hepatocellular carcinoma, 2010, *Cancer Gene Ther.* 17:837-43). Subjects are enrolled, treated and evaluated for 3 months. All safety evaluations are completed for each group prior to enrolling and treating subjects at the next higher dose level. Enrollment and dose escalation continue until a maximum tolerable dose is achieved or the study is terminated.

Cannulation is via the left subclavian artery until tip of catheter is at the proper hepatic artery junction. Cannulation is guided by ultrasonography as described (Lin et al., Clinical effects of intra-arterial infusion chemotherapy with cisplatin, mitomycin C, leucovor and 5-Fluorouracil for unresectable advanced hepatocellular carcinoma, 2004, *J. Chin. Med. Assoc.* 67:602-10).

Primary Outcome Measures

Safety: Systemic and locoregional adverse events are graded according to CTCAS and coded according to MedRA. The adverse events data for all subjects at a single dose range will be evaluated prior to dose escalation. The final safety assessment incorporates data from all dose ranges.

Secondary Outcome Measures

Lesion distribution and retention of LV-FDPS following locoregional administration and subsequent biopsy or necropsy to obtain tissues.

Objective response rate (ORR) in target and measurable non-local lesions (if present) by physical analysis, medical imaging or biopsy during 3 months after treatment.

Levels of LV-FDPS in blood stream during 10 minutes, 30 minutes, 1 hour and 1 day after local injection.

Changes in markers of hepatic function including ALP, ALT, ASAT, total bilirubin and GGT during 3 months after treatment.

Disease free survival beyond historical control (no LV-FDPS) patients in ad hoc analysis.

Inclusion Criteria

Greater than 18 years and including both males and females.

Diagnosis confirmed by histology or cytology or based on currently accepted clinical standards of hepatocellular carcinoma of parenchyma cell origin that is not amenable, at the time of screening, to resection, transplant or other potentially curative therapies.

Treating physician determines that the lesion is amenable to locoregional targeted delivery.

Target lesion must represent measurable disease with a unidimensional longest diameter of ≥1.0 cm by computed tomography; the maximum longest diameter is ≤5.0 cm.

Karnofsky performance score 60-80% of ECOG values.

Life expectancy ≥12 weeks.

Hematopoietic function: WBC ≥2,500/mm$^3$; ANC ≥1000/mm$^3$; Hemoglobin ≥8 g/dL; Platelet count ≥50,000/mm$^3$; Coagulation INR ≤1.3.

AST and ALT ≤5 times ULN; ALPS ≤5 time ULN. Bilirubin ≤1.5 times ULV; Creatine ≤1.5 times ULN and eGFR ≥50.

Thyroid function: Total T3 or free T3, total T4 or free T4 and THC ≤CTCAE Grade 2 abnormality.

Renal, cardiovascular and respiratory function adequate in the opinion of the attending physician.

Immunological function: Circulating Vgamma9Vdelta2+ T cells ≥30/mm$^3$; no immunodeficiency disease.

Negative for HIV by serology and viral RNA test.

Written informed consent.

Exclusion Criteria

Target lesion contiguous with, encompasses or infiltrates blood vessel.

Primary HCC amenable to resection, transplantation or other potentially curative therapies.

Hepatic surgery or chemoembolization within the past 4 months.

Hepatic radiation or whole body radiation therapy within past 4 months.

Chemotherapy with 4 weeks or any use of nitrosourea, mitomycin C or cisplatin.

Current or within past 4 weeks receipt of aminobisphosphonate therapy

Investigational agents within 4 weeks or <5 drug half-lives.

Impaired wound healing due to diabetes.

Significant psychiatric illness, alcohol dependence or illicit drug use.

Unwilling to comply with study protocols and reporting requirements.

Aminobisphosphonate treatment within past 4 months.

Presence of clinically significant cardiovascular, cerebrovascular (stroke), immunological (except hepatitis B or C virus infection, viral hepatitis or cirrhosis), endocrine or central nervous system disorders; current encephalopathy; variceal bleeding requiring hospitalization or transfusion within past 4 months.

History of HIV or acquired immune deficiency syndrome.

Current or prior treatment with antiretroviral medications.

Pregnant, lactating or refusal to adopt barrier or chemical contraceptive use throughout trial and follow-up interval.

LV-FDPS is a Genetic Medicine Delivered by a Lentivirus Vector Via Local Administration to the Site of Late Stage, Non-Resectable Hepatocellular Carcinoma—Adjunct Administration of Aminobisphosphonate

A Phase I clinical trial will test safety and feasibility of delivering LV-FDPS to the site of hepatocellular carcinoma (HCC) using ultrasound guided cannulation of the liver in patients with concomitant aminobisphosphonate chemotherapy. It is rationally predicted that this study will result in the successful treatment of HCC. The study is an open label, 4×3 dose escalation (4 dose ranges, up to 3 subjects per dose) to identify the maximum tolerable dose of LV-FDPS in patients 18 years or older with Stage III/IV non-resectable HCC.

LV-FDPS is a genetic therapy designed to reduce expression in tumor cells of the enzyme farnesyl diphosphate synthase. Experimental studies show that tumor cells modified by LV-FDPS induce the anti-tumor activity of human gamma delta T cells, including the capacity for tumor killing by cellular cytotoxicity. Prior experimental studies also showed the potential for positive interactions of LV-FDPS and specific aminobisphosphonate drugs that may be prescribed in primary or metastatic diseases. For this study, subjects will receive dose escalating amounts of LV-FDPS with continuous standard of care dosing with Aredia® (pamidronate), Zometa® (zoledronic acid) or Actonel® (risedronate) according to physician advice and subject preference.

Subjects with target lesions ≥1 cm in longest diameter (measured by helical CT) and ≤4.9 cm maximum diameter and meeting inclusion and exclusion criteria detailed below, are enrolled and started on aminobisphosphonate therapy. 30 days later size of the target lesion is re-evaluated to ensure subjects still meet starting criteria for LV-FDPS. Subjects without objective clinical response on aminobisphosphonate are enrolled into the next available LV-FDPS dosing category. A maximum of 3 subjects are recruited for each dosage group and all continue on aminobisphosphonate fir the study duration unless otherwise advised by the attending physician. The LV-FDPS dose is a number of transducing units of LV-FDPS as described in the product release criteria, delivered via intrahepatic cannulation in a single bolus with volume not to exceed 25 mL. The minimum dose is $1\times10^9$ transducing units and escalation is 10-fold to a next dose of $1\times10^{10}$ transducing units, the next dose is $1\times10^{11}$ transducing units, and a maximum dose of $1\times10^{12}$ transducing units based on reported experience with recombinant adenovirus therapy for HCC (Sangro, et al., A phase I clinic trial of thymidine kinase-based gene therapy in advanced hepatocellular carcinoma, 2010, *Cancer Gene Ther.* 17:837-43). Subjects are enrolled, treated and evaluated for 3 months. All safety evaluations are completed for each group prior to enrolling and treating subjects at the next higher dose level. Enrollment and dose escalation continue until a maximum tolerable dose is achieved or the study is terminated.

Cannulation is via the left subclavian artery until tip of catheter is at the proper hepatic artery junction. Cannulation is guided by ultrasonography as described (Lin et al., Clinical effects of intra-arterial infusion chemotherapy with cisplatin, mitomycin C, leucovor and 5-Fluorouracil for unresectable advanced hepatocellular carcinoma, 2004, *J. Chin. Med. Assoc.* 67:602-10).

Primary Outcome Measures

Safety: Systemic and locoregional adverse events are graded according to CTCAS and coded according to MedRA. The adverse events data for all subjects at a single dose range will be evaluated prior to dose escalation. The final safety assessment incorporates data from all dose ranges.

Secondary Outcome Measures

- Lesion distribution and retention of LV-FDPS following locoregional administration and subsequent biopsy or necropsy to obtain tissues.
- Objective response rate (ORR) in target and measurable non-local lesions (if present) by physical analysis, medical imaging or biopsy during 3 months after treatment.
- Levels of LV-FDPS in blood stream during 10 minutes, 30 minutes, 1 hour and 1 day after local injection.
- Changes in markers of hepatic function including ALP, ALT, ASAT, total bilirubin and GGT during 3 months after treatment.
- Disease free survival beyond historical control (no LV-FDPS) patients in ad hoc analysis.

Inclusion Criteria

- Greater than 18 years and including both males and females.
- Diagnosis confirmed by histology or cytology or based on currently accepted clinical standards of hepatocellular carcinoma of parenchyma cell origin that is not amenable, at the time of screening, to resection, transplant or other potentially curative therapies.
- Treating physician determines that the lesion is amenable to locoregional targeted delivery.
- Target lesion must represent measurable disease with a unidimensional longest diameter of ≥1.0 cm by computed tomography; the maximum longest diameter is ≤5.0 cm.
- Karnofsky performance score 60-80% of ECOG values.
- Life expectancy ≥12 weeks.
- Hematopoietic function: WBC ≥2,500/mm$^3$; ANC ≥1000/mm$^3$; Hemoglobin ≥8 g/dL; Platelet count ≥50,000/mm$^3$; Coagulation INR ≤1.3.
- AST and ALT ≤5 times ULN; ALPS ≤5 time ULN. Bilirubin ≤1.5 times ULV;
- Creatine ≤1.5 times ULN and eGFR ≥50.
- Thyroid function: Total T3 or free T3, total T4 or free T4 and THC ≤CTCAE Grade 2 abnormality.
- Renal, cardiovascular and respiratory function adequate in the opinion of the attending physician.
- Immunological function: Circulating Vgamma9Vdelta2+ T cells ≥30/mm$^3$; no immunodeficiency disease.
- Negative for HIV by serology and viral RNA test.
- Written informed consent.

Exclusion Criteria

- Intolerant to or unwilling to continue aminobisphosphonate adjunct therapy.
- Objective clinical response after aminobisphosphonate therapy.
- Target lesion contiguous with, encompasses or infiltrates blood vessel.
- Primary HCC amenable to resection, transplantation or other potentially curative therapies.
- Hepatic surgery or chemoembolization within the past 4 months.
- Hepatic radiation or whole body radiation therapy within past 4 months.
- Chemotherapy excluding aminobisphosphonate, within 4 weeks or any use of nitrosourea, mitomycin C or cisplatin.
- Investigational agents within 4 weeks or <5 drug half-lives.
- Impaired wound healing due to diabetes.
- Significant psychiatric illness, alcohol dependence or illicit drug use.
- Unwilling to comply with study protocols and reporting requirements.
- Presence of clinically significant cardiovascular, cerebrovascular (stroke), immunological (except hepatitis B or C virus infection, viral hepatitis or cirrhosis), endocrine or central nervous system disorders; current encephalopathy; variceal bleeding requiring hospitalization or transfusion within past 4 months.
- History of HIV or acquired immune deficiency syndrome.
- Current or prior treatment with antiretroviral medications.
- Pregnant, lactating or refusal to adopt barrier or chemical contraceptive use throughout trial and follow-up interval.

## Example 15—Treatment of a Subject with Chronic Viral Disease(s) of the Liver

LV-FDPS is a Genetic Medicine Delivered by a Lentivirus Vector Via Local Administration to Liver for the Treatment of Hepatitis B Virus, Hepatitis C Virus, HIV or Other Viral Infection of the Liver

A Phase I clinical trial will test safety and feasibility of delivering LV-FDPS to virally infected liver using ultrasound guided cannulation. It is rationally predicted that this study will result in the successful treatment of infections of the liver. The study is an open label, 4×3 dose escalation (4 dose ranges, up to 3 subjects per dose) to identify the maximum tolerable dose of LV-FDPS in patients 18 years or older with chronic viral disease of the liver that is resistant to chemotherapy.

LV-FDPS is a genetic therapy designed to reduce expression in tumor cells of the enzyme farnesyl diphosphate synthase. Experimental studies show that tumor cells modified by LV-FDPS induce human gamma delta T cells, including a capacity for cellular cytotoxicity against virally-infected cells.

Subjects with confirmed viral infection of the liver including hepatitis B virus, hepatitis C virus, HIV or other viruses are enrolled into the next available LV-FDPS dosing category. A maximum of 3 subjects are recruited for each dosage group. The LV-FDPS dose is a number of transducing units of LV-FDPS as described in the product release criteria, delivered via intrahepatic cannulation in a single bolus with volume not to exceed 25 mL. The minimum dose is $1\times10^9$ transducing units and escalation is 10-fold to a next dose of $1\times10^{10}$ transducing units, the next dose is $1\times10^{11}$ transducing units, and a maximum dose of $1\times10^{12}$ transducing units based on reported experience with recombinant adenovirus therapy for HCC (Sangro, et al., A phase I clinic trial of thymidine kinase-based gene therapy in advanced hepatocellular carcinoma, 2010, *Cancer Gene Ther.* 17:837-43). Subjects are enrolled, treated and evaluated for 3 months. All safety evaluations are completed for each group prior to enrolling and treating subjects at the next higher dose level. Enrollment and dose escalation continue until a maximum tolerable dose is achieved or the study is terminated.

Cannulation is via the left subclavian artery until tip of catheter is at the proper hepatic artery junction. Cannulation is guided by ultrasonography as described (Lin et al., Clinical effects of intra-arterial infusion chemotherapy with cisplatin, mitomycin C, leucovor and 5-Fluorouracil for unresectable advanced hepatocellular carcinoma, 2004, J. Chin. Med. Assoc. 67:602-10).

Primary Outcome Measures

Safety: Systemic and locoregional adverse events are graded according to CTCAS and coded according to MedRA. The adverse events data for all subjects at a single dose range will be evaluated prior to dose escalation. The final safety assessment incorporates data from all dose ranges.

Secondary Outcome Measures

- Lesion distribution and retention of LV-FDPS following locoregional administration and subsequent biopsy or necropsy to obtain tissues.
- Objective response rate (ORR) measured as a Sustained Viral Response (SVR) within the organ or systemically during 3 months after treatment.
- Levels of LV-FDPS in blood stream during 10 minutes, 30 minutes, 1 hour and 1 day after local injection.
- Changes in markers of hepatic function including ALP, ALT, ASAT, total bilirubin and GGT during 3 months after treatment.
- Disease free survival beyond historical control (no LV-FDPS) patients in ad hoc analysis.

Inclusion Criteria

- Greater than 18 years and including both males and females.
- Diagnosis confirmed by histology or cytology or based on currently accepted clinical standards of chronic viral infection of the liver that is not amenable, at the time of screening, to resection, transplant or other potentially curative therapies.
- Treating physician determines that the lesion is amenable to locoregional targeted delivery.
- Karnofsky performance score 60-80% of ECOG values.
- Life expectancy ≥12 weeks.
- Hematopoietic function: WBC ≥2,500/mm$^3$; ANC ≥1000/mm$^3$; Hemoglobin ≥8 g/dL; Platelet count ≥50,000/mm$^3$; Coagulation INR ≤1.3.
- AST and ALT ≤5 times ULN; ALPS ≤5 time ULN. Bilirubin ≤1.5 times ULV;
- Creatine ≤1.5 times ULN and eGFR ≥50.
- Thyroid function: Total T3 or free T3, total T4 or free T4 and THC ≤CTCAE Grade 2 abnormality.
- Renal, cardiovascular and respiratory function adequate in the opinion of the attending physician.
- Immunological function: Circulating Vgamma9Vdelta2+ T cells ≥30/mm$^3$; no immunodeficiency disease.
- Negative for HIV by serology and viral RNA test.
- Written informed consent.

Exclusion Criteria

- Chronic viral disease amenable to resection, transplantation or other potentially curative therapies.
- Hepatic surgery or chemoembolization within the past 4 months.
- Hepatic radiation or whole body radiation therapy within past 4 months.
- Investigational agents within 4 weeks or <5 drug half-lives.
- Current (within past 4 weeks) or ongoing receipt of aminobisphosphonate therapy.
- Impaired wound healing due to diabetes.
- Significant psychiatric illness, alcohol dependence or illicit drug use.
- Unwilling to comply with study protocols and reporting requirements.
- Presence of clinically significant cardiovascular, cerebrovascular (stroke), immunological (except virus infection, viral hepatitis or cirrhosis), endocrine or central nervous system disorders; current encephalopathy; variceal bleeding requiring hospitalization or transfusion within past 4 months.
- Pregnant, lactating or refusal to adopt barrier or chemical contraceptive use throughout trial and follow-up interval.

LV-FDPS is a Genetic Medicine Delivered by a Lentivirus Vector Via Local Administration to Liver for the Treatment of Hepatitis B Virus, Hepatitis C Virus, HIV or Other Viral Infection of the Liver—Concomitant Adjunct Aminobisphosphonate Therapy

A Phase I clinical trial will test safety and feasibility of delivering LV-FDPS to virally infected liver using ultrasound guided cannulation. It is rationally predicted that this study will result in the successful treatment of infections of the liver. The study is an open label, 4×3 dose escalation (4 dose ranges, up to 3 subjects per dose) to identify the maximum tolerable dose of LV-FDPS in patients 18 years or older with chronic viral disease of the liver that is resistant to chemotherapy.

LV-FDPS is a genetic therapy designed to reduce expression in tumor cells of the enzyme farnesyl diphosphate synthase. Experimental studies show that tumor cells modified by LV-FDPS induce human gamma delta T cells, including a capacity for cellular cytotoxicity against virally-infected cells. Prior experimental studies also showed the potential for positive interactions of LV-FDPS and specific aminobisphosphonate drugs that may be prescribed during infectious disease. For this study, subjects will receive dose escalating amounts of LV-FDPS with continuous standard of care dosing with Aredia® (pamidronate), Zometa® (zoledronic acid) or Actonel® (risedronate) according to physician advice and subject preference.

Subjects with confirmed viral infection of the liver including hepatitis B virus, hepatitis C virus, HIV or other viruses will initiate aminobisphosphonate therapy for 45 days before re-screening to meet enrollment criteria for LV-FDPS treatment of infectious disease. Eligible subjects are enrolled into the next available LV-FDPS dosing category. A maximum of 3 subjects are recruited for each dosage group. The LV-FDPS dose is a number of transducing units of LV-FDPS as described in the product release criteria, delivered via intrahepatic cannulation in a single bolus with volume not to exceed 25 mL. The minimum dose is $1\times10^9$ transducing units and escalation is 10-fold to a next dose of $1\times10^{10}$ transducing units, the next dose is $1\times10^{11}$ transducing units, and a maximum dose of $1\times10^{12}$ transducing units based on reported experience with recombinant adenovirus therapy for HCC (Sangro, et al., A phase I clinic trial of thymidine kinase-based gene therapy in advanced hepatocellular carcinoma, 2010, *Cancer Gene Ther.* 17:837-43). Subjects are enrolled, treated and evaluated for 3 months. All safety evaluations are completed for each group prior to enrolling and treating subjects at the next higher dose level. Enrollment and dose escalation continue until a maximum tolerable dose is achieved or the study is terminated.

Cannulation is via the left subclavian artery until tip of catheter is at the proper hepatic artery junction. Cannulation is guided by ultrasonography as described (Lin et al., Clinical effects of intra-arterial infusion chemotherapy with cisplatin, mitomycin C, leucovor and 5-Fluorouracil for unresectable advanced hepatocellular carcinoma, 2004, *J. Chin. Med. Assoc.* 67:602-10).

Primary Outcome Measures

Safety: Systemic and locoregional adverse events are graded according to CTCAS and coded according to MedRA. The adverse events data for all subjects at a single dose range will be evaluated prior to dose escalation. The final safety assessment incorporates data from all dose ranges.

Secondary Outcome Measures

Lesion distribution and retention of LV-FDPS following locoregional administration and subsequent biopsy or necropsy to obtain tissues.

Objective response rate (ORR) measured as a Sustained Viral Response (SVR) within the organ or systemically during 3 months after treatment.

Levels of LV-FDPS in blood stream during 10 minutes, 30 minutes, 1 hour and 1 day after local injection.

Changes in markers of hepatic function including ALP, ALT, ASAT, total bilirubin and GGT during 3 months after treatment.

Disease free survival beyond historical control (no LV-FDPS) patients in ad hoc analysis.

Inclusion Criteria

Greater than 18 years and including both males and females.

Diagnosis confirmed by histology or cytology or based on currently accepted clinical standards of chronic viral infection of the liver that is not amenable, at the time of screening, to resection, transplant or other potentially curative therapies.

Treating physician determines that the lesion is amenable to locoregional targeted delivery.

Karnofsky performance score 60-80% of ECOG values.

Life expectancy ≥12 weeks.

Hematopoietic function: WBC ≥2,500/mm$^3$; ANC ≥1000/mm$^3$; Hemoglobin ≥8 g/dL; Platelet count ≥50,000/mm$^3$; Coagulation INR ≤1.3.

AST and ALT ≤5 times ULN; ALPS ≤5 time ULN. Bilirubin ≤1.5 times ULV;

Creatine ≤1.5 times ULN and eGFR ≥50.

Thyroid function: Total T3 or free T3, total T4 or free T4 and THC ≤CTCAE Grade 2 abnormality.

Renal, cardiovascular and respiratory function adequate in the opinion of the attending physician.

Immunological function: Circulating Vgamma9Vdelta2+ T cells ≥30/mm$^3$; no immunodeficiency disease.

Negative for HIV by serology and viral RNA test.

Written informed consent.

Exclusion Criteria

Chronic viral disease amenable to resection, transplantation or other potentially curative therapies.

Hepatic surgery or chemoembolization within the past 4 months.

Hepatic radiation or whole body radiation therapy within past 4 months.

Investigational agents within 4 weeks or <5 drug half-lives.

Impaired wound healing due to diabetes.

Significant psychiatric illness, alcohol dependence or illicit drug use.

Unwilling to comply with study protocols and reporting requirements.

Presence of clinically significant cardiovascular, cerebrovascular (stroke), immunological (except virus infection, viral hepatitis or cirrhosis), endocrine or central nervous system disorders; current encephalopathy; variceal bleeding requiring hospitalization or transfusion within past 4 months.

Pregnant, lactating or refusal to adopt barrier or chemical contraceptive use throughout trial and follow-up interval.

Sequences

The following sequences are referred to herein:

| SEQ ID NO: | Description | Sequence |
|---|---|---|
| 1 | FDPS shRNA sequence #1 | GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATT GTACTCCAGGACTTTTT |
| 2 | FDPS shRNA sequence #2 | GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCTGA ACGAAATCCTGCTTTTT |
| 3 | FDPS shRNA sequence #3 | GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGC CATGTACATGGCTTTTT |
| 4 | FDPS shRNA sequence #4 | GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTC AGCCTCCTTCTGCTTTTT |
| 5 | miR30 FDPS sequence #1 | AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCT CAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAA GGAGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTT CAAGGGGCT |

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| SEQ ID NO: | Description | Sequence |
|---|---|---|
| 6 | miR30 FDPS sequence #2 | AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCT<br>CAGCCTCCTTCTGCGTGAAGCCACAGATGGCAGAA<br>GGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCA<br>AGGGGCT |
| 7 | miR30 FDPS sequence #3 | TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCT<br>GCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAA<br>AGTTGCCTACTGCCTCGGA |
| 8 | miR155 FDPS sequence #1 | CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCT<br>CAGCCTCCTTCTGCTTTTGGCCACTGACTGAGCAGA<br>AGGGCTGAGAAAGTCAGGACACAAGGCCTGTTACT<br>AGCACTCA |
| 9 | miR21 FDPS sequence #1 | CATCTCCATGGCTGTACCACCTTGTCGGGACTTTCTC<br>AGCCTCCTTCTGCCTGTTGAATCTCATGGCAGAAGG<br>AGGCGAGAAAGTCTGACATTTTGGTATCTTTCATCT<br>GACCA |
| 10 | miR185 FDPS sequence #1 | GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTC<br>TCAGCCTCCTTCTGCTGGTCCCCTCCCCGCAGAAGG<br>AGGCTGAGAAAGTCCTTCCCTCCCAATGACCGCGTC<br>TTCGTCG |
| 11 | Rous Sarcoma virus (RSV) promoter | GTAGTCTTATGCAATACTCTTGTAGTCTTGCAACAT<br>GGTAACGATGAGTTAGCAACATGCCTTACAAGGAG<br>AGAAAAAGCACCGTGCATGCCGATTGGTGGAAGTA<br>AGGTGGTACGATCGTGCCTTATTAGGAAGGCAACA<br>GACGGGTCTGACATGGATTGGACGAACCACTGAAT<br>TGCCGCATTGCAGAGATATTGTATTTAAGTGCCTAG<br>CTCGATACAATAAACG |
| 12 | 5' Long terminal repeat (LTR) | GGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGC<br>TCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTC<br>AATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTG<br>CCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCC<br>TCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCA |
| 13 | Psi Packaging signal | TACGCCAAAAATTTTGACTAGCGGAGGCTAGAAGG<br>AGAGAG |
| 14 | Rev response element (RRE) | AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGG<br>AAGCACTATGGGCGCAGCCTCAATGACGCTGACGG<br>TACAGGCCAGACAATTATTGTCTGGTATAGTGCAGC<br>AGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAA<br>CAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAG<br>CAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATA<br>CCTAAAGGATCAACAGCTCC |
| 15 | Central polypurine tract (cPPT) | TTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTG<br>CAGGGGAAAGAATAGTAGACATAATAGCAACAGAC<br>ATACAAACTAAAGAATTACAAAAACAAATTACAAA<br>ATTCAAAATTTTA |
| 16 | Polymerase III shRNA promoters; H1 promoter | GAACGCTGACGTCATCAACCCGCTCCAAGGAATCG<br>CGGGCCCAGTGTCACTAGGCGGGAACACCCAGCGC<br>GCGTGCGCCCTGGCAGGAAGATGGCTGTGAGGGAC<br>AGGGGAGTGGCGCCCTGCAATATTTGCATGTCGCTA<br>TGTGTTCTGGGAAATCACCATAAACGTGAAATGTCT<br>TTGGATTTGGGAATCTTATAAGTTCTGTATGAGACC<br>ACTT |
| 17 | Long WPRE sequence | AATCAACCTCTGATTACAAAATTTGTGAAAGATTGA<br>CTGGTATTCTTAACTATGTTGCTCCTTTTACGCTATG<br>TGGATACGCTGCTTTAATGCCTTTGTATCATGCTATT<br>GCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATA<br>AATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGC<br>CCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGT<br>TTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCA<br>CCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTCCC<br>CCTCCCTATTGCCACGGCGGAACTCATCGCCGCCTG<br>CCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGG<br>CACTGACAATTCCGTGGTGTTGTCGGGGAAATCATC<br>GTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGG<br>ATTCTGCGCGGGACGTCCTTCTGCTACGTCCCTTCG<br>GCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTG |

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| SEQ ID NO: | Description | Sequence |
|---|---|---|
| | | CTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTC GCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCT CCCCGCCT |
| 18 | 3' delta LTR | TGGAAGGGCTAATTCACTCCCAACGAAGATAAGAT CTGCTTTTTGCTTGTACTGGGTCTCTCTGGTTAGACC AGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGA ACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAG TGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACT CTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAG TGTGGAAAATCTCTAGCAGTAGTAGTTCATGTCA |
| 19 | Helper/Rev; Chicken beta actin (CAG) promoter; Transcription | GCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTG CTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCA ATTTTGTATTTATTTATTTTTTAATTATTTTGTGCAGC GATGGGGGCGGGGGGGGGGGGGGCGCGCGCCAGG CGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCG AGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGG CGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCG GCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGC GGGCG |
| 20 | Helper/Rev; HIV Gag; Viral capsid | ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGA ATTAGATCGATGGGAAAAAATTCGGTTAAGGCCAG GGGGAAAGAAAAAATATAAATTAAAACATATAGTA TGGGCAAGCAGGGAGCTAGAACGATTCGCAGTTAA TCCTGGCCTGTTAGAAACATCAGAAGGCTGTAGACA AATACTGGGACAGCTACAACCATCCCTTCAGACAG GATCAGAAGAACTTAGATCATTATATAATACAGTAG CAACCCTCTATTGTGTGCATCAAAGGATAGAGATAA AAGACACCAAGGAAGCTTTAGACAAGATAGAGGAA GAGCAAAACAAAAGTAAGAAAAAAGCACAGCAAG CAGCAGCTGACACAGGACACAGCAATCAGGTCAGC CAAAATTACCCTATAGTGCAGAACATCCAGGGGCA AATGGTACATCAGGCCATATCACCTAGAACTTTAAA TGCATGGGTAAAAGTAGTAGAAGAGAAGGCTTTCA GCCCAGAAGTGATACCCATGTTTTCAGCATTATCAG AAGGAGCCACCCCACAAGATTTAAACACCATGCTA AACACAGTGGGGGGACATCAAGCAGCCATGCAAAT GTTAAAAGAGACCATCAATGAGGAAGCTGCAGAAT GGGATAGAGTGCATCCAGTGCATGCAGGGCCTATT GCACCAGGCCAGATGAGAGAACCAAGGGGAAGTGA CATAGCAGGAACTACTAGTACCCTTCAGGAACAAA TAGGATGGATGACACATAATCCACCTATCCCAGTAG GAGAAATCTATAAAAGATGGATAATCCTGGGATTA AATAAAATAGTAAGAATGTATAGCCCTACCAGCATT CTGGACATAAGACAAGGACCAAAGGAACCCTTTAG AGACTATGTAGACCGATTCTATAAAACTCTAAGAGC CGAGCAAGCTTCACAAGAGGTAAAAAATTGGATGA CAGAAACCTTGTTGGTCCAAAATGCGAACCCAGATT GTAAGACTATTTTAAAAGCATTGGGACCAGGAGCG ACACTAGAAGAAATGATGACAGCATGTCAGGGAGT GGGGGGACCCGGCCATAAAGCAAGAGTTTTGGCTG AAGCAATGAGCCAAGTAACAAATCCAGCTACCATA ATGATACAGAAAGGCAATTTTAGGAACCAAAGAAA GACTGTTAAGTGTTTCAATTGTGGCAAAGAAGGGCA CATAGCCAAAAATTGCAGGGCCCCTAGGAAAAAGG GCTGTTGGAAATGTGGAAAGGAAGGACACCAAATG AAAGATTGTACTGAGAGACAGGCTAATTTTTTAGGG AAGATCTGGCCTTCCCACAAGGGAAGGCCAGGGAA TTTTCTTCAGAGCAGACCAGAGCCAACAGCCCCACC AGAAGAGAGCTTCAGGTTTGGGGAAGAGACAACAA CTCCCTCTCAGAAGCAGGAGCCGATAGACAAGGAA CTGTATCCTTTAGCTTCCCTCAGATCACTCTTTGGCA GCGACCCCTCGTCACAATAA |
| 21 | Helper/Rev; HIV Pol; Protease and reverse transcriptase | ATGAATTTGCCAGGAAGATGGAAACCAAAAATGAT AGGGGGAATTGGAGGTTTTATCAAAGTAGGACAGT ATGATCAGATACTCATAGAAATCTGCGGACATAAA GCTATAGGTACAGTATTAGTAGGACCTACACCTGTC AACATAATTGGAAGAAATCTGTTGACTCAGATTGGC TGCACTTTAAATTTTCCCATTAGTCCTATTGAGACTG TACCAGTAAAATTAAAGCCAGGAATGGATGGCCCA AAAGTTAAACAATGGCCATTGACAGAAGAAAAAAT AAAAGCATTAGTAGAAATTTGTACAGAAATGGAAA AGGAAGGAAAAATTTCAAAAATTGGGCCTGAAAAT |

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| SEQ ID NO: | Description | Sequence |
|---|---|---|
| | | CCATACAATACTCCAGTATTTGCCATAAAGAAAAAA<br>GACAGTACTAAATGGAGAAAATTAGTAGATTTCAG<br>AGAACTTAATAAGAGAACTCAAGATTTCTGGGAAG<br>TTCAATTAGGAATACCACATCCTGCAGGGTTAAAAC<br>AGAAAAAATCAGTAACAGTACTGGATGTGGGCGAT<br>GCATATTTTTCAGTTCCCTTAGATAAAGACTTCAGG<br>AAGTATACTGCATTTACCATACCTAGTATAAACAAT<br>GAGACACCAGGGATTAGATATCAGTACAATGTGCTT<br>CCACAGGGATGGAAAGGATCACCAGCAATATTCCA<br>GTGTAGCATGACAAAAATCTTAGAGCCTTTTAGAAA<br>ACAAAATCCAGACATAGTCATCTATCAATACATGGA<br>TGATTTGTATGTAGGATCTGACTTAGAAATAGGGCA<br>GCATAGAACAAAAATAGAGGAACTGAGACAACATC<br>TGTTGAGGTGGGGATTTACCACACCAGACAAAAAA<br>CATCAGAAAGAACCTCCATTCCTTTGGATGGGTTAT<br>GAACTCCATCCTGATAAATGGACAGTACAGCCTATA<br>GTGCTGCCAGAAAAGGACAGCTGGACTGTCAATGA<br>CATACAGAAATTAGTGGGAAAATTGAATTGGGCAA<br>GTCAGATTTATGCAGGGATTAAAGTAAGGCAATTAT<br>GTAAACTTCTTAGGGGAACCAAAGCACTAACAGAA<br>GTAGTACCACTAACAGAAGAAGCAGAGCTAGAACT<br>GGCAGAAAACAGGGAGATTCTAAAAGAACCGGTAC<br>ATGGAGTGTATTATGACCCATCAAAAGACTTAATAG<br>CAGAAATACAGAAGCAGGGGCAAGGCCAATGGACA<br>TATCAAATTTATCAAGAGCCATTTAAAAATCTGAAA<br>ACAGGAAAATATGCAAGAATGAAGGGTGCCCACAC<br>TAATGATGTGAAACAATTAACAGAGGCAGTACAAA<br>AAATAGCCACAGAAAGCATAGTAATATGGGGAAAG<br>ACTCCTAAATTTAAATTACCCATACAAAAGGAAACA<br>TGGGAAGCATGGTGGACAGAGTATTGGCAAGCCAC<br>CTGGATTCCTGAGTGGGAGTTTGTCAATACCCCTCC<br>CTTAGTGAAGTTATGGTACCAGTTAGAGAAAGAAC<br>CCATAATAGGAGCAGAAACTTTCTATGTAGATGGG<br>GCAGCCAATAGGGAAACTAAATTAGGAAAAGCAGG<br>ATATGTAACTGACAGAGGAAGACAAAAAGTTGTCC<br>CCCTAACGGACACAACAAATCAGAAGACTGAGTTA<br>CAAGCAATTCATCTAGCTTTGCAGGATTCGGGATTA<br>GAAGTAAACATAGTGACAGACTCACAATATGCATT<br>GGGAATCATTCAAGCACAACCAGATAAGAGTGAAT<br>CAGAGTTAGTCAGTCAAATAATAGAGCAGTTAATA<br>AAAAAGGAAAAAGTCTACCTGGCATGGGTACCAGC<br>ACACAAAGGAATTGGAGGAAATGAACAAGTAGATG<br>GGTTGGTCAGTGCTGGAATCAGGAAAGTACTA |
| 22 | Helper Rev; HIV Integrase; Integration of viral RNA | TTTTTAGATGGAATAGATAAGGCCCAAGAAGAACA<br>TGAGAAATATCACAGTAATTGGAGAGCAATGGCTA<br>GTGATTTTAACCTACCACCTGTAGTAGCAAAAGAAA<br>TAGTAGCCAGCTGTGATAAATGTCAGCTAAAAGGG<br>GAAGCCATGCATGGACAAGTAGACTGTAGCCCAGG<br>AATATGGCAGCTAGATTGTACACATTTAGAAGGAA<br>AAGTTATCTTGGTAGCAGTTCATGTAGCCAGTGGAT<br>ATATAGAAGCAGAAGTAATTCCAGCAGAGACAGGG<br>CAAGAAACAGCATACTTCCTCTTAAAATTAGCAGGA<br>AGATGGCCAGTAAAAACAGTACATACAGACAATGG<br>CAGCAATTTCACCAGTACTACAGTTAAGGCCGCCTG<br>TTGGTGGGCGGGGATCAAGCAGGAATTTGGCATTCC<br>CTACAATCCCCAAAGTCAAGGAGTAATAGAATCTAT<br>GAATAAAGAATTAAAGAAAATTATAGGACAGGTAA<br>GAGATCAGGCTGAACATCTTAAGACAGCAGTACAA<br>ATGGCAGTATTCATCCACAATTTTAAAAGAAAAGG<br>GGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAG<br>TAGACATAATAGCAACAGACATACAAACTAAAGAA<br>TTACAAAAACAAATTACAAAAATTCAAAATTTTCGG<br>GTTTATTACAGGGACAGCAGAGATCCAGTTTGGAA<br>AGGACCAGCAAAGCTCCTCTGGAAAGGTGAAGGGG<br>CAGTAGTAATACAAGATAATAGTGACATAAAAGTA<br>GTGCCAAGAAGAAAAGCAAAGATCATCAGGGATTA<br>TGGAAAACAGATGGCAGGTGATGATTGTGTGGCAA<br>GTAGACAGGATGAGGATTAA |
| 23 | Helper/Rev; HIV RRE; Binds Rev element | AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGG<br>AAGCACTATGGGCGCAGCGTCAATGACGCTGACGG<br>TACAGGCCAGACAATTATTGTCTGGTATAGTGCAGC<br>AGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAA |

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| SEQ ID NO: | Description | Sequence |
|---|---|---|
| | | CAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAG<br>CAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATA<br>CCTAAAGGATCAACAGCTCCT |
| 24 | Helper/Rev; HIV Rev; Nuclear export and stabilize viral mRNA | ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAAC<br>TCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATC<br>AAAGCAACCCACCTCCCAATCCCGAGGGGACCCGA<br>CAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAG<br>AGAGACAGAGACAGATCCATTCGATTAGTGAACGG<br>ATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCT<br>GTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACT<br>CTTGATTGTAACGAGGATTGTGGAACTTCTGGGACG<br>CAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATC<br>TCCTACAATATTGGAGTCAGGAGCTAAAGAATAG |
| 25 | Envelope; CMV promoter; Transcription | ACATTGATTATTGACTAGTTATTAATAGTAATCAAT<br>TACGGGGTCATTAGTTCATAGCCCATATATGGAGTT<br>CCGCGTTACATAACTTACGGTAAATGGCCCGCCTGG<br>CTGACCGCCCAACGACCCCCGCCCATTGACGTCAAT<br>AATGACGTATGTTCCCATAGTAACGCCAATAGGGAC<br>TTTCCATTGACGTCAATGGGTGGAGTATTTACGGTA<br>AACTGCCCACTTGGCAGTACATCAAGTGTATCATAT<br>GCCAAGTACGCCCCCTATTGACGTCAATGACGGTAA<br>ATGGCCCGCCTGGCATTATGCCCAGTACATGACCTT<br>ATGGGACTTTCCTACTTGGCAGTACATCTACGTATT<br>AGTCATCGCTATTACCATGGTGATGCGGTTTTGGCA<br>GTACATCAATGGGCGTGGATAGCGGTTTGACTCACG<br>GGGATTTCCAAGTCTCCACCCCATTGACGTCAATGG<br>GAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCC<br>AAAATGTCGTAACAACTCCGCCCCATTGACGCAAAT<br>GGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAA<br>GC |
| 26 | Envelope; VSV-G; Glycoprotein envelope-cell entry | ATGAAGTGCCTTTTGTACTTAGCCTTTTTATTCATTG<br>GGGTGAATTGCAAGTTCACCATAGTTTTTCCACACA<br>ACCAAAAAGGAAACTGGAAAAATGTTCCTTCTAATT<br>ACCATTATTGCCCGTCAAGCTCAGATTTAAATTGGC<br>ATAATGACTTAATAGGCACAGCCTTACAAGTCAAA<br>ATGCCCAAGAGTCACAAGGCTATTCAAGCAGACGG<br>TTGGATGTGTCATGCTTCCAAATGGGTCACTACTTG<br>TGATTTCCGCTGGTATGGACCGAAGTATATAACACA<br>TTCCATCCGATCCTTCACTCCATCTGTAGAACAATG<br>CAAGGAAAGCATTGAACAAACGAAACAAGGAACTT<br>GGCTGAATCCAGGCTTCCCTCCTCAAAGTTGTGGAT<br>ATGCAACTGTGACGGATGCCGAAGCAGTGATTGTCC<br>AGGTGACTCCTCACCATGTGCTGGTTGATGAATACA<br>CAGGAGAATGGGTTGATTCACAGTTCATCAACGGA<br>AAATGCAGCAATTACATATGCCCCACTGTCCATAAC<br>TCTACAACCTGGCATTCTGACTATAAGGTCAAAGGG<br>CTATGTGATTCTAACCTCATTTCCATGGACATCACCT<br>TCTTCTCAGAGGACGGAGAGCTATCATCCCTGGGAA<br>AGGAGGGCACAGGGTTCAGAAGTAACTACTTTGCTT<br>ATGAAACTGGAGGCAAGGCCTGCAAAATGCAATAC<br>TGCAAGCATTGGGGAGTCAGACTCCCATCAGGTGTC<br>TGGTTCGAGATGGCTGATAAGGATCTCTTTGCTGCA<br>GCCAGATTCCCTGAATGCCCAGAAGGGTCAAGTATC<br>TCTGCTCCATCTCAGACCTCAGTGGATGTAAGTCTA<br>ATTCAGGACGTTGAGAGGATCTTGGATTATTCCCTC<br>TGCCAAGAAACCTGGAGCAAAATCAGAGCGGGTCT<br>TCCAATCTCTCCAGTGGATCTCAGCTATCTTGCTCCT<br>AAAAACCCAGGAACCGGTCCTGCTTTCACCATAATC<br>AATGGTACCCTAAAATACTTTGAGACCAGATACATC<br>AGAGTCGATATTGCTGCTCCAATCCTCTCAAGAATG<br>GTCGGAATGATCAGTGGAACTACCACAGAAAGGGA<br>ACTGTGGGATGACTGGGCACCATATGAAGACGTGG<br>AAATTGGACCCAATGGAGTTCTGAGGACCAGTTCA<br>GGATATAAGTTTCCTTTATACATGATTGGACATGGT<br>ATGTTGGACTCCGATCTTCATCTTAGCTCAAAGGCT<br>CAGGTGTTCGAACATCCTCACATTCAAGACGCTGCT<br>TCGCAACTTCCTGATGATGAGAGTTTATTTTTTGGTG<br>ATACTGGGCTATCCAAAAATCCAATCGAGCTTGTAG<br>AAGGTTGGTTCAGTAGTTGGAAAAGCTCTATTGCCT<br>CTTTTTTCTTTATCATAGGGTTAATCATTGGACTATT<br>CTTGGTTCTCCGAGTTGGTATCCATCTTTGCATTAAA<br>TTAAAGCACACCAAGAAAAGACAGATTTATACAGA<br>CATAGAGATGA |

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| SEQ ID NO: | Description | Sequence |
|---|---|---|
| 27 | Helper/Rev; CMV early (CAG) enhancer; Enhance Transcription | TAGTTATTAATAGTAATCAATTACGGGGTCATTAGT<br>TCATAGCCCATATATGGAGTTCCGCGTTACATAACT<br>TACGGTAAATGGCCCGCCTGGCTGACCGCCCAACG<br>ACCCCCGCCCATTGACGTCAATAATGACGTATGTTC<br>CCATAGTAACGCCAATAGGGACTTTCCATTGACGTC<br>AATGGGTGGACTATTTACGGTAAACTGCCCACTTGG<br>CAGTACATCAAGTGTATCATATGCCAAGTACGCCCC<br>CTATTGACGTCAATGACGGTAAATGGCCCGCCTGGC<br>ATTATGCCCAGTACATGACCTTATGGGACTTTCCTA<br>CTTGGCAGTACATCTACGTATTAGTCATC |
| 28 | Helper/Rev; Chicken beta actin intron; Enhance gene expression | GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTC<br>CGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTG<br>ACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGG<br>CCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTTTAA<br>TGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGC<br>CTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGG<br>GGAGCGGCTCGGGGGGTGCGTGCGTGTGTGTGTGC<br>GTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCGGC<br>GGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTG<br>TGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGG<br>GGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGG<br>GAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGG<br>GGTGAGCAGGGGGTGTGGGCGCGGCGGTCGGGCTG<br>TAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGA<br>GCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGG<br>GGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGG<br>TGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCC<br>GCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCGCG<br>GCGGCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGG<br>CGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCG<br>AGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTGGC<br>GGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCT<br>CTAGCGGGCGCGGGCGAAGCGGTGCGGCGCCGGCA<br>GGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCG<br>CCGCGCCGCCGTCCCCTTCTCCATCTCCAGCCTCGG<br>GGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGA<br>CGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGAC<br>CGGCGG |
| 29 | Helper/Rev; Rabbit beta globin poly A; RNA stability | AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACAT<br>CATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATA<br>AAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAA<br>TTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAG<br>GGCAAATCATTTAAAACATCAGAATGAGTATTTGGT<br>TTAGAGTTTGGCAACATATGCCATATGCTGGCTGCC<br>ATGAACAAAGGTGGCTATAAAGAGGTCATCAGTAT<br>ATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCAT<br>AGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATA<br>TTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAA<br>ATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTC<br>CTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCTTC<br>TCTTATGAAGATC |
| 30 | Envelope; Beta globin intron; Enhance gene expression | GTGAGTTTGGGGACCCTTGATTGTTCTTTCTTTTTCG<br>CTATTGTAAAATTCATGTTATATGGAGGGGGCAAAG<br>TTTTCAGGGTGTTGTTTAGAATGGGAAGATGTCCCT<br>TGTATCACCATGGACCCTCATGATAATTTTGTTTCTT<br>TCACTTTCTACTCTGTTGACAACCATTGTCTCCTCTT<br>ATTTTCTTTTCATTTTCTGTAACTTTTTCGTTAAACTT<br>TAGCTTGCATTTGTAACGAATTTTTAAATTCACTTTT<br>GTTTATTTGTCAGATTGTAAGTACTTTCTCTAATCAC<br>TTTTTTTTCAAGGCAATCAGGGTATATTATATTGTAC<br>TTCAGCACAGTTTTAGAGAACAATTGTTATAATTAA<br>ATGATAAGGTAGAATATTTCTGCATATAAATTCTGG<br>CTGGCGTGGAAATATTCTTATTGGTAGAAACAACTA<br>CACCCTGGTCATCATCCTGCCTTTCTCTTTATGGTTA<br>CAATGATATACACTGTTTGAGATGAGGATAAAATAC<br>TCTGAGTCCAAACCGGGCCCCTCTGCTAACCATGTT<br>CATGCCTTCTTCTCTTTCCTACAG |
| 31 | Envelope; Rabbit beta globin poly A; RNA stability | AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACAT<br>CATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATA<br>AAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAA<br>TTTTTTGTGTCTCTCACTCGGAAGGACATATGGGAG<br>GGCAAATCATTTAAAACATCAGAATGAGTATTTGGT |

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| SEQ ID NO: | Description | Sequence |
|---|---|---|
| | | TTAGAGTTTGGCAACATATGCCCATATGCTGGCTGC<br>CATGAACAAAGGTTGGCTATAAAGAGGTCATCAGT<br>ATATGAAACAGCCCCCTGCTGTCCATTCCTTATTCC<br>ATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTA<br>TATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTA<br>AAATTTTCCTTACATGTTTTACTAGCCAGATTTTTCC<br>TCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCT<br>TCTCTTATGGAGATC |
| 32 | Primer | TAAGCAGAATTCATGAATTTGCCAGGAAGAT |
| 33 | Primer | CCATACAATGAATGGACACTAGGCGGCCGCACGAA<br>T |
| 34 | Gag, Pol, Integrase fragment | GAATTCATGAATTTGCCAGGAAGATGGAAACCAAA<br>AATGATAGGGGGAATTGGAGGTTTTATCAAAGTAA<br>GACAGTATGATCAGATACTCATAGAAATCTGCGGA<br>CATAAAGCTATAGGTACAGTATTAGTAGGACCTACA<br>CCTGTCAACATAATTGGAAGAAATCTGTTGACTCAG<br>ATTGGCTGCACTTTAAATTTTCCCATTAGTCCTATTG<br>AGACTGTACCAGTAAAATTAAAGCCAGGAATGGAT<br>GGCCCAAAAGTTAAACAATGGCCATTGACAGAAGA<br>AAAAATAAAAGCATTAGTAGAAATTTGTACAGAAA<br>TGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCT<br>GAAAATCCATACAATACTCCAGTATTTGCCATAAAG<br>AAAAAAGACAGTACTAAATGGAGAAAATTAGTAGA<br>TTTCAGAGAACTTAATAAGAGAACTCAAGATTTCTG<br>GGAAGTTCAATTAGGAATACCACATCCTGCAGGGTT<br>AAAACAGAAAAAATCAGTAACAGTACTGGATGTGG<br>GCGATGCATATTTTTCAGTTCCCTTAGATAAAGACT<br>TCAGGAAGTATACTGCATTTACCATACCTAGTATAA<br>ACAATGAGACACCAGGGATTAGATATCAGTACAAT<br>GTGCTTCCACAGGGATGGAAAGGATCACCAGCAAT<br>ATTCCAGTGTAGCATGACAAAAATCTTAGAGCCTTT<br>TAGAAAACAAAATCCAGACATAGTCATCTATCAAT<br>ACATGGATGATTTGTATGTAGGATCTGACTTAGAAA<br>TAGGGCAGCATAGAACAAAAATAGAGGAACTGAGA<br>CAACATCTGTTGAGGTGGGGATTTACCACACCAGAC<br>AAAAAACATCAGAAAGAACCTCCATTCCTTTGGATG<br>GGTTATGAACTCCATCCTGATAAATGGACAGTACAG<br>CCTATAGTGCTGCCAGAAAAGGACAGCTGGACTGT<br>CAATGACATACAGAAATTAGTGGGAAAATTGAATT<br>GGGCAAGTCAGATTTATGCAGGGATTAAAGTAAGG<br>CAATTATGTAAACTTCTTAGGGGAACCAAAGCACTA<br>ACAGAAGTAGTACCACTAACAGAAGAAGCAGAGCT<br>AGAACTGGCAGAAAACAGGGAGATTCTAAAAGAAC<br>CGGTACATGGAGTGTATTATGACCCATCAAAAGACT<br>TAATAGCAGAAATACAGAAGCAGGGGCAAGGCCAA<br>TGGACATATCAAATTTATCAAGAGCCATTTAAAAAT<br>CTGAAAACAGGAAAGTATGCAAGAATGAAGGGTGC<br>CCACACTAATGATGTGAAACAATTAACAGAGGCAG<br>TACAAAAAATAGCCACAGAAAGCATAGTAATATGG<br>GGAAAGACTCCTAAATTTAAATTACCCATACAAAA<br>GGAAACATGGGAAGCATGGTGGACAGAGTATTGGC<br>AAGCCACCTGGATTCCTGAGTGGGAGTTTGTCAATA<br>CCCCTCCCTTAGTGAAGTTATGGTACCAGTTAGAGA<br>AAGAACCCATAATAGGAGCAGAAACTTTCTATGTA<br>GATGGGGCAGCCAATAGGGAAACTAAATTAGGAAA<br>AGCAGGATATGTAACTGACAGAGGAAGACAAAAAG<br>TTGTCCCCCTAACGGACACAACAAATCAGAAGACT<br>GAGTTACAAGCAATTCATCTAGCTTTGCAGGATTCG<br>GGATTAGAAGTAAACATAGTGACAGACTCACAATA<br>TGCATTGGGAATCATTCAAGCACAACCAGATAAGA<br>GTGAATCAGAGTTAGTCAGTCAAATAATAGAGCAG<br>TTAATAAAAAAGGAAAAAGTCTACCTGGCATGGGT<br>ACCAGCACACAAAGGAATTGGAGGAAATGAACAAG<br>TAGATAAATTGGTCAGTGCTGGAATCAGGAAAGTA<br>CTATTTTTAGATGGAATAGATAAGGCCCAAGAAGA<br>ACATGAGAAATATCACAGTAATTGGAGAGCAATGG<br>CTAGTGATTTTAACCTACCACCTGTAGTAGCAAAAG<br>AAATAGTAGCCAGCTGTGATAAATGTCAGCTAAAA<br>GGGGAAGCCATGCATGGACAAGTAGACTGTAGCCC<br>AGGAATATGGCAGCTAGATTGTACACATTTAGAAG<br>GAAAAGTTATCTTGGTAGCAGTTCATGTAGCCAGTG<br>GATATATAGAAGCAGAAGTAATTCCAGCAGAGACA<br>GGGCAAGAAACAGCATACTTCCTCTTAAAATTAGCA |

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| SEQ ID NO: | Description | Sequence |
|---|---|---|
| | | GGAAGATGGCCAGTAAAAACAGTACATACAGACAA<br>TGGCAGCAATTTCACCAGTACTACAGTTAAGGCCGC<br>CTGTTGGTGGGCGGGGATCAAGCAGGAATTTGGCA<br>TTCCCTACAATCCCCAAAGTCAAGGAGTAATAGAAT<br>CTATGAATAAAGAATTAAAGAAAATTATAGGACAG<br>GTAAGAGATCAGGCTGAACATCTTAAGACAGCAGT<br>ACAAATGGCAGTATTCATCCACAATTTTAAAAGAAA<br>AGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGA<br>ATAGTAGACATAATAGCAACAGACATACAAACTAA<br>AGAATTACAAAAACAAATTACAAAAATTCAAAATT<br>TTCGGGTTTATTACAGGGACAGCAGAGATCCAGTTT<br>GGAAAGGACCAGCAAAGCTCCTCTGGAAAGGTGAA<br>GGGGCAGTAGTAATACAAGATAATAGTGACATAAA<br>AGTAGTGCCAAGAAGAAAAGCAAAGATCATCAGGG<br>ATTATGGAAAACAGATGGCAGGTGATGATTGTGTG<br>GCAAGTAGACAGGATGAGGATTAA |
| 35 | DNA Fragment containing Rev, RRE and rabbit beta globin poly A | TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGA<br>AGAGCTCATCAGAACAGTCAGACTCATCAAGCTTCT<br>CTATCAAAGCAACCCACCTCCCAATCCCGAGGGGA<br>CCCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGG<br>AGAGAGAGACAGAGACAGATCCATTCGATTAGTGA<br>ACGGATCCTTGGCACTTATCTGGGACGATCTGCGGA<br>GCCTGTGCCTCTTCAGCTACCACCGCTTGAGAGACT<br>TACTCTTGATTGTAACGAGGATTGTGGAACTTCTGG<br>GACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGG<br>AATCTCCTACAATATTGGAGTCAGGAGCTAAAGAAT<br>AGAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCA<br>GGAAGCACTATGGGCGCAGCGTCAATGACGCTGAC<br>GGTACAGGCCAGACAATTATTGTCTGGTATAGTGCA<br>GCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGC<br>AACAGCATCTGTTGCAACTCACAGTCTGGGGCATCA<br>AGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGA<br>TACCTAAAGGATCAACAGCTCCTAGATCTTTTTCCC<br>TCTGCCAAAAATTATGGGGACATCATGAAGCCCCTT<br>GAGCATCTGACTTCTGGCTAATAAAGGAAATTTATT<br>TTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCT<br>CACTCGGAAGGACATATGGGAGGGCAAATCATTTA<br>AAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCA<br>ACATATGCCATATGCTGGCTGCCATGAACAAAGGTG<br>GCTATAAAGAGGTCATCAGTATATGAAACAGCCCC<br>CTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGA<br>CTTGAGGTTAGATTTTTTTTATATTTTGTTTTGTGTT<br>ATTTTTTTCTTTAACATCCCTAAAATTTTCCTTACAT<br>GTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTAC<br>TCCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATC<br>CCTCGACCTGCAGCCCAAGCTTGGCGTAATCATGGT<br>CATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCAC<br>AATTCCACACAACATACGAGCCGGAAGCATAAAGT<br>GTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTC<br>ACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAG<br>TCGGGAAACCTGTCGTGCCAGCGGATCCGCATCTCA<br>ATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGC<br>CCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATT<br>CTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGC<br>AGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCA<br>GAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTT<br>TTGCAAAAAGCTAACTTGTTTATTGCAGCTTATAAT<br>GGTTACAAATAAAGCAATAGCATCACAAATTTCAC<br>AAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGT<br>TTGTCCAAACTCATCAATGTATCTTATCAGCGGCCG<br>CCCCGGG |
| 36 | DNA fragment containing the CAG enhancer/ promoter/intron sequence | ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTC<br>ATTAGTTCATAGCCCATATATGGAGTTCCGCGTTAC<br>ATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCC<br>CAACGACCCCCGCCCATTGACGTCAATAATGACGTA<br>TGTTCCCATAGTAACGCCAATAGGGACTTTCCATTG<br>ACGTCAATGGGTGGACTATTTACGGTAAACTGCCCA<br>CTTGGCAGTACATCAAGTGTATCATATGCCAAGTAC<br>GCCCCCTATTGACGTCAATGACGGTAAATGGCCCGC<br>CTGGCATTATGCCCAGTACATGACCTTATGGGACTT<br>TCCTACTTGGCAGTACATCTACGTATTAGTCATCGC<br>TATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCT<br>TCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAAT<br>TTTGTATTTATTTATTTTTTAATTATTTTGTGCAGCG |

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| SEQ ID NO: | Description | Sequence |
|---|---|---|
| | | ATGGGGGCGGGGGGGGGGGGGGCGCGCGCCAGGC<br>GGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGA<br>GGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGC<br>GCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGG<br>CGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCG<br>GGCGGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCC<br>GCTCCGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTG<br>ACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGG<br>ACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGG<br>TTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGA<br>AAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCG<br>GGGGGGAGCGGCTCGGGGGGTGCGTGCGTGTGTGT<br>GTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCC<br>CGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGC<br>TTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGC<br>CGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGA<br>GGGGAACAAAGGCTGCGTGCGGGGTGTGTGCGTGG<br>GGGGGTGAGCAGGGGGTGTGGGCGCGGCGGTCGGG<br>CTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGC<br>TGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGC<br>GGGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGG<br>GGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGG<br>CCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCG<br>CGGCGGCCCCGGAGCGCCGGCGGCTGTCGAGGCGC<br>GGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTG<br>CGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTG<br>GCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCC<br>CTCTAGCGGGCGCGGGCGAAGCGGTGCGGCGCCGG<br>CAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGT<br>CGCCGCGCCGCCGTCCCCTTCTCCATCTCCAGCCTC<br>GGGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGG<br>GACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTG<br>ACCGGCGGGAATTC |
| 37 | DNA fragment containing VSV-G | GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTTAT<br>TCATTGGGGTGAATTGCAAGTTCACCATAGTTTTTC<br>CACACAACCAAAAAGGAAACTGGAAAAATGTTCCT<br>TCTAATTACCATTATTGCCCGTCAAGCTCAGATTTA<br>AATTGGCATAATGACTTAATAGGCACAGCCTTACAA<br>GTCAAAATGCCCAAGAGTCACAAGGCTATTCAAGC<br>AGACGGTTGGATGTGTCATGCTTCCAAATGGGTCAC<br>TACTTGTGATTTCCGCTGGTATGGACCGAAGTATAT<br>AACACATTCCATCCGATCCTTCACTCCATCTGTAGA<br>ACAATGCAAGGAAAGCATTGAACAAACGAAACAAG<br>GAACTTGGCTGAATCCAGGCTTCCCTCCTCAAAGTT<br>GTGGATATGCAACTGTGACGGATGCCGAAGCAGTG<br>ATTGTCCAGGTGACTCCTCACCATGTGCTGGTTGAT<br>GAATACACAGGAGAATGGGTTGATTCACAGTTCATC<br>AACGGAAAATGCAGCAATTACATATGCCCCACTGTC<br>CATAACTCTACAACCTGGCATTCTGACTATAAGGTC<br>AAAGGGCTATGTGATTCTAACCTCATTTCCATGGAC<br>ATCACCTTCTTCTCAGAGGACGGAGAGCTATCATCC<br>CTGGGAAAGGAGGGCACAGGGTTCAGAAGTAACTA<br>CTTTGCTTATGAAACTGGAGGCAAGGCCTGCAAAAT<br>GCAATACTGCAAGCATTGGGGAGTCAGACTCCCATC<br>AGGTGTCTGGTTCGAGATGGCTGATAAGGATCTCTT<br>TGCTGCAGCCAGATTCCCTGAATGCCCAGAAGGGTC<br>AAGTATCTCTGCTCCATCTCAGACCTCAGTGGATGT<br>AAGTCTAATTCAGGACGTTGAGAGGATCTTGGATTA<br>TTCCCTCTGCCAAGAAACCTGGAGCAAAATCAGAG<br>CGGGTCTTCCAATCTCTCCAGTGGATCTCAGCTATC<br>TTGCTCCTAAAAACCCAGGAACCGGTCCTGCTTTCA<br>CCATAATCAATGGTACCCTAAAATACTTTGAGACCA<br>GATACATCAGAGTCGATATTGCTGCTCCAATCCTCT<br>CAAGAATGGTCGGAATGATCAGTGGAACTACCACA<br>GAAAGGGAACTGTGGGATGACTGGGCACCATATGA<br>AGACGTGGAAATTGGACCCAATGGAGTTCTGAGGA<br>CCAGTTCAGGATATAAGTTTCCTTTATACATGATTG<br>GACATGGTATGTTGGACTCCGATCTTCATCTTAGCT<br>CAAAGGCTCAGGTGTTCGAACATCCTCACATTCAAG<br>ACGCTGCTTCGCAACTTCCTGATGATGAGAGTTTAT<br>TTTTTGGTGATACTGGGCTATCCAAAAATCCAATCG<br>AGCTTGTAGAAGGTTGGTTCAGTAGTTGGAAAAGCT<br>CTATTGCCTCTTTTTTCTTTATCATAGGGTTAATCAT |

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| SEQ ID NO: | Description | Sequence |
|---|---|---|
| | | TGGACTATTCTTGGTTCTCCGAGTTGGTATCCATCTT TGCATTAAATTAAAGCACACCAAGAAAAGACAGAT TTATACAGACATAGAGATGAGAATTC |
| 38 | Rev; RSV promoter; Transcription | ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAAC TCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATC AAAGCAACCCACCTCCCAATCCCGAGGGGACCCGA CAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAG AGAGACAGAGACAGATCCATTCGATTAGTGAACGG ATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCT GTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACT CTTGATTGTAACGAGGATTGTGGAACTTCTGGGACG CAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATC TCCTACAATATTGGAGTCAGGAGCTAAAGAATAG |
| 39 | Rev; HIV Rev; Nuclear export and stabilize viral mRNA | ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAAC TCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATC AAAGCAACCCACCTCCCAATCCCGAGGGGACCCGA CAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAG AGAGACAGAGACAGATCCATTCGATTAGTGAACGG ATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCT GTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACT CTTGATTGTAACGAGGATTGTGGAACTTCTGGGACG CAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATC TCCTACAATATTGGAGTCAGGAGCTAAAGAATAG |
| 40 | RSV promoter and HIV Rev | CAATTGCGATGTACGGGCCAGATATACGCGTATCTG AGGGGACTAGGGTGTGTTTAGGCGAAAAGCGGGGC TTCGGTTGTACGCGGTTAGGAGTCCCCTCAGGATAT AGTAGTTTCGCTTTTGCATAGGGAGGGGGAAATGTA GTCTTATGCAATACACTTGTAGTCTTGCAACATGGT AACGATGAGTTAGCAACATGCCTTACAAGGAGAGA AAAAGCACCGTGCATGCCGATTGGTGGAAGTAAGG TGGTACGATCGTGCCTTATTAGGAAGGCAACAGAC AGGTCTGACATGGATTGGACGAACCACTGAATTCCG CATTGCAGAGATAATTGTATTTAAGTGCCTAGCTCG ATACAATAAACGCCATTTGACCATTCACCACATTGG TGTGCACCTCCAAGCTCGAGCTCGTTTAGTGAACCG TCAGATCGCCTGGAGACGCCATCCACGCTGTTTTGA CCTCCATAGAAGACACCGGGACCGATCCAGCCTCCC CTCGAAGCTAGCGATTAGGCATCTCCTATGGCAGGA AGAAGCGGAGACAGCGACGAAGAACTCCTCAAGGC AGTCAGACTCATCAAGTTTCTCTATCAAAGCAACCC ACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAA GGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAG ACAGATCCATTCGATTAGTGAACGGATCCTTAGCAC TTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCA GCTACCACCGCTTGAGAGACTTACTCTTGATTGTAA CGAGGATTGTGGAACTTCTGGGACGCAGGGGGTGG GAAGCCCTCAAATATTGGTGGAATCTCCTACAATAT TGGAGTCAGGAGCTAAAGAATAGTCTAGA |
| 41 | Elongation Factor-1 alpha (EF1-alpha) promoter | CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGG GAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCC GAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGT CGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGC CAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCG GGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGC CTTGAATTACTTCCACGCCCCTGGCTGCAGTACGTG ATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGG GAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCG CCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGG GGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCC TGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAA ATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCA AGATAGTCTTGTAAATGCGGGCCAAGATCTGCACAC TGGTATTTCGGTTTTTGGGGCCGCGGGCGGCGACGG GGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGC GGGGCCTGCGAGCGCGGCCACCGAGAATCGGACGG GGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCCT GGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCG GCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGC GGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGA GCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGG GCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTT TCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGA |

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| SEQ ID NO: | Description | Sequence |
|---|---|---|
| | | GTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTC GAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGA GGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTG GGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTGAT GTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGA TCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAA AGTTTTTTTCTTCCATTTCAGGTGTCGTGA |
| 42 | Promoter; PGK | GGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGG GTTTGCGCAGGGACGCGGCTGCTCTGGGCGTGGTTC CGGGAAACGCAGCGGCGCCGACCCTGGGTCTCGCA CATTCTTCACGTCCGTTCGCAGCGTCACCCGGATCT TCGCCGCTACCCTTGTGGGCCCCCCGGCGACGCTTC CTGCTCCGCCCCTAAGTCGGGAAGGTTCCTTGCGGT TCGCGGCGTGCCGGACGTGACAAACGGAAGCCGCA CGTCTCACTAGTACCCTCGCAGACGGACAGCGCCAG GGAGCAATGGCAGCGCGCCGACCGCGATGGGCTGT GGCCAATAGCGGCTGCTCAGCAGGGCGCGCCGAGA GCAGCGGCCGGGAAGGGGCGGTGCGGGAGGCGGG GTGTGGGGCGGTAGTGTGGGCCCTGTTCCTGCCCGC GCGGTGTTCCGCATTCTGCAAGCCTCCGGAGCGCAC GTCGGCAGTCGGCTCCCTCGTTGACCGAATCACCGA CCTCTCTCCCCAG |
| 43 | Promoter; UbC | GCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCCT CCTCACGGCGAGCGCTGCCACGTCAGACGAAGGGC GCAGGAGCGTTCCTGATCCTTCCGCCCGGACGCTCA GGACAGCGGCCCGCTGCTCATAAGACTCGGCCTTAG AACCCCAGTATCAGCAGAAGGACATTTTAGGACGG GACTTGGGTGACTCTAGGGCACTGGTTTTCTTTCCA GAGAGCGGAACAGGCGAGGAAAAGTAGTCCCTTCT CGGCGATTCTGCGGAGGGATCTCCGTGGGGCGGTG AACGCCGATGATTATATAAGGACGCGCCGGGTGTG GCACAGCTAGTTCCGTCGCAGCCGGGATTTGGGTCG CGGTTCTTGTTTGTGGATCGCTGTGATCGTCACTTGG TGAGTTGCGGGCTGCTGGGCTGGCCGGGGCTTTCGT GGCCGCCGGGCCGCTCGGTGGGACGGAAGCGTGTG GAGAGACCGCCAAGGGCTGTAGTCTGGGTCCGCGA GCAAGGTTGCCCTGAACTGGGGGTTGGGGGGAGCG CACAAAATGGCGGCTGTTCCCGAGTCTTGAATGGAA GACGCTTGTAAGGCGGGCTGTGAGGTCGTTGAAAC AAGGTGGGGGGCATGGTGGGCGGCAAGAACCCAAG GTCTTGAGGCCTTCGCTAATGCGGGAAAGCTCTTAT TCGGGTGAGATGGGCTGGGGCACCATCTGGGGACC CTGACGTGAAGTTTGTCACTGACTGGAGAACTCGGG TTTGTCGTCTGGTTGCGGGGGCGGCAGTTATGCGGT GCCGTTGGGCAGTGCACCCGTACCTTTGGGAGCGCG CGCCTCGTCGTGTCGTGACGTCACCCGTTCTGTTGG CTTATAATGCAGGGTGGGGCCACCTGCCGGTAGGTG TGCGGTAGGCTTTTCTCCGTCGCAGGACGCAGGGTT CGGGCCTAGGGTAGGCTCTCCTGAATCGACAGGCG CCGGACCTCTGGTGAGGGGAGGGATAAGTGAGGCG TCAGTTTCTTTGGTCGGTTTTATGTACCTATCTTCTT AAGTAGCTGAAGCTCCGGTTTTGAACTATGCGCTCG GGGTTGGCGAGTGTGTTTTGTGAAGTTTTTTAGGCA CCTTTTGAAATGTAATCATTTGGGTCAATATGTAAT TTTCAGTGTTAGACTAGTAAA |
| 44 | Poly A; SV40 | GTTTATTGCAGCTTATAATGGTTACAAATAAAGCAA TAGCATCACAAATTTCACAAATAAAGCATTTTTTTC ACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAA TGTATCTTATCA |
| 45 | Poly A; bGH | GACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGC CCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCC ACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATT GCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTG GGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGG ATTGGGAAGACAATAGCAGGCATGCTGGGGATGCG GTGGGCTCTATGG |
| 46 | Envelope; RD114 | ATGAAACTCCCAACAGGAATGGTCATTTTATGTAGC CTAATAATAGTTCGGGCAGGGTTTGACGACCCCCGC AAGGCTATCGCATTAGTACAAAAACAACATGGTAA ACCATGCGAATGCAGCGGAGGGCAGGTATCCGAGG CCCCACCGAACTCCATCCAACAGGTAACTTGCCCAG |

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| SEQ ID NO: | Description | Sequence |
|---|---|---|
| | | GCAAGACGGCCTACTTAATGACCAACCAAAAATGG<br>AAATGCAGAGTCACTCCAAAAAATCTCACCCCTAGC<br>GGGGGAGAACTCCAGAACTGCCCCTGTAACACTTTC<br>CAGGACTCGATGCACAGTTCTTGTTATACTGAATAC<br>CGGCAATGCAGGGCGAATAATAAGACATACTACAC<br>GGCCACCTTGCTTAAAATACGGTCTGGGAGCCTCAA<br>CGAGGTACAGATATTACAAAACCCCAATCAGCTCCT<br>ACAGTCCCCTTGTAGGGGCTCTATAAATCAGCCCGT<br>TTGCTGGAGTGCCACAGCCCCCATCCATATCTCCGA<br>TGGTGGAGGACCCCTCGATACTAAGAGAGTGTGGA<br>CAGTCCAAAAAAGGCTAGAACAAATTCATAAGGCT<br>ATGCATCCTGAACTTCAATACCACCCCTTAGCCCTG<br>CCCAAAGTCAGAGATGACCTTAGCCTTGATGCACGG<br>ACTTTTGATATCCTGAATACCACTTTTAGGTTACTCC<br>AGATGTCCAATTTTAGCCTTGCCCAAGATTGTTGGC<br>TCTGTTTAAAACTAGGTACCCCTACCCCTCTTGCGA<br>TACCCACTCCCTCTTTAACCTACTCCCTAGCAGACTC<br>CCTAGCGAATGCCTCCTGTCAGATTATACCTCCCCT<br>CTTGGTTCAACCGATGCAGTTCTCCAACTCGTCCTG<br>TTTATCTTCCCCTTTCATTAACGATACGGAACAAAT<br>AGACTTAGGTGCAGTCACCTTTACTAACTGCACCTC<br>TGTAGCCAATGTCAGTAGTCCTTTATGTGCCCTAAA<br>CGGGTCAGTCTTCCTCTGTGGAAATAACATGGCATA<br>CACCTATTTACCCCAAAACTGGACAGGACTTTGCGT<br>CCAAGCCTCCCTCCTCCCCGACATTGACATCATCCC<br>GGGGGATGAGCCAGTCCCCATTCCTGCCATTGATCA<br>TTATATACATAGACCTAAACGAGCTGTACAGTTCAT<br>CCCTTTACTAGCTGGACTGGGAATCACCGCAGCATT<br>CACCACCGGAGCTACAGGCCTAGGTGTCTCCGTCAC<br>CCAGTATACAAAATTATCCCATCAGTTAATATCTGA<br>TGTCCAAGTCTTATCCGGTACCATACAAGATTTACA<br>AGACCAGGTAGACTCGTTAGCTGAAGTAGTTCTCCA<br>AAATAGGAGGGGACTGGACCTACTAACGGCAGAAC<br>AAGGAGGAATTTGTTTAGCCTTACAAGAAAAATGCT<br>GTTTTTATGCTAACAAGTCAGGAATTGTGAGAAACA<br>AAATAAGAACCCTACAAGAAGAATTACAAAAACGC<br>AGGGAAAGCCTGGCATCCAACCCTCTCTGGACCGG<br>GCTGCAGGGCTTTCTTCCGTACCTCCTACCTCTCCTG<br>GGACCCCTACTCACCCTCCTACTCATACTAACCATT<br>GGGCCATGCGTTTTCAATCGATTGGTCCAATTTGTT<br>AAAGACAGGATCTCAGTGGTCCAGGCTCTGGTTTTG<br>ACTCAGCAATATCACCAGCTAAAACCCATAGAGTA<br>CGAGCCATGA |
| 47 | Envelope;<br>GALV | ATGCTTCTCACCTCAAGCCCGCACCACCTTCGGCAC<br>CAGATGAGTCCTGGGAGCTGGAAAAGACTGATCAT<br>CCTCTTAAGCTGCGTATTCGGAGACGGCAAAACGA<br>GTCTGCAGAATAAGAACCCCCACCAGCCTGTGACCC<br>TCACCTGGCAGGTACTGTCCCAAACTGGGGACGTTG<br>TCTGGGACAAAAAGGCAGTCCAGCCCCTTTGGACTT<br>GGTGGCCCTCTCTTACACCTGATGTATGTGCCCTGG<br>CGGCCGGTCTTGAGTCCTGGGATATCCCGGGATCCG<br>ATGTATCGTCCTCTAAAAGAGTTAGACCTCCTGATT<br>CAGACTATACTGCCGCTTATAAGCAAATCACCTGGG<br>GAGCCATAGGGTGCAGCTACCCTCGGGCTAGGACC<br>AGGATGGCAAATTCCCCCTTCTACGTGTGTCCCCGA<br>GCTGGCCGAACCCATTCAGAAGCTAGGAGGTGTGG<br>GGGGCTAGAATCCCTATACTGTAAAGAATGGAGTT<br>GTGAGACCACGGGTACCGTTTATTGGCAACCCAAGT<br>CCTCATGGGACCTCATAACTGTAAAATGGGACCAA<br>AATGTGAAATGGGAGCAAAAATTTCAAAAGTGTGA<br>ACAAACCGGCTGGTGTAACCCCCTCAAGATAGACTT<br>CACAGAAAAAGGAAAACTCTCCAGAGATTGGATAA<br>CGGAAAAAACCTGGGAATTAAGGTTCTATGTATATG<br>GACACCCAGGCATACAGTTGACTATCCGCTTAGAGG<br>TCACTAACATGCCGGTTGTGGCAGTGGGCCCAGACC<br>CTGTCCTTGCGGAACAGGGACCTCCTAGCAAGCCCC<br>TCACTCTCCCTCTCTCCCCACGGAAAGCGCCGCCCA<br>CCCCTCTACCCCCGGCGGCTAGTGAGCAAACCCCTG<br>CGGTGCATGGAGAAACTGTTACCCTAAACTCTCCGC<br>CTCCCACCAGTGGCGACCGACTCTTTGGCCTTGTGC<br>AGGGGGCCTTCCTAACCTTGAATGCTACCAACCCAG<br>GGGCCACTAAGTCTTGCTGGCTCTGTTTGGGCATGA<br>GCCCCCCTTATTATGAAGGGATAGCCTCTTCAGGAG<br>AGGTCGCTTATACCTCCAACCATACCCGATGCCACT<br>GGGGGGCCCAAGGAAAGCTTACCCTCACTGAGGTC |

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| SEQ ID NO: | Description | Sequence |
|---|---|---|
| | | TCCGGACTCGGGTCATGCATAGGGAAGGTGCCTCTT ACCCATCAACATCTTTGCAACCAGACCTTACCCATC AATTCCTCTAAAAACCATCAGTATCTGCTCCCCTCA AACCATAGCTGGTGGGCCTGCAGCACTGGCCTCACC CCCTGCCTCTCCACCTCAGTTTTTAATCAGTCTAAAG ACTTCTGTGTCCAGGTCCAGCTGATCCCCCGCATCT ATTACCATTCTGAAGAAACCTTGTTACAAGCCTATG ACAAATCACCCCCCCAGGTTTAAAAGAGAGCCTGCCT CACTTACCCTAGCTGTCTTCCTGGGGTTAGGGATTG CGGCAGGTATAGGTACTGGCTCAACCGCCCTAATTA AAGGGCCCATAGACCTCCAGCAAGGCCTAACCAGC CTCCAAATCGCCATTGACGCTGACCTCCGGGCCCTT CAGGACTCAATCAGCAAGCTAGAGGACTCACTGAC TTCCCTATCTGAGGTAGTACTCCAAAATAGGAGAGG CCTTGACTTACTATTCCTTAAAGAAGGAGGCCTCTG CGCGGCCCTAAAAGAAGAGTGCTGTTTTTATGTAGA CCACTCAGGTGCAGTACGAGACTCCATGAAAAAAC TTAAAGAAAGACTAGATAAAAGACAGTTAGAGCGC CAGAAAAACCAAAACTGGTATGAAGGGTGGTTCAA TAACTCCCCTTGGTTTACTACCCTACTATCAACCATC GCTGGGCCCCTATTGCTCCTCCTTTTGTTACTCACTC TTGGGCCCTGCATCATCAATAAATTAATCCAATTCA TCAATGATAGGATAAGTGCAGTCAAAATTTTAGTCC TTAGACAGAAATATCAGACCCTAGATAACGAGGAA AACCTTTAA |
| 48 | Envelope; FUG | ATGGTTCCGCAGGTTCTTTTGTTTGTACTCCTTCTGG GTTTTTCGTTGTGTTTCGGGAAGTTCCCCATTTACAC GATACCAGACGAACTTGGTCCCTGGAGCCCTATTGA CATACACCATCTCAGCTGTCCAAATAACCTGGTTGT GGAGGATGAAGGATGTACCAACCTGTCCGAGTTCTC CTACATGGAACTCAAAGTGGGATACATCTCAGCCAT CAAAGTGAACGGGTTCACTTGCACAGGTGTTGTGAC AGAGGCAGAGACCTACACCAACTTTGTTGGTTATGT CACAACCACATTCAAGAGAAAGCATTTCCGCCCCAC CCCAGACGCATGTAGAGCCGCGTATAACTGGAAGA TGGCCGGTGACCCCAGATATGAAGAGTCCCTACAC AATCCATACCCCGACTACCACTGGCTTCGAACTGTA AGAACCACCAAAGAGTCCCTCATTATCATATCCCCA AGTGTGACAGATTTGGACCCATATGACAAATCCCTT CACTCAAGGGTCTTCCCTGGCGGAAAGTGCTCAGGA ATAACGGTGTCCTCTACCTACTGCTCAACTAACCAT GATTACACCATTTGGATGCCCGAGAATCCGAGACCA AGGACACCTTGTGACATTTTTACCAATAGCAGAGGG AAGAGAGCATCCAACGGGAACAAGACTTGCGGCTT TGTGGATGAAAGAGGCCTGTATAAGTCTCTAAAAG GAGCATGCAGGCTCAAGTTATGTGGAGTTCTTGGAC TTAGACTTATGGATGGAACATGGGTCGCGATGCAA ACATCAGATGAGACCAAATGGTGCCCTCCAGATCA GTTGGTGAATTTGCACGACTTTCGCTCAGACGAGAT CGAGCATCTCGTTGTGGAGGAGTTAGTTAAGAAAA GAGAGGAATGTCTGGATGCATTAGAGTCCATCATG ACCACCAAGTCAGTAAGTTTCAGACGTCTCAGTCAC CTGAGAAAACTTGTCCCAGGGTTTGGAAAAGCATAT ACCATATTCAACAAAACCTTGATGGAGGCTGATGCT CACTACAAGTCAGTCCGGACCTGGAATGAGATCATC CCCTCAAAAGGGTGTTTGAAAGTTGGAGGAAGGTG CCATCCTCATGTGAACGGGGTGTTTTTCAATGGTAT AATATTAGGGCCTGACGACCATGTCCTAATCCCAGA GATGCAATCATCCCTCCTCCAGCAACATATGGAGTT GTTGGAATCTTCAGTTATCCCCCTGATGCACCCCCT GGCAGACCCTTCTACAGTTTTCAAAGAAGGTGATGA GGCTGAGGATTTTGTTGAAGTTCACCTCCCCGATGT GTACAAACAGATCTCAGGGGTTGACCTGGGTCTCCC GAACTGGGGAAAGTATGTATTGATGACTGCAGGGG CCATGATTGGCCTGGTGTTGATATTTTCCCTAATGA CATGGTGCAGAGTTGGTATCCATCTTTGCATTAAAT TAAAGCACACCAAGAAAAGACAGATTTATACAGAC ATAGAGATGAACCGACTTGGAAAGTAA |
| 49 | Envelope; LCMV | ATGGGTCAGATTGTGACAATGTTTGAGGCTCTGCCT CACATCATCGATGAGGTGATCAACATTGTCATTATT GTGCTTATCGTGATCACGGGTATCAAGGCTGTCTAC AATTTTGCCACCTGTGGGATATTCGCATTGATCAGT TTCCTACTTCTGGCTGGCAGGTCCTGTGGCATGTAC GGTCTTAAGGGACCCGACATTTACAAAGGAGTTTAC |

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| SEQ ID NO: | Description | Sequence |
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| | | CAATTTAAGTCAGTGGAGTTTGATATGTCACATCTG<br>AACCTGACCATGCCCAACGCATGTTCAGCCAACAAC<br>TCCCACCATTACATCAGTATGGGGACTTCTGGACTA<br>GAATTGACCTTCACCAATGATTCCATCATCAGTCAC<br>AACTTTTGCAATCTGACCTCTGCCTTCAACAAAAAG<br>ACCTTTGACCACACACTCATGAGTATAGTTTCGAGC<br>CTACACCTCAGTATCAGAGGGAACTCCAACTATAAG<br>GCAGTATCCTGCGACTTCAACAATGGCATAACCATC<br>CAATACAACTTGACATTCTCAGATCGACAAAGTGCT<br>CAGAGCCAGTGTAGAACCTTCAGAGGTAGAGTCCT<br>AGATATGTTTAGAACTGCCTTCGGGGGGAAATACAT<br>GAGGAGTGGCTGGGGCTGGACAGGCTCAGATGGCA<br>AGACCACCTGGTGTAGCCAGACGAGTTACCAATAC<br>CTGATTATACAAAATAGAACCTGGGAAAACCACTG<br>CACATATGCAGGTCCTTTTGGGATGTCCAGGATTCT<br>CCTTTCCCAAGAGAAGACTAAGTTCTTCACTAGGAG<br>ACTAGCGGGCACATTCACCTGGACTTTGTCAGACTC<br>TTCAGGGGTGGAGAATCCAGGTGGTTATTGCCTGAC<br>CAAATGGATGATTCTTGCTGCAGAGCTTAAGTGTTT<br>CGGGAACACAGCAGTTGCGAAATGCAATGTAAATC<br>ATGATGCCGAATTCTGTGACATGCTGCGACTAATTG<br>ACTACAACAAGGCTGCTTTGAGTAAGTTCAAAGAG<br>GACGTAGAATCTGCCTTGCACTTATTCAAAACAACA<br>GTGAATTCTTTGATTTCAGATCAACTACTGATGAGG<br>AACCACTTGAGAGATCTGATGGGGGTGCCATATTGC<br>AATTACTCAAAGTTTTGGTACCTAGAACATGCAAAG<br>ACCGGCGAAACTAGTGTCCCCAAGTGCTGGCTTGTC<br>ACCAATGGTTCTTACTTAAATGAGACCCACTTCAGT<br>GATCAAATCGAACAGGAAGCCGATAACATGATTAC<br>AGAGATGTTGAGGAAGGATTACATAAAGAGGCAGG<br>GGAGTACCCCCCTAGCATTGATGGACCTTCTGATGT<br>TTTCCACATCTGCATATCTAGTCAGCATCTTCCTGCA<br>CCTTGTCAAAATACCAACACACAGGCACATAAAAG<br>GTGGCTCATGTCCAAAGCCACACCGATTAACCAACA<br>AAGGAATTTGTAGTTGTGGTGCATTTAAGGTGCCTG<br>GTGTAAAAACCGTCTGGAAAAGACGCTGA |
| 50 | Envelope; FPV | ATGAACACTCAAATCCTGGTTTTCGCCCTTGTGGCA<br>GTCATCCCCACAAATGCAGACAAAATTTGTCTTGGA<br>CATCATGCTGTATCAAATGGCACCAAAGTAAACAC<br>ACTCACTGAGAGAGGAGTAGAAGTTGTCAATGCAA<br>CGGAAACAGTGGAGCGGACAAACATCCCCAAAATT<br>TGCTCAAAAGGGAAAAGAACCACTGATCTTGGCCA<br>ATGCGGACTGTTAGGGACCATTACCGGACCACCTCA<br>ATGCGACCAATTTCTAGAATTTTCAGCTGATCTAAT<br>AATCGAGAGACGAGAAGGAAATGATGTTTGTTACC<br>CGGGGAAGTTTGTTAATGAAGAGGCATTGCGACAA<br>ATCCTCAGAGGATCAGGTGGGATTGACAAAGAAAC<br>AATGGGATTCACATATAGTGGAATAAGGACCAACG<br>GAACAACTAGTGCATGTAGAAGATCAGGGTCTTCAT<br>TCTATGCAGAAATGGAGTGGCTCCTGTCAAATACAG<br>ACAATGCTGCTTTCCCACAAATGACAAAATCATACA<br>AAAACACAAGGAGAGAATCAGCTCTGATAGTCTGG<br>GGAATCCACCATTCAGGATCAACCACCGAACAGAC<br>CAAACTATATGGGAGTGGAAATAAACTGATAACAG<br>TCGGGAGTTCCAAATATCATCAATCTTTTGTGCCGA<br>GTCCAGGAACACGACCGCAGATAAATGGCCAGTCC<br>GGACGGATTGATTTTCATTGGTTGATCTTGGATCCC<br>AATGATACAGTTACTTTTAGTTTCAATGGGGCTTTC<br>ATAGCTCCAAATCGTGCCAGCTTCTTGAGGGGAAAG<br>TCCATGGGGATCCAGAGCGATGTGCAGGTTGATGCC<br>AATTGCGAAGGGGAATGCTACCACAGTGGAGGGAC<br>TATAACAAGCAGATTGCCTTTTCAAAACATCAATAG<br>CAGAGCAGTTGGCAAATGCCCAAGATATGTAAAAC<br>AGGAAAGTTTATTATTGGCAACTGGGATGAAGAAC<br>GTTCCCGAACCTTCCAAAAAAAGGAAAAAAAGAGG<br>CCTGTTTGGCGCTATAGCAGGGTTTATTGAAAATGG<br>TTGGGAAGGTCTGGTCGACGGGTGGTACGGTTTCAG<br>GCATCAGAATGCACAAGGAGAAGGAACTGCAGCAG<br>ACTACAAAAGCACCCAATCGGCAATTGATCAGATA<br>ACCGGAAAGTTAAATAGACTCATTGAGAAAACCAA<br>CCAGCAATTTGAGCTAATAGATAATGAATTCACTGA<br>GGTGGAAAAGCAGATTGGCAATTTAATTAACTGGA<br>CCAAAGACTCCATCACAGAAGTATGGTCTTACAATG<br>CTGAACTTCTTGTGGCAATGGAAAACCAGCACACTA<br>TTGATTTGGCTGATTCAGAGATGAACAAGCTGTATG |

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| SEQ ID NO: | Description | Sequence |
|---|---|---|
| | | AGCGAGTGAGGAAACAATTAAGGGAAAATGCTGAA GAGGATGGCACTGGTTGCTTTGAAATTTTTCATAAA TGTGACGATGATTGTATGGCTAGTATAAGGAACAAT ACTTATGATCACAGCAAATACAGAGAAGAAGCGAT GCAAAATAGAATACAAATTGACCCAGTCAAATTGA GTAGTGGCTACAAAGATGTGATACTTTGGTTTAGCT TCGGGGCATCATGCTTTTTGCTTCTTGCCATTGCAAT GGGCCTTGTTTTCATATGTGTGAAGAACGGAAACAT GCGGTGCACTATTTGTATATAA |
| 51 | Envelope; RRV | AGTGTAACAGAGCACTTTAATGTGTATAAGGCTACT AGACCATACCTAGCACATTGCGCCGATTGCGGGGA CGGGTACTTCTGCTATAGCCCAGTTGCTATCGAGGA GATCCGAGATGAGGCGTCTGATGGCATGCTTAAGAT CCAAGTCTCCGCCCAAATAGGTCTGGACAAGGCAG GCACCCACGCCCACACGAAGCTCCGATATATGGCTG GTCATGATGTTCAGGAATCTAAGAGAGATTCCTTGA GGGTGTACACGTCCGCAGCGTGCTCCATACATGGGA CGATGGGACACTTCATCGTCGCACACTGTCCACCAG GCGACTACCTCAAGGTTTCGTTCGAGGACGCAGATT CGCACGTGAAGGCATGTAAGGTCCAATACAAGCAC AATCCATTGCCGGTGGGTAGAGAGAAGTTCGTGGTT AGACCACACTTTGGCGTAGAGCTGCCATGCACCTCA TACCAGCTGACAACGGCTCCCACCGACGAGGAGAT TGACATGCATACACCGCCAGATATACCGGATCGCAC CCTGCTATCACAGACGGCGGGCAACGTCAAAATAA CAGCAGGCGGCAGGACTATCAGGTACAACTGTACC TGCGGCCGTGACAACGTAGGCACTACCAGTACTGA CAAGACCATCAACACATGCAAGATTGACCAATGCC ATGCTGCCGTCACCAGCCATGACAAATGGCAATTTA CCTCTCCATTTGTTCCCAGGGCTGATCAGACAGCTA GGAAAGGCAAGGTACACGTTCCGTTCCCTCTGACTA ACGTCACCTGCCGAGTGCCGTTGGCTCGAGCGCCGG ATGCCACCTATGGTAAGAAGGAGGTGACCCTGAGA TTACACCCAGATCATCCGACGCTCTTCTCCTATAGG AGTTTAGGAGCCGAACCGCACCCGTACGAGGAATG GGTTGACAAGTTCTCTGAGCGCATCATCCCAGTGAC GGAAGAAGGGATTGAGTACCAGTGGGGCAACAACC CGCCGGTCTGCCTGTGGGCGCAACTGACGACCGAG GGCAAACCCCATGGCTGGCCACATGAAATCATTCA GTACTATTATGGACTATACCCCGCCGCCACTATTGC CGCAGTATCCGGGGCGAGTCTGATGGCCCTCCTAAC TCTGGCGGCCACATGCTGCATGCTGGCCACCGCGAG GAGAAAGTGCCTAACACCGTACGCCCTGACGCCAG GAGCGGTGGTACCGTTGACACTGGGGCTGCTTTGCT GCGCACCGAGGGCGAATGCA |
| 52 | Envelope; MLV 10A1 | AGTGTAACAGAGCACTTTAATGTGTATAAGGCTACT AGACCATACCTAGCACATTGCGCCGATTGCGGGGA CGGGTACTTCTGCTATAGCCCAGTTGCTATCGAGGA GATCCGAGATGAGGCGTCTGATGGCATGCTTAAGAT CCAAGTCTCCGCCCAAATAGGTCTGGACAAGGCAG GCACCCACGCCCACACGAAGCTCCGATATATGGCTG GTCATGATGTTCAGGAATCTAAGAGAGATTCCTTGA GGGTGTACACGTCCGCAGCGTGCTCCATACATGGGA CGATGGGACACTTCATCGTCGCACACTGTCCACCAG GCGACTACCTCAAGGTTTCGTTCGAGGACGCAGATT CGCACGTGAAGGCATGTAAGGTCCAATACAAGCAC AATCCATTGCCGGTGGGTAGAGAGAAGTTCGTGGTT AGACCACACTTTGGCGTAGAGCTGCCATGCACCTCA TACCAGCTGACAACGGCTCCCACCGACGAGGAGAT TGACATGCATACACCGCCAGATATACCGGATCGCAC CCTGCTATCACAGACGGCGGGCAACGTCAAAATAA CAGCAGGCGGCAGGACTATCAGGTACAACTGTACC TGCGGCCGTGACAACGTAGGCACTACCAGTACTGA CAAGACCATCAACACATGCAAGATTGACCAATGCC ATGCTGCCGTCACCAGCCATGACAAATGGCAATTTA CCTCTCCATTTGTTCCCAGGGCTGATCAGACAGCTA GGAAAGGCAAGGTACACGTTCCGTTCCCTCTGACTA ACGTCACCTGCCGAGTGCCGTTGGCTCGAGCGCCGG ATGCCACCTATGGTAAGAAGGAGGTGACCCTGAGA TTACACCCAGATCATCCGACGCTCTTCTCCTATAGG AGTTTAGGAGCCGAACCGCACCCGTACGAGGAATG GGTTGACAAGTTCTCTGAGCGCATCATCCCAGTGAC |

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| SEQ ID NO: | Description | Sequence |
|---|---|---|
| | | GGAAGAAGGGATTGAGTACCAGTGGGGCAACAACC<br>CGCCGGTCTGCCTGTGGGCGCAACTGACGACCGAG<br>GGCAAACCCCATGGCTGGCCACATGAAATCATTCA<br>GTACTATTATGGACTATACCCCGCCGCCACTATTGC<br>CGCAGTATCCGGGGCGAGTCTGATGGCCCTCCTAAC<br>TCTGGCGGCCACATGCTGCATGCTGGCCACCGCGAG<br>GAGAAAGTGCCTAACACCGTACGCCCTGACGCCAG<br>GAGCGGTGGTACCGTTGACACTGGGGCTGCTTTGCT<br>GCGCACCGAGGGCGAATGCA |
| 53 | Envelope; Ebola | ATGGGTGTTACAGGAATATTGCAGTTACCTCGTGAT<br>CGATTCAAGAGGACATCATTCTTTCTTTGGGTAATT<br>ATCCTTTTCCAAAGAACATTTTCCATCCCACTTGGA<br>GTCATCCACAATAGCACATTACAGGTTAGTGATGTC<br>GACAAACTGGTTTGCCGTGACAAACTGTCATCCACA<br>AATCAATTGAGATCAGTTGGACTGAATCTCGAAGG<br>GAATGGAGTGGCAACTGACGTGCCATCTGCAACTA<br>AAAGATGGGGCTTCAGGTCCGGTGTCCCACCAAAG<br>GTGGTCAATTATGAAGCTGGTGAATGGGCTGAAAA<br>CTGCTACAATCTTGAAATCAAAAAACCTGACGGGA<br>GTGAGTGTCTACCAGCAGCGCCAGACGGGATTCGG<br>GGCTTCCCCCGGTGCCGGTATGTGCACAAAGTATCA<br>GGAACGGGACCGTGTGCCGGAGACTTTGCCTTCCAC<br>AAAGAGGGTGCTTTCTTCCTGTATGACCGACTTGCT<br>TCCACAGTTATCTACCGAGGAACGACTTTCGCTGAA<br>GGTGTCGTTGCATTTCTGATACTGCCCCAAGCTAAG<br>AAGGACTTCTTCAGCTCACACCCCTTGAGAGAGCCG<br>GTCAATGCAACGGAGGACCCGTCTAGTGGCTACTAT<br>TCTACCACAATTAGATATCAAGCTACCGGTTTTGGA<br>ACCAATGAGACAGAGTATTTGTTCGAGGTTGACAAT<br>TTGACCTACGTCCAACTTGAATCAAGATTCACACCA<br>CAGTTTCTGCTCCAGCTGAATGAGACAATATATACA<br>AGTGGGAAAAGGAGCAATACCACGGGAAAACTAAT<br>TTGGAAGGTCAACCCCGAAATTGATACAACAATCG<br>GGGAGTGGGCCTTCTGGGAAACTAAAAAAACCTCA<br>CTAGAAAAATTCGCAGTGAAGAGTTGTCTTTCACAG<br>CTGTATCAAACAGAGCCAAAAACATCAGTGGTCAG<br>AGTCCGGCGCGAACTTCTTCCGACCCAGGGACCAAC<br>ACAACAACTGAAGACCACAAAATCATGGCTTCAGA<br>AAATTCCTCTGCAATGGTTCAAGTGCACAGTCAAGG<br>AAGGGAAGCTGCAGTGTCGCATCTGACAACCCTTGC<br>CACAATCTCCACGAGTCCTCAACCCCCCACAACCAA<br>ACCAGGTCCGGACAACAGCACCCACAATACACCCG<br>TGTATAAACTTGACATCTCTGAGGCAACTCAAGTTG<br>AACAACATCACCGCAGAACAGACAACGACAGCACA<br>GCCTCCGACACTCCCCCCGCCACGACCGCAGCCGGA<br>CCCCTAAAAGCAGAGAACACCAACACGAGCAAGGG<br>TACCGACCTCCTGGACCCCGCCACCACAACAAGTCC<br>CCAAAACCACAGCGAGACCGCTGGCAACAACAACA<br>CTCATCACCAAGATACCGGAGAAGAGAGTGCCAGC<br>AGCGGGAAGCTAGGCTTAATTACCAATACTATTGCT<br>GGAGTCGCAGGACTGATCACAGGCGGGAGGAGAGC<br>TCGAAGAGAAGCAATTGTCAATGCTCAACCCAAAT<br>GCAACCCTAATTTACATTACTGGACTACTCAGGATG<br>AAGGTGCTGCAATCGGACTGGCCTGGATACCATATT<br>TCGGGCCAGCAGCCGAGGGAATTTACATAGAGGGG<br>CTGATGCACAATCAAGATGGTTTAATCTGTGGGTTG<br>AGACAGCTGGCCAACGAGACGACTCAAGCTCTTCA<br>ACTGTTCCTGAGAGCCACAACCGAGCTACGCACCTT<br>TTCAATCCTCAACCGTAAGGCAATTGATTTCTTGCT<br>GCAGCGATGGGGCGGCACATGCCACATTTTGGGAC<br>CGGACTGCTGTATCGAACCACATGATTGGACCAAG<br>AACATAACAGACAAAATTGATCAGATTATTCATGAT<br>TTTGTTGATAAAACCCTTCCGGACCAGGGGGACAAT<br>GACAATTGGTGGACAGGATGGAGACAATGGATACC<br>GGCAGGTATTGGAGTTACAGGCGTTATAATTGCAGT<br>TATCGCTTTATTCTGTATATGCAAATTTGTCTTTTAG |
| 54 | Polymerase III shRNA promoters; U6 promoter | TTTCCCATGATTCCTTCATATTTGCATATACGATACA<br>AGGCTGTTAGAGAGATAATTGGAATTAATTTGACTG<br>TAAACACAAAGATATTAGTACAAAATACGTGACGT<br>AGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTA<br>AAATTATGTTTTAAAATGGACTATCATATGCTTACC<br>GTAACTTGAAAGTATTTCGATTTCTTGGCTTTATATA<br>TCTTGTGGAAAGGACGAAAC |

-continued

| SEQ ID NO: | Description | Sequence |
|---|---|---|
| 55 | Polymerase III shRNA promoters; 7SK promoter | CTGCAGTATTTAGCATGCCCCACCCATCTGCAAGGC ATTCTGGATAGTGTCAAAACAGCCGGAAATCAAGT CCGTTTATCTCAAACTTTAGCATTTTGGGAATAAAT GATATTTGCTATGCTGGTTAAATTAGATTTTAGTTA AATTTCCTGCTGAAGCTCTAGTACGATAAGCAACTT GACCTAAGTGTAAAGTTGAGATTTCCTTCAGGTTTA TATAGCTTGTGCGCCGCCTGGCTACCTC |
| 56 | FDPS target sequence #1 | GTCCTGGAGTACAATGCCATT |
| 57 | FDPS target sequence #2 | GCAGGATTTCGTTCAGCACTT |
| 58 | FDPS target sequence #3 | GCCATGTACATGGCAGGAATT |
| 59 | FDPS target sequence #4 | GCAGAAGGAGGCTGAGAAAGT |
| 60 | Non-targeting sequence | GCCGCTTTGTAGGATAGAGCTCGAGCTCTATCCTAC AAAGCGGCTTTTT |
| 61 | Forward primer | AGGAATTGATGGCGAGAAGG |
| 62 | Reverse primer | CCCAAAGAGGTCAAGGTAATCA |
| 63 | Forward primer | AGCGCGGCTACAGCTTCA |
| 64 | Reverse primer | GGCGACGTAGCACAGCTTCT |
| 65 | Left Inverted Terminal Repeat (Left ITR) | CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGG CCGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCC TCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGC CAACTCCATCACTAGGGGTTCCT |
| 66 | Right Inverted Terminal Repeat (Right ITR) | GAGCGGCCGCAGGAACCCCTAGTGATGGAGTTGGC CACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGC CGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTG CCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGC TGCCTGCAGG |

While certain of the preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such embodiments. Various modifications may be made thereto without departing from the scope and spirit of the present invention.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 67

<210> SEQ ID NO 1
<211> LENGTH: 53
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: FDPS shRNA sequence #1

<400> SEQUENCE: 1

gtcctggagt acaatgccat tctcgagaat ggcattgtac tccaggactt ttt 53

<210> SEQ ID NO 2
<211> LENGTH: 53
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: FDPS shRNA sequence #2

<400> SEQUENCE: 2

gcaggatttc gttcagcact tctcgagaag tgctgaacga aatcctgctt ttt 53

```
<210> SEQ ID NO 3
<211> LENGTH: 53
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: FDPS shRNA sequence #3

<400> SEQUENCE: 3

gccatgtaca tggcaggaat tctcgagaat tcctgccatg tacatggctt ttt            53


<210> SEQ ID NO 4
<211> LENGTH: 53
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: FDPS shRNA sequence #4

<400> SEQUENCE: 4

gcagaaggag gctgagaaag tctcgagact ttctcagcct ccttctgctt ttt            53


<210> SEQ ID NO 5
<211> LENGTH: 116
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR30 FDPS sequence #1

<400> SEQUENCE: 5

aaggtatatt gctgttgaca gtgagcgaca ctttctcagc ctccttctgc gtgaagccac     60

agatggcaga aggaggctga gaaagtgctg cctactgcct cggacttcaa ggggct        116


<210> SEQ ID NO 6
<211> LENGTH: 114
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR30 FDPS sequence #2

<400> SEQUENCE: 6

aaggtatatt gctgttgaca gtgagcgaca ctttctcagc ctccttctgc gtgaagccac     60

agatggcaga agggctgaga aagtgctgcc tactgcctcg gacttcaagg ggct          114


<210> SEQ ID NO 7
<211> LENGTH: 91
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR30 FDPS sequence #3

<400> SEQUENCE: 7

tgctgttgac agtgagcgac tttctcagcc tccttctgcg tgaagccaca gatggcagaa     60

ggaggctgag aaagttgcct actgcctcgg a                                    91


<210> SEQ ID NO 8
<211> LENGTH: 115
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR155 FDPS sequence #1

<400> SEQUENCE: 8

cctggaggct tgctgaaggc tgtatgctga ctttctcagc ctccttctgc ttttggccac     60

tgactgagca gaagggctga gaaagtcagg acacaaggcc tgttactagc actca         115
```

```
<210> SEQ ID NO 9
<211> LENGTH: 114
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR21 FDPS sequence #1

<400> SEQUENCE: 9

catctccatg gctgtaccac cttgtcggga ctttctcagc ctccttctgc ctgttgaatc     60

tcatggcaga aggaggcgag aaagtctgac attttggtat ctttcatctg acca          114


<210> SEQ ID NO 10
<211> LENGTH: 114
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR185 FDPS sequence #1

<400> SEQUENCE: 10

gggcctggct cgagcagggg gcgagggata ctttctcagc ctccttctgc tggtcccctc     60

cccgcagaag gaggctgaga aagtccttcc ctcccaatga ccgcgtcttc gtcg          114


<210> SEQ ID NO 11
<211> LENGTH: 228
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Rous Sarcoma virus (RSV) promoter

<400> SEQUENCE: 11

gtagtcttat gcaatactct tgtagtcttg caacatggta acgatgagtt agcaacatgc     60

cttacaagga gagaaaaagc accgtgcatg ccgattggtg gaagtaaggt ggtacgatcg    120

tgccttatta ggaaggcaac agacgggtct gacatggatt ggacgaacca ctgaattgcc    180

gcattgcaga gatattgtat ttaagtgcct agctcgatac aataaacg                 228


<210> SEQ ID NO 12
<211> LENGTH: 180
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 5 Long terminal repeat (LTR)

<400> SEQUENCE: 12

ggtctctctg gttagaccag atctgagcct gggagctctc tggctaacta gggaacccac     60

tgcttaagcc tcaataaagc ttgccttgag tgcttcaagt agtgtgtgcc cgtctgttgt    120

gtgactctgg taactagaga tccctcagac ccttttagtc agtgtggaaa atctctagca    180


<210> SEQ ID NO 13
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Psi Packaging signal

<400> SEQUENCE: 13

tacgccaaaa attttgacta gcggaggcta gaaggagaga g                         41


<210> SEQ ID NO 14
<211> LENGTH: 233
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
```

<220> FEATURE:
<223> OTHER INFORMATION: Rev response element (RRE)

<400> SEQUENCE: 14

aggagctttg ttccttgggt tcttgggagc agcaggaagc actatgggcg cagcctcaat 60

gacgctgacg gtacaggcca gacaattatt gtctggtata gtgcagcagc agaacaattt 120

gctgagggct attgaggcgc aacagcatct gttgcaactc acagtctggg gcatcaagca 180

gctccaggca agaatcctgg ctgtggaaag atacctaaag gatcaacagc tcc 233

<210> SEQ ID NO 15
<211> LENGTH: 118
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Central polypurine tract (cPPT)

<400> SEQUENCE: 15

ttttaaaaga aaagggggga ttggggggta cagtgcaggg gaaagaatag tagacataat 60

agcaacagac atacaaacta aagaattaca aaaacaaatt acaaaattca aaatttta 118

<210> SEQ ID NO 16
<211> LENGTH: 217
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Polymerase III shRNA promoters- H1 promoter

<400> SEQUENCE: 16

gaacgctgac gtcatcaacc cgctccaagg aatcgcgggc ccagtgtcac taggcgggaa 60

cacccagcgc gcgtgcgccc tggcaggaag atggctgtga gggacagggg agtggcgccc 120

tgcaatattt gcatgtcgct atgtgttctg ggaaatcacc ataaacgtga aatgtctttg 180

gatttgggaa tcttataagt tctgtatgag accactt 217

<210> SEQ ID NO 17
<211> LENGTH: 590
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Long WPRE sequence

<400> SEQUENCE: 17

aatcaacctc tgattacaaa atttgtgaaa gattgactgg tattcttaac tatgttgctc 60

cttttacgct atgtggatac gctgctttaa tgcctttgta tcatgctatt gcttcccgta 120

tggctttcat tttctcctcc ttgtataaat cctggttgct gtctctttat gaggagttgt 180

ggcccgttgt caggcaacgt ggcgtggtgt gcactgtgtt tgctgacgca acccccactg 240

gttggggcat tgccaccacc tgtcagctcc tttccgggac tttcgctttc cccctcccta 300

ttgccacggc ggaactcatc gccgcctgcc ttgcccgctg ctggacaggg gctcggctgt 360

tgggcactga caattccgtg gtgttgtcgg ggaaatcatc gtcctttcct tggctgctcg 420

cctgtgttgc cacctggatt ctgcgcggga cgtccttctg ctacgtccct tcggccctca 480

atccagcgga ccttccttcc cgcggcctgc tgccggctct gcggcctctt ccgcgtcttc 540

gccttcgccc tcagacgagt cggatctccc tttgggccgc ctccccgcct 590

<210> SEQ ID NO 18
<211> LENGTH: 250
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 3 delta LTR

<400> SEQUENCE: 18

tggaagggct aattcactcc caacgaagat aagatctgct tttgcttgt actgggtctc 60

tctggttaga ccagatctga gcctgggagc tctctggcta actagggaac ccactgctta 120

agcctcaata aagcttgcct tgagtgcttc aagtagtgtg tgcccgtctg ttgtgtgact 180

ctggtaacta gagatccctc agaccctttt agtcagtgtg gaaaatctct agcagtagta 240

gttcatgtca 250

<210> SEQ ID NO 19
<211> LENGTH: 290
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev- Chicken beta actin (CAG) promoter-
Transcription

<400> SEQUENCE: 19

gctattacca tgggtcgagg tgagccccac gttctgcttc actctcccca tctccccccc 60

ctccccaccc ccaattttgt atttatttat tttttaatta ttttgtgcag cgatgggggc 120

gggggggggg ggggcgcgcg ccaggcgggg cggggcgggg cgaggggcgg ggcggggcga 180

ggcggagagg tgcggcggca gccaatcaga gcggcgcgct ccgaaagttt ccttttatgg 240

cgaggcggcg gcggcggcgg ccctataaaa agcgaagcgc gcggcgggcg 290

<210> SEQ ID NO 20
<211> LENGTH: 1503
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev- HIV Gag- Viral capsid

<400> SEQUENCE: 20

atgggtgcga gagcgtcagt attaagcggg ggagaattag atcgatggga aaaaattcgg 60

ttaaggccag ggggaaagaa aaaatataaa ttaaaacata tagtatgggc aagcagggag 120

ctagaacgat tcgcagttaa tcctggcctg ttagaaacat cagaaggctg tagacaaata 180

ctgggacagc tacaaccatc ccttcagaca ggatcagaag aacttagatc attatataat 240

acagtagcaa ccctctattg tgtgcatcaa aggatagaga taaaagacac caaggaagct 300

ttagacaaga tagaggaaga gcaaaacaaa agtaagaaaa aagcacagca agcagcagct 360

gacacaggac acagcaatca ggtcagccaa aattacccta tagtgcagaa catccagggg 420

caaatggtac atcaggccat atcacctaga actttaaatg catgggtaaa agtagtagaa 480

gagaaggctt tcagcccaga agtgataccc atgttttcag cattatcaga aggagccacc 540

ccacaagatt taaacaccat gctaaacaca gtggggggac atcaagcagc catgcaaatg 600

ttaaaagaga ccatcaatga ggaagctgca gaatgggata gagtgcatcc agtgcatgca 660

gggcctattg caccaggcca gatgagagaa ccaaggggaa gtgacatagc aggaactact 720

agtacccttc aggaacaaat aggatggatg acacataatc cacctatccc agtaggagaa 780

atctataaaa gatggataat cctgggatta aataaaatag taagaatgta tagccctacc 840

agcattctgg acataagaca aggaccaaag gaacccttta gagactatgt agaccgattc 900

```
tataaaactc taagagccga gcaagcttca caagaggtaa aaaattggat gacagaaacc    960

ttgttggtcc aaaatgcgaa cccagattgt aagactattt taaaagcatt gggaccagga   1020

gcgacactag aagaaatgat gacagcatgt cagggagtgg ggggacccgg ccataaagca   1080

agagttttgg ctgaagcaat gagccaagta acaaatccag ctaccataat gatacagaaa   1140

ggcaatttta ggaaccaaag aaagactgtt aagtgtttca attgtggcaa agaagggcac   1200

atagccaaaa attgcagggc ccctaggaaa aagggctgtt ggaaatgtgg aaaggaagga   1260

caccaaatga aagattgtac tgagagacag gctaattttt tagggaagat ctggccttcc   1320

cacaagggaa ggccagggaa ttttcttcag agcagaccag agccaacagc cccaccagaa   1380

gagagcttca ggtttgggga agagacaaca actccctctc agaagcagga gccgatagac   1440

aaggaactgt atcctttagc ttccctcaga tcactctttg gcagcgaccc ctcgtcacaa   1500

taa                                                                 1503
```

```
<210> SEQ ID NO 21
<211> LENGTH: 1872
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev- HIV Pol- Protease and reverse
      transcriptase

<400> SEQUENCE: 21

atgaatttgc caggaagatg gaaaccaaaa atgatagggg gaattggagg ttttatcaaa     60

gtaggacagt atgatcagat actcatagaa atctgcggac ataaagctat aggtacagta    120

ttagtaggac ctacacctgt caacataatt ggaagaaatc tgttgactca gattggctgc    180

actttaaatt ttcccattag tcctattgag actgtaccag taaaattaaa gccaggaatg    240

gatggcccaa aagttaaaca atggccattg acagaagaaa aaataaaagc attagtagaa    300

atttgtacag aaatggaaaa ggaaggaaaa atttcaaaaa ttgggcctga aaatccatac    360

aatactccag tatttgccat aaagaaaaaa gacagtacta aatggagaaa attagtagat    420

ttcagagaac ttaataagag aactcaagat ttctgggaag ttcaattagg aataccacat    480

cctgcagggt taaaacagaa aaaatcagta acagtactgg atgtgggcga tgcatatttt    540

tcagttccct tagataaaga cttcaggaag tatactgcat ttaccatacc tagtataaac    600

aatgagacac cagggattag atatcagtac aatgtgcttc cacagggatg gaaaggatca    660

ccagcaatat tccagtgtag catgacaaaa atcttagagc cttttagaaa acaaaatcca    720

gacatagtca tctatcaata catggatgat ttgtatgtag gatctgactt agaaataggg    780

cagcatagaa caaaaataga ggaactgaga caacatctgt tgaggtgggg atttaccaca    840

ccagacaaaa aacatcagaa agaacctcca ttcctttgga tgggttatga actccatcct    900

gataaatgga cagtacagcc tatagtgctg ccagaaaagg acagctggac tgtcaatgac    960

atacagaaat tagtgggaaa attgaattgg gcaagtcaga tttatgcagg gattaaagta   1020

aggcaattat gtaaacttct taggggaacc aaagcactaa cagaagtagt accactaaca   1080

gaagaagcag agctagaact ggcagaaaac agggagattc taaaagaacc ggtacatgga   1140

gtgtattatg acccatcaaa agacttaata gcagaaatac agaagcaggg gcaaggccaa   1200

tggacatatc aaatttatca agagccattt aaaaatctga aaacaggaaa atatgcaaga   1260

atgaagggtg cccacactaa tgatgtgaaa caattaacag aggcagtaca aaaaatagcc   1320

acagaaagca tagtaatatg gggaaagact cctaaattta aattacccat acaaaaggaa   1380
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acatgggaag catggtggac agagtattgg caagccacct ggattcctga gtgggagttt   1440

gtcaataccc ctcccttagt gaagttatgg taccagttag agaaagaacc cataatagga   1500

gcagaaactt tctatgtaga tggggcagcc aatagggaaa ctaaattagg aaaagcagga   1560

tatgtaactg acagaggaag acaaaaagtt gtccccctaa cggacacaac aaatcagaag   1620

actgagttac aagcaattca tctagctttg caggattcgg gattagaagt aaacatagtg   1680

acagactcac aatatgcatt gggaatcatt caagcacaac cagataagag tgaatcagag   1740

ttagtcagtc aaataataga gcagttaata aaaaaggaaa aagtctacct ggcatgggta   1800

ccagcacaca aaggaattgg aggaaatgaa caagtagatg ggttggtcag tgctggaatc   1860

aggaaagtac ta                                                        1872


<210> SEQ ID NO 22
<211> LENGTH: 867
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper Rev- HIV Integrase- Integration of viral
      RNA

<400> SEQUENCE: 22

tttttagatg gaatagataa ggcccaagaa gaacatgaga aatatcacag taattggaga     60

gcaatggcta gtgattttaa cctaccacct gtagtagcaa aagaaatagt agccagctgt    120

gataaatgtc agctaaaagg ggaagccatg catggacaag tagactgtag cccaggaata    180

tggcagctag attgtacaca tttagaagga aaagttatct tggtagcagt tcatgtagcc    240

agtggatata tagaagcaga agtaattcca gcagagacag ggcaagaaac agcatacttc    300

ctcttaaaat tagcaggaag atggccagta aaaacagtac atacagacaa tggcagcaat    360

ttcaccagta ctacagttaa ggccgcctgt tggtgggcgg ggatcaagca ggaatttggc    420

attccctaca atccccaaag tcaaggagta atagaatcta tgaataaaga attaaagaaa    480

attataggac aggtaagaga tcaggctgaa catcttaaga cagcagtaca aatggcagta    540

ttcatccaca attttaaaag aaaagggggg attggggggt acagtgcagg ggaaagaata    600

gtagacataa tagcaacaga catacaaact aaagaattac aaaaacaaat tacaaaaatt    660

caaaattttc gggtttatta cagggacagc agagatccag tttggaaagg accagcaaag    720

ctcctctgga aaggtgaagg ggcagtagta atacaagata atagtgacat aaaagtagtg    780

ccaagaagaa aagcaaagat catcagggat tatggaaaac agatggcagg tgatgattgt    840

gtggcaagta gacaggatga ggattaa                                        867


<210> SEQ ID NO 23
<211> LENGTH: 234
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev- HIV RRE- Binds Rev element

<400> SEQUENCE: 23

aggagctttg ttccttgggt tcttgggagc agcaggaagc actatgggcg cagcgtcaat     60

gacgctgacg gtacaggcca gacaattatt gtctggtata gtgcagcagc agaacaattt    120

gctgagggct attgaggcgc aacagcatct gttgcaactc acagtctggg gcatcaagca    180

gctccaggca agaatcctgg ctgtggaaag atacctaaag gatcaacagc tcct          234
```

```
<210> SEQ ID NO 24
<211> LENGTH: 351
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev- HIV Rev- Nuclear export and
      stabilize viral mRNA

<400> SEQUENCE: 24

atggcaggaa gaagcggaga cagcgacgaa gaactcctca aggcagtcag actcatcaag     60

tttctctatc aaagcaaccc acctcccaat cccgagggga cccgacaggc ccgaaggaat    120

agaagaagaa ggtggagaga gagacagaga cagatccatt cgattagtga acggatcctt    180

agcacttatc tgggacgatc tgcggagcct gtgcctcttc agctaccacc gcttgagaga    240

cttactcttg attgtaacga ggattgtgga acttctggga cgcagggggt gggaagccct    300

caaatattgg tggaatctcc tacaatattg gagtcaggag ctaaagaata g             351


<210> SEQ ID NO 25
<211> LENGTH: 577
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- CMV promoter- Transcription

<400> SEQUENCE: 25

acattgatta ttgactagtt attaatagta atcaattacg gggtcattag ttcatagccc     60

atatatggag ttccgcgtta cataacttac ggtaaatggc ccgcctggct gaccgcccaa    120

cgacccccgc ccattgacgt caataatgac gtatgttccc atagtaacgc caatagggac    180

tttccattga cgtcaatggg tggagtattt acggtaaact gcccacttgg cagtacatca    240

agtgtatcat atgccaagta cgccccctat tgacgtcaat gacggtaaat ggcccgcctg    300

gcattatgcc cagtacatga ccttatggga ctttcctact tggcagtaca tctacgtatt    360

agtcatcgct attaccatgg tgatgcggtt ttggcagtac atcaatgggc gtggatagcg    420

gtttgactca cggggatttc caagtctcca ccccattgac gtcaatggga gtttgttttg    480

gcaccaaaat caacgggact ttccaaaatg tcgtaacaac tccgccccat tgacgcaaat    540

gggcggtagg cgtgtacggt gggaggtcta tataagc                             577


<210> SEQ ID NO 26
<211> LENGTH: 1519
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- VSV-G- Glycoprotein envelope-cell
      entry

<400> SEQUENCE: 26

atgaagtgcc ttttgtactt agccttttta ttcattgggg tgaattgcaa gttcaccata     60

gtttttccac acaaccaaaa aggaaactgg aaaaatgttc cttctaatta ccattattgc    120

ccgtcaagct cagatttaaa ttggcataat gacttaatag gcacagcctt acaagtcaaa    180

atgcccaaga gtcacaaggc tattcaagca gacggttgga tgtgtcatgc ttccaaatgg    240

gtcactactt gtgatttccg ctggtatgga ccgaagtata taacacattc catccgatcc    300

ttcactccat ctgtagaaca atgcaaggaa agcattgaac aaacgaaaca aggaacttgg    360

ctgaatccag gcttccctcc tcaaagttgt ggatatgcaa ctgtgacgga tgccgaagca    420

gtgattgtcc aggtgactcc tcaccatgtg ctggttgatg aatacacagg agaatgggtt    480
```

```
gattcacagt tcatcaacgg aaaatgcagc aattacatat gccccactgt ccataactct    540

acaacctggc attctgacta taaggtcaaa gggctatgtg attctaacct catttccatg    600

gacatcacct tcttctcaga ggacggagag ctatcatccc tgggaaagga gggcacaggg    660

ttcagaagta actactttgc ttatgaaact ggaggcaagg cctgcaaaat gcaatactgc    720

aagcattggg gagtcagact cccatcaggt gtctggttcg agatggctga taaggatctc    780

tttgctgcag ccagattccc tgaatgccca gaagggtcaa gtatctctgc tccatctcag    840

acctcagtgg atgtaagtct aattcaggac gttgagagga tcttggatta ttccctctgc    900

caagaaacct ggagcaaaat cagagcgggt cttccaatct ctccagtgga tctcagctat    960

cttgctccta aaaacccagg aaccggtcct gctttcacca taatcaatgg taccctaaaa   1020

tactttgaga ccagatacat cagagtcgat attgctgctc caatcctctc aagaatggtc   1080

ggaatgatca gtggaactac cacagaaagg gaactgtggg atgactgggc accatatgaa   1140

gacgtggaaa ttggacccaa tggagttctg aggaccagtt caggatataa gtttccttta   1200

tacatgattg gacatggtat gttggactcc gatcttcatc ttagctcaaa ggctcaggtg   1260

ttcgaacatc ctcacattca agacgctgct tcgcaacttc ctgatgatga gagtttattt   1320

tttggtgata ctgggctatc caaaaatcca atcgagcttg tagaaggttg gttcagtagt   1380

tggaaaagct ctattgcctc tttttcttt atcatagggt taatcattgg actattcttg    1440

gttctccgag ttggtatcca tctttgcatt aaattaaagc acaccaagaa aagacagatt   1500

tatacagaca tagagatga                                                1519
```

<210> SEQ ID NO 27
<211> LENGTH: 352
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev- CMV early (CAG) enhancer- EnhanceTranscription

<400> SEQUENCE: 27

```
tagttattaa tagtaatcaa ttacggggtc attagttcat agcccatata tggagttccg     60

cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc cccgcccatt    120

gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca    180

atgggtggac tatttacggt aaactgccca cttggcagta catcaagtgt atcatatgcc    240

aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgcccagta    300

catgacctta tgggactttc ctacttggca gtacatctac gtattagtca tc            352
```

<210> SEQ ID NO 28
<211> LENGTH: 960
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev- Chicken beta actin intron- Enhance gene expression

<400> SEQUENCE: 28

```
ggagtcgctg cgttgccttc gccccgtgcc ccgctccgcg ccgcctcgcg ccgcccgccc     60

cggctctgac tgaccgcgtt actcccacag gtgagcgggc gggacggccc ttctcctccg    120

ggctgtaatt agcgcttggt ttaatgacgg ctcgtttctt ttctgtggct gcgtgaaagc    180

cttaaagggc tccgggaggg ccctttgtgc ggggggggagc ggctcggggg gtgcgtgcgt    240

gtgtgtgtgc gtggggagcg ccgcgtgcgg cccgcgctgc ccggcggctg tgagcgctgc    300
```

```
gggcgcggcg cggggctttg tgcgctccgc gtgtgcgcga ggggagcgcg gccgggggcg    360

gtgccccgcg gtgcgggggg gctgcgaggg gaacaaaggc tgcgtgcggg gtgtgtgcgt    420

gggggggtga gcagggggtg tgggcgcggc ggtcgggctg taaccccccc ctgcaccccc    480

ctccccgagt tgctgagcac ggcccggctt cgggtgcggg gctccgtgcg gggcgtggcg    540

cggggctcgc cgtgccgggc gggggtggc ggcaggtggg ggtgccgggc ggggcggggc    600

cgcctcgggc cggggagggc tcgggggagg ggcgcggcgg ccccggagcg ccggcggctg    660

tcgaggcgcg gcgagccgca gccattgcct tttatggtaa tcgtgcgaga gggcgcaggg    720

acttcctttg tcccaaatct ggcggagccg aaatctggga ggcgccgccg cacccccctct    780

agcgggcgcg ggcgaagcgg tgcggcgccg gcaggaagga aatgggcggg gagggccttc    840

gtgcgtcgcc gcgccgccgt ccccttctcc atctccagcc tcggggctgc cgcaggggga    900

cggctgcctt cgggggggac ggggcagggc ggggttcggc ttctggcgtg tgaccggcgg    960
```

<210> SEQ ID NO 29
<211> LENGTH: 448
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper/Rev- Rabbit beta globin poly A- RNA stability

<400> SEQUENCE: 29

```
agatcttttt ccctctgcca aaaattatgg ggacatcatg aagccccttg agcatctgac     60

ttctggctaa taaaggaaat ttattttcat tgcaatagtg tgttggaatt ttttgtgtct    120

ctcactcgga aggacatatg ggagggcaaa tcatttaaaa catcagaatg agtatttggt    180

ttagagtttg gcaacatatg ccatatgctg gctgccatga acaaaggtgg ctataaagag    240

gtcatcagta tatgaaacag ccccctgctg tccattcctt attccataga aaagccttga    300

cttgaggtta gatttttttt atattttgtt ttgtgttatt tttttcttta acatccctaa    360

aattttcctt acatgtttta ctagccagat ttttcctcct ctcctgacta ctcccagtca    420

tagctgtccc tcttctctta tgaagatc                                       448
```

<210> SEQ ID NO 30
<211> LENGTH: 573
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- Beta globin intron- Enhance gene expression

<400> SEQUENCE: 30

```
gtgagtttgg ggacccttga ttgttctttc tttttcgcta ttgtaaaatt catgttatat     60

ggagggggca aagttttcag ggtgttgttt agaatgggaa gatgtccctt gtatcaccat    120

ggaccctcat gataattttg tttctttcac tttctactct gttgacaacc attgtctcct    180

cttattttct tttcattttc tgtaactttt tcgttaaact ttagcttgca tttgtaacga    240

atttttaaat tcacttttgt ttatttgtca gattgtaagt actttctcta atcacttttt    300

tttcaaggca atcagggtat attatattgt acttcagcac agttttagag aacaattgtt    360

ataattaaat gataaggtag aatatttctg catataaatt ctggctggcg tggaaatatt    420

cttattggta gaaacaacta caccctggtc atcatcctgc ctttctcttt atggttacaa    480

tgatatacac tgtttgagat gaggataaaa tactctgagt ccaaaccggg cccctctgct    540

aaccatgttc atgccttctt ctctttccta cag                                 573
```

<210> SEQ ID NO 31
<211> LENGTH: 450
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- Rabbit beta globin poly A- RNA stability

<400> SEQUENCE: 31

agatcttttt ccctctgcca aaaattatgg ggacatcatg aagccccttg agcatctgac 60

ttctggctaa taaaggaaat ttattttcat tgcaatagtg tgttggaatt ttttgtgtct 120

ctcactcgga aggacatatg ggagggcaaa tcatttaaaa catcagaatg agtatttggt 180

ttagagtttg gcaacatatg cccatatgct ggctgccatg aacaaaggtt ggctataaag 240

aggtcatcag tatatgaaac agccccctgc tgtccattcc ttattccata gaaaagcctt 300

gacttgaggt tagatttttt ttatattttg ttttgtgtta tttttttctt taacatccct 360

aaaattttcc ttacatgttt tactagccag atttttcctc ctctcctgac tactcccagt 420

catagctgtc cctcttctct tatggagatc 450

<210> SEQ ID NO 32
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 32

taagcagaat tcatgaattt gccaggaaga t 31

<210> SEQ ID NO 33
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 33

ccatacaatg aatggacact aggcggccgc acgaat 36

<210> SEQ ID NO 34
<211> LENGTH: 2745
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Gag, Pol, Integrase fragment

<400> SEQUENCE: 34

gaattcatga atttgccagg aagatggaaa ccaaaaatga tagggggaat tggaggtttt 60

atcaaagtaa gacagtatga tcagatactc atagaaatct gcggacataa agctataggt 120

acagtattag taggacctac acctgtcaac ataattggaa gaaatctgtt gactcagatt 180

ggctgcactt taaattttcc cattagtcct attgagactg taccagtaaa attaaagcca 240

ggaatggatg gcccaaaagt taaacaatgg ccattgacag aagaaaaaat aaaagcatta 300

gtagaaattt gtacagaaat ggaaaaggaa ggaaaaattt caaaaattgg gcctgaaaat 360

ccatacaata ctccagtatt tgccataaag aaaaaagaca gtactaaatg gagaaaatta 420

gtagatttca gagaacttaa taagagaact caagatttct gggaagttca attaggaata 480

ccacatcctg cagggttaaa acagaaaaaa tcagtaacag tactggatgt gggcgatgca 540

tatttttcag ttcccttaga taaagacttc aggaagtata ctgcatttac catacctagt 600

-continued

```
ataaacaatg agacaccagg gattagatat cagtacaatg tgcttccaca gggatggaaa    660
ggatcaccag caatattcca gtgtagcatg acaaaaatct tagagccttt tagaaaacaa    720
aatccagaca tagtcatcta tcaatacatg gatgatttgt atgtaggatc tgacttagaa    780
atagggcagc atagaacaaa aatagaggaa ctgagacaac atctgttgag gtggggattt    840
accacaccag acaaaaaaca tcagaaagaa cctccattcc tttggatggg ttatgaactc    900
catcctgata aatggacagt acagcctata gtgctgccag aaaaggacag ctggactgtc    960
aatgacatac agaaattagt gggaaaattg aattgggcaa gtcagattta tgcagggatt   1020
aaagtaaggc aattatgtaa acttcttagg ggaaccaaag cactaacaga agtagtacca   1080
ctaacagaag aagcagagct agaactggca gaaaacaggg agattctaaa agaaccggta   1140
catggagtgt attatgaccc atcaaaagac ttaatagcag aaatacagaa gcaggggcaa   1200
ggccaatgga catatcaaat ttatcaagag ccatttaaaa atctgaaaac aggaaagtat   1260
gcaagaatga agggtgccca cactaatgat gtgaaacaat taacagaggc agtacaaaaa   1320
atagccacag aaagcatagt aatatgggga aagactccta aatttaaatt acccatacaa   1380
aaggaaacat gggaagcatg gtggacagag tattggcaag ccacctggat tcctgagtgg   1440
gagtttgtca atacccctcc cttagtgaag ttatggtacc agttagagaa agaacccata   1500
ataggagcag aaactttcta tgtagatggg gcagccaata gggaaactaa attaggaaaa   1560
gcaggatatg taactgacag aggaagacaa aaagttgtcc ccctaacgga cacaacaaat   1620
cagaagactg agttacaagc aattcatcta gctttgcagg attcgggatt agaagtaaac   1680
atagtgacag actcacaata tgcattggga atcattcaag cacaaccaga taagagtgaa   1740
tcagagttag tcagtcaaat aatagagcag ttaataaaaa aggaaaaagt ctacctggca   1800
tgggtaccag cacacaaagg aattggagga aatgaacaag tagataaatt ggtcagtgct   1860
ggaatcagga aagtactatt tttagatgga atagataagg cccaagaaga acatgagaaa   1920
tatcacagta attggagagc aatggctagt gattttaacc taccacctgt agtagcaaaa   1980
gaaatagtag ccagctgtga taaatgtcag ctaaaagggg aagccatgca tggacaagta   2040
gactgtagcc caggaatatg gcagctagat tgtacacatt tagaaggaaa agttatcttg   2100
gtagcagttc atgtagccag tggatatata gaagcagaag taattccagc agagacaggg   2160
caagaaacag catacttcct cttaaaatta gcaggaagat ggccagtaaa aacagtacat   2220
acagacaatg gcagcaattt caccagtact acagttaagg ccgcctgttg gtgggcgggg   2280
atcaagcagg aatttggcat tccctacaat ccccaaagtc aaggagtaat agaatctatg   2340
aataaagaat taaagaaaat tataggacag gtaagagatc aggctgaaca tcttaagaca   2400
gcagtacaaa tggcagtatt catccacaat tttaaaagaa aaggggggat tggggggtac   2460
agtgcagggg aaagaatagt agacataata gcaacagaca tacaaactaa agaattacaa   2520
aaacaaatta caaaaattca aaattttcgg gtttattaca gggacagcag agatccagtt   2580
tggaaaggac cagcaaagct cctctggaaa ggtgaagggg cagtagtaat acaagataat   2640
agtgacataa aagtagtgcc aagaagaaaa gcaaagatca tcagggatta tggaaaacag   2700
atggcaggtg atgattgtgt ggcaagtaga caggatgagg attaa                  2745
```

<210> SEQ ID NO 35
<211> LENGTH: 1586
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

<220> FEATURE:
<223> OTHER INFORMATION: DNA Fragment containing Rev, RRE and rabbit
beta globin poly A

<400> SEQUENCE: 35

```
tctagaatgg caggaagaag cggagacagc gacgaagagc tcatcagaac agtcagactc     60
atcaagcttc tctatcaaag caacccacct cccaatcccg aggggacccg acaggcccga    120
aggaatagaa gaagaaggtg gagagagaga cagagacaga tccattcgat tagtgaacgg    180
atccttggca cttatctggg acgatctgcg gagcctgtgc ctcttcagct accaccgctt    240
gagagactta ctcttgattg taacgaggat tgtggaactt ctgggacgca gggggtggga    300
agccctcaaa tattggtgga atctcctaca atattggagt caggagctaa agaatagagg    360
agctttgttc cttgggttct tgggagcagc aggaagcact atgggcgcag cgtcaatgac    420
gctgacggta caggccagac aattattgtc tggtatagtg cagcagcaga acaatttgct    480
gagggctatt gaggcgcaac agcatctgtt gcaactcaca gtctggggca tcaagcagct    540
ccaggcaaga atcctggctg tggaaagata cctaaaggat caacagctcc tagatctttt    600
tccctctgcc aaaaattatg gggacatcat gaagcccctt gagcatctga cttctggcta    660
ataaaggaaa tttattttca ttgcaatagt gtgttggaat tttttgtgtc tctcactcgg    720
aaggacatat gggagggcaa atcatttaaa acatcagaat gagtatttgg tttagagttt    780
ggcaacatat gccatatgct ggctgccatg aacaaaggtg gctataaaga ggtcatcagt    840
atatgaaaca gccccctgct gtccattcct tattccatag aaaagccttg acttgaggtt    900
agattttttt tatattttgt tttgtgttat ttttttcttt aacatcccta aaattttcct    960
tacatgtttt actagccaga tttttcctcc tctcctgact actcccagtc atagctgtcc   1020
ctcttctctt atgaagatcc ctcgacctgc agcccaagct tggcgtaatc atggtcatag   1080
ctgtttcctg tgtgaaattg ttatccgctc acaattccac acaacatacg agccggaagc   1140
ataaagtgta aagcctgggg tgcctaatga gtgagctaac tcacattaat tgcgttgcgc   1200
tcactgcccg ctttccagtc gggaaacctg tcgtgccagc ggatccgcat ctcaattagt   1260
cagcaaccat agtcccgccc ctaactccgc ccatcccgcc cctaactccg cccagttccg   1320
cccattctcc gccccatggc tgactaattt tttttattta tgcagaggcc gaggccgcct   1380
cggcctctga gctattccag aagtagtgag gaggcttttt tggaggccta ggcttttgca   1440
aaaagctaac ttgtttattg cagcttataa tggttacaaa taaagcaata gcatcacaaa   1500
tttcacaaat aaagcatttt tttcactgca ttctagttgt ggtttgtcca aactcatcaa   1560
tgtatcttat cagcggccgc cccggg                                        1586
```

<210> SEQ ID NO 36
<211> LENGTH: 1614
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA fragment containing the CAG
enhancer/promoter/intron sequence

<400> SEQUENCE: 36

```
acgcgttagt tattaatagt aatcaattac ggggtcatta gttcatagcc catatatgga     60
gttccgcgtt acataactta cggtaaatgg cccgcctggc tgaccgccca acgacccccg    120
cccattgacg tcaataatga cgtatgttcc catagtaacg ccaataggga ctttccattg    180
acgtcaatgg gtggactatt tacggtaaac tgcccacttg gcagtacatc aagtgtatca    240
```

```
tatgccaagt acgcccccta ttgacgtcaa tgacggtaaa tggcccgcct ggcattatgc    300

ccagtacatg accttatggg actttcctac ttggcagtac atctacgtat tagtcatcgc    360

tattaccatg gtcgaggtg  agccccacgt tctgcttcac tctccccatc tcccccccct    420

ccccaccccc aattttgtat ttatttattt tttaattatt ttgtgcagcg atgggggcgg    480

gggggggggg ggcgcgcgcc aggcggggcg gggcggggcg aggggcgggg cggggcgagg    540

cggagaggtg cggcggcagc caatcagagc ggcgcgctcc gaaagtttcc ttttatggcg    600

aggcggcggc ggcggcggcc ctataaaaag cgaagcgcgc ggcgggcggg agtcgctgcg    660

ttgccttcgc cccgtgcccc gctccgcgcc gcctcgcgcc gcccgccccg gctctgactg    720

accgcgttac tcccacaggt gagcgggcgg gacggccctt ctcctccggg ctgtaattag    780

cgcttggttt aatgacggct cgtttctttt ctgtggctgc gtgaaagcct taaagggctc    840

cgggagggcc ctttgtgcgg gggggagcgg ctcggggggt gcgtgcgtgt gtgtgtgcgt    900

ggggagcgcc gcgtgcggcc cgcgctgccc ggcggctgtg agcgctgcgg gcgcggcgcg    960

gggctttgtg cgctccgcgt gtgcgcgagg ggagcgcggc cgggggcggt gccccgcggt   1020

gcgggggggc tgcgagggga acaaaggctg cgtgcggggt gtgtgcgtgg gggggtgagc   1080

agggggtgtg ggcgcggcgg tcgggctgta accccccct  gcacccccct ccccgagttg   1140

ctgagcacgg cccggcttcg ggtgcggggc tccgtgcggg gcgtggcgcg gggctcgccg   1200

tgccgggcgg ggggtggcgg caggtggggg tgccgggcgg ggcggggccg cctcgggccg   1260

gggagggctc gggggagggg cgcggcggcc ccggagcgcc ggcggctgtc gaggcgcggc   1320

gagccgcagc cattgccttt tatggtaatc gtgcgagagg gcgcagggac ttcctttgtc   1380

ccaaatctgg cggagccgaa atctgggagg cgccgccgca ccccctctag cgggcgcggg   1440

cgaagcggtg cggcgccggc aggaaggaaa tgggcgggga gggccttcgt gcgtcgccgc   1500

gccgccgtcc ccttctccat ctccagcctc ggggctgccg caggggggacg gctgccttcg   1560

gggggacgg  ggcagggcgg ggttcggctt ctggcgtgtg accggcggga attc         1614
```

<210> SEQ ID NO 37
<211> LENGTH: 1531
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DNA fragment containing VSV-G

<400> SEQUENCE: 37

```
gaattcatga agtgcctttt gtacttagcc tttttattca ttggggtgaa ttgcaagttc     60

accatagttt ttccacacaa ccaaaaagga aactggaaaa atgttccttc taattaccat    120

tattgcccgt caagctcaga tttaaattgg cataatgact taataggcac agccttacaa    180

gtcaaaatgc ccaagagtca caaggctatt caagcagacg gttggatgtg tcatgcttcc    240

aaatgggtca ctacttgtga tttccgctgg tatggaccga agtatataac acattccatc    300

cgatccttca ctccatctgt agaacaatgc aaggaaagca ttgaacaaac gaaacaagga    360

acttggctga atccaggctt ccctcctcaa agttgtggat atgcaactgt gacggatgcc    420

gaagcagtga ttgtccaggt gactcctcac catgtgctgg ttgatgaata cacaggagaa    480

tgggttgatt cacagttcat caacggaaaa tgcagcaatt acatatgccc cactgtccat    540

aactctacaa cctggcattc tgactataag gtcaaagggc tatgtgattc taacctcatt    600

tccatggaca tcaccttctt ctcagaggac ggagagctat catccctggg aaaggagggc    660

acagggttca gaagtaacta ctttgcttat gaaactggag gcaaggcctg caaaatgcaa    720
```

```
tactgcaagc attggggagt cagactccca tcaggtgtct ggttcgagat ggctgataag    780

gatctctttg ctgcagccag attccctgaa tgcccagaag ggtcaagtat ctctgctcca    840

tctcagacct cagtggatgt aagtctaatt caggacgttg agaggatctt ggattattcc    900

ctctgccaag aaacctggag caaaatcaga gcgggtcttc caatctctcc agtggatctc    960

agctatcttg ctcctaaaaa cccaggaacc ggtcctgctt tcaccataat caatggtacc   1020

ctaaaatact ttgagaccag atacatcaga gtcgatattg ctgctccaat cctctcaaga   1080

atggtcggaa tgatcagtgg aactaccaca gaaagggaac tgtgggatga ctgggcacca   1140

tatgaagacg tggaaattgg acccaatgga gttctgagga ccagttcagg atataagttt   1200

cctttataca tgattggaca tggtatgttg gactccgatc ttcatcttag ctcaaaggct   1260

caggtgttcg aacatcctca cattcaagac gctgcttcgc aacttcctga tgatgagagt   1320

ttattttttg gtgatactgg gctatccaaa aatccaatcg agcttgtaga aggttggttc   1380

agtagttgga aaagctctat tgcctctttt ttctttatca tagggttaat cattggacta   1440

ttcttggttc tccgagttgg tatccatctt tgcattaaat taaagcacac caagaaaaga   1500

cagatttata cagacataga gatgagaatt c                                  1531
```

```
<210> SEQ ID NO 38
<211> LENGTH: 351
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Rev- RSV promoter- Transcription

<400> SEQUENCE: 38

atggcaggaa gaagcggaga cagcgacgaa gaactcctca aggcagtcag actcatcaag     60

tttctctatc aaagcaaccc acctcccaat cccgagggga cccgacaggc ccgaaggaat    120

agaagaagaa ggtggagaga gagacagaga cagatccatt cgattagtga acggatcctt    180

agcacttatc tgggacgatc tgcggagcct gtgcctcttc agctaccacc gcttgagaga    240

cttactcttg attgtaacga ggattgtgga acttctggga cgcagggggt gggaagccct    300

caaatattgg tggaatctcc tacaatattg gagtcaggag ctaaagaata g             351
```

```
<210> SEQ ID NO 39
<211> LENGTH: 351
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Rev- HIV Rev- Nuclear export and stabilize
      viral mRNA

<400> SEQUENCE: 39

atggcaggaa gaagcggaga cagcgacgaa gaactcctca aggcagtcag actcatcaag     60

tttctctatc aaagcaaccc acctcccaat cccgagggga cccgacaggc ccgaaggaat    120

agaagaagaa ggtggagaga gagacagaga cagatccatt cgattagtga acggatcctt    180

agcacttatc tgggacgatc tgcggagcct gtgcctcttc agctaccacc gcttgagaga    240

cttactcttg attgtaacga ggattgtgga acttctggga cgcagggggt gggaagccct    300

caaatattgg tggaatctcc tacaatattg gagtcaggag ctaaagaata g             351
```

```
<210> SEQ ID NO 40
<211> LENGTH: 884
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
```

<220> FEATURE:
<223> OTHER INFORMATION: RSV promoter and HIV Rev

<400> SEQUENCE: 40

```
caattgcgat gtacgggcca gatatacgcg tatctgaggg gactagggtg tgtttaggcg      60
aaaagcgggg cttcggttgt acgcggttag gagtcccctc aggatatagt agtttcgctt     120
ttgcataggg agggggaaat gtagtcttat gcaatacact tgtagtcttg caacatggta     180
acgatgagtt agcaacatgc cttacaagga gagaaaaagc accgtgcatg ccgattggtg     240
gaagtaaggt ggtacgatcg tgccttatta ggaaggcaac agacaggtct gacatggatt     300
ggacgaacca ctgaattccg cattgcagag ataattgtat ttaagtgcct agctcgatac     360
aataaacgcc atttgaccat tcaccacatt ggtgtgcacc tccaagctcg agctcgttta     420
gtgaaccgtc agatcgcctg gagacgccat ccacgctgtt ttgacctcca tagaagacac     480
cgggaccgat ccagcctccc ctcgaagcta gcgattaggc atctcctatg gcaggaagaa     540
gcggagacag cgacgaagaa ctcctcaagg cagtcagact catcaagttt ctctatcaaa     600
gcaacccacc tcccaatccc gaggggaccc gacaggcccg aaggaataga agaagaaggt     660
ggagagagag acagagacag atccattcga ttagtgaacg gatccttagc acttatctgg     720
gacgatctgc ggagcctgtg cctcttcagc taccaccgct tgagagactt actcttgatt     780
gtaacgagga ttgtggaact tctgggacgc agggggtggg aagccctcaa atattggtgg     840
aatctcctac aatattggag tcaggagcta aagaatagtc taga                      884
```

<210> SEQ ID NO 41
<211> LENGTH: 1104
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Elongation Factor-1 alpha (EF1-alpha) promoter

<400> SEQUENCE: 41

```
ccggtgccta gagaaggtgg cgcggggtaa actgggaaag tgatgtcgtg tactggctcc      60
gcctttttcc cgagggtggg ggagaaccgt atataagtgc agtagtcgcc gtgaacgttc     120
tttttcgcaa cgggtttgcc gccagaacac aggtaagtgc cgtgtgtggt tcccgcgggc     180
ctggcctctt tacgggttat ggcccttgcg tgccttgaat tacttccacg cccctggctg     240
cagtacgtga ttcttgatcc cgagcttcgg gttggaagtg ggtgggagag ttcgaggcct     300
tgcgcttaag gagccccttc gcctcgtgct tgagttgagg cctggcctgg gcgctggggc     360
cgccgcgtgc gaatctggtg gcaccttcgc gcctgtctcg ctgctttcga taagtctcta     420
gccatttaaa atttttgatg acctgctgcg acgctttttt tctggcaaga tagtcttgta     480
aatgcgggcc aagatctgca cactggtatt tcggtttttg gggccgcggg cggcgacggg     540
gcccgtgcgt cccagcgcac atgttcggcg aggcggggcc tgcgagcgcg gccaccgaga     600
atcggacggg ggtagtctca agctggccgg cctgctctgg tgcctggcct cgcgccgccg     660
tgtatcgccc cgccctgggc ggcaaggctg gcccggtcgg caccagttgc gtgagcggaa     720
agatggccgc ttcccggccc tgctgcaggg agctcaaaat ggaggacgcg gcgctcggga     780
gagcgggcgg gtgagtcacc cacacaaagg aaaagggcct ttccgtcctc agccgtcgct     840
tcatgtgact ccacggagta ccgggcgccg tccaggcacc tcgattagtt ctcgagcttt     900
tggagtacgt cgtctttagg ttggggggag gggttttatg cgatggagtt tccccacact     960
gagtgggtgg agactgaagt taggccagct tggcacttga tgtaattctc cttggaattt    1020
```

gcccttttg agtttggatc ttggttcatt ctcaagcctc agacagtggt tcaaagtttt 1080

tttcttccat ttcaggtgtc gtga 1104

<210> SEQ ID NO 42
<211> LENGTH: 511
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Promoter- PGK

<400> SEQUENCE: 42

ggggttgggg ttgcgccttt tccaaggcag ccctgggttt gcgcagggac gcggctgctc 60

tgggcgtggt tccgggaaac gcagcggcgc cgaccctggg tctcgcacat tcttcacgtc 120

cgttcgcagc gtcacccgga tcttcgccgc tacccttgtg ggcccccgg cgacgcttcc 180

tgctccgccc ctaagtcggg aaggttcctt gcggttcgcg gcgtgccgga cgtgacaaac 240

ggaagccgca cgtctcacta gtaccctcgc agacggacag cgccagggag caatggcagc 300

gcgccgaccg cgatgggctg tggccaatag cggctgctca gcagggcgcg ccgagagcag 360

cggccgggaa ggggcggtgc gggaggcggg gtgtggggcg gtagtgtggg ccctgttcct 420

gcccgcgcgg tgttccgcat tctgcaagcc tccggagcgc acgtcggcag tcggctccct 480

cgttgaccga atcaccgacc tctctcccca g 511

<210> SEQ ID NO 43
<211> LENGTH: 1162
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Promoter- UbC

<400> SEQUENCE: 43

gcgccgggtt ttggcgcctc ccgcgggcgc ccccctcctc acggcgagcg ctgccacgtc 60

agacgaaggg cgcaggagcg ttcctgatcc ttccgcccgg acgctcagga cagcggcccg 120

ctgctcataa gactcggcct tagaacccca gtatcagcag aaggacattt taggacggga 180

cttgggtgac tctagggcac tggttttctt tccagagagc ggaacaggcg aggaaaagta 240

gtcccttctc ggcgattctg cggagggatc tccgtggggc ggtgaacgcc gatgattata 300

taaggacgcg ccgggtgtgg cacagctagt tccgtcgcag ccgggatttg ggtcgcggtt 360

cttgtttgtg gatcgctgtg atcgtcactt ggtgagttgc gggctgctgg gctggccggg 420

gctttcgtgg ccgccgggcc gctcggtggg acggaagcgt gtggagagac cgccaagggc 480

tgtagtctgg gtccgcgagc aaggttgccc tgaactgggg gttgggggga gcgcacaaaa 540

tggcggctgt tcccgagtct tgaatggaag acgcttgtaa ggcgggctgt gaggtcgttg 600

aaacaaggtg gggggcatgg tgggcggcaa gaacccaagg tcttgaggcc ttcgctaatg 660

cgggaaagct cttattcggg tgagatgggc tggggcacca tctggggacc ctgacgtgaa 720

gtttgtcact gactggagaa ctcgggtttg tcgtctggtt gcgggggcgg cagttatgcg 780

gtgccgttgg gcagtgcacc cgtacctttg ggagcgcgcg cctcgtcgtg tcgtgacgtc 840

acccgttctg ttggcttata atgcagggtg gggccacctg ccggtaggtg tgcggtaggc 900

ttttctccgt cgcaggacgc agggttcggg cctagggtag gctctcctga atcgacaggc 960

gccggacctc tggtgagggg agggataagt gaggcgtcag tttctttggt cggttttatg 1020

tacctatctt cttaagtagc tgaagctccg gttttgaact atgcgctcgg ggttggcgag 1080 tgtgttttgt gaagtttttt aggcaccttt tgaaatgtaa tcatttgggt caatatgtaa 1140

ttttcagtgt tagactagta aa 1162

<210> SEQ ID NO 44
<211> LENGTH: 120
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Poly A- SV40

<400> SEQUENCE: 44

gtttattgca gcttataatg gttacaaata aagcaatagc atcacaaatt tcacaaataa 60

agcatttttt tcactgcatt ctagttgtgg tttgtccaaa ctcatcaatg tatcttatca 120

<210> SEQ ID NO 45
<211> LENGTH: 227
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Poly A- bGH

<400> SEQUENCE: 45

gactgtgcct tctagttgcc agccatctgt tgtttgcccc tcccccgtgc cttccttgac 60

cctggaaggt gccactccca ctgtcctttc ctaataaaat gaggaaattg catcgcattg 120

tctgagtagg tgtcattcta ttctgggggg tggggtgggg caggacagca agggggagga 180

ttgggaagac aatagcaggc atgctgggga tgcggtgggc tctatgg 227

<210> SEQ ID NO 46
<211> LENGTH: 1695
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- RD114

<400> SEQUENCE: 46

atgaaactcc caacaggaat ggtcatttta tgtagcctaa taatagttcg ggcagggttt 60

gacgaccccc gcaaggctat cgcattagta caaaaacaac atggtaaacc atgcgaatgc 120

agcggagggc aggtatccga ggccccaccg aactccatcc aacaggtaac ttgcccaggc 180

aagacggcct acttaatgac caaccaaaaa tggaaatgca gagtcactcc aaaaaatctc 240

acccctagcg gggagaact ccagaactgc ccctgtaaca ctttccagga ctcgatgcac 300

agttcttgtt atactgaata ccggcaatgc agggcgaata ataagacata ctacacggcc 360

accttgctta aaatacggtc tgggagcctc aacgaggtac agatattaca aaaccccaat 420

cagctcctac agtccccttg taggggctct ataaatcagc ccgtttgctg gagtgccaca 480

gcccccatcc atatctccga tggtggagga cccctcgata ctaagagagt gtggacagtc 540

caaaaaaggc tagaacaaat tcataaggct atgcatcctg aacttcaata ccacccctta 600

gccctgccca aagtcagaga tgaccttagc cttgatgcac ggacttttga tatcctgaat 660

accactttta ggttactcca gatgtccaat tttagccttg cccaagattg ttggctctgt 720

ttaaaactag gtacccctac ccctcttgcg atacccactc cctctttaac ctactcccta 780

gcagactccc tagcgaatgc ctcctgtcag attatacctc ccctcttggt tcaaccgatg 840

cagttctcca actcgtcctg tttatcttcc cctttcatta acgatacgga acaaatagac 900

ttaggtgcag tcacctttac taactgcacc tctgtagcca atgtcagtag tcctttatgt 960

gccctaaacg ggtcagtctt cctctgtgga aataacatgg catacaccta tttaccccaa 1020

```
aactggacag gactttgcgt ccaagcctcc ctcctccccg acattgacat catcccgggg   1080

gatgagccag tccccattcc tgccattgat cattatatac atagacctaa acgagctgta   1140

cagttcatcc ctttactagc tggactggga atcaccgcag cattcaccac cggagctaca   1200

ggcctaggtg tctccgtcac ccagtataca aaattatccc atcagttaat atctgatgtc   1260

caagtcttat ccggtaccat acaagattta caagaccagg tagactcgtt agctgaagta   1320

gttctccaaa ataggagggg actggaccta ctaacggcag aacaaggagg aatttgttta   1380

gccttacaag aaaaatgctg tttttatgct aacaagtcag gaattgtgag aaacaaaata   1440

agaaccctac aagaagaatt acaaaaacgc agggaaagcc tggcatccaa ccctctctgg   1500

accgggctgc agggctttct tccgtacctc ctacctctcc tgggacccct actcaccctc   1560

ctactcatac taaccattgg gccatgcgtt ttcaatcgat tggtccaatt tgttaaagac   1620

aggatctcag tggtccaggc tctggttttg actcagcaat atcaccagct aaaacccata   1680

gagtacgagc catga                                                    1695
```

<210> SEQ ID NO 47
<211> LENGTH: 2013
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- GALV

<400> SEQUENCE: 47

```
atgcttctca cctcaagccc gcaccacctt cggcaccaga tgagtcctgg gagctggaaa     60

agactgatca tcctcttaag ctgcgtattc ggagacggca aaacgagtct gcagaataag    120

aacccccacc agcctgtgac cctcacctgg caggtactgt cccaaactgg ggacgttgtc    180

tgggacaaaa aggcagtcca gcccctttgg acttggtggc cctctcttac acctgatgta    240

tgtgccctgg cggccggtct tgagtcctgg gatatcccgg gatccgatgt atcgtcctct    300

aaaagagtta gacctcctga ttcagactat actgccgctt ataagcaaat cacctgggga    360

gccatagggt gcagctaccc tcgggctagg accaggatgg caaattcccc cttctacgtg    420

tgtccccgag ctggccgaac ccattcagaa gctaggaggt gtgggggct agaatcccta     480

tactgtaaag aatggagttg tgagaccacg ggtaccgttt attggcaacc caagtcctca    540

tgggacctca taactgtaaa atgggaccaa aatgtgaaat gggagcaaaa atttcaaaag    600

tgtgaacaaa ccggctggtg taacccccctc aagatagact tcacagaaaa aggaaaactc   660

tccagagatt ggataacgga aaaaacctgg gaattaaggt tctatgtata tggacaccca    720

ggcatacagt tgactatccg cttagaggtc actaacatgc cggttgtggc agtgggccca    780

gaccctgtcc ttgcggaaca gggacctcct agcaagcccc tcactctccc tctctcccca    840

cggaaagcgc cgcccacccc tctacccccg gcggctagtg agcaaacccc tgcggtgcat    900

ggagaaactg ttaccctaaa ctctccgcct cccaccagtg gcgaccgact ctttggcctt    960

gtgcaggggg ccttcctaac cttgaatgct accaacccag ggccactaag tcttgctgg    1020

ctctgtttgg gcatgagccc ccttattat gaagggatag cctcttcagg agaggtcgct    1080

tatacctcca accatacccg atgccactgg ggggcccaag gaaagcttac cctcactgag   1140

gtctccggac tcgggtcatg catagggaag gtgcctctta cccatcaaca tctttgcaac   1200

cagaccttac ccatcaattc ctctaaaaac catcagtatc tgctcccctc aaaccatagc   1260

tggtgggcct gcagcactgg cctcaccccc tgcctctcca cctcagtttt taatcagtct   1320

aaagacttct gtgtccaggt ccagctgatc ccccgcatct attaccattc tgaagaaacc   1380
```

```
ttgttacaag cctatgacaa atcacccccc aggtttaaaa gagagcctgc ctcacttacc   1440

ctagctgtct tcctggggtt agggattgcg gcaggtatag gtactggctc aaccgcccta   1500

attaaagggc ccatagacct ccagcaaggc ctaaccagcc tccaaatcgc cattgacgct   1560

gacctccggg cccttcagga ctcaatcagc aagctagagg actcactgac ttccctatct   1620

gaggtagtac tccaaaatag gagaggcctt gacttactat tccttaaaga aggaggcctc   1680

tgcgcggccc taaaagaaga gtgctgtttt tatgtagacc actcaggtgc agtacgagac   1740

tccatgaaaa aacttaaaga aagactagat aaaagacagt tagagcgcca gaaaaaccaa   1800

aactggtatg aagggtggtt caataactcc ccttggttta ctaccctact atcaaccatc   1860

gctgggcccc tattgctcct ccttttgtta ctcactcttg ggccctgcat catcaataaa   1920

ttaatccaat tcatcaatga taggataagt gcagtcaaaa ttttagtcct tagacagaaa   1980

tatcagaccc tagataacga ggaaaacctt taa                                2013
```

<210> SEQ ID NO 48
<211> LENGTH: 1530
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- FUG

<400> SEQUENCE: 48

```
atggttccgc aggttctttt gtttgtactc cttctgggtt tttcgttgtg tttcgggaag     60

ttccccattt acacgatacc agacgaactt ggtccctgga gccctattga catacaccat    120

ctcagctgtc caaataacct ggttgtggag gatgaaggat gtaccaacct gtccgagttc    180

tcctacatgg aactcaaagt gggatacatc tcagccatca aagtgaacgg gttcacttgc    240

acaggtgttg tgacagaggc agagacctac accaactttg ttggttatgt cacaaccaca    300

ttcaagagaa agcatttccg ccccacccca gacgcatgta gagccgcgta taactggaag    360

atggccggtg accccagata tgaagagtcc ctacacaatc catacccccga ctaccactgg    420

cttcgaactg taagaaccac caaagagtcc ctcattatca tatccccaag tgtgacagat    480

ttggacccat atgacaaatc ccttcactca agggtcttcc ctggcggaaa gtgctcagga    540

ataacggtgt cctctaccta ctgctcaact aaccatgatt acaccatttg gatgcccgag    600

aatccgagac caaggacacc ttgtgacatt tttaccaata gcagagggaa gagagcatcc    660

aacgggaaca agacttgcgg ctttgtggat gaaagaggcc tgtataagtc tctaaaagga    720

gcatgcaggc tcaagttatg tggagttctt ggacttagac ttatggatgg aacatgggtc    780

gcgatgcaaa catcagatga gaccaaatgg tgccctccag atcagttggt gaatttgcac    840

gactttcgct cagacgagat cgagcatctc gttgtggagg agttagttaa gaaaagagag    900

gaatgtctgg atgcattaga gtccatcatg accaccaagt cagtaagttt cagacgtctc    960

agtcacctga gaaaacttgt cccagggttt ggaaaagcat ataccatatt caacaaaacc   1020

ttgatggagg ctgatgctca ctacaagtca gtccggacct ggaatgagat catcccctca   1080

aaagggtgtt tgaaagttgg aggaaggtgc catcctcatg tgaacggggt gtttttcaat   1140

ggtataatat tagggcctga cgaccatgtc ctaatcccag agatgcaatc atccctcctc   1200

cagcaacata tggagttgtt ggaatcttca gttatccccc tgatgcaccc cctggcagac   1260

ccttctacag ttttcaaaga aggtgatgag gctgaggatt ttgttgaagt tcacctcccc   1320

gatgtgtaca aacagatctc aggggttgac ctgggtctcc cgaactgggg aaagtatgta   1380

ttgatgactg caggggccat gattggcctg gtgttgatat tttccctaat gacatggtgc   1440
```

```
agagttggta tccatctttg cattaaatta aagcacacca agaaaagaca gatttataca   1500

gacatagaga tgaaccgact tggaaagtaa                                    1530


<210> SEQ ID NO 49
<211> LENGTH: 1497
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- LCMV

<400> SEQUENCE: 49

atgggtcaga ttgtgacaat gtttgaggct ctgcctcaca tcatcgatga ggtgatcaac     60

attgtcatta ttgtgcttat cgtgatcacg ggtatcaagg ctgtctacaa ttttgccacc    120

tgtgggatat tcgcattgat cagtttccta cttctggctg gcaggtcctg tggcatgtac    180

ggtcttaagg gacccgacat ttacaaagga gtttaccaat ttaagtcagt ggagtttgat    240

atgtcacatc tgaacctgac catgcccaac gcatgttcag ccaacaactc ccaccattac    300

atcagtatgg ggacttctgg actagaattg accttcacca atgattccat catcagtcac    360

aacttttgca atctgacctc tgccttcaac aaaaagacct ttgaccacac actcatgagt    420

atagtttcga gcctacacct cagtatcaga gggaactcca actataaggc agtatcctgc    480

gacttcaaca atggcataac catccaatac aacttgacat tctcagatcg acaaagtgct    540

cagagccagt gtagaacctt cagaggtaga gtcctagata tgtttagaac tgccttcggg    600

gggaaataca tgaggagtgg ctggggctgg acaggctcag atggcaagac cacctggtgt    660

agccagacga gttaccaata cctgattata caaaatagaa cctgggaaaa ccactgcaca    720

tatgcaggtc cttttgggat gtccaggatt ctcctttccc aagagaagac taagttcttc    780

actaggagac tagcgggcac attcacctgg actttgtcag actcttcagg ggtggagaat    840

ccaggtggtt attgcctgac caaatggatg attcttgctg cagagcttaa gtgtttcggg    900

aacacagcag ttgcgaaatg caatgtaaat catgatgccg aattctgtga catgctgcga    960

ctaattgact acaacaaggc tgctttgagt aagttcaaag aggacgtaga atctgccttg   1020

cacttattca aaacaacagt gaattctttg atttcagatc aactactgat gaggaaccac   1080

ttgagagatc tgatgggggt gccatattgc aattactcaa agttttggta cctagaacat   1140

gcaaagaccg gcgaaactag tgtccccaag tgctggcttg tcaccaatgg ttcttactta   1200

aatgagaccc acttcagtga tcaaatcgaa caggaagccg ataacatgat tacagagatg   1260

ttgaggaagg attacataaa gaggcagggg agtacccccc tagcattgat ggaccttctg   1320

atgttttcca catctgcata tctagtcagc atcttcctgc accttgtcaa aataccaaca   1380

cacaggcaca taaaaggtgg ctcatgtcca aagccacacc gattaaccaa caaaggaatt   1440

tgtagttgtg gtgcatttaa ggtgcctggt gtaaaaaccg tctggaaaag acgctga      1497


<210> SEQ ID NO 50
<211> LENGTH: 1692
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- FPV

<400> SEQUENCE: 50

atgaacactc aaatcctggt tttcgccctt gtggcagtca tccccacaaa tgcagacaaa     60

atttgtcttg gacatcatgc tgtatcaaat ggcaccaaag taaacacact cactgagaga    120

ggagtagaag ttgtcaatgc aacggaaaca gtggagcgga caaacatccc caaaatttgc    180
```

tcaaaaggga aaagaaccac tgatcttggc caatgcggac tgttagggac cattaccgga 240

ccacctcaat gcgaccaatt tctagaattt tcagctgatc taataatcga gagacgagaa 300

ggaaatgatg tttgttaccc ggggaagttt gttaatgaag aggcattgcg acaaatcctc 360

agaggatcag gtgggattga caaagaaaca atgggattca catatagtgg aataaggacc 420

aacggaacaa ctagtgcatg tagaagatca gggtcttcat tctatgcaga aatggagtgg 480

ctcctgtcaa atacagacaa tgctgctttc ccacaaatga caaaatcata caaaaacaca 540

aggagagaat cagctctgat agtctgggga atccaccatt caggatcaac caccgaacag 600

accaaactat atgggagtgg aaataaactg ataacagtcg ggagttccaa atatcatcaa 660

tcttttgtgc cgagtccagg aacacgaccg cagataaatg gccagtccgg acggattgat 720

tttcattggt tgatcttgga tcccaatgat acagttactt ttagtttcaa tggggctttc 780

atagctccaa atcgtgccag cttcttgagg ggaaagtcca tggggatcca gagcgatgtg 840

caggttgatg ccaattgcga aggggaatgc taccacagtg gagggactat aacaagcaga 900

ttgccttttc aaaacatcaa tagcagagca gttggcaaat gcccaagata tgtaaaacag 960

gaaagtttat tattggcaac tgggatgaag aacgttcccg aaccttccaa aaaaaggaaa 1020

aaaagaggcc tgtttggcgc tatagcaggg tttattgaaa atggttggga aggtctggtc 1080

gacgggtggt acggtttcag gcatcagaat gcacaaggag aaggaactgc agcagactac 1140

aaaagcaccc aatcggcaat tgatcagata accggaaagt taaatagact cattgagaaa 1200

accaaccagc aatttgagct aatagataat gaattcactg aggtggaaaa gcagattggc 1260

aatttaatta actggaccaa agactccatc acagaagtat ggtcttacaa tgctgaactt 1320

cttgtggcaa tggaaaacca gcacactatt gatttggctg attcagagat gaacaagctg 1380

tatgagcgag tgaggaaaca attaagggaa aatgctgaag aggatggcac tggttgcttt 1440

gaaatttttc ataaatgtga cgatgattgt atggctagta taaggaacaa tacttatgat 1500

cacagcaaat acagagaaga agcgatgcaa aatagaatac aaattgaccc agtcaaattg 1560

agtagtggct acaaagatgt gatactttgg tttagcttcg ggcatcatg cttttgctt 1620

cttgccattg caatgggcct tgttttcata tgtgtgaaga acggaaacat gcggtgcact 1680

atttgtatat aa 1692

<210> SEQ ID NO 51
<211> LENGTH: 1266
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- RRV

<400> SEQUENCE: 51

agtgtaacag agcactttaa tgtgtataag gctactagac catacctagc acattgcgcc 60

gattgcgggg acgggtactt ctgctatagc ccagttgcta tcgaggagat ccgagatgag 120

gcgtctgatg gcatgcttaa gatccaagtc tccgcccaaa taggtctgga caaggcaggc 180

acccacgccc acacgaagct ccgatatatg gctggtcatg atgttcagga atctaagaga 240

gattccttga gggtgtacac gtccgcagcg tgctccatac atgggacgat gggacacttc 300

atcgtcgcac actgtccacc aggcgactac ctcaaggttt cgttcgagga cgcagattcg 360

cacgtgaagg catgtaaggt ccaatacaag cacaatccat tgccggtggg tagagagaag 420

ttcgtggtta gaccacactt tggcgtagag ctgccatgca cctcatacca gctgacaacg 480

gctcccaccg acgaggagat tgacatgcat acaccgccag atataccgga tcgcaccctg 540

```
ctatcacaga cggcgggcaa cgtcaaaata acagcaggcg gcaggactat caggtacaac    600

tgtacctgcg gccgtgacaa cgtaggcact accagtactg acaagaccat caacacatgc    660

aagattgacc aatgccatgc tgccgtcacc agccatgaca aatggcaatt tacctctcca    720

tttgttccca gggctgatca gacagctagg aaaggcaagg tacacgttcc gttccctctg    780

actaacgtca cctgccgagt gccgttggct cgagcgccgg atgccaccta tggtaagaag    840

gaggtgaccc tgagattaca cccagatcat ccgacgctct tctcctatag gagtttagga    900

gccgaaccgc acccgtacga ggaatgggtt gacaagttct ctgagcgcat catcccagtg    960

acggaagaag ggattgagta ccagtggggc aacaacccgc cggtctgcct gtgggcgcaa   1020

ctgacgaccg agggcaaacc ccatggctgg ccacatgaaa tcattcagta ctattatgga   1080

ctataccccg ccgccactat tgccgcagta tccggggcga gtctgatggc cctcctaact   1140

ctggcggcca catgctgcat gctggccacc gcgaggagaa agtgcctaac accgtacgcc   1200

ctgacgccag gagcggtggt accgttgaca ctggggctgc tttgctgcgc accgagggcg   1260

aatgca                                                              1266
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<210> SEQ ID NO 52
<211> LENGTH: 1266
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- MLV 10A1

<400> SEQUENCE: 52

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agtgtaacag agcactttaa tgtgtataag gctactagac catacctagc acattgcgcc     60

gattgcgggg acgggtactt ctgctatagc ccagttgcta tcgaggagat ccgagatgag    120

gcgtctgatg gcatgcttaa gatccaagtc tccgcccaaa taggtctgga caaggcaggc    180

acccacgccc acacgaagct ccgatatatg gctggtcatg atgttcagga atctaagaga    240

gattccttga gggtgtacac gtccgcagcg tgctccatac atgggacgat gggacacttc    300

atcgtcgcac actgtccacc aggcgactac ctcaaggttt cgttcgagga cgcagattcg    360

cacgtgaagg catgtaaggt ccaatacaag cacaatccat tgccggtggg tagagagaag    420

ttcgtggtta gaccacactt tggcgtagag ctgccatgca cctcatacca gctgacaacg    480

gctcccaccg acgaggagat tgacatgcat acaccgccag atataccgga tcgcaccctg    540

ctatcacaga cggcgggcaa cgtcaaaata acagcaggcg gcaggactat caggtacaac    600

tgtacctgcg gccgtgacaa cgtaggcact accagtactg acaagaccat caacacatgc    660

aagattgacc aatgccatgc tgccgtcacc agccatgaca aatggcaatt tacctctcca    720

tttgttccca gggctgatca gacagctagg aaaggcaagg tacacgttcc gttccctctg    780

actaacgtca cctgccgagt gccgttggct cgagcgccgg atgccaccta tggtaagaag    840

gaggtgaccc tgagattaca cccagatcat ccgacgctct tctcctatag gagtttagga    900

gccgaaccgc acccgtacga ggaatgggtt gacaagttct ctgagcgcat catcccagtg    960

acggaagaag ggattgagta ccagtggggc aacaacccgc cggtctgcct gtgggcgcaa   1020

ctgacgaccg agggcaaacc ccatggctgg ccacatgaaa tcattcagta ctattatgga   1080

ctataccccg ccgccactat tgccgcagta tccggggcga gtctgatggc cctcctaact   1140

ctggcggcca catgctgcat gctggccacc gcgaggagaa agtgcctaac accgtacgcc   1200

ctgacgccag gagcggtggt accgttgaca ctggggctgc tttgctgcgc accgagggcg   1260

aatgca                                                              1266
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<210> SEQ ID NO 53
<211> LENGTH: 2030
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope- Ebola

<400> SEQUENCE: 53

atgggtgtta caggaatatt gcagttacct cgtgatcgat tcaagaggac atcattcttt 60

ctttgggtaa ttatcctttt ccaaagaaca ttttccatcc cacttggagt catccacaat 120

agcacattac aggttagtga tgtcgacaaa ctggtttgcc gtgacaaact gtcatccaca 180

aatcaattga gatcagttgg actgaatctc gaagggaatg gagtggcaac tgacgtgcca 240

tctgcaacta aaagatgggg cttcaggtcc ggtgtcccac caaaggtggt caattatgaa 300

gctggtgaat gggctgaaaa ctgctacaat cttgaaatca aaaaacctga cgggagtgag 360

tgtctaccag cagcgccaga cgggattcgg ggcttccccc ggtgccggta tgtgcacaaa 420

gtatcaggaa cgggaccgtg tgccggagac tttgccttcc acaaagaggg tgctttcttc 480

ctgtatgacc gacttgcttc cacagttatc taccgaggaa cgactttcgc tgaaggtgtc 540

gttgcatttc tgatactgcc ccaagctaag aaggacttct tcagctcaca ccccttgaga 600

gagccggtca atgcaacgga ggacccgtct agtggctact attctaccac aattagatat 660

caagctaccg gttttggaac caatgagaca gagtatttgt tcgaggttga caatttgacc 720

tacgtccaac ttgaatcaag attcacacca cagtttctgc tccagctgaa tgagacaata 780

tatacaagtg ggaaaaggag caataccacg ggaaaactaa tttggaaggt caaccccgaa 840

attgatacaa caatcgggga gtgggccttc tgggaaacta aaaaaacctc actagaaaaa 900

ttcgcagtga agagttgtct ttcacagctg tatcaaacag agccaaaaac atcagtggtc 960

agagtccggc gcgaacttct tccgacccag ggaccaacac aacaactgaa gaccacaaaa 1020

tcatggcttc agaaaattcc tctgcaatgg ttcaagtgca cagtcaagga agggaagctg 1080

cagtgtcgca tctgacaacc cttgccacaa tctccacgag tcctcaaccc cccacaacca 1140

aaccaggtcc ggacaacagc acccacaata cacccgtgta taaacttgac atctctgagg 1200

caactcaagt tgaacaacat caccgcagaa cagacaacga cagcacagcc tccgacactc 1260

cccccgccac gaccgcagcc ggacccctaa aagcagagaa caccaacacg agcaagggta 1320

ccgacctcct ggaccccgcc accacaacaa gtccccaaaa ccacagcgag accgctggca 1380

acaacaacac tcatcaccaa gataccggag aagagagtgc cagcagcggg aagctaggct 1440

taattaccaa tactattgct ggagtcgcag gactgatcac aggcgggagg agagctcgaa 1500

gagaagcaat tgtcaatgct caacccaaat gcaaccctaa tttacattac tggactactc 1560

aggatgaagg tgctgcaatc ggactggcct ggataccata tttcgggcca gcagccgagg 1620

gaatttacat agaggggctg atgcacaatc aagatggttt aatctgtggg ttgagacagc 1680

tggccaacga gacgactcaa gctcttcaac tgttcctgag agccacaacc gagctacgca 1740

ccttttcaat cctcaaccgt aaggcaattg atttcttgct gcagcgatgg ggcggcacat 1800

gccacatttt gggaccggac tgctgtatcg aaccacatga ttggaccaag aacataacag 1860

acaaaattga tcagattatt catgattttg ttgataaaac ccttccggac caggggggaca 1920

atgacaattg gtggacagga tggagacaat ggataccggc aggtattgga gttacaggcg 1980

ttataattgc agttatcgct ttattctgta tatgcaaatt tgtcttttag 2030

<210> SEQ ID NO 54
<211> LENGTH: 237
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Polymerase III shRNA promoters- U6 promoter

<400> SEQUENCE: 54

tttcccatga ttccttcata tttgcatata cgatacaagg ctgttagaga gataattgga 60

attaatttga ctgtaaacac aaagatatta gtacaaaata cgtgacgtag aaagtaataa 120

tttcttgggt agtttgcagt tttaaaatta tgttttaaaa tggactatca tatgcttacc 180

gtaacttgaa agtatttcga tttcttggct ttatatatct tgtggaaagg acgaaac 237

<210> SEQ ID NO 55
<211> LENGTH: 243
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Polymerase III shRNA promoters- 7SK promoter

<400> SEQUENCE: 55

ctgcagtatt tagcatgccc cacccatctg caaggcattc tggatagtgt caaaacagcc 60

ggaaatcaag tccgtttatc tcaaacttta gcattttggg aataaatgat atttgctatg 120

ctggttaaat tagattttag ttaaatttcc tgctgaagct ctagtacgat aagcaacttg 180

acctaagtgt aaagttgaga tttccttcag gtttatatag cttgtgcgcc gcctggctac 240

ctc 243

<210> SEQ ID NO 56
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: FDPS target sequence #1

<400> SEQUENCE: 56

gtcctggagt acaatgccat t 21

<210> SEQ ID NO 57
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: FDPS target sequence #2

<400> SEQUENCE: 57

gcaggatttc gttcagcact t 21

<210> SEQ ID NO 58
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: FDPS target sequence #3

<400> SEQUENCE: 58

gccatgtaca tggcaggaat t 21

<210> SEQ ID NO 59
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: FDPS target sequence #4

<400> SEQUENCE: 59

gcagaaggag gctgagaaag t                                               21


<210> SEQ ID NO 60
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Non-targeting sequence

<400> SEQUENCE: 60

gccgctttgt aggatagagc tcgagctcta tcctacaaag cggctttttt                49


<210> SEQ ID NO 61
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Forward primer

<400> SEQUENCE: 61

aggaattgat ggcgagaagg                                                 20


<210> SEQ ID NO 62
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Reverse primer

<400> SEQUENCE: 62

cccaaagagg tcaaggtaat ca                                              22


<210> SEQ ID NO 63
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Forward primer

<400> SEQUENCE: 63

agcgcggcta cagcttca                                                   18


<210> SEQ ID NO 64
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Reverse primer

<400> SEQUENCE: 64

ggcgacgtag cacagcttct                                                 20


<210> SEQ ID NO 65
<211> LENGTH: 130
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Left Inverted Terminal Repeat (Left ITR)
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<400> SEQUENCE: 65

cctgcaggca gctgcgcgct cgctcgctca ctgaggccgc ccgggcgtcg ggcgaccttt 60

ggtcgcccgg cctcagtgag cgagcgagcg cgcagagagg gagtggccaa ctccatcact 120

aggggttcct 130

<210> SEQ ID NO 66
<211> LENGTH: 151
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Right Inverted Terminal Repeat (Right ITR)

<400> SEQUENCE: 66

gagcggccgc aggaacccct agtgatggag ttggccactc cctctctgcg cgctcgctcg 60

ctcactgagg ccgggcgacc aaaggtcgcc cgacgcccgg gctttgcccg ggcggcctca 120

gtgagcgagc gagcgcgcag ctgcctgcag g 151

<210> SEQ ID NO 67
<211> LENGTH: 1227
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper plasmid without REV

<400> SEQUENCE: 67

tctagaagga gctttgttcc ttgggttctt gggagcagca ggaagcacta tgggcgcagc 60

gtcaatgacg ctgacggtac aggccagaca attattgtct ggtatagtgc agcagcagaa 120

caatttgctg agggctattg aggcgcaaca gcatctgttg caactcacag tctggggcat 180

caagcagctc caggcaagaa tcctggctgt ggaaagatac ctaaaggatc aacagctcct 240

agatcttttt ccctctgcca aaaattatgg ggacatcatg aagccccttg agcatctgac 300

ttctggctaa taaaggaaat ttattttcat tgcaatagtg tgttggaatt ttttgtgtct 360

ctcactcgga aggacatatg ggagggcaaa tcatttaaaa catcagaatg agtatttggt 420

ttagagtttg gcaacatatg ccatatgctg gctgccatga acaaaggtgg ctataaagag 480

gtcatcagta tatgaaacag ccccctgctg tccattcctt attccataga aaagccttga 540

cttgaggtta gatttttttt atattttgtt ttgtgttatt tttttcttta acatccctaa 600

aattttcctt acatgtttta ctagccagat ttttcctcct ctcctgacta ctcccagtca 660

tagctgtccc tcttctctta tgaagatccc tcgacctgca gcccaagctt ggcgtaatca 720

tggtcatagc tgtttcctgt gtgaaattgt tatccgctca caattccaca caacatacga 780

gccggaagca taaagtgtaa agcctggggt gcctaatgag tgagctaact cacattaatt 840

gcgttgcgct cactgcccgc tttccagtcg ggaaacctgt cgtgccagcg gatccgcatc 900

tcaattagtc agcaaccata gtcccgcccc taactccgcc catcccgccc ctaactccgc 960

ccagttccgc ccattctccg ccccatggct gactaatttt ttttatttat gcagaggccg 1020

aggccgcctc ggcctctgag ctattccaga agtagtgagg aggctttttt ggaggcctag 1080

gcttttgcaa aaagctaact tgtttattgc agcttataat ggttacaaat aaagcaatag 1140

catcacaaat ttcacaaata aagcattttt ttcactgcat tctagttgtg gtttgtccaa 1200

actcatcaat gtatcttatc acccggg 1227

What is claimed is:

1. A method of treating a cancer in a subject in need thereof, the method comprising administering or having administered a therapeutically-effective amount of an immunotherapy-based composition to the subject, wherein the immunotherapy-based composition is obtained from an immunotherapy-based viral delivery system comprising:

(i) at least one helper plasmid comprising DNA sequences for expressing a functional protein derived from each of a gag, pol, and rev gene;

(ii) an envelope plasmid comprising a DNA sequence for expressing an envelope protein capable of infecting a target cell; and

(iii) a therapeutic vector comprising:

at least one encoded shRNA that, when expressed, inhibits production of farnesyl diphosphate synthase (FDPS) or,

at least one encoded microRNA that, when expressed, inhibits production of farnesyl diphosphate synthase (FDPS).

2. The method of claim 1, wherein the at least one helper plasmid comprises first and second helper plasmids, wherein the first help helper plasmid comprises DNA sequences for expressing such proteins derived from the gag and pol genes, and the second helper plasmid comprises a DNA sequence for expressing such protein derived from the rev gene.

3. The method of claim 1, wherein the immunotherapy-based viral delivery system is transfected into a packaging cell prior to administering the immunotherapy-based composition to the subject.

4. The method of claim 3, wherein the packaging cell produces the immunotherapy-based composition following transfection with the immunotherapy-based viral delivery system.

5. The method of claim 1, wherein the shRNA comprises a sequence having at least 80 percent identity with SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4; or wherein the microRNA comprises a sequence having at least 80 percent identity with SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10.

6. The method of claim 1, wherein the target cell comprises a cancer cell.

7. The method of claim 6, wherein the cancer cell is capable of activating a gamma delta T cell resident in the subject following infection of the cancer cell with the immunotherapy-based composition.

8. The method of claim 1, further comprising administering or having administered an effective amount of an aminobisphosphonate drug to the subject.

9. The method of claim 8, wherein the aminobisphosphonate drug is administered to the subject separately from the immunotherapy-based composition.

10. The method of claim 8, wherein the aminobisphosphonate drug is administered to the subject together with the immunotherapy-based composition.

11. A method of treating a cancer in a subject in need thereof, the method comprising:

(a) obtaining or having obtained an immunotherapy-based viral delivery system comprising:

(i) at least one helper plasmid comprising DNA sequences for expressing a functional protein derived from each of a gag, pol, and rev gene;

(ii) an envelope plasmid comprising a DNA sequence for expressing an envelope protein capable of infecting a target cell; and

(iii) a therapeutic vector comprising:

at least one encoded shRNA that, when expressed, inhibits production of farnesyl diphosphate synthase (FDPS); or,

at least one encoded microRNA that, when expressed, inhibits production of farnesyl diphosphate synthase (FDPS);

(b) transfecting or having transfected the immunotherapy-based viral delivery system into a packaging cell to produce an immunotherapy-based composition; and

(c) administering or having administered a therapeutically-effective amount of the immunotherapy-based composition to the subject.

12. The method of claim 11, wherein the at least one helper plasmid comprises first and second helper plasmids, wherein the first help helper plasmid comprises DNA sequences for expressing such proteins derived from the gag and pol genes, and the second helper plasmid comprises a DNA sequence for expressing such protein derived from the rev gene.

13. The method of claim 11, wherein the shRNA comprises a sequence having at least 80 percent identity with SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, or SEQ ID NO: 4; or wherein the microRNA comprises a sequence having at least 80 percent identity with SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, or SEQ ID NO: 10.

14. The method of claim 11, further comprising administering an effective amount of an aminobisphosphonate drug to the subject.

15. The method of claim 14, wherein the aminobisphosphonate drug is administered to the subject separately from the immunotherapy-based composition.

16. The method of claim 14, wherein the aminobisphosphonate drug is administered to the subject together with the immunotherapy-based composition.

* * * * *