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(54) **COMBINATION VECTORS AND METHODS FOR TREATING CANCER**

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None
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(57) **ABSTRACT**

A composition for treating cancer is disclosed. The composition includes a lentiviral particle and an aminobisphosphonate drug. The lentiviral particle is capable of infecting a target cell, such as a cancer cell, and includes an envelope protein optimized for targeting such target cell and a viral vector. The viral vector includes a small RNA optimized to target an FDPS mRNA sequence. The aminobisphosphonate drug includes zoledronic acid.

24 Claims, 11 Drawing Sheets

Specification includes a Sequence Listing.

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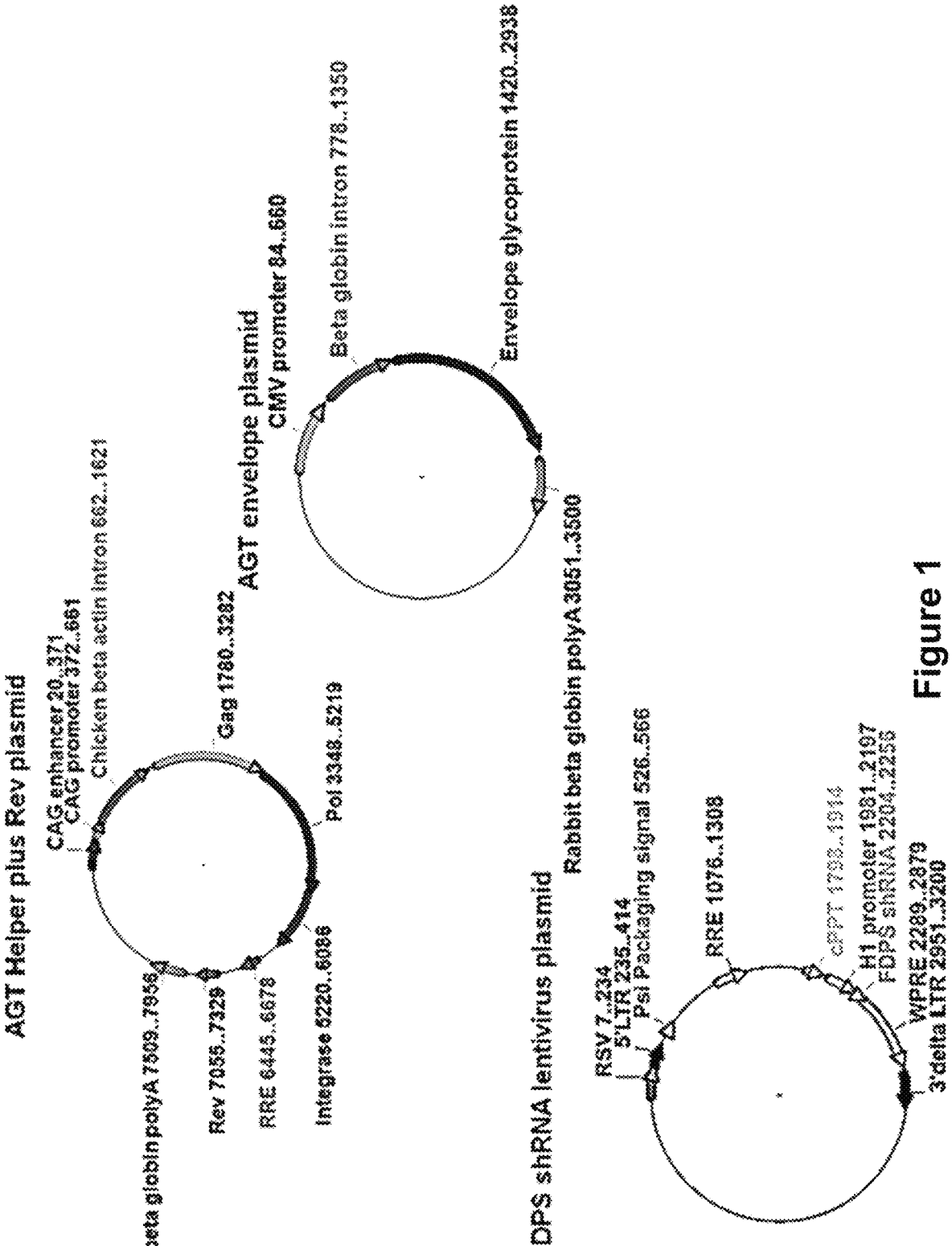
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Rev 7055..7329
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CMV promoter 84..660
Beta globin intron 778..1350
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Rabbit beta globin polyA 3051..3500
FDPS shRNA lentivirus plasmid
RSV 7..234
5'LTR 235..414
Psi Packaging signal 526..566
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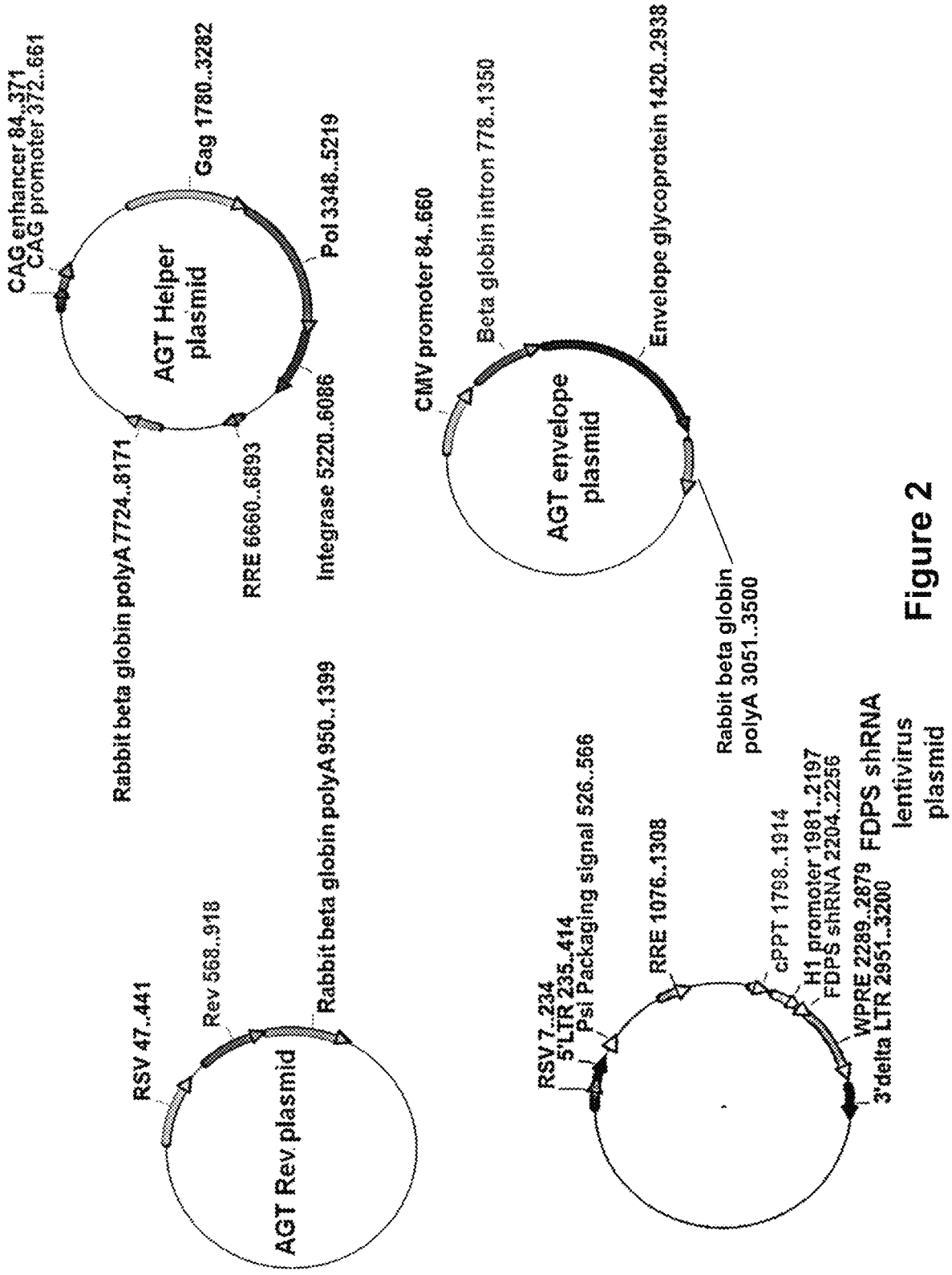
3'delta LTR 2951..3200

Figure 1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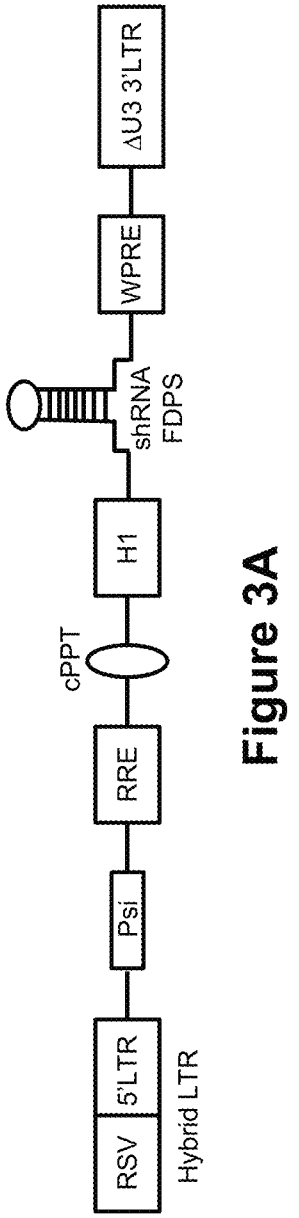

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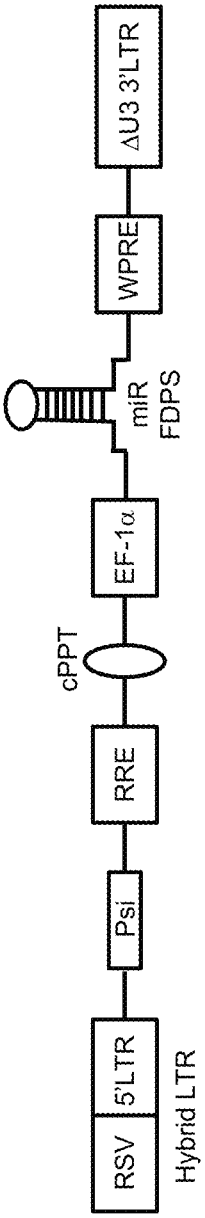

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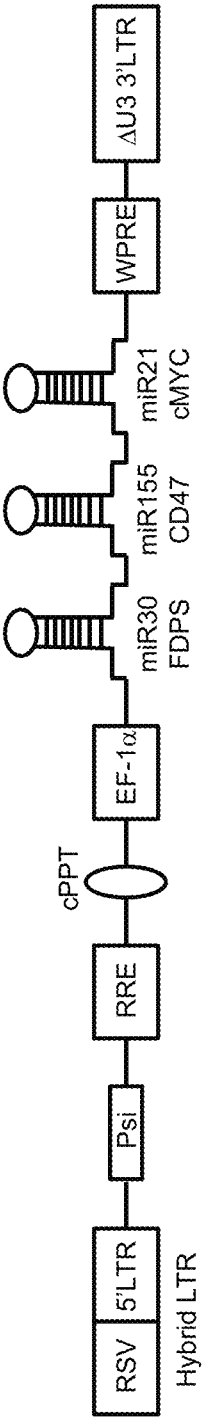

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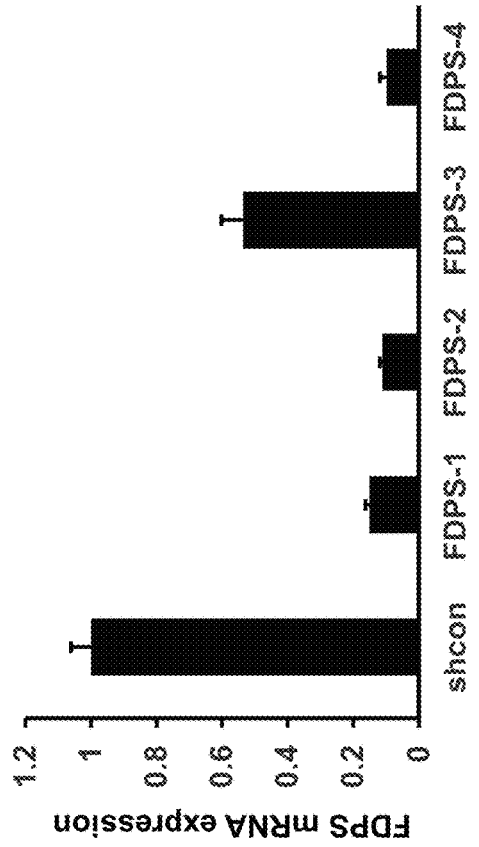

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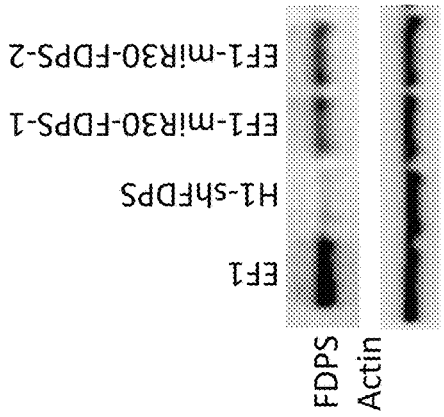

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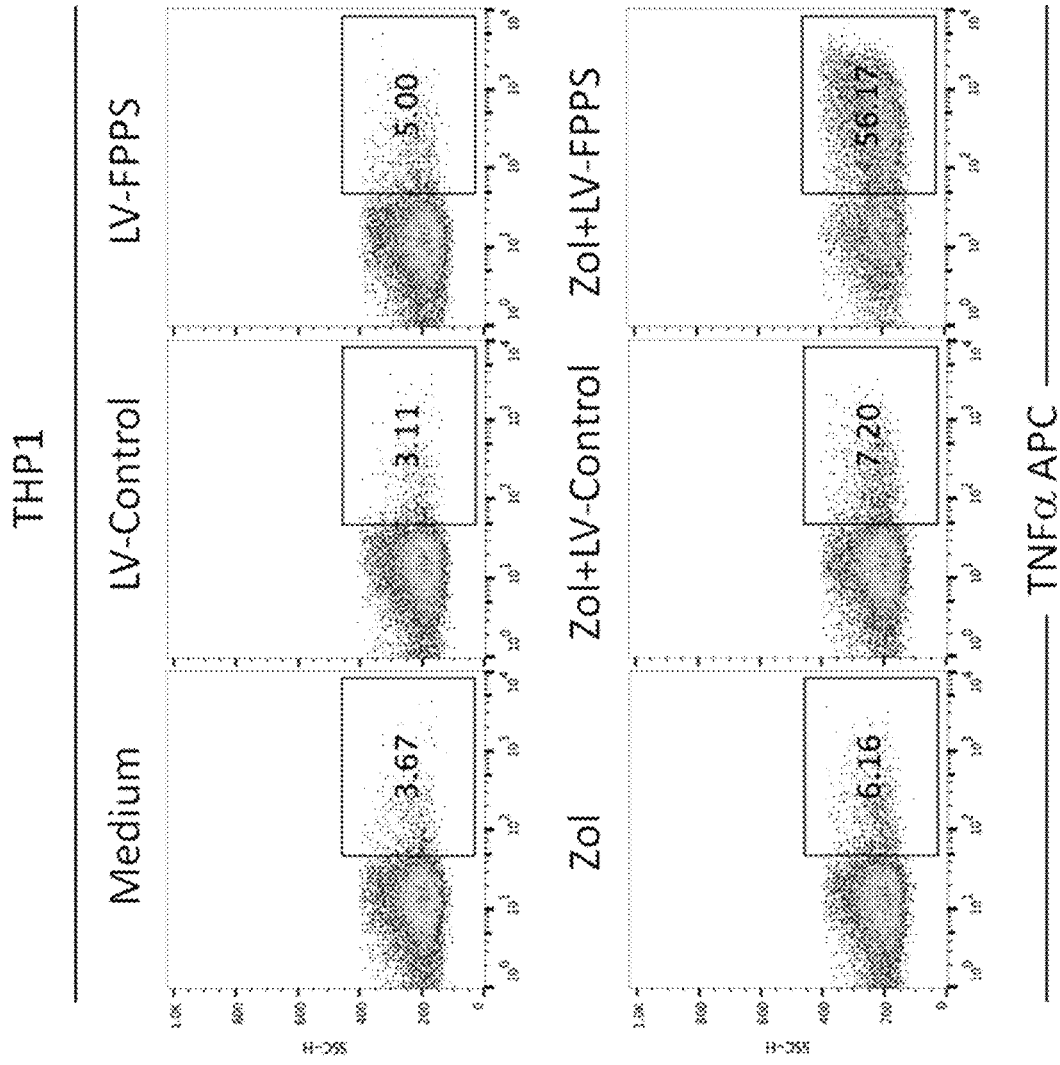
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LV-FPPS
Zol
Zol+LV-Control
Zol+LV-FPPS
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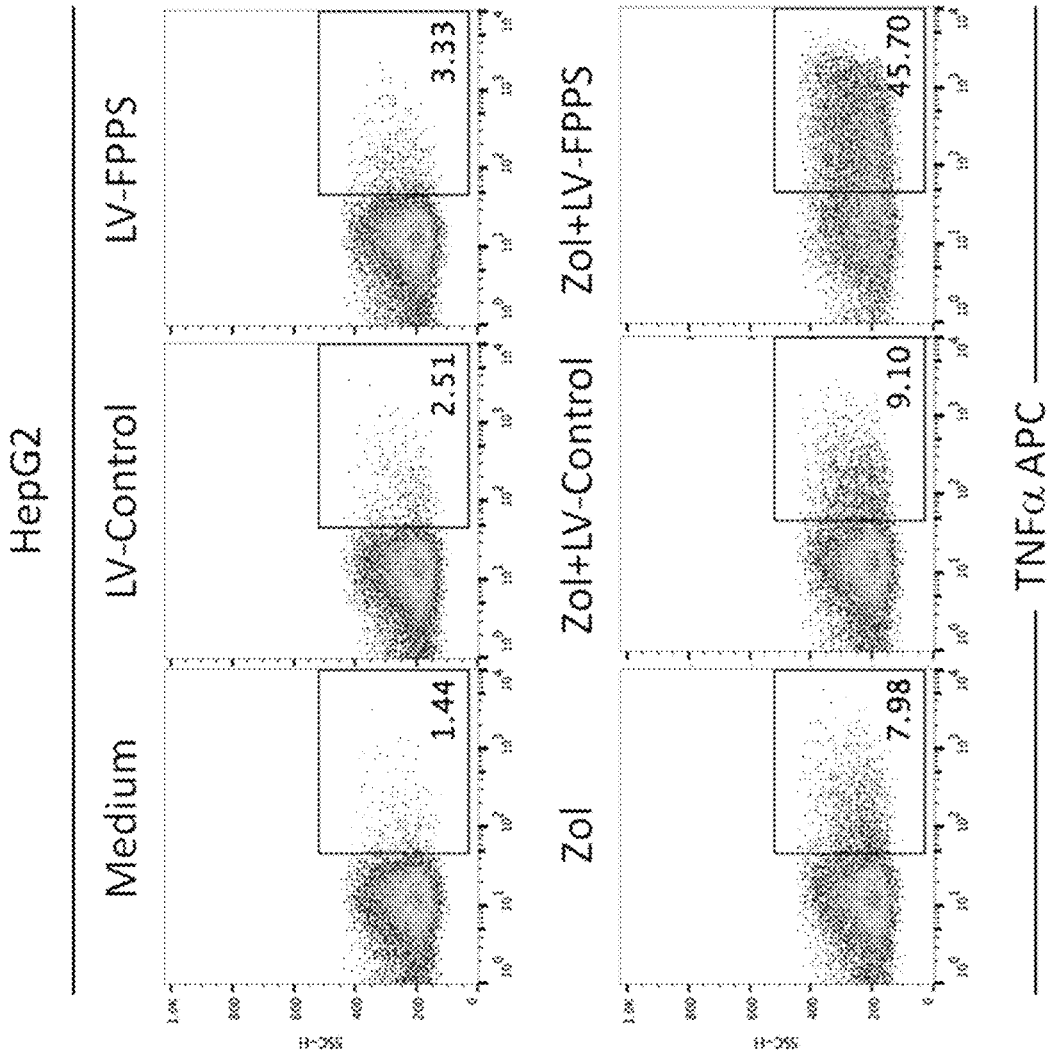
5.00
6.16
7.20
56.17
TNFα APC

Figure 5A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LV-FPPS
Zol
Zol+LV-Control
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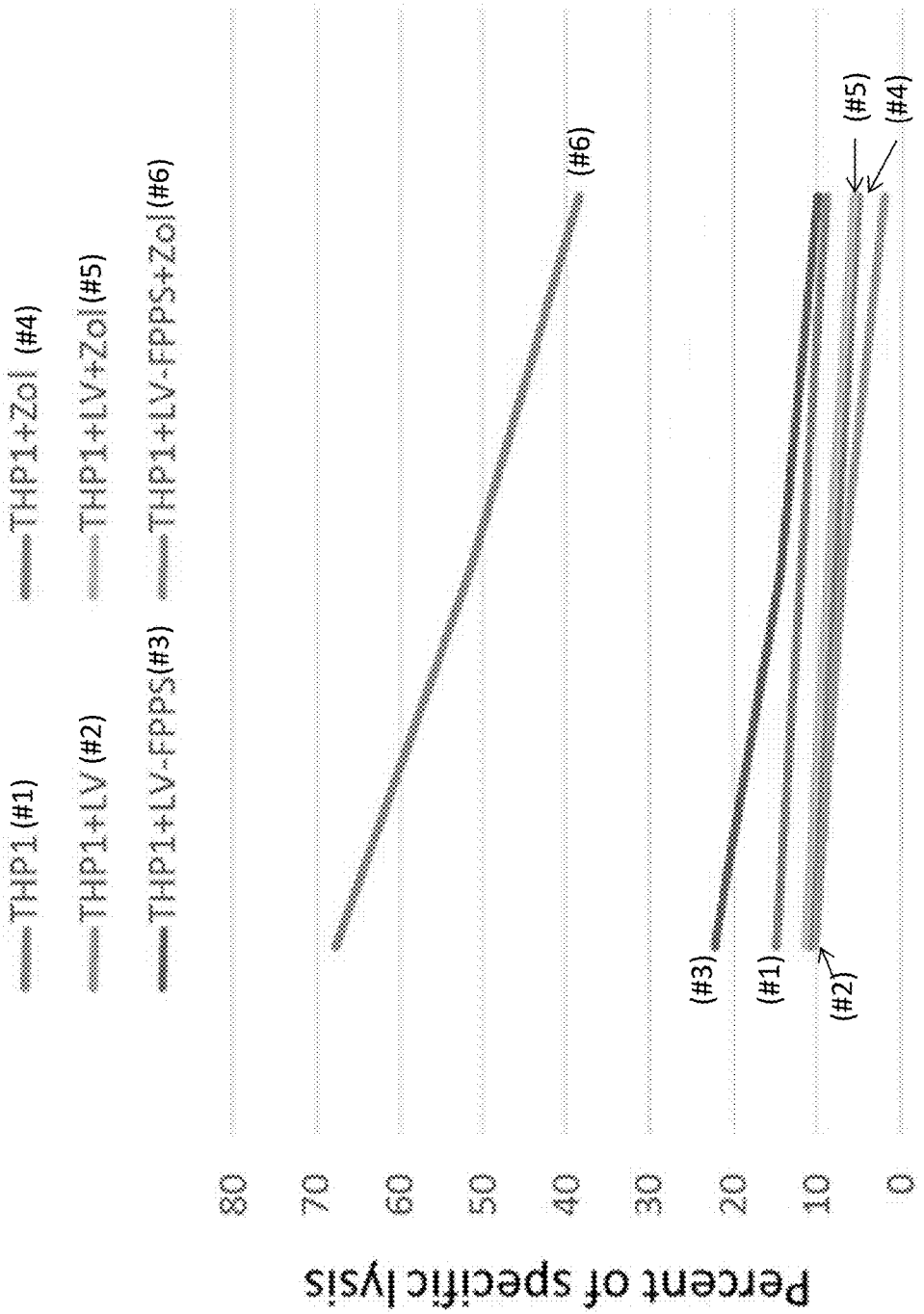
7.98
9.10
45.70
SSC-H
TNFα APC

Figure 5B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THP1+Zol (#4)
THP1+LV (#2)
THP1+LV+Zol (#5)
THP1+LV-FPPS(#3)
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
(#3)
(#1)
(#2)
(#6)
(#5)
(#4)

Figure 6

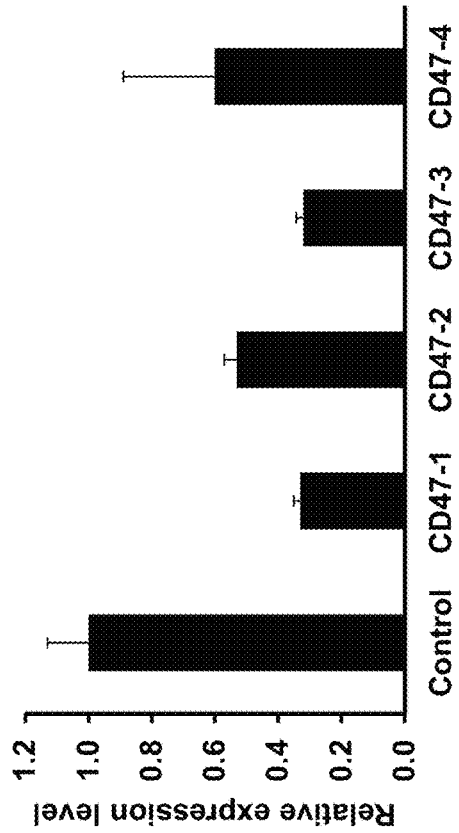


Figure 7A

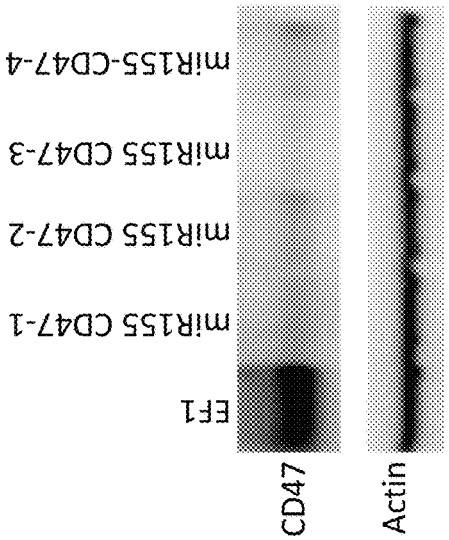


Figure 7B

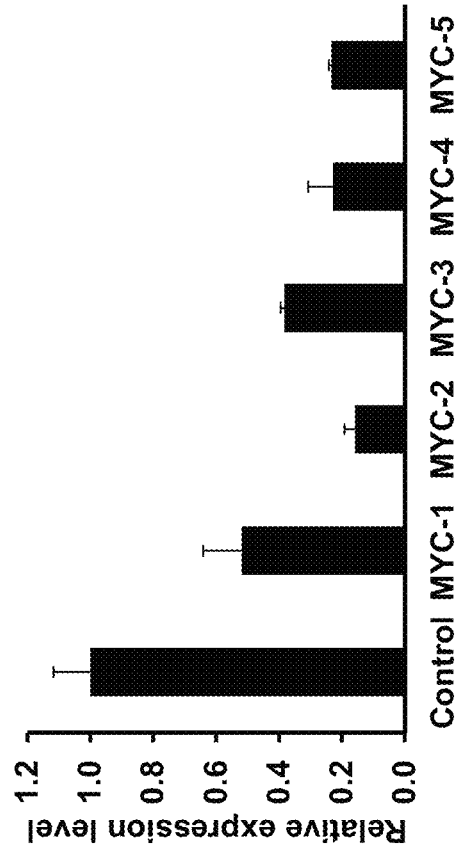


Figure 8A

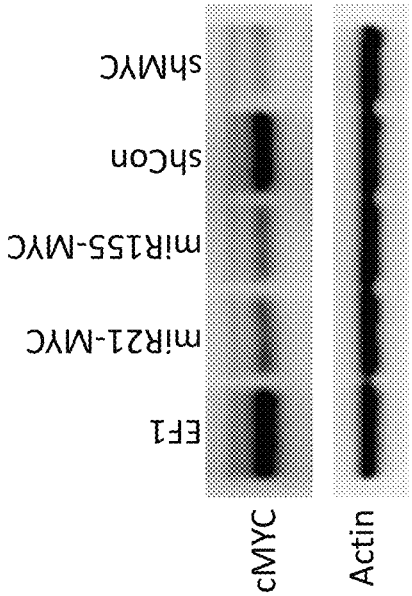


Figure 8B

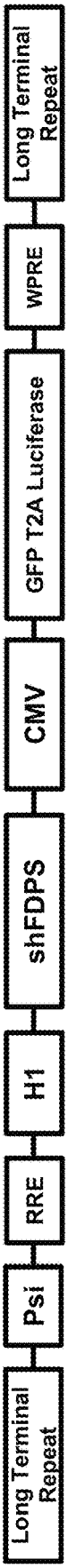
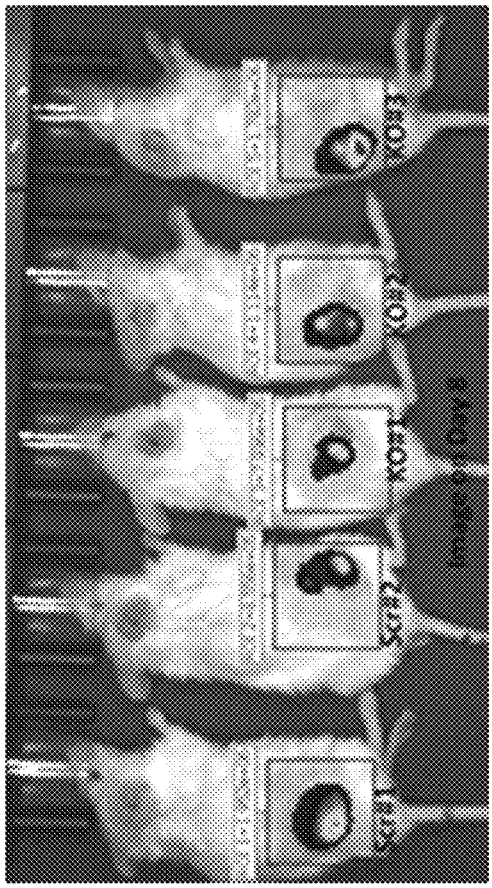

Long Terminal Repeat
Psi
RRE
H1
shFDPS
CMV
GFP T2A Luciferase
WPRE
Long Terminal Repeat

Figure 9



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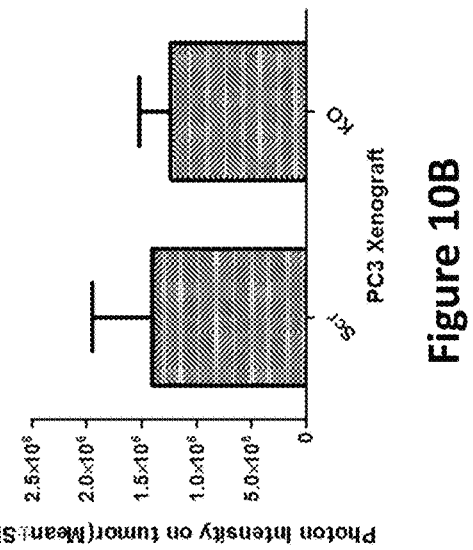

Figure 10B

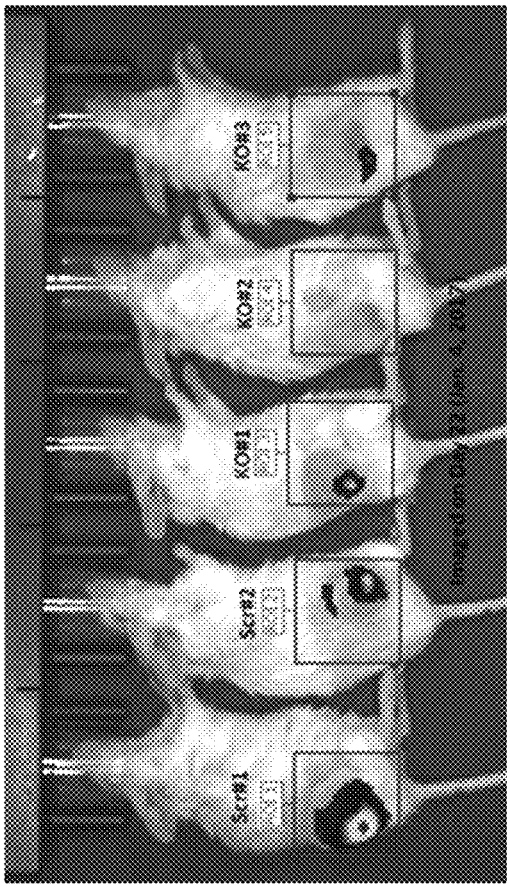

Figure 10C

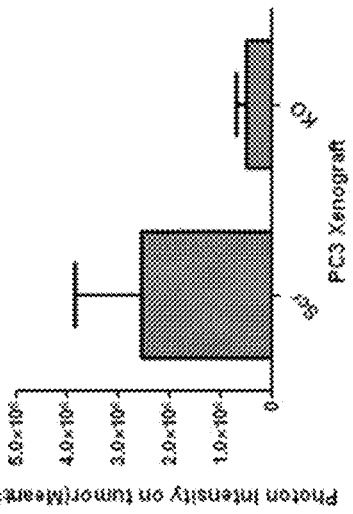

Figure 10D

COMBINATION VECTORS AND METHODS FOR TREATING CANCER

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Patent Application No. 62/305,944, filed on Mar. 9, 2016, and entitled "Combination Vectors and Uses Thereof", which is incorporated herein by reference.

FIELD

Aspects of the present disclosure relate to using vectors to treat cancer. More specifically, aspects of the present disclosure relate to using vectors, including combination vectors, to treat cancer.

BACKGROUND

Cancer is a significant healthcare issue for the world's population. As an example, liver cancer in adult men is the fifth most frequently diagnosed cancer worldwide, and is the second leading cause of cancer-related death in the world. Numerous therapeutic strategies have been employed in an effort to effectively treat cancer. Traditional therapeutic approaches have revolved around the use of chemotherapy and radiation therapy.

Chemotherapy refers to the administration of one or more anti-cancer drugs and/or other agents to a cancer patient by various methods. Broadly, most chemotherapeutic drugs work by impairing mitosis (cell division), effectively targeting fast-dividing cells. However, other fast dividing cells such as those responsible for hair growth and for replacement of the intestinal epithelium (lining) are also affected. Because chemotherapy affects cell division, both normal and cancerous cells are susceptible to the cytotoxic effects of chemotherapeutic agents.

Radiation therapy refers to exposing a patient to high-energy radiation, including x-rays, gamma rays, and neutrons. This type of therapy includes without limitation external-beam therapy, internal radiation therapy, implant radiation, brachytherapy, systemic radiation therapy, and radiotherapy. External beam radiation may include three dimensional conformal radiation therapy, intensity modulated radiation therapy, and conformal proton beam radiation therapy. In practice it is difficult to shield the nearby normal tissue from the cytotoxic effects of the radiation and still deliver a therapeutic dose. An additional complication of radiation is the induction of radiation resistant cells during the course of treatment. Thus, even the best radiotherapeutic techniques often result in incomplete tumor reduction and subsequent recurrence.

More recently, immunotherapeutic approaches have been employed in an attempt to harness the power of the host's immune system to treat cancer. For example, strategies have been employed to target cancer-associated antigens with host-based T cells that specifically recognize such antigens. For example, a recent approach has focused on the development and use of chimeric antigen receptor (CAR) T cells (also known as CAR-T cells). Possible side effects associated with CAR-T cell therapy include chemokine-release syndrome, B cell aplasia, and tumor lysis syndrome. Despite the development of these approaches, cancer remains a significant healthcare issue.

SUMMARY

In an aspect of the disclosure, a viral vector comprising a therapeutic cargo portion is disclosed. The therapeutic cargo portion includes at least one small RNA sequence that is capable of binding to at least one pre-determined complementary mRNA sequence, wherein the at least one complementary mRNA sequence comprises a FDPS mRNA sequence. In embodiments, the therapeutic cargo portion may further include a second small RNA sequence that is capable of binding to a second pre-determined complementary mRNA sequence, wherein the second pre-determined complementary mRNA sequence comprises a CD47 mRNA sequence or a cMyc mRNA sequence. In embodiments, the at least one small RNA sequence is under the control of a first promoter and the second small RNA sequence is under the control of a second promoter. In embodiments, the therapeutic cargo portion may further include a third small RNA sequence that is capable of binding to a third pre-determined complementary mRNA sequence, wherein the third pre-determined complementary mRNA sequence comprises a CD47 mRNA sequence or a cMyc mRNA sequence. In embodiments, the at least one small RNA sequence is under the control of a first promoter, the second small RNA sequence is under the control of a second promoter, and the third small RNA sequence is under the control of a third promoter. In embodiments, the small RNA sequences are under the control of a single promoter. In embodiments, the small RNA sequence is a microRNA (miRNA) or a short hairpin RNA (shRNA).

In another aspect, the small RNA sequence comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with a FDPS small RNA sequence comprising GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTACTCCAGGACTTTTT (SEQ ID NO: 1); GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCTGAACGAAATCCTGCTTTTT (SEQ ID NO: 2); GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGTACATGGCTTTTT (SEQ ID NO: 3); or GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCTTTTT (SEQ ID NO: 4). In embodiments, the small RNA sequence is selected from SEQ ID NOs: 1, 2, 3, or 4.

In another aspect, the second small RNA sequence comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with a CD47 small RNA sequence comprising GGTGAAACGATCATCGAGCCTCGAGGCTCGATGATCGTTTCACCTTTTT (SEQ ID NO: 5); GCTACTGGCCTTGGTTTAACTCGAGTTAAACCAAGGCCAGTAGCTTTTT (SEQ ID NO: 6); CCTCCTTCGTCATTGCCATCTCGAGATGGCAATGACGAAGGAGGTTTTT (SEQ ID NO: 7); GCATGGCCCTCTTCTGATTCTCGAGAATCAGAAGAGGGCCATGCTTTTT (SEQ ID NO: 8); or GGTGAAACGATCATCGAGCTACTCGAGTAGCTCGATGATCGTTTCACCTTTTT (SEQ ID NO: 9) or a cMyc small RNA sequence comprising GCTTCACCAACAGGAACTATGCTCGAGCATAGTTCCTGTTGGTGAAGCTTTT (SEQ ID NO: 10); GCGAACACACAACGTCTTGGACTCGAGTCCAAGACGTTGTGTGTTCGCTTTT (SEQ ID NO: 11); GACATGGTGAACCAGAGTTTCCTCGAGGAAACTCTGGTTCACCATGTCTTTTT (SEQ ID NO: 12); GAGAATGTCAAGAGGCGAACACTCGAGTGTTCGCCTCTTGACATTCTCTTTTT (SEQ ID NO: 13); or GCTCATTTCTGAAGAGGACTTCTCGAGAAGTCCTCTTCAGAAATGAGCTTTTT (SEQ ID NO: 14). In

embodiments, the second small RNA sequence is selected from SEQ ID NOs: 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14.

In another aspect, the third small RNA sequence comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with a CD47 small RNA sequence comprising SEQ ID NOs: 5, 6, 7, 8, or 9 or a cMyc small RNA sequence comprising SEQ ID NOs: 10, 11, 12, 13, or 14. In embodiments, the third small RNA sequence is selected from SEQ ID NOs: 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14.

In another aspect, a viral vector comprising a therapeutic cargo portion is disclosed. The therapeutic cargo portion includes at least one small RNA sequence that is capable of binding to at least one pre-determined complementary mRNA sequence, wherein the at least one complementary mRNA sequence comprises a CD47 mRNA sequence. In embodiments, the therapeutic cargo portion further comprises a second small RNA sequence that is capable of binding to a second pre-determined complementary mRNA sequence, wherein the second pre-determined complementary mRNA sequence comprises a FDPS mRNA sequence or a cMyc mRNA sequence. In embodiments, the at least one small RNA sequence is under the control of a first promoter and the second small RNA sequence is under the control of a second promoter. In embodiments, the therapeutic cargo portion further comprises a third small RNA sequence that is capable of binding to a third pre-determined complementary mRNA sequence, wherein the third pre-determined complementary mRNA sequence comprises a FDPS mRNA sequence or a cMyc mRNA sequence. The small RNA sequence may be a miRNA or a shRNA. In embodiments, the at least one small RNA sequence is under the control of a first promoter, the second small RNA sequence is under the control of a second promoter, and the third small RNA sequence is under the control of a third promoter. In embodiments, the small RNA sequences are under the control of a single promoter.

In another aspect, the small RNA sequence comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with a CD47 small RNA sequence comprising SEQ ID NOs: 5, 6, 7, 8, or 9. In embodiments, the small RNA sequence is selected from SEQ ID NOs: 5, 6, 7, 8, or 9.

In another aspect, the second small RNA sequence comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with a FDPS small RNA sequence comprising SEQ ID NOs: 1, 2, 3, or 4 or a cMyc small RNA sequence comprising SEQ ID NOs: 10, 11, 12, 13, or 14. In embodiments, the second small RNA sequence is selected from SEQ ID NOs: 1, 2, 3, 4, 10, 11, 12, 13, or 14.

In another aspect, the third small RNA comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with a FDPS small RNA sequence comprising SEQ ID NOs: 1, 2, 3, or 4 or a cMyc small RNA sequence comprising SEQ ID NOs: 10, 11, 12, 13, or 14. In embodiments, the third small RNA sequence is selected from SEQ ID NOs: 1, 2, 3, 4, 10, 11, 12, 13, or 14.

In another aspect, a viral vector comprising a therapeutic cargo portion is disclosed. The therapeutic cargo portion comprises a first small RNA sequence that is capable of binding to a first pre-determined complementary mRNA sequence, and at least one additional small RNA sequence that is capable of binding to a second pre-determined complementary mRNA sequence, wherein the first pre-determined complementary mRNA sequence comprises a cMyc mRNA sequence, and the second pre-determined complementary sequence comprises a FDPS mRNA sequence or a CD47 mRNA sequence.

In another aspect, the therapeutic cargo portion further comprises a third small RNA sequence that is capable of binding to a third pre-determined complementary mRNA sequence, wherein the third pre-determined complementary mRNA sequence comprises a FDPS mRNA sequence or a CD47 mRNA sequence. In embodiments, the small RNA sequences are miRNAs or shRNAs. In embodiments, the first small RNA sequence is under the control of a first promoter, the second small RNA sequence is under the control of a second promoter, and the third small RNA sequence is under the control of a third promoter. In embodiments, the small RNA sequences are under the control of a single promoter.

In another aspect, the first small RNA sequence comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with a cMyc small RNA sequence comprising SEQ ID NOs: 10, 11, 12, 13, or 14. In embodiments, the first small RNA sequence is selected from SEQ ID NOs: 10, 11, 12, 13, or 14.

In another aspect, the at least one additional small RNA sequence comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with a FDPS small RNA sequence comprising SEQ ID NOs: 1, 2, 3, or 4 or a CD47 small RNA sequence comprising SEQ ID NOs: 5, 6, 7, 8, or 9. In embodiments, the at least one additional small RNA is selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8 or 9.

In another aspect, the third small RNA sequence comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with a FDPS small RNA sequence comprising SEQ ID NOs: 1, 2, 3, or 4 or a CD47 small RNA sequence comprising SEQ ID NOs: 5, 6, 7, 8, or 9. In embodiments, the third small RNA sequence is selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8 or 9.

In another aspect, the viral vector is a lentiviral vector. In another aspect, a lentiviral particle capable of infecting a target cell is disclosed. The lentiviral particle includes an envelope protein optimized for infecting the target cell, and the viral vector as described herein. In embodiments, the target cell is a tumor cell.

In another aspect, a composition is disclosed comprising the lentiviral particle as described herein, and an aminobisphosphonate drug. In embodiments, the aminobisphosphonate drug is zoledronic acid.

In another aspect, a method of treating cancer in a subject is disclosed. The method comprises administering to the subject a therapeutically effective amount of the composition as detailed herein.

In another aspect, a method of treating cancer in a subject is disclosed. The method comprises administering to the subject a therapeutically effective amount of the lentiviral particle as detailed herein, and a therapeutically effective amount of an aminobisphosphonate drug. In another aspect, a method of preventing cancer in a subject is disclosed. The method comprises administering to the subject a therapeutically effective amount of the lentiviral particle as detailed herein, and a therapeutically effective amount of an aminobisphosphonate drug. In embodiments, the foregoing steps are carried out simultaneously. In embodiments, a defined period of time elapses between the foregoing steps. In embodiments, the aminobisphosphonate drug is zoledronic acid. In embodiments, the therapeutically effective amount of the lentiviral particle comprises a plurality of single doses

of the lentiviral particle. In embodiments, the therapeutically effective amount of the aminobisphosphonate drug comprises a single dose of the aminobisphosphonate drug.

Other aspects and advantages of the inventions described herein will become apparent from the following detailed description, taken in conjunction with the accompanying drawings, which illustrate by way of example the aspects of the inventions.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** depicts an exemplary 3-vector lentiviral system in a circularized form.

FIG. **2** depicts an exemplary 4-vector lentiviral system in a circularized form.

FIG. **3** depicts: (A) a linear map of a lentiviral vector encoding a FDPS shRNA targeting sequence; (B) a linear map of a lentiviral vector encoding a synthetic microRNA (miRNA) with a FDPS targeting sequence; and (C) a linear map of a lentiviral combination vector that encodes a synthetic microRNA (miRNA) with target sequences directed to cMyc, FDPS, and CD47 expression.

FIG. **4** depicts: (A) relative expression levels of human FDPS mRNA in response to various shRNA constructs, as described herein; and (B) that lentiviral-delivered miR-based RNA interference inhibits FDPS expression.

FIG. **5** depicts cytokine expression levels in human peripheral blood gamma delta T cells after exposure to (A) THP1 or (B) HepG2 cells that have been transduced with lentivirus to suppress FDPS.

FIG. **6** depicts percent specific lysis of THP-1 tumor cell line that was modified by lentiviral transduction to suppress FDPS then mixed with normal human gamma delta T cells under a variety of experimental conditions as described herein.

FIG. **7** depicts: (A) relative expression levels of human CD47 mRNA in response to various shRNA constructs, as described herein; (B) that lentiviral-delivered miR-based RNA interference inhibits CD47 expression.

FIG. **8** depicts: (A) the relative expression levels of human cMyc in response to various shRNA constructs, as described herein and (B) that lentiviral-delivered miR-based RNA interference inhibits cMyc expression.

FIG. **9** depicts a linear map of a lentiviral vector encoding a FDPS shRNA targeting sequence as used in Example 6 herein.

FIG. **10** depicts the effect of zoledronic acid treatment of NOD/SCID mice implanted with PC3 cells transduced with LV-shFDPS or control LV as described herein. (A) depicts photographic data at day 8; (B) depicts photon intensity data at day 8; (C) depicts photographic data at day 22; and (D) depicts photon intensity data at day 22.

DETAILED DESCRIPTION

Overview of the Disclosure

The present disclosure relates to therapeutic vectors and delivery of the same to cells. In embodiments, the therapeutic vectors target more than one mRNA target. In embodiments, the therapeutic vectors are provided with small RNAs, including short homology RNAs (shRNAs) or microRNAs (miRNAs) that target FDPS, thereby reducing expression levels of this enzyme. The therapeutic vectors include lentiviral vectors. The present disclosure demonstrates that targeting FDPS, in conjunction with treatment with an aminobisphosphonate drug, can effectively treat cancer.

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 "combination vector" means a therapeutic vector that targets more than one mRNA. For example, a therapeutic vector that contains two shRNAs or two miRNAs directed towards two different mRNAs can be referred to as a "combination vector."

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. A target cell may be a cancer 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 "LV" refers generally to "lentivirus." As an example, reference to "LV-shFDPS" is reference to a lentivirus that expresses an shRNA that targets FDPS.

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 XBLAST 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, word length=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 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 adeno-associated viral (AAV) vector. Additionally, as used herein with reference to the lentiviral vector system, the term "vector" is synonymous with the term "plasmid." For example, the 3-vector and 4-vector systems, which include the 2-vector and 3-vector packaging systems, can also be referred to as 3-plasmid and 4-plasmid systems.

"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 and Embodiments of the Disclosure

In an aspect of the disclosure, a viral vector comprising a therapeutic cargo portion is disclosed. The therapeutic cargo portion includes at least one small RNA sequence that is capable of binding to at least one pre-determined complementary mRNA sequence, wherein the at least one complementary mRNA sequence comprises a FDPS mRNA sequence. In embodiments, the therapeutic cargo portion may further include a second small RNA sequence that is capable of binding to a second pre-determined complementary mRNA sequence, wherein the second pre-determined complementary mRNA sequence comprises a CD47 mRNA sequence or a cMyc mRNA sequence. In embodiments, the therapeutic cargo portion may further include a third small RNA sequence that is capable of binding to a third pre-determined complementary mRNA sequence, wherein the third pre-determined complementary mRNA sequence comprises a CD47 mRNA sequence or a cMyc mRNA sequence. The small RNA sequence may be a microRNA (miRNA) or a short hairpin RNA (shRNA).

In another aspect, the small RNA sequence comprises a sequence having at least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95% or greater percent identity with a FDPS small RNA sequence comprising SEQ ID NOs: 1, 2, 3, or 4. In embodiments, the small RNA sequence is selected from SEQ ID NOs: 1, 2, 3, or 4.

In another aspect, the second small RNA sequence comprises a sequence having at least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95% or greater identity with a CD47 small RNA sequence comprising SEQ ID NOs: 5, 6, 7, 8 or 9 or a cMyc small RNA sequence comprising SEQ ID NOs: 10, 11, 12, 13, or 14. In embodiments, the second small RNA sequence is selected from SEQ ID NOs: 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14.

In another aspect, the third small RNA sequence comprises a sequence having at least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95% or greater identity with a CD47 small RNA sequence comprising SEQ ID NOs: 5, 6, 7, 8 or 9 or a cMyc small RNA sequence comprising SEQ ID NOs: 10, 11, 12, 13, or 14. In embodiments, the third small RNA sequence is selected from SEQ ID NOs: 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14.

In another aspect, a viral vector comprising a therapeutic cargo portion is disclosed. The therapeutic cargo portion includes at least one small RNA sequence that is capable of binding to at least one pre-determined complementary mRNA sequence, wherein the at least one complementary mRNA sequence comprises a CD47 mRNA sequence. In embodiments, the therapeutic cargo portion further comprises a second small RNA sequence that is capable of binding to a second pre-determined complementary mRNA sequence, wherein the second pre-determined complementary mRNA sequence comprises a FDPS mRNA sequence or a cMyc mRNA sequence. In embodiments, the therapeutic cargo portion further comprises a third small RNA sequence that is capable of binding to a third pre-determined complementary mRNA sequence, wherein the third pre-determined complementary mRNA sequence comprises a FDPS mRNA sequence or a cMyc mRNA sequence. In embodiments, the small RNA sequence is a miRNA or a shRNA.

In another aspect, the small RNA sequence comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with a CD47 small RNA sequence comprising SEQ ID NOs: 5, 6, 7, 8 or 9. In embodiments, the small RNA sequence is selected from SEQ ID NOs: 5, 6, 7, 8 or 9.

In another aspect, the second small RNA sequence comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with a FDPS small RNA sequence comprising SEQ ID NOs: 1, 2, 3, or 4 or a cMyc small RNA sequence comprising SEQ ID NOs: 10, 11, 12, 13, or 14. In embodiments, the second small RNA sequence is selected from SEQ ID NOs: 1, 2, 3, 4, 10, 11, 12, 13, or 14.

In another aspect, the third small RNA comprises a sequence having at least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95% or greater identity with a FDPS small RNA sequence comprising SEQ ID NOs: 1, 2, 3, or 4 or a cMyc small RNA sequence comprising SEQ ID NOs: 10, 11, 12, 13, or 14. In embodiments, the third small RNA sequence is selected from SEQ ID NOs: 1, 2, 3, 4, 10, 11, 12, 13, or 14.

In another aspect, a viral vector comprising a therapeutic cargo portion is disclosed. The therapeutic cargo portion comprises a first small RNA sequence that is capable of binding to a first pre-determined complementary mRNA sequence, and at least one additional small RNA sequence that is capable of binding to a second pre-determined complementary mRNA sequence, wherein the first pre-determined complementary mRNA sequence comprises a cMyc mRNA sequence, and the second pre-determined complementary sequence comprises a FDPS mRNA sequence or a CD47 mRNA sequence.

In another aspect, the therapeutic cargo portion further comprises a third small RNA sequence that is capable of binding to a third pre-determined complementary mRNA sequence, wherein the third pre-determined complementary mRNA sequence comprises a FDPS mRNA sequence or a CD47 mRNA sequence. In embodiments, the small RNA sequences are miRNAs or shRNAs.

In another aspect, the first small RNA sequence comprises a sequence having at least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95% or greater identity with a cMyc small RNA sequence comprising SEQ ID NOs: 10, 11, 12, 13, or 14. In embodiments, the first small RNA sequence is selected from SEQ ID NOs: 10, 11, 12, 13, or 14.

In another aspect, the at least one additional small RNA sequence comprises a sequence having at least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95% or greater percent identity with a FDPS small RNA sequence comprising SEQ ID NOs: 1, 2, 3, or 4 or a CD47 small RNA sequence comprising SEQ ID NOs: 5, 6, 7, 8 or 9. In embodiments, the at least one additional small RNA is selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, or 9.

In another aspect, the third small RNA sequence comprises a sequence having at least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95% or greater identity with a FDPS small RNA sequence comprising SEQ ID NOs: 1, 2, 3, or 4 or a CD47 small RNA sequence comprising SEQ ID NOs: 5, 6, 7, 8 or 9. In embodiments, the third small RNA sequence is selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, or 9.

In another aspect, the small RNA sequences referred to herein can comprise a sequence having at least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86%, or at least 87%, or at least 88%, or at least 89%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95% or greater identity with any of the miRNA sequences detailed herein, including: miR30 FDPS sequence #1 (SEQ ID NO: 53), miR30 FDPS sequence #2 (SEQ ID NO: 54). miR30 FDPS sequence #3 (SEQ ID NO: 55), miR155 FDPS sequence #1 (SEQ ID NO: 56), miR21 FDPS sequence #1 (SEQ ID NO: 57), miR185 FDPS sequence #1 (SEQ ID NO: 58), miR155 CD47 sequence #1 (SEQ ID NO: 82; miR155 CD47 target sequence #2 (SEQ ID NO: 66), miR155 CD47 target sequence #3 (SEQ ID NO: 67), miR155 CD47 target sequence #4 (SEQ ID NO: 68), miR21 cMyc sequence (SEQ ID NO: 83); or miR155 cMyc sequence (SEQ ID NO: 70).

In embodiments, the small RNA sequences can comprise any of the miRNA sequences detailed herein, including: miR30 FDPS sequence #1 (SEQ ID NO: 53), miR30 FDPS sequence #2 (SEQ ID NO: 54). miR30 FDPS sequence #3 (SEQ ID NO: 55), miR155 FDPS sequence #1 (SEQ ID NO: 56), miR21 FDPS sequence #1 (SEQ ID NO: 57), miR185 FDPS sequence #1 (SEQ ID NO: 58), miR155 CD47 sequence #1 (SEQ ID NO: 82; miR155 CD47 target sequence #2 (SEQ ID NO: 66), miR155 CD47 target sequence #3 (SEQ ID NO: 67), miR155 CD47 target sequence #4 (SEQ ID NO: 68), miR21 cMyc sequence (SEQ ID NO: 83); or miR155 cMyc sequence (SEQ ID NO: 70).

In another aspect, the viral vector is a lentiviral vector. In another aspect of the disclosure a lentiviral particle capable of infecting a target cell is disclosed. The lentiviral particle includes an envelope protein optimized for infecting the target cell; and the viral vector as described herein. In embodiments, the target cell is a tumor cell.

In another aspect, a composition is disclosed comprising the lentiviral particle as described herein, and an aminobisphosphonate drug. In embodiments, the aminobisphosphonate drug is zoledronic acid.

In another aspect of the disclosure, a method of treating cancer in a subject is disclosed. The method comprises administering to the subject a therapeutically effective amount of the composition as detailed herein.

In another aspect, a method of treating cancer in a subject is disclosed. The method comprises administering to the subject a therapeutically effective amount of the lentiviral particle as detailed herein; and a therapeutically effective amount of an aminobisphosphonate drug. In embodiments, the foregoing steps are carried out simultaneously. In embodiments, a defined period of time elapses between the foregoing steps. In embodiments, the aminobisphosphonate drug is zoledronic acid. In embodiments, the therapeutically effective amount of the lentiviral particle comprises a plurality of single doses of the lentiviral particle. In embodiments, the therapeutically effective amount of the aminobisphosphonate drugs comprises a single dose of the aminobisphosphonate drug.

Additional aspects of the present invention describe the development of multi-gene-targeting vectors for treatment

of cancer, and, as a non-limiting example, for the treatment of hepatocellular carcinoma ("HCC"). These vectors address three concerns in respect of HCC therapy. Firstly, the therapeutic vectors may include inhibitory RNA constructs for reducing the expression of cMyc oncogene protein. The cMyc oncogene protein is responsible for tumorigenesis, tumor growth and immune evasion. The therapeutic vector may include more than just one inhibitory RNA construct for reducing cMyc expression. For example, in embodiments, combination vectors are specifically contemplated when cMyc is a target of the vector. Secondly, vectors have been developed (e.g., through inhibitory RNA constructs) to reduce the expression of farnesyl diphosphate synthase ("FDPS"). By reducing the levels of FDPS, tumor cells are modified, for example, to become stimulatory for gamma delta T cells. These gamma delta T cells are capable of cytotoxic killing of tumor cells. Thirdly, the vectors have been developed to reduce the expression (e.g., through inhibitory RNA constructs) of at least one other gene product. In certain embodiments, the at least one other gene product can be an immune checkpoint regulator. Examples of immune checkpoint regulators include, but are not limited to programmed death-ligand 1 (PD-L1), galactosidase-binding soluble lectin 9 (LGALS9A), tumor necrosis factor receptor super family, member 14 (HVEM), V-set domain containing T cell activation inhibitor 1 (B7-H4), CD276 molecule (B7-H3), CD80 molecule (CD28LG1), and CD86 molecule (CD28LG2). In embodiments, the immune checkpoint regulator is PD-L1. By reducing expression cMyc, levels of PD-L1 are consequently decreased because cMyc is a positive regulator for expression of PD-L1 and other immune evasion genes including CD47, which are expressed in tumor cells. By decreasing the levels of CD47, tumor cell phagocytosis is increased leading to improved T cell responses through cross-presentation of tumor antigens on antigen-presenting cells. By decreasing PD-L1 and potentially other immune checkpoint inhibitory molecules, the efficiency of immune stimulation of T cells, including stimulation of gamma delta T cells, can be improved. While cMyc regulates PD-L1 levels, PD-L1 or other immune checkpoint regulators can be targeted directly using the therapeutic vectors described herein by generating shRNAs or miRNAs that are specifically directed to PD-L1 or the other selected immune checkpoint regulators.

In certain embodiments, the at least one other gene product can be a gene product that influences phagocytosis. For example, the at least one other gene product that influences phagocytosis can be CD47. By reducing the expression of CD47 the block to macrophage phagocytosis of tumor cells is removed. These two mechanisms combine to increase the efficiency and activity of acquired or innate immunity needed to treat or eliminate HCC.

The combination vectors disclosed herein are optimized such that the correct promoter has been selected to best match RNA processing system requirements. Additionally, the therapeutic cargo portion has been designed such that the miRNA or miRNAs are in a cluster so that processing of the first miRNA facilitates processing of the second miRNA and so on. The order of the miRNAs may be important to improve processing fidelity and associated rates so as to ensure that processing is not so rapid that genomic RNA for packaging into lentivirus particles is processed thus decreasing the efficiency of lentivirus manufacturing. Additionally, the combination vectors can be designed such that the therapeutic cargo portion includes multiple shRNAs under the control of discrete promoters.

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. 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.

Therapeutic Vectors

The therapeutic vectors 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 Papovaviri-

dae (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.

With respect to the therapeutic vectors detailed herein, in aspects of the present disclosure, a miRNA or shRNA is under the control of a single promoter. In embodiments, when multiple miRNAs are present in the same therapeutic vector, the miRNAs are under the control of a single promoter, for example a Pol II promoter. In embodiments, the Pol II promoter is EF1-alpha or a CMV promoter.

In embodiments, when multiple shRNAs are present in the same therapeutic vector, the shRNAs are under the control of multiple promoters. For example, a first shRNA is under the control of a first promoter, a second shRNA is under the control of a second promoter, a third shRNA is under the control of a third promoter, and so on. In non-limiting embodiments, the promoters can be selected from H1 (SEQ ID NO: 15), U6 (SEQ ID NO: 16), or 7SK (SEQ ID NO: 17).

As depicted in FIG. **3**C, a non-limiting example of a therapeutic vector includes a therapeutic cargo of three miRNA targeting cMyc, FDPS, and CD47 mRNA. As shown in Table 1 herein, alternate combinations of one to three miRNA sequences can be used in the final form of the therapeutic vector such that the therapeutic vector is a combination vector. While combinations of one to three miRNA sequences can be used in the final therapeutic vector, it is specifically contemplated that up to four, up to five, or up to six, or up to seven, or up to eight or more miRNA sequences could be used in the final therapeutic vector. Further the miRNA sequences may be sequential or randomly arranged (i.e., the first miRNA need not precede the second miRNA etc.). In addition to the combinations selected, all possible orders of miRNA from 5' to 3' end of the sense strand may be utilized for these lentiviral vectors. Vector components are not repeated for each miRNA combination. In developing the vectors containing miRNAs, shRNAs for the genes of interest are first used to prove that the gene of interest will work in the lentivirus construct; thereafter, and once shRNAs are proven to work (as described below), they are assembled into miRNA clusters as shown, for example, in FIG. **3**C herein. The miRNAs preserve targeting sequences but have changes in their overall structure to become better suited for the miRNA processing pathway.

TABLE 1

Combinations of miRNA sequences

Vector 1		miR155FDPS	
Vector 2			miR21CD47
Vector 3	miR30cMyc		
Vector 4	miR30cMyc	miR155FDPS	
Vector 5	miR30cMyc		miR21CD47
Vector 6		miR155FDPS	miR21CD47
Vector 7	miR30cMyc		miR21CD47
Vector 8	miR30cMyc	miR155FDPS	miR21CD47

Combination vectors can also be generated using shRNAs. However, in these circumstances discrete promoters need to be utilized for each target sequence, as is described herein.

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 pol 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 pol 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), flavi viruses (tick-home 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; 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 SFIIV 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. **1**). 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. **2**). 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 the genes targeted by the shRNAs or miRNAs.

In another aspect, the therapeutic vector, can include the following elements: hybrid 5' long terminal repeat (RSV/5' LTR) (SEQ ID NOS: 74-75), Psi sequence (RNA packaging site) (SEQ ID NO: 76), RRE (Rev-response element) (SEQ ID NO: 77), cPPT (polypurine tract) (SEQ ID NO: 78), H1 promoter (SEQ ID NO: 15), FDPS shRNA (e.g., SEQ ID NOS: 1, 2, 3, 4 or variants thereof), Woodchuck Post-Transcriptional Regulatory Element (WPRE) (SEQ ID NO: 79), and 3' Delta LTR (SEQ ID NO: 80). 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: 21); HIV component pol (SEQ ID NO: 22); HIV Int (SEQ ID NO: 23); HIV RRE (SEQ ID NO: 24); and HIV Rev (SEQ ID NO: 25). 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: 27) and vesicular stomatitis virus G glycoprotein (VSV-G) (SEQ ID NO: 29). 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 vector compositions 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 embodiments, vector compositions 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 dosing will be determined for each disease indication, including a specific cancer type, and will depend on toxicity/safety profiles for each individual product or product lot.

Additionally, vector compositions of the present disclosure may be administered periodically, such as once or twice a day, or any other suitable time period. For example, vector compositions 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 embodiments, the disclosed vector compositions are administered as a pharmaceutical composition. In embodiments, the pharmaceutical composition 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 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 vector compositions 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 vector compositions 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 pharmaceutical composition may be a controlled release formulation, sustained release formulation, immediate release formulation, or any combination thereof. Further, the pharmaceutical composition may be a transdermal delivery system.

In embodiments, the pharmaceutical composition 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 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 embodiments, the pharmaceutical composition 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 embodiments, the pharmaceutical composition 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 embodiments, the pharmaceutical composition can be formulated in a liquid dosage form for oral administration, such as suspensions, emulsions or syrups. In 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 embodiments, the composition can be formulated to be suitable for administration to a pediatric patient.

In embodiments, 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 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 embodiments, the treatment of cancer is accomplished by guided direct injection of the disclosed vector constructs into tumors, using needle, or intravascular cannulation. In embodiments, the vectors compositions 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 aspects of 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. **1** (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 transduced 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., which includes 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. **1**. Briefly, and with reference to FIG. **1**, the top-most vector is a helper plasmid, which, in this case, includes Rev. The vector appearing in the middle of FIG. **1** is the envelope plasmid. The bottom-most vector is the therapeutic vector, as described herein.

Referring to FIG. **1**, the Helper plus Rev plasmid includes a CAG enhancer (SEQ ID NO: 18); a CAG promoter (SEQ ID NO: 19); a chicken beta actin intron (SEQ ID NO: 20); a HIV gag (SEQ ID NO: 21); a HIV Pol (SEQ ID NO: 22); a HIV Int (SEQ ID NO: 23); a HIV RRE (SEQ ID NO: 24); a HIV Rev (SEQ ID NO: 25); and a rabbit beta globin poly A (SEQ ID NO: 26).

The Envelope plasmid includes a CMV promoter (SEQ ID NO: 27); a beta globin intron (SEQ ID NO: 28); a VSV-G (SEQ ID NO: 29); and a rabbit beta globin poly A (SEQ ID NO: 30).

Synthesis of a 3-Vector System, which Includes a 2-Vector Lentiviral Packaging System, Consisting of 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: 31) and reverse primer was (5'-CCATACAATGAATGGACACTAGGCGGCCGCACGAAT-3') (SEQ ID NO: 32).

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

(SEQ ID NO: 33)

```
GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGGAAT
TGGAGGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCT
GCGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAAC
ATAATTGGAAGAAATCTGTTGACTCAGATTGGCTGCACTTTAAATTTTCC
CATTAGTCCTATTGAGACTGTACCAGTAAAATTAAAGCCAGGAATGGATG
GCCCAAAAGTTAAACAATGGCCATTGACAGAAGAAAAAATAAAAGCATTA
GTAGAAATTTGTACAGAAATGGAAAAGGAAGGAAAAATTTCAAAAATTGG
GCCTGAAAATCCATACAATACTCCAGTATTTGCCATAAAGAAAAAAGACA
GTACTAAATGGAGAAAATTAGTAGATTTCAGAGAACTTAATAAGAGAACT
CAAGATTTCTGGGAAGTTCAATTAGGAATACCACATCCTGCAGGGTTAAA
ACAGAAAAAATCAGTAACAGTACTGGATGTGGGCGATGCATATTTTTCAG
TTCCCTTAGATAAAGACTTCAGGAAGTATACTGCATTTACCATACCTAGT
ATAAACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACA
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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
```

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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: 34)

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: 35)

ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCC

CATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGC

TGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCC

CATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATT

TACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT

ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGC

CCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTAT

TAGTCATCGCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCTTCAC

TCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTT

TTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGCGCGCGCC

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 deter-

mined by sequencing using a CMV specific primer. The DNA sequence was as follows:

(SEQ ID NO: 29)

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, which includes 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. **2**. Briefly, and with reference to FIG. **2**, 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 therapeutic vector as described herein.

Referring to FIG. **2**, the Helper plasmid includes a CAG enhancer (SEQ ID NO: 18); a CAG promoter (SEQ ID NO: 19); a chicken beta actin intron (SEQ ID NO: 20); a HIV gag (SEQ ID NO: 21); a HIV Pol (SEQ ID NO: 22); a HIV Int (SEQ ID NO: 23); a HIV RRE (SEQ ID NO: 24); and a rabbit beta globin poly A (SEQ ID NO: 26).

The Rev plasmid includes a RSV promoter (SEQ ID NO: 80); a HIV Rev (SEQ ID NO: 25); and a rabbit beta globin poly A (SEQ ID NO: 26).

The Envelope plasmid includes a CMV promoter (SEQ ID NO: 27); a beta globin intron (SEQ ID NO: 28); a VSV-G (SEQ ID NO: 29); and a rabbit beta globin poly A (SEQ ID NO: 30).

Synthesis of a 4-vector system, which includes a 3-vector lentiviral packaging system consisting of 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: 34)

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 sequences were 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: 36)

```
CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGGGGACTAGGGTG

TGTTTAGGCGAAAAGCGGGGCTTCGGTTGTACGCGGTTAGGAGTCCCCTC

AGGATATAGTAGTTTCGCTTTTGCATAGGGAGGGGGAAATGTAGTCTTAT

GCAATACACTTGTAGTCTTGCAACATGGTAACGATGAGTTAGCAACATGC

CTTACAAGGAGAGAAAAAGCACCGTGCATGCCGATTGGTGGAAGTAAGGT

GGTACGATCGTGCCTTATTAGGAAGGCAACAGACAGGTCTGACATGGATT

GGACGAACCACTGAATTCCGCATTGCAGAGATAATTGTATTTAAGTGCCT

AGCTCGATACAATAAACGCCATTTGACCATTCACCACATTGGTGTGCACC

TCCAAGCTCGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCAT

CCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCC

CTCGAAGCTAGCGATTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAG

CGACGAAGAACTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAA

GCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAAGGAATAGA

AGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTCGATTAGTGAACG

GATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCAGC

TACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGGAACT

TCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATCTCCTAC

AATATTGGAGTCAGGAGCTAAAGAATAGTCTAGA
```

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

Promoters: Elongation Factor-1 (EF-1) (SEQ ID NO: 37), phosphoglycerate kinase (PGK) (SEQ ID NO: 38), and ubiquitin C (UbC) (SEQ ID NO: 39) can replace the CMV (SEQ ID NO: 27) 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: 40) and bGH poly A (SEQ ID NO: 41) can replace the rabbit beta globin poly A (SEQ ID NO: 26). 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: 21); HIV Pol (SEQ ID NO: 22); and HIV Int (SEQ ID NO: 23) 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: 42), gibbon ape leukemia virus (GALV) (SEQ ID NO: 43), Rabies (FUG) (SEQ ID NO: 44), lymphocytic choriomeningitis virus (LCMV) (SEQ ID NO: 45), influenza A fowl plague virus (FPV) (SEQ ID NO: 46), Ross River alphavirus (RRV) (SEQ ID NO: 47), murine leukemia virus 10A1 (MLV) (SEQ ID NO: 81), or Ebola virus (EboV) (SEQ ID NO: 48). 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 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'δ 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. Therapeutic Vectors

Exemplary therapeutic vectors have been designed and developed as shown, for example, in FIG. **3**.

Referring first to FIG. **3**A, from left to right, the key genetic elements are as follows: hybrid 5' long terminal repeat (RSV/LTR), Psi sequence (RNA packaging site), RRE (Rev-response element), cPPT (polypurine tract), H1 promoter, an FDPS shRNA sequence including the FDPS shRNA sequences detailed herein, Woodchuck Post-Transcriptional Regulatory Element (WPRE), and LTR with a deletion in the U3 region.

Referring next to FIG. **3**B, from left to right, the key genetic elements are as follows: hybrid 5' long terminal repeat (RSV/LTR), Psi sequence (RNA packaging site), RRE (Rev-response element), cPPT (polypurine tract), EF-1 alpha (EF-1 alpha promoter of gene transcription), a FDPS miR (miRNA) including the FDPS miRNA sequences detailed herein, Woodchuck Post-Transcriptional Regulatory Element (WPRE), and LTR with a deletion in the U3 region.

To produce the vectors outlined generally in FIGS. **3**A and **3**B, the following methods and materials were employed.

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://maidesigner.thermofisher.com/maiexpress/). Individual selected shRNA sequences were inserted into a lentiviral vector immediately 3 prime to a RNA polymerase III promoter H1 (SEQ ID NO: 15) 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-1 alpha 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; SEQ ID NO: 49)
GTCCTGGAGTACAATGCCATT;

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

TTT;

(FDPS target sequence #2; SEQ ID NO: 50)
GCAGGATTTCGTTCAGCACTT;

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

TTT;

(FDPS target sequence #3; SEQ ID NO: 51)
GCCATGTACATGGCAGGAATT;

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

TTT;

(FDPS target sequence #4; SEQ ID NO: 52)
GCAGAAGGAGGCTGAGAAAGT;
and

(FDPS shRNA sequence #4; SEQ ID NO: 4)
GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCTT

TTT.

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: 53)
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGC

GTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCT

CGGACTTCAAGGGGCT

(miR30 FDPS sequence #2; SEQ ID NO: 54)
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGC

GTGAAGCCACAGATGGCAGAAGGGCTGAGAAAGTGCTGCCTACTGCCTCG

GACTTCAAGGGGCT

(miR30 FDPS sequence #3; SEQ ID NO: 55)
TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCGTGAAGCCACA

GATGGCAGAAGGAGGCTGAGAAAGTTGCCTACTGCCTCGGA

(miR155 FDPS sequence #1; SEQ ID NO: 56)
CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCTCAGCCTCCTTCTGC

TTTTGGCCACTGACTGAGCAGAAGGGCTGAGAAAGTCAGGACACAAGGCC

TGTTACTAGCACTCA

(miR21 FDPS sequence #1; SEQ ID NO: 57)
CATCTCCATGGCTGTACCACCTTGTCGGGACTTTCTCAGCCTCCTTCTGC

CTGTTGAATCTCATGGCAGAAGGAGGCGAGAAAGTCTGACATTTTGGTAT

CTTTCATCTGACCA

(miR185 FDPS sequence #1; SEQ ID NO: 58)
GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCTCAGCCTCCTTCTGC

TGGTCCCCTCCCCGCAGAAGGAGGCTGAGAAAGTCCTTCCCTCCCAATGA

CCGCGTCTTCGTCG

Combination vectors, as shown generally in FIG. 3C are also capable of being produced based on the development of the single-target vectors outlined above. An exemplary therapeutic combination vectors is shown in FIG. 3C, and includes from left to right: hybrid 5' long terminal repeat (RSV/LTR), Psi sequence (RNA packaging site), RRE (Rev-response element), cPPT (polypurine tract), EF-1alpha (EF-1 alpha promoter of gene transcription), miR30-FDPS, miR155-CD47, miR21-cMyc, Woodchuck Post-Transcriptional Regulatory Element (WPRE), and LTR with a deletion in the U3 region. The therapeutic vector detailed in FIG. 3C can be produced using the materials and methods described using the following target sequences:

miR30 FDPS sequence #1:
(SEQ ID NO: 53)
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGC

GTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCT

CGGACTTCAAGGGGCT

miR155 CD47 target sequence #1:
(SEQ ID NO: 82)
CCTGGAGGCTTGCTGAAGGCTGTATGCTGTTATCCATCTTCAAAGAGGCA

GTTTTGGCCACTGACTGACTGCCTCTTAAGATGGATAACAGGACACAAGG

CCTGTTACTAGCACTCA

-continued

miR21 cMyc sequence:

(SEQ ID NO: 83)

CATCTCCATGGCTGTACCACCTTGTCGGGTGTTCGCCTCTTGACATTCTC

CTGTTGAATCTCATGGAGAATGTCAAGGGCGAACACTGACATTTTGGTAT

CTTTCATCTGACCA

Example 3. Materials and Methods for FDPS

Inhibitory RNA Design:

The sequence of *Homo sapiens* farnesyl diphosphate synthase (FDPS), transcript variant 1, mRNA (NM_002004.3) 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 the Broad Institute or the BLOCK-iT™ RNAi Designer from Thermo Scientific. A shRNA sequence may be inserted into a lentiviral vector after a RNA polymerase III promoter such as H1, U6, or 7SK to regulate shRNA expression. The RNA sequence may also be embedded within a microRNA backbone to allow for expression by a RNA polymerase II promoter such as CMV or EF-1 alpha. The RNA sequence may also be synthesized as a siRNA oligonucleotide and utilized independently of a lentiviral vector.

Vector Construction:

For FDPS shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by MWG operon. Oligonucleotide sequences were annealed by incubation at 70 degrees Celsius and cooling to room temperature. Annealed oligonucleotides were digested with the restriction enzymes BamHI and EcoRI for one hour at 37 degrees Celsius and then the enzymes were heat-inactivated at 70 degrees Celsius for 20 minutes. In parallel, 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 Invitrogen. The DNA concentration was determined and the vector to oligo sequence was ligated in the ratio 3:1 insert to vector. The ligation reaction was carried out with T4 DNA ligase for 30 minutes at room temperature. 2.5 microliters of the ligation mix was added to 25 microliters of STBL3 competent bacterial cells. Transformation was carried out by heat-shock at 42 degrees Celsius. Bacterial cells were streaked onto agar plates containing ampicillin and then colonies were expanded in LB broth. To check for insertion of the oligo sequences, plasmid DNA was extracted from harvested bacteria cultures with the Invitrogen DNA mini prep kit. Insertion of the shRNA sequence in the lentiviral vector was verified by DNA sequencing using a specific primer for which every promoter is used to regulate shRNA expression. The lentiviral vectors containing a correct FDPS sequence were then used to package lentiviral particles to test for their ability to knockdown FDPS. Mammalian cells were transduced with lentiviral particles either in the presence or absence of polybrene. Cells were collected after 2-4 days and protein and RNA was analyzed for FDPS expression.

Functional Assay for mRNA Reduction:

The effect of different FDPS short homology RNA (shRNA) targeting sequences on FDPS expression was determined by measuring mRNA expression. HepG2 hepatocellular carcinoma cells were transduced with a lentiviral vector containing FDPS shRNA sequences. After 48 hours, cells were lysed and RNA was extracted using the RNeasy mini kit from Qiagen. cDNA was then synthesized from RNA using SuperScript VILO from Invitrogen. The samples were then analyzed by quantitative RT-PCR using an Applied Biosystems StepOne PCR machine. FDPS expression was detected with SYBR Green from Invitrogen using the forward primer (5'-AGGAATTGATGGCGA-GAAGG-3') (SEQ ID NO: 59) and reverse primer (5'-CCCAAAGAGGTCAAGGTAATCA-3') (SEQ ID NO: 60) with standard conditions for polymerase chain reaction analysis. The samples were normalized to the mRNA for beta-actin gene expression using the forward primer (5'-AGCGCGGCTACAGCTTCA-3') (SEQ ID NO: 61) and reverse primer (5'-GGCGACGTAGCACAGCTTCT-3') (SEQ ID NO: 62) with standard conditions for polymerase chain reaction analysis. The relative expression of FDPS was determined by its Ct value normalized to the level of actin for each sample.

Functional Assay for Tumor Cells Modified by LV-FDPS and Used to Activate Cytokine Production in Human Gamma Delta T Cells:

The LV-FDPS vector was also used to treat tumor cells that were then exposed to primary human gamma delta T cells from healthy donors. Combined treatment of tumor cell line with both aminobisphosphonate and vector that suppresses farnesyl pyrophosphate synthase (FDPS) has a synergistic effect on gamma delta T cell production of TNF-alpha. THP1 monocytoid tumor cell line (A) or HepG2 monocytoid tumor cell line (B) were treated with lentiviral control vectors (LV-Control), lentiviral vectors expressing shRNA to down regulate FDPS (LV-FDPS), zoledronic acid (Zol), zoledronic acid plus lentiviral control (Zol+LV-Control), or zoledronic acid plus lentiviral vectors expressing shRNA to down regulate FDPS (Zol+LV-FDPS). Treated cells were mixed with gamma delta T cells at 1:1 ratio for 4 hours. TNF-alpha production by gamma delta T cells was detected by intracellular staining and flow cytometry.

Functional Assay for Tumor Cells Modified by LV-FDPS and Used to Activate Tumor Cell Killing by Human Gamma Delta T Cells:

Monocytoid tumor cells (THP-1) were transduced with lentivirus vector that suppresses FDPS mRNA, then used to activate tumor cell cytotoxicity in normal human gamma delta T cells. The activated gamma delta T cells were recovered after 4 hours of exposure to transduced THP-1 cells, then used in a cytotoxicity assay to kill unmodified THP-1. When gamma delta T cells were stimulated with a combination of transduced THP-1 cells and 10 micromolar zoledronic acid, >70% killing of THP-1 was observed at a ratio of 4 gamma delta T cells to 1 THP-1 cell.

Experimental Data for FDPS

The FDPS shRNA sequences depicted in Table 2 were utilized in the experiments described herein. Further, the sequences detailed in Table 2 can be used in the therapeutic vectors detailed herein.

TABLE 2

FDPS shRNA sequences

Description	shRNA oligonucleotide (sense sequence - loop - antisense sequence	SEQ ID NO
FDPS-1	GTCCTGGAGTACAATGCCATT**CTCGAG**AATGGCATT GTACTCCAGGACTTTTT	1

TABLE 2-continued

FDPS shRNA sequences

Description	shRNA oligonucleotide (sense sequence - loop - antisense sequence	SEQ ID NO
FDPS-2	GCAGGATTTCGTTCAGCACTT**CTCGAG**AAGTGCTGA ACGAAATCCTGCTTTTT	2
FDPS-3	GCCATGTACATGGCAGGAATT**CTCGAG**AATTCCTGC CATGTACATGGCTTTTT	3
FDPS-4	GCAGAAGGAGGCTGAGAAAGT**CTCGAG**ACTTTCTCA GCCTCCTTCTGCTTTTT	4

As shown in FIG. **4**A, the relative expression level of human FDPS following administration of the four different FDPS shRNA sequences was determined. The most significant inhibition of human FDPS expression was found in the FDPS-2 and FDPS-4 samples (as shown in FIG. **4**A, herein).

Further, as shown in FIG. **4**B, a lentiviral-based delivery system was used to target FDPS expression. HepG2 human hepatocellular carcinoma cells were infected with lentiviral vectors containing either the H1 promoter and a FDPS shRNA (SEQ ID NO: 4) sequence or the EF-1alpha promoter and the following miR30-based FDPS sequences:

miR30 FDPS sequence #1:
(SEQ ID NO: 53)
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGC

GTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCT

CGGACTTCAAGGGGCT

miR30 FDPS sequence #2:
(SEQ ID NO: 54)
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGC

GTGAAGCCACAGATGGCAGAAGGGCTGAGAAAGTGCTGCCTACTGCCTCG

GACTTCAAGGGGCT

After 48 hours, cells were lysed and an immunoblot was performed using an anti-FDPS (Thermo Scientific) and an anti-actin (Sigma) antibody for a protein loading control. As shown in FIG. **4**B, treatment with the FDPS shRNA significantly decreased FDPS protein expression. Treatment with the miR30-based FDPS sequences decreased FDPS expression.

As shown in FIG. **5**, monocytoid (THP-1) (FIG. **5**A) or hepatocellular (HepG2) (FIG. **5**B) cancer cells transduced with lentivirus containing shRNA capable of suppressing FDPS mRNA activated cytokine expression in human gamma delta T cells.

This portion of the Example illustrates that knock-down of FDPS in THP1 monocytic leukemia cells by lentiviral (LV)-expressing FDPS shRNA (SEQ ID NO: 4; which is also referred to herein as LV-FDPS shRNA #4) stimulates TNF-α expression in gamma delta T cells, as shown in FIG. **5**A.

THP1 cells (1×10^5 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 5×10^5 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 3.11% 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 56.17%.

The same conditions were used with HepG2 cells and the following data was generated. Without zoledronic acid, LV-control stimulated 2.5% of TNF-α expressing Vγ9Vδ2 T cells and LV-FDPS shRNA #4 stimulated 3.33%. With zoledronic acid treatment, LV-control stimulated 9.1% of TNF-α expressing Vγ9Vδ2 T cells and LV-FDPS shRNA #4 stimulated 45.7%.

Further as shown in FIG. **6**, monocytoid (THP-1) tumor cells transduced with lentivirus capable of suppressing FDPS mRNA activate tumor cell cytotoxicity in normal human gamma delta T cells.

This portion of the Example demonstrates results from mixing treated THP-1 monocytoid tumor cells with cultured human GD T cells, as shown in FIG. **6**.

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. **6**, was: lentiviral control vectors (LV-Control), lentiviral vectors expressing microRNA to down regulate FDPS (LV-FDPS), Zometa (Zol), Zometa plus lentiviral control (Zol+LV-Control), or Zometa plus lentiviral vectors expressing microRNA to down regulate FDPS (Zol+LV-FDPS).

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 4. Materials and Methods for CD47

Inhibitory RNA Selection:

The sequence of *Homo sapiens* CD47 molecule (CD47) mRNA (NM_001777) was used to search for potential siRNA or shRNA candidates capable of reducing CD47 levels in human cells. Potential RNA interference sequences were chosen from candidates selected by siRNA or shRNA design programs such as from the Broad Institute or the BLOCK-iT™ RNAi Designer from Thermo Scientific. Initially, individual selected shRNA sequences were inserted into lentiviral vectors immediately 3' to a RNA polymerase III promoter such as H1, U6, or 7SK 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 CMV or EF-1alpha RNA polymerase II promoters. RNA sequences have also

been synthesized as synthetic siRNA oligonucleotides and introduced directly into cells without using a lentiviral vector.

Vector Construction:

For CD47 shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by Eurofins MWG Operon, LLC. Overlapping sense and antisense oligonucleotide sequences were mixed and annealed during incubation at 70 degrees Celsius before being cooled to room temperature and extending the unpaired ends with DNA polymerase before cooling to room temperature. The extension reaction created double stranded sequences at each end of the oligonucleotide that contain restriction enzyme sites BamHI and EcoRI. The double stranded oligonucleotides were digested with the restriction enzymes BamHI and EcoRI for one hour at 37 degrees Celsius and the enzymes were heat-inactivated at 70 degrees Celsius for 20 minutes. In parallel, 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 Invitrogen. 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, purified and expanded in LB broth. To check for insertion of the oligo sequences, plasmid DNA were extracted from harvested bacteria cultures with the Invitrogen DNA mini prep kit. Insertion of the shRNA sequence in the lentiviral vector was verified by DNA sequencing using a specific primer for the promoter used to regulate shRNA expression.

Functional Assay:

The effect of different CD47 shRNA targeting sequences on CD47 expression was determined by measuring mRNA expression. Hep3B hepatocellular carcinoma cells were transduced with a lentiviral vector containing CD47 shRNA sequences. After 48 hours, cells were lysed and RNA was extracted using the RNeasy mini kit from Qiagen. cDNA was then synthesized from RNA using SuperScript VILO from Invitrogen. The samples were then analyzed by quantitative RT-PCR using an Applied Biosystems StepOne PCR machine. CD47 expression was detected with SYBR Green from Invitrogen using the forward primer (5'-CACTGTCGTCATTCCATGCT-3') (SEQ ID NO: 63) and reverse primer (5'-GCCTCTTGACATTCTCCTC-3') (SEQ ID NO: 64). The samples were normalized by measuring actin expression using the forward primer (5'-AGCGCGGCTACAGCTTCA-3') (SEQ ID NO: 61) and reverse primer (5'-AAAGTCAGTGGGGACAGTGG-3') (SEQ ID NO: 65). The relative expression of CD47 was determined by its Ct value normalized to the level of actin for each sample.

Experimental Data for CD47

The non-limiting examples of CD47 shRNA target sequences depicted in Table 3 were utilized in the experiments described herein. Further, the sequences detailed in Table 3 can be used in the therapeutic vectors detailed herein.

TABLE 3

CD47 shRNA sequences

Description	shRNA oligonucleotide (sense sequence - loop - antisense sequence	SEQ ID NO
CD47 sequence 1	GGTGAAACGATCATCGAGC**CTCGAG**GCTCGATGATC GTTTCACCTTTTT	5
CD47 sequence 2	GCTACTGGCCTTGGTTTAA**CTCGAG**TTAAACCAAGG CCAGTAGCTTTTT	6
CD47 sequence 3	CCTCCTTCGTCATTGCCAT**CTCGAG**ATGGCAATGAC GAAGGAGGTTTTT	7
CD47 sequence 4	GCATGGCCCTCTTCTGATT**CTCGAG**AATCAGAAGAG GGCCATGCTTTTT	8
CD47 sequence 5	GGTGAAACGATCATCGAGCTA**CTCGAG**TAGCTCGAT GATCGTTTCACCTTTTT	9

As shown in FIG. **7**A, the relative expression level of human CD47 following administration of the four different CD47 shRNA sequences was determined. The most significant inhibition of human CD47 expression was found in the shCD47-1 and shCD47-3 samples (as shown in FIG. **7**A, herein).

Further, as shown in FIG. **7**B, a lentiviral-based delivery system was used to target CD47 expression. SNU449 human hepatocellular carcinoma cells were infected with lentiviral vectors containing the following miR155-based CD47 sequences:

miR155 CD47 target sequence #1:
(SEQ ID NO: 82)
CCTGGAGGCTTGCTGAAGGCTGTATGCTGTTATCCATCTTCAAAGAGGCA

GTTTTGGCCACTGACTGACTGCCTCTTAAGATGGATAACAGGACACAAGG

CCTGTTACTAGCACTCA

miR155 CD47 target sequence #2:
(SEQ ID NO: 66)
CCTGGAGGCTTGCTGAAGGCTGTATGCTGTTAGCTCGATGATCGTTTCAC

GTTTTGGCCACTGACTGACGTGAAACGCATCGAGCTAACAGGACACAAGG

CCTGTTACTAGCACTCA

miR155 CD47 target sequence #3:
(SEQ ID NO: 67)
CCTGGAGGCTTGCTGAAGGCTGTATGCTGAAGAATGGCTCCAACAATGAC

GTTTTGGCCACTGACTGACGTCATTGTGAGCCATTCTTCAGGACACAAGG

CCTGTTACTAGCACTCA

miR155 CD47 target sequence #4:
(SEQ ID NO: 68)
CCTGGAGGCTTGCTGAAGGCTGTATGCTGTATACACGCCGCAATACAGAG

GTTTTGGCCACTGACTGACCTCTGTATCGGCGTGTATACAGGACACAAGG

CCTGTTACTAGCACTCA

As shown in FIG. **7**B, treatment with the CD47 shRNA significantly decreased FDPS protein expression. Treatment with the miR155-based CD47 sequences significant decreased CD47 expression.

Example 5. Materials and Methods for cMyc

Inhibitory RNA Design: The mRNA sequence of *Homo sapiens* v-myc avian myelocytomatosis viral oncogene

homolog (MYC) (NM_002467.4) was used to screen for potential shRNA candidates to knock-down MYC expression in hepatocellular cell lines. We obtained five MYC shRNA sequences which can reduce MYC expression. Potential RNA interference sequences were chosen from candidates selected by siRNA or shRNA design programs such as from the Broad Institute or the BLOCK-iT™ RNAi Designer from Thermo Scientific. A shRNA sequence may be inserted into a lentiviral vector after a RNA polymerase III promoter such as H1, U6, or 7SK to regulate shRNA expression. The RNA sequence may also be embedded within a microRNA backbone to allow for expression by a RNA polymerase II promoter such as CMV or EF-1 alpha. The RNA sequence may also be synthesized as a siRNA oligonucleotide and utilized independently of a lentiviral vector.

Vector Construction:

For cMyc shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by MWG operon. Oligonucleotide sequences were annealed by incubation at 70 degrees Celsius and cooling to room temperature. Annealed oligonucleotides were digested with the restriction enzymes BamHI and EcoRI for one hour at 37 degrees Celsius and then the enzymes were heat-inactivated at 70 degrees Celsius for 20 minutes. In parallel, 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 Invitrogen. The DNA concentration was determined and the vector to oligo sequence was ligated in the ratio 3:1 insert to vector. The ligation reaction was carried out with T4 DNA ligase for 30 minutes at room temperature. 2.5 microliters of the ligation mix was added to 25 microliters of STBL3 competent bacterial cells. Transformation was carried out by heat-shock at 42 degrees Celsius. Bacterial cells were streaked onto agar plates containing ampicillin and then colonies were expanded in LB broth. To check for insertion of the oligo sequences, Plasmid DNA was extracted from harvested bacteria cultures with the Invitrogen DNA mini prep kit. Insertion of the shRNA sequence in the lentiviral vector was verified by DNA sequencing using a specific primer for which ever promoter is used to regulate shRNA expression. The lentiviral vectors containing a correct cMyc sequence were then used to package lentiviral particles to test for their ability to knock-down FDPS. Mammalian cells were transduced with lentiviral particles either in the presence or absence of polybrene. Cells were collected after 2-4 days and protein and RNA was analyzed for cMyc expression.

Functional Assay:

The effect of different cMyc shRNA targeting sequences on cMyc expression was determined by measuring mRNA expression. HepG2 hepatocellular carcinoma cells were transduced with a lentiviral vector containing cMyc shRNA sequences. After 48 hours, cells were lysed and RNA was extracted using the RNeasy mini kit from Qiagen. cDNA was then synthesized from RNA using SuperScript VILO from Invitrogen. The samples were then analyzed by quantitative PCR using an Applied Biosystems StepOne PCR machine. cMyc expression was detected with SYBR Green from Invitrogen using the forward primer (5'-GGACTATCCTGCTGCCAA-3') (SEQ ID NO: 69) and reverse primer (5'-GCCTCTTGACATTCTCCTC-3') (SEQ ID NO: 64). The samples were normalized by measuring actin expression using the forward primer (5'-AGCGCGGCTACAGCTTCA-3') (SEQ ID NO: 61) and reverse primer (5'-GGCGACGTAGCACAGCTTCT-3') (SEQ ID NO: 62). The relative expression of cMyc was determined by its Ct value normalized to the level of actin for each sample.

Experimental Data for cMyc

The non-limiting examples of cMyc shRNA sequences depicted in Table 4 below were utilized in the experiments described herein.

TABLE 4

cMyc shRNA sequences

Description	shRNA oligonucleotide (sense sequence - loop - antisense sequence	SEQ ID NO
cMyc shRNA Sequence 1	GCTTCACCAACAGGAACTATG**CTCGAG**CATAGTTCC TGTTGGTGAAGCTTTT	10
cMyc shRNA Sequence 2	GCGAACACACAACGTCTTGGA**CTCGAG**TCCAAGACG TTGTGTGTTCGCTTTT	11
cMyc shRNA Sequence 3	GACATGGTGAACCAGAGTTTC**CTCGAG**GAAACTCTG GTTCACCATGTCTTTTT	12
cMyc shRNA Sequence 4	GAGAATGTCAAGAGGCGAACA**CTCGAG**TGTTCGCCT CTTGACATTCTCTTTTT	13
cMyc shRNA Sequence 5	GCTCATTTCTGAAGAGGACTT**CTCGAG**AAGTCCTCT TCAGAAATGAGCTTTTT	14

As shown in FIG. **8**A, the relative expression level of human cMyc following administration of the five different cMyc shRNA sequences was determined. The most significant inhibition of human cMyc expression was found in the myc-2 sample (as shown in FIG. **8**A, herein).

Further, as shown in FIG. **8**B, SNU449 human hepatocellular carcinoma cells were infected with lentiviral vectors containing either the following miR-based cMYC sequences or a cMyc shRNA:

miR155 cMyc sequence:
(SEQ ID NO: 70)
CCTGGAGGCTTGCTGAAGGCTGTATGCTGTGTTCGCCTCTTGACATTCTC

TTTTGGCCACTGACTGAGAGAATGTAGAGGCGAACACAGGACACAAGGCC

TGTTACTAGCACTCA

miR21 cMyc sequence:
(SEQ ID NO: 83)
CATCTCCATGGCTGTACCACCTTGTCGGGTGTTCGCCTCTTGACATTCTC

CTGTTGAATCTCATGGAGAATGTCAAGGGCGAACACTGACATTTTGGTAT

CTTTCATCTGACCA

The above two cMyc sequences were generated using the below target sequence:

cMyc target sequence:
(SEQ ID NO: 71)
GAGAATGTCAAGAGGCGAACA

cMyc shRNA sequence:
(SEQ ID NO: 13)
GAGAATGTCAAGAGGCGAACACTCGAGTGTTCGCCTCTTGACATTCTCTT

TTT

After 48 hours, cells were lysed and an immunoblot was performed using an anti-cMyc (Santa Cruz) and an anti-actin (Sigma) antibody for a protein loading control. As shown in FIG. **8**B, treatment with the cMyc shRNA significantly

decreased cMyc protein expression. Treatment with the miR-based cMyc sequences also decreased cMyc expression.

Example 6. In Vivo Treatment with FDPS-shRNA and Zoledronic Acid

Protocol Overview for Co-Administration of LV-shRNA-FDPS (Farnesyl Diphosphate Synthase) with or without Zoledronic Acid in Mice Implanted with Human Prostate Cancer Cell Line PC3.

Tumor cells were cultured in vitro, then transduced with lentivirus vector control with a scrambled sequence (non-functional) shRNA insert and an expression cassette for firefly luciferase, or LV-FDPS with a shRNA capable of reducing expression of FDPS mRNA and an expression cassette for firefly luciferase. The transduced tumor cells were implanted on the flank of immune deficient mice by subcutaneous injection. Once tumors reached approximately 200 mm^3 volume, all mice receive a single dose of zoledronic acid (100 micrograms per kilogram body weight, which is similar to a standard human dose) in saline. 7 days after zoledronic acid injection, an imaging study was repeated to measure volume and photon intensity of individual tumors.

The LV-FDPS vector designed, developed, and utilized in this Example is shown diagrammatically in FIG. **9**. The LV-FDPS vector was developed using the methods and materials described herein. The following sequences were used and, as described below, a CMV GFP T2A luciferase sequence was generated and introduced into the therapeutic vector.

CMV promoter sequence:

(SEQ ID NO: 72)

```
ATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATC

TACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACAT

CAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACC

CCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTT

CCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCG

TGTACGGTGGGAGGTTTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGA

TCGCCTGGAGACGCCATCCACGCTGTTTT
```

GFP T2A Luciferase sequence:

(SEQ ID NO: 73)

```
ATGCCCGCCATGAAGATCGAGTGCCGCATCACCGGCACCCTGAACGGCGT

GGAGTTCGAGCTGGTGGGCGGCGGAGAGGGCACCCCCGAGCAGGGCCGCA

TGACCAACAAGATGAAGAGCACCAAAGGCGCCCTGACCTTCAGCCCCTAC

CTGCTGAGCCACGTGATGGGCTACGGCTTCTACCACTTCGGCACCTACCC

CAGCGGCTACGAGAACCCCTTCCTGCACGCCATCAACAACGGCGGCTACA

CCAACACCCGCATCGAGAAGTACGAGGACGGCGGCGTGCTGCACGTGAGC

TTCAGCTACCGCTACGAGGCCGGCCGCGTGATCGGCGACTTCAAGGTGGT

GGGCACCGGCTTCCCCGAGGACAGCGTGATCTTCACCGACAAGATCATCC

GCAGCAACGCCACCGTGGAGCACCTGCACCCCATGGGCGATAACGTGCTG

GTGGGCAGCTTCGCCCGCACCTTCAGCCTGCGCGACGGCGGCTACTACAG

CTTCGTGGTGGACAGCCACATGCACTTCAAGAGCGCCATCCACCCCAGCA
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-continued

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TCCTGCAGAACGGGGGCCCCATGTTCGCCTTCCGCCGCGTGGAGGAGCTG

CACAGCAACACCGAGCTGGGCATCGTGGAGTACCAGCACGCCTTCAAGAC

CCCCATCGCCTTCGCCAGATCTCGAGATATCAGCCATGGCTTCCCGCCGG

CGGTGGCGGCGCAGGATGATGGCACGCTGCCCATGTCTTGTGCCCAGGAG

AGCGGGATGGACCGTCACCCTGCAGCCTGTGCTTCTGCTAGGATCAATGT

GACCGGTGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGA

ATCCCGGCCCTTCCGGTATGGAAGACGCCAAAAACATAAAGAAAGGCCCG

GCGCCATTCTATCCGCTAGAGGATGGAACCGCTGGAGAGCAACTGCATAA

GGCTATGAAGAGATACGCCCTGGTTCCTGGAACAATTGCTTTTACAGATG

CACATATCGAGGTGAACATCACGTACGCGGAATACTTCGAAATGTCCGTT

CGGTTGGCAGAAGCTATGAAACGATATGGGCTGAATACAAATCACAGAAT

CGTCGTATGCAGTGAAAACTCTCTTCAATTCTTTATGCCGGTGTTGGGCG

CGTTATTTATCGGAGTTGCAGTTGCGCCCGCGAACGACATTTATAATGAA

CGTGAATTGCTCAACAGTATGAACATTTCGCAGCCTACCGTAGTGTTTGT

TTCCAAAAAGGGGTTGCAAAAAATTTTGAACGTGCAAAAAAAATTACCAA

TAATCCAGAAAATTATTATCATGGATTCTAAAACGGATTACCAGGGATTT

CAGTCGATGTACACGTTCGTCACATCTCATCTACCTCCCGGTTTTAATGA

ATACGATTTTGTACCAGAGTCCTTTGATCGTGACAAAACAATTGCACTGA

TAATGAACTCCTCTGGATCTACTGGGTTACCTAAGGGTGTGGCCCTTCCG

CATAGAACTGCCTGCGTCAGATTCTCGCATGCCAGAGATCCTATTTTTGG

CAATCAAATCATTCCGGATACTGCGATTTTAAGTGTTGTTCCATTCCATC

ACGGTTTTGGAATGTTTACTACACTCGGATATTTGATATGTGGATTTCGA

GTCGTCTTAATGTATAGATTTGAAGAAGAGCTGTTTTTACGATCCCTTCA

GGATTACAAAATTCAAAGTGCGTTGCTAGTACCAACCCTATTTTCATTCT

TCGCCAAAAGCACTCTGATTGACAAATACGATTTATCTAATTTACACGAA

ATTGCTTCTGGGGGCGCACCTCTTTCGAAAGAAGTCGGGGAAGCGGTTGC

AAAACGCTTCCATCTTCCAGGGATACGACAAGGATATGGGCTCACTGAGA

CTACATCAGCTATTCTGATTACACCCGAGGGGGATGATAAACCGGGCGCG

GTCGGTAAAGTTGTTCCATTTTTTGAAGCGAAGGTTGTGGATCTGGATAC

CGGGAAAACGCTGGGCGTTAATCAGAGAGGCGAATTATGTGTCAGAGGAC

CTATGATTATGTCCGGTTATGTAAACAATCCGGAAGCGACCAACGCCTTG

ATTGACAAGGATGGATGGCTACATTCTGGAGACATAGCTTACTGGGACGA

AGACGAACACTTCTTCATAGTTGACCGCTTGAAGTCTTTAATTAAATACA

AAGGATACCAGGTGGCCCCCGCTGAATTGGAGTCGATATTGTTACAACAC

CCCAACATCTTCGACGCGGGCGTGGCAGGTCTTCCCGACGATGACGCCGG

TGAACTTCCCGCCGCCGTTGTTGTTTTGGAGCACGGAAAGACGATGACGG

AAAAAGAGATCGTGGATTACGTCGCCAGTCAAGTAACAACCGCGAAAAAG

TTGCGCGGAGGAGTTGTGTTTGTGGACGAAGTACCGAAAGGTCTTACCGG

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GCGGAAAGTCCAAATTGTAA
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H1 promoter sequence:

(SEQ ID NO: 15)

GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGCCCAGTGTCAC

TAGGCGGGAACACCCAGCGCGCGTGCGCCCTGGCAGGAAGATGGCTGTGA

GGGACAGGGGAGTGGCGCCCTGCAATATTTGCATGTCGCTATGTGTTCTG

GGAAATCACCATAAACGTGAAATGTCTTTGGATTTGGGAATCTTATAAGT

TCTGTATGAGACCACTT

LV FDPS GFP T2A Luc Construction:

The pGF-1 plasmid (System Biosciences) containing the CMV GFP T2A luciferase sequence was digested with ClaI and KPN1 and the LV-H1-shFDPS plasmid was digested with BstBI and KpnI restriction enzymes (NEB). The DNA was electrophoresed on a 1% agarose gel and the DNA fragments were extracted with a DNA gel extraction kit (Thermo Scientific). The two fragments were ligated with T4 DNA ligase (NEB) and transformed into STBL3 bacteria (Thermo Scientific). Plasmid DNA was extracted from bacteria with a plasmid DNA mini prep kit (Thermo Scientific) and the sequence was verified by DNA sequencing (Eurofins Genomics).

Detailed Experimental Protocol

Day −19: 175 ml flask grown confluently yields 1.87×10^7 ml of PC3 cells; 75 ml flask grown confluently yields 7.5×10^6 ml of PC3 cells.

Day −7: Thaw and grow PC3 cells

Day −4: Material Preparation and Delivery. Prepare lenti-vector control and lenti-shRNA-FDPS transduced PC3 cells.

1. In a 75 ml of flask, 50% confluent PC3 cells, add 12 μl of lenti-control+8 μl of polybrene, incubate for 5 min. then mix with 4 ml of RPMI-10, and cover the surface of PC3 cells.
2. In a 75 ml of flask, 50% confluent PC3 cell, add 20 μl of lenti-FDPS+8 μl of polybrene, incubate for 5 min. then mix with 4 ml of RPMI-10, and cover the surface of PC3 cells.
3. Incubate transduced cells at 37° C. for 8 hr. Add 6 ml of RPMI-10 for overnight culture.

Day −2: Trypsinize 75 ml transduced PC3 cells (confluent 7.5×10^6 cells) and transfer to 175 ml Flask.

Day 0: Material Preparation and Delivery

1. Trypsinize the 80% confluent lenti-vector and lenti-FDPS transduced PC3 cells separately and count cells.
   lenti-vector: 1.5×10^8 cells ($50\times3\times10^{6/5}$ ml) 15 flask
   lenti-FDPS: 1.5×10^8 cells ($50\times3\times10^{6/5}$ ml) 20 flask
2. Resuspend lenti-vector and lenti-FDPS transduced PC3 cells in RPMI without FBS, make the final concentration in 3×10^6 cells/100 μl
   Material: I) 5 ml of PC3-Lenti-vector cells (total 150×10^6 cells) in RPMI without FBS; II) 5 ml of PC3-Lenti-FDPS cells (total 150×10^6 cells) in RPMI without FBS.

Day 0: Subcutaneous injection of PC3 cells. Group I (2 NOD/SCID mice): 0.15 ml of PC3-Lenti-vector cells (0.1 mL of 3×10^6 Lenti-vector in RPMI without FBS+0.05 mL of Matrigel) are subcutaneously inoculated into either the right or left flanks of mice (total 5 ml enough for 50 mice). Group II (3 NOD/SCID mice): 0.15 ml of PC3-Lenti-FDPS KD (0.1 mL of 3×10^6 Lenti-vector in DMEM without FBS+0.05 mL of Matrigel) are subcutaneously inoculated either the right or left flanks of mice (total 5 ml enough for 50 mice).

Day 8: Monitor tumor. Tumor is palpable in the first few days after implantation. Determine tumor size by measuring the perpendicular diameters of tumor with calipers. Tumor size is calculating by following measurement: Tumor volume (mm^3)=d^2 (d=the shortest diameter)×D/2 (D=the longest diameter). Perform bioluminescence imaging to demonstrate tumor location, size and photon intensity as a measure of lentivirus expression of the firefly luciferase gene.

Day 14: Intraperitoneal injection of 100 μg/ml of zoledronic acid (Zol) or PBS to mice when tumor size reaches 200~300 mm^3.

Day 22: Imaging study to measure tumor size.

Effects of LV-shRNA-FDPS with or without Zoledronic Acid on PC3 Tumor Growth in NOD/SCID Mice.

Mice were designated Scr (for scrambled vector control) or KO for LV-shRNA-FDPS. LV used for this study all express the bioluminescence marker firefly luciferase to enable direct visualization of transduced cells and their growth. A bioluminescence imaging study on Day 8 determined the average tumor sizes prior to zoledronic acid treatment (FIG. **10**A). The photon intensity for tumors was measured with a CCD light capture system. The average size of tumor in the Scr animals was slightly larger than was found in the KO animals (FIG. **10**B) but differences were not significant.

6 days after treatment with zoledronic acid (all animals received zoledronic acid by intraperitoneal injection), the imaging study was repeated. Tumor size and location for Scr animals (FIG. **10**C) was similar to earlier observations but there were notable differences in tumor size for animals in the KO group. Tumor volume was reduced sharply in KO #1 and KO #3, and tumor was no longer present in KO #2. Comparing the average photon intensities for Scr and KO groups (FIG. **10**D) revealed a substantial difference with the greatest change seen in the KO group.

These data show that LV-shRNA-FDPS has a small but detectable impact on growth of PC3 tumors in NOD/SCID mice. When combined with a single dose of zoledronic acid, the effect was magnified and eradication of LV-shRNA-FDPS transduced cells was achieved in one case. Thus, light-emitting transduced cells decreased by zoledronic acid only if the LV expressed a shRNA-FDPS. The reduction in tumor mass was not attributable to zoledronic acid treatment because animals with tumors transduced with scrambled control LV showed little or no change in tumor mass after zoledronic acid treatment.

The key to tumor reduction was the combined effect of LV-shRNA-FDPS reducing the levels of FDPS enzyme expression and zoledronic acid inhibiting any residual FDPS activity. As expected, the zoledronic acid was not toxic or mice and had no apparent effects other than reducing tumor mass when combined with LV-shRNA-FDPS. Zoledronic acid is a safe and effective treatment in humans where it is given in high bolus doses or as a chronic therapy for bone demineralization disorders including osteoporosis.

The disclosure of the example embodiments is intended to be illustrative, but not limiting, of the scope of the inventions, which are set forth in the following claims and their equivalents. Although example embodiments of the inventions have been described in some detail for purposes of clarity of understanding, it will be apparent that certain changes and modifications can be practiced within the scope of the following claims. In the following claims, elements and/or steps do not imply any particular order of operation,

unless explicitly stated in the claims or implicitly required by the disclosure.

Sequences

The following sequences are referred to herein:

SEQ ID NO:	Description	Sequence
1	FDPS shRNA sequence #1	GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTAC TCCAGGACTTTTT
2	FDPS shRNA sequence #2	GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCTGAACGA AATCCTGCTTTTT
3	FDPS shRNA sequence #3	GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATG TACATGGCTTTTT
4	FDPS shRNA sequence #4	GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCT CCTTCTGCTTTTT
5	CD47 shRNA sequence #1	GGTGAAACGATCATCGAGCCTCGAGGCTCGATGATCGTTT CACCTTTTT
6	CD47 shRNA sequence #2	GCTACTGGCCTTGGTTTAACTCGAGTTAAACCAAGGCCAG TAGCTTTTT
7	CD47 shRNA sequence #3	CCTCCTTCGTCATTGCCATCTCGAGATGGCAATGACGAAG GAGGTTTTT
8	CD47 shRNA sequence #4	GCATGGCCCTCTTCTGATTCTCGAGAATCAGAAGAGGGCC ATGCTTTTT
9	CD47 shRNA sequence #5	GGTGAAACGATCATCGAGCTACTCGAGTAGCTCGATGATC GTTTCACCTTTTT
10	cMyc shRNA sequence #1	GCTTCACCAACAGGAACTATGCTCGAGCATAGTTCCTGTT GGTGAAGCTTTT
11	cMyc shRNA sequence #2	GCGAACACACAACGTCTTGGACTCGAGTCCAAGACGTTGT GTGTTCGCTTTT
12	cMyc shRNA sequence #3	GACATGGTGAACCAGAGTTTCCTCGAGGAAACTCTGGTTC ACCATGTCTTTTT
13	cMyc shRNA sequence #4	GAGAATGTCAAGAGGCGAACACTCGAGTGTTCGCCTCTTG ACATTCTCTTTTT
14	cMyc shRNA sequence #5	GCTCATTTCTGAAGAGGACTTCTCGAGAAGTCCTCTTCAG AAATGAGCTTTTT
15	H1 promoter	GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGC CCAGTGTCACTAGGCGGGAACACCCAGCGCGCGTGCGCCC TGGCAGGAAGATGGCTGTGAGGGACAGGGGAGTGGCGCC CTGCAATATTTGCATGTCGCTATGTGTTCTGGGAAATCACC ATAAACGTGAAATGTCTTTGGATTTGGGAATCTTATAAGTT CTGTATGAGACCACTT
16	U6 promoter	GAGGGCCTATTTCCCATGATTCCTTCATATTTGCATATACG ATACAAGGCTGTTAGAGAGATAATTGGAATTAATTTGACT GTAAACACAAAGATATTAGTACAAAATACGTGACGTAGA AAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATG TTTTAAAATGGACTATCATATGCTTACCGTAACTTGAAAGT ATTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGA AACACC
17	7SK promoter	CTGCAGTATTTAGCATGCCCCACCCATCTGCAAGGCATTCT GGATAGTGTCAAAACAGCCGGAAATCAAGTCCGTTTATCT CAAACTTTAGCATTTTGGGAATAAATGATATTTGCTATGCT GGTTAAATTAGATTTTAGTTAAATTTCCTGCTGAAGCTCTA GTACGATAAGCAACTTGACCTAAGTGTAAAGTTGAGATTT CCTTCAGGTTTATATAGCTTGTGCGCCGCCTGGCTACCTC
18	CAG enhancer	TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATA GCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAA TGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTG ACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAG GGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTA AACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCA AGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCG CCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCT ACTTGGCAGTACATCTACGTATTAGTCATC
19	CAG promoter	GCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCTTC ACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTA

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		GCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGG
		CGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCG
		CGCGGCGGGCG
20	chicken beta actin intron	GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGC
		CGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTTA
		CTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCGG
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21	HIV gag	ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTA
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22	HIV Pol	ATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGG
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23	HIV Int	TTTTTAGATGGAATAGATAAGGCCCAAGAAGAACATGAGA
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25	HIV Rev	ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAACTCCTC
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26	rabbit beta globin poly A	AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGA
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		ACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATC
27	CMV Promoter	ACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGG
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28	beta globin intron	GTGAGTTTGGGGACCCTTGATTGTTCTTTCTTTTTCGCTATT
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29	VSV-G/DNA fragment containing VSV-G	GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTTATTCAT
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		AGAAGGTTGGTTCAGTAGTTGGAAAAGCTCTATTGCCTCT
		TTTTTCTTTATCATAGGGTTAATCATTGGACTATTCTTGGTT
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30	rabbit beta globin poly A	AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATGA
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		TATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTC
		ACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACAT
		CAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCC
		ATATGCTGGCTGCCATGAACAAAGGTTGGCTATAAAGAGG
		TCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTAT
		TCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATA
		TTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAAATTT
		TCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGA
		CTACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGGAGATC
31	Primer	TAAGCAGAATTCATGAATTTGCCAGGAAGAT
32	Primer	CCATACAATGAATGGACACTAGGCGGCCGCACGAAT
33	Gag, Pol, Integrase fragment	GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAAATG
		ATAGGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTATG
		ATCAGATACTCATAGAAATCTGCGGACATAAAGCTATAGG
		TACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGA
		AGAAATCTGTTGACTCAGATTGGCTGCACTTTAAATTTTCC
		CATTAGTCCTATTGAGACTGTACCAGTAAAATTAAAGCCA
		GGAATGGATGGCCCAAAAGTTAAACAATGGCCATTGACA
		GAAGAAAAAATAAAAGCATTAGTAGAAATTTGTACAGAA
		ATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAA
		AATCCATACAATACTCCAGTATTTGCCATAAAGAAAAAAG
		ACAGTACTAAATGGAGAAAATTAGTAGATTTCAGAGAACT
		TAATAAGAGAACTCAAGATTTCTGGGAAGTTCAATTAGGA
		ATACCACATCCTGCAGGGTTAAAACAGAAAAAATCAGTAA
		CAGTACTGGATGTGGGCGATGCATATTTTTCAGTTCCCTTA
		GATAAAGACTTCAGGAAGTATACTGCATTTACCATACCTA
		GTATAAACAATGAGACACCAGGGATTAGATATCAGTACAA
		TGTGCTTCCACAGGGATGGAAAGGATCACCAGCAATATTC
		CAGTGTAGCATGACAAAAATCTTAGAGCCTTTTAGAAAAC
		AAAATCCAGACATAGTCATCTATCAATACATGGATGATTT
		GTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACA
		AAAATAGAGGAACTGAGACAACATCTGTTGAGGTGGGGA
		TTTACCACACCAGACAAAAAACATCAGAAAGAACCTCCAT
		TCCTTTGGATGGGTTATGAACTCCATCCTGATAAATGGAC
		AGTACAGCCTATAGTGCTGCCAGAAAAGGACAGCTGGACT
		GTCAATGACATACAGAAATTAGTGGGAAAATTGAATTGGG
		CAAGTCAGATTTATGCAGGGATTAAAGTAAGGCAATTATG
		TAAACTTCTTAGGGGAACCAAAGCACTAACAGAAGTAGTA
		CCACTAACAGAAGAAGCAGAGCTAGAACTGGCAGAAAAC
		AGGGAGATTCTAAAAGAACCGGTACATGGAGTGTATTATG
		ACCCATCAAAAGACTTAATAGCAGAAATACAGAAGCAGG
		GGCAAGGCCAATGGACATATCAAATTTATCAAGAGCCATT
		TAAAAATCTGAAAACAGGAAAGTATGCAAGAATGAAGGG
		TGCCCACACTAATGATGTGAAACAATTAACAGAGGCAGTA
		CAAAAAATAGCCACAGAAAGCATAGTAATATGGGGAAAG
		ACTCCTAAATTTAAATTACCCATACAAAAGGAAACATGGG
		AAGCATGGTGGACAGAGTATTGGCAAGCCACCTGGATTCC
		TGAGTGGGAGTTTGTCAATACCCCTCCCTTAGTGAAGTTAT
		GGTACCAGTTAGAGAAAGAACCCATAATAGGAGCAGAAA
		CTTTCTATGTAGATGGGGCAGCCAATAGGGAAACTAAATT
		AGGAAAAGCAGGATATGTAACTGACAGAGGAAGACAAAA
		AGTTGTCCCCCTAACGGACACAACAAATCAGAAGACTGAG
		TTACAAGCAATTCATCTAGCTTTGCAGGATTCGGGATTAG
		AAGTAAACATAGTGACAGACTCACAATATGCATTGGGAAT
		CATTCAAGCACAACCAGATAAGAGTGAATCAGAGTTAGTC
		AGTCAAATAATAGAGCAGTTAATAAAAAAGGAAAAAGTC
		TACCTGGCATGGGTACCAGCACACAAAGGAATTGGAGGA
		AATGAACAAGTAGATAAATTGGTCAGTGCTGGAATCAGGA
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SEQ ID NO:	Description	Sequence
		ACATGAGAAATATCACAGTAATTGGAGAGCAATGGCTAGT GATTTTAACCTACCACCTGTAGTAGCAAAAGAAATAGTAG CCAGCTGTGATAAATGTCAGCTAAAAGGGGAAGCCATGCA TGGACAAGTAGACTGTAGCCCAGGAATATGGCAGCTAGAT TGTACACATTTAGAAGGAAAAGTTATCTTGGTAGCAGTTC ATGTAGCCAGTGGATATATAGAAGCAGAAGTAATTCCAGC AGAGACAGGGCAAGAAACAGCATACTTCCTCTTAAAATTA GCAGGAAGATGGCCAGTAAAAACAGTACATACAGACAAT GGCAGCAATTTCACCAGTACTACAGTTAAGGCCGCCTGTT GGTGGGCGGGGATCAAGCAGGAATTTGGCATTCCCTACAA TCCCCAAAGTCAAGGAGTAATAGAATCTATGAATAAAGAA TTAAAGAAAATTATAGGACAGGTAAGAGATCAGGCTGAA CATCTTAAGACAGCAGTACAAATGGCAGTATTCATCCACA ATTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAG GGGAAAGAATAGTAGACATAATAGCAACAGACATACAAA CTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTT TCGGGTTTATTACAGGGACAGCAGAGATCCAGTTTGGAAA GGACCAGCAAAGCTCCTCTGGAAAGGTGAAGGGGCAGTA GTAATACAAGATAATAGTGACATAAAAGTAGTGCCAAGA AGAAAAGCAAAGATCATCAGGGATTATGGAAAACAGATG GCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATT AA
34	DNA Fragment containing Rev, RRE and rabbit beta globin poly A	TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGAG CTCATCAGAACAGTCAGACTCATCAAGCTTCTCTATCAAA GCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCG AAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGAC AGATCCATTCGATTAGTGAACGGATCCTTGGCACTTATCTG GGACGATCTGCGGAGCCTGTGCCTCTTCAGCTACCACCGC TTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGGAAC TTCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTG GAATCTCCTACAATATTGGAGTCAGGAGCTAAAGAATAGA GGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCA CTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAG ACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTG CTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCA CAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGC TGTGGAAAGATACCTAAAGGATCAACAGCTCCTAGATCTT TTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCC TTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTC ATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGG AAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAA TGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCT GGCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAGT ATATGAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGA AAAGCCTTGACTTGAGGTTAGATTTTTTTTATATTTTGTTTT GTGTTATTTTTTTCTTTAACATCCCTAAAATTTTCCTTACAT GTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCCC AGTCATAGCTGTCCCTCTTCTCTTATGAAGATCCCTCGACC TGCAGCCCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCC TGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATAC GAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAAT GAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCC CGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCGGATCCGC ATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCC GCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTC CGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCC GAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAG GAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAAC TTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATA GCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCAT TCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCA GCGGCCGCCCCGGG
35	DNA fragment containing the CAG enhancer/promoter/ intron sequence	ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTA GTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTAC GGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGC CCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGC CAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTT ACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCAT ATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAAT GGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGA CTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTA TTACCATGGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCT CCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATT TATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGG GGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGG

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SEQ ID NO:	Description	Sequence
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		TCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGG
		CGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGG
		CGGGCGGGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGC
		TCCGCGCCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGAC
		CGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCT
		CCTCCGGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGT
		TTCTTTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGG
		AGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGT
		GCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCG
		CTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGC
		TTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCGGG
		GGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGAACA
		AAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAG
		GGGGTGTGGGCGCGGCGGTCGGGCTGTAACCCCCCCCTGC
		ACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGG
		TGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCGTG
		CCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGG
		GCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGG
		CGCGGCGGCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGG
		CGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAG
		GGCGCAGGGACTTCCTTTGTCCCAAATCTGGCGGAGCCGA
		AATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGG
		GCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGGG
		GAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTCTCCA
		TCTCCAGCCTCGGGGCTGCCGCAGGGGGACGGCTGCCTTC
		GGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGCGTG
		TGACCGGCGGGAATTC
36	RSV promoter and HIV Rev	CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGGG
		GACTAGGGTGTGTTTAGGCGAAAAGCGGGGCTTCGGTTGT
		ACGCGGTTAGGAGTCCCCTCAGGATATAGTAGTTTCGCTTT
		TGCATAGGGAGGGGGAAATGTAGTCTTATGCAATACACTT
		GTAGTCTTGCAACATGGTAACGATGAGTTAGCAACATGCC
		TTACAAGGAGAGAAAAAGCACCGTGCATGCCGATTGGTG
		GAAGTAAGGTGGTACGATCGTGCCTTATTAGGAAGGCAAC
		AGACAGGTCTGACATGGATTGGACGAACCACTGAATTCCG
		CATTGCAGAGATAATTGTATTTAAGTGCCTAGCTCGATAC
		AATAAACGCCATTTGACCATTCACCACATTGGTGTGCACC
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		CGGGACCGATCCAGCCTCCCCTCGAAGCTAGCGATTAGGC
		ATCTCCTATGGCAGGAAGAAGCGGAGACAGCGACGAAGA
		ACTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAA
		AGCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCC
		GAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGA
		CAGATCCATTCGATTAGTGAACGGATCCTTAGCACTTATCT
		GGGACGATCTGCGGAGCCTGTGCCTCTTCAGCTACCACCG
		CTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGGAA
		CTTCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGT
		GGAATCTCCTACAATATTGGAGTCAGGAGCTAAAGAATAG
		TCTAGA
37	Elongation Factor-1 alpha (EF1-alpha) promoter	CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAA
		GTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGG
		GGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTC
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		CGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTATG
		GCCCTTGCGTGCCTTGAATTACTTCCACGCCCCTGGCTGCA
		GTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGG
		TGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGC
		CTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCG
		CCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTCTCGCTG
		CTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGACCT
		GCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGC
		GGGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCC
		GCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTT
		CGGCGAGGCGGGGCCTGCGAGCGCGGCCACCGAGAATCG
		GACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGGTGCC
		TGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCA
		AGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGAT
		GGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAG
		GACGCGGCGCTCGGGAGAGCGGGCGGGTGAGTCACCCAC
		ACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTCGCTTCA
		TGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCG
		ATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGG
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SEQ ID NO:	Description	Sequence
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		ATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATCTTGGTT
		CATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTC
		CATTTCAGGTGTCGTGA
38	Promoter; PGK	GGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTT
		GCGCAGGGACGCGGCTGCTCTGGGCGTGGTTCCGGGAAAC
		GCAGCGGCGCCGACCCTGGGTCTCGCACATTCTTCACGTC
		CGTTCGCAGCGTCACCCGGATCTTCGCCGCTACCCTTGTGG
		GCCCCCCGGCGACGCTTCCTGCTCCGCCCCTAAGTCGGGA
		AGGTTCCTTGCGGTTCGCGGCGTGCCGGACGTGACAAACG
		GAAGCCGCACGTCTCACTAGTACCCTCGCAGACGGACAGC
		GCCAGGGAGCAATGGCAGCGCGCCGACCGCGATGGGCTG
		TGGCCAATAGCGGCTGCTCAGCAGGGCGCGCCGAGAGCA
		GCGGCCGGGAAGGGGCGGTGCGGGAGGCGGGGTGTGGGG
		CGGTAGTGTGGGCCCTGTTCCTGCCCGCGCGGTGTTCCGC
		ATTCTGCAAGCCTCCGGAGCGCACGTCGGCAGTCGGCTCC
		CTCGTTGACCGAATCACCGACCTCTCTCCCCAG
39	Promoter; UbC	GCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCCTCCTC
		ACGGCGAGCGCTGCCACGTCAGACGAAGGGCGCAGGAGC
		GTTCCTGATCCTTCCGCCCGGACGCTCAGGACAGCGGCCC
		GCTGCTCATAAGACTCGGCCTTAGAACCCCAGTATCAGCA
		GAAGGACATTTTAGGACGGGACTTGGGTGACTCTAGGGCA
		CTGGTTTTCTTTCCAGAGAGCGGAACAGGCGAGGAAAAGT
		AGTCCCTTCTCGGCGATTCTGCGGAGGGATCTCCGTGGGG
		CGGTGAACGCCGATGATTATATAAGGACGCGCCGGGTGTG
		GCACAGCTAGTTCCGTCGCAGCCGGGATTTGGGTCGCGGT
		TCTTGTTTGTGGATCGCTGTGATCGTCACTTGGTGAGTTGC
		GGGCTGCTGGGCTGGCCGGGGCTTTCGTGGCCGCCGGGCC
		GCTCGGTGGGACGGAAGCGTGTGGAGAGACCGCCAAGGG
		CTGTAGTCTGGGTCCGCGAGCAAGGTTGCCCTGAACTGGG
		GGTTGGGGGGAGCGCACAAAATGGCGGCTGTTCCCGAGTC
		TTGAATGGAAGACGCTTGTAAGGCGGGCTGTGAGGTCGTT
		GAAACAAGGTGGGGGGCATGGTGGGCGGCAAGAACCCAA
		GGTCTTGAGGCCTTCGCTAATGCGGGAAAGCTCTTATTCG
		GGTGAGATGGGCTGGGGCACCATCTGGGGACCCTGACGTG
		AAGTTTGTCACTGACTGGAGAACTCGGGTTTGTCGTCTGGT
		TGCGGGGGCGGCAGTTATGCGGTGCCGTTGGGCAGTGCAC
		CCGTACCTTTGGGAGCGCGCGCCTCGTCGTGTCGTGACGT
		CACCCGTTCTGTTGGCTTATAATGCAGGGTGGGGCCACCT
		GCCGGTAGGTGTGCGGTAGGCTTTTCTCCGTCGCAGGACG
		CAGGGTTCGGGCCTAGGGTAGGCTCTCCTGAATCGACAGG
		CGCCGGACCTCTGGTGAGGGGAGGGATAAGTGAGGCGTC
		AGTTTCTTTGGTCGGTTTTATGTACCTATCTTCTTAAGTAG
		CTGAAGCTCCGGTTTTGAACTATGCGCTCGGGGTTGGCGA
		GTGTGTTTTGTGAAGTTTTTTAGGCACCTTTTGAAATGTAA
		TCATTTGGGTCAATATGTAATTTTCAGTGTTAGACTAGTAAA
40	Poly A; SV40	GTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGC
		ATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTC
		TAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCA
41	Poly A; bGH	GACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCT
		CCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACT
		GTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCT
		GAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAG
		GACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCAT
		GCTGGGGATGCGGTGGGCTCTATGG
42	Envelope; RD114	ATGAAACTCCCAACAGGAATGGTCATTTTATGTAGCCTAA
		TAATAGTTCGGGCAGGGTTTGACGACCCCCGCAAGGCTAT
		CGCATTAGTACAAAAACAACATGGTAAACCATGCGAATGC
		AGCGGAGGGCAGGTATCCGAGGCCCCACCGAACTCCATCC
		AACAGGTAACTTGCCCAGGCAAGACGGCCTACTTAATGAC
		CAACCAAAAATGGAAATGCAGAGTCACTCCAAAAAATCTC
		ACCCCTAGCGGGGGAGAACTCCAGAACTGCCCCTGTAACA
		CTTTCCAGGACTCGATGCACAGTTCTTGTTATACTGAATAC
		CGGCAATGCAGGGCGAATAATAAGACATACTACACGGCC
		ACCTTGCTTAAAATACGGTCTGGGAGCCTCAACGAGGTAC
		AGATATTACAAAACCCCAATCAGCTCCTACAGTCCCCTTG
		TAGGGGCTCTATAAATCAGCCCGTTTGCTGGAGTGCCACA
		GCCCCCATCCATATCTCCGATGGTGGAGGACCCCTCGATA
		CTAAGAGAGTGTGGACAGTCCAAAAAAGGCTAGAACAAA
		TTCATAAGGCTATGCATCCTGAACTTCAATACCACCCCTTA
		GCCCTGCCCAAAGTCAGAGATGACCTTAGCCTTGATGCAC
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SEQ ID NO:	Description	Sequence
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43	Envelope; GALV	ATGCTTCTCACCTCAAGCCCGCACCACCTTCGGCACCAGA TGAGTCCTGGGAGCTGGAAAAGACTGATCATCCTCTTAAG CTGCGTATTCGGAGACGGCAAAACGAGTCTGCAGAATAAG AACCCCCACCAGCCTGTGACCCTCACCTGGCAGGTACTGT CCCAAACTGGGGACGTTGTCTGGGACAAAAAGGCAGTCCA GCCCCTTTGGACTTGGTGGCCCTCTCTTACACCTGATGTAT GTGCCCTGGCGGCCGGTCTTGAGTCCTGGGATATCCCGGG ATCCGATGTATCGTCCTCTAAAAGAGTTAGACCTCCTGATT CAGACTATACTGCCGCTTATAAGCAAATCACCTGGGGAGC CATAGGGTGCAGCTACCCTCGGGCTAGGACCAGGATGGCA AATTCCCCCTTCTACGTGTGTCCCCGAGCTGGCCGAACCCA TTCAGAAGCTAGGAGGTGTGGGGGGCTAGAATCCCTATAC TGTAAAGAATGGAGTTGTGAGACCACGGGTACCGTTTATT GGCAACCCAAGTCCTCATGGGACCTCATAACTGTAAAATG GGACCAAAATGTGAAATGGGAGCAAAAATTTCAAAAGTG TGAACAAACCGGCTGGTGTAACCCCCTCAAGATAGACTTC ACAGAAAAAGGAAAACTCTCCAGAGATTGGATAACGGAA AAAACCTGGGAATTAAGGTTCTATGTATATGGACACCCAG GCATACAGTTGACTATCCGCTTAGAGGTCACTAACATGCC GGTTGTGGCAGTGGGCCCAGACCCTGTCCTTGCGGAACAG GGACCTCCTAGCAAGCCCCTCACTCTCCCTCTCTCCCCACG GAAAGCGCCGCCCACCCCTCTACCCCCGGCGGCTAGTGAG CAAACCCCTGCGGTGCATGGAGAAACTGTTACCCTAAACT CTCCGCCTCCCACCAGTGGCGACCGACTCTTTGGCCTTGTG CAGGGGGCCTTCCTAACCTTGAATGCTACCAACCCAGGGG CCACTAAGTCTTGCTGGCTCTGTTTGGGCATGAGCCCCCCT TATTATGAAGGGATAGCCTCTTCAGGAGAGGTCGCTTATA CCTCCAACCATACCCGATGCCACTGGGGGGCCCAAGGAAA GCTTACCCTCACTGAGGTCTCCGGACTCGGGTCATGCATA GGGAAGGTGCCTCTTACCCATCAACATCTTTGCAACCAGA CCTTACCCATCAATTCCTCTAAAAACCATCAGTATCTGCTC CCCTCAAACCATAGCTGGTGGGCCTGCAGCACTGGCCTCA CCCCCTGCCTCTCCACCTCAGTTTTTAATCAGTCTAAAGAC TTCTGTGTCCAGGTCCAGCTGATCCCCCGCATCTATTACCA TTCTGAAGAAACCTTGTTACAAGCCTATGACAAATCACCC CCCAGGTTTAAAAGAGAGCCTGCCTCACTTACCCTAGCTG TCTTCCTGGGGTTAGGGATTGCGGCAGGTATAGGTACTGG CTCAACCGCCCTAATTAAAGGGCCCATAGACCTCCAGCAA GGCCTAACCAGCCTCCAAATCGCCATTGACGCTGACCTCC GGGCCCTTCAGGACTCAATCAGCAAGCTAGAGGACTCACT GACTTCCCTATCTGAGGTAGTACTCCAAAATAGGAGAGGC CTTGACTTACTATTCCTTAAAGAAGGAGGCCTCTGCGCGG CCCTAAAAGAAGAGTGCTGTTTTTATGTAGACCACTCAGG TGCAGTACGAGACTCCATGAAAAAACTTAAAGAAAGACT AGATAAAAGACAGTTAGAGCGCCAGAAAAACCAAAACTG GTATGAAGGGTGGTTCAATAACTCCCCTTGGTTTACTACCC TACTATCAACCATCGCTGGGCCCCTATTGCTCCTCCTTTTG TTACTCACTCTTGGGCCCTGCATCATCAATAAATTAATCCA ATTCATCAATGATAGGATAAGTGCAGTCAAAATTTTAGTC

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SEQ ID NO:	Description	Sequence
		CTTAGACAGAAATATCAGACCCTAGATAACGAGGAAAAC CTTTAA
44	Envelope; FUG	ATGGTTCCGCAGGTTCTTTTGTTTGTACTCCTTCTGGGTTTT TCGTTGTGTTTCGGGAAGTTCCCCATTTACACGATACCAGA CGAACTTGGTCCCTGGAGCCCTATTGACATACACCATCTC AGCTGTCCAAATAACCTGGTTGTGGAGGATGAAGGATGTA CCAACCTGTCCGAGTTCTCCTACATGGAACTCAAAGTGGG ATACATCTCAGCCATCAAAGTGAACGGGTTCACTTGCACA GGTGTTGTGACAGAGGCAGAGACCTACACCAACTTTGTTG GTTATGTCACAACCACATTCAAGAGAAAGCATTTCCGCCC CACCCCAGACGCATGTAGAGCCGCGTATAACTGGAAGATG GCCGGTGACCCCAGATATGAAGAGTCCCTACACAATCCAT ACCCCGACTACCACTGGCTTCGAACTGTAAGAACCACCAA AGAGTCCCTCATTATCATATCCCCAAGTGTGACAGATTTG GACCCATATGACAAATCCCTTCACTCAAGGGTCTTCCCTG GCGGAAAGTGCTCAGGAATAACGGTGTCCTCTACCTACTG CTCAACTAACCATGATTACACCATTTGGATGCCCGAGAAT CCGAGACCAAGGACACCTTGTGACATTTTTACCAATAGCA GAGGGAAGAGAGCATCCAACGGGAACAAGACTTGCGGCT TTGTGGATGAAAGAGGCCTGTATAAGTCTCTAAAAGGAGC ATGCAGGCTCAAGTTATGTGGAGTTCTTGGACTTAGACTT ATGGATGGAACATGGGTCGCGATGCAAACATCAGATGAG ACCAAATGGTGCCCTCCAGATCAGTTGGTGAATTTGCACG ACTTTCGCTCAGACGAGATCGAGCATCTCGTTGTGGAGGA GTTAGTTAAGAAAAGAGAGGAATGTCTGGATGCATTAGAG TCCATCATGACCACCAAGTCAGTAAGTTTCAGACGTCTCA GTCACCTGAGAAAACTTGTCCCAGGGTTTGGAAAAGCATA TACCATATTCAACAAAACCTTGATGGAGGCTGATGCTCAC TACAAGTCAGTCCGGACCTGGAATGAGATCATCCCCTCAA AAGGGTGTTTGAAAGTTGGAGGAAGGTGCCATCCTCATGT GAACGGGGTGTTTTTCAATGGTATAATATTAGGGCCTGAC GACCATGTCCTAATCCCAGAGATGCAATCATCCCTCCTCC AGCAACATATGGAGTTGTTGGAATCTTCAGTTATCCCCCTG ATGCACCCCCTGGCAGACCCTTCTACAGTTTTCAAAGAAG GTGATGAGGCTGAGGATTTTGTTGAAGTTCACCTCCCCGA TGTGTACAAACAGATCTCAGGGGTTGACCTGGGTCTCCCG AACTGGGGAAAGTATGTATTGATGACTGCAGGGGCCATGA TTGGCCTGGTGTTGATATTTTCCCTAATGACATGGTGCAGA GTTGGTATCCATCTTTGCATTAAATTAAAGCACACCAAGA AAAGACAGATTTATACAGACATAGAGATGAACCGACTTGG AAAGTAA
45	Envelope; LCMV	ATGGGTCAGATTGTGACAATGTTTGAGGCTCTGCCTCACA TCATCGATGAGGTGATCAACATTGTCATTATTGTGCTTATC GTGATCACGGGTATCAAGGCTGTCTACAATTTTGCCACCT GTGGGATATTCGCATTGATCAGTTTCCTACTTCTGGCTGGC AGGTCCTGTGGCATGTACGGTCTTAAGGGACCCGACATTT ACAAAGGAGTTTACCAATTTAAGTCAGTGGAGTTTGATAT GTCACATCTGAACCTGACCATGCCCAACGCATGTTCAGCC AACAACTCCCACCATTACATCAGTATGGGGACTTCTGGAC TAGAATTGACCTTCACCAATGATTCCATCATCAGTCACAA CTTTTGCAATCTGACCTCTGCCTTCAACAAAAAGACCTTTG ACCACACACTCATGAGTATAGTTTCGAGCCTACACCTCAG TATCAGAGGGAACTCCAACTATAAGGCAGTATCCTGCGAC TTCAACAATGGCATAACCATCCAATACAACTTGACATTCT CAGATCGACAAAGTGCTCAGAGCCAGTGTAGAACCTTCAG AGGTAGAGTCCTAGATATGTTTAGAACTGCCTTCGGGGGG AAATACATGAGGAGTGGCTGGGGCTGGACAGGCTCAGAT GGCAAGACCACCTGGTGTAGCCAGACGAGTTACCAATACC TGATTATACAAAATAGAACCTGGGAAAACCACTGCACATA TGCAGGTCCTTTTGGGATGTCCAGGATTCTCCTTTCCCAAG AGAAGACTAAGTTCTTCACTAGGAGACTAGCGGGCACATT CACCTGGACTTTGTCAGACTCTTCAGGGGTGGAGAATCCA GGTGGTTATTGCCTGACCAAATGGATGATTCTTGCTGCAG AGCTTAAGTGTTTCGGGAACACAGCAGTTGCGAAATGCAA TGTAAATCATGATGCCGAATTCTGTGACATGCTGCGACTA ATTGACTACAACAAGGCTGCTTTGAGTAAGTTCAAAGAGG ACGTAGAATCTGCCTTGCACTTATTCAAAACAACAGTGAA TTCTTTGATTTCAGATCAACTACTGATGAGGAACCACTTGA GAGATCTGATGGGGGTGCCATATTGCAATTACTCAAAGTT TTGGTACCTAGAACATGCAAAGACCGGCGAAACTAGTGTC CCCAAGTGCTGGCTTGTCACCAATGGTTCTTACTTAAATGA GACCCACTTCAGTGATCAAATCGAACAGGAAGCCGATAAC ATGATTACAGAGATGTTGAGGAAGGATTACATAAAGAGG CAGGGGAGTACCCCCCTAGCATTGATGGACCTTCTGATGT TTTCCACATCTGCATATCTAGTCAGCATCTTCCTGCACCTT

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SEQ ID NO:	Description	Sequence
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		GTCCAAAGCCACACCGATTAACCAACAAAGGAATTTGTAG
		TTGTGGTGCATTTAAGGTGCCTGGTGTAAAAACCGTCTGG
		AAAAGACGCTGA
46	Envelope; FPV	ATGAACACTCAAATCCTGGTTTTCGCCCTTGTGGCAGTCAT
		CCCCACAAATGCAGACAAAATTTGTCTTGGACATCATGCT
		GTATCAAATGGCACCAAAGTAAACACACTCACTGAGAGA
		GGAGTAGAAGTTGTCAATGCAACGGAAACAGTGGAGCGG
		ACAAACATCCCCAAAATTTGCTCAAAAGGGAAAAGAACC
		ACTGATCTTGGCCAATGCGGACTGTTAGGGACCATTACCG
		GACCACCTCAATGCGACCAATTTCTAGAATTTTCAGCTGAT
		CTAATAATCGAGAGACGAGAAGGAAATGATGTTTGTTACC
		CGGGGAAGTTTGTTAATGAAGAGGCATTGCGACAAATCCT
		CAGAGGATCAGGTGGGATTGACAAAGAAACAATGGGATT
		CACATATAGTGGAATAAGGACCAACGGAACAACTAGTGC
		ATGTAGAAGATCAGGGTCTTCATTCTATGCAGAAATGGAG
		TGGCTCCTGTCAAATACAGACAATGCTGCTTTCCCACAAA
		TGACAAAATCATACAAAAACACAAGGAGAGAATCAGCTC
		TGATAGTCTGGGGAATCCACCATTCAGGATCAACCACCGA
		ACAGACCAAACTATATGGGAGTGGAAATAAACTGATAAC
		AGTCGGGAGTTCCAAATATCATCAATCTTTTGTGCCGAGTC
		CAGGAACACGACCGCAGATAAATGGCCAGTCCGGACGGA
		TTGATTTTCATTGGTTGATCTTGGATCCCAATGATACAGTT
		ACTTTTAGTTTCAATGGGGCTTTCATAGCTCCAAATCGTGC
		CAGCTTCTTGAGGGGAAAGTCCATGGGGATCCAGAGCGAT
		GTGCAGGTTGATGCCAATTGCGAAGGGGAATGCTACCACA
		GTGGAGGGACTATAACAAGCAGATTGCCTTTTCAAAACAT
		CAATAGCAGAGCAGTTGGCAAATGCCCAAGATATGTAAA
		ACAGGAAAGTTTATTATTGGCAACTGGGATGAAGAACGTT
		CCCGAACCTTCCAAAAAAAGGAAAAAAAGAGGCCTGTTT
		GGCGCTATAGCAGGGTTTATTGAAAATGGTTGGGAAGGTC
		TGGTCGACGGGTGGTACGGTTTCAGGCATCAGAATGCACA
		AGGAGAAGGAACTGCAGCAGACTACAAAAGCACCCAATC
		GGCAATTGATCAGATAACCGGAAAGTTAAATAGACTCATT
		GAGAAAACCAACCAGCAATTTGAGCTAATAGATAATGAAT
		TCACTGAGGTGGAAAAGCAGATTGGCAATTTAATTAACTG
		GACCAAAGACTCCATCACAGAAGTATGGTCTTACAATGCT
		GAACTTCTTGTGGCAATGGAAAACCAGCACACTATTGATT
		TGGCTGATTCAGAGATGAACAAGCTGTATGAGCGAGTGAG
		GAAACAATTAAGGGAAAATGCTGAAGAGGATGGCACTGG
		TTGCTTTGAAATTTTTCATAAATGTGACGATGATTGTATGG
		CTAGTATAAGGAACAATACTTATGATCACAGCAAATACAG
		AGAAGAAGCGATGCAAAATAGAATACAAATTGACCCAGT
		CAAATTGAGTAGTGGCTACAAAGATGTGATACTTTGGTTT
		AGCTTCGGGGCATCATGCTTTTTGCTTCTTGCCATTGCAAT
		GGGCCTTGTTTTCATATGTGTGAAGAACGGAAACATGCGG
		TGCACTATTTGTATATAA
47	Envelope; RRV	AGTGTAACAGAGCACTTTAATGTGTATAAGGCTACTAGAC
		CATACCTAGCACATTGCGCCGATTGCGGGGACGGGTACTT
		CTGCTATAGCCCAGTTGCTATCGAGGAGATCCGAGATGAG
		GCGTCTGATGGCATGCTTAAGATCCAAGTCTCCGCCCAAA
		TAGGTCTGGACAAGGCAGGCACCCACGCCCACACGAAGCT
		CCGATATATGGCTGGTCATGATGTTCAGGAATCTAAGAGA
		GATTCCTTGAGGGTGTACACGTCCGCAGCGTGCTCCATAC
		ATGGGACGATGGGACACTTCATCGTCGCACACTGTCCACC
		AGGCGACTACCTCAAGGTTTCGTTCGAGGACGCAGATTCG
		CACGTGAAGGCATGTAAGGTCCAATACAAGCACAATCCAT
		TGCCGGTGGGTAGAGAGAAGTTCGTGGTTAGACCACACTT
		TGGCGTAGAGCTGCCATGCACCTCATACCAGCTGACAACG
		GCTCCCACCGACGAGGAGATTGACATGCATACACCGCCAG
		ATATACCGGATCGCACCCTGCTATCACAGACGGCGGGCAA
		CGTCAAAATAACAGCAGGCGGCAGGACTATCAGGTACAA
		CTGTACCTGCGGCCGTGACAACGTAGGCACTACCAGTACT
		GACAAGACCATCAACACATGCAAGATTGACCAATGCCATG
		CTGCCGTCACCAGCCATGACAAATGGCAATTTACCTCTCC
		ATTTGTTCCCAGGGCTGATCAGACAGCTAGGAAAGGCAAG
		GTACACGTTCCGTTCCCTCTGACTAACGTCACCTGCCGAGT
		GCCGTTGGCTCGAGCGCCGGATGCCACCTATGGTAAGAAG
		GAGGTGACCCTGAGATTACACCCAGATCATCCGACGCTCT
		TCTCCTATAGGAGTTTAGGAGCCGAACCGCACCCGTACGA
		GGAATGGGTTGACAAGTTCTCTGAGCGCATCATCCCAGTG
		ACGGAAGAAGGGATTGAGTACCAGTGGGGCAACAACCCG
		CCGGTCTGCCTGTGGGCGCAACTGACGACCGAGGGCAAAC
		CCCATGGCTGGCCACATGAAATCATTCAGTACTATTATGG
		ACTATACCCCGCCGCCACTATTGCCGCAGTATCCGGGGCG

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SEQ ID NO:	Description	Sequence
		AGTCTGATGGCCCTCCTAACTCTGGCGGCCACATGCTGCA TGCTGGCCACCGCGAGGAGAAAGTGCCTAACACCGTACGC CCTGACGCCAGGAGCGGTGGTACCGTTGACACTGGGGCTG CTTTGCTGCGCACCGAGGGCGAATGCA
48	Envelope; Ebola	ATGGGTGTTACAGGAATATTGCAGTTACCTCGTGATCGAT TCAAGAGGACATCATTCTTTCTTTGGGTAATTATCCTTTTC CAAAGAACATTTTCCATCCCACTTGGAGTCATCCACAATA GCACATTACAGGTTAGTGATGTCGACAAACTGGTTTGCCG TGACAAACTGTCATCCACAAATCAATTGAGATCAGTTGGA CTGAATCTCGAAGGGAATGGAGTGGCAACTGACGTGCCAT CTGCAACTAAAAGATGGGGCTTCAGGTCCGGTGTCCCACC AAAGGTGGTCAATTATGAAGCTGGTGAATGGGCTGAAAAC TGCTACAATCTTGAAATCAAAAAACCTGACGGGAGTGAGT GTCTACCAGCAGCGCCAGACGGGATTCGGGGCTTCCCCCG GTGCCGGTATGTGCACAAAGTATCAGGAACGGGACCGTGT GCCGGAGACTTTGCCTTCCACAAAGAGGGTGCTTTCTTCCT GTATGACCGACTTGCTTCCACAGTTATCTACCGAGGAACG ACTTTCGCTGAAGGTGTCGTTGCATTTCTGATACTGCCCCA AGCTAAGAAGGACTTCTTCAGCTCACACCCCTTGAGAGAG CCGGTCAATGCAACGGAGGACCCGTCTAGTGGCTACTATT CTACCACAATTAGATATCAAGCTACCGGTTTTGGAACCAA TGAGACAGAGTATTTGTTCGAGGTTGACAATTTGACCTAC GTCCAACTTGAATCAAGATTCACACCACAGTTTCTGCTCCA GCTGAATGAGACAATATATACAAGTGGGAAAAGGAGCAA TACCACGGGAAAACTAATTTGGAAGGTCAACCCCGAAATT GATACAACAATCGGGGAGTGGGCCTTCTGGGAAACTAAA AAAACCTCACTAGAAAAATTCGCAGTGAAGAGTTGTCTTT CACAGCTGTATCAAACAGAGCCAAAAACATCAGTGGTCAG AGTCCGGCGCGAACTTCTTCCGACCCAGGGACCAACACAA CAACTGAAGACCACAAAATCATGGCTTCAGAAAATTCCTC TGCAATGGTTCAAGTGCACAGTCAAGGAAGGGAAGCTGC AGTGTCGCATCTGACAACCCTTGCCACAATCTCCACGAGT CCTCAACCCCCCACAACCAAACCAGGTCCGGACAACAGCA CCCACAATACACCCGTGTATAAACTTGACATCTCTGAGGC AACTCAAGTTGAACAACATCACCGCAGAACAGACAACGA CAGCACAGCCTCCGACACTCCCCCCGCCACGACCGCAGCC GGACCCCTAAAAGCAGAGAACACCAACACGAGCAAGGGT ACCGACCTCCTGGACCCCGCCACCACAACAAGTCCCCAAA ACCACAGCGAGACCGCTGGCAACAACAACACTCATCACCA AGATACCGGAGAAGAGAGTGCCAGCAGCGGGAAGCTAGG CTTAATTACCAATACTATTGCTGGAGTCGCAGGACTGATC ACAGGCGGGAGGAGAGCTCGAAGAGAAGCAATTGTCAAT GCTCAACCCAAATGCAACCCTAATTTACATTACTGGACTA CTCAGGATGAAGGTGCTGCAATCGGACTGGCCTGGATACC ATATTTCGGGCCAGCAGCCGAGGGAATTTACATAGAGGGG CTGATGCACAATCAAGATGGTTTAATCTGTGGGTTGAGAC AGCTGGCCAACGAGACGACTCAAGCTCTTCAACTGTTCCT GAGAGCCACAACCGAGCTACGCACCTTTTCAATCCTCAAC CGTAAGGCAATTGATTTCTTGCTGCAGCGATGGGGCGGCA CATGCCACATTTTGGGACCGGACTGCTGTATCGAACCACA TGATTGGACCAAGAACATAACAGACAAAATTGATCAGATT ATTCATGATTTTGTTGATAAAACCCTTCCGGACCAGGGGG ACAATGACAATTGGTGGACAGGATGGAGACAATGGATAC CGGCAGGTATTGGAGTTACAGGCGTTATAATTGCAGTTAT CGCTTTATTCTGTATATGCAAATTTGTCTTTTAG
49	FDPS target sequence #1	GTCCTGGAGTACAATGCCATT
50	FDPS target sequence #2	GCAGGATTTCGTTCAGCACTT
51	FDPS target sequence #3	GCCATGTACATGGCAGGAATT
52	FDPS target sequence #4	GCAGAAGGAGGCTGAGAAAGT
53	miR30 FDPS sequence #1	AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGC CTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGA GAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT
54	miR30 FDPS sequence #2	AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGC CTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGGCTGAGA AAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT

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SEQ ID NO:	Description	Sequence
55	miR30 FDPS sequence #3	TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCGT GAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTTGCCT ACTGCCTCGGA
56	miR155 FDPS sequence #1	CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCTCAGCC TCCTTCTGCTTTTGGCCACTGACTGAGCAGAAGGGCTGAG AAAGTCAGGACACAAGGCCTGTTACTAGCACTCA
57	miR21 FDPS sequence #1	CATCTCCATGGCTGTACCACCTTGTCGGGACTTTCTCAGCC TCCTTCTGCCTGTTGAATCTCATGGCAGAAGGAGGCGAGA AAGTCTGACATTTTGGTATCTTTCATCTGACCA
58	miR185 FDPS sequence #1	GGGCCTGGCTCGAGCAGGGGGCGAGGGATACTTTCTCAGC CTCCTTCTGCTGGTCCCCTCCCCGCAGAAGGAGGCTGAGA AAGTCCTTCCCTCCCAATGACCGCGTCTTCGTCG
59	Forward primer	AGGAATTGATGGCGAGAAGG
60	Reverse primer	CCCAAAGAGGTCAAGGTAATCA
61	Forward primer	AGCGCGGCTACAGCTTCA
62	Reverse primer	GGCGACGTAGCACAGCTTCT
63	Forward primer	CACTGTCGTCATTCCATGCT
64	Reverse primer	GCCTCTTGACATTCTCCTC
65	Reverse primer	AAAGTCAGTGGGGACAGTGG
66	miR155 CD47 target sequence #2	CCTGGAGGCTTGCTGAAGGCTGTATGCTGTTAGCTCGATG ATCGTTTCACGTTTTGGCCACTGACTGACGTGAAACGCATC GAGCTAACAGGACACAAGGCCTGTTACTAGCACTCA
67	miR155 CD47 target sequence #3	CCTGGAGGCTTGCTGAAGGCTGTATGCTGAAGAATGGCTC CAACAATGACGTTTTGGCCACTGACTGACGTCATTGTGAG CCATTCTTCAGGACACAAGGCCTGTTACTAGCACTCA
68	miR155 CD47 target sequence #4	CCTGGAGGCTTGCTGAAGGCTGTATGCTGTATACACGCCG CAATACAGAGGTTTTGGCCACTGACTGACCTCTGTATCGG CGTGTATACAGGACACAAGGCCTGTTACTAGCACTCA
69	Forward primer	GGACTATCCTGCTGCCAA
70	miR155 cMyc sequence	CCTGGAGGCTTGCTGAAGGCTGTATGCTGTGTTCGCCTCTT GACATTCTCTTTTGGCCACTGACTGAGAGAATGTAGAGGC GAACACAGGACACAAGGCCTGTTACTAGCACTCA
71	cMyc target sequence	GAGAATGTCAAGAGGCGAACA
72	CMV promoter sequence	ATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGG CAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGAT GCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTT GACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCA ATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCC AAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGC GGTAGGCGTGTACGGTGGGAGGTTTATATAAGCAGAGCTC GTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACG CTGTTTT
73	GFP T2A Luciferase sequence	ATGCCCGCCATGAAGATCGAGTGCCGCATCACCGGCACCC TGAACGGCGTGGAGTTCGAGCTGGTGGGCGGCGGAGAGG GCACCCCCGAGCAGGGCCGCATGACCAACAAGATGAAGA GCACCAAAGGCGCCCTGACCTTCAGCCCCTACCTGCTGAG CCACGTGATGGGCTACGGCTTCTACCACTTCGGCACCTAC CCCAGCGGCTACGAGAACCCCTTCCTGCACGCCATCAACA ACGGCGGCTACACCAACACCCGCATCGAGAAGTACGAGG ACGGCGGCGTGCTGCACGTGAGCTTCAGCTACCGCTACGA GGCCGGCCGCGTGATCGGCGACTTCAAGGTGGTGGGCACC GGCTTCCCCGAGGACAGCGTGATCTTCACCGACAAGATCA TCCGCAGCAACGCCACCGTGGAGCACCTGCACCCCATGGG CGATAACGTGCTGGTGGGCAGCTTCGCCCGCACCTTCAGC

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SEQ ID NO:	Description	Sequence
		CTGCGCGACGGCGGCTACTACAGCTTCGTGGTGGACAGCC
		ACATGCACTTCAAGAGCGCCATCCACCCCAGCATCCTGCA
		GAACGGGGGCCCCATGTTCGCCTTCCGCCGCGTGGAGGAG
		CTGCACAGCAACACCGAGCTGGGCATCGTGGAGTACCAGC
		ACGCCTTCAAGACCCCCATCGCCTTCGCCAGATCTCGAGA
		TATCAGCCATGGCTTCCCGCCGGCGGTGGCGGCGCAGGAT
		GATGGCACGCTGCCCATGTCTTGTGCCCAGGAGAGCGGGA
		TGGACCGTCACCCTGCAGCCTGTGCTTCTGCTAGGATCAAT
		GTGACCGGTGAGGGCAGAGGAAGTCTTCTAACATGCGGTG
		ACGTGGAGGAGAATCCCGGCCCTTCCGGTATGGAAGACGC
		CAAAAACATAAAGAAAGGCCCGGCGCCATTCTATCCGCTA
		GAGGATGGAACCGCTGGAGAGCAACTGCATAAGGCTATG
		AAGAGATACGCCCTGGTTCCTGGAACAATTGCTTTTACAG
		ATGCACATATCGAGGTGAACATCACGTACGCGGAATACTT
		CGAAATGTCCGTTCGGTTGGCAGAAGCTATGAAACGATAT
		GGGCTGAATACAAATCACAGAATCGTCGTATGCAGTGAAA
		ACTCTCTTCAATTCTTTATGCCGGTGTTGGGCGCGTTATTT
		ATCGGAGTTGCAGTTGCGCCCGCGAACGACATTTATAATG
		AACGTGAATTGCTCAACAGTATGAACATTTCGCAGCCTAC
		CGTAGTGTTTGTTTCCAAAAAGGGGTTGCAAAAAATTTTG
		AACGTGCAAAAAAAATTACCAATAATCCAGAAAATTATTA
		TCATGGATTCTAAAACGGATTACCAGGGATTTCAGTCGAT
		GTACACGTTCGTCACATCTCATCTACCTCCCGGTTTTAATG
		AATACGATTTTGTACCAGAGTCCTTTGATCGTGACAAAAC
		AATTGCACTGATAATGAACTCCTCTGGATCTACTGGGTTAC
		CTAAGGGTGTGGCCCTTCCGCATAGAACTGCCTGCGTCAG
		ATTCTCGCATGCCAGAGATCCTATTTTTGGCAATCAAATCA
		TTCCGGATACTGCGATTTTAAGTGTTGTTCCATTCCATCAC
		GGTTTTGGAATGTTTACTACACTCGGATATTTGATATGTGG
		ATTTCGAGTCGTCTTAATGTATAGATTTGAAGAAGAGCTG
		TTTTTACGATCCCTTCAGGATTACAAAATTCAAAGTGCGTT
		GCTAGTACCAACCCTATTTTCATTCTTCGCCAAAAGCACTC
		TGATTGACAAATACGATTTATCTAATTTACACGAAATTGCT
		TCTGGGGGCGCACCTCTTTCGAAAGAAGTCGGGGAAGCGG
		TTGCAAAACGCTTCCATCTTCCAGGGATACGACAAGGATA
		TGGGCTCACTGAGACTACATCAGCTATTCTGATTACACCC
		GAGGGGGATGATAAACCGGGCGCGGTCGGTAAAGTTGTTC
		CATTTTTTGAAGCGAAGGTTGTGGATCTGGATACCGGGAA
		AACGCTGGGCGTTAATCAGAGAGGCGAATTATGTGTCAGA
		GGACCTATGATTATGTCCGGTTATGTAAACAATCCGGAAG
		CGACCAACGCCTTGATTGACAAGGATGGATGGCTACATTC
		TGGAGACATAGCTTACTGGGACGAAGACGAACACTTCTTC
		ATAGTTGACCGCTTGAAGTCTTTAATTAAATACAAAGGAT
		ACCAGGTGGCCCCCGCTGAATTGGAGTCGATATTGTTACA
		ACACCCCAACATCTTCGACGCGGGCGTGGCAGGTCTTCCC
		GACGATGACGCCGGTGAACTTCCCGCCGCCGTTGTTGTTTT
		GGAGCACGGAAAGACGATGACGGAAAAAGAGATCGTGGA
		TTACGTCGCCAGTCAAGTAACAACCGCGAAAAAGTTGCGC
		GGAGGAGTTGTGTTTGTGGACGAAGTACCGAAAGGTCTTA
		CCGGAAAACTCGACGCAAGAAAAATCAGAGAGATCCTCA
		TAAAGGCCAAGAAGGGCGGAAAGTCCAAATTGTAA
74	Rous Sarcoma virus (RSV) promoter	GTAGTCTTATGCAATACTCTTGTAGTCTTGCAACATGGTAA
		CGATGAGTTAGCAACATGCCTTACAAGGAGAGAAAAAGC
		ACCGTGCATGCCGATTGGTGGAAGTAAGGTGGTACGATCG
		TGCCTTATTAGGAAGGCAACAGACGGGTCTGACATGGATT
		GGACGAACCACTGAATTGCCGCATTGCAGAGATATTGTAT
		TTAAGTGCCTAGCTCGATACAATAAACG
75	5' Long terminal repeat (LTR)	GGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCT
		GGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCT
		TGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGT
		GACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGT
		GTGGAAAATCTCTAGCA
76	Psi Packaging signal	TACGCCAAAAATTTTGACTAGCGGAGGCTAGAAGGAGAG
		AG
77	Rev response element (RRE)	AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGC
		ACTATGGGCGCAGCCTCAATGACGCTGACGGTACAGGCCA
		GACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTT
		GCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTC
		ACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGG
		CTGTGGAAAGATACCTAAAGGATCAACAGCTCC

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SEQ ID NO:	Description	Sequence
78	Central polypurine tract (cPPT)	TTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGG GGAAAGAATAGTAGACATAATAGCAACAGACATACAAAC TAAAGAATTACAAAAACAAATTACAAAATTCAAAATTTTA
79	Long WPRE sequence	AATCAACCTCTGATTACAAAATTTGTGAAAGATTGACTGG TATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGATACG CTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATG GCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCT CTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCG TGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTTGG GGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGC TTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCT GCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCAC TGACAATTCCGTGGTGTTGTCGGGGAAATCATCGTCCTTTC CTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGG ACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGA CCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTC CGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTT TGGGCCGCCTCCCCGCCT
80	3' delta LTR	TGGAAGGGCTAATTCACTCCCAACGAAGATAAGATCTGCT TTTTGCTTGTACTGGGTCTCTCTGGTTAGACCAGATCTGAG CCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAA GCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGT GCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAG ACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTAGTAGTT CATGTCA
81	Envelope; MLV 10A1	ATGGAAGGTCCAGCGTTCTCAAAACCCCTTAAAGATAAGA TTAACCCGTGGAAGTCCTTAATGGTCATGGGGGTCTATTTA AGAGTAGGGATGGCAGAGAGCCCCCATCAGGTCTTTAATG TAACCTGGAGAGTCACCAACCTGATGACTGGGCGTACCGC CAATGCCACCTCCCTTTTAGGAACTGTACAAGATGCCTTCC CAAGATTATATTTTGATCTATGTGATCTGGTCGGAGAAGA GTGGGACCCTTCAGACCAGGAACCATATGTCGGGTATGGC TGCAAATACCCCGGAGGGAGAAAGCGGACCCGGACTTTTG ACTTTTACGTGTGCCCTGGGCATACCGTAAAATCGGGGTG TGGGGGGCCAAGAGAGGGCTACTGTGGTGAATGGGGTTGT GAAACCACCGGACAGGCTTACTGGAAGCCCACATCATCAT GGGACCTAATCTCCCTTAAGCGCGGTAACACCCCCTGGGA CACGGGATGCTCCAAAATGGCTTGTGGCCCCTGCTACGAC CTCTCCAAAGTATCCAATTCCTTCCAAGGGGCTACTCGAG GGGGCAGATGCAACCCTCTAGTCCTAGAATTCACTGATGC AGGAAAAAAGGCTAATTGGGACGGGCCCAAATCGTGGGG ACTGAGACTGTACCGGACAGGAACAGATCCTATTACCATG TTCTCCCTGACCCGCCAGGTCCTCAATATAGGGCCCCGCAT CCCCATTGGGCCTAATCCCGTGATCACTGGTCAACTACCCC CCTCCCGACCCGTGCAGATCAGGCTCCCCAGGCCTCCTCA GCCTCCTCCTACAGGCGCAGCCTCTATAGTCCCTGAGACT GCCCCACCTTCTCAACAACCTGGGACGGGAGACAGGCTGC TAAACCTGGTAGAAGGAGCCTATCAGGCGCTTAACCTCAC CAATCCCGACAAGACCCAAGAATGTTGGCTGTGCTTAGTG TCGGGACCTCCTTATTACGAAGGAGTAGCGGTCGTGGGCA CTTATACCAATCATTCTACCGCCCCGGCCAGCTGTACGGCC ACTTCCCAACATAAGCTTACCCTATCTGAAGTGACAGGAC AGGGCCTATGCATGGGAGCACTACCTAAAACTCACCAGGC CTTATGTAACACCACCCAAAGTGCCGGCTCAGGATCCTAC TACCTTGCAGCACCCGCTGGAACAATGTGGGCTTGTAGCA CTGGATTGACTCCCTGCTTGTCCACCACGATGCTCAATCTA ACCACAGACTATTGTGTATTAGTTGAGCTCTGGCCCAGAA TAATTTACCACTCCCCCGATTATATGTATGGTCAGCTTGAA CAGCGTACCAAATATAAGAGGGAGCCAGTATCGTTGACCC TGGCCCTTCTGCTAGGAGGATTAACCATGGGAGGGATTGC AGCTGGAATAGGGACGGGGACCACTGCCCTAATCAAAAC CCAGCAGTTTGAGCAGCTTCACGCCGCTATCCAGACAGAC CTCAACGAAGTCGAAAAATCAATTACCAACCTAGAAAAGT CACTGACCTCGTTGTCTGAAGTAGTCCTACAGAACCGAAG AGGCCTAGATTTGCTCTTCCTAAAAGAGGGAGGTCTCTGC GCAGCCCTAAAAGAAGAATGTTGTTTTTATGCAGACCACA CGGGACTAGTGAGAGACAGCATGGCCAAACTAAGGGAAA GGCTTAATCAGAGACAAAAACTATTTGAGTCAGGCCAAGG TTGGTTCGAAGGGCAGTTTAATAGATCCCCCTGGTTTACCA CCTTAATCTCCACCATCATGGGACCTCTAATAGTACTCTTA CTGATCTTACTCTTTGGACCCTGCATTCTCAATCGATTGGT

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SEQ ID NO:	Description	Sequence
		CCAATTTGTTAAAGACAGGATCTCAGTGGTCCAGGCTCTG GTTTTGACTCAACAATATCACCAGCTAAAACCTATAGAGT ACGAGCCATGA
82	miR155 CD47 target sequence #1	CCTGGAGGCTTGCTGAAGGCTGTATGCTGTTATCCATCTTC AAAGAGGCAGTTTTGGCCACTGACTGACTGCCTCTTAAGA TGGATAACAGGACACAAGGCCTGTTACTAGCACTCA
83	miR21 cMyc sequence	CATCTCCATGGCTGTACCACCTTGTCGGGTGTTCGCCTCTT GACATTCTCCTGTTGAATCTCATGGAGAATGTCAAGGGCG AACACTGACATTTTGGTATCTTTCATCTGACCA

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: 84

<210> SEQ ID NO 1
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: FDPS shRNA sequence #1

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<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

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

<400> SEQUENCE: 3

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<210> SEQ ID NO 4
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<220> FEATURE:
<223> OTHER INFORMATION: FDPS shRNA sequence #4

<400> SEQUENCE: 4

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<223> OTHER INFORMATION: CD47 shRNA sequence #1

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<211> LENGTH: 49
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<220> FEATURE:
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<400> SEQUENCE: 7

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<210> SEQ ID NO 8
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 8

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<210> SEQ ID NO 9
<211> LENGTH: 53
<212> TYPE: DNA
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<220> FEATURE:
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<210> SEQ ID NO 10
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<400> SEQUENCE: 10

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<210> SEQ ID NO 11
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<220> FEATURE:
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<210> SEQ ID NO 12
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<400> SEQUENCE: 12

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<210> SEQ ID NO 13
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<400> SEQUENCE: 13

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<210> SEQ ID NO 14
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<220> FEATURE:
<223> OTHER INFORMATION: cMyc shRNA sequence #5

<400> SEQUENCE: 14

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<210> SEQ ID NO 15
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: H1 promoter

<400> SEQUENCE: 15

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cacccagcgc gcgtgcgccc tggcaggaag atggctgtga gggacagggg agtggcgccc 120

tgcaatattt gcatgtcgct atgtgttctg ggaaatcacc ataaacgtga aatgtctttg 180

gatttgggaa tcttataagt tctgtatgag accactt 217

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<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: U6 promoter

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ataattggaa ttaatttgac tgtaaacaca aagatattag tacaaaatac gtgacgtaga 120

aagtaataat ttcttgggta gtttgcagtt ttaaaattat gttttaaaat ggactatcat 180

atgcttaccg taacttgaaa gtatttcgat ttcttggctt tatatatctt gtggaaagga 240

cgaaacacc 249

<210> SEQ ID NO 17
<211> LENGTH: 243
<212> TYPE: DNA
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<220> FEATURE:

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<223> OTHER INFORMATION: 7SK promoter

<400> SEQUENCE: 17

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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 18
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<223> OTHER INFORMATION: CAG enhancer

<400> SEQUENCE: 18

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 19
<211> LENGTH: 290
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CAG promoter

<400> SEQUENCE: 19

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ctccccaccc ccaattttgt atttatttat tttttaatta ttttgtgcag cgatgggggc 120

gggggggggg ggggcgcgcg ccaggcgggg cggggcgggg cgaggggcgg ggcggggcga 180

ggcggagagg tgcggcggca gccaatcaga gcggcgcgct ccgaaagttt cctttttatgg 240

cgaggcggcg gcggcggcgg ccctataaaa agcgaagcgc gcggcgggcg 290

<210> SEQ ID NO 20
<211> LENGTH: 960
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: chicken beta actin intron

<400> SEQUENCE: 20

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cggctctgac tgaccgcgtt actcccacag gtgagcgggc gggacggccc ttctcctccg 120

ggctgtaatt agcgcttggt ttaatgacgg ctcgtttctt ttctgtggct gcgtgaaagc 180

cttaaagggc tccgggaggg ccctttgtgc gggggggagc ggctcggggg gtgcgtgcgt 240

gtgtgtgtgc gtggggagcg ccgcgtgcgg cccgcgctgc ccggcggctg tgagcgctgc 300

gggcgcggcg cggggctttg tgcgctccgc gtgtgcgcga ggggagcgcg gccgggggcg 360

gtgccccgcg gtgcgggggg gctgcgaggg gaacaaaggc tgcgtgcggg gtgtgtgcgt 420

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cggggctcgc cgtgccgggc gggggggtggc 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 21
<211> LENGTH: 1503
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: HIV gag

<400> SEQUENCE: 21

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 gtgggggggac 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 gctaatttttt tagggaagat ctggccttcc 1320

cacaagggaa ggccagggaa ttttcttcag agcagaccag agccaacagc cccaccagaa 1380

gagagcttca ggtttgggga agagacaaca actccctctc agaagcagga gccgatagac 1440

aaggaactgt atcctttagc ttccctcaga tcactctttg gcagcgaccc ctcgtcacaa 1500

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taa 1503

<210> SEQ ID NO 22
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: HIV Pol

<400> SEQUENCE: 22

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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 agaaatagggg 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

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

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: HIV Int

<400> SEQUENCE: 23

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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 aaaaggggg attgggggt 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 24
<211> LENGTH: 234
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: HIV RRE

<400> SEQUENCE: 24

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 25
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: HIV Rev

<400> SEQUENCE: 25

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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 cgcaggggtg gggaagccct 300

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<210> SEQ ID NO 26

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<211> LENGTH: 448
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: rabbit beta globin poly A

<400> SEQUENCE: 26

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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 27
<211> LENGTH: 577
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CMV Promoter

<400> SEQUENCE: 27

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 28
<211> LENGTH: 573
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: beta globin intron

<400> SEQUENCE: 28

gtgagtttgg ggacccttga ttgttctttc tttttcgcta ttgtaaaatt catgttatat 60

ggaggggggca aagttttcag ggtgttgttt agaatgggaa gatgtcccttt gtatcaccat 120

ggaccctcat gataattttg tttctttcac tttctactct gttgacaacc attgtctcct 180

cttatttttct 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

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tgatatacac tgtttgagat gaggataaaa tactctgagt ccaaaccggg cccctctgct 540

aaccatgttc atgccttctt ctctttccta cag 573

<210> SEQ ID NO 29
<211> LENGTH: 1531
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VSV-G / DNA fragment containing VSV-G

<400> SEQUENCE: 29

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

ttatttttttg 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 30
<211> LENGTH: 450
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: rabbit beta globin poly A

<400> SEQUENCE: 30

agatcttttt ccctctgcca aaaattatgg ggacatcatg aagccccttg agcatctgac 60

-continued

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 ttttttttctt taacatccct 360

aaaattttcc ttacatgttt tactagccag atttttcctc ctctcctgac tactcccagt 420

catagctgtc cctcttctct tatggagatc 450

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

<400> SEQUENCE: 31

taagcagaat tcatgaattt gccaggaaga t 31

<210> SEQ ID NO 32
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 32

ccatacaatg aatggacact aggcggccgc acgaat 36

<210> SEQ ID NO 33
<211> LENGTH: 2745
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Gag, Pol, Integrase fragment

<400> SEQUENCE: 33

gaattcatga atttgccagg aagatggaaa ccaaaaatga taggggaat 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

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

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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 ataccctcc 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 tgggggggtac 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 34
<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: 34

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

-continued

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

agatttttt tatattttgt tttgtgttat ttttttcttt aacatcccta aaattttcct 960

tacatgtttt actagccaga tttttcctcc tctcctgact actcccagtc atagctgtcc 1020

ctctttctctt 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 35
<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: 35

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 acgccccta ttgacgtcaa tgacggtaaa tggcccgcct ggcattatgc 300

ccagtacatg accttatggg actttcctac ttggcagtac atctacgtat tagtcatcgc 360

tattaccatg ggtcgaggtg agccccacgt tctgcttcac tctccccatc tccccccct 420

ccccacccc 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

-continued

ttgccttcgc cccgtgcccc gctccgcgcc gcctcgcgcc gcccgccccg gctctgactg 720

accgcgttac tcccacaggt gagcgggcgg gacggccctt ctcctccggg ctgtaattag 780

cgcttggttt aatgacggct cgtttctttt ctgtggctgc gtgaaagcct taaagggctc 840

cgggagggcc ctttgtgcgg gggggagcgg ctcgggggggt gcgtgcgtgt gtgtgtgcgt 900

ggggagcgcc gcgtgcggcc cgcgctgccc ggcggctgtg agcgctgcgg gcgcggcgcg 960

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gcgggggggc tgcgagggga acaaaggctg cgtgcggggt gtgtgcgtgg gggggtgagc 1080

agggggtgtg ggcgcggcgg tcgggctgta acccccccct 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 caggggacg gctgccttcg 1560

gggggacgg ggcagggcgg ggttcggctt ctggcgtgtg accggcggga attc 1614

<210> SEQ ID NO 36
<211> LENGTH: 884
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: RSV promoter and HIV Rev

<400> SEQUENCE: 36

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 37
<211> LENGTH: 1104
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Elongation Factor-1 alpha (EF1-alpha) promoter

<400> SEQUENCE: 37

ccggtgccta gagaaggtgg cgcggggtaa actgggaaag tgatgtcgtg tactggctcc 60

gccttttttcc 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 acgcttttt 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 ttgggggggag gggttttatg cgatggagtt tccccacact 960

gagtgggtgg agactgaagt taggccagct tggcacttga tgtaattctc cttggaattt 1020

gccctttttg agtttggatc ttggttcatt ctcaagcctc agacagtggt tcaaagtttt 1080

tttcttccat ttcaggtgtc gtga 1104

<210> SEQ ID NO 38
<211> LENGTH: 511
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Promoter - PGK

<400> SEQUENCE: 38

ggggttgggg ttgcgccttt tccaaggcag ccctgggttt gcgcagggac gcggctgctc 60

tgggcgtggt tccgggaaac gcagcggcgc cgaccctggg tctcgcacat tcttcacgtc 120

cgttcgcagc gtcacccgga tcttcgccgc taccttgtg 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 39
<211> LENGTH: 1162
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Promoter - UbC

<400> SEQUENCE: 39

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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 40
<211> LENGTH: 120
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Poly A - SV40

<400> SEQUENCE: 40

gtttattgca gcttataatg gttacaaata aagcaatagc atcacaaatt tcacaaataa 60

agcatttttt tcactgcatt ctagttgtgg tttgtccaaa ctcatcaatg tatcttatca 120

<210> SEQ ID NO 41
<211> LENGTH: 227
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Poly A - bGH

<400> SEQUENCE: 41

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 42
<211> LENGTH: 1695
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Envelope - RD114

<400> SEQUENCE: 42

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 ggggagaact 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 43
<211> LENGTH: 2013
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope - GALV

<400> SEQUENCE: 43

atgcttctca cctcaagccc gcaccacctt cggcaccaga tgagtcctgg gagctggaaa 60

agactgatca tcctcttaag ctgcgtattc ggagacggca aaacgagtct gcagaataag 120

aacccccacc agcctgtgac cctcacctgg caggtactgt cccaaactgg ggacgttgtc 180

tgggacaaaa aggcagtcca gccccttggg acttggtggc cctctcttac acctgatgta 240

-continued

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 gtgggggggct 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 gggccactaa gtcttgctgg 1020

ctctgtttgg gcatgagccc cccttattat 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 44
<211> LENGTH: 1530
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope - FUG

<400> SEQUENCE: 44

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

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acaggtgttg tgacagaggc agagacctac accaactttg ttggttatgt cacaaccaca 300

ttcaagagaa agcatttccg ccccacccca gacgcatgta gagccgcgta taactggaag 360

atggccggtg accccagata tgaagagtcc ctacacaatc catacccga 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 45
<211> LENGTH: 1497
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope - LCMV

<400> SEQUENCE: 45

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

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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 46
<211> LENGTH: 1692
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope - FPV

<400> SEQUENCE: 46

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

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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 gggcatcatg ctttttgctt 1620

cttgccattg caatgggcct tgttttcata tgtgtgaaga acggaaacat gcggtgcact 1680

atttgtatat aa 1692

<210> SEQ ID NO 47
<211> LENGTH: 1266
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope - RRV

<400> SEQUENCE: 47

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

<210> SEQ ID NO 48
<211> LENGTH: 2030
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope - Ebola

<400> SEQUENCE: 48

atgggtgtta caggaatatt gcagttacct cgtgatcgat tcaagaggac atcattcttt 60

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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 49
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: FDPS target sequence #1

<400> SEQUENCE: 49

gtcctggagt acaatgccat t 21

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<210> SEQ ID NO 50
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: FDPS target sequence #2

<400> SEQUENCE: 50

gcaggatttc gttcagcact t 21

<210> SEQ ID NO 51
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: FDPS target sequence #3

<400> SEQUENCE: 51

gccatgtaca tggcaggaat t 21

<210> SEQ ID NO 52
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: FDPS target sequence #4

<400> SEQUENCE: 52

gcagaaggag gctgagaaag t 21

<210> SEQ ID NO 53
<211> LENGTH: 116
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR30 FDPS sequence #1

<400> SEQUENCE: 53

aaggtatatt gctgttgaca gtgagcgaca ctttctcagc ctccttctgc gtgaagccac 60

agatggcaga aggaggctga gaaagtgctg cctactgcct cggacttcaa ggggct 116

<210> SEQ ID NO 54
<211> LENGTH: 114
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR30 FDPS sequence #2

<400> SEQUENCE: 54

aaggtatatt gctgttgaca gtgagcgaca ctttctcagc ctccttctgc gtgaagccac 60

agatggcaga agggctgaga aagtgctgcc tactgcctcg gacttcaagg ggct 114

<210> SEQ ID NO 55
<211> LENGTH: 91
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR30 FDPS sequence #3

<400> SEQUENCE: 55

tgctgttgac agtgagcgac tttctcagcc tccttctgcg tgaagccaca gatggcagaa 60

ggaggctgag aaagttgcct actgcctcgg a 91

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<210> SEQ ID NO 56
<211> LENGTH: 115
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR155 FDPS sequence #1

<400> SEQUENCE: 56

cctggaggct tgctgaaggc tgtatgctga ctttctcagc ctccttctgc ttttggccac 60

tgactgagca gaagggctga gaaagtcagg acacaaggcc tgttactagc actca 115

<210> SEQ ID NO 57
<211> LENGTH: 114
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR21 FDPS sequence #1

<400> SEQUENCE: 57

catctccatg gctgtaccac cttgtcggga ctttctcagc ctccttctgc ctgttgaatc 60

tcatggcaga aggaggcgag aaagtctgac attttggtat ctttcatctg acca 114

<210> SEQ ID NO 58
<211> LENGTH: 114
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR185 FDPS sequence #1

<400> SEQUENCE: 58

gggcctggct cgagcagggg gcgagggata ctttctcagc ctccttctgc tggtcccctc 60

cccgcagaag gaggctgaga aagtccttcc ctcccaatga ccgcgtcttc gtcg 114

<210> SEQ ID NO 59
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Forward Primer

<400> SEQUENCE: 59

aggaattgat ggcgagaagg 20

<210> SEQ ID NO 60
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Reverse Primer

<400> SEQUENCE: 60

cccaaagagg tcaaggtaat ca 22

<210> SEQ ID NO 61
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Forward Primer

<400> SEQUENCE: 61

agcgcggcta cagcttca 18

<210> SEQ ID NO 62

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<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Reverse Primer

<400> SEQUENCE: 62

ggcgacgtag cacagcttct 20

<210> SEQ ID NO 63
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Forward Primer

<400> SEQUENCE: 63

cactgtcgtc attccatgct 20

<210> SEQ ID NO 64
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Reverse Primer

<400> SEQUENCE: 64

gcctcttgac attctcctc 19

<210> SEQ ID NO 65
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Reverse Primer

<400> SEQUENCE: 65

aaagtcagtg gggacagtgg 20

<210> SEQ ID NO 66
<211> LENGTH: 117
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR155 CD47 target sequence #2

<400> SEQUENCE: 66

cctggaggct tgctgaaggc tgtatgctgt tagctcgatg atcgtttcac gttttggcca 60

ctgactgacg tgaaacgcat cgagctaaca ggacacaagg cctgttacta gcactca 117

<210> SEQ ID NO 67
<211> LENGTH: 117
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR155 CD47 target sequence #3

<400> SEQUENCE: 67

cctggaggct tgctgaaggc tgtatgctga agaatggctc caacaatgac gttttggcca 60

ctgactgacg tcattgtgag ccattcttca ggacacaagg cctgttacta gcactca 117

<210> SEQ ID NO 68
<211> LENGTH: 117
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: miR155 CD47 target sequence #4

<400> SEQUENCE: 68

cctggaggct tgctgaaggc tgtatgctgt atacacgccg caatacagag gttttggcca 60

ctgactgacc tctgtatcgg cgtgtataca ggacacaagg cctgttacta gcactca 117

<210> SEQ ID NO 69
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Forward primer

<400> SEQUENCE: 69

ggactatcct gctgccaa 18

<210> SEQ ID NO 70
<211> LENGTH: 115
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR155 cMyc sequence

<400> SEQUENCE: 70

cctggaggct tgctgaaggc tgtatgctgt gttcgcctct tgacattctc ttttggccac 60

tgactgagag aatgtagagg cgaacacagg acacaaggcc tgttactagc actca 115

<210> SEQ ID NO 71
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: cMyc target sequence

<400> SEQUENCE: 71

gagaatgtca agaggcgaac a 21

<210> SEQ ID NO 72
<211> LENGTH: 329
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CMV promoter sequence

<400> SEQUENCE: 72

attatgccca gtacatgacc ttatgggact ttcctacttg gcagtacatc tacgtattag 60

tcatcgctat taccatggtg atgcggtttt ggcagtacat caatgggcgt ggatagcggt 120

ttgactcacg gggatttcca agtctccacc ccattgacgt caatgggagt ttgttttggc 180

accaaaatca acgggacttt ccaaaatgtc gtaacaactc cgccccattg acgcaaatgg 240

gcggtaggcg tgtacggtgg gaggtttata taagcagagc tcgtttagtg aaccgtcaga 300

tcgcctggag acgccatcca cgctgtttt 329

<210> SEQ ID NO 73
<211> LENGTH: 2520
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: GFP T2A Luciferase sequence

<400> SEQUENCE: 73

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atgcccgcca tgaagatcga gtgccgcatc accggcaccc tgaacggcgt ggagttcgag 60

ctggtgggcg gcggagaggg cacccccgag cagggccgca tgaccaacaa gatgaagagc 120

accaaaggcg ccctgacctt cagcccctac ctgctgagcc acgtgatggg ctacggcttc 180

taccacttcg gcacctaccc cagcggctac gagaacccct tcctgcacgc catcaacaac 240

ggcggctaca ccaacacccg catcgagaag tacgaggacg gcggcgtgct gcacgtgagc 300

ttcagctacc gctacgaggc cggccgcgtg atcggcgact tcaaggtggt gggcaccggc 360

ttccccgagg acagcgtgat cttcaccgac aagatcatcc gcagcaacgc caccgtggag 420

cacctgcacc ccatgggcga taacgtgctg gtgggcagct tcgcccgcac cttcagcctg 480

cgcgacggcg gctactacag cttcgtggtg gacagccaca tgcacttcaa gagcgccatc 540

caccccagca tcctgcagaa cgggggcccc atgttcgcct tccgccgcgt ggaggagctg 600

cacagcaaca ccgagctggg catcgtggag taccagcacg ccttcaagac ccccatcgcc 660

ttcgccagat ctcgagatat cagccatggc ttcccgccgg cggtggcggc gcaggatgat 720

ggcacgctgc ccatgtcttg tgcccaggag agcgggatgg accgtcaccc tgcagcctgt 780

gcttctgcta ggatcaatgt gaccggtgag ggcagaggaa gtcttctaac atgcggtgac 840

gtggaggaga atcccggccc ttccggtatg gaagacgcca aaaacataaa gaaaggcccg 900

gcgccattct atccgctaga ggatggaacc gctggagagc aactgcataa ggctatgaag 960

agatacgccc tggttcctgg aacaattgct tttacagatg cacatatcga ggtgaacatc 1020

acgtacgcgg aatacttcga aatgtccgtt cggttggcag aagctatgaa acgatatggg 1080

ctgaatacaa atcacagaat cgtcgtatgc agtgaaaact ctcttcaatt ctttatgccg 1140

gtgttgggcg cgttatttat cggagttgca gttgcgcccg cgaacgacat ttataatgaa 1200

cgtgaattgc tcaacagtat gaacatttcg cagcctaccg tagtgtttgt ttccaaaaag 1260

gggttgcaaa aaattttgaa cgtgcaaaaa aaattaccaa taatccagaa aattattatc 1320

atggattcta aaacggatta ccagggattt cagtcgatgt acacgttcgt cacatctcat 1380

ctacctcccg gttttaatga atacgatttt gtaccagagt cctttgatcg tgacaaaaca 1440

attgcactga taatgaactc ctctggatct actgggttac ctaagggtgt ggcccttccg 1500

catagaactg cctgcgtcag attctcgcat gccagagatc ctatttttgg caatcaaatc 1560

attccggata ctgcgatttt aagtgttgtt ccattccatc acggttttgg aatgtttact 1620

acactcggat atttgatatg tggatttcga gtcgtcttaa tgtatagatt tgaagaagag 1680

ctgtttttac gatcccttca ggattacaaa attcaaagtg cgttgctagt accaacccta 1740

ttttcattct tcgccaaaag cactctgatt gacaaatacg atttatctaa tttacacgaa 1800

attgcttctg ggggcgcacc tctttcgaaa gaagtcgggg aagcggttgc aaaacgcttc 1860

catcttccag ggatacgaca aggatatggg ctcactgaga ctacatcagc tattctgatt 1920

acacccgagg gggatgataa accgggcgcg gtcggtaaag ttgttccatt ttttgaagcg 1980

aaggttgtgg atctggatac cgggaaaacg ctgggcgtta atcagagagg cgaattatgt 2040

gtcagaggac ctatgattat gtccggttat gtaaacaatc cggaagcgac caacgccttg 2100

attgacaagg atggatggct acattctgga gacatagctt actgggacga agacgaacac 2160

ttcttcatag ttgaccgctt gaagtcttta attaaataca aaggatacca ggtggccccc 2220

gctgaattgg agtcgatatt gttacaacac cccaacatct tcgacgcggg cgtggcaggt 2280

cttcccgacg atgacgccgg tgaacttccc gccgccgttg ttgttttgga gcacggaaag 2340

acgatgacgg aaaaagagat cgtggattac gtcgccagtc aagtaacaac cgcgaaaaag 2400

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ttgcgcggag gagttgtgtt tgtggacgaa gtaccgaaag gtcttaccgg aaaactcgac 2460

gcaagaaaaa tcagagagat cctcataaag gccaagaagg gcggaaagtc caaattgtaa 2520

<210> SEQ ID NO 74
<211> LENGTH: 228
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Rous Sarcoma virus (RSV) promoter

<400> SEQUENCE: 74

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 75
<211> LENGTH: 180
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 5' Long terminal repeat (LTR)

<400> SEQUENCE: 75

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 76
<211> LENGTH: 41
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Psi Packaging signal

<400> SEQUENCE: 76

tacgccaaaa attttgacta gcggaggcta gaaggagaga g 41

<210> SEQ ID NO 77
<211> LENGTH: 233
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Rev response element (RRE)

<400> SEQUENCE: 77

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 78
<211> LENGTH: 118
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Central polypurine tract (cPPT)

<400> SEQUENCE: 78

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ttttaaaaga aaaggggga ttgggggta cagtgcaggg gaaagaatag tagacataat 60

agcaacagac atacaaacta aagaattaca aaaacaaatt acaaaattca aaatttta 118

<210> SEQ ID NO 79
<211> LENGTH: 590
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Long WPRE sequence

<400> SEQUENCE: 79

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 80
<211> LENGTH: 250
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 3' delta LTR

<400> SEQUENCE: 80

tggaagggct aattcactcc caacgaagat aagatctgct ttttgcttgt actgggtctc 60

tctggttaga ccagatctga gcctgggagc tctctggcta actagggaac ccactgctta 120

agcctcaata aagcttgcct tgagtgcttc aagtagtgtg tgcccgtctg ttgtgtgact 180

ctggtaacta gagatccctc agacccttttt agtcagtgtg gaaaatctct agcagtagta 240

gttcatgtca 250

<210> SEQ ID NO 81
<211> LENGTH: 1938
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope - MLV 10A1

<400> SEQUENCE: 81

atggaaggtc cagcgttctc aaaacccctt aaagataaga ttaacccgtg gaagtcctta 60

atggtcatgg gggtctattt aagagtaggg atggcagaga gcccccatca ggtctttaat 120

gtaacctgga gagtcaccaa cctgatgact gggcgtaccg ccaatgccac ctccctttta 180

ggaactgtac aagatgcctt cccaagatta tattttgatc tatgtgatct ggtcggagaa 240

gagtgggacc cttcagacca ggaaccatat gtcgggtatg gctgcaaata ccccggaggg 300

agaaagcgga cccggacttt tgacttttac gtgtgccctg ggcataccgt aaaatcgggg 360

tgtgggggcc caagagaggg ctactgtggt gaatggggtt gtgaaaccac cggacaggct 420

tactggaagc ccacatcatc atgggaccta atctccctta agcgcggtaa caccccctgg 480

gacacgggat gctccaaaat ggcttgtggc ccctgctacg acctctccaa agtatccaat 540

tccttccaag gggctactcg agggggcaga tgcaaccctc tagtcctaga attcactgat 600

gcaggaaaaa aggctaattg ggacgggccc aaatcgtggg gactgagact gtaccggaca 660

ggaacagatc ctattaccat gttctccctg acccgccagg tcctcaatat agggccccgc 720

atccccattg ggcctaatcc cgtgatcact ggtcaactac ccccctcccg acccgtgcag 780

atcaggctcc ccaggcctcc tcagcctcct cctacaggcg cagcctctat agtccctgag 840

actgccccac cttctcaaca acctgggacg ggagacaggc tgctaaacct ggtagaagga 900

gcctatcagg cgcttaacct caccaatccc gacaagaccc aagaatgttg gctgtgctta 960

gtgtcgggac ctccttatta cgaaggagta gcggtcgtgg gcacttatac caatcattct 1020

accgccccgg ccagctgtac ggccacttcc caacataagc ttaccctatc tgaagtgaca 1080

ggacagggcc tatgcatggg agcactacct aaaactcacc aggccttatg taacaccacc 1140

caaagtgccg gctcaggatc ctactacctt gcagcacccg ctggaacaat gtgggcttgt 1200

agcactggat tgactccctg cttgtccacc acgatgctca atctaaccac agactattgt 1260

gtattagttg agctctggcc cagaataatt taccactccc ccgattatat gtatggtcag 1320

cttgaacagc gtaccaaata taagagggag ccagtatcgt tgaccctggc ccttctgcta 1380

ggaggattaa ccatgggagg gattgcagct ggaataggga cggggaccac tgccctaatc 1440

aaaaacccagc agtttgagca gcttcacgcc gctatccaga cagacctcaa cgaagtcgaa 1500

aaatcaatta ccaacctaga aaagtcactg acctcgttgt ctgaagtagt cctacagaac 1560

cgaagaggcc tagatttgct cttcctaaaa gagggaggtc tctgcgcagc cctaaaagaa 1620

gaatgttgtt tttatgcaga ccacacggga ctagtgagag acagcatggc caaactaagg 1680

gaaaggctta atcagagaca aaaactattt gagtcaggcc aaggttggtt cgaagggcag 1740

tttaatagat ccccctggtt taccacctta atctccacca tcatgggacc tctaatagta 1800

ctcttactga tcttactctt tggaccctgc attctcaatc gattggtcca atttgttaaa 1860

gacaggatct cagtggtcca ggctctggtt ttgactcaac aatatcacca gctaaaacct 1920

atagagtacg agccatga 1938

<210> SEQ ID NO 82
<211> LENGTH: 117
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR155 CD47 target sequence #1

<400> SEQUENCE: 82

cctggaggct tgctgaaggc tgtatgctgt tatccatctt caaagaggca gttttggcca 60

ctgactgact gcctcttaag atggataaca ggacacaagg cctgttacta gcactca 117

<210> SEQ ID NO 83
<211> LENGTH: 114
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: miR21 cMyc sequence

<400> SEQUENCE: 83

catctccatg gctgtaccac cttgtcgggt gttcgcctct tgacattctc ctgttgaatc 60

tcatggagaa tgtcaagggc gaacactgac attttggtat ctttcatctg acca 114

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<210> SEQ ID NO 84
<211> LENGTH: 1227
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Helper plasmid without Rev

<400> SEQUENCE: 84

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 ttatttttcat 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 viral vector comprising a therapeutic cargo portion, wherein the therapeutic cargo portion comprises:

a first small RNA sequence that is capable of binding to a first pre-determined complementary mRNA sequence, wherein the first pre-determined complementary mRNA sequence comprises a FDPS mRNA sequence; and

a second small RNA sequence that is capable of binding to a second pre-determined complementary mRNA sequence, wherein the second pre-determined complementary mRNA sequence comprises a CD47 mRNA sequence or a cMyc mRNA sequence.

2. The viral vector of claim **1**, wherein the first small RNA sequence is under the control of a first promoter, and the second small RNA sequence is under the control of a second promoter.

3. The viral vector of claim **1**, wherein the therapeutic cargo portion further comprises a third small RNA sequence that is capable of binding to a third pre-determined complementary mRNA sequence, wherein the third pre-determined complementary mRNA sequence comprises a CD47 mRNA sequence or a cMyc mRNA sequence.

4. The viral vector of claim **3**, wherein the third small RNA sequence is under the control of a third promoter.

5. The viral vector of claim **3**, wherein the small RNA sequences are under the control of a single promoter.

6. The viral vector of claim **1**, wherein the first small RNA sequence comprises a miRNA or a shRNA.

7. The viral vector of claim **1**, wherein the first small RNA sequence comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with a FDPS small RNA sequence comprising

(SEQ ID NO: 1)
GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTACTCCAGGACTT

TTT;

-continued

(SEQ ID NO: 2)
GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCTGAACGAAATCCTGCTT

TTT;

(SEQ ID NO: 3)
GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGTACATGGCTT

TTT;
or

(SEQ ID NO: 4)
GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCTT

TTT.

8. The viral vector of claim **7**, wherein the first small RNA sequence is selected from SEQ ID NOs: 1, 2, 3, or 4.

9. The viral vector of claim **1**, wherein the second small RNA sequence comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with a CD47 small RNA sequence comprising

(SEQ ID NO: 5)
GGTGAAACGATCATCGAGCCTCGAGGCTCGATGATCGTTTCACCTTTTT;

(SEQ ID NO: 6)
GCTACTGGCCTTGGTTTAACTCGAGTTAAACCAAGGCCAGTAGCTTTTT;

(SEQ ID NO: 7)
CCTCCTTCGTCATTGCCATCTCGAGATGGCAATGACGAAGGAGGTTTTT;

(SEQ ID NO: 8)
GCATGGCCCTCTTCTGATTCTCGAGAATCAGAAGAGGGCCATGCTTTTT;
or

(SEQ ID NO: 9)
GGTGAAACGATCATCGAGCTACTCGAGTAGCTCGATGATCGTTTCACCTT

TTT
or a cMyc small RNA sequence comprising

(SEQ ID NO: 10)
GCTTCACCAACAGGAACTATGCTCGAGCATAGTTCCTGTTGGTGAAGCTT

TT;

(SEQ ID NO: 11)
GCGAACACACAACGTCTTGGACTCGAGTCCAAGACGTTGTGTGTTCGCTT

TT;

(SEQ ID NO: 12)
GACATGGTGAACCAGAGTTTCCTCGAGGAAACTCTGGTTCACCATGTCTT

TTT;

(SEQ ID NO: 13)
GAGAATGTCAAGAGGCGAACACTCGAGTGTTCGCCTCTTGACATTCTCTT

TTT;
or

(SEQ ID NO: 14)
GCTCATTTCTGAAGAGGACTTCTCGAGAAGTCCTCTTCAGAAATGAGCTT

TTT.

10. The viral vector of claim **9**, wherein the second small RNA sequence is selected from SEQ ID NOs: 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14.

11. The viral vector of claim **3**, wherein the third small RNA sequence comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with a CD47 small RNA sequence comprising SEQ ID NOs: 5, 6, 7, 8, or 9 or a cMyc small RNA sequence comprising SEQ ID NOs: 10, 11, 12, 13, or 14.

12. The viral vector of claim **11**, wherein the third small RNA sequence is selected from SEQ ID NOs: 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14.

13. The viral vector of any one of claims **1** and **2-12**, wherein the viral vector is a lentiviral vector.

14. A lentiviral particle capable of infecting a target cell, the lentiviral particle comprising:

a. an envelope protein optimized for infecting the target cell; and

b. the viral vector according to claim **1**.

15. A composition comprising:

a. the lentiviral particle according to claim **14**; and

b. an aminobisphosphonate drug.

16. The composition of claim **15**, wherein the aminobisphosphonate drug is zoledronic acid.

17. A method of treating cancer in a subject, the method comprising administering to the subject a therapeutically effective amount of the composition of claim **15** or **16**.

18. A method of treating cancer in a subject, the method comprising administering to the subject:

a. a therapeutically effective amount of the lentiviral particle according to claim **14**; and

b. a therapeutically effective amount of an aminobisphosphonate drug.

19. A method of preventing cancer in a subject, the method comprising administering to the subject:

a. an effective amount of the lentiviral particle according to claim **14**; and

b. an effective amount of an aminobisphosphonate drug.

20. The method of claim **18** or **19**, wherein the aminobisphosphonate drug is zoledronic acid.

21. The method of claim **18** or **19**, wherein steps (a) and (b) are carried out simultaneously.

22. The method of claim **18** or **19**, wherein a defined period of time elapses between step (a) and step (b).

23. The method of claim **18** or **19**, wherein the therapeutically effective amount of the lentiviral particle comprises a plurality of single doses of the lentiviral particle.

24. The method of claim **18** or **19**, wherein the therapeutically effective amount of the aminobisphosphonate drug comprises a single dose of the aminobisphosphonate drug.

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