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

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(58) **Field of Classification Search**

None

See application file for complete search history.

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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.

18 Claims, 11 Drawing Sheets

Specification includes a Sequence Listing.

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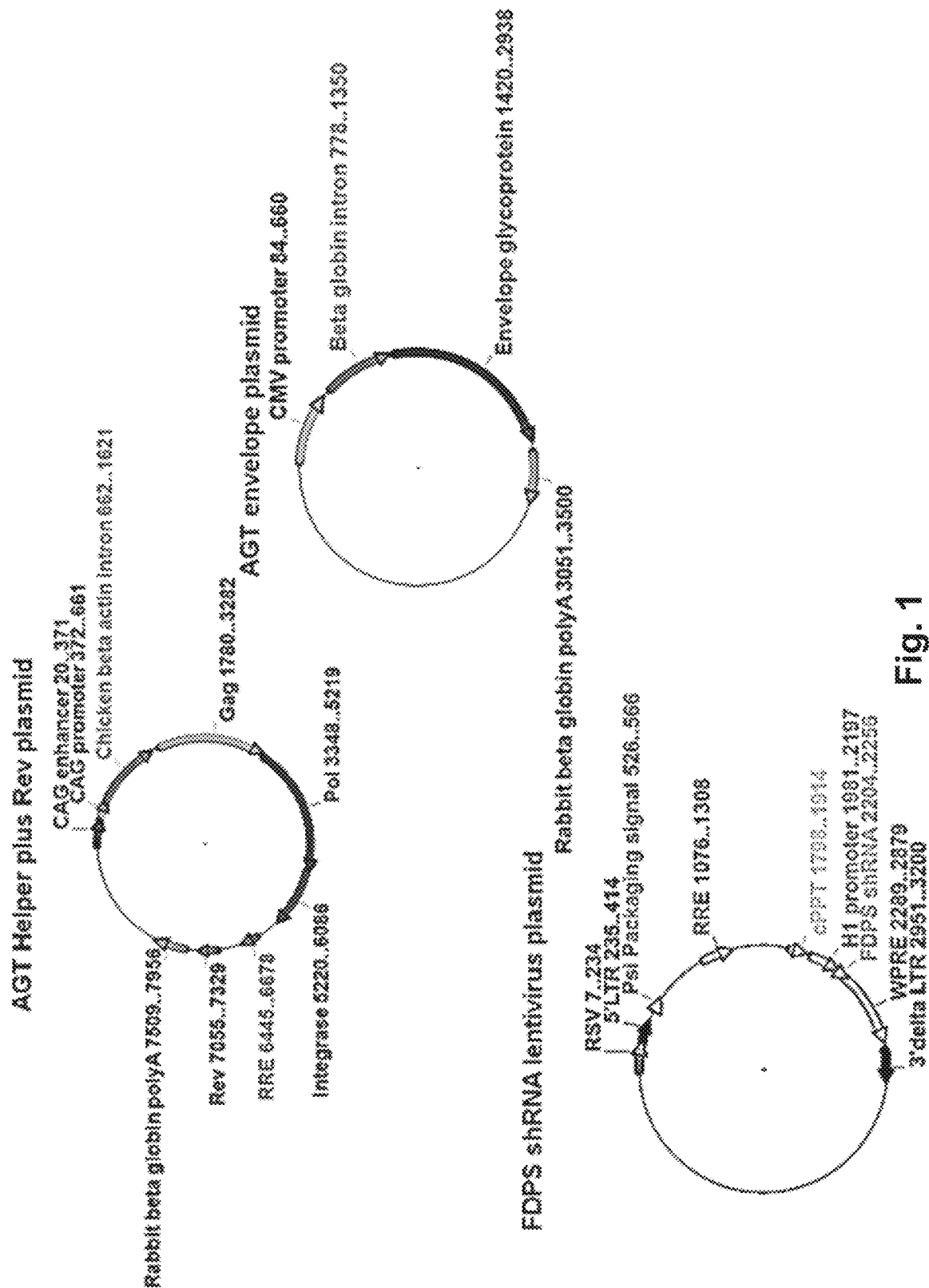


Fig. 1

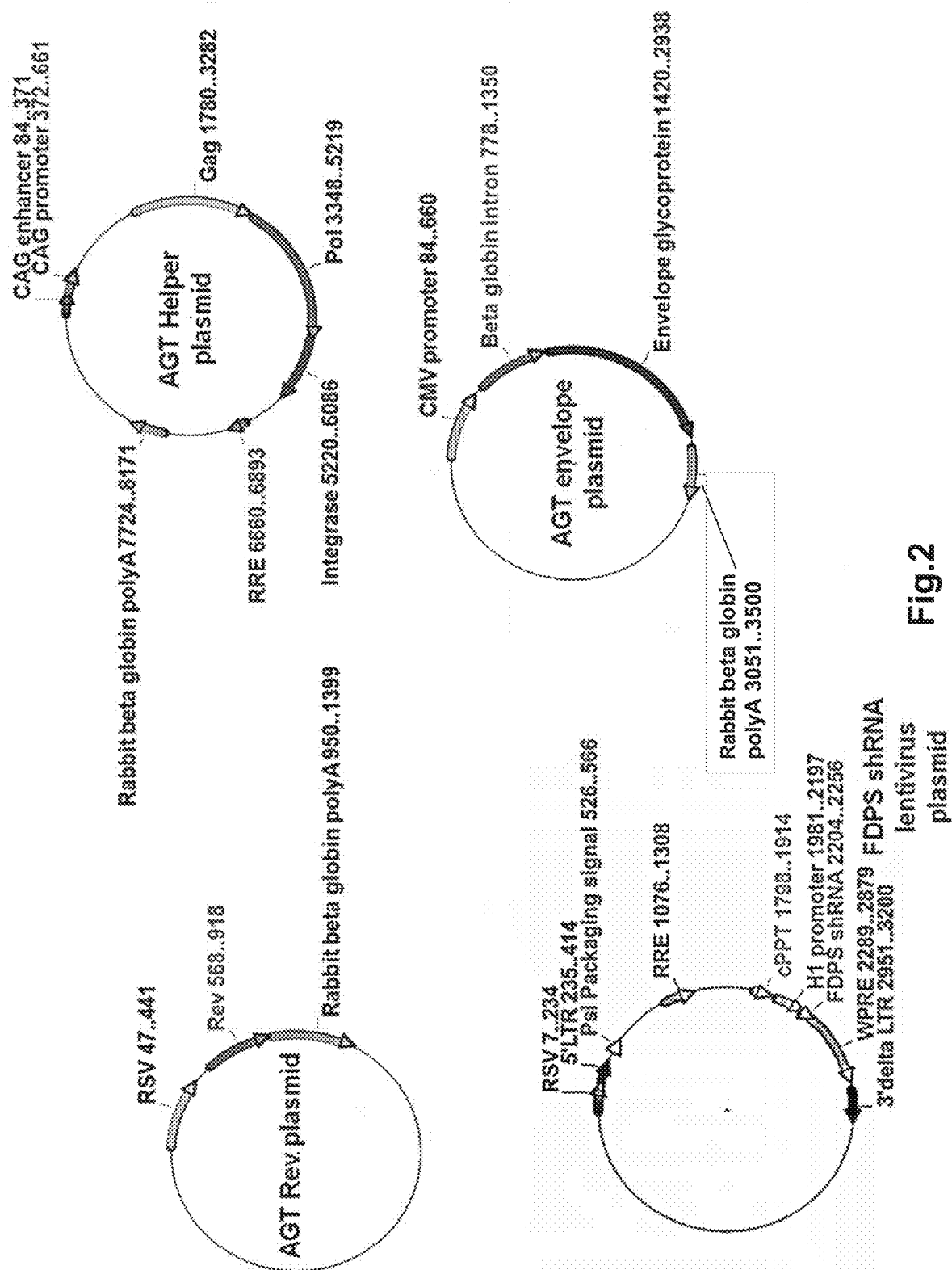


Fig.2

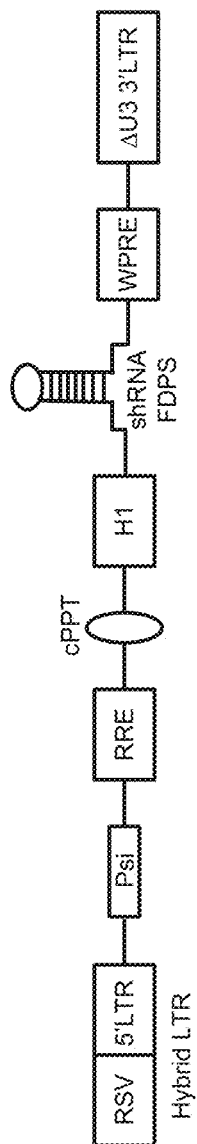


Fig. 3A

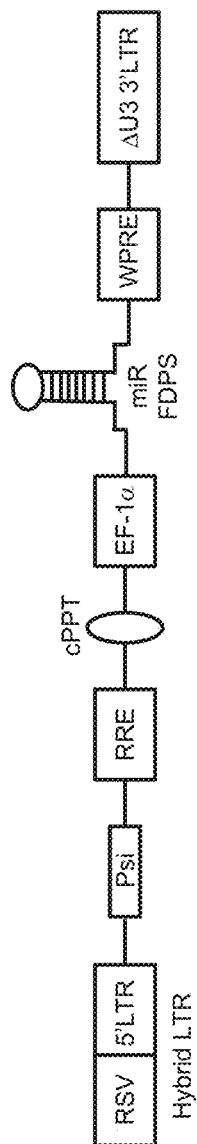


Fig. 3B

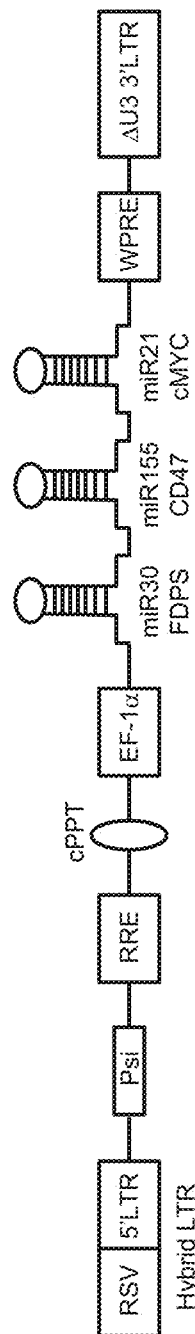


Fig. 3C

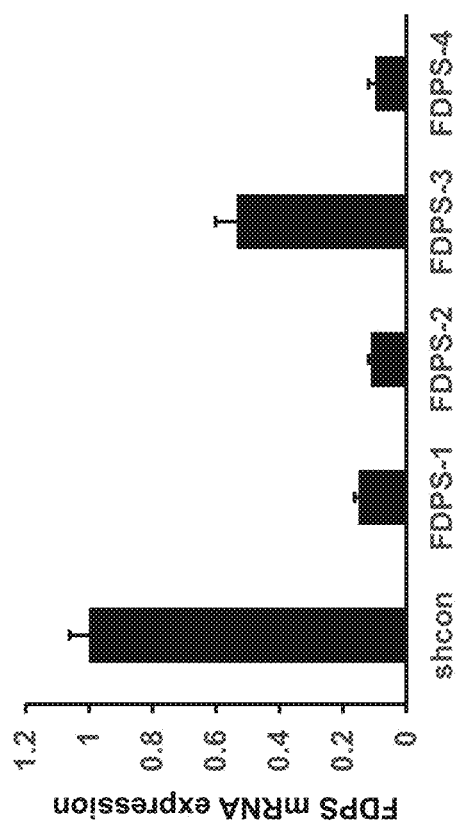


Fig. 4A

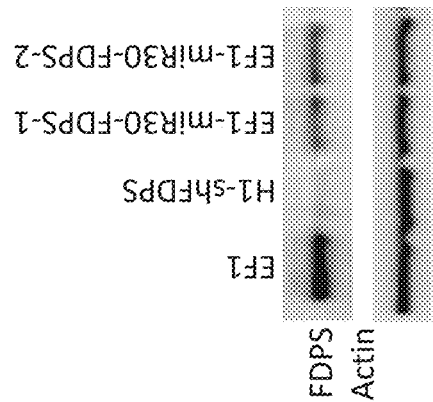
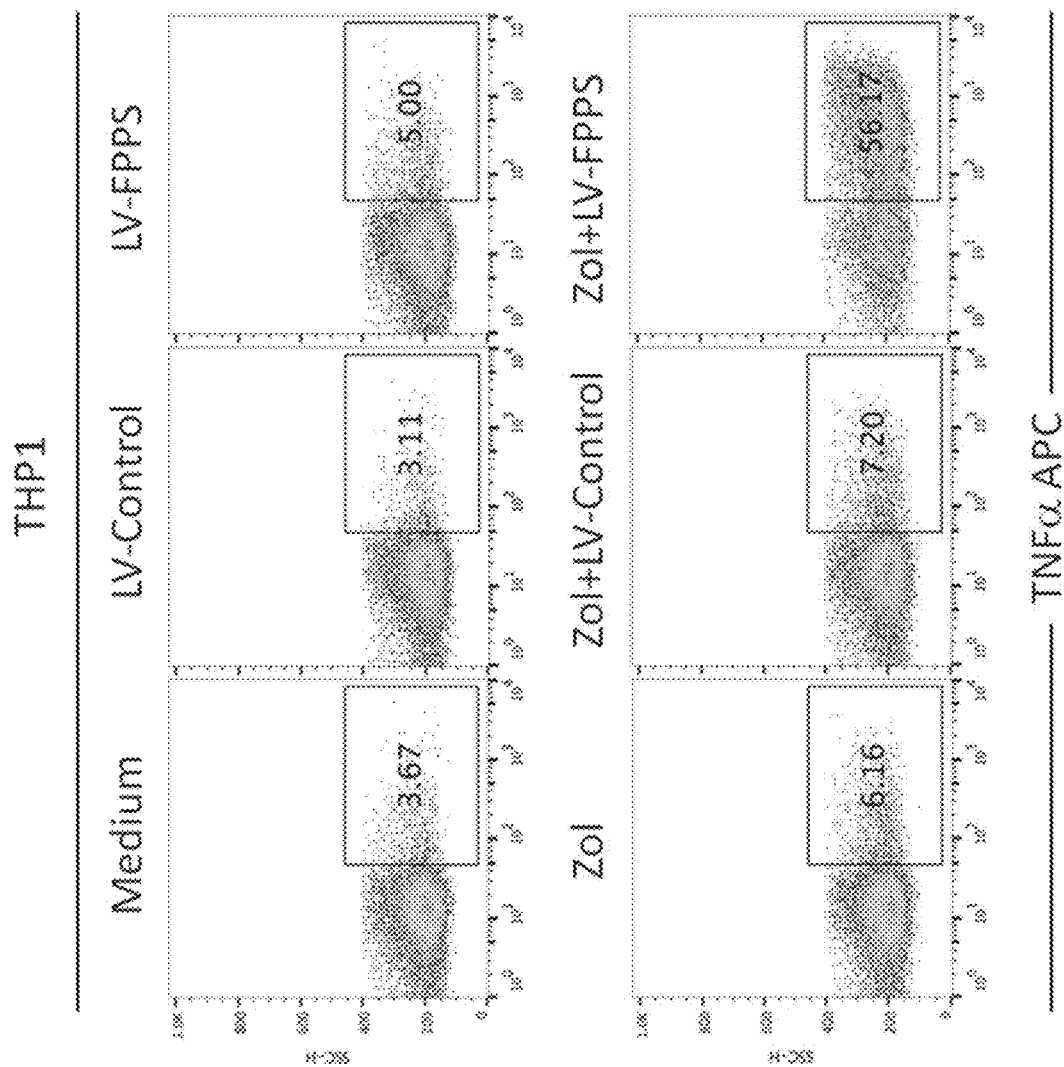


Fig. 4B



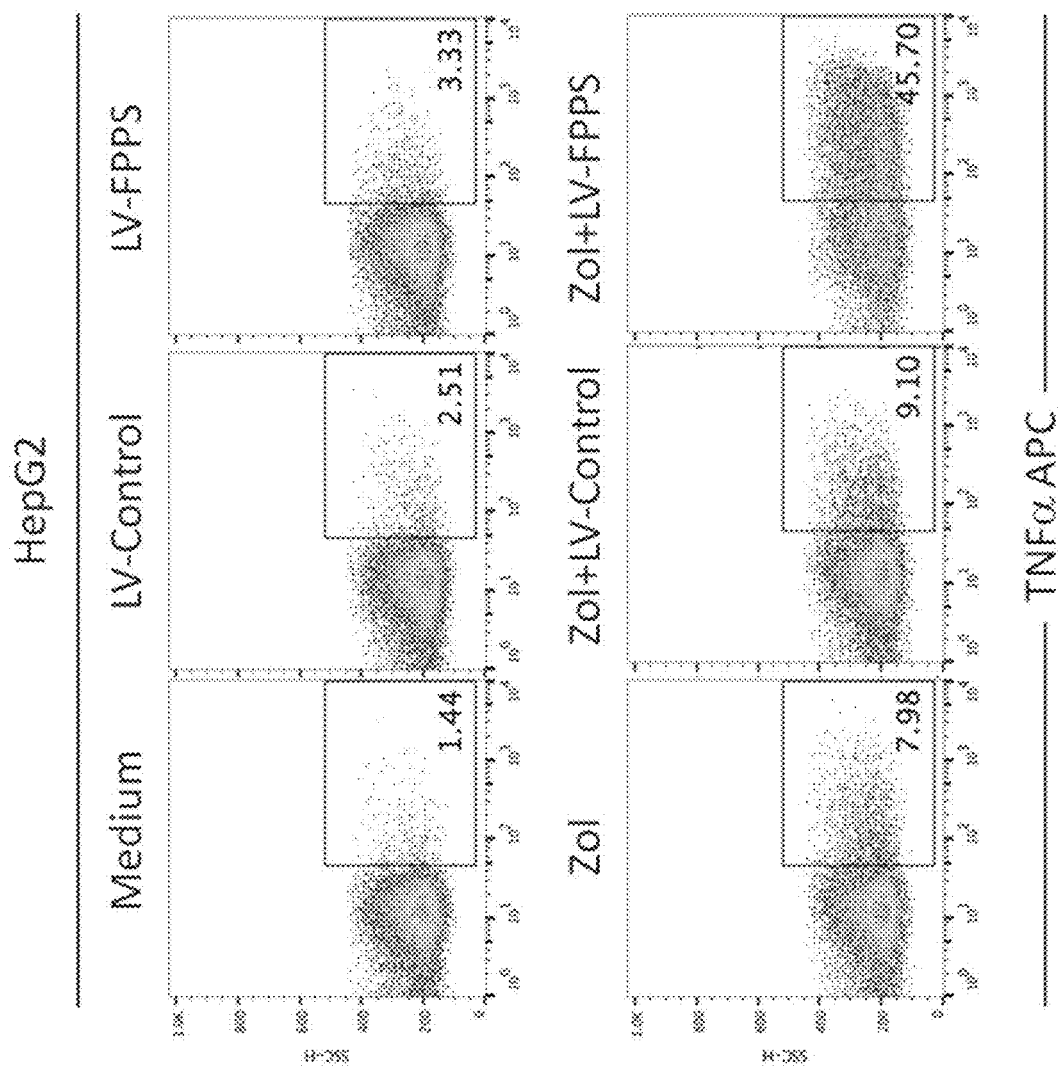


Fig. 5B

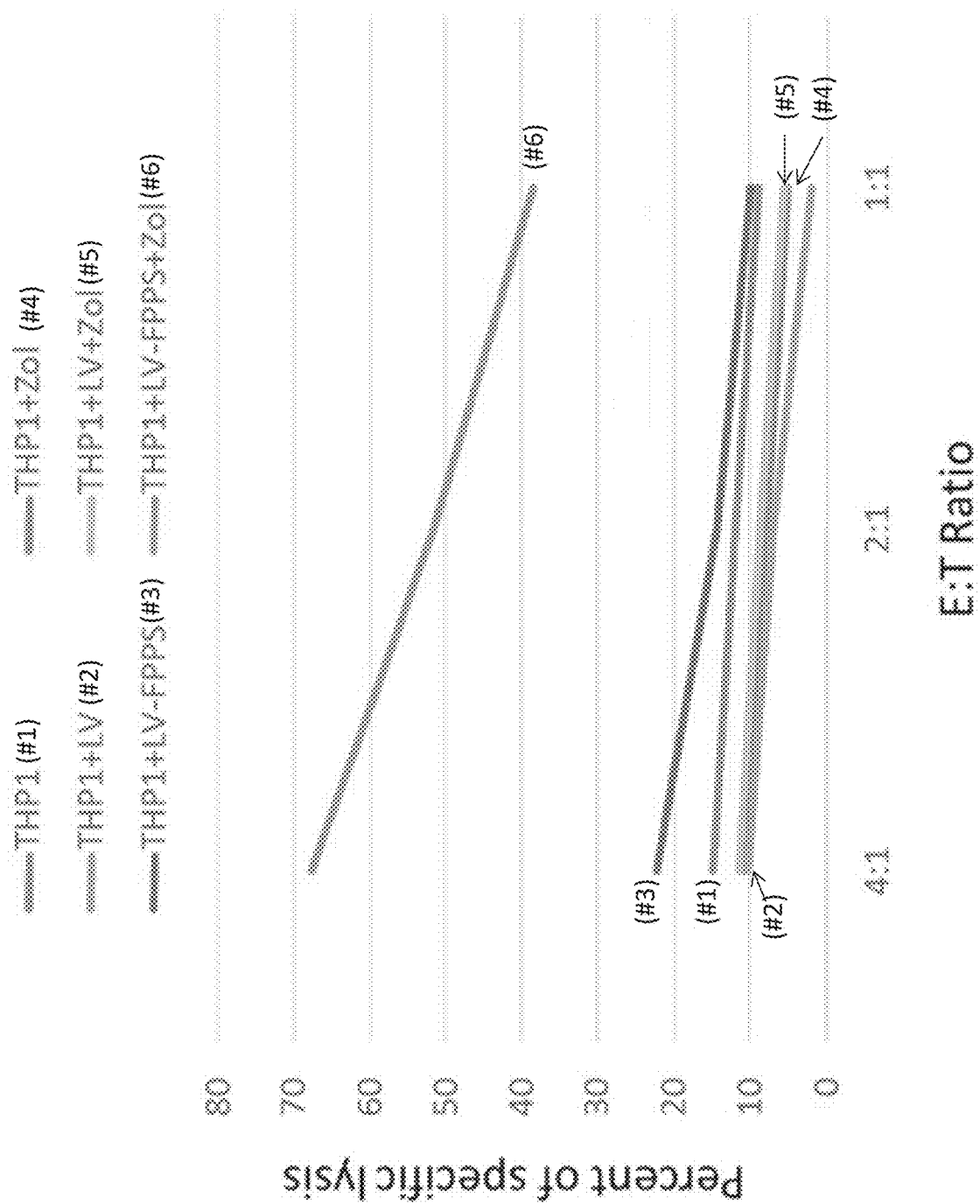


Fig. 6

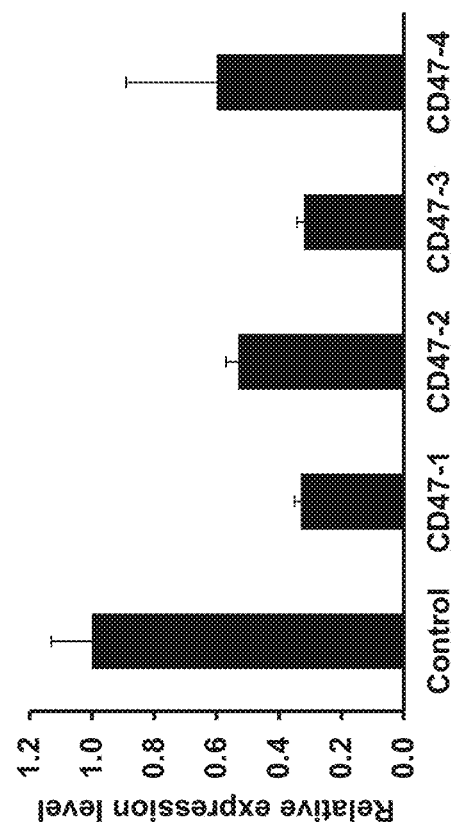


Fig. 7A

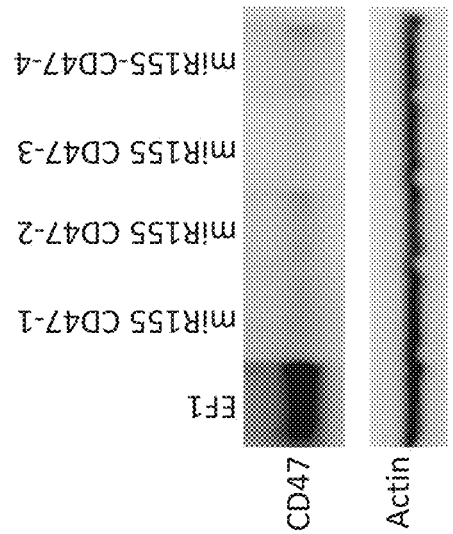


Fig. 7B

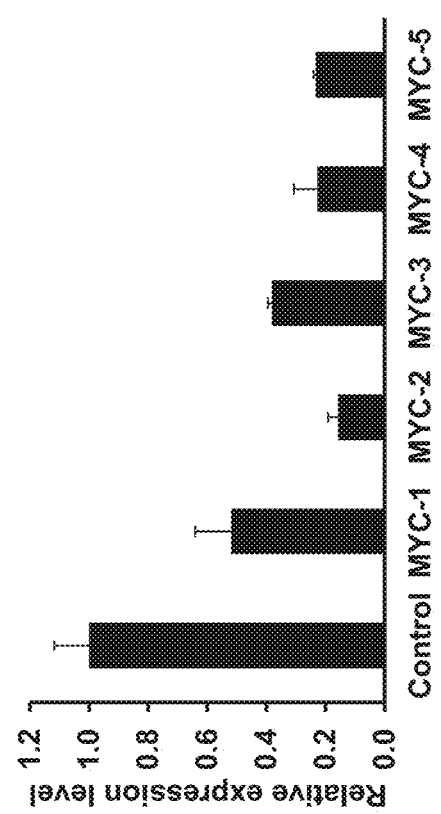


Fig. 8A

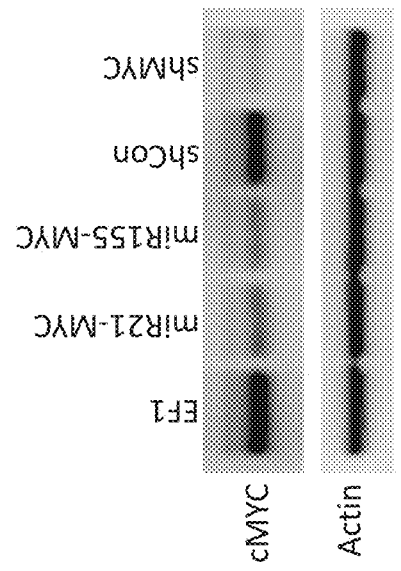


Fig. 8B



Fig. 9

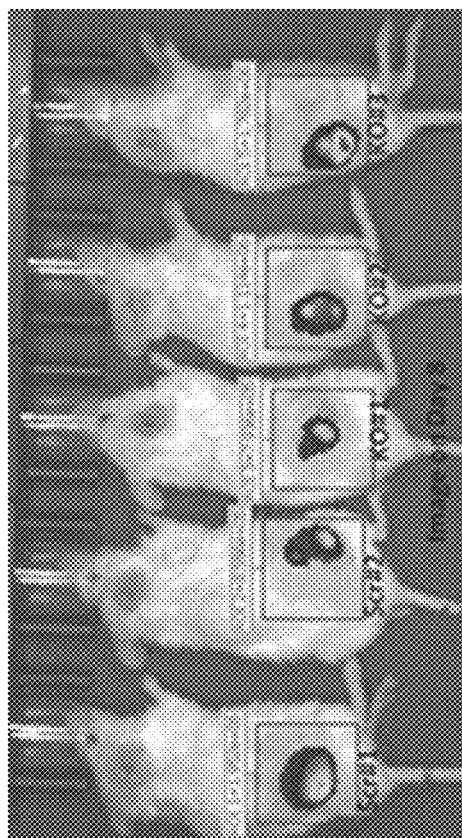


Fig. 10A

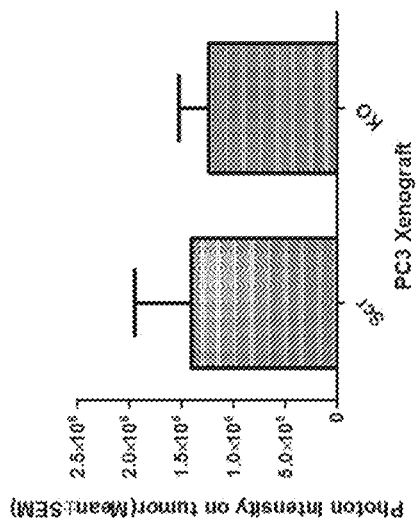


Fig. 10B

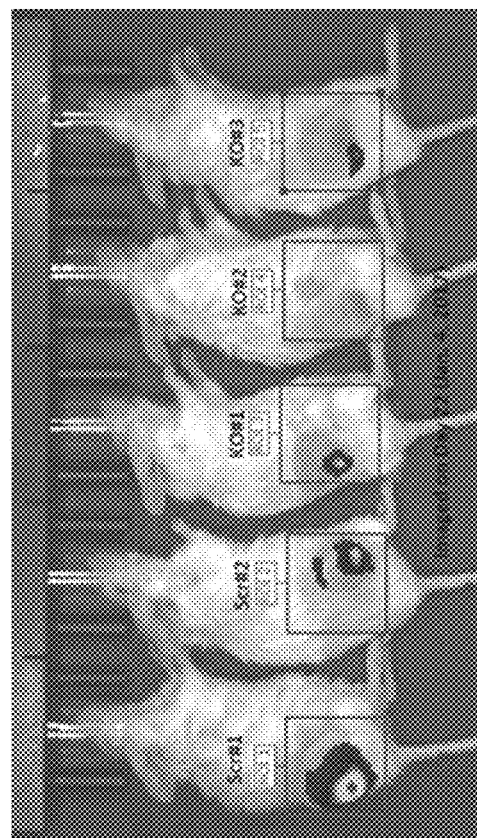


Fig. 10C

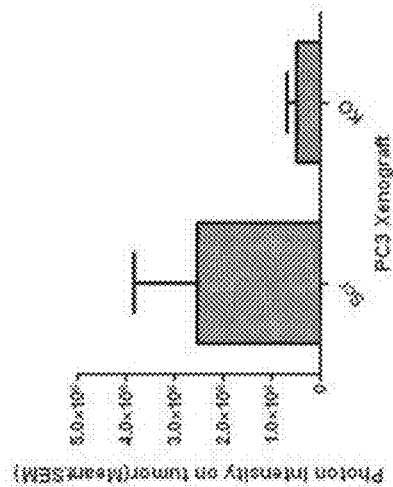


Fig. 10D

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COMBINATION VECTORS AND METHODS FOR TREATING CANCER

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. patent application Ser. No. 16/083,384, filed on Sep. 7, 2018, and entitled "Combination Vectors and Uses Thereof," which is a U.S. national stage application of PCT Patent Application No. PCT/US2017/021639, filed on Mar. 9, 2017 entitled "Combination Vectors and Uses Thereof", which claims priority to U.S. Provisional Patent Application No. 62/305,944, filed on Mar. 9, 2016, and entitled "Combination Vectors and Uses Thereof." These applications are incorporated herein by reference in their entirety.

SEQUENCE LISTING

A Sequence Listing is enclosed with this application and is incorporated herein by reference. The text file of the Sequence Listing is named 7061200517_SL.txt and the file size is 65 kilobytes.

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.

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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 GTCCTGGAGTACAATGC-CATTCTCGAGAATGGCATTGTACTCCAGGACTTTTT (SEQ ID NO: 1); GCAGGAT-TTCGTTTCAGCACTTCTCGAGAAGTGCT-GAACGAAATCCTGCTTTTT (SEQ ID NO: 2); GCCATGTACATGGCAGGAATTCTCGAGAAT-TCCTGCCATGTACATGGCTTTTT (SEQ ID NO: 3); or GCAGAAGGAGGCTGAGAAAGTCTCGA-GACTTTCTCAGCCTCCTTCTGCTTTTT (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 GGTGAAACGAT-CATCGAGCCTCGAGGCTCGATGATCGTTT-CACCTTTTT (SEQ ID NO: 5); GCTACTGGCCTTGTTT-TAACTCGAGTTAAACCAAGGCCAGTAGCTTTTT (SEQ ID NO: 6); CCTCCTTCGTCATTGC-CATCTCGAGATGGCAATGACGAAGGAGGTTTTT (SEQ ID NO: 7); GCATGGCCCTCTTCTGAT-

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TCTCGAGAATCAGAAGAGGGCCATGCTTTTT (SEQ ID NO: 8); or GGTGAAACGATCATCGAGC-TACTCGAGTAGCTCGATGATCGTTTCACCTTTTT (SEQ ID NO: 9) or a cMyc small RNA sequence comprising GCTTCACCAACAGGAACATATGCTCGAGCAT-AGTTCCTGTTGGTGAAGCTTTTT (SEQ ID NO: 10); GCGAACACACAACGTCCTTGGACTCGAGTCCAA-GACGTTGTGTGTTTCGCTTTTT (SEQ ID NO: 11); GACATGGTGAACCAGAGTTTCCTCGAG-GAAACTCTGGTTCACCATGTCTTTTT (SEQ ID NO: 12); GAGAATGT-CAAGAGGCGAACACTCGAGTGTTCGCCTCTTGA-CATTCTCTTTTT (SEQ ID NO: 13); or GCTCATTTC-GAAGAGGACTTCTCGAGAAGTCCTTTCAGAAAT-GAGCTTTTT (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.

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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.

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

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

FIGS. 5A-5B depict cytokine expression levels in human peripheral blood gamma delta T cells after exposure to (FIG. 5A) THP1 or (FIG. 5B) 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.

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

FIGS. 8A-8B depict: (FIG. 8A) the relative expression levels of human cMyc in response to various shRNA constructs, as described herein and (FIG. 8B) 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.

FIGS. 10A-10D depict the effect of zoledronic acid treatment of NOD/SCID mice implanted with PC3 cells transduced with LV-shFDPS or control LV as described herein. (FIG. 10A) depicts photographic data at day 8; (FIG. 10B) depicts photon intensity data at day 8; (FIG. 10C) depicts photographic data at day 22; and (FIG. 10D) 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 $\gamma\delta$ 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, Schwannoma, 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 Papovaviridae (e.g. bovine papillomavirus or BPV) or Herpesviridae (e.g. Epstein Barr Virus or EBV) or Hepadnaviridae (e.g. Hepatitis B Virus or HBV) or pox vectors including vaccinia may be used in the disclosed vectors.

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

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

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

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

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

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. 3C, 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. 3C 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), flaviviruses (tick-borne encephalitis virus, Dengue virus, hepatitis C virus, GB virus), rhabdoviruses (vesicular stomatitis virus, rabies virus), paramyxoviruses (mumps or measles) and orthomyxoviruses (influenza virus). Other envelopes that can preferably be used include those from Moloney Leukemia Virus such as MLV-E, MLV-A and GALV. 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 GP₁ and GP₂ glycoproteins. In another embodiment, one can use different lentiviral capsids with a pseudotyped envelope (for example, FIV or SHIV [U.S. Pat. No. 5,654, 195]). A SHIV pseudotyped vector can readily be used in animal models such as monkeys.

As detailed herein, a lentiviral vector system typically includes at least one helper plasmid comprising at least one of a gag, pol, or rev gene. Each of the gag, pol and rev genes may be provided on individual plasmids, or one or more genes may be provided together on the same plasmid. In one

embodiment, the gag, pol, and rev genes are provided on the same plasmid (e.g., FIG. 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 (WPPE) (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 glycopro-

tein 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

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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 ATGAATTTGCCAG-GAAGAT-3') (SEQ ID NO: 31) and reverse primer was

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(5'-CCATACAAT-GAATGGACACTAGGCGGCCGCACGAAT-3') (SEQ ID NO: 32).

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

(SEQ ID NO: 33)

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GAATTCATGAATTTGCCAGGAAGATGGAACCAAAATGATAGGGGGAAT
10 TGGAGGTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCT
GCGGACATAAAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAAC
ATAATTGGAAGAAATCTGTTGACTCAGATTGGCTGCACCTTTAAATTTTCC
15 CATTAGTCTTATTGAGACTGTACCAGTAAATTAAGCCAGGAATGGATG
GCCCCAAAAGTTAAACAATGGCCATTGACAGAAGAAAAATAAAGCATTAA
GTAGAAATTTGTACAGAAATGGAAGGAAGGAAAAATTTCAAAAATTTGG
20 GCCTGAAAATCCATACAATACTCCAGTATTTGCCATAAAGAAAAAGACA
GTACTAAATGGAGAAAATTAGTAGATTTTCAGAGAACTTAATAAGAGAACT
CAAGATTTCTGGGAAGTTCAATTAGGAATACCACATCCTGCAGGGTTAAA
25 ACAGAAAAAATCAGTAACAGTACTGGATGTGGCGATGCATATTTTTCAG
TTCCCTTAGATAAAGACTTCAGGAAGTATACTGCATTTACCATACCTAGT
ATAACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACA
GGGATGGAAGGATCACCAGCAATATTCAGTGTAGCATGACAAAAATCT
30 TAGAGCCTTTTAGAAAAACAAATCCAGACATAGTCATCTATCAATACATG
GATGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAA
AATAGAGGAAGTGAACAACTCTGTTGAGGTGGGGATTACCACACCAG
35 ACAAAAAACATCAGAAAGAACCTCCATTCTTTGGATGGGTTATGAAGTC
CATCTGTATAAATGGACAGTACAGCCTATAGTGCTGCCAGAAAAGGACAG
CTGGACTGTCAATGACATACAGAAATAGTGGGAAATGAATTGGGCAA
40 GTCAGATTTATGCAGGGATTAAAGTAAGGCAATTATGTAACTTCTTAGG
GGAACCAAGCACTAACAGAAGTAGTACCCTAACAGAAGAAGCAGAGCT
AGAAGTGGCAGAAAAAGGGAGATTCTAAAAGAACCGGTACATGGAGTGT
45 ATTATGACCCATCAAAGACTTAATAGCAGAAATACAGAAGCAGGGGCAA
GGCCAATGGACATATCAATTTATCAAGAGCCATTTAAAAATCTGAAAAAC
AGGAAAGTATGCAAGAAATGAAGGGTGCCACACTAATGATGTGAAACAAT
50 TAACAGAGGCGAGTACAAAAATAGCCACAGAAAGCATAGTAATATGGGGA
AAGACTCCTAAATTTAAATTACCCATACAAAAGGAAACATGGGAAGCATG
GTGGACAGAGTATTGGCAAGCCACCTGGATTCTGAGTGGGAGTTTGTCAT
55 ATACCCCTCCCTTAGTGAAGTTATGGTACCAGTTAGAGAAAGAACCCATA
ATAGGAGCAGAAACTTTCTATGTAGATGGGCGACCAATAGGGAAACTAA
ATTAGGAAAAGCAGGATATGTAAGTACAGAGGAAGACAAAAGTTGTGCC
60 CCCTAACGGACACAAACAAATCAGAAGACTGAGTTACAAGCAATTCATCTA
GCTTTGCGAGGATTCGGGATTAGAAGTAACATAGTGACAGACTCACAATA
TGCATTGGGAATCATTCAAGCACAACAGATAAGAGTGAATCAGAGTTAG
TCAGTCAAATAATAGAGCAGTTAATAAAAAAGGAAAAAGTCTACCTGGCA
65 TGGGTACCAGCACACAAAGGAATTGGAGGAATGAACAGTAGATAAAT

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GGTCAGTGCTGGAATCAGGAAAGTACTATTTTATAGATGGAATAGATAAGG
 CCCAAGAAGAACATGAGAAATATCACAGTAATTGGAGAGCAATGGCTAGT
 GATTTTAACTTACCACCTGTAGTAGCAAAAGAAATAGTAGCCAGCTGTGA
 TAAATGTGTCAGCTAAAAGGGGAGCCATGCATGGACAAGTAGACTGTAGCC
 CAGGAATATGGCAGCTAGATTGTACACATTTAGAAGGAAAAGTTATCTTG
 GTAGCAGTTCATGTAGCCAGTGGATATATAGAAGCAGAAGTAATTCAGC
 AGAGACAGGGCAAGAAACAGCATACTTCTCTTAAAATTAGCAGGAAGAT
 GGCCAGTAAAAACAGTACATACAGACAATGGCAGCAATTTACCAGTACT
 ACAGTTAAGGCCCGCTGTGTGGTGGCGGGATCAAGCAGGAATTTGGCAT
 TCCCTACAATCCCCAAAGTCAAGGAGTAATAGAATCTATGAATAAAGAAT
 TAAAGAAAATTATAGGACAGGTAAGAGATCAGGCTGAACATCTTAAGACA
 GCAGTACAAATGGCAGTATTCATCCACAATTTTAAAGAAAAGGGGGAT
 TGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACAGACA
 TACAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAATTTTCGG
 GTTTATTACAGGGACAGCAGAGATCCAGTTTGGAAGGACCAGCAAGCT
 CCTCTGGAAGGTGAAGGGGAGTGTATATACAAGATAATAGTGACATAA
 AAGTAGTGCCAAAGAAAAGCAAGATCATCAGGGATTATGGAACACAG
 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)

TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAGAGCTCATCAGAAC
 AGTCAGACTCATCAAGCTTCTCTATCAAAGCAACCCACCTCCCAATCCCG
 AGGGGACCCGACAGGCCGGAAGGAATAGAAGAAGAGTGGAGAGAGAGA
 CAGAGACAGATCCATTTCGATTAGTGAACGGATCCTTGGCACTTATCTGG
 ACGATCTGCGGAGCCTGTGCCTCTTCAGCTACACCGCTTGAGAGACTTA
 CTCTTGATTGTAACGAGGATTGTGGAACCTTGGGACGACAGGGGTGGGA
 AGCCCTCAAATATTGGTGAATCTCCTACAATATTGGAGTCAGGAGCTAA
 AGAATAGAGGAGCTTTGTTCTTGGGTTCTTGGGAGCAGCAGGAAGCACT
 ATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTC
 TGGTATAGTGCAGCAGCAGAACAAATTGCTGAGGGCTATTGAGGCGCAAC
 AGCATCTGTTGCAACTCACAGTCTGGGCGATCAAGCAGCTCCAGGCAAGA
 ATCCTGGCTGTGGAAGATACCTAAAGGATCAACAGCTCCTAGATCTTTT
 TCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCTTGAGCATCTGA
 CTTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAAT
 TTTTGTGTCTCTCACTCGAAGGACATATGGGAGGGCAATCATTTAA
 ACATCAGAATGAGTATTGTTTAGAGTTTGGCAACATATGCCATATGCT
 GGCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAGTATATGAACA

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GCCCCCTGCTGTCCATTCCCTATTCCATAGAAAAGCCTTGACTTGAGGTT
 AGATTTTTTTTATATTTTGTGTTTGTGTTATTTTTTCTTTAACATCCCTA
 AAATTTTCCTTACATGTTTACTAGCCAGATTTTCTCTCTCTCTGACT
 ACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATCCCTCGACCTGC
 AGCCCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATTG
 TTATCCGCTCACAATCCACACAACATACGAGCCGGAAGCATAAAGTGTA
 AAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGC
 TCACTGCCCCGCTTTCAGTCGGGAAACCTGTGTCGTCAGCGGATCCGCAT
 CTCAATTAGTCAGCAACCATAGTCCCGCCCCCTAACTCCGCCCATCCCGCC
 CCTAACTCCGCCAGTTCGCCCCATTCTCCGCCCATGGCTGACTAATTT
 TTTTATTTATGTCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAG
 AAGTAGTGAGGAGGCTTTTGGAGGCCCTAGGCTTTTGCAAAAAGCTAAC
 TTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAA
 TTTTCAAAATAAAGCATTTTTTCACTGCATTCTAGTTGTGGTTTGTCCA
 AACTCATCAATGTATCTTATCAGCGGCCGCCCGG

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
 CATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCTGGC
 TGACCGCCCAACGACCCCGCCCATTTGACGTCAATAATGACGTATGTTCC
 CATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATT
 TACGGTAAACTGCCCACTTGGCAGTACATCAAGTGATCATATGCCAAGT
 ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGC
 CCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTAT
 TAGTCATCGCTATTACCATGGGTGAGGTGAGCCCCACGTTCTGCTTAC
 TCTCCCCATCTCCCCCCCCCTCCCCACCCCAATTTGTATTATTTATTT
 TTAAATTATTTGTGTCAGCGATGGGGGCGGGGGGGGGGGCGCGCGCC
 AGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGAGGCGGAGAGGTG
 CGGCGGCAGCCAATCAGAGCGGCGCTCCGAAAGTTTCTTTTATGGCG
 AGGCGGCGGGGCGGGGCGGCGCTATAAAAGCGAAGCGCGGCGGGCGGG
 AGTCGCTGCGTTGCCTTCGCCCGTGCCTCCGCGCGCTCGCGCC
 GCCCGCCCCGCTCTGACTGACCGGTTACTCCACAGGTGAGCGGGCGG
 GACGGCCCTTCTCTCCGGGCTGTAATAGCGCTTGGTTTAAAGCGCT
 CGTTTCTTTTCTGTGGCTGCGTGAAGGCTTAAAGGGCTCCGGGAGGGC
 CTTTGTGCGGGGGGAGCGGCTCGGGGGTGCCTGCGTGTGTGTGCGT
 GGGGAGCGCCGCTGCGGCCGCGCTGCCCGCGGCTGTGAGCGCTGCGG

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GCGCGGCGCGGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGC
 CGGGGGCGGTGCCCCGCGGTGCGGGGGGGTGCAGGGGAACAAAGGCTG
 CGTGGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGTGTGGGCGCGCGG
 TCGGGCTGTAACCCCCCTGCACCCCCCTCCCCGAGTTGTGAGCACGG
 CCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCGG
 TGCCGGGCGGGGGTGGCGGCAGGTGGGGGTGCCGGCGGGGCGGGGCGG
 CCTCGGGCGGGGAGGGCTCGGGGAGGGGCGCGCGCGCCCCGAGCGCC
 GGCGGCTGTGAGGCGCGGCGAGCCGAGCCATTGCCCTTTATGGTAATC
 GTGCGAGAGGGCGCAGGACTTCCTTTGTCCAAATCTGGCGGAGCCGAA
 ATCTGGGAGGCGCGCGCACCCCCCTAGCGGGCGCGGGCGAAGCGGTG
 CGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGC
 GCCGCGTCCCTTCTCCATCTCCAGCCTCGGGGTGCCGCGAGGGGACG
 GCTGCCCTTCGGGGGGGACGGGCGAGGGCGGGTTCGGCTTCTGGCGTGTG
 ACCGGCGGGAATTC

Construction of the VSV-G Envelope Plasmid:

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

(SEQ ID NO: 29)
 GAATTCATGAAGTGCCCTTTGTACTTAGCCCTTTTATTCATTGGGGTGAA
 TTGCAAGTTCACCATAGTTTTCACACAAACAAAAGGAACTGGAAAA
 ATGTTCCCTTCTAATTACCATATTGCCCGTCAAGCTCAGATTAAATTGG
 CATAATGACTTAATAGGCACAGCCTTACAAGTCAAAATGCCCAAGAGTCA
 CAAGGCTATTCAAGCAGACGGTTGGATGTGTCATGCTTCAAATGGGTCA
 CTACTTGTGATTTCCGCTGGTATGGACCGAAGTATATAACACATTCCATC
 CGATCCTTCACTCCATCTGTAGAACAATGCAAGGAAAGCATTGAACAAAC
 GAAACAAGGAACCTTGGCTGAATCCAGGCTTCCCTCCTCAAAGTTGTGGAT
 ATGCAACTGTGACGGATGCCAAGCAGTGATTGTCCAGGTGACTCCTCAC
 CATGTGCTGGTTGATGAATACACAGGAGAATGGGTGATTACAGTTCAT
 CAACGGAAAAATGCAGCAATTACATATGCCCCACTGTCCATACTCTACAA
 CCTGGCATTCTGACTATAAGGTCAAAGGCTATGTGATTCTAACCTCATT
 TCCATGGACATCACCTTCTCTCAGAGGACGGAGAGCTATCATCCCTGGG
 AAAGGAGGGCAGAGGTTTCAAGTAACACTTTTGTCTATGAACTGGAG
 GCAAGGCTGCAAAATGCAATACTGCAAGCATTGGGGAGTCAGACTCCCA
 TCAGGTGTCTGGTTGAGATGGCTGATAAGGATCTCTTTGCTGCAGCCAG
 ATTCCCTGAATGCCAGAAGGTCAGTATCTCTGCTCCATCTCAGACCT
 CAGTGGATGTAAGTCTAATTACAGGACGTTGAGAGGATCTTGATTATTCC
 CTCTGCCAAGAACTGGAGCAAAATCAGAGCGGGTCTTCAATCTCTCC
 AGTGGATCTCAGTATCTTGTCTCTAAACCAGGAACCGGTCTGTCTT

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TCACCATAATCAATGGTACCCTAAAATACTTTGAGACCAGATACATCAGA
 GTCGATATTGCTGCTCCAATCCTCTCAAGAAATGGTCGGAATGATCAGTGG
 AACTACCACAGAAAGGGAACCTGTGGGATGACTGGGCACCATATGAAGACG
 TGGAAATTGGACCCAATGGAGTTCTGAGGACCAGTTCAGGATATAAGTTT
 CCTTTATACATGATTGGACATGGTATGTTGGACTCCGATCTTCATCTTAG
 CTCAAAGGCTCAGGTGTTTCAACATCCTCACATTCAAGACGCTGCTTCGC
 AACTTCCTGATGATGAGAGTTTATTTTTTGGTGATACTGGGCTATCCAAA
 AATCCAATCGAGCTTGTAGAAGGTTGGTTTCACTAGTTGGAAAAGCTCTAT
 TGCCTCTTTTTTCTTTATCATAGGGTTAATCATTTGAGTATCTTTGGTTC
 TCCGAGTTGGTATCCATCTTTGCATTAAATTAAGCACACCAAGAAAAGA
 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)
 TCTAGAAGGAGCTTGTTCCTTGGGTCTTGGGAGCAGCAGGAAGCACTA
 TGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCT
 GGTATAGTGCAGCAGCAGAACAAATTGCTGAGGGCTATTGAGGCGCAACA
 GCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAA
 TCCTGGCTGTGGAAGATACCTAAAGGATCAACAGCTCCTAGATCTTTTT
 CCCTCTGCCAAAATATATGGGACATCATGAAGCCCTTGAGCATCTGAC

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TTCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATT
 TTTTGTGTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTAAAA
 CATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTG
 GCTGCCATGAACAAAGGTGGCTATAAAGAGGTATCAGTATATGAAACAG
 CCCCCTGCTGTCCATTCTTATTCATAGAAAAGCCTTGACTTGAGGTAA
 GATTTTTTTTTATATTTTGTGTTTATTTTTCTTTAACATCCCTAA
 AATTTTCCTTACATGTTTACTAGCCAGATTTTCTCTCTCTGACTA
 CTCCAGTCATAGCTGTCCCTCTTCTTATGAAGATCCCTCGACCTGCA
 GCCCCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATTGT
 TATCCGCTCACAATTCACACAACATACGAGCCGGAAGCATAAAGTGTA
 AGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCT
 CACTGCCCGCTTTCAGTCGGGAAACCTGTCGTGCCAGCGGATCCGCATC
 TCAATTAGTCAGCAACCATAGTCCCGCCCTAACTCCGCCCATCCGCCC
 CTAACTCCGCCAGTTCGCCCATTTCTCGCCCCATGGCTGACTAATTTT
 TTTTATTTATGCGAGGCCGAGGCCCTCGGCCCTGAGCTATTCCAGA
 AGTAGTGAGGAGGCTTTTTGGAGGCCCTAGGCTTTGCAAAAAGCTAACT
 TGTTTATTGCGAGCTTATAATGGTTACAAATAAAGCAATAGCATCAGAAAT
 TTCACAAATAAAGCATTTTTTCTACTGCATTCTAGTTGTGTTTGTCCAA
 ACTCATCAATGTATCTTATCAGCCGGG

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
 TGTTTAGGCGAAAAAGCGGGCTTCGGTTGTACGCGGTTAGGAGTCCCCCTC
 AGGATATAGTAGTTTTCGCTTTTGCATAGGGAGGGGAAATGTAGTCTTAT
 GCAATACACTTGTAGTCTTGCAACATGGTAACGATGAGTTAGCAACATGC
 CTTACAAGGAGAGAAAAAGCACCCTGCATGCCGATTGGTGGAAGTAAGGT
 GGTACGATCGTGCCTTATTAGGAAGGCAACAGACAGGTCTGACATGGATT
 GGACGAACCACTGAATTCGCGATTGCAGAGATAATTGTATTTAAGTGCCCT
 AGCTCGATACAATAAACGCCATTTGACCATTACACATTTGGTGTGCACC
 TCCAAGCTCGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGAGCCCAT
 CCACGCTGTTTGAACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCC
 CTCGAAGCTAGCGATTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAG
 CGACGAAGAACTCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAA
 GCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCGAAGGAATAGA
 AGAAGAAGGTGGAGAGAGAGACAGAGACAGATCCATTGATTAGTGAACG
 GATCCTTAGCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCAGC

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TACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGGAAC
 TCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGAATCTCCTAC
 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' 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. 3A, 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. 3B, 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. 3A and 3B, 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://rnaidesigner.thermofisher.com/maexpress/>). 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)
5 GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTA
TTT;

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

(FDPS shRNA sequence #2; SEQ ID NO: 2)
10 GCAGGATTCGTTTCAGCACTTCTCGAGAAGTGCTGAACGAAATCCTGCTT
TTT;

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

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

TTT;

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

(FDPS shRNA sequence #4; SEQ ID NO: 4)
25 GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTCTCAGCCTCCTTCTGCTT
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)
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCT
GTGAAGCCACAGATGGCAGAAAGGAGGCTGAGAAAGTGTGCTACTGCCT
CGGACTTCAAGGGGCT

(miR30 FDPS sequence #2; SEQ ID NO: 54)
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCT
GTGAAGCCACAGATGGCAGAAAGGCTGAGAAAGTGTGCTACTGCCTCG
50 GACTTCAAGGGGCT

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

(miR155 FDPS sequence #1; SEQ ID NO: 56)
CCTGGAGGCTTGCTGAAGGCTGTATGCTGACTTTCTCAGCCTCCTTCTGCT
TTTTGGCCACTGACTGAGCAGAAAGGCTGAGAAAGTCAAGGACCAAGGCC
TGTTACTAGCACTCA

(miR21 FDPS sequence #1; SEQ ID NO: 57)
CATCTCCATGGCTGTACCACTTGTGCGGACTTTCTCAGCCTCCTTCTGCT
CTGTTGAATCTCATGGCAGAAAGGAGGCTGAGAAAGTGTGCTATTTTGGTAT
65 CTTTCATCTGACCA

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-continued

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

TGGTCCCCCTCCCCGAGAAGGAGGCTGAGAAAGTCCTTCCCTCCCAATGA

CCGCGTCTTCTGTCG

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-1alpha promoter of gene transcription), miR30-FDPS, miR155-CD47, miR21-cMyc, Woodchuck Post-Transcriptional Regulatory Element (WPPE), 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)

AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCT

GTGAAGCCACAGATGGCAGAAAGGAGGCTGAGAAAGTGCTGCTACTGCCT

CGGACTTCAAGGGGCT

miR155 CD47 target sequence #1:

(SEQ ID NO: 82)

CCTGGAGGCTTGCTGAAGGCTGTATGCTGTTATCCATCTTCAAGAGGCA

GTTTTGGCCACTGACTGACTGCCTCTTAAGATGGATAACAGGACACAAGG

CCTGTTACTAGCACTCA

miR21 cMyc sequence:

(SEQ ID NO: 83)

CATCTCCATGGCTGTACCACCTTGTGCGGTGTTGCGCTCTTGACATTCTC

CTGTTGAATCTCATGGAGAATGTCAAGGCGAAGCACTGACATTTTGGTAT

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

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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'-AGGAATTGATGGCGAGAAGG-3') (SEQ ID NO: 59) and reverse primer (5'-CCCAAAGAGGT-CAAGGTAATCA-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- α 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		
De- scrip- tion	shRNA oligonucleotide (sense sequence - loop - antisense sequence)	SEQ ID NO
FDPS-1	GTCTGGAGTACAATGCCATTCTCGAG AATGGCATTGTACTCCAGGACTTTTT	1
FDPS-2	GCAGGATTTCGTTTCAGCACTTCTCGAG AAGTGCTGAACGAAATCCTGCTTTTT	2
FDPS-3	GCCATGTACATGGCAGGAATCTCGAG AATTCCTGCCATGTACATGGCTTTTT	3
FDPS-4	GCAGAAGGAGGCTGAGAAAGTCTCGAG ACTTTCTCAGCCTCCTTCTGCTTTTT	4

As shown in FIG. 4A, 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. 4A, herein).

Further, as shown in FIG. 4B, 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-1 α promoter and the following miR30-based FDPS sequences:

miR30 FDPS sequence #1: (SEQ ID NO: 53)
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGC
GTGAAGCCACAGATGGCAGAAAGGAGGCTGAGAAAGTGCCTACTGCCT
CGGACTTCAAGGGGCT
miR30 FDPS sequence #2: (SEQ ID NO: 54)
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGC
GTGAAGCCACAGATGGCAGAAAGGAGGCTGAGAAAGTGCCTACTGCCTCG
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. 4B, 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. 5A) or hepatocellular (HepG2) (FIG. 5B) 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. 5A.

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 μ M 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 V γ 9V δ 2 T cells. After staining for V γ 9V δ 2 and TNF- α using fluorophore-conjugated anti TCR-V δ 2 and anti-TNF- α antibody, cells were analyzed via flow cytometry. Live cells were gated, and V δ 2+ and TNF- α + cells were selected on a dot blot. The activated cytotoxic V γ 9V δ 2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, LV-control stimulated 3.11% of TNF- α expressing V γ 9V δ 2 T cells and LV-FDPS shRNA #4 stimulated 5%. With zoledronic acid treatment, LV-control stimulated 7.2% of TNF- α expressing V γ 9V δ 2 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 V γ 9V δ 2 T cells and LV-FDPS shRNA #4 stimulated 3.33%. With zoledronic acid treatment, LV-control stimulated 9.1% of TNF- α expressing V γ 9V δ 2 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 treat-

ment 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'-AGCGCGGTACAGCTTCA-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		
De- scrip- tion	shRNA oligonucleotide (sense sequence - loop - antisense sequence)	SEQ ID NO
CD47 sequence 1	GGTGAACGATCATCGAGCCTCGAG GCTCGATGATCGTTTCA CCTTTTT	5
CD47 sequence 2	GCTACTGGCCTTGGTTAACTCGAG TTAAACCAAGGCCAGTA GCTTTTT	6
CD47 sequence 3	CCTCCTTCGTCATTGCCATCTCGAG ATGGCAATGACGAAGGA GGTTTTT	7
CD47 sequence 4	GCATGGCCCTCTTCTGATTCTCGAG AATCAGAAGAGGCCAT GCTTTTT	8
CD47 sequence 5	GGTGAACGATCATCGAGCTACTCGAG TAGCTCGATGATCGT TTCACCTTTTT	9

As shown in FIG. 7A, 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. 7A, herein).

Further, as shown in FIG. 7B, 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)
CCTGGAGGCTTGCTGAAGGCTGTATGCTGTATCCATCTTCAAAGAGGCA
GTTTGGCCACTGACTGACTGCCTCTTAAGATGGATAACAGGACACAAGG
CCTGTTACTAGCACTCA

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-continued

miR155 CD47 target sequence #2: (SEQ ID NO: 66)
 CCTGGAGGCTTGCTGAAGGCTGTATGCTGTAGCTCGATGATCGTTTCAC
 GTTTTGGCCACTGACTGACGTGAAACGCATCGAGCTAACAGGACACAAGG
 CCTGTTACTAGCACTCA

miR155 CD47 target sequence #3: (SEQ ID NO: 67)
 CCTGGAGGCTTGCTGAAGGCTGTATGCTGAAGAATGGCTCCAACATGAC
 GTTTTGGCCACTGACTGACGTGATTGTGAGCCATTCTTCAGGACACAAGG
 CCTGTTACTAGCACTCA

miR155 CD47 target sequence #4: (SEQ ID NO: 68)
 CCTGGAGGCTTGCTGAAGGCTGTATGCTGTATACGCGCGCAATACAGAG
 GTTTTGGCCACTGACTGACCTCTGTATCGCGGTGTATACAGGACACAAGG
 CCTGTTACTAGCACTCA

As shown in FIG. 7B, 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

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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 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 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'-AGCGCGGC-TACAGCTTCA-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		
De- scrip- tion	shRNA oligonucleotide (sense sequence - loop - antisense sequence)	SEQ ID NO
cMyc shRNA Sequence 1	GCTTCACCAACAGGAAGTATGCTCGAG CATAGTTCTCTGTTGGTGAAG CTTTT	10
cMyc shRNA Sequence 2	GCGAACACACAACGTCTTGGACTCGAG TCCAAGACGTTGTGTGTTT GCTTTT	11
cMyc shRNA Sequence 3	GACATGGTGAACAGAGTTTCTCGAG GAAACTCTGGTTCACCATGT CTTTT	12
cMyc shRNA Sequence 4	GAGAATGTCAAGAGGCGAACACTCGAG TGTTGCGCTCTTGACATTCT CTTTT	13
cMyc shRNA Sequence 5	GCTCATTCTGAAGAGGACTTCTCGAG AAGTCTCTTCAGAAATGAG CTTTT	14

As shown in FIG. 8A, 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. 8A, herein).

Further, as shown in FIG. 8B, 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)
 CCTGGAGGCTTGCTGAAGGCTGTATGCTGTGTCGCTCTTGACATTCTC
 TTTTGGCCACTGACTGAGAGAATGTAGAGGCGAACACAGGACACAAGGCC
 TGTTACTAGCACTCA
 miR21 cMyc sequence: (SEQ ID NO: 83)
 CATCTCCATGGCTGTACCACCTTGTGCGGTGTTGCGCTCTTGACATTCTC
 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)
 GAGAATGTCAAGAGGCGAACACTCGAGTGTTCGCTCTTGACATTCTCT
 TTTT

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. 8B, 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 (nonfunctional) 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 mm³ 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
 5 TACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTGGCAGTACAT
 CAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCGAAGTCTCCACC
 CCATTGACGTCAATGGGAGTTTGTGTTTGGCACCAAAATCAACGGGACTTT
 10 CCAAAATGTCGTAACAACTCCGCCCATTTAGCGCAATGGGCGGTAGGCG
 TGTACGGTGGGAGGTTTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGA
 TCGCTTGAGAGCGCCATCCACGCTGTTTT
 GFP T2A Luciferase sequence: (SEQ ID NO: 73)
 ATGCCCCCATGAAGATCGAGTCCGCGATCACCGGCACCTGAACGGCGT
 GGAGTTCGAGCTGGTGGCGCGGAGAGGGCACCCCCGAGCAGGGCCGCA
 TGACCAACAAGATGAAGAGCACCAAGGCGCCCTGACCTTCAGCCCCATC
 20 CTGCTGAGCCACGTGATGGGCTACGGCTTCTACCACTTCGGCACCTACCC
 CAGCGGCTACGAGAACCCCTTCTGCACGCCATCAACAACGGCGGTACAA
 CCAACACCCGCATCGAGAAGTACGAGGACGGCGCGTGTGCACGTGAGC
 25 TTCAGCTACCGCTACGAGGCCGGCCGCGTGATCGGCGACTTCAAGGTGGT
 GGGCACCGGCTTCCCCGAGGACAGCGTGATCTTCAACGACAAGATCATCC
 GCAGCAACGCCACCGTGGAGCACCTGCACCCCATGGGCGATAACGTGCTG
 30 GTGGGCAGCTTCGCCCCGACCTTCAGCTCGCGACGGCGGCTACTACAG
 CTTCTGTTGGTGACGCCACATGCACCTCAAGAGCGCCATCCACCCAGCA
 TCCTGCAGAACGGGGGCCCCATGTTTCGCTTCCGCGCGTGAGGAGCTG
 35 CACAGCAACACCGAGCTGGGCATCGTGGAGTACCAGCACGCCCTTCAAGAC
 CCCCATCGCTTCGCCAGATCTCGAGATATCAGCCATGGCTTCCCCCGCG
 CGGTGGCGGCGAGGATGATGGCACGCTGCCCATGTCTTGTGCCAGGAG
 40 AGCGGGATGGACCGTCACCTGCGAGCTGTGCTTCTGCTAGGATCAATGT
 GACCGGTGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGA
 ATCCCGGCCCTTCCGGTATGGAAGACGCCAAAAACATAAAGAAAGGCCCG
 45 GCGCCATTCTATCCGCTAGAGGATGGAACCGCTGGAGAGCAACTGCATAA
 GGCTATGAAGAGATACGCCCTGGTTCCTGGAACAATTGCTTTTACAGATG
 CACATATCGAGGTGAACATCACGTACGCGGAATACTTCGAATGTCCGTT
 50 CGGTTGGCAGAAGCTATGAAACGATATGGGCTGAATACAAATCACAGAA
 CGTCGTATGCAGTGAAAACCTCTTCAATTCTTTATGCCGTTGTTGGGCG
 CGTTATTTATCGAGTTGCAGTTGCGCCCGGAACGACATTATAATGAA
 55 CGTGAATTGCTCAACAGTATGAACATTTTCGAGCCTACCGTAGTGTGTTGT
 TTCCAAAAGGGGTTGCAAAAAATTTTGAACGTGCAAAAAAATTACCAA
 TAATCCAGAAAATTATTATCATGGATTCTAAACGATTACAGGGGATTT
 CAGTCGATGTACACGTTCTGTCACATCTCATCTACCTCCCGGTTTTAATGA
 60 ATACGATTTTGTACCAGAGTCTTTGATCGTGACAAAAACAATTGCACCTGA
 TAATGAACCTCTCGGATCTACTGGGTTACCTAAGGGTGTGGCCCTTCCG
 CATAGAACTGCCTGCGTCAGATTCTCGCATGCCAGAGATCCTATTTTGG
 65 CAATCAATCATTCCGATACTGCGATTTTAAGTGTGTTCCATTCCATC

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ACGGTTTTTGAATGTTTACTACACTCGGATATTTGATATGTGGATTTCGA
 GTCGTCTTAATGTATAGATTTGAAGAAGAGCTGTTTTTACGATCCCTTCA
 GGATTACAAAATTCAAAGTCGCTGTCTAGTACCAACCTATTTTCATTCT
 TCGCCAAAAGCACTCTGATTGACAAATACGATTTATCTAATTACACGAA
 ATTGCTTCTGGGGCGCACCTCTTTGAAAGAAGTCGGGAAGCGGTTGC
 AAAACGCTTCCATCTTCCAGGATACGACAAGGATATGGGCTCACTGAGA
 CTACATCAGCTATTCTGATTACACCCGAGGGGATGATAAACCGGCGCG
 GTCGGTAAAGTTGTTCCATTTTTTGAAGCGAAGGTTGTGGATCTGGATAC
 CGGGAACCGCTGGCGTTAATCAGAGAGCGAATTATGTGTGAGGAC
 CTATGATTATGTCCGGTTATGTAAACAATCCGGAAGCGACCAACGCCTTG
 ATTGACAAGGATGGATGGCTACATTCTGGAGACATAGCTTACTGGGACGA
 AGACGAACACTTCTTCATAGTTGACCGCTTGAAGTCTTTAATTAATACA
 AAGGATACAGGTGGCCCCGCTGAATTGGAGTCGATATTGTTACAACAC
 CCCAACATCTTCGACGCGGGCGTGGCAGGTCTTCCCGACGATGACGCCGG
 TGAACCTCCCGCCCGCTGTGTGTTTGGAGCACGGAAGACGATGACGG
 AAAAAAGAGATCGTGATTACGTCGCGAGTCAAGTAACAACCGCGAAAAAG
 TTGCGCGGAGGAGTTGTGTTTGTGGACGAAGTACCGAAAGGTCTTACCGG
 AAAACTCGACGCAAGAAAAATCAGAGAGATCCTCATAAGGCCAAGAAGG
 GCGGAAGTCCAAATTGTAA
 H1 promoter sequence: (SEQ ID NO: 15)
 GAACGCTGACGTCAACCCGCTCCAAGGAATCGCGGCCAGTGTCAC
 TAGGCGGGAACACCCAGCGCGCTGCGCCCTGGCAGGAAGATGGCTGTGA
 GGGACAGGGGAGTGGCGCCCTGCAATATTTGCATGTCGCTATGTGTTCTG
 GGAAATCACCATAACGTGAATGTCTTGGATTGGGAATCTTATAAGT
 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 KpnI 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 confluent yields 1.87×10^7 ml of PC3 cells; 75 ml flask grown confluent 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 μ l of lenti-control+8 μ l of polybrene, incubate for 5 min. then mix with 4 ml of RPMI-10, and cover the surface of PC3 cells.

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2. In a 75 ml of flask, 50% confluent PC3 cell, add 20 μ l of lenti-FDPS+8 μ l 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 ($50 \times 3 \times 10^6/5$ ml) 15 flask lenti-FDPS: 1.5×10^8 cells ($50 \times 3 \times 10^6/5$ 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 μ l

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)= $d^2 \times d/2$ (d=the shortest diameter) $\times D/2$ (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 μ g/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. 10A). 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. 10B) 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. 10C) 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. 10D) 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:	De-scription	Sequence
1	FDPS shRNA sequence #1	GTCCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTATC TCCAGGACTTTTT
2	FDPS shRNA sequence #2	GCAGGATTTTCGTTTCAGCACTTCTCGAGAAGTGTGAACGA AATCCTGCCTTTTT
3	FDPS shRNA sequence #3	GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATG TACATGGCTTTTT
4	FDPS shRNA sequence #4	GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCT CCTTCTGCCTTTTT
5	CD47 shRNA sequence #1	GGTGAAACGATCATCGAGCCTCGAGGCTCGATGATCGTTT CACCTTTTT
6	CD47 shRNA sequence #2	GCTACTGGCCTTGGTTTAACTCGAGTTAAACCAAGGCCAG TAGCTTTTT

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SEQ ID NO:	De-scription	Sequence
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	GCGAACACACAACGCTTGGACTCGAGTCCAAGACGTTGT GTGTTTCGCTTTT
12	cMyc shRNA sequence #3	GACATGGTGAACCGAGAGTTTCTCTCGAGAACTCTGGTTC ACCATGTCTTTTT
13	cMyc shRNA sequence #4	GAGAATGTCAAGAGGCGAACACTCGAGTGTTCGCCTCTTG ACATTCTCTTTTT
14	cMyc shRNA sequence #5	GCTCATTTCTGAAGAGGACTTCTCGAGAAGTCTCTTCAG AAATGAGCTTTTT
15	H1 promoter	GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGGC CCAGTGTCACTAGGCGGGAACACCCAGCGCGCGTGCAGCC TGGCAGGAAGATGGCTGTGAGGGACAGGGGAGTGGCGCC CTGCAATATTTGCATGTCGCTATGTGTTCTGGGAAATCAC CATAAACGTGAAATGTCTTTGGATTGGGAATCTTATAAG TTCTGTATGAGACCATT
16	U6 promoter	GAGGGCCTATTTCCCATGATTCTTCATATTTGCATATAC GATACAAGGCTGTTAGAGAGATAATTGGAATTAATTTGAC TGTAACACAAAGATATTAGTACAAAATACGTGACGTAGA AAGTAATAATTTCTTGGTAGTTTGCAGTTTAAATATAT GTTTTAAATGGACTATCATATGCTTACCGTAACTTGAAA GTATTTGATTTCTTGGCTTTATATATCTTGTGGAAAGGA CGAAACACC
17	7SK promoter	CTGCAGTATTTAGCATGCCCCACCCATCTGCAAGGCATTCT TGGATAGTGTCAAAACAGCCGGAAATCAAGTCCGTTTATC TCAAACCTTAGCATTTTGGGAATAAATGATATTGCTATG CTGGTTAAATTAGATTTTAGTTAAATTTCTGCTGAAGCT CTAGTACGATAAGCAACTTGACCTAAGTGTAAGTTGAGA TTTCCTTCAGGTTTATATAGCTTGTGCGCCGCTGGCTAC CTC

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SEQ ID NO:	De-scription	Sequence
18	CAG en-hancer	TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTTCATAGCCCATATATGGAGTTCGCGGTTACATAAATTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCATTTGACGTCATAATGACGTATGTTCCCATAGTAACGCCAATAAGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAACTGGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC
19	CAG promo-ter	GCTATTACCATGGGTGAGGTGAGCCCCACGTTCTGCTTTCCTCTCCCATCTCCCCCCCCCTCCCAACCCCAATTTTGTATTTATTTATTTTAAATATTTTGTGCAGCGATGGGGCGGGGGGGGGGGGGCGCGCGCCAGGCGGGCGGGCGGGGGCAGGGGGCGGGCGGGCGAGCGGAGGTGCGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCTTTTATGGCGAGGCGGGCGGGCGGGCCCTATAAAAGCGAAGCGCGCGCGGGCG
20	chick-en beta actin intron	GGAGTCGCTGCGTTGCCCTTCGCCCCGTCGCCCGCTCCGCGCGCCCTCGCGCCGCGCCCGGCTCTGACTGACCGCGCTACTCCACAGGTGAGCGGGCGGGACGGCCCTCTCTCCGCGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTCTTTTCTGTGGCTGGCTGAAAGCCTTAAAGGGCTCCGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGTGCCTGCGTGTGTGTGCGTGGGAGCGCGCTGCGGCGCGCTGCGCGCGGCTGTGAGCGCTGCGGCGCGGCGCGGGCTTTGTGCGCTCCGCGTGTGCGGAGGGGAGCGCGCGGGGGCGGTGCCCGCGGTGCGGGGGGGCTGCGAGGGGAACAAAGCGTGCCTGCGGGTGTGCGTGGGGGGTGTGAGCGGGGTGTGGGCGCGCGGTGCGGCTGTAAACCCCTTGCACCCCTCTCCCGAGTTGCTGAGCACGGCCCGCTTCGGGTGCGGGCTCCGTGCGGGGCTGGCGCGGGCTCGCGTGCCTGCGGGCGGGGGTGGCGGCAGGTGGGGTGCCTGCGGGCGGGCGGGCGCGCTCGGGCGGGGAGGGCTCGGGGAGGGGCGCGCGCCCCGAGCGCGCGGCTGTGCGAGGCGCGCGGAGCGCGAGCCATTGCGCTTTTATGGTAATCGTGCAGAGGGCGCAGGGACTTCTTTGTCCCAATCTGGCGAGCGCAATCTGGGAGCGCGCGCGACCCCTCTAGCGGGCGCGGCGAAGCGGTGCGGCGCGGAGGAAGAAATGGGCGGGGAGGCGCTTCGTGCGTGCCTGCGCGCGCTTCTCCATCTCCAGCTTCGGGGTGCCTGCGAGGGGACGGCTGCCTTCGGGGGACGGGCGGGGTCGGCTTCTGCGCTGTGACCGGCG
21	HIV gag	ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGAGAATTAATCGATGGGAAAAAATTCGGTTAAGGCGAGGGGAAAAAATAATAAATAAATCAATATAGTATGGCAAGCAGGAGCTAGAACGATTGCGAGTTAATCCTGGCTGTGTAGAAACATCAGAGGGCTGTAGACAAATCTGGGACAGCTACAACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATTATATAACAGTAGCAACCTCTATTGTGTGCATCAAAGGATAGAGATAAAGACACCAAGGAAGCTTTAGACAAGATAGAGGAAGAGCAAAAGTAAGAAAAAGCACAGCAAGCAGCAGTGACACAGGACACAGCAATCAGGTACGCCAAATTACCCCTATAGTGACAGAACATCCAGGGGCAATGGTACATCAGGCCATATCACCTAGAACCTTAAATGATGGGTAAAAGTAGTAGAAGAGAAGGCTTTTCAGCCAGAGTGATACCCATGTTTTCAGCATTTATCAGAAGGAGCCACCCACAAAGATTTAAACACATGCTAAACACAGTGGGGGACATCAAGCAGCATGCAAAATGTTAAAGAGACCATCAATGAGGAAGCTGCAGAATGGGATAGAGTGATCCAGTGATGCAGGGCCTATTGCAACAGGCCAGTAGAGAGAACCAAGGGAAAGTGACATAGCAGAACTACTAGTACCTTCAGGAACAATAGGATGGATGACACATAATCCACCTATCCAGTAGGAGAAATCTATAAAATAGTGATAATCTGGGATTAATAAAATAGTAAGAAATGTATGCCCTACCAAGCATTTCTGGACATAAGACAGGACCAAGGAACCTTTAGAGACTATGTAGACCGATTCTATAAACTCTAGAGCGCGAGCAAGCTTCACAGAGGTAAGAAATTTGGAAGAGAAACCTTTGTTGGTCCAAATGGAACCTAGTTGTAAGACTATTTTAAAGCAATTGGGACAGGAGCGACACTAAGAGAAATGATGACAGCATGTGAGGAGTGGGGGACCCGGCCATAAAGCAAGAGTTTGGCTGAAGCAATGAGCCAAATAACAAATCCAGCTACATAATGATACAGAAAGCAATTTAGGAACCAAGAAAGACTGTTAAGTGTTCATTTGTGG

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SEQ ID NO:	De-scription	Sequence
5		CAAAGAAGGGCACATAGCCAAAAATTCAGGGGCCCTTAGGAAAAAGGGCTGTGGAAATGTGGAAAGGAAGGACACCAATGAAAGATTGTACTGAGAGACAGGCTAATTTTATAGGAAGATCTGGCTTCCCAAGGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAGAGCCACAGCCCAACAGAGAGAAGGCTTCAAGTTTGGGGAAGAGACAACAATCCCTCTCAGAAAGCAGGAGCCGATAGACAAGGAAGTGTATCCTTTAGCTTCCCTCAGATCACTCTTTGGCAGCGACCCCTCGTCACATAA
10		ATGAATTTGCCAGGAAGATGGAACCAAAAAATGATAGGGGGAATTTGGAGGTTTATCAAAGTAGGACAGTATGATCAGATACTCATAGAAATCTCGGGAAGTTCAAGTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAAATTTGAAGAAATCTGTTGACTCAGATTGGCTGCATTTAAATTTTCCATTATGTCCTATTGAGACTGTACCTGCAATTTACCATACCTAGTAATGGATGGCCCAAGTTAAACAATGGCCATTGACAGAAGAAATAAATAAGCATTAGTAGAAATTTGTACAGAAATGGAAAGGAAGGAAAAATTTCAAAAATTTGGGCTGAAAAATCCATACAATACTCCAGTATTTGCCATAAAGAAAAAGACAGTACTAAATGGAGAAAAATAGTAGATTTTCAGAGAACTTAATAAGAACTCAAGATTTCTGGGAAGTTCAAGTATAGGAATACCATCTGCAAGGTTAAAAACAGAAAAATCAGTAACAGTACGGATGTGGGCGATGCATATTTTTCAGTTCCTTAGATAAAGACTTCAGGAAGTATACCTGCAATTTACCATACCTAGTATAACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACAGGGATGGAAGAGATCACCAGCAATATTCAGGTGTAGCATGACAAAAATCTGTAGGCTTTTAGAAAAACAAGTCCAGACATAGTCACTATCAATACATGATGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAAAAGAGGAAGTGTAGCAACACTCTGTGTAGGTTGGGTTACCACACAGACAAAAACATCAGAAAGAACCTCCATTCTTTTGGATGGGTATGAATCCATCTCTGATAAATGGACAGTACAGCTATAGTGTGCTGCCAGAAAGGACAGCTGAGCTGACATGACATACAGAAATTAGTGGGAAATTTGAATTTGGGCAAGTCAAGATTTATGCAAGGATTAAGCTAAGGCAATATGTAAATCTTAGGGGAACCAAGCAATACAGAGAGTAGTACCATAACAGAAAGCAGAGCTAGAACTGGCAGAAAAACAGGGAGATTTCTAAAGAACCGGTACATGGAGTGTATATGACCCATCAAAAGACTTAATAGCAAAATACAGAAAGCAGGGCAAGGCCAATGGACATATCAAAATTTATCAAGAGCCATTTAAAAATCTGAAAAACAGGAAATATGCAAGAAATGAAGGGTGCCCACTAATGATGTGAACAAATTAACAGAGGAGTACAAAAAATAGGCCACAGAAAGCATAGTAATATGGGAAAGACTCTTAAATTTAAATTACCCTACAAAAAGGAACATGGGAAGCATGGTGGACAGAGTATGGCAAGCCACTGGATTCTGAGTGGGAGTTGTCAATACCCCTCCCTTAGTGAAGTTATGGTACAGTTAGAGAAAGAACCATATAGGAGCAGAAATTTCTATGTAGATGGGGCAGCAATAGGAGAACTAAATAGGAAAGCAGGATATGTAACTGACAGAGGAAGACAAAAAGTTGTCCCCCTAACGGACACAACAAATCAGAGAGCTGAGTTACAGCAATTCATCTAGCTTTTGCAGGATTCGGGATTTAGAAATACATAGTAGTACAGAGTACCAATATGCATTGGGAATCATTCAGGCACACAGATAAGAGTGAATCAGAGTTAGTCAGTCAATAATAGAGCAGTTAATAAAAAAGGAAAAAGTCTACCTGGCATGGGTACCAGCACACAAGGAATTTGGAGGAATGAACAAGTAGATGGGTGTGTCAGTGTGGAATCAGGAAAGTACTA
20		ATGAATTTGCCAGGAAGATGGAACCAAAAAATGATAGGGGGAATTTGGAGGTTTATCAAAGTAGGACAGTATGATCAGATACTCATAGAAATCTCGGGAAGTTCAAGTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAAATTTGAAGAAATCTGTTGACTCAGATTGGCTGCATTTAAATTTTCCATTATGTCCTATTGAGACTGTACCTGCAATTTACCATACCTAGTAATGGATGGCCCAAGTTAAACAATGGCCATTGACAGAAGAAATAAATAAGCATTAGTAGAAATTTGTACAGAAATGGAAAGGAAGGAAAAATTTCAAAAATTTGGGCTGAAAAATCCATACAATACTCCAGTATTTGCCATAAAGAAAAAGACAGTACTAAATGGAGAAAAATAGTAGATTTTCAGAGAACTTAATAAGAACTCAAGATTTCTGGGAAGTTCAAGTATAGGAATACCATCTGCAAGGTTAAAAACAGAAAAATCAGTAACAGTACGGATGTGGGCGATGCATATTTTTCAGTTCCTTAGATAAAGACTTCAGGAAGTATACCTGCAATTTACCATACCTAGTATAACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACAGGGATGGAAGAGATCACCAGCAATATTCAGGTGTAGCATGACAAAAATCTGTAGGCTTTTAGAAAAACAAGTCCAGACATAGTCACTATCAATACATGATGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAAAAGAGGAAGTGTAGCAACACTCTGTGTAGGTTGGGTTACCACACAGACAAAAACATCAGAAAGAACCTCCATTCTTTTGGATGGGTATGAATCCATCTCTGATAAATGGACAGTACAGCTATAGTGTGCTGCCAGAAAGGACAGCTGAGCTGACATGACATACAGAAATTAGTGGGAAATTTGAATTTGGGCAAGTCAAGATTTATGCAAGGATTAAGCTAAGGCAATATGTAAATCTTAGGGGAACCAAGCAATACAGAGAGTAGTACCATAACAGAAAGCAGAGCTAGAACTGGCAGAAAAACAGGGAGATTTCTAAAGAACCGGTACATGGAGTGTATATGACCCATCAAAAGACTTAATAGCAAAATACAGAAAGCAGGGCAAGGCCAATGGACATATCAAAATTTATCAAGAGCCATTTAAAAATCTGAAAAACAGGAAATATGCAAGAAATGAAGGGTGCCCACTAATGATGTGAACAAATTAACAGAGGAGTACAAAAAATAGGCCACAGAAAGCATAGTAATATGGGAAAGACTCTTAAATTTAAATTACCCTACAAAAAGGAACATGGGAAGCATGGTGGACAGAGTATGGCAAGCCACTGGATTCTGAGTGGGAGTTGTCAATACCCCTCCCTTAGTGAAGTTATGGTACAGTTAGAGAAAGAACCATATAGGAGCAGAAATTTCTATGTAGATGGGGCAGCAATAGGAGAACTAAATAGGAAAGCAGGATATGTAACTGACAGAGGAAGACAAAAAGTTGTCCCCCTAACGGACACAACAAATCAGAGAGCTGAGTTACAGCAATTCATCTAGCTTTTGCAGGATTCGGGATTTAGAAATACATAGTAGTACAGAGTACCAATATGCATTGGGAATCATTCAGGCACACAGATAAGAGTGAATCAGAGTTAGTCAGTCAATAATAGAGCAGTTAATAAAAAAGGAAAAAGTCTACCTGGCATGGGTACCAGCACACAAGGAATTTGGAGGAATGAACAAGTAGATGGGTGTGTCAGTGTGGAATCAGGAAAGTACTA
23	HIV Int	TTTTTAGATGGAATAGATAAGGCCCAAGAAAGAACATGAGAAATATCAGTAATTTGGAGAGCAATGGCTAGTGAATTTTAACTACCACCTGTAGTAGCAAAAGAAATAGTAGCCAGCTGTGATAAATGTGAGCTAAAAGGGGAAGCCATGCATGGACAAAGTAGACTGTAGCCAGGAATTTGGCAGCTAGATTATACATATTTAGAGGAAAAAGTTATCTGGTAGCAGTTTATGTAGCAGTGGATATATAGAAAGCAGAGTAATTCACAGCAGAGACAGGGCAAGAAACAGCATACTTCTCTTAAATTTAGCAGGAAGATGGCCAGTAAAAACAGTACATACAGACATAGCAGCAATTTTCAACCACTACTACAGTTAAGGCCGCTGTTGGTGGGCGGGGATCAAGCAGGAATTTGGCATTTCCCTACAATCCCCAAGTCAAGGAGTAATAGGAATCTTAAGAAATTAAGAAATTAAGAAAAAGGAAATTAAGAGATCAGGCTGAACATCTTAAGACAGCAGTACAAATGGCAGTATTCATCCCAATTTTAAGAAAAAGGGGGATTGGGGGTACAGTGCAGGGGAAAGTAGTAGACATAATAGCAGACATACAGAACTAAGGAAATTAAGTACAAAAACAATTTACAAAATTTTCGGGT

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SEQ ID NO:	De-scription	Sequence
		TTATTACAGGGACAGCAGAGATCCAGTTTGGAAAGGACCA GCAAAGCTCCTCTGGAAAGGTGAAGGGGCAGTAGTAATA CAAGATAATAGTGACATAAAAGTAGTGCCAAAGAGAAA GCAAAGATCATCAGGGATTATGAAAACAGATGGCAGGT GATGATTGTGTGGCAAGTAGACAGGATGAGGATTAA
24	HIV RRE	AGGAGCTTTGTTCTTGGGTTCTTGGGAGCAGCAGGAAGC ACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCA GACAATTATTGTCTGGTATAGTGACGACGACAGAACATTT GCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTC ACAGTCTGGGGCATCAAGCAGTCCAGGCAAGAATCCTGG CTGTGGAAGATACCTAAAGGATCAACAGCTCCT
25	HIV Rev	ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAACTCCT AAGGCAGTCAGACTCATCAAGTTTCTCTATCAAGCAACC CACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAAGGA ATAGAAGAAGAAGGTGGAGAGAGACAGAGACAGATCC ATTTCGATTAGTGAACGGATCCTTAGCACCTTATCTGGGACG ATCTGCGGAGCCTGTGCCTCTTCAGCTACCCAGCCTTGAG AGACTTACTCTTGATTGTAACGAGGATTGTGGAACCTCTG GGACGCGGGGGTGGGAAGCCCTCAAATATTGGTGAAT CTCCTACAATATTGGAGTCAGGAGCTAAGAATAG
26	rabbit beta globin poly A	AGATCTTTTTCCCTCTGCCAAAAATATGGGACATCATG AAGCCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAAT TTATTTTCATTGCAATAGTGTGTTGGAATTTTTGTGTCT CTCACTCGGAAGGACATATGGGAGGGCAAAATCATTTAAAA CATCAGAAATGAGTATTTGGTTTAGAGTTTGGCAACATATG CCATATGCTGGCTGCCATGAACAAAGGTGGCTATAAAGAG GTCATCAGTATATGAACAGCCCCCTGCTGTCCATTCCTT ATTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTT ATATTTGTGTTTGTGTTATTTTTCTTTAACAATCCCTAA AATTTTCTTACATGTTTTACTAGCCAGATTTTTCTCCT CTCCTGACTACTCCAGTCATAGCTGTCCCTCTTCTCTTA TGAAGATC
27	CMV Promo- ter	ACATTGATTATTGACTAGTTATTAATAGTAATCAATTACG GGGTCAATTAGTTCATAGCCCATATATGGAGTTCCGCGTAT CATAACTTACGGTAAATGGCCGCGCTGGCTGACCCGCCAA CGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCC ATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGG TGGAGTATTTCGGTAACTGCCACTTGGCAGTACATCA AGTGTATCATATGCCAAGTACGCCCTTATTGACGTCAAT GACGGTAAATGGCCGCGCTGGCATTATGCCAGTACATGA CCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATT AGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTAC ATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTT CAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGTTG GCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAA TCCGCCCCATTGACGCAATGGGCGGTAGGCGTGTACGGT GGGAGGTCATATAAGC
28	beta globin intron	GTGAGTTTGGGACCCCTTGATTGTTCTTTCTTTTCGCTA TTGTAAAATTCATGTTATATGGAGGGGGCAAGTTTTCAG GGTGTGTTTGAATGGGAAGATGTCCCTTGATACCAT GGACCTCATGATAATTTGTTTCTTCTACTTTCTACTCT GTTGACAACCATTTGCTCCTCTTATTTCTTTTCAATTTT TGTAACTTTTTCGTTAAACTTTAGCTTGCATTTGTAACGA ATTTTAAATTCATTTTGTGTTTATTTGTGAGATTGTAAGT ACTTTCTCTAAATCACTTTTTTTCAAGGCAATCAGGGTAT ATTATATTGTAATTCAGCACAGTTTATGAGAAACAAATGTT ATAATTAATGATAAGGTAGAATATTTCTGCAATATAAAT CTGGCTGGCGTGGAAATATCTTATTTGGTAGAAACAACTA CACCTGGTCAATCCTCGCTTTCTCTTTATGGTTACAA TGATATACACTGTTTGAATGAGGATAAAATACTCTGAGT CCAAACCGGGCTCTGCTAACCATGTTTATGCTCCTTCTT CTCTTTCTTACAG
29	VSV-G/ DNA frag- ment con- tain- ing	GAATTCATGAAGTGCTTTTGTACTTAGCCTTTTTATTCA TTGGGGTGAATTGCAAGTTTACCATAGTTTTCACACAA CCAAAAGGAACTGGAAAAATGTTCTTCTAATTACCAT TATTGCCCGTCAAGCTCAGATTAAATTGGCATAATGACT TAATAGGCACAGCCTTACAAGTCAAAATGCCAAGAGTCA CAAGGCTATTCAAGCAGACGGTTGGATGTGTCATGCTCC AAATGGGTCACACTTGTGATTTCGCTGGTATGACCGCA

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SEQ ID NO:	De-scription	Sequence
	VSV-G	AGTATATAACACATTCCATCCGATCCTTCACTCCATCTGT AGAACAATGCAAGGAAAGCATTGAACAAACGAAACAAGGA ACTTGGCTGAATCCAGGCTTCCCTCTCAAAGTTGTGGAT ATGCAACTGTGACGGATGCCGAAGCAGTGATTGTCCAGGT GACTCCTCACCATGTGCTGGTTGATGAATACACAGGAGAA TGGGTTGATTTCACAGTTTATCAACGGAATGCAGCAATT ACATATGCCCCACTGTCCATAACTCTACAACCTGGCATT TGACTATAAGGTCAAAGGGCTATGTGATTCTAACCTCATT TCCATGGACATCACCTTTCTTCTCAGAGGACGGAGAGCTAT CATCCTCGGAAAGGAGGGCAGAGGTTTCAAGTAACATA CTTTGCTTATGAAATCGAGGCAAGGCTGCAAAATGCAA TACTGCAAGCATTTGGGAGTCAGATCCCATCAGGTGTCT GGTTCGAGATGGCTGATAAGGATCTCTTTGTGTCAGCCAG ATTCCTCTGAATGCCAGAGGGTCAAGTATCTCTGCTCCA TCTCAGACCTCAGTGGATGATGATTAATTCAGGACGTTG AGAGGATCTTGGATTATTTCCCTCTGCCAAGAAACCTGGAG CAAAATCAGAGCGGGTCTTCCAATCTCTCCAGTGGATCTC AGCTATCTTGCTCTCTAAACACCCAGGAACCGGCTCTGCTT TCACCATAATCAATGGTACCCTAAATACTTTGAGACCAG ATACATCAGAGTCGATATTGTGCTCCAATCTCTCAAGA ATGGTCGGAATGATCAGTGGAACTACACAGAAAGGGAAC TGTGGGATGACTGGGCACCATATGAAGACGTGGAATTTGG ACCCAATGGAGTTCTGAGGACAGGTCAGGATATAAGTTT CCTTTATACATGATTGGACATGGTATGTTGGACTCCGATC TTCATCTTAGCTCAAAGGCTCAGGTGTTTCAACATCTCTCA CATTCAAGACGCTGCTTCGCAACTTCTCGATGATGAGAGT TTATTTTTTGGTGATACATGGGCTATCCAAAATCCAATCG AGCTTGTAGAAGGTTGGTTTCAAGTGTGGAAGCTCTAT TGCCCTCTTTTTCTTTATCATAGGTTTAAATCATTTGAGTA TTCTTGGTTCTCCAGTTTGGTATCCATCTTTCATTAAT TAAAGCACACCAAGAAAAGACAGATTTATACAGACATAGA GATGAGAATTC
	rabbit beta globin poly A	AGATCTTTTTCCCTCTGCCAAAAATATGGGACATCATG AAGCCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAAT TTATTTTCATTGCAATAGTGTGTTGGAATTTTTGTGTCT CTCACTCGGAAGGACATATGGGAGGGCAAAATCATTTAAAA CATCAGAAATGAGTATTTGGTTTAGAGTTTGGCAACATATG CCATATGCTGGCTGCCATGAACAAAGGTGGCTATAAAGAG GTCATCAGTATATGAACAGCCCCCTGCTGTCCATTCCTT ATTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTT ATATTTGTGTTTGTGTTATTTTTCTTTAACAATCCCTAA AATTTTCTTACATGTTTTACTAGCCAGATTTTTCTCCT CTCCTGACTACTCCAGTCATAGCTGTCCCTCTTCTCTTA TGAAGATC
31	Primer	TAAGCAGAATTCATGAATTTGCCAGGAAGAT
32	Primer	CCATACAATGAATGGACACTAGGCGGCCGACGAAT
33	Gag, Pol, Inte- grase frag- ment	GAATTCATGAATTTGCCAGGAAGATGGAACCAAAAAATG ATAGGGGGAATTTGGAGGTTTATCAAAGTAAGACAGTATG ATCAGATACTCATAGAAATCTGCGGACATAAAGCTATAGG TACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGA AGAAATCTGTTGACTCAGATTGGCTGCACCTTAAATTTTC CCATTAGTCCTATTGAGACTGTACCAGTAAAAATTAAGCC AGGAATGGATGGCCCAAAAGTTAAACAATGGCCATTGACA GAAGAAAAAATAAAGCATTAGTAGAAATTTGTACAGAA ATGGAAAAGGAAGGAAAAATTTCAAAAATTTGGGCTCGAA AATCCATACAAATACTCCAGTATTTGCCATAAAGAAAAAG ACAGTACTAAATGGAGAAAAATAGTAGATTTTCAGAGAACT TAATAAGAGAACTCAAGATTTCTGGGAAGTTCAATTAGGA ATACCACATCCTGAGGGTTAAAACAGAAAAATCAGTAA CAGTACTGGATGTGGGCGATGCATATTTTTCAGTTCCCTT AGATAAAGACTTCAGGAAGTATATGCAATTTACCATACCT AGTATAAACAATGAGACACCGAGGATTAGATATCAGTACA ATGTGCTTCCACAGGGATGGAAGGATCACCAGCAATATT CCAGTGTAGCATGACAAAAATCTTAGAGCCTTTTAGAAAA CAAAATCCAGACATAGTATCATTAATACATGGATGATT TGATGTAGGATCTGACTATGAGAAATAGGGCAGCATAGAAC AAAAATAGAGGAACGTGAGACAACATCTGTTGAGGTGGGGA TTTACCACACCGAGCAAAAAACATCAGAAAGAACCTCCAT TCCTTTGGATGGGTTATGAATCCATCCTGATAAATGGAC AGTACAGCCTATAGTGTGCTCCAGAAAGGACAGCTGGACT GTCAATGACATACAGAAATAGTGGGAAAATGAATTGGG

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SEQ ID NO:	De-scription	Sequence
		CAAGTCAGATTTATGCAGGGATTAAAGTAAGGCAATTATG TAAACTTCTTAGGGGAACCAAAGCACTAACAGAAAGTAGTA CCACTAACAGAAAGCAGAGCTAGAAGCTGGCAGAAAAC AGGGAGATTCTAAAAGAACCGGTACATGGAGTGTATTATG ACCCATCAAAAGACTTAATAGCAGAAATACAGAAAGCAGG GGCAAGGCCAATGGACATATCAAATTTATCAAGGCCATT TAAAAATCTGAAAACAGGAAAGTATGCAAGAAATGAAGG TGCCACACTAATGATGTGAAACAATTAACAGAGGCAGTA CAAAAATAGCCACAGAAAGCATAGTAATATGGGAAAG ACTCCTAAATTTAAATTACCCATACAAAAGGAAACATGGG AAGCATGGTGGACAGAGTATTGGCAAGCCACCTGGATTCC TGAGTGGGAGTTTGTCAATACCCCTCCCTTAGTGAAGTTA TGGTACCAGTTAGAGAAAAGACCCATAATAGGAGCAGAAA CTTTCTATGTAGATGGGGCAGCCAATAGGGAACCTAAAT AGGAAAGCAGGATATGTAAGTGAAGAGGAAAGACAAA AGTTGTCCCTTAACGGACACAAACAAATCAGAAGACTGAG TTACAAAGCAATTCATCTAGCTTTGAGGATTCGGGATTAG AAGTAACATAGTGCAGAGCTCACAATATGCATTGGGAAT CATTCAAGCACAAACAGATAAGAGTGAATCAGAGTTAGTC AGTCAAATTAAGAGCAGTTAATAAAAAGGAAAAAGTC TACCTGGCATGGGTACCGACACAAAGGAATTTGGAGGA AATGAACAAGTAGATAAATGGTCAAGTGTGAATCAGGA AAGTACTATTTTAGATGGAAATAGATAAGGCCCAAGAGA ACATGAGAAATATCAGTAATTTGAGAGCAATGGCTAGT GATTTTAACTTACCCTGTAGTAGCAAAAGAAATAGTAG CCAGCTGTGATAAATGTGAGCTAAAGGGGAAGCCATGCA TGGACAAGTAGACTGTAGCCAGGAATATGGCAGCTAGAT TGTACACATTTAGAAGGAAAAGTTATCTTGGTAGCAGTTC ATGTAGCCAGTGGATATATAGAAGCAGAAATTAATCCAGC AGAGACAGGGCAGGAACACAGCATCTTCTCTTAAATTA GCAGGAAGATGGCCAGTAAAAACAGTACATACAGACAAT GGCAGCAATTTACACAGTACTACAGTTAAGGCCCGCTGTT GGTGGCGGGGATCAAGCAGGAATTTGGCATTCCCTACAA TCCCCAAAGTCAAGGAGTAATAGAATCTATGAATAAAGAA TTAAAGAAAATATAGGACAGGTAAGAGATCAGGCTGAA CATCTTAAGACAGCAGTACAAATGGCAGTATTATCCACA ATTTTAAAGAAAAGGGGGATTTGGGGGTACAGTGCAG GGGAAGAATAGTAGACATAATAGCAACAGACATACAAA CTAAAGAATTACAAAACAAATTAACAAAATTAATAATTT TCGGGTTTATTACAGGGACAGCAGAGATCCAGTTTGGAAA GGACAGCAAGCTCCTCTGGAAGAGGTGAAGGGGAGTA GTAATACAAGATAATAGTGACATAAAAGTAGTGCCAAGA AGAAAAGCAAAGATCATCAGGGATTATGGAACACAGATG GCAGGTGATGATTGTGTGGCAAGTAGACAGGATGAGGATT AA
34	DNA Frag- ment con- tain- ing Rev, RRE and rabbit beta globin poly A	CTCAGAAATGGCAGGAAGAGCGGAGACAGCGACGAAGAG CTCATCAGAAACAGTCAGACTCATCAAGCTTCTCATCAAA GCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCG AAGGAATAGAAAGAAAGGTGGAGAGAGAGACAGAGAC AGATCCATTTCGATTAGTGAACGGATCCTTGGCACTTATCT GGGACGATCTCGGAGCCTGTGCCCTTTCAGCTACACCG CTTGAGAGACTTACTCTTGATTGTAAACGAGGATTGTGGAA CTTCTGGGACGCGAGGGGTGGGAAGCCCTCAAAATATTGGT GGAATCTCTACAATATTGGAGTCAGGAGCTAAAGAATAG AGGAGCTTTGTTCTTGGGTTCTTGGGAGCAGCAGGAAGC ACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGGCCA GACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAAATTT GCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTC ACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAAATCCTGG CTGTGGAAGATACCTAAAGGATCAACAGCTCCTAGATCT TTTTCCCTCTGCCAAAATATAGGGGACATCATGAAGCCC CTTGAGCATCTGACTTCTGGCTAATAAGGAAATTTATTT TCATTGCAATAGTGTGTGGAAATTTTGTGTCTCTCACT CGGAAGGACATATGGGAGGGCAATCATTTAAACATCAG AATGAGTATTTGGTTTAGAGTTTGGCAACATATGCCATAT GCTGGCTGCCATGAACAAAGGTGGCTATAAAGAGGTATC AGTATATGAAACAGCCCCCTGCTGTCCATCTCTTATTCCA TAGAAAAGCCTTGAGCTTGAGGTTAGATTTTTTTATATTT TGTTTTGTGTTATTTTTTTCTTTAACTCCCTAAATTTT CCTTACATGTTTTACTAGCCAGATTTTTCTCTCTCTCTG ACTACTCCAGTCATAGCTGTCCCTCTCTCTTATGAAGA TCCCTCGACCTGCAGCCCAAGCTTGGGTAATCATGGTCA TAGCTGTTTTCTGTGTGAAATTTGTTATCCGCTCACAATTC CACACAACATACGAGCCGAAGCATAAAGTGTAAAGCCTG

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SEQ ID NO:	De-scription	Sequence
		GGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTG CGCTCACTGCCCGCTTCCAGTCGGGAAACCTGTCGTGCC AGCGGATCCGCATCTCAATTAGTCAGCAACCATAGTCCCG CCCCTAACCTCCGCCCATCCCGCCCCCTAAGTCCGCCAGTT CCGCCCATTTCTCCGCCCATGGCTGACTAATTTTTTTAT TTATGCAGAGGCCGAGGCCGCTCGGCCCTCAGAGCTATT CAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCCTAGGCTTTT GCAAAAGCTAACTTGTATTATGCAGCTTATAATGGTTAC AAATAAGCAATAGCATCACAAATTTACAAAATAAGCAT TTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAACTCAT CAATGTATCTTATCAGCGGCCGCCCGGG
35	DNA frag- ment con- tain- ing the CAG enhanc- er/ promo- ter/ intron se- quence	ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTA GTTTCATAGCCCATATATGGAGTTCGCGTTACATAACTTA CGGTAATGGCCGCTCTAGCTGACCGCTCAAGACCCCCG CCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACG CCAATAGGGACTTTCATTGACGTCAATGGGTGGACTATT TACGTAATCTGCCCATTTGGCAGTACATCAAGTGTATCA TATGCCAAGTACGCCCTTATGACGTCAATGACGGTAAA TGGCCCGCTGGCATTATGCCAGTACATGACCTTATGGG ACTTTCTACTTGGCAGTACATCTAGTATTAGTCATCGC TATTACCATGGGTGAGGTGAGCCCCACGTCTTCTGCTTAC TCTCCCCATCTCCCCCTCCACCCCAATTTTGTAT TTATTTATTTTTTAATTTATTTGTGCGAGCATGGGGCGG GGGGGGGGGGGGCGCGCCAGGCGGGGGCGGGGGCGGCG AGGGGCGGGGGCGGGCGAGGCGGAGAGGTGCGCGCGCAGC CAATCAGAGCGCGCGCTCCGAAAGTTTCTTTTATGGCG AGGCGGGCGGGCGGGCGCCCTATAAAAAGCGAAGCGCGC GGCGGGCGGGAGTGCCTGCTTGCCTTCGCCCTGTCGCC GCTCGCGCGCTCGCGCTCGGCCCTCCCGCTCTGACTG ACCGCGTTACTCCACAGGTGAGCGGGCGGGACGGCCCTT CTCCTCCGGGCTGTAAATAGCGCTTGGTTAATGACGGCT CGTTTCTTTTCTGTGGCTGCTGAGGCTTAAAGGGCTC CGGGAGGGCCCTTTGTGCGGGGGGAGCGGCTCGGGGGGT CGGTGCGTGTGTGTGCGTGGGAGCGCCGCTGCGGCC CGCGCTGCCCGCGGCTGTCGCGCTGCGGGCGCGCGCG GGGCTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGG CGGGGGCGGTGCCCGCGGTGCGGGGGGCTGCGAGGGGA ACAAAGGCTGCGTGGGGGTGTGCGCTGGGGGGTGGG AGGGGGTGTGGGCGCGCGGTGCGGCTGTAAACCCCCCT GCACCCCCCTCCCGAGTTGCTGAGCACGGCCGCTTCG GGTGGGGGCTCCGTGCGGGGCTGCGGGGCGGGGCTCGCG TGCCGGGCGGGGGTGGCGGAGGTGGGGGTGCGGGGCG GGCGGGGCGCTCGGGCGGGGAGGGCTCGGGGAGGGG CGCGCGGCCCGGAGCGCGCGGCTGTGAGGCGCG CGAGCCGAGCCATTGCCTTTATGGTAATCGTGCAGAG GGCGCAGGACTTCTTTGTCCCAATCTGCGGAGGCGGA AATCTGGGAGGCGCGCGCGCCGACCCCTCTAGGGCGCGG GCGAAGCGGTGCGCGCGCGCGAGGAAGAAATGGGCGGG GAGGGCTTCTGCGCTGCGCGCGCGCGCTCCCTTCTCC ATCTCCAGCTCGGGGCTGCGCGAGGGGAGCGGCTGCTT CGGGGGGAGCGGGGAGGGGGTTCGGCTTCTGGCGTG TGACCGGCGGAATTC
36	RSV promo- ter and HIV Rev	CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGGG GACTAGGGTGTGTTTAGGCGAAAAGCGGGCTTCGGTTGT ACGCGTTAGGAGTCCCTCAGGATATAGTAGTTTCGCTT TTGCATAGGAGGGGGAATGTAGTCTTATGCAATACACT TGATGCTTGCACATGTGAACGATGAGTTAGCAACATGC CTTACAAGGAGAGAAAAGCAACCGTGCATGCGATTGGTG GAAGTAAGTGGTACGATCGTCCCTTATTAGGAAGGCAAC AGACAGGTCTGACATGGAATGGAGCAACCACTGAATCCG CATTGCAAGATAATTGTATTTAAGTGCCCTAGCTCGATAC AATAAACGCCATTTGACCATTCACACATTTGGTGTGACC TCCAAGCTCAGCTCGTTTAGTGAACCGTCAGATCGCTG GAGACGCCATCCACGCTGTTTGAACCTCCATAGAAGACAC CGGACCGATCCAGCTCCCTCCGAAGCTCAGGATTAGG ATCTCTATGGCAGGAAGAAGCGGAGACAGCAGCAAGA ACTCTCAAGGCAGTCAGACTCATCAAGTTCTCTATCAA AGCAACCCACCTCCCAATCCGAGGGGACCCGACAGGCC GAAGGAATAGAAGAAGAAGGTGGAGAGAGACAGAGA CAGATCCATTGATTAGTGAACGGATCTTAGCACTTATC TGGGACGATCTGCGGAGCTGTGCTCTTCACTTACCACC GCTTGAGAGACTTACTCTGATTGTAAACGAGGATTGGGA ACTTCTGGGACGAGGGGGTGGGAAGCCCTCAATATTGG

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SEQ ID NO:	De-scription	Sequence
		TGGAATCTCTACAATATTGGAGTCAGGAGCTAAAGAATA GTCTAGA
37	Elon- gation Fac- tor-1 alpha (EF1- alpha) promo- ter	CCGGTGCCTAGAGAAGGTGGCGGGGTAAACTGGGAAA GTGATGTCGTACTGGCTCCGCCCTTTTCCCGAGGGTGG GGGAGAACCCTATATAAGTGCAGTAGTCGCCGTGAACGTT CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTG CCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTACGGGTTA TGGCCCTTGGCTGGCTTGAATTACTTCCACGCCCTGGCT GCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGT GGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCCT CGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGG CCGCGCGCTGCGAATCTGGTGGCACCTTCGCGCCTGTCTC GCTGCTTTCGATAAGTCTCTAGCCATTTAAATTTTGGAT GACCTGCTGCGACGCTTTTCTGGCAAGATAGTCTTGT AAATGCGGGCCAGATCTGCACACTGGTATTTTCGGTTTTT GGGGCCGCGGGCGGACGCGGGCCCGTGCCTCCAGCGCA CATGTTCCGCGAGGCGGGGCCCTGCGAGCGCGGCCACCGAG AATCGGACGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTG GTGCTGGCCTCGCCCGCGCTGTATCGCCCGCCCTGGG CGGCAAGGCTGGCCCGGTGCGCACAGTTGCGTGAGCGGA AAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAA TGGAGGACGCGCGCTCGGAGAGCGGGCGGGTGAGTCAC CCACACAAAGGAAAAGGGCCTTTCGCTCTCAGCCGTGCG TTCATGTGACTCCAGGAGTACCGGCGCGCTCCAGGCAC CTCGATTAGTTCTCGAGCTTTTGGAGTACGTGCTCTTAG GTTGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACAC TGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCACTTG ATGTAATTCTCCTTGAATTTGCCCTTTTGGAGTTTGGAT CTTGTTTCAATCTCAAGCCTCAGACAGTGGTTCAAAGTTT TTTTCTTCCATTTCAAGTGTCGTGA
38	Promo- ter; PGK	GGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTT GCGCAGGGACGCGGCTGCTCTGGGCGTGGTTCCGGGAAAC GCAGCGGCGCCGACCTCGGCTCAGCACATTTCTCACGTTC CGTTTCGACGCTCACCCGGATCTTCGCCGCTACCCCTTG GGCCCCCGGCGACGCTTCTGCTCCGCCCTAAGTCGGG AAGGTTCTTTCGGTTTCGCGGCTGCCGACGTGACAAAC GGAAGCCGACGCTCTCACTAGTACCTCGCAGACGGACAG CGCCAGGGAGCAATGGCAGCGCGCCGACCGCATGGGCTG TGGCCAATAGCGGCTGCTCAGCAGGGCGCGCGAGAGCA GCGGCGCGGAAAGGGCGGTGCGGGAGGCGGGGTGTGGG CGGTAGTGTGGGCTGTCTTCTCGCCGCGCGGTGTTCGCG ATTCTGCAAGCCTCCGGAGCGCACGTGCGCAGTCGGCTCC CTCGTTGACCGAATACCGACCTCTCTCCCCAG
39	Promo- ter; UbC	GCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCTCTCTC ACGGCGAGCGCTGCCACGTGACAGCAAGGGCGCAGGAGC GTTCTGTATCCTTCCGCCCGGACGCTCAGGACAGCGGCC GCTGCTCATAAGACTCGGCCTTAGAACCCAGTATCAGCA GAAGGACATTTTAGGACGGGACTTGGGTGACTCTAGGGCA CTGGTTTTCTTTCAGAGAGCGGAACAGGCGAGGAAAAGT AGTCCCTTCTCGGCGATTCTGCGGAGGGATCTCCGTGGG CGGTGAACCGCATGATTATATAAGGACGCGCCGGGTGTG GCACAGCTAGTTCCTGCGAGCCGGATTGGGTGCGCGT TCTTGTGTGGATCGCTGTGATCGTCACTTGGTGAGTTG CGGGCTGCTGGGCTGGCGGGGCTTTCGTGGCGCGCGGG CGCTCGGTGGGACGGAAGCGTGTGGAGAGACCGCAAGGG CTGTAGCTTGGGTCCGCGAGCAAGGTGCGCTGAACTGGG GGTTGGGGGAGCGCACAAAATGGCGGCTTTCCTCGAGTC TTGAATGGAAGACGCTTGAAGGCGGGCTGTGAGGTCTGT GAAAACAAGGTGGGGGCGATGGTGGGCGGCAAGAACCCAA GGTCTTGAGGCCCTTCGCTAATGCGGGAAGCTCTTATTCC GGTGAGATGGGCTGGGCGACCATCTGGGGACCTGACGTG AAGTTTGTCACTGACTGGAGAAGCTCGGGTTTGTGCTCTGG TTGCGGGGGCGGCGAGTTATGCGGTGCCGTTGGGCGAGTGA CCCGTACCTTTGGGAGCGCGCCCTCGTGTGCTGTGACG TCACCCGTTCTGTTGGCTTATAATGCAGGTTGGGGCCACC TGCCGGTAGGTGTGCGGTAGGCTTTCTCCGTGCGAGGAC GCAGGGTTCCGGCTTAGGTTAGGCTCTCTCTGAATCGACAG CGCGCGGACCTCTGTGAGGGGAGGGATAAGTGAGGCGTCT AGTTTCTTTGGTGGTTTTATGTACCTATCTCTTAAGTA

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SEQ ID NO:	De-scription	Sequence
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40	Poly A; SV40	GTTTATTGCAGCTTATAATGGTTACAAAATAAGCAATAGC ATCACAAATTTACAAATAAGCATTTTTTTCACTGCATT CTAGTTGTGGTTGTGCCAACTCATCAATGTATCTTATCA
41	Poly A; bGH	GACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCC TCCCCCGTGCCTTCTTGACCTTGGAAGGTGCCATCCCA CTGTCTTTTCTTAATAAAATGAGAAATGTCATCCGATTG TCTGAGTAGGTGTCTTCTATTCTGGGGGTTGGGTGGGG CAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAG GCATGCTGGGGATGCGGTGGGCTCTATGG
42	Enve- lope; RD114	ATGAAACTCCCAACAGGAATGGTCATTTTATGTAGCCTAA TAATAGTTCGGGCAGGGTTTGACGACCCCGCAAGGCTAT CGCATTAGTACAAAAACAACATGGTAAACCATGCGAATGC AGCGGAGGGCAGGTATCCGAGGCCCGACCGAATCCATCC AACAGGTAACCTGCCCAGGCAAGCGGCTACTTATGAC CAACCAAAAATGGAATGCAGAGTCACTCCAAAAAATCTC ACCCCTAGCGGGGAGAACTCCAGAACTGCCCTGTAAACA CTTTCAGGACTCGATGCAAGTTCTTGTATATACTGAATA CCGGCAATGCAGGGCGAATAATAAGACATACTACACGGCC ACCTTGTCTTAAATAACGCTCTGGGAGCCTCAACGAGGTAC AGATATTACAAAACCCCAATCAGCTCCTACAGTCCCCTTG TAGGGGCTCTATAAATCAGCCCGTTTGTGAGTGCCACA GCCCCCATCCATATCTCGATGGTGGAGGACCCCTCGATA CTAAGAGAGTGTGGACAGTCCAAAAAAGGCTAGAACAAA TTCATAAGGCTATGCATCCTGAACCTCAATACCCCCCTT AGCCCTGCCCAAAGTCAGAGATGACCTTAGCCTTGATGCA CGGACTTTTGATATCTCTGAATACCACTTTTAGGTTACTCC AGATGTCCAATTTTAGCCTTGCCCAAGATTGTTGGCTCTG TTTAAAACTAGGTACCCCTACCCCTCTTGCATACCCACT CCCTCTTTAACCCTACTCCCTAGCAGTCCCTAGCGAATG CCTCTGTGAGATTATACCTCCCCCTCTTGGTTCAACCGAT GCAGTTCTCAAACCTGCTCTGTTATCTTCCCTTTTCAAT AACGATACGGAACAAATAGCACTAGGTGCACTCACTTTA CTAACTGCACCTCTGTAGCCAATGTGAGTGTCTTTATG TGCCCTAAACGGGTGAGTCTTCTCTGTGGAATAACATG GCATACACCTATTATCCCAAACTGGACAGGACTTTTGGC TCCAAGCCTCCCTCTCCCGGACATTGACATCATCCCGGG GGATGAGCCAGTCCCATCTTCTGCAATGATCATTATATA CATAGACTAAACGAGCTGTACAGTTTCACTCTTACTAG CTGGACTGGGAATCACCGCAGCTTACCACCGGAGCTAC AGGCCTAGGTGTCTCCGTACCCAGTATACAAAATATCC CATCAGTTAATATCTGATGTCCAAGTCTTATCCGGTACCA TACAAGATTTACAAGACAGGTAGACTCGTTAGCTGAAGT AGTTCTCAGAAATAGGAGGGGACTGGACCTACTAACGGCA GAACAAGGAGGAATTTGTTTAGGCTTACAAGAAAATGCT GTTTTTATGCTAACAAGTCAGGAATTTGTGAGAAACAAAAT AAGAACCCTACAGAAGAAATACAAAACGCGAGGGAAGC CTGGCATCCAACCTCTCTGAGCCGGGCTGCAGGGCTTTC TTCCGTACCTCTACCTCTCTCGGACCCCTACTCACCTT CTTACTCATATAACCATTTGGGCCATGCGTTTTCAATCGA TTGGTCCAATTTGTTAAAGACAGGATCTCAGTGGTCCAGG CTCTGGTTTTGACTCAGCAATATCACCAGCTAAAACCCAT AGAGTACGAGCCATGA
43	Enve- lope; GALV	ATGCTTCTCACCTCAAGCCCGCACCACTTCCGCGACCCAGA TGAGTCTTGGGAGCTGGAAGAGACTGATCATCTCTTAAG CTGCGTATTTCGAGACGCGCAACAGCTTTCGAGAATAAG AACCCCAACCGCCTGTGACCTCAGCTGGCAGGTACTGT CCCAAACTGGGACGTTGTCTGGGACAAAAGGCAAGTCCA GCCCCCTTGGACTTGGTGGCCCTCTCTTACACCTGATGTA TGTGCCCTGGCGGCGGCTTGTAGTCTTGGGATATCCCGG GATCCGATGTATCGTCTCTAAAAGAGTTAGACCTCTCTGA TTCAGACTATACTGCCGCTTATAAGCAAAATCAGCTGGGGA GCCATAGGGTGCAGCTACCCCTCGGCTAGGACAGGATGG CAAATCCCCCTTCTACGTGTGTCCCGAGCTGGCCGAAC CCATTGAGAAGCTAGGAGGTGTGGGGGCTAGAATCCCTA TACTGTAAAGAATGGAGTTGTGAGACCACGGGTACCGTTT ATTGGCAACCCAGTCAAGTCCGACCTCAGGACCTCAACTGTA ATGGGACCAAAATGTGAATGGGAGCAAAAATTTCAAAG

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SEQ ID NO:	De-scription	Sequence
		TGTGAACAAACCGGCTGGTGTAAACCCCTCAAGATAGACT TCACAGAAAAAGGAACTCTCCAGAGATTGGATAACGGA AAAAACCTGGGAATTAAGGTTCTATGTATATGGACACCCA GGCATAACAGTTGACTATCCGCTTAGAGGTCACTAACATGC CGGTTGTGGCAGTGGGCCAGACCCCTGTCTTGGCGGAACA GGGACCTCCTAGCAAGCCCTCACTCTCCCTCTCTCCCA CGGAAAGCGCCGCCACCCCTCTACCCCGGCGGCTAGTG AGCAAAACCCCTGCGGTGCATGGAGAACTGTTACCTAAA CTCTCCGCTCCACCAGTGGCGACCGACTCTTTGGCCTT GTGCAAGGGGCTTCTTAACCTTGAATGCTACCAACCCAG GGGCCACTAAGCTTTGCTGGCTCTGTTTGGGCATGAGCCC CCCTTATTATGAAGGGATAGCCTCTTACAGGAGGTGCT TATACCTCCAACCATACCCGATGCCACTGGGGGGCCAAAG GAAAGCTTACCCTCACTGAGGTCTCCGGACTCGGGTCATG CATAGGGAAGGTGCTCTTACCCATCAACATCTTTGCAAC CAGACCTTACCCTCACTCAATCTCTTAAACCATCAGTATC TGCTCCCCCTCAAACCATAGCTGGTGGGCTGCAGCACTGG CCTCACCCCTGCCTCTCCACCTCAGTTTTTAATCAGTCT AAAGACTTCTGTGCCAGTCCAGCTGATCCCCGCATCT ATTACCATCTGAAGAAACCTTGTACAAAGCCTATGACAA ATCACCCCCAGGTTTAAAGAGAGCCTGCCTCACTTACC CTAGCTGTCTTCTGGGTTAGGGATTGCGGCAGGTATAG GTACTGGCTCAACCCCTTAATTAAGGGCCATAGACCT CCAGCAAGGCCAACCAGCCTCCAAATCGCCATTGACGCT GACCTCCGGGCCCTTCAGACTCAATCAGCAAGCTAGAGG ACTCACTGACTTCCCTATCTGAGGTAGTACTCCAAATAG GAGAGGCCTTGACTTACTATTCTTAAAGAGGAGGCCTC TGCGCGGCCCTAAAAGAGAGTGTGTTTTATGTAGACC ACTCAGGTGCAGTACGAGACTCCATGAAAAAATCTAAAGA AAGACTAGATAAAAGACAGTTAGAGCGCCAGAAAAACCAA AACTGGTATGAAGGTGGTTCAATAACTCCCTTGGTTTA CTACCTACTATCAACCATCGCTGGGCCCTATTGCTCCT CCTTTTGTACTCACTCTTGGGCCCTGCATCATCAATAAA TTAATCCAATTATCAATGATAGGATAAGTGCAGTCAAAA TTTTAGTCTTTAGACAGAAATATCAGACCTTAGATAACGA GGAAAAACCTTTAA
44	Enve- lope; FUG	ATGGTTCCGAGGTCTTTTGTGTGTTACTCCTTCTGGGTT TTTCGTTGTGTTTCGGGAAGTTCCCCATTTACACGATACC AGACGAACCTTGCTCCTGGAGCCCTATTGACATACACCAT CTCAGCTGTCCAAATAACCTGGTTGTGGAGGATGAAGGAT GTACCAACCTGTCCGAGTTCTCCTACATGGAACCTCAAAGT GGGATACATCTCAGCCATCAAAGTGAACGGGTTCACTTGC ACAGGTGTGTGACAGAGGCAGAGACCTACACCAACTTTG TTGGTTATGTACACACCATTAAGAGAAAGCATTTCCG CCCCACCCAGACGCATGTAGAGCCGCGTATAACTGGAAG ATGGCCCGGTGACCCAGATATGAAGAGTCCCTACACAATC CATACCCGACTACCACTGGCTTCACTGTAAGAACCAC CAAAGAGTCCCTCATTATCATATCCCCAAGTGTGACAGAT TTGGACCCATATGACAAATCCCTTCACTCAAGGGTCTTCC CTGGCGGAAGTGTCTCAGGAATAACGGTGTCTCTACCTA CTGCTCAACTAACCATGATTACACCATTTGGATGCCCGAG AATCCGAGACCAAGGACACCTTGTGACATTTTACCAATA GCAGAGGGAAGAGAGCATCCAACGGGAACAAGACTTGC CGCTTTGTGATGAAAGAGGCTGTATAAGTCTCTAAAGGA GCATGCGAGCTCAAGTTATGTGGAGTCTTGGACTTAGAC TTATGGATGGAACATGGGTGCGGATGCAACATCAGATGA GACCAAAATGGTGCCCTCCAGATCAGTTGGTGAATTTGCAC GACTTTCGCTCAGACGAGATCAGCATCTCGTTGTGGAGG AGTTAGTTAAGAAAGAGAGGAATGTCTGGATGCATTAGA GTCCATCATGACACCAAGTCAAGTAAAGTTTCAAGCTCTC AGTCACCTGAGAAACTTGTCCAGGGTTTGGAAAGCAT ATACCATATTTCAACAAACCTTGATGGAGGCTGATGCTCA CTACAAGTCAGTCCGACCTGGAATGAGATCATCCCTCA AAAGGGTGTGTTGAAAGTTGGAGGAAGTGCCATCTCATG TGAAACGGGTGTTTTTCAATGTTAATAATTAGGGCTTCA CGACCATGTCTTAATCCAGAGATGCAATCATCCCTCTC CAGCAACATATGGAGTTGTGGAATCTTCAGTTATCCCCC TGATGCACCCCTGGCAGACCTTCTACAGTTTTCAAAGA AGGTGATGAGGCTGAGGATTTTGTGAAGTTTCACTCCCC GATGTGTACAAACAGATCTCAGGGTTGACCTGGGTCTCC CGAATGGGGAAGTATGTATTGATGACTGCAGGGGCCAT

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SEQ ID NO:	De-scription	Sequence
		GATTGGCCTGGTGTGATATTTTCCCTAATGACATGGTGC AGAGTTGGTATCCATCTTTGCATTAAATTAAGCACACCA AGAAAAGACAGATTTATACAGACATAGAGATGAACCGACT TGGAAAGTAA
45	Enve- lope; LCMV	ATGGGTCAGATTGTGACAATGTTTGGAGCTCTGCCTCACA TCATCGATGAGGTGATCAACATTGTCTATTGTGCTTAT CGTGATCAGGGTATCAAGGCTGTCTACAATTTTGCCACC TGTGGGATATTGCGATTGATCAGTTTCCCTACTTCTGGCTG GCAGGTCCTGTGGCATGTACGGTCTTAAGGGACCCGACAT TTACAAAGGAGTTTACCAATTTAAGTCAGTGGAGTTGAT ATGTCACTCTGAACCTGACCTGCCAACCGCATGTTTCA CCAACAACCTCCACCATTACATCAGTATGGGACTTCTGG ACTAGAATTGACCTTCAACATGATTCCATCATCAGTCAC AACTTTTGCAACTGACCTCTGCCTTCAACAAAAAGACCT TTGACCACACACTCATGAGTATAGTTTCGAGCCTACACCT CAGTATCAGAGGGAACCTCAACTATAAGGCAGTATCCTGC GACTTCAACAATGGCATAAACATCCAATACAATTGACAT TCTCAGATCGACAAAGTGTCTCAGAGCCAGTGTAGAACCTT CAGAGGTAGAGTCTTAGATATGTTTGAAGTGCCTTCCGG GGGAATATACATGAGGATGCTGGGCTGGACCTGACCTCAG ATGGCAAGACCACCTGGTGTAGCCAGACGAGTTACCAATA CCTGATTATACAAAATAGAACCTGGGAAAACCACTGCACA TATGCAAGTCTTTTGGGATGTCAGGATTTCTCTTTCCC AAGAGAAGACTAAGTTCTTCACTAGGAGACTAGCGGGCAC ATTCACCTGGACTTTGTGACACTCTTCAAGGGTGGAGAAT CCAGGTGGTTATTGCTCTGACCTGACCAATGGATGATTCTGCTG CAGAGCTTAAGTGTTCGGGAACACAGCAGTTGCGAAATG CAATGTAATCATGATGCCGAATTTCTGTGACATGCTGCGA CTAATTGACTACAAACAGGCTGCTTTGAGTAAGTTCAAAG AGGACGTAGAATCTGCCTTGCACTTATTCAAACAACAGT GAATTTCTTGATTTCAGATCAACTACTGATGAGGAACAC TTGAGAGATCTGATGGGGTGCATATTGCAATTACTCAA AGTTTTGTGACCTAGAATGCAAGACCGCGGAAACTAG TGTCCTCAAGTGTCTGGCTGTGACCAATGGTTCTTACTTA AATGAGACCCACTTCAGTGTCAATCGAACAGGAAGCCG ATAACATGATTACAGAGATGTTGAGGAAGGATTACATAAA GAGGCGAGGGAGTACCCCTTAGCATTTGATGACCTTCTG ATGTTTTCCACATCTGCATATCTAGTCAGTCTTCTCTGC ACCTTGTCAAATACCAACACACAGGCACATAAAAGGTGG CTCATGTCCAAAGCCACACCGATTAAACCAACAAAGGAATT TGATGTTGTGGTGCATTTAAGGTGCTTGGTGTAAAAACCG TCTGGAAAAAGACGCTGA
46	Enve- lope; FPV	ATGAACACTCAAATCCTGGTTTTTCGCCCTTGTGGCAGTCA TCCCCACAAATGCAGACAAAATTTGTCTTGGACATCATGC TGTATCAATGGCACCAAGTAAACACACTCACTGAGAGA GGAGTAGAAGTTGTCAATGCAACGGAAACAGTGGAGCGG ACAAACATCCCCAAATTTGCTCAAAAGGGAAGAAACC ACTGATCTTGGCCAATGCGGACTGTAGGGACATTACCG GACCCTCAATGCGACCAATTTCTAGAATTTTTCAGCTGA TCTAATAATCGAGAGACGAGAAGGAATGATGTTGTTTAC CCGGGAAGTTTGTTAATGAAGAGGCATTCGACAAAATCC TCAGAGGATCAGGTGGGATTGACAAAGAAACAAATGGGATT CACATATAGTGAATAAGGACCAACCGAACCACTAGTGC ATGTAGAAGATCAGGCTCTTCACTTCTATGACAGAAATGGAG TGGCTCCTGTCAAATACAGACAAATGCTGCTTTCCCAAAA TGACAAAATCATACAAAACACAAGGAGAGAAATCAGCTC TGATAGTCTGGGGAATCCACCATTCAGGATCAACCCACGA ACAGACCAAACTATATGGGAGTGGAAATAAATGATAAC AGTCGGGAGTTCCAAATATCATCAATCTTTTGTGCCGAGT CCAGGAACACGACCGCAGATAAATGGCCAGTCCGACCGGA TTGATTTTCAATGGTTGATCTTGGATCCCAATGATACAGT TACTTTTAGTTTCAATGGGCTTTTATAGCTCCAAATCGT GCCAGCTTCTTGGGGGAAGTCCATGGGGATTCAGAGCG ATGTGCAGTTGATGCCAATTCGCAAGGGGAATGCTACCA CAGTGGAGGGACTATAACAGAGATGCTGCTTTTCAAAC ATCAATAGCAGAGCAGTTGGCAAATGCCCAAGATATGTAA AACAGGAAGTTTATTTATGGCAACTGGGATGAAGAACGT TCCCGAACCTTCCAAAAAAGGAAAGAAAGAGGCTGTTT GGCGCTATAGCAGGGTTTATTTGAAAAAGTTGGGAAGGTC TGGTCGACGGGTGGTACGGTTTCAGGCATCAGAAATGCACA AGGAGAAGGAATGCAGCAGACTACAAAAGCACCAATC GGCAATTGATCAGATAACCGGAAGTTAAATAGACTATT GAGAAAACCAACCGCAATTTGAGCTAATAGATAATGAAT

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SEQ ID NO:	De-scription	Sequence
		TCACTGAGGTGGAAAAGCAGATTGGCAATTTAATTAAGTCTG GACCAAAGACTCCATCACAGAAGTATGGTCTTACAATGCT GAACCTCTTGTGGCAATGGAAAACAGCACACTATTGATT TGGCTGATTGAGAGATGAACAAGCTGTATGAGCGAGTGAG GAAACAATTAAGGGAAAATGCTGAAGAGGATGGCACTGG TTGCTTTGAAATTTTTCATAAATGTGACGATGATTGATG GCTAGTATAAGGAACAATACTTATGATCACAGCAAATACA GAGAAGAAGCGATGCAAAATAGAATACAAATTGACCCAGT CAAATTTGAGTAGTGGCTACAAAGATGTGATACCTTTGGTTT AGCTTCGGGGCATCATGCTTTTGGCTTCTTGCCATTGCAA TGGGCCTTTGTTTTCATATGTGTGAAGAACGAAACATGCG GTGCACTATTTGTATATAA
47	Enve- lope; RRV	AGTGTAAACAGAGCACTTAAATGTGTATAAGGCTACTAGAC CATACCTAGCACATTGCGCCGATTGCGGGGACGGGTACTT CTGCTATAGCCCACTGCTATCGAGGAGATCCGAGATGAG GCGTCTGATGGGATGCTTAAGATCCAAGTCTCCGCCAAA TAGGTCCTGGACAAAGCGAGGCACCCAGCCACACGAAGCT CCGATATATGGCTGGTCATGATGTTCCAGGAATCTAAGAGA GATTCCTTGAGGGGTACACGTCGCGAGCGTGTCTCCATAC ATGGGACGATGGGACACTTCATCGTCGCACACTGTCCACC AGGCGACTACCTCAAGGTTTCGTTTCGAGGACGAGATTTCG CACGTGAAGGCATGTAAGGTCCAATACAGCACAAATCCAT TGCCGTTGGGTAGAGAGAAGTTTCGTGGTTAGACCACTT TGGCGTAGAGCTGCCATGCACCTCATACCAGCTGACAACG GCTCCACCGAGAGGAGATTGACATGCATACACCGCCAG ATATACCGGATCGCACCTGCTATCACAGACGGCGGGCAA CGTCAAAAACACAGCAGGCGCGGAGGACTATCAGGTACAA CTGTACCTGCGGCCGTGACAACGTAAGGCACTACCACTACT GACAAGACCATCAACACATGCAAGATTGACCAATGCCATG CTGCCGTACCCAGCCATGACAAATGGCAATTTACCTCTCC ATTTGTTCCAGGGGTGATCAGACAGCTAGGAAAGGCAAG GTACAGCTTCCGTTCCCTCTGACTAACGTCACCTGCCGAG TGCCGTTGGCTCGAGCGCCGATGCCACCTATGGTAAGAA GGAGGTGACCCTGAGATTACACCCAGATCATCCGACGCTC TTCTCCTATAGGAGTTTAGGAGCCGAACCGCACCCGTACG AGGAATGGGTTGACAAGTTCTCTGAGCGCATCATCCAGT GACGGAAGAAGGGATTGAGTACCACTGGGGCAACACCCG CCGGTCCTGCTGTGGGCGCAACTGACGACCGAGGGCAAA CCCATGGCTGGCCACATGAAATCATTAGTACTATTATGG ACTATACCCCGCCGCACTATTGCCGCGATATCCGGGGCG AGTCTGATGGCCCTCCTAACTCTGGCGGCCACATGCTGCA TGCTGGCCACCGCGAGGAGAAAGTGCCTAACACCGTACGC CCTGACGCGAGGAGCGGTGGTACCGTTGACACTGGGGCTG CTTTGCTGCGCACCGAGGGCGAATGCA
48	Enve- lope; Ebola	ATGGGTGTACAGGAATATTGCACTTACCTCGTGATCGAT TCAAGAGGACATCATCTTTCTTTGGGTAATTATCTCTTT CCAAAGAACATTTTCCATCCCACTTGGAGTCATCCACAAT AGCACAATTACAGGTTAGTGATGTCGACAAACTGGTTTGGC GTGACAAACTGTCATCCACAAATCAATTGAGATCAGTTGG ACTGAATCTCGAAGGGAATGGAGTGGCAACTGACGTGCCA TCTGCAACTAAAGATGGGGCTTCAGGTCCGGTGTCCAC CAAAGGTGGTCAATTATGAAGCTGGTGAATGGGCTGAAAA CTGCTACAATCTTGAAATCAAAAAACCTGACGGGAGTGAG TGTCTACCAAGCAGCGCCAGACGGGATTCGGGGCTTCCCC GGTGCCGGTATGTGCACAAAGTATCAGGAACGGGACCGTG TGCCGGAGACTTTTGCTTCCACAAAGAGGGTGTCTTCTTC CTGTATGACCGACTTGCTTCCACAGTTATCTACCGAGGAA CGACTTTCGCTGAAGGTGTCGTTGCAATTTCTGATACGCG CCAAGCTAAGAAGGACTTCTTCAGCTCACACCCCTTGAGA GAGCCGGTCAATGCAACGGAGGACCCGTCTAGTGGCTACT ATTTCTACCACAATTAGATATCAAGCTACCGGTTTGGAAAC CAATGAGACAGAGTATTGTTTCGAGGTTGACAATTTGACC TACGTCFCAACTTGAATCAAGATTACACACAGTTTCTGCG TCCAGCTGAATGAGACAATATATACAAGTGGGAAAAGGAG CAATACCAAGGGAACAACTAATTGGAAGGTCAACCCCGAA ATTGATACAACAATCGGGAGTGGGCTTCTTGGGAAACTA AAAAAACCTCACTAGAAAAATTCGAGTGAAGAGTTGTCT TTCAACAGCTGTATCAAAACAGAGCCAAAAACATCAGTGGT CAGAGTCCGGCGCGAACTTCTTCCGACCCAGGACCAACAC AACAACCTGAAGACCAAAATCATGGCTTCAGAAAAATTC TCTGCAATGGTTCAAGTGCACAGTCAAGGAAGGGAAGCTG CAGTGTGCGATCTGACAACCCCTTGCCACAATCTCCACGAG TCCTCAACCCCCACACCAAAACAGGTCGGGACACAGC

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SEQ ID NO:	De-scription	Sequence
5		ACCCAACAATACACCCGTGTATAAACTTGACATCTCTGAGG CAACTCAAGTTGAACAACATCACCCGAGAACAGACAACGA CAGCACAGCCTCCGACACTCCCCCGCCACGACCGCAGCC GGACCCCTAAAAGCAGAGAACCCACACGAGCAAGGT ACCGACCTCCTGGACCCCGCCACCACAACAGTCCCCAAA ACCACAGCGAGACCGCTGGCAACAACAACACTCATCACC AGATACCGGAGAAGAGAGTGCACGAGCGGGAAGCTAGG CTTAATTACCAATACTATTGCTGGAGTCGAGGACTGATC ACAGCGGGGAGGAGAGCTCGAAGAGAAGCAATTTGCAAT GCTCAACCCAAATGCAACCCCTAATTTACATTACTGGACT CTCAGGATGAAGGTGCTGCAATCGGACTGGCCTGGATACC ATATTTTCGGGCCAGCAGCCGAGGGAATTTACATAGAGGGG CTGATGCACAATCAAGATGGTTAATCTGTGGGTTGAGAC AGCTGGCCACGAGACGACTCAAGCTCTTCAACTGTTCTCT GAGAGCCACAACCGAGCTACGCACCTTTTCAATCCTCAAC CGTAAGGCAATTGATTTCTTGCTGACGCGATGGGCGGGCA CATGCCACATTTTGGGACCGGACTGCTGTATCGAACCACA TGATTGGACCAAGAACATAACAGACAAAATTGATCAGATT ATTCATGATTTTGTGTATAAAACCTTCCGGACCAAGGGG ACAATGACAATTGGTGGACAGGATGGAGACAATGGATAC CGGCAGGTATTGGAGTTACAGCGCTTATAATTGCAAGTTAT CGCTTTATTCTGTATATGCAAAATTTGTCTTTTAG
49	FDPS target se- quence #1	GTCCTGGAGTACAATGCCATT
50	FDPS target se- quence #2	GCAGGATTTCGTTGAGCACTT
51	FDPS target se- quence #3	GCCATGTACATGGCAGGAATT
52	FDPS target se- quence #4	GCAGAAGGAGGCTGAGAAAGT
53	miR30 FDPS se- quence #1	AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGC CTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTGA GAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT
54	miR30 FDPS se- quence #2	AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGC CTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGCTGAGA AAGTGTGCTACTGCCTCGGACTTCAAGGGGCT
55	miR30 FDPS se- quence #3	TGCTGTTGACAGTGAGCGACTTTCTCAGCCTCCTTCTGCG TGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTTGCCT ACTGCCTCGA
56	miR155 FDPS se- quence #1	CCTGGAGGCTTGTGTAAGGCTGTATGCTGACTTTCTCAGC CTCCTTCTGCTTTTGGCCACTGACTGAGCAGAAGGGCTGA GAAAGTCAGGACACAAGGCTGTACTAGCACTCA

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SEQ ID NO:	De-scription	Sequence
57	miR21 FDPS se-quence #1	CATCTCCATGGCTGTACCACCTTGTCTGGGACTTTCTCAGCCTCCTTCTGCCTGTTGAATCTCATGGCAGAAGGAGGCGAGAAAGTCTGACATTTTGGTATCTTTCATCTGACCA
58	miR185 FDPS se-quence #1	GGGCCTGGCTCGAGCAGGGGGCGAGGGATACCTTTCTCAGCCTCCTTCTGCTGGTCCCCCTCCCCGAGAAGGAGGCTGAGAAGTCTCTCCCTCCCAATGACCGCTCTTCGTCTG
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	AAAGTCAGTGGGACAGTGG
66	miR155 CD47 target se-quence #2	CCTGGAGGCTTGTCTGAAGGCTGTATGCTGTTAGCTCGATGATCGTTTCACGTTTTGGCCACTGACTGACGTGAAACGCATCGAGCTAACAGGACACAAGGCCTGTTACTAGCACTCA
67	miR155 CD47 target se-quence #3	CCTGGAGGCTTGTCTGAAGGCTGTATGCTGAAGAATGGCTCCAACAATGACGTTTTGGCCACTGACTGACGTCTATTGTGAGCCATTCTTCAGGACACAAGGCCTGTTACTAGCACTCA
68	miR155 CD47 target se-quence #4	CCTGGAGGCTTGTCTGAAGGCTGTATGCTGTATACACGCCGCAATACAGAGGTTTTGGCCACTGACTGACCTCTGTATCGGCGTGATATACAGGACACAAGGCCTGTTACTAGCACTCA
69	Forward primer	GGACTATCCTGCTGCCAA
70	miR155 cMyc se-quence	CCTGGAGGCTTGTCTGAAGGCTGTATGCTGTGTTTCGCCTCTTGACATTCTTTTTGGCCACTGACTGAGAGAATGTAGAGCGAACACAGGACACAAGGCCTGTTACTAGCACTCA
71	cMyc target se-quence	GAGAATGTCAAGAGGCGAACA

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SEQ ID NO:	De-scription	Sequence
72	CMV promoter se-quence	ATTATGCCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGATTTCCAAGTCTCCACCCCATTTGACGTCATGGGAGTTTGTGTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACCTCCGCCCATTTGACGCAATGGGCGGTAGGCGTGTACGGTGGGAGGTTTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCCATCCACGTGTTTT
73	GFP T2A Luciferase se-quence	ATGCCCGCCATGAAGATCGAGTGC CGCATCACCCGGCACCCGAGTACCGGTTCGAGCTGGTGGGCGCGGAGAGGGCACCCTCGAGCAGGGCCGCATGACCAACAAGATGAAGAGCACCAGGCGCCCTGACCTTCAGCCCCCTACCTGCTGAGCCACGTGATGGCTACGGCTTCTACCACTTCGGCACCTACCCCAGCGCTACGAGAACCCCTTCTGTCACGCCATCAACAACGGCGGTACACCAACACCCGCATCGAGAAGTACGAGGACGGCGCGTGTGTCAGTGTGAGCTTCAGCTACCGTACGAGGCGCGCGTGTATCGGCGACTTCAAGGTGGTGGGCACCGCTTCCCCGAGGACAGCGTGATCTTCAACGACAAGATCATCCGACGCAACGCCACCGTGGAGCACCTGCACCCCATGGGCGATAACGTGCTGGTGGGCGAGCTTCGCCCGCACCTTCAGCCTGCGCGACGGCGGTACTACAGCTTCGTGGTGGACAGCCACATGCATTTCAAGAGCGCCATCCACCCAGCATCTCGCAGAACGGGGGCCCATGTTTCGCCCTTCGCCCGCGTGGAGGAGCTGCACAGCAACACCGAGCTGGGCATCGTGGAGTACCAGCACGCCCTTCAAGACCCCCATCGCTTCGCCAGATCTCGAGATATCAGCCATGGCTTCCCGCCGGCGGTGGCGGCGCAGGATGATGGCACGCTGCCCATGCTTGTGTCGCCAGGAGAGCGGGAATGGACCGTCAACCCTGCAGCTGTGCTTCTGTAGGATCAATGTGACCGGTGAGGGCAGAGGAAGTCTTCAACATGCGGTGACGTGGAGGAGAATCCCGGCCCTTCGGTATGGAAGACGCCAAAACATAAAGAAAGGCCCGCGCCATTCTATCCGCTAGAGGATGGAACCGCTGGAGAGCAACTGCATAAGGCTATGAAGAGATACGCCCTGGTTCCTGGAACAATTGCTTTTACAGATGCACATATCGAGGTGAACATCAGTACGCGGAATACTTCGAAATGTCCGTTCCGTTGGCAGAAGCTATGAACGATATGGGCTGAATACAAATCACAGAATCGTCGTATCGAGTGAATACTCTTCAATTCTTTATGCCGTTGGGCGCGTTATTATCGGAGTTGCAGTTGCGCCCGCGCAACGACATTTATAATGAACGTGAATTGCTCAACAGTATGAACATTTTCGACGCCCTACGTAAGTGTGTTTCCAAAAGGGGTTGCAAAAATTTTGAACGTGCAAAAATTTACCAATAATCCAGAAAATTTATATCATGGATTCTAAACCGGATTACAGGGATTTCAGTCGATGTACACGTTTCGTACATCTCATCTACCTCCCGGTTTAAATGAATACGATTTTGTACAGAGTCTTTGATCGTGACAAAACAATTGCACTGATAATGAATCCTCTGGATCTACTGGGTACCTAAGGGTGTGGCCCTTCGCATAGAACTGCCTGCGTCAGATTCTCGCATGCCAGAGATCCTATTTTGGCAATCAATCATTCGCGATACTGCGATTTTAAGTGTGTTCCATTCCATCACGGTTTTGGAATGTTTACTACACTCGGATATTGATATGTGGATTTCGAGTCGTCTTAATGTATAGATTGAAGAAAGAGCTGTTTTTACGATCCCTTCAGGATTACAAAATTCAAAGTGCCTGCTAGTACCAACCCATTTTTCATTCTTCGCCAAAGCACTCTGATTGACAAATACGATTTATCTAATTTACACGAAATGCTTCTGGGGCGCACCTCTTCGAAAGAAGTCGGGAAGCGGTTGCAAAACGCTTCCATCTTCCAGGATACGACAAGGATATGGGCTCACTGAGACTACATCAGCTATTCTGATTACACCCGAGGGGATGATAAACCGGGCGCGGTGCGTAAGTTGTTCCATTTTTTGAAGCGAAGGTTGTGGATCTGGAATACCGGAAAACGCTGGGCGTTAATCAGAGAGGCGAATTAATGTGTGAGAGGACCTATGATTATGTCGGGTTATGTAAACAATCCGAAGCGACCAACGCCCTTGATTGACAAGGATGGATGCTACATTCTGGAGACATAGCTTACTGGGACGAAGACGAACTTCTTCATAGTTGACCGCTTGAAGTCTTTAATTAATACAAAGGATACAGGTGGCCCCCGCTGAATTGGAGTCGATATTGTTACAACACCCCAACATCTTCGACGCGGGCGTGCGAGGTCTTCCCGACGATGACGCCGTTGAACCTCCCGCCGCCGTTGTTGTTTGGAGCACGGAAAGACGATGACGGAAAAAGAGATCGTGGAATTACGTGCGCAGTCAAGTAACAACCGCGAAAAGTTGCGCGGAGGAGTTGTGTTTGTGGACGAAGTACCGAAGGTTTACCGGAAAACCTGACGCGAAGAAAAATCAGAGATCTCTATAAGGCCAAGAAGGCGGAAAGTCCAAATTTGTA

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SEQ ID NO:	De-scription	Sequence
74	Rous Sarcoma virus (RSV) promoter	GTAGTCTTATGCAATACTCTTGTAGTCTTGCAACATGGTAACGATGAGTTAGCAACATGCCTTACAAGGAGAGAAAAAGCACCGTGCATGCCGATTGGTGGAAAGTAAGGTGGTACGATCGTGCCCTATTAGGAAGGCAACAGACGGGTCTGACATGGATTGGACGAACCACTGAATTGCCGATTGCAGAGATATTGTATTTAAGTGCCTAGCTCGATACAATAAACG
75	5' Long terminal repeat (LTR)	GGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCCTTGAGTGCTTCAAGTAGTGTGTGCCGTCTGTGTGTGACTCTGGTAAGTAGATCCCTCAGACCCTTTTAGTCAAGTGTGAAAATCTCTAGCA
76	Psi Packaging signal	TACGCCAAAAATTTTACTAGCGGAGGCTAGAAGGAGAGAG
77	Rev response element (RRE)	AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGCGCAGCCTCAATGACGCTGACGGTACAGGCCAGACAATTATGTCTGGTATAGTGACGACGAGAACAAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTGC
78	Central poly purine tract (cPPT)	TTTTAAAAAGAAAGGGGGGATTGGGGGGTACAGTGCAGGGAAAGAAATAGTAGACATAATAGCAACAGACATACAACATAAGAAATTACAAAACAAATTACAAAATTCAAAATTTTA
79	Long WPRE sequence	AATCAACCTCTGATTACAAAATTTGTGAAGATTGACTGGTATCTTAACATATGTGCTCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTAGGCTTTCAATTTCTCCTCCTGTATAAATCCTGGTTGCTGTCTTTATGAGGAGTTGTGGCCGTTGTGAGCAACGTGGCGTGGTGTGCACTGTGTTTGTGACGCAACCCCACTGTGTTGGGCATTGCCACCACCTGTCAGCTCCTTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACATCAGCCGCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTGGGCACTGACAATCCGTGGTGTGTGCGGGAAATCATCGTCCCTTCCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGCTCCTTCTGCTACGTCCTTTCGGCCCTCATCCAGCGGACCTTCTTCCCGCGGCTGCTGCCGGCTCTGCGGCTCTTCCGCGTCTTTCGCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCCTCCCGCCT
80	3' delta LTR	TGGAAGGGCTAATTAACCTCCCAACGAAGATAAGATCTGCTTTTGTCTGTACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAGCCTCAATAAAGCTTGCCCTTGTGCTTCAAGTAGTGTGTGCCGCTCTGTTGTGACTCTGGTAAGTAGATCCCTCAGACCTTTTAGTCAAGTGTGGAATACTCTAGCAGTAGTAGTTTATGTCA
81	Envelope; MLV 10A1	ATGGAAGGTCCAGCGTTCTCAAAACCCCTTAAAGATAAGATTAAACCGTGGAAAGTCTTAATGGTCAATGGGGTCTATTTAAGAGTAGGATGGCAGAGAGCCCATCAGGTCTTTAATGTAACCTGGAGAGTCAACACCTGATGACTGGGCGTACCGCAATGCCACCTCCCTTTTAGGAAGTGTACAAGATGCCTTCCCAAGATTATATTTGTATCTATGTGATCTGGTCGGAGAA

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SEQ ID NO:	De-scription	Sequence
5		GAGTGGGACCCCTTCCAGACGAGGAACCATATGTCGGGTATGGCTGCAAAATACCCCGGAGGGAGAAAGCGGACCCGGACTTTTGACTTTTACGTGTGCCCTGGGCATACCGTAAAAATCGGGGTGTGGGGGGCCAAAGAGAGGGCTACTGTGGTGAATGGGGTTGTGAAACCACCGGACAGGCTTACTGGAAGCCACATCATCATGGGACCTAATCTCCCTTAAGCGCGGTAAACACCCCTGGGACAGGGATGCTCCAAAATGGCTTGTGGCCCTGCTACGACCTCTCCAAAGTATCCAATTCCTTCCAGGGGCTACTCGAGGGGCGAGATGCAACCTCTAGTCTTAGAATTCAGTATGCAGGAAAAAGGCTAATTTGGGACGGGCCCCAAATCGTGGGACTGAGACTGTACCGGACAGGAACAGATCCTATTACCATGTCTCCCTGACCCGCGAGGTCTCTAATATAGGGCCCGCATCCCCATTGGGCCATAATCCCGTGATCACTGGTCAACTACCCCCCTCCCGACCCGTGCAGATCAGGCTCCCGAGGCTCTTCAGCTCCTCTACAGGCGCAGCTCTATAGTCCCTGAGACTGCCCCACCTTCTCAACAACCTGGGACGGGAGACAGGCTGCTAAACCTGGTAGAAGGAGCTATCAGGCGCTTAACCTCACCAATCCCGACAGACCCCAAGATGTTGGCTGTGCTTAGTGTGGGACCTCCTATTACGAAGGAGTAGCGGTGCGTGGCAGCTTATACCAATCATCTACCGCCCGGCGAGCTGTACGGCCACTTCCCAACATAAGCTTACCCTATCTGAAGTGACAGGACAGGGCTATGCATGGGAGCACTACCTAAACTACAGGCCCTTATGTAAACACCCAAAGTGCCGGCTCAGGATCTACTACCTTGACGACCCCGCTGGAACATGTGGGCTGTAGCACTGGATTGACTCCCTGCTTGTGCCACCACGATGCTCAATCTAACACAGACTATTGTGTATTAGTTGAGCTCTGGCCAGAAATAATTTACCACTCCCCCGATTATATGTATGGTCAGCTTGAACAGCGTACCAAAATATAAGAGGGAGCCAGTATCGTTGACCTGGCCCTTCTGTAGGAGGATTAACCATGGGAGGATTGACAGCTGGAATAGGACAGGGACCACTGCCCTAATCAAAACCCAGCAGTTTGTAGCAGCTTACGCCGCTATCCAGACAGCTCAACGAAGTCAAAAAATCAATTACCAACCTAGAAAGTCACTGACCTCGTTGTCTGAAGTAGTCTCAGAACCGAAGAGGCTAGATTGCTCTTCTCAAAAGAGGGAGGTCTCTGCGAGCCCTAAAAGAAAGATGTGTTTTATGCAGACACACGGGACTAGTGAGAGACAGCATGGCCAAACTAAGGAAAGGCTAATCAGAGACAAAACTATTGAGTCAGGCCAAGGTTGGTTTCAAGGGGAGTTAATAGATCCCCCTGGTTTACCACCTAATCTCCACCATCAGGACCTCTAATAGTACTCTACTGATCTTACTCTTGGACCCCTGCATTCTCAATCGATTGGTCCAATTTGTTAAAGACAGGATCTCAGTGGTCCAGGCTCTGGTTTTGACTCAACAATATCACCAGCTAAAACCTATAGAGTACGAGCCATGA
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35		
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50		
82	miR155 CD47 target sequence #1	CCTGGAGGCTTGTGAAGGCTGTATGCTGTTATCCATCTTCAAAGAGGCAGTTTGGCCACTGACTGACTGCCTCTTAAGATGGATAACAGGACCAAGGCTGTGTACTAGACTCA
83	miR21 cMyc target sequence	CATCTCCATGGCTGTACCACCTTGTGGGTGTTCCGCTCTTGACATTCTCCTGTTGAATCTCATGGAGAATGTCAAGGGCGAACACTGACATTTTGGTATCTTTTACTGACCA

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

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<211> LENGTH: 53

<212> TYPE: DNA

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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
<211> LENGTH: 53
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: FDPS shRNA sequence #3

<400> SEQUENCE: 3

gccatgtaca tggcaggaat tctcgagaat tcctgccatg tacatggctt ttt 53

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

<400> SEQUENCE: 4

gcagaaggag gctgagaaaag tctcgagact ttctcagcct cttctgctt ttt 53

<210> SEQ ID NO 5
<211> LENGTH: 49
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CD47 shRNA sequence #1

<400> SEQUENCE: 5

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<210> SEQ ID NO 6
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CD47 shRNA sequence #2

<400> SEQUENCE: 6

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CD47 shRNA sequence #3

<400> SEQUENCE: 7

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<210> SEQ ID NO 8
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<212> TYPE: DNA
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<400> SEQUENCE: 8
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<210> SEQ ID NO 9
<211> LENGTH: 53
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CD47 shRNA sequence #5

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<210> SEQ ID NO 10
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: cMyc shRNA sequence #1

<400> SEQUENCE: 10
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: cMyc shRNA sequence #2

<400> SEQUENCE: 11
gcgaacacac aacgtcttgg actcgagtcc aagacgttgt gtgttcgctt tt 52

<210> SEQ ID NO 12
<211> LENGTH: 53
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: cMyc shRNA sequence #3

<400> SEQUENCE: 12
gacatggtga accagagttt cctcgaggaa actctgggtc accatgtctt ttt 53

<210> SEQ ID NO 13
<211> LENGTH: 53
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: cMyc shRNA sequence #4

<400> SEQUENCE: 13
gagaatgtca agaggcgaac actcgagtgt tcgcctcttg acattctctt ttt 53

<210> SEQ ID NO 14
<211> LENGTH: 53
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: cMyc shRNA sequence #5

<400> SEQUENCE: 14

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<210> SEQ ID NO 15

<211> LENGTH: 217

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: H1 promoter

<400> SEQUENCE: 15

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caccagcgcg gcgtgcgccc tggcaggaag atggctgtga gggacagggg agtggcgccc 120

tgcaatatatt gcattgtcgt atgtgtttctg ggaaatcacc ataaacgtga aatgtctttg 180

gatttgggaa tcttataagt tctgtatgag accactt 217

<210> SEQ ID NO 16

<211> LENGTH: 249

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: U6 promoter

<400> SEQUENCE: 16

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ataattggaa ttaatttgac tgtaaacaca aagatattag taaaaatac gtgacgtaga 120

aagtaataat ttcttgggta gtttgcagtt taaaattat gttttaaaat ggactatcat 180

atgcttaccg taacttgaaa gtatttcgat ttcttggctt tatatatctt gtggaaagga 240

cgaaacacc 249

<210> SEQ ID NO 17

<211> LENGTH: 243

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: 7SK promoter

<400> SEQUENCE: 17

ctgcagtatt tagcatgcc caccatctg caaggcattc tggatagtgt caaacagcc 60

ggaaatcaag tccgtttatc tcaaaactta gcattttggg aataaatgat atttgctatg 120

ctgggttaaat tagatttttag ttaaatttcc tgctgaagct ctagtacgat aagcaacttg 180

acctaaagtgt aaagttgaga ttcccttcag gtttatatag cttgtgcgcc gcctggctac 240

ctc 243

<210> SEQ ID NO 18

<211> LENGTH: 352

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: CAG enhancer

<400> SEQUENCE: 18

tagttattaa tagtaatcaa ttacgggggc attagtcat agcccatata tggagttccg 60

cgttacataa cttacggtaa atggcccgcc tggctgaccg cccaacgacc ccgcccatt 120

gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca 180

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atgggtggac tatttacggt aaactgccca cttggcagta catcaagtgt atcatatgcc 240
aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgccagta 300
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<210> SEQ ID NO 19
<211> LENGTH: 290
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CAG promoter

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<400> SEQUENCE: 19

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gctattacca tgggtcgagg tgagcccccac gttctgcttc actctcccca tctccccccc 60
ctcccccccc ccaattttgt atttatttat tttttaatta ttttgtgcag cgatgggggc 120
gggggggggg ggggcgcgcg ccaggcgggg cggggcgggg cgaggggcgg ggcggggcga 180
ggcggagagg tgcggcgcca gccaatcaga gcggcgcgct ccgaaagtct ccttttatgg 240
cgagggcgcg gcggcgggcg cctataaaaa agcgaagcgc gcggcgggcg 290

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

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<400> SEQUENCE: 20

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ggagtgcgtg cgttgcttc gccccgtgcc ccgctccgcg ccgcctcgcg ccgcccgccc 60
cggctctgac tgaccgcgtt actccacag gtgagcgggc gggacggccc ttctcctccg 120
ggctgtaatt agcgttgggt ttaatgacgg ctcgtttctt ttctgtggct gcgtgaaagc 180
cttaaagggc tccgggaggg ccttttgtgc gggggggagc ggctcggggg gtgcgtgcgt 240
gtgtgtgtgc gtggggagcg ccgcgtgcgg ccgcgcgtgc ccggcggtcg tgagcgtgc 300
gggcgcggcg cggggctttg tgcgtccgc gtgtgcgcga ggggagcgcg gccgggggcg 360
gtgcccccg gtgcgggggg gctgcgaggg gaacaaagcg tgctgcggg gtgtgtgcgt 420
gggggggtga gcaggggggt tgggcgcggc ggtcgggctg taaccccccc ctgcaccccc 480
ctccccgagt tgctgagcac ggcgcggctt cgggtgcggg gctccgtcg ggcgtggcg 540
cggggctcgc cgtgccgggc ggggggtggc ggcaggtggg ggtgcgggc ggggcggggc 600
cgccctcggc cggggagggc tcgggggagg ggcgcggcg ccccgagcg ccggcggctg 660
tcgaggcgcg gcgagccga gccattgcct tttatggtaa tcgtgcgaga gggcgaggg 720
acttcctttg tcccaaatct ggcgagcg aaatctggga ggcgcgcgc cccccctct 780
agcgggcgcg ggcgaagcgg tcggcgccg gcaggaagga aatgggcggg gagggccttc 840
gtgcgtcgcc gcgcgcgct ccccttctcc atctccagcc tcggggctgc cgcaggggga 900
cggctgcctt cgggggggac ggggcagggc ggggttcggc ttctggcggtg tgaccggcg 960

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<210> SEQ ID NO 21
<211> LENGTH: 1503
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: HIV gag

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<400> SEQUENCE: 21

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atgggtgcga gagcgtcagt attaagcggg ggagaattag atcgatggga aaaaattcgg	60
ttaaggccag ggggaaagaa aaaatataaa ttaaacata tagtatgggc aagcagggag	120
ctagaacgat tcgcagttaa tctggcctg ttagaaacat cagaaggctg tagacaaata	180
ctgggacagc tacaaccatc ccttcagaca ggatcagaag aacttagatc attatataat	240
acagtagcaa ccctctattg tgtgcatcaa aggatagaga taaaagacac caaggaagct	300
ttagacaaga tagaggaaga gcaaaacaaa agtaagaaaa aagcacagca agcagcagct	360
gacacaggac acagcaatca ggtcagccaa aattacccta tagtgcagaa catccagggg	420
caaatggtac atcaggccat atcacctaga actttaaatg catgggtaaa agtagtagaa	480
gagaaggctt tcagcccaga agtgataccc atgttttcag cattatcaga aggagccacc	540
ccacaagatt taaacaccat gctaaacaca gtggggggac atcaagcagc catgcaaatg	600
ttaaagaga ccatcaatga ggaagctgca gaatgggata gagtgcattc agtgcattgca	660
gggcctattg caccaggcca gatgagagaa ccaaggggaa gtgacatagc aggaactact	720
agtacccttc aggaacaaat aggatggatg acacataatc cacctatccc agtaggagaa	780
atctataaaa gatggataat cctgggatta aataaaatag taagaatgta tagccctacc	840
agcattcttg acataagaca aggaccaaag gaacccttta gagactatgt agaccgattc	900
tataaaactc taagagccga gcaagcttca caagaggtaa aaaattggat gacagaaacc	960
ttgttggtcc aaaatgcgaa ccagattgt aagactattt taaaagcatt gggaccaggga	1020
gagacactag aagaaatgat gacagcatgt caggagtggt ggggacccgg ccataaagca	1080
agagtttttg ctgaagcaat gagccaagta acaaatccag ctaccataat gatacagaaa	1140
ggcaatttta ggaaccaaag aaagactgtt aagtgtttca attgtggcaa agaagggcac	1200
atagccaaaa attgcagggc ccttaggaaa aagggtgtgt ggaaatgttg aaaggaagga	1260
caccaaataa aagattgtac tgagagacag gctaattttt tagggaagat ctggccttcc	1320
cacaagggaa ggccagggaa ttttcttcag agcagaccag agccaacagc cccaccagaa	1380
gagagcttca ggtttgggga agagacaaca actccctctc agaagcagga gccgatagac	1440
aaggaaactgt atccttttagc ttcccctaga tcactctttg gcagcgaccc ctctgcacaa	1500
taa	1503

<210> SEQ ID NO 22

<211> LENGTH: 1872

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: HIV Pol

<400> SEQUENCE: 22

atgaatttgc caggaagatg gaaacccaaa atgatagggg gaattggagg ttttatcaaa	60
gtaggacagt atgatcagat actcatagaa atctgcggac ataaagctat aggtacagta	120
ttagtaggac ctacacctgt caacataatt ggaagaaatc tgttgactca gattggctgc	180
actttaaat ttccatttag 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 taaacagaa aaaatcagta acagtactgg atgtggcgca tgcataat	540

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tcagttccct tagataaaga cttcaggaag tatactgcat ttaccatacc tagtataaac	600
aatgagacac cagggattag atatcagtac aatgtgcttc cacagggatg gaaaggatca	660
ccagcaatat tccagtgtag catgacaaaa atcttagagc cttttagaaa acaaaatcca	720
gacatagtca tctatcaata catggatgat ttgtatgtag gatctgactt agaaataggg	780
cagcatagaa caaaaataga ggaactgaga caacatctgt tgaggtgggg atttaccaca	840
ccagacaaaa aacatcagaa agaacctcca ttcccttgga tgggttatga actccatcct	900
gataaatgga cagtacagcc tatagtgtctg ccagaaaagg acagctggac tgtcaatgac	960
atacagaaat tagtgggaaa attgaattgg gcaagtcaga tttatgcagg gattaaagta	1020
aggcaattat gtaaaactct 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 gggaaatcatt caagcacaac cagataagag tgaatcagag	1740
ttagtcagtc aaataataga gcagttaata aaaaaggaaa aagtctacct ggcatgggta	1800
ccagcacaca aaggaattgg aggaaatgaa caagtagatg gggttggtcag tgctggaatc	1860
aggaaagtac ta	1872

<210> SEQ ID NO 23

<211> LENGTH: 867

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: HIV Int

<400> SEQUENCE: 23

tttttagatg gaatagataa ggccaagaa gaacatgaga aatatcacag taattggaga	60
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gataaatgtc agctaaaagg ggaagccatg catggacaag tagactgtag ccaggaata	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 tgggtggcgg ggatcaagca ggaatttggc	420
attccctaca atcccaaaag tcaaggagta atagaatcta tgaataaaga attaaagaaa	480
attataggac aggtaagaga tcaggctgaa catcttaaga cagcagtaca aatggcagta	540
ttcatccaca attttaaaag aaaagggggg attggggggg acagtgcagg ggaaagaata	600
gtagacataa tagcaacaga cataaaaact aaagaattac aaaaacaaat tacaaaaatt	660

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caaaattttc gggtttatta cagggacagc agagatccag tttggaaagg accagcaaag	720
ctcctctgga aaggtgaagg ggcagtagta atacaagata atagtgcacat 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

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gctgagggct attgagggcg aacagcatct gttgcaactc acagtctggg gcatcaagca	180
gctccaggca agaatcctgg ctgtggaaag atacctaaag gatcaacagc tcct	234

<210> SEQ ID NO 25
 <211> LENGTH: 351
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: HIV Rev

<400> SEQUENCE: 25

atggcaggaa gaagcggaga cagcgacgaa gaactcctca aggcagtcag actcatcaag	60
tttctctatc aaagcaaccc acctcccaat cccgagggga cccgacaggc ccgaaggaa	120
agaagaagaa ggtggagaga gagacagaga cagatccatt cgattagtga acggatcctt	180
agcacttata tgggacgata tgcggagcct gtgcctcttc agctaccacc gcttgagaga	240
cttactcttg attgtaacga ggattgtgga acttctggga cgcagggggg gggaaagcct	300
caaatattgg tggaaatctc tacaatattg gagtcaggag ctaaagaata g	351

<210> SEQ ID NO 26
 <211> LENGTH: 448
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: rabbit beta globin poly A

<400> SEQUENCE: 26

agatcttttt ccctctgcca aaaattatgg ggacatcatg aagccccttg agcatctgac	60
ttctggctaa taaaggaaat ttattttcat tgcaatagtg tgttgaatt ttttgtgtct	120
ctcactcgga aggacatatg ggagggcaaa tcatttaaaa catcagaatg agtatttggt	180
ttagagtttg gcaacatatg ccatatgctg gctgccatga acaaagggtg ctataaagag	240
gtcatcagta tatgaaacag cccctgctg tccattcctt attccataga aaagccttga	300
cttgagggtta gatttttttt atattttgtt ttgtgttatt ttttcttta acatccctaa	360
aattttcctt acatgtttta ctagccagat ttttctcct ctctgacta ctcccagtca	420
tagctgtccc tcttctctta tgaagatc	448

<210> SEQ ID NO 27
 <211> LENGTH: 577
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence

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<220> FEATURE:

<223> OTHER INFORMATION: CMV Promoter

<400> SEQUENCE: 27

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acattgatta ttgactagtt attaatagta atcaattacg gggtcattag ttcataagccc    60
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cgacccccgc ccattgacgt caataatgac gtatgttccc atagtaacgc caatagggac    180
tttcattga cgtcaatggg tggagtattt acggtaaaact gcccacttgg cagtacatca    240
agtgtatcat atgccaagta cgcgccctat tgacgtcaat gacggtaaat ggccgcctg    300
gcattatgcc cagtacatga ccttatggga ctttcctact tggcagtaca tctacgtatt    360
agtcacgctc attaccatgg tgatgcgggt ttggcagtag atcaatgggc gtggatagcg    420
gtttgactca cggggatttc caagtctcca cccattgac gtcaatggga gttgttttg    480
gcacaaaaat caacgggact ttccaaaatg tcgtaacaac tccgcccac tgacgcaaat    540
gggcggtagg cgtgtacggg gggaggtcta tataagc                                577

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<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 ttgttcttcc ttttctgcta ttgtaaaatt catgttatat    60
ggagggggca aagttttcag ggtgtgtgtt agaatgggaa gatgtccctt gtatcaccat    120
ggaccctcat gataattttg tttctttcac tttctactct gttgacaacc attgtctcct    180
cttattttct tttcattttc tgtaactttt tcgttaaaact ttagcttgca ttgtaacga    240
atttttaaat tcacttttgt ttatttgtca gattgtaagt actttctcta atcacttttt    300
tttcaaggca atcaggggat attatatgtt acttcagcac agtttttagag aacaattgtt    360
ataattaaat gataaggtag aatattttct catataaaatt ctggctggcg tggaaatatt    420
cttatttgta gaaacaacta caccctggtc atcactctgc ctttctcttt atggttacaa    480
tgatatacac tgtttgagat gaggataaaa tactctgagt ccaaaccggg cccctctgct    540
aaccatgttc atgccttctt ctcttttcta cag                                573

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<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
tattgcccggt 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 cctcctcaa agttgtggat atgcaactgt gacggatgcc    420

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gaagcagtga ttgtccaggt gactctcac catgtgctgg ttgatgaata cacaggagaa	480
tgggttgatt cacagttcat caacggaaaa tgcagcaatt acatatgcc cactgtccat	540
aactctacaa cctggcattc tgactataag gtcaaagggc tatgtgattc taacctcatt	600
tccatggaca tcaccttctt ctacaggagc ggagagctat catccctggg aaaggagggc	660
acagggttca gaagtaacta ctttgcttat gaaactggag gcaaggcctg caaaatgcaa	720
tactgcaagc attggggagt cagactccca tcaggtgtct ggctcgagat ggctgataag	780
gatctctttg ctgcagccag attccctgaa tgcccagaag ggtcaagtat ctctgtcca	840
tctcagacct cagtggatgt aagtctaatt caggacgttg agaggatctt ggattattcc	900
ctctgccaa gaaactggag caaaatcaga gcgggtcttc caatctctcc agtggatctc	960
agctatcttg ctctaaaaa cccaggaacc ggtcctgctt tcaccataat caatggtacc	1020
ctaaaatact ttgagaccag atacatcaga gtcgatattg ctgctccaat cctctcaaga	1080
atggtcggaa tgatcagtgg aactaccaca gaaagggaa tgtgggatga ctgggcacca	1140
tatgaagacg tggaaattgg acccaatgga gttctgagga ccagttcagg atataagttt	1200
cctttataca tgattggaca tggatgtgtg gactcogac ttcatcttag ctcaaaggct	1260
cagggtgttg aacatctca cattcaagac gctgcttcgc aacttctga tgatgagagt	1320
ttattttttg gtgatactgg gctatccaaa aatccaatcg agctttaga aggttggttc	1380
agtagttgga aaagctctat tgctctttt ttctttatca tagggttaat cattggacta	1440
ttcttggttc tccagattgg 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
ttctggctaa taaaggaaat ttattttcat tgcaatagtg tgttggaatt ttttgtgtct	120
ctcactcgga aggacatag ggagggcaaa tcatttaaaa catcagaatg agtatttggt	180
ttagagtttg gcaacatag cccatagct ggctgccatg aacaaagggt ggctataaag	240
aggatcatag tatatgaac agcccctgc tgtccattcc ttattccata gaaaagcctt	300
gacttgaggt tagatttttt ttatattttg ttttgtgtta ttttttctt 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
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<210> SEQ ID NO 32
 <211> LENGTH: 36

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<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 tagggggaat tggaggtttt 60
 atcaaagtaa gacagtatga tcagatactc atagaaatct gcggacataa agctataggt 120
 acagtattag taggacctac acctgtcaac ataattggaa gaaatctgtt gactcagatt 180
 ggctgcactt taaattttcc cattagtctt attgagactg taccagtaaa attaaagcca 240
 ggaatggatg gcccaaaagt taaacaatgg ccattgacag aagaaaaaat aaaagcatta 300
 gtagaaattt gtacagaaat ggaaaaggaa ggaaaaattt caaaaattgg gcctgaaaat 360
 ccatacaata ctccagtatt tgccataaag aaaaagaca gtactaaatg gagaaaatta 420
 gtagatttca gagaacttaa taagagaact caagatttct gggaagtcca attaggaata 480
 ccacatcctg cagggttaaa acagaaaaaa tcagtaacag tactggatgt gggcgatgca 540
 tatttttctg ttcccttaga taaagacttc aggaagtata ctgcatttac catacctagt 600
 ataaacaatg agacaccagg gattagatat cagtacaatg tgcttcaca gggatggaaa 660
 ggatcaccag caattatcca gtgtagcatg acaaaaatct tagagccttt tagaaaacaa 720
 aatccagaca tagtcatcta tcaatacatg gatgatttgt atgtaggatc tgacttagaa 780
 atagggcagc atagaacaaa aatagaggaa ctgagacaac atctgttgag gtggggattt 840
 accacaccag acaaaaaaca tcagaagaa cctccattcc tttggatggg ttatgaactc 900
 catcctgata aatggacagt acagcctata gtgctgccag aaaaggacag ctggactgtc 960
 aatgacatac agaaattagt gggaaaattg aattgggcaa gtcagattta tgcagggtt 1020
 aaagtaaggc aattatgtaa acttcttagg ggaaccaaag cactaacaga agtagtacca 1080
 ctaacagaag aagcagagct agaactggca gaaaacaggg agattctaaa agaaccggt 1140
 catggagtgt attatgacct atcaaaagac ttaatagcag aaatacagaa gcaggggcaa 1200
 ggccaatgga catatcaaat ttatcaagag ccatttaaaa atctgaaaac aggaaagtat 1260
 gcaagaatga aggggtgcca cactaatgat gtgaaacaat taacagaggc agtacaaaaa 1320
 atagccacag aaagcatagt aatatgggga aagactccta aatttaaatt acccatataa 1380
 aaggaaacat gggaagcatg gtggacagag tattggcaag ccacctggat tcctgagtgg 1440
 gagtttgta ataccctcc cttagtgaag ttatggtacc agttagagaa agaaccata 1500
 ataggagcag aaactttcta ttagatggg gcagccaata gggaaactaa attaggaaaa 1560
 gcaggatatg taactgacag aggaagacaa aaagttgtcc ccctaacgga cacaacaaat 1620
 cagaagactg agttacaagc aattcatcta gctttgcagg attcgggatt agaagtaaac 1680
 atagtgcag actcacaata tgcattggga atcattcaag cacaaccaga taagagtga 1740
 tcagagttag tcagtcaaat aatagagcag ttaataaaaa aggaaaaagt ctacctggca 1800

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tgggtaccag cacacaaagg aattggagga aatgaacaag tagataaatt ggtcagtgtc	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 catacttctt cttaaaatta gcaggaagat ggccagtaaa aacagtacat	2220
acagacaatg gcagcaatct caccagtact acagttaagg ccgcctgttg gtgggcgggg	2280
atcaagcagg aatttggcat tccttacaat ccccaaagtc aaggagtaat agaattctatg	2340
aataaagaat taaagaaaat tataggacag gtaagagatc aggtgaaca tcttaagaca	2400
gcagtacaaa tggcagtatt catccacaat tttaaaagaa aaggggggat tgggggggtac	2460
agtgccaggg aaagaatagt agacataata gcaacagaca taaaactaa agaattacaa	2520
aaacaaatta caaaaattca aaattttcgg gtttattaca gggacagcag agatccagtt	2580
tggaaaggac cagcaaagct cctctggaaa ggtgaagggg cagtagtaat acaagataat	2640
agtgacataa aagtagtgcc aagaagaaaa gcaaagatca tcagggatta tggaaaacag	2700
atggcaggtg atgatttgtg 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 caaccacct cccaatcccg aggggacccg acaggcccga	120
aggaatagaa gaagaagggtg gagagagaga cagagacaga tccattcgat tagtgaacgg	180
atccttggca cttatctggg acgatctgag gagcctgtgc ctcttcagct accaccgctt	240
gagagactta ctcttgattg taacgaggat tgtggaactt ctgggacgca gggggtggga	300
agccctcaaa tattggtgga atctcctaca atattggagt caggagctaa agaatagagg	360
agctttgttc cttgggttct tgggagcagc aggaagcact atgggcgcag cgtcaatgac	420
gctgacggta caggccagac aattattgtc tggatatagt cagcagcaga acaatttgct	480
gagggtctatt gaggcgcaac agcatctgtt gcaactcaca gtctggggca tcaagcagct	540
ccaggcaaga atcctggctg tggaaagata cctaaaggat caacagctcc tagatctttt	600
tccctctgcc aaaaattatg gggacatcat gaagcccctt gagcatctga cttctggcta	660
ataaagggaaa tttattttca ttgcaatagt gtgttggaat tttttgtgct tctcactcgg	720
aaggacatat gggaggggcaa atcatttaaa acatcagaat gagtatttgg tttagagttt	780
ggcaacatat gccatatgct ggctgccatg aacaaagggt gctataaaga ggtcatcagt	840
atatgaaaca gccccctgct gtccattcct tattccatag aaaagccttg acttgaggtt	900
agattttttt tatattttgt tttgtgttat tttttctttt aacatcccta aaattttcct	960
tacatgtttt actagccaga tttttcctcc tctcctgact actcccagtc atagctgtcc	1020
ctcttctctt atgaagatcc ctgcacctgc agcccaagct tggcgtaatc atggtcatag	1080

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ctgtttctctg tgtgaaattg ttatccgctc acaattccac acaacatacg agccggaagc	1140
ataaagtgtg aagcctgggg tgccaatga gtgagctaac tcacattaat tgcgttgccg	1200
tcaactgccg ctttccagtc gggaaacctg tcgtgccagc ggatccgcac ctcaattagt	1260
cagcaaccat agtcccgcgc ctaactccgc ccatccgcgc cctaactccg cccagttccg	1320
cccattctcc gccccatggc tgactaatgt tttttattta tgcagaggcc gagggccgct	1380
cggcctctga gctattccag aagtagtgag gaggcttttt tggaggccta ggcttttgca	1440
aaaagctaac ttgtttattg cagcttataa tggttacaaa taaagcaata gcatcacaaa	1500
tttcacaaat aaagcatttt ttctactgca ttctagttgt ggtttgtcca aactcatcaa	1560
tgatctttat 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
gttcgcggtt acataactta cggtaaatgg cccgcctggc tgaccgccca acgacccccg	120
cccattgacg tcaataatga cgtatgttcc catagtaacg ccaataggga ctttccattg	180
acgtcaatgg gtggactatt tacggtaaac tgcccacttg gcagtacatc aagtgtatca	240
tatgccaaagt acgcccccta ttgacgtcaa tgacggtaaa tggcccgctt ggcattatgc	300
ccagtacatg accttatggg actttccctac ttggcagtag atctacgtat tagtcatcgc	360
tattaccatg ggtcgagggt agccccacgt tctgcttcac tctccccatc tccccccct	420
ccccaccccc aattttgtat ttattttatt tttaattatt ttgtgcagcg atggggcgcg	480
gggggggggg ggcgcgcgcgc aggcggggcg ggcggggcg aggggcgggg cggggcgagg	540
cggagagggt cggcggcagc caatcagagc ggcgcgctcc gaaagtttcc ttttatggcg	600
agggcgcggc ggcggcggcgc ctataaaaag cgaagcgcgc ggcgggcggg agtcgctgcg	660
ttgccttcgc cccgtgcccc gctccgcgcgc gcctcgcgcgc gcccgccccg gctctgactg	720
accgcgttac tcccacaggt gagcggggcg gacggccctt ctctccggg ctgtaattag	780
cgttggtgtt aatgacggct cgtttctttt ctgtggctgc gtgaaagcct taaagggctc	840
cgggaggggc ctttgtgcgg gggggagcgg ctcggggggt gcgtgcgtgt gtgtgtgcgt	900
ggggagcgcc gcgtgcggcc cgcgctgccc ggcgctgtg agcgtgcgg gcgcggcgcg	960
gggcttttgt cgctcccgct gtgcgcgagg ggagcgcggc cgggggcggg gcccccggt	1020
gcgggggggc tgcgagggga acaaaggctg cgtgcggggt gtgtgcgtgg gggggtgagc	1080
agggggtgtg ggcgcggcgg tcgggctgta accccccctt gcacccccct ccccgagttg	1140
ctgagcacgg cccggcttcg ggtgcggggc tccgtgcggg gcgtggcgcg gggctcgccg	1200
tgccggggcg ggggtggcgg caggtggggg tgccggggcg ggcggggcgc cctcgggcgc	1260
gggagggctc gggggagggg cgcggcgccc ccggagcgcc ggcggctgtc gaggcgcggc	1320
gagccgcagc cattgccttt tatggtaatc gtgcgagagg gcgcagggac ttctttgtc	1380
ccaaatctgg cggagccgaa atctgggagg cgcgcgcgca cccctctag cgggcgcggg	1440
cgaagcgggt cggcgcgggc aggaaggaaa tgggcgggga gggccttcgt gcgtcgccgc	1500

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gccgcgctcc ccttctccat ctccagctc ggggctgccg cagggggacg gctgccttcg 1560
gggggggacgg ggcaggggcg ggttcggctt ctggcggtgtg accggcgga attc 1614

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<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

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<400> SEQUENCE: 36
caattgcgat gtacgggcca gatatacgcg tatctgaggg gactaggggtg tgtttaggcg 60
aaaagcgggg cttegggtgt acgcgggttag gagtccctc aggatatagt agtttcgctt 120
ttgcataggg agggggaaat gtagtcttat gcaatacact ttagtcttg caacatggta 180
acgatgagtt agcaacatgc cttacaagga gagaaaaagc accgtgcatg ccgattgggtg 240
gaagtaaggt ggtacgatcg tgccttatta ggaaggcaac agacaggtct gacatggatt 300
ggacgaacca ctgaattccg cattgcagag ataattgtat ttaagtgcct agctcgatac 360
aataaacgcc atttgacatc tcaccacatt ggtgtgcacc tccaagctcg agctcgttta 420
gtgaaccgtc agatcgctcg gagacgcat ccacgtggtt ttgacctcca tagaagacac 480
cgggaccgat ccagcctccc ctgcaagcta gcgattagcg atctcctatg gcaggaagaa 540
gcggagacag cgacgaagaa ctctcaagg cagtcagact catcaagttt ctctatcaaa 600
gcaaccaccc tccaatccc gaggggaccc gacaggcccg aaggaataga agaagaaggt 660
ggagagagag acagagacag atccattcga ttagtgaacg gatccttagc acttatctgg 720
gacgatctgc ggagcctgtg cctcttcagc taccaccgct tgagagactt actcttgatt 780
gtaacgagga ttgtggaact tctgggacgc aggggggtggg aagccctcaa atattgggtgg 840
aatctcttac aatattggag tcaggagcta aagaatagtc taga 884

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<210> SEQ ID NO 37
<211> LENGTH: 1104
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Elongation Factor-1 alpha (EF1-alpha) promoter

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<400> SEQUENCE: 37
ccggtgccta gagaaggtgg cgcggggtaa actgggaaag tgatgtcgtg tactggctcc 60
gcctttttcc cgagggtggg ggagaaccgt atataagtgc agtagtcgcc gtgaacgttc 120
tttttcgcaa cgggtttgcc gccagaacac aggtaagtgc cgtgtgtggt tcccgcgggc 180
ctggcctctt tacgggttat ggcccttcgc tgccttgaat tacttccacg cccctggctg 240
cagtacgtga ttcttgatcc cgagcttcgg gttggaagtg ggtgggagag ttcgaggcct 300
tgcgcttaag gagcccttc gcctcgtgct tgagttgagg cctggcctgg gcgctggggc 360
cgccgcgtgc gaatctgggt gcaccttcgc gcctgtctcg ctgctttcga taagtctcta 420
gccatttaaa atttttgatg acctgctgcg acgctttttt tctggcaaga tagtcttgta 480
aatgcgggcc aagatctgca cactggtatt tcggtttttg gggccgcggg cggcgacggg 540
gcccgtgcgt cccagcgcac atgttcggcg agggggggcc tgcgagcgcg gccaccgaga 600
atcggaaggg ggtagtctca agctggccgg cctgctctgg tgctggcct cgcgcggccg 660
tgtatcgccc cgccctgggc ggcaaggctg gcccggtcgg caccagttgc gtgagcgga 720
agatggccgc tcccggccc tgctgcaggg agctcaaaat ggaggacgag gcgctcgga 780

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gagcgggagg gtagtcacc cacacaaagg aaaagggcct ttcgctcctc agcgcgcgt	840
tcatgtgact ccacggagta ccggggcgccg tccaggcacc tcgattagtt ctgcagcttt	900
tggagtacgt cgtcttttagg ttgggggggag ggggttttatg cgatggagtt tccccacact	960
gagtgggtgg agactgaagt taggccagct tggcacttga tgtaattctc cttggaattt	1020
gccctttttg agtttgatc ttggttcatt ctcaagcctc agacagtggg 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

gggggtgggg ttgcgccttt tccaaggcag ccttgggttt gcgcaggac gcggctgctc	60
tgggcgtggt tccgggaaac gcagcggcgc cgaccctggg tctcgacat tcttcacgtc	120
cgttcgcagc gtcaccggga tcttcgcgc tacccttggt gggcccccg cgacgcttcc	180
tgctccgccc ctaagtggg aaggttcctt gcggttcgcg gcgtgccga cgtgacaaac	240
ggaagccgca cgtctcacta gtaccctgc agacggacag cggcaggag caatggcagc	300
gcgccgaccg cgatgggctg tggccaatag cggctgctca gcaggcgcg ccgagagcag	360
cggccgggaa ggggcggtgc gggaggcggg gtgtggggcg gtagtggtgg ccctgttcct	420
gcccgcgcgg tgttcgcgat 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

gcgcggggtt ttggcgctc ccgcggggcg cccctcctc acggcgagcg ctgccacgtc	60
agacgaaggg cgcaggagcg ttctgatcc ttcgccccg acgtcagga cagcgccccg	120
ctgctcataa gactcggcct tagaacccca gtatcagcag aaggacattt taggacggga	180
cttgggtgac tctagggcac tggttttctt tccagagagc ggaacaggcg aggaaaagta	240
gtcccttctc ggcgattctg cggaggatc tccgtggggc ggtgaacgcc gatgattata	300
taaggacgcg ccgggtgtgg cacagctagt tccgtcgag ccgggatttg ggtcgcggtt	360
cttgtttggt gatcgctgtg atcgtcactt ggtgagttgc gggctgctgg gctggccggg	420
gctttcgtgg ccgcggggcc gctcgggtgg acggaagcgt gtggagagac cgccaagggc	480
tgtagtctgg gtccgcgagc aaggttgccc tgaactgggg gttgggggga gcgcacaaaa	540
tggcggtgtg tcccgagtct tgaatggaag acgcttgtaa ggcgggctgt gaggtcgttg	600
aaacaaggtg gggggcatgg tggggcgcaa gaacccaagg tcttgaggcc ttcgctaata	660
cgggaaagct cttattcggg tgagatgggc tggggcacca tctggggacc ctgacgtgaa	720
gtttgtcact gactggagaa ctccgggttg tcgtctggtt gcggggcgcg cagttatgcg	780
gtgccgttgg gcagtgcacc cgtaccttg ggagcgcgcg cctcgtcgtg tcgtgacgtc	840

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acccgttctg ttgcttata atgcagggtg gggccacctg ccggtagggtg tgcggtaggc	900
ttttctccgt cgcaggacgc aggggttcggg cctagggtag gctctcctga atcgacaggc	960
gccggacctc tggtagggg agggataagt gaggcgtcag tttctttggt cggttttatg	1020
tacctatctt ctttaagtagc 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 tcaactgcatt ctagtgtggg ttgtccaaa 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 tccccgtgc ctctctgac	60
cctggaaggt gccactccca ctgtccttcc ctaataaaat gaggaattg catcgcatg	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:
 <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 ttgccaggc	180
aagacggcct acttaatgac caacaaaaaa tggaaatgca gagtcaactcc aaaaaatctc	240
acccctagcg ggggagaact ccagaactgc ccctgtaaca ctttccagga ctcgatgcac	300
agttcttggt atactgaata ccggcaatgc agggcgaata ataagacata ctacacggcc	360
accttgctta aaatacggtc tgggagcctc aacgaggtag agatattaca aaaccccaat	420
cagctcctac agtccccttg tagggctctc ataaatcagc ccgtttgctg gagtgcacac	480
gccccatcc atactccga tggtaggagga cccctcgata ctaagagagt gtggacagtc	540
caaaaaaggc tagaacaat tcataaggct atgcacactg aacttcaata ccacccctta	600
gccctgccca aagtcagaga tgaccttagc cttgatgcac ggacttttga tctctgaat	660
accactttta gggtactcca gatgtccaat tttagccttg cccaagattg ttggctctgt	720
ttaaaactag gtacccctac ccctcttgcg ataccactc cctctttaac ctactcccta	780

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gcagactccc tagcgaatgc ctctgtcag attatacctc cctcttgggt tcaaccgatg      840
cagttctcca actcgtcctg tttatcttcc cctttcatta acgatacggg acaaatagac      900
ttaggtgcag tcacctttac taactgcacc tctgtagcca atgtcagtag tcctttatgt      960
gccctaaaacg ggtcagtcctt cctctgtgga aataacatgg catacaccta tttaccccaa    1020
aactggacag gactttgcgt ccaagcctcc ctccctcccg 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 actggacctc ctaacggcag aacaaggagg aatttgttta    1380
gccttacaag aaaaatgctg tttttatgct aacaagtcag gaattgtgag aaacaaaata    1440
agaaccctac aagaagaatt acaaaaacgc agggaaagcc tggcatccaa ccctctctgg    1500
accgggctgc agggctttct tccgtacctc ctacctctcc tgggacctcc actcaccctc    1560
ctactcatac taaccattgg gccatgcgtt ttcaatcgat tggccaatt tgtaaagac     1620
aggatctcag tggccaggc tctggttttg actcagcaat atcaccagct aaaaccata     1680
gagtacgagc catga                                           1695

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<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

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atgcttctca cctcaagccc gcaccacctt cggcaccaga tgagtccctg gagctggaaa      60
agactgatca tcctcttaag ctgcgtattc ggagacggca aaacgagtct gcagaataag     120
aacccccacc agcctgtgac cctcacctgg caggtaactgt cccaaactgg ggacgttgct     180
tgggacaaaa aggcagtcca gcccttttgg acttggtggc cctctcttac acctgatgta     240
tgtgccctgg cggccggtct tgagtcctgg gatatcccg gatccgatgt atcgtcctct     300
aaaagagtta gacctctga ttcagactat actgccgctt ataagcaaat cacctgggga     360
gccatagggt gcagctaccc tcgggctagg accaggatgg caaattcccc cttctacgtg     420
tgtccccgag ctggccgaac ccattcagaa gctaggaggt gtggggggct agaatcccta     480
tactgtaaag aatggagtgt tgagaccacg ggtaccgttt attggcaacc caagtctca     540
tgggacctca taactgtaaa atgggaccaa aatgtgaaat gggagcaaaa atttcaaaag     600
tgtgaacaaa ccgctggtg taacccccctc aagatagact tcacagaaaa aggaaaaactc     660
tccagagatt ggataacgga aaaaacctgg gaattaaggt tctatgtata tggacaccca     720
ggcatacagt tgactatccg cttagaggtc actaacatgc cggttgtggc agtgggcccc     780
gacctgtccc ttgcggaaca gggacctcct agcaagcccc tcactctccc tctctcccca     840
cggaaagcgc cgcccacccc tctacccccg gcggctagtg agcaaacccc tgcggtgcat     900
ggagaaactg ttaccctaaa ctctccgcct cccaccagtg gcgaccgact ctttgacctt     960
gtgcaggggg ccttcctaac cttgaatgct accaaccagc gggccactaa gtcttgctgg    1020
ctctgttttg gcatgagccc cccttattat gaagggatag cctcttcagg agaggctcgt    1080

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tatacctcca accatacccg atgccactgg ggggcccag gaaagcttac cctcactgag	1140
gtctccggac tcgggtcatg catagggaag gtgcctctta cccatcaaca tctttgcaac	1200
cagaccttac ccatcaattc ctctaaaaac catcagtatc tgctccctc aaaccatagc	1260
tgggtgggct gcagcactgg cctcaccccc tgctctctca cctcagtttt taatcagtct	1320
aaagacttct gtgtccaggt ccagctgac ccccgcatct attaccattc tgaagaaacc	1380
ttgttacaag cctatgacaa atcacccccc aggtttaaaa gagagcctgc ctcacttacc	1440
ctagctgtct tcctgggggt agggattgct gcaggtatag gtactggctc aaccgccta	1500
attaagggc ccatagacct ccagcaaggc ctaaccagcc tccaaatcgc cattgacgct	1560
gacctccggg cccttcagga ctcaatcagc aagctagagg actcactgac ttcctatct	1620
gaggtagtac tccaaaatag gagaggcctt gacttactat tccttaaga aggaggctc	1680
tgcgcgcccc taaaagaaga gtgctgtttt tatgtagacc actcaggctc agtacgagac	1740
tccatgaaaa aacttaaga aagactagat aaaagacagt tagagcgcca gaaaaacca	1800
aactggatg aagggtggtt caataactcc ccttggttta ctacctact atcaaccatc	1860
gtcgggcccc tattgtctct cctttgttta ctactcttg ggccctgcat catcaataaa	1920
ttaatccaat tcataatga taggataagt gcagtcaaaa ttttagtct tagacagaaa	1980
tatcagacc 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 ttctgtgtg ttctgggaag	60
ttccccattt acacgatacc agacgaactt ggtccctgga gccctattga catacaccat	120
ctcagctgtc caaataacct ggttgtggag gatgaaggat gtaccaacct gtccgagttc	180
tcctacatgg aactcaaagt gggatacatc tcagccatca aagtgaacgg gtctacttgc	240
acagggtgtg tgacagaggc agagacctac accaactttg ttggttatgt cacaaccaca	300
ttcaagagaa agcatttccg cccccccca gacgcatgta gagccgcgta taactggaag	360
atggccggtg accccagata tgaagagtcc ctacacaatc cataccccga ctaccactgg	420
cttcgaactg taagaaccac caaagagtcc ctcatattca tatcccaag tgtgacagat	480
ttggaccat atgacaaatc ccttcactca aggtctctcc ctggcggaat gtgctcagga	540
ataacggtgt cctctaccta ctgctcaact aaccatgatt acaccatttg gatgcccag	600
aatccgagac caaggacacc ttgtgacatt ttaccaata gcagaggga gagagcatcc	660
aacgggaaca agacttgcgg ctttgtggat gaaagaggcc tgtataagtc tctaaaagga	720
gcatgcaggc tcaagttatg tggagtctt ggacttagac ttatggatgg aacatgggtc	780
gcgatgcaaa catcagatga gaccaaagtg tgccctccag atcagttggt gaatttgac	840
gactttcgct cagacgagat cgagcatctc gttgtggagg agttagttaa gaaaagagag	900
gaatgtctgg atgcattaga gtccatcatg accaccaagt cagtaagttt cagacgtctc	960
agtcacctga gaaaacttgt ccagggttt ggaaaagcat ataccatatt caacaaaacc	1020
ttgatggagg ctgatgtca ctacaagtca gtccggacct ggaatgagat catcccctca	1080
aaagggtgtt tgaagtttg aggaaggtgc catcctcatg tgaacggggt gtttttcaat	1140

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ggataatat tagggcctga cgaccatgtc ctaatcccag agatgcaatc atccctcctc 1200
cagcaacata tggagttggt ggaatcttca gttatcccc tgatgcaccc cctggcagac 1260
ccttctacag ttttcaaaga aggtgatgag gctgaggatt ttgttgaagt tcacctcccc 1320
gatgtgtaca aacagatctc aggggttgac ctgggtctcc cgaactgggg aaagtatgta 1380
ttgatgactg cagggggccat gattggcctg gtgttgatat tttccctaata gacatgggtgc 1440
agagttggta tccatctttg cattaaatta aagcacacca agaaaagaca gattttatata 1500
gacatagaga tgaaccgact tggaaagtaa 1530

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<210> SEQ ID NO 45
<211> LENGTH: 1497
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Envelope - LCMV

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<400> SEQUENCE: 45

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atgggtcaga ttgtgacaat gtttgaggct ctgcctcaca tcatcgatga ggtgatcaac 60
attgtcatta ttgtgcttat cgtgatcacg ggtatcaagg ctgtctacaa ttttgccacc 120
tgtgggatat tcgcattgat cagtttcccta cttctggctg gcaggctcctg tggcatgtac 180
ggctcttaagg gacccgacat ttacaaagga gtttaccat ttaagtcagt ggagtttgat 240
atgtcacatc tgaacctgac catgcccac gcattgttcag ccaacaactc ccaccattac 300
atcagtatgg ggacttcttg actagaattg accttcacca atgattccat catcagtcac 360
aacttttgca atctgacctc tgccctcaac aaaaagacct ttgaccacac actcatgagt 420
atagtttcga gcctacacct cagtatcaga gggaactcca actataaggc agtatcctgc 480
gacttcaaca atggcataac catccaatac aacttgacat tctcagatcg acaaagtgtc 540
cagagccagt gtagaacctt cagaggtaga gtcctagata tgtttagaac tgccctcggg 600
gggaaataca tgaggagtgg ctggggctgg acaggctcag atggcaagac cacctgggtg 660
agccagacga gttaccaata cctgattata caaaatagaa cctgggaaaa ccactgcaca 720
tatgcaggtc cttttgggat gtccaggatt ctcctttccc aagagaagac taagttcttc 780
actaggagac tagcgggcac attcacctgg actttgtcag actcttcagg ggtggagaat 840
ccagtggtt attgcctgac caaatggatg attcttctg 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 tgcctccaag tgctggcttg tcaccaatgg ttcttactta 1200
aatgagaccc acttcagtga tcaaatcgaa caggaagccg ataacatgat tacagagatg 1260
ttgaggaagg attacataaa gaggcagggg agtaccctcc tagcattgat ggaccttctg 1320
atgttttcca catctgcata tctagtcagc atcttctgc accttgtaa aataccaaca 1380
cacaggcaca taaaagggtg ctcatgtcca aagccacacc gattaacca caaaggaatt 1440
tgtagttgtg gtgcatttaa ggtgcctggt gtaaaaaccg tctggaaaag acgctga 1497

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<210> SEQ ID NO 46
<211> LENGTH: 1692
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Envelope - FPV

<400> SEQUENCE: 46

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atgaacactc aaatcctggt ttctgcctt gtggcagtca tccccacaaa tgcagacaaa    60
atttgtcttg gacatcatgc tgtatcaaat ggcaccaaag taaacacact cactgagaga    120
ggagtagaag ttgtcaatgc aacggaaaca gtggagcgga caaacatccc caaaatttgc    180
tcaaagggga aaagaaccac tgatcttggc caatgcggac tgtagggac cattaccgga    240
ccacctcaat gcgaccaatt tctagaatct tcagctgatc taataatcga gagacgagaa    300
ggaaatgatg tttgttacc gggaagttt gttaatgaag aggcattgcg acaaatcctc    360
agaggatcag gtgggattga caaagaaaca atgggattca catatagtgg aataaggacc    420
aacggaacaa ctagtgcatt tagaagatca gggctctcat tctatgcaga aatggagtgg    480
ctctgtctaa atacagacaa tgctgcttcc ccacaaatga caaaatcata caaaaacaca    540
aggagagaat cagctctgat agtctgggga atccaccatt caggatcaac caccgaacag    600
accaaactat atgggagtgg aaataaactg ataacagtcg ggagtcccaa atatcatcaa    660
tcttttgtgc cgagtccagg aacacgaccg cagataaatg gccagtcagg acggattgat    720
tttcattggt tgatcttggg tcccaatgat acagttactt ttagtttcaa tggggcttcc    780
atagctccaa atcgtgccag ctctctgagg 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
aaaagcacc ccaatcggaat tgatcagata accggaaagt taaatagact cattgagaaa   1200
accaaccagc aatttgagct aatagataat gaattcactg aggtggaaaa gcagattggc   1260
aatttaatta actggacca agactccatc acagaagtat ggtcttaca tgctgaactt   1320
cttgtggcaa tggaaaacca gcacactatt gatttggctg attcagagat gaacaagctg   1380
tatgagcgag tgaggaaaca attaaggga aatgctgaag aggatggcac tggttgcttt   1440
gaaatttttc ataaatgtga cgatgattgt atggctagta taaggaacaa tacttatgat   1500
cacagcaaat acagagaaga agcgatgcaa aatagaatac aaattgaccc agtcaaatg   1560
agtagtggct acaaagatgt gatactttgg tttagcttcg gggcatcatg ctttttgctt   1620
cttgccattg caatgggcct tgttttcata tgtgtgaaga acggaacat gcggtgcact   1680
atttgtatat aa                                     1692

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<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

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agtgtaacag agcactttta tgtgtataag gctactagac catacctage acattgcgcc    60
gattgcgggg acgggtactt ctgctatagc ccagttgcta tcgaggagat ccgagatgag    120
gcgtctgatg gcatgcttaa gatccaagtc tccgcccacaa taggtctgga caaggcaggc    180

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acccacgccc acacgaagct ccgatatatg gctggtcatg atgttcagga atctaagaga	240
gattccttga ggggtgtacac gtccgcagcg tgctccatac atgggacgat gggacacttc	300
atcgtcgcac actgtccacc aggcgactac ctcaaggttt cggttcgagga cgcagattcg	360
cacgtgaagg catgtaaggt ccaatacaag cacaatccat tgccggtggg tagagagaag	420
ttcgtggta gaccacactt tggcgtagag ctgccatgca cctcatacca gctgacaacg	480
gtcccccag acgaggagat tgacatgcat acaccgccag atataccgga tcgcaccctg	540
ctatcacaga cggcgggcaa cgtcaaaata acagcaggcg gcaggactat caggtagaac	600
tgtacctgag gccgtgacaa cgtaggcact accagtactg acaagacat caacacatgc	660
aagattgacc aatgccatgc tgccgtcacc agccatgaca aatggcaatt tacctctcca	720
tttgttccca gggctgatca gacagctagg aaaggcaagg tacacgttcc gttccctctg	780
actaacgtca cctgccgagt gccgttggct cgagcgccgg atgccaccta tgtaagaag	840
gaggtgaccc tgagattaca ccagatcat ccgacgctct tctcctatag gagtttagga	900
gccgaaccgc acccgtaga ggaatgggtt gacaagttct ctgagcgcat catccagtgc	960
acggaagaag ggattgagta ccagtggggc aacaaccgc cggctctgct gtggcgcaa	1020
ctgacgaccg agggcaaac ccattggctgg ccacatgaaa tcattcagta ctattatgga	1080
ctataccccc ccgcactat tgccgcagta tccggggcga gtctgatggc cctcctaact	1140
ctggcgcca catgctgcat gctggccacc gcgaggagaa agtgccaac accgtacgcc	1200
ctgacgccag gacgggtggt accgttgaca ctggggctgc tttgctgagc accgaggcg	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
ctttgggtta ttatcctttt ccaagaaca ttttccatcc cacttgagat catccacaat	120
agcacattac aggttagtga tgcgacaaa ctggtttgcc gtgacaaact gtcattccaca	180
aatcaattga gatcagttgg actgaatctc gaagggaatg gagtggcaac tgacgtgcca	240
tctgcaacta aaagatgggg ctccaggtcc ggtgtccac caaagggtgt caattatgaa	300
gctggtgaat gggctgaaaa ctgctacaat ctgaaatca aaaaacctga cgggagtgag	360
tgtctaccag cagcgccaga cgggattcgg ggcttcccc ggtgccggtg tgtgcacaaa	420
gtatcaggaa cgggaccgtg tgccggagac tttgccttc acaagagggt tgctttcttc	480
ctgtatgacc gacttgcttc cacagttatc taccaggaaa cgactttcgc tgaagggtgc	540
gttgcatctc tgatactgcc ccaagctaag aaggacttct tcagctcaca ccccttgaga	600
gagccggtca atgcaacgga ggaccgtct agtggtact attctaccac aattagatat	660
caagctaccg gttttggaac caatgagaca gagtatttgt tcgaggttga caatttgacc	720
tacgtccaac ttgaatcaag attcacacca cagtttctgc tccagctgaa tgagacaata	780
tatacaagtg ggaaggag caataccacg ggaactaa tttggaaggt caaccccgaa	840
attgatacaa caatcgggga gtgggccttc tgggaaacta aaaaacctc actagaaaaa	900

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ttcgcagtga agagttgtct ttcacagctg tatcaaacag agccaaaaac atcagtggtc 960
agagtccggc gcgaacttct tccgaccag ggaccaaac aacaactgaa gaccacaaaa 1020
tcatggcttc agaaaattcc tctgcaatgg ttcaagtga cagtcaagga agggaagctg 1080
cagtgtcgca tctgacaacc cttgccacaa tctccacgag tctcaaccc cccacaacca 1140
aaccaggtcc ggacaacagc acccacaata caccctgtga taaacttgac atctctgagg 1200
caactcaagt tgaacaacat caccgcagaa cagacaacga cagcacagcc tccgacactc 1260
cccccgccac gaccgcagcc ggaccacctaa aagcagagaa caccaacacg agcaagggtg 1320
ccgacctcct ggaccccgcc accacaacaa gtccccaaaa ccacagcgag accgctggca 1380
acaacaacac tcatcaccaa gataccggag aagagagtgc cagcagcggg aagctaggct 1440
taattaccaa tactattgct ggagtcgcag gactgatcac aggcgggagg agagctcgaa 1500
gagaagcaat tgtcaatgct caaccctaat gcaaccctaa tttacattac tggactactc 1560
aggatgaagg tgctgcaatc ggactggcct ggataccata tttcgggcca gcagccgagg 1620
gaatttacat agaggggctg atgcacaatc aagatgggtt 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 ccttcgggac cagggggaca 1920
atgacaattg gtggacagga tggagacaat ggataccggc aggtattgga gttacaggcg 1980
ttataattgc agttatcgct ttattctgta tatgcaaatt tgtcttttag 2030

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<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

```

```

<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

```

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```

<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 gaaagtgtg 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 aagtgtgcc 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

<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 ttttgccac        60
tgactgagca gaagggtga gaaagtcagg acacaaggcc tggtactagc 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 ctgttgaac        60
tcatggcaga aggaggcgag aaagtcgtac attttggtat ctttcatctg acca          114

<210> SEQ ID NO 58

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<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 ctctttctgc tggteccctc 60
cccgacagaag gaggtgaga 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 tcaagtaat 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
<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

-continued

<400> SEQUENCE: 64

gcctcttgac attctctc

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 gggacagtg

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 agaattggctc 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

<220> FEATURE:

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

<400> SEQUENCE: 68

cctggaggct tgctgaaggc tgtatgctgt atacacgccg caatacagag gttttggcca

60

ctgactgacc tctgtatcgg cgtgtatata 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

-continued

<400> SEQUENCE: 70

cctggaggct tgcgaaggc tgtatgctgt gttcgctct tgacattctc ttttgccac 60

tgactgagag aatgtagagg cgaacacagg acacaaggcc tggtactagc 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 taccatgggtg atgcggtttt ggcagtacat caatgggcgt ggatagcggg 120

ttgactcacg gggatttcca agtctccacc ccattgacgt caatgggagt ttgttttgcc 180

acaaaaatca acgggacttt ccaaaatgtc gtaacaactc cgccccattg acgcaaatgg 240

gcggtaggcg tgtacggtgg gaggtttata taagcagagc tcgttttagtg 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

atgcccgcca tgaagatcga gtgccgcac accggcacc tgaacggcgt ggagtccgag 60

ctgggtggcg gcggagaggg ccccccgag caggggccga tgaccaaca gatgaagagc 120

accaaaggcg ccctgacctt cagccctac ctgctgagcc acgtgatggg ctacggcttc 180

taccacttcg gcacctacc cagcggtac gagaacctt tcctgcacgc catcaacaac 240

ggcggttaca ccaacaccg catcgagaag tacgaggacg gcggcgtgct gcacgtgagc 300

ttcagctacc gctacgagc cgcccgctg atcgcgact tcaagggtgt gggcaccggc 360

ttccccgagg acagcgtgat cttcaccgac aagatcatcc gcagcaacgc caccgtggag 420

cacctgcacc ccatgggcga taactgtgtg gtgggcagct tcgcccgcac cttcagcctg 480

cgcgacggcg gctactacag cttcgtggtg gacagccaca tgcaattcaa gacgcccac 540

caccaccgca tcctgcagaa cgggggcccc atgttcgcct tccgcccgt ggaggagctg 600

cacagcaaca ccgagctggg catcgtggag taccagcag cttcaagac ccccatcgcc 660

ttcgccagat ctcgagatat cagccatggc ttcccgcgg cggtggcggc gcaggatgat 720

ggcacgtgc ccatgtcttg tgcccaggag agcgggatgg accgtcacc tgcagcctgt 780

gcttctgcta ggatcaatgt gaccggtgag ggcagaggaa gtcttctaac atgcggtgac 840

gtggaggaga atcccgccc ttccggtatg gaagacgcca aaacataaa gaaaggcccg 900

-continued

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gagccattct atccgctaga ggatggaacc gctggagagc aactgcataa ggctatgaag   960
agatacgcgc tggttcctgg aacaattgct tttacagatg cacatatcga ggtgaacatc  1020
acgtacgcgg aataactcga 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 caatcaaadc  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 gacaaaacg atttatctaa ttacacgaa  1800
attgcttctg ggggcgcacc tctttcgaaa gaagtcgggg aagcggttgc aaaacgcttc  1860
catcttcag ggatacgaca aggatatggg ctactgaga ctacatcagc tattctgatt  1920
acacccgagg gggatgataa accgggcgcg gtcggtaaaag ttgttccatt ttttgaagcg  1980
aaggttgttg atctggatac cgggaaaacg ctgggcgtta atcagagagg cgaattatgt  2040
gtcagaggac ctatgattat gtccggttat gtaaacatc cggaagcgac caacgccttg  2100
attgacaagg atggatggct acattctgga gacatagctt actgggacga agacgaacac  2160
ttcttcatag ttgaccgctt gaagtcttta attaaataca aaggatacca ggtggccccc  2220
gctgaattgg agtcgatatt gttacaacac cccaacatct tcgacgcggg cgtggcaggt  2280
cttcccgcgc atgacgcggg tgaacttccc gccgcgcttg ttgttttga gcacggaaaag  2340
acgatgacgg aaaaagagat cgtggattac gtcgccagtc aagtaacaac cgcgaaaaag  2400
ttgcgcggag gagttgtgtt tgtggacgaa gtaccgaaag gtcttaccgg aaaactcgac  2460
gcaagaaaaa tcagagagat cctcataaag gccagaagg gcggaaagtc caaattgtaa  2520

```

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<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 ttagtcttg caacatggta acgatgagtt agcaacatgc   60
cttacaagga gagaaaaagc accgtgcacg ccgattgggt gaagtaaggt ggtacgatcg  120
tgcttatta 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:

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<223> OTHER INFORMATION: 5' Long terminal repeat (LTR)

<400> SEQUENCE: 75

```

ggctctctctg gtttagaccag atctgagcct gggagctctc tggctaacta gggaaccac      60
tgcttaagcc tcaataaagc ttgccttgag tgcttcaagt agtgtgtgcc cgtctgttgt      120
gtgactctgg taactagaga tccttcagac 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
gacgtgacg gtacaggcca gacaattatt gtctgggata gtgcagcagc agaacaattt      120
gctgagggct attgaggcgc aacagcatct gttgcaactc acagtctggg gcatcaagca      180
gtccaggca 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

```

ttttaaaaga aaagggggga ttgggggggta cagtgcaggg gaaagaatag tagacataat      60
agcaacagac atacaaaacta 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 tcatgtatt gcttcccgta      120
tggctttcat tttctctccc ttgtataaat cctggttgct gtctctttat gaggagtgtt      180
ggccccgttg caggcaacgt ggcgtgggtg gcaactgtgtt tgctgacgca acccccactg      240
gttggggcat tgccaccacc tgctcagctcc ttccggggac ttctgcttcc cccctcccta      300
tgccacggc ggaactcacc gccgcctgcc ttgccgctg ctggacaggg gctcggtgtt      360
tgggcactga caattccgtg gtgttgctgg ggaatcacc gtcctttcct tggtgctcg      420
cctgtgttgc cacctggatt ctgcgcggga cgtccttctg ctacgtccct tcggccctca      480

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atccagcgga ccttccttcc cgcggcctgc tgccggctct gcggcctatt ccggtcttc 540

gccttcgccc tcagacgagt cggatctccc ttggggccgc ctccccgct 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

tggaagggt aattcactcc caacgaagat aagatctgct ttttcttgt actgggtctc 60

tctggtaga ccagatctga gctctgggagc tctctggcta actagggaac ccactgctta 120

agcctcaata aagcttgctc tgagtgttc aagtagtgtg tgcccgctg ttgtgtgact 180

ctggttaacta gagatccctc agaccctttt 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 cagcgtcttc aaaaccctt aaagataaga ttaaccctg gaagtcctta 60

atgggtcatgg ggggtctattt aagagtaggg atggcagaga gccccatca ggtctttaat 120

gtaacctgga gagtcaccaa cctgatgact gggcgtaccg ccaatgccac ctccctttta 180

ggaactgtac aagatgcctt cccaagatta tattttgatc tatgtgatct ggtcggagaa 240

gagtgggacc cttcagaaca ggaaccatat gtcgggtatg gctgcaaata ccccgagggg 300

agaaagcgga cccggacttt tgacttttac gtgtgccctg ggcataccgt aaaatcgggg 360

tgtggggggc caagagaggg ctactgtggt gaatggggtt gtgaaaccac cggacaggct 420

tactggaagc ccacatcatc atgggaccta atctccctta agcgcggtaa cccccctgg 480

gacacgggat gtcctaaaat ggcttggtgc cctgtctacg acctctcaa agtatccaat 540

tccttccaag gggctactcg agggggcaga tgcaaccctc tagtctaga attcactgat 600

gcaggaaaaa aggctaattg ggacggggcc aaatcgtggg gactgagact gtaccggaca 660

ggaacagatc ctattaccat gttctccctg acccgccagg tcctcaatat agggccccgc 720

atccccattg ggctaatacc cgtgatcact ggtcaactac cccctccccg acccgtgcag 780

atcaggctcc ccaggctcc tcagctcct cctacaggcg cagcctctat agtcctgag 840

actgccccac cttctcaaca acctgggacg ggagacaggc tgctaaacct ggtagaagga 900

gcctatcagg cgcttaacct caccaatccc gacaagacc aagaatgttg gctgtgctta 960

gtgtcgggac ctcttatta cgaaggagta gcggtcgtgg gcacttatac caatattct 1020

accgccccgg ccagctgtac ggccacttcc caacataagc ttacctatc tgaagtgaca 1080

ggacagggcc tatgcatggg agcactacct aaaactcacc aggccttatg taacaccacc 1140

caaagtgcg gctcaggatc ctactacct gcagcaccg ctggaacaat gtgggcttgt 1200

agcactggat tgactccctg cttgtccacc acgatgtca atctaaccac agactattgt 1260

gtattagttg agctctggcc cagaataatt taccactccc ccgattatat gtatggtcag 1320

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cttgaacagc gtaccaaata taagaggag ccagtatcgt tgacctggc ccttctgcta 1380
ggaggattaa ccatgggagg gattgcagct ggaatagga cggggaccac tgcctaatac 1440
aaaaccagc agtttgagca gcttcacgcc gctatccaga cagacctcaa cgaagtcgaa 1500
aaatcaatta ccaacctaga aaagtcactg acctcgttgt ctgaagtagt cctacagaac 1560
cgaagaggcc tagatttgct cttcctaaaa gagggaggtc tctgcgcagc cctaaaagaa 1620
gaatgttggt tttatgcaga ccacacggga ctagtgcagc acagcatggc caaactaagg 1680
gaaaggctta atcagagaca aaaactatgt gagtcaggcc aaggttggtt cgaagggcag 1740
tttaatatgat ccccttggtt taccacctta atctccacca tcatgggacc tctaatagta 1800
ctcttactga tcttactctt tggacctgc attctcaatc gattggtcca atttgtaaa 1860
gacaggatct cagtgggtcca ggctctgggt 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

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```

<400> SEQUENCE: 82

```

```

cctggaggct tgcgaaggc tgcgtgctgt 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 gttcgctctt tgacattctc ctgttgaatc 60
tcatggagaa tgtcaagggc gaacactgac attttggtat ctttcatctg acca 114

```

```

<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 tcttggtgtg ggaaagatac ctaaaggatc aacagctcct 240
agatcttttt ccctctgcc aaaaattatg ggacatcatg aagccccttg agcatctgac 300
ttctggctaa taaaggaaat ttattttcat tgcaatagtg tgttggaatt ttttgtgtct 360
ctcactcgga aggacatatg ggagggcaaa tcatttaaaa catcagaatg agtatttggt 420
ttagagtttg gcaacatatg ccatatgctg gctgccatga acaaagggtg ctataaagag 480
gtcatcagta tatgaaacag cccctctgctg tccattcctt attccataga aaagccttga 540
cttgagggtta gatttttttt atattttgtt ttgtgttatt ttttcttta acatccctaa 600

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```

aattttcctt acatgtttta ctageccagat ttttctcct ctectgacta ctcccagtc 660
tagctgtccc tcttctctta tgaagatccc tcgacctgca gcccagctt ggcgtaatca 720
tggtcatagc tgtttcctgt gtgaaattgt tatccgctca caattccaca caacatacga 780
gccggaagca taaagtgtaa agcctggggt gcctaataag tgagctaact cacattaatt 840
gcgttgcgct cactgccgcg tttccagtcg ggaaacctgt cgtgccagcg gateccgcatc 900
tcaattagtc agcaaccata gtcccgcgcc taactccgcc catccgcgcc ctaactccgc 960
ccagttccgc ccattctcgc ccccatggct gactaatatt ttttatttat gcagaggccg 1020
agggcgctc ggctctgag ctattccaga agtagtgagg aggctttttt ggaggcctag 1080
gcttttgc aaagctaact tgtttattgc agcttataat ggttacaaat aaagcaatag 1140
catcacaat ttcacaaata aagcattttt ttcactgcat tctagttgtg gtttgtccaa 1200
actcatcaat gtatcttata acccggg 1227

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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 CD47 mRNA sequence,

wherein the first small RNA sequence comprises a sequence having at least about 80%, or at least about 85%, or at least about 90%, or at least about 95% identity with SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or SEQ ID NO: 9; 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 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 first small RNA sequence and the second small RNA sequence are under the control of a single promoter.

4. The viral vector of claim 1, wherein the small RNA sequences comprise a miRNA or a shRNA.

5. The viral vector of claim 1, wherein the viral vector is a lentiviral vector.

6. The viral vector of claim 1, wherein the first small RNA sequence comprises SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or SEQ ID NO: 9.

7. 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 CD47 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 cMyc mRNA sequence,

wherein the second small RNA sequence comprises a sequence having at least about 80%, or at least about 85%, or at least about 90%, or at least about 95% identity with SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14.

8. The viral vector of claim 7, wherein the second small RNA sequence comprises SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, or SEQ ID NO: 14.

9. A viral vector comprising a therapeutic cargo portion, wherein the therapeutic cargo portion comprises: a small RNA sequence that is capable of binding to a pre-determined complementary mRNA sequence, wherein the pre-determined complementary mRNA sequence comprises a CD47 mRNA sequence,

wherein the small RNA sequence comprises a sequence having at least about 80%, or at least about 85%, or at least about 90%, or at least about 95% identity with SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or SEQ ID NO: 9.

10. The viral vector of claim 9 wherein the small RNA sequence comprises a miRNA or a shRNA.

11. The viral vector of claim 9, wherein the viral vector is a lentiviral vector.

12. The viral vector of claim 9, wherein the small RNA sequence comprises SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or SEQ ID NO: 9.

13. A method of treating cancer in a subject using an immunotherapy-based composition, the method comprising: administering to the subject a therapeutically effective amount of a lentiviral particle comprising the viral vector of claim 1.

14. The method of claim 13, further comprising administering to the subject an effective amount of an amino-bisphosphonate drug.

15. A method of treating cancer in a subject using an immunotherapy-based composition, the method comprising administering to the subject a therapeutically effective amount of a lentiviral particle comprising the viral vector of claim 9.

16. The method of claim 15, further comprising administering to the subject an effective amount of an amino-bisphosphonate drug.

17. A method of treating cancer in a subject using an immunotherapy-based composition, the method comprising:

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administering to the subject a therapeutically effective amount of a lentiviral particle comprising the viral vector of claim 7.

18. A method of treating cancer in a subject using an immunotherapy-based composition, the method comprising: 5

(i) administering to the subject a therapeutically effective amount of a lentiviral particle comprising 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 10

a first pre-determined complementary mRNA sequence, wherein the first pre-determined complementary mRNA sequence comprises a CD47 mRNA sequence; and

a second small RNA sequence that is capable of bind- 15

ing to a second pre-determined complementary mRNA sequence, wherein the second pre-determined complementary mRNA sequence comprises a cMyc mRNA sequence; and

(ii) administering to the subject an effective amount of an 20 aminobisphosphonate drug.

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