(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
20 December 2018 (20.12.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/232359 A1

(51) International Patent Classification:
C12N 9/10 (2006.01) *C12N 5/0783* (2010.01)
C12N 15/113 (2010.01) *G01N 33/573* (2006.01)
C12N 15/86 (2006.01)

(21) International Application Number:
PCT/US2018/037924

(22) International Filing Date:
15 June 2018 (15.06.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/521,274 16 June 2017 (16.06.2017) US
62/633,461 21 February 2018 (21.02.2018) US

(71) Applicant: **AMERICAN GENE TECHNOLOGIES INTERNATIONAL INC.** [US/US]; 9640 Medical Center Drive, Rockville, MD 20850 (US).

(72) Inventors: **PAUZA, Charles, David**; 9640 Medical Center Drive, Rockville, MD 20850 (US). **LAHUSEN, Tyler**; 9640 Medical Center Drive, Rockville, MD 20850 (US). **LI, Haishan**; 9640 Medical Center Drive, Rockville, MD 20850 (US). **LIOU, Mei-Ling**; 9640 Medical Center Drive, Rockville, MD 20850 (US). **XIAO, Lingzhi**; 9640 Medical Center Drive, Rockville, MD 20850 (US).

(74) Agent: **MULHOLLAND, William, F.**; Snell & Wilmer LLP, One Arizona Center, 400 E. Van Buren, Phoenix, AZ 85004 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,

(54) Title: METHODS AND COMPOSITIONS FOR THE ACTIVATION OF TUMOR CYTOTOXICITY VIA HUMAN GAMMA-DELTA T-CELLS

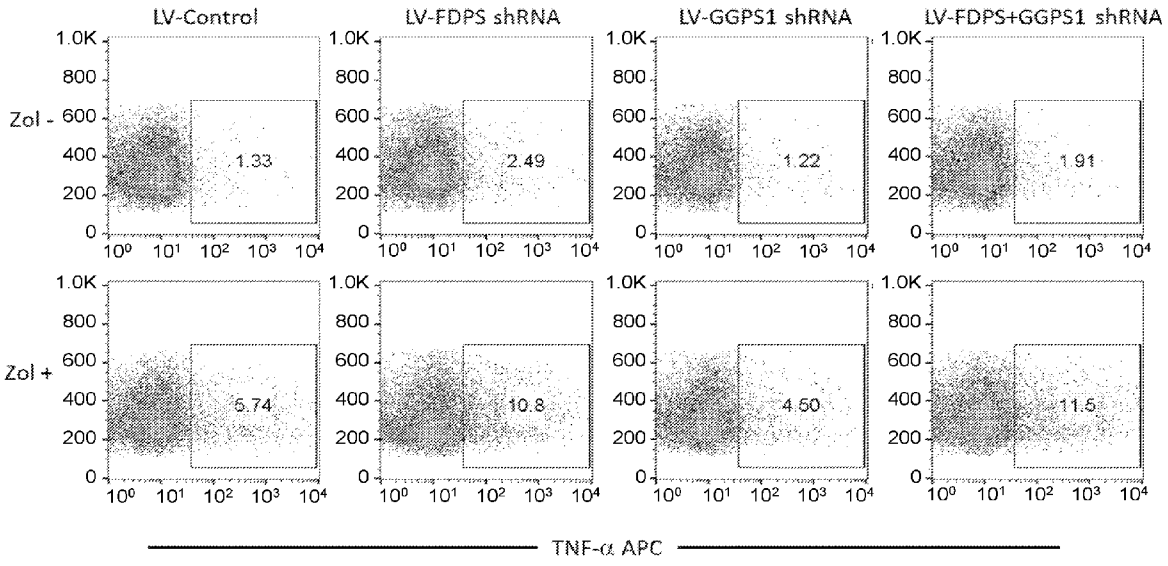


Figure 25

(57) Abstract: The present disclosure relates generally to methods and compositions for activating gamma-delta (GD) T cells. Such methods and compositions can be used to treat cancer.

[Continued on next page]

WO 2018/232359 A1

TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

PCT PATENT APPLICATION

METHODS AND COMPOSITIONS FOR THE ACTIVATION OF TUMOR CYTOTOXICITY VIA HUMAN GAMMA-DELTA T-CELLS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to: U.S. Provisional Patent Application No. 62/521,274, filed on June 16, 2017, and entitled "Methods and Compositions for the Activation of Tumor Cytotoxicity Via Human Gamma-Delta T-Cells", and U.S. Provisional Patent Application No. 62/633,461, filed on February 21, 2018, and entitled "Methods and Compositions for the Activation of Tumor Cytotoxicity Via Human Gamma-Delta T-Cells", which are each incorporated herein by reference.

FIELD

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

BACKGROUND

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

Another subset of GD T cells, Vδ1+, make up a much smaller percentage of the T cells circulating in the blood, but Vδ+1 cells are most commonly found in the epithelial mucosa and the skin. Minor cell populations express other Vδ chains and may be associated with specific responses during allergy, transplantation or viral and bacterial diseases.

In general, GD T cells have several functions, including killing tumor cells and pathogen-infected cells. Stimulation through their unique T cell receptor ("TCR") composed of two glycoprotein chains, γ and δ that interact with CD3 complex proteins to create a functional TCR, improves the capacity for cellular cytotoxicity, cytokine secretion and other effector functions. The TCRs of GD T cells have unique specificities and the cells themselves occur in high clonal frequencies, thus allowing rapid innate-like responses to tumors and

pathogens.

Bisphosphonate drugs and other inhibitors of farnesyl diphosphate synthase ("FDPS"), which are downstream from isopentenyl pyrophosphate ("IPP") in the mevalonate pathway (see, *e.g.*, Figure 1), have been used to treat various diseases, including cancers, specifically those involving bone metastasis. Bisphosphonate drugs include, for example, trade names such as Zometa® (Novartis), Actonel® (Procter & Gamble), Aredia® (Novartis) and Fosamax® (Merck).

Certain bisphosphonates have also been investigated for stimulation of GD T cells. This may be because inhibition of FDPS in myeloid or tumor cells, blocks the conversion of IPP to farnesyl diphosphate causing IPP to accumulate while simultaneously reducing levels of geranylgeranyl pyrophosphate ("GGPP"), a downstream product of FDPS that normally suppresses activation of the NLRP3 inflammasome pathway. The reduction in GGPP removes an inhibitor of the caspase-dependent inflammasome pathway and allows secretion of cytokines including interleukin-1 beta and interleukin-18, the latter being especially important for gamma delta T cell activation.

Thus, when FDPS is blocked, the increased IPP and decreased GGPP modify the myeloid or tumor cells and the modified cells gain an increased capacity for activating GD T cells and specifically the Vδ2+ subset. Activated Vδ2+ cells proliferate rapidly, express multiple cytokines and chemokines, and can function to cytotoxically destroy tumor cells or pathogen-infected cells. GD T cell effector activities include secretion of IFN-gamma, which activates macrophages and antigen-presenting cells, secretion of TNF-alpha among other cytokines and chemokines that activate other innate and acquired immune mechanisms, activation of granzyme B that attacks and destroys target cells and cell surface expression of FasL that triggers cellular apoptosis in Fas+ target cells.

A significant problem with traditional cancer treatment is that patients become insensitive to chemotherapy treatments. Chemo-resistant tumor cells in particular become very difficult to treat. As an alternative therapy to treat chemo-resistant patients, or as a primary therapy in place of chemotherapy and/or radiation therapy the present application proposes the use of a recombinant lentivirus to express genes at the tumor site, where manipulation of proteins that impact GD T cell activity may slow down tumor growth and activate the patient's own innate immune response to recognize and kill cancers.

SUMMARY OF THE INVENTION

In an aspect of the disclosure, a viral vector comprising first and second encoded genetic elements is disclosed. The first encoded genetic element comprises at least one small RNA capable of inhibiting production of at least one enzyme involved in the mevalonate pathway, and the second encoded genetic element comprises one of a butyrophilin family member, a cytokine, or a chemokine. In embodiments, the viral vector also includes a third encoded genetic element, wherein the third encoded genetic element comprises one of a butyrophilin family member, a cytokine, or a chemokine. In embodiments, the viral vector also includes a fourth encoded genetic element, wherein the fourth encoded genetic element comprises one of a butyrophilin family member, a cytokine, or a chemokine. In embodiments, the at least one enzyme is farnesyl diphosphate synthase (FDPS), geranylgeranyl-diphosphate synthase 1 (GGPS1), isopentyl-disphosphate delta isomerase 1 (IDI1), or farnesyl transferase (F-Tase). In embodiments, the first encoded genetic element comprises a microRNA or a shRNA.

In embodiments, the microRNA comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with: AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAA GCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCA AGGGGCT (SEQ ID NO: 68), or
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAA GCCACAGATGGCAGAAGGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAG GGGCT (SEQ ID NO: 69).

In embodiments, the microRNA comprises
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAA GCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCA AGGGGCT (SEQ ID NO: 68), or
AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAA GCCACAGATGGCAGAAGGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAG GGGCT (SEQ ID NO: 69).

In embodiments, the shRNA comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 95% or more than 95% percent identity with GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTACTCCAGGACTTTTT (SEQ ID NO: 1);

GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCTGAACGAAATCCTGCTTTTT (SEQ ID NO: 2); GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGTACATGGCTTTTT (SEQ ID NO: 3); GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCTTTTT (SEQ ID NO: 4). In embodiments, the shRNA comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 95% or more than 95% percent identity with SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 76.

In embodiments, the shRNA comprises: GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTACTCCAGGACTTTTT (SEQ ID NO: 1); GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCTGAACGAAATCCTGCTTTTT (SEQ ID NO: 2); GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGTACATGGCTTTTT (SEQ ID NO: 3); or GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCTTTTT (SEQ ID NO: 4). In embodiments, the shRNA comprises SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, or SEQ ID NO: 76.

In embodiments, the butyrophilin family member comprises BTN3A3, BTN3A2, or BTN3A1 or variants thereof. In embodiments, the butyrophilin family member comprises BTN3A3 (R381H). In embodiments, the cytokine comprises IL-1, IL-1β, IL-2, IL-4, IL-7, IL-12, IL-15, IL-17, IL-18, IL-23, IL-33, IL-36, TNF-α, or interferon-γ. In embodiments, the chemokine comprises a CC chemokine, a CXC chemokine, a CX3C chemokine, a C chemokine, or a XC chemokine. In further embodiments, the CC chemokine comprises RANTES. In embodiments, the viral vector is a lentiviral vector.

In another aspect, a lentiviral vector system for expressing a lentiviral particle is disclosed. The system includes a lentiviral vector as detailed herein; at least one envelope plasmid for expressing an envelope protein optimized for infecting a target cell; and at least one helper plasmid for expressing gag, pol, and rev genes, wherein when the lentiviral vector, the at least one envelope plasmid, and the at least one helper plasmid are transfected into a packaging cell, the lentiviral particle is produced by the packaging cell, wherein the lentiviral

particle is capable of infecting the target cell and inhibiting the at least one enzyme involved in the mevalonate pathway within the target cell.

In another aspect, a lentiviral particle capable of infecting a target cell is disclosed. The lentiviral particle comprises an envelope protein optimized for infecting the target cell, and a lentiviral vector as detailed herein. In embodiments, the target cell is a cancer cell.

In another aspect, a method of activating a gamma delta (GD) T cell is disclosed. The method includes infecting, or having infected, in the presence of the GD T cell, a target cell with a lentiviral particle, wherein the lentiviral particle comprises a viral vector comprising first and second encoded genetic elements wherein the first encoded genetic element comprises at least one small RNA capable of inhibiting production of at least one enzyme involved in the mevalonate pathway, and the second encoded genetic element comprises one of a butyrophilin family member, a cytokine, or a chemokine, wherein when the at least one enzyme is inhibited in the target cell, the target cell activates the GD T cell. In embodiments, the target cell is a cancer cell. In embodiments, the method further comprises contacting, or having contacted, the target cell and the GD T cell with an amount of an aminobisphosphonate drug. In embodiments, the aminobisphosphonate drug is zoledronic acid. In embodiments, the at least one enzyme is farnesyl diphosphate synthase (FDPS), geranylgeranyl-diphosphate synthase 1 (GGPS1), isopentenyl-diphosphate delta isomerase 1 (IDI1), or farnesyl transferase (F-Tase).

In another aspect, a method of treating cancer in a subject is disclosed. The method includes administering, or having administered, to the subject a therapeutically effective amount of a lentiviral particle wherein the lentiviral particle comprises a viral vector comprising first and second encoded genetic elements wherein the first encoded genetic element comprises at least one small RNA capable of inhibiting production of at least one enzyme involved in the mevalonate pathway, and the second encoded genetic element comprises one of a butyrophilin family member, a cytokine, or a chemokine, wherein when the at least one enzyme is inhibited in a cancer cell in the presence of a GD T cell, the target cell activates the GD T cell, to thereby treat the cancer. In embodiments, the method further comprises contacting, or having contacted, the target cell and the GD T cell with an amount of an aminobisphosphonate drug. In embodiments, the aminobisphosphonate drug is zoledronic acid. In embodiments, the butyrophilin family member includes BTN3A3 (SEQ ID NO: 17) or BTN3A3 (R381H) (SEQ ID NO: 54). In further embodiments, the cytokine includes IL-1, IL-2, IL-12, IL-15, IL-17, IL-18, IL-23, or IL-36.

In another aspect, a viral vector is disclosed. The viral vector comprises a first small

RNA that targets a first target of the mevalonate pathway and is capable of increasing a first product of the mevalonate pathway, and a second small RNA that targets a second target of the mevalonate pathway and is capable of decreasing a second product of the mevalonate pathway. In embodiments, the first target is a first enzyme of the mevalonate pathway and the second target is a second enzyme of the mevalonate pathway. In embodiments, at least one of the first enzyme and the second enzyme comprises farnesyl diphosphate synthase (FDPS), geranylgeranyl-diphosphate synthase 1 (GGPS1), isopentenyl-diphosphate delta isomerase 1 (IDI1), or farnesyl transferase (F-Tase). In embodiments, the first product of the mevalonate pathway comprises isopentenyl pyrophosphate (IPP). In embodiments, the second product of the mevalonate pathway comprises geranylgeranyl pyrophosphate (GGPP).

In another aspect, a method of treating cancer in a subject is disclosed. The method comprises administering, or having administered, to the subject a therapeutically effective amount of a lentiviral particle wherein the lentiviral particle comprises a viral vector as described herein. In embodiments, the method further comprises administering, or having administered, to the subject a therapeutically effective amount of an aminobisphosphonate drug.

BRIEF DESCRIPTION OF THE DRAWINGS

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

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

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

Figure 4 depicts various linear maps of lentiviral vectors expressing a FDPS shRNA targeting sequence in combination with BTN3A3 and/or IL-2, IL-15, and IL-18.

Figure 5 depicts FACS data demonstrating activation of Vδ2+ T cells by PC3 prostate carcinoma cells with a lentivirus expressing BTN3A3 (R381H) or BTN3A3 (WT), as described herein.

Figure 6 depicts FACS data demonstrating activation of Vδ2+ T cells HepG2 cells with a lentivirus expressing BTN3A3 (R381H) or both BTN3A3 (R381H) and shRNA #4, as described herein.

Figure 7 depicts FACS data demonstrating activation of Vδ2+ T cells by PC3 prostate carcinoma cells with a lentivirus expressing BTN3A3 (R381H) or both BTN3A3 (R381H) and shRNA #4, as described herein.

Figure 8 depicts FACS data demonstrating activation of Vδ2+ T cells by HepG2 cells with a lentivirus expressing shFDPS-IL-2, as described herein.

Figure 9 depicts FACS data demonstrating activation of Vδ2+ T cells by PC3 cells with a lentivirus expressing shFDPS-IL-2, as described herein.

Figure 10 depicts FACS data demonstrating activation of Vδ2+ T cells by PC3 cells with a lentivirus expressing shFDPS-IL-15, as described herein.

Figure 11 depicts data demonstrating the extracellular expression of BTN3A3 in PC3 and HepG2 cells with a lentivirus expressing BTN3A3 (R381H) or BTN3A3 (R381H) and shFDPS.

Figure 12 depicts FACS data demonstrating activation of Vδ2+ T cells by HepG2 cells transduced with a lentivirus expressing Lv-shFDPS, as described herein.

Figure 13 depicts data demonstrating delayed growth of tumors in mice injected with PC3 cells transduced with a lentivirus expressing Lv-shFDPS, as described herein.

Figure 14 depicts data demonstrating survival of mice injected with a lentivirus expressing Lv-shFDPS and subsequently treated with PBMC and/or zoledronic acid.

Figure 15 depicts data demonstrating tumor volume of mice injected with a lentivirus expressing Lv-shFDPS and subsequently treated with PBMC and/or zoledronic acid.

Figure 16 depicts the gross appearance of Lv-shFDPS PC3 xenografted tumors treated and untreated with PBMC.

Figure 17 depicts a lentiviral vector containing a H1 promoter with a synthetic shRNA sequence targeting FDPS, GGPS1, or IDI1, and a lentiviral vector containing an elongation factor 1 alpha promoter with a synthetic microRNA having a FDPS targeting sequence.

Figure 18 depicts data demonstrating reduction of FDPS protein expression in HepG2 cells transduced with lentivirus expressing shFDPS #1 (SEQ ID NO: 1) or shFDPS #4 (SEQ ID NO: 4) and treated with or without zoledronic acid, as described herein.

Figures 19A and 19B depict data demonstrating reduction of FDPS RNA (Figure 19A) and protein expression (Figure 19B) in PC3 cells transduced with lentivirus expressing shFDPS-A (SEQ ID NO: 64), shFDPS-R (SEQ ID NO: 65), shFDPS-TT (SEQ ID NO: 66), and shFDPS-L (SEQ ID NO: 67), as described herein.

Figure 20 depicts reduction of FDPS protein expression in HepG2 cells transduced

with lentivirus expressing shFDPS-4 (SEQ ID NO: 4), miR30-FDPS-1 (SEQ ID NO: 68) and miR30-FDPS-3 (SEQ ID NO: 69), as described herein.

Figure 21 depicts FACS data demonstrating activation of Vδ2+ T cells by THP-1 cells transduced with a lentivirus expressing miR30-FDPS #1 (SEQ ID NO: 68) and treated with or without zoledronic acid, as described herein.

Figure 22 depicts reduction of GGPS1 protein expression in HeLa cells transduced with lentivirus expressing shGGPS1 #1 (SEQ ID NO: 70), shGGPS1 #2 (SEQ ID NO: 71), and shGGPS1 #3 (SEQ ID NO: 73), as described herein.

Figure 23 depicts FACS data demonstrating activation of Vδ2+ T cells by PC3 cells transduced with a lentivirus expressing shFDPS sequence #4 (SEQ ID NO: 4) or shGGPS1 sequence #1 (SEQ ID NO: 70) and treated with or without zoledronic acid, as described herein.

Figure 24 depicts FACS data demonstrating activation of Vδ2+ T cells by HepG2 cells transduced with a lentivirus expressing shFDPS sequence #4 (SEQ ID NO: 4) or shGGPS1 sequence #1 (SEQ ID NO: 70) and treated with or without zoledronic acid, as described herein.

Figure 25 depicts FACS data demonstrating activation of Vδ2+ T cells by THP-1 cells transduced with a lentivirus expressing shFDPS sequence #4 (SEQ ID NO: 4) and/or shGGPS1 sequence #1 (SEQ ID NO: 70) and treated with or without zoledronic acid, as described herein.

Figure 26 depicts reduction of IDI1 protein expression in PC3 cells transduced with lentivirus expressing shIDI1 (SEQ ID NO: 76), as described herein.

Figure 27 depicts FACS data demonstrating activation of Vδ2+ T cells by PC3 cells transduced with a lentivirus expressing shFDPS sequence #4 (SEQ ID NO: 1) or shIDI1 sequence #1 (SEQ ID NO: 76), as described herein.

Figure 28 depicts data demonstrating activation of Vδ2+ T cells by THP-1 cells treated with zoledronic acid, FTI277, or zaragozic acid, as described herein.

Figure 29 depicts FACS data demonstrating activation of Vδ2+ T cells by PC3 cells transduced with a lentivirus expressing shFDPS sequence #4 (SEQ ID NO: 4) and treated with zoledronic acid, FTI277, or zaragozic acid, as described herein.

Figure 30 depicts FACS data demonstrating activation of Vδ2+ T cells by HepG2 cells transduced with a lentivirus expressing shFDPS sequence #4 (SEQ ID NO: 4) and treated with zoledronic acid, FTI277, or zaragozic acid, as described herein.

DETAILED DESCRIPTION

Overview of Disclosure

The present disclosure relates to gene therapy constructs and delivery of the same to cells, resulting in suppression of Farnesyl diphosphate synthase ("FDPS") or other enzymes of the mevalonate pathway, which are necessary to convert isopentenyl phosphate (IPP) to farnesyl diphosphate (FDP) and other downstream products of the mevalonate pathway, as shown, for example, in Figure 1. In embodiments, one or more viral vectors are provided with microRNAs or short hairpin RNAs (shRNA) that target one or more of FDPS, GGPS1, IDI1, F-Tase, or squalene synthase, thereby reducing expression levels of these enzymes. The viral vectors include lentiviral vectors and AAV vectors. A consequence of modulating expression of FDPS and other enzymes of the mevalonate pathway is to increase the accumulation of IPP, which is a stimulator of GD T cell proliferation and differentiation. A consequence of modulating expression of GGPS1 and other enzymes of the mevalonate pathway is to decrease GGPP levels, which allows secretion of cytokines including interleukin-1 beta and interleukin-18. Accordingly, the constructs provided herein are used to activate GD T cells, and are used to treat cancers and infectious diseases.

Definitions and Interpretation

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

Sons, Inc. (2003). Any enzymatic reactions or purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclature used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art.

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

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

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

As used herein, the terms "administration of" or "administering" refer to providing an active agent to a 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 "butyrophilin 3A" may be referred to herein as "BTN3A". Further, "butyrophilin 3A1" may be referred to herein as "BTN3A1", and may include the BTN3A1 portion of SEQ ID NO: 53. Butyrophilin 3A3 may be referred to herein as "BTN3A3" (SEQ ID NO: 17). Variants of BTN3A3, include, but are not limited to, BTN3A3 (R381H), and may include the BTN3A3 portion of SEQ ID NO: 54 or SEQ ID NO: 55 or SEQ ID NO: 59. Reference to "R381H" is reference to an arginine (R) amino acid being substituted by a histidine (H) amino acid at amino acid position 381. This convention for defining amino acid substitutions may be used for other positions and other amino acids herein.

As used herein, the term "CA19-9" refers to carbohydrate antigen 19-9. As used herein, the term "CC chemokine" refers to a class of chemokine proteins characterized by having two adjacent cysteines near their amino terminus. The term "CXC chemokine" refers to a class of chemokine proteins characterized by having two cysteines separated by one amino acid near their amino terminus. The term "CX3C chemokine" refers to a class of chemokine proteins characterized by having two cysteines separated by three amino acids near their amino terminus. The term "XC chemokine" refers to a class of chemokine proteins characterized by having one cysteine adjacent an amino acid near their amino terminus.

As used herein, the term "CD" refers to a cluster of differentiation protein. Examples of such proteins include, but are not limited to CD4 and CD8. Reference, for example, to CD4+ indicates that the CD4 protein is positively expressed.

As used herein, the term "CEA" refers to carcinoembryonic antigen.

As used herein, the terms "bisphosphonates" and "bisphosphonate drugs" refer to therapeutic agents of various embodiments, and encompass any of aminobisphosphonates, diphosphonates, biphosphonic acids, and diphosphonic acids, as well as pharmaceutically acceptable salts and derivatives thereof. The use of a specific nomenclature in referring to bisphosphonates is not meant to limit the scope of the present invention, unless specifically indicated.

As used herein, the terms "co-administration" or "combined administration" or "combined use" or "combination therapy" or the like as utilized herein refer to administration of a therapeutic vector or a lentiviral particle and a bisphosphonate drug or any combination of these to a single subject in need thereof (*e.g.*, a patient), and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration and/or at the same time.

As used herein, the term "fixed combination" refers to two or more active ingredients or components, including any of their respective compositions, formulations or drug forms, *e.g.*, a therapeutic vector or a lentiviral particle and a bisphosphonate drug or any combination of these, that are administered essentially in combination to a patient, for example essentially simultaneously, in the form of a single entity or dosage or combined entities or dosages, *e.g.*, in one tablet or in one capsule or in combined tablets or capsules or combined liquid forms.

As used herein, the term "non-fixed combination" refers to two or more active ingredients or components, including any of their respective compositions, formulations or drug forms, *e.g.*, a therapeutic vector or a lentiviral particle and a bisphosphonate drug or any

combination of these, that are administered in combination to a patient as separate entities either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides therapeutically effective levels of the active components in the patient. The non-fixed combination can be dosed independently of each other or by use of different fixed combinations *e.g.,* simultaneously or at different time points. The active components may be administered as separate pharmaceutical dosage forms or pharmaceutical formulations that may be, for example, sold independently of each other, with or without label instructions concerning the possibility of a combined use. Such instructions may be provided in the package equipment, *e.g.,* leaflet or the like, or in other information, *e.g.,* provided to physicians and medical staff. A non-fixed combination, its respective active ingredients or components, including any of their respective compositions, formulations or drug forms, or the parts thereof, can be administered simultaneously or chronologically staggered, *e.g.,* at different time points and with equal or different time intervals for any part of the administration. Such time intervals may be chosen such that the effect on the treated disease, when treated in combination, is more effective than would be obtained by use of only any one of the active components.

As used herein, the terms "combination," "in combination" and "combination therapies," may refer generally to any or both of the "fixed combination" and "non-fixed combination" definitions and embodiments described above.

As used herein, the transitional term "comprising," when used to define compositions and methods, means that the compositions and methods include the recited elements, but does not exclude others. As used herein, "consisting essentially of," when used to define compositions and methods, means that the composition and methods include additional elements, but only if those additional elements do not materially affect the basic and novel characteristics of the composition or methods. As used herein, "consisting of," when used to define compositions and methods, means that the compositions and methods exclude more than trace elements of other ingredients for compositions and substantial method steps. Embodiments defined by each of these transitional terms are within the scope of this disclosure. For example, 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, the terms "expression," "expressed," or "encodes" refer to a 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.

As used herein, 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.

As used herein, the term "gamma delta T cell" may also be referred to herein as a γδ T cell, a Vγ9Vδ2 T cell, a Vgamma9Vdelta2 T cell, a Vγ2Vδ2 T cell, a Vgamma2Vdelta2 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 as killing a target cell or assisting another effector cell to kill a target cell.

As used herein, the term "F-Tase" refers to farnesyl transferase.

As used herein, the term "GGPP" refers to geranylgeranyl pyrophosphate, and may also be referred to herein as geranylgeranyl diphosphate.

As used herein, the terms "GGDPS," "GGPPS," "GGDPS1," "GGPS1" and "GGPPS1" refer to geranylgeranyl diphosphate synthase 1, and may also be referred to herein as geranylgeranyl pyrophosphate synthase or geranylgeranyl-diphosphate synthase.

As used herein, the term "HER-2" refers to human epidermal growth factor receptor 2.

As used herein, cytokines such as "interleukin 2" may also be referred to as "IL-2," "IL2" and the like. IL-2 can also include reference to SEQ ID NO: 56. In a related manner, "interleukin 15" can also include reference to SEQ ID NO: 57. In a related manner, "interleukin 18" can also include reference to SEQ ID NO: 58. In a related manner, "interleukin 23" can also include reference to SEQ ID NO: 60. In a related manner, "interleukin 36" can also include reference to any of SEQ ID NOs: 61-63. In general, the prefix "IL" refers to an interleukin.

As used herein, the term "IDI1" refers to isopentenyl-diphosphate delta isomerase 1.

As used herein, the term "IFN" refers to interferon, and the terms IFN-gamma and IFN-γ refer to interferon-gamma.

As used herein, the terms "individual," "subject," and "patient" are used

interchangeably herein, and refer to any individual mammal subject, *e.g.*, bovine, canine, feline, equine, and/or human.

As used herein, the term "IPP" refers to isopentenyl pyrophosphate.

As used herein, the term "M2-PK" refers to pyruvate kinase isoenzyme type M2.

As used herein, the term "MHC" refers to a major histocompatibility complex.

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

As used herein, the term "NK cell" or "NK receptor family" refers to a "natural killer cell" or "natural killer cell receptor family", respectively.

As used herein, the term "packaging cell line" refers to any cell line that can be used to express a lentiviral particle.

As used herein, the term "PBMC" refers to peripheral blood mononuclear cells.

As used herein, the term "homology" refers to the percentage number of amino acids, nucleic acids, or analogs thereof, that are identical or constitute conservative substitutions. Homology may be determined using sequence comparison programs such as GAP (Deveraux et al., 1984, Nucleic Acids Research 12, 387-395). In this way sequences of a similar or substantially different length to those cited herein could be compared by insertion of gaps into the alignment, such gaps being determined, for example, by the comparison algorithm used by GAP.

As used herein, the term "sequence identity," which also may appear in the non-limiting context of "a sequence 50% identical to," and "having at least 80%, or at least 85%, or at least 90%, or at least 95% identity with" a given sequence, as similar pharasings, as used herein, refers to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity" may be calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.*, A, T, C, G, I) or the identical amino acid residue (*e.g.*, Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. Optimal alignment of sequences for aligning a comparison window may be conducted by computerized implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science

Drive Madison, Wis., USA) or by inspection and the best alignment (*i.e.*, resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul et al., Nucl. Acids Res. 25:3389, 1997.

As used here, the term "percent identity," which may be used interchangeably with the term "sequence 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*).

Suitable algorithms for determining percent sequence identity include 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, wordlength = 3 to obtain amino acid sequences homologous to the protein molecules of the disclosure. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.* Nucleic Acids Res. 25(17):3389-3402 (1997). 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, the term "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.* J Pharm Sci 66:1-19) (1977).

As used herein, the term "pharmaceutically acceptable salt" refers to derivatives of compounds or other active ingredients, wherein the parent compound or active ingredient is modified by converting an existing acid or base moiety to its salt form. Non-limiting

examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; alkali metal, alkaline metal, ammonium, and mono-, di, tri-, or tetra-C1-C30-alkyl-substituted ammonium; and the like. The pharmaceutically acceptable salts of various embodiments include the conventional non-toxic salts of the compound or active ingredient formed, for example, from nontoxic inorganic or organic acids. Suitable organic acids are, *e.g.*, carboxylic acids or sulfonic acids, such as acetic acid, succinic acid, fumaric acid or methansulfonic acid. The pharmaceutically acceptable salts herein can be synthesized from the parent compound or active ingredient which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418 and Journal of Pharmaceutical Science, 66, 2 (1977), each of which is incorporated herein by reference in its entirety.

As used herein, the term "PSA" refers to prostate-specific antigen.

As used herein, the term "RANTES" is synonymous with chemokine (C-C motif) ligand 5, which is also synonymous with CCL5.

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 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). In embodiments, "small RNA" are capable of inhibiting or knocking-down gene expression of a target gene, generally through pathways that result in the inhibition or destruction of the target gene mRNA.

As used herein, the term "TCR" refers to a T cell receptor, and the term "TCRs" refers to the plural form thereof.

As used herein, 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, and a lentivirus plasmid as mentioned, for example in Figures 2 and 3 herein.

As used herein, the term "TNF" refers to tumor necrosis factor, and reference to TNF-alpha or TNF-α refers to tumor necrosis factor-alpha.

As used herein, the terms "treatment" and "treating" refer to the intended targeting of a disease state and combatting of it, *i.e.*, ameliorating or preventing the disease state. A 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.

As used herein, the terms "treatment" or "treating" generally refer 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.

As used herein, the term "VSVG" or "VSV-G" refers to vesicular stomatitis virus G envelope glycoprotein.

Description of Aspects of the Disclosure

In an aspect of the disclosure, a viral vector comprising first and second encoded genetic elements is disclosed. The first encoded genetic element comprises a small RNA capable of inhibiting production of an enzyme involved in the mevalonate pathway, and the second encoded genetic element comprises one of a butyrophilin family member, a cytokine, or a chemokine. In embodiments, the viral vector includes a third encoded genetic element, wherein the third encoded genetic element comprises one of a butyrophilin family member, a cytokine, or a chemokine. In embodiments, the viral vector includes a fourth encoded genetic

element, wherein the fourth encoded genetic element comprises one of a butyrophilin family member, a cytokine, or a chemokine. In embodiments, the enzyme is farnesyl diphosphate synthase (FDPS) or a functional variant thereof. In embodiments, the first encoded genetic element comprises a microRNA or a shRNA. In embodiments, the shRNA comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 95% percent identity or more with

GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTACTCCAGGACTTTTT (SEQ ID NO: 1);

GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCTGAACGAAATCCTGCTTTTT (SEQ ID NO: 2);

GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGTACATGGCTTTTT (SEQ ID NO: 3); or

GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCTTTTT (SEQ ID NO: 4).

In embodiments, the shRNA comprises:

GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTACTCCAGGACTTTTT (SEQ ID NO: 1);

GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCTGAACGAAATCCTGCTTTTT (SEQ ID NO: 2);

GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGTACATGGCTTTTT (SEQ ID NO: 3); or

GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCTTTTT (SEQ ID NO: 4).

In embodiments, the shRNA comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96% , at least 97% , at least 98% , at least 99%, or 100% identity with SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, or SEQ ID NO: 67.

In embodiments, the miRNA comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96% , at least 97% , at least 98% , at least 99%, or 100% identity with SEQ ID NO: 68 or SEQ ID NO: 69.

In embodiments, the enzyme is GGPS1 or a functional variant thereof. In embodiments, the shRNA comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96% , at least 97% , at least 98% , at least 99%, or 100% identity with SEQ ID NO: 70, SEQ ID NO: 71, or SEQ ID NO: 72.

In embodiments, the enzyme is IDI1 or a functional variant thereof. In embodiments, the shRNA comprises a sequence having at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96% , at least 97% , at least 98% , at least 99%, or 100% identity with SEQ ID NO: 76.

In embodiments, the enzyme is F-Tase, or squalene synthase, or functional variants thereof.

In embodiments, the butyrophilin family member comprises BTN3A3, BTN3A3, or BTN3A1. In embodiments, the butyrophilin family member comprises BTN3A3 (R381H). In embodiments, the butyrophilin family member comprises a butyrophilin-like molecule. In embodiments, the butyrophilin-like molecule comprises BTNL3 or BTNL8. In embodiments, the cytokine comprises IL-1, IL-1β, IL-2, IL-4, IL-7, IL-12, IL-15, IL-17, IL-18, IL-23, IL-33, IL-36, TNF-α, or interferon-γ.

In embodiments, the chemokine comprises a CC chemokine, a CXC chemokine, a CX3C chemokine, a C chemokine, or a XC chemokine. In further embodiments, the CC chemokine comprises RANTES. In embodiments, the viral vector is a lentiviral vector. In further embodiments, the C chemokine comprises XCL1 (Lymphotactin).

In another aspect, a lentiviral vector system for expressing a lentiviral particle is disclosed. The system includes a lentiviral vector as detailed herein; at least one envelope plasmid for expressing an envelope protein optimized for infecting a target cell; and at least one helper plasmid for expressing gag, pol, and rev genes, or functional variants thereof, wherein when the lentiviral vector, the at least one envelope plasmid, and the at least one helper plasmid are transfected into a packaging cell, a lentiviral particle is produced by the packaging cell, wherein the lentiviral particle is capable of infecting the target cell and inhibiting an enzyme involved in the mevalonate pathway within the target cell.

In embodiments, the lentiviral particle is capable of causing increased levels of a first product of the mevalonate pathway. In embodiments, the first product comprises IPP. In embodiments, the lentiviral particle is capable of causing decreased levels of a second

product of the mevalonate pathway. In embodiments, the second product comprises GGPP. In embodiments, the lentiviral product increases the first product and decreases the second product.

In embodiments, the lentiviral particle encodes a small RNA capable of targeting a first target of the mevalonate pathway. In embodiments, the lentiviral particle further encodes a small RNA capable of targeting a second target of the mevalonate pathway. In embodiments, at least one of the first target and the second target is an enzyme. In embodiments, at least one of the first target and the second target is FDPS, GGPS1, IDI1, F-Tase, or squalene synthase.

In embodiments, targeting of the first target by the small RNA causes an increase in the presence, level, or concentration of a first product of the mevalonate pathway. In embodiments, the presence, level, or concentration of the first product of the mevalonate pathway is increased by up to 10% over a first product control, wherein the first product control can mean the presence, level, or concentration of the first product when the first target is not targeted by the small RNA. In embodiments, the presence, level, or concentration of the first product of the mevalonate pathway is increased by up to 10% to up to 20% over the first product control, as described herein. In embodiments, the presence, level, or concentration of the first product of the mevalonate pathway is increased by up to 20% to up to 30% over the first product control, as described herein. In embodiments, the presence, level, or concentration of the first product of the mevalonate pathway is increased by up to 30% to up to 40% over the first product control, as described herein. In embodiments, the presence, level, or concentration of the first product of the mevalonate pathway is increased by up to 40% to up to 50% over the first product control, as described herein. In embodiments, the presence, level, or concentration of the first product of the mevalonate pathway is increased by more than 50% over the first product control, as described herein. In embodiments, the first product of the mevalonate pathway comprises IPP.

In embodiments, targeting of the second target by the small RNA causes a decrease in the presence, level, or concentration of a second product of the mevalonate pathway. In embodiments, the presence, level, or concentration of the second product of the mevalonate pathway is decreased by up to 10% of a second product control, wherein the second product control can mean the presence, level, or concentration of the second product when the second target is not targeted by the small RNA. In embodiments, the presence, level, or concentration of the second product of the mevalonate pathway is decreased by up to 10% to up to 20% of the second product control, as described herein. In embodiments, the presence,

level, or concentration of the second product of the mevalonate pathway is decreased by up to 20% to up to 30% of the second product control, as described herein. In embodiments, the presence, level, or concentration of the second product of the mevalonate pathway is decreased by up to 30% to up to 40% of the second product control, as described herein. In embodiments, the presence, level, or concentration of the second product of the mevalonate pathway is decreased by up to 40% to up to 50% of the second product control, as described herein. In embodiments, the presence, level, or concentration of the second product of the mevalonate pathway is decreased by more than 50% of the second product control, as described herein. In embodiments, the second product of the mevalonate pathway comprises GGPP.

In embodiments, the increase in the presence, level, or concentration of the first product of the mevalonate pathway causes an increase in gamma delta (GD) T cell activation. In embodiments, GD T cell activation is increased by up to 10% over a first activation control, wherein the first activation control can mean the level of GD T cell activation when the first target is not targeted by the small RNA. In embodiments, GD T cell activation caused by modulation of the first product is increased by up to 10% to up to 20% over the first activation control, as described herein. In embodiments, GD T cell activation caused by modulation of the first product is inceased by up to 20% to up to 30% over the first activation control, as described herein. In embodiments, GD T cell activation caused by modulation of the first product is increased by up to 30% to up to 40% over the first activation control, as described herein. In embodiments, GD T cell activation caused by modulation of the first product is increased by up to 40% to up to 50% over the first activation control, as described herein. In embodiments, GD T cell activation caused by modulation of the first product is increased by 50% or more over first activation control, as described herein.

In embodiments, the decrease in the presence, level, or concentration of the second product of the mevalonate pathway causes an increase in gamma delta (GD) T cell activation. In embodiments, GD T cell activation caused by modulation of the second product is increased by up to 10% over a second activation control, wherein the second activation control can mean the level of GD T cell activation when the second target is not targeted by the small RNA. In embodiments, GD T cell activation caused by modulation of the second product is increased by up to 10% to up to 20% over the second activation control, as described herein. In embodiments, GD T cell activation caused by modulation of the second product is increased by up to 20% to up to 30% over the second activation control, as described herein. In embodiments, GD T cell activation caused by modulation of the second

product is increased by up to 30% to up to 40% over the second activation control, as described herein. In embodiments, GD T cell activation caused by modulation of the second product is increased by up to 40% to up to 50% over the second activation control, as described herein. In embodiments, GD T cell activation caused by modulation of the second product is increased by 50% or more over the second activation control, as described herein.

In another aspect, a lentiviral particle capable of infecting a target cell is disclosed. The lentiviral particle comprises an envelope protein optimized for infecting the target cell, and a lentiviral vector as detailed herein. In embodiments, the target cell is a cancer cell.

In another aspect, a method of activating a gamma delta (GD) T cell is disclosed. The method includes infecting, in the presence of the GD T cell, a target cell with a lentiviral particle, wherein the lentiviral particle comprises a viral vector comprising first and second encoded genetic elements wherein the first encoded genetic element comprises a small RNA capable of inhibiting production of an enzyme involved in the mevalonate pathway, and the second encoded genetic element comprises one of a butyrophilin family member, a cytokine, or a chemokine, wherein when the enzyme is inhibited in the target cell, the target cell activates the GD T cell. In embodiments, the enzyme comprises at least one of FDPS, GGPS1, IDI1, F-Tase, and/or squalene synthase, or functional variants thereof.

In embodiments, the target cell is a cancer cell. In embodiments, the method further comprises contacting the target cell and the GD T cell with an amount of 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 includes administering to the subject a therapeutically effective amount of a lentiviral particle wherein the lentiviral particle comprises a viral vector comprising first and second encoded genetic elements, wherein the first encoded genetic element comprises a small RNA capable of inhibiting production of an enzyme involved in the mevalonate pathway, and the second encoded genetic element comprises one of a butyrophilin family member, a cytokine, or a chemokine, wherein when the enzyme is inhibited in a cancer cell in the presence of a GD T cell, the target cell activates the GD T cell, to thereby treat the cancer. In embodiments, the enzyme comprises at least one of FDPS, GGPS1, IDI1, F-Tase, squalene synthase, and/or functional variants thereof.

In embodiments, the method further comprises contacting the target cell and the GD T cell with an amount of an aminobisphosphonate drug. In embodiments, the method includes administering to the subject a therapeutically effective amount of a lentiviral particle wherein

the lentiviral particle comprises a viral vector comprising first, second and third encoded genetic elements wherein the first encoded genetic element comprises a small RNA or RNAs capable of inhibiting production of an enzyme or enzymes involved in the mevalonate pathway, the second encoded genetic element comprises a butyrophilin family member, and the third genetic element encodes a cytokine or a chemokine, wherein when the enzyme is inhibited in a cancer cell in the presence of a GD T cell, the target cell activates the GD T cell, the butyrophilin increases efficiency of activating GD T cells, the cytokine increases GD T cell activation and proliferation, and the chemokine increases the presence of the GD T cells at a tumor site to thereby treat the cancer. In embodiments, the method further comprises exposure of the target cell and the GD T cell with an amount of an aminobisphosphonate drug. In embodiments, the aminobisphosphonate drug is zoledronic acid.

In embodiments, the butyrophilin family member includes BTN3A3 (SEQ ID NO: 17) or BTN3A3 (R381H) (SEQ ID NO: 54). In embodiments, the cytokine includes IL-2, IL-12, IL-15, IL-18, IL-23, or IL-36 but can also include other cytokines which are known to activate immune cells, such as T cells. In embodiments, the chemokine may include chemokine (C-C motif) ligand 5 encoded by the CCL5 gene, or other chemokines known to be recognized by GD T cell receptors and known to be capable of attracting GD T cells to sites of tumor growth.

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, and can also include a cancer cell population from any of the foregoing, and can be associated with one or more of carcinomas, sarcomas, myelomas, leukemias, lymphomas, mixed types or mixtures of the foregoing. In still a further aspect cancer includes, but is not limited to astrocytoma, acute myeloid leukemia, anaplastic large cell lymphoma, acute lymphoblastic

leukemia, angiosarcoma, B-cell lymphoma, Burkitt's lymphoma, breast carcinoma, bladder carcinoma, carcinoma of the head and neck, cervical carcinoma, chronic lymphoblastic leukemia, chronic myeloid leukemia, colorectal carcinoma, endometrial carcinoma, esophageal squamous cell carcinoma, Ewing's sarcoma, fibrosarcoma, glioma, glioblastoma, gastrinoma, gastric carcinoma, hepatoblastoma, hepatocellular carcinoma, Kaposi's sarcoma, Hodgkin lymphoma, laryngeal squamous cell carcinoma, larynx carcinoma, leukemia, leiomyosarcoma, lipoma, liposarcoma, melanoma, mantle cell lymphoma, medulloblastoma, mesothelioma, myxofibrosarcoma, myeloid leukemia, mucosa-associated lymphoid tissue B cell lymphoma, multiple myeloma, high-risk myelodysplastic syndrome, nasopharyngeal carcinoma, neuroblastoma, neurofibroma, high-grade non-Hodgkin lymphoma, non-Hodgkin lymphoma, lung carcinoma, non-small cell lung carcinoma, ovarian carcinoma, esophageal carcinoma, osteosarcoma, pancreatic carcinoma, pheochromocytoma, prostate carcinoma, renal cell carcinoma, retinoblastoma, rhabdomyosarcoma, salivary gland tumor, schwanomma, small cell lung cancer, squamous cell carcinoma of the head and neck, testicular tumor, thyroid carcinoma, urothelial carcinoma, and Wilms tumor.

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

Infectious Diseases

The compositions and methods disclosed herein can be used to treat infectious diseases. The term “infectious disease” includes any disease that is caused by an infectious agent. An “infectious agent” includes any exogenous pathogen including, without limitation, bacteria, fungi, viruses, mycoplasma, and parasites. Infectious agents that may be treated with compositions provided for in this disclosure include any art-recognized infectious organisms that cause pathogenesis in an animal, including such organisms as bacteria that are gram-negative or gram-positive cocci or bacilli, DNA and RNA viruses, including, but not limited to, DNA viruses such as papilloma viruses, parvoviruses, adenoviruses, herpesviruses and vaccinia viruses, and RNA viruses, such as arenaviruses, coronaviruses, rhinoviruses, respiratory syncytial viruses, influenza viruses, picomaviruses, paramyxoviruses, reoviruses, retroviruses, and rhabdoviruses. Examples of fungi that may be treated with the compositions and methods of the disclosure include fungi that grow as molds or are yeastlike, including, for example, fungi that cause diseases such as ringworm, histoplasmosis, blastomycosis,

aspergillosis, cryptococcosis, sporotrichosis, coccidioidomycosis, paracoccidio-idomycosis, and candidiasis. Compositions and methods provided for herein may be utilized to treat parasitic infections including, but not limited to, infections caused by somatic tapeworms, blood flukes, tissue roundworms, ameba, and *Plasmodium, Trypanosoma, Leishmania*, and *Toxoplasma* species.

Methods of GD T Cell Activation

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

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

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

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

In embodiments, the disclosed compositions and methods provided herein comprise a form of gene therapy for activating GD T cells at the site of tumor. In an aspect, the compositions and methods provided herein activate GD T cells and support their

proliferation, differentiation, and functional capacities by promoting the production of specific cytokines needed for cytolytic activity capable of killing cancer cells or treating infectious diseases.

In embodiments, the gene therapy sequences (*e.g.*, FDPS shRNAs, FDPS miRNAs, GGPS1 shRNAs, IDI1 shRNAs, F-Tase small RNAs, or squalene synthase small RNAs) are carried by therapeutic vectors, including but not limited to viral vectors such as lentiviruses or adeno-associated viruses, although other viral vectors can also be suitable. Gene therapy constructs may also be delivered in the form of DNA or RNA, including but not limited to plasmid forms. In embodiments, the disclosed gene therapy constructs may also be delivered in the form of protein-nucleic acid complexes or lipid nucleic acid complexes and mixtures of these formulations. For instance, a protein-nucleic acid complex can comprise nucleic acids of interest in a complex with cationic peptides such as lysine and arginine. Lipid-nucleic acids complexes can comprise lipid emulsions, micelles, liposomes, and/or mixtures of neutral and cationic lipids such as DOTMA, DOSPA, DOTAP, and DMRIE.

In embodiments, therapeutic vectors may comprise a single construct or at least two, at least three, at least four, or at least five different constructs. When more than one construct is present in a vector the constructs may be identical, or they may be different. For instance, the constructs may vary in terms of their promoters, the presence or absence of integrating elements, and/or their sequences.

In embodiments, a therapeutic vector will comprise at least one construct that encodes a small RNA capable of knocking down the expression of at least one of FDPS, GGPS1, IDI1, F-Tase, squalene synthase, and/or functional variants thereof. In embodiments, the therapeutic vector will also encode a specific cytokine(s) and/or chemokine(s), including but not limited to TNF-α, interferon-γ, IL-1, IL-1β, IL-2, IL-4, IL-7, IL-12, IL-15, IL-17, IL-18, IL-23, IL-33, IL-36, or RANTES. In embodiments, a single construct may encode both small RNAs capable of knocking down the expression of FDPS and specific cytokines or chemokines, including but not limited to TNF-α, interferon-γ, IL-1, IL-1β, IL-2, IL-4, IL-7, IL-12, IL-15, IL-17, IL-18, IL-23, IL-33, IL-36, or RANTES.

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

In embodiments, the disclosed constructs and vectors comprise short hairpin RNA ("shRNA"), micro RNA ("miRNA"), or siRNA capable of reducing or knocking down expression of FDPS, geranyl pyrophosphate synthase ("GPPS"), farnesyl transferase ("F-

Tase"), IDI1, and/or squalene synthase genes. By down regulating these genes, which control steroid and isoprenoid synthesis, isopentenyl pyrophosphate ("IPP") levels are elevated and/or GGPP levels are decreased. Elevation and accumulation of IPP is a mechanism for increasing GD T cells activation. Further, down regulation of these pyrophosphate synthase genes removes an important negative regulator of inflammasome function that in turn results in increased expression of cytokines that are important for GD T cell activation and effector cell function. BTN3A3 on the cancer cell surface and higher cytoplasmic levels of IPP potently stimulate Vgamma9Vdelta2 T cells (also referred to herein as Vγ9Vδ2 T cells).

In embodiments, the disclosed constructs are regulated by specific promoters that are capable of producing interleukin-2 and/or interleukin-15 to sustain GD T cell proliferation. However, as noted herein, other cytokines including IL-18, IL-23, and IL-36 can also be selected and used. In addition, the disclosed constructs may be regulated by specific promoters that are capable of producing interleukin-1 beta and/or interleukin-18 and/or interferon-gamma required for GD T cell differentiation and acquisition of all effector cell function. Desirable effector cell functions include the capacity for direct cytotoxic cell killing of tumors and/or infected cells, secretion of beneficial cytokines and/or chemokines, increased expression of NK receptors required to recognize cancerous or cells, and increased expression of Fc receptors needed to bind targeting antibodies in order to co-localize GD T cells with cancerous or infected cell targets.

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

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

In embodiments, the disclosed methods for activation of GD T cells further comprise a step of suppressing pathologic inflammatory responses that may include cellular

proliferation leading to atherosclerosis, chronic immune activation that stimulates tumor growth, autoimmune diseases including psoriasis and other presentations in the epidermis, inflammatory diseases of the central nervous system, and arthritis and other diseases of unregulated immune responses.

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

In embodiments, therapeutic vectors are administered in combination with bisphosphonate drugs. In various embodiments, such combinations achieve synergistic, positive or heightened activation of gamma delta T cells. Such positive activation may allow alternate, modified or reduced doses of bisphosphonates and may decrease adverse reactions to bisphosphonates including acute inflammatory responses and chronic diseases. Combinations of therapeutic vectors with bisphosphonates may be together or separate, with or without instructions for combined use or to combination products. The therapeutic vectors and/or bisphosphonates may be administered entirely separately and may be formulated in entirely distinct pharmaceutical dosage forms. The therapeutic vectors and/or bisphosphonates may be sold independently of each other, with or without label instructions concerning the possibility of a combined use. Such instructions also may be provided in the package equipment, *e.g.*, leaflet or the like, or in other information *e.g.*, provided to physicians and medical staff (*e.g.*, oral communications, communications in writing or the like). Such labels or other instructions can refer to either a fixed combination in one dosage unit form, or a non-fixed combination as a kit of parts for the combined administration where the therapeutic vector may be administered independently of the bisphosphonate drug, at the same time, or separately within time intervals. In various embodiments, the combination exhibits a cooperative or joint effect, or a decrease in toxicity or complications of treatment. In one embodiment the effect of the combination is synergistic. A synergistic effect is achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately. A synergistic effect may be attained when the active ingredients are: (1) co-formulated and administered or delivered simultaneously in a combined, unit dosage formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect may be attained when the compounds are administered or delivered

sequentially, *e.g.,* by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, i.e., serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together, albeit subject to potential variances in timing as detailed herein.

The combinations herein may be manufactured and/or formulated by the same or different manufacturers. The active ingredients may be brought together into a combination therapy: (i) prior to release of the combination product to physicians (*e.g.,* in the case of a kit comprising the compound of the disclosure and the other therapeutic agent); (ii) by the treating physician (or under the guidance of a physician) shortly before administration; (iii) in the actual patient, *e.g.,* during sequential administration of the active ingredients disclosed herein.

In embodiments, a therapeutically effective amount of each of the combinations may be administered simultaneously or sequentially and in any order, and the components may be administered together or separate. For example, the method of treating a proliferative disease according to the disclosure may comprise (i) administration of a first agent such as a therapeutic vector that forms part of a lentiviral particle and/or (ii) administration of a second agent such as a bisphosphonate drug in free or pharmaceutically acceptable salt form. The administration of agents (i), and/or (ii) may be simultaneous or sequential in any order, in therapeutically effective amounts, preferably in cooperative, jointly effective, and/or synergistically effective, amounts, *e.g.,* in daily or intermittent dosages corresponding to the amounts described herein. The combinations may be administered separately at different times during the course of therapy or concurrently in divided or single drug forms. Furthermore, the term "administering" also encompasses the use of a pro-drug of a combination partner that converts in vivo to the combination partner as such. The instant disclosure is therefore to be understood as embracing all such regimens of simultaneous or alternating treatment and the term "administering" is to be interpreted accordingly.

In embodiments, agents (i) and (ii) 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 For example, a therapeutic vector and/or bisphosphonate drug may be administered intravenously. Further, agents (i) and (ii) 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. For example, a bisphosphonate drug may be formulated into a tablet and administered orally.

A combination therapy according to the disclosure can besides or in addition be administered especially for cancer therapy in combination with chemotherapy, radiotherapy, immunotherapy, surgical intervention, or a combination of these. Long-term therapy is equally possible as is adjuvant therapy in the context of other treatment strategies, as described above. Other possible treatments are therapy to maintain the patient's status after tumor regression, or even chemo-preventive therapy, for example in patients at risk.

Constructs for GD T Cell Activation

Inhibition of FDPS, GGPS1, IDI1, and/or functional variants thereof may result in IPP accumulation and/or diminished GGPP levels, resulting in activation of Vdelta2+ GD T cells and expression of interferon-gamma, TNF-alpha, and IL-18, which are also important in activating GD T cells. Inhibition of farnesyl transferase and/or squalene synthase results in decreased prenylation of proteins. The disclosed constructs can be transfected or transduced into specific target cells, like tumor cells or infected cells, where they can express RNA sequences (*i.e.,* siRNA, shRNA or microRNA) that will inhibit translation of FDPS, GGPS1, IDI1, F-Tase, squalene synthase, and/or functional variants thereof, as well as encode and express cytotoxic cytokines or chemokines.

Disclosed herein are constructs for decreasing expression of FDPS, GGPS1, IDI1, F-Tase, squalene synthase, and/or functional variants thereof, increasing expression of cytokines, and increasing expression of chemokines including RANTES. For instance, in embodiments the constructs may encode for interferon-gamma, IL-1, IL-1β, IL-2, IL-4, IL-7, IL-12, IL-15, IL-17, IL-18, IL-23, IL-33, IL-36, or TNF-α.

Expression of cytokines and chemokines, like those listed above, will result in localized cytotoxic destruction of tumor cells or cells infected with pathogenic organisms. Accordingly, expression of such constructs by a tumor cell can result in the tumor cells assisting in their own destruction and activating an immune mechanism capable of destroying other tumor cells not genetically modified by the lentivirus vector. The capacity for genetically modified cells to activate GD T cells involves the GD T cell receptor, butyrophilin recognition, and the activation of GD T cell receptors for common gamma chain cytokines. Killing of tumor cells relies on a family of GD T cell surface receptors generally

described as members of the NK receptor family that distinguish tumor cells from normal cells and provide for selectivity in the cell killing process. Consequently, a small number of genetically modified tumor cells can activate a sufficient number of GD T cells to achieve broad destruction of tumors including killing of both genetically-modified and non-modified cells in the same or distant tumors. Accordingly, expression of such constructs by a tumor cell or an infected cell will result in the unwanted cells assisting in its own destruction.

Likewise, if the disclosed constructs are expressed in a tumor cell or infected cell, decreasing the expression of FDPS, GGPS1, IDI1, F-Tase, squalene synthase, and/or functional variants thereof may result in activation and recruitment of GD T cells to the tumor site of site of cell infection. Increasing expression of RANTES will further attract GD T cells to intended tissue location. Because GD T cells can kill a broad range of tumors of epithelial origin as well as many leukemias and lymphomas, and are further able to produce high levels of the anti-tumor cytokine, IFNγ, recruitment of GD T cells to the site of a tumor can be a particularly effective means of inducing anti-tumor immunity.

Decreased expression of FDPS, GGPS1, IDI1, F-Tase, squalene synthase, and/or functional variants thereof can be achieved via shRNA, microRNA, siRNA, or other means known in the art. For instance, shRNAs according to SEQ ID NOs: 1, 2, 3, or 4, or variants thereof can be used in the disclosed constructs and methods, although this example is not limiting. shRNAs according to SEQ ID NOs: 64-67, 70-72, 76, or variants thereof can be used in the disclosed constructs and methods, although this example is not limiting. miRNAs according to SEQ ID NOs: 68 or 69, or variants thereof can be used in the disclosed constructs and methods, although this example is not limiting. The coding regions for RNAs to decrease expression of FDPS, GGPS1, IDI1, F-Tase, squalene synthase, and/or functional variants thereof, and the coding regions of cytokine and chemokines may be in the same construct or on different constructs.

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

maintenance of the recombinant gene that may exceed the therapeutic interval.

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

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

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

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

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

Constructs may comprise specific promoters for expressing cytokines involved in the maintenance of GD T cells (*i.e.,* IL-2, IL-7, IL-12, IL-15, IL-17, IL-18, IL-23, or IL-36). For example, promoters that may be incorporated into the disclosed constructs include but are not limited to TATA-box promoters, CpG-box promoters, CCAAT-box promoters, TTGACA-box promoters, BRE-box promoters, INR-box promoters, AT-based promoters, CG-based

promoters, ATCG-compact promoters, ATCG-balanced promoters, ATCG-middle promoters, ATCG-less promoters, AT-less promoters, CG-less promoters, AT-spike promoters, and CG-spike promoters. See, for *e.g.*: Gagniuc and Ionescu-Tirgoviste, Eukaryotic genomes may exhibit up to 10 generic classes of gene promoters, BMC GENOMICS 13:512 (2012).

Therapeutic Vectors

The construct can be delivered via known transfection and/or transduction vectors, including but not limited to lentiviral vectors, adeno-associated virus, poxvirus, herpesvirus vectors, protein and/or lipid complexes, liposomes, micelles, bacterially-produced vesicles, eukaryotic cell-produced vesicles, exosomes 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, or lymphocytes). 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, Vesicular Stomatitis Virus G (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 and herpesvirus vectors target to macrophages, dendritic cells and epithelial cells, measles virus vectors may target to B cells, rabies viral vectors may target to neural cells.

Lentiviral Vector System

A lentiviral virion (particle) is expressed by a vector system encoding the necessary viral proteins to produce a virion (viral particle). There is at least one vector containing a nucleic acid sequence encoding the lentiviral pol proteins necessary for reverse transcription and integration, operably linked to a promoter. In another embodiment, the 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 embodiments, 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 GP, and GP_2 glycoproteins. In another embodiment, one can use different lentiviral capsids with a pseudotyped envelope (for example, FIV or SHIV [U.S. Patent 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, or functional variants thereof. Each of the gag, pol and rev genes, or functional variants thereof, 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.*, Figure 2).

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

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

In another aspect, and as detailed in Figure 2, the lentiviral vector, which is also referred to herein as a therapeutic vector, can include the following elements: hybrid 5' long terminal repeat (RSV/5' LTR) (SEQ ID NOs: 5-6), Psi sequence (RNA packaging site) (SEQ ID NO: 7), RRE (Rev-response element) (SEQ ID NO: 8), cPPT (polypurine tract) (SEQ ID NO: 9), H1 promoter (SEQ ID NO: 10), shFDPS (SEQ ID NOs: 1, 2, 3, 4), CMV (SEQ ID NO: 19), BTN3A3 (R381H) - T2A - IL-2 (collectively, SEQ ID NO: 55), Woodchuck Post-Transcriptional Regulatory Element (WPRE) (SEQ ID NO: 11), and 3' Delta LTR (SEQ ID NO: 12). 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: a CMV (CAG) enhancer (SEQ ID NO: 21); a Chicken beta actin (CAG) promoter (SEQ ID NO: 13); a chicken beta actin intron (SEQ ID NO: 22); a HIV gag (SEQ ID NO: 14); a HIV Pol (SEQ ID NO: 15); a HIV Int (SEQ ID NO: 16); a HIV RRE (SEQ ID NO: 8); a HIV Rev (SEQ ID NO: 18); and a rabbit beta globin poly A (SEQ ID NO: 23). 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: 19) and vesicular stomatitis virus G glycoprotein (VSV-G) (SEQ ID NO: 20). In another aspect, sequence variation, by way of substitution, deletion, addition, or mutation can be used to modify the sequences references herein.

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

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

Doses and Dosage Forms

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

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

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

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

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

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

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

In embodiments, the pharmaceutical composition comprising a vector can be

formulated in a solid dosage form for oral administration, and the solid dosage form can be powders, granules, capsules, tablets or pills. In 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 embodiments, the oral dosage form can be immediate release, or a modified release form. Modified release dosage forms include controlled or extended release, enteric release, and the like. The excipients used in the modified release dosage forms are commonly known to a person of ordinary skill in the art.

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

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

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

In 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 propylene glycol, polyethylene glycol, 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 disclosed vectors are administered into the cerebrospinal fluid, blood or lymphatic circulation by venous or arterial cannulation or injection, intradermal delivery, intramuscular delivery or injection into a draining organ near the site of disease.

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

Examples

Example 1 - Development of a Lentiviral Vector System.

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

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

Referring more specifically to Figure 2, the Helper plus Rev plasmid includes a CMV (CAG) enhancer (SEQ ID NO: 21); a Chicken beta actin (CAG) promoter (SEQ ID NO: 13); a chicken beta actin intron (SEQ ID NO: 22); a HIV gag (SEQ ID NO: 14); a HIV Pol (SEQ ID NO: 15); a HIV Int (SEQ ID NO: 16); a HIV RRE (SEQ ID NO: 8); a HIV Rev (SEQ ID NO: 18); and a rabbit beta globin poly A (SEQ ID NO: 23).

The Envelope plasmid includes a CMV promoter (SEQ ID NO: 19); a beta globin intron (SEQ ID NO: 24); a VSV-G (SEQ ID NO: 20); and a rabbit beta globin poly A (SEQ ID NO: 25).

Synthesis of a 2-vector lentiviral packaging system including Helper (plus Rev) and Envelope plasmids.

Materials and Methods:

Construction of the helper plasmid: The helper plasmid was constructed by initial PCR amplification of a DNA fragment from the pNL4-3 HIV plasmid (NIH Aids Reagent Program) containing Gag, Pol, and Integrase genes. Primers were designed to amplify the fragment with EcoRI and NotI restriction sites which could be used to insert at the same sites in the pCDNA3.1 plasmid (Invitrogen). The forward primer was (5'-TAAGCAGAATTC ATGAATTTGCCAGGAAGAT-3') (SEQ ID NO: 26) and reverse primer was (5'-CCATACAATGAATGGACACTAGGCGGCCGCACGAAT-3') (SEQ ID NO: 27).

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

GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGGGGAATTGGA
GGTTTTATCAAAGTAAGACAGTATGATCAGATACTCATAGAAATCTGCGGACATA
AAGCTATAGGTACAGTATTAGTAGGACCTACACCTGTCAACATAATTGGAAGAA
ATCTGTTGACTCAGATTGGCTGCACTTTAAATTTTCCCATTAGTCCTATTGAGACT
GTACCAGTAAAATTAAAGCCAGGAATGGATGGCCCAAAAGTTAAACAATGGCCA
TTGACAGAAGAAAAAATAAAAGCATTAGTAGAAATTTGTACAGAAATGGAAAAG
GAAGGAAAAATTTCAAAAATTGGGCCTGAAAATCCATACAATACTCCAGTATTT
GCCATAAAGAAAAAAGACAGTACTAAATGGAGAAAATTAGTAGATTTCAGAGAA
CTTAATAAGAGAACTCAAGATTTCTGGGAAGTTCAATTAGGAATACCACATCCTG
CAGGGTTAAAACAGAAAAAATCAGTAACAGTACTGGATGTGGGCGATGCATATT
TTTCAGTTCCCTTAGATAAAGACTTCAGGAAGTATACTGCATTTACCATACCTAG
TATAAACAATGAGACACCAGGGATTAGATATCAGTACAATGTGCTTCCACAGGG
ATGGAAAGGATCACCAGCAATATTCCAGTGTAGCATGACAAAAATCTTAGAGCC
TTTTAGAAAACAAAATCCAGACATAGTCATCTATCAATACATGGATGATTTGTAT
GTAGGATCTGACTTAGAAATAGGGCAGCATAGAACAAAAATAGAGGAACTGAG
ACAACATCTGTTGAGGTGGGGATTTACCACACCAGACAAAAAACATCAGAAAGA
ACCTCCATTCCTTTGGATGGGTTATGAACTCCATCCTGATAAATGGACAGTACAG
CCTATAGTGCTGCCAGAAAAGGACAGCTGGACTGTCAATGACATACAGAAATTA
GTGGGAAAATTGAATTGGGCAAGTCAGATTTATGCAGGGATTAAAGTAAGGCAA

TTATGTAAACTTCTTAGGGGAACCAAAGCACTAACAGAAGTAGTACCACTAACA
GAAGAAGCAGAGCTAGAACTGGCAGAAAACAGGGAGATTCTAAAAGAACCGGT
ACATGGAGTGTATTATGACCCATCAAAAGACTTAATAGCAGAAATACAGAAGCA
GGGGCAAGGCCAATGGACATATCAAATTTATCAAGAGCCATTTAAAAATCTGAA
AACAGGAAAGTATGCAAGAATGAAGGGTGCCCACACTAATGATGTGAAACAATT
AACAGAGGCAGTACAAAAAATAGCCACAGAAAGCATAGTAATATGGGGAAAGA
CTCCTAAATTTAAATTACCCATACAAAAGGAAACATGGGAAGCATGGTGGACAG
AGTATTGGCAAGCCACCTGGATTCCTGAGTGGGAGTTTGTCAATACCCCTCCCTT
AGTGAAGTTATGGTACCAGTTAGAGAAAGAACCCATAATAGGAGCAGAAACTTT
CTATGTAGATGGGGCAGCCAATAGGGAAACTAAATTAGGAAAAGCAGGATATGT
AACTGACAGAGGAAGACAAAAAGTTGTCCCCCTAACGGACACAACAAATCAGAA
GACTGAGTTACAAGCAATTCATCTAGCTTTGCAGGATTCGGGATTAGAAGTAAAC
ATAGTGACAGACTCACAATATGCATTGGGAATCATTCAAGCACAACCAGATAAG
AGTGAATCAGAGTTAGTCAGTCAAATAATAGAGCAGTTAATAAAAAAGGAAAAA
GTCTACCTGGCATGGGTACCAGCACACAAAGGAATTGGAGGAAATGAACAAGTA
GATAAATTGGTCAGTGCTGGAATCAGGAAAGTACTATTTTTAGATGGAATAGATA
AGGCCCAAGAAGAACATGAGAAATATCACAGTAATTGGAGAGCAATGGCTAGTG
ATTTTAACCTACCACCTGTAGTAGCAAAAGAAATAGTAGCCAGCTGTGATAAATG
TCAGCTAAAAGGGGAAGCCATGCATGGACAAGTAGACTGTAGCCCAGGAATATG
GCAGCTAGATTGTACACATTTAGAAGGAAAAGTTATCTTGGTAGCAGTTCATGTA
GCCAGTGGATATATAGAAGCAGAAGTAATTCCAGCAGAGACAGGGCAAGAAAC
AGCATACTTCCTCTTAAAATTAGCAGGAAGATGGCCAGTAAAAACAGTACATAC
AGACAATGGCAGCAATTTCACCAGTACTACAGTTAAGGCCGCCTGTTGGTGGGC
GGGGATCAAGCAGGAATTTGGCATTCCCTACAATCCCCAAAGTCAAGGAGTAAT
AGAATCTATGAATAAAGAATTAAAGAAAATTATAGGACAGGTAAGAGATCAGGC
TGAACATCTTAAGACAGCAGTACAAATGGCAGTATTCATCCACAATTTTAAAAGA
AAAGGGGGGATTGGGGGGTACAGTGCAGGGGAAAGAATAGTAGACATAATAGC
AACAGACATACAAACTAAAGAATTACAAAAACAAATTACAAAAATTCAAAATTT
TCGGGTTTATTACAGGGACAGCAGAGATCCAGTTTGGAAAGGACCAGCAAAGCT
CCTCTGGAAAGGTGAAGGGGCAGTAGTAATACAAGATAATAGTGACATAAAAGT
AGTGCCAAGAAGAAAAGCAAAGATCATCAGGGATTATGGAAAACAGATGGCAG
GTGATGATTGTGTGGCAAGTAGACAGGATGAGGATTAA (SEQ ID NO: 28)

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 Eurofins

Genomics. The DNA fragment was then inserted into the plasmid at the XbaI and XmaI restriction sites The DNA sequence was as follows:

TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGAGCTCATCAGAACAGT
CAGACTCATCAAGCTTCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGACC
CGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGAT
CCATTCGATTAGTGAACGGATCCTTGGCACTTATCTGGGACGATCTGCGGAGCCT
GTGCCTCTTCAGCTACCACCGCTTGAGAGACTTACTCTTGATTGTAACGAGGATT
GTGGAACTTCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGGAATCTC
CTACAATATTGGAGTCAGGAGCTAAAGAATAGAGGAGCTTTGTTCCTTGGGTTCT
TGGGAGCAGCAGGAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTACAGG
CCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAATTTGCTGAGGGCTAT
TGAGGCGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCATCAAGCAGCTCCA
GGCAAGAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCCTAGATCT
TTTTCCCTCTGCCAAAAATTATGGGGACATCATGAAGCCCCTTGAGCATCTGACT
TCTGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTG
TCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAAACATCAGAATGA
GTATTTGGTTTAGAGTTTGGCAACATATGCCATATGCTGGCTGCCATGAACAAAG
GTGGCTATAAAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCCTTA
TTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTTTTATATTTTGTTTTGTGTT
ATTTTTTTCTTTAACATCCCTAAAATTTTCCTTACATGTTTTACTAGCCAGATTTTT
CCTCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCTTCTCTTATGAAGATCCC
TCGACCTGCAGCCCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAA
ATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAA
AGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTG
CCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCGGATCCGCATCTCAATTAGTC
AGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGT
TCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGA
GGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGC
CTAGGCTTTTGCAAAAAGCTAACTTGTTTATTGCAGCTTATAATGGTTACAAATA
AAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGT
TGTGGTTTGTCCAAACTCATCAATGTATCTTATCAGCGGCCGCCCCGGG (SEQ ID NO: 29)

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 Eurofins Genomics. The DNA fragment was then inserted into the plasmid at the MluI and EcoRI restriction sites. The DNA sequence was as follows:

ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATAT
ATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCA
ACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAAT
AGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTG
GCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACG
GTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTAC
TTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGGTCGAGGTGAGCCC
CACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTT
ATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGCGCGC
GCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTG
CGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGC
GGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGGGAGTCGCT
GCGTTGCCTTCGCCCCGTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCCCCGGC
TCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCC
GGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTG
AAAGCCTTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGCGGCTCGGGG
GGTGCGTGCGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCGCTGCCCG
GCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCG
CGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGG
GAACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGGGGTGTGG
GCGCGGCGGTCGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTTGCTGAGC
ACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCGCCG
TGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCCT
CGGGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCGGAGCGCCGGCGGC
TGTCGAGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGG
GCGCAGGGACTTCCTTTGTCCCAAATCTGGCGGAGCCGAAATCTGGGAGGCGCC
GCCGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGTGCGGCGCCGGCAGGAAGG
AAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTCTCCATCT
CCAGCCTCGGGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGG
GCGGGGTTCGGCTTCTGGCGTGTGACCGGCGGGAATTC (SEQ ID NO: 30)

Construction of the VSV-G Envelope plasmid:

The vesicular stomatitis Indiana virus glycoprotein (VSV-G) sequence was synthesized by Eurofins Genomics 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:

GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTTATTCATTGGGGTGAATTGCAA
GTTCACCATAGTTTTTCCACACAACCAAAAAGGAAACTGGAAAAATGTTCCTTCT
AATTACCATTATTGCCCGTCAAGCTCAGATTTAAATTGGCATAATGACTTAATAG
GCACAGCCTTACAAGTCAAAATGCCCAAGAGTCACAAGGCTATTCAAGCAGACG
GTTGGATGTGTCATGCTTCCAAATGGGTCACTACTTGTGATTTCCGCTGGTATGG
ACCGAAGTATATAACACATTCCATCCGATCCTTCACTCCATCTGTAGAACAATGC
AAGGAAAGCATTGAACAAACGAAACAAGGAACTTGGCTGAATCCAGGCTTCCCT
CCTCAAAGTTGTGGATATGCAACTGTGACGGATGCCGAAGCAGTGATTGTCCAG
GTGACTCCTCACCATGTGCTGGTTGATGAATACACAGGAGAATGGGTTGATTCAC
AGTTCATCAACGGAAAATGCAGCAATTACATATGCCCCACTGTCCATAACTCTAC
AACCTGGCATTCTGACTATAAGGTCAAAGGGCTATGTGATTCTAACCTCATTTCC
ATGGACATCACCTTCTTCTCAGAGGACGGAGAGCTATCATCCCTGGGAAAGGAG
GGCACAGGGTTCAGAAGTAACTACTTTGCTTATGAAACTGGAGGCAAGGCCTGC
AAAATGCAATACTGCAAGCATTGGGGAGTCAGACTCCCATCAGGTGTCTGGTTCG
AGATGGCTGATAAGGATCTCTTTGCTGCAGCCAGATTCCCTGAATGCCCAGAAGG
GTCAAGTATCTCTGCTCCATCTCAGACCTCAGTGGATGTAAGTCTAATTCAGGAC
GTTGAGAGGATCTTGGATTATTCCCTCTGCCAAGAAACCTGGAGCAAAATCAGA
GCGGGTCTTCCAATCTCTCCAGTGGATCTCAGCTATCTTGCTCCTAAAAACCCAG
GAACCGGTCCTGCTTTCACCATAATCAATGGTACCCTAAAATACTTTGAGACCAG
ATACATCAGAGTCGATATTGCTGCTCCAATCCTCTCAAGAATGGTCGGAATGATC
AGTGGAACTACCACAGAAAGGGAACTGTGGGATGACTGGGCACCATATGAAGAC
GTGGAAATTGGACCCAATGGAGTTCTGAGGACCAGTTCAGGATATAAGTTTCCTT
TATACATGATTGGACATGGTATGTTGGACTCCGATCTTCATCTTAGCTCAAAGGC
TCAGGTGTTCGAACATCCTCACATTCAAGACGCTGCTTCGCAACTTCCTGATGAT
GAGAGTTTATTTTTTGGTGATACTGGGCTATCCAAAAATCCAATCGAGCTTGTAG
AAGGTTGGTTCAGTAGTTGGAAAAGCTCTATTGCCTCTTTTTTCTTTATCATAGGG
TTAATCATTGGACTATTCTTGGTTCTCCGAGTTGGTATCCATCTTTGCATTAAATT
AAAGCACACCAAGAAAAGACAGATTTATACAGACATAGAGATGAGAATTC (SEQ

ID NO: 31)

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

Referring, in part, to Figure 3, the Helper plasmid includes a CMV (CAG) enhancer (SEQ ID NO: 21); a Chicken beta actin (CAG) promoter (SEQ ID NO: 13); a chicken beta actin intron (SEQ ID NO: 22); a HIV gag (SEQ ID NO: 14); a HIV Pol (SEQ ID NO: 15); a HIV Int (SEQ ID NO: 16); a HIV RRE (SEQ ID NO: 8); and a rabbit beta globin poly A (SEQ ID NO: 23).

The Rev plasmid includes a RSV promoter and a HIV Rev (SEQ ID NO: 33); and a rabbit beta globin poly A (SEQ ID NO: 23).

The Envelope plasmid includes a CMV promoter (SEQ ID NO: 19); a beta globin intron (SEQ ID NO: 24); a VSV-G (SEQ ID NO: 20); and a rabbit beta globin poly A (SEQ ID NO: 23).

Synthesis of a 3-vector lentiviral packaging system including Helper, Rev, and Envelope plasmids.

Materials and Methods:

Construction of the Helper plasmid without Rev:

The Helper plasmid without Rev was constructed by inserting a DNA fragment containing the RRE and rabbit beta globin poly A sequence. This sequence was synthesized by Eurofins Genomics 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:

TCTAGAAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAGCACTATGGGC
GCAGCGTCAATGACGCTGACGGTACAGGCCAGACAATTATTGTCTGGTATAGTGC
AGCAGCAGAACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCAAC
TCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAAGAT
ACCTAAAGGATCAACAGCTCCTAGATCTTTTTCCCTCTGCCAAAAATTATGGGGA
CATCATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTATTTTC
ATTGCAATAGTGTGTTGGAATTTTTTGTGTCTCTCACTCGGAAGGACATATGGGA
GGGCAAATCATTTAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATA

TGCCATATGCTGGCTGCCATGAACAAAGGTGGCTATAAAGAGGTCATCAGTATAT
GAAACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGT
TAGATTTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAAAATTTT
CCTTACATGTTTTACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCCCAGTCATA
GCTGTCCCTCTTCTCTTATGAAGATCCCTCGACCTGCAGCCCAAGCTTGGCGTAAT
CATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAAC
ATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAA
CTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGT
GCCAGCGGATCCGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCG
CCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACT
AATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAG
AAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTAACTTGTT
TATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAAT
AAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATC
TTATCACCCGGG (SEQ ID NO: 32)

Construction of the Rev plasmid:

The RSV promoter and HIV Rev sequence was synthesized as a single DNA fragment by Eurofins Genomics 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:

CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGGGGACTAGGGTGTGTTT
AGGCGAAAAGCGGGGCTTCGGTTGTACGCGGTTAGGAGTCCCCTCAGGATATAG
TAGTTTCGCTTTTGCATAGGGAGGGGGAAATGTAGTCTTATGCAATACACTTGTA
GTCTTGCAACATGGTAACGATGAGTTAGCAACATGCCTTACAAGGAGAGAAAAA
GCACCGTGCATGCCGATTGGTGGAAGTAAGGTGGTACGATCGTGCCTTATTAGGA
AGGCAACAGACAGGTCTGACATGGATTGGACGAACCACTGAATTCCGCATTGCA
GAGATAATTGTATTTAAGTGCCTAGCTCGATACAATAAACGCCATTTGACCATTC
ACCACATTGGTGTGCACCTCCAAGCTCGAGCTCGTTTAGTGAACCGTCAGATCGC
CTGGAGACGCCATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCC
AGCCTCCCCTCGAAGCTAGCGATTAGGCATCTCCTATGGCAGGAAGAAGCGGAG
ACAGCGACGAAGAACTCCTCAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAA
GCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAAGGAATAGAAGA

AGAAGGTGGAGAGAGAGACAGAGACAGATCCATTCGATTAGTGAACGGATCCTT
AGCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCTTCAGCTACCACCGCTTG
AGAGACTTACTCTTGATTGTAACGAGGATTGTGGAACTTCTGGGACGCAGGGGGT
GGGAAGCCCTCAAATATTGGTGGAATCTCCTACAATATTGGAGTCAGGAGCTAA
AGAATAGTCTAGA (SEQ ID NO: 33)

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

Promoters: Elongation Factor-1 (EF-1) (SEQ ID NO: 34), phosphoglycerate kinase (PGK) (SEQ ID NO: 35), and ubiquitin C (UbC) (SEQ ID NO: 36) can replace the CMV (SEQ ID NO: 19) or Chicken beta actin (CAG) promoter (SEQ ID NO: 13). These sequences can also be further varied by addition, substitution, deletion or mutation.

Poly A sequences: SV40 poly A (SEQ ID NO: 37) and bGH poly A (SEQ ID NO: 38) can replace the rabbit beta globin poly A (SEQ ID NO: 23). 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: 14); HIV Pol (SEQ ID NO: 15); and HIV Int (SEQ ID NO: 16) 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: 39), gibbon ape leukemia virus (GALV) (SEQ ID NO: 40), Rabies (FUG) (SEQ ID NO: 41), lymphocytic choriomeningitis virus (LCMV) (SEQ ID NO: 42), influenza A fowl plague virus (FPV) (SEQ ID NO: 43), Ross River alphavirus (RRV) (SEQ ID NO: 44), murine leukemia virus 10A1 (MLV) (SEQ ID NO: 45), or Ebola virus (EboV) (SEQ ID NO: 46). Sequences for these envelopes are identified in the sequence portion herein. Further, these sequences can also be further varied by addition, substitution, deletion or mutation.

In summary, the 3-vector versus 4-vector systems can be compared and contrasted, in part, as follows. The 3-vector lentiviral vector system contains: 1. Helper plasmid: HIV Gag, Pol, Integrase, and Rev/Tat; 2. Envelope plasmid: VSV-G/FUG envelope; and 3. Therapeutic vector: RSV, 5'LTR, Psi Packaging Signal, RRE, cPPT, H1, shFDPS, CMV,

BTN3A3 (R381H) T2A IL-2, WPRE, and 3'δ LTR. The 4-vector lentiviral vector system contains: 1. Helper plasmid: HIV Gag, Pol, and Integrase; 2. Rev plasmid: Rev; 3. Envelope plasmid: VSV-G/FUG envelope; and 4. Therapeutic vector: RSV, 5'LTR, Psi Packaging Signal, RRE, cPPT, H1, shFDPS, CMV, BTN3A3 (R381H) T2A IL-2, WPRE, and 3'δ LTR. Sequences corresponding with the above elements are identified in the sequence listings portion herein.

Example 2 - Development of a Lentiviral Vector that Inhibits FDPS.

The purpose of this Example was to develop an FDPS-inhibiting lentivirus vector, which is also referred to herein as LV-shFDPS.

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 identified by siRNA or shRNA design programs such as from GPP Web Portal hosted by the Broad Institute (http://portals.broadinstitute.org/gpp/public/) or the BLOCK-iT RNAi Designer from Thermo Scientific (https://rnaidesigner.thermofisher.com/rnaiexpress/). Individual selected shRNA sequences were inserted into a lentiviral vector immediately 3 prime to a RNA polymerase III promoter such as H1 (SEQ ID NO: 10), U6 (SEQ ID NO: 47), or 7SK (SEQ ID NO: 48) to regulate shRNA expression. These lentivirus shRNA constructs were used to transduce cells and measure the change in specific mRNA levels. The shRNA most potent for reducing mRNA levels were embedded individually within a microRNA backbone to allow for expression by either the EF-1alpha or CMV RNA polymerase II promoters. The microRNA backbone was selected from mirbase.org. RNA sequences were also synthesized as synthetic siRNA oligonucleotides and introduced directly into cells without using a lentiviral vector.

Lentiviral Vector Construction: For FDPS shRNA, oligonucleotide sequences containing BamHI and EcoRI restriction sites were synthesized by Eurofins Genomics. 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:

GTCCTGGAGTACAATGCCATT

(FDPS target sequence #1; SEQ ID NO: 49);

GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTACTCCAGGACTTTTT

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

GCAGGATTTCGTTCAGCACTT

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

GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCTGAACGAAATCCTGCTTTTT

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

GCCATGTACATGGCAGGAATT

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

GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGTACATGGCTTTTT

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

GCAGAAGGAGGCTGAGAAAGT

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

GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTGCTTTTT

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

Without limiting any of the foregoing, therapeutic vectors (which are also referred to herein as lentiviral plasmids) can be constructed as detailed in Figures 2-4. With continued reference to Figure 4:

Vector 1 includes from left to right, a 5' LTR sequence; a Psi sequence; a RRE sequence; a CMV sequence; a BTN3A1 sequence; a WPRE sequence; and a 3' LTR sequence.

Vector 2 includes from left to right, a 5' LTR sequence; a Psi sequence; a RRE sequence; a CMV sequence; a BTN3A3 sequence; a WPRE sequence; and a 3' LTR sequence.

Vector 3 includes from left to right, a 5' LTR sequence; a Psi sequence; a RRE sequence; a CMV sequence; a BTN3A3 (R381H) sequence; a WPRE sequence; and a 3' LTR sequence.

Vector 4 includes from left to right, a 5' LTR sequence; a Psi sequence; a RRE sequence; a H1 sequence; a shFDPS sequence; a CMV sequence; a BTN3A1 sequence; a WPRE sequence; and a 3' LTR sequence.

Vector 5 includes from left to right, a 5' LTR sequence; a Psi sequence; a RRE sequence; a H1 sequence; a shFDPS sequence; a CMV sequence; a BTN3A3 sequence; a WPRE sequence; and a 3' LTR sequence.

Vector 6 includes from left to right, a 5' LTR sequence; a Psi sequence; a RRE sequence; a H1 sequence; a shFDPS sequence; a CMV sequence; a BTN3A3 (R381H) sequence; a WPRE sequence; and a 3' LTR sequence.

Vector 7 includes from left to right, a 5' LTR sequence; a Psi sequence; a RRE sequence; a H1 sequence; a shFDPS sequence; an AFP sequence; a BTN3A3 (R381H) sequence, a WPRE sequence; and a 3' LTR sequence.

Vector 8 includes from left to right, a 5' LTR sequence; a Psi sequence; a RRE sequence; a H1 sequence; a shFDPS sequence; a CMV sequence; an IL-2 sequence; a WPRE sequence; and a 3' LTR sequence.

Vector 9 includes from left to right, a 5' LTR sequence; a Psi sequence; a RRE sequence; a H1 sequence; a shFDPS sequence; a CMV sequence; an IL-15 sequence; a WPRE sequence; and a 3' LTR sequence.

Vector 10 includes from left to right, a 5' LTR sequence; a Psi sequence; a RRE sequence; a H1 sequence; a shFDPS sequence; a CMV sequence; an IL-18 sequence; a WPRE sequence; and a 3' LTR sequence.

Vector 11 includes from left to right, a 5' LTR sequence; a Psi sequence; a RRE sequence; a H1 sequence; a shFDPS sequence; a CMV sequence; a BTN3A3 (R381H) sequence; a T2A sequence; an IL-2 sequence; a WPRE sequence; and a 3' LTR sequence.

Example 3 - Expression of BTN3A3 (R381H) or BTN3A3 (WT) in PC3 prostate carcinoma cells.

This Example illustrates that expression of BTN3A3 (R381H) or BTN3A3 (WT) in PC3 cells by lentiviral (LV)-expressing BTN3A3 (R381H) or BTN3A3 (WT) stimulates TNF-α expression in GD T cells, as shown in Figure 5.

PC3 cells were transduced with either LV-vector, LV-BTN3A3 (R381H), or LV-BTN3A3 (WT). Three days after transduction, cells were treated with or without 1μM zoledronic acid. After 24 hours, the transduced PC3 cells were co-cultured with $5x10^5$ PBMC cells and IL-2 in a round bottom 96 well plate for 4 hours. The PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated, and Vδ2+ and TNF-α+ cells were selected on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, the percent of TNF-α expressing Vγ9Vδ2 T cells was 0.37% with LV-vector, 26.7% with BTN3A3 (R381H), and 0.44% with LV-BTN3A3 (WT). With zoledronic acid, the percent of TNF-α expressing Vγ9Vδ2 T cells was 8.91% with LV-vector, 35.2% with BTN3A3 (R381H), and 8.76% with LV-BTN3A3 (WT).

Example 4 – Expression of BTN3A3 (R381H) and knock-down of FDPS in HepG2 liver carcinoma cells by shRNA #4.

This Example illustrates that expression of BTN3A3 (R381H) and knock-down of FDPS cells by lentiviral (LV)-expressing BTN3A3 (R381H) and FDPS shRNA #4 stimulates TNF-α expression in GD T cells, as shown in Figure 6.

HepG2 cells were transduced with LV-vector, LV-BTN3A3 (R381H), or LV-shFDPS-BTN3A3 (R381H). Three days after transduction, cells were treated with or without 1μM zoledronic acid. After 24 hours, the transduced HepG2 cells were co-cultured with $5x10^5$ PBMC cells and IL-2 in a round bottom 96 well plate for 4 hours. The PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated, and Vδ2+ and TNF-α+ cells were selected on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, the percent of TNF-α expressing Vγ9Vδ2 T cells was 0.6% with LV-vector, 9.5% with BTN3A3 (R381H), and 13.2% with the combination of LV-shFDPS-BTN3A3 (R381H). With zoledronic acid, the percent of TNF-α expressing Vγ9Vδ2 T cells was 7.2% with LV-vector,

17.8% with BTN3A3 (R381H), and 30.1% with the combination of LV-shFDPS-BTN3A3 (R381H).

Example 5 - Expression of BTN3A3 (R381H) and knock-down of FDPS in PC3 prostate carcinoma cells by shRNA #4.

This Example illustrates that expression of BTN3A3 (R381H) and knock-down of FDPS cells by lentiviral (LV)-expressing BTN3A3 (R381H) and FDPS shRNA #4 stimulates TNF-α expression in GD T cells, as shown in Figure 7.

PC3 cells were transduced with LV-vector, LV-BTN3A3 (R381H), or LV-shFDPS-BTN3A3 (R381H). Three days after transduction, cells were treated with or without 1μM zoledronic acid. After 24 hours, the transduced PC3 cells were co-cultured with $5x10^5$ PBMC cells and IL-2 in a round bottom 96 well plate for 4 hours. The PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated, and Vδ2+ and TNF-α+ cells were selected on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of flow cytograms. Without zoledronic acid, the percent of TNF-α expressing Vγ9Vδ2 T cells was 0.1% with LV-vector, 21.1% with BTN3A3 (R381H), and 18.2% with the combination of LV-shFDPS-BTN3A3 (R381H). With zoledronic acid, the percent of TNF-α expressing Vγ9Vδ2 T cells was 13.6% with LV-vector, 25.5% with BTN3A3 (R381H), and 39.6% with the combination of LV-shFDPS-BTN3A3 (R381H).

Example 6 - Expression of IL-2 and knock-down of FDPS in HepG2 liver carcinoma cells by shRNA #4.

This Example illustrates that expression of IL-2 and knock-down of FDPS cells by lentiviral (LV)-expressing IL-2 and FDPS shRNA #4 stimulates TNF-α expression in GD T cells, as shown in Figure 8.

HepG2 cells were transduced with LV-shFDPS or LV-shFDPS-IL-2. Three days after transduction, cells were treated with or without 1μM zoledronic acid. After 24 hours, the transduced HepG2 cells were co-cultured with $5x10^5$ PBMC cells and with or without IL-2 in a round bottom 96 well plate for 4 hours. The PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated, and Vδ2+ and TNF-α+ cells were

selected on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of flow cytograms. With only zoledronic acid, the percent of TNF-α expressing Vγ9Vδ2 T cells was 7.5% with LV-shFDPS and 20.1% with LV-shFDPS-IL-2. With zoledronic acid and IL-2, the percent of TNF-α expressing Vγ9Vδ2 T cells was 27.8% with LV-shFDPS and 24.7% with LV-shFDPS-IL-2.

Example 7 - Expression of IL-2 and knock-down of FDPS in PC3 carcinoma cells by shRNA #4.

This Example illustrates that expression of IL-2 and knock-down of FDPS cells by lentiviral (LV)-expressing IL-2 and FDPS shRNA #4 stimulates TNF-α expression in GD T cells, as shown in Figure 9.

PC3 cells were transduced with LV-shFDPS or LV-shFDPS-IL-2. Three days after transduction, cells were treated with or without 1μM zoledronic acid. After 24 hours, the transduced PC3 cells were co-cultured with $5x10^5$ PBMC cells and with or without IL-2 in a round bottom 96 well plate for 4 hours. The PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated, and Vδ2+ and TNF-α+ cells were selected on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of flow cytograms. With only zoledronic acid, the percent of TNF-α expressing Vγ9Vδ2 T cells was 24.6% with LV-shFDPS and 46.8% with LV-shFDPS-IL-2. With zoledronic acid and IL-2, the percent of TNF-α expressing Vγ9Vδ2 T cells was 48.6% with LV-shFDPS and 41% with LV-shFDPS-IL-2.

Example 8 - Expression of IL-15 and knock-down of FDPS in PC3 carcinoma cells by shRNA #4.

This Example illustrates that expression of IL-15 and knock-down of FDPS cells by lentiviral (LV)-expressing IL-15 and FDPS shRNA #4 stimulates TNF-α expression in GD T cells, as shown in Figure 10.

PC3 cells were transduced with LV-vector, LV-shFDPS, or LV-shFDPS-IL-15. Three days after transduction, cells were treated with or without 1μM zoledronic acid. After 24 hours, the transduced PC3 cells were co-cultured with $5x10^5$ PBMC cells and with or without IL-2 in a round bottom 96 well plate for 4 hours. The PBMC cells were pre-stimulated with zoledronic acid and IL-2 for 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2

and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated, and Vδ2+ and TNF-α+ cells were selected on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of flow cytograms. With only zoledronic acid, the percent of TNF-α expressing Vγ9Vδ2 T cells was 10% with LV-vector, 13% with LV-shFDPS, and 14.6% with LV-shFDPS-IL-15. With zoledronic acid and IL-2, the percent of TNF-α expressing Vγ9Vδ2 T cells was 14.5% with LV-vector, 21.7% with LV-shFDPS, and 21% with LV-shFDPS-IL-15.

Example 9 – Expression of BTN3A3 in PC3 and HepG2 carcinoma cells by LV-BTN3A3 (R381H) and LV-shFDPS-BTN3A3 (R381H).

This Example illustrates that lentivirus (LV)-expressing BTN3A3 (R381H) alone and with shFDPS increases BTN3A3 expression in PC3 and HepG2 carcinoma cells as shown in Figure 11.

PC3 prostate or HepG2 liver carcinoma cells were transduced with LV-vector or LV-BTN3A3 (R381H) for 3 days, as shown in Figures 11A and 11B. After staining for BTN3A3 using a fluorophore-conjugated anti BTN3A3 (CD277) antibody, cells were analyzed via flow cytometry. There was an increase in the mean fluorescence intensity (MFI) from 6 to 21 and from 3 to 10 in PC3 and HepG2 cells, respectively. HepG2 liver carcinoma cells were transduced with LV-vector or LV-shFDPS-BTN3A3 (R381H) for 3 days, as shown in Figure 11C. There was an increase in the mean fluorescence intensity (MFI) from 4 to 18.

Example 10 – Stimulation of Cytotoxic Vγ2Vδ2 Cells by Lv-shFDPS Transduced Cells and Zoledronic Acid.

This Example illustrates that activation of cytotoxic Vγ2Vδ2 cells is increased by treatment with lentivirus expressing shFDPS (LV-shFDPS) and zoledronic acid, as shown in Figure 12.

HepG2 liver carcinoma cells were transduced with Lv-shFDPS, Lv-FDPS-IL-15 (expressing both shRNAFDPS and the human cytokine interleukin 15) or Lv-control and cultured for 72 hours. Zoledronic acid (1 μM) was added to (1) cells transduced with Lv-shFDPS, (2) cells transduced with Lv-FDPS-IL-15, and (3) HepG2 cells transduced with Lv-control. Treated cells were cultured for 24 hours. Lv-shFDPS transduced cells, Lv-FDPS-IL-15 transduced cells, and Lv-control transduced cells were co-cultured with PBMC enriched for Vγ9Vδ2 cells plus a protein transport inhibitor (BD GolgiStop) for 4 hours. After 4 hours

of stimulation, cells were collected and labeled with Vδ2 phycoerythrin (PE) and TNFαallophycocyanin (APC), and the labeled cells were analyzed by flow cytometry.

Results showed that the frequency of responding Vγ9Vδ2 T cells (expressing TNF-α measured by intracellular cytokine staining) in the presence of 1 μM zoledronic acid, was higher in Lv-FDPS than in Lv-control and was increased further by Lv-FDPS-IL-15. Adding 100 Units/ml of interleukin-2 (IL-2) increased activation of Vγ9Vδ2 T cells by HepG2 transduced with Lv-FDPS compared to Lv-control, but IL-2 substitute for IL-15 and reduced the differences between Lv-FDPS and Lv-FDPS-IL-15 treated HepG2 for activating Vγ9Vδ2 T cells.

Example 11 – Growth Curve of Lv-shFDPS Versus Lv-control Transduced PC3 Tumors in Mice.

This Example illustrates that the rate of human prostate cancer (PC3) cell tumor growth in mice is slowed after treatment with Lv-shFDPS, as shown in Figure 13.

NSG™ mice were subcutaneously injected with Matrigel® and 3 million PC3 cells that were transduced with one of Lv-shFDPS or Lv-control. Tumors were monitored and measured twice a week. Tumor size was determined by measuring the perpendicular diameter of each tumor with calipers. Tumor volume (mm^3) was calculated with the following formula: d^2 x (D/2), where d = the shortest diameter, and D = the longest diameter.

Xenografted PC3 tumors treated and/or transduced with Lv-shFDPS showed slower growth compared to the growth of xenografted PC3 tumors treated and/or transduced with Lv-control. For example, it took 21 days for Lv-control xenografted PC3 tumors to grow to 300 mm^3, but it took 30 day for Lv-shFDPS xenografted PC3 tumors to grow to 300 mm^3.

Example 12 – Tumor Growth and Survival of Mice Xenografted With Lv-FDPS or Lv-control Transduced PC3 Tumors When Treated With or Without Vγ9Vδ2 T cells and/or Zoledronic Acid.

This Example illustrates that treatment of xenografted PC3 tumors transduced with Lv-shFDPS and subsequently treated with Vγ9Vδ2 T cells, slows tumor growth and increases survival, with or without zoledronic acid treatment.

NSG™ mice were subcutaneously injected in the right flank with Matrigel® and 3 million PC3 cells transduced with one of Lv-shFDPS (also referred to in Figures 14 and 15 as FDPS knockdown or "FDPS KD") or Lv-control (also referred to in Figures 14 and 15 as

"Lv" or "control"). Tumors were monitored and measured until the tumor size reached about 300mm^3. Tumor size was determined by measuring the perpendicular diameter of each tumor with calipers. Tumor volume (mm^3) was calculated with the following formula: d^2 x (D/2), where d = the shortest diameter, and D = the longest diameter.

When the resulting tumors reached a size of 300 mm^3, the mice were randomized and grouped into eight groups: four groups of Lv-shFDPS-transduced mice and four groups of Lv-control-transduced mice. One group from each of the Lv-shFDPS-transduced mice and the Lv-control-transduced mice were treated with intraperitoneal injections of PBMCs once per week for 4 weeks. One group from each of the Lv-shFDPS-transduced mice and the Lv-control-transduced mice were treated with 100 μg/kg of zoledronic acid. One group from each of the Lv-shFDPS-transduced mice and the Lv-control-transduced mice were treated with a combination of PBMCs and zoledronic acid. One group from each of the Lv-shFDPS-transduced mice and the Lv-control-transduced mice were treated with intraperitoneal injections of PBS once per week for 4 weeks (control). Mouse survival was observed for the shorter of 95 days or when the tumor size reached 2000 mm^3. Tumors were excised and observed at the end of the study.

As shown in Figure 14, a Kaplan Meier survival curve showed a significant survival advantage achieved by Lv-shFDPS PC3 xenografted mice compared to Lv-control PC3 xenografted (scramble) mice. The survival rate of Lv-shFDPS xenografted mice treated with PBMCs (many of which were Vγ9Vδ2 cells) was greater than the survival rate of Lv-shFDPS xenografted mice not treated with PBMCs. Treatment of Lv-control PC3 xenografted mice with PBMCs did not substantially affect survival.

As shown in Figure 15, gross observation of Lv-shFDPS xenografted PC3 tumors showed smaller tumor volumes compare to that of Lv-control xenografted PC3 tumors. Tumor volume of Lv-shFDPS xenografted PC3 tumors treated with PBMCs (many of which were Vγ9Vδ2 cells) was largely decreased as compare to the tumor volume of Lv-shFDPS PC3 tumors not treated with PBMCs. No significant difference was observed between Lv-control xenografted mice treated or untreated with PBMCs, and the constituents of these groups were sacrificed when tumors reached a size of 2000 mm^3. Treatment Lv-shFDPS-transduced mice and Lv-control-transduced mice with 100 μg/kg of zoledronic acid showed no obvious effect on tumor size or survival.

As shown in Figure 16, gross observation of the appearance of Lv-shFDPS xenografted PC3 tumors showed that the volume of tumors treated with PBMCs was largely

decreased compare to that of Lv-shFDPS PC3 tumors untreated with PBMCs. Some Lv-shFDPS PC3 tumors treated with PBMCs showed unmeasurable tumors.

Example 13 – Development of Lentiviral Vectors that Inhibit FDPS, GGPS1, and IDI1

This Example illustrates development of lentiviral vectors that inhibit FDPS, GGPS1, and IDI1, as shown in Figure 17.

Cloning of shRNA sequences: Potential RNA interference sequences were identified with the shRNA design program from the Broad institute (http://portals.broadinstitute.org/gpp/public/seq/search) of the BLOCK-iT RNAi Designer (https://rnaidesigner.thermofisher.com/rnaiexpress/) from Thermo Scientific. Short-hairpin oligonucleotide sequences containing BamHI and EcoRI restriction sites or microRNA sequences containing BsrGI and EcoRI restriction sites were synthesized by Eurofins Genomics. Oligonucleotide sequences were annealed by incubating at 70 degrees Celsius then cooling to room temperature for 1 hour. In parallel, the lentiviral vectors were digested with the restriction enzymes BamHI and EcoRI or BsrGI and EcoRI for one hour at 37 degrees Celsius. The digested lentiviral vectors were purified by agarose gel electrophoresis and extracted from the gel using a DNA gel extraction kit (Thermo Scientific). The DNA concentration was determined for each and 50 ng of vector were added to 2 microliters of annealed oligo. The ligation reactions were done with T4 DNA ligase for 30 minutes at room temperature. 2.5 microliters of the ligation mix were added to 25 microliters of StbI3 competent bacterial cells. Transformations were done with a heat-shock step at 42 degrees Celsius. Bacterial cells were streaked onto agar plates containing ampicillin and selected colonies were expanded in LB broth. To check for insertion of the oligo sequences, plasmid DNA were extracted from harvested bacterial cultures with a DNA mini prep kit (Thermo Scientific). Insertions of the shRNA sequence in the lentiviral vector were verified by DNA sequencing using H1 or EF-1 primers. Lentiviral vectors containing correct shRNA sequences were used to package lentiviral particles for testing their ability to knock-down mRNA. Cells were transduced with lentiviral particles and collected after 3 days; both protein and mRNA were analyzed.

Identification of FDPS shRNA sequences. The sequence of *Homo sapiens* farnesyl diphosphate synthase (FDPS) (NM_002004.3) mRNA were used to search for potential shRNA candidates to reduce FDPS levels in human cells. In addition to FDPS shRNA

sequences #1-4, as discussed above, the following exemplary shRNA and microRNA sequences were determined to knock down FDPS:

ACTTTCTCAGCCTCCTTCTGCCTCGAGGCAGAAGGAGGCTGAGAAAGTTTTTT

(FDPS shRNA sequence #4A; SEQ ID NO: 64);

GCAGAAGGAGGCTGAGAAAGTGAGCTCACTTTCTCAGCCTCCTTCTG

(FPDS shRNA sequence #4R; SEQ ID NO: 65);

GCAGAAGGAGGCTGAGAAAGTTTACTTTCTCAGCCTCCTTCTGCTTTTT

(FDPS shRNA sequence #4TT; SEQ ID NO: 66);

GCAGAAGGAGGCTGAGAAAGTACTTTCTCAGCCTCCTTCTGCTTTTT

(FDPS shRNA sequence #4L; SEQ ID NO: 67);

AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAA GCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCA AGGGGCT

(FDPS miR30 sequence #1; SEQ ID NO: 68);

AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCTGCGTGAA GCCACAGATGGCAGAAGGGCTGAGAAAGTGCTGCCTACTGCCTCGGACTTCAAG GGGCT

(FDPS miR30 sequence #3; SEQ ID NO: 69).

Identification of GGPS1 shRNA sequences. The sequences of *Homo sapiens* geranylgeranyl pyrophosphate synthase (GGPS1) (NM_001037277.1) mRNA were used to search for potential shRNA candidates to reduce GGPS1 in human cells. Using the following target sequences, exemplary shRNA sequences were determined to knock-down GGPS1:

GCTTGAAGCTAAAGCCTATAA

(GGPS1 target sequence #1; SEQ ID NO: 73);

GCTTGAAGCTAAAGCCTATAACTCGAGTTATAGGCTTTAGCTTCAAGCTTTTT

(GGPS1 shRNA sequence #1; SEQ ID NO: 70);

GTACATTATCTTGAGGATGTA

(GGPS1 target sequence #2; SEQ ID NO: 74);

GTACATTATCTTGAGGATGTACTCGAGTACATCCTCAAGATAATGTACTTTTT

(GGPS1 shRNA sequence #2; SEQ ID NO: 71);

CCTGAGCTAGTAGCCTTAGTA

(GGPS1 target sequence #3; SEQ ID NO: 75); and

CCTGAGCTAGTAGCCTTAGTACTCGAGTACTAAGGCTACTAGCTCAGGTTTTT

(GGPS1 shRNA sequence #3; SEQ ID NO: 72).

Identification of IDI1 shRNA sequences. The sequence of *Homo sapiens* isopentenyl-diphosphate delta-isomerase 1 (IDI1) (NM_004508.3) mRNA was used to search for potential shRNA candidates to reduce IDI1 levels in human cells. Using the following target sequence, an exemplary shRNA sequence was determined to knock-down IDI1:

GCCAGTGGTGAAATTAAGATA

(IDI1 target sequence; SEQ ID NO: 77); and

GCCAGTGGTGAAATTAAGATACTCGAGTATCTTAATTTCACCACTGGCTTTTT

(IDI1 shRNA sequence; SEQ ID NO: 76).

Example 14 – FDPS RNA and protein expression in HepG2 hepatocellular carcinoma cells transduced with lentiviruses expressing shFDPS.

This Example illustrates reduction of FDPS RNA and protein expression by shFDPS in HepG2 cells.

Figure 18 shows that FDPS protein expression is reduced by shFDPS. HepG2 cells were infected at 5 MOI with lentiviral vectors containing either shCon or two different FDPS shRNA sequences, LV-shFDPS #1 (SEQ ID NO: 1) or LV-shFDPS #4 (SEQ ID NO: 4). After 48 hours, the cells were treated with or without 1 μM zoledronic acid (Zol). After 72 hours, the cells were lysed and an immunoblot was performed using an anti-FDPS and an anti-actin antibody as a protein loading control. The densitometry of the immunoblot bands were quantified and LV-shControl was set as 1 (100%). There was a 62% (LV-shFDPS #1 (SEQ ID NO: 1)), 48% (LV-shFDPS #1+Zol (SEQ ID NO: 1)), 44% (LV-shFDPS #4 (SEQ ID NO: 4)), and 32% (LV-shFDPS #4+Zol (SEQ ID NO: 4)) reduction of FDPS protein expression.

Example 15 – FDPS protein expression in PC3 prostate carcinoma cells transduced with lentiviruses expressing shFDPS hairpin-loop variations.

This Example illustrates that shFDPS hairpin-loop variations; A (antisense-loop-sense), R (sense-reverse loop-antisense), TT (sense-TT-antisense), and L (sense-antisense) are effective in reducing FDPS protein expression in PC3 cells.

PC3 cells were infected, at 5 MOI, with lentiviral vectors containing a non-targeting sequence (shCon) or different variations of shFDPS, namely, shFDPS #4 (SEQ ID NO: 4), shFDPS-A (SEQ ID NO: 64), shFDPS-R (SEQ ID NO: 65), shFDPS-TT (SEQ ID NO: 66),

or shFDPS-L (SEQ ID NO: 67). After 72 hours, cells were lysed and RNA was extracted using the RNeasy mini kit. cDNA was synthesized from RNA using the SuperScript VILO cDNA synthesis kit. PCR reactions were performed using the TaqMan Fast Advanced Master Mix and the samples were then analyzed by quantitative PCR (qPCR) using an Applied Biosystems QuantStudio3 qPCR machine (Thermo Scientific).

Expression of FDPS cDNA was determined by quantitative PCR using a TaqMan FDPS probe and FDPS primers. For Figure 19A, FDPS expression was detected with Fam-labeled TaqMan probe (5'-TAGCATCTCCTATCTCTGGGTGCCC-3') (SEQ ID NO: 78), using the FDPS forward primer (5'-GTGCTGACTGAGGATGAGATG-3') (SEQ IF NO: 79) and reverse primer (5'-CCGGTTATACTTGCCTCCAAT-3') (SEQ ID NO: 80). The samples were normalized to actin expression. Actin was detected with a Fam-labeled TaqMan probe (5'-AGCGGGAAATCGTGCGTGAC-3') (SEQ ID NO: 81) using the Actin forward primer (5'-GGACCTGACTGACTACCTCAT-3') (SEQ ID NO: 82) and reverse primer (5'-CGTAGCACAGCTTCTCCTTAAT-3') (SEQ ID NO: 83). The relative FDPS RNA expression of the shCon sample is set at 100%. There was a 95% (shFDPS #4 (SEQ ID NO: 4)), 75% (shFDPS-A (SEQ ID NO: 64)), 95% (shFDPS-R (SEQ ID NO: 65)), 90% (shFDPS-TT (SEQ ID NO: 66)) and a 72% (FDPS-L (SEQ ID NO: 67)) decrease in FDPS expression, as shown in Figure 19A.

To examine the effects of the shFDPS variations on protein expression, PC3 cells were infected at 5 MOI with lentiviral vectors containing either shControl or variations of shFDPS #4. After 72 hours, cells were lysed and an immunoblot was performed using an anti-FDPS and an anti-actin antibody as a protein loading control. The densitometry of the immunoblot bands were quantified and LV-shControl was set as 1 (100%). As shown in Figure 19B, there was an 87% (LV-shFDPS #4 (SEQ ID NO: 4)), 13% (LV-shFDPS-A (SEQ ID NO: 64)), 88% (LV-shFDPS-R (SEQ ID NO: 65)), 81% (LV-FDPS-TT (SEQ ID NO: 66)), and 37% (LV-FDPS-L (SEQ ID NO: 67)) reduction of FDPS protein expression.

Example 16 – FDPS protein expression in HepG2 hepatocellular carcinoma cells transduced with lentiviruses expressing miR30-FDPS.

This Example illustrates decrease in FDPS protein expression in cells transduced with lentiviruses expressing miR30-FDPS.

To measure FDPS protein expression, HepG2 human hepatocellular carcinoma cells were infected, at 5 MOI, with lentiviral vectors containing either a shControl, shFDPS #3 (SEQ ID NO: 3), miR30-FDPS #1 (SEQ ID NO: 68), or miR30-FDPS #3 (SEQ ID NO: 69).

After 72 hours, cells were lysed with NP-40 lysis buffer and proteins were measured with the Bio-Rad protein assay reagent. Protein samples at 50 micrograms were electrophoresed on 4-12% Bis-Tris gels (Thermo Scientific) and transferred to PVDF membranes. The blots were blocked in 5% blotting grade blocker. An immunoblot was performed using an anti-FDPS antibody (Bethyl Laboratories) and an anti-actin antibody (Millipore Sigma) as a protein loading control. Antibodies were bound with HRP-conjugated secondary antibodies (Thermo Scientific) and detected with a Licor c-DiGit Blot scanner using the Immobilon Western ECL reagent (Millipore Sigma). The densitometry of the immunoblot bands were quantified with the NIH image software, and LV-Control was set as 1 (100%). As shown in Figure 20, there was an 85% (LV-shFDPS #4 (SEQ ID NO: 4)), 59% (LV-miR30-FDPS #1 (SEQ ID NO: 68)), and 53% (LV-miR30-FDPS #3 (SEQ ID NO: 69)) reduction of FDPS protein expression, respectively.

Example 17 – Activation of Vγ9Vδ2 T cells by THP-1 monocytic leukemia carcinoma cells transduced with a lentivirus expressing miR30-FDPS #1.

This Example illustrates that knock-down of FDPS for 7 days in THP-1 monocytic leukemia carcinoma cells by LV-expressing miR30-FDPS miRNA #1 (SEQ ID NO: 68) and treatment with or without zoledronic acid stimulates TNF-α expression in Vγ9Vδ2 T cells, as shown in Figure 21.

THP-1 cells ($2x10^5$ cells) were transduced with LV-control or LV-miR30 FDPS #1 (SEQ ID NO: 68) for 7 days. Cells were treated with or without 1 μM zoledronic acid. After 24 hours, the transduced THP-1 cells were co-cultured for 4 hours with $2x10^5$ PBMC cells in 5 mL round-bottom tubes. The PBMC cells had been pre-stimulated with zoledronic acid plus IL-2 for at least 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated; Vδ2+ and TNF-α+ cells were identified on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of the flow cytograms. Without zoledronic acid, LV-control stimulated 2.44% of TNF-α expressing Vγ9Vδ2 T cells and LV-miR30 FDPS #1 (SEQ ID NO: 68) stimulated 28.4%. With zoledronic acid treatment, LV-control stimulated 23.8% of TNF-α expressing Vγ9Vδ2 T cells and LV-miR30 FDPS #1 (SEQ ID NO: 68) stimulated 61.4%.

Example 18 – GGPS1 protein expression in HeLa cervical carcinoma cells transduced with lentiviruses expressing shGGPS1.

HeLa cells were infected at 5 MOI with lentiviral vectors containing either a shControl or three different GGPS1 shRNA sequences, namely LV-shGGPS1 #1 (SEQ ID NO: 70), LV-shGGPS1 #2 (SEQ ID NO: 71), or LV-shGGPS1 #3 (SEQ ID NO: 73). After 72 hours, cells were lysed and an immunoblot was performed using an anti-GGPS1 antibody from Santa Cruz Biotechnology (Cat. No. sc-271680) and an anti-actin antibody as a protein loading control. The densitometry of the immunoblot bands were quantified, and LV-shControl was set as 1 (100%). As shown in Figure 22, there was a 54% (LV-shGGPS1 #1(SEQ ID NO: 70)), 69% (LV- shGGPS1 #2 (SEQ ID NO: 71)), and 51% (LV- shGGPS1 #3(SEQ ID NO: 72)) reduction of FDPS protein expression, respectively.

Example 19 – Activation of Vγ9Vδ2 T cells by PC3 prostate carcinoma cells transduced with a lentivirus expressing shFDPS or shGGPS1 and treated with zoledronic acid.

This Example illustrates that knock-down of FDPS or GGPS1 for 3 days in PC3 cells transduced with LV-expressing FDPS shRNA #4 (SEQ ID NO: 4) or GGPS1 shRNA #1 (SEQ ID NO: 1) and treatment with zoledronic acid stimulates TNF-α expression in Vγ9Vδ2 T cells, as shown in Figure 23.

PC3 cells were transduced with LV-control or LV-FDPS shRNA #4 (SEQ ID NO: 4) or LV-GGPS1 shRNA #1 (SEQ ID NO: 70) for 3 days. Two days after transduction, cells were treated with or without 1 μM zoledronic acid. After 24 hours, the transduced PC3 cells were co-cultured for 4 hours with $5x10^5$ PBMC cells in a round bottom 96 well plate. The PBMC cells had been pre-stimulated with zoledronic acid plus IL-2 for at least 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated; Vδ2+ and TNF-α+ cells were identified on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of the flow cytograms. Without zoledronic acid, LV-control stimulated 2.78% of TNF-α expressing Vγ9Vδ2 T cells whereas LV-FDPS shRNA #4 (SEQ ID NO: 4) stimulated 0.77% and LV-GGPS1 #1 shRNA (SEQ ID NO: 70) stimulated 1.23%. With zoledronic acid treatment, LV-control stimulated 5.71% of TNF-α expressing Vγ9Vδ2 T cells, whereas LV-FDPS shRNA #4 (SEQ ID NO: 4) stimulated 11.4% and LV-GGPS1 #1 shRNA (SEQ ID NO: 70) stimulated 10%.

Example 20 – Activation of Vδ2+ T cells by HepG2 hepatocellular carcinoma cells transduced with a lentivirus expressing shFDPS or shGGPS1 and treated with zoledronic acid.

This Example illustrates that knock-down of FDPS or GGPS1 for 3 days in HepG2 cells transduced with LV-expressing FDPS shRNA #4 (SEQ ID NO: 4) or GGPS1 shRNA #1 (SEQ ID NO: 70) and treatment with zoledronic acid stimulates TNF-α expression in Vγ9Vδ2 T cells, as shown in Figure 24.

HepG2 cells were transduced with LV-control or LV-FDPS shRNA #4 (SEQ ID NO: 4) or LV-GGPS1 shRNA #1 (SEQ ID NO: 70) for 3 days. Two days after transduction, cells were treated with or without 1 μM zoledronic acid. After 24 hours, the transduced HepG2 cells were co-cultured for 4 hours with $5x10^5$ PBMC cells in a round bottom 96 well plate. The PBMC cells had been pre-stimulated with zoledronic acid plus IL-2 for at least 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated; Vδ2+ and TNF-α+ cells were identified on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of the flow cytograms. Without zoledronic acid, LV-control stimulated 0.36% of TNF-α expressing Vγ9Vδ2 T cells whereas LV-FDPS shRNA #4 (SEQ ID NO: 4) stimulated 0.9% and LV-GGPS1 #1 (SEQ ID NO: 70) shRNA stimulated 0.58%. With zoledronic acid treatment, LV-control stimulated 6.88% of TNF-α expressing Vγ9Vδ2 T cells, whereas LV-FDPS shRNA #4 (SEQ ID NO: 4) stimulated 21.1% and LV-GGPS1 #1 shRNA (SEQ ID NO: 70) stimulated 12%.

Example 21 – Activation of Vγ9Vδ2 T cells by THP-1 cells transduced with a lentivirus expressing shFDPS or shGGPS1 and treated with zoledronic acid.

This Example illustrates that knock-down of FDPS or GGPS1 for 3 days in THP-1 cells transduced with Lv-expressing FDPS shRNA #4 (SEQ ID NO: 4) and/or GGPS1 shRNA #1 (SEQ ID NO: 70) and treatment with zoledronic acid stimulates TNF-α expression in Vγ9Vδ2 T cells, as shown in Figure 25.

THP-1 cells were transduced with LV-control or LV-FDPS shRNA #4 (SEQ ID NO: 4) or Lv-GGPS1 shRNA #1 (SEQ ID NO: 70) for 3 days. Two days after transduction, cells were treated with or without 1 μM zoledronic acid. After 24 hours, the transduced THP-1 cells were co-cultured for 4 hours with $5x10^5$ PBMC cells in a round bottom 96 well plate. The PBMC cells had been pre-stimulated with zoledronic acid plus IL-2 for at least 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated; Vδ2+ and TNF-α+ cells were identified on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of the flow cytograms.

Without zoledronic acid, LV-control stimulated 1.33% of TNF-α expressing Vγ9Vδ2 T cells whereas Lv-FDPS shRNA #4 (SEQ ID NO: 4) stimulated 2.49%, Lv-GGPS1 #1 shRNA (SEQ ID NO: 70) stimulated 1.22%, and both combined stimulated 1.91%. With zoledronic acid treatment, Lv-control stimulated 5.74% of TNF-α expressing Vγ9Vδ2 T cells, whereas Lv-FDPS shRNA #4 (SEQ ID NO: 4) stimulated 10.8%, Lv-GGPS1 shRNA #1 (SEQ ID NO: 70) stimulated 4.5%, and both combined stimulated 11.5%.

Example 22 – IDI1 protein expression in PC3 prostate carcinoma cells transduced with lentiviruses expressing shIDI1.

This Example illustrates the effects of transduction with a lentiviral vector encoding the IDI1 shRNA sequence on IDI1 expression, as determined by immunoblot analyses.

PC3 cells were infected, at 5 MOI, with lentiviral vectors containing either a shControl or an IDI1 shRNA sequence (SEQ ID NO: 76). After 72 hours, cells were lysed and an immunoblot was performed using an anti-IDI1 antibody from Thermo Fisher (Cat. No. PA5-44207) and an anti-actin antibody as a protein loading control. The densitometry of the immunoblot bands were quantified, and Lv-shControl was set as 1 (100%). As shown in Figure 26, there was an 88% reduction of IDI1 protein expression.

Example 23 – Activation of Vγ9Vδ2 T cells by PC3 prostate carcinoma cells transduced with a lentivirus expressing shFDPS or shIDI1 and treated with zoledronic acid.

This Example illustrates that knock-down of FDPS or IDI1 for 3 days in PC3 cells transduced with Lv-expressing FDPS shRNA #4 (SEQ ID NO: 4) or IDI1 shRNA #1 (SEQ ID NO: 76) and treatment with zoledronic acid stimulates TNF-α expression in Vγ9Vδ2 T cells, as shown in Figure 27.

PC3 cells were transduced with Lv-control or Lv-FDPS shRNA #4 (SEQ ID NO: 4) or LV-IDI1 shRNA #1 (SEQ ID NO: 76) for 3 days. Two days after transduction, cells were treated with or without 1 μM zoledronic acid. After 24 hours, the transduced PC3 cells were co-cultured for 4 hours with $5x10^5$ PBMC cells in a round bottom 96 well plate. The PBMC cells had been pre-stimulated with zoledronic acid plus IL-2 for at least 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated; Vδ2+ and TNF-α+ cells were identified on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of the flow cytograms. Without zoledronic acid, LV-control stimulated 3.82% of TNF-α expressing Vγ9Vδ2 T cells whereas LV-FDPS

shRNA #4 (SEQ ID NO: 4) stimulated 2.28% and LV-IDI1 shRNA #1 (SEQ ID NO: 76) stimulated 1.92%. With zoledronic acid treatment, LV-control stimulated 8.66% of TNF-α expressing Vγ9Vδ2 T cells, whereas LV-FDPS shRNA #4 (SEQ ID NO: 4) stimulated 36.9% and LV-IDI1 shRNA #1 (SEQ ID NO: 76) stimulated 12.9%.

Example 24 – Activation of Vγ9Vδ2 T cells by THP-1 acute monocytic leukemia cells treated with zoledronic acid (Zol), FTI277 (FTI), or zaragozic acid (ZA).

This Example illustrates that treatment with zoledronic acid stimulates TNF-α expression in Vγ9Vδ2 T cells, as shown in Figure 28.

ZA is a small molecule inhibitor of squalene synthase in the pathway committed to sterol synthesis. THP-1 cells were treated with either the FDPS inhibitor Zol (10 μM), the farnesyl transferase inhibitor FTI (10 μM), or ZA (50 μM) for 24 hours. THP-1 cells ($2.5x10^5$) were co-cultured with $2.5x10^5$ PBMC cells in a round bottom 96 well plate for 5 hours. The PBMC cells had been pre-stimulated with zoledronic acid plus IL-2 for at least 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated; Vδ2+ and TNF-α+ cells were identified on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of the flow cytograms. Untreated cells stimulated 3.08% of TNF-α expressing Vγ9Vδ2 T cells, whereas zoledronic acid treatment stimulated 40.1%, FTI277 treatment stimulated 11.7%, and zaragozic acid stimulated 2.13%.

Example 25 – Activation of Vγ9Vδ2 T cells by PC3 prostate carcinoma cells transduced with a lentivirus expressing shFDPS and treated with zoledronic acid (Zol), FTI277 (FTI), or zaragozic acid (ZA).

This Example illustrates that treatment of PC3 cells transduced with LV-expressing FDPS shRNA #4, with zoledronic acid stimulates TNF-α expression in Vγ9Vδ2 T cells, as shown in Figure 29.

PC3 cells were transduced with LV-control or LV-FDPS shRNA #4 (SEQ ID NO: 4) for 3 days. Two days after transduction, cells were treated with or without 1 μM zoledronic acid, 1 μM FTI277, or 5 μM zaragozic acid. After 24 hours, the transduced PC3 cells were co-cultured for 4 hours with $5x10^5$ PBMC cells in a round bottom 96 well plate. The PBMC cells had been pre-stimulated with zoledronic acid plus IL-2 for at least 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti

TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated; Vδ2+ and TNF-α+ cells were identified on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of the flow cytograms. In LV-control transduced cells, untreated cells stimulated 1.73% of TNF-α expressing Vγ9Vδ2 T cells, whereas zoledronic acid treatment stimulated 2.87%, FTI277 stimulated 1.64%, and zaragozic acid stimulated 1.57%. In LV-FDPS shRNA #4 (SEQ ID NO: 4) transduced cells, untreated cells stimulated 1.77% of TNF-α expressing Vγ9Vδ2 T cells, whereas zoledronic acid stimulated 50.3%, FTI277 stimulated 2.44% and zaragozic acid stimulated 2.66%.

Example 26 – Activation of Vγ9Vδ2 T cells by HepG2 hepatocellular carcinoma cells transduced with a lentivirus expressing shFDPS and treated with zoledronic acid (Zol), FTI277 (FTI), or zaragozic acid (ZA).

This Example illustrates that treatment of HepG2 cells transduced with LV-expressing FDPS shRNA #4, with zoledronic acid stimulates TNF-α expression in Vγ9Vδ2 T cells, as shown in Figure 30.

HepG2 cells were transduced with LV-control or LV-FDPS shRNA #4 (SEQ ID NO: 4) for 3 days. Two days after transduction, cells were treated with or without 1 μM zoledronic acid, 1 μM FTI277, or 5 μM zaragozic acid. After 24 hours, the transduced HepG2 cells were co-cultured for 4 hours with $5x10^5$ PBMC cells in a round bottom 96 well plate. The PBMC cells had been pre-stimulated with zoledronic acid plus IL-2 for at least 11 days to expand Vγ9Vδ2 T cells. After staining for Vγ9Vδ2 and TNF-α using fluorophore-conjugated anti TCR-Vδ2 and anti-TNF-α antibody, cells were analyzed via flow cytometry. Live cells were gated; Vδ2+ and TNF-α+ cells were identified on a dot blot. The activated cytotoxic Vγ9Vδ2 T cells appeared in the upper right quadrant of the flow cytograms. In LV-control transduced cells, untreated cells stimulated 1.82% of TNF-α expressing Vγ9Vδ2 T cells, whereas zoledronic acid treatment stimulated 3.02%, FTI277 stimulated 1.72%, and zaragozic acid stimulated 1.63%. In LV-FDPS shRNA #4 (SEQ ID NO: 4) transduced cells, untreated cells stimulated 1.86% of TNF-α expressing Vγ9Vδ2 T cells, whereas zoledronic acid stimulated 50.8%, FTI277 stimulated 2.69% and zaragozic acid stimulated 2.82%.

While certain preferred embodiments of the present disclosure have been described and specifically exemplified above, it is not intended that any invention be limited to such embodiments.

Sequences

The following sequences are referred to herein and, as such, are incorporated into this disclosure:

SEQ ID NO:	Description	Sequence
1	FDPS shRNA sequence #1	GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTAC TCCAGGACTTTTT
2	FDPS shRNA sequence #2	GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCTGAACG AAATCCTGCTTTTT
3	FDPS shRNA sequence #3	GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCAT GTACATGGCTTTTT
4	FDPS shRNA sequence #4	GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCC TCCTTCTGCTTTTT
5	Rous Sarcoma virus (RSV) promoter	GTAGTCTTATGCAATACTCTTGTAGTCTTGCAACATGGTA ACGATGAGTTAGCAACATGCCTTACAAGGAGAGAAAAAG CACCGTGCATGCCGATTGGTGGAAGTAAGGTGGTACGAT CGTGCCTTATTAGGAAGGCAACAGACGGGTCTGACATGG ATTGGACGAACCACTGAATTGCCGCATTGCAGAGATATT GTATTTAAGTGCCTAGCTCGATACAATAAACG
6	5' Long terminal repeat (LTR)	GGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTC TGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAG CTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTG TGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGT CAGTGTGGAAAATCTCTAGCA
7	Psi Packaging signal	TACGCCAAAAATTTTGACTAGCGGAGGCTAGAAGGAGAG AG
8	Rev response element (RRE)	AGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAGGAAG CACTATGGGCGCAGCCTCAATGACGCTGACGGTACAGGC CAGACAATTATTGTCTGGTATAGTGCAGCAGCAGAACAA TTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGTTGCA ACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAAGAAT CCTGGCTGTGGAAAGATACCTAAAGGATCAACAGCTCC
9	Central polypurine tract (cPPT)	TTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGTGCAGG GGAAAGAATAGTAGACATAATAGCAACAGACATACAAA CTAAAGAATTACAAAAACAAATTACAAAATTCAAAATTT TA
10	Polymerase III shRNA promoters; H1 promoter	GAACGCTGACGTCATCAACCCGCTCCAAGGAATCGCGGG CCCAGTGTCACTAGGCGGGAACACCCAGCGCGCGTGCGC CCTGGCAGGAAGATGGCTGTGAGGGACAGGGGAGTGGC GCCCTGCAATATTTGCATGTCGCTATGTGTTCTGGGAAAT CACCATAAACGTGAAATGTCTTTGGATTTGGGAATCTTAT AAGTTCTGTATGAGACCACTT
11	Long WPRE	AATCAACCTCTGATTACAAAATTTGTGAAAGATTGACTG

	sequence	GTATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGATAC GCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTAT GGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGT CTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGG CGTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGT TGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTT TCGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGC CGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTG GGCACTGACAATTCCGTGGTGTTGTCGGGGAAATCATCG TCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCT GCGCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAAT CCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGC GGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCG GATCTCCCTTTGGGCCGCCTCCCCGCCT
12	3’ delta LTR	TGGAAGGGCTAATTCACTCCCAACGAAGATAAGATCTGC TTTTTGCTTGTACTGGGTCTCTCTGGTTAGACCAGATCTG AGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCT TAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTG TGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCC TCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTAG TAGTTCATGTCA
13	Helper/Rev; Chicken beta actin (CAG) promoter; Transcription	GCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCTTC ACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTA TTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCG GGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGG GCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCG GCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTT ATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCG AAGCGCGCGGCGGGCG
14	Helper/Rev; HIV Gag; Viral capsid	ATGGGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTA GATCGATGGGAAAAAATTCGGTTAAGGCCAGGGGGAAA GAAAAAATATAAATTAAAACATATAGTATGGGCAAGCAG GGAGCTAGAACGATTCGCAGTTAATCCTGGCCTGTTAGA AACATCAGAAGGCTGTAGACAAATACTGGGACAGCTACA ACCATCCCTTCAGACAGGATCAGAAGAACTTAGATCATT ATATAATACAGTAGCAACCCTCTATTGTGTGCATCAAAG GATAGAGATAAAAGACACCAAGGAAGCTTTAGACAAGA TAGAGGAAGAGCAAAACAAAAGTAAGAAAAAAGCACAG CAAGCAGCAGCTGACACAGGACACAGCAATCAGGTCAGC CAAAATTACCCTATAGTGCAGAACATCCAGGGGCAAATG GTACATCAGGCCATATCACCTAGAACTTTAAATGCATGG GTAAAAGTAGTAGAAGAGAAGGCTTTCAGCCCAGAAGTG ATACCCATGTTTTCAGCATTATCAGAAGGAGCCACCCCAC AAGATTTAAACACCATGCTAAACACAGTGGGGGGACATC AAGCAGCCATGCAAATGTTAAAAGAGACCATCAATGAGG AAGCTGCAGAATGGGATAGAGTGCATCCAGTGCATGCAG GGCCTATTGCACCAGGCCAGATGAGAGAACCAAGGGGA AGTGACATAGCAGGAACTACTAGTACCCTTCAGGAACAA ATAGGATGGATGACACATAATCCACCTATCCCAGTAGGA GAAATCTATAAAAGATGGATAATCCTGGGATTAAATAAA ATAGTAAGAATGTATAGCCCTACCAGCATTCTGGACATA AGACAAGGACCAAAGGAACCCTTTAGAGACTATGTAGAC

		CGATTCTATAAAACTCTAAGAGCCGAGCAAGCTTCACAA GAGGTAAAAAATTGGATGACAGAAACCTTGTTGGTCCAA AATGCGAACCCAGATTGTAAGACTATTTTAAAAGCATTG GGACCAGGAGCGACACTAGAAGAAATGATGACAGCATG TCAGGGAGTGGGGGGACCCGGCCATAAAGCAAGAGTTTT GGCTGAAGCAATGAGCCAAGTAACAAATCCAGCTACCAT AATGATACAGAAAGGCAATTTTAGGAACCAAAGAAAGA CTGTTAAGTGTTTCAATTGTGGCAAAGAAGGGCACATAG CCAAAAATTGCAGGGCCCCTAGGAAAAAGGGCTGTTGGA AATGTGGAAAGGAAGGACACCAAATGAAAGATTGTACTG AGAGACAGGCTAATTTTTTAGGGAAGATCTGGCCTTCCC ACAAGGGAAGGCCAGGGAATTTTCTTCAGAGCAGACCAG AGCCAACAGCCCCACCAGAAGAGAGCTTCAGGTTTGGGG AAGAGACAACAACTCCCTCTCAGAAGCAGGAGCCGATAG ACAAGGAACTGTATCCTTTAGCTTCCCTCAGATCACTCTT TGGCAGCGACCCCTCGTCACAATAA
15	Helper/Rev; HIV Pol; Protease and reverse transcriptase	ATGAATTTGCCAGGAAGATGGAAACCAAAAATGATAGGG GGAATTGGAGGTTTTATCAAAGTAGGACAGTATGATCAG ATACTCATAGAAATCTGCGGACATAAAGCTATAGGTACA GTATTAGTAGGACCTACACCTGTCAACATAATTGGAAGA AATCTGTTGACTCAGATTGGCTGCACTTTAAATTTTCCCA TTAGTCCTATTGAGACTGTACCAGTAAAATTAAAGCCAG GAATGGATGGCCCAAAAGTTAAACAATGGCCATTGACAG AAGAAAAAATAAAAGCATTAGTAGAAATTTGTACAGAAA TGGAAAAGGAAGGAAAAATTTCAAAAATTGGGCCTGAA AATCCATACAATACTCCAGTATTTGCCATAAAGAAAAAA GACAGTACTAAATGGAGAAAATTAGTAGATTTCAGAGAA CTTAATAAGAGAACTCAAGATTTCTGGGAAGTTCAATTA GGAATACCACATCCTGCAGGGTTAAAACAGAAAAAATCA GTAACAGTACTGGATGTGGGCGATGCATATTTTTCAGTTC CCTTAGATAAAGACTTCAGGAAGTATACTGCATTTACCAT ACCTAGTATAAACAATGAGACACCAGGGATTAGATATCA GTACAATGTGCTTCCACAGGGATGGAAAGGATCACCAGC AATATTCCAGTGTAGCATGACAAAAATCTTAGAGCCTTTT AGAAAACAAAATCCAGACATAGTCATCTATCAATACATG GATGATTTGTATGTAGGATCTGACTTAGAAATAGGGCAG CATAGAACAAAAATAGAGGAACTGAGACAACATCTGTTG AGGTGGGGATTTACCACACCAGACAAAAAACATCAGAAA GAACCTCCATTCCTTTGGATGGGTTATGAACTCCATCCTG ATAAATGGACAGTACAGCCTATAGTGCTGCCAGAAAAGG ACAGCTGGACTGTCAATGACATACAGAAATTAGTGGGAA AATTGAATTGGGCAAGTCAGATTTATGCAGGGATTAAAG TAAGGCAATTATGTAAACTTCTTAGGGGAACCAAAGCAC TAACAGAAGTAGTACCACTAACAGAAGAAGCAGAGCTA GAACTGGCAGAAAACAGGGAGATTCTAAAAGAACCGGT ACATGGAGTGTATTATGACCCATCAAAAGACTTAATAGC AGAAATACAGAAGCAGGGGCAAGGCCAATGGACATATC AAATTTATCAAGAGCCATTTAAAAATCTGAAAACAGGAA AATATGCAAGAATGAAGGGTGCCCACACTAATGATGTGA AACAATTAACAGAGGCAGTACAAAAAATAGCCACAGAA AGCATAGTAATATGGGGAAAGACTCCTAAATTTAAATTA CCCATACAAAAGGAAACATGGGAAGCATGGTGGACAGA GTATTGGCAAGCCACCTGGATTCCTGAGTGGGAGTTTGTC

		AATACCCCTCCCTTAGTGAAGTTATGGTACCAGTTAGAGA AAGAACCCATAATAGGAGCAGAAACTTTCTATGTAGATG GGGCAGCCAATAGGGAAACTAAATTAGGAAAAGCAGGA TATGTAACTGACAGAGGAAGACAAAAAGTTGTCCCCCTA ACGGACACAACAAATCAGAAGACTGAGTTACAAGCAATT CATCTAGCTTTGCAGGATTCGGGATTAGAAGTAAACATA GTGACAGACTCACAATATGCATTGGGAATCATTCAAGCA CAACCAGATAAGAGTGAATCAGAGTTAGTCAGTCAAATA ATAGAGCAGTTAATAAAAAAGGAAAAAGTCTACCTGGCA TGGGTACCAGCACACAAAGGAATTGGAGGAAATGAACA AGTAGATGGGTTGGTCAGTGCTGGAATCAGGAAAGTACT A
16	Helper Rev; HIV Integrase; Integration of viral RNA	TTTTTAGATGGAATAGATAAGGCCCAAGAAGAACATGAG AAATATCACAGTAATTGGAGAGCAATGGCTAGTGATTTT AACCTACCACCTGTAGTAGCAAAAGAAATAGTAGCCAGC TGTGATAAATGTCAGCTAAAAGGGGAAGCCATGCATGGA CAAGTAGACTGTAGCCCAGGAATATGGCAGCTAGATTGT ACACATTTAGAAGGAAAAGTTATCTTGGTAGCAGTTCAT GTAGCCAGTGGATATATAGAAGCAGAAGTAATTCCAGCA GAGACAGGGCAAGAAACAGCATACTTCCTCTTAAAATTA GCAGGAAGATGGCCAGTAAAAACAGTACATACAGACAA TGGCAGCAATTTCACCAGTACTACAGTTAAGGCCGCCTGT TGGTGGGCGGGGATCAAGCAGGAATTTGGCATTCCCTAC AATCCCCAAAGTCAAGGAGTAATAGAATCTATGAATAAA GAATTAAAGAAAATTATAGGACAGGTAAGAGATCAGGCT GAACATCTTAAGACAGCAGTACAAATGGCAGTATTCATC CACAATTTTAAAAGAAAAGGGGGGATTGGGGGGTACAGT GCAGGGGAAAGAATAGTAGACATAATAGCAACAGACAT ACAAACTAAAGAATTACAAAAACAAATTACAAAAATTCA AAATTTTCGGGTTTATTACAGGGACAGCAGAGATCCAGT TTGGAAAGGACCAGCAAAGCTCCTCTGGAAAGGTGAAGG GGCAGTAGTAATACAAGATAATAGTGACATAAAAGTAGT GCCAAGAAGAAAAGCAAAGATCATCAGGGATTATGGAA AACAGATGGCAGGTGATGATTGTGTGGCAAGTAGACAGG ATGAGGATTAA
17	Lenti-BTN3A3 (“BTN3A3”)	ATGAAAATGGCAAGTTCCCTGGCTTTCCTTCTGCTCAACT TTCATGTCTCCCTCTTCTTGGTCCAGCTGCTCACTCCTTGC TCAGCTCAGTTTTCTGTGCTTGGACCCTCTGGGCCCATCC TGGCCATGGTGGGTGAAGACGCTGATCTGCCCTGTCACCT GTTCCCGACCATGAGTGCAGAGACCATGGAGCTGAGGTG GGTGAGTTCCAGCCTAAGGCAGGTGGTGAACGTGTATGC AGATGGAAAGGAAGTGGAAGACAGGCAGAGTGCACCGT ATCGAGGGAGAACTTCGATTCTGCGGGATGGCATCACTG CAGGGAAGGCTGCTCTCCGAATACACAACGTCACAGCCT CTGACAGTGGAAAGTACTTGTGTTATTTCCAAGATGGTGA CTTCTACGAAAAAGCCCTGGTGGAGCTGAAGGTTGCAGC ATTGGGTTCTGATCTTCACATTGAAGTGAAGGGTTATGAG GATGGAGGGATCCATCTGGAGTGCAGGTCCACTGGCTGG TACCCCCAACCCCAAATAAAGTGGAGCGACGCCAAGGGA GAGAACATCCCGGCTGTGGAAGCACCTGTGGTTGCAGAT

		GGAGTGGGCCTGTATGCAGTAGCAGCATCTGTGATCATG AGAGGCAGCTCTGGTGGGGGTGTATCCTGCATCATCAGA AATTCCCTCCTCGGCCTGGAAAAGACAGCCAGCATATCC ATCGCAGACCCCTTCTTCAGGAGCGCCCAGCCCTGGATC GCGGCCCTGGCAGGGACCCTGCCTATCTCGTTGCTGCTTC TCGCAGGAGCCAGTTACTTCTTGTGGAGACAACAGAAGG AAAAAATTGCTCTGTCCAGGGAGACAGAAAGAGAGCGA GAGATGAAAGAAATGGGATACGCTGCAACAGAGCAAGA AATAAGCCTAAGAGAGAAGCTCCAGGAGGAACTCAAGT GGAGGAAAATCCAGTACATGGCTCGTGGAGAGAAGTCTT TGGCCTATCATGAATGGAAAATGGCCCTCTTCAAACCTGC GGATGTGATTCTGGATCCAGACACGGCAAACGCCATCCT CCTTGTTTCTGAGGACCAGAGGAGTGTGCAGCGTGCTGA AGAGCCGCGGGATCTGCCAGACAACCCTGAGAGATTTGA ATGGCGTTACTGTGTCCTTGGCTGTGAAAACTTCACATCA GGGAGACATTACTGGGAGGTGGAAGTGGGGGACAGAAA AGAGTGGCATATTGGGGTATGTAGTAAGAACGTGGAGAG GAAAAAAGGTTGGGTCAAAATGACACCGGAGAACGGAT ACTGGACTATGGGCCTGACTGATGGGAATAAGTATCGGG CTCTCACTGAGCCCAGAACCAACCTGAAACTTCCTGAGC CTCCTAGGAAAGTGGGGATCTTCCTGGACTATGAGACTG GAGAGATCTCGTTCTATAATGCCACAGATGGATCTCATAT CTACACCTTTCCGCACGCCTCTTTCTCTGAGCCTCTATATC CTGTTTTCAGAATTTTGACCTTGGAGCCCACTGCCCTGAC CATTTGCCCAATACCAAAAGAAGTAGAGAGTTCCCCCGA TCCTGACCTAGTGCCTGATCATTCCCTGGAGACACCACTG ACCCCGGGCTTAGCTAATGAAAGTGGGGAGCCTCAGGCT GAAGTAACATCTCTGCTTCTCCCTGCCCACCCTGGAGCTG AGGTCTCCCCTTCTGCAACAACCAATCAGAACCATAAGC TACAGGCACGCACTGAAGCACTTTACTGA
18	Helper/Rev; HIV Rev; Nuclear export and stabilize viral mRNA	ATGGCAGGAAGAAGCGGAGACAGCGACGAAGAACTCCT CAAGGCAGTCAGACTCATCAAGTTTCTCTATCAAAGCAA CCCACCTCCCAATCCCGAGGGGACCCGACAGGCCCGAAG GAATAGAAGAAGAAGGTGGAGAGAGAGACAGAGACAGA TCCATTCGATTAGTGAACGGATCCTTAGCACTTATCTGGG ACGATCTGCGGAGCCTGTGCCTCTTCAGCTACCACCGCTT GAGAGACTTACTCTTGATTGTAACGAGGATTGTGGAACTT CTGGGACGCAGGGGGTGGGAAGCCCTCAAATATTGGTGG AATCTCCTACAATATTGGAGTCAGGAGCTAAAGAATAG
19	Envelope; CMV promoter; Transcription	ACATTGATTATTGACTAGTTATTAATAGTAATCAATTACG GGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTA CATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCA ACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCC CATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATG GGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACA TCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTC AATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTAC ATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACG

		TATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCA GTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGG ATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTG TTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTA ACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTG TACGGTGGGAGGTCTATATAAGC
20	Envelope; VSV-G; Glycoprotein envelope-cell entry	ATGAAGTGCCTTTTGTACTTAGCCTTTTTATTCATTGGGGT GAATTGCAAGTTCACCATAGTTTTTCCACACAACCAAAA AGGAAACTGGAAAAATGTTCCTTCTAATTACCATTATTGC CCGTCAAGCTCAGATTTAAATTGGCATAATGACTTAATAG GCACAGCCTTACAAGTCAAAATGCCCAAGAGTCACAAGG CTATTCAAGCAGACGGTTGGATGTGTCATGCTTCCAAATG GGTCACTACTTGTGATTTCCGCTGGTATGGACCGAAGTAT ATAACACATTCCATCCGATCCTTCACTCCATCTGTAGAAC AATGCAAGGAAAGCATTGAACAAACGAAACAAGGAACT TGGCTGAATCCAGGCTTCCCTCCTCAAAGTTGTGGATATG CAACTGTGACGGATGCCGAAGCAGTGATTGTCCAGGTGA CTCCTCACCATGTGCTGGTTGATGAATACACAGGAGAAT GGGTTGATTCACAGTTCATCAACGGAAAATGCAGCAATT ACATATGCCCCACTGTCCATAACTCTACAACCTGGCATTC TGACTATAAGGTCAAAGGGCTATGTGATTCTAACCTCATT TCCATGGACATCACCTTCTTCTCAGAGGACGGAGAGCTAT CATCCCTGGGAAAGGAGGGCACAGGGTTCAGAAGTAACT ACTTTGCTTATGAAACTGGAGGCAAGGCCTGCAAAATGC AATACTGCAAGCATTGGGGAGTCAGACTCCCATCAGGTG TCTGGTTCGAGATGGCTGATAAGGATCTCTTTGCTGCAGC CAGATTCCCTGAATGCCCAGAAGGGTCAAGTATCTCTGCT CCATCTCAGACCTCAGTGGATGTAAGTCTAATTCAGGAC GTTGAGAGGATCTTGGATTATTCCCTCTGCCAAGAAACCT GGAGCAAAATCAGAGCGGGTCTTCCAATCTCTCCAGTGG ATCTCAGCTATCTTGCTCCTAAAAACCCAGGAACCGGTCC TGCTTTCACCATAATCAATGGTACCCTAAAATACTTTGAG ACCAGATACATCAGAGTCGATATTGCTGCTCCAATCCTCT CAAGAATGGTCGGAATGATCAGTGGAACTACCACAGAAA GGGAACTGTGGGATGACTGGGCACCATATGAAGACGTGG AAATTGGACCCAATGGAGTTCTGAGGACCAGTTCAGGAT ATAAGTTTCCTTTATACATGATTGGACATGGTATGTTGGA CTCCGATCTTCATCTTAGCTCAAAGGCTCAGGTGTTCGAA CATCCTCACATTCAAGACGCTGCTTCGCAACTTCCTGATG ATGAGAGTTTATTTTTTGGTGATACTGGGCTATCCAAAAA TCCAATCGAGCTTGTAGAAGGTTGGTTCAGTAGTTGGAA AAGCTCTATTGCCTCTTTTTTCTTTATCATAGGGTTAATCA TTGGACTATTCTTGGTTCTCCGAGTTGGTATCCATCTTTGC ATTAAATTAAAGCACACCAAGAAAAGACAGATTTATACA GACATAGAGATGA
21	Helper/Rev; CMV early (CAG) enhancer; Enhance Transcription	TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCAT AGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAA ATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCAT TGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAAT AGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGG TAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGC CAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGC CCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTT

		TCCTACTTGGCAGTACATCTACGTATTAGTCATC
22	Helper/Rev; Chicken beta actin intron; Enhance gene expression	GGAGTCGCTGCGTTGCCTTCGCCCCGTGCCCCGCTCCGCG CCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTT ACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCC GGGCTGTAATTAGCGCTTGGTTTAATGACGGCTCGTTTCT TTTCTGTGGCTGCGTGAAAGCCTTAAAGGGCTCCGGGAG GGCCCTTTGTGCGGGGGGGAGCGGCTCGGGGGGTGCGTG CGTGTGTGTGTGCGTGGGGAGCGCCGCGTGCGGCCCGCG CTGCCCGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGG CTTTGTGCGCTCCGCGTGTGCGCGAGGGGAGCGCGGCCG GGGGCGGTGCCCCGCGGTGCGGGGGGGCTGCGAGGGGA ACAAAGGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAG CAGGGGGTGTGGGCGCGGCGGTCGGGCTGTAACCCCCCC CTGCACCCCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTT CGGGTGCGGGGCTCCGTGCGGGGCGTGGCGCGGGGCTCG CCGTGCCGGGCGGGGGGTGGCGGCAGGTGGGGGTGCCG GGCGGGGCGGGGCCGCCTCGGGCCGGGGAGGGCTCGGG GGAGGGGCGCGGCGGCCCCGGAGCGCCGGCGGCTGTCG AGGCGCGGCGAGCCGCAGCCATTGCCTTTTATGGTAATC GTGCGAGAGGGCGCAGGGACTTCCTTTGTCCCAAATCTG GCGGAGCCGAAATCTGGGAGGCGCCGCCGCACCCCCTCT AGCGGGCGCGGGCGAAGCGGTGCGGCGCCGGCAGGAAG GAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGC CGTCCCCTTCTCCATCTCCAGCCTCGGGGCTGCCGCAGGG GGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTT CGGCTTCTGGCGTGTGACCGGCGG
23	Helper/Rev; Rabbit beta globin poly A; RNA stability	AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATG AAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAA TTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCT CTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAA ACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATAT GCCATATGCTGGCTGCCATGAACAAAGGTGGCTATAAAG AGGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATTCC TTATTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTTTT TTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCTAA AATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCCTC TCCTGACTACTCCCAGTCATAGCTGTCCCTCTTCTCTTATG AAGATC
24	Envelope; Beta globin intron; Enhance gene expression	GTGAGTTTGGGGACCCTTGATTGTTCTTTCTTTTTCGCTAT TGTAAAATTCATGTTATATGGAGGGGGCAAAGTTTTCAG GGTGTTGTTTAGAATGGGAAGATGTCCCTTGTATCACCAT GGACCCTCATGATAATTTTGTTTCTTTCACTTTCTACTCTG TTGACAACCATTGTCTCCTCTTATTTTCTTTTCATTTTCTG TAACTTTTTCGTTAAACTTTAGCTTGCATTTGTAACGAATT TTTAAATTCACTTTTGTTTATTTGTCAGATTGTAAGTACTT TCTCTAATCACTTTTTTTTCAAGGCAATCAGGGTATATTA TATTGTACTTCAGCACAGTTTTAGAGAACAATTGTTATAA TTAAATGATAAGGTAGAATATTTCTGCATATAAATTCTGG CTGGCGTGGAAATATTCTTATTGGTAGAAACAACTACAC CCTGGTCATCATCCTGCCTTTCTCTTTATGGTTACAATGAT ATACACTGTTTGAGATGAGGATAAAATACTCTGAGTCCA AACCGGGCCCCTCTGCTAACCATGTTCATGCCTTCTTCTC

		TTTCCTACAG
25	Envelope; Rabbit beta globin poly A; RNA stability	AGATCTTTTTCCCTCTGCCAAAAATTATGGGGACATCATG AAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAGGAAA TTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCT CTCACTCGGAAGGACATATGGGAGGGCAAATCATTTAAA ACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAACATAT GCCCATATGCTGGCTGCCATGAACAAAGGTTGGCTATAA AGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTCCATT CCTTATTCCATAGAAAAGCCTTGACTTGAGGTTAGATTTT TTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACATCCCT AAAATTTTCCTTACATGTTTTACTAGCCAGATTTTTCCTCC TCTCCTGACTACTCCCAGTCATAGCTGTCCCTCTTCTCTTA TGGAGATC
26	Primer	TAAGCAGAATTCATGAATTTGCCAGGAAGAT
27	Primer	CCATACAATGAATGGACACTAGGCGGCCGCACGAAT
28	Gag, Pol, Integrase fragment	GAATTCATGAATTTGCCAGGAAGATGGAAACCAAAAATG ATAGGGGGAATTGGAGGTTTTATCAAAGTAAGACAGTAT GATCAGATACTCATAGAAATCTGCGGACATAAAGCTATA GGTACAGTATTAGTAGGACCTACACCTGTCAACATAATT GGAAGAAATCTGTTGACTCAGATTGGCTGCACTTTAAATT TTCCCATTAGTCCTATTGAGACTGTACCAGTAAAATTAAA GCCAGGAATGGATGGCCCAAAAGTTAAACAATGGCCATT GACAGAAGAAAAAATAAAAGCATTAGTAGAAATTTGTAC AGAAATGGAAAAGGAAGGAAAAATTTCAAAAATTGGGC CTGAAAATCCATACAATACTCCAGTATTTGCCATAAAGA AAAAAGACAGTACTAAATGGAGAAAATTAGTAGATTTCA GAGAACTTAATAAGAGAACTCAAGATTTCTGGGAAGTTC AATTAGGAATACCACATCCTGCAGGGTTAAAACAGAAAA AATCAGTAACAGTACTGGATGTGGGCGATGCATATTTTTC AGTTCCCTTAGATAAAGACTTCAGGAAGTATACTGCATTT ACCATACCTAGTATAAACAATGAGACACCAGGGATTAGA TATCAGTACAATGTGCTTCCACAGGGATGGAAAGGATCA CCAGCAATATTCCAGTGTAGCATGACAAAAATCTTAGAG CCTTTTAGAAAACAAAATCCAGACATAGTCATCTATCAAT ACATGGATGATTTGTATGTAGGATCTGACTTAGAAATAG GGCAGCATAGAACAAAAATAGAGGAACTGAGACAACAT CTGTTGAGGTGGGGATTTACCACACCAGACAAAAAACAT CAGAAAGAACCTCCATTCCTTTGGATGGGTTATGAACTCC ATCCTGATAAATGGACAGTACAGCCTATAGTGCTGCCAG AAAAGGACAGCTGGACTGTCAATGACATACAGAAATTAG TGGGAAAATTGAATTGGGCAAGTCAGATTTATGCAGGGA TTAAAGTAAGGCAATTATGTAAACTTCTTAGGGGAACCA AAGCACTAACAGAAGTAGTACCACTAACAGAAGAAGCA GAGCTAGAACTGGCAGAAAACAGGGAGATTCTAAAAGA ACCGGTACATGGAGTGTATTATGACCCATCAAAAGACTT AATAGCAGAAATACAGAAGCAGGGGCAAGGCCAATGGA CATATCAAATTTATCAAGAGCCATTTAAAAATCTGAAAA CAGGAAAGTATGCAAGAATGAAGGGTGCCCACACTAATG ATGTGAAACAATTAACAGAGGCAGTACAAAAAATAGCCA CAGAAAGCATAGTAATATGGGGAAAGACTCCTAAATTTA AATTACCCATACAAAAGGAAACATGGGAAGCATGGTGGA

		CAGAGTATTGGCAAGCCACCTGGATTCCTGAGTGGGAGT TTGTCAATACCCCTCCCTTAGTGAAGTTATGGTACCAGTT AGAGAAAGAACCCATAATAGGAGCAGAAACTTTCTATGT AGATGGGGCAGCCAATAGGGAAACTAAATTAGGAAAAG CAGGATATGTAACTGACAGAGGAAGACAAAAAGTTGTCC CCCTAACGGACACAACAAATCAGAAGACTGAGTTACAAG CAATTCATCTAGCTTTGCAGGATTCGGGATTAGAAGTAA ACATAGTGACAGACTCACAATATGCATTGGGAATCATTC AAGCACAACCAGATAAGAGTGAATCAGAGTTAGTCAGTC AAATAATAGAGCAGTTAATAAAAAAGGAAAAAGTCTACC TGGCATGGGTACCAGCACACAAAGGAATTGGAGGAAATG AACAAGTAGATAAATTGGTCAGTGCTGGAATCAGGAAAG TACTATTTTTAGATGGAATAGATAAGGCCCAAGAAGAAC ATGAGAAATATCACAGTAATTGGAGAGCAATGGCTAGTG ATTTTAACCTACCACCTGTAGTAGCAAAAGAAATAGTAG CCAGCTGTGATAAATGTCAGCTAAAAGGGGAAGCCATGC ATGGACAAGTAGACTGTAGCCCAGGAATATGGCAGCTAG ATTGTACACATTTAGAAGGAAAAGTTATCTTGGTAGCAG TTCATGTAGCCAGTGGATATATAGAAGCAGAAGTAATTC CAGCAGAGACAGGGCAAGAAACAGCATACTTCCTCTTAA AATTAGCAGGAAGATGGCCAGTAAAAACAGTACATACAG ACAATGGCAGCAATTTCACCAGTACTACAGTTAAGGCCG CCTGTTGGTGGGCGGGGATCAAGCAGGAATTTGGCATTC CCTACAATCCCCAAAGTCAAGGAGTAATAGAATCTATGA ATAAAGAATTAAAGAAAATTATAGGACAGGTAAGAGATC AGGCTGAACATCTTAAGACAGCAGTACAAATGGCAGTAT TCATCCACAATTTTAAAAGAAAAGGGGGGATTGGGGGGT ACAGTGCAGGGGAAAGAATAGTAGACATAATAGCAACA GACATACAAACTAAAGAATTACAAAAACAAATTACAAAA ATTCAAAATTTTCGGGTTTATTACAGGGACAGCAGAGAT CCAGTTTGGAAAGGACCAGCAAAGCTCCTCTGGAAAGGT GAAGGGGCAGTAGTAATACAAGATAATAGTGACATAAA AGTAGTGCCAAGAAGAAAAGCAAAGATCATCAGGGATT ATGGAAAACAGATGGCAGGTGATGATTGTGTGGCAAGTA GACAGGATGAGGATTAA
29	DNA Fragment containing Rev, RRE and rabbit beta globin poly A	TCTAGAATGGCAGGAAGAAGCGGAGACAGCGACGAAGA GCTCATCAGAACAGTCAGACTCATCAAGCTTCTCTATCAA AGCAACCCACCTCCCAATCCCGAGGGGACCCGACAGGCC CGAAGGAATAGAAGAAGAAGGTGGAGAGAGAGACAGAG ACAGATCCATTCGATTAGTGAACGGATCCTTGGCACTTAT CTGGGACGATCTGCGGAGCCTGTGCCTCTTCAGCTACCAC CGCTTGAGAGACTTACTCTTGATTGTAACGAGGATTGTGG AACTTCTGGGACGCAGGGGGTGGGAAGCCCTCAAATATT GGTGGAATCTCCTACAATATTGGAGTCAGGAGCTAAAGA ATAGAGGAGCTTTGTTCCTTGGGTTCTTGGGAGCAGCAG GAAGCACTATGGGCGCAGCGTCAATGACGCTGACGGTAC AGGCCAGACAATTATTGTCTGGTATAGTGCAGCAGCAGA ACAATTTGCTGAGGGCTATTGAGGCGCAACAGCATCTGT TGCAACTCACAGTCTGGGGCATCAAGCAGCTCCAGGCAA GAATCCTGGCTGTGGAAAGATACCTAAAGGATCAACAGC TCCTAGATCTTTTTCCCTCTGCCAAAAATTATGGGGACAT CATGAAGCCCCTTGAGCATCTGACTTCTGGCTAATAAAG GAAATTTATTTTCATTGCAATAGTGTGTTGGAATTTTTTGT

		GTCTCTCACTCGGAAGGACATATGGGAGGGCAAATCATT TAAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGCAA CATATGCCATATGCTGGCTGCCATGAACAAAGGTGGCTA TAAAGAGGTCATCAGTATATGAAACAGCCCCCTGCTGTC CATTCCTTATTCCATAGAAAAGCCTTGACTTGAGGTTAGA TTTTTTTTATATTTTGTTTTGTGTTATTTTTTTCTTTAACAT CCCTAAAATTTTCCTTACATGTTTTACTAGCCAGATTTTTC CTCCTCTCCTGACTACTCCCAGTCATAGCTGTCCCTCTTCT CTTATGAAGATCCCTCGACCTGCAGCCCAAGCTTGGCGTA ATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCG CTCACAATTCCACACAACATACGAGCCGGAAGCATAAAG TGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACA TTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAA ACCTGTCGTGCCAGCGGATCCGCATCTCAATTAGTCAGCA ACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAA CTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACT AATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCC TCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAG GCCTAGGCTTTTGCAAAAAGCTAACTTGTTTATTGCAGCT TATAATGGTTACAAATAAAGCAATAGCATCACAAATTTC ACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTT TGTCCAAACTCATCAATGTATCTTATCAGCGGCCGCCCCG GG
30	DNA fragment containing the CAG enhancer/promoter/ intron sequence	ACGCGTTAGTTATTAATAGTAATCAATTACGGGGTCATTA GTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTA CGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCC GCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAAC GCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTA TTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTAT CATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGT AAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTAT GGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCAT CGCTATTACCATGGGTCGAGGTGAGCCCCACGTTCTGCTT CACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGT ATTTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGC GGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGG GGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGC GGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTT TATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCG AAGCGCGCGGCGGGCGGGAGTCGCTGCGTTGCCTTCGCC CCGTGCCCCGCTCCGCGCCGCCTCGCGCCGCCCGCCCCG GCTCTGACTGACCGCGTTACTCCCACAGGTGAGCGGGCG GGACGGCCCTTCTCCTCCGGGCTGTAATTAGCGCTTGGTT TAATGACGGCTCGTTTCTTTTCTGTGGCTGCGTGAAAGCC TTAAAGGGCTCCGGGAGGGCCCTTTGTGCGGGGGGGAGC GGCTCGGGGGGTGCGTGCGTGTGTGTGTGCGTGGGGAGC GCCGCGTGCGGCCCGCGCTGCCCGGCGGCTGTGAGCGCT GCGGGCGCGGCGCGGGGCTTTGTGCGCTCCGCGTGTGCG CGAGGGGAGCGCGGCCGGGGGCGGTGCCCCGCGGTGCG GGGGGGCTGCGAGGGGAACAAAGGCTGCGTGCGGGGTG TGTGCGTGGGGGGGTGAGCAGGGGGTGTGGGCGCGGCG GTCGGGCTGTAACCCCCCCCTGCACCCCCCTCCCCGAGTT GCTGAGCACGGCCCGGCTTCGGGTGCGGGGCTCCGTGCG

		GGGCGTGGCGCGGGGCTCGCCGTGCCGGGCGGGGGGTGG CGGCAGGTGGGGGTGCCGGGCGGGGCGGGGCCGCCTCG GGCCGGGGAGGGCTCGGGGGAGGGGCGCGGCGGCCCCG GAGCGCCGGCGGCTGTCGAGGCGCGGCGAGCCGCAGCCA TTGCCTTTTATGGTAATCGTGCGAGAGGGCGCAGGGACTT CCTTTGTCCCAAATCTGGCGGAGCCGAAATCTGGGAGGC GCCGCCGCACCCCCTCTAGCGGGCGCGGGCGAAGCGGTG CGGCGCCGGCAGGAAGGAAATGGGCGGGGAGGGCCTTC GTGCGTCGCCGCGCCGCCGTCCCCTTCTCCATCTCCAGCC TCGGGGCTGCCGCAGGGGGACGGCTGCCTTCGGGGGGGA CGGGGCAGGGCGGGGTTCGGCTTCTGGCGTGTGACCGGC GGGAATTC
31	DNA fragment containing VSV-G	GAATTCATGAAGTGCCTTTTGTACTTAGCCTTTTTATTCAT TGGGGTGAATTGCAAGTTCACCATAGTTTTTCCACACAAC CAAAAAGGAAACTGGAAAAATGTTCCTTCTAATTACCAT TATTGCCCGTCAAGCTCAGATTTAAATTGGCATAATGACT TAATAGGCACAGCCTTACAAGTCAAAATGCCCAAGAGTC ACAAGGCTATTCAAGCAGACGGTTGGATGTGTCATGCTT CCAAATGGGTCACTACTTGTGATTTCCGCTGGTATGGACC GAAGTATATAACACATTCCATCCGATCCTTCACTCCATCT GTAGAACAATGCAAGGAAAGCATTGAACAAACGAAACA AGGAACTTGGCTGAATCCAGGCTTCCCTCCTCAAAGTTGT GGATATGCAACTGTGACGGATGCCGAAGCAGTGATTGTC CAGGTGACTCCTCACCATGTGCTGGTTGATGAATACACA GGAGAATGGGTTGATTCACAGTTCATCAACGGAAAATGC AGCAATTACATATGCCCCACTGTCCATAACTCTACAACCT GGCATTCTGACTATAAGGTCAAAGGGCTATGTGATTCTA ACCTCATTTCCATGGACATCACCTTCTTCTCAGAGGACGG AGAGCTATCATCCCTGGGAAAGGAGGGCACAGGGTTCAG AAGTAACTACTTTGCTTATGAAACTGGAGGCAAGGCCTG CAAAATGCAATACTGCAAGCATTGGGGAGTCAGACTCCC ATCAGGTGTCTGGTTCGAGATGGCTGATAAGGATCTCTTT GCTGCAGCCAGATTCCCTGAATGCCCAGAAGGGTCAAGT ATCTCTGCTCCATCTCAGACCTCAGTGGATGTAAGTCTAA TTCAGGACGTTGAGAGGATCTTGGATTATTCCCTCTGCCA AGAAACCTGGAGCAAAATCAGAGCGGGTCTTCCAATCTC TCCAGTGGATCTCAGCTATCTTGCTCCTAAAAACCCAGGA ACCGGTCCTGCTTTCACCATAATCAATGGTACCCTAAAAT ACTTTGAGACCAGATACATCAGAGTCGATATTGCTGCTCC AATCCTCTCAAGAATGGTCGGAATGATCAGTGGAACTAC CACAGAAAGGGAACTGTGGGATGACTGGGCACCATATGA AGACGTGGAAATTGGACCCAATGGAGTTCTGAGGACCAG TTCAGGATATAAGTTTCCTTTATACATGATTGGACATGGT ATGTTGGACTCCGATCTTCATCTTAGCTCAAAGGCTCAGG TGTTCGAACATCCTCACATTCAAGACGCTGCTTCGCAACT TCCTGATGATGAGAGTTTATTTTTTGGTGATACTGGGCTA TCCAAAAATCCAATCGAGCTTGTAGAAGGTTGGTTCAGT AGTTGGAAAAGCTCTATTGCCTCTTTTTTCTTTATCATAG GGTTAATCATTGGACTATTCTTGGTTCTCCGAGTTGGTAT CCATCTTTGCATTAAATTAAAGCACACCAAGAAAAGACA GATTTATACAGACATAGAGATGAGAATTC
32	DNA fragment of	TCTAGAAGGAGCTTTGTTCCTTGGGTTCTTGGGAGC

	Helper plasmid without Rev containing RRE and rabbit beta globin poly A	AGCAGGAAGCACTATGGGCGCAGCGTCAATGACGC TGACGGTACAGGCCAGACAATTATTGTCTGGTATAG TGCAGCAGCAGAACAATTTGCTGAGGGCTATTGAGG CGCAACAGCATCTGTTGCAACTCACAGTCTGGGGCA TCAAGCAGCTCCAGGCAAGAATCCTGGCTGTGGAAA GATACCTAAAGGATCAACAGCTCCTAGATCTTTTTC CCTCTGCCAAAAATTATGGGGACATCATGAAGCCCC TTGAGCATCTGACTTCTGGCTAATAAAGGAAATTTA TTTTCATTGCAATAGTGTGTTGGAATTTTTTGTGTCT CTCACTCGGAAGGACATATGGGAGGGCAAATCATTT AAAACATCAGAATGAGTATTTGGTTTAGAGTTTGGC AACATATGCCATATGCTGGCTGCCATGAACAAAGGT GGCTATAAAGAGGTCATCAGTATATGAAACAGCCCC CTGCTGTCCATTCCTTATTCCATAGAAAAGCCTTGAC TTGAGGTTAGATTTTTTTTATATTTTGTTTTGTGTTAT TTTTTTCTTTAACATCCCTAAAATTTTCCTTACATGTT TTACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCC CAGTCATAGCTGTCCCTCTTCTCTTATGAAGATCCCT CGACCTGCAGCCCAAGCTTGGCGTAATCATGGTCAT AGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAAT TCCACACAACATACGAGCCGGAAGCATAAAGTGTA AAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACAT TAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGG AAACCTGTCGTGCCAGCGGATCCGCATCTCAATTAG TCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATC CCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGC CCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGC CGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGTA GTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAA AAAGCTAACTTGTTTATTGCAGCTTATAATGGTTACA AATAAAGCAATAGCATCACAAATTTCACAAATAAAG CATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAA ACTCATCAATGTATCTTATCACCCGGG
33	RSV promoter and HIV Rev	CAATTGCGATGTACGGGCCAGATATACGCGTATCTGAGG GGACTAGGGTGTGTTTAGGCGAAAAGCGGGGCTTCGGTT GTACGCGGTTAGGAGTCCCCTCAGGATATAGTAGTTTCGC TTTTGCATAGGGAGGGGGAAATGTAGTCTTATGCAATAC ACTTGTAGTCTTGCAACATGGTAACGATGAGTTAGCAAC ATGCCTTACAAGGAGAGAAAAAGCACCGTGCATGCCGAT TGGTGGAAGTAAGGTGGTACGATCGTGCCTTATTAGGAA GGCAACAGACAGGTCTGACATGGATTGGACGAACCACTG AATTCCGCATTGCAGAGATAATTGTATTTAAGTGCCTAGC TCGATACAATAAACGCCATTTGACCATTCACCACATTGGT GTGCACCTCCAAGCTCGAGCTCGTTTAGTGAACCGTCAG ATCGCCTGGAGACGCCATCCACGCTGTTTTGACCTCCATA GAAGACACCGGGACCGATCCAGCCTCCCCTCGAAGCTAG CGATTAGGCATCTCCTATGGCAGGAAGAAGCGGAGACAG CGACGAAGAACTCCTCAAGGCAGTCAGACTCATCAAGTT TCTCTATCAAAGCAACCCACCTCCCAATCCCGAGGGGAC CCGACAGGCCCGAAGGAATAGAAGAAGAAGGTGGAGAG

		AGAGACAGAGACAGATCCATTCGATTAGTGAACGGATCC TTAGCACTTATCTGGGACGATCTGCGGAGCCTGTGCCTCT TCAGCTACCACCGCTTGAGAGACTTACTCTTGATTGTAAC GAGGATTGTGGAACTTCTGGGACGCAGGGGGTGGGAAGC CCTCAAATATTGGTGGAATCTCCTACAATATTGGAGTCAG GAGCTAAAGAATAGTCTAGA
34	Elongation Factor-1 alpha (EF1-alpha) promoter	CCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAA AGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTG GGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACG TTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAG TGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGT TATGGCCCTTGCGTGCCTTGAATTACTTCCACGCCCCTGG CTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAA GTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCC CTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTG GGGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTG TCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTT GATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCT TGTAAATGCGGGCCAAGATCTGCACACTGGTATTTCGGTT TTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAG CGCACATGTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCA CCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCT GCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGC CCTGGGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGCGT GAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGA GCTCAAAATGGAGGACGCGGCGCTCGGGAGAGCGGGCG GGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCC TCAGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCG CCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGAGTA CGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGG AGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTTAGGC CAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCT TTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACA GTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGA
35	Promoter; PGK	GGGGTTGGGGTTGCGCCTTTTCCAAGGCAGCCCTGGGTTT GCGCAGGGACGCGGCTGCTCTGGGCGTGGTTCCGGGAAA CGCAGCGGCGCCGACCCTGGGTCTCGCACATTCTTCACGT CCGTTCGCAGCGTCACCCGGATCTTCGCCGCTACCCTTGT GGGCCCCCCGGCGACGCTTCCTGCTCCGCCCCTAAGTCGG GAAGGTTCCTTGCGGTTCGCGGCGTGCCGGACGTGACAA ACGGAAGCCGCACGTCTCACTAGTACCCTCGCAGACGGA CAGCGCCAGGGAGCAATGGCAGCGCGCCGACCGCGATG GGCTGTGGCCAATAGCGGCTGCTCAGCAGGGCGCGCCGA GAGCAGCGGCCGGGAAGGGGCGGTGCGGGAGGCGGGGT GTGGGGCGGTAGTGTGGGCCCTGTTCCTGCCCGCGCGGT GTTCCGCATTCTGCAAGCCTCCGGAGCGCACGTCGGCAG TCGGCTCCCTCGTTGACCGAATCACCGACCTCTCTCCCCA G
36	Promoter; UbC	GCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCCTCCTC ACGGCGAGCGCTGCCACGTCAGACGAAGGGCGCAGGAG CGTTCCTGATCCTTCCGCCCGGACGCTCAGGACAGCGGCC CGCTGCTCATAAGACTCGGCCTTAGAACCCCAGTATCAG

		CAGAAGGACATTTTAGGACGGGACTTGGGTGACTCTAGG GCACTGGTTTTCTTTCCAGAGAGCGGAACAGGCGAGGAA AAGTAGTCCCTTCTCGGCGATTCTGCGGAGGGATCTCCGT GGGGCGGTGAACGCCGATGATTATATAAGGACGCGCCGG GTGTGGCACAGCTAGTTCCGTCGCAGCCGGGATTTGGGT CGCGGTTCTTGTTTGTGGATCGCTGTGATCGTCACTTGGT GAGTTGCGGGCTGCTGGGCTGGCCGGGGCTTTCGTGGCC GCCGGGCCGCTCGGTGGGACGGAAGCGTGTGGAGAGACC GCCAAGGGCTGTAGTCTGGGTCCGCGAGCAAGGTTGCCC TGAACTGGGGGTTGGGGGGAGCGCACAAAATGGCGGCTG TTCCCGAGTCTTGAATGGAAGACGCTTGTAAGGCGGGCT GTGAGGTCGTTGAAACAAGGTGGGGGGCATGGTGGGCGG CAAGAACCCAAGGTCTTGAGGCCTTCGCTAATGCGGGAA AGCTCTTATTCGGGTGAGATGGGCTGGGGCACCATCTGG GGACCCTGACGTGAAGTTTGTCACTGACTGGAGAACTCG GGTTTGTCGTCTGGTTGCGGGGGCGGCAGTTATGCGGTGC CGTTGGGCAGTGCACCCGTACCTTTGGGAGCGCGCGCCT CGTCGTGTCGTGACGTCACCCGTTCTGTTGGCTTATAATG CAGGGTGGGGCCACCTGCCGGTAGGTGTGCGGTAGGCTT TTCTCCGTCGCAGGACGCAGGGTTCGGGCCTAGGGTAGG CTCTCCTGAATCGACAGGCGCCGGACCTCTGGTGAGGGG AGGGATAAGTGAGGCGTCAGTTTCTTTGGTCGGTTTTATG TACCTATCTTCTTAAGTAGCTGAAGCTCCGGTTTTGAACT ATGCGCTCGGGGTTGGCGAGTGTGTTTTGTGAAGTTTTTT AGGCACCTTTTGAAATGTAATCATTTGGGTCAATATGTAA TTTTCAGTGTTAGACTAGTAAA
37	Poly A; SV40	GTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGC ATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATT CTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCA
38	Poly A; bGH	GACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCC TCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCA CTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTG TCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGG GCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCA GGCATGCTGGGGATGCGGTGGGCTCTATGG
39	Envelope; RD114	ATGAAACTCCCAACAGGAATGGTCATTTTATGTAGCCTA ATAATAGTTCGGGCAGGGTTTGACGACCCCCGCAAGGCT ATCGCATTAGTACAAAAACAACATGGTAAACCATGCGAA TGCAGCGGAGGGCAGGTATCCGAGGCCCCACCGAACTCC ATCCAACAGGTAACTTGCCCAGGCAAGACGGCCTACTTA ATGACCAACCAAAAATGGAAATGCAGAGTCACTCCAAAA AATCTCACCCCTAGCGGGGGAGAACTCCAGAACTGCCCC TGTAACACTTTCCAGGACTCGATGCACAGTTCTTGTTATA CTGAATACCGGCAATGCAGGGCGAATAATAAGACATACT ACACGGCCACCTTGCTTAAAATACGGTCTGGGAGCCTCA ACGAGGTACAGATATTACAAAACCCCAATCAGCTCCTAC AGTCCCCTTGTAGGGGCTCTATAAATCAGCCCGTTTGCTG GAGTGCCACAGCCCCCATCCATATCTCCGATGGTGGAGG ACCCCTCGATACTAAGAGAGTGTGGACAGTCCAAAAAAG GCTAGAACAAATTCATAAGGCTATGCATCCTGAACTTCA ATACCACCCCTTAGCCCTGCCCAAAGTCAGAGATGACCTT AGCCTTGATGCACGGACTTTTGATATCCTGAATACCACTT

		TTAGGTTACTCCAGATGTCCAATTTTAGCCTTGCCCAAGA TTGTTGGCTCTGTTTAAAACTAGGTACCCCTACCCCTCTT GCGATACCCACTCCCTCTTTAACCTACTCCCTAGCAGACT CCCTAGCGAATGCCTCCTGTCAGATTATACCTCCCCTCTT GGTTCAACCGATGCAGTTCTCCAACTCGTCCTGTTTATCT TCCCCTTTCATTAACGATACGGAACAAATAGACTTAGGTG CAGTCACCTTTACTAACTGCACCTCTGTAGCCAATGTCAG TAGTCCTTTATGTGCCCTAAACGGGTCAGTCTTCCTCTGT GGAAATAACATGGCATACACCTATTTACCCCAAAACTGG ACAGGACTTTGCGTCCAAGCCTCCCTCCTCCCCGACATTG ACATCATCCCGGGGGATGAGCCAGTCCCCATTCCTGCCAT TGATCATTATATACATAGACCTAAACGAGCTGTACAGTTC ATCCCTTTACTAGCTGGACTGGGAATCACCGCAGCATTCA CCACCGGAGCTACAGGCCTAGGTGTCTCCGTCACCCAGT ATACAAAATTATCCCATCAGTTAATATCTGATGTCCAAGT CTTATCCGGTACCATACAAGATTTACAAGACCAGGTAGA CTCGTTAGCTGAAGTAGTTCTCCAAAATAGGAGGGGACT GGACCTACTAACGGCAGAACAAGGAGGAATTTGTTTAGC CTTACAAGAAAAATGCTGTTTTTATGCTAACAAGTCAGG AATTGTGAGAAACAAAATAAGAACCCTACAAGAAGAATT ACAAAAACGCAGGGAAAGCCTGGCATCCAACCCTCTCTG GACCGGGCTGCAGGGCTTTCTTCCGTACCTCCTACCTCTC CTGGGACCCCTACTCACCCTCCTACTCATACTAACCATTG GGCCATGCGTTTTCAATCGATTGGTCCAATTTGTTAAAGA CAGGATCTCAGTGGTCCAGGCTCTGGTTTTGACTCAGCAA TATCACCAGCTAAAACCCATAGAGTACGAGCCATGA
40	Envelope; GALV	ATGCTTCTCACCTCAAGCCCGCACCACCTTCGGCACCAGA TGAGTCCTGGGAGCTGGAAAAGACTGATCATCCTCTTAAG CTGCGTATTCGGAGACGGCAAAACGAGTCTGCAGAATAA GAACCCCCACCAGCCTGTGACCCTCACCTGGCAGGTACTG TCCCAAACTGGGGACGTTGTCTGGGACAAAAAGGCAGTC CAGCCCCTTTGGACTTGGTGGCCCTCTCTTACACCTGATG TATGTGCCCTGGCGGCCGGTCTTGAGTCCTGGGATATCCC GGGATCCGATGTATCGTCCTCTAAAAGAGTTAGACCTCCT GATTCAGACTATACTGCCGCTTATAAGCAAATCACCTGGG GAGCCATAGGGTGCAGCTACCCTCGGGCTAGGACCAGGA TGGCAAATTCCCCCTTCTACGTGTGTCCCCGAGCTGGCCG AACCCATTCAGAAGCTAGGAGGTGTGGGGGGCTAGAATC CCTATACTGTAAAGAATGGAGTTGTGAGACCACGGGTAC CGTTTATTGGCAACCCAAGTCCTCATGGGACCTCATAACT GTAAAATGGGACCAAAATGTGAAATGGGAGCAAAAATTT CAAAAGTGTGAACAAACCGGCTGGTGTAACCCCCTCAAG ATAGACTTCACAGAAAAAGGAAAACTCTCCAGAGATTGG ATAACGGAAAAAACCTGGGAATTAAGGTTCTATGTATAT GGACACCCAGGCATACAGTTGACTATCCGCTTAGAGGTC ACTAACATGCCGGTTGTGGCAGTGGGCCCAGACCCTGTCC TTGCGGAACAGGGACCTCCTAGCAAGCCCCTCACTCTCCC TCTCTCCCCACGGAAAGCGCCGCCCACCCCTCTACCCCCG GCGGCTAGTGAGCAAACCCCTGCGGTGCATGGAGAAACT GTTACCCTAAACTCTCCGCCTCCCACCAGTGGCGACCGAC TCTTTGGCCTTGTGCAGGGGGCCTTCCTAACCTTGAATGC TACCAACCCAGGGGCCACTAAGTCTTGCTGGCTCTGTTTG GGCATGAGCCCCCCTTATTATGAAGGGATAGCCTCTTCAG

		GAGAGGTCGCTTATACCTCCAACCATACCCGATGCCACTG GGGGGCCCAAGGAAAGCTTACCCTCACTGAGGTCTCCGG ACTCGGGTCATGCATAGGGAAGGTGCCTCTTACCCATCAA CATCTTTGCAACCAGACCTTACCCATCAATTCCTCTAAAA ACCATCAGTATCTGCTCCCCTCAAACCATAGCTGGTGGGC CTGCAGCACTGGCCTCACCCCCTGCCTCTCCACCTCAGTT TTTAATCAGTCTAAAGACTTCTGTGTCCAGGTCCAGCTGA TCCCCCGCATCTATTACCATTCTGAAGAAACCTTGTTACA AGCCTATGACAAATCACCCCCCAGGTTTAAAAGAGAGCC TGCCTCACTTACCCTAGCTGTCTTCCTGGGGTTAGGGATT GCGGCAGGTATAGGTACTGGCTCAACCGCCCTAATTAAA GGGCCCATAGACCTCCAGCAAGGCCTAACCAGCCTCCAA ATCGCCATTGACGCTGACCTCCGGGCCCTTCAGGACTCAA TCAGCAAGCTAGAGGACTCACTGACTTCCCTATCTGAGGT AGTACTCCAAAATAGGAGAGGCCTTGACTTACTATTCCTT AAAGAAGGAGGCCTCTGCGCGGCCCTAAAAGAAGAGTGC TGTTTTTATGTAGACCACTCAGGTGCAGTACGAGACTCCA TGAAAAAACTTAAAGAAAGACTAGATAAAAGACAGTTAG AGCGCCAGAAAAACCAAAACTGGTATGAAGGGTGGTTCA ATAACTCCCCTTGGTTTACTACCCTACTATCAACCATCGCT GGGCCCCTATTGCTCCTCCTTTTGTTACTCACTCTTGGGCC CTGCATCATCAATAAATTAATCCAATTCATCAATGATAGG ATAAGTGCAGTCAAAATTTTAGTCCTTAGACAGAAATATC AGACCCTAGATAACGAGGAAAACCTTTAA
41	Envelope; FUG	ATGGTTCCGCAGGTTCTTTTGTTTGTACTCCTTCTGGGTTT TTCGTTGTGTTTCGGGAAGTTCCCCATTTACACGATACCA GACGAACTTGGTCCCTGGAGCCCTATTGACATACACCATC TCAGCTGTCCAAATAACCTGGTTGTGGAGGATGAAGGAT GTACCAACCTGTCCGAGTTCTCCTACATGGAACTCAAAGT GGGATACATCTCAGCCATCAAAGTGAACGGGTTCACTTGC ACAGGTGTTGTGACAGAGGCAGAGACCTACACCAACTTT GTTGGTTATGTCACAACCACATTCAAGAGAAAGCATTTCC GCCCCACCCCAGACGCATGTAGAGCCGCGTATAACTGGA AGATGGCCGGTGACCCCAGATATGAAGAGTCCCTACACA ATCCATACCCCGACTACCACTGGCTTCGAACTGTAAGAAC CACCAAAGAGTCCCTCATTATCATATCCCCAAGTGTGACA GATTTGGACCCATATGACAAATCCCTTCACTCAAGGGTCT TCCCTGGCGGAAAGTGCTCAGGAATAACGGTGTCCTCTAC CTACTGCTCAACTAACCATGATTACACCATTTGGATGCCC GAGAATCCGAGACCAAGGACACCTTGTGACATTTTTACCA ATAGCAGAGGGAAGAGAGCATCCAACGGGAACAAGACTT GCGGCTTTGTGGATGAAAGAGGCCTGTATAAGTCTCTAAA AGGAGCATGCAGGCTCAAGTTATGTGGAGTTCTTGGACTT AGACTTATGGATGGAACATGGGTCGCGATGCAAACATCA GATGAGACCAAATGGTGCCCTCCAGATCAGTTGGTGAATT TGCACGACTTTCGCTCAGACGAGATCGAGCATCTCGTTGT GGAGGAGTTAGTTAAGAAAAGAGAGGAATGTCTGGATGC ATTAGAGTCCATCATGACCACCAAGTCAGTAAGTTTCAGA CGTCTCAGTCACCTGAGAAAACTTGTCCCAGGGTTTGGAA AAGCATATACCATATTCAACAAAACCTTGATGGAGGCTG ATGCTCACTACAAGTCAGTCCGGACCTGGAATGAGATCAT CCCCTCAAAAGGGTGTTTGAAAGTTGGAGGAAGGTGCCA TCCTCATGTGAACGGGGTGTTTTTCAATGGTATAATATTA

		GGGCCTGACGACCATGTCCTAATCCCAGAGATGCAATCAT CCCTCCTCCAGCAACATATGGAGTTGTTGGAATCTTCAGT TATCCCCCTGATGCACCCCCTGGCAGACCCTTCTACAGTT TTCAAAGAAGGTGATGAGGCTGAGGATTTTGTTGAAGTTC ACCTCCCCGATGTGTACAAACAGATCTCAGGGGTTGACCT GGGTCTCCCGAACTGGGGAAAGTATGTATTGATGACTGC AGGGGCCATGATTGGCCTGGTGTTGATATTTTCCCTAATG ACATGGTGCAGAGTTGGTATCCATCTTTGCATTAAATTAA AGCACACCAAGAAAAGACAGATTTATACAGACATAGAGA TGAACCGACTTGGAAAGTAA
42	Envelope; LCMV	ATGGGTCAGATTGTGACAATGTTTGAGGCTCTGCCTCACA TCATCGATGAGGTGATCAACATTGTCATTATTGTGCTTAT CGTGATCACGGGTATCAAGGCTGTCTACAATTTTGCCACC TGTGGGATATTCGCATTGATCAGTTTCCTACTTCTGGCTG GCAGGTCCTGTGGCATGTACGGTCTTAAGGGACCCGACAT TTACAAAGGAGTTTACCAATTTAAGTCAGTGGAGTTTGAT ATGTCACATCTGAACCTGACCATGCCCAACGCATGTTCAG CCAACAACTCCCACCATTACATCAGTATGGGGACTTCTGG ACTAGAATTGACCTTCACCAATGATTCCATCATCAGTCAC AACTTTTGCAATCTGACCTCTGCCTTCAACAAAAAGACCT TTGACCACACACTCATGAGTATAGTTTCGAGCCTACACCT CAGTATCAGAGGGAACTCCAACTATAAGGCAGTATCCTG CGACTTCAACAATGGCATAACCATCCAATACAACTTGACA TTCTCAGATCGACAAAGTGCTCAGAGCCAGTGTAGAACCT TCAGAGGTAGAGTCCTAGATATGTTTAGAACTGCCTTCGG GGGGAAATACATGAGGAGTGGCTGGGGCTGGACAGGCTC AGATGGCAAGACCACCTGGTGTAGCCAGACGAGTTACCA ATACCTGATTATACAAAATAGAACCTGGGAAAACCACTG CACATATGCAGGTCCTTTTGGGATGTCCAGGATTCTCCTT TCCCAAGAGAAGACTAAGTTCTTCACTAGGAGACTAGCG GGCACATTCACCTGGACTTTGTCAGACTCTTCAGGGGTGG AGAATCCAGGTGGTTATTGCCTGACCAAATGGATGATTCT TGCTGCAGAGCTTAAGTGTTTCGGGAACACAGCAGTTGCG AAATGCAATGTAAATCATGATGCCGAATTCTGTGACATGC TGCGACTAATTGACTACAACAAGGCTGCTTTGAGTAAGTT CAAAGAGGACGTAGAATCTGCCTTGCACTTATTCAAAAC AACAGTGAATTCTTTGATTTCAGATCAACTACTGATGAGG AACCACTTGAGAGATCTGATGGGGGTGCCATATTGCAATT ACTCAAAGTTTTGGTACCTAGAACATGCAAAGACCGGCG AAACTAGTGTCCCCAAGTGCTGGCTTGTCACCAATGGTTC TTACTTAAATGAGACCCACTTCAGTGATCAAATCGAACAG GAAGCCGATAACATGATTACAGAGATGTTGAGGAAGGAT TACATAAAGAGGCAGGGGAGTACCCCCCTAGCATTGATG GACCTTCTGATGTTTTCCACATCTGCATATCTAGTCAGCAT CTTCCTGCACCTTGTCAAAATACCAACACACAGGCACATA AAAGGTGGCTCATGTCCAAAGCCACACCGATTAACCAAC AAAGGAATTTGTAGTTGTGGTGCATTTAAGGTGCCTGGTG TAAAAACCGTCTGGAAAAGACGCTGA
43	Envelope; FPV	ATGAACACTCAAATCCTGGTTTTCGCCCTTGTGGCAGTCA TCCCCACAAATGCAGACAAAATTTGTCTTGGACATCATGC TGTATCAAATGGCACCAAAGTAAACACACTCACTGAGAG AGGAGTAGAAGTTGTCAATGCAACGGAAACAGTGGAGCG GACAAACATCCCCAAAATTTGCTCAAAAGGGAAAAGAAC

		CACTGATCTTGGCCAATGCGGACTGTTAGGGACCATTACC GGACCACCTCAATGCGACCAATTTCTAGAATTTTCAGCTG ATCTAATAATCGAGAGACGAGAAGGAAATGATGTTTGTT ACCCGGGGAAGTTTGTTAATGAAGAGGCATTGCGACAAA TCCTCAGAGGATCAGGTGGGATTGACAAAGAAACAATGG GATTCACATATAGTGGAATAAGGACCAACGGAACAACTA GTGCATGTAGAAGATCAGGGTCTTCATTCTATGCAGAAAT GGAGTGGCTCCTGTCAAATACAGACAATGCTGCTTTCCCA CAAATGACAAAATCATACAAAAACACAAGGAGAGAATCA GCTCTGATAGTCTGGGGAATCCACCATTCAGGATCAACCA CCGAACAGACCAAACTATATGGGAGTGGAAATAAACTGA TAACAGTCGGGAGTTCCAAATATCATCAATCTTTTGTGCC GAGTCCAGGAACACGACCGCAGATAAATGGCCAGTCCGG ACGGATTGATTTTCATTGGTTGATCTTGGATCCCAATGAT ACAGTTACTTTTAGTTTCAATGGGGCTTTCATAGCTCCAA ATCGTGCCAGCTTCTTGAGGGGAAAGTCCATGGGGATCC AGAGCGATGTGCAGGTTGATGCCAATTGCGAAGGGGAAT GCTACCACAGTGGAGGGACTATAACAAGCAGATTGCCTT TTCAAAACATCAATAGCAGAGCAGTTGGCAAATGCCCAA GATATGTAAAACAGGAAAGTTTATTATTGGCAACTGGGA TGAAGAACGTTCCCGAACCTTCCAAAAAAAGGAAAAAAA GAGGCCTGTTTGGCGCTATAGCAGGGTTTATTGAAAATGG TTGGGAAGGTCTGGTCGACGGGTGGTACGGTTTCAGGCAT CAGAATGCACAAGGAGAAGGAACTGCAGCAGACTACAA AAGCACCCAATCGGCAATTGATCAGATAACCGGAAAGTT AAATAGACTCATTGAGAAAACCAACCAGCAATTTGAGCT AATAGATAATGAATTCACTGAGGTGGAAAAGCAGATTGG CAATTTAATTAACTGGACCAAAGACTCCATCACAGAAGT ATGGTCTTACAATGCTGAACTTCTTGTGGCAATGGAAAAC CAGCACACTATTGATTTGGCTGATTCAGAGATGAACAAGC TGTATGAGCGAGTGAGGAAACAATTAAGGGAAAATGCTG AAGAGGATGGCACTGGTTGCTTTGAAATTTTTCATAAATG TGACGATGATTGTATGGCTAGTATAAGGAACAATACTTAT GATCACAGCAAATACAGAGAAGAAGCGATGCAAAATAG AATACAAATTGACCCAGTCAAATTGAGTAGTGGCTACAA AGATGTGATACTTTGGTTTAGCTTCGGGGCATCATGCTTT TTGCTTCTTGCCATTGCAATGGGCCTTGTTTTCATATGTGT GAAGAACGGAAACATGCGGTGCACTATTTGTATATAA
44	Envelope; RRV	AGTGTAACAGAGCACTTTAATGTGTATAAGGCTACTAGAC CATACCTAGCACATTGCGCCGATTGCGGGGACGGGTACTT CTGCTATAGCCCAGTTGCTATCGAGGAGATCCGAGATGA GGCGTCTGATGGCATGCTTAAGATCCAAGTCTCCGCCCAA ATAGGTCTGGACAAGGCAGGCACCCACGCCCACACGAAG CTCCGATATATGGCTGGTCATGATGTTCAGGAATCTAAGA GAGATTCCTTGAGGGTGTACACGTCCGCAGCGTGCTCCAT ACATGGGACGATGGGACACTTCATCGTCGCACACTGTCCA CCAGGCGACTACCTCAAGGTTTCGTTCGAGGACGCAGATT CGCACGTGAAGGCATGTAAGGTCCAATACAAGCACAATC CATTGCCGGTGGGTAGAGAGAAGTTCGTGGTTAGACCAC ACTTTGGCGTAGAGCTGCCATGCACCTCATACCAGCTGAC AACGGCTCCCACCGACGAGGAGATTGACATGCATACACC GCCAGATATACCGGATCGCACCCTGCTATCACAGACGGC GGGCAACGTCAAAATAACAGCAGGCGGCAGGACTATCAG

		GTACAACTGTACCTGCGGCCGTGACAACGTAGGCACTAC CAGTACTGACAAGACCATCAACACATGCAAGATTGACCA ATGCCATGCTGCCGTCACCAGCCATGACAAATGGCAATTT ACCTCTCCATTTGTTCCCAGGGCTGATCAGACAGCTAGGA AAGGCAAGGTACACGTTCCGTTCCCTCTGACTAACGTCAC CTGCCGAGTGCCGTTGGCTCGAGCGCCGGATGCCACCTAT GGTAAGAAGGAGGTGACCCTGAGATTACACCCAGATCAT CCGACGCTCTTCTCCTATAGGAGTTTAGGAGCCGAACCGC ACCCGTACGAGGAATGGGTTGACAAGTTCTCTGAGCGCA TCATCCCAGTGACGGAAGAAGGGATTGAGTACCAGTGGG GCAACAACCCGCCGGTCTGCCTGTGGGCGCAACTGACGA CCGAGGGCAAACCCCATGGCTGGCCACATGAAATCATTC AGTACTATTATGGACTATACCCCGCCGCCACTATTGCCGC AGTATCCGGGGCGAGTCTGATGGCCCTCCTAACTCTGGCG GCCACATGCTGCATGCTGGCCACCGCGAGGAGAAAGTGC CTAACACCGTACGCCCTGACGCCAGGAGCGGTGGTACCG TTGACACTGGGGCTGCTTTGCTGCGCACCGAGGGCGAATG CA
45	Envelope; MLV 10A1	ATGGAAGGTCCAGCGTTCTCAAAACCCCTTAAAGATAAG ATTAACCCGTGGAAGTCCTTAATGGTCATGGGGGTCTATT TAAGAGTAGGGATGGCAGAGAGCCCCCATCAGGTCTTTA ATGTAACCTGGAGAGTCACCAACCTGATGACTGGGCGTA CCGCCAATGCCACCTCCCTTTTAGGAACTGTACAAGATGC CTTCCCAAGATTATATTTTGATCTATGTGATCTGGTCGGA GAAGAGTGGGACCCTTCAGACCAGGAACCATATGTCGGG TATGGCTGCAAATACCCCGGAGGGAGAAAGCGGACCCGG ACTTTTGACTTTTACGTGTGCCCTGGGCATACCGTAAAAT CGGGGTGTGGGGGGCCAAGAGAGGGCTACTGTGGTGAAT GGGGTTGTGAAACCACCGGACAGGCTTACTGGAAGCCCA CATCATCATGGGACCTAATCTCCCTTAAGCGCGGTAACAC CCCCTGGGACACGGGATGCTCCAAAATGGCTTGTGGCCCC TGCTACGACCTCTCCAAAGTATCCAATTCCTTCCAAGGGG CTACTCGAGGGGGCAGATGCAACCCTCTAGTCCTAGAATT CACTGATGCAGGAAAAAAGGCTAATTGGGACGGGCCCAA ATCGTGGGGACTGAGACTGTACCGGACAGGAACAGATCC TATTACCATGTTCTCCCTGACCCGCCAGGTCCTCAATATA GGGCCCCGCATCCCCATTGGGCCTAATCCCGTGATCACTG GTCAACTACCCCCCTCCCGACCCGTGCAGATCAGGCTCCC CAGGCCTCCTCAGCCTCCTCCTACAGGCGCAGCCTCTATA GTCCCTGAGACTGCCCCACCTTCTCAACAACCTGGGACGG GAGACAGGCTGCTAAACCTGGTAGAAGGAGCCTATCAGG CGCTTAACCTCACCAATCCCGACAAGACCCAAGAATGTTG GCTGTGCTTAGTGTCGGGACCTCCTTATTACGAAGGAGTA GCGGTCGTGGGCACTTATACCAATCATTCTACCGCCCCGG CCAGCTGTACGGCCACTTCCCAACATAAGCTTACCCTATC TGAAGTGACAGGACAGGGCCTATGCATGGGAGCACTACC TAAAACTCACCAGGCCTTATGTAACACCACCCAAAGTGCC GGCTCAGGATCCTACTACCTTGCAGCACCCGCTGGAACAA TGTGGGCTTGTAGCACTGGATTGACTCCCTGCTTGTCCAC CACGATGCTCAATCTAACCACAGACTATTGTGTATTAGTT GAGCTCTGGCCCAGAATAATTTACCACTCCCCCGATTATA TGTATGGTCAGCTTGAACAGCGTACCAAATATAAGAGGG AGCCAGTATCGTTGACCCTGGCCCTTCTGCTAGGAGGATT

		AACCATGGGAGGGATTGCAGCTGGAATAGGGACGGGGAC CACTGCCCTAATCAAAACCCAGCAGTTTGAGCAGCTTCAC GCCGCTATCCAGACAGACCTCAACGAAGTCGAAAAATCA ATTACCAACCTAGAAAAGTCACTGACCTCGTTGTCTGAAG TAGTCCTACAGAACCGAAGAGGCCTAGATTTGCTCTTCCT AAAAGAGGGAGGTCTCTGCGCAGCCCTAAAAGAAGAATG TTGTTTTTATGCAGACCACACGGGACTAGTGAGAGACAGC ATGGCCAAACTAAGGGAAAGGCTTAATCAGAGACAAAAA CTATTTGAGTCAGGCCAAGGTTGGTTCGAAGGGCAGTTTA ATAGATCCCCCTGGTTTACCACCTTAATCTCCACCATCAT GGGACCTCTAATAGTACTCTTACTGATCTTACTCTTTGGA CCCTGCATTCTCAATCGATTGGTCCAATTTGTTAAAGACA GGATCTCAGTGGTCCAGGCTCTGGTTTTGACTCAACAATA TCACCAGCTAAAACCTATAGAGTACGAGCCATGA
46	Envelope; Ebola	ATGGGTGTTACAGGAATATTGCAGTTACCTCGTGATCGAT TCAAGAGGACATCATTCTTTCTTTGGGTAATTATCCTTTTC CAAAGAACATTTTCCATCCCACTTGGAGTCATCCACAATA GCACATTACAGGTTAGTGATGTCGACAAACTGGTTTGCCG TGACAAACTGTCATCCACAAATCAATTGAGATCAGTTGGA CTGAATCTCGAAGGGAATGGAGTGGCAACTGACGTGCCA TCTGCAACTAAAAGATGGGGCTTCAGGTCCGGTGTCCCAC CAAAGGTGGTCAATTATGAAGCTGGTGAATGGGCTGAAA ACTGCTACAATCTTGAAATCAAAAAACCTGACGGGAGTG AGTGTCTACCAGCAGCGCCAGACGGGATTCGGGGCTTCC CCCGGTGCCGGTATGTGCACAAAGTATCAGGAACGGGAC CGTGTGCCGGAGACTTTGCCTTCCACAAAGAGGGTGCTTT CTTCCTGTATGACCGACTTGCTTCCACAGTTATCTACCGA GGAACGACTTTCGCTGAAGGTGTCGTTGCATTTCTGATAC TGCCCCAAGCTAAGAAGGACTTCTTCAGCTCACACCCCTT GAGAGAGCCGGTCAATGCAACGGAGGACCCGTCTAGTGG CTACTATTCTACCACAATTAGATATCAAGCTACCGGTTTT GGAACCAATGAGACAGAGTATTTGTTCGAGGTTGACAAT TTGACCTACGTCCAACTTGAATCAAGATTCACACCACAGT TTCTGCTCCAGCTGAATGAGACAATATATACAAGTGGGA AAAGGAGCAATACCACGGGAAAACTAATTTGGAAGGTCA ACCCCGAAATTGATACAACAATCGGGGAGTGGGCCTTCT GGGAAACTAAAAAAACCTCACTAGAAAAATTCGCAGTGA AGAGTTGTCTTTCACAGCTGTATCAAACAGAGCCAAAAA CATCAGTGGTCAGAGTCCGGCGCGAACTTCTTCCGACCCA GGGACCAACACAACAACTGAAGACCACAAAATCATGGCT TCAGAAAATTCCTCTGCAATGGTTCAAGTGCACAGTCAAG GAAGGGAAGCTGCAGTGTCGCATCTGACAACCCTTGCCA CAATCTCCACGAGTCCTCAACCCCCCACAACCAAACCAG GTCCGGACAACAGCACCCACAATACACCCGTGTATAAAC TTGACATCTCTGAGGCAACTCAAGTTGAACAACATCACCG CAGAACAGACAACGACAGCACAGCCTCCGACACTCCCCC CGCCACGACCGCAGCCGGACCCCTAAAAGCAGAGAACAC CAACACGAGCAAGGGTACCGACCTCCTGGACCCCGCCAC CACAACAAGTCCCCAAAACCACAGCGAGACCGCTGGCAA CAACAACACTCATCACCAAGATACCGGAGAAGAGAGTGC CAGCAGCGGGAAGCTAGGCTTAATTACCAATACTATTGCT GGAGTCGCAGGACTGATCACAGGCGGGAGGAGAGCTCGA AGAGAAGCAATTGTCAATGCTCAACCCAAATGCAACCCT

		AATTTACATTACTGGACTACTCAGGATGAAGGTGCTGCAA TCGGACTGGCCTGGATACCATATTTCGGGCCAGCAGCCGA GGGAATTTACATAGAGGGGCTGATGCACAATCAAGATGG TTTAATCTGTGGGTTGAGACAGCTGGCCAACGAGACGACT CAAGCTCTTCAACTGTTCCTGAGAGCCACAACCGAGCTAC GCACCTTTTCAATCCTCAACCGTAAGGCAATTGATTTCTT GCTGCAGCGATGGGGCGGCACATGCCACATTTTGGGACC GGACTGCTGTATCGAACCACATGATTGGACCAAGAACAT AACAGACAAAATTGATCAGATTATTCATGATTTTGTTGAT AAAACCCTTCCGGACCAGGGGGACAATGACAATTGGTGG ACAGGATGGAGACAATGGATACCGGCAGGTATTGGAGTT ACAGGCGTTATAATTGCAGTTATCGCTTTATTCTGTATAT GCAAATTTGTCTTTTAG
47	Polymerase III shRNA promoters; U6 promoter	TTTCCCATGATTCCTTCATATTTGCATATACGATACAAGG CTGTTAGAGAGATAATTGGAATTAATTTGACTGTAAACAC AAAGATATTAGTACAAAATACGTGACGTAGAAAGTAATA ATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAA TGGACTATCATATGCTTACCGTAACTTGAAAGTATTTCGA TTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAAC
48	Polymerase III shRNA promoters; 7SK promoter	CTGCAGTATTTAGCATGCCCCACCCATCTGCAAGGCATTC TGGATAGTGTCAAAACAGCCGGAAATCAAGTCCGTTTATC TCAAACTTTAGCATTTTGGGAATAAATGATATTTGCTATG CTGGTTAAATTAGATTTTAGTTAAATTTCCTGCTGAAGCT CTAGTACGATAAGCAACTTGACCTAAGTGTAAAGTTGAG ATTTCCTTCAGGTTTATATAGCTTGTGCGCCGCCTGGCTAC CTC
49	FDPS target sequence #1	GTCCTGGAGTACAATGCCATT
50	FDPS target sequence #2	GCAGGATTTCGTTCAGCACTT
51	FDPS target sequence #3	GCCATGTACATGGCAGGAATT
52	FDPS target sequence #4	GCAGAAGGAGGCTGAGAAAGT
53	Lenti-BTN3A1 (“LV-BTN3A1, lentivirus expressing BTN3A1”)	ATGAAAATGGCAAGTTTCCTGGCCTTCCTTCTGCTCAACT TTCGTGTCTGCCTCCTTTTGCTTCAGCTGCTCATGCCTCAC TCAGCTCAGTTTTCTGTGCTTGGACCCTCTGGGCCCATCCT GGCCATGGTGGGTGAAGACGCTGATCTGCCCTGTCACCTG TTCCCGACCATGAGTGCAGAGACCATGGAGCTGAAGTGG GTGAGTTCCAGCCTAAGGCAGGTGGTGAACGTGTATGCA GATGGAAAGGAAGTGGAAGACAGGCAGAGTGCACCGTAT CGAGGGAGAACTTCGATTCTGCGGGATGGCATCACTGCA GGGAAGGCTGCTCTCCGAATACACAACGTCACAGCCTCT GACAGTGGAAAGTACTTGTGTTATTTCCAAGATGGTGACT TCTATGAAAAAGCCCTGGTGGAGCTGAAGGTTGCAGCAC TGGGTTCTGATCTTCACGTTGATGTGAAGGGTTACAAGGA TGGAGGGATCCATCTGGAGTGCAGGTCCACTGGCTGGTA CCCCCAACCCCAAATACAGTGGAGCAACAACAAGGGAGA GAACATCCCGACTGTGGAAGCACCTGTGGTTGCAGACGG

		AGTGGGCCTGTATGCAGTAGCAGCATCTGTGATCATGAG AGGCAGCTCTGGGGAGGGTGTATCCTGTACCATCAGAAG TTCCCTCCTCGGCCTGGAAAAGACAGCCAGCATTTCCATC GCAGACCCCTTCTTCAGGAGCGCCCAGAGGTGGATCGCC GCCCTGGCAGGGACCCTGCCTGTCTTGCTGCTGCTTCTTG GGGGAGCCGGTTACTTCCTGTGGCAACAGCAGGAGGAAA AAAAGACTCAGTTCAGAAAGAAAAAGAGAGAGCAAGAG TTGAGAGAAATGGCATGGAGCACAATGAAGCAAGAACAA AGCACAAGAGTGAAGCTCCTGGAGGAACTCAGATGGAGA AGTATCCAGTATGCATCTCGGGGAGAGAGACATTCAGCC TATAATGAATGGAAAAAGGCCCTCTTCAAGCCTGCGGAT GTGATTCTGGATCCAAAAACAGCAAACCCCATCCTCCTTG TTTCTGAGGACCAGAGGAGTGTGCAGCGTGCCAAGGAGC CCCAGGATCTGCCAGACAACCCTGAGAGATTTAATTGGC ATTATTGTGTTCTCGGCTGTGAGAGCTTCATATCAGGGAG ACATTACTGGGAGGTGGAGGTAGGGGACAGGAAAGAGTG GCATATAGGGGTGTGCAGTAAGAATGTGCAGAGAAAAGG CTGGGTCAAAATGACACCTGAGAATGGATTCTGGACTAT GGGGCTGACTGATGGGAATAAGTATCGGACTCTAACTGA GCCCAGAACCAACCTGAAACTTCCTAAGCCCCCTAAGAA AGTGGGGGTCTTCCTGGACTATGAGACTGGAGATATCTCA TTCTACAATGCTGTGGATGGATCGCATATTCATACTTTCCT GGACGTCTCCTTCTCTGAGGCTCTATATCCTGTTTTCAGAA TTTTGACCTTGGAGCCCACGGCCCTGACTATTTGTCCAGC GTGA
54	Lenti-BTN3A3 (R381H)	ATGAAAATGGCAAGTTCCCTGGCTTTCCTTCTGCTCAACT TTCATGTCTCCCTCTTCTTGGTCCAGCTGCTCACTCCTTGC TCAGCTCAGTTTTCTGTGCTTGGACCCTCTGGGCCCATCCT GGCCATGGTGGGTGAAGACGCTGATCTGCCCTGTCACCTG TTCCCGACCATGAGTGCAGAGACCATGGAGCTGAGGTGG GTGAGTTCCAGCCTAAGGCAGGTGGTGAACGTGTATGCA GATGGAAAGGAAGTGGAAGACAGGCAGAGTGCACCGTAT CGAGGGAGAACTTCGATTCTGCGGGATGGCATCACTGCA GGGAAGGCTGCTCTCCGAATACACAACGTCACAGCCTCT GACAGTGGAAAGTACTTGTGTTATTTCCAAGATGGTGACT TCTACGAAAAAGCCCTGGTGGAGCTGAAGGTTGCAGCAT TGGGTTCTGATCTTCACATTGAAGTGAAGGGTTATGAGGA TGGAGGGATCCATCTGGAGTGCAGGTCCACTGGCTGGTA CCCCCAACCCCAAATAAAGTGGAGCGACACCAAGGGAGA GAACATCCCGGCTGTGGAAGCACCTGTGGTTGCAGATGG AGTGGGCCTGTATGCAGTAGCAGCATCTGTGATCATGAG AGGCAGCTCTGGTGGGGGTGTATCCTGCATCATCAGAAAT TCCCTCCTCGGCCTGGAAAAGACAGCCAGCATATCCATCG CAGACCCCTTCTTCAGGAGCGCCCAGCCCTGGATCGCGGC CCTGGCAGGGACCCTGCCTATCTCGTTGCTGCTTCTCGCA GGAGCCAGTTACTTCTTGTGGAGACAACAGAAGGAAAAA ATTGCTCTGTCCAGGGAGACAGAAAGAGAGCGAGAGATG AAAGAAATGGGATACGCTGCAACAGAGCAAGAAATAAG CCTAAGAGAGAAGCTCCAGGAGGAACTCAAGTGGAGGAA AATCCAGTACATGGCTCGTGGAGAGAAGTCTTTGGCCTAT

		CATGAATGGAAAATGGCCCTCTTCAAACCTGCGGATGTG ATTCTGGATCCAGACACGGCAAACGCCATCCTCCTTGTTT CTGAGGACCAGAGGAGTGTGCAGCGTGCTGAAGAGCCGC GGGATCTGCCAGACAACCCTGAGAGATTTGAATGGCACT ACTGTGTCCTTGGCTGTGAAAACTTCACATCAGGGAGACA TTACTGGGAGGTGGAAGTGGGGGACAGAAAAGAGTGGCA TATTGGGGTATGTAGTAAGAACGTGGAGAGGAAAAAAGG TTGGGTCAAAATGACACCGGAGAACGGATACTGGACTAT GGGCCTGACTGATGGGAATAAGTATCGGGCTCTCACTGA GCCCAGAACCAACCTGAAACTTCCTGAGCCTCCTAGGAA AGTGGGGATCTTCCTGGACTATGAGACTGGAGAGATCTC GTTCTATAATGCCACAGATGGATCTCATATCTACACCTTT CCGCACGCCTCTTTCTCTGAGCCTCTATATCCTGTTTTCAG AATTTTGACCTTGGAGCCCACTGCCCTGACCATTTGCCCA ATACCAAAAGAAGTAGAGAGTTCCCCCGATCCTGACCTA GTGCCTGATCATTCCCTGGAGACACCACTGACCCCGGGCT TAGCTAATGAAAGTGGGGAGCCTCAGGCTGAAGTAACAT CTCTGCTTCTCCCTGCCCACCCTGGAGCTGAGGTCTCCCCT TCTGCAACAACCAATCAGAACCATAAGCTACAGGCACGC ACTGAAGCACTTTACTGA
55	BTN3A3-FDPSsh-IL-2 (“BTN3A3 (R381H) T2A IL-2”)	ATGAAAATGGCAAGTTCCCTGGCTTTCCTTCTGCTCAACT TTCATGTCTCCCTCTTCTTGGTCCAGCTGCTCACTCCTTGC TCAGCTCAGTTTTCTGTGCTTGGACCCTCTGGGCCCATCCT GGCCATGGTGGGTGAAGACGCTGATCTGCCCTGTCACCTG TTCCCGACCATGAGTGCAGAGACCATGGAGCTGAGGTGG GTGAGTTCCAGCCTAAGGCAGGTGGTGAACGTGTATGCA GATGGAAAGGAAGTGGAAGACAGGCAGAGTGCACCGTAT CGAGGGAGAACTTCGATTCTGCGGGATGGCATCACTGCA GGGAAGGCTGCTCTCCGAATACACAACGTCACAGCCTCT GACAGTGGAAAGTACTTGTGTTATTTCCAAGATGGTGACT TCTACGAAAAAGCCCTGGTGGAGCTGAAGGTTGCAGCAT TGGGTTCTGATCTTCACATTGAAGTGAAGGGTTATGAGGA TGGAGGGATCCATCTGGAGTGCAGGTCCACTGGCTGGTA CCCCCAACCCCAAATAAAGTGGAGCGACACCAAGGGAGA GAACATCCCGGCTGTGGAAGCACCTGTGGTTGCAGATGG AGTGGGCCTGTATGCAGTAGCAGCATCTGTGATCATGAG AGGCAGCTCTGGTGGGGGTGTATCCTGCATCATCAGAAAT TCCCTCCTCGGCCTGGAAAAGACAGCCAGCATATCCATCG CAGACCCCTTCTTCAGGAGCGCCCAGCCCTGGATCGCGGC CCTGGCAGGGACCCTGCCTATCTCGTTGCTGCTTCTCGCA GGAGCCAGTTACTTCTTGTGGAGACAACAGAAGGAAAAA ATTGCTCTGTCCAGGGAGACAGAAAGAGAGCGAGAGATG AAAGAAATGGGATACGCTGCAACAGAGCAAGAAATAAG CCTAAGAGAGAAGCTCCAGGAGGAACTCAAGTGGAGGAA AATCCAGTACATGGCTCGTGGAGAGAAGTCTTTGGCCTAT CATGAATGGAAAATGGCCCTCTTCAAACCTGCGGATGTG ATTCTGGATCCAGACACGGCAAACGCCATCCTCCTTGTTT CTGAGGACCAGAGGAGTGTGCAGCGTGCTGAAGAGCCGC GGGATCTGCCAGACAACCCTGAGAGATTTGAATGGCACT ACTGTGTCCTTGGCTGTGAAAACTTCACATCAGGGAGACA

		TTACTGGGAGGTGGAAGTGGGGGACAGAAAAGAGTGGCA TATTGGGGTATGTAGTAAGAACGTGGAGAGGAAAAAAGG TTGGGTCAAAATGACACCGGAGAACGGATACTGGACTAT GGGCCTGACTGATGGGAATAAGTATCGGGCTCTCACTGA GCCCAGAACCAACCTGAAACTTCCTGAGCCTCCTAGGAA AGTGGGGATCTTCCTGGACTATGAGACTGGAGAGATCTC GTTCTATAATGCCACAGATGGATCTCATATCTACACCTTT CCGCACGCCTCTTTCTCTGAGCCTCTATATCCTGTTTTCAG AATTTTGACCTTGGAGCCCACTGCCCTGACCATTTGCCCA ATACCAAAAGAAGTAGAGAGTTCCCCCGATCCTGACCTA GTGCCTGATCATTCCCTGGAGACACCACTGACCCCGGGCT TAGCTAATGAAAGTGGGGAGCCTCAGGCTGAAGTAACAT CTCTGCTTCTCCCTGCCCACCCTGGAGCTGAGGTCTCCCCT TCTGCAACAACCAATCAGAACCATAAGCTACAGGCACGC ACTGAAGCACTTTACCGTAGACGAAAGCGCGGAAGCGGA GAGGGCAGAGGAAGTCTGCTAACATGCGGTGACGTCGAG GAGAATCCTGGACCTATGTACAGGATGCAACTCCTGTCTT GCATTGCACTAAGTCTTGCACTTGTCACAAACAGTGCACC TACTTCAAGTTCTACAAAGAAAACACAGCTACAACTGGA GCATTTACTGCTGGATTTACAGATGATTTTGAATGGAATT AATAATTACAAGAATCCCAAACTCACCAGGATGCTCACA TTTAAGTTTTACATGCCCAAGAAGGCCACAGAACTGAAA CATCTTCAGTGTCTAGAAGAAGAACTCAAACCTCTGGAG GAAGTGCTAAATTTAGCTCAAAGCAAAAACTTTCACTTAA GACCCAGGGACTTAATCAGCAATATCAACGTAATAGTTCT GGAACTAAAGGGATCTGAAACAACATTCATGTGTGAATA TGCTGATGAGACAGCAACCATTGTAGAATTTCTGAACAG ATGGATTACCTTTTGTCAAAGCATCATCTCAACACTGACT TGA
56	Cytokine IL-2 (“IL-2, or IL2”)	ATGTACAGGATGCAACTCCTGTCTTGCATTGCACTAAGTC TTGCACTTGTCACAAACAGTGCACCTACTTCAAGTTCTAC AAAGAAAACACAGCTACAACTGGAGCATTTACTGCTGGA TTTACAGATGATTTTGAATGGAATTAATAATTACAAGAAT CCCAAACTCACCAGGATGCTCACATTTAAGTTTTACATGC CCAAGAAGGCCACAGAACTGAAACATCTTCAGTGTCTAG AAGAAGAACTCAAACCTCTGGAGGAAGTGCTAAATTTAG CTCAAAGCAAAAACTTTCACTTAAGACCCAGGGACTTAAT CAGCAATATCAACGTAATAGTTCTGGAACTAAAGGGATC TGAAACAACATTCATGTGTGAATATGCTGATGAGACAGC AACCATTGTAGAATTTCTGAACAGATGGATTACCTTTTGT CAAAGCATCATCTCAACACTGACTTGA
57	Cytokine IL-15 (“IL15”, or “IL-15”)	ATGAGAATTTCGAAACCACATTTGAGAAGTATTTCCATCC AGTGCTACTTGTGTTTACTTCTAAACAGTCATTTTCTAACT GAAGCTGGCATTCATGTCTTCATTTTGGGCTGTTTCAGTG CAGGGCTTCCTAAAACAGAAGCCAACTGGGTGAATGTAA TAAGTGATTTGAAAAAAATTGAAGATCTTATTCAATCTAT GCATATTGATGCTACTTTATATACGGAAAGTGATGTTCAC CCCAGTTGCAAAGTAACAGCAATGAAGTGCTTTCTCTTGG AGTTACAAGTTATTTCACTTGAGTCCGGAGATGCAAGTAT

		TCATGATACAGTAGAAAATCTGATCATCCTAGCAAACAA CAGTTTGTCTTCTAATGGGAATGTAACAGAATCTGGATGC AAAGAATGTGAGGAACTGGAGGAAAAAAATATTAAAGA ATTTTTGCAGAGTTTTGTACATATTGTCCAAATGTTCATCA ACACTTCTTGA
58	Cytokine IL-18 (“IL-18”, or “IL18”)	CTGGACAGTCAGCAAGGAATTGTCTCCCAGTGCATTTTGC CCTCCTGGCTGCCAACTCTGGCTGCTAAAGCGGCTGCCAC CTGCTGCAGTCTACACAGCTTCGGGAAGAGGAAAGGAAC CTCAGACCTTCCAGATCGCTTCCTCTCGCAACAAACTATT TGTCGCAGGAATAAAGATGGCTGCTGAACCAGTAGAAGA CAATTGCATCAACTTTGTGGCAATGAAATTTATTGACAAT ACGCTTTACTTTATAGCTGAAGATGATGAAAACCTGGAAT CAGATTACTTTGGCAAGCTTGAATCTAAATTATCAGTCAT AAGAAATTTGAATGACCAAGTTCTCTTCATTGACCAAGGA AATCGGCCTCTATTTGAAGATATGACTGATTCTGACTGTA GAGATAATGCACCCCGGACCATATTTATTATAAGTATGTA TAAAGATAGCCAGCCTAGAGGTATGGCTGTAACTATCTCT GTGAAGTGTGAGAAAATTTCAACTCTCTCCTGTGAGAACA AAATTATTTCCTTTAAGGAAATGAATCCTCCTGATAACAT CAAGGATACAAAAAGTGACATCATATTCTTTCAGAGAAG TGTCCCAGGACATGATAATAAGATGCAATTTGAATCTTCA TCATACGAAGGATACTTTCTAGCTTGTGAAAAAGAGAGA GACCTTTTTAAACTCATTTTGAAAAAAGAGGATGAATTGG GGGATAGATCTATAATGTTCACTGTTCAAAACGAAGACTA G
59	Lenti-AFP tumor-specific promoter (BTN3A3) [“LTSP-AFP BTN3A3”]	CGATAGTTTGAGGAGAATATTTGTTATATTTGCAAAATAA AATAAGTTTGCAAGTTTTTTTTTCTGCCCCAAAGAGCTCT GTGTCCTTGAACATAAAATACAAATAACCGCTATGCTGTT AATTATTGGCAAATGTCCCATTTTCAACCTAAGGAAATAC CATAAAGTAACAGATATACCAACAAAAGGTTACTAGTTA ACAGGCATTGCCTGAAAAGAGTATAAAAGAATTTCAGCA TGATTTTCCATATTGTGCTTCCACCACTGCCAATAACACG
60	Cytokine IL-23 (“IL-23”, or “IL23”) alpha subunit p19	AGAGCCAGCCAGATTTGAGAAGAAGGCAAAAAGATGCTG GGGAGCAGAGCTGTAATGCTGCTGTTGCTACTGCCCTGGA CAGCTCAGGGCAGAGCTGTGCCTGGGGGCAGCAGCCCTG CCTGGACTCAGTGCCAGCAGCTTTCACAGAAGCTCTGCAC ACTGGCCTGGAGTGCACATCCACTAGTGGGACACATGGA TCTAAGAGAAGAGGGAGATGAAGAGACTACAAATGATGT TCCCCATATCCAGTGTGGAGATGGCTGTGACCCCCAAGGA CTCAGGGACAACAGTCAGTTCTGCTTGCAAAGGATCCACC AGGGTCTGATTTTTTATGAGAAGCTGCTAGGATCGGATAT TTTCACAGGGGAGCCTTCTCTGCTCCCTGATAGCCCTGTG GGCCAGCTTCATGCCTCCCTACTGGGCCTCAGCCAACTCC TGCAGCCTGAGGGTCACCACTGGGAGACTCAGCAGATTC CAAGCCTCAGTCCCAGCCAGCCATGGCAGCGTCTCCTTCT CCGCTTCAAGATCCTTCGCAGCCTCCAGGCCTTTGTGGCC

		GTAGCCGCCCGGGTCTTTGCCCATGGAGCAGCAACCCTGA GTCCCTAAAGGCAGCAGCTCAAG
61	Cytokine IL-36A (“IL-36A”, or “IL36A”) alpha	AAAACCCAAGTGCAGTAGAAGCCATTGTTCATAATGGTA GGGATACAGGGTCCTTCGTAACAGATTATCAGTGTGGCCT ATGCTGGAAAGTCTGGTGACCTCTGATTTTTTTTGCTTCCA GGTCTTTGGCCTTGGCACTCTTTGTCATATTAGAGTTCCTG GGTCTAGGCCTGGGCAGGATTCATAGGTGCAGCTGCTTCT GCTGGAGGTAGACTGCATCCAACAAAGTAAGGGTGCTGG GTGAGTTCTGGGAGTATAGATTCTGACTGGGGTCACTGCT GGGCTGGCCGCCAGTCTTTCATCTGACCCAGGGTTAAACT GTGGCTTGGGACTGACTCAGGTCCTCTCTTGGGGTCGGTC TGCACATAAAAGGACTCCTATCCTTGGCAGTTCTGAAACA ACACCACCACAATGGAAAAAGCATTGAAAATTGACACAC CTCAGCAGGGGAGCATTCAGGATATCAATCATCGGGTGT GGGTTCTTCAGGACCAGACGCTCATAGCAGTCCCGAGGA AGGACCGTATGTCTCCAGTCACTATTGCCTTAATCTCATG CCGACATGTGGAGACCCTTGAGAAAGACAGAGGGAACCC CATCTACCTGGGCCTGAATGGACTCAATCTCTGCCTGATG TGTGCTAAAGTCGGGGACCAGCCCACACTGCAGCTGAAG GAAAAGGATATAATGGATTTGTACAACCAACCCGAGCCT GTGAAGTCCTTTCTCTTCTACCACAG CCAGAGTGGCAGG AACTCCACCTTCGAGTCTGTGGCTTTCCCTGGCTGGTTCAT CGCTGTCAGCTCTGAAGGAGGCTGTCCTCTCATCCTTACC CAAGAACTGGGGAAAGCCAACACTACTGACTTTGGG TTAACTATGCTGTTTTAA
62	Cytokine IL-36B (“IL-36B”, or “IL36B”) beta	CACGGGTTCCTCCCCACTCTGTCTTTCTCACCTCTCCTTCA CTTTTCCTAGCCTCCTCACCACCATCTGATCTATCTTGTTC TCTTCACAAAAGGCTCTGAAGACATCATGAACCCACAAC GGGAGGCAGCACCCAAATCCTATGCTATTCGTGATTCTCG ACAGATGGTGTGGGTCCTGAGTGGAAATTCTTTAATAGCA GCTCCTCTTAGCCGCAGCATTAAGCCTGTCACTCTTCATTT AATAGCCTGTAGAGACACAGAATTCAGTGACAAGGAAAA GGGTAATATGGTTTACCTGGGAATCAAGGGAAAAGATCT CTGTCTCTTCTGTGCAGAAATTCAGGGCAAGCCTACTTTG CAGCTTAAGCTTCAGGGCTCCCAAGATAACATAGGGAAG GACACTTGCTGGAAACTAGTTGGAATTCACACATGCATAA ACCTGGATGTGAGAGAGAGCTGCTTCATGGGAACCCTTG ACCAATGGGGAATAGGAGTGGGTAGAAAGAAGTGGAAG AGTTCCTTTCAACATCACCATCTCAGGAAGAAGGACAAA GATTTCTCATCCATGCGGACCAACATAGGAATGCCAGGA AGGATGTAGAAATAAGGGGAGGAAGATTCCCATCTCTAC AATCTTTGAGTGGGTTTGCTATCAATGAAATGCTACAAAT GGAATAAGTTGCAGAAATTTTTCTCTTTTCTTGGGTTCTGG AGAGTTTGTAAAACAAGGACACTATGTATTTTTAAAGAGT TGGTAAATCTTACCTGTAAAGCTAGAGAAGGTCGGAGTCT TTTTAGGAGTAGATTTGGACTACATAACCTGTAAATGTGT TTTGTCCAGTCCTTAGAGTGTTTTTTTAAAAAATTGT AAAGTCAAGGTTTTCATGAAAAATGGGAAGATCAGACAA CATTGCTCCTGAATTCCCACAGAGCAGCAAGCTACTAGAG CTCAATCTGTTATTTCTTTTCCTGATGTACAGGGGTTAAGT CCTATGGAAGAAACAGCAGAATTATTCAAAATTATTTACA TAATGTGCAATTATTCACTAGAGCATGAGGAGTGAAACG CTCTGTTTAGTATGTATAACTTAAAAGGAACACATACAAT

		TAAAAGTAATTGAAAGACATTTCTTCTTAAAAATTCTATA ATCTTACACTGGTAAAATAAACTAGTTTTTCCCATGT
63	Cytokine IL-36G ("IL-36G", or "IL-36G") gamma	GAAGCTGCTGGAGCCACGATTCAGTCCCCTGGACTGTAG ATAAAGACCCTTTCTTGCCAGGTGCTGAGACAACCACACT ATGAGAGGCACTCCAGGAGACGCTGATGGTGGAGGAAGG GCCGTCTATCAATCAATCACTGTTGCTGTTATCACATGCA AGTATCCAGAGGCTCTTGAGCAAGGCAGAGGGGATCCCA TTTATTTGGGAATCCAGAATCCAGAAATGTGTTTGTATTG TGAGAAGGTTGGAGAACAGCCCACATTGCAGCTAAAAGA GCAGAAGATCATGGATCTGTATGGCCAACCCGAGCCCGT GAAACCCTTCCTTTTCTACCGTGCCAAGACTGGTAGGACC TCCACCCTTGAGTCTGTGGCCTTCCCGGACTGGTTCATTG CCTCCTCCAAGAGAGACCAGCCCATCATTCTGACTTCAGA ACTTGGGAAGTCATACAACACTGCCTTTGAATTAAATATA AATGACTGAACTCAGCCTAGAGGTGGCAGCTTGGTCTTTG TCTTAAAGTTTCTGGTTCCCAATGTGTTTTCGTCTACATTT TCTTAGTGTCATTTTCACGCTGGTGCTGAGACAGGGGCAA GGCTGCTGTTATCATCTCATTTTATAATGAAGAAGAAGCA ATTACTTCATAGCAACTGAAGAACAGGATGTGGCCTCAG AAGCAGGAGAGCTGGGTGGTATAAGGCTGTCCTCTCAAG CTGGTGCTGTGTAGGCCACAAGGCATCTGCATGAGTGACT TTAAGACTCAAAGACCAAACACTGAGCTTTCTTCTAGGGG TGGGTATGAAGATGCTTCAGAGCTCATGCGCGTTACCCAC GATGGCATGACTAGCACAGAGCTGATCTCTGTTTCT GTTTTGCTTTATTCCCTCTTGGGATGATATCATCCAGTCTT TATATGTTGCCAATATACCTCATTGTGTGTAATAGAACCT TCTTAGCATTAAGACCTTGTAAACAAAAATAATTCTTGTG TTAAGTTAAATCATTTTTGTCCTAATTGTAATGTGTAATCT TAAAGTTAAATAAACTTTGTGTATTTATATAATAATAAAG CTAAAACTGATATAAAATAAAGAAAGAGTAAACTG
64	FDPS shRNA sequence #4A	ACTTTCTCAGCCTCCTTCTGCCTCGAGGCAGAAGGAGGCT GAGAAAGTTTTTT
65	FDPS shRNA sequence #4R	GCAGAAGGAGGCTGAGAAAGTGAGCTCACTTTCTCAGCC TCCTTCTG
66	FDPS shRNA sequence #4TT	GCAGAAGGAGGCTGAGAAAGTTTACTTTCTCAGCCTCCTT CTGCTTTTT
67	FDPS sequence #4L	GCAGAAGGAGGCTGAGAAAGTACTTTCTCAGCCTCCTTCT GCTTTTT
68	FDPS miR30 sequence #1	AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGC CTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGAGGCTG AGAAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT
69	FDPS miR30 sequence #3	AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGC CTCCTTCTGCGTGAAGCCACAGATGGCAGAAGGGCTGAG AAAGTGCTGCCTACTGCCTCGGACTTCAAGGGGCT
70	GGPS1 shRNA sequence #1	GCTTGAAGCTAAAGCCTATAACTCGAGTTATAGGCTTTAG CTTCAAGCTTTTT
71	GGPS1 shRNA sequence #2	GTACATTATCTTGAGGATGTACTCGAGTACATCCTCAAGA TAATGTACTTTTT

72	GGPS1 shRNA sequence 3	CCTGAGCTAGTAGCCTTAGTACTCGAGTACTAAGGCTACT AGCTCAGGTTTTT
73	GGPS1 target sequence #1	GCTTGAAGCTAAAGCCTATAA
74	GGPS1 target sequence #2	GTACATTATCTTGAGGATGTA
75	GGPS1 target sequence #3	CCTGAGCTAGTAGCCTTAGTA
76	IDI1 shRNA sequence	GCCAGTGGTGAAATTAAGATACTCGAGTATCTTAATTTCA CCACTGGCTTTTT
77	IDI1 target sequence	GCCAGTGGTGAAATTAAGATA
78	Fam-labeled TaqMan probe	TAGCATCTCCTATCTCTGGGTGCCC
79	FDPS forward primer	GTGCTGACTGAGGATGAGATG
80	FDPS reverse primer	CCGGTTATACTTGCCTCCAAT
81	Fam-labeled TaqMan probe	AGCGGGAAATCGTGCGTGAC
82	Actin forward primer	GGACCTGACTGACTACCTCAT
83	Actin reverse primer	CGTAGCACAGCTTCTCCTTAAT

CLAIMS

WHAT IS CLAIMED IS:

1. A viral vector comprising first and second encoded genetic elements wherein the first encoded genetic element comprises at least one small RNA capable of inhibiting production of at least one enzyme involved in the mevalonate pathway, and the second encoded genetic element comprises one of a butyrophilin family member, a cytokine, or a chemokine.

2. The viral vector of claim 1, further comprising a third encoded genetic element, wherein the third encoded genetic element comprises one of a butyrophilin family member, a cytokine, or a chemokine.

3. The viral vector of claim 2, further comprising a fourth encoded genetic element, wherein the fourth encoded genetic element comprises one of a butyrophilin family member, a cytokine, or a chemokine.

4. The viral vector of claim 1, wherein the at least one enzyme is farnesyl diphosphate synthase (FDPS), geranylgeranyl-diphosphate synthase 1 (GGPS1), isopentenyl-diphosphate delta isomerase 1 (IDI1), or farnesyl transferase (F-Tase).

5. The viral vector of claim 1, wherein the first encoded genetic element comprises a microRNA or a shRNA.

6. The viral vector of claim 5, wherein the microRNA comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with:

a) AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCT GCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTA CTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 68); or

b) AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCT GCGTGAAGCCACAGATGGCAGAAGGGCTGAGAAAGTGCTGCCTACT GCCTCGGACTTCAAGGGGCT (SEQ ID NO: 69).

7. The viral vector of claim 6, wherein the microRNA comprises:

a) AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCT GCGTGAAGCCACAGATGGCAGAAGGAGGCTGAGAAAGTGCTGCCTA CTGCCTCGGACTTCAAGGGGCT (SEQ ID NO: 68); or

b) AAGGTATATTGCTGTTGACAGTGAGCGACACTTTCTCAGCCTCCTTCT GCGTGAAGCCACAGATGGCAGAAGGGCTGAGAAAGTGCTGCCTACT GCCTCGGACTTCAAGGGGCT (SEQ ID NO: 69).

8. The viral vector of claim 5, wherein the shRNA comprises a sequence having at least 80%, or at least 85%, or at least 90%, or at least 95% percent identity with:

a) GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTACTCCAGGA CTTTTT (SEQ ID NO: 1);

b) GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCTGAACGAAATCCTG CTTTTT (SEQ ID NO: 2);

c) GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGTACATGG CTTTTT (SEQ ID NO: 3);

d) GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTG CTTTTT (SEQ ID NO: 4);

e) ACTTTCTCAGCCTCCTTCTGCCTCGAGGCAGAAGGAGGCTGAGAAAG TTTTTT (SEQ ID NO: 64);

f) GCAGAAGGAGGCTGAGAAAGTGAGCTCACTTTCTCAGCCTCCTTCTG (SEQ ID NO: 65);

g) GCAGAAGGAGGCTGAGAAAGTTTACTTTCTCAGCCTCCTTCTGCTTTT T (SEQ ID NO: 66); or

h) GCAGAAGGAGGCTGAGAAAGTACTTTCTCAGCCTCCTTCTGCTTTTT (SEQ ID NO: 67).

9. The viral vector of claim 8, wherein the shRNA comprises:

a) GTCCTGGAGTACAATGCCATTCTCGAGAATGGCATTGTACTCCAGGA CTTTTT (SEQ ID NO: 1);

b) GCAGGATTTCGTTCAGCACTTCTCGAGAAGTGCTGAACGAAATCCTG CTTTTT (SEQ ID NO: 2);

c) GCCATGTACATGGCAGGAATTCTCGAGAATTCCTGCCATGTACATGG CTTTTT (SEQ ID NO: 3);

d) GCAGAAGGAGGCTGAGAAAGTCTCGAGACTTTCTCAGCCTCCTTCTG CTTTTT (SEQ ID NO: 4);

e) ACTTTCTCAGCCTCCTTCTGCCTCGAGGCAGAAGGAGGCTGAGAAAG TTTTTT (SEQ ID NO: 64);

f) GCAGAAGGAGGCTGAGAAAGTGAGCTCACTTTCTCAGCCTCCTTCTG (SEQ ID NO: 65);

g) GCAGAAGGAGGCTGAGAAAGTTTACTTTCTCAGCCTCCTTCTGCTTTT T (SEQ ID NO: 66); or

h) GCAGAAGGAGGCTGAGAAAGTACTTTCTCAGCCTCCTTCTGCTTTTT (SEQ ID NO: 67).

10. The viral vector of claim 1, wherein the butyrophilin family member comprises BTN3A3, BTN3A2, or BTN3A1.

11. The viral vector of claim 1, wherein the butyrophilin family member comprises BTN3A3 (R381H).

12. The viral vector of claim 1, wherein the cytokine comprises IL-1, IL-1β, IL-2, IL-4, IL-7, IL-12, IL-15, IL-17, IL-18, IL-23, IL-33, IL-36, TNF-α, or interferon-γ.

13. The viral vector of claim 1, wherein the chemokine comprises a CC chemokine, a CXC chemokine, a CX3C chemokine, a C chemokine, or a XC chemokine.

14. The viral vector of claim 13, wherein the CC chemokine comprises RANTES.

15. The viral vector of any one of claims 1, wherein the viral vector is a lentiviral vector.

16. A lentiviral vector system for expressing a lentiviral particle comprising:

a lentiviral vector according to claim 15;

at least one envelope plasmid for expressing an envelope protein optimized for infecting a target cell; and

at least one helper plasmid for expressing gag, pol, and rev genes,

wherein when the lentiviral vector, the at least one envelope plasmid, and the at least one

helper plasmid are transfected into a packaging cell, the lentiviral particle is produced by

the packaging cell, wherein the lentiviral particle is capable of infecting the target cell

and inhibiting the at least one enzyme involved in the mevalonate pathway within the target cell.

17. A lentiviral particle capable of infecting a target cell, the lentiviral particle comprising an envelope protein optimized for infecting the target cell, and a lentiviral vector according to claim 15.

18. The lentiviral particle of claim 17, wherein the target cell is a cancer cell.

19. A method of activating a gamma delta (GD) T cell comprising:

infecting, or having infected, in the presence of the GD T cell, a target cell with a lentiviral particle,

wherein the lentiviral particle comprises a viral vector comprising first and second encoded genetic elements,

wherein the first encoded genetic element comprises at least one small RNA capable of inhibiting production of at least one enzyme involved in the mevalonate pathway, and the second encoded genetic element comprises one of a butyrophilin family member, a cytokine, or a chemokine, and

wherein when the at least one enzyme is inhibited in the target cell, the target cell activates the GD T cell.

20. The method of claim 19, wherein the target cell is a cancer cell.

21. The method of claim 19, further comprising contacting, or having contacted, the target cell and the GD T cell with an amount of an aminobisphosphonate drug.

22. The method of claim 21, wherein the aminobisphosphonate drug is zoledronic acid.

23. The method of claim 19 or claim 21, wherein the at least one enzyme is farnesyl diphosphate synthase (FDPS), geranylgeranyl-diphosphate synthase 1 (GGPS1), isopentenyl-diphosphate delta isomerase 1 (IDI1), or farnesyl transferase (F-Tase).

24. A method of treating cancer in a subject, the method comprising administering, or having administered, to the subject a therapeutically effective amount of a lentiviral particle wherein the lentiviral particle comprises a viral vector comprising first and second encoded genetic elements wherein the first encoded genetic element comprises at least one small RNA capable of inhibiting production of at least one enzyme involved in the mevalonate pathway, and the second encoded genetic element comprises one of a butyrophilin family member, a cytokine, or a chemokine, wherein when the at least one enzyme is inhibited in a cancer cell in the presence of a GD T cell, the target cell activates the GD T cell, to thereby treat the cancer.

25. The method of claim 24, further comprising administering, or having administered, to the subject a therapeutically effective amount of an aminobisphosphonate drug.

26. The method of claim 25, wherein the aminobisphosphonate drug is zoledronic acid.

27. The method of claim 24 or claim 25, wherein the at least one enzyme is farnesyl diphosphate synthase (FDPS), geranylgeranyl-diphosphate synthase 1 (GGPS1), isopentenyl-diphosphate delta isomerase 1 (IDI1), or farnesyl transferase (F-Tase).

28. The method of claim 24, wherein the butyrophilin family member comprises

BTN3A3 or BTN3A3 (R381H).

29. A viral vector comprising:

a first small RNA that targets a first target of the mevalonate pathway and is capable of increasing a first product of the mevalonate pathway; and

a second small RNA that targets a second target of the mevalonate pathway and is capable of decreasing a second product of the mevalonate pathway.

30. The viral vector of claim 29, wherein the first target is a first enzyme of the mevalonate pathway and the second target is a second enzyme of the mevalonate pathway.

31. The viral vector of claim 30, wherein at least one of the first enzyme and the second enzyme comprises farnesyl diphosphate synthase (FDPS), geranylgeranyl-diphosphate synthase 1 (GGPS1), isopentenyl-diphosphate delta isomerase 1 (IDI1), or farnesyl transferase (F-Tase).

32. The viral vector of claim 29, wherein the first product of the mevalonate pathway comprises isopentenyl pyrophosphate (IPP).

33. The viral vector of claim 29, wherein the second product of the mevalonate pathway comprises geranylgeranyl pyrophosphate (GGPP).

34. A method of treating cancer in a subject, the method comprising administering, or having administered, to the subject a therapeutically effective amount of a lentiviral particle wherein the lentiviral particle comprises the viral vector of claim 29.

35. The method of claim 34, further comprising administering, or having administered, to the subject a therapeutically effective amount of an aminobisphosphonate drug.

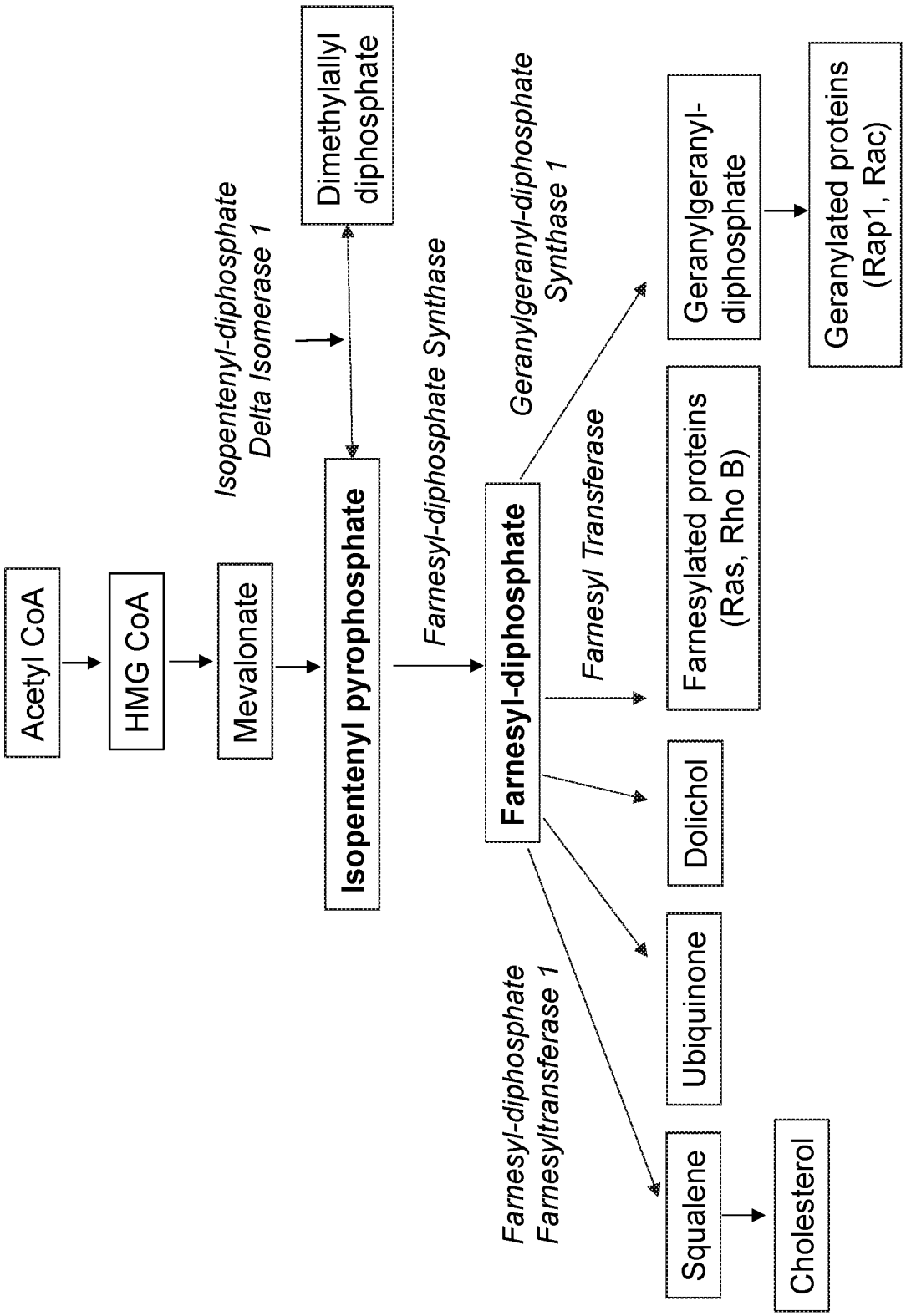

Acetyl CoA
HMG CoA
Mevalonate
Isopentenyl-diphosphate Delta Isomerase 1
Dimethylallyl diphosphate
Isopentenyl pyrophosphate
Farnesyl-diphosphate Synthase
Farnesyl-diphosphate
Geranylgeranyl-diphosphate Synthase 1
Farnesyl Transferase
Farnesyl-diphosphate Farnesyltransferase 1
Farnesylated proteins (Ras, Rho B)
Geranylgeranyl-diphosphate
Geranylated proteins (Rap1, Rac)
Dolichol
Ubiquinone
Squalene
Cholesterol

Figure 1

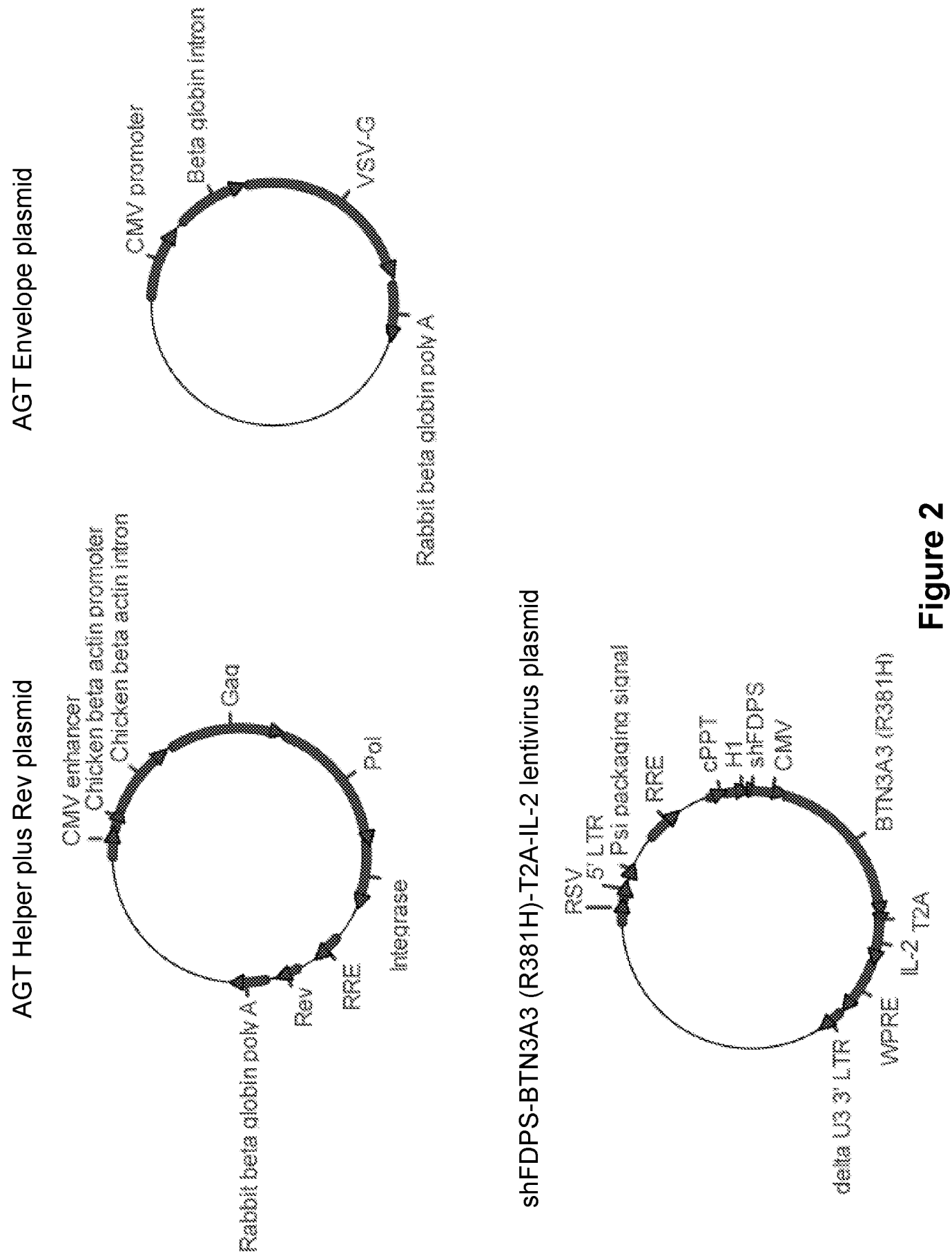
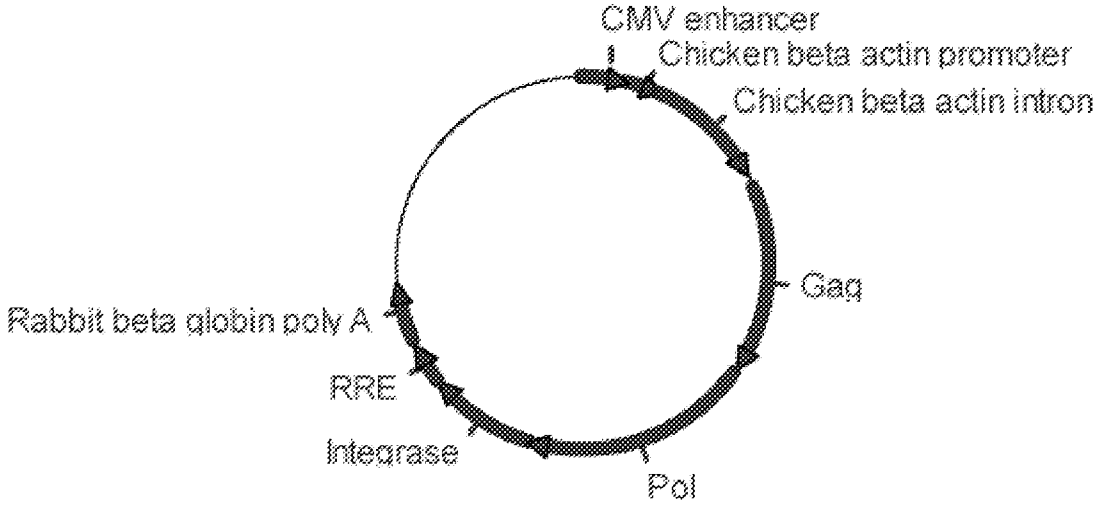
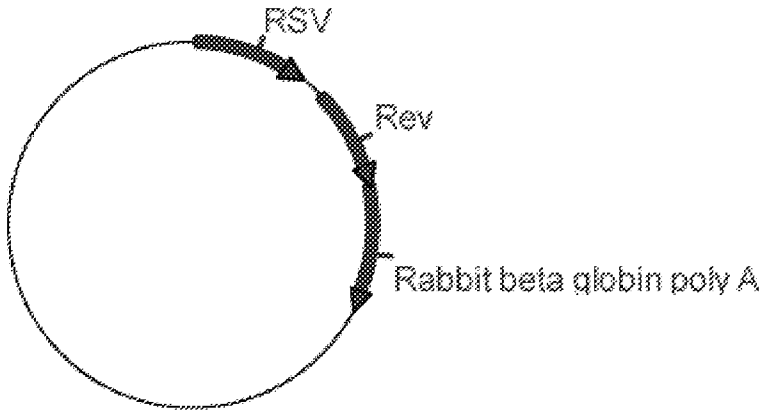

AGT Helper plus Rev plasmid
CMV enhancer
Chicken beta actin promoter
Chicken beta actin intron
Gag
Pol
Integrase
RRE
Rev
Rabbit beta globin poly A
AGT Envelope plasmid
CMV promoter
Beta globin intron
VSV-G
Rabbit beta globin poly A
shFDPS-BTN3A3 (R381H)-T2A-IL-2 lentivirus plasmid
RSV
5' LTR
Psi packaging signal
RRE
cPPT
H1
shFDPS
CMV
BTN3A3 (R381H)
T2A
IL-2
WPRE
delta U3 3' LTR

Figure 2

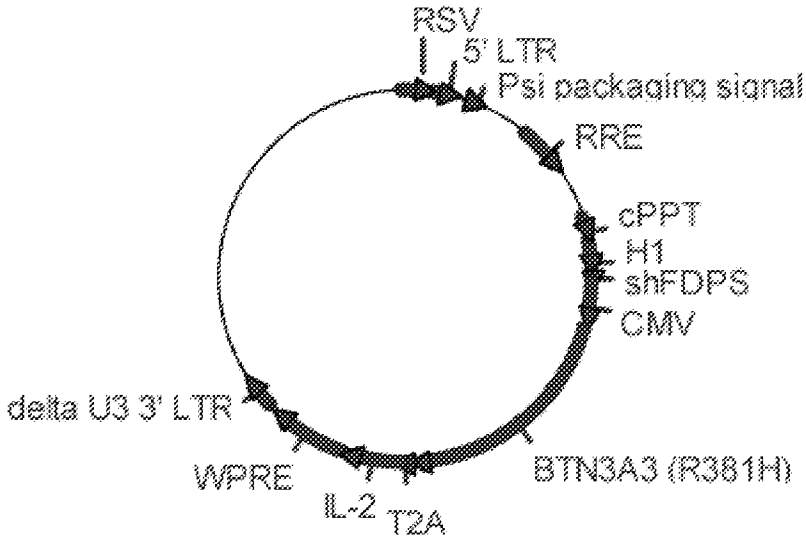
AGT Helper plasmid



AGT Rev plasmid



shFDPS-BTN3A3 (R381H)-T2A-IL-2 lentivirus plasmid



AGT Envelope plasmid

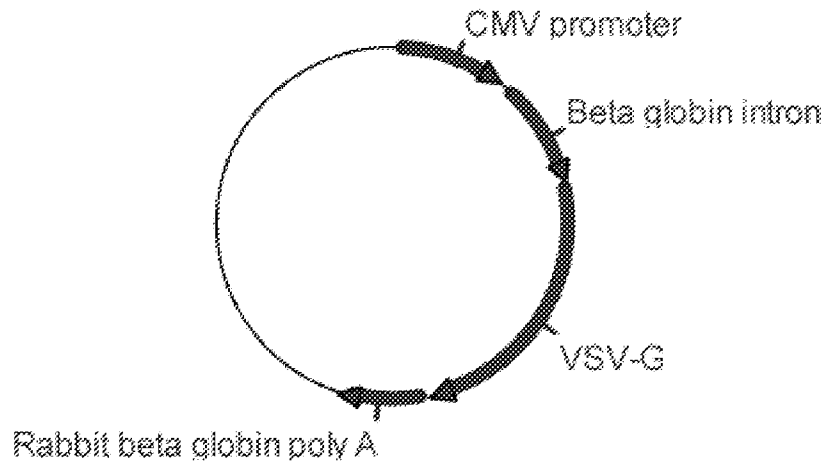


Figure 3

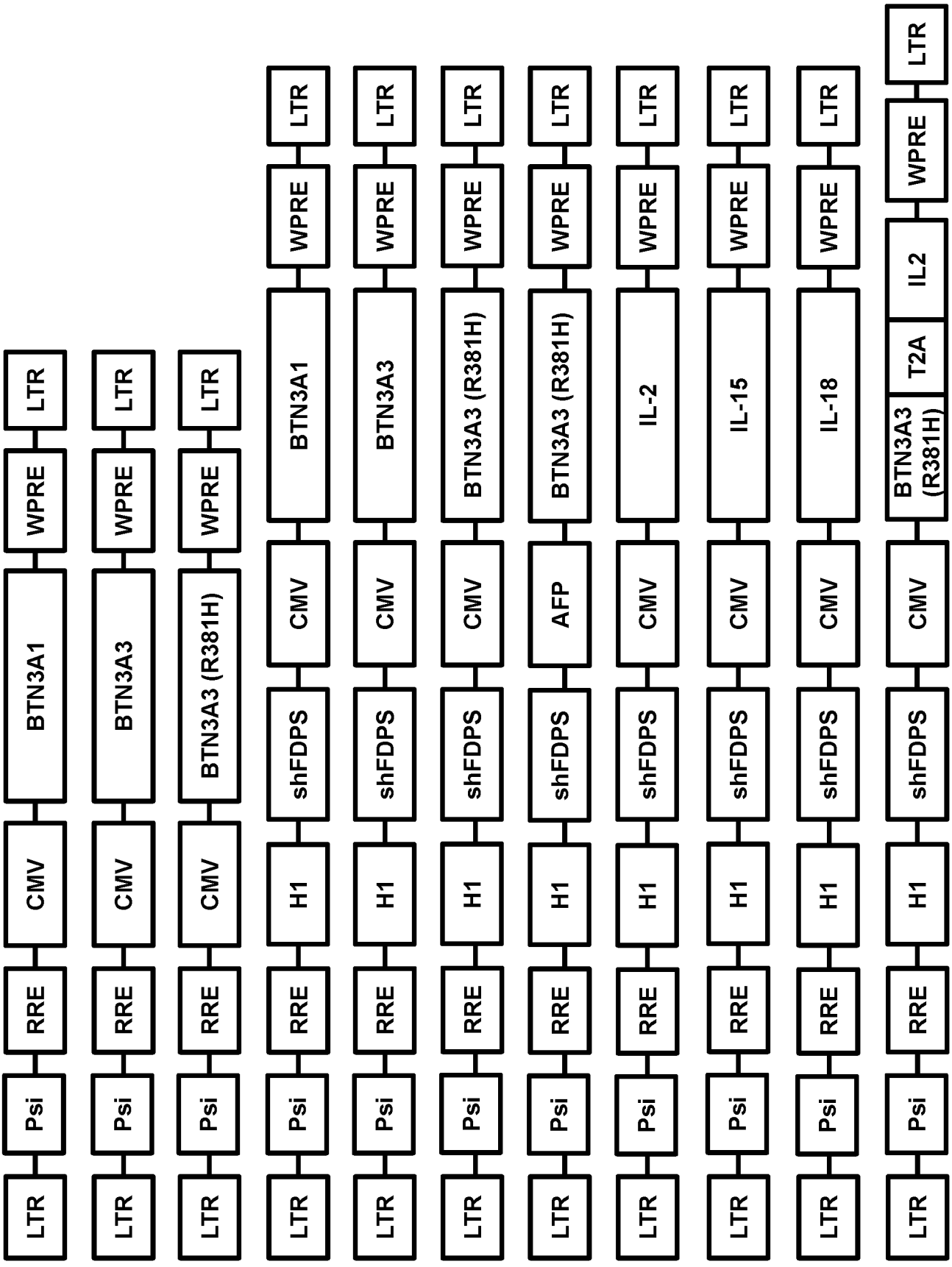

Vector 1
LTR
Psi
RRE
CMV
BTN3A1
WPRE
LTR
Vector 2
LTR
Psi
RRE
CMV
BTN3A3
WPRE
LTR
Vector 3
LTR
Psi
RRE
CMV
BTN3A3 (R381H)
WPRE
LTR
Vector 4
LTR
Psi
RRE
H1
shFDPS
CMV
BTN3A1
WPRE
LTR
Vector 5
LTR
Psi
RRE
H1
shFDPS
CMV
BTN3A3
WPRE
LTR
Vector 6
LTR
Psi
RRE
H1
shFDPS
CMV
BTN3A3 (R381H)
WPRE
LTR
Vector 7
LTR
Psi
RRE
H1
shFDPS
AFP
BTN3A3 (R381H)
WPRE
LTR
Vector 8
LTR
Psi
RRE
H1
shFDPS
CMV
IL-2
WPRE
LTR
Vector 9
LTR
Psi
RRE
H1
shFDPS
CMV
IL-15
WPRE
LTR
Vector 10
LTR
Psi
RRE
H1
shFDPS
CMV
IL-18
WPRE
LTR
Vector 11
LTR
Psi
RRE
H1
shFDPS
CMV
BTN3A3 (R381H)
T2A
IL2
WPRE
LTR

Figure 4

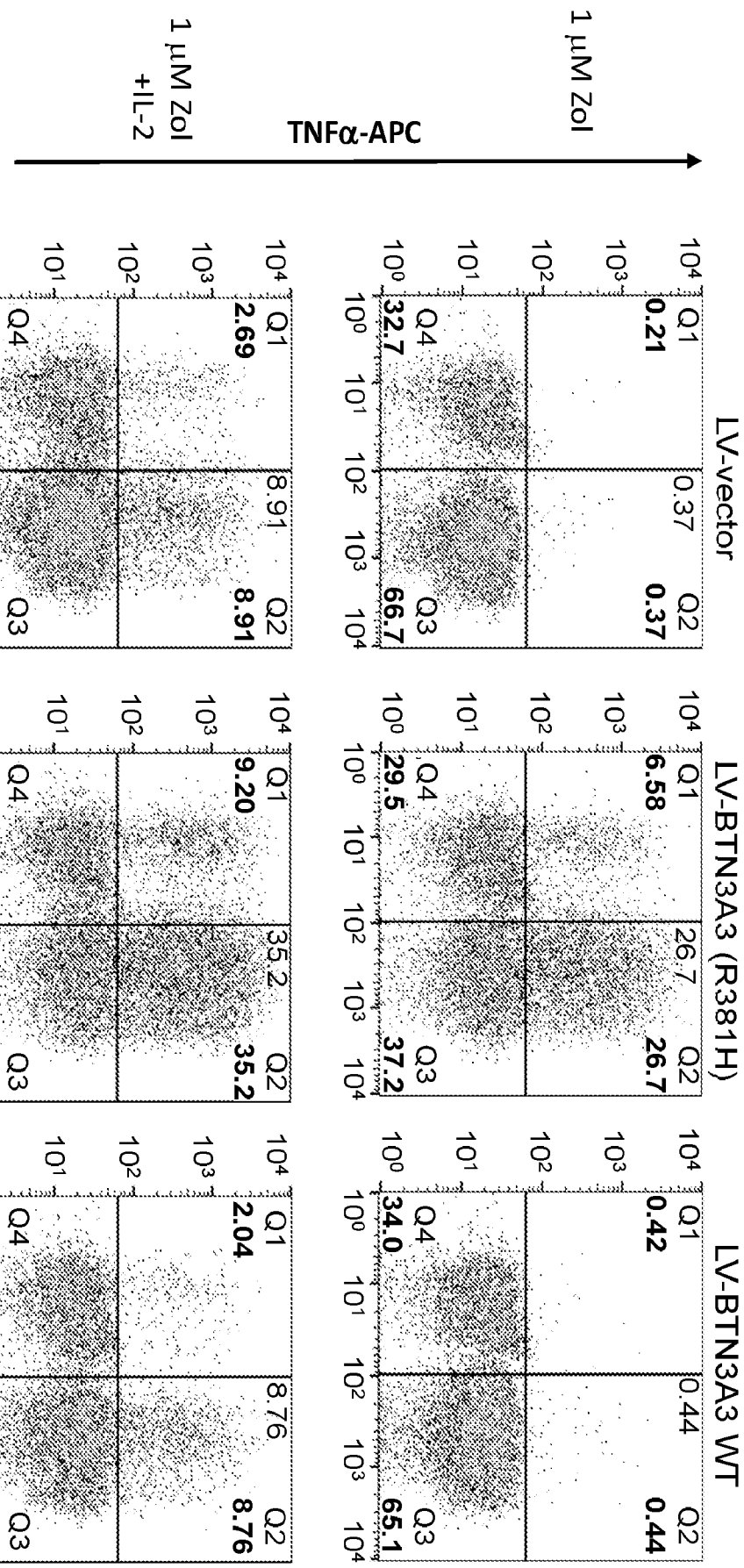

LV-vector
LV-BTN3A3 (R381H)
LV-BTN3A3 WT
1 μM Zol
1 μM Zol +IL-2
TNFα-APC
Vδ2-PE
Q1 0.21
Q2 0.37
Q3 66.7
Q4 32.7
0.37
Q1 2.69
Q2 8.91
Q3 57.6
Q4 30.8
8.91
Q1 6.58
Q2 26.7
Q3 37.2
Q4 29.5
26.7
Q1 9.20
Q2 35.2
Q3 31.3
Q4 24.3
35.2
Q1 0.42
Q2 0.44
Q3 65.1
Q4 34.0
0.44
Q1 2.04
Q2 8.76
Q3 54.8
Q4 34.4
8.76

Figure 5

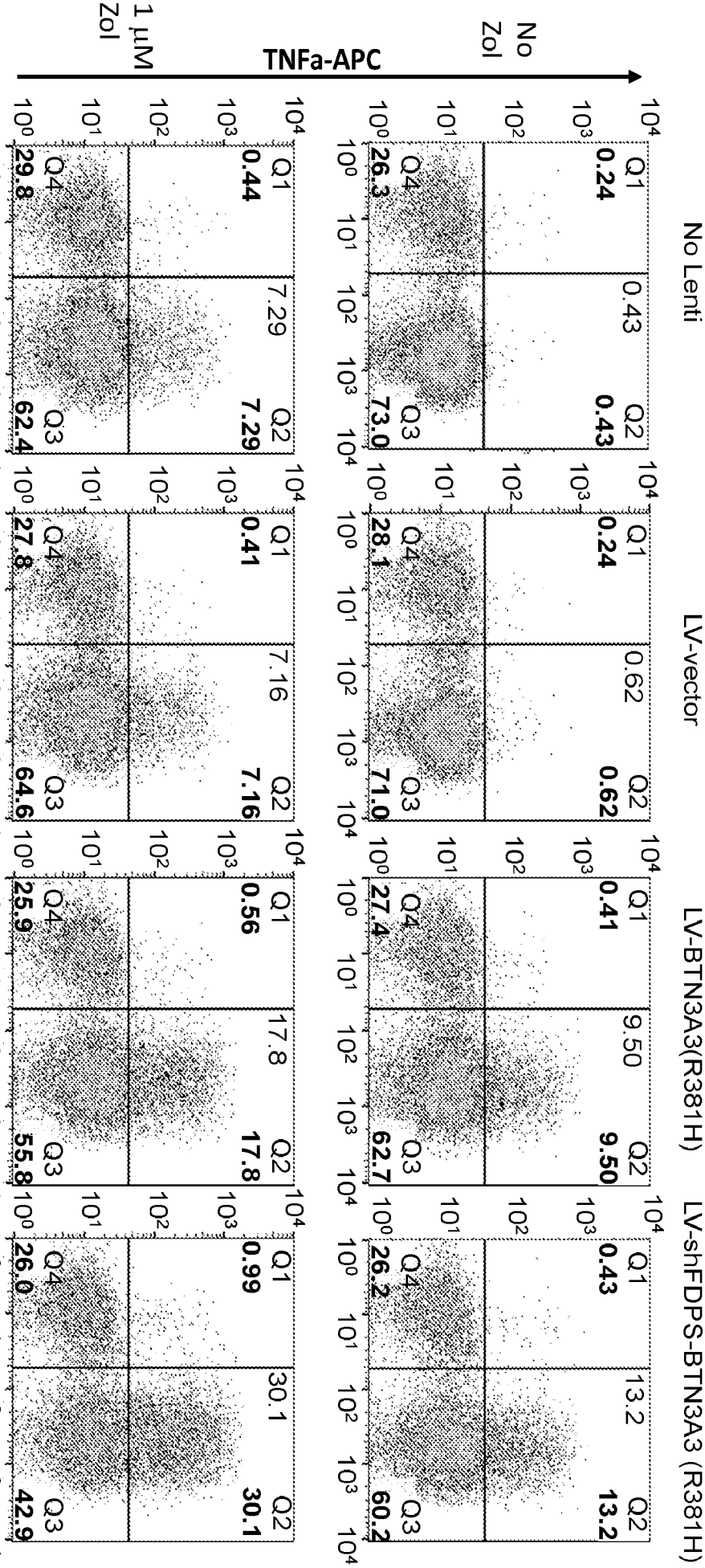

No Lenti
LV-vector
LV-BTN3A3(R381H)
LV-shFDPS-BTN3A3 (R381H)
No Zol
1 μM Zol
TNFa-APC
Vδ2-PE
Q1 0.24
Q2 0.43
Q3 73.0
Q4 26.3
Q1 0.44
Q2 7.29
Q3 62.4
Q4 29.8
Q1 0.24
Q2 0.62
Q3 71.0
Q4 28.1
Q1 0.41
Q2 7.16
Q3 64.6
Q4 27.8
Q1 0.41
Q2 9.50
Q3 62.7
Q4 27.4
Q1 0.56
Q2 17.8
Q3 55.8
Q4 25.9
Q1 0.43
Q2 13.2
Q3 60.2
Q4 26.2
Q1 0.99
Q2 30.1
Q3 42.9
Q4 26.0

Figure 6

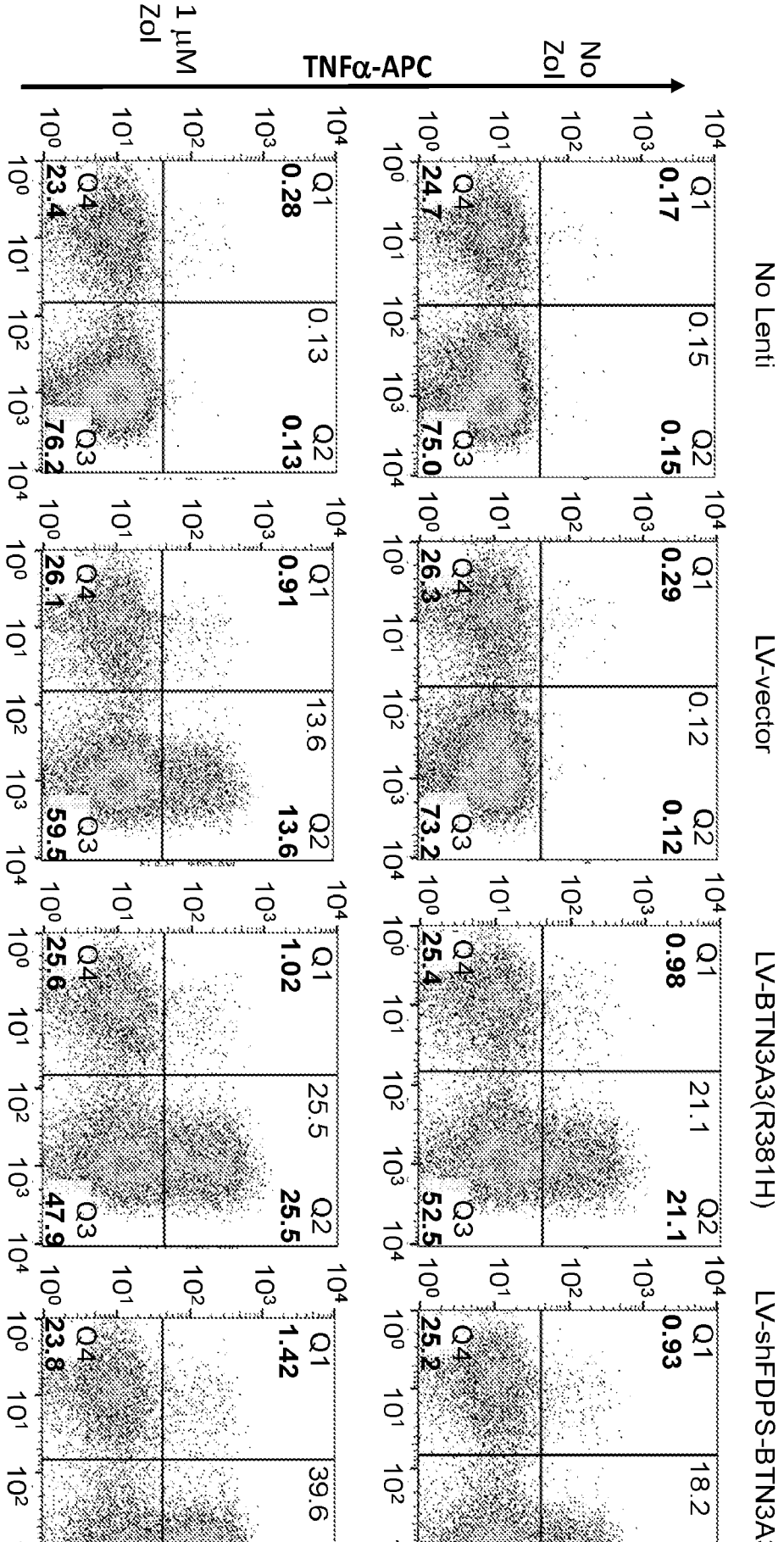

No Lenti
LV-vector
LV-BTN3A3(R381H)
LV-shFDPS-BTN3A3(R381H)
No Zol
1 μM Zol
TNFα-APC
Vδ2-PE
Q1 0.17
Q2 0.15
Q3 75.0
Q4 24.7
Q1 0.28
Q2 0.13
Q3 76.2
Q4 23.4
Q1 0.29
Q2 0.12
Q3 73.2
Q4 26.3
Q1 0.91
Q2 13.6
Q3 59.5
Q4 26.1
Q1 0.98
Q2 21.1
Q3 52.5
Q4 25.4
Q1 1.02
Q2 25.5
Q3 47.9
Q4 25.6
Q1 0.93
Q2 18.2
Q3 55.7
Q4 25.2
Q1 1.42
Q2 39.6
Q3 35.2
Q4 23.8

Figure 7

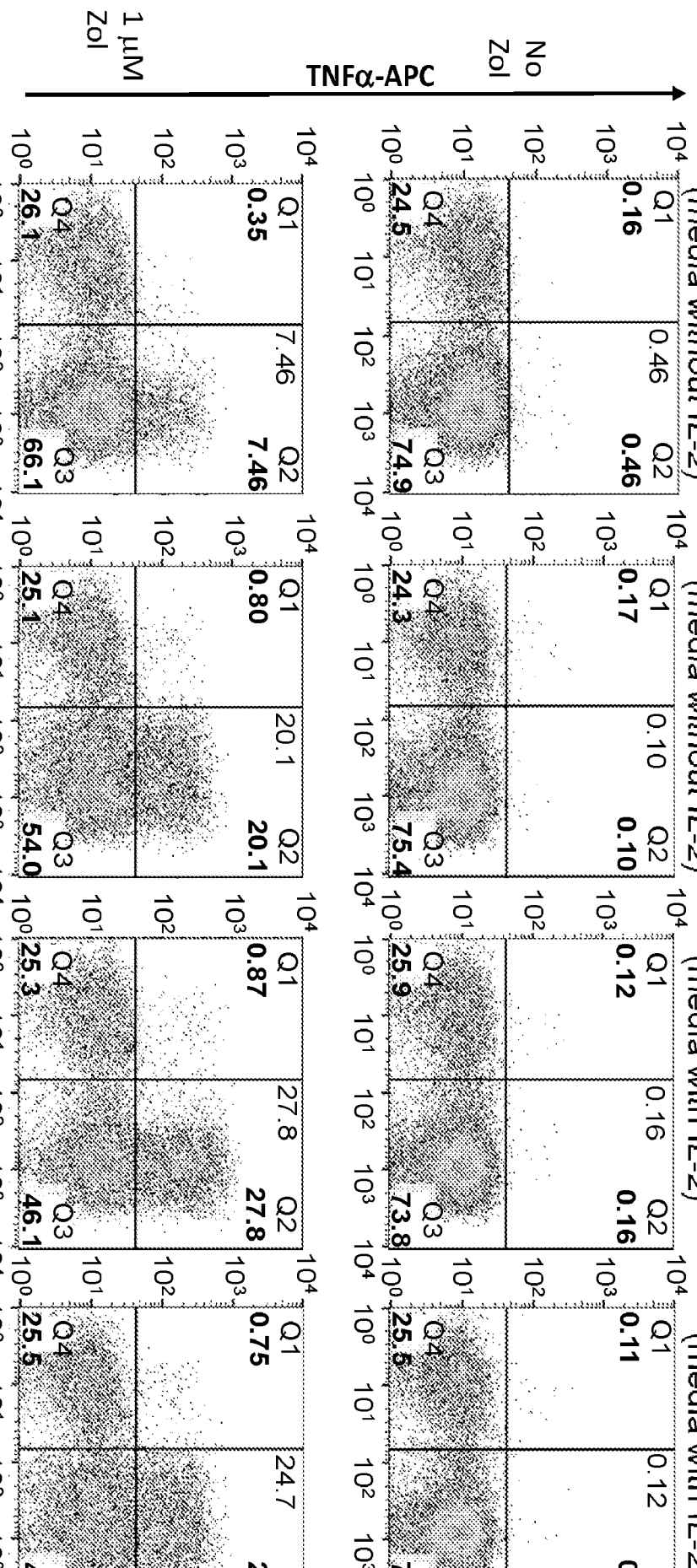

LV-shFDPS (media without IL-2)
LV-shFDPS-IL-2 (media without IL-2)
LV-shFDPS (media with IL-2)
LV-shFDPS-IL-2 (media with IL-2)
No Zol
1 μM Zol
TNFα-APC
Vδ2-PE
Q1 0.16
Q2 0.46
Q4 24.5
Q3 74.9
Q1 0.35
Q2 7.46
Q4 26.1
Q3 66.1
Q1 0.17
Q2 0.10
Q4 24.3
Q3 75.4
Q1 0.80
Q2 20.1
Q4 25.1
Q3 54.0
Q1 0.12
Q2 0.16
Q4 25.9
Q3 73.8
Q1 0.87
Q2 27.8
Q4 25.3
Q3 46.1
Q1 0.11
Q2 0.12
Q4 25.6
Q3 74.2
Q1 0.75
Q2 24.7
Q4 25.5
Q3 49.1

Figure 8

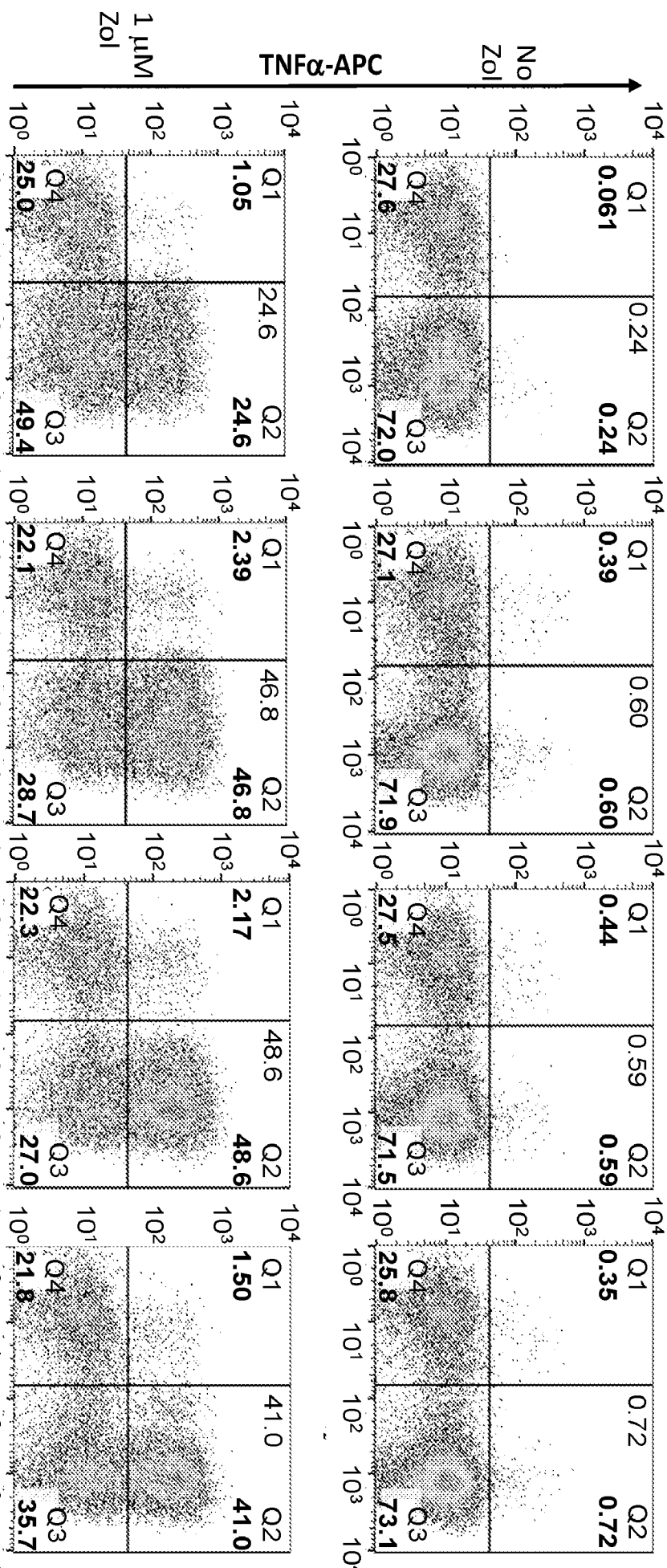

LV-shFDPS (medium without IL-2)
LV-shFDPS-IL2 (media without IL-2)
LV-shFDPS (media with IL-2)
LV-shFDPS-IL2 (media with IL-2)
TNFα-APC
No Zol
1 μM Zol
Vδ2-PE
Q1 0.061
Q2 0.24
Q3 72.0
Q4 27.6
Q1 1.05
Q2 24.6
Q3 49.4
Q4 25.0
Q1 0.39
Q2 0.60
Q3 71.9
Q4 27.1
Q1 2.39
Q2 46.8
Q3 28.7
Q4 22.1
Q1 0.44
Q2 0.59
Q3 71.5
Q4 27.5
Q1 2.17
Q2 48.6
Q3 27.0
Q4 22.3
Q1 0.35
Q2 0.72
Q3 73.1
Q4 25.8
Q1 1.50
Q2 41.0
Q3 35.7
Q4 21.8

Figure 9

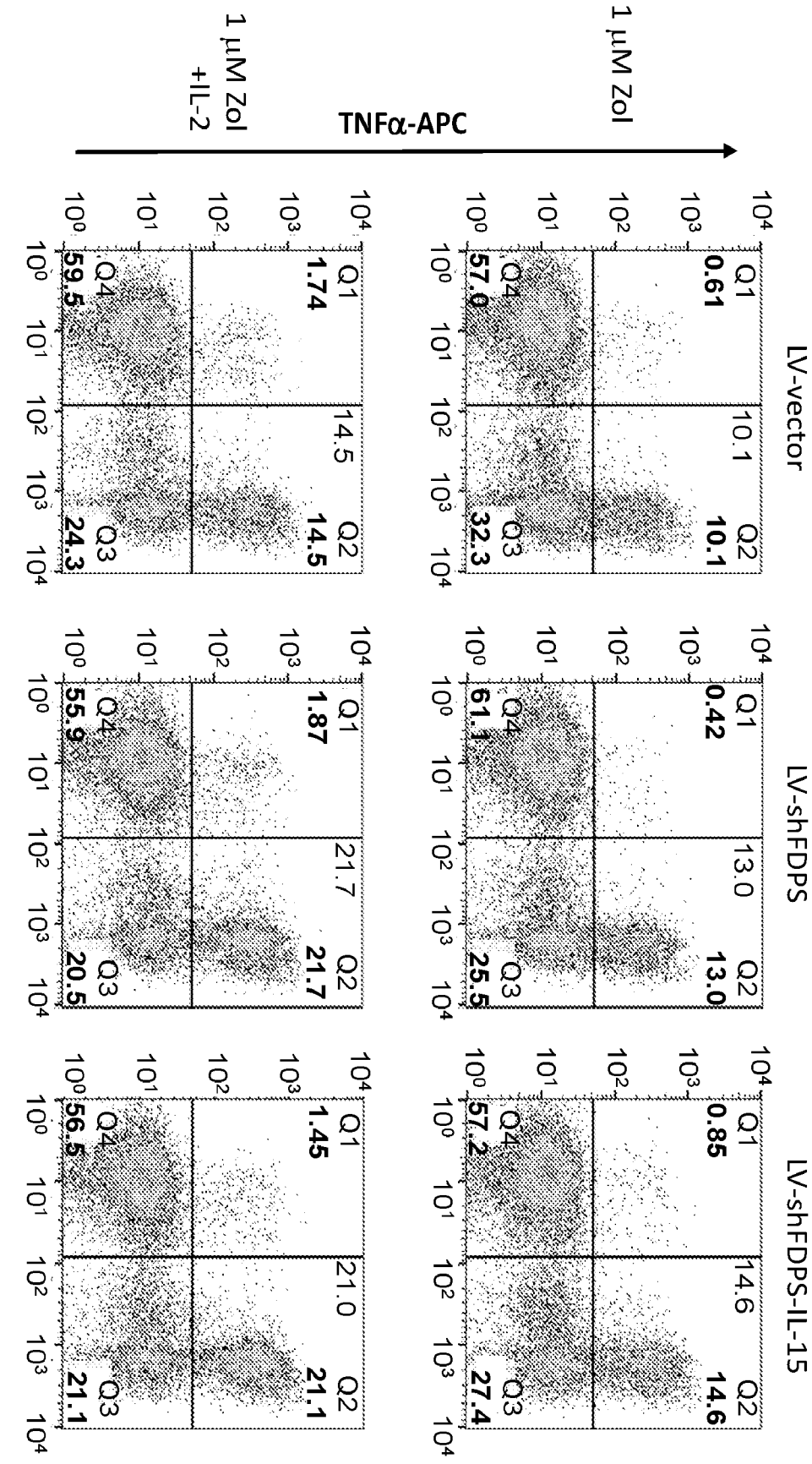

LV-vector
LV-shFDPS
LV-shFDPS-IL-15
1 μM Zol
1 μM Zol +IL-2
TNFα-APC
Vδ2-PE
Q1 0.61
Q2 10.1
Q3 32.3
Q4 57.0
Q1 1.74
Q2 14.5
Q3 24.3
Q4 59.5
Q1 0.42
Q2 13.0
Q3 25.5
Q4 61.1
Q1 1.87
Q2 21.7
Q3 20.5
Q4 55.9
Q1 0.85
Q2 14.6
Q3 27.4
Q4 57.2
Q1 1.45
Q2 21.1
Q3 21.1
Q4 56.5

Figure 10

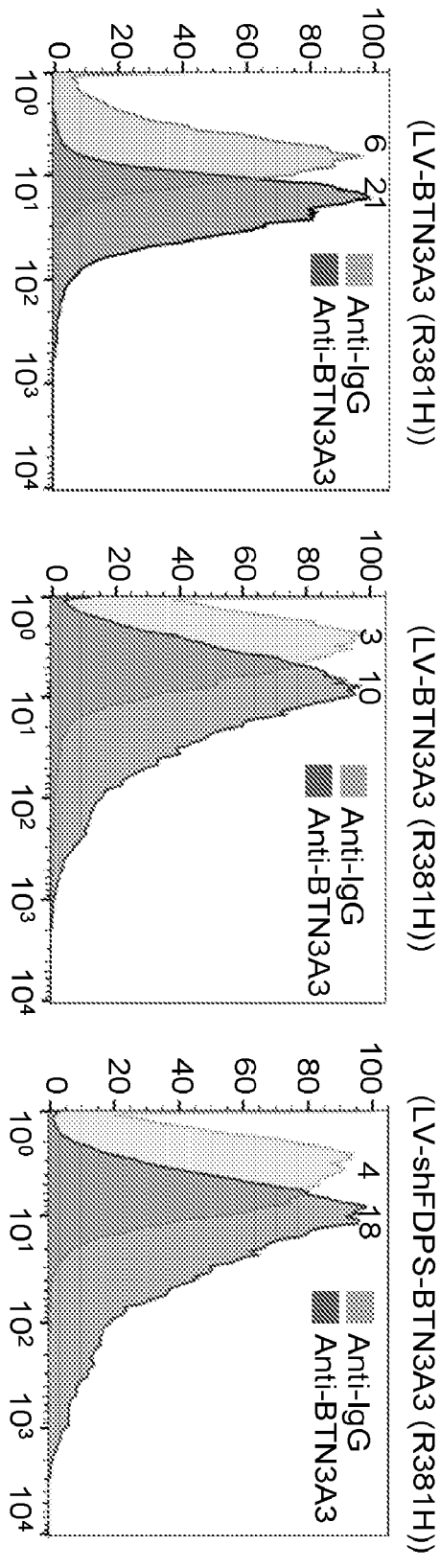

PC3
(LV-BTN3A3 (R381H))
HepG2
(LV-BTN3A3 (R381H))
HepG2
(LV-shFDPS-BTN3A3 (R381H))
Anti-IgG
Anti-BTN3A3
6
21
3
10
4
18
0
20
40
60
80
100
10^0
10^1
10^2
10^3
10^4
A
B
C

Figure 11

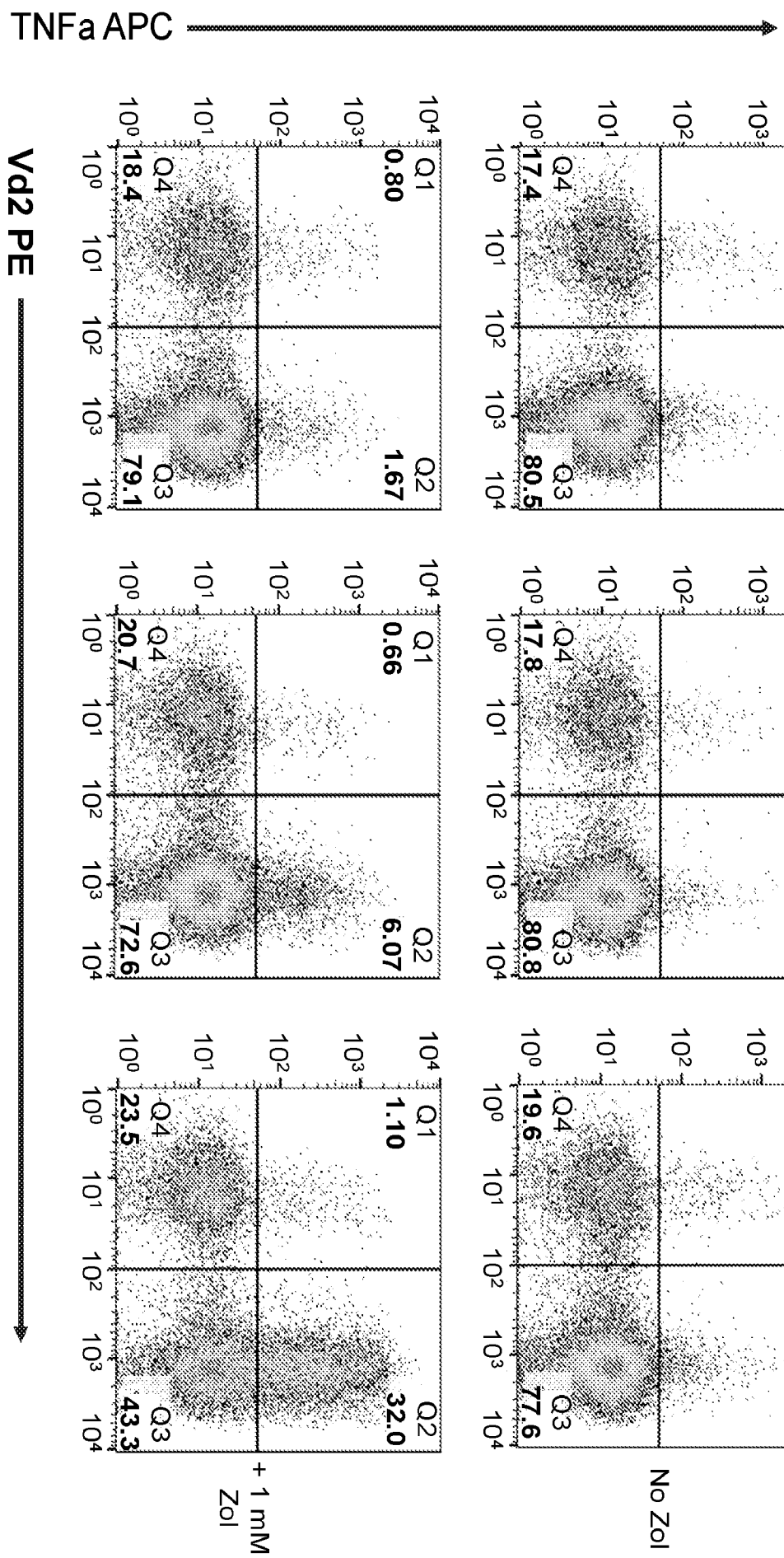

TNFa APC
Vd2 PE
no Lv
Lv-control
Lv-FDPSsh
No Zol
+ 1 mM Zol
Q1 0.67
Q2 1.36
Q4 17.4
Q3 80.5
Q1 0.80
Q2 1.67
Q4 18.4
Q3 79.1
Q1 0.48
Q2 0.89
Q4 17.8
Q3 80.8
Q1 0.66
Q2 6.07
Q4 20.7
Q3 72.6
Q1 0.81
Q2 2.04
Q4 19.6
Q3 77.6
Q1 1.10
Q2 32.0
Q4 23.5
Q3 43.3

Figure 12

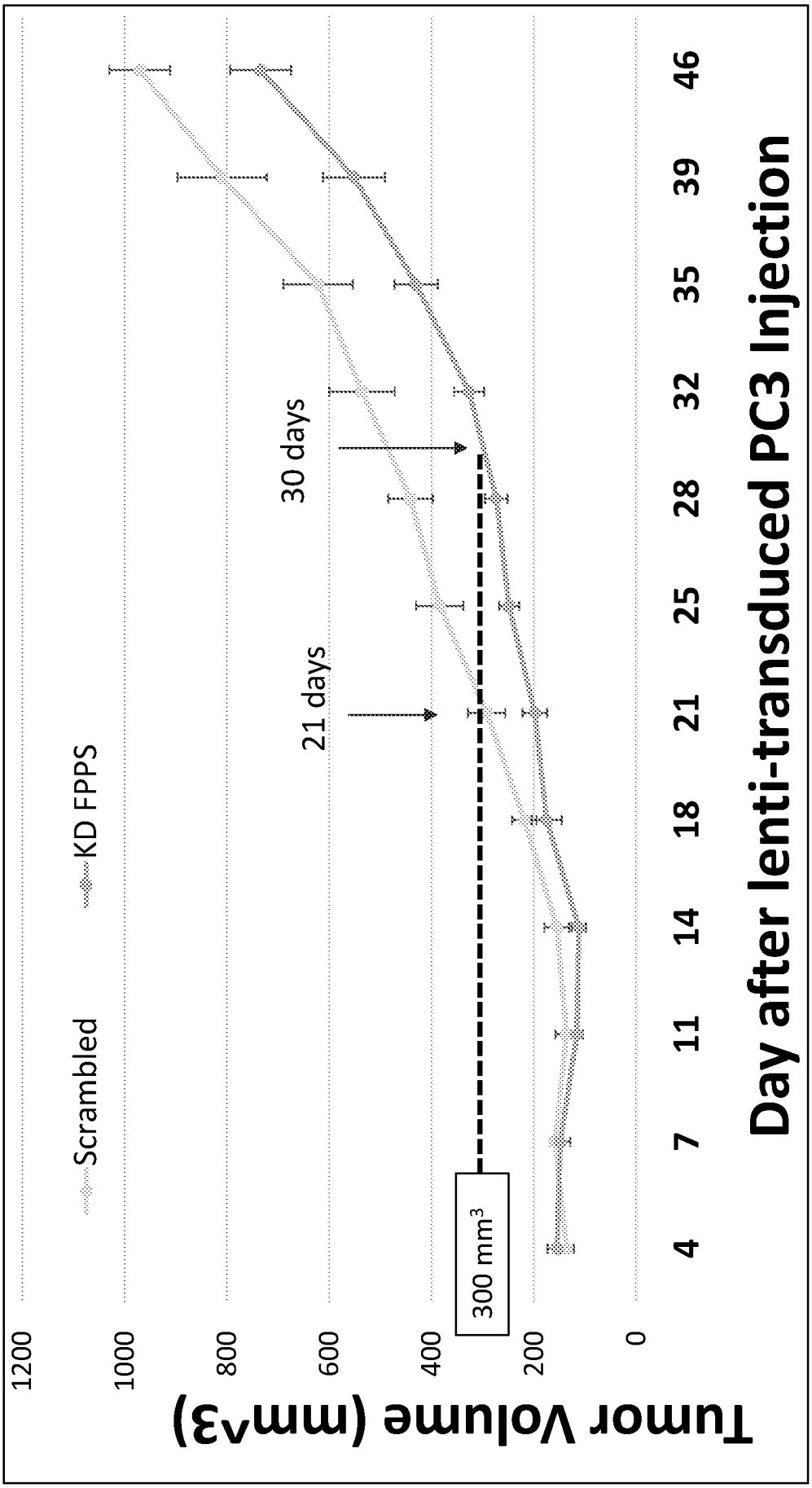

Tumor Volume (mm^3)
1200
1000
800
600
400
200
0
4
7
11
14
18
21
25
28
32
35
39
46
Day after lenti-transduced PC3 Injection
Scrambled
KD FPPS
21 days
30 days
300 mm3

Figure 13

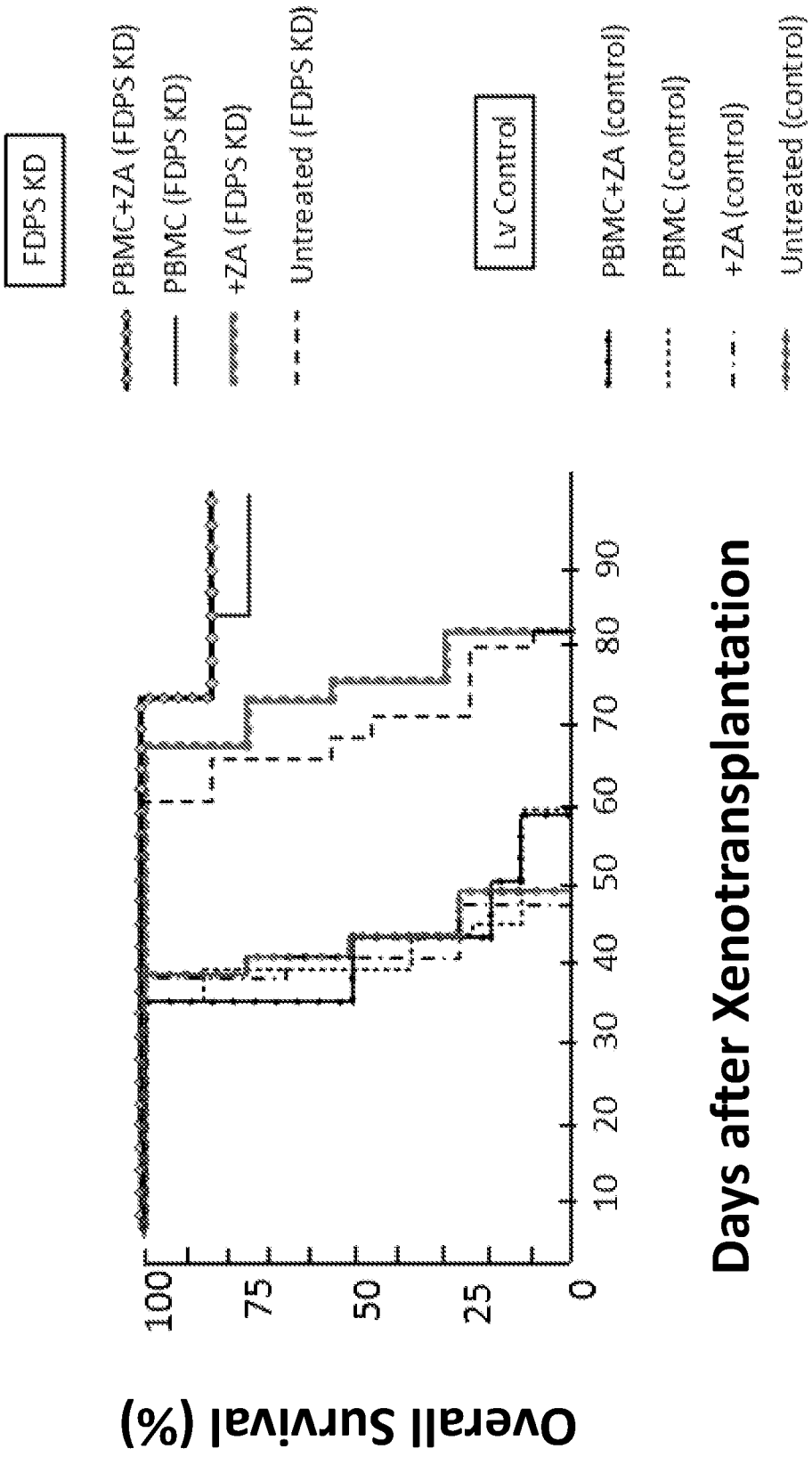
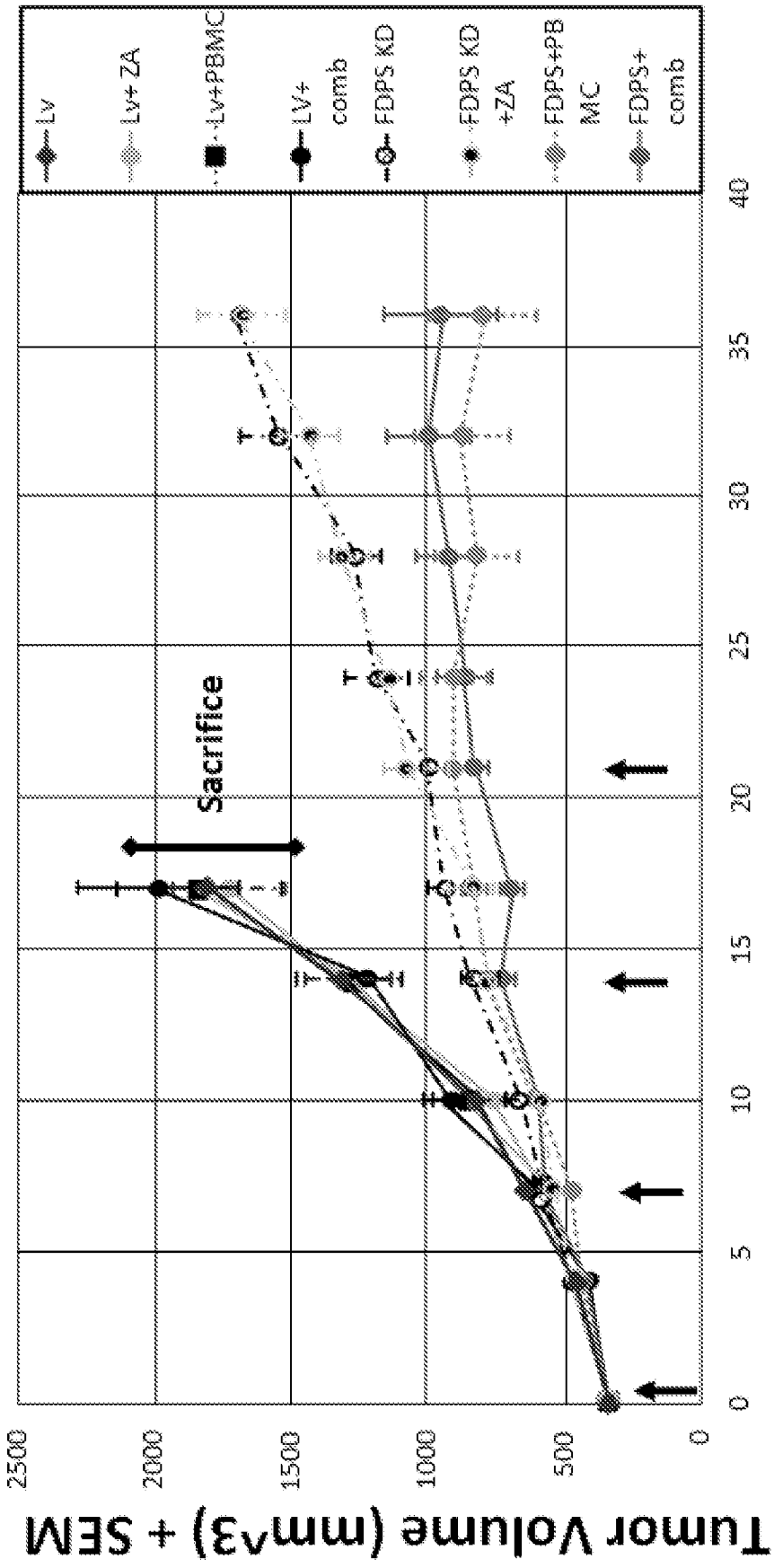

FDPS KD
PBMC+ZA (FDPS KD)
PBMC (FDPS KD)
+ZA (FDPS KD)
Untreated (FDPS KD)
Lv Control
PBMC+ZA (control)
PBMC (control)
+ZA (control)
Untreated (control)
Overall Survival (%)
100
75
50
25
0
10
20
30
40
50
60
70
80
90
Days after Xenotransplantation

Figure 14



LV = lentivirus control; FDPS = Lentivirus expressing shRNA against FDPS; ZA = zoledronic acid; comb = PBMC + ZA

Figure 15

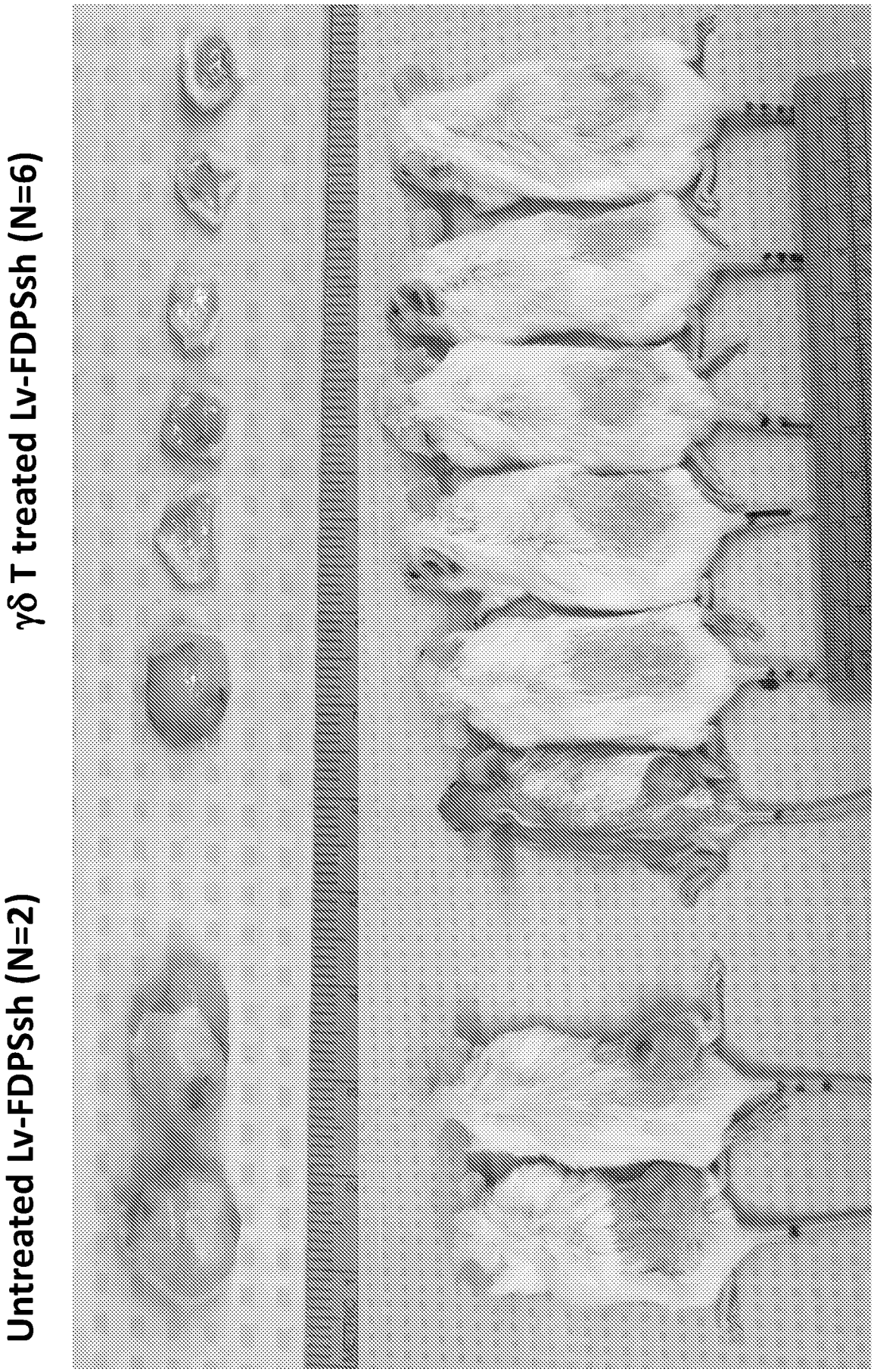


Figure 16

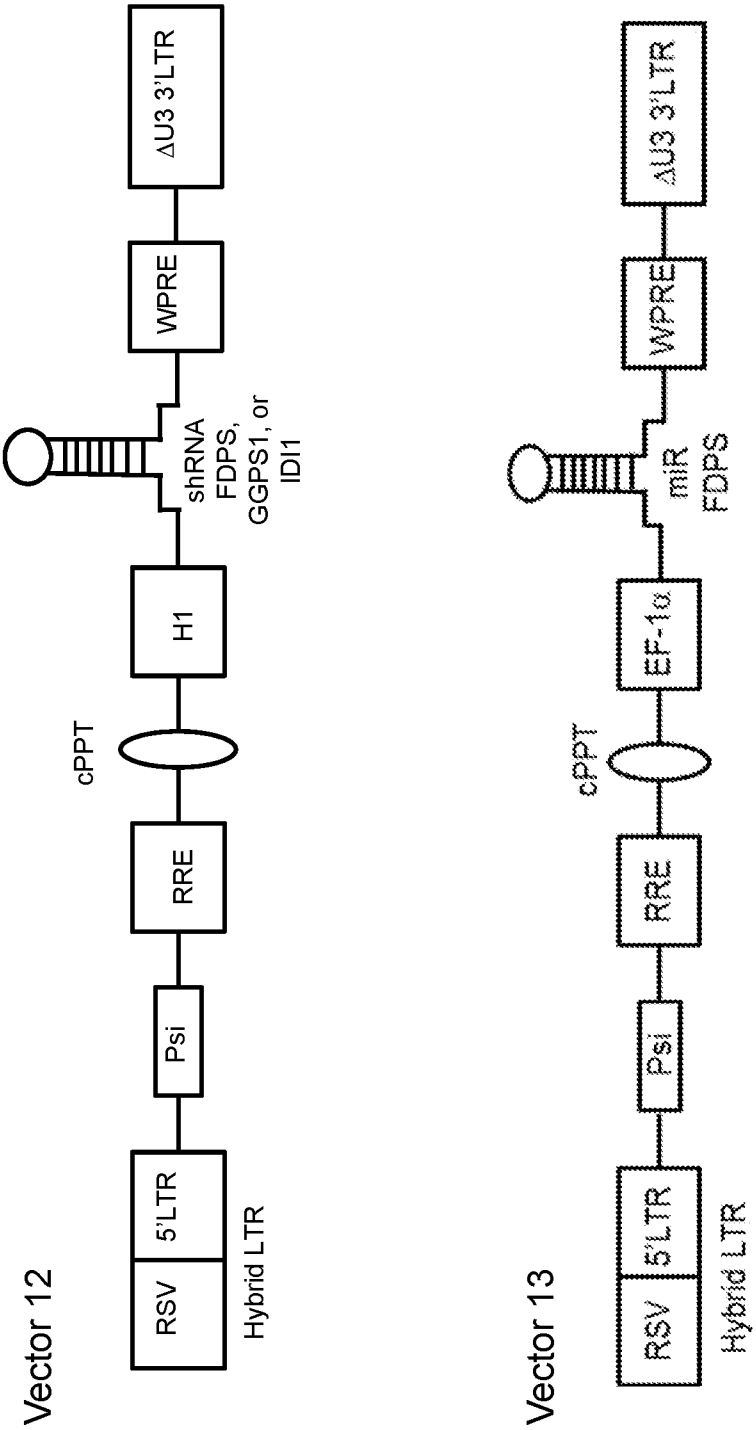

Vector 12
RSV
5'LTR
Hybrid LTR
Psi
RRE
cPPT
H1
shRNA
FDPS,
GGPS1, or
IDI1
WPRE
ΔU3 3'LTR
Vector 13
RSV
5'LTR
Hybrid LTR
Psi
RRE
cPPT
EF-1α
miR
FDPS
WPRE
ΔU3 3'LTR

Figure 17

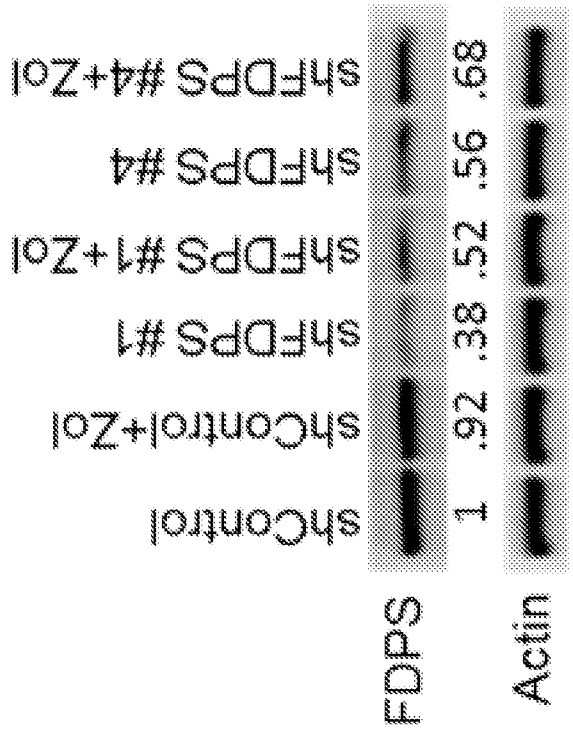

shControl
shControl+Zol
shFDPS #1
shFDPS #1+Zol
shFDPS #4
shFDPS #4+Zol
1 .92 .38 .52 .56 .68
FDPS
Actin

Figure 18

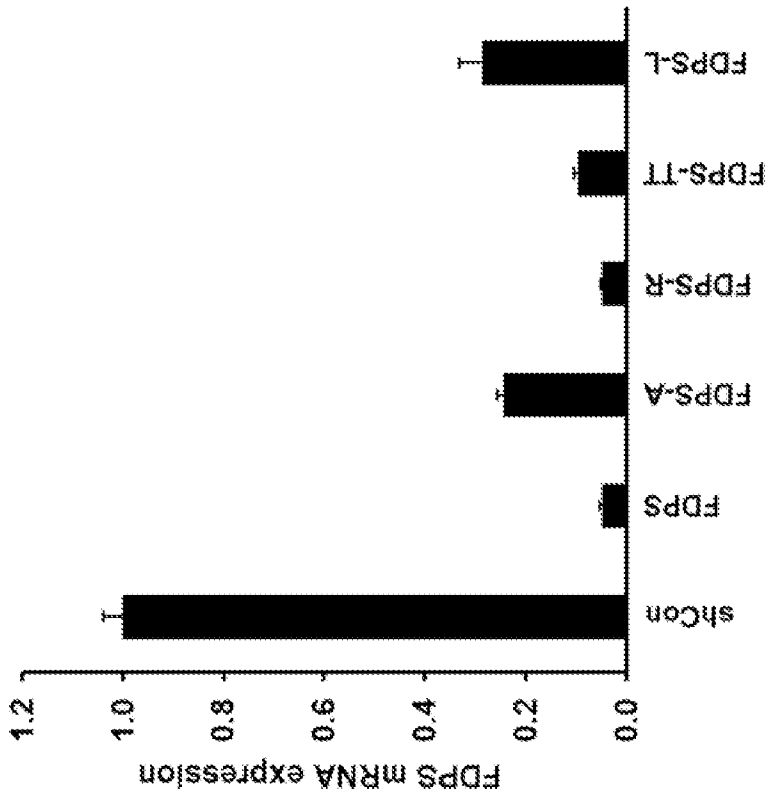


Figure 19A

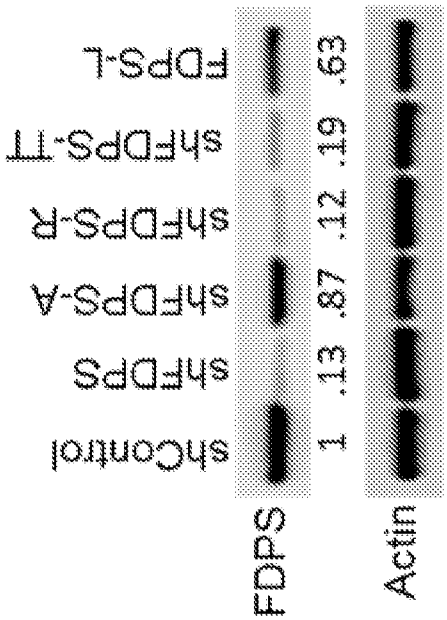


Figure 19B

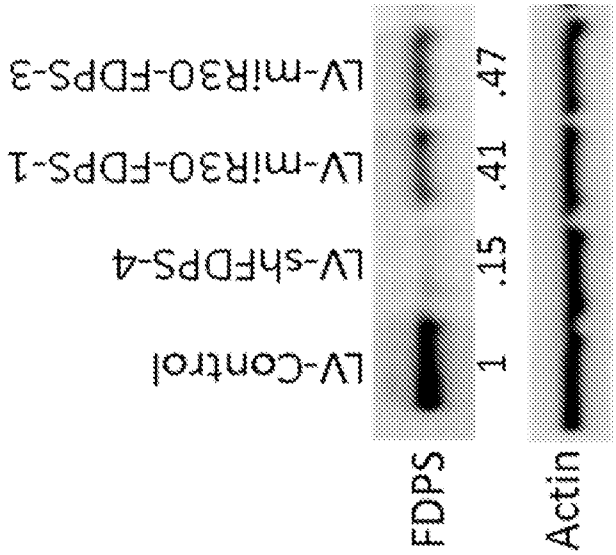

FDPS
Actin
1
.15
.41
.47
LV-Control
LV-shFDPS-4
LV-miR30-FDPS-1
LV-miR30-FDPS-3

Figure 20

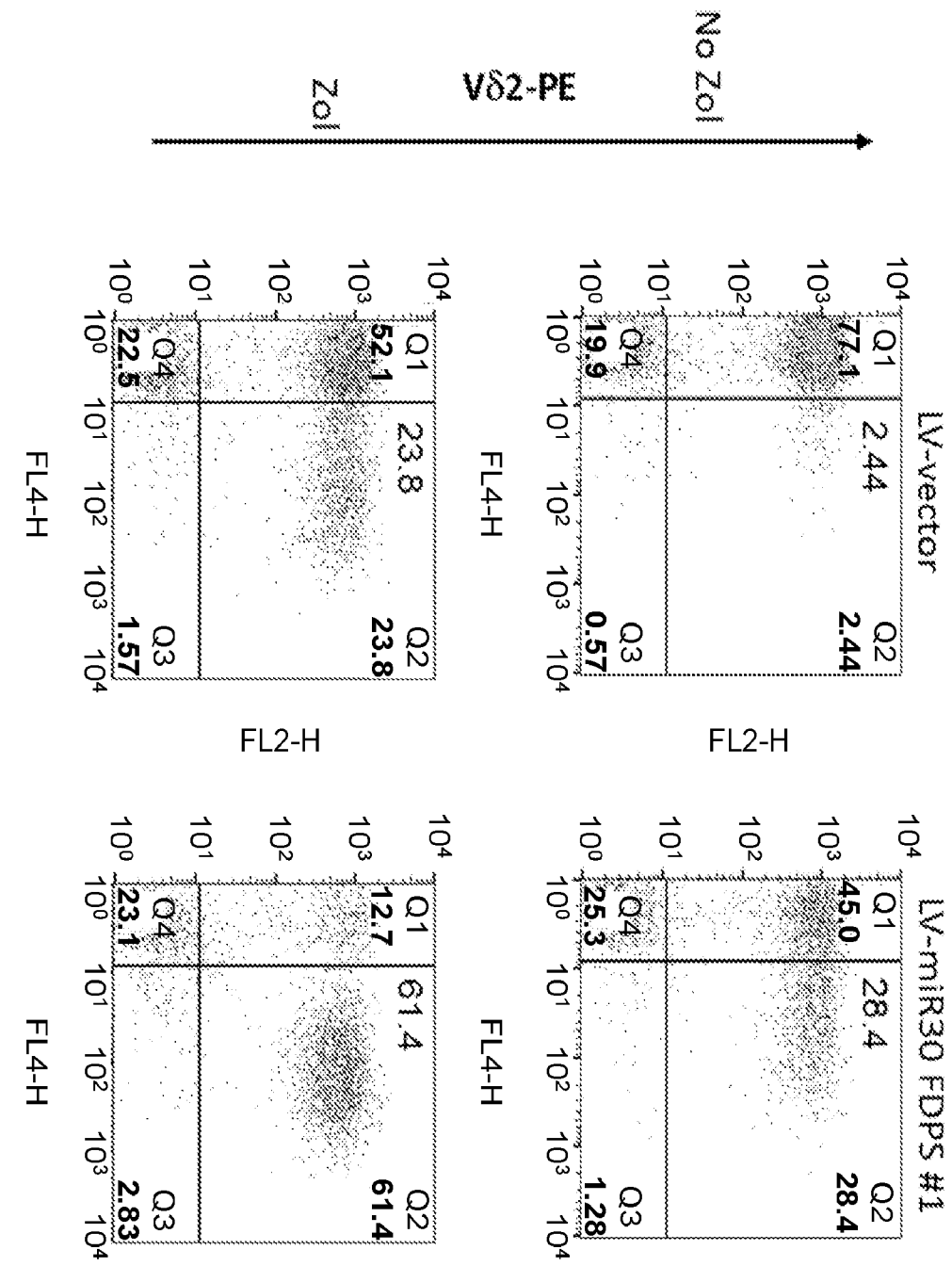

No Zol
Vδ2-PE
Zol
LV-vector
LV-miR30 FDPS #1
FL2-H
FL4-H
Q1
Q2
Q3
Q4
77.1
2.44
19.9
0.57
45.0
28.4
25.3
1.28
52.1
23.8
22.5
1.57
12.7
61.4
23.1
2.83
TNFα-APC

Figure 21

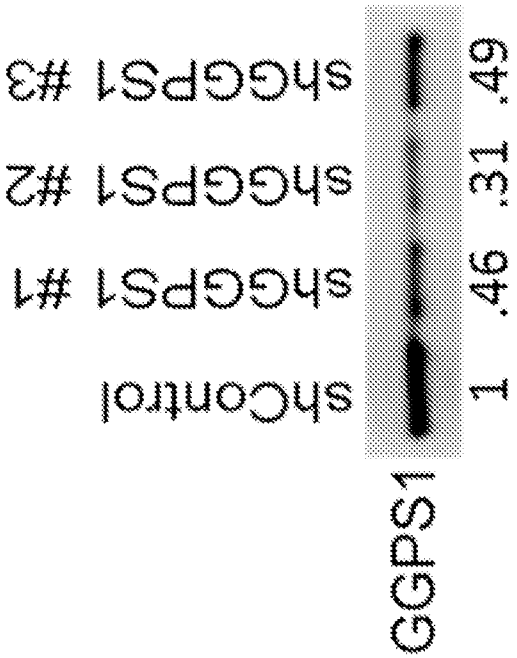


Figure 22

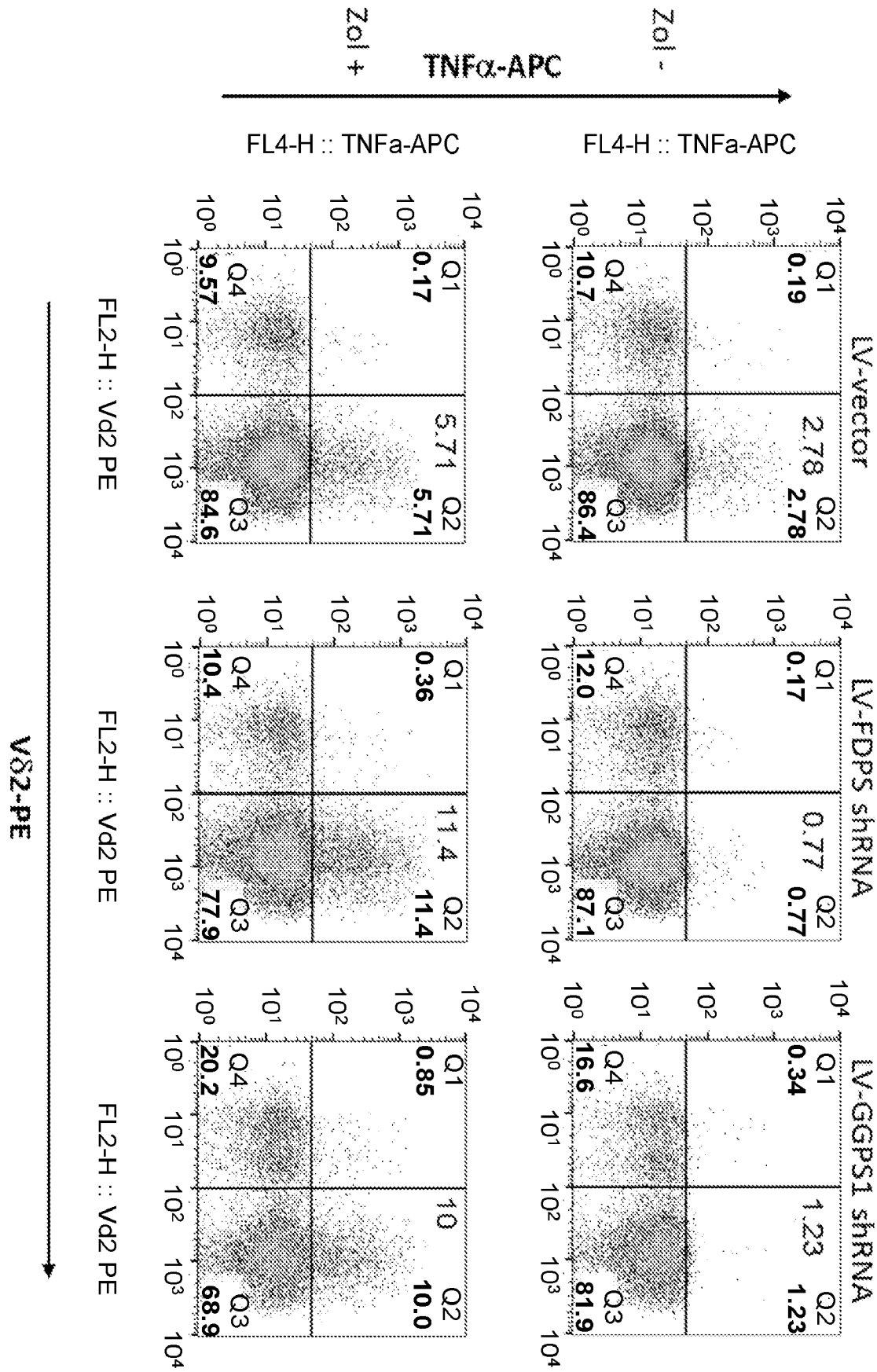

Zol -
Zol +
TNFα-APC
FL4-H :: TNFa-APC
LV-vector
LV-FDPS shRNA
LV-GGPS1 shRNA
Q1 0.19
2.78 Q2 2.78
Q4 10.7
Q3 86.4
Q1 0.17
0.77 Q2 0.77
Q4 12.0
Q3 87.1
Q1 0.34
1.23 Q2 1.23
Q4 16.6
Q3 81.9
Q1 0.17
5.71 Q2 5.71
Q4 9.57
Q3 84.6
Q1 0.36
11.4 Q2 11.4
Q4 10.4
Q3 77.9
Q1 0.85
10 Q2 10.0
Q4 20.2
Q3 68.9
FL2-H :: Vd2 PE
Vδ2-PE

Figure 23

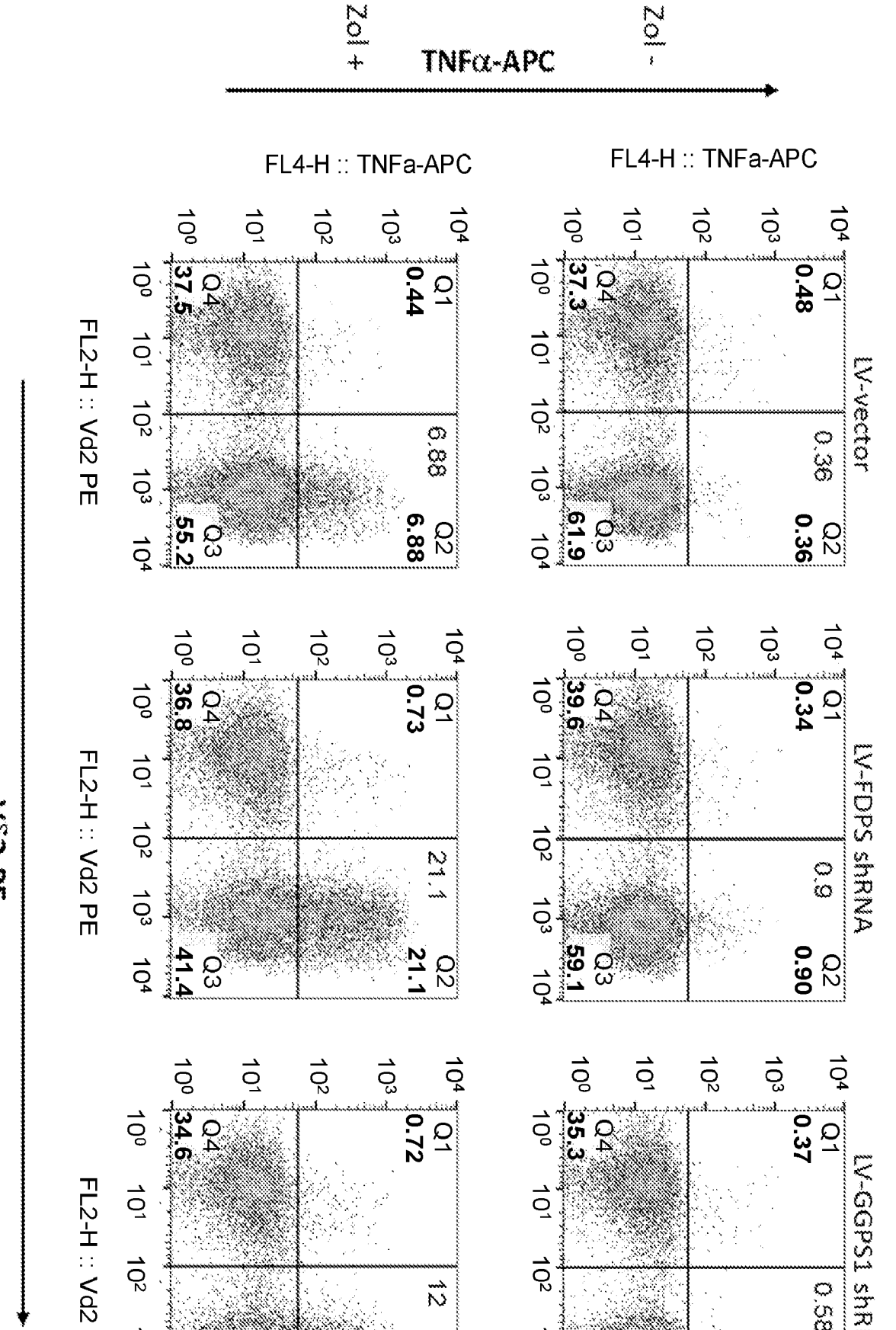

Zol +
TNFα-APC
Zol -
FL4-H :: TNFa-APC
LV-vector
LV-FDPS shRNA
LV-GGPS1 shRNA
Q1 0.48
Q2 0.36
Q3 61.9
Q4 37.3
Q1 0.44
Q2 6.88
Q3 55.2
Q4 37.5
Q1 0.34
Q2 0.90
Q3 59.1
Q4 39.6
Q1 0.73
Q2 21.1
Q3 41.4
Q4 36.8
Q1 0.37
Q2 0.58
Q3 63.7
Q4 35.3
Q1 0.72
Q2 12.0
Q3 52.7
Q4 34.6
FL2-H :: Vd2 PE
Vδ2-PE

Figure 24

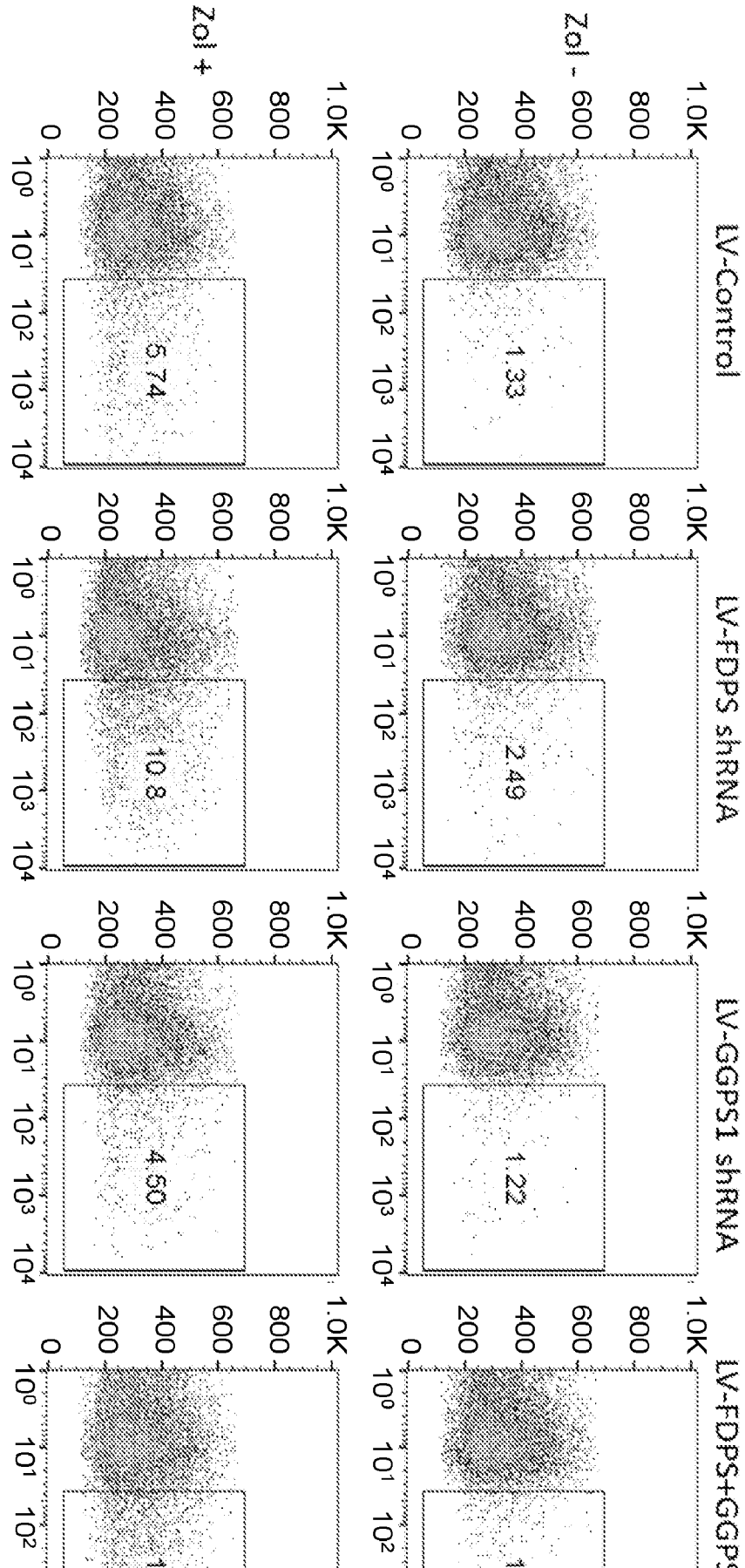

LV-Control
LV-FDPS shRNA
LV-GGPS1 shRNA
LV-FDPS+GGPS1 shRNA
Zol -
Zol +
1.33
2.49
1.22
1.91
5.74
10.8
4.50
11.5
TNF-α APC

Figure 25

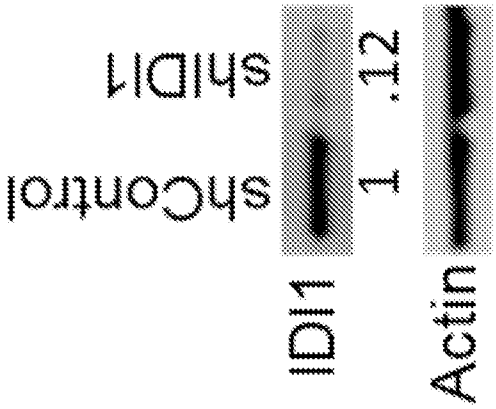


Figure 26

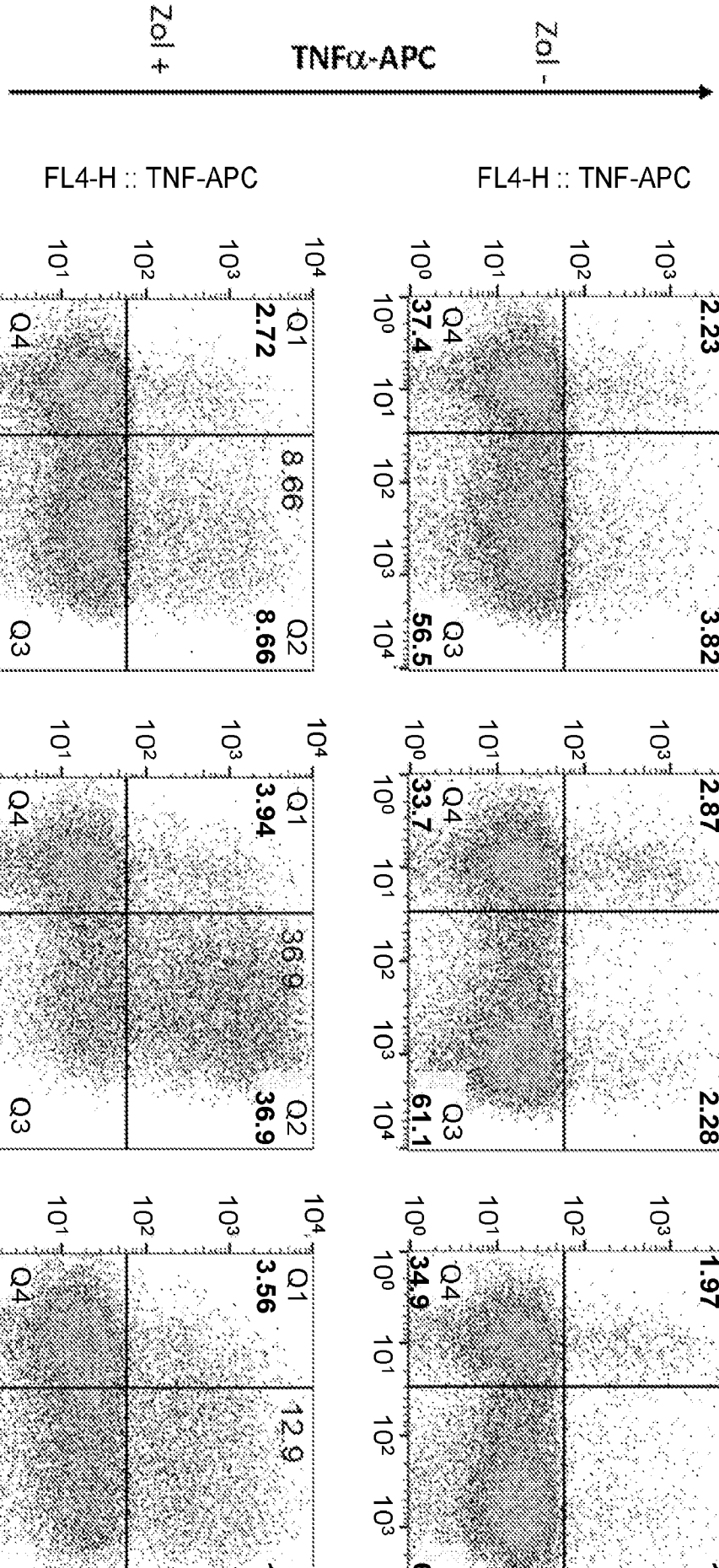

Zol +
TNFα-APC
Zol -
FL4-H :: TNF-APC
FL4-H :: TNF-APC
LV-vector
LV-FDPS shRNA
LV-IDI1 shRNA
Q1 2.23
3.82
Q2 3.82
Q4 37.4
Q3 56.5
Q1 2.87
2.28
Q2 2.28
Q4 33.7
Q3 61.1
Q1 1.97
1.92
Q2 1.92
Q4 34.9
Q3 61.2
Q1 2.72
8.66
Q2 8.66
Q4 40.6
Q3 48.0
Q1 3.94
36.9
Q2 36.9
Q4 36.8
Q3 22.3
Q1 3.56
12.9
Q2 12.9
Q4 44.2
Q3 39.4
FL2-H :: PE-Vd2
FL2-H :: PE-Vd2
FL2-H :: PE-Vd2
Vδ2-PE

Figure 27

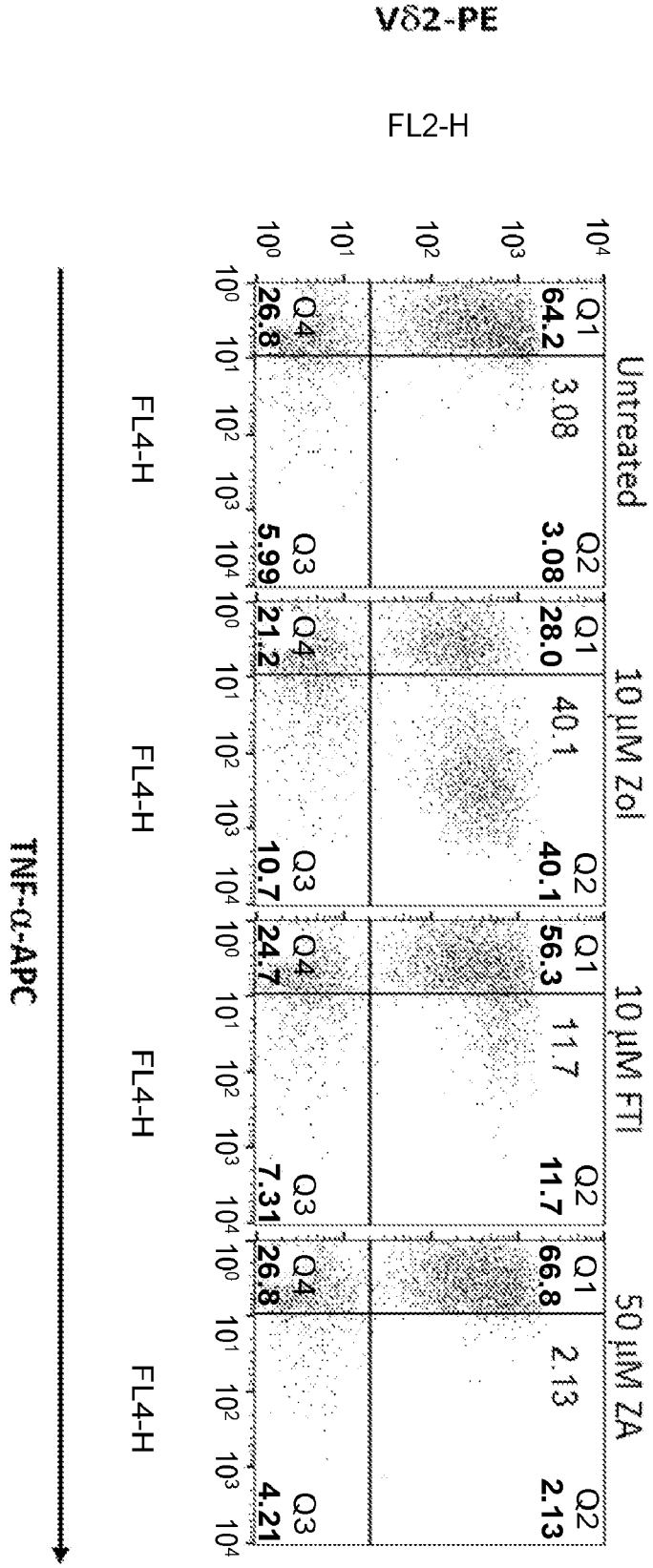

Vδ2-PE
FL2-H
Untreated
10 μM Zol
10 μM FTI
50 μM ZA
Q1 64.2
Q2 3.08
Q3 5.99
Q4 26.8
Q1 28.0
Q2 40.1
Q3 10.7
Q4 21.2
Q1 56.3
Q2 11.7
Q3 7.31
Q4 24.7
Q1 66.8
Q2 2.13
Q3 4.21
Q4 26.8
FL4-H
TNF-α-APC

Figure 28

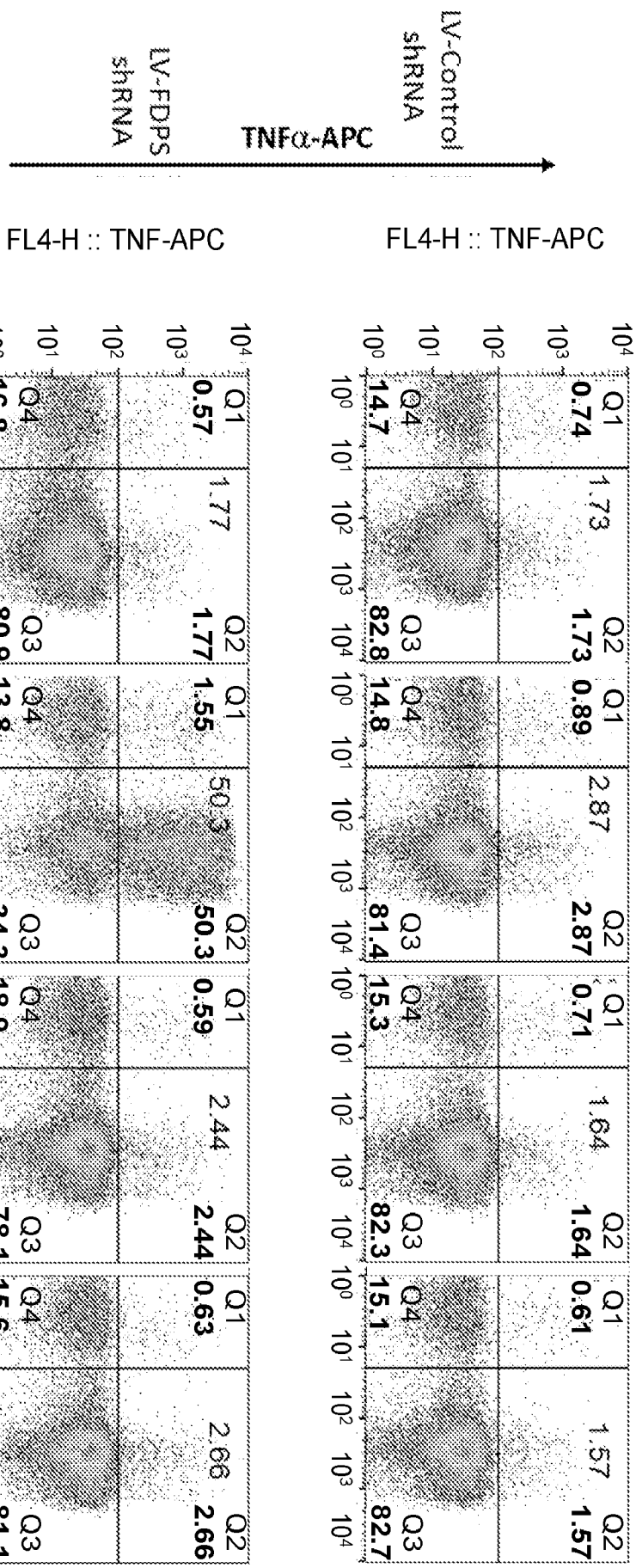

LV-Control shRNA
LV-FDPS shRNA
TNFα-APC
FL4-H :: TNF-APC
Untreated
1 μM Zol
1 μM FTI
5 μM ZA
Vδ2-PE
FL2-H :: PE-Vd2

Figure 29

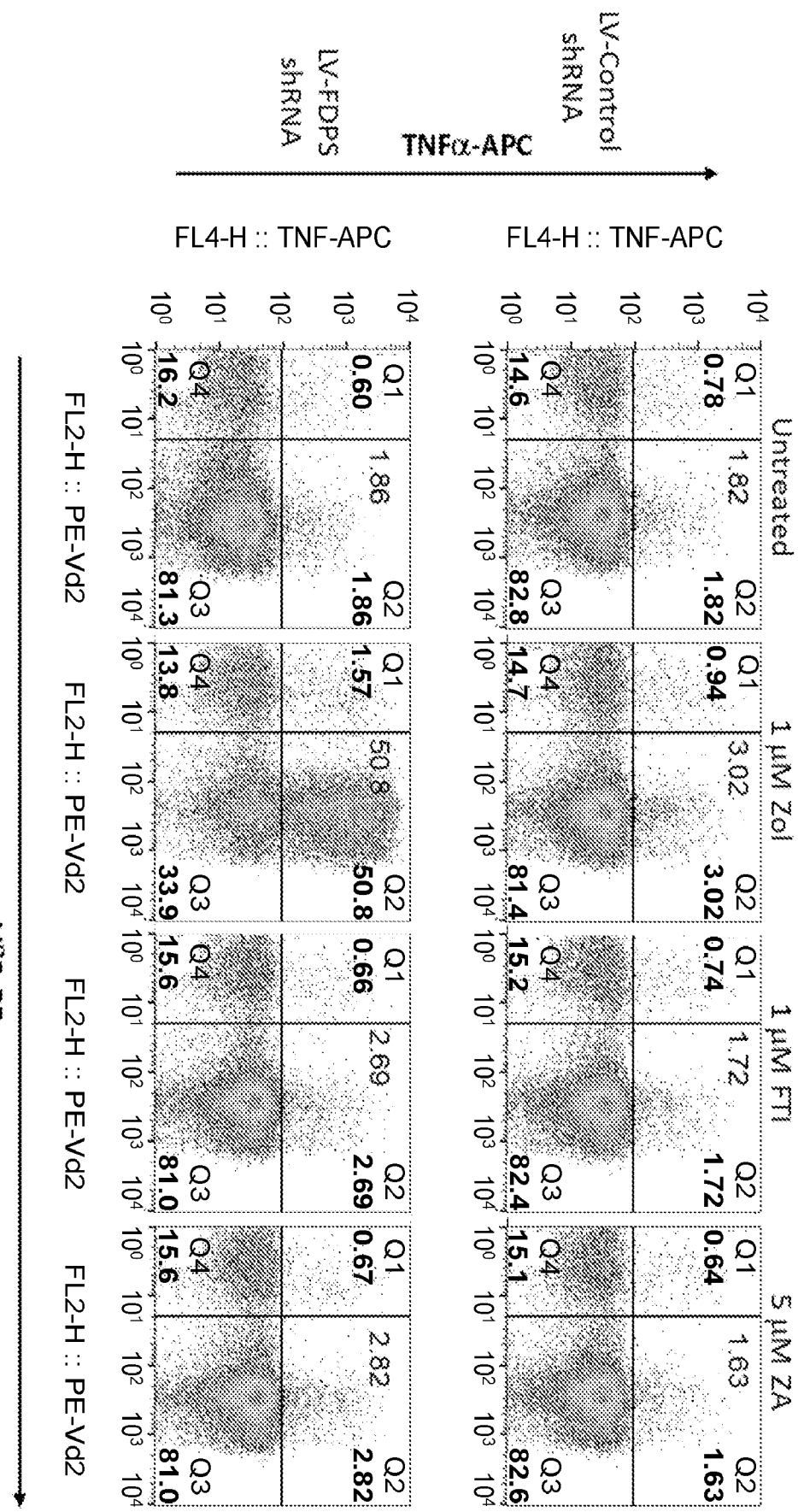

LV-Control shRNA
LV-FDPS shRNA
TNFα-APC
FL4-H :: TNF-APC
Untreated
1 μM Zol
1 μM FTI
5 μM ZA
Vδ2-PE
FL2-H :: PE-Vd2
Q1 0.78
Q2 1.82
Q3 82.8
Q4 14.6
Q1 0.94
Q2 3.02
Q3 81.4
Q4 14.7
Q1 0.74
Q2 1.72
Q3 82.4
Q4 15.2
Q1 0.64
Q2 1.63
Q3 82.6
Q4 15.1
Q1 0.60
Q2 1.86
Q3 81.3
Q4 16.2
Q1 1.57
Q2 50.8
Q3 33.9
Q4 13.8
Q1 0.66
Q2 2.69
Q3 81.0
Q4 15.6
Q1 0.67
Q2 2.82
Q3 81.0
Q4 15.6

Figure 30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/37924

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/37924

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-***- Please See Within the Next Supplemental Page -***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos..
Group 1+ Claims 1, 4-7, 10-11, 15-35; SEQ ID NO: 68; (microRNA); farnesyl diphosphatate synthase (mevalonate pathway enzyme); BTN3A3 (butyrophilin family member)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/37924

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 9/10, 15/113, 15/86, 5/0783; G01N 33/573 (2018.01)

CPC - C12N 9/10, 15/113, 15/86, 5/0636; G01N 33/573

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	(WANG, H et al.) Butyrophilin 3A1 Plays an Essential Role in Prenyl Pyrophosphate Stimulation of Human Vg2Vd2 T Cells. Journal of Immunology. 05 July, 2013; Vol. 191, No. 3; pages 1029-1042; page 1030, column 2, paragraph 4; page 1031, column 1, paragraph 2; page 1032, column 2, paragraphs 1-3; DOI: 10.4049/jimmunol.1300658	1, 4-5, 10-11, 15-22, 23/19, 23/21, 24-26, 27/24-25, 28, 32
Y	(JIANG, X et al.) A Novel EST-derived RNAi Screen Reveals a Critical Role for Farnesyl Diphosphate Synthase in Beta2-adrenergic Receptor Internalization and Down-Regulation. FASEB Journal. 25 January, 2012; Vol. 26, No. 5; pages 1-13; page 3, column 1, paragraph 5 – column 2, paragraph 1; DOI: 10.1096/fj.11-193870	1, 4-5, 10-11, 15-22, 23/19, 23/21, 24-26, 27/24-25, 28-35
Y	(MIETTINEN, TP et al.) Mevalonate Pathway Regulates Cell Size Homeostasis and Proteostasis through Autophagy. Cell Reports. December 2015; Vol. 13, No. 11; pages 2610-2620; figure 2A; DOI: 10.1016/j.celrep.2015.11.045	29-35
Y	(TOLMACHOV,) Designing Lentiviral Gene Vectors. Viral Gene Therapy. 2011; Chapter 13; pages 263-284; page 265, paragraph 2; page 266, paragraphs 2-3; page 267, paragraph 1	16-22, 23/19, 23/21
Y	WO 2013/096455 A1 (DANA-FARBER CANCER INSTITUTE, INC., et al.) 27 June, 2013; paragraphs [008], [00145]; claims 50, 74	24-26, 27/24-25, 28
A	US 2015/0018539 A1 (MIRIMUS, INC.) 15 January, 2015; figure 5A; paragraph [0036]	6-7

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 October 2018 (26.10.2018)	09 NOV 2018
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/37924

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	(TRACEY, A) Human DNA Sequence from Clone RP1-288M22 on Chromosome 6q12-13, Complete Sequence. National Center for Biotechnology Information. Genbank Entry. [retrieved on 24 January, 2013]. Retrieved from the Internet: < https://www.ncbi.nlm.nih.gov/nucleotide/AL035467.23?report=genbank&log $=nucltop&blast_rank=1&RID=UUD4GX2D014>; pages 1-34	6-7
PX	US 2018/0142257 A1 (AMERICAN GENE TECHNOLOGIES INTERNATIONAL INC.,) 24 May, 2018; whole document	1, 4-7, 10-11, 15-22, 23/19, 23/21, 24-26, 27/24-25, 28-35

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US18/37924

-***-Continued from Box III Observations where unity of invention is lacking -***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-35, SEQ ID NO: 68, farnesyl diphosphatate synthase, and BTN3A3 are directed toward viral vectors and methods for their use in activating a gamma delta T cell and treating cancer; wherein the viral vector comprises first and second encoded genetic elements incuding a small RNA for inhibiting mevalonate pathway enzyme wherein cells targeted by the viral vector activate gamma delta T cells.

The vectors and methods will be searched to the extent that they encompass SEQ ID NO: 68; (first exemplary microRNA), farnesyl diphosphatate synthase (FDPS) (first exemplary mevalonate pathway enzyme), and BTN3A3 (first exemplary butyrophilin family member). Applicant is invited to elect additional RNA oligonucleotide(s), with specified SEQ ID NO: for each, such that the sequence of each elected species is fully specified (i.e. no optional or variable residues or substituents); and/or additional mevalonate pathway enzyme(s) and/or additional butrophylin family member(s), to be searched. Additional oligonucleotide sequence(s), enzyme(s), or butrophylin family member(s) will be searched upon the payment of additional fees. It is believed that claims 1 (in-part), 4-7 (each in-part) 10 (in-part), 11 (in-part), 15-28 (each in-part), 29, 30, 31 (in-part), and 32-35 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 68; (microRNA); farnesyl diphosphatate synthase (mevalonate pathway enzyme); BTN3A3 (butyrophilin family member). Applicants must specify the claims that encompass any additionally elected oligonucleotide sequence(s), and/or mevalonate pathway enzyme(s), and/or butyrophilin family member(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be SEQ ID NO: 69 (microRNA).

No technical features are shared between the oligonucletide sequences, mevalonate pathway enzymes or butyrophilin family members of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features of: a viral vector comprising first and second encoded genetic elements wherein the first encoded genetic element comprises at least one small RNA capable of inhibiting production of at least one enzyme involved in the mevalonate pathway, and the second encoded genetic element comprises one of a butyrophilin family member, a cytokine, or a chemokine; a method of activating a gamma delta (GD) T cell comprising: infecting, or having infected, in the presence of the GD T cell, a target cell with a lentiviral particle, wherein the lentiviral particle comprises a viral vector comprising said first and second encoded genetic elements, and wherein when the at least one enzyme is inhibited in the target cell, the target cell activates the GD T cell; a method of treating cancer in a subject, the method comprising administering, or having administered, to the subject a therapeutically effective amount of the lentiviral particle wherein the lentiviral particle comprises a viral vector comprising said first and second encoded genetic elements, wherein when the at least one enzyme is inhibited in a cancer cell in the presence of a GD T cell, the target cell activates the GD T cell, to thereby treat the cancer; a viral vector comprising: a first small RNA that targets a first target of the mevalonate pathway and is capable of increasing a first product of the mevalonate pathway; and a second small RNA that targets a second target of the mevalonate pathway and is capable of decreasing a second product of the mevalonate pathway; and a method of treating cancer in a subject, the method comprising administering, or having administered, to the subject a therapeutically effective amount of the lentiviral particle wherein the lentiviral particle comprises the viral vector; however these shared technical features are previously disclosed by US 2014/0178340 A1 Tocagen, Inc (hereinafter 'Tocagen') in view of WO 2012/048303 A2 to Columbia University (hereinafter 'Columbia') and US 2010/0316676 A1 to Sanders et al. (hereinafter 'Sanders').

Tocagen discloses a viral vector (lentiviral vector; paragraphs [0051], [0064]) comprising first and second encoded genetic elements (polynucleotide encoding miRNA, siRNA, RNAi, cytokines or combinations; paragraphs [0051], [0052]), and wherein the second encoded genetic element comprises one of a butyrophilin family member, a cytokine (paragraphs [0051], [0052]), or a chemokine; infecting, or having infected, a target cell with a lentiviral particle (retroviral, including lentiviral, infection of a target cell; paragraphs [0051], [0059]), wherein the lentiviral particle comprises a viral vector (lentiviral vector; paragraphs [0051], [0064]) comprising said first and second encoded genetic elements (polynucleotide encoding miRNA, siRNA, RNAi, cytokines or combinations; paragraphs [0051], [0052]), and wherein at least one enzyme is inhibited in the target cell (inhibition of PPR or IFN pathway enzymes; paragraph [0050]); a method of treating cancer in a subject (abstract; paragraph [0006]), the method comprising administering (administering a retroviral vector; paragraphs [0045], [0051]), or having administered, to the subject a therapeutically effective amount (effective doses of vector; paragraph [0048]) of the lentiviral particle (lentiviral vector; paragraphs [0051], [0064]) wherein the lentiviral particle comprises a viral vector (lentiviral vector; paragraphs [0051], [0064]) comprising said first and second encoded genetic elements (polynucleotide encoding miRNA, siRNA, RNAi, cytokines or combinations; paragraphs [0051], [0052]), to thereby treat the cancer (abstract; paragraph [0006]); a viral vector comprising: a first small RNA (polynucleotide encoding miRNA, siRNA, RNAi; paragraphs [0051], [0052]); and a second small RNA (polynucleotide encoding miRNA, siRNA, RNAi or combinations; paragraphs [0051], [0052]); and a method of treating cancer in a subject (abstract; paragraph [0006]), the method comprising administering (administering a retroviral vector; paragraphs [0045], [0051]), or having administered, to the subject a therapeutically effective amount (effective doses of vector; paragraph [0048]) of the lentiviral particle (lentiviral vector; paragraphs [0051], [0064]) wherein the lentiviral particle comprises the viral vector (lentiviral vector; paragraphs [0051], [0064]). Tocagen does not disclose wherein the first encoded genetic element comprises at least one small RNA capable of increasing a first product of the mevalonate pathway and wherein a second small RNA is capable of decreasing a second product of at least one enzyme involved in the mevalonate pathway; a method of activating a gamma delta (GD) T cell comprising: infecting, or having infected, in the presence of the GD T cell, a target cell with a lentiviral particle; wherein when the at least one enzyme is inhibited in the target cell, the target cell activates the GD T cell; or wherein when the at least one enzyme is inhibited in a cancer cell in the presence of a GD T cell, the target cell activates the GD T cell.

-***-Continued Within the Next Supplemental Box-***-

-***-Continued from Previous Supplemental Page:

Columbia discloses vectors for the expression of small RNAs (p53 shRNA; paragraphs [0008], [0104]) wherein the small RNAs are capable of increasing a product of mevalonate pathway enzymes of the mevalonate pathway (mutant p53 upregulation of mevalonate pathway; paragraphs [0054], [0146], [0149]) or capable of decreasing (inhibition of) mevalonate pathway enzymes (down-regulation of mevalonate pathway enzymes; paragraphs [0010], [0022]) in the treatment of cancer (paragraph [0010]).

Sanders discloses treating cancer (paragraphs [0006], [0098]) using compounds that inhibit the mevalonate pathway (paragraph [0132]) which compounds also activate gamma delta (GD) T cells (abstract; paragraphs [0101], [0132]) and further wherein administration of a retrovirus can be used to stimulate activation of gamma delta T cells (paragraphs [0100], [0101]).

It would have been obvious to one of ordinary skill in the art at the time of the invention to have modified the disclosure of Tocagen to provide wherein the first encoded genetic element comprises at least one small RNA capable of increasing a first product of the mevalonate pathway and wherein a second small RNA is capable of decreasing a second product of at least one enzyme involved in the mevalonate pathway; and to provide a method of activating a gamma delta (GD) T cell comprising: infecting, or having infected, in the presence of the GD T cell, a target cell with a lentiviral particle; wherein when the at least one enzyme is inhibited in the target cell, the target cell activates the GD T cell; and to provide wherein when the at least one enzyme is inhibited in a cancer cell in the presence of a GD T cell, the target cell activates the GD T cell, because the ability to inhibit or upregulate enzymes of the mevalonate pathway using small inhibitory RNAs such as shRNA carried in lentiviral vectors as disclosed by Columbia would have been expected to motivate the use of said small RNAs in combination with polynucleotides encoding cytokines to as previously disclosed by Tocagen to increase the effectiveness of cancer therapy. Further, since the use of compounds that inhibit the mevalonate pathway also act as activators of gama delta (GD) T cells and that retroviral administration can be used to deliver said compounds that activate GD T cells as previously disclosed by Sanders, the skilled artisan would have been motivated to modify the disclosure of Tocagen to provide a method of activating a gamma delta (GD) T cell comprising infecting, in the presence of the GD T cell, a target cell with a lentiviral particle carrying the polynucleotide(s) encoding small RNAs to inhibit mevalonate pathway enzymes as previously disclosed by Columbia.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the combination of the Tocagen, Columbia and Sanders references, unity of invention is lacking.