



## (51) International Patent Classification:

C12N 15/864 (2006.01) C07K 14/805 (2006.01)  
C12N 15/85 (2006.01)

## (21) International Application Number:

PCT/US2016/018815

## (22) International Filing Date:

19 February 2016 (19.02.2016)

## (25) Filing Language:

English

## (26) Publication Language:

English

## (30) Priority Data:

62/118,095 19 February 2015 (19.02.2015) US  
62/118,139 19 February 2015 (19.02.2015) US  
62/118,114 19 February 2015 (19.02.2015) US

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(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, DK, DM,  
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,  
HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,  
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, 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))
- with sequence listing part of description (Rule 5.2(a))

## (54) Title: RECOMBINANT AAV VECTORS FOR GENE THERAPY OF HUMAN HEMATOPOIETIC DISORDERS

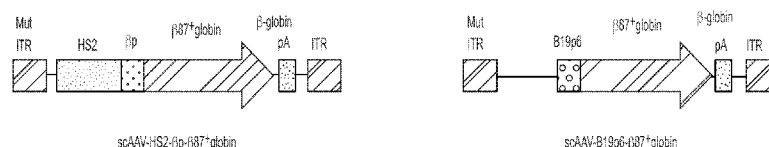


FIG. 1

(57) Abstract: Provided herein are recombinant AAV (rAAV) particles comprising a nucleic acid vector comprising a parvovirus B 19p6 promoter operatively linked to a heterologous gene, such as a human globin gene, and rAAV capsid proteins comprising one or more amino acid substitutions in a surface exposed loop of the capsid protein that result, e.g., in increased P antigen binding compared to a corresponding un-mutated AAV capsid protein. Also provided are methods and compositions related to such capsid proteins, methods of targeting gene expression to a cell of erythroid lineage, methods of treating a hemoglobinopathy using such rAAV particles, and methods for efficient transduction of a host cell suspension with a rAAV.



## RECOMBINANT AAV VECTORS FOR GENE THERAPY OF HUMAN HEMATOPOIETIC DISORDERS

### RELATED APPLICATIONS

5 This application claims the benefit under 35 U.S.C. § 119(e) of U.S. provisional application number 62/118,095, filed February 19, 2015, U.S. provisional application number 62/118,139, filed February 19, 2015, and U.S. provisional application number 62/118,114, filed February 19, 2015, the contents of each of which are incorporated herein by reference in their entirety.

### GOVERNMENT SUPPORT

10 This invention was made with government support under HL-097088 and EB-015684 awarded by the National Institutes of Health. The government has certain rights in the invention.

### BACKGROUND

15 Different recombinant adeno-associated virus (rAAV) serotypes have tropisms for different tissues and cell types. There remains a need to develop serotypes that can selectively target tissues and cell types of interest. Additionally, methods for infecting cells with recombinant adeno-associated virus (rAAV) remain limited in their ability to be efficient  
20 in certain cell types. Accordingly, methods are needed to address the limited infection rates in those cells.

### SUMMARY

25 Aspects of the application relate to compositions and methods for treating disorders relating to the hematopoietic system using recombinant AAV (rAAV). Aspects of the application include cell-specific expression, cell-specific targeting, efficient rAAV transduction, and combinations thereof.

In some aspects, the application provides rAAV particles and nucleic acid vectors that

comprise a parvovirus B19p6 promoter operatively linked to a heterologous gene, such as a human globin gene. Also provided are various methods that utilize such particles and nucleic acid vectors, such as methods of treating hemoglobinopathies.

In some aspects, the disclosure provides an rAAV particle comprising a nucleic acid vector comprising a parvovirus B19p6 promoter operatively linked to a heterologous gene. In some embodiments, the rAAV particle is not AAV2. In some embodiments, the rAAV particle is AAV2. In some embodiments, the rAAV particle is AAV6.

In some embodiments, the heterologous gene is a globin gene. In some embodiments, the globin gene is selected from the group consisting of a  $\beta$ -globin gene, an anti-sickling  $\beta$ -globin gene, and a  $\gamma$ -globin gene. In some embodiments, the globin gene is a human globin gene. In some embodiments, the globin gene is a human  $\beta$ -globin gene or human anti-sickling  $\beta$ -globin gene.

In some embodiments, the rAAV particle is a AAV6 particle. In some embodiments, the AAV6 particle comprises a modified capsid protein comprising a non-tyrosine residue at a position that corresponds to a surface-exposed tyrosine residue in a wild-type AAV6 capsid protein, a non-threonine residue at a position that corresponds to a surface-exposed threonine residue in the wild-type AAV6 capsid protein, a non-lysine residue at a position that corresponds to a surface-exposed lysine residue in the wild-type AAV6 capsid protein, a non-serine residue at a position that corresponds to a surface-exposed serine residue in the wild-type AAV6 capsid protein, or a combination of two or more thereof.

In some embodiments, the modified capsid protein comprises a non-tyrosine residue and/or a non-threonine residue at one or more of or each of Y705, Y731, and T492 of a wild-type AAV6 capsid protein. In some embodiments, the non-tyrosine residue is phenylalanine and the non-threonine residue is valine.

In some embodiments, the nucleic acid vector further comprises AAV2 or AAV6 inverted terminal repeat sequences (ITRs) flanking the parvovirus B19p6 promoter operatively linked to the heterologous gene.

Other aspects of the disclosure relate to a nucleic acid vector comprising a parvovirus B19p6 promoter operatively linked to a globin gene. In some embodiments, the globin gene

is selected from the group consisting of a  $\beta$ -globin gene, an anti-sickling  $\beta$ -globin gene, and a  $\gamma$ -globin gene. In some embodiments, the globin gene is a human globin gene. In some embodiments, the globin gene is a human  $\beta$ -globin gene or human anti-sickling  $\beta$ -globin gene.

5 Further aspects of the disclosure relate to rAAV capsid proteins comprising one or more amino acid substitutions in a surface-exposed loop region. In some embodiments, the substitutions result in improved targeting of a tissue or cell of interest, e.g., a cell expressing P antigen.

10 Aspects of the disclosure relate to an rAAV capsid protein comprising one or more amino acid substitutions that result in increased P antigen binding compared to a corresponding un-mutated AAV capsid protein, wherein the one or more amino acid substitutions are in a surface exposed loop of the capsid protein. Other aspects of the disclosure relate to an rAAV capsid protein comprising one or more amino acid substitutions that introduce a P antigen binding site into a surface exposed loop of the capsid protein. In  
15 some embodiments, the surface exposed loop is loop VIII. In some embodiments, a surface exposed loop is replaced by a B19 P antigen binding site. In some embodiments, the B19 P antigen binding site comprises the amino acid sequence QQYTDQIE (SEQ ID NO: 1). In some embodiments, the rAAV capsid protein is a variant of an AAV6 capsid protein.

20 Other aspects of the disclosure provide a method of increasing rAAV tropism for hematopoietic stem cells, the method comprising altering a surface exposed loop of an AAV capsid protein to introduce one or more amino acid substitutions that result in increased P antigen binding compared to a corresponding un-mutated AAV capsid protein. Yet other aspects of the disclosure provide a method of increasing rAAV tropism for hematopoietic stem cells, the method comprising altering a surface exposed loop of an AAV capsid protein  
25 to introduce one or more amino acid substitutions that introduce a P antigen binding site into a surface exposed loop of the capsid protein. In some embodiments, the surface exposed loop is loop VIII. In some embodiments, a surface exposed loop is replaced by a B19 P antigen binding site. In some embodiments, the B19 P antigen binding site comprises the amino acid

sequence QQYTDQIE (SEQ ID NO: 1). In some embodiments, the rAAV capsid protein is a variant of an AAV6 capsid protein.

Other aspects of the disclosure relate to a method of delivering an rAAV to a cell, the method comprising administering an rAAV particle comprising an rAAV capsid protein of any one of the embodiments above or described herein. In some embodiments, the cell is a hematopoietic stem cell, a megakaryocyte, an endothelial cell, a cardiomyocyte, a hepatocyte, or a trophoblast. In some embodiments, the cell is a hematopoietic stem cell. In some embodiments, the subject is a human subject.

Other aspects of the disclosure relate to an rAAV particle comprising an rAAV capsid protein of any one of the embodiments above or described herein.

Yet further aspects of the disclosure relate to a nucleic acid encoding an rAAV capsid protein of any one of the embodiments above or described herein. In some embodiments, the nucleic acid is a plasmid.

In some aspects, the disclosure provides an rAAV particle comprising a nucleic acid vector comprising a parvovirus B19p6 promoter operatively linked to a heterologous gene, wherein the rAAV particle capsid protein comprises one or more amino acid substitutions that result in increased P antigen binding compared to a corresponding un-mutated AAV capsid protein.

Other aspects of the disclosure relate to a method of targeting gene expression to a cell of erythroid lineage in a subject, the method comprising administering the rAAV particle of any one of the embodiments above or described herein or the nucleic acid vector of any one of the embodiments above or described herein to a subject. In some embodiments, the subject is a human subject. In some embodiments, the cell of erythroid lineage is a hematopoietic stem cell. In some embodiments, the cell of erythroid lineage is a CD36+ burst-forming units-erythroid (BFU-E) cell or a colony-forming unit-erythroid (CFUE-E) progenitor cell.

Yet other aspects of the disclosure relate to a method of treating a hemoglobinopathy, the method comprising administering the rAAV particle of any one of the embodiments above or described herein or the nucleic acid vector of any one of the embodiments above or

described herein to a subject having a hemoglobinopathy. In some embodiments, the subject is a human subject. In some embodiments, the hemoglobinopathy is  $\beta$ -thalassemia or sickle cell disease.

Also provided herein are methods of achieving efficient rAAV transduction of host cells that are grown suspension, such as hematopoietic stem and progenitor cells (e.g., bone marrow-derived cells, cord blood-derived cells, CD34+ cells, and CD36+ cells) and other cell types that are grown under non-adherent conditions. As described herein, it has been shown that cell suspensions grown at high density (e.g., at 200,000 cell per 50 microliters or greater) showed improved transduction efficiency of rAAV particles compared to cell suspensions grown at low densities.

In some aspects, the disclosure provides a method for efficient AAV transduction of a host cell suspension, the method comprising contacting a host cell suspension with a recombinant AAV (rAAV) particle composition, wherein the host cell suspension has a density of greater than 4,000 cells per microliter.

In some embodiments, the rAAV particle composition is a AAV2 or AAV6 particle composition. In some embodiments, the recombinant AAV (rAAV) particle within the composition comprises a modified capsid protein comprising a non-tyrosine residue at a position that corresponds to a surface-exposed tyrosine residue in a wild-type AAV2 or AAV6 capsid protein, a non-threonine residue at a position that corresponds to a surface-exposed threonine residue in the wild-type AAV2 or AAV6 capsid protein, a non-lysine residue at a position that corresponds to a surface-exposed lysine residue in the wild-type AAV2 or AAV6 capsid protein, a non-serine residue at a position that corresponds to a surface-exposed serine residue in the wild-type AAV2 or AAV6 capsid protein, or a combination thereof. In some embodiments, the modified capsid protein comprises a non-tyrosine residue and/or a non-threonine residue at one or more of or each of Y705, Y731, and T492 of a wild-type AAV6 capsid protein. In some embodiments, the modified capsid protein comprises a non-tyrosine residue and/or a non-threonine residue at one or more of or each of Y444, Y500, Y731, and T491 of a wild-type AAV2 capsid protein. In some

embodiments, the non-tyrosine residue is phenylalanine and the non-threonine residue is valine.

In some embodiments, the rAAV particle composition contains  $3 \times 10^3$ - $1 \times 10^4$  vector genomes(vg)/mL of rAAV particles. In some embodiments, the rAAV particle composition contains  $1 \times 10^2$ - $1 \times 10^6$ ,  $1 \times 10^3$ - $1 \times 10^6$ ,  $1 \times 10^3$ - $1 \times 10^5$ , or  $1 \times 10^3$ - $1 \times 10^4$  vg/mL of rAAV particles.

In some embodiments, the recombinant AAV (rAAV) particle within the composition comprises a nucleic acid vector that encodes a therapeutic protein.

In some embodiments, the rAAV particle within the composition comprises a nucleic acid vector comprising a parvovirus B19p6 promoter operatively linked to a heterologous gene. In some embodiments, the rAAV particle within the composition comprises an rAAV capsid protein comprising one or more amino acid substitutions that result in increased P antigen binding compared to a corresponding un-mutated AAV capsid protein.

In some embodiments, the host cell suspension comprises stem cells. In some embodiments, the host cell suspension comprises human cells. In some embodiments, the host cell suspension comprises hematopoietic stem cells. In some embodiments, the host cell suspension is a non-adherent host cell suspension. In some embodiments, the method further comprises administering host cells of the host cell suspension to a subject. In some embodiments, host cells of the host cell suspension are obtained from a subject.

## BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure, which can be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

**FIG. 1** is a non-limiting illustration of recombinant AAV nucleic acid vectors.

**FIG. 2** shows a structural alignment of loop VIII of an AAV6 capsid protein with the P antigen binding site. The sequences from top to bottom correspond to SEQ ID NOs.: 1 and 28, respectively.

**FIG. 3** is a non-limiting schematic representation of AAV vector-mediated transduction of HEK293 (3A, 3C), K562 (3B, 3D), M07e (3E, 3F), and Raji (3G, 3H) cells at low and high cell densities, respectively.

**FIG. 4** shows non-limiting results of transductions efficiencies of rAAV2 and rAAV6 particles at  $3 \times 10^3$ - $1 \times 10^4$  vector genomes (vgs)/cell at low (20,000 or 60,000 cells in 50 microliters) or high (200,000 or 580,000 cells in 50 microliters) cell densities. The particles tested contained wild-type (WT) or mutated capsid proteins (for AAV2: Capsid-modified quadruple-mutant (4444F+Y500F+Y731F+T491V) and for AAV6: Capsid-modified triple-mutant (Y705F+Y731F+T492V)).

**FIG. 5** shows non-limiting results of transduction efficiencies of AAV in human hematopoietic cells at various cell densities. K562 cells were transduced at various indicated cell densities at MOIs of 3,000 or 30,000 vgs/mL with WT scAAV6-CBAp-EGFP (5A). K562 cells were also transduced at low or high cell densities with TM scAAV6-CBAp-EGFP (5B). The vector genome copy numbers/cell were determined 2 hours post-transduction by qPCR and data were normalized to  $\beta$ -actin DNA copy number (5C). K562 cells were transduced at low or high cell densities with TM scAAV6-CBAp-Gluc, and transgene expression and mean fluorescence intensity were determined in the culture supernatants (5D). K562 cells were transduced at low or high cell densities with QM scAAV2-CBAp-EGFP (5E). Primary human bone marrow-derived CD34+ cells were transduced at low or high densities with indicated AAV6 or AAV2, EGFP-expressing cells were visualized under a fluorescence microscope 48 hours post-transduction (5F). The vector genome copy numbers/cell (5G).

**FIG. 6** shows non-limiting data depicting the effect of initial cell-cell contact. Schematics of the experiment design (6A). K562 cells and 8 replicate cultures were transduced at low-density (L) with scAAV6-TM-CBAp-EGFP and subsequently pooled together to reach high-density (L→H), and conversely, cells were transduced at high-density (H), and were subsequently diluted to low-density (H→L). Transgene expression in each cell population was analyzed by fluorescence microscopy 48 hours post-transduction (6B). Mean fluorescence intensity of transgene expression is additionally depicted (6C).

**FIG. 7** shows non-limiting data depicting the effect of culture volume. Schematics of the experiment design (7A). A fixed number of K562 cells were transduced with viruses in various indicated volumes and subsequently diluted to 2.0 mL. Transgene expression in each cell population was analyzed by fluorescence microscopy (7B). Mean fluorescence intensity of transgene expression is additionally depicted (7C).

**FIG. 8** shows non-limiting data depicting the transduction efficiency of AAV in various human hematopoietic cell lines at low and high cell densities. (A) Human K562, M07e, and Raji cells were transduced with scAAV2-CBAp-EGFP at either low or high cell density (8A). Mean fluorescence intensity of transgene expression in each cell type is depicted (8B). FACS analyses of the level of expression of membrane heparin sulfate proteoglycan in various human cell types (8C). Each cell type was transduced with scAAV6-TM-CBAp-EGFP at high cell density, and transgene expression was analyzed 48 hours post-transduction (8D). Mean fluorescence intensity of transgene expression in each cell type is additionally depicted (8E).

#### DETAILED DESCRIPTION

In various aspects, the application provides compositions and methods for treating disorders relating to the hematopoietic system with recombinant AAV (rAAV). Aspects of the application include cell-specific expression, cell-specific targeting, efficient rAAV transduction, and combinations thereof. In some aspects, the disclosure provides methods that are useful in the preparation of therapeutic compositions. In some aspects, the disclosure provides compositions that are useful in therapeutic applications. As described herein, such methods and compositions are useful in treating hematopoietic diseases and disorders (e.g., hemoglobinopathies).

In some aspects, the disclosure relates to recombinant AAV (rAAV) particles and nucleic acid vectors that comprise a parvovirus B19p6 promoter operatively linked to a heterologous gene, such as a human globin gene. As described herein, such particles and vectors are useful for targeting cells, such as cells of the erythroid lineage. As used herein,

the term “vector” can refer to a nucleic acid vector (e.g., a plasmid or recombinant viral genome) or a viral vector (e.g., an rAAV particle comprising a recombinant genome).

Other aspects of the disclosure relate to targeting gene expression to a cell, such as a cell of erythroid lineage. In some embodiments, the method comprises administering a  
5 rAAV particle described herein or a nucleic acid vector described herein to a cell. The administration may be *ex vivo* (e.g., to a cell in a culture) or *in vivo* (e.g., in a subject).

In some embodiments, the cell of erythroid lineage is a hematopoietic stem cell. In some embodiments, the hematopoietic stem cell is a CD34<sup>+</sup>, *lin*<sup>-</sup> HSC. In some  
10 embodiments, the cell of erythroid lineage is a CD36<sup>+</sup> burst-forming units-erythroid (BFU-E) cell or a colony-forming unit-erythroid (CFUE-E) progenitor cell. In some embodiments, the cells are identified as being CD36<sup>+</sup> and/or glycophorin A<sup>+</sup>. HSCs and other cell types expressing particular markers, such as CD34, *lin*, CD36, or glycophorin A, can be detected, sorted, and collected using any method known in the art, e.g., by single-cell sorting methods such as fluorescence-activated cell sorting.

15 Other aspects of the disclosure relate to a method of treating a hemoglobinopathy. In some embodiments, the method comprises administering a rAAV particle described herein or a nucleic acid vector described herein to a subject (e.g., a human subject) having a hemoglobinopathy (e.g., a  $\beta$ -thalassemia or sickle cell disease).

Other aspects of the disclosure relate to a method of increasing rAAV tropism for a  
20 tissue or cell of interest. In some embodiments, the method comprises altering a surface exposed loop of an AAV capsid protein to introduce one or more amino acid substitutions that result in increased binding to a cell or tissue of interest compared to a corresponding unmutated AAV capsid protein.

In some aspects, the disclosure relates to methods of targeting rAAV particles by  
25 modifying one or more surface exposed loops, e.g., by replacing all or part of the loop with a sequence that enhances binding to a cell or tissue of interest. Related compositions, host cells, nucleic acids, and rAAV particles are also provided.

In some aspects, the disclosure relates to an rAAV capsid protein comprising one or more amino acid substitutions in a surface exposed loop of the capsid protein. In some

embodiments, the one or more amino acid substitutions result in increased P antigen binding compared to a corresponding unmutated AAV capsid protein. In some embodiment, a rAAV capsid protein is provided comprising one or more amino acid substitutions that introduce a P antigen binding site into a surface exposed loop of the capsid protein. In some embodiments, the surface exposed loop is any one of loops I to IX. In some embodiments, the surface exposed loop is loop VIII.

In some embodiments, a surface exposed loop is replaced by a B19 P antigen binding site. In some embodiments, the B19 P antigen binding site comprises the amino acid sequence QQYTDQIE, or a fragment or variant thereof that is capable of binding to P antigen. A P antigen binding site can be identified, e.g., by mutagenesis of known P antigen binding sites, e.g., using phage display or site-directed mutagenesis in combination with a binding assay such as a surface plasmon resonance, ELISA, or co-immunoprecipitation assay. In some embodiments, the rAAV capsid protein is an AAV6 capsid protein comprising the one or more amino acid substitutions in a surface exposed loop. In some embodiments, AAV6 loop VIII (residues 592 to 598) are substituted with a P antigen-binding site (e.g., residues 399 to 406, QQYTDQIE, of human parvovirus B19). An exemplary wild-type AAV6 capsid protein is provided below. Loops I-IX are underlined and bolded. Loop VIII is underlined, bolded and italicized.

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1  MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD DGRGLVLPGY
51 KYLGPFNGLD KGEPVNAADA AALEHDKAYD QQLKAGDNPY LRYNHADAEF
101 QERLQEDTSF GGNLGRAVFQ AKKRVLEPFG LVEEGAKTAP GKGRPVEQSP
151 QEPDSSSGIG KTGQQPAKKR LNFGQTGDSE SVPDPQPLGE PPATPAAVGP
201 TTMASGGGAP MADNNEGADG VGNASGNWHC DSTWLGDRVI TTSTRTWALP
251 TYNNHLYKQI SSASTGASND NHYFGYSTPW GYFDFNRFHC HFSPRDWQRL
301 INNNWGFRPK RLNFKLFNIQ VKEVTTNDGV TTIANNLTST VQVFSDSEYQ
351 LPYVLGSAHQ GCLPPFPADV FMIPQYGYLT LNNGSQAVGR SSFYCLEYFP
401 SQMLRTGNNF TFSYTFEDVP FHSSYAHSQS LDRLMNPLID QYLYYLNRTQ
451 NQSGSAQNKD LLFSRCSPAG MSVQPKNWLP GPCYRQQRVS KTKTDNNNSN
501 FTWTGASKYN LNGRESIINP GTAMASHKDD KDKFFPMSGV MIFGKESAGA

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551 SNTALDENVMI TDEEEIKATN PVATERFGTV AVNLQSSSTD PATGADVHVMG  
 601 ALPGMVWQDR DVYLQGPWA KIPHTDGHFH PSPLMGGFGL KHPPQILIK  
 651 NTPVPANPPA EFSATKFASF ITQYSTGQVS VEIEWELQKE NSKRWNPEVQ  
 701 YTSNYAKSAN VDFTVDDNGL YTEPRPIGTR YLTRPL (SEQ ID NO: 21)

5

In some embodiments, the cell of interest is a cell expressing P antigen (e.g., a hematopoietic stem cell), and the method comprises altering a surface exposed loop of an AAV capsid protein to introduce one or more amino acid substitutions that result in increased P antigen binding compared to a corresponding un-mutated AAV capsid protein. In some  
 10 embodiments, the method comprises altering a surface exposed loop of an AAV capsid protein to introduce one or more amino acid substitutions that introduce a P antigen binding site into a surface exposed loop of the capsid protein. In some embodiments, the surface exposed loop is loop VIII. In some embodiments, the surface exposed loop is replaced by a B19 P antigen binding site. In some embodiments, the B19 P antigen binding site comprises  
 15 the amino acid sequence QQYTDQIE (SEQ ID NO: 1), or a fragment or variant thereof that is capable of binding to P antigen.

In some embodiments, the rAAV capsid protein is an AAV6 capsid protein comprising the one or more amino acid substitutions in a surface exposed loop. AAV6 capsid proteins are further described herein. In some embodiments, AAV6 loop VIII  
 20 (residues 592 to 598) is substituted with a P antigen-binding site (e.g., residues 399 to 406, QQYTDQIE (SEQ ID NO: 1), of human parvovirus B19).

Other aspects of the disclosure relate to a method of delivering an rAAV to a cell, the method comprising administering an rAAV particle comprising an rAAV capsid protein as described herein. In some embodiments, the cell is *ex vivo*. In some embodiments, the cell is  
 25 *in vivo*, e.g., in a subject as described herein, such as a human subject.

In some embodiments, the cell is a cell expressing P antigen. A cell expressing P antigen can be identified, e.g., by Western blot, ELISA, or another immunoassay known in the art utilizing a P antigen antibody or antigen-binding fragment thereof. An exemplary human P antigen amino acid sequence is provided below:

NP\_001033717.1| UDP-GalNAc:beta-1,3-N-acetylgalactosaminyltransferase 1 [Homo sapiens]  
 MASALWTVLPSRMSLRSLKWSLLLLSLLSFFVMWYLSLPHYNVIERVNWMYFYEYEPYIRQ  
 DFHFTLREHSNCSHQNPFLVILVTSHPSDVKARQAIRVTWGEKKSWWGYEVLTFLLGQEA  
 5 KEDKMLALSLEDEHLLYGDIIRQDFLDTYNNLTTLKTIMAFRWVTEFCPNAKYVMKTDTDVFI  
 NTGNLVKYLLNLNHSEKFFTGYPLIDNYSYRGFYQKTHISYQEYPPFKVFPPYCSGLGYIMSRD  
 LVPRIYEMMGHVKPIKFEDVYVGICLNLKVNHIPEDTNLFFLYRIHLDVCQLRRVIAAHGFS  
 SKEITFWQVMLRNTTCHY (SEQ ID NO: 2)

10 Exemplary cells that express P antigen include hematopoietic stem cells, megakaryocytes, endothelial cells, cardiomyocytes, hepatocytes, and trophoblasts. In some embodiments, the cell is a hematopoietic stem cell.

In some embodiments, an rAAV particle comprising an rAAV capsid protein as described herein, e.g., comprising one or more amino acid substitutions in a surface-exposed  
 15 binding loop, is administered to a subject, e.g., to treat a disease, such as a hemoglobinopathy. In some embodiments, the method comprises administering a rAAV particle described herein to a subject (e.g., a human subject) having a hemoglobinopathy (e.g.,  $\beta$ -thalassemia or sickle cell disease).

Yet other aspects of the disclosure relate to a method for efficient AAV transduction  
 20 of a host cell suspension. In some embodiments, the method comprises contacting a host cell suspension with a recombinant AAV (rAAV) particle composition, wherein the host cell suspension has a density of greater than 4,000 cells per microliter (e.g., greater than 4,000 cells per microliter, greater than 5,000 cells per microliter, greater than 6,000 cells per microliter, greater than 7,000 cells per microliter, greater than 8,000 cells per microliter,  
 25 greater than 9,000 cells per microliter, greater than 10,000 cells per microliter, greater than 11,000 cells per microliter, greater than 12,000 cells per microliter, greater than 13,000 cells per microliter, greater than 14,000 cells per microliter, or greater than 15,000 cells per microliter). In some embodiments, the host cell suspension has a density of 4,000 cells per microliter to 15,000 cells per microliter.

30 In some embodiments, a host cell suspension is a culture of cells that are in

suspension (e.g., containing less than 10%, less than 5%, or less than 1% cells that are adhered to a solid substrate). The host cell suspension may contain non-adherent host cells or adherent host cells that have been treated such that they are no longer adherent (e.g., treated with trypsin or another protease or other molecule that disrupts adherence). In some  
5 embodiments, the host cell suspension is a non-adherent host cell suspension. In some embodiments, the non-adherent host cell is a human cell, such as a human stem cell. In some embodiments, the non-adherent host cell is a hematopoietic stem cell. In some embodiments, the host cell is obtained from a subject as described herein (e.g., is a primary cell). In some embodiments, the host cell is obtained from a cell line.

10 In some embodiments, the host cell suspension comprises culture medium, such as serum-free culture medium. Exemplary culture media includes Dulbecco's Modified Eagle Medium (DMEM), RPMI 1640, F10 Nutrient Mixture, Ham's F12 Nutrient Mixture, and Minimum Essential Media, all of which are known in the art and commercially available (see, e.g., products available from Life Technologies).

15 In some embodiments, an rAAV particle composition contacted with a host cell suspension contains  $1 \times 10^2$ - $1 \times 10^6$ ,  $1 \times 10^3$ - $1 \times 10^6$ ,  $1 \times 10^3$ - $1 \times 10^5$ , or  $1 \times 10^3$ - $1 \times 10^4$  vector genomes(vgs)/mL of rAAV particles.

In some embodiments, host cells that have been transduced with an rAAV particle composition, e.g., by a method described herein, are administered to a subject. In some  
20 embodiments, the host cells are obtained from the subject, transduced with the rAAV particle composition, and then administered to the subject.

Other aspects of the disclosure relate to a method of treating a hemoglobinopathy, e.g., by administering host cells produced by a method described herein. In some  
25 embodiments, the method comprises administering an rAAV particle composition described herein to a host cell of a subject (e.g., a human subject) having a hemoglobinopathy (e.g.,  $\beta$ -thalassemia or sickle cell disease) and subsequently administering the host cell to the subject.

Other aspects of the disclosure relate to a method of treating a disease involving blood cells, e.g., by administering host cells produced by a method described herein. In some  
embodiments, the method comprises administering an rAAV particle composition described

herein to a host cell of a subject (e.g., a human subject) having the disease and subsequently administering the host cell to the subject. Exemplary blood cells include T cell, B cells, dendritic cells, macrophages, monocytes, and hematopoietic stem cells. In some embodiments, the disease is a blood cell cancer, e.g., a leukemia (such as Acute lymphocytic leukemia, Acute myelogenous leukemia, Chronic lymphocytic leukemia, or Chronic myelogenous leukemia), lymphoma (such as Hodgkin lymphoma or non-Hodgkin lymphoma), or myeloma (such as multiple myeloma). Other exemplary diseases involving blood cells include anemia, hemophilia, myelodysplastic syndrome, sickle cell disease, thalassemia, deep vein thrombosis, von Willebrand disease, factor II, V, VII, X, or XII deficiency, Polycythemia vera, thrombocytopenia and Idiopathic thrombocytopenic purpura.

Other aspects of the disclosure relate to a method of treating cancer, e.g., by administering host cells produced by a method described herein. In some embodiments, the method comprises administering an rAAV particle composition described herein to a host cell of a subject (e.g., a human subject) having cancer and subsequently administering the host cell to the subject. Exemplary cancers include breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, myeloma, lung cancer and the like.

The rAAV particle, nucleic acid vector, or host cell may be delivered in the form of a composition, such as a composition comprising the active ingredient, such as a rAAV particle, nucleic acid, or host cell described herein, and a therapeutically or pharmaceutically acceptable carrier. The rAAV particles, nucleic acid vectors, or host cells may be prepared in a variety of compositions, and may also be formulated in appropriate pharmaceutical vehicles for administration to human or animal subjects.

The disclosure also provides compositions comprising one or more of the disclosed nucleic acid vectors, rAAV particles, or host cells. As described herein, such compositions may further comprise a pharmaceutical excipient, buffer, or diluent, and may be formulated for administration to an animal, and particularly a human being. Such compositions may further optionally comprise a liposome, a lipid, a lipid complex, a microsphere, a microparticle, a nanosphere, or a nanoparticle, or may be otherwise formulated for

administration to the cells, tissues, organs, or body of a subject in need thereof. Such compositions may be formulated for use in a variety of therapies, such as for example, in the amelioration, prevention, and/or treatment of conditions such as peptide deficiency, polypeptide deficiency, peptide overexpression, polypeptide overexpression, including for  
5 example, conditions which result in diseases or disorders as described herein.

In some embodiments, the number of rAAV particles administered to a cell or a subject may be on the order ranging from  $10^6$  to  $10^{14}$  particles/mL or  $10^3$  to  $10^{15}$  particles/mL, or any values therebetween for either range, such as for example, about  $10^6$ ,  $10^7$ ,  $10^8$ ,  $10^9$ ,  $10^{10}$ ,  $10^{11}$ ,  $10^{12}$ ,  $10^{13}$ , or  $10^{14}$  particles/mL. In one embodiment, rAAV particles of higher  
10 than  $10^{13}$  particles/mL are administered. In some embodiments, the number of rAAV particles administered to a subject may be on the order ranging from  $10^6$  to  $10^{14}$  vector genomes(vgs)/mL or  $10^3$  to  $10^{15}$  vgs/mL, or any values therebetween for either range, such as for example, about  $10^6$ ,  $10^7$ ,  $10^8$ ,  $10^9$ ,  $10^{10}$ ,  $10^{11}$ ,  $10^{12}$ ,  $10^{13}$ , or  $10^{14}$  vgs/mL. In one  
15 embodiment, rAAV particles of higher than  $10^{13}$  vgs/mL are administered. The rAAV particles can be administered as a single dose, or divided into two or more administrations as may be required to achieve therapy of the particular disease or disorder being treated. In some embodiments, 0.0001 mL to 10 mLs are delivered to a subject.

In some embodiments, the disclosure provides formulations of one or more rAAV-based compositions disclosed herein in pharmaceutically acceptable solutions for  
20 administration to a cell or an animal, either alone or in combination with one or more other modalities of therapy, and in particular, for therapy of human cells, tissues, and diseases affecting man.

If desired, rAAV particle or nucleic acid vectors may be administered in combination with other agents as well, such as, e.g., proteins or polypeptides or various pharmaceutically-  
25 active agents, including one or more systemic or topical administrations of therapeutic polypeptides, biologically active fragments, or variants thereof. In fact, there is virtually no limit to other components that may also be included, given that the additional agents do not cause a significant adverse effect upon contact with the target cells or host tissues. The rAAV particles may thus be delivered along with various other agents as required in the

particular instance. Such compositions may be purified from host cells or other biological sources, or alternatively may be chemically synthesized as described herein.

Formulation of pharmaceutically-acceptable excipients and carrier solutions is well-known to those of skill in the art, as is the development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including e.g., oral, parenteral, intravenous, intranasal, intra-articular, and intramuscular administration and formulation.

Typically, these formulations may contain at least about 0.1% of the therapeutic agent (e.g., rAAV particle or host cell) or more, although the percentage of the active ingredient(s) may, of course, be varied and may conveniently be between about 1 or 2% and about 70% or 80% or more of the weight or volume of the total formulation. Naturally, the amount of therapeutic agent(s) (e.g., rAAV particle) in each therapeutically-useful composition may be prepared in such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

In certain circumstances it will be desirable to deliver an rAAV particle or host cell in suitably formulated pharmaceutical compositions disclosed herein either subcutaneously, intraocularly, intravitreally, parenterally, subcutaneously, intravenously, intracerebro-ventricularly, intramuscularly, intrathecally, orally, intraperitoneally, by oral or nasal inhalation, or by direct injection to one or more cells, tissues, or organs by direct injection.

The pharmaceutical forms of the rAAV particle or host cell compositions suitable for injectable use include sterile aqueous solutions or dispersions. In some embodiments, the form is sterile and fluid to the extent that easy syringability exists. In some embodiments, the form is stable under the conditions of manufacture and storage and is preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, saline, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures

thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the rAAV particle or host cell is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum oil such as mineral oil, vegetable oil such as peanut oil, soybean oil, and sesame oil, animal oil, or oil of synthetic origin. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers.

The compositions of the present disclosure can be administered to the subject being treated by standard routes including, but not limited to, pulmonary, intranasal, oral, inhalation, parenteral such as intravenous, topical, transdermal, intradermal, transmucosal, intraperitoneal, intramuscular, intracapsular, intraorbital, intravitreal, intracardiac, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection.

For administration of an injectable aqueous solution, for example, the solution may be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, intravitreal, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, and the general safety and purity standards as required by, e.g., FDA Office of Biologics standards.

Sterile injectable solutions are prepared by incorporating the rAAV particles or host

cells in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those  
5 enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Ex vivo delivery of cells (e.g., host cells) transduced with rAAV particles is also  
10 contemplated herein. Ex vivo gene delivery may be used to transplant rAAV-transduced host cells back into the host. A suitable ex vivo protocol may include several steps. For example, a segment of target tissue or an aliquot of target fluid may be harvested from the host and rAAV particles may be used to transduce a nucleic acid vector into the host cells in the tissue or fluid. These genetically modified cells may then be transplanted back into the host. Several  
15 approaches may be used for the reintroduction of cells into the host, including intravenous injection, intraperitoneal injection, or in situ injection into target tissue. Autologous and allogeneic cell transplantation may be used according to the disclosure.

The amount of rAAV particle, nucleic acid vector, or host cell compositions and time of administration of such compositions will be within the purview of the skilled artisan  
20 having benefit of the present teachings. It is likely, however, that the administration of therapeutically-effective amounts of the disclosed compositions may be achieved by a single administration, such as for example, a single injection of sufficient numbers of infectious particles to provide therapeutic benefit to the patient undergoing such treatment.

Alternatively, in some circumstances, it may be desirable to provide multiple, or successive  
25 administrations of the rAAV particle or host cell compositions, either over a relatively short, or a relatively prolonged period of time, as may be determined by the medical practitioner overseeing the administration of such compositions.

The composition may include rAAV particles or host cells, either alone, or in combination with one or more additional active ingredients, which may be obtained from

natural or recombinant sources or chemically synthesized. In some embodiments, rAAV particles are administered in combination, either in the same composition or administered as part of the same treatment regimen, with a proteasome inhibitor, such as Bortezomib, or hydroxyurea.

5 To "treat" a disease as the term is used herein, means to reduce the frequency or severity of at least one sign or symptom of a disease or disorder experienced by a subject. The compositions described above are typically administered to a subject in an effective amount, that is, an amount capable of producing a desirable result. The desirable result will depend upon the active agent being administered. For example, an effective amount of a  
10 rAAV particle may be an amount of the particle that is capable of transferring a heterologous nucleic acid to a host organ, tissue, or cell.

Toxicity and efficacy of the compositions utilized in methods of the disclosure can be determined by standard pharmaceutical procedures, using either cells in culture or experimental animals to determine the LD50 (the dose lethal to 50% of the population). The  
15 dose ratio between toxicity and efficacy the therapeutic index and it can be expressed as the ratio LD50/ED50. Those compositions that exhibit large therapeutic indices are preferred. While those that exhibit toxic side effects may be used, care should be taken to design a delivery system that minimizes the potential damage of such side effects. The dosage of compositions as described herein lies generally within a range that includes an ED50 with  
20 little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

### **Recombinant AAV (rAAV) Particles and Nucleic Acid Vectors**

Aspects of the disclosure relate to recombinant adeno-associated virus (rAAV)  
25 particles for delivery of one or more nucleic acid vectors comprising a sequence encoding a protein or polypeptide of interest into various tissues, organs, and/or cells. In some embodiments, the rAAV particles comprise a rAAV capsid protein as described herein, e.g., comprising one or more amino acid substitutions in a surface-exposed binding loop.

The wild-type AAV genome is a single-stranded deoxyribonucleic acid (ssDNA), either positive- or negative-sensed. The genome comprises two inverted terminal repeats (ITRs), one at each end of the DNA strand, and two open reading frames (ORFs): *rep* and *cap* between the ITRs. The *rep* ORF comprises four overlapping genes encoding Rep proteins required for the AAV life cycle. The *cap* ORF comprises overlapping genes encoding capsid proteins: VP1, VP2 and VP3, which interact together to form the viral capsid. VP1, VP2 and VP3 are translated from one mRNA transcript, which can be spliced in two different manners: either a longer or shorter intron can be excised resulting in the formation of two isoforms of mRNAs: a ~2.3 kb- and a ~2.6 kb-long mRNA isoform. The capsid forms a supramolecular assembly of approximately 60 individual capsid protein subunits into a non-enveloped, T-1 icosahedral lattice capable of protecting the AAV genome. The mature capsid is composed of VP1, VP2, and VP3 (molecular masses of approximately 87, 73, and 62 kDa respectively) in a ratio of about 1:1:10.

Recombinant AAV (rAAV) particles may comprise a nucleic acid vector, which may comprise at a minimum (a) one or more heterologous nucleic acid regions comprising a sequence encoding a protein or polypeptide of interest (e.g., a globin gene) or an RNA of interest (e.g., a siRNA or microRNA) and (b) one or more regions comprising inverted terminal repeat (ITR) sequences (e.g., wild-type ITR sequences or engineered ITR sequences) flanking the one or more heterologous nucleic acid regions. In some embodiments, the nucleic acid vector is between 4kb and 5kb in size (e.g., 4.2 to 4.7 kb in size). This nucleic acid vector may be encapsidated by a viral capsid, such as an AAV2 or AAV6 capsid, which may comprise a modified capsid protein as described herein. In some embodiments, the nucleic acid vector is circular. In some embodiments, the nucleic acid vector is single-stranded. In some embodiments, the nucleic acid vector is double-stranded. In some embodiments, a double-stranded nucleic acid vector may be, for example, a self-complimentary vector that contains a region of the nucleic acid vector that is complementary to another region of the nucleic acid vector, initiating the formation of the double-strandedness of the nucleic acid vector.

Accordingly, in some embodiments, an rAAV particle comprises a viral capsid and a

nucleic acid vector as described herein, which is encapsidated by the viral capsid. In some embodiments, the nucleic acid vector comprises (1) one or more heterologous nucleic acid regions comprising a sequence encoding a protein or polypeptide of interest (e.g., a globin gene), (2) one or more nucleic acid regions comprising a sequence that facilitates expression of the heterologous nucleic acid region (e.g., a parvovirus B19p6 promoter), and (3) one or more nucleic acid regions comprising a sequence that facilitate integration of the heterologous nucleic acid region (optionally with the one or more nucleic acid regions comprising a sequence that facilitates expression) into the genome of the subject. In some embodiments, viral sequences that facilitate integration comprise Inverted Terminal Repeat (ITR) sequences. In some embodiments, the nucleic acid vector comprises one or more heterologous nucleic acid regions comprising a sequence encoding a protein or polypeptide of interest operably linked to a promoter, wherein the one or more heterologous nucleic acid regions are flanked on each side with an ITR sequence. The ITR sequences can be derived from any AAV serotype (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) or can be derived from more than one serotype. In some embodiments, the ITR sequences are derived from AAV2 or AAV6. ITR sequences and plasmids containing ITR sequences are known in the art and commercially available (see, e.g., products and services available from Vector Biolabs, Philadelphia, PA; Cellbiolabs, San Diego, CA; Agilent Technologies, Santa Clara, Ca; and Addgene, Cambridge, MA; and Gene delivery to skeletal muscle results in sustained expression and systemic delivery of a therapeutic protein. Kessler PD, Podsakoff GM, Chen X, McQuiston SA, Colosi PC, Matelis LA, Kurtzman GJ, Byrne BJ. Proc Natl Acad Sci U S A. 1996 Nov 26;93(24):14082-7; and Curtis A. Machida. Methods in Molecular Medicine™. Viral Vectors for Gene TherapyMethods and Protocols. 10.1385/1-59259-304-6:201 © Humana Press Inc. 2003. Chapter 10. Targeted Integration by Adeno-Associated Virus. Matthew D. Weitzman, Samuel M. Young Jr., Toni Cathomen and Richard Jude Samulski; U.S. Pat. Nos. 5,139,941 and 5,962,313, all of which are incorporated herein by reference).

In some embodiments, the nucleic acid vector comprises a pTR-UF-11 plasmid backbone, which is a plasmid that contains AAV2 ITRs. This plasmid is commercially available from the American Type Culture Collection (ATCC MBA-331).

Exemplary ITR sequences for AAV2, AAV3, AAV5, and AAV6 are provided below.

**AAV2:**

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCC  
 5 CGACGCCCGGGCTTTGCCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGT  
 GGCCAACTCCATCACTAGGGGTTTCCT (SEQ ID NO: 3)

**AAV3:**

TTGGCCACTCCCTCTATGCGCACTCGCTCGCTCGGTGGGGCCTGGCGACCAAAGGTCGCC  
 10 AGACGGACGTGCTTTGCACGTCCGGCCCCACCGAGCGAGCGAGTGCGCATAGAGGGAGT  
 GGCCAACTCCATCACTAGAGGTATGGC (SEQ ID NO: 4)

**AAV5:**

CTCTCCCCCCTGTGCGGTTTCGCTCGCTCGCTGGCTCGTTTGGGGGGGTGGCAGCTCAAAG  
 15 AGCTGCCAGACGACGGCCCTCTGGCCGTCGCCCCCCCCAAACGAGCCAGCGAGCGAGCGA  
 ACGCGACAGGGGGGAGAGTGCCACACTCTCAAGCAAGGGGGGTTTTGTA (SEQ ID NO: 5)

**AAV6:**

TTGCCCACTCCCTCTATGCGCGCTCGCTCGCTCGGTGGGGCCTGCGGACCAAAGGTCCGC  
 20 AGACGGCAGAGCTCTGCTCTGCCGGCCCCACCGAGCGAGCGAGCGCGCATAGAGGGAGT  
 GGGCAACTCCATCACTAGGGGTA (SEQ ID NO: 6)

In some embodiments, the nucleic acid vector comprises one or more regions comprising a sequence that facilitates expression of the heterologous nucleic acid, e.g.,  
 25 expression control sequences operatively linked to the heterologous nucleic acid. Numerous such sequences are known in the art. Non-limiting examples of expression control sequences include promoters, insulators, silencers, response elements, introns, enhancers, initiation sites, termination signals, and poly(A) tails. Any combination of such control sequences is contemplated herein (e.g., a promoter and an enhancer).

To achieve appropriate expression levels of the protein or polypeptide of interest, any

of a number of promoters suitable for use in the selected host cell may be employed. In some embodiments, the promoter is a parvovirus B19p6 promoter. An exemplary sequence of the parvovirus B19p6 promoter is provided below:

```

5      1 CCAACCCTAA TTCCGGAAGT CCCGCCCACC GGAAGTGACG TCACAGGAAA TGACGTCACA
      61 GGAAATGACG TAATTGTCCG CCATCTTGTA CCGGAAGTCC CGCCTACCGG CGGCGACCGG
      121 CGGCATCTGA TTTGGTGTCT TCTTTTAAAT TTTAGCGGGC TTTTTCCTCG CCTTATGCAA
      181 ATGGGCAGCC ATTTTAAGTG TTTTACTATA ATTTTATTGG TTAGTTTTGT AACGGTTAAA
      241 ATGGGCGGAG CGTAGGCGGG GACTACAGTA TATATAGCAC GGCCTGCGG CAGCTCTTTC
10    301 TTTCTGGGCT GCTTTTTCCT GGACTTCTT GCTGTTTTTT GTGAGCTAAC TAACAGGTAT
      361 TTATACTACT TGTTAACATA CTAA (SEQ ID NO: 7)

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The promoter may be, for example, a constitutive promoter, tissue-specific promoter, inducible promoter, or a synthetic promoter. For example, constitutive promoters of different strengths can be used. A nucleic acid vector described herein may include one or more

15 constitutive promoters, such as viral promoters or promoters from mammalian genes that are generally active in promoting transcription. Non-limiting examples of constitutive viral promoters include the Herpes Simplex virus (HSV), thymidine kinase (TK), Rous Sarcoma Virus (RSV), Simian Virus 40 (SV40), Mouse Mammary Tumor Virus (MMTV), Ad E1A

20 and cytomegalovirus (CMV) promoters. Non-limiting examples of constitutive mammalian promoters include various housekeeping gene promoters, as exemplified by the  $\beta$ -actin promoter.

Inducible promoters and/or regulatory elements may also be contemplated for achieving appropriate expression levels of the protein or polypeptide of interest. Non-

25 limiting examples of suitable inducible promoters include those from genes such as cytochrome P450 genes, heat shock protein genes, metallothionein genes, and hormone-inducible genes, such as the estrogen gene promoter. Another example of an inducible promoter is the tetVP16 promoter that is responsive to tetracycline.

Tissue-specific promoters and/or regulatory elements are also contemplated herein.

30 Non-limiting examples of such promoters that may be used include the parvovirus B19p6

promoter, promoters that are myeloid and erythroid cell-specific, dendritic cell-specific, macrophage- and monocyte-specific, T- and B-lymphocyte-specific, specific for hematopoietic stem or progenitor cells, dendritic cells, macrophages or monocytes.

Synthetic promoters are also contemplated herein. A synthetic promoter may  
 5 comprise, for example, regions of known promoters, regulatory elements, transcription factor binding sites, enhancer elements, repressor elements, and the like.

In some embodiments, a nucleic acid vector described herein may also contain marker or reporter genes, e.g., LacZ or a fluorescent protein.

In some embodiments, the nucleic acid vector comprises one or more heterologous  
 10 nucleic acid regions comprising a sequence encoding a protein or polypeptide of interest, such as a globin gene. Exemplary globin genes include, but are not limited to, a  $\beta$ -globin gene (e.g., a human  $\beta$ -globin gene), an anti-sickling  $\beta$ -globin gene (e.g., a human anti-sickling  $\beta$ -globin gene), and a  $\gamma$ -globin gene (e.g., a human  $\gamma$ -globin gene). Exemplary nucleic acid and protein sequences for each globin gene mentioned above are provided below.

15 Human  $\beta$ -globin protein:

MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  
 VKAHGKKVLGAFSDGLAHLNKLKGTFA TLSELHCDKLHVDPENFRLLGNVLVCVLAHHFG  
 KEFTPPVQAAYQKVVAGVANALAHKYH (SEQ ID NO: 8)

20 Human  $\gamma$ -globin protein:

MGHFTEEDKATITSLWGKVNVEDAGGETLGRLLVVYPWTQRFFDSFGNLSSASAIM  
 GNPVKVKAHGKKVLTSLGDAIKHLDDLKGTFAQLSELHCDKLHVDPENFKLLGNVLVTVLAI  
 HFGKEFTPEVQASWQKMVTGVASALSSRYH (SEQ ID NO: 9)

25 Human anti-sickling  $\beta$ -globin gene nucleic acid sequence:

#### **Exon 1**

ATGGTGCACCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAA  
 CGTGGATGAAGTTGGTGGTGAGGCCCTGGGCA (SEQ ID NO: 10)

#### **Intron 1**

GGTTGGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCATGTGGA  
GACAGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCTCTCTGCCTATTGGTCTATTTT  
CCCACCCTTA (SEQ ID NO: 11)

### Exon 2

5 GGCTGCTGGTGGTCTACCCTTGGACCCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCA  
CTCCTGATGCTGTTATGGGCAACCCTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGT  
GCCTTTAGTGATGGCCTGGCTCACCTGGACAACCTCAAGGGCACCTTTGCC\*CAG\*CTGA  
GTGAGCTGCACTGTGACAAGCTGCACGTGGATCCTGAGAACTTCAGG (SEQ ID NO: 12)

### Delta 12 Intron (372 bp-deletion in Intron 2)

10 GTGAGTCTATGGGACCCTTGATGTTTTCTTTCCCCTTCTTTTCTATGGTTAAGTTCATGTCA  
TAGGAAGGGGAGAAGTAACAGGGTACACATATTGACCAAATCAGGGTAATTTTGCATTT  
GTAATTTTAAAAAATGCTTTCTTCTTTTAATATACTTTTTTGTATTCTTATTTCTAATACT  
TTCCCTAATCTCTTTCTTTTCAAGGGCAATAATGATACAATGTATCATGCCTCTTTGCACCAT  
TCTAAAGAATAACAGTGATAATTTCTGGGTAAAGGCAATAGCAATATTTCTGCATATAAA  
15 TATTTCTGCATATAAATTGTAAGTATGTAAGAGGTTTCATATTGCTAATAGCAGCTACA  
ATCCAGCTACCATTTCTGCTTTTATTTTATGGTTGGGATAAGGCTGGATTATTCTGAGTCCA  
AGCTAGGCCCTTTTGCTAATCATGTTTCATACCTCTTATCTTCCTCCCACAG (SEQ ID NO:  
13)

### Exon 3

20 CTCCTGGGCAACGTGCTGGTCTGTGTGCTGGCCCATCACTTTGGCAAAGAATTCACCCCA  
CCAGTGCAGGCTGCCTATCAGAAAGTGGTGGCTGGTGTGGCTAATGCCCTGGCCCACAA  
GTATCACTAA (SEQ ID NO: 14)

\*CAG\* = T87Q

25 In some embodiments, the sequence encoding the globin gene is provided with  
introns. In some embodiments, the sequence encoding the globin gene is provided without  
introns.

Human anti-sickling  $\beta$ -globin gene protein sequence:

30 MVHLTPEEKSAVTALWGKVNVDDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDVAMGNPK  
VKAHGKKVLGAFSDGLAHLNLTGKFAQLSELHCDKLHVDPENFRLLGNVLVCVLAHHFG

KEFTPPVQAAYQKVVAGVANALAHKYH (SEQ ID NO: 15)

The protein or polypeptide of interest may be, e.g., a polypeptide or protein of interest provided in Table 1. The sequences of the polypeptide or protein of interest may be obtained, e.g., using the non-limiting National Center for Biotechnology Information (NCBI) Protein IDs or SEQ ID NOs from patent applications provided in Table 1

Table 1. Non-limiting examples of proteins or polypeptides of interest and associated diseases

| <b>Protein or Polypeptide</b>                                        | <b>Non-limiting Exemplary diseases</b> | <b>Non-limiting NCBI Protein IDs or Patent SEQ ID NOs</b>                                                   |
|----------------------------------------------------------------------|----------------------------------------|-------------------------------------------------------------------------------------------------------------|
| acid alpha-glucosidase (GAA)                                         | Pompe                                  | NP_000143.2, NP_001073271.1, NP_001073272.1                                                                 |
| Methyl CpG binding protein 2 (MECP2)                                 | Rett syndrome                          | NP_001104262.1, NP_004983.1                                                                                 |
| Aromatic L-amino acid decarboxylase (AADC)                           | Parkinson's disease                    | NP_000781.1, NP_001076440.1, NP_001229815.1, NP_001229816.1, NP_001229817.1, NP_001229818.1, NP_001229819.1 |
| Glial cell-derived neurotrophic factor (GDNF)                        | Parkinson's disease                    | NP_000505.1, NP_001177397.1, NP_001177398.1, NP_001265027.1, NP_954701.1                                    |
| Cystic fibrosis transmembrane conductance regulator (CFTR)           | Cystic fibrosis                        | NP_000483.3                                                                                                 |
| Tumor necrosis factor receptor fused to an antibody Fc (TNFR:Fc)     | Arthritis, Rheumatoid arthritis        | SEQ ID NO. 1 of WO2013025079                                                                                |
| HIV-1 gag-pro $\Delta$ rt (tgAAC09)                                  | HIV infection                          | SEQ ID NOs. 1-5 of WO2006073496                                                                             |
| Sarcoglycan alpha, beta, gamma, delta, epsilon, or zeta (SGCA, SGCB, | Muscular dystrophy                     | SGCA NP_000014.1,                                                                                           |

|                                                           |                                                        |                                                                                                                                                                                                                                |
|-----------------------------------------------------------|--------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| SGCG, SGCD, SGCE, or SGCZ)                                |                                                        | NP_001129169.1<br><br>SGCB<br>NP_000223.1<br><br>SGCG<br>NP_000222.1<br><br>SGCD<br>NP_000328.2,<br>NP_001121681.1,<br>NP_758447.1<br><br>SGCE<br>NP_001092870.1,<br>NP_001092871.1,<br>NP_003910.1<br><br>SGCZ<br>NP_631906.2 |
| Alpha-1-antitrypsin (AAT)                                 | Hereditary emphysema or Alpha-1-antitrypsin deficiency | NP_000286.3,<br>NP_001002235.1,<br>NP_001002236.1,<br>NP_001121172.1,<br>NP_001121173.1,<br>NP_001121174.1,<br>NP_001121175.1,<br>NP_001121176.1,<br>NP_001121177.1,<br>NP_001121178.1,<br>NP_001121179.1                      |
| Glutamate decarboxylase 1(GAD1)                           | Parkinson's disease                                    | NP_000808.2,<br>NP_038473.2                                                                                                                                                                                                    |
| Glutamate decarboxylase 2 (GAD2)                          | Parkinson's disease                                    | NP_000809.1,<br>NP_001127838.1                                                                                                                                                                                                 |
| Aspartoacylase (ASPA)                                     | Canavan's disease                                      | NP_000040.1,<br>NP_001121557.1                                                                                                                                                                                                 |
| Nerve growth factor (NGF)                                 | Alzheimer's disease                                    | NP_002497.2                                                                                                                                                                                                                    |
| Granulocyte-macrophage colonystimulating factory (GM-CSF) | Prostate cancer                                        | NP_000749.2                                                                                                                                                                                                                    |
| Cluster of Differentiation 86 (CD86 or B7-2)              | Malignant melanoma                                     | NP_001193853.1,<br>NP_001193854.1,                                                                                                                                                                                             |

|                                                                   |                                                                  |                                                                                                                                                                                                                                                                             |
|-------------------------------------------------------------------|------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                   |                                                                  | NP_008820.3,<br>NP_787058.4,<br>NP_795711.1                                                                                                                                                                                                                                 |
| Interleukin 12 (IL-12)                                            | Malignant melanoma                                               | NP_000873.2,<br>NP_002178.2                                                                                                                                                                                                                                                 |
| neuropeptide Y (NPY)                                              | Parkinson's disease, epilepsy                                    | NP_000896.1                                                                                                                                                                                                                                                                 |
| ATPase, Ca++ transporting, cardiac muscle, slow twitch 2 (SERCA2) | Chronic heart failure                                            | NP_001672.1,<br>NP_733765.1                                                                                                                                                                                                                                                 |
| Dystrophin or Minidystrophin                                      | Muscular dystrophy                                               | NP_000100.2,<br>NP_003997.1,<br>NP_004000.1,<br>NP_004001.1,<br>NP_004002.2,<br>NP_004003.1,<br>NP_004004.1,<br>NP_004005.1,<br>NP_004006.1,<br>NP_004007.1,<br>NP_004008.1,<br>NP_004009.1,<br>NP_004010.1,<br>NP_004011.2,<br>NP_004012.1,<br>NP_004013.1,<br>NP_004014.1 |
| Ceroid lipofuscinosis neuronal 2 (CLN2)                           | Late infantile neuronal ceroidlipofuscinosis or Batten's disease | NP_000382.3                                                                                                                                                                                                                                                                 |
| Neurturin (NRTN)                                                  | Parkinson's disease                                              | NP_004549.1                                                                                                                                                                                                                                                                 |
| N-acetylglucosaminidase, alpha (NAGLU)                            | Sanfilippo syndrome (MPSIIIB)                                    | NP_000254.2                                                                                                                                                                                                                                                                 |
| Iduronidase, alpha-1 (IDUA)                                       | MPSI-Hurler                                                      | NP_000194.2                                                                                                                                                                                                                                                                 |
| Iduronate 2-sulfatase (IDS)                                       | MPSII-Hunter                                                     | NP_000193.1,<br>NP_001160022.1,<br>NP_006114.1                                                                                                                                                                                                                              |
| Glucuronidase, beta (GUSB)                                        | MPSVII-Sly                                                       | NP_000172.2,<br>NP_001271219.1                                                                                                                                                                                                                                              |
| Hexosaminidase A, $\alpha$ polypeptide (HEXA)                     | Tay-Sachs                                                        | NP_000511.2                                                                                                                                                                                                                                                                 |
| Retinal pigment epithelium-specific protein 65kDa (RPE65)         | Leber congenital amaurosis                                       | NP_000320.1                                                                                                                                                                                                                                                                 |
| Factor IX (FIX)                                                   | Hemophilia B                                                     | NP_000124.1                                                                                                                                                                                                                                                                 |
| Adenine nucleotide translocator                                   | progressive external                                             | NP_001142.2                                                                                                                                                                                                                                                                 |

|                                                                                                                                                                  |                                                                                                                                                                     |                                                                             |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| (ANT-1)                                                                                                                                                          | ophthalmoplegia                                                                                                                                                     |                                                                             |
| ApaLI                                                                                                                                                            | mitochondrial heteroplasmy, myoclonic epilepsy with ragged red fibers (MERRF) or mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) | YP_007161330.1                                                              |
| NADH ubiquinone oxidoreductase subunit 4 (ND4)                                                                                                                   | Leber hereditary optic                                                                                                                                              | YP_003024035.1                                                              |
| very long-acyl-CoA dehydrogenase (VLCAD)                                                                                                                         | very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency                                                                                                           | NP_000009.1, NP_001029031.1, NP_001257376.1, NP_001257377.1                 |
| short-chain acyl-CoA dehydrogenase (SCAD)                                                                                                                        | short-chain acyl-CoA dehydrogenase (SCAD) deficiency                                                                                                                | NP_000008.1                                                                 |
| medium-chain acyl-CoA dehydrogenase (MCAD)                                                                                                                       | medium-chain acyl-CoA dehydrogenase (MCAD) deficiency                                                                                                               | NP_000007.1, NP_001120800.1, NP_001272971.1, NP_001272972.1, NP_001272973.1 |
| Myotubularin 1 (MTM1)                                                                                                                                            | X-linked myotubular myopathy                                                                                                                                        | NP_000243.1                                                                 |
| Myophosphorylase (PYGM)                                                                                                                                          | McArdle disease (glycogen storage disease type V, myophosphorylase deficiency)                                                                                      | NP_001158188.1, NP_005600.1                                                 |
| Lipoprotein lipase (LPL)                                                                                                                                         | LPL deficiency                                                                                                                                                      | NP_000228.1                                                                 |
| sFLT01 (VEGF/PIGF (placental growth factor) binding domain of human VEGFR1/Flt-1 (hVEGFR1) fused to the Fc portion of human IgG(1) through a polyglycine linker) | Age-related macular degeneration                                                                                                                                    | SEQ ID NO: 2, 8, 21, 23, or 25 of WO2009105669                              |
| Glucocerebrosidase (GC)                                                                                                                                          | Gaucher disease                                                                                                                                                     | NP_000148.2, NP_001005741.1, NP_001005742.1, NP_001165282.1, NP_001165283.1 |
| UDP glucuronosyltransferase 1 family, polypeptide A1 (UGT1A1)                                                                                                    | Crigler–Najjar syndrome                                                                                                                                             | NP_000454.1                                                                 |
| Glucose 6-phosphatase (G6Pase)                                                                                                                                   | GSD-Ia                                                                                                                                                              | NP_000142.2, NP_001257326.1                                                 |
| Ornithine carbamoyltransferase (OTC)                                                                                                                             | OTC deficiency                                                                                                                                                      | NP_000522.3                                                                 |

|                                                     |                                                |                                                                                                                                             |
|-----------------------------------------------------|------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| Cystathionine-beta-synthase (CBS)                   | Homocystinuria                                 | NP_000062.1,<br>NP_001171479.1,<br>NP_001171480.1                                                                                           |
| Factor VIII (F8)                                    | Haemophilia A                                  | NP_000123.1,<br>NP_063916.1                                                                                                                 |
| Hemochromatosis (HFE)                               | Hemochromatosis                                | NP_000401.1,<br>NP_620572.1,<br>NP_620573.1,<br>NP_620575.1,<br>NP_620576.1,<br>NP_620577.1,<br>NP_620578.1,<br>NP_620579.1,<br>NP_620580.1 |
| Low density lipoprotein receptor (LDLR)             | Phenylketonuria (PKU)                          | NP_000518.1,<br>NP_001182727.1,<br>NP_001182728.1,<br>NP_001182729.1,<br>NP_001182732.1                                                     |
| Galactosidase, alpha (AGA)                          | Fabry disease                                  | NP_000160.1                                                                                                                                 |
| Phenylalanine hydroxylase (PAH)                     | Hypercholesterolaemia or Phenylketonuria (PKU) | NP_000268.1                                                                                                                                 |
| Propionyl CoA carboxylase, alpha polypeptide (PCCA) | Propionic acidaemias                           | NP_000273.2,<br>NP_001121164.1,<br>NP_001171475.1                                                                                           |

Other exemplary polypeptides or proteins of interest include adrenergic agonists, anti-apoptosis factors, apoptosis inhibitors, cytokine receptors, cytokines, cytotoxins, erythropoietic agents, glutamic acid decarboxylases, glycoproteins, growth factors, growth factor receptors, hormones, hormone receptors, interferons, interleukins, interleukin receptors, kinases, kinase inhibitors, nerve growth factors, netrins, neuroactive peptides, neuroactive peptide receptors, neurogenic factors, neurogenic factor receptors, neuropilins, neurotrophic factors, neurotrophins, neurotrophin receptors, N-methyl-D-aspartate antagonists, plexins, proteases, protease inhibitors, protein decarboxylases, protein kinases, protein kinase inhibitors, proteolytic proteins, proteolytic protein inhibitors, semaphorin a semaphorin receptors, serotonin transport proteins, serotonin uptake inhibitors, serotonin receptors, serpins, serpin receptors, and tumor suppressors. In some embodiments, the

polypeptide or protein of interest is a human protein or polypeptide.

The rAAV particle may be of any AAV serotype, including any derivative or pseudotype (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 2/1, 2/5, 2/8, or 2/9). As used herein, the serotype of an rAAV viral vector (e.g., an rAAV particle) refers to the serotype of the capsid proteins of the recombinant virus. In some embodiments, the rAAV particle is not AAV2. In some embodiments, the rAAV particle is AAV2. In some embodiments, the rAAV particle is AAV6. In some embodiments, the rAAV particle is an AAV6 serotype comprising an rAAV capsid protein as described herein. Non-limiting examples of derivatives and pseudotypes include rAAV2/1, rAAV2/5, rAAV2/8, rAAV2/9, AAV2-AAV3 hybrid, AAVrh.10, AAVhu.14, AAV3a/3b, AAVrh32.33, AAV-HSC15, AAV-HSC17, AAVhu.37, AAVrh.8, CHt-P6, AAV2.5, AAV6.2, AAV2i8, AAV-HSC15/17, AAVM41, AAV9.45, AAV6(Y445F/Y731F), AAV2.5T, AAV-HAE1/2, AAV clone 32/83, AAVShH10, AAV2 (Y->F), AAV8 (Y733F), AAV2.15, AAV2.4, AAVM41, and AAVr3.45. Such AAV serotypes and derivatives/pseudotypes, and methods of producing such derivatives/pseudotypes are known in the art (see, e.g., Mol Ther. 2012 Apr;20(4):699-708. doi: 10.1038/mt.2011.287. Epub 2012 Jan 24. The AAV vector toolkit: poised at the clinical crossroads. Asokan A1, Schaffer DV, Samulski RJ.). In some embodiments, the rAAV particle is a pseudotyped rAAV particle, which comprises (a) a nucleic acid vector comprising ITRs from one serotype (e.g., AAV2) and (b) a capsid comprised of capsid proteins derived from another serotype (e.g., AAV1, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, or AAV10). Methods for producing and using pseudotyped rAAV vectors are known in the art (see, e.g., Duan et al., J. Virol., 75:7662-7671, 2001; Halbert et al., J. Virol., 74:1524-1532, 2000; Zolotukhin et al., Methods, 28:158-167, 2002; and Auricchio et al., Hum. Molec. Genet., 10:3075-3081, 2001).

In some embodiments, the rAAV particle comprises a capsid that includes modified capsid proteins (e.g., capsid proteins comprising a modified VP3 region and/or one or more amino acid substitutions in a surface exposed loop, such as by replacing loop VIII with a B19 P antigen binding site) optionally further modified to replace one or more surface exposed tyrosine, lysine, serine, or threonine residues (e.g., in a VP3 region of a capsid protein, see,

e.g., U.S. Patent Publication Number US20130310443, which is incorporated herein by reference in its entirety). In some embodiments, the rAAV particle comprises a modified capsid protein comprising a non-tyrosine residue (e.g., a phenylalanine) at a position that corresponds to a surface-exposed tyrosine residue in a wild-type capsid protein, a non-threonine residue (e.g., a valine) at a position that corresponds to a surface-exposed threonine residue in the wild-type capsid protein, a non-lysine residue (e.g., a glutamic acid) at a position that corresponds to a surface-exposed lysine residue in the wild-type capsid protein, a non-serine residue (e.g., valine) at a position that corresponds to a surface-exposed serine residue in the wild-type capsid protein, or a combination thereof. Exemplary surface-exposed lysine residues include positions that correspond to K258, K321, K459, K490, K507, K527, K572, K532, K544, K549, K556, K649, K655, K665, or K706 of the wild-type AAV2 capsid protein. Exemplary surface-exposed serine residues include positions that correspond to S261, S264, S267, S276, S384, S458, S468, S492, S498, S578, S658, S662, S668, S707, or S721 of the wild-type AAV2 capsid protein. Exemplary surface-exposed threonine residues include positions that correspond to T251, T329, T330, T454, T455, T503, T550, T592, T581, T597, T491, T671, T659, T660, T701, T713, or T716 of the wild-type AAV2 capsid protein. Exemplary surface-exposed tyrosine residues include positions that correspond to Y252, Y272, Y444, Y500, Y700, Y704, or Y730 of the wild-type AAV2 capsid protein.

Exemplary, non-limiting wild-type capsid protein sequences are provided below.

#### Exemplary AAV1 capsid protein

```

1  MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD DGRGLVLPGY
51  KYLGPFENGLD KGEPVNAADA AALEHDKAYD QQLKAGDNPY LRYNHADA EF
101 QERLQEDTSF GGNLGRAVFQ AKKRVLEPLG LVEEGAKTAP GKKRPVEQSP
25  151 QEPDSSSGIG KTGQQPAKKR LNFGQTGDSE SVPDPQPLGE PPATPAAVGP
201 TTMASGGGAP MADNNEGADG VGNASGNWHC DSTWLGDRVI TTSTRTWALP
251 TYNNHLYKQI SSASTGASND NHYFGYSTPW GYFDFNRFHC HFSPRDWQRL
301 INNNWGFRPK RLNFKLFNIQ VKEVTTNDGV TTIANNLTST VQVFSDSEYQ
351 LPYVLGSAHQ GCLPPFPADV FMIPQYGYLT LNNGSQAVGR SSFYCLEYFP
30  401 SQMLRTGNNF TFSYTFEEVP FHSSYAHSQS LDRLMNPLID QYLYYLNRTQ

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451 NQSGSAQNKD LLFSRGSPAG MSVQPKNWLP GPCYRQQRVS KTKTDNNNSN  
 501 FTWTGASKYN LNGRESIINP GTAMASHKDD EDKFFPMSGV MIFGKESAGA  
 551 SNTALDNVMI TDEEEIKATN PVATERFGTV AVNFQSSSTD PATGDVHAMG  
 601 ALPGMVWQDR DVYLQGPIWA KIPHTDGHFH PSPLMGGFGL KNPPPQILIK  
 5 651 NTPVPANPPA EFSATKFASF ITQYSTGQVS VEIEWELQKE NSKRWNPEVQ  
 701 YTSNYAKSAN VDFTVDNNGL YTEPRPIGTR YLTRPL\* (SEQ ID NO: 16)

#### Exemplary AAV2 capsid protein

1 MAADGYLPDW LEDTLSEGIR QWWKLKPGPP PPKPAERHKD DSRGLVLPGY  
 10 51 KYLGPFNGLD KGEPVNEADA AALEHDKAYD RQLDSGDNPY LKYNHADA EF  
 101 QERLKEDTSF GGNLGRAVFQ AKKRVLEPLG LVEEPVKTAP GKKRPVEHSP  
 151 VEPDSSSGTG KAGQQPARKR LNFGQTGDAD SVPDPQPLGQ PPAAPSGLGT  
 201 NTMATGSGAP MADNNEGADG VGNSSGNWHC DSTWMGDRVI TTSTRTWALP  
 251 TYNNHLYKQI SSQSGASNDN HYFGYSTPWG YFDFNRFHCH FSPRDWQRLI  
 15 301 NNNWGFPRPKR LNFKLFNIQV KEVTQNDGTT TIANNLTSTV QVFTDSEYQL  
 351 PYVLGSAHQG CLPPFPADV F MVPQYGYLTL NNGSQAVGRS SFYCLEYFPS  
 401 QMLRTGNFT FSYTFEDVPF HSSYAHSQSL DRLMNPLIDQ YLYYLSRTNT  
 451 PSGTTTQSRL QFSQAGASDI RDQSRNWLPG PCYRQQRVSK TSADNNNSEY  
 501 SWTGATKYHL NGRDSL VNPG PAMASHKDDE EKFFPQSGVL IFGKQGSEKT  
 20 551 NVDIEKVMIT DEEEIRTTNP VATEQYGSVS TNLQRGNRQA ATADVNTQGV  
 601 LPGMVWQDRD VYLQGPIWAK IPHTDGHFHP SPLMGGFGLK HPPPQILIKN  
 651 TPVPANPSTT FSAAKFASFI TQYSTGQVSV EIEWELQKEN SKRWNPEIQY  
 701 TSNYNKSVNV DFTVDTNGVY SEPRPIGTRY LTRNL\* (SEQ ID NO: 17)

#### Exemplary AAV3 capsid protein

1 MAADGYLPDW LEDNLSEGIR EWWALKPGVP QPKANQQHQD NRRGLVLPGY  
 51 KYLGPGNGLD KGEPVNEADA AALEHDKAYD QQLKAGDNPY LKYNHADA EF  
 101 QERLQEDTSF GGNLGRAVFQ AKKRILEPLG LVEEAAKTAP GKKGAVDQSP  
 151 QEPDSSSGVG KSGKQPARKR LNFGQTGDSE SVPDPQPLGE PPAAPTSLGS  
 30 201 NTMASGGGAP MADNNEGADG VGNSSGNWHC DSQWLGDRI TTSTRTWALP  
 251 TYNNHLYKQI SSQSGASNDN HYFGYSTPWG YFDFNRFHCH FSPRDWQRLI

301 NNNWGFRPKK LSFKLFNIQV RGV TQNDGTT TIANNLTSTV QVFTDSEYQL  
 351 PYVLGSAHQG CLPPFPADV F MVPQYGYLTL NNGSQA VGRS SFYCLEYFPS  
 401 QMLRTGNNFQ FSYTFEDVPF HSSYAHSQSL DRLMNPLIDQ YLYYLNRTQG  
 451 TTSGTTNQSR LLFSQAGPQS MSLQARNWLP GPCYRQQRLS KTANDNNNSN  
 5 501 FPWTAASKYH LNGRDSL VNP GPAMASHKDD EEKFFPMHGN LIFGKEGT TA  
 551 SNAELDNVMI TDEEEIRTTN PVATEQYGT V ANN LQSSNTA PTTGT VNHQG  
 601 ALPGMVWQDR DVYLQGPIWA KIPHTDGHFH PSPLMGGFGL KHPPQIMIK  
 651 NTPVPANPPT TFSPAKFASF ITQYSTGQVS VEIEWELQKE NSKRWNPEIQ  
 701 YTSNYNKSVN VDFTVDTNGV YSEPRPIGTR YLTRNL\* (SEQ ID NO: 18)

10

#### Exemplary AAV4 capsid protein

1 MTDGYLPDWL EDNLSEGVRE WWALQPGAPK PKANQQHQDN ARGLVLPGYK  
 51 YLGPGNGLDK GEPVNAADAA ALEHDKAYDQ QLKAGDNPYL KYNHADA EFQ  
 101 QRLQGDT SFG GNLGRA VFQA KKRVL EPLGL VEQAGETAPG KKRPLIESPQ  
 15 151 QPDSSTGIGK KGKQPAKKKL VFEDET GAGD GPPEGSTSGA MSDDSEMRAA  
 201 AGGAAVEGGQ GADGVGNASG DWHCDSTWSE GHVTTTSTRT WVLPTYNNHL  
 251 YKRLGESLQS NTYNGFSTPW GYFDFNRFHC HFSPRDWQRL INNNWGM RPK  
 301 AMRVKIFNIQ VKEVTTSNGE TTVANNLTST VQIFADSSYE LPYVMDAGQE  
 351 GSLPPFPNDV FMVPQYGYCG LVTGNTSQQQ TDRNAFYCLE YFPSQMLRTG  
 20 401 NNFEITYSFE KVPFHSMYAH SQSLDRLMNP LIDQYLWGLQ STTTGTTLNA  
 451 GTATTNFTKL RPTNFSNFKK NWLPGPSIKQ QGFSKTANQN YKIPATGSDS  
 501 LIKYETHSTL DGRWSALTPG PPMATAGPAD SKFSNSQLIF AGPKQNGNTA  
 551 TVPGTLIFTS EEELAATNAT DTDMWGNLPG GDQSNSNLPT VDRLTALGAV  
 601 PGMVWQNRDI YYQGPIWAKI PHTDGHFHPS PLIGGFGLKH PPPQIFIKNT  
 25 651 PVPANPATTF SSTPVNSFIT QYSTGQVSVQ IDWEIQKERS KRWNPEVQFT  
 701 SNYGQQNSLL WAPDAAGKYT EPRAIGTRYL THHL\* (SEQ ID NO: 19)

#### Exemplary AAV5 capsid protein

1 MSFVDHPPDW LEEVGEGLRE FLGLEAGPPK PKPNQQHQDQ ARGLVLPGYN  
 30 51 YLGPGNGLDR GEPVNRAD E V AREHDISYNE QLEAGDNPYL KYNHADA EFQ  
 101 EKLADDT SFG GNLGKAVFQA KKRVL EPGFL VEEGAKTAPT GKRIDDHFPK

151 RKKARTEEDS KPSTSSDAEA GPSGSQQLQI PAQPASSLGA DTMSAGGGGP  
 201 LGDNNQGADG VGNASGDWHC DSTWMGDRVV TKSTRTWVLP SYN NHQYREI  
 251 KSGSVDGSNA NAYFGYSTPW GYFDFNRFHS HWSRPDWQRL INNYWGFRPR  
 301 SLRVKIFNIQ VKEVTVQDST TTIANNLTST VQVFTDDDYQ LPYVVGNGTE  
 5 351 GCLPAFPQV FTL PQYGYAT LNRDNTENPT ERSSFFCLEY FPSKMLRTGN  
 401 NFEFTYNFEE VPFHSSFAPS QNLFKLANPL VDQYLRFVS TNNTGGVQFN  
 451 KNLAGRYANT YKNWFPGPMG RTQGWNLGSG VNRASVSAFA TTNRMELEGA  
 501 SYQVPPQPNG MTNNLQGSNT YALENTMIFN SQPANPGTTA TYLEGNMLIT  
 551 SESETQPVNR VAYNVGGQMA TNNQSSTTAP ATGTYNLQEI VPGSVWMERD  
 10 601 VYLQGPWAK IPETGAHFHP SPAMGGFGLK HPPPMMLIKN TPVPGNITSF  
 651 SDVPVSSFIT QYSTGQVTVE MEWELKKENS KRWNPEIQYT NNYNDPQFVD  
 701 FAPDSTGEYR TTRPIGTRYL TRPL\* (SEQ ID NO: 20)

#### Exemplary AAV6 capsid protein

15 1 MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD DGRGLVLPGY  
 51 KYLGPFNGLD KGEPVNAADA AALEHDKAYD QQLKAGDNPY LRYNHADA EF  
 101 QERLQEDTSF GGNLGRAVFQ AKKRVLEPFG LVEEGA KTAP GK KRPVEQSP  
 151 QEPDSSSGIG KTGQQPAKKR LNFGQTGDSE SVPDPQPLGE PPATPAAVGP  
 201 TT MASGGGAP MADNNEGADG VGNASGNWHC DSTWLGDRVI TTSTRTWALP  
 20 251 TYN NHLYKQI SSASTGASND NHYFGYSTPW GYFDFNRFHC HFSPRDWQRL  
 301 INNNWGFRPK RLNFKLFNIQ VKEVTTNDGV TTIANNLTST VQVFSDSEYQ  
 351 LPYVLGSAHQ GCLPPFPADV FMIPQYGYLT LNNGSQAVGR SSFYCLEYFP  
 401 SQMLRTGNNF TFSYTFEDVP FHSSYAHSQS LDRLMNPLID QYLYYLNRTQ  
 451 NQSGSAQNKD LLFSRGSPAG MSVQPKNWLP GPCYRQQRVS KTKTDNNNSN  
 25 501 FTWTGASKYN LNGRESIINP GTAMASHKDD KDKFFPMSGV MIFGKESAGA  
 551 SNTALDNVMI TDEEEIKATN PVATERFGTV AVNLQSSSTD PATGDVHVMG  
 601 ALPGMVWQDR DVYLQGPWA KIPHTDGHFH PSPLMGGFGL KHPPQILIK  
 651 NTPVPANPPA EFSATKFASF ITQYSTGQVS VEIEWELQKE NSKRWNPEVQ  
 701 YTSNYAKSAN VDFTVDNGL YTEPRIGTR YLTRPL\* (SEQ ID NO: 21)

#### Exemplary AAV7 capsid protein

1 MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD NGRGLVLPGY  
 51 KYLGPFNGLD KGEPVNAADA AALEHDKAYD QQLKAGDNPY LRYNHADA EF  
 101 QERLQEDTSF GGNLGRAVFQ AKKRVLEPLG LVEEGAKTAP AKKRPVEPSP  
 151 QRSPDSSTGI GKKGQQPARK RLNFGQTGDS ESVPDPQPLG EPPAAPSSVG  
 5 201 SGTVAAGGGA PMADNNEGAD GVGNASGNWH CDSTWLGDRV ITTSTRTWAL  
 251 PTYNNHLYKQ ISSETAGSTN DNTYFGYSTP WGYFDFNRFH CHFSPRDWQR  
 301 LINNNWGFRP KKLRFKLFNI QVKEVTTNDG VTTIANNLTS TIQVFSDSEY  
 351 QLPYVLGSAH QGCLPPFPAD VFMIPQYGYL TLNNGSQSVG RSSFYCLEYF  
 401 PSQMLRTGNN FEFSYSFEDV PFHSSYAHSQ SLDRLMNPLI DQYLYYLART  
 10 451 QSNPGGTAGN RELQFYQGGP STMAEQAKNW LPGPCFRQQR VSKTLDQNNN  
 501 SNFAWTGATK YHLNGRNSLV NPGVAMATHK DDED RFFPSS GVLIFGKTGA  
 551 TNKTTLENVL MTNEEEIRPT NPVATEEYGI VSSNLQAANT AAQTQVVNNQ  
 601 GALPGMVWQN RDVYLQGPIW AKIPHTDGNF HPSPLMGGFG LKHPPPQILI  
 651 KNTVPANPP EVFTPAKFAS FITQYSTGQV SVEIEWELQK ENSKRWNPEI  
 15 701 QYTSNFEKQT GVDFAVDSQG VYSEPRPIGT RYLTRNL\* (SEQ ID NO: 22)

Exemplary AAV8 capsid protein

1 MAADGYLPDW LEDNLSEGIR EWWALKPGAP KPKANQQKQD DGRGLVLPGY  
 51 KYLGPFNGLD KGEPVNAADA AALEHDKAYD QQLQAGDNPY LRYNHADA EF  
 20 101 QERLQEDTSF GGNLGRAVFQ AKKRVLEPLG LVEEGAKTAP GKKRPVEPSP  
 151 QRSPDSSTGI GKKGQQPARK RLNFGQTGDS ESVPDPQPLG EPPAAPSGVG  
 201 PNTMAAGGGA PMADNNEGAD GVGSSSGNWH CDSTWLGDRV ITTSTRTWAL  
 251 PTYNNHLYKQ ISNGTSGGAT NDNTYFGYST PWGYFDFNRF HCHFSPRDWQ  
 301 RLINNNWGFR PKRLSFKLFN IQVKEVTQNE GTKTIANNLT STIQVFTDSE  
 25 351 YQLPYVLGSA HQGCLPPFPA DVFMIPQYGY LTLNNGSQAV GRSSFYCLEY  
 401 FPSQMLRTGN NFQFTYTFED VPFHSSYAHS QSLDRLMNPL IDQYLYYLSR  
 451 TQTTGGTANT QTLGFSQGGP NTMANQAKNW LPGPCYRQQR VSTTTGQNNN  
 501 SNFAWTAGTK YHLNGRNSLA NPGIAMATHK DDEERFFPSN GILIFGKQNA  
 551 ARDNADYSDV MLTSEEEIKT TNPVATEEYG IVADNLQQQN TAPQIGTVNS  
 30 601 QGALPGMVWQ NRDVYLQGPI WAKIPHTDGN FHPSPLMGGF GLKHPPPQIL  
 651 IKNTVPADP PTTFNQSKLN SFITQYSTGQ VSVEIEWELQ KENSKRWNPE  
 701 IQYTSNYYKS TSVDFAVNTE GUYSEPRPIG TRYLTRNL\* (SEQ ID NO: 23)

Exemplary AAV9 capsid protein

1 MAADGYLPDW LEDNLSEGIR EWWALKPGAP QPKANQQHQD NARGLVLPGY  
 51 KYLGPGNGLD KGEPVNAADA AALEHDKAYD QQLKAGDNPY LKYNHADA EF  
 5 101 QERLKEDTSF GGNLGRAVFQ AKKRLLEPLG LVEEAAKTAP GKKRPVEQSP  
 151 QEPDSSAGIG KSGAQPAKKR LNFGQTGDTE SVPDPQPIGE PPAAPSGVGS  
 201 LTMASGGGAP VADNNEGADG VGSSSGNWHC DSQWLGD RVI TTSTRTWALP  
 251 TYNNHLYKQI SNSTSGGSSN DNAYFGYSTP WGYFDFNRFH CHFSPRDWQR  
 301 LINNNWGFRP KRLNFKLFNI QVKEVTDNNG VKTIANNLTS TVQVFTDSDY  
 10 351 QLPYVLGSAH EGCLPPFPAD VFMI PQYGYL TLNDGSQAVG RSSFYCLEYF  
 401 PSQMLRTGNN FQFSYEFENV PFHSSYAHSQ SLDRLMNPLI DQYLYYLSKT  
 451 INSGSQNQQT LKFSVAGPSN MAVQGRNYIP GPSYRQQRVS TTVTQNNNSE  
 501 FAWPGASSWA LNGRNSLMNP GPAMASHKEG EDRFFPLSGS LIFGKQGTGR  
 551 DNVDADKVM I TNEEEIKTTN PVATESYGQV ATNHQSAQAQ AQTGWVQNQG  
 15 601 ILPGMVWQDR DVYLQGPIWA KIPHTDGNFH PSPLMGGFGM KHPPPQILIK  
 651 NTPVPADPPT AFNKDKLNSF ITQYSTGQVS VEIEWELQKE NSKRWNPEIQ  
 701 YTSNYYKSN N VEFVNTGEGV YSEPRPIGTR YLTRNL\* (SEQ ID NO: 24)

Exemplary AAV10 capsid protein

20 1 MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD DGRGLVLPGY  
 51 KYLGPFNGLD KGEPVNAADA AALEHDKAYD QQLKAGDNPY LRYNHADA EF  
 101 QERLQEDTSF GGNLGRAVFQ AKKRVLEPLG LVEEGA KTAP GKKRPVEPSP  
 151 QRSPDSSTGI GKKGQQPAKK RLNFGQTGDS ESVPDPQPIG EPPAGPSGLG  
 201 SGTMAAGGGA PMADNNEGAD GVGSSSGNWH CDSTWLGD RVI ITTSTRTWAL  
 25 251 PTYNNHLYKQ ISNGTSGGST NDNTYFGYST PWGYFDFNRF HCHFSPRDWQ  
 301 RLINNNWGFR PKRLNFKLFN IQVKEVTQNE GTKTIANNLT STIQVFTDSE  
 351 YQLPYVLGSA HQGCLPPFPA DVFMIPQYGY LTLNNGSQAV GRSSFYCLEY  
 401 FPSQMLRTGN NFEFSYQFED VPFHSSYAHS QSLDRLMNPL IDQYLYYLSR  
 451 TQSTGGTAGT QQLLFSQAGP NNMSAQAKNW LPGPCYRQQR VSTTLSQNNN  
 30 501 SNFAWTGATK YHLNGRDSL V NPGVAMATHK DDEERFFPSS GVLMFGKQGA  
 551 GKDNVDYSSV MLTSEEEIKT TNPVATEQYG VVADNLQQQN AAPIVGAVNS  
 601 QGALPGMVWQ NRDVYLQGPI WAKIPHTDGN FHPSPLMGGF GLKHPPPQIL

651 IKNTPVPADP PTTFSQAKLA SFITQYSTGQ VSVEIEWELQ KENSKRWNPE  
 701 IQYTSNYYKS TNVDFAVNTD GTYSEPRPIG TRYLTRNL\* (SEQ ID NO: 25)

In some embodiments, the modified capsid protein comprises a non-tyrosine residue and/or a non-threonine residue at one or more of or each of Y705, Y731, and T492 of a wild-type AAV6 capsid protein (see sequence below with Y705, Y731, and T492 positions underlined, bolded and italicized). In some embodiments, the non-tyrosine residue is phenylalanine and the non-threonine residue is valine.

1 MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD DGRGLVLPGY  
 51 KYLGPFNGLD KGEPVNAADA AALEHDKAYD QQLKAGDNPY LRYNHADAEF  
 101 QERLQEDTSF GGNLGRAVFQ AKKRVLEPFG LVEEGAKTAP GKCRPVEQSP  
 151 QEPDSSSGIG KTGQQPAKKR LNFGQTGDSE SVPDPQPLGE PPATPAAVGP  
 201 TTMASGGGAP MADNNEGADG VGNASGNWHC DSTWLGDRVI TTSTRTWALP  
 251 TYNNHLYKQI SSASTGASND NHYFGYSTPW GYFDFNRFHC HFSPRDWQRL  
 301 INNNWGFRPK RLNFKLFIQ VKEVTTNDGV TTIANNTST VQVFSDSEYQ  
 351 LPYVLGSAHQ GCLPPFPADV FMIPQYGYLT LNNGSQAVGR SSFYCLEYFP  
 401 SQMLRTGNNF TFSYTFEDVP FHSSYAHSQS LDRLMNPLID QYLYFLNRTQ  
 451 NQSGSAQNKD LLFSRGSPAG MSVQPKNWLP GPCYRQQRVS **K**TKTDNNNSN  
 501 FTWTGASKYN LNGRESIINP GTAMASHKDD KDKFFPMSGV MIFGKESAGA  
 551 SNTALDNVMI TDEEEIKATN PVATERFGTV AVNLQSSSTD PATGDVHVMG  
 601 ALPGMVWQDR DVYLQGPIWA KIPHTDGHFH PSPLMGGFGL KHPPPQILIK  
 651 NTPVPANPPA EFSATKFASF ITQYSTGQVS VEIEWELQKE NSKRWNPEVQ  
 701 YTSNYAKSAN VDFTVDNNGL YTEPRPIGTR YLTRPL (SEQ ID NO: 21)

In some embodiments, the modified capsid protein comprises a non-tyrosine residue and/or a non-threonine residue at one or more of or each of Y444, Y500, Y731, and T491 of a wild-type AAV2 capsid protein (see sequence below with Y444, Y500, Y731, and T491 positions underlined, bolded and italicized). In some embodiments, the non-tyrosine residue is phenylalanine and the non-threonine residue is valine.

1 MAADGYLPDW LEDTLSEGIR QWWKLKPGPP PPKPAERHKD DSRGLVLPGY  
 5 151 KYLGPFNGLD KGEPVNEADA AALEHDKAYD RQLDSGDNPY LKYNHADA EF  
 101 QERLKEDTSF GGNLGRAVFQ AKKRVLEPLG LVEEPVKTAP GK KRPVEHSP  
 151 VEPDSSSGTG KAGQQPARKR LNFGQTGDAD SVPDPQPLGQ PPAAPSGLGT  
 5 201 NTMATGSGAP MADNNEGADG VGNSSGNWHC DSTWMGDRVI TTSTRTWALP  
 251 TYNNHLYKQI SSQSGASNDN HYFGYSTPWG YFDFNRFHCH FSPRDWQRLI  
 301 NNNWGFPRPKR LNFKLFNIQV KEVTQNDGTT TIANNLTSTV QVFTDSEYQL  
 351 PYVLGSAHQG CLPPFPADV F MVPQYGYLTL NNGSQAVGRS SFYCLEYFPS  
 401 QMLRTGNNFT FSYTFEDVPF HSSYAHSQSL DRLMNPLIDQ YLYYLSRTNT  
 10 451 PSGTTTQSRL QFSQAGASDI RDQSRNWLPG PCYRQQRVSK TSADNNNSEY  
 501 SWTGATKYHL NGRDSL VNPG PAMASHKDDE EKFFPQSGVL IFGKQGSEKT  
 551 NVDIEKVMIT DEEEIRTTNP VATEQYGSVS TNLQRGNRQA ATADVNTQGV  
 601 LPGMVWQDRD VYLQGPIWAK IPHTDGHFHP SPLMGGFGLK HPPPQILIKN  
 651 TPVPANPSTT FSAAKFASFI TQYSTGQVSV EIEWELQKEN SKRWNPEIQY  
 15 701 TSNYNKSVNV DFTVDTNGVY SEPRPIGTRYLTRNL (SEQ ID NO: 17)

Other aspects of the disclosure relate to the nucleic acid vector. In some  
 embodiments, the nucleic acid vector is provided in a form suitable for inclusion in a rAAV  
 particle, such as a single-stranded or self-complementary nucleic acid. In some  
 20 embodiments, the nucleic acid vector is provided in a form suitable for use in a method of  
 producing rAAV particles. For example, in some embodiments, the nucleic acid vector is a  
 plasmid (e.g., comprising an origin of replication (such as an E. coli ORI) and optionally a  
 selectable marker (such as an Ampicillin or Kanamycin selectable marker)). In some  
 embodiments, the nucleic acid vector comprises a parvovirus B19p6 promoter operatively  
 25 linked to a globin gene, wherein the promoter and gene are flanked by ITR sequences, such  
 as AAV2 or AAV6 ITR sequences. In some embodiments, the nucleic acid vector comprises  
 the sequence as shown below (which is annotated based on the regions of the nucleic acid as  
 shown in brackets. In some embodiments, the nucleic acid vector comprises the sequence as  
 shown below without the introns.

30

## AAV2-ITR

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCC  
CGACGCCCCGGGCTTTGCCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGT  
GGCCAACTCCATCACTAGGGGTTTCCT (SEQ ID NO: 3)

5

## B19p6 Promoter

CCAACCCTAATTCCGGAAGTCCCGCCACCGGAAGTGACGTCACAGGAAATGACGTCAC  
AGGAAATGACGTAATTGTCCGCCATCTTGTACCGGAAGTCCCGCCTACCGGCGGGCGACC  
GGCGGCATCTGATTTGGTGTCTTCTTTTAAATTTTAGCGGGCTTTTTTCCCGCCTTATGCA  
AATGGGCAGCCATTTTAAGTGTTTTACTATAATTTTATTGGTTAGTTTTGTAAACGGTAAA  
ATGGGCGGAGCGTAGGCGGGGACTACAGTATATATAGCACGGCACTGCCGCAGCTCTTT  
CTTTCTGGGCTGCTTTTTCTGGACTTTCTTGCTGTTTTTTGTGAGCTAACTAACAGGTATT  
TATACTACTTGTTAACATACTAA (SEQ ID NO: 7)

10

15 Human anti-sickling  $\beta$ -globin gene**Exon 1**

ATGGTGCACCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAA  
CGTGGATGAAGTTGGTGGTGAGGCCCTGGGCA (SEQ ID NO: 10)

**Intron 1**

20 GGTTGGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAACTGGGCATGTGGA  
GACAGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCTCTCTGCCTATTGGTCTATTTT  
CCCACCCTTA (SEQ ID NO: 11)

**Exon 2**

25 GGCTGCTGGTGGTCTACCCTTGGACCCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCA  
CTCCTGATGCTGTTATGGGCAACCCTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGT  
GCCTTTAGTGATGGCCTGGCTCACCTGGACAACCTCAAGGGCACCTTTGCC\*CAG\*CTGA  
GTGAGCTGCACTGTGACAAGCTGCACGTGGATCCTGAGAACTTCAGG (SEQ ID NO: 12)

\*CAG\* = T87Q

**Delta 12 Intron (372 bp-deletion in Intron 2)**

30 GTGAGTCTATGGGACCCTTGATGTTTTCTTTCCCCTTCTTTTCTATGGTTAAGTTCATGTCA  
TAGGAAGGGGAGAAGTAACAGGGTACACATATTGACCAAATCAGGGTAATTTTGCATTT

GTAATTTTAAAAAATGCTTTCTTCTTTTAATATACTTTTTTGTTTATCTTATTTCTAATACT  
TTCCCTAATCTCTTTCTTTCAGGGCAATAATGATACAATGTATCATGCCTCTTTGCACCAT  
TCTAAAGAATAACAGTGATAATTTCTGGGTAAAGGCAATAGCAATATTTCTGCATATAAA  
TATTTCTGCATATAAATTGTAAGTATGTAAGAGGTTTCATATTGCTAATAGCAGCTACA  
5 ATCCAGCTACCATTTCTGCTTTTATTTTATGGTTGGGATAAAGGCTGGATTATTCTGAGTCCA  
AGCTAGGCCCTTTTGCTAATCATGTTTCATACCTCTTATCTTCCTCCCACAG (SEQ ID NO:  
13)

**Exon 3**

CTCCTGGGCAACGTGCTGGTCTGTGTGCTGGCCCATCACTTTGGCAAAGAATTCACCCCA  
10 CCAGTGCAGGCTGCCTATCAGAAAGTGGTGGCTGGTGTGGCTAATGCCCTGGCCCACAA  
GTATCACTAA (SEQ ID NO: 14)

**Poly A sequence**

GCTCGCTTTCTTGCTGTCCAATTTCTATTAAAGGTTCCCTTTGTTCCCTAAGTCCAACACTACT  
AAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTT  
15 ATTTTCATTGCAATGATGTATTTAAATTATTTCTGAATATTTTACTAAAAAGGGAATGTGG  
GAGGTCAGTGCATTTAAACATAAAGAAATGAAGAGCTAGTTCAAACCTTGGGAAAATA  
CACTATATCTTAAACTCCATGAAAGAAGGTGAGGCTGCAAACAGCTAATGCACATTGGC  
AACAGCCCCTGATGCCTATGCCTTATTCATCCCTCAGAAAAGGATTCAAGTAGAGGCTTG  
ATTTGGAGGTTAAAGTTTTGCTATGCTGTATTTTACATTACTTATTGTTTTAGCTGTCTCA  
20 TGAATGTCTTTTCACTACCCATTTGCTTATCCTGCATCTCTCAGCCTTGACTCCACTCAGTT  
CTCTTGCTTAGAGATAACACCTTTCCCCTGAAGTGTTCCCTTCCATGTTTTACGGCGAGATG  
GTTTCTCCTCGCCTGGCCACTCAGCCTTAGTTGTCTCTGTTGTCTTATAGAGGTCTACTTG  
AAGAAGGAAAAACAGGGGGCATGGTTTGACTGTCCTGTGAGCCCTTCTTCCCTGCCTCCC  
CCACTCACAGTGACCCGGAATCTGCAGTGCTAGTCTCCCGGAATCACTCTTTACAG  
25 TCTGCTTTGGAAGGACTGGGCTTAGTATGAAAAGTTAGGACTGAGAAGAATTTGAAAGG  
GGGCTTTTTGTAGCTTGATATTCATACTGTCTTATTACCCTATCATAGGCCACCCCAA  
TGGAAGTCCCATTCTTCCTCAGGATGTTTAAGATTAGCATTGAGGAAGAGATCAGAGGTC  
TGCTGGCTCCCTTATCATGTCCCTTATGGTGCTTCTGGCTCTGCAGTTATTAGCATAGTGT  
TACCATCAACCACCTTAACCTTCATTTTTCTTATTCAATACCTAGCGCGTATCGCGGGATCC  
30 ACTAGTTCT (SEQ ID NO: 26)

AAV2-ITR

AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGG  
 CCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCCGGCCTCAGTGAGCGAG  
 CGAGCGCGCAGAGAGGGAGTGGCCAA (SEQ ID NO: 27)

5 In some embodiments of the above sequence, the AAV2 ITRs are replaced with AAV6 ITRs, the B19p6 promoter is replaced with an HS2 enhancer and  $\beta$ -globin promoter, and/or the human anti-sickling  $\beta$ -globin gene is replaced with a human  $\gamma$ -globin gene.

Methods of producing rAAV particles and nucleic acid vectors are also known in the art and commercially available (see, e.g., Zolotukhin et al. Production and purification of serotype 1, 2, and 5 recombinant adeno-associated viral vectors. Methods 28 (2002) 158–167; 10 and U.S. Patent Publication Numbers US20070015238 and US20120322861, which are incorporated herein by reference; and plasmids and kits available from ATCC and Cell Biolabs, Inc.). For example, a plasmid containing the nucleic acid vector may be combined with one or more helper plasmids, e.g., that contain a rep gene (e.g., encoding Rep78, Rep68, 15 Rep52 and Rep40) and a cap gene (e.g., encoding VP1, VP2, and VP3, including a modified VP3 region as described herein), and transfected into a producer cell line such that the rAAV particle can be packaged and subsequently purified.

In some embodiments, the one or more helper plasmids include a first helper plasmid comprising a rep gene and a cap gene (e.g., encoding a rAAV capsid protein as described 20 herein) and a second helper plasmid comprising a E1a gene, a E1b gene, a E4 gene, a E2a gene, and a VA gene. In some embodiments, the rep gene is a rep gene derived from AAV2 or AAV6 and the cap gene is derived from AAV2 or AAV6 and may include modifications to the gene in order to produce the modified capsid protein described herein. Helper plasmids, and methods of making such plasmids, are known in the art and commercially available (see, 25 e.g., pDM, pDG, pDP1rs, pDP2rs, pDP3rs, pDP4rs, pDP5rs, pDP6rs, pDG(R484E/R585E), and pDP8.ape plasmids from PlasmidFactory, Bielefeld, Germany; other products and services available from Vector Biolabs, Philadelphia, PA; Cellbiolabs, San Diego, CA; Agilent Technologies, Santa Clara, Ca; and Addgene, Cambridge, MA; pxx6; Grimm et al. (1998), Novel Tools for Production and Purification of Recombinant Adenoassociated Virus

Vectors, Human Gene Therapy, Vol. 9, 2745-2760; Kern, A. et al. (2003), Identification of a Heparin-Binding Motif on Adeno-Associated Virus Type 2 Capsids, Journal of Virology, Vol. 77, 11072-11081.; Grimm et al. (2003), Helper Virus-Free, Optically Controllable, and Two-Plasmid-Based Production of Adeno-associated Virus Vectors of Serotypes 1 to 6, Molecular Therapy, Vol. 7, 839-850; Kronenberg et al. (2005), A Conformational Change in the Adeno-Associated Virus Type 2 Capsid Leads to the Exposure of Hidden VP1 N Termini, Journal of Virology, Vol. 79, 5296-5303; and Moullier, P. and Snyder, R.O. (2008), International efforts for recombinant adeno-associated viral vector reference standards, Molecular Therapy, Vol. 16, 1185-1188).

10 An exemplary, non-limiting, rAAV particle production method is described next.

One or more helper plasmids are produced or obtained, which comprise rep and cap ORFs for the desired AAV serotype and the adenoviral VA, E2A (DBP), and E4 genes under the transcriptional control of their native promoters. The cap ORF may also comprise one or more modifications to produce a modified capsid protein as described herein. HEK293 cells (available from ATCC®) are transfected via CaPO4-mediated transfection, lipids or polymeric molecules such as Polyethylenimine (PEI) with the helper plasmid(s) and a plasmid containing a nucleic acid vector described herein. The HEK293 cells are then incubated for at least 60 hours to allow for rAAV particle production. Alternatively, in another example Sf9-based producer stable cell lines are infected with a single recombinant baculovirus containing the nucleic acid vector. As a further alternative, in another example HEK293 or BHK cell lines are infected with a HSV containing the nucleic acid vector and optionally one or more helper HSVs containing rep and cap ORFs as described herein and the adenoviral VA, E2A (DBP), and E4 genes under the transcriptional control of their native promoters. The HEK293, BHK, or Sf9 cells are then incubated for at least 60 hours to allow for rAAV particle production. The rAAV particles can then be purified using any method known the art or described herein, e.g., by iodixanol step gradient, CsCl gradient, chromatography, or polyethylene glycol (PEG) precipitation.

The disclosure also contemplates host cells that comprise at least one of the disclosed rAAV particles or nucleic acid vectors. Such host cells include mammalian host cells, with

human host cells being preferred, and may be either isolated, in cell or tissue culture. In the case of genetically modified animal models (e.g., a mouse), the transformed host cells may be comprised within the body of a non-human animal itself. In some embodiments, the host cell is a cell of erythroid lineage, such as a CD36<sup>+</sup> burst-forming units-erythroid (BFU-E) cell or a colony-forming unit-erythroid (CFUE-E) progenitor cell.

### **Subjects**

Aspects of the disclosure relate to methods for use with a subject, such as human or non-human primate subjects. Non-limiting examples of non-human primate subjects include macaques (e.g., cynomolgus or rhesus macaques), marmosets, tamarins, spider monkeys, owl monkeys, vervet monkeys, squirrel monkeys, baboons, gorillas, chimpanzees, and orangutans. In some embodiments, the subject is a human subject. Other exemplary subjects include domesticated animals such as dogs and cats; livestock such as horses, cattle, pigs, sheep, goats, and chickens; and other animals such as mice, rats, guinea pigs, and hamsters.

In some embodiments, the subject has or is suspected of having a disease that may be treated with gene therapy. In some embodiments, the subject has or is suspected of having a hemoglobinopathy. A hemoglobinopathy is a disease characterized by one or more mutation(s) in the genome that results in abnormal structure of one or more of the globin chains of the hemoglobin molecule. Exemplary hemoglobinopathies include hemolytic anemia, sickle cell disease, and thalassemia. Sickle cell disease is characterized by the presence of abnormal, sickle-shaped hemoglobins, which can result in severe infections, severe pain, stroke, and an increased risk of death. Subjects having sickle cell disease can be identified, e.g., using one or more of a complete blood count, a blood film, hemoglobin electrophoresis, and genetic testing. Thalassemias are a group of autosomal recessive diseases characterized by a reduction in the amount of hemoglobin produced. Symptoms include iron overload, infection, bone deformities, enlarged spleen, and cardiac disease. The subgroups of thalassemias include alpha-thalassemia, beta-thalassemia, and delta thalassemia. Subjects having a thalassemia may be identified, e.g., using one or more of complete blood count, hemoglobin electrophoresis, Fe Binding Capacity, urine urobilin and urobilinogen,

peripheral blood smear, hematocrit, and genetic testing.

In some embodiments, a host cell is derived from a subject and use to produce a host cell suspension as described herein.

In some embodiments, the subject has or is suspected of having a disease involving  
5 blood cells (e.g., a disease caused by a defect, such as a genetic mutation, in one or more blood cell types). Exemplary blood cells include T cell, B cells, dendritic cells, macrophages, monocytes, and hematopoietic stem cells. In some embodiments, the disease is a blood cell cancer, e.g., a leukemia (such as Acute lymphocytic leukemia, Acute myelogenous leukemia, Chronic lymphocytic leukemia, or Chronic myelogenous leukemia), lymphoma (such as  
10 Hodgkin lymphoma or non-Hodgkin lymphoma), or myeloma (such as multiple myeloma). Other exemplary diseases involving blood cells include anemia, hemophilia, myelodysplastic syndrome, sickle cell disease, thalassemia, deep vein thrombosis, von Willebrand disease, factor II, V, VII, X, or XII deficiency, Polycythemia vera, thrombocytopenia and Idiopathic thrombocytopenic purpura. Subjects having such diseases can be identified by the skilled  
15 practitioner according to methods known in the art, e.g., using one or more of a complete blood count, platelet aggregation test, bleeding time test, genetic testing, and biomarker assays.

In some embodiments, the subject has or is suspected of having cancer. Exemplary cancers include breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer,  
20 pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, myeloma, lung cancer and the like. Subjects having cancer can be identified by the skilled practitioner according to methods known in the art, e.g., using one or more of a biopsy, x-ray, CT scan, Magnetic Resonance Imaging (MRI), ultrasound, genetic testing, and biomarker assays.

25 Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present disclosure to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are

incorporated by reference for the purposes or subject matter referenced herein.

## EXAMPLES

### Example 1: Recombinant AAV-Parvovirus B19 Hybrid Vectors for Gene Therapy of Human Hemoglobinopathies

The generation of a hybrid human parvovirus containing the adeno-associated virus 2 (AAV2) capsid and the human parvovirus B19 genome was previously described (Proc. Natl. Acad. Sci., USA, 86: 8078-8082, 1989). Subsequently, the parvovirus B19 promoter at map unit 6 (B19p6) was shown to be necessary and sufficient to confer human erythroid cell-tropism to the AAV2-B19p6 hybrid virus (Proc. Natl. Acad. Sci., USA, 92: 12416-12420, 1995). These studies led to the development of AAV2-B19p6 hybrid vectors with which erythroid lineage-restricted transgene expression from the B19p6 promoter could be achieved following stable transduction of murine hematopoietic stem cells (HSCs). In more recent studies, it was observed that of the 10 most commonly used AAV serotypes, AAV6 was the most efficient in transducing human HSCs (Cytotherapy, 15: 986-996, 2013). When the B19p6 promoter-driven expression cassette was encapsidated in tyrosine-mutant AAV6 capsids, erythroid lineage-restricted, high-level expression of the reporter transgene was achieved following stable transduction of human HSCs, both in vitro as well as in a murine xenograft model in vivo (PLoS One, 8: e58757, 2013). More interestingly, the level of the reporter transgene expression from the B19p6 promoter was significantly higher than that from the human  $\beta$ -globin gene promoter.

As described herein, recombinant AAV6 was generated containing the human  $\beta$ -globin gene driven by either the B19p6 promoter or the  $\beta$ -globin gene promoter (Figure 1). Studies are currently underway to determine whether high levels the  $\beta$ -globin protein can be expressed, which would be expected to lead to phenotypic correction of both  $\beta$ -thalassemia and sickle cell disease. The sequence of the B19p6 promoter is provided below:

```

1 CCAACCCTAA TTCCGGAAGT CCCGCCACC GGAAGTGACG TCACAGGAAA TGACGTCACA
61 GGAAATGACG TAATTGTCCG CCATCTTGTA CCGGAAGTCC CGCCTACCGG CGGCGACCGG
121 CGGCATCTGA TTTGGTGTCT TCTTTTAAAT TTTAGCGGGC TTTTTCCTCG CCTTATGCAA

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181 ATGGGCAGCC ATTTTAAGTG TTTTACTATA ATTTTATTGG TTAGTTTTGT AACGGTTAAA  
 241 ATGGGCGGAG CGTAGGCGGG GACTACAGTA TATATAGCAC GGCAC TGCCG CAGCTCTTTC  
 301 TTTCTGGGCT GCTTTTTCCT GGACTTTCTT GCTGTTTTTT GTGAGCTAAC TAACAGGTAT  
 361 TTATACTACT TGTTAACATA CTAA (SEQ ID NO: 7)

5

In this context, it is important to note that the use of recombinant lentiviral vectors in a clinical trial led to transfusion-independence in a young patient with  $\beta$ -thalassemia, but also activated a cellular proto-oncogene, frequently associated with preleukemia (Nature, 467: 318-322, 2010).

10

Thus, the recombinant AAV6-B19p6- $\beta$ -globin vectors promise to prove to be a safer alternative for the potential gene therapy of human hemoglobinopathies in general, and  $\beta$ -thalassemia and sickle cell disease in particular.

#### Example 2: Development of Chimeric AAV6-B19 Vectors for in vivo Targeting of Human

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#### Hematopoietic Stem Cells

20

Pathogenic human parvovirus B19, which has a remarkable tropism for primary human erythroid progenitor cells in the bone marrow, utilizes activated  $\alpha 5 \beta 1$  integrin as a cellular co-receptor to gain entry into target cells (*Blood*, 102: 3927-3933, 2003), following binding to the erythrocyte P antigen as receptor, which is expressed abundantly on these cells as well as on mature red blood cells (RBCs). However, RBCs lack the expression of  $\alpha 5 \beta 1$  integrin, and as a consequence, B19 fails to enter these cells, but effectively utilizes mature RBCs to traffic to the bone marrow, where the target erythroid progenitor cells reside.

25

Although of the 10 most commonly used AAV serotypes, AAV6 is the most efficient serotype for transducing primary human hematopoietic stem cells (HSCs), both *in vitro* and in murine xenograft models *in vivo* (*Cytherapy*, 15: 986-998, 2013; *PLoS One*, 8(3): e58757, 2013), AAV6 vector promiscuity makes it difficult to target HSCs *in vivo*. Here, the plan is to exploit the RBC-binding property of B19, mediated by the P antigen-binding site on the B19 capsid, to develop a chimeric AAV-B19 vector with the proven safety and efficacy of AAV6, and the target-specificity of B19, by inserting the P antigen-binding site into the AAV6 capsid. Based on AAV crystal structure, combined with various site-directed and

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insertion mutagenesis studies of the capsid gene, specific regions of the capsid viral proteins were identified that are surface-exposed and tolerant to insertion of the peptides (FIG. 2). Amino acids in the AAV6 loop VIII (residues 592 to 598) are substituted with that of the B19 P antigen-binding site (residues 399 to 406). Alternatively, the entire loop VIII of AAV6 (residues 572 to 603) is substituted with the entire P antigen-binding site (residues 383 to 411). It is hypothesized that these study will result in development of safe and efficient vectors for targeting primary human HSCs directly in the patient's bone marrow. The current treatment of human hemoglobinopathies involve bone marrow harvest, HSC isolation and purification, *ex vivo* transduction, and HSC transplantation, using lentivirus-based vectors. The major disadvantages of these treatments include cumbersome procedures, high patient care costs, and the potential risk of initiating preleukemia associated with lentiviral vectors (*Nature*, 467: 318-322, 2010).

Thus, the ability to deliver the chimeric AAV6-B19 therapeutic vector (e.g., rAAV) directly to the patient's bone marrow to achieve high-efficiency transduction of HSCs should circumvent each of the problems associated with the use of lentiviral vectors. The availability of these novel AAV6-B19 chimeras should prove useful in the potential gene therapy of human hematopoietic disorders in general, and human hemoglobinopathies in particular.

### Example 3: Strategies to Achieve High-Efficiency Transduction of Human Hematopoietic Stem Cells with Recombinant AAV

Unlike most adherent cells, human hematopoietic stem cells (HSCs), grown in suspension, are not transduced efficiently by AAV2 serotype vectors (e.g., rAAV), although these cells express both heparin sulfate proteoglycan (HSPG) and Fibroblast growth factor receptor 1 (FGFR1), albeit at low levels. It was reasoned that the lack of proximity of HSPG and FGFR1 on these cells might account for the suboptimal transduction of these cells, and it was hypothesized that if the transduction was performed at high cell density, presumably allowing for HSPG on one cell to come in close proximity to FGFR1 on the neighboring cell, then AAV2 bound to HSPG on one cell could utilize FGFR1 on the neighboring cell to gain entry in the latter, and vice versa, thus leading to increased transduction. To test this

hypothesis, primary human HSCs were transduced at either low or high cell density. Whereas only ~5% of these cells were transduced at low-density, the transduction efficiency increased up to ~20% at high-density. Thus, these studies have revealed a novel mechanism, which has been termed “cross-transduction” (FIG. 3), in which AAV vectors (e.g., particles) exploit to gain entry into target cells. Of the 10 commonly used AAV serotypes, AAV6 is the most efficient in transducing primary human HSCs, both in vitro and in murine xenograft models in vivo (*Cytotherapy*, 15: 986-998, 2013; *PLoS One*, 8(3): e58757, 2013). However, the transduction efficiency of these vectors ranged between ~6-87% in HSCs obtained from several different donors (n=11). Such a wide range of transduction efficiency of AAV6 vectors is presumably due to different levels of expression of the putative receptors and/or co-receptors on these cells. In the present study, the transduction efficiency of AAV2 vectors could be augmented both by performing transduction of hematopoietic stem cells (HSCs) with the wild-type (wt)-AAV2 vectors at high cell density, or by using capsid-modified Y444F+Y500F+Y731F+T491V-mutant AAV2 vectors. It was examined whether similar strategies could also be employed to increase the transduction efficiency of HSCs from donors that are not transduced efficiently by AAV6 vectors. Primary human HSCs were transduced with AAV6 vectors either at low or at high density. Whereas only ~14% of the cells transduced at low-density with high multiplicity of infection (MOI) expressed the transgene, the transduction efficiency at high-density increased up to ~20% and 25%, at low, and high MOIs, respectively, also with a significant increase in the mean fluorescence intensity, thus corroborating that the initial cell-cell contact was a critical factor in achieving increased transduction. Next, the transduction efficiencies of the wild-type (wt) and the capsid-modified triple-mutant (Y705F+Y731F+T491V) AAV6 vectors were compared. Again, the wt- and the capsid-modified quadruple-mutant (Y444F+Y500F+Y731F+T491V) AAV2 vectors were used for comparison. Again, ~27% transduction efficiency of the wt-AAV6 vectors was increased by up to ~45% with the capsid-modified AAV6 vectors, with a concomitant increase in the mean fluorescence intensity (FIG. 4).

Additional data were obtained from a series of experiments that were performed to further investigate the relationship between cell density and transduction efficiency. The

results, as analyzed by flow cytometry 48 hours post-transduction, indicated that, compared to the conventionally used  $6 \times 10^5$  cells/mL, increased cell density, up to  $1.0 \times 10^7$  cells/mL, dramatically enhanced the scAAV6-mediated transgene expression, in both the EGFP-positivity and EGFP mean fluorescence intensity (Figure 5A), presumably due to the increased probability of more efficient rAAV attachment to the cell receptor and/or co-receptor. Next, K562 cells were transduced with the optimized TM-scAAV6-CBAP-EGFP vectors either at low-density ( $1 \times 10^6$  cells/mL) or high-density ( $1 \times 10^7$  cells/mL). Whereas only ~25% of K562 cells were transduced at low-density, the transduction efficiency at high-density increased up to 77%, and the EGFP mean value increased to 160% (Figure 5B). The enhancement of transgene expression also correlated with a significant increase in the intra-cellular viral genome copy number (Figure 5C), as determined by qPCR of total DNA isolated 2 hours post-transduction. Similar results were obtained with the TM-scAAV6 rAAV expressing the Guassia luciferase (Gluc) transgene (Figure 5D), as well as when the optimized AAV2-CBAP-EGFP vectors containing the quadruple mutation (Y444F+Y500F+Y731F+T491V; QM-scAAV2) were used (Figure 5E). Similar results were also obtained when these serotypes were used to transduce HSPCs from a donor, which are transduced extremely poorly under conventional conditions (Figure 5F). These studies further corroborate the novel mechanism of “cross-transduction” by recombinant AAV of human cells in general, and HSCs in particular, wherein initial cell-cell contact is critical in achieving high-efficiency transduction.

That the initial cell-cell contact was critical in achieving high-efficiency transduction, was further corroborated by experiments in which cells were transduced at low-density, and subsequently pooled together to reach high-density, and conversely, cells were transduced at high-density, and soon after transduction, were diluted to low-density (FIG. 6A). The increased transduction was observed only under the latter condition (FIG. 6B). In the second set of experiments, a fixed number of K562 cells were infected with rAAV in various volume for 2 hours and subsequently diluted in the same volume of 2 mL (FIG. 7A). Once again, the increased transduction efficiency was observed only under the condition of high cell density (FIG. 7B, 7C), accompanied with a significantly increased intra-cellular viral genome copy

numbers (FIG. 7D).

These studies were extended to include two additional human hematopoietic cell lines, M07e and Raji, which express low to extremely low levels of heparin sulfate proteoglycan (HSPG), the primary receptor for AAV2, and consequently, are transduced extremely poorly by AAV2. Under the condition of high cell density, significantly enhanced transduction of M07e cells, but not Raji cells, was observed (FIG. 8A, 8B), since M07e cells express high levels of AAV2 co-receptor and fibroblast growth factor receptor 1 (FIG. 8C). Raji cells, by comparison, express undetectable levels of both HSPG and FGFR117. To address the possibility whether alternative receptors/co-receptors were being used under the condition of high cell density, K562 cells were transduced with scAAV2 in the absence or the presence of heparin, which is known to compete for AAV2 cellular entry. Heparin at 5 µg/mL significantly reduced the transduction efficiency of scAAV2 under the condition of high cell density for each of the cell types tested (FIG. 8D, 8E). These results strongly suggest that the putative receptors/co-receptors for viral entry remain unaltered under the condition of high cell density.

The efficacy of AAV-mediated transduction of primary HSPCs derived from bone marrow (BM) as well as from umbilical cord blood (CB) was further evaluated. BM-derived CD34+ cells from individual donors (or a mixture from 10 donors) were purchased from a commercial source (AllCells, LLC, Alameda, CA, USA), and were used to transduce with different scAAV-CBAp-EGFP at an MOI of 10,000 vgs/cell without fetal bovine serum (FBS). Transgene expression was evaluated by flow cytometry 48 hours post-transduction. As shown in Table 2, consistent with our previously published studies, whereas scAAV6 transduced human HSPCs more efficiently than scAAV2, capsid modification on both rAAVs further enhanced their transduction efficiency. The transgene expression at high cell density was consistently higher than that at low cell density. The increased transduction efficiency in human HSPCs at high cell density also correlated with a significantly increased intra-cellular viral genome copy number 2 hours post-viral transduction. However, the extent of transgene expression declined over time, and in none of the cell populations tested, the viral genome copy number was below the detection limit of qPCR 14 days post-transduction.

Table 2. Transduction efficiency of AAV in primary human HSPCs from various donors.

|                 | <b>WT-<br/>AAV2</b>     | <b>WT-<br/>AAV6</b> | <b>TM-<br/>AAV6-</b> | <b>WT-<br/>AAV2</b>      | <b>QM-<br/>AAV2-</b> | <b>WT-<br/>AAV6</b> | <b>TM-<br/>AAV6-</b> |
|-----------------|-------------------------|---------------------|----------------------|--------------------------|----------------------|---------------------|----------------------|
|                 | <b>Low Cell Density</b> |                     |                      | <b>High Cell Density</b> |                      |                     |                      |
| <b>Donor #1</b> | ND                      | 1.3%<br>5,090       | ND                   | ND                       | ND                   | 19.4%<br>9,080      | ND                   |
| <b>Donor #2</b> | 1.4%,<br>4184           | 9.8%<br>6904        | ND                   | 7.2%<br>5810             | 31.1%<br>15672       | 27.0%<br>9994       | 45.2%<br>25373       |
| <b>Donor #3</b> | ND                      | ND                  | 28.6%,<br>8,246      | ND                       | ND                   | ND                  | 58.5%<br>23,846      |
| <b>Donor #4</b> | 4.4%,<br>3,720          | 9.5%,<br>6,512      | ND                   | 11.6%,<br>6,686          | 19.6%,<br>13,001     | 23.9%,<br>15,434    | ND                   |

Data are presented as % EGFP positive cells, and EGFP mean fluorescence intensities.

ND = Not done.

## OTHER EMBODIMENTS

All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present disclosure, and without departing from the spirit and scope thereof, can make various changes and modifications of the disclosure to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

## EQUIVALENTS

While several inventive embodiments have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the function and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to

be within the scope of the inventive embodiments described herein. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the inventive teachings is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific inventive embodiments described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, inventive embodiments may be practiced otherwise than as specifically described and claimed. Inventive embodiments of the present disclosure are directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the inventive scope of the present disclosure.

All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, i.e., “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements

specifically identified. Thus, as a non-limiting example, a reference to “A and/or B”, when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (i.e. “one or the other but not both”) when preceded by terms of exclusivity, such as “either,” “one of,” “only one of,” or “exactly one of.” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one,

optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of” shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03. It should be appreciated that embodiments described in this document using an open-ended transitional phrase (e.g., “comprising”) are also contemplated, in alternative embodiments, as “consisting of” and “consisting essentially of” the feature described by the open-ended transitional phrase. For example, if the disclosure describes “a composition comprising A and B”, the disclosure also contemplates the alternative embodiments “a composition consisting of A and B” and “a composition consisting essentially of A and B”.

Claims

What is claimed is:

1. A recombinant AAV (rAAV) particle comprising a nucleic acid vector comprising a  
5 parvovirus B19p6 promoter operatively linked to a heterologous gene, wherein the rAAV  
particle is not AAV2.
2. The rAAV particle of any prior claim, wherein the heterologous gene is a globin gene.
- 10 3. The rAAV particle of claim 2, wherein the globin gene is selected from the group  
consisting of a  $\beta$ -globin gene, an anti-sickling  $\beta$ -globin gene, and a  $\gamma$ -globin gene.
4. The rAAV particle of any prior claim, wherein the globin gene is a human globin gene.
- 15 5. The rAAV particle of any prior claim, wherein the globin gene is a human  $\beta$ -globin gene  
or human anti-sickling  $\beta$ -globin gene.
6. The rAAV particle of any prior claim, wherein the rAAV particle is a AAV6 particle.
- 20 7. The rAAV particle of claim 6, wherein the AAV6 particle comprises a modified capsid  
protein comprising a non-tyrosine residue at a position that corresponds to a surface-exposed  
tyrosine residue in a wild-type AAV6 capsid protein, a non-threonine residue at a position  
that corresponds to a surface-exposed threonine residue in the wild-type AAV6 capsid  
protein, a non-lysine residue at a position that corresponds to a surface-exposed lysine residue  
25 in the wild-type AAV6 capsid protein, a non-serine residue at a position that corresponds to a  
surface-exposed serine residue in the wild-type AAV6 capsid protein, or a combination  
thereof.
8. The rAAV particle of claim 7, wherein the modified capsid protein comprises a non-

tyrosine residue and/or a non-threonine residue at one or more of or each of Y705, Y731, and T492 of a wild-type AAV6 capsid protein.

9. The rAAV particle of claim 7 or 8, wherein the non-tyrosine residue is phenylalanine and the non-threonine residue is valine.

10. The rAAV particle of any prior claim, wherein the nucleic acid vector further comprises AAV2 or AAV6 inverted terminal repeat sequences (ITRs) flanking the parvovirus B19p6 promoter operatively linked to the heterologous gene.

11. A nucleic acid vector comprising a parvovirus B19p6 promoter operatively linked to a globin gene.

12. The nucleic acid vector of any prior claim, wherein the globin gene is selected from the group consisting of a  $\beta$ -globin gene, an anti-sickling  $\beta$ -globin gene, and a  $\gamma$ -globin gene.

13. The nucleic acid vector of any prior claim, wherein the globin gene is a human globin gene.

14. The nucleic acid vector of any prior claim, wherein the globin gene is a human  $\beta$ -globin gene or human anti-sickling  $\beta$ -globin gene.

15. The nucleic acid vector of any prior claims, wherein the vector further comprises inverted terminal repeats (ITRs) flanking the parvovirus B19p6 promoter operatively linked to the globin gene.

16. A method of targeting gene expression to a cell of erythroid lineage in a subject, the method comprising administering the rAAV particle of any prior claim or the nucleic acid vector of any prior claim to a subject.

17. The method of the prior claim, wherein the subject is a human subject.

18. The method of the prior claim, wherein the cell of erythroid lineage is a hematopoietic stem cell.

19. The method of the prior claim, wherein the cell of erythroid lineage is a CD36<sup>+</sup> burst-forming units-erythroid (BFU-E) cell or a colony-forming unit-erythroid (CFUE-E) progenitor cell.

20. A method of treating a hemoglobinopathy, the method comprising administering the rAAV particle of any prior claim or the nucleic acid vector of any prior claim to a subject having a hemoglobinopathy.

21. The method of the prior claim, wherein the subject is a human subject.

22. The method of the prior claim, wherein the hemoglobinopathy is  $\beta$ -thalassemia or sickle cell disease.

23. An rAAV capsid protein comprising one or more amino acid substitutions that result in increased P antigen binding compared to a corresponding un-mutated AAV capsid protein, wherein the one or more amino acid substitutions are in a surface exposed loop of the capsid protein.

24. An rAAV capsid protein comprising one or more amino acid substitutions that introduce a P antigen binding site into a surface exposed loop of the capsid protein.

25. The rAAV capsid protein of any one of claims 23-24, wherein the surface exposed loop is loop VIII.

26. The rAAV capsid protein of any one of claims 23-25, wherein a surface exposed loop is replaced by a B19 P antigen binding site.

5 27. The rAAV capsid protein of claim 26, wherein the B19 P antigen binding site comprises the amino acid sequence QQYTDQIE (SEQ ID NO: 1).

28. The rAAV capsid protein of any one of claims 23-27, wherein the rAAV capsid protein is a variant of an AAV6 capsid protein.

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29. A method of increasing rAAV tropism for hematopoietic stem cells, the method comprising altering a surface exposed loop of an AAV capsid protein to introduce one or more amino acid substitutions that result in increased P antigen binding compared to a corresponding un-mutated AAV capsid protein.

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30. A method of increasing rAAV tropism for hematopoietic stem cells, the method comprising altering a surface exposed loop of an AAV capsid protein to introduce one or more amino acid substitutions that introduce a P antigen binding site into a surface exposed loop of the capsid protein.

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31. The method of any one of claims 29-30, wherein the surface exposed loop is loop VIII.

32. The method of any one of claims 29-31, wherein a surface exposed loop is replaced by a B19 P antigen binding site.

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33. The method of claim 32, wherein the B19 P antigen binding site comprises the amino acid sequence QQYTDQIE (SEQ ID NO: 1).

34. The method of any one of claims 29-33, wherein the rAAV capsid protein is a variant of

an AAV6 capsid protein.

35. A method of delivering an rAAV to a cell, the method comprising administering an rAAV particle comprising an rAAV capsid protein of any one of claims 23-34 to a subject.

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36. The method of claim 35, wherein the cell is a hematopoietic stem cell, a megakaryocyte, an endothelial cell, a cardiomyocyte, a hepatocyte, or a trophoblast.

37. The method of claim 36, wherein the cell is a hematopoietic stem cell.

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38. The method of any one of claims 35 to 37, wherein the subject is a human subject.

39. An rAAV particle comprising an rAAV capsid protein of any one of claims 23-38.

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40. A nucleic acid encoding an rAAV capsid protein of any one of claims 23-39.

41. The nucleic acid of claim 40, wherein the nucleic acid is a plasmid.

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42. A method for efficient AAV transduction of a host cell suspension, the method comprising contacting a host cell suspension with a recombinant AAV (rAAV) particle composition, wherein the host cell suspension has a density of greater than 4,000 cells per microliter.

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43. The method of claim 42, wherein the rAAV particle composition is a AAV2 or AAV6 particle composition.

44. The method of any one of claims 42-43, wherein the rAAV particle composition contains  $3 \times 10^3$ - $1 \times 10^4$  vector genomes(vg)/mL of rAAV particles.

45. The method of any one of claims 42-44, wherein the recombinant AAV (rAAV) particle within the composition comprises a nucleic acid vector that encodes a therapeutic protein.

46. The method of any one of claims 42-45, wherein the recombinant AAV (rAAV) particle within the composition comprises a modified capsid protein comprising a non-tyrosine residue at a position that corresponds to a surface-exposed tyrosine residue in a wild-type AAV2 or AAV6 capsid protein, a non-threonine residue at a position that corresponds to a surface-exposed threonine residue in the wild-type AAV2 or AAV6 capsid protein, a non-lysine residue at a position that corresponds to a surface-exposed lysine residue in the wild-type AAV2 or AAV6 capsid protein, a non-serine residue at a position that corresponds to a surface-exposed serine residue in the wild-type AAV2 or AAV6 capsid protein, or a combination thereof.

47. The method of claim 46, wherein the modified capsid protein comprises a non-tyrosine residue and/or a non-threonine residue at one or more of or each of Y705, Y731, and T492 of a wild-type AAV6 capsid protein.

48. The method of claim 46, wherein the modified capsid protein comprises a non-tyrosine residue and/or a non-threonine residue at one or more of or each of Y444, Y500, Y731, and T491 of a wild-type AAV2 capsid protein.

49. The method of claim 47 or 48, wherein the non-tyrosine residue is phenylalanine and the non-threonine residue is valine.

50. The method of any one of claims 42-49, wherein the host cell suspension comprises stem cells.

51. The method of any one of claims 42-50, wherein the host cell suspension comprises human cells.

52. The method of any one of claims 42-51, wherein the host cell suspension comprises hematopoietic stem cells.

5 53. The method of any one of claims 42-52, wherein the host cell suspension is a non-adherent host cell suspension.

54. The method of any one of claims 42-53, wherein the method further comprises administering host cells of the host cell suspension to a subject.

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55. The method of any one of claims 42-54, wherein host cells of the host cell suspension are obtained from a subject.

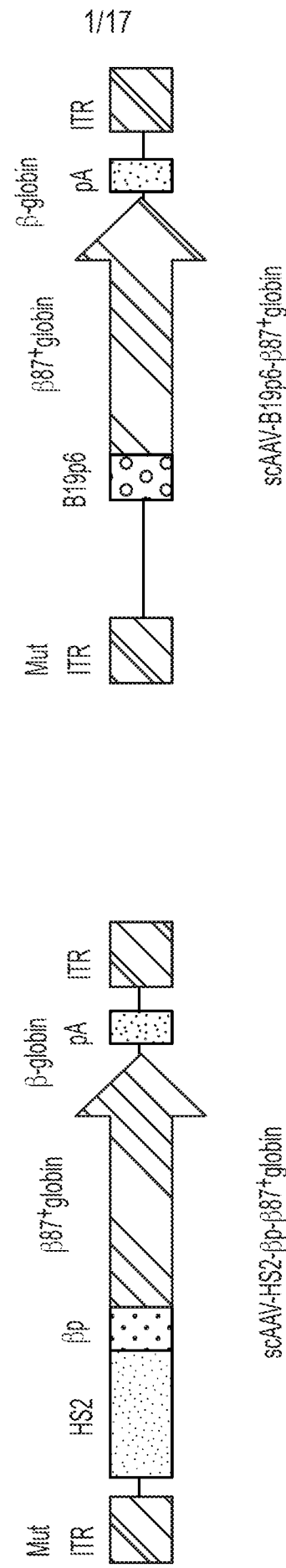


FIG. 1

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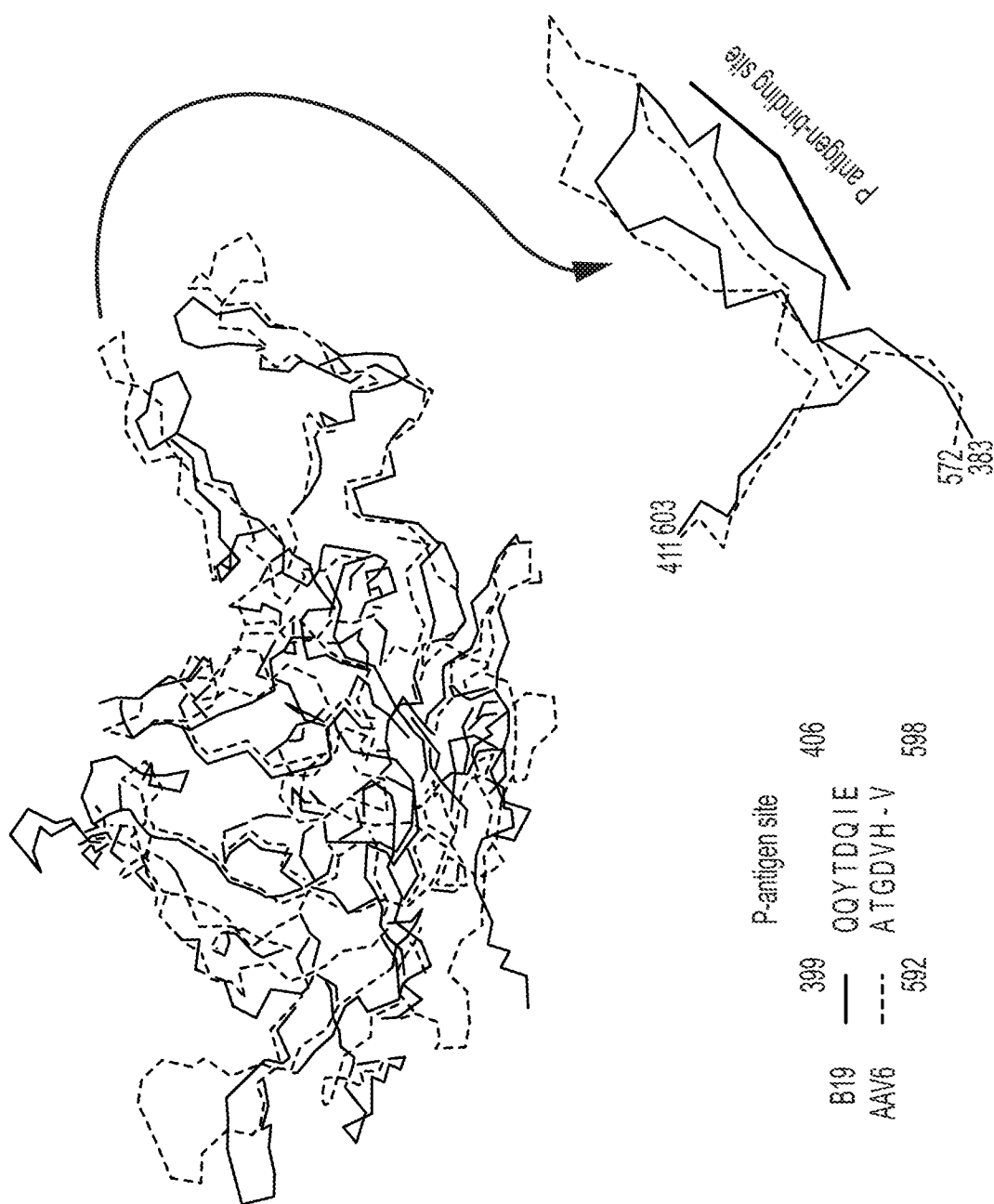


FIG. 2

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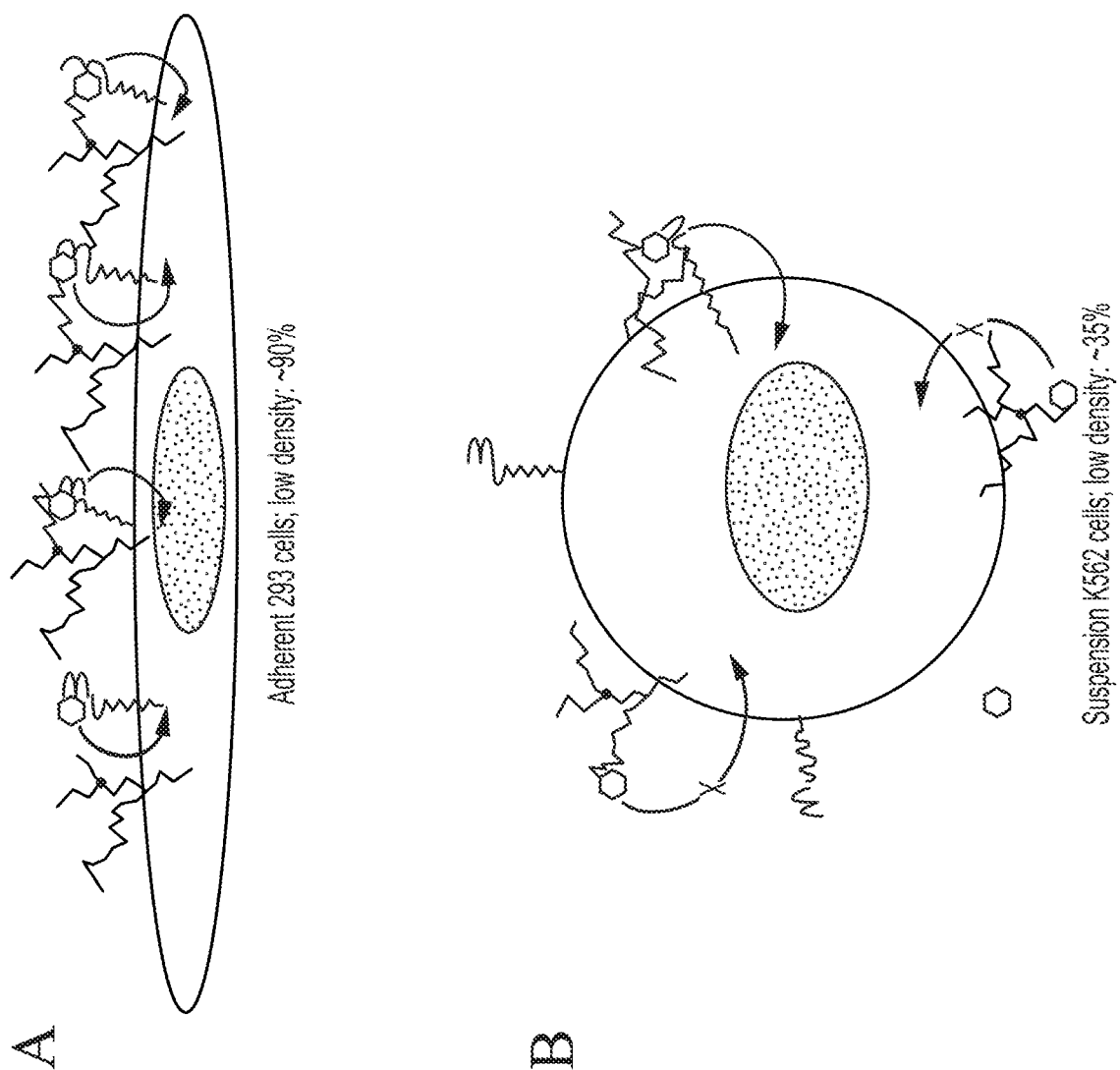


FIG. 3

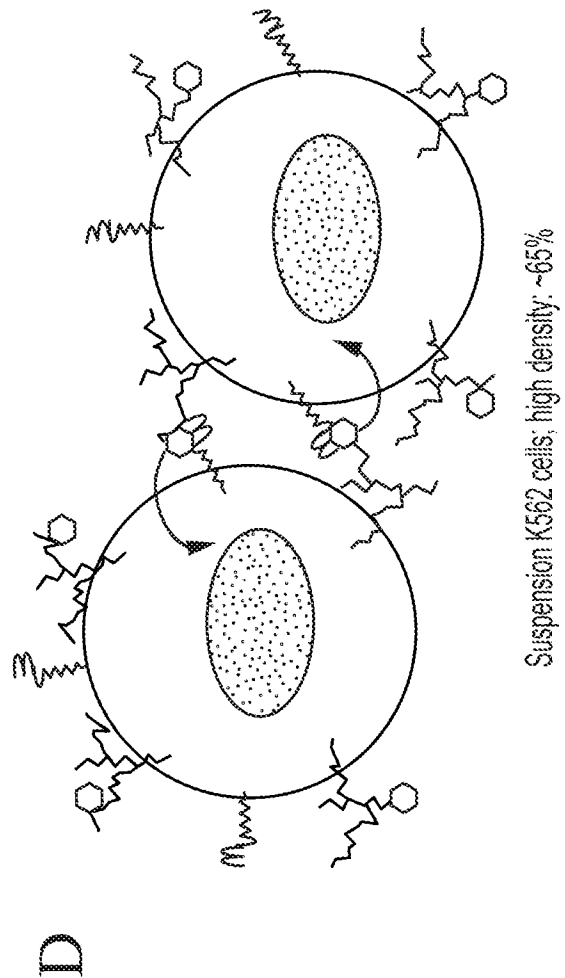
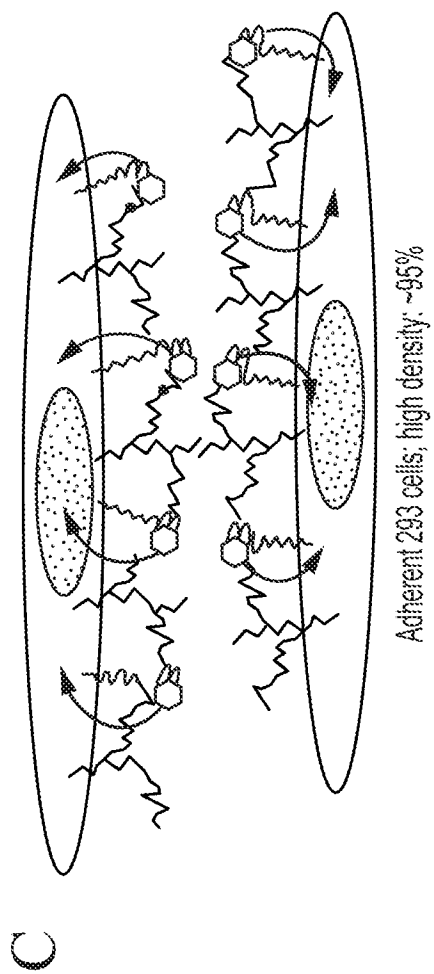


FIG. 3 (Continued)

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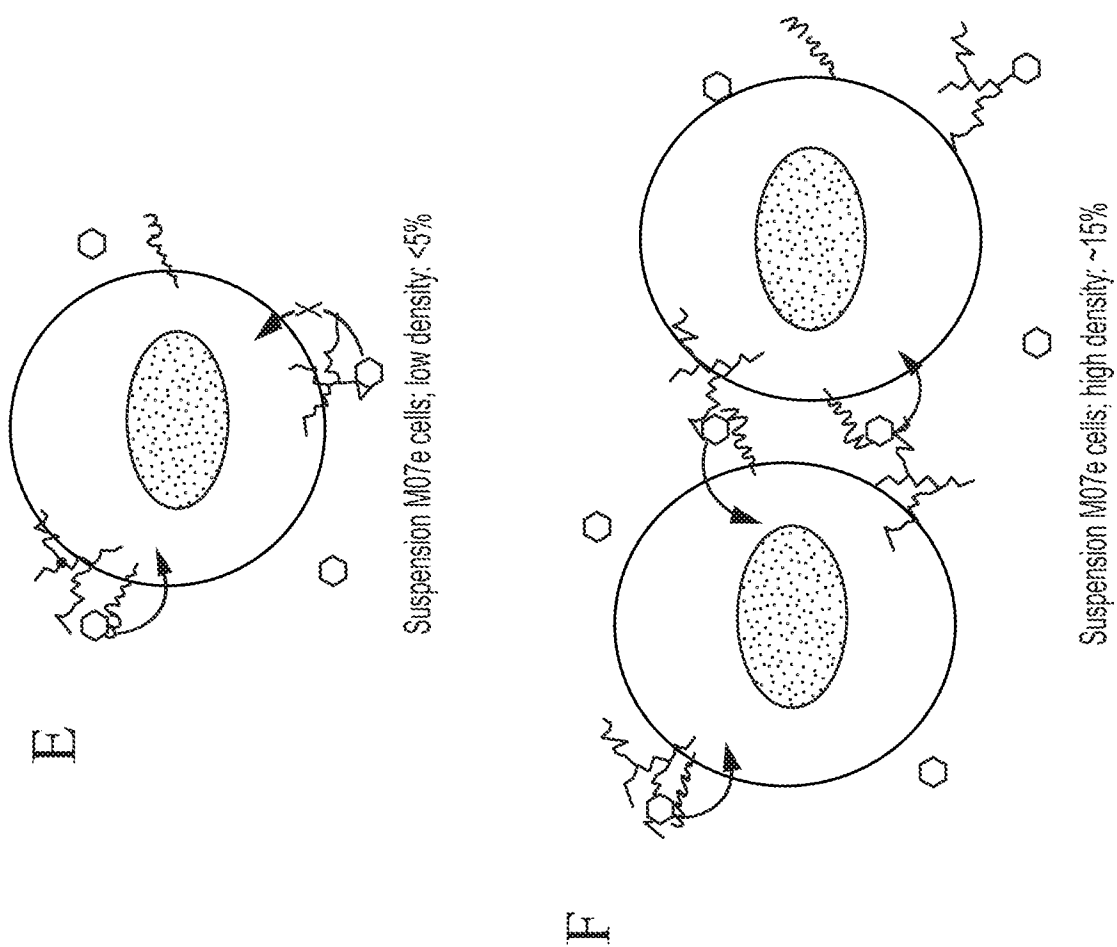
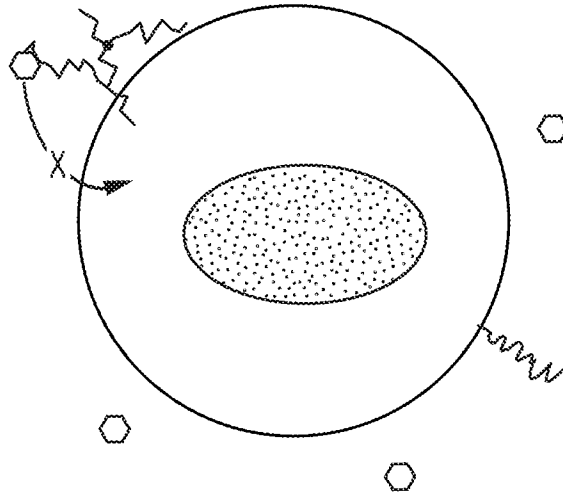


FIG. 3 (Continued)

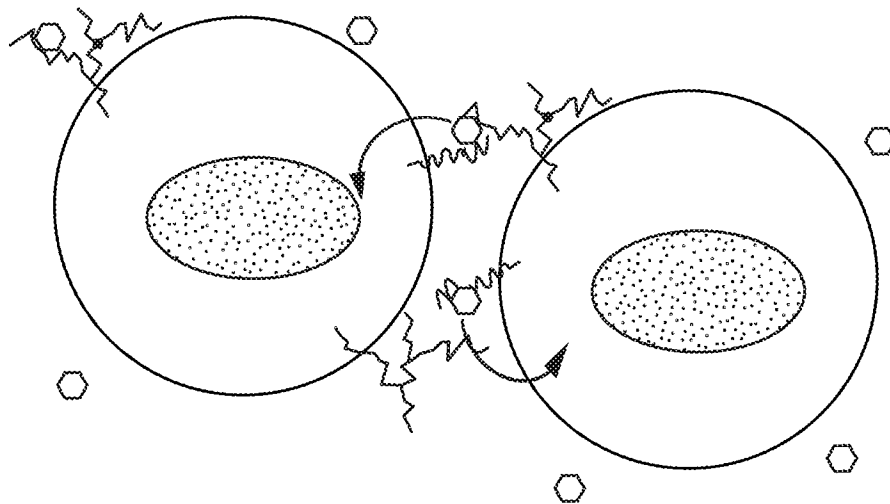
6/17

G



Suspension Raji cells; low density: &lt;1%

H



Suspension Raji cells; high density: ~5%

FIG. 3 (Continued)

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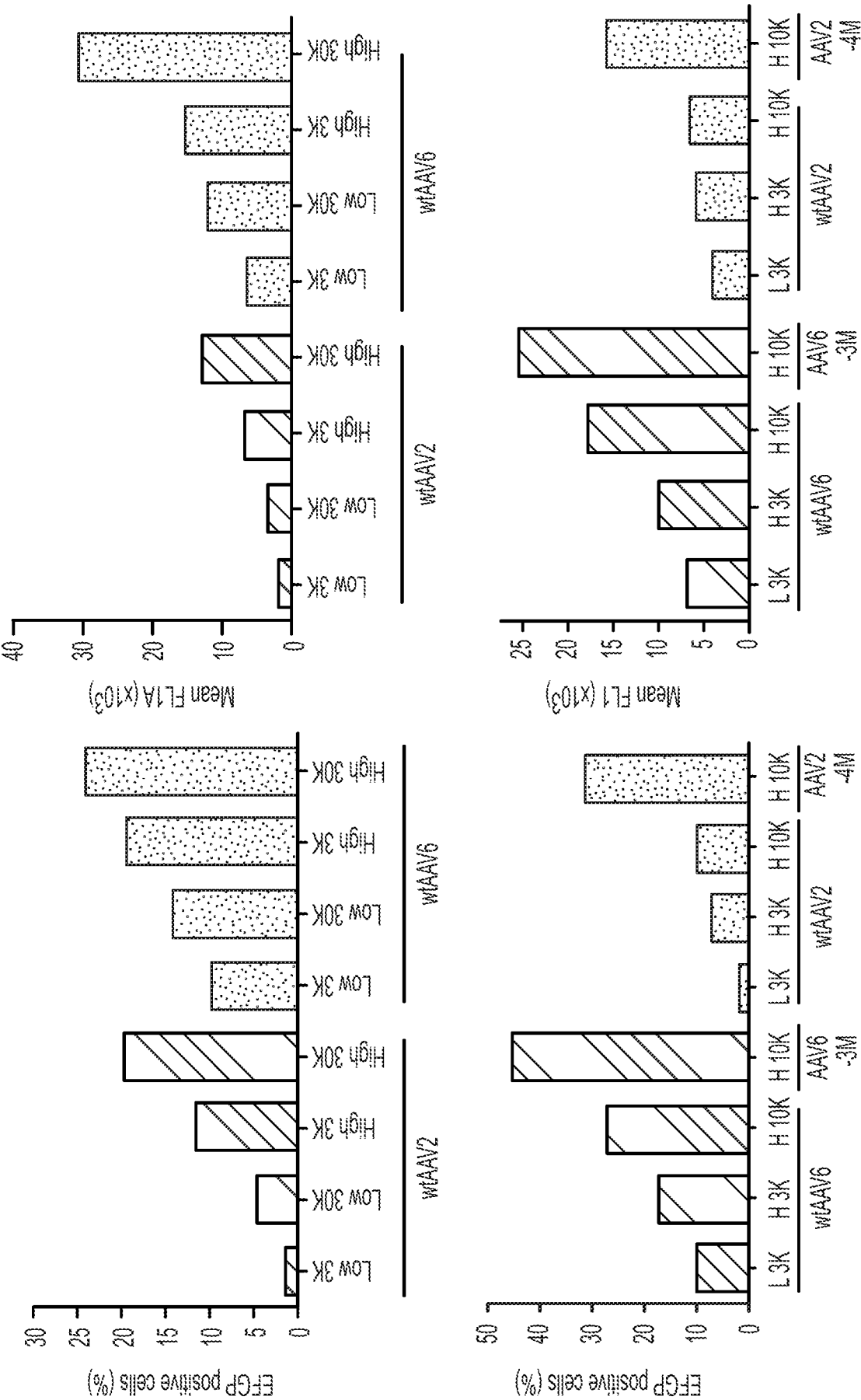


FIG. 4

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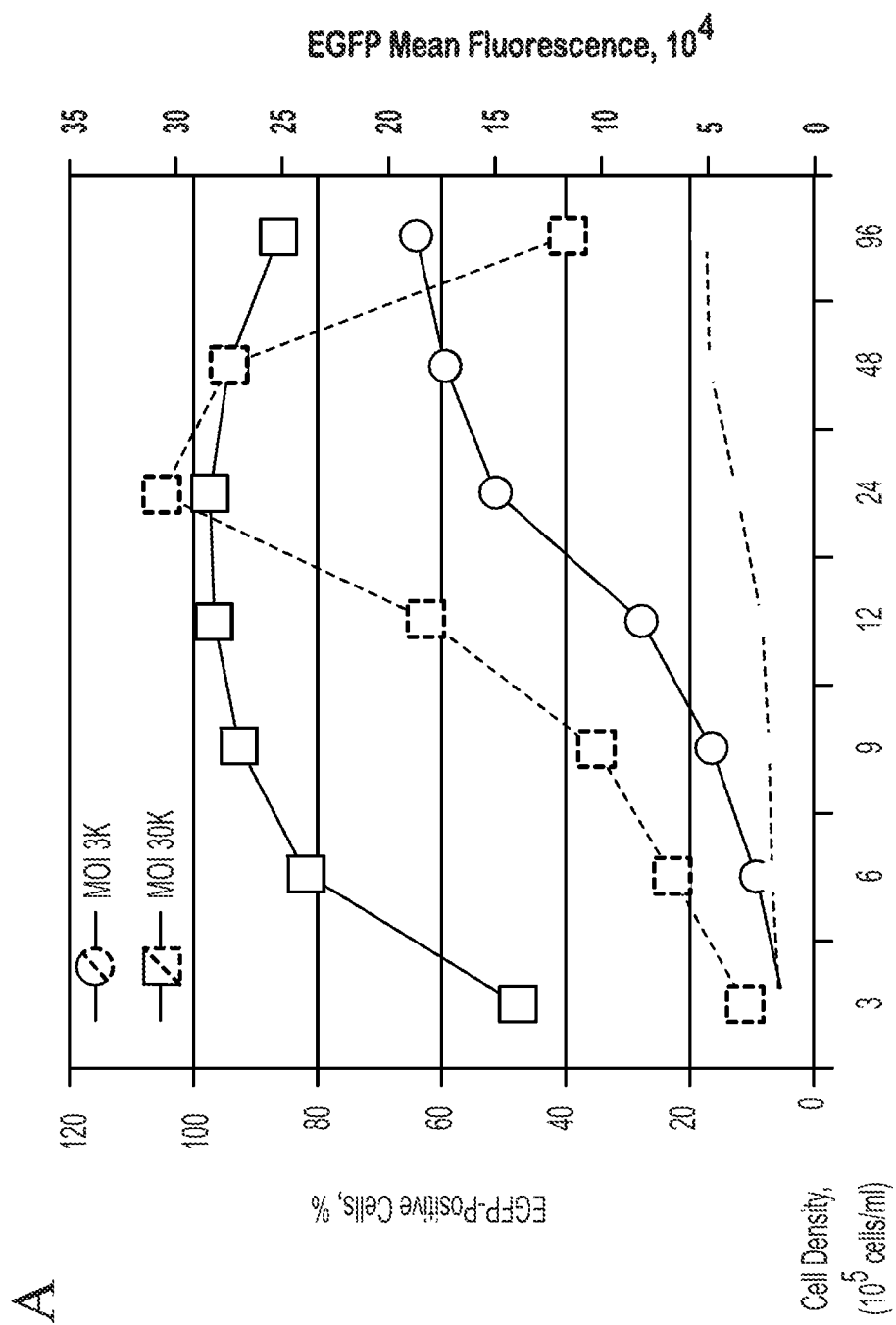


FIG. 5

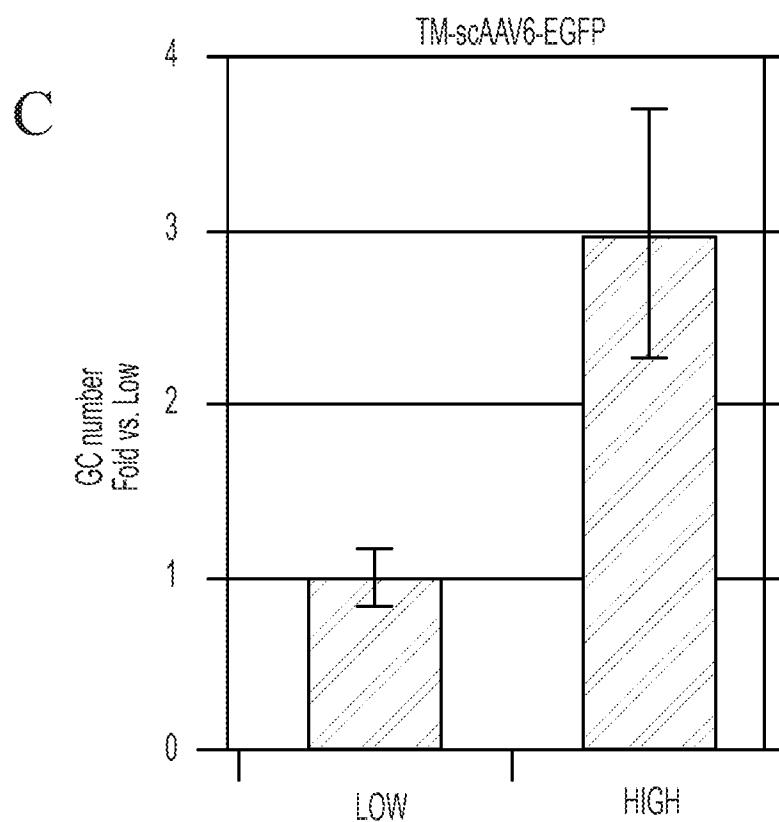
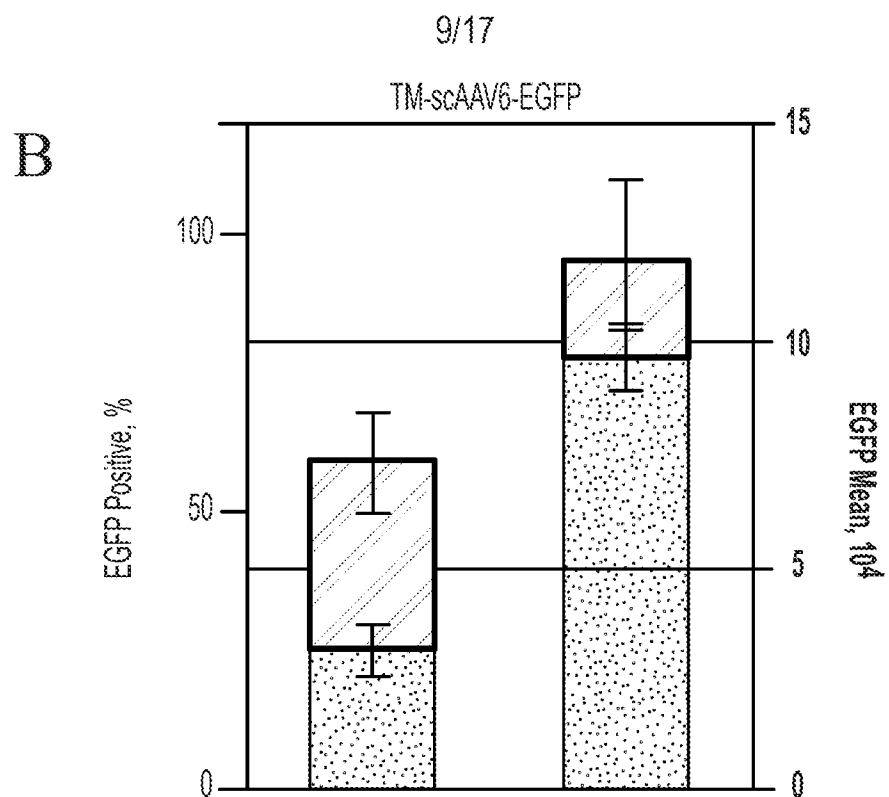
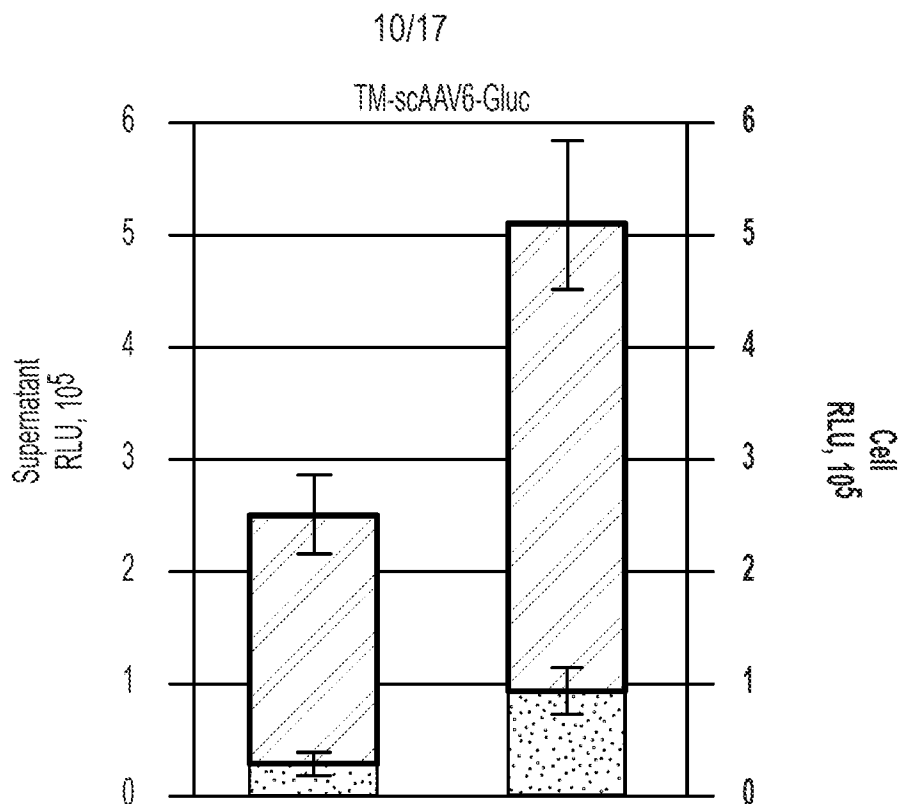


FIG. 5 (Continued)

D



E

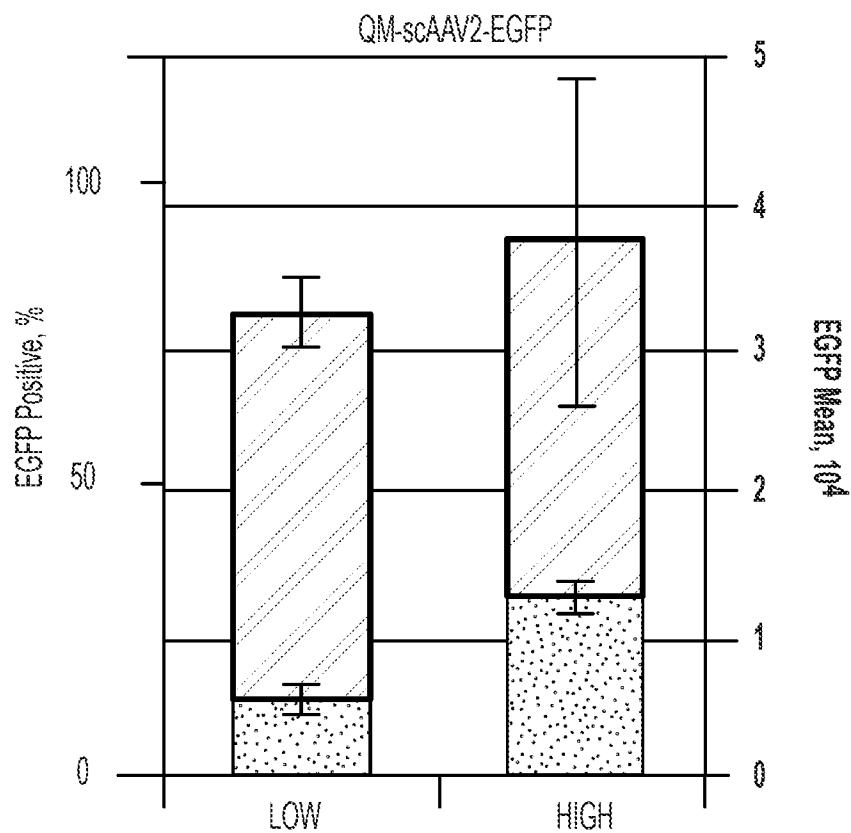


FIG. 5 (Continued)  
SUBSTITUTE SHEET (RULE 26)

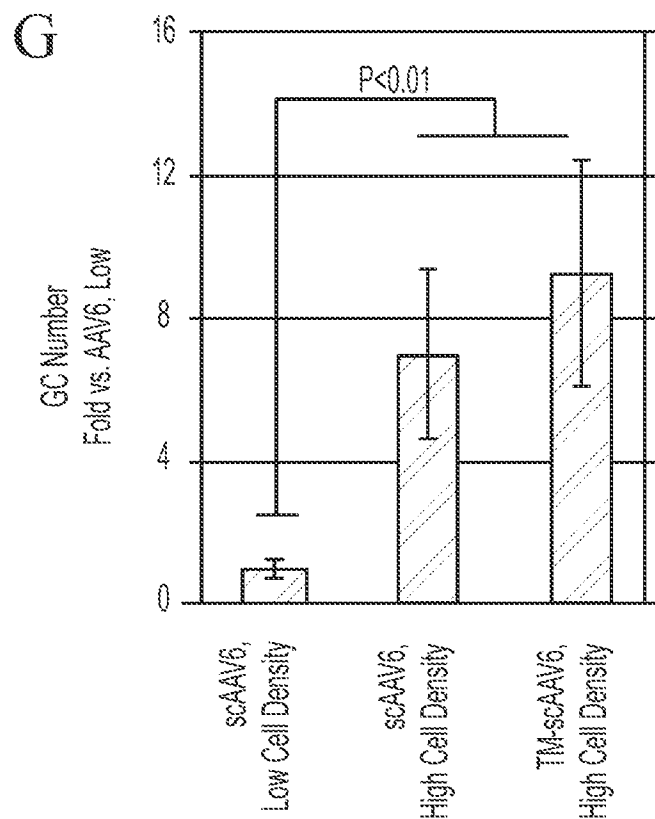
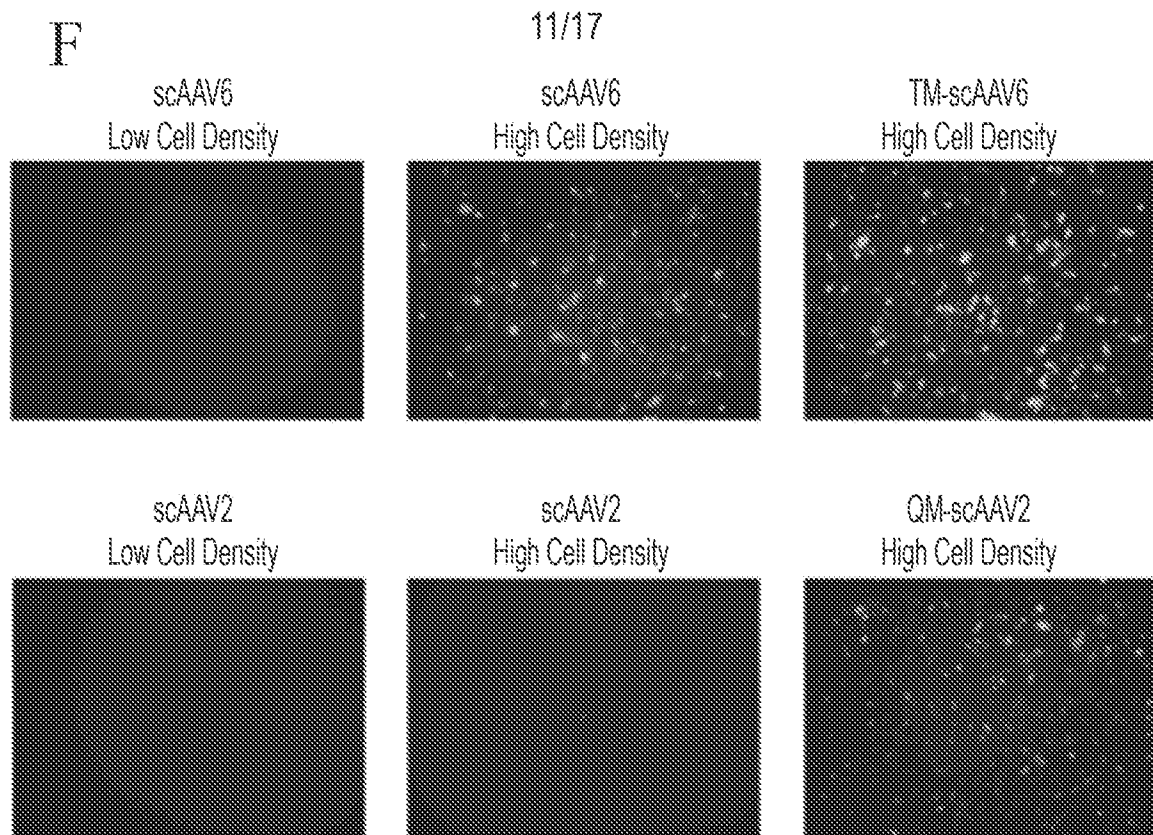


FIG. 5 (Continued)

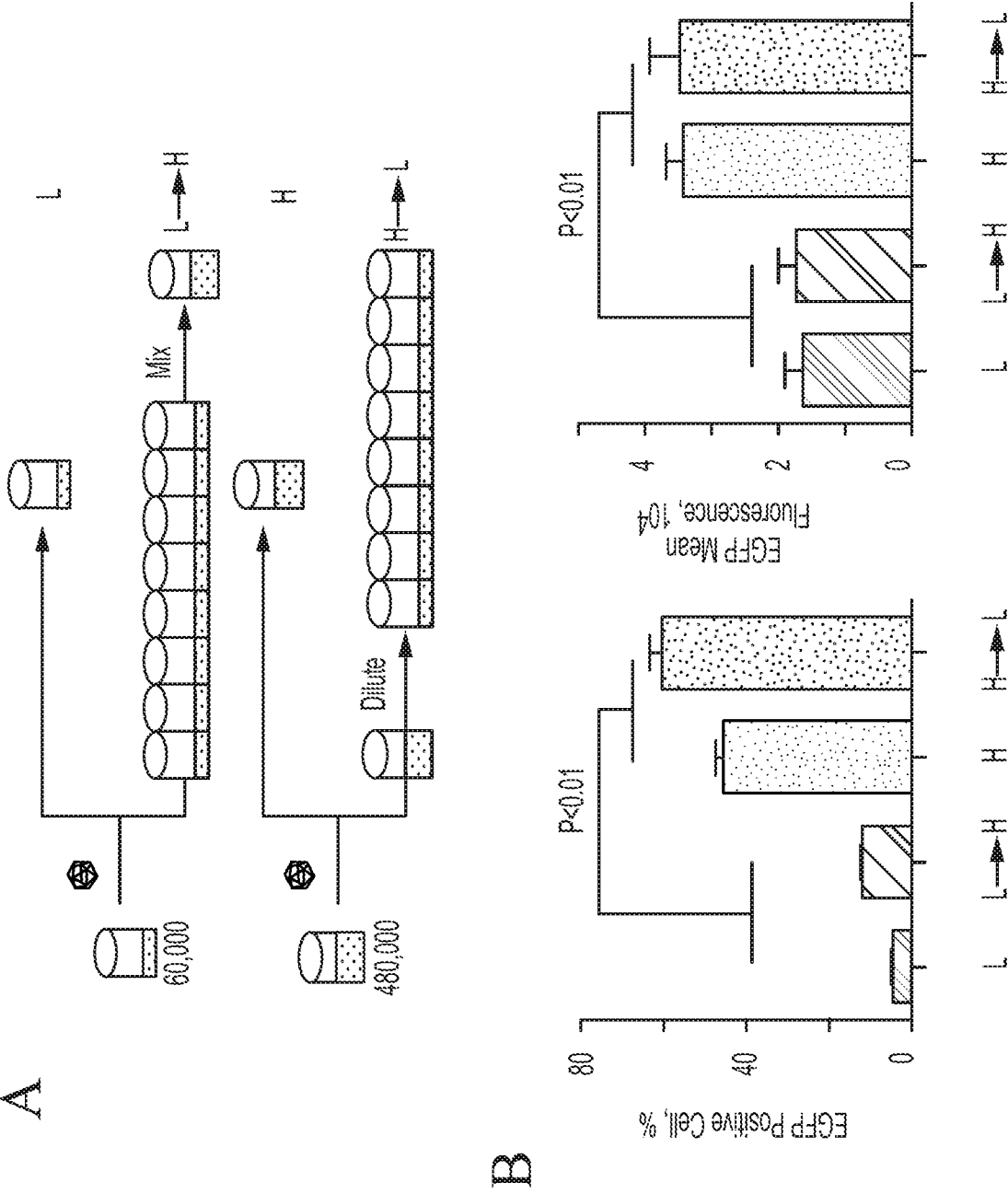


FIG. 6

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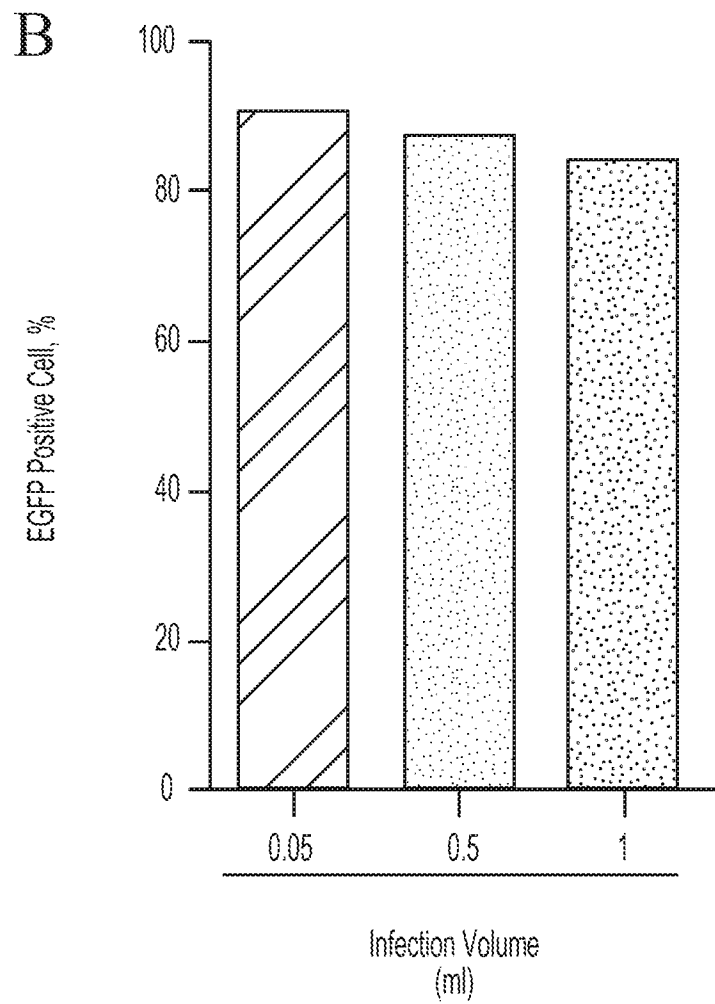
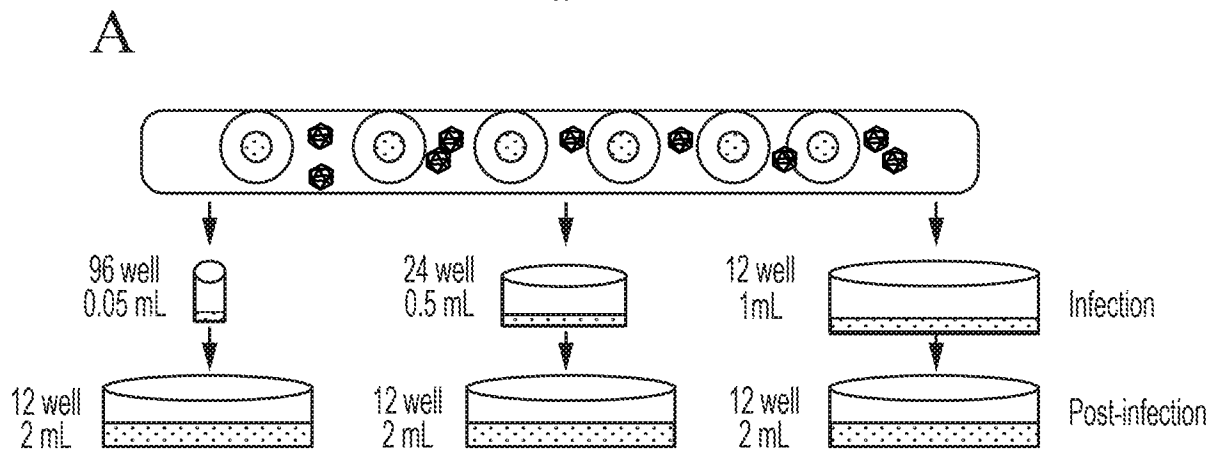


FIG. 7

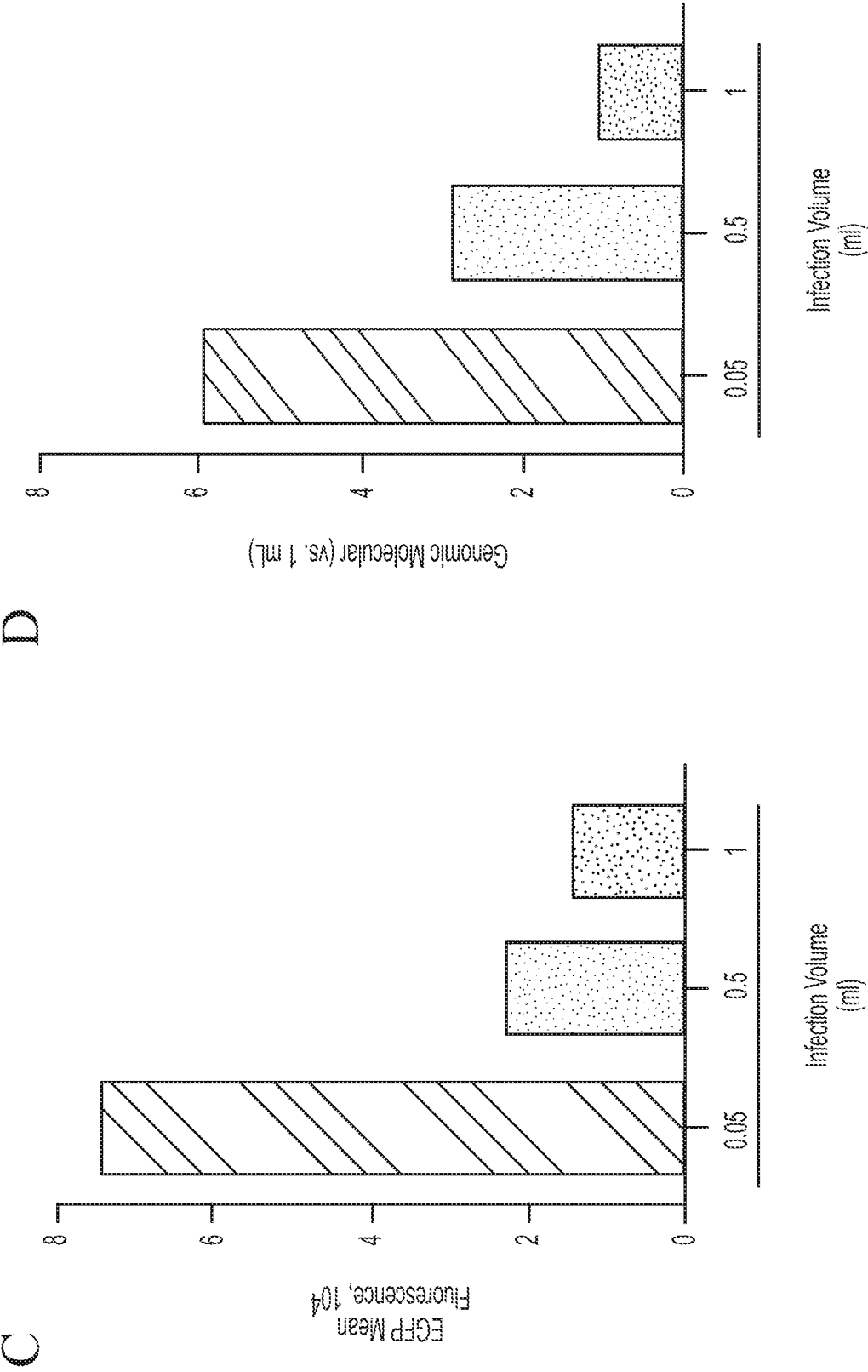


FIG. 7 (Continued)

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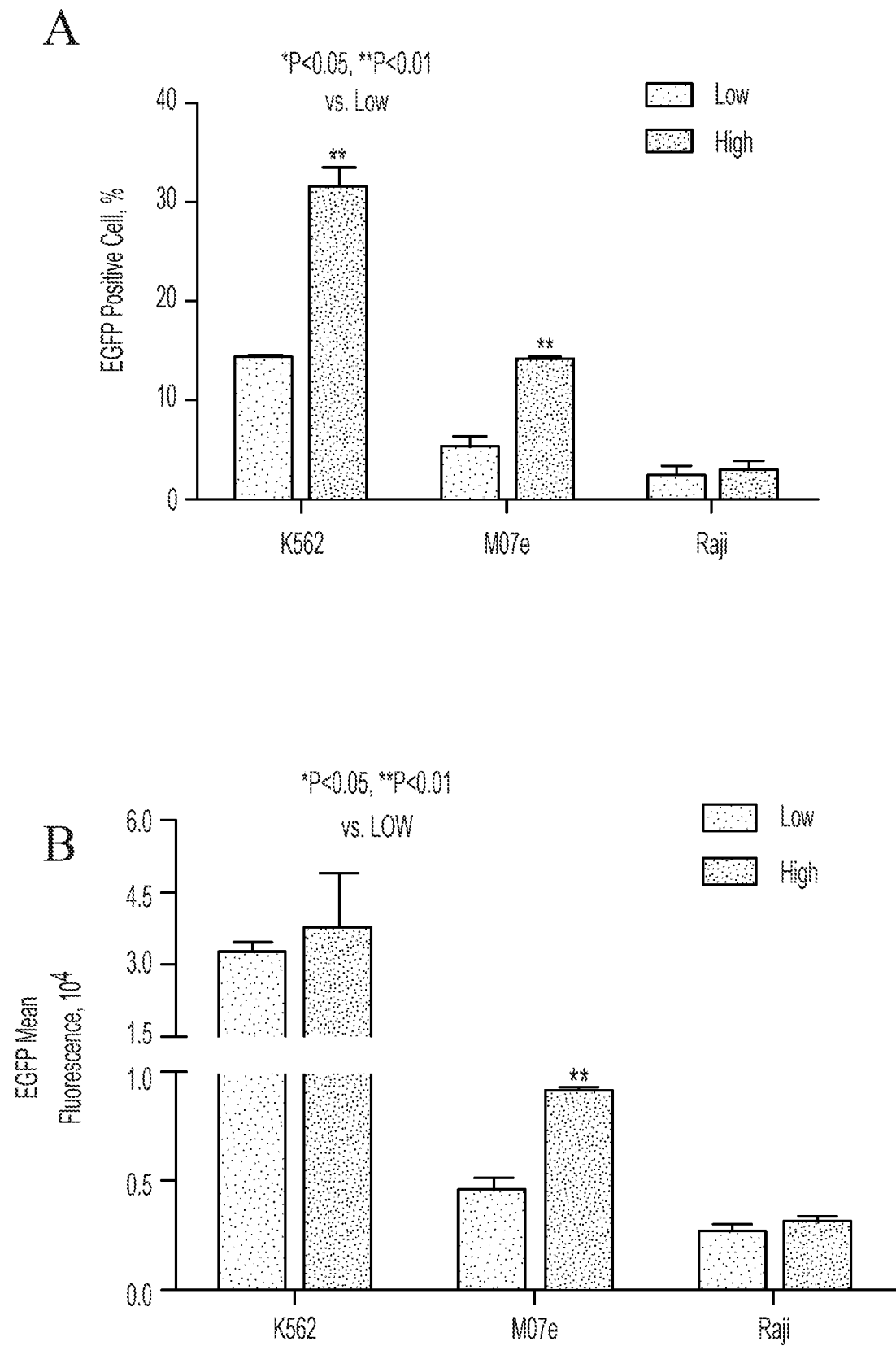


FIG. 8

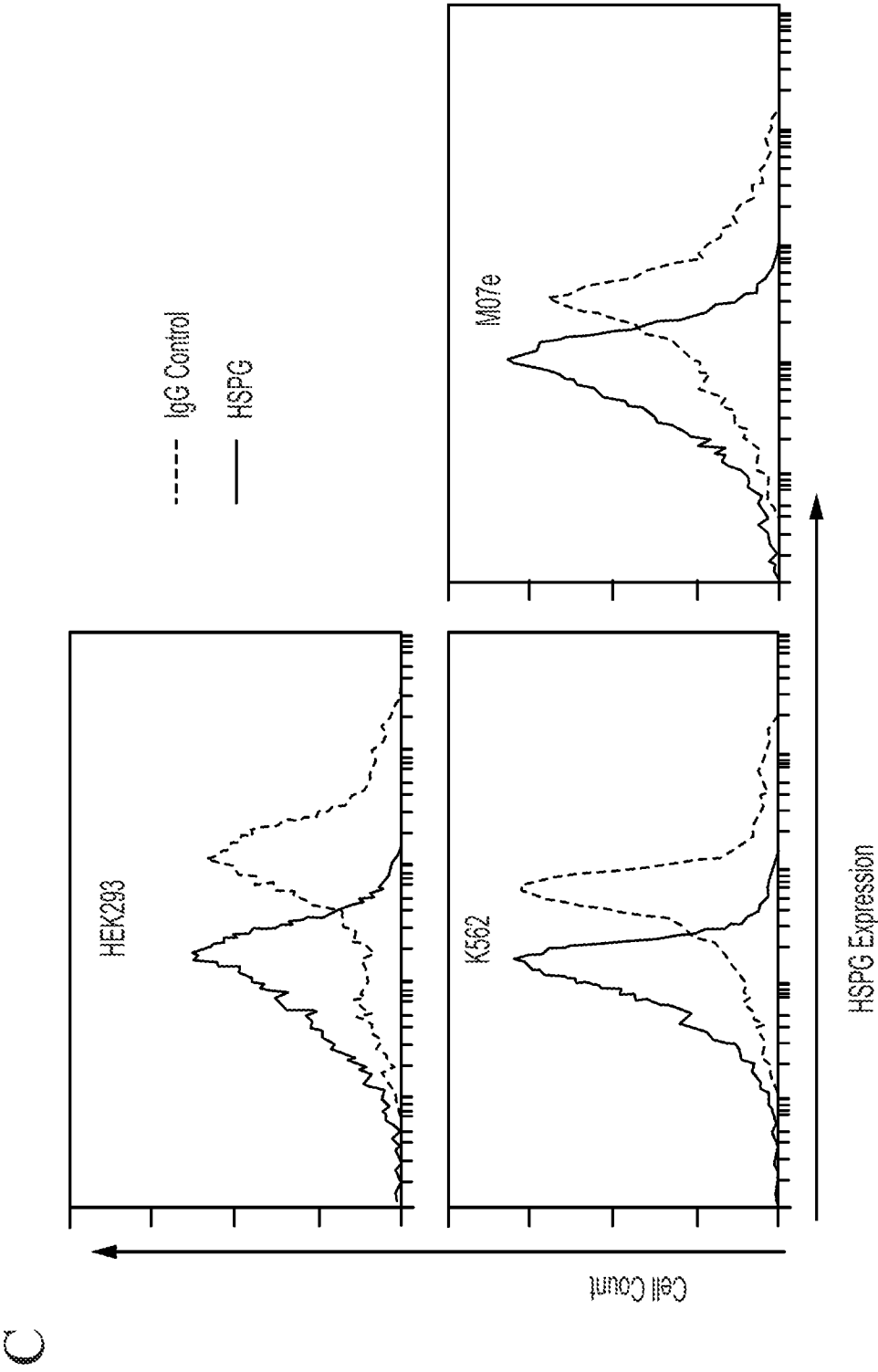


FIG. 8 (Continued)

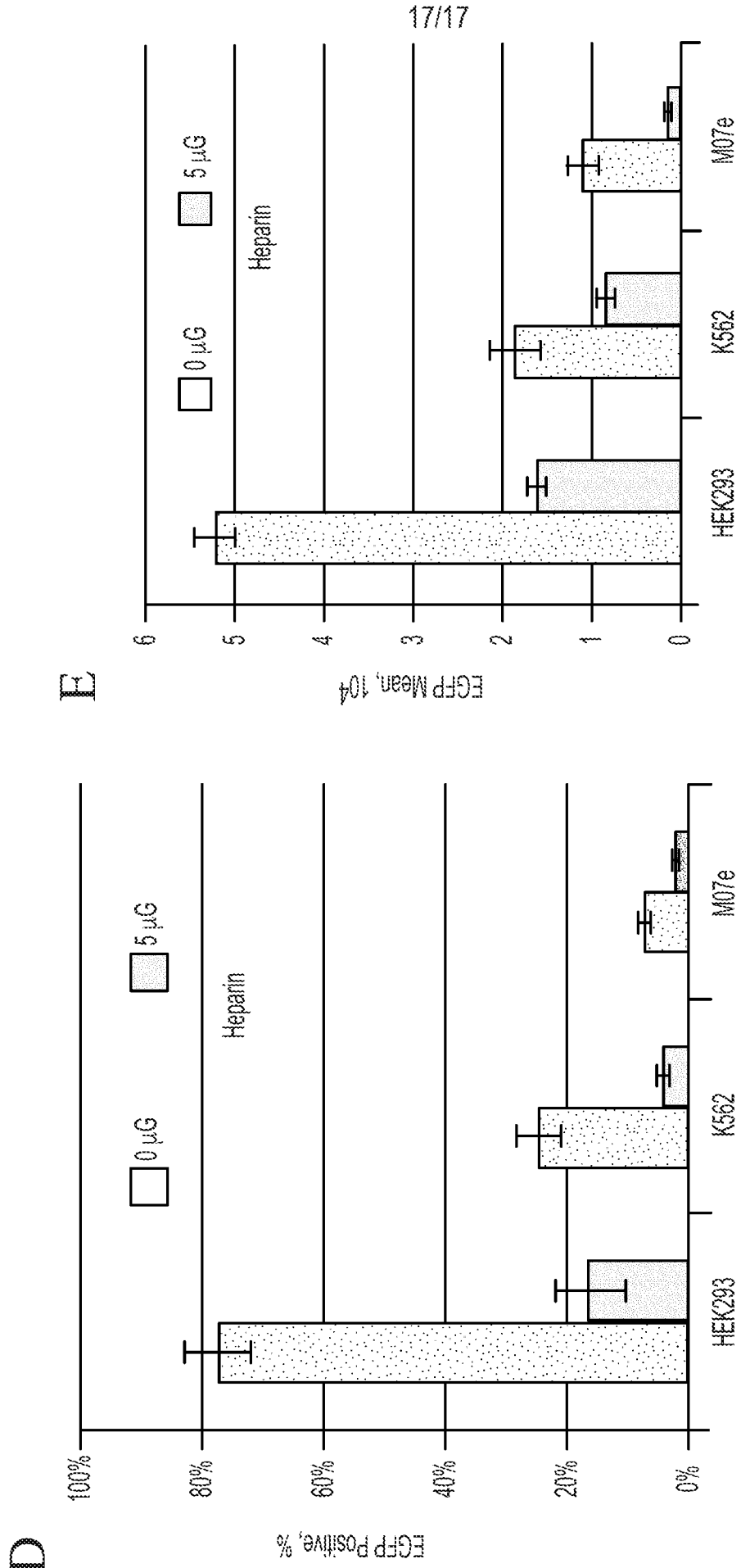


FIG. 8 (Continued)

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/18815

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/864, 15/85; C07K 14/805 (2016.01)

CPC - C12N 15/864, 15/8645, 15/85; C07K 14/805

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)

IPC(8) Classifications: C07K 14/005, 14/805, 14/015; A61K 38/42; C12N 7/00, 15/86, 15/864, 15/79, 15/85, 15/09, 15/63 (2016.01)

CPC Classifications: C07K 14/005, 14/805, 14/015; A61K 38/42, 48/00; C12N 7/00, 15/86, 15/864, 15/8645, 15/79, 15/85, 15/09, 15/63

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

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

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Google; Google Scholar; PubMed; EBSCO; Recombinant\*, AAV\*, rAAV\*, 'adeno-associated virus', Nucle\*, acid\*, vector\*, Viral\*, Plasmid\*, genom\*, Parvovirus\*, B19p6, 'B19 promoter at map unit 6', Link\*, Coupl\*, Join\*, bound\*, Heterolog\*, globin\*, gen\*, particl\*, 'University of Florida Research Foundation'

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                   | Relevant to claim No. |
|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X         | US 2014/0341852 A1 (UNIVERSITY OF FLORIDA RESEARCH FOUNDATION, INC.) 20 November 2014; paragraphs [0025], [0027], [0035], [0037], [0058], [0060], [0113], [0371], [0373], [0383], [0397], [0399], [0436], [0450]; claim 16.                                                                                                                          | 1-3, 11, 42, 43       |
| ---       |                                                                                                                                                                                                                                                                                                                                                      | ---                   |
| Y         |                                                                                                                                                                                                                                                                                                                                                      | 44/42-43              |
| Y         | (BARKER, SE et al.) Subretinal Delivery Of Adeno-Associated Virus Serotype 2 Results In Minimal Immune Responses That Allow Repeat Vector Administration In Immunocompetent Mice. The Journal of Gene Medicine. 1 April 2009, Vol. 11, pages 486-497, DOI: 10.1002/jgm.1327; abstract; page 489, first paragraph; page 496, second-third paragraphs. | 44/42-43              |

☐ 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

"&amp;" document member of the same patent family

Date of the actual completion of the international search

10 June 2016 (10.06.2016)

Date of mailing of the international search report

25 JUL 2016

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

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/18815

## 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.: 4-10, 12-22, 26-28, 32-41, 45-55  
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 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.:  
1-3, 11, 42-44

### 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**  
Information on patent family members

International application No.

PCT/US16/18815

----Continued from Box No. 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.

Group I, Claims 1-3, 11 and 42-44 are directed toward a recombinant AAV (rAAV) particle comprising a nucleic acid vector comprising a parvovirus B19p6 promoter operatively linked to a globin gene; and a method for efficient AAV transduction of a host cell suspension.

Group II, Claims 23-25 and 29-31 are directed toward an rAAV capsid protein comprising one or more amino acid substitutions that result in increased P antigen binding compared to a corresponding un-mutated AAV capsid protein, wherein the one or more amino acid substitutions are in a surface exposed loop of the capsid protein; and a method of increasing rAAV tropism for hematopoietic stem cells therewith.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include a B19p6 promoter, not present in Group II; the special technical features of Group II include an rAAV capsid protein comprising substitutions that increase P antigen binding, not present in Group I.

Groups I and II share the technical features including: rAAV; and host cells.

However, these shared technical features are previously disclosed by the abstract of the article 'Rationally designed capsid and transgene cassette of AAV6 vectors for dendritic cell-based cancer Immunotherapy' by Pandya et al. (hereinafter 'Pandya').

Pandya discloses rAAV (capsid-optimized adeno-associated virus (rAAV); abstract); and host cells (monocyte-derived dendritic cells (host cells); abstract).

Since none of the special technical features of the Groups I and II inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Pandya reference, unity of invention is lacking.