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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 August 2023 (17.08.2023)

WIPO | PCT

(10) International Publication Number
WO 2023/154763 A3

(51) International Patent Classification:
A61K 38/17 (2006.01) *C12N 15/86* (2006.01)

(21) International Application Number:
PCT/US2023/062231

(22) International Filing Date:
08 February 2023 (08.02.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/307,963 08 February 2022 (08.02.2022) US

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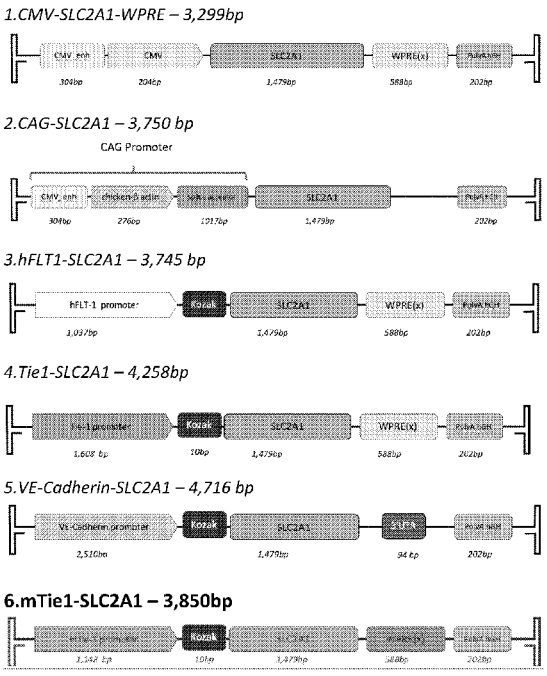
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, 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, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, 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, ME, 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).

(54) Title: ADENO-ASSOCIATED VIRAL VECTOR FOR GLUT1 EXPRESSION AND USES THEREOF

FIG. 1



(57) Abstract: Provided herein is a gene therapy for GLUT1 Deficiency Syndrome and related disorders using a recombinant adeno-associated virus (rAAV) virion as a vector to express an GLUT1 protein or functional variant thereof. The capsid may be an AAV-BR1 capsid or a functional variant thereof. Other promoters or capsids may be used. The rAAV virion may use an endothelial-specific promoter, *e.g.*, a FLT-1 promoter. Further provided are methods of treatment, such as by intracerebrally and/or intravenously of the rAAV virion, and other compositions and methods.

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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))*

(88) Date of publication of the international search report:
28 September 2023 (28.09.2023)

ADENO-ASSOCIATED VIRAL VECTOR FOR GLUT1 EXPRESSION AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of priority to U.S. Provisional Patent Application No. 63/307,963, filed February 8, 2022, the disclosure of which is incorporated herein by reference in its entirety for all purposes.

STATEMENT REGARDING THE SEQUENCE LISTING

[0002] The Sequence Listing XML associated with this application is provided in XML file format and is hereby incorporated by reference into the specification. The name of the XML file containing the Sequence Listing XML is ROPA_027_01WO_ST26.xml. The XML file is 223m012 bytes, and created on February 8, 2023, and is being submitted electronically via USPTO Patent Center.

BACKGROUND

[0003] Mutations in the *SLC2A1* gene encoding glucose transporter 1 (GLUT1) are associated with a neurodevelopmental disorder termed GLUT1 Deficiency Syndrome (GLUT1 DS). GLUT1 DS is an autosomal-dominant disorder which is often presents as a sporadic disease with *de novo* mutations producing haploinsufficiency and conferring symptomatic heterozygosity.

[0004] GLUT1 is an insulin-independent glucose transporter. Patients with classic GLUT1 DS, also known as De Vivo disease, suffer low brain glucose levels and exhibit a phenotype characterized by: early-onset seizures (median 12 months), delayed development, acquired microcephaly (decelerating head growth), complex movement disorders (spasticity, ataxia, dystonia); paroxysmal eye-head movements; and hypoglycorrhachia, or low glucose concentration in cerebrospinal fluid (CSF). The clinical course of the disease reveals the importance of early treatment. Alter et al. *J. Child Neurol.* 30(2):160-169 (2015). GLUT1 has been implicated in the function of endothelial cells, including transport of glucose across the blood-brain barrier (BBB), angiogenesis and maintenance of the BBB. However, studies in haploinsufficient mouse models have provided conflicting evidence concerning the role of GLUT1 in maintaining the physical integrity of the BBB. Although an endothelial cell

lineage-specific knockout of GLUT1 reduces endothelial energy availability and reduces proliferation without affecting migration, thereby delaying developmental angiogenesis (Veys et al., *Circ. Res.* 2020; 127:466-482), the effect of restoring GLUT1 expression specifically in endothelial cells has not been tested.

[0005] Therapeutic strategies for the disease are reviewed in Tang et al. *Ann. Clin. Trans. Neurol.* 2019; 6(9):1923-1932. Current standard of care is ketogenic diet, which raises the levels of ketones, which substitute for glucose, in the blood to make them available to the brain. Treatment with the triglyceride Triheptanoin has been proposed as an alternative to ketogenic diet. Gene therapy using adeno-associated virus (AAV) vectors have also been attempted. Targeting GLUT1 deficiency in neurons, AAV9 vectors encoding GLUT1 under the control of a neuron-specific promoter (*e.g.*, synapsin) have been tested in a young postnatal mouse model. Other studies employed a constitutive promoter (*e.g.*, CMV promoter) or the promoter of the endogenous GLUT1 gene. Various small molecules have also been tested, including the anticonvulsant carbonic anhydrase inhibitor acetazolamide and others.

[0006] While haploinsufficiency of GLUT1 arrests brain angiogenesis resulting in a relatively diminutive cerebral microvasculature, which may be related to glucose-dependence of endothelial tip cells, Tang et al. have observed that whether low GLUT1 in endothelial cells triggers this pathology remains to be investigated. The GLUT1 protein is expressed in additional brain cells including oligodendrocytes, microglia, and ependymal cells.

[0007] There is an unmet need for therapy for GLUT1 Deficiency Syndrome. The gene therapies provided herein address this need.

SUMMARY

[0008] The present invention relates generally to gene therapy for neurological disease or disorders using adeno-associated virus (AAV)-based delivery of a polynucleotide encoding GLUT1 or a functional variant thereof.

[0009] Although GLUT1 Deficiency Syndrome (DS) is a neurodevelopmental disorder with clinical manifestations rooted in lack of appropriate neuronal function, the present gene therapy may, without being bound by theory, target endothelial cells responsible for guiding the angiogenesis and development of the vasculature in the central nervous system (CNS).

Delivery of AAV to the developing central nervous system CNS vasculature either via intravenous, direct delivery to intracerebroventricular system, or by a combination of both routes, with subsequent GLUT1 protein expression in endothelial tip cells, may promote vascular growth and formation throughout the CNS during a critical window of angiogenesis and neurodevelopment. In addition, delivery of AAV to the CNS vasculature, with subsequent GLUT1 protein expression in mature endothelial cells may promote increased availability of glucose to neurons by increasing the transport of glucose across the blood-brain barrier.

[0010] In one aspect, the disclosure provides a recombinant adeno-associated virus (rAAV) virion, comprising a vector genome and a capsid, wherein the vector genome comprises an expression cassette, flanked by 5′ and 3′ inverted terminal repeats (ITRs), wherein the expression cassette comprises a polynucleotide sequence encoding GLUT1 or a functional variant thereof, operatively linked to a promoter, and wherein the capsid is a BR1 capsid or a functional variant thereof.

[0011] In some embodiments, the capsid is a BR1 capsid.

[0012] In some embodiments, the capsid comprises the polypeptide sequence motif XDGXXWX, wherein each X is any amino acid (SEQ ID NO: 107).

[0013] In some embodiments, the capsid comprises the polypeptide sequence ADGVQWT (SEQ ID NO:108), DDGVSWK (SEQ ID NO:109), SDGLTWS (SEQ ID NO:110) or SDGLAWV (SEQ ID NO:111).

[0014] In some embodiments, the capsid comprises the polypeptide sequence NRGTEWD or a functional variant having 1, 2, 3, or more substitutions thereto.

[0015] In some embodiments, the capsid comprises the polypeptide sequence NRGTEWD (SEQ ID NO:112).

[0016] In some embodiments, the capsid comprises an insertion of the polypeptide sequence NRGTEWD in the GH loop compared to an AAV2 VP1 reference sequence as set forth in SEQ ID NO 76.

[0017] In some embodiments, the capsid comprises a VP3 polypeptide that shares at least 98%, at least 99%, or 100% identity to an AAV2 VP3 polypeptide sequence as set forth in SEQ ID NO: 106.

[0018] In some embodiments, the capsid comprises a VP2 polypeptide that shares at least 98%, at least 99%, or 100% identity to an AAV2 VP2 polypeptide sequence as set forth in SEQ ID NO: 105.

[0019] In some embodiments, the capsid comprises a VP1 polypeptide that shares at least 98%, at least 99%, or 100% identity to an AAV2 VP1 polypeptide sequence as set forth in SEQ ID NO: 104.

[0020] In some embodiments, the promoter is a FLT-1 promoter.

[0021] In some embodiments, the FLT-1 promoter is a human FLT-1 (hFLT-1) promoter.

[0022] In some embodiments, the hFLT-1 promoter shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity with SEQ ID NO: 1.

[0023] In some embodiments, the expression cassette comprises a polyA signal, optionally a human growth hormone (hGH) polyA.

[0024] In some embodiments, the expression cassette comprises a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), optionally a WPRE(x).

[0025] In some embodiments, the expression cassette comprises a 3′ untranslated region (3′ UTR) comprising a sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity with SEQ ID NO: 4.

[0026] In some embodiments, the polynucleotide sequence encoding GLUT1 is a SLC2A1 polynucleotide.

[0027] In some embodiments, the SLC2A1 polynucleotide is a human SLC2A1 polynucleotide.

[0028] In some embodiments, the the polynucleotide sequence encoding GLUT1 shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity with SEQ ID NO: 5.

[0029] In some embodiments, the expression cassette is flanked by 5′ and 3′ inverted terminal repeats (ITRs), optionally AAV2 ITRs, optionally an ITR that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity with SEQ ID NO: 6 or SEQ ID NO: 7.

[0030] In some embodiments, the expression cassette shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity with any one of SEQ ID NOs: 8-16, SEQ ID NO: 97, SEQ ID NO: 99, and SEQ ID NO: 101.

[0031] In some embodiments, the rAAV virion is not an AAV2 virion.

[0032] In some embodiments, the disclosure provides a method of treating and/or preventing a disease or disorder in a subject in need thereof, comprising administering the rAAV virion to the subject.

[0033] In some embodiments, the disease or disorder is a neurological disorder.

[0034] In some embodiments, the disease or disorder is Glucose transporter 1 deficiency syndrome (GLUT1DS) or De Vivo Disease.

[0035] In some embodiments, the rAAV virion is administered by intracerebroventricular (ICV) injection.

[0036] In some embodiments, the rAAV virion is administered by an intravenous (IV) injection.

[0037] In some embodiments, the rAAV virion is administered by ICV injection in combination with an IV injection.

[0038] In some embodiments, the administration results in expression of the polynucleotide sequence encoding GLUT1 in the brain, optionally at increased levels compared to a reference rAAV virion.

[0039] In some embodiments, the administration results in an increase in expression of GLUT1 protein in the brain and/or an increase in glucose levels and/or lactate levels in the CSF, optionally at increased levels compared to a reference rAAV virion, wherein optionally the increases is an increase of at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or 100%.

[0040] In some embodiments, the reference rAAV virion is a variant of an AAV2 virion.

[0041] In some embodiments, the reference rAAV virion is an AAV-BR1 virion.

[0042] In some embodiments, the rAAV virion is administered at a dose of 1×10^{12} vector genomes (vg), 1×10^{13} vg, 1×10^{14} vg, or 3×10^{14} vg.

[0043] In some embodiments, the method causes increased glucose uptake by cerebral microvasculature endothelial cells compared to a method performed using an endogenous Glut1 promoter or a ubiquitous promoter.

[0044] In some embodiments, the disclosure provides a method of expressing GLUT1 in a cell, comprising contacting the cells with the rAAV virion.

[0045] In some embodiments, the cell is an endothelial cell.

[0046] In some embodiments, the endothelial cell is a cerebral microvasculature endothelial cell.

[0047] In some embodiments, the endothelial cell is an in vivo endothelial cell.

[0048] In some embodiments, the cell is a neuron.

[0049] In some embodiments, the neuron is an in vivo neuron.

[0050] In some embodiments, the method comprises in vivo administration of the rAAV virion to a subject.

[0051] In some embodiments, the rAAV virion causes increased glucose uptake by the cell compared to a cell contacted with a rAAV virion comprising an endogenous Glut1 promoter or a ubiquitous promoter.

[0052] In some embodiments, the disclosure provides a pharmaceutical composition comprising the rAAV virion.

[0053] In some embodiments, the disclosure provides a kit comprising the rAAV virion or a pharmaceutical composition and optionally instructions for use.

[0054] In further aspects, the disclosure provides polynucleotides (*e.g.*, vector genomes), pharmaceutical compositions, kits, and other compositions and methods.

[0055] Various other aspects and embodiments are disclosed in the detailed description that follows. The invention is limited solely by the appended claims.

BRIEF DESCRIPTION OF FIGURES

[0056] **FIG. 1** shows a vector diagrams for various non-limiting examples of a vector genome.

[0057] **FIG. 2** shows a vector diagram of a non-limiting example of a vector genome. The full polynucleotide sequence of the vector genome is SEQ ID NO: 17. The capitalized portion is the expression cassette (SEQ ID NO: 8).

[0058] **FIG. 3** shows a vector diagram of a non-limiting example of a vector genome. The full polynucleotide sequence of the vector genome is SEQ ID NO: 19. The capitalized portion is the expression cassette (SEQ ID NO: 10).

[0059] **FIG. 4** shows a vector diagram of a non-limiting example of a vector genome. The full polynucleotide sequence of the vector genome is SEQ ID NO: 21. The capitalized portion is the expression cassette (SEQ ID NO: 12).

[0060] **FIG. 5** shows a vector diagram of a non-limiting example of a vector genome. The full polynucleotide sequence of the vector genome is SEQ ID NO: 96. The capitalized portion is the expression cassette (SEQ ID NO: 97). An alternative of the full polynucleotide sequence of the vector genome is SEQ ID NO: 23. An alternative of the expression cassette is SEQ ID NO: 14.

[0061] **FIG. 6** shows a vector diagram of a non-limiting example of a vector genome. The full polynucleotide sequence of the vector genome is SEQ ID NO: 25. The capitalized portion is the expression cassette (SEQ ID NO: 16).

[0062] **FIG. 7** shows a vector diagram of a non- limiting example of a vector genome. The full polynucleotide sequence of the vector genome is SEQ ID NO: 98. The capitalized portion is the expression cassette (SEQ ID NO: 99).

[0063] **FIG. 8** shows a vector diagram of a non- limiting example of a vector genome. The full polynucleotide sequence of the vector genome is SEQ ID NO: 100. The capitalized portion is the expression cassette (SEQ ID NO: 101).

[0064] **FIG. 9**. AAV9-mediated Expression of hGlut1 protein CHO-Lec2 Cells. CHO-Lec2 cells were transduced with AAV9 vectors expressing the hGlut1 transgene protein driven by one of several endothelial-specific promoters (i.e., hFLT1, mTie1 or hGlut1) or by the ubiquitous CMV promoter. [SLC2A1 = GLUT1 Gene].

[0065] **FIGs. 10A-10C.** Expression of transgene protein (Glut1-GFP) following transfection of human cerebral microvasculature endothelial cells (hCMEC/d3s).

[0066] **FIG. 10A**. GFP fluorescence 72 hours following transfection with constructs containing one of several endothelial cell promoters driving expression of Glut1-GFP transgene.

[0067] **FIG. 10B**. GFP fluorescence 72 hours following transfection with constructs containing one of two ubiquitous promoters (CMV or CAG), control vector without Glut1 (CMV-GFP) or no transfection (No NFX). Images obtained using Operetta CLS™ (PerkinElmer®).

[0068] **FIG. 10C**. Diagram of expression cassette containing the promoter of interest (hFLT1, mTie, hTie or hGlut1) and the GLUT1 (SLC2A1) gene (T2A linked-GFP) and regulatory elements flanked by AAV2 inverted terminal repeats (ITRs).

[0069] **FIGs. 11A-11C**. 2-Deoxy-D-glucose (glucose) Uptake in hCMEC/d3 cells following expression of human GLUT1 (SLC2A1). Human cerebromicrovascular endothelial cells (hCMEC/d3s) were transfected with plasmids expressing either CAG-GFP (negative control) or with a hGLUT1-t2A-eGFP transgene driven by one of several endothelial-specific

promoters (i.e., hFLT1, mTie1 or hGlut1) or by the ubiquitous CMV promoter. Glucose uptake was measured using a luminescence-based kit (Promega®) with 0.5 mM 2-Deoxy-D-glucose (2-DG) in culture media. Glucose uptake was normalized by Total Cells using phase-contrast imaging [Error bars represent S.E.M; n=6 replicates per condition].

[0070] **FIG. 11A**. Glucose (2-DG) uptake was measured at 72 hours post-transfection in a first experiment.

[0071] **FIG. 11B**. Glucose (2-DG) uptake was measured at 72 hours post-transfection in a second experiment.

[0072] **FIG. 11C**. Glucose (2-DG) uptake was measured at 96 hours post-transfection.

[0073] **FIGS 12A-12B**. 2-Deoxy-D-glucose (glucose) Uptake in hCMEC/d3 cells following expression of human GLUT1 (SLC2A1). Human cerebromicrovascular endothelial cells (hCMEC/d3s) were transfected with plasmids expressing a hGLUT1-t2A-eGFP transgene driven by one of several endothelial-specific promoters (i.e., hFLT1, mTie1 or hGlut1) or by the ubiquitous CMV promoter. Non-transfected hCMEC/d3 served as controls (CON). Glucose uptake was measured using a luminescence-based kit (Promega®) with varying concentrations (0 mM, 0.1 mM, 0.5 mM or 1.0 mM) of 2-Deoxy-D-glucose in the culture media. Glucose uptake was normalized on a per cell basis through multiplexing with the RealTime-Glo MT Cell Viability Assay (Promega®), performed according to the manufacturer's recommendations.

[0074] **FIG. 12A**. shows glucose uptake in hCMEC/d3 cells following expression of human Glut1 (*SLC2A1*) at a 72-hour time point.

[0075] **FIG. 12B**. shows glucose uptake in hCMEC/d3 cells following expression of human Glut1 (*SLC2A1*) at a 96-hour time point.

[0076] **FIG. 13.** 2-Deoxy-D-glucose (glucose) Uptake Following AAV9-mediated Expression of hGLUT1 (SLC2A1) in hCMEC/d3 Cells. Human cerebromicrovascular endothelial cells (hCMEC/d3s) were transduced with AAV9 vectors (3×10^5 vector genomes/cell) expressing either CAG-GFP (negative control) or the hGLUT1 transgene driven by one of several endothelial-specific promoters (i.e., hFLT1, mTie1 or hGlut1) or by the ubiquitous CMV promoter. Glucose (2-DG) uptake was measured 72 hours post-

transduction using the luminescence-based Glucose Uptake-Glo kit (Promega®) and normalized per cell using the RealTime-Glo MT Cell Viability Assay (Promega®) [Error bars represent S.E.M; n=4 replicates per condition].

DETAILED DESCRIPTION OF THE INVENTION

[0077] The present disclosure provided gene therapy vectors for GLUT1 that deliver a polynucleotide encoding GLUT1 or a functional variant thereof with an AAV virion having tropism for endothelial cells, such as AAV-BR1, along with methods of use, and other compositions and methods. In some embodiments, the AAV vector genome includes an endothelial cell-specific promoter, such as an FLT-1 promoter.

[0078] This disclosure further provides methods of treating a disease or disorder in a subject by administering a gene therapy vector of the disclosure. In some embodiments, the disease or disorder is Glucose transporter 1 deficiency syndrome (GLUT1 DS) or De Vivo Disease.

[0079] In accordance with the present invention, a polynucleotide encoding a GLUT1 or functional variant thereof may be employed in generating a gene therapy vector. The resulting vector may be employed in treating diseases or disorders, e.g. Glucose transporter 1 deficiency syndrome (GLUT1 DS), De Vivo Disease or others.

DEFINITIONS

[0080] The section headings are for organizational purposes only and are not to be construed as limiting the subject matter described to particular aspects or embodiments.

[0081] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are expressly incorporated by reference in their entirety. In cases of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples described herein are illustrative only and are not intended to be limiting.

[0082] All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as an acknowledgment, or any form of suggestion, that they constitute valid prior art or form part of the common general knowledge in any country in the world.

[0083] In the present description, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. The term "about", when immediately preceding a number or numeral, means that the number or numeral ranges plus or minus 10%. It should be understood that the terms "a" and "an" as used herein refer to "one or more" of the enumerated components unless otherwise indicated. The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. The term "and/or" should be understood to mean either one, or both of the alternatives. As used herein, the terms "include" and "comprise" are used synonymously.

[0084] The term "sequence identity" refers to the percentage identity of a polypeptide or polynucleotide sequence of interest to a reference sequence, calculated as 100 times the number of exact matches in an optimal alignment of the sequence of interest to the reference sequence divided by the total length of the reference sequence (including gaps). An optimal alignment of the sequences may be generated using the European Molecular Biology Open Software Suite (EMBOSS) needle program available at www.ebi.ac.uk, as described in Maderia et al. *Nucleic Acids Res.* 47(W1): W636-W641 (2019). When comparing DNA and RNA sequences, thymine (T) and uracil (U) are counted as a match.

[0085] As used herein, an "AAV vector" or "rAAV vector" refers to a recombinant vector comprising one or more polynucleotides of interest (or transgenes) that are flanked by AAV terminal repeat sequences (ITRs). Such AAV vectors can be replicated and packaged into infectious viral particles when present in a host cell that has been transfected with a plasmid encoding and expressing *rep* and *cap* gene products. Alternatively, AAV vectors can

be packaged into infectious particles using a host cell that has been stably engineered to express *rep* and *cap* genes.

[0086] As used herein, an "AAV virion" or "AAV viral particle" or "AAV vector particle" refers to a viral particle composed of at least one AAV capsid protein and an encapsidated polynucleotide AAV vector. As used herein, if the particle comprises a heterologous polynucleotide (*i.e.*, a polynucleotide other than a wild-type AAV genome such as a transgene to be delivered to a mammalian cell), it is typically referred to as an "AAV vector particle" or simply an "AAV vector." Thus, production of AAV vector particle necessarily includes production of AAV vector, as such a vector is contained within an AAV vector particle.

[0087] As used herein, "GH loop" refers to the loop created between the G and H strands of the jelly-roll β-barrel of the AAV capsid protein VP1, as described in Xie et al. *PNAS* 99(16):10405-10410 (2002).

[0088] As used herein, "promoter" refers to a polynucleotide sequence capable of promoting initiation of RNA transcription from a polynucleotide in a eukaryotic cell.

[0089] As used herein, "vector genome" refers to the polynucleotide sequence packaged by the vector (*e.g.*, an rAAV virion), including flanking sequences (in AAV, inverted terminal repeats). The terms "expression cassette" and "polynucleotide cassette" refer to the portion of the vector genome between the flanking ITR sequences. "Expression cassette" implies that the vector genome comprises at least one gene encoding a gene product operably linked to an element that drives expression (*e.g.*, a promoter).

[0090] As used herein, the term "patient in need" or "subject in need" refers to a patient or subject at risk of, or suffering from, a disease, disorder or condition that is amenable to treatment or amelioration with a recombinant gene therapy vector or gene editing system disclosed herein. A patient or subject in need may, for instance, be a patient or subject diagnosed with a disorder associated with central nervous system. A subject may have a mutation in an *SLC2A1* gene or deletion of all or a part of *SLC2A1* gene, or of gene regulatory sequences, that causes aberrant expression of the GLUT1 protein. "Subject" and "patient" are used interchangeably herein. The subject treated by the methods described herein may be an adult or a child. Subjects may range in age.

[0091] As used herein, the term "variant" or "functional variant" refer, interchangeably, to a protein that has one or more amino-acid substitutions, insertions, or deletions compared to a parental protein that retains one or more desired activities of the parental protein.

[0092] As used herein, "genetic disruption" refers to a partial or complete loss of function or aberrant activity in a gene. For example, a subject may suffer from a genetic disruption in expression or function in the *SLC2A1* gene that decreases expression or results in loss or aberrant function of the GLUT1 protein in at least some cells (*e.g.*, endothelial cells and/or neurons) of the subject.

[0093] As used herein, "treating" refers to ameliorating one or more symptoms of a disease or disorder. The term "preventing" refers to delaying or interrupting the onset of one or more symptoms of a disease or disorder or slowing the progression of *SLC2A1*-related neurological disease or disorder, *e.g.*, GLUT1 Deficiency Syndrome (GLUT1 DS).

GLUT1 PROTEIN OR POLYNUCLEOTIDE

[0094] The present disclosure contemplates compositions and methods of use related to glucose transporter 1 (GLUT1) protein. Various mutations in *SLC2A1*are known to be associated with GLUT1 DS. Both inherited and *de novo* mutations have been observed. In some cases, a heterozygous missense mutation is sufficient to cause disease.

[0095] The polypeptide sequence of GLUT1 is as follows:

MEPSSKKLTGRLMLAVGGAVLGSLQFGYNTGVINAPQKVIEEFYNQTWVHRYGESILPTTLTTLWSLSVAIFSVG
GMIGSFSVGLFVNRFGRRNSMLMMNLLAFVSAVLMGFSKLGKSFEMLILGRFIIGVYCGLTTGFVPMYVGEVSPT
ALRGALGTLHQLGIVVGILIAQVFGLDSIMGNKDLWPLLLSIIFIPALLQCIVLPFCPESPRFLLINRNEENRAK
SVLKKLRGTADVTHDLQEMKEESRQMMREKKVTILELFRSPAYRQPILIAVVLQLSQQLSGINAVFYYSTSIFEK
AGVQQPVYATIGSGIVNTAFTVVSLFVVERAGRRTLHLIGLAGMAGCAILMTIALALLEQLPWMSYLSIVAIFGF
VAFFEVGPGPIPWFIVAELFSQGPRPAAIAVAGFSNWTSNFIVGMCFQYVEQLCGPYVFIIFTVLLVLFFIFTYF
KVPETKGRTFDEIASGFRQGGASQSDKTPEELFHPLGADSQV

(SEQ ID NO: 26).

[0096] In some embodiments, the GLUT1 protein comprises a polypeptide sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 26).

[0097] In some embodiments, the disclosure provides a recombinant adeno-associated virus (rAAV) virion, comprising a capsid and a vector genome, wherein the vector genome comprises a polynucleotide sequence encoding the GLUT1 protein or a functional variant thereof, operatively linked to a promoter. In some embodiments, the disclosure provides a recombinant adeno-associated virus (rAAV) virion, comprising a capsid and genome, wherein the rAAV virion genome comprises a polynucleotide sequence encoding an GLUT1 protein, operatively linked to a promoter. The polynucleotide encoding the GLUT1 protein may comprise a polynucleotide sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to:

ATGGAGCCCAGCAGCAAGAAGCTGACGGGTCGCCTCATGCTGGCCGTGGGAGGAGCAGTGCTTGGCTCCCTGCAG
TTTGGCTACAACACTGGAGTCATCAATGCCCCCCAGAAGGTGATCGAGGAGTTCTACAACCAGACATGGGTCCAC
CGCTATGGGGAGAGCATCCTGCCCACCACGCTCACCACGCTCTGGTCCCTCTCAGTGGCCATCTTTTCTGTTGGG
GGCATGATTGGCTCCTTCTCTGTGGGCCTTTTCGTTAACCGCTTTGGCCGGCGGAATTCAATGCTGATGATGAAC
CTGCTGGCCTTCGTGTCCGCCGTGCTCATGGGCTTCTCGAAACTGGGCAAGTCCTTTGAGATGCTGATCCTGGGC
CGCTTCATCATCGGTGTGTACTGCGGCCTGACCACAGGCTTCGTGCCCATGTATGTGGGTGAAGTGTCACCCACA
GCCCTTCGTGGGGCCCTGGGCACCCTGCACCAGCTGGGCATCGTCGTCGGCATCCTCATCGCCCAGGTGTTCGGC
CTGGACTCCATCATGGGCAACAAGGACCTGTGGCCCCTGCTGCTGAGCATCATCTTCATCCCGGCCCTGCTGCAG
TGCATCGTGCTGCCCTTCTGCCCCGAGAGTCCCCGCTTCCTGCTCATCAACCGCAACGAGGAGAACCGGGCCAAG
AGTGTGCTAAAGAAGCTGCGCGGGACAGCTGACGTGACCCATGACCTGCAGGAGATGAAGGAAGAGAGTCGGCAG
ATGATGCGGGAGAAGAAGGTCACCATCCTGGAGCTGTTCCGCTCCCCCGCCTACCGCCAGCCCATCCTCATCGCT
GTGGTGCTGCAGCTGTCCCAGCAGCTGTCTGGCATCAACGCTGTCTTCTATTACTCCACGAGCATCTTCGAGAAG
GCGGGGGTGCAGCAGCCTGTGTATGCCACCATTGGCTCCGGTATCGTCAACACGGCCTTCACTGTCGTGTCGCTG
TTTGTGGTGGAGCGAGCAGGCCGGCGGACCCTGCACCTCATAGGCCTCGCTGGCATGGCGGGTTGTGCCATACTC
ATGACCATCGCGCTAGCACTGCTGGAGCAGCTACCCTGGATGTCCTATCTGAGCATCGTGGCCATCTTTGGCTTT
GTGGCCTTCTTTGAAGTGGGTCCTGGCCCCATCCCATGGTTCATCGTGGCTGAACTCTTCAGCCAGGGTCCACGT
CCAGCTGCCATTGCCGTTGCAGGCTTCTCCAACTGGACCTCAAATTTCATTGTGGGCATGTGCTTCCAGTATGTG
GAGCAACTGTGTGGTCCCTACGTCTTCATCATCTTCACTGTGCTCCTGGTTCTGTTCTTCATCTTCACCTACTTC
AAAGTTCCTGAGACTAAAGGCCGGACCTTCGATGAGATCGCTTCCGGCTTCCGGCAGGGGGGAGCCAGCCAAAGT
GACAAGACACCCGAGGAGCTGTTCCATCCCCTGGGGGCTGATTCCCAAGTG

(SEQ ID NO: 5).

[0098] In some embodiments, the polynucleotide sequence encoding the GLUT1 protein is a codon-optimized sequence. The polynucleotide encoding the GLUT1 protein may comprise a polynucleotide sequence at least 75%, at least 80%, at least 85%, at least 90%, at

least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to:

ATGGAACCATCATCCAAAAAGCTGACCGGACGACTGATGCTTGCAGTTGGCGGTGCGGTCTTGGGGAGCCTGCAG
TTTGGGTACAATACTGGCGTAATCAATGCCCCGCAGAAGGTTATTGAAGAATTTTACAATCAAACGTGGGTACAT
CGCTACGGTGAATCCATTCTTCCTACAACTCTGACCACACTCTGGAGCCTTTCTGTAGCGATTTTTTCCGTCGGG
GGCATGATAGGATCATTTTCCGTCGGTCTTTTTGTGAACCGCTTTGGCCGGAGAAATTCCATGCTGATGATGAAT
CTTCTCGCTTTCGTGAGTGCCGTCCTCATGGGATTTAGTAAACTGGGTAAATCTTTCGAGATGTTGATACTGGGG
AGATTTATTATCGGCGTGTATTGTGGTTTGACCACGGGCTTTGTACCAATGTATGTTGGCGAGGTTTCTCCGACA
GCATTGAGAGGTGCACTCGGGACCTTGCACCAGTTGGGCATCGTAGTAGGAATCCTTATAGCGCAAGTTTTCGGG
CTCGATTCCATCATGGGGAACAAAGATCTCTGGCCATTGCTCCTCTCAATAATTTTTATACCGGCATTGCTTCAG
TGTATTGTTCTTCCTTTTTGCCCAGAGTCCCCTAGGTTCCTGCTCATAAACAGGAATGAGGAGAATCGCGCTAAG
TCCGTGTTGAAAAAACTTAGGGGAACTGCAGACGTTACTCACGATTTGCAAGAGATGAAGGAGGAATCTAGGCAA
ATGATGCGCGAGAAGAAGGTTACCATACTCGAACTCTTCCGCTCCCCCGCGTACAGGCAGCCCATTCTTATCGCG
GTCGTCTTGCAGTTGTCACAACAGTTGAGTGGGATTAATGCAGTTTTCTATTATAGCACGTCCATATTTGAAAAA
GCAGGCGTCCAACAACCTGTCTATGCAACTATAGGCTCAGGCATTGTAAACACAGCGTTTACTGTAGTATCACTG
TTTGTCGTTGAGCGGGCTGGTCGAAGGACCTTGCACCTCATAGGACTGGCGGGCATGGCGGGCTGTGCGATTCTT
ATGACAATTGCGCTCGCGCTGTTGGAACAGCTTCCGTGGATGTCCTATCTCTCTATAGTAGCAATATTTGGATTT
GTTGCATTTTTTGAAGTTGGGCCCGGACCTATCCCCTGGTTCATCGTCGCGGAGCTCTTTTCCCAAGGCCCAAGA
CCGGCTGCCATTGCTGTTGCAGGCTTCTCAAACTGGACGAGTAATTTCATAGTAGGTATGTGTTTCCAGTATGTT
GAACAGCTCTGTGGGCCCTATGTCTTTATCATCTTTACTGTGTTGCTCGTGTTGTTCTTTATCTTCACTTATTTC
AAAGTACCCGAGACAAAGGGCAGGACGTTTGACGAGATTGCATCTGGTTTTAGACAAGGAGGTGCCTCACAGAGT
GATAAAACCCCGGAGGAATTGTTTCATCCGCTGGGAGCCGACTCACAGGTC

(SEQ ID NO: 27)

[0099] Optionally, the polynucleotide sequence encoding the AAV virion genome may comprise a Kozak sequence, including but not limited to GCCACCATGG (SEQ ID NO: 28). Kozak sequence may overlap the polynucleotide sequence encoding an GLUT1 protein or a functional variant thereof. For example, the AAV virion genome may comprise a polynucleotide sequence (with Kozak underlined) at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to:

gccaccATGGAGCCCAGCAGCAAGAAGCTGACGGGTCGCCTCATGCTGGCCGTGGGAGGAGCAGTGCTTGGCTCC
CTGCAGTTTGGCTACAACACTGGAGTCATCAATGCCCCCCAGAAGGTGATCGAGGAGTTCTACAACCAGACATGG
GTCCACCGCTATGGGGAGAGCATCCTGCCCACCACGCTCACCACGCTCTGGTCCCTCTCAGTGGCCATCTTTTCT
GTTGGGGGCATGATTGGCTCCTTCTCTGTGGGCCTTTTCGTTAACCGCTTTGGCCGGCGGAATTCAATGCTGATG
ATGAACCTGCTGGCCTTCGTGTCCGCCGTGCTCATGGGCTTCTCGAAACTGGGCAAGTCCTTTGAGATGCTGATC

CTGGGCCGCTTCATCATCGGTGTGTACTGCGGCCTGACCACAGGCTTCGTGCCCATGTATGTGGGTGAAGTGTCA
CCCACAGCCCTTCGTGGGGCCCTGGGCACCCTGCACCAGCTGGGCATCGTCGTCGGCATCCTCATCGCCCAGGTG
TTCGGCCTGGACTCCATCATGGGCAACAAGGACCTGTGGCCCCTGCTGCTGAGCATCATCTTCATCCCGGCCCTG
CTGCAGTGCATCGTGCTGCCCTTCTGCCCCGAGAGTCCCCGCTTCCTGCTCATCAACCGCAACGAGGAGAACCGG
GCCAAGAGTGTGCTAAAGAAGCTGCGCGGGACAGCTGACGTGACCCATGACCTGCAGGAGATGAAGGAAGAGAGT
CGGCAGATGATGCGGGAGAAGAAGGTCACCATCCTGGAGCTGTTCCGCTCCCCCGCCTACCGCCAGCCCATCCTC
ATCGCTGTGGTGCTGCAGCTGTCCCAGCAGCTGTCTGGCATCAACGCTGTCTTCTATTACTCCACGAGCATCTTC
GAGAAGGCGGGGGTGCAGCAGCCTGTGTATGCCACCATTGGCTCCGGTATCGTCAACACGGCCTTCACTGTCGTG
TCGCTGTTTGTGGTGGAGCGAGCAGGCCGGCGGACCCTGCACCTCATAGGCCTCGCTGGCATGGCGGGTTGTGCC
ATACTCATGACCATCGCGCTAGCACTGCTGGAGCAGCTACCCTGGATGTCCTATCTGAGCATCGTGGCCATCTTT
GGCTTTGTGGCCTTCTTTGAAGTGGGTCCTGGCCCCATCCCATGGTTCATCGTGGCTGAACTCTTCAGCCAGGGT
CCACGTCCAGCTGCCATTGCCGTTGCAGGCTTCTCCAACTGGACCTCAAATTTCATTGTGGGCATGTGCTTCCAG
TATGTGGAGCAACTGTGTGGTCCCTACGTCTTCATCATCTTCACTGTGCTCCTGGTTCTGTTCTTCATCTTCACC
TACTTCAAAGTTCCTGAGACTAAAGGCCGGACCTTCGATGAGATCGCTTCCGGCTTCCGGCAGGGGGGAGCCAGC
CAAAGTGACAAGACACCCGAGGAGCTGTTCCATCCCCTGGGGGCTGATTCCCAAGTG

(SEQ ID NO: 29).

[0100] In some embodiments, the Kozak sequence is an alternative Kozak sequence comprising or consisting of any one of:

(gcc)gccRccAUGG (SEQ ID NO: 30);

AGNNAUGN;

ANNAUGG;

ACCAUGG; and

GACACCAUGG (SEQ ID NO: 31).

[0101] In some embodiments, the AAV virion genome comprises no Kozak sequence. The polynucleotide sequence may be codon-optimized.

AAV VIRION GENOME

[0102] The AAV virions of the disclosure comprise a vector genome. The vector genome may comprise an expression cassette (or a polynucleotide cassette for gene-editing applications not requiring expression of the polynucleotide sequence). Any suitable inverted

terminal repeats (ITRs) may be used. The ITRs may be from the same serotype as the capsid or a different serotype (*e.g.*, AAV2 ITRs may be used).

[0103] In some embodiments, the 5′ ITR comprises a polynucleotide sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to:

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTT
GGTCGCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCT

(SEQ ID NO: 32)

[0104] In some embodiments, the 5′ ITR comprises a polynucleotide sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to:

GCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGT
GAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTTGTAGTTAATGATTAACCCGCC
ATGCTACTTATCTACGTA

(SEQ ID NO: 6)

[0105] In some embodiments, the 5′ ITR comprises a polynucleotide sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to:

CTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCA
GTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTTGTAGTTAATGATTAACCCG
CCATGCTACTTATCTACGTA

(SEQ ID NO: 33)

[0106] In some embodiments, the 3′ ITR comprises a polynucleotide sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to:

AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGG
TCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAA

(SEQ ID NO: 34)

[0107] In some embodiments, the 3′ ITR comprises a polynucleotide sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to:

TACGTAGATAAGTAGCATGGCGGGTTAATCATTAACTACAAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCT
CTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCA
GTGAGCGAGCGAGCGCGC

(SEQ ID NO: 7)

[0108] In some embodiments the AAV virion genome comprises one or more filler sequences, *e.g.*, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to:

GCGGCAATTCAGTCGATAACTATAACGGTCCTAAGGTAGCGATTTAAATACGCGCTCTCTTAAGGTAGCCCCGGG
ACGCGTCAATTGACTACAAACCGAGTATCTGCAGAGGGCCCTGCGTATG

(SEQ ID NO: 35);

CTTCTGAGGCGGAAAGAACCAGATCCTCTCTTAAGGTAGCATCGAGATTTAAATTAGGGATAACAGGGTAATGGC
GCGGGCCGC

(SEQ ID NO: 36); or

GTTACCCAGGCTGGAGTGCAGTGGCACATTTCTGCTCACTGCAACCTCCTCCTCCCTGGGTTC

(SEQ ID NO: 37).

Promoters

[0109] In some embodiments, the polynucleotide sequence encoding an GLUT1 protein or functional variant thereof is operably linked to a promoter.

[0110] The present disclosure contemplates use of various promoters. Promoters useful in embodiments of the present disclosure include, without limitation, a cytomegalovirus (CMV) promoter, phosphoglycerate kinase (PGK) promoter, or a promoter sequence comprised of the CMV enhancer and portions of the chicken beta-actin promoter and the rabbit beta-globin

gene (CAG). In some cases, the promoter may be a synthetic promoter. Exemplary synthetic promoters are provided by Schlabach et al. *PNAS USA*. 107(6):2538–43 (2010). In some embodiments, the promoter comprises a polynucleotide sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to:

ACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCC
CATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGT
ACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGC
CCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGA
GGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTT
TTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGC
GGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGA
GGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCGG

(SEQ ID NO: 38)

[0111] In some embodiments, a polynucleotide sequence encoding an GLUT1 protein or functional variant thereof is operatively linked to an inducible promoter. An inducible promoter may be configured to cause the polynucleotide sequence to be transcriptionally expressed or not transcriptionally expressed in response to addition or accumulation of an agent or in response to removal, degradation, or dilution of an agent. The agent may be a drug. The agent may be tetracycline or one of its derivatives, including, without limitation, doxycycline. In some cases, the inducible promoter is a tet-on promoter, a tet-off promoter, a chemically-regulated promoter, a physically-regulated promoter (*i.e.*, a promoter that responds to presence or absence of light or to low or high temperature). Inducible promoters include heavy metal ion inducible promoters (such as the mouse mammary tumor virus (mMTV) promoter or various growth hormone promoters), and the promoters from T7 phage which are active in the presence of T7 RNA polymerase. This list of inducible promoters is non-limiting.

[0112] In some cases, the promoter is a tissue-specific promoter, such as a promoter capable of driving expression in an endothelial cell to a greater extent than in a non-endothelial cell. In some embodiments, tissue-specific promoter is an endothelial-specific promoter. In some embodiments, tissue-specific promoter is a selected from any various endothelial-specific promoters including but not limited to FLT1 (Vascular Endothelial Growth Factor Receptor 1), Tie1 (Tyrosine Kinase With Immunoglobulin Like And EGF

Like Domains 1), VE-Cadherin (Vascular Endothelial Cadherin), hSYN1 (human synapsin), INA (alpha-internexin), NES (nestin), TH (tyrosine hydroxylase), FOXA2 (Forkhead box A2), CaMKII (calmodulin-dependent protein kinase II), and NSE (neuron-specific enolase). In some cases, the promoter is a ubiquitous promoter. A "ubiquitous promoter" refers to a promoter that is not tissue-specific under experimental or clinical conditions. In some cases, the ubiquitous promoter is any one of CMV, CAG, UBC, PGK, EF1-alpha, GAPDH, SV40, HBV, chicken beta-actin, and human beta-actin promoters.

[0113] In some embodiments, the promoter sequence is selected from Table 3. In some embodiments, the promoter comprises a polynucleotide sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to any one of SEQ ID NOS 1-3 and 39-51.

Table 3

PROMOTER	SEQUENCE	SEQ ID NO:
Human FLT-1 (hFLT-1)	TTTGCTTCTAGGAAGCAGAAGACTGAGGAAATGACTTGGGCGGGTG CATCAATGCGGCCAAAAAAGACACGGACACGCTCCCCTGGGACCT GAGCTGGTTCGCAGTCTTCCCAAAGGTGCCAAGCAAGCGTCAGTTC CCCTCAGGCGCTCCAGGTTCAGTGCCTTGTGCCGAGGGTCTCCGGT GCCTTCCTAGACTTCTCGGGACAGTCTGAAGGGGTCAGGAGCGGCG GGACAGCGCGGGAAGAGCAGGCAAGGGGAGACAGCCGGACTGCG CCTCAGTCCTCCGTGCCAAGAACACCGTCGCGGAGGCGCGGCCAGC TTCCCTTGGATCGGACTTTCCGCCCCTAGGGCCAGGCGGCGGAGCT TCAGCCTTGTCCCTTCCCCAGTTTCGGGCGGCCCCCAGAGCTGAGT AAGCCGGGTGGAGGGAGTCTGCAAGGATTTCCTGAGCGCGATGGG CAGGAGGAGGGGCAAGGGCAAGAGGGCGCGGAGCAAAGACCCTG AACCTGCCGGGGCCGCGCTCCCGGGCCCGCGTCGCCAGCACCTCCC CACGCGCGCTCGGCCCCGGGCCACCCGCCCTCGTCGGCCCCCGCCC CTCTCCGTAGCCGCAGGGAAGCGAGCCTGGGAGGAAGAAGAGGGT AGGTGGGGAGGCGGATGAGGGGTGGGGGACCCCTTGACGTCACCA GAAGGAGGTGCCGGGGTAGGAAGTGGGCTGGGGAAAGGTTATAAA TCGCCCCCGCCCTCGGCTGCTCTTCATCGAGGTCCGCGGGAGGCTC GGAGCGCGCCAGGCGGACACTCCTCTCGGCTCCTCCCCGGCAGCGG CGGCGGCTCGGAGCGGGCTCCGGGGCTCGGGTGCAGCGGCCAGCG GGCGCCTGGCGGCGAGGATTACCCGGGGAAGTGGTTGTCTCCTGGC TGGAGCCGCGAGACGGGCGCTCAGGGCGCGGGGCCGGCGGCGGCG AACAAGAGGACGGACTCTGGCGGCCGGGTCGTTGGCCGCGGGGAG CGCGGGCACCGGGCGAGCAGGCCGCGTCGCGCTCACC	1
Human FLK-1 (hFLK-1)	TGGAGCCGCCAAATATTTTGGGAAATAGCGGGAATGTTGGCGAACT GGGCAAGTGCGTTTTCTGATTAAGAGCAACCAGATTCAGCTTTTTA AACTACAATTATACTGGCCAAACAAAATACCCTTATACAAAAACCA AAACTACTGGCAGGAGTCGCTGCCAGCTTGCGACCCGGCATACTTG GCTGAGTATCCGCTTCTCCCTTGTGGCTCCAAACTGCTGCAGATTCT CGGCCACTTCAGACGCGCGCGATGGCGAAGAGGGTCCTGCACTTTG ACGCGCCTGGTGAGGGAGCGCTGCTCTTCGCAGCGCTCCTGGTGAT GCTCCCCAAATTTCGGGGACCGGCAAGCGATTAAATCTTGGAGTTG CTCAGCGCCCGTTACCGAGTACTTTTTATTTACACCAGAAACAAAG TTGTTGCTCTGGGATGTTCTCTCCTGGGCGACTTGGGGCCCAGCGCA	85

	GTCCAGTTGTGTGGGGAAATGGGGAGATGTAAATGGGCTTGGGGA GCTGGAGATCGCCGCCGGGTACCCGGGTGAGGGGCGGGGCTGGCC GCACGGGAGAGCCCCTCCTCCGCTCCGGCCCCGCCCCGCATGGCCC CGCCTCCGCGCTCTAGAGTTTCGGCACCAGCTCCCACCCTGCACTG AGTCCCGGGACCCCGGGAGAGCGGTCAATGTGTGGTCGCTGCGTTT CCTCTGCCTGCGCCGGGCATCACTTGCGCGCCGCAGAAAGTCCGTC TGGCAGCCTGGATATCCTCTCCTACCGGCACCCGCAGACGCCCCTG CAGCCGCGGTCGGCGCCCGGGCTCCCTAGCCCTGTGCGCTCAACTG TCCTGCGCTGCGGGGTGCCGCGAGTTCCACCTCCGCGCCTCCTTCTC TAGACAGGCGCTGGGAGAAAGAACCGGCTCCCGAGTTCTGGGCAT TTCGCCCGGCTCGAGGTGCA	
Human Tie-1 (hTIE-1)	AGCTCCTCCCAGCCTCAGGCCCAGGAATGGGAATCTCTGTGGGTCA CACATCAGTAGGGAGGTCTTTCCCGATCCTTTTCTATGCTACTCCAG GAGTCAAAGCGTCTCCTGGGACTTTTCAGGGCGCTTCAGAAGAGCC CTGGGCCTAAACCAGCTCAACCAAGCTGCAGGGACCCAGCCTCCTG AGAAAAGTGAATGTGAGCCCGGTGCATTCAGAGGAGAATGAAGCC TTCACCCAGAACACACTCTGGGAAGATGTCCCAGGCCCAGGGGGA GGGTTTGTACTACCAGACCTAAGTCACCTAAACTGACACCAAGTCT CATCCATCCCAACCATTCCATTCCGGGTCAGAGGGGTCATCGATTT AACCAGCAAGGCTGCCCATCCAACGGTTGCTCCCTCTGCTCCCTGG AAGGGCCTCCTCGTGGGCGTTCTGTACCTACAGGTCTTGTTCCGTTC TGGGAACTGCCAGTGGTGGCAAGAGGTGGAGCAACGGGTGCCAGG GCAGGGAGAGGTGAGTCTGGGAGGGAAGCAGAGGCAAGATCCATG GGGCTTTAGAGACTTTGCCAAAGCAGTGCGACTGCTCCCAGGTTGT TGTCAGCCGTCAAGAGTGAGTGCACCTCCCTGGGCAGACTTCTGCT GCCCCAGTGCCCAGGAATAGGCAGGGGTTTGCCGCAAAATGAATG ACACCTGGCAGACAATAAGCTGAAGCTTTCATTAGCAGCTTAAGCT GAGGACTATCTATGCAACCGATACTCCCTGTGTGCTCCCCGGGACT GCTTAATGTGAGCCCTTGTGGAGCGATTGGCACCAAGAAAGCAAG GACTAAGTCAGAAGTTCAAGTCCCAGCCTTGCCACAGCCTCAGGGT GCCCTCGAGCACAGCAAGCCTCAGTTTTCCCATCTGTACAATGAGA GAGGTACACAAGGTAGACTCGAAGGCTCTTTGTTGCCAGGGCCCTG TGTTCCTTTGAGTGTATGTGCTTCTCAGGCCCACAGAGGTCCTTTGT GTTTCGTATGTGAACTGCTCTCTAGGAAACCCATGTAACTGTCTGTG TCCTGGGGCACATACATGAGGACTCATGTGGGCCGTATTGTGTGTT TGTGCCGGGGGGAGGGGAGACCCCAGAACAATGTCCCCCACCCCA CCCCCCTCCTCAATAGGCGGAAGCCACTGGCTTCCTCCCTTTCCTGC CTCCTGCCTCCTTTGTGCCAGCAAGACTGAGTACTGGAGAGAGACA GGGGATGGGAAAAATCAGTCCAGCTGTCCCCAGGTCTGCCCTTACC ATAACCTTCCCCCCACCTCAAGTGACTCCTCCCAGGCCACACCCAT CCCCAGCCTTGTGGGGGCCAGATTGGGGGGCCTAGAGGCTCAAAG GCAGAATGAGTCCTCCCACCCCCTACCCTGCCACCCCTCCCACCCA AGCCACCTCATTTCCTCTTCCTCCCCAGCACCGACCCACACTGACCA ACACAGGCTGAGCAGTCAGGCCCACAGCATCTGACCCCAGGCCCA GCTCGTCCTGGCTGGCCTGGGTCGGCCTCTGGAGT	2
Mouse Tie-1 (mTIE-1)	AAGCTTCCGACCGTTAGTCAGAGAACTGTAAGTGCTCAGAGCCTGG CTGACAATGATCTGGAATGAACCAGATAACAACATAATAAAATCTC AGTAAAATAATTTAACAGTTAGCTTGGAAGCTGGTCAGCTCTGGGG AAATCAGGGTAAATTGTGCTGTCATGAACTGTCCCACACTGACATC GGCCAAAGTGAATATGAACTTTGGTAGATCCAATGCCTGTTCTATT TATTTTTCCAGTGAAAAGTATTTTGATAGAGCTTTTCATTTTGTAAA TACACTGAGTTAACCAAAATATCATGGATTTCCGTTTGTTCTTAAGA CATGCAACTCGTCTACGGCTATACCACTCTGAACGCGCCCGATCTC GGAAGACATGCAACTCAAATGTAAATACAGTAGAATATTACTTAGG TAGAAACTCCTGGTGATTTTAAAAGATTGGAAAAGAATATGAGGA AGAGTTGAATAATGCAAATTCTAGTGTGTGTGCTACCGAAGTGAAC ACTTAATGCACAGTCTACAGACTAGGACATTTTATCGTGTGTTGTA AAATTGGGTAGAAACTTGTGTTTGTGAAAACTGAGCATTAAAACCT TACAGAGACCGTTTCTTGTTTACTTTTGAAAAAAAAAAGAGTCACG TGAGCCTCATTTTGTATTTGTGTGTGTGTGTGTGTGTGTCTCCCCT	86

	CCTCCCAGCGTGTGTGTGCTGGGAGGAGGGGAGACCCCAGAACAA TGTCCTGCCTCCAAACCTTCTCAATAGGCGGAAGCCACTGGCTTCCT CCCTTTCCTGTCTCCCGTGCTCCAGCAATGCAGATGGAAGGGACCG AAGGGATGGGAGAGAGAGCCCAACCATCCCCAGATCTGTCCTTGTC ACAACCTGCCTCCCACCTCTAATGCCCCCCCTTCCAGAGACTTCCAG GCCACACCCATCCCGGGCTTGTGGGGGCTGGACACGGGAGGACTA CAGGCGACAACTCTTCCCACCCTCTCTCCCTGCCACCCCTCCTACCC TAACCATCATTTCCTCTTCCTCCCCAGCACCGAGGTGCACTGAGCTG GACAGGCTGAACACTCAGACCCACAGCAACTGACCCCGGGCCCAG CTGGCCTTGGCTGGCCCAGGGCAGCTTCCAGAGT	
Human Tie-2 (hTIE-2)	GCTGGAGTGCAGTGGCACGATCTCGGCTCACTGCAACCTCTGCCTC CCAGGTTCAAACAATTCTCCTGCCTCAGCCTCCAGAGTAGCTGGGG TTACAGGTGCACGCCAGCAAGCACAGCTAAATTTTGTATTTTTAGT AGAGATGGGGTTTTGCCATGTTGGCCAGGCTGGTCTCAAACTCCTG ACCTCAGGTGATCCACTCCCAAAGTGCTGGGATTATAGGCGTGAGC CACTGTGCCAGGCCCACTGTTTTTGTTTTTTTTTTTCGTGATGACAA ATTTAAAGTCATCTCATAGGAATAGAAAATAGCTTTTTAGTAGAAG CTCTTGGAATTTAAATTGAGACTGAATGGAAAGATGAAAGAAAAT AAACTTATTAACATTTAATGAGAACCTTCAAAGAACTAGGCATAGT ACCAAATGGTTTTATATTTTTAAACCTCATTTATTCCTCTCAAAACA CCTGGGAAGGAGATATTTTTGCCATTTCACAGCTGTTGAAACTGAG GCTCAAAAAGACTAAGTAACTTTTCTCAGCTACACATGTGGCTGAG CCAGTATTTGAACCCAGTTCTGTTTGCAGACAGAACCTGGGCTTTTT CACACCTGCAAACTGGAAACATTAATTGGTTCTTAAGATCATCATC GATGTGATAAAACCTGGGACAGAAATTAGTCAAGACTAGCTGCATC TGCCTTTTCCTCTGGTGGGTAGGAAAAGGAGGAGTATAATGATTTC CTCAGGCATGAAGGTCGATGATGAGCAAAGTGTATACTCTCTAATC TAATGTCATAATTCATATTGTGGAGTAATTATCTGGATAAGTGTAG GGTCTCTGACCTCATTCTAGATATTGTACATTCCATGGCTATTTTCA TTTTGGTCCATGAACTCTCTTTGCTCTCATGAGCACCATTTTTATCCC AATCTAATCCTGTATGTTTGTGTTTTTACACAGATTAGTTTTTAAAT GTTATATATAATTTGCTTCTGAAACACCATTGCTCAATGACTACCAA ATCTTTCTCATTACCAAAATCCTTCTATGCCAACTTCTTCAAGAAAT TTGATCACCTTTAGATGAATTGTTAATGAAAATTAAAGCTATAGCC GGCAACATGGGTATCTTTGGGCTAATGGCCAACCAACAGGCCATCT GTGTGAAAGAAAACAGGCTAACAATTTTGGACTCTGGTCTCTTGGG GCTACATTGAGCATTGACCTCACCGGTGCTCACTGAAATTAATTGC TTTTCAGGTTGTATTTTCTCATCACGGAAACCTTCTTCTCCCAATTC AAACCATGTGGGTTAAAATGAGAAAACAAAAGCCAAAACGGCTTC CCACACCCAAAAGCTCCTTCTGTCAGAGATCCCAGTAGCCCCGGGA GAGCTGTTAGAAGTCTGAGAAGGATTGGTCATCATCGCATACCATA CATAGGTGGAGGGCTTGTTATTCTCAGTTTCCCGCCTATGAGAGGA TACCCCTATTGTTTCTGAAAATGCTGACCGGGACCCACACTTCCAA CAAAAATTCCTCTGCCCCTACAGCAGCAGCAAAAGCAGCAGCAGA AGCAACAGCAACAGATAAGTGTTTTGATGAATTGCGAGATGGATA GGGCTTGAGTGCCCCCAGCCCTGCTGATACCAAATGCCTTTAAGAT ACAGCCTTTCCCATCCTAATCTACAAAGGAAACAGGAAAAAGGAA CTTAAAACTCCCTGTGCTCAGACAGAAATGAGACTGTTACAGCCTG CTTCTGTGCTGTTCCTTCTTGCCTCTAACTTGTAAACAAGACGTAGT AGGACGATGCTAATGGAAAGTCACAAACCGCTGGGTTTTTGAAAG GATCCTTGGGACCTCATGCACATTTGTGGAAACTGGATGGAGAGAT TTGGGGAAGCATGGACTCTTTAGCCAGCTTAGTTCTCTGTGGAGTC AGCTTGCTCCTTTCTGGTAAGGTTTGGCTTTATTTTTTTTAATTTAGT ATTTTAAAAAACAGAGTTAGTGATTTCTGGGTGCTCTCCCCAAATCT CATCAGTGCTGATGAACAAGGGGTGGCTGTAGCAAAGGCACCATTT C	
Human VE-cadherin (hVE-caderin)	CTAGTAGCAGAAACAAGGTCCTCTGGAAGAGCAACTGATGCTCTTA GGTACTGAAGCATCATCCTGCCCCAGAGACCACTCGCATATGAAGC ACACATATTCAGTCTGCCTTACTTGTGTTAATGATTGCCAGTGTCCC TCTGACCTCCTAGCCCTGAAAAGTGTGGCCTGAAGGTCATTTCAGA	3

	GACGGGGAGAGCTGCTCAGAGAAGCCAATCGGCGAGTCTAGGACA CACAGACAGGATCTAGTCCCAGAGTTCGCTAGCCTAGGTGAGCGTC CCCTGGCCCCTTATACCACTTCCTTCTCCAGCTTGCATCTAATCTGC TCTGGCAGACCATCGTGTTTCCTGTCTTCCTGGCAGCCTCCAGCACG CTCAGTGCTACTCCCTGCGCATGCGCCCTCCTCCCAGTACCTTCTCT GACTCCAGTGGGCTTGGAGTGCGAGGAGGAAGGGTGAGGAAGGGG TGAAATCAGGTATTGGATCCACAGGGGGTCTGAAGAGCACTAGCCT GGCCTTTTGGGACTGAACTTCTGCTATGAAGACCTCCACTGCCATCC CTGGAGTCCGGGGCACATCCAAGGCTTGCTGTCCATCGTTTACTGTT TACAGATGACAACAATGACTGTGTTCGGGGCAGAAATATCCACCAG GGCTAGAGTACAAAAGGAGTTTGCATTGATGGCCGGACAGGCCCT GTCCCTGGCAGCCTGCCAGCGCTGAGTATGAGACCCAGCGGGAAGT GCTACCCTGGCAGACGTGTCCACTGAGTACACAGACCACCAAGGCA GGCAGCTCTCGGGGAAGCTGTCTATGCTGGGCCAGCCCACCTTGAG GGCAGGGAACAGAACAGATTGTGGCAGAGAGGAAAATGTGGAGCT TCTGTTTGTTCACAGACACACGCACTCGCCCACGCACGCACGCACG CACGCACGCACGCACGAATGCACGCACGCAGTAGTTGAATGCTATG GATTCCGCTCAGAGCTGAGAACAGCCCCAGCGACAGTTCCCTGGCC TCTCTCCTTACTCTGATGTCCTCATCTGTCTTCACATGGTCTCAGGA CGCTAATACTCCATCCTAATGTACACTCCTTTCCCTGGGCCTCCGTT CCAGTTCAGTTCTCAGAGGACCTGGAGGGAGTGATTGGCTACACCA ACTTTGCTTTCGTTCACCAAGCCCATGTCTCTACTTGGGTGTCTAAT GGGCATCTCCAACATTACCTACCCCAAACAGAAAACCCTTTCTTCC CCCCAACCACACCCCACCCTACCCCCACAGTATTTTCTCCATGCCCG GAAAGATCTGCTCTCTTATGGTCCCTCTTTGCCTCACTGAAAAGCAG GACAAGTTGGGGACTTCCCAAACTTTTATGCATGAAGAAACCCAGG CAATTTGCCAAAAGGTACACTCTGGGGGTCTGTCATTTACTCTGAG CCAGAACCCTGAAATTTTTACTAACCCATCACATAATGAATGAAGA GAATCTTTTTCTTTTTTTTTTTTTTTCTTTTTTTTTGGTTTTTCGAGAC AGGGTTTCTCTGTATAGCCCTGGCTATCCTGGAACACACTCTGTAG ACCAGGCTGGCCTCGAACTCAGAAATCCACCTGCCTCTGCCTCCCG AGTGCTGGGATTAAAGGCGTGCGCCACCACGCCTGGCTGAATGAA GAGAATCTTGACCTCATCTCCCCAGCCTCTTGGTCCTGAGGGACCCT GGTCTACCTACTGCTTTGCTGTCTTCTTAGCTCTTCTTACTTTTTTGC TGACTCAGACCTATGGCTATCTCCATTATACAGATGAGGAGACTGA GGCATGGATCCCTGGTTGGTCCATGGTCACGTGAAGCCCATCACCC AGTATTTGTAAAGTGAGATGGGCCAGGCTGGTACCTTGGAACTGAA ACTCACACTGCCCTACCTGGAAGAATCTGACAGGCAAAATCTGCTG CTGAAAGTGATTGTCTGTCACGTTTCTCAGCTGCCCGACTCTGAGA ACTCCACAGCCCCCTTTCGTTCCACCATACTACAGAGTCGCCACGG AAAGCCGGCTCTGTGGAGAAGCTGAGGTAGCTGGGTTTCTGTCTGG GTTACTCTGTCCAGCGAGGAAACAAGTACCTTAGACCCACTAAGCC TCTGCTTTCTGAACTGTAAAGTGGGGGATATGACACCTGCCTCCCA GGGATGGCTGAATGCTCTGGCAGAAGCTTAGAGCCCCCACAGCTAC CCCTAGGCTCACAGCTCCTCCGATGAGACCTAGAATTGAGGTATGA GTTGAATACCCCAGGCAGGTCCAAGGCTTCCACGGGCCCAGGCTGA CCAAGCTGAGGCCGCCCACCGTAGGGCTTGCCTATCTGCAGGCAGC TCACAAAGGAACAATAACAGGAAACCATCCCGAGGGGAAGTGGGC CAGGGCCAGTTGGAAAACCTGCCTCCCTCCCAGCCTGGGTGTGGCT CCCCTCTCCCCTCCTGAGGCAATCAACTGTGCTCTCCACAAAGCTCG GCCCTGGACAGACT	
Human VE-cadherin (hVE-caderin) (short version)	CATCCATGCCCATGGCCTCAGATGCCAGCCATAAGCTGTTGGGTTC CAAACCTCGACTCCAGGCTGGACTCACCCCTGTCTCCCCCACCAGC CTGACACCTCCACCTGGGTATCTAACGAGCATCTCAAACTCAACCT GCCTGAGACAGAGGAATCACTATCCCCTCCTCCTCCAAAAATATCC TTCCATCACACTCCCCATCTTGTGCTCTGATTTACTAAACGGCCCTG GGCCCTCTCTTTCTCAGGGTCTCTGCTTGCCCAGCTATATAATAAAA CAAGTTTGGGACTTCCCAACCATTCACCCATGGAAAAACAGAAGCA ACTCTTCAAAGGACAGATTCCCAGGATCTGCCCTGGGAGATTCCAA ATCAGTTGATCTGGGGTGAGCCCAGTCCTCTGTAGTTTTTAGAAGCT	88

	CCTCCTATGTCTCTCCTGGTCAGCAGAATCTTGGCCCCTCCCTTCCC CCCAGCCTCTTGGTTCTTCTGGGCTCTGATCCAGCCTCAGCGTCACT GTCTTCCACGCCCCTCTTTGATTCTCGTTTATGTCAAAAGCCTTGTG AGGATGAGGCTGTGATTATCCCCATTTTACAGATGAGGAAACTGTG GCTCCAGGATGACACAACTGGCCAGAGGTCACATCAGAAGCAGAG CTGGGTCACTTGACTCCACCCAATATCCCTAAATGCAAACATCCCC TACAGACCGAGGCTGGCACCTTAGAGCTGGAGTCCATGCCCGCTCT GACCAGGAGAAGCCAACCTGGTCCTCCAGAGCCAAGAGCTTCTGTC CCTTTCCCATCTCCTGAAGCCTCCCTGTCACCTTTAAAGTCCATTCC CACAAAGACATCATGGGATCACCACAGAAAATCAAGCTCTGGGGC TAGGCTGACCCCAGCTAGATTTTTGGCTCTTTTATACCCCAGCTGGG TGGACAAGCACCTTAAACCCGCTGAGCCTCAGCTTCCCGGGCTATA AAATGGGGGTGATGACACCTGCCTGTAGCATTCCAAGGAGGGTTAA ATGTGATGCTGCAGCCAAGGGTCCCCACAGCCAGGCTCTTTGCAGG TGCTGGGTTCAGAGTCCCAGAGCTGAGGCCGGGAGTAGGGGTTCA AGTGGGGTGCCCCAGGCAGGGTCCAGTGCCAGCCCTCTGTGGAGAC AGCCATCCGGGGCCGAGGCAGCCGCCCACCGCAGGGCCTGCCTATC TGCAGCCAGCCCAGCCCTCACAAAGGAACAATAACAGGAAACCAT CCCAGGGGGAAGTGGGCCAGGGCCAGCTGGAAAACCTGAAGGGGA GGCAGCCAGGCCTCCCTCGCCAGCGGGGTGTGGCTCCCCTCCAAAG ACGGTCGGCTGACAGGCTCCACAGAGCTCCACTCACGCTCAGCCCT GGACGGACAGGCAGTCCAACGGAACAGAAACATCCCTCAGCCCAC AGGCACGGTGAGTGGGGGCTCCCACACTCCCCTCCACCCCAAACCC GCCACCCTGCGCCCAAGATGGGAGGGTCCTCAGCTTCCCCATCTGT AGAATGGGCATCGTCCCACTCCCATGACAGAGAGGCTCC	
Human Intercellular Adhesion Molecule 2 (hICAM-2)	GTCTCCCAGGCATGACTCCAACAATGCATCCCATGGGATTTGGGGT TCCCCAGATCTGGGGCTTGTAGGCCTGACTCTCCCCTGTGCACACGT CTCATACACGCATGCGTGCACCCATTGCCTGCCCCGCCCCTTGCAC AGGGAGTCAGCAGGGAGGACTGGGTTATGCCCTGCTTATCAGCAGC TTCCCAGCTTCCTCTGCCTGGATTCTTAGAGGCCTGGGGTCCTAGAA CGAGCTGGTGCACGTGGCTTCCCAAAGATCTCTCAGATAATGAGAG GAAATGCAGTCATCAGTTTGCAGAAGGCTAGGGATTCTGGGCCATA GCTCAGACCTGCGCCCACCATCTCCCTCCAGGCAGCCCTTGGCTGG TCCCTGCGAGCCCGTGGAGACTGCCAGTC	89
Human Von Willebrand Factor (hVWF)	ATCTTTAGCCGATCCATTCAACCCTGGCCAGGATCCAAATGGACTG TTTTTGTCAGGGCCAGGACCGGATCCTTCATACCTGGGGTGCATAG GAAGTGTTAGTACTCCCCTTCCTCCAAACACAGCAGCAAAATTGGC TCAGGTTGAGGTGTTTTTCTCAACTTCCCTGGAGTCCAGCCCTGGAA GCTGGATCAGGAAGCTGTGTTGTTCTACTGTGATTCCCCCTGGCCTG TATCAGCTTGCCCTGAAACAACCAGCATTCCTGGTTATCCCACACA GGTGGGGCACTCTAGGAAGACCAGGGATCAAGTGTGGGGGTGTAG GGATAGGGGGTGTTTGGGGAGGGCAAGGCAGTTAATTAAGGCAGC TGCCAGGAGGTCTCCCTCCAAACTCTACAAAGCTTTATCAGCTTGG AGGTACTTCTAATACCATTTCCTTTCATTGTTTCCTTTTGGTAATTAA AAGGAGGCCAATCCCCTGTTGTGGCAGCTCACAGCTATTGTGGTGG GAAAGGGAGGGTGGTTGGTGGATGTCACAGCTTGGGCTTTATCTCC CCCAGCAGTGGGGACTCCACAGCCCCTGGGCTACATAACAGCAAG ACAGTCCGGAGCTGTAGCAGACCTGATTGAGCCTTTGCAGCAGCTG AGAGCATGGCCTAGGGTGGGCGGCACCATTGTCCAGCAGCTGAGTT TCCCAGGGACCTTGGAGATAGCCGCAGCCCTCATTTGCAGGGGA	90
Ple261 (Human Claudin5)	TGGCTTCCGGAGGGTGGCCTGGGGGCTGGGGTGCCAGGGACACCA TCGCCACTGGTGGGAGGGCAGGGCACAGCCCCTCCGTGTCCCTTTG TCTCTCCTGTCTGAAGGCCAGAGCAGGCTGCTAGGCCTGGGGCCAC CACTGCCCCTGGGTGCTACACCCAGTGTGCTGGGTCACTGGGAACT TCCTGAAGTGGTGTCACCTGAACTGGGCCCCCAAGGATGGGGTGCG GGCAGTACCGCAGGAAGAGGAGCAGCCCCTGTGAAGATTGAGAGG TCTGGGAAGCCCCTGCGGCTTGGGAGAGTGGGGGTCGCCAGGCAG GGGGAAAGCCCCTGTGCCACCGCTTTTTGCCAGAGACTCAGGCTCC AGAGAGGCAGTGAGTGGCATGGGGGGTGAGGCTGGGGCCCTGGGC CTGACCTCCACACGCCTGCCTGGCCTCTCTGTTTGCCATGGGATGAG	91

	AGAGACAGTGCTGGGACTCAGAGCGGGGCTGGAGAGTGAGAGTGC GAGAAAGGGCCTGGGTGGGGCTTGGACCCCGGGGCGGGCTTTCTG GAGAGCCCCCCTACGAGGGCCTCTACGGCGGTGACGGGGTGGGGG GCTTCTGCAAACCTTGGTCAGGGAAGTGGAGCTGGCTCGAGTGGAA GAGACCACCCGGCTCAGTCGGGGATGTGGGAGTGGACTGGGTGGT GCAGACTGGGGGTCGAGCGCCTTCTGAAGTGACGGGGCCGGGACG CGCAGGGAGGCGGCCCAAGAAGCGCGCCCTAGGCCAGCCCAGAAT GCGCTCGGCCGCGACTAGGACAACGGCGGGTGGGGCTGGGGGCGG CTGCCGGGCGGGGAGCGGTCCCGCGCCCTCAGCTACCCCTCAAGAG CCGTTGTTTCCCTAACTTCAGCTGCCAGAGGCTCTGTGATTGGCTGC GGCACGATGACCCGCGCACGGATTGGCTGCTTCGGGCCGGGGGGC CGGGCCCGGGGGACAGAATCCGCCCCCGAACCTTCAAAGAGGGTA CCCCCCGGCAGGAGCTGGCAGACCCAGGAGGTGCGACAGACCCGC GGGGCAAACGGACTGGGGCCAAGAGCCGGGAGCGCGGGCGCAAA GGCACCAGGGCCCGCCCAGGGCGCCGCGCAGCACGGCCTTGG	
Human Endoglin (hENG)	CGCCTTGCTGTGCCACTTTGGGACTTCCCTCCCTAGCCTGAGCTTCA GTTTTCCTGCCTGTTAGGCAGCCCCATGTCAACTGCACTTAGTAGGC CGGGTTTGATGCCCGACAAGACGTGAAGTGGTGGAGGTGGGCAGG ATCCCAGCGCTACCATCTTCTTGAACCAGTGATCTCAACACATCGG ATTTCTGTTTCCTCATCTGCAAAATGGGATCAGTGAGCTCAGGTGG GTCACAAATTCTACAGGAACTACTTTAGCCAAGCCCGGCCCCCTGA AAGTTCCCCTCGGTGGGCTGTTAGGGTGATTGTTTTCATCTGTGGGG CTCCCTGATGCGTCCCACCCACCAGCCTTGGAGAGGGTGGGATGGG AGGGTGGGGTGCTTGGGGAGACAAGCCTAGAGCCTGGGCCCTCCC ACCCCACTGCCTCCCCCCATCCCAGGGCCCCCCACCCAGTGACAAA GCCCGTGGCACTTCCTCTACCCGGTTGGCAGGCGGCCTGGCCCAGC CCCTTCTCTAAGGAAGCGCATTTCCTGCCTCCCTGGGCCGGCCGGG CTGGATGAGCCGGGAGCTCCCTGCTGCCGGTCATACCACAGCCTTC ATCTGCGCCCTGGGGCCAGGACTGCTGCTGTCACTGCCATCCATTG GAGCCCAGCACCCCCTCCCCGCCCATCCTTCGGACAGCAACTCCAG CCCAGCCCCGCGTCCCTGTGTCCACTTCTCCTGACCCCTCGGCCGCC ACCCCAGAAGGCTGGAGCAGGGACGCCGTCGCTCCGGCCGCCTGCT CCCCTCGGGTCCCCGTGCGAGCCCACGCCGGCCCCGGTGCCCGCCC GCAGCCCTGCCACTGGACACAGGATAAGGCCCAGCGCACAGGCCC CCACGTGGACACC	92
Human Platelet Derived Growth Factor Subunit B (hPDGFB	GCCCAGGCTGGAGTGCAGTGGCACAGTCACAACTCACTGCAGCCTC AAACTCCTGGGCTCAAAACGATCCACAGTCTCCTGAGTAGCTGGGA CTACAGGAGCTTGTTACCACACCCAGCTCCAGTTTATAAATTCATCT CCAGTTTATAAAGGAGGAAACCGAGGTACTGAGAGGTTAAAAAAC CTTCCTGCAGACACTTGTCCAGCAAGTGGCCACTCCAGGATTTGGA CCAAGGTGATGTGTCTTCAGGCTGTGTCTCTGCCACTGTGCCACGCT GCTGGGTGGTAGGCAGCAGTGGGTGGGTGCCTGCAGTGGTCTGTAA AGACCACCTGAGATGTCCTTCCTCCTCTGTTCCACCCTGTCCAGGTC CAAGAAGACAGTCTATGAAGAGAGAGCAGGTGTGACTCTCTCAGT GTGCTCCTCTGTGAGAAGCAGGCTGACATCCCAAAGGGAAGGGCG GATAACAGAGACAGTGCAAGCGGAGGAGATGAGGGTGCCTCAAAG CCGGGAGGCTGGGTGATGCAGGAGCCTGCGTGTCCCGAGGGGGGT GCTGGGCCCAGTGTGAGTACGTGTGACTGTGACTGAGACAGTGTGA CTGCTGAAGGCAGGGACACAGCAGCTCCCTGACTGGGGGCAGAAG GCGTTAACTGTGTGAAGGCTGGTTGTGGGTGGGTGGGCTCTGGGCC TCGAACCCGGGGGCTGAGGGAGATAGTAAACAGCAGGGTGACTGA CGGGAAGATCATGTTGGTAGCCCTGCGAAGATGCTGCAGGGCTGTG GGGGTTTGTGTGACTTTGCAGTTCAACAAATTCAAATTCAGCCAAC GCTGGCAGGGCCTGTTGTGCCAGGCAACCAGCTAGGAGGAGGAGA CTCGGACCCAGCTTGCAGCTGAAGGGCGCTGGCTGCCGGGTTCTGT GGGTTCACCTTGCGGTGTCTTCCCTTGCTAACACTGAGTCCTTACAA TAGCCCCATCTCCAGGTTGAGGCTAGATGGAGGGGACAGAGGGAA GTGACTTGCCCAAGGTGACCCAAGCTCCCGAGTGCCAGGGCAGGAT CTGAATTCAGGCTCTCAGACTGCAGAGCCTGAGTCCCTCCCTGCCA TGCCTGTGCCAGGGTGGAAATGTCTGGTCCTGGAGGGGAGCGTGGA	93

	CTCCTGGCCTTGGCTCTGGAGACATCCCCCTAGACCACGTGGGCTC CTAACCTGTCCATGGTCACTGTGCTGAGGGGCGGGACGGTGGGTCA CCCCTAGTTCTTTTTTCCCCAGGGCCAGATTCATGGACTGAAGGGTT GCTCGGCTCTCAGAGACCCCCTAAGCGCCCCGCCCTGGCCCCAAGC CCTCCCCCAGCTCCCGCGTCCCCCCCCCTCCTGGCGCTGACTCCGGGC CAGAAGAGGAAAGGCTGTCTCCACCCACCTCTCGCACTCTCCCTTC TCCTTTATAAAGGCCGGAACAGCTGAAAGGGTGGCAACTTCTCCTC CTGCAGCCGGGAGCGGCCTGCCTGCCTCCCTGCGCACCCGCAGCCT CCCCCGCTGCCTCCCTAGGGCTCCCCTCCGGCCGCCAGCGCCCATTT TTCATTCCCTAGATAGAGATACTTTGCGCGCACACACATACATACG CGCGCAAAAAGGAAAAAAAAAAAAAAAAGCCCACCCTCCAGCCTC GCTGC	
Human Endothelial Cell Specific Molecule 1 (hESM1)	ACATCCAATGCCCGCTCTGCCTCATCTTCTATGGGAAACAAGAATT TTAGAGGTCAGGTAGCCTAACACCATCAATTCTCAAAAGAGGAAGC TGAGGCCAAGAGAAGTCCTGTGAATTTCTTACAGCTCATTTGTGAC AGACCAAGAATTACCCACTTTACTGGGTTGTTATTTACTAAGTGAC AGTGAGTCTATATCTCTTTTGACAAGTGAGGTGGGGGCATGGAATT CGGCATGTGGTTGGTGTAAGAACTCCCCTCTCTCCTCTTTAACCTTA CTTAATAAGACCCTGGCACAGTTGATATTTTAAGAGGGCTACTCTG TTTTCCCAGAGGGACCTAGGCACGGTAACCCTCTTAGCATGCAGAC CTTGTTTCCTGAGGGGTAATGTTTCCCTTCCCTGTGACTTGTTTCTTG GGGGCTGTGTTCTGATTTTCCTGCTGAGCCACTTGTTGCCTTGGGCT GGCTGCCGCGCTTGGCAGTTTTTAGTGAGGGCTCTGATAGATGCCA GGAGGTGAGGGGAAGGGCTCTGGGTGGACTCCGTCATTGGACAAG CAGACTTAGTGATGGATGAGCCTTCCCCTGAGGAAGTTTTGGATCA GAAGTCCAACTGATAAGTTTTTCCAGAATTGAGTAACCCAGAAGCA GTGCCGAAAGGATCTTACCTCTCTTGTGGCTTTTTGTATTGATTTTA AAAGAAATTCTCAGAGGCAGTTCCACATTGTACTGGAAGCACAGCT ATATCCACAATAGGCTTAGATATATGTAACATGAATTGCTTTAGAA ATAACATTTGAGGAGAGGGGTGAGAGGAAGGAAGAGAGGGTCTTA AAAAATAGCCCTATCAAAATATTTTCTTTCTTCTAAGTATTGAAAAG ACACAATATAACCCTTTCTTCTTTCAAATGATCTCATAGCTATTTGT TGAGGGGAAATACCAAATGTTTATTATTTTTTTTGAAGAAGCTTCTT CGGTCCTGATGATTCATGTTGATATCATTTTCCTCCTGACTACAGAG GCTCTGAGACAAAGCTACACCTCAAGTGATATGCCAGGGTCAGAAC AATTCCCGTCCTGAAGGAGGGTGTGCAACCTTCTTTATCCCTCCTTC ACAGACGTCCTTGAGCCCTTGAGACGGATGTGAGTGAGTTTTTCAG TCCTCATGCAAAACAACCATCTAAACATAACAGATGACATCAGCTT GGGCTTTTCAATTCCTGGATGGCAGCAGCGTGTTAATCCAGCCTTC ATCCTGGATTTCATAAACCAAAACAAGAGAGCCTGGCAGGAGGAC AGCGCTGCTGCTGGGTTGAGGAAATTGATGACGGGAAAGCATGCG GGCAACCCAGTGTATAAAACTCATAAACGTGTAGGCAGAGGCTCA GCTACCAGTTTGGACGGCTGCTTCCCACCAGCAAAGACCACGACTG GAGAGCCGAGCCGGAGGCAGCTGG	94
Human Apelin (hAPLN)	TGGCACACACGCACCCTGTCCAATGTATCTTTTGTGTAAATCTGGAC TTAACACTTCAAGCAAACTGCCTGGCTTGCTGAAAGGTGGAGACAC CTTTCGATTCAGTCTTTTAATATGTGTTGAGTGCCACCTATGTGCAG AGCAAGATATTGGGGACTTTGGAGAGATCCAGAAGAGTGAGAAGA CAGTATCCTACCTTAGGGGGTTCCCAGTCCAATGAGGGAAGCAGCC CCATGCCTTGGGAGCTCCCAAGCTATAGAAGCAGCTAACAATCGAG TCTGGAAAGGCAAACAACTTCAGGACCCGCTTCTAAAGCGGAATCG CAAGTACACGCAAAATGAATCCAGCCTTGACTGTGTGGAGTTGGGT AAACCACCTGCCTCTTACGTTGATGGGGAACTAGAATGAGGACAGC TCCAGGGAACAAGAAAGGGTAGACCATAGGAGCTGTCCCATGTCC CAACAGTGGGGAGGAGCTGATGGGCGGCCCCTGCTGGATTAGTGTT ATCCTGAGAAGGCTTCTGGATGCGATGGGATTTGAGGTGCTGCTGC AAAGAATGAATTGCTCACGGAAGGGTGGGGTGGGGGCATTCCAGG TAGAGGGTGCCTCCTGGGGGATGCAGGGAACATGAGGGGCCTGGG CAATTAATCAAGCCTTGGGCACAAGCCTAGGCAGTCACCCCCAATT CAAAGCCAGTTGAAAATGCAGAGGAGAGAGGAGGGCCAGTGTTTG	95

	GTTGTCTTGACCAAACCCTTGAAGCTGGCCAGCGGCAAGGGCAAGG ACCAGGGTCAGAGGTAGAGGGCGTGAGTGAAGGCAACCCAGACTG AGTCCTTCCCTAAGCGCCCAGGTTTCCTGACAGCTGTTAAGGAAGC AAGGTGAGAAAGGGTTAAGTGTGCCCCTCCACCGCCCCAAATGCTT CCTGTGTTTGAAATCCTTCAGGTCTCTGCAAACCCTCTGGCCCCCGG CCAGGCGGGCATTGTCCGGGGAGCGGTTGTAGGTTGTCAGAGAGG CCGCGCAGCCTTTGTTGTGGGGCCACCTCGGGGTTCCCTCTCGCGCT CACGCTCGGGCTGGGGCTGCAGAGTGCGTGCCTGGAGGGGGGCGG TGCGGGAGGCTCGCTCCCTCTCCCTCTTCCTGCCCCCCCTCTAGCCC TCCCGATGACCACATGACCAAGTGGGCTCGCGGCCAAGCCACAAG CTACAAAATGCAGCCCCTGGAGTGAGCGGGGAGCATTCTCTCTGGC AGCCGGGGTCACGGGCAGTTGCAGCCGCGGCCGAGCAGCCAGCCG CTAAGAAAGAGCTCGCCGCTGCCGCTCCCGGAGCCGCCGAGGCCA GCTTCGCGGCGCTGCCCCGCGGCGGGAGAGGAGGCTGCAGAAGAG CGGAGGCGGCCAGCGG	
Human beta-actin (HuBa)	GCCCAGCACCCCAAGGCGGCCAACGCCAAAACTCTCCCTCCTCCTC TTCCTCAATCTCGCTCTCGCTCTTTTTTTTTTTCGCAAAAGGAGGGG AGAGGGGGTAAAAAAATGCTGCACTGTGCGGCGAAGCCGGTGAGT GAGCGGCGCGGGGCCAATCAGCGTGCGCCGTTCCGAAAGTTGCCTT TTATGGCTCGAGCGGCCGCGGCGGCGCCCTATAAAACCCAGCGGCG CGACGCGCCACCACCGCCGAGTC	39
Chicken beta-actin (CBA)	GGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCC CCTCCCCACCCCCAATTTTGTATTTATTTATTTTTTAATTATTTTGTG CAGCGATGGGGGCGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGC GGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGG CGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGC GAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCG GGCGGGA	40
Cytomegalovirus (CMV)	TGGTGATGCGGTTTTGGCAGTACACCAATGGGCGTGGATAGCGGTT TGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGG AGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTA ATAACCCCGCCCCGTTGACGCAAATGGGCGGTAGGCGTGTACGGTG GGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCG	41
Cytomegalovirus (CMV) (second version)	TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCAT ATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGG CTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTAT GTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGG TGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTA TCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGG CCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTAC TTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGC GGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACG GGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTT GGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGC CCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTAT ATAAGCAGAGCTGGTTTAGTGAACCGT	42
Cytomegalovirus (CMV) (third version)	CGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAAC GACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACG GTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATT ATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATC TACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTA CATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGT CTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATC AACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCA AATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGC T	43
CAG promoter (first version)	ACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGC CCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAG	44

	GGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGC CCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCT ATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGT ACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTA GTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCAC TCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTAT TTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGCG CGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGA GGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAA AGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAA GCGAAGCGCGCGGCGGGCGG	
CAG promoter (second version)	CGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAAC GACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACG GTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGT ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATT ATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATC TACGTATTAGTCATCGCTATTACCATGTCGAGGTGAGCCCCACGTTC TGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTAT TTATTTATTTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGG GGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGC GGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCG CTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCT ATAAAAAGCGAAGCGCGCGGCGGGCG	45
Human EF1-alpha (EF1-α)	CAACCTTTGGAGCTAAGCCAGCAATGGTAGAGGGAAGATTCTGCAC GTCCCTTCCAGGCGGCCTCCCCGTCACCACCCCCCCCAACCCGCCC CGACCGGAGCTGAGAGTAATTCATACAAAAGGACTCGCCCCTGCCT TGGGGAATCCCAGGGACCGTCGTTAAACTCCCACTAACGTAGAACC CAGAGATCGCTGCGTTCCCGCCCCCTCACCCGCCCGCTCTCGTCATC ACTGAGGTGGAGAATAGCATGCGTGAGGCTCCGGTGCCCGTCAGTG GGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGG GGTCGGCAATTGAACGGGTGCCTAGAGAAGGTGGCGCGGGGTAAA CTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGT GGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT	46
Human Synapsin1 (Syn), short version	AGTGCAAGTGGGTTTTAGGACCAGGATGAGGCGGGGTGGGGGTGC CTACCTGACGACCGACCCCGACCCACTGGACAAGCACCCAACCCCC ATTCCCCAAATTGCGCATCCCCTATCAGAGAGGGGGAGGGGAAAC AGGATGCGGCGAGGCGCGTGCGCACTGCCAGCTTCAGCACCGCGG ACAGTGCCTTCGCCCCCGCCTGGCGGCGCGCGCCACCGCCGCCTCA GCACTGAAGGCGCGCTGACGTCACTCGCCGGTCCCCCGCAAACTCC CCTTCCCGGCCACCTTGGTCGCGTCCGCGCCGCCGCCGGCCCAGCC GGACCGCACCACGCGAGGCGCGAGATAGGGGGGCACGGGCGCGAC CATCTGCGCTGCGGCGCCGGCGACTCAGCGCTGCCTC	47
Human Synapsin1 (Syn) with 3′ extension	AGTGCAAGTGGGTTTTAGGACCAGGATGAGGCGGGGTGGGGGTGC CTACCTGACGACCGACCCCGACCCACTGGACAAGCACCCAACCCCC ATTCCCCAAATTGCGCATCCCCTATCAGAGAGGGGGAGGGGAAAC AGGATGCGGCGAGGCGCGTGCGCACTGCCAGCTTCAGCACCGCGG ACAGTGCCTTCGCCCCCGCCTGGCGGCGCGCGCCACCGCCGCCTCA GCACTGAAGGCGCGCTGACGTCACTCGCCGGTCCCCCGCAAACTCC CCTTCCCGGCCACCTTGGTCGCGTCCGCGCCGCCGCCGGCCCAGCC GGACCGCACCACGCGAGGCGCGAGATAGGGGGGCACGGGCGCGAC CATCTGCGCTGCGGCGCCGGCGACTCAGCGCTGCCTCAGTCTGCGG TGGGCAGCGGAGGAGTCGTGTCGTGCCTGAGAGCGCAG	48
Human Synapsin1 (Syn) with 5′ extension	CTGCAGAGGGCCCTGCGTATGAGTGCAAGTGGGTTTTAGGACCAGG ATGAGGCGGGGTGGGGGTGCCTACCTGACGACCGACCCCGACCCA CTGGACAAGCACCCAACCCCCATTCCCCAAATTGCGCATCCCCTAT CAGAGAGGGGGAGGGGAAACAGGATGCGGCGAGGCGCGTGCGCA CTGCCAGCTTCAGCACCGCGGACAGTGCCTTCGCCCCCGCCTGGCG GCGCGCGCCACCGCCGCCTCAGCACTGAAGGCGCGCTGACGTCACT CGCCGGTCCCCCGCAAACTCCCCTTCCCGGCCACCTTGGTCGCGTCC	49

	GCGCCGCCGCCGGCCCAGCCGGACCGCACCACGCGAGGCGCGAGA TAGGGGGGCACGGGCGCGACCATCTGCGCTGCGGCGCCGGCGACT CAGCGCTGCCTC	
Human CamKIIa (CaMKIIa)	ACTTGTGGACAAAGTTTGCTCTATTCCACCTCCTCCAGGCCCTCCTT GGGTCCATCACCCCAGGGGTGCTGGGTCCATCCCACCCCCAGGCCC ACACAGGCTTGCAGTATTGTGTGCGGTATGGTCAGGGCGTCCGAGA GCAGGTTTCGCAGTGGAAGGCAGGCAGGTGTTGGGGAGGCAGTTA CCGGGGCAACGGGAACAGGGCGTTTTGGAGGTGGTTGCCATGGGG ACCTGGATGCTGACGAAGGCTCGCGAGGCTGTGAGCAGCCACAGT GCCCTGC	50
eSYN promoter	GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTC ATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACG GTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGA CGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTT CCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTG GCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACG TCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGAC CTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCG CTATTACCATGGCTGCAGAGGGCCCTGCGTATGAGTGCAAGTGGGT TTTAGGACCAGGATGAGGCGGGGTGGGGGTGCCTACCTGACGACC GACCCCGACCCACTGGACAAGCACCCAACCCCCATTCCCCAAATTG CGCATCCCCTATCAGAGAGGGGGAGGGGAAACAGGATGCGGCGAG GCGCGTCGCGACTGCCAGCTTCAGCACCGCGGACAGTGCCTTCGCC CCCGCCTGGCGGCGCGCGCCACCGCCGCCTCAGCACTGAAGGCGCG CTGACGTCACTCGCCGGTCCCCCGCAAACTCCCCTTCCCGGCCACCT TGGTCGCGTCCGCGCCGCCGCCGGCCCAGCCGGACCGCACCACGCG AGGCGCGAGATAGGGGGGCACGGGCGCGACCATCTGCGCTGCGGC GCCGGCGACTCAGCGCTGCCTCAGTCTGCGGTGGGCAGCGGAGGA GTCGTGTCGTGCCTGAGAGCGCAGG	51
hGLUT1 promoter	ACCATTTTGCTAGAGAAGGCCGCGGAGGCTCAGAGAGGTGCGCAC ACTTGCCCTGAGTCACACAGCGAATGCCCTCCGCGGTCCCAACGCA GAGAGAACGAGCCGATCGGCAGCCTGAGCGAGGCAGTGGTTAGGG GGGGCCCCGGCCCCGGCCACTCCCCTCACCCCCTCCCCGCAGAGCG CCGCCCAGGACAGGCTGGGCCCCAGGCCCCGCCCCGAGGTCCTGCC CACACACCCCTGACACACCGGCGTCGCCAGCCAATGGCCGGGGTCC TATAAACGCTACGGTCCGCGCGCTCTCT	102

[0114] In some embodiments, the AAV virion genome comprises a polynucleotide sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 1. In some embodiments, the AAV virion genome comprises a polynucleotide sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 2. In some embodiments, the AAV virion genome comprises a polynucleotide sequence at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to SEQ ID NO: 3.

[0115] Further illustrative examples of promoters are the SV40 late promoter from simian virus 40, the Baculovirus polyhedron enhancer/promoter element, Herpes Simplex Virus

thymidine kinase (HSV tk), the immediate early promoter from cytomegalovirus (CMV) and various retroviral promoters including LTR elements. A large variety of other promoters are known and generally available in the art, and the sequences of many such promoters are available in sequence databases such as the GenBank database.

Other Regulatory Elements

[0116] In some cases, AAV virions of the present disclosure further comprise one or more regulatory elements selected from the group consisting of an enhancer, an intron, a poly-A signal, a 2A peptide encoding sequence, a WPRE (Woodchuck hepatitis virus posttranscriptional regulatory element), and a HPRE (Hepatitis B posttranscriptional regulatory element).

[0117] In some embodiments, the rAAV virion genome comprises a CMV enhancer.

[0118] In certain embodiments, the rAAV virion genome comprises one or more enhancers. In particular embodiments, the enhancer is a CMV enhancer sequence, a GAPDH enhancer sequence, a β-actin enhancer sequence, or an EF1-α enhancer sequence. Sequences of the foregoing are known in the art. For example, the sequence of the CMV immediate early (IE) enhancer is:

CGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGC
CCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCC
ATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCA
AGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCC
GCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACA
TCTACGTATTAGTCATCGCTATTACCATG

(SEQ ID NO: 52)

[0119] In certain embodiments, the rAAV virion genome comprises one or more introns. In particular embodiments, the intron is a rabbit globin intron sequence, a chicken β-actin intron sequence, a synthetic intron sequence, or an EF1-α intron sequence.

[0120] In certain embodiments, the rAAV virion genome comprises a polyA sequence. In particular embodiments, the polyA sequence is a rabbit globin polyA sequence, a human growth hormone polyA sequence, a bovine growth hormone polyA sequence, a PGK polyA

sequence, an SV40 polyA sequence, or a TK polyA sequence. In some embodiments, the poly-A signal may be a bovine growth hormone polyadenylation signal (bGHpA).

[0121] In certain embodiments, the rAAV virion genome comprises one or more transcript stabilizing element. In particular embodiments, the transcript stabilizing element is a WPRE sequence, a HPRE sequence, a scaffold-attachment region, a 3′ UTR, or a 5′ UTR. In particular embodiments, the rAAV virion genome comprises both a 5′ UTR and a 3′ UTR.

[0122] In some embodiments, the rAAV virion genome comprises a 5′ untranslated region (UTR) selected from Table 4. In some embodiments, the rAAV virion genome comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID NOS 53-61.

Table 4

5′ UNTRANSLATED REGION	**SEQUENCE**	**SEQ ID NO:**
Human beta-actin exon/intron	CGCGTCCGCCCGCGAGCACAGAGCCTCGCCTTTGCCGATC CGCCGCCCGTCCACACCCGCCGCCAGGTAAGCCCGGCCAG CCGACCGGGGCATGCGGCCGCGGCCCTTCGCCCGTGCAGA GCCGCCGTCTGGGCCGCAGCGGGGGGCGCATGGGGCGGA ACCGGACCGCCGTGGGGGGCGCGGGAGAAGCCCCTGGGC CTCCGGAGATGGGGGACACCCCACGCCAGTTCGCAGGCG CGAGGCCGCGCTCGGGCGGGCGCGCTCCGGGGGTGCCGC TCTCGGGGCGGGGGCAACCGGCGGGGTCTTTGTCTGAGCC GGGCTCTTGCCAATGGGGATCGCACGGTGGGCGCGGCGTA GCCCCCGTCAGGCCCGGTGGGGGCTGGGGCGCCATGCGC GTGCGCGCTGGTCCTTTGGGCGCTAACTGCGTGCGCGCTG GGAATTGGCGCTAATTGCGCGTGCGCGCTGGGACTCAATG GCGCTAATCGCGCGTGCGTTCTGGGGCCCGGGCGCTTGCG CCACTTCCTGCCCGAGCCGCTGGCGCCCGAGGGTGTGGCC GCTGCGTGCGCGCGCGCGACCCGGTCGCTGTTTGAACCGG GCGGAGGCGGGGCTGGCGCCCGGTTGGGAGGGGGTTGGG GCCTGGCTTCCTGCCGCGCGCCGCGGGGACGCCTCCGACC AGTGTTTGCCTTTTATGGTAATAACGCGGCCGGCCCGGCT	53

	TCCTTTGTCCCCAATCTGGGCGCGCCGGCGCCCCCTGG CGGCCTAAGGACTCGGCGCGCCGGAAGTGGCCAGGGCGG CAGCGGCTGCTCTTGGCGGCCCCGAGGTGACTATAGCCTT CTTTTGTGTCTTGATAGTTCGCCAGCCTCTGCTAACCATGT TCATGCCTTCTTCTTTTTCCTACAGCTCCTGGGCAACGTGC TGGTTATTGTGCTGTCTCATCATTTTGGCAAAGAATTC	
Chicken beta-actin exon/intron + rabbit globin intron	GTCGCTGCGCGCTGCCTTCGCCCCGTGCCCCGCTCCGCCG CCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTT ACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCG GGCTGTAATTAGCGCTTGGTTTAATGACGGCTTGTTTCTTT TCTGTGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGC CCTTTGTGCGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGT GTGTGTGCGTGGGGAGCGCCGCGTGCGGCTCCGCGCTGCC CGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGT GCGCTCCGCAGTGTGCGCGAGGGGAGCGCGGCCGGGGGC GGTGCCCCGCGGTGCGGGGGGGGCTGCGAGGGGAACAAA GGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGG GGTGTGGGCGCGTCGGTCGGGCTGCAACCCCCCCTGCACC CCCCTCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGC GGGGCTCCGTACGGGGCGTGGCGCGGGGCTCGCCGTGCC GGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGC GGGGCCGCCTCGGGCCGGGGAGGGCTCGGGGGAGGGGCG CGGCGGCCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGC GAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGG GCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGA AATCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGG GGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGG GGAGGGCCTTCGTGCGTCGCCGCGCCGCCGTCCCCTTCTC CCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGCTGCC TTCGGGGGGGACGGGGCAGGGCGGGGTTCGGCTTCTGGC GTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCA TGCCTTCTTCTTTTTCCTACAGCTCCTGGGCAACGTGCTGG TTATTGTGCTGTCTCATCATTTTGGCAAAGAATTC	54
5′ UTR-Syn1 Hs	AGTCTGCGGTGGGCAGCGGAGGAGTCGTGTCGTGCCTGAG AGCGCAGCTGTGCTCCTGGGCACCGCGCAGTCCGCCCCCG	55

	CGGCTCCTGGCCAGACCACCCCTAGGACCCCCTGCCCCAA GTCGCA	
CMV IE exon	TCAGATCGCCTGGAGAGGCCATCCACGCTGTTTTGACCTC CATAGTGGACACCGGGACCGATCCAGCCTCCGCGGCCGG GAACGGTGCATTGGAACGCGGATTCCCCGTGCCAAGAGTG AC	56
TPL-eMLP *(adenovirus derived enhancer element)*	CTCACTCTCTTCCGCATCGCTGTCTGCGAGGGCCAGCTGTT GGGCTCGCGGTTGAGGACAAACTCTTCGCGGTCTTTCCAG TACTCTTGGATCGGAAACCCGTCGGCCTCCGAACGGTACT CCGCCACCGAGGGACCTGAGCGAGTCCGCATCGACCGGA TCGGAAAACCTCTCGAGAAAGGCGTCTAACCAGTCACAGT CGCAAGGTAGGCTGAGCACCGTGGCGGGCGGCAGCGGGT GGCGGTCGGGGTTGTTTCTGGCGGAGGTGCTGCTGATGAT GTAATTAAAGTAGGCGGTCTTGAGACGGCGGATGGTCGA GGTGAGGTGTGGCAGGCTTGAGATCCAGCTGTTGGGGTGA GTACTCCCTCTCAAAAGCGGGCATTACTTCTGCGCTAAGA TTGTCAGTTTCCAAAAACGAGGAGGATTTGATATTCACCT GGCCCGATCTGGCCATACACTTGAGTGACAATGACATCCA CTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAG	57
Human EF1-α intron/exon	CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTG CCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTAT GGCCCTTGCGTGCCTTGAATTACTTCCACCTGGCTCCAGTA CGTGATTCTTGATCCCGAGCTGGAGCCAGGGGCGGGCCTT GCGCTTTAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGC CTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGG CACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGC CATTTAAAATTTTTGATGACGTGCTGCGACGCTTTTTTTCT GGCAAGATAGTCTTGTAAATGCGGGCCAGGATCTGCACAC TGGTATTTCGGTTTTTGGGCCCGCGGCCGGCGACGGGGCC CGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCCTGC GAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAG CTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTG TATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCA CCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTG	58

	CTCCAGGGGGCTCAAAATGGAGGACGCGGCGCTCGGGAG AGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCT TTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTAC CGGGCGCCGTCCAGGCACCTCGATTAGTTCTGGAGCTTTT GGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGC GATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTT AGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTG GCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAG ACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAG	
Human EF1-α, intron A	GTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTA CGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACCTGG CTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAA GTGGGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCC TTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGG GGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTC TCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGA TGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGT AAATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTT GGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGC ACATGTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCG AGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTC TGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTG GGCGGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGC GGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCA AAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAG TCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCG TCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAG GCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTT TAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCA CACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCAC TTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGG ATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTT TTTTTCTTCCATTTCAG	59

5' UTR human CamKIIa	TCAGAAGCCCCGGGCTCGTCAGTCAAACCGGTTCTCTGTT TGCACTCGGCAGCACGGGCAGGCAAGTGGTCCCTAGGTTC GGG	60
B-globin intron	GTGAGTCTATGGGACCCTTGATGTTTTCTTTCCCCTTCTTTT CTATGGTTAAGTTCATGTCATAGGAAGGGGAGAAGTAACA GGGTACACATATTGACCAAATCAGGGTAATTTTGCATTTG TAATTTTAAAAAATGCTTTCTTCTTTTAATATACTTTTTTGT TTATCTTATTTCTAATACTTTCCCTAATCTCTTTCTTTCAGG GCAATAATGATACAATGTATCATGCCTCTTTGCACCATTCT AAAGAATAACAGTGATAATTTCTGGGTTAAGGCAATAGCA ATATTTCTGCATATAAATATTTCTGCATATAAATTGTAACT GATGTAAGAGGTTTCATATTGCTAATAGCAGCTACAATCC AGCTACCATTCTGCTTTTATTTTATGGTTGGGATAAGGCTG GATTATTCTGAGTCCAAGCTAGGCCCTTTTGCTAATCATGT TCATACCTCTTATCTTCCTCCCACAG	61

[0123] In some embodiments, the rAAV virion genome comprises a 3′ untranslated region selected from Table 5. In some embodiments, the rAAV virion genome comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID NOS 62-70.

Table 5

3′ UNTRANSLATED REGION	**SEQUENCE**	**SEQ ID NO:**
WPRE(x) (mutated woodchuck hepatitis regulatory element – version 1)	AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACT GGTATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGAT ACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGT ATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCT GTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGT GGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTG GTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGAC TTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATC GCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGT	62

	TGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAATCAT CGTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATT CTGCGCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCA ATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCT GCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGT CGGATCTCCCTTTGGGCCGCCTCCCCGC	
WPRE(x) (mutated woodchuck hepatitis regulatory element – version 2)	TCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGT ATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGATACG CTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATG GCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTC TCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGC GTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTT GGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTT CGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGCC GCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGG GCACTGACAATTCCGTGGTGTTGTCGGGGAAATCATCGTC CTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGC GCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCC AGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGG CCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGA TCTCCCTTTGGGCCGCCTCCCCGCA	63
WPRE(x) (mutated woodchuck hepatitis regulatory element – version 3)	TTCCTGTTAATCAACCTCTGGATTACAAAATTTGTGAAAG ATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTAT GTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGC TTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCT GGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAG GCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAAC CCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTT TCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGG AACTCATCGCCGCCTGCCTTGCCCGCTGCTGGACAGGGG CTCGGCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGG GAAGCTGACGTCCTTTCCGCGGCTGCTCGCCTGTGTTGCC ACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCCTT CGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCT GCCGGCTCTGCGGCCTCTTCCGCCTCTTCGCCTTCGCCCT	64

	CAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCCCA TGTATCTTTTTCACCTGTGCCTTGTTTTTGCCTGTGTTCCG CGTCCTACTTTTCAAGCCTCCAAGCTGTGCCTTGGGCGGC TTTGGGGCATGGACATAGATCCCTATAAAGAATTTGGTTC ATCTTATCAGTTGTTGAATTTTCTTCCTTTGGAC	
CAAX	TGTGTGATAATG	65
EES	CTGTTCTCATCACATCATATCAAGGTTATATACCATCAAT ATTGCCACAGATGTTACTTAGCCTTTTAATATTTCTCTAAT TTAGTGTATATGCAATGATAGTTCTCTGATTTCTGAGATT GAGTTTCTCATGTGTAATGATTATTTAGAGTTTCTCTTTCA TCTGTTCAAATTTTTGTCTAGTTTTATTTTTTACTGATTTG TAAGACTTCTTTTTATAATCTGCATATTACAATTCTCTTTA CTGGGGTGTTGCAAATATTTTCTGTCATTCTATGGCCTGA CTTTTCTTAATGGTTTTTTAATTTTAAAAATAAGTCTTAAT ATTCATGCAATCTAATTAACAATCTTTTCTTTGTGGTTAG GACTTTGAGTCATAAGAAATTTTTCTCTACACTGAAGTCA TGATGGCATGCTTCTATATTATTTTCTAAAAGATTTAAAG TTTTGCCTTCTCCATTTAGACTTATAATTCACTGGAATTTT TTTGTGTGTATGGTATGACATATGGGTTCCCTTTTATTTTT TACATATAAATATATTTCCCTGTTTTTCTAAAAAAGAAAA AGATCATCATTTTCCCATTGTAAAATGCCATATTTTTTTCA TAGGTCACTTACATATATCAATGGGTCTGTTTCTGAGCTC TACTCTATTTTATCAGCCTCACTGTCTATCCCCACACATCT CATGCTTTGCTCTAAATCTTGATATTTAGTGGAACATTCT TTCCCATTTTGTTCTACAAGAATATTTTTGTTATTGTCTTT GGGCTTTCTATATACATTTTGAAATGAGGTTGACAAGTTA	66
HPRE	ATAACAGGCCTATTGATTGGAAAGTTTGTCAACGAATTGT GGGTCTTTTGGGGTTTGCTGCCCCTTTTACGCAATGTGGA TATCCTGCTTTAATGCCTTTATATGCATGTATACAAGCAA AACAGGCTTTTACTTTCTCGCCAACTTACAAGGCCTTTCT CAGTAAACAGTATATGACCCTTTACCCCGTTGCTCGGCAA CGGCCTGGTCTGTGCCAAGTGTTTGCTGACGCAACCCCCA CTGGTTGGGGCTTGGCCATAGGCCATCAGCGCATGCGTG GAACCTTTGTGTCTCCTCTGCCGATCCATACTGCGGAACT CCTAGCCGCTTGTTTTGCTCGCAGCAGGTCTGGAGCAAAC CTCATCGGGACCGACAATTCTGTCGTACTCTCCCGCAAGT ATACATCGTTTCCATGGCTGCTAGGCTGTGCTGCCAACTG GATCCTGCGCGGGACGTCCTTTGTTTACGTCCCGTCGGCG CTGAATCCCGCGGACGACCCCTCCCGGGGCCGCTTGGGG CTCTACCGCCCGCTTCTCCGTCTGCCGTACCGTCCGACCA CGGGGCGCACCTCTCTTTACGCGGACTCCCCGTCTGTGCC TTCTCATCTGCCGGACCGTGTGCACTTCGCTTCACCTCTG CACGTCGCATGGAGGCCACCGTGAACGCCCACCGGAACC TGCCCAAGGTCTTGCATAAGAGGACTCTTGGACTTTCAGC AATGTCATC	67

R2V17 *(HepB derived enhancer element)*	TTCCTGTAAACAGGCCTATTGATTGGAAAGTTTGTCAACG AATTGTGGGTCTTTTGGGGTTTGCTGCCCCTTTTACGCAA TGTGGATATCCTGCTTTAATGCCTTTATATGCATGTATAC AAGCAAAACAGGCTTTTACTTTCTCGCCAACTTACAAGGC CTTTCTCAGTAAACAGTATATGACCCTTTACCCCGTTGCT CGGCAACGGCCTGGTCTGTGCCAAGTGTTTGCTGACGCA ACCCCCACTGGTTGGGGCTTGGCCATAGGCCATCAGCGC ATGCGTGGAACCTTTGTGTCTCCTCTGCCGATCCATACTG CGGAACTCCTAGCCGCTTGTTTTGCTCGCAGCTGGACTGG AGCAAACCTCATCGGGACCGACAATTCTGTCGTACTCTCC CGCAAGCACTCACCGTTTCCGCGGCTGCTCGCCTGTGTTG CCACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCC TTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTG CTGCCGGCTCTGCGGCCTCTTCCGCCTCTTCGCCTTCGCC CTCAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCC CATGTATCTTTTTCACCTGTGCCTTGTTTTTGCCTGTGTTC CGCGTCCTACTTTTCAAGCCTCCAAGCTGTGCCTTGGGCG GCTTTGGGGCATGGACATAGATCCCTATAAAGAATTTGG TTCATCTTATCAGTTGTTGAATTTTCTTCCTTTGGAC	68
3'UTR(globin)	GCTGGAGCCTCGGTAGCCGTTCCTCCTGCCCGCTGGGCCT CCCAACGGGCCCTCCTCCCCTCCTTGCACCGGCCCTTCCT GGTCTTTGAATAAA	69
WPRE(r)	ATTCGAGCATCTTACCGCCATTTATTCCCATATTTGTTCTG TTTTTCTTGATTTGGGTATACATTTAAATGTTAATAAAAC AAAATGGTGGGGCAATCATTTACATTTTTAGGGATATGTA ATTACTAGTTCAGGTGTATTGCCACAAGACAAACATGTTA AGAAACTTTCCCGTTATTTACGCTCTGTTCCTGTTAATCA ACCTCTGGATTACAAAATTTGTGAAAGATTGACTGATATT CTTAACTATGTTGCTCCTTTTACGCTGTGTGGATATGCTG CTTTAATGCCTCTGTATCATGCTATTGCTTCCCGTACGGCT TTCGTTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCT TTATGAGGAGTTGTGGCCCGTTGTCCGTCAACGTGGCGTG GTGTGCTCTGTGTTTGCTGACGCAACCCCCACTGGCTGGG GCATTGCCACCACCTGTCAACTCCTTTCTGGGACTTTCGC	70

	TTTCCCCCTCCCGATCGCCACGGCAGAACTCATCGCCGCC TGCCTTGCCCGCTGCTGGACAGGGGCTAGGTTGCTGGGC ACTGATAATTCCGTGGTGTTGTCGGGGAAGGGCC	

[0124] In some embodiments, the rAAV virion genome comprises a polyadenylation (polyA) signal selected from Table 6. In some embodiments, the polyA signal comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to any one of SEQ ID NOS 71-75.

Table 6

POLY-ADENYLATION SITE	**SEQUENCE**	**SEQ ID NO:**
Rabbit globin (pAGlobin-Oc)	TGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTG TTGGAATTTTTTGTGTCTCTCACTCGGAAGAACATATGG GAGGGCAAATCATTTAAAACATCAGAATGAGTATTTGGT TTAGAGTTTGGCAACATATGCCCATATGCTGGCTGCCAT GAACAAAGGTTGGCTATAAAGAGGTCATCAGTATATGA AACAGCCCCCTGCTGTCCATTCCTTATTCCATAGAAAAG CCTTGACTTGAGGTTAGATTTTTTTTATATTTTGTTTTGTG TTATTTTTTTCTTTAACATCCCTAAAATTTTCCTTACATGT TTTACTAGCCAGATTTTTCCTCCTCTCCTGACTACTCCCA GTCATAGCTGTCCCTCTTCTCTTATGGAGATC	71
Bovine growth hormone (pAGH-Bt – version 1)	TTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCC TTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAA TAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGT CATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAA GGGGGAGGATTGGGAATACAATAGCAGGCATGCTGGGG ATGCGGTGGGCTCTATGGGTACCCAGGTGCTGAAGAATT GACCCGGTTCCTCCTGGG	72
Bovine growth hormone (pAGH-Bt – version 2)	TTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCC TTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAA TAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGT CATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAA	73

	GGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGG ATGCGGTGGGCTCTATGGGTACCCAGGTGCTGAAGAATT GACCCGGTTCCTCCTGGG	
Bovine growth hormone (pAGH-Bt – version 3)	CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTC CCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCAC TGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTG TCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGG GCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGC AGGCATGCTGGGGATGCGGTGGGCTCTATGG	74
Human growth hormone (pAGH-Hs)	CTGCCCGGGTGGCATCCCTGTGACCCCTCCCCAGTGCCT CTCCTGGCCCTGGAAGTTGCCACTCCAGTGCCCACCAGC CTTGTCCTAATAAAATTAAGTTGCATCATTTTGTCTGACT AGGTGTCCTTCTATAATATTATGGGGTGGAGGGGGGTGG TATGGAGCAAGGGGCCCAAGTTGGGAAGAAACCTGTAG GGCCTGC	75

[0125] Illustrative rAAV virion genomes are depicted in FIG. 2-8 and provided as SEQ ID NOs: 17-25. The capitalized portion of each sequence is the expression cassette (SEQ ID NOs: 8-16, SEQ ID NO: 97, SEQ ID NO: 99, and SEQ ID NO: 101). In some embodiments, the rAAV virion genome comprises, consists essentially of, or consists of a polynucleotide sequence that shares at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to any one of SEQ ID NOs: 8-16, SEQ ID NO: 97, SEQ ID NO: 99, and SEQ ID NO: 101, optionally with or without the ITR sequences in lowercase. The coding sequence is underlined. The expression cassette is capitalized.

ADENO-ASSOCIATED VIRUS GENOME

[0126] Adeno-associated virus (AAV) is a replication-deficient parvovirus, the single-stranded DNA genome of which is about 4.7 kb in length including two ~145-nucleotide inverted terminal repeat (ITRs). There are multiple known variants of AAV, also sometimes called serotypes when classified by antigenic epitopes. The nucleotide sequences of the genomes of the AAV serotypes are known. For example, the complete genome of AAV-1 is provided in GenBank Accession No. NC_002077; the complete genome of AAV-2 is provided in GenBank Accession No. NC_001401 and Srivastava et al., J. Virol., 45: 555-564

(1983); the complete genome of AAV-3 is provided in GenBank Accession No. NC_1829; the complete genome of AAV-4 is provided in GenBank Accession No. NC_001829; the AAV-5 genome is provided in GenBank Accession No. AF085716; the complete genome of AAV-6 is provided in GenBank Accession No. NC_00 1862; at least portions of AAV-7 and AAV-8 genomes are provided in GenBank Accession Nos. AX753246 and AX753249, respectively; the AAV-9 genome is provided in Gao et al., J. Virol., 78: 6381-6388 (2004); the AAV-10 genome is provided in Mol. Ther., 13(1): 67-76 (2006); and the AAV-11 genome is provided in Virology, 330(2): 375-383 (2004). The sequence of the AAVrh.74 genome is provided in U.S. Patent 9,434,928, incorporated herein by reference. Cis-acting sequences directing viral DNA replication (rep), encapsidation/packaging and host cell chromosome integration are contained within the AAV ITRs. Three AAV promoters (named p5, p19, and p40 for their relative map locations) drive the expression of the two AAV internal open reading frames encoding rep and cap genes. The two rep promoters (p5 and p19), coupled with the differential splicing of the single AAV intron (at nucleotides 2107 and 2227), result in the production of four rep proteins (rep78, rep68, rep52, and rep40) from the rep gene. Rep proteins possess multiple enzymatic properties that are ultimately responsible for replicating the viral genome. The cap gene is expressed from the p40 promoter and it encodes the three capsid proteins VP1, VP2, and VP3. Alternative splicing and non-consensus translational start sites are responsible for the production of the three related capsid proteins. A single consensus polyadenylation site is located at map position 95 of the AAV genome. The life cycle and genetics of AAV are reviewed in Muzyczka, Current Topics in Microbiology and Immunology, 158: 97-129 (1992).

[0127] AAV possesses unique features that make it attractive as a vehicle for delivering foreign DNA to cells, for example, in gene therapy. AAV infection of cells in culture is noncytopathic, and natural infection of humans and other animals is silent and asymptomatic. Moreover, AAV infects many mammalian cells allowing the possibility of targeting many different tissues in vivo. Moreover, AAV transduces slowly dividing and non-dividing cells, and can persist essentially for the lifetime of those cells as a transcriptionally active nuclear episome (extrachromosomal element). The AAV proviral genome is inserted as cloned DNA in plasmids, which makes construction of recombinant genomes feasible. Furthermore, because the signals directing AAV replication and genome encapsidation are contained within the ITRs of the AAV genome, some or all of the internal approximately 4.3 kb of the genome (encoding replication and structural capsid proteins, rep-cap) may be replaced with

foreign DNA. To generate rAAV vector genomes, the rep and cap proteins may be provided in trans. Another significant feature of AAV is that it is an extremely stable and hearty virus. It easily withstands the conditions used to inactivate adenovirus (56° to 65°C for several hours), making cold preservation of AAV less critical. AAV may even be lyophilized. Finally, AAV-infected cells are not resistant to superinfection.

[0128] Recombinant AAV virions useful in the practice of the present invention can be constructed utilizing methodologies well known in the art of molecular biology. Typically, viral vectors carrying transgenes are assembled from polynucleotides encoding the transgene, suitable regulatory elements and elements necessary for production of viral proteins, which mediate cell transduction. Such rAAV virions may be produced by techniques known in the art, e.g., by transfecting packaging cells or by transient transfection with helper plasmids or viruses. Typical examples of virus packaging cells include but are not limited to HeLa cells, SF9 cells (optionally with a baculovirus helper vector), 293 cells, etc. A Herpesvirus-based system can be used to produce AAV vectors, as described in US20170218395A1. Detailed protocols for producing such replication-defective recombinant viruses may be found for instance in W095/14785, W096/22378, U.S. Pat. No. 5,882,877, U.S. Pat. No. 6,013,516, U.S. Pat. No. 4,861,719, U.S. Pat. No. 5,278,056 and W094/19478, the complete contents of each of which is hereby incorporated by reference.

[0129] AAV vectors useful in the practice of the present invention can be packaged into AAV virions (viral particles) using various systems including adenovirus-based and helper-free systems. Standard methods in AAV biology include those described in Kwon and Schaffer. *Pharm Res.* 25(3):489-99 (2008); Wu et al. *Mol. Ther.* 14(3):316-27 (2006); Burger et al. *Mol. Ther.* 10(2):302-17 (2004); Grimm et al. *Curr Gene Ther.* 3(4):281-304 (2003); Deyle DR, Russell DW. *Curr Opin Mol Ther.* 11(4):442-447 (2009); McCarty et al. *Gene Ther.* 8(16):1248-54 (2001); and Duan et al. *Mol Ther.* 4(4):383-91 (2001). Helper-free systems included those described in US 6,004,797; US 7,588,772; and US 7,094,604.

[0130] AAV DNA in the rAAV vector genomes may be from any AAV variant or serotype for which a recombinant virus can be derived including, but not limited to, AAV variants or serotypes AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, and AAV13. Production of pseudotyped rAAV is disclosed in, for example, WO 01/83692. Other types of rAAV variants, for example rAAV with capsid mutations, are also contemplated. *See, for example*, Marsic et al., Molecular Therapy, 22(11):

1900-1909 (2014). The nucleotide sequences of the genomes of various AAV serotypes are known in the art.

[0131] In some cases, the rAAV comprises a self-complementary genome. As defined herein, an rAAV comprising a "self-complementary" or "double stranded" genome refers to an rAAV which has been engineered such that the coding region of the rAAV is configured to form an intra-molecular double-stranded DNA template, as described in McCarty et al. Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis. Gene Therapy. 8 (16): 1248–54 (2001). The present disclosure contemplates the use, in some cases, of an rAAV comprising a self-complementary genome because upon infection (such transduction), rather than waiting for cell mediated synthesis of the second strand of the rAAV vector genome, the two complementary halves of scAAV will associate to form one double stranded DNA (dsDNA) unit that is ready for immediate replication and transcription. It will be understood that instead of the full coding capacity found in rAAV (4.7-6kb), rAAV comprising a self-complementary genome can only hold about half of that amount (≈2.4kb).

[0132] In other cases, the rAAV vector genome is a single stranded genome. As defined herein, a "single standard" genome refers to a genome that is not self-complementary. In most cases, non-recombinant AAVs have singled stranded DNA genomes. There have been some indications that rAAVs should be scAAVs to achieve efficient transduction of cells. The present disclosure contemplates, however, rAAV vector genomes may have singled stranded genomes, rather than self-complementary genomes, with the understanding that other genetic modifications of the rAAV vector genome may be beneficial to obtain optimal gene transcription in target cells. In some cases, the present disclosure relates to single-stranded rAAV vector genomes capable of achieving efficient gene transfer to anterior segment in the mouse eye. *See* Wang et al. Single stranded adeno-associated virus achieves efficient gene transfer to anterior segment in the mouse eye. PLoS ONE 12(8): e0182473 (2017).

[0133] In preferred embodiments, the AAV is an AAV variant having a high degree of tropism for endothelial cells , such as the AAV2 variant BR1. Production of pseudotyped rAAV is disclosed in, for example, WO 01/83692. Other types of rAAV variants, for example rAAV with capsid mutations, are also contemplated. *See, for example*, Marsic et al., Molecular Therapy, 22(11): 1900-1909 (2014). In some embodiments, said rAAV vector

genome is of serotype AAV-BR1 and comprises a single-stranded genome. In some embodiments, a rAAV vector genome comprises the inverted terminal repeat (ITR) sequences of AAV2. In some embodiments, the rAAV vector genome comprises an AAV2 genome.

[0134] AAV genomes may comprise wild-type AAV sequence or they may comprise one or more modifications to a wild-type AAV sequence. In certain embodiments, an AAV genome comprises one or more amino acid modifications, e.g., substitutions, deletions, or insertions, within a capsid protein, e.g., VP1, VP2 and/or VP3. In particular embodiments, the modification provides for reduced immunogenicity when the AAV genome is provided to a subject.

[0135] Capsid proteins of a rAAV may be modified so that the rAAV is targeted to a particular target tissue of interest such as endothelial cells and/or endothelial tip cells. In some embodiments, the rAAV is directly injected into the intracerebroventricular space, injected intravenously or by combined intracerebroventicular and intravenous delivery to the subject.

[0136] In some embodiments, the rAAV virion is a variant of an AAV2 rAAV virion. The capsid many be an AAV2 capsid or functional variant thereof. The polypeptide sequence of the AAV2 VP1 is provided as SEQ ID NO: 76. In some embodiments, the AAV2 capsid shares at least 95% to a reference AAV2 capsid, *e.g.*,

MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFNGLDKGEPVNEADAAALEH
DKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSP
VEPDSSSGTGKAGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGTNTMATGSGAPMADNNEGADGVGNSS
GNWHCDSTWMGDRVITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLI
NNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQY
GYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTNT
PSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVNPGPAMAS
HKDDEEKFFPQSGVLIFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGV
LPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYST
GQVSVEIEWELQKENSKRWNPEIQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL

(SEQ ID NO: 76)

[0137] In some embodiments, the capsid protein is encoded by a polynucleotide supplied on a plasmid *in trans* to the transfer plasmid. In some embodiments, the polynucleotide

sequence of AAV-BR1 *cap* shares at least 98%, at least 99%, or 100% identity SEQ ID NO: 103:

ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACACTCTCTCTGAAGGAATAAGACAGTGGTGGAAGCTC
AAACCTGGCCCACCACCACCAAAGCCCGCAGAGCGGCATAAGGACGACAGCAGGGGTCTTGTGCTTCCTGGGTAC
AAGTACCTCGGACCCTTCAACGGACTCGACAAGGGAGAGCCGGTCAACGAGGCAGACGCCGCGGCCCTCGAGCAC
GACAAAGCCTACGACCGGCAGCTCGACAGCGGAGACAACCCGTACCTCAAGTACAACCACGCCGACGCGGAGTTT
CAGGAGCGCCTTAAAGAAGATACGTCTTTTGGGGGCAACCTCGGACGAGCAGTCTTCCAGGCGAAAAAGAGGGTT
CTTGAACCTCTGGGCCTGGTTGAGGAACCTGTTAAGACGGCTCCGGGAAAAAAGAGGCCGGTAGAGCACTCTCCT
GTGGAGCCAGACTCCTCCTCGGGAACCGGAAAGGCGGGCCAGCAGCCTGCAAGAAAAAGATTGAATTTTGGTCAG
ACTGGAGACGCAGACTCAGTACCTGACCCCCAGCCTCTCGGACAGCCACCAGCAGCCCCCTCTGGTCTGGGAACT
AATACGATGGCTACAGGCAGTGGCGCACCAATGGCAGACAATAACGAGGGCGCCGACGGAGTGGGTAATTCCTCG
GGAAATTGGCATTGCGATTCCACATGGATGGGCGACAGAGTCATCACCACCAGCACCCGAACCTGGGCCCTGCCC
ACCTACAACAACCACCTCTACAAACAAATTTCCAGCCAATCAGGAGCCTCGAACGACAATCACTACTTTGGCTAC
AGCACCCCTTGGGGGTATTTTGACTTCAACAGATTCCACTGCCACTTTTCACCACGTGACTGGCAAAGACTCATC
AACAACAACTGGGGATTCCGACCCAAGAGACTCAACTTCAAGCTCTTTAACATTCAAGTCAAAGAGGTCACGCAG
AATGACGGTACGACGACGATTGCCAATAACCTTACCAGCACGGTTCAGGTGTTTACTGACTCGGAGTACCAGCTC
CCGTACGTCCTCGGCTCGGCGCATCAAGGATGCCTCCCGCCGTTCCCAGCAGACGTCTTCATGGTGCCACAGTAT
GGATACCTCACCCTGAACAACGGGAGTCAGGCAGTAGGACGCTCTTCATTTTACTGCCTGGAGTACTTTCCTTCT
CAGATGCTGCGTACCGGAAACAACTTTACCTTCAGCTACACTTTTGAGGACGTTCCTTTCCACAGCAGCTACGCT
CACAGCCAGAGTCTGGACCGTCTCATGAATCCTCTCATCGACCAGTACCTGTATTACTTGAGCAGAACAAACACT
CCAAGTGGAACCACCACGCAGTCAAGGCTTCAGTTTTCTCAGGCCGGAGCGAGTGACATTCGGGACCAGTCTAGG
AACTGGCTTCCTGGACCCTGTTACCGCCAGCAGCGAGTATCAAAGACATCTGCGGATAACAACAACAGTGAATAC
TCGTGGACTGGAGCTACCAAGTACCACCTCAATGGCAGAGACTCTCTGGTGAATCCGGGCCCGGCCATGGCAAGC
CACAAGGACGATGAAGAAAAGTTTTTTCCTCAGAGCGGGGTTCTCATCTTTGGGAAGCAAGGCTCAGAGAAAACA
AATGTGGACATTGAAAAGGTCATGATTACAGACGAAGAGGAAATCAGGACAACCAATCCCGTGGCTACGGAGCAG
TATGGTTCTGTATCTACCAACCTCCAGAGAGGCCAGAGAGGCAATCGGGGGACTGAGTGGGATGCCCAGGCGGCC
ACCGCAGATGTCAACACACAAGGCGTTCTTCCAGGCATGGTCTGGCAGGACAGAGATGTGTACCTTCAGGGGCCC
ATCTGGGCAAAGATTCCACACACGGACGGACATTTTCACCCCTCTCCCCTCATGGGTGGATTCGGACTTAAACAC
CCTCCTCCACAGATTCTCATCAAGAACACCCCGGTACCTGCGAATCCTTCGACCACCTTCAGTGCGGCAAAGTTT
GCTTCCTTCATCACACAGTACTCCACGGGACAGGTCAGCGTGGAGATCGAGTGGGAGCTGCAGAAGGAAAACAGC
AAACGCTGGAATCCCGAAATTCAGTACACTTCCAACTACAACAAGTCTGTTAATGTGGACTTTACTGTGGACACT
AATGGCGTGTATTCAGAGCCTCGCCCCATTGGCACCAGATACCTGACTCGTAATCTGTAA

(SEQ ID NO: 103)

[0138] In some embodiments, the disclosure further provides protein sequences for AAV-BR1 VP1 and functional variants thereof. The sequence of the AAV-BR1 VP1 polypeptide is provided as SEQ ID NO: 104. In some embodiments, the sequence of AAV VP1 shares at least 98%, at least 99%, or 100% identity SEQ ID NO: 104:

MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFNGLDKGEPVNEADAAALEH
DKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTAPGKKRPVEHSP
VEPDSSSGTGKAGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGTNTMATGSGAPMADNNEGADGVGNSS
GNWHCDSTWMGDRVITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLI
NNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQY
GYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTNT
PSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVNPGPAMAS
HKDDEEKFFPQSGVLIFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGQRGNRGTEWDAQAA
TADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPSTTFSAAKF
ASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL

(SEQ ID NO: 104)

[0139] In some embodiments, the disclosure further provides protein sequences for AAV-BR1 VP2 and functional variants thereof. The sequence of the AAV-BR1 VP1 polypeptide is provided as SEQ ID NO: 105. In some embodiments, the sequence of AAV VP2 shares at least 98%, at least 99%, or 100% identity SEQ ID NO: 105:

MAPGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGTNTMATGSGAPMA
DNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRF
HCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCL
PPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPL
IDQYLYYLSRTNTPSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNG
RDSLVNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGQ
RGNRGTEWDAQAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPV
PANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYNKSVNVDFTVDTNGVYSEPRPIGT
RYLTRNL

(SEQ ID NO: 105)

[0140] In some embodiments, the disclosure further provides protein sequences for AAV-BR1 VP3 and functional variants thereof. The sequence of the AAV-BR1 VP1 polypeptide is provided as SEQ ID NO: 106. In some embodiments, the sequence of AAV VP3 shares at least 98%, at least 99%, or 100% identity SEQ ID NO: 106:

MATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYST
PWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPY
VLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHS
QSLDRLMNPLIDQYLYYLSRTNTPSGTTTQSRLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSW
TGATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQYG
SVSTNLQRGQRGNRGTEWDAQAATADVNTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPP

PQILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYNKSVNVDFTVDTNG
VYSEPRPIGTRYLTRNL

(SEQ ID NO: 106)

[0141] In some embodiments, the capsid comprises an insertion in the GH loop, that is between approximately amino acid residue 588 and residue 589, in the AAV2 VP1 reference sequence set forth in SEQ ID NO 76. In some embodiments, the insertion sequence shares at least 98%, at least 99%, or 100% identity SEQ ID NO: 107:

XDGXXWX, wherein each X is any amino acid.

(SEQ ID NO: 107)

[0142] In some embodiments, the capsid comprises an insertion in the GH loop between amino acids 588 and 589 compared to a VP1 reference sequence as set forth in SEQ ID NO 76. In some embodiments, the insertion sequence shares at least 98%, at least 99%, or 100% identity SEQ ID NO: 108:

ADGVQWT

(SEQ ID NO: 108)

[0143] In some embodiments, the capsid comprises an insertion in the GH loop between amino acids 588 and 589 compared to a VP1 reference sequence as set forth in SEQ ID NO 76. In some embodiments, the insertion sequence shares at least 98%, at least 99%, or 100% identity SEQ ID NO: 109:

DDGVSWK

(SEQ ID NO: 109)

[0144] In some embodiments, the capsid comprises an insertion in the GH loop between amino acids 588 and 589 compared to a VP1 reference sequence as set forth in SEQ ID NO 76. In some embodiments, the insertion sequence shares at least 98%, at least 99%, or 100% identity SEQ ID NO: 110:

SDGLTWS

(SEQ ID NO: 110)

[0145] In some embodiments, the capsid comprises an insertion in the GH loop between amino acids 588 and 589 compared to a VP1 reference sequence as set forth in SEQ ID NO 76. In some embodiments, the insertion sequence shares at least 98%, at least 99%, or 100% identity SEQ ID NO: 111:

SDGLAWV

(SEQ ID NO: 111)

[0146] In some embodiments, the capsid comprises an insertion in the GH loop between amino acids 588 and 589 compared to a VP1 reference sequence as set forth in SEQ ID NO 76. In some embodiments, the insertion sequence shares at least 98%, at least 99%, or 100% identity SEQ ID NO: 112:

NRGTEWD

(SEQ ID NO: 112)

[0147] Further AAV capsids used in the rAAV virions of the disclosure include those disclosed in Pat. Pub. Nos. WO 2009/012176 A2 and WO 2015/168666 A2.

[0148] Use of an AAV-BR1 may result in endothelial-cell specific expression of the Glut1 transgene and, accordingly, increased efficacy of the transgene.

[0149] In some embodiments, rAAV vector genome is not an AAV2 genome. Without being bound by theory, use of an AAV2 genome results in transduction of neuronal cells in addition to or instead of endothelial cells. Without being bound by theory, spread of AAV2 genome within the CNS and associated cerebrovasculature is limited by its interaction with Heparan Sulfate Proteoglycan (HSPG) receptors.

PHARMACEUTICAL COMPOSITIONS AND KITS

[0150] In an aspect, the disclosure provides pharmaceutical compositions comprising the rAAV virion of the disclosure and one or more pharmaceutically acceptable carriers, diluents, or excipients.

[0151] For purposes of administration, e.g., by injection, various solutions can be employed, such as sterile aqueous solutions. Such aqueous solutions can be buffered, if desired, and the liquid diluent first rendered isotonic with saline or glucose. Solutions of

rAAV as a free acid (DNA contains acidic phosphate groups) or a pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as Poloxamer 188, *e.g.*, at least 0.001% or 0.01%. A dispersion of rAAV can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. In this connection, the sterile aqueous media employed are all readily obtainable by standard techniques well-known to those skilled in the art.

[0152] The pharmaceutical forms suitable for injectable use include but are not limited to sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form is sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating actions of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can 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 a dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by use of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0153] Sterile injectable solutions may be prepared by incorporating rAAV in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating the sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the methods of preparation are vacuum drying and the freeze-drying technique that yield a powder of the active ingredient plus any additional desired ingredient from the previously sterile-filtered solution thereof.

[0154] In another aspect, the disclosure comprises a kit comprising an rAAV virion of the disclosure and instructions for use.

METHODS OF USE

[0155] In an aspect, the disclosure provides a method of increasing GLUT1 activity in a cell, comprising contacting the cell with an rAAV of the disclosure. In another aspect, the disclosure provides a method of increasing GLUT1 activity in a subject, comprising administering to an rAAV of the disclosure. In some embodiments, the cell and/or subject is deficient in *SLC2A1* messenger RNA or GLUT1 protein expression levels and/or activity and/or comprises a loss-of-function mutation in *SLC2A1*. The cell may be an endothelial cell, *e.g.* an endothelial tip cell or a cerebral vascular endothelial cell.

[0156] In some embodiments, the method restores normal function of endothelial tip cells. In some embodiments, the method restores GLUT1 transporter protein expression levels in cell culture and/or *in vivo*. In some embodiments, the method restores normal glucose transport and metabolism (*e.g.* glycolysis, lactate production) in cell culture and/or *in vivo*. In some embodiments, the method restores normal angiogenesis and/or development of the microvasculature in central nervous system (CNS).

[0157] In some embodiments, the method restores normal function of cerebral vascular endothelial cells. In some embodiments, the method restores GLUT1 transporter protein expression levels in cell culture and/or *in vivo*. In some embodiments, the method restores normal glucose transport and metabolism (*e.g.* glycolysis, lactate production) in cell culture and/or *in vivo*. In some embodiments, the method restores normal angiogenesis and/or development of the microvasculature in central nervous system (CNS).

METHODS OF TREATMENT

[0158] In another aspect, the disclosure provides a method of treating a disease or disorder in a subject in need thereof, comprising administering to the subject an effective amount of an rAAV virion of the disclosure. In some embodiments, the disease or disorder is a neurological disease or disorder. In some embodiments, the subject suffers from a genetic disruption in *SLC2A1* expression or function. In some embodiments, the disease or disorder is a GLUT1 Deficiency Syndrome (GLUT1 DS).

[0159] The AAV-mediated delivery of GLUT1 protein to the vasculature of the CNS may increase life span, prevent, diminish, mitigate, or attenuate neuronal degeneration, early-onset seizures, delayed development, acquired microcephaly (decelerating head growth), complex movement disorders (spasticity, ataxia, dystonia), paroxysmal eye-head movements, and/or low lactate and/or glucose concentration in cerebrospinal fluid (hypoglycorrhachia). In some embodiments, the method provides treatment early in the course of disease, *e.g.*, in a newborn, infant, or juvenile.

[0160] In some embodiments, the methods of disclosure result in an increase (e.g., an increase of about 5% to about 10%, about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 70%, or about 70% to about 100%) in wildtype GLUT1 protein expression in the subject. In certain embodiments, the methods of the disclosure result in an increase (e.g., an increase of about 5% to about 25%, about 25% to about 50%, about 50% to about 100%, or about 100% to about 200%) in the ratio of wildtype to mutant GLUT1 protein in the subject.

[0161] In some embodiments, the methods of disclosure result in an increase (e.g., an increase of about 5% to about 10%, about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 50% to about 70%, or about 70% to about 100%) in wildtype GLUT1 protein expression in an endothelial cell. In certain embodiments, the methods of the disclosure result in an increase (e.g., an increase of about 5% to about 25%, about 25% to about 50%, about 50% to about 100%, or about 100% to about 200%) in the ratio of wildtype to mutant GLUT1 protein in an endothelial cell.

[0162] The methods disclosed herein may provide efficient biodistribution in the brain and/or the vasculature of CNS. They may result in sustained expression in all, or a substantial fraction of, endothelial cells (*e.g.*, endothelial tip cells). Notably, the methods disclosed herein may provide long-lasting expression of GLUT1 protein throughout development and aging of the subject following rAAV virion administration.

[0163] Combination therapies are also contemplated by the invention. Combinations of methods of the invention with standard medical treatments (e.g., corticosteroids or topical pressure reducing medications) are specifically contemplated, as are combinations with novel therapies. In some cases, a subject may be treated with a steroid and/or combination of

immune suppressing agents to prevent or to reduce an immune response to administration of a rAAV described herein.

[0164] A therapeutically effective amount of the rAAV vector genome, *e.g.* for intracerebroventricular (ICV),intra-cisterna magna (ICM) injection, or intravenous (IV) injection, is a dose of rAAV ranging from about 1×10^{12} vg/kg to about 5×10^{12} vg/kg, or about 1×10^{13} vg/kg to about 5×10^{13} vg/kg, or about 1×10^{14} vg/kg to about 5×10^{14} vg/kg, or about 1×10^{15} vg/kg to about 5×10^{15} vg/kg, by brain weight. The invention also comprises compositions comprising these ranges of rAAV vector genomes.

[0165] For example, in particular embodiments, a therapeutically effective amount of rAAV vector genome is a dose of about 1×10^{10} vg, about 2×10^{10} vg, about 3×10^{10} vg, about 4×10^{10} vg, about 5×10^{10} vg, about 6×10^{10} vg, about 7×10^{10} vg, about 8×10^{10} vg, about 9×10^{10} vg, about 1×10^{12} vg, about 2×10^{12} vg, about 3×10^{12} vg, about 4×10^{12} vg, about 4×10^{13} vg, and about 4×10^{14} vg. The invention also comprises compositions comprising these doses of rAAV vector genome.

[0166] In some embodiments, for example where ICV injection is performed, a therapeutically effective amount of rAAV vector genome is a dose in the range of 1×10^{10} vg/hemisphere to 2×10^{14} vg/hemisphere, or about 1×10^{10} vg/hemisphere, about 1×10^{11} vg/hemisphere, about 1×10^{12} vg/hemisphere, 1×10^{13} vg/hemisphere, or about 1×10^{14} vg/hemisphere. In some embodiments, for example where ICM injection is performed, a therapeutically effective amount of rAAV vector genome is a dose in the range of 2×10^{10} vg total to 2×10^{14} vg total, or about 2×10^{10} vg total, about 2×10^{11} vg total, about 2×10^{12} vg total, about 2×10^{13} vg total, or about 2×10^{14} vg total.

[0167] In some embodiments, the therapeutic composition comprises more than about 1×10^{9}, 1×10^{10}, or 1×10^{11} genomes of the rAAV vector genome per volume of therapeutic composition injected. In embodiments cases, the therapeutic composition comprises more than approximately 1×10^{11}, 1×10^{12}, 1×10^{13}, or 1×10^{14} genomes of the rAAV per mL. In certain embodiments, the therapeutic composition comprises less than about 1×10^{14}, 1×10^{13} or 1×10^{12} genomes of the rAAV per mL.

[0168] Evidence of functional improvement, clinical benefit or efficacy in patients may be assessed by the analysis of paroxysmal eye-head movements, surrogate markers of reduction in seizure frequency (generalized tonic clonic and myoclonic seizures), lactate and/or glucose

concentration in cerebrospinal fluid (CSF), assessment of developmental delay, chorea, dystonia, and microcephaly. Measures in cognition, motor, speech and language function using standard disease rating scales, such as Columbia Neurological Score, Composite Intellectual Estimate, Adaptive Behavior Composite, verbal and nonverbal cognitive skills and visuomotor integration, and Six Minute Walk Test. Cognitive and Developmental Assessments including the Peabody Developmental Motor Scales 2nd edition (PDMS-2) and Bayley Scales of Infant Development, 3rd edition applied as appropriate to level of child's disability. Gross motor function measure (GFMF-88), Pediatric Evaluation of Disability Inventory (PEDI). These or similar scales, as well as patient-reported outcomes on quality of life such as Caregiver Global Impression of Change in Seizure Duration (CGICSD) on a 3-point scale (decrease, no change, or increase in average duration), Pediatric Quality of Life Inventory (PedsQL™) and Vineland Adaptive Behavior Scales-2nd may demonstrate improvements in components of the disease. Baseline and post treatment Brain magnetic resonance imaging may show improvements or normalized brain volume for age of patient compared to age-matched patient control data and historical data from GLUT1 Deficiency patients.

[0169] Clinical benefit could be observed as increase in life-span, meeting normal neurodevelopmental milestones, normalized glucose concentration in CSF, decreases in frequency or magnitude paroxysmal eye-head movements, decrease or absence of epileptic seizure activity (including myoclonic, clonic, generalized tonic-clonic and/or epileptic spasm), improvement in, or lack of development of complex movement disorders such as spasticity, dystonia, and/or ataxia, and improved or normal performance in Columbia Neurological Score and/or Six Minute Walk Test. Evidence of neuroprotective and/or neurorestorative effects may be evident on all of the prior mentioned metrics and/or on magnetic resonance imaging (MRI) by characterizing overall brain size, lack of microcephaly and/or cortical and/or cerebellar atrophy.

[0170] In some embodiments, method causes increased glucose uptake by cells compared to cells contacted with, or of cells of a subject administered, a vector comprising an endogenous Glut1 promoter or a ubiquitous promoter. In some cases, the increase is at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, or at least 50%. In some cases, the increase is at least 1.1-fold, at least 1.2-fold, at least 1.3-fold, at least 1.4-fold, at least 1.5-fold, at least 1.6-fold, at least 1.7-fold, or at least 1.8-fold. The vector may be any vector disclosed herein. The cell may be an endothelial cell or a neuronal cell. For example,

the method may increase glucose uptake by human cerebral microvasculature endothelial cells, either *in vitro* or *in vivo*.

ADMINISTRATION OF COMPOSITIONS

[0171] Administration of an effective dose of the compositions may be by routes standard in the art including, but not limited to, intravenous, intracerebral, intrathecal, intracisternal, or intra-cerebroventricular administration. In some cases, administration comprises intravenous, intracerebral, intrathecal, intracisternal or intracerebroventricular injection. Administration may be performed by intrathecal injection with or without Trendelenberg tilting. Intracisterna magna (ICM) delivery may be achieved via catheter entry at the intrathecal (IT) space. Intracerebroventricular injection(s) may be achieved via magnetic resonance imaging (MRI) guided neurosurgical targeting.

[0172] In some embodiments, the disclosure provides for systemic administration of an effective dose of rAAV and compositions of the invention. For example, systemic administration may be administration into the circulatory system so that the entire body is affected. Systemic administration includes intravenous administration through injection or infusion.

[0173] In some embodiments, administration of rAAV of the present invention may be accomplished by using any physical method that will transport the rAAV recombinant vector into the target tissue of an animal. Administration includes, but is not limited to, injection into the central nervous system (CNS) or cerebrospinal fluid (CSF) and/or intravenous injection.

[0174] In some embodiments, the methods of the disclosure comprise intracerebroventricular, intracisterna magna, intrathecal, or intraparenchymal delivery. Infusion may be performed using specialized cannula, catheter, syringe/needle using an infusion pump. Optionally, targeting of the injection site may be accomplished with MRI-guided imaging. Administration may comprise delivery of an effective amount of the rAAV virion, or a pharmaceutical composition comprising the rAAV virion, to the CNS. These may be achieved, *e.g.*, via unilateral intraventricular injection, bilateral intraventricular injection, intracisternal magna infusion with Trendelenburg tilting procedure, or intracisternal magna infusion without Trendelenburg tilting procedure, intrathecal infusion with Trendelenburg tilting procedure, or intrathecal infusion without Trendelenburg tilting procedure. The compositions of the disclosure may further be administered intravenously.

[0175] In some embodiments, the methods of the disclosure comprise administration of rAAV by intravenous (IV) injection. In some embodiments, the methods of the disclosure comprise administration of rAAV by intracerebroventricular (ICV) injection. In some embodiments, the methods of the disclosure comprise administration of rAAV by IV injection in combination with ICV injection.

[0176] Direct delivery to the CNS could involve targeting the intraventricular space, either unilaterally or bilaterally, specific neuronal regions or more general brain regions containing neuronal targets. Individual patient intraventricular space, brain region and/or neuronal target(s) selection and subsequent intraoperative delivery of AAV could by accomplished using a number of imaging techniques (MRI, CT, CT combined with MRI merging) and employing any number of software planning programs (*e.g.*, Stealth System, Clearpoint Neuronavigation System, Brainlab, Neuroinspire etc). Intraventricular space or brain region targeting and delivery could involve use of standard stereotactic frames (Leksell, CRW) or using frameless approaches with or without intraoperative MRI. Actual delivery of AAV may be by injection through needle or cannulae with or without inner lumen lined with material to prevent adsorption of rAAV (e.g. Smartflow cannulae, MRI Interventions cannulae). Delivery device consists of syringe(s) and automated infusion or microinfusion pumps with preprogrammed infusion rates and volumes. A syringe/needle combination or just a guide cannulae for the needle may be interfaced directly with the stereotactic frame. Infusion may include constant flow rate or varying rates with convection enhanced delivery.

EXAMPLES

EXAMPLE 1: PRE-CLINICAL BIOACTIVITY AND EFFICACY

[0177] Recombinant AAV virions with an AAV-BR1 capsid are produced using the vector genomes disclosed in FIGS. 2-8. These are evaluated in mouse models of disease as a consequence of GLUT1 deficiency disease. One model employs a *flox*-ed GLUT1 gene crossed to a transgenic animal that expresses *Cre/lox* from a constitutive promoter or an endothelial-specific promoter (*e.g.*, Tie-2). The resulting mice are heterozygous *null* at the GLUT1 locus and exhibit a developmental phenotype that mimics human disease. A second mouse model of GLUT1 DS is a heterozygous haploinsufficient mouse generated by targeted disruption of the promoter and exon 1 regions of the mouse GLUT-1 gene (GLUT-1 $^{+/-}$ mice).

Additional animal models may include a GLUT1 DS model where the GLUT1 gene has a S324P point mutation.

[0178] Gene expression and dose-response is evaluated *in vitro* (using endothelial and neuronal cell lines) and *in vivo* (using wild-type and GLUT1 DS model mice). Cultured cells (Human Embryonic Kidney cells 293, HEK293; human umbilical vein endothelial cells, HUVEC; human brain-derived endothelial cells, bEND3; human brain microvasculature endothelial cells, HBEC-5i; human brain microvascular endothelial cell line, hCMEC/D3 (Blood-Brain Barrier model); human glial oligodendrocytic hybrid cells, MO3.13; human neuroblastoma, SH-SY5Y) transfected with SLC2A1 expression vectors will reveal transduction efficiency by quantitative real-time PCR analyses, GLUT1 levels by ELISA and/or Western blot. Proof of concept and efficacy of AAV vector construct(s) will be revealed *in vivo* using GLUT1 DS mice by expression of transgene (GLUT1 protein) in the CNS and CNS vasculature by immunolabeling, enhanced brain capillary density and/or increase in blood vessel size in CNS, increase in brain glucose uptake using positron emission tomography (PET), increase in CSF glucose levels or lactate levels and/or in CSF/blood glucose ratio, increase in CSF lactate levels, and improvement in motor performance using standard assays such as rotarod and/or vertical pole assay, relative to GLUT1 DS mutant mouse controls. Gene expression and efficacy *in vivo* using GLUT1 DS mouse model(s) will be evident following delivery of AAV vector construct(s) by intravenous or direct injection to the intracerebroventricular space, while employing these routes of administration either alone and/or in combination.

EXAMPLE 2: IN VITRO EVALUATION OF GLUT1 EXPRESSION USING ENDOTHELIAL PROMOTERS

[0179] Gene expression was evaluated *in vitro* using human cerebral microvasculature endothelial cells (hCMEC/D3). Expression of Glut1 by hCMEC/D3 cells transfected with AAV9 vectors encoding SLC2A1 under the control of a hFLT1, mTIE1, hGlut1, or CMV promoter (diagramed in **FIG. 10C**) was evaluated (**FIG. 9**). Expression from the endothelial promoters (hFLT1 and mTIE1) was comparable to expression from the Glut1 promoter, and much lower than expression from the CMV promoter. A similar pattern of expression levels between these constructs was observed by immunofluorescence microscopy (**FIG. 10A** and **FIG. 10B**).

[0180] Surprisingly, 2-Deoxy-D-glucose (2-DG) uptake by human cerebral microvasculature endothelial cells transfected or transduced with the gene under the control of the endothelial promoters was greater than the control Glut1 promoter, with the hFLT-1 promoter demonstrating the highest level of 2-DG (glucose) uptake (**FIGs. 11A-11C**, **FIG. 12**, and **FIG. 13**). This finding of greater 2-DG (glucose) uptake with the hFLT-1 promoter construct was also observed across a range of 2-DG concentrations (**FIG 12A**; 0, 0.1, 0.5, and 1 mM) and varying time points following transfection (**FIG 12B**) and in some instances was found to be comparable or slightly greater than that observed with the CMV promoter (**FIG. 11A-11C**; **FIG. 12A, 12B; FIG. 13**).

[0181] **FIG. 9** Expression of transgene protein (Glut1-GFP) following transfection of human cerebral microvasculature endothelial cells (hCMEC/d3s).

[0182] **FIG. 10A**. GFP fluorescence 72 hours following transfection with constructs containing one of several endothelial cell promoters driving expression of Glut1-GFP transgene.

[0183] **FIG. 10B**. GFP fluorescence 72 hours following transfection with constructs containing one of two ubiquitous promoters (CMV or CAG), control vector without Glut1 (CMV-GFP) or no transfection (No NFX). Images obtained using Operetta CLS™ (PerkinElmer®).

[0184] **FIG. 10C**. Diagram of expression cassette containing the promoter of interest (hFLT1, mTie, hTie or hGlut1) and the GLUT1 (SLC2A1) gene (T2A linked-GFP) and regulatory elements flanked by AAV2 inverted terminal repeats (ITRs).

[0185] **FIGs. 11A-11C**. 2-Deoxy-D-glucose (glucose) Uptake in hCMEC/d3 cells following expression of human GLUT1 (SLC2A1). Human cerebromicrovascular endothelial cells (hCMEC/d3s) were transfected with plasmids expressing either CAG-GFP (CON; negative control) or with a hGLUT1-t2A-eGFP transgene driven by one of several endothelial-specific promoters (i.e., hFLT1, mTie, hTie or hGlut1) or by the ubiquitous CMV or CAG promoters. Glucose uptake was measured using a luminescence-based kit (Promega®) with 0.5 mM 2-Deoxyglucose (2-DG) in culture media. Glucose (2-DG) uptake was normalized by Total Cells using phase-contrast imaging [Error bars represent S.E.M; n=6 replicates per condition].

[0186] **FIG. 11A**. Glucose (2-DG) uptake was measured at 72 hours post-transfection in a first experiment.

[0187] **FIG. 11B**. Glucose (2-DG) uptake was measured at 72 hours post-transfection in a second experiment.

[0188] **FIG. 11C**. Glucose (2-DG) uptake was measured at 96 hours post-transfection.

[0189] **FIG. 12A**. shows glucose (2-DG) uptake in hCMEC/D3 cells following expression of human Glut1 (*SLC2A1*) at a 72-hour time point.

[0190] **FIG. 12B**. shows glucose (2-DG) uptake in hCMEC/D3 cells following expression of human Glut1 (*SLC2A1*) at a 96-hour time point.

[0191] **FIG. 13.** 2-Deoxy-D-glucose (glucose) Uptake Following AAV9-mediated Expression of hGLUT1 (SLC2A1) in hCMEC/D3 cells. Human cerebromicrovascular endothelial cells (hCMEC/d3s) were transduced with AAV9 vectors (3 x 10^5 vector genomes/cell) expressing either CAG-GFP (negative control) or the hGLUT1 transgene driven by one of several endothelial-specific promoters (i.e., hFLT1, mTie1 or hGlut1) or by the ubiquitous CMV promoter. Glucose (2-DG) uptake was measured 72 hours post-transduction using the luminescence-based Glucose Uptake-Glo kit (Promega®) and normalized per cell using the RealTime-Glo MT Cell Viability Assay (Promega®) [Error bars represent S.E.M; n=4 replicates per condition].

EXAMPLE 3: IN VIVO EVALUATION OF AAV-BR1 CAPSID-MEDIATED GLUT1 EXPRESSION USING ENDOTHELIAL PROMOTERS IN AN ANIMAL MODEL OF GLUT1 DEFICIENCY

[0191] A series of experiments evaluating the *in vivo* effects of AAV-BR1 capsid-mediated expression of Glut1 transporter protein in the mouse model of GLUT1 Deficiency Syndrome (DS) will be performed. This model employs a mouse that is heterozygous haploinsufficient due to a targeted disruption of the promoter and exon 1 regions of the mouse GLUT-1 gene (GLUT-1 +/- mice) and displays the characteristic features of human GLUT DS such as seizure activity, hypoglycorrhachia, microencephaly and impairments in motor function (Wang et al, Hum Mol Gen, 2006; Tang et al., Nat Comm, 2016). AAV-BR1 virions will be evaluated at different doses and different routes of administration (intravenous or intracerebroventricular) with expression of the GLUT1 transgene driven by either a

ubiquitous promoter (CMV) or one of several endothelial cell promoters (*e.g.*, hFLT-1). The extent to which endothelial cell promoter-mediated GLUT1 transgene expression following delivery using an AAV-BR1 virion can prevent or mitigate the functional and pathological deficits in this mouse model will be evaluated. Potential beneficial effects of AAV-BR1-mediated Glut1 protein expression when administered to the heterozygous haploinsufficient mouse will be revealed by comparisons to untreated GLUT-1 +/- control mice and consist of improved or normalized body weight gain, behavioral performance on motor tests (e.g. rotarod, vertical pole assay), CSF glucose levels, brain weight, and integrity and size of brain microvasculature (e.g. brain capillary density, vessel size, number of vessel branch points).

CLAIMS

1. A recombinant adeno-associated virus (rAAV) virion, comprising a vector genome and a capsid,

wherein the vector genome comprises an expression cassette, flanked by 5′ and 3′ inverted terminal repeats (ITRs),

wherein the expression cassette comprises a polynucleotide sequence encoding GLUT1 or a functional variant thereof, operatively linked to a promoter, and

wherein the capsid is a BR1 capsid or a functional variant thereof.

2. The rAAV virion of claim 1, wherein the capsid is a BR1 capsid.

3. The rAAV virion of claim 1, wherein the capsid comprises the polypeptide sequence motif XDGXXWX, wherein each X is any amino acid (SEQ ID NO: 107).

4. The rAAV virion of claim 1, wherein the capsid comprises the polypeptide sequence ADGVQWT (SEQ ID NO:108), DDGVSWK (SEQ ID NO:109), SDGLTWS (SEQ ID NO:110) or SDGLAWV (SEQ ID NO:111).

5. The rAAV virion of claim 1, wherein the capsid comprises the polypeptide sequence NRGTEWD or a functional variant having 1, 2, 3, or more substitutions thereto.

6. The rAAV virion of claim 1, wherein the capsid comprises the polypeptide sequence NRGTEWD (SEQ ID NO:112).

7. The rAAV virion of claim 1, wherein the capsid comprises an insertion of the polypeptide sequence NRGTEWD in the GH loop compared to an AAV2 VP1 reference sequence as set forth in SEQ ID NO 76.

8. The rAAV virion of claim 1, wherein the capsid comprises a VP3 polypeptide that shares at least 98%, at least 99%, or 100% identity to an AAV-BR1 VP3 polypeptide sequence as set forth in SEQ ID NO:106.

9. The rAAV virion of claim 1, wherein the capsid comprises a VP2 polypeptide that shares at least 98%, at least 99%, or 100% identity to an AAV-BR1 VP2 polypeptide sequence as set forth in SEQ ID NO:105.

10. The rAAV virion of claim 1, wherein the capsid comprises a VP1 polypeptide that shares at least 98%, at least 99%, or 100% identity to an AAV-BR1 VP1 polypeptide sequence as set forth in SEQ ID NO: 104.

11. The rAAV virion of any one of claims 1 to 10, wherein the promoter is a FLT-1 promoter.

12. The rAAV virion of claim 11, wherein the FLT-1 promoter is a human FLT-1 (hFLT-1) promoter.

13. The rAAV virion of claim 12, wherein the hFLT-1 promoter shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity with SEQ ID NO: 1.

14. The rAAV virion of any one of claims 1 to 13, wherein the expression cassette comprises a polyA signal, optionally a human growth hormone (hGH) polyA.

15. The rAAV virion of any one of claims 1 to 14, wherein the expression cassette comprises a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), optionally a WPRE(x).

16. The rAAV virion of any one of claims 1 to 15, wherein the expression cassette comprises a 3′ untranslated region (3′ UTR) comprising a sequence that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity with SEQ ID NO: 4.

17. The rAAV virion of any one of claims 1 to 16, wherein the polynucleotide sequence encoding GLUT1 is a *SLC2A1* polynucleotide.

18. The rAAV virion of claim 17, wherein the *SLC2A1* polynucleotide is a human *SLC2A1* polynucleotide.

19. The rAAV virion of any one of claim 16 to 18, wherein the polynucleotide sequence encoding GLUT1 shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity with SEQ ID NO: 5.

20. The rAAV virion of any one of claims 1 to 19, wherein the expression cassette is flanked by 5′ and 3′ inverted terminal repeats (ITRs), optionally AAV2 ITRs, optionally an

ITR that shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity with SEQ ID NO: 6 or SEQ ID NO: 7.

21. The rAAV virion of any one of claim 1 to 20, wherein the expression cassette shares at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identity with any one of SEQ ID NOs: 8-16, SEQ ID NO: 97, SEQ ID NO: 99, and SEQ ID NO: 101.

22. The rAAV virion of claim 20 or claim 21, wherein the rAAV virion is not an AAV2 virion.

23. A method of treating and/or preventing a disease or disorder in a subject in need thereof, comprising administering the rAAV virion of any one of claims 19 to 22 to the subject.

24. The method of claim 23, wherein the disease or disorder is a neurological disorder.

25. The method of claim 23 or claim 24, wherein the disease or disorder is Glucose transporter 1 deficiency syndrome (GLUT1DS) or De Vivo Disease.

26. The method of any one of claim 23 to 25, wherein the rAAV virion is administered by intracerebroventricular (ICV) injection.

27. The method of any one of claim 23 to 25, wherein the rAAV virion is administered by an intravenous (IV) injection.

28. The method of any one of claim 23 to 25, wherein the rAAV virion is administered by intracerebroventricular (ICV) injection in combination with an intravenous (IV) injection.

29. The method of any one of claims 23 to 28, wherein the administration results in expression of the polynucleotide sequence encoding GLUT1 in the brain, optionally at increased levels compared to a reference rAAV virion.

30. The method of any one of claims 23 to 29, wherein the administration results in an increase in expression of GLUT1 protein in the brain and/or an increase in glucose levels and/or lactate levels in the CSF, optionally at increased levels compared to a reference rAAV virion, wherein optionally the increases is an increase of at least about 10%, at least about

20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or 100%.

31. The method of claim 30, wherein the reference rAAV virion is an AAV2 virion.

32. The method of claim 30, wherein the reference rAAV virion is an AAV9 virion.

33. The method of any one of claims 23 to 32, wherein the rAAV virion is administered at a dose of 1×10^{12} vector genomes (vg), 1×10^{13} vg, 1×10^{14} vg, or 3×10^{14} vg.

34. The method of any one of claims 23 to 33, wherein the method causes increased glucose uptake by cerebral microvasculature endothelial cells compared to a method performed using an endogenous Glut1 promoter or a ubiquitous promoter.

35. A method of expressing GLUT1 in a cell, comprising contacting the cells with the rAAV virion of any one of claims 23 to 34.

36. The method of claim 35, wherein the cell is an endothelial cell.

37. The method of claim 36, wherein the endothelial cell is a cerebral microvasculature endothelial cell.

38. The method of claim 36 or claim 37, wherein the endothelial cell is an *in vivo* endothelial cell.

39. The method of claim 35, wherein the cell is a neuron.

40. The method of claim 39, wherein the neuron is an *in vivo* neuron.

41. The method of any one of claims 35 to 40, wherein the method comprises *in vivo* administration of the rAAV virion to a subject.

42. The method of any one of claims 35 to 41, wherein the rAAV virion causes increased glucose uptake by the cell compared to a cell contacted with a rAAV virion comprising an endogenous Glut1 promoter or a ubiquitous promoter.

43. A pharmaceutical composition comprising the rAAV virion of any one of claims 23 to 34.

44. A kit comprising the rAAV virion of any one of claims 23 to 34 or the pharmaceutical composition of claim 43 and optionally instructions for use.

FIG. 1

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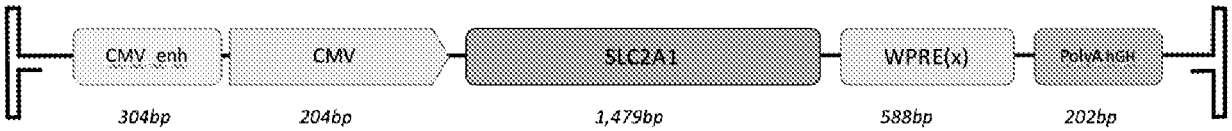

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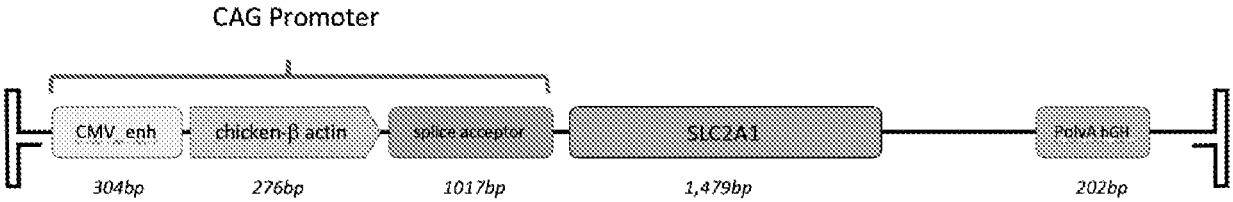

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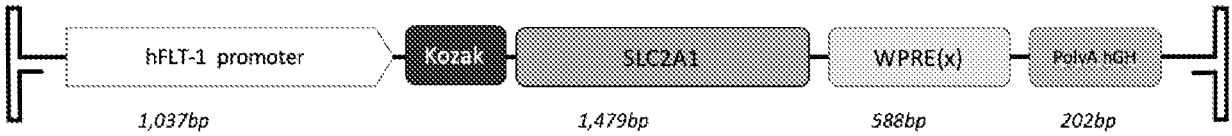

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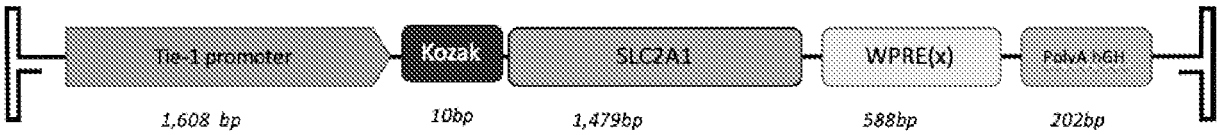

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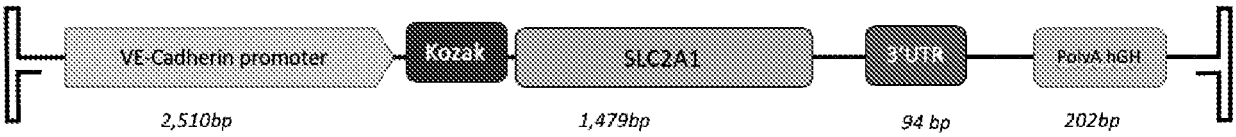

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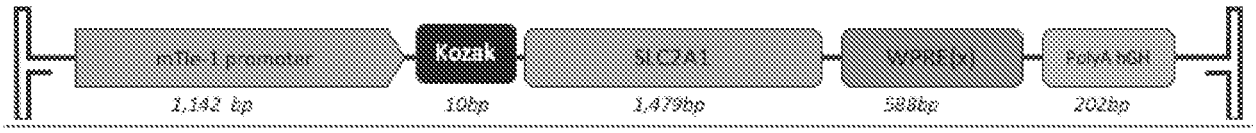

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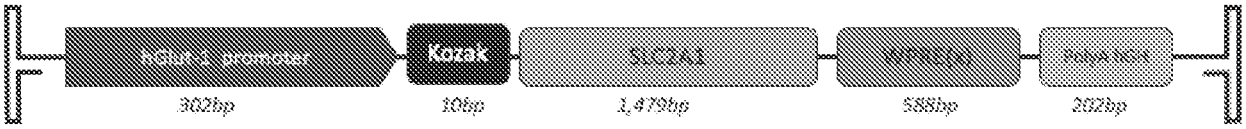

FIG. 1 (con't)

FIG. 2

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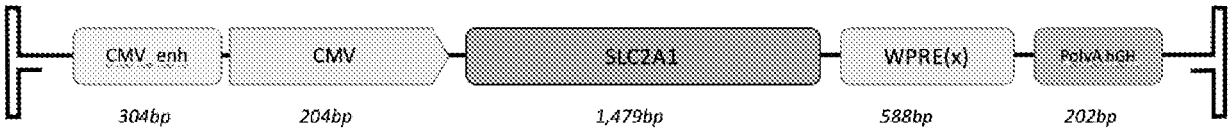

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FIG. 2 (con't)

FIG. 3

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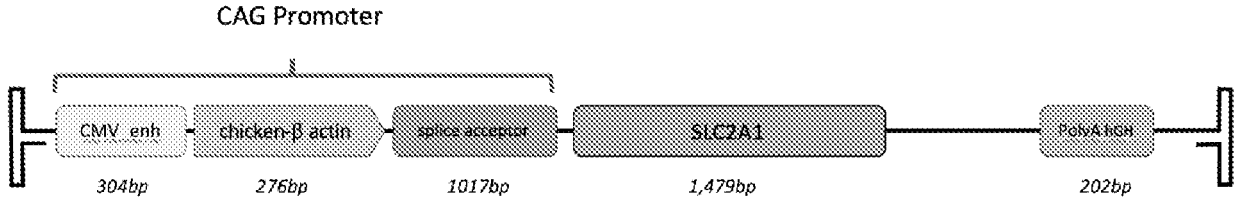

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FIG. 3 (con't)

FIG. 4

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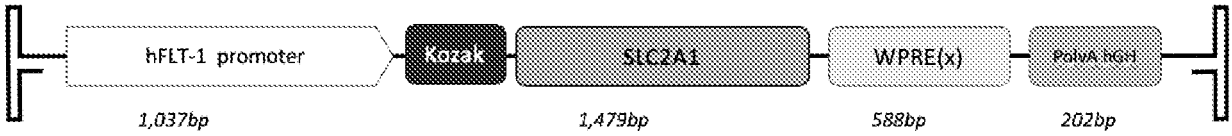

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FIG. 4 (con't)

FIG. 5

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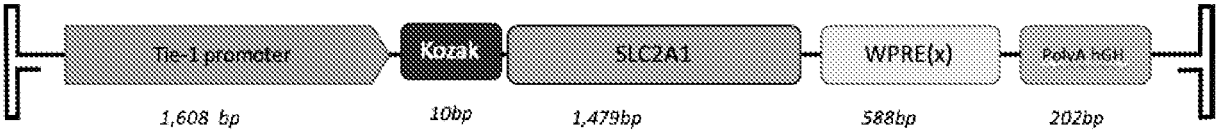

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FIG. 5 (con't)

FIG. 6

VE-Cadherin-SLC2A1 – 4,716 bp

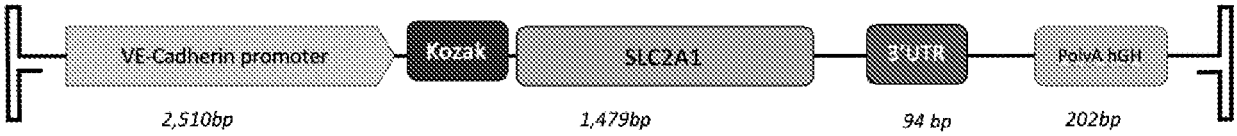

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FIG. 6 (con't)

FIG. 7

mTie1-SLC2A1 – 3,850 bp

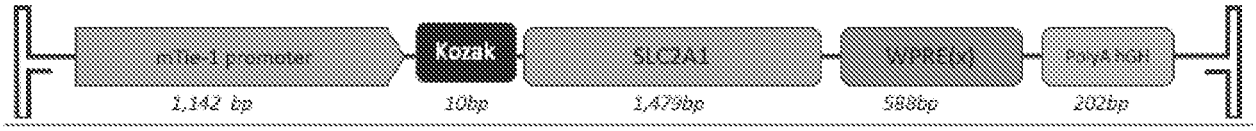

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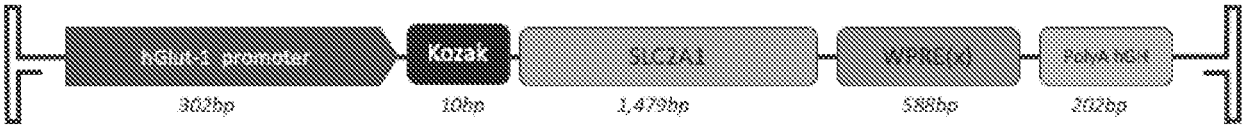
FIG. 7 (con't)

FIG. 8

hGLUT-SLC2A1 – 3,010 bp



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gagtgcagtggcacatttctgctcactgcaacctcctcctccctgggttctacgtagataagtagcatggcgggttaatcattaactac
aaggaacccctagtgatggagttggccactccctctctgcgcgctcgctcgctcactgaggccgggcgaccaaaggtcgcccgacg
cccgggctttgcccgggcggcctcagtgagcgagcgagcgcgc

FIG. 8 (con't)

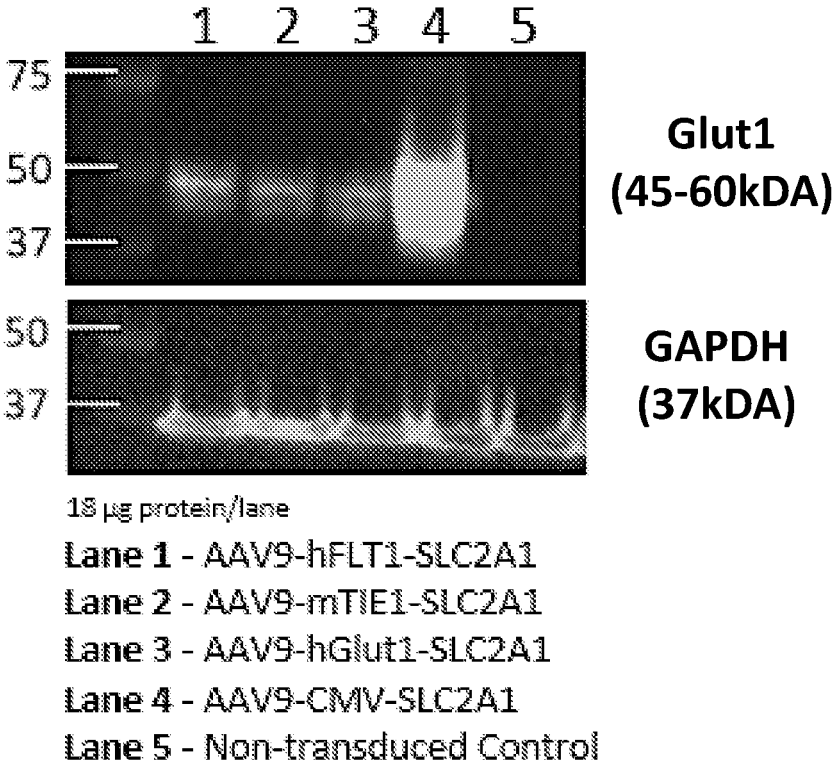
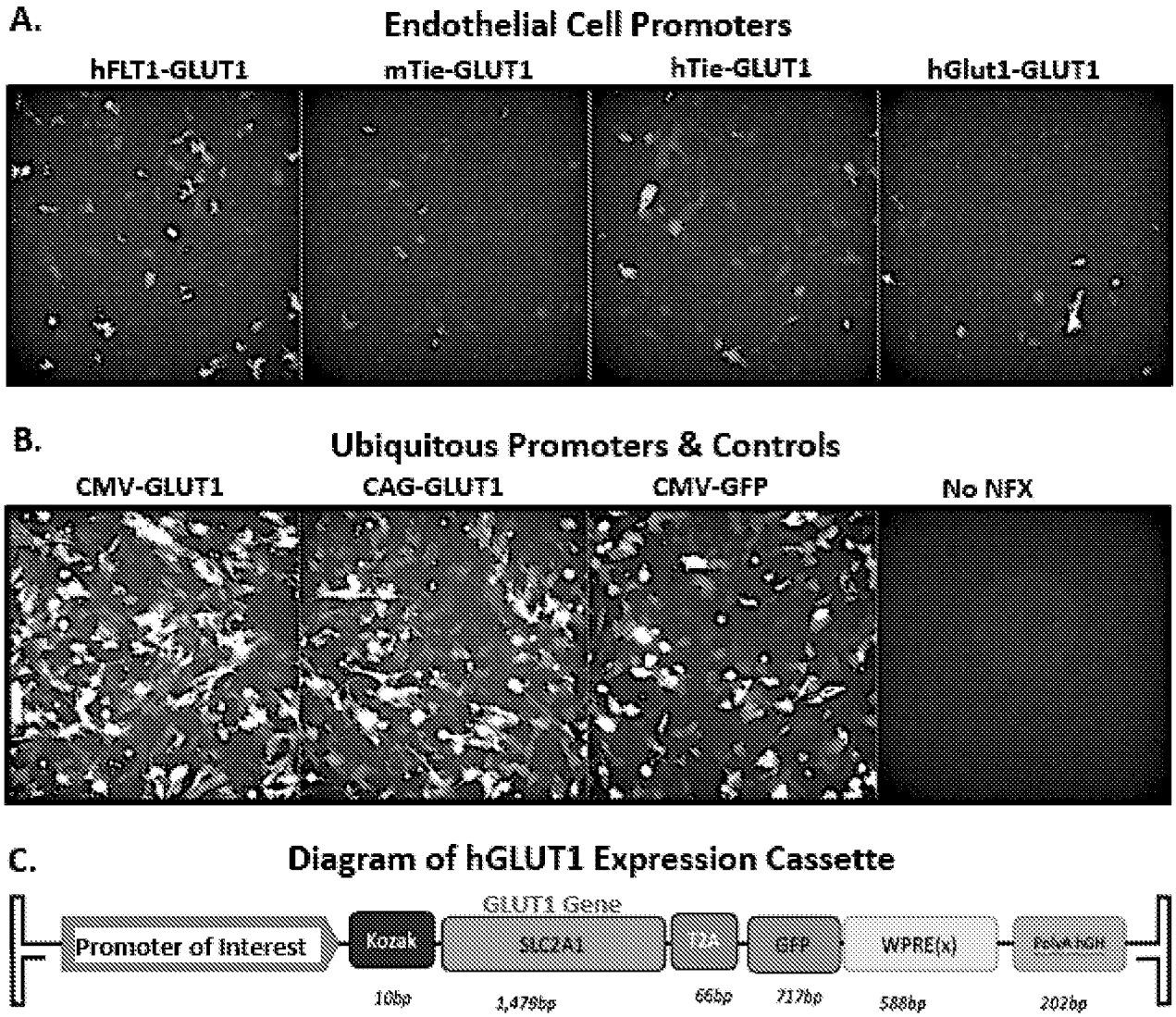
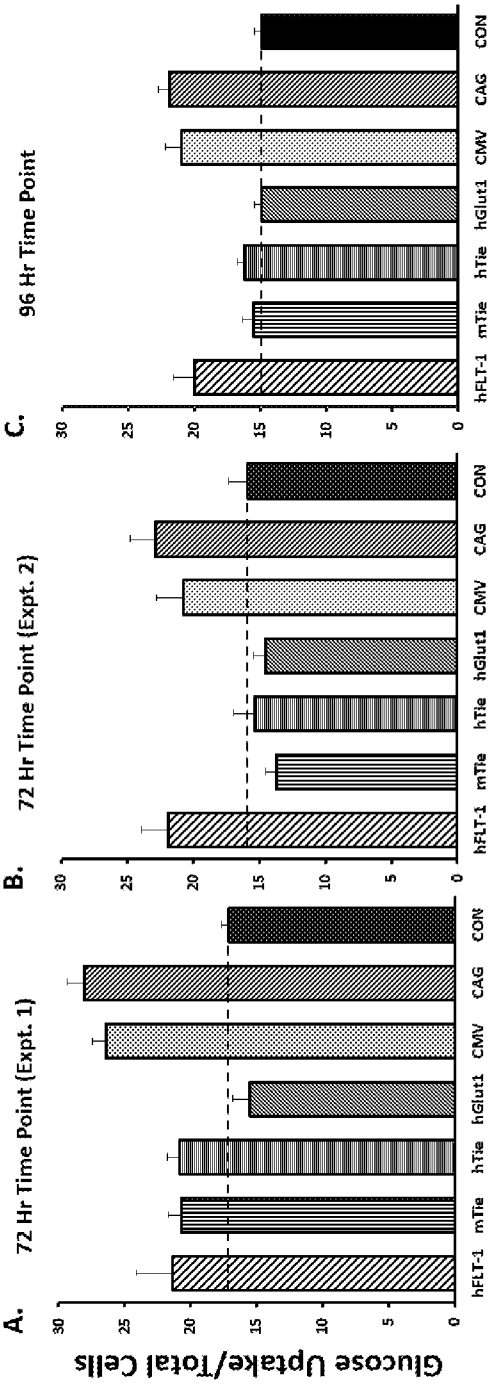

1 2 3 4 5
75
50
37
Glut1
(45-60kDA)
50
37
GAPDH
(37kDA)
18 µg protein/lane
Lane 1 - AAV9-hFLT1-SLC2A1
Lane 2 - AAV9-mTIE1-SLC2A1
Lane 3 - AAV9-hGlut1-SLC2A1
Lane 4 - AAV9-CMV-SLC2A1
Lane 5 - Non-transduced Control

FIG. 9


A.
Endothelial Cell Promoters
hFLT1-GLUT1
mTie-GLUT1
hTie-GLUT1
hGlut1-GLUT1
B.
Ubiquitous Promoters & Controls
CMV-GLUT1
CAG-GLUT1
CMV-GFP
No NFX
C.
Diagram of hGLUT1 Expression Cassette
GLUT1 Gene
Promoter of Interest
Kozak
SLC2A1
2A
GFP
WPRE(x)
PolyA hGH
10bp
1,479bp
66bp
717bp
588bp
202bp

FIGs. 10A-10C


A.
72 Hr Time Point (Expt. 1)
B.
72 Hr Time Point (Expt. 2)
C.
96 Hr Time Point
Glucose Uptake/Total Cells
0
5
10
15
20
25
30
hFLT-1
mTie
hTie
hGlut1
CMV
CAG
CON

FIGs. 11A-11C

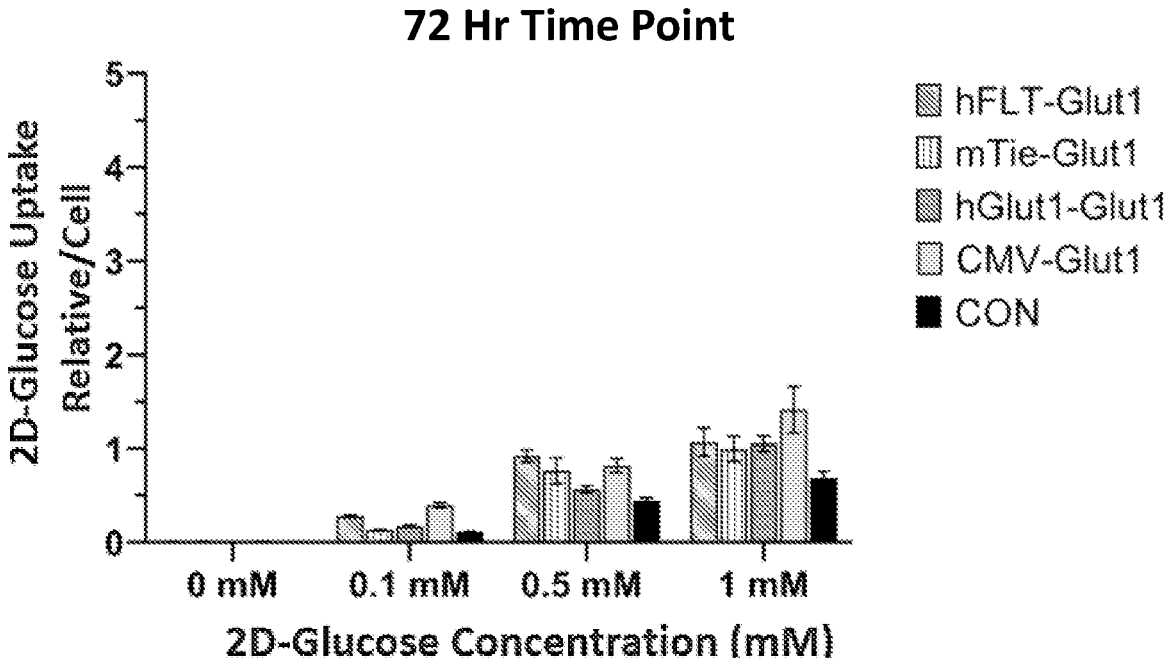


FIG. 12A

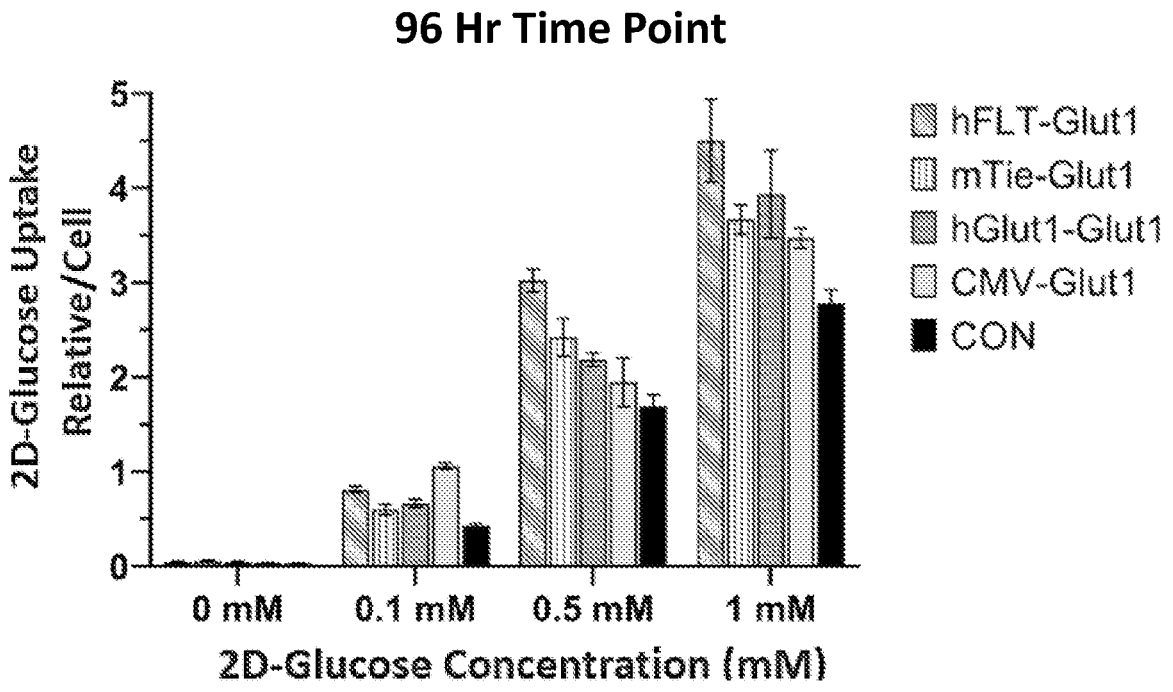


FIG. 12B

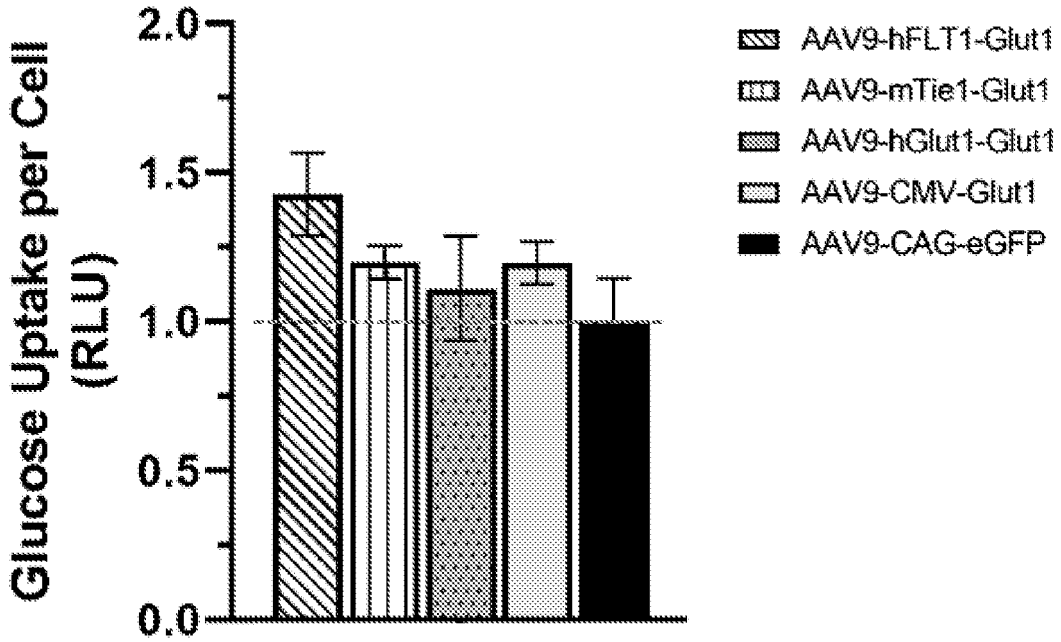

Glucose Uptake per Cell (RLU)
2.0
1.5
1.0
0.5
0.0
AAV9-hFLT1-Glut1
AAV9-mTie1-Glut1
AAV9-hGlut1-Glut1
AAV9-CMV-Glut1
AAV9-CAG-eGFP

FIG. 13