

## ADENO-ASSOCIATED VIRAL VECTOR FOR GLUT1 EXPRESSION AND USES THEREOF

### CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Application No. 63/061,726, filed on August 5, 2021, the contents of which are incorporated by reference herein in their entireties.

### STATEMENT REGARDING THE SEQUENCE LISTING

[0002] The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is ROPA\_018\_01WO\_ST25.txt. The text file is about 190 KB, created on August 3, 2021, and is being submitted electronically via EFS-Web.

### 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 hypoglycorrachia, or low glucose concentration in cerebrospinal fluid (CSF). The clinical course of diseases 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 angiogenesis and maintenance of the blood-brain barrier (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]** Multiple challenges to addressing GLUT1 DS by gene therapy exist. The degree of CNS coverage with vector that will be needed and what therapeutic levels of GLUT1 are required to achieve clinically meaningful effects are both highly unpredictable.

**[0008]** There is an unmet need for therapy for GLUT1 Deficiency Syndrome. The gene therapies provided herein address this need.

## SUMMARY

**[0009]** 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.

**[0010]** 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). Direct delivery of AAV to the developing central nervous system CNS vasculature, 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.

**[0011]** In one aspect, the disclosure provides an expression cassette, comprising a polynucleotide sequence encoding GLUT1 or a functional variant thereof, operatively linked to a promoter.

**[0012]** In some embodiments, the promoter is an endothelial promoter, optionally a Tie-1 promoter, Tie-2 (TEK) promoter, FLT-1 promoter, FLK-1(KDR) promoter, ICAM-2 promoter, VE-Cadherin (CDH5) promoter, VWF promoter, ENG promoter, PDGFB promoter, ESM1 promoter, APLN promoter, or Claudin-5 (Ple261) promoter, provided the endothelial promoter is not a Glut1 promoter.

**[0013]** In some embodiments, the promoter is a FLT-1 promoter.

**[0014]** In some embodiments, the FLT-1 promoter is a human FLT-1 (hFLT-1) promoter.

**[0015]** In some embodiments, the hFLT-1 promoter shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 1.

**[0016]** In some embodiments, the promoter is a Tie-1 promoter.

**[0017]** In some embodiments, the Tie-1 promoter is a human Tie-1 (hTie-1) promoter.

**[0018]** In some embodiments, the hTie-1 promoter shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 2.

**[0019]** In some embodiments, the promoter is a vascular endothelial-cadherin (VE-cadherin) promoter.

**[0020]** In some embodiments, the VE-cadherin promoter is a human VE-cadherin (hVE-cadherin) promoter.

- [0021] In some embodiments, the hVE-cadherin promoter shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 3.
- [0022] In some embodiments, the promoter is a ubiquitous promoter.
- [0023] In some embodiments, the promoter is a CMV promoter.
- [0024] In some embodiments, the promoter is a CAG promoter.
- [0025] In some embodiments, the expression cassette comprises a polyA signal, optionally a human growth hormone (hGH) polyA.
- [0026] In some embodiments, the expression cassette comprises a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), optionally a WPRE(x).
- [0027] In some embodiments, the expression cassette comprises a 3' untranslated region (3' UTR) comprising a sequence that shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 4.
- [0028] In some embodiments, the polynucleotide sequence encoding GLUT1 is a *SLC2A1* polynucleotide.
- [0029] In some embodiments, the *SLC2A1* polynucleotide is a human *SLC2A1* polynucleotide.
- [0030] In some embodiments, the polynucleotide sequence encoding GLUT1 shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 5.
- [0031] In some embodiments, the expression cassette is flanked by 5' and 3' inverted terminal repeats (ITRs), optionally AAV2 ITRs.
- [0032] In some embodiments, the expression cassette shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 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.
- [0033] In another aspect, the disclosure provides a gene therapy vector, comprising any one of the expression cassettes of the disclosure.

**[0034]** In some embodiments, the gene therapy vector is a recombinant adeno-associated virus (rAAV) vector.

**[0035]** In some embodiments, the rAAV vector is an AAV6, AAV8, AAV9, or AAVrh.74, AAVrh.10 vector, or a functional variant thereof.

**[0036]** In some embodiments, the rAAV vector is not an AAV2 vector.

**[0037]** In some embodiments, the rAAV vector comprises a capsid protein that shares 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to any one of SEQ ID NOs: 76-82.

**[0038]** In another aspect, the disclosure provides a method of treating and/or preventing a disease or disorder in a subject in need thereof, comprising administering any one of the vectors of the disclosure to the subject.

**[0039]** In some embodiments, the disease or disorder is a neurological disorder.

**[0040]** In some embodiments, the disease or disorder is Glucose transporter 1 deficiency syndrome (GLUT1 DS) or De Vivo Disease.

**[0041]** In some embodiments, the vector is administered by intracerebroventricular (ICV) injection.

**[0042]** In some embodiments, the administration results in an increase in expression of the polynucleotide sequence encoding GLUT1 in the brain and/or an increase in glucose levels or lactate levels in the CSF, optionally at increased levels compared to a reference rAAV vector, wherein optionally the increases is an increase of at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% or higher.

**[0043]** In some embodiments, the administration results in expression of GLUT1 protein in the brain, optionally at increased levels compared to a reference rAAV vector.

**[0044]** In some embodiments, the vector is administered at a dose of 1E11 vector genomes (vg), 1E12 vg, 1E13, 1E14, 2E14 or 3E14.

**[0045]** In another aspect, the disclosure provides a method of expressing GLUT1 in a cell, comprising contacting the cells with any one of the vectors of the disclosure.

- [0046] In some embodiments, the cell is an 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 vector to a subject
- [0051] In further aspects, the disclosure provides polynucleotides (*e.g.*, vector genomes), pharmaceutical compositions, kits, and other compositions and methods.
- [0052] 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

- [0053] **FIG. 1** shows a vector diagrams for various non-limiting examples of a vector genome.
- [0054] **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).
- [0055] **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).
- [0056] **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).
- [0057] **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.

**[0058]** **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).

**[0059]** **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).

**[0060]** **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).

**[0061]** **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].

**[0062]** **FIGs. 10A-10C.** Expression of transgene protein (Glut1-GFP) following transfection of human cerebral microvasculature endothelial cells (hCMEC/d3s).

**[0063]** **FIG. 10A.** GFP fluorescence 72 hours following transfection with constructs containing one of several endothelial cell promoters driving expression of Glut1-GFP transgene.

**[0064]** **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®).

**[0065]** **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).

[0066] **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].

[0067] **FIG. 11A.** Glucose (2-DG) uptake was measured at 72 hours post-transfection in a first experiment.

[0068] **FIG. 11B.** Glucose (2-DG) uptake was measured at 72 hours post-transfection in a second experiment.

[0069] **FIG. 11C.** Glucose (2-DG) uptake was measured at 96 hours post-transfection.

[0070] **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.

[0071] **FIG. 12A.** shows glucose uptake in hCMEC/d3 cells following expression of human Glut1 (*SLC2A1*) at a 72-hour time point.

[0072] **FIG. 12B.** shows glucose uptake in hCMEC/d3 cells following expression of human Glut1 (*SLC2A1*) at a 96-hour time point.

[0073] **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 ( $3 \times 10^5$  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

### DEFINITIONS

**[0074]** 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.

**[0075]** 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.

**[0076]** 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.

**[0077]** 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.

**[0078]** As used herein, the terms “identity” and “identical” refer, with respect to a polypeptide or polynucleotide sequence, to the percentage of exact matching residues in an alignment of that “query” sequence to a “subject” sequence, such as an alignment generated by the BLAST algorithm. Identity is calculated, unless specified otherwise, across the full length of the subject sequence. Thus a query sequence “shares at least x% identity to” a subject sequence if, when the query sequence is aligned to the subject sequence, at least x% (rounded down) of the residues in the subject sequence are aligned as an exact match to a corresponding residue in the query sequence. Where the subject sequence has variable positions (*e.g.*, residues denoted X), an alignment to any residue in the query sequence is counted as a match.

**[0079]** 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.

**[0080]** 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.

**[0081]** As used herein, “promoter” refers to a polynucleotide sequence capable of promoting initiation of RNA transcription from a polynucleotide in a eukaryotic cell.

**[0082]** 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).

**[0083]** 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 a newborn, infant, juvenile or adult.

**[0084]** As used herein, the term “variant” or “functional variant” refer, interchangeably, to a protein that has one or more amino-acid substitutions, insertions, or deletion compared to a parental protein that retains one or more desired activities of the parental protein.

**[0085]** 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.

**[0086]** 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

[0087] 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.

[0088] The polypeptide sequence of GLUT1 is as follows:

MEPSSKKLTGRLMLAVGGAVLGSLQFGYNTGVINAPQKVIEEFYNQTWVHRYGESI  
LPTTLTTLWSLSVAIFSVGGMIGSFVGLFVNRFGRRNSMLMMNLLAFVSAVLMGFS  
KLGKSFEMILGRFIIGVYCGLTTGFVPMYVGEVSPTALRGALGTLHQLGIVVGILIA  
QVFGLD SIMGNKDLWPLLLSIIFIPALLQCIVLPFCPESPRFLLINRNEENRAKSVLKKL  
RGTADVTHDLQEMKEESRQMMREKKVTILELFRSPAYRQPILIAVVLQLSQQLSGIN  
AVFYYSTSIFEKAGVQQPVYATIGSGIVNTAFTVVSLFVVERAGRRTLHLIGLAGMA  
GCAILMTIALALLEQLPWMSYLSIVAIFGFVAFFEVGPGPIWFIVAELFSQGPRPAAIA  
VAGFSNWT SNFIVGMC FYVEQLCGPYVFHIFTVLLVLFFIFTYFKVPETKGRTFDEIA  
SGFRQGGASQSDKTPEELFHPLGADSQV

(SEQ ID NO: 26).

[0089] In some embodiments, the GLUT1 protein comprises a polypeptide sequence at least 75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 26).

[0090] 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 a vector genome, wherein the vector 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%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to:

ATGGAGCCCAGCAGCAAGAAGCTGACGGGTCGCCTCATGCTGGCCGTGGGAGGA  
GCAGTGCTTGGCTCCCTGCAGTTTGGCTACAACACTGGAGTCATCAATGCCCCC  
AGAAGGTGATCGAGGAGTTCTACAACCAGACATGGGTCCACCGCTATGGGGAGA  
GCATCCTGCCACCACGCTCACCACGCTCTGGTCCCTCTCAGTGGCCATCTTTTCT  
GTTGGGGGCATGATTGGCTCCTTCTCTGTGGGCCTTTTCGTTAACCGCTTTGGCCG  
GCGGAATTCAATGCTGATGATGAACCTGCTGGCCTTCGTGTCCGCCGTGCTCATG  
GGCTTCTCGAACTGGGCAAGTCCTTTGAGATGCTGATCCTGGGCCGCTTCATCA  
TCGGTGTGTACTGCGGCCTGACCACAGGCTTCGTGCCCATGTATGTGGGTGAAGT  
GTCACCCACAGCCCTTCGTGGGGCCCTGGGCACCCTGCACCAGCTGGGCATCGTC  
GTCGGCATCCTCATCGCCAGGTGTTCCGGCCTGGACTCCATCATGGGCAACAAGG  
ACCTGTGGCCCCTGCTGCTGAGCATCATCTTCATCCCGGCCCTGCTGCAGTGCAT  
CGTGCTGCCCTTCTGCCCCGAGAGTCCCCGCTTCCTGCTCATCAACCGCAACGAG  
GAGAACCGGGCCAAGAGTGTGCTAAAGAAGCTGCGCGGGACAGCTGACGTGAC  
CCATGACCTGCAGGAGATGAAGGAAGAGAGTCGGCAGATGATGCGGGAGAAGA  
AGGTCACCATCCTGGAGCTGTTCCGCTCCCCCGCCTACCGCCAGCCATCCTCAT  
CGCTGTGGTGCTGCAGCTGTCCCAGCAGCTGTCTGGCATCAACGCTGTCTTCTAT  
TACTCCACGAGCATCTTCGAGAAGGCGGGGGTGCAGCAGCCTGTGTATGCCACC  
ATTGGCTCCGGTATCGTCAACACGGCCTTCACTGTCGTGTCGCTGTTTGTGGTGG  
AGCGAGCAGGCCGGCGGACCCTGCACCTCATAGGCCTCGCTGGCATGGCGGGTT  
GTGCCATACTCATGACCATCGCGCTAGCACTGCTGGAGCAGCTACCCTGGATGTC  
CTATCTGAGCATCGTGGCCATCTTTGGCTTTGTGGCCTTCTTTGAAGTGGGTCCTG  
GCCCCATCCCATGGTTCATCGTGGCTGAACTCTTCAGCCAGGGTCCACGTCCAGC  
TGCCATTGCCGTTGCAGGCTTCTCCAACCTGGACCTCAAATTCATTGTGGGCATG  
TGCTTCCAGTATGTGGAGCAACTGTGTGGTCCCTACGTCTTCATCATCTTCACTGT  
GCTCCTGGTTCTGTTCTTCATCTTCACCTACTTCAAAGTTCCTGAGACTAAAGGCC  
GGACCTTCGATGAGATCGCTTCCGGCTTCCGGCAGGGGGGAGCCAGCCAAAGTG  
ACAAGACACCCGAGGAGCTGTTCCATCCCCTGGGGGCTGATTCCCAAGTG

(SEQ ID NO: 5).

**[0091]** 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%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to:

ATGGAACCATCATCCAAAAAGCTGACCGGACGACTGATGCTTGCAGTTGGCGGT  
GCGGTCTTGGGGAGCCTGCAGTTTGGGTACAATACTGGCGTAATCAATGCCCCGC  
AGAAGGTTATTGAAGAATTTTACAATCAAACGTGGGTACATCGCTACGGTGAATC  
CATTCTTCCTACAACCTCTGACCACACTCTGGAGCCTTCTGTAGCGATTTTTTCCG  
TCGGGGGCATGATAGGATCATTTTCCGTCGGTCTTTTTGTGAACCGCTTTGGCCG  
GAGAAATTCCATGCTGATGATGAATCTTCTCGCTTTCGTGAGTGCCGTCTCATG  
GGATTTAGTAAACTGGGTAAATCTTTCGAGATGTTGATACTGGGGAGATTTATTA  
TCGGCGTGTATTGTGGTTTGACCACGGGCTTTGTACCAATGTATGTTGGCGAGGT  
TTCTCCGACAGCATTGAGAGGTGCACTCGGGACCTTGCACCAGTTGGGCATCGTA  
GTAGGAATCCTTATAGCGCAAGTTTTCGGGCTCGATTCCATCATGGGGAACAAAG  
ATCTCTGGCCATTGCTCCTCTCAATAATTTTTATACCGGCATTGCTTCAGTGTATT  
GTTCTTCCTTTTTTGCCAGAGTCCCCTAGGTTCTGCTCATAAACAGGAATGAGG  
AGAATCGCGCTAAGTCCGTGTTGAAAAAACTTAGGGGAAGTGCAGACGTTACTC  
ACGATTTGCAAGAGATGAAGGAGGAATCTAGGCAAATGATGCGCGAGAAGAAG  
GTTACCATACTCGAACTCTTCCGCTCCCCCGCGTACAGGCAGCCCATTCTTATCG  
CGGTCGTCTTGCAGTTGTCACAACAGTTGAGTGGGATTAATGCAGTTTTCTATTAT  
AGCACGTCCATATTTGAAAAAGCAGGCGTCCAACAACCTGTCTATGCAACTATA  
GGCTCAGGCATTGTAAACACAGCGTTTACTGTAGTATCACTGTTTGTCTGTTGAGC  
GGGCTGGTCGAAGGACCTTGCACCTCATAGGACTGGCGGGCATGGCGGGCTGTG  
CGATTCTTATGACAATTGCGCTCGCGCTGTTGGAACAGCTTCCGTGGATGTCCTA  
TCTCTCTATAGTAGCAATATTTGGATTTGTTGCATTTTTTTGAAGTTGGGCCCCGAC  
CTATCCCCTGGTTCATCGTCGCGGAGCTCTTTTCCAAGGCCCAAGACCGGCTGC  
CATTGCTGTTGCAGGCTTCTCAAACCTGGACGAGTAATTTCATAGTAGGTATGTGT  
TTCCAGTATGTTGAACAGCTCTGTGGGCCCTATGTCTTTATCATCTTTACTGTGTT  
GCTCGTGTTGTTCTTTATCTTCACTTATTTCAAAGTACCCGAGACAAAGGGCAGG  
ACGTTTGACGAGATTGCATCTGGTTTTAGACAAGGAGGTGCCTCACAGAGTGATA  
AAACCCCGGAGGAATTGTTTCATCCGCTGGGAGCCGACTCACAGGTC

(SEQ ID NO: 27)

[0092] Optionally, the polynucleotide sequence encoding the vector 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 vector genome may comprise a polynucleotide sequence (with Kozak underlined) at least 75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to:

gccaccATGGAGCCCAGCAGCAAGAAGCTGACGGGTCGCCTCATGCTGGCCGTGGG  
AGGAGCAGTGCTTGGCTCCCTGCAGTTTGGCTACAACACTGGAGTCATCAATGCC  
CCCCAGAAGGTGATCGAGGAGTTCTACAACCAGACATGGGTCCACCGCTATGGG  
GAGAGCATCCTGCCCACCACGCTCACCACGCTCTGGTCCCTCTCAGTGGCCATCT  
TTTCTGTTGGGGGCATGATTGGCTCCTTCTCTGTGGGCCTTTTCGTAAACCGCTTT  
GGCCGGCGGAATTCAATGCTGATGATGAACCTGCTGGCCTTCGTGTCCGCCGTGC  
TCATGGGCTTCTCGAACTGGGCAAGTCCTTTGAGATGCTGATCCTGGGCCGCTT  
CATCATCGGTGTGTACTGCGGCCTGACCACAGGCTTCGTGCCCATGTATGTGGGT  
GAAGTGTCACCCACAGCCCTTCGTGGGGCCCTGGGCACCCTGCACCAGCTGGGC  
ATCGTCGTCGGCATCCTCATCGCCCAGGTGTTTCGGCCTGGACTCCATCATGGGCA  
ACAAGGACCTGTGGCCCCCTGCTGCTGAGCATCATCTTCATCCCGGCCCTGCTGCA  
GTGCATCGTGCTGCCCTTCTGCCCCGAGAGTCCCCGCTTCCTGCTCATCAACCGC  
AACGAGGAGAACCGGGCCAAGAGTGTGCTAAAGAAGCTGCGCGGGACAGCTGA  
CGTGACCCATGACCTGCAGGAGATGAAGGAAGAGAGTCGGCAGATGATGCGGG  
AGAAGAAGGTCACCATCCTGGAGCTGTTCCGCTCCCCCGCCTACCGCCAGCCCAT  
CCTCATCGCTGTGGTGCTGCAGCTGTCCCAGCAGCTGTCTGGCATCAACGCTGTC  
TTCTATTACTCCACGAGCATCTTCGAGAAGGCGGGGGTGCAGCAGCCTGTGTATG  
CCACCATTGGCTCCGGTATCGTCAACACGGCCTTCACTGTCGTGCTGCTGTTTGTG  
GTGGAGCGAGCAGGCCGGCGGACCCTGCACCTCATAGGCCTCGCTGGCATGGCG  
GGTTGTGCCATACTCATGACCATCGCGCTAGCACTGCTGGAGCAGCTACCCTGGA  
TGTCTATCTGAGCATCGTGGCCATCTTTGGCTTTGTGGCCTTCTTTGAAGTGGGT  
CCTGGCCCCATCCCATGGTTCATCGTGGCTGAACTCTTCAGCCAGGGTCCACGTC  
CAGCTGCCATTGCCGTTGCAGGCTTCTCCAACCTGGACCTCAAATTTATTGTGGG  
CATGTGCTTCCAGTATGTGGAGCAACTGTGTGGTCCCTACGTCTTCATCATCTTCA  
CTGTGCTCCTGGTTCTGTTCTTCATCTTCACCTACTTCAAAGTTCCTGAGACTAAA  
GGCCGGACCTTCGATGAGATCGCTTCCGGCTTCCGGCAGGGGGGAGCCAGCCAA  
AGTGACAAGACACCCGAGGAGCTGTTCCATCCCCTGGGGGCTGATTCCCAAGTG

(SEQ ID NO: 29).

**[0093]** 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).

**[0094]** In some embodiments, the vector genome comprises no Kozak sequence.

## VECTOR GENOME

**[0095]** 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).

**[0096]** In some embodiments, the 5' ITR comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to:

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTC  
GCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGGGAGTGGCCAACTCCATCACTAGGGGTTTCCT

(SEQ ID NO: 32)

**[0097]** In some embodiments, the 5' ITR comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to:

GCGCGCTCGCTCGCTCACTGAGGCCGCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCTCAGT  
GAGCGAGCGAGCGCGCAGAGGGAGTGGCCAACTCCATCACTAGGGGTTTCCTTGTAGTTAATGATTAACCCGCC  
ATGCTACTTATCTACGTA

(SEQ ID NO: 6)

**[0098]** In some embodiments, the 5' ITR comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to:

CTGCGCGCTCGCTCGCTCACTGAGGCCGCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCCGGCCTCA  
GTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAATCCATCACTAGGGGTTCCCTTGATGTTAATGATTAACCCG  
CCATGCTACTTATCTACGTA

(SEQ ID NO: 33)

**[0099]** In some embodiments, the 3' ITR comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to:

AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGG  
TCGCCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG

(SEQ ID NO: 34)

**[0100]** In some embodiments, the 3' ITR comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to:

TACGTAGATAAGTAGCATGGCGGGTTAATCATTAATACTACAAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCT  
CTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCA  
GTGAGCGAGCGAGCGCGC

(SEQ ID NO: 7)

**[0101]** In some embodiments the vector genome comprises one or more filler sequences, *e.g.*, at least 75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to:

GCGGCAATTCAGTCGATAACTATAACGGTCCTAAGGTAGCGATTTAAATACGCG  
CTCTCTTAAGGTAGCCCCGGGACGCGTCAATTGACTACAAACCGAGTATCTGCAG  
AGGGCCCTGCGTATG (SEQ ID NO: 35);

CTTCTGAGGCGGAAAGAACCAGATCCTCTCTTAAGGTAGCATCGAGATTTAAATT  
AGGGATAACAGGGTAATGGCGCGGGCCGC (SEQ ID NO: 36); or

GTTACCCAGGCTGGAGTGCAGTGGCACATTTCTGCTCACTGCAACCTCCTCCTCC  
CTGGGTTC (SEQ ID NO: 37).

### Promoters

**[0102]** In some embodiments, the polynucleotide sequence encoding an GLUT1 protein or functional variant thereof is operably linked to a promoter.

**[0103]** 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%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to:

ACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCGCCCATTTGACGTCAATAATGACGTATGTTCC  
CATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGT  
ACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGC  
CCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGA  
GGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCAATTTTGTATTTATTTATTTT  
TTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGC  
GGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGA  
GGCGGGCGGGCGGGCGGGCCCTATAAAAAGCGAAGCGCGCGGGCGGGCGG

(SEQ ID NO: 38)

**[0104]** 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.

**[0105]** In some cases, the promoter is a tissue-specific promoter, such as a promoter capable of driving expression in a neuron to a greater extent than in a non-neuronal cell. In some embodiments, tissue-specific promoter is a neuron-specific promoter. In some embodiments, tissue-specific promoter is selected from any various neuron-specific promoters including but not limited to 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.

**[0106]** In some embodiments, the promoter sequence is selected from Table 3. In some embodiments, the promoter 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 1-3 and 39-51.

**Table 3**

| PROMOTER             | SEQUENCE                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | SEQ ID NO: |
|----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| Human FLT-1 (hFLT-1) | TTTGCTTCTAGGAAGCAGAAGACTGAGGAAATGACTTGGGCGGGTG<br>CATCAATGCGGCCAAAAAAGACACGGACACGCTCCCCTGGGACCT<br>GAGCTGGTTCGCAGTCTTCCCAAAGGTGCCAAGCAAGCGTCAGTTC<br>CCCTCAGGCGCTCCAGGTTCAAGTGCCCTTGTGCCGAGGGTCTCCGGT<br>GCCTTCCTAGACTTCTCGGGACAGTCTGAAGGGGTCAGGAGCGGCG<br>GGACAGCGCGGGAAGAGCAGGCAAGGGGAGACAGCCGGACTGCG<br>CCTCAGTCCTCCGTGCCAAGAACACCGTCGCGGAGGCGCGGCCAGC<br>TTCCCTTGATCGGACTTTCCGCCCCCTAGGGCCAGGCGGCGGAGCT<br>TCAGCCTTGTCCCTTCCCCAGTTTCGGGCGGCCCCCAGAGCTGAGT<br>AAGCCGGGTGGAGGGAGTCTGCAAGGATTTCTGAGCGCGATGGG<br>CAGGAGGAGGGGCAAGGGCAAGAGGGCGCGGAGCAAAGACCTTG<br>AACCTGCCGGGGCCGCGTCCCGGGCCCGCTCGCCAGCACCTCCC | 1          |

|                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |    |
|-------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
|                         | CACGCGCGCTCGGCCCCGGGCCACCCGCCCTCGTCGGCCCCCGCCC<br>CTCTCCGTAGCCGCAGGGAAGCGAGCCTGGGAGGAAGAAGAGGGT<br>AGGTGGGGAGGCGGATGAGGGGTGGGGGACCCCTTGACGTCACCA<br>GAAGGAGGTGCCGGGGTAGGAAGTGGGCTGGGGAAAGGTTATAAA<br>TCGCCCCCGCCCTCGGCTGCTCTTCATCGAGGTCCGCGGGAGGCTC<br>GGAGCGCGCCAGGCGGACACTCCTCTCGGCTCCTCCCCGGCAGCGG<br>CGGCGGCTCGGAGCGGGCTCCGGGGCTCGGGTGCAGCGGCCAGCG<br>GGCGCTTGGCGGCGAGGATTACCCGGGGAAGTGGTTGTCTCCTGGC<br>TGGAGCCGCGAGACGGGCGCTCAGGGCGCGGGGCCGCGGCGGCG<br>AACAAGAGGACGGA CTCTGGCGGCCGGGTCGTTGGCCGCGGGGAG<br>CGCGGGCACCGGGCGAGCAGGCCGCGCTCGCGCTCACC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |    |
| Human FLK-1<br>(hFLK-1) | TGGAGCCGCCAAATATTTTGGGAAATAGCGGGAATGTTGGCGAACT<br>GGGCAAGTGCGTTTTTCTGATTAAGAGCAACCAGATTGAGCTTTTAA<br>AACTACAATTATACTGGCCAAACAAAATACCCCTATACAAAAACCA<br>AACTACTGGCAGGAGTCGCTGCCAGCTTGCGACCCGGCATACTTG<br>GCTGAGTATCCGCTTCTCCCTTGTGGCTCCAACTGCTGCAGATTCT<br>CGGCCACTTCAGACGCGCGCGATGGCGAAGAGGGTCTGCACTTTG<br>ACGCGCCTGGTGAGGGAGCGCTGCTCTTCGCAGCGCTCCTGGTGAT<br>GCTCCCCAAATTTTCGGGGACCGGCAAGCGATTAAATCTTGGAGTTG<br>CTCAGCGCCCCGTTACCGAGTACTTTTTATTTACACCAGAAACAAAG<br>TTGTTGCTCTGGGATGTTCTCTCCTGGGCGACTTGGGGCCCAGCGCA<br>GTCCAGTTGTGTGGGAAATGGGGAGATGTAAATGGGCTTGGGGA<br>GCTGGAGATCGCCGCCGGGTACCCGGGTGAGGGGCGGGGCTGGCC<br>GCACGGGAGAGCCCCCTCCTCCGCTCCGGCCCCCGCCCGCATGGCCC<br>CGCCTCCGCGCTCTAGAGTTTCGGCACCAAGCTCCCAACCTGCACTG<br>AGTCCCGGGACCCCGGGAGAGCGGTCAATGTGTGGTTCGCTGCGTTT<br>CCTCTGCCTGCGCCGGGCATCACTTGCGCGCCGCAGAAAGTCCGTC<br>TGGCAGCCTGGATATCCTCTCTACCGGCACCCGCAGACGCCCTG<br>CAGCCGCGGTGCGCGCCCGGGCTCCCTAGCCCTGTGCGCTCAACTG<br>TCCTGCGCTGCGGGGTGCCGCGAGTTCACCTCCGCGCCTCCTTCTC<br>TAGACAGGCGCTGGGAGAAAGAACC GGCTCCCGAGTTCTGGGCAT<br>TTCGCCCCGGCTCGAGGTGCA | 85 |
| Human Tie-1<br>(hTIE-1) | AGCTCCTCCCAGCCTCAGGCCCAGGAATGGGAATCTCTGTGGGTCA<br>CACATCAGTAGGGAGGTCTTTCCCGATCCTTTTCTATGCTACTCCAG<br>GAGTCAAAGCGTCTCCTGGGACTTTTCAGGGCGCTTCAGAAGAGCC<br>CTGGGCCTAAACCAGCTCAACCAAGCTGCAGGGACCCAGCCTCCTG<br>AGAAAAGTGAATGTGAGCCCGGTGCATTGAGAGGAGAATGAAGCC<br>TTCACCCAGAACACACTCTGGGAAGATGTCCCAGGCCAGGGGGA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 2  |

|                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |    |
|-------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
|                         | GGGTTTGTACTACCAGACCTAAGTCACCTAAACTGACACCAAGTCT<br>CATCCATCCCAACCATTCCATTCCGGGTCAGAGGGGTCATCGATTT<br>AACCAGCAAGGCTGCCCATCCAACGGTTGCTCCCTCTGCTCCCTGG<br>AAGGGCCTCCTCGTGGGCGTTCTGTACCTACAGGTCTTGTTCCGTTT<br>TGGGAACTGCCAGTGGTGGCAAGAGGTGGAGCAACGGGTGCCAGG<br>GCAGGGAGAGGTGAGTCTGGGAGGGAAGCAGAGGCAAGATCCATG<br>GGGCTTTAGAGACTTTGCCAAAGCAGTGCGACTGCTCCAGGTGT<br>TGTCAGCCGTCAAGAGTGAGTGCACCTCCCTGGGCAGACTTCTGCT<br>GCCCCAGTGCCCAGGAATAGGCAGGGGTTTGCCGAAAATGAATG<br>ACACCTGGCAGACAATAAGCTGAAGCTTTCATTAGCAGCTTAAGCT<br>GAGGACTATCTATGCAACCGATACTCCCTGTGTGCTCCCCGGGACT<br>GCTTAATGTGAGCCCTTGTGGAGCGATTGGCACCAAGAAAGCAAG<br>GACTAAGTCAGAAGTTCAAGTCCCAGCCTTGCCACAGCCTCAGGGT<br>GCCCTCGAGCACAGCAAGCCTCAGTTTTCCCATCTGTACAATGAGA<br>GAGGTACACAAGGTAGACTCGAAGGCTCTTTGTTGCCAGGGCCCTG<br>TGTTCTTTGAGTGTATGTGCTTCTCAGGCCCACAGAGGTCTTTGT<br>GTTTCGTATGTGAACTGCTCTCTAGGAAACCCATGTAAGTGTCTGTG<br>TCCTGGGGCACATACATGAGGACTCATGTGGGGCCGTATTGTGTGTT<br>TGTGCCGGGGGAGGGGAGACCCAGAACAATGTCCCCACCCCA<br>CCCCCTCCTCAATAGGCGGAAGCCACTGGCTTCTCCTTTCTGTC<br>CTCCTGCCTCCTTTGTGCCAGCAAGACTGAGTACTGGAGAGAGACA<br>GGGGATGGGAAAAATCAGTCCAGCTGTCCCCAGGTCTGCCCTTACC<br>ATAACCTTCCCCCACCTCAAGTGACTCCTCCCAGGCCACACCCAT<br>CCCCAGCCTTGTGGGGGCCAGATTGGGGGGCCTAGAGGCTCAAAG<br>GCAGAATGAGTCCTCCCACCCCTACCCTGCCACCCCTCCCACCCA<br>AGCCACCTCATTTCTCTTCTCCCCAGCACCGACCCACACTGACCA<br>ACACAGGCTGAGCAGTCAGGCCCACAGCATCTGACCCAGGCCCA<br>GCTCGTCTGGCTGGCCTGGGTGCGCCTCTGGAGTATGGTCTGGCG<br>GGTGCCCCCTTCTTGCTCCCCATCCTCTTCTTGGCTTCTCATGTGG |    |
| Mouse Tie-1<br>(mTIE-1) | AAGCTTCCGACCGTTAGTCAGAGAACTGTAAGTGCTCAGAGCCTGG<br>CTGACAATGATCTGGAATGAACCAGATAACAACATAATAAAATCTC<br>AGTAAAATAATTTAACAGTTAGCTTGAAGCTGGTCAGCTCTGGGG<br>AAATCAGGGTAAATTGTGCTGTCATGAACTGTCCCACTGACATC<br>GGCCAAAGTGAATATGAACTTTGGTAGATCCAATGCCTGTTCTATT<br>TATTTTCCAGTGAAAAGTATTTTGATAGAGCTTTTCATTTTGAAA<br>TACACTGAGTTAACCAAAATATCATGGATTTCGTTTGTTCTTAAGA<br>CATGCAACTCGTCTACGGCTATACCACTCTGAACGCGCCCCGATCTC<br>GGAAGACATGCAACTCAAATGTAAATACAGTAGAATATTACTTAGG<br>TAGAAACTCCTGGTGATTTTAAAGATTGGAAAAGAATATGAGGA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 86 |

|                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |  |
|-------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
|                         | AGAGTTGAATAATGCAAATTCTAGTGTGTGTGCTACCGAAGTGAAC<br>ACTTAATGCACAGTCTACAGACTAGGACATTTTATCGTGTGTTGTA<br>AAATTGGGTAGAACTTGTGTTTGTGAAAACTGAGCATTAAAACCT<br>TACAGAGACCGTTTCTTGTTTACTTTTGAAAAAAAAGAGTCACG<br>TGAGCCTCATTTTGTATTTGTGTGTGTGTGTGTGTGTGTGTCTCCCT<br>CCTCCCAGCGTGTGTGTGCTGGGAGGAGGGGAGACCCAGAACAA<br>TGTCTGCCTCCAAACCTTCTCAATAGGCGGAAGCCACTGGCTTCCT<br>CCCTTTCTGTCTCCCGTGCTCCAGCAATGCAGATGGAAGGGACCG<br>AAGGGATGGGAGAGAGAGGCCCAACCATCCCCAGATCTGTCTTGTC<br>ACAACCTGCCTCCCACCTCTAATGCCCCCCTTCCAGAGACTTCCAG<br>GCCACCCCATCCCGGGCTTGTGGGGGCTGGACACGGGAGGACTA<br>CAGGCGACAACTCTTCCACCTCTCTCCCTGCCACCCCTCCTACCC<br>TAACCATCATTTCTCTTCTCCCCAGCACCGAGGTGCACTGAGCTG<br>GACAGGCTGAACACTCAGACCCACAGCAACTGACCCCGGGCCCAG<br>CTGGCCTTGGCTGGCCAGGGCAGCTTCCAGAGT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |  |
| Human Tie-2<br>(hTIE-2) | GCTGGAGTGCAGTGGCACGATCTCGGCTCACTGCAACCTCTGCCTC<br>CCAGGTTCAAACAATTCTCCTGCCTCAGCCTCCAGAGTAGCTGGGG<br>TTACAGGTGCACGCCAGCAAGCACAGCTAAATTTTGTATTTTAGT<br>AGAGATGGGGTTTTGCCATGTTGGCCAGGCTGGTCTCAAACCTCTG<br>ACCTCAGGTGATCCACTCCCAAAGTGCTGGGATTATAGGCGTGAGC<br>CACTGTGCCAGGCCCCACTGTTTTTGTTTTTTTTTTCGTGATGACAA<br>ATTTAAAGTCATCTCATAGGAATAGAAAATAGCTTTTTAGTAGAAG<br>CTCTTGGAATTTAAATTGAGACTGAATGGAAAGATGAAAGAAAAT<br>AAACTTATTAACATTTAATGAGAACCTTCAAAGAACTAGGCATAGT<br>ACCAAATGGTTTTATTTTTTAAACCTCATTTATTCCTCTCAAAACA<br>CCTGGGAAGGAGATATTTTTGCCATTTACAGCTGTTGAAACTGAG<br>GCTCAAAAAGACTAAGTAACTTTTCTCAGCTACACATGTGGCTGAG<br>CCAGTATTTGAACCCAGTTCTGTTTGACAGACAGAACCTGGGCTTTTT<br>CACACCTGCAAACTGGAAACATTAATTGGTTCTTAAGATCATCATC<br>GATGTGATAAAACCTGGGACAGAAATTAGTCAAGACTAGCTGCATC<br>TGCCTTTTCTCTGGTGGGTAGGAAAAGGAGGAGTATAATGATTTC<br>CTCAGGCATGAAGGTCGATGATGAGCAAAGTGTATACTCTCTAATC<br>TAATGTCATAATTCATATTGTGGAGTAATTATCTGGATAAGTGTAG<br>GGTCTCTGACCTCATTCTAGATATTGTACATTCCATGGCTATTTTCA<br>TTTTGGTCCATGAACTCTTTTGCTCTCATGAGCACCATTTTATCCC<br>AATCTAATCCTGTATGTTTGTGTTTTACACAGATTAGTTTTTAAAT<br>GTTATATATAATTTGCTTCTGAAACACCATTGCTCAATGACTACCAA<br>ATCTTTCTCATTACCAAAATCCTTCTATGCCAACTCTTCAAGAAAT<br>TTGATCACCTTTAGATGAATTGTTAATGAAAATTAAAGCTATAGCC |  |

|                                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |   |
|---------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---|
|                                 | GGCAACATGGGTATCTTTGGGCTAATGGCCAACCAACAGGCCATCT<br>GTGTGAAAGAAAACAGGCTAACAATTTTGGACTCTGGTCTCTTGGG<br>GCTACATTGAGCATTGACCTCACCGGTGCTCACTGAAATTAATTGC<br>TTTTCAGGTTGTATTTTCTCATCACGGAACCTTCTTCTCCCAATTC<br>AAACCATGTGGGTAAATGAGAAAACAAAAGCCAAAACGGCTTC<br>CCACACCCAAAAGCTCCTTCTGTCAGAGATCCCAGTAGCCCCGGGA<br>GAGCTGTTAGAAGTCTGAGAAGGATTGGTCATCATCGCATACCATA<br>CATAGGTGGAGGGCTTGTATTCTCAGTTTCCCGCTATGAGAGGA<br>TACCCCTATTGTTTCTGAAAATGCTGACCGGGACCCACACTTCCAA<br>CAAAAATTCCTCTGCCCCACAGCAGCAGCAAAAGCAGCAGCAGA<br>AGCAACAGCAACAGATAAGTGTTTTGATGAATTGCGAGATGGATA<br>GGGCTTGAGTGCCCCCAGCCCTGCTGATACCAAATGCCTTTAAGAT<br>ACAGCCTTTCCCATCCTAATCTACAAAGGAAACAGGAAAAAGGAA<br>CTTAAAACTCCCTGTGCTCAGACAGAAATGAGACTGTTACAGCCTG<br>CTTCTGTGCTGTTCTTCTTGCTCTAACTTGTAACAAGACGTAAGT<br>AGGACGATGCTAATGGAAAGTCACAAACCGCTGGGTTTTTGAAAG<br>GATCCTTGGGACCTCATGCACATTTGTGGAACTGGATGGAGAGAT<br>TTGGGGAAGCATGGACTCTTTAGCCAGCTTAGTTCTCTGTGGAGTC<br>AGCTTGCTCCTTTCTGGTAAGGTTTGGCTTTATTTTTTTAATTTAGT<br>ATTTTAAAAAACAGAGTTAGTGATTTCTGGGTGCTCTCCCCAAATCT<br>CATCAGTGCTGATGAACAAGGGGTGGCTGTAGCAAAGGCACCATTT<br>C |   |
| Human VE-cadherin (hVE-caderin) | CTAGTAGCAGAAACAAGGTCCTCTGGAAGAGCAACTGATGCTCTTA<br>GGTACTGAAGCATCATCTGCCCCAGAGACCACTCGCATATGAAGC<br>ACACATATTAGTCTGCCTTACTTGTGTTAATGATTGCCAGTGTCCC<br>TCTGACCTCCTAGCCCTGAAAAGTGTGGCCTGAAGGTCATTTGAGA<br>GACGGGGAGAGCTGCTCAGAGAAGCCAATCGGCGAGTCTAGGACA<br>CACAGACAGGATCTAGTCCCAGAGTTCGCTAGCCTAGGTGAGCGTC<br>CCCTGGCCCCCTTATAACCACTTCCTTCTCCAGCTTGATCTAATCTGC<br>TCTGGCAGACCATCGTGTTTCTGTCTTCTTGGCAGCCTCCAGCACG<br>CTCAGTGCTACTCCCTGCGCATGCGCCCTCCTCCCAGTACCTTCTCT<br>GACTCCAGTGGGCTTGGAGTGCAGGAGGAAGGGTGAGGAAGGGG<br>TGAAATCAGGTATTGGATCCACAGGGGGTCTGAAGAGCACTAGCCT<br>GGCCTTTTGGGACTGAACTTCTGCTATGAAGACCTCCACTGCCATCC<br>CTGGAGTCCGGGGCACATCCAAGGCTTGCTGTCCATCGTTTACTGTT<br>TACAGATGACAACAATGACTGTGTTCCGGGGCAGAAATATCCACCAG<br>GGCTAGAGTACAAAAGGAGTTTGCATTGATGGCCGGACAGGCCCT<br>GTCCCTGGCAGCCTGCCAGCGCTGAGTATGAGACCCAGCGGGAAGT<br>GCTACCCTGGCAGACGTGTCCACTGAGTACACAGACCACCAAGGCA                                                                                                                                                                                                   | 3 |

|  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |  |
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|  | GGCAGCTCTCGGGGAAGCTGTCTATGCTGGGCCAGCCCACCTTGAG<br>GGCAGGGAACAGAACAGATTGTGGCAGAGAGGAAAATGTGGAGCT<br>TCTGTTTGTTCACAGACACACGCACTCGCCACGCACGCACGCACG<br>CACGCACGCACGCACGAATGCACGCACGCAGTAGTTGAATGCTATG<br>GATTCCGCTCAGAGCTGAGAACAGCCCCAGCGACAGTTCCCTGGCC<br>TCTCTCCTTACTCTGATGTCCTCATCTGTCTTCACATGGTCTCAGGA<br>CGCTAATACTCCATCCTAATGTACACTCCTTCCCTGGGCCTCCGTT<br>CCAGTTCAGTTCTCAGAGGACCTGGAGGGAGTGATTGGCTACACCA<br>ACTTTGCTTTCGTTACCAAGCCCATGTCTCTACTTGGGTGTCTAAT<br>GGGCATCTCCAACATTACCTACCCCAAACAGAAAACCCTTTCTTCC<br>CCCCAACACACCCCAACCTACCCCAACAGTATTTTCTCCATGCCCG<br>GAAAGATCTGCTCTCTTATGGTCCCTCTTTCCTCACTGAAAAGCAG<br>GACAAGTTGGGGACTTCCCAAACCTTTATGCATGAAGAAACCCAGG<br>CAATTTGCCAAAAGGTACACTCTGGGGGTCTGTCATTTACTCTGAG<br>CCAGAACCCTGAAATTTTTACTAACCCTATCACATAATGAATGAAGA<br>GAATCTTTTTCTTTTTTTTTTTTTTTTTTTTTTTTGGTTTTTCGAGAC<br>AGGGTTTCTCTGTATAGCCCTGGCTATCCTGGAACACACTCTGTAG<br>ACCAGGCTGGCCTCGAACTCAGAAATCCACCTGCCTCTGCCTCCCG<br>AGTGCTGGGATTAAAGGCGTGCGCCACCACGCCTGGCTGAATGAA<br>GAGAATCTTGACCTCATCTCCCCAGCCTCTTGGTCCTGAGGGACCT<br>GGTCTACCTACTGCTTTGCTGTCTTCTTAGCTCTTCTTACTTTTTTGC<br>TGACTCAGACCTATGGCTATCTCCATTATACAGATGAGGAGACTGA<br>GGCATGGATCCCTGGTTGGTCCATGGTCACGTGAAGCCCATCACCC<br>AGTATTTGTAAAGTGAGATGGGCCAGGCTGGTACCTTGGAACCTGAA<br>ACTCACACTGCCCTACCTGGAAGAATCTGACAGGCAAAATCTGCTG<br>CTGAAAGTGATTGTCTGTACGTTTCTCAGCTGCCCCACTCTGAGA<br>ACTCCACAGCCCCCTTTCGTTCCACCATACTACAGAGTCGCCACGG<br>AAAGCCGGCTCTGTGGAGAAGCTGAGGTAGCTGGGTTTCTGTCTGG<br>GTTACTCTGTCCAGCGAGGAAACAAGTACCTTAGACCCACTAAGCC<br>TCTGCTTTCTGAACTGTAAAGTGGGGGATATGACACCTGCCTCCCA<br>GGGATGGCTGAATGCTCTGGCAGAAGCTTAGAGCCCCACAGCTAC<br>CCCTAGGCTCACAGCTCCTCCGATGAGACCTAGAATTGAGGTATGA<br>GTTGAATACCCACAGGCAAGTCCAAGGCTTCCACGGGGCCAGGCTGA<br>CCAAGCTGAGGCCGCCACCGTAGGGCTTGCTATCTGCAGGCAGC<br>TCACAAAGGAACAATAACAGGAAACCATCCCGAGGGGAAGTGGGC<br>CAGGGCCAGTTGAAAACCTGCCTCCCTCCCAGCCTGGGTGTGGCT<br>CCCCTCTCCCCTCTGAGGCAATCAACTGTGCTCTCCACAAAGCTCG<br>GCCCTGGACAGACT |  |
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|---------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Human VE-cadherin (hVE-cadherin) (short version)  | <p>CATCCATGCCCATGGCCTCAGATGCCAGCCATAAGCTGTTGGGTTC<br/> CAAACCTCGACTCCAGGCTGGACTCACCCCTGTCTCCCCACCAGC<br/> CTGACACCTCCACCTGGGTATCTAACGAGCATCTCAAACCTCAACCT<br/> GCCTGAGACAGAGGAATCACTATCCCCCTCTCCTCCAAAAATATCC<br/> TTCCATCACACTCCCCATCTTGTGCTCTGATTTACTAAACGGCCCTG<br/> GGCCCTCTCTTTCTCAGGGTCTCTGCTTGCCCAGCTATATAATAAAA<br/> CAAGTTTGGGACTTCCCAACCATTCACCCATGGAAAAACAGAAGCA<br/> ACTCTTCAAAGGACAGATTCCCAGGATCTGCCCTGGGAGATTCCAA<br/> ATCAGTTGATCTGGGGTGAGCCAGTCCTCTGTAGTTTTTAGAAGCT<br/> CCTCCTATGTCTCTCCTGGTCAGCAGAATCTTGGCCCCCTCCCTTCCC<br/> CCCAGCCTCTTGGTTCTTCTGGGCTCTGATCCAGCCTCAGCGTCACT<br/> GTCTTCCACGCCCCCTCTTTGATTCTCGTTTATGTCAAAAGCCTTGTG<br/> AGGATGAGGCTGTGATTATCCCCATTTTACAGATGAGGAACTGTG<br/> GCTCCAGGATGACACAACTGGCCAGAGGTCACATCAGAAGCAGAG<br/> CTGGGTCACTTGACTCCACCCAATATCCCTAAATGCAAAACATCCCC<br/> TACAGACCGAGGCTGGCACCTTAGAGCTGGAGTCCATGCCCCGTCT<br/> GACCAGGAGAAGCCAACCTGGTCCTCCAGAGCCAAGAGCTTCTGTC<br/> CCTTTCCCATCTCCTGAAGCCTCCCTGTCACCTTTAAAGTCCATTCC<br/> CACAAAGACATCATGGGATCACCACAGAAAATCAAGCTCTGGGGC<br/> TAGGCTGACCCAGCTAGATTTTTGGCTCTTTTATACCCAGCTGGG<br/> TGGACAAGCACCTTAAACCCGCTGAGCCTCAGCTTCCCGGGCTATA<br/> AAATGGGGGTGATGACACCTGCCTGTAGCATTCCAAGGAGGGTTAA<br/> ATGTGATGCTGCAGCCAAGGGTCCCCACAGCCAGGCTCTTTGCAGG<br/> TGCTGGGTTCAGAGTCCCAGAGCTGAGGCCGGGAGTAGGGGTTCA<br/> AGTGGGGTGCCCCAGGCAGGGTCCAGTGCCAGCCCTCTGTGGAGAC<br/> AGCCATCCGGGGCCGAGGCAGCCGCCACCGCAGGGCCTGCCTATC<br/> TGCAGCCAGCCAGCCCTCACAAGGAACAATAACAGGAAACCAT<br/> CCCAGGGGGAAGTGGGCCAGGGCCAGCTGGAAAACTGAAGGGGA<br/> GGCAGCCAGGCCTCCCTCGCCAGCGGGGTGTGGCTCCCCTCCAAAG<br/> ACGGTCGGCTGACAGGCTCCACAGAGCTCCACTCACGCTCAGCCCT<br/> GGACGGACAGGCAGTCCAACGGAACAGAAACATCCCTCAGCCCAC<br/> AGGCACGGTGAGTGGGGGCTCCCACACTCCCCTCCACCCCAAACCC<br/> GCCACCCTGCGCCCAAGATGGGAGGGTCCTCAGCTTCCCCATCTGT<br/> AGAATGGGCATCGTCCCACTCCCATGACAGAGAGGCTCC</p> | 88 |
| Human Intercellular Adhesion Molecule 2 (hlCAM-2) | <p>GTCTCCAGGCATGACTCCAACAATGCATCCCATGGGATTTGGGGT<br/> TCCCCAGATCTGGGGCTTGTAGGCCTGACTCTCCCCTGTGCACACGT<br/> CTCATACACGCATGCGTGACCCATTGCCTGCCCCGCCCCCTTGAC<br/> AGGGAGTCAGCAGGGAGGACTGGGTTATGCCCTGCTTATCAGCAGC<br/> TTCCCAGCTTCTCTGCCTGGATTCTTAGAGGCCTGGGGTCCTAGAA<br/> CGAGCTGGTGACGCTGGCTTCCCAAAGATCTCTCAGATAATGAGAG<br/> GAAATGCAGTCATCAGTTTGAGAAGGCTAGGGATTCTGGGCCATA</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 89 |

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|------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
|                                          | GCTCAGACCTGCGCCACCATCTCCCTCCAGGCAGCCCTTGGCTGG<br>TCCCTGCGAGCCCGTGGAGACTGCCAGTC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |    |
| Human Von<br>Willebrand Factor<br>(hVWF) | ATCTTTAGCCGATCCATTCAACCCTGGCCAGGATCCAAATGGACTG<br>TTTTTGTGTCAGGGCCAGGACCGGATCCTTCATACCTGGGGTGCATAG<br>GAAGTGTTAGTACTCCCTTCTCTCAAACACAGCAGCAAAATTGGC<br>TCAGGTTGAGGTGTTTTTCTCAACTTCCCTGGAGTCCAGCCCTGGAA<br>GCTGGATCAGGAAGCTGTGTTGTTCTACTGTGATTCCCCCTGGCCTG<br>TATCAGCTTGCCCTGAAACAACCAGCATTCTGGTTATCCACACA<br>GGTGGGGCACTCTAGGAAGACCAGGGATCAAGTGTGGGGGTGTAG<br>GGATAGGGGGTGTGTTGGGGAGGGCAAGGCAGTTAATTAAGGCAGC<br>TGCCAGGAGGTCTCCCTCCAAACTCTACAAAGCTTTATCAGCTTGG<br>AGGTACTTCTAATAACATTTCTTTTCATTGTTTCTTTTGGTAATTAA<br>AAGGAGGCCAATCCCCTGTTGTGGCAGCTCACAGCTATTGTGGTGG<br>GAAAGGGAGGGTGGTTGGTGGATGTCACAGCTTGGGCTTTATCTCC<br>CCCAGCAGTGGGGACTCCACAGCCCCTGGGCTACATAACAGCAAG<br>ACAGTCCGGAGCTGTAGCAGACCTGATTGAGCCTTTCAGCAGCTG<br>AGAGCATGGCCTAGGGTGGGCGGCACCATTGTCCAGCAGCTGAGTT<br>TCCCAGGGACCTTGGAGATAGCCGCAGCCCTCATTTGCAGGGGA                                                                                                                                                                                                                                           | 90 |
| Ple261 (Human<br>Claudin5)               | TGGCTTCCGGAGGGTGGCCTGGGGGCTGGGGTGCCAGGGACACCA<br>TCGCCACTGGTGGGAGGGCAGGGCACAGCCCCTCCGTGTCCCTTTG<br>TCTCTCCTGTCTGAAGGCCAGAGCAGGCTGCTAGGCCTGGGGCCAC<br>CACTGCCCCTGGGTGCTACACCCAGTGTGCTGGGTCACTGGGAACT<br>TCCTGAAGTGGTGTCACTGAACTGGGCCCCCAAGGATGGGGTGCG<br>GGCAGTACCGCAGGAAGAGGAGCAGCCCCTGTGAAGATTGAGAGG<br>TCTGGGAAGCCCCTGCGGCTTGGGAGAGTGGGGGTCGCCAGGCAG<br>GGGGAAAGCCCCTGTGCCACCGCTTTTGGCAGAGACTCAGGCTCC<br>AGAGAGGCAGTGAGTGGCATGGGGGTGAGGCTGGGGCCCTGGGC<br>CTGACCTCCACACGCCTGCCTGGCCTCTCTGTTTGCCATGGGATGAG<br>AGAGACAGTGCTGGGACTCAGAGCGGGGCTGGAGAGTGAGAGTGC<br>GAGAAAGGGCCTGGGTGGGGCTTGGACCCCGGGCGGGCTTTCTG<br>GAGAGCCCCCTACGAGGGCCTCTACGGCGGTGACGGGGTGGGGG<br>GCTTCTGCAAACCTTGGTCAGGGAAGTGGAGCTGGCTCGAGTGAA<br>GAGACCACCCGGCTCAGTCGGGGATGTGGGAGTGGACTGGGTGGT<br>GCAGACTGGGGGTCGAGCGCCTTCTGAAGTGACGGGGCCGGGACG<br>CGCAGGGAGGCGGCCAAGAAGCGCGCCCTAGGCCAGCCCAGAAT<br>GCGCTCGGCCGCGACTAGGACAACGGCGGGTGGGGCTGGGGGCGG<br>CTGCCGGGCGGGGAGCGGTCCCGCGCCCTCAGCTACCCCTCAAGAG<br>CCGTTGTTTCCCTAACTTCAGCTGCCAGAGGCTCTGTGATTGGCTGC<br>GGCACGATGACCCGCGCACGGATTGGCTGCTTCGGGCGGGGGG | 91 |

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|------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
|                                                                  | CGGGCCCGGGGACAGAATCCGCCCCGAACCTTCAAAGAGGGTA<br>CCCCCGGCAGGAGCTGGCAGACCCAGGAGGTGCGACAGACCCGC<br>GGGGCAAACGGA CTGGGGCCAAGAGCCGGGAGCGCGGGCGCAAA<br>GGCACCAGGGCCCCGCCAGGGCGCCGCGCAGCACGGCCTTGG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |    |
| Human Endoglin<br>(hENG)                                         | CGCCTTGCTGTGCCACTTTGGGACTTCCCTCCCTAGCCTGAGCTTCA<br>GTTTTCTGCTGT TAGGCAGCCCCATGTCAACTGCACTTAGTAGGC<br>CGGGTTTGATGCCCCACAAGACGTGAAGTGGTGGAGGTGGGCAGG<br>ATCCCAGCGCTACCATCTTCTTGAACCAAGTATCTCAACACATCGG<br>ATTTCTGTTTCTCATCTGCAAAATGGGATCAGTGAGCTCAGGTGG<br>GTCACAAATTCTACAGGAACTACTTTAGCCAAGCCCGCCCCCTGA<br>AAGTTCCCTCGGTGGGTGTTAGGGTGATTGTTTTCTATCTGTGGGG<br>CTCCCTGATGCGTCCCACCCACCAGCCTTGGAGAGGGTGGGATGGG<br>AGGGTGGGGTGCTTGGGGAGACAAGCCTAGAGCCTGGGCCCTCCC<br>ACCCCACTGCCTCCCCCATCCCAGGGCCCCCACCAGTGACAAA<br>GCCCCGTGGCACTTCCTCTACCCGGTTGGCAGGCGGCCTGGCCAGC<br>CCCTTCTCTAAGGAAGCGCATTTCTGCTCCCTGGGCGGCGGG<br>CTGGATGAGCCGGGAGCTCCCTGCTGCCGGTCATACCACAGCCTTC<br>ATCTGCGCCCTGGGGCCAGGACTGCTGCTGTCACTGCCATCCATTG<br>GAGCCCAGCACCCCTCCCCGCCATCCTTCGGACAGCAACTCCAG<br>CCCAGCCCCGCTCCCTGTGTCCACTTCTCCTGACCCCTCGGCCGCC<br>ACCCAGAAAGGCTGGAGCAGGGACGCCGTCGCTCCGGCCGCCTGCT<br>CCCCTCGGGTCCCCGTGCGAGCCCACGCCGGCCCCGGTGCCCGCCC<br>GCAGCCCTGCCACTGGACACAGGATAAGGCCAGCGCACAGGCC<br>CCACGTGGACACC | 92 |
| Human Platelet<br>Derived Growth<br>Factor Subunit B<br>(hPDGFB) | GCCCAGGCTGGAGTGCA GTGGCACAGTCACA ACTCACTGCAGCCTC<br>AAATCCTGGGCTCAAAACGATCCACAGTCTCCTGAGTAGCTGGGA<br>CTACAGGAGCTTGTTACCACACCCAGCTCCAGTTTATAAATTCATCT<br>CCAGTTTATAAAGGAGGAAACCGAGGTACTGAGAGGTTAAAAAAC<br>CTTCCTGCAGACACTTGTCCAGCAAGTGGCCACTCCAGGATTTGGA<br>CCAAGGTGATGTGTCTTCAAGGCTGTGTCTCTGCCACTGTGCCACGCT<br>GCTGGGTGGTAGGCAGCAGTGGGTGGGTGCCTGCAGTGGTCTGTAA<br>AGACCACCTGAGATGTCTTCCTCCTCTGTTCCACCCTGTCCAGGTC<br>CAAGAAGACAGTCTATGAAGAGAGAGCAGGTGTGACTCTCTCAGT<br>GTGCTCCTCTGTGAGAAGCAGGCTGACATCCCAAAGGGAAGGGCG<br>GATAACAGAGACAGTGCAAGCGGAGGAGATGAGGGTGCCTCAAAG<br>CCGGGAGGCTGGGTGATGCAGGAGCCTGCGTGTCCGAGGGGGGT<br>GCTGGGCCCAGTGTGAGTACGTGTGACTGTGACTGAGACAGTGTGA<br>CTGCTGAAGGCAGGGACACAGCAGCTCCCTGACTGGGGGCAGAAAG                                                                                                                                                                                                                                                               | 93 |

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|-------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
|                                                             | <p> GCGTTAACTGTGTGAAGGCTGGTTGTGGGTGGGTGGGCTCTGGGCC<br/> TCGAACCCGGGGGCTGAGGGAGATAGTAAACAGCAGGGTGACTGA<br/> CGGGAAGATCATGTTGGTAGCCCTGCGAAGATGCTGCAGGGCTGTG<br/> GGGGTTTGTGTGACTTTGCAGTTCAACAAATTCAAATTCAGCCAAC<br/> GCTGGCAGGGCCTGTTGTGCCAGGCAACCAGCTAGGAGGAGGAGA<br/> CTCGGACCCAGCTTGCAGCTGAAGGGCGCTGGCTGCCGGGTCTGT<br/> GGGTTACCTTGCGGTGTCTTCCCTTGCTAACACTGAGTCCTTACAA<br/> TAGCCCCATCTCCAGGTTGAGGCTAGATGGAGGGGACAGAGGGAA<br/> GTGACTTGCCCAAGGTGACCCAAGCTCCCGAGTGCCAGGGCAGGAT<br/> CTGAATTCAGGCTCTCAGACTGCAGAGCCTGAGTCCCTCCCTGCCA<br/> TGCCTGTGCCAGGGTGGAATGTCTGGTCTTGAGAGGGAGCGTGGA<br/> CTCCTGGCCTTGGCTCTGGAGACATCCCCCTAGACCACGTGGGCTC<br/> CTAACCTGTCCATGGTCACTGTGCTGAGGGGCGGGACGGTGGGTCA<br/> CCCCTAGTTCTTTTTTCCCCAGGGCCAGATTCATGGACTGAAGGGTT<br/> GCTCGGCTCTCAGAGACCCCCCTAAGCGCCCCGCCCTGGCCCCAAGC<br/> CCTCCCCCAGCTCCCGCGTCCCCCCCCCTCCTGGCGCTGACTCCGGGC<br/> CAGAAGAGGAAAGGCTGTCTCCACCCACCTCTCGCACTCTCCCTTC<br/> TCCTTTATAAAGGCCGGAACAGCTGAAAGGGTGGCAACTTCTCCTC<br/> CTGCAGCCGGGAGCGGCCTGCCTGCCTCCCTGCGCACCCGCGAGCCT<br/> CCCCCGCTGCCTCCCTAGGGCTCCCCTCCGGCCGCCAGCGCCATTT<br/> TTCATTCCCTAGATAGAGATACTTTGCGCGCACACACATACATACG<br/> CGCGCAAAAAGGAAAAAAGCCACCCTCCAGCCTC<br/> GCTGC </p> |    |
| Human<br>Endothelial Cell<br>Specific Molecule<br>1 (hESM1) | <p> ACATCCAATGCCCGCTCTGCCTCATCTTCTATGGGAAACAAGAATT<br/> TTAGAGGTCAGGTAGCCTAACACCATCAATTCTCAAAAGAGGAAGC<br/> TGAGGCCAAGAGAAGTCCTGTGAATTTCTTACAGCTCATTTGTGAC<br/> AGACCAAGAATTACCCACTTTACTGGGTGTTATTTACTAAGTGAC<br/> AGTGAGTCTATATCTCTTTTGACAAGTGAGGTGGGGGCATGGAATT<br/> CGGCATGTGGTTGGTGTAAGAACTCCCCTCTCTCCTCTTTAACCTTA<br/> CTTAATAAGACCCTGGCACAGTTGATATTTAAGAGGGCTACTCTG<br/> TTTTCCAGAGGGACCTAGGCACGGTAACCCTCTTAGCATGCAGAC<br/> CTTGTTTCCTGAGGGGTAATGTTTCCCTTCCCTGTGACTTGTTTCTTG<br/> GGGGCTGTGTTCTGATTTTCTGCTGAGCCACTTGTTGCCTTGGGCT<br/> GGCTGCCGCGCTTGGCAGTTTTTAGTGAGGGCTCTGATAGATGCCA<br/> GGAGGTGAGGGGAAGGGCTCTGGGTGGACTCCGTCATTGGACAAG<br/> CAGACTTAGTGATGGATGAGCCTTCCCCTGAGGAAGTTTGGATCA<br/> GAAGTCCAAGTATAAGTTTTTCCAGAATTGAGTAACCCAGAAGCA<br/> GTGCCGAAAGGATCTTACCTCTCTTGTGGCTTTTTGTATTGATTTA<br/> AAAGAAATTCTCAGAGGCAGTTCCACATTGTAAGTGAAGCACAGCT </p>                                                                                                                                                                                                                                                                                                                           | 94 |

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|-------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
|                         | <p>ATATCCACAATAGGCTTAGATATATGTAACATGAATTGCTTTAGAA<br/> ATAACATTTGAGGAGAGGGGTGAGAGGAAGGAAGAGAGGGTCTTA<br/> AAAAATAGCCCTATCAAAATATTTCTTTCTTCTAAGTATTGAAAAG<br/> ACACAATATAACCCCTTTCTTTCTTTCAAATGATCTCATAGCTATTTGT<br/> TGAGGGGAAATACCAAATGTTTATTATTTTTTTGAAGAAGCTTCTT<br/> CGGTCCTGATGATTCATGTTGATATCATTTTCCTCCTGACTACAGAG<br/> GCTCTGAGACAAAGCTACACCTCAAGTGATATGCCAGGGTCAGAAC<br/> AATTCCCGTCCTGAAGGAGGGTGTGCAACCTTCTTTATCCCTCCTTC<br/> ACAGACGTCCTTGAGCCCTTGAGACGGATGTGAGTGAGTTTTTCAG<br/> TCCTCATGCAAAACAACCATCTAAACATAACAGATGACATCAGCTT<br/> GGGCTTTTCAATTCTGGATGGCAGCAGCGTGTTAATCCAGCCTTC<br/> ATCCTGGATTTTATAAACCAAAACAAGAGAGCCTGGCAGGAGGAC<br/> AGCGCTGCTGCTGGGTTGAGGAAATTGATGACGGGAAAGCATGCG<br/> GGCAACCCAGTGTATAAACTCATAAACGTGTAGGCAGAGGCTCA<br/> GCTACCAGTTTGGACGGCTGCTTCCCACCAGCAAAGACCACGACTG<br/> GAGAGCCGAGCCGGAGGCAGCTGG</p>                                                                                                                                                                                                                                                                                                                                                                                           |    |
| Human Apelin<br>(hAPLN) | <p>TGGCACACACGCACCCTGTCCAATGTATCTTTTGTGTAAATCTGGAC<br/> TTAACACTTCAAGCAAAGCTGCTGGCTTGCTGAAAGGTGGAGACAC<br/> CTTTTCGATTCACTCTTTTAATATGTGTTGAGTGCCACCTATGTGCAG<br/> AGCAAGATATTGGGGACTTTGGAGAGATCCAGAAGAGTGAGAAGA<br/> CAGTATCCTACCTTAGGGGGTTCCAGTCCAATGAGGGAAGCAGCC<br/> CCATGCCTTGGGAGCTCCCAAGCTATAGAAGCAGCTAACAATCGAG<br/> TCTGGAAAGGCAAACAACCTCAGGACCCGCTTCTAAAGCGGAATCG<br/> CAAGTACACGCAAAATGAATCCAGCCTTGACTGTGTGGAGTTGGGT<br/> AAACCACCTGCCTCTTACGTTGATGGGGAAGTGAATGAGGACAGC<br/> TCCAGGGAACAAGAAAGGGTAGACCATAGGAGCTGTCCCATGTCC<br/> CAACAGTGGGGAGGAGCTGATGGGCGGCCCCCTGCTGGATTAGTGTT<br/> ATCCTGAGAAGGCTTCTGGATGCGATGGGATTTGAGGTGCTGCTGC<br/> AAAGAATGAATTGCTCACGGAAGGGTGGGGTGGGGGCATTCCAGG<br/> TAGAGGGTGCCTCCTGGGGGATGCAGGGAACATGAGGGGCTGGG<br/> CAATTAATCAAGCCTTGGGCACAAGCCTAGGCAGTCACCCCCAATT<br/> CAAAGCCAGTTGAAAATGCAGAGGAGAGAGGAGGGCCAGTGTTTG<br/> GTTGTCTTGACCAAACCTTGAAGCTGGCCAGCGCAAGGGCAAGG<br/> ACCAGGGTCAGAGGTAGAGGGCGTGAGTGAAGGCAACCCAGACTG<br/> AGTCCTTCCCTAAGCGCCAGGTTTCTGACAGCTGTAAAGGAAGC<br/> AAGGTGAGAAAGGGTTAAGTGTGCCCCCTCACCGCCCCAAATGCTT<br/> CCTGTGTTTGAAATCCTTCAGGTCTCTGCAAACCTCTGGCCCCCGG<br/> CCAGGCGGGCATTGTCCGGGGAGCGGTTGTAGGTTGTCAGAGAGG<br/> CCGCGCAGCCTTTGTTGTGGGGCCACCTCGGGGTTCCCTCTCGCGCT</p> | 95 |

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|----------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
|                                              | CACGCTCGGGCTGGGGCTGCAGAGTGCGTGCCTGGAGGGGGGCGG<br>TGCGGGAGGCTCGCTCCCTCTCCCTCTTCCTGCCCCCCTCTAGCCC<br>TCCCGATGACCACATGACCAAGTGGGCTCGCGGCCAAGCCACAAG<br>CTACAAAATGCAGCCCCTGGAGTGAGCGGGGAGCATTCTCTCTGGC<br>AGCCGGGGTACGGGCAGTTGCAGCCGCGGCCGAGCAGCCAGCCG<br>CTAAGAAAGAGCTCGCCGCTGCCGCTCCCGGAGCCGCCGAGGCCA<br>GCTTCGCGGCGCTGCCCCGCGGCGGGAGAGGAGGCTGCAGAAGAG<br>CGGAGGCGGCCAGCGG                                                                                                                                                                                              |    |
| Human beta-actin<br>(HuBa)                   | GCCCAGCACCCCAAGGCGGCCAACGCCAAAACCTCTCCCTCCTCCTC<br>TTCCTCAATCTCGCTCTCGCTCTTTTTTTTTTCGAAAAGGAGGGG<br>AGAGGGGGTAAAAAATGCTGCACTGTGCGGCGAAGCCGGTGAGT<br>GAGCGGCGCGGGGCCAATCAGCGTGCGCCGTTCCGAAAGTTGCCTT<br>TTATGGCTCGAGCGGCCGCGGCGGCGCCCTATAAAACCCAGCGGCG<br>CGACGCGCCACCACCGCCGAGTC                                                                                                                                                                                                                                                                                       | 39 |
| Chicken beta-actin<br>(CBA)                  | GGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCC<br>CCTCCCCACCCCAATTTTGTATTTATTTATTTTAAATTATTTGTG<br>CAGCGATGGGGGCGGGGGGGGGGGGGCGCGCGCCAGGCGGGGC<br>GGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGG<br>CGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGC<br>GAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCG<br>GGCGGGA                                                                                                                                                                                                                                                          | 40 |
| Cytomegalovirus<br>(CMV)                     | TGGTGATGCGGTTTTGGCAGTACACCAATGGGCGTGGATAGCGGTT<br>TGACTACGGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGG<br>AGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTA<br>ATAACCCCGCCCCGTTGACGCAAAATGGGCGGTAGGCGTGACGGTG<br>GGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCG                                                                                                                                                                                                                                                                                                                            | 41 |
| Cytomegalovirus<br>(CMV) (second<br>version) | TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCAT<br>ATATGGAGTTCGCGTTACATAACTTACGGTAAATGGCCCGCCTGG<br>CTGACCGCCCAACGACCCCCGCCCATTTGACGTCAATAATGACGTAT<br>GTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGG<br>TGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTA<br>TCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGG<br>CCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCTAC<br>TTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGC<br>GGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACG<br>GGGATTTCCAAGTCTCCACCCCATTTGACGTCAATGGGAGTTTGTTTT<br>GGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGC | 42 |

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|---------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
|                                             | CCCATTGACGCAAATGGGCGGTAGGCGGTACGGTGGGAGGTCTAT<br>ATAAGCAGAGCTGGTTTAGTGAACCGT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |    |
| Cytomegalovirus<br>(CMV) (third<br>version) | CGTTACATAAATTACGGTAAATGGCCCGCCTGGCTGACCGCCCAAC<br>GACCCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAA<br>CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACG<br>GTAAACTGCCCACCTTGGCAGTACATCAAGTGTATCATATGCCAAGT<br>ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATT<br>ATGCCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATC<br>TACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTA<br>CATCAATGGGCGTGGATAGCGGTTTGA CTACGGGGATTTC AAGT<br>CTCCACCCCATTTGACGTCAATGGGAGTTTGT TTTGGCACCAAAATC<br>AACGGGACTTTCCAAAATGTCGTAACA ACTCCGCCCCATTGACGCA<br>AATGGGCGGTAGGCGGTACGGTGGGAGGTCTATATAAGCAGAGC<br>T                                                                  | 43 |
| CAG promoter<br>(first version)             | ACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGC<br>CCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAG<br>GGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGC<br>CCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCT<br>ATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCACT<br>ACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTA<br>GTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCAC<br>TCTCCCCATCTCCCCCCCCCTCCCCACCCCAATTTTGTATTTATTTAT<br>TTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGCG<br>CGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGA<br>GGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAA<br>AGTTTCCTTTTATGGCGAGGCGGCGGGCGGCGGCGGCCCTATAAAAA<br>GCGAAGCGCGCGGCGGGCGG | 44 |
| CAG promoter<br>(second version)            | CGTTACATAAATTACGGTAAATGGCCCGCCTGGCTGACCGCCCAAC<br>GACCCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTAA<br>CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACG<br>GTAAACTGCCCACCTTGGCAGTACATCAAGTGTATCATATGCCAAGT<br>ACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATT<br>ATGCCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATC<br>TACGTATTAGTCATCGCTATTACCATGTCGAGGTGAGCCCCACGTTT<br>TGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCAATTTTGTAT<br>TTATTTATTTTAAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGG<br>GGGGGCGCGGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCG<br>GGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCG                                                                         | 45 |

|                                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |    |
|-----------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
|                                               | CTCCGAAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCCCT<br>ATAAAAAGCGAAGCGCGCGGCGGGCG                                                                                                                                                                                                                                                                                                                                                                                                                            |    |
| Human EF1-alpha<br>(EF1- $\alpha$ )           | CAACCTTTGGAGCTAAGCCAGCAATGGTAGAGGGAAGATTCTGCAC<br>GTCCCTTCCAGGCGGCCTCCCCGTCACCACCCCCCAACCCGCCC<br>CGACCGGAGCTGAGAGTAATTCATACAAAAGGACTCGCCCCTGCCT<br>TGGGGAATCCCAGGGACCGTCGTTAAACTCCCCTAACGTAGAACC<br>CAGAGATCGCTGCGTTCCCGCCCCCTCACCAGCCGCTCTCGTCATC<br>ACTGAGGTGGAGAATAGCATGCGTGAGGCTCCGGTGCCCGTCAGTG<br>GGCAGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGGAGG<br>GGTCGGCAATTGAACGGGTGCCTAGAGAAGGTGGCGCGGGGTAAA<br>CTGGGAAAGTGATGTCGTGTAAGTGGCTCCGCCTTTTCCCAGGGT<br>GGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT | 46 |
| Human Synapsin1<br>(Syn), short<br>version    | AGTGCAAGTGGGTTTTAGGACCAGGATGAGGCGGGGTGGGGGTGC<br>CTACCTGACGACCGACCCGACCCACTGGACAAGCACCCAACCCCC<br>ATTCCCCAAATTGCGCATCCCCTATCAGAGAGGGGGAGGGGAAAC<br>AGGATGCGGCGAGGCGCGTGCGCACTGCCAGCTTCAGCACCGCGG<br>ACAGTGCCTTCGCCCCCGCTGGCGGCGCGCGCCACCGCCGCCTCA<br>GCACTGAAGGCGCGCTGACGTCACTCGCCGGTCCCCCGCAAACCTCC<br>CCTTCCCGGCCACCTTGGTTCGCGTCCGCGCCGCGCCGCGGCCAGCC<br>GGACCGCACACGCGAGGCGCGAGATAGGGGGGCACGGGCGCGAC<br>CATCTGCGCTGCGGCGCCGGCGACTCAGCGCTGCCTC                                                      | 47 |
| Human Synapsin1<br>(Syn) with 3'<br>extension | AGTGCAAGTGGGTTTTAGGACCAGGATGAGGCGGGGTGGGGGTGC<br>CTACCTGACGACCGACCCGACCCACTGGACAAGCACCCAACCCCC<br>ATTCCCCAAATTGCGCATCCCCTATCAGAGAGGGGGAGGGGAAAC<br>AGGATGCGGCGAGGCGCGTGCGCACTGCCAGCTTCAGCACCGCGG<br>ACAGTGCCTTCGCCCCCGCTGGCGGCGCGCGCCACCGCCGCCTCA<br>GCACTGAAGGCGCGCTGACGTCACTCGCCGGTCCCCCGCAAACCTCC<br>CCTTCCCGGCCACCTTGGTTCGCGTCCGCGCCGCGCCGCGGCCAGCC<br>GGACCGCACACGCGAGGCGCGAGATAGGGGGGCACGGGCGCGAC<br>CATCTGCGCTGCGGCGCCGGCGACTCAGCGCTGCCTCAGTCTGCGG<br>TGGGCAGCGGAGGAGTCGTGTCGTGCCTGAGAGCGCAG   | 48 |
| Human Synapsin1<br>(Syn) with 5'<br>extension | CTGCAGAGGGCCCTGCGTATGAGTGCAAGTGGGTTTTAGGACCAGG<br>ATGAGGCGGGGTGGGGGTGCCTACCTGACGACCGACCCGACCCA<br>CTGGACAAGCACCCAACCCCCATTCCCCAAATTGCGCATCCCCTAT<br>CAGAGAGGGGGAGGGGAAACAGGATGCGGCGAGGCGCGTGCGCA<br>CTGCCAGCTTCAGCACCGCGGACAGTGCCTTCGCCCCCGCTGGCG<br>GCGCGCGCCACCGCCGCCTCAGCACTGAAGGCGCGCTGACGTCACT                                                                                                                                                                                                   | 49 |

|                            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |     |
|----------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
|                            | CGCCGGTCCCCGCAAACCTCCCCTTCCCGGCCACCTTGGTCGCGTCC<br>GCGCCGCCCGGCCAGCCGGACCGCACCACGCGAGGCGCGAGA<br>TAGGGGGGCACGGGCGCGACCATCTGCGCTGCGGCGCCGGCGACT<br>CAGCGCTGCCTC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |     |
| Human CamKIIa<br>(CaMKIIa) | ACTTGTGGACAAAGTTTGTCTATTCCACCTCCTCCAGGCCCTCCTT<br>GGGTCCATCACCCCAGGGGTGCTGGGTCCATCCCACCCCAGGCCC<br>ACACAGGCTTGCAGTATTGTGTGCGGTATGGTCAGGGCGTCCGAGA<br>GCAGGTTTCGCAGTGGAAGGCAGGCAGGTGTTGGGGAGGCAGTTA<br>CCGGGGCAACGGGAACAGGGCGTTTTGGAGGTGTTGCCATGGGG<br>ACCTGGATGCTGACGAAGGCTCGCGAGGCTGTGAGCAGCCACAGT<br>GCCCTGC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | 50  |
| eSYN promoter              | GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTC<br>ATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACG<br>GTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCGCCCATTGA<br>CGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTT<br>CCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTG<br>GCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACG<br>TCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGAC<br>CTTATGGGACTTTTCTACTTGGCAGTACATCTACGTATTAGTCATCG<br>CTATTACCATGGCTGCAGAGGGCCCTGCGTATGAGTGCAAGTGGGT<br>TTTAGGACCAGGATGAGGCGGGGTGGGGGTGCCTACCTGACGACC<br>GACCCCGACCCACTGGACAAGCACCCAACCCCATTCCCCAAATTG<br>CGCATCCCCTATCAGAGAGGGGGAGGGGAAACAGGATGCGGCGAG<br>GCGCGTCGCGACTGCCAGCTTCAGCACCGCGGACAGTGCCTTCGCC<br>CCCGCCTGGCGGCGCGCGCCACCGCCGCCTCAGCACTGAAGGCGCG<br>CTGACGTCACTCGCCGGTCCCCCGCAAACCTCCCCTTCCCGGCCACCT<br>TGGTCGCGTCCGCGCCGCCCGCGCCAGCCGACCGCACCACGCG<br>AGGCGCGAGATAGGGGGGCACGGGCGCGACCATCTGCGCTGCGGC<br>GCCGGCGACTCAGCGCTGCCTCAGTCTGCGGTGGGCAGCGGAGGA<br>GTCGTGTCGTGCCTGAGAGCGCAGG | 51  |
| hGLUT1<br>promoter         | ACCATTTTGCTAGAGAAGGCCGCGGAGGCTCAGAGAGGTGCGCAC<br>ACTTGCCCTGAGTCACACAGCGAATGCCCTCCGCGGTCCCAACGCA<br>GAGAGAACGAGCCGATCGGCAGCCTGAGCGAGGCAGTGTTAGGG<br>GGGGCCCCGGCCCCGGCCACTCCCCTCACCCCTCCCCGAGAGCG<br>CCGCCCAGGACAGGCTGGGCCCCAGGCCCCGCCCCGAGGTCCTGCC<br>CACACACCCCTGACACACCGGCGTCGCCAGCCAATGGCCGGGGTCC<br>TATAAACGCTACGGTCCGCGCGCTCTCT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 102 |

[0107] In a preferred embodiment, the vector genome comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 1. In a preferred embodiment, the vector genome comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 2. In a preferred embodiment, the vector genome comprises a polynucleotide sequence at least 75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to SEQ ID NO: 3.

[0108] 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**

[0109] In some cases, vectors 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).

[0110] In some embodiments, the vector comprises a CMV enhancer.

[0111] In certain embodiments, the vectors comprise one or more enhancers. In particular embodiments, the enhancer is a CMV enhancer sequence, a GAPDH enhancer sequence, a  $\beta$ -actin enhancer sequence, or an EF1- $\alpha$  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)

**[0112]** In certain embodiments, the vectors comprise one or more introns. In particular embodiments, the intron is a rabbit globin intron sequence, a chicken  $\beta$ -actin intron sequence, a synthetic intron sequence, or an EF1- $\alpha$  intron sequence.

**[0113]** In certain embodiments, the vectors comprise 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).

**[0114]** In certain embodiments, the vectors comprise 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 vectors comprise both a 5' UTR and a 3' UTR.

**[0115]** In some embodiments, the vector comprises a 5' untranslated region (UTR) selected from Table 4. In some embodiments, the vector 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**

| <b>5' UNTRANSLATED REGION</b> | <b>SEQUENCE</b>                                                                                                                                                                                                                                                                                                                                                                                       | <b>SEQ ID NO:</b> |
|-------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|
| Human beta-actin exon/intron  | CGCGTCCGCCCCGCGAGCACAGAGCCTCGCCTTTGCCGATC<br>CGCCGCCCCGTCCACACCCGCCGCCAGGTAAGCCCGGCCAG<br>CCGACCGGGGCATGCGGCCGCGGCCCTTCGCCCCGTGCAGA<br>GCCGCCGTCTGGGCCGCAGCGGGGGCGCATGGGGCGGA<br>ACCGGACCGCCGTGGGGGGCGCGGGAGAAGCCCCTGGGC<br>CTCCGGAGATGGGGGACACCCACGCCAGTTCGCAGGCG<br>CGAGGCCGCGCTCGGGCGGGCGCGCTCCGGGGGTGCCGC<br>TCTCGGGGCGGGGGCAACCGGCGGGGTCTTTGTCTGAGCC<br>GGGCTCTTGCCAATGGGGATCGCACGGTGGGCGCGGCGTA | 53                |

|                                                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |    |
|-------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
|                                                             | GCCCCCGTCAGGCCCGGTGGGGGCTGGGGCGCCATGCGC<br>GTGCGCGCTGGTCCTTTGGGCGCTAACTGCGTGCGCGCTG<br>GGAATTGGCGCTAATTGCGCGTGCGCGCTGGGACTCAATG<br>GCGCTAATCGCGCGTGCGTTCTGGGGCCCGGGCGCTTGCG<br>CCACTTCCTGCCCCGAGCCGCTGGCGCCCGAGGGTGTGGCC<br>GCTGCGTGCGCGCGCGGACCCGGTCGCTGTTTGAACCGG<br>GCGGAGGCGGGGCTGGCGCCCGGTTGGGAGGGGGTTGGG<br>GCCTGGCTTCCTGCCGCGCGCCGCGGGGACGCCTCCGACC<br>AGTGTTCGCTTTTATGGTAATAACGCGGCCGCGCCCGGCT<br>TCCTTTGTCCCCAATCTGGGCGCGCGCCGCGCCCCCTGG<br>CGGCCTAAGGACTCGGCGCGCCGGAAGTGGCCAGGGCGG<br>CAGCGGCTGCTCTTGCGGCCCCGAGGTGACTATAGCCTT<br>CTTTTGTGTCTTGATAGTTCGCCAGCCTCTGCTAACCATGT<br>TCATGCCTTCTTCTTTTCTACAGCTCCTGGGCAACGTGC<br>TGGTTATTGTGCTGTCTCATCATTTTGGCAAAGAATTC                                                                                                                                                                                                                                                                           |    |
| Chicken beta-actin<br>exon/intron + rabbit<br>globin intron | GTCGCTGCGCGCTGCCTTCGCCCCGTGCCCCGCTCCGCCG<br>CCGCCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTT<br>ACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCG<br>GGCTGTAATTAGCGCTTGGTTTAATGACGGCTTGTTCCTTT<br>TCTGTGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGC<br>CCTTTGTGCGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGT<br>GTGTGTGCGTGCGGAGCGCCGCGTGCGGCTCCGCGCTGCC<br>CGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGT<br>GCGCTCCGCAGTGTGCGCGAGGGGAGCGCGGCCGGGGGCG<br>GGTGCCCCGCGGTGCGGGGGGGGCTGCGAGGGGAACAAA<br>GGCTGCGTGCGGGGTGTGTGCGTGCGGGGGGTGAGCAGGG<br>GGTGTGGGCGCGTTCGGTTCGGGCTGCAACCCCCCTGCACC<br>CCCCCCCCGAGTTGCTGAGCACGGCCCCGGCTTCGGGTGC<br>GGGGCTCCGTACGGGGCGTGCGCGGGGGCTCGCCGTGCC<br>GGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGC<br>GGGGCCGCCTCGGGCCGGGAGGGCTCGGGGGAGGGGCG<br>CGGCGGCCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGC<br>GAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGG<br>GCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGA<br>AATCTGGGAGGCGCCGCCGACCCCCCTCTAGCGGGCGCGG<br>GGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGG | 54 |

|                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |    |
|------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
|                                                      | GGAGGGCCTTCGTGCGTCGCCGCGCCGCGTCCCCTTCTC<br>CCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGGTGCC<br>TTCGGGGGGGACGGGGCAGGGCGGGGTTGGCTTCTGGC<br>GTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCA<br>TGCCTTCTTCTTTTTCCTACAGCTCCTGGGCAACGTGCTGG<br>TTATTGTGCTGTCTCATCATTTTGGCAAAGAATTC                                                                                                                                                                                                                                                                                                                 |    |
| 5' UTR-Syn1 Hs                                       | AGTCTGCGGTGGGCAGCGGAGGAGTCGTGTCGTGCCTGAG<br>AGCGCAGCTGTGCTCCTGGGCACCGCGCAGTCCGCCCCCG<br>CGGCTCCTGGCCAGACCACCCCTAGGACCCCTGCCCCAA<br>GTCGCA                                                                                                                                                                                                                                                                                                                                                                                                                                     | 55 |
| CMV IE exon                                          | TCAGATCGCCTGGAGAGGCCATCCACGCTGTTTTGACCTC<br>CATAGTGGACACCGGGACCGATCCAGCCTCCGCGGCCGG<br>GAACGGTGCATTGGAACGCGGATTCCCCGTGCCAAGAGTG<br>AC                                                                                                                                                                                                                                                                                                                                                                                                                                         | 56 |
| TPL-eMLP<br>(adenovirus derived<br>enhancer element) | CTCACTCTCTCCGCATCGCTGTCTGCGAGGGCCAGCTGTT<br>GGGCTCGCGGTTGAGGACAACTCTTCGCGGTCTTTCCAG<br>TACTCTTGGATCGGAAACCCGTCGGCCTCCGAACGGTACT<br>CCGCCACCGAGGGACCTGAGCGAGTCCGCATCGACCGGA<br>TCGGAAAACCTCTCGAGAAAGGCGTCTAACCAGTCACAGT<br>CGCAAGGTAGGCTGAGCACCGTGGCGGGCGGCAGCGGGT<br>GGCGGTGCGGGTTGTTTCTGGCGGAGGTGCTGCTGATGAT<br>GTAATTAAAGTAGGCGGTCTTGAGACGGCGGATGGTCGA<br>GGTGAGGTGTGGCAGGCTTGAGATCCAGCTGTTGGGGTGA<br>GTACTCCCTCTCAAAAGCGGGCATTACTTCTGCGCTAAGA<br>TTGTCAGTTTCCAAAAACGAGGAGGATTTGATATTACCT<br>GGCCCGATCTGGCCATACACTTGAGTGACAATGACATCCA<br>CTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAG | 57 |
| Human EF1- $\alpha$<br>intron/exon                   | CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTG<br>CCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTAT<br>GGCCCTTGCGTGCCTTGAATTACTTCCACCTGGCTCCAGTA<br>CGTGATTCTTGATCCCGAGCTGGAGCCAGGGGCGGGCCTT<br>GCGCTTTAGGAGCCCTTCGCCTCGTGCTTGAGTTGAGGC<br>CTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGG<br>CACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGC                                                                                                                                                                                                                                                            | 58 |

|                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |    |
|--------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
|                                | <p> CATTTAAAATTTTGGATGACGTGCTGCGACGCTTTTTTCT<br/> GGCAAGATAGTCTTGTAATGCGGGCCAGGATCTGCACAC<br/> TGGTATTTTCGGTTTTTGGGCCCCGCGGCCGGCGACGGGGCC<br/> CGTGCGTCCCAGCGCACATGTTTCGGCGAGGCGGGGCCTGC<br/> GAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAG<br/> CTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTG<br/> TATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTTCGGCA<br/> CCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTG<br/> CTCCAGGGGGCTCAAAATGGAGGACGCGGCGCTCGGGAG<br/> AGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCT<br/> TTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTAC<br/> CGGGCGCCGTCCAGGCACCTCGATTAGTTCTGGAGCTTTT<br/> GGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGC<br/> GATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTT<br/> AGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTG<br/> GCCTTTTTGAGTTTGGATCTTGGTTCAATTCTCAAGCCTCAG<br/> ACAGTGGTTCAAAGTTTTTTTCTTCCATTTTCAG </p>                                                                                                    |    |
| Human EF1- $\alpha$ , intron A | <p> GTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTA<br/> CGGGTTATGGCCCTTGCCTGCTTGAATTACTTCCACCTGG<br/> CTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTGGAA<br/> GTGGGTGGGAGAGTTTCGAGGCCTTTCGCTTAAGGAGCCCC<br/> TTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGG<br/> GGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTC<br/> TCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGA<br/> TGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGT<br/> AAATGCGGGCCAAGATCTGCACACTGGTATTTTCGGTTTTT<br/> GGGGCCGCGGGCGGCGACGGGGCCCGTTCGCTCCCAGCGC<br/> ACATGTTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCG<br/> AGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTC<br/> TGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTG<br/> GGCGGCAAGGCTGGCCCGGTTCGGCACCAAGTTGCGTGAGC<br/> GGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCA<br/> AAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAG<br/> TCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCG<br/> TCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAG<br/> GCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTT </p> | 59 |

|                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |    |
|-------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
|                         | TAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCA<br>CACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCAC<br>TTGATGTAATTCTCCTTGGAATTTGCCCTTTTGGAGTTTGG<br>ATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTT<br>TTTTCTTCCATTTAG                                                                                                                                                                                                                                                                                                                                    |    |
| 5' UTR human<br>CamKIIa | TCAGAAGCCCCGGGCTCGTCAGTCAAACCGGTTCTCTGTT<br>TGCACTCGGCAGCACGGGCAGGCAAGTGGTCCCTAGGTTC<br>GGG                                                                                                                                                                                                                                                                                                                                                                                                                                          | 60 |
| B-globin intron         | GTGAGTCTATGGGACCCTTGATGTTTTCTTTCCCTTCTTTT<br>CTATGGTTAAGTTCATGTCATAGGAAGGGGAGAAGTAACA<br>GGGTACACATATTGACCAAATCAGGGTAATTTGCATTTG<br>TAATTTTAAAAAATGCTTTCTTCTTTTAATATACTTTTTGT<br>TTATCTTATTTCTAATACTTTCCCTAATCTCTTTCTTTTCAGG<br>GCAATAATGATACAATGTATCATGCCTCTTTGCACCATTTCT<br>AAAGAATAACAGTGATAATTTCTGGGTAAAGGCAATAGCA<br>ATATTTCTGCATATAAATATTTCTGCATATAAATTGTAAC<br>GATGTAAGAGGTTTCATATTGCTAATAGCAGCTACAATCC<br>AGCTACCATTCTGCTTTTATTTTATGGTTGGGATAAGGCTG<br>GATTATTCTGAGTCCAAGCTAGGCCCTTTTGCTAATCATGT<br>TCATACCTCTTATCTTCTCCACAG | 61 |

[0116] In some embodiments, the vector comprises a 3' untranslated region selected from Table 5. In some embodiments, the vector 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<br>REGION                                                     | SEQUENCE                                                                                                                                                                      | SEQ<br>ID<br>NO: |
|-------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|
| WPRE(x) (mutated<br>woodchuck hepatitis<br>regulatory element –<br>version 1) | AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACT<br>GGTATTCTTAATCTATGTTGCTCCTTTTACGCTATGTGGAT<br>ACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGT<br>ATGGCTTTCATTTCTCCTCCTTGTATAAATCCTGGTTGCT | 62               |

|                                                                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |    |
|----------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
|                                                                      | GTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGT<br>GGCGTG GTGTGCACTGTGTTTGCTGACGCAACCCCCACTG<br>GTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGAC<br>TTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATC<br>GCCGCCTGCCTTGCCCCGCTGCTGGACAGGGGCTCGGCTGT<br>TGGGCACTGACAATTCCGTGGTGTGTCGGGGAAATCAT<br>CGTCCTTTCTTGCTGCTCGCCTGTGTTGCCACCTGGATT<br>CTGCGCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCA<br>ATCCAGCGGACCTTCCTTCCCGCGGCCCTGCTGCCGGCTCT<br>GCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGT<br>CGGATCTCCCTTTGGGCGCCTCCCCGC                                                                                                                                                                                  |    |
| WPRE(x) (mutated woodchuck hepatitis regulatory element – version 2) | TCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGT<br>ATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGATACG<br>CTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATG<br>GCTTTTCATTTTCTCCTCCTTGTATAAAATCCTGGTTGCTGTC<br>TCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGC<br>GTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTT<br>GGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTT<br>CGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGCC<br>GCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGG<br>GCACTGACAATTCCGTGGTGTGTCGGGGAAATCATCGTC<br>CTTTCCTTGCTGCTCGCCTGTGTTGCCACCTGGATTCTGC<br>GCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCC<br>AGCGGACCTTCCTTCCCGCGGCCCTGCTGCCGGCTCTGCGG<br>CCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGA<br>TCTCCCTTTGGGCGCCTCCCCGCA | 63 |
| WPRE(x) (mutated woodchuck hepatitis regulatory element – version 3) | TTCCTGTTAATCAACCTCTGGATTACAAAATTTGTGAAAG<br>ATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTAT<br>GTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGC<br>TTCCCGTATGGCTTTTCATTTTCTCCTCCTTGTATAAAATCCT<br>GGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAG<br>GCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAAC<br>CCCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTT<br>TCCGGGACTTTTCGCTTTCCCCCTCCCTATTGCCACGGCGG<br>AACTCATCGCCGCTGCCTTGCCCGCTGCTGGACAGGGG                                                                                                                                                                                                                                                          | 64 |

|      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |    |
|------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
|      | CTCGGCTGTTGGGCACTGACAATTCCGTGGTGTGTGCGG<br>GAAGCTGACGTCTTTCCGCGGCTGCTCGCCTGTGTTGCC<br>ACCTGGATTCTGCGCGGGACGTCTTCTGCTACGTCCCTT<br>CGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCT<br>GCCGGCTCTGCGGCCTCTTCCGCCTCTTCGCCTTCGCCCT<br>CAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCCCA<br>TGTATCTTTTTACCTGTGCCTTGTTTTGCCTGTGTTCCG<br>CGTCCTACTTTTCAAGCCTCCAAGCTGTGCCTTGGGCGGC<br>TTTGGGGCATGGACATAGATCCCTATAAAGAATTGGTTC<br>ATCTTATCAGTTGTTGAATTTTCTTCCTTTGGAC                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |    |
| CAAX | TGTGTGATAATG                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 65 |
| EES  | CTGTTCTCATCACATCATATCAAGGTTATATACCATCAAT<br>ATTGCCACAGATGTTACTTAGCCTTTTAATATTTCTCTAAT<br>TLAGTGATATGCAATGATAGTTCTCTGATTTCTGAGATT<br>GAGTTTCTCATGTGTAATGATTATTTAGAGTTTCTCTTTCA<br>TCTGTTCAAATTTTTGTCTAGTTTTATTTTTACTGATTTG<br>TAAGACTTCTTTTTATAATCTGCATATTACAATTCTCTTTA<br>CTGGGGTGTGCAAATATTTTCTGTCAATTCTATGGCCTGA<br>CTTTTCTTAATGGTTTTTTAATTTTAAAAATAAGTCTTAAT<br>ATTCATGCAATCTAATTAACAATCTTTTCTTTGTGGTTAG<br>GACTTTGAGTCATAAGAAATTTTTCTCTACACTGAAGTCA<br>TGATGGCATGCTTCTATATTATTTCTAAAAGATTTAAAG<br>TTTTGCCTTCTCCATTTAGACTTATAATTCAGTGAATTTT<br>TTTGTGTGTATGGTATGACATATGGGTTCCCTTTTATTTTT<br>TACATATAAATATATTTCCCTGTTTTTCTAAAAAAGAAAA<br>AGATCATCATTTTCCCATTTGTAATGCCATATTTTTTTCA<br>TAGGTCACCTACATATATCAATGGGTCTGTTTCTGAGCTC<br>TACTCTATTTTATCAGCCTCACTGTCTATCCCCACACATCT<br>CATGCTTTGCTCTAAATCTTGATATTTAGTGGAACATTCT<br>TTCCCATTTTGTCTACAAGAATATTTTTGTTATTGTCTTT<br>GGGCTTTCTATATACATTTTGAAATGAGGTTGACAAGTTA | 66 |
| HPRE | ATAACAGGCCTATTGATTGGAAAGTTTGTCAACGAATTGT<br>GGGTCTTTTGGGGTTTGCTGCCCTTTTACGCAATGTGGA<br>TATCCTGCTTTAATGCCTTTATATGCATGTATACAAGCAA<br>AACAGGCTTTTACTTTCTCGCCAACTTACAAGGCCTTTCT<br>CAGTAAACAGTATATGACCCTTTACCCCGTTGCTCGGCAA<br>CGGCCTGGTCTGTGCCAAGTGTGCTGACGCAACCCCCA<br>CTGGTTGGGGCTTGCCATAGGCCATCAGCGCATGCGTG<br>GAACCTTTGTGTCTCTCTGCCGATCCATACTGCGGAAC<br>CCTAGCCGCTTGTTTTGCTCGCAGCAGGTCTGGAGCAAAC<br>CTCATCGGGACCGACAATTCTGTCTGACTCTCCCGCAAGT<br>ATACATCGTTTCCATGGCTGCTAGGCTGTGCTGCCAACTG<br>GATCCTGCGCGGGACGTCTTTGTTTACGTCCCGTCGGCG<br>CTGAATCCCGCGGACGACCCCTCCCGGGGCCGCTTGGGG<br>CTCTACCGCCCGCTTCTCCGTCTGCCGTACCGTCCGACCA<br>CGGGGCGCACCTCTCTTTACGCGGACTCCCGTCTGTGCC                                                                                                                                                                                                                                           | 67 |

|                                                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |    |
|--------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
|                                                  | TTCTCATCTGCCGGACCGTGTGCACTTCGCTTCACCTCTG<br>CACGTGCGCATGGAGGCCACCGTGAACGCCCACCGGAACC<br>TGCCCAAGGTCTTGCATAAGAGGACTCTTGGACTTTCAGC<br>AATGTCATC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |    |
| R2V17 ( <i>HepB</i> derived<br>enhancer element) | TTCCTGTAAACAGGCCTATTGATTGGAAAGTTTGTCAACG<br>AATTGTGGGTCTTTTGGGGTTTGCTGCCCCCTTTACGCAA<br>TGTGGATATCCTGCTTTAATGCCTTTATATGCATGTATAC<br>AAGCAAAACAGGCCTTTTACTTTCTCGCCAACTTACAAGGC<br>CTTTCTCAGTAAACAGTATATGACCCTTTACCCCGTTGCT<br>CGGCAACGGCCTGGTCTGTGCCAAGTGTGTTGCTGACGCA<br>ACCCCCACTGGTTGGGGCTTGCCCATAGGCCATCAGCGC<br>ATGCGTGGAACCTTTGTGTCTCCTCTGCCGATCCATACTG<br>CGGAACTCCTAGCCGCTTGTTTTGCTCGCAGCTGGACTGG<br>AGCAAACCTCATCGGGACCGACAATTCTGTCTACTCTCC<br>CGCAAGCACTACCGTTTCCGCGGCTGCTCGCCTGTGTTG<br>CCACCTGGATTCTGCGCGGGACGTCTTCTGCTACGTCCC<br>TTCGGCCCTCAATCCAGCGGACCTTCTTCCCGCGGCCCTG<br>CTGCCGGCTCTGCGGCCTCTTCCGCCTCTTCGCCTTCGCC<br>CTCAGACGAGTCGGATCTCCCTTTGGGCCGCTCCCCGCC<br>CATGTATCTTTTTACCTGTGCCTTGTTTTGCCTGTGTTT<br>CGCGTCCTACTTTTCAAGCCTCCAAGCTGTGCCTTGGGCG<br>GCTTTGGGGCATGGACATAGATCCCTATAAAGAATTTGG<br>TTCATCTTATCAGTTGTTGAATTTCTTCCTTTGGAC | 68 |
| 3'UTR(globin)                                    | GCTGGAGCCTCGGTAGCCGTTCTCCTGCCCCGCTGGGCCT<br>CCCAACGGGGCCCTCCTCCCCCTCCTTGCAACGGGCCCTTCT<br>GGTCTTTGAATAAA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | 69 |
| WPRe(r)                                          | ATTCGAGCATCTTACCGCCATTTATTCCCATATTTGTTCTG<br>TTTTCTTGATTTGGGTATACATTTAAATGTAAATAAAAC<br>AAAATGGTGGGGCAATCATTTACATTTTATAGGGATATGTA<br>ATTACTAGTTCAGGTGTATTGCCACAAGACAAACATGTTA<br>AGAAACTTTCCCGTTATTTACGCTCTGTTCTGTTAATCA<br>ACCTCTGGATTACAAAATTTGTGAAAGATTGACTGATATT<br>CTTAACCTATGTTGCTCCTTTTACGCTGTGTGGATATGCTG<br>CTTTAATGCCTCTGTATCATGCTATTGCTTCCCGTACGGCT<br>TTCGTTTTCTCCTCCTTGATAAATCCTGGTTGCTGTCTCT                                                                                                                                                                                                                                                                                                                                                                                                                                             | 70 |

|  |                                                                                                                                                                                                                                                                |  |
|--|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
|  | TTATGAGGAGTTGTGGCCCGTTGTCCGTCAACGTGGCGTG<br>GTGTGCTCTGTGTTTGCTGACGCAACCCCCACTGGCTGGG<br>GCATTGCCACCACCTGTCAACTCCTTTCTGGGACTTTTCGC<br>TTTCCCCCTCCCGATCGCCACGGCAGAACTCATCGCCGCC<br>TGCCTTGCCCGCTGCTGGACAGGGGCTAGGTTGCTGGGC<br>ACTGATAATTCCGTGGTGTGTGTCGGGAAGGGCC |  |
|--|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|

**[0117]** In some embodiments, the vector 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-<br>ADENYLATION<br>SITE                      | SEQUENCE                                                                                                                                                                                                                                                                                                                                                                                                                             | SEQ<br>ID<br>NO: |
|---------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|
| Rabbit globin<br>(pAGlobin-Oc)                    | TGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTG<br>TTGGAATTTTTTGTGTCTCTCACTCGGAAGAACATATGG<br>GAGGGCAAATCATTTAAACATCAGAATGAGTATTTGGT<br>TTAGAGTTTGGCAACATATGCCCATATGCTGGCTGCCAT<br>GAACAAAGGTTGGCTATAAAGAGGTCATCAGTATATGA<br>AACAGCCCCCTGCTGTCCATTCTTATTCCATAGAAAAG<br>CCTTGACTTGAGGTTAGATTTTTTTTATATTTTGTGTTGTG<br>TTATTTTTTTCTTTAACATCCCTAAAATTTTCCTTACATGT<br>TTTACTAGCCAGATTTTTCTCCTCTCCTGACTACTCCCA<br>GTCATAGCTGTCCCTCTTCTCTTATGGAGATC | 71               |
| Bovine growth<br>hormone (pAGH-Bt –<br>version 1) | TTGCCAGCCATCTGTTGTTTGCCCTCCCCCGTGCCTTCC<br>TTGACCTGGAAGGTGCCACTCCCACTGTCCTTTCTAA<br>TAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGT<br>CATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAA<br>GGGGGAGGATTGGGAATACAATAGCAGGCATGCTGGGG<br>ATGCGGTGGGCTCTATGGGTACCCAGGTGCTGAAGAATT<br>GACCCGGTTCCTCCTGGG                                                                                                                                                     | 72               |

|                                             |                                                                                                                                                                                                                                                                                     |    |
|---------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----|
| Bovine growth hormone (pAGH-Bt – version 2) | TTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTCC<br>TTGACCCTGGAAGGTGCCACTCCCCTGTCCTTTCCTAA<br>TAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGT<br>CATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAA<br>GGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGG<br>ATGCGGTGGGCTCTATGGGTACCCAGGTGCTGAAGAATT<br>GACCCGGTTCCTCTCTGGG | 73 |
| Bovine growth hormone (pAGH-Bt – version 3) | CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTC<br>CCCCGTGCCTTTCCTTGACCCTGGAAGGTGCCACTCCCAC<br>TGTCTTTCCTAATAAAATGAGGAAATTGCATCGCATTG<br>TCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGG<br>GCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGC<br>AGGCATGCTGGGGATGCGGTGGGCTCTATGG                               | 74 |
| Human growth hormone (pAGH-Hs)              | CTGCCCCGGTGGCATCCCTGTGACCCCTCCCCAGTGCCT<br>CTCCTGGCCCTGGAAGTTGCCACTCCAGTGCCCACCAGC<br>CTTGTCCTAATAAAATTAAGTTGCATCATTTTGTCTGACT<br>AGGTGTCCTTCTATAATATTATGGGGTGGAGGGGGGTGG<br>TATGGAGCAAGGGGCCCAAGTTGGGAAGAAACCTGTAG<br>GGCCTGC                                                      | 75 |

[0118] Illustrative vector 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 vector genome comprises, consists essentially of, or consists of a polynucleotide sequence that shares at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 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 VECTOR

[0119] 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\_001862; 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).

**[0120]** AAV possesses unique features that make it attractive as a vector 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 AAV vectors, 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.

**[0121]** AAV DNA in the rAAV 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 AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV- 10, AAV-11, AAV- 12, AAV-13 and AAVrh10. 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.

**[0122]** 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 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).

**[0123]** In other cases, the rAAV vector comprises 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 are 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 vectors that maybe have singled stranded genomes, rather than self-complementary genomes, with the understanding that other genetic modifications of the rAAV vector may be beneficial to obtain optimal gene transcription in target cells. In some cases, the present disclosure relates to single-stranded rAAV vectors 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).

[0124] In some cases, the rAAV vector is of the serotype AAV1, AAV2, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV13, AAVrh10, or AAVrh74. 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 cases, the rAAV vector is of the serotype AAV9. In some embodiments, said rAAV vector is of serotype AAV9 and comprises a single stranded genome. In some embodiments, said rAAV vector is of serotype AAV9 and comprises a self-complementary genome. In some embodiments, a rAAV vector comprises the inverted terminal repeat (ITR) sequences of AAV2. In some embodiments, the rAAV vector comprises an AAV2 genome, such that the rAAV vector is an AAV-2/9 vector, an AAV-2/6 vector, or an AAV-2/8 vector.

[0125] Full-length sequences and sequences for capsid genes for most known AAVs are provided in US Patent No. 8,524,446, which is incorporated herein in its entirety.

[0126] AAV vectors 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 vector 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 vector is provided to a subject.

[0127] 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 or more particularly endothelial tip cells. In some embodiments, the rAAV is directly injected into the intracerebroventricular space of the subject.

**[0128]** In some embodiments, the rAAV virion is an AAV2 rAAV virion. The capsid may be an AAV2 capsid or functional variant thereof. In some embodiments, the AAV2 capsid shares at least 98%, 99%, or 100% identity to a reference AAV2 capsid, *e.g.*,

MAADGYLPDWLEDTLSEGI RQWWKLKPGPPPKPAERHKDDSRGLVLPGYKYLGPFGNLDKGEPVNEADAAALEH  
DKAYDRQLDSGDNPYLYKNHADA EFQERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTA PGKKRPVEHSP  
VEPDSSSGTGKAGQQPARKRLNFGQTGDADSVDPDQPLGQPPAAPSGLTNTMATGSGAPMADNNEGADGVGNSS  
GNWHCDSTWMGDRVITTTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDNRFHCHFS PRDWQR LI  
NNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQY  
GYLT LNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLSRTNT  
PSGTTTQSR LQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSL VNPGPAMAS  
HKDDEEKFFPQSGVLI FGKQGSSEKTNVDIEKVMITDEEEI RTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGV  
LPGMVWQDRDVYLQGP IWAKI PHTDGHFHPSPLMGGFGLKHPPPQILIKNT PVPANPSTTFSAAKFASFITQYST  
GQVSVEIEWELQKENS KRWNPEIQYTSN YNKS VNVDFTVDTNGVYSEPRPIGTRYLTRNL

(SEQ ID NO: 76)

**[0129]** In some embodiments, the rAAV virion is an AAV9 rAAV virion. The capsid may be an AAV9 capsid or functional variant thereof. In some embodiments, the AAV9 capsid shares at least 98%, 99%, or 100% identity to a reference AAV9 capsid, *e.g.*,

MAADGYLPDWLEDNLSEGI REWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPFGNLDKGEPVNAADAAALEH  
DKAYDQQLKAGDNPYLYKNHADA EFQERLKEDTSFGGNLGRAVFQAKKRLLLEPLGLVEEAAKTAPGKKRPVEQSP  
QEPDSSAGIGKSGAQPAKKRLNFGQTGDTE SVDPDQPIGEPPAAPSGVGS LTMA SGGGAPVADNNEGADGVGSSS  
GNWHCD SQWL GDRVITTTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDNRFHCHFS PRDWQR  
LINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVLGSAHEGCLPPFPADVFMI P  
QYGYLT LNDSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENVPFHSSYAHSQSLDRLMNPLIDQYLYLSKT  
INGSGONQQT LKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWP GASSWALNGRNSLMNPGPAMA  
SHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVMITNEEEI KTTNPVATESYGVATNHQSAQAQAQTGWVQNG  
ILPGMVWQDRDVYLQGP IWAKI PHTDGNFHPSPLMGGFGMKHPPPQILIKNT PVPADPPTAFNKDKLNSFITQYS  
TGQVSVEIEWELQKENS KRWNPEIQYTSNYYKSNNVEFAVNTG VYSEPRPIGTRYLTRNL

(SEQ ID NO: 77)

**[0130]** In some embodiments, the rAAV virion is an AAV6 rAAV virion. The capsid may be an AAV6 capsid or functional variant thereof. In some embodiments, the AAV6 capsid shares at least 98%, 99%, or 100% identity to a reference AAV6 capsid, *e.g.*,

MAADGYLPDWLEDNLSEGI REWWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFGNLDKGEPVNAADAAALEH  
DKAYDQQLKAGDNPYLYRNHADA EFQERLQEDTSFGGNLGRAVFQAKKRVLEPFG LVEEGA KTAPGKKRPVEQSP  
QEPDSSSGIGKTGQQPAKKRLNFGQTGDSESVDPDQPLGEPPATPAAVGPTTMA SGGGAPMADNNEGADGVGNAS

GNWHCDSTWLGDRVITTTSTRTWALPTYNNHLYKQISSASTGASNDNHYFGYSTPWGYFDNRFHCHFSRWDQRL  
 INNNWGFRPKRLNFKLFNIQVKEVTTNDGVTTIANNLSTVQVFSQDSEYQLPYVLGSAHQGCLPPFPADVFMIPQ  
 YGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNTFTSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLNRTO  
 NQSGSAQNKDLLFSRGSFAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESIINPGTAMA  
 SHKDDKDKFFPMGVMIFGKESAGASNTALDNVMTDEEEIKATNPVATERFGTVAVNLQSSSTD PATGDVHVMG  
 ALPGMVWQDRDVYLQGPWAKIPHTDGHFHSPLMGGFGLKHPPPQILIKNTPVPANPPAEFSATKFASFITQYS  
 TGQVSVEIEWELQKENS KRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRYLTRPL

(SEQ ID NO: 78)

**[0131]** In some embodiments, the rAAV virion is an AAVrh.10 rAAV virion. The capsid many be an AAVrh.10 capsid or functional variant thereof. In some embodiments, the AAVrh.10 capsid shares at least 98%, 99%, or 100% identity to a reference AAVrh.10 capsid, *e.g.*,

MAADGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEPVNAADAAALEH  
 DKAYDQQLKAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSP  
 QRSFDSSTGIGKKGQQPAKRLNFGQTDSESVDPQPFIGEPPAGPSGLSGTMAAGGGAPMADNNEGADGVGSS  
 SGNWHCDSTWLGDRVITTTSTRTWALPTYNNHLYKQISNGTSGGSTNDNTYFGYSTPWGYFDNRFHCHFSRWDQ  
 RLINNNWGFRPKRLNFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQ  
 YGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNTFTSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLSR  
 TQSTGGTAGTQQLFSQAGPNNMSAQAKNWLPGPCYRQQRVSTTSLQNNNSNFAWTGATKYHLNGRDSLVPNGVA  
 MATHKDDEERFFPSSGVLMTFGKQAGKDNVDYSSVMLTSEEEIKTNPVATEQYGVVADNLQQQNAAPIVGAVNS  
 QGALPGMVWQNRDVYLQGPWAKIPHTDGNFHSPLMGGFGLKHPPPQILIKNTPVPADPPTTFSQAKLASFITQ  
 YSTGQVSVEIEWELQKENS KRWNPEIQYTSNYKSTNVDFAVNTDGTYSERPIGTRYLTRNL

(SEQ ID NO: 79)

**[0132]** In some embodiments, the rAAV virion is an AAV8 rAAV virion. The capsid many be an AAV8 capsid or functional variant thereof. In some embodiments, the AAV8 capsid shares at least 98%, 99%, or 100% identity to a reference AAV8 capsid, *e.g.*,

MAADGYLPDWLEDNLSEGIREWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEPVNAADAAALEH  
 DKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAKTAPGKKRPVEPSP  
 QRSFDSSTGIGKKGQQPARKRLNFGQTDSESVDPQPLGEPPAAPSGVGPNTMAAGGGAPMADNNEGADGVGSS  
 SGNWHCDSTWLGDRVITTTSTRTWALPTYNNHLYKQISNGTSGGATNDNTYFGYSTPWGYFDNRFHCHFSRWDQ  
 RLINNNWGFRPKRLSFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQ  
 YGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNTFTSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYLSR  
 TQTTGGTANTQTLGFSQGGPNTMANQAKNWLPGPCYRQQRVSTTTGQNNNSNFAWTAGTKYHLNGRNSLANPGIA  
 MATHKDDEERFFPSNGILIFGQNAARDNADYSDVMLTSEEEIKTNPVATEEYGVVADNLQQQNTAPQIGTVNS

QGALPGMVWQNRDVYLQGPWAKIPHTDGNFHPSPMLGGFGLKHPPPQILIKNTVPVADPPTTFNQSKLNSFITQ  
YSTGQVSVIEIEWELQKENS KRWNPEIQYTSNYYKSTSVDFAVNTEGVYSEPRPIGTRYLTRNL

(SEQ ID NO: 80)

**[0133]** In some embodiments, the rAAV virion is an AAVrh.74 rAAV virion. The capsid may be an AAVrh.74 capsid or functional variant thereof. In some embodiments, the AAVrh.74 capsid shares at least 98%, 99%, or 100% identity to a reference AAVrh.74 capsid, *e.g.*,

MAADGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQDNGRGLVLPGYKYLGPFGNLDKGEVNAADAAALEH  
DKAYDQQLQAGDNPYLRYNHADAQEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVESPVKTA PGKKRPVEPSP  
QRSPDSSTGIGKKGQPAKKRLNFGQTGDSESVDPQPIGEPPAGPSGLSGTMAAGGGAPMADNNEGADGVGSS  
SGNWHCDSTWLGD RVITTTSTRTWALPTYNNHLYKQISNGTSGGSTNDNTYFGYSTPWGYFDNRFHCHFS PRDWQ  
RLINNNWGFPRKRLNFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVEMI  
PQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNEFEFSYNFEDVPFHSSYAHSQSLDRLMNP LIDQYLYLSR  
TQSTGGTAGTQQLLFSQAGPNNMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFAWTGATKYHLNGRDSLVPNGVA  
MATHKDDEERFFPSSGVL MFGKQGAGKDNVDYSSVMLTSEEEIKTTNPVATEQYGVVADNLQQQNAAPIVGAVNS  
QGALPGMVWQNRDVYLQGPWAKIPHTDGNFHPSPMLGGFGLKHPPPQILIKNTVPVADPPTTFNQAKLASFITQ  
YSTGQVSVIEIEWELQKENS KRWNPEIQYTSNYYKSTNVDFAVNTEGTYSEPRPIGTRYLTRNL

(SEQ ID NO: 81)

**[0134]** In some embodiments, the rAAV virion is an AAV-PHP.B rAAV virion or a neutrotrophic variant thereof, such as, without limitation, those disclosed in Int'l Pat. Pub. Nos. WO 2015/038958 A1 and WO 2017/100671 A1. For example, the AAV capsid may comprise at least 4 contiguous amino acids from the sequence TLAVPFK (SEQ ID NO:83) or KFPVALT (SEQ ID NO:84), *e.g.*, inserted between a sequence encoding for amino acids 588 and 589 of AAV9.

**[0135]** The capsid may be an AAV-PHP.B capsid or functional variant thereof. In some embodiments, the AAV-PHP.B capsid shares at least 98%, 99%, or 100% identity to a reference AAV-PHP.B capsid, *e.g.*,

MAADGYLPDWLEDNLSEGIREWDLKPGAPQPKANQQHQDNARGLVLPGYKYLGPFGNLDKGEVNAADAAALEH  
DKAYDQQLKAGDNPYLYKNHADAQEFQERLKEDTSFGGNLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSP  
QEPDSSAGIGKSGAQPAKKRLNFGQTGDTE SVDPQPIGEPPAAPSGVGS LTMAAGGGAPVADNNEGADGVGSSS  
GNWHCDSQWLGD RVITTTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDNRFHCHFS PRDWQR  
LINNNWGFPRKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVLGSAHEGCLPPFPADVFMIP

QYGYLTLDGSGQAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENVFPFHSSYAHSQSLDRLMNPIDQYLYLSRT  
 INSGSQNQQT LKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNSEFAWPGASSWALNGRNSLMNPGPAMA  
 SHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYGGQVATNHQSAQTLAVPFKAQAQT  
 GWVQNQGILPGMVWQDRDVYLQGPWAKIPHTDGNFHPSPMLGGFGMKHPPQILIKNTPVPADPPTAFNKDKLN  
 SFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYYKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL

(SEQ ID NO: 82)

**[0136]** 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.

**[0137]** Without being bound by theory, the present inventors have determined that an AAV9 vector or an AAVrh.10 vector will confer broad CNS distribution of vector. Without being bound by theory, the present inventors have further determined that an AAV6 vector may provide some specificity to targeted endothelial cells. Other vector serotypes including but not limited to AAV8 and AAVrh.10 may be used.

**[0138]** In some embodiments, rAAV vector is not an AAV2 vector. Without being bound by theory, the present inventors have determined that, in some cases, use of an AAV2 vector results in transduction of neuronal cells in addition to or instead of endothelial cells. Without being bound by theory, the present inventors have further determined that the spread of AAV2 vector within the CNS is limited by its interaction with Heparan Sulfate Proteoglycan (HSPG) receptors.

#### PHARMACEUTICAL COMPOSITIONS AND KITS

**[0139]** 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.

**[0140]** 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 at, e.g., 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.

**[0141]** 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.

**[0142]** 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 preferred 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.

**[0143]** In another aspect, the disclosure comprises a kit comprising an rAAV virion of the disclosure and instructions for use.

**METHODS OF USE**

[0144] 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.

[0145] 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).

**METHODS OF TREATMENT**

[0146] 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).

[0147] The AAV-mediated delivery of GLUT1 protein to 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 (hypoglycorrachia). In some embodiments, the method provides treatment early in the course of disease, *e.g.*, in a newborn, infant, or juvenile.

[0148] The methods disclosed herein may provide efficient biodistribution in the brain and/or the 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.

**[0149]** 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.

**[0150]** A therapeutically effective amount of the rAAV vector, *e.g.* for intracerebroventricular (ICV) or intra-cisterna magna (ICM) injection, is a dose of rAAV ranging from about  $1 \times 10^{12}$  vg/kg to about  $5 \times 10^{12}$  vg/kg, or about  $1 \times 10^{13}$  vg/kg to about  $5 \times 10^{13}$  vg/kg, or about  $1 \times 10^{14}$  vg/kg to about  $5 \times 10^{14}$  vg/kg, or about  $1 \times 10^{15}$  vg/kg to about  $5 \times 10^{15}$  vg/kg, by brain weight. The invention also comprises compositions comprising these ranges of rAAV vector.

**[0151]** For example, in particular embodiments, a therapeutically effective amount of rAAV vector is a dose of about  $1 \times 10^{10}$  vg, about  $2 \times 10^{10}$  vg, about  $3 \times 10^{10}$  vg, about  $4 \times 10^{10}$  vg, about  $5 \times 10^{10}$  vg, about  $6 \times 10^{10}$  vg, about  $7 \times 10^{10}$  vg, about  $8 \times 10^{10}$  vg, about  $9 \times 10^{10}$  vg, about  $1 \times 10^{12}$  vg, about  $2 \times 10^{12}$  vg, about  $3 \times 10^{12}$  vg, about  $4 \times 10^{12}$  vg, about  $4 \times 10^{13}$  vg, and about  $4 \times 10^{14}$  vg. The invention also comprises compositions comprising these doses of rAAV vector.

**[0152]** In some embodiments, for example where ICV injection is performed, a therapeutically effective amount of rAAV vector is a dose in the range of  $1 \times 10^{10}$  vg/hemisphere to  $2 \times 10^{14}$  vg/hemisphere, or about  $1 \times 10^{10}$  vg/hemisphere, about  $1 \times 10^{11}$  vg/hemisphere, about  $1 \times 10^{12}$  vg/hemisphere,  $1 \times 10^{13}$  vg/hemisphere, or about  $1 \times 10^{14}$  vg/hemisphere. In some embodiments, for example where ICM injection is performed, a therapeutically effective amount of rAAV vector is a dose in the range of  $2 \times 10^{10}$  vg total to  $2 \times 10^{14}$  vg total, or about  $2 \times 10^{10}$  vg total, about  $2 \times 10^{11}$  vg total, about  $2 \times 10^{12}$  vg total, about  $2 \times 10^{13}$  vg total, or about  $2 \times 10^{14}$  vg total.

**[0153]** In some embodiments, the therapeutic composition comprises more than about  $1 \times 10^9$ ,  $1 \times 10^{10}$ , or  $1 \times 10^{11}$  genomes of the rAAV vector per volume of therapeutic composition injected. In embodiments cases, the therapeutic composition comprises more than approximately  $1 \times 10^{11}$ ,  $1 \times 10^{12}$ ,  $1 \times 10^{13}$ , or  $1 \times 10^{14}$  genomes of the rAAV vector per mL. In certain

embodiments, the therapeutic composition comprises less than about  $1 \times 10^{14}$ ,  $1 \times 10^{13}$  or  $1 \times 10^{12}$  genomes of the rAAV vector per mL.

**[0154]** 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 2<sup>nd</sup> edition (PDMS-2) and Bayley Scales of Infant Development, 3<sup>rd</sup> 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<sup>TM</sup>) and Vineland Adaptive Behavior Scales-2<sup>nd</sup> 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.

**[0155]** 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.

**[0156]** 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

**[0157]** 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.

**[0158]** 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.

**[0159]** In particular, 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 directly into the brain.

**[0160]** 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.

**[0161]** 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 be 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 AAV vector (*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

**[0162]** Recombinant AAV virions 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 *flax*-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 <sup>+/-</sup> mice). Additional animal models may include a GLUT1 DS model where the GLUT1 gene has a S324P point mutation.

[0163] 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 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**

[0164] 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**).

[0165] 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**).

[0166] **FIG. 9** Expression of transgene protein (Glut1-GFP) following transfection of human cerebral microvasculature endothelial cells (hCMEC/d3s).

[0167] **FIG. 10A.** GFP fluorescence 72 hours following transfection with constructs containing one of several endothelial cell promoters driving expression of Glut1-GFP transgene.

[0168] **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®).

[0169] **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).

[0170] **FIGs. 11A-11C.** 2-Deoxy-D-glucose (glucose) Uptake in hCMEC/d3 cells following expression of human GLUT1 (SLC2A1). Human cerebrovascular 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].

[0171] **FIG. 11A.** Glucose (2-DG) uptake was measured at 72 hours post-transfection in a first experiment.

[0172] **FIG. 11B.** Glucose (2-DG) uptake was measured at 72 hours post-transfection in a second experiment.

[0173] **FIG. 11C.** Glucose (2-DG) uptake was measured at 96 hours post-transfection.

[0174] **FIG. 12A.** shows glucose (2-DG) uptake in hCMEC/D3 cells following expression of human Glut1 (*SLC2A1*) at a 72-hour time point.

[0175] **FIG. 12B.** shows glucose (2-DG) uptake in hCMEC/D3 cells following expression of human Glut1 (*SLC2A1*) at a 96-hour time point.

[0176] **FIG. 13.** 2-Deoxy-D-glucose (glucose) Uptake Following AAV9-mediated Expression of hGLUT1 (SLC2A1) in hCMEC/D3 cells. Human cerebrovascular endothelial cells (hCMEC/d3s) were transduced with AAV9 vectors ( $3 \times 10^5$  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 AAV9-MEDIATED GLUT1 EXPRESSION USING ENDOTHELIAL PROMOTERS IN AN ANIMAL MODEL OF GLUT1 DEFICIENCY**

A series of experiments evaluating the *in vivo* effects of AAV9-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). AAV9 constructs 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 (hFLT-1, mTie, hGlut1). The extent to which endothelial cell promoter-mediated GLUT1 transgene expression following delivery using an AAV9 vector can prevent or mitigate the functional and pathological deficits in this mouse

model will be evaluated. Potential beneficial effects of AAV9-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).