

CSRP3 (CYSTEINE AND GLYCINE RICH PROTEIN 3) GENE THERAPY**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims priority to U.S. Application No. 63/061,727, filed on August 5, 2020, 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_020_01WO_ST25.txt. The text file is about 120 KB, created on August 3, 2021, and is being submitted electronically via EFS-Web.

BACKGROUND

[0003] Cysteine and glycine rich protein 3 (*CSRP3*) encodes Muscle LIM Protein (MLP). Genetic defects in *CSRP3* are associated with autosomal dominant cardiomyopathy, both hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM), as Autosomal dominant mutations in different domains of the protein are linked with different phenotypes. Loss-of-function mutations that decrease MLP levels can cause protein mislocalization and proteasome-mediated degradation, resulting in disruption of normal signaling pathways in cardiac and skeletal muscle. Changes in MLP levels or intracellular localization are also associated with skeletal myopathies, including facioscapulohumeral muscular dystrophy, nemaline myopathy, and limb girdle muscular dystrophy type 2B. Changes in levels of the isoform MLP-b protein or dysregulation of the MLP:MLP-b ratio have been detected in limb girdle muscular dystrophy type 2A, Duchenne muscular dystrophy, and dermatomyositis patients.

[0004] *CSRP3* patients exhibit variable symptoms depending on the specific mutation, but general symptoms include obstructive HCM or DCM, ventricular hypertrophy (with interventricular septum in the range of 14-32mm), ventricular tachycardia, exercise intolerance, angina. Mild NYHA (New York Heart Association) scores of I-II are common. Sudden cardiac death has been observed, for example in a family carrying the C58G

mutation. In one study, the majority of C58G carriers who provided muscle biopsies complained of exertional myalgias and cramps at presentation.

[0005] There is an unmet need for therapy for *CSRP3*-related diseases or disorders. The gene therapies provided herein address this need.

SUMMARY

[0006] The present invention relates generally to gene therapy for a disease or disorder, *e.g.*, a cardiac disease or disorder, using a vector expressing MLP or a functional variant thereof.

[0007] In one aspect, the disclosure provides polynucleotide, comprising an expression cassette and optionally flanking adeno-associated virus (AAV) inverted terminal repeats (ITRs), wherein the polynucleotide comprises a polynucleotide sequence encoding Muscle LIM Protein (MLP) or a functional variant thereof, operatively linked to a promoter.

[0008] In some embodiments, the promoter is a cardiac-specific promoter.

[0009] In some embodiments, the promoter is a muscle-specific promoter.

[0010] In some embodiments, the promoter is a cardiomyocyte-specific promoter.

[0011] In some embodiments, the promoter is a MHCK7 promoter.

[0012] In some embodiments, the MHCK7 promoter shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 31.

[0013] In some embodiments, the promoter is a cardiac troponin T (hTNNT2) promoter.

[0014] In some embodiments, the hTNNT2 promoter shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 32.

[0015] In some embodiments, the expression cassette comprises exon 1 of the cardiac troponin T (hTNNT2) gene, wherein optionally the hTNNT2 promoter and exon 1 together share at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 32.

[0016] In some embodiments, the promoter is a ubiquitous promoter, optionally a CMV promoter or a CAG promoter.

[0017] In some embodiments, the expression cassette comprises a polyA signal.

[0018] In some embodiments, the polyA signal is a human growth hormone (hGH) polyA.

[0019] In some embodiments, the expression cassette comprises a Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element (WPRE), optionally a WPRE(x).

[0020] In some embodiments, the Muscle LIM Protein (MLP) or a functional variant thereof is an MLP.

[0021] In some embodiments, the MLP is a human MLP.

[0022] In some embodiments, the MLP is MLP isoform A.

[0023] In some embodiments, the MLP shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 1.

[0024] In some embodiments, the MLP is MLP isoform B.

[0025] In some embodiments, the MLP shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 2.

[0026] In some embodiments, the MLP shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 3.

[0027] In some embodiments, the MLP 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 MLP is a Cysteine And Glycine Rich Protein 3 (*CSRP3*) polynucleotide.

[0029] In some embodiments, the *CSRP3* polynucleotide is a human *CSRP3* polynucleotide.

[0030] In some embodiments, the polynucleotide sequence encoding MLP 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 polynucleotide sequence encoding MLP shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 7.

[0032] In some embodiments, the polynucleotide comprises at least about 2.4 kb, at most about 2.6 kb, or between about 2.4 kb and about 2.6 kb.

[0033] In some embodiments, the polynucleotide comprises at least about 3.0 kb, at most about 3.3 kb, or between about 3.0 kb and about 3.3 kb.

[0034] In some embodiments, the polynucleotide comprises at least about 2.4 kb, least about 2.6 kb, least about 3.0 kb, at least about 3.3 kb, at least about 3.5 kb, at least about 3.7 kb, at least about 3.9 kb, at least about 4.1 kb., or at least about 4.3 kb.

[0035] In some embodiments, the polynucleotide comprises least about 2.6 kb, least about 3.0 kb, at most about 3.3 kb, at most about 3.5 kb, at most about 3.7 kb, at most about 3.9 kb, at most about 4.1 kb., at most about 4.3 kb, or at most about 4.5 kb.

[0036] 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: 8-11.

[0037] In some embodiments, the polynucleotide shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with any one of SEQ ID NOs: 12-15.

[0038] In some embodiments, the expression cassette is flanked by 5' and 3' inverted terminal repeats (ITRs), optionally AAV2 ITRs, optionally ITRs that shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with any one of SEQ ID NO: 20-26.

[0039] In some embodiments, the polynucleotide is self-complementary.

[0040] In some embodiments, the polynucleotide comprises the expression cassette and a reverse complement of the expression cassette.

[0041] In some embodiments, the expression cassette and the reverse complement of the expression cassette are flanked by 5' and 3' inverted terminal repeats (ITRs), optionally

AAV2 ITRs, optionally an ITR that shares at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identity with SEQ ID NO: 23 or SEQ ID NO: 26.

[0042] In another aspect, the disclosure provides a gene therapy vector, comprising a polynucleotide of the disclosure.

[0043] In some embodiments, the gene therapy vector is a recombinant adeno-associated virus (rAAV) vector.

[0044] In some embodiments, the rAAV vector is an AAV9 or a functional variant thereof.

[0045] 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 NO: 77.

[0046] In some embodiments, the rAAV vector is an AAVrh10 or a functional variant thereof.

[0047] 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 NO: 79.

[0048] In some embodiments, the rAAV vector is an AAV6 or a functional variant thereof.

[0049] 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 NO: 78.

[0050] In some embodiments, the rAAV vector is an AAVrh74 or a functional variant thereof.

[0051] 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 NO: 80.

[0052] In some embodiments, the rAAV vector is a self-complementary AAV vector.

[0053] 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 a vector of the disclosure to the subject.

[0054] In some embodiments, the disease or disorder is a cardiac disorder.

[0055] In some embodiments, the disease or disorder is heart failure.

[0056] In some embodiments, the disease or disorder is hypertrophic cardiomyopathy.

[0057] In some embodiments, the disease or disorder is dilated cardiomyopathy.

[0058] In some embodiments, the subject is a mammal.

[0059] In some embodiments, the subject is a primate.

[0060] In some embodiments, the subject is a human.

[0061] In some embodiments, the subject has a mutation in the *CSRP3* gene that causes an amino acid substitution selected from C58G, L44P, S54R, E55G, and/or K69R, relative to a human *CSRP3* encoding a human MLP having the sequence of SEQ ID NO: 1.

[0062] In some embodiments, the vector is administered by intravenous injection, intracardiac injection, intracardiac infusion, and/or cardiac catheterization.

[0063] In some embodiments, the administration increases MLP expression by at least about 5%.

[0064] In some embodiments, the administration increases MLP expression by at least about 30%.

[0065] In some embodiments, the administration increases MLP expression by at least about 70%.

[0066] In some embodiments, the administration increases MLP expression by about 5% to about 10%.

[0067] In some embodiments, the administration increases MLP expression by about 30% to about 50%.

[0068] In some embodiments, the administration increases MLP expression by about 70% to about 100%.

[0069] In some embodiments, the method treats and/or prevents the disease or disorder.

[0070] In another aspect, the disclosure provides a pharmaceutical composition comprising a vector of the disclosure.

[0071] In another aspect, the disclosure provides a kit comprising a vector or pharmaceutical composition of the disclosure, and optionally instructions for use.

[0072] In another aspect, the disclosure provides a use of a composition of the disclosure in treating a disease or disorder, optionally according to any of the methods disclosed herein.

[0073] In another aspect, the disclosure provides a composition of the disclosure for use in treating a disease or disorder, optionally according to any of the methods disclosed herein.

[0074] In another aspect, the disclosure provides a method of expressing Muscle LIM Protein (MLP) or a functional variant thereof, comprising contacting a cell with a vector of the disclosure.

[0075] In some embodiments, the cell is a cardiomyocyte.

[0076] In some embodiments, the cardiomyocyte is a human cardiomyocyte.

[0077] In some embodiments, the promoter is an MHCK7 promoter and wherein the expression level of the MLP is at least 2-fold greater than the expression level of MLP in a cell transduced with a vector having an hTNNT2 promoter.

[0078] In some embodiments, the promoter is an MHCK7 promoter and wherein the expression level of the MLP is between 2-fold greater and 10-fold greater than the expression level of MLP in a cell transduced with a vector having an hTNNT2 promoter.

[0079] 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

[0080] **FIG. 1** 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: 12. The capitalized portion is the expression cassette (SEQ ID NO: 8).

[0081] **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: 13. The capitalized portion is the expression cassette (SEQ ID NO: 9).

[0082] **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: 14. The capitalized portion is the expression cassette (SEQ ID NO: 10).

[0083] **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: 15. The capitalized portion is the expression cassette (SEQ ID NO: 11).

[0084] **FIG. 5A** shows CSRP3 expression in transduced CHO-Lec2.

[0085] **FIG. 5B** shows CSRP3 expression in transduced cardiomyocytes (differentiated AC16 cell line – Sigma-Aldrich® cat# SCC109). The cells were transduced with 3E5 MOI from each vector; after 6 days the cells lysates were collected, and a Western Blot performed using an anti-CSRP3 Polyclonal antibody (Thermo-Fisher® PA5-29155 1:1000).

DETAILED DESCRIPTION OF THE INVENTION

[0086] The present disclosure provided gene therapy vectors for *CSRP3* that delivery a polynucleotide encoding MLP, along with method of use, and other compositions and methods. Treatment of CSRP3-related disorder is complicated by autosomal dominant nature of most forms of CSRP3-related disorders and evidence suggesting that the level of protein expression and balance between MLP isoforms is crucial to normal function in healthy subjects. Moreover, successful gene therapy in the heart is unpredictable. Cardiomyocytes are a particularly challenging cell type to target with gene therapy. The compositions and methods disclosed herein address this problem.

DEFINITIONS

[0087] 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.

[0088] 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.

[0089] 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.

[0090] 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.

[0091] 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.

[0092] 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.

[0093] 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.

[0094] As used herein, “promoter” refers to a polynucleotide sequence capable of promoting initiation of RNA transcription from a polynucleotide in a eukaryotic cell.

[0095] 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 operable linked to an element that drives expression (*e.g.*, a promoter).

[0096] 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 heart. A subject may have a mutation in an *CSRP3* gene or deletion of all or a part of *CSRP3* gene, or of gene regulatory sequences, that causes aberrant expression of the MLP protein. “Subject” and “patient” are used interchangeably herein. The subject treated by the methods described herein may be an adult or a child. Subjects may range in age.

[0097] 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.

[0098] 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 *CSRP3* gene that decreases expression or results in loss or aberrant function of the MLP protein in at least some cells (*e.g.*, cardiac cells) of the subject.

[0099] 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 *CSRP3*-related disease or disorder, *e.g.*, hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), or skeletal myopathy.

[0100]

MLP PROTEIN OR POLYNUCLEOTIDE

[0101] The present disclosure contemplates compositions and methods of use related to Muscle LIM Protein (MLP) protein. Various mutations in *CSRP3* are known to be associated with hypertrophic cardiomyopathy (HCM) or dilated cardiomyopathy (DCM). Both inherited

and *de novo* mutations have been observed. In some cases, a heterozygous missense mutation is sufficient to cause disease.

[0102] The polypeptide sequence of MLP is as follows:

MPNWGGGAKCGACEKTVYHAEIQCNGRSFHKTCFHCMACRKALDSTTVAAHESE
IYCKVCYGRRYGPKGIGYGQGAGCLSTDTGEHLGLQFQQSPKPARSVTTSNPSKFTA
KFGESSEKPCRCGKSVYAAEKVMGGGKPPWHKTCFRCAICGKSLESTNVTDKDGELYC
KVCIYAKNFGPTGIGFGGLTQQVEKKE

[0103] (SEQ ID NO: 1).

[0104] A second isoform of MLP has the following polypeptide sequence:

MPNWGGGAKCGACEKTVYHAEIQCNGRSFHKTCFHCSQRSRHAQLPPATLPNSLR
SLESPRSALDVASQSMLLRRLWEVASLGTRPVSAPSVGRVWSPQMSLTGMGNFIA
KFAMPKILAPRVLGLEALHNKWKRKNEEVRRFSDFLRA

[0105] (SEQ ID NO: 2).

[0106] Another isoform of MLP has the following polypeptide sequence:

MPNWGGGAKCGACEKTVYHAEIQCNGRSFHKTCFHCLC

[0107] (SEQ ID NO: 3).

[0108] Another isoform of MLP has the following polypeptide sequence:

MPNWGGGAKCGACEKTVYHAEIQCNGRSFHKTCFHCTLAQDLFPLCHLWEESGV
HKC

[0109] (SEQ ID NO: 4).

[0110] In some embodiments, the MLP protein comprises a polypeptide 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: 1-4.

[0111] 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 MLP 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 MLP, operatively linked to a promoter. The polynucleotide encoding the MLP may comprise a polynucleotide sequence at least 75%, 80%, 85%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to:

ATGCCAACTGGGGCGGAGGCGCAAAATGTGGAGCCTGTGAAAAGACCGTCTAC
CATGCAGAAGAAATCCAGTGCAATGGAAGGAGTTTCCACAAGACGTGTTTCCAC
TGCATGGCCTGCAGGAAGGCTCTTGACAGCACGACAGTCGCGGCTCATGAGTCG
GAGATCTACTGCAAGGTGTGCTATGGGCGCAGATATGGCCCCAAAGGGATCGGG
TATGGACAAGGCGCTGGCTGTCTCAGCACAGACACGGGCGAGCATCTCGGCCTG
CAGTTCCAACAGTCCCCAAAGCCGGCACGCTCAGTTACCACCAGCAACCCTTCCA
AATTCAGTGCAGAGTTTGGAGAGTCCGAGAAGTGCCCTCGATGTGGCAAGTCAG
TCTATGCTGCTGAGAAGGTTATGGGAGGTGGCAAGCCTTGGCACAAGACCTGTTT
CCGCTGTGCCATCTGTGGGAAGAGTCTGGAGTCCACAAATGTCACTGACAAAGA
TGGGGAAGTTTATTGCAAAGTTTGCTATGCCAAAAATTTTGGCCCCACGGGTATT
GGGTTTGGAGGCCTTACACAACAAGTGGAAGAAAGAA

(SEQ ID NO: 5).

[0112] Optionally, the polynucleotide sequence encoding the vector genome may comprise a Kozak sequence, including but not limited to GCCACCATGG (SEQ ID NO: 6). Kozak sequence may overlap the polynucleotide sequence encoding an MLP 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:

gccaccATGCCAACTGGGGCGGAGGCGCAAAATGTGGAGCCTGTGAAAAGACCGT
CTACCATGCAGAAGAAATCCAGTGCAATGGAAGGAGTTTCCACAAGACGTGTTT
CCACTGCATGGCCTGCAGGAAGGCTCTTGACAGCACGACAGTCGCGGCTCATGA
GTCGGAGATCTACTGCAAGGTGTGCTATGGGCGCAGATATGGCCCCAAAGGGAT
CGGGTATGGACAAGGCGCTGGCTGTCTCAGCACAGACACGGGCGAGCATCTCGG
CCTGCAGTTCCAACAGTCCCCAAAGCCGGCACGCTCAGTTACCACCAGCAACCCT
TCCAAATTCAGTGCAGAGTTTGGAGAGTCCGAGAAGTGCCCTCGATGTGGCAAG

TCAGTCTATGCTGCTGAGAAGGTTATGGGAGGTGGCAAGCCTTGGCACAAGACC
 TGTTTCCGCTGTGCCATCTGTGGGAAGAGTCTGGAGTCCACAAATGTCACTGACA
 AAGATGGGGAACTTTATTGCAAAGTTTGCTATGCCAAAAATTTTGGCCCCACGGG
 TATTGGGTTTGGAGGCCTTACACAACAAGTGGAAAAGAAAGAA

(SEQ ID NO: 7).

[0113] In some embodiments, the Kozak sequence is an alternative Kozak sequence comprising or consisting of any one of:

(gcc)gccRccAUGG (SEQ ID NO: 16);

(gcc)gccRccAUGC (SEQ ID NO: 17);

AGNNAUGN;

ANNAUGG;

ANNAUGC;

ACCAUGG;

ACCAUGC;

GACACCAUGG (SEQ ID NO: 18); and

GACACCAUGC (SEQ ID NO: 19).

[0114] In some embodiments, the vector genome comprises no Kozak sequence.

VECTOR GENOME

[0115] 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).

[0116] 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:

CCTGCAGGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTC
GCCCCGCCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTTCCT

(SEQ ID NO: 20)

[0117] 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:

GCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCCTCAGT
GAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTTCCTTGTAGTTAATGATTAACCCGCC
ATGCTACTTATCTACGTA

(SEQ ID NO: 21)

[0118] 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:

CTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTTGGTCGCCCGGCCCTCA
GTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTTCCTTGTAGTTAATGATTAACCCG
CCATGCTACTTATCTACGTA

(SEQ ID NO: 22)

[0119] 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:

TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCGGGCGTCGGGCGACCTTT
GGTCGCCCGGCCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTTCCT

(SEQ ID NO: 23)

[0120] 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
TCGCCCCAGCGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGCTGCCTGCAGG

(SEQ ID NO: 24)

[0121] 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:

TACGTAGATAAGTAGCATGGCGGGTTAATCATTAAC TACAAGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCT
CTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCACGCCCGGGCTTTGCCCGGGCGGCCTCA
GTGAGCGAGCGAGCGCGC

(SEQ ID NO: 25)

[0122] 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:

AGGAACCCCTAGTGATGGAGACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCC
GACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGT

(SEQ ID NO: 26)

[0123] 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:

GCGGCAATTCAGTCGATAACTATAACGGTCCTAAGGTAGCGATTAAATACGCG
CTCTCTTAAGGTAGCCCCGGGACGCGTCAATTGACTACAAACCGAGTATCTGCAG
AGGGCCCTGCGTATG (SEQ ID NO: 27);

CTTCTGAGGCGGAAAGAACCAGATCCTCTCTTAAGGTAGCATCGAGATTAAATT
AGGGATAACAGGGTAATGGCGCGGGCCGC (SEQ ID NO: 28); or

GTTACCCAGGCTGGAGTGCAGTGGCACATTTCTGCTCACTGCAACCTCCTCCTCC
CTGGGTTC (SEQ ID NO: 29).

Promoters

[0124] In some embodiments, the polynucleotide sequence encoding an MLP protein or functional variant thereof is operably linked to a promoter.

[0125] 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:

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ACTTACGGTAAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCATTTGACGTCAATAATGACGTATGTTCC
CATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGT
ACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCTGGCATTATGC
CCAGTACATGACCTTATGGGACTTTCTTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCTGA
GGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCCTCCCCACCCCAATTTTGTTATTTATTTATTTT
TTAATTATTTTGTGTCAGCGATGGGGGCGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCG
GGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCTTTTATGGCGA
GGCGGCGGGGCGGGGCGGCCCTATAAAAAGCGAAGCGCGCGGGCGGGCGG
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(SEQ ID NO: 30)

[0126] In some embodiments, a polynucleotide sequence encoding an MLP 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.

[0127] In some cases, the promoter is a tissue-specific promoter, such as a promoter capable of driving expression in a cardiac cell to a greater extent than in a non-cardiac cell. In some embodiments, tissue-specific promoter is a selected from any various cardiac cell-specific promoters including but not limited to, desmin (Des), alpha-myosin heavy chain (α -MHC), myosin light chain 2 (MLC-2), cardiac troponin C (cTnC), cardiac troponin T (hTNNT2), muscle creatine kinase (CK) and combinations of promoter/enhancer regions

thereof, such as MHCK7. 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.

[0128] 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 31-51.

Table 3

PROMOTER	SEQUENCE	SEQ ID NO:
MHCK7	ACCCTTCAGATTAAAAATAACTGAGGTAAGGGCCTGGGTAG GGGAGGTGGTGTGAGACGCTCCTGTCTCTCCTCTATCTGCCC ATCGGCCCTTTGGGGAGGAGGAATGTGCCCAAGGACTAAAA AAAGGCCATGGAGCCAGAGGGGCGAGGGCAACAGACCTTTC ATGGGCAAACCTTGGGGCCCTGCTGTCTAGCATGCCCCACTA CGGGTCTAGGCTGCCCATGTAAGGAGGCAAGGCCTGGGGAC ACCCGAGATGCCTGGTTATAATTAACCCAGACATGTGGCTGC CCCCCCCCCCAACACCTGCTGCCTCTAAAAATAACCCTGT CCCTGGTGGATCCCCTGCATGCGAAGATCTTCGAACAAGGCT GTGGGGGACTGAGGGCAGGCTGTAACAGGCTTGGGGGCCAG GGCTTATACGTGCCTGGGACTCCCAAAGTATTACTGTTCCAT GTTCCCGGCGAAGGGCCAGCTGTCCCCGCCAGCTAGACTCA GCACTTAGTTTATAGGAACCAAGTGAGCAAGTCAGCCCTTGGGGC AGCCCATACAAGGCCATGGGGCTGGGCAAGCTGCACGCCTG GGTCGGGGTGGGCACGGTGCCCGGGCAACGAGCTGAAAGC TCATCTGCTCTCAGGGGCCCCCTCCCTGGGGACAGCCCCTCCT GGCTAGTCACACCCTGTAGGCTCCTCTATATAACCCAGGGGC ACAGGGGCTGCCCTCATTCTACCACCACCTCCACAGCACAGA CAGACACTCAGGAGCCAGCCAG	31
Human cardiac troponin T	CTCAGTCCATTAGGAGCCAGTAGCCTGGAAGATGTCTTTACC CCCAGCATCAGTTCAAGTGAGCAGCACATAACTCTTGCCCT	33

PROMOTER	SEQUENCE	SEQ ID NO:
promoter (without exon 1) hTnnT2 / HTNNT2	CTGCCTTCCAAGATTCTGGTGCTGAGACTTATGGAGTGTCTT GGAGGTTGCCTTCTGCCCCCAACCCTGCTCCCAGCTGGCCC TCCCAGGCCTGGGTTGCTGGCCTCTGCTTTATCAGGATTCTCA AGAGGGACAGCTGGTTTATGTTGCATGACTGTTCCCTGCATA TCTGCTCTGGTTTTAAATAGCTTATCTGAGCAGCTGGAGGAC CACATGGGCTTATATGGCGTGGGGTACATGTTCCCTGTAGCCT TGTCCCTGGCACCTGCCAAAATAGCAGCCAACACCCCCCACC CCCACCGCCATCCCCCTGCCCCACCCGTCCCCTGTGCGACAT TCCTCCCTCCGCAGGGCTGGCTCACCAGGCCCCAGCCCACAT GCCTGCTTAAAGCCCTCTCCATCCTCTGCCTCACCCAGT	
Human cardiac troponin T promoter (with exon 1, underlined) hTnnT2 / HTNNT2	CTCAGTCCATTAGGAGCCAGTAGCCTGGAAGATGTCTTTACC CCCAGCATCAGTTCAAGTGGAGCAGCACATAACTCTTGCCCT CTGCCTTCCAAGATTCTGGTGCTGAGACTTATGGAGTGTCTT GGAGGTTGCCTTCTGCCCCCAACCCTGCTCCCAGCTGGCCC TCCCAGGCCTGGGTTGCTGGCCTCTGCTTTATCAGGATTCTCA AGAGGGACAGCTGGTTTATGTTGCATGACTGTTCCCTGCATA TCTGCTCTGGTTTTAAATAGCTTATCTGAGCAGCTGGAGGAC CACATGGGCTTATATGGCGTGGGGTACATGTTCCCTGTAGCCT TGTCCCTGGCACCTGCCAAAATAGCAGCCAACACCCCCCACC CCCACCGCCATCCCCCTGCCCCACCCGTCCCCTGTGCGACAT TCCTCCCTCCGCAGGGCTGGCTCACCAGGCCCCAGCCCACAT GCCTGCTTAAAGCCCTCTCCATCCTCTGCCTCACCCAGT <u>CCCC</u> <u>GCTGAGACTGAGCAGACGCCTCCAGGATCTGTGCGCAG</u>	32
Mouse α - cardiac myosin heavy chain promoter (α MHC)	GGTACCGGATCCTGCAAGGTCACACAAGGGTCTCCACCCACC AGGTGCCCTAGTCTCAATTTTCAGTTTCCATGCCTTGTTCTCAC AATGCTGGCCTCCCCAGAGCTAATTTGGACTTTGTTTTTATTT CAAAAGGGCCTGAATGAGGAGTAGATCTTGTGCTACCCAGC TCTAAGGGTGCCCGTGAAGCCCTCAGACCTGGAGCCTTTGCA ACAGCCCTTTAGGTGGAAGCAGAATAAAGCAATTTTCCTTAA AGCCAAAATCCTGCCTCTAGACTCTTCTTCTCTGACCTCGGTC CCTGGGCTCTAGGGTGGGGAGGTGGGGCTTGGAAGAAGAAG GTGGGGAAGTGGCAAAGCCGATCCCTAGGGCCCTGTGAAG	34

PROMOTER	SEQUENCE	SEQ ID NO:
	TTCGGAGCCTTCCCTGTACAGCACTGGCTCATAGATCCTCCT CCAGCCAAACATAGCAAGAAGTGATACCTCCTTTGTGACTTC CCCAGGCCCAGTACCTGTGAGGTTGAAACAGGATTTAGAGA AGCCTCTGAACTCACCTGAACTCTGAAGCTCATCCACCAAGC AAGCACCTAGGTGCCACTGCTAGTTAGTATCCTACGCTGATA ATATGCAGAGCTGGGCCACAGAAGTCCTGGGGTGTAGGAAC TGACCAGTGACTTTTCAGTCGGCAAAGGTATGACCCCTCAG CAGATGTAGTAATGTCCCCTTAGATCCCATCCCAGGCAGGTC TCTAAGAGGACATGGGATGAGAGATGTAGTCATGTGGCATT CCAAACACAGCTATCCACAGTGTCCTTGCCCCCTCCACTTA GCCAGGAGGACAGTAACCTTAGCCTATCTTTCTTCCTCCCCA TCCTCCCAGGACACACCCCTGGTCTGCAGTATTCATTTCTTC CTTCACGTCCCCTCTGTGACTTCCATTTGCAAGGCTTTTGACC TCTGCAGCTGCTGGAAGATAGAGTTTGCCCTAGGTGTGGCA AGCCATCTCAAGAGAAAGCAGACAACAGGGGGACCAGATTT TGGAAGGATCAGGAACTAAATCACTGGCGGGCCTGGGGGTA GAAAAAAGAGTGAGTGAGTCCGCTCCAGCTAAGCCAAGCTA GTCCCCGAGATACTCTGCCACAGCTGGGCTGCTCGGGGTAGC TTTAGGAATGTGGGTCTGAAAGACAATGGGATTGGAAGACA TCTCTTTGAGTCTCCCCTCAACCCACCTACAGACACACTCGT GTGTGGCCAGACTCCTGTTCAACAGCCCTCTGTGTTCTGACC ACTGAGCTAGGCAACCAGAGCATGGGCCCTGTGCTGAGGAT GAAGAGTTGGTTACCAATAGCAAAAACAGCAGGGGAGGGAG AACAGAGAACGAAATAAGGAAGGAAGAAGGAAAGGCCAGT CAATCAGATGCAGTCAGAAGAGATGGGAAGCCAACACACAG CTTGAGCAGAGGAAACAGAAAAGGGAGAGATTCTGGGCATA AGGAGGCCACAGAAAGAAGAGCCCAGGCCCCCAAGTCTCC TCTTTATACCCTCATCCCGTCTCCCAATTAAGCCCACTCTTCT TCCTAGATCAGACCTGAGCTGCAGCGAAGAGACCCGTAGGG AGGATCACACTGGATGAAGGAGATGTGTGGAGAAGTCCAGG GAACCTAAGAGCCAGAGCCTAAAAGAGCAAGAGATAAAGGT GCTTCAAAGGTGGCCAGGCTGTGCACACAGAGGGTCGAGGA CTGGTGGTAGAGCCTCAAGATAAGGATGATGCTCAGAATGG GCGGGGGGGGGGATTCTGGGGGGGGGAGAGAGAAGGTGAG	

PROMOTER	SEQUENCE	SEQ ID NO:
	AAGGAGCCTGGAACAGAGAATCTGGAAGCGCTGGAAACGAT ACCATAAAGGGAAGAACCCAGGCTACCTTTAGATGTAAATC ATGAAAGACAGGGAGAAGGGAAGCTGGAGAGAGTAGAAGG ACCCCGGGGCAAGACATTGAAGCAAGGACAAGCCAGGTTGA GCGCTCCGTGAAATCAGCCTGCTGAAGGCAGAGCCCTGGTAT GAGCACCAGAACAGCAGAGGCTAGGGTTAATGTCTGAGACAG GGAACAGAAGGTAGACACAGGAACAGACAGAGACGGGGGA GCCAGGTAACAAAGGAATGGTCCTTCTCACCTGTGGCCAGA GCGTCCATCTGTGTCCACATACTCTAGAATGTTTCATCAGACT GCAGGGCTGGCTTGGGAGGCAGCTGGAAAGAGTATGTGAGA GCCAGGGGAGACAAGGGGGCCTAGGAAAGGAAGAAGAGGG CAAACCAGGCCACACAAGAGGGCAGAGCCCAGAACTGAGTT AACTCCTTCCTTGTTCATCTTCCATAGGAGGCAGTGGGAAC TCTGTGACCACCATCCCCCATGAGCCCCCACTACCCATACCA AGTTTGGCCTGAGTGGCATTCTAGGTTCCCTGAGGACAGAGC CTGGCCTTTGTCTCTTGGACCTGACCCAAGCTGACCCAATGT TCTCAGTACCTTATCATGCCCTCAAGAGCTTGAGAACCAGGC AGTGACATATTAGGCCATGGGCTAACCCTGGAGCTTGACACAC AGGAGCCTCAAGTGACCTCCAGGGACACAGCTGCAGACAGG TGGCCTTTATCCCCAAAGAGCAACCATTTGGCATAGGTGGCT GCAAATGGGAATGCAAGGTTGAATCAGGTCCCTTCAAGAAT ACTGCATGCAAGACCTAAGACCCCTGGAGAGAGGGGTATGC TCCTGCCCCCACCACCATAAGGGGAGTGAACCTATCCTAGGG GGCTGGCGACCTTGGGGAGACACCACATTACTGAGAGTGCT GAGCCCAGAAAACTGACCGCCCTGTGTCCTGCCACCTCCA CACTCTAGAGCTATATTGAGAGGTGACAGTAGATAGGGTGG GAGCTGGTAGCAGGGAGAGTGTTCTGGGTGTGAGGGTGTA GGGGAAAGCCAGAGCAGGGGAGTCTGGCTTTGTCTCCTGAA CACAATGTCTACTTAGTTATAACAGGCATGACCTGCTAAAGA CCCAACATCTACGACCTCTGAAAAGACAGCAGCCCTGGAGG ACAGGGGTGTCTCTGAGCCTTGGGTGCTTGATGGTGCCACA AAGGAGGGCATGAGTGTGAGTATAAGGCCCCAGGAGCGTTA GAGAAGGGCACTTGGGAAGGGGTCAGTCTGCAGAGCCCCTA TCCATGGAATCTGGAGCCTGGGGCCAACCTGGTGTAATCTCT	

PROMOTER	SEQUENCE	SEQ ID NO:
	GGGCCTGCCAGGCATTCAAAGCAGCACCTGCATCCTCTGGCA GCCTGGGGAGGCGGAAGGGAGCAACCCCCCACTTATACCCT TTCTCCCTCAGCCCCAGGATTAACACCTCTGGCCTTCCCCCTT CCCACCTCCCATCAGGAGTGGAGGGTTGCAGAGGGAGGGTA AAAACCTACATGTCCAAACATCATGGTGCACGATATATGGAT CAGTATGTGTAGAGGCAAGAAAGGAAATCTGCAGGCTTAAC TGGGTTAATGTGTAAAGTCTGTGTGCATGTGTGTGTGTCTGA CTGAAAACGGGCATGGCTGTGCAGCTGTTCAGTTCTGTGCGT GAGGTTACCAGACTGCAGGTTTGTGTGTAAATTGCCCAAGGC AAAGTGGGTGAATCCCTTCCATGGTTTAAAGAGATTGGATGA TGGCCTGCATCTCAAGGACCATGGAAAATAGAATGGACACT CTATATGTGTCTCTAAGCTAAGGTAGCAAGGTCCTTGGAGGA CACCTGTCTAGAGATGTGGGCAACAGAGACTACAGACAGTA TCTGTACAGAGTAAGGAGAGAGAGGAGGGGGTGTAGAATTC TCTTACTATCAAAGGGAACTGAGTCGTGCACCTGCAAAGTG GATGCTCTCCCTAGACATCATGACTTTGTCTCTGGGGAGCCA GCACTGTGGAACCTTCAGGTCTGAGAGAGTAGGAGGCTCCCCCT CAGCCTGAAGCTATGCAGATAGCCAGGGTTGAAAGGGGGAA GGGAGAGCCTGGGATGGGAGCTTGTGTGTTGGAGGCAGGGG ACAGATATTAAGCCTGGAAGAGAAGGTGACCCCTACCCAGT TGTTCAACTCACCTTCAGATTAATAAATAACTGAGGTAAGGG CCTGGGTAGGGGAGGTGGTGTGAGACGCTCCTGTCTCTCCTC TATCTGCCCATCGGCCCTTTGGGGAGGAGGAATGTGCCAAG GACTAAAAAAGGCCATGGAGCCAGAGGGGCGAGGGCAAC AGACCTTTCATGGGCAAACCTTGGGGCCCTGCTGTCCTCCTG TCACCTCCAGAGCCAAGGGATCAAAGGAGGAGGAGCCAGGA CAGGAGGGAAGTGGGAGGGAGGGTCCCAGCAGAGGACTCC AAATTTAGGCAGCAGGCATATGGGATGGGATATAAAGGGGC TGGAGCACTGAGAGCTGTCAGAGATTTCTCCAACCCAGGTAA GAGGGAGTTTCGGGTGGGGGCTCTTACCCACACCAGACCTC TCCCCACCTAGAAGGAACTGCCTTTCCTGGAAGTGGGGTTC AGGCCGGTCAGAGATCTGACAGGGTGGCCTTCCACCAGCCT GGGAAGTTCTCAGTGGCAGGAGGTTTCCACAAGAAACACTG GATGCCCCCTTCCCTTACGCTGTCTTCTCCATCTTCCTCCTGGG	

PROMOTER	SEQUENCE	SEQ ID NO:
	GATGCTCCTCCCCGTCTTGGTTTATCTTGGCTCTTCGTCTTCA GCAAGATTTGCCCTGTGCTGTCCACTCCATCTTTCTCTACTGT CTCCGTGCCTTGCCCTTGCCCTTCTTGCGTGTCTTCCTTTCCAC CCATTTCTCACTTCACCTTTTTCTCCCCCTTCTCATTTGTATTCAT CCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTC CTTTCTCCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTCCTTC TTCTGTGTGTCAGAGTGCTGAGAATCACACCTGGGGTTCCCAC CCTTATGTAAACAATCTTCCAGTGAGCCACAGCTTCAGTGCT GCTGGGTGCTCTCTTACCTTCCTCACCCCTGGCTTGTCTGT TCCATCCTGGTCAGGATCTCTAGATTGGTCTCCCAGCCTCTGC TACTCCTCTTCCTGCCTGTTCTCTCTCTGTCCAGCTGCGCCA CTGTGGTGCCTCGTTCCAGCTGTGGTCCACATTCTTCAGGATT CTCTGAAAAGTTAACCAGGTGAGAATGTTTCCCCTGTAGACA GCAGATCACGATTCTCCCGGAAGTCAGGCTTCCAGCCCTCTC TTTCTCTGCCCAGCTGCCCCGGCACTCTTAGCAAACCTCAGGC ACCCTTACCCACATAGACCTCTGACAGAGAAGCAGGCACTT TACATGGAGTCCTGGTGGGAGAGCCATAGGCTACGGTGTA AAGAGGCAGGGAAGTGGTGGTGTAGGAAAGTCAGGACTTCA CATAGAAGCCTAGCCCACACCAGAAATGACAGACAGATCCC TCCTATCTCCCCCATAAGAGTTTGAGTCGACCCGCGGCCCCG AATTG	
Chicken cardiac troponin T promoter (cTnT)	GGGATAAAAGCAGTCTGGGCTTTCACATGACAGCATCTGGG GCTGCGGCAGAGGGTTCGGTCCGAAGCGCTGCCTTATCAGC GTCCCCAGCCCTGGGAGGTGACAGCTGGCTGGCTTGTGTCAG CCCCTCGGGCACTCACGTATCTCCGTCCGACGGGTTTAAAAT AGCAAACTCTGAGGCCACACAATAGCTTGGGCTTATATGG GCTCCTGTGGGGGAAGGGGAGCACGGAGGGGGCCGGGGCC GCTGCTGCCAAAATAGCAGCTCACAAGTGTTCATTCTCTC TGGGCGCCGGGCACATTCTGTGGCTCTGCCCCCCCCGGGG TGGGCGCCGGGGGACCTTAAAGCCTCTGCCCCCAAGGAG CCCTTCCCAGACAGCCGCGGCACCCACCGCTCCGTGGGA	35

PROMOTER	SEQUENCE	SEQ ID NO:
Human Creatine Kinase M (hCKM)	CTCTCAGCCCTGGAAGTCCTTGCTCACAGCCGAGGCGCCGAG AGCGCTTGCTCTGCCAGATCTGCGCGAGTCTGGCGCCCGCG CTCTGAACGGCGTCGCTGCCCAGCCCCCTTCCCCGGGAGGTG GGAGCGGCCACCCAGGGCCCCCGTGGCTGCCCTTGTAAGGAG GCGAGGCCCCGAGGACACCCGAGACGCCCCGGTTATAATTAAC CAGGACACGTGGCGAACCCCCCTCCAACACCTGCCCCCGAA CCCCCCCATAACCCAGCGCCTCGGGTCTCGGCCTTTGCGGCAG AGGAGACAGCAAAGCGCCCTCTAAAAATAACTCCTTTCCCG GCGACCGAGACCCTCCCTGTCCCCCGCACAGCGGAAATCTCC CAGTGGCACCGAGGGGGCGAGGGTTAAGTGGGGGGGAGGGT GACCACCGCCTCCCACCCTTGCCCTGAGTTTGAATCTCTCCA ACTCAGCCAGCCTCAGTTTCCCCTCCACTCAGTCCCTAGGAG GAAGGGGCGCCCAAGCGCGGGTTTCTGGGGTTAGACTGCCC TCCATTGCAATTGGTCCTTCTCCCGGCCCTCTGCTTCCTCCAGC TCACAGGGTATCTGCTCCTCCTGGAGCCACACCTTG GTTCCC CGAGGTGCCGCTGGGACTCGGGTAGGGGTGAGGGCCCAGGG GGCACAGGGGGAGCCGAGGGCCACAGGAAGGGCTGGTGGCT GAAGGAGACTCAGGGGCCAGGGGACGGTGGCTTCTACGTGC TTGGGACGTTCCCAGCCACCGTCCCATGTTCCCGGCGGGGGG CCAGCTGTCCCCACCGCCAGCCCAACTCAGCACTTGGTCAGG GTATCAGCTTGGTGGGGGGGCGTGAGCCCAGCCCCTGGGGC GGCTCAGCCCATAAAGGCCATGGGGCTGGGCGCAAAGCAT GCCTGGGTTCAGGGTGGGTATGGTGCGGGAGCAGGGAGGTG AGAGGCTCAGCTGCCCTCCAGAACTCCTCCCTGGGGACAACC CCTCCCAGCCAATAGCACAGCCTAGGTCCCCCTATATAAGGC CACGGCTGCTGGCCCTTCCTTTGGGTCAGTGTACCTCCAGG ATACAGACA	36
Human beta-actin (HuBa)	GCCCAGCACCCCAAGGCGGCCAACGCCAAAACCTCTCCCTCCT CCTCTTCCTCAATCTCGCTCTCGCTCTTTTTTTTTTTTCGAAAA GGAGGGGAGAGGGGGTAAAAAAATGCTGCACTGTGCGGCGA AGCCGGTGAGTGAGCGGCGCGGGCCAATCAGCGTGCGCCG TTCCGAAAGTTGCCTTTTATGGCTCGAGCGGCCCGCGGCGCG	37

PROMOTER	SEQUENCE	SEQ ID NO:
	CCCTATAAAACCCAGCGGCGCGACGCGCCACCACCGCCGAGTC	
Chicken beta-actin (CBA)	GGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCTCCCCACCCCAATTTTGTATTTATTTATTTTAAATATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGGCGGGCGGCCCTATAAAAGCGAAGCGCGCGGGCGGGCGGGA	38
Cytomegalovirus (CMV)	TGGTGATGCGGTTTTTGGCAGTACACCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCACAAATGTCGTAATAACCCCGCCCCGTTGACGCAAATGGGCGGTAGGCGGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCG	39
Cytomegalovirus (CMV) (second version)	TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCGCCCATTTGACGTCATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGATTTCCAAGTCTCCACCCCATTTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCACAAATGTCGTAACAACCTCCGCCCATTTGACGCAAATGGGCGGTAGGCGGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGTGAACCGT	40

PROMOTER	SEQUENCE	SEQ ID NO:
Cytomegalovirus (CMV) (third version)	CGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCC CAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCC CATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGT GGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGT GTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGG TAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATG GGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCT ATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGT GGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCC ATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGG GACTTTCACCAAAATGTCGTAACAACCTCCGCCCCATTGACGCAA ATGGGCGGTAGGCGTGACGGTGGGAGGTCTATATAAGCAG AGCT	41
CAG promoter (first version)	ACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCC CCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAAC GCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTT ACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATAT GCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCC CGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTC TACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCAT GGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTC CCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTAAAT TATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGGGGCGCGC GCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCG AGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCT CCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCC CTATAAAAAGCGAAGCGCGCGGCGGCGGCGG	42
CAG promoter (second version)	CGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCC CAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCC CATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGT GGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGT GTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGG	43

PROMOTER	SEQUENCE	SEQ ID NO:
	TAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATG GGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCT ATTACCATGTGCGAGGTGAGCCCCACGTTCTGCTTCACTCTCC CCATCTCCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTAT TTTTTAATTATTTTGTGCAGCGATGGGGGCGGGGGGGGGGGG GGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGGGGCGGGG CGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGC GGCGCGCTCCGAAAGTTTCCTTTTATGGCGAGGCGGCGGCGG CGGCGGCCCTATAAAAAGCGAAGCGCGCGGGCGGGCG	
Human EF1- alpha (EF1- α)	CAACCTTTGGAGCTAAGCCAGCAATGGTAGAGGGAAGATTC TGCACGTCCCTTCCAGGCGGCCTCCCCGTCACCAACCCCCCCC AACCCGCCCCGACCGGAGCTGAGAGTAATTCATACAAAAGG ACTCGCCCCTGCCTTGGGGAATCCCAGGGACCGTCGTAAAC TCCCCTAACGTAGAACCCAGAGATCGCTGCGTTCCCGCCCC CTCACCCGCCCCGCTCTCGTCATCACTGAGGTGGAGAATAGCA TGCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACAT CGCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAAT TGAACGGGTGCCTAGAGAAGGTGGCGCGGGGTAACTGGGA AAGTGATGTGCGTGTACTGGCTCCGCCTTTTCCCGAGGGTGG GGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT	44
Human Synapsin I (Syn), short version	AGTGCAAGTGGGTTTTAGGACCAGGATGAGGCGGGGTGGGG GTGCCTACCTGACGACCGACCCCGACCCACTGGACAAGCAC CCAACCCCCATTCCCCAAATTGCGCATCCCCTATCAGAGAGG GGGAGGGGAAACAGGATGCGGCGAGGCGCGTGCGCACTGCC AGCTTCAGCACCGCGGACAGTGCCTTCGCCCCCGCCTGGCGG CGCGCGCCACCGCCGCCTCAGCACTGAAGGCGCGCTGACGT CACTCGCCGGTCCCCCGCAAACCTCCCTTCCCGGCCACCTTG GTCGCGTCCGCGCCGCGCGGCCAGCCGGACCGCACCCAC GCGAGGCGCGAGATAGGGGGGCACGGGCGCGACCATCTGCG CTGCGGCGCCGGCGACTCAGCGCTGCCTC	45
Human Synapsin I (Syn)	AGTGCAAGTGGGTTTTAGGACCAGGATGAGGCGGGGTGGGG GTGCCTACCTGACGACCGACCCCGACCCACTGGACAAGCAC	46

PROMOTER	SEQUENCE	SEQ ID NO:
with 3' extension	CCAACCCCCATTCCCCAAATTGCGCATCCCCTATCAGAGAGG GGGAGGGGAAACAGGATGCGGCGAGGCGCGTGCGCACTGCC AGCTTCAGCACCGCGGACAGTGCCCTTCGCCCCCGCCTGGCGG CGCGCGCCACCGCCGCCTCAGCACTGAAGGCGCGCTGACGT CACTCGCCGGTCCCCCGCAAACCTCCCCTTCCCGGCCACCTTG GTCGCGTCCGCGCCGCCGCGGCCAGCCGGACCGCACCAC GCGAGGCGCGAGATAGGGGGGCACGGGCGCGACCATCTGCG CTGCGGCGCCGGCGACTCAGCGCTGCCTCAGTCTGCGGTGGG CAGCGGAGGAGTCGTGTCGTGCCTGAGAGCGCAG	
Human Synapsin I (Syn) with 5' extension	CTGCAGAGGGCCCTGCGTATGAGTGCAAGTGGGTTTTAGGAC CAGGATGAGGCGGGGTGGGGGTGCCTACCTGACGACCGACC CCGACCCACTGGACAAGCACCCAACCCCCATTCCCCAAATTG CGCATCCCCTATCAGAGAGGGGGAGGGGAAACAGGATGCGG CGAGGCGCGTGCGCACTGCCAGCTTCAGCACCGCGGACAGT GCCTTCGCCCCCGCCTGGCGGGCGCGGCCACCGCCGCCTCAG CACTGAAGGCGCGCTGACGTCACCTCGCCGGTCCCCCGCAAAC TCCCCTTCCCGGCCACCTTGGTCGCGTCCGCGCCGCCGCGG CCCAGCCGGACCGCACACGCGAGGCGCGAGATAGGGGGGC ACGGGCGCGACCATCTGCGCTGCGGGCGCCGGCGACTCAGCG CTGCCTC	47
Human CamKIIa (CaMKIIa)	ACTTGTGGACAAAGTTTGCTCTATTCCACCTCCTCCAGGCCCT CCTTGGGTCCATACCCCAGGGGTGCTGGGTCCATCCCACCC CCAGGCCCACACAGGCTTGCAAGTATTGTGTGCGGTATGGTCA GGGCGTCCGAGAGCAGGTTTCGCAAGTGAAGGCAGGCAGGT GTTGGGGAGGCAGTTACCGGGGCAACGGGAACAGGGCGTTT TGGAGGTGGTTGCCATGGGGACCTGGATGCTGACGAAGGCT CGCGAGGCTGTGAGCAGCCACAGTGCCCTGC	48
eSYN promoter	GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGG GGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACAT AACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACC CCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATT	49

PROMOTER	SEQUENCE	SEQ ID NO:
	TACGGTAAACTGCCCACCTTGGCAGTACATCAAGTGTATCATA TGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGC CCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTC CTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCAT GGCTGCAGAGGGCCCTGCGTATGAGTGCAAGTGGGTTTTAG GACCAGGATGAGGCGGGGTGGGGTGCCTACCTGACGACCG ACCCCGACCCACTGGACAAGCACCCAACCCCCATTCCCCAAA TTGCGCATCCCCTATCAGAGAGGGGGAGGGGAAACAGGATG CGGCGAGGCGCGTTCGCGACTGCCAGCTTCAGCACCGCGGAC AGTGCCTTCGCCCCCGCCTGGCGGCGCGCGCCACCGCCGCCT CAGCACTGAAGGCGCGCTGACGTCACTCGCCGGTCCCCCGCA AACTCCCCCTTCCCGGCCACCTTGGTCGCGTCCGCGCCGCCGC CGGCCCAGCCGGACCGCACACGCGAGGCGCGAGATAGGGG GGCACGGGCGCGACCATCTGCGCTGCGGCGCCGGCGACTCA GCGCTGCCTCAGTCTGCGGTGGGCAGCGGAGGAGTCGTGTC GTGCCTGAGAGCGCAGG	

[0129] 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: 31. 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: 32. 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: 33.

[0130] 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

[0131] 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).

[0132] In some embodiments, the vector comprises a CMV enhancer.

[0133] 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 β -actin enhancer sequence, or an EF1- α enhancer sequence. Sequences of the foregoing are known in the art. For example, the sequence of the CMV immediate early (IE) enhancer is:

ACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTTGACG
TCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTC
AATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCA
TATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCAT
TATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATT
AGTCATCGCTATTACCA

(SEQ ID NO: 50)

[0134] In certain embodiments, the vectors comprise one or more introns. In particular embodiments, the intron is a rabbit globin intron sequence, a chicken β -actin intron sequence, a synthetic intron sequence, an SV40 intron, or an EF1- α intron sequence.

[0135] 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).

[0136] 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.

[0137] 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 51-61.

Table 4

5' UNTRANSLATED REGION	SEQUENCE	SEQ ID NO:
Human beta-actin exon/intron	CGCGTCCGCCCCGCGAGCACAGAGCCTCGCCTTTGCCGATC CGCCGCCCCGTCCACACCCGCCGCCAGGTAAGCCCGGCCAG CCGACCGGGGCATGCGGCCGCGGCCCTTCGCCCCTGCAGA GCCGCCGTCTGGGCCGAGCGGGGGGCGCATGGGGCGGA ACCGGACCGCCGTGGGGGGCGCGGGAGAAGCCCCTGGGC CTCCGGAGATGGGGGACACCCACGCCAGTTCGAGGCG CGAGGCCGCGCTCGGGCGGGCGCGCTCCGGGGGTGCCGC TCTCGGGGCGGGGGCAACCGGCGGGGTCTTTGTCTGAGCC GGGCTCTTGCCAATGGGGATCGCACGGTGGGCGCGGCGTA GCCCCCGTCAGGCCCGGTGGGGGCTGGGGCGCCATGCGC GTGCGCGCTGGTCCTTTGGGCGCTAACTGCGTGCGCGCTG GGAATTGGCGCTAATTGCGCGTGCGCGCTGGGACTCAATG GCGCTAATCGCGCGTGCGTTCTGGGGCCCCGGGCGCTTGCG CCACTTCCTGCCCCGAGCCGCTGGCGCCCCGAGGGTGTGGCC GCTGCGTGCGCGCGCGGACCCGGTCGCTGTTTGAACCGG GCGGAGGCGGGGCTGGCGCCCGGTTGGGAGGGGGTTGGG GCCTGGCTTCCTGCCGCGCGCCGCGGGGACGCCTCCGACC AGTGTTTGCCTTTTATGGTAATAACGCGGCCGGCCCCGGCT TCCTTTGTCCCCAATCTGGGCGCGCGCCGGCGCCCCCTGG CGGCCTAAGGACTCGGCGCGCCGGAAGTGGCCAGGGCGG CAGCGGCTGCTCTTGCGGGCCCCGAGGTGACTATAGCCTT CTTTGTGTCTTGATAGTTCGCCAGCCTCTGCTAACCATGT	51

	TCATGCCTTCTTCTTTTTCTACAGCTCCTGGGCAACGTGC TGGTTATTGTGCTGTCTCATCATTTTGGCAAAGAATTC	
Chicken beta-actin exon/intron + rabbit globin intron	GTCGCTGCGCGCTGCCTTCGCCCCGTGCCCCGCTCCGCCG CCGCCTCGCGCCGCCGCCCGGCTCTGACTGACCGCGTT ACTCCCACAGGTGAGCGGGCGGGACGGCCCTTCTCCTCCG GGCTGTAATTAGCGCTTGGTTTAATGACGGCTTGTTCCTTT TCTGTGGCTGCGTGAAAGCCTTGAGGGGCTCCGGGAGGGC CCTTTGTGCGGGGGGAGCGGCTCGGGGGGTGCGTGCGTGT GTGTGTGCGTGGGAGCGCCGCGTGCGGCTCCGCGCTGCC CGGCGGCTGTGAGCGCTGCGGGCGCGGCGCGGGGCTTTGT GCGCTCCGCAGTGTGCGCGAGGGGAGCGCGGCCGGGGG GGTGCCCCGCGGTGCGGGGGGGGCTGCGAGGGGAACAAA GGCTGCGTGCGGGGTGTGTGCGTGGGGGGGTGAGCAGGG GGTGTGGGCGCGTCGGTTCGGGCTGCAACCCCCCTGCACC CCCCCCCCGAGTTGCTGAGCACGGCCCGGCTTCGGGTGC GGGGCTCCGTACGGGGCGTGGCGCGGGGCTCGCCGTGCC GGGCGGGGGGTGGCGGCAGGTGGGGGTGCCGGGCGGGGC GGGGCCGCTCGGGCCGGGAGGGCTCGGGGGAGGGGCG CGGCGGCCCCCGGAGCGCCGGCGGCTGTCGAGGCGCGGC GAGCCGCAGCCATTGCCTTTTATGGTAATCGTGCGAGAGG GCGCAGGGACTTCCTTTGTCCCAAATCTGTGCGGAGCCGA AATCTGGGAGGCGCCGCCGACCCCCCTCTAGCGGGCGCGG GGCGAAGCGGTGCGGCGCCGGCAGGAAGGAAATGGGCGG GGAGGGCCTTCGTGCGTCCCGCGCCGCGTCCCCCTTCTC CCTCTCCAGCCTCGGGGCTGTCCGCGGGGGGACGGGTGCC TTCGGGGGGGACGGGGCAGGGCGGGGTTTCGGCTTCTGGC GTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTCA TGCCCTTCTTCTTTTTCTACAGCTCCTGGGCAACGTGCTGG TTATTGTGCTGTCTCATCATTTTGGCAAAGAATTC	52
Chimeric intron sequence	GGTAAGTTTAGTCTTTTGTCTTTTATTTACAGTCCCGGAT CCGGTGGTGGTGCAAATCAAAGAACTGCTCCTCAGTGGAT GTTGCCTTTACTTCTAGGCCTGTACGGAAGTGTTACTTCTG CTCTAAAAGCTGCGGAATTGTACCCGC	53

5' UTR-Syn1 Hs	AGTCTGCGGTGGGCAGCGGAGGAGTCGTGTCGTGCCTGAG AGCGCAGCTGTGCTCCTGGGCACCGCGCAGTCCGCCCCCG CGGCTCCTGGCCAGACCACCCCTAGGACCCCCTGCCCAA GTCGCA	54
CMV IE exon	TCAGATCGCCTGGAGAGGCCATCCACGCTGTTTTGACCTC CATAGTGGACACCGGGACCGATCCAGCCTCCGCGGCCGG GAACGGTGCATTGGAACGCGGATTCCCCGTGCCAAGAGTG AC	55
TPL-eMLP (adenovirus derived enhancer element)	CTCACTCTCTTCCGCATCGCTGTCTGCGAGGGCCAGCTGTT GGGCTCGCGGTTGAGGACAACTCTTCGCGGTCTTTCCAG TACTCTTGGATCGGAAACCCGTCGGCCTCCGAACGGTACT CCGCCACCGAGGGACCTGAGCGAGTCCGCATCGACCGGA TCGGAACCTCTCGAGAAAGGCGTCTAACCAGTCACAGT CGCAAGGTAGGCTGAGCACCGTGGCGGGCGGCAGCGGGT GGCGGTCGGGGTTGTTTCTGGCGGAGGTGCTGCTGATGAT GTAATTAAAGTAGGCGGTCTTGAGACGGCGGATGGTCGA GGTGAGGTGTGGCAGGCTTGAGATCCAGCTGTTGGGGTGA GTACTCCCTCTCAAAAGCGGGCATTACTTCTGCGCTAAGA TTGTCAGTTTCAAAAACGAGGAGGATTTGATATTACCT GGCCCGATCTGGCCATACTTGAGTGACAATGACATCCA CTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAG	56
Human EF1- α intron/exon	CTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTG CCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTACGGGTTAT GGCCCTTGCGTGCCTTGAATTACTTCCACCTGGCTCCAGTA CGTGATTCTTGATCCCGAGCTGGAGCCAGGGGCGGGCCTT GCGCTTTAGGAGCCCCCTTCGCCTCGTGCTTGAGTTGAGGC CTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTGG CACCTTCGCGCCTGTCTCGCTGCTTTGATAAGTCTCTAGC CATTTAAAATTTTGTGATGACGTGCTGCGACGCTTTTTTCT GGCAAGATAGTCTTGTAATGCGGGCCAGGATCTGCACAC TGGTATTTTCGGTTTTTGGGCCCCGCGCCGGCGACGGGGCC CGTGCGTCCCAGCGCACATGTTTCGGCGAGGCGGGGCCTGC GAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAG CTGGCCGGCCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTG	57

	TATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTTCGGCA CCAGTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTG CTCCAGGGGGCTCAAAATGGAGGACGCGGCGCTCGGGAG AGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCT TTCCGTCCTCAGCCGTCGCTTCATGTGACTCCACGGAGTAC CGGGCGCCGTCCAGGCACCTCGATTAGTTCTGGAGCTTTT GGAGTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGC GATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGTT AGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTG GCCTTTTTGAGTTTGGATCTTGGTTCATTCTCAAGCCTCAG ACAGTGGTTCAAAGTTTTTTTCTTCCATTTTCAG	
Human EF1- α , intron A	GTAAGTGCCGTGTGTGGTTCCCGCGGGCCTGGCCTCTTTA CGGGTTATGGCCCTTGCGTGCTTGAATTACTTCCACCTGG CTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTGGAA GTGGGTGGGAGAGTTTCGAGGCCTTGCCTTAAGGAGCCCC TTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGG GGCCGCCGCGTGCGAATCTGGTGGCACCTTCGCGCCTGTC TCGCTGCTTTTCGATAAGTCTCTAGCCATTAAATTTTTGA TGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGT AAATGCGGGCCAAGATCTGCACACTGGTATTTTCGGTTTTT GGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGC ACATGTTTCGGCGAGGCGGGGCCTGCGAGCGCGGCCACCG AGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTC TGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTG GGCGGCAAGGCTGGCCCGGTTCGGCACCAGTTGCGTGAGC GGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCA AAATGGAGGACGCGGCGCTCGGGAGAGCGGGCGGGTGAG TCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCG TCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAG GCACCTCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTT TAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCA CACTGAGTGGGTGGAGACTGAAGTTAGGCCAGCTTGGCAC TTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGG ATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTT TTTTTCTTCCATTTTCAG	58

5' UTR human CamKIIa	TCAGAAGCCCCGGGCTCGTCAGTCAAACCGGTTCTCTGTT TGCACTCGGCAGCACGGGCAGGCAAGTGGTCCCTAGGTTC GGG	59
B-globin intron	GTGAGTCTATGGGACCCTTGATGTTTTCTTTCCCTTCTTTT CTATGGTTAAGTTCATGTCATAGGAAGGGGAGAAGTAACA GGGTACACATATTGACCAAATCAGGGTAATTTTGCATTTG TAATTTTAAAAAATGCTTTCTTCTTTTAATATACTTTTTTGT TTATCTTATTTCTAATACTTTCCCTAATCTCTTTCTTTTCAGG GCAATAATGATACAATGTATCATGCCTCTTTGCACCATTTCT AAAGAATAACAGTGATAATTTCTGGGTAAAGGCAATAGCA ATATTTCTGCATATAAATATTTCTGCATATAAATTGTAAC GATGTAAGAGGTTTCATATTGCTAATAGCAGCTACAATCC AGCTACCATTCTGCTTTTATTTTATGGTTGGGATAAGGCTG GATTATTCTGAGTCCAAGCTAGGCCCTTTTGCTAATCATGT TCATACCTCTTATCTTCTCCACAG	60
SV40 intron	TCTAGAGGATCCGGTACTCGAGGAACTGAAAAACCAGAA AGTTAACTGGTAAGTTTAGTCTTTTTGTCTTTTATTTTCAGG TCCCGGATCCGGTGGTGGTGCAAATCAAAGAACTGCTCCT CAGTGGATGTTGCCTTTACTTCTAGGCCTGTACGGAAGTG TTACTTCTGCTCTAAAAGCTGCGGAATTGTACCCGC	61

[0138] 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 REGION	SEQUENCE	SEQ ID NO:
WPRE(x) (mutated woodchuck hepatitis	AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACT GGTATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGAT ACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGT	62

regulatory element – version 1)	ATGGCTTTCATTTTCTCCTCCTTGATAAAATCCTGGTTGCT GTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGT GGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTG GTTGGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGAC TTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATC GCCGCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGT TGGGCACTGACAATTCCGTGGTGTGTCGGGGAAATCAT CGTCCTTTTCTTGCTGCTCGCCTGTGTTGCCACCTGGATT CTGCGCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCA ATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCT GCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGT CGGATCTCCCTTTGGGCGCCTCCCCGC	
WPRE(x) (mutated woodchuck hepatitis regulatory element – version 2)	TCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGT ATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGATACG CTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATG GCTTTTCATTTTCTCCTCCTTGATAAAATCCTGGTTGCTGTC TCTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGC GTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTT GGGGCATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTT CGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGCC GCCTGCCTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGG GCACTGACAATTCCGTGGTGTGTCGGGGAAATCATCGTC CTTTCCTTGCTGCTCGCCTGTGTTGCCACCTGGATTCTGC GCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCC AGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGG CCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGA TCTCCCTTTGGGCGCCTCCCCGCA	63
WPRE(x) (mutated woodchuck hepatitis regulatory element – version 3)	TTCCTGTTAATCAACCTCTGGATTACAAAATTTGTGAAAG ATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTAT GTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGC TTCCCGTATGGCTTTCATTTTCTCCTCCTTGATAAAATCCT GGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAG GCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAAC CCCCACTGGTTGGGGCATTGCCACCACCTGTCAGCTCCTT TCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGG	64

	AACTCATCGCCGCTGCCTTGCCCGCTGCTGGACAGGGG CTCGGCTGTTGGGCACTGACAATTCCGTGGTGTGTGCGGG GAAGCTGACGTCCTTTCCGCGGCTGCTCGCCTGTGTTGCC ACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCCTT CGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCT GCCGGCTCTGCGGCCTCTTCCGCCTCTTCGCCTTCGCCCT CAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCCCA TGTATCTTTTTACCTGTGCCTTGTTTTGCCTGTGTTCCG CGTCCTACTTTTCAAGCCTCCAAGCTGTGCCTTGGGCGGC TTTGGGGCATGGACATAGATCCCTATAAAGAATTTGGTTC ATCTTATCAGTTGTTGAATTTTCTTCCTTTGGAC	
CAAX	TGTGTGATAATG	65
EES	CTGTTCTCATCACATCATATCAAGGTTATATACCATCAAT ATTGCCACAGATGTTACTTAGCCTTTTAATATTTCTCTAAT TTAGTGATATGCAATGATAGTTCTCTGATTTCTGAGATT GAGTTTCTCATGTGTAATGATTATTTAGAGTTTCTCTTTCA TCTGTTCAAATTTTTGTCTAGTTTTATTTTTACTGATTTG TAAGACTTCTTTTTATAATCTGCATATTACAATTCCTTTA CTGGGGTGTGCAAATATTTTCTGTCATTCTATGGCCTGA CTTTTCTTAATGGTTTTTTAATTTTAAAAATAAGTCTTAAT ATTCATGCAATCTAATTAACAATCTTTTCTTTGTGGTTAG GACTTTGAGTCATAAGAAATTTTTCTCTACACTGAAGTCA TGATGGCATGCTTCTATATTATTTTCTAAAAGATTTAAAG TTTTGCCTTCTCCATTTAGACTTATAATTCAGTGGAAATTT TTTGTGTGTATGGTATGACATATGGGTTCCCTTTTATTTTT TACATATAAATATATTTCCCTGTTTTTCTAAAAAAGAAAA AGATCATCATTTTCCCATTGTAAAATGCCATATTTTTTTCA TAGGTCACCTACATATATCAATGGGTCTGTTTCTGAGCTC TACTCTATTTTATCAGCCTCACTGTCTATCCCCACACATCT CATGCTTTGCTCTAAATCTTGATATTTAGTGGAACATTCT TTCCCATTTTGTCTACAAGAATATTTTGTATTGTCTTT GGGCTTTCTATATACATTTTGAAATGAGGTTGACAAGTTA	66
HPRE	ATAACAGGCCTATTGATTGGAAAGTTTGTCAACGAATTGT GGGTCTTTTGGGGTTTGCTGCCCCTTTTACGCAATGTGGA TATCCTGCTTTAATGCCTTTATATGCATGTATACAAGCAA AACAGGCTTTTACTTTCTCGCCAACTTACAAGGCCTTTCT CAGTAAACAGTATATGACCCTTTACCCCGTTGCTCGGCAA CGGCCTGGTCTGTGCCAAGTGTGCTGACGCAACCCCCA CTGGTTGGGGCTTGCCCATAGGCCATCAGCGCATGCGTG GAACCTTTGTGTCTCCTCTGCCGATCCATACTGCGGAACT CCTAGCCGCTTGTTTTGCTCGCAGCAGGTCTGGAGCAAAC CTCATCGGGACCGACAATTCTGTCTGACTCTCCCGCAAGT ATACATCGTTTCCATGGCTGCTAGGCTGTGCTGCCAACTG GATCCTGCGCGGGACGTCCTTTGTTTACGTCCCGTCGGCG CTGAATCCCGCGGACGACCCCTCCCGGGGCCGCTTGGGG	67

	CTCTACCGCCCGCTTCTCCGTCTGCCGTACCGTCCGACCA CGGGGCGCACCTCTCTTTACGCGGACTCCCCGTCTGTGCC TTCTCATCTGCCGGACCGTGTGCACTTCGCTTCACCTCTG CACGTGCGATGGAGGCCACCGTGAACGCCACCGGAACC TGCCCAAGGTCTTGCATAAGAGGACTCTTGGACTTTCAGC AATGTCATC	
R2V17 (<i>HepB</i> derived enhancer element)	TTCCTGTAAACAGGCCTATTGATTGGAAAGTTTGTCAACG AATTGTGGGTCTTTTGGGGTTTGCTGCCCCCTTTACGCAA TGTGGATATCCTGCTTTAATGCCTTTATATGCATGTATAC AAGCAAAACAGGCCTTTTACTTTCTCGCCAACTTACAAGGC CTTTCTCAGTAAACAGTATATGACCCTTTACCCCGTTGCT CGGCAACGGCCTGGTCTGTGCCAAGTGTGTTGCTGACGCA ACCCCCACTGGTTGGGGCTTGGCCATAGGCCATCAGCGC ATGCGTGGAACCTTTGTGTCTCCTCTGCCGATCCATACTG CGGAACCTCCTAGCCGCTTGTGTTGCTCGCAGCTGGACTGG AGCAAACCTCATCGGGACCGACAATTCTGTGCTACTCTCC CGCAAGCACTCACCGTTTCCGCGGCTGCTCGCCTGTGTTG CCACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCC TTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCGCTG CTGCCGGCTCTGCGGCCTCTTCCGCCTCTTCGCCTTCGCC CTCAGACGAGTCGGATCTCCCTTTGGGCCGCTCCCCGCC CATGTATCTTTTTACCTGTGCCTTGTTTTGCCTGTGTTT CGCGTCCCTACTTTTCAAGCCTCCAAGCTGTGCCTTGGGCG GCTTTGGGGCATGGACATAGATCCCTATAAAGAATTTGG TTCATCTTATCAGTTGTTGAATTTTCTTCCTTTGGAC	68
3'UTR(globin)	GCTGGAGCCTCGGTAGCCGTTCTCCTGCCCGCTGGGCCT CCCAACGGGGCCCTCCTCCCCCTCCTTGACCGGCCCTTCCT GGTCTTTGAATAAA	69
WPRe(r)	ATTCGAGCATCTTACCGCCATTTATTCCCATATTTGTTCTG TTTTTCTTGATTTGGGTATACATTTAAATGTTAATAAAAC AAAATGGTGGGGCAATCATTTTACATTTTATAGGGATATGTA ATTACTAGTTCAGGTGTATTGCCACAAGACAAACATGTTA AGAAACTTTCCCGTTATTTACGCTCTGTTCTGTTAATCA ACCTCTGGATTACAAAATTTGTGAAAGATTGACTGATATT CTTAACCTATGTTGCTCCTTTTACGCTGTGTGGATATGCTG CTTTAATGCCTCTGTATCATGCTATTGCTTCCCGTACGGCT	70

	TTCGTTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCT TTATGAGGAGTTGTGGCCCGTTGTCCGTCAACGTGGCGTG GTGTGCTCTGTGTTTGCTGACGCAACCCCCACTGGCTGGG GCATTGCCACCACCTGTCAACTCCTTTCTGGGACTTTTCGC TTTCCCCCTCCCGATCGCCACGGCAGAACTCATCGCCGCC TGCCTTGCCCGCTGCTGGACAGGGGCTAGGTTGCTGGGC ACTGATAATTCCGTGGTGTGTGTCGGGGAAGGGCC	
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[0139] 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- ADENYLATION SITE	SEQUENCE	SEQ ID NO:
Rabbit globin (pAGlobin-Oc)	TGGCTAATAAAGGAAATTTATTTTCATTGCAATAGTGTG TTGGAATTTTTTGTGTCTCTCACTCGGAAGAACATATGG GAGGGCAAATCATTTAAACATCAGAATGAGTATTTGGT TTAGAGTTTGGCAACATATGCCCATATGCTGGCTGCCAT GAACAAAGGTTGGCTATAAAGAGGTCATCAGTATATGA AACAGCCCCCTGCTGTCCATTCTTATTCCATAGAAAAG CCTTGACTTGAGGTTAGATTTTTTTTTATATTTTGTTTTGTG TTATTTTTTCTTTAACATCCCTAAAATTTTCCTTACATGT TTTACTAGCCAGATTTTTCTCCTCTCCTGACTACTCCCA GTCATAGCTGTCCCTCTTCTCTTATGGAGATC	71
Bovine growth hormone (pAGH-Bt – version 1)	TTGCCAGCCATCTGTTGTTTGCCCTCCCCCGTGCCTTCC TTGACCTGGAAGGTGCCACTCCCCTGTCCTTTCCTAA TAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGT CATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAA GGGGGAGGATTGGGAATACAATAGCAGGCATGCTGGGG ATGCGGTGGGCTCTATGGGTACCCAGGTGCTGAAGAATT GACCCGGTTCCTCCTGGG	72

Bovine growth hormone (pAGH-Bt – version 2)	TTGCCAGCCATCTGTTGTTTGGCCCTCCCCCGTGCCTTCC TTGACCCTGGAAGGTGCCACTCCCAGTGCCTTTCCTAA TAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGT CATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAA GGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGG ATGCGGTGGGCTCTATGGGTACCCAGGTGCTGAAGAATT GACCCGGTTCCTCTCTGGG	73
Bovine growth hormone (pAGH-Bt – version 3)	CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGGCCCTC CCCCGTGCCTTTCCTTGACCCTGGAAGGTGCCACTCCCAC TGTCTTTTCCTAATAAAATGAGGAAATTGCATCGCATTG TCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGG GCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGC AGGCATGCTGGGGATGCGGTGGGCTCTATGG	74
Human growth hormone (pAGH-Hs)	CTGCCCCGGTGGCATCCCTGTGACCCCTCCCCAGTGCCT CTCCTGGCCCTGGAAGTTGCCACTCCAGTGCCACCAGC CTTGTCCTAATAAAATTAAGTTGCATCATTTTGTCTGACT AGGTGTCCTTCTATAATATTATGGGGTGGAGGGGGGTGG TATGGAGCAAGGGGCCCAAGTTGGGAAGAAACCTGTAG GGCCTGC	75

[0140] Illustrative vector genomes are depicted in FIG. 1-4 and provided as SEQ ID NOs: 12-15. The expression cassette of each sequence, capitalized, is SEQ ID NOs: 8-11. 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: 8-11, optionally with or without the ITR sequences in lowercase. The coding sequence is capitalized.

ADENO-ASSOCIATED VIRUS VECTOR

[0141] 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).

[0142] 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.

[0143] Gene delivery viral vectors useful in the practice of the present invention can be constructed utilizing methodologies well known in the art of molecular biology. Typically, viral vectors carrying transgenes are assembled from polynucleotides encoding the transgene, suitable regulatory elements and elements necessary for production of viral proteins, which mediate cell transduction. Such recombinant viruses may be produced by techniques known in the art, *e.g.*, by transfecting packaging cells or by transient transfection with helper plasmids or viruses. Typical examples of virus packaging cells include but are not limited to HeLa cells, SF9 cells (optionally with a baculovirus helper vector), 293 cells, etc. A Herpesvirus-based system can be used to produce AAV vectors, as described in US20170218395A1. Detailed protocols for producing such replication-defective recombinant viruses may be found for instance in W095/14785, W096/22378, U.S. Pat. No. 5,882,877, U.S. Pat. No. 6,013,516, U.S. Pat. No. 4,861,719, U.S. Pat. No. 5,278,056 and W094/19478, the complete contents of each of which is hereby incorporated by reference.

[0144] AAV vectors useful in the practice of the present invention can be packaged into AAV virions (viral particles) using various systems including adenovirus-based and helper-free systems. Standard methods in AAV biology include those described in Kwon and Schaffer. *Pharm Res.* (2008) 25(3):489-99; Wu et al. *Mol. Ther.* (2006) 14(3):316-27. Burger et al. *Mol. Ther.* (2004) 10(2):302-17; Grimm et al. *Curr Gene Ther.* (2003) 3(4):281-304; Deyle DR, Russell DW. *Curr Opin Mol Ther.* (2009) 11(4):442-447; McCarty et al. *Gene Ther.* (2001) 8(16):1248-54; and Duan et al. *Mol Ther.* (2001) 4(4):383-91. Helper-free systems included those described in US 6,004,797; US 7,588,772; and US 7,094,604;

[0145] 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.

[0146] 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 (≈ 2.4 kb).

[0147] 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).

[0148] 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.

[0149] 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.

[0150] 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.

[0151] 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.

[0152] 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.*,

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MAADGYLPDWLEDTLSEGIQWWKLKPGPPPKPAERHKDDSRGLVLPGYKYLGPENGLDKGEPVNEADAAALEH
DKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTA PGKKRPVEHSP
VEPDSSSGTGKAGQQPARKRLNFGQTGDADSVDPDQPLGQPPAAPSGLGTNTMATGSGAPMADNNEGADGVGNSS
GNWHCDSTWMGDRVITTTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDNRFHCHFS PRDWQRLI
NNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQY
GYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTNT
PSGTTTQSRQLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSL VNPGPAMAS
HKDDEEKFFPQSGVLI FGKQGSEKTNVDIEKVMITDEEEI RTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGV
LPGMVWQDRDVYLQGPWIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTVPVPANPSTTFSAAKFASFITQYST
GQVSVEIEWELQKENS KRWNPEIQYTSNYNKS NVNDFTVDTNGVYSEPRPIGTRYLTRNL
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(SEQ ID NO: 76)

[0153] 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.*,

MAADGYLPDWLEDNLSEGIREWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPNGLDKGEFVNAADAAALEH
DKAYDQQLKAGDNPYLYKNHADAQERLQEDTSFGGNLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSP
QEPDSSAGIGKSGAQPAKKRLNFGQTGDTSVDPDPQPIGEPPAAPSGVGSMTMASGGGAPVADNNEGADGVGSSS
GNWHCDSQLGDRVITTTSTRTWALPTYNNHLYKQISNSTSGSSNDNAYFGYSTPWGYFDFNRHCHFSRQWQR
LINNNWGFPRKRLNFKLFNIQVKEVTDNNGVKTIANNLSTVQVFTSDYQLPYVLGSAHEGCLPFPFADVFMI P
QYGYLTLDNGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENVFPFHSSYAHSSQLDRLMNLIDQYLYYLSKT
INGSGQNNQTLKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNSEFAWPGASSWALNGRNSLMNPGPAMA
SHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNGG
ILPGMVWQDRDVYLQGPWAKIPHTDGNFHPSPLMGGFGMKHPPQILIKNTVPADPPTAFNKDKLNSFITQYS
TGQVSVEIEWELQKENS KRWNPEIQYTSNYKSNVFAVNTGEGVSEPRPIGTRYLTRNL

(SEQ ID NO: 77)

[0154] In some embodiments, the rAAV virion is an AAV6 rAAV virion. The capsid may be an AAV9 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.*,

MAADGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFGNLDKGEFVNAADAAALEH
DKAYDQQLKAGDNPYLYRNHADAQERLQEDTSFGGNLGRAVFQAKKRVLEPFGGLVEEAAKTAPGKKRPVEQSP
QEPDSSSGIGKGTGQPAKKRLNFGQTGDSESVDPDPQLGEPPATPAAVGPTTMASGGGAPMADNNEGADGVGNAS
GNWHCDSTWLGDRVITTTSTRTWALPTYNNHLYKQISSASTGASNDNHFGYSTPWGYFDFNRHCHFSRQWQRL
INNNWGFPRKRLNFKLFNIQVKEVTTNDGVTTIANNLSTVQVFSDEYQLPYVLGSAHQGCLPFPFADVFMI PQ
YGYLTLDNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPFHSSYAHSSQLDRLMNLIDQYLYYLNRTQ
NQSGSAQNKDLLFSRGS PAGMSVQPKNWLPGPCYRQQRVSKTKTDNNNSNFTWTGASKYNLNGRESI INPGTAMA
SHKDDKDKFFPMSGVMI FGKESAGASNTALDNVMITDEEEIKATNPVATERFGTVAVNLQSSSTDPATGDVHVMG
ALPGMVWQDRDVYLQGPWAKIPHTDGHFHPSPLMGGFGLKHPPQILIKNTVPANPPAEFSATKFASFITQYS
TGQVSVEIEWELQKENS KRWNPEVQYTSNYAKSANVDFTVDNNGLYTEPRPIGTRYLTRPL

(SEQ ID NO: 78)

[0155] In some embodiments, the rAAV virion is an AAVrh.10 rAAV virion. The capsid may be an AAV9 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.*,

MAADGYLPDWLEDNLSEGIREWDLKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFGNLDKGEFVNAADAAALEH
DKAYDQQLKAGDNPYLYRNHADAQERLQEDTSFGGNLGRAVFQAKKRVLEPFGGLVEEAAKTAPGKKRPVEPSP
QRSPDSSTGIGKKGQPAKKRLNFGQTGDSESVDPDPQPIGEPPAGPSGLSGTMAAGGGAPMADNNEGADGVGSS

SGNWHCDSTWLGDREVITTTSTRTWALPTYNNHLYKQISNGTSGGSTNDNTYFGYSTPWGYFDFNRFHCHFSPRDWQ
RLINNNWGFRPKRLNFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVMI
PQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNEFFSYQFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSR
TQSTGGTAGTQQLLFSQAGPNMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFAWTGATKYHLNGRDSLVPNGVA
MATHKDDEERFFPSSGVLMEFGKQGAGKDNVDYSSVMLTSEEEIKTTNPVATEQYGVVADNLQQNAAPIVGAVNS
QGALPGMVWQNRDVYLQGPWAKIPHTDGNFHPSPLMGGFGLKHPPQILIKNTVPADPPTTFSQAKLASFITQ
YSTGQVSVEIEWELQKENSQRWNPEIQYTSNYKSTNVDFAVNTDGTYSERPIGTRYLTRNL

(SEQ ID NO: 79)

[0156] In some embodiments, the capsid protein is encoded by a polynucleotide supplied on a plasmid *in trans* to the transfer plasmid. The polynucleotide sequence of wild-type AAVrh74 *cap* is as follows:

AAVrh74 capsid coding sequence (SEQ ID NO: 80)

[0157] ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTCTCT
GAGGGCATTTCGCGAGTGGTGGGACCTGAAACCTGGAGCCCCGAAACCCAAAGCC
AACCAGCAAAAGCAGGACAACGGCCGGGGTCTGGTGCTTCCTGGCTACAAGTAC
CTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGGCGGACGCA
GCGGCCCTCGAGCACGACAAGGCCTACGACCAGCAGCTCCAAGCGGGTGACAAT
CCGTACCTGCGGTATAATCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAA
GATACGTCTTTTGGGGGCAACCTCGGGCGCGCAGTCTTCCAGGCCAAAAAGCGG
GTTCTCGAACCTCTGGGCCTGGTTGAATCGCCGGTTAAGACGGCTCCTGGAAAGA
AGAGACCGGTAGAGCCATCACCCCAGCGCTCTCCAGACTCCTCTACGGGCATCG
GCAAGAAAGGCCAGCAGCCCGCAAAAAAGAGACTCAATTTTGGGCAGACTGGC
GACTCAGAGTCAGTCCCCGACCCTCAACCAATCGGAGAACCACCAGCAGGCCCC
TCTGGTCTGGGATCTGGTACAATGGCTGCAGGCGGTGGCGCTCCAATGGCAGAC
AATAACGAAGGCGCCGACGGAGTGGGTAGTTCTCAGGAAATTGGCATTGCGAT
TCCACATGGCTGGGCGACAGAGTCATCACCACCAGCACCCGCACCTGGGGCCCTG
CCCACCTACAACAACCACCTCTACAAGCAAATCTCCAACGGGACCTCGGGAGGA
AGCACCAACGACAACACCTACTTCGGCTACAGCACCCCCTGGGGGTATTTTGACT
TCAACAGATTCCACTGCCACTTTTCACCACGTGACTGGCAGCGACTCATCAACAA
CAACTGGGGATTCCGGCCCAAGAGGCTCAACTTCAAGCTCTTCAACATCCAAGTC
AAGGAGGTCACGCAGAATGAAGGCACCAAGACCATCGCCAATAACCTTACCAGC
ACGATTCAGGTCTTTACGGACTCGGAATACCAGCTCCCGTACGTGCTCGGCTCGG

CGCACCAGGGCTGCCTGCCTCCGTTCCCGGCGGACGTCTTCATGATTCCTCAGTA
CGGGTACCTGACTCTGAACAATGGCAGTCAGGCTGTGGGCCGGTCGTCCTTCTAC
TGCCTGGAGTACTTTCCTTCTCAAATGCTGAGAACGGGCAACAACCTTTGAATTCA
GCTACAACCTCGAGGACGTGCCCTTCCACAGCAGCTACGCGCACAGCCAGAGCC
TGGACCGGCTGATGAACCCTCTCATCGACCAGTACTTGTACTACCTGTCCCGGAC
TCAAAGCACGGGCGGTACTGCAGGAACCTCAGCAGTTGCTATTTTCTCAGGCCGG
GCCTAACAACATGTCGGCTCAGGCCAAGAACTGGCTACCCGGTCCCTGCTACCG
GCAGCAACGCGTCTCCACGACACTGTCTGCAGAACAAACAGCAACTTTGCCTG
GACGGGTGCCACCAAGTATCATCTGAATGGCAGAGACTCTCTGGTGAATCCTGG
CGTTGCCATGGCTACCCACAAGGACGACGAAGAGCGATTTTTTCCATCCAGCGG
AGTCTTAATGTTTGGGAAACAGGGAGCTGGAAAAGACAACGTGGACTATAGCAG
CGTGATGCTAACCCAGCGAGGAAGAAATAAAGACCACCAACCCAGTGGCCACAG
AACAGTACGGCGTGGTGGCCGATAACCTGCAACAGCAAAACGCCGCTCCTATTG
TAGGGGCCGTC AATAGTCAAGGAGCCTTACCTGGCATGGTGTGGCAGAACCGGG
ACGTGTACCTGCAGGGTCCCATCTGGGCCAAGATTCTCATACGGACGGCAACTT
TCATCCCTCGCCGCTGATGGGAGGCTTTGGACTGAAGCATCCGCCTCCTCAGATC
CTGATTA AAAACACACCTGTTCCCGCGGATCCTCCGACCACCTTCAATCAGGCCA
AGCTGGCTTCTTTTCATCACGCAGTACAGTACCGGCCAGGTCAGCGTGGAGATCGA
GTGGGAGCTGCAGAAGGAGAACAGCAAACGCTGGAACCCAGAGATTCAGTACA
CTTCCAAC TACTACAAATCTACAAATGTGGACTTTGCTGTCAATACTGAGGGTAC
TTATTCCGAGCCTCGCCCCATTGGCACCCGTTACCTCACCCGTAATCTGTAA

[0158] The disclosure further provides protein sequences for AAVrh74 VP1, VP2, and VP3, including SEQ ID NOs: 2-4, and homologs or functional variants thereof.

AAVrh74 VP1 (SEQ ID NO: 81)

[0159] MAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGD RVITTSTRTWALP
TYNNHLYKQISNGTSGGSTNDNTYFGYSTPWGYFDFNRFHCHFS PRDWQRLINNNW
GFRPKRLNFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFTDSEYQLPYVLGSAHQGCL
PPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGN NFEFSYNFEDVPF
HSSYAHSQSLDRLMNPLIDQYLYLSRTQSTGGTAGTQQLFSQAGPNNMSAQAKN
WLPGPCYRQQRVSTLSQNNNSNFAWTGATKYHLNGRDSL VNPGVAMATHKDDEE
RFFPSSGVL MFGKQGAGKDNVDYSSVMLTSEEEIKTTNPVATEQYGVVADNLQQQN
AAPIVGAVNSQ GALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPP

PQILIKNTPVPADPPTTFNQAKLASFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTS
 NYYKSTNVDFAVNTEGTYSEPRPIGTRYLTRNL

AAVrh74 VP2 (SEQ ID NO: 82)

[0160] STIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAV
 GRSSFYCLEYFPSQMLRTGNNFEFSYNFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYL
 SRTQSTGGTAGTQQLLFSQAGPNNMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFA
 WTGATKYHLNGRDSL VNPGVAMATHKDDEERFFPSSGVL MFGKQGAGKDNVDYS
 SVMLTSEEEIKTTNPVATEQYGVVADNLQQQNAAPIVGAVNSQGALPGMVWQNRD
 VYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQAKLAS
 FITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYYKSTNVDFAVNTEGTYSEPRPI
 GTRYLTRNL

AAVrh74 VP3 (SEQ ID NO: 83)

[0161] RTGNNFEFSYNFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQSTGGT
 AGTQQLLFSQAGPNNMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFAWTGATKYH
 LNGRDSL VNPGVAMATHKDDEERFFPSSGVL MFGKQGAGKDNVDYSSVMLTSEEEI
 KTTNPVATEQYGVVADNLQQQNAAPIVGAVNSQGALPGMVWQNRDVYLQGPIWA
 KIPHTDGNFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQAKLASFITQYSTGQV
 SVEIEWELQKENS KRWNPEIQYTSNYYKSTNVDFAVNTEGTYSEPRPIGTRYLTRNL

[0162] In certain cases, the AAVrh74 capsid comprises the amino acid sequence set forth in SEQ ID NO: 2. In some embodiments, the rAAV vector comprises a polypeptide that comprises, or consists essentially of, or yet further consists of a sequence, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89%, more typically 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to amino acid sequence of AAVrh74 VP1 which is set forth in SEQ ID NO: 2. In some embodiments, the rAAV vector comprises a polypeptide that comprises, or consists essentially of, or yet further consists of a sequence, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89%, more typically 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to amino acid sequence of AAVrh74 VP2 which is set forth in SEQ ID NO: 3. In some embodiments, the rAAV vector comprises a polypeptide that comprises, or consists essentially of, or yet further consists of a sequence, *e.g.*, at least 65%, at least 70%, at least 75%, at least 80%, 81%, 82%,

83%, 84%, 85%, 86%, 87%, 88%, or 89%, more typically 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to amino acid sequence of AAVrh74 VP3 which is set forth in SEQ ID NO: 4.

[0163] 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:85) or KFPVALT (SEQ ID NO:86), *e.g.*, inserted between a sequence encoding for amino acids 588 and 589 of AAV9.

[0164] 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.*,

MAADGYLPDWLEDNLSEGIREWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPNGLDKGEFVNAADAAALEH
DKAYDQQLKAGDNPYLYKNHADADEFQERLKEDTSFGGNLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSP
QEPDSSAGIGKSGAQPAKKRLNFGQTGDTESVPDPQPIGEPPAAPSGVGSMTMASGGGAPVADNNEGADGVGSSS
GNWHCDSQWLGDREVITTTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDNRFHCHFSRDPWQR
LINNNWGRFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSQYQLPYVLGSAHEGCLPFPFADVFMI P
QYGYLTLDGSAQAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENVFPFHSSYAHSSQSLDRLMNPLIDQYLYLSRT
INGSGQNQQT LKFSVAGPSNMAVQGRNYIPGPSYRQQRVSTTTVTQNNSEFAWP GASSWALNGRNSLMNPGPAMA
SHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYGGQVATNHQSAQTLAVPFKAQAQT
GWWQNQGILPGMVWQDRDVYLQGPWIWAKIPHTDGNFHPSPLMGGFGMKHPPQIILIKNTFVPADFPPTAFNKDKLN
SFITQYSTGQVSVEIEWELQKENS KRWNPEIQYTSNYKSNVFEAVNTEGVYSEPRPIGTRYLTRNL

(SEQ ID NO: 84)

[0165] 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.

[0166] Without being bound by theory, the present inventors have determined that an AAV9 vector, AAVrh.74, or an AAVrh.10 vector will confer desirable cardiac tropism on the vector. Without being bound by theory, the present inventors have further determined that an AAV9 vector, AAVrh.74, or an AAVrh.10 vector may provide desired specificity to cardiac cells.

PHARMACEUTICAL COMPOSITIONS AND KITS

[0167] 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.

[0168] For purposes of administration, e.g., by injection, various solutions can be employed, such as sterile aqueous solutions. Such aqueous solutions can be buffered, if desired, and the liquid diluent first rendered isotonic with saline or glucose. Solutions of rAAV as a free acid (DNA contains acidic phosphate groups) or a pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as Poloxamer 188, e.g., at 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.

[0169] 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.

[0170] 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.

[0171] In another aspect, the disclosure comprises a kit comprising an rAAV virion of the disclosure and instructions for use.

METHODS OF USE

[0172] In an aspect, the disclosure provides a method of increasing MLP activity in a cell, comprising contacting the cell with an rAAV of the disclosure. In another aspect, the disclosure provides a method of increasing MLP activity in a subject, comprising administering to an rAAV of the disclosure. In some embodiments, the cell and/or subject is deficient in *CSRP3* messenger RNA or MLP protein expression levels and/or activity and/or comprises a loss-of-function mutation in *CSRP3*. The cell may be a cardiac cell, *e.g.* a cardiomyocyte cell.

[0173] In some embodiments, the method promotes survival of cardiac cell, *e.g.* a cardiomyocyte cell, in cell culture and/or *in vivo*. In some embodiments, the method promotes and/or restores function of the heart.

METHODS OF TREATMENT

[0174] 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 cardiac disease or disorder. Illustrative cardiac disorders include heart failure, hypertrophic cardiomyopathy, and dilated cardiomyopathy. In some embodiments, the subject suffers from a genetic disruption in *CSRP3* expression or function. In some embodiments, the disease or disorder is HCM or DCM. In some embodiments, the disease or disorder is familial

hypertrophic cardiomyopathy-12 (CMH12). In some embodiments, the disease or disorder is dilated cardiomyopathy-1M (CMD1M). In some embodiments, the disease or disorder is a skeletal myopathy. In some embodiments, the disease or disorder is facioscapulohumeral muscular dystrophy, nemaline myopathy, or limb girdle muscular dystrophy type 2B. In some embodiments, the disease or disorder is limb girdle muscular dystrophy type 2A, Duchenne muscular dystrophy, or dermatomyositis.

[0175] The AAV-mediated delivery of MLP protein to the heart may increase life span, prevent or attenuate cardiac cell degeneration, heart failure, scarring, reduced ejection fraction, arrhythmia, angina, obstructive HCM or DCM, ventricular hypertrophy (IVS: range 14-32mm), ventricular tachycardia, Mild NYHA scores I-II common, exercise intolerance, angina (chest pain), sudden cardiac death, exertional myalgias and cramps.

[0176] The methods disclosed herein may provide efficient biodistribution in the heart. They may result in sustained expression in all, or a substantial fraction of, cardiac cells, *e.g.*, cardiomyocytes. Notably, the methods disclosed herein may provide long-lasting expression of MLP protein throughout the life of the subject following AAV vector administration.

[0177] 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.

[0178] In some embodiments, the AAV vector is administered at a dose of between about 1×10^{12} and 5×10^{14} vector genomes (vg) of the AAV vector per kilogram (kg) of total body mass of the subject (vg/kg). In some embodiments, the AAV vector is administered at a dose of between about 1×10^{13} and 5×10^{14} vg/kg. In some embodiments, the AAV vector is administered at a dose of between about 5×10^{13} and 3×10^{14} vg/kg. In some embodiments, the AAV vector is administered at a dose of between about 5×10^{13} and 1×10^{14} vg/kg. In some embodiments, the AAV vector is administered at a dose of less than about 1×10^{12} vg/kg, less than about 3×10^{12} vg/kg, less than about 5×10^{12} vg/kg, less than about 7×10^{12} vg/kg, less than about 1×10^{13} vg/kg, less than about 3×10^{13} vg/kg, less than about 5×10^{13} vg/kg, less than

about 7×10^{13} vg/kg, less than about 1×10^{14} vg/kg, less than about 3×10^{14} vg/kg, less than about 5×10^{14} vg/kg, less than about 7×10^{14} vg/kg, less than about 1×10^{15} vg/kg, less than about 3×10^{15} vg/kg, less than about 5×10^{15} vg/kg, or less than about 7×10^{15} vg/kg.

[0179] In some embodiments, the AAV vector is administered at a dose of about 1×10^{12} vg/kg, about 3×10^{12} vg/kg, about 5×10^{12} vg/kg, about 7×10^{12} vg/kg, about 1×10^{13} vg/kg, about 3×10^{13} vg/kg, about 5×10^{13} vg/kg, about 7×10^{13} vg/kg, about 1×10^{14} vg/kg, about 3×10^{14} vg/kg, about 5×10^{14} vg/kg, about 7×10^{14} vg/kg, about 1×10^{15} vg/kg, about 3×10^{15} vg/kg, about 5×10^{15} vg/kg, or about 7×10^{15} vg/kg.

[0180] In some embodiments, the AAV vector is administered at a dose of 1×10^{12} vg/kg, 3×10^{12} vg/kg, 5×10^{12} vg/kg, 7×10^{12} vg/kg, 1×10^{13} vg/kg, 3×10^{13} vg/kg, 5×10^{13} vg/kg, 7×10^{13} vg/kg, 1×10^{14} vg/kg, 3×10^{14} vg/kg, 5×10^{14} vg/kg, 7×10^{14} vg/kg, 1×10^{15} vg/kg, 3×10^{15} vg/kg, 5×10^{15} vg/kg, or 7×10^{15} vg/kg.

[0181] In some embodiments, the AAV vector is administered systemically at a dose of between about 1×10^{12} and 5×10^{14} vector genomes (vg) of the AAV vector per kilogram (vg) of total body mass of the subject (vg/kg). In some embodiments, the AAV vector is administered systemically at a dose of between about 1×10^{13} and 5×10^{14} vg/kg. In some embodiments, the AAV vector is administered systemically at a dose of between about 5×10^{13} and 3×10^{14} vg/kg. In some embodiments, the AAV vector is administered systemically at a dose of between about 5×10^{13} and 1×10^{14} vg/kg. In some embodiments, the AAV vector is administered systemically at a dose of less than about 1×10^{12} vg/kg, less than about 3×10^{12} vg/kg, less than about 5×10^{12} vg/kg, less than about 7×10^{12} vg/kg, less than about 1×10^{13} vg/kg, less than about 3×10^{13} vg/kg, less than about 5×10^{13} vg/kg, less than about 7×10^{13} vg/kg, less than about 1×10^{14} vg/kg, less than about 3×10^{14} vg/kg, less than about 5×10^{14} vg/kg, less than about 7×10^{14} vg/kg, less than about 1×10^{15} vg/kg, less than about 3×10^{15} vg/kg, less than about 5×10^{15} vg/kg, or less than about 7×10^{15} vg/kg.

[0182] In some embodiments, the AAV vector is administered systemically at a dose of about 1×10^{12} vg/kg, about 3×10^{12} vg/kg, about 5×10^{12} vg/kg, about 7×10^{12} vg/kg, about 1×10^{13} vg/kg, about 3×10^{13} vg/kg, about 5×10^{13} vg/kg, about 7×10^{13} vg/kg, about 1×10^{14} vg/kg, about 3×10^{14} vg/kg, about 5×10^{14} vg/kg, about 7×10^{14} vg/kg, about 1×10^{15} vg/kg, about 3×10^{15} vg/kg, about 5×10^{15} vg/kg, or about 7×10^{15} vg/kg.

[0183] In some embodiments, the AAV vector is administered systemically at a dose of 1×10^{12} vg/kg, 3×10^{12} vg/kg, 5×10^{12} vg/kg, 7×10^{12} vg/kg, 1×10^{13} vg/kg, 3×10^{13} vg/kg, 5×10^{13} vg/kg, 7×10^{13} vg/kg, 1×10^{14} vg/kg, 3×10^{14} vg/kg, 5×10^{14} vg/kg, 7×10^{14} vg/kg, 1×10^{15} vg/kg, 3×10^{15} vg/kg, 5×10^{15} vg/kg, or 7×10^{15} vg/kg.

[0184] In some embodiments, the AAV vector is administered intravenously at a dose of between about 1×10^{12} and 5×10^{14} vector genomes (vg) of the AAV vector per kilogram (vg) of total body mass of the subject (vg/kg). In some embodiments, the AAV vector is administered intravenously at a dose of between about 1×10^{13} and 5×10^{14} vg/kg. In some embodiments, the AAV vector is administered intravenously at a dose of between about 5×10^{13} and 3×10^{14} vg/kg. In some embodiments, the AAV vector is administered intravenously at a dose of between about 5×10^{13} and 1×10^{14} vg/kg. In some embodiments, the AAV vector is administered intravenously at a dose of less than about 1×10^{12} vg/kg, less than about 3×10^{12} vg/kg, less than about 5×10^{12} vg/kg, less than about 7×10^{12} vg/kg, less than about 1×10^{13} vg/kg, less than about 3×10^{13} vg/kg, less than about 5×10^{13} vg/kg, less than about 7×10^{13} vg/kg, less than about 1×10^{14} vg/kg, less than about 3×10^{14} vg/kg, less than about 5×10^{14} vg/kg, less than about 7×10^{14} vg/kg, less than about 1×10^{15} vg/kg, less than about 3×10^{15} vg/kg, less than about 5×10^{15} vg/kg, or less than about 7×10^{15} vg/kg.

[0185] In some embodiments, the AAV vector is administered intravenously at a dose of about 1×10^{12} vg/kg, about 3×10^{12} vg/kg, about 5×10^{12} vg/kg, about 7×10^{12} vg/kg, about 1×10^{13} vg/kg, about 3×10^{13} vg/kg, about 5×10^{13} vg/kg, about 7×10^{13} vg/kg, about 1×10^{14} vg/kg, about 3×10^{14} vg/kg, about 5×10^{14} vg/kg, about 7×10^{14} vg/kg, about 1×10^{15} vg/kg, about 3×10^{15} vg/kg, about 5×10^{15} vg/kg, or about 7×10^{15} vg/kg.

[0186] In some embodiments, the AAV vector is administered intravenously at a dose of 1×10^{12} vg/kg, 3×10^{12} vg/kg, 5×10^{12} vg/kg, 7×10^{12} vg/kg, 1×10^{13} vg/kg, 3×10^{13} vg/kg, 5×10^{13} vg/kg, 7×10^{13} vg/kg, 1×10^{14} vg/kg, 3×10^{14} vg/kg, 5×10^{14} vg/kg, 7×10^{14} vg/kg, 1×10^{15} vg/kg, 3×10^{15} vg/kg, 5×10^{15} vg/kg, or 7×10^{15} vg/kg.

[0187] Evidence of functional improvement, clinical benefit or efficacy in patients may be revealed by change in New York Heart Association functional classification (NYHA Class), pathological electrocardiogram, left ventricular end-diastolic/end-systolic diameter, maximal interventricular wall thickness, maximal posterior wall thickness, Peak E and Peak A Velocity, peak early and peak late transmitral filling velocities, early diastolic and late diastolic tissue

Doppler velocities, hypertension and degree of cardiac hypertrophy. Additional myocardial histology would reveal AAV-mediated MLP benefit showing reduction in hypertrophy of cardiomyocytes, reduced myocyte array and reduced interstitial and perivascular fibrosis and scarring compared to baseline or disease-matched control patients.

[0188] Administration of Compositions

[0189] Administration of an effective dose of the compositions may be by routes standard in the art including, but not limited to, systemic, local, direct injection, intravenous, intracardiac administration. In some cases, administration comprises systemic, local, direct injection, intravenous, intracardiac injection. Administration may be performed by cardiac catheterization.

[0190] In some embodiments, the disclosure provides for local administration and 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 parental administration through injection, infusion or implantation. Routes of administration for the compositions disclosed herein include intravenous (“IV”) administration, intraperitoneal (“IP”) administration, intramuscular (“IM”) administration, intralesional administration, or subcutaneous (“SC”) administration, or the implantation of a slow-release device, *e.g.*, a mini-osmotic pump, a depot formulation, etc. In some embodiments, the methods of the disclosure comprise administering an AAV vector of the disclosure, or pharmaceutical composition thereof by intravenous, intramuscular, intraarterial, intrarenal, intraurethral, intracardiac, intracoronary, intramyocardial, intradermal, epidural, subcutaneous, intraperitoneal, intraventricular, ionophoretic or intracranial administration.

[0191] 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 heart.

[0192] In some embodiments, the methods of the disclosure comprise intracardiac delivery. Infusion may be performed using specialized cannula, catheter, syringe/needle using an infusion pump. Administration may comprise delivery of an effective amount of the rAAV virion, or a pharmaceutical composition comprising the rAAV virion, to the heart. These may

be achieved, *e.g.*, via intravenous, intramuscular, intraarterial, intrarenal, intraurethral, intracardiac, intracoronary, intramyocardial, intradermal, epidural, subcutaneous, intraperitoneal, intraventricular, ionophoretic or intracranial administration. The compositions of the disclosure may further be administered intravenously.

[0193] The method of treatment disclosed herein may reduce and/or prevent one or more symptoms including but not limited to ventricular hypertrophy, ventricular tachycardia, mild NYHA scores I-II common, exercise intolerance, and angina.

EXAMPLES

EXAMPLE 1: PRE-CLINICAL BIOACTIVITY AND EFFICACY

Vectors illustrated in FIGs. 1-4 are tested *in vitro* using cultured cardiomyocytes (*e.g.*, induced pluripotent stem cell cardiomyocytes, iPSC-CMs). Expression of MLP is assessed by immunofluorescence and Western blot. Phosphorylation assays reveal a reduction in protein kinase C-alpha (PKC-A) autophosphorylation.

[0194] Selected vectors are tested *in vivo* using an MLP-deficient or MLP-mutant mouse models of cardiomyopathy (*e.g.*, C58G knock-in (KI) model or W4R KI model). Efficacy is determined by measuring left ventricular ejection fraction (LVEF) and/or left ventricular end-diastolic dimension (LVED) using echocardiography, reduction in overall heart weight (*e.g.*, normalized to tibia length), invasive haemodynamic assessments of left ventricular performance on dP/dt_{max} , dP/dt_{min} , and relaxation constant Tau, or reduction in left and/or right ventricular hypertrophy upon histologic evaluation. Additionally, *in vivo* efficacy in the mouse model is assessed by measuring biomarkers including but not limited to atrial natriuretic factor (Nppa) gene expression, brain natriuretic peptide (Nppb) gene expression, and beta-myosin heavy chain protein expression. Physiological efficacy is determined by testing for protein kinase C-alpha (PKC-A) activity, phosphorylated MLP in heart, ubiquitin proteasome degradation activity. Normalization or mitigation in response to treatment is observed for AAV vectors.

EXAMPLE 2: PROTEIN EXPRESSION IN HUMAN CARDIOMYOCYTES

[0195] Vectors illustrated in FIGs. 1-4 were tested *in vitro* using a control cell line (CHO-Lec2; **FIG. 5A**) and cultured cardiomyocytes (differentiated AC16 cell line; **FIG. 5B**).

Expression of muscle LIM protein (MLP; the protein encoded by *CSRP3*) was assessed by Western blot.

[0196] FIG. 5A shows CSRP3 expression in transduced CHO-Lec2. FIG. 5B shows CSRP3 expression in transduced cardiomyocytes (differentiated AC16 cell line – Sigma-Aldrich® cat# SCC109). The cells were transduced with 3E5 MOI from each vector; after 6 days the cells lysates were collected, and a Western Blot performed using an anti-CSRP3 Polyclonal antibody (Thermo-Fisher® PA5-29155 1:1000).

[0197] Expression of the MLP protein from the *CSRP3* transgene is higher when the MHCK7 promoter is used than when the hTNNT2 (“hTnT”) promoter is used. Both AAV9 and AAVrh74 serotypes of AAV vector are capable of transducing the cardiomyocyte cell line. Expression of the MLP protein is apparently higher with the AAVrh74 vector than with the AAV9 vector based on data in FIG 5B.