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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 June 2023 (15.06.2023)

WIPO | PCT

(10) International Publication Number
WO 2023/108157 A1

(51) International Patent Classification:
A61K 48/00 (2006.01) *C12N 15/864* (2006.01)
C12N 15/86 (2006.01)

(21) International Application Number:
PCT/US2022/081321

(22) International Filing Date:
09 December 2022 (09.12.2022)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/288,519 10 December 2021 (10.12.2021) US

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: METHODS AND COMPOSITIONS FOR TREATING MYBPC3 RELATED HYPERTROPHIC CARDIOMYOPATHY WITH A VIRAL VECTOR

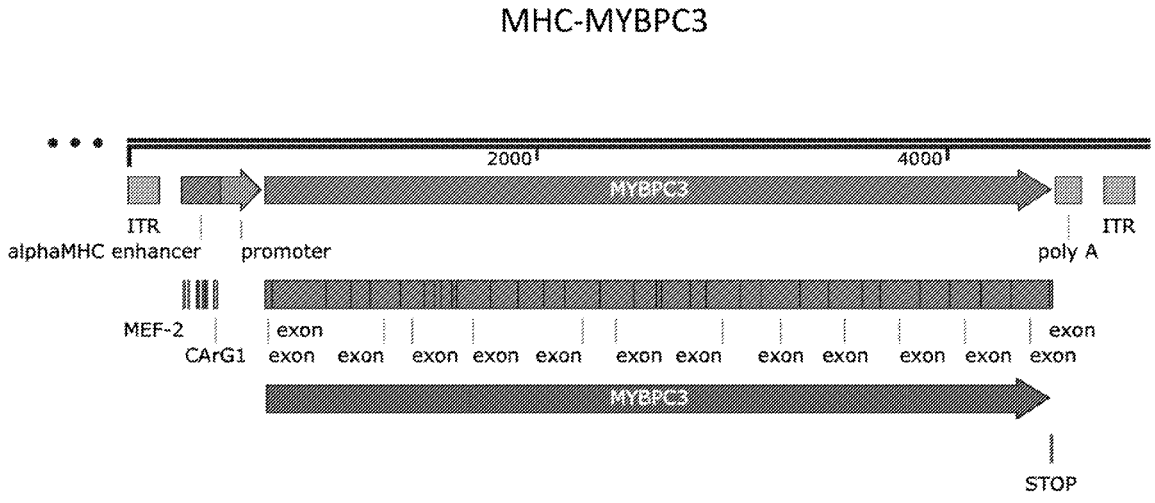

FIG. 1

(57) Abstract: In several embodiments, the present disclosure relates to nucleic acids, compositions, and methods for the delivery of a therapeutic gene to a subject. In several embodiments, the therapeutic gene is through the use of a viral vector. In several embodiments, the viral vector is an adeno-associated virus. In several embodiments, the therapeutic gene is delivered to treat a cardiac disease, injury or other disorder.

[Continued on next page]

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Published:

— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

METHODS AND COMPOSITIONS FOR TREATING MYBPC3 RELATED HYPERTROPHIC CARDIOMYOPATHY WITH A VIRAL VECTOR

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Patent Application No. 63/288519, filed December 10, 2021, the entire contents of which are incorporated by reference herein.

INCORPORATION BY REFERENCE OF MATERIAL IN SEQUENCE LISTING

[0002] This application incorporates the material provided in the accompanying XML file entitled U120270087WO00-SEQ-PRW.xml, created December 8, 2022, which is 67,554 bytes in size.

BACKGROUND

[0003] Cardiomyopathy represents a collection of diverse conditions of the heart muscle and is the second most common cause of heart disease in subjects and medical management of the secondary signs is the only therapeutic option. These diseases have many causes, symptoms, and treatments, and can affect people of all ages and races. When cardiomyopathy occurs, the normal muscle in the heart can thicken, stiffen, thin out, or fill with substances the body produces that do not belong in the heart muscle. As a result, the heart muscle's ability to pump blood is reduced, which can lead to irregular heartbeats, the backup of blood into the lungs or rest of the body, and heart failure. Cardiomyopathy can be acquired or inherited. The cause isn't always known but there is an increasing understanding of the genetic underpinnings of inherited forms of disease.

[0004] Gene transfer strategies have been shown to ameliorate heart disease.

SUMMARY

[0005] Cardiomyopathy is a class of disease of heart muscle that adversely impacts the hearts ability to circulate blood through the cardiovascular system. Various types of cardiomyopathies exist, including dilated cardiomyopathy, hypertrophic cardiomyopathy, and restrictive cardiomyopathy. Cardiomyopathy in human populations is a major medical burden and treatment needs are currently unmet, despite cardiomyopathies in human populations being particularly desirable to treat.

[0006] Dilated cardiomyopathy (DCM) is one of the most common types of human cardiomyopathy, occurring mostly in adults 20 to 60. DCM affects the heart's ventricles and atria, the lower and upper chambers of the heart, respectively. Most forms of DCM are acquired forms from a number of causes that include coronary heart disease, heart attack, high blood pressure, diabetes, thyroid disease, viral hepatitis and viral infections that inflame the heart muscle. Alcohol abuse and certain drugs, such as cocaine and amphetamines, as well as at least two drugs used to treat cancer (doxorubicin and daunorubicin), can also lead to DCM. In addition, there are a number of genetic forms of DCM, including, but not limited to the DCM associated with Duchenne and Becker muscular dystrophies. In the case of certain forms of Becker muscular dystrophy, as well as in most cases of Duchenne muscular dystrophy, the cardiomyopathy can ultimately limit the patient's survival.

[0007] Hypertrophic cardiomyopathy (HCM) occurs when the walls of the heart muscle become abnormally thick. The increase in wall thickness may increase cardiac complications, as well as block or obstruct blood flowing in the heart. Hypertrophic cardiomyopathy presents with otherwise unexplained left ventricular hypertrophy that may lead to arrhythmias, heart failure, and in certain cases, sudden cardiac death (SCD). HCM is also the leading cause of sudden cardiac death in adolescents, with a risk of 0.5-2% per year. Notably, MYBPC3 mutations are commonly associated with HCM, contributing to ~40% of gene-identified cases and exhibiting 50-75% penetrance.

[0008] Restrictive cardiomyopathy (RCM) is a condition leading to a stiffening of the chambers of the heart over time. While the heart's ability to contract remains largely unaffected, the cardiac muscle does not fully relax between beats of the heart. This restricts the ability of the ventricles to fill with blood and causes blood to back up in the circulatory system.

[0009] Heart function is critically dependent upon calcium-dependent signaling. During heart disease, malfunctioning of calcium channels within cardiac cells promotes calcium cycling abnormalities, further inhibiting heart function. Gene transfer strategies to reduce calcium cycling abnormalities are reported to ameliorate heart disease in small and large animal models, as well as in human clinical trials.

[00010] Disclosed herein are gene delivery approaches for treatment of human subjects with one or more types of cardiomyopathy or symptoms thereof.

[00011] Accordingly, some aspects of the present disclosure provide recombinant adeno- associated virus (rAAV) vectors for delivering transgenes into the heart of a subject. Such rAAV vectors may include, from 5' to 3', in order, a first adeno-associated virus (AAV) inverted terminal repeat (ITR) sequence, a promoter operably linked to the one or more transgene, and a

second AAV inverted terminal repeat (ITR) sequence. In some embodiments, the rAAV vector includes, in addition to a promoter, a regulatory element which modifies expression, e.g., in a manner that provides physiologically relevant expression levels and/or restricts expression to a particular cell type or tissue. In some embodiments, the regulatory element comprises one or more of an enhancer, a 5' untranslated region (UTR), and a 3' UTR. In some embodiments, the rAAV vector also includes at least one polyadenylation signal (e.g., positioned 3' of the transgene). In some embodiments, two transgenes are operably linked to the same single promoter. In some embodiments, each transgene is operably linked to a separate promoter. In some embodiments in which multiple transgenes are provided, the rAAV vector also includes at least one polyadenylation signal (e.g., positioned 3' of two transgenes expressed from a single promoter or 3' of one or both transgenes expressed from different promoters). Aspects of the disclosure provide recombinant adeno-associated virus (rAAV) nucleic acid vector for delivering two or more transgenes into the heart of a subject, wherein said vector comprises, from 5' to 3', a first adeno-associated virus (AAV) inverted terminal repeat (ITR) sequence, two or more transgenes and a promoter operably linked to the two or more transgenes, a polyadenylation signal, and a second AAV inverted terminal repeat (ITR) sequence

[00012] In some embodiments, the therapeutic transgene is encoded by a polynucleotide having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 9 or 29 or 43. In some embodiments, one or more of the transgenes of the present disclosure are naturally-occurring sequences. In some embodiments, one or more transgenes are engineered to be species-specific. In some embodiments, one or more transgenes are codon-optimized for expression in a species of interest, e.g. human. For example, in several embodiments, the therapeutic transgene (e.g. the MYBPC transgene) are codon-optimized.

[00013] Further provided herein are rAAV particles containing the rAAV vectors disclosed herein, encapsidated in AAV capsids. Other aspects of the present disclosure include compositions containing the rAAV particles described herein. In several embodiments, such compositions may be administered to a subject for gene therapy for cardiomyopathy. In additional embodiments, such compositions may be administered to a subject for gene therapy for heart disease. In some embodiments, the heart disease causes heart failure in the subject.

[00014] The compositions of the present disclosure may be administered to the subject via different routes. In some embodiments, the composition is administered via intravenous injection into the subject. In some embodiments, the administration of the composition results in expression of the transgene (or, if multiple transgenes are used, expression of two or more

transgenes) in the subject's heart. In various embodiments, the step of administering the composition results in improved cardiac function in the subject, such as improved cardiac function in the subject for more than 10 months. In some embodiments, administration results in improved cardiac function for more than 12 months, more than 14 months, more than 16 months, more than 17 months, more than 20 months, more than 22 months, or more than 24 months. In several embodiments, improved cardiac function is represented by an increase in left ventricular ejection fraction (LVEF). In several embodiments, the LVEF (as compared to a pre-therapy measurement) increases by at least about 1%, about 2%, about 3%, about 4%, about 5% or more (including any amount between those listed). In several embodiments, LVEF is measured by echocardiography. In some embodiments, administration results in improved cardiac physiology (e.g., structural features) for more than 12 months, more than 14 months, more than 16 months, more than 17 months, more than 20 months, more than 22 months, or more than 24 months. In several embodiments, the improved cardiac physiology is represented by a decrease in left ventricular wall thickness. In several embodiments, left ventricular wall thickness is reduced by at least about 1%, about 2%, about 3%, about 4%, about 5% or more (including any amount between those listed). In several embodiments, the left ventricular wall thickness is measured by cardiac magnetic resonance imaging (MRI) or transthoracic echocardiography (TTE).

[00015] In some embodiments, described herein are compositions comprising AAV vectors, virions, viral particles, and pharmaceutical formulations thereof, useful in methods for delivering genetic material encoding one or more beneficial or therapeutic product(s) to mammalian cells and tissues. The rAAV vectors, rAAV particles, or the composition comprising the rAAV particles of the present disclosure, may be used for gene therapy for heart diseases in a subject in need thereof, such as one or more types of cardiomyopathy.

[00016] Additionally, provided herein are compositions, as well as therapeutic and/or diagnostic kits that include one or more of the disclosed AAV compositions, formulated with one or more additional ingredients, or prepared with one or more instructions for their use.

[00017] In some embodiments, described herein is a nucleic acid comprising an expression construct comprising a human MYBPC3 coding sequence and, optionally an enhancer element (such as an alpha MHC enhancer) operably linked to a promoter, wherein the expression construct is flanked on each side by an inverted terminal repeat sequence. In some embodiments, described herein is a nucleic acid comprising an expression construct comprising a human MYBPC3 coding sequence, an enhancer element (such as an alpha MHC enhancer) operably linked to a promoter, and a Kozak sequence, wherein the Kozak sequence enhances transgene

expression in the heart, wherein the expression construct is flanked on each side by an inverted terminal repeat sequence, wherein the Kozak sequence is non-native to the human MYBPC3 coding sequence and/or non-native to the promoter. In several embodiments, the Kozak sequence is a synthetic sequence. In some embodiments, the human MYBPC3 coding sequence is codon-optimized for expression in human cells. In some embodiments, the promoter comprises a cardiac specific promotor. In some embodiments, the promoter is one or more of CMV, mini CMV, CBA, HSV, TK, RSV, SV40, MMTV, Ad E1A and combinations thereof. In some embodiments, the cardiac specific enhancer or regulatory element comprises an alphaMHC enhancer. In some embodiments, the promoter is CBA (Chicken Beta-Actin). In some embodiments, the nucleic acid is a recombinant adeno-associated virus (rAAV) vector. In some embodiments, the nucleic acid is a single-stranded or self-complementary rAAV nucleic acid vector. In some embodiments, the rAAV particle is an AAV9 particle. In some embodiments, the rAAV particle is an rh74 particle. In some embodiments, the rAAV particle is an rh10 particle. In some embodiments, a composition comprising a plurality of rAAV particles is provided. In some embodiments, the plurality of rAAV particle may further comprise a pharmaceutically acceptable carrier. In some embodiments, the rh74 particle comprises at least one capsid protein encoded by a polynucleotide having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 18, or a portion of SEQ ID NO. 29 (for example, SEQ ID NO: 29 encodes the rh74 VP1, VP2, and VP3 proteins – thus, in several embodiments, an rh74 particle according to embodiments disclosed herein comprises at least one capsid protein encoded by a polynucleotide having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to a subpart of the nucleotide sequence of SEQ ID NO: 18). In some embodiments, the rh74 particle comprises an amino acid sequence least about 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to the amino acid sequence set forth as SEQ ID NO: 16, or a portion of SEQ ID NO. 16 (for example, SEQ ID NO: 16 is the amino acid sequence of rh74 VP1, VP2, and VP3 proteins – thus, in several embodiments, an rh74 particle according to embodiments disclosed herein comprises at least one capsid protein having at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to a subpart of the amino acid sequence of SEQ ID NO: 16). In some embodiments, the AAV9 particle comprises an amino acid sequence least about 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to the amino acid sequence set forth as SEQ ID NO: 7.

[00018] In some embodiments, a method of treating hypertrophic cardiomyopathy is described, the method comprising administering a therapeutically effective amount of rAAV comprising a nucleic acid expression construct comprising a human MYBPC3 coding sequence and an enhancer element operably linked to a promoter, wherein the expression construct is flanked on each side by an inverted terminal repeat sequence, and wherein said administration results in expression of a therapeutically effective amount of human MYBPC3, thereby treating the hypertrophic cardiomyopathy. In some embodiments, the rAAV is administered via intravenous injection. In some embodiments, between about 0.5 and about 5 rAAV vector genomes per cell are administered. In some embodiments, between about 0.5 and about 2 rAAV vector genomes per cell are administered. In some embodiments, between about 1×10^{13} and about 3×10^{14} vector genomes per kilo (vgs/kg) are administered.

[00019] Also described herein is a method of inducing increased expression of human MYBPC3 in a target cell, comprising contacting a target cell with a plurality of rAAV particles comprising a nucleic acid expression construct comprising a human MYBPC3 coding sequence and an enhancer element (such as an alpha MHC enhancer) operably linked to a promoter, wherein the expression construct is flanked on each side by an inverted terminal repeat sequence, and wherein said contacting results in the target cell increasing expression of human MYBPC3 as compared to prior to the contacting, thereby increasing the expression of human MYBPC3. In some embodiments, the contacting is in vivo. In some embodiments, the method is used for the treatment of hypertrophic cardiomyopathy. In some embodiments, the nucleic acids, the rAAV particles, the compositions, or the methods of manufacture described herein can be used for the treatment of hypertrophic cardiomyopathy.

BRIEF DESCRIPTION OF THE DRAWINGS

[00020] FIG. 1 shows a gene construct mas for a non-limiting example of a construct including a sequence for MYBPC3.

[00021] FIG. 2 shows a gene construct map for a non-limiting example of a construct including a sequence for MYBPC3.

[00022] FIG. 3 shows a gel assessing RT mediated detection of MYBPC3 RNA.

[00023] FIGS. 4A-4D show multiple qPCR plots demonstrating hMYBPC3 expression with respect to a non-limiting example of a construct described herein. FIG. 4A shows expression of GAPDH in heart tissue. FIG. 4B shows expression of MYBPC3 in heart tissue. FIG 4C shows expression of MYBPC3 in heart tissue normalized to GAPDH. FIG 4D shows MYBPC3 expression based on the respective AAV9 samples.

[00024] FIG. 5 shows a gel assessing detection of MYBPC3 DNA.

[00025] FIGS. 6A show multiple qPCR plots demonstrating hMYBPC3 expression with respect to a non-limiting example of a construct described herein. FIG. 6A shows expression of GAPDH in heart tissue. FIG. 6B shows expression of MYBPC3 in heart tissue. FIG 6C shows expression of MYBPC3 in heart tissue normalized to GAPDH. FIG 6D shows MYBPC3 expression based on the respective AAV9 samples

DETAILED DESCRIPTION

[00026] Reference is made to particular features and/or non-limiting embodiments of the invention. It is to be understood that the disclosure of the invention in this specification includes all possible combinations of such particular features. For example, where a particular feature is disclosed in the context of a particular aspect or embodiment of the invention, or a particular claim, that feature can also be used, to the extent possible, in combination with and/or in the context of other particular aspects and embodiments of the invention, and in the invention generally.

[00027]

Definitions

[00028] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art. All patents, applications, published applications and other publications referenced herein are incorporated by reference in their entirety unless stated otherwise. In the event that there are a plurality of definitions for a term herein, those in this section prevail unless stated otherwise.

[00029] A "subject" refers to mammal that is the object of treatment using a method or composition as provided for herein.. "Mammal" includes, without limitation, mice, rats, rabbits, guinea pigs, dogs, cats, sheep, goats, cows, horses, primates, such as monkeys, chimpanzees, and apes, and humans. In some embodiments, the subject is human.

[00030] The terms "treating," "treatment," "therapeutic," or "therapy" do not necessarily mean total cure or abolition of the disease or condition. Any alleviation of any undesired signs or symptoms of a disease or condition, to any extent can be considered treatment and/or therapy. To "treat" a disease as the term is used herein, means to reduce the frequency or severity of at least one sign or symptom of a disease or disorder experienced by a subject.

[00031] The term "effective amount," as used herein, refers to an amount that is capable of treating or ameliorating a disease or condition or otherwise capable of producing an intended therapeutic effect, such as reducing the frequency or severity of at least one sign or symptom of a disease or disorder experienced by a subject.

[00032] A "nucleic acid" sequence refers to a deoxyribonucleic acid (DNA) or or ribonucleic acid (RNA) sequence. The term captures sequences that include any of the known base analogues of DNA and RNA such as, but not limited to 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinylcytosine, pseudoisocytosine, 5-(carboxyhydroxyl- methyl) uracil, 5-fluorouracil, 5-bromouracil, 5- carboxymethylaminomethyl-2-thiouracil, 5-carboxymethylaminomethyluracil, dihydrouracil, inosine, N6-isopentenyladenine, 1-methyladenine, 1- methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxy- aminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarbonylmethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5- methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N- uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine.

[00033] The term “polynucleotide,” refers to a polymeric form of nucleotides of any length, including DNA, RNA, or analogs thereof. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs, and may be interrupted by non-nucleotide components. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The term polynucleotide, as used herein, refers interchangeably to double- and single-stranded molecules. Unless otherwise specified or required, any embodiment of the invention described herein that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

[00034] The term “isolated” when referring to a nucleotide sequence, means that the indicated molecule is present in the substantial absence of other biological macromolecules of the same type. Thus, an “isolated nucleic acid molecule which encodes a particular polypeptide” refers to a nucleic acid molecule which is substantially free of other nucleic acid molecules that do not encode the subject polypeptide; however, the molecule may include some additional bases or moieties which do not materially affect the basic characteristics of the composition.

[00035] The term “identity” refers to an exact nucleotide-to-nucleotide or amino acid-to-amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Two or more sequences (polynucleotide or amino acid) can be compared by determining their "percent identity." The percent identity of two sequences, whether nucleic acid or amino acid

sequences, is the number of exact matches between two aligned sequences divided by the length of the shorter sequences and multiplied by 100.

[00036] For the purpose of describing the relative position of nucleotide sequences in a particular nucleic acid molecule throughout the instant application, such as when a particular nucleotide sequence is described as being situated "upstream," "downstream," "3'," or "5'" relative to another sequence, it is to be understood that it is the position of the sequences in the "sense" or "coding" strand of a DNA molecule that is being referred to as is conventional in the art.

[00037] Sequence identity can be determined by aligning sequences using algorithms, such as BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, Wis.), using default gap parameters, or by inspection, and the best alignment (*i.e.*, resulting in the highest percentage of sequence similarity over a comparison window). Percentage of sequence identity is calculated by comparing two optimally aligned sequences over a window of comparison, determining the number of positions at which the identical residues occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of matched and mismatched positions not counting gaps in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. Unless otherwise indicated the window of comparison between two sequences is defined by the entire length of the shorter of the two sequences.

[00038] The term "recombinant," as applied to a polynucleotide means that the polynucleotide is the product of various combinations of cloning, restriction or ligation steps, and other procedures that result in a construct that is distinct from a polynucleotide found in nature and/or a combination of polynucleotides and viral proteins that is not found in nature. A recombinant virus is a viral particle comprising a recombinant polynucleotide. The terms respectively include replicates of the original polynucleotide construct and progeny of the original virus construct.

[00039] The term "gene," refers to a polynucleotide containing at least one open reading frame that is capable of encoding a particular gene product. Any of the polynucleotide sequences described herein may be used to identify larger fragments or full-length coding sequences of the genes with which they are associated. Methods of isolating larger fragment sequences are known to those of skill in the art.

[00040] The term "transgene," as used herein, refers to a nucleic acid sequence to be positioned within a viral vector and encoding a polypeptide, protein or other product of interest.

In some embodiments, one rAAV vector may comprise a sequence encoding one or more transgenes (which can optionally be the same gene, or different genes). For example, one rAAV vector may comprise the coding sequence for 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 transgenes. The transgenes of the present disclosure relate to the improvement of one or more heart conditions, such as cardiomyopathies as provided for herein.

[00041] The terms “gene transfer” or “gene delivery” refer to methods or systems for inserting DNA, such as a transgene, into host cells, such as those of a subject afflicted with a cardiomyopathy. In several embodiments, gene transfer yields transient expression of non-integrated transferred DNA, extrachromosomal replication and expression of transferred replicons (e.g., episomes). In additional embodiments, gene transfer results in integration of transferred genetic material into the genomic DNA of host cells.

[00042] The terms “regulatory element” or “regulatory sequence”, or variations thereof, refer to a nucleotide sequence that participates in functional regulation of a polynucleotide, including replication, duplication, transcription, splicing, translation, or degradation of the polynucleotide. Regulatory elements can be enhancing or inhibitory in nature, depending on the embodiment. Non-limiting examples of regulatory elements include transcriptional regulatory sequences such as promoter sequences, polyadenylation signals, transcription termination sequences, upstream regulatory domains, origins of replication, internal ribosome entry sites ("IRES"), enhancers, and the like. These elements collectively provide for the replication, transcription and translation of a coding sequence in a recipient cell, though not all of these sequences need always be present. It shall be appreciated that the structural components of a rAAV vector as provided for herein may be listed in individual paragraphs solely for clarity and may be used together in combination. For example, any regulatory element or other component can be used in combination with any transgene (or transgenes) provided for herein.

[00043] A “promoter” is a polynucleotide that interacts with an RNA polymerase and initiates transcription of a coding region (e.g., a transgene) usually located downstream (in the 3′ direction) from the promoter.

[00044] The term “operably linked” refers to an arrangement of elements wherein the components are configured to perform a function. For example, regulatory sequences operably linked to a coding sequence result in the expression of the coding sequence. Depending on the embodiment, a regulatory sequence need not be contiguous with the coding sequence. Thus, for example, one or more untranslated, yet transcribed, sequences can be present between a promoter

sequence and a coding sequence, with those two sequence still being considered "operably linked".

[00045] The term "vector" means any molecular vehicle, such as a plasmid, phage, transposon, cosmid, chromosome, virus, viral particle, virion, etc. which can transfer gene sequences (e.g., a transgene) to or between cells of interest.

[00046] An "expression vector" is a vector comprising a region of nucleic acid (e.g., a transgene) which encodes a gene product (e.g., a polypeptide or protein) of interest. As disclosed herein, vectors are used for achieving expression, e.g., stable expression, of a protein in an intended target cell. An expression vector may also comprise control elements operatively linked to the transgene to facilitate expression of the encoded protein in the target cell. A combination of one or more regulatory elements and a gene or genes to which they are operably linked for expression may be referred to herein as an "expression cassette."

[00047] The term "AAV" is an abbreviation for adeno-associated virus, and may be used to refer to the virus itself or derivatives thereof. The term covers all subtypes and both naturally occurring and recombinant forms, unless otherwise indicated. The abbreviation "rAAV" refers to recombinant adeno-associated virus, also referred to as a recombinant AAV vector (or "rAAV vector"), which refers to AAV comprising a polynucleotide sequence not of AAV origin (e.g. a transgene).. The term "AAV" includes AAV serotype 1 (AAV-1), AAV serotype 2 (AAV-2), AAV serotype 3 (AAV-3), AAV serotype 4 (AAV-4), AAV serotype 5 (AAV-5), AAV serotype 6 (AAV-6), AAV serotype 7 (AAV-7), AAV serotype 8 (AAV-8), AAV serotype 9 (AAV-9), serotype rh10 AAV, serotype rh74 AAV, or a pseudotyped rAAV (e.g., AAV2/9, referring an AAV vector with the genome of AAV2 (e.g., the ITRs of AAV2) and the capsid of AAV9). In several embodiments, the preferred serotype for delivery to human patients affected by a cardiomyopathy is one of AAV-9, serotype rh74, serotype rh10, or AAV-8. In several embodiments, an rh74 AAV is mutated to advantageously enhance delivery to cardiac tissue, for example by a tryptophan to arginine mutation at amino acid 505 of VP1 capsid, or other mutations, as described in PCT Publication WO 2019/178412, which is incorporated in its entirety by reference herein.

[00048] The term "AAV virus" or "AAV viral particle" or "rAAV vector particle" refers to a viral particle composed of at least AAV capsid protein and an encapsidated polynucleotide.

[00049] The term "heterologous" refers to genotypically distinct origins. For example, a heterologous polynucleotide is one derived from a different species as compared to a reference species (for example a human gene inserted into a viral plasmid is a heterologous gene). A

promoter removed from its native coding sequence and operatively linked to a coding sequence with which it is not naturally found linked is a heterologous promoter.

[00050] As used herein, the term "kit" may be used to describe variations of the portable, self-contained enclosure that includes at least one set of components to conduct one or more of the diagnostic or therapeutic methods of the present disclosure.

[00051] The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the rAAV particle or preparation, and/or rAAV vectors is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum oil such as mineral oil, vegetable oil such as peanut oil, soybean oil, and sesame oil, animal oil, or oil of synthetic origin. Saline solutions and aqueous dextrose and glycerol solutions may also be employed as liquid carriers.

[00052] In some embodiments, sequences recited herein are CpG depleted, and cDNA codon optimized. In some embodiments, the sequences encoding MYBPC3 are optionally CpG depleted.

[00053] Terms and phrases used in this application, and variations thereof, especially in the appended claims, unless otherwise expressly stated, should be construed as open ended as opposed to limiting. As examples of the foregoing, the term 'including' should be read to mean 'including, without limitation,' 'including but not limited to,' or the like; the term 'comprising' as used herein is synonymous with 'including,' 'containing,' or 'characterized by,' and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps; the term 'having' should be interpreted as 'having at least;' the term 'includes' should be interpreted as 'includes but is not limited to;' the term 'example' is used to provide exemplary instances of the item in discussion, not an exhaustive or limiting list thereof; and use of terms like 'preferably,' 'preferred,' 'desired,' or 'desirable,' and words of similar meaning should not be understood as implying that certain features are critical, essential, or even important to the structure or function, but instead as merely intended to highlight alternative or additional features that may or may not be utilized in a particular embodiment. In addition, the term "comprising" is to be interpreted synonymously with the phrases "having at least" or "including at least". When used in the context of a process, the term "comprising" means that the process includes at least the recited steps, but may include additional steps. When used in the context of a compound, composition or device, the term "comprising" means that the compound, composition, or device includes at least the recited features or components, but may also include additional features or components. Likewise, a group of items linked with the conjunction 'and' should not be read as requiring that each and every one of those items be present in the grouping, but rather should be read as 'and/or'

unless expressly stated otherwise. Similarly, a group of items linked with the conjunction 'or' should not be read as requiring mutual exclusivity among that group, but rather should be read as 'and/or' unless expressly stated otherwise.

[00054] With respect to the use of substantially any plural and/or singular terms herein, those having skill in the art can translate from the plural to the singular and/or from the singular to the plural as is appropriate to the context and/or application. The various singular/plural permutations may be expressly set forth herein for sake of clarity. The indefinite article "a" or "an" does not exclude a plurality. A single processor or other unit may fulfill the functions of several items recited in the claims. The mere fact that certain measures are recited in mutually different dependent claims does not indicate that a combination of these measures cannot be used to advantage. Any reference signs in the claims should not be construed as limiting the scope.

[00055] The ranges disclosed herein also encompass any and all overlap, sub-ranges, and combinations thereof. Language such as "up to," "at least," "greater than," "less than," "between," and the like includes the number recited. Numbers preceded by a term such as "about" or "approximately" include the recited numbers. For example, "about 90%" includes "90%." In some embodiments, at least 95% homologous or identical includes 96%, 97%, 98%, 99%, and 100% homologous or identical to the reference sequence. In addition, when a sequence is disclosed as "comprising" a nucleotide or amino acid sequence, such a reference shall also include, unless otherwise indicated, that the sequence "comprises", "consists of" or "consists essentially of" the recited sequence.

[00056] Sequence Listing

SEQ ID:	**Elements (5' -> 3')**	**Nt sequence**
1	ITR-L	TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGG CCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCC GGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGA GTGGCCAACTCCATCACTAGGGGTTCCT
2	spacer	CTAGAGGTACCTGTACA
3	alphaMHC enhancer	CCTTCAGATTAAAAATAACTAAGGTAAGGGCCATGTGGG TAGGGGAGGTGGTGTGAGACGGTCCTGTCTCTCCTCTAT CTGCCCATCGGCCCTTTGGGGAGGAGGAATGTGCCCAAG GACTAAAAAAAGGCCCTGGAGCCAGAGGGGCGAGGGCA GCAGACCTTTCATGGGCAAACCTCAGGGCTGCTGTC
4	spacer	GTCGA
5	Mini CMV promoter	GGTAGGCGTGTACGGTGGGAGGCCTATATAAGCAGAGC T
6	spacer	CGTTTAGTGAACCGTCAGATCGCCTGGAGGAATTC
7	"sd/sa"	GTAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCA ATAGAAACTGGGCTTGTCGAGACAGAGAAGACTCTTGC

		GTTTCTGATAGGCACCTATTGGTCTTACTGACATCCACTT TGCCTTTCTCTCCACAG
8	spacer	AAGCTTGCTAGCGTTTAAACTTAAGCTTGCCACC
9	MYBPC3	ATGCCTGAGCCTGGCAAGAAACCTGTGTCCGCCTTCAGC AAAAAGCCCCGCTCTGTGGAAGTGGCCGCTGGATCTCCT GCCGTGTTTGAGGCCGAAACAGAACGCGCTGGCGTGAA AGTTCGATGGCAGAGAGGCGGCAGCGATATCAGCGCCA GCAACAAATATGGCCTGGCCACCGAGGGCACCAGACAC ACACTGACAGTCAGAGAAGTGGGCCCTGCCGATCAGGG CTCTTATGCCGTGATTGCCGGCAGCAGCAAAGTGAAGTT CGACCTGAAAGTGATCGAGGCCGAGAAGGCCGAGCCTA TGCTTGCTCCTGCTCCAGCTCCTGCTGAAGCTACAGGTG CTCCTGGCGAAGCTCCAGCACCAGCTGCTGAACTGGGAG AGTCTGCCCCATCTCCTAAGGGCTCTAGCAGCGCCGCTC TGAATGGACCTACACCTGGCGCTCCCGATGATCCCATCG GCCTGTTTGTTATGAGGCCCCAGGATGGCGAAGTGACAG TCGGCGGCAGCATCACCTTTAGCGCTAGAGTTGCAGGCG CCAGCCTGCTGAAACCTCCTGTGGTCAAGTGGTTCAAAG GCAAATGGGTCGACCTGTCCTCCAAAGTGGGCCAGCATC TGCAGCTCCACGACAGCTACGATAGAGCCAGCAAGGTG TACCTGTTCGAGCTGCACATCACAGACGCCCAGCCAGCC TTTACCGGCAGCTACAGATGTGAAGTGTCCACCAAGGAC AAGTTCGACTGCAGCAACTTCAACCTGACCGTGCACGAG GCCATGGGCACAGGCGATCTGGATCTGCTGAGCGCCTTC AGAAGAACATCTCTGGCTGGCGGAGGCAGACGGATCAG CGATTCTCACGAGGATACCGGCATCCTGGACTTCAGCAG CCTGCTCAAGAAGCGGGACAGCTTCAGAACCCCTCGGG ACAGCAAACTGGAAGCCCCTGCCGAGGAAGATGTCTGG GAGATCCTGAGACAGGCCCCTCCTAGCGAGTACGAGAG AATCGCCTTTCAGTACGGCGTGACCGACCTGAGGGGCAT GCTGAAAAGGCTGAAGGGCATGCGCCGGGACGAGAAGA AGTCCACAGCCTTCCAGAAAAAGCTGGAACCCGCCTACC AGGTGTCCAAGGGCCACAAGATCAGACTGACCGTGGAA CTGGCCGACCACGACGCCGAAGTGAAGTGGCTGAAGAA CGGCCAAGAGATTCAGATGAGCGGCAGCAAGTACATCT TCGAGAGCATCGGCGCCAAGCGGACCCTGACAATCAGC CAGTGTAGCCTGGCCGATGACGCCGCCTATCAGTGTGTT GTTGGCGGCGAGAAGTGCAGCACCGAGCTGTTCGTGAA AGAACCTCCAGTGCTGATCACCCGGCCTCTGGAAGATCA GCTGGTCATGGTCGGACAGCGCGTGGAATTCGAGTGCGA GGTTTCCGAAGAGGGCGCCCAAGTCAAATGGCTGAAAG ACGGCGTCGAGCTGACCAGAGAGGAAACCTTCAAGTAC CGGTTCAAGAAGGACGGCCAGCGGCACCACCTGATCAT CAATGAAGCCATGCTGGAAGATGCCGGCCACTACGCCTT GTGTACATCTGGTGGACAGGCCCTGGCCGAGCTGATTGT GCAAGAGAAGAAACTCGAGGTGTACCAGTCTATCGCCG ACCTGATGGTCGGAGCCAAAGACCAGGCCGTGTTCAAGT GCGAAGTGTCCGACGAGAATGTGCGCGGCGTGTGGCTG AAAAATGGCAAAGAACTGGTGCCCGACAGCCGGATCAA GGTGTCCCACATTGGCAGAGTGCACAAGCTGACCATCGA CGACGTGACCCCTGCTGACGAGGCCGATTACAGCTTTGT

		GCCTGAGGGCTTCGCCTGTAACCTGAGCGCCAAGCTGCA CTTTATGGAAGTCAAGATCGACTTCGTGCCCCGGCAAGA GCCTCCTAAGATCCACCTGGATTGCCCTGGCAGAATCCC CGACACAATCGTGGTGGTGGCCGGAAACAAGCTGAGAC TGGATGTGCCCATCAGCGGCGATCCTGCTCCTACAGTGA TCTGGCAGAAGGCCATCACACAGGGCAACAAGGCCCCT GCTAGACCCGCTCCTGATGCTCCTGAAGATACCGGCGAT AGCGACGAGTGGGTGTTCGACAAGAAACTGCTGTGCGA GACAGAGGGAAGAGTGCGAGTGGAAACCACAAAGGACC GCAGCATCTTCACCGTGGAAGGCGCCGAGAAAGAGGAC GAGGGCGTCTACACAGTGACCGTGAAGAATCCCGTGGG CGAAGATCAAGTGAACCTGACAGTGAAAGTCATCGACG TGCCCGACGCTCCTGCCGCTCCAAAGATCTCTAACGTGG GAGAAGATAGCTGCACCGTGCAGTGGGAGCCTCCAGCTT ATGATGGCGGACAGCCTATCCTGGGCTACATCCTGGAAC GCAAGAAGAAAAAGTCCTACCGGTGGATGCGGCTGAAC TTCGATCTGATCCAAGAGCTGAGCCACGAAGCCAGACG GATGATCGAAGGCGTGGTGTACGAGATGAGAGTGTACG CCGTGAACGCCATCGGCATGAGCAGACCTTCTCCAGCCT CTCAGCCCTTCATGCCTATCGGCCCTCCAAGCGAACCTA CACACCTGGCCGTTGAGGACGTGTCCGATACCACCGTGT CTCTGAAATGGCGGCCTCCTGAGAGAGTTGGAGCTGGCG GACTGGATGGCTACAGCGTGGAATACTGTCCAGAGGGCT GTAGCGAGTGGGTTGCAGCTCTGCAGGGACTGACCGAG CACACCTCTATCCTGGTCAAGGACCTGCCTACCGGCGCT AGACTGCTGTTTAGAGTGCGGGCCCACAACATGGCTGGA CCAGGTGCACCAGTGACCACCACAGAACCCGTGACAGT GCAAGAAATCCTGCAGCGGCCAAGACTGCAGCTGCCCA GACACCTGAGGCAGACCATCCAGAAGAAAGTCGGCGAG CCCGTGAACCTGCTGATCCCATTTCAAGGCAAGCCCAGA CCTCAAGTGACCTGGACCAAAGAGGGACAGCCTCTGGC CGGCGAAGAGGTGTCCATCAGAAACAGCCCCACCGACA CCATCCTGTTCATCAGAGCCGCCAGAAGGGTGCACTCCG GCACCTATCAAGTGACTGTGCGGATCGAGAACATGGAA GATAAGGCCACACTGGTGCTGCAGGTCGTGGACAAACC CAGTCCTCCTCAGGACCTGAGAGTGACAGATGCCTGGGG ACTGAACGTGGCCCTGGAATGGAAACCTCCACAGGACG TGGGCAACACAGAGCTGTGGGGCTATACCGTGCAGAAA GCCGACAAAAAGACCATGGAATGGTTCACCGTGCTGGA ACACTACCGGCGGACCCATTGTGTGGTGCCTGAGCTGAT CATCGGCAACGGCTACTACTTCCGGGTGTTCAGCCAGAA TATGGTCGGATTCAGCGACAGGGCCGCCACCACAAAAG AACCTGTGTTCATCCCCAGACCTGGCATCACCTACGAGC CTCCAAACTACAAGGCCCTGGATTTCAGCGAGGCCCCTA GATTCACACAGCCCCTGGTCAACAGAAGCGTGATCGCCG GCTACACCGCCATGCTGTGTTGTGCCGTTAGAGGCAGCC CCAAGCCTAAGATCTCCTGGTTCAAAAACGGCCTGGACC TCGGCGAGGACGCCCGGTTTAGAATGTTTAGCAAGCAGG GCGTGCTGACCCTGGAAATCAGAAAGCCCTGTCCTTTCG ACGGCGGCATCTACGTGTGCAGAGCCACCAATCTGCAGG

		GCGAAGCTAGATGCGAGTGCAGACTGGAAGTTCGGGTG CCCCAG
10	STOP	TGATGATGAGC
11	spacer	GGCCGCCGGCCG
12	polyA	AATAAAAGATCCTTATTTTCATTGGATCTGTGTGTTGGTT TTTTGTGTG
13	spacer	GTCGACTCTAG
14	ITR-R	AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGC GCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCG CCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCG AGCGAGCGCGCAGAGAGGGAGTGGCCAA
18	RH74 VP1, VP2, VP3	ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGAC AACCTCTCTGAGGGCATTCGCGAGTGGTGGGACCTGAAA CCTGGAGCCCCGAAACCCAAAGCCAACCAGCAAAAGCA GGACAACGGCCGGGGTCTGGTGCTTCCTGGCTACAAGTA CCTCGGACCCTTCAACGGACTCGACAAGGGGGAGCCCGT CAACGCGGCGGACGCAGCGGCCCTCGAGCACGACAAGG CCTACGAC CAGCAGCTCCAAGCGGGTGACAATCCGTACCTGCGGTAT AATCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGA AGATACGTCTTTTGGGGGCAACCTCGGGCGCGCAGTCTT CCAGGCCAAAAAGCGGGTTCTCGAACCTCTGGGCCTGGT TGAATCGCCGGTTAAGACGGCTCCTGGAAAGAAGAGAC CGGTAGAGCCATCACCCCAGCGCTCTCCAGACTCCTCTA CGGGCATC GGCAAGAAAGGCCAGCAGCCCGCAAAAAAGAGACTCAA TTTTGGGCAGACTGGCGACTCAGAGTCAGTCCCCGACCC TCAACCAATCGGAGAACCACCAGCAGGCCCCTCTGGTCT GGGATCTGGTACAATGGCTGCAGGCGGTGGCGCTCCAAT GGCAGACAATAACGAAGGCGCCGACGGAGTGGGTAGTT CCTCAGGAAATTGGCATTGCGATTCCACATGGCTGGGCG ACAGAGTCATCACCACCAGCACCCGCACCTGGGCCCTGC CCACCTACAACAACCACCTCTACAAGCAAATCTCCAACG GGACCTCGGGAGGAAGCACCAACGACAACACCTACTTC GGCTACAGCACCCCCTGGGGGTATTTTGACTTCAACAGA TTCCACTGCCACTTTTCACCACGTGACTGGCAGCGACTC ATCAACAACAACTGGGGATTCCGGCCCAAGAGGCTCAA CTTCAAGCTCTTCAAC ATCCAAGTCAAGGAGGTCACGCAGAATGAAGGCACCAA GACCATCGCCAATAACCTTACCAGCACGATTCAGGTCTT TACGGACTCGGAATACCAGCTCCCGTACGTGCTCGGCTC GGCGCACCAGGGCTGCCTGCCTCCGTTCCCGGCGGACGT CTTCATGATTCCTCAGTACGGGTACCTGACTCTGAACAA TGGCAGTCAGGCTGTGGGCCGGTCGTCCTTCTACTGCCT GGAGTAC TTTCCTTCTCAAATGCTGAGAACGGGCAACAACTTTGAA TTCAGCTACAACTTCGAGGACGTGCCCTTCCACAGCAGC TACGCGCACAGCCAGAGCCTGGACCGGCTGATGAACCCT CTCATCGACCAGTACTTGTACTACCTGTCCCGGACTCAA

		AGCACGGGCGGTACTGCAGGAACTCAGCAGTTGCTATTT TCTCAGGCCGGGCCTAACAACATGTCGGCTCAGGCCAAG AACTGG CTACCCGGTCCCTGCTACCGGCAGCAACGCGTCTCCACG ACACTGTCGCAGAACAACAACAGCAACTTTGCCTGGACG GGTGCCACCAAGTATCATCTGAATGGCAGAGACTCTCTG GTGAATCCTGGCGTTGCCATGGCTACCCACAAGGACGAC GAAGAGCGATTTTTTCCATCCAGCGGAGTCTTAATGTTT GGGAAACAGGGAGCTGGAAAAGACAACGTGGACTATAG CAGCGTGATGCTAACCAGCGAGGAAGAAATAAAGACCA CCAACCCAGTGGCCACAGAACAGTACGGCGTGGTGGCC GATAACCTGCAACAGCAAAACGCCGCTCCTATTGTAGGG GCCGTCAATAGTCAAGGAGCCTTACCTGGCATGGTGTGG CAGAACCGGGACGTGTACCTGCAGGGTCCCATCTGGGCC AAGATTCCTCATACGGACGGCAACTTTCATCCCTCGCCG CTGATGGGAGGCTTTGGACTGAAGCATCCGCCTCCTCAG ATCCTGATTAAAAACACACCTGTTCCCGCGGATCCTCCG ACCACCTTCAATCAGGCCAAGCTGGCTTCTTTCATCACG CAGTACAGTACCGGCCAGGTCAGCGTGGAGATCGAGTG GGAGCTGCAGAAGGAGAACAGCAAACGCTGGAACCCAG AGATTCAGTACACTTCCAACTACTACAAATCTACAAATG TGGACTTTGCTGTCAATACTGAGGGTACTTATTCCGAGC CTCGCCCCATTGGCACCCGTTACCTCACCCGTAATCTGTA A
19	Whole Construct 1	TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAA AGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGCGAG CGCGCAGAGAGGGAGTGGCCAACTCCATCACTAGGGGTTCCTCTAGAGGTA CCTGTACACCTTCAGATTAAAAATAACTAAGGTAAGGGCCATGTGGGTAGG GGAGGTGGTGTGAGACGGTCCTGTCTCTCCTCTATCTGCCCATCGGCCCTTT GGGGAGGAGGAATGTGCCCAAGGACTAAAAAAAGGCCCTGGAGCCAGAGG GGCGAGGGCAGCAGACCTTTCATGGGCAAACCTCAGGGCTGCTGTCGTCGA GGTAGGCGTGTACGGTGGGAGGCCTATATAAGCAGAGCTCGTTTAGTGAAC CGTCAGATCGCCTGGAGGAATTCGTAAGTATCAAGGTTACAAGACAGGTTTA AGGAGACCAATAGAAACTGGGCTTGTCGAGACAGAGAAGACTCTTGCGTTT CTGATAGGCACCTATTGGTCTTACTGACATCCACTTTGCCTTTCTCTCCACAGA AGCTTGCTAGCGTTTAAACTTAAGCTTGCCACCATGCCTGAGCCTGGCAAGA AACCTGTGTCCGCCTTCAGCAAAAAGCCCCGCTCTGTGGAAGTGGCCGCTGG ATCTCCTGCCGTGTTTGAGGCCGAAACAGAACGCGCTGGCGTGAAAGTTCG ATGGCAGAGAGGCGGCAGCGATATCAGCGCCAGCAACAAATATGGCCTGGC CACCGAGGGCACCAGACACACACTGACAGTCAGAGAAGTGGGCCCTGCCGA TCAGGGCTCTTATGCCGTGATTGCCGGCAGCAGCAAAGTGAAGTTCGACCTG AAAGTGATCGAGGCCGAGAAGGCCGAGCCTATGCTTGCTCCTGCTCCAGCTC CTGCTGAAGCTACAGGTGCTCCTGGCGAAGCTCCAGCACCAGCTGCTGAACT GGGAGAGTCTGCCCCATCTCCTAAGGGCTCTAGCAGCGCCGCTCTGAATGGA CCTACACCTGGCGCTCCCGATGATCCCATCGGCCTGTTTGTTATGAGGCCCCA GGATGGCGAAGTGACAGTCGGCGGCAGCATCACCTTTAGCGCTAGAGTTGC AGGCGCCAGCCTGCTGAAACCTCCTGTGGTCAAGTGGTTCAAAGGCAAATG GGTCGACCTGTCCTCCAAAGTGGGCCAGCATCTGCAGCTCCACGACAGCTAC GATAGAGCCAGCAAGGTGTACCTGTTCGAGCTGCACATCACAGACGCCCAG CCAGCCTTTACCGGCAGCTACAGATGTGAAGTGTCCACCAAGGACAAGTTCG ACTGCAGCAACTTCAACCTGACCGTGCACGAGGCCATGGGCACAGGCGATC

		TGGATCTGCTGAGCGCCTTCAGAAGAACATCTCTGGCTGGCGGAGGCAGAC GGATCAGCGATTCTCACGAGGATACCGGCATCCTGGACTTCAGCAGCCTGCT CAAGAAGCGGGACAGCTTCAGAACCCCTCGGGACAGCAAACTGGAAGCCCC TGCCGAGGAAGATGTCTGGGAGATCCTGAGACAGGCCCCTCCTAGCGAGTA CGAGAGAATCGCCTTTCAGTACGGCGTGACCGACCTGAGGGGCATGCTGAA AAGGCTGAAGGGCATGCGCCGGGACGAGAAGAAGTCCACAGCCTTCCAGA AAAAGCTGGAACCCGCCTACCAGGTGTCCAAGGGCCACAAGATCAGACTGA CCGTGGAACTGGCCGACCACGACGCCGAAGTGAAGTGGCTGAAGAACGGC CAAGAGATTCAGATGAGCGGCAGCAAGTACATCTTCGAGAGCATCGGCGCC AAGCGGACCCTGACAATCAGCCAGTGTAGCCTGGCCGATGACGCCGCCTATC AGTGTGTTGTTGGCGGCGAGAAGTGCAGCACCGAGCTGTTCGTGAAAGAAC CTCCAGTGCTGATCACCCGGCCTCTGGAAGATCAGCTGGTCATGGTCGGACA GCGCGTGGAATTCGAGTGCGAGGTTTCCGAAGAGGGCGCCCAAGTCAAATG GCTGAAAGACGGCGTCGAGCTGACCAGAGAGGAAACCTTCAAGTACCGGTT CAAGAAGGACGGCCAGCGGCACCACCTGATCATCAATGAAGCCATGCTGGA AGATGCCGGCCACTACGCCTTGTGTACATCTGGTGGACAGGCCCTGGCCGA GCTGATTGTGCAAGAGAAGAAACTCGAGGTGTACCAGTCTATCGCCGACCT GATGGTCGGAGCCAAAGACCAGGCCGTGTTCAAGTGCGAAGTGTCCGACGA GAATGTGCGCGGCGTGTGGCTGAAAAATGGCAAAGAACTGGTGCCCGACA GCCGGATCAAGGTGTCCCACATTGGCAGAGTGCACAAGCTGACCATCGACG ACGTGACCCCTGCTGACGAGGCCGATTACAGCTTTGTGCCTGAGGGCTTCGC CTGTAACCTGAGCGCCAAGCTGCACTTTATGGAAGTCAAGATCGACTTCGTG CCCCGGCAAGAGCCTCCTAAGATCCACCTGGATTGCCCTGGCAGAATCCCCG ACACAATCGTGGTGGTGGCCGGAAACAAGCTGAGACTGGATGTGCCCATCA GCGGCGATCCTGCTCCTACAGTGATCTGGCAGAAGGCCATCACACAGGGCA ACAAGGCCCCTGCTAGACCCGCTCCTGATGCTCCTGAAGATACCGGCGATAG CGACGAGTGGGTGTTCGACAAGAAACTGCTGTGCGAGACAGAGGGAAGAG TGCGAGTGGAAACCACAAAGGACCGCAGCATCTTCACCGTGGAAGGCGCCG AGAAAGAGGACGAGGGCGTCTACACAGTGACCGTGAAGAATCCCGTGGGC GAAGATCAAGTGAACCTGACAGTGAAAGTCATCGACGTGCCCGACGCTCCT GCCGCTCCAAAGATCTCTAACGTGGGAGAAGATAGCTGCACCGTGCAGTGG GAGCCTCCAGCTTATGATGGCGGACAGCCTATCCTGGGCTACATCCTGGAAC GCAAGAAGAAAAAGTCCTACCGGTGGATGCGGCTGAACTTCGATCTGATCC AAGAGCTGAGCCACGAAGCCAGACGGATGATCGAAGGCGTGGTGTACGAG ATGAGAGTGTACGCCGTGAACGCCATCGGCATGAGCAGACCTTCTCCAGCCT CTCAGCCCTTCATGCCTATCGGCCCTCCAAGCGAACCTACACACCTGGCCGTT GAGGACGTGTCCGATACCACCGTGTCTCTGAAATGGCGGCCTCCTGAGAGA GTTGGAGCTGGCGGACTGGATGGCTACAGCGTGGAATACTGTCCAGAGGGC TGTAGCGAGTGGGTTGCAGCTCTGCAGGGACTGACCGAGCACACCTCTATCC TGGTCAAGGACCTGCCTACCGGCGCTAGACTGCTGTTTAGAGTGCGGGCCCA CAACATGGCTGGACCAGGTGCACCAGTGACCACCACAGAACCCGTGACAGT GCAAGAAATCCTGCAGCGGCCAAGACTGCAGCTGCCCAGACACCTGAGGCA GACCATCCAGAAGAAAGTCGGCGAGCCCGTGAACCTGCTGATCCCATTTCAA GGCAAGCCCAGACCTCAAGTGACCTGGACCAAAGAGGGACAGCCTCTGGCC GGCGAAGAGGTGTCCATCAGAAACAGCCCCACCGACACCATCCTGTTCATCA GAGCCGCCAGAAGGGTGCACTCCGGCACCTATCAAGTGACTGTGCGGATCG AGAACATGGAAGATAAGGCCACACTGGTGCTGCAGGTCGTGGACAAACCCA GTCCTCCTCAGGACCTGAGAGTGACAGATGCCTGGGGACTGAACGTGGCCC TGGAATGGAAACCTCCACAGGACGTGGGCAACACAGAGCTGTGGGGCTATA CCGTGCAGAAAGCCGACAAAAAGACCATGGAATGGTTCACCGTGCTGGAAC ACTACCGGCGGACCCATTGTGTGGTGCCTGAGCTGATCATCGGCAACGGCTA CTACTTCCGGGTGTTCAGCCAGAATATGGTCGGATTCAGCGACAGGGCCGCC

		ACCACAAAAGAACCTGTGTTCATCCCCAGACCTGGCATCACCTACGAGCCTC CAAACTACAAGGCCCTGGATTTCAGCGAGGCCCCTAGATTCACACAGCCCCT GGTCAACAGAAGCGTGATCGCCGGCTACACCGCCATGCTGTGTTGTGCCGTT AGAGGCAGCCCCAAGCCTAAGATCTCCTGGTTCAAAAACGGCCTGGACCTCG GCGAGGACGCCCGGTTTAGAATGTTTAGCAAGCAGGGCGTGCTGACCCTGG AAATCAGAAAGCCCTGTCCTTTCGACGGCGGCATCTACGTGTGCAGAGCCAC CAATCTGCAGGGCGAAGCTAGATGCGAGTGCAGACTGGAAGTTCGGGTGCC CCAGTGATGATGAGCGGCCGCCGGCCGAATAAAAGATCCTTATTTTCATTGG ATCTGTGTGTTGGTTTTTTGTGTGGTCGACTCTAGAGGAACCCCTAGTGATG GAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGAC CAAAGGTCGCCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCGAGC GAGCGCGCAGAGAGGGAGTGGCCAA
20	ITR-L	TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGG CCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCC GGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGA GTGGCCAACTCCATCACTAGGGGTTCCT
21	spacer	CTAGAGGTACCTGTACA
22	alphaMHC enhancer	CCTTCAGATTAAAAATAACTAAGGTAAGGGCCATGTGGG TAGGGGAGGTGGTGTGAGACGGTCCTGTCTCTCCTCTAT CTGCCCATCGGCCCTTTGGGGAGGAGGAATGTGCCCAAG GACTAAAAAAAGGCCCTGGAGCCAGAGGGGCGAGGGCA GCAGACCTTTCATGGGCAAACCTCAGGGCTGCTGTC
23	spacer	GTCGA
24	Mini CMV promoter	GGTAGGCGTGTACGGTGGGAGGCCTATATAAGCAGAGC T
25	spacer	CGTTTAGTGAACCGTCAGATCGCCTGGAGGAATTC
26	sd/sa	GTAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCA ATAGAAACTGGGCTTGTCGAGACAGAGAAGACTCTTGC GTTTCTGATAGGCACCTATTGGTCTTACTGACATCCACTT TGCCTTTCTCTCCACAG
27	spacer	AAGCTTGCTAGCGTTTAAACTTAAG
28	Kozac sequence	AGCCCCAAC
29	MYBPC3	ATGCCTGAGCCTGGCAAGAAACCTGTGTCTGCCTTCAGC AAGAAGCCCAGATCTGTTGAGGTGGCAGCTGGCAGCCCT GCTGTGTTTGAGGCTGAGACAGAAAGAGCTGGGGTCAA AGTCAGATGGCAGAGAGGAGGCTCTGACATCTCTGCCA GCAACAAATATGGCCTGGCCACAGAGGGCACCAGACAC ACCCTGACAGTTAGAGAAGTGGGCCCTGCTGACCAGGG CAGCTATGCTGTGATTGCTGGCTCCAGCAAAGTGAAGTT TGACCTGAAAGTGATTGAGGCAGAGAAGGCTGAGCCCA TGCTTGCTCCAGCTCCAGCACCAGCTGAAGCTACTGGTG CTCCTGGGGAAGCTCCTGCTCCTGCTGCTGAACTTGGAG AGTCTGCCCCATCTCCTAAGGGCTCTAGCTCTGCTGCCCT GAATGGACCTACACCTGGGGCTCCAGATGACCCCATTGG CCTGTTTGTGATGAGGCCCCAGGATGGGGAAGTGACAGT TGGAGGCAGCATCACCTTTTCTGCCAGAGTGGCTGGGGC CAGCCTGCTGAAACCTCCTGTGGTCAAGTGGTTCAAAGG CAAATGGGTTGACCTGTCCTCCAAAGTGGGCCAGCACCT CCAGCTGCATGACAGCTATGATAGGGCCAGCAAGGTGT

		ACCTGTTTGAGCTGCACATCACAGATGCCCAGCCAGCCT TCACAGGCAGCTACAGATGTGAAGTGTCCACCAAGGAC AAGTTTGACTGCAGCAACTTCAACCTGACAGTGCATGAG GCCATGGGCACAGGGGACCTTGATCTGCTGTCAGCCTTT AGAAGAACCAGCCTGGCTGGTGGTGGCAGAAGAATCTC TGACAGCCATGAGGACACAGGCATCCTGGACTTCAGCTC CCTGCTGAAGAAGAGAGACAGCTTCAGAACCCCTAGAG ACAGCAAGCTGGAAGCCCCAGCTGAGGAAGATGTCTGG GAGATTCTGAGACAGGCCCCTCCATCTGAGTATGAGAGA ATTGCCTTCCAGTATGGGGTCACAGACCTGAGAGGCATG CTGAAGAGACTGAAGGGCATGAGAAGAGATGAGAAGAA GTCCACAGCCTTCCAGAAGAAGCTGGAACCTGCCTACCA GGTGTCCAAGGGCCACAAGATCAGACTGACAGTGGAAC TGGCTGACCATGATGCTGAAGTGAAGTGGCTGAAGAAT GGCCAAGAGATCCAAATGTCTGGCAGCAAGTACATCTTT GAGAGCATTGGAGCCAAGAGGACCCTGACCATCAGCCA GTGTAGCCTGGCAGATGATGCAGCCTATCAGTGTGTTGT TGGTGGTGAAAAGTGCAGCACAGAGCTGTTTGTCAAAG AGCCTCCAGTCCTGATCACCAGACCTCTGGAAGATCAGC TGGTCATGGTTGGACAGAGGGTTGAGTTTGAGTGTGAAG TCTCTGAAGAGGGTGCCCAAGTCAAATGGCTGAAAGAT GGGGTTGAGCTGACCAGAGAGGAAACCTTCAAGTACAG GTTCAAGAAGGATGGCCAGAGGCACCACCTGATCATCA ATGAAGCCATGCTGGAAGATGCTGGCCACTATGCCCTGT GCACAAGTGGTGGACAAGCCCTGGCTGAGCTGATTGTGC AAGAAAAGAAACTGGAAGTGTACCAGAGCATTGCTGAC CTGATGGTTGGAGCTAAGGACCAGGCTGTGTTTAAATGT GAAGTTTCAGATGAGAATGTCAGAGGAGTGTGGCTCAA AAATGGCAAAGAACTGGTGCCTGACTCCAGGATCAAGG TGTCCCACATTGGCAGAGTGCACAAGCTGACAATTGATG ATGTGACCCCTGCTGATGAGGCTGACTACAGCTTTGTGC CTGAGGGCTTTGCCTGCAACCTGTCTGCCAAGCTGCACT TCATGGAAGTCAAGATTGACTTTGTGCCCAGGCAAGAGC CACCTAAGATCCACCTGGATTGCCCTGGCAGAATCCCAG ACACCATTGTGGTTGTGGCTGGCAACAAGCTGAGACTGG ATGTGCCCATCTCTGGGGACCCTGCTCCTACAGTGATTT GGCAGAAGGCCATCACACAGGGCAACAAGGCTCCAGCC AGACCAGCTCCTGATGCTCCTGAGGATACTGGGGACTCT GATGAGTGGGTGTTTGACAAGAAACTGCTGTGTGAAACT GAGGGCAGAGTCAGAGTGGAAACCACAAAGGACAGATC CATCTTCACAGTGGAAGGGGCTGAGAAAGAGGATGAAG GGGTCTACACAGTGACAGTGAAGAACCCTGTGGGAGAA GATCAAGTGAACCTGACTGTGAAAGTCATTGATGTGCCA GATGCTCCAGCAGCTCCCAAGATCAGCAATGTTGGAGAG GACAGCTGCACAGTGCAGTGGGAGCCACCAGCCTATGA TGGTGGACAGCCTATCCTGGGCTACATCCTGGAAAGAAA GAAGAAAAAGTCCTACAGATGGATGAGGCTCAACTTTG ATCTGATCCAAGAGCTGAGCCATGAAGCTAGAAGGATG ATTGAAGGGGTTGTGTATGAGATGAGAGTGTATGCAGTG AATGCCATTGGCATGAGCAGACCCTCTCCAGCCTCTCAG CCTTTCATGCCCATTGGACCACCATCTGAGCCCACACAC

		CTGGCAGTGGAAGATGTGTCTGACACCACAGTGTCCCTG AAGTGGAGGCCACCTGAGAGAGTTGGAGCTGGAGGCCT GGATGGCTACTCTGTGGAATACTGCCCAGAGGGCTGCTC TGAGTGGGTTGCAGCTCTGCAGGGACTGACAGAGCACA CCTCCATCCTGGTCAAGGATCTGCCTACAGGGGCTAGAC TGCTGTTCAGAGTTAGGGCCCACAACATGGCTGGACCAG GGGCTCCTGTGACAACCACAGAACCTGTGACTGTGCAAG AGATTCTGCAGAGGCCCAGACTGCAGCTGCCTAGACACC TGAGGCAGACCATCCAAAAGAAAGTGGGAGAGCCTGTG AACCTGCTGATCCCATTCCAAGGCAAGCCCAGACCTCAA GTGACCTGGACAAAAGAGGGACAGCCCCTGGCTGGGGA AGAAGTCTCCATCAGAAACAGCCCCACTGACACCATCCT GTTCATCAGAGCTGCCAGAAGAGTGCATTCTGGCACCTA CCAAGTGACTGTCAGAATTGAGAACATGGAAGATAAGG CCACACTGGTGCTGCAGGTTGTGGATAAGCCCAGTCCTC CTCAGGATCTGAGAGTGACAGATGCCTGGGGACTGAAT GTGGCCCTGGAATGGAAACCTCCACAGGATGTGGGCAA CACTGAGCTGTGGGGCTATACAGTGCAGAAGGCAGATA AGAAAACCATGGAATGGTTCACAGTGCTGGAACACTAT AGAAGGACCCACTGTGTGGTGCCAGAGCTGATCATTGGC AATGGCTACTACTTCAGGGTGTTCAGCCAGAATATGGTT GGATTCTCTGATAGGGCAGCCACCACAAAAGAACCAGT GTTCATCCCCAGACCTGGCATCACCTATGAGCCTCCAAA CTACAAGGCCCTGGATTTCTCTGAGGCCCCTAGCTTCAC CCAGCCTCTGGTCAATAGATCAGTGATTGCAGGCTATAC TGCCATGCTGTGCTGTGCAGTCAGAGGCAGCCCAAAGCC TAAGATCTCCTGGTTTAAGAATGGACTGGACCTTGGGGA AGATGCCAGATTCAGAATGTTCAGCAAGCAAGGGGTGC TGACCCTGGAAATCAGAAAGCCCTGTCCTTTTGATGGTG GTATCTATGTGTGCAGGGCTACCAACCTCCAGGGTGAAG CAAGATGTGAATGCAGGCTGGAAGTTAGAGTGCCCCAG TGATGATGAGCGGCCGC
30	STOP	TGATGATGAGC
31	spacer	GGCCGCCGGCCG
32	polyA	AATAAAAGATCCTTATTTTCATTGGATCTGTGTGTTGGTT TTTTGTGTG
33	spacer	GTCGACTCTAG
34	ITR-R	AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGC GCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCG CCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCG AGCGAGCGCGCAGAGAGGGAGTGGCCAA

[00057] aMHC-mCMV-MYBPC3 Construct

35	ITR-L	TTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGG CCGGGCGACCAAAGGTCGCCCGACGCCCGGGCTTTGCCC GGGCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGA GTGGCCAACTCCATCACTAGGGGTTCCT
36	spacer	tctagaggcgcgccaagcttggtacCTGTACA
37	alphaMHC enhancer	CCTTCAGATTAAAAATAACTAAGGTAAGGGCCATGTGGG TAGGGGAGGTGGTGTGAGACGGTCCTGTCTCTCCTCTAT

		CTGCCCATCGGCCCTTTGGGGAGGAGGAATGTGCCCAAG GACTAAAAAAAGGCCCTGGAGCCAGAGGGGCGAGGGCA GCAGACCTTTCATGGGCAAACCTCAGGGCTGCTGTC
38	spacer	GTCGA
39	Mini CMV promoter	GGTAGGCGTGTACGGTGGGAGGCCTATATAAGCAGAGC TCGTTTAGTGAACCGTCAGATCGCCTGGAG
40	spacer	GAATTC
41	sd/sa Chimeric Intron	GTAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCA ATAGAAACTGGGCTTGTCGAGACAGAGAAGACTCTTGC GTTTCTGATAGGCACCTATTGGTCTTACTGACATCCACTT TGCCTTTCTCTCCACAG
42	spacer	AAGCTTGCCACC
43	MYBPC3	ATGCCTGAGCCTGGCAAGAAACCTGTGTCTGCCTTCAGC AAGAAGCCCAGATCTGTTGAGGTGGCAGCTGGCAGCCCT GCTGTGTTTGAGGCTGAGACAGAAAGAGCTGGGGTCAA AGTCAGATGGCAGAGAGGAGGCTCTGACATCTCTGCCA GCAACAAATATGGCCTGGCCACAGAGGGCACCAGACAC ACCCTGACAGTTAGAGAAGTGGGCCCTGCTGACCAGGG CAGCTATGCTGTGATTGCTGGCTCCAGCAAAGTGAAGTT TGACCTGAAAGTGATTGAGGCAGAGAAGGCTGAGCCCA TGCTTGCTCCAGCTCCAGCACCAGCTGAAGCTACTGGTG CTCCTGGGGAAGCTCCTGCTCCTGCTGCTGAACTTGGAG AGTCTGCCCCATCTCCTAAGGGCTCTAGCTCTGCTGCCCT GAATGGACCTACACCTGGGGCTCCAGATGACCCCATTGG CCTGTTTGTGATGAGGCCCCAGGATGGGGAAGTGACAGT TGGAGGCAGCATCACCTTTTCTGCCAGAGTGGCTGGGGC CAGCCTGCTGAAACCTCCTGTGGTCAAGTGGTTCAAAGG CAAATGGGTTGACCTGTCCTCCAAAGTGGGCCAGCACCT CCAGCTGCATGACAGCTATGATAGGGCCAGCAAGGTGT ACCTGTTTGAGCTGCACATCACAGATGCCCAGCCAGCCT TCACAGGCAGCTACAGATGTGAAGTGTCCACCAAGGAC AAGTTTGACTGCAGCAACTTCAACCTGACAGTGCATGAG GCCATGGGCACAGGGGACCTTGATCTGCTGTCAGCCTTT AGAAGAACCAGCCTGGCTGGTGGTGGCAGAAGAATCTC TGACAGCCATGAGGACACAGGCATCCTGGACTTCAGCTC CCTGCTGAAGAAGAGAGACAGCTTCAGAACCCCTAGAG ACAGCAAGCTGGAAGCCCCAGCTGAGGAAGATGTCTGG GAGATTCTGAGACAGGCCCCTCCATCTGAGTATGAGAGA ATTGCCTTCCAGTATGGGGTCACAGACCTGAGAGGCATG CTGAAGAGACTGAAGGGCATGAGAAGAGATGAGAAGAA GTCCACAGCCTTCCAGAAGAAGCTGGAACCTGCCTACCA GGTGTCCAAGGGCCACAAGATCAGACTGACAGTGGAAC TGGCTGACCATGATGCTGAAGTGAAGTGGCTGAAGAAT GGCCAAGAGATCCAAATGTCTGGCAGCAAGTACATCTTT GAGAGCATTGGAGCCAAGAGGACCCTGACCATCAGCCA GTGTAGCCTGGCAGATGATGCAGCCTATCAGTGTGTTGT TGGTGGTGAAAAGTGCAGCACAGAGCTGTTTGTCAAAG AGCCTCCAGTCCTGATCACCAGACCTCTGGAAGATCAGC TGGTCATGGTTGGACAGAGGGTTGAGTTTGAGTGTGAAG TCTCTGAAGAGGGTGCCCAAGTCAAATGGCTGAAAGAT

		GGGGTTGAGCTGACCAGAGAGGAAACCTTCAAGTACAG GTTCAAGAAGGATGGCCAGAGGCACCACCTGATCATCA ATGAAGCCATGCTGGAAGATGCTGGCCACTATGCCCTGT GCACAAGTGGTGGACAAGCCCTGGCTGAGCTGATTGTGC AAGAAAAGAAACTGGAAGTGTACCAGAGCATTGCTGAC CTGATGGTTGGAGCTAAGGACCAGGCTGTGTTTAAATGT GAAGTTTCAGATGAGAATGTCAGAGGAGTGTGGCTCAA AAATGGCAAAGAACTGGTGCCTGACTCCAGGATCAAGG TGTCCCACATTGGCAGAGTGCACAAGCTGACAATTGATG ATGTGACCCCTGCTGATGAGGCTGACTACAGCTTTGTGC CTGAGGGCTTTGCCTGCAACCTGTCTGCCAAGCTGCACT TCATGGAAGTCAAGATTGACTTTGTGCCCAGGCAAGAGC CACCTAAGATCCACCTGGATTGCCCTGGCAGAATCCCAG ACACCATTGTGGTTGTGGCTGGCAACAAGCTGAGACTGG ATGTGCCCATCTCTGGGGACCCTGCTCCTACAGTGATTT GGCAGAAGGCCATCACACAGGGCAACAAGGCTCCAGCC AGACCAGCTCCTGATGCTCCTGAGGATACTGGGGACTCT GATGAGTGGGTGTTTGACAAGAAACTGCTGTGTGAAACT GAGGGCAGAGTCAGAGTGGAAACCACAAAGGACAGATC CATCTTCACAGTGGAAGGGGCTGAGAAAGAGGATGAAG GGGTCTACACAGTGACAGTGAAGAACCCTGTGGGAGAA GATCAAGTGAACCTGACTGTGAAAGTCATTGATGTGCCA GATGCTCCAGCAGCTCCCAAGATCAGCAATGTTGGAGAG GACAGCTGCACAGTGCAGTGGGAGCCACCAGCCTATGA TGGTGGACAGCCTATCCTGGGCTACATCCTGGAAAGAAA GAAGAAAAAGTCCTACAGATGGATGAGGCTCAACTTTG ATCTGATCCAAGAGCTGAGCCATGAAGCTAGAAGGATG ATTGAAGGGGTTGTGTATGAGATGAGAGTGTATGCAGTG AATGCCATTGGCATGAGCAGACCCTCTCCAGCCTCTCAG CCTTTCATGCCCATTGGACCACCATCTGAGCCCACACAC CTGGCAGTGGAAGATGTGTCTGACACCACAGTGTCCCTG AAGTGGAGGCCACCTGAGAGAGTTGGAGCTGGAGGCCT GGATGGCTACTCTGTGGAATACTGCCCAGAGGGCTGCTC TGAGTGGGTTGCAGCTCTGCAGGGACTGACAGAGCACA CCTCCATCCTGGTCAAGGATCTGCCTACAGGGGCTAGAC TGCTGTTCAGAGTTAGGGCCCACAACATGGCTGGACCAG GGGCTCCTGTGACAACCACAGAACCTGTGACTGTGCAAG AGATTCTGCAGAGGCCCAGACTGCAGCTGCCTAGACACC TGAGGCAGACCATCCAAAAGAAAGTGGGAGAGCCTGTG AACCTGCTGATCCCATTCCAAGGCAAGCCCAGACCTCAA GTGACCTGGACAAAAGAGGGACAGCCCCTGGCTGGGGA AGAAGTCTCCATCAGAAACAGCCCCACTGACACCATCCT GTTCATCAGAGCTGCCAGAAGAGTGCATTCTGGCACCTA CCAAGTGACTGTCAGAATTGAGAACATGGAAGATAAGG CCACACTGGTGCTGCAGGTTGTGGATAAGCCCAGTCCTC CTCAGGATCTGAGAGTGACAGATGCCTGGGGACTGAAT GTGGCCCTGGAATGGAAACCTCCACAGGATGTGGGCAA CACTGAGCTGTGGGGCTATACAGTGCAGAAGGCAGATA AGAAAACCATGGAATGGTTCACAGTGCTGGAACACTAT AGAAGGACCCACTGTGTGGTGCCAGAGCTGATCATTGGC AATGGCTACTACTTCAGGGTGTTCAGCCAGAATATGGTT

		GGATTCTCTGATAGGGCAGCCACCACAAAAGAACCAGT GTTCATCCCCAGACCTGGCATCACCTATGAGCCTCCAAA CTACAAGGCCCTGGATTTCTCTGAGGCCCCTAGCTTCAC CCAGCCTCTGGTCAATAGATCAGTGATTGCAGGCTATAC TGCCATGCTGTGCTGTGCAGTCAGAGGCAGCCCAAAGCC TAAGATCTCCTGGTTTAAGAATGGACTGGACCTTGGGGA AGATGCCAGATTCAGAATGTTCAGCAAGCAAGGGGTGC TGACCCTGGAAATCAGAAAGCCCTGTCCTTTTGATGGTG GTATCTATGTGTGCAGGGCTACCAACCTCCAGGGTGAAG CAAGATGTGAATGCAGGCTGGAAGTTAGAGTGCCCCAG TGAT
44	STOP	TGATGATGA
45	spacer	GCGGCCGCtcgag
46	bGH polyA	CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTC CCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCAC TGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTG TCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGG GCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCA GGCATGCTGGGGA
47	spacer	GTCGACGCGCCGGCGTCTAGA
48	ITR-R	AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGC GCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCG CCCGACGCCCGGGCTTTGCCCGGGCGGCCTCAGTGAGCG AGCGAGCGCGCAGAGAGGGAGTGGCCAA

[00058]

SEQ ID:	**Description**	**Protein Sequence**
15	MYBC	MPEPGKKPVSAFSKKPRSVEVAAGSPAVFEAETERAGV KVRWQRGGSDISASNKYGLATEGTRHTLTVREVGPADQ GSYAVIAGSSKVKFDLKVIEAEKAEPMLAPAPAPAEATG APGEAPAPAAELGESAPSPKGSSSAALNGPTPGAPDDPIG LFVMRPQDGEVTVGGSITFSARVAGASLLKPPVVKWFK GKWVDLSSKVGQHLQLHDSYDRASKVYLFELHITDAQP AFTGSYRCEVSTKDKFDCSNFNLTVHEAMGTGDLDLLS AFRRTSLAGGGRRISDSHEDTGILDFSSLLKKRDSFRTPR DSKLEAPAEEDVWEILRQAPPSEYERIAFQYGVTDLRGM LKRLKGMRRDEKKSTAFQKKLEPAYQVSKGHKIRLTVE LADHDAEVKWLKNGQEIQMSGSKYIFESIGAKRTLTISQ CSLADDAAYQCVVGGEKCSTELFVKEPPVLITRPLEDQL VMVGQRVEFECEVSEEGAQVKWLKDGVELTREETFKY RFKKDGQRHHLIINEAMLEDAGHYALCTSGGQALAELIV QEKKLEVYQSIADLMVGAKDQAVFKCEVSDENVRGVW LKNGKELVPDSRIKVSHIGRVHKLTIDDVTPADEADYSF VPEGFACNLSAKLHFMEVKIDFVPRQEPPKIHLDCPGRIP DTIVVVAGNKLRLDVPISGDPAPTVIWQKAITQGNKAPA RPAPDAPEDTGDSDEWVFDKKLLCETEGRVRVETTKDR SIFTVEGAEKEDEGVYTVTVKNPVGEDQVNLTVKVIDVP DAPAAPKISNVGEDSCTVQWEPPAYDGGQPILGYILERK KKKSYRWMRLNFDLIQELSHEARRMIEGVVYEMRVYA

		VNAIGMSRPSPASQPFMPIGPPSEPTHLAVEDVSDTTVSL KWRPPERVGAGGLDGYSVEYCPEGCSEWVAALQGLTE HTSILVKDLPTGARLLFRVRAHNMAGPGAPVTTTEPVTV QEILQRPRLQLPRHLRQTIQKKVGEPVNLLIPFQGKPRPQ VTWTKEGQPLAGEEVSIRNSPTDTILFIRAARRVHSGTYQ VTVRIENMEDKATLVLQVVDKPSPPQDLRVTDAWGLNV ALEWKPPQDVGNTELWGYTVQKADKKTMEWFTVLEH YRRTHCVVPELIIGNGYYFRVFSQNMVGFSDRAATTKEP VFIPRPGITYEPPNYKALDFSEAPRFTQPLVNRSVIAGYTA MLCCAVRGSPKPKISWFKNGLDLGEDARFRMFSKQGVL TLEIRKPCPFDGGIYVCRATNLQGEARCECRLEVRVPQ
16	Rh74 VP1 (VP2, VP3)	MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQ KQDNGRGLVLPGYKYLGPFNGLDKGEPVNAADAAALEH DKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN LGRAVFQAKKRVLEPLGLVESPVKTAPGKKRPVEPSPQR SPDSSTGIGKKGQQPAKKRLNFGQTGDSESVPDPQPIGEPP AGPSGLGSGTMAAGGGAPMADNNEGADGVGSSSGNWH CDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGS TNDNTYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNW GFRPKRLNFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFT DSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNG SQAVGRSSFYCLEYFPSQMLRTGNNFEFSYNFEDVPFHSS YAHSQSLDRLMNPLIDQYLYYLSRTQSTGGTAGTQQLLF SQAGPNNMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNF AWTGATKYHLNGRDSLVNPGVAMATHKDDEERFFPSSG VLMFGKQGAGKDNVDYSSVMLTSEEEIKTTNPVATEQY GVVADNLQQQNAAPIVGAVNSQGALPGMVWQNRDVYL QGPIWAKIPHTDGNFHPSPLMGGFGLKHPPPQILIKNTPVP ADPPTTFNQAKLASFITQYSTGQVSVEIEWELQKENSKRW NPEIQYTSNYYKSTNVDFAVNTEGTYSEPRPIGTRYLTRN L
17	AAV9 VP1	MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQ HQDNARGLVLPGYKYLGPGNGLDKGEPVNAADAAALE HDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGG NLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQ EPDSSAGIGKSGAQPAKKRLNFGQTGDTESVPDPQPIGEP PAAPSGVGSLTMASGGGAPVADNNEGADGVGSSSGNWH CDSQWLGDRVITTSTRTWALPTYNNHLYKQISNSTSGGSS NDNAYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWG FRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTD SDYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGS QAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENVPFHSSY AHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQTLKFSV AGPSNMAVQGRNYIPGPSYRQQRVSTTVTQNNNSEFAWP GASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIF GKQGTGRDNVDADKVMITNEEEIKTTNPVATESYGQVAT NHQSAQAQAQTGWVQNQGILPGMVWQDRDVYLQGPIW AIPHTDGNFHPSPLMGGFGMKHPPPQILIKNTPVPADPPTA FNKDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEIQY TSNYYKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL

[00059] Kozak Sequences

SEQ ID NO:	Gene	Kozak sequence
49	*MYH7*	GGCACAGCC
50	*ACTC1*	TGTGCCAAG
51	*TNNI3*	AGTCTCAGC
52	*MYL7*	GCAGAGAGA
53	*NPPA*	TCCAGAGAC
54	*NPPB*	TCCAGAGAC
55	*TNNI2*	GACCTCAGG
56	*MYBPC3*	TCTCTCAGG
57	*MYL4*	CAAGACAAC
58	*MYBPHL*	AGGCCCAGC
59	*MYH6*	AGCACCAAG
60	*LRRC10*	AGCCTCCGC
61	*ACTC1*	TGTGCCAAG
62	*RD3L*	AGGCTAAAA

The Transgene

[00060] A transgene may be employed to correct, reduce, eliminate, or otherwise ameliorate gene deficiencies, which may include deficiencies in which normal genes are expressed at less than normal levels, are expressed at normal or near-normal levels but having a gene product with abnormal activity, or deficiencies in which the functional gene product is not expressed. In several embodiments, the transgene sequence encodes a therapeutic protein or polypeptide which is to be expressed in a host cell. Embodiments of the present disclosure also include using multiple transgenes.

[00061] The MYBPC3 gene provides instructions for making cardiac myosin binding protein C (cardiac MyBP-C). Cardiac MyBP-C is found in cardiac muscle cells, where it plays a role in sarcomere contraction. Muscle contraction depends heavily on the activity of sarcomeres resident in myocytes. Mutations in MYBPC3 are common causes of familial hypertrophic cardiomyopathy, accounting for up to 30% of all cases. Though some individuals have no obvious health effects, all affected individuals possess an increased risk of heart failure and sudden death. MYBPC3 mutations generally present phenotypically shorter or otherwise altered MyBP-C proteins. Reduced MyBPC in the sarcomere disrupts myosin conformations, which may contribute to various cardiac disease states.

Regulatory Elements

[00062] In some embodiments, the rAAV vector comprises one or more regions comprising a sequence that facilitates expression of the heterologous nucleic acid, *e.g.*, expression regulatory sequences operatively linked to the heterologous nucleic acid. A promoter drives transcription of the nucleic acid sequence that it regulates, thus, it is typically located at or near the transcriptional start site of a gene. A promoter may have, for example, a length of 100 to 1000 nucleotides. In some embodiments, a promoter is operably linked to a nucleic acid, or a sequence of a nucleic acid (nucleotide sequence). A promoter is considered to be "operably linked" to a sequence of nucleic acid that it regulates when the promoter is in a correct functional location and orientation relative to the sequence such that the promoter regulates (e.g., to control ("drive") transcriptional initiation and/or expression of) that sequence. Numerous such sequences are known in the art.

[00063] Promoters that may be used in accordance with the present disclosure may comprise any promoter that can drive the expression of the transgenes in the heart of the subject. In some embodiments, the promoter may be a tissue- specific promoter. A "tissue-specific promoter", as used herein, refers to promoters that can only function in a specific type of tissue, e.g., the heart. Thus, a "tissue- specific promoter" is not able to drive the expression of the transgenes in other types of tissues. In some embodiments, the promoter that may be used in accordance with the present disclosure is a cardiac-restricted promoter. Non-limiting examples of Tissue-specific promoters and/or regulatory elements that may be used include (1) desmin, creatine kinase, myogenin, alpha myosin heavy chain, and natriuretic peptide, specific for muscle cells, and (2) albumin, alpha-1-antitrypsin, hepatitis B virus core protein promoters, specific for liver cells. Non-limiting examples of cardiac-restricted promoter selected from cardiac troponin C, cardiac troponin I, and cardiac troponin T (cTnT). In treating cardiomyopathies as provided for herein, cardiac-restricted promoters are advantageous at least due to the reduced possibility of off-target expression of the transgene(s), thereby effectively increasing the delivered dose to the heart and enhancing therapy. Non-limiting examples of expression regulatory sequences include promoters, insulators, silencers, response elements, introns, enhancers, initiation sites, termination signals, and poly(A) tails. Any combination of such regulatory sequences is contemplated herein (*e.g.*, a promoter and an enhancer).

[00064] Alternatively, the promoter may be, without limitation, a promoter from one of the following genes: α-myosin heavy chain gene, 6- myosin heavy chain gene, myosin light chain 2v (MLC-2v) gene, myosin light chain 2a gene, CARP gene, cardiac α-actin gene, cardiac m2 muscarinic acetylcholine gene, atrial natriuretic factor gene (ANF), cardiac sarcoplasmic

reticulum Ca-ATPase gene, skeletal α-actin gene; or an artificial cardiac promoter derived from MLC-2v gene.

[00065] To achieve appropriate expression levels of the nucleic acid, protein, or polypeptide of interest, any of a number of promoters suitable for use in the selected host cell may be employed. The promoter may be, for example, a constitutive promoter, tissue-specific promoter, inducible promoter, or a synthetic promoter. For example, constitutive promoters of different strengths can be used. An rAAV vector described herein may include one or more constitutive promoters, such as viral promoters or promoters from mammalian genes that are generally active in promoting transcription. Non-limiting examples of constitutive viral promoters include the Herpes Simplex virus (HSV), thymidine kinase (TK), Rous Sarcoma Virus (RSV), Simian Virus 40 (SV40), Mouse Mammary Tumor Virus (MMTV), Ad E1A and cytomegalovirus (CMV) promoters. Non-limiting examples of non-viral constitutive promoters include various housekeeping gene promoters, as exemplified by the β-actin promoter, including the chicken β-actin promoter (CBA).

[00066] Inducible promoters and/or regulatory elements may also be contemplated for achieving appropriate expression levels of the protein or polypeptide of interest. Non-limiting examples of suitable inducible promoters include those from genes such as cytochrome P450 genes, heat shock protein genes, metallothionein genes, and hormone-inducible genes, such as the estrogen gene promoter. Another example of an inducible promoter is the tetVP16 promoter that is responsive to tetracycline.

[00067] Synthetic promoters are also contemplated herein. A synthetic promoter may comprise, for example, regions of known promoters, regulatory elements, transcription factor binding sites, enhancer elements, repressor elements, and the like.

[00068] Enhancer elements can function in combination with other regulatory elements to increase the expression of a transgene. In several embodiments, the enhancer elements are upstream (positioned 5') of the transgene. Non-limiting embodiments of enhancer elements include nucleotide sequences comprising, for example, a 100 base pair element from Simian virus 40 (SV40 late 2XUSE), a 35 base pair element from Human Immunodeficiency Virus 1(HIV-1 USE), a 39 base pair element from ground squirrel hepatitis virus (GHV USE), a 21 base pair element from adenovirus (Adenovirus L3 USE), a 21 base pair element from human prothrombin (hTHGB USE), a 53 base pair element from human C2 complement gene (hC2 USE), truncations of any of the foregoing, and combinations of the foregoing. In some embodiments the enhancer is derived from the α-myosin heavy chain (αMHC) gene. In some embodiments the αMHC enhancer comprises SEQ ID NO: 3.

[00069] Non-limiting polyadenylation signals include nucleotide sequences comprising, for example, a 624 base pair polyadenylation signal from human growth hormone (hGH), a 135 base pair polyadenylation signal from simian virus 40 (sV40 late), a 49 base pair synthetic polyadenylation signal from rabbit beta-globin (SPA), a 250 base pair polyadenylation signal from bovine growth hormone (bGH), truncations of any of the foregoing, and combinations of the foregoing.

[00070] In some embodiments of the disclosed rAAV vectors, the two or more transgenes are operably controlled by a single promoter. In some embodiments, each of the two or more transgenes are operably controlled by a distinct promoter.

[00071] In some embodiments, the rAAV vectors of the present disclosure further comprise an Internal Ribosome Entry Site (IRES). An IRES is a nucleotide sequence that allows for translation initiation in the middle of a messenger RNA (mRNA) sequence as part of the greater process of protein synthesis. Usually, in eukaryotes, translation can be initiated only at the 5' end of the mRNA molecule, since 5' cap recognition is required for the assembly of the initiation complex. In some embodiments, the IRES is located between the transgenes.

[00072] In such embodiments, the proteins encoded by different transgenes are translated individually (i.e., versus translated as a fusion protein).

[00073] In some embodiments, the rAAV vectors of the present disclosure comprise at least, in order from 5' to 3', a first adeno-associated vims (AAV) inverted terminal repeat (ITR) sequence, a promoter operably linked to a first transgene, an IRES operably linked to a second transgene, a polyadenylation signal, and a second AAV inverted terminal repeat (ITR) sequence.

[00074] In some embodiments, the rAAV vectors of the present disclosure further comprise a polyadenylation (pA) signal.

Expression Cassette

[00075] The expression cassette is composed of, at a minimum, a transgene and its regulatory sequences. Where the cassette is designed to be expressed from a rAAV, the expression cassette further contains 5′ and 3′ AAV ITRs. These ITR's may be full-length, or one or both of the ITRs may be truncated. In one embodiment, the rAAV is pseudotyed, i.e., the AAV capsid is from a different source AAV than that the AAV which provides the ITRs. In one embodiment, the ITRs of AAV serotype 2 are used. In additional embodiments, the ITRs of AAV serotype 1 are used. However, ITRs from other suitable sources may be selected.

[00076] FIG. 1 depicts a non-limiting embodiment of the construct described herein. At the 5’ end, an AAV ITR and alphaMHC enhancer are positioned upstream from a promoter.

Following the promoter, the MYBPC3 transgene, consisting of multiple exons is depicted. The construct further includes a polyadenylated site following the MYBPC3 transgene. Within the structural sequences described in the aforementioned construct, at least one or a plurality of spacer sequences may be inserted at any point within the construct. Additionally, any number of promoter or regulatory sequences may comprise a construct to alter or change the expression of MYBPC3.

[00077] FIG. 2 depicts a non-limiting embodiment of a construct described herein. At the 5' end, an AAV ITR and alphaMHC enhancer are positioned upstream from a CMV promoter. Following the promoter, the MYBPC3 transgene is depicted. The construct further includes a bGH polyadenylated site following the MYBPC3 transgene. Within the structural sequences described in the aforementioned construct, at least one or a plurality of spacer sequences may be inserted at any point within the construct. Additionally, any number of promoter or regulatory sequences may comprise a construct to alter or change the expression of MYBPC3.

The Vector

[00078] Further provided herein are rAAV viral particles or rAAV preparations containing such particles. In several embodiments, rAAV particles comprise a viral capsid and one or more transgenes as described herein, which is encapsidated by the viral capsid. Methods of producing rAAV particles are known in the art and are commercially available (see, e.g., Zolotukhin el al. Production and purification of serotype 1, 2, and 5 recombinant adeno-associated viral vectors. Methods 28 (2002) 158-167; and U.S. Patent Application Publication Numbers US 2007/0015238 and US 2012/0322861, which are incorporated herein by reference; and plasmids and kits available from ATCC and Cell Biolabs, Inc.). For example, a plasmid containing the rAAV vector may be combined with one or more helper plasmids, e.g., that contain a rep gene (e.g., encoding Rep78, Rep68, Rep52 and Rep40) and a cap gene (encoding VP1, VP2, and VP3, including a modified VP3 region as described herein), and transfected into a producer cell line such that the rAAV particle can be packaged and subsequently purified.

[00079] The rAAV particles or particles within an rAAV preparation disclosed herein, may be of any AAV serotype, including any derivative or pseudotype (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 2/1, 2/5, 2/8, 2/9, 3/1, 3/5, 3/8, or 3/9). As used herein, the serotype of an rAAV an rAAV particle refers to the serotype of the capsid proteins of the recombinant virus. In some embodiments, the rAAV particle is rAAV6 or rAAV9. In some embodiments, the rAAV particle is AAVrh.74. In a preferred embodiment, the rAAV particle is AAVrh74. In an additional preferred embodiment, the rAAV is AAV9. In several embodiments, an rh74 AAV is mutated to

advantageously enhance delivery to cardiac tissue, for example by a tryptophan to arginine mutation at amino acid 505 of VP1 capsid, or other mutations, as described in PCT Publication WO 2019/1784412, which is incorporated in its entirety by reference herein. Non-limiting examples of derivatives, pseudotypes, and/or other vector types include, but are not limited to, AAVrh.10, AAVrh.74, AAV2/1, AAV2/5, AAV2/6, AAV2/8, AAV2/9, AAV2-AAV3 hybrid, AAVhu.14, AAV3a/3b, AAVrh32.33, AAV-HSC15, AAV- HSC17, AAVhu.37, AAVrh.8, CHt-P6, AAV2.5, AAV6.2, AAV2i8, AAV-HSC15/17, AAVM41, AAV9.45, AAV6(Y445F/Y731F), AAV2.5T, AAV-HAE1/2, AAV clone 32/83, AAVShHIO, AAV2 (Y->F), AAV8 (Y733F), AAV2.15, AAV2.4, AAVM41, and AAVr3.45.

[00080] Such AAV serotypes and derivatives/pseudotypes, and methods of producing such derivatives/pseudotypes are known in the art (see, e.g., Mol Ther. 2012 Apr;20(4):699- 708. doi: 10.1038/mt.2011.287. Epub 2012 Jan 24. The AAV vector toolkit: poised at the clinical crossroads. Asokan Al, Schaffer DV, Samulski RJ.). In particular embodiments, the capsid of any of the herein disclosed rAAV particles is of the AAVrh.10 serotype. In some embodiments, the capsid is of the AAV2/6 serotype. In some embodiments, the rAAV particle is a pseudotyped rAAV particle, which comprises (a) an rAAV vector comprising ITRs from one serotype (e.g., AAV2, AAV3) and (b) a capsid comprised of capsid proteins derived from another serotype (e.g., AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, or AAV10). Methods for producing and using pseudotyped rAAV vectors are known in the art (see, e.g., Duan et al, J. Virol., 75:7662-7671, 2001; Halbert et al, J. Virol., 74:1524-1532, 2000; Zolotukhin et al, Methods, 28:158-167, 2002; and Auricchio et al., Hum. Molec. Genet., 10:3075-3081, 2001). rAAV Gene Therapy for Heart Diseases

[00081] In some embodiments, the rAAV vectors of the present disclosure further comprise a polyadenylation (pA) signal. For example, in preferred embodiments the pA signal comprises one or more of the following sequences: 12, or 11-13 in sequence, or 12-13 in sequence, or 32, or 46.

[00082] In some embodiments, the rAAV vectors of the present disclosure comprise at least, in order from 5' to 3', a first adeno-associated vims (AAV) inverted terminal repeat (ITR) sequence, a promoter operably linked to a transgene, a polyadenylation signal, and a second AAV inverted terminal repeat (ITR) sequence.

[00083] In some embodiments, the rAAV vector genome is circular. In some embodiments, the rAAV vector genome is linear. In some embodiments, the rAAV vector genome is single-stranded. In some embodiments, the rAAV vector genome is double- stranded. In some embodiments, the rAAV vector genome is a self- complementary rAAV vector.

[00084] Described herein are non-limiting examples of rAAV vectors. The vectors illustrated below comprise the linearized plasmid sequences set forth as SEQ ID NOs: 1-14, or 20-34, or 35-48. The vectors of the disclosure may comprise nucleotide sequences that have at least 70% identity, at least about 80% identity, at least about 90% identity, at least about 95% identity, at least about 96% identity, at least about 97% identity, at least about 98% identity, at least about 99% identity, at least about 99.5% identity, or at least about 99.9% identity to the sequences set forth as SEQ ID NOs: 1-14, or 20-34, or 35-48. In several embodiments, the rAAV comprises one or more nucleotide sequences that has 100% identity to one or more of the sequences set forth as SEQ ID NOs 1-14, or 20-34, or 35-48.

[00085] In some embodiments, any of the disclosed rAAV nucleic acid vector sequences comprise truncations at the 5' or 3' end relative to the sequences of any one of SEQ ID NOs: 1-14, or 20-34, or 35-48. In some embodiments, any of the rAAV vectors comprise a nucleotide sequence that differs from the sequence of any one of SEQ ID NOs: 1-14, or 20-34, or 35-48 by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or more than 18 nucleotides.

Recombinant Adeno-Associated Virus Vectors and Therapeutic Use Thereof

[00086] Many serotypes of AAV have been cloned and sequenced. Serotypes 1 and 6 share >99% amino acid homology in their capsid proteins. Of the first six AAV serotypes, serotype 2 is widely characterized and therefore often used in gene transfer studies, however according to embodiments disclosed herein, other AAV serotypes are also used, such as AAV9, AAV20, AAVrh74, AAVrh10, and the like. In several embodiments, repeat administration of a given serotype that would be expected to elicit a humoral immune response is performed in connection with an immune management regimen. In several embodiments, an immune management regimen comprises administration of one or more agents that function as B-cell depletors, alone, or in conjunction with one or more agents that inhibit one or more aspects of the mTOR pathway. In one embodiment, an antiCD20 antibody is administered and rapamycin is administered. In several embodiments, this allows for the repeat administration of a given serotype rAAV with reduced, limited or no immune response to a subsequent dosing of the rAAV. Further information about immune management can found in United States Patent Application No. 15/306,139, the entire contents of which is incorporated by reference herein.

[00087] The therapeutic rAAV vectors, therapeutic rAAV particles, or the composition comprising the therapeutic rAAV particles of the present disclosure, may be used for gene therapy for heart diseases in a human subject in need thereof, such as cardiomyopathies as provided for herein. Examples of heart disease that may be treated using the methods and compositions of the

present disclosure include, but are not limited to, cardiomyopathy and acute ischemia. In some embodiments, cardiomyopathy is hypertrophic cardiomyopathy or dilated cardiomyopathy. In some embodiments, the cardiomyopathy is hypertrophic cardiomyopathy and is caused by or associated with reduced or non-existent expression and/or function of MYBPC3. The therapeutic rAAV vectors, particles, and compositions comprising the therapeutic rAAV particles may be used for treatment of such heart failure (e.g., heart failure secondary to cardiomyopathy)when administered to a subject in need thereof, e.g., via vascular delivery into the coronary arteries and/or direct injection to the heart. In some embodiments, administration is via systemic delivery to a subject in need thereof. The therapeutic rAAV vectors, particles, and compositions comprising the rAAV particles drive the concurrent expression of MYBPC3 in the cardiomyocytes of the subject.

[00088] The amino acid sequence of the therapeutic MyBP-C encoded by the MYBPC3 transgene is at least about 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to the amino acid sequence set forth as SEQ ID NO: _15__.

[00089] In some embodiments, there are provided amino acid sequences that correspond to any of the nucleic acids disclosed herein (and/or included in the accompanying sequence listing), while accounting for degeneracy of the nucleic acid code. Furthermore, those sequences (whether nucleic acid or amino acid) that vary from those expressly disclosed herein (and/or included in the accompanying sequence listing), but have functional similarity or equivalency are also contemplated within the scope of the present disclosure. The foregoing includes mutants, truncations, substitutions, or other types of modifications.

[00090] In accordance with some embodiments described herein, any of the sequences may be used, or a truncated or mutated form of any of the sequences disclosed herein (and/or included in the accompanying sequence listing) may be used and in any combination.

[00091] [The promoter driving expression of the therapeutic nucleic acid can be, but is not limited to, a constitutive promoter, an inducible promoter, a tissue-specific promoter, a neuronal- specific promoter, a muscle-specific promoter, or a synthetic promoter. In some embodiments, the promoter is a neuronal-specific promoter or a muscle-specific promoter. A constitutive promoter can be, but is not limited to, a Herpes Simplex virus (HSV) promoter, a thymidine kinase (TK) promoter, a Rous Sarcoma Virus (RSV) promoter, a Simian Virus 40 (SV40) promoter, a Mouse Mammary Tumor Virus (MMTV) promoter, an Adenovirus E1A promoter, a cytomegalovirus (CMV) promoter, a mammalian housekeeping gene promoter, or a β-actin promoter. An inducible promoter can be, but is not limited to, a cytochrome P450 gene

promoter, a heat shock protein gene promoter, a metallothionein gene promoter, a hormone-inducible gene promoter, an estrogen gene promoter, or a tetVP16 promoter that is responsive to tetracycline. A muscle-specific promoter can be, but is not limited to, desmin promoter, a creatine kinase promoter, a myogenin promoter, an alpha myosin heavy chain promoter, or a natriuretic peptide promoter.

[00092] In some embodiments, the therapeutic rAAV promoter comprises a neuronal- or cardiomuscle-specific promoter.

[00093] The therapeutic rAAV can be serotype 1, serotype 2, serotype 3, serotype 4, serotype 5, serotype 6, serotype 7, serotype 8, serotype 9, serotype 10, serotype 11, serotype 12, serotype rh10, or serotype rh74. The therapeutic rAAV can also be a pseudo-type rAAV.

[00094] In some embodiments, the therapeutic rAAV has a sequence sharing at least 85% sequence identity to SEQ ID NO: 1-14, or 20-34, or 35-48 when assembled in sequence.

[00095] In some embodiments, the therapeutic rAAV has a sequence sharing at least 95% sequence identity to SEQ ID NO: 1-14, or 20-34, or 35-48 when assembled in sequence.

Pharmaceutical Formulations and Administration

[00096] Compositions described herein may further comprise a pharmaceutical excipient, buffer, or diluent, and may be formulated for administration to host cell *ex vivo* or *in situ* in an animal, and particularly a human being. Such compositions may further optionally comprise a liposome, a lipid, a lipid complex, a microsphere, a microparticle, a nanosphere, or a nanoparticle, or may be otherwise formulated for administration to the cells, tissues, organs, or body of a subject in need thereof. Such compositions may be formulated for use in a variety of therapies, such as for example, in the amelioration, prevention, and/or treatment of conditions such as peptide deficiency, polypeptide deficiency, peptide overexpression, polypeptide overexpression, including for example, conditions which result in diseases or disorders as described herein.

[00097] Formulations comprising pharmaceutically-acceptable excipients and/or carrier solutions are well-known to those of skill in the art, as is the development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including e.g., oral, parenteral, intravenous, intranasal, intra-articular, and intramuscular administration and formulation.

[00098] Typically, these formulations may contain at least about 0.1% of the therapeutic agent (e.g., therapeutic rAAV particle or preparation) or more, although the percentage of the active ingredient(s) may, of course, be varied and may conveniently be between

about 1 or 2% and about 70% or 80% or more of the weight or volume of the total formulation. Naturally, the amount of therapeutic agent(s) in each therapeutically-useful composition may be prepared in such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art when preparing such pharmaceutical formulations. Additionally, a variety of dosages and treatment regimens may be desirable.

[00099] In certain circumstances, it will be desirable to deliver the therapeutic rAAV particles or preparations, in suitably formulated pharmaceutical compositions disclosed herein; either subcutaneously, intracardially, intraocularly, intravitreally, parenterally, subcutaneously, intravenously, intracerebro-ventricularly, intramuscularly, intrathecally, orally, intraperitoneally, by oral or nasal inhalation, or by direct injection to one or more cells (e.g., cardiomyocytes and/or other heart cells), tissues, or organs. In some embodiments, the therapeutic rAAV particles or the composition comprising the therapeutic rAAV particles of the present invention are delivered systemically via intravenous injection, particularly in those for treating a human. In some embodiments, the therapeutic rAAV particles or the composition comprising the therapeutic rAAV particles of the present invention are injected directly into the heart of the subject. Direct injection to the heart may comprise injection into one or more of the myocardial tissues, the cardiac lining, or the skeletal muscle surrounding the heart, e.g., using a needle catheter. In several embodiments, direct injection to human heart is preferred, for example, if delivery is performed concurrently with a surgical procedure whereby access to the heart is improved.

[000100] The pharmaceutical formulations of the compositions suitable for injectable use include sterile aqueous solutions or dispersions. In some embodiments, the formulation is sterile and fluid to the extent that easy syringability exists. In some embodiments, the form is stable under the conditions of manufacture and storage, and is preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier may be a solvent or dispersion medium containing, for example, water, saline, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, vegetable oils or other pharmaceutically acceptable carriers such as those that are Generally Recognized as Safe (GRAS) by the United States Food and Drug Administration. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In fact, there is virtually no limit to other components that may also be included, as long as the additional agents do not cause a significant adverse effect upon contact with the target cells or host tissues. The

therapeutic rAAV particles or preparations may thus be delivered along with various other pharmaceutically acceptable agents as required in the particular instance. Such compositions may be purified from host cells or other biological sources, or alternatively may be chemically synthesized as described herein.

[000101] The amount of therapeutic rAAV particle or preparation, and/or therapeutic rAAV vector compositions and time of administration of such compositions will be within the purview of the skilled artisan having benefit of the present teachings. It is likely, however, that the administration of therapeutically- effective amounts of the compositions of the present disclosure may be achieved by a single administration, such as for example, a single injection of sufficient numbers of infectious particles to provide therapeutic benefit to the patient undergoing such treatment. In some circumstances, it may be desirable to provide multiple or successive administrations of the rAAV particle or preparation, and/or rAAV vector compositions, either over a relatively short, or a relatively prolonged period of time, as may be determined by the medical practitioner overseeing the administration of such compositions.

[000102] Toxicity and efficacy of the compositions utilized in methods of the present invention may be determined by standard pharmaceutical procedures, using either cells in culture or experimental animals to determine the LD_{50} (the dose lethal to 50% of the population). The dose ratio between toxicity and efficacy the therapeutic index and it may be expressed as the ratio LD_{50}/ED_{50}. Those compositions that exhibit large therapeutic indices are preferred. While compositions that exhibit toxic side effects may be used, care should be taken to design a delivery system that minimizes the potential damage of such side effects. The dosage of compositions as described herein lies generally within a range that includes an ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

[000103] Other aspects of the present disclosure relate to methods and preparations for use with a subject, such as human or non-human subjects, a host cell in situ in a subject, or a host cell derived from a subject. In some embodiments, the subject is a mammal. In some embodiments, the subject is a companion animal. "A companion animal", as used herein, refers to pets and other domestic animals. Non-limiting examples of companion animals include dogs and cats; livestock such as horses, cattle, pigs, sheep, goats, and chickens; and other animals such as mice, rats, guinea pigs, and hamsters. In some embodiments, the subject is a human subject.

[000104] In some embodiments, one or more pharmaceutically acceptable excipients (including vehicles, carriers, diluents, and/or delivery polymers) are added to the pharmaceutical

compositions including a therapeutic, thereby forming a pharmaceutical formulation suitable for in vivo delivery to a subject, such as a human.

[000105] pharmaceutical composition or medicament includes a pharmacologically effective amount of at least one of the therapeutic and optionally one or more pharmaceutically acceptable excipients. Pharmaceutically acceptable excipients (excipients) are substances other than the Active Pharmaceutical ingredient (API, therapeutic product) that are intentionally included in the drug delivery system. Excipients do not exert or are not intended to exert a therapeutic effect at the intended dosage. Excipients may act to a) aid in processing of the drug delivery system during manufacture, b) protect, support or enhance stability, bioavailability or patient acceptability of the API, c) assist in product identification, and/or d) enhance any other attribute of the overall safety, effectiveness, of delivery of the API during storage or use. A pharmaceutically acceptable excipient may or may not be an inert substance.

[000106] Excipients include, but are not limited to: absorption enhancers, anti-adherents, anti-foaming agents, anti-oxidants, binders, buffering agents, carriers, coating agents, colors, delivery enhancers, delivery polymers, dextran, dextrose, diluents, disintegrants, emulsifiers, extenders, fillers, flavors, glidants, humectants, lubricants, oils, polymers, preservatives, saline, salts, solvents, sugars, suspending agents, sustained release matrices, sweeteners, thickening agents, tonicity agents, vehicles, water-repelling agents, and wetting agents.

[000107] The pharmaceutical compositions can contain other additional components commonly found in pharmaceutical compositions. Such additional components can include, but are not limited to: anti-pruritics, astringents, local anesthetics, or anti-inflammatory agents (*e.g.*, antihistamine, diphenhydramine, etc.).

[000108] The carrier can be, but is not limited to, a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol), and suitable mixtures thereof. A carrier may also contain adjuvants such as preservatives, wetting agents, emulsifying agents, and dispersing agents. A carrier may also contain isotonic agents, such as sugars, polyalcohols, sodium chloride, and the like into the compositions.

[000109] Pharmaceutically acceptable refers to those properties and/or substances which are acceptable to the subject from a pharmacological/toxicological point of view. The phrase pharmaceutically acceptable refers to molecular entities, compositions, and properties that are physiologically tolerable and do not typically produce an allergic or other untoward or toxic reaction when administered to a subject. In some embodiments, a pharmaceutically acceptable

compound is approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals and more particularly in humans.

[000110] The The rAAVs or pharmaceutical compositions as described herein, may be formulated for administration to host cell ex vivo or in situ in an animal, and particularly a human being. The rAAVs or pharmaceutical compositions can be administered by a variety of routes. Administration routes included, but are not limited to, intravenous, intra-arterial, subcutaneous, intramuscular, intrahepatic, intraperitoneal and/or local delivery to a target tissue. In some embodiments, a plurality of injections, or other administration types, are provided, for example 2, 3, 4, 5, 6, 7, 8, 9, 10 or more injections. Routes of administration may be combined, if desired. Depending on the embodiment, the first and second rAAV need not be administered the same number of times (*e.g.*, the first rAAV may be administered 1 time, and the second vector may be administered three times). In some embodiments, the dosing is intramuscular administration.

[000111] In some embodiments, the number of rAAV particles administered to a subject may be on the order ranging from about 10^6 to about 10^{14} particles/mL or about 10^3 to about 10^{13} particles/mL, or any values in between for either range, such as for example, about 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, or 10^{14} particles/mL. In some embodiments, the number of rAAV particles administered to a subject may be on the order ranging from about 10^6 to about 10^{14} vector genomes(vgs)/mL or 10^3 to 10^{15} vgs/mL, or any values in between for either range, such as for example, about 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, or 10^{14} vgs/mL. In some embodiments, between about 0.5 and about 5 rAAV vector genomes per cell are administered. In some embodiments, between about 0.5 and about 2 rAAV vector genomes per cell are administered. In some embodiments, between about 1 x 10^{13} and about 3 x 10^{14} vector genomes per kilo (vgs/kg) are administered. In some embodiments, dosing is based on the mass of the subject's cardiac muscle. In some embodiments, dosing is based on body weight. In some embodiments, dosing is based on body surface area. The rAAV particles can be administered as a single dose, or divided into two or more administrations as may be required to achieve therapy of the particular disease or disorder being treated. In some embodiments, doses ranging from about 0.0001 mL to about 10 mLs are delivered to a subject.

[000112] For administration of an injectable aqueous solution, for example, the solution may be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, intravitreal, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of

the present disclosure. For example, one dosage may be dissolved in 1 mL of isotonic NaCl solution and either added to 1000 mL of hypodermoclysis fluid or injected at the proposed site of infusion, (see, for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). In several embodiments, the rAAV formulation will comprise, consist of, or consist essentially of active rAAV ingredient, a mono-basic buffer (e.g., sodium phosphate mono-basic buffer, a di-basic salt (e.g., sodium phosphate di-basic), a sodium-based tonicifier (e.g., sodium chloride tonicifier), a non-sodium tonicifier (e.g., magnesium chloride hexahydrate tonicifier), a surfactant (e.g., poloxamer 188 surfactant), and water. In several embodiments, the rAAV formulation will comprise, consist of, or consist essentially of active rAAV ingredient, sodium phosphate mono-basic buffer, sodium phosphate di-based, sodium chloride tonicifier, magnesium chloride hexahydrate tonicifier, poloxamer 188 surfactant, and water. In several embodiments, the active rAAV ingredient is present in the formulation according to the vector genome amounts provided for herein. In several embodiments, the mono-basic buffer (e.g., sodium phosphate mono-basic buffer) is present in the formulation at a concentration between about 0.2 mg/mL and about 0.5 mg/mL. In several embodiments, the di-basic salt (e.g., sodium phosphate di-basic) is present in the formulation at a concentration between about 1.5 mg/mL and about 4 mg/mL. In several embodiments, the sodium-based tonicifier (e.g., sodium chloride tonicifier) is present in the formulation at a concentration between about 8 mg/mL and about 12 mg/mL. In several embodiments, the non-sodium tonicifier (e.g., magnesium chloride hexahydrate tonicifier) is present in the formulation at a concentration between about 0.1 mg/mL and about 0.35 mg/mL. In several embodiments, the surfactant (e.g., poloxamer 188 surfactant) is present in the formulation at a concentration between about 0.05 mg/mL and about 0.8 mg/mL. In several embodiments, water is present to bring the volume of the formulation (e.g. a dosage unit) to 1 mL.

[000113] Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, and the general safety and purity standards as required by, e.g., FDA Office of Biologies standards.

[000114] Sterile injectable solutions are prepared by incorporating the rAAV particles or preparations, in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle that contains the basic dispersion medium and the 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 freeze-drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[000115] The amount of rAAV particle or preparation, and time of administration of such particle or preparation will be within the purview of the skilled artisan having benefit of the present teachings. It is likely, however, that the administration of therapeutically-effective amounts of the AAV particles or preparation of the present disclosure may be achieved by a single administration, such as for example, a single injection of sufficient numbers of infectious particles to provide therapeutic benefit to the patient undergoing such treatment. Alternatively, in some circumstances, it may be desirable to provide multiple or successive administrations of the rAAV particle or preparation, either over a relatively short, or a relatively prolonged period of time, as may be determined by the medical practitioner overseeing the administration of such compositions.

[000116] If desired, rAAV particles may be administered in combination with other agents as well, such as, e.g., proteins or polypeptides or various pharmaceutically- active agents, including one or more administrations of therapeutic polypeptides, biologically active fragments, or variants thereof. In fact, there is virtually no limit to other components that may also be included, as long as the additional agents do not cause a significant adverse effect upon contact with the target cells or host tissues. The rAAV particles or preparations may thus be delivered along with various other pharmaceutically acceptable agents as required in the particular instance. Such compositions may be purified from host cells or other biological sources, or alternatively may be chemically synthesized as described herein.

[000117] In some embodiments, treatment of a subject with a rAAV particles as described herein achieves one, two, three, four, or more of the following effects, including, for example: (i) reduction or amelioration the severity of disease or symptom associated therewith; (ii) reduction in the duration of a symptom associated with a disease; (iii) protection against the progression of a disease or symptom associated therewith; (iv) regression of a disease or symptom associated therewith; (v) protection against the development or onset of a symptom associated with a disease; (vi) protection against the recurrence of a symptom associated with a disease; (vii) reduction in the hospitalization of a subject; (viii) reduction in the hospitalization length; (ix) an increase in the survival of a subject with a disease; (x) a reduction in the number of symptoms associated with a disease; (xi) an enhancement, improvement, supplementation, complementation, or augmentation of the prophylactic or therapeutic effect(s) of another therapy.

[000118] As is apparent to those skilled in the art in view of the teachings of this specification, an effective amount of viral vector to be added can be empirically determined. Administration can be administered in a single dose, a plurality of doses, continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosages of administration are well known to those of skill in the art and will vary with the viral vector, the composition of the therapy, the target cells, and the subject being treated. Single and multiple administrations can be carried out with the dose level and pattern being selected by the treating physician.

Kits

[000119] Herein are described compositions including one or more of the disclosed rAAV vectors comprised within a kit for diagnosing, preventing, treating or ameliorating one or more symptoms of a heart disease or condition, such as a cardiomyopathy. Such kits may be useful in the diagnosis, prophylaxis, and/or therapy or a human disease, and may be particularly useful in the treatment, prevention, and/or amelioration of one or more symptoms of heart disease, such as a cardiomyopathy. In some embodiments, the heart disease is caused by cardiomyopathy. In some embodiments, the heart disease is caused by hypertrophic cardiomyopathy or dilated cardiomyopathy. In some embodiments, the heart disease is hypertrophic cardiomyopathy.

[000120] Kits comprising one or more of the disclosed rAAV vectors (as well as one or more virions, viral particles, transformed host cells or pharmaceutical compositions comprising such vectors); and instructions for using such kits in one or more therapeutic, diagnostic, and/or prophylactic clinical embodiments are also provided according to several embodiments. Such kits may comprise one or more reagents, restriction enzymes, peptides, therapeutics, pharmaceutical compounds, or means for delivery of the composition(s) to host cells, or to an animal (e.g., syringes, injectables, and the like). Depending on the embodiment, kits include those for treating, preventing, or ameliorating the symptoms of a disease, deficiency, dysfunction, and/or injury, or may include components for the large-scale production of the viral vectors themselves.

[000121] In some embodiments, a kit comprises one or more containers or receptacles comprising one or more doses of any of the described therapeutic. Such kits may be therapeutic in nature. In some embodiments, the kit contains a unit dosage, meaning a predetermined amount of a composition comprising, for example, a described therapeutic with or without one or more additional agents.

[000122] One or more of the components of a kit can be provided in one or more liquid or frozen solvents. The solvent can be aqueous or non-aqueous. The formulation in the kit can

also be provided as dried powder(s) or in lyophilized form that can be reconstituted upon addition of an appropriate solvent.

[000123] In some embodiments, a kit comprises a label, marker, package insert, bar code and/or reader indicating directions of suitable usage of the kit contents. In some embodiments, the kit may comprise a label, marker, package insert, bar code and/or reader indicating that the kit contents may be administered in accordance with a certain dosage or dosing regimen to treat a subject.

[000124] In addition, a kit may also contain various reagents, including, but not limited to, wash reagents, elution reagents, and concentration reagents. Such reagents may be readily selected from among the reagents described herein, and from among conventional concentration reagents.

[000125] As used herein, the term "kit" may be used to describe variations of the portable, self-contained enclosure that includes at least one set of components to conduct one or more of the diagnostic or therapeutic methods of the invention.

Combination therapies

[000126] Multiple embodiments of the present disclosure are directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the inventive scope of the present disclosure.

[000127] The compositions of the present disclosure may include rAAV particles or preparations, and/or rAAV vectors, either alone or in combination with one or more additional active ingredients, which may be obtained from natural or recombinant sources or chemically synthesized. In some embodiments, rAAV particles or preparations are administered in combination, either in the same composition or administered as part of the same treatment regimen, with a proteasome inhibitor, such as Bortezomib, or hydroxyurea.

[000128] If desired, rAAV particles and rAAV vectors may be administered in combination with other agents as well, such as, e.g., proteins or polypeptides or various pharmaceutically-active agents. This may, in some embodiments, reflect for example one or more administrations of therapeutic polypeptides, (e.g., a recombinant form of a functional peptide or protein that aids to replace or supplement the rAAV-based production of protein encoded by the transgene) biologically active fragments, or variants thereof. The rAAV particles or preparations may thus be delivered along with various other pharmaceutically acceptable

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

[000129] In some embodiments, the additional therapeutic agent comprises an anti-inflammatory agent. The anti-inflammatory agent can be, but is not limited to, a corticosteroid, cortisone hydrocortisone, hydrocortisone-21-monoesters (e.g., hydrocortisone-21-acetate, hydrocortisone-21-butyrate, hydrocortisone-21-propionate, hydrocortisone-21-valerate, etc.), hydrocortisone-17,21-diesters (e.g., hydrocortisone-17,21-diacetate, hydrocortisone-17-acetate-21-butyrate, hydrocortisone-17,21-dibutyrate, etc.), alclometasone, dexamethasone, flumethasone, prednisolone, methylprednisolone, betamethasone, typically as betamethasone benzoate or betamethasone diproprionate; fluocinonide; prednisone; and triamcinolone, typically as triamcinolone acetonide. In some embodiments, the anti-inflammatory agent is a mast cell degranulation inhibitor, such as, without limitation, cromolyn (5,5′-(2-hydroxypropane-1,3-diyl)bis(oxy)bis(4-oxo-4H-chromene-2-carboxylic acid) (also known as cromoglycate), and 2-carboxylatochromon-5′-yl-2-hydroxypropane derivatives such as bis(acetoxymethyl), disodium cromoglycate, nedocromil (9-ethyl-4,6-dioxo-10-propyl-6,9-dihydro-4H-pyrano[3,2-g]quinoline- 2,8-dicarboxylic acid) and tranilast (2-{[(2E)-3-(3,4-dimethoxyphenyl)prop-2-enoyl]amino}), and lodoxamide (2-[2-chloro-5-cyano-3-(oxaloamino)anilino]-2-oxoacetic acid). In some embodiments, the anti-inflammatory agent is a nonsteroidal anti-inflammatory drugs (NSAIDs), such as, without limitation, aspirin compounds (acetylsalicylates), non-aspirin salicylates, diclofenac, diflunisal, etodolac, fenoprofen, flurbiprofen, ibuprofen, indomethacin, ketoprofen, meclofenamate, naproxen, naproxen sodium, phenylbutazone, sulindac, and tometin.

[000130] In some embodiments, the anti-inflammatory agent comprises an antihistamine. The antihistamine can be, but is not limited to, clemastine, clemastine fumarate (2(R)-[2-[1-(4-Chlorophenyl)-1-phenyl-ethoxy]ethyl-1-methylpyrrolidine), dexmedetomidine, doxylamine, loratidine, desloratidine and promethazine, and diphenhydramine, or pharmaceutically acceptable salts, solvates or esters thereof. In some embodiments, the antihistamine includes, without limitation, azatadine, azelastine, burfroline, cetirizine, cyproheptadine, doxantrozole, etodroxizine, forskolin, hydroxyzine, ketotifen, oxatomide, pizotifen, proxicromil, N,N′- substituted piperazines or terfenadine. In some embodiments, the antihistamine is an H1 antagonist, such as, but not limited to, cetirizine, chlorpheniramine, dimenhydrinate, diphenhydramine, fexofenadine, hydroxyzine, orphenadrine, pheniramine, and doxylamine. In some embodiments, the antihistamine is an H2 antagonist, such as, but not limited to, cimetidine, famotidine, lafutidine, nizatidine, ranitidine, and roxatidine.

[000131] In some embodiments, the additional therapeutic agent comprises an antiviral agent, including antiretroviral agents. Suitable antiviral agents include, without limitation, remdesivir, acyclovir, famcyclovir, ganciclovir, foscarnet, idoxuridine, sorivudine, trifluorothymidine, valacyclovir, vidarabine, didanosine, dideoxyinosine, stavudine, zalcitabine, zidovudine, amantadine, interferon alpha, ribavirin and rimantadine.

[000132] In some embodiments, the additional therapeutic agent comprises an antibiotic. Non-limiting examples of suitable antibiotics include beta-lactams such as penicillins, aminopenicillins (e.g., amoxicillin, ampicillin, hetacillin, etc.), penicillinase resistant antibiotics (e.g., cloxacillin, dicloxacillin, methicillin, nafcillin, oxacillin, etc.), extended spectrum antibiotics (e.g., axlocillin, carbenicillin, mezlocillin, piperacillin, ticarcillin, etc.); cephalosporins (e.g., cefadroxil, cefazolin, cephalixin, cephalothin, cephapirin, cephradine, cefaclor, cefacmandole, cefmetazole, cefonicid, ceforanide, cefotetan, cefoxitin, cefprozil, cefuroxime, loracarbef, cefixime, cefoperazone, cefotaxime, cefpodoxime, ceftazidime, ceftiofur, ceftizoxime, ceftriaxone, moxalactam, etc.); monobactams such as aztreonam; Carbapenems such as imipenem and eropenem; quinolones (e.g., ciprofloxacin, enrofloxacin, difloxacin, orbifloxacin, marbofloxacin, etc.); chloramphenicols (e.g., chloramphenicol, thiamphenicol, florfenicol, etc.); tetracyclines (e.g., chlortetracycline, tetracycline, oxytetracycline, doxycycline, minocycline, etc.); macrolides (e.g., erythromycin, tylosin, tlimicosin, clarithromycin, azithromycin, etc.); lincosamides (e.g., lincomycin, clindamycin, etc.); aminoglycosides (e.g., gentamicin, amikacin, kanamycin, apramycin, tobramycin, neomycin, dihydrostreptomycin, paromomycin, etc.); sulfonamides (e.g., sulfadmethoxine, sfulfamethazine, sulfaquinoxaline, sulfamerazine, sulfathiazole, sulfasalazine, sulfadiazine, sulfabromomethazine, suflaethoxypyridazine, etc.); glycopeptides (e.g., vancomycin, teicoplanin, ramoplanin, and decaplanin; and other antibiotics (e.g., rifampin, nitrofuran, virginiamycin, polymyxins, tobramycin, etc.)).

[000133] In some embodiments, the additional therapeutic agent comprises an antifungal agent, such as, but not limited to, itraconazole, ketoconazole, fluoconazole, and amphotericin B. In some embodiments, the therapeutic agent is an antiparasitic agents, such as, but not limited to, the broad spectrum antiparasitic medicament nitazoxanide; antimalarial drugs and other antiprotozoal agents (*e.g.*, artemisins, mefloquine, lumefantrine, tinidazole, and miltefosine); anthelminthics such as mebendazole, thiabendazole, and ivermectin; and antiamoebic agents such as rifampin and amphotericin B.

[000134] In some embodiments, the additional therapeutic agent comprises an analgesic agent, including, without limitation, opioid analgesics such as alfentanil, buprenorphine,

butorphanol, codeine, drocode, fentanyl, hydrocodone, hydromorphone, levorphanol, meperidine, methadone, morphine, nalbuphine, oxycodone, oxymorphone, pentazocine, propoxyphene, sufentanil, and tramadol; and nonopioid analgesics such as apazone, etodolac, diphenpyramide, indomethacin, meclofenamate, mefenamic acid, oxaprozin, phenylbutazone, piroxicam, and tolmetin.

[000135] The disclosure herein of any particular feature, aspect, method, property, characteristic, quality, attribute, element, or the like in connection with an embodiment can be used in all other embodiments set forth herein. Accordingly, it should be understood that various features and aspects of the disclosed embodiments can be combined with or substituted for one another in order to form varying modes of the disclosed inventions. Thus, it is intended that the scope of the present inventions herein disclosed should not be limited by the particular disclosed embodiments described above. Moreover, while the invention is susceptible to various modifications, and alternative forms, specific examples thereof have been shown in the drawings and are herein described in detail. It should be understood, however, that the invention is not to be limited to the particular forms or methods disclosed, but to the contrary, the invention is to cover all modifications, equivalents, and alternatives falling within the spirit and scope of the various embodiments described and the appended claims. Any methods disclosed herein need not be performed in the order recited. The methods disclosed herein include certain actions taken by a practitioner; however, they can also include any third-party instruction of those actions, either expressly or by implication. In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[000136] Any titles or subheadings used herein are for organization purposes and should not be used to limit the scope of embodiments disclosed herein.

EXAMPLES

[000137] The following examples are illustrative only and are not intended to be a limitation on the scope of the invention.

MATERIALS AND METHODS

CONSTRUCT DESIGN.

[000138] MYBPC3 cDNA was codon optimized for expression in human tissues and was subcloned into a plasmid backbone suitable for production of AAV. Constructs comprising single stranded AAV genomes were engineered to comprise the elements as provided in Table 1

below, with other designs and alternative configurations noted. A schematic representation of a construct is provided in FIG. 1.

Table 1

Elements (5' -> 3')	Nt sequence	AA sequence (as applicable)
ITR-L	TTGGCCACTCCCTCTCTG CGCGCTCGCTCGCTCACT GAGGCCGGGCGACCAAA GGTCGCCCGACGCCCGG GCTTTGCCCGGGCGGCCT CAGTGAGCGAGCGAGCG CGCAGAGAGGGAGTGGC CAACTCCATCACTAGGG GTTCCT (SEQ ID NO: 20)	
spacer	CTAGAGGTACCTGTACA(SEQ ID NO: 21)	
alphaMHC enhancer	CCTTCAGATTAAAAATA ACTAAGGTAAGGGCCAT GTGGGTAGGGGAGGTGG TGTGAGACGGTCCTGTCT CTCCTCTATCTGCCCATC GGCCCTTTGGGGAGGAG GAATGTGCCCAAGGACT AAAAAAAGGCCCTGGAG CCAGAGGGGCGAGGGCA GCAGACCTTTCATGGGC AAACCTCAGGGCTGCTG TC (SEQ ID NO: 22)	
spacer	GTCGA (SEQ ID NO: 23)	
Mini CMV promoter	GGTAGGCGTGTACGGTG GGAGGCCTATATAAGCA GAGCT (SEQ ID NO: 24)	
spacer	CGTTTAGTGAACCGTCAG ATCGCCTGGAGGAATTC (SEQ ID NO: 25)	
sd/sa	GTAAGTATCAAGGTTAC AAGACAGGTTTAAGGAG ACCAATAGAAACTGGGC TTGTCGAGACAGAGAAG ACTCTTGCGTTTCTGATA GGCACCTATTGGTCTTAC TGACATCCACTTTGCCTT TCTCTCCACAG (SEQ ID NO: 26)	
spacer	AAGCTTGCTAGCGTTTAA ACTTAAG (SEQ ID NO: 27)	
Kozac sequence	CTTGCCACC (SEQ ID NO: 28)	

MYBPC3	ATGCCTGAGCCTGGCAA	MPEPGKKPVSAFSKKPRSVEVAAG
	GAAACCTGTGTCTGCCTT	SPAVFEAETERAGVKVRWQRGGS
	CAGCAAGAAGCCCAGAT	DISASNKYGLATEGTRHTLTVREV
	CTGTTGAGGTGGCAGCT	GPADQGSYAVIAGSSKVKFDLKVI
	GGCAGCCCTGCTGTGTTT	EAEKAEPMLAPAPAPAEATGAPGE
	GAGGCTGAGACAGAAAG	APAPAAELGESAPSPKGSSSAALN
	AGCTGGGGTCAAAGTCA	GPTPGAPDDPIGLFVMRPQDGEVT
	GATGGCAGAGAGGAGGC	VGGSITFSARVAGASLLKPPVVKW
	TCTGACATCTCTGCCAGC	FKGKWVDLSSKVGQHLQLHDSYD
	AACAAATATGGCCTGGC	RASKVYLFELHITDAQPAFTGSYR
	CACAGAGGGCACCAGAC	CEVSTKDKFDCSNFNLTVHEAMG
	ACACCCTGACAGTTAGA	TGDLDLLSAFRRTSLAGGGRRISDS
	GAAGTGGGCCCTGCTGA	HEDTGILDFSSLLKKRDSFRTPRDS
	CCAGGGCAGCTATGCTG	KLEAPAEEDVWEILRQAPPSEYERI
	TGATTGCTGGCTCCAGCA	AFQYGVTDLRGMLKRLKGMRRD
	AAGTGAAGTTTGACCTG	EKKSTAFQKKLEPAYQVSKGHKIR
	AAAGTGATTGAGGCAGA	LTVELADHDAEVKWLKNGQEIQM
	GAAGGCTGAGCCCATGC	SGSKYIFESIGAKRTLTISQCSLADD
	TTGCTCCAGCTCCAGCAC	AAYQCVVGGEKCSTELFVKEPPVL
	CAGCTGAAGCTACTGGT	ITRPLEDQLVMVGQRVEFECEVSE
	GCTCCTGGGGAAGCTCCT	EGAQVKWLKDGVELTREETFKYR
	GCTCCTGCTGCTGAACTT	FKKDGQRHHLIINEAMLEDAGHY
	GGAGAGTCTGCCCCATCT	ALCTSGGQALAELIVQEKKLEVYQ
	CCTAAGGGCTCTAGCTCT	SIADLMVGAKDQAVFKCEVSDEN
	GCTGCCCTGAATGGACCT	VRGVWLKNGKELVPDSRIKVSHIG
	ACACCTGGGGCTCCAGA	RVHKLTIDDVTPADEADYSFVPEG
	TGACCCCATTGGCCTGTT	FACNLSAKLHFMEVKIDFVPRQEP
	TGTGATGAGGCCCCAGG	PKIHLDCPGRIPDTIVVVAGNKLRL
	ATGGGGAAGTGACAGTT	DVPISGDPAPTVIWQKAITQGNKA
	GGAGGCAGCATCACCTT	PARPAPDAPEDTGDSDEWVFDKK
	TTCTGCCAGAGTGGCTGG	LLCETEGRVRVETTKDRSIFTVEG
	GGCCAGCCTGCTGAAAC	AEKEDEGVYTVTVKNPVGEDQVN
	CTCCTGTGGTCAAGTGGT	LTVKVIDVPDAPAAPKISNVGEDS
	TCAAAGGCAAATGGGTT	CTVQWEPPAYDGGQPILGYILERK
	GACCTGTCCTCCAAAGTG	KKKSYRWMRLNFDLIQELSHEAR
	GGCCAGCACCTCCAGCT	RMIEGVVYEMRVYAVNAIGMSRP
	GCATGACAGCTATGATA	SPASQPFMPIGPPSEPTHLAVEDVS
	GGGCCAGCAAGGTGTAC	DTTVSLKWRPPERVGAGGLDGYS
	CTGTTTGAGCTGCACATC	VEYCPEGCSEWVAALQGLTEHTSI
	ACAGATGCCCAGCCAGC	LVKDLPTGARLLFRVRAHNMAGP
	CTTCACAGGCAGCTACA	GAPVTTTEPVTVQEILQRPRLQLPR
	GATGTGAAGTGTCCACC	HLRQTIQKKVGEPVNLLIPFQGKPR
	AAGGACAAGTTTGACTG	PQVTWTKEGQPLAGEEVSIRNSPT
	CAGCAACTTCAACCTGA	DTILFIRAARRVHSGTYQVTVRIEN
	CAGTGCATGAGGCCATG	MEDKATLVLQVVDKPSPPQDLRV
	GGCACAGGGGACCTTGA	TDAWGLNVALEWKPPQDVGNTEL
	TCTGCTGTCAGCCTTTAG	WGYTVQKADKKTMEWFTVLEHY
	AAGAACCAGCCTGGCTG	RRTHCVVPELIIGNGYYFRVFSQN
	GTGGTGGCAGAAGAATC	MVGFSDRAATTKEPVFIPRPGITYE
	TCTGACAGCCATGAGGA	PPNYKALDFSEAPSFTQPLVNRSVI
	CACAGGCATCCTGGACTT	AGYTAMLCCAVRGSPKPKISWFK

	CAGCTCCCTGCTGAAGA AGAGAGACAGCTTCAGA ACCCCTAGAGACAGCAA GCTGGAAGCCCCAGCTG AGGAAGATGTCTGGGAG ATTCTGAGACAGGCCCCT CCATCTGAGTATGAGAG AATTGCCTTCCAGTATGG GGTCACAGACCTGAGAG GCATGCTGAAGAGACTG AAGGGCATGAGAAGAGA TGAGAAGAAGTCCACAG CCTTCCAGAAGAAGCTG GAACCTGCCTACCAGGT GTCCAAGGGCCACAAGA TCAGACTGACAGTGGAA CTGGCTGACCATGATGCT GAAGTGAAGTGGCTGAA GAATGGCCAAGAGATCC AAATGTCTGGCAGCAAG TACATCTTTGAGAGCATT GGAGCCAAGAGGACCCT GACCATCAGCCAGTGTA GCCTGGCAGATGATGCA GCCTATCAGTGTGTTGTT GGTGGTGAAAAGTGCAG CACAGAGCTGTTTGTCAA AGAGCCTCCAGTCCTGAT CACCAGACCTCTGGAAG ATCAGCTGGTCATGGTTG GACAGAGGGTTGAGTTT GAGTGTGAAGTCTCTGA AGAGGGTGCCCAAGTCA AATGGCTGAAAGATGGG GTTGAGCTGACCAGAGA GGAAACCTTCAAGTACA GGTTCAAGAAGGATGGC CAGAGGCACCACCTGAT CATCAATGAAGCCATGC TGGAAGATGCTGGCCAC TATGCCCTGTGCACAAGT GGTGGACAAGCCCTGGC TGAGCTGATTGTGCAAG AAAAGAAACTGGAAGTG TACCAGAGCATTGCTGA CCTGATGGTTGGAGCTA AGGACCAGGCTGTGTTT AAATGTGAAGTTTCAGA TGAGAATGTCAGAGGAG TGTGGCTCAAAAATGGC AAAGAACTGGTGCCTGA	NGLDLGEDARFRMFSKQGVLTLEI RKPCPFDGGIYVCRATNLQGEARC ECRLEVRVPQ (SEQ ID NO: 15)

	CTCCAGGATCAAGGTGT CCCACATTGGCAGAGTG CACAAGCTGACAATTGA TGATGTGACCCCTGCTGA TGAGGCTGACTACAGCTT TGTGCCTGAGGGCTTTGC CTGCAACCTGTCTGCCAA GCTGCACTTCATGGAAGT CAAGATTGACTTTGTGCC CAGGCAAGAGCCACCTA AGATCCACCTGGATTGCC CTGGCAGAATCCCAGAC ACCATTGTGGTTGTGGCT GGCAACAAGCTGAGACT GGATGTGCCCATCTCTGG GGACCCTGCTCCTACAGT GATTTGGCAGAAGGCCA TCACACAGGGCAACAAG GCTCCAGCCAGACCAGC TCCTGATGCTCCTGAGGA TACTGGGGACTCTGATG AGTGGGTGTTTGACAAG AAACTGCTGTGTGAAAC TGAGGGCAGAGTCAGAG TGGAAACCACAAAGGAC AGATCCATCTTCACAGTG GAAGGGGCTGAGAAAGA GGATGAAGGGGTCTACA CAGTGACAGTGAAGAAC CCTGTGGGAGAAGATCA AGTGAACCTGACTGTGA AAGTCATTGATGTGCCA GATGCTCCAGCAGCTCCC AAGATCAGCAATGTTGG AGAGGACAGCTGCACAG TGCAGTGGGAGCCACCA GCCTATGATGGTGGACA GCCTATCCTGGGCTACAT CCTGGAAAGAAAGAAGA AAAAGTCCTACAGATGG ATGAGGCTCAACTTTGAT CTGATCCAAGAGCTGAG CCATGAAGCTAGAAGGA TGATTGAAGGGGTTGTGT ATGAGATGAGAGTGTAT GCAGTGAATGCCATTGG CATGAGCAGACCCTCTCC AGCCTCTCAGCCTTTCAT GCCCATTGGACCACCATC TGAGCCCACACACCTGG CAGTGGAAGATGTGTCT	

	GACACCACAGTGTCCCT GAAGTGGAGGCCACCTG AGAGAGTTGGAGCTGGA GGCCTGGATGGCTACTCT GTGGAATACTGCCCAGA GGGCTGCTCTGAGTGGG TTGCAGCTCTGCAGGGA CTGACAGAGCACACCTC CATCCTGGTCAAGGATCT GCCTACAGGGGCTAGAC TGCTGTTCAGAGTTAGGG CCCACAACATGGCTGGA CCAGGGGCTCCTGTGAC AACCACAGAACCTGTGA CTGTGCAAGAGATTCTGC AGAGGCCCAGACTGCAG CTGCCTAGACACCTGAG GCAGACCATCCAAAAGA AAGTGGGAGAGCCTGTG AACCTGCTGATCCCATTC CAAGGCAAGCCCAGACC TCAAGTGACCTGGACAA AAGAGGGACAGCCCCTG GCTGGGGAAGAAGTCTC CATCAGAAACAGCCCCA CTGACACCATCCTGTTCA TCAGAGCTGCCAGAAGA GTGCATTCTGGCACCTAC CAAGTGACTGTCAGAAT TGAGAACATGGAAGATA AGGCCACACTGGTGCTG CAGGTTGTGGATAAGCC CAGTCCTCCTCAGGATCT GAGAGTGACAGATGCCT GGGGACTGAATGTGGCC CTGGAATGGAAACCTCC ACAGGATGTGGGCAACA CTGAGCTGTGGGGCTAT ACAGTGCAGAAGGCAGA TAAGAAAACCATGGAAT GGTTCACAGTGCTGGAA CACTATAGAAGGACCCA CTGTGTGGTGCCAGAGCT GATCATTGGCAATGGCT ACTACTTCAGGGTGTTCA GCCAGAATATGGTTGGA TTCTCTGATAGGGCAGCC ACCACAAAAGAACCAGT GTTCATCCCCAGACCTGG CATCACCTATGAGCCTCC AAACTACAAGGCCCTGG	

	ATTTCTCTGAGGCCCCTA GCTTCACCCAGCCTCTGG TCAATAGATCAGTGATTG CAGGCTATACTGCCATGC TGTGCTGTGCAGTCAGA GGCAGCCCAAAGCCTAA GATCTCCTGGTTTAAGAA TGGACTGGACCTTGGGG AAGATGCCAGATTCAGA ATGTTCAGCAAGCAAGG GGTGCTGACCCTGGAAA TCAGAAAGCCCTGTCCTT TTGATGGTGGTATCTATG TGTGCAGGGCTACCAAC CTCCAGGGTGAAGCAAG ATGTGAATGCAGGCTGG AAGTTAGAGTGCCCCAG TGATGATGAGCGGCCGC (SEQ ID NO: 29)	
STOP	TGATGATGAGC (SEQ ID NO: 30)	
spacer	GGCCGCCGGCCG (SEQ ID NO: 31)	
polyA	AATAAAAGATCCTTATTT TCATTGGATCTGTGTGTT GGTTTTTTGTGTG (SEQ ID NO: 32)	
spacer	GTCGACTCTAG (SEQ ID NO: 33)	
ITR-R	AGGAACCCCTAGTGATG GAGTTGGCCACTCCCTCT CTGCGCGCTCGCTCGCTC ACTGAGGCCGGGCGACC AAAGGTCGCCCGACGCC CGGGCTTTGCCCGGGCG GCCTCAGTGAGCGAGCG AGCGCGCAGAGAGGGAG TGGCCAA (SEQ ID NO: 34)	

[000139] *In silico* derivation of consensus Kozak sequence for enhanced expression in cardiac tissues

[000140] An analysis of highly expressed genes in human heart tissues was performed to design a novel synthetic Kozak sequence to enhance transgene expression in the heart. Genes were selected from the Human Protein Atlas and Kozak sequences for each were identified in NCBI, as show in Table 2 below. A consensus sequence was derived using Weblogo

(https://weblogo.berkeley.edu/logo.cgi). The consensus sequence (**AGCCCCAAC** (SEQ ID NO: 28)) was then utilized in the design of the transgene constructs provided herein.

Table 2.

Gene	**Kozak sequence**	**SEQ ID NO:**
MYH7	GGCACAGCC	49
ACTC1	TGTGCCAAG	50
TNNI3	AGTCTCAGC	51
MYL7	GCAGAGAGA	52
NPPA	TCCAGAGAC	53
NPPB	TCCAGAGAC	54
TNNI2	GACCTCAGG	55
MYBPC3	TCTCTCAGG	56
MYL4	CAAGACAAC	57
MYBPHL	AGGCCCAGC	58
MYH6	AGCACCAAG	59
LRRC10	AGCCTCCGC	60
ACTC1	TGTGCCAAG	61
RD3L	AGGCTAAAA	62
Consensus Sequence	**AGCCCCAAC**	**28**

[000141] Alternative constructs were designed with various promoters and the *in silico* derived sequence, as shown below in Table 3.

Table 3.

Elements (5' -> 3')	**Nt sequence**	**AA sequence (as applicable)**
ITR-L	TTGGCCACTCCCTCTCTG CGCGCTCGCTCGCTCACT GAGGCCGGGCGACCAAA GGTCGCCCGACGCCCGG GCTTTGCCCGGGCGGCCT CAGTGAGCGAGCGAGCG CGCAGAGAGGGAGTGGC CAACTCCATCACTAGGG GTTCCT(SEQ ID NO: 20)	
spacer	CTAGAGGTACCTGTACA(SEQ ID NO: 21)	
alphaMHC enhancer	CCTTCAGATTAAAAATA ACTAAGGTAAGGGCCAT GTGGGTAGGGGAGGTGG TGTGAGACGGTCCTGTCT CTCCTCTATCTGCCCATC	

	GGCCCTTTGGGGAGGAG GAATGTGCCCAAGGACT AAAAAAAGGCCCTGGAG CCAGAGGGGCGAGGGCA GCAGACCTTTCATGGGC AAACCTCAGGGCTGCTG TC(SEQ ID NO: 22)	
spacer	GTCGA(SEQ ID NO: 23)	
Mini CMV promoter	GGTAGGCGTGTACGGTG GGAGGCCTATATAAGCA GAGCT (SEQ ID NO: 24)	
spacer	CGTTTAGTGAACCGTCAG ATCGCCTGGAGGAATTC (SEQ ID NO: 25)	
sd/sa	GTAAGTATCAAGGTTAC AAGACAGGTTTAAGGAG ACCAATAGAAACTGGGC TTGTCGAGACAGAGAAG ACTCTTGCGTTTCTGATA GGCACCTATTGGTCTTAC TGACATCCACTTTGCCTT TCTCTCCACAG (SEQ ID NO: 26)	
spacer	AAGCTTGCTAGCGTTTAA ACTTAAG (SEQ ID NO: 27)	
Kozac sequence	AGCCCCAAC (SEQ ID NO: 28)	
MYBPC3	ATGCCTGAGCCTGGCAA GAAACCTGTGTCTGCCTT CAGCAAGAAGCCCAGAT CTGTTGAGGTGGCAGCT GGCAGCCCTGCTGTGTTT GAGGCTGAGACAGAAAG AGCTGGGGTCAAAGTCA GATGGCAGAGAGGAGGC TCTGACATCTCTGCCAGC AACAAATATGGCCTGGC CACAGAGGGCACCAGAC ACACCCTGACAGTTAGA GAAGTGGGCCCTGCTGA CCAGGGCAGCTATGCTG TGATTGCTGGCTCCAGCA AAGTGAAGTTTGACCTG AAAGTGATTGAGGCAGA GAAGGCTGAGCCCATGC TTGCTCCAGCTCCAGCAC CAGCTGAAGCTACTGGT GCTCCTGGGGAAGCTCCT GCTCCTGCTGCTGAACTT GGAGAGTCTGCCCCATCT	MPEPGKKPVSAFSKKPRSVEVAAG SPAVFEAETERAGVKVRWQRGGS DISASNKYGLATEGTRHTLTVREV GPADQGSYAVIAGSSKVKFDLKVI EAEKAEPMLAPAPAPAEATGAPGE APAPAAELGESAPSPKGSSSAALN GPTPGAPDDPIGLFVMRPQDGEVT VGGSITFSARVAGASLLKPPVVKW FKGKWVDLSSKVGQHLQLHDSYD RASKVYLFELHITDAQPAFTGSYR CEVSTKDKFDCSNFNLTVHEAMG TGDLDLLSAFRRTSLAGGGRRISDS HEDTGILDFSSLLKKRDSFRTPRDS KLEAPAEEDVWEILRQAPPSEYERI AFQYGVTDLRGMLKRLKGMRRD EKKSTAFQKKLEPAYQVSKGHKIR LTVELADHDAEVKWLKNGQEIQM SGSKYIFESIGAKRTLTISQCSLADD AAYQCVVGGEKCSTELFVKEPPVL ITRPLEDQLVMVGQRVEFECEVSE EGAQVKWLKDGVELTREETFKYR FKKDGQRHHLIINEAMLEDAGHY ALCTSGGQALAELIVQEKKLEVYQ

	CCTAAGGGCTCTAGCTCT GCTGCCCTGAATGGACCT ACACCTGGGGCTCCAGA TGACCCCATTGGCCTGTT TGTGATGAGGCCCCAGG ATGGGGAAGTGACAGTT GGAGGCAGCATCACCTT TTCTGCCAGAGTGGCTGG GGCCAGCCTGCTGAAAC CTCCTGTGGTCAAGTGGT TCAAAGGCAAATGGGTT GACCTGTCCTCCAAAGTG GGCCAGCACCTCCAGCT GCATGACAGCTATGATA GGGCCAGCAAGGTGTAC CTGTTTGAGCTGCACATC ACAGATGCCCAGCCAGC CTTCACAGGCAGCTACA GATGTGAAGTGTCCACC AAGGACAAGTTTGACTG CAGCAACTTCAACCTGA CAGTGCATGAGGCCATG GGCACAGGGGACCTTGA TCTGCTGTCAGCCTTTAG AAGAACCAGCCTGGCTG GTGGTGGCAGAAGAATC TCTGACAGCCATGAGGA CACAGGCATCCTGGACTT CAGCTCCCTGCTGAAGA AGAGAGACAGCTTCAGA ACCCCTAGAGACAGCAA GCTGGAAGCCCCAGCTG AGGAAGATGTCTGGGAG ATTCTGAGACAGGCCCCT CCATCTGAGTATGAGAG AATTGCCTTCCAGTATGG GGTCACAGACCTGAGAG GCATGCTGAAGAGACTG AAGGGCATGAGAAGAGA TGAGAAGAAGTCCACAG CCTTCCAGAAGAAGCTG GAACCTGCCTACCAGGT GTCCAAGGGCCACAAGA TCAGACTGACAGTGGAA CTGGCTGACCATGATGCT GAAGTGAAGTGGCTGAA GAATGGCCAAGAGATCC AAATGTCTGGCAGCAAG TACATCTTTGAGAGCATT GGAGCCAAGAGGACCCT GACCATCAGCCAGTGTA	SIADLMVGAKDQAVFKCEVSDEN VRGVWLKNGKELVPDSRIKVSHIG RVHKLTIDDVTPADEADYSFVPEG FACNLSAKLHFMEVKIDFVPRQEP PKIHLDCPGRIPDTIVVVAGNKLRL DVPISGDPAPTVIWQKAITQGNKA PARPAPDAPEDTGDSDEWVFDKK LLCETEGRVRVETTKDRSIFTVEG AEKEDEGVYTVTVKNPVGEDQVN LTVKVIDVPDAPAAPKISNVGEDS CTVQWEPPAYDGGQPILGYILERK KKKSYRWMRLNFDLIQELSHEAR RMIEGVVYEMRVYAVNAIGMSRP SPASQPFMPIGPPSEPTHLAVEDVS DTTVSLKWRPPERVGAGGLDGYS VEYCPEGCSEWVAALQGLTEHTSI LVKDLPTGARLLFRVRAHNMAGP GAPVTTTEPVTVQEILQRPRLQLPR HLRQTIQKKVGEPVNLLIPFQGKPR PQVTWTKEGQPLAGEEVSIRNSPT DTILFIRAARRVHSGTYQVTVRIEN MEDKATLVLQVVDKPSPPQDLRV TDAWGLNVALEWKPPQDVGNTEL WGYTVQKADKKTMEWFTVLEHY RRTHCVVPELIIGNGYYFRVFSQN MVGFSDRAATTKEPVFIPRPGITYE PPNYKALDFSEAPSFTQPLVNRSVI AGYTAMLCCAVRGSPKPKISWFK NGLDLGEDARFRMFSKQGVLTLEI RKPCPFDGGIYVCRATNLQGEARC ECRLEVRVPQ (SEQ ID NO: 15)

	GCCTGGCAGATGATGCA GCCTATCAGTGTGTTGTT GGTGGTGAAAAGTGCAG CACAGAGCTGTTTGTCAA AGAGCCTCCAGTCCTGAT CACCAGACCTCTGGAAG ATCAGCTGGTCATGGTTG GACAGAGGGTTGAGTTT GAGTGTGAAGTCTCTGA AGAGGGTGCCCAAGTCA AATGGCTGAAAGATGGG GTTGAGCTGACCAGAGA GGAAACCTTCAAGTACA GGTTCAAGAAGGATGGC CAGAGGCACCACCTGAT CATCAATGAAGCCATGC TGGAAGATGCTGGCCAC TATGCCCTGTGCACAAGT GGTGGACAAGCCCTGGC TGAGCTGATTGTGCAAG AAAAGAAACTGGAAGTG TACCAGAGCATTGCTGA CCTGATGGTTGGAGCTA AGGACCAGGCTGTGTTT AAATGTGAAGTTTCAGA TGAGAATGTCAGAGGAG TGTGGCTCAAAAATGGC AAAGAACTGGTGCCTGA CTCCAGGATCAAGGTGT CCCACATTGGCAGAGTG CACAAGCTGACAATTGA TGATGTGACCCCTGCTGA TGAGGCTGACTACAGCTT TGTGCCTGAGGGCTTTGC CTGCAACCTGTCTGCCAA GCTGCACTTCATGGAAGT CAAGATTGACTTTGTGCC CAGGCAAGAGCCACCTA AGATCCACCTGGATTGCC CTGGCAGAATCCCAGAC ACCATTGTGGTTGTGGCT GGCAACAAGCTGAGACT GGATGTGCCCATCTCTGG GGACCCTGCTCCTACAGT GATTTGGCAGAAGGCCA TCACACAGGGCAACAAG GCTCCAGCCAGACCAGC TCCTGATGCTCCTGAGGA TACTGGGGACTCTGATG AGTGGGTGTTTGACAAG AAACTGCTGTGTGAAAC	

	TGAGGGCAGAGTCAGAG TGGAAACCACAAAGGAC AGATCCATCTTCACAGTG GAAGGGGCTGAGAAAGA GGATGAAGGGGTCTACA CAGTGACAGTGAAGAAC CCTGTGGGAGAAGATCA AGTGAACCTGACTGTGA AAGTCATTGATGTGCCA GATGCTCCAGCAGCTCCC AAGATCAGCAATGTTGG AGAGGACAGCTGCACAG TGCAGTGGGAGCCACCA GCCTATGATGGTGGACA GCCTATCCTGGGCTACAT CCTGGAAAGAAAGAAGA AAAAGTCCTACAGATGG ATGAGGCTCAACTTTGAT CTGATCCAAGAGCTGAG CCATGAAGCTAGAAGGA TGATTGAAGGGGTTGTGT ATGAGATGAGAGTGTAT GCAGTGAATGCCATTGG CATGAGCAGACCCTCTCC AGCCTCTCAGCCTTTCAT GCCCATTGGACCACCATC TGAGCCCACACACCTGG CAGTGGAAGATGTGTCT GACACCACAGTGTCCCT GAAGTGGAGGCCACCTG AGAGAGTTGGAGCTGGA GGCCTGGATGGCTACTCT GTGGAATACTGCCCAGA GGGCTGCTCTGAGTGGG TTGCAGCTCTGCAGGGA CTGACAGAGCACACCTC CATCCTGGTCAAGGATCT GCCTACAGGGGCTAGAC TGCTGTTCAGAGTTAGGG CCCACAACATGGCTGGA CCAGGGGCTCCTGTGAC AACCACAGAACCTGTGA CTGTGCAAGAGATTCTGC AGAGGCCCAGACTGCAG CTGCCTAGACACCTGAG GCAGACCATCCAAAAGA AAGTGGGAGAGCCTGTG AACCTGCTGATCCCATTC CAAGGCAAGCCCAGACC TCAAGTGACCTGGACAA AAGAGGGACAGCCCCTG	

	GCTGGGGAAGAAGTCTC CATCAGAAACAGCCCCA CTGACACCATCCTGTTCA TCAGAGCTGCCAGAAGA GTGCATTCTGGCACCTAC CAAGTGACTGTCAGAAT TGAGAACATGGAAGATA AGGCCACACTGGTGCTG CAGGTTGTGGATAAGCC CAGTCCTCCTCAGGATCT GAGAGTGACAGATGCCT GGGGACTGAATGTGGCC CTGGAATGGAAACCTCC ACAGGATGTGGGCAACA CTGAGCTGTGGGGCTAT ACAGTGCAGAAGGCAGA TAAGAAAACCATGGAAT GGTTCACAGTGCTGGAA CACTATAGAAGGACCCA CTGTGTGGTGCCAGAGCT GATCATTGGCAATGGCT ACTACTTCAGGGTGTTCA GCCAGAATATGGTTGGA TTCTCTGATAGGGCAGCC ACCACAAAAGAACCAGT GTTCATCCCCAGACCTGG CATCACCTATGAGCCTCC AAACTACAAGGCCCTGG ATTTCTCTGAGGCCCCTA GCTTCACCCAGCCTCTGG TCAATAGATCAGTGATTG CAGGCTATACTGCCATGC TGTGCTGTGCAGTCAGA GGCAGCCCAAAGCCTAA GATCTCCTGGTTTAAGAA TGGACTGGACCTTGGGG AAGATGCCAGATTCAGA ATGTTCAGCAAGCAAGG GGTGCTGACCCTGGAAA TCAGAAAGCCCTGTCCTT TTGATGGTGGTATCTATG TGTGCAGGGCTACCAAC CTCCAGGGTGAAGCAAG ATGTGAATGCAGGCTGG AAGTTAGAGTGCCCCAG TGATGATGAGCGGCCGC (SEQ ID NO: 29)	
STOP	TGATGATGAGC (SEQ ID NO: 30)	
spacer	GGCCGCCGGCCG (SEQ ID NO: 31)	

polyA	AATAAAAGATCCTTATTT TCATTGGATCTGTGTGTT GGTTTTTTGTGTG (SEQ ID NO: 32)	
spacer	GTCGACTCTAG (SEQ ID NO: 33)	
ITR-R	AGGAACCCCTAGTGATG GAGTTGGCCACTCCCTCT CTGCGCGCTCGCTCGCTC ACTGAGGCCGGGCGACC AAAGGTCGCCCGACGCC CGGGCTTTGCCCGGGCG GCCTCAGTGAGCGAGCG AGCGCGCAGAGAGGGAG TGGCCAA (SEQ ID NO: 34)	

[000142] Additional constructs comprising were designed to include the following alternative promoters:

TNNC1

GATCACTGGGACCAGAGGAGGGGCTGGAGGATACTACACGCAGGGGTGGGCTGG GCTGGGCTGGGCTGGGCCAGGAATGCAGCGGGGCAGGGCTATTTAAGTCAAGGG CCGGCTGGCAACCCCAGCAAGCTGTCCTGTGAG (SEQ ID NO: 63)

MHC

CAAGGCTGTGGGGGACTGAGGGCAGGCTGTAACAGGCTTGGGGGCCAGGGCTTAT ACGTGCCTGGGACTCCCAAAGTATTACTGTTCCATGTTCCCGGCGAAGGGCCAGCT GTCCCCCGCCAGCTAGACTCAGCACTTAGTTTAGGAACCAGTGAGCAAGTCAGCC CTTGGGGCAGCCCATACAAGGCCATGGGGCTGGGCAAGCTGCACGCCTGGGTCCG GGGTGGGCACGGTGCCCGGGCAACGAGCTGAAAGCTCATCTGCTCTCAGGGGCCC CTCCCTGGGGACAGCCCCTCCTGGCTAGTCACACCCTGTAGGCTCCTCTATATAAC CCAGGGGCACAGGGGCTGCCCTC (SEQ ID NO: 64)

AAV production.

[000143] Recombinant AAV (rAAV) particles comprising each of the constructs are made by suspension transfection of Expi293F cells with the MYBPC3constructs and other plasmids needed for rAAV production (e.g., comprising rep and cap expression cassettes) to generate three groups of rAAV comprising (1) AAV9 capsid proteins (2) rh74 capsid proteins; and (3) rh74 variant capsid proteins comprising a tryptophan to arginine mutation at amino acid

505 of the rh74 VP1 capsid protein. Vector is isolated using a capture column followed by an anion exchange column and purified using a cesium chloride gradient to a titer of 2-5E+13 vg/ml.

Example 1. *In vitro* expression study

[000144] The three groups of rAAV comprising the MYBPC3 constructs is made as described above and delivered to HEK293, C2C12 or cardiomyocytes derived from human induced pluripotent stem cells. Whole cell lysates are generated and probed for expression of MYBPC3 by ELISA and/or immunoblotting.

Example 2. *In vivo* expression study

[000145] The three groups of rAAV comprising the MYBPC3 constructs is made as described above and administered via the facial vein to newborn C57BL/6 mice (n=6-10/group) at 5E+13 vg/kg. Two to four weeks after rAAV dosing, heart, diaphragm and skeletal muscle tissues are harvested and whole cell lysates are analyzed for MYBPC3 expression using ELISA and/or immunoblot.

[000146] [The three groups of rAAV comprising the MYBPC3 constructs is made as described above and administered via the jugular vein to 5-7 weeks old C57BL/6 mice (n=6-10/group) at three different doses: 1E+13 vg/kg, 5E+13 vg/kg or 1+E14 vg/kg. One month after rAAV dosing, heart, diaphragm and skeletal muscle tissues are harvested and whole cell lysates are analyzed for MYBPC3 expression using ELISA and/or immunoblot.

Example 3. Restoration of MYBPC3 expression *in vivo*

[000147] Constitutive homozygous MYBPC3 knockout mice ($Mybpc3^{tm1Rmos}$; MGI: 3526881) rapidly develop a severe, early-onset hypertrophic cardiomyopathy-related phenotype that remains relatively stable over time with survival well into adulthood (>1 year) (Harris et al., 2002).

[000148] Mybpc3-floxed (Mybpc3 fl/fl) mice ($Mybpc3^{tm2.1Rmos}$; MGI: 5523781) are utilized to create tissue-specific MYBPC3 null animals (Chen et al., 2012). To generate MYBPC3 cardiomyocyte-specific knockout (CKO) mice, Mybpc3 fl/fl mice are crossed with α-myosin heavy chain-transgenic (αMHC-Cre) mice.

[000149] rAAV comprising the MYBPC3 constructs is made as described above and delivered via a single IV injection to presymptomatic and/or symptomatic MYBPC3 mutant mice using different doses. Endpoints include survival as well as cardiac function monitored by echocardiography. Upon necropsy, heart tissues are collected and whole tissue lysates are

analyzed for AAV biodistribution by ddPCR and for human MYBPC3 expression by ELISA and/or immunoblot. In addition, tissue sections are analyzed for histopathology. Therapeutic effects of the rAAV are assessed via the measured endpoints and/or histopathology assessments.

Example 4. MYBPC3 DNA and mRNA detection from treated mice

[000150] Two groups of rAAV comprising (1) AAV9 capsid proteins and (2) rh74 capsid proteins were administered via intraperitoneal injection to neonatal mouse models according to Table 4. After at least 25 days (avg. necroscopy at 28 days), Post-treatment mouse heart tissue was isolated from treated mice and harvested hearts separated into three separate sections: upper heart tissue, middle heart tissue, and lower heart tissue. The upper heart tissue was fixed in formalin for paraffin embedding (FFPE) tissue processing, while middle and lower heart tissues were frozen immediately at -80C. Frozen heart tissues were processed and homogenized via bead beating, and resulting homogenized lysates were split into two fractions: a first fraction for RNA extraction, and a second fraction for DNA extraction.

Table 4

Group Number	Treatment	Animal IDs
1	Control (Untreated)	1-1
		1-2
2	rAAV9-aMHCmCMV-MYBPC3	2-1
		2-2
		2-3
		2-4
		2-5
		2-6
		2-7
3	rAAVrh74-aMHCmCMV-MYBPC3	3-1
		3-2
		3-3
		3-4
		3-5
		3-6

RNA Extraction

[000151] RNA was extracted using extraction reagents from Promega, and purified RNA was analyzed for quantity and quality via Tapestation and nanodrop. RT-PCR was run to detect codon optimized hMYBPC3 mRNA. FIG. 3 demonstrates detection of mRNA signal for hMYBPC3 mRNA from isolated tissue lysates. As can be appreciated, RT positive samples shows mRNA hMYBPC3 signal for AAV9 and RH74 mediated vectors at the predicted size of 260 bp. FIG. 4 shows RT-qPCR plots comparing A: GAPDH control, B: MYBPC3, C: hMYBPC3 RNA normalized to GAPDH, and D: hMYBPC3 RNA normalized with respect to AAV9 samples. Samples 2-2 and 3-5 expressed the high relative fold change for hMYBPC3. The results confirm the detection of hMYBPC3 RNA expression in treated mouse heart samples using AAV9 and RH74 mediated vectors.

DNA Extraction and Assays for hMYBPC3

[000152] DNA was extracted from homogenized tissue lysates similarly to RNA extraction methods and analyzed for quantity and quality via Tapestation and nanodrop. Total DNA was used as template in PCR reaction with primers designed to specifically recognized codon optimized human MYBPC3 DNA. FIG. 5 shows rAAV9 replicates A-D and RH74 replicates E-H, as well as non-treated mock control. The presence of an expected 260 bp band indicates that hMYBPC3 DNA was detectable in treated mice in both rAAV9 and RH74 based constructs. FIG. 6 shows qPCR plots comparing A: GAPDH control, B: MYBPC3, C: hMYBPC3 DNA normalized to GAPDH, and D: hMYBPC3 DNA normalized with respect to AAV9 samples. As can be appreciated, samples 2-2 and 3-5 expressed between 4 to 6 fold increased levels of hMYBPC3 DNA detected in tissue samples. Results confirm the detection of co-hMYBPC3 DNA in treated mouse heart samples using AAV9 and RH74 mediated vectors.

WHAT IS CLAIMED IS:

1. A nucleic acid comprising an expression construct comprising a human MYBPC3 coding sequence, operably linked to a cardiac specific enhancer or regulatory element and a promoter, wherein the expression construct is flanked on each side by an inverted terminal repeat sequence.

2. The nucleic acid of Claim 1, wherein the human MYBPC3 coding sequence is codon-optimized for expression in human cells.

3. The nucleic acid of Claim 1, wherein the human MYBPC3 coding sequence has at least about 85% sequence identity to the sequence of SEQ ID NO: 9, 29 or 43.

4. The nucleic acid of Claim 1, wherein the promoter comprises a cardiac specific promotor.

5. The nucleic acid of Claim 1, wherein the promoter is selected from the group consisting of: CMV, mini-CMV, CBA, HSV, TK, RSV, SV40, MMTV, Ad E1Aand combinations thereof, and wherein the cardiac specific enhancer or regulatory element comprises an alphaMHC enhancer.

6. The nucleic acid of Claim 4, wherein the MYBPC3 promoter sequence has at least about 85% sequence identity to the sequence of SEQ ID NO: 5, 24, or 39.

7. The nucleic acid of Claim 1, wherein the expression construct has at least about 85% sequence identity to the sequence of SEQ ID NO: 1-14, or 20-34, or 35-48 when assembled in sequence.

8. The nucleic acid of Claim 7, wherein the expression construct comprises the sequence SEQ ID NO: 1-14, or 20-34, or 35-48 when assembled in sequence.

9. The nucleic acid of Claim 1, wherein the nucleic acid is a recombinant adeno-associated virus (rAAV) vector.

10. The nucleic acid of Claim 9, wherein the nucleic acid is a single-stranded or self-complementary rAAV nucleic acid vector.

11. A recombinant adeno-associated virus (rAAV) particle comprising the nucleic acid of and one of Claims 1 to 10.

12. The rAAV particle of Claim 11, wherein the rAAV particle is an rh74 particle.

13. The rAAV particle of Claim 11, wherein the rAAV particle is an AAV9 particle.

14. The rAAV particle of Claim 11, wherein the rAAV particle is an rh10 particle.

15. A composition comprising a plurality of the rAAV particle of any one of Claims 12, 13, or 14.

16. The composition of Claim 15, further comprising a pharmaceutically acceptable carrier.

17. A method of treating hypertrophic cardiomyopathy, the method comprising:
administering a therapeutically effective amount of rAAV comprising a nucleic acid expression construct comprising a human MYBPC3 coding sequence operably linked to a cardiac specific enhancer or regulatory element and a promoter, wherein the expression construct is flanked on each side by an inverted terminal repeat sequence, and wherein said administration results in expression of a therapeutically effective amount of human MyBP-C thereby treating the hypertrophic cardiomyopathy.

18. The method of Claim 17, wherein the rAAV is administered via intravenous injection.

19. The method of Claim 17, wherein between about 0.5 and about 5 rAAV vector genomes per cell are administered.

20. The method of Claim 19, wherein between about 0.5 and about 2 rAAV vector genomes per cell are administered.

21. A method of inducing increased expression of human MyBP-C in a target cell, comprising:

contacting a target cell with a plurality of rAAV particles comprising a nucleic acid expression construct comprising a human MYBPC coding sequence operably linked to a cardiac specific enhancer or regulatory element and a promoter, wherein the expression construct is flanked on each side by an inverted terminal repeat sequence, and

wherein said contacting results in the target cell increasing expression of human MyBP-C as compared to prior to the contacting, thereby increasing the expression of human MyBP-C

22. The method of Claim 21, wherein the contacting is in vivo.

23. The method of Claim 21 or 22, for the treatment of hypertrophic cardiomyopathy.

24. Use of the nucleic acid of any one of Claims 1 to 9, the rAAV particle of any one of Claims 11 to 14, or the composition of Claims 15 or 16 in the manufacture of a medicament for the treatment of hypertrophic cardiomyopathy.

25. Use of the nucleic acid of any one of Claims 1 to 9, the rAAV particle of any one of Claims 11 to 14, or the composition of Claims 15 or 16 for the treatment of hypertrophic cardiomyopathy.

26. A nucleic acid comprising an expression construct comprising:

a human MYBPC3 coding sequence;

a cardiac enhancer element operably linked to a promoter; and

a Kozak sequence, wherein the Kozak sequence enhances transgene expression in the heart, wherein the expression construct is flanked on each side by an inverted terminal repeat sequence, wherein the Kozak sequence is non-native with respect to the human MYBPC3 coding sequence, the cardiac enhancer element, and/or the promoter.

27. The nucleic acid of claim 26, wherein the Kozak sequence is a synthetic sequence and has at least 85% sequence identity to the sequence of SEQ ID NO: 28

28. The nucleic acid of Claim 26 to 27, wherein the human MYBPC coding sequence is codon-optimized for expression in human cells.

29. The nucleic acid of Claim 26 to 28, wherein the human MYBPC coding sequence has at least about 85% sequence identity to the sequence of SEQ ID NO: 9, 29 or 43.

30. The nucleic acid of any one of Claims 26 to 29, wherein the promoter comprises a cardiac specific promotor.

31. The nucleic acid of any one of Claims 26 to 29, wherein the promoter is selected from the group consisting of: CMV, mini-CMV, CBA, HSV, TK, RSV, SV40, MMTV, Ad E1Aand combinations thereof, and wherein the cardiac specific enhancer or regulatory element comprises an alphaMHC enhancer.

32. The nucleic acid of Claim 30 or 31, wherein the MYBPC3 promoter sequence has at least about 85% sequence identity to the sequence of SEQ ID NO: 5, 24, or 39.

33. The nucleic acid of any one of Claims 26 to 32, wherein the expression construct has at least about 85% sequence identity to the sequence of SEQ ID NO: 1-14, or 20-34, or 35-48 arranged in sequence.

34. The nucleic acid of Claim 33, wherein the expression construct comprises the sequence of SEQ ID NO: 1-14, or 20-34, or 35-48 arranged in sequence.

35. The nucleic acid of any one of Claims 26 to 34, wherein the nucleic acid is a recombinant adeno-associated virus (rAAV) vector.

36. The nucleic acid of Claim 35, wherein the nucleic acid is a single-stranded or self-complementary rAAV nucleic acid vector.

37. A recombinant adeno-associated virus (rAAV) particle comprising the nucleic acid of Claim 35 or Claim 36.

38. The rAAV particle of Claim 37, wherein the rAAV particle is an AAV9 particle.

39. The rAAV particle of Claim 37, wherein the rAAV particle is an rh74 particle.

40. The rAAV particle of Claim 37, wherein the rAAV particle is an rh10 particle.

41. A composition comprising a plurality of the rAAV particle of any one of Claims 38, 39, or 40.

42. The composition of Claim 41, further comprising a pharmaceutically acceptable carrier.

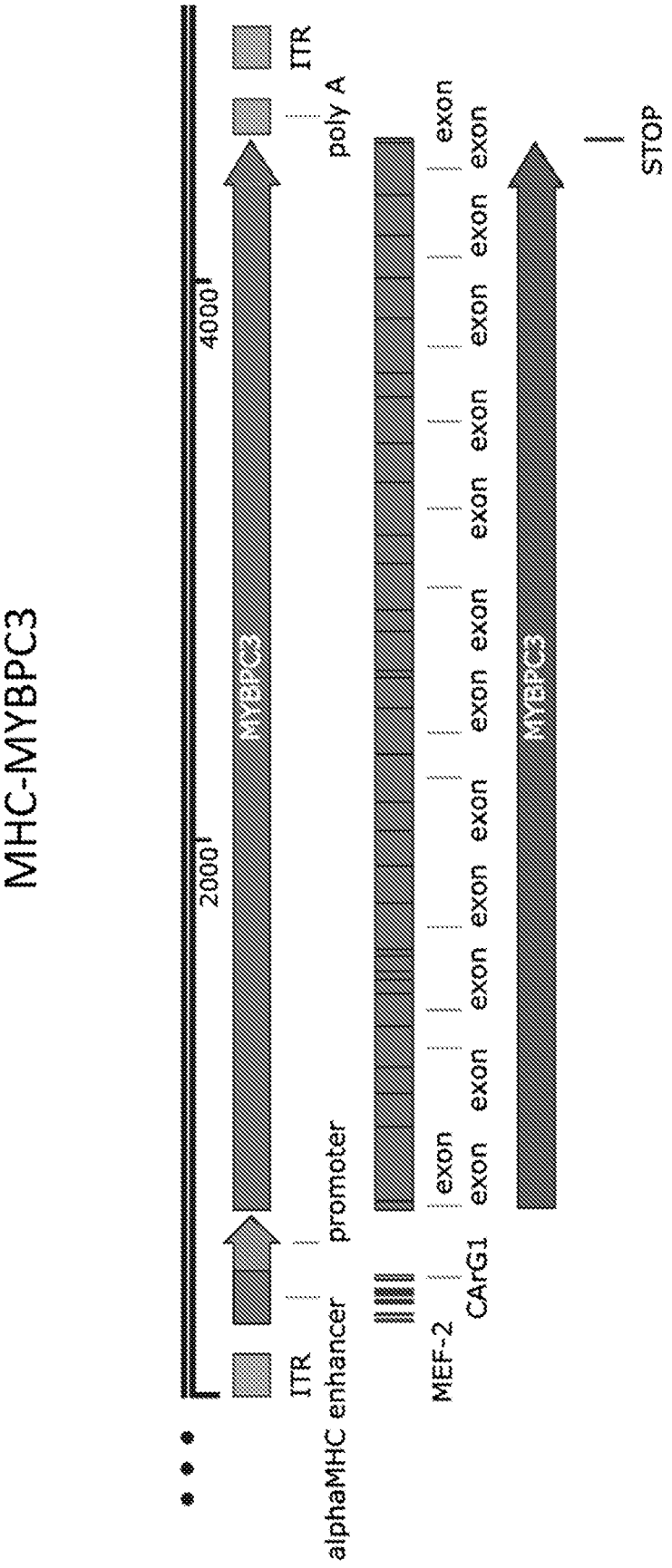

MHC-MYBPC3
2000
4000
MYBPC3
ITR
alphaMHC enhancer
promoter
MEF-2
CArG1
exon
exon exon exon exon exon exon exon exon exon exon exon
MYBPC3
ITR
poly A
STOP

FIG. 1

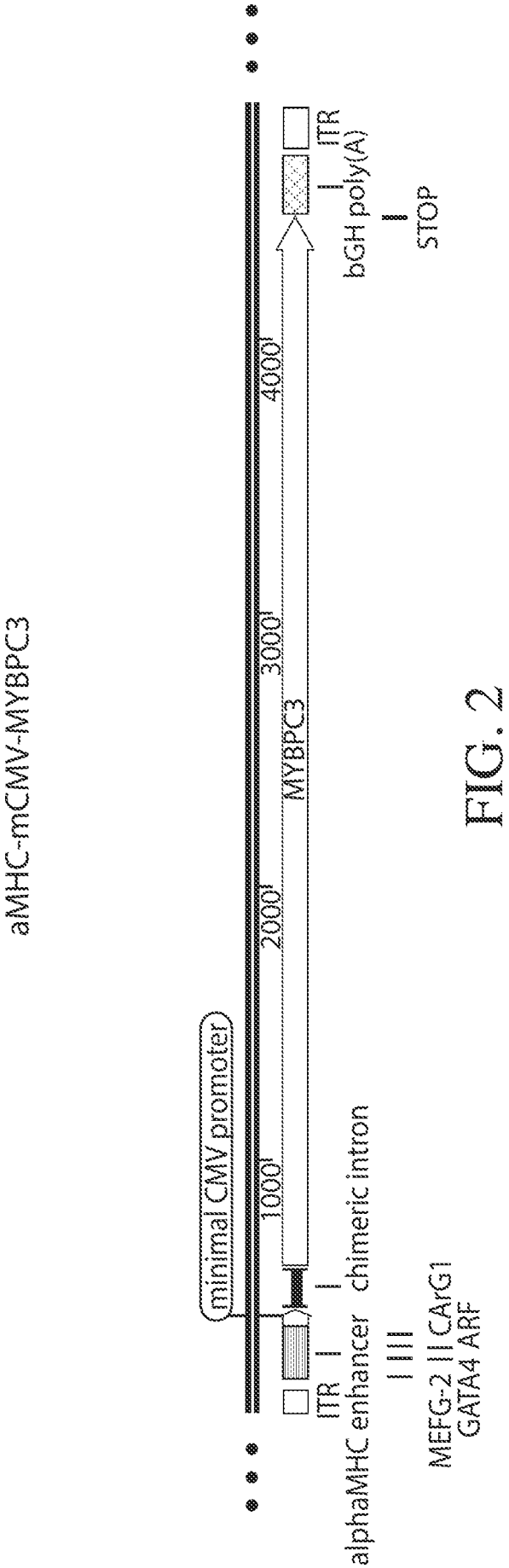
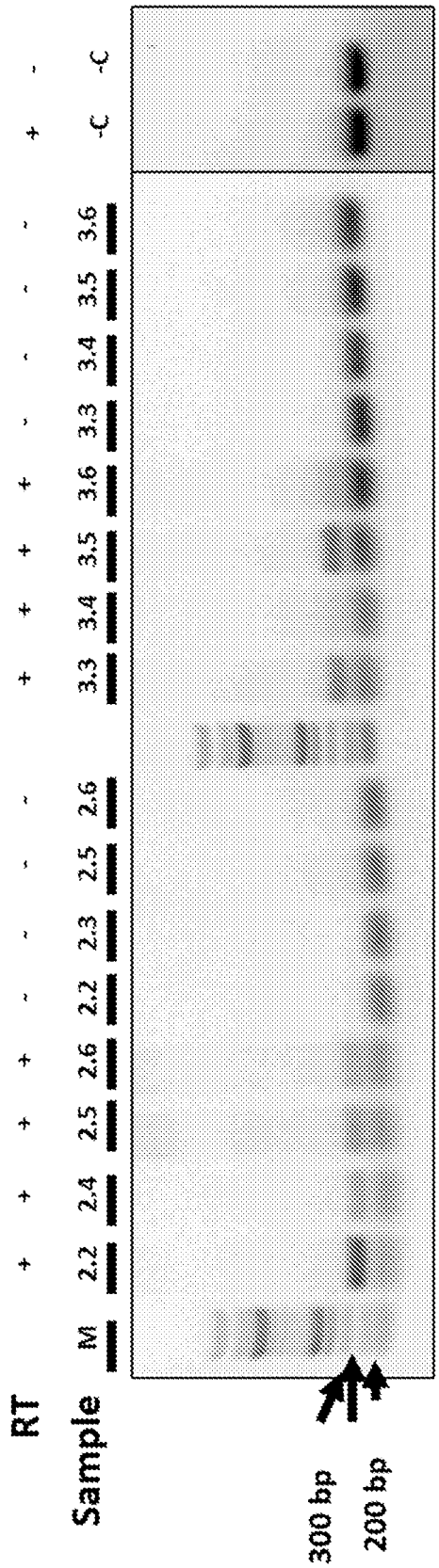

aMHC-mCMV-MYBPC3
minimal CMV promoter
1000'
2000'
MYBPC3
3000'
4000'
ITR
alphaMHC enhancer
chimeric intron
MEFG-2
CArG1
GATA4
ARF
ITR
bGH poly(A)
STOP

FIG. 2


RT
Sample
M
2.2
2.4
2.5
2.6
2.2
2.3
2.5
2.6
3.3
3.4
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3.6
3.3
3.4
3.5
3.6
-C
-C
300 bp
200 bp

FIG. 3

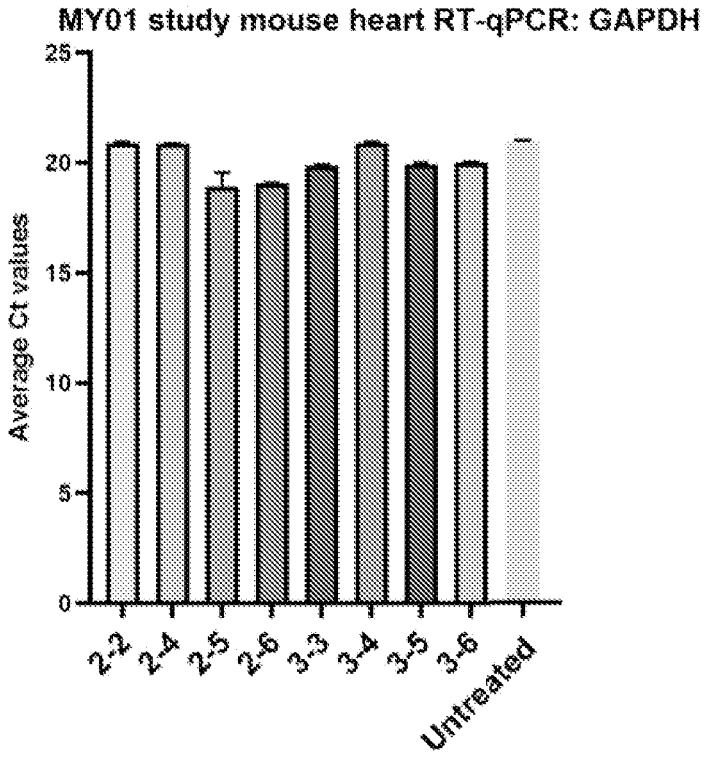
MY01 study mouse heart RT-qPCR: GAPDH
Average Ct values
25
20
15
10
5
0
2-2
2-4
2-5
2-6
3-3
3-4
3-5
3-6
Untreated

FIG. 4A

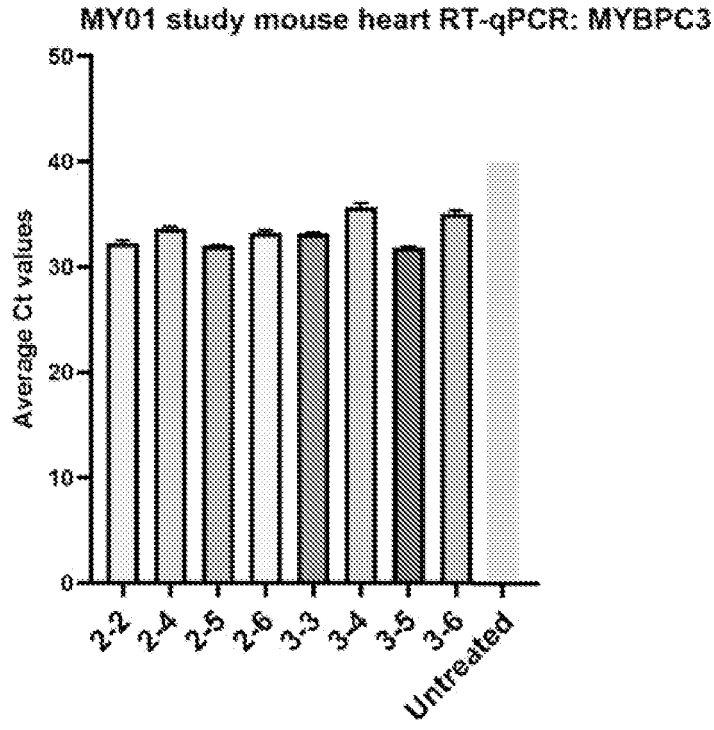
MY01 study mouse heart RT-qPCR: MYBPC3
Average Ct values
50
40
30
20
10
0
2-2
2-4
2-5
2-6
3-3
3-4
3-5
3-6
Untreated

FIG. 4B

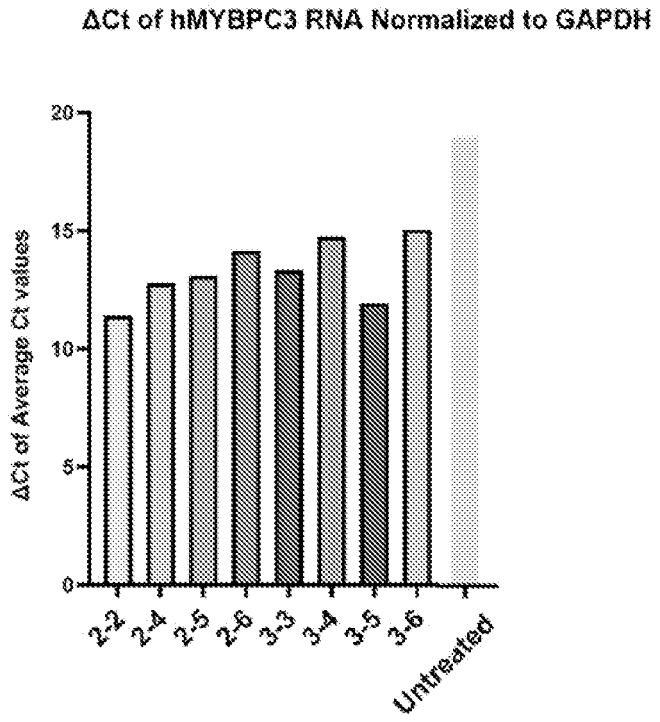
ΔCt of hMYBPC3 RNA Normalized to GAPDH
ΔCt of Average Ct values
20
15
10
5
0
2-2
2-4
2-5
2-6
3-3
3-4
3-5
3-6
Untreated

FIG. 4C

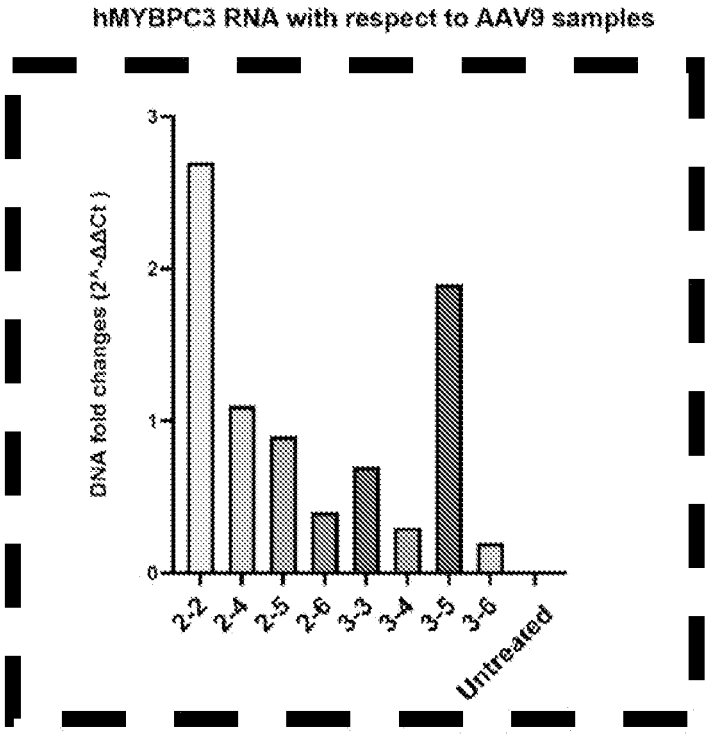
hMYBPC3 RNA with respect to AAV9 samples
DNA fold changes (2^-ΔΔCt)
3
2
1
0
2-2
2-4
2-5
2-6
3-3
3-4
3-5
3-6
Untreated

FIG. 4D

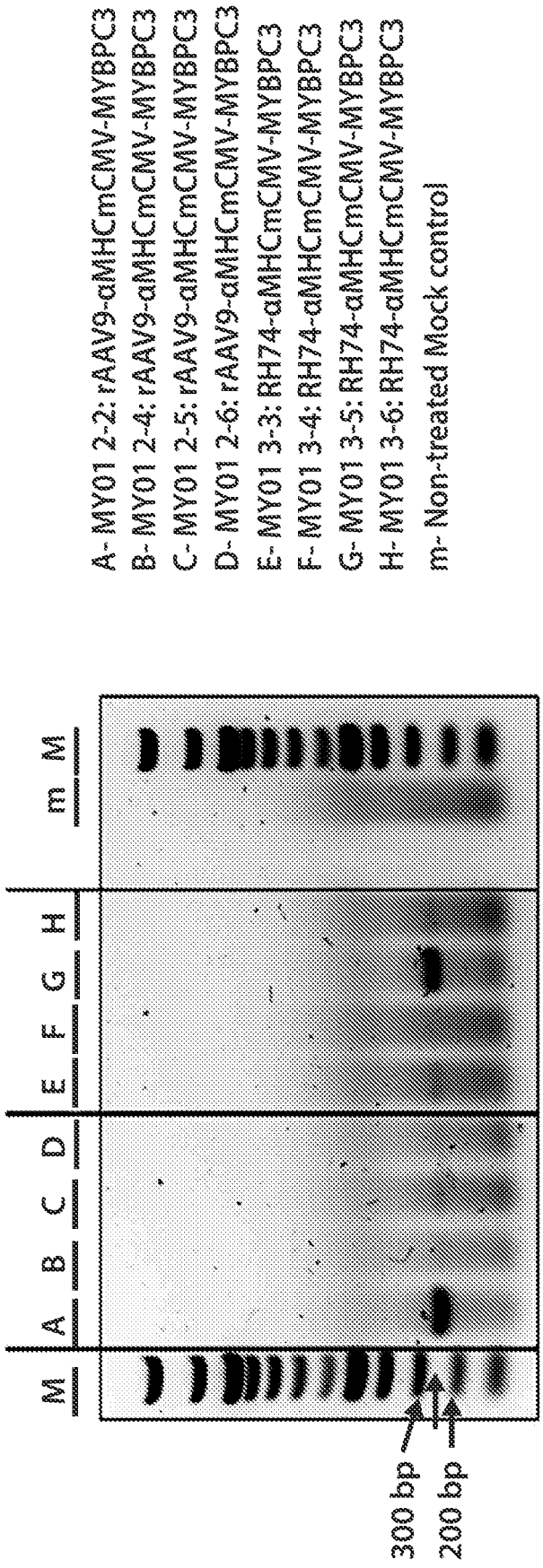
A- MY01 2-2: rAAV9-aMHCmCMV-MYBPC3
B- MY01 2-4: rAAV9-aMHCmCMV-MYBPC3
C- MY01 2-5: rAAV9-aMHCmCMV-MYBPC3
D- MY01 2-6: rAAV9-aMHCmCMV-MYBPC3
E- MY01 3-3: RH74-aMHCmCMV-MYBPC3
F- MY01 3-4: RH74-aMHCmCMV-MYBPC3
G- MY01 3-5: RH74-aMHCmCMV-MYBPC3
H- MY01 3-6: RH74-aMHCmCMV-MYBPC3
m- Non-treated Mock control
M
A
B
C
D
E
F
G
H
m
M
300 bp
200 bp

FIG. 5

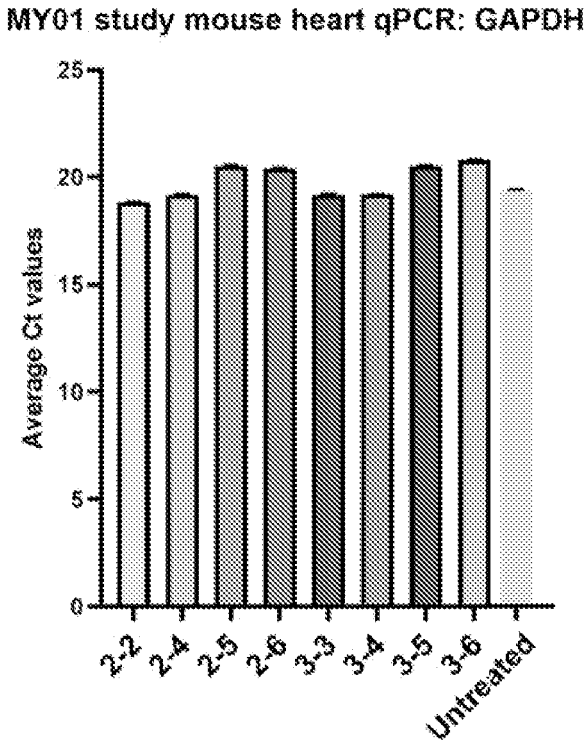
MY01 study mouse heart qPCR: GAPDH
Average Ct values
25
20
15
10
5
0
2-2
2-4
2-5
2-6
3-3
3-4
3-5
3-6
Untreated

FIG. 6A

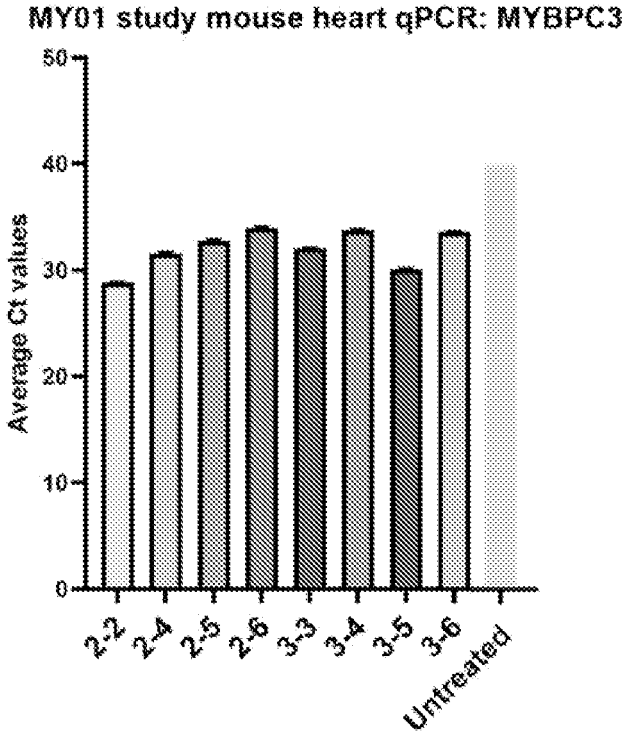
MY01 study mouse heart qPCR: MYBPC3
Average Ct values
50
40
30
20
10
0
2-2
2-4
2-5
2-6
3-3
3-4
3-5
3-6
Untreated

FIG. 6B

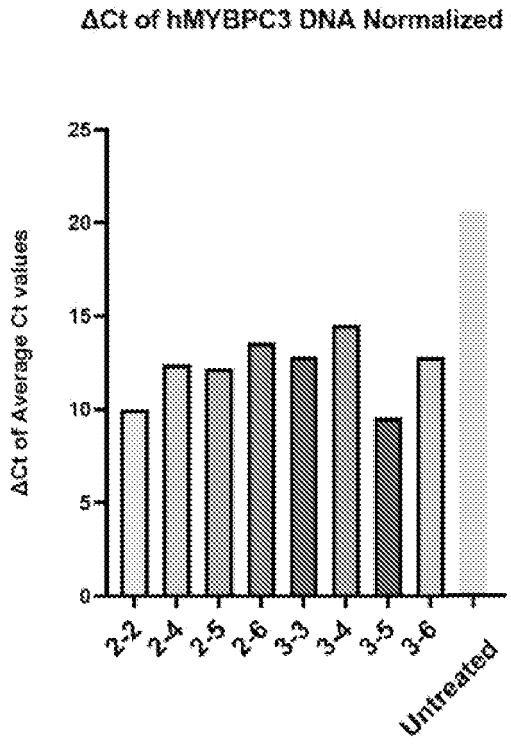
ΔCt of hMYBPC3 DNA Normalized to GAPDH
ΔCt of Average Ct values
25
20
15
10
5
0
2-2
2-4
2-5
2-6
3-3
3-4
3-5
3-6
Untreated

FIG. 6C

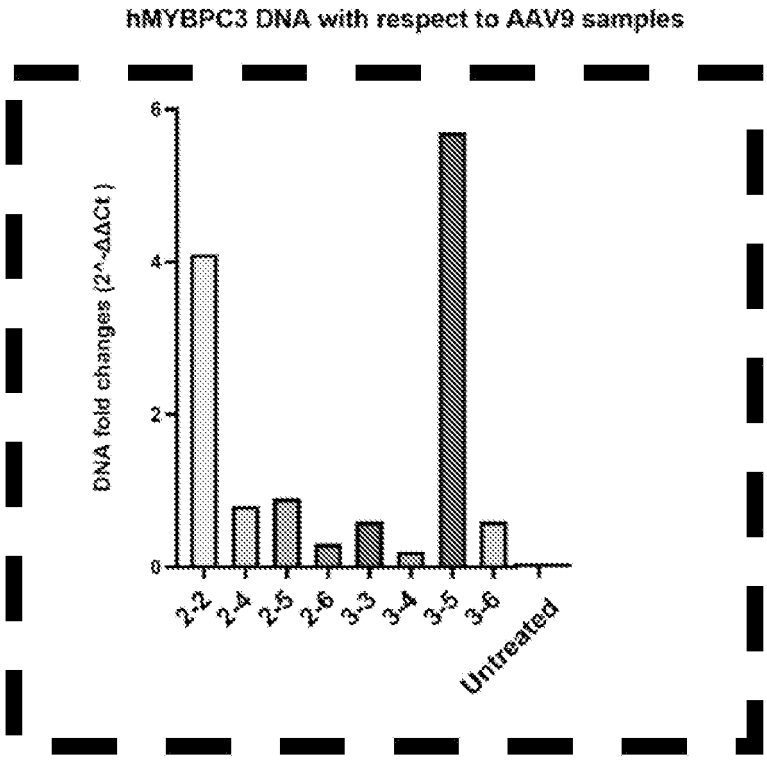
hMYBPC3 DNA with respect to AAV9 samples
DNA fold changes (2^-ΔΔCt)
6
4
2
0
2-2
2-4
2-5
2-6
3-3
3-4
3-5
3-6
Untreated

FIG. 6D