GENE THERAPY VECTORS FOR TREATING HEART DISEASE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Patent Application Serial No. 63/047,633 filed on July 2, 2020 and U.S. Provisional Patent Application Serial No. 62/976,160 filed on February 13, 2020, the contents of which are hereby incorporated by reference in their entireties.

TECHNICAL FIELD

[0002] The present disclosure relates to compositions and methods for the treatment or prevention of heart disease (*e.g.*, cardiomyopathy) in a subject. In particular, the present disclosure relates to a vector comprising a cardiac-specific promoter operability linked to a therapeutic gene product for the treatment of heart disease (*e.g.*, cardiomyopathy).

REFERENCE TO SEQUENCE LISTING

[0003] This application is being filed electronically via EFS-Web and includes an electronically submitted sequence listing in .txt format. The .txt file contains a sequence listing entitled "TENA_015_02WO_SeqList_ST25.txt" created on February 9, 2021 and having a size of 824 kilobytes. The sequence listing contained in this .txt file is part of the specification and is incorporated herein by reference in its entirety.

BACKGROUND

[0004] Gene therapy approaches for the treatment of heart disease often employ vectors configured to effectively transduce cardiac cells and to express a transgene in a cardiac-tissue specific manner. Adeno-associated virus (AAV) vectors, cardiac-specific promoters, or both in combination, may be used to deliver a polynucleotide encoding a gene product (*e.g.* a therapeutic protein) to heart tissue and thereby express the gene product in that tissue to treat the heart disease. Cardiac-specific promoters include desmin (Des), alpha-myosin heavy chain (α-MHC), myosin light chain 2 (MLC-2) and cardiac troponin C (TNNC1 or cTnC) promoters, as well as the 600

base pair cardiac troponin T (TNNT2) promoter. The delivery of polynucleotides encoding large proteins remains challenging, however, due in part to the packaging limit of viral vectors.

[0005] Given these challenges, there remains a need in the art for improved gene therapy vectors for heart disease.

SUMMARY

[0006] The present disclosure relates generally to compositions and methods for the treatment or prevention of heart disease (*e.g.* cardiomyopathy). In a first ~~aspect~~embodiment, the present disclosure provides vectors comprising a promoter, optionally a cardiac-specific promoter, operably linked to a polynucleotide encoding a therapeutic gene product for the treatment of prevention of heart disease, *e.g.*, cardiomyopathy. The vector may be an adeno-associated viral (AAV) vector.

[0007] In some ~~aspects~~embodiments, the present disclosure provides a cardiac troponin T promoter, comprising a polynucleotide having between 300 bp and 500 bp. In some embodiments, the polynucleotide comprises a sequence that shares at least 80%, at least 90%, or at least 100% identity to any one of SEQ ID NOs: 1-85. In some embodiments, the polynucleotide comprises a sequence that shares at least 80%, at least 90%, or at least 100% identity to SEQ ID NO: 1. In some embodiments, the polynucleotide comprises a sequence that shares at least 80%, at least 90%, or at least 100% identity to SEQ ID NO: 3. In some embodiments, the polynucleotide shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% sequence identity with a genomic polynucleotide sequence upstream of and including the transcription start site of a troponin T gene. In some embodiments, the polynucleotide shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% sequence identity with a genomic polynucleotide sequence -450 bp to +1 bp relative to the transcription start site of a troponin T gene. In some embodiments, the polynucleotide shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% sequence identity with a genomic polynucleotide sequence -350 bp to +1 bp relative to the transcription start site of a troponin T gene. In some embodiments, the polynucleotide shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% sequence identity with a genomic polynucleotide sequence -250 bp to +1 bp relative to the transcription start site of a troponin T gene. In some embodiments, the polynucleotide shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% sequence identity with a genomic polynucleotide sequence -450 bp to +50 bp relative to the transcription

start site of a troponin T gene. In some embodiments, the polynucleotide shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% sequence identity with a genomic polynucleotide sequence -350 bp to +50 bp relative to the transcription start site of a troponin T gene. In some embodiments, the polynucleotide shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% sequence identity with a genomic polynucleotide sequence -250 bp to +50 bp relative to the transcription start site of a troponin T gene. In some embodiments, the troponin T gene is a human troponin T gene.

[0008] In some embodiments, the promoter is a muscle-specific promoter. In some embodiments, the promoter is a cardiac cell-specific promoter. In some embodiments, the promoter is a cardiomyocyte-specific promoter. In some embodiments, the promoter has the same cell-type specificity as a native troponin T promoter of about 600 bp. In some embodiments, the promoter described herein has the same cell-type specificity as a reference promoter comprising SEQ ID NO: 1. In some embodiments, the promoter expresses a gene product operatively linked thereto at least about 10%, at least about 20%, at least about 30% more than a native troponin T promoter. In some embodiments, the promoter described herein expresses a gene product operatively linked thereto at least about 10%, at least about 20%, at least about 30% more than a reference promoter comprising SEQ ID NO: 1.

[0009] In some ~~aspects~~embodiments, the present disclosure provides a vector comprising any one of the promoters described herein operatively linked to a polynucleotide encoding a gene product. In some embodiments the vector is a viral vector. In some embodiments, the viral vector is an adeno-associated virus vector (AAV). In some embodiments, the viral vector has a packaging limit of at most about 5.5 kb.

[0009a] Disclosed herein is a recombinant vector comprising a promoter, wherein the promoter is a modified human cardiac troponin T promoter consisting of a polynucleotide having between 350 bp and 450 bp, wherein the polynucleotide comprises a sequence that shares at least 80% identity, at least 90% or 100% identity to SEQ ID NO: 3.

[0009b] Disclosed herein is a recombinant vector, comprising an expression cassette comprising or consisting of, in 5′ to 3′ order, a 5′ segment comprising a promoter, a polynucleotide encoding a gene product, and a 3′ segment comprising a polyA signal, wherein: a) the polynucleotide encoding the gene product comprises between 3 kb and 11 kb, between 3 kb and 5kb, between 3.5 kb and 4.5kb, or between 3.7 kb and 4 kb, b) the 5’ segment and the 3’ segment together are at

most 0.8 kb or at most 0.9 kb, and c) the vector does not comprise a woodchuck hepatitis virus post-transcriptional element (WPRE).

[0009c] Disclosed herein is a recombinant adeno-associated virus (AAV) vector, comprising an expression cassette comprising or consisting of, in 5′ to 3′ order, a 5′ segment comprising a promoter, a polynucleotide encoding a gene product, and a 3′ segment comprising a polyA signal, the expression cassette optionally flanked by one or both of a 5′ inverted terminal repeat (ITR) and a 3′ ITR, wherein: a) the polynucleotide encoding the gene product comprises between 3 kb and 5kb, between 3.5 kb and 4.5kb, or between 3.7 kb and 4 kb; b) the 5′ segment and the 3′ segment together are at most 0.8 kb or at most 0.9 kb; c) the 5′ ITR, the 5′ segment, the 3′ segment, and the 3′ ITR together are at most 1.2 kb; and/or d) the vector genome is at most 4.7 kb, at most 4.8 kb, or at most 4.9 kb.

[0010] In some embodiments, the gene product is selected from MYBPC3, KCNH2, TRPM4, DSG2, and ATP2A2 protein. In some embodiments, the gene product is selected from CACNA1C, DMD, DMPK, EPG5, EVC, EVC2, FBN1, NF1, SCN5A, SOS1, NPR1, ERBB4, VIP, and MYH7 proteins. In some embodiments, the gene product is a Cas9, optionally selected from SpCas9, St1Cas9, and SaCas9.

[0011] In some embodiments, the vector described herein comprises a polynucleotide encoding a second gene product. In some embodiments, the second gene product is a functional RNA, optionally a microRNA or a guide RNA.

[0012] In some ~~aspects~~embodiments, the present disclosure provides an isolated cell comprising any one of the promoters described herein. In some embodiments, the isolated cell is an induced pluripotent stem cell or an isolated cardiomyocyte.

[0012a] Disclosed herein is an isolated cell comprising the vector or the rAAV virion as defined herein, optionally wherein the isolated cell is an induced pluripotent stem cell, a cardiac cell, or a cardiomyocyte.

[0013] In some ~~aspects~~embodiments, the present disclosure provides a pharmaceutical composition comprising any one of the vectors described herein.

[0014] In some ~~aspects~~embodiments, the present disclosure provides a cell therapy composition comprising any one of the isolated cells described herein.

[0014a] Disclosed herein is a cell therapy composition comprising the cell as defined herein.

[0015] In some ~~aspects~~embodiments, the present disclosure provides a recombinant adeno-associated virus (AAV) vector genome, comprising a *MYBPC3* polynucleotide encoding a MYBPC3 protein, or a functional variant thereof, and a promoter, wherein the promoter is a polynucleotide having between 300 bp and 500 bp. In some embodiments, the polynucleotide comprises a sequence that shares at least 80%, at least 90%, or at least 100% identity to any one of SEQ ID NOs: 1-85. In some embodiments, the polynucleotide comprises a sequence that shares at least 80%, at least 90%, or at least 100% identity to any one of SEQ ID NO: 1. In some embodiments, the polynucleotide comprises a sequence that shares at least 80%, at least 90%, or at least 100% identity to any one of SEQ ID NO: 3. In some embodiments, the polynucleotide shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% sequence identity with a genomic polynucleotide sequence upstream of and including the transcription start site of a troponin T gene. In some embodiments, the polynucleotide shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% sequence identity with a genomic polynucleotide sequence -450 bp to +1 bp relative to the transcription start site of a troponin T gene. In some embodiments, the polynucleotide shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% sequence identity with a genomic polynucleotide sequence -350 bp to +1 bp relative to the transcription start site of a troponin T gene. In some embodiments, the polynucleotide shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% sequence identity with a genomic polynucleotide sequence -250 bp to +1 bp relative to the transcription start site of a troponin T gene. In some embodiments, the polynucleotide shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% sequence identity with a genomic polynucleotide sequence -450 bp to +50 bp relative to the transcription start site of a troponin T gene. In some embodiments, the polynucleotide shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% sequence identity with a genomic polynucleotide sequence -350 bp to +50 bp relative to the transcription start site of a troponin T gene. In some embodiments, the polynucleotide shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% sequence identity with a genomic polynucleotide sequence -250 bp to +50 bp relative to the transcription start site of a troponin T gene. In some embodiments, the troponin T gene is a human troponin T gene.

[0016] In some embodiments, the promoter is a muscle-specific promoter. In some embodiments, the promoter is a cardiac cell-specific promoter. In some embodiments, the promoter is a cardiomyocyte-specific promoter. In some embodiments, the promoter has the same cell-type

specificity as a native troponin T promoter of about 600 bp. In some embodiments, the promoter has the same cell-type specificity as a reference promoter comprising SEQ ID NO: 1. In some embodiments, the promoter expresses a gene product operatively linked thereto at least about 10%, at least about 20%, at least about 30% more than a native troponin T promoter. In some embodiments, the promoter expresses a gene product operatively linked thereto at least about 10%, at least about 20%, at least about 30% more than a reference promoter comprising SEQ ID NO: 1.

[0017] In some embodiments, the recombinant adeno-associated virus (AAV) vector genome described herein comprises a *MYBPC3* polynucleotide encoding a MYBPC3 protein. In some embodiments, the *MYBPC3* polynucleotide comprises at least about 3.5 kB. In some embodiments, the *MYBPC3* polynucleotide comprises about 3.8 kB. In some embodiments, the MYBPC3 is a full-length MYBPC3. In some embodiments, the MYBPC3 is a truncated MYBPC3.

[0018] In some embodiments, the rAAV vector genome described herein expresses MYBPC3. In some embodiments, the rAAV vector genome expresses MYBPC3 at about the same level as a reference AAV vector comprising a native troponin T promoter of about 600 bp. In some embodiments, the rAAV vector genome expresses MYBPC3 at a level at least about 10% greater than a reference AAV vector comprising a native troponin T promoter of about 600 bp. In some embodiments, the rAAV vector genome expresses MYBPC3 at a level at least about 20% greater than a reference AAV vector comprising a native troponin T promoter of about 600 bp.

[0019] In some ~~aspects~~embodiments, the present disclosure provides a recombinant adeno-associated virus (AAV) vector genome, comprising an expression cassette comprising, in 5′ to 3′ order, a 5′ segment comprising a promoter; a polynucleotide encoding a gene product; and a 3′ segment comprising a polyA signal, the expression cassette optionally flanked by one or both of a 5′ inverted terminal repeat (ITR) and a 3′ ITR, wherein the polynucleotide encoding the gene product comprises between 3 kb and 11kb, between 3 kb and 5kb, between 3.5 kb and 4.5kb, or between 3.7 kb and 4 kb; and wherein: a) the 5′ segment and the 3′ segment together comprise at most 0.8 kbp or at most 0.9 kbp; b) the 5′ ITR, 5′ segment, the 3′ segment, and 3′ ITR together comprise or at most 1.2 kbp, at most 1.3 kbp; and/or c) the vector genome comprises at most 4.7 kbp, at most 4.8 kbp, at most 4.9 kbp, or at most 5.0 kbp. In some embodiments, the 5′ segment comprises at most 500 bp or at most 480 bp. In some embodiments, the 3′ segment comprises at most 200 bp or at most 150 bp.

[0020] In some embodiments, the rAAV vector genome comprises a polynucleotide encoding a gene product comprising 3.7 kbp to 3.9 kbp, optionally 3.8 kbp. In some embodiments, the gene product is MYBPC3, or a functional variant thereof. In some embodiments, the gene product is MYBPC3. In some embodiments, the polynucleotide encoding MYBPC3 shares at least 90% identity to SEQ ID NO: 86. In some embodiments, the polynucleotide encoding MYBPC3 share at least 95% identify to SEQ ID NO: 86. In some embodiments the polynucleotide encoding MYBPC3 is SEQ ID NO: 86. In some embodiments, MYBPC3 shares at least 90% identity to the polypeptide sequence of SEQ ID NO: 103. In some embodiments, MYBPC3 shares at least 95% identity to the polypeptide sequence of SEQ ID NO: 103. In some embodiments, MYBPC3 shares 100% identity to the polypeptide sequence of SEQ ID NO: 103.

[0021] In some embodiments, the rAAV vector genome comprises a promoter, wherein the promoter is a polynucleotide having between 300 bp and 500 bp. In some embodiments the promoter comprises a sequence that shares at least 80% identity to SEQ ID NO:1-85 or SEQ ID NO: 91. In some embodiments the promoter comprises a sequence that shares at least 90% identity to SEQ ID NO:1-85 or SEQ ID NO: 91. In some embodiments the promoter comprises a sequence that shares at least 100% identity to SEQ ID NO:1-85 or SEQ ID NO: 91.

[0022] In some embodiments, the rAAV vector genome comprises a polyA signal. In some embodiments, the polyA signal comprises, consists essentially of, or consists of a sequence that shares at least 90% identity to SEQ ID NO: 92. In some embodiments, the polyA signal comprises, consists essentially of, or consists of a sequence that shares at least 95% identity to SEQ ID NO: 92. In some embodiments, the polyA signal is SEQ ID NO: 92.

[0023] In some embodiments, the rAAV vector genome comprises a 5′ segment. In some embodiments, the 5′ segment shares at least 80% identity to SEQ ID NO: 93. In some embodiments, the 5′ segment shares at least 90% identity to SEQ ID NO: 93. In some embodiments, the 5′ segment shares at least 95% identity to SEQ ID NO: 93. In some embodiments, the 5′ segment is SEQ ID NO: 93.

[0024] In some embodiments, the rAAV vector genome comprises a 3′ segment. In some embodiments, the 3′ segment shares at least 80% identity to SEQ ID NO: 94. In some embodiments, the 3′ segment shares at least 90% identity to SEQ ID NO: 94. In some embodiments, the 3′ segment shares at least 95% identity to SEQ ID NO: 94. In some embodiments, the 3′ segment is SEQ ID NO: 94.

[0025] In some embodiments, the rAAV vector genome comprises an expression cassette. In some embodiments, the expression cassette shares at least 80% identity to SEQ ID NO: 95. In some embodiments, the expression cassette shares at least 90% identity to SEQ ID NO: 95. In some embodiments, the expression cassette shares at least 95% identity to SEQ ID NO: 95. In some embodiments, the expression cassette is SEQ ID NO: 95.

[0026] In some embodiments, the rAAV genome comprises an expression cassette that is flanked by one or both of a 5′ inverted terminal repeat (ITR) and a 3′ ITR. In some embodiments, the 5′ ITR comprises a sequence that shares 95% identity to SEQ ID NO: 96. In some embodiments, the 3′ ITR comprises a sequence that shares at least 95% identity to SEQ ID NO: 97.

[0027] In some embodimentsaspects, the present disclosure provides a recombinant AAV (rAAV) virion. In some embodiments the rAAV virion comprises any one of the rAAV vector genomes described herein and an AAV capsid protein.

[0027a] Disclosed herein is a recombinant adeno-associated virus (rAAV) virion, comprising a vector genome comprising an expression cassette comprising a polynucleotide encoding MYBPC3 operatively linked to a promoter, the expression cassette flanked by a 5′ inverted terminal repeat (ITR) and a 3′ ITR; wherein the vector genome comprises at most 4.8 kb.

[0028] In some aspectsembodiments, the present disclosure provides a method of expressing a MYBPC3 protein in a cell, comprising transducing the cell with an rAAV virion described herein or any one of the rAAV vector genomes described herein. In some embodiments, the cell is a *MYBPC3*$^{-/-}$ cell. In some embodiments, the cell comprises an inactivating mutation in one or both copies of the endogenous *MYBPC3* gene.

[0028a] Disclosed herein is an *in vitro* or *ex vivo* method of expressing a MYBPC3 protein in a cell, comprising transducing the cell with the vector as defined herein, or the rAAV virion as defined herein.

[0029] In some aspectsembodiments, the present disclosure provides a method of treating and/or preventing a cardiomyopathy in a subject in need thereof, comprising administering the rAAV virion described herein or any one of the rAAV vector genomes described herein to the subject, wherein optionally the subject suffers from or is at risk for cardiomyopathy.

[0029a] Disclosed herein is the use of the vector as defined herein, the rAAV virion as defined herein, or the pharmaceutical composition as defined herein, in the manufacture of a medicament for treating and/or preventing a cardiomyopathy in a subject.

[0030] In some ~~aspects~~embodiments, the present disclosure provides a method of expressing a MYBPC3 protein in the heart of a subject in need thereof, comprising administering the rAAV virion described herein or any one of the rAAV vector genomes described herein to the subject, optionally a subject suffering from or at risk for cardiomyopathy, wherein optionally the subject suffers from or is at risk for cardiomyopathy.

[0031] In some embodiments, administration of the AAV vector causes specific expression of MYBPC3 in the heart of the subject. In some embodiments, administration of the AAV vector causes low or undetectable expression of MYBPC3 in the skeletal tissue, brain, and/or liver of the subject, wherein optionally the subject suffers from or is at risk for cardiomyopathy.

[0032] In some ~~aspects~~embodiments, the present disclosure provides a method of treating a disease caused by a MYBPC3 mutation in a subject in need thereof, comprising administering the rAAV virion described or any one of the rAAV vector genomes described herein to the subject, wherein optionally the subject suffers from or is at risk for cardiomyopathy.

[0032a] Disclosed herein is the use of the vector as defined herein, the rAAV virion as defined herein, or the pharmaceutical composition as defined herein, in the manufacture of a medicament for treating and/or preventing a disease or disorder caused by a MYBPC3 mutation in a subject.

[0033] In some ~~aspects~~embodiments, the present disclosure provides a method of increasing MYBPC3 activity and/or increasing cardiac function in the heart of a subject in need thereof, comprising administering the rAAV virion described herein or any one of the rAAV vector genomes described herein to the subject, wherein optionally the subject suffers from or is at risk for cardiomyopathy.

[0034] In some embodiments, the methods described herein treats the cardiomyopathy. In some embodiments, the methods described herein prevents the cardiomyopathy. In some embodiments, the cardiomyopathy is hypertrophic cardiomyopathy.

[0035] In some embodiments, the methods described herein comprise intravenous administration of the rAAV virion described herein or any one of the rAAV vector genomes described herein to the subject. In some embodiments, the methods described herein comprise intracardiac administration of the rAAV virion described herein or any one of the rAAV vector genomes described herein to the subject. In some embodiments, the methods described herein comprise direct injection of the rAAV virion described herein or any one of the rAAV vector genomes

described herein to the subject. In some embodiments, the methods described herein comprise administering a dose of about 10^{11} to about 10^{14} rAAV virions per kg or viral genomes per kg.

[0036] In some embodiments, the subject is a mammal. In some embodiments, the subject is a human. In some embodiments, the subject is an adult.

[0037] In some embodiments, the pharmaceutical compositions described herein are for use as a medicament in therapeutic or prophylactic treatment of heart disease, *e.g.* cardiomyopathies.

BRIEF DESCRIPTION OF THE DRAWINGS

[0038] FIG. 1A shows maps of insert sequences for AAV vector genomes adapted for large cargoes, showing deletion or truncation of two *cis*-regulatory elements.

[0039] FIG. 1B shows flow cytometry analysis, two days post-infection, of human cardiac fibroblasts (n = 2), MOI 160,000 with AAV-packaged constructs.

[0040] FIG. 1C shows a map of an insert sequence for an AAV vector genome adapted for large cargoes, showing deletion or truncation of two *cis*-regulatory elements, deletion of the intron, and partial deletion of the sequence 3′ to the 5′ ITR.

[0041] FIG. 2A shows a schematic of the original and altered versions of a viral genome comprising the cardiac-specific troponin (TNNT2) promoter and myosin binding protein C (MYBPC3) transgene.

[0042] FIG. 2B shows detection of MYBPC3 protein by immunofluorescence in *MYBPC3*$^{-/-}$ iPSC-derived cardiomyocytes transduced with AAV6-packaged constructs encoding MYBPC3 driven by the cardiac-specific TNNT2 promoter.

[0043] FIG. 3A shows detection of MYBPC3 protein by Western blot in *MYBPC3*$^{-/-}$ iPSC-derived cardiomyocytes transduced with AAV6-packaged constructs encoding human MYBPC3 driven by various sizes (400-600 bp) of the cardiac-specific TNNT2 promoter. GAPDH was used as a loading control.

[0044] FIG. 3B shows detection of MYBPC3 protein by Western blot in *MYBPC3*$^{-/-}$ iPSC-derived cardiomyocytes transduced with AAV6-packaged constructs encoding human MYBPC3 driven by various sizes (400 or 600 bp) of the human cardiac TNNT2 promoter. No Kozak sequence was used as a negative control and GAPDH was used as a loading control.

[0045] FIG. 3C shows detection of MYBPC3 protein by Western blot in *MYBPC3*$^{-/-}$ iPSC-derived cardiomyocytes transfected with AAV6 plasmids encoding human MYBPC3 driven by various

sizes (400 or 600 bp) of the human cardiac TNNT2 promoter. GAPDH was used as a loading control.

[0046] FIG. 4A shows a map of the introns of the *Mybpc3* gene and a dot blot of MYBPC3 protein expression in the founder mouse of the KO line.

[0047] FIG. 4B shows a bar graph of body weight of wild-type (WT) or KO mice (*Mybpc3*$^{-/-}$) littermates at two weeks of age. WT (n = 11), *Mybpc3*$^{+/-}$ (n = 7) and *Mybpc3*$^{-/-}$ (n = 13).

[0048] FIG. 4C shows a bar graph of ejection fraction (%) measured by echocardiography in wild-type (WT), heterozygous KO mice (*Mybpc3*$^{+/-}$), or homozygous KO mice (*Mybpc3*$^{-/-}$) at two weeks of age.

[0049] FIG. 4D shows a bar graph of fractional shortening measured by echocardiography in wild-type (WT), heterozygous KO mice (*Mybpc3*$^{+/-}$), or homozygous KO mice (*Mybpc3*$^{-/-}$) at two weeks of age.

[0050] FIG. 4E shows a bar graph of left ventricular (LV) mass normalized by body weight (BW) in wild-type (WT), heterozygous KO mice (*Mybpc3*$^{+/-}$), or homozygous KO mice (*Mybpc3*$^{-/-}$) at two weeks of age.

[0051] FIG. 4F shows a bar graph of Left ventricular internal diameter during systole (LVIDs) normalized by body weight in wild-type (WT), heterozygous KO mice (*Mybpc3*$^{+/-}$), or homozygous KO mice (*Mybpc3*$^{-/-}$) at two weeks of age.

[0052] FIG. 4G shows a bar graph of Left ventricular internal diameter during diastole (LVIDd) normalized by body weight in wild-type (WT), heterozygous KO mice (*Mybpc3*$^{+/-}$), or homozygous KO mice (*Mybpc3*$^{-/-}$) at two weeks of age.

[0053] FIG. 5 shows detection of MYBPC3 mRNA by qRT-PCR in heart, skeletal, brain and liver tissue harvested from mice retro-orbitally injected with E12 GC AAV9-packaged constructs encoding human MYBPC3 driven by various sizes (400 or 600 bp) of the human cardiac TNNT2 promoter.

[0054] FIG. 6A shows a bar graph showing absolute quantification of vector genomes per microgram of genomic DNA in the heart and liver of adult mice 4 weeks after intravenous administration with an AAV9 vector containing the 400 bp modified TNNT2 promoter cassette.

[0055] FIG. 6B shows a bar graph showing fold increase over vehicle of transgene RNA in the heart and liver of adult mice 4 weeks after intravenous administration with an AAV9 vector containing the 400 bp modified TNNT2 promoter cassette.

[0056] FIG. 7 shows a western blot of MYBPC3 protein expression in homozygous *Mybpc3*$^{-/-}$ mice injected retro-orbitally at two weeks of age with 1E14 vg·kg^{-1} test vector encoding *Mybpc3* or vehicle, HBSS.

[0057] FIG. 8 shows a bar graph showing MYBPC3 expression in homozygous *Mybpc3*$^{-/-}$ mice injected retro-orbitally at two weeks of age 3E13 vg·kg^{-1} and 1E14 vg·kg^{-1} test vector encoding *Mybpc3* or vehicle, HBSS.

[0058] FIG. 9A shows a bar graph showing left ventricular mass normalized to body weight (LVM/BM) in homozygous *Mybpc3*$^{-/-}$ mice 6 weeks after they were injected retro-orbitally at two weeks of age with 1E13 vg·kg-1, 3E13 vg·kg^{-1}, and 1E14 vg·kg^{-1} of test vector encoding *Mybpc3* or vehicle, HBSS.

[0059] FIG. 9B shows a bar graph showing FAS expressed as % percentage change in LV internal dimensions between systole and diastole in homozygous *Mybpc3*$^{-/-}$ mice 6 weeks after they were injected retro-orbitally at two weeks of age with 1E13 vg·kg^{-1}, 3E13 vg·kg^{-1}, and 1E14 vg·kg-1 of test vector encoding *Mybpc3* or vehicle, HBSS.

[0060] FIG. 9C shows a bar graph showing ejection fraction in homozygous *Mybpc3*$^{-/-}$ mice 6 weeks after they were injected retro-orbitally at two weeks of age with 1E13 vg·kg^{-1}, 3E13 vg·kg^{-1}, and 1E14 vg·kg-1 of test vector encoding *Mybpc3* or vehicle, HBSS.

[0061] FIG. 9D shows a bar graph showing left ventricular mass normalized to body weight (LVM/BM) in homozygous *Mybpc3*$^{-/-}$ mice 31 weeks after they were injected retro-orbitally at two weeks of age with 1E13 vg·kg-1, 3E13 vg·kg^{-1}, and 1E14 vg·kg^{-1} of test vector encoding *Mybpc3* or vehicle, HBSS.

[0062] FIG. 9E shows a bar graph showing FAS expressed as % percentage change in LV internal dimensions between systole and diastole in homozygous *Mybpc3*$^{-/-}$ mice 31 weeks after they were injected retro-orbitally at two weeks of age with 1E13 vg·kg^{-1}, 3E13 vg·kg^{-1}, and 1E14 vg·kg^{-1} of test vector encoding *Mybpc3* or vehicle, HBSS.

[0063] FIG. 9F shows a bar graph showing ejection fraction in homozygous *Mybpc3*$^{-/-}$ mice 31 weeks after they were injected retro-orbitally at two weeks of age with 1E13 vg·kg^{-1}, 3E13 vg·kg^{-1}, and 1E14 vg·kg^{-1} of test vector encoding *Mybpc3* or vehicle, HBSS.

[0064] FIG. 10A is an illustration of an AAV9 vector encoding *Mybpc3* in the context of the 5.4 kbp or 4.7 kbp expression cassettes.

[0065] FIG. 10B is a bar graph showing ejection fraction in homozygous *Mybpc3*$^{-/-}$ mice 18 weeks after they were injected retro-orbitally at three months of age with 3E13 vg·kg^{-1} or 1E14 vg·kg^{-1} of AAV9 vector encoding *Mybpc3* in the context of the 5.4 kbp or 4.7 kbp cassettes, or injected with vehicle control, HBSS.

[0066] FIG. 10C is a plot showing ejection fraction progression in homozygous *Mybpc3*$^{-/-}$ mice after they were injected retro-orbitally at three months of age with 3E13 vg·kg^{-1} or 1E14 vg·kg^{-1} of AAV9 vector encoding *Mybpc3* in the context of the 5.4 kbp or 4.7 kbp cassettes, or injected with vehicle control, HBSS.

[0067] FIG. 10D is a bar graph showing left ventricular mass normalized to body weight (LVM/BM) in homozygous *Mybpc3*$^{-/-}$ mice 18 weeks after they were injected retro-orbitally at three months of age with 3E13 vg·kg^{-1} or 1E14 vg·kg^{-1} of AAV9 vector encoding *Mybpc3* in the context of the 5.4 kbp or 4.7 kbp cassettes, or injected with vehicle control, HBSS.

[0068] FIG. 11A is a bar graph showing GFP expression in cardiac tissue following systemic delivery of an AAV9 capsid variant, CR9-10, with a GFP-encoding cassette or AAV9 with a GFP-encoding cassette in adult mice ($p < 0.05$, One-way ANOVA; Dunnett's multiple comparison test).

[0069] FIG. 11B is a bar graph showing ejection fraction in *Mybpc3*$^{-/-}$ mice injected retro-orbitally at two weeks of age with 1E13 vg·kg-1 and 3E13 vg·kg-1of AAV9 vector encoding *Mybpc3*, CR9-10 vector encoding *Mybpc3*, or vehicle control HBSS.

[0070] FIG. 11C is a bar graph showing ejection fraction compared to pre-dose baseline (ΔEF) in *Mybpc3*$^{-/-}$ mice injected retro-orbitally at two weeks of age with 1E13 vg·kg-1 and 3E13 vg·kg-1of AAV9 vector encoding an expression cassette *Mybpc3* gene, CR9-10 vector encoding an expression cassette *Mybpc3* gene, or vehicle control HBSS.

[0071] FIG. 12A is a bar graph showing GFP expression in the left ventricle of non-human primates one month after intravenous delivery of 1E13 vg·kg-1 dose of AAV vector encoding GFP packaged in one of fourteen different capsid proteins.

[0072] FIG. 12B is a bar graph showing GFP expression in the liver of non-human primates one month after intravenous delivery of 1E13 vg·kg-1 dose of AAV vector encoding GFP packaged in one of fourteen different capsid proteins.

[0073] FIG. 12C is a bar graph showing the ratio of GFP expression in the left ventricle:liver of non-human primates one month after intravenous delivery of 1E13 vg·kg^{-1} dose of AAV vector encoding GFP packaged in one of fourteen different capsid proteins.

[0074] FIG. 13A is a plot showing ejection fraction progression in homozygous *Mybpc3*$^{-/-}$ mice that were injected retro-orbitally at two weeks of age with AAV9 encoding the mouse *Mybpc3* gene (mMybpc3) (at 1E14 vg·kg^{-1}), AAV9 encoding the human *MYBPC3* gene (hMYBPC3) (at 1E14 vg·kg^{-1}), or vehicle, HBSS.

[0075] FIG. 13B is a plot showing left ventricular mass normalized to body weight (LVM/BW) progression in homozygous *Mybpc3*$^{-/-}$ mice that were injected retro-orbitally at two weeks of age with AAV9 encoding the mouse *Mybpc3* gene (mMybpc3) (at 1E14 vg·kg^{-1}), AAV9 encoding the human *MYBPC3* gene (hMYBPC3) (at 1E14 vg·kg^{-1}), or vehicle, HBSS.

[0076] FIG. 13C is a bar graph left ventricular posterior wall thickness during diastole (LVPW;d) in homozygous *Mybpc3*$^{-/-}$ mice that were injected retro-orbitally at two weeks of age with AAV9 encoding the mouse *Mybpc3* gene (mMybpc3) (at 1E14 vg·kg^{-1}), AAV9 encoding the human *MYBPC3* gene (hMYBPC3) (at 1E14 vg·kg^{-1}), or vehicle, HBSS.

[0077] FIG. 14A is a bar graph showing ejection fraction in homozygous *Mybpc3*$^{-/-}$ mice that were injected retro-orbitally at two weeks of age with 1E13 vg·kg^{-1}, 1E14 vg·kg^{-1}, and 3E14 vg·kg^{-1} of test vector encoding the human *MYBPC3* gene or vehicle, HBSS.

[0078] FIG. 14B is a bar graph showing ejection fraction compared to pre-dose baseline (ΔEF) in homozygous *Mybpc3*$^{-/-}$ mice that were injected retro-orbitally at two weeks of age with 1E13 vg·kg^{-1}, 1E14 vg·kg^{-1}, and 3E14 vg·kg^{-1} of test vector encoding the human *MYBPC3* gene or vehicle, HBSS.

[0079] FIG. 14C is a bar graph showing left ventricular mass normalized to body weight (LVM/BW) in homozygous *Mybpc3*$^{-/-}$ mice that were injected retro-orbitally at two weeks of age with 1E13 vg·kg^{-1}, 1E14 vg·kg^{-1}, and 3E14 vg·kg^{-1} of test vector encoding the human *MYBPC3* gene or vehicle, HBSS.

DETAILED DESCRIPTION

[0080] The present disclosure provides compositions and methods for gene therapy in cardiac cells and/or with large genes. The disclosed polynucleotides and vectors may be used in treatment or prevention of disease (*e.g.* heart disease, such as cardiomyopathy). The present disclosure provides cardiac-specific promoters, expression cassettes, recombinant adeno-associated virus (rAAV) viral genomes, rAAV virions, pharmaceutical compositions, and methods of use. The expression

cassettes and rAAV viral genomes may comprise a cardiac-specific promoter operably linked to a polynucleotide encoding a gene product. The gene product may be a therapeutic gene product, such therapeutic gene product used to treat and/or prevent heart disease, *e.g.* cardiomyopathy. The disclosure further provides rAAV viral genomes and expression cassettes engineered to deliver and express a large gene product. In some embodiments, the vector genome comprises a polynucleotide encoding a MYBPC3 polypeptide, or a functional variant thereof and a promoter. In some embodiments, the promoter is a cardiac troponin T promoter (*i.e.*, a TNNT2 promoter). In some embodiments, the rAAV vector genome comprises an expression cassette comprising a polynucleotide encoding a gene product, *e.g.* MYBPC3 and a promoter, *e.g.* TNNT2 promoter, flanked by one or more inverted terminal repeat polynucleotide sequences. In some embodiments, the rAAV virion comprises a polynucleotide comprising an rAAV vector genome as described herein and an AAV capsid protein. The present disclosure also provides pharmaceutical compositions comprising the vector genomes, rAAV vector genomes, and rAAV virions described herein. Also provided are methods of treating and/or preventing a cardiomyopathy in a subject comprising administering the rAAV virions or vector genomes described herein.

[0081] Other embodiments, features, and advantages of the invention will be apparent from and encompassed by the following detailed description and claims.

I. Definitions

[0082] As used in this specification and the appended claims, the singular forms “a,” “an” and “the” include plural references unless the content clearly dictates otherwise.

[0083] As used in this specification, the term “and/or” is used in this disclosure to mean either “and” or “or” unless indicated otherwise.

[0084] Throughout this specification, unless the context requires otherwise, the words “comprise”, or variations such as “comprises” or “comprising”, will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

[0085] As used in this application, the terms “about” and “approximately” are used as equivalents. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art. In certain embodiments, the term “approximately” or “about” refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or

less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0086] The terms "polynucleotide" and "nucleic acid," used interchangeably herein, refer to a polymeric form of nucleotides of more than about 100 nucleotides, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. "Oligonucleotide" generally refers to polynucleotides of between about 5 and about 100 nucleotides of single- or double-stranded DNA. However, for the purposes of this disclosure, there is no upper limit to the length of an oligonucleotide. Oligonucleotides are also known as "oligomers" or "oligos" and may be isolated from genes, or chemically synthesized by methods known in the art. The terms "polynucleotide" and "nucleic acid" should be understood to include, as applicable to the embodiments being described, single-stranded (such as sense or antisense) and double-stranded polynucleotides.

[0087] The term "promoter" as used herein refers a polynucleotide sequence that has one or more recognition site(s) to which an RNA polymerase binds, such that in a host or target cell, an RNA polymerase may initiate and transcribe a polynucleotide sequence "downstream" of the promoter into an RNA. Similarly stated, a "promoter" is operably linked or operatively linked to a polynucleotide sequence if in a host or target cell in which the promoter is active, an RNA polymerase initiates transcription of the polynucleotide at a transcription state site. Promoters operative in mammalian cells generally comprise an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated and/or another sequence found 70 to 80 bases upstream from the start of transcription, a CNCAAT region where N may be any nucleotide.

[0088] The terms "upstream" and "upstream end" refer to a portion of a polynucleotide that is, with reference to a transcription start site (TSS), 5′ to the TSS on the sense strand (or coding strand) of the polynucleotide; and 3′ to the TSS on the antisense strand of the polynucleotide. The terms "downstream" and "downstream end" refer to a portion of a polynucleotide that is, with reference to a TSS, 3′ to TSS on the sense strand (or coding strand) of the polynucleotide; and 5′ to the TSS on the antisense strand of the polynucleotide. Thus, a deletion from the upstream end of a promoter

is a deletion of one or more base pairs in the non-transcribed region of the polynucleotide, 5′ to the TSS on the sense strand (or equivalently, 3′ to the TSS on the antisense strand). A deletion from the downstream end of a promoter is a deletion of one or more base pairs in the transcribed region of the polynucleotide, 3′ to the TSS on the sense strand (or equivalently, 5′ to the TSS on the antisense strand).

[0089] As used herein, the term "transgene" refers to a nucleic acid sequence encoding a protein or RNA (*e.g.*, a therapeutic protein), which is partly or entirely heterologous, *i.e.*, foreign, to the transgenic animal or cell into which it is introduced, or, is homologous to an endogenous gene of the transgenic animal or cell into which it is introduced, but which is designed to be inserted, or is inserted, into the animal's genome in such a way as to alter the genome of the cell into which it is inserted (*e.g.*, it is inserted at a location which differs from that of the natural gene or its insertion results in a knockout). A transgene can include one or more transcriptional regulatory sequences and any other nucleic acid, such as introns, that may be necessary for optimal expression of a selected nucleic acid.

[0090] The term "sequence identity" refers to the percentage of bases or amino acids between two polynucleotide or polypeptide sequences that are the same, and in the same relative position. As such one polynucleotide or polypeptide sequence has a certain percentage of sequence identity compared to another polynucleotide or polypeptide sequence. For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. The term "reference sequence" refers to a molecule to which a test sequence is compared.

[0091] Methods of sequence alignment for comparison and determination of percent sequence identity is well known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the homology alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson and Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), by manual alignment and visual inspection (see, *e.g.*, Brent *et al.*, Current Protocols in Molecular Biology (2003)), by use of algorithms know in the art including the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., *Nuc. Acids Res.* 25:3389-3402 (1977); and Altschul et al., *J. Mol. Biol.* 215:403-410 (1990), respectively. Software

for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

[0092] In some embodiments, the determination of the percentage of sequence identity may take place after a local alignment. Such alignments are well known in the art, for instance the service EMBOSS Matcher identifies local similarities between two sequences using an algorithm based on the LALIGN application, version 2.0u4. In an example, the identity between two nucleic acid sequences may be calculated using the service Matcher (EMBOSS) set to the default parameters, e.g. matrix (DNAfull), gap open (16), gap extend (4), alternative matches (1).

[0093] An "expression cassette" or "expression construct" refers to a DNA polynucleotide sequence operably linked to a promoter. "Operably linked" or "operatively linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For instance, a promoter is operably linked to a polynucleotide sequence if the promoter affects the transcription or expression of the polynucleotide sequence.

[0094] As used herein, the term "delivery", which is used interchangeably with "transduction," refers to the process by which exogenous nucleic acid molecules are transferred into a cell such that they are located inside the cell. Delivery of nucleic acids is a distinct process from expression of nucleic acids.

[0095] The term "modified" refers to a substance or compound (*e.g.*, a cell, a polynucleotide sequence, and/or a polypeptide sequence) that has been altered or changed as compared to the corresponding unmodified substance or compound.

[0096] The term "sample" refers to a biological composition (*e.g.*, a cell or a portion of a tissue) that is subjected to analysis and/or genetic modification. In some embodiments, a sample is a "primary sample" in that it is obtained directly from a subject; in some embodiments, a "sample" is the result of processing of a primary sample, for example to remove certain components and/or to isolate or purify certain components of interest.

[0097] The term "gene" or "recombinant gene" refers to a nucleic acid comprising an open reading frame encoding a polypeptide, including both exon and (optionally) intron sequences.

[0098] The term "transfection" refers to the uptake of foreign DNA by a cell. A cell has been "transfected" when exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are generally known in the art. *See, e.g.*, Graham et al., *Virology* 52:456 (1973); Sambrook et al., Molecular Cloning: A Laboratory Manual (1989); Davis et al., Basic

Methods in Molecular Biology (1986); Chu et al., *Gene* 13:197 (1981). Such techniques can be used to introduce one or more exogenous DNA moieties, such as a nucleotide integration vector and other nucleic acid molecules, into suitable host cells. The term captures chemical and electrical transfection procedures.

[0099] The term "expression" refers to the process by which a nucleic acid is translated into peptides or is transcribed into RNA, which, for example, can be translated into peptides, polypeptides or proteins. If the nucleic acid is derived from genomic DNA, expression may, if an appropriate eukaryotic host cell or organism is selected, include splicing of the mRNA. For heterologous nucleic acid to be expressed in a host cell, it must initially be delivered into the cell and then, once in the cell, ultimately reside in the nucleus.

[0100] The term "gene therapy" involves the transfer of heterologous DNA to cells of a mammal, particularly a human, with a disorder or conditions for which therapy or diagnosis is sought. The DNA is introduced into the selected target cells in a manner such that the heterologous DNA is expressed and a therapeutic product encoded thereby is produced. Alternatively, the heterologous DNA may in some manner mediate expression of DNA that encodes the therapeutic product; it may encode a product, such as a peptide or RNA that in some manner mediates, directly or indirectly, expression of a therapeutic product. Gene therapy may also be used to deliver nucleic acid encoding a gene product to replace a defective gene or supplement a gene product produced by the mammal or the cell in which it is introduced. The introduced nucleic acid may encode a therapeutic gene product that is not normally produced in the mammalian host or that is not produced in therapeutically effective amounts or at a therapeutically useful time. The heterologous DNA encoding the therapeutic product may be modified prior to introduction into the cells of the afflicted host in order to enhance or otherwise alter the product or expression thereof.

[0101] As used herein, a "heterologous" polynucleotide or nucleic acid refers to a polynucleotide or portion of a polynucleotide derived from a source other than the host organism or, for a viral vector, the native, non-recombinant virus. Examples of heterologous DNA include, but are not limited to, DNA that encodes traceable marker proteins, such as a protein that confers drug resistance, DNA that encodes therapeutically effective substances, such as anti-cancer agents, enzymes and hormones, and DNA that encodes other types of proteins, such as antibodies.

[0102] The term "wild type" refers to the naturally-occurring polynucleotide sequence encoding a protein, or a portion thereof, or protein sequence, or portion thereof, respectively, as it normally exists in vivo in a normal or healthy subject.

[0103] The term "variant" refers to a protein or nucleic acid having one or more genetic changes (*e.g.* insertions, deletions, substitutions, or the like) that returns all or substantially all of the functions of the reference protein or nucleic acid. For example, a variant of a therapeutic protein retains the same or substantially the same activity and/or provides the same or substantially the same therapeutic benefit to a subject in need thereof. A variant of a promoter sequence retains the ability to initiate transcription at the same or substantially the same level as the reference promoter, and retains the same or substantially the same cell type specificity. In particular embodiments, polynucleotides variants have at least or about 50%, 55%, 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%,76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%,85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a reference sequence. In particular embodiments, protein variants have at least or about 50%, 55%, 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%,76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%,85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to a reference sequence.

[0104] The term "subject" includes animals, such as *e.g.* mammals. In some embodiments, the mammal is a primate. In some embodiments, the mammal is a human. In some embodiments, subjects are livestock such as cattle, sheep, goats, cows, swine, and the like; or domesticated animals such as dogs and cats. In some embodiments (*e.g.*, particularly in research contexts) subjects are rodents (*e.g.*, mice, rats, hamsters), rabbits, primates, or swine such as inbred pigs and the like. The terms "subject" and "patient" are used interchangeably herein.

[0105] The term "administering" to a subject is a procedure by which one or more delivery agents, together or separately, are introduced into or applied onto a subject such that target cells which are present in the subject are eventually contacted with the agent.

[0106] "Treating" as used herein refers to delivering an agent or composition to a subject to affect a physiologic outcome.

[0107] As used herein, the term "gene product" refers to the a protein or nucleic acid produced by the transcription of a polynucleotide and, in the case of a protein gene product, the subsequent translation of transcript into a protein. A "therapeutic gene product" refers to a gene product that

provides a therapeutic physiological effect or benefit to a subject in need when expressed in a therapeutic amount in a subject.

[0108] As used herein, the term "therapeutic protein" refers to a protein or polypeptide that provides a therapeutic physiological effect or benefit to a subject in need when expressed or administered in a therapeutic amount in a subject. In some embodiments, treatment with a therapeutic protein or a vector that expresses a therapeutic protein provides a therapeutic physiological effect or benefit to a subject with heart disease (*e.g.*, a subject with cardiomyopathy). Illustrative therapeutic proteins for the treatment of heart disease are provided in Table 2.

[0109] As used herein, the term "cardiomyopathy" refers to the deterioration of the function of the myocardium (*i.e.*, the actual heart muscle) for any reason. Subjects with cardiomyopathy are often at risk of arrhythmia or sudden cardiac death or both.

[0110] As used herein, the term "hypertrophic cardiomyopathy" refers to a disease of the heart and myocardium in which a portion of the myocardium is hypertrophied.

[0111] As used herein, the term "familial hypertrophic cardiomyopathy" refers to a genetic disorder characterized by increased growth (*i.e.*, hypertrophy) in thickness of the wall of the left ventricle.

[0112] As used herein, the term "effective amount" refers to the minimum amount of an agent or composition required to result in a particular physiological effect. The effective amount of a particular agent may be represented in a variety of ways based on the nature of the agent, such as mass/volume, # of cells/volume, particles/volume, (mass of the agent)/(mass of the subject), # of cells/(mass of subject), or particles/(mass of subject). The effective amount of a particular agent may also be expressed as the half-maximal effective concentration (EC_{50}), which refers to the concentration of an agent that results in a magnitude of a particular physiological response that is half-way between a reference level and a maximum response level.

II. Polynucleotides

[0113] In some embodiments, the present disclosure provides polynucleotide sequences for the treatment and/or prevention of heart disease (*e.g.*, cardiomyopathy). In some embodiments, the polynucleotide sequences comprise a cardiac-specific promoter operatively linked to a polynucleotide encoding one or more therapeutic gene products for the treatment and/or prevention of cardiomyopathy.

[0114] Polynucleotides refer to a polymeric form of nucleotides of at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, at least 100, at least 200, at least 300, at least 400, at least 500, at least 1000, at least 5000, at least 10000, or at least 15000 or more nucleotides in length, either ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide, as well as all intermediate lengths. "Intermediate lengths," in this context, means any length between the quoted values, such as 6, 7, 8, 9, *etc.*, 101, 102, 103, *etc.*; 151, 152, 153, *etc.*; 201, 202, 203, *etc.*

[0115] As a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide, or fragment of variant thereof, as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated in particular embodiments, for example polynucleotides that are optimized for human and/or primate codon selection. Further, alleles of the genes comprising the polynucleotide sequences provided herein may also be used. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides.

[0116] The polynucleotides contemplated herein, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters and/or enhancers, untranslated regions (UTRs), signal sequences, Kozak sequences, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, internal ribosomal entry sites (IRES), recombinase recognition sites (*e.g.,* LoxP, FRT, and Att sites), termination codons, transcriptional termination signals, and polynucleotides encoding self-cleaving polypeptides, epitope tags, as disclosed elsewhere herein or as known in the art.

[0117] Polynucleotides can be prepared, manipulated and/or expressed using any of a variety of well-established techniques known and available in the art.

[0118] In some embodiments, the polynucleotide sequence is a promoter. In some embodiments, the polynucleotide sequence is a promoter operatively linked to a polynucleotide encoding a therapeutic gene product for the treatment or prevention of heart disease (*e.g.*, cardiomyopathy).

[0119] In some embodiments, the vector comprises a cardiac-specific promoter which is operably linked to a polynucleotide encoding a therapeutic gene product (*e.g.*, encoding a therapeutic protein, *e.g.*, MYBPC3 protein). As used herein, a "cardiac-specific promoter" refers to a promoter whose activity in cardiac cells is at least 2-fold higher than in any other non-cardiac cell type.

Preferably, a cardiac-specific promoter suitable for being used in the vector of the invention has an activity in cardiac cells which is at least 5-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, or at least 50-fold higher compared to its activity in a non-cardiac cell type.

[0120] In some embodiments, the vector comprises a cardiomyocyte-specific promoter which is operably linked to a polynucleotide encoding a therapeutic gene product (*e.g.*, MYBPC3 protein). A "cardiomyocyte-specific promoter", as used herein, specifies a promoter whose activity in cardiomyocytes is at least 2-fold higher than in any other non-cardiac cell type or cardiac cell which is not a cardiomyocyte. Preferably, a cardiomyocyte-specific promoter suitable for being used in the vector of the present disclosure has an activity in cardiomyocytes which is at least 5-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, or at least 50-fold higher compared to its activity in a non-cardiac cell type or a cardiac cell type which is not a cardiomyocyte.

[0121] In some embodiments, the cardiac-specific or cardiomyocyte-specific promoter is a human promoter. Examples of cardiac-specific or cardiomyocyte-specific promoter include, but are not limited to, the alpha myosin heavy chain promoter, the myosin light chain 2v promoter, the alpha myosin heavy chain promoter, the alpha-cardiac actin promoter, the alpha-tropomyosin promoter, the cardiac troponin C promoter, the cardiac troponin I promoter, the cardiac myosin-binding protein C promoter, and the sarco/endoplasmic reticulum Ca^{2+}ATPase (SERCA) promoter (*e.g.* isoform 2 of SERCA2).

[0122] In some embodiments, the cardiac-specific promoter is the cardiac TNNT2 promoter. In some embodiments, the cardiac TNNT2 promoter is modified, *e.g.*, by the deletion, insertion, or substitution of polynucleotides. Illustrative polynucleotide sequences of the cardiac TNNT2 promoter are shown in **Table 1** below. The transcription start site (TSS) of the TNNT2 promoters are bolded and underlined.

Table 1. Illustrative TNNT2 promoters

Name	DNA Sequence	SEQ ID NO.
TNNT2p-600	GTCATGGAGAAGACCCACCTTGCAGATGTCCTCACTGGGGCTGGCAGAGCCG GCAACCTGCCTAAGGCTGCTCAGTCCATTAGGAGCCAGTAGCCTGGAAGATG TCTTTACCCCCAGCATCAGTTCAAGTGGAGCAGCACATAACTCTTGCCCTCT GCCTTCCAAGATTCTGGTGCTGAGACTTATGGAGTGTCTTGGAGGTTGCCTT CTGCCCCCCAACCCTGCTCCCAGCTGGCCCTCCCAGGCCTGGGTTGCTGGCC TCTGCTTTATCAGGATTCTCAAGAGGGACAGCTGGTTTATGTTGCATGACTG TTCCCTGCATATCTGCTCTGGTTTTAAATAGCTTATCTGAGCAGCTGGAGGA CCACATGGGCTTATATGGCGTGGGGTACATGTTCCTGTAGCCTTGTCCCTGG CACCTGCCAAAATAGCAGCCAACACCCCCCACCCCCACCGCCATCCCCCTGC CCCACCCGTCCCCTGTCGCACATTCCTCCCTCCGCAGGGCTGGCTCACCAGG CCCCAGCCCACATGCCTGCTTAAAGCCCT**CTCCATC**CTCTGCCTCACCCAGT CCCCGCTGAGACTGAGCAGACGCCTCCA	1
TNNT2p-500	GATGTCTTTACCCCCAGCATCAGTTCAAGTGGAGCAGCACATAACTCTTGCC CTCTGCCTTCCAAGATTCTGGTGCTGAGACTTATGGAGTGTCTTGGAGGTTG CCTTCTGCCCCCCAACCCTGCTCCCAGCTGGCCCTCCCAGGCCTGGGTTGCT GGCCTCTGCTTTATCAGGATTCTCAAGAGGGACAGCTGGTTTATGTTGCATG ACTGTTCCCTGCATATCTGCTCTGGTTTTAAATAGCTTATCTGAGCAGCTGG AGGACCACATGGGCTTATATGGCGTGGGGTACATGTTCCTGTAGCCTTGTCC CTGGCACCTGCCAAAATAGCAGCCAACACCCCCCACCCCCACCGCCATCCCC CTGCCCCACCCGTCCCCTGTCGCACATTCCTCCCTCCGCAGGGCTGGCTCAC CAGGCCCCAGCCCACATGCCTGCTTAAAGCCCT**CTCCATC**CTCTGCCTCACC CAGTCCCCGCTGAGACTGAGCAGACGCCTCCA	2
TNNT2p-400	GTTGCCTTCTGCCCCCCAACCCTGCTCCCAGCTGGCCCTCCCAGGCCTGGGT TGCTGGCCTCTGCTTTATCAGGATTCTCAAGAGGGACAGCTGGTTTATGTTG CATGACTGTTCCCTGCATATCTGCTCTGGTTTTAAATAGCTTATCTGAGCAG CTGGAGGACCACATGGGCTTATATGGCGTGGGGTACATGTTCCTGTAGCCTT GTCCCTGGCACCTGCCAAAATAGCAGCCAACACCCCCCACCCCCACCGCCAT CCCCCTGCCCCACCCGTCCCCTGTCGCACATTCCTCCCTCCGCAGGGCTGGC TCACCAGGCCCCAGCCCACATGCCTGCTTAAAGCCCT**CTCCATC**CTCTGCCT CACCCAGTCCCCGCTGAGACTGAGCAGACGCCTCCA	3
TNNT2p-300	GTTGCATGACTGTTCCCTGCATATCTGCTCTGGTTTTAAATAGCTTATCTGA GCAGCTGGAGGACCACATGGGCTTATATGGCGTGGGGTACATGTTCCTGTAG CCTTGTCCCTGGCACCTGCCAAAATAGCAGCCAACACCCCCCACCCCCACCG CCATCCCCCTGCCCCACCCGTCCCCTGTCGCACATTCCTCCCTCCGCAGGGC TGGCTCACCAGGCCCCAGCCCACATGCCTGCTTAAAGCCCT**CTCCATC**CTCT GCCTCACCCAGTCCCCGCTGAGACTGAGCAGACGCCTCCA	4

[0123] In some embodiments, the cardiac TNNT2 promoter is modified to comprise a polynucleotide sequence of between about 200 and 500 base pairs, between about 250 and 500 base pairs, between about 300 to 500 base pairs, between about 350 to 500 base pairs, between about 400 to 500 base pairs, between about 450 to 500 base pairs, between about 200 and 450 base pairs, between about 200 and 400 base pairs, between about 200 and 350 base pairs, between about

200 and 300 base pairs, and between about 200 and 250 base pairs in length. In some embodiments, the modified cardiac TNNT2 promoter comprises a polynucleotide sequence of between about 350 base pairs to about 450 base pairs, between about 375 base pairs to about 425 base pairs, between about 375 base pairs to about 400 base pairs, between about 375 base pairs to about 425 base pairs, between about 400 base pairs to about 425 base pairs, or between about 400 base pairs to about 450 base pairs. In some embodiments, the cardiac TNNT2 promoter comprises a polynucleotide sequence of about 400 base pairs.

[0124] In a particular embodiment, the modified cardiac troponin T promoter comprises between 300 bp and 500 bp of SEQ ID NO: 1. For instance, the modified cardiac troponin T promoter may comprise SEQ ID NO: 3. In some examples, the 300 bp-500 bp sequence may be linked to further polynucleotide sequences but may not be linked to additional sequences derived from SEQ ID NO: 1. For example, in an embodiment, the modified cardiac troponin T promoter may include no more than 500 bp of SEQ ID NO: 1 but may include additional unrelated polynucleotide sequences. In another example, the modified cardiac troponin T promoter may include SEQ ID NO: 3, no additional sequences derived from SEQ ID NO: 1, but may include additional unrelated polynucleotide sequences.

[0125] In some embodiments, the cardiac TNNT2 promoter is modified by the deletion of polynucleotides. A modification may include one, two, three or more internal deletions. Each deletion may be a deletion of 1 base pair, 2 base pairs, 3 base pairs, 4 base pairs, 5 base pairs, 10 base pairs, 15 base pairs, 20 base pairs, 25 base pairs, 30 base pairs, 40 base pairs, 50 base pairs, 60 base pairs, 70 base pairs, 80 base pairs, 90 base pairs, 100 base pairs, 125 base pairs, 150 base pairs, 175 base pairs, 200 base pairs, 225 base pairs, 250 base pairs, 275 base pairs, or 300 base pairs with respect to a reference cardiac TNNT2 promoter (SEQ ID NO: 1) having about 600 base pairs.

[0126] In some embodiments, the TNNT2 promoter is modified by the deletion of polynucleotides from the upstream end of the promoter with respect to a reference cardiac TNNT2 promoter (SEQ ID NO: 1) having about 600 base pairs. A modification may include the deletion of 1 base pair, 2 base pairs, 3 base pairs, 4 base pairs, 5 base pairs, 10 base pairs, 15 base pairs, 20 base pairs, 25 base pairs, 30 base pairs, 40 base pairs, 50 base pairs, 60 base pairs, 70 base pairs, 80 base pairs, 90 base pairs, 100 base pairs, 125 base pairs, 150 base pairs, 175 base pairs, 200 base pairs, 225 base pairs, 250 base pairs, 275 base pairs, or 300 base pairs from the upstream end of the promoter

with respect to a reference cardiac TNNT2 promoter (SEQ ID NO: 1) having about 600 base pairs. In some embodiments, the modification is a 200 base pair deletion from the upstream end of the promoter with respect to a reference cardiac TNNT2 promoter (SEQ ID NO: 1) having about 600 base pairs.

[0127] In some embodiments, the cardiac TNNT2 promoter is modified by the deletion of polynucleotides from the downstream end of the promoter with respect to a reference cardiac TNNT2 promoter (SEQ ID NO: 1) having about 600 base pairs. A modification may include the deletion of 1 base pair, 2 base pairs, 3 base pairs, 4 base pairs, 5 base pairs, 10 base pairs, 15 base pairs, 20 base pairs, 25 base pairs, 30 base pairs, 40 base pairs, 50 base pairs, 60 base pairs, 70 base pairs, 80 base pairs, 90 base pairs, 100 base pairs, 125 base pairs, 150 base pairs, 175 base pairs, 200 base pairs, 225 base pairs, 250 base pairs, 275 base pairs, or 300 base pairs from the downstream end of the promoter with respect to a reference cardiac TNNT2 promoter (SEQ ID NO: 1) having about 600 base pairs.

[0128] In some embodiments, the cardiac TNNT2 promoter is modified by an internal deletion of polynucleotides. A modification may include the internal deletion of 1 base pair, 2 base pairs, 3 base pairs, 4 base pairs, 5 base pairs, 10 base pairs, 15 base pairs, 20 base pairs, 30 base pairs, 40 base pairs, 50 base pairs, 60 base pairs, 70 base pairs, 80 base pairs, 90 base pairs, 100 base pairs, 125 base pairs, 150 base pairs, 175 base pairs, 200 base pairs, 225 base pairs, 250 base pairs, 275 base pairs, or 300 base pairs with respect to a reference cardiac TNNT2 promoter (SEQ ID NO: 1).

[0129] In some embodiments, the cardiac TNNT2 promoter is modified by the insertion of polynucleotides. A modification may include the insertion of 1 base pair, 2 base pairs, 3 base pairs, 4 base pairs, 5 base pairs, 10 base pairs, 15 base pairs, 20 base pairs, 25 base pairs, 30 base pairs, 35 base pairs, 40 base pairs, 45 base pairs, 50 base pairs, 55 base pairs, 60 base pairs, 65 base pairs, 70 base pairs, 75 base pairs, 80, base pairs, 85 base pairs, 90 base pairs, 100 base pairs, 125 base pairs, 150 base pairs, 175 base pairs, 200 base pairs, 225 base pairs, 250 base pairs, 275 base pairs, or 300 base pairs with respect to a reference cardiac TNNT2 promoter (SEQ ID NO: 1) .

[0130] In some embodiments, the cardiac TNNT2 promoter is modified by the substitution of polynucleotides. A modification may include the substitution of 1 base pair, 2 base pairs, 3 base pairs, 4 base pairs, 5 base pairs, 6 base pairs, 7 base pairs, 8 base pairs, 9 base pairs, or 10 base pairs with respect to a reference cardiac TNNT2 promoter (SEQ ID NO: 1).

[0131] In some embodiments, the polynucleotide sequence of the TNNT2 promoter shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the polynucleotide sequence -450 base pairs to +1 base pairs relative to the transcription start site of the human TNNT2 gene. In some embodiments, the polynucleotide sequence of the TNNT2 promoter shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% sequence identity with the polynucleotide sequence -350 base pairs to +1 base pairs relative to the transcription start site of the human TNNT2 gene. In some embodiments, the polynucleotide sequence of the TNNT2 promoter shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% sequence identity with the polynucleotide sequence -250 base pairs to +1 base pairs relative to the transcription start site of the human TNNT2 gene.

[0132] In some embodiments, the polynucleotide sequence of the cardiac TNNT2 promoter shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% sequence identity with the polynucleotide sequence -450 base pairs to +50 base pairs relative to the transcription start site of the TNNT2 gene. In some embodiments, the polynucleotide sequence of the cardiac TNNT2 promoter shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% sequence identity with the polynucleotide sequence -350 base pairs to +50 base pairs relative to the transcription start site of the TNNT2 gene. In some embodiments, the polynucleotide sequence of the cardiac TNNT2 promoter shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% sequence identity with the polynucleotide sequence -250 base pairs to +5 base pairs relative to the transcription start site of the TNNT2 gene.

[0133] In some embodiments, the cardiac TNNT2 promoter comprises a polynucleotide comprising a sequence that shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% identity to any one of SEQ ID NOs: 1-85. In some embodiments, the polynucleotide comprises a sequence that shares at least 80% identity to any one of SEQ ID NOS: 1-85. In some embodiments, the polynucleotide comprises a sequence that shares at least 90% identity to any one of SEQ ID NOS: 1-85. In some embodiments, the polynucleotide comprises a sequence that shares at least 100% identity to any one of SEQ ID NOS: 1-85. In some embodiments, the polynucleotide comprises a sequence that shares at least 80% identity to SEQ ID NO: 1. In some embodiments, the polynucleotide comprises a sequence that shares at least 90% identity to SEQ ID NO: 1. In some embodiments, the polynucleotide comprises a sequence that shares at least 100% identity to SEQ ID NO: 1. In some embodiments, the polynucleotide comprises a sequence that shares at least

80% identity to SEQ ID NO: 3. In some embodiments, the polynucleotide comprises a sequence that shares at least 90% identity to SEQ ID NO: 3. In some embodiments, the polynucleotide comprises a sequence that shares at least 100% identity to SEQ ID NO: 3.

B. Illustrative Gene Products (Proteins)

[0134] The promoters of the disclosure may be operatively linked to a polynucleotide comprising a sequence encoding a gene product (*e.g.*, protein or nucleic acid). In some embodiments, the gene product is a therapeutic protein. The therapeutic protein may be any of the native human proteins listed in Table 2, or functional homologs or variants thereof. The promoters of the disclosure are particularly suited for use with large genes that may otherwise be expressed at low levels or not be expressed when delivered by a viral vector. An advantage of some embodiments disclosed herein lies in the ability to express a therapeutic protein (particularly a large therapeutic protein) in a viral vector having limited packaging capacity, *e.g.*, an AAV vector. A "large" protein is any protein whose size impacts expression in a selected vector. Generally, "large" therapeutic proteins comprise at least about 1000 or more amino acids – that is, the protein is encoded by a polynucleotide sequence of about 3 kbps or greater. Illustrative proteins, including large proteins, are provided in **Table 2** below.

Table 2. Illustrative Proteins

Gene Name	Gene Symbol	NCBI Gene ID	UniProt ID
Myosin-binding protein C	MYBPC3	4607	Q14896
Potassium voltage-gated channel subfamily H member 2	KCNH2	3757	Q12809
Transient receptor potential cation channel subfamily M member 4	TRPM4	54795	Q8TD43
Desmoglein-2	DSG2	1829	Q14126
ATPase sarcoplasmic/endoplasmic reticulum calcium transporting 2	ATP2A2	488	P16615
Calcium voltage-gated channel subunit alpha 1C	CACNA1C	775	Q13936
Dystrophin	DMD	1756	P11532
DM1 protein kinase	DMPK	1760	Q09013
Ectopic P granules protein 5 homolog	EPG5	57724	Q9HCE0
EvC ciliary complex subunit 1	EVC	2121	P57679
Limbin	EVC2	132884	Q86UK5

Gene Name	Gene Symbol	NCBI Gene ID	UniProt ID
Fibrillin-1	FBN1	2200	P35555
Neurofibromin	NF1	4763	P21359
Sodium channel protein type 5 subunit alpha	SCN5A	6331	Q14524
Son of sevenless homolog 1	SOS1	6654	Q07889
Natriuretic peptide receptor 1	NPR1	4881	P16066
Receptor tyrosine-protein kinase erbB-4	ERBB4	2066	Q15303
Vasoactive intestinal peptide	VIP	7432	P01282
Beta-myosin heavy chain	MYH7	4625	P12883

[0135] Various therapeutic polynucleotides, or therapeutic proteins encoded by polynucleotides, having lengths of 3 kilobases or greater are expressed more effectively when operatively linked to a modified TNNT2 promoter of the disclosure compared to a TNNT2 promoter of about 600 base pairs. The promoters of the disclosure are useful in expression of, at least, the following: a) large genes in which loss-of-function mutations result in cardiomyopathy (gene replacement therapy); b) large genes whose expression in cardiomyocytes is cardioprotective; c) combinations of genes whose co-expression in cardiomyocytes is beneficial; and d) tools for cardiomyocyte-specific genome editing. A “large” gene is any gene whose size impacts expression in a selected vector. Generally, “large” therapeutic genes encode proteins that comprise at least about 1000 or more amino acids – that is, the gene comprises a polynucleotide sequence of about 3 kbps or greater. In further embodiments, the vectors and promoters of the disclosure are used in treatment of the diseases or disorders list in **Table 3**, where the polynucleotide encodes the therapeutic protein indicated in the table.

Table 3. Illustrative Therapeutic Gene Products for Heart Disease

Condition	Therapeutic Gene Product	Gene Size (kb)
Timothy syndrome	CACNA1C	6.663
Becker muscular dystrophy	DMD	11.055
Duchenne muscular dystrophy	DMD	11.055
Myotonic dystrophy type 1	DMPK	4.653
Vici syndrome	EPG5	7.737
Ellis-Van Creveld syndrome	EVC	2.976
Ellis-Van Creveld syndrome	EVC2	3.924
Marfan syndrome	FBN1	8.613

Long QT Syndrome	KCNH2	3.477
Neurofibromatosis Noonan syndrome	NF1	3.517
Brugada Syndrome	SCN5A	6.048
Long QT Syndrome	SCN5A	6.048
Paroxysmal ventricular fibrillation 1	SCN5A	6.048
Progressive familial heart block type 1A	SCN5A	6.048
Noonan syndrome	SOS1	3.999
Progressive familial heart block type 1B	TRPM4	3.642
Acute decompensated heart failure (ADHF)	NPR1	3.183
Congestive Heart Failure (CHF)	ERBB4	3.924
Congestive Heart Failure (CHF)	VIP analog*	3.729
Hypertrophic cardiomyopathy	MYBPC3	3.822
Left Ventricular Noncompaction Cardiomyopathy	MYBPC3	3.822
Hypertrophic cardiomyopathy	MYH7	5.805
Left Ventricular Noncompaction Cardiomyopathy	MYH7	5.805

*VIP fused to an ELP biopolymer (*e.g.*, PB1046) to increase stability in vivo

[0136] *MYBPC3* is a gene expressed in cardiac cells. Various mutations in *MYBPC3* are known to cause hypertrophic cardiomyopathy. Almost half of all mutations causative for hypertrophic cardiomyopathy result in truncations, via nonsense, frameshift or splice-site mutations (Marian and Braunwald, Circ. Res. 121:749-770 (2017); Walsh et al., Genet. Med. 19:192-203 (2017). mRNAs containing premature stop codons are subjected to surveillance and degradation by nonsense-mediated decay machinery. This is consistent with decreased levels of mutant RNAs in analysis of cardiac tissue from hypertrophic cardiomyopathy patients who have received myectomies (Marston et al., Circ. Res. 105:219-222 (2009); van Dijk et al., Circulation 119:1473-1483 (2009); Helms et al., Circ. Cardiovasc. Genet. 7:434-443 (2014). Further, any resultant truncated polypeptides appear sensitive to the ubiquitin-proteasome degradation system. In patient myectomy samples, no truncated protein was observed for nine distinct mutations (Rottbauer et al., J. Clin. Invest. 100:475-482 (1997); Moolman et al., Circulation 101:1396-1402 (2000); Marston et al., Circ. Res. 105:219-222 (2009); van Dijk et al., Circ. Heart Fail 5:36-46 (2012)). Even though it appears that the wild-type *MYBPC3* allele in heterozygous patients is slightly upregulated, the total amount of MYBPC3 protein incorporated into sarcomeres falls significantly below normal at ~65% (Marston et al., Circ. Res. 105:219-222 (2009); van Dijk et al., Circ. Heart Fail 5:36-46 (2012); McNamara et al., PLoS One 12:e0180064 (2017)). Thus, the sarcomeric pathophysiology of hypertrophic cardiomyopathy patients with *MYBPC3* truncating mutations appears to be due to haploinsufficiency.

[0137] In some embodiments, the disclosure provides a vector comprising a polynucleotide sequence that encodes the MYBPC3 protein, operatively linked to a modified cardiac TNNT2 promoter. Similarly stated, in some embodiments, the polynucleotide sequence operatively linked to the cardiac-specific promoter (*e.g.*, a modified cardiac TNNT2 promoter) is *MYBPC3*, or a mutant, variant, or fragment thereof. In humans, the *MYBPC3* gene encodes the MYBPC3 protein (also known as MyBP-C), which regulates the cardiac sarcomere, the basic unit of muscle contraction. The cardiac muscle sarcomere consists of thick and thin filaments, and MYBPC3 attaches to the thick filaments to prevent premature degradation. Illustrative MYBPC3 polynucleotide sequences are shown in **Table 4A** below. In some embodiments, the polynucleotide encoding MYBPC3 shares at least 85%, 90%, 95%, 99%, or 100% identity to any one of SEQ ID NOs: 86-89. Illustrative MYBPC3 protein sequences are shown in **Table 4B**. In some embodiments, the vector genome encodes an MYBPC3 protein that shares at least 85%, 90%, 95%, 99%, or 100% identity to any one of SEQ ID NOs: 103-106.

Table 4A. Illustrative MYBPC3 Polynucleotide Sequences

Name	DNA Sequence	SEQ ID NO.
MYBPC3	ATGCCTGAGCCGGGGAAGAAGCCAGTCTCAGCTTTTAGCAAGAAGCCACGGTCAGTGGA AGTGGCCGCAGGCAGCCCTGCCGTGTTCGAGGCCGAGACAGAGCGGGCAGGAGTGAAGG TGCGCTGGCAGCGCGGAGGCAGTGACATCAGCGCCAGCAACAAGTACGGCCTGGCCACA GAGGGCACACGGCATACGCTGACAGTGCGGGAAGTGGGCCCTGCCGACCAGGGATCTTA CGCAGTCATTGCTGGCTCCTCCAAGGTCAAGTTCGACCTCAAGGTCATAGAGGCAGAGA AGGCAGAGCCCATGCTGGCCCCTGCCCCTGCCCCTGCTGAGGCCACTGGAGCCCCTGGA GAAGCCCCGGCCCCAGCCGCTGAGCTGGGAGAAAGTGCCCCAAGTCCCAAAGGGTCAAG CTCAGCAGCTCTCAATGGTCCTACCCCTGGAGCCCCCGATGACCCCATTGGCCTCTTCG TGATGCGGCCACAGGATGGCGAGGTGACCGTGGGTGGCAGCATCACCTTCTCAGCCCGC GTGGCCGGCGCCAGCCTCCTGAAGCCGCCTGTGGTCAAGTGGTTCAAGGGCAAATGGGT GGACCTGAGCAGCAAGGTGGGCCAGCACCTGCAGCTGCACGACAGCTACGACCGCGCCA GCAAGGTCTATCTGTTCGAGCTGCACATCACCGATGCCCAGCCTGCCTTCACTGGCAGC TACCGCTGTGAGGTGTCCACCAAGGACAAATTTGACTGCTCCAACTTCAATCTCACTGT CCACGAGGCCATGGGCACCGGAGACCTGGACCTCCTATCAGCCTTCCGCCGCACGAGCC TGGCTGGAGGTGGTCGGCGGATCAGTGATAGCCATGAGGACACTGGGATTCTGGACTTC AGCTCACTGCTGAAAAAGAGAGACAGTTTCCGGACCCCGAGGGACTCGAAGCTGGAGGC ACCAGCAGAGGAGGACGTGTGGGAGATCCTACGGCAGGCACCCCCATCTGAGTACGAGC GCATCGCCTTCCAGTACGGCGTCACTGACCTGCGCGGCATGCTAAAGAGGCTCAAGGGC ATGAGGCGCGATGAGAAGAAGAGCACAGCCTTTCAGAAGAAGCTGGAGCCGGCCTACCA GGTGAGCAAAGGCCACAAGATCCGGCTGACCGTGGAACTGGCTGACCATGACGCTGAGG TCAAATGGCTCAAGAATGGCCAGGAGATCCAGATGAGCGGCAGCAAGTACATCTTTGAG TCCATCGGTGCCAAGCGTACCCTGACCATCAGCCAGTGCTCATTGGCGGACGACGCAGC CTACCAGTGCGTGGTGGGTGGCGAGAAGTGTAGCACGGAGCTCTTTGTGAAAGAGCCCC CTGTGCTCATCACGCGCCCCTTGGAGGACCAGCTGGTGATGGTGGGGCAGCGGGTGGAG TTTGAGTGTGAAGTATCGGAGGAGGGGGCGCAAGTCAAATGGCTGAAGGACGGGGTGGA	86

	GCTGACCCGGGAGGAGACCTTCAAATACCGGTTCAAGAAGGACGGGCAGAGACACCACC TGATCATCAACGAGGCCATGCTGGAGGACGCGGGGCACTATGCACTGTGCACTAGCGGG GGCCAGGCGCTGGCTGAGCTCATTGTGCAGGAAAAGAAGCTGGAGGTGTACCAGAGCAT CGCAGACCTGATGGTGGGCGCAAAGGACCAGGCGGTGTTCAAATGTGAGGTCTCAGATG AGAATGTTCGGGGTGTGTGGCTGAAGAATGGGAAGGAGCTGGTGCCCGACAGCCGCATA AAGGTGTCCCACATCGGGCGGGTCCACAAACTGACCATTGACGACGTCACACCTGCCGA CGAGGCTGACTACAGCTTTGTGCCCGAGGGCTTCGCCTGCAACCTGTCAGCCAAGCTCC ACTTCATGGAGGTCAAGATTGACTTCGTACCCAGGCAGGAACCTCCCAAGATCCACCTG GACTGCCCAGGCCGCATACCAGACACCATTGTGGTTGTAGCTGGAAATAAGCTACGTCT GGACGTCCCTATCTCTGGGGACCCCGCTCCCACTGTGATCTGGCAGAAGGCTATCACGC AGGGGAATAAGGCCCCAGCCAGGCCAGCCCCAGATGCCCCAGAGGACACAGGTGACAGC GATGAGTGGGTGTTTGACAAGAAGCTGCTGTGTGAGACCGAGGGCCGGGTCCGCGTGGA GACCACCAAGGACCGCAGCATCTTCACGGTCGAGGGGGCAGAGAAGGAAGATGAGGGCG TCTACACGGTCACAGTGAAGAACCCTGTGGGCGAGGACCAGGTCAACCTCACAGTCAAG GTCATCGACGTGCCAGACGCACCTGCGGCCCCCAAGATCAGCAACGTGGGAGAGGACTC CTGCACAGTACAGTGGGAGCCGCCTGCCTACGATGGCGGGCAGCCCATCCTGGGCTACA TCCTGGAGCGCAAGAAGAAGAAGAGCTACCGGTGGATGCGGCTGAACTTCGACCTGATT CAGGAGCTGAGTCATGAAGCGCGGCGCATGATCGAGGGCGTGGTGTACGAGATGCGCGT CTACGCGGTCAACGCCATCGGCATGTCCAGGCCCAGCCCTGCCTCCCAGCCCTTCATGC CTATCGGTCCCCCCAGCGAACCCACCCACCTGGCAGTAGAGGACGTCTCTGACACCACG GTCTCCCTCAAGTGGCGGCCCCCAGAGCGCGTGGGAGCAGGAGGCCTGGATGGCTACAG CGTGGAGTACTGCCCAGAGGGCTGCTCAGAGTGGGTGGCTGCCCTGCAGGGGCTGACAG AGCACACATCGATACTGGTGAAGGACCTGCCCACGGGGGCCCGGCTGCTTTTCCGAGTG CGGGCACACAATATGGCAGGGCCTGGAGCCCCTGTTACCACCACGGAGCCGGTGACAGT GCAGGAGATCCTGCAACGGCCACGGCTTCAGCTGCCCAGGCACCTGCGCCAGACCATTC AGAAGAAGGTCGGGGAGCCTGTGAACCTTCTCATCCCTTTCCAGGGCAAGCCCCGGCCT CAGGTGACCTGGACCAAAGAGGGGCAGCCCCTGGCAGGCGAGGAGGTGAGCATCCGCAA CAGCCCCACAGACACCATCCTGTTCATCCGGGCCGCTCGCCGCGTGCATTCAGGCACTT ACCAGGTGACGGTGCGCATTGAGAACATGGAGGACAAGGCCACGCTGGTGCTGCAGGTT GTTGACAAGCCAAGTCCTCCCCAGGATCTCCGGGTGACTGACGCCTGGGGTCTTAATGT GGCTCTGGAGTGGAAGCCACCCCAGGATGTCGGCAACACGGAACTCTGGGGGTACACAG TGCAGAAAGCCGACAAGAAGACCATGGAGTGGTTCACCGTCTTGGAGCATTACCGCCGC ACCCACTGCGTGGTGCCAGAGCTCATCATTGGCAATGGCTACTACTTCCGCGTCTTCAG CCAGAATATGGTTGGCTTTAGTGACAGAGCGGCCACCACCAAGGAGCCCGTCTTTATCC CCAGACCAGGCATCACCTATGAGCCACCCAACTATAAGGCCCTGGACTTCTCCGAGGCC CCAAGCTTCACCCAGCCCCTGGTGAACCGCTCGGTCATCGCGGGCTACACTGCTATGCT CTGCTGTGCTGTCCGGGGTAGCCCCAAGCCCAAGATTTCCTGGTTCAAGAATGGCCTGG ACCTGGGAGAAGACGCCCGCTTCCGCATGTTCAGCAAGCAGGGAGTGTTGACTCTGGAG ATTAGAAAGCCCTGCCCCTTTGACGGGGGCATCTATGTCTGCAGGGCCACCAACTTACA GGGCGAGGCACGGTGTGAGTGCCGCCTGGAGGTGCGAGTGCCTCAGTAA	
MYBPC3 -delC3	ATGCCTGAGCCGGGGAAGAAGCCAGTCTCAGCTTTTAGCAAGAAGCCACGGTCAGTGGA AGTGGCCGCAGGCAGCCCTGCCGTGTTCGAGGCCGAGACAGAGCGGGCAGGAGTGAAGG TGCGCTGGCAGCGCGGAGGCAGTGACATCAGCGCCAGCAACAAGTACGGCCTGGCCACA GAGGGCACACGGCATACGCTGACAGTGCGGGAAGTGGGCCCTGCCGACCAGGGATCTTA CGCAGTCATTGCTGGCTCCTCCAAGGTCAAGTTCGACCTCAAGGTCATAGAGGCAGAGA AGGCAGAGCCCATGCTGGCCCCTGCCCCTGCCCCTGCTGAGGCCACTGGAGCCCCTGGA GAAGCCCCGGCCCCAGCCGCTGAGCTGGGAGAAAGTGCCCCAAGTCCCAAAGGGTCAAG CTCAGCAGCTCTCAATGGTCCTACCCCTGGAGCCCCCGATGACCCCATTGGCCTCTTCG TGATGCGGCCACAGGATGGCGAGGTGACCGTGGGTGGCAGCATCACCTTCTCAGCCCGC GTGGCCGGCGCCAGCCTCCTGAAGCCGCCTGTGGTCAAGTGGTTCAAGGGCAAATGGGT GGACCTGAGCAGCAAGGTGGGCCAGCACCTGCAGCTGCACGACAGCTACGACCGCGCCA GCAAGGTCTATCTGTTCGAGCTGCACATCACCGATGCCCAGCCTGCCTTCACTGGCAGC TACCGCTGTGAGGTGTCCACCAAGGACAAATTTGACTGCTCCAACTTCAATCTCACTGT CCACGAGGCCATGGGCACCGGAGACCTGGACCTCCTATCAGCCTTCCGCCGCACGAGCC TGGCTGGAGGTGGTCGGCGGATCAGTGATAGCCATGAGGACACTGGGATTCTGGACTTC	87

	AGCTCACTGCTGAAAAAGAGAGACAGTTTCCGGACCCCGAGGGACTCGAAGCTGGAGGC ACCAGCAGAGGAGGACGTGTGGGAGATCCTACGGCAGGCACCCCCATCTGAGTACGAGC GCATCGCCTTCCAGTACGGCGTCACTGACCTGCGCGGCATGCTAAAGAGGCTCAAGGGC ATGAGGCGCGATGAGAAGAAGAGCACAGCCTTTCAGAAGAAGCTGGAGCCGGCCTACCA GGTGAGCAAAGGCCACAAGATCCGGCTGACCGTGGAACTGGCTGACCATGACGCTGAGG TCAAATGGCTCAAGAATGGCCAGGAGATCCAGATGAGCGGCAGCAAGTACATCTTTGAG TCCATCGGTGCCAAGCGTACCCTGACCATCAGCCAGTGCTCATTGGCGGACGACGCAGC CTACCAGTGCGTGGTGGGTGGCGAGAAGTGTAGCACGGAGCTCTTTGTGAAAGAGCCCC CTGTGTACCAGAGCATCGCAGACCTGATGGTGGGCGCAAAGGACCAGGCGGTGTTCAAA TGTGAGGTCTCAGATGAGAATGTTCGGGGTGTGTGGCTGAAGAATGGGAAGGAGCTGGT GCCCGACAGCCGCATAAAGGTGTCCCACATCGGGCGGGTCCACAAACTGACCATTGACG ACGTCACACCTGCCGACGAGGCTGACTACAGCTTTGTGCCCGAGGGCTTCGCCTGCAAC CTGTCAGCCAAGCTCCACTTCATGGAGGTCAAGATTGACTTCGTACCCAGGCAGGAACC TCCCAAGATCCACCTGGACTGCCCAGGCCGCATACCAGACACCATTGTGGTTGTAGCTG GAAATAAGCTACGTCTGGACGTCCCTATCTCTGGGGACCCCGCTCCCACTGTGATCTGG CAGAAGGCTATCACGCAGGGGAATAAGGCCCCAGCCAGGCCAGCCCCAGATGCCCCAGA GGACACAGGTGACAGCGATGAGTGGGTGTTTGACAAGAAGCTGCTGTGTGAGACCGAGG GCCGGGTCCGCGTGGAGACCACCAAGGACCGCAGCATCTTCACGGTCGAGGGGGCAGAG AAGGAAGATGAGGGCGTCTACACGGTCACAGTGAAGAACCCTGTGGGCGAGGACCAGGT CAACCTCACAGTCAAGGTCATCGACGTGCCAGACGCACCTGCGGCCCCCAAGATCAGCA ACGTGGGAGAGGACTCCTGCACAGTACAGTGGGAGCCGCCTGCCTACGATGGCGGGCAG CCCATCCTGGGCTACATCCTGGAGCGCAAGAAGAAGAAGAGCTACCGGTGGATGCGGCT GAACTTCGACCTGATTCAGGAGCTGAGTCATGAAGCGCGGCGCATGATCGAGGGCGTGG TGTACGAGATGCGCGTCTACGCGGTCAACGCCATCGGCATGTCCAGGCCCAGCCCTGCC TCCCAGCCCTTCATGCCTATCGGTCCCCCCAGCGAACCCACCCACCTGGCAGTAGAGGA CGTCTCTGACACCACGGTCTCCCTCAAGTGGCGGCCCCCAGAGCGCGTGGGAGCAGGAG GCCTGGATGGCTACAGCGTGGAGTACTGCCCAGAGGGCTGCTCAGAGTGGGTGGCTGCC CTGCAGGGGCTGACAGAGCACACATCGATACTGGTGAAGGACCTGCCCACGGGGGCCCG GCTGCTTTTCCGAGTGCGGGCACACAATATGGCAGGGCCTGGAGCCCCTGTTACCACCA CGGAGCCGGTGACAGTGCAGGAGATCCTGCAACGGCCACGGCTTCAGCTGCCCAGGCAC CTGCGCCAGACCATTCAGAAGAAGGTCGGGGAGCCTGTGAACCTTCTCATCCCTTTCCA GGGCAAGCCCCGGCCTCAGGTGACCTGGACCAAAGAGGGGCAGCCCCTGGCAGGCGAGG AGGTGAGCATCCGCAACAGCCCCACAGACACCATCCTGTTCATCCGGGCCGCTCGCCGC GTGCATTCAGGCACTTACCAGGTGACGGTGCGCATTGAGAACATGGAGGACAAGGCCAC GCTGGTGCTGCAGGTTGTTGACAAGCCAAGTCCTCCCCAGGATCTCCGGGTGACTGACG CCTGGGGGTCTTAATGTGGCTCTGGAGTGGAAGCCACCCCAGGATGTCGGCAACACGGAA CTCTGGGGGTACACAGTGCAGAAAGCCGACAAGAAGACCATGGAGTGGTTCACCGTCTT GGAGCATTACCGCCGCACCCACTGCGTGGTGCCAGAGCTCATCATTGGCAATGGCTACT ACTTCCGCGTCTTCAGCCAGAATATGGTTGGCTTTAGTGACAGAGCGGCCACCACCAAG GAGCCCGTCTTTATCCCCAGACCAGGCATCACCTATGAGCCACCCAACTATAAGGCCCT GGACTTCTCCGAGGCCCCAAGCTTCACCCAGCCCCTGGTGAACCGCTCGGTCATCGCGG GCTACACTGCTATGCTCTGCTGTGCTGTCCGGGGTAGCCCCAAGCCCAAGATTTCCTGG TTCAAGAATGGCCTGGACCTGGGAGAAGACGCCCGCTTCCGCATGTTCAGCAAGCAGGG AGTGTTGACTCTGGAGATTAGAAAGCCCTGCCCCTTTGACGGGGGCATCTATGTCTGCA GGGCCACCAACTTACAGGGCGAGGCACGGTGTGAGTGCCGCCTGGAGGTGCGAGTGCCT CAGTAA	
MYBPC3 -delC4	ATGCCTGAGCCGGGGAAGAAGCCAGTCTCAGCTTTTAGCAAGAAGCCACGGTCAGTGGA AGTGGCCGCAGGCAGCCCTGCCGTGTTCGAGGCCGAGACAGAGCGGGCAGGAGTGAAGG TGCGCTGGCAGCGCGGAGGCAGTGACATCAGCGCCAGCAACAAGTACGGCCTGGCCACA GAGGGCACACGGCATACGCTGACAGTGCGGGAAGTGGGCCCTGCCGACCAGGGATCTTA CGCAGTCATTGCTGGCTCCTCCAAGGTCAAGTTCGACCTCAAGGTCATAGAGGCAGAGA AGGCAGAGCCCATGCTGGCCCCTGCCCCTGCCCCTGCTGAGGCCACTGGAGCCCCTGGA GAAGCCCCGGCCCCAGCCGCTGAGCTGGGAGAAAGTGCCCCAAGTCCCAAAGGGTCAAG CTCAGCAGCTCTCAATGGTCCTACCCCTGGAGCCCCCGATGACCCCATTGGCCTCTTCG TGATGCGGCCACAGGATGGCGAGGTGACCGTGGGTGGCAGCATCACCTTCTCAGCCCGC GTGGCCGGCGCCAGCCTCCTGAAGCCGCCTGTGGTCAAGTGGTTCAAGGGCAAATGGGT	88

	GGACCTGAGCAGCAAGGTGGGCCAGCACCTGCAGCTGCACGACAGCTACGACCGCGCCA GCAAGGTCTATCTGTTCGAGCTGCACATCACCGATGCCCAGCCTGCCTTCACTGGCAGC TACCGCTGTGAGGTGTCCACCAAGGACAAATTTGACTGCTCCAACTTCAATCTCACTGT CCACGAGGCCATGGGCACCGGAGACCTGGACCTCCTATCAGCCTTCCGCCGCACGAGCC TGGCTGGAGGTGGTCGGCGGATCAGTGATAGCCATGAGGACACTGGGATTCTGGACTTC AGCTCACTGCTGAAAAAGAGAGACAGTTTCCGGACCCCGAGGGACTCGAAGCTGGAGGC ACCAGCAGAGGAGGACGTGTGGGAGATCCTACGGCAGGCACCCCCATCTGAGTACGAGC GCATCGCCTTCCAGTACGGCGTCACTGACCTGCGCGGCATGCTAAAGAGGCTCAAGGGC ATGAGGCGCGATGAGAAGAAGAGCACAGCCTTTCAGAAGAAGCTGGAGCCGGCCTACCA GGTGAGCAAAGGCCACAAGATCCGGCTGACCGTGGAACTGGCTGACCATGACGCTGAGG TCAAATGGCTCAAGAATGGCCAGGAGATCCAGATGAGCGGCAGCAAGTACATCTTTGAG TCCATCGGTGCCAAGCGTACCCTGACCATCAGCCAGTGCTCATTGGCGGACGACGCAGC CTACCAGTGCGTGGTGGGTGGCGAGAAGTGTAGCACGGAGCTCTTTGTGAAAGAGCCCC CTGTGCTCATCACGCGCCCCTTGGAGGACCAGCTGGTGATGGTGGGGCAGCGGGTGGAG TTTGAGTGTGAAGTATCGGAGGAGGGGGCGCAAGTCAAATGGCTGAAGGACGGGGTGGA GCTGACCCGGGAGGAGACCTTCAAATACCGGTTCAAGAAGGACGGGCAGAGACACCACC TGATCATCAACGAGGCCATGCTGGAGGACGCGGGGCACTATGCACTGTGCACTAGCGGG GGCCAGGCGCTGGCTGAGCTCATTGTGCAGGAAAAGAAGCTGGAGCCTCCCAAGATCCA CCTGGACTGCCCAGGCCGCATACCAGACACCATTGTGGTTGTAGCTGGAAATAAGCTAC GTCTGGACGTCCCTATCTCTGGGGACCCCGCTCCCACTGTGATCTGGCAGAAGGCTATC ACGCAGGGGAATAAGGCCCCAGCCAGGCCAGCCCCAGATGCCCCAGAGGACACAGGTGA CAGCGATGAGTGGGTGTTTGACAAGAAGCTGCTGTGTGAGACCGAGGGCCGGGTCCGCG TGGAGACCACCAAGGACCGCAGCATCTTCACGGTCGAGGGGGCAGAGAAGGAAGATGAG GGCGTCTACACGGTCACAGTGAAGAACCCTGTGGGCGAGGACCAGGTCAACCTCACAGT CAAGGTCATCGACGTGCCAGACGCACCTGCGGCCCCCAAGATCAGCAACGTGGGAGAGG ACTCCTGCACAGTACAGTGGGAGCCGCCTGCCTACGATGGCGGGCAGCCCATCCTGGGC TACATCCTGGAGCGCAAGAAGAAGAAGAGCTACCGGTGGATGCGGCTGAACTTCGACCT GATTCAGGAGCTGAGTCATGAAGCGCGGCGCATGATCGAGGGCGTGGTGTACGAGATGC GCGTCTACGCGGTCAACGCCATCGGCATGTCCAGGCCCAGCCCTGCCTCCCAGCCCTTC ATGCCTATCGGTCCCCCCAGCGAACCCACCCACCTGGCAGTAGAGGACGTCTCTGACAC CACGGTCTCCCTCAAGTGGCGGCCCCCAGAGCGCGTGGGAGCAGGAGGCCTGGATGGCT ACAGCGTGGAGTACTGCCCAGAGGGCTGCTCAGAGTGGGTGGCTGCCCTGCAGGGGCTG ACAGAGCACACATCGATACTGGTGAAGGACCTGCCCACGGGGGCCCGGCTGCTTTTCCG AGTGCGGGCACACAATATGGCAGGGCCTGGAGCCCCTGTTACCACCACGGAGCCGGTGA CAGTGCAGGAGATCCTGCAACGGCCACGGCTTCAGCTGCCCAGGCACCTGCGCCAGACC ATTCAGAAGAAGGTCGGGGAGCCTGTGAACCTTCTCATCCCTTTCCAGGGCAAGCCCCG GCCTCAGGTGACCTGGACCAAAGAGGGGCAGCCCCTGGCAGGCGAGGAGGTGAGCATCC GCAACAGCCCCACAGACACCATCCTGTTCATCCGGGCCGCTCGCCGCGTGCATTCAGGC ACTTACCAGGTGACGGTGCGCATTGAGAACATGGAGGACAAGGCCACGCTGGTGCTGCA GGTTGTTGACAAGCCAAGTCCTCCCCAGGATCTCCGGGTGACTGACGCCTGGGGTCTTA ATGTGGCTCTGGAGTGGAAGCCACCCCAGGATGTCGGCAACACGGAACTCTGGGGGTAC ACAGTGCAGAAAGCCGACAAGAAGACCATGGAGTGGTTCACCGTCTTGGAGCATTACCG CCGCACCCACTGCGTGGTGCCAGAGCTCATCATTGGCAATGGCTACTACTTCCGCGTCT TCAGCCAGAATATGGTTGGCTTTAGTGACAGAGCGGCCACCACCAAGGAGCCCGTCTTT ATCCCCAGACCAGGCATCACCTATGAGCCACCCAACTATAAGGCCCTGGACTTCTCCGA GGCCCCAAGCTTCACCCAGCCCCTGGTGAACCGCTCGGTCATCGCGGGCTACACTGCTA TGCTCTGCTGTGCTGTCCGGGGTAGCCCCAAGCCCAAGATTTCCTGGTTCAAGAATGGC CTGGACCTGGGAGAAGACGCCCGCTTCCGCATGTTCAGCAAGCAGGGAGTGTTGACTCT GGAGATTAGAAAGCCCTGCCCCTTTGACGGGGGCATCTATGTCTGCAGGGCCACCAACT TACAGGGCGAGGCACGGTGTGAGTGCCGCCTGGAGGTGCGAGTGCCTCAGTAA	

MYBPC3 -delC4b	ATGCCTGAGCCGGGGAAGAAGCCAGTCTCAGCTTTTAGCAAGAAGCCACGGTCAGTGGA AGTGGCCGCAGGCAGCCCTGCCGTGTTCGAGGCCGAGACAGAGCGGGCAGGAGTGAAGG TGCGCTGGCAGCGCGGAGGCAGTGACATCAGCGCCAGCAACAAGTACGGCCTGGCCACA GAGGGCACACGGCATACGCTGACAGTGCGGGAAGTGGGCCCTGCCGACCAGGGATCTTA CGCAGTCATTGCTGGCTCCTCCAAGGTCAAGTTCGACCTCAAGGTCATAGAGGCAGAGA AGGCAGAGCCCATGCTGGCCCCTGCCCCTGCCCCTGCTGAGGCCACTGGAGCCCCTGGA GAAGCCCCGGCCCCAGCCGCTGAGCTGGGAGAAAGTGCCCCAAGTCCCAAAGGGTCAAG CTCAGCAGCTCTCAATGGTCCTACCCCTGGAGCCCCCGATGACCCCATTGGCCTCTTCG TGATGCGGCCACAGGATGGCGAGGTGACCGTGGGTGGCAGCATCACCTTCTCAGCCCGC GTGGCCGGCGCCAGCCTCCTGAAGCCGCCTGTGGTCAAGTGGTTCAAGGGCAAATGGGT GGACCTGAGCAGCAAGGTGGGCCAGCACCTGCAGCTGCACGACAGCTACGACCGCGCCA GCAAGGTCTATCTGTTCGAGCTGCACATCACCGATGCCCAGCCTGCCTTCACTGGCAGC TACCGCTGTGAGGTGTCCACCAAGGACAAATTTGACTGCTCCAACTTCAATCTCACTGT CCACGAGGCCATGGGCACCGGAGACCTGGACCTCCTATCAGCCTTCCGCCGCACGAGCC TGGCTGGAGGTGGTCGGCGGATCAGTGATAGCCATGAGGACACTGGGATTCTGGACTTC AGCTCACTGCTGAAAAAGAGAGACAGTTTCCGGACCCCGAGGGACTCGAAGCTGGAGGC ACCAGCAGAGGAGGACGTGTGGGAGATCCTACGGCAGGCACCCCCATCTGAGTACGAGC GCATCGCCTTCCAGTACGGCGTCACTGACCTGCGCGGCATGCTAAAGAGGCTCAAGGGC ATGAGGCGCGATGAGAAGAAGAGCACAGCCTTTCAGAAGAAGCTGGAGCCGGCCTACCA GGTGAGCAAAGGCCACAAGATCCGGCTGACCGTGGAACTGGCTGACCATGACGCTGAGG TCAAATGGCTCAAGAATGGCCAGGAGATCCAGATGAGCGGCAGCAAGTACATCTTTGAG TCCATCGGTGCCAAGCGTACCCTGACCATCAGCCAGTGCTCATTGGCGGACGACGCAGC CTACCAGTGCGTGGTGGGTGGCGAGAAGTGTAGCACGGAGCTCTTTGTGAAAGAGCCCC CTGTGCTCATCACGCGCCCCTTGGAGGACCAGCTGGTGATGGTGGGGCAGCGGGTGGAG TTTGAGTGTGAAGTATCGGAGGAGGGGGCGCAAGTCAAATGGCTGAAGGACGGGGTGGA GCTGACCCGGGAGGAGACCTTCAAATACCGGTTCAAGAAGGACGGGCAGAGACACCACC TGATCATCAACGAGGCCATGCTGGAGGACGCGGGGCACTATGCACTGTGCACTAGCGGG GGCCAGGCGCTGGCTGAGCTCATTGTGCAGGAAAAGAAGCTGGAGCCCAGGCAGGAACC TCCCAAGATCCACCTGGACTGCCCAGGCCGCATACCAGACACCATTGTGGTTGTAGCTG GAAATAAGCTACGTCTGGACGTCCCTATCTCTGGGGACCCCGCTCCCACTGTGATCTGG CAGAAGGCTATCACGCAGGGGAATAAGGCCCCAGCCAGGCCAGCCCCAGATGCCCCAGA GGACACAGGTGACAGCGATGAGTGGGTGTTTGACAAGAAGCTGCTGTGTGAGACCGAGG GCCGGGTCCGCGTGGAGACCACCAAGGACCGCAGCATCTTCACGGTCGAGGGGGCAGAG AAGGAAGATGAGGGCGTCTACACGGTCACAGTGAAGAACCCTGTGGGCGAGGACCAGGT CAACCTCACAGTCAAGGTCATCGACGTGCCAGACGCACCTGCGGCCCCCAAGATCAGCA ACGTGGGAGAGGACTCCTGCACAGTACAGTGGGAGCCGCCTGCCTACGATGGCGGGCAG CCCATCCTGGGCTACATCCTGGAGCGCAAGAAGAAGAAGAGCTACCGGTGGATGCGGCT GAACTTCGACCTGATTCAGGAGCTGAGTCATGAAGCGCGGCGCATGATCGAGGGCGTGG TGTACGAGATGCGCGTCTACGCGGTCAACGCCATCGGCATGTCCAGGCCCAGCCCTGCC TCCCAGCCCTTCATGCCTATCGGTCCCCCCAGCGAACCCACCCACCTGGCAGTAGAGGA CGTCTCTGACACCACGGTCTCCCTCAAGTGGCGGCCCCCAGAGCGCGTGGGAGCAGGAG GCCTGGATGGCTACAGCGTGGAGTACTGCCCAGAGGGCTGCTCAGAGTGGGTGGCTGCC CTGCAGGGGCTGACAGAGCACACATCGATACTGGTGAAGGACCTGCCCACGGGGGCCCG GCTGCTTTTCCGAGTGCGGGCACACAATATGGCAGGGCCTGGAGCCCCTGTTACCACCA CGGAGCCGGTGACAGTGCAGGAGATCCTGCAACGGCCACGGCTTCAGCTGCCCAGGCAC CTGCGCCAGACCATTCAGAAGAAGGTCGGGGAGCCTGTGAACCTTCTCATCCCTTTCCA GGGCAAGCCCCGGCCTCAGGTGACCTGGACCAAAGAGGGGCAGCCCCTGGCAGGCGAGG AGGTGAGCATCCGCAACAGCCCCACAGACACCATCCTGTTCATCCGGGCCGCTCGCCGC GTGCATTCAGGCACTTACCAGGTGACGGTGCGCATTGAGAACATGGAGGACAAGGCCAC GCTGGTGCTGCAGGTTGTTGACAAGCCAAGTCCTCCCCAGGATCTCCGGGTGACTGACG CCTGGGGGTCTTAATGTGGCTCTGGAGTGGAAGCCACCCCAGGATGTCGGCAACACGGAA CTCTGGGGGTACACAGTGCAGAAAGCCGACAAGAAGACCATGGAGTGGTTCACCGTCTT GGAGCATTACCGCCGCACCCACTGCGTGGTGCCAGAGCTCATCATTGGCAATGGCTACT ACTTCCGCGTCTTCAGCCAGAATATGGTTGGCTTTAGTGACAGAGCGGCCACCACCAAG GAGCCCGTCTTTATCCCCAGACCAGGCATCACCTATGAGCCACCCAACTATAAGGCCCT GGACTTCTCCGAGGCCCCAAGCTTCACCCAGCCCCTGGTGAACCGCTCGGTCATCGCGG	89

	GCTACACTGCTATGCTCTGCTGTGCTGTCCGGGGTAGCCCCAAGCCCAAGATTTCCTGG TTCAAGAATGGCCTGGACCTGGGAGAAGACGCCCGCTTCCGCATGTTCAGCAAGCAGGG AGTGTTGACTCTGGAGATTAGAAAGCCCTGCCCCTTTGACGGGGGCATCTATGTCTGCA GGGCCACCAACTTACAGGGCGAGGCACGGTGTGAGTGCCGCCTGGAGGTGCGAGTGCCT CAGTAA	

Table 4B. Illustrative MYBPC3 Protein Sequences

Name	Protein Sequence	SEQ ID NO.
MYBPC3	MPEPGKKPVSAFSKKPRSVEVAAGSPAVFEAETERAGVKVRWQRGGSDISASNKYGLAT EGTRHTLTVREVGPADQGSYAVIAGSSKVKFDLKVIEAEKAEPMLAPAPAPAEATGAPG EAPAPAAELGESAPSPKGSSSAALNGPTPGAPDDPIGLFVMRPQDGEVTVGGSITFSAR VAGASLLKPPVVKWFKGKWVDLSSKVGQHLQLHDSYDRASKVYLFELHITDAQPAFTGS YRCEVSTKDKFDCSNFNLTVHEAMGTGDLDLLSAFRRTSLAGGGRRISDSHEDTGILDF SSLLKKRDSFRTPRDSKLEAPAEEDVWEILRQAPPSEYERIAFQYGVTDLRGMLKRLKG MRRDEKKSTAFQKKLEPAYQVSKGHKIRLTVELADHDAEVKWLKNGQEIQMSGSKYIFE SIGAKRTLTISQCSLADDAAYQCVVGGEKCSTELFVKEPPVLITRPLEDQLVMVGQRVE FECEVSEEGAQVKWLKDGVELTREETFKYRFKKDGQRHHLIINEAMLEDAGHYALCTSG GQALAELIVQEKKLEVYQSIADLMVGAKDQAVFKCEVSDENVRGVWLKNGKELVPDSRI KVSHIGRVHKLTIDDVTPADEADYSFVPEGFACNLSAKLHFMEVKIDFVPRQEPPKIHL DCPGRIPDTIVVVAGNKLRLDVPISGDPAPTVIWQKAITQGNKAPARPAPDAPEDTGDS DEWVFDKKLLCETEGRVRVETTKDRSIFTVEGAEKEDEGVYTVTVKNPVGEDQVNLTVK VIDVPDAPAAPKISNVGEDSCTVQWEPPAYDGGQPILGYILERKKKKSYRWMRLNFDLI QELSHEARRMIEGVVYEMRVYAVNAIGMSRPSPASQPFMPIGPPSEPTHLAVEDVSDTT VSLKWRPPERVGAGGLDGYSVEYCPEGCSEWVAALQGLTEHTSILVKDLPTGARLLFRV RAHNMAGPGAPVTTTEPVTVQEILQRPRLQLPRHLRQTIQKKVGEPVNLLIPFQGKPRP QVTWTKEGQPLAGEEVSIRNSPTDTILFIRAARRVHSGTYQVTVRIENMEDKATLVLQV VDKPSPPQDLRVTDAWGLNVALEWKPPQDVGNTELWGYTVQKADKKTMEWFTVLEHYRR THCVVPELIIGNGYYFRVFSQNMVGFSDRAATTKEPVFIPRPGITYEPPNYKALDFSEA PSFTQPLVNRSVIAGYTAMLCCAVRGSPKPKISWFKNGLDLGEDARFRMFSKQGVLTLE IRKPCPFDGGIYVCRATNLQGEARCECRLEVRVPQ	103
MYBPC3 -delC3	MPEPGKKPVSAFSKKPRSVEVAAGSPAVFEAETERAGVKVRWQRGGSDISASNKYGLAT EGTRHTLTVREVGPADQGSYAVIAGSSKVKFDLKVIEAEKAEPMLAPAPAPAEATGAPG EAPAPAAELGESAPSPKGSSSAALNGPTPGAPDDPIGLFVMRPQDGEVTVGGSITFSAR VAGASLLKPPVVKWFKGKWVDLSSKVGQHLQLHDSYDRASKVYLFELHITDAQPAFTGS YRCEVSTKDKFDCSNFNLTVHEAMGTGDLDLLSAFRRTSLAGGGRRISDSHEDTGILDF SSLLKKRDSFRTPRDSKLEAPAEEDVWEILRQAPPSEYERIAFQYGVTDLRGMLKRLKG MRRDEKKSTAFQKKLEPAYQVSKGHKIRLTVELADHDAEVKWLKNGQEIQMSGSKYIFE SIGAKRTLTISQCSLADDAAYQCVVGGEKCSTELFVKEPPVYQSIADLMVGAKDQAVFK CEVSDENVRGVWLKNGKELVPDSRIKVSHIGRVHKLTIDDVTPADEADYSFVPEGFACN LSAKLHFMEVKIDFVPRQEPPKIHLDCPGRIPDTIVVVAGNKLRLDVPISGDPAPTVIW QKAITQGNKAPARPAPDAPEDTGDSDEWVFDKKLLCETEGRVRVETTKDRSIFTVEGAE KEDEGVYTVTVKNPVGEDQVNLTVKVIDVPDAPAAPKISNVGEDSCTVQWEPPAYDGGQ PILGYILERKKKKSYRWMRLNFDLIQELSHEARRMIEGVVYEMRVYAVNAIGMSRPSPA SQPFMPIGPPSEPTHLAVEDVSDTTVSLKWRPPERVGAGGLDGYSVEYCPEGCSEWVAA LQGLTEHTSILVKDLPTGARLLFRVRAHNMAGPGAPVTTTEPVTVQEILQRPRLQLPRH LRQTIQKKVGEPVNLLIPFQGKPRPQVTWTKEGQPLAGEEVSIRNSPTDTILFIRAARR	104

	VHSGTYQVTVRIENMEDKATLVLQVVDKPSPPQDLRVTDAWGLNVALEWKPPQDVGNTE LWGYTVQKADKKTMEWFTVLEHYRRTHCVVPELIIGNGYYFRVFSQNMVGFSDRAATTK EPVFIPRPGITYEPPNYKALDFSEAPSFTQPLVNRSVIAGYTAMLCCAVRGSPKPKISW FKNGLDLGEDARFRMFSKQGVLTLEIRKPCPFDGGIYVCRATNLQGEARCECRLEVRVP Q	
MYBPC3 -delC4	MPEPGKKPVSAFSKKPRSVEVAAGSPAVFEAETERAGVKVRWQRGGSDISASNKYGLAT EGTRHTLTVREVGPADQGSYAVIAGSSKVKFDLKVIEAEKAEPMLAPAPAPAEATGAPG EAPAPAAELGESAPSPKGSSSAALNGPTPGAPDDPIGLFVMRPQDGEVTVGGSITFSAR VAGASLLKPPVVKWFKGKWVDLSSKVGQHLQLHDSYDRASKVYLFELHITDAQPAFTGS YRCEVSTKDKFDCSNFNLTVHEAMGTGDLDLLSAFRRTSLAGGGRRISDSHEDTGILDF SSLLKKRDSFRTPRDSKLEAPAEEDVWEILRQAPPSEYERIAFQYGVTDLRGMLKRLKG MRRDEKKSTAFQKKLEPAYQVSKGHKIRLTVELADHDAEVKWLKNGQEIQMSGSKYIFE SIGAKRTLTISQCSLADDAAYQCVVGGEKCSTELFVKEPPVLITRPLEDQLVMVGQRVE FECEVSEEGAQVKWLKDGVELTREETFKYRFKKDGQRHHLIINEAMLEDAGHYALCTSG GQALAELIVQEKKLEPPKIHLDCPGRIPDTIVVVAGNKLRLDVPISGDPAPTVIWQKAI TQGNKAPARPAPDAPEDTGDSDEWVFDKKLLCETEGRVRVETTKDRSIFTVEGAEKEDE GVYTVTVKNPVGEDQVNLTVKVIDVPDAPAAPKISNVGEDSCTVQWEPPAYDGGQPILG YILERKKKKSYRWMRLNFDLIQELSHEARRMIEGVVYEMRVYAVNAIGMSRPSPASQPF MPIGPPSEPTHLAVEDVSDTTVSLKWRPPERVGAGGLDGYSVEYCPEGCSEWVAALQGL TEHTSILVKDLPTGARLLFRVRAHNMAGPGAPVTTTEPVTVQEILQRPRLQLPRHLRQT IQKKVGEPVNLLIPFQGKPRPQVTWTKEGQPLAGEEVSIRNSPTDTILFIRAARRVHSG TYQVTVRIENMEDKATLVLQVVDKPSPPQDLRVTDAWGLNVALEWKPPQDVGNTELWGY TVQKADKKTMEWFTVLEHYRRTHCVVPELIIGNGYYFRVFSQNMVGFSDRAATTKEPVF IPRPGITYEPPNYKALDFSEAPSFTQPLVNRSVIAGYTAMLCCAVRGSPKPKISWFKNG LDLGEDARFRMFSKQGVLTLEIRKPCPFDGGIYVCRATNLQGEARCECRLEVRVPQ	105
MYBPC3 -delC4b	MPEPGKKPVSAFSKKPRSVEVAAGSPAVFEAETERAGVKVRWQRGGSDISASNKYGLAT EGTRHTLTVREVGPADQGSYAVIAGSSKVKFDLKVIEAEKAEPMLAPAPAPAEATGAPG EAPAPAAELGESAPSPKGSSSAALNGPTPGAPDDPIGLFVMRPQDGEVTVGGSITFSAR VAGASLLKPPVVKWFKGKWVDLSSKVGQHLQLHDSYDRASKVYLFELHITDAQPAFTGS YRCEVSTKDKFDCSNFNLTVHEAMGTGDLDLLSAFRRTSLAGGGRRISDSHEDTGILDF SSLLKKRDSFRTPRDSKLEAPAEEDVWEILRQAPPSEYERIAFQYGVTDLRGMLKRLKG MRRDEKKSTAFQKKLEPAYQVSKGHKIRLTVELADHDAEVKWLKNGQEIQMSGSKYIFE SIGAKRTLTISQCSLADDAAYQCVVGGEKCSTELFVKEPPVLITRPLEDQLVMVGQRVE FECEVSEEGAQVKWLKDGVELTREETFKYRFKKDGQRHHLIINEAMLEDAGHYALCTSG GQALAELIVQEKKLEPRQEPPKIHLDCPGRIPDTIVVVAGNKLRLDVPISGDPAPTVIW QKAITQGNKAPARPAPDAPEDTGDSDEWVFDKKLLCETEGRVRVETTKDRSIFTVEGAE KEDEGVYTVTVKNPVGEDQVNLTVKVIDVPDAPAAPKISNVGEDSCTVQWEPPAYDGGQ PILGYILERKKKKSYRWMRLNFDLIQELSHEARRMIEGVVYEMRVYAVNAIGMSRPSPA SQPFMPIGPPSEPTHLAVEDVSDTTVSLKWRPPERVGAGGLDGYSVEYCPEGCSEWVAA LQGLTEHTSILVKDLPTGARLLFRVRAHNMAGPGAPVTTTEPVTVQEILQRPRLQLPRH LRQTIQKKVGEPVNLLIPFQGKPRPQVTWTKEGQPLAGEEVSIRNSPTDTILFIRAARR VHSGTYQVTVRIENMEDKATLVLQVVDKPSPPQDLRVTDAWGLNVALEWKPPQDVGNTE LWGYTVQKADKKTMEWFTVLEHYRRTHCVVPELIIGNGYYFRVFSQNMVGFSDRAATTK EPVFIPRPGITYEPPNYKALDFSEAPSFTQPLVNRSVIAGYTAMLCCAVRGSPKPKISW FKNGLDLGEDARFRMFSKQGVLTLEIRKPCPFDGGIYVCRATNLQGEARCECRLEVRVP Q	106

[0138] In some embodiments, the disclosure provides a vector comprising a polynucleotide sequence that encodes the potassium voltage gated channel subfamily H member 2 (KCNH2) protein, operatively linked to a modified cardiac TNNT2 promoter. Similarly stated, in some embodiments, the polynucleotide sequence operatively linked to the cardiac-specific promoter

(*e.g.*, a modified cardiac TNNT2 promoter) is *KCNH2*, or a mutant, variant, or fragment thereof (*e.g.*, SEQ ID NO: 107). In humans, the *KCNH2* gene encodes the KCNH2 protein (also known as hERG1, *e.g.*, SEQ ID NO: 108), which forms a potassium channel with other KCNH2 proteins to transport potassium out of cells. KCHN2 proteins are abundantly expressed in cardiac muscle, which function to recharge the cardiac tissue after each heartbeat to maintain regular rhythm. In some embodiments, the polynucleotide encoding KCNH2 shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 107. In some embodiments, the KCNH2 protein shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 108.

[0139] In some embodiments, the disclosure provides a vector comprising a polynucleotide sequence that encodes the transient receptor potential cation channel subfamily M membrane 4 (TRPM4) protein, operatively linked to a modified cardiac TNNT2 promoter. Similarly stated, in some embodiments, the polynucleotide sequence operatively linked to the cardiac-specific promoter (*e.g.*, a modified cardiac TNNT2 promoter) is *TRPM4*, or a mutant, variant, or fragment thereof (*e.g.*, SEQ ID NO: 109). In humans, the *TRPM4* gene encodes the TRPM4 protein (*e.g.*, SEQ ID NO: 110), which functions as a channel to control the flow of cations into and out of cells. The TRPM4 channel is abundantly expressed in cardiac cells and plays a key role in generating and transmitting electrical signals. In some embodiments, the polynucleotide encoding TRPM4 shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 109. In some embodiments, the TRPM4 protein shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 110.

[0140] In some embodiments, the disclosure provides a vector comprising a polynucleotide sequence that encodes the desmoglein 2 (DSG2) protein, operatively linked to a modified cardiac TNNT2 promoter. Similarly stated, in some embodiments, the polynucleotide sequence operatively linked to the cardiac-specific promoter (*e.g.*, a modified cardiac TNNT2 promoter) is *DSG2*, or a mutant, variant, or fragment thereof (*e.g.*, SEQ ID NO: 111). In humans, the *DSG2* gene encodes DSG2 protein (*e.g.*, SEQ ID NO: 112), which is a transmembrane glycoprotein and component of desmosomes. Desmosomes are intercellular junctions that provide strong adhesion between cells giving mechanical strength to tissues. In some embodiments, the polynucleotide encoding DSG2 shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 111. In some embodiments, the DSG2 protein shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 112.

[0141] In some embodiments, the disclosure provides a vector comprising a polynucleotide sequence that encodes the ATPase sarcoplasmic/endoplasmic reticulum calcium transporting 2 (ATP2A2) protein, operatively linked to a modified cardiac TNNT2 promoter. Similarly stated, in some embodiments, the polynucleotide sequence operatively linked to the cardiac-specific promoter (*e.g.*, a modified TNNT2 promoter) is *ATP2A2*, or a mutant, variant, or fragment thereof (*e.g.*, SEQ ID NO: 113). In humans, the *ATP2A2* gene encodes sarco(endo)plasmic reticulum calcium-ATPase 2 (SERCA2) protein (*e.g.*, SEQ ID NO: 114), which catalyzes the hydrolysis of ATP coupled with the translocation of calcium from the cytosol into the sarcoplasmic reticulum lumen. The regulation of calcium ions into and out of the sarcoplasmic reticulum assists with muscle contraction and relaxation. In some embodiments, the polynucleotide encoding ATP2A2 shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 113. In some embodiments, the ATP2A2 protein shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 114.

[0142] In some embodiments, the disclosure provides a vector comprising a polynucleotide sequence that encodes the calcium voltage-gated channel subunit alpha 1C (CACNA1C) protein, operatively linked to a modified cardiac TNNT2 promoter. Similarly stated, in some embodiments, the polynucleotide sequence operatively linked to the cardiac-specific promoter (*e.g.*, a modified TNNT2 promoter) is *CACNA1C*, or a mutant, variant, or fragment thereof (*e.g.*, SEQ ID NO: 115). In humans, the *CACNA1C* gene encodes the alpha 1 subunit of a voltage-dependent calcium channel protein (*e.g.*, SEQ ID NO: 116), which functions to mediate the influx of calcium ions into the cell upon membrane polarization. In some embodiments, the polynucleotide encoding CACNA1C shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 115. In some embodiments, the CACNA1C protein shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 116.

[0143] In some embodiments, the disclosure provides a vector comprising a polynucleotide sequence that encodes the dystrophin (DMD) protein, operatively linked to a modified cardiac TNNT2 promoter. Similarly stated, in some embodiments, the polynucleotide sequence operatively linked to the cardiac-specific promoter (*e.g.*, a modified TNNT2 promoter) is *DMD*, or a mutant, variant, or fragment thereof (*e.g.*, SEQ ID NO: 117). In humans, the *DMD* gene encodes the DMD protein (*e.g.*, SEQ ID NO: 118), which forms a component of the dystrophin-glycoprotein complex (DGC). The DGC acts as an anchor, connecting the cytoskeleton with the extracellular matrix, thereby strengthening muscle fibers and protecting them from injury as

muscles contract and relax. In some embodiments, the polynucleotide encoding DMD shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 117. In some embodiments, the DMD protein shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 118.

[0144] In some embodiments, the disclosure provides a vector comprising a polynucleotide sequence that encodes the DM1 protein kinase (DMPK) protein, operatively linked to a modified cardiac TNNT2 promoter. Similarly stated, in some embodiments, the polynucleotide sequence operatively linked to the cardiac-specific promoter (*e.g.*, a modified TNNT2 promoter) is *DMPK*, or a mutant, variant, or fragment thereof (*e.g.*, SEQ ID NO: 119). In humans, the *DMPK* gene encodes myotonic dystrophy protein kinase protein (*e.g.*, SEQ ID NO: 120), which plays an important role in brain, muscle and heart development and homeostasis. Myotonic dystrophy protein kinase inhibits myosin phosphatase, which plays a role in muscle tensing and relaxation. In some embodiments, the polynucleotide encoding DMPK shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 119. In some embodiments, the DMPK protein shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 120.

[0145] In some embodiments, the disclosure provides a vector comprising a polynucleotide sequence that encodes the ectopic P granules protein 5 homolog (EPG5) protein, operatively linked to a modified cardiac TNNT2 promoter. Similarly stated, in some embodiments, the polynucleotide sequence operatively linked to the cardiac-specific promoter (*e.g.*, a modified TNNT2 promoter) is *EPG5*, or a mutant, variant, or fragment thereof (*e.g.*, SEQ ID NO: 121). In humans, the *EPG5* gene encodes the EPG5 protein (*e.g.*, SEQ ID NO: 122), which functions in autophagy to promote the interaction between autophagosomes and lysosomes. In some embodiments, the polynucleotide encoding EPG5 shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 121. In some embodiments, the EPG5 protein shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 122.

[0146] In some embodiments, the disclosure provides a vector comprising a polynucleotide sequence that encodes the EvC ciliary complex subunit 1 (EVC) protein, operatively linked to a modified cardiac TNNT2 promoter. Similarly stated, in some embodiments, the polynucleotide sequence operatively linked to the cardiac-specific promoter (*e.g.*, a modified TNNT2 promoter) is *EVC*, or a mutant, variant, or fragment thereof (*e.g.*, SEQ ID NO: 123). In humans, the *EVC* gene encodes the EVC protein (*e.g.*, SEQ ID NO: 124), which is found primarily in cilia, and functions to transmit information between cells. The EVC protein also regulates Sonic Hedgehog,

which plays a role in cell growth and differentiation. In some embodiments, the polynucleotide encoding EVC shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 123. In some embodiments, the EVC protein shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 124.

[0147] In some embodiments, the disclosure provides a vector comprising a polynucleotide sequence that encodes the limbin protein, operatively linked to a modified cardiac TNNT2 promoter. Similarly stated, in some embodiments, the polynucleotide sequence operatively linked to the cardiac-specific promoter (*e.g.*, a modified TNNT2 promoter) is *EVC2*, or a mutant, variant, or fragment thereof (*e.g.*, SEQ ID NO: 125). In humans, the *EVC2* gene encodes the limbin protein (*e.g.*, SEQ ID NO: 126). While the function of limbin is unknown, it is important for normal growth and development, particularly the development of bones and teeth. In some embodiments, the polynucleotide encoding limbin shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 125. In some embodiments, the limbin protein shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 126.

[0148] In some embodiments, the disclosure provides a vector comprising a polynucleotide sequence that encodes the fibrillin-1 protein, operatively linked to a modified cardiac TNNT2 promoter. Similarly stated, in some embodiments, the polynucleotide sequence operatively linked to the cardiac-specific promoter (*e.g.*, a modified TNNT2 promoter) is *FBN1*, or a mutant, variant, or fragment thereof (*e.g.*, SEQ ID NO: 127). In humans, the *FBN1* gene encodes fibrillin-1 and asprosin proteins (*e.g.*, SEQ ID NO: 128). Fibrillin-1 is a glycoprotein that serves as a structural component of calcium-binding microfibrils, which provide force-bearing support in elastic and nonelastic connective tissue throughout the body. Asprosin is a hormone normally secreted by white adipose tissue to regulate glucose homeostasis. In some embodiments, the polynucleotide encoding fibrillin-1 shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 127. In some embodiments, the fibrillin-1 protein shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 128.

[0149] In some embodiments, the disclosure provides a vector comprising a polynucleotide sequence that encodes the neurofibromin (NF1) protein, operatively linked to a modified cardiac TNNT2 promoter. Similarly stated, in some embodiments, the polynucleotide sequence operatively linked to the cardiac-specific promoter (*e.g.*, a modified TNNT2 promoter) is *NF1*, or a mutant, variant, or fragment thereof (*e.g.*, SEQ ID NO: 129). In humans, the *NF1* gene encodes

the NF1 protein (*e.g.*, SEQ ID NO: 130), which functions as a tumor suppressor and negative regulator of the Ras signaling pathway that stimulates cell growth and division. In some embodiments, the polynucleotide encoding NF1 shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 129. In some embodiments, the NF1 protein shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 130.

[0150] In some embodiments, the disclosure provides a vector comprising a polynucleotide sequence that encodes the sodium channel protein type 5 subunit alpha (SCN5A) protein, operatively linked to a modified cardiac TNNT2 promoter. Similarly stated, in some embodiments, the polynucleotide sequence operatively linked to the cardiac-specific promoter (*e.g.*, a modified TNNT2 promoter) is *SCN5A*, or a mutant, variant, or fragment thereof (*e.g.*, SEQ ID NO: 131). In humans, the *SCN5A* gene encodes the SCN5A protein (*e.g.*, SEQ ID NO: 132), which is a tetrodotoxin-resistant voltage-gated sodium channel subunit. SCN5A is found primarily in cardiac muscle and is responsible for the initial upstroke of the action potential in an electrocardiogram. In some embodiments, the polynucleotide encoding SCN5A shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 131. In some embodiments, the SCN5A protein shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 132.

[0151] In some embodiments, the disclosure provides a vector comprising a polynucleotide sequence that encodes the son of sevenless homolog 1 (SOS1) protein, operatively linked to a modified cardiac TNNT2 promoter. Similarly stated, in some embodiments, the polynucleotide sequence operatively linked to the cardiac-specific promoter (*e.g.*, a modified TNNT2 promoter) is *SOS1*, or a mutant, variant, or fragment thereof (*e.g.*, SEQ ID NO: 133). In humans, the *SOS1* gene encodes the SOS1 protein (*e.g.*, SEQ ID NO: 134), which functions as a component of a trimeric complex that participates in transduction signals from Ras to Rac by promoting Rac-specific guanine nucleotide exchange factor activity. In some embodiments, the polynucleotide encoding SOS1 shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 133. In some embodiments, the SOS1 protein shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 134.

[0152] In some embodiments, the disclosure provides a vector comprising a polynucleotide sequence that encodes the natriuretic peptide receptor 1 (NPR1) protein, operatively linked to a modified cardiac TNNT2 promoter. Similarly stated, in some embodiments, the polynucleotide sequence operatively linked to the cardiac-specific promoter (*e.g.*, a modified TNNT2 promoter)

is *NPR1*, or a mutant, variant, or fragment thereof (*e.g.*, SEQ ID NO: 135). The *NPR1* gene in humans encodes the NPR1 protein (also referred to as GC-A) (*e.g.*, SEQ ID NO: 136), which is a transmembrane catalytic receptor with intracellular guanylyl cyclase activity. NPR1 serves as a receptor for both atrial and brain natriuretic peptides, which are vasoactive hormones that play a key role in cardiovascular homeostasis. In some embodiments, the polynucleotide encoding NPR1 shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 135. In some embodiments, the NPR1 protein shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 136.

[0153] In some embodiments, the disclosure provides a vector comprising a polynucleotide sequence that encodes the receptor tyrosine-protein kinase erbB-4 (ERBB4) protein, operatively linked to a modified cardiac TNNT2 promoter. Similarly stated, in some embodiments, the polynucleotide sequence operatively linked to the cardiac-specific promoter (*e.g.*, a modified TNNT2 promoter) is *ERBB4*, or a mutant, variant, or fragment thereof (*e.g.*, SEQ ID NO: 137). The *ERBB4* gene in humans encodes the ERBB4 protein in humans (*e.g.*, SEQ ID NO: 138), which is a transmembrane receptor in the epidermal growth factor family. Signaling through the ERBB4 receptor induces a variety of cellular responses, including mitogenesis and differentiation. In some embodiments, the polynucleotide encoding ERBB4 shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 137. In some embodiments, the ERBB4 protein shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 138.

[0154] In some embodiments, the disclosure provides a vector comprising a polynucleotide sequence that encodes vasoactive intestinal peptide (VIP), operatively linked to a modified TNNT2 promoter. Similarly stated, in some embodiments, the polynucleotide sequence operatively linked to the cardiac-specific promoter (*e.g.*, a modified TNNT2 promoter) is *VIP*, or a mutant, variant, or fragment thereof (*e.g.*, SEQ ID NO: 139). In humans, the *VIP* gene encodes the vasoactive intestinal peptide (*e.g.*, SEQ ID NO: 140), which functions as a neuromodulator and neurotransmitter. VIP is a potent vasodilator, regulates smooth muscle activity, epithelial cell secretion and blood flow in the gastrointestinal tract. In some embodiments, the polynucleotide encoding VIP shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 139. In some embodiments, the VIP protein shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 140.

[0155] In some embodiments, the disclosure provides a vector comprising a polynucleotide sequence that encodes the beta-myosin heavy chain (MyHC-β), operatively linked to a modified

cardiac TNNT2 promoter. Similarly stated, in some embodiments, the polynucleotide sequence operatively linked to the cardiac-specific promoter (*e.g.*, a modified TNNT2 promoter) is *MYH7*, or a mutant, variant, or fragment thereof (*e.g.*, SEQ ID NO: 141). In humans, the *MYH7* gene encodes the MyHC-β protein (*e.g.*, SEQ ID NO: 142), which is a hexameric, asymmetric motor forming the majority of the thick filaments in cardiac muscle. The enzymatic activity of the ATPase in the myosin head hydrolyzes ATP, fueling the process of shortening sarcomeres in order to generate intraventricular pressure and power. In some embodiments, the polynucleotide encoding MyHC-β shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 141. In some embodiments, the MyHC-β protein shares at least 90%, 95%, 99%, or 100% identity to SEQ ID NO: 142.

III. Vectors

[0156] In some embodiments, the disclosure provides vectors for the treatment or prevention of heart disease. In particular, the vectors described herein comprise a cardiac-specific promoter operatively linked to a polynucleotide that encodes a therapeutic protein, wherein expression of the therapeutic protein treats a subject in need thereof (*e.g.*, a subject having cardiomyopathy). For example, in some embodiments, the vector is an AAV-based vector comprising the cardiac TNNT2 promoter operatively linked to a polynucleotide that encodes the MYBPC3 protein for the treatment or prevention of cardiomyopathy.

[0157] In some embodiments, the vector comprises, in addition to the cardiac-specific promoters (*e.g.*, a modified cardiac troponin T promoter) and therapeutic gene products (*e.g.*, MYBPC3 protein) described herein, a marker gene that facilitates identification or selection of cells that have been transfected, transduced or infected. Examples of marker genes include, but are not limited to, genes encoding fluorescent proteins, *e.g.*, enhanced green fluorescent protein, Ds-Red (DsRed: Discosoma sp. red fluorescent protein (RFP); Bevis et al. (2002) Nat. Biotechnol. 20(11):83-87), yellow fluorescent protein, mCherry, and cyanofluorescent protein; and genes encoding proteins conferring resistance to a selection agent, *e.g.*, a neomycin resistance gene, a puromycin resistance gene, a blasticidin resistance gene, and the like.

[0158] In some embodiments, the vector comprises a polynucleotide sequence having a size of at most about 4.0 kilobases, at most about 4.5 kilobases, at most about 5 kilobases, at most about 5.1 kilobases, at most about 5.2 kilobases, at most about 5.3 kilobases, at most about 5.4 kilobases, or

at most about 5.5 kilobases. In some embodiments, the vector comprises a polynucleotide sequence having a size of at most about 4.5 kilobases. In some embodiments, the vector comprises a polynucleotide sequence having a size of at most about 5 kilobases. In some embodiments, the vector comprises a polynucleotide sequence having a size of at most about 5.5 kilobases. In some embodiments, the vector comprises a polynucleotide sequence having a size of at most about 6 kilobases.

[0159] Methods of introducing polynucleotides into a host cell are known in the art, and any known method can be used to introduce the polynucleotides described herein into a cell. Suitable methods include *e.g.*, viral or bacteriophage infection, transfection, conjugation, protoplast fusion, lipofection, electroporation, calcium phosphate precipitation, polyethyleneimine (PEI)-mediated transfection, DEAE-dextran mediated transfection, liposome-mediated transfection, particle gun technology, calcium phosphate precipitation, direct micro injection, nanoparticle-mediated nucleic acid delivery, microfluidics delivery methods, and the like.

A. Non-viral vectors

[0160] In some embodiments, the polynucleotides described herein are delivered to a cell in a non-viral vector, such as a transposon, a nanoparticle (*e.g.*, a lipid nanoparticle), a liposome, an exosome, an attenuated bacterium, or a virus-like particle. In some embodiments, the non-viral vector is a mammalian virus-like particle. For example, mammalian virus-like particle can be generated (*e.g.*, by purification of the "empty" mammalian virus-like particle followed by *ex vivo* assembly of the mammalian virus-like particle with the desired cargo). The non-viral vector can also be engineered to incorporate targeting ligands to alter target tissue specificity.

B. Viral vectors

[0161] In some embodiments, the vector is a viral vector. In some embodiments, the viral vector is a retroviral vector, *e.g.*, a lentiviral vector. As used herein, the term "retrovirus" or "retroviral" refers an RNA virus that reverse transcribes its genomic RNA into a linear double-stranded DNA copy and subsequently covalently integrates its genomic DNA into a host genome. Retrovirus vectors are a common tool for gene delivery (Miller, *Nature*. 357: 455-460 (2000)). Once the virus is integrated into the host genome, it is referred to as a "provirus." The provirus serves as a template for RNA polymerase II and directs the expression of RNA molecules encoded by the virus. In

some embodiments, a retroviral vector is altered so that it does not integrate into the host cell genome.

[0162] Illustrative retroviruses (family Retroviridae) include, but are not limited to: (1) genus gammaretrovirus, such as, Moloney murine leukemia virus (M-MuLV), Moloney murine sarcoma virus (MoMSV), murine mammary tumor virus (MuMTV), gibbon ape leukemia virus (GaLV), and feline leukemia virus (FLV), (2) genus spumavirus, such as, simian foamy virus, (3) genus lentivirus, such as, human immunodeficiency virus-1 and simian immunodeficiency virus.

[0163] As used herein, the term "lentiviral" or "lentivirus" refers to a group (or genus) of complex retroviruses. Illustrative lentiviruses include, but are not limited to: HIV (human immunodeficiency virus; including HIV type 1, and HIV type 2; visna-maedi virus (VMV) virus; the caprine arthritis-encephalitis virus (CAEV); equine infectious anemia virus (EIAV); feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV).

[0164] In some embodiments, the viral vector is an adenoviral vector. The genetic organization of adenovirus includes an approximate 36 kb, linear, double-stranded DNA virus, which allows substitution of large pieces of adenoviral DNA with foreign sequences up to 7 kb (Grunhaus et al., *Seminar in Virology* 200(2):535-546, 1992)).

[0165] In some embodiments, the viral vector is an adeno-associated viral (AVV) vector, such as an AAV vector selected from the group consisting of serotype 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 or chimeric AAV derived thereof.

[0166] In some embodiments, the AAV expression vector is pseudotyped to enhance targeting. A pseudotyping strategy can promote gene transfer and sustain expression in a target cell type. For example, the AAV2 genome can be packaged into the capsid of another AAV serotype such as AAV5, AAV7, or AAV8, producing pseudotyped vectors such as AAV2/5, AAV2/7, and AAV2/8 respectively, as described in Balaji et al. *J Surg Res.* Sep; 184(1): 691–698 (2013). In some embodiments, an AAV9 may be used to target expression in myofibroblast-like lineages, as described in Piras et al. *Gene Therapy* 23:469–478 (2016). In some embodiments, AAV1, AAV6, or AAV9 is used, and in some embodiments, the AAV is engineered, as described in Asokari et al. *Hum Gene Ther*. Nov; 24(11): 906–913 (2013); Pozsgai et al. Mol Ther. Apr 5; 25(4): 855–869 (2017); Kotterman, M.A. and D.V. Schaffer Engineering Adeno-Associated Viruses for Clinical Gene Therapy. *Nature Reviews Genetics*, 15:445-451 (2014); and US20160340393A1 to Schaffer

et al. In some embodiments, the viral vector is AAV engineered to increase target cell infectivity as described in US20180066285A1.

C. Regulatory Elements

[0167] In some embodiments, the disclosure provides a vector comprising one or more regulatory elements operatively linked to a polynucleotide encoding a therapeutic protein or nucleic acid. In some embodiments, the regulatory element is a cardiac-specific promoter (*e.g.*, a modified TNNT2 promoter) that is operatively linked to a therapeutic protein or nucleic acid for the treatment of heart disease.

[0168] As used herein, the term "regulatory element" refers those non-translated regions of the vector (*e.g.*, origin of replication, selection cassettes, promoters, enhancers, translation initiation signals (Shine Dalgarno sequence or Kozak sequence) introns, a polyadenylation sequence, 5′ and 3′ untranslated regions) which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. The transcriptional regulatory element may be functional in either a eukaryotic cell (*e.g.*, a mammalian cell) or a prokaryotic cell (*e.g.*, bacterial or archaeal cell). In some embodiments, a polynucleotide sequence encoding the therapeutic gene products (*e.g.*, a therapeutic protein or nucleic acid) described herein is operably linked to multiple control elements that allow expression of the polynucleotide in both prokaryotic and eukaryotic cells.

[0169] As used herein, the term "transcription start site" or "TSS" refers to the first base pair transcribed by an RNA polymerase when the RNA polymerase initiates transcription. A TSS is different from the start codon (canonically, ATG), which must be downstream of the TSS in the transcribed region of the polynucleotide. The location of a transcription start site can be determined experimentally or by prediction using any of various prediction algorithms. Annotated TSSs are available from the Eukaryotic Promoter Database and the UCSC Genome Browser. Multiple TSSs for TNNT2 are identified in the UCSC Genome Browser.

[0170] As used herein, the TSS for TNNT2 is defined to be the sequence identified by the C at the 5′ end of the motif identified by dbTSS: **C**TCCATC.

[0171] The term "modified cardiac TNNT2 promoter" as used herein refers to a promoter that comprises a polynucleotide sequence of at least 200 base pairs that comprises one or more continuous or discontinuous polynucleotide segments each sharing 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a corresponding segment of the TNNT2p-600 segment

provided in Table 1 as SEQ ID NO: 1. As it is a "promoter," a modified cardiac TNNT2 promoter must be capable of promoting initiation of transcription by an RNA polymerase in a host or target cell at or near a TSS within the promoter (*i.e.* at or near the TTS of TNNT2 as defined herein) or, if the endogenous TSS of TNNT2 is not present in the modified cardiac TNNT2 promoter then at a heterologous TSS at most 100 base pairs downstream (3′ on the sense strand) to the downstream (3′) end of the modified cardiac TNNT2 promoter. Similarly stated, a modified cardiac TNNT2 promoter may comprise only sequences upstream of the TSS of TNNT2 or more comprise the TSS of TNNT2.

[0172] The length of a promoter (*e.g.*, a modified cardiac TNNT2 promoter), a promoter "having" so many base pairs, as used herein, is defined according to the number of base pairs in the polynucleotide sequence of the promoter from its 5′ end to its 3′ end, inclusive of the endpoints, and inclusive of any intervening sequences that do not align to a reference promoter sequence (*e.g.*, the endogenous cardiac TNNT2 promoter of a human or other organism). The 5′ end and the 3′ end of the promoter are defined as the last base pair in either direction to match a corresponding sequence in a reference promoter sequence when the sequence are aligned by the BLAST algorithm or the equivalent. Thus, the length of a promoter in a vector can be determined by searching a nucleotide database containing a genome of a reference organism using the polynucleotide sequence of the vector and identifying one or more aligned regions that encompass or are within about 1-5 kb of an endogenous gene, or by aligning the vector to a predetermined reference promoter. If the promoter aligns to the reference genome or reference promoter sequence as a continuous segment, then the length of promoter is the length alignment reported (the 3′ end position minus the 5′ end position, + 1 unless the TSS is included). If the promoter aligns in multiple segments (*e.g.*, 2, 3, 4, or 5 segments), then the length of the promoter can be calculated by the 3′ end position of the 3′-most segment of the reference genome or reference promoter sequence, minus the 5′ end position of the 5′-most segment of reference genome or reference promoter sequence, plus 1 unless the TSS is included (such that the calculated length includes both end points). For example, the length of a promoter that extends from a base pair 100 bp before the TSS (-100 bp) to 5 bp before the TSS (-5 bp) is -5-(-100) + 1 = 100 - 5 + 1 = 96 bp. The TSS is numbered +1 bp. Therefore, the length of a promoter that extends from a base pair 100 bp before the TSS (-100 bp) to 5 bp after the TSS (+5 bp) is +5-(-100) = 100 + 5 = 105 bp.

[0173] The term "enhancer" refers to a segment of DNA which contains sequences capable of providing enhanced transcription and in some instances can function independent of their orientation relative to another control sequence. An enhancer can function cooperatively or additively with promoters and/or other enhancer elements. An enhancer may overlap with a promoter or be upstream or downstream of the promoter. In some embodiments, the modified cardiac TNNT2 promoter comprises one or more enhancers. In some embodiments, the modified cardiac TNNT2 promoter comprises no enhancer.

[0174] In addition to or instead of a modified cardiac TNNT2 promoter, some embodiments employ other eukaryotic promoters, including but not limited to: cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, a viral simian virus 40 (SV40) (*e.g.,* early and late SV40), a spleen focus forming virus (SFFV) promoter, long terminal repeats (LTRs) from retrovirus (*e.g.*, a Moloney murine leukemia virus (MoMLV) LTR promoter or a Rous sarcoma virus (RSV) LTR), a herpes simplex virus (HSV) (thymidine kinase) promoter, H5, P7.5, and P11 promoters from vaccinia virus, an elongation factor 1-alpha (EF1α) promoter, early growth response 1 (EGR1) promoter, a ferritin H (FerH) promoter, a ferritin L (FerL) promoter, a Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) promoter, a eukaryotic translation initiation factor 4A1 (EIF4A1) promoter, a heat shock 70kDa protein 5 (HSPA5) promoter, a heat shock protein 90kDa beta, member 1 (HSP90B1) promoter, a heat shock protein 70kDa (HSP70) promoter, a β-kinesin (β-KIN) promoter, the human ROSA 26 locus (Irions *et al.*, *Nature Biotechnology* 25, 1477-1482 (2007)), a Ubiquitin C (UBC) promoter, a phosphoglycerate kinase-1 (PGK) promoter, a cytomegalovirus enhancer/chicken β-actin (CAG) promoter, a β-actin promoter and a myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer-binding site substituted (MND) promoter, and mouse metallothionein-l. The vector may also contain a ribosome binding site for translation initiation and a transcription terminator. The vector may also include polynucleotide sequences for amplifying expression. The vector may also include polynucleotide sequences encoding protein tags (*e.g.*, 6xHis tag, hemagglutinin tag, green fluorescent protein, etc.) that are fused to the site-directed modifying polypeptide, thus resulting in a chimeric polypeptide.

[0175] In some embodiments, the promoters of the disclosure are tissue-specific. The term "tissue-specific promoter" means a polynucleotide sequence that serves as a promoter, *i.e.*, regulates expression of a selected polynucleotide sequence operably linked to the promoter, and which

affects expression of the selected polynucleotide sequence in specific cells of a tissue, such as myocytes or myocardial cells. In some embodiments, the tissue-specific promoter is a cardiac-specific promoter. In some embodiments, the cardiac-specific promoter is TNNT2 or a modified TNNT2 promoter. A tissue-specific promoter causes expression of an operatively linked polynucleotide, or a gene product encoded by that polynucleotide, at 5x, 10x, 20x, 25x or greater levels in the tissue of interest than in a reference tissue.

[0176] In some embodiments, the vectors described herein comprise a transcription termination signal. Elements directing the efficient termination and polyadenylation of the heterologous nucleic acid transcripts increases heterologous gene expression. Transcription termination signals are generally found downstream of the polyadenylation signal. In some embodiments, vectors comprise a polyadenylation sequence 3′ of a polynucleotide encoding a polypeptide to be expressed. The term "polyA site" or "polyA sequence" as used herein denotes a DNA sequence which directs both the termination and polyadenylation of the nascent RNA transcript by RNA polymerase II. Polyadenylation sequences can promote mRNA stability by addition of a polyA tail to the 3′ end of the coding sequence and thus, contribute to increased translational efficiency. Cleavage and polyadenylation is directed by a poly(A) sequence in the RNA. The core poly(A) sequence for mammalian pre-mRNAs has two recognition elements flanking a cleavage-polyadenylation site. Typically, an almost invariant AAUAAA hexamer lies 20-50 nucleotides upstream of a more variable element rich in U or GU residues. Cleavage of the nascent transcript occurs between these two elements and is coupled to the addition of up to 250 adenosines to the 5′ cleavage product. In particular embodiments, the core poly(A) sequence is an ideal polyA sequence (*e.g.*, AATAAA, ATTAAA, AGTAAA). In particular embodiments, the poly(A) sequence is an SV40 polyA sequence, a bovine growth hormone polyA sequence (BGHpA), a rabbit β-globin polyA sequence (rβgpA), variants thereof, or another suitable heterologous or endogenous polyA sequence known in the art.

IV. Recombinant adeno-associated virus (rAAV) viral genome, expression cassette, and rAAV virions

[0177] The disclosure provides an expression cassette comprising a polynucleotide encoding a transgene, *e.g.* a sequence encoding a MYBPC3 polypeptide, or functional variant thereof. The transgene polynucleotide sequence in an expression cassette can be, for example, an open reading frame encoding a protein. The expression cassette may comprise, optionally, a promoter

operatively linked to the transgene, optionally an intron region, optionally a polyadenylation (poly(A)) signal, optionally a woodchuck hepatitis virus post-transcriptional element (WPRE), and optionally a transcription termination signal. The expression cassette may be flanked by one or more inverted terminal repeats (ITRs). An expression cassette flanked by one or more ITRs is herein referred to as a "viral genome." The ITRs in an expression cassette serve as markers used for viral packaging of the expression cassette (Clark et al. *Hum Gene Ther.* 6:1329-41 (1995)). Illustrative and non-limiting embodiments of viral genomes of the disclosure are shown in **FIG. 1A, FIG. 1C, and FIG. 2A**. The polynucleotide encoding the expression cassette provides the function of expressing the transgene within a host cell. The expression cassette can be integrated into the host cell genome by, for example, infecting the host cell with an rAAV virion comprising capsid protein and a viral genome comprising an expression cassette.

[0178] The promoter sequence of the expression cassette, when present, controls expression of the polynucleotide encoding the transgene, *e.g.* a sequence encoding MYBPC3 or functional variant thereof. Various promoters can be used. The promoter may be cell-type specific. Constitutive promoters are used in expression cassettes and can be, for example, the cytomegalovirus enhancer fused to the chicken β-actin promoter (CAG), simian virus 40 (SV40) promoter, and the herpes simplex virus thymidine kinase (HSV-TK) promoter (Damdindorj et al. *PLoS One*. 9:e106472 (2014)). Other cell-type specific promoters may also be used. Cardiac cell specific promoters can be, for example, the MLC2v promoter (Phillips et al. *Hypertension*. 39:651-5 (2002)) and the cardiac Troponin-T (cTnT) promoter (Konkalmatt et al. *Circ Cardiovasc Imaging*. 6:478–486 (2013)).

[0179] In some aspects, the disclosure provides promoters have been optimized for cardiac cell-specific expression and length to accommodate transgenes of specified size. In one embodiment, the promoter of an rAAV vector genome described herein is a polynucleotide having between 300 bp and 500 bp.

[0180] Exemplary expression cassette and viral genome sequences of the disclosure can be found in Table 5. In some embodiments, the expression cassette comprises a polynucleotide sequence that shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 95, SEQ ID NO: 99, or SEQ ID NO: 101. In some embodiments, the viral genome comprises a polynucleotide sequence that shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 98, SEQ ID NO: 100, or SEQ ID NO: 102. In another embodiment, an expression

cassette can be segmented according to the polynucleotide regions flanking the transgene. The polynucleotide sequence spanning the 5′ end of the cassette to the 5′ end of the transgene is herein referred to as the 5′ segment of the expression cassette. The polynucleotide sequence spanning the 3′ end of the transgene to the 3′ end of the expression cassette is herein referred to as the 3′ segment of the expression cassette. In one embodiment, the 5′ segment of the expression cassette comprises a polynucleotide sequence that shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 93. In one embodiment, the 3′ segment of the expression cassette comprises a polynucleotide sequence that shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 94.

[0181] The ability to express large transgenes delivered by rAAV vectors or rAAV virions is limited. rAAV vector genome sizes have a maximum sequence length of about 5 kb, thus providing a limit for the length of all elements required in an expression cassette including regulatory elements, *e.g.* promoter, and a transgene, *e.g.* MYBPC3. rAAV vector genomes exceeding 5 kb result in vector genome truncation during rAAV virion packaging and reduce or ablate transgene expression (Wu et al. *Mol Ther.* 18:80-86 (2010)). In some embodiments, the present disclosure provides rAAV vector genomes that are optimized for carrying large transgenes. Elements of the vector genome have been reduced in length in order to accommodate a larger transgene. In one embodiment, the 5′ segment and 3′ segment of an expression cassette together comprise at most 0.8 kbp or at most 0.9 kbp. In another embodiment, the 5′ ITR, the 5′ segment, the 3′ segment, and 3′ ITR together comprise comprise 1.2 kbp or at most comprise 1.3 kbp. In one embodiment, the 5′ segment comprises at most 500 bp or at most 480 bp. In one embodiment, the 3′ segment comprises at most 200 bp or at most 150 bp. In another embodiment, the vector genome comprises at most 4.7 kbp. 4.8 kbp, 4.9 kbp, or 5.0 kbp. In some embodiments, the polynucleotide encoding the gene product comprises between 3 kb and 11 kb, between 3 kbp and 5 kbp, between 3.5 kbp and 4.5 kbp, or between 3.7 kbp and 4 kbp. In some embodiments, the polynucleotide encoding the gene product comprises 3.7 kbp to 3.9 kbp. In some embodiments, the polynucleotide encoding the gene product comprises 3.8 kbp.

Table 5. Illustrative expression cassette and viral genomes

Name	DNA Sequence	SEQ ID NO.

viral genome (600 bp promoter)	Ctgcgcgctcgctcgctcactgaggccgcccgggcaaagcccgggcgtcgggcgacc tttggtcgcccggcctcagtgagcgagcgagcgcgcagagagggagtggccaactcc atcactaggggttccttgtagttaatgattaacccgccatgctacttatctacgtag ccatgctctaggaagatcggaattcgcccttaagtcatggagaagacccaccttgca gatgtcctcactggggctggcagagccggcaacctgcccaaggctgctcagtccatt aggagccagtagcctggaagatgtctttaccccagcatcagttcaagtggagcagc acataactcttgccctctgccttccaagattctggtgctgagacttatggagtgtct tggaggttgccttctgccccccaaccctgctcccagctggccctcccaggcctgggt tgctggcctctgctttatcaggattctcaagagggacagctggtttatgttgcatga ctgttccctgcatatctgctctggttttaaatagcttatctgagcagctggaggacc acatgggcttatatggcgtggggtacatgttcctgtagccttgtccctggcacctgc caaaatagcagccaacacccccccaccccccaccgccatcccccctgccccacccgtccc ctgtcgcacattcctccctccgcagggctggctcaccaggccccagcccacatgcct gcttaaagccctctccatcctctgcctcacccagtccccgctgagactgagcagacg cctccagccaccaagcttaataaaagatctttattttcattagatctgtgtgttggt tttttgtgtgctggggactcgagttaagggcgaattcccgataaggatcttcctaga gcatggctacgtagataagtagcatggcgggttaatcattaactacaaggaacccct agtgatggagttggccactccctctctgcgcgctcgctcgctcactgaggccgggcg accaaaggtcgcccgacgcccgggctttgcccgggcggcctcagtgagcgagcgagc gcgcag	98
expression cassette (600 bp promoter)	Tgtagttaatgattaacccgccatgctacttatctacgtagccatgctctaggaaga tcggaattcgcccttaagtcatggagaagacccaccttgcagatgtcctcactgggg ctggcagagccggcaacctgcccaaggctgctcagtccattaggagccagtagcctg gaagatgtctttaccccagcatcagttcaagtggagcagcacataactcttgccct ctgccttccaagattctggtgctgagacttatggagtgtcttggaggttgccttctg ccccccaaccctgctcccagctggccctcccaggcctgggttgctggcctctgcttt atcaggattctcaagagggacagctggtttatgttgcatgactgttccctgcatatc tgctctggttttaaatagcttatctgagcagctggaggaccacatgggcttatatgg cgtggggtacatgttcctgtagccttgtccctggcacctgccaaaatagcagccaac acccccccaccccccaccgccatcccccctgccccacccgtcccctgtcgcacattcctc cctccgcagggctggctcaccaggccccagcccacatgcctgcttaaagccctctcc atcctctgcctcacccagtccccgctgagactgagcagacgcctccagccaccaagc ttaataaaagatctttattttcattagatctgtgtgttggtttttgtgtgctgggg actcgagttaagggcgaattcccgataaggatcttcctagagcatggctacgtagat aagtagcatggcgggttaatcattaactacaa	99
viral genome (400 bp promoter)	Ctgcgcgctcgctcgctcactgaggccgcccgggcaaagcccgggcgtcgggcgacc tttggtcgcccggcctcagtgagcgagcgagcgcgcagagagggagtggccaactcc atcactaggggttccttgtagttaatgattaacccgccatgctacttatctacgtag ccatgctctaggaagatcggaattcgcccttaagttgccttctgccccccaaccctg ctcccagctggccctcccaggcctgggttgctggcctctgctttatcaggattctca agagggacagctggtttatgttgcatgactgttccctgcatatctgctctggtttta aatagcttatctgagcagctggaggaccacatgggcttatatggcgtggggtacatg ttcctgtagccttgtccctggcacctgccaaaatagcagccaacacccccccacccccc accgccatccccctgccccacccgtcccctgtcgcacattcctccctccgcagggct ggctcaccaggccccagcccacatgcctgcttaaagccctctccatcctctgcctca cccagtccccgctgagactgagcagacgcctccagccaccaagcttaataaaagatc tttattttcattagatctgtgtgttggtttttgtgtgctggggactcgagttaagg gcgaattcccgataaggatcttcctagagcatggctacgtagataagtagcatggcg ggttaatcattaactacaaggaacccctagtgatggagttggccactccctctctgc gcgctcgctcgctcactgaggccgggcgaccaaaggtcgcccgacgcccgggctttg cccgggcggcctcagtgagcgagcgagcgcgcag	100

expression cassette (400 bp promoter)	Tgtagttaatgattaacccgccatgctacttatctacgtagccatgctctaggaaga tcggaattcgcccttaagttgccttctgccccccaaccctgctcccagctggccctc ccaggcctgggttgctggcctctgctttatcaggattctcaagagggacagctggtt tatgttgcatgactgttccctgcatatctgctctggttttaaatagcttatctgagc agctggaggaccacatgggcttatatggcgtggggtacatgttcctgtagccttgtc cctggcacctgccaaaatagcagccaacacccccccacccccaccgccatcccccctgc cccacccgtcccctgtcgcacattcctccctccgcagggctggctcaccaggcccca gcccacatgcctgcttaaagccctctccatcctctgcctcacccagtccccgctgag actgagcagacgcctccagccaccaagcttaataaaagatctttattttcattagat ctgtgtgttggttttttgtgtgctggggactcgagttaagggcgaattcccgataag gatcttcctagagcatggctacgtagataagtagcatggcgggttaatcattaacta ca	101
viral genome + MYBPC3 transgene (400 bp promoter)	ctgcgcgctcgctcgctcactgaggccgcccgggcaaagcccgggcgtcgggcgacc tttggtcgcccggcctcagtgagcgagcgagcgcgcagagagggagtggccaactcc atcactaggggttccttgtagttaatgattaacccgccatgctacttatctacgtag ccatgctctaggaagatcggaattcgcccttaagttgccttctgccccccaaccctg ctcccagctggccctcccaggcctgggttgctggcctctgctttatcaggattctca agagggacagctggtttatgttgcatgactgttccctgcatatctgctctggtttta aatagcttatctgagcagctggaggaccacatgggcttatatggcgtggggtacatg ttcctgtagccttgtccctggcacctgccaaaatagcagccaacacccccccacccc accgccatccccctgccccacccgtcccctgtcgcacattcctccctccgcagggct ggctcaccaggccccagcccacatgcctgcttaaagccctctccatcctctgcctca cccagtccccgctgagactgagcagacgcctccagccaccatgcctgagccggggaa gaagccagtctcagcttttagcaagaagccacggtcagtggaagtggccgcaggcag ccctgccgtgttcgaggccgagacagagcgggcaggagtgaaggtgcgctggcagcg cggaggcagtgacatcagcgccagcaacaagtacggcctggccacagagggcacacg gcatacgctgacagtgcgggaagtgggccctgccgaccagggatcttacgcagtcat tgctggctcctccaaggtcaagttcgacctcaaggtcatagaggcagagaaggcaga gcccatgctggcccctgcccctgcccctgctgaggccactggagcccctggagaagc cccggccccagccgctgagctgggagaaagtgccccaagtcccaaagggtcaagctc agcagctctcaatggtcctacccctggagcccccgatgaccccattggcctcttcgt gatgcggccacaggatggcgaggtgaccgtgggtggcagcatcaccttctcagcccg cgtggccggcgccagcctcctgaagccgcctgtggtcaagtggttcaagggcaaatg ggtggacctgagcagcaaggtgggccagcacctgcagctgcacgacagctacgaccg cgccagcaaggtctatctgttcgagctgcacatcaccgatgcccagcctgccttcac tggcagctaccgctgtgaggtgtccaccaaggacaaatttgactgctccaacttcaa tctcactgtccacgaggccatgggcaccggagacctggacctcctatcagccttccg ccgcacgagcctggctggaggtggtcggcggatcagtgatagccatgaggacactgg gattctggacttcagctcactgctgaaaaagagagacagtttccggaccccgaggga ctcgaagctggaggcaccagcagaggaggacgtgtgggagatcctacggcaggcacc cccatctgagtacgagcgcatcgccttccagtacggcgtcactgacctgcgcggcat gctaaagaggctcaagggcatgaggcgcgatgagaagaagagcacagcctttcagaa gaagctggagccggcctaccaggtgagcaaaggccacaagatccggctgaccgtgga actggctgaccatgacgctgaggtcaaatggctcaagaatggccaggagatccagat gagcggcagcaagtacatctttgagtccatcggtgccaagcgtaccctgaccatcag ccagtgctcattggcggacgacgcagcctaccagtgcgtggtgggtggcgagaagtg tagcacggagctctttgtgaaagagccccctgtgctcatcacgcgccccttggagga ccagctggtgatggtggggcagcgggtggagtttgagtgtgaagtatcggaggaggg ggcgcaagtcaaatggctgaaggacggggtggagctgacccgggaggagaccttcaa ataccggttcaagaaggacgggcagagacaccacctgatcatcaacgaggccatgct ggaggacgcggggcactatgcactgtgcactagcgggggccaggcgctggctgagct cattgtgcaggaaaagaagctggaggtgtaccagagcatcgcagacctgatggtggg cgcaaaggaccaggcggtgttcaaatgtgaggtctcagatgagaatgttcggggtgt gtggctgaagaatgggaaggagctggtgcccgacagccgcataaaggtgtcccacat cgggcgggtccacaaactgaccattgacgacgtcacacctgccgacgaggctgacta cagctttgtgcccgagggcttcgcctgcaacctgtcagccaagctccacttcatgga	102

	ggtcaagattgacttcgtacccaggcaggaacctcccaagatccacctggactgccc aggccgcataccagacaccattgtggttgtagctggaaataagctacgtctggacgt ccctatctctggggaccccgctcccactgtgatctggcagaaggctatcacgcaggg gaataaggccccagccaggccagccccagatgccccagaggacacaggtgacagcga tgagtgggtgtttgacaagaagctgctgtgtgagaccgagggccgggtccgcgtgga gaccaccaaggaccgcagcatcttcacggtcgaggggggcagagaaggaagatgaggg cgtctacacggtcacagtgaagaaccctgtgggcgaggaccaggtcaacctcacagt caaggtcatcgacgtgccagacgcacctgcggcccccaagatcagcaacgtgggaga ggactcctgcacagtacagtgggagccgcctgcctacgatggcgggcagcccatcct gggctacatcctggagcgcaagaagaagaagagctaccggtggatgcggctgaactt cgacctgattcaggagctgagtcatgaagcgcggcgcatgatcgagggcgtggtgta cgagatgcgcgtctacgcggtcaacgccatcggcatgtccaggcccagccctgcctc ccagcccttcatgcctatcggtccccccagcgaacccacccacctggcagtagagga cgtctctgacaccacggtctccctcaagtggcggcccccagagcgcgtgggagcagg aggcctggatggctacagcgtggagtactgcccagagggctgctcagagtgggtggc tgccctgcaggggctgacagagcacacatcgatactggtgaaggacctgcccacggg ggcccggctgcttttccgagtgcgggcacacaatatggcagggcctggagcccctgt taccaccacggagccggtgacagtgcaggagatcctgcaacggccacggcttcagct gcccaggcacctgcgccagaccattcagaagaaggtcggggagcctgtgaaccttct catccctttccagggcaagccccggcctcaggtgacctggaccaaagaggggcagcc cctggcaggcgaggaggtgagcatccgcaacagccccacagacaccatcctgttcat ccgggccgctcgccgcgtgcattcaggcacttaccaggtgacggtgcgcattgagaa catggaggacaaggccacgctggtgctgcaggttgttgacaagccaagtcctcccca ggatctccgggtgactgacgcctggggtcttaatgtggctctggagtggaagccacc ccaggatgtcggcaacacggaactctgggggtacacagtgcagaaagccgacaagaa gaccatggagtggttcaccgtcttggagcattaccgccgcacccactgcgtggtgcc agagctcatcattggcaatggctactacttccgcgtcttcagccagaatatggttgg ctttagtgacagagcggccaccaccaaggagcccgtctttatccccagaccaggcat cacctatgagccacccaactataaggccctggacttctccgaggccccaagcttcac ccagcccctggtgaaccgctcggtcatcgcgggctacactgctatgctctgctgtgc tgtccggggtagccccaagcccaagatttcctggttcaagaatggcctggacctggg agaagacgcccgcttccgcatgttcagcaagcagggagtgttgactctggagattag aaagccctgccccttgacgggggcatctatgtctgcagggccaccaacttacaggg cgaggcacggtgtgagtgccgcctggaggtgcgagtgcctcagtaaagcttaataaa agatctttattttcattagatctgtgtgttggtttttgtgtgctggggactcgagt taagggcgaattcccgataaggatcttcctagagcatggctacgtagataagtagca tggcgggttaatcattaactacaaggaacccctagtgatggagttggccactccctc tctgcgcgctcgctcgctcactgaggccgggcgaccaaaggtcgcccgacgcccggg ctttgcccgggcggcctcagtgagcgagcgagcgcgcag	
expression cassette + MYBPC3 transgene (400 bp promoter)	tgtagttaatgattaacccgccatgctacttatctacgtagccatgctctaggaaga tcggaattcgcccttaagttgccttctgccccccaaccctgctcccagctggccctc ccaggcctgggttgctggcctctgctttatcaggattctcaagagggacagctggtt tatgttgcatgactgttccctgcatatctgctctggttttaaatagcttatctgagc agctggaggaccacatgggcttatatggcgtggggtacatgttcctgtagccttgtc cctggcacctgccaaaatagcagccaacacccccccaccccccaccgccatcccctgc cccacccgtcccctgtcgcacattcctccctccgcagggctggctcaccaggcccca gcccacatgcctgcttaaagccctctccatcctctgcctcacccagtccccgctgag actgagcagacgcctccagccaccatgcctgagccggggaagaagccagtctcagct tttagcaagaagccacggtcagtggaagtggccgcaggcagccctgccgtgttcgag gccgagacagagcgggcaggagtgaaggtgcgctggcagcgcggaggcagtgacatc agcgccagcaacaagtacggcctggccacagagggcacacggcatacgctgacagtg cgggaagtgggccctgccgaccagggatcttacgcagtcattgctggctcctccaag gtcaagttcgacctcaaggtcatagaggcagagaaggcagagcccatgctggccccct gcccctgcccctgctgaggccactggagcccctggagaagccccggccccagccgct gagctgggagaaagtgccccaagtcccaaagggtcaagctcagcagctctcaatggt cctacccctggagcccccgatgaccccattggcctcttcgtgatgcggccacaggat	95

	ggcgaggtgaccgtgggtggcagcatcaccttctcagcccgcgtggccggcgccagc ctcctgaagccgcctgtggtcaagtggttcaagggcaaatgggtggacctgagcagc aaggtgggccagcacctgcagctgcacgacagctacgaccgcgccagcaaggtctat ctgttcgagctgcacatcaccgatgcccagcctgccttcactggcagctaccgctgt gaggtgtccaccaaggacaaatttgactgctccaacttcaatctcactgtccacgag gccatgggcaccggagacctggacctcctatcagccttccgccgcacgagcctggct ggaggtggtcggcggatcagtgatagccatgaggacactgggattctggacttcagc tcactgctgaaaaagagagacagtttccggaccccgagggactcgaagctggaggca ccagcagaggaggacgtgtgggagatcctacggcaggcacccccatctgagtacgag cgcatcgccttccagtacggcgtcactgacctgcgcggcatgctaaagaggctcaag ggcatgaggcgcgatgagaagaagagcacagcctttcagaagaagctggagccggcc taccaggtgagcaaaggccacaagatccggctgaccgtggaactggctgaccatgac gctgaggtcaaatggctcaagaatggccaggagatccagatgagcggcagcaagtac atctttgagtccatcggtgccaagcgtaccctgaccatcagccagtgctcattggcg gacgacgcagcctaccagtgcgtggtgggtggcgagaagtgtagcacggagctcttt gtgaaagagccccctgtgctcatcacgcgccccttggaggaccagctggtgatggtg gggcagcgggtggagtttgagtgtgaagtatcggaggaggggcgcaagtcaaatgg ctgaaggacggggtggagctgacccgggaggagaccttcaaataccggttcaagaag gacgggcagagacaccacctgatcatcaacgaggccatgctggaggacgcggggcac tatgcactgtgcactagcgggggccaggcgctggctgagctcattgtgcaggaaaag aagctggaggtgtaccagagcatcgcagacctgatggtgggcgcaaaggaccaggcg gtgttcaaatgtgaggtctcagatgagaatgttcggggtgtgtggctgaagaatggg aaggagctggtgcccgacagccgcataaaggtgtcccacatcgggcgggtccacaaa ctgaccattgacgacgtcacacctgccgacgaggctgactacagctttgtgcccgag ggcttcgcctgcaacctgtcagccaagctccacttcatggaggtcaagattgacttc gtacccaggcaggaacctcccaagatccacctggactgcccaggccgcataccagac accattgtggttgtagctggaaataagctacgtctggacgtccctatctctggggac cccgctcccactgtgatctggcagaaggctatcacgcaggggaataaggccccagcc aggccagccccagatgccccagaggacacaggtgacagcgatgagtgggtgtttgac aagaagctgctgtgtgagaccgagggccgggtccgcgtggagaccaccaaggaccgc agcatcttcacggtcgagggggcagagaaggaagatgagggcgtctacacggtcaca gtgaagaaccctgtgggcgaggaccaggtcaacctcacagtcaaggtcatcgacgtg ccagacgcacctgcggcccccaagatcagcaacgtgggagaggactcctgcacagta cagtgggagccgcctgcctacgatggcgggcagcccatcctgggctacatcctggag cgcaagaagaagaagagctaccggtggatgcggctgaacttcgacctgattcaggag ctgagtcatgaagcgcggcgcatgatcgagggcgtggtgtacgagatgcgcgtctac gcggtcaacgccatcggcatgtccaggcccagccctgcctcccagcccttcatgcct atcggtccccccagcgaacccacccacctggcagtagaggacgtctctgacaccacg gtctccctcaagtggcggcccccagagcgcgtgggagcaggaggcctggatggctac agcgtggagtactgcccagagggctgctcagagtgggtggctgccctgcaggggctg acagagcacacatcgatactggtgaaggacctgcccacgggggcccggctgcttttc cgagtgcgggcacacaatatggcagggcctggagcccctgttaccaccacggagccg gtgacagtgcaggagatcctgcaacggccacggcttcagctgcccaggcacctgcgc cagaccattcagaagaaggtcggggagcctgtgaaccttctcatccctttccagggc aagccccggcctcaggtgacctggaccaaagaggggcagcccctggcaggcgaggag gtgagcatccgcaacagccccacagacaccatcctgttcatccgggccgctcgccgc gtgcattcaggcacttaccaggtgacggtgcgcattgagaacatggaggacaaggcc acgctggtgctgcaggttgttgacaagccaagtcctccccaggatctccgggtgact gacgcctggggtcttaatgtggctctggagtggaagccaccccaggatgtcggcaac acggaactctgggggtacacagtgcagaaagccgacaagaagaccatggagtggttc accgtcttggagcattaccgccgcacccactgcgtggtgccagagctcatcattggc aatggctactacttccgcgtcttcagccagaatatggttggctttagtgacagagcg gccaccaccaaggagcccgtctttatccccagaccaggcatcacctatgagccaccc aactataaggccctggacttctccgaggccccaagcttcacccagcccctggtgaac cgctcggtcatcgcgggctacactgctatgctctgctgtgctgtccggggtagcccc aagcccaagatttcctggttcaagaatggcctggacctgggagaagacgcccgcttc	

	cgcatgttcagcaagcagggagtgttgactctggagattagaaagccctgccccttt gacgggggcatctatgtctgcagggccaccaacttacagggcgaggcacggtgtgag tgccgcctggaggtgcgagtgcctcagtaaagcttataaaagatctttattttcat tagatctgtgtgttggttttttgtgtgctggggactcgagttaagggcgaattcccg ataaggatcttcctagagcatggctacgtagataagtagcatggcgggttaatcatt aactaca	
5′ segment – partial viral genome (400 bp promoter)	Tgtagttaatgattaacccgccatgctacttatctacgtagccatgctctaggaaga tcggaattcgcccttaagttgccttctgccccccaaccctgctcccagctggccctc ccaggcctgggttgctggcctctgctttatcaggattctcaagagggacagctggtt tatgttgcatgactgttccctgcatatctgctctggttttaaatagcttatctgagc agctggaggaccacatgggcttatatggcgtggggtacatgttcctgtagccttgtc cctggcacctgccaaaatagcagccaacacccccccacccccaccgccatcccctgc cccacccgtcccctgtcgcacattcctccctccgcagggctggctcaccaggcccca gcccacatgcctgcttaaagccctctccatcctctgcctcacccagtccccgctgag actgagcagacgcctccagccacc	93
3′ segment – partial viral genome	Agcttaataaaagatctttattttcattagatctgtgtgttggtttttgtgtgctg ggactcgagttaagggcgaattcccgataaggatcttcctagagcatggctacgta gataagtagcatggcgggttaatcattaactaca	94

[0182] In some aspects of the disclosure, an rAAV virion is used to deliver the expression cassettes described herein to cardiac cells of a subject, *e.g.* to treat cardiomyopathy. Accordingly, the disclosure provides an rAAV virion, the rAAV virion comprising an AAV capsid and an expression cassette comprising a polynucleotide encoding a transgene operatively linked to a promoter.

[0183] The rAAV virions of the disclosure comprise a capsid protein. Capsid proteins are structural proteins that make up the assembled icosahedral packaging of the rAAV virion that contains the expression cassette. Capsid proteins are classified by the serotype. Wild type capsid serotypes in rAAV virions can be, for example, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, or AAV12 (Naso et al. *BioDrugs* 31:317–334 (2017)). Engineered capsid types include chimeric capsids and mosaic capsids (Choi et al. *Curr Gene Ther.* 5: 299–310 (2005)). Capsids are selected for rAAV virions based on their ability to transduce specific tissue or cell types (Liu et al. *Curr Pharm Des.* 21:3248-56 (2015)).

[0184] Any capsid protein that can facilitate rAAV virion transduction into cardiac cells for delivery of a transgene, as described herein, can be used. Capsid proteins used in rAAV virions for transgene delivery to cardiac cells that result in high expression can be, for example, AAV4, AAV6, AAV7, AAV8, and AAV9 (Zincarelli et al. *Mol. Ther.* 16:P1073-1080 (2008)). Artificial

capsids, such as chimeric capsids generated through combinatorial libraries, can also be used for transgene delivery to cardiac cells that results in high expression (see US 63/012,703, the contents of which are herein incorporated by reference). Other capsid proteins with various features can also be used in the rAAV virions of the disclosure. AAV vectors and capsids are provided in U.S. Pat. Pub. Nos. US10011640B2; US7892809B2, US8632764B2, US8889641B2, US9475845B2, US10889833B2, US10480011B2, and US10894949B2, the contents of which are herein incorporated by reference; and Int'l Pat. Pub. Nos. WO2020198737A1, WO2019028306A2, WO2016054554A1, WO2018152333A1, WO2017106236A1, WO2008124724A1, WO2017212019A1, WO2020117898A1, WO2017192750A1, WO2020191300A1, and WO2017100671A1, the contents of which are herein incorporated by reference.

[0185] In some embodiments, the rAAV virions of the disclosure comprise an engineered capsid protein. Engineered capsid proteins can be derived from a parental, *e.g.* wild type, capsid and include, for example, variant polypeptide sequence with respect to a parental capsid sequence at one or more sites. For example, variant sites of the parental capsid can occur at the VR-IV site, VR-V site, VR-VII site and/or VR-VIII site (see, *e.g.* Büning and Srivastava. *Mol Ther Methods Clin Dev*. 12:248-265 (2019)).

[0186] In some embodiments, the capsid protein is an AAV5/AAV9 chimeric capsid protein. In some embodiments, the chimeric capsid protein comprises at least 1, 2, 3, 4, 5 or more polypeptide segments that are derived from AAV5 capsid protein (SEQ ID NO. 144). In some embodiments, the chimeric capsid protein comprises at least 1, 2, 3, 4, 5 or more polypeptide segments that are derived from AAV9 capsid protein (SEQ ID NO: 143). In some embodiments, at least one polypeptide segment is derived from the AAV5 capsid protein and at least one polypeptide segment is derived from the AAV9 capsid protein.

[0187] In some embodiments, the capsid protein is a combinatory capsid proteins. As used herein, "combinatory capsid protein" refers to a AAV5/AAV9 chimeric capsid protein, which further comprises amino acid variations with respect to the chimeric parental sequence at one or more sites. In some embodiments, the one or more sites of the chimeric parental sequence are selected from those equivalent to the VR-IV site, the VR-V site, the VR-VII site and the VR-VIII site of the AAV9 capsid protein.

[0188] In some embodiments, the rAAV virions comprise an engineered capsid protein selected from Table 6.

Table 6. Engineered Capsid Proteins

Engineered Capsid	SEQ ID NO:
CR9-01	145
CR9-07	146
CR9-08	147
CR9-09	148
CR9-10	149
CR9-11	150
CR9-13	151
CR9-14	152
CR9-15	153
CR9-16	154
CR9-17	155
CR9-20	156
CR9-21	157
CR9-22	158
ZC23	159
ZC24	160
ZC25	161
ZC26	162
ZC27	163
ZC28	164
ZC29	165
ZC30	166
ZC31	167
ZC32	168
ZC33	169
ZC34	170
ZC35	171

Engineered Capsid	SEQ ID NO:
ZC40	172
ZC41	173
ZC42	174
ZC43	175
ZC44	176
ZC45	177
ZC46	178
ZC47	179
ZC48	180
ZC49	181
ZC50	182
TN47-07	183
TN47-10	184
TN47-13	185
TN47-14	186
TN47-17	187
TN47-22	188
TN40-07	189
TN40-10	190
TN40-13	191
TN40-14	192
TN40-17	193
TN40-22	194
TN44-07	195
TN44-10	196
TN44-13	197
TN44-14	198

Engineered Capsid	SEQ ID NO:
TN44-17	199

Engineered Capsid	SEQ ID NO:
TN44-22	200

[0189] In some embodiments, the rAAV is replication defective, in that the rAAV virion cannot independently further replicate and package its genome. For example, when a cardiac cell is targeted with rAAV virions, the transgene is expressed in the targeted cardiac cell, however, due to the fact that the targeted cardiac cell lacks AAV rep and cap genes and accessory function genes, the rAAV is not able to replicate.

[0190] In some embodiments, rAAV virions of the present disclosure encapsulating the expression cassettes as described herein, can be produced using helper-free production. rAAVs are replication-deficient viruses and normally require components from a live helper virus, such as adenovirus, in a host cell for packaging of infectious rAAV virions. rAAV helper-free production systems allow the production of infectious rAAV virions without the use of a live helper virus. In the helper-free system, a host packaging cell line is co-transfected with three plasmids. A first plasmid may contain adenovirus gene products (*e.g.* E2A, E4, and VA RNA genes) needed for the packaging of rAAV virions. A second plasmid may contain required AAV genes (*e.g.*, REP and CAP genes). A third plasmid contains the polynucleotide sequence encoding the transgene of interest and a promoter flanked by ITRs. A host packaging cell line can be, for example, AAV-293 host cells. Suitable host cells contain additional components required for packaging infectious rAAV virions that are not supplied by the plasmids. In some embodiments, the CAP genes can encode, for example, AAV capsid proteins as described herein.

IV. Methods of Treatment

[0191] The present disclosure also provides pharmaceutical compositions comprising the rAAV vector genomes or rAAV virions disclosed herein and one or more pharmaceutically acceptable carriers, diluents or excipients. In particular embodiments, the pharmaceutical composition comprises an rAAV vector genome or rAAV virion as described herein, comprising a polynucleotide sequence that encodes a therapeutic protein or nucleic acid, operatively linked to a cardiac-specific promoter (*e.g.*, a modified TNNT2 promoter). For example, in some embodiments, the pharmaceutical composition is an AAV9 vector comprising the modified cardiac

TNNT2 promoter (SEQ ID NO: 3) operatively linked to the MYBPC3 protein (SEQ ID NO: 86). Provided are pharmaceutical compositions, *e.g.*, for use in preventing or treating cardiomyopathy, which comprises a therapeutically effective amount of a vector that comprises a polynucleotide sequence encoding a therapeutic protein or nucleic acid that can restore contractile function in the heart.

[0192] The disclosure provides methods for expressing a polynucleotide a cell. The method may comprise, for example, transducing a target cell with the rAAV virions, rAAV vector genomes, or expression cassettes described herein. A target cell can be, for example and without limitation, a cardiac cell, a muscle cell, an induced pluripotent stem cell-derived cardiomyocyte (iPSC-CM), a cardiomyocyte, or a *MYBPC3*$^{-/-}$ iPSC-CM. In one aspect, a method of expressing a MYBPC3 protein in a cell comprises transducing a target cell or population of target cells with an rAAV virion or rAAV vector genomes described herein. In one embodiment, the cell is a *MYBPC3*$^{-/-}$ cell. In one embodiment, the cell comprises an inactivating mutation in one or both copies of the endogenous *MYBPC3* gene.

[0193] The compositions that are described herein can be employed in a method of treating a subject with a cardiac disease or condition. "Treating" or "treatment of a condition or subject in need thereof" refers to (1) taking steps to obtain beneficial or desired results, including clinical results such as the reduction of symptoms; (2) preventing the disease, for example, causing the clinical symptoms of the disease not to develop in a patient that may be predisposed to the disease, but does not yet experience or display symptoms of the disease; (3) inhibiting the disease, for example, arresting or reducing the development of the disease or its clinical symptoms; (4) relieving the disease, for example, causing regression of the disease or its clinical symptoms; or (5) delaying the disease. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, promoting cardiac sarcomere contraction.

[0194] Subjects in need of treatment using the compositions and methods of the present disclosure include, but are not limited to, individuals having a congenital heart defect, individuals suffering from a degenerative muscle disease, individuals suffering from a condition that results in ischemic heart tissue (*e.g.*, individuals with coronary artery disease), and the like. In some examples, a method is useful to treat a degenerative muscle disease or condition (*e.g.*, familial cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, or coronary artery disease with resultant ischemic cardiomyopathy). In some examples, a subject method is

useful to treat individuals having a cardiac or cardiovascular disease or disorder, for example, cardiovascular disease, aneurysm, angina, arrhythmia, atherosclerosis, cerebrovascular accident (stroke), cerebrovascular disease, congenital heart disease, congestive heart failure, myocarditis, valve disease coronary, artery disease dilated, diastolic dysfunction, endocarditis, high blood pressure (hypertension), cardiomyopathy, hypertrophic cardiomyopathy, restrictive cardiomyopathy, coronary artery disease with resultant ischemic cardiomyopathy, mitral valve prolapse, myocardial infarction (heart attack), or venous thromboembolism. In some examples, the subject is suffering from or at risk for cardiomyopathy.

[0195] In some embodiments, the compositions and methods disclosed herein can be used for the prevention and/or treatment of cardiomyopathies in a subject. In some embodiments, the compositions and methods described herein can be used to treat cardiomyopathies affiliated with mutations in cardiac myosin binding protein C (MYBPC3), such as hypertrophic cardiomyopathy and familial hypertrophic cardiomyopathy. The cardiomyopathy treated by the compositions and methods described herein can also include cardiomyopathies associated with a pulmonary embolus, a venous thrombosis, a myocardial infarction, a transient ischemic attack, a peripheral vascular disorder, atherosclerosis, ischemic cardiac disease and/or other myocardial injury or vascular disease. In certain embodiments, the cardiomyopathies treated by the compositions and methods described herein can include cardiac diseases associated with myocardial tissue hypercontractility, such as heart failure related to left ventricular hypercontractility.

[0196] In some embodiments, the compositions and methods described herein can induce detectable expression of a therapeutic protein or nucleic acid (*e.g.*, MYBPC3 protein), or a mutant, variant, or fragment thereof, to modulate contractile function of the myocardial tissue in a subject in need thereof. In some embodiments, the amount, concentration, and volume of the composition that modulates contractile function in myocardial tissue administered to a subject can be controlled and/or optimized to substantially improve the functional parameters of the heart while mitigating adverse side effects.

[0197] The amount of the composition that modulates contractile function administered to myocardial tissue can also be an amount required to result in the detectable expression of a therapeutic protein or nucleic acid (*e.g.*, MYBPC3 protein) or a mutant, variant, or fragment thereof in the heart; preserve and/or improve contractile function; delay the emergence of cardiomyopathy or reverse the pathological course of the disease; increase myocyte viability;

improve myofilament function; inhibit left ventricular hypertrophy; cardiac hypertrophy regression, normalize systolic and diastolic function in heart; and restore normal cross-bridge behavior at the myofilament level.

[0198] In some embodiments, the compositions and methods disclosed herein results in detectable expression of MYBPC3 protein, or a mutant, variant, or fragment thereof, in a cardiac cell of the subject being treated. In some embodiments, administration of an rAAV vector genome or rAAV virion described herein causes specific expression of MYBPC3 protein in the heart of the subject. In some embodiments, administration of rAAV vector genome or rAAV virion described herein causes low or undetectable expression of MYBPC3 in the skeletal tissue, brain, and/or liver of the subject, wherein optionally the subject suffers from or is at risk for cardiomyopathy.

[0199] In some embodiments, the compositions and methods disclosed herein results in detectable expression of KCNH2 protein, or a mutant, variant, or fragment thereof, in a cardiac cell of the subject being treated.

[0200] In some embodiments, the compositions and methods disclosed herein results in detectable expression of TRPM4 protein, or a mutant, variant, or fragment thereof, in a cardiac cell of the subject being treated.

[0201] In some embodiments, the compositions and methods disclosed herein results in detectable expression of DSG2 protein, or a mutant, variant, or fragment thereof, in a cardiac cell of the subject being treated.

[0202] In some embodiments, the compositions and methods disclosed herein results in detectable expression of ATP2A2 protein, or a mutant, variant, or fragment thereof, in a cardiac cell of the subject being treated.

[0203] In some embodiments, the compositions and methods disclosed herein results in detectable expression of CACNA1C, DMD, DMPK, EPG5, EVC, EVC2, FBN1, NF1, SCN5A, SOS1, NPR1, ERBB4, VIP, or MYH7, or a mutant, variant, or fragment thereof, in a cardiac cell of the subject being treated.

[0204] "Detectable expression" typically refers to expression at least 5%, 10%, 15%, 20% or more compared to a control subject or tissue not treated with the vector. In some embodiments, detectable expression means expression at 1.5-fold, 2-fold, 2.5-fold, or 3-fold greater than a no-vector control. Expression can be assess by Western blot, as described in the example that follows, or enzyme-linked immunosorbent assay (ELISA), or other methods known in the art. In some

cases, expression is measured quantitatively using a standard curve. Standard curves can be generated using purified protein, *e.g.* purified MYBPC3 protein, by methods described in the examples or known in the art. Alternatively, expression of the therapeutic gene product can be assessed by quantification of the corresponding mRNA.

[0205] In some embodiments, the detectable expression of the therapeutic gene product in heart tissue occurs at doses, in vector genomes (vg) per kilogram weight of subject (kg), of 3×10^{14} vg/kg or less, 2×10^{14} vg/kg or less, 1×10^{14} vg/kg or less, 9×10^{13} vg/kg or less, 8×10^{13} vg/kg or less, 7×10^{13} vg/kg or less, 6×10^{13} vg/kg or less, 5×10^{13} vg/kg or less, 4×10^{13} vg/kg or less, 3×10^{13} vg/kg or less, 2×10^{13} vg/kg or less, or 1×10^{13} vg/kg or less.

[0206] In various embodiments, the compositions described herein contain the rAAV virions or vector genomes described herein and one or more pharmaceutically acceptable excipients. Pharmaceutically acceptable excipients can include vehicles (*e.g.*, carriers, diluents and excipients) that are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions. Illustrative pharmaceutical forms suitable for injectable use include, *e.g.*, sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions.

[0207] In various embodiments, the pharmaceutical compositions of the disclosure comprise about 1×10^{8} genome copies per milliliter (GC/mL), about 5×10^{8} GC/mL, about 1×10^{9} GC/mL, about 5×10^{9} GC/mL, about 1×10^{10} GC/mL, about 5×10^{10} GC/mL, about 1×10^{11} GC/mL, about 5×10^{11} GC/mL, about 1×10^{12} GC/mL, about 5×10^{12} GC/mL, about 5×10^{13} GC/mL, about 1×10^{14} GC/mL, or about 5×10^{14} GC/mL of the viral vector (*e.g.* rAAV virion).

[0208] In various embodiments, the pharmaceutical compositions of the disclosure comprise about 1×10^{8} viral genomes per milliliter (vg/mL), about 5×10^{8} vg/mL, about 1×10^{9} vg/mL, about 5×10^{9} vg/mL, about 1×10^{10} vg/mL, about 5×10^{10} vg/mL, about 1×10^{11} vg/mL, about 5×10^{11} vg/mL, about 1×10^{12} vg/mL, about 5×10^{12} vg/mL, about 5×10^{13} vg/mL, about 1×10^{14} vg/mL, or about 5×10^{14} vg/mL of the viral vector (*e.g.* rAAV virion).

[0209] In some embodiments, the pharmaceutical compositions of the disclosure are administered in a total volume of about 1 mL, 5 mL, 10 mL, about 20 mL, about 25mL, about 30 mL, about 35 mL, about 40 mL, about 45 mL, about 50 mL, about 55 mL, about 60 mL, 65 mL, about 70 mL, about 75 mL, about 80 mL, about 85 mL, about 90 mL, about 95 mL, about 100 mL, about 105 mL, about 110 mL, about 115 mL, about 120 mL, about 125 mL, about 130 mL, about 135 mL, about 140 mL, about 145 mL, about 150 mL, about 155 mL, about 160 mL, about 165 mL, about 170 mL, about 175 mL, about 180 mL, about 185 mL, about 190 mL, about 200 mL, about 205 mL, about 210 mL, about 215 mL, or about 220 mL.

[0210] In some embodiments, the methods of the disclosure comprise administering an rAAV virion encoding MYBPC3 at a dose of about 1×10^{8} genome copies per milliliter (GC/mL), about 5×10^{8} GC/mL, about 1×10^{9} GC/mL, about 5×10^{9} GC/mL, about 1×10^{10} GC/mL, about 5×10^{10} GC/mL, about 1×10^{11} GC/mL, about 5×10^{11} GC/mL, about 1×10^{12} GC/mL, about 5×10^{12} GC/mL, about 5×10^{13} GC/mL, about 1×10^{14} GC/mL, or about 5×10^{14} GC/mL of the rAAV virion.

[0211] In preferred embodiments, the methods of the disclosure comprise intravenously administering an rAAV virion encoding MYBPC3 at a dose of about 3×10^{12} GC/mL, about 3×10^{13} GC/mL, about 1×10^{14} GC/mL, or about 3×10^{14} GC/mL of the rAAV virion.

[0212] In preferred embodiments, the methods of the disclosure comprise administering, by localized delivery to the heart, an rAAV virion encoding MYBPC3 at a dose of about 3×10^{11} GC/mL, about 3×10^{12} GC/mL, about 1×10^{13} GC/mL, or about 3×10^{13} GC/mL of the rAAV virion.

[0213] In some embodiments, the methods of the disclosure comprise administering an rAAV virion encoding MYBPC3 at a dose of about 1×10^{8} viral genomes per milliliter (vg/mL), about 5×10^{8} vg/mL, about 1×10^{9} vg/mL, about 5×10^{9} vg/mL, about 1×10^{10} vg/mL, about 5×10^{10} vg/mL, about 1×10^{11} vg/mL, about 5×10^{11} vg/mL, about 1×10^{12} vg/mL, about 5×10^{12} vg/mL, about 5×10^{13} vg/mL, about 1×10^{14} vg/mL, or about 5×10^{14} vg/mL of the rAAV virion.

[0214] In preferred embodiments, the methods of the disclosure comprise intravenously administering an rAAV virion encoding MYBPC3 at a dose of about 3×10^{12} vg/mL, about 3×10^{13} vg/mL, about 1×10^{14} vg/mL, or about 3×10^{14} vg/mL of the rAAV virion.

[0215] In preferred embodiments, the methods of the disclosure comprise administering, by localized delivery to the heart, an rAAV virion encoding MYBPC3 at a dose of about 3×10^{11} vg/mL, about 3×10^{12} vg/mL, about 1×10^{13} vg/mL, or about 3×10^{13} vg/mL of the rAAV virion.

[0216] Genome copies per milliliter can be determined by quantitative polymerase change reaction (qPCR) using a standard curve generated with a reference sample having a known concentration of the polynucleotide genome of the virus. For AAV, the reference sample used is often the transfer plasmid used in generation of the rAAV virion but other reference samples may be used.

[0217] Alternatively or in addition, the concentration of a viral vector can be determined by measuring the titer of the vector on a cell line. Viral titer is typically expressed as viral particles (vp) per unit volume (*e.g.*, vp/mL). In various embodiments, the pharmaceutical compositions of the disclosure comprise about 1×10^8 viral particles per milliliter (vp/mL), about 5×10^8 vp/mL, about 1×10^9 vp/mL, about 5×10^9 vp/mL, about 1×10^{10} vp/mL, about 5×10^{10} vp/mL, about 1×10^{11} vp/mL, about 5×10^{11} vp/mL, about 1×10^{12} vp/mL, about 5×10^{12} vp/mL, about 5×10^{13} vp/mL, or about 1×10^{14} vp/mL, or about 5×10^{14} of the viral vector (*e.g.*, rAAV virion).

[0218] In one embodiment, the present disclosure provides a kit comprising a container housing a pharmaceutical composition as described herein.

[0219] The rAAV virions or vector genomes of the present disclosure can be administered to a subject in need thereof by systemic application, *e.g.*, by intravenous, intra-arterial or intraperitoneal delivery of a vector in analogy to what has been shown in animal models (Katz et al., 2012, *Gene Ther*. 19:659-669. In some embodiments, the rAAV virions or vector genomes of the present disclosure treat or prevent hypertrophic cardiomyopathy, wherein the vector is administered systemically.

[0220] In some embodiments, the rAAV virions or vector genomes of the present disclosure can be delivered by direct administration to the heart tissue, *e.g.* by intracoronary administration. In some embodiments, the vectors are administered as a single dose by antegrade epicardial coronary artery infusion over a 10-minute period in a cardiac catheterization laboratory after angiography (percutaneous intracoronary delivery without vessel balloon occlusion) with the use of standard 5F or 6F guide or diagnostic catheters (Jaski et al., 2009, *J Card Fail*. 15: 171-181).

[0221] Subjects who are suitable for treatment using the compositions, compositions and methods of the present disclosure include individuals (*e.g.*, mammalian subjects, such as humans, non-human primates, domestic mammals, experimental non-human mammalian subjects such as mice, rats, etc.) having a cardiac condition.

[0222] In some embodiments, the rAAV virions or vector genomes of the present disclosure can be used to treat a subject in need thereof. In some embodiments, the viral vector can be

administered to the subject in need to treat a cardiovascular disease. In some embodiments, the rAAV virions or vector genomes are administered to a subject to treat cardiomyopathy. In some embodiments, the viral vector is administered systemically. In other embodiments, the viral vector is delivered by direct administration to the heart tissue.

[0223] rAAV virions or vector genomes can be administered by various routes, including without limitation direct injection into the heart or cardiac catheterization. In a preferred embodiment, a pharmaceutical composition comprising an rAAV virion encoding MYBPC3 is administered by intracardiac catheter delivery via retrograde coronary sinus infusion (RCSI). Alternatively, the viral vector can be administered systemically such as by intravenous infusion. When direct injection is used, it may be performed either by open-heart surgery or by minimally invasive surgery. In some cases, the viral vector is delivered to the pericardial space by injection or infusion.

[0224] The viral vector administered to the subject can be traced by a variety of methods. For example, recombinant viruses labeled with or expressing a marker (such as green fluorescent protein, or beta-galactosidase) can readily be detected. The recombinant viruses may be engineered to cause the target cell to express a marker protein, such as a surface-expressed protein or a fluorescent protein. Alternatively, the infection of target cells with recombinant viruses can be detected by their expression of a cell marker that is not expressed by the animal employed for testing (for example, a human-specific antigen when injecting cells into an experimental animal). The presence and phenotype of the target cells can be assessed by fluorescence microscopy (*e.g.*, for green fluorescent protein, or beta-galactosidase), by immunohistochemistry (*e.g.*, using an antibody against a human antigen), by ELISA (using an antibody against a human antigen), or by RT-PCR analysis using primers and hybridization conditions that cause amplification to be specific for RNA indicative of a cardiac phenotype.

[0225] All patents, patent publications, and other publications referenced and identified in the present specification are individually and expressly incorporated herein by reference in their entirety for all purposes.

EXAMPLES

Example 1: Design of Vector Genome for Large Cargos

[0226] The purpose of this study was to evaluate a vector having deletions in non-coding portions of the vector to a parental vector. It demonstrates that, surprisingly, deletion in non-coding regions increases potency of the vector.

[0227] With two intact flanking ITR sequences (each ~130 bp), promoter, intron, WPRE and polyadenylation signal, and standard *cis*-regulatory sequences for optimal transgene expression, typical AAV vector genomes require about 1.8-2.0 kbp of non-coding DNA sequence. Trangenes of about 3.0 kbp or greater, like the 3.8 kbp transgene MYBPC3, cause the vector genome to exceed 5.0 kbp. For example, Mearini et al., *Nat Commun* 5:5515 (2014) reports an AAV vector encoding MYBPC3 having a vector genome size of 5.4 kbp. Without ITR sequences, this is about 5.2 kbp.

[0228] A reporter system was generated to test whether an AAV vector would tolerate shortened non-coding regions. A conventional AAV vector having a CAG promoter, intron, WPRE, and standard polyA sequence was modified to remove the WPRE (589 bp) and to shorten the polyA sequence (removing 170 bp) **(FIG. 1A**). Expression of a GFP reporter cloned into the multiple cloning site (MCS) was maintained but slightly decreased when these vector elements were deleted or shortened (**FIG. 1B**). The vector was further truncated by deletion of the intron and a portion of the sequence 3′ to the 5′ ITR (**FIG. 1C**). The final vector genome was about 1.1 kpb (0.8 kpb without ITRs).

Example 2: MYBPC3 transgene expression in induced cardiomyocytes in vitro

[0229] The purpose of this study was to provide an improved tissue-specific promoter for expression of a therapeutic gene product in induced cardiomyocytes *in vitro* using an AAV-based vector system. Human induced pluripotent stem cells (iPSCs) were differentiated into cardiomyocytes using mesoderm induction, cardiac specification and metabolic selection as previously described (Tohyama et al. *Cell Stem Cell.* 2013;12(1):127-37; Lian et al. *Proc Natl Acad Sci U S A.* 2012;109(27):E1848-57; Burridge PW, Holmstrom A, Wu JC. *Curr Protoc Hum Genet.* 2015;87:21 3 1-15.) iPSC-CM viral transductions were performed with AAV6 at the indicated multiplicities of infection.

[0230] The gene expression cassettes depicted in **FIG. 2A** were constructed for an AAV-based vector system for the treatment of cardiomyopathy. The AAV vector, based on the vector depicted in **FIG. 1C**, comprised several *cis*-regulatory elements, including two inverted terminal repeats (ITRs, 260 bp each), a polyadenylation signal (A, 49 bp), and a full-length (SEQ ID NO: 1) or modified cardiac troponin T (TNNT2) promoter (SEQ ID NO: 2-4). No WPRE was included, and the polyA signal and the sequence 3′ to the 5′ ITR were both shortened. The modified TNNT2 promoters contained 100-200 bp deletions at the 5´ (upstream) end of the wild-type TNNT2 promoter. Human myosin binding protein C (MYBPC3, SEQ ID NO: 86) with a polynucleotide sequence length of 3.825 kb was tested as the therapeutic gene product in iPSC-derived cardiomyocytes.

[0231] To determine whether wild-type or modified TNNT2 promoters could induce detectable expression of the MYBPC3 protein, *MYBPC3*$^{-/-}$ iPSC-derived cardiomyocytes were transduced with AAV6 particles at 6×10^4 MOI. Cells were analyzed for MYBPC3 protein expression by immunofluorescence or Western blot 5-15 days post-infection.

[0232] FIG. 2B shows that the AAV vector comprising the wild-type TNNT2 promoter (SEQ ID NO: 1) drives expression of MYBPC3 protein in the sarcomeres of *MYBPC3*$^{-/-}$ iPSC-derived cardiomyocytes.

[0233] FIG. 3A – FIG. 3C show that the AAV vector comprising the 400 bp modified TNNT2 promoter (SEQ ID NO: 3) drives higher MYBPC3 protein expression than either the 600 bp wild-type TNNT2 (SEQ ID NO: 1) or 500 bp modified TNNT2 (SEQ ID NO: 2) promoters in transduced *MYBPC3*$^{-/-}$ iPSC-derived cardiomyocytes. In contrast, *MYBPC3*$^{-/-}$ iPSC-derived cardiomyocytes transfected with a plasmid (rather than transduced with virus) encoding MYBPC3 under the control of either the 600 bp wild-type TNNT2 (SEQ ID NO:1) or 400 bp modified TNNT2 promoter (SEQ ID NO: 3) showed similar MYBPC3 protein expression.

Example 3: *Mybpc3*$^{-/-}$ mice model hypertrophic severe cardiomyopathy

[0234] Homozygous *Mybpc3* knockout mice (KO) were generated on a C57Bl/6 background by a CRISPR-Cas9 paired gRNA deletion of exons one and two (**FIG. 4A**). KO mice exhibited severe deficits in cardiac function (**FIG. 4C** and **FIG. 4D**) and pronounced cardiac hypertrophy (**FIGs. 4E-4G**) at two weeks of age, despite normal Mendelian ratios and comparable body weight to wild-type littermates (**FIG. 4B**). This model has more severe cardiac hypertrophy than other models (Schlossarek et al. *Basic Res. Cardiol.* 107:1-13 (2012)). Our KO mice exhibit severe,

early-onset HCM in juveniles (two-week-old mice) that models pediatric onset of HCM in humans, as well as late-stage HCM in adults. *See* Lekanne Deprez et al., *J Med Genet* 43:829-832 (2006); Xin et al., *Am J Med Genet Part A* 143A:2662-2667 (2007); Zahka et al., *Heart* 94:1326-1330 (2008); Marziliano et al., *Neonatology* 102:254-258 (2012); Wessels et al., *Eur J Hum Genet* 23:922-928 (2015).

Example 4: MYBPC3 transgene expression in heart tissue *in vivo*

[0235] The purpose of this study was to examine therapeutic protein expression *in vivo* using an AAV-based vector system comprising a modified cardiac-specific promoter.

[0236] Adult mice were retro-orbitally injected with AAV9 recombinant virus comprising either the 600 bp wild-type TNNT2 (SEQ ID NO: 1) or 400 bp modified TNNT2 (SEQ ID NO: 3) promoter operatively linked to a polynucleotide that encodes *MYBPC3*. Tissue samples from heart, skeletal muscle (tibialis anterior), liver and whole brain were harvested 2 weeks post-infection. RNA was extracted from all tissues, synthesized to cDNA and analyzed by qRT-PCR using primers specific to human *MYBPC3*.

[0237] As shown in **FIG. 5**, mice injected with the AAV9-based vector comprising either the wild-type or modified TNNT2 promoter showed high levels of *MYBPC3* mRNA in heart tissue compared to skeletal, brain or liver tissue. The 400 bp modified TNNT2 promoter showed increased expression of *MYBPC3* mRNA in heart tissue compared to the 600 bp wild-type TNNT2 promoter.

[0238] As shown in FIGs. 6A-6B, adult mice were intravenously dosed via tail vein injection with an AAV9 vector with the 400 bp modified TNNT2 promoter cassette. Tissue samples from heart and liver were harvested 4 weeks post-injection. Absolute quantification of viral genomes per microgram of genomic DNA was assessed by qPCR using linearized standards. RNA was extracted from all tissues, synthesized to cDNA and analyzed by qRT-PCR using primers specific to human *MYBPC3*. Surprisingly, the 400 bp TNNT2 promoter retains high selectivity for the heart: despite the 100-fold greater vector genomes detected in the liver than the heart 4 weeks post-injection in the adult-dosed animals (**FIG. 6A**, logarithmic scale), liver expression of the transgene was less than 1/10,000th of cardiac expression (**FIG. 6B**, logarithmic scale).

[0239] Collectively, these results indicate that a 200 bp deletion from the wild-type TNNT2 promoter, *i.e.*, the 400 bp modified TNNT2 promoter (SEQ ID NO: 3), effectively drives

expression of MYBPC3 protein in cardiomyocytes with high selectivity despite deletion of a substantial portion of the promoter sequence.

Example 5: Rescue of Cardiac Function in *Mybpc3* null Mice

[0240] This example demonstrates functional rescue of loss of function in the *Mybpc3* in mice, using the vector designed for large cargoes described in Example 1 and the 400 bp modified hTNNT2 promoter described in Examples 2 and 3.

[0241] The 400 bp hTNNT2 promoter and murine *Mybpc3* gene were cloned into the vector shown in **FIG. 1C**. This vector was packaged using an AAV9 capsid to generate the test vector.

[0242] In a first experiment, homozygous *Mybpc3*$^{-/-}$ mice were injected retro-orbitally at two weeks of age with 1E14 vg·kg^{-1} test vector encoding *Mybpc3* or vehicle, HBSS. Cardiac tissue was harvested two weeks later (at four weeks), along with that of wild-type littermates. The experimental vector achieved wild-type levels of MYBPC3 protein expression in *Mybpc3*$^{-/-}$ mice at two weeks post-injection (**FIG. 7**). We conclude the test vector was capable of expressing MYBPC3 at physiological levels in juvenile animals at a dose as low as 1E14 vg·kg^{-1}

[0243] In a second experiment, the first experiment was repeated at a lower dose, 3E13 vg·kg^{-1}. Homozygous *Mybpc3*$^{-/-}$ mice were injected retro-orbitally at two weeks of age with 3E13 vg·kg^{-1} and 1E14 vg·kg^{-1} test vector encoding *Mybpc3* or vehicle, HBSS. Wild-type levels of cardiac MYBPC3 protein expression were detected by ELISA in *Mybpc3*$^{-/-}$ mice at two and six weeks post-injection (**FIG. 8**) We conclude the test vector was capable of expressing MYBPC3 at physiological levels in juvenile animals at a dose as low as 3E13 vg·kg^{-1}

[0244] In a third experiment, cardiac function was assessed using assays relevant to hypertrophic cardiomyopathy. Hypertrophic cardiomyopathy presents physiologically as (1) an increase in heart size, measured as reported as the ratio of left ventricular mass/total body mass (LVM/BW) in mg·g^{-1}; and (2) as fractional shortening (FAS), measured by echocardiography.

[0245] Homozygous *Mybpc3*$^{-/-}$ mice were injected retro-orbitally at two weeks of age with 1E13 vg·kg^{-1}, 3E13 vg·kg^{-1}, and 1E14 vg·kg^{-1} of test vector encoding *Mybpc3* or vehicle, HBSS. Dose-dependent rescue of cardiac function was observed at all tested doses (1E13 vg·kg^{-1}, 3E13 vg·kg^{-1}, and 1E14 vg·kg^{-1}). LVM/BM was decreased from vehicle control at all tested doses six weeks post-injection (**FIG. 9A**). FAS (expressed as % percentage change in LV internal dimensions between systole and diastole, **FIG. 9B**) and ejection fraction (**FIG. 9C**) were increased from vehicle control at all tested doses six weeks post-injection. Even greater improvements in

LVM/BM (**FIG. 9D**), FAS (**FIG. 9E**), and EF (**FIG. 9F**) were observed 31 weeks following injection. Consistent with equal levels of MYBPC3 protein expression observed at 3E13 $vg \cdot kg^{-1}$ and 1E14 $vg \cdot kg^{-1}$ doses (*see* **FIG. 8**), animals treated with 3E13 $vg \cdot kg^{-1}$ or 1E14 $vg \cdot kg^{-1}$ exhibit similar improvements in hypertrophy, FAS or EF improvements. Even at only 1E13 $vg \cdot kg^{-1}$ dose, hypertrophy, FAS and EF are all improved compared to vehicle control.

[0246] We conclude the test vector was capable of rescue of cardiac function in juvenile animals at a dose as low as 1E13 $vg \cdot kg^{-1}$.

[0247] Rescue of function in symptomatic juvenile mice is, in the case of hypertrophic cardiomyopathy, more challenging than prevention of decline in function in infants, because hypertrophic cardiomyopathy is a progressive disorder. Older animals exhibit more severe disease than younger animals. To our knowledge, rescue of MYBPC3 loss of function in symptomatic juvenile animals has never been demonstrated before with AAV. Our model is also a complete loss of function caused by deletion of the *Mybpc3* gene, not a partial loss of function due to mutation.

[0248] We compared our results to those reported in Mearini et al., *Nat. Commun.* 5:5515 (2014), which used a 5.4 kb expression cassette encoding *Mybpc3* in mice having a single nucleotide polymorphism in the endogenous *Mybpc3* gene. Mearni et al. report prevention of high LVM/BW at two-weeks of age in mice injected as neonates (not symptomatic juveniles) with very high doses (1E12 vg and 3E12 vg, which corresponds to 7E14 $vg \cdot kg^{-1}$ and 2E15 $vg \cdot kg^{-1}$, based on an average neonate mass of 1.5 g) of an AAV9 vector encoding the same *Mybpc3* gene. Mearni et al. used a 550 bp hTNNT2 promoter, rather than the present 400 bp modified hTNNT2 promoter. The vector Mearini et al. does not significantly prevent FAS decline, even at 2E15 $vg \cdot kg^{-1}$. By contrast, the present vector demonstrates improvement in physiological parameters in juveniles animals (not only neonates) at doses at least as low as 1E13 $vg \cdot kg^{-1}$ to1E14 $vg \cdot kg^{-1}$.

[0249] The vector and promoter modifications dramatically and surprisingly increase potency of the vector.

Example 6: Direct comparison of 5.4 kbp cassette to 4.7 kbp cassette

[0250] This example directly compares a 5.4 kbp cassette encoding the *Mybpc3* gene to a 4.7 kbp cassette encoding the *Mybpc3* gene in mature (2.5 months of age) homozygous mice with advanced disease.

[0251] Homozygous *Mybpc3*$^{-/-}$ mice were injected retro-orbitally with 3E13 vg·kg^{-1} or 1E14 vg·kg^{-1} of AAV9 vector encoding *Mybpc3* in the context of the 5.4 kbp or 4.7 kbp cassettes (**FIG 10A**), or injected with vehicle control, HBSS. Even when dosed at this advanced stage of cardiac decline, the 4.7 kbp cassette significantly improved cardiac function based on ejection fraction (EF) (**FIG. 10B**), with clear restoration of function above pre-dose baseline (**FIG. 10C**), unlike animals treated with vehicle (Veh) or the 5.4 kbp cassette. Further, compared to the 5.4 kbp cassette, the 4.7 kbp cassette was also able to significantly decrease hypertrophy, as indicated by LVM/BM, eighteen weeks post-injection (**FIG. 10D**).

[0252] This example demonstrates functional rescue of loss of function in the *Mybpc3* in mice, using the vector backbone and promoter modifications described in Examples 1-3.

[0253] This example also demonstrates that, in a challenging model of disease – adult, homozygous *Mybpc3*$^{-/-}$ mice – a 5.4 kbp vector (SEQ ID NO: 201) at low dose fails to generate any physiological improvement; whereas a 4.7 kbp vector according to the present disclosure causes statistically significant improvement in physiological parameters related to cardiomyopathy at doses as low as 3E13 vg·kg^{-1}.

Example 7: Greater Efficacy with an Improved AAV Capsid encoding MYBPC3

[0254] This example demonstrates how the improved potency of the large cargo vector (Example 1) and modified promoter (Examples 2 and 3) based on rescue of *Mybpc3*$^{-/-}$ mice (Example 5) can be further improved by use of an engineered AAV capsid.

[0255] An AAV9 capsid variant, CR9-10 exhibited significantly higher cardiac transduction upon systemic delivery in adult mice than AAV9 with a GFP-encoding cassette as determined by ELISA ($p < 0.05$, One-way ANOVA; Dunnett's multiple comparison test) (**FIG. 11A**).

[0256] In a second experiment, the expression cassette encoding the murine *Mybpc3* gene was packaged into either AAV9 or CR9-10 and the potency of cardiac rescue in *Mybpc3*$^{-/-}$ mice compared. Homozygous *Mybpc3*$^{-/-}$ mice were injected retro-orbitally at two weeks of age with 1E13 vg·kg^{-1} and 3E13 vg·kg^{-1} of AAV9 vector, CR9-10 vector, or vehicle control HBSS. All test articles significantly improved cardiac function based on ejection fraction (EF) (**FIG. 11B**), with clear restoration of function above pre-dose baseline (ΔEF) (**FIG. 11C**). Consistent with improved cardiac transduction, CR9-10 resulted in greater EF improvement than AAV9.

Example 8: Non-human primate study of engineered AAV capsid variants

[0257] Biodistribution of AAV vectors having engineered capsids (described in U.S. Provisional Patent Appl. No. 63/012,703, which is incorporated herein in its entirety), were assessed in male cynomolgus macaques (Macaca fascicularis) following intravenous delivery.

[0258] AAV vector generated with fourteen different capsids, including AAV9, were pooled and injected into NHPs at a 1E13 vg·kg-1 dose (n=3). Viral DNA was extracted from left ventricle and liver one month after systemic delivery. Consistent with the murine results, CR9-10 exhibited increased cardiac transduction compared to AAV9 (**FIG. 12A**). Additionally, many variants decreased liver viral burden relative to AAV9 (**FIG. 12B**), improving the ratio of left ventricle transduction to liver infection (**FIG. 12C**).

Example 10: Rescue of Cardiac Function with the Human MYBPC3 Gene in Mybpc3 null Mice

[0259] This example demonstrates the ability of human *MYBPC3* gene to rescue *Mybpc3*$^{-/-}$ mice.

[0260] Homozygous *Mybpc3*$^{-/-}$ mice were injected retro-orbitally at two weeks of age with AAV9 encoding the mouse *Mybpc3* gene (mMybpc3) (at 1E14 vg·kg-1), AAV9 encoding the human *MYBPC3* gene (hMYBPC3) (at 1E14 vg·kg-1), or vehicle, HBSS. Cardiac size and function were monitored by echocardiography up to eight months post-injection. The results indicate that AAV9-mediated cardiac MYBPC3 replacement of either mMybpc3 and hMYBPC3 in *Mybpc3*$^{-/-}$ mice resulted in recovery of cardiac size and function. Ejection fraction (EF) was significantly improved by both the human and mouse orthologs of MYBPC3, with mMybpc3 yielding greater improvement in EF compared to hMYBPC3 (**FIG. 13A**). Importantly, hMYBPC3 was just as potent as mMybpc3 at reducing cardiac hypertrophy over time, as evidenced by left ventricular mass normalized to body weight (LVM/BW) (**FIG. 13B**). This was further validated by comparable decreases in left ventricular posterior wall thickness during diastole (LVPW;d) (**FIG. 13C**). Critically, all improvements exhibited robust stability out to 8 months post-injection.

[0261] In a second study, the efficacy of hMYBPC3 was assessed over a range of viral doses. Homozygous *Mybpc3*$^{-/-}$ mice were injected retro-orbitally at two weeks of age with 1E13 vg·kg-1, 1E14 vg·kg-1, and 3E14 vg·kg-1 of test vector encoding the human *MYBPC3* gene or vehicle, HBSS. Dose-dependent improvement of cardiac function was observed for all tested doses (1E13 vg·kg-1, 1E14 vg·kg-1, and 3E14 vg·kg-1) fourteen weeks post-injection, as indicated by EF (**FIG. 14A**), with significant improvement above pre-dose baseline for 1E14 vg·kg-1 and 3E14 vg·kg-1 treatments (**FIG. 14B**). Significant reduction in cardiac hypertrophy was also observed for 1E14

vg·kg-1 and 3E14 vg·kg-1 treatments, based on LVM/BW (**FIG. 14C**). Thus, we conclude the hMYBPC3 test vector was capable of preserving cardiac function in adult animals at a dose as low as 1E13 vg·kg-1.

Example 11: Treatment of Hypertrophic cardiomyopathy (HCM)

[0262] Cardiomyopathy is the number-one cause of sudden cardiac arrest in children under 18. Hypertrophic cardiomyopathy (HCM) affects 0.5 million Americans, potentially resulting in heart failure or sudden death. Loss-of-function mutations in *Myosin Binding Protein C3*, *MYBPC3*, are the most common genetic cause of HCM. The majority of *MYBPC3* mutations causative for HCM result in truncations, via nonsense, frameshift or splice-site mutations. The sarcomeric pathophysiology of the majority of HCM patients with *MYBPC3* mutations appears to be due to haploinsufficiency, as the total amount of MYBPC3 protein incorporated into sarcomeres falls significantly below normal. Decreased sarcomeric levels of MYBPC3 result in decreased myosin inhibition with more myosin heads engaged on the actin filament, resulting in hypercontractility.

[0263] The clearest path to the treatment of haploinsufficiency is the restoration of the insufficient gene product; in this case wild-type MYBPC3. Thus, we have successfully engineered an AAV vector (TN-201) with superior properties for selective restoration of MYBPC3 to cardiomyocytes upon systemic delivery. Critically, we have demonstrated for the first time with AAV the ability of both a mouse surrogate and TN-201 to reverse cardiac dysfunction and hypertrophy in a symptomatic murine model of disease.

[0264] Dose-ranging efficacy studies exhibited restoration of wild-type MYBPC3 protein levels and saturation of cardiac improvement at the clinically relevant dose of 3E13 vg/kg. Further, pilot safety studies in adult and infant mice injected with >10X an efficacious dose exhibited no clinical observations, no alterations in cardiac function, and no histopathological findings. Importantly, we have determined that TN-201 produced utilizing the highly scalable Sf9 platform results in similarly potent efficacy in a *Mybpc3*$^{-/-}$ model of disease. Finally, we have established that our observed efficacy is sufficiently meaningful for stable benefit up to 8 months post-injection, as well as reversal of cardiac dysfunction even in late-stage homozygote disease.

Example 12: Clinical Studies

[0265] A pharmaceutical composition comprising rAAV virions encoding MYBPC3, as described herein, is administered by intravenously or by retrograde coronary sinus (RCSI). Functional efficacy is determined by cardiac functional status assessments (*e.g.*, New York Heart Association

Functional Classification, NYHA; Cardiopulmonary exercise test, CPET), quality of life questionnaires (*e.g.*, Kansas City Cardiomyopathy Questionnaire Clinical Quality Score, KCCQ-CSS), cardiac imaging (*e.g.*, echocardiography), cardiac biomarkers (e.g. troponin and NT-proBNP), cardiac rhythm and immunologic assessments, cardiac functional status assessments (*e.g.*, Pediatric Interagency Registry for Mechanically Assisted Circulatory Support, PEDIMACS; Ross classifications), and/or Major Adverse Cardiac Events (MACE) (total death, cardiac transplantation, initiation of inotropes, initiation of ventilatory, or mechanical circulatory support). Clinical studies may include monitoring safety and continued efficacy (*e.g.*, adverse events, severe adverse events, electrocardiogram, cardiac enzymes, biomarkers, functional status, left ventricular (LV) function/mass, quality of life, serum chemistries, liver function tests) on an annual basis for up to 10 years.

CLAIMS

~~1. A modified cardiac troponin T promoter, comprising a polynucleotide having between 300 bp and 500 bp.~~

~~2. The promoter of claim 1, wherein the polynucleotide comprises a sequence that shares at least 80%, at least 90%, or 100% identity to any one of SEQ ID NOs: 1-85.~~

~~3. The promoter of claim 1, wherein the polynucleotide comprises a sequence that shares at least 80%, at least 90%, or 100% identity to SEQ ID NO: 3.~~

~~4. The promoter of claim 1, wherein the polynucleotide shares at least 80%, 90%, 95%, 96%, 97%, 98%, 99%, or and 100% sequence identity with a genomic polynucleotide sequence:~~

~~(i) upstream of and including the transcription start site of a troponin T gene; or~~

~~(ii) -450 bp to +1 bp, -350 bp to +1 bp, -250 bp to +1 bp, -450 bp to +50 bp, -350 bp to +50 bp, or -250 bp to +50 bp relative to the transcription start site of a troponin T gene;~~

~~optionally wherein the troponin T gene is a human troponin T gene.~~

~~5. The promoter of any one of claims 1-4, wherein the promoter is:~~

~~(i) a muscle-specific promoter; and/or~~

~~(ii) a cardiac cell-specific promoter; and/or~~

~~(iii) a cardiomyocyte-specific promoter.~~

~~6. The promoter of any one of claims 1-5, wherein the promoter has the same cell-type specificity as:~~

~~(i) a native troponin T promoter of about 600 bp; and/or~~

~~(ii) a reference promoter comprising SEQ ID NO: 1.~~

~~7. The promoter of any one of claims 1-6, wherein the promoter expresses a gene product operatively linked thereto at least about 10%, at least about 20%, at least about 30% more than a native troponin T promoter; optionally wherein the native troponin T promoter is a reference promoter comprising SEQ ID NO: 1.~~

~~8. A vector comprising the promoter of any one of claims 1-7 operatively linked to a polynucleotide encoding a gene product.~~

~~9. The vector of claim 8, wherein the vector is a viral vector; optionally wherein:~~

~~the viral vector is an adeno-associated virus vector (AAV) and/or wherein the viral vector has a packaging limit of at most about 5.5 kB;~~

~~optionally wherein the AAV vector is AAV9 or a variant thereof.~~

~~10. The vector of claim 8 or claim 9, wherein the gene product is selected from:~~

~~(i) MYBPC3, KCNH2, TRPM4, DSG2, and ATP2A2 proteins; or~~

~~(ii) CACNA1C, DMD, DMPK, EPG5, EVC, EVC2, FBN1, NF1, SCN5A, SOS1, NPR1, ERBB4, VIP, and MYH7 proteins.~~

~~11. The vector of any one of claims 8-10, wherein the gene product is a Cas9, optionally selected from SpCas9, St1Cas9, and SaCas9.~~

~~12. The vector of any one of claims 8-11, wherein the vector comprises a polynucleotide encoding second gene product; optionally wherein the second gene product is a functional RNA, optionally a microRNA or a guide RNA.~~

~~13. An isolated cell comprising the promoter of any one of claims 1-7.~~

~~14. The isolated cell of claim 13, wherein the isolated cell is an induced pluripotent stem cell or an isolated cardiomyocyte.~~

~~15. A pharmaceutical composition comprising the vector of any one of claims 8-12.~~

~~16. A cell therapy composition comprising the cell of claim 13 or claim 14.~~

~~17. A recombinant adeno-associated virus (AAV) vector genome, comprising a *MYBPC3* polynucleotide encoding a MYBPC3 protein, or a functional variant thereof, and a promoter, wherein the promoter is the modified cardiac troponin T promoter according to any one of claims 1 to 7.~~

~~18. The rAAV vector genome of claim 17, wherein the *MYBPC3* polynucleotide comprises at least about 3.5 kB, comprises about 3.8 kB, is a full-length MYBPC3, or is a truncated MYBPC3.~~

~~19. The rAAV vector genome of claim 17 or claim 18, wherein the MYBPC3 is a human MYBPC3.~~

~~20. The rAAV vector genome of any one of claims 17-19, wherein the vector expresses MYBPC3 at about the same level as, at least about 10% greater than, or at least about 20% greater than a reference AAV vector comprising a native troponin T promoter of about 600 bp.~~

~~21. A recombinant adeno-associated virus (AAV) vector genome, comprising an expression cassette comprising, in 5′ to 3′ order, a 5′ segment comprising a promoter; a polynucleotide encoding a gene product; and a 3′ segment comprising a polyA signal, the expression cassette optionally flanked by one or both of a 5′ inverted terminal repeat (ITR) and a 3′ ITR,~~

~~wherein the polynucleotide encoding the gene product comprises between 3 kb and 11kb, between 3 kb and 5kb, between 3.5 kb and 4.5kb, or between 3.7 kb and 4 kb; and~~

~~wherein:~~

~~a) the 5′ segment and the 3′ segment together comprise at most 0.8 kbp or at most 0.9 kbp;~~

~~b) the 5′ ITR, 5′ segment, the 3′ segment, and 3′ ITR together comprise or at most 1.2 kbp, at most 1.3 kbp; and/or~~

~~c) the vector genome comprises at most 4.7 kbp, at most 4.8 kbp, at most 4.9 kbp, at most 5.0 kbp, or at most 5.2 kbp.~~

~~22. The rAAV vector genome of claim 21, wherein the 5′ segment comprises at most 500 bp or at most 480 bp and/or wherein 3′ segment comprises at most 200 bp or at most 150 bp.~~

~~23. The rAAV vector genome of claim 21 or claim 22, wherein the polynucleotide encoding the gene product comprises 3.7 kbp to 3.9 kbp, optionally 3.8 kbp.~~

~~24. The rAAV vector genome of any one of claims 21-23, wherein the gene product is MYBPC3, or functional variant thereof; optionally wherein~~

~~the polynucleotide encoding MYBPC3 shares at least 90% identity to SEQ ID NO: 86, at least 95% identity to SEQ ID NO: 86, or is SEQ ID NO: 86; and /or~~

~~MYBPC shares at least 90% identity to the polypeptide sequence of SEQ ID NO: 103, at least 95% identity to the polypeptide sequence of SEQ ID NO: 103, or is the polypeptide sequence of SEQ ID NO: 103.~~

~~25. The rAAV vector genome of any one of claim 21-24, wherein the promoter is promoter is the modified cardiac troponin T promoter according to any one of claims 1 to 8.~~

~~26. The rAAV vector genome of claim 25, wherein:~~

~~(i) the promoter comprises a sequence that shares at least 80% identity, at least 90% identity, or 100% identity to SEQ ID NO: 91; and/or~~

~~(ii) the polyA signal comprises, consists essentially of, or consists of a sequence that shares at least 90% identity to, at least 95% identity to, or is SEQ ID NO: 92.~~

~~(iii) the 5′ segment shares at least 80% identity to, at least 90% identity to, at least 95% identity to, or is SEQ ID NO: 93; and/or~~

~~(iv) the 3′ segment shares at least 80% identity, at least 90% identity to, shares at least 95% identity to, or is SEQ ID NO: 94.~~

~~27. The rAAV vector genome of any one of claims 21-26, wherein the expression cassette shares at least 80% identity to, at least 90% identity to, at least 95% identity to, or is SEQ ID NO: 95; and/or wherein the rAAV vector genome shares at least 80% identity to, at least 90% identity to, at least 95% identity to, or is SEQ ID NO: 102.~~

~~28. The AAV vector genome of any one of claims 21-27, wherein the 5′ ITR comprises a sequence that shares 95% identity to SEQ ID NO: 96 and/or the 3′ ITR comprises a sequence that shares at least 95% identity to SEQ ID NO: 97.~~

~~29. A recombinant AAV (rAAV) virion, comprising the rAAV vector genome of any one of claims 17-28, and an AAV capsid protein,~~

~~optionally wherein the rAAV virion is a serotype AAV9 virion, or a variant thereof, and/or the AAV capsid protein is an AAV9 capsid protein or variant thereof.~~

30. An *in vitro* or *ex vivo* method of expressing a MYBPC3 protein in a cell, comprising transducing the cell with the rAAV virion of claim 29 or the rAAV vector genome of any one of claims 17-28.

31. The method of claim 30, wherein the cell is a *MYBPC3*$^{-/-}$ cell.

32. The method of claim 30 or claim 31, wherein the cell comprises an inactivating mutation in one or both copies of the endogenous *MYBPC3* gene.

33. An rAAV virion of claim 29 or an rAAV vector genome of any one of claims 17-28, for use as a medicament.

34. An rAAV virion of claim 29 or an rAAV vector genome of any one of claims 17-28, for use in a method of treating and/or preventing a cardiomyopathy in a subject; optionally wherein the cardiomyopathy is hypertrophic cardiomyopathy.

35. An rAAV virion of claim 29 or an rAAV vector genome of any one of claims 17-28, for use in a method of treating and/or preventing a disease or disorder caused by a MYBPC3 mutation in a subject.

36. The rAAV virion or rAAV vector genome for use according to claim 34 or claim 35, wherein the method comprises expressing a MYBPC3 protein and/or increasing MYBPC3 activity and/or increasing cardiac function in the heart of the subject.

37. The rAAV virion or rAAV vector genome for use according to any one of claims 33-36, wherein administration of the rAAV virion or rAAV vector genome causes specific expression of MYBPC3 in the heart of the subject.

38. The rAAV virion or rAAV vector genome for use according to any one of claims 33-37, wherein administration of the rAAV virion or rAAV vector genome causes low or undetectable expression of MYBPC3 in the skeletal tissue, brain, and/or liver of the subject.

39. The rAAV virion or rAAV vector genome for use according to any one of claims 34-38, wherein the subject comprises a MYBPC3 mutation.

~~40. The rAAV virion or rAAV vector genome for use according to any one of claims 34-39, wherein the method comprises:~~

~~(i) intravenous administration of the rAAV virion or rAAV vector genome to the subject;~~

~~(ii) intracardiac administration of the rAAV virion or rAAV vector genome to the subject;~~

~~(iii) direct injection of the rAAV virion or rAAV vector genome to the subject.~~

~~41. The rAAV virion or rAAV vector genome for use according to any one of claims 34-40, wherein the subject is a mammal; optionally wherein the subject is a human.~~

~~42. The rAAV virion or rAAV vector genome for use according to any one of claims 34-41, wherein the subject is a juvenile.~~

~~43. The rAAV virion or rAAV vector genome for use according to any one of claims 34-41, wherein the subject is an adult.~~

~~44. The rAAV virion or rAAV vector genome for use according to any one of claims 33-43, wherein the rAAV virion or rAAV vector genome is administered at a dose of about 10^{11} vg/kg to about 10^{14} vg/kg.~~

1. A recombinant vector comprising a promoter, wherein the promoter is a modified human cardiac troponin T promoter consisting of a polynucleotide having between 350 bp and 450 bp, wherein the polynucleotide comprises a sequence that shares at least 80% identity, at least 90% or 100% identity to SEQ ID NO: 3.

2. The vector of claim 1, wherein the polynucleotide has 400 bp.

3. The vector of claim 2, wherein the polynucleotide has the sequence set forth in SEQ ID NO:3.

4. The vector of any one of claims 1-3, wherein the promoter is operatively linked to a polynucleotide encoding a gene product.

5. The vector of claim 4, wherein the promoter: (i) is a cardiac cell-specific promoter, (ii) is a cardiomyocyte-specific promoter, (iii) has the same cell-type specificity as the native human cardiac troponin T promoter of SEQ ID NO: 1, and/or (iv) expresses the gene product at least about

10%, at least about 20%, at least about 30% more than a native human cardiac troponin T promoter and/or a promoter comprising SEQ ID NO: 1.

6. The vector of claim 4 or claim 5, wherein the gene product is selected from the group consisting of: myosin binding protein C (MYBPC3), KCNH2, TRPM4, DSG2, ATP2A2, CACNA1C, DMD, DMPK, EPG5, EVC, EVC2, FBN1, NF1, SCN5A, SOS1, NPR1, ERBB4, VIP, MYH7 and Cas9 proteins, optionally wherein the Cas9 is selected from the group consisting of SpCas9, St1Cas9 and SaCas9.

7. The vector of claim 4 or claim 5, wherein the gene product is human MYBPC3 or a functional variant thereof, wherein the polynucleotide encoding MYBPC3 comprises at least about 3.5 kb or 3.8 kb, or encodes full-length human MYBPC3, optionally wherein the polynucleotide encoding MYBPC3 shares at least 80%, 90%, 95% or 100% identity to SEQ ID NO:86 and/or human MYBPC shares at least 90%, 95% or 100% identity to the polypeptide sequence of SEQ ID NO:103.

8. The vector of any one of claims 1-7, wherein the vector is a viral vector, optionally wherein the viral vector is a retroviral vector, a lentiviral vector, an adenoviral vector, or an adeno-associated viral (AAV) vector, optionally wherein the vector has a packaging limit of at most about 5.5 kB.

9. The vector of any one of claims 1-8, wherein the vector is an AAV vector, optionally wherein the AAV vector is AAV9 or a variant thereof.

10. A recombinant vector, comprising an expression cassette comprising or consisting of, in 5′ to 3′ order, a 5′ segment comprising a promoter, a polynucleotide encoding a gene product, and a 3′ segment comprising a polyA signal, wherein:

a) the polynucleotide encoding the gene product comprises between 3 kb and 11 kb, between 3 kb and 5kb, between 3.5 kb and 4.5kb, or between 3.7 kb and 4 kb,

b) the 5’ segment and the 3’ segment together are at most 0.8 kb or at most 0.9 kb, and

c) the vector does not comprise a woodchuck hepatitis virus post-transcriptional element (WPRE).

11. The vector of claim 10, wherein the vector does not comprise an intron.

12. The vector of claim 10 or claim 11, wherein the gene product is selected from the group consisting of: myosin binding protein C (MYBPC3), KCNH2, TRPM4, DSG2, ATP2A2, CACNA1C, DMD, DMPK, EPG5, EVC, EVC2, FBN1, NF1, SCN5A, SOS1, NPR1, ERBB4, VIP, MYH7 and Cas9 proteins, optionally wherein the Cas9 is selected from the group consisting of SpCas9, St1Cas9 and SaCas9.

13. The vector of claim 10 or claim 11, wherein the gene product is human MYBPC3 or a functional variant thereof, wherein the polynucleotide encoding MYBPC3 comprises at least about 3.5 kb or 3.8 kb, or encodes full-length human MYBPC3, optionally wherein the polynucleotide encoding MYBPC3 shares at least 80%, 90%, 95% or 100% identity to SEQ ID NO:86 and/or human MYBPC shares at least 90%, 95% or 100% identity to the polypeptide sequence of SEQ ID NO:103.

14. The vector of claim 7 or claim 13, wherein the gene product is full-length human MYBPC3.

15. The vector of any one of claims 1-14, wherein the vector is a viral vector, optionally wherein the viral vector is a retroviral vector, an adenoviral vector, or an adeno-associated viral (AAV) vector, optionally wherein the vector has a packaging limit of at most about 5.5 kB.

16. The vector of claim 15, wherein the vector is a retroviral vector, and the retroviral vector is a lentiviral vector.

17. The vector of claim 15, wherein the vector is an AAV, optionally wherein the AAV is AAV9 or a variant thereof.

18. A recombinant adeno-associated virus (AAV) vector, comprising an expression cassette comprising or consisting of, in 5′ to 3′ order, a 5′ segment comprising a promoter, a polynucleotide encoding a gene product, and a 3′ segment comprising a polyA signal, the expression cassette optionally flanked by one or both of a 5′ inverted terminal repeat (ITR) and a 3′ ITR, wherein:

a) the polynucleotide encoding the gene product comprises between 3 kb and 5kb, between 3.5 kb and 4.5kb, or between 3.7 kb and 4 kb;

b) the 5′ segment and the 3′ segment together are at most 0.8 kb or at most 0.9 kb;

c) the 5′ ITR, the 5′ segment, the 3′ segment, and the 3′ ITR together are at most 1.2 kb; and/or

d) the vector genome is at most 4.7 kb, at most 4.8 kb, or at most 4.9 kb.

19. The vector of claim 18, wherein the vector does not comprise a WPRE.

20. The vector of claim 18 or claim 19, wherein the vector does not comprise an intron.

21. The vector of any one of claims 18-20, wherein the 5′ ITR has a sequence that shares 95% or 100% identity to SEQ ID NO: 96 and/or the 3′ ITR has a sequence that shares at least 95% or 100% identity to SEQ ID NO: 97.

22. The vector of any one of claims 10-21, wherein the 5′ segment is at most 500 bp or at most 480 bp.

23. The vector of any one of claims 10-22, wherein the promoter is a cardiac troponin T promoter.

24. The vector of claim 23, wherein the cardiac troponin T promoter is a human cardiac troponin T promoter of SEQ ID NO:1.

25. The vector of claim 23, wherein the cardiac troponin T promoter is a modified human cardiac troponin T promoter consisting of a polynucleotide having between 350 bp and 450 bp, or between 400 bp and 500 bp, and wherein the polynucleotide comprises a sequence that shares at least 80% identity, at least 90% or 100% identity to SEQ ID NO: 3.

26. The vector of claim 25, wherein the polynucleotide has 400 bp.

27. The vector of claim 26, wherein the polynucleotide has the sequence set forth in SEQ ID NO: 3.

28. The vector of any one of claims 10-27, wherein the 5′ segment shares at least 90% identity to, at least 95% identity to, or 100% identity to SEQ ID NO: 93.

29. The vector of any one of claims 10-28, wherein the 3′ segment comprises at most 200 bp or at most 150 bp.

30. The vector of any one of claims 10-29, wherein the 3′ segment shares at least 80% identity, at least 90% identity to, at least 95% identity to, or 100% identity to SEQ ID NO: 94.

31. The vector of any one of claims 10-30, wherein the polyA signal comprises, consists essentially of, or consists of, a sequence that shares at least 90% identity to, at least 95% identity to, or 100% identity to SEQ ID NO: 92.

32. The vector of any one of claims 10-31, wherein:

(i) the promoter consists of a sequences that shares at least 90% identity or 100% identity to SEQ ID NO: 3;

(ii) the 5′ segment consists of a sequence that shares at least 95% identity or 100% identity to SEQ ID NO: 93;

(iii) the polyA signal consists of a sequence that shares at least at least 95% identity or 100% identity to SEQ ID NO: 92; and

(iv) the 3′ segment consist of a sequence that shares at least 95% identity or 100% identity to SEQ ID NO: 94.

33. The vector of any one of claims 10-32, wherein the polynucleotide encoding the gene product comprises 3.7 kb to 3.9 kb, and/or wherein the vector genome comprises at most 4.8 kb.

34. The vector of any one of claims 18-33, wherein the gene product is MYBPC3, or a functional variant thereof, and:

(i) the polynucleotide encoding MYBPC3 shares at least 90% identity to SEQ ID NO: 86, at least 95% identity to SEQ ID NO: 86, or 100% identity to SEQ ID NO: 86; and /or

(ii) MYBPC shares at least 90% identity to the polypeptide sequence of SEQ ID NO: 103, at least 95% identity to the polypeptide sequence of SEQ ID NO: 103, or 100% identity to the polypeptide sequence of SEQ ID NO: 103.

35. The vector of claim 34, wherein the MYBPC3 is a full-length human MYBPC3.

36. The vector of any one of claims 18-35, wherein the expression cassette shares at least 90% identity, at least 95% identity, or 100% identity to SEQ ID NO: 95; and/or wherein the vector genome shares at least 90% identity to, at least 95% identity to or 100% identity to SEQ ID NO: 102.

37. A recombinant AAV (rAAV) virion, comprising the vector of any one of claims 1-36, and an AAV capsid protein.

38. The rAAV virion of claim 37, wherein the rAAV virion is a serotype AAV9 virion, or a variant thereof, and/or the AAV capsid protein is an AAV9 capsid protein or a variant thereof.

39. A recombinant adeno-associated virus (rAAV) virion, comprising a vector genome comprising an expression cassette comprising a polynucleotide encoding MYBPC3 operatively linked to a promoter, the expression cassette flanked by a 5′ inverted terminal repeat (ITR) and a 3′ ITR; wherein the vector genome comprises at most 4.8 kb.

40. The rAAV virion of claim 39, wherein the promoter is a cardiac troponin T promoter.

41. The rAAV virion of claim 40, wherein the cardiac troponin T promoter consists of about 400 bp.

42. The rAAV virion of claim 40 or claim 41, wherein the cardiac troponin T promoter shares at least 80% sequence identity or at least 90% sequence identity to the polynucleotide sequence of SEQ ID NO: 3.

43. The rAAV virion of claim 42, wherein the cardiac troponin T promoter has the polynucleotide sequence of SEQ ID NO: 3.

44. The rAAV virion of any one of claims 39-43, wherein the MYBPC3 shares at least 90% sequence identity or at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 103.

45. The rAAV virion of any one of claims 39-44, wherein the MYBPC3 comprises the amino acid sequence of SEQ ID NO: 103.

46. The rAAV virion of any one of claims 39-45, wherein the polynucleotide encoding MYBPC3 shares at least 80% sequence identity, at least 90% sequence identity, at least 95% sequence identity, or 100% sequence identity to the polynucleotide sequence of SEQ ID NO: 86.

47. The rAAV virion of any one of claims 39-46, wherein the 5′ ITR comprises a sequence that shares at least 95% or 100% sequence identity to the polynucleotide sequence of SEQ ID NO: 96; and the 3′ ITR comprises a sequence that shares at least 95% or 100% sequence identity to the polynucleotide sequence of SEQ ID NO: 97.

48. The rAAV virion of any one of claims 39-47, wherein the expression cassette shares at least 80% sequence identity, at least 90% of sequence identity, or at least 95% sequence identity to the polynucleotide sequence of SEQ ID NO: 95.

49. The rAAV virion of any one of claims 39-48, wherein the expression cassette comprises the polynucleotide sequence of SEQ ID NO: 95.

50. The rAAV virion of any one of claims 39-49, wherein the vector genome is at most 4.7 kb or at most 4713 base pairs, or wherein the vector genome is 4.7 kb or 4713 base pairs.

51. The rAAV virion of any one of claims 39-50, wherein the vector genome shares at least 80% sequence identity, at least 85% sequence identity, at least 90% sequence identity, or at least 95% sequence identity to the polynucleotide sequence of SEQ ID NO: 102.

52. The rAAV virion of any one of claims 39-51, wherein the vector genome shares 100% sequence identity to the polynucleotide sequence of SEQ ID NO: 102.

53. The rAAV virion of any one of claims 39-52, wherein the rAAV virion is a serotype AAV9 virion.

54. A pharmaceutical composition comprising the vector of any one of claims 1-36 or the rAAV virion of any one of claims 37-53, and a pharmaceutically acceptable excipient.

55. The vector of any one of claims 1-36, the rAAV virion of any one of claims 37-53, or the pharmaceutical composition of claim 54, for use as a medicament.

56. An isolated cell comprising the vector of any one of claims 1-36 or the rAAV virion of any one of claims 37-53, optionally wherein the isolated cell is an induced pluripotent stem cell, a cardiac cell, or a cardiomyocyte.

57. A cell therapy composition comprising the cell of claim 56.

58. An *in vitro* or *ex vivo* method of expressing a MYBPC3 protein in a cell, comprising transducing the cell with the vector of any one of claims 7, 13, 34 or 35, or the rAAV virion of any one of claims 39-53.

59. The method of claim 58, wherein the cell is a cardiac cell.

60. The method of claim 58 or claim 59, wherein the cell is a *MYBPC3*$^{-/-}$ cell or comprises an inactivating mutation in one or both copies of the endogenous *MYBPC3* gene.

61. Use of the vector of any one of claims 7, 13, 34 or 35, the rAAV virion of any one of claims 39-53, or the pharmaceutical composition of claim 54, in the manufacture of a medicament for treating and/or preventing a cardiomyopathy in a subject.

62. The use of claim 61, wherein the cardiomyopathy is hypertrophic cardiomyopathy.

63. The use of claim 61 or claim 62, wherein the cardiomyopathy is caused by a MYBPC3 mutation.

64. Use of the vector of any one of claims 7, 13, 34 or 35, the rAAV virion of any one of claims 39-53, or the pharmaceutical composition of claim 54, in the manufacture of a medicament for treating and/or preventing a disease or disorder caused by a MYBPC3 mutation in a subject.

65. The use according to any one of claims 61-64, wherein administration of the rAAV virion, the vector or the pharmaceutical composition to the subject causes specific expression of MYBPC3 in the heart of the subject, and causes low or undetectable expression of MYBPC3 in the skeletal tissue, brain, and/or liver of the subject.

66. The use according to any one of claims 61-65, wherein the subject has a MYBPC3 mutation.

67. The use according to claim 66, wherein the MYBPC3 mutation is a homozygous *MYBPC3*$^{-/-}$ mutation.

68. The use according to any one of claims 61-67, wherein:

(i) the vector, the rAAV virion or the pharmaceutical composition is to be intravenously administered to the subject;

(ii) the vector, the rAAV virion or the pharmaceutical composition is to be administered by intracardiac catherization to the subject; or

(iii) the vector, the rAAV virion or the pharmaceutical composition is to be directly injected into the heart tissue of the subject.

69. The use according to any one of claims 61-68, wherein the vector, the rAAV virion or the pharmaceutical composition is to be administered to the subject at a dose of about 10^{11} vg/kg to about 10^{14} vg/kg.

70. The use according to any one of claims 61-69, wherein the subject is a human.

71. The use according to any one of claims 61-70, wherein the use increases ejection fraction in the subject.