SUPPRESSION-REPLACEMENT GENE THERAPY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of priority from U.S. Provisional Application Serial No. 63/132,316, filed December 30, 2020, U.S. Provisional Application Serial No. 63/179,083, filed April 23, 2021, U.S. Provisional Application Serial No. 63/208,556, filed June 9, 2021, and U.S. Provisional Application Serial No. 63/270,388, filed October 21, 2021. The disclosures of the prior applications are considered part of (and are incorporated by reference in) the disclosure of this application.

TECHNICAL FIELD

This document relates to methods and materials for treating a mammal having a congenital disease (e.g., a congenital heart disease such as congenital long QT syndrome). For example, this document provides methods and materials for generating and using nucleic acids that can be administered to a mammal having a congenital disease, and can suppress expression of mutant disease-related alleles in the mammal while providing a replacement cDNA that does not contain the disease-related mutation(s).

BACKGROUND

Congenital long QT syndrome (LQTS) is an autosomal dominant disorder characterized by delayed repolarization of the myocardium that is associated with a prolonged QT interval on electrocardiogram (ECG). Patients with LQTS have increased risk for torsadogenic syncope/seizures and sudden cardiac death (SCD). The prevalence of LQTS is about 1 in 2000, and when untreated, higher risk patients have an estimated 10-year mortality of 50% (Schwartz *et al.*, *Circulation,* 120:1761-1767 (2009); and Schwartz and Ackerman, *Eur. Heart J.*, 34:3109-3116 (2013)).

LQTS is caused by pathogenic variants in cardiac ion channels or their interacting regulatory proteins (Giudicessi *et al.*, *Trends Cardiovasc. Med.*, 28:453-464 (2018)). Type 1 LQTS (LQT1) is the most common form of LQTS, accounting for about 35% of

cases (Ackerman *et al.*, *Heart Rhythm.*, 8:1308-1339 (2011)). LQT1 is caused by loss-of-function variants in *KCNQ1*, which encodes the α-subunit of the $K_v7.1$ voltage-gated potassium channel that is responsible for the slow delayed rectifier current (I_{Ks}) during repolarization of the cardiac action potential. Because the *KCNQ1*-encoded α-subunits tetramerize during $K_v7.1$ channel assembly, pathogenic missense variants commonly exhibit a dominant-negative effect due to interference with the wild-type (WT) subunits translated from the non-affected allele. Another common form of LQTS is LQT2, which accounts for about 30% of cases. Patients with LQT2 host loss-of-function mutations in the *KCNH2*-encoded I_{Kr} (Kv11.1) potassium channel that, like *KCNQ1*, plays a role in cardiac action potential duration (APD) (Tester *et al.*, *Heart Rhythm.*, 2(5):507-517 (2005); Giudicessi *et al.*, *Trends Cardiovasc. Med.*, 28:453-464 (2018); and Ackerman e*t al.*, *Heart Rhythm.*, 8:1308-1339 (2011)). Pathogenic variants in *KCNQ1* or *KCNH2* that lead to a gain-of-function and an abnormal increase in I_{Ks} or I_{Kr} current density, respectively, can lead to short QT syndrome (SQTS). The third most common form of LQTS is LQT3, which accounts for about 10% of cases. Patients with LQT3 host gain-of-function mutations in the SCN5A-encoded I_{Na} (Nav1.5) sodium channel that also plays a role in the cardiac APD (Tester *et al.*, *J. Am. Coll. Cardiol. EP*, 4:569-579 (2018)). Pathogenic variants in *SCN5A* that lead to a loss-of-function and a decrease in I_{Na} can cause Brugada syndrome (Wilde and Amin, *J. Am. Coll. Cardiol. EP*, 4:569-579 (2018)).

Current therapies for management of LQTS include beta-blockers, which provide a first line treatment, as well as more invasive therapies such as left cardiac sympathetic denervation (LCSD) or implantation of a cardioverter defibrillator (ICD). These, however, can have limitations including noncompliance, breakthrough cardiac events, or infection (Rohatgi *et al.*, *J. Am. Coll. Cardiol.*, 70:453-462 (2017); Priori *et al.*, *Heart Rhythm.*, 10:1932-1963 (2013); Al-Khatib *et al.*, *Heart Rhythm.*, 15:e190-e252 (2018); Schwartz *et al.*, *Circulation*, 109:1826-1833 (2004); Bos *et al.*, *Circ. Arrhythm. Electrophysiol.*, 6:705-711 (2013); Schwartz *et al.*, *Circulation*, 122:1272-1282 (2010); Horner *et al.*, *Heart Rhythm.*, 7:1616-1622 (2010); and Kleemann *et al.*, *Circulation*, 115:2474-2480 (2007)), and they do not treat the underlying pathogenic substrate.

RNA interference (RNAi) technology, such as small interfering RNA (siRNA), utilizes endogenous gene silencing to knock down gene expression. Attempts to overcome dominant-negative *KCNH2* variants in LQT2 have used allele-specific siRNAs to selectively knock down the mutant allele (Lu *et al.*, *Heart Rhythm*, 10:128-136 (2013); and Matsa *et al.*, *Eur. Heart J.*, 35:1078-1087 (2014)). The best possible outcome of this method would be haploinsufficiency, however. In addition, it would be necessary to engineer and validate a separate siRNA for each unique LQT2-causative variant, which would be impractical in *KCNQ1*, *KCNH2*, and *SCN5A*, as there are hundreds of LQT1-, LQT2-, and LQT3-causative variants (Landrum *et al.*, *Nucleic Acids Res.*, 46:D1062-D1067 (2018)).

SUMMARY

This document is based, at least in part, on the development of a dual-component "suppression-and-replacement" *KCNQ1* (KCNQ1-SupRep) gene therapy approach for LQT1, in which a *KCNQ1* shRNA is used to suppress expression of the endogenous *KCNQ1* alleles and a codon-altered "shRNA-immune" copy of *KCNQ1* is used for gene replacement. As described herein, the "KCNQ1-SupRep" system was successfully used to rescue the prolonged action potential duration in induced pluripotent stem cell (iPSC) cardiomyocytes derived from fibroblasts or PBMCs from four patients with unique LQT1-causative *KCNQ1* variants. This document therefore describes successful preclinical hybrid gene therapy in LQT1, and demonstrates that the system provided herein is capable of complete rescue of *KCNQ1* function. Theoretically, KCNQ1-SupRep is applicable to essentially any patient with LQT1, because it targets the whole *KCNQ1* gene rather than specific mutations.

This document also is based, at least in part, on the development of a "suppression-and-replacement" *KCNH2* (KCNH2-SupRep) gene therapy approach for LQT2, in which a *KCNQ2* shRNA is used to suppress expression of the endogenous *KCNH2* alleles and a codon-altered "shRNA-immune" copy of *KCNH2* is used for gene replacement.

In addition, this document is based, at least in part, on the development of a "suppression-and-replacement" *SCN5A* (SCN5A-SupRep) gene therapy approach for LQT3, in which a *SCN5A* shRNA is used to suppress expression of the endogenous SCN5A alleles and a codon-altered "shRNA-immune" copy of *SCN5A* is used for gene replacement.

Having the ability to reduce the myocardium repolarization time (e.g., by shortening the APD) using the methods and materials described herein can allow clinicians and patients (e.g., LQTS patients) to achieve cardiac function that more closely resembles the function of a healthy heart, when compared to the function of an untreated LQTS patient's heart. In some cases, having the ability to reduce the myocardium repolarization time in LQTS patients using the methods and materials described herein can allow clinicians and patients to reduce LQTS symptoms and/or reverse LQTS progression. For example, delivery of a nucleic acid or virus construct provided herein to heart tissue can rescue cardiac defects and increase survival in LQTS patients.

In one aspect, this document features a nucleic acid construct. The nucleic acid construct can include (a) a first nucleotide sequence encoding an RNAi molecule capable of hybridizing to a target sequence encoding an endogenous KCNQ1 polypeptide within a cell and suppressing expression of the endogenous KCNQ1 polypeptide within the cell, and (b) a second nucleotide sequence encoding a KCNQ1 polypeptide, where the second nucleotide sequence includes a target sequence identical to the target sequence of the first nucleotide sequence with the exception that the target sequence of the second nucleotide sequence comprises 1 to 13 wobble position variants as compared to the target sequence of the first nucleotide sequence, and where the RNAi molecule does not suppress expression of the KCNQ1 polypeptide from the second nucleotide sequence within the cell. The first nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, or SEQ ID NO:36, and the second nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:9. The first nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:36 and the second nucleotide sequence can include, consist essentially of, or consist of the sequence set

forth in SEQ ID NO:9. The first nucleotide sequence can be operably linked to a first promoter, and the second nucleotide sequence can be operably linked to a second promoter. The first and second promoters can be the same or can be different. The first promoter can be a U6 promoter, and the second promoter can be a cytomegalovirus immediate-early (CMV) promoter. The nucleic acid construct can further include a nucleotide sequence encoding a reporter. The reporter can be a fluorescent polypeptide. The nucleotide sequence encoding the reporter can be downstream of the second nucleotide sequence encoding the KCNQ1 polypeptide (e.g., a cDNA encoding the KCNQ1 polypeptide), and can be separated from the second nucleotide sequence by an internal ribozyme entry sequence (IRES) or P2A self-cleaving peptide sequence. The nucleic acid construct can be within a viral vector. The viral vector can be an adeno-associated virus (AAV) vector (e.g., an AAV serotype 9 vector or an AAV2/9 vector). The cell can be a cardiomyocyte.

In another aspect, this document features a virus particle containing a nucleic acid construct described herein (e.g., a nucleic acid construct of the preceding paragraph).

In another aspect, this document features a method for treating a mammal having a congenital cardiac disease. The method can include administering to the mammal a nucleic acid construct containing (a) a first nucleotide sequence encoding an RNAi molecule capable of hybridizing to a target sequence encoding an endogenous KCNQ1 polypeptide within a cell of the mammal and suppressing expression of the endogenous KCNQ1 polypeptide within the cell, and (b) a second nucleotide sequence encoding a KCNQ1 polypeptide, where the second nucleotide sequence comprises a target sequence identical to the target sequence of the first nucleotide sequence with the exception that the target sequence of the second nucleotide sequence comprises 1 to 13 wobble position variants as compared to the target sequence of the first nucleotide sequence, and where the RNAi molecule does not suppress expression of the KCNQ1 polypeptide from the second nucleotide sequence within the cell. The congenital cardiac disease can be long QT syndrome (LQTS) or short QT syndrome (SQTS). The congenital cardiac disease can be LQT1. The first nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, or SEQ ID

NO:36, and the second nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:9. The first nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:36, and the second nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:9. The first nucleotide sequence can be operably linked to a first promoter, and the second nucleotide sequence can be operably linked to a second promoter. The first and second promoters can be the same or can be different. The first promoter can be a U6 promoter ,and the second promoter can be a CMV promoter. The nucleic acid construct can further include a nucleotide sequence encoding a reporter. The reporter can be a fluorescent polypeptide. The nucleotide sequence encoding the reporter can be downstream of the second nucleotide sequence encoding the KCNQ1 polypeptide (e.g., a cDNA encoding the KCNQ1 polypeptide), and can be separated from the second nucleotide sequence by an IRES. The nucleic acid construct can be within a viral vector. The viral can be an AAV vector (e.g., an AAV serotype 9 vector or an AAV2/9 vector). The cell can be a cardiomyocyte.

In another aspect, this document features a method for reducing the action potential duration (APD) in cardiac cells within a mammal. The method can include administering to the mammal a nucleic acid construct containing (a) a first nucleotide sequence encoding an RNAi molecule capable of hybridizing to a target sequence encoding an endogenous KCNQ1 polypeptide within cardiac cells of the mammal and suppressing expression of the endogenous KCNQ1 polypeptide within the cardiac cells, and (b) a second nucleotide sequence encoding a KCNQ1 polypeptide, where the second nucleotide sequence comprises a target sequence identical to the target sequence of the first nucleotide sequence with the exception that the target sequence of the second nucleotide sequence comprises 1 to 13 wobble position variants as compared to the target sequence of the first nucleotide sequence, and where the RNAi molecule does not suppress expression of the KCNQ1 polypeptide from the second nucleotide sequence within the cell. The first nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, or SEQ ID NO:36, and the second nucleotide sequence can include, consist essentially of, or consist

of the sequence set forth in SEQ ID NO:9. The first nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:36, and the second nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:9. The first nucleotide sequence can be operably linked to a first promoter, and the second nucleotide sequence can be operably linked to a second promoter. The first and second promoters can be the same or can be different. The first promoter can be a U6 promoter, and the second promoter can be a CMV promoter. The nucleic acid construct can be within a viral vector. The viral vector can be an AAV vector (e.g., an AAV serotype 9 vector or an AAV2/9 vector).

In another aspect, this document features a method for reducing one or more symptoms of LQTS in a mammal. The method can include administering to the mammal a nucleic acid construct containing (a) a first nucleotide sequence encoding an RNAi molecule capable of hybridizing to a target sequence encoding an endogenous KCNQ1 polypeptide within a cell of the mammal and suppressing expression of the endogenous KCNQ1 polypeptide within the cell, and (b) a second nucleotide sequence encoding a KCNQ1 polypeptide, where the second nucleotide sequence comprises a target sequence identical to the target sequence of the first nucleotide sequence with the exception that the target sequence of the second nucleotide sequence comprises 1 to 13 wobble position variants as compared to the target sequence of the first nucleotide sequence, and where the RNAi molecule does not suppress expression of the KCNQ1 polypeptide from the second nucleotide sequence within the cell. The LQTS can be LQT1. The first nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, or SEQ ID NO:36, and the second nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:9. The first nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:36, and the second nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:9. The first nucleotide sequence can be operably linked to a first promoter, and the second nucleotide sequence can be operably linked to a second promoter. The first and second promoters can be the same or can be different. The first promoter can be a U6 promoter, and the

second promoter can be a CMV promoter. The nucleic acid construct can be within a viral vector. The viral vector can be an AAV vector (e.g., an AAV serotype 9 vector or an AAV2/9 vector). The cell can be a cardiomyocyte.

In another aspect, this document features a nucleic acid construct that can include (a) a first nucleotide sequence encoding an RNAi molecule capable of hybridizing to a target sequence encoding an endogenous KCNH2 polypeptide within a cell and suppressing expression of the endogenous KCNH2 polypeptide within the cell, and (b) a second nucleotide sequence encoding a KCNH2 polypeptide, where the second nucleotide sequence comprises a target sequence identical to the target sequence of the first nucleotide sequence with the exception that the target sequence of the second nucleotide sequence comprises 1 to 13 wobble position variants as compared to the target sequence of the first nucleotide sequence, and where the RNAi molecule does not suppress expression of the KCNH2 polypeptide from the second nucleotide sequence within the cell. The first nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:27, and the second nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:29. The first nucleotide sequence can be operably linked to a first promoter, and the second nucleotide sequence can be operably linked to a second promoter. The first and second promoters can be the same or can be different. The first promoter can be a U6 promoter, and the second promoter can be a CMV promoter. The nucleic acid construct can further include a nucleotide sequence encoding a reporter. The reporter can be a fluorescent polypeptide. The nucleotide sequence encoding the reporter can be downstream of the second nucleotide sequence encoding the KCNH2 polypeptide (e.g., a cDNA encoding the KCNH2 polypeptide), and can be separated from the second nucleotide sequence by an IRES or P2A self-cleaving peptide sequence. The nucleic acid construct can be within a viral vector. The viral vector can be an AAV vector (e.g., an AAV serotype 9 vector or an AAV2/9 vector). The cell can be a cardiomyocyte.

In another aspect, this document features a virus particle containing a nucleic acid construct described herein (e.g., a nucleic acid construct described in the preceding paragraph).

In still another aspect, this document features a method for treating a mammal having a congenital cardiac disease. The method can include administering to the mammal a nucleic acid construct containing (a) a first nucleotide sequence encoding an RNAi molecule capable of hybridizing to a target sequence encoding an endogenous KCNH2 polypeptide within a cell of the mammal and suppressing expression of the endogenous KCNH2 polypeptide within the cell, and (b) a second nucleotide sequence encoding a KCNH2 polypeptide, where the second nucleotide sequence comprises a target sequence identical to the target sequence of the first nucleotide sequence with the exception that the target sequence of the second nucleotide sequence comprises 1 to 13 wobble position variants as compared to the target sequence of the first nucleotide sequence, and where the RNAi molecule does not suppress expression of the KCNH2 polypeptide from the second nucleotide sequence within the cell. The congenital cardiac disease can be LQTS or SQTS. The congenital cardiac disease can be LQT2. The first nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:27, and the second nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:29. The first nucleotide sequence can be operably linked to a first promoter, and the second nucleotide sequence can be operably linked to a second promoter. The first and second promoters can be the same or can be different. The first promoter can be a U6 promoter, and the second promoter can be a CMV promoter. The nucleic acid construct can further include a nucleotide sequence encoding a reporter. The reporter can be a fluorescent polypeptide. The nucleotide sequence encoding the reporter can be downstream of the second nucleotide sequence encoding the KCNH2 polypeptide (e.g., a cDNA encoding the KCNH2 polypeptide), and can be separated from the second nucleotide sequence by an IRES. The nucleic acid construct can be within a viral vector. The viral vector can be an AAV vector (e.g., an AAV serotype 9 vector or an AAV2/9 vector). The cell can be a cardiomyocyte.

In another aspect, this document features a method for reducing the APD in cardiac cells within a mammal. The method can include administering to the mammal a nucleic acid construct containing (a) a first nucleotide sequence encoding an RNAi

molecule capable of hybridizing to a target sequence encoding an endogenous KCNH2 polypeptide within cardiac cells of the mammal and suppressing expression of the endogenous KCNH2 polypeptide within the cardiac cells, and (b) a second nucleotide sequence encoding a KCNH2 polypeptide, where the second nucleotide sequence comprises a target sequence identical to the target sequence of the first nucleotide sequence with the exception that the target sequence of the second nucleotide sequence comprises 1 to 13 wobble position variants as compared to the target sequence of the first nucleotide sequence, and where the RNAi molecule does not suppress expression of the KCNH2 polypeptide from the second nucleotide sequence within the cell. The first nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:27, and the second nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:29. The first nucleotide sequence can be operably linked to a first promoter, and the second nucleotide sequence can be operably linked to a second promoter. The first and second promoters can be the same or can be different. The first promoter can be a U6 promoter, and the second promoter can be a CMV promoter. The nucleic acid construct can be within a viral vector. The viral vector can be an AAV vector (e.g., an AAV serotype 9 vector or an AAV2/9 vector).

In yet another aspect, this document features a method for reducing one or more symptoms of LQTS in a mammal. The method can include administering to the mammal a nucleic acid construct containing (a) a first nucleotide sequence encoding an RNAi molecule capable of hybridizing to a target sequence encoding an endogenous KCNH2 polypeptide within a cell of the mammal and suppressing expression of the endogenous KCNH2 polypeptide within the cell, and (b) a second nucleotide sequence encoding a KCNH2 polypeptide, where the second nucleotide sequence comprises a target sequence identical to the target sequence of the first nucleotide sequence with the exception that the target sequence of the second nucleotide sequence comprises 1 to 13 wobble position variants as compared to the target sequence of the first nucleotide sequence, and where the RNAi molecule does not suppress expression of the KCNH2 polypeptide from the second nucleotide sequence within the cell. The LQTS can be LQT2. The first nucleotide

sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:27, and the second nucleotide sequence can include, consist essentially of, or consist of the sequence set forth in SEQ ID NO:29. The first nucleotide sequence can be operably linked to a first promoter, and the second nucleotide sequence can be operably linked to a second promoter. The first and second promoters can be the same or can be different. The first promoter can be a U6 promoter, and the second promoter can be a CMV promoter. The nucleic acid construct can be within a viral vector. The viral vector can be an AAV vector (e.g., an AAV serotype 9 vector or an AAV2/9 vector). The cell can be a cardiomyocyte.

In another aspect, this document features a nucleic acid construct for treating a congenital heart disease caused by an endogenous cardiac polypeptide containing one or more mutations causative of the congenital heart disease, where the construct can include (a) a first nucleotide sequence encoding an RNAi molecule capable of hybridizing to a target sequence encoding the endogenous cardiac polypeptide within a cell and suppressing expression of the endogenous cardiac polypeptide within the cell, and (b) a second nucleotide sequence encoding a replacement version of the endogenous cardiac polypeptide that lacks the one or more mutations causative of the congenital heart disease, wherein the second nucleotide sequence comprises a target sequence identical to the target sequence of the first nucleotide sequence with the exception that the target sequence of the second nucleotide sequence comprises 1 to 13 wobble position variants as compared to the target sequence of the first nucleotide sequence, and wherein the RNAi molecule does not suppress expression of the replacement version of the endogenous cardiac polypeptide that lacks the one or more mutations causative of the congenital heart disease from the second nucleotide sequence within the cell. The first nucleotide sequence can be operably linked to a first promoter and the second nucleotide sequence can be operably linked to a second promoter. The first and second promoters can be the same, or the first and second promoters can be different. The first promoter can be a U6 promoter and the second promoter can be a CMV promoter. The nucleic acid construct can further include a nucleotide sequence encoding a reporter. The reporter can be a fluorescent polypeptide. The nucleotide sequence encoding the reporter can be

downstream of the second nucleotide sequence encoding the cDNA, and can be separated from the second nucleotide sequence by an IRES or P2A self-cleaving peptide sequence. The nucleic acid construct can be within a viral vector. The viral vector can be an AAV vector (e.g., an AAV serotype 9 vector or an AAV2/9 vector). The cell can be a cardiomyocyte.

In another aspect, this document features a virus particle containing the nucleic acid construct described herein (e.g., a nucleic acid construct described in the preceding paragraph).

In still another aspect, this document features a method for treating a mammal having a congenital cardiac disease. The method can include administering to the mammal a nucleic acid construct containing (a) a first nucleotide sequence encoding an RNAi molecule capable of hybridizing to a target sequence encoding the endogenous cardiac polypeptide within a cell and suppressing expression of the endogenous cardiac polypeptide within the cell, and (b) a second nucleotide sequence encoding a replacement version of the endogenous cardiac polypeptide that lacks the one or more mutations causative of the congenital heart disease, wherein the second nucleotide sequence comprises a target sequence identical to the target sequence of the first nucleotide sequence with the exception that the target sequence of the second nucleotide sequence comprises 1 to 13 wobble position variants as compared to the target sequence of the first nucleotide sequence, and wherein the RNAi molecule does not suppress expression of the replacement version of the endogenous cardiac polypeptide that lacks the one or more mutations causative of the congenital heart disease from the second nucleotide sequence within the cell. The first nucleotide sequence can be operably linked to a first promoter and the second nucleotide sequence can be operably linked to a second promoter. The first and second promoters can be the same, or the first and second promoters can be different. The first promoter can be a U6 promoter and the second promoter can be a CMV promoter. The nucleic acid construct can further include a nucleotide sequence encoding a reporter. The reporter can be a fluorescent polypeptide. The nucleotide sequence encoding the reporter can be downstream of the second nucleotide sequence encoding the cDNA, and can be separated from the second nucleotide sequence by an

IRES. The nucleic acid construct can be within a viral vector. The viral vector can be an AAV vector (e.g., an AAV serotype 9 vector or an AAV2/9 vector). The cell can be a cardiomyocyte.

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

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

FIG. 1A is a diagram of an exemplary KCNQ1-P2A AAV construct, and **FIG. 1B** shows the DNA sequence (SEQ ID NO:1029) for the construct. **FIG. 1C** shows a *KCNQ1* target sequence (sh#5; SEQ ID NO:102), a corresponding shIMM *KCNQ1* sequence (SEQ ID NO:103), a wild type *KCNQ1* nucleotide sequence (SEQ ID NO:1030, with the sh#5 sequence underlined), a corresponding shIMM *KCNQ1* nucleotide sequence (SEQ ID NO:1031, with the shIMM sequence underlined), and a KCNQ1 amino acid sequence (SEQ ID NO:1032).

FIG. 2A is a diagram of an exemplary KCNH2-P2A AAV construct, and **FIG. 2B** shows the DNA sequence (SEQ ID NO:1033) for the construct. The encoded AmpR amino acid sequence (SEQ ID NO:2784) also is shown. **FIG. 2C** shows a *KCNH2* target sequence (RAB_sh#4; SEQ ID NO:27), a corresponding shIMM *KCNH2* sequence (SEQ ID NO:29), a wild type *KCNH2* nucleotide sequence (SEQ ID NO:1034, with the RAB_sh#4 sequence underlined), a corresponding shIMM *KCNH2* nucleotide sequence

(SEQ ID NO:1035, with the shIMM sequence underlined), and a KCNH2 amino acid sequence (SEQ ID NO:1036).

FIG. 3A is a diagram of an exemplary SCN5A-P2A Lenti construct, and **FIG. 3B** shows the DNA sequence (SEQ ID NO:1041) for the construct. **FIG. 3C** shows a *SCN5A* target sequence (sh#4; SEQ ID NO:30), a corresponding shIMM *SCN5A* sequence (SEQ ID NO:32), a wild type *SCN5A* nucleotide sequence (SEQ ID NO:1042, with the sh#5 sequence underlined), a corresponding shIMM *SCN5A* nucleotide sequence (SEQ ID NO:1043, with the shIMM sequence underlined), and a SCN5A amino acid sequence (SEQ ID NO:1044).

FIG. 4A is a diagram of an exemplary PKP2-P2A AAV construct, and **FIG. 4B** shows the DNA sequence (SEQ ID NO:1037) for the construct. **FIG. 4C** shows a *PKP2* target sequence (sh#36; SEQ ID NO:52), a corresponding shIMM *PKP2* sequence (SEQ ID NO:993), a wild type *PKP2* nucleotide sequence (SEQ ID NO:1038, with the sh#5 sequence underlined), a corresponding shIMM *PKP2* nucleotide sequence (SEQ ID NO:1039, with the shIMM sequence underlined), and a PKP2 amino acid sequence (SEQ ID NO:1040).

FIGS. 5A-5C show results obtained from experiments used to test *KCNQ1* shRNAs for the KCNQ1-SupRep vector. TSA201 cells were co-transfected with KCNQ1-WT and various *KCNQ1* shRNAs or a non-targeting scrambled shRNA control (shCT). **FIG. 5A** includes a graph (top) plotting *KCNQ1* expression for cells co-transfected with four commercial shRNAs (sh#1-4), normalized to GAPDH, measured by qRT-PCR. An image of a representative western blot of KCNQ1 with cofilin housekeeping control also is shown (bottom). **FIG. 5B** is a graph plotting ImageJ quantification of western blot relative pixel density. *KCNQ1* sh#4 was selected for the final KCNQ1-SupRep gene therapy vector, and is referred to as shKCNQ1 in the further studies described herein. Results and representative images were obtained from three independent experiments (defined as three identical repeats of each experiment conducted from start to finish on separate weeks with one biological replicate per treatment group per run). Graphs show mean ± S.D. One-way ANOVA with post-hoc Tukey's test for multiple comparisons also was used. *$p<0.05$. **FIG. 5C** is a graph plotting knockdown of

KCNQ1 in TSA201 cells co-transfected with various custom shRNAs (sh#5-sh#8), normalized to GAPDH, determined using qPCR.

FIGS. 6A and 6B depict the design for the *KCNQ1* suppression-replacement (KCNQ1-SupRep) vector. **FIG. 6A** shows a sequence alignment of the target sequence portion of shKCNQ1 (SEQ ID NO:7) to KCNQ1-WT cDNA (SEQ ID NO:8) (top) and "shRNA-immune" *KCNQ1* (KCNQ1-shIMM, bottom) (SEQ ID NO:9), which includes 10 wobble base synonymous variants (underlined). The amino acid sequence shown is KCNQ1 p.V458-P469 (c.1372-1407, NM_000218.2) (SEQ ID NO:10). **FIG. 6B** is a schematic of representative KCNQ1-SupRep vector maps. (U6) U6 promoter; (CMV) cytomegalovirus promoter; (MHC) alpha-myosin heavy chain promoter, (MLC) myosin light chain 2 promoter, (TnC) cardiac troponin C promoter, (TnT) cardiac troponin T promoter, (E) calsequestrin-2 cardiomyocyte-specific transcriptional cis-regulatory enhancer motif, (IRES) internal ribosome entry site; and (CFP) cyan fluorescent protein.

FIGS. 7A and 7B show that shKCNQ1 knocks down KCNQ1-WT but not KCNQ1-shIMM in TSA201 cells co-transfected with KCNQ1-WT or KCNQ1-shIMM and shCT, shKCNQ1, or KCNQ1-SupRep. **FIG. 7A** is a graph (top) plotting relative *KCNQ1* expression normalized to GAPDH measured by allele-specific qRT-PCR quantifying KCNQ1-WT (white) and KCNQ1-shIMM (grey). Results were confirmed with western blotting (bottom) for KCNQ1 with cofilin as housekeeping control. **FIG. 7B** is a graph plotting ImageJ quantification of western blot pixel density. Results and representative images were obtained from three independent experiments (defined as three identical repeats of each experiment conducted from start to finish on separate weeks with one biological replicate per treatment group per run). Both graphs show mean ± S.D. For relative *KCNQ1*, one-way ANOVA with post-hoc Tukey's test for multiple comparisons was used in both **FIG. 7A and FIG. 7B**. For the sample treated with KCNQ1-SupRep in **FIG. 7A**, an unpaired 2-tailed student's t-test was used to compare the proportion of KCNQ1-WT compared to KCNQ1-shIMM (vertical bracket). *$p<0.05$.

FIG. 8 is a graph plotting relative *KCNQ1* levels, indicating that suppression and replacement of KCNQ1-WT by shKCNQ1 and KCNQ1-SupRep was dose-dependent. TSA201 cells were co-transfected with 100 fmol KCNQ1-WT and a range (0-300 fmol)

of shCT, shKCNQ1, or KCNQ1-SupRep. *KCNQ1* expression was measured by allele-specific qRT-PCR and normalized to *GAPDH*. Markers represent the total *KCNQ1*. For KCNQ1-SupRep treatment when both KCNQ1-WT and -shIMM were present simultaneously, the allele-specific proportions of KCNQ1-WT (light grey shading) and KCNQ1-shIMM (dark grey shading) are shown.

FIG. 9 is a graph plotting relative *KCNQ1* levels during activation of the two components of KCNQ1-SupRep showing that both shKCNQ1 and KCNQ1-shIMM activate at essentially the same rate. TSA201 cells were co-transfected with 100 fmol KCNQ1-WT and 100 fmol of shCT, shKCNQ1, KCNQ1-shIMM, or KCNQ1-SupRep and RNA harvested at different time points from 0 hours to 72 hours. *KCNQ1* expression was measured by allele-specific qRT-PCR and normalized to *GAPDH*. Markers represent the total *KCNQ1*. For KCNQ1-SupRep treatment when both KCNQ1-WT and -shIMM were present simultaneously, the allele-specific proportion of KCNQ1-WT (light grey shading) and KCNQ1-shIMM (dark grey shading) are shown. Cells treated with KCNQ1-WT and shCT have nearly identical total *KCNQ1* compared to cells treated with KCNQ1-WT and KCNQ1-SupRep, however in KCNQ1-SupRep, the proportion of KCNQ1-WT (light grey shading) is strongly suppressed while the proportion of KCNQ1-shIMM (dark grey shading) becomes the predominant form of KCNQ1 present.

FIGS. 10A-10C show patch clamp analysis of I_{Ks} in TSA201 cells co-transfected with KCNQ1-WT, KCNQ1-shIMM, or KCNQ1-variants and the Kv7.1 beta-subunit, *KCNE1*. **FIG. 10A** shows representative voltage clamp I_{Ks} traces for the indicated constructs, determined from a holding potential of -80mV and test potentials from -40mV to +80mV in 10mV increments with 4s duration. KCNQ1-shIMM produced WT I_{Ks} current (top). KCNQ1-Y171X, KCNQ1-V254M, and KCNQ1-I567S produced no I_{Ks} current (bottom). **FIG. 10B** is a graph plotting peak current density in the transfected cells. Error bars represent standard error of the mean (S.E.M.). **FIG. 10C** is a graph plotting peak current density at the +80mV depolarization step. Error bars represent standard deviation (S.D.). One-way ANOVA with post-hoc Tukey's test for multiple comparisons also was used. *$p<0.05$.

FIG. 11 is a series of representative images showing immunofluorescence of TSA201 cells transfected with KCNQ1-WT, KCNQ1-shIMM, or KCNQ1-variants. KCNQ1-shIMM and KCNQ1-WT both trafficked to the cell membrane. KCNQ1-Y171X resulted in a premature stop codon and no expressed protein, while KCNQ1-V254M correctly trafficked to the cell membrane. KCNQ1-I567S created detectable protein, although seemingly at a lower expression level consistent with qPCR and western blot results. DAPI was used to stain nuclei, KCNQ1 (green), and merge. Representative images were obtained from three independent experiments (defined as three identical repeats of this experiment conducted from start to finish on separate weeks with one biological replicate per treatment group per run). Scale bars = 20 μm.

FIG. 12 includes a graph (top) and a western blot (bottom) showing that KCNQ1-SupRep knocked down LQT1 disease-causing *KCNQ1* variants, including both nonsense and missense variants, and replaced the variants with KCNQ1-shIMM. TSA201 cells were co-transfected with KCNQ1-WT or KCNQ1-variants and shCT, shKCNQ1, or KCNQ1-SupRep. shKCNQ1 knocks down *KCNQ1* in a variant-independent manner. KCNQ1-SupRep knocks down *KCNQ1* variants via shKCNQ1 and expresses KCNQ1-shIMM, which is knockdown immune. The graph at the top of **FIG. 12** demonstrates proportional expression of KCNQ1-WT/variants and KCNQ1-shIMM, detected using allele-specific qRT-PCR to measure KCNQ1-WT/variant (white) and KCNQ1-shIMM (gray). Overall KCNQ1 expression (not allele-specific) was validated by western blotting with cofilin as a housekeeping control (**FIG. 12**, bottom). Results and representative images were obtained from three independent experiments (defined as three identical repeats of each experiment conducted from start to finish on separate weeks with one biological replicate per treatment group per run). The graph shows mean ± S.D. For relative *KCNQ1*, a separate one-way ANOVA with post-hoc Tukey's test for multiple comparisons was conducted for each *KCNQ1* variant to compare the three treatments and avoid extraneous comparisons between variants. In samples treated with KCNQ1-SupRep, an unpaired two-tailed student's t-test was used to compare the proportion of KCNQ1-WT compared to KCNQ1-shIMM (vertical brackets). *$p<0.05$.

FIGS. 13A-13D show quality control of iPSCs derived from four patients with LQT1, an unrelated healthy control, and two CRISPR-Cas9 corrected isogenic control iPSCs generated from two of the LQT1 patient iPSCs (KCNQ1-V254M and KCNQ1-A344A/spl). **FIG. 13A** shows Sanger sequencing confirmation of LQT1-causative *KCNQ1* variants in iPSCs derived from patients with LQT1 (middle), from an unrelated healthy control (top), and from isogenic controls (bottom). **FIGS. 13B-13D** show representative quality control studies completed for all iPSC lines, including normal karyotype (**FIG. 13B**), bright field image of an iPSC colony with normal morphology (**FIG. 13C**), and immunofluorescence microscopy (**FIG. 13D**) for markers of pluripotency including DAPI nuclear stain, Tra-1-60 or SSEA-4, Nanog or Oct-4, and a merged image. Scale bars = 20 μM. (spl) splice; (*) silent variant introduced during CRISPR-Cas9 correction to prevent reintroduction of double-strand breaks after successful editing of the transfected target cell.

FIG. 14 includes representative images showing immunofluorescence of iPSC-CMs derived from a patient with KCNQ1-V254M mediated LQT1, one week after transduction with lentiviral shCT or KCNQ1-SupRep. The patient-derived iPSC-CMs were stained with three separate antibodies to demonstrate (1) the presence of cardiomyocytes (cardiac troponin T, CTNT), (2) transduction by lentivirus as indicated by the turboGFP reporter (GFP) in shCT or by the CFP reporter in KCNQ1-SupRep, and (3) the presence of KCNQ1 either endogenously or as the result of treatment with KCNQ1-SupRep. The results showed that high purity populations of cardiomyocytes were evenly transduced with lentiviral shCT or KCNQ1-SupRep. With shCT, there was weak staining for KCNQ1, but when cells were treated with KCNQ1-SupRep, KCNQ1 staining was bright, indicating robust expression. Cells were counterstained with DAPI for nuclear stain. The figure shows representative images of iPSC-CMs from one LQT1 variant (KCNQ1-V254M). Immunofluorescence results for iPSC-CMs derived from the unrelated control and other three LQT1 variants (KCNQ1-Y171X, -I567S, and -A344A/spl) are found in **FIGS. 15A-15D**. Scale bars 50 μm.

FIGS. 15A-15D show immunofluorescence images from the iPSC-CMs not shown in **FIG. 14**, including the unrelated control (**FIG. 15A**) and three LQT1 variants

(KCNQ1-Y171X, -I567S, and -A344A/spl; **FIGS. 15B, 15C, and 15D**, respectively). Immunofluorescence images were acquired one week after transduction with lentiviral shCT or KCNQ1-SupRep. The patient-derived iPSC-CMs were stained with three separate antibodies to demonstrate (1) presence of cardiomyocytes (cardiac troponin T; CTNT), (2) transduction by lentivirus as indicated by the turboGFP reporter in shCT (GFP or CFP in KCNQ1-SupRep), and (3) the presence of KCNQ1, either endogenous or as the result of treatment with KCNQ1-SupRep. The results showed high purity populations of cardiomyocytes that were evenly transduced with lentiviral shCT or KCNQ1-SupRep. In shCT, there was weak staining for KCNQ1, but in treatment with KCNQ1-SupRep, KCNQ1 staining was bright and indicated robust expression. Cells were counterstained with DAPI for nuclear stain. Scale bars = 50 μm.

FIGS. 16A and 16B show that action potential duration (APD) was shortened in LQT1 iPSC-CMs treated with lentivirus containing KCNQ1-SupRep compared to shCT. **FIG. 16A** includes a series of representative traces showing three consecutive FluoVolt™ voltage dye optical action potentials paced at 1 Hz for untreated, unrelated healthy control and KCNQ1-Y171X, KCNQ1-V254M, KCNQ1-I567S, and KCNQ1-A344A/spl iPSC-CMs treated with shCT or KCNQ1-SupRep. **FIG. 16B** includes a series of graphs plotting APD_{90} and APD_{50} values for untreated, unrelated healthy control and KCNQ1-Y171X, KCNQ1-V254M, KCNQ1-I567S, and KCNQ1-A344A/spl iPSC-CMs treated with shCT or KCNQ1-SupRep. Action potential trace videos were obtained for a 20 second duration at 50 fps with 1 Hz pacing. Regions of interest containing flashing cells were identified, and the changes in fluorescence intensity over time were measured to produce optical action potentials from which APD_{90} and APD_{50} values were determined. APD_{90} and APD_{50} values for all action potentials within a 20 second trace were averaged to produce a single data point. The total number of measurements (n) is shown. Box plots show median and interquartile range with whiskers extending to minimum and maximum values. Baseline APD_{90} and APD_{50} values were assessed by one-way ANOVA with post-hoc Dunnett's test comparing each *KCNQ1* variant treated with shCT to the untreated, unrelated control (**TABLE 5**). APD shortening due to KCNQ1-SupRep compared to

treatment with shCT was assessed by unpaired two-tailed student's t-tests at both the APD_{90} and APD_{50} levels separately for each variant. *$p<0.0001$.

FIGS. 17A and 17B show that CRISPR-Cas9 corrected isogenic controls serve as a marker for "perfect" correction of the cardiac APD. FluoVolt™ voltage dye measurement of the cardiac APD was conducted in isogenic control iPSC-CMs generated from two of the four LQT1 iPSCs (KCNQ1-V254M and KCNQ1-A344A/spl). Data for treatment with shCT or KCNQ1-SupRep was shown here unchanged from **FIGS. 16A and 16B**. Both isogenic control iPSC-CMs had significantly shorter APD_{90} and APD_{50} than the LQT1 iPSC-CMs treated with shCT, which indicated that correction of the single pathogenic LQT1 variant in KCNQ1 was able to rescue the disease phenotype *in vitro*. As with the unrelated control, the isogenic controls were measured untreated as to provide the purest signal for a normal APD. Treatment of LQT1 iPSC-CMs with KCNQ1-SupRep resulted in APD shortening, although the degree of shortening was variable. For KCNQ1-V254M, KCNQ1-SupRep undercorrected the prolonged APD_{90} and overcorrected the APD_{50}. In KCNQ1-A344A/spl, ideal correction for the APD_{90} was achieved and matched the isogenic control APD_{90}, but overcorrection of the APD_{50} also occurred. **FIG. 17A** includes representative traces showing three consecutive action potentials paced at 1 Hz. **FIG. 17B** includes a pair of graphs plotting APD_{90} and APD_{50} values for untreated, isogenic controls, and KCNQ1-V254M and KCNQ1-A344A/spl iPSC-CMs treated with shCT or KCNQ1-SupRep. Action potential trace videos were obtained for a 20 second duration at 50 fps with 1 Hz pacing. Regions of interest containing flashing cells were identified, and the changes in fluorescence intensity over time were measured to produce optical action potentials from which APD_{90} and APD_{50} values were determined. APD_{90} and APD_{50} values for all action potentials within a 20 second trace were averaged to produce a single data point. The total number of measurements (n) is shown. Box plots show median and interquartile range with whiskers extending to minimum and maximum values. A one-way ANOVA with post-hoc Tukey's test comparing all pairs for APD_{90} and all pairs for APD_{50} was used for each *KCNQ1* variant tested. *$p<0.0001$, unless indicated by a specific p-value in the figure.

FIGS. 18A-18D show that use of iPSC-CM 3D organoid culture system can achieve results similar to those obtained in standard syncytial monolayer culture. To assess whether culture in 3D organoid or syncytial monolayer yields findings similar to monolayer culture, the iPSC-CMs from one of the four patients with LQT1 (KCNQ1-Y171X) were dissociated and plated into a round mold containing thick collagenous MATRIGEL® to form a spheroid. After 2-3 days, the iPSC-CMs formed a strong beating syncytium in 3D, and were used as the organoid model for this study. The organoids were treated with KCNQ1-SupRep, shCT, or left untreated as control. Seven days post viral transduction, the organoids were assayed by immunofluorescence or FluoVolt™ voltage dye. **FIG. 18A** is an image of a beating iPSC-CM organoid suspended in media in a 24-well culture plate, with a zoomed in image shown in the inset. **FIG. 18B** includes representative images of organoids that were fixed, cryosectioned, and stained for immunofluorescence using the cardiomyocyte marker cardiac troponin T (CTNT; top) and the lentiviral transduction marker as indicated by the turboGFP reporter in shCT (GFP; middle) or by the CFP reporter in KCNQ1-SupRep (bottom). **FIG. 18C** is a representative trace of FluoVolt™ voltage dye in the untreated LQT1 organoid or the LQT1 organoid treated with KCNQ1-SupRep. **FIG. 18D** is a graph plotting overall APD_{90} and APD_{50} values for untreated and KCNQ1-SupRep treated organoids from KCNQ1-Y171X iPSC-CMs. *$p<0.0001$.

FIGS. 19A-19F provide a summary of the LQT1 and LQT2 transgenic rabbit phenotype. Shown in **FIG. 19A** are schematic representations of pathogenic variants (KCNQ1-Y315S and KCNH2-G628S) in the KCNQ1-encoded potassium channel subunit (left) and KCNH2-encoded potassium channel subunit polypeptides (right) and the transgenic constructs (bottom). **FIG. 19B** includes representative electrocardiogram traces showing the differences in QT interval between wild-type (WT), LQT1, and LQT2 rabbits. **FIG. 19C** is a bar graph showing the significant difference in QT interval duration between WT and LQT1 or LQT2 rabbits. **FIG. 19D** shows the spontaneous torsades de pointes (TdP) in a oestradiol-treated LQT2 rabbit initiated by short-long-short sequence. **FIG. 19E** includes representative cellular cardiac action potential traces that demonstrated prolonged action potential durations in LQT1 and LQT2 rabbit

cardiomyocytes compared with cardiomyocytes from WT rabbits. **FIG. 19F** shows IV-curves of I_{Ks} and I_{Kr} currents in cardiomyocytes isolated form WT, LQT1, and LQT2 rabbit hearts, indicating the loss of I_{Ks} in LQT1 rabbits and loss of I_{Kr} in LQT2 rabbits.

FIGS. 20A-20C demonstrate generation and confirmation of KCNH2-G604S and KCNH2-N633S iPSC lines. **FIG. 20A** is an image of a karyotype, showing that each clone had a normal karyotype for their respective sex. **FIG. 20B** is an image showing phase-contrast light images of iPSC colonies from each of the patient cell lines used for the study. **FIG. 20C** contains representative Sanger sequencing chromatograms for the patent cell lines. The boxes indicate the relevant codon, and the stars indicate the exact nucleotide of interest. Scale bars = 50 μm.

FIG. 21 is an image showing immunocytochemistry for p.G604S clone #1, p.G604S clone #2, p.N633S clone #1, and p.N633S clone #2. Each of the respective clones for each line was demonstrated to express Nanog and SSEA4 pluripotency markers. Scale bars = 20 μm.

FIG. 22 is a graph plotting knockdown of *KCNH2* in TSA201 cells with various shRNAs, determined using qPCR.

FIG. 23 is a graph plotting the results of FluoVolt™ studies using CRISPR-Cas9 corrected isogenic controls as a marker for correction of cardiac APD in N633S iPSC-CMs and isogenic control iPSC-CMs generated from LQT2 iPSCs (N633S). APD_{90B} and APD_{50B} values were determined for isogenic control treated with shCT, and for KCNH2-N633S variant treated with shCT or KCNH2-SupRep. Action potential trace videos were obtained for a 20 second duration at 50 fps with 1 Hz pacing. Regions of interest containing flashing cells were identified, and the changes in fluorescence intensity over time were measured to produce optical action potentials from which APD_{90} and APD_{50} values were determined. APD_{90} and APD_{50} values for all action potentials within a 20 second trace were averaged to produce a single data point, and Bazett corrected APD_{90B} and APD_{50B} values were plotted. The total number of measurements (n) and medians (horizontal black lines) are indicated. A one-way ANOVA with post-hoc Tukey's test comparing all pairs for APD_{90B} and all pairs for APD_{50B} was used.

FIG. 24 is a graph plotting the results of FluoVolt™ voltage dye measurement of cardiac APD in N633S iPSC-CMs and isogenic control iPSC-CMs generated from LQT2 iPSCs (N633S). APD_{90B} and APD_{50B} values for the untreated (UT) KCNH2-N633S variant, the SupRep treated isogenic control, and the untreated (UT) isogenic control are shown. Action potential trace videos were obtained for a 20 second duration at 50 fps with 1 Hz pacing. Regions of interest containing flashing cells were identified, and the changes in fluorescence intensity over time were measured to produce optical action potentials from which APD_{90} and APD_{50} values were determined. APD_{90} and APD_{50} values for all action potentials within a 20 second trace were averaged to produce a single data point. Bazett corrected APD_{90B} and APD_{50B} values are shown, and the total number of measurements (n) is indicated. Dot plots show median (horizontal black line). A one-way ANOVA with post-hoc Tukey's test comparing all pairs for APD_{90B} and all pairs for APD_{50B} was used.

FIG. 25 is a graph plotting the results of FluoVolt™ voltage dye measurement of cardiac APD in G604S iPSC-CMs. APD_{90} and APD_{50} values for KCNH2-G604S variant treated with shCT and KCNH2-G604S variant treated with KCNH2-SupRep are shown. Treatment of LQT2 iPSC-CMs with KCNH2-SupRep results in significant APD_{90} and APD_{50} shortening compared to those treated with shCT. Action potential trace videos were obtained for a 20 second duration at 50 fps with 1 Hz pacing. Regions of interest containing flashing cells were identified, and the changes in fluorescence intensity over time were measured to produce optical action potentials from which APD_{90} and APD_{50} values were determined. APD_{90} and APD_{50} values for all action potentials within a 20 second trace were averaged to produce a single data point. The total number of measurements (n) is shown. Dot plots show median (horizontal black line). A student's t-test comparing all pairs for APD_{90B} and all pairs for APD_{50B} was used.

FIG. 26 is a graph plotting APD_{90} and APD_{50} values for the KCNH2-G604S variant treated with shCT (1), and KCNH2-SupRep (2), or CRISPR-Cas9 corrected isogenic control treated with shCT (3). Treatment of the KCNH2-G604S iPSC-CMs with KCNH2-SupRep resulted in significant APD_{90} shortening compared to treatment with shCT. Action potential trace videos were obtained for 20 second durations at 50 fps with

1 Hz pacing. Regions of interest containing flashing cells were identified, and the changes in fluorescence intensity over time were measured to produce optical action potentials from which APD_{90} and APD_{50} values were determined. APD_{90} and APD_{50} values for all action potentials within a 20 second trace were averaged to produce a single data point. The total number of measurements (n) is shown. The graph also shows the medians (horizontal black lines). A one-way ANOVA with post-hoc Tukey's test was usd to compare all pairs for APD_{90} and all pairs for APD_{50}.

FIG. 27 is a graph plotting APD_{90} and APD_{50} values for the KCNH2-G628S variant treated with shCT (1), KCNH2-SupRep (2), or CRISPR-Cas9 corrected isogenic control treated with shCT (3). Treatment of the KCNH2-G628S iPSC-CMs with KCNH2-SupRep resulted in significant APD_{90} shortening compared to treatment with shCT. Action potential trace videos were obtained for 20 second durations at 50 fps with 1 Hz pacing. Regions of interest containing flashing cells were identified, and the changes in fluorescence intensity over time were measured to produce optical action potentials from which APD_{90} values were determined. APD_{90} values for all action potentials within a 20 second trace were averaged to produce a single data point. The total number of measurements (n) is shown. The graph also shows the medians (horizontal black lines). A one-way ANOVA with post-hoc Tukey's test was used to compare all pairs for APD_{90}.

FIGS. 28A and 28B show that KCNH2-SupRep knocked down LQT2 disease-causing KCNH2 missense variants and replaced them with KCNH2-shIMM. TSA201 cells were co-transfected with KCNH2-WT or KCNH2-variants and shCT, shKCNH2, or KCNH2-SupRep. shKCNH2 knocked down KCNH2 in a variant-independent manner. **FIG. 28A** is a graph plotting proportional expression of KCNH2-WT/variants and KCNH2-shIMM, which were detected using allele-specific qRT-PCR to measure KCNH2-WT/variant (white) and KCNH2-shIMM (grey). **FIG. 28B** is an image of a western blot showing overall KCNH2 expression (not allele-specific), with GAPDH as a housekeeping control.

FIGS. 29A and 29B show that shKCNH2 knocked down KCNH2-WT but not KCNH2-shIMM in TSA201 cells co-transfected with KCNH2-WT or KCNH2-shIMM and shCT, shKCNH2, or KCNH2-SupRep. **FIG. 29A** is a graph plotting relative KCNH2

expression normalized to GAPDH, as measured by allele-specific qRT-PCR to quantify KCNH2-WT (white) and KCNH2-shIMM (grey). Results were confirmed with western blotting (**FIG. 29B**) for KCNH2, with GAPDH as a housekeeping control.

FIGS. 30A-30D show that KCNH2-AAV-P2A CTnC-EGFP did not generate KCNH2 current in heterologous TSA 201 cells. **FIG. 30A** is a plot of representative whole cell I_{Kr} tracings from TSA201 cells expressing KCNH2-WT with KCNE2, determined from a holding potential of -80 mV and testing potentials from -40 mV to +60 mV in 10 mV increments with a 3 second duration. **FIG. 30B** shows representative whole cell outward tracings from TSA201 cells expressing KCNH2-AAV-P2A CTnC-EGFP, determined from a holding potential of -80 mV and testing potentials from -40 mV to +60 mV in 10 mV increments with a 3 second duration. **FIG. 30C** is a graph plotting current-voltage relationship for KCNH2-pIRES2-EGFP with KCNE2-pIRES2-dsRed2 (n=9) and KCNH2-AAV-P2A CTnC-EGFP (n=8). All values represent mean ± SEM. **FIG. 30D** is a graph plotting peak current density at +10 mV for KCNH2-pIRES2-EGFP with KCNE2-pIRES2-dsRed2 (n=9) and KCNH2-AAV-P2A CTnC-EGFP (n=8). All values represent mean ± SEM.

FIGS. 31A-31E show that KCNH2-AAV-P2A CTnC-EGFP generated E-4031 sensitive outward current in H9C2 cells. **FIG. 31A** includes representative whole cell outward current tracings from empty H9C2 cells (upper panel), H9C2 cells expressing KCNH2-AAV-P2A CTnC-EGFP before E-4031 (middle panel), and H9C2 cells expressing KCNH2-AAV-P2A CTnC-EGFP after E-4031 (lower panel) determined from a holding potential of -80 mV and testing potentials from -40 mV to +60 mV in 10 mV increments with a 3 second duration. **FIG. 31B** is a graph plotting current-voltage relationship for outward current from empty H9C2 cells and H9C2 cells expressing KCNH2-AAV-P2A CTnC-EGFP (n=9). All values represent mean ± SEM. **FIG. 31C** is a graph plotting peak current density at +60 mV from empty H9C2 cells and H9C2 cells expressing KCNH2-AAV-P2A CTnC-EGFP (n=9). All values represent mean ± SEM. **FIG. 31D** is a graph plotting current-voltage relationship for H9C2 cells expressing KCNH2-AAV-P2A CTnC-EGFP, before and after E-4031(n=6). All values represent mean ± SEM. **FIG. 31E** is a graph plotting peak current density at +60 mV from H9C2

cells expressing KCNH2-AAV-P2A CTnC-EGFP, before and after E-4031 (n=6). All values represent mean ± SEM.

FIG. 32 is a graph plotting APD_{90} and APD_{50} values for the KCNH2-N588K variant treated with shCT (1), KCNH2-SupRep (2), or isogenic control treated with shCT (3). Treatment of SQT1 iPSC-CMs with KCNH2-SupRep resulted in significant APD_{90} prolongation compared to treatment with shCT. Action potential trace videos were obtained for 20 second durations at 50 fps with 1 Hz pacing. Regions of interest containing flashing cells were identified, and the changes in fluorescence intensity over time were measured to produce optical action potentials from which APD_{90} and APD_{50} values were determined. APD_{90} and APD_{50} values for all action potentials within a 20 second trace were averaged to produce a single data point. The total number of measurements (n) is shown. The graph also shows medians (horizontal black line). A one-way ANOVA with post-hoc Tukey's test was used to compare all pairs for APD_{90} and APD_{50} was used.

FIGS. 33A-33D show quality control for iPSCs derived from a patient with the SCN5A-F1760C variant. **FIG. 33A** is a bright field image of an iPSC colony with normal morphology. **FIG. 33B** shows the Sanger sequencing confirmation (SEQ ID NO:1047) of the LQT3-causing SCN5A-F1760C variant in iPSCs derived from the patient. **FIG. 33C** is an image showing a normal karyotype for the iPSC line generated from the patient's blood sample. **FIG. 33D** includes images of immunofluorescence microscopy for markers of pluripotency, including DAPI nuclear stain, Tra-1-60 or SSEA-4, Nanog or Oct-4, and a merged image.

FIG. 34 is a graph plotting knockdown of *SCN5A* in TSA201 cells with various shRNAs, determined using qPCR.

FIG. 35 is a schematic showing representative SCN5A-SupRep vector maps. (CMV) cytomegalovirus promoter; (MCS) multiple cloning site; (U6) U6 promoter; (ChlorR) chloramphenicol resistance gene; (Ori) origin of replication; (WPRE) Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element; (GFP) green fluorescent protein; (P2A) a member of 2A self-cleaving peptide family; (HA) tag

derived from the human influenza hemagglutinin molecule corresponding to amino acids 98-106.

FIGS. 36A and 36B show that the APD was shortened in LQT3 SCN5A-F1760C iPSC-CMs treated with lentivirus containing SCN5A-SupRep, compared to untreated cells. **FIG. 36A** includes representative traces showing five consecutive FLUOVOLT™ voltage dye optical action potentials paced at 1 Hz for untreated and SCN5A-SupRep treated SCN5A-F1760C iPSC-CMs. **FIG. 36B** is a graph plotting APD_{90} and APD_{50} values for untreated and SCN5A-SupRep treated SCN5A-F1760C iPSC-CMs. Action potential trace videos were obtained for a 20 second duration at 50 fps with 1 Hz pacing. Regions of interest containing flashing cells were identified, and the changes in fluorescence intensity over time were measured to produce optical action potentials from which APD_{90} and APD_{50} values were determined. APD_{90} and APD_{50} values for all action potentials within a 20 second trace were averaged to produce a single data point.

FIG. 37 is a graph plotting knockdown of *MYH7* in TSA201 cells with various shRNAs, determined using qPCR.

FIG. 38 is a graph plotting knockdown of *PKP2* in TSA201 cells with various shRNAs, determined by qRT-PCR.

FIGS. 39A-39D show quality control of iPSCs derived from a patient with a PKP2-c2146-1G>C variant. **FIG. 39A** includes bright field images of iPSC colonies with normal morphology. **FIG. 39B** shows Sanger sequencing confirmation of the ACM-causative PKP2-c2146-1G>C variant in iPSCs derived from the patient with ACM. **FIG. 39C** shows a normal karyotype for clones from the iPSC line generated from the patient's blood sample. **FIG. 39D** includes images of immunofluorescence microscopy for DAPI nuclear stain and markers of pluripotency, including Tra-1-60 or SSEA-4, Nanog or Oct-4, and a merged image.

FIG. 40 includes a series of graphs showing that calcium transient duration (CTD) and decay were shortened in ACM iPSC-CMs treated with lentivirus containing PKP2-SupRep compared to untreated cells. Given that PKP2-mediated ACM-associated arrhythmic events are often associated with exertion, calcium handling measurements were performed under both baseline and following treatment with the adrenergic agonist,

isoproterenol (Iso). Trace videos were obtained for a 20 second duration at 50 fps with 0.5 Hz pacing. Regions of interest containing flashing cells were identified, and the changes in fluorescence intensity over time were measured to produce calcium transient traces from which the values were determined. All values of calcium transients within a 20 second trace were averaged to produce a single data point for all the parameters except for calcium amplitude, where only the first value was taken for analysis.

FIG. 41 is a graph plotting knockdown of *DSP* in TSA201 cells with various shRNAs, determined by qRT-PCR.

FIG. 42 is a graph plotting knockdown of *MYBPC3* in TSA201 cells with various shRNAs, determined by qRT-PCR.

FIG. 43 is a graph plotting knockdown of *RBM20* in TSA201 cells with various shRNAs, determined by qRT-PCR.

FIG. 44 is a graph plotting knockdown of *CACNA1C* in TSA201 cells with various shRNAs, determined by qRT-PCR.

FIG. 45 is a graph plotting knockdown of CALM1 in TSA201 cells with various shRNAs, determined by qRT-PCR.

FIG. 46 is a graph plotting knockdown of CALM2 in TSA201 cells with various shRNAs, determined by qRT-PCR.

FIG. 47 is a graph plotting knockdown of CALM3 in TSA201 cells with various shRNAs, determined by qRT-PCR.

FIG. 48 is a graph plotting knockdown of KCNJ2 in TSA201 cells with various shRNAs, determined by qRT-PCR.

FIG. 49 is a graph plotting knockdown of CASQ2 in TSA201 cells with various shRNAs, determined by qRT-PCR.

FIG. 50 is a graph plotting knockdown of DSG2 in TSA201 cells with various shRNAs, determined by qRT-PCR.

FIG. 51 is a graph plotting knockdown of TNNT2 in TSA201 cells with various shRNAs, determined by qRT-PCR.

FIG. 52 is a graph plotting knockdown of TPM1 in TSA201 cells with various shRNAs, determined by qRT-PCR.

FIG. 53 is a graph plotting knockdown of LMNA in TSA201 cells with various shRNAs, determined by qRT-PCR.

FIG. 54 is a graph plotting knockdown of PLN in TSA201 cells with various shRNAs, determined by qRT-PCR.

DETAILED DESCRIPTION

This document provides methods and materials for treating a mammal having a congenital disease (e.g., a congenital heart disease such as a LQTS or, more specifically, LQT1, LQT2, or LQT3) through suppression of endogenous causative allele(s) and replacement with/expression of a non-mutant (non-causative), non-suppressed coding sequence. In general, the methods and materials provided herein involve the use of nucleic acid constructs that contain one or more suppressive components (e.g., an RNAi nucleic acid such as a shRNA) designed to suppress the expression of one or more disease-associated alleles (or their transcribed RNAs) within one or more types of cells (e.g., cardiomyocytes) present within a mammal (e.g., the heart of a mammal such as a human having LQTS, or more specifically, LQT1, LQT2, or LQT3), in combination with one or more corrective components (e.g., a nucleic acid encoding a version of the disease-associated allele that encodes a wild type polypeptide and is immune to the suppressive component). The methods and materials provided herein can be used to reduce one or more symptoms or effects of the disease caused by allele(s) targeted by the suppressive component.

In some cases, this document provides a suppression-and-replacement (SupRep) nucleic acid that can be used to treat a mammal having a congenital disorder. Disorders that can be treated according to the methods provided herein include, without limitation, LQTS (e.g., LQT1, LQT2, LQT3, LQT4, LQT5, LQT6, LQT7, LQT8, LQT9, LQT10, LQT11, LQT12, LQT13, LQT14, LQT15, LQT16, or LQT17), Brugada syndrome (BrS), catecholaminergic polymorphic ventricular tachycardia (CPVT), arrhythmogenic cardiomyopathy (ACM), hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), SQTS, Timothy syndrome, left ventricular non-compaction cardiomyopathy (LVNC), skeletal myopathy, Andersen-Tawil syndrome (ATS), familial

hypercholesterolemia (FH), cardiomyopathies, atrial fibrillation, and Triadin knockout syndrome (TKOS).

The nucleic acids provided herein include two main components – a suppressive gene therapy component that can suppress the expression of a selected disease-associated allele, and a corrective gene therapy component encoding a corrected version of the selected disease-associated allele that is immune to the suppressive gene therapy component.

The suppressive component can be, for example, an RNAi nucleic acid such as a shRNA, siRNA, or a micro RNA (miRNA). The suppressive component can have any appropriate length. For example, the suppressive component can be from about 10 to 40 nucleotides in length (e.g., from about 10 to about 20, from about 15 to about 30, from about 18 to about 22, from about 20 to about 30, or from about 30 to about 40 nucleotides in length).

The suppressive component can be designed to target a region of a disease-associated allele that does not contain the pathogenic mutation(s) (e.g., LQTS-causative mutations) or other genetic polymorphisms. In this manner, the suppressive component can reduce the expression of numerous versions of the endogenous alleles, including wild type alleles, alleles containing disease-associated mutations, or alleles containing other polymorphisms that are not causative of the disorder to be treated.

In some cases, the suppressive component can be designed to target a region of a disease-associated allele that contains one or more pathogenic mutations (e.g., one or more LQTS-causative mutations) or other genetic polymorphisms.

The corrective component can be a nucleic acid that encodes a corrected version of the disease-associated allele that lacks the pathogenic mutation(s), and may encode a wild type polypeptide. The corrective component also contains base substitutions as compared to the endogenous version of the targeted gene, such that the corrective component is immune to (e.g., not suppressed by) the suppressive gene therapy component. For example, the region of the corrective component that would otherwise be targeted by the suppressive component can include from about 1 to about 13 (e.g., from about 1 to about 3, from about 2 to about 4, from about 3 to about 5, from about 4 to

about 6, from about 5 to about 7, from about 6 to about 8, from about 7 to about 9, from about 8 to about 10, from about 9 to about 11, from about 10 to about 12, or from about 11 to about 13) wobble base synonymous variants that do not change the amino acid sequence encoded by the corrective component, as compared to the corresponding wild type sequence. In some cases, the region of the corrective component that would otherwise be targeted by the suppressive component can include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 wobble base synonymous variants that do not change the amino acid sequence encoded by the corrective component, as compared to the corresponding wild type sequence (e.g., wild type, non-pathogenic sequence). Due to the presence of the synonymous variants, expression of the suppressive component will not reduce the expression of the corrective component.

Other suppressive component/corrective component combinations also can be used. For example, in some cases, the suppressive component can be designed to target the 5′ untranslated region (UTR) or 3′ UTR, since the corrective cDNA does not contain the UTRs but endogenous transcription of mRNA does contain the UTRs. In such cases, the corrective component does not need to contain silent variants since the suppressive component (e.g., RNAi) is targeted to a UTR. In some cases, the suppressive component can target a sequence near the 5′ or 3′ end of the coding sequence, and the corrective component can include a truncated cDNA that does not contain the sequence targeted by the suppressive component.

In some cases, the corrective component may encode a polypeptide that is not 100% identical to the wild type polypeptide at the amino acid sequence level, but has activity at a level sufficient to treat the disorder. Amino acid substitutions can be made, in some cases, by selecting substitutions that do not differ significantly in their effect on maintaining (a) the structure of the peptide backbone in the area of the substitution, (b) the charge or hydrophobicity of the molecule at particular sites, or (c) the bulk of the side chain. For example, naturally occurring residues can be divided into groups based on side-chain properties: (1) hydrophobic amino acids (methionine, alanine, valine, leucine, and isoleucine); (2) neutral hydrophilic amino acids (cysteine, serine, and threonine); (3) acidic amino acids (aspartic acid and glutamic acid); (4) basic amino acids (asparagine,

glutamine, histidine, lysine, and arginine); (5) amino acids that influence chain orientation (glycine and proline); and (6) aromatic amino acids (tryptophan, tyrosine, and phenylalanine). Substitutions made within these groups can be considered conservative substitutions. Non-limiting examples of conservative substitutions that can be encoded within a corrective component of a SupRep construct provided herein include, without limitation, substitution of valine for alanine, lysine for arginine, glutamine for asparagine, glutamic acid for aspartic acid, serine for cysteine, asparagine for glutamine, aspartic acid for glutamic acid, proline for glycine, arginine for histidine, leucine for isoleucine, isoleucine for leucine, arginine for lysine, leucine for methionine, leucine for phenyalanine, glycine for proline, threonine for serine, serine for threonine, tyrosine for tryptophan, phenylalanine for tyrosine, and/or leucine for valine.

In some cases, a SupRep construct provided herein also can encode or contain a reporter. Any appropriate reporter can be used. In some cases, for example, a fluorescent reporter (e.g., green fluorescent protein, red fluorescent protein, or yellow fluorescent protein) can be used. In some cases, a non-fluorescent tag can be included. Any appropriate non-fluorescent tag can be used, including, without limitation, hemagglutinin, FLAG® tag, His6, and V5.

A non-limiting example of a SupRep construct provided herein is a SupRep *KCNQ1* gene therapy vector that can be used for treating of mammals having LQT1. As described in the Examples herein, the therapeutic efficacy of the SupRep *KCNQ1* gene therapy vector is supported by results obtained using two *in vitro* model systems. Again, the SupRep strategy has two components that occur in tandem. First, for *KCNQ1* and LQT1, suppression of *both* endogenous *KCNQ1* alleles (the WT allele and the LQT1 mutant-containing allele) occurs via a *KCNQ1* shRNA. The second component involves replacement of *KCNQ1* via expression of a shRNA-immune (shIMM) *KCNQ1* cDNA that contains synonymous variants at the wobble base of each codon within the shRNA's binding sequence. As noted above, these synonymous variants did not alter the WT amino acid sequence, but did prevent knock down (KD) by the shRNA – thereby rendering it "immune" to the shRNA. KCNQ1-SupRep can be mutation-independent,

eliminating the need to design multiple RNAi since the shRNA targets the gene itself rather than discrete mutations.

Nucleic acid molecules encoding a suppressive component and a corrective component can be produced by techniques including, without limitation, common molecular cloning, polymerase chain reaction (PCR), chemical nucleic acid synthesis techniques, and combinations of such techniques. For example, PCR can be used with oligonucleotide primers designed to amplify nucleic acid (e.g., genomic DNA or RNA) encoding a selected polypeptide (e.g., KCNQ1).

This document also provides methods for using the SupRep constructs described herein to treat a mammal identified as having a congenital disorder. As described in the Examples herein, for example, a KCNQ1-SupRep gene therapy vector was generated, and its ability to suppress and replace *KCNQ1* was validated via heterologous expression in TSA201 cells. In addition, the LQT1 disease phenotype was rescued by shortening of the cardiac action potential duration (APD) in an *in vitro* cardiac model using patient-specific, induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) generated from four patients with distinct LQT1-causative variants. Further, the studies described herein demonstrated that the KCNQ1-SupRep gene therapy approximated a "therapeutic cure," in terms of APD normalization, when compared to the gold standard of a patient's own corrected isogenic control cells.

Any appropriate mammal can be treated as described herein. For example, mammals including, without limitation, humans, monkeys, dogs, cats, cows, horses, pigs, sheep, rabbits, rats, and mice having a congenital disorder (e.g., a congenital heart disorder such as a LQTS, or more specifically LQT1) can be treated as described herein. In some cases, a mammal (e.g., a human) having a congenital disease (e.g., a congenital cardiac disease such as a LQTS, or more specifically LQT1) can be treated by administering a SupRep nucleic acid construct to the mammal (e.g., to the heart muscle of the mammal) in a manner that suppresses expression of endogenous disease-associated alleles and provides a replacement wild type cDNA (or a cDNA that does not include disease-associated polymorphisms). A mammal can be identified as having a congenital disorder using any appropriate diagnostic technique. Non-limiting examples include,

without limitation, genetic screening for one or more disease-associated alleles and assessment of organ (e.g., heart) function deficits (e.g., by electrocardiogram, echocardiogram, exercise stress test, and/or lidocaine challenge).

In some cases, the mammal can have LQT1 or SQTS, and the gene to be suppressed and replaced can be *KCNQ1*. An example of a KCNQ1 construct is shown in **FIGS. 1A and 1B**. An exemplary *KCNQ1* sequence is set forth in NCBI RefSeq accession number NM_000218 (e.g., version NM_000218.2 or NM_00218.3) (**FIG. 1C**). A KCNQ1 polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_000209 (e.g., version NP_000209.2) (**FIG. 1C**).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *KCNQ1* are set forth in **TABLE 1A**.

TABLE 1A

Representative *KCNQ1* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GGCTGGAAATGCTTCGTTTACCACT	54	GGGTGGAAGTGTTTTGTATATCATT	55
GCTGGAAATGCTTCGTTTACCACTT	56	GGTGGAAGTGTTTTGTATATCATTT	57
GGAAATGCTTCGTTTACCACT	58	GGAAGTGTTTTGTATATCATT	59
GGAAATGCTTCGTTTACCA	60	GGAAGTGTTTTGTATATCA	61
GAAATGCTTCGTTTACCACTT	62	GAAGTGTTTTGTATATCATTT	63
TTCCTCATCGTCCTGGTCTGCCTCATCTT	64	TTTCTGATTGTGCTCGTGTGTCTGATTTT	65
GCGTGCTGTCCACCATCGAGCAGTATGCC	66	GTGTCCTCTCGACGATTGAACAATACGCG	67
GTCCACCATCGAGCAGTAT	68	CTCGACGATTGAACAATAC	69
TCCACCATCGAGCAGTATGCC	70	TCGACGATTGAACAATACGCG	71
GTGTTCTTCGGGACGGAGTACGTGGTCCG	72	GTCTTTTTTGGCACCGAATATGTCGTGCG	73
CTCATCGTGGTCGTGGCCTCCATGGTGGT	74	CTGATTGTCGTGGTCGCGTCGATGGTCGT	75
GGGCAGGTGTTTGCCACGTCGGCCATCAG	76	GGCCAAGTCTTCGCGACCTCCGCGATTAG	77
ACCGCCAGGGAGGCACCTGGAGGCTCCTG	78	ATCGGCAAGGTGGGACGTGGAGACTGCTC	79
TGGTCTTCATCCACCGCCAGGAGCTGATA	80	TCGTGTTTATTCATCGGCAAGAACTCATT	81
TGGTCTTCATCCACCGCCAGG	82	TCGTGTTTATTCATCGGCAAG	83
GCTGATAACCACCCTGTACAT	84	ACTCATTACGACGCTCTATAT	85
ACCACCCTGTACATCGGCTTCCTGGGCCT	86	ACGACGCTCTATATTGGGTTTCTCGGGCT	87
ACCACCCTGTACATCGGCTTC	88	ACGACGCTCTATATTGGGTTT	89
CTGGCTGAGAAGGACGCGGTGAACGAGTC	90	CTCGCAGAAAAAGATGCCGTCAATGAATC	91
CTGTGGTGGGGGGTGGTCACAGTCACCAC	92	CTCTGGTGGGGCGTCGTGACTGTGACGAC	93
AGACCATCGCCTCCTGCTTCTCTGTCTTT	94	AAACGATTGCGTCGTGTTTTTCAGTGTTC	95

AGCAGAAGCAGAGGCAGAAGCACTTCAAC	96	AACAAAAACAAAGACAAAAACATTTTAAT	97
GAAGCAGAGGCAGAAGCACTT	98	AAAACAAAGACAAAAACATTT	99
CCCAAACCCAAGAAGTCTGTGGTGGTAAA	100	CCGAAGCCGAAAAAATCAGTCGTCGTTAA	101
GTTCAAGCTGGACAAAGACAATGGGGTGA	102	ATTTAAACTCGATAAGGATAACGGCGTCA	103
GTTCAAGCTGGACAAAGACAA	104	ATTTAAACTCGATAAGGATAA	105
TGGACAAAGACAATGGGGTGA	106	TCGATAAGGATAACGGCGTCA	107
GAGAGAAGATGCTCACAGT	108	GTGAAAAAATGCTGACTGT	109
GACAGTTCTGTAAGGAAGAGCCCAACACT	110	GATAGCTCAGTTAGAAAAAGTCCTACTCT	111
GTTCTGTAAGGAAGAGCCCAACACT	112	GCTCAGTTAGAAAAAGTCCTACTCT	113
GCCCAACACTGCTGGAAGTGAGCATGCCC	114	GTCCTACTCTCCTCGAGGTCAGTATGCCG	115
GCCCAACACTGCTGGAAGTGA	116	GTCCTACTCTCCTCGAGGTCA	117
TGAGAACCAACAGCTTCGCCGAGGACCTG	118	TGAGGACGAATAGTTTTGCGGAAGATCTC	119
GGGCCACCATTAAGGTCAT	120	GCGCGACGATAAAAGTGAT	121
GGCCACCATTAAGGTCATT	122	CGCGACGATAAAAGTGATA	123
CGCATGCAGTACTTTGTGGCCAAGAAGAA	124	CGGATGCAATATTTCGTCGCGAAAAAAAA	125
AAGAAATTCCAGCAAGCGCGGAAGCCTTA	126	AAAAAGTTTCAACAGGCCCGCAAACCATA	127
AGGGCCACCTCAACCTCATGGTGCGCATC	128	AAGGGCATCTGAATCTGATGGTCCGGATT	129
GTCCATTGGGAAGCCCTCACTGTTCATCT	130	ATCGATAGGCAAACCGTCTCTCTTTATTT	131
GGAAGCCCTCACTGTTCATCT	132	GCAAACCGTCTCTCTTTATTT	133
GCCTGAACCGAGTAGAAGA	134	GGCTCAATCGTGTTGAGGA	135
GAAGACAAGGTGACGCAGCTGGACCAGAG	136	GAGGATAAAGTCACCCAACTCGATCAAAG	137

In some cases, the mammal can have LQT2 or SQTS, and the gene to be suppressed and replaced can be *KCNH2*. An example of a KCNH2 construct is shown in **FIGS. 2A and 2B**. An exemplary *KCNH2* sequence is set forth in NCBI RefSeq accession number NM_000238 (e.g., version NM_000238.4; **FIG. 2C**). A *KCNH2* polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_000229 (e.g., version NP_000229.1; **FIG. 2C**).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *KCNH2* are set forth in **TABLE 1B**.

TABLE 1B

Representative *KCNH2* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
CACCTTCCTGGACACCATCATCCGCAAGT	138	TACGTTTCTCGATACGATTATTCGGAAAT	139
CACCTTCCTGGACACCATCAT	140	TACGTTTCTCGATACGATTAT	141

TGGACACCATCATCCGCAAGT	142	TCGATACGATTATTCGGAAAT	143
TGGGCGCCGAGGAGCGCAAAGTGGAAATC	144	TCGGGGCGGAAGAACGGAAGGTCGAGATT	145
GATGGGAGCTGCTTCCTATGT	146	GACGGCAGTTGTTTTCTTTGC	147
GGAGCTGCTTCCTATGTCT	148	GCAGTTGTTTTCTTTGCCT	149
GGGCTGTCATCATGTTCAT	150	GCGCAGTGATTATGTTTAT	151
GCTGTCATCATGTTCATCCTCAATT	152	GCAGTGATTATGTTTATTCTGAACT	153
TCGTGCGCTACCGCACCATTAGCAAGATT	154	TGGTCCGGTATCGGACGATAAGTAAAATA	155
ATCACCCTCAACTTTGTGGACCTCAAGGG	156	ATTACGCTGAATTTCGTCGATCTGAAAGG	157
GTGACCGTGAGATCATAGCACCTAAGATA	158	GCGATCGAGAAATTATTGCTCCAAAAATT	159
GATCATAGCACCTAAGATAAA	160	AATTATTGCTCCAAAAATTAA	161
GATCATAGCACCTAAGATA	162	AATTATTGCTCCAAAAATT	163
GAGCGAACCCACAATGTCA	164	GAACGTACGCATAACGTGA	165
GTGGGACTGGCTCATCCTGCTGCTGGTCA	166	CTGGGATTGGCTGATTCTCCTCCTCGTGA	167
GGTCATCTACACGGCTGTCTT	168	CGTGATTTATACCGCAGTGTT	169
GTGGACATCCTCATCAACT	170	GTCGATATTCTGATTAATT	171
GACATCCTCATCAACTTCCGCACCACCTA	172	GATATTCTGATTAATTTTCGGACGACGTA	173
GAAGCTGGATCGCTACTCAGA	174	CAAACTCGACCGGTATTCTGA	175
GAAGCTGGATCGCTACTCA	176	CAAACTCGACCGGTATTCT	177
GCCCCTCCATCAAGGACAAGTATGT	178	GGCCGTCGATTAAAGATAAATACGT	179
CTGACATCTGCCTGCACCTGAACCGCTCA	180	CAGATATTTGTCTCCATCTCAATCGGTCT	181
CTGACATCTGCCTGCACCTGAACCGCTCA	182	CAGATATTTGTCTCCATCTCAATCGGTCT	183
TGAAGTTCAAGACCACACATGCACCGCCA	184	TGAAATTTAAAACGACTCACGCTCCCCCT	185
CTTCTGGTCCAGCCTGGAGATCACCTTCA	186	TTTTTGGTCGAGTCTCGAAATTACGTTTA	187
CACGGAGCAGCCAGGGGAGGTGTCGGCCT	27	TACCGAACAACCTGGCGAAGTCTCCGCGT	29
CACGGAGCAGCCAGGGGAGGT	188	TACCGAACAACCTGGCGAAGT	189
AGCCAGGGGAGGTGTCGGCCT	190	AACCTGGCGAAGTCTCCGCGT	191
CTGCAGCTGCTACAGAGGCAGATGACGCT	192	CTCCAACTCCTTCAAAGACAAATGACCCT	193
CGACGCCTCTCCCTACCGGGCCAGCTGGG	194	CGTCGGCTGTCGCTTCCCGGGCAACTCGG	195
CGACGCCTCTCCCTACCGGGCCAGCTGGG	196	CGTCGGCTGTCGCTTCCCGGGCAACTCGG	197

In some cases, the mammal can have LQT3 or BrS, and the gene to be suppressed and replaced can be *SCN5A* (which encodes sodium channel protein type 5 subunit alpha isoform b). An example of a SCN5A construct is shown in **FIGS. 3A and 3B**. An exemplary *SCN5A* sequence is set forth in NCBI RefSeq accession number NM_000335 (e.g., version NM_000335.5; **FIG. 3C**). A *SCN5A* polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_000326 (e.g., version NP_000326.2; **FIG. 3C**).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *SCN5A* are set forth in **TABLE 1C**.

TABLE 1C

Representative *SCN5A* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GGCAAACTTCCTATTACCT	198	GGCTAATTTTCTTTTGCCA	199
GACCATCTTCCGGTTCAGT	200	AACGATTTTTCGCTTTAGC	201
GTTCAGTGCCACCAACGCCTTGTAT	202	CTTTAGCGCGACGAATGCGTTATAC	203
GGTTCACTCGCTCTTCAACATGCTCATCA	30	CGTACATTCCCTGTTTAATATGCTGATTA	32
GGTTCACTCGCTCTTCAACAT	204	CGTACATTCCCTGTTTAATAT	205
GTTCACTCGCTCTTCAACATGCTCATCAT	206	GTACATTCCCTGTTTAATATGCTGATTAT	207
GCTCTTCAACATGCTCATCAT	208	CCTGTTTAATATGCTGATTAT	209
GCTCTTCATGGGCAACCTAAGGCACAAGT	210	ACTGTTTATGGGGAATCTTAGACATAAAT	211
GGCAACCTAAGGCACAAGT	212	GGGAATCTTAGACATAAAT	213
GGAATCCCTGGACCTTTACCT	214	GGAGTCGCTCGATCTATATCT	215
GGACCTTTACCTCAGTGAT	216	CGATCTATATCTGAGCGAC	217
GGGCCTTTCTTGCACTCTT	218	GGGCGTTCCTAGCTCTGTT	219
GATCTTCTTCATGCTTGTCAT	220	GATTTTTTTTATGCTAGTGAT	221
GGAGGCCATGGAAATGCTCAAGAAA	222	AGAAGCGATGGAGATGCTGAAAAAG	223
GGCCATGGAAATGCTCAAGAA	224	AGCGATGGAGATGCTGAAAAA	225
GCCATGGAAATGCTCAAGAAA	226	GCGATGGAGATGCTGAAAAAG	227
GCCATGGAAATGCTCAAGA	228	GCGATGGAGATGCTGAAAA	229
GCCCCAGTAAACAGCCATGAGAGAA	230	GCGCCTGTTAATAGTCACGAAAGGA	231
GATGGTCCCAGAGCAATGAAT	232	GACGGACCGAGGGCTATGAAC	233
GTCCCAGAGCAATGAATCA	234	GACCGAGGGCTATGAACCA	235
GGAAGAGTTAGAGGAGTCTCGCCACAAGT	236	CGAGGAATTGGAAGAATCACGGCATAAAT	237
GGAAGAGTTAGAGGAGTCT	238	CGAGGAATTGGAAGAATCA	239
GTCCATCAAGCAGGGAGTGAA	240	GTCGATTAAACAAGGTGTCAA	241
GACCTCACCATCACTATGT	242	GATCTGACGATTACAATGT	243
GCGCTGGAGCACTACAACATGACAA	244	GCCCTCGAACATTATAATATGACTA	245
GCTGGAGCACTACAACATGACAAGT	246	CCTCGAACATTATAATATGACTAGC	247
GGAGCACTACAACATGACA	248	CGAACATTATAATATGACT	249
GAGCACTACAACATGACAAGT	250	GAACATTATAATATGACTAGC	251
GAGCACTACAACATGACAA	252	GAACATTATAATATGACTA	253
GCACTACAACATGACAAGT	254	ACATTATAATATGACTAGC	255
GACAAGTGAATTCGAGGAGAT	256	GACTAGCGAGTTTGAAGAAAT	257
GTCGGAAACCTGGTCTTCACA	258	GTGGGTAATCTCGTGTTTACT	259
GTCGGAAACCTGGTCTTCA	260	GTGGGTAATCTCGTGTTTA	261

GCTGGCACATGATGGACTTCTTTCA	262	GGTGGCATATGATGGATTTTTTCCA	263
GCTGGCACATGATGGACTTCT	264	GGTGGCATATGATGGATTTTT	265
GCTGGCACATGATGGACTT	266	GGTGGCATATGATGGATTT	267
GGCACATGATGGACTTCTT	268	GGCATATGATGGATTTTTT	269
GCACATGATGGACTTCTTTCA	270	GCATATGATGGATTTTTTCCA	271
GCACATGATGGACTTCTTT	272	GCATATGATGGATTTTTTC	273
GCCTGCTGGTCTTCTTGCTTGTTAT	274	GTCTCCTCGTGTTTTTACTAGTAAT	275
GCTGGTCTTCTTGCTTGTTAT	276	CCTCGTGTTTTTACTAGTAAT	277
GCCCCTGATGAGGACAGAGAGATGAACAA	278	GCGCCAGACGAAGATAGGGAAATGAATAA	279
GGAAGACCATCAAGGTTCT	280	GCAAAACGATTAAAGTACT	281
GCCTCATCTTCTGGCTCATCT	282	GTCTGATTTTTTGGCTGATTT	283
GCCAGTGTGAGTCCTTGAACT	284	GTCAATGCGAATCGTTAAATT	285
GCCCTTCTGCAGGTGGCAACATTTA	286	GCGCTACTCCAAGTCGCTACTTTCA	287
GCAGGTGGCAACATTTAAA	288	CCAAGTCGCTACTTTCAAG	289
GAAGAGCAGCCTCAGTGGGAATACA	290	GAGGAACAACCACAATGGGAGTATA	291
GAGCAGCCTCAGTGGGAATACAACCTCTA	292	GAACAACCACAATGGGAGTATAATCTGTA	293
GCAGCCTCAGTGGGAATACAACCTCTACA	294	ACAACCACAATGGGAGTATAATCTGTATA	295
GCAGCCTCAGTGGGAATACAA	296	ACAACCACAATGGGAGTATAA	297
GCCTCAGTGGGAATACAACCTCTACATGT	298	ACCACAATGGGAGTATAATCTGTATATGT	299
GTGGGAATACAACCTCTACAT	300	ATGGGAGTATAATCTGTATAT	301
GGGAATACAACCTCTACATGT	302	GGGAGTATAATCTGTATATGT	303
GGGAATACAACCTCTACAT	304	GGGAGTATAATCTGTATAT	305
AAGTACTACAATGCCATGAAG	306	AAATATTATAACGCGATGAAA	307
GTACCAGGGCTTCATATTCGACATTGTGA	308	ATATCAAGGGTTTATTTTTGATATAGTCA	309
GGGCTTCATATTCGACATTGT	310	AGGGTTTATTTTTGATATAGT	311
GGCTTCATATTCGACATTGTGACCA	312	GGGTTTATTTTTGATATAGTCACGA	313
GCTTCATATTCGACATTGTGA	314	GGTTTATTTTTGATATAGTCA	315
GCTTCATATTCGACATTGT	316	GGTTTATTTTTGATATAGT	317
GCTGCTGCTCTTCCTCGTCATGTTCATCT	318	CCTCCTCCTGTTTCTGGTGATGTTTATTT	319
GCTGCTCTTCCTCGTCATGTTCATCTACT	320	CCTCCTGTTTCTGGTGATGTTTATTTATT	321
GCTGCTCTTCCTCGTCATGTT	322	CCTCCTGTTTCTGGTGATGTT	323
GCTCTTCCTCGTCATGTTCAT	324	CCTGTTTCTGGTGATGTTTAT	325
GAGGCTGGCATCGACGACATGTTCAACTT	326	GAAGCAGGGATTGATGATATGTTTAATTT	327
GCTGGCATCGACGACATGTTCAACT	328	GCAGGGATTGATGATATGTTTAATT	329
GGCATCGACGACATGTTCA	330	GGGATTGATGATATGTTTA	331
GCATCGACGACATGTTCAACT	332	GGATTGATGATATGTTTAATT	333
GCATCGACGACATGTTCAA	334	GGATTGATGATATGTTTAA	335
GACGACATGTTCAACTTCCAGACCT	336	GATGATATGTTTAATTTTCAAACGT	337
GGGCATCCTCTTCTTCACCACCTACATCA	338	CGGGATTCTGTTTTTTACGACGTATATTA	339

GGCATCCTCTTCTTCACCACCTACATCAT	340	GGGATTCTGTTTTTTACGACGTATATTAT	341
GGCATCCTCTTCTTCACCACCTACA	342	GGGATTCTGTTTTTTACGACGTATA	343
GCATCCTCTTCTTCACCACCTACAT	344	GGATTCTGTTTTTTACGACGTATAT	345
GGTCTGACTACAGCCACAGTGAAGA	346	GCTCAGATTATAGTCATAGCGAGGA	347
GTCTGACTACAGCCACAGTGA	348	CTCAGATTATAGTCATAGCGA	349

In some cases, the mammal can have HCM or DCM, and the gene to be suppressed and replaced can be *MYH7* (which encodes myosin heavy chain 7). An exemplary *MYH7* sequence is set forth in NCBI RefSeq accession number NM_000257 (e.g., version NM_000257.4). A MYH7 polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_000248 (e.g., version NP_000248.2).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *MYH7* are set forth in **TABLE 1D**.

TABLE 1D

Representative *MYH7* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GACCTCAAGAAGGATGTCT	350	GATCTGAAAAAAGACGTGT	351
GTGTCACCGTCAACCCTTACA	352	GCGTGACGGTGAATCCATATA	353
GTCACCGTCAACCCTTACA	354	GTGACGGTGAATCCATATA	355
GTCAACACCAAGAGGGTCATCCAGTACTT	356	GTGAATACGAAAAGAGTGATTCAATATTT	357
GAGGGTCATCCAGTACTTT	358	AAGAGTGATTCAATATTTC	359
GCTGAAAGCAGAGAGAGATTATCACATTT	33	ACTCAAGGCTGAAAGGGACTACCATATAT	35
GGAGCTCATGGCCACTGATAA	360	AGAACTGATGGCGACAGACAA	361
GAGCTCATGGCCACTGATA	362	GAACTGATGGCGACAGACA	363
GGGCTTCACTTCAGAGGAGAA	364	CGGGTTTACATCTGAAGAAAA	365
GGCTTCACTTCAGAGGAGAAA	366	GGGTTTACATCTGAAGAAAAG	367
GGCTTCACTTCAGAGGAGA	368	GGGTTTACATCTGAAGAAA	369
GCTTCACTTCAGAGGAGAA	370	GGTTTACATCTGAAGAAAA	371
GGGCAGAATGTCCAGCAGGTGATAT	372	GGCCAAAACGTGCAACAAGTCATTT	373
GGCAGAATGTCCAGCAGGTGATATA	374	GCCAAAACGTGCAACAAGTCATTTA	375
GCAGAATGTCCAGCAGGTGAT	376	CCAAAACGTGCAACAAGTCAT	377
GAATGTCCAGCAGGTGATATA	378	AAACGTGCAACAAGTCATTTA	379
GAATGTCCAGCAGGTGATA	380	AAACGTGCAACAAGTCATT	381
GGCCAAGGCAGTGTATGAGAGGATGTTCA	382	CGCGAAAGCTGTCTACGAAAGAATGTTTA	383

GGCTGATGCGCCTATTGAGAA	384	CGCAGACGCCCCAATAGAAAA	385
GCTGATGCGCCTATTGAGA	386	GCAGACGCCCCAATAGAAA	387
GAAGGGCAAAGGCAAGGCCAAGAAA	388	AAAAGGGAAGGGGAAAGCGAAAAAG	389
GGCAAAGGCAAGGCCAAGAAA	390	GGGAAGGGGAAAGCGAAAAAG	391
GAGACTCCCTGCTGGTAAT	392	GGGATTCGCTCCTCGTTAT	393
GTCAAGAATTGGCCCTGGATGAAGCTCTA	394	GTGAAAAACTGGCCGTGGATGAAACTGTA	395
GGAGAGCATCATGGACCTGGAGAAT	396	AGAAAGTATTATGGATCTCGAAAAC	397
GTCCGTGCAGATCGAGATGAA	398	CTCGGTCCAAATTGAAATGAA	399
GTGCAGATCGAGATGAACA	400	GTCCAAATTGAAATGAATA	401
GCAGATCGAGATGAACAAGAA	402	CCAAATTGAAATGAATAAAAA	403
GAGCAGATCATCAAGGCCAAGGCTAACCT	404	GAACAAATTATTAAAGCGAAAGCAAATCT	405
GCCAAGGCTAACCTGGAGAAGATGT	406	GCGAAAGCAAATCTCGAAAAAATGT	407
GCTAACCTGGAGAAGATGT	408	GCAAATCTCGAAAAAATGT	409
GTGGAGGCTGTTAATGCCAAGTGCT	410	GTCGAAGCAGTAAACGCGAAATGTT	411
GCTGTTAATGCCAAGTGCT	412	GCAGTAAACGCGAAATGTT	413
GCCCAGAAGCAAGTCAAGA	414	GCGCAAAAACAGGTGAAAA	415
AAGAGCCTCCAGAGCTTGTTG	416	AAAAGTCTGCAAAGTTTATTA	417
GCATCAAGGAGCTCACCTA	418	GGATTAAAGAACTGACGTA	419
GCTAAAGGTCAAGGCCTACAA	420	ACTTAAAGTGAAAGCGTATAA	421

In some cases, the mammal can have ACM, and the gene to be suppressed and replaced can be *DSP* (which encodes desmoplakin). An exemplary *DSP* sequence is set forth in NCBI RefSeq accession number NM_004415 (e.g., version NM_004415.4). A DSP polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_004406 (e.g., version NP_004406.2).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *DSP* are set forth in **TABLE 1E**.

TABLE 1E

Representative *DSP* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GGAGATGGAATACAACTGACT	422	GGTGACGGTATTCAGCTCACA	423
GGAAATCCTCGACAGCTTGATCAGA	424	GGAGATTCTGGATAGTTTAATTAGG	425
GAAATCCTCGACAGCTTGATCAGAGAGAT	426	GAGATTCTGGATAGTTTAATTAGGGAAAT	427
GCAAATGCGAGCCCTTTATAA	428	ACAGATGCGTGCGCTATACAA	429
GGATGAGTTCACCAAACATGT	430	GGACGAATTTACGAAGCACGT	431
GGATGAGTTCACCAAACAT	432	GGACGAATTTACGAAGCAC	433

GTCCTCAATCAGCATCCAGCTTCAGACAA	434	GTGCTGAACCAACACCCTGCATCTGATAA	435
GCATCCAGCTTCAGACAAA	436	ACACCCTGCATCTGATAAG	437
GCAGACGCAGTGGAGTTGGATTCTTCAGA	438	CCAAACCCAATGGAGCTGGATACTACAAA	439
GCAGTGGAGTTGGATTCTT	440	CCAATGGAGCTGGATACTA	441
GGAACAGATCAAGGAGCTGGAGAAA	442	CGAGCAAATTAAAGAACTCGAAAAG	443
GGTGCAGAACTTGGTAAACAA	444	AGTCCAAAATTTAGTTAATAA	445
GTGCAGAACTTGGTAAACA	446	GTCCAAAATTTAGTTAATA	447
GAGCTCTCTGTGACTACAA	448	GGGCACTGTGCGATTATAA	449
GGCTCTGTGGAACCAGCTCTACATCAACA	450	AGCACTCTGGAATCAACTGTATATTAATA	451
GCACTACTGCATGATTGACATAGAGAAGA	44	GCATTATTGTATGATAGATATTGAAAAAA	452
GCATGATTGACATAGAGAAGA	453	GTATGATAGATATTGAAAAAA	454
GCATGATTGACATAGAGAA	455	GTATGATAGATATTGAAAA	456
GGAACCTGCCAAGATGTCAACCATAATAA	457	GGTACGTGTCAGGACGTGAATCACAACAA	458
GACCAGGGATCTTCTCACCACATCACAGT	459	GATCAAGGTTCATCACATCATATTACTGT	460
GACCAGGGATCTTCTCACCACATCA	461	GATCAAGGTTCATCACATCATATTA	462
GCTTAAGAGTGTGCAGAATGA	463	ACTAAAAAGCGTCCAAAACGA	464
GCCTGGACCTGGATAAAGT	465	GTCTCGATCTCGACAAGGT	466
GTTGGCCACTATGAAGACAGA	467	ATTAGCGACAATGAAAACTGA	468
GTTGGCCACTATGAAGACA	469	ATTAGCGACAATGAAAACT	470
GGCCACTATGAAGACAGAACT	471	AGCGACAATGAAAACTGAGCT	472
GGCCACTATGAAGACAGAA	473	AGCGACAATGAAAACTGAG	474
GCCACTATGAAGACAGAACTA	475	GCGACAATGAAAACTGAGCTT	476
GCAGATCCACTCTCAGACT	477	ACAAATTCATTCACAAACA	478
GGCTTTCTGCAAGTGGCTCTATGAT	479	AGCATTTTGTAAATGGCTGTACGAC	480
GCTTTCTGCAAGTGGCTCTAT	481	GCATTTTGTAAATGGCTGTAC	482
GTGGCTCTATGATGCTAAA	483	ATGGCTGTACGACGCAAAG	484
GCTCGGTACATTGAACTACTT	485	GCACGCTATATAGAGCTTCTA	486
GAACTACTTACAAGATCTGGAGACTATTA	487	GAGCTTCTAACTAGGTCAGGTGATTACTA	488
GAACTACTTACAAGATCTGGAGACT	489	GAGCTTCTAACTAGGTCAGGTGATT	490
GGCAGAGTGTTCCCAGTTCAA	491	AGCTGAATGCTCGCAATTTAA	492
GCAGAGTGTTCCCAGTTCAAA	493	GCTGAATGCTCGCAATTTAAG	494
GCAGAGTGTTCCCAGTTCA	495	GCTGAATGCTCGCAATTTA	496
GGCAAAGGTAAGAAACCACTA	497	CGCTAAAGTTAGGAATCATTA	498
GCAAAGGTAAGAAACCACTAT	499	GCTAAAGTTAGGAATCATTAC	500
GACCACCATCAAGGAGATA	501	AACGACGATTAAAGAAATT	502
GAAGGAAGAGGATACCAGT	503	AAAAGAGGAAGACACGAGC	504
GGAGCTTATCTGAAGAAAT	505	GAAGTTTGTCAGAGGAGAT	506
GAGCTTATCTGAAGAAATA	507	AAGTTTGTCAGAGGAGATT	508
GATCGACAAAGAAACAAATGA	509	CATTGATAAGGAGACTAACGA	510

GATCGACAAAGAAACAAAT	511	CATTGATAAGGAGACTAAC	512
GCAGAAAGCAAACAGTAGT	513	CCAAAAGGCTAATAGCAGC	514
GGAGAGGACTGTGAAGGACCAGGATATCA	515	AGAAAGAACAGTCAAAGATCAAGACATTA	516
GACTGTGAAGGACCAGGATAT	517	AACAGTCAAAGATCAAGACAT	518
GTGAAGGACCAGGATATCA	519	GTCAAAGATCAAGACATTA	520
GAAGCAGAAGGTGGAAGAGGA	521	AAAACAAAAAGTCGAGGAAGA	522
GGAGCAGGCATCCATTGTT	523	CGAACAAGCTTCGATAGTA	524
GGAACAGGAAAGTGTCAAA	525	AGAGCAAGAGAGCGTGAAG	526
GAAATTGAGAGGCTGCAGTCT	527	GAGATAGAAAGACTCCAATCA	528
GAACCTGACCAAGGAGCACTT	529	AAATCTCACGAAAGAACATTT	530
GGAGCACTTGATGTTAGAA	531	AGAACATTTAATGTTGGAG	532
GAGCACTTGATGTTAGAAGAA	533	GAACATTTAATGTTGGAGGAG	534
GCACTTGATGTTAGAAGAA	535	ACATTTAATGTTGGAGGAG	536
GCAACCATCTTGGAACTAA	537	GCTACGATTTTAGAGCTTA	538
GAGGAGGCTATTAGGAAGATA	539	GAAGAAGCAATAAGAAAAATT	540
GGAGGCTATTAGGAAGATA	541	AGAAGCAATAAGAAAAATT	542
GGAGTGAGATCGAAAGACT	543	GAAGCGAAATTGAGAGGCT	544
GAGGATTCTACCAGGGAGACA	545	GAAGACTCAACGAGAGAAACT	546
GGATTCTACCAGGGAGACA	547	AGACTCAACGAGAGAAACT	548
GGAGATTGATAAACTCAGACA	549	AGAAATAGACAAGCTGAGGCA	550
GGAGATTGATAAACTCAGA	551	AGAAATAGACAAGCTGAGG	552
GCTGAGGAAGAAGGTGACA	553	CCTCAGAAAAAAAGTCACT	554
GAGGCCAAGAGAAAGAAATTAATCA	555	GAAGCGAAAAGGAAAAAGTTGATTA	556
GAGGCCAAGAGAAAGAAATTA	557	GAAGCGAAAAGGAAAAAGTTG	558
GAGGCCAAGAGAAAGAAAT	559	GAAGCGAAAAGGAAAAAGT	560
GGCCAAGAGAAAGAAATTAAT	561	AGCGAAAAGGAAAAAGTTGAT	562
GGCCAAGAGAAAGAAATTA	563	AGCGAAAAGGAAAAAGTTG	564
GCCAAGAGAAAGAAATTAA	565	GCGAAAAGGAAAAAGTTGA	566
GAAATTAATCAGCCCAGAATCCACAGTCA	567	AAAGTTGATTAGTCCTGAGTCGACTGTGA	568
GCCCAGAATCCACAGTCAT	569	GTCCTGAGTCGACTGTGAT	570
GGTATAATTGATCCCCATCGGAATGAGAA	571	GGAATTATAGACCCGCACCGCAACGAAAA	572
GATCCCCATCGGAATGAGA	573	GACCCGCACCGCAACGAAA	574
AAGAAGGTCAGTTACGTGCAG	575	AAAAAAGTGAGCTATGTCCAA	576
GGTCTGCTCTTGCTTTCAGTA	577	GGACTCCTGTTACTATCTGTT	578
GTCTGCTCTTGCTTTCAGT	579	GACTCCTGTTACTATCTGT	580
GCTTTCAGTACAGAAGAGA	581	ACTATCTGTTCAAAAAAGG	582
GCATAGCAGGCATATACAA	583	GTATTGCTGGGATTTATAA	584
GGCATTTATGAGGCCATGAAA	585	GGGATATACGAAGCGATGAAG	586
GCAACTTGAGGTTACCAGT	587	GTAATTTAAGATTGCCTGT	588

GCAGAACGAGCTGTCACTGGGTATAATGA	589	GCTGAGCGTGCAGTGACAGGCTACAACGA	590
GAGCTGTCACTGGGTATAA	591	GTGCAGTGACAGGCTACAA	592
GCTGTCACTGGGTATAATGAT	593	GCAGTGACAGGCTACAACGAC	594
GGGTATAATGATCCTGAAACA	595	GGCTACAACGACCCAGAGACT	596
GAAACAGGAAACATCATCTCT	597	GAGACTGGTAATATTATTTCA	598
GAAACAGGAAACATCATCT	599	GAGACTGGTAATATTATTT	600
GGGCCACGGTATTCGCTTATTAGAA	601	AGGGCATGGAATACGGTTGTTGGAG	602
GGGCCACGGTATTCGCTTATT	603	AGGGCATGGAATACGGTTGTT	604
GGCCACGGTATTCGCTTATTA	605	GGGCATGGAATACGGTTGTTG	606
GCCACGGTATTCGCTTATT	607	GGCATGGAATACGGTTGTT	608
GACCCAAAGGAGAGCCATCGTTTACCAGT	609	GATCCTAAAGAAAGTCACCGATTGCCTGT	610
GGAGAGCCATCGTTTACCAGT	611	AGAAAGTCACCGATTGCCTGT	612
GGAGAGCCATCGTTTACCA	613	AGAAAGTCACCGATTGCCT	614
GAGCCATCGTTTACCAGTTGACATA	615	AAGTCACCGATTGCCTGTAGATATT	616
GAGCCATCGTTTACCAGTTGA	617	AAGTCACCGATTGCCTGTAGA	618
GAGCCATCGTTTACCAGTT	619	AAGTCACCGATTGCCTGTA	620
GCCATCGTTTACCAGTTGACA	621	GTCACCGATTGCCTGTAGATA	622
GCCATCGTTTACCAGTTGA	623	GTCACCGATTGCCTGTAGA	624
GTTTACCAGTTGACATAGCATATAA	625	GATTGCCTGTAGATATTGCTTACAA	626
GTTGACATAGCATATAAGA	627	GTAGATATTGCTTACAAAA	628
GATTCTCTCAGATCCAAGTGATGAT	629	AATACTGTCTGACCCTAGCGACGAC	630
GATTCTCTCAGATCCAAGTGA	631	AATACTGTCTGACCCTAGCGA	632
GATCCAAGTGATGATACCA	633	GACCCTAGCGACGACACGA	634
GCTCTGTCTTCTGCCTCTGAA	635	CCTGTGCCTACTCCCACTCAA	636
GGAAGCGTAGAGTGGTCATAGTTGA	637	GAAAACGAAGGGTCGTGATTGTAGA	638
GGAAGCGTAGAGTGGTCAT	639	GAAAACGAAGGGTCGTGAT	640
GAAGCGTAGAGTGGTCATAGT	641	AAAACGAAGGGTCGTGATTGT	642
GAAGCGTAGAGTGGTCATA	643	AAAACGAAGGGTCGTGATT	644
GCGTAGAGTGGTCATAGTTGA	645	ACGAAGGGTCGTGATTGTAGA	646
GCGTAGAGTGGTCATAGTT	647	ACGAAGGGTCGTGATTGTA	648
GTTGACCCAGAAACCAATAAA	649	GTAGATCCTGAGACGAACAAG	650
GACCCAGAAACCAATAAAGAAATGT	651	GATCCTGAGACGAACAAGGAGATGT	652
GACCCAGAAACCAATAAAGAA	653	GATCCTGAGACGAACAAGGAG	654
GTCTGTTCAGGAGGCCTACAA	655	GTCAGTACAAGAAGCGTATAA	656
GCCTACAAGAAGGGCCTAATT	657	GCGTATAAAAAAGGGCTTATA	658
GCAGGAATGTGAATGGGAAGAAATA	659	ACAAGAGTGCGAGTGGGAGGAGATT	660
GGAATGTGAATGGGAAGAAAT	661	AGAGTGCGAGTGGGAGGAGAT	662
GAATGTGAATGGGAAGAAATA	663	GAGTGCGAGTGGGAGGAGATT	664
GGGAAGAAATAACCATCACGGGATCAGAT	665	GGGAGGAGATTACGATTACCGGTTCTGAC	666

GCAGTCAGTATGATATTCAAGATGCTATT	667	GGAGCCAATACGACATACAGGACGCAATA	668
GCAGTCAGTATGATATTCA	669	GGAGCCAATACGACATACA	670
GCCTCAGCCTCACTCAATT	671	GTCTGAGTCTGACACAGTT	672
GCTGACATGATCTCCTTGAAA	673	GCAGATATGATTTCGTTAAAG	674
GCTCCCGACATGAATCAGTAA	675	GTTCGCGTCACGAGTCTGTTA	676
GCTCCCGACATGAATCAGT	677	GTTCGCGTCACGAGTCTGT	678
GCGTCAGGAATTTAACCATAA	679	GTGTGAGAAACTTGACGATTA	680
GTCAGGAATTTAACCATAA	681	GTGAGAAACTTGACGATTA	682
GTGTGATTGACCAAGACAT	683	GAGTCATAGATCAGGATAT	684
GCAGCAGAGGCAGTGAAAGAA	685	GCTGCTGAAGCTGTCAAGGAG	686
GGAAGTGCATGGGAGGATA	687	CGAGGTCCACGGCAGAATT	688
GAAGTGCATGGGAGGATAA	689	GAGGTCCACGGCAGAATTA	690
GCTCCATGGTAGAAGATATCA	691	GGTCGATGGTTGAGGACATTA	692
GACGCCACAGGGAATTCTT	693	GATGCGACTGGCAACTCAT	694
GAATTCTTCCTACTCTTAT	695	CAACTCATCGTATTCATAC	696

In some cases, the mammal can have HCM, and the gene to be suppressed and replaced can be *MYBPC3* (which encodes myosin binding protein C3). An exemplary *MYBPC3* sequence is set forth in NCBI RefSeq accession number NM_000256 (e.g., version NM_000256.3). A MYBPC3 polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_000247 (e.g., version NP_000247.2).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *MYBPC3* are set forth in **TABLE 1F**.

TABLE 1F

Representative *MYBPC3* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GCTCCTCCAAGGTCAAGTT	697	GGTCGTCGAAAGTGAAATT	698
GCTCCAACTTCAATCTCACTGTCCA	699	GTTCGAATTTTAACCTGACAGTGCA	700
GCCATGAGGACACTGGGATTCTGGACTT	701	GTCACGAAGATACAGGCATACTCGATTT	702
GAGGACACTGGGATTCTGGACTTCA	703	GAAGATACAGGCATACTCGATTTTA	704
GGACACTGGGATTCTGGACTT	705	AGATACAGGCATACTCGATTT	706
GAGAAGAAGAGCACAGCCTTTCAGA	707	GAAAAAAAAAGTACTGCGTTCCAAA	708
GAGAAGAAGAGCACAGCCTTTCAGAAGA	709	GAAAAAAAAAGTACTGCGTTCCAAAAAA	710
GGTGAGCAAAGGCCACAAGAT	711	AGTCAGTAAGGGGCATAAAAT	712

GAGGTCAAATGGCTCAAGAAT	713	GAAGTGAAGTGGCTGAAAAAC	714
GAGGTCAAATGGCTCAAGA	715	GAAGTGAAGTGGCTGAAAA	716
GGTCAAATGGCTCAAGAAT	717	AGTGAAGTGGCTGAAAAAC	718
GCTCAAGAATGGCCAGGAGATCCAGATGA	719	GCTGAAAAACGGGCAAGAAATTCAAATGA	720
GCTCAAGAATGGCCAGGAGAT	721	GCTGAAAAACGGGCAAGAAAT	722
GGAGGAGACCTTCAAATACCGGTTCAAGA	46	CGAAGAAACGTTTAAGTATCGCTTTAAAA	723
AAGGACCGCAGCATCTTCACG	724	AAAGATCGGAGTATTTTTACC	725
GGGCAGAGAAGGAAGATGA	726	GCGCTGAAAAAGAGGACGA	727
GAGAAGGAAGATGAGGGCGTCTACA	728	GAAAAAGAGGACGAAGGGGTGTATA	729
GAAGATGAGGGCGTCTACA	730	GAGGACGAAGGGGTGTATA	731
GCTACATCCTGGAGCGCAAGAAGAA	732	GGTATATTCTCGAACGGAAAAAAAA	733
GCGCAAGAAGAAGAAGAGCTA	734	ACGGAAAAAAAAAAAAAGTTA	735
GCAAGAAGAAGAAGAGCTA	736	GGAAAAAAAAAAAAAGTTA	737
GCGCCAGACCATTCAGAAGAA	738	CCGGCAAACGATACAAAAAAA	739
GCCAGACCATTCAGAAGAA	740	GGCAAACGATACAAAAAAA	741
GGCATCACCTATGAGCCACCCAACTATAA	742	GGGATTACGTACGAACCTCCGAATTACAA	743
GTAGCCCCAAGCCCAAGATTT	744	GAAGTCCGAAACCGAAAATAT	745
GCCCAAGATTTCCTGGTTCAAGAAT	746	ACCGAAAATATCGTGGTTTAAAAAC	747
GCCCAAGATTTCCTGGTTCAA	748	ACCGAAAATATCGTGGTTTAA	749
GATTTCCTGGTTCAAGAAT	750	AATATCGTGGTTTAAAAAC	751
GTTGACTCTGGAGATTAGA	752	CTTAACACTCGAAATAAGG	753

In some cases, the mammal can have DCM, and the gene to be suppressed and replaced can be *RBM20* (which encodes RNA binding motif protein 20). An exemplary *RBM20* sequence is set forth in NCBI RefSeq accession number NM_001134363 (e.g., version NM_001134363.3). A RBM20 polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_001127835 (e.g., version NP_001127835.2).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *RBM20* are set forth in **TABLE 1G**.

TABLE 1G

Representative *RBM20* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GCCATGTCCCAGCCTCTCTTCAATCAACT	754	GCGATGTCGCAACCACTGTTTAACCAGCT	755
GTCCCAGCCTCTCTTCAATCA	756	GTCGCAACCACTGTTTAACCA	757

GCCCTGAAACAGATGGTCA	758	GGCCAGAGACTGACGGACA	759
GCCAAAGCAAGCCTGATCTCA	760	GGCAGAGTAAACCAGACCTGA	761
GCCAAAGCAAGCCTGATCT	762	GGCAGAGTAAACCAGACCT	763
GCCGCATATCTGTAGCATCTGTGACAAGA	764	ACCCCACATTTGCAGTATTTGCGATAAAA	765
GCATATCTGTAGCATCTGTGA	766	CCACATTTGCAGTATTTGCGA	767
GCATATCTGTAGCATCTGT	768	CCACATTTGCAGTATTTGC	769
GTAGCATCTGTGACAAGAA	770	GCAGTATTTGCGATAAAAA	771
GCATCTGTGACAAGAAGGTGT	772	GTATTTGCGATAAAAAAGTCT	773
GAAAGGGAAGCTGCACGCTCAGAAA	774	CAAGGGCAAACTCCATGCACAAAAG	775
GGAAGCTGCACGCTCAGAAAT	776	GCAAACTCCATGCACAAAAGT	777
GAAGCTGCACGCTCAGAAA	778	CAAACTCCATGCACAAAAG	779
GCTCAGAAATGCCTGGTCT	780	GCACAAAAGTGTCTCGTGT	781
GCTGGCATCCGGTGTATACTT	782	GCAGGGATTCGCTGCATTCTA	783
GCTGTTTATAACCCTGCTGGGAATGAAGA	784	GCAGTATACAATCCAGCAGGCAACGAGGA	785
GCCCATTCCAGCAAGGTCATTCACTCAGT	786	CCCGATACCTGCTAGATCTTTTACACAAT	787
GCCCATTCCAGCAAGGTCATT	788	CCCGATACCTGCTAGATCTTT	789
GCAAGGTCATTCACTCAGTCA	790	GCTAGATCTTTTACACAATCT	791
GCAAGGTCATTCACTCAGT	792	GCTAGATCTTTTACACAAT	793
GGTCATTCACTCAGTCAAGCCCCACATTT	48	GATCTTTTACACAATCTAGTCCGACTTTC	794
GAAGGAAGCTGCACTGAGAAT	795	GAGGGTAGTTGTACAGAAAAC	796
GAAGGAAGCTGCACTGAGA	797	GAGGGTAGTTGTACAGAAA	798
GAAGCTGCACAGGCCATGGTCCAGTATTA	799	GAGGCAGCTCAAGCGATGGTGCAATACTA	800
GCTGCACAGGCCATGGTCCAGTATTATCA	801	GCAGCTCAAGCGATGGTGCAATACTACCA	802
GCACAGGCCATGGTCCAGTATTATCAAGA	803	GCTCAAGCGATGGTGCAATACTACCAGGA	804
GGCCATGGTCCAGTATTATCAAGAA	805	AGCGATGGTGCAATACTACCAGGAG	806
GGCCATGGTCCAGTATTATCA	807	AGCGATGGTGCAATACTACCA	808
GGCCATGGTCCAGTATTAT	809	AGCGATGGTGCAATACTAC	810
GCCATGGTCCAGTATTATCAAGAAA	811	GCGATGGTGCAATACTACCAGGAGA	812
GGTCCAGTATTATCAAGAA	813	GGTGCAATACTACCAGGAG	814
GTCCAGTATTATCAAGAAA	815	GTGCAATACTACCAGGAGA	816
GCTGTGATCAATGGTGAGA	817	GCAGTCATTAACGGAGAAA	818
GAAGTTGCTCATTCGGATGTCCAAGAGAT	819	AAAATTACTGATACGCATGTCGAAAAGGT	820
GTTGCTCATTCGGATGTCCAAGAGATACA	821	ATTACTGATACGCATGTCGAAAAGGTATA	822
GTTGCTCATTCGGATGTCCAAGAGA	823	ATTACTGATACGCATGTCGAAAAGG	824
GGATGTCCAAGAGATACAA	825	GCATGTCGAAAAGGTATAA	826
GGAATTGCAGCTCAAGAAA	827	AGAGTTACAACTGAAAAAG	828
GTGGGCAGACAGGAGAAAGAAGCAGAGTT	829	GTCGGGAGGCAAGAAAAGGAGGCTGAATT	830
GTGGGCAGACAGGAGAAAGAA	831	GTCGGGAGGCAAGAAAAGGAG	832
GGGCAGACAGGAGAAAGAA	833	CGGGAGGCAAGAAAAGGAG	834

GCAGACAGGAGAAAGAAGCAGAGTT	835	GGAGGCAAGAAAAGGAGGCTGAATT	836
AAGAAGCAGAGTTCTCTGATC	837	AGGAGGCTGAATTTTCAGACC	838
GAGCTGGAAGAAATTGTGCCCATTGACCA	839	GAACTCGAGGAGATAGTCCCGATAGATCA	840
GCCCATTGACCAGAAAGACAA	841	CCCGATAGATCAAAAGGATAA	842
GTGTGACAACCACCTTAGACT	843	GCGTCACTACGACGTTGGATT	844
GTGTGACAACCACCTTAGA	845	GCGTCACTACGACGTTGGA	846
GTGACAACCACCTTAGACTTA	847	GTCACTACGACGTTGGATTTG	848
GTGACAACCACCTTAGACT	849	GTCACTACGACGTTGGATT	850
GACAACCACCTTAGACTTA	851	CACTACGACGTTGGATTTG	852
GCAGAAATCAGCCTCAAGTCA	853	GCTGAGATTAGTCTGAAATCT	854
GCAGAAATCAGCCTCAAGT	855	GCTGAGATTAGTCTGAAAT	856
GAAATCAGCCTCAAGTCACCCAGAGAACT	857	GAGATTAGTCTGAAATCTCCGAGGGAGCT	858
GCAAAGGGAGTGGAGAGCTCAGATGTTCA	859	GCTAAAGGTGTCGAAAGTTCTGACGTACA	860
GGAGTGGAGAGCTCAGATGTT	861	GGTGTCGAAAGTTCTGACGTA	862
GAGTGGAGAGCTCAGATGT	863	GTGTCGAAAGTTCTGACGT	864
GTGGATGATTGCAAGACCA	865	GTCGACGACTGTAAAACGA	866
GAGGTCACCAGAGTACACT	867	CAGATCTCCTGAATATACA	868

In some cases, the mammal can have LQTS or Timothy syndrome, and the gene to be suppressed and replaced can be *CACNA1C* (which encodes calcium voltage-gated channel subunit alpha1 C). An exemplary *CACNA1C* sequence is set forth in NCBI RefSeq accession number NM_000719 (e.g., version NM_000719.7). A CACNA1C polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_000710 (e.g., version NP_000710.5).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *CACNA1C* are set forth in **TABLE 1H**.

TABLE 1H

Representative *CACNA1C* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GTCAATGAGAATACGAGGATGTACA	869	GTGAACGAAAACACCAGAATGTATA	870
GGAACGAGTGGAATATCTCTTTCTCATAA	50	CGAGCGTGTCGAGTACCTGTTCCTGATTA	871
GGAGAAGCAGCAGCTAGAAGA	872	CGAAAAACAACAACTTGAGGA	873
GGAGAAGCAGCAGCTAGAA	874	CGAAAAACAACAACTTGAG	875
GCAGCAGCTAGAAGAGGATCTCAAA	876	ACAACAACTTGAGGAAGACCTGAAG	877
GCAGCAGCTAGAAGAGGATCT	878	ACAACAACTTGAGGAAGACCT	879

GCAGCTAGAAGAGGATCTCAA	880	ACAACTTGAGGAAGACCTGAA	881
GCTAGAAGAGGATCTCAAA	882	ACTTGAGGAAGACCTGAAG	883
GATTGGATCACTCAGGCCGAAGACA	884	GACTGGATTACACAAGCGGAGGATA	885
GCTCCTTCTCCTCTTCCTCTTCATCATCA	886	CCTGCTACTGCTGTTTCTGTTTATTATTA	887
AAGTTCAACTTTGATGAGATG	888	AAATTTAATTTCGACGAAATG	889
GGACTGGAATTCGGTGATGTA	890	AGATTGGAACTCCGTCATGTA	891
GACTGGAATTCGGTGATGTAT	892	GATTGGAACTCCGTCATGTAC	893
GACTGGAATTCGGTGATGT	894	GATTGGAACTCCGTCATGT	895
GGAATTCGGTGATGTATGA	896	GGAACTCCGTCATGTACGA	897
GGAGGAGGAAGAGGAGAAGGAGAGAAAGA	898	AGAAGAAGAGGAAGAAAAAGAAAGGAAAA	899
GCCGGAACTACTTCAACAT	900	GTCGCAATTATTTTAATAT	901
GTCCAGTGCAATCAATGTCGTGAAGATCT	902	ATCGAGCGCTATTAACGTGGTCAAAATTT	903
GCTCTTCAAGGGAAAGCTGTACACCTGTT	904	ACTGTTTAAAGGTAAACTCTATACGTGCT	905
GGGAGCAGGAGTACAAGAACTGTGA	906	GCGAACAAGAATATAAAAATTGCGA	907
GGGAGCAGGAGTACAAGAACT	908	GCGAACAAGAATATAAAAATT	909
GAGCAGGAGTACAAGAACTGT	910	GAACAAGAATATAAAAATTGC	911
GCAGGAGTACAAGAACTGTGA	912	ACAAGAATATAAAAATTGCGA	913
GGAACAACAACTTTCAGACCT	914	GCAATAATAATTTCCAAACGT	915
GAAGCCAAGGGTCGTATCAAA	916	GAGGCGAAAGGACGAATTAAG	917
GAAGCCAAGGGTCGTATCA	918	GAGGCGAAAGGACGAATTA	919

In some cases, the mammal can have ACM, and the gene to be suppressed and replaced can be *PKP2* (which encodes plakophilin 2). An example of a PKP2 construct is shown in **FIGS. 4A and 4B**. An exemplary *PKP2* sequence is set forth in NCBI RefSeq accession number NM_001005242 (e.g., version NM_001005242.3; **FIG. 4C**). A PKP2 polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_001005242 (e.g., version NP_001005242.2; **FIG. 4C**).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *PKP2* are set forth in **TABLE 1I**.

TABLE 1I

Representative *PKP2* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GCAGTGTTCCTGAGTATGTCTACAA	920	GTAGCGTACCAGAATACGTGTATAA	921
GCAGTGTTCCTGAGTATGT	922	GTAGCGTACCAGAATACGT	923
GTGTTCCTGAGTATGTCTACA	924	GCGTACCAGAATACGTGTATA	925

GTTCCTGAGTATGTCTACAACCTACACTT	926	GTACCAGAATACGTGTATAATCTTCATTT	927
GTTCCTGAGTATGTCTACA	928	GTACCAGAATACGTGTATA	929
GCTAAAGGCTGGCACAACT	930	GCTTAAAGCAGGGACTACA	931
GCACAACTGCCACTTATGA	932	GGACTACAGCGACATACGA	933
GGGAAGAGGAACAGCACAGTA	934	GGGTAGGGGTACTGCTCAATA	935
GAAGAGGAACAGCACAGTACA	936	GTAGGGGTACTGCTCAATATA	937
GAAGAGGAACAGCACAGTA	938	GTAGGGGTACTGCTCAATA	939
CTCTGAGGAGACTGGAGATTT	940	CACTCAGAAGGCTCGAAATAT	941
GAGGAGACTGGAGATTTCT	942	CAGAAGGCTCGAAATATCA	943
GCTCACTACACGCACAGCGATTACCAGTA	944	GCACATTATACCCATAGTGACTATCAATA	945
GTACCAGCATGGCTCTGTT	946	ATATCAACACGGGTCAGTA	947
GGCAACCTCTTGGAGAAGGAGAACTACCT	948	GGGAATCTGTTAGAAAAAGAAAATTATCT	949
GGAATGCAGACATGGAGATGACTCT	950	GCAACGCTGATATGGAAATGACACT	951
GGAATGCAGACATGGAGATGA	952	GCAACGCTGATATGGAAATGA	953
GGGCCTTGAGAAACTTAGT	954	GCGCGTTAAGGAATTTGGT	955
GGCCTTGAGAAACTTAGTA	956	CGCGTTAAGGAATTTGGTT	957
GACAACAAATTGGAGGTGGCTGAACTAAA	958	GATAATAAGTTAGAAGTCGCAGAGCTTAA	959
GCTGAAGCAAACCAGAGACTTGGAGACTA	960	CCTCAAACAGACGAGGGATTTAGAAACAA	961
GCTGAAGCAAACCAGAGACTT	962	CCTCAAACAGACGAGGGATTT	963
GAAGCAAACCAGAGACTTGGAGACT	964	CAAACAGACGAGGGATTTAGAAACA	965
GCAAACCAGAGACTTGGAGACTAAA	966	ACAGACGAGGGATTTAGAAACAAAG	967
GGATGCCTAAGAAACATGAGT	968	GGTTGTCTTAGGAATATGAGC	969
GGATGCCTAAGAAACATGA	970	GGTTGTCTTAGGAATATGA	971
GATGCCTAAGAAACATGAGTT	972	GTTGTCTTAGGAATATGAGCT	973
GAGAAGATGTGACGGACTCAT	974	GAGGAGGTGCGATGGTCTGAT	975
GAAGATGTGACGGACTCAT	976	GGAGGTGCGATGGTCTGAT	977
GAGGAACCATTGCAGATTA	978	GGGGTACGATAGCTGACTA	979
GGAACCATTGCAGATTACCAGCCAGATGA	980	GGTACGATAGCTGACTATCAACCTGACGA	981
GAACCATTGCAGATTACCA	982	GTACGATAGCTGACTATCA	983
GATGACAAGGCCACGGAGAAT	984	GACGATAAAGCGACCGAAAAC	985
GCATTCTTCATAACCTCTCCTACCA	986	GTATACTACACAATCTGTCGTATCA	987
GCATTCTTCATAACCTCTCCT	988	GTATACTACACAATCTGTCGT	989
GCAGAGCTCCCAGAGAAATAT	52	GCTGAACTGCCTGAAAAGTAC	990
GGCAGTCGAAGCAGGAAAGTA	991	GGGAGCCGTAGTAGAAAGGTT	992
GCAGTCGAAGCAGGAAAGTAA	993	GGAGCCGTAGTAGAAAGGTTA	994
GTCGAAGCAGGAAAGTAAA	995	GCCGTAGTAGAAAGGTTAA	996
GTGGCTGTGGCATTCCATTGTTATA	997	ATGGCTCTGGCACTCGATAGTAATT	998
GGCTGTGGCATTCCATTGTTA	999	GGCTCTGGCACTCGATAGTAA	1000
GCTGTGGCATTCCATTGTTAT	1001	GCTCTGGCACTCGATAGTAAT	1002

GCTGTGGCATTCCATTGTT	1003	GCTCTGGCACTCGATAGTA	1004
GTGGCATTCCATTGTTATA	1005	CTGGCACTCGATAGTAATT	1006
AAGACAGCCATCTCGCTGCTG	1007	AAAACTGCGATTTCCCTCCTC	1008
GCTGAGGAATCTGTCCCGGAATCTT	1009	CCTCAGAAACCTCTCGCGCAACCTA	1010
GAGGAATCTGTCCCGGAATCTTTCT	1011	CAGAAACCTCTCGCGCAACCTATCA	1012
GGAATCTGTCCCGGAATCTTT	1013	GAAACCTCTCGCGCAACCTAT	1014
GAATCTGTCCCGGAATCTT	1015	AAACCTCTCGCGCAACCTA	1016
GAAGGCTCAGTTTAAGAAGACAGAT	1017	AAAAGCACAATTCAAAAAAACTGAC	1018
AAGGCTCAGTTTAAGAAGACA	1019	AAAGCACAATTCAAAAAAACT	1020
GGCTCAGTTTAAGAAGACAGA	1021	AGCACAATTCAAAAAAACTGA	1022
GGCTCAGTTTAAGAAGACA	1023	AGCACAATTCAAAAAAACT	1024
GCTCAGTTTAAGAAGACAGAT	1025	GCACAATTCAAAAAAACTGAC	1026
GGACTGCCAAAGCCTACCACTCCCTTAAA	1027	GCACAGCGAAGGCGTATCATTCGCTAAAG	1028

In some cases, the mammal can have ACM, and the gene to be suppressed and replaced can be *DSG2* (which encodes desmoglein 2). An exemplary *DSG2* sequence is set forth in NCBI RefSeq accession number NM_001943 (e.g., version NM_001943.5). A DSG2 polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_001934 (e.g., version NP_001934.2).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *DSG2* are set forth in **TABLE 1J**.

TABLE 1J

Representative *DSG2* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GTGGACTTCACTTACAGGT	1048	GCGGTCTACATTTGCAAGT	1049
GGGAGGGAGAGGATCTGTCCAAGAAGAAT	1050	GCGAAGGTGAAGACCTCTCGAAAAAAAAC	1051
GAGGGAGAGGATCTGTCCAAGAAGA	1052	GAAGGTGAAGACCTCTCGAAAAAAA	1053
GGGAGAGGATCTGTCCAAGAAGAAT	1054	AGGTGAAGACCTCTCGAAAAAAAAC	1055
GAGGATCTGTCCAAGAAGAAT	1056	GAAGACCTCTCGAAAAAAAAC	1057
GAGGATCTGTCCAAGAAGA	1058	GAAGACCTCTCGAAAAAAA	1059
GGATCTGTCCAAGAAGAAT	1060	AGACCTCTCGAAAAAAAAC	1061
GTCCAAGAAGAATCCAATTGCCAAGATA	1062	CTCGAAAAAAAAACCCTATAGCGAAAATT	1063
GAATCCAATTGCCAAGATA	1064	AAACCCTATAGCGAAAATT	1065
GCAGAAGAAAGAGGACTCAAA	1066	GCTGAGGAGAGGGGTCTGAAG	1067
GCAGAAGAAAGAGGACTCA	1068	GCTGAGGAGAGGGGTCTGA	1069
GGAGAACTGAATGTTACCA	1070	GGTGAGCTCAACGTAACGA	1071

GGATGCAAGAGGAAACAATGT	1072	AGACGCTAGGGGTAATAACGT	1073
GGATGCAAGAGGAAACAAT	1074	AGACGCTAGGGGTAATAAC	1075
GATGCAAGAGGAAACAATGTAGAGA	1076	GACGCTAGGGGTAATAACGTTGAAA	1077
GATGCAAGAGGAAACAATGTA	1078	GACGCTAGGGGTAATAACGTT	1079
GAGGAAACAATGTAGAGAA	1080	GGGGTAATAACGTTGAAAA	1081
GTTCTACCTAAATAAAGATACAGGAGAGA	1082	CTTTTATCTTAACAAGGACACTGGTGAAA	1083
GTGTTACCTTGGACAGAGA	1084	GCGTAACGTTAGATAGGGA	1085
GATGCAGATGAAATAGGTTCT	1086	GACGCTGACGAGATTGGATCA	1087
GATGCAGATGAAATAGGTT	1088	GACGCTGACGAGATTGGAT	1089
GATGAAATAGGTTCTGATA	1090	GACGAGATTGGATCAGACA	1091
GGAGGTTATTTCCACATAGAA	1092	GGTGGATACTTTCATATTGAG	1093
GAGGTTATTTCCACATAGA	1094	GTGGATACTTTCATATTGA	1095
GAAACAGATGCTCAAACTA	1096	GAGACTGACGCACAGACAA	1097
GTTAGCGAGAGCATGGATAGA	1098	GTAAGTGAAAGTATGGACAGG	1099
GATCAAGCAAAGGCCAAATAA	1100	GGTCTAGTAAGGGGCAGATTA	1101
GTGGCCATATCAGAAGATTATCCTAGAAA	1102	GTCGCGATTTCTGAGGACTACCCAAGGAA	1103
GTGGCCATATCAGAAGATTAT	1104	GTCGCGATTTCTGAGGACTAC	1105
GTGGCCATATCAGAAGATT	1106	GTCGCGATTTCTGAGGACT	1107
GGCCATATCAGAAGATTATCCTAGA	1108	CGCGATTTCTGAGGACTACCCAAGG	1109
GGCCATATCAGAAGATTAT	1110	CGCGATTTCTGAGGACTAC	1111
GCCATATCAGAAGATTATCCTAGAA	1112	GCGATTTCTGAGGACTACCCAAGGA	1113
GCCATATCAGAAGATTATCCT	1114	GCGATTTCTGAGGACTACCCA	1115
GGCACAGTCCTTATCAATGTT	1116	GGGACTGTGCTAATTAACGTA	1117
GCACAGTCCTTATCAATGT	1118	GGACTGTGCTAATTAACGT	1119
GGATGGACACCCAAACAGT	1120	CGACGGTCATCCTAATAGC	1121
GCTGCTGCAACAAAGTGAGAA	1122	CCTCCTCCAGCAGAGCGAAAA	1123
GCTGCAACAAAGTGAGAAA	1124	CCTCCAGCAGAGCGAAAAG	1125
GGGAAGCACAGCATGACTCCTATGT	1126	GAGAGGCTCAACACGATTCGTACGT	1127
GGAAGCACAGCATGACTCCTA	1128	AGAGGCTCAACACGATTCGTA	1129
GAAGCACAGCATGACTCCTAT	1130	GAGGCTCAACACGATTCGTAC	1131
GAAGCACAGCATGACTCCT	1132	GAGGCTCAACACGATTCGT	1133
GCTGCATCCTTGGAATAATGA	1134	GCTCCACCCATGGAACAACGA	1135
GCTGCATCCTTGGAATAAT	1136	GCTCCACCCATGGAACAAC	1137
GCATCCTTGGAATAATGAA	1138	CCACCCATGGAACAACGAG	1139
GAGCACCACCTGAAGACAA	1140	GTGCTCCTCCAGAGGATAA	1141
GCCATCATTTCTGCCAGTGGATCAA	1142	CCCTTCTTTCCTCCCTGTCGACCAG	1143
GCCATCATTTCTGCCAGTGGA	1144	CCCTTCTTTCCTCCCTGTCGA	1145
GGGCAGTCTAGTAGGAAGAAA	1146	CGGGAGCCTTGTTGGTAGGAA	1147
GGCAGTCTAGTAGGAAGAAAT	1148	GGGAGCCTTGTTGGTAGGAAC	1149

GCAGTCTAGTAGGAAGAAATGGAGTAGGA	1150	GGAGCCTTGTTGGTAGGAACGGTGTTGGT	1151
GCAGTCTAGTAGGAAGAAA	1152	GGAGCCTTGTTGGTAGGAA	1153
GAAATGGAGTAGGAGGTAT	1154	GGAACGGTGTTGGTGGAAT	1155
GCCAAGGAAGCCACGATGAAA	1156	GCGAAAGAGGCGACCATGAAG	1157
GAAGCCACGATGAAAGGAAGTAGCT	1158	GAGGCGACCATGAAGGGTAGCAGTT	1159
GCTGCTGTTGCACTGAACGAAGAAT	1160	GCAGCAGTAGCTCTCAATGAGGAGT	1161
GTTGCACTGAACGAAGAAT	1162	GTAGCTCTCAATGAGGAGT	1163
GAATCGCTGAATGCTTCTATT	1164	GAGTCCCTCAACGCATCAATA	1165
GGAAATAGTCACTGAAAGA	1166	AGAGATTGTGACAGAGAGG	1167
GAAATAGTCACTGAAAGATCT	1168	GAGATTGTGACAGAGAGGTCA	1169
GAAATAGTCACTGAAAGAT	1170	GAGATTGTGACAGAGAGGT	1171
GAAATGTGATAGCAACAGA	1172	GGAACGTCATTGCTACTGA	1173
GATCGAATCCTCTGGAAGGCACTCA	1174	GTTCCAACCCACTCGAGGGGACACA	1175
GAATCCTCTGGAAGGCACTCA	1176	CAACCCACTCGAGGGGACACA	1177
AAGGCACTCAGCATCTTCAAG	1178	AGGGGACACAACACCTACAGG	1179

In some cases, the mammal can have ACM, DCM, left ventricular non-compaction cardiomyopathy (LVNC), or skeletal myopathy, and the gene to be suppressed and replaced can be *DES* (which encodes desmin). An exemplary *DES* sequence is set forth in NCBI RefSeq accession number NM_001927 (e.g., version NM_001927.4). A DES polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_001918 (e.g., version NP_001918.3).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *DES* are set forth in **TABLE 1K**.

TABLE 1K

Representative *DES* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GTGAACCAGGAGTTTCTGA	1180	GTCAATCAAGAATTCCTCA	1181
GAGCTCAATGACCGCTTCGCCAACTACAT	1182	GAACTGAACGATCGGTTTGCGAATTATAT	1183
GTTGAAGGAAGAAGCAGAGAACAAT	1184	ATTAAAAGAGGAGGCTGAAAATAAC	1185
GTTGAAGGAAGAAGCAGAGAA	1186	ATTAAAAGAGGAGGCTGAAAA	1187
GAAGGAAGAAGCAGAGAACAA	1188	AAAAGAGGAGGCTGAAAATAA	1189
GGAAGAAGCAGAGAACAATTT	1190	AGAGGAGGCTGAAAATAACTT	1191
GGAAGAAGCAGAGAACAAT	1192	AGAGGAGGCTGAAAATAAC	1193
GGAGCGCAGAATTGAATCTCT	1194	CGAACGGAGGATAGAGTCACT	1195

GGAGCGCAGAATTGAATCT	1196	CGAACGGAGGATAGAGTCA	1197
GAAAGTGCATGAAGAGGAGAT	1198	AAAGGTCCACGAGGAAGAAAT	1199
GAACATTTCTGAAGCTGAGGAGTGGTACA	1200	AAATATATCAGAGGCAGAAGAATGGTATA	1201
GAAGCTGAGGAGTGGTACA	1202	GAGGCAGAAGAATGGTATA	1203
GCTGAGGAGTGGTACAAGT	1204	GCAGAAGAATGGTATAAAT	1205
GGAATACCGACACCAGATCCAGTCCTACA	1206	GGAGTATCGTCATCAAATTCAATCGTATA	1207
GACACCAGATCCAGTCCTACA	1208	GTCATCAAATTCAATCGTATA	1209
GTCCTACACCTGCGAGATTGA	1210	ATCGTATACGTGTGAAATAGA	1211
GCACTAACGATTCCCTGATGA	1212	GGACAAATGACTCGCTCATGA	1213
GTGGCTACCAGGACAACAT	1214	GCGGGTATCAAGATAATAT	1215
GACCTACTCTGCCCTCAACTT	1216	AACGTATTCAGCGCTGAATTT	1217
GGTTCTGAGGTCCATACCA	1218	GGATCAGAAGTGCACACGA	1219
GAGGTCCATACCAAGAAGACGGTGATGAT	1220	GAAGTGCACACGAAAAAAACCGTCATGAT	1221
GAGGTCCATACCAAGAAGA	1222	GAAGTGCACACGAAAAAAA	1223
GTCCATACCAAGAAGACGGTGATGA	1224	GTGCACACGAAAAAAACCGTCATGA	1225

In some cases, the mammal can have Andersen-Tawil syndrome (ATS) or CPVT, and the gene to be suppressed and replaced can be *KCNJ2* (which encodes potassium inwardly rectifying channel subfamily J member 2). An exemplary *KCNJ2* sequence is set forth in NCBI RefSeq accession number NM_000891 (e.g., version NM_000891.3). A KCNJ2 polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_000882 (e.g., version NP_000882.1).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *KCNJ2* are set forth in **TABLE 1L**.

TABLE 1L

Representative *KCNJ2* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GAACCAACCGCTACAGCATCGTCTCTTCA	1226	GTACGAATCGGTATAGTATTGTGTCATCT	1227
GGGAAGAGTAAAGTCCACACCCGACAACA	1228	GGCAAAAGCAAGGTGCATACGCGTCAGCA	1229
GAAGAGTAAAGTCCACACCCGACAACAGT	1230	CAAAAGCAAGGTGCATACGCGTCAGCAAT	1231
GCACAGCTCCTCAAATCCAGAATTACTT	1232	GCTCAACTGCTGAAGTCGAGGATAACAT	1233
GGATCAAATAGACATCAAT	1234	CGACCAGATTGATATTAAC	1235
GGTGTCCCCAATCACTATA	1236	CGTCTCGCCTATTACAATT	1237
GAAATAGATGAAGACAGTCCTTTATATGA	1238	GAGATTGACGAGGATAGCCCATTGTACGA	1239
GATGAAGACAGTCCTTTATAT	1240	GACGAGGATAGCCCATTGTAC	1241

GAAGACAGTCCTTTATATGAT	1242	GAGGATAGCCCATTGTACGAC	1243
GTGCCGTAGCTCTTATCTAGCAAATGAAA	1244	ATGTCGAAGTTCATACCTTGCTAACGAGA	1245
GAAGAGAAGCACTACTACA	1246	GAGGAAAAACATTATTATA	1247
AAGCACTACTACAAAGTGGAC	1248	AAACATTATTATAAGGTCGAT	1249
GAGGAAGACGACAGTGAAA	1250	GAAGAGGATGATAGCGAGA	1251
GCGAGAGTCGGAGATATGA	1252	CCGTGAATCCGAAATTTGA	1253

In some cases, the mammal can have CPVT, and the gene to be suppressed and replaced can be *CASQ2* (which encodes calsequestrin 2). An exemplary *CASQ2* sequence is set forth in NCBI RefSeq accession number NM_001232 (e.g., version NM_001232). A CASQ2 polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_001223.2 (e.g., version NP_001223.2).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *CASQ2* are set forth in **TABLE 1M**.

TABLE 1M

Representative *CASQ2* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GGGCTTAATTTCCCCACATAT	1254	GGCCTAAACTTTCCGACTTAC	1255
GCTTAATTTCCCCACATATGA	1256	CCTAAACTTTCCGACTTACGA	1257
GCTTAATTTCCCCACATAT	1258	CCTAAACTTTCCGACTTAC	1259
GATGGGAAGGACCGAGTGGTAAGTCTTT	1260	GACGGCAAAGATCGTGTCGTTAGCCTAT	1261
GGGAAGGACCGAGTGGTAAGTCTTT	1262	GGCAAAGATCGTGTCGTTAGCCTAT	1263
GAAGGACCGAGTGGTAAGTCT	1264	CAAAGATCGTGTCGTTAGCCT	1265
GAAGGACCGAGTGGTAAGT	1266	CAAAGATCGTGTCGTTAGC	1267
GGACCGAGTGGTAAGTCTT	1268	AGATCGTGTCGTTAGCCTA	1269
GACCGAGTGGTAAGTCTTT	1270	GATCGTGTCGTTAGCCTAT	1271
GGCCCAGGTCCTTGAACATAA	1272	CGCGCAAGTGCTAGAGCACAA	1273
GCCCAGGTCCTTGAACATA	1274	GCGCAAGTGCTAGAGCACA	1275
GTCCTTGAACATAAAGCTATA	1276	GTGCTAGAGCACAAGGCAATT	1277
GGTGGATGCCAAGAAAGAA	1278	GGTCGACGCGAAAAAGGAG	1279
GATGAAGAAGGAAGCCTGTAT	1280	GACGAGGAGGGTAGTCTCTAC	1281
GAAGAAGGAAGCCTGTATATT	1282	GAGGAGGGTAGTCTCTACATA	1283
GAAGAAGGAAGCCTGTATA	1284	GAGGAGGGTAGTCTCTACA	1285
AAGGAAGCCTGTATATTCTTA	1286	AGGGTAGTCTCTACATACTAA	1287
GGAAGCCTGTATATTCTTA	1288	GGTAGTCTCTACATACTAA	1289
GAAGCCTGTATATTCTTAA	1290	GTAGTCTCTACATACTAAA	1291

GGTGATCGCACAATAGAGT	1292	GGAGACCGGACTATTGAAT	1293
GGTGGAGTTCCTCTTGGATCTAATT	1294	AGTCGAATTTCTGTTAGACCTTATA	1295
GTGGAGTTCCTCTTGGATCTA	1296	GTCGAATTTCTGTTAGACCTT	1297
GGAGTTCCTCTTGGATCTAAT	1298	CGAATTTCTGTTAGACCTTAT	1299
GGAGTTCCTCTTGGATCTA	1300	CGAATTTCTGTTAGACCTT	1301
GAGTTCCTCTTGGATCTAATT	1302	GAATTTCTGTTAGACCTTATA	1303
GAGTTCCTCTTGGATCTAA	1304	GAATTTCTGTTAGACCTTA	1305
GTTCCTCTTGGATCTAATTGA	1306	ATTTCTGTTAGACCTTATAGA	1307
GTTCCTCTTGGATCTAATT	1308	ATTTCTGTTAGACCTTATA	1309
GAAGACCCAGTGGAGATCA	1310	GAGGATCCTGTCGAAATTA	1311
GACCCAGTGGAGATCATCA	1312	GATCCTGTCGAAATTATTA	1313
GCCTTACATCAAATTCTTT	1314	ACCATATATTAAGTTTTTC	1315
GGTTGCAAAGAAATTATCT	1316	CGTAGCTAAAAAGTTGTCA	1317
GAAGATGAATGAGGTTGACTT	1318	AAAAATGAACGAAGTAGATTT	1319
GATGAATGAGGTTGACTTCTA	1320	AATGAACGAAGTAGATTTTTA	1321
GAATGAGGTTGACTTCTAT	1322	GAACGAAGTAGATTTTTAC	1323
GAGCCCATTGCCATCCCCAACAAACCTTA	1324	GAACCGATAGCGATTCCGAATAAGCCATA	1325
GCCCATTGCCATCCCCAACAAACCTTACA	1326	ACCGATAGCGATTCCGAATAAGCCATATA	1327
GCAGAGAAGAGTGATCCAGATGGCTACGA	1328	GCTGAAAAAAGCGACCCTGACGGGTATGA	1329
GACAATACTGACAACCCCGATCTGA	1330	GATAACACAGATAATCCGGACCTCA	1331
GTTGCCTACTGGGAGAAGACTTTCAAGAT	1332	GTAGCGTATTGGGAAAAAACATTTAAAAT	1333
GTTGCCTACTGGGAGAAGACT	1334	GTAGCGTATTGGGAAAAAACA	1335
GTTGCCTACTGGGAGAAGA	1336	GTAGCGTATTGGGAAAAAA	1337
GCCTACTGGGAGAAGACTTTCAAGA	1338	GCGTATTGGGAAAAAACATTTAAAA	1339
GCCTACTGGGAGAAGACTT	1340	GCGTATTGGGAAAAAACAT	1341
GGGAGAAGACTTTCAAGAT	1342	GGGAAAAAACATTTAAAAT	1343
GGAGAAGACTTTCAAGATT	1344	GGAAAAAACATTTAAAATA	1345
GGAAAGATAAACACTGAAGAT	1346	GGTAAAATTAATACAGAGGAC	1347
GGAAAGATAAACACTGAAGATGATGATGA	1348	GGTAAAATTAATACAGAGGACGACGACGA	1349
GAAAGATAAACACTGAAGATGATGA	1350	GTAAAATTAATACAGAGGACGACGA	1351
GAAAGATAAACACTGAAGA	1352	GTAAAATTAATACAGAGGA	1353
GAAGAGGATAATGATGACAGT	1354	GAGGAAGACAACGACGATAGC	1355
GAGGATAATGATGACAGTGAT	1356	GAAGACAACGACGATAGCGAC	1357
GGATAATGATGACAGTGAT	1358	AGACAACGACGATAGCGAC	1359

In some cases, the mammal can have DCM, and the gene to be suppressed and replaced can be *LMNA* (which encodes lamin A/C). An exemplary *LMNA* sequence is set forth in NCBI RefSeq accession number NM_170707 (e.g., version NM_170707.4). A

LMNA polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_733821 (e.g., version NP_733821.1).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *LMNA* are set forth in **TABLE 1N**.

TABLE 1N

Representative *LMNA* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GAGCTCAATGATCGCTTGGCGGTCTACAT	1360	GAACTGAACGACCGGTTAGCCGTGTATAT	1361
GGAGCTGAGCAAAGTGCGTGAGGAGTTTA	1362	CGAACTCAGTAAGGTCCGAGAAGAATTCA	1363
GAGCTGAGCAAAGTGCGTGAGGAGTTTAA	1364	GAACTCAGTAAGGTCCGAGAAGAATTCAA	1365
GCTGAGCAAAGTGCGTGAGGAGTTT	1366	ACTCAGTAAGGTCCGAGAAGAATTC	1367
GAGCAAAGTGCGTGAGGAGTT	1368	CAGTAAGGTCCGAGAAGAATT	1369
GCAAAGTGCGTGAGGAGTTTA	1370	GTAAGGTCCGAGAAGAATTCA	1371
GCAAAGTGCGTGAGGAGTT	1372	GTAAGGTCCGAGAAGAATT	1373
GCAATACCAAGAAGGAGGGTGACCT	1374	GGAACACGAAAAAAGAAGGAGATCT	1375
GCATGAGGACCAGGTGGAGCAGTATAAGA	1376	ACACGAAGATCAAGTCGAACAATACAAAA	1377
GAGGACCAGGTGGAGCAGTATAAGA	1378	GAAGATCAAGTCGAACAATACAAAA	1379
GGACCAGGTGGAGCAGTATAA	1380	AGATCAAGTCGAACAATACAA	1381
GACCAGGTGGAGCAGTATA	1382	GATCAAGTCGAACAATACA	1383
AAGCTGGACAATGCCAGGCAG	1384	AAACTCGATAACGCGAGACAA	1385
GACCAGTCCATGGGCAATTGGCAGATCAA	1386	GATCAATCGATGGGGAACTGGCAAATTAA	1387
GGCAGATCAAGCGCCAGAATGGAGATGA	1388	GGCAAATTAAACGGCAAAACGGTGACGA	1389
GCGCCAGAATGGAGATGAT	1390	ACGGCAAAACGGTGACGAC	1391
GATGATCCCTTGCTGACTT	1392	GACGACCCGTTACTCACAT	1393
GGATGAGGATGGAGATGACCT	1394	AGACGAAGACGGTGACGATCT	1395

In some cases, the mammal can have DCM, and the gene to be suppressed and replaced can be *TPM1* (which encodes tropomyosin 1). An exemplary *TPM1* sequence is set forth in NCBI RefSeq accession number NM_001018005 (e.g., version NM_001018005.2). A TPM1 polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_001018005 (e.g., version NP_001018005.1).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *TPM1* are set forth in **TABLE 1O**.

TABLE 1O

Representative *TPM1* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GAAGACAGGAGCAAGCAGCTGGAAGATGA	1396	GAGGATAGAAGTAAACAACTCGAGGACGA	1397
AAGCTGAGAAGGCAGCAGATG	1398	AGGCAGAAAAAGCTGCTGACG	1399
GAGAAGGCAGCAGATGAGAGTGAGA	1400	GAAAAAGCTGCTGACGAAAGCGAAA	1401
GAGAAGGCAGCAGATGAGA	1402	GAAAAAGCTGCTGACGAAA	1403
GAAGGCAGCAGATGAGAGTGA	1404	AAAAGCTGCTGACGAAAGCGA	1405
GCAGCAGATGAGAGTGAGA	1406	GCTGCTGACGAAAGCGAAA	1407
GCAGATGAGAGTGAGAGAGGCATGAAAGT	1408	GCTGACGAAAGCGAAAGGGGGATGAAGGT	1409
GCAAATGTGCCGAGCTTGAAGAAGAAT	1410	GGAAGTGCGCGGAACTAGAGGAGGAGT	1411
GAAGGAAGACAGATATGAGGAAGAGATCA	1412	AAAAGAGGATAGGTACGAAGAGGAAATTA	1413
AAGACGAGCTGTACGCTCAGA	1414	AGGATGAACTCTATGCACAAA	1415

In some cases, the mammal can have DCM or ACM, and the gene to be suppressed and replaced can be *PLN* (which encodes phospholamban). An exemplary *PLN* sequence is set forth in NCBI RefSeq accession number NM_002667 (e.g., version NM_002667.5). A PLN polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_002658 (e.g., version NP_002658.1).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *PLN* are set forth in **TABLE 1P**.

TABLE 1P

Representative *PLN* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
AAGAGCCTCAACCATTGAAAT	1416	GAGGGCGTCTACGATAGAGAT	1417
AACCATTGAAATGCCTCAACA	1418	TACGATAGAGATGCCACAGCA	1419
AATGCCTCAACAAGCACGTCA	1420	GATGCCACAGCAGGCTCGACA	1421
TCAATTTCTGTCTCATCTTAA	1422	TTAACTTTTGCCTGATTTTGA	1423
TGTCTCTTGCTGATCTGTATC	1424	TGCCTGTTACTCATTTGCATT	1425
GTCTCTTGCTGATCTGTAT	1426	GCCTGTTACTCATTTGCAT	1427

In some cases, the mammal can have familial hypercholesterolemia (FH), and the gene to be suppressed and replaced can be *LDLR* (which encodes the low density lipoprotein receptor). An exemplary *LDLR* sequence is set forth in NCBI RefSeq

accession number NM_000527 (e.g., version NM_000527.5). A LDLR polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_000518 (e.g., version NP_000518.1).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *LDLR* are set forth in **TABLE 1Q**.

TABLE 1Q

Representative *LDLR* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GTGCCAAGACGGGAAATGCATCTCCTACA	1428	ATGTCAGGATGGCAAGTGTATTTCGTATA	1429
GCCAAGACGGGAAATGCATCTCCTA	1430	GTCAGGATGGCAAGTGTATTTCGTA	1431
GACGGGAAATGCATCTCCTACAAGT	1432	GATGGCAAGTGTATTTCGTATAAAT	1433
GACGGGAAATGCATCTCCT	1434	GATGGCAAGTGTATTTCGT	1435
GGGAAATGCATCTCCTACA	1436	GGCAAGTGTATTTCGTATA	1437
GGAAATGCATCTCCTACAAGT	1438	GCAAGTGTATTTCGTATAAAT	1439
GGAAATGCATCTCCTACAA	1440	GCAAGTGTATTTCGTATAA	1441
GTCAACCGCTGCATTCCTCAGTTCT	1442	GTGAATCGGTGTATACCACAATTTT	1443
GCAGTTCGTCTGTGACTCA	1444	CCAATTTGTGTGCGATTCT	1445
GAAGATGGCTCGGATGAGT	1446	GAGGACGGGTCCGACGAAT	1447
GACGAATTCCAGTGCTCTGAT	1448	GATGAGTTTCAATGTTCAGAC	1449
GGACATGAGCGATGAAGTT	1450	AGATATGAGTGACGAGGTA	1451
GCGAATGCATCACCCTGGACAAAGT	1452	GGGAGTGTATTACGCTCGATAAGGT	1453
GCATCACCCTGGACAAAGT	1454	GTATTACGCTCGATAAGGT	1455
GCTACAAGTGCCAGTGTGA	1456	GGTATAAATGTCAATGCGA	1457
GACCTGTCCCAGAGAATGA	1458	GATCTCTCGCAAAGGATGA	1459
GAGAATGATCTGCAGCACCCAGCTTGACA	1460	AAGGATGATTTGTAGTACGCAACTAGATA	1461
GGATCCACAGCAACATCTACT	1462	GGATTCATAGTAATATTTATT	1463
GGATCCACAGCAACATCTA	1464	GGATTCATAGTAATATTTA	1465
GCAACATCTACTGGACCGACTCTGT	1466	GTAATATTTATTGGACGGATTCAGT	1467
GCTTCATGTACTGGACTGACT	1468	GGTTTATGTATTGGACAGATT	1469
GCTTCATGTACTGGACTGA	1470	GGTTTATGTATTGGACAGA	1471
GGACATCTACTCGCTGGTGACTGAA	1472	CGATATTTATTCCCTCGTCACAGAG	1473
GCATCACCCTAGATCTCCT	1474	GGATTACGCTTGACCTGCT	1475
GACGTTGCTGGCAGAGGAAATGAGAAGAA	1476	GATGTAGCAGGGAGGGGTAACGAAAAAAA	1477
GACGTTGCTGGCAGAGGAAATGAGA	1478	GATGTAGCAGGGAGGGGTAACGAAA	1479
GTTGCTGGCAGAGGAAATGAGAAGA	1480	GTAGCAGGGAGGGGTAACGAAAAAA	1481
GAACATCAACAGCATCAACTT	1482	AAATATTAATAGTATTAATTT	1483
GAGGATGAGGTCCACATTT	1484	GAAGACGAAGTGCATATAT	1485

In some cases, the mammal can have FH, and the gene to be suppressed and replaced can be *PCSK9* (which encodes proprotein convertase subtilisin/kexin type 9). An exemplary *PCSK9* sequence is set forth in NCBI RefSeq accession number NM_174936 (e.g., version NM_174936.4). A PCSK9 polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_777596 (e.g., version NP_777596.2).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *PCSK9* are set forth in **TABLE 1R**.

TABLE 1R

Representative *PCSK9* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GAGGTGTATCTCCTAGACA	1486	GAAGTCTACCTGCTTGATA	1487
GGTCTGGAATGCAAAGTCA	1488	GGACTCGAGTGTAAGGTGA	1489
AATGCAAAGTCAAGGAGCATG	1490	AGTGTAAGGTGAAAGAACACG	1491
AAAGTCAAGGAGCATGGAATC	1492	AAGGTGAAAGAACACGGTATT	1493
AAGGATCCGTGGAGGTTGCCT	2757	AAAGACCCCTGGAGATTACCA	2758
AAGATCCTGCATGTCTTCCAT	2759	AAAATTCTCCACGTGTTTCAC	2760
GGTCACCGACTTCGAGAATGT	2761	GGTGACGGATTTTGAAAACGT	2762
GCACCCTCATAGGCCTGGAGTTTAT	2763	GGACGCTGATTGGGCTCGAATTCAT	2764
GAGTTGAGGCAGAGACTGA	2765	GAATTAAGACAAAGGCTCA	2766
GAGGCAGAGACTGATCCACTT	2767	AAGACAAAGGCTCATTCATTT	2768
GGCAGAGACTGATCCACTTCT	2769	GACAAAGGCTCATTCATTTTT	2770
GGCAGAGACTGATCCACTT	2771	GACAAAGGCTCATTCATTT	2772
AACTGCAGCGTCCACACAGCT	2773	AATTGTAGTGTGCATACTGCA	2774

In some cases, the mammal can have HCM or DCM, and the gene to be suppressed and replaced can be *TNNT2* (which encodes cardiac type troponin T2). An exemplary *TNNT2* sequence is set forth in NCBI RefSeq accession number NM_001276345 (e.g., version NM_001276345.2). A TNNT2 polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_001263274 (e.g., version NP_001263274.1).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *TNNT2* are set forth in **TABLE 1S**.

TABLE 1S

Representative *TNNT2* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GGAGCAGGAAGAAGCAGCTGTTGAA	1494	AGAACAAGAGGAGGCTGCAGTAGAG	1495
GAGCAGGAAGAAGCAGCTGTTGAAGAAGA	1496	GAACAAGAGGAGGCTGCAGTAGAGGAGGA	1497
GCAGGAAGAAGCAGCTGTTGA	1498	ACAAGAGGAGGCTGCAGTAGA	1499
GGAAGAAGCAGCTGTTGAA	1500	AGAGGAGGCTGCAGTAGAG	1501
AAGAGGAGGACTGGAGAGAGG	1502	AGGAAGAAGATTGGAGGGAAG	1503
GGAGACCAGGGCAGAAGAAGATGAA	1504	AGAAACGAGAGCTGAGGAGGACGAG	1505
GACCAGGGCAGAAGAAGATGAAGAA	1506	AACGAGAGCTGAGGAGGACGAGGAG	1507
GACCAGGGCAGAAGAAGATGAAGAAGAA	1508	AACGAGAGCTGAGGAGGACGAGGAGGAG	1509
GGGCAGAAGAAGATGAAGAAGAAGA	1510	GAGCTGAGGAGGACGAGGAGGAGGA	1511
GGCAGAAGAAGATGAAGAAGA	1512	AGCTGAGGAGGACGAGGAGGA	1513
GGCAGAAGAAGATGAAGAA	1514	AGCTGAGGAGGACGAGGAG	1515
GCAGAAGAAGATGAAGAAGAA	1516	GCTGAGGAGGACGAGGAGGAG	1517
AAGATGAAGAAGAAGAGGAAG	1518	AGGACGAGGAGGAGGAAGAGG	1519
GAAGAAGAAGAGGAAGCAAAG	1520	GAGGAGGAGGAAGAGGCTAAA	1521
AAGCAAAGGAGGCTGAAGATG	1522	AGGCTAAAGAAGCAGAGGACG	1523
GAAGCGCATGGAGAAGGACCTGAATGAGT	1524	CAAACGGATGGAAAAAGATCTCAACGAAT	1525
AGCTGTGGCAGAGCATCTATA	1526	AACTCTGGCAAAGTATTTACA	1527

In some cases, the mammal can have LQTS or CPVT, and the gene to be suppressed and replaced can be *CALM1* (which encodes calmodulin 1). An exemplary *CALM1* sequence is set forth in NCBI RefSeq accession number NM_006888 (e.g., version NM_006888.6). A CALM1 polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_008819 (e.g., version NP_008819.1).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *CALM1* are set forth in **TABLE 1T**.

TABLE 1T

Representative *CALM1* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
AAGAACAGATTGCTGAATTCA	1528	AGGAGCAAATAGCAGAGTTTA	1529
GAAAGATACAGATAGTGAAGAAGAA	1530	GAAGGACACTGACAGCGAGGAGGAG	1531
AAGATACAGATAGTGAAGAAG	1532	AGGACACTGACAGCGAGGAGG	1533
GATGAAGAAGTAGATGAAATGATCAGAGA	1534	GACGAGGAGGTTGACGAGATGATTAGGGA	1535
GATGAAGAAGTAGATGAAATGATCA	1536	GACGAGGAGGTTGACGAGATGATTA	1537
AAGTAGATGAAATGATCAGAG	1538	AGGTTGACGAGATGATTAGGG	1539

In some cases, the mammal can have LQTS or CPVT, and the gene to be suppressed and replaced can be *CALM2* (which encodes calmodulin 2). An exemplary *CALM2* sequence is set forth in NCBI RefSeq accession number NM_001743 (e.g., version NM_001743.6). A CALM2 polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_001734 (e.g., version NP_001734.1).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *CALM2* are set forth in **TABLE 1U**.

TABLE 1U

Representative *CALM2* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GGCTGACCAACTGACTGAA	1540	GGCAGATCAGCTCACAGAG	1541
AAGAGCAGATTGCAGAATTCA	1542	AGGAACAAATAGCTGAGTTTA	1543
GAGCAGATTGCAGAATTCAAA	1544	GAACAAATAGCTGAGTTTAAG	1545
GAGCAGATTGCAGAATTCA	1546	GAACAAATAGCTGAGTTTA	1547
GCAGATTGCAGAATTCAAAGA	1548	ACAAATAGCTGAGTTTAAGGA	1549
GCAGATTGCAGAATTCAAA	1550	ACAAATAGCTGAGTTTAAG	1551
GACAAAGATGGTGATGGAACTATAA	1552	GATAAGGACGGAGACGGTACAATTA	1553
AAAGATGGTGATGGAACTATA	1554	AAGGACGGAGACGGTACAATT	1555
AAGATGGTGATGGAACTATAA	1556	AGGACGGAGACGGTACAATTA	1557
GGCAGAATCCCACAGAAGCAGAGTT	1558	GCCAAAACCCGACTGAGGCTGAATT	1559
GCAGAATCCCACAGAAGCAGAGTTA	1560	CCAAAACCCGACTGAGGCTGAATTG	1561
GAATCCCACAGAAGCAGAGTT	1562	AAACCCGACTGAGGCTGAATT	1563
GAAGTAGATGCTGATGGTAAT	1564	GAGGTTGACGCAGACGGAAAC	1565
AAGTAGATGCTGATGGTAATG	1566	AGGTTGACGCAGACGGAAACG	1567

GCTGATGGTAATGGCACAATTGACT	1568	GCAGACGGAAACGGGACTATAGATT	1569
GCTGATGGTAATGGCACAATT	1570	GCAGACGGAAACGGGACTATA	1571
GGTAATGGCACAATTGACT	1572	GGAAACGGGACTATAGATT	1573
GGAGAGAAGTTAACAGATGAAGAAGTTGA	1574	GGTGAAAAATTGACTGACGAGGAGGTAGA	1575
GAGAGAAGTTAACAGATGAAGAAGT	1576	GTGAAAAATTGACTGACGAGGAGGT	1577
GAGAAGTTAACAGATGAAGAAGTTGATGA	1578	GAAAAATTGACTGACGAGGAGGTAGACGA	1579

In some cases, the mammal can have LQTS or CPVT, and the gene to be suppressed and replaced can be *CALM3* (which encodes calmodulin 3). An exemplary *CALM3* sequence is set forth in NCBI RefSeq accession number NM_005184 (e.g., version NM_005184.4). A CALM3 polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_005175.2 (e.g., version NP_005175.2).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *CALM3* are set forth in **TABLE 1V**.

TABLE 1V

Representative *CALM3* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GCTGACTGAGGAGCAGATTGCAGAGTTCA	1580	ACTCACAGAAGAACAAATAGCTGAATTTA	1581
GAGCAGATTGCAGAGTTCA	1582	GAACAAATAGCTGAATTTA	1583
GACAAGGATGGAGATGGCACTATCA	1584	GATAAAGACGGTGACGGGACAATTA	1585
AAGGATGGAGATGGCACTATC	1586	AAAGACGGTGACGGGACAATT	1587
GATGGAGATGGCACTATCA	1588	GACGGTGACGGGACAATTA	1589
GGAGATGGCACTATCACCACCAAGGAGTT	1590	GGTGACGGGACAATTACGACGAAAGAATT	1591
AAGCAGAGCTGCAGGATATGA	1592	AGGCTGAACTCCAAGACATGA	1593
GCTGCAGGATATGATCAATGA	1594	ACTCCAAGACATGATTAACGA	1595
AAAGATGAAGGACACAGACAG	1596	GAAAATGAAAGATACTGATAG	1597
AAGATGAAGGACACAGACAGT	1598	AAAATGAAAGATACTGATAGC	1599
AAGGACACAGACAGTGAGGAG	1600	AAAGATACTGATAGCGAAGAA	1601
AAGCTGACCGATGAGGAGGTG	1602	AAACTCACGGACGAAGAAGTC	1603
GACCGATGAGGAGGTGGATGAGATGATCA	1604	CACGGACGAAGAAGTCGACGAAATGATTA	1605
GATGAGGAGGTGGATGAGATGATCA	1606	GACGAAGAAGTCGACGAAATGATTA	1607
GAGGAGGTGGATGAGATGA	1608	GAAGAAGTCGACGAAATGA	1609
GAGGTGGATGAGATGATCA	1610	GAAGTCGACGAAATGATTA	1611

In some cases, the mammal can have Triadin Knockout Syndrome (TKOS), and the gene to be suppressed and replaced can be *TRDN* (which encodes triadin). An exemplary *TRDN* sequence is set forth in NCBI RefSeq accession number NM_006073 (e.g., version NM_006073.4). A TRDN polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_006064 (e.g., version NP_006064.2).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *CALM3* are set forth in **TABLE 1W**.

TABLE 1W

Representative *TRDN* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GTGCTGAAGAGGACAGTCACAGAAGACAT	1612	GTCCTCAAAAGAACTGTGACTGAGGATAT	1613
GTGCTGAAGAGGACAGTCACA	1614	GTCCTCAAAAGAACTGTGACT	1615
GTGCTGAAGAGGACAGTCA	1616	GTCCTCAAAAGAACTGTGA	1617
GCTGAAGAGGACAGTCACA	1618	CCTCAAAAGAACTGTGACT	1619
GAAGAGGACAGTCACAGAAGACATA	1620	CAAAAGAACTGTGACTGAGGATATT	1621
GAAGAGGACAGTCACAGAAGA	1622	CAAAAGAACTGTGACTGAGGA	1623
GAGGACAGTCACAGAAGACAT	1624	AAGAACTGTGACTGAGGATAT	1625
GGACAGTCACAGAAGACAT	1626	GAACTGTGACTGAGGATAT	1627
GACAGTCACAGAAGACATAGT	1628	AACTGTGACTGAGGATATTGT	1629
GACAGTCACAGAAGACATA	1630	AACTGTGACTGAGGATATT	1631
GCCTGGCTTCTGGTCATTGCCCTGATAAT	1632	GCGTGGCTACTCGTGATAGCGCTCATTAT	1633
GGCTTCTGGTCATTGCCCTGATAAT	1634	GGCTACTCGTGATAGCGCTCATTAT	1635
GATTGGCTCAGATCCTTTAAA	1636	AATAGGGTCTGACCCATTGAA	1637
GCTATGGAGGAAACCACGGACTGGATCTA	1638	GCAATGGAAGAGACGACCGATTGGATTTA	1639
GGAGGAAACCACGGACTGGATCTAT	1640	GGAAGAGACGACCGATTGGATTTAC	1641
GGAAACCACGGACTGGATCTA	1642	AGAGACGACCGATTGGATTTA	1643
GAAACCACGGACTGGATCTAT	1644	GAGACGACCGATTGGATTTAC	1645
GGCAAGAAGCACATGCAGTGA	1646	GGGAAAAAACATATGCAATGA	1647

In some cases, the mammal can have CPVT, and the gene to be suppressed and replaced can be *RYR2* (which encodes ryanodine receptor 2). An exemplary *RYR2* sequence is set forth in NCBI RefSeq accession number NM_001035 (e.g., version

NM_001035.3). A RYR2 polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_001026 (e.g., version NP_001026.2).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *RYR2* are set forth in **TABLE 1X**.

TABLE 1X

Representative *RYR2* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GAGAACATGGTGAAGAGCAGCGGAGAACT	1648	GTGAGCACGGAGAGGAACAACGCAGGACA	1649
GAACATGGTGAAGAGCAGCGGAGAACTGT	1650	GAGCACGGAGAGGAACAACGCAGGACAGT	1651
GAGCATTTAGAGCATGAAGACAAACAGAA	1652	GAACACTTGGAACACGAGGATAAGCAAAA	1653
GCATTTAGAGCATGAAGACAAACAGAACA	1654	ACACTTGGAACACGAGGATAAGCAAAATA	1655
GTTGCAGTCCGTTCTAACCAGCATCTCAT	1656	GTAGCTGTGCGATCAAATCAACACCTGAT	1657
GTCCGTTCTAACCAGCATCTCATCTGTGA	1658	GTGCGATCAAATCAACACCTGATTTGCGA	1659
GGGCGTCAGTGAAGGTTCTGCTCAGTATA	1660	CGGGGTGAGCGAGGGATCAGCACAATACA	1661
GGCGTCAGTGAAGGTTCTGCTCAGTATAA	1662	GGGGTGAGCGAGGGATCAGCACAATACAA	1663
GATGGCCTCTTCTTTCCAGTCGTTAGTTT	1664	GACGGGCTGTTTTTCCCTGTGGTAAGCTT	1665
GCCTCTTCTTTCCAGTCGTTAGTTTCTCT	1666	GGCTGTTTTTCCCTGTGGTAAGCTTTTCA	1667
GTCCGGTTAGAGATGACAACAAGAGACAA	1668	GACCCGTAAGGGACGATAATAAAAGGCAG	1669
GAAGAAATCCTCGCCTTGTTCCCTACACT	1670	GGAGGAACCCACGGCTAGTACCGTATACA	1671
GAAATCCTCGCCTTGTTCCCTACACTCTT	1672	GGAACCCACGGCTAGTACCGTATACACTA	1673
GCGGGATTATTCAAGAGTGAGCACAAGAA	1674	GCCGGTTTGTTTAAAAGCGAACATAAAAA	1675
GGATCCTCTGCAGTTCATGTCTCTTCATA	1676	AGACCCACTCCAATTTATGTCACTACACA	1677
GATCCTCTGCAGTTCATGTCTCTTCATAT	1678	GACCCACTCCAATTTATGTCACTACACAT	1679
GCCATGTGGATGAACCTCAGCTCCTCTAT	1680	GTCACGTCGACGAGCCACAACTGCTGTAC	1681
GCTCCTCTATGCCATTGAGAACAAGTACA	1682	ACTGCTGTACGCGATAGAAAATAAATATA	1683
GCTGGCTACTATGACCTGCTGATTGACAT	1684	GCAGGGTATTACGATCTCCTCATAGATAT	1685
GAGGACTTGAAGCACATCTTGCAGTTGAT	1686	GAAGATTTAAAACATATTTTACAATTAAT	1687
GCAAGCCTTAAACATGTCAGCTGCACTCA	1688	GCAGGCGTTGAATATGTCTGCAGCTCTGA	1689
GATGCCTCTTAAACTGCTGACAAATCATT	1690	AATGCCACTAAAGCTCCTCACTAACCACT	1691
GCCCTATGATACACTGACAGCCAAAGAGA	1692	CCCGTACGACACTCTCACTGCGAAGGAAA	1693
GACCTGGAACTGGACACGCCTTCTATTGA	1694	GATCTCGAGCTCGATACCCCATCAATAGA	1695
GGTGGCAGCAGAGGCAAAGGAGAACATTT	1696	GGAGGGAGTAGGGGGAAGGGTGAGCACTT	1697
GGAGGACATGCTTCCAACAAAGAGAAAGA	1698	GGTGGTCACGCATCGAATAAGGAAAAGGA	1699
GAGGACATGCTTCCAACAAAGAGAAAGAA	1700	GTGGTCACGCATCGAATAAGGAAAAGGAG	1701
GGACATGCTTCCAACAAAGAGAAAGAAAT	1702	GGTCACGCATCGAATAAGGAAAAGGAGAT	1703
GGAGTTCTTGTCAGGCATAGGATTTCACT	1704	GGTGTACTAGTGAGACACAGAATATCTCT	1705
GAGTTCTTGTCAGGCATAGGATTTCACTA	1706	GTGTACTAGTGAGACACAGAATATCTCTT	1707

GGCCAGCATCAGTTCGGAGAAGACCTAAT	1708	GGGCAACACCAATTTGGTGAGGATCTTAT	1709
GCCAGCATCAGTTCGGAGAAGACCTAATA	1710	GGCAACACCAATTTGGTGAGGATCTTATT	1711
GTGGAGAGGCAACGTTCTGCATTAGGAGA	1712	GTCGAAAGACAGCGATCAGCTTTGGGTGA	1713
GCTATTAGATGGCAAATGGCTCTTTACAA	1714	GCAATAAGGTGGCAGATGGCACTATATAA	1715
GCTGTCAATCTCTTTCTTCAGGGATATGA	1716	GCAGTGAACCTGTTCCTACAAGGTTACGA	1717
GGCCTATGCAGATATTATGGCAAAGAGTT	1718	GGCGTACGCTGACATAATGGCTAAAAGCT	1719
GCAGATATTATGGCAAAGAGTTGTCATGA	1720	GCTGACATAATGGCTAAAAGCTGCCACGA	1721
GGATGGTGACAGAGGAAGGATCAGGAGAA	1722	GCATGGTCACTGAAGAGGGTTCTGGTGAG	1723
GATGGTGACAGAGGAAGGATCAGGAGAAA	1724	CATGGTCACTGAAGAGGGTTCTGGTGAGA	1725
GAGAATGAAACCCTCGACTACGAAGAGTT	1726	GAAAACGAGACGCTGGATTATGAGGAATT	1727
GGATCTGAAGAGAGAAGGAGGACAGTACA	1728	CGACCTCAAAAGGGAGGGTGGTCAATATA	1729
GAAAGCCAAGGAAGACAAGGGCAAACAAA	1730	AAAGGCGAAAGAGGATAAAGGGAAGCAGA	1731
GCTACATGGAGCCCACGTTGCGTATCTTA	1732	GGTATATGGAACCGACCTTACGAATTTTG	1733
GATGATATTAAAGGCCAGTGGGATAGACT	1734	GACGACATAAAGGGGCAATGGGACAGGCT	1735
GAAGACCCAGCAGGAGATGAATATGAGAT	1736	GAGGATCCTGCTGGTGACGAGTACGAAAT	1737

In some cases, the mammal can have FH, and the gene to be suppressed and replaced can be *APOB* (which encodes apolipoprotein B). An exemplary *APOB* sequence is set forth in NCBI RefSeq accession number NM_000384 (e.g., version NM_000384.3). An APOB polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_000375 (e.g., version NP_000375.3).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *APOB* are set forth in **TABLE 1Y**.

TABLE 1Y

Representative *APOB* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GGGACTGCTGATTCAAGAAGT	1738	GGCACAGCAGACTCTAGGAGC	1739
GGGACTGCTGATTCAAGAA	1740	GGCACAGCAGACTCTAGGA	1741
GACTGCTGATTCAAGAAGT	1742	CACAGCAGACTCTAGGAGC	1743
GATTCAAGAAGTGCCACCAGGATCA	1744	GACTCTAGGAGCGCGACGAGAATTA	1745
GAAAGATGAACCTACTTACAT	1746	AAAGGACGAGCCAACATATAT	1747
GAACCTACTTACATCCTGAACATCA	1748	GAGCCAACATATATTCTCAATATTA	1749
GGAAACTGCTCCACTCACTTT	1750	GGTAATTGTTCGACACATTTC	1751
GGAAACTGCTCCACTCACT	1752	GGTAATTGTTCGACACATT	1753
GAAACTGCTCCACTCACTT	1754	GTAATTGTTCGACACATTT	1755
GAAGCCATCTGCAAGGAGCAACACCTCTT	1756	GAGGCGATTTGTAAAGAACAGCATCTGTT	1757

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GGCCAACACTTACTTGAAT	2318	AGCGAATACATATTTAAAC	2319
GCCAACACTTACTTGAATT	2320	GCGAATACATATTTAAACT	2321
GCAAGTCAGCCCAGTTCCTTCCATGATTT	2322	GCTAGCCAACCGAGCTCGTTTCACGACTT	2323
GCCCAGTTCCTTCCATGATTT	2324	ACCGAGCTCGTTTCACGACTT	2325
GTTCCTTCCATGATTTCCCTGACCT	2326	GCTCGTTTCACGACTTTCCAGATCT	2327
GTGGCCCTGAATGCTAACACT	2328	GTCGCGCTCAACGCAAATACA	2329
GTGGCCCTGAATGCTAACA	2330	GTCGCGCTCAACGCAAATA	2331
GGCCCTGAATGCTAACACTAA	2332	CGCGCTCAACGCAAATACAAA	2333
GGCCCTGAATGCTAACACT	2334	CGCGCTCAACGCAAATACA	2335
GCCCTGAATGCTAACACTA	2336	GCGCTCAACGCAAATACAA	2337
GGTTCCATCGTGCAAACTTGA	2338	AGTACCTTCCTGTAAGCTAGA	2339
GGTTCCATCGTGCAAACTT	2340	AGTACCTTCCTGTAAGCTA	2341
GTTCCATCGTGCAAACTTGACTTCA	2342	GTACCTTCCTGTAAGCTAGATTTTA	2343
GTTCCATCGTGCAAACTTGACTTCAGAGA	2344	GTACCTTCCTGTAAGCTAGATTTTAGGGA	2345
GTGCAAACTTGACTTCAGAGA	2346	CTGTAAGCTAGATTTTAGGGA	2347
GTGCAAACTTGACTTCAGA	2348	CTGTAAGCTAGATTTTAGG	2349
GCAAACTTGACTTCAGAGAAA	2350	GTAAGCTAGATTTTAGGGAGA	2351
GCAAACTTGACTTCAGAGA	2352	GTAAGCTAGATTTTAGGGA	2353
GCTGAGAACTTCATCATTT	2354	ACTCAGGACATCTTCTTTC	2355
GTACCTGCTGGAATTGTCA	2356	GTTCCAGCAGGTATAGTGA	2357
GTGACTTCAGTGCAGAATA	2358	GAGATTTTAGCGCTGAGTA	2359
GTGCAGAATATGAAGAAGA	2360	GCGCTGAGTACGAGGAGGA	2361
GATGGCAAATATGAAGGACTT	2362	GACGGGAAGTACGAGGGTCTA	2363
GCTTCTGGCTTGCTAACCTCTCTGA	2364	GCATCAGGGTTACTTACGTCACTCA	2365
GCTTCTGGCTTGCTAACCTCTCTGAAAGA	2366	GCATCAGGGTTACTTACGTCACTCAAGGA	2367
GCTTCTGGCTTGCTAACCT	2368	GCATCAGGGTTACTTACGT	2369
GGCTTGCTAACCTCTCTGAAA	2370	GGGTTACTTACGTCACTCAAG	2371
GGCTTGCTAACCTCTCTGAAAGACA	2372	GGGTTACTTACGTCACTCAAGGATA	2373
GGCTTGCTAACCTCTCTGA	2374	GGGTTACTTACGTCACTCA	2375
GCTTGCTAACCTCTCTGAAAGACAA	2376	GGTTACTTACGTCACTCAAGGATAA	2377
GCTTGCTAACCTCTCTGAA	2378	GGTTACTTACGTCACTCAA	2379
GCTAACCTCTCTGAAAGACAA	2380	ACTTACGTCACTCAAGGATAA	2381

GGGCCATTAGGCAAATTGA	2382	GCGCGATAAGACAGATAGA	2383
GGCCATTAGGCAAATTGATGA	2384	CGCGATAAGACAGATAGACGA	2385
GGCCATTAGGCAAATTGAT	2386	CGCGATAAGACAGATAGAC	2387
GGACCTACCAAGAGTGGAAGGACAA	2388	GCACGTATCAGGAATGGAAAGATAA	2389

In some cases, the mammal can have DCM or HCM, and the gene to be suppressed and replaced can be *TNNI3* (which encodes cardiac type Troponin I3). An exemplary *TNNI3* sequence is set forth in NCBI RefSeq accession number NM_000363 (e.g., version NM_000363.5). A TNNI3 polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number Q59H18 (e.g., version Q59H18.3).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *TNNI3* are set forth in **TABLE 1Z**.

TABLE 1Z

Representative *TNNI3* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GTGGACAAGGTGGATGAAGAGAGAT	2390	CTGGACTAGATGGATGAAAAGGGAC	2391
GACAAGGTGGATGAAGAGA	2392	GACTAGATGGATGAAAAGG	2393
GACCTTCGAGGCAAGTTTA	2394	GATCTACGTGGGAAATTCA	2395

In some cases, the mammal can have DCM or HCM, and the gene to be suppressed and replaced can be *TNNC1* (which encodes slow skeletal and cardiac type Troponin C1). An exemplary *TNNC1* sequence is set forth in NCBI RefSeq accession number NM_003280 (e.g., version NM_003280.3). A TNNC1 polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_003271 (e.g., version NP_003271.1).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *TNNC1* are set forth in **TABLE 1AA**.

TABLE 1AA

Representative *TNNC1* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GGTAGAGCAGCTGACAGAA	2396	CGTTGAACAACTCACTGAG	2397
AGGAGCTGCAGGAGATGAT	2398	AAGAACTCCAAGAAATGAT	2399
GATGGTTCGGTGCATGAAG	2400	GATGGTACGCTGTATGAAA	2401
GCATGAAGGACGACAGCAAAGGGAAATCT	2402	GTATGAAAGATGATAGTAAGGGCAAGTCA	2403
ATGAAGGACGACAGCAAAGGGAAATCTGA	2404	ATGAAAGATGATAGTAAGGGCAAGTCAGA	2405
GAAGGACGACAGCAAAGGGAAATCT	2406	GAAAGATGATAGTAAGGGCAAGTCA	2407
AGGACGACAGCAAAGGGAAATCTGA	2408	AAGATGATAGTAAGGGCAAGTCAGA	2409
GGACGACAGCAAAGGGAAATCTGAG	2410	AGATGATAGTAAGGGCAAGTCAGAA	2411
GACGACAGCAAAGGGAAATCT	2412	GATGATAGTAAGGGCAAGTCA	2413
ACAGCAAAGGGAAATCTGA	2414	ATAGTAAGGGCAAGTCAGA	2415
GCAAAGGGAAATCTGAGGAGGAGCTGTCT	2416	GTAAGGGCAAGTCAGAAGAAGAACTCTCA	2417
AAAGGGAAATCTGAGGAGGAGCTGTCTGA	2418	AAGGGCAAGTCAGAAGAAGAACTCTCAGA	2419
AGGGAAATCTGAGGAGGAGCTGTCT	2420	GGGCAAGTCAGAAGAAGAACTCTCA	2421
GGAAATCTGAGGAGGAGCTGTCTGA	2422	GCAAGTCAGAAGAAGAACTCTCAGA	2423
GAAATCTGAGGAGGAGCTGTCTGAC	2424	CAAGTCAGAAGAAGAACTCTCAGAT	2425
AAATCTGAGGAGGAGCTGTCT	2426	AAGTCAGAAGAAGAACTCTCA	2427
ATCTGAGGAGGAGCTGTCT	2428	GTCAGAAGAAGAACTCTCA	2429
AGGAGCTGTCTGACCTCTTCCGCATGTTT	2430	AAGAACTCTCAGATCTGTTTCGGATGTTC	2431
AGGAGCTGTCTGACCTCTT	2432	AAGAACTCTCAGATCTGTT	2433
GCTGTCTGACCTCTTCCGCATGTTT	2434	ACTCTCAGATCTGTTTCGGATGTTC	2435
ATCGACCTGGATGAGCTGAAGATAA	2436	ATTGATCTCGACGAACTCAAAATTA	2437
GACCTGGATGAGCTGAAGATA	2438	GATCTCGACGAACTCAAAATT	2439
GACCTGGATGAGCTGAAGA	2440	GATCTCGACGAACTCAAAA	2441
ACCTGGATGAGCTGAAGATAA	2442	ATCTCGACGAACTCAAAATTA	2443
ACCTGGATGAGCTGAAGAT	2444	ATCTCGACGAACTCAAAAT	2445
GGATGAGCTGAAGATAATG	2446	CGACGAACTCAAAATTATG	2447
GAGCTGAAGATAATGCTGCAGGCTACAGG	2448	GAACTCAAAATTATGCTCCAAGCAACTGG	2449
AGGACGACATCGAGGAGCTCATGAA	2450	AAGATGATATTGAAGAACTGATGAA	2451
ACGACATCGAGGAGCTCATGA	2452	ATGATATTGAAGAACTGATGA	2453
ATCGAGGAGCTCATGAAGGACGGAGACAA	2454	ATTGAAGAACTGATGAAAGATGGTGATAA	2455
GAGGAGCTCATGAAGGACGGAGACAAGAA	2456	GAAGAACTGATGAAAGATGGTGATAAAAA	2457
AGGAGCTCATGAAGGACGGAGACAA	2458	AAGAACTGATGAAAGATGGTGATAA	2459
GAGCTCATGAAGGACGGAGACAAGAACAA	2460	GAACTGATGAAAGATGGTGATAAAAATAA	2461
GAGCTCATGAAGGACGGAGACAAGA	2462	GAACTGATGAAAGATGGTGATAAAA	2463
AGCTCATGAAGGACGGAGACAAGAA	2464	AACTGATGAAAGATGGTGATAAAAA	2465

AGCTCATGAAGGACGGAGACA	2466	AACTGATGAAAGATGGTGATA	2467
GCTCATGAAGGACGGAGACAAGAAC	2468	ACTGATGAAAGATGGTGATAAAAAT	2469
GCTCATGAAGGACGGAGACAA	2470	ACTGATGAAAGATGGTGATAA	2471
GAAGGACGGAGACAAGAACAA	2472	GAAAGATGGTGATAAAAATAA	2473
GAAGGACGGAGACAAGAAC	2474	GAAAGATGGTGATAAAAAT	2475
AGGACGGAGACAAGAACAA	2476	AAGATGGTGATAAAAATAA	2477
GGACGGAGACAAGAACAAC	2478	AGATGGTGATAAAAATAAT	2479

In some cases, the mammal can have HCM or DCM, and the gene to be suppressed and replaced can be *MYL2* (which encodes myosin light chain 2). An exemplary *MYL2* sequence is set forth in NCBI RefSeq accession number NM_000432 (e.g., version NM_000432.4). A MYL2 polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_000423 (e.g., version NP_000423.2).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *MYL2* are set forth in **TABLE 1BB**.

TABLE 1BB

Representative *MYL2* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GGCACCTAAGAAAGCAAAGAA	2480	GGCTCCAAAAAAGGCTAAAAA	2481
GGCACCTAAGAAAGCAAAGAAGAGA	2482	GGCTCCAAAAAAGGCTAAAAAAAGG	2483
GCCAACTCCAACGTGTTCT	2484	GCGAATTCGAATGTCTTTT	2485
GGAGGCCTTCACTATCATGGACCAGAACA	2486	AGAAGCGTTTACAATTATGGATCAAAATA	2487
GCCTTCACTATCATGGACCAGAACA	2488	GCGTTTACAATTATGGATCAAAATA	2489
GGACCAGAACAGGGATGGCTTCATT	2490	GGATCAAAATAGAGACGGGTTTATA	2491
GGACCAGAACAGGGATGGCTTCATTGACA	2492	GGATCAAAATAGAGACGGGTTTATAGATA	2493
GGGATGGCTTCATTGACAAGA	2494	GAGACGGGTTTATAGATAAAA	2495
GGATGGCTTCATTGACAAGAA	2496	AGACGGGTTTATAGATAAAAA	2497
GGATGGCTTCATTGACAAGAACGATCTGA	2498	AGACGGGTTTATAGATAAAAATGACCTCA	2499
GATGGCTTCATTGACAAGA	2500	GACGGGTTTATAGATAAAA	2501
GGCTTCATTGACAAGAACGATCTGA	2502	GGGTTTATAGATAAAAATGACCTCA	2503
GGCTTCATTGACAAGAACGATCTGAGAGA	2504	GGGTTTATAGATAAAAATGACCTCAGGGA	2505
GAACGATCTGAGAGACACCTT	2506	AAATGACCTCAGGGATACGTT	2507
GAGGCTCCGGGTCCAATTAACTTTACTGT	2508	GAAGCACCCGGACCTATAAATTTCACAGT	2509
GGCTCCGGGTCCAATTAACTT	2510	AGCACCCGGACCTATAAATTT	2511
GGCTCCGGGTCCAATTAACTTTACT	2512	AGCACCCGGACCTATAAATTTCACA	2513

GCTCCGGGTCCAATTAACTTT	2514	GCACCCGGACCTATAAATTTC	2515
GGGTCCAATTAACTTTACT	2516	CGGACCTATAAATTTCACA	2517
GGGTCCAATTAACTTTACTGT	2518	CGGACCTATAAATTTCACAGT	2519
GTCCAATTAACTTTACTGT	2520	GACCTATAAATTTCACAGT	2521
GAGGAAACCATTCTCAACGCATTCAAAGT	2522	GAAGAGACGATACTGAATGCTTTTAAGGT	2523
GGAAACCATTCTCAACGCATTCAAA	2524	AGAGACGATACTGAATGCTTTTAAG	2525
GAAACCATTCTCAACGCATTCAAAGTGTT	2526	GAGACGATACTGAATGCTTTTAAGGTCTT	2527
GGGTGCTGAAGGCTGATTA	2528	GCGTCCTCAAAGCAGACTA	2529
GGTGCTGAAGGCTGATTACGT	2530	CGTCCTCAAAGCAGACTATGT	2531
GGCTGATTACGTTCGGGAAATGCTGACCA	2532	AGCAGACTATGTACGCGAGATGCTCACGA	2533
GTTCGGGAAATGCTGACCA	2534	GTACGCGAGATGCTCACGA	2535
GGAGGAGGTTGACCAGATGTT	2536	AGAAGAAGTAGATCAAATGTT	2537
GAGGAGGTTGACCAGATGT	2538	GAAGAAGTAGATCAAATGT	2539
GACGTGACTGGCAACTTGGACTACA	2540	GATGTCACAGGGAATTTAGATTATA	2541
GACTGGCAACTTGGACTACAA	2542	CACAGGGAATTTAGATTATAA	2543
GGACTACAAGAACCTGGTGCACATCATCA	2544	AGATTATAAAAATCTCGTCCATATTATTA	2545
GTGCACATCATCACCCACGGAGAAGAGAA	2546	GTCCATATTATTACGCATGGTGAGGAAAA	2547

In some cases, the mammal can have HCM or DCM, and the gene to be suppressed and replaced can be *MYL3* (which encodes myosin light chain 3). An exemplary *MYL3* sequence is set forth in NCBI RefSeq accession number NM_000258 (e.g., version NM_000258.3). An MYL3 polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_000249 (e.g., version NP_000249.1).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *MYL3* are set forth in **TABLE 1CC**.

TABLE 1CC

Representative *MYL3* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GATGCTTCCAAGATCAAGA	2548	GACGCATCGAAAATTAAAA	2549
GATGAAGATCACCTACGGGCAGTGT	2550	AATGAAAATTACGTATGGCCAATGC	2551
GAAGCCAAGACAGGAAGAGCT	2552	CAAACCTAGGCAAGAGGAACT	2553
GAAGCCAAGACAGGAAGAGCTCAATACCA	2554	CAAACCTAGGCAAGAGGAACTGAACACGA	2555
GAAGAGCTCAATACCAAGATGATGGACTT	2556	GAGGAACTGAACACGAAAATGATGGATTT	2557

GAGCTCAATACCAAGATGA	2558	GAACTGAACACGAAAATGA	2559
GCTCAATACCAAGATGATGGACTTT	2560	ACTGAACACGAAAATGATGGATTTC	2561
GAGGCTGACAGAAGACGAAGTGGAGAAGT	2562	AAGACTCACTGAGGATGAGGTCGAAAAAT	2563
GCTGACAGAAGACGAAGTGGA	2564	ACTCACTGAGGATGAGGTCGA	2565
GCTGACAGAAGACGAAGTGGAGAAGTTGA	2566	ACTCACTGAGGATGAGGTCGAAAAATTAA	2567
GAAGACGAAGTGGAGAAGT	2568	GAGGATGAGGTCGAAAAAT	2569
GACGAAGTGGAGAAGTTGA	2570	GATGAGGTCGAAAAATTAA	2571
GCAAGAGGACTCCAATGGCTGCATCAACT	2572	CCAGGAAGATTCGAACGGGTGTATTAATT	2573
GAGGACTCCAATGGCTGCATCAACT	2574	GAAGATTCGAACGGGTGTATTAATT	2575

In some cases, the mammal can have HCM or DCM, and the gene to be suppressed and replaced can be *JPH2* (which encodes junctophilin 2). Exemplary *JPH2* sequences are set forth in NCBI RefSeq accession number NM_020433 (e.g., version NM_020433.5) and NCBI RefSeq accession number NM_175913 (e.g., version NM_175913.4). A JPH2 polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_065166 (e.g., version NP_065166.2) or NCBI RefSeq accession number NP_787109 (e.g., version NP_787109.2).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *JPH2* are set forth in **TABLE 1DD**.

TABLE 1DD

Representative *JPH2* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GCGAATACTCTGGCTCCTGGAACTT	2576	GGGAGTATTCAGGGTCGTGGAATTT	2577
GCCGTGTCAGCTTCCTTAAGA	2578	GTCGAGTGAGTTTTCTAAAAA	2579
GCCAACCAGGAGTCCAACATT	2580	GCGAATCAAGAATCGAATATA	2581
GTCCAACATTGCTCGCACTTT	2582	ATCGAATATAGCACGGACATT	2583
GACTTCTACCAGCCAGGTCCGGAATATCA	2584	GATTTTTATCAACCTGGACCCGAGTACCA	2585
GCATGGTGATCCTGCTGAACA	2586	GTATGGTCATTCTCCTCAATA	2587

In some cases, the mammal can have LQTS, HCM, or limb-girdle muscular dystrophy (LGMD), and the gene to be suppressed and replaced can be *CAV3* (which encodes caveolin 3). Exemplary *CAV3* sequences are set forth in NCBI RefSeq accession number NM_033337 (e.g., version NM_033337.3) and NCBI RefSeq accession number NM_001234 (e.g., version NM_001234.5). A CAV3 polypeptide can, in some cases,

have the amino acid sequence set forth in NCBI RefSeq accession number NP_203123 (e.g., version NP_203123.1) or NCBI RefSeq accession number NP_001225 (e.g., version NP_001225.1).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *CAV3* are set forth in **TABLE 1EE**.

TABLE 1EE

Representative *CAV3* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GGCCCAGATCGTCAAGGATAT	2588	AGCGCAAATTGTGAAAGACAT	2589
GGTGAACCGAGACCCCAAGAACATT	2590	CGTCAATCGTGATCCGAAAAATATA	2591
GTGAACCGAGACCCCAAGAACATTA	2592	GTCAATCGTGATCCGAAAAATATAA	2593
GAACCGAGACCCCAAGAACAT	2594	CAATCGTGATCCGAAAAATAT	2595
GAGACCCCAAGAACATTAACGAGGACATA	2596	GTGATCCGAAAAATATAAATGAAGATATT	2597
GACCCCAAGAACATTAACGAGGACA	2598	GATCCGAAAAATATAAATGAAGATA	2599
GACCCCAAGAACATTAACGAGGACATAGT	2600	GATCCGAAAAATATAAATGAAGATATTGT	2601
GAACATTAACGAGGACATA	2602	AAATATAAATGAAGATATT	2603
GAACATTAACGAGGACATAGT	2604	AAATATAAATGAAGATATTGT	2605
GAGCTACACCACCTTCACT	2606	CAGTTATACGACGTTTACA	2607
GAGCTACACCACCTTCACTGT	2608	CAGTTATACGACGTTTACAGT	2609
GCTACACCACCTTCACTGT	2610	GTTATACGACGTTTACAGT	2611
GCTACACCACCTTCACTGTCT	2612	GTTATACGACGTTTACAGTGT	2613
GCATCTCCTTCTGCCACATCT	2614	GTATTTCGTTTTGTCATATTT	2615
GCCATGCATTAAGAGCTACCT	2616	CCCTTGTATAAAAAGTTATCT	2617

In some cases, the mammal can have LQTS or CPVT, and the gene to be suppressed and replaced can be *TECRL* (which encodes trans-2,3-enoyl-CoA reductase like protein). Exemplary *TECRL* sequences are set forth in NCBI RefSeq accession number NM_001010874 (e.g., version NM_001010874.5) and NCBI RefSeq accession number NM_001363796 (e.g., version NM_001363796.1). A TECRL polypeptide can, in some cases, have the amino acid sequence set forth in NCBI RefSeq accession number NP_001010874 (e.g., version NP_001010874.2) or NCBI RefSeq accession number NP_001350725 (e.g., version NP_001350725.1).

Examples of shRNA sequences and corresponding shIMM sequences targeted to *TECRL* are set forth in **TABLE 1FF**.

TABLE 1FF

Representative *TECRL* shRNA and shIMM sequences

shRNA Sequence	SEQ ID	shIMM Sequence	SEQ ID
GGAACGCAAGAGAGCATTACT	2618	CGAGCGGAAAAGGGCTTTGCT	2619
GAACGCAAGAGAGCATTACTT	2620	GAGCGGAAAAGGGCTTTGCTA	2621
GAGCTACACGGTTCATACT	2622	GGGCAACTCGCTTTATTCT	2623
GAGCTACACGGTTCATACTGA	2624	GGGCAACTCGCTTTATTCTCA	2625
GCTACACGGTTCATACTGA	2626	GCAACTCGCTTTATTCTCA	2627
GAAGGATGATATGAGAAAT	2628	CAAAGACGACATGAGGAAC	2629
GCCCTCTAAGACCAACTCCAGCAGTCAAA	2630	GGCCACTTAGGCCTACACCTGCTGTGAAG	2631
GACCAACTCCAGCAGTCAA	2632	GGCCTACACCTGCTGTGAA	2633
GATGCTCAAACAAGGAAACAGATAT	2634	GACGCACAGACTAGAAAGCAAATTT	2635
GCTCAAACAAGGAAACAGA	2636	GCACAGACTAGAAAGCAAA	2637
GCTCAAACAAGGAAACAGATA	2638	GCACAGACTAGAAAGCAAATT	2639
GCTCAAACAAGGAAACAGATATGTATTCT	2640	GCACAGACTAGAAAGCAAATTTGCATACT	2641
GGAAACAGATATGTATTCT	2642	GAAAGCAAATTTGCATACT	2643
GAAGGACTACATTACCATTCAAAGT	2644	AAAAGATTATATAACGATACAGAGC	2645
GGACTACATTACCATTCAA	2646	AGATTATATAACGATACAG	2647
GGACTACATTACCATTCAAAGTATT	2648	AGATTATATAACGATACAGAGCATA	2649
GCAGCTTCCTCCATTGTCA	2650	GCTGCATCGTCGATAGTGA	2651
GCAGCTTCCTCCATTGTCACA	2652	GCTGCATCGTCGATAGTGACT	2653
GCAGCTTCCTCCATTGTCACACTGT	2654	GCTGCATCGTCGATAGTGACTCTCT	2655
GTCAGTTGGACCACAGTGT	2656	GTGAGCTGGACGACTGTCT	2657
GGACCTCTGCTAATATACCTCCTCT	2658	GGTCCACTCCTTATTTATCTGCTGT	2659
GACCTCTGCTAATATACCT	2660	GTCCACTCCTTATTTATCT	2661
GACCTCTGCTAATATACCTCCTCTT	2662	GTCCACTCCTTATTTATCTGCTGTT	2663
GCTAATATACCTCCTCTTT	2664	CCTTATTTATCTGCTGTTC	2665
GAGGATCCCATGTATATAT	2666	AAGAATTCCTTGCATTTAC	2667
GGCTTGCTTCTGTCATTGT	2668	AGCATGTTTTTGCCACTGC	2669
GGCTTGCTTCTGTCATTGTAT	2670	AGCATGTTTTTGCCACTGCAT	2671
GCTTGCTTCTGTCATTGTA	2672	GCATGTTTTTGCCACTGCA	2673
GCTTGCTTCTGTCATTGTATA	2674	GCATGTTTTTGCCACTGCATT	2675
GGGATTTACTTCTTGGATTGCCTACTACA	2676	GGGTTTCACATCATGGATAGCGTATTATA	2677
GGATTTACTTCTTGGATTGCCTACTACAT	2678	GGTTTCACATCATGGATAGCGTATTATAT	2679
GATTTACTTCTTGGATTGCCTACTA	2680	GTTTCACATCATGGATAGCGTATTA	2681
GATTTACTTCTTGGATTGCCTACTACATT	2682	GTTTCACATCATGGATAGCGTATTATATA	2683

GATTGCCTACTACATTAAT	2684	GATAGCGTATTATATAAAC	2685
GCCTACTACATTAATCATCCACTAT	2686	GCGTATTATATAAACCACCCTCTTT	2687
GAAACAGGCAAATCACAGT	2688	GTAATAGACAGATTACTGT	2689
GGCAAATCACAGTATCTGCTATCAA	2690	GACAGATTACTGTTTCAGCAATTAA	2691
GCAAATCACAGTATCTGCTATCAAT	2692	ACAGATTACTGTTTCAGCAATTAAC	2693
GCTGGGAATCATTTCATCA	2694	GCAGGCAACCACTTTATTA	2695
GCTGGGAATCATTTCATCAAT	2696	GCAGGCAACCACTTTATTAAC	2697
GCCTGTTTCCCAAGTCCAAATTATA	2698	GCGTGCTTTCCTAGCCCTAACTACA	2699
GTTTCCCAAGTCCAAATTA	2700	GCTTTCCTAGCCCTAACTA	2701
GTTTCCCAAGTCCAAATTATA	2702	GCTTTCCTAGCCCTAACTACA	2703
GGTTTCATGTCCTAACTACACCTAT	2704	CGTATCTTGCCCAAATTATACGTAC	2705
GTTTCATGTCCTAACTACA	2706	GTATCTTGCCCAAATTATA	2707
GTCCTAACTACACCTATGA	2708	GCCCAAATTATACGTACGA	2709
GTCCTAACTACACCTATGAGA	2710	GCCCAAATTATACGTACGAAA	2711
GAGATTGGATCATGGATTAGT	2712	GAAATAGGTTCTTGGATAAGC	2713
GAGATTGGATCATGGATTAGTTTCACAGT	2714	GAAATAGGTTCTTGGATAAGCTTTACTGT	2715
GATTGGATCATGGATTAGT	2716	AATAGGTTCTTGGATAAGC	2717
GATTGGATCATGGATTAGTTT	2718	AATAGGTTCTTGGATAAGCTT	2719
GATTGGATCATGGATTAGTTTCACA	2720	AATAGGTTCTTGGATAAGCTTTACT	2721
GATTGGATCATGGATTAGTTTCACAGTCA	2722	AATAGGTTCTTGGATAAGCTTTACTGTGA	2723
GGATCATGGATTAGTTTCA	2724	GGTTCTTGGATAAGCTTTA	2725
GGATCATGGATTAGTTTCACA	2726	GGTTCTTGGATAAGCTTTACT	2727
GGATCATGGATTAGTTTCACAGTCA	2728	GGTTCTTGGATAAGCTTTACTGTGA	2729
GATCATGGATTAGTTTCACAGTCAT	2730	GTTCTTGGATAAGCTTTACTGTGAT	2731
GGATTAGTTTCACAGTCAT	2732	GGATAAGCTTTACTGTGAT	2733
GGATTAGTTTCACAGTCATGA	2734	GGATAAGCTTTACTGTGATGA	2735
GATGAGTATCCAGATGTCT	2736	CATGAGCATTCAAATGTCA	2737

Any appropriate method can be used to deliver a SupRep nucleic acid construct to cells (e.g., cardiac cells) within a living mammal. For example, a SupRep construct containing a suppressive component and a replacement component can be administered to a mammal using one or more vectors, such as viral vectors. In some cases, vectors for administering SupRep nucleic acids can be used for transient expression of the suppressive and corrective components. In some cases, vectors for administering SupRep nucleic acids can be used for stable expression of the suppressive and corrective components. In some cases, where a vector for administering nucleic acid can be used for

stable expression, the vector can be engineered to integrate nucleic acid designed to express the suppressive component and/or nucleic acid designed to express the corrective component into the genome of a cell. In such cases, any appropriate method can be used to integrate the nucleic acid(s) into the genome of a cell. For example, gene therapy techniques can be used to integrate nucleic acid designed to express a suppressive component (e.g., a shRNA) and/or nucleic acid designed to express a corrective component (e.g., a wild type polypeptide that is immune to the suppressive component) into the genome of a cell. In some cases, stable expression does not necessarily require integration into the genome. Using AAV9, for example, the SupRep DNA can persist on its own in the cell, without integrating into the human genome. Non-integrated DNA typically is destroyed as genomic DNA replicates, but in non-dividing cells such as cardiomyocytes or neurons, the SupRep DNA can persist indefinitely since the cells do not replicate or divide to remove the SupRep DNA.

Vectors for administering SupRep nucleic acids to cells can be prepared using standard materials (e.g., packaging cell lines, helper viruses, and vector constructs). *See*, for example, Gene Therapy Protocols (Methods in Molecular Medicine), edited by Jeffrey R. Morgan, Humana Press, Totowa, NJ (2002), and Viral Vectors for Gene Therapy: Methods and Protocols, edited by Curtis A. Machida, Humana Press, Totowa, NJ (2003). Virus-based nucleic acid delivery vectors typically are derived from animal viruses, such as adenoviruses, adeno-associated viruses (AAVs), retroviruses, lentiviruses, vaccinia viruses, herpes viruses, and papilloma viruses. In some cases, a SupRep nucleic acid construct can be delivered to cells using adeno-associated virus vectors (e.g., an AAV serotype 1 viral vector, an AAV serotype 2 viral vector, an AAV serotype 3 viral vector, an AAV serotype 4 viral vector, an AAV serotype 5 viral vector, an AAV serotype 6 viral vector, an AAV serotype 7 viral vector, an AAV serotype 8 viral vector, an AAV serotype 9 viral vector, an AAV serotype 10 viral vector, an AAV serotype 11 viral vector, an AAV serotype 12 viral vector, or a recombinant AAV serotype viral vector such as an AAV serotype 2/9 viral vector in which the AAV2 inverted terminal repeats and genome are contained within the AAV9 capsid, which can result in AAV9 tropism for cardiomyocytes), lentiviral vectors, retroviral vectors,

adenoviral vectors, herpes simplex virus vectors, or poxvirus vector. In some cases, an AAV9 vector can be used to deliver one or more SupRep nucleic acids to cells.

In addition to nucleic acid encoding a suppressive component and nucleic acid encoding a corrective component, a viral vector can contain regulatory elements operably linked to the nucleic acid encoding the suppressive component and the corrective component. As used herein, "operably linked" refers to positioning of a regulatory element in a vector relative to a nucleic acid in such a way as to permit or facilitate expression of the encoded RNA and/or polypeptide. Such regulatory elements can include promoter sequences, enhancer sequences, response elements, signal peptides, internal ribosome entry sequences (IRES), P2A self-cleaving peptide sequences, polyadenylation signals, terminators, or inducible elements that modulate expression (e.g., transcription or translation) of a nucleic acid. The choice of element(s) that may be included in a viral vector depends on several factors, including, without limitation, inducibility, targeting, and the level of expression desired. For example, a promoter can be included in a viral vector to facilitate transcription of a nucleic acid encoding a suppressive component (e.g., a shRNA) and a corrective component (e.g., a WT polypeptide that is immune to the suppression by the suppressive component). A promoter can be constitutive or inducible (e.g., in the presence of tetracycline or rapamycin), and can affect the expression of a nucleic acid encoding a shRNA or a polypeptide in a general or tissue-specific manner. Examples of promoters that can be used to drive expression of suppressive and corrective components (e.g., in cardiomyocyte cells) include, without limitation, a U6 promoter, a H1 promoter a cytomegalovirus immediate-early (CMV) promoter, an alpha-myosin heavy chain promoter, a myosin light chain 2 promoter, cardiac troponin T, and a cardiac troponin C promoter.

As used herein, the term "AAV particle" refers to packaged capsid forms of the AAV virus that transmits its nucleic acid genome to cells. The term "viral genome" refers to one copy of a virus genome. Each virus particle contains one viral genome, and each AAV vector contains one viral genome. In some cases, a composition containing an AAV particle encoded by an AAV vector as provided herein can be administered at a

concentration from about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL (e.g., from about 10^{10} AAV particles/mL to about 10^{11} AAV particles/mL, from about 10^{10} AAV particles/mL to about 10^{12} AAV particles/mL, from about 10^{10} AAV particles/mL to about 10^{13} AAV particles/mL, from about 10^{11} AAV particles/mL to about 10^{12} AAV particles/mL, from about 10^{11} AAV particles/mL to about 10^{13} AAV particles/mL, from about 10^{11} AAV particles/mL to about 10^{14} AAV particles/mL, from about 10^{12} AAV particles/mL to about 10^{13} AAV particles/mL, from about 10^{12} AAV particles/mL to about 10^{14} AAV particles/mL, or from about 10^{13} AAV particles/mL to about 10^{14} AAV particles/mL). In some cases, a composition containing an AAV particle encoded by an AAV vector as provided herein can be administered at a concentration from about 10^{10} viral genomes per kilogram body weight (vg/kg) to about 10^{15} vg/kg (e.g., from about 10^{10} to about 10^{11} vg/kg, from about 10^{10} to about 10^{12} vg/kg, from about 10^{10} to about 10^{13} vg/kg, from about 10^{11} to about 10^{12} vg/kg, from about 10^{11} to about 10^{13} vg/kg, from about 10^{11} to about 10^{14} vg/kg, from about 10^{12} to about 10^{13} vg/kg, from about 10^{12} to about 10^{14} vg/kg, or from about 10^{13} to about 10^{14} vg/kg).

In some cases, a SupRep nucleic acid construct can be administered to a mammal using a non-viral vector. Methods of using non-viral vectors for nucleic acid delivery are described elsewhere. *See*, for example, Gene Therapy Protocols (Methods in Molecular Medicine), edited by Jeffrey R. Morgan, Humana Press, Totowa, NJ (2002). For example, a SupRep nucleic acid encoding a suppressive component and a corrective component can be administered to a mammal by direct injection of nucleic acid molecules (e.g., plasmids) comprising SupRep nucleic acid, or by administering nucleic acid molecules complexed with lipids, polymers, or nanospheres. In some cases, a SupRep nucleic acid designed to express a suppressive component and a corrective component can be delivered to cells (e.g., cardiomyocytes) via direct injection (e.g., into the myocardium), intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills.

When KCNQ1-SupRep and/or KCNH2-SupRep and/or SCN5A-SupRep gene therapy is efficiently delivered to the majority of cardiomyocytes, the gene therapy-

mediated restoration of repolarization reserve may distribute via gap junctions to partially or completely compensate for neighboring untransduced cardiomyocytes. From the studies described herein, it was noteworthy that during measurement of optical action potentials, no arrhythmic activity was observed in electrically coupled iPSC-CMs that had been transduced with KCNQ1-SupRep – suggesting that efficient transduction of cells may be sufficient to maintain normal rhythm and compensate for untransduced neighboring cells.

Any appropriate amount of a SupRep nucleic acid can be administered to a mammal (e.g., a human) having a congenital disorder. An effective amount of a SupRep nucleic acid can reduce one or more symptoms of the disorder being treated. In some cases, for example, effective suppression-and-replacement of *KCNQ1* (e.g., for patients having LQT1, severe cases where multiple pathogenic variants in *KCNQ1* are inherited such as autosomal recessive LQT1 and Jervell and Lange-Nielsen syndrome (JLNS), or type 2 SQTS (SQT2)) using KCNQ1-SupRep gene therapy can produce I_{Ks} current density similar to that of a healthy individual (e.g., within about 50%, about 25%, about 20%, about 15%, about 10%, or about 5% of the I_{Ks} current density of a healthy individual). Pathogenic variants in *KCNQ1* that lead to a gain-of-function and an abnormal increase in I_{Ks} current density can lead to SQTS. In some cases, a therapeutically effective amount can provide enough I_{Ks} to ameliorate the LQTS phenotype without overcompensating and causing SQTS. In LQT1 and JLNS, disease severity correlates with the degree of lost I_{Ks} (Moss *et al.*, *Circulation*, 115:2481-2489 (2007)). Heterozygous nonsense or frameshift mutations cause haploinsufficiency and typically result in mild LQT1 with ~50% I_{Ks}. Dominant-negative missense mutations reduce I_{Ks} beyond 50% and are more strongly associated with breakthrough cardiac events. In the most severe cases, patients with JLNS inherit two mutant alleles that result in either minimal (<10%) or no I_{Ks} (Bhuiyan *et al.*, *Prog. Biophys. Mol. Biol.*, 98:319-327 (2008)). Conversely, *KCNQ1* variants with substantial gain of function cause SQT2, though the exact degree of increased I_{Ks} is not well established (Chen *et al.*, *Science*, 299:251-254 (2003); Bellocq *et al.*, *Circulation*, 109:2394-2397 (2004); Hong *et al.*, *Cardiovasc. Res.*, 68:433-440 (2005); and Das *et al.*, *Heart Rhythm*, 6:1146-1153

(2009)). Thus, the therapeutic window for KCNQ1-SupRep in humans may be relatively wide, allowing flexibility for achieving optimal efficacy. In some cases, KCNQ1-SupRep dosing can be modified by the promoters and/or enhancers driving expression, or by the amount of viral particles delivered to the mammal. In some cases, a therapeutically effective amount of a KCNQ1-SupRep construct can increase I_{Ks} by at least 25% (e.g., at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 90%, at least 100%, or at least 200%), as compared to the I_{Ks} prior to treatment.

In some cases, effective suppression-and-replacement of *KCNH2* (e.g., for patients having LQT2 or type 1 short QT syndrome (SQT1)) using KCNH2-SupRep gene therapy can produce I_{Kr} current density similar to that of a healthy individual (e.g., within about 50%, about 25%, about 20%, about 15%, about 10%, or about 5% of the I_{Kr} current density of a healthy individual). In some cases, a therapeutically effective amount can provide enough I_{Kr} to ameliorate the LQTS phenotype without overcompensating and causing SQTS. Like LQT1, in LQT2, disease severity correlates with the degree of lost I_{Kr} (Moss *et al.*, *Circulation*, 105:794-799 (2002)). Heterozygous nonsense or frameshift mutations cause haploinsufficiency and typically result in LQT2 with ~50% I_{Kr}. Dominant-negative missense mutations reduce I_{Kr} beyond 50% and are more strongly associated with cardiac events, especially when localized to the pore region of the channel (Moss *et al.*, *supra*). Conversely, *KCNH2* variants with substantial gain of function can cause SQT1 (Brugada *et al.*, *Circulation*, 109:30-35 (2004); and Sun *et al.*, *JMCC*, 50:433-441 (2011)). Thus, the therapeutic window for KCNH2-SupRep in humans may be relatively wide, allowing flexibility for achieving optimal efficacy. In some cases, KCNH2-SupRep dosing can be modified by the promoters and/or enhancers driving expression, or by the amount of viral particles delivered to the mammal. In some cases, a therapeutically effective amount of a KCNH2-SupRep construct can increase I_{Kr} by at least 25% (e.g., at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 90%, at least 100%, or at least 200%), as compared to the I_{Kr} prior to treatment.

In some cases, effective suppression-and-replacement of *SCN5A* (e.g., for patients having LQT3, multifocal ectopic premature Purkinje-related contraction (MEPPC) syndrome, SCN5A-mediated dilated cardiomyopathy, recessive sick sinus syndrome, or

BrS) using SCN5A-SupRep gene therapy can produce I_{Na} current density and sodium channel kinetics similar to that of a healthy individual (e.g., within about 50%, about 25%, about 20%, about 15%, about 10%, or about 5% of the I_{Na} current density of a healthy individual). In some cases, SCN5A-SupRep dosing can be modified by the promoters and/or enhancers driving expression, or by the amount of viral particles delivered to the mammal. In some cases, a therapeutically effective amount of a SCN5A-SupRep construct can suppress the amount of pathological increase in I_{Na} late current or window current by at least 25% (e.g., at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 90%, at least 100%, or at least 200%), as compared to the I_{Na} late current prior to treatment.

The typical QT range is about 350-450 ms for men and about 350-460 ms for women, but QT above about 430-440 generally is considered to be borderline high. The QT for males having LQTS is typically greater than 450 ms, and the QT for women having LQTS is typically greater than 460 ms. Most LQTS patients top out at less than 520 ms. In some cases, an effective amount of a KCNQ1-SupRep construct and/or a KCNH2-SupRep construct and/or a SCN5A-SupRep construct administered to a mammal (e.g., a human) having LQT1 and/or LQT2 and/or LQT3 can shorten the APD to a length similar to that of a healthy individual, such that the APD is within the normal range. In some cases, an effective amount of a KCNQ1-SupRep construct and/or a KCNH2-SupRep construct and/or a SCN5A-SupRep construct administered to a mammal (e.g., a human) having LQT1 and/or LQT2 and/or LQT3 can shorten the APD to a length that is within about 10% (e.g., within about 8%, about 5%, or about 3%, of the APD of a healthy individual). In some cases, a therapeutically effective amount of a KCNQ1-SupRep construct and/or a KCNH2-SupRep construct and/or a SCN5A-SupRep construct to a mammal (e.g., a human) having LQT1 and/or LQT2 and/or LQT3 can shorten the APD by at least 10% (e.g., at least 15%, at least 20%, at least 25%, at least 30%, at least 40%, or at least 50%), as compared to the APD prior to treatment.

In some cases, symptoms can be assessed on the day of treatment, 1 day post treatment, 3 months post treatment, 6 months post treatment, 1 year post treatment and every year thereafter post treatment. In some cases, symptoms can be assessed between 1 day post

treatment and 7 days post treatment (e.g., between 1 day and 2 days post treatment, between 1 day and 3 days post treatment, between 1 day and 4 days post treatment, between 2 days and 3 days post treatment, between 2 days and 4 days post treatment, between 2 days and 5 days post treatment, between 3 days and 4 days post treatment, between 3 days and 5 days post treatment, 3 days and 6 days post treatment, between 4 days and 5 days post treatment, between 4 days and 6 days post treatment, between 4 days and 7 days post treatment, between 5 days and 6 days post treatment, between 5 days and 7 days post treatment, or between 6 days and 7 days post treatment). In some cases, symptoms can be assessed between 1 week post treatment and 4 weeks post treatment (e.g., between 1 week and 2 weeks post treatment, between 1 week and 3 weeks post treatment, between 1 week and 4 weeks post treatment, between 2 weeks and 3 weeks post treatment, between 2 weeks and 4 weeks post treatment, or between 3 weeks and 4 weeks post treatment). In some cases, symptoms can be assessed between 1 month post treatment and 12 months post treatment (e.g., between 1 month and 2 months post treatment, between 1 month and 3 months post treatment, between 1 month and 4 months post treatment, between 2 months and 3 months post treatment, between 2 months and 4 months post treatment, between 2 months and 5 months post treatment, between 3 months and 4 months post treatment, between 3 months and 5 months post treatment, between 3 months and 6 months post treatment, between 4 months and 5 months post treatment, between 4 and 6 months post treatment, between 4 months and 7 months post treatment, between 5 months and 6 months post treatment, between 5 months and 7 months post treatment, between 5 months and 8 months post treatment, between 6 months and 7 months post treatment, between 6 months and 8 months post treatment, between 6 months and 9 months post treatment, between 7 months and 8 months post treatment, between 7 months and 9 months post treatment, between 7 months and 10 months post treatment, between 8 months and 9 months post treatment, between 8 months and 10 months post treatment, between 8 months and 11 months post treatment, between 9 months and 10 months post treatment, between 9 months and 11 months post treatment, between 9 months and 12 months post treatment, between 10 months and 11 months post treatment, between 10 months and 12 months post treatment, or between 11 months and 12 months post treatment). In some cases, symptoms can be assessed between 1

year post treatment and about 20 years post treatment (e.g., between 1 year and 5 years post treatment, between 1 year and 10 years post treatment , between 1 year and 15 years post treatment, between 5 years and 10 years post treatment, between 5 years and 15 years post treatment, between 5 years and 20 years post treatment, between 10 years and 15 years post treatment, between 10 years and 20 years post treatment, or between 15 years and 20 years post treatment).

In some cases, a treatment as provided herein can be administered to a mammal (e.g., a human) having a congenital disease (e.g., a congenital heart disease such as LQTS, or more specifically, LQT1 or LQT2 or LQT3) in a single dose, without further administration.

In some cases, a treatment as provided herein can be administered to a mammal (e.g., a human) having a congenital disease (e.g., a congenital heart disease such as LQTS, or more specifically, LQT1) at least once daily, or at least once weekly for at least two consecutive days or weeks. In some cases, a treatment as provided herein is administered to a mammal (e.g., a human) having a congenital disease at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 consecutive days or weeks. In some cases, a treatment as provided herein is administered to a mammal (e.g., a human) having a congenital disease at least once daily or at least once weekly for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 consecutive weeks. In some cases, a treatment as provided herein is administered to a mammal (e.g., a human) having a congenital disease at least once daily or at least once weekly for at most 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 consecutive days or weeks. In some cases, a treatment as provided herein is administered to a mammal (e.g., a human) having a congenital disease at least once weekly for at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 consecutive weeks or months. In some cases, a treatment as provided herein is administered to a mammal (e.g., a human) having a congenital disease at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 consecutive months or years, chronically for a subject's entire life span, or an indefinite period of time.

In one embodiment, a mammal having LQT1 or SQTS associated with a pathogenic mutation in the *KCNQ1* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a

pathogenic mutation in the *KCNQ1* gene. Pathogenic mutations in or encoded by or encoded by the *KCNQ1* gene include, without limitation, c.421G>A (p.V141M), c.919G>C (p.V307L), c.513C>A (p.Y171X), c.760G>A (p.V254M), c.1700T>G (p.I567S), c.1377C>T (p.D459D), c.1380C>A (p.G460G), c.1383T>C (p.Y461Y), c.1386C>T (p.D462D), c.1389T>C (p.S463S), c.1392T>C (p.S464S), c.1395A>C (p.V465V), c.1398G>A (p.R466R), c.1401G>A (p.K467K), and c.1404C>T (p.S468S). *See*, also, Wu et al., *J Arrhythm*. 2016, 32(5):381-388; Hedley et al., *Hum Mutat*. 2009, 30:1486-1551; and Morita et al., *Lancet* 2008, 372:750-763. SupRep constructs targeted to mutant *KCNQ1* alleles can be designed to suppress the mutant *KCNQ1* alleles and replace them with a wild type *KCNQ1* allele. SupRep constructs targeted to mutant *KCNQ1* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *KCNQ1* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *KCNQ1* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *KCNQ1* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *KCNQ1* allele and replace it with a wild type *KCNQ1* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *KCNQ1* construct and a shKCNQ1 construct, and measuring *KCNQ1* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *KCNQ1* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *KCNQ1* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *KCNQ1* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration,

or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *KCNQ1* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of LQT1 or SQTS, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having LQT1 or SQTS associated with a pathogenic mutation in *KCNQ1* can result in a reduction in symptoms such as rapid heartbeat, fainting, and/or seizures. In some cases, effective SupRep treatment of a mammal having LQT1 or SQTS associated with a pathogenic *KCNQ1* mutation can result in an I_{Ks} current density and/or cardiac APD that is similar to the I_{Ks} current density and/or cardiac APD of a healthy individual.

In another embodiment, a mammal having LQT2 or SQTS associated with a pathogenic mutation in the *KCNH2* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *KCNH2* gene. Pathogenic mutations in or encoded by the *KCNH2* gene include, without limitation, c.1764C>G (p.N588K), c.82A>G (p.K28E), c.2893G>T (p.G965X), c.3036_3048del (p.R1014fs), and c.3107_3111dup (p.V1038fs). *See*, also, Hedley et al., *Hum Mutat*. 2009, 30:1486-1551; Curran et al., *Cell* 1995, 80:795-803; and Smith et al., *J Arrhythm*. 2016, 32(5):373-380. SupRep constructs targeted to mutant *KCNH2* alleles can be designed to suppress the mutant *KCNH2* alleles and replace them with a wild type *KCNH2* allele. SupRep constructs targeted to mutant *KCNH2* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *KCNH2* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *KCNH2* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *KCNH2* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *KCNH2* allele and

replace it with a wild type *KCNH2* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *KCNH2* construct and a shKCNH2 construct, and measuring *KCNH2* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *KCNH2* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *KCNH2* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *KCNH2* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *KCNH2* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of LQT2 or SQTS, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having LQT2 or SQTS associated with a pathogenic mutation in *KCNH2* can result in a reduction in symptoms such as rapid heartbeat, fainting (e.g., during periods of strenuous exercise or emotional distress), and/or seizures. In some cases, effective SupRep treatment of a mammal having LQT2 or SQTS associated with a pathogenic *KCNH2* mutation can result in shortening of the APD to a length similar to that of a healthy individual, such that the APD is within the normal range.

In another embodiment, a mammal having LQT3 or BrS associated with a pathogenic mutation in the *SCN5A* gene can be identified by, for example, analyzing a

biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *SCN5A* gene. Pathogenic mutations in or encoded by the *SCN5A* gene include, without limitation, c.100C>T (p.R34C), c.1571C>A (p.S524Y), c.1673A>G (p.H558R), c.3308C>A (p.S1103Y), c.3578G>A (p.R1193Q), c.3908C>T (p.T1304M), c.4509_4516del (p.1505–1507del), c.4865G>A (p.R1623Q), and c.5851G>T (p.V1951L). *See*, also, Kapa et al., *Circulation* 2009, 120:1752-1760; and Hedley et al., *Hum Mutat*. 2009, 30:1486-1551. SupRep constructs targeted to mutant *SCN5A* alleles can be designed to suppress the mutant *SCN5A* alleles and replace them with a wild type *SCN5A* allele. SupRep constructs targeted to mutant *SCN5A* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *SCN5A* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *SCN5A* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *SCN5A* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *SCN5A* allele and replace it with a wild type *gene* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *SCN5A* construct and a shSCN5A construct, and measuring *SCN5A* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *SCN5A* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *SCN5A* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *SCN5A* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or

drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *SCN5A* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of LQT3 or BrS, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having LQT3 or BrS associated with a pathogenic mutation in *SCN5A* can result in a reduction in symptoms such as fainting and/or seizures. In some cases, effective SupRep treatment of a mammal having LQT3 or BrS associated with a pathogenic *SCN5A* mutation can result in shortening of the APD to a length similar to that of a healthy individual, such that the APD is within the normal range.

In another embodiment, a mammal having HCM or DCM associated with a pathogenic mutation in the *MYH7* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *MYH7* gene. Pathogenic mutations in or encoded by the *MYH7* gene include, without limitation, c.1156T>C (p.Y386H), c.1680T>C (p.S532P), c.1816G>A (p.V606M), c.2602G>C (p.A868P), c.2945T>C (p.M982T), c.4258A>T (p.R1420W), and c.5779A>T (p.I1927F). *See*, also, Millat et al., *Eur J Med Genet.* 2010, 53:261-267; Van Driest et al., *Mayo Clin Proc* 2005, 80(4):463-469; references. SupRep constructs targeted to mutant *MYH7* alleles can be designed to suppress the mutant *MYH7* alleles and replace them with a wild type *MYH7* allele. SupRep constructs targeted to mutant *MYH7* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *MYH7* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *MYH7* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *MYH7* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *MYH7* allele

and replace it with a wild type *gene* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *MYH7* construct and a shMYH7 construct, and measuring *MYH7* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *MYH7* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *MYH7* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *MYH7* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *MYH7* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of HCM or DCM, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having HCM or DCM associated with a pathogenic mutation in *MYH7* can result in a reduction in symptoms such as dyspnea, fatigue, edema of the legs and/or ankles, chest pain, arrhythmia, fainting, lightheadedness, and/or heart palpitations. In some cases, effective SupRep treatment of a mammal having HCM or DCM associated with a pathogenic *MYH7* mutation can result in reduced cardiac hypertrophy and cardiomyocyte size, and/or decreased interstitial fibrosis and myocardial disarray.

In another embodiment, a mammal having ACM associated with a pathogenic mutation in the *DSP* gene can be identified by, for example, analyzing a biological

sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *DSP* gene. Pathogenic mutations in or encoded by the *DSP* gene include, without limitation, c.151C>T (p.N51X), c.478C>T (p. R160X), c.897C>G (p.S299R), c.1264G>A (p.E422K), c.1333A>G (p.I445V), c.3160_3169delAAGAACAA (p.K1052fsX26), c.3337C>T (p. R1113X), c.4775A>G (p.K1592R), c.5212C>T (p. R1738X), c.6478C>T (p.R2160X), and c.6496C>T (p.R2166X). *See*, also, Bhonsale et al., *Eur Heart J.* 2015, 36(14):847-855; Sen-Chowdhry et al., *Circulation* 2007, 115:1710-1720; and Norman et al., *Circulation* 2005, 112:636-642. SupRep constructs targeted to mutant *DSP* alleles can be designed to suppress the mutant *DSP* alleles and replace them with a wild type *DSP* allele. SupRep constructs targeted to mutant *DSP* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *DSP* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *DSP* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *DSP* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *DSP* allele and replace it with a wild type *DSP* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *DSP* construct and a shDSP construct, and measuring *DSP* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *DSP* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *DSP* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *DSP* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration,

intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *DSP* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of ACM, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having ACM associated with a pathogenic mutation in *DSP* can result in a reduction in symptoms such as fibrofatty replacement of the myocardium, ventricular arrhythmias, syncope, sustained ventricular tachycardia (VT) or fibrillation (VF), and/or heart failure. In some cases, effective SupRep treatment of a mammal having ACM associated with a pathogenic *DSP* mutation can result a reduction in LV inflammation, fibrosis, and/or systolic dysfunction.

In another embodiment, a mammal having HCM associated with a pathogenic mutation in the *MYBPC3* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *MYBPC3* gene. Pathogenic mutations in or encoded by the *MYBPC3* gene include, without limitation, c.3535G>A (p.E1179K), c.3413G>A (p.R1138H), c.3392T>C (p.I1131T), c.3106C>T (p.R1036C), c.3004C>T (p.R1002W), c.2992C>G (p.Q998E), c.2870C>G (p.T957S), c.2686G>A (p.V896M), c.2498C>T (p.A833V), c.2497G>A (p.A833T), c.1144C>T (p.R382TW), c.977G>A (p.R326Q), c.706A>G (p.S236G), and c.472G>A (p.V158M). *See*, also, Helms et al.,. *Circ: Gen Precision Med.* 2020, 13:396-405; Carrier et al., *Gene.* 2015, 573(2):188-197; Millat et al., *supra*; and Page et al., *Circ Cardiovasc Genet.* 2012, 5:156-166. SupRep constructs targeted to mutant *MYBPC3* alleles can be designed to suppress the mutant *MYBPC3* alleles and replace them with a wild type *MYBPC3* allele. SupRep constructs targeted to mutant *MYBPC3* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to

target a *MYBPC3* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *MYBPC3* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *MYBPC3* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *MYBPC3* allele and replace it with a wild type *MYBPC3* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *MYBPC3* construct and a shMYBPC3 construct, and measuring *MYBPC3* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *MYBPC3* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *MYBPC3* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *MYBPC3* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *MYBPC3* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of HCM, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having HCM associated with a pathogenic mutation in *MYBPC3* can result in a reduction in symptoms such as dyspnea, rapid heartbeat, chest pain, fainting, dizziness, and/or fatigue. In some cases, effective SupRep treatment of a

mammal having HCM associated with a pathogenic *MYBPC3* mutation can result in reduced contractility, improved relaxation, and/or reduced energy consumption.

In another embodiment, a mammal having DCM associated with a pathogenic mutation in the *RBM20* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *RBM20* gene. Pathogenic mutations in or encoded by the *RBM20* gene include, without limitation, c.1913C>T (p.P638L), c.1901G>A (p.R634Q), c.1906C>A (p.R636S), c.1907G>A (p.R636H), c.1909A>G (p.S637G), c.1661G>A (p.V535I), c.1958C>T (p.R634W), c.1964C>T (p.R636C), and c.2205G>A (p.R716Q). *See*, also, Brauch et al., *J Am Coll Cardiol*. 2009, 54:930-941; Li et al., *Clin Transl Sci*. 2010, 3:90-97; and Refaat et al., *Heart Rhythm*. 2012, 9:390-396. SupRep constructs targeted to mutant *RBM20* alleles can be designed to suppress the mutant *RBM20* alleles and replace them with a wild type *RBM20* allele. SupRep constructs targeted to mutant *RBM20* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *RBM20* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *RBM20* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *RBM20* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *RBM20* allele and replace it with a wild type *RBM20* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *RBM20* construct and a shRBM20 construct, and measuring *RBM20* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *RBM20* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *RBM20* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *RBM20* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about

10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *RBM20* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of DCM, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having DCM associated with a pathogenic mutation in *RBM20* can result in a reduction in symptoms such as dyspnea, fatigue, edema of the legs and/or ankles, chest pain, arrhythmia, fainting, lightheadedness, and/or heart palpitations. In some cases, effective SupRep treatment of a mammal having DCM associated with a pathogenic *RBM20* mutation can result in normalization of LV size and/or strengthening of the LV.

In another embodiment, a mammal having LQTS or Timothy syndrome associated with a pathogenic mutation in the *CACNA1C* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *CACNA1C* gene. Pathogenic mutations in or encoded by the *CACNA1C* gene include, without limitation, c.2570C>G (p.P857R), c.2500A>G (p.K834Q), c.2570C>T (p.P857L), c.5717G>A (p.R1906Q), c.82G>A (p.A28T), c.2578C>G (p.R860G), c.3497T>C (p.I166T), c.3496A>G (p.I1166V), c.4425C>G (p.I1475M), and c.4486G>A (p.E1496K). *See*, also, Boczek et al., *Circ Cardiovasc Genet.* 2013, 6(3):279-289; Wemhöner et al., *J Mol Cell Cardiol.* 2015, 80:186-195; references. SupRep constructs targeted to mutant *CACNA1C* alleles can be designed to suppress the mutant *CACNA1C* alleles and replace them with a wild type *CACNA1C* allele. SupRep constructs targeted to mutant *CACNA1C* alleles can be

designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *CACNA1C* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *CACNA1C* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *CACNA1C* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *CACNA1C* allele and replace it with a wild type *CACNA1C* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *CACNA1C* construct and a shCACNA1C construct, and measuring *CACNA1C* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *CACNA1C* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *CACNA1C* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *CACNA1C* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *CACNA1C* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of LQTS or Timothy syndrome, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having LQTS or Timothy syndrome associated with a pathogenic mutation in *CACNA1C* can result in a reduction

in symptoms such as rapid heartbeat, fainting, seizures, episodes of hypoglycemia, and/or episodes of hypothermia. In some cases, effective SupRep treatment of a mammal having LQTS or Timothy syndrome associated with a pathogenic *CACNA1C* mutation can result in an I_{Ks} current density and/or cardiac APD that is similar to the I_{Ks} current density and/or cardiac APD of a healthy individual.

In another embodiment, a mammal having ACM associated with a pathogenic mutation in the *PKP2* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *PKP2* gene. Pathogenic mutations in or encoded by the *PKP2* gene include, without limitation, c.235C>T (p.R79X), c.397C>T (p.Q133X), c.2386T>C (p.C796R), c.2011delC (p.P671fsX683), c.1368delA (p.N456fsX458), c.145-148delCAGA (p.S50fsX110), c.2509delA (p.V837fsX930), c.2489+1G>A (p.mutant splice product), c.1171-2A>G (p.mutant splice product), c.2146-1G>C (p.mutant splice product), c.2197-2202insGdelCACACC (p.A733fsX740), c.1613G>A (p.W538X), c.1271T>C (p.F424S), c.1642delG (p.V548fsX562), and c.419C>T (p.S140F). *See*, also, Dalal et al., *Circulation*. 2006, 113:1641-1649; van Tintelen et al., *Circulation*. 2006, 113(13):1650-1658; and Fressart et al., *Europace*. 2010, 12(6):861-868. SupRep constructs targeted to mutant *PKP2* alleles can be designed to suppress the mutant *PKP2* alleles and replace them with a wild type *PKP2* allele. SupRep constructs targeted to mutant *PKP2* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *PKP2* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *PKP2* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *PKP2* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *PKP2* allele and replace it with a wild type *PKP2* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *PKP2* construct and a shPKP2 construct, and measuring *PKP2* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *PKP2* expression (e.g., the

ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *PKP2* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *PKP2* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *PKP2* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of ACM, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having ACM associated with a pathogenic mutation in *PKP2* can result in a reduction in symptoms such as fibrofatty replacement of the myocardium, ventricular arrhythmias, syncope, sustained VT or VF, and/or heart failure. In some cases, effective SupRep treatment of a mammal having ACM associated with a pathogenic *PKP2* mutation can result in reduction in LV inflammation, fibrosis, and/or systolic dysfunction.

In another embodiment, a mammal having ACM associated with a pathogenic mutation in the *DSG2* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *DSG2* gene. Pathogenic mutations in or encoded by the *DSG2*gene include, without limitation, c.378+1G>T (p.mutant splice product), c.560A>G (p.D187G), c.146 G>A (p.R49H), c.560 A>G (p.D187G), c.1520 G>A (p.C507Y),

c.1003A>G (p.T335A), and c.961 T>A (p.F321I), as well as mutations resulting in p.K294E, p.D154E, p.V392I, p.L772X, and p.R773K. *See*, also, Brodehl et al., *Int J Mol Sci.* 2021, 22(7):3786; Debus et al., *J Mol Cell Cardiol.* 2019, 129:303-313; and Xu et al., *J Am Coll Cardiol.* 2010, 55(6):587-597. SupRep constructs targeted to mutant *DSG2* alleles can be designed to suppress the mutant *DSG2* alleles and replace them with a wild type *DSG2* allele. SupRep constructs targeted to mutant *DSG2* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *DSG2* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *DSG2* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *DSG2* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *DSG2* allele and replace it with a wild type *DSG2* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *DSG2* construct and a shDSG2 construct, and measuring *DSG2* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *DSG2* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *DSG2* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *DSG2* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *DSG2* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal,

intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of ACM, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having ACM associated with a pathogenic mutation in *DSG2* can result in a reduction in symptoms such as fibrofatty replacement of the myocardium, ventricular arrhythmias, syncope, sustained VT or VF, and/or heart failure. In some cases, effective SupRep treatment of a mammal having ACM associated with a pathogenic *DSG2* mutation can result in reduction in LV inflammation, fibrosis, and/or systolic dysfunction.

In another embodiment, a mammal having ACM, DCM, LVNC, or skeletal myopathy associated with a pathogenic mutation in the *DES* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *DES* gene. Pathogenic mutations in or encoded by the *DES* gene include, without limitation, c.407C>T (p.L136P), c.1009G>C (p.A337P), c.1013T>G (p.L338R), c.1195G>T (p.D399Y), and c.1201G>A (p.E401K). *See*, also, Brodehl et al., *J Mol Cell Cardiol.* 2016, 91:207-214; Goudeau et al., *Hum Mutat.* 2006, 27(9):906–913; references. SupRep constructs targeted to mutant *DES* alleles can be designed to suppress the mutant *DES* alleles and replace them with a wild type *DES* allele. SupRep constructs targeted to mutant *DES* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *DES* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *DES* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *DES* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *DES* allele and replace it with a wild type *DES* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *DES* construct and a shDES construct, and measuring *DES* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *DES* expression (e.g., the ability to knock down at

least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *DES* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *DES* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *DES* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of ACM, DCM, LVNC, or skeletal myopathy, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having ACM, DCM, LVNC, or skeletal myopathy associated with a pathogenic mutation in *DES* can result in a reduction in symptoms such as fibrofatty replacement of the myocardium, ventricular arrhythmias, fainting, sustained VT or VF, dyspnea, fatigue, edema of the legs and/or ankles, chest pain, lightheadedness, heart palpitations, and/or heart failure. In some cases, effective SupRep treatment of a mammal having ACM, DCM, LVNC, or skeletal myopathy associated with a pathogenic *DES* mutation can result in reduction in LV inflammation, fibrosis, systolic dysfunction, and/or endomyocardial trabeculations, as well as normalization of LV size, and/or strengthening of the LV.

In another embodiment, a mammal having ATS (also referred to as LQT7) or CPVT associated with a pathogenic mutation in the *KCNJ2* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the

sample includes a pathogenic mutation in the *KCNJ2* gene. Pathogenic mutations in or encoded by the *KCNJ2* gene include, without limitation, c.199C>T (p.R67W), c.271_282del12 (p.A91_L94del), c.653G>A (p.R218Q), c.953A>G (p.N318S), c.966G>C (p.W322C), and c.1244C>T (p.P415L). *See*, also, Limberg et al., *Basic Res Cardiol*. 2013, 108:353; Andelfinger et al., *Am J Hum Genet*. 2002, 71(3):663-668; and Tristani-Firouzi et al., *J Clin Invest*. 2002, 110(3):381-388. SupRep constructs targeted to mutant *KCNJ2* alleles can be designed to suppress the mutant *KCNJ2* alleles and replace them with a wild type *KCNJ2* allele. SupRep constructs targeted to mutant *KCNJ2* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *KCNJ2* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *KCNJ2* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *KCNJ2* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *KCNJ2* allele and replace it with a wild type *KCNJ2* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *KCNJ2* construct and a shKCNJ2 construct, and measuring *KCNJ2* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *KCNJ2* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *KCNJ2* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *KCNJ2* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *KCNJ2* can be administered to a mammal in a non-viral

vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of ATS or CPVT, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having ATS or CPVT associated with a pathogenic mutation in *KCNJ2* can result in a reduction in symptoms such as muscle weakness, fainting, lightheadedness, dizziness, periodic paralysis, and/or arrhythmia (e.g., VT). In some cases, effective SupRep treatment of a mammal having ATS or CPVT associated with a pathogenic *KCNJ2* mutation can result in normalization and/or regulation of the heart rhythm.

In another embodiment, a mammal having CPVT associated with a pathogenic mutation in the *CASQ2* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *CASQ2* gene. Pathogenic mutations in or encoded by the *CASQ2* gene include, without limitation, c.62delA (p.E21Gfs*15), c.97C>T (p.R33*), c.98G>A (p.R33Q), c.115G>T (p.E39*), c.115G>A (p.E39K), c.158G>T (p.C53F), c.164A>G (p.Y55C), c.199C>T (p.Q67*), c.204delA (p.K68Nfs*5), c.213delA (p.Q71Hfs*2), c.230T>C (p.L77P), c.234+2T>C (p.mutant splice site), c.259A>T (p.K87*), c.339-354del (p.S113Rfs*6), c.500T>A (p.L167H), c.518G>T (p.S173I), c.532+1G>A (p. mutant splice site), c.539A>G (p.K180R), c.545T>C (p.F182S), c.546delT (p.F182Lfs*28), c.572C>T (p.P191L), c.603delA (p.V203Lfs*7), c.618A>C (p.K206N), and c.691C>T (p.P231S). *See*, also, Ng et al., *Circulation*. 2020, 142(10):932-947; and Gray et al., *Heart Rhythm*. 2016, 13(8):1652-1660. SupRep constructs targeted to mutant *CASQ2* alleles can be designed to suppress the mutant *CASQ2* alleles and replace them with a wild type *CASQ2* allele. SupRep constructs targeted to mutant *CASQ2* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *CASQ2* allele containing a pathogenic mutation, either by targeting a region of

a disease-associated *CASQ2* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *CASQ2* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *CASQ2* allele and replace it with a wild type *CASQ2* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *CASQ2* construct and a shCASQ2 construct, and measuring *CASQ2* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *CASQ2* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *CASQ2* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *CASQ2* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *CASQ2* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of CPVT, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having CPVT associated with a pathogenic mutation in *CASQ2* can result in a reduction in symptoms such as dizziness, lightheadedness, fainting, and/or VT. In some cases, effective SupRep treatment of a mammal having CPVT associated with a pathogenic *CASQ2* mutation can result in normalization and/or regulation of the heart rhythm.

In another embodiment, a mammal having DCM associated with a pathogenic mutation in the *LMNA* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *LMNA* gene. Pathogenic mutations in or encoded by the *LMNA* gene include, without limitation, c.481G>A (p.E161K), c.1130G>A (p.R377H), c.1621C>T (p.R541C), c.1621C>G (p.R541G), c.266G>T (p.R89L), c.736C>T (p.Q246*), c.1197_1240del44 (p.G400Rfs*11), c.1292C>G (p.S431*), 1526_1527insC (p.T510Yfs*42), c.1443C>G (p.Y481*), and c.767 T>G (p.V256G). *See*, also, Saj et al., *BMC Med Genet*. 2013, 14:55; Sebillon et al., *J Med Genet*. 2003, 40:560-567; and Parks et al., *Am Heart* J. 2008, 156(1):161-169. SupRep constructs targeted to mutant *LMNA* alleles can be designed to suppress the mutant *LMNA* alleles and replace them with a wild type *LMNA* allele. SupRep constructs targeted to mutant *LMNA* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *LMNA* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *LMNA* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *LMNA* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *LMNA* allele and replace it with a wild type *LMNA* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *LMNA* construct and a shLMNA construct, and measuring *LMNA* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *LMNA* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *LMNA* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *LMNA* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into

a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *LMNA* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of DCM, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having DCM associated with a pathogenic mutation in *LMNA* can result in a reduction in symptoms such as dyspnea, fatigue, edema of the legs and/or ankles, chest pain, arrhythmia, fainting, lightheadedness, and/or heart palpitations. In some cases, effective SupRep treatment of a mammal having DCM associated with a pathogenic *LMNA* mutation can result in normalization of LV size and/or strengthening of the LV.

In another embodiment, a mammal having DCM associated with a pathogenic mutation in the *TPM1* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *TPM1* gene. Pathogenic mutations in or encoded by the *TPM1* gene include, without limitation, c.688G>A (p. D230N), c.688G>A (p.D230N), c.23T>G (p.M8R), c.632C>G (p.A211G), c.725C>T (p.A242V), c.163G>A (p.D55N), c.337C>G (p.L113V), c.341A>G (p.E114G), c.275T>C (p.I92T), c.423G>C (p.M141I), and c.416A>T (p.E139V). *See*, also, Pugh et al., *Genet Med.* 2014, 16:601-608; and McNally and Mestroni, *Circ Res.* 2017, 121:731-748. SupRep constructs targeted to mutant *TPM1* alleles can be designed to suppress the mutant *TPM1* alleles and replace them with a wild type *TPM1* allele. SupRep constructs targeted to mutant *TPM1* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *TPM1* allele containing a

pathogenic mutation, either by targeting a region of a disease-associated *TPM1* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *TPM1* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *TPM1* allele and replace it with a wild type *TPM1* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *TPM1* construct and a shTPM1 construct, and measuring *TPM1* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *TPM1* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *TPM1* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *TPM1* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *TPM1* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of DCM, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having DCM associated with a pathogenic mutation in *TPM1* can result in a reduction in symptoms such as dyspnea, fatigue, edema of the legs and/or ankles, chest pain, arrhythmia, fainting, lightheadedness, and/or heart palpitations. In some cases, effective SupRep treatment of a mammal having DCM

associated with a pathogenic *TPM1* mutation can result in normalization of LV size and/or strengthening of the LV.

In another embodiment, a mammal having DCM or ACM associated with a pathogenic mutation in the *PLN* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *PLN* gene. Pathogenic mutations in or encoded by the *PLN* gene include, without limitation, c.40_42delAGA (p.R14del), c.116T>G (p.L39X), and c.25C > T (p.R9C). *See*, also, te Rijdt et al., *Cardiovasc Pathol.* 2019, 40:2-6; Groeneweg et al., *Am J Cardiol.* 2013, 112:1197-1206; Fish et al., *Sci Rep.* 2016, 22235; and Haghighi et al., *J Clin Invest.* 2003, 111(6):869-876. SupRep constructs targeted to mutant *PLN* alleles can be designed to suppress the mutant *PLN* alleles and replace them with a wild type *PLN* allele. SupRep constructs targeted to mutant *PLN* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *PLN* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *PLN* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *PLN* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *PLN* allele and replace it with a wild type *PLN* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *PLN* construct and a shPLN construct, and measuring *PLN* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *PLN* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *PLN* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *PLN* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the

myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *PLN* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of DCM or ACM, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having DCM or ACM associated with a pathogenic mutation in *PLN* can result in a reduction in symptoms such as dyspnea, fatigue, edema of the legs and/or ankles, chest pain, arrhythmia, fainting, lightheadedness, heart palpitations, fibrofatty replacement of the myocardium, sustained VT or VF, and/or heart failure. In some cases, effective SupRep treatment of a mammal having DCM or ACM associated with a pathogenic *PLN* mutation can result in normalization of LV size, strengthening of the LV, reduction in LV inflammation, reduction in fibrosis, and/or reduction in systolic dysfunction.

In another embodiment, a mammal having FH associated with a pathogenic mutation in the *LDLR* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *LDLR* gene. Pathogenic mutations in or encoded by the *LDLR* gene include, without limitation, c.1845+2T>C, c.1012T >A (p.C338S), c.1297G>C (p.D433H), c.1702C>G (p.L568V), and c.2431A>T (p.K811*), c.97C>T (p.Q33X), c.357delG (p.K120fs), c.428G>A (p.C143Y), c.517T>C (p.C173R), c.1448G>A (p.W483X), c.1744C>T (p.L582F), c.1757C>A (p.S586X), and c.1879G>A (p.A627T). *See*, also, Tada et al., *J Clin Lipidol*. 2020, 14(3):346-351; Wang et al., *J Geriatr Cardiol*. 2018, 15(6):434-440; Hori et al., *Atherosclerosis*. 2019, 289:101-108; and Galicia-Garcia et al., *Sci Rep*. 2020, 10:1727. SupRep constructs targeted to mutant *LDLR* alleles can be

designed to suppress the mutant *LDLR* alleles and replace them with a wild type *LDLR* allele. SupRep constructs targeted to mutant *LDLR* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *LDLR* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *LDLR* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *LDLR* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *LDLR* allele and replace it with a wild type *LDLR* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *LDLR* construct and a shLDLR construct, and measuring *LDLR* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *LDLR* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *LDLR* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *LDLR* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *LDLR* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of FH, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having FH associated with a pathogenic mutation in *LDLR* can result in a reduction in

symptoms such as elevated total and LDL cholesterol levels, angina, and/or xanthomas. In some cases, effective SupRep treatment of a mammal having FH associated with a pathogenic *LDLR* mutation can alleviate cerebrovascular disease and/or peripheral vascular disease associated with the FH.

In another embodiment, a mammal having FH associated with a pathogenic mutation in the *PCSK9* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *PCSK9* gene. Pathogenic mutations in or encoded by the *PCSK9* gene include, without limitation, c.381T>A (p.S127R), c.644G>A (p.R215H), c.646T>C (p.F216L), c.1120G>T (p.D374Y), and c.1486C>T (p.R496W), as well as p.N157K, p.R218S, p.R237W, p.E670G, p.R218S, p.R357H, p.R469W, p.A443T, p.R496W, p.N425S, p.D374H, p.D129G, p.A168E, p.G236S, p.N354I, p.A245T, p.R272Q, p.R272Q, and p.A245T. *See*, also, Hori et al., *supra*; Youngblom et al., "Familial Hypercholesterolemia," 2014 Jan 2 (Updated 2016 Dec 8), In: Adam et al., eds., GENEREVIEWS® University of Washington, Seattle; and Guo et al., *Front Genet.* 2020, 11:1020. SupRep constructs targeted to mutant *PCSK9* alleles can be designed to suppress the mutant *PCSK9* alleles and replace them with a wild type *PCSK9* allele. SupRep constructs targeted to mutant *PCSK9* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *PCSK9* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *PCSK9* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *PCSK9* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *PCSK9* allele and replace it with a wild type *PCSK9* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *PCSK9* construct and a shPCSK9 construct, and measuring *PCSK9* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *PCSK9* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90

percent, at least 95 percent, at least 98 percent, or at least 99 percent of *PCSK9* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *PCSK9* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *PCSK9* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of FH, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having FH associated with a pathogenic mutation in *PCSK9* can result in a reduction in symptoms such as elevated total and LDL cholesterol levels, angina, and/or xanthomas. In some cases, effective SupRep treatment of a mammal having FH associated with a pathogenic *PCSK9* mutation can alleviate cerebrovascular disease and/or peripheral vascular disease associated with the FH.

In another embodiment, a mammal having HCM or DCM associated with a pathogenic mutation in the *TNNT2* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *TNNT2* gene. Pathogenic mutations in or encoded by the *TNNT2* gene include, without limitation, c.421C>T (p.R141W), and c.835C>T (p.Q279X), as well as p.P80S, p.D86A, p.R92L, p.K97N, p.K124N, p.R130C, p.R134G, and p.R144W. *See*, also, Long et al., *J Am Heart Assoc*. 2015, 4(12):e002443; Gao et al., *Medicine*. 2020, 99(34):e21843; Millat et al., *supra*; and Hershberger et al., *Circ*

Cardiovasc Genet. 2009, 2:306-313. SupRep constructs targeted to mutant *TNNT2* alleles can be designed to suppress the mutant *TNNT2* alleles and replace them with a wild type *TNNT2* allele. SupRep constructs targeted to mutant *TNNT2* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *TNNT2* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *TNNT2* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *TNNT2* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *TNNT2* allele and replace it with a wild type *TNNT2* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *TNNT2* construct and a shTNNT2 construct, and measuring *TNNT2* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *TNNT2* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *TNNT2* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *TNNT2* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *TNNT2* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of HCM or DCM, to determine whether one or more symptoms of the disorder are diminished. For

example, effective SupRep treatment of a mammal having HCM or DCM associated with a pathogenic mutation in *TNNT2* can result in a reduction in symptoms such as dyspnea, rapid heartbeat, chest pain, fainting, dizziness, fatigue, edema of the legs and/or ankles, arrhythmia, lightheadedness, and/or heart palpitations. In some cases, effective SupRep treatment of a mammal having HCM or DCM associated with a pathogenic *TNNT2* mutation can result in reduced contractility, improved relaxation, reduced energy consumption, normalization of LV size, and/or strengthening of the LV.

In another embodiment, a mammal having LQTS or CPVT associated with a pathogenic mutation in the *CALM1* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *CALM1* gene. Pathogenic mutations in or encoded by the *CALM1* gene include, without limitation, p.N54I, p.F90L, p.N98S, p.E105A, p.D130G, p.D132V, p.E141G, and p.F142L. *See*, also, Jensen et al., *Front Mol Neurosci.* 2018, 11:396; and Boczek et al., *Circ Cardiovasc Genet.* 2016, 9:136-146. SupRep constructs targeted to mutant *CALM1* alleles can be designed to suppress the mutant *CALM1* alleles and replace them with a wild type *CALM1* allele. SupRep constructs targeted to mutant *CALM1* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *CALM1* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *CALM1* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *CALM1* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *CALM1* allele and replace it with a wild type *CALM1* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *CALM1* construct and a shgene construct, and measuring *CALM1* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *CALM1* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *CALM1* expression at the mRNA and/or protein level) can be selected. The

selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *CALM1* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *CALM1* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of LQTS or CPVT, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having LQTS or CPVT associated with a pathogenic mutation in *CALM1* can result in a reduction in symptoms such as rapid heartbeat, fainting, seizures, dizziness, lightheadedness, and/or VT. In some cases, effective SupRep treatment of a mammal having LQTS or CPVT associated with a pathogenic *CALM1* mutation can result in an normalization of IKs current density, normalization of cardiac APD, and/or regulation of heart rhythm.

In another embodiment, a mammal having LQTS or CPVT associated with a pathogenic mutation in the *CALM2* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *CALM2* gene. Pathogenic mutations in or encoded by the *CALM2* gene include, without limitation, p.D96V, p.N98I, p.N98S, p.D130G, p.D130V, p.E132E, p.D132H, p.D134H, and p.Q136P. *See*, also, Jensen et al., *supra*; and Boczek et al. *supra*. SupRep constructs targeted to mutant *CALM2* alleles can be designed to suppress the mutant *CALM2* alleles and replace them with a wild type *CALM2* allele.

SupRep constructs targeted to mutant *CALM2* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *CALM2* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *CALM2* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *CALM2* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *CALM2* allele and replace it with a wild type *CALM2* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *CALM2* construct and a shgene construct, and measuring *CALM2* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *CALM2* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *CALM2* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *CALM2* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *CALM2* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of LQTS or CPVT, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having LQTS or CPVT associated with a pathogenic mutation in *CALM2* can result in a reduction in symptoms such as

rapid heartbeat, fainting, seizures, dizziness, lightheadedness, and/or VT. In some cases, effective SupRep treatment of a mammal having LQTS or CPVT associated with a pathogenic *CALM2* mutation can result in an normalization of IKs current density, normalization of cardiac APD, and/or regulation of heart rhythm.

In another embodiment, a mammal having LQTS or CPVT associated with a pathogenic mutation in the *CALM3* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *CALM3* gene. Pathogenic mutations in or encoded by the *CALM3* gene include, without limitation, p.D96H, p.A103V, p.D130G, and p.F142L. *See*, also, Jensen et al., *supra*; and Boczek et al. *supra*. SupRep constructs targeted to mutant *CALM3* alleles can be designed to suppress the mutant *CALM3* alleles and replace them with a wild type *CALM3* allele. SupRep constructs targeted to mutant *CALM3* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *CALM3* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *CALM3* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *CALM3* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *CALM3* allele and replace it with a wild type *CALM3* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *CALM3* construct and a shgene construct, and measuring *CALM3* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *CALM3* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *CALM3* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *CALM3* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of

administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *CALM3* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of LQTS or CPVT, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having LQTS or CPVT associated with a pathogenic mutation in *CALM3* can result in a reduction in symptoms such as rapid heartbeat, fainting, seizures, dizziness, lightheadedness, and/or VT. In some cases, effective SupRep treatment of a mammal having LQTS or CPVT associated with a pathogenic *CALM3* mutation can result in an normalization of IKs current density, normalization of cardiac APD, and/or regulation of heart rhythm.

In another embodiment, a mammal having TKOS associated with a pathogenic mutation in the *TRDN* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *TRDN* gene. Pathogenic mutations in or encoded by the *TRDN* gene include, without limitation, c.613C>T (p.Q205X), c.22+29A>G (p.N9fs*5), c.438_442delTAAGA (p.K147fs*0), c.53_56delACAG (p.D18fs*13), c.423delA (p.E142fs*33), c.502G>T (p.E168X), c.503G>T (p.E168X), c.545_546insA (p.K182fs*10), c.420delA (p.K140fs*34), c.176C>G (p.T59R), c.613C>T (p.Q205X), c.53_56delACAG (p.D18fs*13), c.618delG (p.A208fs*15), and c.232+2T>A. *See*, also, Clemens et al., Circulation: *Gen Precision Med.* 12(2): e002419; and Altmann et al., *Circulation.* 2015, 131(23):2051-2060. SupRep constructs targeted to mutant *TRDN* alleles can be designed to suppress the mutant *TRDN* alleles and replace them with a wild

type *TRDN* allele. SupRep constructs targeted to mutant *TRDN* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *TRDN* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *TRDN* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *TRDN* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *TRDN* allele and replace it with a wild type *TRDN* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *TRDN* construct and a shTRDN construct, and measuring *TRDN* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *TRDN* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *TRDN* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *TRDN* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *TRDN* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of TKOS, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having TKOS associated with a pathogenic mutation in *TRDN* can result in a reduction in symptoms such as fainting, skeletal

myopathy, and/or proximal muscle weakness. In some cases, effective SupRep treatment of a mammal having TKOS associated with a pathogenic *TRDN* mutation can result in correction of T-wave inversions and/or QT prolongation.

In another embodiment, a mammal having CPVT associated with a pathogenic mutation in the *RYR2* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *RYR2* gene. Pathogenic mutations in or encoded by the *RYR2* gene include, without limitation, c.1258C>T (p.R420W), c.1259G>A (p.R420Q), c.1519G>A (p.V507I), c.3407C>T (p.A1136V), c.5170G>A (p.E1724K), c.5654G>A (p.G1885E), c.5656G>A (p.G1886S), c.6504C>G (p.H2168Q), c.7158G>A (p.A2387T), c.8874A>G (p.Q2958R), c.12533A>G (p.N4178S), c.13528G>A (p.A4510T), c.14311G>A (p.V4771I), c.14542G>A (p.I4848V), and c.14876G>A (p.R4959Q). *See*, also, Medeiros-Domingo et al., *J Am Coll Cardiol.* 2009, 54(22):2065-2074; and Jiang et al., *Proc Natl Acad Sci USA.* 2004, 101(35): 13062-13067. SupRep constructs targeted to mutant *RYR2* alleles can be designed to suppress the mutant *RYR2* alleles and replace them with a wild type *RYR2* allele. SupRep constructs targeted to mutant *RYR2* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *RYR2* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *RYR2* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *RYR2* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *RYR2* allele and replace it with a wild type *RYR2* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *RYR2* construct and a shRYR2 construct, and measuring *RYR2* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *RYR2* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *RYR2* expression at the mRNA and/or protein level) can be selected. The selected

construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *RYR2* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *RYR2* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of CPVT, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having CPVT associated with a pathogenic mutation in *RYR2* can result in a reduction in symptoms such as dizziness, lightheadedness, fainting, and/or VT. In some cases, effective SupRep treatment of a mammal having CPVT associated with a pathogenic *RYR2* mutation can result in normalization and/or regulation of the heart rhythm.

In another embodiment, a mammal having FH associated with a pathogenic mutation in the *APOB* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *APOB* gene. Pathogenic mutations in or encoded by the *APOB* gene include, without limitation, c.10093C>G (p.H3365D), c.4163G>A (p.R1388H), c.10579C>T (p.R3527W), p.P994L, and p.T3826M. *See*, also, Alves et al., *Atherosclerosis*. 2018, 277:P448-456; Sun et al., *Lipids Health Dis*. 2018, 17:252; and Cui et al., *Clin Cardiol*. 2019, 42:385-390. SupRep constructs targeted to mutant *APOB* alleles can be designed to suppress the mutant *APOB* alleles and replace them with a wild type *APOB* allele. SupRep constructs targeted to mutant *APOB* alleles can be designed

and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *APOB* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *APOB* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *APOB* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *APOB* allele and replace it with a wild type *APOB* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *APOB* construct and a shAPOB construct, and measuring *APOB* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *APOB* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *APOB* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *APOB* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *APOB* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of FH, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having FH associated with a pathogenic mutation in *APOB* can result in a reduction in symptoms such as elevated total and LDL cholesterol levels, angina, and/or xanthomas. In some cases, effective SupRep treatment

of a mammal having FH associated with a pathogenic *APOB* mutation can alleviate cerebrovascular disease and/or peripheral vascular disease associated with the FH.

In another embodiment, a mammal having DCM or HCM associated with a pathogenic mutation in the *TNNI3* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *TNNI3* gene. Pathogenic mutations in or encoded by the *TNNI3* gene include, without limitation, p.K36Q, p.N185K, and $p.98_{truncation}$, c.407G>A (p.R136Q), c.433C>T (p.R145W), c.448A>T (p.S150C), c.549G>T (p.K183N), and c.557G>A (p.R186Q). *See*, also, Bollen et al., *J Physiol.* 2017, 595(14):4677-4693; and Millat et al., *supra*. SupRep constructs targeted to mutant *TNNI3* alleles can be designed to suppress the mutant *TNNI3* alleles and replace them with a wild type *TNNI3* allele. SupRep constructs targeted to mutant *TNNI3* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *TNNI3* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *TNNI3* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *TNNI3* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *TNNI3* allele and replace it with a wild type *TNNI3* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *TNNI3* construct and a shTNNI3 construct, and measuring *TNNI3* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *TNNI3* expression (e.g., the ability to knock down at least 50 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of TNNI3 expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *TNNI3* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the

myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *TNNI3* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of DCM or HCM, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having DCM or HCM associated with a pathogenic mutation in *TNNI3* can result in a reduction in symptoms such as dyspnea, rapid heartbeat, chest pain, fainting, dizziness, fatigue, edema of the legs and/or ankles, arrhythmia, lightheadedness, and/or heart palpitations. In some cases, effective SupRep treatment of a mammal having DCM or HCM associated with a pathogenic *TNNI3* mutation can result in reduced contractility, improved relaxation, reduced energy consumption, normalization of LV size, and/or strengthening of the LV.

In another embodiment, a mammal having DCM or HCM associated with a pathogenic mutation in the *TNNC1* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *TNNC1* gene. Pathogenic mutations in or encoded by the *TNNC1* gene include, without limitation, c.91G>T (p.A31S), p.Y5H, p.M103I, p.I148V, p.A8V, p.L29Q, p.C84Y, p.E134D, p.D145E, and p.Q122AfsX30. *See*, also, Parvatiyar et al., *J Biol Chem*. 2012, 287(38):31845-31855; and Veltri et al., *Front Physiol*. 2017, 8:221. SupRep constructs targeted to mutant *TNNC1* alleles can be designed to suppress the mutant *TNNC1* alleles and replace them with a wild type *TNNC1* allele. SupRep constructs targeted to mutant *TNNC1* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *TNNC1* allele containing a pathogenic mutation, either by targeting a

region of a disease-associated *TNNC1* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *TNNC1* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *TNNC1* allele and replace it with a wild type *TNNC1* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *TNNC1* construct and a shTNNC1 construct, and measuring *TNNC1* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *TNNC1* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *TNNC1* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *TNNC1* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *TNNC1* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of DCM or HCM, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having DCM or HCM associated with a pathogenic mutation in *TNNC1* can result in a reduction in symptoms such as dyspnea, rapid heartbeat, chest pain, fainting, dizziness, fatigue, edema of the legs and/or ankles, arrhythmia, lightheadedness, and/or heart palpitations. In some cases, effective SupRep treatment of a mammal having DCM or HCM associated with a pathogenic *TNNC1* mutation can result in reduced

contractility, improved relaxation, reduced energy consumption, normalization of LV size, and/or strengthening of the LV.

In another embodiment, a mammal having DCM or HCM associated with a pathogenic mutation in the *MYL2* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *MYL2* gene. Pathogenic mutations in or encoded by the *MYL2* gene include, without limitation, p.D94A, p.D166A, p.P95A, and p.I158L. *See*, also, Huang et al., *FEBS J.* 2015, 282(12):2379-2393; . Alvarez-Acosta et al., *J Cardiovasc Dis.* 2014, 2; and Poetter et al., *Nat Genet.* 1996, 13:63-69. SupRep constructs targeted to mutant *MYL2* alleles can be designed to suppress the mutant *MYL2* alleles and replace them with a wild type *MYL2* allele. SupRep constructs targeted to mutant *MYL2* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *MYL2* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *MYL2* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *MYL2* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *MYL2* allele and replace it with a wild type *MYL2* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *MYL2* construct and a shMYL2 construct, and measuring *MYL2* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *MYL2* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *MYL2* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *MYL2* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via

intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *MYL2* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of DCM or HCM, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having DCM or HCM associated with a pathogenic mutation in *MYL2* can result in a reduction in symptoms such as dyspnea, rapid heartbeat, chest pain, fainting, dizziness, fatigue, edema of the legs and/or ankles, arrhythmia, lightheadedness, and/or heart palpitations. In some cases, effective SupRep treatment of a mammal having DCM or HCM associated with a pathogenic *MYL2* mutation can result in reduced contractility, improved relaxation, reduced energy consumption, normalization of LV size, and/or strengthening of the LV.

In another embodiment, a mammal having DCM or HCM associated with a pathogenic mutation in the *MYL3* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *MYL3* gene. Pathogenic mutations in or encoded by the *MYL3* gene include, without limitation, c.170C>G (p.A57G), c.530 A>G, c.2155C>T (p. R719W), c.77C>T (p.A26V), c.2654A>C (p.N885T), and c.1987C>T (p.R663C). *See*, also, Poetter et al., *supra*; and Zhao et al., *Int J Mol Med.* 2016, 37:1511-1520. SupRep constructs targeted to mutant *MYL3* alleles can be designed to suppress the mutant *MYL3* alleles and replace them with a wild type *MYL3* allele. SupRep constructs targeted to mutant *MYL3* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *MYL3* allele containing a pathogenic mutation, either by targeting a region of a

disease-associated *MYL3* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *MYL3* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *MYL3* allele and replace it with a wild type *MYL3* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *MYL3* construct and a shMYL3 construct, and measuring *MYL3* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *MYL3* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *MYL3* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *MYL3* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *MYL3* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of DCM or HCM, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having DCM or HCM associated with a pathogenic mutation in *MYL3* can result in a reduction in symptoms such as dyspnea, rapid heartbeat, chest pain, fainting, dizziness, fatigue, edema of the legs and/or ankles, arrhythmia, lightheadedness, and/or heart palpitations. In some cases, effective SupRep treatment of a mammal having DCM or HCM associated with a pathogenic *MYL3* mutation can result in reduced contractility,

improved relaxation, reduced energy consumption, normalization of LV size, and/or strengthening of the LV.

In another embodiment, a mammal having DCM or HCM associated with a pathogenic mutation in the *JPH2* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *JPH2* gene. Pathogenic mutations in or encoded by the *JPH2* gene include, without limitation, p.S101R, p.Y141H, p.S165F, p.T161K, and p.E641X. *See*, also, Landstrom et al., *J Mol Cell Cardiol.* 2007, 42:1026-1035; and Jones et al., *Sci Rep*. 2019, 9:9038. SupRep constructs targeted to mutant *JPH2* alleles can be designed to suppress the mutant *JPH2* alleles and replace them with a wild type *JPH2* allele. SupRep constructs targeted to mutant *JPH2* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *JPH2* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *JPH2* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *JPH2* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *JPH2* allele and replace it with a wild type *JPH2* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *JPH2* construct and a shJPH2 construct, and measuring *JPH2* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *JPH2* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *JPH2* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *JPH2* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration,

intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *JPH2* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of DCM or HCM, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having DCM or HCM associated with a pathogenic mutation in *JPH2* can result in a reduction in symptoms such as dyspnea, rapid heartbeat, chest pain, fainting, dizziness, fatigue, edema of the legs and/or ankles, arrhythmia, lightheadedness, and/or heart palpitations. In some cases, effective SupRep treatment of a mammal having DCM or HCM associated with a pathogenic *JPH2* mutation can result in reduced contractility, improved relaxation, reduced energy consumption, normalization of LV size, and/or strengthening of the LV.

In another embodiment, a mammal having LQTS, HCM, or LGMD associated with a pathogenic mutation in the *CAV3* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *CAV3* gene. Pathogenic mutations in or encoded by the *CAV3* gene include, without limitation, c.233 C>T (p.T78M), c.253 G>A (p.A85T), c.290 T>G (p.F97C), c.423 C>G (p.S141R), p.P104L, and p.R27Q. *See*, also, Shah et al., *J Cachexia Sarcopenia Muscle* 2020, 11(3):838-858; and Vatta et al., *Circulation.* 2006, 114:2104-2112. SupRep constructs targeted to mutant *CAV3* alleles can be designed to suppress the mutant *CAV3* alleles and replace them with a wild type *CAV3* allele. SupRep constructs targeted to mutant *CAV3* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *CAV3* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *CAV3* allele that contains a

pathogenic mutation, or by targeting a region of a disease-associated *CAV3* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *CAV3* allele and replace it with a wild type *CAV3* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *CAV3* construct and a shCAV3 construct, and measuring *CAV3* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *CAV3* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *CAV3* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *CAV3* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *CAV3* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of LQTS, HCM, or LGMD, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having LQTS, HCM, or LGMD associated with a pathogenic mutation in *CAV3* can result in a reduction in symptoms such as dyspnea, rapid heartbeat, arrhythmia, chest pain, fainting, dizziness, seizures, fatigue, atrophy and/or weakness of muscles in the hip and shoulder areas, cardiomyopathy. In some cases, effective SupRep treatment of a mammal having LQTS, HCM, or LGMD associated with a pathogenic *CAV3*

mutation can result in reduced contractility, improved relaxation, and/or reduced energy consumption.

In another embodiment, a mammal having LQTS or CPVT associated with a pathogenic mutation in the *TECRL* gene can be identified by, for example, analyzing a biological sample (e.g., analyzing a blood sample using PCR and/or DNA sequencing methods) obtained from the mammal to determine whether DNA in the sample includes a pathogenic mutation in the *TECRL* gene. Pathogenic mutations in or encoded by the *TECRL* gene include, without limitation, p.R196Q, c.331+1G>A, p.Q139X, p.P290H, p.S309X, and p.V298A. *See*, also, Devalla et al., *EMBO Mol Med.* 2016, 8(12):1390-1408; and Moscu-Gregor et al., *J Cardiovasc Electrophysiol.* 2020, 31(6):1527-1535. SupRep constructs targeted to mutant *TECRL* alleles can be designed to suppress the mutant *TECRL* alleles and replace them with a wild type *TECRL* allele. SupRep constructs targeted to mutant *TECRL* alleles can be designed and prepared using methods described, for example, in the Examples herein. For example, a SupRep construct can be generated to target a *TECRL* allele containing a pathogenic mutation, either by targeting a region of a disease-associated *TECRL* allele that contains a pathogenic mutation, or by targeting a region of a disease-associated *TECRL* allele that does not contain a pathogenic mutation. The SupRep constructs can be tested for their ability to suppress a mutant *TECRL* allele and replace it with a wild type *TECRL* allele. For example, constructs can be tested in an *in vitro* model system by co-transfecting cultured cells with a wild type *TECRL* construct and a shTECRL construct, and measuring *TECRL* expression with qRT-PCR and/or western blotting. A construct having a relatively high ability to knock down *TECRL* expression (e.g., the ability to knock down at least 70 percent, at least 75 percent, at least 80 percent, at least 85 percent, at least 90 percent, at least 95 percent, at least 98 percent, or at least 99 percent of *TECRL* expression at the mRNA and/or protein level) can be selected. The selected construct can be packaged in a virus particle (e.g., an AAV particle) and delivered to a mammal identified as having a pathogenic mutation in the *TECRL* gene at a dose of, for example, about 10^{10} vg/kg to about 10^{15} vg/kg, or about 10^{10} AAV particles/mL to about 10^{15} AAV particles/mL, using any appropriate route of administration (e.g., via direct injection into a tissue such as the myocardium, or via

intraperitoneal administration, intranasal administration, intravenous administration, intrathecal administration, intracerebral administration, intraparenchymal administration, or oral delivery in nanoparticles and/or drug tablets, capsules, or pills). In some cases, a SupRep construct targeted to *TECRL* can be administered to a mammal in a non-viral vector (e.g., in a plasmid or in a nucleic acid molecule complexed with lipids, polymers, or nanospheres), and can be delivered by direct injection to a tissue (e.g., the myocardium), or by intraperitoneal, intranasal, intravenous, intrathecal, intracerebral, or intraparenchymal administration, or by oral delivery. After administration, the mammal can be monitored for symptoms of LQTS or CPVT, to determine whether one or more symptoms of the disorder are diminished. For example, effective SupRep treatment of a mammal having LQTS or CPVT associated with a pathogenic mutation in *TECRL* can result in a reduction in symptoms such as rapid heartbeat, fainting, seizures, dizziness, lightheadedness, and/or VT. In some cases, effective SupRep treatment of a mammal having LQTS or CPVT associated with a pathogenic *TECRL* mutation can result in an normalization of IKs current density, normalization of cardiac APD, and/or regulation of heart rhythm.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1 – Materials and Methods

Samples: Human samples were obtained from patients with LQT1 and from an unrelated healthy control (**TABLE 2**).

Plasmids and Cloning of KCNQ1-SupRep: WT *KCNQ1* cDNA (NM_000218.2) was subcloned into pIRES2-EGFP (Clontech; Mountain View, CA) using *Nhe*I and *Bam*HI restriction sites. The QuikChange II XL site-directed mutagenesis kit (Agilent; Santa Clara, CA) was used to introduce two missense variants (p. T66S and p. Y67W) into the chromophore domain of EGFP, converting it to a cyan fluorescent protein and creating pIRES2-CFP-KCNQ1-WT. A second round of site-directed mutagenesis was completed using pIRES2-CFP-KCNQ1-WT to introduce the KCNQ1 variants p.Y171X,

p.V254M, and p.I567S (c.513C>A, c.760G>A, and c.1700T>G, respectively). Four pre-designed *KCNQ1* shRNAs (sh#1-4) were purchased from OriGene (Rockville, MD) in the pGFP-C-shLenti backbone along with a non-targeting scramble shRNA control (shCT). The shRNA sequences are listed in **TABLE 3A**. *KCNQ1* sh#4 was selected for the final KCNQ1-SupRep gene therapy vector and is referred to throughout this document as shKCNQ1. A DNA fragment containing ten synonymous variants within the *KCNQ1* sh#4 (shKCNQ1) target sequence of the KCNQ1-WT cDNA: c.1377C>T, c.1380C>A, c.1383T>C, c.1386C>T, c.1389T>C, c.1392T>C, c.1395A>C, c.1398G>A, c.1401G>A, and c.1404C>T (KCNQ1: p.D459D, p.G460G, p.Y461Y, p.D462D, p.S463S, p.S464S, p.V465V, p.R466R, p.K467K, and p.S468S, respectively) was synthesized and cloned into pIRES2-CFP-KCNQ1-WT using *Bgl*II and *Pvu*I restriction sites to create KCNQ1-shIMM (pIRES2-CFP-KCNQ1-shIMM) (GenScript; Piscataway, NJ). KCNQ1-shIMM and the CFP reporter were then PCR subcloned into the pGFP-C-shLenti backbone containing shKCNQ1 using 5′ *Mlu*I and 3′ *Bsr*GI+reverseBsaI restriction sites, excising the original GFP in the process to create the final KCNQ1-SupRep (pCFP-C-shLenti-shKCNQ1-KCNQ1-shIMM). Primers used for PCR cloning were: 5′-GGCACGCGTTTATGGCCGCGGCCTCCTC-3′ (forward primer; SEQ ID NO:1) and 5′-GCCGGTCTCTGTACACCGCTTTACTTGTACAGCTCGTCC-3′ (reverse primer; SEQ ID NO:2).

LQT1 and Unrelated Control Patient Selection for iPSC Generation: Patients were evaluated by a genetic cardiologist and LQTS specialist. Dermal fibroblasts or peripheral blood mononuclear cells (PBMCs) were collected by 4 mm skin punch biopsy or blood sample, respectively. Samples were obtained from nearly 1200 patients diagnosed with a variety of inherited cardiac channelopathies and their affected or unaffected family members, including 236 patients with LQT1. Four LQT1 patients were selected to span a variety of variant types (one nonsense, two missense, one synonymous splice) and phenotypes. These four patients included a lifelong asymptomatic patient and three patients with strong LQT1 phenotypes, defined as having at least one ECG with QTc greater than 500 ms, a positive history of LQTS-related symptoms (syncope, seizure, near drowning, sudden cardiac arrest), and a positive family history of LQTS-

related symptoms. A presumably healthy, unaffected father of a patient hosting a *de novo* variant was selected as an unrelated control.

Fibroblast/PBMCs Reprogramming into iPSCs and Quality Control: Fibroblasts or PBMCs were reprogrammed by Sendai virus transduction using the CytoTune-iPS 2.0 reprogramming kit (Thermo; Waltham, MA) or electroporation with four episomal DNA plasmids containing the Yamanaka factors: pCXLE-hUL, pCXLE-hSK, pCXLE-hOCT3/4-shp53-F, and pCXWB-EBNA1 (Addgene; Watertown, MA). At least two colonies were picked within 21 days post-induction and clonally expanded. All iPSCs were cultured in mTeSR™1 (STEMCELL®; Vancouver, Canada) supplemented with 1% penicillin/streptomycin on MATRIGEL®-coated (Corning; Corning, NY) 6 cm culture dishes in a 5% CO_2 incubator at 37°C. At 85% confluence, iPSCs were passaged using ReLeSR (STEMCELL®). Each clone was then karyotyped.

All lines had normal karyotype except the patient with KCNQ1-V254M (and subsequent isogenic control), which had a reprogramming-induced balanced translocation between chromosomes 13 and 22. No genes encoding ion channels critical to the cardiac action potential are located on chromosomes 13 or 22, so these cells were still included in the study. *KCNQ1* variant confirmation was conducted by Sanger sequencing of PCR-amplicons from genomic DNA. Expression of pluripotent markers in all iPSC clones was confirmed by confocal immunofluorescence microscopy using primary antibodies against Oct4 (Thermo, PA5-27438), Nanog (Thermo, PA1-097), Tra-1-60 (Santa Cruz; Dallas, TX; sc-21705), and SSEA-4 (Thermo, MA1-021) at a 1:250 dilution. Secondary antibodies were ALEXA FLUOR® 488 goat-anti-mouse (Thermo, A-11001) and ALEXA FLUOR® 594 goat-anti-rabbit (Thermo, A-11037). Counterstaining with DAPI (Thermo) was used at a 1:2000 dilution from a 5mg/mL stock. Images were acquired on a Zeiss LSM 780 confocal microscope.

iPSC-CM Culture, Differentiation, and Dissociation: When iPSCs were 85% confluent, differentiation into cardiomyocytes (CMs) was induced as described elsewhere (Schwartz 2009, *supra*; and Schwartz 2013, *supra*). Differentiation was initiated (day 0) by changing the culture medium to RPMI 1640 GlutaMAX™ plus 25 mM HEPES ((4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)) supplemented with B27-minus

insulin (RPMI/B27-ins) (Thermo) containing 5 μM CHIR99021 (MilliporeSigma; St. Louis, MO). On day 2, the medium was changed to RPMI/B27-ins containing 5 μM IWP-2 (MilliporeSigma). On day 4, the medium was changed back to the maintenance medium RPMI/B27-ins. Spontaneous beating typically began on days 6-7, with expansion to remaining cells by days 10-12. The iPSC-CMs were allowed to mature until at least day 30, changing the media twice per week. After day 30, iPSC-CMs were dissociated enzymatically using STEMdiff™ cardiomyocyte dissociation kit (STEMCELL®). Briefly, cells were rinsed with PBS (without Ca^{2+}/Mg^{2+}) and placed in dissociation medium for 10 minutes at 37°C, and then deactivated by addition of STEMdiff™ Cardiomyocyte Support Medium (STEMCELL®). Cells were triturated, transferred to a 15 mL conical tube, and pelleted by centrifugation at 300 rcf for 3 minutes. The supernatant was aspirated and the cells suspended in Cardiomyocyte Support Medium before transfer to appropriate MATRIGEL®-coated culture ware. After 24 hours, the medium was changed back to RPMI/B27-ins. Dissociation resulted in a mixture of single cells and small-to-medium sized iPSC-CM clusters, depending on cell density before and after plating. Spontaneous beating generally returned 24 hours after dissociation, with strong electrical coupling and syncytia formation between days 3-7.

CRISPR-Cas9 Corrected Isogenic Control iPSC: Genome editing of iPSC cell lines was contracted through Applied Stem Cell (Milpitas, CA). Isogenic "variant corrected" control iPSC cell lines were created for the two patient-specific LQT1 cells lines harboring KCNQ1-V254M (c.760G>A) and KCNQ1-A344A/spl (c.1032G>A). Guide RNAs (gRNAs) were designed using the gRNA design tool by Applied Stem Cell. Based on proximity to the target site and off-target profile, two gRNAs were selected for assessment of gRNA activity by next generation sequencing. Based on these results, the gRNAs 5′-CTGGCGGTGGATGAAGACCA-3′ (KCNQ1-V254M; SEQ ID NO:3) and 5′-CCCAGCAGTAGGTGCCCCGT-3′ (KCNQ1-A344A/spl; SEQ ID NO:4) were selected. Single-stranded oligodeoxynucleotide donors (ssODNs) were designed to be used as the repair template at the gRNA cut sites during homology directed repair. The isogenic control ssODNs were: 5′-CAGATCCTGAGGATGCTACACGTCGACCGCC AGGGAGGCACCTGGAGGCTGCTGGGCTCGGTGGTCTTCATCCACCGCCAGgtg

ggtggcccgggttaggggtgcggggcccag-3′ (KCNQ1-V254M; SEQ ID NO:5) and 5′- gtgcagcca ccccaggaccccagctgtccaaggagccagggaaaacgcacacacggggcacctacCGCTGGGAGCGCAAA GAAGGAGATGGCAAAGACAGAGAAGCAGGAGGCGAT-3' (KCNQ1-A344A/spl; SEQ ID NO:6), where uppercase = exon, lowercase = intron, underline = synonymous variant to prevent re-cutting after successful editing, and underline + bold + italic = WT nucleotide to replace target variant.

The gRNA was cloned into the expression vector pBT-U6-Cas9-2A-GFP, and the resulting plasmid was transfected into iPSCs along with the ssODN. Parental iPSCs ($5x10^5$) were plated on six-well plates and transfected by electroporation using 1100V, 30ms, 1P in the Neon Transfection System (Thermo). The iPSC population was subjected to limiting dilution for cloning and genotype analysis. Genomic DNA was extracted from each iPSC clone and analyzed by Sanger sequencing for the absence of the KCNQ1-V254M and KCNQ1-A344A/spl variants, respectively.

TSA201 Cell Culture and Transfection: TSA201 cells (passage 20 or lower) were maintained in Dulbecco's Modified Eagle Medium (Corning) supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin/streptomycin in a 5% CO_2 incubator at 37°C. For *patch clamp*, cells were split into T25 flasks. After 24 hours, heterologous expression of the $K_V7.1$ channel (*KCNQ1* α-subunit plus *KCNE1* β-subunit) was achieved using 5μL LIPOFECTAMINE® 2000 (Thermo) to co-transfect 1 μg of pIRES2-CFP-KCNQ1-WT, -shIMM, -Y171X, -V254M, or -I567S and 1 μg of pIRES2-dsRED2-KCNE1-WT in OPTI-MEM® (Thermo). After 4-6 hours, the medium was replaced with the maintenance medium for 48 hours before patch clamp electrophysiology experiments. For *allele-specific qRT-PCR*, *western blot*, and *trafficking immunofluorescence microscopy*, $5x10^5$ cells (or $1.5x10^6$ cells for the activation kinetics time course in **FIG. 9**) were plated per well in 6-well plates. After 24 hours, cells were co-transfected in maintenance medium using 10 μL EFFECTENE® (Qiagen; Hilden, Germany) with 100 fmol (between 0.3-0.7 μg) equimolar amounts (or as otherwise indicated) of each plasmid pIRES2-CFP-KCNQ1-WT or -variant, pGFP-C-shLenti-shKCNQ1(#1-#4) or -shCT, pCFP-C-shLenti-KCNQ1-SupRep, or pIRES2-dsRED2-

KCNE1-WT, as indicated by each figure. Endpoint assays were conducted as described in the appropriate methods sections.

Cell Membrane Trafficking Immunofluorescence Microscopy: TSA201 cells were co-transfected with KCNQ1-WT, -shIMM, or -variants and KCNE1-WT as above. After 24 hours, cells were dissociated using TrypLE™ Express (Thermo) and plated into 8-chamber culture slides (CELLTREAT®; Pepperell, MA). After another 24 hours, cells were fixed with 4% paraformaldehyde for 10 minutes and washed 3 times with PBS. Cells were blocked with 0.2% Tween-20/5% goat serum in PBS for 1 hour and incubated at 4°C overnight using a primary antibody against KCNQ1 (Santa Cruz, sc-365186) at a 1:100 dilution. Cells were washed 3 times for 15 minutes each with PBS-0.2% TWEEN®-20 and incubated in secondary ALEXA FLUOR® 488 goat-anti-mouse (Thermo) at a dilution of 1:250 for 1 hour before washing again 3 times for 15 minutes each. DAPI (4′,6-diamidino-2-phenylindole) counterstain was added during the first wash at a concentration of 1:2000 as before. VECTASHIELD® mounting media (Vector Labs; Burlingame, CA) was diluted 1:10 in PBS and used as mounting solution, and images were acquired on a Zeiss LSM 780 confocal microscope. Results shown in the figures herein are representative of three independent experiments (defined throughout the study as "three identical repeats of each experiment conducted from start to finish on separate weeks with one biological replicate per treatment group per run").

Western Blotting: TSA201 cells were co-transfected with KCNQ1-WT, -shIMM, or -variants and shKCNQ1(#1-4), -shCT, or KCNQ1-SupRep as described above. After 48 hours, cells were lysed in 1X RIPA buffer with protease and phosphatase inhibitors and chilled on ice for 10 minutes. Lysates were sonicated for 10 seconds at 50% amplitude and the cell debris was pelleted at 21,000 rcf for 15 minutes at 4°C. The supernatant was collected and the protein concentration quantified by BCA assay (Thermo) before mixing 1:1 with loading buffer (2X Laemmli buffer with 1:20 β-mercaptoethanol). Importantly, the lysates were NOT denatured at 95°C, which would have caused irreversible SDS-resistant high molecular weight aggregates of the KCNQ1 proteins (Sagné *et al.*, *Biochem. J.*, 316(Pt 3):825-831 (1996); and Little, "Amplification-refractory mutation system (ARMS) analysis of point mutations," *Curr. Protoc. Hum.*

Genet., Chapter 9:Unit 9.8 (2001)). Proteins (10 μg/lane) were run on a 4-15% TGX gel (Bio-Rad; Hercules, CA) and transferred to a PVDF membrane using a Trans-Blot Turbo Transfer System (Bio-Rad). Membranes were blocked for 1 hour in tris-buffered saline (TBS) with 0.1% TWEEN®-20/3% bovine serum albumin and incubated at 4°C overnight with primary antibodies against KCNQ1 (Santa Cruz, sc-365186) and Cofilin (Santa Cruz, sc-376476) as a housekeeping control at a 1:1000 dilution in blocking solution. The membrane was washed 3 times for 15 minutes each with TBS-0.1% TWEEN®-20 prior to addition of secondary antibody HRP-conjugated goat-anti-mouse (R&D Systems; Minneapolis, MN; HAF007) at a dilution of 1:5000 in blocking solution. The membrane was washed 3 times for 15 minutes each with TBS and incubated in SuperSignal™ West Pico PLUS chemiluminescent ECL substrate (Thermo) for 3 minutes and exposed using autoradiography film. Pixel density was quantified using freely available ImageJ software. All western blots presented herein are representative images of three independent experiments.

Allele-Specific qRT-PCR: Allele-specific primers were developed for qRT-PCR to specifically amplify (1) total *KCNQ1*, (2) endogenous *KCNQ1* (includes KCNQ1-WT and -variants, but excludes KCNQ1-shIMM), and (3) KCNQ1-shIMM, by adapting allele-specific genotyping methods described elsewhere (**TABLE 4**) (Rohatgi *et al.*, *supra*; and Priori *et al.*, *supra*). For total *KCNQ1*, primers were purchased from IDT (Coralville, IA; PRIMETIME™ qPCR Primer Assay, Hs.PT.58.41042304). Allele-specific primers were created by designing two forward primers spanning the shKCNQ1 target site, with one complementary to endogenous *KCNQ1* (allele-specific for KCNQ1-WT and -variants) and the other complementary to KCNQ1-shIMM (allele-specific for KCNQ1-shIMM). A common reverse primer was used with both allele-specific forward primers. *GAPDH* primers were purchased from IDT (PRIMETIME™ qPCR Primer Assay, Hs.PT.39a.22214836) as a housekeeping control. A standard curve was used to correct for PCR amplification bias. TSA201 cells were co-transfected with KCNQ1-WT, -shIMM, or -variants and shKCNQ1(#1-4), -shCT, or KCNQ1-SupRep as above. After 48 hours (or at the indicated time for the activation kinetics time-course in **FIG. 9**), RNA was harvested using an RNeasy kit (Qiagen) and quantified using a NanoDrop ND-1000

spectrophotometer (Thermo). Complementary DNA (cDNA) was generated by loading 500 ng RNA in the SuperScript™ IV VILO™ Master Mix reverse transcription kit (Thermo). For each sample, four qRT-PCR reactions were run using the SYBR Green Master Mix kit (Qiagen) with the four sets of primers as described. Data was analyzed using the ΔΔCT method by first normalizing *KCNQ1* to *GAPDH* and then comparing the relative fold change to the KCNQ1-WT and shCT treatment group. All qRT-PCR experiments (except the dose-response curve in **FIG. 8** and the time-course in **FIG. 9**) are the results of three independent experiments.

I_{Ks} Whole Cell Patch Clamp Electrophysiology: A standard whole-cell patch clamp technique was used to measure the slow delayed rectifier current, I_{Ks}, produced by KCNQ1-WT, -shIMM, and -variants at room temperature (22-24°C) with the use of Axopatch 200B amplifier, Digidata 1440A system, and pCLAMP version 10.7 software (Axon Instruments; Sunnyvale, CA). The extracellular (bath) solution contained the following (mmol/L): 150 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 1 Na-pyruvate, and 15 HEPES. The pH was adjusted to 7.4 with NaOH. The intracellular (pipette) solution contained the following (mmol/L): 20 KCl, 125 K-aspartate, 1 $MgCl_2$, 10 EGTA, 5 Mg-ATP, 5 HEPES, 2 Na_2-phosphocreatine, and 2 Na_2-GTP. The pH was adjusted to 7.2 with KOH (Al-Khatib *et al.*, *supra*). Microelectrodes were pulled on a P-97 puller (Sutter Instruments; Novato, CA) and fire polished to a final resistance of 2-3MΩ. The series resistance was compensated by 80-85%. Currents were filtered at 1 kHz and digitized at 5 kHz with an 8-pole Bessel filter. The voltage dependence of activation was determined using voltage-clamp protocols described in the description of **FIGS. 10A-10C**. Data were analyzed using Clampfit (Axon Instruments) and Excel (Microsoft; Redmond, WA) and fitted with GraphPad Prism 8 software (GraphPad; San Diego, CA).

Lentivirus Generation and Transduction of iPSC-CMs: For application of KCNQ1-SupRep to iPSC-CMs (or shCT as a treatment control), lentivirus was used. Lentiviral particles were generated from pCFP-C-shLenti-shKCNQ1-shIMM (KCNQ1-SupRep) and pGFP-C-shLenti-shCT (shCT), using the pPACKH1 HIV Lentivector Packaging kit (SBI System Biosciences; Palo Alto, CA). Lentiviral titers were quantified by two methods, including qRT-PCR (~$1x10^{11}$ viral genomes/mL) to determine the total

number of viral particles, and by transducing TSA201 cells in serial dilution to define the number of functional infectious particles (~$5x10^8$ infectious units/mL). Lentivirus was applied to iPSC-CMs at a multiplicity of infection (MOI) of 20-25 infectious units/cell (4,000-5,000 viral genomes/cell). After reaching at least day 30 post-induction of differentiation, iPSC-CMs derived from the healthy unrelated control, the four patients with LQT1, or two isogenic controls, were dissociated and plated into MATRIGEL®-coated 35mm dishes with glass-bottom insets for FluoVolt™ (MatTek; Ashland, MA) or 8-chamber culture slides for immunofluorescence (CELLTREAT®) as described above. After 24-48 hours of recovery, iPSC-CMs were left untreated or were transduced with lentiviral particles containing KCNQ1-SupRep or shCT treatment control at an MOI of 20-25. To increase transduction efficiency, Polybrene infection reagent (MilliporeSigma) was added during transduction to a final concentration of 8 μg/mL and the iPSC-CMs were centrifuged at 250 rcf for 1.5 hours at room temperature in the 35 mm dishes. After 24 hours post-transduction, the medium was exchanged for fresh maintenance medium, RPMI/B27-ins.

Immunofluorescence in iPSC-CMs: Immunofluorescence was conducted 7 days post-transduction of iPSC-CMs with lentiviral particles containing either KCNQ1-SupRep or shCT. Cells were fixed with 4% paraformaldehyde for 10 minutes and washed 3 times with PBS. Cells were blocked with 0.1% Triton X-100/5% donkey serum in PBS for 1 hour and incubated at 4°C overnight using primary antibodies against cTnT (abcam; Cambridge, UK, ab45932), turboGFP for treatment with shCT (OriGene, TA150041) or eCFP for treatment with KCNQ1-SupRep (MyBioSource; San Diego, CA, MBS9401609), and KCNQ1 (Santa Cruz, sc-10646) at a 1:100 dilution each in blocking solution. Cells were washed 3 times for 15 minutes each with PBS-0.1% Triton X-100 and incubated in secondary ALEXA FLUOR PLUS® 488 donkey-anti-goat (Thermo, A32814), ALEXA FLUOR PLUS® 594 donkey-anti-mouse (Thermo, A32744), and ALEXA FLUOR PLUS® 647 donkey-anti-rabbit (Thermo, A32795) at a dilution of 1:250 each in blocking solution for 1 hour before washing again 3 times for 15 minutes each. DAPI counterstain was added during the first wash at a concentration of 1:2000 as before. VECTASHIELD® mounting media (Vector Labs) was diluted 1:10 in PBS and

used as mounting solution, and images were acquired on a Zeiss LSM 780 confocal microscope using identical settings between images.

Voltage Dye Optical Action Potentials in iPSC-CMs: Voltage dye experiments were conducted between 3-7 days post-transduction of iPSC-CMs with lentiviral particles containing either KCNQ1-SupRep or shCT. Unrelated control cells and isogenic controls were not transduced with lentivirus, but rather were left untreated to provide an ideal normal baseline representing a "healthy" APD. On the day of imaging, iPSC-CMs were rinsed with pre-warmed (37°C) HEPES-buffered Tyrode's solution (Alfa Aesar; Haverhill, MA). Using the FluoVolt™ Membrane Potential kit (Thermo), 0.125 μL FluoVolt™ dye and 1.25 μL PowerLoad were added to 0.5 mL Tyrode's solution for each 35 mm glass-bottom dish and incubated at 37°C for 20 minutes. Excess dye was removed in three rinses with pre-warmed Tyrode's solution, and a final 2 mL Tyrode's solution was added to the iPSC-CMs for imaging. During imaging, the dishes were kept in a heated 37°C stage-top chamber (Live Cell Instrument; Seoul, South Korea) with 5% CO_2. Using a Nikon Eclipse Ti light microscope (Nikon; Tokyo, Japan) under 40X-water objective magnification, optical action potentials were recorded in 20s fast time-lapse videos at a rate of 50 frames/sec (fps, 20ms exposure time) with LED illumination at 5% power. iPSC-CMs were paced at 1 Hz (9 ms pulse duration, 25V) using a MyoPacer field stimulator (Ion Optix; Westwood, MA) to eliminate beat-rate dependent effects on the APD. Videos were focused on electrically-coupled syncytial areas of iPSC-CMs (clusters and monolayers) since these areas of cells best follow the pacing stimulus and produce the greatest signal-to-noise represented by large changes in fluorescence intensity (often ~8-12%). For analysis, rectangular regions of interest were drawn over flashing areas of cells, and NIS-Elements software (Nikon) was used to quantify the fluorescence intensity over time within each region of interest, resulting in optical action potential traces. Using a custom in-house Excel-based program, traces were corrected for photobleaching and the amplitude was normalized as change in fluorescence divided by the baseline minimum florescence ($\Delta F/F_{min}$). In a semi-automated manner, common action potential parameters including APD_{90}, APD_{50}, amplitude, rise time, upstroke velocity, etc. were detected for each individual optical action potential and averaged across all beats within a

20 second trace. The average of all beats within a 20 second trace represents a single data point. For representative traces, the maximum amplitude was further normalized to 1.0 to allow for accurate visualization of APD differences.

3D iPSC-CM Organoid Culture, Immunofluorescence, and Optical Action Potentials: 3D-organoids were generated based on a protocol described elsewhere (Zimmerman *et al.*, *Circ. Res.*, 90:223-230 (2002)). Briefly, a spontaneously beating syncytial monolayer of iPSC-CMs from a patient with KCNQ1-Y171X was dissociated as described above. The pelleted iPSC-CMs were resuspended in a mixture of 80% ice cold undiluted MATRIGEL® (Corning) with 20% fetal bovine serum with 1 million iPSC-CMs per 15 μL. Aliquots of 15 μL (containing 1 million iPSC-CMs each) were transferred to an organoid embedding sheet (STEMCELL®) at 37°C in a 5% CO_2 incubator for 30 minutes to solidify in a spherical shape. The organoids were then transferred to individual wells of a 24-well plate in RPMI/B27-ins. Organoids were allowed to mature for a minimum of 7 days before transducing with lentiviral shCT or KCNQ1-SupRep. After seven days post-transduction, organoids were fixed for immunofluorescence or live-imaged for electrophysiology using FluoVolt™ voltage dye. For immunofluorescence, organoids were rinsed with PBS, fixed in 4% paraformaldehyde for 10 minutes on ice, and washed three times with PBS. Organoids were suspended in Tissue-Plus™ optimal cutting temperature (O.C.T.) compound (Thermo), transferred to disposable base molds (Thermo), and frozen quickly on dry ice. Frozen organoids were cryosectioned and mounted on slides for imaging. Immunofluorescence was conducted as described above using 0.1% Triton X-100/5% goat serum in PBS as blocking solution, primary antibodies against cTnT (abcam, ab45932) and turboGFP for treatment with shCT (OriGene, TA150041) or eCFP for treatment with KCNQ1-SupRep (MyBioSource, MBS9401609) at a 1:100 dilution each. Secondary antibodies were ALEXA FLUOR PLUS® 488 goat-anti-mouse (Thermo, A32723) and ALEXA FLUOR PLUS® 594 goat-anti-rabbit (Thermo, A32740) at a dilution of 1:250 each. For FluoVolt™, the experiment was conducted as above using whole organoids instead of syncytial monolayers.

Statistical Analysis: GraphPad Prism 8 was used for all statistical analysis and to fit all data for figures. Individual data points are shown wherever practical along with the mean. Error bars represent standard deviation (S.D.) unless otherwise indicated in the figure legend. Specific statistical methods are indicated in each figure legend. Briefly, one-way ANOVA with post-hoc Tukey's or Dunnett's test for multiple comparisons was performed for comparisons among three or more groups as appropriate. An unpaired two-tailed student's t-test was performed to determine statistical significance between two groups when indicated. A $p<0.05$ was considered to be significant.

Example 2 - Generation of a KCNQ1-SupRep gene therapy construct

To make KCNQ1-SupRep, four candidate *KCNQ1* shRNAs (sh#1-4) in the pGFP-C-shLenti lentiviral backbone were purchased from OriGene, along with a non-targeting scrambled control shRNA (shCT, **TABLE 3A**). The KD efficiency of each *KCNQ1* shRNA was determined by co-transfecting TSA201 cells with KCNQ1-WT and sh#1-4. Expression of *KCNQ1* was measured by quantitative reverse transcription PCR (qRT-PCR, **FIG. 5A**) and confirmed by western blot (**FIGS. 5A and 5B**). Of the four shRNAs tested, sh#1, sh#2, and sh#4 all resulted in significant KD of *KCNQ1* (mRNA: 69-78% KD, protein: 50-77% KD) with no statistically significant differences between the three shRNAs. Any of these shRNAs could in theory have been used as part of the final KCNQ1-SupRep gene therapy vector. To select a final shRNA from the three potential candidates, by raw average KD, *KCNQ1* sh#4 provided the strongest KD of *KCNQ1* on both the mRNA (78%, $p=0.004$) and protein (77%, $p<0.004$) levels. Further, at the time of selection, the *KCNQ1* sh#4 target sequence (nucleotides c.1376-1404, exon 10-11 boundary) was assessed using the Genome Aggregation Database (gnomAD) and ClinVar, and was found to be devoid of both common genetic polymorphisms and all known pathogenic LQT1-causative mutations that may interfere with KD efficiency. *KCNQ1* sh#4 therefore was selected for the final KCNQ1-SupRep and is referred to herein as "shKCNQ1."

Four additional, custom-made shRNAs were subsequently tested (sh#5-sh#8; sequences in **TABLE 3B**). TSA201 cells were co-transfected with KCNQ1-WT and

sh#5-sh#8) or non-targeting scrambled shRNA control (shCT). KCNQ1 expression normalized to GAPDH was measured by qRT-PCR. sh#5 had the strongest knockdown (95%) by raw value **(FIG. 5C)**.

To create the replacement shRNA-immune version of *KCNQ1*, called KCNQ1-shIMM, ten synonymous variants were introduced into the WT *KCNQ1* cDNA at the wobble base of each codon within shKCNQ1's target site, nucleotides c.1376-1404 (**FIG. 6A**). KCNQ1-shIMM was then cloned into the shKCNQ1-containing vector, pGFP-C-shLenti, downstream of the CMV promoter. In this step, the original GFP reporter (which remained the reporter for shCT) was exchanged for an internal ribosome entry site (IRES) with CFP. The final KCNQ1-SupRep gene therapy vector used in this *in vitro* study is illustrated in **FIG. 6B**.

Example 3 – KCNQ1-SupRep gene therapy both suppresses and replaces KCNQ1-WT

To confirm that KCNQ1-shIMM is indeed immune to KD by shKCNQ1, TSA201 cells were co-transfected with KCNQ1-WT or KCNQ1-shIMM and shKCNQ1. The expression of KCNQ1-WT versus KCNQ1-shIMM was quantified using allele-specific qRT-PCR. Each sample was run in four separate reactions, using a unique set of allele-specific primers (**TABLE 4**), to quantify (1) total *KCNQ1*, (2) endogenous *KCNQ1*, which includes WT or variant-containing alleles, but excludes KCNQ1-shIMM, (3) KCNQ1-shIMM, and (4) *GAPDH* as a housekeeping control. Commercial primers were used to amplify total *KCNQ1*. For exclusive amplification of endogenous *KCNQ1* or KCNQ1-shIMM, two forward primers were designed within the shKCNQ1 target site, one complementary to the WT sequence and the other complementary to the unique, modified sequence engineered to create KCNQ1-shIMM. A common reverse primer was used for both reactions, and a standard curve was used to correct for PCR amplification bias.

Compared to shCT, shKCNQ1 caused significant (87%) suppression of KCNQ1-WT ($p<0.0001$), but was unable to suppress KCNQ1-shIMM ($p=0.997$, **FIG. 7A**). Notably, there was no difference in the expression of KCNQ1-WT compared to KCNQ1-shIMM ($p>0.9999$), indicating that introduction of the synonymous variants in KCNQ1-

shIMM did not disturb its expressivity as a result of uneven bias in the use of human codons. Next, KCNQ1-SupRep was co-transfected with KCNQ1-WT, which resulted in 52% suppression of KCNQ1-WT with 255% replacement of KCNQ1-shIMM ($p<0.0001$, **FIG. 7A**). The dual component KCNQ1-SupRep vector had less potent suppression compared to shKCNQ1 alone, but exhibited stronger expression of KCNQ1-shIMM than KCNQ1-shIMM alone. While the reason for this is unclear, varying amounts of KCNQ1-SupRep were transfected and shown to cause dose-dependent suppression and replacement, suggesting that KCNQ1-SupRep expression can be adjusted as needed (**FIG. 8**). Results obtained by qRT-PCR were confirmed by western blotting, which demonstrated that shKCNQ1 was able to significantly KD KCNQ1-WT ($p=0.037$) but not KCNQ1-shIMM ($p=0.61$, **FIGS. 7A and 7B**). As a safety metric for onset of the gene therapy, allele-specific qRT-PCR was used to measure the activation kinetics of KCNQ1-SupRep in a three day time course of TSA201 cells co-transfected with WT-KCNQ1 and shCT, shKCNQ1, KCNQ1-shIMM, or KCNQ1-SupRep. Compared to treatment with shCT, KCNQ1-SupRep caused reduction of KCNQ1-WT that was replaced with KCNQ1-shIMM, but the total *KCNQ1* was not altered at any time during the three day onset, avoiding over- or under-expression (**FIG. 9**).

Example 4 – Selection of patients with LQT1-causative variants in *KCNQ1*

Four patients with LQT1 hosting unique variants, KCNQ1-Y171X, KCNQ1-V254M, KCNQ1-I567S, and KCNQ1-A344A/spl were selected for this study. All four KCNQ1 variants were classified as pathogenic (LQT1-causative) by current American College of Medical Genetics guidelines (Richards *et al.*, *Genet. Med.*, 17:405-424 (2015)). This gene therapy pilot study therefore included a nonsense, premature truncation variant (KCNQ1-Y171X) producing haploinsufficiency in a patient with a mild phenotype, as well as two dominant-negative missense variants (KCNQ1-V254M and KCNQ1-I567S) and a synonymous splice variant (KCNQ1-A344A/spl) that causes skipping of exon 7 (Tsuji *et al.*, *J. Mol. Cell Cardiol.*, 24:662-669 (2007)), in three patients with a strong LQT1 phenotype including documented QTc greater than 500 ms, a

positive history of LQTS-related symptoms (syncope, seizure, near drowning, sudden cardiac arrest), and a positive family history of LQTS-related symptoms (**TABLE 2**).

All four variants have been described elsewhere, though only KCNQ1-V254M and KCNQ1-A344A/spl have been characterized functionally as dominant-negative mutations (Tsjui *et al.*, *supra*; Piippo *et al.*, *J. Am. Coll. Cardiol.*, 37:562-568 (2001); Wang *et al.*, *J. Cardiovasc. Electrophysiol.*, 10:817-826 (1999); and Choi *et al.*, *Circulation*, 110:2119-2124 (2004)). Site-directed mutagenesis was used to introduce three of the four LQT1 patient variants (KCNQ1-Y171X, -V254M, and -I567S) into KCNQ1-WT to evaluate the ability of KCNQ1-SupRep to suppress and replace *KCNQ1* variants in a mutation-independent manner. KCNQ1-A344A/spl was not included for heterologous expression studies in TSA201 cells since the KCNQ1-WT is a full length cDNA and does not contain the introns necessary to evaluate a splicing variant like KCNQ1-A344A/spl.

Example 5 – Validation of function for KCNQ1-shIMM and KCNQ1 pathogenic variants

KCNQ1-WT and -shIMM, and LQT1-causative variants KCNQ1-Y171X, -V254M, and -I567S were co-transfected into TSA201 cells with the $K_V7.1$ channel β-subunit, *KCNE1*. The resulting I_{Ks} current was measured by standard whole cell patch clamp. Representative traces are shown in **FIG. 10A**. Importantly, KCNQ1-shIMM produced robust I_{Ks} current with no significant difference from KCNQ1-WT ($p=0.28$, **FIGS. 10B and 10C**). All three LQT1 variants (KCNQ1-Y171X, -V254M, and -I567S) resulted in no functional I_{Ks} current beyond the minimal background ion channel activity of TSA201 cells, consistent with complete loss of function (**FIGS. 10A-10C**). Null current was expected for a nonsense variant like KCNQ1-Y171X and additionally for KCNQ1-V254M, whose null status was in concordance with data described elsewhere (Wang *et al.*, *supra*). Total lack of current from KCNQ1-I567S was a novel finding, but was consistent with the patient's clinically definitive LQT1 and the fact that most LQT1-causative variants are missense variants.

To evaluate trafficking of KCNQ1 to the cell membrane, transfected TSA201 cells were assessed by immunofluorescence microscopy using a KCNQ1 antibody. Both

KCNQ1-WT and KCNQ1-shIMM produced bright staining along the cell membrane, indicating that the synonymous variants in KCNQ1-shIMM did not interfere with correct trafficking (**FIG. 11**). Of the LQT1 variants, KCNQ1-Y171X produced no detectable protein as a result of premature truncation, while KCNQ1-V254M and KCNQ1-I567S exhibited normal cell membrane trafficking, though the overall expression of KCNQ1-I567S appeared to be decreased. Taken together, these results indicated that KCNQ1-shIMM has WT function and that KCNQ1-Y171X, -V254M, and -I567S are LQT1-causative variants with total loss of function.

Example 6 – KCNQ1-SupRep gene therapy both suppresses and replaces KCNQ1 variants in a mutation-independent manner

To confirm that treatment with KCNQ1-SupRep gene therapy can suppress and replace LQT1-causative variants in a mutation-independent manner, TSA201 cells were co-transfected with the three *KCNQ1* variants and shKCNQ1, KCNQ1-SupRep, or shCT control. All three LQT1-causative variants were suppressed by shKCNQ1, ranging from 87% to 93% KD relative to KCNQ1-WT as measured by allele-specific qRT-PCR (**FIG. 12**, top). While the suppression was visibly marked for each of the three variants, suppression by shKCNQ1 did not reach statistical significance for KCNQ1-Y171X and KCNQ1-I567S, presumably due to lower baseline expression of these variants. Despite not reaching statistical significance, it is noteworthy that very few mRNA transcripts were detectable in any sample (WT or variant) that was treated with shKCNQ1. Notably, KCNQ1-Y171X had substantially decreased expression at baseline, likely due to its premature stop codon and predicted subsequent nonsense-mediated decay of mRNA transcripts (Hug *et al.*, *Nucleic Acids Res.*, 44:1483-1495 (2016)).

Results obtained by qRT-PCR were confirmed by western blotting. KCNQ1-Y171X produced no detectable protein as a result of its premature truncation, while KCNQ1-V254M was suppressed by shKCNQ1, and KCNQ1-I567S had faint baseline expression that also was suppressed by shKCNQ1 (**FIG. 12**, bottom). Overall, KCNQ1-SupRep caused suppression and replacement of three LQT1-causative *KCNQ1* variants, validating its ability to suppress and replace *KCNQ1* in a mutation-independent manner.

Example 7 – Generation of iPSC-CMs from four patients with LQT1

From the 236 patients with LQT1 in the iPSC biorepository, four patients with distinct LQT1 mutations were selected to have their iPSCs differentiated into iPSC-CMs, in order to test the APD-shortening potential of this KCNQ1-SupRep gene therapy. A healthy unrelated individual was included as a control, and two isogenic controls were created by CRISPR-Cas9 correction of KCNQ1-V254M and KCNQ1-I567S, respectively. These isogenic controls served as the gold standard for a possible therapeutic cure, thereby providing a marker for the "ideal" rescue/normalization of the prolonged APD and indicating how close to this ideal did treatment with KCNQ1-SupRep gene therapy reach.

Dermal fibroblasts or peripheral blood mononuclear cells (PBMCs) were collected from each patient and were used to generate iPSCs. Standard quality control assays were performed on each iPSC line, including Sanger sequencing of the LQT1-causative variant, karyotyping, bright field morphology, and immunofluorescence microscopy for pluripotent markers including Tra-1-60, Nanog, SSEA-4, and Oct4 (**FIGS. 13A-13D**). Differentiation of iPSCs was induced by methods described elsewhere to generate spontaneously beating iPSC-CMs (Burridge *et al.*, *Nat. Methods*, 11:855-860 (2014); and Mummery *et al.*, *Circ. Res.*, 111:344-358 (2012)). Since the cardiac APD is known to shorten as iPSC-CMs mature over time, all experiments were conducted at least 30 days after the induction of differentiation (Shaheen *et al.*, *Stem Cell Reports*, 10:1879-1894 (2018)).

Example 8 – KCNQ1-SupRep gene therapy increases KCNQ1 in LQT1 iPSC-CMs

To assess the ability of lentiviral KCNQ1-SupRep to transduce iPSC-CMs and increase WT KCNQ1 expression, unrelated control and LQT1 iPSC-CMs were transduced with lentiviral KCNQ1-SupRep or shCT and evaluated using immunofluorescence microscopy. Cardiac troponin T (cTnT) was used as a marker of cardiomyocytes. Antibodies targeting the lentiviral reporters (turboGFP for shCT or CFP for KCNQ1-SupRep) were used to identify transduced cells, and KCNQ1 was stained to

visualize the effects of KCNQ1-SupRep on overall expression of KCNQ1. Results for KCNQ1-V254M iPSC-CMs (**FIG. 14**) and remaining unrelated control and LQT1 iPSC-CMs (**FIGS. 15A-15D**) showed high purity cardiomyocytes within the iPSC-CM cultures that had been evenly transduced with lentiviral KCNQ1-SupRep or shCT. At baseline in iPSC-CMs treated with shCT, KCNQ1 was only faintly detectable by confocal microscopy, whereas iPSC-CMs treated with KCNQ1-SupRep displayed robust staining for KCNQ1 (**FIGS. 14 and 15A-15D**). This suggests that in iPSC-CMs, treatment with KCNQ1-SupRep gene therapy drives substantial overexpression of KCNQ1-shIMM.

Example 9 – KCNQ1-SupRep gene therapy shortens the cardiac APD in LQT1 iPSC-CMs as measured by FluoVolt™ voltage dye

Further studies were conducted to test whether treatment with KCNQ1-SupRep gene therapy is able to rescue the pathognomonic feature of LQT1 by shortening the pathologically prolonged APD. FluoVolt™ voltage dye was used to measure optical action potentials in iPSC-CMs derived from four patients with LQT1 (stemming from KCNQ1-Y171X, -V254M, -I567S, or -A344A/spl) treated with either the lentiviral shCT control or KCNQ1-SupRep gene therapy. The unrelated control was measured without any treatment as a measure for a healthy APD. All iPSC-CMs were paced at 1 Hz during recording to eliminate beat rate-dependent changes to the APD. Representative optical action potentials are shown in **FIG. 16A**. When treated with shCT, all LQT1 iPSC-CMs had significantly longer APD at 90% repolarization (APD_{90}) and three of the four also had significantly longer APD at 50% repolarization (APD_{50}) compared to untreated unrelated healthy control iPSC-CMs, validating the LQT1 iPSC-CMs as an *in vitro* model of LQT1.

A full summary of APD_{90} and APD_{50} values and APD shortening due to KCNQ1-SupRep is shown in **TABLE 5**. APD_{90} and APD_{50} values were assessed by one-way ANOVA with post-hoc Dunnett's test comparing each KCNQ1 variant treated with shCT or KCNQ1-SupRep to the untreated, unrelated control (brackets in **TABLE 5**). All four LQT1 iPSC-CMs treated with shCT had significantly longer APD_{90} than the unrelated control, and two of the three had significantly longer APD_{50} as well, confirming that

these LQT1 lines display prolonged APD – the hallmark feature of LQT1. APD shortening due to KCNQ1-SupRep compared to treatment with shCT was then assessed by unpaired two-tailed student's t-tests at both the APD_{90} and APD_{50} levels separately for each variant. KCNQ1-SupRep resulted in statistically significant attenuation of both APD_{90} and APD_{50} in all four LQT1 iPSC-CMs (**TABLE 5** and **FIG. 16B**). When treated with KCNQ1-SupRep, the APD_{90} and APD_{50} of both LQT1 lines shortened significantly. In particular, the APD_{90} shortened by 117 ms in KCNQ1-Y171X, by 111 ms in KCNQ1-V254M, by 85 ms in KCNQ1-I567S, and by 210 ms in KCNQ1-A344A/spl (**TABLE 5** and **FIG. 16B**).

To determine whether the observed APD shortening due to KCNQ1-SupRep represents complete rescue to WT or if the shorter APD values were incomplete or overcorrection, two CRISPR-Cas9 corrected isogenic controls were created from the KCNQ1-V254M and KCNQ1-A344A/spl parent LQT1 iPSC cell lines. When measured by FluoVolt™, and plotted against the shCT and KCNQ1-SupRep treatment data from **FIG. 16B**, both isogenic controls had significantly shorter APD_{90} and APD_{50} compared to their shCT-treated counterparts (**FIGS. 17A and 17B**).

Isogenic correction of KCNQ1-V254M shortened the APD_{90} by 200 ms to 380 ± 112 ms ($n=58$, $p<0.0001$), and isogenic correction of KCNQ1-A344A/spl shortened the APD_{90} by 176 ms ($n=57$, $p<0.001$). A full summary of the APD_{90} and APD_{50} values for KCNQ1-V254M and KCNQ1-A344A/spl with isogenic controls is shown in **TABLE 6**. Comparing the shortened APD values of the KCNQ1-V254M and KCNQ1-A344A/spl iPSC-CMs treated with KCNQ1-SupRep gene therapy to the APD values of the isogenic controls, there was apparent variability in the actual degree of rescue. In KCNQ1-V254M, there was statistically significant incomplete shortening of the APD_{90} and concomitant overcorrection of the APD_{50} while in KCNQ1-A344A/spl the APD_{90} had complete rescue with no significant difference, but did show overcorrection of the APD_{50}. Despite this variability, treatment with KCNQ1-SupRep gene therapy demonstrated the ability to completely rescue the prolonged action potential in LQT1 iPSC-CMs.

Example 10 – KCNQ1-SupRep gene therapy shortens the cardiac APD in 3D-organoid culture of LQT1 iPSC-CMs

To determine whether the APD-shortening ability of KCNQ1-SupRep is translatable from 2D syncytial monolayer iPSC-CM culture to a three-dimensional environment, LQT1 iPSC-CM 3D-organoids were generated from one of the four LQT1 variants using the KCNQ1-Y171X iPSC-CMs. The KCNQ1-Y171X iPSC-CMs were dissociated and embedded in a MATRIGEL® spheroid mold and allowed to reorganize naturally on the collagenous extracellular architecture to create a 3D-cardiac organoid (**FIG. 18A**). The organoids were treated with shCT or KCNQ1-SupRep, cryosectioned, and stained for immunofluorescence using cardiac troponin T (cTnT) to mark cardiomyocytes and the lentiviral reporters (turboGFP for shCT and CFP for KCNQ1-SupRep) to mark infected cells. Immunofluorescence revealed networks of cardiomyocytes and prominent staining of turboGFP and CFP, indicating even transduction by shCT and KCNQ1-SupRep (**FIG. 18B**). The APD of untreated and KCNQ1-SupRep treated organoids were assessed by FluoVolt™, revealing that KCNQ1-SupRep resulted in statistically significant shortening of the APD_{90} and APD_{50} (**FIGS. 18C and 18D**), and suggesting that KCNQ1-SupRep retained APD-shortening ability in a simple 3D organoid environment.

Taken together, the studies described above used two *in vitro* model systems to engineer and validate the APD-attenuating effect of a hybrid suppression-and-replacement gene therapy construct for LQTS, and LQT1 in particular. The results of these studies indicated that suppression-replacement gene therapy can be used to directly target the pathogenic substrate and ameliorating the resultant disease not only for LQT1 specifically, but also for LQTS in general, and perhaps for almost any sudden death-predisposing autosomal dominant genetic heart disease.

TABLE 2

Summary of subjects selected for generation of iPSCs for iPSC-CM studies

Subject	Sex	Age at Sample Collection	KCNQ1 Variant(s)	Average QTc (ms) [Range]	LQTS-Related Symptoms	Family History	Treatment	iPSC Source; Generation Method
LQT1 #1	Female	41	Y171X (c.513C>A)	No ECG Available	Asymptomatic	Daughter – JLNS	BB	PBMC; Episomal DNA
LQT1 #2	Female	28	V254M (c.760G>A)	512 [486-533]	Near drowning (x2)	Mother – near drowning	BB, ICD	Fibroblasts; Sendai
LQT1 #3	Female	59	I567S (c.1700T>G)	488 [465-512]	Cardiogenic syncope, ICD storm	Sister – syncope while swimming (x2) Father – sudden death (80-years-old)	BB, LCSD, ICD	Fibroblasts; Episomal DNA
LQT1 #4	Male	12	A344A/spl (c.1032G>A)	536 [444-604]	Potential cardiogenic syncope (x2)	Great-great aunt – sudden death (30-years-old)	BB, LCSD	Fibroblasts; Sendai
Unrelated Control	Male	47	—	No ECG Available	Asymptomatic	—	—	Fibroblasts; Sendai

KCNQ1 variants are listed as the resulting change on the protein level with cDNA change in parenthesis. (QTc) Bazett-corrected QT interval; (ECG) electrocardiogram; (JLNS) Jervell and Lange-Nielsen syndrome; (BB) beta-blocker; (ICD) implantable cardioverter defibrillator; (PBMC) peripheral blood mononuclear cells.

TABLE 3A

KCNQ1 shRNA sequences

shRNA	**Target sequence (sense)***	**Hairpin Loop**	**Antisense sequence**	***KCNQ1* Location**
shCT	GCACTACCAGAGCTAACTCAGATAGTACT	TCAAGAG	AGTACTATCTGAGTTAGCTCTGGTAGTGC	Non-targeting
KCNQ1 sh#1 (DNA)	CACTCATTCAGACCGCATGGAGGTGCTAT	TCAAGAG	ATAGCACCTCCATGCGGTCTGAATGAGTG	Exon 8-9 boundary
KCNQ1 sh#1 (RNA)	CACUCAUUCAGACCGCAUGGAGGUGCUAU	UCAAGAG	AUAGCACCUCCAUGCGGUCUGAAUGAGUG	
KCNQ1 sh#2 (DNA)	TGACTCCTGGAGAGAAGATGCTCACAGTC	TCAAGAG	GACTGTGAGCATCTTCTCTCCAGGAGTCA	Exon 10
KCNQ1 sh#2 (RNA)	UGACUCCUGGAGAGAAGAUGCUCACAGUC	UCAAGAG	GACUGUGAGCAUCUUCUCUCCAGGAGUCA	
KCNQ1 sh#3 (DNA)	AGTTCTGTGAAACGCTCCAGTGGTTACAC	TCAAGAG	GTGTAACCACTGGAGCGTTTCACAGAACT	Intron 1
KCNQ1 sh#3 (RNA)	AGUUCUGUGAAACGCUCCAGUGGUUACAC	UCAAGAG	GUGUAACCACUGGAGCGUUUCACAGAACU	
KCNQ1 sh#4 (DNA)	ACGGCTATGACAGTTCTGTAAGGAAGAGC	TCAAGAG	GCTCTTCCTTACAGAACTGTCATAGCCGT	Exon 10-11 boundary
KCNQ1 sh#4 (RNA)	ACGGCUAUGACAGUUCUGUAAGGAAGAGC	UCAAGAG	GCUCUUCCUUACAGAACUGUCAUAGCCGU	

*shCT = SEQ ID NO:11

KCNQ1 sh#1 (DNA) = SEQ ID NO:12; *KCNQ1* sh#1 (RNA) = SEQ ID NO:16
KCNQ1 sh#2 (DNA) = SEQ ID NO:13; *KCNQ1* sh#2 (RNA) = SEQ ID NO:17
KCNQ1 sh#3 (DNA) = SEQ ID NO:14; *KCNQ1* sh#3 (RNA) = SEQ ID NO:18
KCNQ1 sh#4 (DNA) = SEQ ID NO:15; *KCNQ1* sh#4 (RNA) = SEQ ID NO:19

TABLE 3B

KCNQ1 shRNA sequences

shRNA	Target sequence (sense)*	Hairpin Loop	Antisense sequence	*KCNQ1* Location
KCNQ1 sh#5 (DNA)	GTTCAAGCTGGACAAAGACAATGGGGTGA	TCAAGAG	TCACCCCATTGTCTTTGTCCAGCTTGAAC	Exon 10
KCNQ1 sh#5 *(RNA)*	GUUCAAGCUGGACAAAGACAAUGGGGUGA	UCAAGAG	UCACCCCAUUGUCUUUGUCCAGCUUGAAC	
KCNQ1 sh#6 (DNA)	GACAGTTCTGTAAGGAAGAGCCCAACACT	TCAAGAG	AGTGTTGGGCTCTTCCTTACAGAACTGTC	Exon 10-11
KCNQ1 sh#6 (RNA)	GACAGUUCUGUAAGGAAGAGCCCAACACU	UCAAGAG	AGUGUUGGGCUCUUCCUUACAGAACUGUC	
KCNQ1 sh#7 (DNA)	AGACCATCGCCTCCTGCTTCTCTGTCTTT	TCAAGAG	AAAGACAGAGAAGCAGGAGGCGATGGTCT	Exon 7
KCNQ1 sh#7 (RNA)	AGACCAUCGCCUCCUGCUUCUCUGUCUUU	UCAAGAG	AAAGACAGAGAAGCAGGAGGCGAUGGUCU	
KCNQ1 sh#8 (DNA)	CCCAAACCCAAGAAGTCTGTGGTGGTAAA	TCAAGAG	TTTACCACCACAGACTTCTTGGGTTTGGG	Exon 9-10
KCNQ1 sh#8 (RNA)	CCCAAACCCAAGAAGUCUGUGGUGGUAAA	UCAAGAG	UUUACCACCACAGACUUCUUGGGUUUGGG	

**KCNQ1* sh#5 (DNA) = SEQ ID NO:36; *KCNQ1* sh#5 (RNA) = SEQ ID NO:40
KCNQ1 sh#6 (DNA) = SEQ ID NO:37; *KCNQ1* sh#6 (RNA) = SEQ ID NO:41
KCNQ1 sh#7 (DNA) = SEQ ID NO:38; *KCNQ1* sh#7 (RNA) = SEQ ID NO:42
KCNQ1 sh#8 (DNA) = SEQ ID NO:39; *KCNQ1* sh#8 (RNA) = SEQ ID NO:43

TABLE 4

qRT-PCR primers

Primer Set	**Amplifies:**	**Forward Primer (5′→3′)**	**Reverse Primer (5′→3′)**	**Location (FW, RV)**
Total	KCNQ1-ALL	GAGCCACACTCTGCTGTC (SEQ ID NO:20)	GGAGAGAAGATGCTCACAGTC (SEQ ID NO:21)	Exon 9, Exon 10
Allele-Specific Endogenous	KCNQ1-WT KCNQ1-variants	GACGGCTATGACAGTTCTGTAAGGAAGAGC (SEQ ID NO:22)	TGTGAGATGTGGGTGATGGGTGTCAGCAGA (SEQ ID NO:23)	Exon 10, Exon 11
Allele-Specific shIMM	KCNQ1-shIMM	GATGGATACGATAGCTCCGTCAGAAAAAGT (SEQ ID NO:24)	TGTGAGATGTGGGTGATGGGTGTCAGCAGA (SEQ ID NO:23)	Exon 10, Exon 11
GAPDH	GAPDH-ALL	ACATCGCTCAGACACCATG (SEQ ID NO:25)	TGTAGTTGAGGTCAATGAAGGG (SEQ ID NO:26)	Exon 2, Exon 3

TABLE 5

Summary of FIGS. 16A and 16B FluoVolt™ optical action potential data

iPSC-CMs	shCT ADP_{90} (ms)	SupRep APD_{90} (ms)	ΔAPD_{90} (ms)	p-value (SupRep v. shCT)	shCT APD_{50} (ms)	SupRep APD_{50} (ms)	ΔAPD_{50} (ms)	p-value (SupRep v. shCT)
Unrelated Control	[Untreated] 332±53 (n=50)	-	-	-	[Untreated] 184±23 (n=50)	-	-	-
KCNQ1-Y171X	585±77 (n=52) p<0.0001****	468±43 (n=63) p<0.0001****	-117	p<0.0001****	230±26 (n=52) p=0.0015**	181±23 (n=63) $p=0.9997^{n.s.}$	-49	p<0.0001****
KCNQ1-V254M	580±56 (n=42) p<0.0001****	469±89 (n=55) p<0.0001****	-111	p<0.0001****	353±112 (n=42) p<0.0001****	224±96 (n=55) p=0.0073**	-129	p<0.0001****
KCNQ1-I567S	452±72 (n=45) p<0.0001****	367±60 (n=45) $p=0.1945^{n.s.}$	-85	p<0.0001****	184±24 (n=45) $p>0.9999^{n.s.}$	149±24 (n=45) p<0.0424*	-35	p<0.0001****
KCNQ1-A344A/spl	553±98 (n=61) p<0.0001****	343±133 (n=63) $p<0.9757^{n.s.}$	-210	p<0.0001****	350±94 (n=61) p<0.0001****	142±47 (n=63) p<0.0033**	-208	p<0.0001****

APD_{90} and APD_{50} values were assessed by one-way ANOVA with post-hoc Dunnett's test to compare each KCNQ1 variant treated with shCT or KCNQ1-SupRep to the untreated, unrelated control (all p-values except those listed in the SupRep v. shCT columns). All four LQT1 iPSC-CMs treated with shCT had significantly longer APD_{90} than the unrelated control, and three of the four had significantly longer APD_{50} as well. APD shortening due to KCNQ1-SupRep compared to treatment with shCT was assessed by unpaired two-tailed student's t-tests at both the APD_{90} and APD_{50} levels separately for each variant. KCNQ1-SupRep resulted in statistically significant attenuation of both APD_{90} and APD_{50} in all four LQT1 iPSC-CMs. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, (n.s.) not significant.

TABLE 6

Summary of FIGS. 17A and 17B FluoVolt™ optical action potential data

iPSC-CMs	shCT ADP_{90} (ms)	SupRep APD_{90} (ms)	ΔAPD_{90} (ms)	p-value (SupRep v. shCT)	shCT APD_{50} (ms)	SupRep APD_{50} (ms)	ΔAPD_{50} (ms)	p-value (SupRep v. shCT)
KCNQ1-V254M	580±56 (n=42) p<0.0001****	469±89 (n=55) p<0.0001****	-111	p<0.0001****	353±112 (n=42) p<0.0001****	224±96 (n=55) p=0.0303*	-129	p<0.0001****
Isogenic Control for V254M	(Untreated) 380±112 (n=58)	-	-	-	(Untreated) 267±60 (n=58)	-	-	-
KCNQ1-A344A/spl	553±98 (n=61) p<0.0001****	343±133 (n=63) p=0.2450$^{n.s.}$	-210	p<0.0001****	350±94 (n=61) p<0.0001***	142±47 (n=63) p<0.0001****	-208	p<0.0001****
Isogenic Control for A344A/spl	(Untreated) 377±105 (n=57)	-	-	-	(Untreated) 231±68 (n=57)	-	-	-

APD_{90} and APD_{50} values for KCNQ1-V254M and KCNQ1-A344A/spl were compared to their respective isogenic controls by one-way ANOVA with post-hoc Tukey's test (all p-values except those listed in the SupRep v. shCT columns). The APD values for the isogenic controls served as a benchmark for the "ideal" rescue of APD for each of the two variants, KCNQ1-V254M and KCNQ1-A344A/spl. Treatment of the LQT1 iPSC-CMs with KCNQ1-SupRep resulted in shortening of the APD for each set of LQT1 iPSC-CMs tested, bringing the APD closer to the respective isogenic control for each variant. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, (n.s.) not significant.

Example 11 – Restoring normal cellular electrophysiology in a transgenic LQT1 rabbit model

Experiments are conducted to evaluate the effect of AAV9-based gene delivery of KCNQ1-SupRep gene therapy to reverse QT/APD-prolongation and arrhythmia susceptibility in an established humanized rabbit model of LQT1 with an LQT1-causing human pathogenic KCNQ1-p.Y315S variant. Animals are treated with AAV9-KCNQ1-SupRep for whole animal arrhythmia phenotyping and molecular/cellular electrophysiological phenotyping in acutely isolated rabbit ventricular CMs, to determine the effects of AAV9-mediated delivery of the KCNQ1-SupRep vector on restoring normal molecular, cellular, whole heart, and whole animal electrophysiological phenotypes and preventing ventricular arrhythmias. Rabbits and humans share similar K+ currents underlying cardiac repolarization (Nerbonne, *J. Physiol.*, 525(2):285-298 (2000)), such that transgenic rabbit models are useful for investigating human arrhythmogenic diseases with impaired repolarization. The transgenic LQT1 and LQT2 rabbit models for use in these studies selectively over-express either loss-of-function, dominant-negative pore-localizing variants of human KCNQ1 (LQT1, KCNQ1-Y315S, loss of IKs) or KCNH2 (LQT2, KCNH2-G628S, loss of IKr) in the heart, respectively. These LQT1 and LQT2 rabbits mimic the human LQTS phenotype with QT-prolongation, spontaneous Torsade-de-Pointes (TdP) ventricular tachycardia, and SCD (**FIGS. 19A-19F**) (Brunner *et al.*, *J. Clin. Invest.*, 118:2246-2259 (2008); and Odening *et al.*, *Heart Rhythm*, 9:823-832 (2012)). The KCNQ1-Y315S and KCNH2-G628S mutations are expressed in the rabbit hearts under control of the rabbit beta-myosin heavy chain (β-MyHC) promoter (**FIG. 19A**) to produce LQT1 and LQT2 phenotypes in the rabbit models, respectively. The rabbits exhibit significant prolongation of QT (**FIGS. 19B and 19C**), a propensity to develop spontaneous torsades de pointes (TdP) following treatment with ostradiol (**FIG. 19D**), and action potential duration (**FIG. 19E**) due to elimination of IKs or IKr currents, respectively (**FIG. 19F**). Detailed methods for generation and phenotypic assessment of the rabbits are described elsewhere (Brunner *et al.*, *supra*). Given the similarity to the human LQTS phenotype, these models have unique advantages for investigating novel LQTS therapies *in vivo* and on the whole heart level.

To focus on treatment of the LQT1 transgenic rabbit model, the QTc/APD-attenuating effects of AAV9-KCNQ1-SupRep are investigated in detail *in vivo*, *ex vivo* (whole-heart), and *in vitro* (rabbit cardiomyocyte) in the LQT1 rabbits. The anti-arrhythmic properties of AAV9-KCNQ1-SupRep are assessed *ex vivo* in Langendorff-perfused LQT1 rabbit hearts in which arrhythmias are facilitated by AV-node ablation and hypokalemia, to evaluate the ability of KCNQ1SupRep gene therapy delivery to reverse the pathogenic LQT1 phenotype in KCNQ1-Y315S transgenic rabbits. Following protocols described elsewhere (Odening *et al.*, *Eur. Heart J.*, 40:842-853 (2019)), all experiments are performed in female (f) and male (m) adult rabbits (aged 4-7 months). For *in vivo* experiments (surgery, surface ECG), rabbits are anesthetized with S-ketamine and xylazine (12.5 mg/kg / 3.5 mg/kg IM, followed by IV infusion). After surgery, analgetic therapy with buprenorphine is maintained for 3 days. Beating heart excision (for action potential recordings and arrhythmia assessments in Langendorff-perfused hearts, and cellular patch clamping) are performed after additional injection of heparin (500 IE IV) and thiopental-sodium (40 mg/kg IV). *In vivo* cardiac phenotyping is performed using surface ECG35 (Odening *et al.* 2019, *supra*) on KCNQ1-Y315S transgenic rabbits after AAV9 delivery of KCNQ1-SupRep or AAV9-sham vectors. Similarly, molecular and cellular electrophysiological characterization of AAV9-KCNQ1-SupRep and AAV9-shCT treated rabbits is performed as described elsewhere (Brunner *et al.*, *supra*; and Odening *et al.* 2019, *supra*).

The transgenic LQT1 rabbit expresses two endogenous wild-type rabbit KCNQ1 alleles and a single transgenic human KCNQ1 mutant (p.Y315S) allele. The human and rabbit KCNQ1 cDNA are 73% homologous overall. shRNAs having 100% homology between rabbit and human KCNQ1 (such that both rabbit and human alleles are suppressed simultaneously in the LQT1 rabbit model) are designed and tested, and virus particles are produced.

Analogous experiments are carried out using one or more KCNH2-SupRep constructs in a LQT2 rabbit model.

AAV9-KCNQ1-SupRep gene transfer in isolated LQT1 CMs: The functionality of the AAV9-KCNQ1-SupRep gene transfer is tested in isolated ventricular CMs from

LQT1 rabbits before the constructs are tested in LQT1 rabbits *in vivo*. In particular, left ventricular CMs are obtained from the hearts of transgenic LQT1 rabbits (n=5) by standard collagenase digestion (Brunner *et al.*, *supra*; and Odening *et al.* 2019, *supra*). CMs are maintained in culture for 48 hours, and half of the cell cultures are incubated with AAV9-KCNQ1-SupRep. Functional consequences on cellular APD and IKs current densities are then analyzed (compared to sham-treated LQT1 CMs) using standard voltage and current mode patch clamping (see below).

AAV9-KCNQ1-SupRep gene transfer in vivo via lateral thoracotomy: For *in vivo* gene transfer, lateral thoracotomy is performed and AAV9-KCNQ1-SupRep or AAV9-shCT constructs are painted on the epicardial surface of both ventricles and both atria. Adult LQT1 rabbits of both sexes (LQT1-KCNQ1-SupRep and LQT1-AAV9-shCT controls, split into groups and used for *in vivo* and *ex vivo* whole heart experiments or cellular electrophysiology) are anesthetized with S-ketamine and xylazine. Rabbits are intubated to guarantee proper ventilation during open chest surgery, and left lateral thoracotomy is performed. After thorough painting of AAV9-KCNQ1-SupRep or AAV9-shCT on the surface of the whole heart, the chest is closed and the rabbit is awakened. After at least 1-2 weeks of post-surgery recovery, experiments are performed to investigate the electrophysiological consequences of the KCNQ1-SupRep gene therapy in LQT1 rabbits.

12-lead ECG recording in vivo: Adult LQT1-KCNQ1-SupRep (female and male) and LQT1-AAV9-shCT sham-controls (female and male) rabbits are subjected to conventional 12-lead surface ECG recordings to determine the effect of KCNQ1-SupRep gene therapy on restoring normal QT duration and diminishing pro-arrhythmic markers. ECG is performed under general anesthesia with S-ketamine and xylazine, as this anesthetic regimen does not impact cardiac repolarization (Odening *et al.*, *Am. J. Physiol. Heart Circ. Physiol.*, 295:H2264-2272 (2008)). KCNQ1 gene-transfer mediated changes in QT, heart rate corrected QT, and Tpeak-Tend (Tp-e) and beat-to-beat variability of QT (short term variability of the QT interval; STVQT) are calculated to assess changes in spatial and temporal heterogeneity of repolarization.

Monophasic Action Potential (MAP) measurements in Langendorff-perfused hearts ex vivo: MAP is performed as described elsewhere (Odening *et al.* 2019, *supra*). Briefly, adult LQT1-KCNQ1-SupRep (female and male) and LQT1-AAV9-shCT sham-control (female and male) rabbits are anesthetized as described above. Following euthanasia with thiopental-sodium (40 mg/kg) IV, hearts are excised rapidly, mounted on a Langendorff-perfusion set-up (IH5, Hugo Sachs Electronic-Harvard Apparatus), retrogradely perfused via the cannulated aorta ascendens with warm (37°C), pre-oxygenated (95% O_2 and 5% CO_2), modified Krebs-Henseleit solution at the constant flow rate of 50 mL/minute. Action potential duration at 90%, 75%, and 30% of repolarization (APD90, 75, 30) is assessed, and AP triangulation (APD90-APD30) and APD restitution (based on APD90 values at 2 and 4Hz stimulation) are calculated for each LV region.

Arrhythmia experiments in Langendorff-perfused hearts ex vivo: The anti-arrhythmic effect of KCNQ1-SupRep gene therapy is assessed *ex vivo* in AV-node-ablated Langendorff perfused LQT1-KCNQ1-SupRep (female and male) and LQT1-AAV9-shCT (female and male) hearts, beating spontaneously with stable ventricular escape rhythm (VER) at a constant rate of around 60-80 beats/minute (Hornyik *et al.*, *Br. J. Pharmacol.*, 177:3744-3759 (2020)). After 10 minutes of baseline (arrhythmia-free) recording, hearts are perfused with 2 mM low K^+ containing KH solution (10 minutes) to provoke arrhythmias. In a second step, 10 μM of IK1-blocker $BaCl_2$ are added to the 2 mM low K^+ containing KH solution and perfused (10 minutes) to reduce repolarization reserve and further increase susceptibility to arrhythmia formation. ECGs are recorded continuously and the duration (%) and incidence (average number of events) of arrhythmias are measured off-line. Arrhythmias are defined as ventricular extra beats (VEB), bigeminy, ventricular tachycardia (VT), and ventricular fibrillation (VF). Arrhythmia rates are very high (in the range of 60-80%) in LQT1 hearts, while even in low K^+ KH combined with $BaCl_2$, no serious ventricular arrhythmias occur in normal wild type hearts (Hornyik *et al.*, *supra*).

Electrophysiological recording in rabbit CMs: Left ventricular CMs are obtained from the hearts of KCNQ1SupRep-treated transgenic LQT1 rabbits and sham control

transgenic LQT1 rabbits by standard collagenase digestion (Brunner et al., *supra*; and Odening *et al.* 2019, *supra*). Whole cell currents (IKs, IKr, Ito, and IK1) and action potentials are recorded using Axopatch 200B patch clamp amplifier (Molecular Devices), digitized at a sampling frequency of 10 kHz with Digidata 1440A interface and acquired with pCLAMP software as described elsewhere (Odening *et al.*, 2019, *supra*).

Data interpretation: For normally distributed values, Student's t test (unpaired) is used to compare the means of 2 groups, and Mann-Whitney and Wilcoxon matched pairs test are used for values not normally distributed. Fisher's exact test is used for categorical variables such as arrhythmia incidences. In I-V-curves, differences are assessed using repeated-measure ANOVA, complemented by Bonferroni post-hoc analyses. Cellular electrophysiology data are evaluated using pClamp 9.0 and Origin 7.0 software, and results are given as mean ± SEM. All other analyses are performed with Prism 5.01 for Windows (Graph-Pad), and their data are presented as mean ± SD, with n indicating the number of experiments/animals, tests being 2-tailed, and $p < 0.05$ considered significant. All experiments in the rabbits are performed and analyzed in a blinded fashion.

Example 12 – Materials and methods for LQT2 SupRep

Cloning of KCNH2-SupRep: WT *KCNH2* cDNA (NM_000238.3) was subcloned into pIRES2-EGFP (Clontech; Mountain View, CA) to generate pIRES2-EGFP-KCNH2-WT. The p.G604S and p.N633S variants in pIRES2-EGFP-KCNH2-WT were produced by GenScript (Piscataway, NJ). DNA Sanger sequencing was used to confirm vector integrity. Five custom-designed *KCNH2* shRNAs (sh#1-5) were ordered from OriGene (Rockville, MD) in the pGFP-C-shLenti backbone along with a non-targeting scrambled shRNA control (shCT). For the final KCNH2-SupRep gene therapy vector, *KCNH2* sh#4 was selected as the lead candidate and is referred to as shKCNH2. A DNA fragment containing ten synonymous variants within the *KCNH2* sh#4 (shKCNQ2) target sequence of the KCNH2-WT cDNA: c.2694C>T, c.2697G>C, c.2700G>A, c.2703G>A, c.2706A>T, c.2709G>C, c.2712G>A, c.2715G>C, c.2718G>C, and c.2721C>G (KCNH2: p.D898D, p.T899T, p.E900E, p.Q901Q, p.P902P, p.G903G, p.E904E, p.V905V, p.S906S, and p.A907A, respectively) was synthesized and cloned into pIRES2-

EGFP-KCNH2-WT to create KCNH2-shIMM (pIRES2-EGFP-KCNH2-shIMM) (GenScript; Piscataway, NJ). KCNH2-shIMM was subcloned into the pGFP-C-shLenti backbone containing shKCNH2 to create the final KCNH2-SupRep.

KCNH2 mammalian expression vectors for patch clamp experiments: Wild-type *KCNH2* cDNA was subcloned into pIRES2-EGFP (Clontech, Mountain View, CA) and AAV-P2A CTnC-EGFP (GenScript; Piscataway, NJ) to produce KCNH2-pIRES2-EGFP and KCNH2-AAV-P2A CTnC-EGFP.

TSA 201 and H9C2 cell culture and transfection for patch clamp experiments: TSA 201 and H9C2 cells were cultured in Dulbecco's Modification of Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 1.0% L-glutamine, and 1.2% penicillin/streptomycin solution in a 5% CO_2 incubator at 37°C. Heterologous expression of *KCNH2* was accomplished by using 5 µl or 3 µl of Lipofectamine (Invitrogen) to transfect 1.0 µg of pIRES2-KCNH2-EGFP along with 1.0 µg KCNE2-pIRES2-dsRed2 or 1.0 µg KCNH2-AAV-P2A CTnC-EGFP in OPTI-MEM media. The transfected cells were incubated for 48 hours before electrophysiological experiments.

Electrophysiological measurements: A standard whole-cell patch clamp technique was used to measure pIRES2-KCNH2-WT-EGFP with KCNE2-pIRES2-dsRed2 and KCNH2-AAV-P2A CTnC-EGFP currents at room temperature (RT) using an Axopatch 200B amplifier, Digidata 1440A, and pclamp version 10.4 software (Axon Instruments, Sunnyvale, CA). The extracellular (bath) solution contained (mmol/L): 150 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 1 Na-Pyruvate, and 15 HEPES. The pH was adjusted to 7.4 with NaOH. The intracellular (pipette) solution contained (mmol/L): 150 KCl, 5 NaCl, 2 $CaCl_2$, 5 EGTA, 5 MgATP, 10 HEPES, pH adjusted to 7.2 with KOH. Microelectrodes were fire polished to a final resistance of 2-3 MΩ after being pulled using a P-97 puller (Sutter Instruments, Novato, CA). Series resistance was compensated by 80-85%. Currents were filtered at 1 kHz and digitized at 5 kHz with an eight-pole Bessel filter. The voltage dependence of activation was determined using voltage-clamp protocols described for **FIGS. 30A and 31A**. Data were analyzed using Clampfit (Axon Instruments, Sunnyvale, CA), Excel (Microsoft, Redmond, WA) and graphed with GraphPad Prism 8.3 (GraphPad Software, San Diego, CA).

LQT2 Patient Selection for iPSC Generation: All patients were evaluated by a single genetic cardiologist and LQTS specialist. Dermal fibroblasts and peripheral blood mononuclear cells (PBMCs) were collected by 4 mm skin punch biopsy and blood sample, respectively. Samples were obtained from 212 patients with LQT2. For this study, two LQT2 patients (13-year-old male and 12-year-old female) with two different LQT2-causative missense variants were selected based on a strong LQT2 phenotype defined as at least one ECG with QTc greater than 500 ms, positive history of LQTS-related symptoms (syncope, seizure, sudden cardiac arrest), and positive family history of LQTS-related symptoms (**TABLE 7**). PBMCs or fibroblasts were reprogrammed into induced pluripotent stem cells (iPSCs) by Sendai virus transduction using the Cytotune 2.0 reprogramming kit. Colonies were picked within 21 days post infection with Yamanaka factors. For each variant line, two representative clones were generated, characterized, and analyzed for quality control as described elsewhere (O'Hare et al., *Circ Genom Precis Med.* 13:466-475 (2020)). Karyotyping for each of the patient-specific iPSC clones was completed by the Mayo Clinic Cytogenetics Laboratory, and all mutant iPSC clones that were tested demonstrated normal karyotypes (**FIG. 20A**). Genomic DNA was isolated from each iPSC clone (**FIG. 20B**) and the variant's presence and integrity of the rest of the KCNH2 sequence was confirmed by Sanger sequencing (**FIG. 20C**). All iPSC clones were confirmed to express Nanog (ThermoFisher PA1-097X) and SSEA-4 (ThermoFisher, MA1-021) pluripotent markers **(FIG. 21).** All iPSCs were cultured in mTeSR-Plus medium (STEMCELL®) supplemented with 1% antibiotic/antimycotic solution on MATRIGEL®-coated (Corning; Corning, NY) 6 cm culture dishes in a 5% CO_2 incubator at 37°C. At 85% confluence, iPSCs were passaged using ReLeSR (STEMCELL®).

iPSC-CM Culture, Differentiation, and Dissociation: iPSCs were differentiated into cardiomyocytes (CMs) after reaching ~85% confluency, using a protocol described elsewhere (Burridge et al., *supra*; and Mummery et al., *supra*). On day 0, differentiation was initiated by changing the culture medium from mTeSR-Plus to RPMI 1640 GlutaMAX plus 25mM HEPES supplemented with B27-minus insulin (RPMI/B27-ins; Thermo) containing 5μM CHIR99021 (MilliporeSigma; St. Louis, MO). After 48 hours

(day 2), the medium was changed to RPMI/B27-ins containing 5 μM IWP-2 (MilliporeSigma). On day 4, the medium was changed back to the RPMI/B27-ins maintenance medium. Spontaneous beating began on days 6-7. From days 10-16, iPSC-CMs were cultured in selection medium containing 500 μg/ml of recombinant human albumin, 217 μg/ml of L-ascorbic acid 2-phosphate, and 5 mM of DL-Lactate in RPMI 1640 medium (without glucose). Post selection, iPSC-CMs were dissociated enzymatically using a STEMdiff cardiomyocyte dissociation kit (STEMCELL®) as described elsewhere (Dotzler et al., *Circulation*. 143:1411-1425 (2021)). After 24 hours, cells were maintained in RPMI/B27-ins medium. For all experiments, cells were used after at least 30 days post differentiation.

Generation of CRISPR-Cas9 Corrected Isogenic Control iPSCs: Genome editing of iPSC cell lines was contracted through Applied Stem Cell (Milpitas, CA). Using CRISPR-Cas9 technology, isogenic "variant corrected" control iPSC cell lines were created for both LQT2 patient cell lines (p.G604S and p.N633S). Briefly, two guide RNAs (gRNAs) for each variant line were designed and validated *in vivo*. Based on specificity score, cutting efficiency, and off-target profile, one candidate gRNA was chosen for genome editing on each patient iPSC line. A single-stranded oligodeoxynucleotide (ssODN) was designed to be used as a repair template, and a silent mutation in the gRNA binding site was introduced into the ssODN to prevent re-cutting. The LQT2 patient iPSC line was transfected with the gRNA construct and ssODN using a Neon system, and transfected iPSCs were subjected to puromycin selection. Single-cell colonies were picked for genotyping, and two clones with variant correction were expanded for further studies.

TSA201 Cell Culture and Transfection for Western Blot and qRT-PCR: TSA201 cells were maintained at 37°C using Dulbecco's Modified Eagle Medium (Corning) supplemented with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin/ streptomycin in a 5% CO_2 incubator. For allele-specific qRT-PCR and western blot experiments, $5x10^5$ cells were plated per well in 6-well plates. After 24 hours, cells were co-transfected in maintenance medium using 10 μL Effectene (Qiagen; Hilden, Germany) with 100 fmol (0.3-0.7 μg) equimolar amounts of each plasmid (pIRES2-EGFP-KCNH2-

WT or -variant, pGFP-C-shLenti-shKCNH2(#1-#5) or -shCT, KCNH2-shIMM, or pGFP-C-shLenti-KCNH2-SupRep).

Western Blotting: TSA201 cells were co-transfected with KCNH2-WT, -shIMM, or -variants and shKCNH2(#1-5), -shCT, or KCNH2-SupRep as described above. After 48 hours, cells were lysed using 1X RIPA buffer with protease and phosphatase inhibitors. Lysates were chilled on ice for 10 minutes and then sonicated for 10 seconds at 50% amplitude, and the cell debris was pelleted at 21,000 rcf for 15 minutes at 4°C. The supernatant was transferred to a new tube and the protein concentration was measured using the Pierce BCA Protein Assay Kit (ThermoFisher) before mixing 1:1 with loading buffer (2X Laemmli buffer with 1:20 β-mercaptoethanol). Proteins (10 µg/lane) were run on a 4-15% TGX gel (Bio-Rad; Hercules, CA) and transferred to a PVDF membrane using a Trans-Blot Turbo Transfer System (Bio-Rad). After blocking for 1 hour in tris-buffered saline (TBS) with 0.1% Tween-20/3% bovine serum albumin, the membrane was incubated at 4°C overnight with primary antibodies against KCNH2 (Alomone) and GAPDH housekeeping control (Santa Cruz, sc-376476) at 1:500 and 1:5000 dilutions, respectively, in blocking solution. The membrane was then washed in TBS-T for 3 x 15 minutes and incubated in secondary antibody HRP-conjugated goat-anti-rabbit (Invitrogen) at a dilution of 1:5000 in blocking solution. After 1 hour, the membrane was washed in TBS-T for 3 x 15 minutes. Finally, the membrane was incubated in SuperSignal™ West Pico PLUS chemiluminescent ECL substrate (ThermoFisher) and exposed to HyBlot CL autoradiography film (Denville Scientific Inc., E3012). Pixel density was quantified using freely available ImageJ software.

Allele-Specific qRT-PCR: Allele-specific primers were designed for qRT-PCR to specifically amplify total *KCNH2*, endogenous *KCNH2* including KCNH2-WT and -variants, but excluding KCNH2-shIMM, and KCNH2-shIMM, by adapting allele-specific genotyping methods described elsewhere (Rohatgi et al., *J Am Coll Cardiol.* 2017, 70:453-462; and Priori et al., *Heart Rhythm.* 2013, 10:1932-1963). For total *KCNH2*, primers were purchased from IDT (Coralville, IA). For allele-specific primers, two reverse primers spanning the shKCNH2 target site with one complementary to endogenous *KCNH2* (allele-specific for KCNH2-WT and -variants) and the other

complementary to KCNH2-shIMM (allele-specific for KCNH2-shIMM) were used. A common forward primer was used for both allele-specific forward primers. *GAPDH* primers (IDT) were used as housekeeping controls. A standard curve was used to correct for PCR amplification bias. TSA201 cells were co-transfected as described above. After 48 hours, RNA was harvested using the RNeasy kit (Qiagen) and measured using the NanoDrop ND-1000 spectrophotometer (Thermo). Complementary DNA (cDNA) was generated by loading 500 ng RNA in the SuperScript IV VILO Master Mix reverse transcription kit (Thermo). Four qRT-PCR reactions were run per sample using the SYBR Green Master Mix kit (Qiagen) with the four sets of primers described above. Data were analyzed using the ΔΔCT method by first normalizing *KCNH2* to *GAPDH* and then comparing the relative fold change to the KCNH2-WT and shCT treatment groups.

Lentivirus Generation and Transduction of iPSC-CMs: Lentivirus was used for application of KCNH2-SupRep or shCT (treatment control) to iPSC-CMs. Lentiviral particles were generated from pGFP-C-shLenti-shKCNH2-shIMM (KCNH2-SupRep) and pGFP-C-shLenti-shCT (shCT), using the pPACKH1 HIV Lentivector Packaging kit (SBI System Biosciences; Palo Alto, CA). After more than 30 days post-induction of differentiation, iPSC-CMs derived from two patients with LQT2 and their respective isogenic controls were dissociated and plated into MATRIGEL®-coated 35 mm dishes with glass-bottom insets for FluoVolt (MatTek; Ashland, MA) as described above. After 48 hours of recovery, iPSC-CMs were transduced with lentiviral particles containing KCNH2-SupRep or shCT. Polybrene (8μg/mL) infection reagent (MilliporeSigma) was added to increase transduction efficiency and the iPSC-CMs were centrifuged at 250 rcf for 1.5 hours at room temperature in the 35 mm dishes. After 24 hours post-transduction, the medium was exchanged for fresh maintenance medium, RPMI/B27-ins.

Voltage Dye Optical Action Potentials in iPSC-CMs: Voltage dye experiments were conducted between 3-7 days post-transduction of iPSC-CMs with lentiviral particles containing either KCNH2-SupRep or shCT. On the day of imaging, iPSC-CMs were washed with pre-warmed (37°C) HEPES-buffered Tyrode's solution (Alfa Aesar; Haverhill, MA). Each 35 mm glass-bottom dish was incubated at 37°C for 20 minutes with 0.125 μL FluoVolt dye, 1.25 μL PowerLoad, and 0.5 mL Tyrode's solution

(FluoVolt Membrane Potential kit, Thermo). Excess dye was rinsed thrice with Tyrode's solution, and a final 2 mL of Tyrode's solution was added to the iPSC-CMs for imaging. During imaging, the dishes were kept in a heated 37°C stage-top chamber (Live Cell Instrument; Seoul, South Korea) with 5% CO_2. Under 40X-water objective magnification using a Nikon Eclipse Ti light microscope (Nikon; Tokyo, Japan), optical action potentials were recorded in 20 second fast time-lapse videos at a rate of 50 frames/second (fps, 20 ms exposure time) with LED illumination at 5% power. iPSC-CMs were paced at 1Hz (9 ms pulse duration, 25V) using a MyoPacer field stimulator (Ion Optix; Westwood, MA) to eliminate beat-rate dependent effects on the APD. Rectangular regions of interest were drawn over flashing areas of cells for analysis. NIS-Elements software (Nikon) was used to measure the fluorescence intensity over time within each region of interest, resulting in optical action potential traces. The traces were corrected for photobleaching and the amplitude was normalized as change in fluorescence divided by the baseline minimum fluorescence ($\Delta F/F_{min}$) using a custom Excel program. In a semi-automated manner, common action potential parameters including APD_{90}, APD_{50}, amplitude, rise time, upstroke velocity, etc. were detected for each individual optical action potential and averaged across all beats within a 20 second trace. The average of all beats within a 20 second trace represented a single data point. For representative traces, the maximum amplitude was further normalized to 1.0 to allow for accurate visualization of APD differences.

Statistics: All statistical analysis was done using GraphPad Prism 9. Individual data points are shown where applicable along with the mean. Differences between group means of normally distributed parameters were assessed using a one-way analysis of variance (ANOVA) for comparisons among $\geq$3 groups. For multiple post-hoc ANOVA analyses, Tukey's test was used. A value of $P \leq 0.05$ was considered statistically significant. For patch clamp experiments, data points are shown as the mean value and bars represent the standard error of the mean. GraphPad Prism 8.3 (GraphPad Software, San Diego, CA) was used for t-test. A Student's t-test was performed to determine statistical significance between two groups. A paired t-test was performed to determine statistical significance before and after E-4031. $P<0.05$ was considered to be significant.

TABLE 7

Summary of subjects selected for generation of iPSCs for iPSC-CM studies

Subject	Sex	Age at Sample Collection	KCNH2 Variant(s)	Average QTc (ms) [Range]	LQTS-Related Symptoms	Family History	Treatment	iPSC Source; Generation Method
LQT2 #1	Male	13.1	G604S (c.1810G>A)	517 [439-581]	Aborted cardiac arrest, cardiogenic syncope	-	LCSD, Mexiletine, Nadolol	PBMC; Episomal DNA
LQT2 #2	Female	12.6	N633S (c.1898A>G)	545 [525-600]	Recurrent syncope, Torsade de Pointes	Father passed away from SCD	Transvenous ICD, LCSD, Nadolol	Fibroblasts; Sendai

KCNH2 variants are listed as the resulting change on the protein level with cDNA change in parenthesis.

QTc, Bazett-corrected QT interval; ICD, implantable cardioverter defibrillator; LCSD, left cardiac sympathic denervation; PBMC, peripheral blood mononuclear cells; SCD, sudden cardiac death.

Example 13 – shRNA knockdown of *KCNH2*

Seventeen (17) unique shRNAs targeting *KCNH2* were tested, and one candidate shRNA (designated Rab_sh4) was identified that suppressed the endogenous *KCNH2* alleles (both mutant and wild-type) in TSA201 cells with about 80% knockdown efficiency (**FIG. 22**). The shRNA (5′-CACGGAGCAGCCAGGGGAGGTGTCGGCCT-3′; SEQ ID NO:27) (RNA sequence 5′-CACGGAGCAGCCAGGGGAGGUGUCGG CCU-3′; SEQ ID NO:28) was completely homologous with the rabbit sequence and the human sequence. The shRNA was designed in a lentivirus backbone (pGFP-C-shLenti) and in an AAV9 backbone (pGFP-A-shAAV). Once this shRNA was identified, a SupRep construct containing the shRNA (SEQ ID NO:28) and an "shRNA-immune" (5′-TACCGAACAACCTGGCGAAGTCTCCGCGT-3′; SEQ ID NO:29) version of the *KCNH2* cDNA was generated (the shRNA for knocking down the endogenous *KCNH2* alleles, and the shRNA-immune for simultaneously providing a replacement wild-type *KCNH2* allele). As with *KCNQ1*, the shIMM sequence had alterations at the wobble base of each codon within the shRNA target sequence, which prevented knockdown by the shRNA, but did not change the encoded amino acid sequence. The SupRep construct was designed in both a lentivirus backbone (pGFP-C-shLenti) and an AAV9 backbone (pGFP-A-shAAV), with five SupRep constructs generated in the lentivirus backbone and five in the AAV9 backbone. These constructs differed in the reporter sequences (P2A, Fusion-GFP, IRES, HA-Tag, and No reporter) that they contained. The 10 total constructs were as follows:

shLenti-SupRep-P2A
shLenti-SupRep-Fusion-GFP
shLenti-SupRep-IRES
shLenti-SupRep-HA Tag
shLenti-SupRep-No Reporter
shAAV-SupRep-P2A
shAAV-SupRep-Fusion-GFP
shAAV-SupRep-IRES
shAAV-SupRep-HA Tag

shAAV-SupRep-No Reporter

The SupRep constructs contained a CMV promoter and a human growth hormone (HGH) polyadenylation signal, but can be modified to include other promoters/enhancers. For example, the CMV promoter can be replaced with a cTnC promoter, which is smaller than the CMV promoter and more cardiac-specific. Additionally, the HGH polyadenylation signal can be replaced with a smaller SV40 terminator sequence. These modifications reduce the size of the SupRep construct and allow it to be packaged into AAV9 with greater efficiency.

Example 14 – SupRep correction of KCNH2 *in vitro*

CRISPR-Cas9 corrected isogenic controls were used as a marker for "ideal" correction of the cardiac APD. FluoVolt™ voltage dye was used to measure the cardiac APD in N633S iPSC-CMs and isogenic control iPSC-CMs generated from LQT2 iPSCs (N633S). APD_{90B} and APD_{50B} values for isogenic control treated with shCT and KCNH2-N633S variant treated with shCT or KCNH2-SupRep are plotted in **FIG. 23**. The isogenic control iPSC-CMs had significantly shorter APD_{90B} and APD_{50B} than the LQT2 iPSC-CMs treated with shCT, indicating that correction of the single pathogenic LQT2 variant in KCNH2 was able to rescue the disease phenotype *in vitro*. Treatment of LQT2 iPSC-CMs with KCNH2-SupRep resulted in APD_{90B} shortening that was not significantly different from the APD_{90B} of the isogenic control treated with shCT. For KCNH2-N633S, KCNH2-SupRep achieved "ideal" correction of the prolonged APD_{90B} and overcorrected the APD_{50B}.

In further studies, CRISPR-Cas9 corrected isogenic controls again served as a marker for correction of cardiac APD. Results from FluoVolt™ voltage dye measurement of cardiac APD in N633S iPSC-CMs and isogenic control iPSC-CMs generated from LQT2 iPSCs (N633S) are plotted in **FIG. 24**. APD_{90B} and APD_{50B} values for the untreated (UT) KCNH2-N633S variant, the SupRep treated isogenic control, and the untreated (UT) isogenic control are plotted. The treated and untreated isogenic control iPSC-CMs had significantly shorter APD_{90B} and APD_{50B} than the untreated LQT2 iPSC-CMs, again indicating that correction of the single pathogenic LQT2 variant in KCNH2

was able to rescue the disease phenotype *in vitro*. Treatment of the isogenic control iPSC-CMs with KCNH2-SupRep resulted in overcorrection in APD_{90B} and APD_{50B} shortening, compared to the untreated isogenic control.

Results from FluoVolt™ voltage dye measurement of cardiac APD in G604S iPSC-CMs are plotted in **FIG. 25**. APD_{90} and APD_{50} values for KCNH2-G604S variant treated with shCT or SupRep are plotted. Treatment of LQT2 iPSC-CMs with SupRep resulted in significant APD_{90} and APD_{50} shortening compared to those treated with shCT.

In additional studies, CRISPR-Cas9 corrected isogenic controls served as a marker for "ideal" correction of the cardiac APD. FluoVolt voltage dye measurement of the cardiac APD in G604S iPSC-CMs and isogenic control iPSC-CMs generated from LQT2 iPSCs (G604S) are shown in **FIG. 26**. APD_{90} and APD_{50} values for isogenic controls treated with shCT (3) and KCNH2-G604S variant treated with shCT (1) or KCNH2-SupRep (2) are shown. The isogenic control iPSC-CMs had significantly shorter APD_{90} and APD_{50} than the LQT2 iPSC-CMs treated with shCT, indicating that correction of the single pathogenic LQT2 variant in KCNH2 was able to rescue the disease phenotype *in vitro*. Treatment of LQT2 iPSC-CMs with KCNH2-SupRep resulted in APD_{90} shortening. For KCNH2-G604S, KCNH2-SupRep overcorrected the prolonged APD_{90} and APD_{50} as compared to isogenic control treated with shCT.

CRISPR-Cas9 also was used to insert KCNH2-G628S into wild type cells that served as isogenic controls that provided a marker for "ideal" cardiac APD. FluoVolt voltage dye measurement of the cardiac APD in G628S iPSC-CMs and isogenic control iPSC-CMs are shown in **FIG. 27**. APD_{90} values for isogenic controls treated with shCT (3) and KCNH2-G628S variant treated with shCT (1) or KCNH2-SupRep (2) are shown. The isogenic control iPSC-CMs had significantly shorter APD_{90} than the LQT2 iPSC-CMs treated with shCT, indicating that insertion of a single pathogenic LQT2 variant in KCNH2 was able to show the disease phenotype *in vitro*. Treatment of LQT2 iPSC-CMs with KCNH2-SupRep resulted in APD_{90} shortening. For KCNH2-G628S, KCNH2-SupRep overcorrected the prolonged APD_{90} as compared to isogenic control treated with shCT.

Example 15 - KCNH2-SupRep gene therapy both suppresses and replaces KCNH2-WT

To test whether KCNH2-shIMM is indeed immune to KD by shKCNH2 (sh#4), TSA201 cells were co-transfected with KCNH2-WT or KCNH2-shIMM and shKCNH2. The expression of KCNH2-WT versus KCNH2-shIMM was quantified using allele-specific qRT-PCR. Each sample was run in four separate reactions, using a unique set of allele-specific primers, to quantify (1) total KCNH2, (2) endogenous KCNH2, which included WT and variant-containing alleles, but excluded KCNH2-shIMM, (3) KCNH2-shIMM, and (4) GAPDH as a housekeeping control. Commercial primers were used to amplify total KCNH2. For exclusive amplification of endogenous KCNH2 or KCNH2-shIMM, two reverse primers were designed within the shKCNH2 target site, one complementary to the WT sequence and the other complementary to the unique, modified sequence engineered to create KCNH2-shIMM. A common forward primer was used for both reactions, and a standard curve was used to correct for PCR amplification bias. Results showed that shKCNH2 knocked down KCNH2-WT but not KCNH2-shIMM in TSA201 cells co-transfected with KCNH2-WT or KCNH2-shIMM and shCT, shKCNH2, or KCNH2-SupRep (**FIG. 28A)**. Relative *KCNH2* expression normalized to GAPDH was measured by allele-specific qRT-PCR quantifying KCNH2-WT (white) and KCNH2-shIMM (grey). Results were confirmed by western blotting for KCNH2 with GAPDH as a housekeeping control (**FIG. 28B**).

Example 16 - Validation of variant-independent suppression and replacement using KCNH2-SupRep

To test whether the KCNH2-SupRep gene therapy knocked down and replaced KCNH2 in a variant independent manner, TSA201 cells were co-transfected with KCNH2-WT or KCNH2-variants and shCT, shKCNH2, or KCNH2-SupRep. Results showed that KCNH2-SupRep knocked down LQT2 disease-causing *KCNH2* missense variants and replaced them with KCNH2-shIMM. shKCNH2 knocked down *KCNH2* in a variant-independent manner. KCNH2-SupRep knocked down *KCNH2* variants via shKCNH2 and expressed KCNH2-shIMM which was knockdown immune. The graph in

FIG. 29A shows proportional expression of KCNH2-WT/variants and KCNH2-shIMM detected using allele-specific qRT-PCR to measure KCNH2-WT/variant (white) and KCNH2-shIMM (grey). **FIG. 29B** shows overall KCNH2 expression (not allele-specific) validated by western blotting with GAPDH as a housekeeping control.

Example 17 - KCNH2-AAV-P2A CTnC-EGFP generated E-4031 sensitive outward current in H9C2 cells

To determine whether the cardiac specific KCNH2-AAV-P2A CTnC-EGFP was only expressed in cardiomyocytes and not in non-cardiomyocytes, heterologous expression and patch-clamp studies were performed in TSA201 cells for both KCNH2-pIRES2-EGFP with KCNE2-pIRES2-dsRed2 and KCNH2-AAV-P2A CTnC-EGFP. Co-expression of KCNH2-pIRES2-EGFP along with KCNE2-pIRES2-dsRed2 revealed robust I_{kr} current. However, expression of KCNH2-AAV-P2A CTnC-EGFP only exhibited endogenous outward current from TSA201 cells, not typical KCNH2 current (**FIGS. 30A and 30B**) indicating that KCNH2-AAV-P2A CTnC-EGFP was not expressed in TSA201 cells. Peak current density was significantly smaller lower the voltage range from -10 mV to +20 mV for KCNH2-AAV-P2A CTnC-EGFP expression (**FIG. 30C**). At +10 mV, the peak current density was 41.4 ± 3.4 pA/pF (KCNH2-pIRES2-EGFP, n=9) and 23.2 ± 3.0 pA/pF (KCNH2-AAV-P2A CTnC-EGFP**,** n=8, $p < 0.05$ vs. KCNH2-pIRES2-EGFP) (**FIG. 30D**).

To determine whether cardiac specific KCNH2-AAV-P2A CTnC-EGFP was expressed in cardiomyocytes and could generate KCNH2 current, heterologous expression and patch-clamp studies were performed in H9C2 cells, which are rat neonatal cardiomyocytes. Empty H9C2 cells only exhibited a small outward current (**FIG. 31A**, upper panel), whereas with KCNH2-AAV-P2A CTnC-EGFP expression, robust outward current was revealed (**FIG. 31A**, middle panel). This outward current was inhibited by a specific KCNH2 channel blocker (500 nM E-4031) (**FIG. 31A**, lower panel). The peak current density was significantly increased across the voltage range from -20 mV to +60 mV for KCNH2-AAV-P2A CTnC-EGFP expression ($P<0.05$ vs. empty H9C2) (**FIG. 31B**). At +60 mV, the peak current density was 17.3 ± 3.8 pA/pF (empty H9C2, n=9) and

29.8 ± 3.6 pA/pF (KCNH2-AAV-P2A CTnC-EGFP, n=9, $p < 0.05$ vs. empty H9C2) (**FIG. 31C**). 500 nM E-4031 significantly inhibited KCNH2-AAV-P2A CTnC-EGFP expressed outward current across the voltage range from +10 mV to +60 mV ($P<0.05$ vs. before E-4031) (**FIG. 31D**). At +60 mV, peak current density was 29.8 ± 5.4 pA/pF (before E-4031, n=6) and 19.1 ± 3.2 pA/pF (after E-4031, n=6, $p < 0.05$ vs. before E-4031) (**FIG. 31E**).

Example 18 - KCNH2-SupRep Prolongs the Pathologically Shortened Cardiac APD in SQT1 iPSC-CMs as Measured by FluoVolt Voltage Dye

To show that KCNH2-SupRep can rescue both LQT2 and type 1 short QT (SQT1) disease phenotypes, CRISPR-Cas9 was used to insert KCNH2-N588K, a known SQT1 variant, into wildtype cells which serve as the isogenic control (**FIG. 32**). Isogenic controls served as markers for "ideal" cardiac APD. FluoVolt voltage dye measurement of the cardiac APD in N588K iPSC-CMs and isogenic control iPSC-CMs are plotted in **FIG. 32**. APD_{90} and APD_{50} values for isogenic control treated with shCT and KCNH2-N588K variant treated with shCT (1) or KCNH2-SupRep (2) are shown. The isogenic control iPSC-CMs (3) had significantly longer APD_{90} and APD_{50} than the SQT1 iPSC-CMs treated with shCT, which indicated that insertion of a single pathogenic type 1 short QT (SQT1) variant in KCNH2 was able to show the disease phenotype *in vitro*. Treatment of SQT1 iPSC-CMs with KCNH2-SupRep resulted in APD_{90} prolongation. For KCNH2-N588K, KCNH2-SupRep corrected the shortened APD_{90} and APD_{50} as compared to isogenic control treated with shCT.

Example 19 – Materials and Methods for LQT3 SupRep

LQT3 Patient Selection for iPSC Generation: Patients were evaluated by a genetic cardiologist and LQTS specialist. Dermal fibroblasts and PBMCs were collected by 4 mm skin punch biopsy and blood sample, respectively. Samples were obtained from nearly 1200 patients diagnosed with a variety of inherited cardiac channelopathies and their affected or unaffected family members, including 80 patients with LQT3. For

generation of iPSCs, four LQT3 patients bearing mutations resulting in the following changes on the protein level were selected: P1332L, R1623Q, and F1760C (**TABLE 8**).

Fibroblast/PBMCs Reprogramming into iPSCs and Quality Control: Fibroblasts or PBMCs were reprogrammed by Sendai virus transduction using the CytoTune-iPS 2.0 reprogramming kit (Thermo; Waltham, MA) or electroporation with four episomal DNA plasmids containing the Yamanaka factors: pCXLE-hUL, pCXLE-hSK, pCXLE-hOCT3/4-shp53-F, and pCXWB-EBNA1 (Addgene; Watertown, MA). At least two colonies were picked within 21 days post-induction and clonally expanded. All iPSCs were cultured in mTeSR™1 (STEMCELL®) supplemented with 1% penicillin/streptomycin on MATRIGEL®-coated (Corning) 6 cm culture dishes in a 5% CO_2 incubator at 37°C. At 85% confluence, iPSCs were passaged using ReLeSR (STEMCELL®). Each clone was then karyotyped.

All lines had a normal karyotype. *SCN5A* variant confirmation was conducted by Sanger sequencing of PCR-amplicons from genomic DNA. Expression of pluripotent markers in all iPSC clones was confirmed by confocal immunofluorescence microscopy using primary antibodies against Oct4 (Thermo, PA5-27438), Nanog (Thermo, PA1-097), Tra-1-60 (Santa Cruz; Dallas, TX; sc-21705), and SSEA-4 (Thermo, MA1-021) at a 1:250 dilution. Secondary antibodies were ALEXA FLUOR® 488 goat-anti-mouse (Thermo, A-11001) and ALEXA FLUOR® 594 goat-anti-rabbit (Thermo, A-11037). Counterstaining with DAPI (Thermo) was used at a 1:2000 dilution from a 5mg/mL stock. Images were acquired on a Zeiss LSM 980 confocal microscope.

Quality control for iPSCs: Standard quality control assays were performed on SCN5A-F1760C iPSC line, including Sanger sequencing of the LQT3-causative variant, karyotyping, bright field morphology, and immunofluorescence microscopy for pluripotent markers including Tra-1-60, Nanog, SSEA-4, and Oct4 (**FIG. 33A-33D**). Differentiation of iPSCs was induced by methods described elsewhere to generate spontaneously beating iPSC-CMs (Burridge et al., *supra*; and Mummery et al., *supra*). Since the cardiac APD is known to shorten as iPSC-CMs mature over time, all experiments were conducted at least 30 days after the induction of differentiation (Shaheen et al., *supra*).

iPSC-CM Culture, Differentiation, and Dissociation: When iPSCs were 85% confluent, differentiation into cardiomyocytes (CMs) was induced as described elsewhere (Schwartz 2009, *supra*; and Schwartz 2013, *supra*). Differentiation was initiated (day 0) by changing the culture medium to RPMI 1640 GlutaMAX™ plus 25 mM HEPES ((4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)) supplemented with B27-minus insulin (RPMI/B27-ins) (Thermo) containing 5 μM CHIR99021 (MilliporeSigma; St. Louis, MO). On day 2, the medium was changed to RPMI/B27-ins containing 5 μM IWP-2 (MilliporeSigma). On day 4, the medium was changed back to the maintenance medium RPMI/B27-ins. Spontaneous beating typically began on days 6-7, with expansion to remaining cells by days 10-12. The iPSC-CMs were allowed to mature until at least day 30, changing the media twice per week. After day 30, iPSC-CMs were dissociated enzymatically using STEMdiff™ cardiomyocyte dissociation kit (STEMCELL®). Briefly, cells were rinsed with PBS (without Ca^{2+}/Mg^{2+}) and placed in dissociation medium for 10 minutes at 37°C, and then deactivated by addition of STEMdiff™ Cardiomyocyte Support Medium (STEMCELL®). Cells were triturated, transferred to a 15 mL conical tube, and pelleted by centrifugation at 300 rcf for 3 minutes. The supernatant was aspirated and the cells suspended in Cardiomyocyte Support Medium before transfer to appropriate MATRIGEL®-coated culture ware. After 24 hours, the medium was changed back to RPMI/B27-ins. Dissociation resulted in a mixture of single cells and small-to-medium sized iPSC-CM clusters, depending on cell density before and after plating. Spontaneous beating generally returned 24 hours after dissociation, with strong electrical coupling and syncytia formation between days 3-7.

CRISPR-Cas9 Corrected Isogenic Control iPSC: Isogenic "variant corrected" control iPSC cell lines were commercially created for the three patient-specific LQT3 cells lines harboring either SCN5A-R1623Q, SCN5A-P1332L, or SCN5A-F1760C mutation. These isogenic controls serve as the gold standard for a possible therapeutic cure, thereby providing a marker for the "ideal" rescue/normalization of the prolonged APD and indicating how close to this ideal did treatment with SCN5A-SupRep gene therapy reach.

Lentivirus Generation and Transduction of iPSC-CMs: Lentivirus was used for application of SCN5A-SupRep to iPSC-CMs (or shCT as a treatment control). Lentiviral particles were generated from shLenti-shSCN5A-shIMM-P2A-GFP (SCN5A-GFP-SupRep) and shLenti-shSCN5A-shIMM-HA (SCN5A-HA-SupRep), using the pPACKH1 HIV Lentivector Packaging kit (SBI System Biosciences; Palo Alto, CA). After reaching at least day 30 post-induction of differentiation, iPSC-CMs patient with LQT3 were dissociated and plated into MATRIGEL®-coated 35mm dishes with glass-bottom insets for FLUOVOLT™ (MatTek) or 10-well culture reaction slides for immunofluorescence (Marienfeld SUPERIOR™) as described above. After 24-48 hours of recovery, iPSC-CMs were left untreated or were transduced with lentiviral particles containing SCN5A-SupRep. To increase transduction efficiency, Polybrene infection reagent (MilliporeSigma) was added during transduction to a final concentration of 8 μg/mL and the iPSC-CMs were centrifuged at 250 rcf for 1.5 hours at room temperature in the 35 mm dishes. At 24 hours post-transduction, the medium was exchanged for fresh maintenance medium, RPMI/B27-ins.

Voltage Dye Optical Action Potentials in iPSC-CMs: Voltage dye experiments were conducted between 3-7 days post-transduction of iPSC-CMs with lentiviral particles containing SCN5A-SupRep. On the day of imaging, iPSC-CMs were rinsed with pre-warmed (37°C) HEPES-buffered Tyrode's solution (Alfa Aesar). Using the FLUOVOLT™ Membrane Potential kit (Thermo), 0.125 μL FLUOVOLT™ dye and 1.25 μL PowerLoad were added to 0.5 mL Tyrode's solution for each 35 mm glass-bottom dish and incubated at 37°C for 20 minutes. Excess dye was removed in three rinses with pre-warmed Tyrode's solution, and a final 2 mL Tyrode's solution was added to the iPSC-CMs for imaging. During imaging, the dishes were kept in a heated 37°C stage-top chamber (Live Cell Instrument) with 5% CO_2. Using a Nikon Eclipse Ti light microscope (Nikon) under 40X-water objective magnification, optical action potentials were recorded in 20 second fast time-lapse videos at a rate of 50 frames/second (fps, 20 ms exposure time) with LED illumination at 5% power. iPSC-CMs were paced at 1 Hz (9 ms pulse duration, 25V) using a MyoPacer field stimulator (Ion Optix; Westwood, MA) to eliminate beat-rate dependent effects on the APD. Videos were focused on electrically-

coupled syncytial areas of iPSC-CMs (clusters and monolayers) since these areas of cells best follow the pacing stimulus and produce the greatest signal-to-noise represented by large changes in fluorescence intensity (often ~8-12%). For analysis, rectangular regions of interest were drawn over flashing areas of cells, and NIS-Elements software (Nikon) was used to quantify the fluorescence intensity over time within each region of interest, resulting in optical action potential traces. Using a custom Excel-based program, traces were corrected for photobleaching and the amplitude was normalized as change in fluorescence divided by the baseline minimum florescence ($\Delta F/F_{min}$). In a semi-automated manner, common action potential parameters including APD_{90}, APD_{50}, amplitude, rise time, upstroke velocity, etc. were detected for each individual optical action potential and averaged across all beats within a 20 second trace. The average of all beats within a 20 second trace represented a single data point. For representative traces, the maximum amplitude was further normalized to 1.0 to allow for accurate visualization of APD differences.

Statistics: GraphPad Prism 9 was used for all statistical analysis and to fit all data for figures. Individual data points are shown wherever practical along with the mean. Error bars represent standard error of the mean (SEM). An unpaired two-tailed student's t-test was performed to determine statistical significance between two groups when indicated. $p<0.05$ was considered to be significant.

TABLE 8

Summary of subjects selected for generation of iPSCs for iPSC-CM studies

Subject	Sex	Age at Sample Collection	SCN5A Variant(s)	Average QTc (ms)	LQTS-Related Symptoms	Family History	Treatment	iPSC S Gener Metl
LQT3 #1	Female	4.5	P1332L (c.3995C>T)	583	Cardiac arrest at age 25 months	-	Nadolol and mexiletine	PBM Episc DN
LQT3 #2	Male	3.1	R1623Q (c.4868G>A)	480	Recurrent episodes of spontaneously terminating and ICD-terminating VF	Symptomatic LQTS in twin brother	Propranolol, mexiletine, ICD implant, RCSD, LCSD, Heart transplant	Fibroblasts; Sendai
LQT3 #3	Female	2 months	F1760C (c.5279T>C)	680	Several long episodes of torsade de pointes, 3:1 AV block SCD at age 5 months	-	Mexiletine, LCSD, ICD,	PBMC; Episomal DNA

SCN5A variants are listed as the resulting change on the protein level with cDNA change in parenthesis.

QTc, Bazett-corrected QT interval; ICD, implantable cardioverter defibrillator; PBMC, peripheral blood mononuclear cells; LCSD, left cardiac sympathic denervation; LCSD, right cardiac sympathic denervation; SCD, sudden cardiac death.

Example 20 – shRNA knockdown of *SCN5A*

To make SCN5A-SupRep, six candidate *SCN5A* shRNAs (sh#1-6) in the pGFP-C-shLenti lentiviral backbone were tested. The KD efficiency of each *SCN5A* shRNA was determined by co-transfecting TSA201 cells with SCN5A-WT and sh#1-6. Expression of *SCN5A* was measured by quantitative reverse transcription PCR (qRT-PCR, **FIG. 34**). Of the six shRNAs tested, sh#1, sh#3, sh#4 and sh#5 all resulted in significant KD of *SCN5A* (mRNA: 78-91% KD). Thus, any of these shRNAs could have been used as part of the final SCN5A-SupRep gene therapy vector. By raw KD, however, *SCN5A* sh#1 (5′-GGTTCACTCGCTCTTCAACATGCTCATCA-3′; SEQ ID NO:30) (RNA sequence 5′-GGUUCACUCGCUCUUCAACAUGCUCAUCA-3′; SEQ ID NO:31) provided the strongest KD of *SCN5A*, suppressing the endogenous SCN5A alleles (both mutant and wild-type) in TSA201 cells with about 91% knockdown efficiency (**FIG. 34**). Further, at the time of selection, the *SCN5A* sh#1 target sequence was assessed using the Genome Aggregation Database (gnomAD) and ClinVar, and was found to be devoid of both common genetic polymorphisms and all known pathogenic LQT3-causative mutations that may interfere with KD efficiency. *SCN5A* sh#1 therefore was selected for the final SCN5A-SupRep and is referred to as "shSCN5A."

The shRNA was designed in a lentivirus backbone (pGFP-C-shLenti). Once this shRNA was identified, a SupRep construct containing the shRNA (SEQ ID NO:31) and an "shRNA-immune" (5′- CGTACATTCCCTGTTTAATATGCTGATTA-3′; SEQ ID NO:32) version of the *SCN5A* cDNA was generated (the shRNA for knocking down the endogenous *SCN5A* alleles, and the shRNA-immune for simultaneously providing a replacement wild-type *SCN5A* allele). As with *KCNQ1*, the shIMM sequence had alterations at the wobble base of each codon within the shRNA target sequence, which prevented knockdown by the shRNA, but did not change the encoded amino acid sequence. The SupRep construct was designed in a lentivirus backbone (pGFP-C-shLenti), with three SupRep constructs generated in the lentivirus backbone. These constructs differed in the reporter sequences (P2A, HA-Tag, and No reporter) that they contained. The 3 total constructs were as follows:

shLenti-SupRep-P2A

shLenti-SupRep-HA Tag

shLenti-SupRep-No Reporter

The final SCN5A-SupRep gene therapy vector used in this *in vitro* study is illustrated in **FIG. 35**. The SupRep constructs contained a CMV promoter and a human growth hormone (HGH) polyadenylation signal, but can be modified to include other promoters/enhancers. For example, the CMV promoter can be replaced with a cTnC promoter, which is smaller than the CMV promoter and more cardiac-specific. Additionally, the HGH polyadenylation signal can be replaced with a smaller SV40 terminator sequence. These modifications reduce the size of the SupRep construct and allow it to be packaged into AAV9 with greater efficiency.

Example 21 - SCN5A-SupRep Gene Therapy Shortens the Cardiac APD in LQT3 iPSC-CMs as Measured by FLUOVOLT™ Voltage Dye

Action potential analyses were conducted to test whether treatment with SCN5A-SupRep gene therapy was able to rescue the pathognomonic feature of LQT3 by shortening the pathologically prolonged APD. FLUOVOLT™ voltage dye was used to measure optical action potentials in iPSC-CMs derived from a patient with LQT3-causing SCN5A-F1760C treated with SCN5A-SupRep gene therapy. All iPSC-CMs were paced at 1 Hz during recording to eliminate beat rate-dependent changes to the APD. Representative optical action potentials are shown in **FIG. 36A**. When untreated, SCN5A-F1760C iPSC-CMs had a significantly longer APD at 90% repolarization (APD_{90}) and had a significantly longer APD at 50% repolarization (APD_{50}) compared to untreated unrelated healthy control iPSC-CMs, validating the SCN5A-F1760C iPSC-CMs as an *in vitro* model of LQT3. APD shortening due to SCN5A-SupRep compared to untreated SCN5A-F1760C iPSC-CMs was then assessed by unpaired two-tailed student's t-tests at both the APD_{90} and APD_{50} levels separately for each variant. SCN5A-SupRep resulted in statistically significant attenuation of both APD_{90} and APD_{50} in SCN5A-F1760C iPSC-CMs (**FIG. 36B**). When treated with SCN5A-SupRep, the APD_{90} and APD_{50} of SCN5A-F1760C lines shortened significantly. These results indicated that suppression-

replacement gene therapy is a promising strategy for directly targeting the pathogenic substrate and ameliorating the resultant disease for LQT3.

Example 22 – shRNA knockdown of *MYH7*

Six (6) unique shRNAs targeting *MYH7* were tested, and one candidate shRNA (designated sh2) was identified that suppressed the endogenous *MYH7* alleles (both mutant and wild-type) in TSA201 cells with about 85% knockdown efficiency (**FIG. 37**). The shRNA (5′-GCTGAAAGCAGAGAGAGATTATCACATTT-3′; SEQ ID NO:33) (RNA sequence 5′-GCUGAAAGCAGAGAGAGAUUAUCACAUUU-3′; SEQ ID NO:34) was completely homologous with the human sequence. The shRNA was designed in a lentivirus backbone (pGFP-C-shLenti). Once this shRNA was identified, a SupRep construct containing the shRNA (SEQ ID NO:34) and an "shRNA-immune" (5′-ACTCAAGGCTGAAAGGGACTACCATATAT-3′; SEQ ID NO:35) version of the *MYH7* cDNA was generated (the shRNA for knocking down the endogenous *MYH7* alleles, and the shRNA-immune for simultaneously providing a replacement wild-type *MYH7* allele). As with *KCNQ1*, the shIMM sequence had alterations at the wobble base of each codon within the shRNA target sequence, which prevented knockdown by the shRNA, but did not change the encoded amino acid sequence. The SupRep construct was designed in a lentivirus backbone (pGFP-C-shLenti), with three SupRep constructs generated in the lentivirus backbone. These constructs differed in the reporter sequences (P2A, HA-Tag, and No reporter) that they contained. The 3 total constructs were as follows:

shLenti-SupRep-P2A

shLenti-SupRep-HA Tag

shLenti-SupRep-No Reporter

The SupRep constructs contained a CMV promoter and a human growth hormone (HGH) polyadenylation signal, but can be modified to include other promoters/enhancers. For example, the CMV promoter can be replaced with a cTnC promoter, which is smaller than the CMV promoter and more cardiac-specific. Additionally, the HGH polyadenylation signal can be replaced with a smaller SV40 terminator sequence. These

modifications reduce the size of the SupRep construct and allow it to be packaged into AAV9 with greater efficiency.

Example 23 – Materials and methods for PKP2 SupRep

Generation of a PKP2-SupRep gene therapy construct: To make PKP2-SupRep, eight candidate *PKP2* shRNAs (sh#1-8) in the pGFP-C-shLenti lentiviral backbone were tested. The KD efficiency of each *PKP2* shRNA was determined by co-transfecting TSA201 cells with PKP2-WT and sh#1-8. Expression of *PKP2,* normalized to *GAPDH*, was measured by qRT-PCR (**FIG. 38**). Of the eight shRNAs tested, sh#2, sh#4, sh#6 and sh#7 all resulted in significant KD of *PKP2* (mRNA: 75-90% KD). Any of these shRNAs could in theory have been used as part of the final PKP2-SupRep gene therapy vector. To select a final shRNA from the four potential candidates, by raw KD, *PKP2* sh#7 (5′-GCAGAGCTCCCAGAGAAATAT-3′; SEQ ID NO:52) (RNA sequence 5′–GCAGAGCUCCCAGAGAAAUAU-3′; SEQ ID NO:53) provided the strongest KD of *PKP2* on both the mRNA (90%) levels. Further, at the time of selection, the *PKP2* sh#7 target sequence was assessed using the Genome Aggregation Database (gnomAD) and ClinVar, and was found to be devoid of both common genetic polymorphisms and all known pathogenic ACM-causative mutations that may interfere with KD efficiency. *PKP2* sh#7 therefore was selected for the final PKP2-SupRep and is referred to as "shPKP2."

To create the replacement shRNA-immune version of *PKP2*, called PKP2-shIMM, ten synonymous variants were introduced into the WT *PKP2* cDNA (NM_004572.4) at the wobble base of each codon within the shPKP2 target site (5′-GCTGAACTGCCTGAAAAGTAC-3′; SEQ ID NO:990). PKP2-shIMM was then cloned into the shPKP2-containing vector, pGFP-C-shLenti, downstream of the CMV promoter. Three variations of the construct were made: shLenti-SupRep-P2A-GFP, shLenti-SupRep-HA Tag, shLenti-SupRep-No Reporter.

PKP2 Patient Selection for iPSC Generation: Patients were evaluated by a genetic cardiologist. Dermal fibroblasts and PBMCs were collected by 4 mm skin punch biopsy and blood sample, respectively. Samples were obtained from nearly 1200 patients

diagnosed with a variety of inherited cardiac channelopathies and their affected or unaffected family members, including 29 patients with *PKP2* variants. Four patients with *PKP2* variants were selected for generation of iPSCs: R79X, E149X, Q457X, c.2146-1G>C.

Fibroblast/PBMCs Reprogramming into iPSCs and Quality Control: Fibroblasts or PBMCs were reprogrammed by Sendai virus transduction using the CytoTune-iPS 2.0 reprogramming kit (Thermo) or electroporation with four episomal DNA plasmids containing the Yamanaka factors: pCXLE-hUL, pCXLE-hSK, pCXLE-hOCT3/4-shp53-F, and pCXWB-EBNA1 (Addgene; Watertown, MA). At least two colonies were picked within 21 days post-induction and clonally expanded. All iPSCs were cultured in mTeSR™1 (STEMCELL®) supplemented with 1% penicillin/streptomycin on MATRIGEL®-coated (Corning) 6 cm culture dishes in a 5% CO_2 incubator at 37°C. At 85% confluence, iPSCs were passaged using ReLeSR (STEMCELL®). Each clone was then karyotyped.

All lines had a normal karyotype. *PKP2* variant confirmation was conducted by Sanger sequencing of PCR-amplicons from genomic DNA. Expression of pluripotent markers in all iPSC clones was confirmed by confocal immunofluorescence microscopy using primary antibodies against Oct4 (Thermo, PA5-27438), Nanog (Thermo, PA1-097), Tra-1-60 (Santa Cruz; Dallas, TX; sc-21705), and SSEA-4 (Thermo, MA1-021) at a 1:250 dilution. Secondary antibodies were ALEXA FLUOR® 488 goat-anti-mouse (Thermo, A-11001) and ALEXA FLUOR® 594 goat-anti-rabbit (Thermo, A-11037). Counterstaining with DAPI (Thermo) was used at a 1:2000 dilution from a 5mg/mL stock. Images were acquired on a Zeiss LSM 980 confocal microscope.

Quality control for iPSCs: Standard quality control assays were performed on c.2146-1G>C iPSC line, including Sanger sequencing of the ACM-causative variant, karyotyping, bright field morphology, and immunofluorescence microscopy for pluripotent markers including Tra-1-60, Nanog, SSEA-4, and Oct4 (**FIG. 39A-39D**). Differentiation of iPSCs was induced by methods described elsewhere to generate spontaneously beating iPSC-CMs (Burridge et al., *supra*; and Mummery et al., *supra*). Since the cardiac APD is known to shorten as iPSC-CMs mature over time, all

experiments were conducted at least 30 days after the induction of differentiation (Shaheen et al., *supra*).

iPSC-CM Culture, Differentiation, and Dissociation: When iPSCs were 85% confluent, differentiation into cardiomyocytes (CMs) was induced as described elsewhere (Schwartz 2009, *supra*; and Schwartz 2013, *supra*). Differentiation was initiated (day 0) by changing the culture medium to RPMI 1640 GLUTAMAX™ plus 25 mM HEPES ((4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)) supplemented with B27-minus insulin (RPMI/B27-ins) (Thermo) containing 5 μM CHIR99021 (MilliporeSigma). On day 2, the medium was changed to RPMI/B27-ins containing 5 μM IWP-2 (MilliporeSigma). On day 4, the medium was changed back to the maintenance medium RPMI/B27-ins. Spontaneous beating typically began on days 6-7, with expansion to remaining cells by days 10-12. The iPSC-CMs were allowed to mature until at least day 30, changing the media twice per week. After day 30, iPSC-CMs were dissociated enzymatically using STEMDIFF™ cardiomyocyte dissociation kit (STEMCELL®). Briefly, cells were rinsed with PBS (without Ca^{2+}/Mg^{2+}) and placed in dissociation medium for 10 minutes at 37°C, and then deactivated by addition of STEMDIFF™ Cardiomyocyte Support Medium (STEMCELL®). Cells were triturated, transferred to a 15 mL conical tube, and pelleted by centrifugation at 300 rcf for 3 minutes. The supernatant was aspirated, and the cells suspended in Cardiomyocyte Support Medium before transfer to appropriate MATRIGEL®-coated culture ware. After 24 hours, the medium was changed back to RPMI/B27-ins. Dissociation resulted in a mixture of single cells and small-to-medium sized iPSC-CM clusters, depending on cell density before and after plating. Spontaneous beating generally returned 24 hours after dissociation, with strong electrical coupling and syncytia formation between days 3-7.

Lentivirus Generation and Transduction of iPSC-CMs: Lentivirus was used for application of PKP2-SupRep to iPSC-CMs. Lentiviral particles were generated from shLenti-shPKP2-shIMM-P2A-GFP (PKP2-GFP-SupRep) and shLenti-shPKP2-shIMM-HA (PKP2-HA-SupRep), using the pPACKH1 HIV Lentivector Packaging kit (SBI System Biosciences; Palo Alto, CA). After reaching at least day 30 post-induction of differentiation, iPSC-CMs from a patient with ACM were dissociated and plated into

MATRIGEL®-coated 35mm dishes with glass-bottom insets for Fluo-4 AM (Invitrogen; cat# F14201) or 10-well culture reaction slides for immunofluorescence (Marienfeld SUPERIOR™) as described above. After 24-48 hours of recovery, iPSC-CMs were left untreated or were transduced with lentiviral particles containing PKP2-SupRep. To increase transduction efficiency, Polybrene infection reagent (MilliporeSigma) was added during transduction to a final concentration of 8 μg/mL and the iPSC-CMs were centrifuged at 250 rcf for 1.5 hours at room temperature in the 35 mm dishes. After 24 hours post-transduction, the medium was exchanged for fresh maintenance medium, RPMI/B27-ins.

Intracellular Calcium Assay in iPSC-CMs: Intracellular calcium assay experiments were conducted between 3-7 days post-transduction of iPSC-CMs with lentiviral particles containing PKP2-SupRep. On the day of imaging, iPSC-CMs were rinsed with pre-warmed (37°C) HEPES-buffered Tyrode's solution (Alfa Aesar). Fluo-4 AM dye (Invitrogen) was dissolved in 50 μL DMSO, then 5 μL Fluo-4 AM and 2 μL PLURONIC™ F-127 (Invitrogen) were added to 1 mL Tyrode's solution for each 35 mm glass-bottom dish and incubated at 37°C for 30 minutes. Excess dye was removed in one rinse and two 5-minute washes with pre-warmed Tyrode's solution, and a final 1.5 mL Tyrode's solution was added to the iPSC-CMs for imaging. During imaging, the dishes were kept in a heated 37°C stage-top chamber (Live Cell Instrument) with 5% CO_2. Using a Nikon Eclipse Ti light microscope (Nikon) under 40X-water objective magnification, calcium transients were recorded in 20 second fast time-lapse videos at a rate of 50 frames/second (fps, 20ms exposure time) with LED illumination at 5% power. iPSC-CMs were paced at 0.5 Hz (9 ms pulse duration, 25V) using a MyoPacer field stimulator (Ion Optix; Westwood, MA) to eliminate beat-rate dependent effects on the calcium transient. Videos were focused on electrically-coupled syncytial areas of iPSC-CMs (clusters and monolayers) since these areas of cells best follow the pacing stimulus and produce the greatest signal-to-noise represented by large changes in fluorescence intensity. For analysis, rectangular regions of interest were drawn over flashing areas of cells, and NIS-Elements software (Nikon) was used to quantify the fluorescence intensity over time within each region of interest, resulting in traces of calcium transients. Using a

custom Excel-based program, traces were corrected for photobleaching and the amplitude was normalized as change in fluorescence divided by the baseline minimum florescence ($\Delta F/F_{min}$). In a semi-automated manner, common calcium transient parameters including Ca^{2+} amplitude, 50% and 90% Ca^{2+} transient duration (CTD), peak to 50% and peak to 90% decay, upstroke time, upstroke velocity, Vmax, etc. were detected for each individual calcium transient and averaged across all beats within a 20 second trace, except in case of Ca^{2+} amplitude where the value was taken only for the first beat. For all parameters, except for Ca^{2+} amplitude, the average of all beats within a 20 second trace represented a single data point. Upon recording the baseline measurements, 0.5 ml 400 nM isoproterenol was added to cells to make a final concentration of 100 nM and calcium transient recordings were taken every one minute for a total of 10 minutes. Traces were analyzed the same was as described above for baseline measurement.

Statistics: GraphPad Prism 9 was used for all statistical analysis and to fit all data for figures. Individual data points are shown wherever practical along with the mean. Error bars represent standard error of the mean (SEM). Two-way ANOVA with post-hoc Tukey's test for multiple comparisons also was used. A $p<0.05$ was considered to be significant.

Example 24 - PKP2-SupRep Gene Therapy Shortens Transient Duration and Decay Time in ACM iPSC-CMs as Measured by Fluo-4 AM

Calcium transient analyses were conducted to test whether treatment with PKP2-SupRep gene therapy was able to rescue the abnormal calcium handling feature of ACM. Fluo-4 AM dye was used to measure calcium transients in iPSC-CMs derived from patient with c.2146-1G>C PKP2 variant treated with PKP2-SupRep gene therapy. All iPSC-CMs were paced at 0.5 Hz during recording to eliminate beat rate-dependent changes to the calcium transient. Prolonged Ca^{2+} decay time is a key pathophysiology of ARVC, and may lead to remodeling of cardiac tissue into myopathic state, such as elevation of fibrosis and aseptic inflammation mediated exacerbation of desmosome alteration. Further, prolongation of Ca^{2+} decay time can accelerate arrhythmic potential through maladaption of sarcolemmal channel functions such as NCX1, LTCC, and Na^{+}

channels which elicit DAD and EAD. These studies demonstrated that SupRep successfully rescued arrhythmic potential with one delivery of therapeutic regimen (**FIG. 40**).

Example 25 – shRNA knock down of *DSP*

TSA201 cells were co-transfected with DSP-WT and six custom *DSP* shRNAs (sh1-6) or a non-targeting scrambled shRNA control (shCT). *DSP* expression normalized to *GAPDH* was measured by qRT-PCR. sh5 (5′-GCACTACTGCATGATTGACATAG AGAAGA-3′; SEQ ID NO:44) (RNA sequence 5'- GCACUACUGCAUGAUUGACA UAGAGAAGA-3′; SEQ ID NO:45) had the strongest knockdown by raw value (**FIG. 41**), with about 88% knockdown efficiency.

Example 26 – shRNA knock down of *MYBPC3*

TSA201 cells were co-transfected with MYBPC3-WT and six custom *MYBPC3* shRNAs (sh1-6) or non-targeting scramble shRNA control (shCT). *MYBPC3* expression normalized to *GAPDH* was measured by qRT-PCR. sh4 (5′-GGAGGAGACCTTCAAAT ACCGGTTCAAGA-3′; SEQ ID NO:46) (RNA sequence 5'- GGAGGAGACCUUCAAA UACCGGUUCAAGA-3′; SEQ ID NO:47) had the strongest knockdown by raw value (**FIG. 42**), with about 82% knockdown efficiency.

Example 27 – shRNA knock down of *RMB20*

TSA201 cells were co-transfected with RBM20-WT and six custom *RBM20* shRNAs (sh1-6) or non-targeting scramble shRNA control (shCT). *RBM20* expression normalized to *GAPDH* was measured by qRT-PCR. sh5 (5′-GGTCATTCACTCAGTC AAGCCCCACATTT-3′; SEQ ID NO:48) (RNA sequence 5'- GGUCAUUCACUCAGU CAAGCCCCACAUUU-3′; SEQ ID NO:49) had the strongest knockdown by raw value (**FIG. 43**), with about 82% knockdown efficiency.

Example 28 – shRNA knock down of *CACNA1C*

TSA201 cells were co-transfected with CACNA1C-WT and six custom *CACNA1C* shRNAs (sh1-6) or non-targeting scramble shRNA control (shCT). *CACNA1C* expression normalized to *GAPDH* was measured by qRT-PCR. sh1 (5′-GGAACGAGTGGAATATCTCTTTCTCATAA-3′; SEQ ID NO:50) (RNA sequence 5'-GGAACGAGUGGAAUAUCUCUUUCUCAUAA-3′; SEQ ID NO:51) had the strongest knockdown by raw value (**FIG. 44**), with about 92% knockdown efficiency.

Example 29 – Testing *CALM1* shRNA

TSA201 cells were co-transfected with CALM1-WT and six custom *CALM1* shRNAs (sh1-6) or non-targeting scramble shRNA control (shCT). *CALM1* expression normalized to *GAPDH* was measured by qRT-PCR. Sh2 (5′-GAAAGATACAGATAGTGAAGAAGAA-3′; SEQ ID NO:2738) (RNA sequence 5′–GAAAGAUACAGAUAGUGAAGAAGAA-3′; SEQ ID NO:2739) had the strongest knockdown by raw value (**FIG. 45**), with about 89% knockdown efficiency.

Example 30 – Testing *CALM2* shRNA

TSA201 cells were co-transfected with CALM2-WT and six custom *CALM2* shRNAs (sh1-6) or non-targeting scramble shRNA control (shCT). *CALM2* expression normalized to *GAPDH* was measured by qRT-PCR. Sh3 (5′-GCTGATGGTAATGGCACAATTGACT-3′; SEQ ID NO:2740) (RNA sequence 5′–GCUGAUGGUAAUGGCACAAUUGACU-3′; SEQ ID NO:2741) had the strongest knockdown by raw value (**FIG. 46**), with about 70% knockdown efficiency.

Example 31 – Testing *CALM3* shRNA

TSA201 cells were co-transfected with CALM3-WT and six custom *CALM3* shRNAs (sh1-6) or non-targeting scramble shRNA control (shCT). *CALM3* expression normalized to *GAPDH* was measured by qRT-PCR. Sh6 (5′-GATGAGGAGGTGGATGAGATGATCA-3′; SEQ ID NO:2742) (RNA sequence 5′–

GAUGAGGAGGUGGAUGAGAUGAUCA-3′; SEQ ID NO:2743) had the strongest knockdown by raw value (**FIG. 47**), with about 87% knockdown efficiency.

Example 32 – Testing *KCNJ2* shRNA

TSA201 cells were co-transfected with KCNJ2-WT and six custom *KCNJ2* shRNAs (sh1-6) or non-targeting scramble shRNA control (shCT). *KCNJ2* expression normalized to *GAPDH* was measured by qRT-PCR. Sh5 (5′-GTGCCGTAGCTCTTATCTAGCAAATGAAA-3′; SEQ ID NO:2744) (RNA sequence 5′– GUGCCGUAGCUCUUAUCUAGCAAAUGAAA-3′; SEQ ID NO:2745) had the strongest knockdown by raw value (**FIG. 48**), with about 74% knockdown efficiency.

Example 33 – Testing *CASQ2* shRNA

TSA201 cells were co-transfected with CASQ2-WT and six custom *CASQ2* shRNAs (sh1-6) or non-targeting scramble shRNA control (shCT). *CASQ2* expression normalized to *GAPDH* was measured by qRT-PCR. Sh2 (5′-AAGGAAGCCTGTATATTCTTA-3′; SEQ ID NO:2746) (RNA sequence 5′–AAGGAAGCCUGUAUAUUCUUA-3′; SEQ ID NO:2747) had the strongest knockdown by raw value (**FIG. 49**), with about 89% knockdown efficiency.

Example 34 – Testing *DSG2* shRNA

TSA201 cells were co-transfected with DSG2-WT and six custom *DSG2* shRNAs (sh1-6) or non-targeting scramble shRNA control (shCT). *DSG2* expression normalized to *GAPDH* was measured by qRT-PCR. Sh5 (5′-GCAGTCTAGTAGGAAGAAATGGAGTAGGA-3′; SEQ ID NO:2748) (RNA sequence 5′– GCAGUCUAGUAGGAAGAAAUGGAGUAGGA-3′; SEQ ID NO:2749) had the strongest knockdown by raw value (**FIG. 50**), with about 70% knockdown efficiency.

Example 35 – Testing *TNNT2* shRNA

TSA201 cells were co-transfected with TNNT2-WT and seven custom *TNNT2* shRNAs (sh1-7) or non-targeting scramble shRNA control (shCT). *TNNT2* expression

normalized to *GAPDH* was measured by qRT-PCR. Sh4 (5′-GAAGAAGAAGAGGAAGCAAAG-3′; SEQ ID NO:2750) (RNA sequence 5′–GAAGAAGAAGAGGAAGCAAAG-3′; SEQ ID NO:2750) had the strongest knockdown by raw value (**FIG. 51**), with about 90% knockdown efficiency.

Example 36 – Testing *TPM1* shRNA

TSA201 cells were co-transfected with TPM1-WT and six custom *TPM1* shRNAs (sh1-6) or non-targeting scramble shRNA control (shCT). *TPM1* expression normalized to *GAPDH* was measured by qRT-PCR. Sh2 (5′- AAGCTGAGAAGGCAGCAGATG-3′; SEQ ID NO:2751) (RNA sequence 5′– AAGCUGAGAAGGCAGCAGAUG-3′; SEQ ID NO:2752) had the strongest knockdown by raw value (**FIG. 52**), with about 85% knockdown efficiency.

Example 37 – Testing *LMNA* shRNA

TSA201 cells were co-transfected with LMNA-WT and six custom *LMNA* shRNAs (sh1-6) or non-targeting scramble shRNA control (shCT). *LMNA* expression normalized to *GAPDH* was measured by qRT-PCR. Sh5 (5′-GGCAGATCAAGCGCCAGAATGGAGATGA-3′; SEQ ID NO:2753) (RNA sequence 5′– GGCAGAUCAAGCGCCAGAAUGGAGAUGA-3′; SEQ ID NO:2754) had the strongest knockdown by raw value (**FIG. 53**), with about 75% knockdown efficiency.

Example 38 – Testing *PLN* shRNA

TSA201 cells were co-transfected with LMNA-WT and six custom *PLN* shRNAs (sh1-6) or non-targeting scramble shRNA control (shCT). *PLN* expression normalized to *GAPDH* was measured by qRT-PCR. Sh5 (5′- TGTCTCTTGCTGATCTGTATC-3′; SEQ ID NO:2755) (RNA sequence 5′– UGUCUCUUGCUGAUCUGUAUC-3′; SEQ ID NO:2756) had the strongest knockdown by raw value (**FIG. 54**), with about 80% knockdown efficiency.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A nucleic acid construct comprising:

(a) a first nucleotide sequence encoding an RNAi molecule capable of hybridizing to a target sequence encoding an endogenous KCNQ1 polypeptide within a cell and suppressing expression of said endogenous KCNQ1 polypeptide within said cell, and

(b) a second nucleotide sequence encoding a KCNQ1 polypeptide, wherein said second nucleotide sequence comprises a target sequence identical to said target sequence of said first nucleotide sequence with the exception that said target sequence of said second nucleotide sequence comprises 1 to 13 wobble position variants as compared to said target sequence of said first nucleotide sequence, and wherein said RNAi molecule does not suppress expression of said KCNQ1 polypeptide from said second nucleotide sequence within said cell.

2. The nucleic acid construct of claim 1, wherein said first nucleotide sequence comprises the sequence set forth in SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, or SEQ ID NO:36, and wherein said second nucleotide sequence comprises the sequence set forth in SEQ ID NO:9.

3. The nucleic acid construct of claim 1, wherein said first nucleotide sequence comprises the sequence set forth in SEQ ID NO:36 and said second nucleotide sequence comprises the sequence set forth in SEQ ID NO:9.

4. The nucleic acid construct of any one of claims 1 to 3, wherein said first nucleotide sequence is operably linked to a first promoter and said second nucleotide sequence is operably linked to a second promoter.

5. The nucleic acid construct of claim 4, wherein said first and second promoters are the same.

6. The nucleic acid construct of claim 4, wherein said first and second promoters are different.

7. The nucleic acid construct of claim 6, wherein said first promoter is a U6 promoter and said second promoter is a cytomegalovirus immediate-early (CMV) promoter.

8. The nucleic acid construct of any one of claims 1 to 7, further comprising a nucleotide sequence encoding a reporter.

9. The nucleic acid construct of claim 8, wherein said reporter is a fluorescent polypeptide.

10. The nucleic acid construct of claim 8 or claim 9, wherein said nucleotide sequence encoding said reporter is downstream of said second nucleotide sequence encoding said cDNA, and is separated from said second nucleotide sequence by an internal ribozyme entry sequence (IRES) or P2A self-cleaving peptide sequence.

11. The nucleic acid construct of any one of claims 1 to 10, wherein said nucleic acid construct is within a viral vector.

12. The nucleic acid construct of claim 11, wherein said viral vector is an adeno-associated virus (AAV) vector.

13. The nucleic acid construct of claim 12, wherein said AAV vector is an AAV serotype 9 vector or an AAV2/9 vector.

14. The nucleic acid construct of any one of claims 1 to 13, wherein said cell is a cardiomyocyte.

15. A virus particle comprising the nucleic acid construct of any one of claims 1 to 14.

16. A method for treating a mammal having a congenital cardiac disease, said method comprising administering to said mammal a nucleic acid construct comprising:

(a) a first nucleotide sequence encoding an RNAi molecule capable of hybridizing to a target sequence encoding an endogenous KCNQ1 polypeptide within a cell of said mammal and suppressing expression of said endogenous KCNQ1 polypeptide within said cell, and

(b) a second nucleotide sequence encoding a KCNQ1 polypeptide, wherein said second nucleotide sequence comprises a target sequence identical to said target sequence of said first nucleotide sequence with the exception that said target sequence of said second nucleotide sequence comprises 1 to 13 wobble position variants as compared to said target sequence of said first nucleotide sequence, and wherein said RNAi molecule does not suppress expression of said KCNQ1 polypeptide from said second nucleotide sequence within said cell.

17. The method of claim 16, wherein said congenital cardiac disease is long QT syndrome (LQTS) or short QT syndrome (SQTS).

18. The method of claim 16, wherein said congenital cardiac disease is LQT1.

19. The method of any one of claims 16 to 18, wherein said first nucleotide sequence comprises the sequence set forth in SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, or SEQ ID NO:36, and wherein said second nucleotide sequence comprises the sequence set forth in SEQ ID NO:9.

20. The method of any one of claims 16 to 18, wherein said first nucleotide sequence comprises the sequence set forth in SEQ ID NO:36 and said second nucleotide sequence comprises the sequence set forth in SEQ ID NO:9.

21. The method of any one of claims 16 to 20, wherein said first nucleotide sequence is operably linked to a first promoter and said second nucleotide sequence is operably linked to a second promoter.

22. The method of claim 21, wherein said first and second promoters are the same.

23. The method of claim 21, wherein said first and second promoters are different.

24. The method of claim 21, wherein said first promoter is a U6 promoter and said second promoter is a CMV promoter.

25. The method of any one of claims 16 to 24, wherein said nucleic acid construct further comprises a nucleotide sequence encoding a reporter.

26. The method of claim 25, wherein said reporter is a fluorescent polypeptide.

27. The method of claim 25 or claim 26, wherein said nucleotide sequence encoding said reporter is downstream of said second nucleotide sequence encoding said cDNA, and is separated from said second nucleotide sequence by an IRES.

28. The method of any one of claims 16 to 27, wherein said nucleic acid construct is within a viral vector.

29. The method of claim 28, wherein said viral vector is an AAV vector.

30. The method of claim 29, wherein said AAV vector is an AAV serotype 9 vector or an AAV2/9 vector.

31. The method of any one of claims 16 to 30, wherein said cell is a cardiomyocyte.

32. A method for reducing the action potential duration (APD) in cardiac cells within a mammal, said method comprising administering to said mammal a nucleic acid construct comprising:

(a) a first nucleotide sequence encoding an RNAi molecule capable of hybridizing to a target sequence encoding an endogenous KCNQ1 polypeptide within cardiac cells of said mammal and suppressing expression of said endogenous KCNQ1 polypeptide within said cardiac cells, and

(b) a second nucleotide sequence encoding a KCNQ1 polypeptide, wherein said second nucleotide sequence comprises a target sequence identical to said target sequence of said first nucleotide sequence with the exception that said target sequence of said second nucleotide sequence comprises 1 to 13 wobble position variants as compared to

said target sequence of said first nucleotide sequence, and wherein said RNAi molecule does not suppress expression of said KCNQ1 polypeptide from said second nucleotide sequence within said cell.

33. The method of claim 32, wherein said first nucleotide sequence comprises the sequence set forth in SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, or SEQ ID NO:36, and wherein said second nucleotide sequence comprises the sequence set forth in SEQ ID NO:9.

34. The method of claim 32, wherein said first nucleotide sequence comprises the sequence set forth in SEQ ID NO:36 and said second nucleotide sequence comprises the sequence set forth in SEQ ID NO:9.

35. The method of any one of claims 32 to 34, wherein said first nucleotide sequence is operably linked to a first promoter and said second nucleotide sequence is operably linked to a second promoter.

36. The method of claim 35, wherein said first and second promoters are the same.

37. The method of claim 35, wherein said first and second promoters are different.

38. The method of claim 37, wherein said first promoter is a U6 promoter and said second promoter is a CMV promoter.

39. The method of any one of claims 32 to 38, wherein said nucleic acid construct is within a viral vector.

40. The method of claim 39, wherein said viral vector is an AAV vector.

41. The method of claim 40, wherein said AAV vector is an AAV serotype 9 vector or an AAV2/9 vector.

42. A method for reducing one or more symptoms of LQTS in a mammal, said method comprising administering to said mammal a nucleic acid construct comprising:

(a) a first nucleotide sequence encoding an RNAi molecule capable of hybridizing to a target sequence encoding an endogenous KCNQ1 polypeptide within a cell of said mammal and suppressing expression of said endogenous KCNQ1 polypeptide within said cell, and

(b) a second nucleotide sequence encoding a KCNQ1 polypeptide, wherein said second nucleotide sequence comprises a target sequence identical to said target sequence of said first nucleotide sequence with the exception that said target sequence of said second nucleotide sequence comprises 1 to 13 wobble position variants as compared to said target sequence of said first nucleotide sequence, and wherein said RNAi molecule does not suppress expression of said KCNQ1 polypeptide from said second nucleotide sequence within said cell.

43. The method of claim 42, wherein said LQTS is LQT1.

44. The method of claim 42 or claim 43, wherein said first nucleotide sequence comprises the sequence set forth in SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:15, or SEQ ID NO:36, and wherein said second nucleotide sequence comprises the sequence set forth in SEQ ID NO:9.

45. The method of claim 42 or claim 43, wherein said first nucleotide sequence comprises the sequence set forth in SEQ ID NO:36 and said second nucleotide sequence comprises the sequence set forth in SEQ ID NO:9.

46. The method of any one of claims 42 to 45, wherein said first nucleotide sequence is operably linked to a first promoter and said second nucleotide sequence is operably linked to a second promoter.

47. The method of claim 46, wherein said first and second promoters are the same.

48. The method of claim 46, wherein said first and second promoters are different.

49. The method of claim 48, wherein said first promoter is a U6 promoter and said second promoter is a CMV promoter.

50. The method of any one of claims 42 to 49, wherein said nucleic acid construct is within a viral vector.

51. The method of claim 50, wherein said viral vector is an AAV vector.

52. The method of claim 51, wherein said AAV vector is an AAV serotype 9 vector or an AAV2/9 vector.

53. The method of any one of claims 42 to 52, wherein said cell is a cardiomyocyte.

54. A nucleic acid construct comprising:

(a) a first nucleotide sequence encoding an RNAi molecule capable of hybridizing to a target sequence encoding an endogenous KCNH2 polypeptide within a cell and suppressing expression of said endogenous KCNH2 polypeptide within said cell, and

(b) a second nucleotide sequence encoding a KCNH2 polypeptide, wherein said second nucleotide sequence comprises a target sequence identical to said target sequence of said first nucleotide sequence with the exception that said target sequence of said second nucleotide sequence comprises 1 to 13 wobble position variants as compared to said target sequence of said first nucleotide sequence, and wherein said RNAi molecule does not suppress expression of said KCNH2 polypeptide from said second nucleotide sequence within said cell.

55. The nucleic acid construct of claim 54, wherein said first nucleotide sequence comprises the sequence set forth in SEQ ID NO:27 and said second nucleotide sequence comprises the sequence set forth in SEQ ID NO:29.

56. The nucleic acid construct of claim 54 or claim 55, wherein said first nucleotide sequence is operably linked to a first promoter and said second nucleotide sequence is operably linked to a second promoter.

57. The nucleic acid construct of claim 56, wherein said first and second promoters are the same.

58. The nucleic acid construct of claim 56, wherein said first and second promoters are different.

59. The nucleic acid construct of claim 58, wherein said first promoter is a U6 promoter and said second promoter is a CMV promoter.

60. The nucleic acid construct of any one of claims 54 to 59, further comprising a nucleotide sequence encoding a reporter.

61. The nucleic acid construct of claim 60, wherein said reporter is a fluorescent polypeptide.

62. The nucleic acid construct of claim 60 or claim 61, wherein said nucleotide sequence encoding said reporter is downstream of said second nucleotide sequence encoding said cDNA, and is separated from said second nucleotide sequence by an IRES or P2A self-cleaving peptide sequence.

63. The nucleic acid construct of any one of claims 54 to 62, wherein said nucleic acid construct is within a viral vector.

64. The nucleic acid construct of claim 63, wherein said viral vector is an AAV vector.

65. The nucleic acid construct of claim 64, wherein said AAV vector is an AAV serotype 9 vector or an AAV2/9 vector.

66. The nucleic acid construct of any one of claims 54 to 65, wherein said cell is a cardiomyocyte.

67. A virus particle comprising the nucleic acid construct of any one of claims 54 to 66.

68. A method for treating a mammal having a congenital cardiac disease, said method comprising administering to said mammal a nucleic acid construct comprising:

(a) a first nucleotide sequence encoding an RNAi molecule capable of hybridizing to a target sequence encoding an endogenous KCNH2 polypeptide within a cell of said mammal and suppressing expression of said endogenous KCNH2 polypeptide within said cell, and

(b) a second nucleotide sequence encoding a KCNH2 polypeptide, wherein said second nucleotide sequence comprises a target sequence identical to said target sequence of said first nucleotide sequence with the exception that said target sequence of said second nucleotide sequence comprises 1 to 13 wobble position variants as compared to said target sequence of said first nucleotide sequence, and wherein said RNAi molecule does not suppress expression of said KCNH2 polypeptide from said second nucleotide sequence within said cell.

69. The method of claim 68, wherein said congenital cardiac disease is LQTS or SQTS.

70. The method of claim 68, wherein said congenital cardiac disease is LQT2.

71. The method of any one of claims 68 to 70, wherein said first nucleotide sequence comprises the sequence set forth in SEQ ID NO:27 and said second nucleotide sequence comprises the sequence set forth in SEQ ID NO:29.

72. The method of any one of claims 68 to 71, wherein said first nucleotide sequence is operably linked to a first promoter and said second nucleotide sequence is operably linked to a second promoter.

73. The method of claim 72, wherein said first and second promoters are the same.

74. The method of claim 72, wherein said first and second promoters are different.

75. The method of claim 74, wherein said first promoter is a U6 promoter and said second promoter is a CMV promoter.

76. The method of any one of claims 68 to 75, wherein said nucleic acid construct further comprises a nucleotide sequence encoding a reporter.

77. The method of claim 76, wherein said reporter is a fluorescent polypeptide.

78. The method of claim 76 or claim 77, wherein said nucleotide sequence encoding said reporter is downstream of said second nucleotide sequence encoding said cDNA, and is separated from said second nucleotide sequence by an IRES.

79. The method of any one of claims 68 to 78, wherein said nucleic acid construct is within a viral vector.

80. The method of claim 79, wherein said viral vector is an AAV vector.

81. The method of claim 80, wherein said AAV vector is an AAV serotype 9 vector or an AAV2/9 vector.

82. The method of any one of claims 68 to 81, wherein said cell is a cardiomyocyte.

83. A method for reducing the APD in cardiac cells within a mammal, said method comprising administering to said mammal a nucleic acid construct comprising:

(a) a first nucleotide sequence encoding an RNAi molecule capable of hybridizing to a target sequence encoding an endogenous KCNH2 polypeptide within cardiac cells of said mammal and suppressing expression of said endogenous KCNH2 polypeptide within said cardiac cells, and

(b) a second nucleotide sequence encoding a KCNH2 polypeptide, wherein said second nucleotide sequence comprises a target sequence identical to said target sequence of said first nucleotide sequence with the exception that said target sequence of said second nucleotide sequence comprises 1 to 13 wobble position variants as compared to said target sequence of said first nucleotide sequence, and wherein said RNAi molecule does not suppress expression of said KCNH2 polypeptide from said second nucleotide sequence within said cell.

84. The method of claim 83, wherein said first nucleotide sequence comprises the sequence set forth in SEQ ID NO:27 and said second nucleotide sequence comprises the sequence set forth in SEQ ID NO:29.

85. The method of claim 83 or claim 84, wherein said first nucleotide sequence is operably linked to a first promoter and said second nucleotide sequence is operably linked to a second promoter.

86. The method of claim 85, wherein said first and second promoters are the same.

87. The method of claim 85, wherein said first and second promoters are different.

88. The method of claim 87, wherein said first promoter is a U6 promoter and said second promoter is a CMV promoter.

89. The method of any one of claims 83 to 88, wherein said nucleic acid construct is within a viral vector.

90. The method of claim 89, wherein said viral vector is an AAV vector.

91. The method of claim 90, wherein said AAV vector is an AAV serotype 9 vector or an AAV2/9 vector.

92. A method for reducing one or more symptoms of LQTS in a mammal, said method comprising administering to said mammal a nucleic acid construct comprising:

(a) a first nucleotide sequence encoding an RNAi molecule capable of hybridizing to a target sequence encoding an endogenous KCNH2 polypeptide within a cell of said mammal and suppressing expression of said endogenous KCNH2 polypeptide within said cell, and

(b) a second nucleotide sequence encoding a KCNH2 polypeptide, wherein said second nucleotide sequence comprises a target sequence identical to said target sequence of said first nucleotide sequence with the exception that said target sequence of said second nucleotide sequence comprises 1 to 13 wobble position variants as compared to

said target sequence of said first nucleotide sequence, and wherein said RNAi molecule does not suppress expression of said KCNH2 polypeptide from said second nucleotide sequence within said cell.

93. The method of claim 92, wherein said LQTS is LQT2.

94. The method of claim 92 or claim 93, wherein said first nucleotide sequence comprises the sequence set forth in SEQ ID NO:27 and said second nucleotide sequence comprises the sequence set forth in SEQ ID NO:29.

95. The method of any one of claims 92 to 94, wherein said first nucleotide sequence is operably linked to a first promoter and said second nucleotide sequence is operably linked to a second promoter.

96. The method of claim 95, wherein said first and second promoters are the same.

97. The method of claim 95, wherein said first and second promoters are different.

98. The method of claim 97, wherein said first promoter is a U6 promoter and said second promoter is a CMV promoter.

99. The method of any one of claims 92 to 98, wherein said nucleic acid construct is within a viral vector.

100. The method of claim 99, wherein said viral vector is an AAV vector.

101. The method of claim 100, wherein said AAV vector is an AAV serotype 9 vector or an AAV2/9 vector.

102. The method of any one of claims 92 to 101, wherein said cell is a cardiomyocyte.

103. A nucleic acid construct for treating a congenital heart disease caused by an endogenous cardiac polypeptide containing one or more mutations causative of said congenital heart disease, wherein said construct comprises:

(a) a first nucleotide sequence encoding an RNAi molecule capable of hybridizing to a target sequence encoding said endogenous cardiac polypeptide within a cell and suppressing expression of said endogenous cardiac polypeptide within said cell, and

(b) a second nucleotide sequence encoding a replacement version of said endogenous cardiac polypeptide that lacks said one or more mutations causative of said congenital heart disease, wherein said second nucleotide sequence comprises a target sequence identical to said target sequence of said first nucleotide sequence with the exception that said target sequence of said second nucleotide sequence comprises 1 to 13 wobble position variants as compared to said target sequence of said first nucleotide sequence, and wherein said RNAi molecule does not suppress expression of said replacement version of said endogenous cardiac polypeptide that lacks said one or more mutations causative of said congenital heart disease from said second nucleotide sequence within said cell.

104. The nucleic acid construct of claim 103, wherein said first nucleotide sequence is operably linked to a first promoter and said second nucleotide sequence is operably linked to a second promoter.

105. The nucleic acid construct of claim 104, wherein said first and second promoters are the same.

106. The nucleic acid construct of claim 104, wherein said first and second promoters are different.

107. The nucleic acid construct of claim 106, wherein said first promoter is a U6 promoter and said second promoter is a CMV promoter.

108. The nucleic acid construct of any one of claims 103 to 107, further comprising a nucleotide sequence encoding a reporter.

109. The nucleic acid construct of claim 108, wherein said reporter is a fluorescent polypeptide.

110. The nucleic acid construct of claim 108 or claim 109, wherein said nucleotide sequence encoding said reporter is downstream of said second nucleotide sequence encoding said cDNA, and is separated from said second nucleotide sequence by an internal ribozyme entry sequence (IRES) or P2A self-cleaving peptide sequence.

111. The nucleic acid construct of any one of claims 103 to 110, wherein said nucleic acid construct is within a viral vector.

112. The nucleic acid construct of claim 111, wherein said viral vector is an AAV vector.

113. The nucleic acid construct of claim 112, wherein said AAV vector is an AAV serotype 9 vector or an AAV2/9 vector.

114. The nucleic acid construct of any one of claims 103 to 113, wherein said cell is a cardiomyocyte.

115. A virus particle comprising the nucleic acid construct of any one of claims 103 to 114.

116. A method for treating a mammal having a congenital cardiac disease, said method comprising administering to said mammal a nucleic acid construct comprising:

(a) a first nucleotide sequence encoding an RNAi molecule capable of hybridizing to a target sequence encoding said endogenous cardiac polypeptide within a cell and suppressing expression of said endogenous cardiac polypeptide within said cell, and

(b) a second nucleotide sequence encoding a replacement version of said endogenous cardiac polypeptide that lacks said one or more mutations causative of said congenital heart disease, wherein said second nucleotide sequence comprises a target sequence identical to said target sequence of said first nucleotide sequence with the exception that said target sequence of said second nucleotide sequence comprises 1 to 13 wobble position variants as compared to said target sequence of said first nucleotide sequence, and wherein said RNAi molecule does not suppress expression of said

replacement version of said endogenous cardiac polypeptide that lacks said one or more mutations causative of said congenital heart disease from said second nucleotide sequence within said cell.

117. The method of claim 116, wherein said first nucleotide sequence is operably linked to a first promoter and said second nucleotide sequence is operably linked to a second promoter.

118. The method of claim 117, wherein said first and second promoters are the same.

119. The method of claim 117, wherein said first and second promoters are different.

120. The method of claim 119, wherein said first promoter is a U6 promoter and said second promoter is a CMV promoter.

121. The method of any one of claims 116 to 120, wherein said nucleic acid construct further comprises a nucleotide sequence encoding a reporter.

122. The method of claim 121, wherein said reporter is a fluorescent polypeptide.

123. The method of claim 121 or claim 122, wherein said nucleotide sequence encoding said reporter is downstream of said second nucleotide sequence encoding said cDNA, and is separated from said second nucleotide sequence by an IRES.

124. The method of any one of claims 116 to 123 wherein said nucleic acid construct is within a viral vector.

125. The method of claim 124, wherein said viral vector is an AAV vector.

126. The method of claim 125, wherein said AAV vector is an AAV serotype 9 vector or an AAV2/9 vector.

127. The method of any one of claims 116 to 126, wherein said cell is a cardiomyocyte.