Makarenko *et al.*, *Circulation research.* Oct 1 2004;95(7):708-716; Jaber *et al.*. *Circulation. Heart failure.* Sep 2008;1(3):192-199]. Sarcomeric passive tension is reduced by approximately 30% in DCM adult patients and this loss of tension results from loss of titin and increased fibrosis [Makarenko et al, supra]. Recently, 5,200 patients underwent sequencing of titin to identify pathogenic mutations leading to DCM [Roberts *et al.*,*Science translational medicine.* Jan 14 2015;7(270):270ra276] and it was identified that 30% of familial DCM cases are due to titin-based mutations, specifically C-terminus truncating mutations [Herman *et al.*, The *New England Journal of Medicine.* 2012;366(7):619-628; Gerull *et al.*, *supra*; Itoh-Satoh *et al.*, *Biochemical and Biophysical Research Communications.* 2002;291(2):385-393; Gerull *et al.*, *Journal of Molecular Medicine.* 2006;84(6):478-483]. Non-ischemic DCM occurs in 1 of 250 people and it is an important cause of heart arrhythmias, heart failure and heart transplants.

[0008] Since there has previously been no cure or treatment for titin-based myopathies or other titinopathies, there exists a need in the art to provide products and methods for treating titin-based myopathies and/or other titinopathies.

Summary

[0009] Provided herein are *ex vivo* and *in vivo* methods for creating permanent changes in the genome by correcting one or more mutations in the titin gene by genome editing and restoring titin protein activity, which can be used to treat titin-based cardiomyopathies and other titinopathies, as well as components, kits and compositions for performing such methods, and cells produced by them.

[0009a] In one aspect, the present disclosure refers to an *ex vivo or in vitro* method for increasing functional titin protein expression in an isolated human cell containing a mutation in the human titin gene, wherein the mutation is in exon 219, in exon 326, a c.32854G>C mutation in exon 219, or a c.37112G>A mutation in intron 242,
the method comprising introducing into the cell

(a) a Cas9 endonuclease or a homolog, codon-optimized, or modified version thereof that effects a break within the titin gene, or a nucleic acid that encodes the Cas9 endonuclease or a homolog, codon-optimized, or modified version thereof that effects a break within the titin gene; and

(b) (i) when the mutation is in exon 219, a nucleic acid encoding a guide ribonucleic acid (gRNA) that is complementary to a target sequence that comprises a sequence set forth in SEQ ID NO: 19, 23, or 27-41;

(ii) when the mutation is in exon 326, a nucleic acid encoding a gRNA that is complementary to a target sequence that comprises a sequence set forth in SEQ ID NO: 64, 66, 68, 70, 72, 74, or 76;

(iii) when the mutation is the c.32854G>C mutation in exon 219, a nucleic acid encoding a gRNA that is complementary to a target sequence that comprises a sequence set forth in SEQ ID NO: 1, 5, 9, or 13 and a nucleic acid comprising a donor template region comprising SEQ ID NO: 18; or

(iv) when the mutation is the c.37112G>A mutation in intron 242, a nucleic acid encoding a gRNA that is complementary to a target sequence that comprises asequence set forth in SEQ ID NO: 57, 59, or 61 and a nucleic acid comprising a donor template region comprising SEQ ID NO: 63

resulting in a repair of the human titin gene resulting in an increased expression of functional titin protein in the cell.

[0009b] In another aspect, the present disclosure refers to a use of a Cas9 endonuclease or a homolog, codon-optimized, or modified version thereof that effects a break within the titin gene, or a nucleic acid that encodes the Cas9 endonuclease or a homolog, codon-optimized, or modified version thereof that effects a break within the titin gene and a nucleic acid encoding a guide ribonucleic acid (gRNA) in the manufacture of a medicament for treating a subject with a titin-based cardiomyopathy or other titinopathy by editing the titin gene in a cell of the subject or isolated from the subject containing a mutation in the human titin gene comprising:

using the medicament comprising the Cas9 endonuclease or a homolog, codon-optimized, or modified version thereof that effects a break within the titin gene, or a nucleic acid that encodes the Cas9 endonuclease or a homolog, codon-optimized, or modified version thereof that effects a break within the titin gene and the nucleic acid encoding the gRNA to edit the titin gene and increase expression of functional titin

protein by the cell, wherein the mutation is in exon 219, in exon 326, a c.32854G>C mutation in exon 219, or a c.37112G>A mutation in intron 242; and

(i) when the mutation is in exon 219, a nucleic acid encoding a gRNA that is complementary to a target sequence that comprises a sequence set forth in SEQ ID NO: 19, 23, or 27-41;

(ii) when the mutation is in exon 326, a nucleic acid encoding a gRNA that is complementary to a target sequence that comprises a sequence set forth in SEQ ID NO: 64, 66, 68, 70, 72, 74, or 76;

(iii) when the mutation is the c.32854G>C mutation in exon 219, a nucleic acid encoding a gRNA that is complementary to a target sequence that comprises a sequence set forth in SEQ ID NO: 1, 5, 9, or 13 and a nucleic acid comprising a donor template region comprising SEQ ID NO: 18; or

(iv) when the mutation is the c.37112G>A mutation in intron 242, a nucleic acid encoding a gRNA that is complementary to a target sequence that comprises a sequence set forth in SEQ ID NO: 57, 59, or 61 and a nucleic acid comprising a donor template region comprising SEQ ID NO: 63.

[0009c] In another aspect, the present disclosure refers to a nucleic acid encoding a guide ribonucleic acid (gRNA) that is complementary to a target sequence that comprises a sequence set forth in SEQ ID NO: 1, 5, 9, 13, 19, 23, 27-41, 57, 59, 61, 64, 66, 68, 70, 72, 74, or 76.

[0010] In one example, provided herein are methods for editing the titin gene in a human cell by genome editing comprising introducing into the cell one or more deoxyribonucleic acid (DNA) endonucleases to effect one or more double-strand breaks (DSBs) within the titin gene that results in permanent correction of one or more mutations within the titin gene and restoration of titin protein activity.

[0011] In some examples, methods provided herein are *ex vivo* methods for treating a patient with a titin-based cardiomyopathy or other titinopathy comprising the steps of: performing a biopsy of the patient's heart; isolating a cardiac progenitor cell, including but not limited to an endogenous cardiac stem cell (eCSC) or primary

Claims

What is claimed is:

1. An *ex vivo or in vitro* method for increasing functional titin protein expression in an isolated human cell containing a mutation in the human titin gene, wherein the mutation is in exon 219, in exon 326, a c.32854G>C mutation in exon 219, or a c.37112G>A mutation in intron 242, the method comprising introducing into the cell

(a) a Cas9 endonuclease or a homolog, codon-optimized, or modified version thereof that effects a break within the titin gene, or a nucleic acid that encodes the Cas9 endonuclease or a homolog, codon-optimized, or modified version thereof that effects a break within the titin gene; and

(b) (i) when the mutation is in exon 219, a nucleic acid encoding a guide ribonucleic acid (gRNA) that is complementary to a target sequence that comprises a sequence set forth in SEQ ID NO: 19, 23, or 27-41;

(ii) when the mutation is in exon 326, a nucleic acid encoding a gRNA that is complementary to a target sequence that comprises a sequence set forth in SEQ ID NO: 64, 66, 68, 70, 72, 74, or 76;

(iii) when the mutation is the c.32854G>C mutation in exon 219, a nucleic acid encoding a gRNA that is complementary to a target sequence that comprises a sequence set forth in SEQ ID NO: 1, 5, 9, or 13 and a nucleic acid comprising a donor template region comprising SEQ ID NO: 18; or

(iv) when the mutation is the c.37112G>A mutation in intron 242, a nucleic acid encoding a gRNA that is complementary to a target sequence that comprises a sequence set forth in SEQ ID NO: 57, 59, or 61 and a nucleic acid comprising a donor template region comprising SEQ ID NO: 63

resulting in a repair of the human titin gene resulting in an increased expression of functional titin protein in the cell.

2. Use of a Cas9 endonuclease or a homolog, codon-optimized, or modified version thereof that effects a break within the titin gene, or a nucleic acid that encodes the Cas9 endonuclease

or a homolog, codon-optimized, or modified version thereof that effects a break within the titin gene and a nucleic acid encoding a guide ribonucleic acid (gRNA) in the manufacture of a medicament for treating a subject with a titin-based cardiomyopathy or other titinopathy by editing the titin gene in a cell of the subject or isolated from the subject containing a mutation in the human titin gene comprising:

using the medicament comprising the Cas9 endonuclease or a homolog, codon-optimized, or modified version thereof that effects a break within the titin gene, or a nucleic acid that encodes the Cas9 endonuclease or a homolog, codon-optimized, or modified version thereof that effects a break within the titin gene and the nucleic acid encoding the gRNA to edit the titin gene and increase expression of functional titin protein by the cell, wherein the mutation is in exon 219, in exon 326, a c.32854G>C mutation in exon 219, or a c.37112G>A mutation in intron 242; and

(i) when the mutation is in exon 219, a nucleic acid encoding a gRNA that is complementary to a target sequence that comprises a sequence set forth in SEQ ID NO: 19, 23, or 27-41;

(ii) when the mutation is in exon 326, a nucleic acid encoding a gRNA that is complementary to a target sequence that comprises a sequence set forth in SEQ ID NO: 64, 66, 68, 70, 72, 74, or 76;

(iii) when the mutation is the c.32854G>C mutation in exon 219, a nucleic acid encoding a gRNA that is complementary to a target sequence that comprises a sequence set forth in SEQ ID NO: 1, 5, 9, or 13 and a nucleic acid comprising a donor template region comprising SEQ ID NO: 18; or

(iv) when the mutation is the c.37112G>A mutation in intron 242, a nucleic acid encoding a gRNA that is complementary to a target sequence that comprises a sequence set forth in SEQ ID NO: 57, 59, or 61 and a nucleic acid comprising a donor template region comprising SEQ ID NO: 63.

3. The method of claim 1 or the use of claim 2, wherein the gRNA is a single-molecule gRNA (sgRNA).

4. The method of claim 1 or the use of claim 2, wherein the gRNA is a modified gRNA or a modified single-molecule gRNA (sgRNA).

5. The method of claim 1 or the use of claim 2, wherein the nucleic acid

(a) is a DNA or RNA; and/or

(b) comprises a modified polynucleotide or a modified RNA.

6. The method of claim 1 or the use of claim 2, wherein the cell is a cardiomyocyte or a cardiac stem cell.

7. The method of claim 1 or the use of claim 2, wherein the subject suffers from a titinopathy or a titin-based cardiomyopathy.

8. The method of claim 1 or the use of claim 2, wherein the one or more DNA endonucleases is pre-complexed with one or more gRNAs or sgRNAs.

9. The method of claim 1 or the use of claim 2, wherein

(a) the Cas9 endonuclease or the homolog, codon-optimized, or modified version thereof that effects a break within the titin gene, or the nucleic acid that encodes the Cas9 endonuclease or a homolog, codon-optimized, or modified version thereof that effects a break within the titin gene;

(b) the nucleic acid encoding the gRNA; and/or

(c) the nucleic acid comprising the donor template

are each formulated separately into a lipid nanoparticle.

10. The method of claim 1 or the use of claim 2, wherein

(a) the Cas9 endonuclease or the homolog, codon-optimized, or modified version thereof that effects a break within the titin gene, or the nucleic acid that encodes the Cas9

endonuclease or a homolog, codon-optimized, or modified version thereof that effects a break within the titin gene;

(b) the nucleic acid encoding the gRNA; and/or

(c) the nucleic acid comprising the donor template

are co-formulated together into a lipid nanoparticle.

11. The method of claim 1 or the use of claim 2, wherein

(a) the nucleic acid that encodes the Cas9 endonuclease or a homolog, codon-optimized, or modified version thereof that effects a break within the titin gene;

(b) the nucleic acid encoding the gRNA; and/or

(c) the nucleic acid comprising the donor template

is provided in a vector.

12. The method of claim 1 or the use of claim 2, wherein

(a) the nucleic acid that encodes the Cas9 endonuclease or a homolog, codon-optimized, or modified version thereof that effects a break within the titin gene;

(b) the nucleic acid encoding the gRNA; and/or

(c) the nucleic acid comprising the donor template

is provided in the same vector.

13. The method or the use of claim 11 or 12, wherein the vector is adeno-associated virus (AAV).

14. The method of claim 1 or the use of claim 2, wherein the nucleic acid that encodes the Cas9 endonuclease or a homolog, codon-optimized, or modified version thereof that effects a break within the titin gene is formulated into a lipid nanoparticle, and the nucleic acid encoding the gRNA and/or the nucleic acid comprising the donor template are provided in a vector.

15. The method of claim 1 or the use of claim 2, wherein the titin gene is located on Chromosome 2q31 (Genome Reference Consortium – GRCh38/hg38).

16. The use of claim 2, wherein the medicament is for treating a subject with a titin-based cardiomyopathy or other titinopathy.

17. A nucleic acid encoding a guide ribonucleic acid (gRNA) that is complementary to a target sequence that comprises a sequence set forth in SEQ ID NO: 1, 5, 9, 13, 19, 23, 27-41, 57, 59, 61, 64, 66, 68, 70, 72, 74, or 76.

18. The nucleic acid of claim 17, wherein the gRNA is a single-molecule gRNA (sgRNA).

19. The nucleic acid of claim 17, wherein the gRNA is a modified gRNA or a modified single-molecule gRNA (sgRNA).