2698, 2701, 2755, 2758, 2763, 2785, 2805, 3341, 3344, 3403, 3404, 3405, 3410, 3421, 3422, 3430, 3432, 3435, 3437, 3464, 3472, 3475, 3479, 3485, 3496, 3503, 3519, 3523, 3524, 3531, 3534, 3542, 3549, 3556, 3558, and 3592. In another aspect, the present disclosure refers to an *ex vivo* method for inserting the C9ORF72 gene in a human cell by genome editing, the method comprising the step of: introducing into the human cell one or more Cas9 endonucleases and one or more guide ribonucleic acids (gRNAs) to effect one or more single-strand breaks (SSBs) or double-strand breaks (DSBs) within or near a safe harbor locus that results in a permanent insertion of the C9ORF72 gene or minigene, and results in restoration of the C9orf72 protein activity, wherein the one or more gRNAs comprise a spacer sequence selected from the group consisting of SEQ ID NOs 2669, 2673, 2681, 2687, 2689, 2694, 2697, 2698, 2701, 2755, 2758, 2763, 2785, 2805, 3341, 3344, 3403, 3404, 3405, 3410, 3421, 3422, 3430, 3432, 3435, 3437, 3464, 3472, 3475, 3479, 3485, 3496, 3503, 3519, 3523, 3524, 3531, 3534, 3542, 3549, 3556, 3558, and 3592. In another aspect of the present disclosure, there is provided one or more guide ribonucleic acids (gRNAs) comprising a spacer sequence selected from SEQ ID NOs: 2669, 2673, 2681, 2689, 2697, 2698, 2755, 2758, 2763, 2785, 2805, 3341, 3344, 3403, 3404, 3405, 3410, 3421, 3422, 3430, 3432, 3435, 3437, 3464, 3472, 3475, 3479, 3485, 3496, 3503, 3519, 3523, 3524, 3531, 3534, 3542, 3549, 3556, 3558 and 3592. In yet another aspect, the present disclosure refers to an *ex vivo* method of editing the C9ORF72 gene in a cell from a patient with amyotrophic lateral sclerosis (ALS) or frontotemporal lobular dementia (FTLD), said method comprises usingone or more guide ribonucleic acids (gRNAs) as disclosed herein, and one or more Cas9 endonucleases.

[0017] Provided herein are cellular, *ex vivo* and *in vivo* methods for creating permanent changes to the genome by deleting, inserting or correcting the expanded hexanucleotide repeat within or near the C9ORF72 gene or other DNA sequences that encode regulatory elements of the C9ORF72 gene or knocking in C9ORF72 cDNA or minigene into a safe harbor locus by genome editing and restoring C9orf72 protein activity, which can be used to treat amyotrophic lateral sclerosis (ALS) or frontotemporal lobular dementia (FTLD), as well as components, kits and compositions for performing such methods, and cells produced by them.

Claims

What is claimed is:

1. An *ex vivo* method for editing the C9ORF72 gene in a human cell by genome editing comprising introducing into the cell one or more Cas9 endonucleases and one or more guide ribonucleic acids (gRNAs) to effect one or more single-strand breaks (SSBs) or double-strand breaks (DSBs) within or near the C9ORF72 gene or other DNA sequences that encode regulatory elements of the C9ORF72 gene that results in permanent deletion, insertion, or correction of the expanded hexanucleotide repeat within or near the C9ORF72 gene and results in restoration of C9orf72 protein activity, wherein the one or more gRNAs comprise a spacer sequence selected from the group consisting of SEQ ID NOs 2669, 2673, 2681, 2687, 2689, 2694, 2697, 2698, 2701, 2755, 2758, 2763, 2785, 2805, 3341, 3344, 3403, 3404, 3405, 3410, 3421, 3422, 3430, 3432, 3435, 3437, 3464, 3472, 3475, 3479, 3485, 3496, 3503, 3519, 3523, 3524, 3531, 3534, 3542, 3549, 3556, 3558, and 3592.

2. An *ex vivo* method for inserting the C9ORF72 gene in a human cell by genome editing, the method comprising the step of: introducing into the human cell one or more Cas9 endonucleases and one or more guide ribonucleic acids (gRNAs) to effect one or more single-strand breaks (SSBs) or double-strand breaks (DSBs) within or near a safe harbor locus that results in a permanent insertion of the C9ORF72 gene or minigene, and results in restoration of the C9orf72 protein activity, wherein the one or more gRNAs comprise a spacer sequence selected from the group consisting of SEQ ID NOs 2669, 2673, 2681, 2687, 2689, 2694, 2697, 2698, 2701, 2755, 2758, 2763, 2785, 2805, 3341, 3344, 3403, 3404, 3405, 3410, 3421, 3422, 3430, 3432, 3435, 3437, 3464, 3472, 3475, 3479, 3485, 3496, 3503, 3519, 3523, 3524, 3531, 3534, 3542, 3549, 3556, 3558, and 3592.

3. The method of claim 1 or 2, wherein the one or more Cas9 endonucleases are encoded on one or more polynucleotides.

4. The method of any one of claims 1-3, wherein the one or more Cas9 endonucleases are encoded on one or more ribonucleic acids (RNAs).

5. The method according to claim 3 or 4, wherein the one or more polynucleotides or one or more RNAs are one or more modified polynucleotides or one or more modified RNAs.

6. The method of claim 1 or 2, wherein the one or more Cas9 endonucleases are proteins or polypeptides.

7. The method according to any one of the preceding claims, wherein the one or more gRNAs are single-molecule guide RNA (sgRNAs).

8. The method according to any one of the preceding claims, wherein the one or more gRNAs or one or more sgRNAs are one or more modified gRNAs or one or more modified sgRNAs.

9. The method of any one of claims 1-8, wherein the one or more Cas9 endonucleases and one or more gRNAs are either each formulated separately into lipid nanoparticles or all co-formulated into a lipid nanoparticle.

10. The method of any one of claims 1-8, wherein the one or more Cas9 endonucleases are formulated into a lipid nanoparticle, and the one or more gRNA are delivered by a viral vector.

11. The method of claim 10, wherein the viral vector is an adeno-associated virus (AAV) vector.

12. The method of claim 11, wherein the AAV vector is an AAV6 vector.

13. One or more guide ribonucleic acids (gRNAs) comprising a spacer sequence selected from SEQ ID NOs: 2669, 2673, 2681, 2689, 2697, 2698, 2755, 2758, 2763, 2785, 2805, 3341, 3344, 3403, 3404, 3405, 3410, 3421, 3422, 3430, 3432, 3435, 3437, 3464, 3472, 3475, 3479, 3485, 3496, 3503, 3519, 3523, 3524, 3531, 3534, 3542, 3549, 3556, 3558, and 3592.

14. The one or more gRNAs of claim 13, wherein the one or more gRNAs are one or more single-molecule guide RNAs (sgRNAs).

15. The one or more gRNAs or sgRNAs of claim 13 or 14, wherein the one or more gRNAs or one or more sgRNAs is one or more modified gRNAs or one or more modified sgRNAs.

16. An *ex vivo* method of editing the C9ORF72 gene in a cell from a patient with amyotrophic lateral sclerosis (ALS) or frontotemporal lobular dementia (FTLD), said method comprises using one or more guide ribonucleic acids (gRNAs) according to claims 13-15, and one or more Cas9 endonucleases.

17. The method of claim 16, wherein the one or more gRNAs are one or more single-molecule guide RNAs (sgRNAs).

18. The method of claim 16 or 17, wherein the one or more gRNAs or one or more sgRNAs is one or more modified gRNAs or one or more modified sgRNAs.