correction of a gene carrying a harmful mutation, for example, or to explore the function of a gene. Early technologies developed to insert a transgene into a living cell were often limited by the random nature of the insertion of the new sequence into the genome. Random insertions into the genome may result in disrupting normal regulation of neighboring genes leading to severe unwanted effects. Furthermore, random integration technologies offer little reproducibility, as there is no guarantee that the sequence would be inserted at the same place in two different cells. Recent genome engineering strategies, such as ZFNs, TALENs, HEs and MegaTALs, enable a specific area of the DNA to be modified, thereby increasing the precision of the correction or insertion compared to early technologies. These newer platforms offer a much larger degree of reproducibility.

[0015] Despite efforts from researchers and medical professionals worldwide who have been trying to address ALS and FTLD, and despite the promise of genome engineering approaches, there still remains a critical need for developing safe and effective treatments for ALS and FTLD.

[0016] Currently, all therapies for addressing ALS and FTLD aim at slowing the progression of the disease, rather than curing the disease. In contrast, the present invention presents an approach to correct the genetic basis of the disease. By using genome engineering tools to create permanent changes to the genome that can restore the C9ORF72 hexanucleotide repeat expansion with a single treatment, the resulting therapy should stop the disease progression completely.

Summary

[0016a] In one aspect, the present disclosure refers to 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. 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, 2688, 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, 3590 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.

[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.

Provided herein is a method for editing the C9ORF72 gene in a human cell by genome editing, the method comprising the step of introducing into the human cell one or more deoxyribonucleic acid (DNA) endonucleases to effect one or more single-strand breaks (SSBs) or one or more 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 at least one of a permanent insertion, correction, or modulation of expression or function of one or more mutations or exons within or near or affecting the expression or function of the C9ORF72 gene, or within or near a safe harbor locus that results in a permanent insertion of the C9ORF72 gene and results in restoration of C9orf72 protein activity.

[0018] Also provided herein is a 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 deoxyribonucleic acid (DNA) endonucleases 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 C9orf72 protein activity.

[0019] In one example, disclosed herein is a method for editing the C9ORF72 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 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 or within or near a safe harbor locus that results in permanent insertion of the C9ORF72 gene or minigene, and restoration of C9orf72 protein activity.

[0020] In another example, disclosed herein is an *ex vivo* method for treating a patient with amyotrophic lateral sclerosis (ALS) or frontotemporal lobular dementia (FTLD) comprising the steps of: creating a patient specific induced pluripotent stem cell (iPSC); editing within or near the C9ORF72 gene of the iPSC or other DNA sequences that encode regulatory elements of the C9ORF72 gene of the iPSC or editing within or near a safe harbor locus of the iPSC; differentiating the genome edited iPSC into a hematopoietic progenitor cell, a neural progenitor cell, or a neural

cell; and implanting the hematopoietic progenitor cell, neural progenitor cell, or neural cell into the patient.

[0021] In some examples, the step of creating a patient specific induced pluripotent stem cell comprises: isolating a somatic cell from the patient; and introducing a set of pluripotency-associated genes into the somatic cell to induce the cell to become a pluripotent stem cell. In some examples, the somatic cell is a fibroblast. In some examples, the set of pluripotency-associated genes is one or more of the genes selected from the group consisting of OCT4, SOX2, KLF4, Lin28, NANOG and cMYC.

[0022] The step of editing within or near the C9ORF72 gene or other DNA sequences that encode regulatory elements of the C9ORF72 gene or of editing within or near a safe harbor locus or of editing within or near a locus of the first exon of the C9ORF72 gene can comprise introducing into the iPSC one or more deoxyribonucleic acid (DNA) endonucleases 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 a permanent insertion, correction, or modulation of expression or function of one or more mutations or exons within or near or affecting the expression or function of the C9ORF72 gene or within or near a safe harbor locus that results in a permanent insertion of the C9ORF72 gene and results in restoration of C9orf72 protein activity. The safe harbor locus can be selected from the group consisting of: AAVS1 (PPP1R12C), ALB, Angptl3, ApoC3, ASGR2, CCR5, FIX (F9), G6PC, Gys2, HGD, Lp(a), Pcsk9, Serpina1, TF, and TTR. The safe harbor locus can be selected from the group consisting of: exon 1-2 of AAVS1 (PPP1R12C), exon 1-2 of ALB, exon 1-2 of Angptl3, exon 1-2 of ApoC3, exon 1-2 of ASGR2, exon 1-2 of CCR5, exon 1-2 of FIX (F9), exon 1-2 of G6PC, exon 1-2 of Gys2, exon 1-2 of HGD, exon 1-2 of Lp(a), exon 1-2 of Pcsk9, exon 1-2 of Serpina1, exon 1-2 of TF, and exon 1-2 of TTR.

[0023] In some examples, the step of editing the C9ORF72 gene of the iPSC comprises introducing into the iPSC one or more deoxyribonucleic acid (DNA) endonucleases to effect one or more double-strand breaks (DSBs) within or near the C9ORF72 gene that results in permanent deletion, insertion, or correction of the expanded hexanucleotide repeat within or near the C9ORF72 gene.

[0024] In some examples, the step of differentiating the genome edited iPSC into hematopoietic progenitor cell, a neural progenitor cell, or neural cell comprises one or more of the following: treatment with a combination of small molecules or delivery of master transcription factors.

[0025] In some examples, the step of implanting the hematopoietic progenitor cell, neural progenitor cell, or neural cell into the patient comprises implanting the hematopoietic progenitor cell, neural progenitor cell, or neural cell into the patient by transplantation, local injection, systemic infusion, or combinations thereof.

[0026] In another example, disclosed herein is an *ex vivo* method for treating a patient with amyotrophic lateral sclerosis (ALS) or frontotemporal lobular dementia (FTLD) comprising the steps of: isolating a white blood cell from the patient; editing the C9ORF72 gene of the white blood cell; and implanting the edited white blood cell into the patient.

[0027] In some examples, step of isolating a white blood cell comprises: cell differential centrifugation and cell culturing.

[0028] The step of editing within or near the C9ORF72 gene or other DNA sequences that encode regulatory elements of the C9ORF72 gene or of editing within or near a safe harbor locus or of editing within or near a locus of the first exon of the C9ORF72 gene can comprise introducing into the white blood cell one or more deoxyribonucleic acid (DNA) endonucleases 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 a permanent insertion, correction, or modulation of expression or function of one or more mutations or exons within or near or affecting the expression or function of the C9ORF72 gene or within or near a safe harbor locus that results in a permanent insertion of the C9ORF72 gene and results in restoration of C9orf72 protein activity. The safe harbor locus can be selected from the group consisting of: AAVS1 (PPP1R12C), ALB, Angptl3, ApoC3, ASGR2, CCR5, FIX (F9), G6PC, Gys2, HGD, Lp(a), Pcsk9, Serpina1, TF, and TTR. The safe harbor locus can be selected from the group consisting of: exon 1-2 of AAVS1 (PPP1R12C), exon 1-2 of ALB, exon 1-2 of Angptl3, exon 1-2 of ApoC3, exon 1-2 of ASGR2, exon 1-2 of CCR5, exon 1-2 of FIX (F9), exon 1-2 of G6PC, exon 1-2 of Gys2, exon 1-2 of HGD, exon

1-2 of Lp(a), exon 1-2 of Pcsk9, exon 1-2 of Serpina1, exon 1-2 of TF, and exon 1-2 of TTR.

[0029] In some examples, the step of editing the C9ORF72 gene of the neural cell comprises introducing into the neural cell one or more deoxyribonucleic acid (DNA) endonucleases to effect one or more double-strand breaks (DSBs) within or near the C9ORF72 gene that results in permanent deletion, insertion, or correction of the expanded hexanucleotide repeat within or near the C9ORF72 gene.

[0030] In some examples, the step of implanting the white blood cell into the patient comprises implanting the white blood cell into the patient by transplantation, local injection, systemic infusion, or combinations thereof.

[0031] In an example, disclosed herein is an *ex vivo* method for treating a patient with amyotrophic lateral sclerosis (ALS) or frontotemporal lobular dementia (FTLD) comprising the steps of: optionally, performing a biopsy of the patient's bone marrow; isolating a mesenchymal stem cell; editing within or near the C9ORF72 gene of the stem cell or other DNA sequences that encode regulatory elements of the C9ORF72 gene or editing within or near a safe harbor locus of the C9ORF72 gene; differentiating the stem cell into a hematopoietic progenitor cell, neural progenitor cell, or neural cell; and implanting the hematopoietic progenitor cell, neural progenitor cell, or neural cell into the patient.

[0032] In some examples, the step of isolating a mesenchymal stem cell comprises: aspiration of bone marrow and isolation of mesenchymal cells by density centrifugation using Percoll™.

[0033] The step of editing within or near the C9ORF72 gene or other DNA sequences that encode regulatory elements of the C9ORF72 gene or of editing within or near a safe harbor locus or of editing within or near a locus of the first exon of the C9ORF72 gene can comprise introducing into the mesenchymal stem cell one or more deoxyribonucleic acid (DNA) endonucleases 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 a permanent insertion, correction, or modulation of expression or function of one or more mutations or exons within or near or affecting the expression or function of the C9ORF72 gene or within or near a safe harbor locus that results in

a permanent insertion of the C9ORF72 gene and results in restoration of C9orf72 protein activity. The safe harbor locus can be selected from the group consisting of: AAVS1 (PPP1R12C), ALB, Angptl3, ApoC3, ASGR2, CCR5, FIX (F9), G6PC, Gys2, HGD, Lp(a), Pcsk9, Serpina1, TF, and TTR. The safe harbor locus can be selected from the group consisting of: exon 1-2 of AAVS1 (PPP1R12C), exon 1-2 of ALB, exon 1-2 of Angptl3, exon 1-2 of ApoC3, exon 1-2 of ASGR2, exon 1-2 of CCR5, exon 1-2 of FIX (F9), exon 1-2 of G6PC, exon 1-2 of Gys2, exon 1-2 of HGD, exon 1-2 of Lp(a), exon 1-2 of Pcsk9, exon 1-2 of Serpina1, exon 1-2 of TF, and exon 1-2 of TTR.

[0034] In some examples, the step of editing the C9ORF72 gene of the stem cell comprises introducing into the stem cell one or more deoxyribonucleic acid (DNA) endonucleases to effect one or more double-strand breaks (DSBs) within or near the C9ORF72 gene that results in permanent deletion, insertion, or correction of the expanded hexanucleotide repeat within or near the C9ORF72 gene.

[0035] In some examples, the step of differentiating the stem cell into a hematopoietic progenitor cell, neural progenitor cell, or neural cell comprises one or more of the following to differentiate the genome edited stem cell into a neural cell: treatment with a combination of small molecules or delivery of master transcription factors.

[0036] In some examples, step of implanting the cell into the patient comprises implanting the cell into the patient by transplantation, local injection, systemic infusion, or combinations thereof.

[0037] In another example, disclosed herein is an *ex vivo* method for treating a patient with amyotrophic lateral sclerosis (ALS) or frontotemporal lobular dementia (FTLD) comprising the steps of: optionally treating the patient with granulocyte colony stimulating factor (GCSF); isolating a hematopoietic progenitor cell from the patient; editing within or near the C9ORF72 gene of the hematopoietic progenitor cell or other DNA sequences that encode regulatory elements of the C9ORF72 gene or editing within or near a safe harbor locus of the C9ORF72 gene of the hematopoietic progenitor cell; and implanting the cell into the patient.

[0038] In some examples, the step of treating the patient with granulocyte colony stimulating factor (GCSF) is performed in combination with Plerixaflor.

[0039] In some examples, the step of isolating a hematopoietic progenitor cell from the patient comprises isolating CD34+ cells.

[0040] The step of editing within or near the C9ORF72 gene or other DNA sequences that encode regulatory elements of the C9ORF72 gene or of editing within or near a safe harbor locus or of editing within or near a locus of the first exon of the C9ORF72 gene can comprise introducing into the hematopoietic progenitor cell one or more deoxyribonucleic acid (DNA) endonucleases 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 a permanent insertion, correction, or modulation of expression or function of one or more mutations or exons within or near or affecting the expression or function of the C9ORF72 gene or within or near a safe harbor locus that results in a permanent insertion of the C9ORF72 gene and results in restoration of C9orf72 protein activity. The safe harbor locus can be selected from the group consisting of: AAVS1 (PPP1R12C), ALB, Angptl3, ApoC3, ASGR2, CCR5, FIX (F9), G6PC, Gys2, HGD, Lp(a), Pcsk9, Serpina1, TF, and TTR. The safe harbor locus can be selected from the group consisting of: exon 1-2 of AAVS1 (PPP1R12C), exon 1-2 of ALB, exon 1-2 of Angptl3, exon 1-2 of ApoC3, exon 1-2 of ASGR2, exon 1-2 of CCR5, exon 1-2 of FIX (F9), exon 1-2 of G6PC, exon 1-2 of Gys2, exon 1-2 of HGD, exon 1-2 of Lp(a), exon 1-2 of Pcsk9, exon 1-2 of Serpina1, exon 1-2 of TF, and exon 1-2 of TTR.

[0041] In some examples, the step of editing the C9ORF72 gene of the hematopoietic progenitor cell comprises introducing into the stem cell one or more deoxyribonucleic acid (DNA) endonucleases to effect one or more double-strand breaks (DSBs) within or near the C9ORF72 gene or other DNA sequences that encode regulatory elements of the C9ORF72 gene or editing within or near a safe harbor locus of the C9ORF72 gene that results in permanent deletion, insertion, or correction of the expanded hexanucleotide repeat within or near the C9ORF72 gene and restoration of C9orf72 protein activity.

[0042] In some examples, the step of implanting the cell into the patient comprises implanting the neural cell into the patient by transplantation, local injection, systemic infusion, or combinations thereof.

[0043] In another example, disclosed herein is an *in vivo* method for treating a patient with amyotrophic lateral sclerosis (ALS) or frontotemporal lobular dementia (FTLD) comprising the step of editing the C9ORF72 gene in a cell of the patient.

[0044] The step of editing within or near the C9ORF72 gene or other DNA sequences that encode regulatory elements of the C9ORF72 gene or of editing within or near a safe harbor locus or of editing within or near a locus of the first exon of the C9ORF72 gene can comprise introducing into the cell one or more deoxyribonucleic acid (DNA) endonucleases 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 a permanent insertion, correction, or modulation of expression or function of one or more mutations or exons within or near or affecting the expression or function of the C9ORF72 gene or within or near a safe harbor locus that results in a permanent insertion of the C9ORF72 gene and results in restoration of C9orf72 protein activity. The safe harbor locus can be selected from the group consisting of: AAVS1 (PPP1R12C), ALB, Angptl3, ApoC3, ASGR2, CCR5, FIX (F9), G6PC, Gys2, HGD, Lp(a), Pcsk9, Serpina1, TF, and TTR. The safe harbor locus can be selected from the group consisting of: exon 1-2 of AAVS1 (PPP1R12C), exon 1-2 of ALB, exon 1-2 of Angptl3, exon 1-2 of ApoC3, exon 1-2 of ASGR2, exon 1-2 of CCR5, exon 1-2 of FIX (F9), exon 1-2 of G6PC, exon 1-2 of Gys2, exon 1-2 of HGD, exon 1-2 of Lp(a), exon 1-2 of Pcsk9, exon 1-2 of Serpina1, exon 1-2 of TF, and exon 1-2 of TTR.

[0045] In some examples, the step of editing the C9ORF72 gene in a cell of the patient comprises introducing into the cell one or more deoxyribonucleic acid (DNA) endonucleases to effect one or more double-strand breaks (DSBs) within or near the C9ORF72 gene that results in permanent deletion, insertion, or correction of the expanded hexanucleotide repeat within or near the C9ORF72 gene.

[0046] In some *in vivo* examples, the cell is a neural cell, a bone marrow cell, a hematopoietic progenitor cell, or a CD34+ cell.

[0047] In some examples, the one or more DNA endonucleases is a Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas100, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2,

Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, or Cpf1 endonuclease; or a homolog, codon-optimized, or modified version thereof.

[0048] In some examples, the method comprises introducing into the cell one or more polynucleotides encoding the one or more DNA endonucleases. In some examples, the method comprises introducing into the cell one or more ribonucleic acids (RNAs) encoding the one or more DNA endonucleases. In some examples, the one or more polynucleotides or one or more RNAs is one or more modified polynucleotides or one or more modified RNAs.

[0049] In some examples, the method further comprises introducing into the cell one or more DNA endonucleases, wherein the DNA endonuclease is a protein or polypeptide.

[0050] In some examples, the method further comprises introducing into the cell one or more guide ribonucleic acids (gRNAs). In some examples, the one or more gRNAs are single-molecule guide RNA (sgRNAs). In some examples, the one or more gRNAs or one or more sgRNAs is one or more modified gRNAs or one or more modified sgRNAs.

[0051] In some examples, the one or more DNA endonucleases is pre-complexed with one or more gRNAs or one or more sgRNAs.

[0052] In some examples, the method further comprises introducing into the cell a polynucleotide donor template comprising a part of the wild-type C9ORF72 gene or cDNA. In some examples, the part of the wild-type C9ORF72 gene or cDNA is the entire C9ORF72 gene or cDNA, or the cDNA of natural Variant 1, Variant 2, or Variant 3. In some examples, the donor template is either single or double stranded. In some examples, the donor template has homologous arms to the 9p21.2 region.

[0053] In some examples, the method further comprises introducing into the cell one guide ribonucleic acid (gRNA) and a polynucleotide donor template comprising at least a portion of the wild-type C9ORF72 gene. In some examples, the method further comprises introducing into the cell one guide ribonucleic acid (gRNA) and a polynucleotide donor template comprising at least a portion of a codon-optimized or modified C9ORF72 gene. In some examples, the one or more DNA endonucleases

is one or more Cas9 or Cpf1 endonucleases that effect one single-strand break (SSB) or one double-strand break (DSB) at a locus within or near the C9ORF72 gene or other DNA sequences that encode regulatory elements of the C9ORF72 gene that facilitates insertion of a new sequence from the polynucleotide donor template into the chromosomal DNA at the DSB locus that results in permanent insertion or correction of a part of the chromosomal DNA of the C9ORF72 gene proximal to the locus or safe harbor locus, and wherein the gRNA comprises a spacer sequence that is complementary to a segment of the locus or safe harbor locus and restoration of C9orf72 protein activity. In some examples, proximal means nucleotides both upstream and downstream of the DSB locus.

[0054] In some examples, the method further comprises introducing into the cell two guide ribonucleic acid (gRNAs) and a polynucleotide donor template comprising at least a portion of the wild-type C9ORF72 gene, and wherein the one or more DNA endonucleases is two or more Cas9 or Cpf1 endonucleases that effect or create at least two (e.g., a pair) of single-strand breaks (SSBs) or a pair of double-strand breaks (DSBs), the first at a 5' locus and the second at a 3' locus, within or near the C9ORF72 gene or other DNA sequences that encode regulatory elements of the C9ORF72 gene or within or near a safe harbor locus that facilitates insertion of a new sequence from the polynucleotide donor template into the chromosomal DNA between the 5' locus and the 3' locus that results in permanent insertion or correction of the chromosomal DNA between the 5' locus and the 3' locus within or near the C9ORF72 gene or other DNA sequences that encode regulatory elements of the C9ORF72 gene or within or near a safe harbor locus, and wherein the first guide RNA comprises a spacer sequence that is complementary to a segment of the 5' locus and the second guide RNA comprises a spacer sequence that is complementary to a segment of the 3' locus.

[0055] In some examples, the one or two gRNAs are one or two single-molecule guide RNA (sgRNAs). In some examples, the one or two gRNAs or one or two sgRNAs is one or two modified gRNAs or one or two modified sgRNAs.

[0056] In some examples, the one or more DNA endonucleases is pre-complexed with one or two gRNAs or one or two sgRNAs.

[0057] In some examples, the part of the wild-type C9ORF72 gene or cDNA is the entire C9ORF72 gene or cDNA; the cDNA of natural Variant 1, Variant 2, or Variant 3; or about 30 hexanucleotide repeats.

[0058] In some examples, the donor template is either single or double stranded. In some examples, the donor template has homologous arms to the 9p21.2 region.

[0059] In some examples, the DSB, or 5’ DSB and 3’ DSB are in the first intron of the C9ORF72 gene.

[0060] The donor template can be either single or double stranded polynucleotide. The donor template can have homologous arms to a safe harbor locus, such as, for example, exon 1-2 of AAVS1 (PPP1R12C), exon 1-2 of ALB, exon 1-2 of Angptl3, exon 1-2 of ApoC3, exon 1-2 of ASGR2, exon 1-2 of CCR5, exon 1-2 of FIX (F9), exon 1-2 of G6PC, exon 1-2 of Gys2, exon 1-2 of HGD, exon 1-2 of Lp(a), exon 1-2 of Pcsk9, exon 1-2 of Serpina1, exon 1-2 of TF, and exon 1-2 of TTR.

[0061] In some examples, the insertion or correction is by homology directed repair (HDR).

[0062] In some examples, the method further comprises introducing into the cell two guide ribonucleic acid (gRNAs), and wherein the one or more DNA endonucleases is two or more Cas9 or Cpf1 endonucleases that effect a pair of double-strand breaks (DSBs), the first at a 5’ DSB locus and the second at a 3’ DSB locus, within or near the C9ORF72 gene that causes a deletion of the chromosomal DNA between the 5’ DSB locus and the 3’ DSB locus that results in permanent deletion of the chromosomal DNA between the 5’ DSB locus and the 3’ DSB locus within or near the C9ORF72 gene, and wherein the first guide RNA comprises a spacer sequence that is complementary to a segment of the 5' DSB locus and the second guide RNA comprises a spacer sequence that is complementary to a segment of the 3' DSB locus.

[0063] In some examples, both the 5’ DSB and 3’ DSB are in or near either the first exon, first intron, or second exon of the C9ORF72 gene.

[0064] In some examples, the deletion is a deletion of the expanded hexanucleotide repeat.

[0065] In some examples, the Cas9 or Cpf1 mRNA, gRNA, and donor template are either each formulated separately into lipid nanoparticles or all co-formulated into a lipid nanoparticle.

[0066] In some examples, the Cas9 or Cpf1 mRNA, gRNA, and donor template are formulated into separate exosomes or are co-formulated into an exosome.

[0067] In some examples, the Cas9 or Cpf1 mRNA is formulated into a lipid nanoparticle, and both the gRNA and donor template are delivered to the cell by a viral vector. In some examples, the viral vector is an adeno-associated virus (AAV) vector. In some examples, the AAV vector is an AAV6 vector.

[0068] The Cas9 or Cpf1 mRNA can be formulated into a lipid nanoparticle, and the gRNA can be delivered to the cell by electroporation and donor template can be delivered to the cell by a viral vector. The viral vector can be an adeno-associated virus (AAV) vector. The AAV vector can be an AAV6 vector.

[0069] In some examples, the C9ORF72 gene is located on Chromosome 9: 27,546,542 – 27,573,863 (Genome Reference Consortium – GRCh38/hg38).

[0070] In some examples, the restoration of C9orf72 protein activity is compared to wild-type or normal C9orf72 protein activity.

[0071] In another example, disclosed herein is one or more guide ribonucleic acids (gRNAs) comprising a spacer sequence selected from the group consisting of the nucleic acid sequences in SEQ ID NOs: 1-18807 for editing the C9ORF72 gene in a cell from a patient with amyotrophic lateral sclerosis (ALS) or frontotemporal lobular dementia (FTLD). In some examples, the one or more gRNAs are one or more single-molecule guide RNAs (sgRNAs). In some examples, the one or more gRNAs or one or more sgRNAs is one or more modified gRNAs or one or more modified sgRNAs.

[0072] Also disclosed herein is one or more guide ribonucleic acids (gRNAs) comprising a spacer sequence selected from the group consisting of the nucleic acid sequences in SEQ ID NOs: 18810-73668 for editing a safe harbor locus in a cell from a patient with amyotrophic lateral sclerosis (ALS) or frontotemporal lobular dementia (FTLD), wherein the safe harbor locus is selected from the group consisting of AAVS1 (PPP1R12C), ALB, Angptl3, ApoC3, ASGR2, CCR5, FIX (F9), G6PC, Gys2, HGD, Lp(a), Pcsk9, Serpina1, TF, and TTR. In some examples, the

one or more gRNAs are one or more single-molecule guide RNAs (sgRNAs). In some examples, the one or more gRNAs or one or more sgRNAs is one or more modified gRNAs or one or more modified sgRNAs.

[0073] Also disclosed herein are cells that have been modified by the preceding methods to permanently correct one or more mutations within the C9ORF72 gene, and restore C9orf72 protein activity. Further disclosed herein are methods for ameliorating amyotrophic lateral sclerosis (ALS) or frontotemporal lobular dementia (FTLD) by the administration of cells that have been modified by the preceding methods to an ALS or FTLD patient.

[0074] In some examples, the methods and compositions of the disclosure comprise one or more modified guide ribonucleic acids (gRNAs). Non-limiting examples of modifications can comprise one or more nucleotides modified at the 2' position of the sugar, in some examples a 2'-O-alkyl, 2'-O-alkyl-O-alkyl, or 2'-fluoro-modified nucleotide. In some examples, RNA modifications include 2'-fluoro, 2'-amino or 2' O-methyl modifications on the ribose of pyrimidines, abasic residues, desoxy nucleotides, or an inverted base at the 3' end of the RNA.

[0075] In some examples, the one or more modified guide ribonucleic acids (gRNAs) comprise a modification that makes the modified gRNA more resistant to nuclease digestion than the native oligonucleotide. Non-limiting examples of such modifications include those comprising modified backbones, for example, phosphorothioates, phosphorothyos, phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages.

[0076] In another example, disclosed herein is a method for treating a patient with amyotrophic lateral sclerosis (ALS) or frontotemporal lobular dementia (FTLD) comprising transplanting the bone marrow from a donor to the patient.

[0077] Various other aspects are described and exemplified herein.

Brief Description of the Drawings

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, 2688, 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, 3590 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 usingone or more guide ribonucleic acids (gRNAs) according to claims 13-15.

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.