

72(*C9orf72*) gene target nucleic acid sequences. The Class 2 Type V CRISPR proteins and guide nucleic acids are modified for passive entry into target cells. The Class 2 Type V CRISPR proteins and guide nucleic acids are useful in a variety of methods for target nucleic acid modification of *C9orf72*-related diseases, which methods are also provided.

[0007] In one example, the present disclosure relates to CasX:guide nucleic acid systems (CasX:gNA system) and methods used to alter a target nucleic acid comprising the *C9orf72* gene with one or more mutations or that comprises the hexanucleotide repeat sequence expansion (HRS) in cells. In some examples of the disclosure, the CasX:gNA system has utility in knocking-down or knocking-out a *C9orf72* gene with one or more mutations or that comprises the hexanucleotide repeat sequence expansion (HRS), in order to reduce or eliminate expression of the *C9orf72* gene product, accumulation of the RNA from the HRS, and/or the DPR in subjects having a *C9orf72*-related disease. In other examples, the CasX:gNA system has utility in correcting the *C9orf72* gene comprising the HRS.

[0007a] In one aspect, the present disclosure refers to a system comprising a CasX variant protein and a guide nucleic acid (gNA), wherein the CasX variant protein comprises a sequence having at least 90% sequence identity to SEQ ID NO: 138 and exhibits one or more improved characteristics relative to a CasX reference protein of any one of SEQ ID NOS: 1-3, wherein the CasX variant protein is capable of forming a ribonuclear protein complex (RNP) with the gNA, wherein the gNA comprises a targeting sequence complementary to a target nucleic acid sequence comprising a *chromosome 9 open reading frame 72 (C9orf72)* gene, and wherein the CasX variant protein comprises a non-target strand binding (NTSB) domain derived from amino acids 101-191 of SEQ ID NO:1 and a helical 1b domain derived from amino acids 192-332 of SEQ ID NO:1.

[0008] In some examples of the system, the gNA is a gRNA, or a gDNA, or a chimera of RNA and DNA, and may be a single-molecule gNA or a dual-molecule gNA. In other examples, the CasX:gNA system gNA has a targeting sequence complementary to a target nucleic acid sequence comprising a region within the *C9orf72* gene. In some examples, the targeting sequence of the gNA is selected from the group consisting of SEQ ID NOS: 309-343, 363-2100, 2295-2185, or a sequence having at least about 65%, at least about 75%, at least about 85%, or at least about 95% identity thereto. The gNA can comprise a targeting sequence comprising 14 to 30 consecutive nucleotides. In some examples, the targeting sequence of the gNA consists of 21 nucleotides. In other examples, the targeting sequence of the gNA consists of 20 nucleotides. In other examples, the targeting sequence consists of 19 nucleotides, the targeting sequence of the gNA having a sequence selected from the group consisting of SEQ ID NOS: 309-343, 363-2100, 2295-2185 with a single nucleotide removed from the 3' end of the sequence. In other examples, the targeting sequence consists of 18 nucleotides, having a sequence selected from the group consisting of SEQ ID NOS: 309-343, 363-2100, 2295-2185 with two nucleotides removed from the 3' end of the sequence. In other examples, the targeting sequence consists of 17 nucleotides, having a sequence selected from the group consisting of SEQ ID NOS: 309-343, 363-2100, 2295-2185 with three nucleotides removed from the 3' end of the sequence. In other examples, the targeting sequence consists of 16 nucleotides, having a sequence selected from the group consisting of

is selected from the group consisting of a *C9orf72* exon, a *C9orf72* intron, a *C9orf72* intron-exon junction, a *C9orf72* regulatory element, or combinations thereof, wherein the donor template is used to knock down or knock out the *C9orf72* gene or is used to correct the mutation in the *C9orf72* gene. In some examples, the donor template comprises a hexanucleotide repeat of a GGGGCC sequence wherein the number of repeats ranges from 10 to about 30 repeats and is utilized to replace the hexanucleotide repeat expansion of the mutant *C9orf72* gene. In some cases, the donor sequence is a single-stranded DNA template or a single stranded RNA template. In other cases, the donor template is a double-stranded DNA template.

[0013] In other examples, the disclosure relates to nucleic acids encoding the systems of any of the examples described herein, as well as vectors comprising the nucleic acids. In some examples, the vector is selected from the group consisting of a retroviral vector, a lentiviral vector, an adenoviral vector, an adeno-associated viral (AAV) vector, a herpes simplex virus (HSV) vector, a plasmid, a minicircle, a nanoplasmid, and an RNA vector. In other examples, the vector is a virus-like particle (VLP) comprising an RNP of a CasX and gNA of any of the examples described herein and, optionally, a donor template nucleic acid and a targeting moiety such as a viral-derived glycoprotein.

[0013a] In one aspect, the present disclosure refers to a nucleic acid comprising a sequence encoding: (a) a gNA comprising a targeting sequence complementary to a target nucleic acid sequence comprising a chromosome 9 open reading frame 72 (*C9orf72*) gene; and (b) a CasX variant protein comprising a sequence having at least 90% sequence identity to SEQ ID NO: 138 and exhibits one or more improved characteristics relative to a CasX reference protein of any one of SEQ ID NOS: 1-3, wherein the CasX variant protein is capable of forming a ribonuclear protein complex (RNP) with the gRNA, wherein the CasX variant protein further comprises one or more nuclear localization signals (NLS), and wherein the CasX variant protein comprises a non-target strand binding (NTSB) domain derived from amino acids 101-191 of SEQ ID NO:1 and a helical 1b domain derived from amino acids 192-332 of SEQ ID NO:1.

[0013b] In one aspect, the present disclosure refers to a vector comprising the nucleic acid disclosed herein, wherein the vector is selected from the group consisting of a retroviral vector, a lentiviral vector, an adenoviral vector, an adeno-associated viral (AAV) vector, a herpes simplex virus (HSV) vector, a virus-like particle (VLP), a plasmid, a minicircle, a nanoplasmid, and an RNA vector.

[0013c] In one aspect, the present disclosure refers to a host cell comprising the vector disclosed herein, wherein the host cell is selected from the group consisting of BHK, HEK293, HEK293T, NS0, SP2/0, YO myeloma cells, P3X63 mouse myeloma cells, PER, PER.C6, NIH3T3, COS, HeLa, CHO, and yeast cells.

[0014] In other examples, the disclosure provides a method of modifying a *C9orf72* target nucleic acid sequence of cells of a population, wherein said method comprises introducing into the cell: a) CasX:gNA system of any of the examples disclosed herein; b) the nucleic acid of any of the examples disclosed herein; c) the vector of any of the examples disclosed herein; d) the VLP of any of the examples disclosed herein; or e) a combination of the foregoing, wherein the *C9orf72* gene target nucleic acid sequence of the cells targeted by the first gNA is modified by the CasX protein, introducing a single- or double-stranded break in the target nucleic acid sequence. In some examples of the method, the method further comprises a second gNA or a nucleic acid encoding the second gNA, wherein the second gNA has a targeting sequence complementary to a different portion of the target nucleic acid sequence. In some examples of the method, the modifying comprises introducing an insertion, deletion, substitution, duplication, or inversion of one or more nucleotides in the target nucleic acid sequence as compared to the wild-type sequence. In some cases, the method further comprises contacting the target nucleic acid with a donor template nucleic acid of any of the examples disclosed herein. In some examples, the target *C9orf72* gene for modification comprises more than 30, more than 100, more than 500, more than 700, more than 1000, or more than 1600

copies of a hexanucleotide repeat sequence GGGGCC. In some examples of the method, the donor template comprises a nucleic acid comprising at least a portion of a *C9orf72* gene for correcting (by knocking in) the mutation of the *C9orf72* gene, or comprises a sequence comprising a mutation or heterologous sequence for knocking-down or knocking out the mutant *C9orf72* such that expression of the HRS or the DPR by the cells of the population is reduced by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% in comparison to a cell where the that has not been modified. In some cases, the modifying of the target nucleic acid sequence occurs *in vivo*. In some examples, the cell is a eukaryotic cell selected from the group consisting of a rodent cell, a mouse cell, a rat cell, a primate cell, a and a non-human primate cell. In some examples, the cell is a human cell. In some examples, the cell is a selected from the group consisting of a Purkinje cell, frontal cortex neuron, motor cortex neuron, hippocampus neuron, cerebellum neuron, upper motor neuron, spinal cord neuron, spinal cord motor neuron, glial cell, and astrocytes.

[0014a] In one aspect, the present disclosure refers to a method of modifying a *chromosome 9 open reading frame 72 (C9orf72)* gene target nucleic acid sequence in a population of cells *in vitro* or *ex vivo*, the method comprising the steps of, introducing into the population of cells: a. the system disclosed herein; b. the nucleic acid disclosed herein; c. the vector disclosed herein; or d. a combination thereof; and modifying the *C9orf72* gene target nucleic acid sequence in the population of cells targeted by the gNA and the CasX variant protein, wherein the modifying comprises: (i) introducing a double-stranded break in the *C9orf72* target nucleic acid sequence; and/or (ii) introducing an insertion, deletion, substitution, duplication, or inversion of one or more nucleotides in the *C9orf72* target nucleic acid sequence.

[0014b] In one aspect, the present disclosure refers to a population of cells modified *in vitro* or *ex vivo* by: a. the system disclosed herein; b. the nucleic acid disclosed herein; c. the vector disclosed herein; or d. a combination thereof, wherein the population of cells once modified include at least one of the following characteristics relative to a population of unmodified cells: (i) at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% do not express a detectable level of dipeptide repeat protein (DPR), (ii) expression of a functional *C9orf72* protein is increased by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90 %, and/or (iii) mutation of the *C9orf72* gene is corrected, resulting in expression of a functional *C9orf72* protein, wherein cells of the population of cells are selected from the group consisting of Purkinje cell, frontal cortex neuron, motor cortex neuron, hippocampus neuron, cerebellum neuron, upper motor neuron, spinal cord neuron, spinal cord motor neuron, glial cell, and astrocytes.

[0015] In other examples, the disclosure provides methods of modifying a *C9orf72* target nucleic acid in a population of cells of a subject wherein the target cells are contacted using vectors encoding the CasX protein and one or more gNAs comprising a targeting sequence complementary to the *C9orf72* gene, and optionally further comprising a donor template. In some cases, the vector is an Adeno-Associated Viral (AAV) vector selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV 44.9, AAV-Rh74, or AAVRh10. In other cases, the vector is a lentiviral vector. In other examples, the disclosure provides methods wherein the target cells are contacted using a vector wherein the vector is a virus-like particle (VLP) comprising an RNP of a CasX and gNA of any of the examples described herein and, optionally, a donor template nucleic acid. In some examples of the method, the vector is administered to a subject at a therapeutically effective dose. The subject can be a mouse, rat, pig, non-human primate, or a human. The dose can be administered by a route of administration selected from the group consisting of subcutaneous, intradermal, intraneural, intranodal, intramedullary, intramuscular, intralumbar, intrathecal, subarachnoid, intraventricular, intracapsular, intravenous, intralymphatic, or intraperitoneal routes, wherein the administering method is injection, transfusion, or implantation.

[0016] In other examples, the disclosure provides a method of treating a *C9orf72*-related disorder in a subject, comprising modifying a gene encoding *C9orf72* gene in cells of the subject, the modifying comprising contacting said cells with: a) CasX:gNA system of any of the examples disclosed herein; b) the nucleic acid of any of the examples disclosed herein; c) the vector of any of the examples disclosed herein; d) the VLP of any of the examples disclosed herein; or e) a combination of the foregoing wherein the *C9orf72* gene of the cells targeted by the first gNA is modified by the CasX protein. In some examples, the subject is selected from the group consisting of mouse, rat, pig, non-human primate, and human. In some examples, the *C9orf72*-related disorder is ALS or FTD. In some cases, the methods of treating a subject with a *C9orf72*-related disease result in improvement in at least one clinically-relevant parameter. In other cases, the methods of treating a subject with a *C9orf72*-related disease result in improvement in at least two clinically-relevant parameters.

[0017] In other examples, the disclosure provides compositions for use in a method of treating a *C9orf72*-related disorder in a subject. In some examples, the method comprises modifying a gene encoding *C9orf72* gene in cells of the subject, the modifying comprising contacting said cells with compositions selected from: a) CasX:gNA system of any of the examples disclosed herein; b) the nucleic acid of any of the examples disclosed herein; c) the vector of any of the examples disclosed herein; d) the VLP of any of the examples disclosed herein; or e) a combination of the foregoing wherein the *C9orf72* gene of the cells targeted by the first gNA is modified by the CasX protein. In some examples, the subject is selected from the group consisting of mouse, rat, pig, non-human primate, and human. In some examples, the *C9orf72*-related disorder is ALS or FTD. In some cases, the methods of treating a subject with a *C9orf72*-related disease result in improvement in at least one clinically-relevant parameter. In other cases, the methods of treating a subject with a *C9orf72*-related disease result in improvement in at least two clinically-relevant parameters.

[0017a] In one aspect, the present disclosure refers to a composition for use in treating a *chromosome 9 open reading frame 72 (C9orf72)*-related disorder, wherein the disorder is amyotrophic lateral sclerosis (ALS) or frontotemporal dementia (FTD), in a subject in need thereof, wherein cells of the subject are to be contacted with a therapeutically effective dose of the composition such that a *C9orf72* gene in the cells is to be modified, wherein the composition is selected from: a. the system disclosed herein; b. the nucleic acid disclosed herein; c. the vector disclosed herein; or d. combinations thereof, and wherein the *C9orf72* gene of the cells targeted by the gNA is modified by the CasX variant protein.

[0017b] In one aspect, the present disclosure refers to use of a composition in the manufacture of a medicament for treating a *chromosome 9 open reading frame 72 (C9orf72)*-related disorder, wherein the disorder is amyotrophic lateral sclerosis (ALS) or frontotemporal dementia (FTD), in a subject in need thereof, wherein cells of the subject are to be contacted with a therapeutically effective dose of the composition such that a *C9orf72* gene in the cells is to be modified, wherein the composition is selected from: a. the system disclosed herein; b. the nucleic acid disclosed herein; c. the vector disclosed herein; or d. combinations thereof, and wherein the *C9orf72* gene of the cells targeted by the gNA is modified by the CasX variant protein.

INCORPORATION BY REFERENCE

[0018] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. The contents of WO 2020/247882, filed on June 5, 2020, and U.S. provisional applications

CLAIMS

What is claimed is:

1. A system comprising a CasX variant protein and a guide nucleic acid (gNA), wherein the CasX variant protein comprises a sequence having at least 90% sequence identity to SEQ ID NO: 138 and exhibits one or more improved characteristics relative to a CasX reference protein of any one of SEQ ID NOS: 1-3, wherein the CasX variant protein is capable of forming a ribonuclear protein complex (RNP) with the gNA, wherein the gNA comprises a targeting sequence complementary to a target nucleic acid sequence comprising a *chromosome 9 open reading frame 72 (C9orf72)* gene, and wherein the CasX variant protein comprises a non-target strand binding (NTSB) domain derived from amino acids 101-191 of SEQ ID NO:1 and a helical 1b domain derived from amino acids 192-332 of SEQ ID NO:1.
2. The system of claim 1, wherein the *C9orf72* gene comprises one or more mutations, or wherein the *C9orf72* gene mutation comprises more than 30, more than 100, more than 500, more than 700, more than 1000, or more than 1600 copies of a hexanucleotide repeat sequence GGGGCC in a hexanucleotide repeat sequence expansion (HRS).
3. The system of claim 1, wherein:
 - (a) the gNA is a single-molecule guide RNA (gRNA) comprising a scaffold sequence and a targeting sequence, and wherein the scaffold sequence comprises a scaffold stem loop sequence of CCAGCGACUAUGUCGUAGUGG (SEQ ID NO: 32);
 - (b) the targeting sequence of the gNA comprises a sequence selected from the group consisting of SEQ ID NOS: 309-343, 363-2100 and 2295-21835, or a sequence having at least 90% identity thereto; and/or
 - (c) the gNA has a scaffold comprising a sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 2238.
4. The system of claim 1, further comprising a second gNA, wherein the second gNA has a targeting sequence complementary to a different or overlapping portion of the target nucleic acid sequence compared to the targeting sequence of the gNA.
5. The system of claim 4, wherein the gNA is complementary to a sequence that is 5' to the HRS and the targeting sequence of the second gNA is complementary to a sequence that is 5' or 3' to the HRS.
6. The system of claim 1, wherein the CasX variant protein further comprises one or more nuclear localization signals (NLS), wherein the one or more NLS are selected from the

group of sequences consisting of PKKKRKV (SEQ ID NO: 165), KRPAATKAGQAKKKK (SEQ ID NO: 166), PAAKRVKLD (SEQ ID NO: 167), RQRRNELKRSP (SEQ ID NO: 168), NQSSNFGPMKGGNFGGRSSGPYGGGGQYFAKPRNQGGY (SEQ ID NO: 169), RMRIZFKNKGKDTAELRRRRVEVSVELRKAKKDEQILKRRNV (SEQ ID NO: 170), VSRKRPRP (SEQ ID NO: 171), PPKKARED (SEQ ID NO: 172), PQPKKKPL (SEQ ID NO: 173), SALIKKKKKMAP (SEQ ID NO: 174), DRLRR (SEQ ID NO: 175), PKQKKRK (SEQ ID NO: 176), RKLKKKIKKL (SEQ ID NO: 177), REKKKFLKRR (SEQ ID NO: 178), KRKGDEVDGVDEVAKKK SKK (SEQ ID NO: 179), RKCLQAGMNLEARKTKK (SEQ ID NO: 180), PRPRKIPR (SEQ ID NO: 181), PPRKKRTVV (SEQ ID NO: 182), NLSKKKKRKREK (SEQ ID NO: 183), RRPSRPFRKP (SEQ ID NO: 184), KRPRSPSS (SEQ ID NO: 185), KRGINDRNFWRGENERKTR (SEQ ID NO: 186), PRPPKMARYDN (SEQ ID NO: 187), KRSFSKAF (SEQ ID NO: 188), KLKIKRPVK (SEQ ID NO: 189), PKTRRRPRRS QRKRPPT (SEQ ID NO: 191), RRKKRRPRRKRR (SEQ ID NO: 194), PKKKSRRPKKKSRK (SEQ ID NO: 195), HKKKHPDASVNFSEFSK (SEQ ID NO: 196), QRP GPYDRPQRP GPYDRP (SEQ ID NO: 197), LSPSLSPLLSPSLSP (SEQ ID NO: 198), RGKGGKGLGKGGAKRHRK (SEQ ID NO: 199), PKRGRGRPKRGRGR (SEQ ID NO: 200), MSRRRKANPTKLSENAKKLAKEVEN (SEQ ID NO: 192), PKKKRKVPPPPAAKRVKLD (SEQ ID NO: 190), PKKKRKVPPPPKKKKRKV (SEQ ID NO: 201), PAKRARRGYKC (SEQ ID NO: 202), KLGPRKATGRW (SEQ ID NO: 203), and PRRKREE (SEQ ID NO: 204), and wherein:

(i) the one or more NLS are within 50 amino acids of a C-terminus of the CasX variant protein,

(ii) the one or more NLS are within 50 amino acids of a N-terminus of the CasX variant protein, and/or

(iii) the CasX variant protein comprises at least two NLS that are within 50 amino acids of the N-terminus and the C-terminus of the CasX variant protein.

7. The system of claim 1, wherein the CasX variant protein and the gNA exhibit at least one or more improved characteristics as compared to the reference CasX protein of any one of SEQ ID NOS: 1-3 and a reference gNA of any one of SEQ ID NOS: 4-16, and

wherein the improved characteristic is selected from the group consisting of improved folding of the CasX variant protein, improved binding affinity to the gNA, improved binding affinity to a target DNA, improved ability to utilize a greater spectrum of one or more protospacer adjacent motif (PAM) sequences including ATC, CTC, GTC or TTC in the

editing of target DNA, improved unwinding of the target DNA, increased editing activity, improved editing efficiency, improved editing specificity, increased nuclease activity, increased target strand loading for double strand cleavage, decreased target strand loading for single strand nicking, decreased off-target cleavage, improved binding of non-target DNA strand, improved protein stability, improved protein solubility, improved protein:gNA RNP stability, improved protein:gNA complex solubility, improved protein yield, improved protein expression, and improved fusion characteristics, and

wherein the improved characteristic of the RNP of the CasX variant protein is at least 1.1 to 100,000-fold improved relative to the RNP of the reference CasX protein of any one of SEQ ID NOS: 1-3 and the reference gRNA comprising any one of SEQ ID NOS: 4-16.

8. The system of claim 1, wherein the CasX variant protein comprises a nuclease domain having double-stranded cleavage activity.

9. A nucleic acid comprising a sequence encoding:

(a) a gNA comprising a targeting sequence complementary to a target nucleic acid sequence comprising a chromosome 9 open reading frame 72 (*C9orf72*) gene; and

(b) a CasX variant protein comprising a sequence having at least 90% sequence identity to SEQ ID NO: 138 and exhibits one or more improved characteristics relative to a CasX reference protein of any one of SEQ ID NOS: 1-3, wherein the CasX variant protein is capable of forming a ribonuclear protein complex (RNP) with the gRNA, wherein the CasX variant protein further comprises one or more nuclear localization signals (NLS), and wherein the CasX variant protein comprises a non-target strand binding (NTSB) domain derived from amino acids 101-191 of SEQ ID NO:1 and a helical 1b domain derived from amino acids 192-332 of SEQ ID NO:1.

10. A vector comprising the nucleic acid of claim 9, wherein the vector is selected from the group consisting of a retroviral vector, a lentiviral vector, an adenoviral vector, an adeno-associated viral (AAV) vector, a herpes simplex virus (HSV) vector, a virus-like particle (VLP), a plasmid, a minicircle, a nanoplasmid, and an RNA vector.

11. The vector of claim 10, wherein the vector is an AAV vector selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12, AAV44.9, AAV-Rh74, and AAVRh10.

12. A host cell comprising the vector of claim 10, wherein the host cell is selected from the group consisting of BHK, HEK293, HEK293T, NS0, SP2/0, YO myeloma cells, P3X63 mouse myeloma cells, PER, PER.C6, NIH3T3, COS, HeLa, CHO, and yeast cells.

13. A method of modifying a *chromosome 9 open reading frame 72 (C9orf72)* gene target nucleic acid sequence in a population of cells *in vitro* or *ex vivo*, the method comprising the steps of,

introducing into the population of cells:

- a. the system of claim 1;
- b. the nucleic acid of claim 9;
- c. the vector of claim 10; or
- d. a combination thereof; and

modifying the *C9orf72* gene target nucleic acid sequence in the population of cells targeted by the gNA and the CasX variant protein, wherein the modifying comprises:

(i) introducing a double-stranded break in the *C9orf72* target nucleic acid sequence; and/or

(ii) introducing an insertion, deletion, substitution, duplication, or inversion of one or more nucleotides in the *C9orf72* target nucleic acid sequence.

14. The method of claim 13, wherein the cell is a eukaryotic cell selected from the group consisting of a rodent cell, a mouse cell, a rat cell, a pig cell, a primate cell, and a non-human primate cell.

15. The method of claim 14, wherein the population of cells is selected from the group consisting of Purkinje cell, frontal cortex neuron, motor cortex neuron, hippocampus neuron, cerebellum neuron, upper motor neuron, spinal cord neuron, spinal cord motor neuron, glial cell, and astrocytes.

16. A population of cells modified *in vitro* or *ex vivo* by:

- a. the system of claim 1;
- b. the nucleic acid of claim 9;
- c. the vector of claim 10; or
- d. a combination thereof,

wherein the population of cells once modified include at least one of the following characteristics relative to a population of unmodified cells:

- (i) at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% do not express a detectable level of dipeptide repeat protein (DPR),
- (ii) expression of a functional *C9orf72* protein is increased by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90 %, and/or

(iii) mutation of the *C9orf72* gene is corrected, resulting in expression of a functional *C9orf72* protein,

wherein cells of the population of cells are selected from the group consisting of Purkinje cell, frontal cortex neuron, motor cortex neuron, hippocampus neuron, cerebellum neuron, upper motor neuron, spinal cord neuron, spinal cord motor neuron, glial cell, and astrocytes.

17. A composition for use in treating a *chromosome 9 open reading frame 72 (C9orf72)*-related disorder, wherein the disorder is amyotrophic lateral sclerosis (ALS) or frontotemporal dementia (FTD), in a subject in need thereof, wherein cells of the subject are to be contacted with a therapeutically effective dose of the composition such that a *C9orf72* gene in the cells is to be modified, wherein the composition is selected from:

- a. the system of claim 1;
- b. the nucleic acid of claim 9;
- c. the vector of claim 10; or
- d. combinations thereof, and

wherein the *C9orf72* gene of the cells targeted by the gNA is modified by the CasX variant protein.

18. Use of a composition in the manufacture of a medicament for treating a *chromosome 9 open reading frame 72 (C9orf72)*-related disorder, wherein the disorder is amyotrophic lateral sclerosis (ALS) or frontotemporal dementia (FTD), in a subject in need thereof, wherein cells of the subject are to be contacted with a therapeutically effective dose of the composition, such that a *C9orf72* gene in the cells is to be modified, wherein the composition is selected from:

- a. the system of claim 1;
- b. the nucleic acid of claim 9;
- c. the vector of claim 10; or
- d. combinations thereof, and

wherein the *C9orf72* gene of the cells targeted by the gNA is modified by the CasX variant protein.