

What is claimed is:

1. A fusion protein comprising a zinc finger protein (ZFP) domain and a transcription repressor domain, wherein the ZFP domain binds to a target region in an intronic segment between exons 1a and 1b on a mutant allele of a human *C9orf72* gene, wherein the target region comprises more than 30 tandem repeats of G₄C₂ (SEQ ID NO:1), and wherein the ZFP domain comprises six recognition helix regions as shown in a single row of the table below, wherein “^” indicates that arginine is substituted with glutamine at the fourth position upstream of the first amino acid in the indicated helix, and wherein the SEQ ID NO of each sequence is indicated in parentheses:

RSDHLSE (33)	SSRYRTK (56)	RSDHLSE (33)	SSRYRTK (56)	RSDHLSE (33)	SSRYRTK (56)
REQDLKQ (57)	HRKSLSR (41)	REQDLKQ (57)	HRKSLSR (41)	REQDLKQ (57)	HRKSLSR (41)
DRSHLTR ^(53)	DSSTRKT (54)	DRSHLTR (53)	DSSTRKT (54)	DRSHLTR (53)	DSSTRKT (54)
DKRDLAR ^(55)	RSADRKT (45)	DKRDLAR (55)	RSADRKT (45)	DKRDLAR (55)	RSADRKT (45)
ERRDLRR ^(77)	RSADRKT (45)	ERRDLRR (77)	RSADRKT (45)	ERRDLRR (77)	RSADRKT (45)
RSDHLSE (33)	DRSHLAR ^(34)	RSDHLSE ^(33)	DRSHLAR (34)	RSDHLSE ^(33)	DRSHLAR (34)
RSDHLSE ^(33)	DRSHLAR ^(34)	RSDHLSE ^(33)	DRSHLAR (34)	RSDHLSE ^(33)	DRSHLAR (34)
RSDHLSE ^(33)	DRSHLAR (34)	RSDHLSE ^(33)	DRSHLAR ^(34)	RSDHLSE (33)	DRSHLAR ^(34)
RSAHLSR ^(29)	HRKSLSR (41)	RSAHLSR ^(29)	HRKSLSR (41)	RSAHLSR (29)	HRKSLSR (41)
RSAHLSR (29)	HRKSLSR (41)	RSAHLSR ^(29)	HRKSLSR (41)	RSAHLSR ^(29)	HRKSLSR (41)
RSAHLSR (29)	HRKSLSR (41)	RSAHLSR ^(29)	HRKSLSR (41)	RSAHLSR (29)	HRKSLSR ^(41)
RSAHLSR (29)	HRKSLSR ^(41)	RSAHLSR ^(29)	HRKSLSR (41)	RSAHLSR ^(29)	HRKSLSR (41)
RSAHLSR (29)	HRKSLSR ^(41)	RSAHLSR (29)	HRKSLSR ^(41)	RSAHLSR (29)	HRKSLSR ^(41)
REQDLKQ ^(57)	HRKSLSR ^(41)	REQDLKQ ^(57)	HRKSLSR ^(41)	REQDLKQ (57)	HRKSLSR (41)
REQDLKQ (57)	HRKSLSR ^(41)	REQDLKQ (57)	HRKSLSR ^(41)	REQDLKQ (57)	HRKSLSR (41)
RNADRIT ^(46)	HRKSLSR ^(41)	RNADRIT ^(46)	HRKSLSR (41)	RNADRIT ^(46)	HRKSLSR (41)

2. The fusion protein of claim 1, wherein the fusion protein represses transcription of repeat-containing mRNA from the mutant allele and does not repress transcription of wildtype mRNA from the gene.

3. The fusion protein of claim 1 or 2, wherein the ZFP domain binds to a sense sequence

in the target region, wherein the sense sequence comprises one to three tandem repeats of hexanucleotide GGGGCC (SEQ ID NO:1), GGGCCG (SEQ ID NO:2), GGCCGG (SEQ ID NO:3), GCCGGG (SEQ ID NO:4), CCGGGG (SEQ ID NO:5), or CGGGGC (SEQ ID NO:6).

4. The fusion protein of any one of claims 1–3, wherein the fusion protein represses sense transcription from the mutant *C9orf72* allele in a human cell.

5. The fusion protein of claim 4, wherein the fusion protein represses sense transcription from the *C9orf72* 1a promoter and does not repress sense transcription from the *C9orf72* 1b promoter.

6. The fusion protein of claim 1 or 2, wherein the ZFP domain binds to an antisense sequence in the target region, wherein the antisense sequence comprises one to three tandem repeats of hexanucleotide GGCCCC (SEQ ID NO:7), GCCCCG (SEQ ID NO:8), CCCC GG (SEQ ID NO:9), CCCGGC (SEQ ID NO:10), CCGGCC (SEQ ID NO:11), or CGGCCC (SEQ ID NO:12).

7. The fusion protein of any one of the preceding claims, wherein the fusion protein represses antisense transcription from the mutant *C9orf72* allele in a human cell.

8. The fusion protein of any one of the preceding claims, wherein the fusion protein represses both sense transcription and antisense transcription from the mutant *C9orf72* allele in a human cell.

9. The fusion protein of any one of the preceding claims, wherein the fusion protein represses sense and/or antisense transcription from the mutant *C9orf72* allele by at least 30%, 40%, 75%, 90%, or 95%.

10. The fusion protein of claim 9, wherein the fusion protein does not repress sense transcription from the *C9orf72* 1b promoter.

11. The fusion protein of any one of the preceding claims, wherein the transcription repressor domain comprises a KRAB domain amino acid sequence from human KOX1.

12. The fusion protein of any one of the preceding claims, wherein the ZFP domain is linked to the transcription repressor domain through a peptide linker.
13. The fusion protein of claim 1, wherein the ZFP DNA-binding domain comprises six fingers respectively comprising SEQ ID NOs: 33, 34, 33, 34, 33, and 34 and wherein the ZFP DNA-binding domain comprises an arginine to glutamine substitution at the fourth position upstream of each of the second, third, and fifth fingers.
14. The fusion protein of claim 1, wherein the ZFP DNA-binding domain comprises six fingers respectively comprising SEQ ID NOs: 33, 34, 33, 34, 33, and 34, and wherein the ZFP DNA-binding domain comprises an arginine to glutamine substitution at the fourth position upstream of each of the first, second, third, and fifth fingers.
15. The fusion protein of claim 1, wherein the ZFP DNA-binding domain comprises six fingers respectively comprising SEQ ID NOs: 33, 34, 33, 34, 33, and 34, and wherein the ZFP DNA-binding domain comprises an arginine to glutamine substitution at the fourth position upstream of each of the first, third, fourth, and sixth fingers.
16. The fusion protein of claim 1, wherein the ZFP DNA-binding domain comprises six fingers respectively comprising SEQ ID NOs: 29, 41, 29, 41, 29, and 41, and wherein the ZFP DNA-binding domain comprises an arginine to glutamine substitution at the fourth position upstream of each of the first and third fingers.
17. The fusion protein of claim 1, wherein the ZFP DNA-binding domain comprises six fingers respectively comprising SEQ ID NOs: 29, 41, 29, 41, 29, and 41, and wherein the ZFP DNA-binding domain comprises an arginine to glutamine substitution at the fourth position upstream of each of the third and fifth fingers.
18. The fusion protein of claim 1, wherein the ZFP DNA-binding domain comprises six fingers respectively comprising SEQ ID NOs: 29, 41, 29, 41, 29, and 41, and wherein the ZFP DNA-binding domain comprises an arginine to glutamine substitution at the fourth position upstream of each of the third and sixth fingers.

19. The fusion protein of claim 1, wherein the ZFP DNA-binding domain comprises six fingers respectively comprising SEQ ID NOs: 53, 54, 53, 54, 53, and 54 , and wherein the ZFP DNA-binding domain comprises an arginine to glutamine substitution at the fourth position upstream of the first finger.
20. The fusion protein of claim 1, wherein the ZFP DNA-binding domain comprises six fingers respectively comprising SEQ ID NOs: 55, 45, 55, 45, 55, and 45 , and wherein the ZFP DNA-binding domain comprises an arginine to glutamine substitution at the fourth position upstream of the first finger.
21. The fusion protein of claim 1, wherein the ZFP DNA-binding domain comprises six fingers respectively comprising SEQ ID NOs: 77, 45, 77, 45, 77, and 45 , and wherein the ZFP DNA-binding domain comprises an arginine to glutamine substitution at the fourth position upstream of the first finger.
22. The fusion protein of claim 1, wherein the ZFP DNA-binding domain comprises six fingers respectively comprising SEQ ID NOs: 33, 56, 33, 56, 33, and 56.
23. The fusion protein of claim 1, wherein the ZFP DNA-binding domain comprises six fingers respectively comprising SEQ ID NOs: 57, 41, 57, 41, 57, and 41.
24. The fusion protein of claim 1, wherein the ZFP DNA-binding domain comprises six fingers respectively comprising SEQ ID NOs: 46, 41, 46, 41, 46, and 41, and wherein the ZFP DNA-binding domain comprises an arginine to glutamine substitution at the fourth position upstream of each of the first, second, third, and fifth fingers.
25. A nucleic acid construct comprising a coding sequence for the fusion protein of any one of claims 1–24, wherein the coding sequence is linked operably to a transcription regulatory element.
26. The nucleic acid construct of claim 25, wherein the transcription regulatory element is a mammalian promoter that is constitutively active or inducible in a brain cell, optionally wherein the promoter is a human synapsin I promoter.

27. The nucleic acid construct of claim 25 or 26, wherein the construct is a viral construct, optionally wherein the viral construct is a recombinant adeno-associated viral construct.
28. A host cell comprising the nucleic acid construct of any one of claims 25–27.
29. The host cell of claim 28, wherein the host cell is a human cell.
30. The host cell of claim 29, wherein the human cell is a neuron or a pluripotent stem cell, wherein the stem cell is optionally an embryonic stem cell or an inducible pluripotent stem cell (iPSC).
31. A recombinant virus comprising the nucleic acid construct of claim 27, optionally wherein the recombinant virus is a recombinant adeno-associated virus (rAAV).
32. A pharmaceutical composition comprising the nucleic acid construct of any one of claims 25–27, or the recombinant virus of claim 31, and a pharmaceutically acceptable carrier.
33. A method of inhibiting transcription of a mutant allele of a *C9orf72* gene in a human cell *in vitro*, wherein the mutant allele comprises an expanded G₄C₂ (SEQ ID NO:1) repeat region in an intronic segment between exons 1a and 1b, the method comprising introducing to the cell a fusion protein of any one of claims 1–24, a nucleic acid construct of any one of claims 25–27, a recombinant virus of claim 31, or a pharmaceutical composition of claim 32.
34. The method of claim 33, wherein the human cell is a neuron, a glial cell, an ependymal cell, or a neuroepithelial cell.
35. Use of a fusion protein of any one of claims 1–24, a nucleic acid construct of any one of claims 25–27, a recombinant virus of claim 31, or a pharmaceutical composition of claim 32 in the manufacture of a medicament for treating a patient suffering from amyotrophic lateral sclerosis (ALS) or C9 familial frontotemporal dementia (C9FTD), wherein the fusion protein,

the nucleic acid construct, the recombinant virus, or the pharmaceutical composition is to be introduced to the patient.

36. The use of claim 35, wherein the fusion protein is to be introduced through a recombinant virus that expresses the fusion protein.

37. The use of claim 36, wherein the recombinant virus is an adeno-associated virus (AAV), optionally of serotype 9 or pseudotype AAV2/9 or AAV2/6/9.

38. The use of claim 36 or 37, wherein the recombinant virus is to be administered via an intracerebroventricular, intrathecal, intracranial, retro-orbital (RO), intravenous, intranasal and/or intracisternal route to the patient.

39. The use of any one of claims 35–38, wherein two or more fusion proteins are to be introduced, optionally wherein the coding sequences for the two or more fusion proteins are on the same recombinant viral vector.

40. Use of a nucleic acid construct of any one of claims 25–27 or a recombinant virus of claim 31 for the manufacture of a medicament for treating a patient suffering from amyotrophic lateral sclerosis (ALS) or C9 familial frontotemporal dementia (C9FTD).